Molecular studies of Asphalt, Mummy and Kassel earth pigments

their characterisation, identification and effect on the drying of traditional oil paint

Georgiana M. Languri

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Prof. Dr. P. J. Schoenmakers
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Faculteit der Natuurwetenschappen, Wiskunde en Informatica.

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MOLART and MOLART Reports

MOLART - Molecular Aspects of Ageing of Painted Art - was a 5-year cooperative project between art historians, restorers, analytical chemists and technical physicists funded by the Dutch Organisation for Scientific Research (NWO). Technical support and advice was given by Shell-SRTCA (Amsterdam), AKZO-NOBEL (Arnhem), Instituut Collectie Nederland (ICN, Amsterdam) and the Dutch art museums. The project was launched on 1 February 1995 and ended in early 2003. The object of MOLART was to contribute to the development of a scientific framework for the conservation of painted art on the molecular level. The focus of MOLART was the determination of the present chemical and physical condition of works of art produced in the period from the 15th to the 20th century. Studies of historical paint manufacturing and workshop practice must give insight into the nature of the painters' media and the painting technique used originally. Fundamental studies on varnishes, paint, and colorants are undertaken to understand the molecular aspects of ageing since this is thought to be a main cause for the continued need to treat paintings.

This report is the ninth in a series of MOLART reports that will summarise all research results obtained in the course of the project. Information about MOLART can be obtained from the project co-ordinator Prof. Dr. J.J. Boon, FOM-Institute for Atomic and Molecular Physics, Kruislaan 407, 1098 SJ Amsterdam, The Netherlands, boon@amolf.nl.

1. *Molecular studies of fresh and aged triterpenoid varnishes*, Gisela A. van der Doelen, 1999. ISBN 90-801704-3-7
2. *A mathematical study on craquelure and other mechanical damage in paintings*, Petri de Willigen, 1999. ISBN 90-407-1946-2
3. *Solvent extractable components of oil paint films, Kenneth R. Sutherland*, 2001. ISBN 90-801704-4-5
4. *Molecular changes in egg tempera paint dosimeters as tools to monitor the museum environment,* Oscar F. van den Brink, 2001. ISBN 90-801704-6-1
5. *Discoloration in renaissance and baroque oil paintings*, Margriet van Eikema Hommes, 2002. In Press: Archetype Publications, London.
6. *Analytical chemical studies on traditional linseed oil paints*, Jorrit D.J. van den Berg, 2002. ISBN *90*-801704-7-X
7. *Microspectroscopic analysis of traditional oil paint*, Jaap van der Weerd, 2002. ISBN 90-801704-8-8

8: *Laser Desorption Mass Spectrometric Studies of Artists' Organic Pigments,* Nicolas Wyplosz, 2003. ISBN 90-77209-02-6

Forthcoming

10: *Analysis of diterpenoid resins and polymers in paint media and varnishes: with an atlas of mass spectra,* Klaas Jan van den Berg (forthcoming 2004).

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Chapter 2

Languri, G.M., van der Horst, J., and Boon, J.J., 'Characterisation of a "unique" asphalt sample from the early 19th century Hafkenscheid painting materials collection by analytical pyrolysis MS and GC/MS', *Journal of Analytical and Applied Pyrolysis*, 2002, **63**, 171-196.

Languri, G.M., van der Horst, J., and Boon, J.J., Erratum to 'Characterisation of a "unique" asphalt sample from the early 19th century Hafkenscheid painting materials collection by analytical pyrolysis MS and GC/MS', *Journal of Analytical and Applied Pyrolysis*, 2002, **64**, 123-124.

Languri, G.M., and Boon, J.J., 'Asphalt-Preliminary results', *MOLART Evaluation*, Amsterdam, The Netherlands, 1998, 75-83.

Chapter 3

Languri, G.M. and Boon, J.J, 'Between Myth and Reality: On the Mummy Pigment from the 19th century Hafkenscheid Collection', submitted to Studies in Conservation (2003).

Chapter 4

Languri, G.M., and Boon, J.J., 'Mass spectrometry as a micro-analytical method to study Kassel earth (Vandyke brown) pigments in oil paint', in R. Van Grieken et al, *7th International Conference of Non-destructive Testing and Microanalysis for the diagnostics and Conservation of the Cultural and Environmental Heritage*, Antwerp, Belgium, 2002.

Languri, G.M., Eijkel, G.B., and Boon, J.J., 'Characterisation of molecular markers and biomarkers in a series of Kassel earth pigments', in preparation.

Chapter 5

Languri, G.M., van den Berg, J.D.J., Boon, J.J., 'Effects of additions of mastic, Copaiba Balsam, asphalt or earth pigments on the chemical drying of oil and oil paint', in A. Phenix, *Deterioration of Artists' Paints: Effects and Analysis*, a joint meeting of ICOM-CC Working Groups Paintings 1 and 2 and The Paintings Section, UKIC, London, UK, 2001, 25-26.

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Languri, G.M., et. al., 'On poorly drying 19th-century oil paints: reference materials, model systems and paintings', in preparation.

Other publications

Boitelle, R., van den Berg, K.J., Geldof, M., and Languri, G.M., 'Descending into details of Théodore Rousseau's *La descente des vaches* (Museum Mesdag, the Hague), Technical Research of a Darkened Painting', in preparation.

Boitelle, R., van den Berg, K.J., Geldof, M., and Languri, G.M., 'Descending into details of Théodore Rousseau's *La descente des vaches* (Museum Mesdag, the Hague), Technical Research of a Darkened Painting', in A. Phenix, *Deterioration of Artists' Paints: Effects and Analysis*, a joint meeting of ICOM-CC Working Groups Paintings 1 and 2 and The Paintings Section, UKIC, London, UK, 2001, 27-32.

Contents

List of Abbreviations

AmE	Alcohol methyl ethers
AMOLF	FOM Institute for Atomic and Molecular Physics
amu	Atomic mass unit
BP	Base Peak
CCI	Canadian Conservation Institute
DA	Discriminant Analysis
da	dicarboxylic acid, diacid
Dalton	see amu
DCM	Dichloromethane
DHA	Dehydroabietic acid
DTMS	Direct Temperature resolved Mass Spectrometry
DTMS/MS	DTMS tandem Mass Spectrometry
EI	Electron Ionisation
ESI	Electro Spray Ionisation
ESI- FTICR-MS	Electro Spray Ionisation Fourier Transform Ion Cyclotron Resonance Mass Spectrometry
EtOH	Ethanol
FAB/MS	Fast Atom Bombardment Mass Spectrometry
FAB/MS/MS	FAB tandem Mass Spectrometry
fame (FAmE)	Fatty acid methyl ester
FOM	Fundamenteel Onderzoek der Materie
FTIR	Fourier Transform Infrared Spectroscopy
GC	Gas Chromatography
GC/C-IRMS	GC/combustion/isotope ratio Mass Spectrometry
GC/MS	Gas Chromatography/Mass Spectrometry
GLC	Gas Liquid Chromatography
HMW	High Molecular Weight
HPLC	High Pressure Liquid Chromatography
HPSEC	High Pressure Size Exclusion Chromatography
HR FAB/MS	High Resolution Fast Atom Bombardment Mass Spectrometry
IR	Infrared
LC	Liquid Chromatography
LcFA	Long chain Fatty Acid
LMW	Low Molecular Weight
M	Molecular ion
Me	Methyl group
MOLART	Molecular aspects of ageing in painted art
MPLC	Medium Pressure Liquid Chromatography

MS	Mass Spectrometry
MW	Molecular Weight
NWO	Nederlandse Organisatie voor Wetenschappelijk Onderzoek
PCA	Principal Component Analysis
Py-	Pyrolysis
Py-GC/MS	Pyrolysis Gas Chromatography Mass Spectrometry
Py-TMAH-GC/MS	Pyrolysis Transesterification Gas Chromatography Mass Spectrometry
ScFA	Short chain Fatty Acid
SEC	Size Exclusion Chromatography
SEM / EDX	Scanning Electron Microscopy / Energy Dispersive X-ray Analyser
SRAL	Stichting Restauratie Atelier Limburg
TAG	Triglyceride
TD-GC/MS	Thermal Desorption Gas Chromatography Mass Spectrometry
TIC	Total Ion Current/ Total Ion Chromatogram
TLC	Thin Layer Chromatography
TMAH	Tetramethylammonium hydroxide
TMTFTH	Trimethyl-trifluoro-toluyl ammonium hydroxide
UV	Ultra-violet
UvA	University of Amsterdam
VI	Von Imhoff
VIS	Visible (light)
XRD	X-ray Diffraction

1. General introduction

Introduction

The pigments: Asphalt, Kassel earth and Mummy

The black-brown pigments asphalt, Kassel earth and Mummy have been used in paintings for a long time. Kassel earth and mummy pigments are described as used in oil paint since the 16th century [1-4] and asphalt probably since the middle of the 16th century [5, 6]. Their peak of use in painting was reached in the late 18th century and in the 19th century their use was reported to result in disastrous effects, such as migration of layers and formation of drying cracks or darkening. Fading of paint is said to occur for paints containing Kassel earth-like pigments. Carlyle reviewed the use of the above mentioned pigments in Britain in the late 18th century and in the 19th century [7, 8]. Regarding their organic or inorganic nature, asphalt is mentioned as an organic pigment that could be natural or synthetic [7-9], mummy pigment is described as having an organic nature [3], while Kassel earth too is described as an organic pigment. Kassel earth is found as inorganic or a mixture of organic and inorganic, when adulterated or pretreated [1, 7, 8]. (More details are given in chapter 2, 3 and 4.) J. Townsend et al. also talk about evidence for additions and substitutions in the later 19th-century black-brown pigments [10].

This study focuses on the genuine (see also below) asphalt, Kassel earth[1] and mummy[2] pigments, especially in relation to 19th-century sources, as well as their use in 19th-century oil paintings. Their use has resulted in paint film defects and gained those pigments a bad reputation, although this letter point is scientifically unexplained as yet!

Asphalt is a mixture of aliphatic and aromatic low and high molecular weight hydrocarbons [11-13]. It can be natural: from sediments and rocks, reffered to as “real asphalt” [7, 8, 14, 15], or it can be artificial, i.e. derived from petroleum, coal tar, water-gas tars, and their pitches [11-13]. This illustrates the diversity of asphalt pigments. True mummy pigment is made of parts of mummies ground to a powder. This implies an extremely complex and diverse composition for the pigment considering the variety of materials used for embalming, for example asphalt[3], and

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[1] i.e. the asphalt pigment made of “real” asphalt and Kassel earth pigment of organic-lignitic nature, as described in more detail in chapter 2, repectively in chapter 4

[2] a real mummy pigment is more difficult to identify as being derived from a real mummy (including the possible Egyptian provenance and asphaltic composition) as discussed in chapter 3

[3] in Arabic ‘mumiya’ means ‘bitumen’ (=asphalt) [2, 16, 17]

besides that different parts of the body of a mummy were used for making the pigment [3, 18]. Kassel earth is discussed in the literature as a pigment consisting of a mixture of humic (i.e. plant derived) substances from soil [1]. More details about the exact provenance of these pigments is given in chapter 2, chapter 3, and chapter 4, respectively. Natural sediments of asphalt in the 19th century are mentioned to be present in Egypt, the Dead Sea area, France, Germany, Switzerland, China, Italy and Trinidad [7, 8, 15, 19-21]. The source of mummy pigment is rather vague as the lack of Egyptian mummies was filled with fake mummies made from corpses in order to have the raw material for producing the pigment [3, 16, 22, 23]. Sources of Kassel earth pigment are associated with deposits of brown coal in Europe (Germany, Switzerland, England, Czechoslovakia, Italy) but also in the US (Wyoming, New Mexico, Montana) [1].) All three pigments were known and sold under several names indicating the real or suggested origin, and the manufacturer. For example: Antwerp brown, Engelsche betume, Bitume von Ottoz, Brun Vibert for asphalt [1, 7, 8, 14, 15], Egyptian brown for mummy [3], Vandyke brown, Cologne/Collin's earth or Cassel earth for Kassel earth pigments [1, 7, 8].

Due to its beautiful brown warm mellow tone and transparency when applied in thin layers in paintings asphalt was used as a pigment, for glazing and in underpaint [7, 8, 14, 15]. The mummy pigment was used for glazing and shadows [3]. Blackish-brown, with a purple tint and high transparency but a low hiding power in oil, Kassel earth was recommended for shadows of flesh, blacks and browns and as a glaze [1]. Beside their use in oil paint, asphalt, mummy and Kassel earth pigments had several other utilisations (for more details see chapter 2, 3 and 4) [3, 16, 20, 21, 24, 25].

All three pigments were occasionally adulterated (including pretreatments) or substituted with other materials. The artificial "asphalt" obtained from the manufacture of lampblack or pitch possibly contained drying balsams [9]. A more common 'asphalt' was the coal tar type, which is mentioned as an 'inferior' variety that would result in paint that never dries completely [9]. According to some authors the asphalt pigment was quite often adulterated or replaced by Kassel earth or ivory black like pigments [7, 8, 14]. However, Feller thinks the replacement of asphalt with Kassel earth pigments is unlikely [1]. Apart from its inconsistent composition, the mummy pigment seems to have been substituted with Prussian brown (i.e. calcined Prussian blue) [4, 7, 8] or Kassel earth like pigments [19]. Mixtures of asphalt and lime are also mentioned as "mummy pigment" [3]. Calcinated cork and wood, carbon black pigments and iron oxide were used as replacements for Kassel earth [1, 7, 8]. Sometimes ochre was added to Kassel earth pigments [1].

With respect to preparation methods it seems that most of the time the "real" asphalt could not be used directly in oil, because of solubility problems. Its use in oil paint required a roasting step or melting with resin [7, 8, 15]. In contrast, the "artificial" asphalt was soluble in plant oil [9]. The mummy pigment was ground in nut oil, mixed with amber varnish and other colours[4] and drying oil, but less oil than for making asphalt paint [2-4]. Kassel earth pigment was used as such in the preparation of paint, sometimes after drying and removal of the sand [1].

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[4] ultramarine, glazing colours (*e.g.* madder lake), ivory black [3]

Conservation problems of 18-19th-century paintings (and their relation with asphalt, mummy or Kassel earth pigments)

Paintings from the middle of the 18th and the 19th century often have a ruinous appearance [9, 10, 15], for examples paintings by Sir J. Reynolds, W. Hilton, H.P. Briggs, Sir D. Wilkie, J.C. Horsley, Th. Rousseau, J. Israels, etc. [9, 26-32]. Such paintings can be faded or darkened completely, show drying cracks, migrating or exfoliating layers, and corrugated surfaces, which makes the exhibition of the paintings in museums difficult or undesirable. The ruinous appearance is explained as due to the use of a dark palette (including the above mentioned black-brown pigments) and an extensive use of new materials such as megilp[5]-like media, balsams, waxes, starch, gums, egg, soap and/or the development of a new painting technique [7-9, 15, 34, 35]. The defects can be aggravated by unsuitable restoration techniques [34]. Carlyle reports on differences between the theory and the craft of oil painting with respect to 19th-century British instruction manuals on oil painting [36].

Paint defects in the 18-19th-century paintings, seen as darkening and the formation of premature drying cracks are often thought to be associated with the use of asphalt pigments. Such drying cracks are known as “craquelure anglais”, a name that points towards an excessive use of asphalt pigments in 19th-century Britain [7-9]. The drying qualities and fastness to light of the mummy pigment are reported to be sometimes better and other times worse than for asphalt and related to the quality of the pigment [2, 3, 7, 8]. The lightfastness of the organic Kassel earth is reported as worse compared to its inorganic substitutes[6] [1, 7, 8]. For modern Kassel earth pigment, when used in glazes the lightfastness is only intermediary [1].

Status quo of the analytical evidence of asphalt, mummy and Kassel earth in (18-19th-century) paintings

Raymond White was the first person to use the distribution of hopanes in extracts from paint samples as marker compounds for asphaltic materials [37]. More detailed methods for the study of asphalt in paintings have been developed by Languri *et al.* [38, 39] (chapter 2), which show that analytical results of asphalt as a painting material and in paintings are limited by its complexity. This is shown further in chapter 2 and 5 [see also 2, 34, 40, 41]. Although molecular evidence for asphalt — in most cases from the Dead Sea or of Middle Eastern origin — in samples from real mummies was obtained with multiple analytical methods (true also for geological samples of asphalt), as discussed in chapter 3, no mass spectrometric results are known to be available for mummy pigment samples. Considering its complex composition it is quite difficult to firmly identify it in paint samples [2]. In a similar way not many analytical investigations are available related to the Kassel earth pigments or their presence in paintings [1]. The existing identification is based on IR spectroscopy, wet chemical methods, optical microscopy or X-ray fluorescence analyses [1]. These, together with more modern methods like Neutron Activation and Mössbauer spectroscopy applied to modern Kassel earth pigments identified humic substances and polysaccharides from soil, certain types of plants detected from pollen analysis, Fe, Ca, and a little Mn [1]. However insufficient molecular evidence for Kassel earth pigments in paints has been obtained up till now.

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[5] mixture of drying oil made with a lead drier and mastic varnish [9]; not recommended [7-9, 33]

[6] When charred or carbonised the pigment becomes darker, duller, and less fugitive [1].

Rationale and aims of this thesis

Clearly there is a need to fill the existing gap in the characterisation and identification of the asphalt, Kassel earth and mummy pigments. An accurate characterisation implies knowledge about the pigments at a molecular level that would also provide indications on its mechanism when used in paint. This thesis discusses aspects related to the genuine pigments[7] mentioned above; other materials used as substitutes or adulterants are outside the scope of this research.

It is important as well to have a method available for the identification of such pigments in small samples like those from paintings. A requirement would be that this method is suitable for complex materials and preferably in one analytical step.

This has been achieved by using mass spectrometric techniques (DTMS, DTMS/MS, Py-(TMAH)-GC/MS, ESI-FTICRMS), infrared spectroscopy (FTIR), optical and electronic microscopy (SEM-EDX), and size exclusion chromatography (HPSEC) applied to fresh and naturally and artificially aged samples.

Chapter 2, 3 and 4: Nature of the pigments by characterisation at molecular level

Asphalt, Kassel earth and mummy pigments were characterised at a molecular level using mass spectrometric (MS) techniques (see chapter 2, 3 and 4). This has resulted in a set of markers and biomarkers specific for the particular black-brown organic pigments that are further used to identify their presence in historical paint material collections or other reference samples of interest. In chapter 2 results on an ‘asphalt’ sample from the 19th-century paint material collection are given. The sample is identified as real asphalt (by comparison with a Dead Sea asphalt reference sample) and a possible source of this asphalt is suggested. Chapter 3 deals with mass spectrometric characterisation of an historical ‘mummy’ pigment sample in order to assess a possible Egyptian origin of mummy pigment samples, seen as a Dead Sea asphalt containing samples. Chapter 4 reports on the characterisation of Kassel earth samples and the identification of such samples as Kassel earth type. An attempt is made to relate the marker components of these pigment samples to botanical sources. A set of fingerprint markers is derived for each of the three investigated materials: asphalt, mummy, and Kassel earth.

Chapter 5: On poorly drying 19th-century oil paints-reference materials, model systems and paintings

Chapter 5 treats aspects of poorly drying 19th-century oil paints, based on a series of reference materials, model systems and on samples taken from original paintings. The first part of the chapter studies the fate of the fingerprint markers of asphalt pigment when this is used as a component of oil paint. The influence of high temperature on asphalt markers and biomarkers is discussed in relation to the boiling or roasting pre-treatment of asphalt when preparing the oil paint in the 19th century. The solvent sensitivity of asphalt marker compounds is further discussed. The

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[7] the mummy pigment is interesting not only for its general composition but also for its possible asphalt content (“as cause of defects in paint films”) and its possible relation with real mummies.

influence of artificial light ageing on asphalt markers from asphalt-linseed oil solutions (in dichloromethane) is reported. The last part of this section deals with asphalt markers and biomarkers in asphalt oil paints. Similar aspects related to the fingerprint markers of Kassel earth pigments when used as a component of oil paint are reported in the annex to chapter 5.

The second part of chapter 5 treats the effect of asphalt and Kassel earth on the composition of oil paint. The aspect of fatty acids and dicarboxylic acids in ageing oil paint is discussed. The kinetic aspect of drying of an asphalt-oil mixture studied by FTIR is presented. The effect of asphalt on the drying of oil in the studied artificial light aged asphalt-linseed oil solutions (in dichloromethane) is discussed. Asphalt and Kassel earth containing oil paints are examined with respect to the status of the drying of oil. A comparison is made between the effect of asphalt, Kassel earth, an ivory black containing oil paint, and a lead white containing oil paint. Lastly chapter 5 deals with samples from 19th-century paintings supposedly containing asphalt or Kassel earth pigments. Samples from three 19th-century paintings were selected as relevant for the phenomena thought to be caused by the use of asphalt in paint.

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2. Asphalt: Characterisation of the 19th-century Hafkenscheid asphalt pigment. Markers and biomarkers

Abstract

Asphalt has been used since antiquity for various purposes and by painters since the 17th century. Asphalt as a pigment material with a known pre 19th century provenance is extremely rare. The analytical pyrolysis techniques of Direct Temperature resolved Mass Spectrometry (DTMS) and Pyrolysis Gas liquid Chromatography coupled with Mass Spectrometry (Py-GC/MS) were applied to characterise a unique "asphalt" sample from a painting materials collection dating from the early 19th century. The provenance of this sample was investigated by comparison with a sample from present day floating asphalt from the Dead Sea.

DTMS demonstrated a complex envelope of ions from hydrocarbons ranging past m/z 1000 in both samples. Both samples showed evaporating sets of compounds as well pyrolysing materials evolving from asphaltenes. Asphaltene network polymers were marked by aliphatic cross-links releasing alkenes and alkanes, and aromatic centres releasing alkylbenzenes, alkylnaphthalenes, alkylanthracenes, alkyl-phenanthrenes, alkylbenzothiophenes. Hopanoid hydrocarbons and C-ring mono-aromatic steroid hydrocarbons were among the biomarker compounds identified. Particularly striking were homologous series of alkylbenzothiophenes and alkyl-dibenzothiophenes with long hydrocarbon chains that could be traced with DTMS and confirmed by DTMS/MS.

The results indicate that the early 19th century 'asphalt' sample from the Hafkenscheid collection is indeed natural asphalt that shows many similarities to the Dead Sea asphalt sample, which is therefore proposed as the possible region of provenance.

Introduction

Asphalts as classified by Yen [1] are divided in “natural asphalts”, found in sediments and rocks, and “artificial asphalts, mainly petroleum-derived asphalts and to a lesser extent, coal tar, water-gas tars, and their pitches”. The “recorded” history of asphalt use starts about 38,000 BC in the Paleolithic period [2]. The varied utilisation of asphaltic material includes: its use in agriculture and ship building as an adhesive and waterproofing agent, its use in construction as mortar and cement, its use for lighting and heating or as a weapon (e.g. as component of Greek fire), the decorative use as a coating of art objects or in the making of paints or varnishes and its utilisation in medicine and embalming [2, 3, 4, 5]. Many of its uses disappeared in the course of history. In the 19th century with the development of photography, asphalt came back as a light sensitive substance in the making of “heliographs” [6]. Since early in the 20th century, the presence of asphalt in sediments is used as an indicator of the existence of buried oil fields [4].

Asphaltic materials have been used in paintings at least since the 17th century, but the pinnacle of its use was reached in the 18-19th century. Paint defects in the 18-19th century paintings, seen as darkening and the formation of premature drying cracks are often associated with its use. Recently, Bothe [7] has reviewed the complexity of asphalt utilised in paintings from the 18th and 19th century. In some cases asphaltic materials were used as such i.e. directly mixed with cold oil. In other cases various preparatory measures were taken (heating, specific solvents, addition of resins) to ensure that the asphaltic particles dissolved completely. In some cases, the asphalt was roasted before use (Mérimée recipe) [8]. The variety of preparatory methods for use in paint makes it difficult for the analytical chemist to develop suitable analytical methodology. White has used the distribution of hopanes in extracts from paint samples as marker compounds on the assumption that these are specific for asphaltic materials [9]. Hopanes are primarily part of the solvent extractable maltene fraction of asphalts, and may be lost from the painting by solvent cleaning. Hopanes are of low abundance in the asphaltene fraction, which has a better chance of surviving in a painting that has been cleaned several times. For these reasons, hopanes are not completely reliable markers for the presence of asphalt in paintings. It is also unclear whether hopanes are always present in an asphaltic oil paint considering the many different recipes and preparation methods.

Asphalts are emulsions of cross-linked flat sheets of polycyclic aromatic and heterocyclic hydrocarbons (asphaltenes) “dissolved” in a mixture of lower molecular weight aliphatic and alicyclic hydrocarbons (maltenes) [1]. Depending on the geological origin, there may be a substantial amount of sulphur compounds present, presumably contributing S-linkages to the network polymer system. Aliphatic hydrocarbon chains with variable chain length (C_2-C_{40}) link most of the aromatic centres. Pyrolysis of asphalt results in the thermal dissociation of the asphaltene fraction which breaks the cross-links resulting in sets of straight chain alkanes and alkenes and a large number of isomers of alkylated mono- and polycyclic aromatic hydrocarbons [10]. Maltene in such an asphalt will evaporate in the low temperature pyrolysis experiments. The maltene fraction contains hopanoid hydrocarbons, aromatic steroids, alicyclic and aliphatic hydrocarbons that are used as biomarker molecules by geochemists [11]. The traditional way to analyse asphalt samples consists of fractionation of the complete sample, by solvent extraction, into maltenes and asphaltenes. The maltenes are then analysed by on-column GC/MS, while the asphaltenes are investigated by Py-GC/MS.

Direct Temperature Mass Spectrometry, a fast fingerprinting method particularly suited for the recognition of many classes of compounds, achieves a physical separation between low molecular weight compounds by evaporation and cross-linked fractions of a sample by gradually raising the temperature of the filament probe [12, 13]. An asphalt sample will be physically separated during DTMS into a fraction evaporating at low temperature equivalent to the maltenes and fractions relating to the asphaltenes at higher temperature. It has been shown before that the thermal extract data of a sample (LT of DTMS) is equivalent to the classic solvent extract DTMS data [14]. The analytical process at high temperature under DTMS conditions is equivalent to in source pyrolysis mass spectrometry [12]. Under Py-GC/MS conditions in the Curie point pyrolysis system available at FOM-AMOLF these evaporating and pyrolysing fractions are injected directly into the GC column, analysed collectively and usually not as separate fractions [15]. The analytical approaches used provide complementary information.

Numerous citations exist which describe asphalt as the cause of the "craquelure anglais" in the paintings of 19th century [16, 8]. Artists liked the beautiful brown warm mellow tone and the glaze-like transparency of asphalt containing paint when applied in thin layers. This may be the reasons for its purported extensive use. In the 18th century, two varieties of 'asphalt' are mentioned: natural or native asphalt and artificial 'asphalt' [16]. In early 19th century England, a third variety was introduced, an 'asphalt' originating from coal tar [16]. The natural asphalt was described as a brownish-black [16] solid material, in fact always deep black in colour (when pure, [17]) and viscous at relatively early stages of degradation, while brown and friable in more advanced stages of alteration [18]. Asphalt was first roasted and ground because it could not be dissolved in oil directly [16]. Carlyle et al. call this kind of asphalt "real asphalt". Artificial 'asphalt' was said to be a by-product of the manufacture of lampblack or pitch, with possibly additions of poor drying balsams. This variety is soluble in vegetable oils. The most common 'asphalt', the coal tar type, is mentioned as an 'inferior' variety that would result in paint that never dries completely [16]. Paint manufacturers and/or painters themselves [19] also adulterated asphalt with other geo-materials during paint preparation.

It is not clear whether all of the asphalt marker compounds that can be identified with analytical methods will survive on a painting considering the oil paint preparation process, the strongly oxidising conditions during ageing and the loss by evaporation and the various solvent cleaning methods used by conservators and restorers. Perhaps the more cross-linked fractions have a better chance of survival under these conditions. It is unclear what kind of asphalt could have been available in the 19th century. Egypt, the Dead Sea area, France, Germany, Switzerland, China, Italy and Trinidad are mentioned as sources of real asphalt. [8] Gettens and Stout [20] have suggested that asphalt from the Dead Sea area has probably been used in European painting. Petroleum production started after 1860 so asphalt in earlier paintings must have relied on open pit sources [7] or floating blocks such as those from the Dead Sea area [4, 6].

The Hafkenscheid collection of pigments [21, 22] dating from the late 18th and early 19th century provided the opportunity to analyse an asphaltic material that was available to colourmen and painters of that period.

The collection represents the product assortment supplied by the Amsterdam firm "Michiel Hafkenscheid and Son" in "Painting Materials, Turpentine and Gums" during the first three decades of the 19th century. Nowadays, the Hafkenscheid Collection is kept in the custody of the Teylers Museum in Haarlem, The Netherlands. The collection consists of about 370 samples, inorganic materials (ores, powdered pigments, abrasives, inert fillers) and organic materials (unprepared vegetable and animal dyes, tannins, organic pigments, gums and resins, several glues). A great number of the analysed materials proved to be early 19th century standard pigments [21, 22]. The Hafkenscheid collection is one of the few surviving collections of pigments and paint materials for painters from the early 19th century and unique in the Netherlands. The single "asphalt" sample present in the collection had not been characterised. Although some oxidation may have occurred in the last 200 years of exposure to the atmosphere, the sample is treated as a natural intact asphalt for analytical purposes.

In this paper, the analytical pyrolysis methodology presented is designed to provide information on the presence of asphalt in oil paint. Comparative analytical data on asphalt containing oil paint samples are not the subject of this chapter and will be published elsewhere. Attention is given to the distribution of marker compounds released by Py-GC/MS and DTMS techniques from the complete sample as is the usual practice when microgram amounts of oil painting sample are analysed. The objective is to identify the sample from the Hafkenscheid collection as an asphalt and to correlate its composition with possible sources. The supposition of a Middle Eastern origin of the Hafkenscheid sample was tested by comparison with a reference sample from a floating asphalt block collected in the Dead Sea (Israel) analysed under the same conditions and by comparison with data from the literature. The asphalt sample from the Hafkenscheid collection (sample HS) and a Dead Sea reference asphalt (sample DS) were also compared with the analytical pyrolysis data from large MOLART reference collection of asphalts, but reporting of these data is outside the scope of this paper.

Experimental

Samples

The 'asphalt' from the Hafkenscheid Collection [21, 22] was kindly made available by the Teylers Museum in Haarlem (The Netherlands). Labelled as sample HS, the Hafkenscheid sample consisted of small amorphous lumps of black glossy material. Sample HS was compared with a type I Dead Sea [4] asphalt sample (sample DS), provided by Dr. Arie Nissenbaum (Weizmann Institute of Science, Israel). Portions of both samples were completely soluble in dichloromethane (DCM).

Sample preparation

The samples (5-10 µg) for DTMS were dissolved in 15-25 µl of DCM. An aliquot of 1-2 µl of this solution was added on the Pt/ Rh filament of a direct insertion probe for in-source analysis. Typically 10 µg of sample is used for Py-GC/MS. The samples were dissolved in DCM, applied to a ferromagnetic wire (Curie point 770 ºC) and dried in vacuo. After drying the sample wire was inserted into a glass liner, placed in the cold compartment of the pyrolysis unit, flushed with helium and then moved into the pyrolysis chamber (220 ºC) of the FOM 5LX Curie point pyrolysis unit [23].

Direct Temperature-resolved Mass Spectrometry (DTMS) and Direct Temperature-resolved Mass Spectrometry Mass Spectrometry (DTMS/MS)

DTMS experiments were carried out on a JEOL JMS SX-102 double focussing mass spectrometer (B/E). A direct insertion probe equipped with a resistively heated Pt/Rh filament was used. The filament (Pt/Rh 9:1, 100 µm) was heated with a rate of 0.5 A/min to an end temperature of about 800 ºC. Ions were generated by EI (16 eV) in an ionisation chamber kept at 190 ºC, accelerated to 8 kV, analysed from m/z 20-1000 (about 1 s cycle time) and post-accelerated to 10 kV. A JEOL MS-MP 9020D data system was used for data acquisition and processing. The DTMS spectra were obtained under low energy (16 eV) electron impact conditions to minimise fragmentation reactions of the ions.

DTMS/MS experiments were carried out on a JEOL JMS-SX/SX 102A tandem mass spectrometer (B/E/B/E). Collision Induced Dissociation (CID) was performed in the third Field Free Region using helium as a collision gas (1.5 10-3 Pa). The voltage of the collision cell was 2 kV. The same conditions as for DTMS were used concerning the probe, ion generation, acceleration and post-acceleration of ions and acquisition. The ions were analysed from m/z 0 to 375 at about 1 s cycle time. The resolution used was 3000.

Pyrolysis Gas Chromatography Mass Spectrometry (Py-GC/MS)

The experiments were carried out with a FOM-5LX Curie-point pyrolysis unit mounted on a Carlo Erba series 8565 HRGC MEGA 2 gas chromatograph. For separation a fused silica SGE BPX5 column (25 m, 0.32 mm i. d., 0.25 µm film thickness) used with helium as a carrier gas at a flow rate of 2 ml/min. For both samples the oven temperature was programmed from the initial 35 ºC (for 0 min.) to a final 320 ºC (for 10 min), with a ramp of 4 ºC /min. The column was interfaced directly to a JEOL JMS DX-303 double focussing (E/B) mass spectrometer or a JEOL JMS-SX/SX 102A tandem mass spectrometer (B/E/B/E), using a home-built high temperature interface. Ions were generated by electron impact ionisation (70 eV) in the ionisation chamber, accelerated to 3 kV, respectively 8 kV, mass separated and post-accelerated to 10 kV before detection. The mass range was scanned from m/z 35-500, respectively m/z 40-800, with a cycle time of 1 s. A JEOL MS-MP 9020D data system was used for data acquisition and processing.

Results and discussion

Bulk analysis: $\delta\ ^{13}C$ isotopic values

$\delta\ ^{13}C$ Isotopic measurements on archaeological asphalt samples have been used as a proxy for their provenance [4, 5, 24]. The $\delta\ ^{13}C$ data on the Hafkenscheid (HS) and Dead Sea (DS) asphalt samples are listed in Table 1 together with values from the literature. The isotopic values of HS matches that of DS very closely. Comparison with values of other Dead Sea asphalts published by Nissenbaum [4, 24] show a good match. Comparison with literature carbon isotopic data on asphalt and asphalt containing artefacts from the Mesopotamian area [5] shows significantly different values.

Table 1. Stable carbon isotope data (δC^{13}) for sample HS and DS and asphalts available in the 19th century [5, 24].

Asphalt sample	**δC^{13}** (VPDB units for HS and DS, PDB units for the other samples)
HS	**-29.26 ± 0.10 %**
DS	-29.31 ± 0.10 %
Asphaltenes (*i.e.* the C^{13} enriched fraction) of Middle Eastern asphalts; [5]	
Israel (Dead Sea)	
floating blocks	-29.4
archaeological	(-29.1) – (-29.5)
Iraq	
archaeological	(-27.6) – (-28.6)
Syria	
archaeological	(-25.0) – (-26.7)
Iran	(-26.1) – (-28.0)

The data obtained support the hypothesis that the Hafkenscheid asphalt may have its origin in present day Israel. However, according to Nissenbaum [25] asphalt from Hasbeya, Southern Lebanon, is “very similar geochemically to Dead Sea asphalt and most certainly has the same source and thermal history. This deposit has also been used since ancient times, and travellers to the area said it was quarried even in the 19th century”. As far as know to the current researcher, no recent detailed geochemical work has been done on the Hasbeya asphalt.

Analytical pyrolysis studies

The results from the DTMS and Py-GC/MS analysis of the asphalt sample from the Hafkenscheid collection (sample HS) are presented in Fig. 1, Fig. 4a, Fig. 5, Fig. 7a, Fig. 8a, Fig. 9a and Fig. 10a. Similar results obtained for the Dead Sea asphalt (sample DS) are shown in Fig. 2, Fig. 3, Fig. 4b, Fig. 6, Fig. 7b, Fig. 8b, Fig. 9b and Fig. 10b. Identified compounds are listed in Table 2. The number of compounds in pyrolysates of asphalt is very high, so mass chromatography of selected compounds was used to trace specific compounds and their distribution. Peaks were identified on the basis of their 70 eV mass spectrum and retention time. In some cases the identification of the mass spectra was complicated due to co-elution and/or their relatively low abundance of certain compounds.

DTMS and DTMS/MS data

The spectra of sample HS (Fig. 1b and 1c) and DS (Fig. 2b and 2c) show the classical characteristics of an asphaltic material: unresolved envelopes of hydrocarbon fragment ion series extending beyond mass 1000 (truncated at m/z 700 in the figures). The TIC of both asphaltic samples show fractions evaporating at relatively low temperature (maltenes) as well as fractions evolved by pyrolysis of cross-linked fractions (asphaltenes) at higher temperature (see Fig. 1a and Fig. 2a).

In the DTMS spectrum of the maltene fraction of HS (Fig. 1b), ions of specific compounds e.g. m/z 253 indicative of C- ring monoaromatic steroids (and m/z 267 from methylated isomers) are observed above the ion envelope. These compounds are well known markers for mature oil [11] and their presence is confirmed by GC/MS (see below). Other specific fragment ions of lower abundance in HS (Fig. 1b) are m/z 191 from hopanoid and related pentacyclic hydrocarbons, m/z 217 from regular steranes and m/z 231 (and m/z 245 from methylated isomers) from triaromatic steroids. At mass ranges higher than m/z 300, a series of peaks of low relative abundance are observed above the ion envelope suggesting the presence of several homologous compound series.

In the DTMS spectrum of the evaporated fraction of DS (Fig. 2b) the highest peak above the ion envelope is m/z 175 accompanied by m/z 161 and m/z 189 at mass differences of 14 amu. The second high peak is observed at m/z 253 for C- ring monoaromatic steroids accompanied by a peak at m/z 267 (from methylated isomers). Other peaks of interest including peaks at m/z 191 for hopanes, 217 for regular steranes, 231 for triaromatic steroids and its methylated isomer at m/z 245 are of much lower abundance. After m/z 300 several series of peaks with mass increments of 14 amu are observed. The most predominant of these series shows peaks at m/z 288 + m(CH_2), in which m varies from 1 to 20. The series starts with a few peaks of a relatively high abundance, followed peaks maximising at m/z 372 and then diminishing towards 568 (see details in Fig. 2d). The m/z values of this series in Fig. 2d suggest a homologous series that can be assigned to alkylated benzothiophenes, regular steranes or triaromatic steranes. DTMS/MS of m/z 358 shown in Fig. 3 shows the characteristic fragment ions (m/z 161, 175 and 189) of an alkylbenzothiophene with an alkyl chain length $C_{12}H_{25}$. Other ions in this series analysed by DTMS/MS and information obtained by Py-GC/MS confirm that the peak series m/z 288 + m(CH_2) belongs to a series of alkylbenzothiophenes. The high peaks in the DTMS profile (Fig. 2b) at m/z 161, 175 and 189 are the fragment ions corresponding to alkylbenzothiophenes (see Table 2). Two other series are seen in the DTMS spectrum of DS one of which shows peaks at m/z 310 + k(CH_2), in which k= 1-14 has the highest relative abundance but still quite low when compared to the previous series. The envelope of the series diminishes towards higher m/z range. DTMS/MS and Py-GC/MS identified this series as due to alkylated dibenzothiophenes. The series showing peaks at m/z 370, 398, 412, 426, 440, 454, 468, 482, 496, are of a much lower abundance in the spectrum (Fig 2b and 2d), but are tentatively identified as a series of hopanes based on the Py-GC/MS data.

The homologous series of compounds in the DTMS of the DS asphalt are not very prominent in the HS asphalt. However, DTMS/MS and Py-GC/MS studies of the HS asphalt confirm the presence of a series alkylbenzothiophenes with peaks at m/z 330 + n(CH_2), where n= 1-10. The distribution follows the shape of the ion envelope, with slightly higher values at m/z 358 and m/z 428 (Fig. 1d). From the series of hopanes, as suggested for DS, just a few peaks are present in the data of HS. Only the

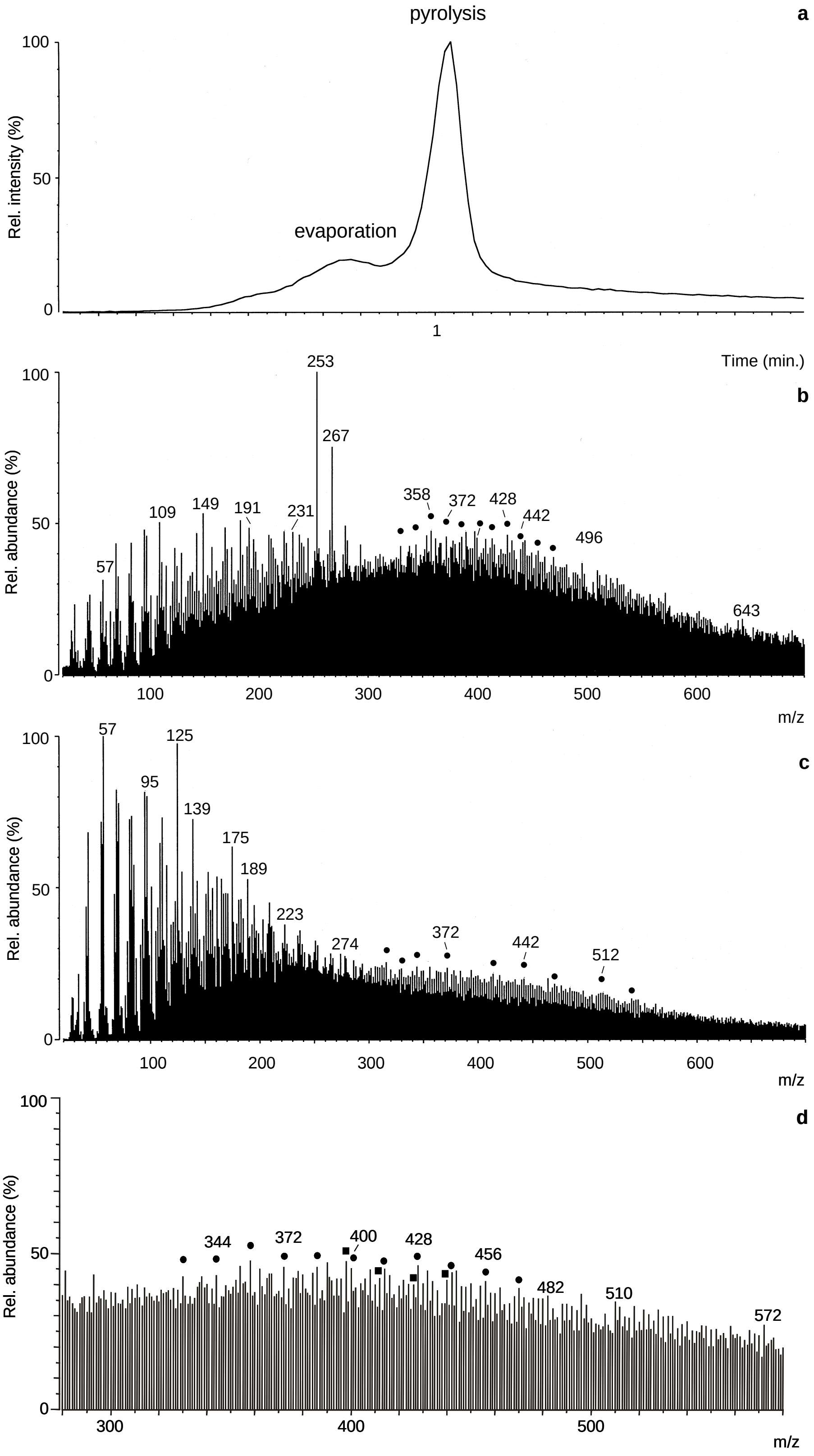

Fig. 1. DTMS fractionation of sample HS: (a) TIC of DTMS spectrum, (b) evaporation fraction, (c) the high temperature fraction, (d) detail of a series of compounds seen in (b); '●' was used to mark the series of alkylbenzothiophenes and '■' for a series of hopanes.

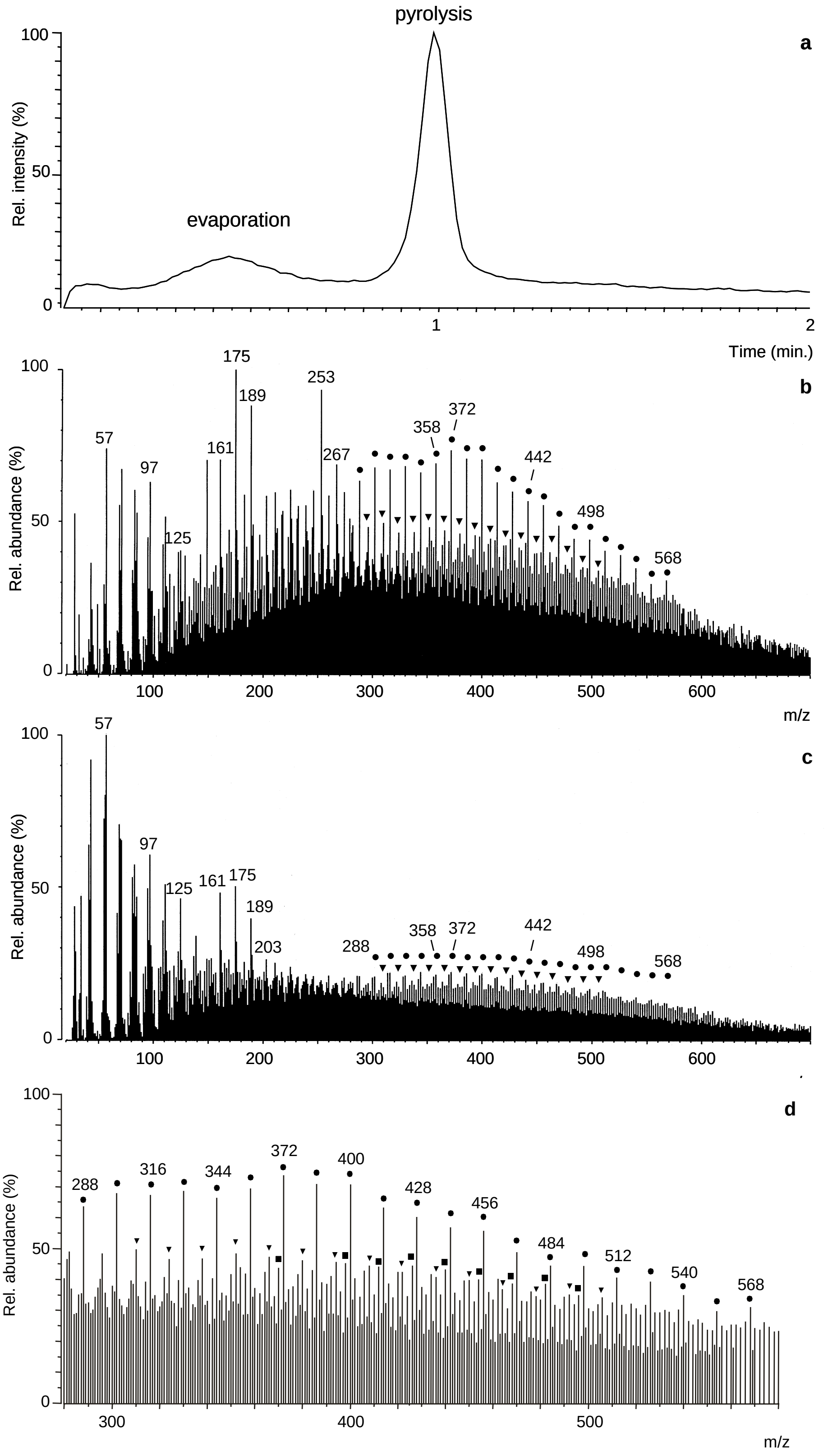

Fig. 2. DTMS fractionation of sample DS: (a) TIC of DTMS spectrum, (b) evaporation fraction, (c) the high temperature fraction, (d) detail of series of compounds seen in (b); ‘●’ was used to mark the series of alkylbenzothiophenes, ‘▼’ for series alkyldibenzothiophenes and ‘■’ for series of hopanes.

peaks at m/z 398, 412, 426 and 440 with a relatively low abundance are visible above the envelope.

Both DS and HS DTMS evaporation spectra (Fig. 1b and 2b) show fragment ions of alkanes (m/z 43, 57, 71) and alkenes (m/z 55, 69, 83, 97, 111, 125). Other fragment ions like m/z 95, 109, 123 or m/z 129, 143, 157, 171 or m/z 169, 183, which differ by 14 amu, are observed but their significance is not clear.

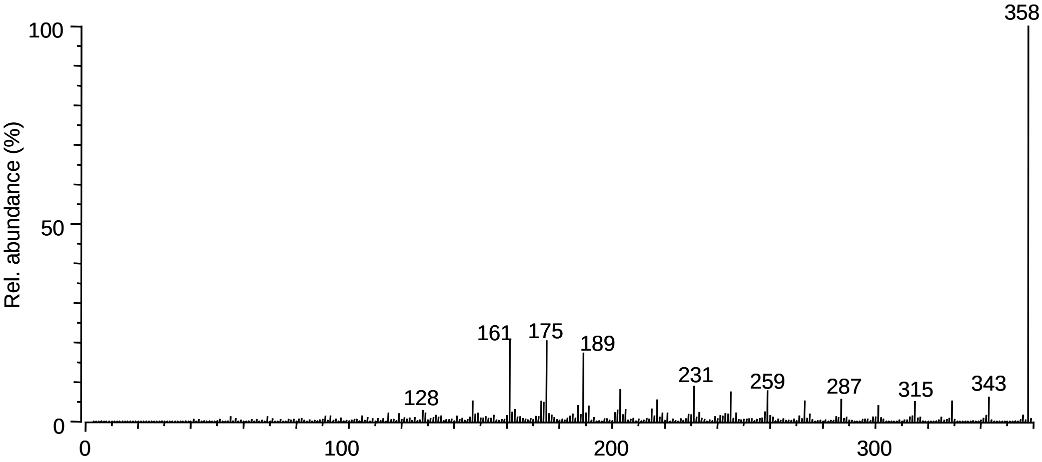

Fig. 3. MS/MS of m/z 358 of sample DS.

The high temperature part of the DTMS spectra of HS and DS (Fig. 1c and 2c) mainly show fragment ions from paraffinic hydrocarbons (alkanes, alkenes and alkadienes) resulting from the thermal degradation of the paraffinic links between the aromatic sheets structures. The pyrolysed fractions in the HT part of the DTMS spectrum of HS and DS show less variation compared to their evaporated fraction.

The highest peaks above the ion envelope in the DTMS data of the high temperature fraction of HS (Fig. 1c) are observed as fragment ions for alkanes (m/z 43, 57, 71, 85), alkenes (m/z 41, 55, 69, 83, 97, 111, 125, 139, 153), alkadienes (m/z 67, 81, 95, 109, 123, 137) and a very high peak at m/z 125, possibly from alkylthiophenes. Above m/z 300 a few peaks of very low relative abundance can be still observed at m/z 412, 440, 454 and also at m/z 316, 344, 372, 414, 442, 470 and 512. These mass peaks are originating from the same series as seen in the evaporated fraction.

In the high temperature DTMS of DS (Fig. 2c) the highest peaks observed are the fragment ions for alkanes (m/z 43, 57, 71, 85), alkenes (m/z 41, 55, 69, 83, 97, 111, 125, 139) and alkadienes (m/z 67, 81, 95, 109, 123, 137). Other peaks of higher abundance above the ion envelope are fragment ions at mass increments of 14 amu from alkylbenzothiophenes at m/z 161, 175, 189 and 203. Peaks of low relative abundance at higher than 300 m/z range form two series observed or in part already observed in the evaporated fraction. A first series shows peaks differing by 14 amu starting at m/z 288 till m/z 568, which is identical the alkylbenzothiophenes series to the highest series seen in the evaporated fraction. This suggests that these compounds are partly adsorbed or encapsulated in the asphaltene network. Another series displaying peaks between m/z 300 and m/z 566, differing by 14 amu, are tentatively identified as an unsaturated alkylbenzothiophenes series originating from the asphaltene network by thermal cleavage.

Py-GC/MS data

The Py-GC/MS data of HS and DS show homologous series of straight chain hydrocarbons of normal alkenes and alkanes (see the TIC in Fig. 4a and 4b, Table 2). At low elution temperature the n-alkenes and n-alkanes are separated (marked in Fig. 4a and 4b and Table 2 as ‘c: 1’/’c: 0’ in which ‘c’ is the number of carbon atoms present in the molecule).

Table 2. List of compounds identified in the TIC of Py-GC/MS data of the sample HS and sample DS.

Classes of compounds	**MW**	**Important EI fragment ions (m/z)**	**Label**	**Sample HS**	**Sample DS**
Aliphatic cross links					
Alkanes		85	**c: 0**	+ (C_7-C_{35})	+ (C_7-C_{34})
Alkenes		83	**c: 1**	+ (C_6-C_{31})[1]	+ (C_6-C_{31})[2]
Alkylbenzenes					
Benzene	78	78, 77, 63, 51	**B**	+	+
C_1-benzene (toluene)[3]	92	92, 91, 65, 51	**B_1**	+	+
C_2-benzene[4]	106	106, 105, 91, 77, 65	**B_2**	+	+
C_3-benzene[5]	120	120, 119, 105, 91, 77	**B_3**	+	+
Alkylnaphthalenes					
Naphthalene	128	128, 102	**N**	+	+
C_1-naphthalene[6]	142	142, 115	**N_1**	+	+
C_2-naphthalene[7]	156	156, 141, 115	**N_2**	+	+
C_3-naphthalene[8]	170	170, 155	**N_3**	+	+
Alkylbenzothiophenes					
C_1-benzothiophene	148	148, 147	**BT_1**	+	+
C_2-benzothiophene	162	162, 161, 147	**BT_2**	+	+
C_3-benzothiophene	176	176, 175, 161, 147	**BT_3**	+	+
C_4-benzothiophene	190	190, 189, 175, 161, 147,115	**BT_4**	+	+
Alkyldibenzothiophenes					
C_1-dibenzothiophene	198	198, 197, 165	**DBT_1**	+	+
C_2-dibenzothiophene	212	212, 211, 197, 184	**DBT_2**	+	+
C_3-dibenzothiophene	226	226, 225, 211	**DBT_3**	+	+
C_4-dibenzothiophene	240	240, 239, 225	**DBT_4**	+	+
Hopanoid compounds					
C_{30}-hopane[9]	412	191	**H_{30}**	+	+
C_{31}-hopane[10]	426	191	**H_{31}**	+ (22S, 22R)	+ (22S, 22R)?

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[1] up to C_{26} the alkene-alkane peaks are resolved

[2] up to C_{26} the alkene-alkane peaks are resolved

[3] detected as methyl-substituted benzene

[4] detected as dimethyl-/ ethyl- substituted benzene

[5] detected as trimethyl-/ dimethyl, ethyl- / normal or isopropyl- substituted benzene

[6] detected as α and β methyl- naphthalene

[7] detected as α-α, α-α‘, β-β, β-β’, α-β dimethyl- naphthalene

[8] detected as trimethyl- naphthalene

[9] detected as 17α, 21β (H)-30-hopane

[10] detected as 17α, 21β (H)-29-homohopane, isomers 22S and 22R

Table 2 continued.

Classes of compounds	MW	Important EI fragment ions (m/z)	Label	Sample HS	Sample DS
C_{32}-hopane[11]	440	191	**H_{32}**	+ (22S, 22R)	+ (22S, 22R)?
Terpanes					
Gammacerane (C_{30})	412	191	**G**[12]	+	+?
C-ring monoaromatic steroids					
C_{27}-monoaromatic steroid[13]	366	253	**MA_{27}**	+?	+?
C_{28}-monoaromatic steroid[14]	380	253	**MA_{28}**	+?	+?
C_{29}-monoaromatic steroid[15]	394	253	**MA_{29}**	+?	+?

At higher retention temperatures, separation is not achieved and the n- alkene + alkane peaks are simply marked with their carbon number. HS shows a distribution of straight chain resolved hydrocarbons in Fig. 4a ranging from C_6 to C_{31} (alkenes) and from C_7 to C_{35} (alkanes), on top of an unresolved envelope of other hydrocarbon compounds starting near the C_{15} hydrocarbon. The GC conditions chosen leave compounds unresolved till C_7 in the Py-GC/MS data. The alkenes are resolved from alkanes till $C_{26}H_{54}$. A similar distribution is observed for sample DS with aliphatic chains ranging from C_6 to C_{31} (alkenes) and from C_7 to C_{34} (alkanes) (Fig. 4b). The straight chain hydrocarbons result from the aliphatic cross bridges in the asphaltene macromolecule [1] by thermally induced radical cleavage processes during pyrolysis leading to homologous series of alkanes and alkenes. The isoprenoidic compounds phytane and pristane are absent in the Py-GC/MS data of both samples suggesting that biodegradation processes have been severe [11].

Mass chromatogram generation was used to trace specific biomarker compounds (Fig. 5-6) and alkyl-aromatic sheet structures (Fig. 7-10). The relative distribution of the compounds identified in this way allows the tracing of the original source and the geothermal maturity of an asphalt. Connan et al. [5] has shown this successfully for archaeological materials from Mesopotamia. The identified compounds are given in Table 1 with structures of typical markers (aliphatic cross-links such as alkanes and alkenes, aromatic sheet compounds such as alkylbenzenes, alkylnaphthalenes and alkylbenzothiophenes) and biomarkers (steroid and hopanoid compounds) present in asphalt illustrated in Scheme 1.

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[11] detected as 17α, 21β (H)-29-bishomohopane, isomers 22S and 22R

[12] detected as gammacerane (G)

[13] tentatively identified as $C_{27}H_{42}$ C-ring monoaromatic steroids, probably isomers (I 20S and/or V 20S) in the 1st group of peaks, (I 20R +V 20R and/or II 20S) in the 2nd group of peaks, (II 20R) in the 3rd group of peaks [11]

[14] tentatively identified as $C_{28}H_{44}$ C-ring monoaromatic steroids, probably isomers (I 20S and/or V 20S) in the 2nd group of peaks, (II 20S and/or I 20R + V 20R) in the 3rd group of peaks, (II 20S and/or II 20R) in the 4th group of peaks [11]

[15] tentatively identified as $C_{29}H_{46}$ C-ring monoaromatic steroids, probably isomers (I 20S + V 20S) in the 3rd group of peaks, (I 20R + V 20R) in the 4th group of peaks, (II 20R) in the 5th group of peaks [11]

Mass chromatograms of characteristic fragment ions of C-ring monoaromatic steroids MAx (m/z 253), various steroid skeletons (m/z 217 + 231 + 245 +253 + 267) and hopanes Hi (m/z 191) are plotted in Fig. 5 of HS and Fig. 6 of DS. M/z 217 is a common fragment ion for 14α (H)–steranes. M/z 231 and m/z 253 (245 and 267 for their methyl substituted compounds) represent common fragments for triaromatic steroids and monoaromatic steroids (MA), [11]. Only the C-ring monoaromatic steranes, m/z 253 form a predominant mass chromatogram profile in the Py-GC/MS data of HS (Fig. 5b and 5c). The other steranes form an unresolved envelope. This is also observed in DS (Fig. 6b and 6c).

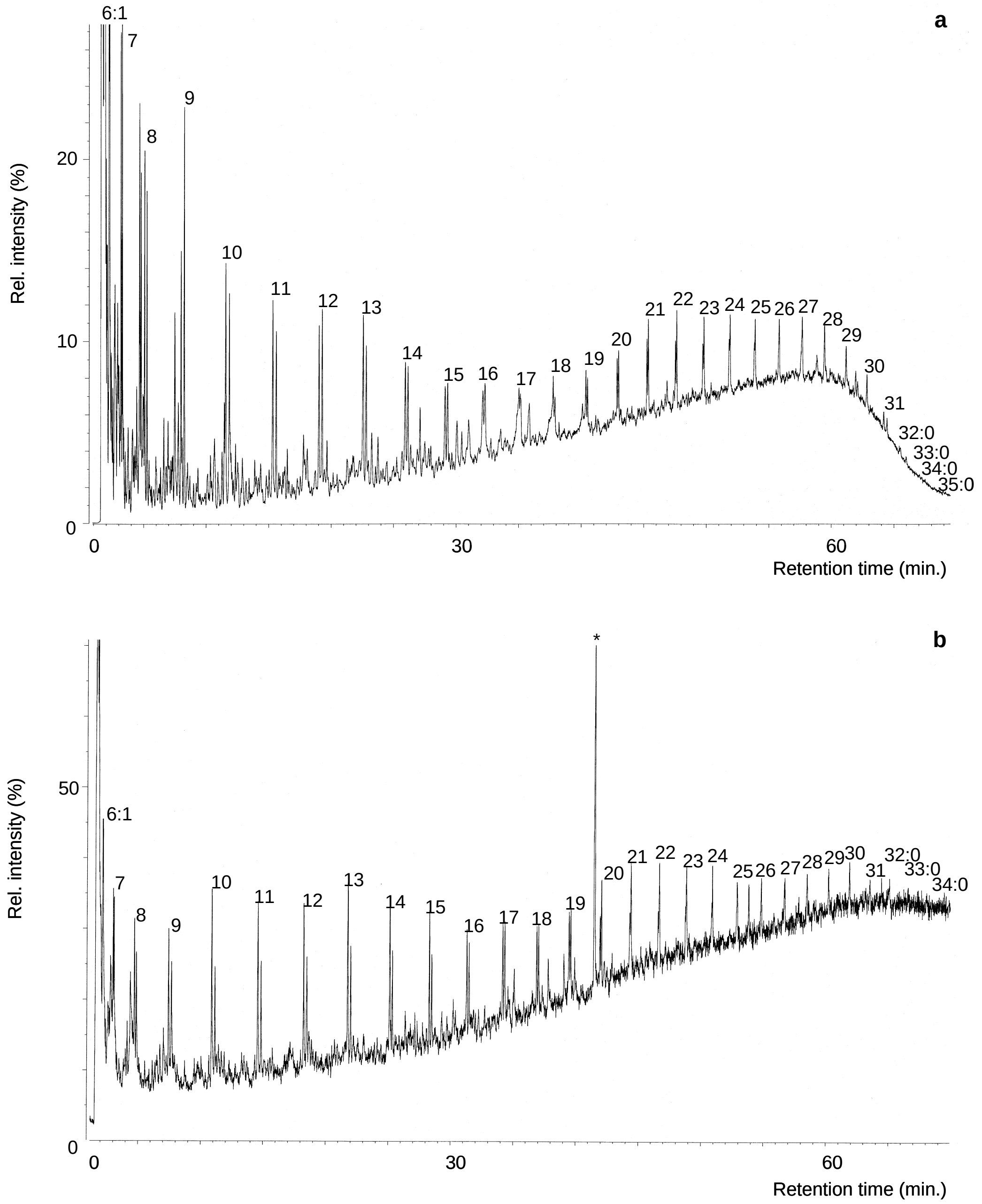
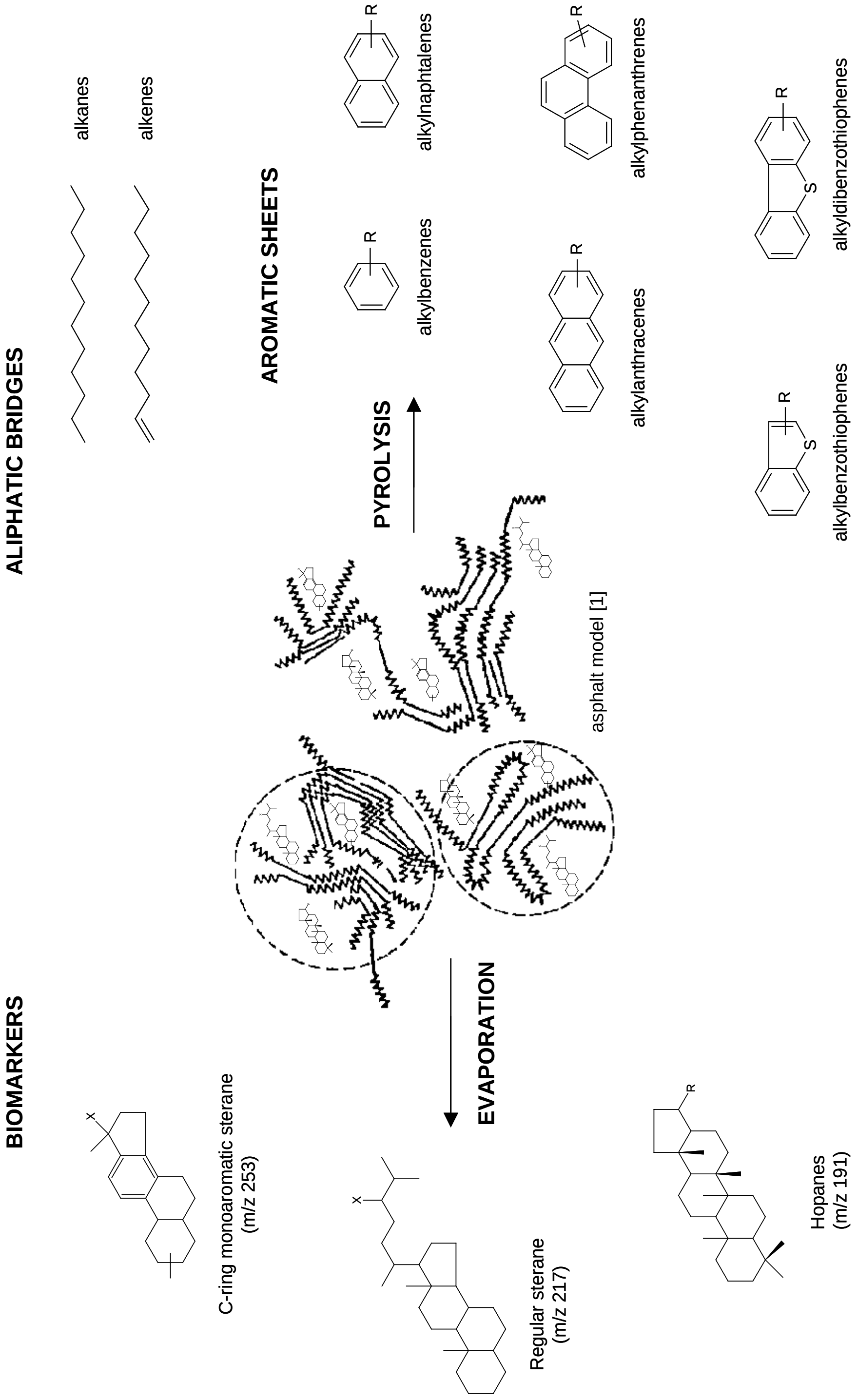


Fig. 4. Py-GC/MS TIC of sample HS (a) and sample DS (b); the presence of dibutyl phthalate is marked with ''.*



Scheme 1. Structures of specific asphalt markers and biomarkers.

Although there could be a bias about the influence of the thermal exposure during the analytical process on the steroid distribution, no influence was found when distribution in maltenes analysed by on-column GC/MS and thermally extracted fractions in Curie point Py-GC/MS of a standard reference asphalt provided by Shell were compared (chapter 5). The ion profiles of the monoaromatic steroids in HS and DS are almost identical to the literature [11, 26] and show a series

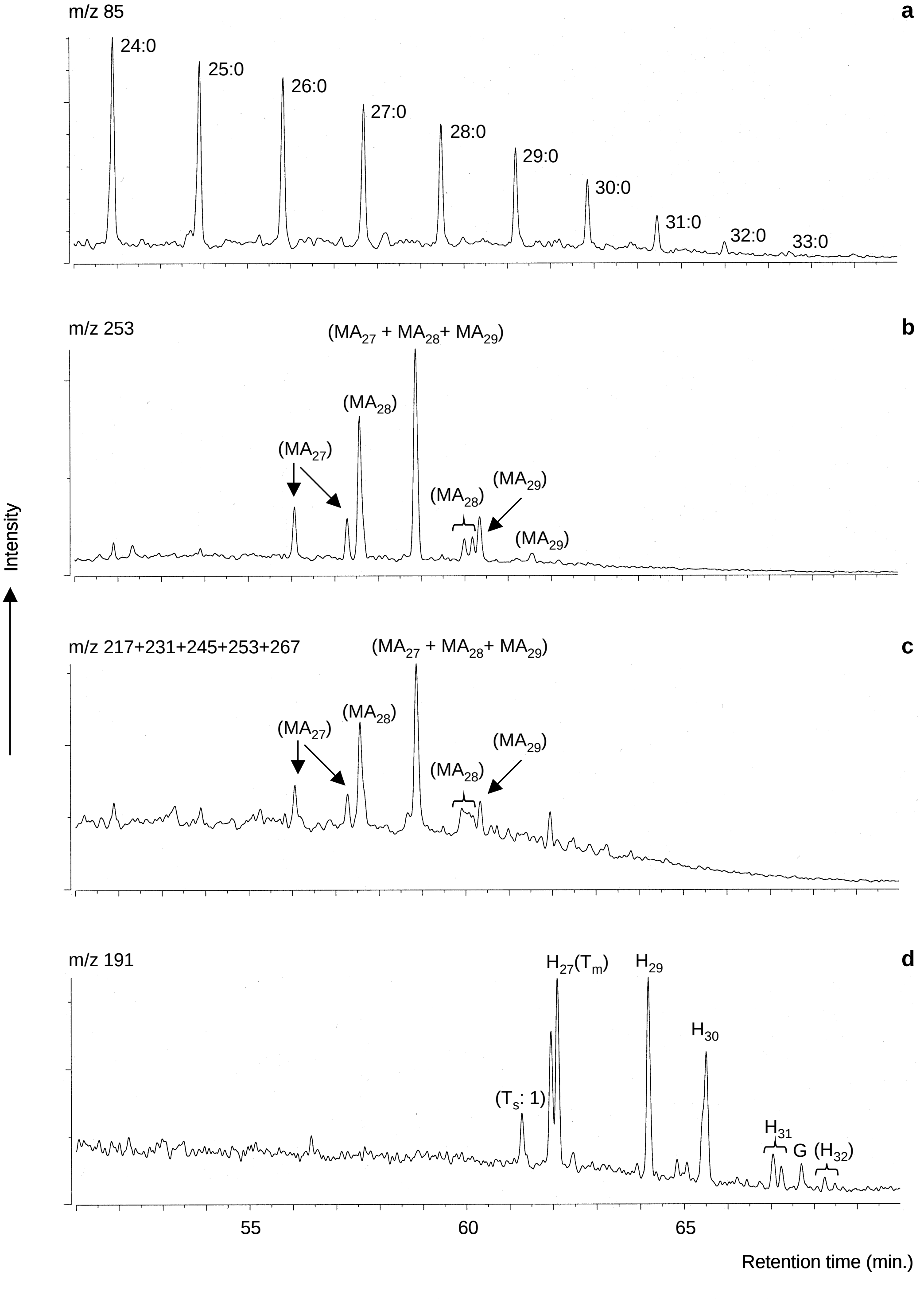


Fig. 5. Partial mass chromatograms of (a) alkanes as markers for aliphatic cross-links (m/z 85), (b) C-ring monoaromatic steroids MA_i (m/z 253), (c) steroid hydrocarbons (m/z 217+231+245 +253+267) and biomarker (d) hopanes H_i (m/z 191) in Py-GC/MS data of sample HS.

of non-rearranged steranes and rearranged C-ring monoaromatic steranes ranging from C_{27} to C_{29}. Peters and Moldowan [11] list eight known compounds in petroleum. Four of them match the non-rearranged 5(H), 10(CH_3) C-ring monoaromatic steranes: I (5β, 10β), II (5α, 10β), III (5α, 10α), IV (5β, 10α). The other four structures, V (5β, 10β), VI (5β, 10α), VII (5α, 10α) and VIII (5α, 10β) correspond to the rearranged 5(CH3), 10(H) C-ring monoaromatic steranes.

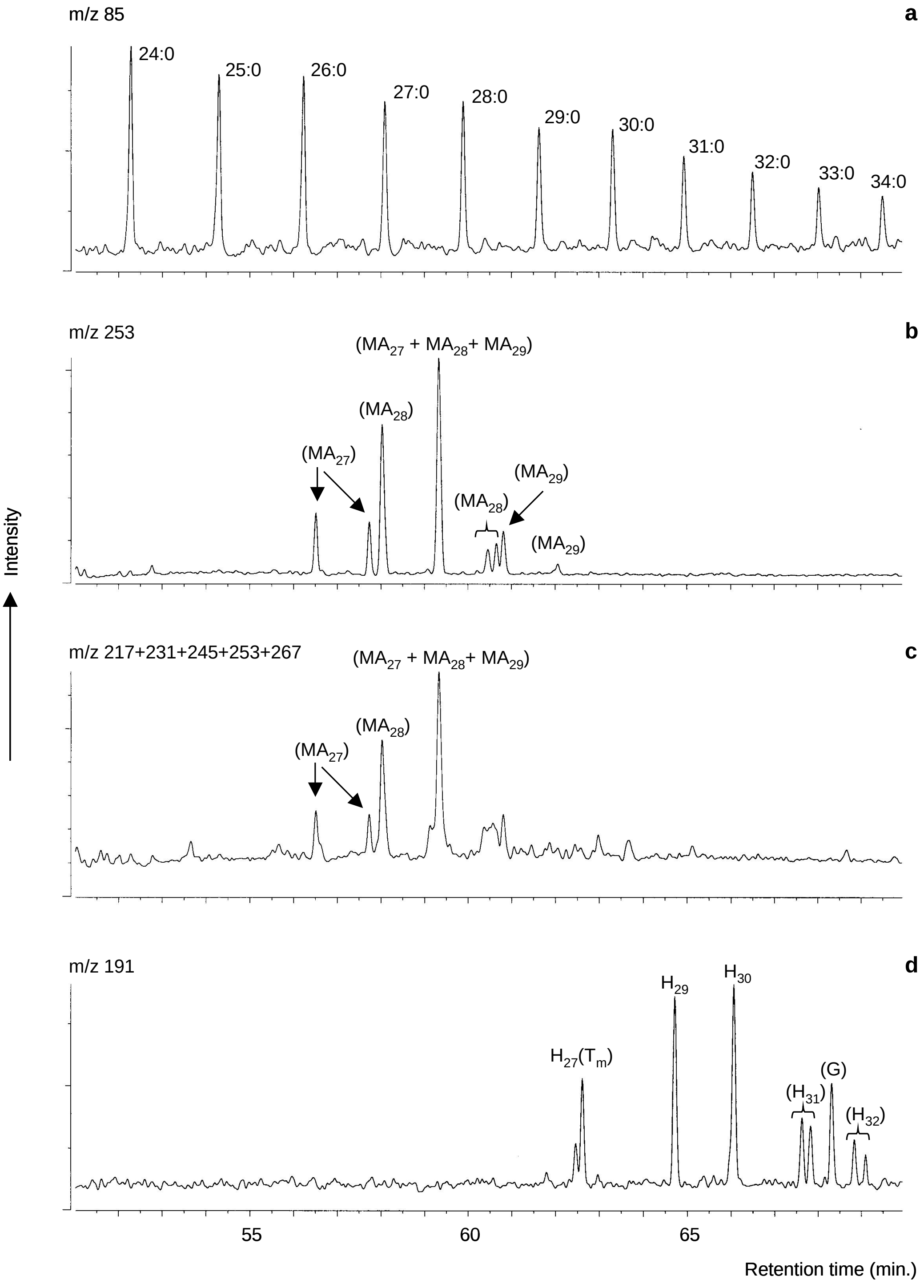
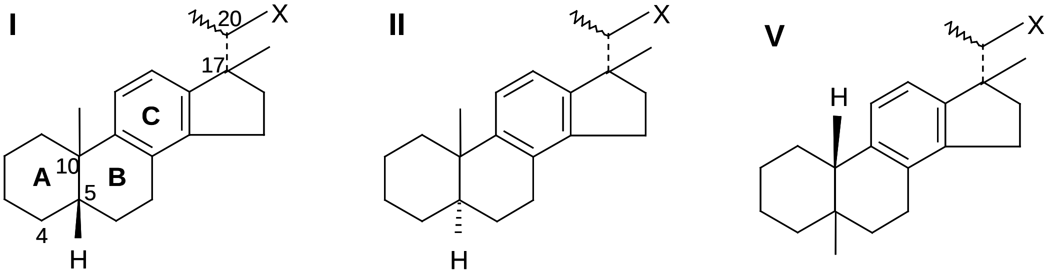


Fig. 6. Partial mass chromatograms of (a) alkanes as markers for aliphatic cross-links (m/z 85), (b) C-ring monoaromatic steroids MA_i (m/z 253), (c) steroid hydrocarbons (m/z 217+231+245 +253+267) and biomarker (d) hopanes H_i (m/z 191) in Py-GC/MS data of sample DS.

They specify that only structures I, II and V, shown in Scheme 2, are the most common and used in quantification of MA steroids [11].



5β (H), 10β (CH_3) 5α (H), 10β (CH_3) 5β (H), 10β (CH_3)

*Scheme 2. Most common structures of non-rearranged C-ring monoaromatic steroids (structures **I** and **I I**) and rearranged C-ring monoaromatic steroids (structure **V**). [11, 26]*

According to the literature five groups of peaks are expected and symmetrically distributed around the middle one. The first group contains two peaks of the C_{27} homologues. The second group has four peaks, the first two for the C_{27} homologues and the last two for the C_{28} homologue, possibly reversed [11]. The third group includes a C_{27} homologue, two peaks for the C_{28} homologues and one peak for the C_{29} homologues. The fourth group has two C_{28} homologues and one peak for the C_{29} homologues. The last group is in fact just one peak, a C_{29} homologue. The corresponding isomers for each homologue in the group are given in Table 3.

Table 3. Isomers of C-ring MA steroids. [11]

Group	**C_{27}**	**C_{28}**	**C_{29}**
1st	I 20S, V 20 S	-	-
2nd	I 20R + V 20R, II 20S	I 20S, V 20S	-
3rd	II 20R	II 20S, I 20R + V 20R	I 20S + V 20S
4th	-	II 20S, II 20R	I 20R + V 20R
5th	-	-	II 20R

In the data from both HS and DS, these five groups of peaks are observed, with a similar relative distribution around the middle group as mentioned above. The peaks shown in the m/z 253 partial mass chromatogram (Fig. 5 and 6) and listed in Table 2 are tentatively identified as C-ring MA steranes on the basis of the presence of the specific fragment m/z 253 and the rather good match of their relative distribution with the data from literature. Due to their low relative abundance and the complexity of the asphaltene compound envelope, the corresponding parent ions could not be discriminated from isobaric alkylbenzothiophenes molecular ions. The C–ring MA's in Fig. 5 and 6 were tentatively identified as the first group being C_{27} homologues, the second group C_{27} and C_{28} homologues, the third group C_{27}, C_{28} and

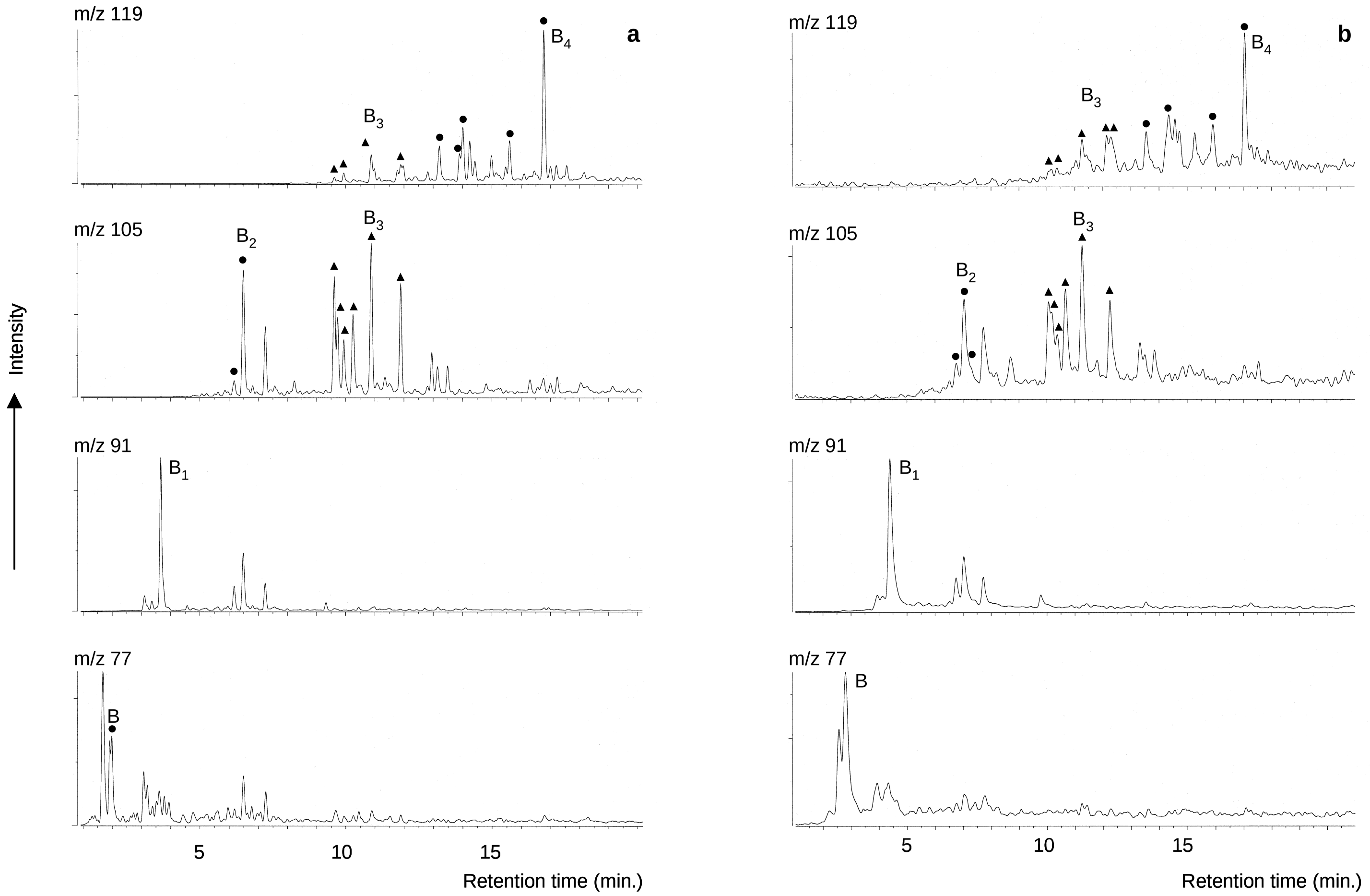


Fig. 7. Partial mass chromatograms of alkylbenzenes $\mathbf{B}_i$ *of sample HS (a) and sample DS (b) in Py-GC/MS data; the isomers are marked with '●' and '▲'to distinguish between two consecutive homologues.*

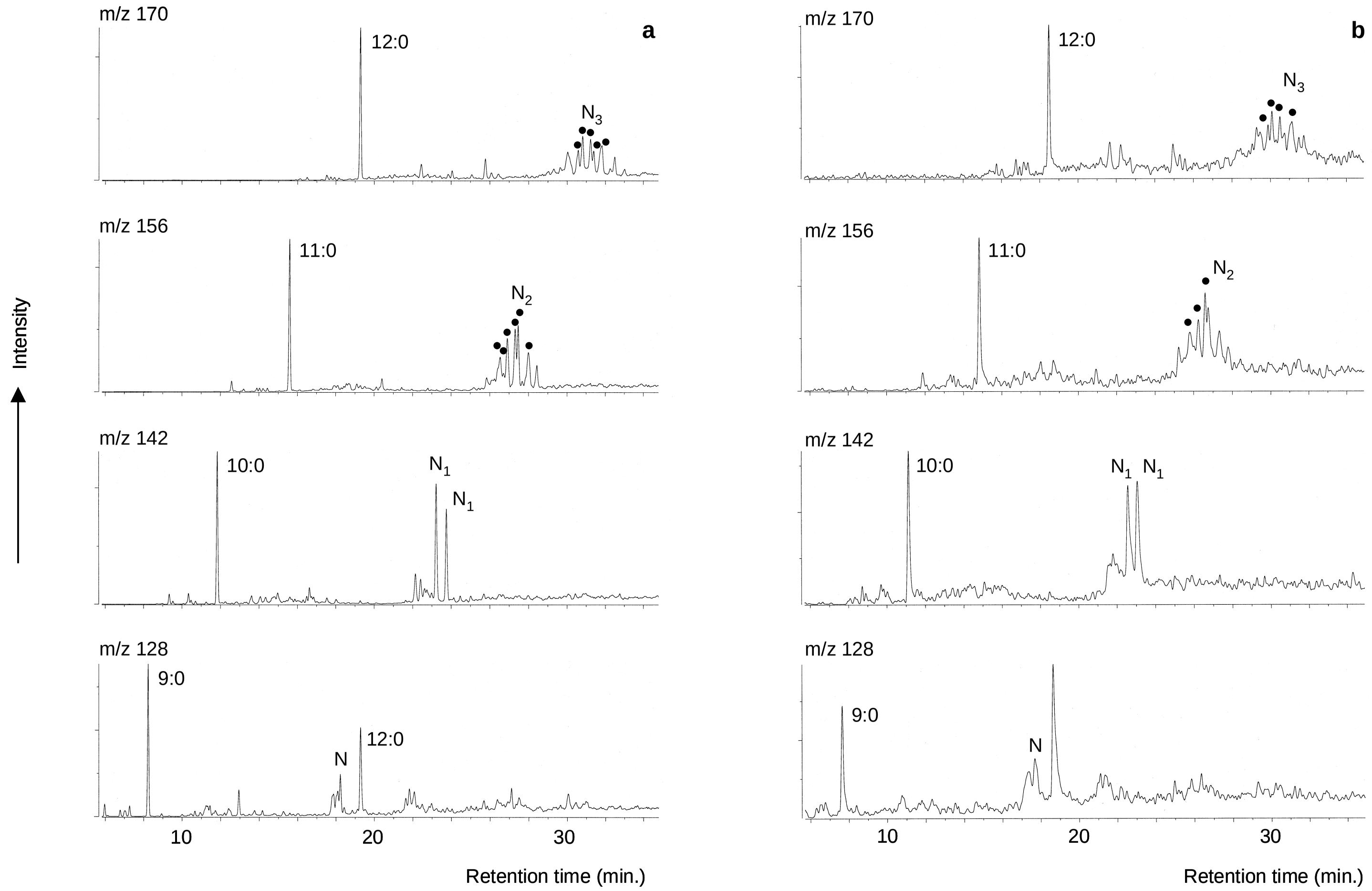


Fig. 8. Partial mass chromatograms of alkylnaphthalenes **N$_i$** *of sample HS (a) and sample DS (b) in Py-GC/MS data; the isomers are marked with '●'.*

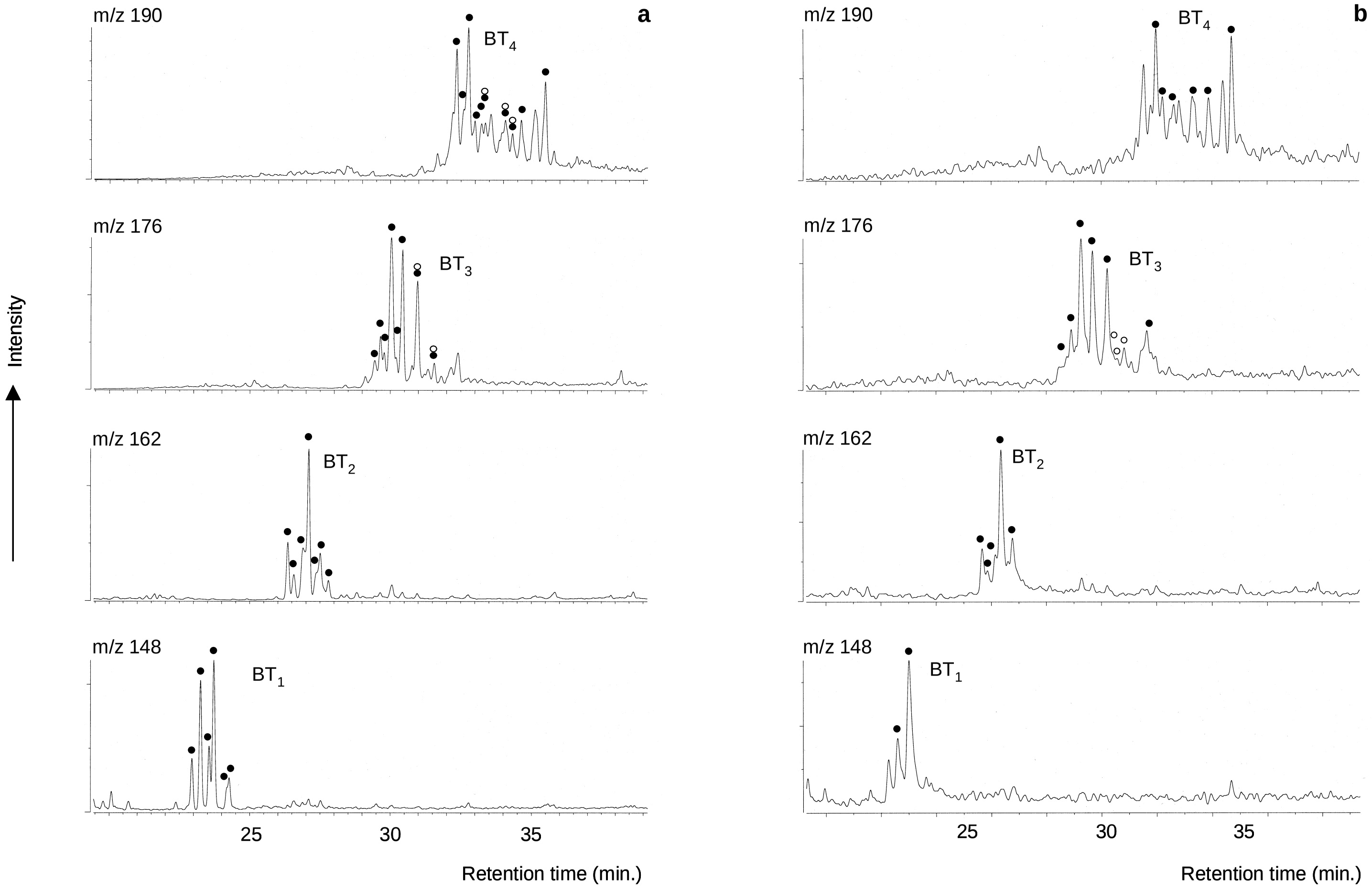


Fig. 9. Partial mass chromatograms of alkylbenzothiophenes ***BT$_i$*** *of sample HS (a) and sample DS (b) in Py-GC/MS data; '●' is used to mark the saturated compounds and 'o' the unsaturated homologues.*

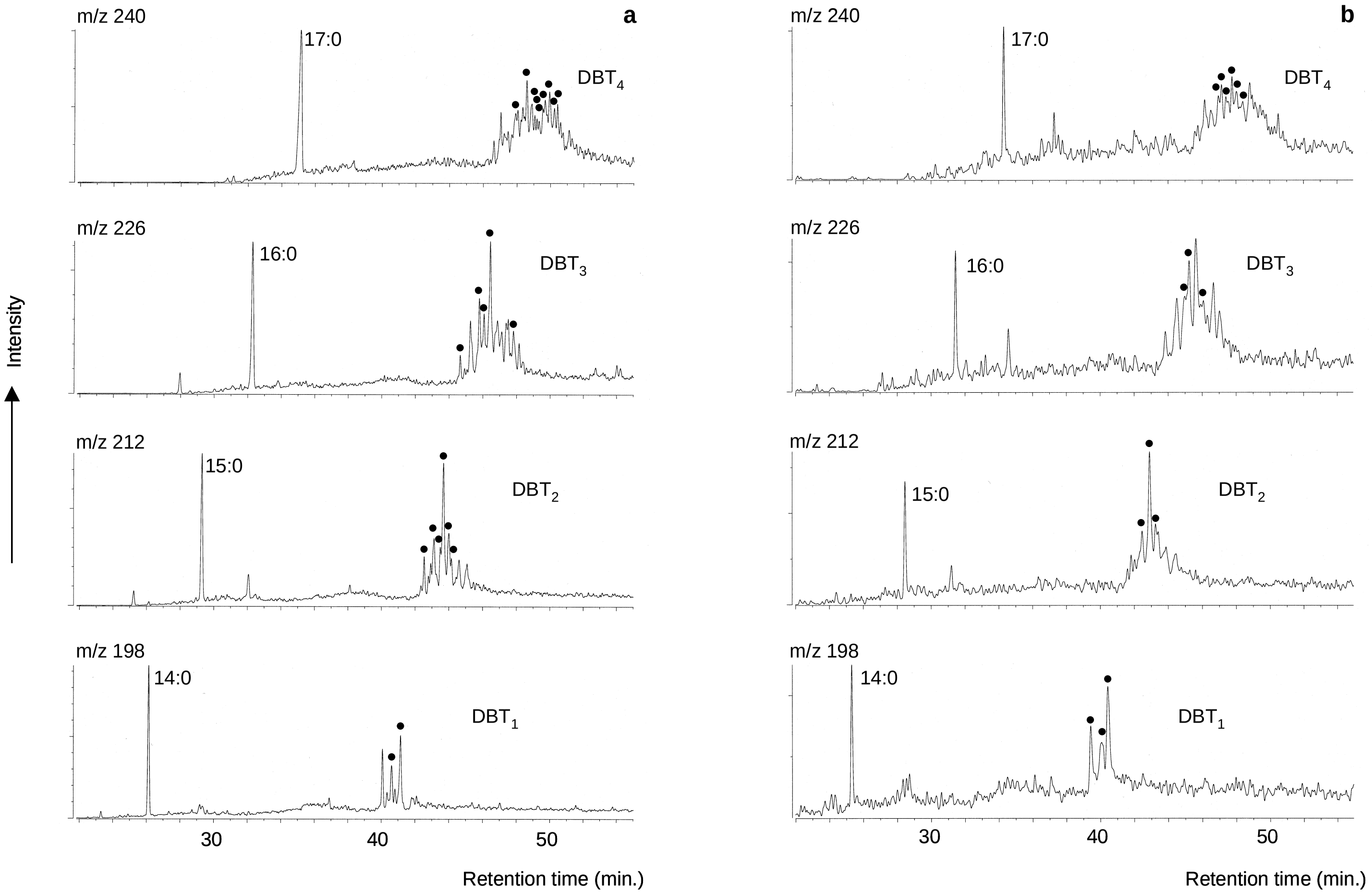


Fig. 10. Partial mass chromatograms of alkyldibenzothiophenes DBT_i of sample HS (a) and sample DS (b) in Py-GC/MS data; the isomers are marked with '●'.

C_{29} homologues, the fourth group C_{28} and C_{29} homologues and the fifth group as the C_{29} homologues. No diasteranes (m/z 259) are present in sample HS and DS but these compounds are not expected in a Dead Sea asphalt type [5].

The hopanes in HS (C_{27} to C_{32}) have a low relative abundance with respect to the MA's but their mass spectra are recognisable. Relatively abundant in Fig. 5 are the Tm (C_{27}) trisnorhopane isomer, norhopane (C_{29}), hopane (C_{30}), homohopane (C_{31}) 22S and 22R isomers and the tentatively identified bishomohopane (C32) 22S and 22R isomers. The T_s trisnorhopane isomer is not present, but a peak with a similar relative retention time but a parent ion at m/z 368 appears before the T_m. The peak between the C_{31} and C_{32} hopanoid homologues could be identified as gammacerane. [11]. The abundance of hopanes in DS (C_{29} to C_{31}) is relatively low compared to the data available in the literature on other Dead Sea asphalt samples [5, 27]. In Fig. 6 the trisnorhopane isomer T_m (C_{27}), norhopane (C_{29}), hopane (C_{30}) are positively identified, while homohopane (C_{31}) 22S and 22R isomers and bishomohopane (C_{32}) 22S and 22R isomers are tentatively identified on the basis of relative retention time and some characteristic mass peaks. The peak between the C_{31} and C_{32} hopanoid homologues is tentatively identified as gammacerane.

The Dead Sea asphalt sample analysed and the asphalt sample from the Hafkenscheid collection contain a large number of algal-derived steroid biomarkers mainly present as C-ring monoaromatic steroid hydrocarbons. Both samples have a low abundance of bacterially derived hopanoid markers.

Aromatic hydrocarbons released from asphaltenes in HS and DS are present as alkylbenzenes, alkylnaphthalenes, alkylanthracenes, alkylphenanthrenes, alkylbenzothiophenes and alkyldibenzothiophenes. Alkylbenzenes (m/z 91, 105, 119) are shown in Fig. 7a and 7b. Alkylnaphthalenes (m/z 142, 156, 170) are given in Fig. 8a and 8b. Alkylbenzothiophenes (m/z 148, 162, 176) are shown in Fig. 9a and 9b. Alkyldibenzothiophenes (m/z 198, 212, 226, 240) are shown in Fig. 10a and 10b. Alkylanthracenes and alkylphenanthrenes are not shown. There is good degree of similarity in the distribution of all these marker compounds between HS and DS. These aromatic subunits are typical for asphaltenes [5] and good evidence that the Hafkenscheid sample was indeed a natural asphalt. The data obtained for the HS and DS samples were compared to several other asphalts kindly provided by Shell Research and Technology Centre in Amsterdam. All marker compounds are present in these samples but their relative distribution can differ between asphalts of different origins. The presence of the sulphur containing benzothiophenes (BT) and dibenzothiophenes (DBT) [5] is another indication for the asphaltic nature of the Hafkenscheid sample. Both dibenzothiophenes and aromatic steroids are considered to be the most reliable markers for asphalt by Connan et al. [5] due to their resistance against bacterial degradation.

Conclusions

The DTMS and Py-GC/MS data confirm that the Hafkenscheid "asphalt" is indeed a natural asphalt sample. The distribution of the "biomarker" compounds is not unlike asphalts from the Middle Eastern region, but further comparative studies on natural asphalts available in the 19th century should be performed to assure its provenance. The match with the Dead Sea reference sample is good considering the history of the samples. The Hafkenscheid sample has been exposed to air for about 200 years and it is not unlikely that this will have an effect on the sulphur containing compounds in the asphalt. Moreover studies by Connan et al. [5] have shown that asphalts near the Dead Sea may differ in composition due to exposure or thermal history. The methodology applied exposes a large number of marker compounds that may be very useful for asphalt characterisation in asphalt pigmented paints from paintings. Preliminary studies of asphalt pigmented paints in the FOM-AMOLF laboratory have already shown that such markers can be traced. The proposed methodology is also very applicable to the extremely small samples that may be obtained from paintings. A complicating factor in painting studies will be the paint manufacturing process. The data show that many characteristic markers for asphalt can be evaporated or released by pyrolysis. So any paint manufacturing pretreatment involving roasting of the asphalt will likely result extensive or even complete loss of the marker compounds identified. In addition, restoration treatment of paintings often implies exposure to solvents, which may displace or even extract solvent sensitive materials such as the maltene fraction. In the case of asphalt, the solvent extractable biomarker compounds are especially sensitive, putting a bias on the validity of hopanes as markers of asphalt in paintings with an unknown history of preservation. The asphaltene fractions of asphalt are much less likely to be effected by solvent cleaning and thus may provide a rich potential source of asphalt markers in paintings.

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3. Mummy: Characterisation of molecular markers and biomarkers in the 19th-century Hafkenscheid mummy pigment

Abstract

A mummy pigment from the 19th-century Hafkenscheid collection of painting materials is analysed by various analytical mass spectrometric techniques using Direct Temperature resolved Mass Spectrometry (DTMS) and Pyrolysis-Gas Chromatography coupled with Mass Spectrometry (Py-GC/MS), Py-tetramethylammonium hydroxide-GC/MS (Py-TMAH-GC/MS), and on-column GC/MS. Results were compared with data from megilp and an asphalt sample from the Hafkenscheid collection analysed as reference materials under similar conditions. The mummy pigment investigated has a complex nature and it was found to contain mainly slightly aged mastic resin and a poorly oxidised linseed oil with additions of beeswax, asphalt, pine resin and another fat. Molecular markers for asphalt in the mummy sample were identified by Py-GC/MS, finding various hopanes, benzo- and dibenzothiophenes but the asphalt components seem to have undergone some pre-treatment that modified its composition. Long chain normal fatty acids indicative of drying oil and beeswax were identified by Py-TMAH-GC/MS. In the same way two diterpenoid acid methyl esters indicative of slightly oxidised pine resin were discovered. Other fatty acids, e.g. myristic acid and monounsaturated fatty acids (C_6-C_{16} and C_{18}), suggest an additional fat component in the mummy pigment. The presence of iso and anteiso fatty acids points to a bacterial and/or fungal lipid contribution or even contribution from human tissues. The degraded asphalt, the unusual fat component and the beeswax are possibly originating from mummies, but an Egyptian origin cannot be confirmed.

The mummy pigment in the Hafkenscheid collection seems to be a medium-like material with the black appearance of asphalt. It is postulated that is consists of a megilp used as base material with additions of black mummy powder or a combination of materials with similar properties. A connection between Egyptian mummies and the Hafkenscheid mummy pigments cannot be proven.

Introduction

Mummies are "bodies embalmed or treated for burial with preservatives after the manner of the ancient Egyptians" [1]. As a pigment, mummy[1] is described as a "brown, bituminous pigment […] prepared from the bones and bodily remains of Egyptian mummies, which have been embalmed with asphaltum" [2]. A "mummy pigment" sample from the 19th-century Hafkenscheid collection (see below) is the object of this study. Does it contain asphalt[2]? How would its composition relate to Egyptian mummies[3]? The strategy adopted in this research consists of qualitative mass spectrometric analysis of the mummy pigment, and its comparison to suitable reference samples.

a) Mummification in Egypt: on the use of asphalt as an embalming material

In his review Lucas [7] has shown mummification to be a common practice in Egyptian culture covering a period of more than 3000 years [7, 8]. Embalming techniques used in ancient Egypt involved the removal of internal organs, and the preservation of the body using natron (i.e. sodium salts), the application of resin or asphalt-resin mixtures to stop bacterial growth, with bandages and sawdust inside the body to preserve its original shape as much as possible. Wrapping of the body required other materials like resins, herbs, onions, gums, and asphalt to soak the bandages in, or to put in between the several bandage layers [7, 9].

Egyptians used asphalt for embalming only for a limited period, no more than from 1000 B.C. to 400 A.D. [6, 10-13]. Buckley et al. and White place it ca 400 B.C. - 0 [6, 10]. Wisseman and Proefke et al. confine the use of asphalt to the Roman period, 31 B.C.- A.D. 395 [11, 12]. Connan estimates the period to be longer, 1000 B.C. - 400 A.D., including the Intermediate, Ptolemaic and Roman periods[4] [13]. Harrell and Lewan suggested that a local Egyptian source of asphalt was possibly available from around 900 B.C. Comparative studies on mummies [5, 13, 14] have shown that the asphalt used for embalming came mostly from the Dead Sea area being a readily available resource as floating blocks of asphalt that could be transported by camels. Other sources of asphalt have been suggested to be in Egypt itself or from Hit in Mesopotamia [13, 14].

b) From embalmed bodies to the mummy pigment used in painting

Egyptian mummies have been dug out and traded for centuries, because they generated a great interest for medical, historical, scientific and often more trivial

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[1] in this chapter the term "mummy" is used for the embalmed bodies and "mummy pigment" for the pigment.

[2] the term "asphalt" has often been used as if interchangeable with "bitumen"; however it is more appropiate to consider "asphalt" as the source rock and "bitumen" as the organic fraction extracted from asphalt [3]; in this research the term "asphalt" for asphalt/ bitumen material is used.

[3] 'mumiya' means 'wax' in Persian and 'bitumen' in Arabic [4-6].

[4] Egyptian mummies from the Queen valley and in museums in Lyon, Hanover, and Paris, showed that Dead Sea bitumen was the most common found in balsams. Another possible source suggested is Hit Iraq. [13]

reasons. Material from mummies has been used as fertiliser, as fuel, in paper manufacture, and as medicine as well as pigment [15].

The ageing of embalming materials gave mummies a darkened-blackened appearance suggestive of asphalt. Asphalt and crude oil were used for internal and external medical treatment [16]. The idea of the curative properties of asphalt was transferred to mummies. In consequence dark material was scraped from linen or from the interior cavities of mummies, ground into powder and used as a medicine, e.g. in Europe between the 12th and the 18th century [4, 6, 8, 11, 15-20]. Replacement mummies made from inadequately cleansed corpses of slaves and criminals used to cover the extensive need of mummy-medicine is thought to have caused the spread of black death in Western Europe and meant the end of use for this medicine [4, 7, 8, 15].

The "asphalt containing" mummy-medicine is described as a pigment from the 12th century to the end of the 19th century and as oil paint pigment since the 16th century [15, 18, 21-24]. It was possibly also used as a watercolour pigment [15, 18, 23]. Used for glazing and shadows, the mummy pigment or "Egyptian brown" was ground in drying oil such as nut oil, and mixed with amber varnish and/or other colours[5] [6, 15, 18]. Prussian brown[6] and Kassel earth/Vandyke brown[7] pigments or asphalt-lime mixtures are suggested as substitutes or adulterants for the mummy pigment [2, 15, 18]. The drying qualities and fastness to light of mummy pigment, so important for painters and restorers, are reported to be sometimes better and other times worse than asphalt depending on the mummy pigment composition [6, 15, 21, 22].

c) Analytical evidence for asphalt in mummies

The composition of mummy pigment is expected to be complex and related to the composition of real mummies, i.e. dependent on the embalming materials and techniques, the origin of the mummy and the provenance of the sample used for preparation of the pigment [7, 15, 25]. Woodcock mentions the use of different parts of the mummies for making the pigment. The muscles and flesh parts were preferred, but bones and bandages were included [15, 25]. A certain degree of compositional variability is expected as the constituent materials themselves (e.g. body, bandage, etc.) can differ significantly.

Spielmann, Lucas, Benson and Petit, who tried to identify asphalt in mummies in the beginning of the 20th century with more simple analytical means, had great difficulties in its identification. Their methods were based on solubility tests, sulphur content, characteristic smell, fluorescence, spectrographic analysis of elements for presence of the characteristic metals such as nickel, vanadium, molybdenum [7, 26-28]. Others also tried infrared spectroscopy, X-ray diffraction (XRD) and differential thermal analysis (DTA) [9, 29].

Currently used techniques for molecular evidence of asphalt in samples from mummies are mainly chromatographic and mass spectrometric techniques[8] or

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[5] ultramarine, glazing colours (*e.g.* madder lake), ivory black [15]

[6] i.e. calcined Prussian blue [21, 22]

[7] i.e. "a brown organic pigment […] derived from earthly substances similar to lignite or brown coal" [2]; later in the text referred as Kassel earth

[8] where LC= Liquid Chromatography, HPLC= High Pressure LC, MPLC= Medium Pressure LC, GC= Gas Chromatography, MS= Mass Spectrometry, GC/MS= GC coupled with MS, TD-GC/MS= Thermal Desorption GC/MS, Py-GC/MS= Pyrolysis GC/MS, GC/C-IRMS= GC/combustion/isotope ratio MS,

combinations of both, like HPLC or MPLC combined with GC/MS, GC, TD-GC/MS, Py-GC/MS, isotopic chemistry of carbon and hydrogen GC/C-IRMS, FAB/MS, HR FAB/MS, FAB/MS/MS, GC/MS and Py-MS [5, 6, 10, 12, 13, 30-35]. Plasma atomic emission spectroscopy was also used to identify asphalt in mummy samples by trace metal analysis of nickel, vanadium and molybdenum [26, 32, 36]. Techniques such as Raman spectroscopy combined with GC/MS and SEM[9], and radiocarbon dating, TLC and GLC reported in literature for analysis of mummy samples exclude the search for asphalt [25, 27, 32, 35, 37-40].

The success of GC/MS techniques used for identification of asphalt and complex organic composition of mummy samples is extensively reported in the literature. The presence of *lipids* from mainly plant (oil, waxes, resins) but also animal and human origin and *fossil hydrocarbons* (*asphalt biomarkers*) derived from embalming materials was demonstrated. In the *lipid* group long chain even[10] carbon fatty acids[11] from vegetable oils or animal fat tallow, their oxidative degradation products (dicarboxylic fatty acids), and fatty acids originating from human tissues were found [6, 10, 30, 31, 35, 37]. Wax derived n-alkanes[12], with a odd-over-even carbon number predominance typical for epicuticular waxes of higher land plants, hydroxy wax esters[13] and wax esters[14] of even carbon number have been retrieved [5, 10, 13, 30, 33, 35]. Resin plant derived compounds such as hydroxyaromatic acids[15], cadalenes and retenes, monoterpenoids and sesquiterpenoids are also reported [5, 10, 30]. Derivatives of abietic acid[16] from coniferous resins are said to be a major component of mummy samples [6, 31, 35]. Another type of resin found in mummy samples is the *Pistacia* genus resin (mastic), containing oxidised and dehydrogenated triterpenoid compounds[17] with oleanic and ursanic structures [10, 30, 41-44]. The *fossil hydrocarbons* (*asphalt biomarkers*) found in mummy samples consist of n-alkanes with no carbon number predominance[18], steroid hydrocarbons as steranes (m/z 217+218), sterenes (m/z 215) and aromatic steroids, polycyclic triterpanoid hydrocarbons as hopanoid C-skeletons and gammacerane (both traced by m/z 191), and aromatic hydrocarbons like phenantrenes, naphtalenes and methyl- naphtalenes [5, 10, 12, 13, 30, 33, 35, 45-49]. Asphalt biomarkers are prone to oxidation[19] under special conditions like biodegradation, evaporation, water washing, abiotic oxidation or roasting [50, 51].

In a number of cases, analysis of samples from mummies has revealed the presence of asphalt from the Dead Sea or of other Middle Eastern origin [5, 12-14, 30,

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FAB/MS= Fast Atom Bombardment MS, HR FAB/MS= High Resolution FAB/MS, FAB/MS/MS= FAB tandem Mass Spectrometry, Py-MS= Pyrolysis MS

[9] SEM= Scaning electronin Microscopy, TLC= Thin Layer Chromatography, GLC= Gas Liquid Chromatography

[10] i.e. of biosynthetic origin

[11] (e.g. C_{10}-C_{24})

[12] (e.g. C_{25}-C_{33}, C_{21}-C_{33} with max at C_{27}, C_{23}-C_{31}, C_{23}-C_{31})

[13] (e.g. C_{42}-C_{54})

[14] (e.g. C_{40}-C_{50}, C_{40}, C_{40}-C_{48})

[15] 4-hydroxy-3 methoxy benzoic acid, p-hydroxybenzoic acid as from aromatic benzoate of balsamic resins or ferulic acid (*Umbelliferae*) [10]

[16] like dehydroabietic acid (DHA) but mostly oxidised homologues such as 7-oxo dehydroabietic and 15-OH-7-oxo dehydroabietic acids [35]

[17] dammarenes [10]; oleanonic and (iso)masticadienoic acids [30]

[18] the even carbon number alkanes are hardly synthesised by land plants; (e.g. C_{18}-C_{36} with a max at C_{24}, C_{19}-C_{33}, C_{16}-C_{19})

[19] to benzylic ketones (from oxidation of aromatic steroids and secohopanoids), carboxylic acids (from oxidation of steroids, trycyclic terpenoids, hopanoids and benzothiophenes) and sulfones (from oxidation of steroid, terpenoid or hopanoid sulfides) [50]

32, 33]. In other cases, no asphalt has been found [6, 10, 34]. White states that it is unlikely for mummy to be firmly identified in paint samples, except under exceptional circumstances [6]. To the knowledge of the current researcher no analytical data are available for the mummy pigment.

d) Provenance and relevance of the Hafkenscheid mummy pigment sample

The mummy pigment sample under consideration here originates from a 19th-century paint material collection, the Hafkenscheid Collection[20] [23, 52-54]. The collection represents the available paint making materials from the Painting Materials, Turpentine and Gums business owned by the Hafkenscheid family. It is one of the few surviving collections of paint materials from the early 19th century. The collection includes approximately 370 samples of inorganic and organic materials. A large number of the materials have been analysed already and proved to be standard early 19th-century pigments [23, 45, 46, 52].

The presence in the collection of the still uncharacterised mummy pigment [52] gave us an excellent opportunity to check the possible asphaltic nature of such a pigment and its composition in general, to verify any possible relation to Egyptian mummies, and to get an idea of the nature of mummy pigments available to painters in the 19th century using an analytical protocol applied before to characterise the asphalt from this collection [45, 46].

Experimental

Samples

In this study results are shown for the mummy pigment from the Hafkenscheid Collection [23, 52] (labelled “MP”). A 19th-century asphalt sample from the same collection (labelled “A”) was analysed and used as a reference for evaluating the asphaltic nature of the MP-sample. Preliminary results for the MP were somewhat similar to megilp[21]. A megilp sample present in the MOLART collection as a gift of Dr. J. Townsend (Tate Gallery, London) was therefore analysed as well (labelled as “MG”). The MP-sample consists of small black amorphous lumps of glossy material and looks very similar to the A-sample [45, 46]. The MG-sample is a transparent-yellowish and sticky material.

Direct Temperature Mass Spectrometry (DTMS) [56, 57]

For DTMS analysis, 5-10 μg of sample were dissolved in 15-25 μl of solvent (ethanol, or dichloromethane, DCM). An aliquot of 1-2 μl of this solution was placed on the Pt/ Rh filament (Pt/Rh 9:1, 100 μm) of a direct insertion probe for in-source analysis and DTMS experiments were carried out on a JEOL JMS SX-102 double

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[20] nowadays in the custody of the Teylers Museum in Haarlem, The Netherlands [23, 52]; see also Chapter 2

[21] megilp= mixture of oil, mastic resin and a lead drier [21, 22, 55]

focussing mass spectrometer (B/E). The filament was heated with a rate of 0.5 A/min to an end temperature of about 800 °C. Ions were generated by 16 eV EI to minimise fragmentation reactions of the ions in an ionisation chamber kept at 190 °C, accelerated to 8 kV, analysed from m/z 20-1000 (about 1 s cycle time) and post-accelerated to 10 kV. A JEOL MS-MP 9020D data system was used for data acquisition and processing.

(Off-line transmethylation) Pyrolysis Gas Chromatography Mass Spectrometry, Py-(TMAH)-GC/MS, and on-line transmethylation Gas Chromatography Mass Spectrometry, on-column GC/MS

Typically 10 µg of sample is used for *Py-(TMAH)-GC/MS*. The samples were dissolved in DCM or ground with tetramethylammonium hydroxide (TMAH), applied to a ferromagnetic wire (Curie point 770 °C) and dried *in vacuo*. After drying the sample wire was inserted into a glass liner, placed in the cold compartment of the pyrolysis unit, flushed with helium and then moved into the pyrolysis chamber (220 °C) of the FOM 5LX Curie point pyrolysis unit [58]. For *on-column GC/MS* a methanolic extract of the sample (5 µg/ µl) was methylated according to the Hashimoto et al. method [41, 59]. An aliquot of 16 µl of the methanolic extract was evaporated to dryness and aliquots of 250 µl of methanol, 25 µl of toluene and 10 µl of TMS-diazomethane were added. The mixture was left at room temperature for 30 minutes. After evaporation to dryness, the sample was dissolved in 1 ml of DCM. For separation a fused silica SGE BPX5 column was used (25 m, 0.32 mm i. d., 0.25 µm film thickness) with helium as a carrier gas at a flow rate of 2 ml/min, in a Carlo Erba series 8565 HRGC MEGA 2 gas chromatograph. For the Pyrolysis experiments the oven temperature was programmed from the initial 35 °C (for 0 min.) to a final 320 °C (for 10 min), with a ramp of 4 °C /min. For the on-column measurement the oven temperature was programmed from the initial 50 °C (for 2 min.), with a ramp of 8 °C /min to 250 °C (for 0 min), to a final 350 °C (for 0 min), with a ramp of 3 °C /min. The column was interfaced directly to a JEOL JMS DX-303 double focussing (E/B) mass spectrometer using a home built high temperature interface. Ions were generated by electron impact ionisation (70 eV) in the ionisation chamber, accelerated to 3 kV or 8 kV respectively, mass separated and post-accelerated to 10 kV before detection. The mass range was scanned from m/z 35-500 or m/z 40-800 respectively, with a cycle time of 1 s. A JEOL MS-MP 9020D data system was used for data acquisition and processing.

Results and discussion

a) DTMS of the mummy pigment and reference materials

The general composition of the mummy pigment (MP) was determined by DTMS and is shown together with the mass spectra of the asphalt (A) and megilp (MG) reference samples in Fig. 1. The interpretation of the peaks present in the mass spectra is given in Table 1. The mass spectra in Fig. 1 (0-700 m/z) show a strong similarity of the MP with MG and no similarity with the A. The DTMS of the MP-sample, in Fig. 1a, shows three groups of peaks. The first group, m/z 100-300 m/z, contains mostly fragment ions of terpenoids ("○") and waxes, and molecular ions of fatty acids ("□"). The second group, m/z 350-500, shows molecular ions of triterpenoids ("○") and the third one, m/z 590-700, includes molecular ions of wax esters ("◆"). The DTMS of the MG-sample, Fig. 1b, displays also three groups of peaks, a first group ranging from m/z 100-300 displays fragment ions of chemically dried oil ("□") and terpenoids ("○"), the second group ranging from m/z 350-500, shows molecular ions of triterpenoids ("○") and a third mass range showing low intensity ions of m/z 590-700 are fragment ions of diglycerides deriving from oil.

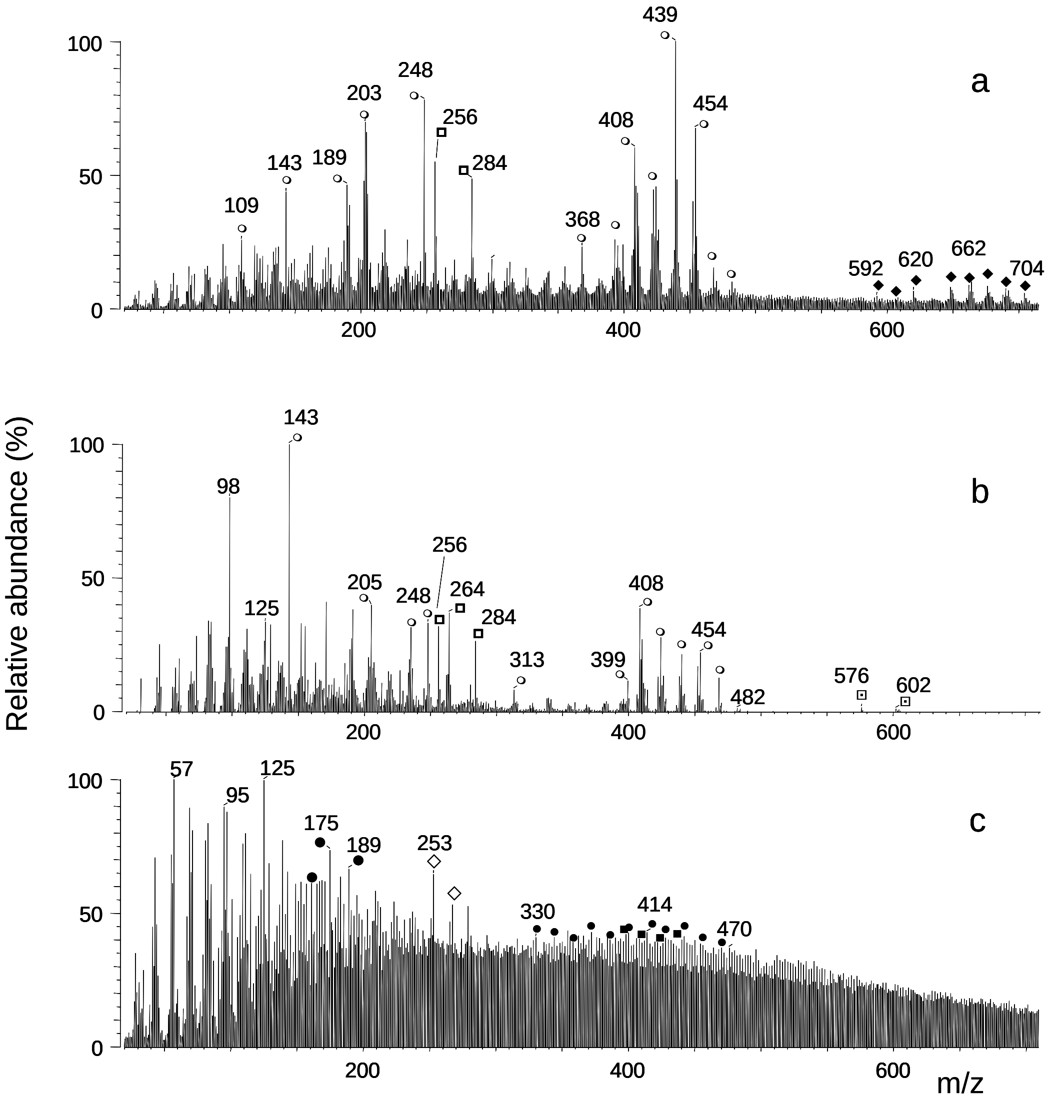

Fig. 1. DTMS summation spectra of: (a) Hafkenscheid mummy pigment sample (MP), (b) Hafkenscheid asphalt sample (A) and (c) megilp sample (MG).

*Triterpenoids (mastic) are marked with ○, fatty acids (oil/fat) are marked with □, diglycerides (oil/fat) are marked with ⊡, diterpenoids (pine resin) are marked with *, (beeswax) esters are marked with ◆, alkylbenzothiophenes (asphalt) are marked with ●, hopanoids (asphalt) are marked with ■, monoaromatic steroids (asphalt) are marked with ◇.*

The mass spectrum of the MP-sample shows fragment ions for triterpenoids of a high relative abundance such as m/z 109, 143, 163, 189, 191, 203, 204, 235, 248, 355, 399, 409, 439 and the molecular ions of these compounds such as m/z 408, 414, 422, 424, 426, 440, 452, 454, 468 (Fig. 1a), as explained in Table 1 and the references therein [10, 41-43, 48, 60-68]. Similar peaks (m/z 163, 191, 205, 235, 248, 313, 399, 408, 410, 422, 424, 426, 438, 440, 452, 454, 468, 482) but with different relative ratios are seen for the MG-sample (Fig. 1b) that is known to contain oil and mastic resin [69]. An argument in favour of mastic resin in the MP-sample is the presence of peaks such as m/z 163, 235, 439 (Table 1), typical of mastic resin [41-43]. Peaks such as m/z 143, 163, 189, 205, 248 are indicative of aged triterpenoid resin but only slightly aged since some peaks at m/z 203, 232, high 248, and high 439 (base peak in the spectrum) are characteristic for relatively fresh mastic [41-43]. The highest peak in the spectrum is observed at m/z 143 (base peak of ocotillone), m/z value characteristic for aged triterpenoids.

Table 1. List of most characteristic m/z values in the DTMS summation spectra of the Hafkenscheid mummy pigment sample (MP) (Fig. 1a). TTP= triterpenoid compound, FI= fragment ion, m= mastic resin, FA= fatty acid, DTP= diterpenoid compound, MI= molecular ion, BP= base peak, B= beeswax, t= tentatively identified.

M/z range	**M/z**	**Type compound**	**Explanation**	**Type ion**
0-400	109	TTP	Side chain cleavage product of dammarane skeletal compounds [41-43]	FI
	143	TTP	FI of ocotillone-type molecules, i.e. the hydroxyisopropylmethyltetrahydrofuran side chain [41-43, 60]	FI
	163	TTP (m)	Marker for aged mastic, characteristic FI of 28-norolean-17-en-3-one [41-43, 61]	FI
	189	TTP	Peak observed in aged mastic and dammar [41-43]	FI
	191	TTP	Peak characteristic for triterpenoids [48]	FI
	203	TTP	FI of compounds with oleanane or ursane skeleton with an aldehyde/ acid group at C_{28} [41-43]	FI
	204	TTP	Peak observed in triterpenoids [62]	
	205	TTP	Peak observed in aged mastic and dammar [41-43]	FI
	235	TTP (m)	FI of C-ring cleavage of 3-oxo-olean-18-en-28-oic (moronic) acid [41-43]	FI
	248	TTP	FI of compounds with oleanane or ursane skeleton with an acid group at C_{28} characteristic for aged mastic and dammar [41-43]	FI
	256	FA	Hexadecanoic (palmitic) acid [63]	MI
	257	FI	Palmitic acid moiety in beeswax esters	FI
	284	FA	Octadecanoic (stearic) acid [64]	MI
	355	TTP	Characteristic for 20-hydroxy-24-dammaren-3-one (hydroxydammarenone) [41-43]	FI
	368	FA	Tetracosanoic (lignoceric) fatty acid [65]	
	399	TTP	FI of 20,24-epoxy-25-hydroxy-dammaran-3-one (ocotillone) [41-43]	FI
	408	TTP	FI of 3β-hydroxy-dammara-20,24-diene (dammaradienol) and 20-dammar-24-ene-3β,20-diol (dammarenediol) [41-43]/ unidentified oleanadiene [44]	FI
	409	TTP	FI of compounds with oleanane or ursane skeleton with an aldehyde/ acid group at C_{28} [41-43]	FI
	422	TTP	MI of ocotillone-type molecules (t) [41-43]	

Table 1 continued

M/z range	M/z	Type compound	Explanation	Type ion
400-500	424	TTP	FI of hydroxydammarenone, MI of 3-oxo-dammara-20(21),24-diene (dammaradienone) or 3-oxo-olean-12-ene (β-amyrone) [41-43, 61, 62, 66-68]	FI/ MI
	426	TTP	FI of dammarenediol and MI of dammaradienol [41-43]	MI
	439	TTP	FI of 3-oxo-13α,14β,17βH,20αH-lanosta-8,24-dien-26-oic acid and 3-oxo-13α,14β,17βH,20αH-lanosta-7,24-dien-26-oic (isomasticadienoic and masticadienoic) acid from mastic resin [41-43]	BP, FI
	440	TTP	3-hydroxy-olean-12-en-28-al (oleanolic aldehyde) or 3-hydroxy-urs-12-en-28-al (ursolic aldehyde) (t) [41-43]	MI
	454	TTP	MI of 3-oxo-olean-12-en-28-oic (oleanonic) acid, 3-oxo-12-ursen-28-oic (ursonic), moronic acid [41-43]	MI
	468	TTP	11-oxo ursonic/ oleanonic acid [41-43]	MI
500-700	592	B	Ester of the tetracosanoic alcohol (C_{24}) with the palmitic acid [65]	MI
	620	B	Ester of the hexacosanoic alcohol (C_{26}) with the palmitic acid [65]	MI
	648	B	Ester of the octacosanoic alcohol (C_{28}) with the palmitic acid [65]	MI
	662	B	Ester of the nonacosanoic alcohol (C_{29}) with the palmitic acid [65]	MI
	664	B	Ester of the octacosanoic alcohol (with the hydroxypalmitic acid [10, 65]	MI
	676	B	Ester of the triacontanoic (melissyl) alcohol (C_{30}) with the palmitic acid [65]	MI
	690	B	Ester of the hentriacontanoic alcohol (C_{31}) with the palmitic acid [65]	MI
	692	B	Ester of the triacontanoic alcohol with the hydroxypalmitic acid [10, 65]	MI
	704	B	Ester of the dotriacontanoic alcohol (C_{32}) with the palmitic acid [65]	MI
	732	B	Ester of the tetracontanoic alcohol (C_{34}) with the palmitic acid [65]	MI

Apart from peaks characteristic for a triterpenoid mastic resin in the MG, other peaks characteristic for the oil used in the preparation of the megilp are present in the DTMS spectrum such as the molecular ions of palmitic and stearic acid at m/z 256 and m/z 284. The oil is relatively fresh as pointed out by the presence of the peak at m/z 264 typical for the loss of water from the monounsaturated C_{18} fatty acid, small peaks indicative for polyunsaturated C_{18} fatty acid at m/z 280 and diacylglyceride peaks at m/z 576 (C_{16}-$C_{18:1}$) and 602 ($C_{18:1}$-$C_{18:1}$) [70]. Some oxidation of the lipids has occurred, which is reflected in the presence of average height fragment ions for dicarboxylic acids at m/z 98, 152, and fragment ions of midchain oxidised stearic acids at m/z 155, 171 [70].

Molecular ions indicative of fatty acids from oil or fat are observed in the spectrum of the MP-sample as peaks with a rather high relative abundance for palmitic acid, m/z 256, and for stearic acid, m/z 284. The peak at m/z 368 is probably the molecular ion of the lignoceric (tetracosanoic) acid from beeswax [65]. An electron ionisation fragment ion of lower relative intensity is observed in the mass spectrum of the MP-sample at m/z 257, a typical fragment ion of the palmitic acid moiety in beeswax esters. The peaks over m/z 500 at m/z 592, 620, 648, 662, 676, 690, 704, 732 are molecular ions for beeswax esters or beeswax hydroxy-esters at m/z 664 and 692 [10, 65, 71, 72]. No tracers for cellulose polysaccharide (m/z 57, 60, 73, 98, 126, 144) or phosphorus from bones (m/z 62, 124) are identified.

The DTMS of the A-sample (Fig. 1c) shows one unresolved envelope of mass peaks with characteristic fragment ions of aliphatic and aromatic hydrocarbons and steroids ("◇") in the range of m/z 50-300. Fragment ions characteristic for aliphatic compounds like alkanes (m/z 43, 57, 71, 85, 155, 169, 183, 197), alkenes and possibly alkylthiophenes (m/z 41, 55, 69, 83, 97, 111, 125, 139, 153, 167, 181, 195, 209, 223) and alkynes (m/z 67, 81, 95, 109, 123, 137, 151, 165, 193) are present [73]. More specific fragment ions are observed at m/z 161, 175 and 189 characteristic of alkylbenzothiophenes [17] and at m/z 253 and 267 characteristic for monoaromatic steroids and methylated monoaromatic steroids [48]. Mass peaks representing the molecular ion region of alkylbenzothiophenes at m/z 330, 344, 358, 372, 386, 400, 414, 428, 442, 456, 470 ("●") and hopanes at m/z 370, 398, 412, 426, 440, 454, 468 ("■") are indicated in the range of m/z 300-450 [45, 46].

The composition of the mummy pigment MP as determined by DTMS can be summarised as a mixture of a slightly aged mastic resin, with oil or fat and beeswax as other components. The combination mastic and oil could be interpreted as a megilp component[22]. If asphalt would be present as a minor component its signature could be hidden under the strong signal of the resin and oil components of the MP-sample. In order to explore this hypothesis the sample was further investigated by Py-GC/MS.

b) Search for asphalt markers in the MP by Py-GC/MS

The presence of asphalt was easily demonstrated when a specific search for asphalt biomarkers was done as shown in Fig. 2 following the protocol for selective identification of asphalt described in Chapter 2 [45, 46].

b1) Search for hopanoid biomarkers

In the partial mass chromatograms of the fragment ion m/z 191 (Fig. 2b) typical for hopanoids, peaks corresponding to such compounds were observed. The identification was based on mass spectrum and retention time in relation to alkanes (m/z 85). Homologues H_{27}, H_{29}-H_{31} were observed. Two peaks were seen for H_{27}. One is tentatively identified as 18αH)-22,29,30 trisnorneohopene ($H_{27:\,1}/T_{s:\,1}$) and the other one identified as 17α H)-22,29,30 trisnorhopane (H_{27}/T_m). The H_{29} and H_{30} homologues were identified as 17α 21βH)-30-norhopane, respectively 17α21βH)-30-hopane. The two H_{31} homologues were identified as 17α21βH)-29-homohopane (isomers 22S and 22R, this last one only tentatively identified) [48]. The hopanoid peaks have a low relative intensity when compared to the fragment ion m/z 191 of the M_2 (28-nor-Δ^{17} Δ^{12}-olean-dien-3-one) and M_1 (a nor-homologue of M_2) mastic resin compounds [74, 75]. The C_{29}, C_{31} and C_{33} alkanes and C_{30} and C_{32} unsaturated

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[22] megilp was often added to paint in the 19th century [21, 22]

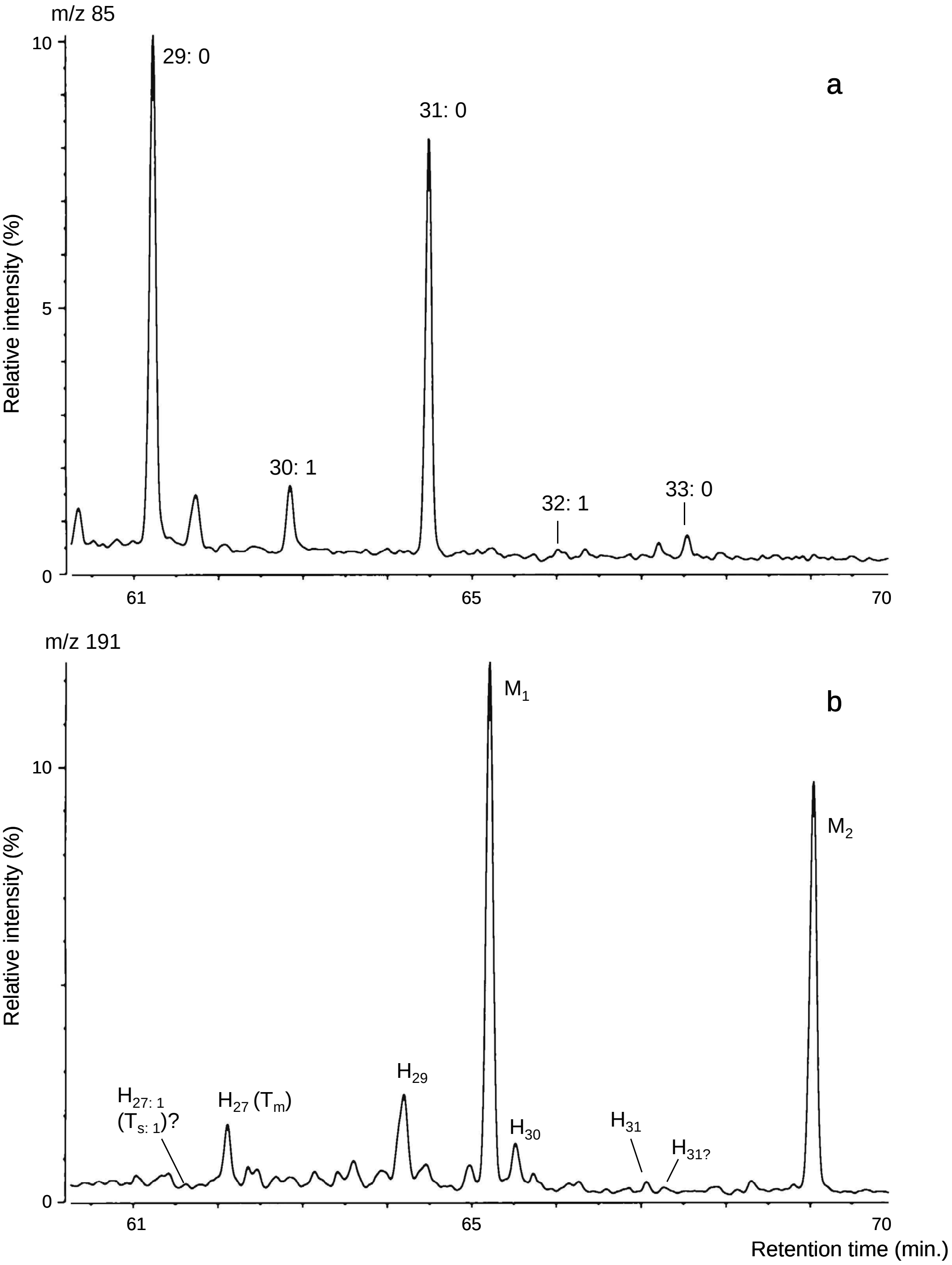

Fig. 2. Py-GC/MS partial mass chromatogram of (a) alkanes as markers for aliphatic cross-links (m/z 85) and (b) hopanoid compounds, H_i (m/z 191) in the Hafkenscheid mummy pigment sample (MP); M_1 and M_2 are isotopic contributions of mastic resin components to m/z 191.

homologues observed in the displayed window for aliphatic moieties identified by the mass chromatogram of m/z 85 (Fig. 2a), are considered to be derived from beeswax [71].

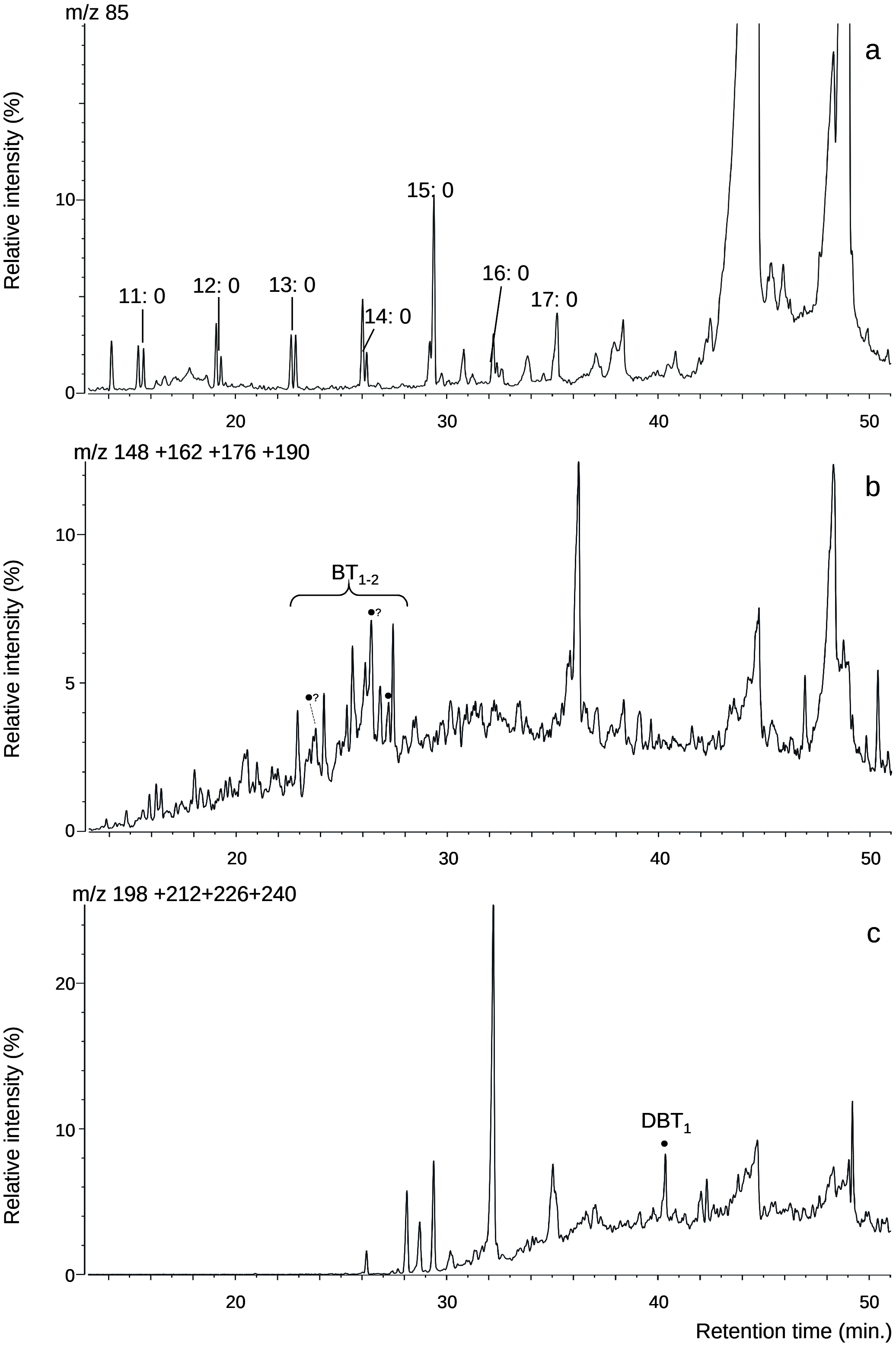

Fig. 3. Py-GC/MS partial mass chromatogram of (a) alkanes as markers for aliphatic cross-links (m/z 85) (b) alkyl-benzothiophenes (m/z 148+ 162+ 176+ 190) and (c) alkyl-dibenzothiophenes (m/z 198+ 212+ 226+ 240) in the Hafkenscheid mummy pigment sample (MP).

b2) Search for steroid biomarkers and alkyl(di)benzothiophene markers

A search was also made for steroid compounds (not shown) by running partial mass chromatograms for (methyl) monoaromatic steroids, m/z 253 and 267, for (methyl) triaromatic steroids, m/z 231 and 245, and for 14α(H) and 14β(H) steranes, m/z 217 and 218, but with negative results. The sulphur containing asphalt markers are present in the MP-sample as shown in Fig. 3, where partial mass chromatograms for alkylbenzothiophenes, m/z 148+162+176+190, Fig. 3b, and alkydibenzothiophenes, m/z 198+212+226+240, Fig. 3c, are presented. The compounds have been identified on the basis of their retention time and mass spectrum. A few benzothiophenes with an alkyl chain length of 1-2 carbon atoms, $BT_{1\text{-}2}$, and one dibenzothiophene with one carbon atom alkyl chain, DBT_1, were identified. The first window in Fig. 3a, shows the $C_{11:0}$- $C_{17:0}$ alkanes as being in the range of aliphatics (m/z 85) where the benzothiophenic compounds are eluting.

b3) The aliphatic profile of alkanes and alkenes

The complete range of aliphatic hydrocarbons in the Py-GC/MS measurement of the MP-sample starts at the $C_{8:0}$ and goes up to the C_{33} alkane homologue. The profile of alkanes at lower retention times with the C_8-C_{17} saturated and the C_9-C_{16} unsaturated homologues is more reminiscent of decarboxylated fatty acids from the oil/fat component of MP than of the interconnecting aliphatic chains present in an asphaltic constituent of MP [3, 45, 46]. The profile of aliphatic chains of the A-sample covers a range from C_7-C_{35} alkanes and from C_6-C_{31} for alkenes. At higher retention times in the Py-GC/MS run of MP, the alkanes have an odd carbon chain ranging from C_{25}-C_{33}. They have the same profile as the alkanes in beeswax. A C_{21} alkane of unknown significance is observed coeluting with the stearic acid. Even alkenes (C_{24}-C_{32}) in the Py-GC/MS data are thought to represent the alcoholic moieties of the beeswax ester [71] after their pyrolytic decomposition.

b4) Search for lignite markers

An additional search in the Py-GC/MS data was performed for the presence of Kassel earth that was possibly used as a substitute or an adulterant for the pigment. Kassel earth pigment of the Hafkenscheid collection was investigated (Chapter 4) and several marker compounds have been identified [51]. The absence of the main marker compounds such as phenol (m/z 94) and guaiacol (m/z 124) in Py-GC/MS data of the MP sample demonstrates that Kassel earth was not used as substitute pigment.

In summary, Py-GC/MS data are showing the occurrence of asphalt identified by benzo- and dibenzothiophenes and hopanoids as markers as a minor constituent of mummy sample MP. The absence of steroid biomarkers could point to the use of degraded asphalt due to heating or other pre-treatments in the embalming, burial or during preparation of the mummy pigment [21, 22, 50].

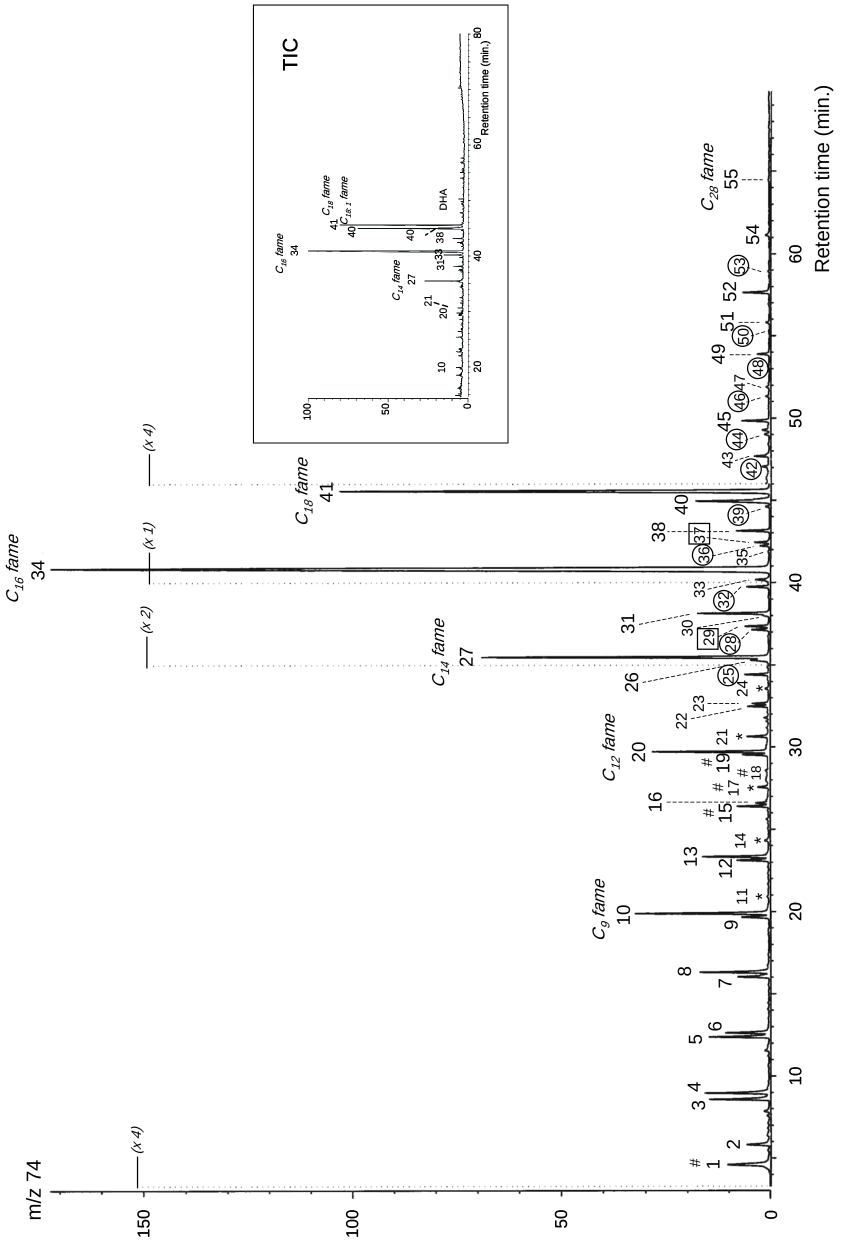

Fig. 4. Py-TMAH-GC/MS partial mass chromatogram of fatty acid components (m/z 74) of the Hafkenscheid mummy pigment sample (MP), with an insert of the TIC.

c) Investigation of the oil fat and diterpenoid components of MP

Transmethylation followed by Py-GC/MS analysis of MP enabled us to obtain more information on the polar components of the sample (i.e. the type of oil/fat and its degree of oxidation). The partial mass chromatogram for m/z 74 in Fig. 4 and Table 2 are showing and list the fatty acids[23] as methyl esters and dicarboxylic acids as dimethyl esters in the MP-sample. The insert in Fig. 4 represents the total ion chromatogram (TIC) of the Py-TMAH-GC/MS run. The fatty acid profile of the MP-sample (Fig. 4-insert) is clearly dominated by the hexadecanoic (nr 34: palmitic) and the octadecanoic (nr 41: stearic) fatty acids, with a ratio suggesting linseed oil [76]. The octadecenoic fatty acids (nr 40) are the third and the fifth in height in the TIC suggesting rather fresh oil. The fourth peak in height is myristic acid suggesting other lipid sources than oil alone. The peak at retention time 50.24 min in the TIC (insert Fig. 4) was identified as dehydroabietic acid (DHA). Only one other diterpenoid peak in the TIC (retention time 52.56 min) is tentatively identified as 7-oxo-dehydroabietic acid methyl ester enol with a very low intensity.

*Table 2. Identified compounds presented in the m/z 74 partial mass chromatogram (Fig. 4) of the Py-TMAH-GC/MS data (insert Fig. 4) of the Hafkenscheid mummy pigment sample (MP). Compounds other than the normal fatty acids are marked as follows: ***** for dicarboxylic acids methyl esters, **O** for iso fatty acids methyl esters, for anteiso fatty acids methyl esters, and **#** for other compounds; t= tentatively identified.*

Label	**Name compound**	**MW**	**RT (min)**
	Normal fatty acids, as methyl esters		
2	(t) pentanoic acid methyl ester (5 fame)	116	5.84
3	hexenoic acid methyl ester (6: 1 fame)	128	8.62
4	hexanoic acid methyl ester (6 fame)	130	8.99
5	heptenoic acid methyl ester (7: 1 fame)	142	12.39
6	heptanoic acid methyl ester (7 fame)	144	12.03
7	octenoic acid methyl ester (8: 1 fame)	156	16.03
8	octanoic acid methyl ester (8 fame)	158	16.34
9	nonenoic acid methyl ester (9: 1 fame)	170	19.65
10	nonanoic acid methyl ester (9 fame)	172	19.92
12	decenoic acid methyl ester (10:1 fame)	184	23.11
13	caproic (decanoic) acid methyl ester (10 fame)	186	23.37
15	undecenoic acid methyl ester (11: 1 fame)	198	26.38
16	undecanoic acid methyl ester (11 fame)	200	26.59
19	dodecenoic acid methyl ester (12: 1 fame)	212	29.52
20	lauric (dodecanoic) acid methyl ester (12 fame)	214	29.74
22	tridecenoic acid methyl ester (13: 1 fame)	226	32.47
23	tridecanoic acid methyl ester (13 fame)	228	32.66
26	tetradecenoic acid methyl ester (14: 1 fame)	240	35.28
27	myristic (tetradecanoic) acid methyl ester (14 fame)	242	35.47
30	pentadecenoic acid methyl ester (15: 1 fame)	254	37.99
31	pentadecanoic acid methyl ester (15 fame)	256	38.14
33	hexadecenoic acid methyl ester (16: 1 fame)	268	40.19

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[23] the terms “fatty acid” and “dicarboxylic acid” used in the text mean their methyl esters (see Table 2)

Table 2 continued.

Label	Name compound	MW	RT (min)
34	palmitic (hexadecanoic) acid methyl ester (16 fame)	270	40.78
38	margaric (heptadecanoic) acid methyl ester (17 fame)	284	43.13
40	*Cis* octadecenoic acid methyl ester (18: 1 fame)	296	44.91
40	*Trans* octadecenoic acid methyl ester (18: 1 fame)	296	45.08
41	stearic (octadecanoic) acid methyl ester (18 fame)	298	45.53
43	nonadecanoic acid methyl ester (19 fame)	312	47.7
45	arachidic (eicosanoic) acid methyl ester (20 fame)	326	49.83
47	heneicosanoic acid methyl ester (21 fame)	340	51.88
49	docosanoic acid methyl ester (22 fame)	354	53.9
51	tricosanoic acid methyl ester (23 fame)	368	55.79
52	tetracosanoic acid methyl ester (24 fame)	382	57.67
54	hexacosanoic acid methyl ester (26 fame)	410	61.15
55	octacosanoic acid methyl ester (28 fame)	438	64.46
	Dicarboxylic acids, as dimethyl esters (* in Fig. 4)		
11	hexanoic dicarboxylic acid dimethyl ester (6 dadime)	174	20.92
14	heptanoic dicarboxylic acid dimethyl ester (7 dadime)	188	24.34
17	octanoic dicarboxylic acid dimethyl ester (8 dadime)	202	27.56
21	nonanoic dicarboxylic acid dimethyl ester (9 dadime)	216	30.65
24	decanoic dicarboxylic acid dimethyl ester (10 dadime)	230	33.57
	Iso fatty acids, as methyl esters (**O** in Fig. 4)		
25	iso myristic (tetradecanoic) acid methyl ester (i-14 fame)	242	34.43
28	iso pentadecanoic acid methyl ester (i-15 fame)	256	37.15
32	iso palmitic (hexadecanoic) acid methyl ester (i-16 fame)	270	39.75
36	iso margaric (heptadecanoic) acid methyl ester (i-17 fame)	284	42.22
39	iso stearic (octadecanoic) acid methyl ester (i-18 fame)	298	44.60
42	iso nonadecanoic acid methyl ester (i-19 fame)	312	47.06
44	(t) iso arachidic (eicosanoic) acid methyl ester (i-20 fame)	326	49.00
46	iso heneicosanoic acid methyl ester (i-21 fame)	340	51.30
48	(t) iso docosanoic acid methyl ester (i-22 fame)	354	53.14
50	iso tricosanoic acid methyl ester (i-23 fame)	368	55.26
53	(t) iso pentacosanoic acid methyl ester (i-25 fame)	396	58.91
	Anteiso fatty acids, as methyl esters (□ in Fig. 4)		
29	anteiso pentadecanoic acid methyl ester (ai-15 fame)	256	37.37
37	anteiso margaric heptadecanoic acid methyl ester (ai-17 fame)	284	42.43
	Other compounds (# in Fig. 4)		
1	Unidentified (83, 84, 91, 74, 92, 67, 101)	?	4.61
15	C_2-substituted naphthalene[24] (N_2)	156	26.38
17	C_2-substituted naphthalene (N_2)	156	27.56
18	Pentadecene (15: 1)	210	28.56
19	(t) C_3-substituted naphtalene[25] (N_3)	170	29.52
19	(t) 3-guaiacyl-prop-2-enol (cis) (G)	194	29.52

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[24] C_2- dimethyl- or ethyl- substituents

[25] C_3- trimethyl- or methyl, ethyl- or (iso)propyl- substituents

The mass spectrum of DHA is given in Fig. 5. The absence of more oxidised diterpenoid acids points to a relatively small contribution of a relatively fresh diterpenoid/pine resin [77].

The relative distribution of the fatty acids is given by the m/z 74 partial mass chromatogram in Fig. 4. The height of certain peaks was multiplied in Fig. 4 (by 2x or 4x as indicated) in order to show the complete range of fatty acid components. Note that dimethylester have a relatively low m/z 74 fragment ion.

Normal fatty acids are present from the C_5 homologue (pentanoic) up to the C_{28} (octacosanoic) homologue and have a low intensity except for the palmitic, stearic, myristic, lauric (dodecanoic) and the nonanoic acids. Monunsaturated fatty acids range from the C_6 homologue to the C_{16} homologue. Another homologue present is the C_{18} monounsaturated octodecenoic fatty acid. No squalene (molecular weight 410) often associated with monounsaturated fatty acids in case of handling contamination was observed, indicating another origin for these fatty acids. The relatively large relative abundance of saturated fatty acids does not preclude an input from mummies in the form of adipocire. The relative ratios between the main fatty acids is interpreted as linseed oil as the main oil component of the MP-sample with some additions of fat [76].

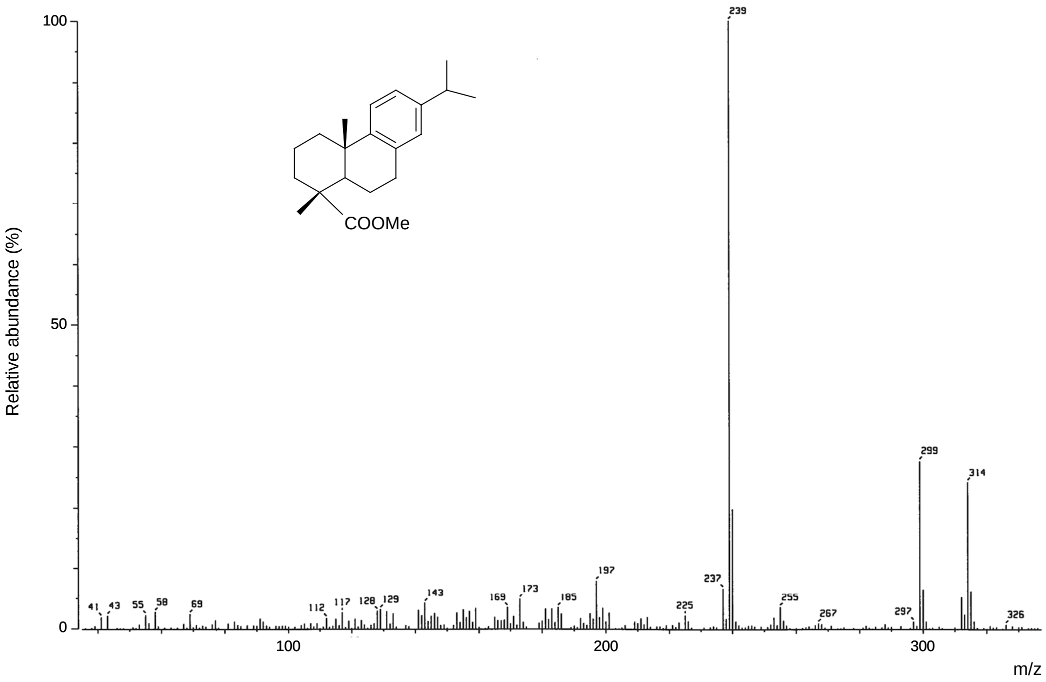

Fig. 5. Mass spectrum and formula of dehydroabietic acid (DHA) in Py-TMAH-GC/MS data of the Hafkenscheid mummy pigment sample (MP).

Dicarboxylic acids, the C_6-C_{10} homologues, are present at a very low relative intensity in both the partial mass chromatogram (as expected) of Fig. 4, but also in the TIC, insert Fig. 4. This suggests that the lipids have a saturated fat origin although some antioxidant activity from the mastic and asphalt on an oil constituent can be considered.

A special feature in the partial mass chromatogram and TIC is the occurrence of some other peaks albeit at very low relative abundance identified as the *iso fatty acids* and *anteiso fatty acids* being the C_{14}-C_{23}, C_{25} homologues, and the C_{15} and C_{17} homologues respectively. Iso fatty acids (odd and even) and odd anteiso fatty acids can be found in bacteria and in some fungi but originally they are all bacteria derived [78]. Such branched chain fatty acids have been observed before in samples from mummies and have been interpreted as a contribution from the human adipose tissue rather than from bacteria due to their low relative abundance and the simultaneous presence of myristic, monounsaturated C_{16} and C_{18} fatty acids in the samples investigated [37, 71]. A similar situation is observed in the MP-sample where small amounts of iso and anteiso fatty acids, a high peak for the myristic fatty acid, and monounsaturated fatty acids from C_6-C_{16} are observed. This suggests that apart from a linseed oil an additional source of fatty acids has to be postulated in the MP-sample with a bacterial, fungal or maybe even human origin.

Some *other compounds* with a small contribution to m/z 74 (Fig. 4) listed in Table 2 were also identified in the MP-sample. These are three alkyl substituted naphtalenes (two N_2 isomers, one N_3) and one guaiacylpropenol isomer. The naphtalenes could be derived from the asphalt. The significance of the guaiacylpropenol is unknown but a plant origin is likely.

Summarising, the fatty acid profile observed in MP is not only oil derived. There are too many non-oil features suggesting other sources of fats. These could be derived from bacterial or fungal sources possibly in association with mummies.

d) Identification of the mastic resin by on-column GC/MS

The triterpenoid resin component of the MP was investigated by on-column GC/MS to determine the type of resin and its degree of oxidation. Results are given in Fig. 6 as the TIC time window where triterpenoid compounds usually elute.

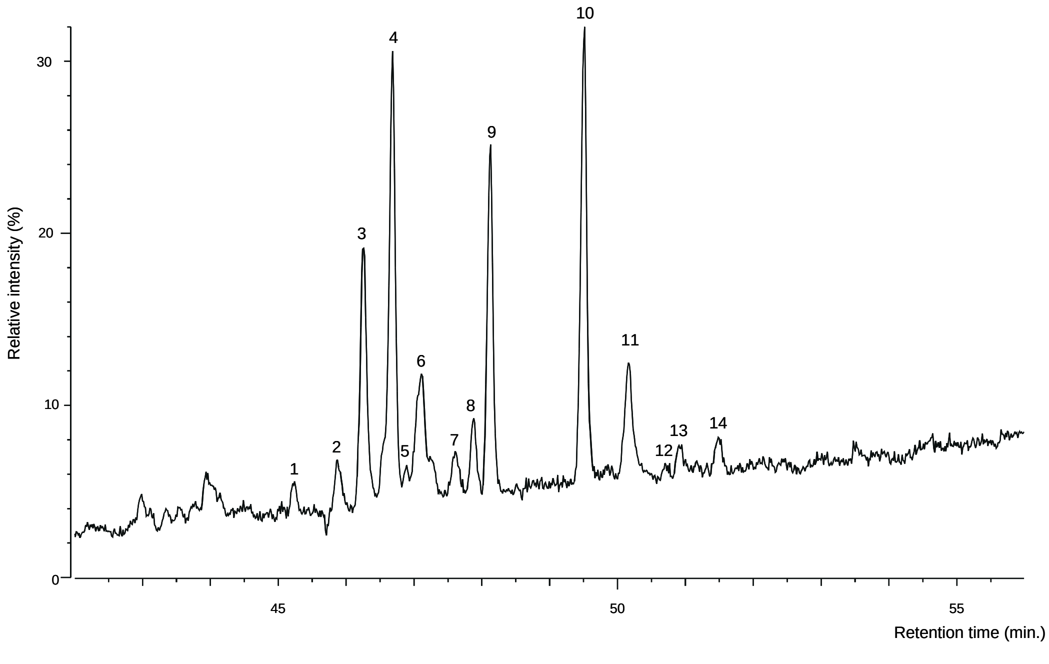

Fig. 6. On-column GC/MS retention window of the methylated triterpenoid compounds from mastic in the Hafkenscheid mummy pigment sample (MP).

The presence of compounds such as oleanonic acid methyl ester, ocotillone, hydroxydammarenone and 11-oxo-oleanonic acid methyl ester in Fig. 6 and listed in Table 3 and the references therein are indicative for a triterpenoid resin [41-43, 79-82]. Compounds exclusive for a mastic resin like the methyl esters of moronic acid and the isomers of the masticadecanoic acid positively identify mastic in the MP-sample [41-43]. The relative ratios of the peaks with low moronic acid and higher (iso)masticadecanoic acids points in the direction of an only slightly aged mastic resin [41-43]. This on-column measurement confirms the earlier supposition made on the basis of the DTMS data that a slightly aged mastic resin was used in preparation of the mummy pigment of the Hafkenscheid collection.

Table 3. Identified compounds presented in the on-column GC/MS retention window of the methylated triterpenoid compounds for mastic in the Hafkenscheid mummy pigment sample (MP) (Fig. 6); t= tentatively identified.

Label	Name compound	MW	RT (min)
1	Unidentified (391, 299, 406, 407, 411, 189, 239)	426 (t)	45,24
2	Unidentified (202, 413, 408, 107, 132, 220, 393)	428 (t)	45,87
3	3-oxo-olean-18-en-28-oic (moronic) acid methyl ester [41-43,79]	468	46,26
4	3-oxo-olean-12-en-28-oic (oleanonic) acid methyl ester [41-43,79]	468	46,67
4	20,24-epoxy-25-hydroxy-dammaran-3-one (ocotillone) [41-43,80]	458	
5	(t) Isomer of oleanonic acid methyl ester (189, 143, 44, 468, 203, 262)	468	46,88
6	20-hydroxy-24-dammaren-3-one (hydroxydammarenone) [41-43, 81]	442	47,08
7	Unidentified (109, 216, 81, 69, 466, 55)	466	47,61
8	Unidentified (466, 95, 244, 309, 121, 157, 451)	466	47,87
9	3-oxo-13α,14β,17βH,20αH-lanosta-8,24-dien-26-oic acid or 3-oxo-13α,14β,17βH,20αH-lanosta-7,24-dien-26-oic ((iso) masticadienoic) acid methyl ester [41-43, 79, 82]	468	48,11
10	(Iso) masticadienoic acid methyl ester	468	49,50
11	Unidentified (471, 369, 453, 102, 119, 145, 249, 251, 439, 421)	486 (t)	50,15
12	11-oxo-oleanonic acid methyl ester	482	50,70
13	Unidentified (496, (208), 95, 123, 163)	496	50,92
14	Isomer of (iso) masticadienoic acid methyl ester? (453, 55, 95, 109, 468, 421, 123)	468 (t)	51,48

Composition of the Hafkenscheid Mummy pigment

The composition of the mummy pigment from the 19th-century Hafkenscheid Collection is shown to be complex. The mass spectrometric approach indicates the mummy pigment sample consists of drying oil (possibly linseed) in connection with slightly aged mastic resin, asphalt, fat and some pine resin as additional components. Such materials have been shown before in mass spectrometric results of samples from mummies while megilp medium – a mastic oil gel - was often mixed into paint[26] in the 19th century [5, 6, 10, 12, 13, 21, 22, 30, 31, 33, 35, 37, 69]. Wax derived compounds and diterpenoid resins however have not always been retrieved in mummy samples [6, 30]. The presence of branched chain fatty acids in the mummy

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[26] e.g. in preparation of asphalt pigments, according to Carlyle and Southall [21, 22, 55]

pigment sample points to a bacterial, fungal or even human tissue contribution as shown for real mummies by Buckley et al. [37, 71, 78]. No traces of polysaccharide or bones (phosphorus) have been found in the Hafkenscheid mummy pigment sample.

The analysed mummy pigment contains asphalt, which could explain the black colour of the sample. The presence of asphalt in the sample is supported by mass spectrometric identification of asphalt (bio)markers, hopanoids, benzo- and dibenzothiophenes, shown for asphalt containing mummy samples [5, 10, 12, 13, 30, 33, 35]. The absence of steroid biomarkers and higher homologues of the hopanoid compounds is thought to result from the biodegradation of the asphalt due to heating or other pre-treatments [21, 22, 50]. The absence of gammacerane, a compound very abundant in Dead Sea asphalt [17] excludes the provenance of the asphalt from this specific Middle Eastern source (gammacerane survives the roasting treatment used by Merrimée and Williams to prepare asphalt containing media- see Chapter 5) [51, 83-85].

The mixture of asphalt, oil, fat, beeswax, conifer and mastic resin as found in the Hafkenscheid mummy pigment could belong to an embalming mixture used in the ancient Egypt [7, 9]. Some of the materials present in this mummy pigment like asphalt, beeswax and the fat component *could* therefore have originated from real mummies but an Egyptian provenance is hard to prove. On the other hand the rather low degree of oxidation of the oil and resins in the analysed mummy pigment sample *would* suggest these materials could have been introduced at a later time, i.e. the 19th century, by the manufacturer in the technological process of the pigment. In that case, a megilp could have been used as binding medium for the black pigment powder.

This research shows that material not dis*similar* to mummies embalmed with asphalt was present as a black pigment preparation in the 19th century. The composition of this material contains so many features common to other paint materials in the 19th century [21, 22] that it will be hard to trace it in paintings as indeed Raymond White already has postulated [6].

Conclusions

The composition of the *mummy pigment* from the 19th-century Hafkenscheid paint material collection is complex. It consists of poorly oxidised linseed oil and slightly aged mastic resin as main components with additions of beeswax, asphalt, pine resin and an unidentified fat component. The combination of drying oil and mastic points to a 19th-century megilp as base material. The asphalt, beeswax and fat component of the “mummy pigment” could point to mummy as a constituent of the pigment sample. The origin of the unidentified fat is possibly bacterial and/or fungal or even human indicating a possible relation to mummies. Mummy components could have been added to the megilp base to create a mummy medium with the appearance of asphalt. An Egyptian origin of mummy components can’t be confirmed on the basis of molecular characteristics of the sample in the Hafkenscheid collection.

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4. Kassel earth: Characterisation of molecular markers and biomarkers in a series of Kassel earth pigments

Abstract

Kassel earth pigments and their 'homologues', Cologne earth or Vandyke brown, were used in oil paint since the 16th century. Kassel earth is considered to originate from areas related to mining of brown coal (lignite). The pigment could be used as such or could be mixed with other materials. The main purpose of this work is to study the molecular aspects of the composition of Kassel earth pigments. The analysed samples originate from a variety of private, commercial and museum collection sources from the 18th, the 19th century and 20th century. Di- and triterpenoids, waxes, aliphatic and aromatic compounds were identified by DTMS. The assignment of the wax esters as montan wax esters is made by DTMS/MS. The samples are sometimes richer in di-, triterpenoids and wax esters (compounds less chemically bound to the network) and sometimes richer in (methoxy) phenolic compounds (present in the cross-linked network). Less chemically bound compounds are solvent extractable. Principal Component Analysis of the DTMS data shows a grouping in samples richer in fossil leaf material and samples richer in fossil wood derived material. This grouping coincides with Kassel earth and Cologne earth on the one hand and Vandyke brown on the other hand.

The molecular chemistry of the Kassel earth samples studied by Py-TMAH-GC/MS revealed lipid derived fatty acids and lignin and soil derived aromatic compounds. The main identified fatty components are methyl derivatives of long-chain fatty acids (C_{22}, C_{24}, C_{26}, C_{28}, C_{30})*, of long-chain alcohols* (C_{24}, C_{26}, C_{28}) *and some ω-hydroxyfatty acids from higher land plants. The α-ω dicarboxylic acids* (C_6 *to* C_{10}) *derive from oxidation of lipids and the short-chain fatty acids* (C_5-C_{12}) *are possibly derived from microorganisms. The main identified aromatic compounds are methyl derivatives of benzoic and benzene-dicarboxylic acids, (mono-, di- and tri-)methoxy benzaldehyde, acetophenone, benzoic acid and benzene propenoic acid, alkylated benzenes, benzene (methoxyphenols) and bisphenol. Plant terpenoids (e.g. phyllocladane, fridelin, olean/urs-12-ene, etc.) observed in GC/MS data are more characteristic for samples with a high abundance of fossil leaf material (Kassel earth), while bacterial terpenoids (e.g. 17α, 21β-homohopane, etc.) are more abundant in samples with more fossil wood remains (Vandyke brown). Pristene is identified in all the samples. The Kassel earth pigments are clearly made from deposits rich in fossil plant material of a diverse composition. This study connects the world of the conservation of art to that of the geochemistry of coal.*

Introduction

Kassel earth was used as pigment since the 16th century, for shadows of flesh, blacks and browns and in underpaint. It was used as pigment in oil but also in wax (encaustic) or water media [1-3]. Its history of use as a pigment is not only mentioned in European sources (German, French, Italian, English, Spanish) but also in US sources [1]. Kassel earth pigment is said to have been used in brown grounds and in shadows of some 18th and 19th century American paintings [1].

In this chapter the name “Kassel earth” will be used to include the pigments known as Kassel earth, Cologne earth and Vandyke brown (and with all the different spelling of their names) [1-3]. Within the chapter the pigments will be refered as “Kassel earth-like pigments ”, “Kassel earth pigments” or only “Kassel earth”.

Generally speaking the supposed occurrence of Kassel earth pigment in paintings relies more on the information found in the literature than actual analytical evidence [1]. According to the review of Feller and Feller, Kassel earth seems to have been a component of the palette of several painters like D. Velásquez and G. David in the 15-16th century; P.P. Rubens, A. van Dyck and Rembrandt van Rijn in the 16-17th century; F. Guardi, T. Gainsborough, J.-L. David, J.A.D. Ingres, T. Gericault, E. Delacroix and J.-B.-C. Corot in the 18-19th century; and A-W. Bouguereau, A. Derain and G. De Chirico in the 19-20th century. Kassel earth pigment is described as present in some 19th century pigment collections and in certain water colour boxes [1, 4-8]. Kassel earth is considered to be a cause of failing of brown paint in 19th century paintings causing defects such as fading [1-3]. This is thought to be directly related to the composition of the pigment and its interaction with the oil network.

The molecular aspects of the composition of Kassel earth pigment are the main purpose of this study. The pigment is said to be a complex organic material of fossil plant origin [1, 9]. However it is also found to be substituted and occasionally adulterated with other materials sometimes of inorganic nature like iron oxides [1-3, 6, 7]. Kassel earth is mentioned as a substitute for asphalt pigments [2, 3]. Feller thinks that this is not possible considering their different appearance [1]. Few analytical investigations are available related to the presence of Kassel earth brown in paintings and other art objects [1]. Feller used IR spectroscopy in the 1970’s to identify Kassel earth pigment in brown ground and shadows of some 18th and 19th century American paintings [1]. However, IR as an identification method has severe limitations for the identification of Kassel earth in an oil paint matrix because of the convolution of the IR absorption peaks. At the Doerner Institute in Munich the pigment was identified as humic acids in 61 paintings, mostly 19th century paintings, using wet chemical methods. Other identifications have relied on optical microscopy and some spectrochemical analyses or X-ray fluorescence analyses [1]. Modern Kassel earth pigments have been investigated by means of physical methods such as optical microscopy, XRD, IR spectroscopy, Neutron Activation and Mössbauer spectroscopy and also using wet chemical methods [1]. Results point to humic substances and polysaccharides from soil, certain types of plants detected from pollen analysis, and some metals like Fe, Ca, and little Mn [1]. Polarized-light microscopy, XRD or emissions spectroscopy are generally insufficient to identify Kassel earth in oil paint samples that supposedly contain the pigment. It is the opinion of the current researcher that molecular evidence is required to support the identification of a pigment with a complex organic nature like Kassel earth.

Kassel earth is considered to come from the Cologne area and the region between Bruhl and Bonn near Cologne[1], in Germany [1]. When these sources were exhausted, others in the vicinity of Kassel (spelled also as Cassel) were exploited. Feller reports one source as still active in 1997 [1]. Eisenach, a city close to Kassel, is also mentioned as a source in the 19th century [1, 10]. According to Feller two of the places close to Cologne, Reincassel (spelling as by Feller [1]) and Obercassel-on-Rhein are possibly responsible for the Kassel earth name [1]. 19th century sources also indicate deposits in the province of Sachsen, Thuringen, in Germany [1]. In his review Feller talks about other European or American sources[2] of Kassel earth pigments as well.

A first correlation with fossil wood deposits is supported by Tertiary formation of brown coal mentioned at Habichesberg and Hohen Meissners [10] at beginning of the 20th century near Kassel [1, 10]. The first mine was active till 1972 and the second was still mined in 1986 [1]. Habichesberg is probably inside the present day nature park Habichtswald [10].

Selection of samples and the analytical approach

The analysed samples originate from a variety of private, commercial and museum collection sources. Samples were available from the 19th century collection of Hafkenscheid, the 20th century pigment collections of Feller (reported in *Artists' Pigments*) and the collection of paints and pigments of Von Imhoff. Three other samples originating from historical collections, two samples from the 18th century Vigani collection (Queen's College, Cambridge, UK) and one sample from the Winsor & Newton archive were available for this study at a later stage for that reason only some analyses were possible in the given time. A few other samples were available from commercial or geological origin. More details about the samples are given in the experimental section.

Mass spectrometric techniques were used such as DTMS, DTMS/MS and Py-(TMAH)-GC/MS in order to get an insight in to the analytical chemistry of Kassel earth pigments and to investigate the diversity in composition of the Kassel earth samples by applying multivariate analysis to the mass spectrometric data. In a paint sample or a sample from paintings, the analytical information of the pigment and of the oil matrix around it is often additive. The deconvolution of both sets of information requires a good characterisation of the pigment at the molecular level. The importance and advantages of the methods used in this analytical approach for small samples in general has been explained before (see Chapter 2). In this chapter will be shown typical mass spectrometric fingerprints for Kassel earth pigments and will be pointed out which markers and biomarkers are specific for such materials. Studies on the effect of Kassel earth-like pigments on the drying of oil paint have been reported elsewhere by Languri and Boon [9]. For oil paints that supposedly contain Kassel earth, molecular evidence is essential to confirm the presence of the

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[1] Frechen [10], Bensberg [10], Kirdorf, Liblar, Hermuhlecin, Obercassel-on Rhein, Pfaffroth, Reincassel, Rogendorf [1]

[2] Europe (Switzerland, England- Birmingham, Mendip Hills mentioned in the 18th century, Czechoslovakia, Italy- Nocera), US (Hanna- Wyoming, Putnam- New Mexico, Box Elder-Montana mentioned in 1929) [1]

pigment. A firm identification of the pigment and knowledge about its diversity in composition may also help to understand the supposed relationship of the degradation of brown areas in paintings with the presence of Kassel earth pigments.

Experimental

Samples

Twenty Kassel earth and Vandyke brown samples listed in Table 1 are the object of this study. With the exception of the lignite sample of geological origin, which preserves the structure of the original wood, all the other samples were received as dark brown powders. Of high interest especially for this thesis are the samples from historical collections. This was the reason to add the later arrived samples from the Vigani collection and Winsor & Newton archive. The two samples from the 18th-century Vigani collection, V_{1-2}, were provided by Lisa Wagner during her investigations of the Vigani cabinet from the Queen's College, Cambridge, UK [11, 12]. These samples can be representative for the material sold as Kassel earth in the 18th century and most probably used by the painters of that time, too [11, 12]. The H_1-H_4 samples were obtained from Mr. M. van Hoorn, the curator of the 19th-century Hafkenscheid collection of the Teylers museum, Haarlem, The Netherlands. In the Hafkenscheid collection are paint materials kept by the Dutch firm *Hafkenscheid en Zoon*, firm that traded such materials in that time period [4, 5, 13, 14]. These materials are thought to be representive for 19th-century paint materials [4, 5, 13, 14]. Another Kassel earth sample, WN_1 provided by Dr. Carlyle originates from the Winsor & Newton archive, London, UK. Winsor & Newton[3] is one of the English companies that traded paint materials in the 19th century [2, 3]. Seven other Kassel earth-like samples, F_{1-7}, were kindly provided by Dr. Feller. These samples are real Kassel earth materials considered to be representative for the 20th-century pigment industry and not necessarily representative for the material used in the 19th and 20th C for painting [15]. These samples were obtained from the Kassel area at the end of the 1960's [1]. The same samples have been characterised by Feller and Feller by several analytical methods but not by mass spectrometric techniques [1]. Paint restorer Mr. H.C. von Imhoff, Switzerland, made available two 20th-century Kassel earth pigments, I_{1-2}, that are the same in origin but have been stored in two different places and most probably also under different conditions [16-18]. These Kassel earth samples were used in reconstruction experiments of oil paints in 1972 and thus are very valuable for comparing data obtained on the Kassel earth pigments with data obtained on the oil paints containing these pigments [9, 16-18]. Two commercial samples, one from the German firm Schmincke, C_1, and one from the Belgian firm Blockx, C_2, were available in the MOLART collection of paint materials and are analysed during this study. Paint restorer A. Boersma from the Boymans van Beuningen museum, Rotterdam, The Netherlands, provided another 20th-century Kassel earth-like sample, coded as O. One geological sample of fossil wood, a Jülich brown coal, coded G, was included in this study to make the connection stronger between Kassel earth-like materials and lignites. Dehmer characterised the sample before using Py-GC/MS [19].

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[3] nowadays owned by Colart Fine Art & Graphics, Whitefriars Avenue Harrow, Middlesex, HA3 5RH, UK.

Table 1. Kassel earth samples, their name, age and provenance.

Sample	**Label**	**Provenance**
18th-century *Vigani collection*		
Cullens earth1 (S1119)	V_1	Vigani's Materia Medica Cabinet, Queens'College, Cambridge
Colens earth. Terra Lemni (S1115)	V_2	Vigani's Materia Medica Cabinet, Queens'College, Cambridge
19th-century *Hafkenscheid collection*		
Kassel arde (S906)	H_1	Hafkenscheid collection, Teylers Museum, Haarlem
Keulse aarde gemalen (S907)	H_2	Hafkenscheid collection, Teylers Museum, Haarlem
Keulse aarde (S908)	H_3	Hafkenscheid collection, Teylers Museum, Haarlem
Van Dijkbruin (S909)	H_4	Hafkenscheid collection, Teylers Museum, Haarlem
Winsor & Newton archive (?)		
Cologne earth (S1116)	WN_1	Winsor & Newton archive
20th-century *Artists' Pigments 'collection'*		
Vandyke brown (S910)	F_1	Scholz&Co. (Germany?)
Vandyke brown (S911)	F_2	Bakelite AG (Frielendorf, Germany?)
Vandyke brown (S912)	F_3	W. Hawley and Son Ltd., UK(1968, Czechoslovakia)
Kasseler Braun (S913)	F_4	Wilhelm Urban & Co. (1968, Germany, pp.165)
Cassel earth (S914)	F_5	G.&E. Habich' s Farbenfabrik GmbH, (1973, Germany)
HS Kasseler Braun (S915)	F_6	G.&E. Habich' s Farbenfabrik GmbH, (1968, Germany)
Cassel earth (S916)	F_7	BLOCKX, Terwagne, Belgium
Von Imhoff-Canadian Conservation Institute		
Kassel earth (S893)	I_1	Mülfellner-Rupf, Seidengasse 14 Zurich 1
Kassel earth (S922)	I_2	Mülfellner-Rupf, Seidengasse 14 Zurich 1
Commercial samples		
Vandyck brown, (S552)	C_1	Schmincke
Cassel earth (S568)	C_2	Blockx
Geological samples		
Julich brown coal (S570)	G	Brown coal deposits near Julich (Lower Rhine area, Germany)
Other samples		
Van Dijk's bruin (S353)	O	Luitwieler

Extraction

The H_1, F_7 and I_1 (the last two more abundant than H_1 and quite similar in composition) Kassel earth samples have been extracted with apolar and polar solvents in the following order hexane, dichloromethane (DCM) and ethanol (EtOH). Hexane was added to the sample and mixed using a VORTEX-GENIE mixer model K-550-GE (USA) and then for 5 minutes in a BRANSON ultrasonic cleaner, model B-2200 E4 (USA). The mixture was then centrifuged for 5 minutes in a Micro 20 Hettich centrifuge, type 200424 (Germany) at 50 rot/min x 100, the solvent and extractables were removed with a glass Pasteur pipette. DCM was added to the hexane residue and the operation was repeated. Afterwards, EtOH was added to the DCM residue and the operation was repeated again. The clean residue and all the extracts were subsequently analysed by DTMS, with and without addition of TMAH 2.5 % solution in water [20].

Direct Temperature-resolved Mass Spectrometry (DTMS) and Direct Temperature-resolved Mass Spectrometry Mass Spectrometry (DTMS/MS)

For DTMS analysis, 5-10 µg of sample was dissolved in 15-25 µl of DCM. An aliquot of 1-2 µl of this solution was placed on the Pt/ Rh filament (Pt/Rh 9:1, 100 µm) of a direct insertion probe for in-source analysis [21-23]. DTMS experiments were carried out on a JEOL JMS SX-102A double focussing mass spectrometer (B/E). The filament was heated at a rate of 0.5 A/min to an end temperature of about 800 °C. Ions were generated by 16 eV electron ionisation (EI) to minimise fragmentation reactions of the ions in an ionisation chamber kept at 190 °C, accelerated to 8 kV, analysed from m/z 20-1000 (about 1 s cycle time) and post-accelerated to 10 kV. A JEOL MS-MP 9020D data system was used for data acquisition and processing.

DTMS/MS experiments were carried out on a JEOL JMS-SX/SX 102A tandem mass spectrometer (B/E/B/E). Collision Induced Dissociation (CID) was performed in the third Field Free Region using helium as a collision gas ($1.5\ 10^{-3}$ Pa). The voltage of the collision cell was 2 kV. The same conditions as for DTMS were used concerning the probe, ion generation, acceleration and post-acceleration of ions and acquisition. The ions were analysed from m/z 0 to 375 at about a 1 s cycle time. The resolution used was 3000.

Py-(TMAH)-GC/MS Pyrolysis Transmethylation Gas Chromatography Mass Spectrometry

Typically 10 µg of sample is used for Py-(TMAH)-GC/MS. The samples were dissolved in DCM or ground with a 2.5 % tetramethylammonium hydroxide (TMAH) in water. Aliquots were applied to a ferromagnetic wire (Curie point 770 °C or 358 °C when TMAH was used) and dried in vacuo. After drying, the sample wire was inserted into a glass liner, placed in the cold compartment of the pyrolysis unit, flushed with helium and then moved into the pyrolysis chamber (220 °C) of the FOM-AMOLF 5LX Curie point pyrolysis unit [24]. Pyrolysis time was 9 s (770 wire) or 6 s (358 wire). For separation, a fused silica SGE BPX5 column (25 m, 0.32 mm i. d., 0.25 µm film thickness) was used in a Carlo Erba series 8565 HRGC MEGA 2 gas chromatograph using helium as the carrier gas at a flow rate of 2 ml/min. The oven temperature was programmed from the initial 35 °C (for 0 min.) to a final 320 °C (for 10 min), with a ramp of 4 °C /min. The column was interfaced directly to a JEOL JMS DX-303 or the SX102A/102 mass spectrometer using a home built high temperature interface. Ions were generated by electron ionisation (70 eV EI) in the ionisation chamber, accelerated to 3 kV (DX-MS) or 8 kV (SX-MS) respectively, mass separated and post-accelerated to 10 kV before detection. The mass range was scanned from m/z 35-500 or m/z 40-800 respectively, with a cycle time of 1 s. A JEOL MS-MP 9020D data system was used for data acquisition and processing.

Principal Component Analysis (PCA)

Mass spectra were numerically analysed by PCA with the FOMpyroMAP multivariate analysis programme, a modified version of the ARTHUR package from Infometrix Inc. (Seattle, USA; 1978 release) and with the FOM developed Matlab® (The Mathworks Inc., Natick, MA, USA) toolbox ChemomeTricks, developed by Gert B. Eijkel, FOM-AMOLF. [23, 25-33].

Results and Discussion

DTMS of Kassel earth pigments

The samples listed in Table 1 were analysed by DTMS and the summation spectra were compared by PCA. The PCA was performed at a stage when the Vigani and Winsor & Newton samples were not yet available. The PCA map in Fig. 1 shows the diversity of sample composition. Variance after PCA is 60% for PC1 and 10% for PC2. The PCA map groups the samples on the positive side (F_2, F_3, F_1, C_2, I_2, C_1, G, H_4) and on the negative side (I_1, O, H_1, F_6, H_3, H_2, F_4, F_5, F_7). The chemical meaning of this separation is revealed in the positive and negative spectra of PC1 shown in Fig. 2a, b and Table 2.

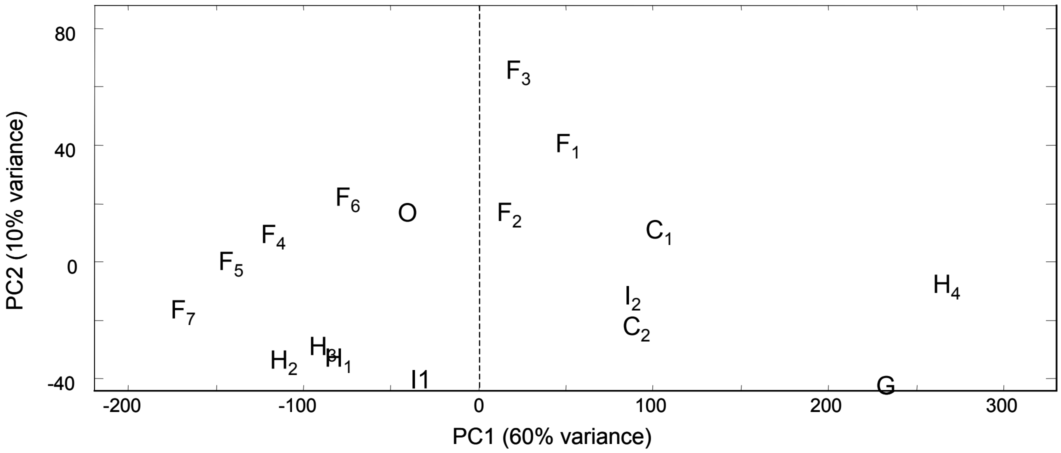

Fig. 1. PCA map of DTMS data of the analysed Kassel earth-like samples. (The labels of the samples are explained in Table 1)

The assignments in Table 2 are confirmed by Py-GC/MS work reported further below and by comparison with earlier GC/MS data on brown coal (lignite and/or subbituminous coal) reported in the literature [19, 32, 23, 36-38]. The following ions were assigned in this way to compounds classes of: diterpenoids (m/z 123, 274, 259), aromatic and olefinic diterpenoids (m/z 123, 246), triterpanes (m/z 109, 143, 189, 248, 408, 424, 442, 452), aromatic triterpenes (m/z 145, 376, 378), other triterpenes (m/z 109, 204, 218, 410), polyaromatic triterpenes (m/z 268, 322, 324, 342, 356), hopanoids (m/z 191), demethylated hopanoids (m/z 177), methylated hopanoids (m/z 205), phenols (m/z 94, 108), methoxyphenols (m/z 124, 138, 150, 152, 164, 178, 180, 182), (alkyl)benzenes (m/z 77, 78, 91), alkanes/alkenes (m/z 57 + n x 14 and m/z 55+ n x 14), dihydroxybenzenes (m/z 110), long chain wax esters (m/z 676, 704, 732, 760, 788, 816, 844, 872, 900, 928) and fatty acids (m/z 256, 396, 410, 424, 452, 480, 508). M/z 28 and 44 are representative for decarboxylation of organic acids in the samples.

Table 2. List of the characteristic classes of compounds, m/z values and relative abundance in the reconstructed spectra of the PC1, positive and negative side and in samples F_4, C_1 and G.

Classes of compounds	**m/z**	**Peak abundance (%)**			
		PC1	**F_4**	**C_1**	**G**
Aliphatic compounds					
Alkanes	57, 71, 95	34*(57)*	16,20,31	39,35,38	22,18,19
Alkenes	55, 69, 93	27*(69)*	9,18,9	18,32,15	9,16,8
Fatty acids [34]					
C_{16} fatty acid	256	19	16	20	14
C_{26} fatty acid	396	55	35	18	4
C_{27} fatty acid	410	50	49	31	4
C_{28} fatty acid	424	85	80	38	3
C_{30} fatty acid	452	44	23	7	1
C_{32} fatty acid	480	26	1	3	-
C_{34} fatty acid	508	11	6	1	-
Wax esters (odd not shown in the table)					
C_{46} wax ester	676	4	-	-	-
C_{48} wax ester	704	5	2	1	-
C_{50} wax ester	732	7	2	1	-
C_{52} wax ester	760	9	3	2	-
C_{54} wax ester	788	10	3	2	-
C_{56} wax ester	816	9	3	2	-
C_{58} wax ester	844	7	3	2	-
C_{60} wax ester	872	6	-	2	-
C_{62} wax ester	900	-	-	1	-
Aromatic compounds					
Alkylbenzenes	77, 78, 91	14,17,25	-,-,10	-,-,16	11,19,28
Phenol	94	32	15	-	28
Alkylmethoxyphenols	124	93	-	-	85
	138	77	-	-	72
	152	30	-	-	30
	164	23	-	-	26
	178	23	-	-	19
	180	14	-	-	14

Table 2 continued

Classes of compounds	**m/z**	**Peak abundance (%)**			
		PC1	**F_4**	**C_1**	**G**
	182	12	-	-	11
Alkyldihydroxybenzenes	110	73	-	43	65
Carbon dioxide					
decarboxylation of organic matter	28, 44	-, 96	27,33	100, 100	100, 57
Diterpenes					
Phyllocladane [19]	274, 123	33,45	57,42	28,45	-,-
Dihydromuen [19]	274, 259	33,-	57,26	28,18	-,-
Triterpanes					
Fragment ion	109	25	38	-	-
Fragment ion	143	9	43	30	-
Fragment ion	189	22	60	35	-
Fragment ion	248	22	45	19	7
Molecular ion	408	41	38	26	3
Molecular ion	424	85	80	38	3
Molecular ion	442	39	41	18	-
Molecular ion	452	44	23	7	-
Aromatic triterpenes					
Component X [19]	376, 145	-	19,36	28,53	4,14
Triterpenes					
Olean-18-ene [19]	410, 109	50,25	42,38	31,42	-
Olean-12-ene [19]	410, 218	50,37	42,73	31,51	4,10
Polyaromatic triterpenes					
Tetramethyloctahydropicene [19]	342, 342	7,7	22,22	73,73	7,7
1,2,9-trimethyltetrahydropicene[19]	324, 324	7,7	32,32	53,53	7,7
Component G [19]	322, 322	7,7	11,11	23,23	5,5
2,2,9-trimethyltetrahydropicene[19]	324, 268	7,-	11,11	53,14	7,10
Component E [19]	356, 169	-,7	42,38	20,20	-
Hopanoids [19, 35]					
18 α(H)-22,29,30 trisnorneohopene ($T_{s:1}$)	368, 191	23,87	19,78	10,64	-
17α(H)-22,29,30 trisnorhopane (T_m)	370, 191	14,87	15,78	11,64	-
Hop-17(21)-ene and/or Hop-22(29)-ene	410, 191	50,87	42,78	31,64	4,25
17α, 21β-homohopane	426, 191	91,87	100,78	65,64	3,25
17β, 21β-homohopane	426, 205	91,44	100,78	65,49	-
Homomoretane	426,191/205	91,87/44	100, 78/78	65, 64/49	-
Fragment ion of norhopanes and normoretanes	177	-	31	27	-
Methylethylcyclopentenochrysene	310, 281	-	-	22,14	-

The reconstructed spectrum of the samples on the positive side in Fig. 2a shows peaks characteristic of decarboxylation of organic matter (m/z 28, 44), phenol (m/z 94) and methylphenols (m/z 108), alkylmethoxyphenols (m/z 124, 138, 150, 152, 164, 178, 180, 182) and dihydroxybenzene isomers (m/z 110) representative of fossil wood material [23, 39]. The peaks at m/z 49, 51, 84, 86 are due to the solvent, DCM. M/z 256 is a lipid derived peak (palmitic acid). The reconstructed spectrum of the samples on the negative side in Fig. 2b shows peaks characteristic of high molecular weight waxes (m/z 732, 760, 788, 816, 844), diterpenoids (m/z 274) and various triterpenoids (m/z 143, 191, 205, 218, 248, 410, 424, 426, 452, 454, 480, 508) of bacterial or plant origin representative of fossil leaf material [20, 32, 39-41]. Some

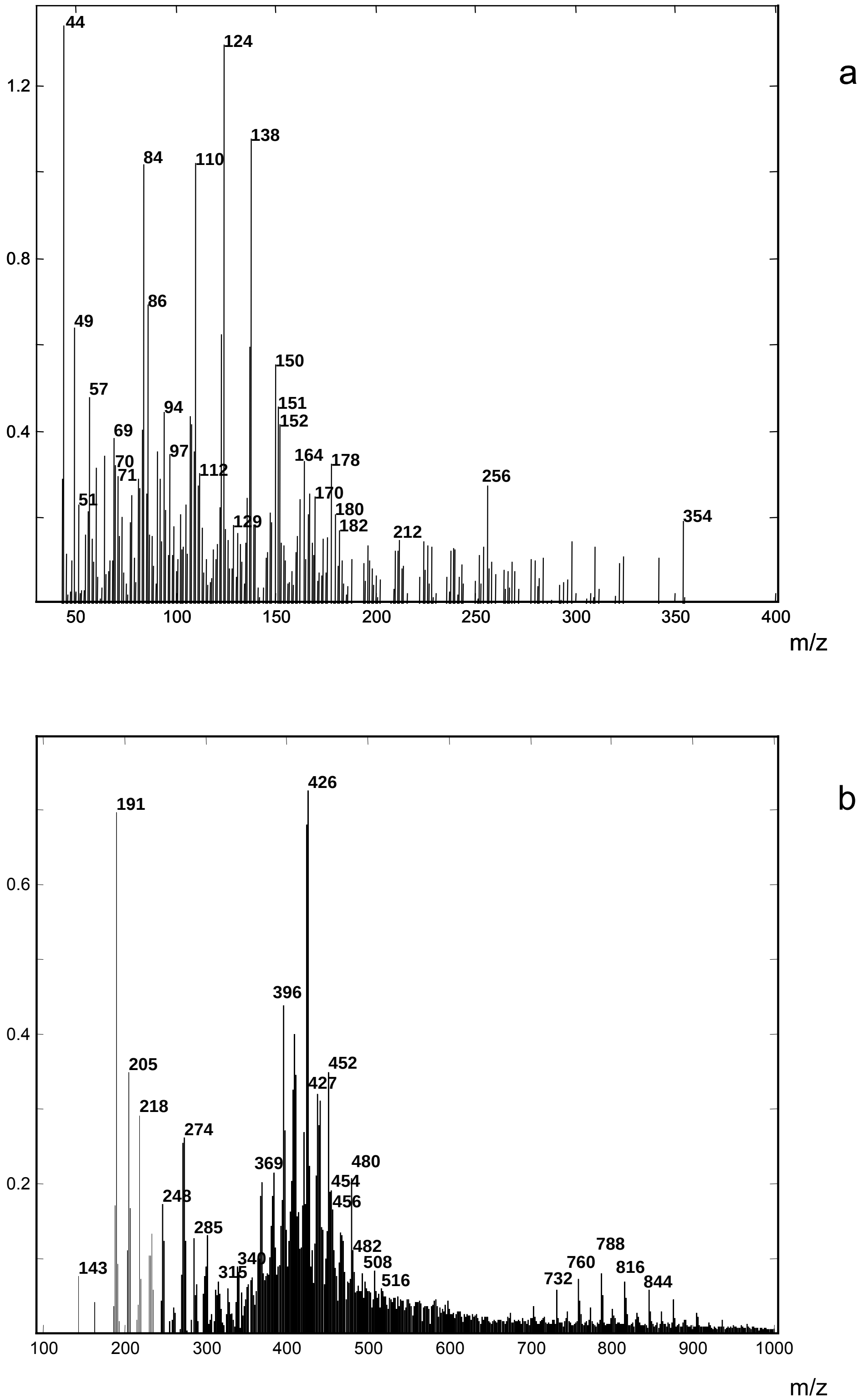

Fig. 2. Positive (a) and negative (b) spectra of the first principal component (PC1) of the DTMS data obtained from the analysed Kassel earth-like samples.

peaks like m/z 396 and 410 are probably lipid derived and related to the wax [42, 43]. No indications for polysaccharides were found [20] in the reconstructed spectrum neither in the mass spectra of the samples. Polysaccharides are present in fresh leaf

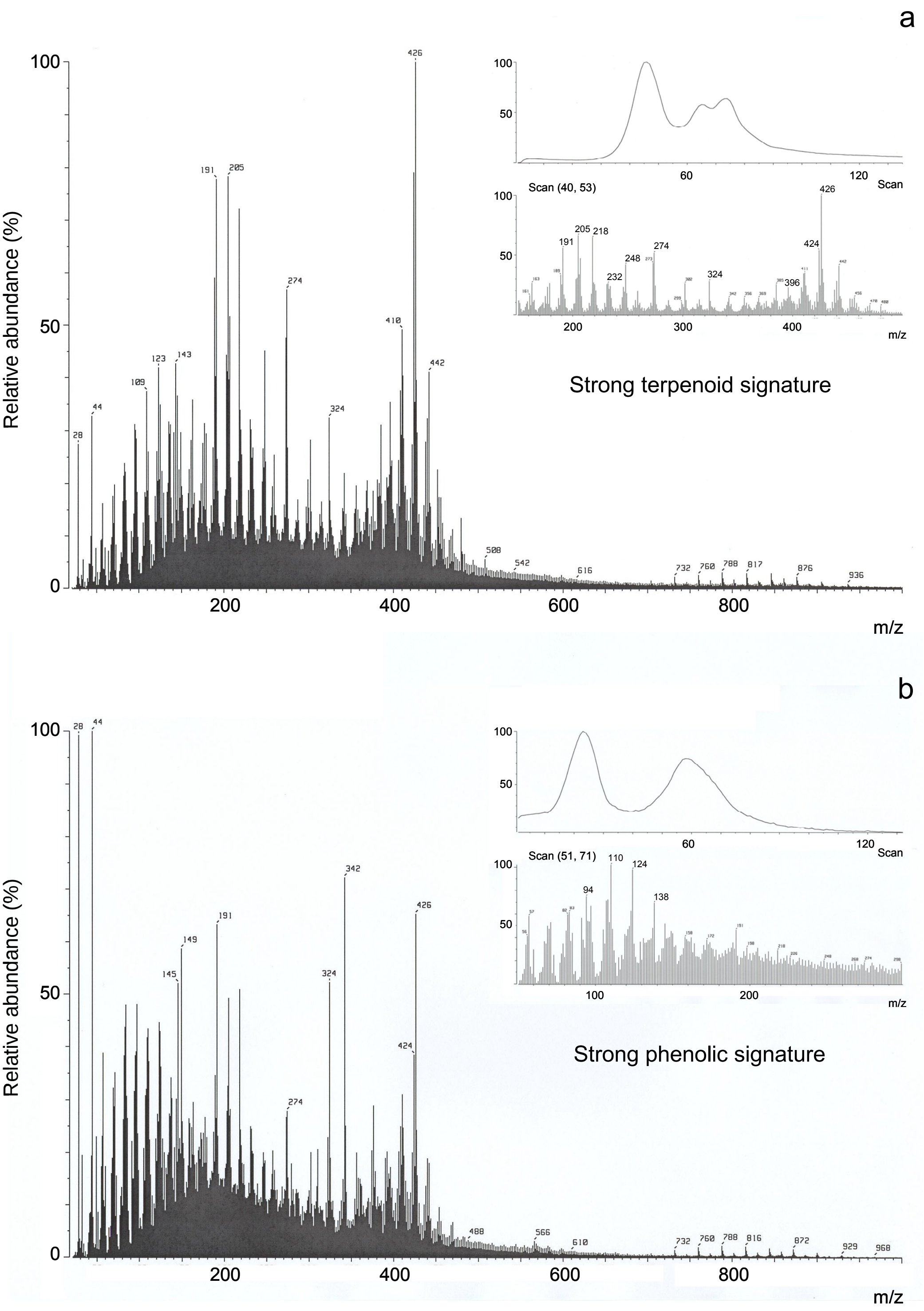

Fig. 3. Typical DTMS spectra of F_4, a Kassel earth-like sample with a strong terpenoid signature (a) and C_1, a Kassel earth-like sample with a strong phenolic signature (b). The inserts show the TIC of the measurements (upper window) and the mass spectrum of the terpenoid signature (scan 40-53 sec) in Fig. 3a, respectively the mass spectrum of the phenolic signature (scan 51-71 sec) in Fig. 3b.

and wood cells as cellulose and hemicellulose. Their absence suggests that the analysed samples have reached the lignite stage [23, 39].

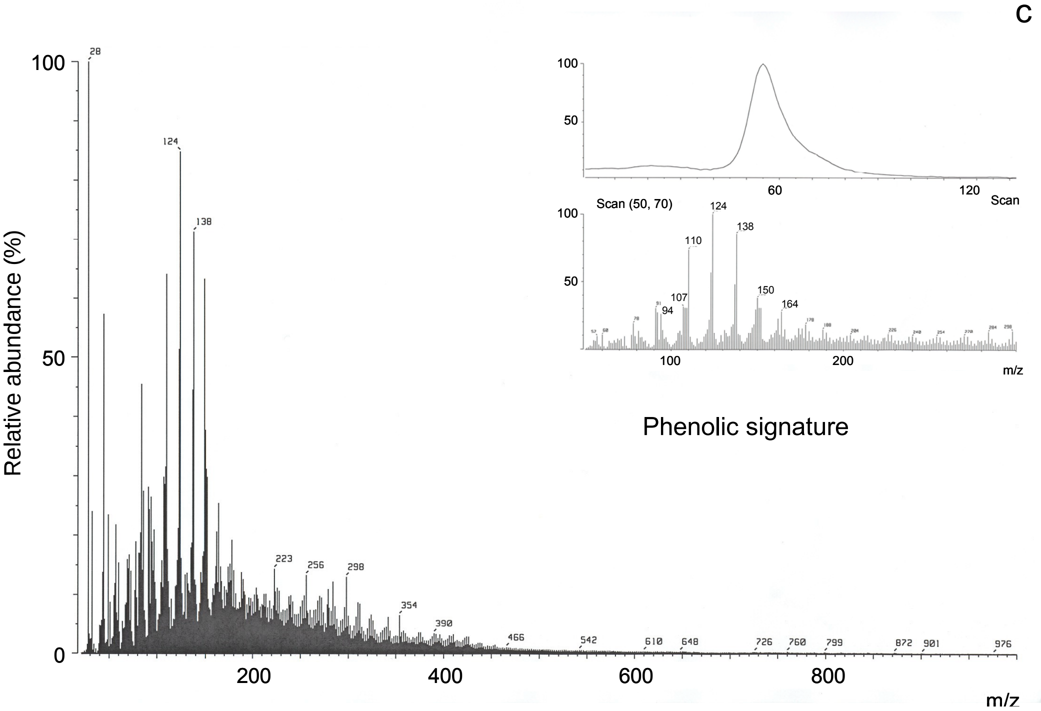

Fig. 3 continued. Typical DTMS spectra of G, a fossil wood sample with a strong phenolic signature (c). The insert shows the TIC of the measurements (upper window) and the mass spectrum of the phenolic signature (scan 51-71 sec).

The grouping in Fig. 1 suggests a higher content of fossil leaf material in Kassel earth and Cologne earth samples (H_1-H_3, F_4-F_7- samples with known origin, I_1) and a high fossil wood material content in Vandyke brown samples (H_4, $F_{1\text{-}3}$- samples with known origin, C_1). A few of the samples for which the deposit source is not known but only the supplier classify in a different way. For example the Kassel earth samples with woody characteristics (I_2, C_2) and Vandyke brown samples with leaf characteristics (O). Sample G of known geological origin is classified with the Kassel earth sample on the positive PC1 space. This underpins the idea that Kassel earth-like pigments are in fact brown coals, i.e. lignite or subbituminous coals [44]. Typical DTMS spectra for the observations by PCA are shown in Fig. 3 for samples with a terpenoid signature like F_4 (PC1-), Fig. 3a, and samples with a phenolic signature, for example C_1 (PC1+), Fig. 3b, respectively G (PC1+), Fig. 3c, where G is an extreme case of this second group of samples. The chemical significance of the m/z values seen in Fig. 3a, b, c and in Fig. 2a, b is given in Table 2. The full mass spectrum, a relevant partial mass spectrum and the DTMS total ion current trace are shown for each case. In the low m/z range of m/z 20-250 of the DTMS summation spectra in Fig. 3a, b and c, mostly fragment ions are seen, while at higher m/z values molecular ions around m/z 400 and around m/z 800 are observed. The peaks have a different relative abundance in the three samples. In Fig. 3a of sample F_4, the highest peaks are observed in the area of molecular ions of terpenoids (around m/z 400) and their corresponding fragment ions (around m/z 200). Peaks with a lower relative abundance resulting from decarboxylation of organic matter and other ones characteristic for waxes are present at the beginning, and the end of the mass spectrum, respectively.

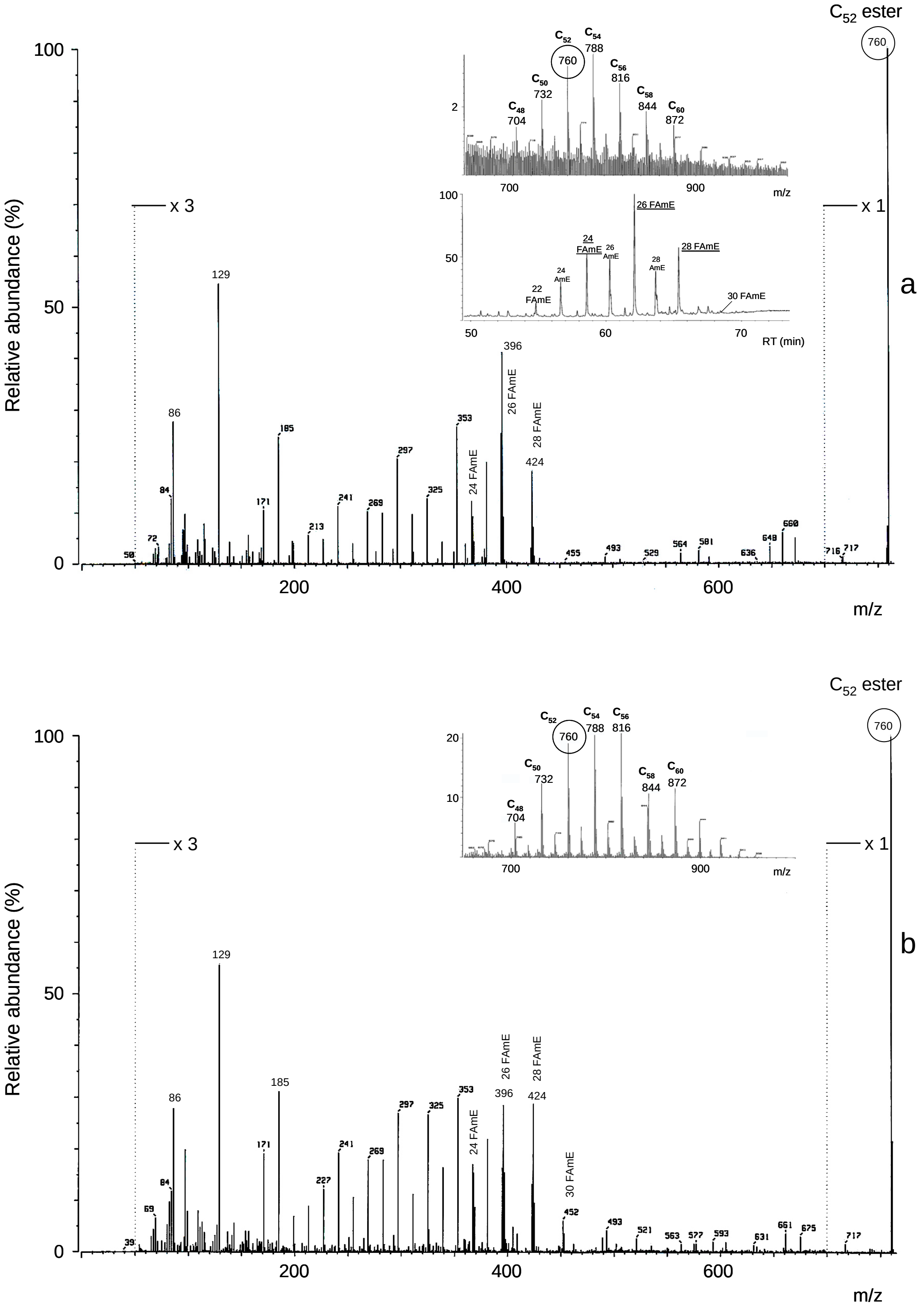

Fig. 4. DTMS/MS of m/z 760 peak in the F$_7$ Kassel earth like sample (a) and in a German montan wax reference sample (b). The DTMS distribution of wax esters in the two samples is shown in the (higher) inserts in the figure. The GC/MS distribution of the fatty acid methyl esters (fame) and alcohol methyl ethers (AmE) of the F$_7$ sample in the Py-TMAH-GC/MS TIC is shown in the lower insert of (a).

The mass peak profile is somewhat different in Fig. 3b of sample C_1, where the highest peaks are m/z 28 and 44 from organic matter decarboxylation followed in relative intensity by peaks corresponding to polyaromatic triterpenes (m/z 324, 342), terpenoids (around m/z 400 and m/z 200) and wax peaks (around m/z 800) respectively. A peculiar group of peaks observed in Fig. 3b and c around m/z 100 corresponds to fragment ions of aliphatic compounds and/or molecular ions of small aromatic compounds like phenols. Aromatic compounds typical for fossil wood are predominant in the G sample (Fig. 3c) and will be addressed separately below. M/z 28 and 44 interpreted as decarboxylation products from acid moieties due to pyrolysis are also abundant in the DTMS of G in Fig. 3c. The m/z area corresponding to terpenoids or waxes is empty implying the absence of these compounds in sample G. The DTMS spectra obtained for the Vigani samples would classify them together with the samples with a terpenoid signature on the negative side of PC1. The DTMS of the Winsor & Newton sample points towards a phenolic signature with similarity to the samples on the positive side of PC1.

The high molecular weight wax esters present in the DTMS spectra of most of the Kassel earth samples are characteristic compounds (biomarkers) for Kassel earth-like pigments. These compounds shown to be partly sequestered in the network are possibly a way to trace Kassel earth pigments in oil paint samples as shown earlier [9]. DTMS/MS was performed to obtain a more solid identification and to derive the fatty acid and alcohol moieties from specific fragment ions. The similarity of the wax ester peaks found in the investigated pigment samples with peaks from known montan waxes was investigated. The DTMS distributions of presumed wax esters for example in the F_7 Kassel earth sample (insert of Fig. 4a) and in a German montan wax reference sample (insert of Fig. 4b) range from m/z 704 (C_{48}) to m/z 928 (C_{64}) and are very much alike. (DTMS work on waxes has been described before [45-47]). The second insert in Fig 4a shows the distribution of fatty acids as methyl esters (C_{22}, C_{24}, C_{26}, C_{28} and C_{30} FAmE) and alcohols as methyl ethers (C_{24}, C_{26}, C_{28} AmE) in F_7 as revealed by Py-TMAH-GC/MS. (GC/MS work on waxes is reported by Matthies [48] and Asperger et al. [49].) The collision induced fragmentation data of the m/z 760 peak, i.e. the C_{52} ester in F_7 (Fig. 4a) and in the German wax (Fig. 4b) shows some even peaks of high intensity and many peaks with odd mass numbers with mass differences of 14 Dalton. Peaks m/z 368, 396 and 424 in Fig 4a are considered to be the result of a γH-rearrangement releasing the fatty acid (fa) moiety with a chain length of C_{24}, C_{26} and C_{28} respectively. The montan wax spectrum also shows a peak at m/z 452 from a C_{30} fatty acid moiety. The odd peaks are interpreted as α-cleavage products of the fatty acid moieties with m/z 129 and 185 as main fragment peaks. Evidence for the alcoholic moiety could not be identified in the MS/MS spectrum. To obtain a C_{52} wax ester (MW 760) combinations of C_{24} fatty acid with C_{28} alcohol, C_{26} fatty acid with C_{26} alcohol and C_{28} fatty acid with C_{24} alcohol are required. These alcohols were indeed observed by GC/MS of the whole wax mixture. Since several of the other wax ester peaks in the DTMS/MS of the Kassel earth and montan wax showed similar ion patterns, the identity of the wax ester peaks in the DTMS is sufficiently confirmed. Montan waxes (fossilised leaf waxes) are known to be associated with lignite deposits [43, 50].

DTMS also allows the investigation of the form in which organic matter is present in a sample. For example, volatile compounds will desorb very quickly while cross-linked materials require a much higher temperature. The TIC inserts in Fig. 3a, b and c present a desorption evaporation/ pyrolysis profile of the samples. The mass spectrum insert in Fig 3a (scan< 50) corresponds to the desorption part of the DTMS data. Peaks characteristic to di- and triterpenoids are observed (m/z 274, 324, 191, 205, 218, 248, 410, 424, 426, 442). The limited mass range cuts off the presentation of the higher mass range with the corresponding peaks of the wax esters. The m/z values are explained in Table 2. The mass spectrum insert in Fig 3b and c corresponds to the pyrolysis part of the DTMS data and show peaks characteristic of phenolic compounds (scan> 50) generated from a cross-linked network. The mass spectrum of the terpenoid features in sample F_4 (PC1-), and the phenolic features, sample C_1 and G (both PC1+), are very much similar to the PCA spectra in Fig. 2a and Fig. 2b respectively implying that sample F_4 and C_1 are compositionally representative for the two different groups with primarily fossilised leaf/cutin-derived or wood/lignin-derived material, respectively.

The ratio between volatile and bound material is expressed in the diversity of the desorption profiles of all samples investigated as shown in Fig. 5. Samples from the 19^{th} century as well as those from the 18^{th} century are grouped together and lined by a box. The PCA-space classification corresponding to wood derived or leaf derived material is marked by (PC1+) and (PC1-) respectively. For the Vigani and Winsor & Newton samples that have not been included in the PCA the assignment to the positive or negative side of the PC1 is only tentative. Some of the samples are richer in the more volatile material like F_1, F_3, F_4-F_6, C_1. Other samples H_1-H_2, WN_1, F_2, F_7, I_1-I_2, O, C_2, G, V_1-V_2 contain more cross-linked/bound material. The appearance of the TIC of sample H_4 is explained by the primarily inorganic composition of the sample (an example of adulteration?). Very volatile compounds are released in the first 20 scans of the DTMS measurement of most of the samples.

The more volatile compounds not are only extractable by the increase of temperature in a DTMS experiment as shown in Fig. 5, but also by solvents. This aspect was further investigated by extraction of sample F_7, I_1 and H_1. Extractions were performed with organic solvents: hexane, DCM and ethanol. The residues and extracts were then analysed by DTMS before and after transesterification with TMAH. An illustrative example is shown in Fig. 6, which shows the DTMS before (a) and after (b) the solvent extractions of H_1. The spectra and TIC (not shown) differ substantially showing that many of the chemical compounds of H_1 are solvent sensitive. The residue of H_1 releases large amounts of CO_2 (m/z 44 and 28) that suggests strong decarboxylation of the compounds present in the residual network. DTMS in the presence of TMAH indeed shows that the decarboxylation process is preventable by prior methylation of the residue. For example, the methylated vanillic acid (m/z 196) is an intense mass peak in the DTMS-TMAH experiment, whereas this compound is hardly observable in the direct DTMS. An interesting feature is the partial extraction of the high molecular weight waxes. Their presence in the residue suggests that they are very well embedded in the Kassel earth matrix perhaps creating a very hydrophobic local environment. These waxes are also not transesterifiable by the TMAH. The McLafferty rearrangement ion at m/z 74 is also a minor ion in the overall spectrum with TMAH suggesting that aliphatic moieties with carboxylic groups are no main feature of the Kassel earth pigment residue. The DTMS of the residue is devoid of evidence for diterpenoid and triterpenoids resins although some of the peaks at m/z 408, 424 and 452 might be assigned a small amount of residual unextractable triterpenoids. Hopanoid compounds with a strong m/z 191 and 205 are also no more visible in the residue. These features are removed by DCM extraction. The DTMS signature of the residue is characterised by m/z 94, 108, 110, 122, 134, 136 etc.

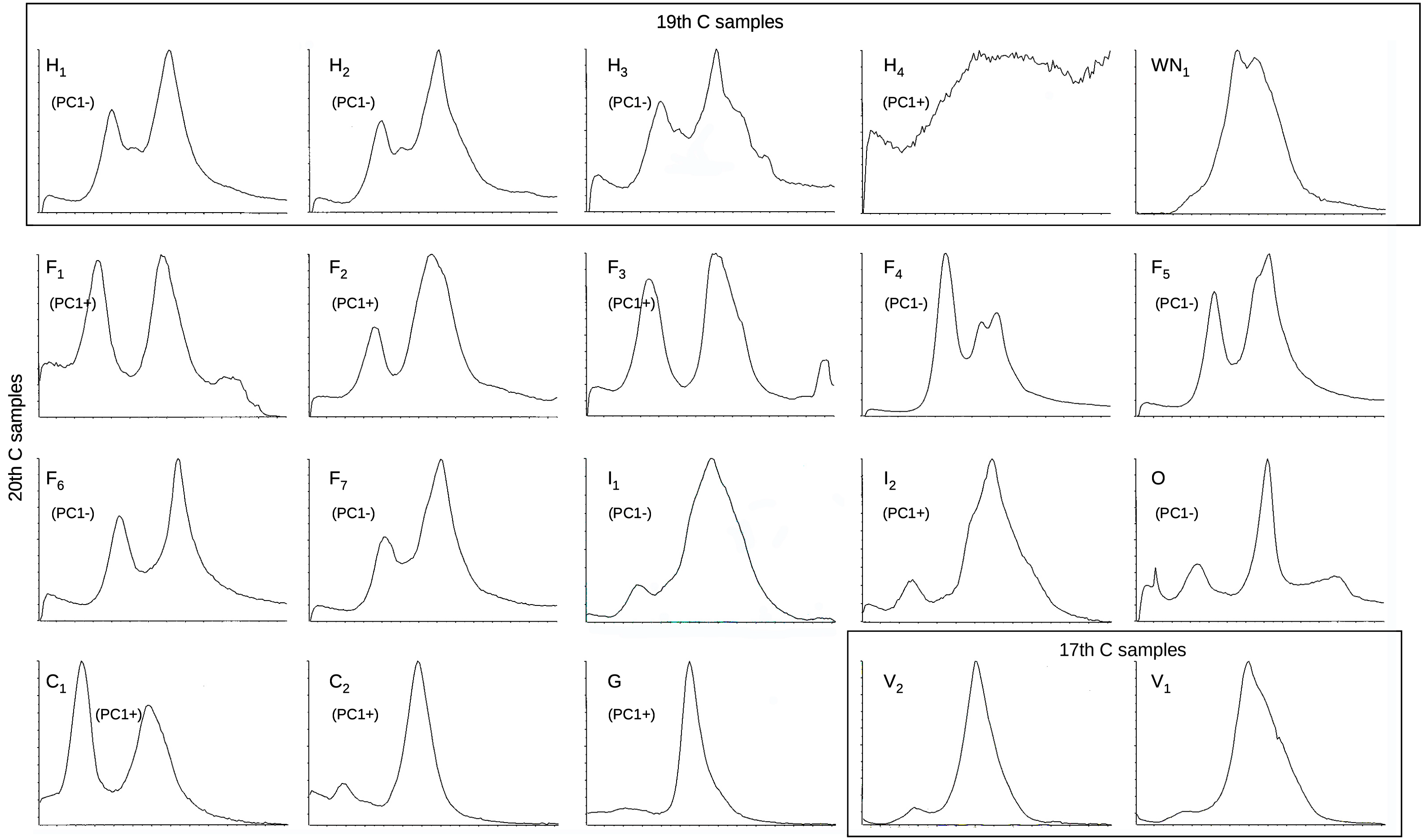

Fig. 5. TICs of the DTMS measurements of the investigated Kassel earth-like samples, grouped as 18th-century samples, 19th-century samples and 20th-century samples.

suggesting that phenolic moieties are part of the residual network. These features are also visible in the DTMS of the residue after TMAH treatment pointing towards a chemical bond to the network via the phenolic OH.

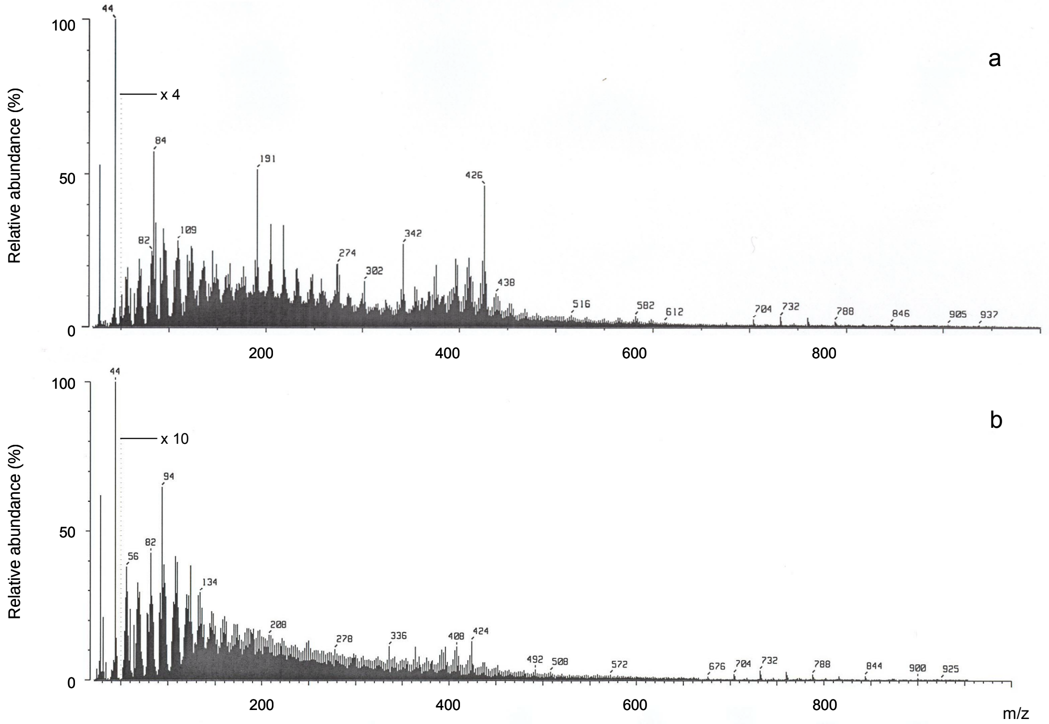

Fig. 6. Summation spectra of the H_1 Kassel earth sample before (a) and after extraction (b) with hexane, dichloromethane and ethanol.

Transmethylation studies of Kassel earth pigments by Py-TMAH-GC/MS. a) Lipid derived compounds in Py-TMAH-GC/MS data

Fat compounds in lignites do not have only one source. There is always a combination of lipids from plants and microorganisms, fat compounds of microbial origin and other ones derived from the plant polyesters cutin and suberin including their fossil forms as cutan and suberan [41]. Del Río and Hatcher [51] report a TMAH approach in combination with GC/MS applied to biopolymer from plants, like cutin (present in leaves) and suberin (the cutin homologue for roots and barks). The main identified compounds were methyl derivatives of long-chain fatty acids, hydroxyfatty acids, dicarboxylic acids and long-chain alcohols [51].

The lignitic composition of the Kassel earth samples, expressed by the presence of cutin and lignin derived compounds was further surveyed by Py-TMAH-GC/MS. Partial mass chromatograms of methyl ester of aliphatic and aromatic compounds are shown in Fig. 7a (m/z 74) and Fig. 8a (m/z 77) respectively, Fig. 7b and 8b show the TIC's of the Py-TMAH-GC/MS measurement, i.e. the relative abundance of the compounds of interest in the samples. Table 3 and Table 4 give information about the identified compounds and their occurrence in all the samples.

M/z 74 (Fig. 7a) is a fragment ion characteristic of fatty acid methyl esters [52] but this ion is not very strong in methyl esters of dicarboxylic acids and other

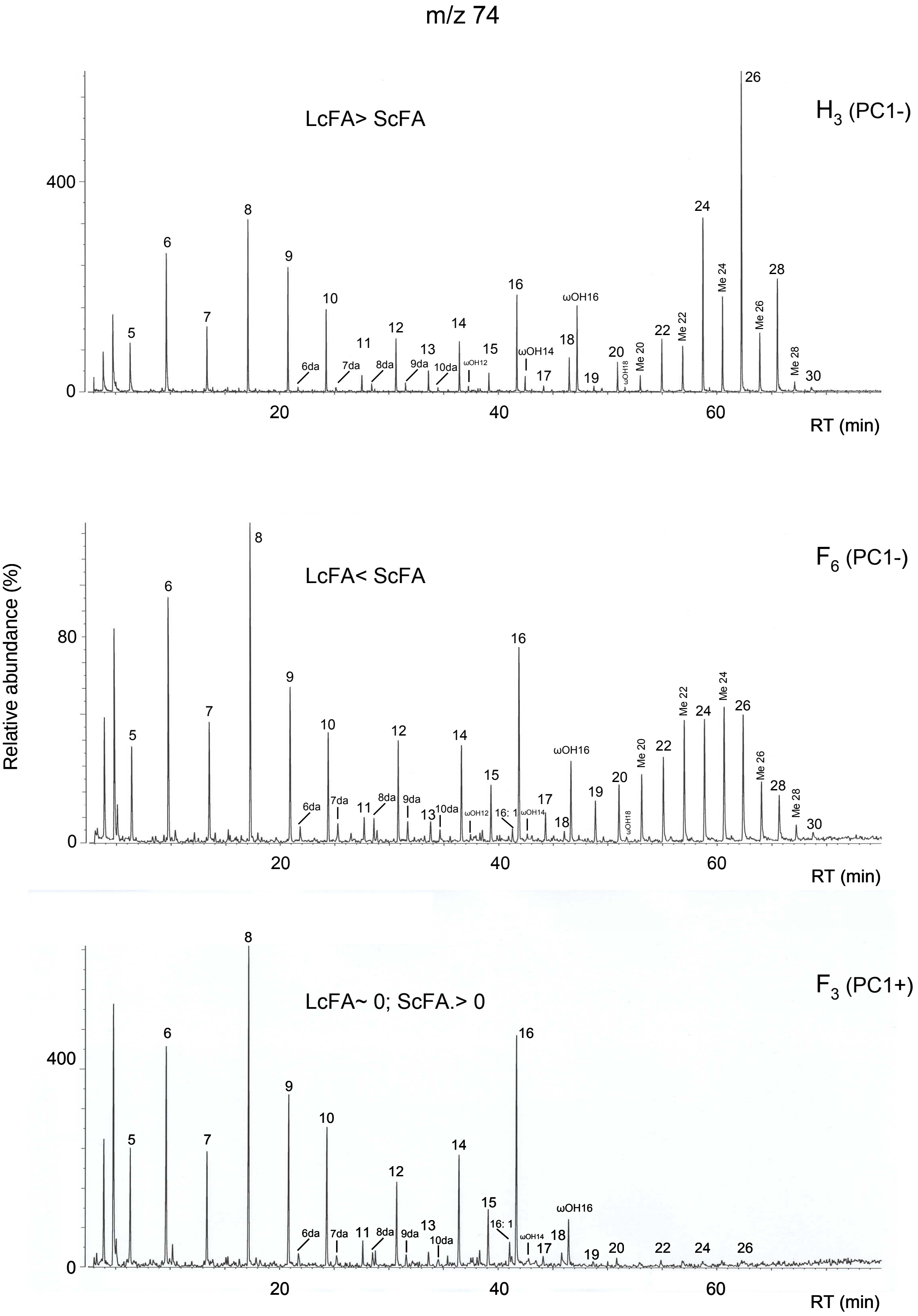

Fig. 7a. Partial mass chromatogram of m/z 74 from Py-TMAH-GC/MS data for H_3, a Kassel earth-like sample with a strong signature of long chain fatty acids, F_6, a Kassel earth-like sample with a weaker signature of long chain fatty acids and F_3, a Kassel earth-like sample with almost no long chain fatty acids. Example of notation: 16 for C_{16} fatty acid methyl ester, 9da for C_9 dicarboxylic acid dimethyl ester, ωOH16 for ω-hydroxydecanoic acid methyl ester, Me 22 for methyl docosanoic acid methyl ester.

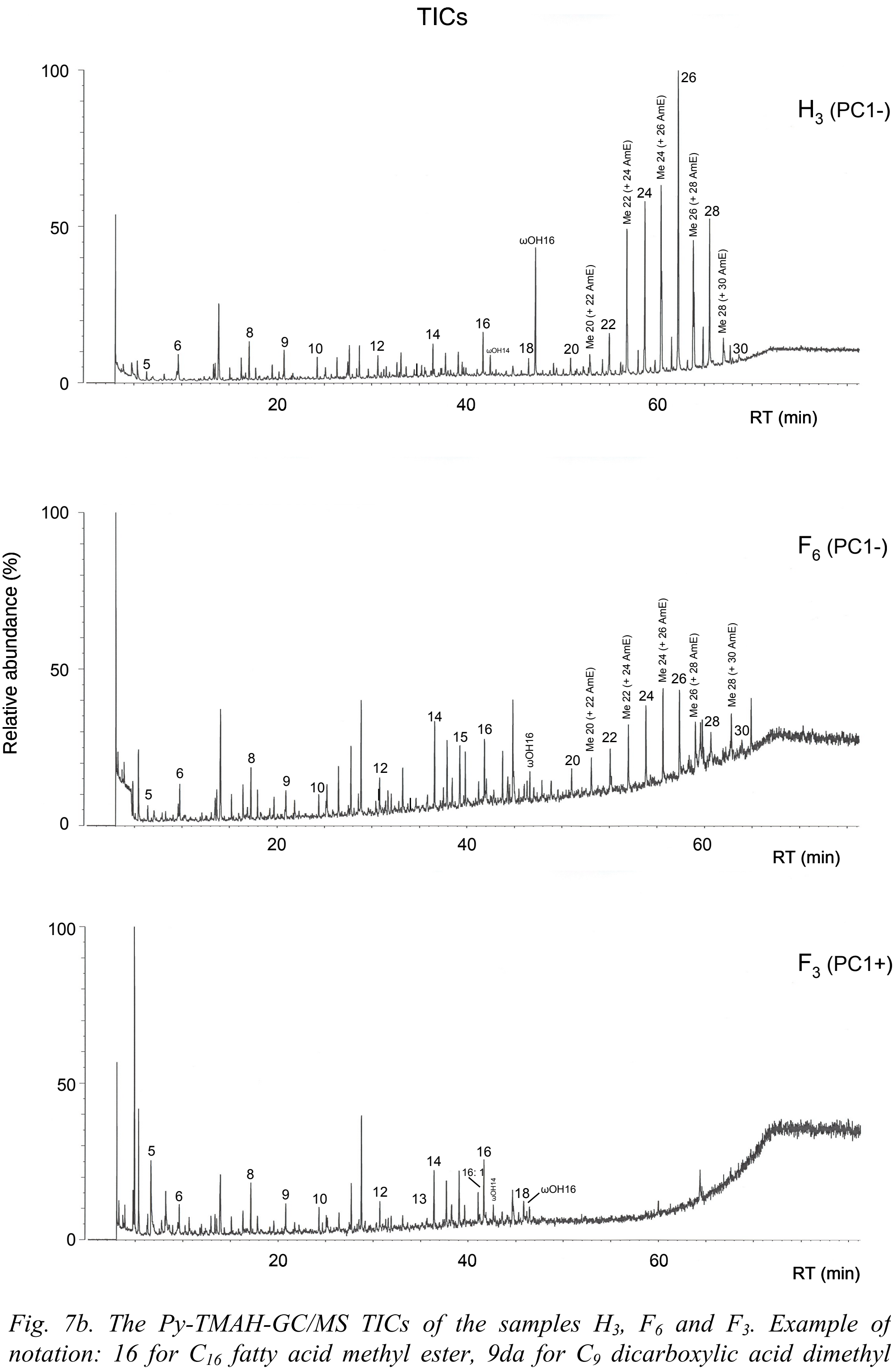

Fig. 7b. The Py-TMAH-GC/MS TICs of the samples H_3, F_6 and F_3. Example of notation: 16 for C_{16} fatty acid methyl ester, 9da for C_9 dicarboxylic acid dimethyl ester, ωOH16 for ω-hydroxydecanoic acid methyl ester, Me 22 for methyl docosanoic acid methyl ester, 22 AmE for docosanol methyl ether.

types of acids. The profiles of these compounds are therefore to be compared with the TIC to get an impression of their relative abundance. The mass chromatogram of m/z 74 simplified the comparison of the Py-TMAH-GC/MS data of all the samples. Sample H_3, F_6 from the negative PC1 and F_3 from the positive PC1 are shown in Fig 7a.

The fatty acid methyl ester (FAmE) profile in Fig. 7a depicts that long-chain fatty acids (LcFA) are higher in relative abundance than the shorter fatty acids in H_3 and oppositely that the short-chain fatty acids (ScFA) are more abundant than the long-chain ones in F_6. An extreme case of the second example is given by sample F_3, where the long-chain fatty acids are almost absent. It is noteworthy to point out that the shorter chain fatty acids can not be observed in regular GC/MS using wet chemical workup or in case where TMTFTH is used because of volatilisation of these methyl esters due to blowing off of the solvents and/or the reagent. The direct injection on the column of the methyl esters under the analytical conditions used for this research (formation of the methyl esters from fatty acid ammonium salts in an on-line mode) prevents their loss and enables their analysis. The long-chain FAmE i.e. C_{20}-C_{30} are derived from the montan wax esters [51, 53], while the origin of the short-chain FAmE i.e. C_5-C_{10} is less clear. These latter FAmE are also abundant in the fossil wood sample G suggesting some relationship to wood degrading microorganisms. The C_8 homologue is the highest in relative abundance (in the m/z 74 PMC) in almost all samples. Other highly abundant homologues are the C_6 and C_9. Fatty acids in the C_8-C_{24} range have been reported before by Smeerdijk and Boon as a suberin component of *Ericaceae* rootlets [36]. So root remains may be another source of the short chain fatty acids. The FAmE from C_{12}-C_{20} are not unusual as fat components from plants, animal and bacteria [41, 42, 54]. Long-chain alcohols coelute with odd chain FAmE and contribute to the high molecular weight compound profile in the TIC. However, the m/z 74 mass chromatogram filters out the methyl alcohol contribution showing that very long odd chain FAmE are constituents of the Kassel earth pigment samples.
The "α-ω" dicarboxylic acid methyl esters were detected ranging from C_6 to C_{10}. These compounds are usually oxidation products of polyunsaturated fatty acid moieties in lipids such as plant oils [55]. Martín et al. [41] suggests that dicarboxylic acids may act as a bridge in the plant cross-linked network. Nierop detected these dicarboxylic acids in soil samples using a Py-TMAH-GC/MS [56, 57]. The C_{16} hydroxy FAmE is the highest compound of this class in the m/z 74 trace but moieties with C_{12}, C_{14} and C_{18} chain length were also detected. The first two homologues have been found before in pine needles [56]. The C_{18} homologue is typical for angiosperms [51]. ω-Hydroxyfatty acid homologues with more than 18 carbon atoms characteristic for suberin [56] were not observed in the samples currently under discussion. The hydroxyfatty acids are analysed as methoxy-FAmE because the TMAH also methylates the hydroxy group. Their mass spectra are characterised by the loss of 32 Dalton (M-32) and subsequent loss of 28 Dalton, which is also observed in methoxyethers. Hydroxyfatty acids have various origins depending on the position of the hydroxy group. Present day cutins contain ω-hydroxy fatty acids with a chain length of C_{16} and C_{18}, while the C_{18} moieties also have a midchain hydroxy group [56]. Bacteria contain β-hydroxy fatty acids ranging from C_{13}-C_{18} [56]. Since, the position of the hydroxy group cannot be identified in methoxy derivatives, the origin of the hydroxy fatty acids can not be specified. However the elution of the C_{16} hydroxyhomologue just after the C_{18} FAmE suggests it could be the ω-16 hydroxy C_{16} FAmE as reported by del Río and Hatcher [51].

Lignites are residues from forest ecosystems with rivers and small lakes. The lignites in Germany are thought to be the residues of subtropical forests [19, 58, 59]. Depending on the conditions, wood and leaf remains accumulate while in lakes contributions by the aquatic flora can be expected as well. Bacterial contributions to the organic matter are the result of the preservation of bacterial remains. The high intensity distribution of the long-chain fatty acids in sample H_3 with an even over odd predominance suggests a lignite deposit formed by oxidative decay of subtropical forests rich in plant material with leaf waxes [19, 58, 59]. In general, a maximum in distribution is expected around C_{28} or C_{30} FAmEs [51, 53]. In this case, the maximum depends on the sample: C_{28} (FAmE) is observed as maximum in 6 samples, C_{26} (FAmE) in another 6 samples, C_{24} (FAmE) in one case and even C_{25} (FAmE) in one other case (Table 3). The notation in Table 3 are done as follows: "+" for present, "**h**" (bold) for higher intensity, "**m**" (bold) for the highest in intensity in the group, "-" for absence. The C_{24}, C_{26}, C_{28}, C_{30} FAmEs seem to be always the most abundant FAmEs in the distribution. A low intensity or the absence of these compounds like in sample F_6 and F_3 respectively, suggests a low input of higher land plant derived input material. Odd very long-chain fatty acids are unusual for plant derived waxes. However midchain-branched esters have been mentioned as present in less common classes of wax [60]. The presence and/or the predominance of odd long-chains is known for paraffins (note: these long-chains are n-alkanes with a maximum around C_{23}-C_{25}) [53]. In the case where the FAmE profile resembles a Gaussian distribution as for example in sample F_6, the distribution suggests a possible contribution of a series of oil-derived alkanes (which are often Gaussian distributed) that oxidised during or after deposition [53]. In most of the cases, the plant-derived even chain very long-chain fatty acids are predominant.

The difference in ratios between LcFA (long-chain fatty acids) and ScFA (short-chain fatty acids) and their supposedly different origin, from plant and microorganisms respectively, gives interesting information about the conditions of coalification in the source deposits from where the Kassel earth-like samples have been originally obtained. A high relative abundance in cuticular wax from higher land plants is seen in the Kassel earth samples classified in the positive PC1 such as H_1, F_4, F_5, F_7 and I_1 (LcFA>ScFa), H_2 and H_3 (LcFA≈ScFA). Samples classified as richer in fossil woody remains from the negative PC1 like F_2, I_2 and C_1 (LcFA>ScFa), F_1 and C_2 (LcFA≈ScFA) also show long chain plant derived fatty acids. It needs to be noted that DTMS in combination with PCA bases a classification on differences in relative abundance. Inspection of the DTMS data on sample richer in woody components indeed shows that montan waxes are sometimes present.

The Kassel earth samples F_6 and O from the negative PC1 space and the fossil wood G from the positive PC1 space are samples with a Gaussian distribution of the odd and even wax peaks with sometimes a predominance of the odd peaks and a LcFA<ScFa. The H_4 Kassel earth (in PC1-) and F_3 Vandyke brown sample (in PC1+) show a total lack of LcFA.

It appears that Kassel earth lignites are richer in montan waxes compared to the lignites used for Vandyke browns, but there are exceptions such as F_1, F_2, C_1 with in higher content in LcFA.

Table 3. Fatty acid methyl esters (fame) and dicarboxylic acid dimethylesters (da) in the m/z 74 partial mass chromatogram of the Py-TMAH-GC/MS measurements (Py $_{temperature}$= 353 °C) of the Kassel earth samples (see Fig. 7a, b).

*Classification of samples according to predominance of long-chain fatty acids (LcFA, i.e. >12 fame), respectively short-chain fatty acids (ScFA, i.e. <12 fame). Example of notation: "+" for present, "**h**" (bold) for higher intensity, "**m**" (bold) for the highest in intensity in the group, "-" for absence.*

Compound (lable)	**MW**	**RT** (min)	**Occurrence**																
			LcFA>ScFA								**LcFA≈ScFA**				**LcFA<ScFA**			**ScFA>0; LcFA= 0**	
			H_1	*F_2*	*F_4*	*F_5*	*F_7*	*I_1*	*I_2*	*C_1*	*H_2*	*H_3*	*F_1*	*C_2*	*F_6*	*O*	*G*	*H_4*	*F_3*
Short-chain FamE + da																			
pentanoic acid methyl ester (**5**)	116	6.35	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
hexanoic acid methyl ester (**6**)	130	9.63	**h**	**h**	**h**	**h**	**h**	**m**	**m**	+	**h**	**h**	**h**	**h**	**h**	**h**	+	**h**	+
heptanoic acid methyl ester (**7**)	144	13.33	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
octanoic acid methyl ester (**8**)	158	17.13	**m**	**m**	**m**	**m**	**m**	**h**	**h**	**m**	**m**	**m**	**m**	**m**	**m**	**m**	**m**	**m**	**m**
nonanoic acid methyl ester (**9**)	172	20.76	**h**	+	+	+	+	**h**	+	+	+	**h**	+	+	+	+	**h**	+	**h**
*hexanoic dicarboxylic acid dimethyl ester (**6 da**)*	*174*	*21.69*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
caproic (decanoic) acid methyl ester (**10**)	186	24.25	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
*heptanoic dicarboxylic acid dimethyl ester (**7 da**)*	*188*	*25.14*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
undecanoic acid methyl ester (**11**)	200	27.53	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
*octanoic dicarboxylic acid dimethyl ester (**8 da**)*	*202*	*28.43*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
lauric (dodecanoic) acid methyl ester (**12**)	214	30.65	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
*nonanoic dicarboxylic acid dimethyl ester (**9 da**)*	*216*	*31.54*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Long-chain FAmE + ω FAmE																			
tridecanoic acid methyl ester (**13**)	228	33.61	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
*decanoic dicarboxylic acid dimethyl ester (**10 da**)*	*230*	*34.46*	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-
myristic (tetradecanoic) acid methyl ester (**14**)	242	36.44	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ω-hydroxydodecanoic acid methyl ester (**ωOH12**)/ *undecanoic dicarboxylic acid dimethyl ester (**11 da**)*	244	37.26	+	+?	+	+	+	+	+	+	+	+	-	+	+	+	-	+	+
iso pentadecanoic acid methyl ester (**i-15**)	256	38.12	+	+	-	+	+	+	+	-	+	+	+	+	+	+	-	+	+
pentadecanoic acid methyl ester (**15**)	256	39.11	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Table 3 continued.

Compound (**lable**)	**MW**	**RT** (min)	**Occurrence**																	
			LcFA>ScFA								**LcFA≈ScFA**				**LcFA<ScFA**			**ScFA>0; LcFA= 0**	
			H_1	F_2	F_4	F_5	F_7	I_1	I_2	C_1	H_2	H_3	F_1	C_2	F_6	O	G	H_4	F_3
hexadecenoic acid methyl ester (**16: 1**)	268	41.27	+	+	+	+	-	+	+	-	-	-	+	+	+	+	+	+	+
palmitic (hexadecanoic) acid methyl ester (**16**)	270	41.70	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ω-hydroxytetradecanoic acid methyl ester (**ωOH14**)	272	42.48	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-
margaric (heptadecanoic) acid methyl ester (**17**)	284	44.17	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
octadecenoic acid methyl ester (**18: 1**)	296	45.85	+	-	-	+	-	+	-	-	-	-	+	+	+	+	+	+	+
stearic (octadecanoic) acid methyl ester (**18**)	298	46.48	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ω-hydroxyhexadecanoic acid methyl ester (**ωOH16**)	300	47.20	**h**	**h**	**h**	**h**	**h**	**h**	**h**	**h**	**h**	**h**	+	**h**	+	-	-	-	-
nonadecanoic acid methyl ester (**19**)	312	48.73	-	+	+	+	+	+	+	+	-	+	+	+	+	+	-	-	-
arachidic (eicosanoic) acid methyl ester (**20**)	326	50.88	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-
ω-hydroxyoctadecanoic acid methyl ester (**ωOH18**)	328	51.56	-	+	+	+	+	-	-	+	+	+	-	+	+	+	-	-	-
methyl eicosanoic acid methyl ester (**Me 20**)	340	52.97	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-
docosanoic acid methyl ester (**22**)	354	54.98	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-
methyl docosanoic acid methyl ester (**Me 22**)	368	56.98	+	+	+	+	+	+	+	+	+	+	+	+	**h**	+	+	-	-
tetracosanoic acid methyl ester (**24**)	382	58.76	**m**	+	+	**h**	**h**	**h**	**h**	**h**	+	**h**	+	**h**	+	+	+	-	-
methyl tetracosanoic acid methyl ester (**Me 24**)	396	60.52	+	+	+	+	+	+	+	+	+	+	+	+	**m**	**h**	+	-	-
hexacosanoic acid methyl ester (**26**)	410	62.26	**h**	**h**	**h**	**h**	**m**	**m**	**m**	**h**	**h**	**m**	**h**	**m**	**h**	**m**	**h**	-	-
methyl hexacosanoic acid methyl ester (**Me 26**)	424	63.91	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-
octacosanoic acid methyl ester (**28**)	438	65.55	+	**m**	**m**	**m**	**h**	**h**	**h**	**m**	**m**	**h**	**m**	**h**	+	**h**	**m**	-	-
methyl octacosanoic acid methyl ester (**Me 28**)	452	67.11	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-
triacontanoic acid methyl ester (**30**)	466	68.66	+	**h**	**h**	**h**	+	+	+	**h**	+	+	**h**	+	+	+	**h**	-	-
methyl triacontanoic acid methyl ester (**Me 30**)	480	70.00	-	+	+	+	-	-	-	+	+	-	+	-	-	-	+	-	-
dotriacontanoic acid methyl ester (**32**)	494	71.25	-	+	+	+	-	-	-	+	-	-	+	-	-	-	+	-	-
methyl dotriacontanoic acid methyl ester (**Me 32**)	508	72.85	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
tetratriacontanoic acid methyl ester (**34**)	522	74.70	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-

b) Lignin derived compounds in Py-TMAH-GC/MS data

TMAH analytical work has been used for analysis of lignin derived compounds for example in the group of del Río, Hatcher or Nierop [41, 51, 56, 61-64]. Lignins and fossil woody tissues were analysed earlier by the more classical Py-GC/MS (see for example Stout et al., Smeerdijk and Boon, Nip et al., Tromp et al., Demirbaş, Dijkstra et al.) [19, 23, 36-38, 65, 66]. However, such an approach would not give enough details on the more polar compounds such as functionalised aromatic acids. The analytical solution has been to transesterify the sample before performing GC/MS analysis, by pyrolysis or on-column TMAH-GC/MS analysis. In some studies the Py-TMAH-GC/MS results have been compared with the results obtained from Py-GC/MS work [56, 63, 67]. The Py-TMAH-GC/MS experiments of Martín et. al on soil humic acids [41] are such an example. After reaction with TMAH, the samples were pyrolysed for 10 s at 500°C and GC analysed from 40 to 300 °C. The aromatic compounds consists of methoxy derivatives[4] of benzoic acid methyl ester and benzene propenoic acid methyl ester and benzene. Martín et. al discuss the presence of these compounds in terms of p-coumaryl, guaiacyl and syringyl units and their provenance from grass, gymnosperms, respectively angiosperms [41]. The analytical TMAH approach makes the transmethylation an ideal solution for killing two birds with one stone: i.e. analysing long chain aliphatic fatty acids and alcohols from montan waxes as shown earlier in this chapter, and the lignin derived compounds in the same analytical run.

The aromatic compounds observed after Py-TMAH-GC/MS of the Kassel earth-like samples are shown as the m/z 77 partial mass chromatograms in Fig. 8a for samples C_1 (PC1+) and H_3 (PC1-). The m/z 77 profile was chosen to pull out some aromatic features highly associated with the lignin precursor of the lignites in the analysed samples, [41, 61]. However it has to be specified that not all characteristic compounds can be extracted from the data in this way. The other aromatic compounds identified in the Kassel earth-like samples are shown in Fig. 2 and Fig.3 and explained in Table 2. The TICs of the two examples are shown in Fig. 8b, for a better insight in the abundance of the compounds of interest in the sample.

The m/z 77 survey points towards samples rich in aromatic compounds as C_1 observed on the positive side of the first principal component and other ones with less aromatic compounds such as H_3, a representative sample from the fossil leaf rich material shown on the negative side of the same first principal component (Fig. 1). The TMAH reagent will methylate aromatic acid and alcoholic groups, except the ones possibly resulting from the cleavage of the β-O-4 bonds [61, 63]. The low temperature Curie point wire (353 °C) used to evaporate the methylated compounds is not expected to decompose the lignin network polymer. It is unknown to what extent lignin remnants substantially weakened by diagenesis can contribute to the compound profile. The aromatic compounds characterised by m/z 77 with a relative abundance higher than 6 % in the partial mass chromatogram (PMC) have been identified using libraries of mass spectra [68, 69] (see Table 4) and are confirmed by comparative

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[4] 4-methoxybenzenecarboxylic acid methyl ester (1), 1,3,4-trimethoxybenzene (2), benzenedicarboxylic acid dimethyl ester (3), 3,4-dimethoxybenzenecarboxylic acid methyl ester and its methyl derivative (4, 5), 4-methoxy- and 3,4 dimethoxybenzenepropenoic acid methyl ester (6, 9), 3,4,5-trimethoxybenzenecarboxylic acid methyl ester (7). The notation from the original paper is given as 1, 2, etc., between the brackets.

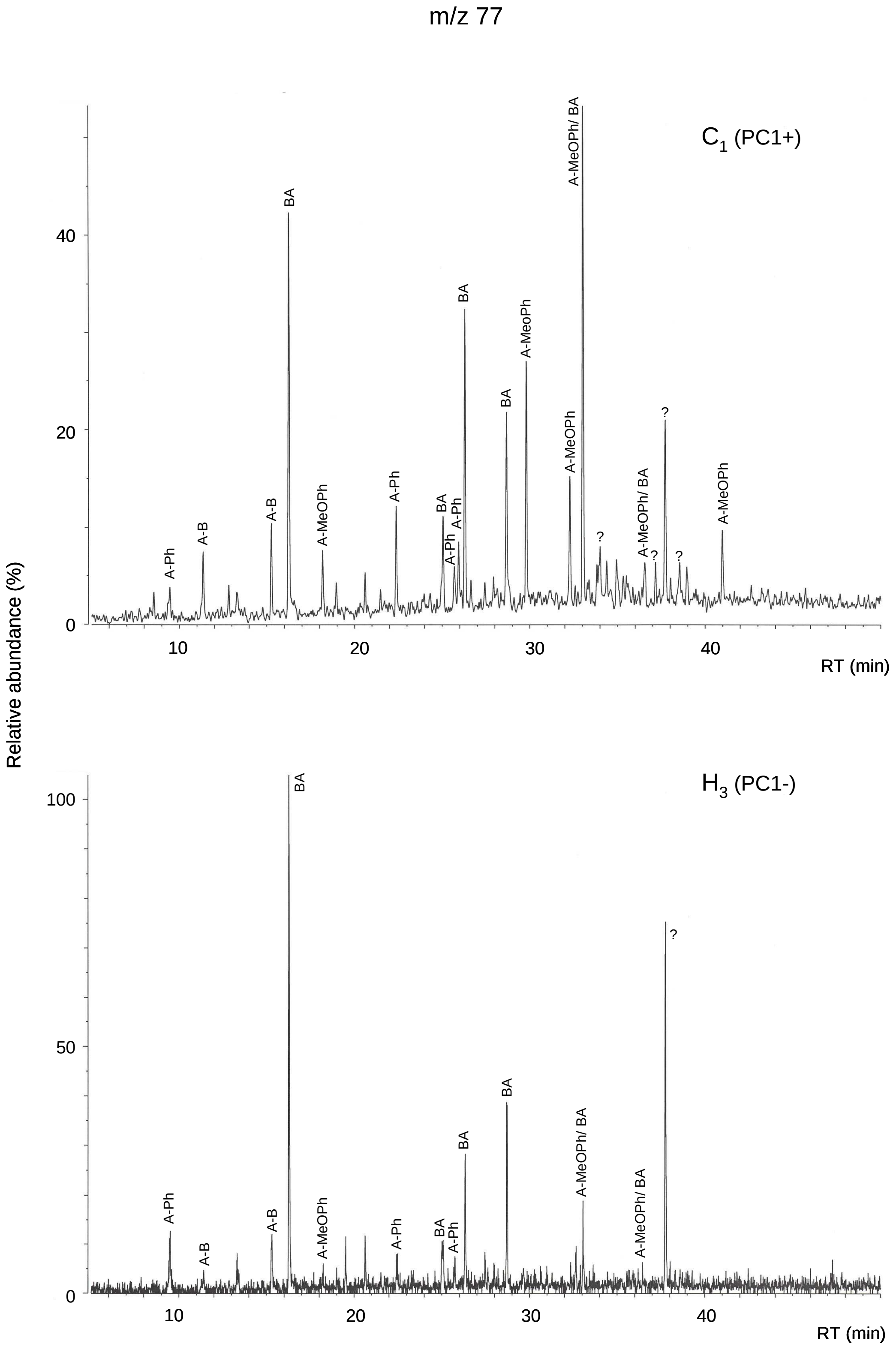


Fig. 8a. Partial mass chromatogram of m/z 77 from Py-TMAH-GC/MS data of C_1, a Kassel earth-like sample with a strong signature of aromatic compounds and H_3, a Kassel earth-like sample with a weaker signature of aromatic compounds. Example of notation: A-Ph for alkylphenols, A-B for alkylbenzenes, BA for benzoic acid methyl esters, A-MeOPh for alkylmethoxyphenols.

Table 4. Aromatic compounds indicative for wood (W) or leaf (L) derived polyphenolic materials in Kassel earth and Van Dyck brown samples traced by m/z 77 partial mass chromatogram in the Py-TMAH-GC/MS data (Py $_{temperature}$= 353 °C).

Where "A-B" are alkylbenzenes, "A-Ph" are alkylphenols, "A-MeOPh" are alkylmethoxyphenols and "BA" are benzoic acids. "M" are molecular ions and "BP" base peaks in the mass spectra. In bold are shown compounds with very high intensity in most of the measurements. Example of notation: if present "+" (relative abundance higher than 50 %) or " (+)" (relative abundance lower than 50 %) and "-" if absent. Tentatively identified compounds are marked with "T".

Identification of compounds [67-69]	**Presumed precursor**	**MW**	**RT (min)**	**Leaf derived polyphenolics**										**Wood derived polyphenolics**						
				H1	**H_2**	**H_3**	**H_4**	**F_4**	**F_5**	**F_6**	**F_7**	**I_1**	**O**	**F_1**	F_2	F_3	C_1	C_2	G	I_2
anisole (methoxybenzene)	phenol (A-Ph)	108	9.33	-	(+)	(+)	-	-	(+)	(+)	(+?)	(+)	-	(+)	-	-	(+)	(+)	-	-
C_2- alkylbenzene	unknown (A-B)	106	11.36	-	-	-	-	-	-	-	(+)	(+)	-	(+)	-	-	-	(+)	(+?)	-
C_3- alkylbenzene	unknown (A-B)	120	15.26	(+)	-	(+)	-	-	-	-	(+)	(+)	-	-	-	-	(+)	(+)	(+?)	-
benzoic acid methyl ester	benzoic acid (BA)	136	16.26	(+)	+	+	(+)	+	+	(+)	+	+	(+)	+	(+)	(+)	(+)	+	**+**	**(+)**
methoxymethylphenol [63]	methoxyphenol (A-MeOPh)	138	18.16	-	-	-	-	-	-	-	-	-	-	-	-	-	(+)	(+)	(+)	-
p methoxybenzaldehyde [62,63]	hydroxy-benzaldehyde (A-Ph)	136	22.38	(+)	-	(+)	-	-	-	-	(+)	-	-	-	-	-	(+)	(+)	(+)	-
p methoxybenzoic acid methyl ester [63, 64]	p hydroxybenzoic acid (BA)	166	25.00	(+)	(+)	(+)	-	(+)	+	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
methyl methoxybenzene ketone (methoxyacetophenone)	methyl phenol ketone (A-Ph)	150	25.69	(+)	-	-	-	-	-	-	(+)	(+)	-	-	-	-	-	(+)	-	-
C_4- alkyl methoxybenzene	C_4- alkylphenol (A-Ph)	164	25.92	-	-	-	-	(+)	-	-	-	-	-	-	-	-	(+)	(+)	-	-
methoxy benzoic acid methyl ester [41]	hydroxy benzoic acid (BA)	166	26.29	(+)	(+)	(+)	-	-	+	(+)	(+)	(+)	(+)	(+)	(+)	-	(+)	+	+	(+)
dicarboxylic acid dimethylester benzene (1, 2 dicarboxylic acid dimethylester benzene), [41, 63, 64]	benzene dicarboxylic acid (BA)	194	28.66	(+)	+	(+)	(+)	+	+	(+)	(+)	(+)	(+)	+	(+)	(+)	(+)	+	+	+
3, 4 dimethoxy-1 benzaldehyde [56, 61-63]	p phenol- 3 methoxy- 1 benzaldehyde (A-MeOPh)	166	29.75	(+)	-	-	-	-	-	-	(+)	-	-	-	-	-	(+)	(+)	+	-

Table 4 continued.

Identification of compounds [67-69]	**Presumed precursor**	**MW**	**RT (min)**	**Leaf derived polyphenolics**										**Wood derived polyphenolics**						
				H1	**H_2**	**H_3**	**H_4**	**F_4**	**F_5**	**F_6**	**F_7**	**I_1**	**O**	**F_1**	F_2	F_3	C_1	C_2	G	I_2
C_3-2-methoxy phenol- T 180(M), 165(BP), 137, 122, 109, 77	idem (A-MeOPh)	180	32.28	(H_1)							(F_7)			-	-	-	(+)	(+)	(+)	-
***vanillic acid methyl ester* (1, 2 dimethoxy- carboxylic acid methyl ester) or 2, 4 dimethoxy-carboxylic acid methyl ester** [41, 56, 61, 63]	vanillic acid/methoxy hydroxy benzoic acid (A-MeOPh/ BA)	196	33.02	+	(+)	(+)	-	(+)	(+)	(+)	-	(+)	-	(+)	(+)	-	+	+	+	(+)
194(M, BP), 179, 165, 151, 121, 91	unknown	194	34.36	-	-	-	-	-	-	-	-	-	-	-	-	-	(+)	-	-	-
1, 2, 3 trimethoxy 4 benzoic acid methyl ester (1, 2, 3 trimethoxy phenyl formic acid methyl ester) [41, 56]	idem/ dimethoxy, hydroxybenzoic acid (A-MeOPh/ BA)	226	36.54	(+)	-	-	-	-	-	(+)	-	-	-	-	-	-	(+)	-	(+)	-
224(M), 193(BP), 165, 150, 91, 77	unknown	224	37.31	-	-	-	-	-	-	-	-	-	-	-	-	-	(+)	-	-	-
methoxybisphenyl-**T** **184(M, BP), 141, 77**	hydroxybisphenyl-T	184	37.70	(+)	+	+	(+)	(+)	+	+	(+)	(+)	(+)	+	(+)	(+)	(+)	+	(+)	(+)
238(M?), 224, 209(BP), 178, 77	unknown	238?	38.52	-	-	-	-	-	-	-	-	-	-	-	-	-	(+)	-	(+)	-
1 (3, 4 dimethoxy) phenyl-propenoic acid methyl ester or veratry propenoic acid methyl ester [41, 56, 61, 63]	1 (3 methoxy, 4 hydroxy) phenyl-propenoic acid (A-MeOPh)	222	40.99	-	-	(+)	-	(+)	(+)	-	-	-	-	-	(+)	-	(+)	-	-	-

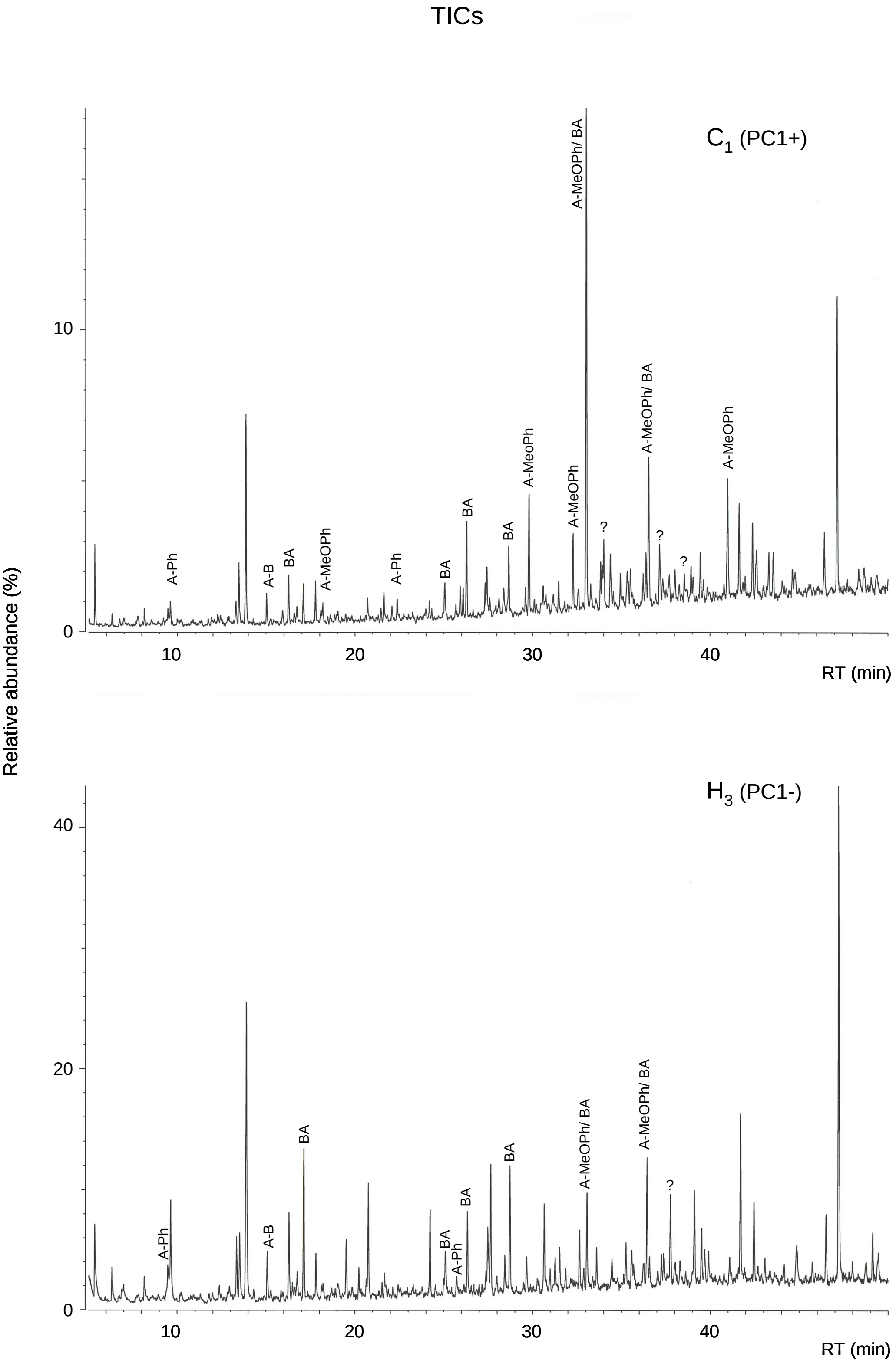
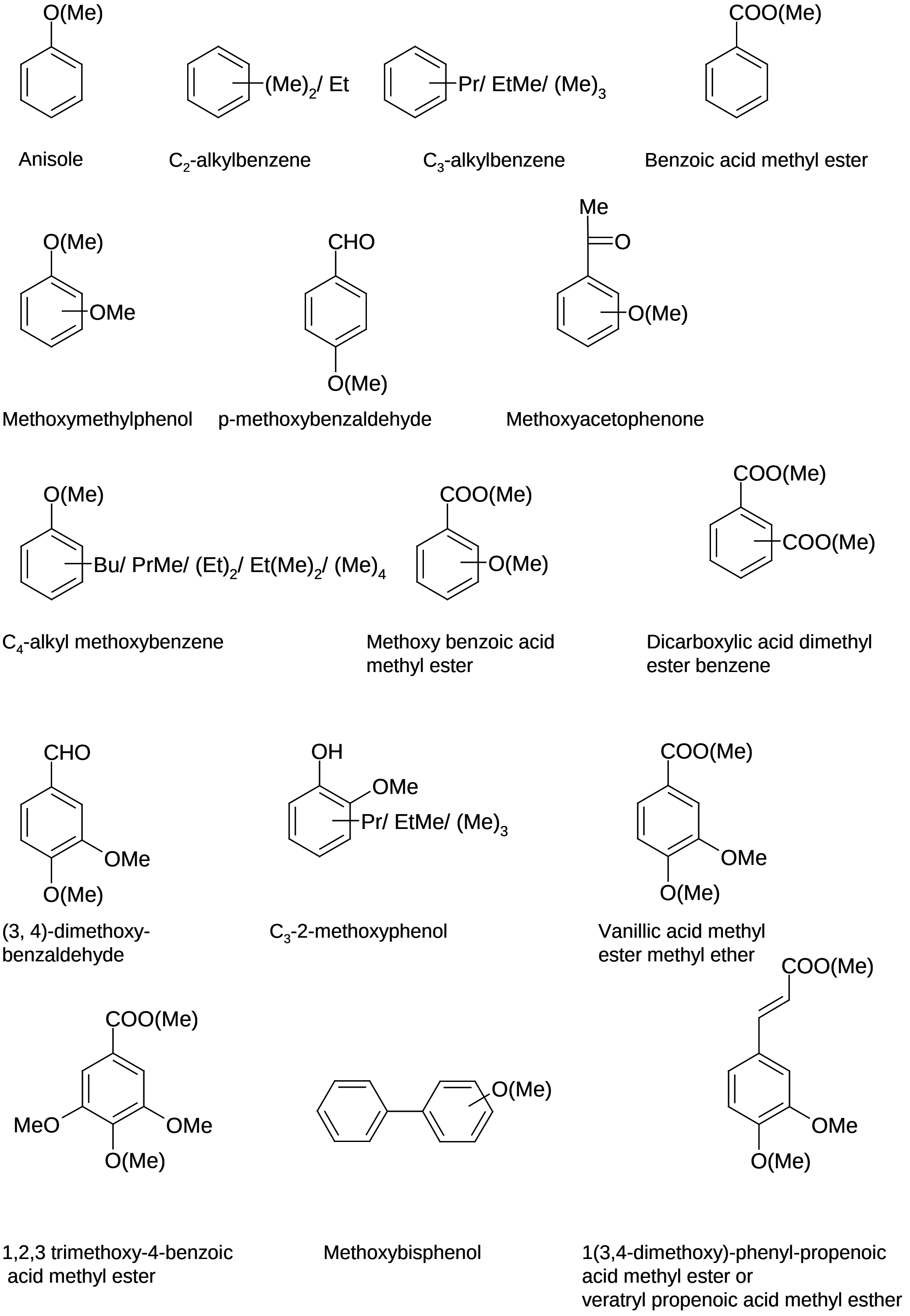


Fig. 8b. The Py-TMAH-GC/MS TICs of the samples C_1 and H_3. Example of notation: A-Ph for alkylphenols, A-B for alkylbenzenes, BA for benzoic acid methyl esters, A-MeOPh for alkylmethoxyphenols (idem Fig. 8a).

information available in the literature [41, 56, 61-64, 67]. Alkylated benzenes, benzoic and dicarboxylic acids methyl esters, methoxy, di- and tri methoxy derivatives of benzaldehyde, acetophenone, benzoic acid methyl ester and benzene propenoic acid methyl ester, benzene (methoxyphenols) and bisphenol are identified in the analysed samples. The unknowns are given as the m/z values of the peaks



Anisole

C_2-alkylbenzene

C_3-alkylbenzene

Benzoic acid methyl ester

Methoxymethylphenol

p-methoxybenzaldehyde

Methoxyacetophenone

C_4-alkyl methoxybenzene

Methoxy benzoic acid methyl ester

Dicarboxylic acid dimethyl ester benzene

(3, 4)-dimethoxy-benzaldehyde

C_3-2-methoxyphenol

Vanillic acid methyl ester methyl ether

1,2,3 trimethoxy-4-benzoic acid methyl ester

Methoxybisphenol

1(3,4-dimethoxy)-phenyl-propenoic acid methyl ester or veratryl propenoic acid methyl esther

Scheme 1. Chemical structures of aromatic compounds characterised by the presence of their m/z 77 peak, identified in the Kassel earth-like samples.

observed in the mass spectrum (Table 4). In Table 4 the following coding system was used: “A-B” for alkylbenzenes, “A-Ph” for alkylphenols, “A-MeOPh” for alkylmethoxyphenols, “BA” for benzoic acids, “M” for molecular ions and “BP” for base peaks in the mass spectra. Depending on the relative abundance in the PMC the occurrence of the compounds in the whole range of samples is given as for example C_1 if the relative abundance of the compounds is higher than 50 % and between brackets, (C_1), if the relative abundance is lower than 50 %. The structures of the identified compounds are shown in Scheme 1. The higher abundance of derivatives of benzoic acid (the compounds with the highest intensity in bold in Table 4) apparently in all the samples points to differences in the degree of oxidation of the input material in the deposit and possibly to early stages of diagenesis in the development to lignite [61]. The rather high amount of vanillic acid (a degradation product of coniferyl alcohol with vanillin as intermediate in lignin [70]) in the samples is directly related to the input material as this compound is marker for fungal activity in soils [56, 66]. The occurrence of the aromatic compounds in the Kassel earth-like samples is shown in the extreme right column of Table 4. From the Py-TMAH-GC/MS results no big difference regarding the abundance in aromatic compounds is observed between the samples classified on the positive side of PC1 and the samples from the negative side of PC1. This is interpreted as indicating that some deposits were richer in leaf containing material than other deposits. The hypothesis of a subtropical forest as the starting ecosystem for the lignite deposits at least in Germany (the exact deposit source for the samples here discussed is not known) is supported by the low amount of the syringyl units (trimethoxy aromatic compounds). These syringyl compounds are characteristic for angiosperms and were found in the samples currently under discussion, in conditions of low pyrolysis temperatures analysis [41, 56]. Work by Stout and Boon on identified woody tissues from peat-forming sediments and lignites has shown that syringyl and coniferyl moieties in lignins are prone to demethoxylation thus diminishing the relative amount and ratio of the methoxyalkylaromatic compounds [23].

Plant terpenoids and bacterial hopanoids.

The same classification as used by Dehmer [19] was used in this section to discuss the presence of terpenoids observed as diterpanes, triterpanes, aromatic- and polyaromatic triterpenes, other triterpenes and hopanoids. Partial mass chromatograms of m/z 191 and m/z 205 in the Py-GC/MS data are used to search for triterpenoids. An example is shown in Fig. 9 for sample H_1, in which m/z 191 is used to trace hopanoids and m/z 205 is used to trace 4-methyl-hopanoids. In the process it was clear that many other plant derived triterpenoid compounds were also traced in this way. Table 5 identifies the compounds, lists the retention time, traces the potential origin of the compounds and lists the occurrence in the mass chromatograms of the samples analysed. The compounds with the higher abundance are given in bold. Compounds marked with “T” are tentatively identified. “**m**” (Bold) stays for compounds with the highest relative abundance in the sample, “+”for presence and “-“ for absence. The mass spectrum of the identified compounds was compared with published mass spectra [19, 72-74]. When a peak could not be identified, the most prominent masses were listed. In some cases more than one potential molecular mass was observed pointing to unresolved compounds. These peaks are marked by an M with index in brackets. The base peak is marked as (BP). The origin of the compounds is either bacterial (B) in the case of hopanoids triterpanes or higher plant (P) [40]. It is observed (Table 5) that either compounds of plant origin or with a bacterial origin are the most characteristic in the samples.

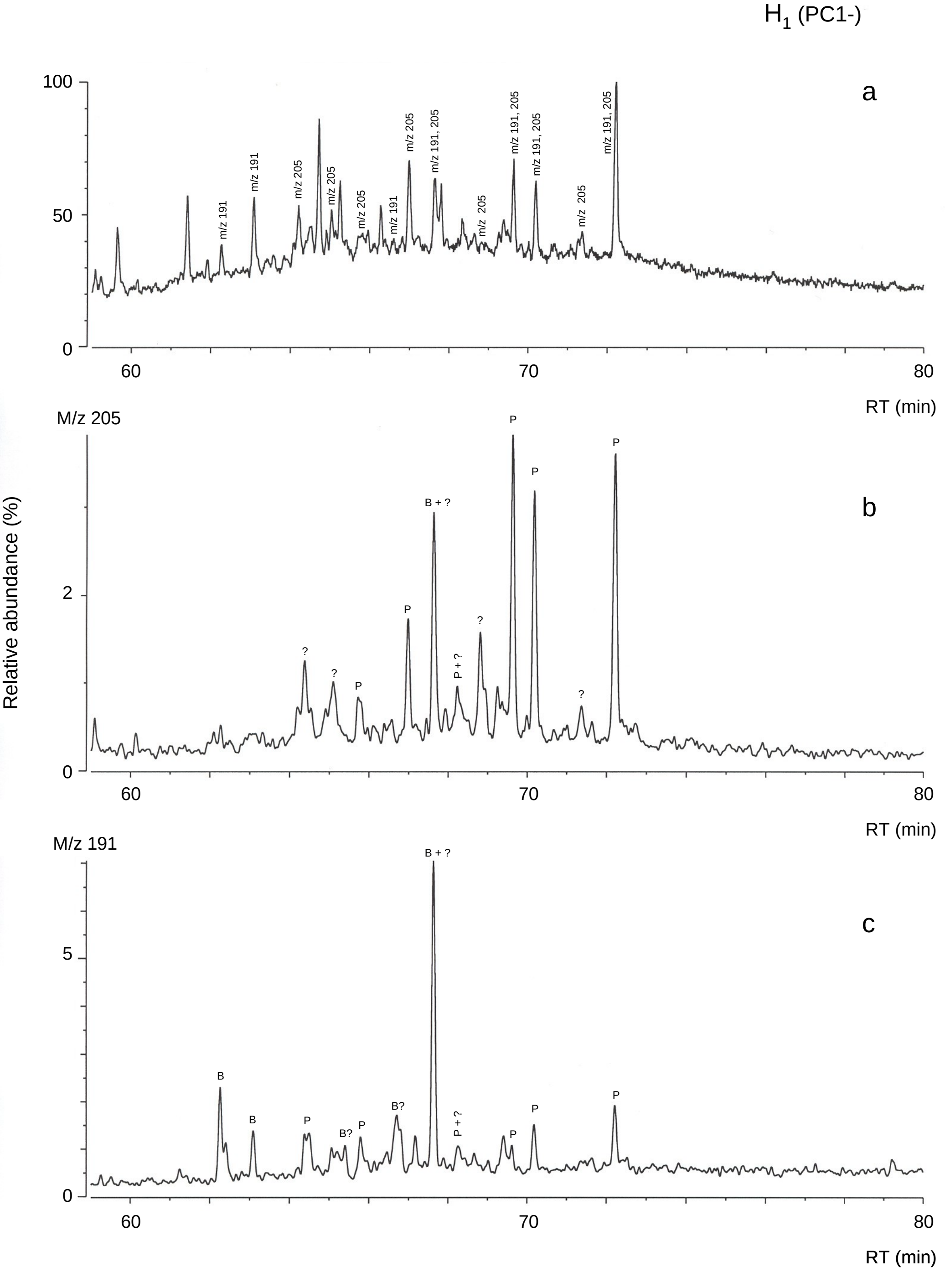


Fig. 9. Detail of the Py-TMAH-GC/MS TIC (a) of the H_1- Kassel earth sample in the elution range of bacteria (B) and plant derived (P) triterpenoid compounds: (b), the partial mass chromatogram of m/z 205 and the partial mass chromatogram of m/z 191 (c).

Such examples are the fridelin and olean/urs-12-ene plant terpenoids as the highest peak in H_1, H_2, F_4, F_5, F_7, I_1 and O samples (all PC1-) but also in F_1, F_3, C_2 (PC1+) and 17α(H), 21β(H)-homohopane in H_3 and F_6 (PC1-), F_2, C_1 and G (PC1+).

Table 5. Terpenoids from plant (P) or bacterial (B) origin characterised by m/z 191 and m/z 205 in the Kassel earth samples [19, 71-74].

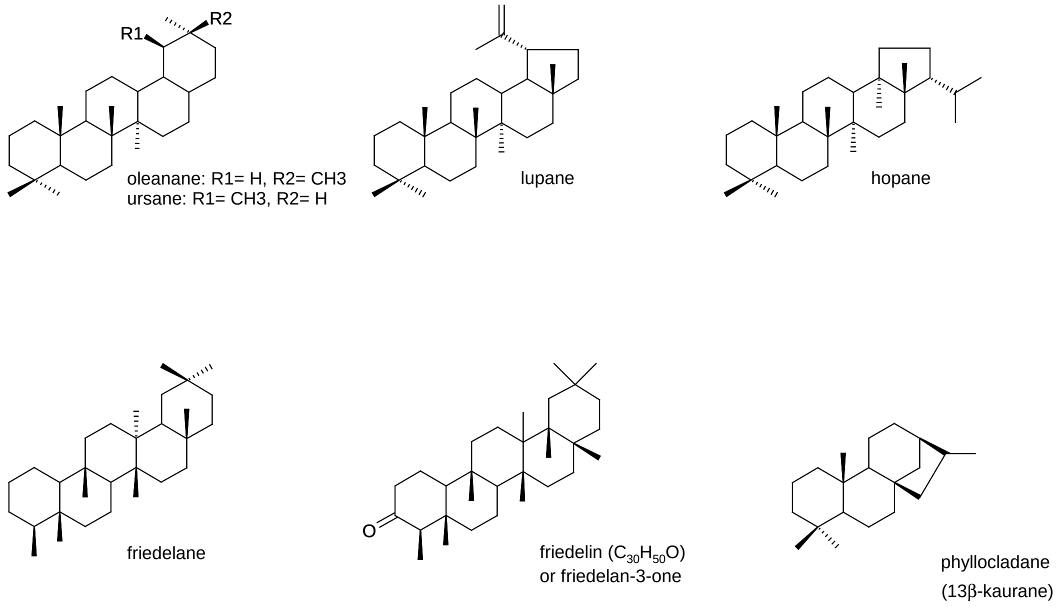
*The compounds with the higher abundance are given in bold. Compounds marked with "T" are tentatively identified. "**m**" (Bold) stays for compounds with the highest relative abundance in the sample, "+"for presence and "-" for absence. "M" stays for molecular ions and "BP" for the base peak in the mass spectra.*

Compound	Origin	RT (min)	Occurrence																
			PC1-										PC1+						
			H_1	H_2	H_3	H_4	F_4	F_5	F_6	F_7	I_1	O	F_1	F_2	F_3	I_2	C_1	C_2	G
22,29, 30-trisnorhop—17(21)-ene	B	62.29	+	+	+	-	+	?	+	+	+	+	+	+	+	-	+	+	+
17α(H)-22, 29, 30 trisnorhopane	B	63.06	+	+	+	-	+	?	+	+	+	+	+	+	+	-	+	?	+
218(BP), 205, 191, 203, 189, 410(M_1), 408(M_2)- T, 424(M_3)- T	unknown	64.36	+	+	+	-	?	+	+	+	+	**m**	+	+	**m**	-	+	+	+
olean/urs-12-ene	P	64.45	+	-	?	-	+	+	+	+	+	**m**	+	+	**m**	-	-	-	+
145(BP), 376(M_1), 361, 158, 189, 410(M_2), 191, 416(M_3)	unknown	65.09	+	-	+	-	-	-	-	-	+	-	?	+	-	-	-	+	+
191(BP), 189**?**, 177, 408(M_1)?, 360, 398(M_2)	(B?)	65.39	+	?	+	-	+	?	+	+	+	+	+	+	+	-	-	-	+
95(BP), 141, 109, 189, 191, 410(M_1), 299, 408(M_2), 205, 203, 218, 424(M_3), 274, 394(M_4), 259	P	65.80	+	?	+	-	+	?	?	+	+	+	-	+	+	-	-	**m**	?
191(BP), 69, 95, 175, 189, 177, 424(M_1), 396(M_2), 398(M_3)- T	(B?)	66.75	+	+	+	-	+	?	+	+	+	?	+	+	+	-	-	+	+
tetramethyloctahydropicene [19]	P	66.97	+	?	-	-	-	-	+	+	+	-	+	-	?	-	+	-	-
1,2,3,4,4a,5,6,14b- octahydro-2,2,4a,9-tetramethylpicene-T/lupane- T [72]	P	67.19	+	-	-	-	+	-	-	-	+	-	+	-	+	-	-	-	+
17α(H), 21β(H)-homohopane	B	67.65	+	+	**m**	-	+	+	**m**	+	+	+	+	**m**	+	-	**m**	+	**m**
195(BP), 374(M), 163	unknown	67.65	+	+	+	-	+	+	+	+	+	+	+	+	+	-	+	+	+
55(BP), 95, 69, 191, 143, 109, 205, 189, 424(M_1), 392(M_2), 450(M_3- C_{32} methyl ketone), 388(M_4), 396(M_5)	unknown	68.28	+	-	+	-	+	?	+	+	+	-	+	+	+	-	-	-	+
143(BP), 125, 205, no 399, no 458 (M) ocotillone (20,24-Epoxy-25-hydroxy-dammaran-3-one)?	P	68.28	+	-	+	-	+	?	+	+	+	-	+	+	+	-	-	-	+
205/95(BP), 424 (M_1), 218, 109, 135, 189, 191, 143, 177,409, 396 (M_2)- T	unknown	68.86	+	-	-	-	+	-	+	+	+	-	+	+	+	-	-	-	+
231(BP), 414(M_1), 191, 95, 109, 163, 205, 412 (M_2), 218	unknown	69.38	+	?	+	-	+	-	+	+	+	+	+	+	+	-	-	-	-

Table 5 continued.

Compound	**Origin**	**RT (min)**	**Occurrence**																
			PC1-										**PC1+**						
			H_1	H_2	H_3	H_4	F_4	F_5	F_6	F_7	I_1	O	F_1	F_2	F_3	I_2	C_1	C_2	G
424(BP, M), 205, 109, 95, 189, 218, 191, 409, 232, 245, 368(M_2)- T methylolean-13(18)-ene- T [73]	P	69.61	+	-	-	-	+	+	-	+	+	-	+	+	-	-	-	-	+
205(BP), 95, 191, 424(M), 245, 218, 410(M_2), 396(M_3) methylolean-13(18)-ene- T	P	70.19	+	+	?	-	+	+	+	+	+	+	+	+	+	-	-	-	?
274(BP,M), 259 trymethyltetrahydrochrysene/ dihydroimuene [72]	P/P	70.19	+	+	?	-	+	+	+	+	+	+	+	+	+	-	-	-	?
191, 426(M_1), 205, 218, 478(M_2)- T, 424(M_3)-T, 396(M_4) -T	unknown	71.33	+	-	+	-	+	+	-	+	+	-	+	+	-	-	-	-	-
friedelin [71] 426(BP,M), 125, 95, 123, 69, 109, 205, 273, 302, 218, 246, 179, 411	P	72.22	**m**	**m**	-	-	**m**	m	-	**m**	**m**	-	m	+	-	-	-	+	-

Other plant terpenoids tentatively identified as 1,2,3,4,4a,5,6,14b-octahydro-2,2,4a,9-tetramethyl-picene vs. lupane and ocotillone, and other hopanoid compounds like 22,29,30-trisnorhop-17(21)-ene, 17α(H)-22, 29, 30 trisnorhopane are evenly distributed on the positive an negative side of PC1. It seems that plant terpenoids are stronger in the samples from the negative space of PC1 (material said already to be richer in fossil leaves), while hopanoids are more abundant in the samples plotted in the positive space of PC1 (material richer in fossil wood remains). Phyllocladanes are present as well in samples on the negative side of PC1 (H_1, F_4, F_6, F_7, O) and in one sample from the positive side (F_3) [19, 32, 40, 75]. Pristene (MW 266) was identified in all the samples except H_4. Other terpenoids present in the samples have been specified in Table 2 and were discussed when the DTMS results were reported above.



Scheme 2. Chemical structures of terpenoid-type (oleanane, ursane, lupane, hopane, friedelane, friedelin, phyllocladane) of biomarkers present in Kassel earth and Vandyke brown samples.

Plant terpenoids and bacterial hopanoids are important biomarkers in the analysis of subbituminous coal. Kumari found in coals of lower rank than lignites (e.g. peats) triterpenoids such as taraxerene, hop-12-ene, hop-22(29)-ene, neo-hop-12-ene, taraxerone (friedoolean-14-en-3-one) and taraxerol (friedoolean-14-en-3β-ol) [76]. Compounds with a friedoleanan structures were also reported in rootlets and peat by Smeerdijk and Boon [36]. Lignites and fossil woods from the late Miocene in the Hausruck district (Alpine Foreland Basin, Austria) showed presence of the saturated and monounsaturated sesquiterpenoids: cadalane and drimane; the diterpenoids: pimarane, abietane and phyllocladane (alfa-phyllocladane and pimarane being the main ones) and the triterpenoids: tetra- and pentacyclic oleanane, ursane, and lupane compounds as well as mono- tri- and tetraaromatic pentacyclic terpenoids[5] [40, 77]. The diversity of land plants terpenoids indicated both angiosperms and

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[5] saturated hydrocarbons : des-A-oleananes, des-A-urs-12-ene, des-A-lupan, olean-12-ene, olen-13(180-ene, and urs-12-ene; aromatic hydrocarbons: trimethyl-tetrahydro-chrysenes, tetramethyl-octahydro-chrysenes, dinoroleanatetraene, dinorursaatetraene, dinorolupatriene [40]

conifers (gymnosperms) to be involved in formation of these deposits [40]. Phyllocladane-type of diterpenoids are common constituents of conifer resins, while the structures with a oleanane, ursane or lupane skeleton (Scheme 2) are known as biomarkers for angiosperms [40 (and its bibliography)]. Triterpenoids with ursane, oleanane and hopane skeletons were identified also in soil samples by Martín et al. [41] and in deep-sea Miocene sediments [78]. Another type of terpenoids are phenolic terpenoids reported by Simoneit et al.[79] in Eocene fossil bark remnants from Geiseltal lignites (Germany), as mono- and triaromatic hydroxyterpenoids of the oleanane, ursane and lupane type of compounds. However such compounds were not found in the samples under consideration here. The pristene isoprenoid is a pyrolysis product of tocopherols (vitamin E) or chromanes [80] and can be considered as an indicator of plant matter.

Regarding the range of hopanoids, Bechtel et al. found in the analysed samples of lignite and fossil wood consistently the following types: ββ- and αβ-hopanes from C_{27} to C_{31} (the C_{28} hopanes were absent). The main identified hopanoids were the αβ-C_{31} hopane (22R) and the hop-17(21)-ene. Other hopanoids were the C_{27} and C_{28} hop-17(21)-enes. The composition in hopanoids is said to be comparable with those obtained by Dehmer for Miocene lignites in Germany. The occurrence of 17α, 21β(H)-homohopane is favoured by acidic conditions in the soil [40 (and its bibliography)]. The hopanes in the coal are derived from aerobic and anaerobic bacteria, fungi and kryptogames [40 (and its bibliography)].

Discussion

This chapter elucidates the organic chemical composition of Kassel earth and Vandyke brown pigments. It is focussed on molecular level aspects that can be determined with mass spectrometric techniques. Mass spectrometry was used for the classification of a number of samples available for analysis and also for determination of biomarker compounds and other molecular material characteristics. This focus could be chosen because Feller and Johnston- Feller have determined many of the physical and some of the chemical properties of the organic brown pigments with optical microscopy, wet chemical methods, XRD, IR spectroscopy, identification of metals, neutron activation analysis and Mössbauer spectroscopy. Samples from the Cologne area (Lower Rhine area) and Kassel area (North of Hessen) studied by Dr. Feller were made available for this study to make a direct comparison possible with samples from other sources. The samples from the Hafkenscheid collection were important to make the comparison with material available in the early 19th century. The problem in general is that Kassel earth and the Vandyke brown are mixtures of materials, which makes it virtually impossible to define a specific or unique composition. The results described illustrate this diversity in composition and certainly diversity on the molecular level. This study also bridges the world of conservation of art with the geochemistry of coal.

European Kassel earth and Vandyke browns were mined from areas with mainly Miocene and possibly Eocene lignite deposits in Germany. A flora with a mixture of gymnosperms (softwoods) and angiosperms (hardwoods) in the subtropical climatic conditions of the Miocene in Germany contributed organic material that was preserved in relatively small terrestrial depositional environments as extensively discussed by Stach et al., Pietz, Teichmüller, Knobloch, Kvaček et. al and reviewed by Dehmer [19, 44, 58, 59, 81-83]. The direct consequence at molecular level of these contributions is revealed by the presence of diterpenoid biomarkers and guaiacyl lignin units (methoxyphenols) characteristic for conifers, and triterpenoids and syringyl lignin units (dimethoxyphenols) present in angiosperms. A third contribution to the deposits is microbial and fungal activity, which cause the decay of the original plant material and result in for example specific fatty acids and also hopanoid biomarkers. Modifications in the organic matter are to be expected depending on soil conditions, conditions in the lake environments and after burial as well. Considering the floral, temporal and spatial diversity in the deposits, it would be rather surprising if one consistent pigment product could be obtained from these deposits. This diversity in composition is indeed transferred to the later Kassel earth pigments and possibly was to some extent mapped and used by paint manufacturers and painters (e.g., the different terminology [1-3]). Feller mentions a fine rich brown, of a very good strength for foregrounds [1]. Carlyle mentions that Kassel earth is reported to be lighter in colour and more reddish than Cologne earth [2, 3]. Although Kassel earth and Vandyke brown pigments are treated as similar in the literature, this study suggests that they are compositionally different to some extent. Kassel earth materials are more aliphatic, i.e. richer in cuticular waxes and terpenoids (e.g. F_4-F_7), while Vandyke browns are more aromatic showing a stronger phenolic signature (e.g. F_1-F_3). This general classification is in agreement with the name of the samples with only three exceptions: samples C_2, I_2, and O. These differences must be related to the flora and the conditions under which the deposits of brown coal had formed. It was not clear for the current researcher whether there was any awareness of qualitative differences. It would be worthwhile to examine literary sources and painters' manuals for information about this topic.

An important feature of Kassel earth and Vandyke brown is the absence of sulphur. No organic or inorganic sulphur compounds (sulphur is seen in mass spectra as m/z 32 and 64) were found in any of the samples (except some in the primarily inorganic H_4). Lignites could contain S only when the deposits are marine in origin or have been under marine water for some time [32]. Pietz thinks the deposits of coal from Kassel and Cologne have not been covered by marine water in Miocene [59]. The deposits more eastward in Germany have suffered from marine incursions and are richer in sulphur. The sulphur present as sulphide is introduced by sulphate reducing bacteria. Although none of the sample investigated here have been exposed to marine conditions, it is still important to be aware that sulphur rich lignites exist and could have been used for the preparation of pigment. Carlyle reports about the quality of pigment being bituminous as related to a foetid smell, which means a smell characteristic of S containing compounds [2, 3]. This would imply that there was at least a qualitative criterion available in the past.

It is suspected that the quality of the Kassel earth pigments has an influence on the drying properties of the oil paint. A precise characterisation of the pigments is the first requirement to understand its possible impact on the oxidation and cross-linking of oil paint. Are these pigments antioxidative or sicattive? This is hard to say. Kassel earth is a "mixture" of terpenoids, waxes and various phenolic compounds. Di- and triterpenoids are prone to oxidation and hopanoids can oxidise as well [84]. 2, 5-Isobutyl substituted phenols are used as antioxidants for varnishes [85], so it may be possible that some of the phenolic compounds in Kassel earth pigments have antioxidising properties. The easiest approach is to study oil paints prepared with Kassel earth pigments. Some aspects previously reported [9] are discussed in the last chapter of this thesis. The Kassel earth pigment used by Von Imhoff to prepare a Kassel earth oil paint, was found not to have any negative effect in the drying of the oil paint. So a pure Kassel earth pigment gives a well drying paint. However, have these pigments always been used in pure form? Feller has studied the colorimetric aspect of oil paints prepared with Kassel earth pigments (the same as reported here under F_{1-7}) and concludes fading is only intermediary [1]. The results presented in this chapter also do not suggest that fading or discoloration of Kassel earth is expected. However, Feller also admits that Kassel earth was sometimes substituted or adulterated with bone black [1]. Recent findings suggest that black paints containing bone black can undergo photo-oxidation and discolour to bone white [86]. This implies that fading of an oil paint that supposedly contains Kassel earth, could be due to the presence of another pigment or just be another pigment.

Kassel earth pigment contains solvent extractable fractions, which was shown in the desorption of the MS analysis and by solvent extraction experiments. Apolar solvents can potentially remove montan waxes and other characteristic markers of the pigment or pigmented paint, which makes identification much more difficult. The alkyl(methoxy)phenols as lignin derived units originate from highly bound fractions in Kassel earth, so these fractions are more likely to be preserved on solvent cleaned and lined paintings. Since these fractions are also the colour-carrying matrix, they are probably the best targets for analytical purposes. Another group of potentially stable identifying compounds worth investigation more are the short chain fatty acids, which appear to be rather unique features although some of these compounds are also expected from the oil component in mature oil paint. The latter compounds can only been observed by direct on-column pyrolysis-transesterification GC/MS (Py-TMAH-GC/MS), however. This approach is rarely used in conservation science laboratories.

Conclusions

The molecular chemical composition of a series of Kassel earth-like pigments has been determined. Plant terpenoids (pristene, phyllocladane, fridelin, olean/urs-12-ene, picene and lupane derivatives, ocotillone), bacterial-derived triterpenoids (17α,21β-homohopane, 22,29,30-trisnorhop-17(21)-ene, 17α-22,29,30 trisnorhopane), montan waxes, aliphatic compounds (ω-hydroxyfatty acids, α-ω dicarboxylic acids from gymnosperms and angiosperms, short-chain fatty acids from microorganisms) and aromatic compounds (methyl esters of benzoic and dicarboxylic acids, methoxy derivatives of benzaldehyde, acetophenone, benzoic acid and benzene propenoic acid and phenol, alkylated benzenes, bisphenol) have been found by DTMS, DTMS/MS, Py-TMAH-GC/MS and Py-GC/MS. All MS results suggest that Kassel earth-like pigments are in fact lignites or brown coal and can be treated as lignites in analytical investigations.

Certain diversity in composition is observed but many pigment sample fall into two categories. The first group to which most of the samples under the name of Kassel and Cologne earth belong is characterised by a high abundance in montan waxes (from fossil leaves) and higher plant terpenoids. The second group to which most of the samples under the name of Vandyke brown belong is characterised by a higher abundance of aromatic compounds (derived from wood plant tissues).

Of the identified markers some seem to be unique for lignite: montan waxes, vanillic acid (a benzoic acid methyl ester) and other lignin derived phenolic and methoxyphenolic compounds, phyllocladane (not observed in varnishes in paintings) and fridelin (an angiosperm-derived terpenoid). However, only the phenolics seem to be strongly bound to the cross-linked network of the samples. The possibility of loosing the other markers due to restoration and conservation procedures may make the identification of Kassel earth in oil paints more problematic.

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5. On poorly drying 19th-century oil paints: reference materials, model systems and paintings

Abstract

Poor drying of oil paint observed in the paint of 19th-century paintings is often considered to be caused by the presence of geomaterials like asphalt or Kassel earth pigments. The objective of this chapter is to investigate in what way the pigments of asphalt and Kassel earth influence the drying of oil paint. The asphalt pigment itself, light exposed dichloromethane test solutions of the pigment in linseed oil and various reconstructed paints are investigated and the results are compared with 19th-century paint samples from paintings by Sir J. Reynolds, Th. Rousseau and J. Israëls. This chapter is divided into three main sections focusing on (A) the fate of marker compounds in oil paint containing asphalt pigment, (B) the effects of asphalt and Kassel earth on the constituents in oil paint and (C) paint sample from paintings suspected of containing asphaltic materials.

Pretreatments of asphalt pigment are shown to affect the characteristic asphalt markers. Only hopanes and alkyl aromatics are preserved. Apolar solvents extract most of the biomarkers (i.e. the maltenes) from an asphalt sample. Asphalt specific compounds in light aged asphalt-linseed oil dichloromethane (DCM) solutions are rather well preserved. Artificially aged asphalt containing oil paint reconstructions show only the preservation of some hopanes and some alkyl aromatics.

A kinetic FTIR study of linseed oil and linseed oil with asphalt shows that when asphalt is present a much longer induction time is necessary before the chemical drying of linseed oil starts. DTMS, Py-TMAH-GC/MS and ESI-FTICR-MS measurements point to a slower drying of the oil in the presence of asphalt in DCM solutions of asphalt and linseed oil compared to DCM linseed oil solutions alone. The chemical drying of the oil is monitored using ratios of $C_{18:1}$ and $C_{18:0}$ fatty acids, and the azelaic acid and stearic acid. Less oxidation and more cross-linking are observed by ESI-FTICR-MS and HPSEC for the oil triglycerides in the asphalt-oil solution. The data suggest that chemical interactions forming cross links occur between asphalt and linseed oil as a function of the light exposure time, i.e. the light ageing period.

The chemical drying of the naturally aged Von Imhoff painted reconstructions (without drier) shows that asphalt retards the drying of linseed oil while Kassel earth does affect the drying. The oil seems to dry much better in the presence of driers and other materials as observed in the artificially aged oil asphalt paints prepared after 19th-century recipes. The 19th-century oil paint samples seem to have a complex composition (diterpenoid and triterpenoids resins, wax, vermilion, lead driers) with indications for lignite and possibly some degraded asphalt. The poor drying of the oil in these paints that follows from the fatty acid ratios determined seems to be more related to the overall paint composition, painting technique and restoration techniques than to the presence of a single culprit like asphalt.

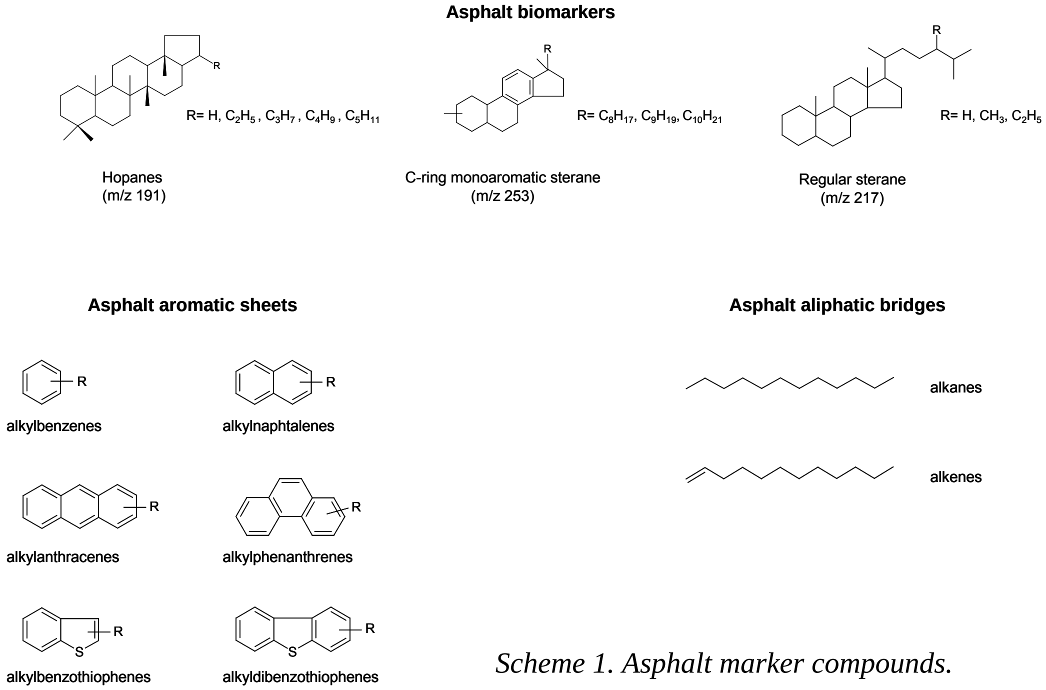
Introduction

Defects in paintings such as darkening of the paint, premature drying cracks, migration or sinking-in of brown-black layers disturb the surface characteristics of paintings and therefore their aesthetic quality. These phenomena have been often observed in 19th-century paintings [1-10]. According to many authors these defects are considered to be caused by the addition of asphalt or asphaltic components to the paint [3-7, 11-16]. Specifically Roelofs in his book, intended to teach artists about oil painting technique, warns painters that asphalt is detrimental to a painting in the long run despite its short-term attractive effect. Further studies into the nature of these asphaltic components and related materials, and their effect on the drying of oil and oil paint are the main rationale for this chapter.

Paint samples from brown-black areas in 19th-century paintings displaying premature cracks and migration of layers were suspected to be asphalt containing oil paints and were investigated in an early stage of this study [7, 17-22]. As asphalt was not found to be an obvious main component in these paint samples, the research spawned a series of studies designed to understand the fate of asphalt derived organic matter in oil paintings. This is a main focus of the chapter. A previous study in chapter 2 describes the specific compounds that characterise asphalt in 19th-century pigment samples of defined origin that contain asphalt. The analytical protocol developed was applied in this chapter to samples from paintings. In this process some marker compounds pointed to lignitic material moving this investigation also into the direction of chemical markers of Kassel earth and Vandyke brown. These markers were discussed in a previous chapter (chapter 4). Effects of Kassel earth on oil paints are reported in this chapter. Whether Kassel earth was used as a substitute for asphalt is not clear [4, 5, 23], but other brown or black pigments certainly have been introduced on the palette of the painters and were used in the 19th century under the name of asphalt as discovered by Carlyle [4, 5]. Carbon black pigments e.g. ivory black and bone black are also mentioned as common substitute or adulterant for asphalt and Kassel earth [4, 5, 23]. Carbon black is not easily detected with the present techniques of analytical organic mass spectrometry however. Their effects on the oil paint itself are easier to detect.

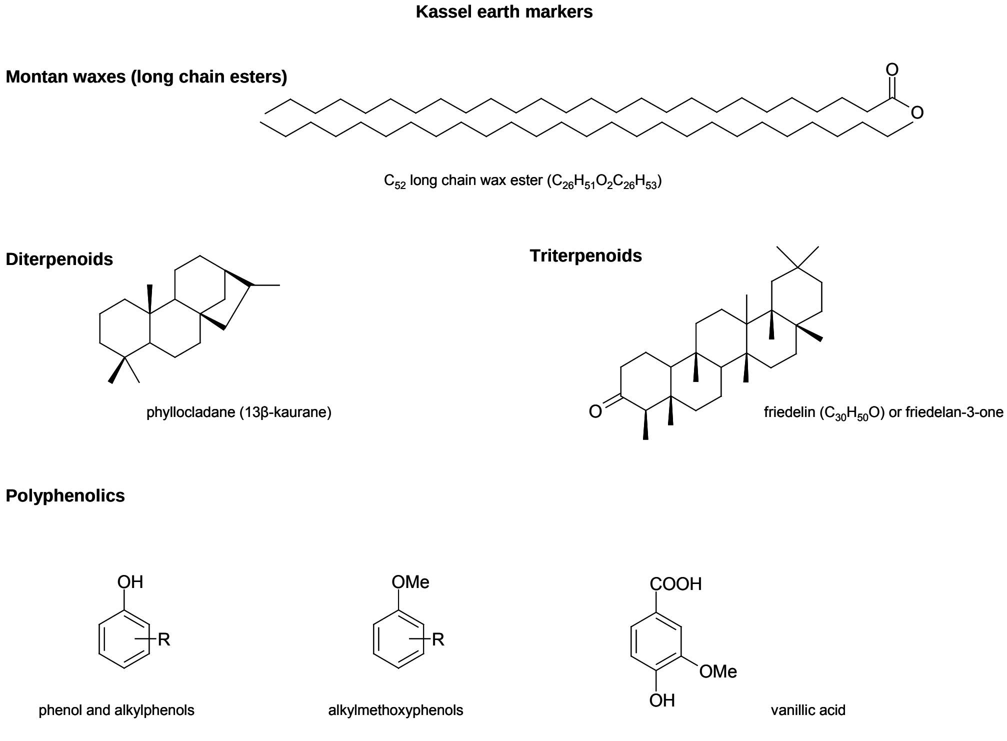
Asphalt can be identified by organic mass spectrometry using pyrolysis gas chromatography mass spectrometry on the basis of the presence of marker compounds such as series of long chain alkanes and alkenes, alkylbenzenes, alkylnaphtalenes, alkylbenzothiophenes, alkyldibenzothiophenes and so-called biomarkers such as 17α, 21β hopanoids and gammacerane, C-ring monoaromatic steroids, 14α/β steranes and triaromatic steroid compounds (see chapter 2 and Scheme 1). In similar way characteristic markers of Kassel earth (Scheme 2) like alkylphenols, alkylmethoxyphenols and specific markers such as vanillic acid, montan wax derived acids and alcohols, and diterpenoids such as phyllocladane were defined as tools to trace this pigment (see chapter 4). Although this approach works fine on pure reference samples, quite a few of these characteristics could not be detected in the analysed samples from the selected 19th-century paintings. Some of the asphalt markers were observed in one sample and some of the Kassel earth in some other samples. (Results are discussed in the appropriate section.)

The rather scarce results obtained after a direct search for markers and biomarkers particularly in the 19th-century brown-black samples suggested that a closer look at the influence of the pigment on the oil paint matrix pigmented with asphalt or Kassel earth was required.



Scheme 1. Asphalt marker compounds.

This is another main focus of the chapter. Recipes for asphalt paint preparation in the 19th century point out that asphalt paint is not made simply mixing asphalt and oil together but requires several preparation steps [4, 5, 24] that might affect its overall chemistry. Similarly, Kassel earth also seems to have undergone pretreatment



Scheme 2. Kassel earth marker compounds.

sometimes [23]. Asphaltic oil paints reconstructed according to these recipes were prepared, painted out and artificially light aged in the course of the MOLART project by R. Boitelle [24, 25]. Results from a selection of these samples are reported in this chapter.

This chapter presents results on the VEGA asphalt, representative asphalt rich material donated by Shell quite similar in its properties to the asphalt from the Dead Sea used in the 19th century. Its chemical fate after roasting, exposure to solvents, accelerated ageing as such and in the presence of drying oil, and incorporation into oil paint as well as the chemical effect of the asphalt on the drying of the oil is discussed. This last aspect is investigated also for naturally aged reconstructions prepared with another asphalt material. A similar complete set-up for Kassel earth was not available but some aspects of these different conditions were tested as well and are reported in this chapter. Results from these experiments were used to put the analytical data from the 19th-century paintings into perspective, the concluding aim of this chapter.

Experimental

Samples and sample preparation

The untreated and roasted VEGA asphalt was analysed. Furthermore it was investigated as a fresh mixture of asphalt and linseed oil (5 % asphalt in oil). It was analysed as well as an asphalt-linseed oil mixture in dichloromethane (2: 1 weight/weight asphalt: linseed oil, per 1 ml solvent) and also after incorporation into paint according to 19th-century recipes, in both cases before and after artificial light ageing. For the dichloromethane (DCM) solutions samples of asphalt in DCM and linseed oil in the same solvent were prepared and aged in the same way for comparison. Control solutions of the latter solutions were prepared. A naturally aged asphalt containing oil paint, prepared however with another asphalt pigment (analysed by us but not reported here), was also investigated for reasons explained in the results and discussion section.

The untreated Kassel earth pigment used by Von Imhoff to prepare oil paints was analysed as reported in chapter 4. A naturally aged oil paint prepared with the same pigment and from the same series as the above-mentioned asphalt paint sample was investigated in this study. Moreover two other samples of the same series of naturally aged samples, an ivory black oil paint and a lead white oil paint were used for comparison.

Brown-black paint samples from selected 19th-century paintings by Rousseau, Israëls and Reynolds were regarded as appropriate for the current study, i.e. suspected to contain asphalt, because these three painters are said to have used asphalt pigments [7, 17-21, 26-28] while the defects observed on the areas of the paintings where the samples were taken, correspond to the defects associated in the literature with the presence of asphalt [3-7, 11-16]. Details about the symbols used for the samples, their appearance and the methods and materials used for their preparation as well as information on their ageing treatments are given below, in Table 1 (reference samples and oil paint reconstruction samples) and in Table 2 (19th-century oil paint samples).

Table 1. The label, composition and appearance of samples used for the asphalt paint preparation.

Legend: OP_i stands for oil paint, "c"- control samples (initial time), "a"- aged samples, VI stands for Von Imhoff [35-39].

Label, Composition	**Appearance**
Original material	
A, VEGA asphalt	black, glossy, sticky solid mass
Roasted asphalt	
A_{ro}, roasted VEGA asphalt	brownish, mat, not sticky
Asphalt extract and residue	
A_e, heptane extract (maltene) of A	colorless solution
A_{re}, heptane residue (asphaltenes) of A	dark brown-black residue
Asphalt-linseed oil dichloromethane (DCM) test solution	
LoAc, linseed oil: A, 1:2 (mg)/ml DCM	dark brown solution
LoAi, linseed oil: A	artificially aged for 1-12 weeks (i= 1-4, 8, 12); dark brown solution
Asphalt oil paint reconstructions	
OP_{1c}, William's "Antwerp brown" (see below)	Tiny particles in yellow-brown medium; elastic (skin like) before ageing
OP_{2c}, Merimée's English method (see below)	Dark black, grain structure like before ageing.
OP_{3c}, Merimée's other method (see below)	Dark black and elastic (skin like) with visible warm brown particles in it before ageing.
OP_{1a}, William's "Antwerp brown" : VEGA asphalt boiled to cinder, mixed cold with drying oil (linseed oil and litharge/ lead oxide) applied on glass support; lead white linseed oil ground [4, 5 , 24]	Artificially aged for 3 months, [40, 41] Tiny particles in yellow-brown medium; elastic (skin like); after ageing small lumps, semi-transparent and soft, have become visible carrying on top particles of white ground disrupted from the lead white ground
OP_{2a}, Merimée's English method: VEGA asphalt dissolved in oil of turpentine, mixed with mastic varnish (1: 2, mastic resin: gum terpentine) and drying oil (see above); ground and support as above [4, 5 , 24]	Artificially aged for 3 months, [40, 41] Dark black, irregular structure
OP_{3a}, Merimée's other method: VEGA asphalt and shellac added to heated turpentine and drying oil (see above), mixed with beeswax; ground and support as above [4, 5, 24]	Artificially aged for 3 months [40, 41] Dark black and elastic (rubbery texture) with visible warm brown particles in it; particles of ground have protruded to the surface and there cracked open like a crust of bread [24]

Table 1 continued.

Label, Composition	Appearance
A-VI, Von Imhoff asphalt paint sample: Düll asphalt, cold pressed linseed oil (standoil); wood support; gypsum-chalk-whiting ground [35-38]	Natural aged for 25 years
Ke-VI, Von Imhoff Kassel earth paint sample: Mülfellner-Rupf Kasselerbrown, standoil; ground and support as above [35-38]	Natural aged for 27 years
PbW-VI, Von Imhoff lead white paint sample: Düll lead white, standoil; ground and support as above [35-38]	Natural aged for 25 years
IvB-VI, Von Imhoff ivory black paint sample: Düll ivory black, standoil; ground and support as above [35-38]	Natural aged for 25 years

Table 2. The label, sample description and condition of the 19th-century paintings from which the samples originate.

Sample's label	Description	
	sample	**painting**
Ro1 (286/1b)	brown paint + varnish	Unrelined, not known if cleaned, with severely darkened drying cracks and top layers shrunken into thick islands. *Restorer question:* What is the composition of the oil paint and the relation with poor drying of the oil? Is this an asphalt / Kassel earth containing paint?
Ro2 (286/5)	brownish glossy material; dark brown material	
Ro3 (287/2a)	brown paint + varnish	Wax-resin relined, cleaned, varnished, showing small drying cracks in the blue and brown areas. *Restorer question:* What is the composition of the oil paint and the relation with poor drying of the oil? Is this an asphalt / Kassel earth containing paint?
Re1 (1029/20)	bituminous paint, over red	Glue-lined, partly cleaned, condition of painting variable in different areas. *Restorer question:* What is the composition of the oil paint and the relation with poor drying of the oil? Is this an asphalt containing paint?
I1 (154/5)	light brown paint	Showing drying cracks, and severely darkened. *Restorer question:* What is the composition of the oil paint and the relation with poor drying of the oil? Is this an asphalt containing paint?
I2 (154/2)	black paint	

VEGA asphalt

For the analysis of the untreated VEGA asphalt a lump of the black sticky solid mass was analysed: sample A. The roasted asphalt, sample A_{ro}, was prepared by heating the asphalt on a glass slide above a gas torch (t_{flame}~ 400 °C). The roasting of the asphalt was intended in order to investigate the difference in composition after exposure to a high temperature. The sensitivity of asphalt to solvents was tested according to the protocol used by Behar et al. for asphalt fractionation [29]. A few mg of VEGA asphalt were introduced in a gas chromatography vial of 2.5 ml and dissolved with a few ml of chloroform. The mixture was stirred using a VORTEX-GENIE mixer model K-550-GE (USA). Heptane was added to the mixture causing the precipitation of asphaltenes. The new mixture was centrifuged for 5 minutes in a CHRIST JOTA-RVC Gefriertrocknungsanlagen, type 100005 (Germany), the extract (maltene fraction) was removed with a Pasteur pipette and the procedure repeated. Heptane was added to the extract, the solution was centrifuged and the extract removed again. The procedure was repeated using the extract till no precipitate was formed, which is three times. The extract (maltene fraction), sample A_e, was concentrated by evaporation of part of the solvent under a stream of nitrogen, then subjected to on-column GC/MS measurement. The asphaltene residue, sample A_{re}, was dissolved in DCM and subjected afterwards to pyrolysis gas chromatography coupled with mass spectrometry (Py-GC/MS).

For DTMS analysis the VEGA asphalt was ground in DCM and the solution was applied to the Pt/Rh wire of the DTMS probe (see Experimental, DTMS).

Asphalt-oil dichloromethane test solutions

DCM test solutions of pigment and linseed oil are easy to prepare and require only a few weeks of artificial light ageing instead of months as necessary for artificial ageing of painted reconstructions. It was previously shown that such solutions produce similar changes as observed for atmosphere exposed drying of oil paint [30-33]. The rate of oxidation and cross-linking of the linseed oil in solution is much higher than observed for example in painted reconstructions. Natural ageing of painted reconstructions takes many years, and natural ageing of real paintings requires hundreds of years.

Test solutions of linseed oil (Lo), asphalt (A) and linseed oil-asphalt (LoA) in DCM were prepared, stored in glass vials, and artificially light aged for 12 weeks in a home-made light ageing device at FOM-AMOLF, Amsterdam, The Netherlands. Samples notations as reported in this chapter are: Loc, Loi (i= 1-4, 8, 12), LoAc, LoAi (idem as for Lo) and A12. The code numbers are equivalent to the number of weeks of artificial ageing. More details on the ratio “pigment: oil: solvent” are given in Table 1. The vials were opened daily to allow interaction of the solutions with atmospheric oxygen. The control samples and the several aged solutions were kept sealed under argon, at -18 °C. The following conditions were used for ageing of the samples: a halogen lamp (Philips Compact Plusline double ended lamp type 100 T3 Q/CL/CP 230V 100W R7s; 1600lm output, 2900K correlated temperature), radiant incidence 150 mW/m^2 (UV), 3000 lux (VIS), UVA (ultraviolet/visible ratio) 50W/lm, 315 nm wavelength, ca. 30 °C [34]. The test solutions were analysed as such by mass spectrometric techniques.

Asphalt and Kassel earth oil paint reconstructions

Fourier Transform Infrared Spectroscopy was used to determine the induction time observed for the drying of linseed oil and mixtures of linseed oil and VEGA asphalt or smalt (a well drying pigment when used in oil paint). A ratio of 5 % weight/weight ratio of asphalt, or smalt to oil was used.

Painted test panels considered to be representative of the natural ageing of oil paints were sampled for the current investigations. These test panels had been naturally aged for about 25 years at Canadian Conservation Institute (CCI). H.C. von Imhoff has prepared these oil paints [35-37] using cold-pressed linseed oil (Mühlfellner-Rupf, Zurich, Switzerland) that was allowed to stand in dishes of 4 mm height for 3 weeks. The oil was mixed with the pigment until a workable paint was obtained, after the skin of the oil had been removed. The paints were then applied on sized and primed lime-wood and aged. Samples of four pigmented oil paints, without ground layers, were selected for this study: asphalt, Kassel earth, ivory black and lead white (the last two samples for comparison with the first two). The samples were labelled as follows: A-VI, Ke-VI, IvB-VI and PbW-VI, respectively. The test panel oil paints have undergone natural ageing at CCI: 22 ± 2 °C, RH 45.66 ± 5 %, radiant incidence 7950 mW/m^2 (UV), 318 lux (VIS), UVA 25 W/lumen for 3500 hrs/year since 1973. The panels were stored in wooden box at CCI from 1973 until 1990. In 1990 the panels were removed from their wooden boxes and hanged vertically on an interior wall of the Analytical Research Laboratory of CCI (1030 Innes Road, Ottawa, Canada) where they are still stored [38, 39].

Artificially aged paint reconstructions containing asphalt were available for this study. René Boitelle set up the painted reconstructions containing VEGA asphalt, as a part of the MOLART subproject “Drying problems in 19th-century paintings and the role of bitumen in the darkening of paint” [24]. After 8-10 months of curing, the reconstructions were artificially aged for 3 months in the ageing device at Stichting Restauratie Atelier Limburg (SRAL), Maastricht, The Netherlands. This time equals approximately 40 years of light conditions in a museum[1] [40-42]. The asphalt containing oil paints were prepared after three recipes from the 19th century. These are William’s “Antwerp brown” recipe, Merimée’s English method and Merimée’s other method [4, 5, 24]. The samples were labelled as OP_1, OP_2 and OP_3, respectively. More details about preparation of the oil paints are given in Table 1. The reconstructions were artificially aged at a temperature of max 29 °C, 27.5 % relative humidity, 10000 lux (VIS), at a wavelength of the light of 350-850 nm. More details about the ageing device at SRAL are given elsewhere [40, 41].

In the current research only the brown layer of all the oil paint reconstruction samples was analysed by mass spectrometry. Separation of layers of the oil paints was done under the microscope on a glass slide using a scalpel and a needle, cleaned before with ethanol. Suspensions of the samples in ethanol (EtOH) were analysed by DTMS and Py-GC/MS. Samples were ground in the TMAH transesterification agent instead of solvent for Py-TMAH-GC/MS. More details are available further in the Experimental’s Analytical techniques section.

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[1] 200 lux for 7hrs/day is considered the average light intensity in a museum. At SRAL the samples are exposed to 10^4 lux for 24 hrs/day; 92 days of exposure at SRAL imply 24/7 x 92 days of exposure x $10^4/200$ = 15771 days of 200 lux/day for 7 hrs= 43.2 years.

19th-century oil paint samples

Samples from 19th-century paintings with defects that would match the description of paints prepared with asphalt pigment were investigated for this study. These samples originate from areas of the paintings that have darkened and show drying cracks specific to a poor drying of the oil. The samples had a dark brown-blackish appearance (see Table 2) and were provided by several people. Joyce Townsend (Tate Gallery, London) provided a sample from a darkened painting by Sir Joshua Reynolds supposedly containing asphalt. The painting represents the '*Death of Dido*', was painted around 1781 and belongs to the Royal Collection, London, United Kingdom (catalogue number 1029) [7]. In this experiments the sample's code was Re_1. J. Townsend described the sample before [28]. R. Boitelle provided two samples, coded I_1 and I_2, from '*Alone*', a painting by Josef Israëls that was painted around 1880. The painting belongs to the Mesdag Museum, The Hague, The Netherlands (catalogue number 154). This painting also shows signs of darkening and poor drying of the oil paint [9, 21]. Van den Berg and Boon have been described the samples before [27]. Three other similar samples originate from a painting and a sketch by Théodore Rousseau, both hanging in the Mesdag Museum and both having the same name, '*La descente des vaches*'. The sketch (catalogue number 287) was painted between 1834-1835, while the painting (catalogue number 286) was painted about one year later, between 1835-1836. Unlike the sketch that shows small drying cracks in the blue and brown areas, the painting had severely darkened and the top layers had shrunken into thick islands of paint. The samples coded Ro_1 and Ro_2 were obtained from the painting's darkened foreground. Sample Ro_3 comes from a brown spot in the yellow foreground of the sketch. Some details are given in Table 2 and more details about the samples have been discussed elsewhere [26, 43].

Only the brown layers of the paint samples were analysed, after separation of layers as described for the oil paint reconstructions. In some situations traces of the varnish could not be entirely removed.

Analytical techniques

Direct Temperature-resolved Mass Spectrometry (DTMS)

The samples (pigments and oil paints) were analysed by DTMS as solutions or suspensions. These were prepared using 5-10 µg of sample and 15-25 µl of solvent (DCM for the pigments and EtOH for the oil paint samples). The test solutions in DCM were analysed as such. An aliquot of 1-2 µl of the suspension/ solution was applied to the filament of a direct insertion probe for in-source analysis. DTMS experiments were carried out on a JEOL JMS SX-102 double focussing mass spectrometer (B/E) and a JEOL JMS-SX/SX 102A tandem mass spectrometer (B/E/B/E) [44, 45]. A resistively heated Pt/Rh filament (Pt/Rh 9:1, 100 µm) was used for the direct insertion probe. The filament was heated at a rate of 0.5 A/min to an end temperature of about 800 °C. Ions generated by electron ionisation (EI) in an ionisation chamber kept at 190 °C, were accelerated to 8 kV, analysed from m/z 20-1000 (about 1 s cycle time) and post-accelerated to 10 kV. A mass resolution of 1000 was used. A JEOL MS-MP 9020D data system was used for data acquisition and processing. To minimise the fragmentation reactions of the ions the DTMS spectra were acquired under low energy EI conditions at 16 eV. The test solution samples were analysed in triplicate for discriminant analysis, and the spectra were summed over the TIC.

Discriminant Analysis of the DTMS data

Mass spectra were numerically analysed by discriminant analysis (DA) with the FOMpyroMAP multivariate analysis programme, a modified version of the ARTHUR package from Infometrix Inc. (Seattle, USA; 1978 release) and with the FOM developed Matlab® (The Mathworks Inc., Natick, MA, USA) toolbox ChemomeTricks [46-49].

Gas Chromatography Mass Spectrometry (GC/MS) analysis in various modes[2]: Py-GC/MS, Py-TMAH-GC/MS and on-column GC/MS

Typical amounts of sample used for GC/MS experiments were 10 µg for solid samples, or 10 µl solution for pyrolysis GC/MS measurements and 10 µl solution for on column GC/MS measurements. Solid samples were ground in DCM for Py-GC/MS experiments. For Py-TMAH-GC/MS experiments, the samples were homogenised in tetramethylammonium hydroxide (TMAH) 2.5% in water, and aliquots were applied to a ferromagnetic wire and dried *in vacuo*. The Curie point of the wires is given in Table 3.

Table 3. GC/MS methods and oven programs used for analysis of the samples.

GC/MS method	Samples	Details
Py-GC/MS	A, A_{ro}, A_{re}, A-VI	35(0)-4-320(10); 770 °C wire, 9 s Py
Py-TMAH-GC/MS	Lo, LoA, OP_{1-3},	50(2)-6-320(10); 610 °C wire, 6 s Py
	A-VI, PbW-VI, IvB-VI	50(2)-6-320(0); 610 °C wire, 6 s Py
	Ke-VI	50(2)-6-320(10); 610 °C wire, 6 s Py
	Ro_{1-3}	35(0)-4-320(10); 770 °C wire, 9 s Py
	Re_1	50(2)-6-320(2); 610 °C wire, 6 s Py
	I_{1-2}	40(2)-6-352(6); 770 °C wire, 6 s Py
On column GC/MS	A_e	110(2)-3-320(10)

After drying of the sample, the wire was inserted into a glass liner, placed in the cold compartment of the pyrolysis unit, flushed with helium and then moved into the pyrolysis chamber (220 °C) of the FOM 5LX Curie point pyrolysis unit [50]. The experiments were carried out with a FOM-5LX Curie-point pyrolysis unit [50] mounted on a Carlo Erba series 8565 HRGC MEGA 2 gas chromatograph. For on-column GC/MS the sample was directly injected on column. For separation a fused silica SGE BPX5 column (25 m, 0.32 mm i. D., 0.25 µm film thickness) used with helium as a carrier gas at a flow rate of 2 ml/min. The oven temperature was

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[2] Py-GC/MS stands for on line Pyrolysis GC/MS; Py-TMAH-GC/MS stands for on line transesterification Pyrolysis GC/MS.

programmed as shown in Table 3. For analysis of samples known to contain asphalt a longer oven program was chosen as shown in Table 3 to have a good separation of the asphalt markers and biomarkers. The paint samples are usually analysed with a oven program that follows the standard method for paint samples (see as well Table 3). The GC column was interfaced directly to a JEOL JMS DX-303 double focussing (E/B) mass spectrometer or a JEOL JMS-SX/SX 102A tandem mass spectrometer (B/E/B/E), using a home built interface kept at 300 °C. Ions were generated by electron ionisation (70 eV) in the ionisation chamber, accelerated to 3 kV (DX303) or 8 kV (SX/SX 102A), mass separated and post-accelerated to 10 kV before detection. The mass range was scanned from m/z 35-500, or m/z 40-800 respectively, with a cycle time of 1 s. A JEOL MS-MP 9020D data system was used for data acquisition and processing.

Electrospray Ionisation Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (ESI-FTICR-MS)

A modified 7.0e FTICR-MS instrument (Bruker-Spectrospin, Fällanden, Switzerland) equipped with a 7-Tesla superconducting magnet and an in-house designed external ion source was used for ESI-FTICR-MS measurements of the test solutions. Details of the instrumental parameters have been described before [51, 52]. The ICR cell used was a home-built open cell. The samples were mixed with DCM: EtOH (7:3, v/v) (1/100 µl) and 10mM NH_4Ac was added before analysis. Ions were generated using an atmospheric pressure electrospray ionisation interface, maintained at 3-4 kV, desolvated in a heated capillary, transported through several differentially pumped sections till trapping in the ICR cell at ~5 x 10^{-9} mbar. Data were processed using XMASS 5.0 software from Bruker Daltonik GmbH, Bremen, Germany.

High Pressure Size Exclusion Chromatography (HPSEC)

HPSEC or SEC was used only for the test solution in DCM. The samples were measured using a Shimadzu LC10 system, consisting of a SCL-10AD *vp* control panel, a LC-10AD *vp* pump, a DGU-14A degasser, a SIL-10AD *vp* autoinjector, a CTO-10AS column oven and a FRC-10A fraction collector (Shimadzu Benelux, 's-Hertogenbosch, The Netherlands). Separation was achieved on two columns used in series: PL gel Mixed D 5 µm (300 x 7.5 mm) and PL gel 5 µm 10^3Å (300 x 7.5 mm) of Polymer Laboratories, Heerlen, The Netherlands. Three different detectors, connected in series, were used for detection: a SPD-10A *vp* UV/VIS-detector (Shimadzu) operated at 240 nm, a RID-10A refractive index detector (Shimadzu) and a 996 Photo Diode Array (PDA)–detector (Waters™ Chromatography B.V., Etten Leur, The Netherlands) operated at 195-500 nm, in combination with Class *vp* 5.03 (Shimadzu) and Millenium 32 (waters) software, respectively. The system was operated at a temperature of 40 °C or 30 °C with a flow rate of 1 ml/min and DCM was used as a mobile phase. The analysis time was of 25 min. Calibration was performed with polystyrene standards (Polymer Laboratories) with an average mass ranging from 570 to 370,000.

Fourier Transform Infrared Spectrometry (FTIR)

Initial chemical changes in a 5 % (w/w) asphalt-linseed oil mixture were investigated using a FTS-6000 Bio-Rad FTIR imaging system (Bio-Rad, Cambridge, MA, USA), consisting of a Michelson interferometer (Bio-Rad FTS-6000), an IR microscope (Bio-Rad UMA-500) and a MCT narrow band detector. The fresh asphalt paint was applied as a thin film on a ZnSe disc. The disc was placed in a sample compartment of the BioRad FTS 6000 spectrometer. Spectra were averaged with a 30 minutes time resolution. The resolution of the spectra is 4 cm^{-1} and the mirror speed was 5 kHz. Data were processed using WIN-IR Pro 2.5 software of Bio-Rad.

Results and discussion

A) Fate of marker compounds in oil paint that contains asphalt

The experiments planned required a relatively large amount of natural asphalt. A representative sample that would match the chemical properties of the Dead Sea asphalt was received from Shell. As discussed in chapter 2, the asphalt from the Dead Sea was considered to be the main source of asphalt in the past. The VEGA asphalt was investigated using the analytical Py-GC/MS protocol reported in detail in chapter 2. The full inventory of chemical marker compounds found made VEGA a good testing material to measure the effects of roasting and other 19th-century pretreatments on the chemical structure of the asphalt. VEGA was also used to make paint and to test the effect of ageing of the paint. The results of these investigations are reported in the next six sections. Apart from these paint studies, VEGA was also used to investigate its reactivity with drying oil. A similar but more limited approach for Kassel earth and Kassel earth containing oil paints is reported by Languri and Boon in Annex A of chapter 5 [53]. The main focus there is on the pigment and its fate in oil paint reconstructions. Some aspects of the effect of solvent extraction are also tested.

VEGA asphalt

The TIC and selected mass chromatograms for the hopanoid, monoaromatic steroid, all steroid biomarkers and aliphatic chain elements are depicted in Fig. 1a and those from the aromatic alkylbenzenes, alkylnaphtalenes, alkylbenzothiophenes and alkyldibenzothiophenes markers are presented in Fig. 1b. The same notation for markers as introduced in chapter 2 was used. Ratios of biomarkers calculated from their intensity in the partial mass chromatograms are shown in Table 4 and Fig. 2. The bar graphs in Fig. 2 were obtained by integration of the area of the peaks of interest (markers and biomarkers)[3] in the partial mass chromatograms from the Py-(TMAH)-GC/MS data. The comparison between the abundance of the markers and biomarkers is absolute (bound to m/z values used) within each sample and relative from sample to sample.

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[3] The whole range of alkanes (m/z 85), hopanes (m/z 191), monoaromatic steroids (m/z 253), alkylbenzenes (m/z 77+91+105+119), alkylnaphtalenes (m/z 128+142+156+170), alkylbenzothiophenes (m/z 148+162+176+190), alkyldibenzothiophenes (m/z 198+212+226+240)

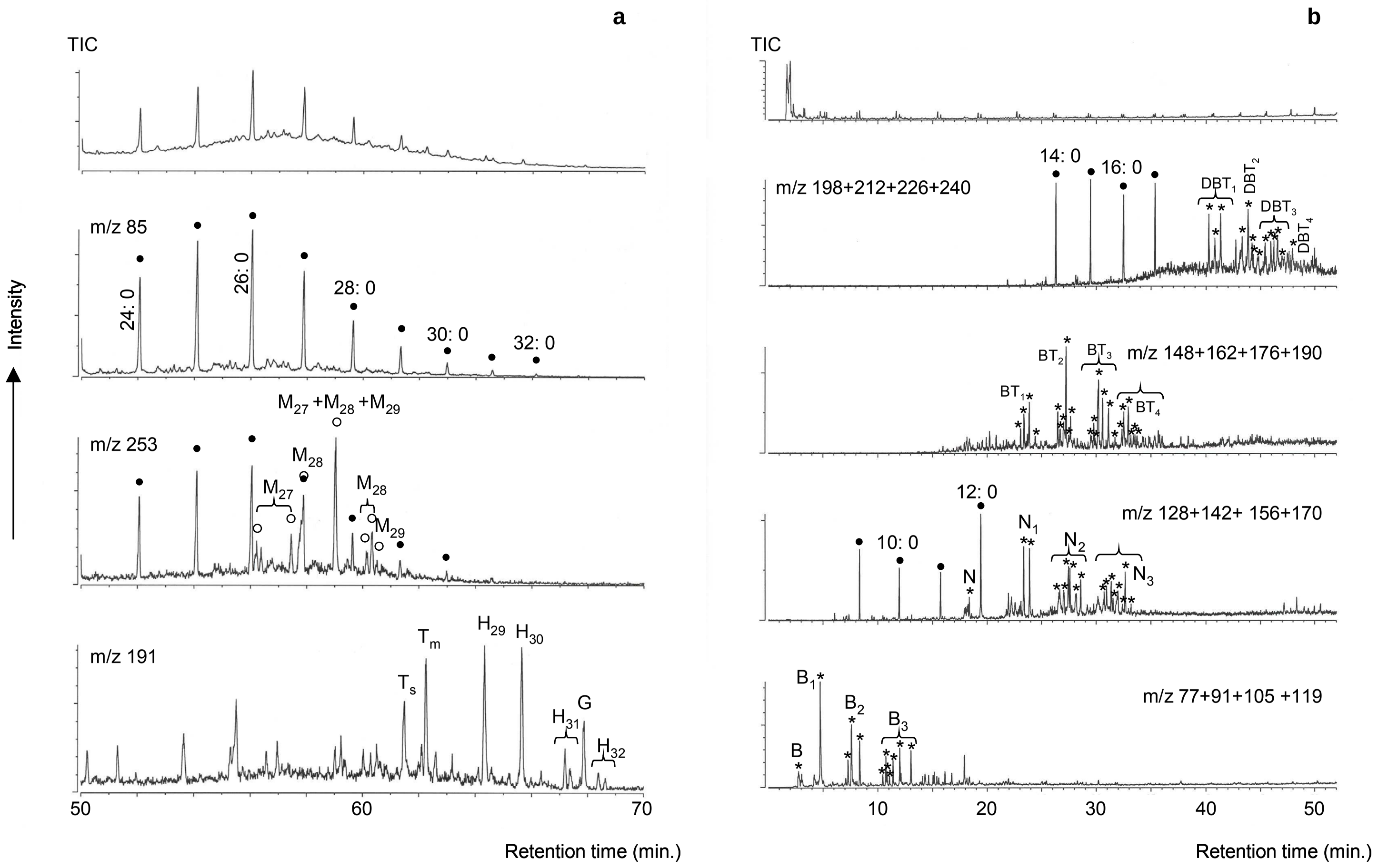

Fig. 1. Biomarkers (a) and markers (b) of the VEGA asphalt sample. Biomarkers: hopanoids (m/z 191), monoaromatic steroids (m/z 253) and the C_{23}-C_{35} *n-alkane retention time window (m/z 85). Markers: alkylbenzenes (m/z 77+91+105+119), alkylnaphtalenes (m/z 128+142+156+170), alkylbenzothiophenes (m/z 148+162+176+190) and alkyldibenzothiophenes (m/z 198+212 +226+240).*

Table 4. Presence and absence of asphalt marker molecules and biomarkers in asphalt containing samples.

Legend: "i" stands for no. of C atoms in the alkyl chain, H_i stands for hopane homologues (H_{27-37}), T_s and T_m for the H_{27} hopane homologues, G for gammacerane, MA_i for monoaromatic steroids, C_i for alkanes (i= 6- max 38), B for benzene, B_i for alkylbenzenes (i= 1-3), N for naphthalene, N_i for alkylnaphthalene (i= 1-3), BT_i for alkybenzothiophenes (i=1-4), DBT_i for alkyldibenzothiophenes (i=1-4). The eluting (alkane) windows are as follows: C_{29}-$C_{36/38}$ alkanes for the hopanes, C_{26}-C_{29} alkanes for the monoaromatic steroids, $C_{6/7}$-C_{11} alkanes for the alkylbenzenes, C_{11}-C_{17} alkanes for the alkynaphtalenes, C_{13}-C_{17} alkanes for the alkylbenzothiophenes, C_{18}-C_{21} alkanes for the alkyldibenzothiophenes.

Sample	**Markers and biomarkers**	
	Present	**Absent**
A	hopanes: $H_{27, 29-32}$ (T_s, T_m, H_{29}, H_{30}, H_{31}, H_{32}) gammacerane: G monoaromatic steroids: MA_{27-29} alkanes: C_7-C_{35} aromatics: B-B_{1-3}, N-N_3, BT_{1-4},DBT_{1-4}	
A_{ro}	hopanes: $T_{s: 1}$, T_m, H_{29-32} ($T_{s: 1}$, T_m, H_{29}, H_{30}, H_{31}, H_{32}) gammacerane: G alkanes: C_7-C_{29} aromatics: B_{1-3}, N-N_3, BT_{1-4}, DBT_{1-4}	monoaromatic steroids
A_e	hopanes: $H_{27, 29-37}$ (T_s, T_m, H_{29}, H_{30}, H_{31}, H_{32}, H_{33}, H_{34}, H_{35}, H_{36}, H_{37}) gammacerane: G monoaromatic steroids: MA_{27-29} alkanes: C_{12}-C_{39}	B-B_3, N-N_3, BT_{1-4}, DBT_{1-4}
A_{re}	hopanes: $T_{s: 1}$, T_m, H_{29-33} gammacerane: G monoaromatic steroids: MA_{27-29} alkanes: C_7-C_{33} aromatics: B-B_3, N-N_3, BT_{1-4}, DBT_{1-3}	DBT_4
LoAc	hopanes: $H_{27, 29-35}$ (T_s, T_m, H_{29}, H_{30}, H_{31}, H_{32}, H_{33}, H_{34}, H_{35}) gammacerane: G monoaromatic steroids: MA_{27-29} alkanes: C_7-C_{34} aromatics: B_{1-3}, N-N_3, BT_{1-4}, DBT_{1-2}	B, DBT_{3-4}
LoAa	hopanes: $H_{27, 29-35}$ (T_s, T_m, H_{29}, H_{30}, H_{31}, H_{32}, H_{33}, H_{34}, H_{35}) gammacerane: G monoaromatic steroids: MA_{27-29} alkanes: C_7-C_{34} aromatics: B_{1-3}, N-N_3, BT_{1-4}, DBT_{1-3}	B, DBT_4
OP_{1c}	hopanes: T_m, $H_{29-31(22S)}$ aromatics: B_1, B_{2-3}?, N_3	T_s, $H_{31(22R)}$, G, monoaromatic steroids, alkanes, B, N-N_2, BT_{1-4}, DBT_{1-4}
OP_{1a}	hopanes: T_m, $H_{29-31(22S)}$ aromatics: B_{2-3}, N_3	T_s, $H_{31(22R)}$, G, monoaromatic steroids, alkanes, B-B_1, N-N_2,BT_{1-4}, DBT_{1-4}

Table 4 continued.

Sample	Markers and biomarkers	
	Present	**Absent**
OP_{2c}	hopanes: H_{29}, H_{30} aromatics: $B_{1\text{-}3}$, N?, N_2	T_s,T_m, $H_{31(22S,\,22R)}$, G, monoaromatic steroids, alkanes, B, N_1, N_3, $BT_{1\text{-}4}$, $DBT_{1\text{-}4}$
OP_{2a}	hopanes: $T_{s:\,1}$, T_m, H_{29}, H_{30} aromatics: B2?, B3, N?, $N_{1\text{-}3}$	T_m, $H_{31(22S,\,22R)}$, G, monoaromatic steroids, alkanes, B-B_1, $BT_{1\text{-}4}$,$DBT_{1\text{-}4}$
OP_{3c}	hopanes: T_m, H_{29}, H_{30} beeswax alkanes: C_{25}, C_{27}, C_{29}, C_{31} aromatics: $B_{1\text{-}3}$, N?, N_1, N_1 (2nd isomer)?	T_s, $H_{31(22S)}$, $H_{31(22R)}$, G, monoaromatic steroids, asphaltic alkanes, B, $N_{2\text{-}3}$, $BT_{1\text{-}4}$, $DBT_{1\text{-}4}$
OP_{3a}	hopanes: T_m, H_{29}, H_{30} beeswax alkanes: C_{27}, C_{29}, C_{31} aromatics: $B_{1\text{-}3}$, N_1	T_s, $H_{31(22S)}$, $H_{31(22R)}$, G, monoaromatic steroids, asphaltic alkanes, B, N, $N_{2\text{-}3}$, $BT_{1\text{-}4}$, $DBT_{1\text{-}4}$

The n-alkanes observed in the VEGA asphalt sample range from C_6 to C_{35}. It is obvious by comparison of the TIC with the mass chromatogram m/z 85 of alkanes that the aliphatic compounds have a stronger relative intensity than hopanoids and steroid compounds.

The hopanoid and steroid elution windows are delimited by C_{26}-C_{36} alkanes (m/z 85).

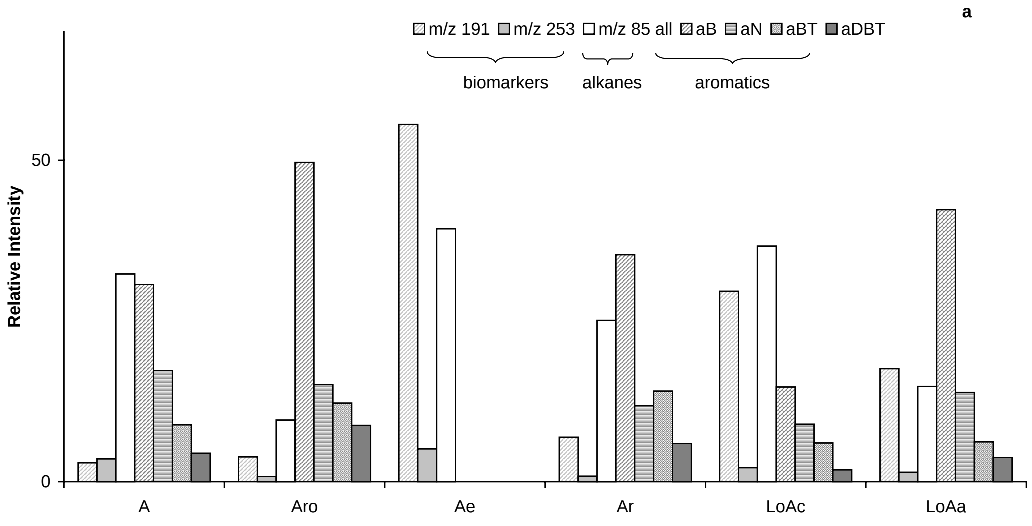

Fig. 2a. Ratios of markers and biomarkers in VEGA asphalt samples and VEGA asphalt-oil test solutions. The labels are explained in Table 1.

In the lowest mass chromatogram of Fig. 1a the 17α, 21β(H) hopanoids (T_s and T_m, H_{27} homologues, H_{29}-H_{32} homologues; Scheme 1) characterised by the m/z 191 fragment ion are displayed. Gammacerane, the oleanane type of pentacyclic hydrocarbon considered being highly characteristic of Dead Sea asphalt [54, chapter 2] is marked with G. The relative ratios of the hopanoid compounds are shown in Fig. 2 and Table 4, from where it appears that the T_m, H_{29} and H_{30} homologues are the highest in intensity. The middle mass chromatogram of Fig. 1a with the profile of monoaromatic steroids (m/z 253), tentatively identified as the MA_{27}, MA_{28} and MA_{29} homologues, shows a similar pattern as observed in the Dead Sea asphalts [54, chapter 2]. Note that the mass chromatogram of m/z 253 also pulls out a series of alkane homologues. Other steroid hydrocarbons identified were 14α(H) steranes (m/z 217), 14β(H) steranes (m/z 218), monoaromatic and methyl-monoaromatic steroids (m/z 253, m/z 267), triaromatic steroids and methyl triaromatic steroids (m/z 231, m/z 245), but these will not be considered in the presented comparative studies.

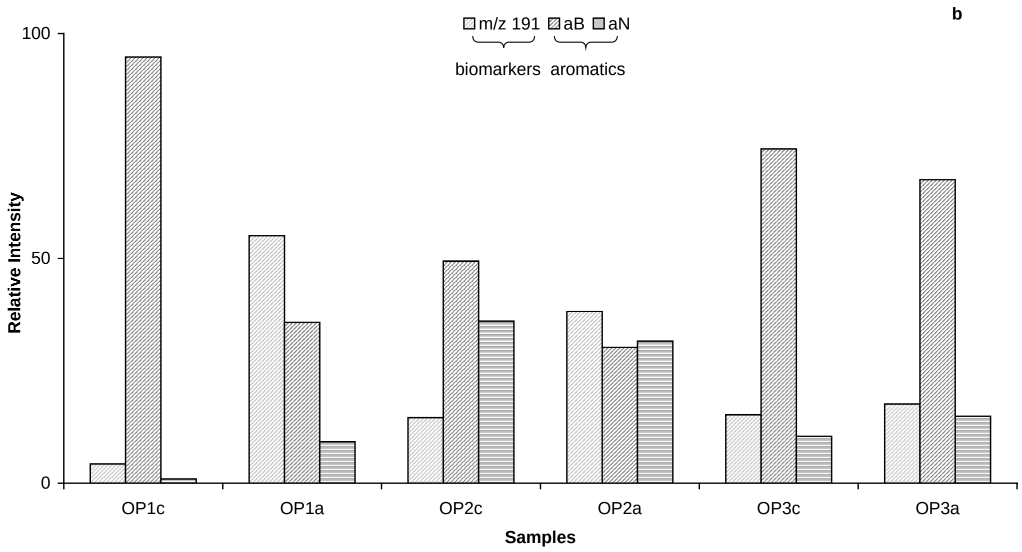

Fig. 2b. Ratios of markers and biomarkers in fresh and artificially aged oil paint reconstructions containing VEGA asphalt. The labels are explained in Table1.

The aromatic compounds displayed by mass chromatograms in Fig. 1b, exhibit the whole range of homologues of alkylbenzenes coded B-B_3 (m/z 77+91+105+119), alkylnaphtalenes coded N-N_3 (m/z 128+142+156+170), alkyl-benzothiophenes coded BT_{1-4} (m/z 148+162+176+190), and alkyldibenzothiophenes coded DBT_{1-4} (m/z 198+212+226+240). The relative ratio between these markers is shown in Fig. 2 and Table 4 showing the alkylbenzenes as the highest in intensity of the aromatic compounds.

Effect of roasting on the composition of asphalt

The 19th-century pretreatment of roasting affects the composition of the asphalt as can be deduced from the (bio)marker profiles in Fig. 3a and b. The TIC points out that the n-alkanes have almost completely disappeared. Some of the peaks at high elution times in the TIC are from hopanoid compounds as can be deduced from m/z 191 and the corresponding mass spectra. The mass chromatogram of m/z 253 shows that the monoaromatic steroids are also strongly affected. It is still possible to trace some of the n-alkanes (up to C_{29}) in the C_{26}-C_{36} elution window. The hopanoids and gammacerane are present but some in diminished relative abundance as shown in Fig. 2 and Table 4 (T_m and H_{29} are still high in intensity, while the intensity of H_{30} has decreased). The changes in the ratios of these different homologues suggest that these compounds are present in different chemically bonded forms. The relative ratio between the classes of biomarkers in the roasted VEGA

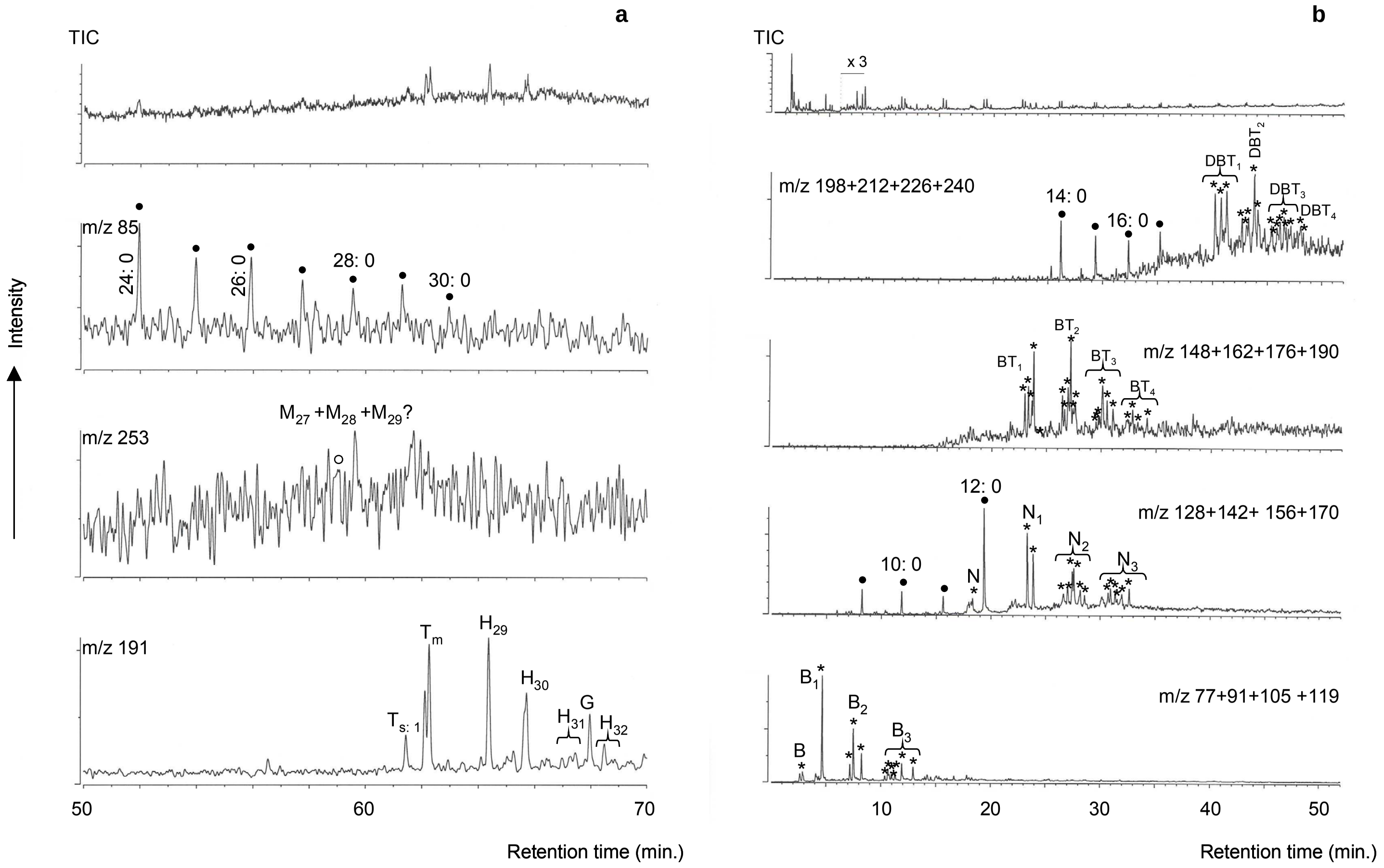

Fig. 3. Biomarkers (a) and markers (b) of the roasted VEGA asphalt sample. Biomarkers: hopanoids (m/z 191), monoaromatic steroids (m/z 253) and the C_{23}-C_{35} n-alkane retention time window (m/z 85). Markers: alkylbenzenes (m/z 77+91+105+119), alkylnaphtalenes (m/z 128+142+156+170), alkylbenzothiophenes (m/z 148+162+176+190) and alkyldibenzothiophenes (m/z 198+212 +226+240).

asphalt mass chromatograms from Fig. 2, shows the hopanoids as the most resistant of the biomarkers as already suggested by Moldowan [55].

The aromatic compounds in Fig. 3b show preservation of the B-B_3, N-N_3, BT_{1-4}, DBT_{1-4} homologues. The alkylbenzene signature remains the strongest one, all the other aromatic compounds being decreased in intensity, as seen in Fig. 2.

Roasting clearly affects some of the (bio)markers like monoaromatic steroids, some of the hopanoids and the higher alkane homologues. The persistence of some of the hopanoid hydrocarbons and alkylaromatics is striking.

Solvent sensitivity of asphalt marker compounds

Since the roasting process affects the composition of the asphalt, the action of solvents on the composition of asphalt was also investigated. The effect of mixing in hot melts of resins was not investigated. Asphaltenes of VEGA were precipitated from a chloroform solution with heptane. The heptane solubles of the VEGA asphalt, i.e. the maltene fraction [29, 56], was subjected to on-column GC/MS. The relative ratios of markers and specific markers of the VEGA maltene fraction (not shown as partial mass chromatograms) are given in Fig. 2 and in Table 4. The asphaltene fraction [29, 56, 57] was analysed by Py-GC/MS and results are shown in Fig. 4a, b. The distribution of markers and biomarkers in the two fractions of the asphalt is shown in Fig. 2 and Table 4.

The n-alkanes in the maltene fraction range from C_{12} (under the current experimental conditions) to C_{39}. Alkylaromatics were not detected. Monoaromatic steroid alkanes searched by the mass chromatogram of m/z 253 show the MA_{27}, MA_{28} and MA_{29} homologues. The series of hopanoids observed in the maltene fraction by the mass chromatogram of m/z 191 goes up to at least homologue H_{35}. Gammacerane is detected as the highest compound in the m/z 191 mass chromatogram.

The asphaltenes show a profile of n-alkanes after pyrolysis ranging up to C_{32}. The asphaltene fraction shows a full range of aromatic compounds similar to those observed earlier for the unfractionated VEGA asphalt. The profile of monoaromatic steroid hydrocarbons in Fig 4a resembles the distribution profile in the unfractionated sample but the ratio of the compounds differs from the maltene fraction. The hopanoids of the asphaltenes in the elution window from C_{26}-C_{36} show a strongly differing profile compared to the maltenes. Trisnorhopanes are relatively prominent while the gammacerane is much lower in intensity. The whole profile resembles the unfractionated sample more closely. All the observations suggest that the maltene fraction is quantitatively smaller than the asphaltene fraction.

Aromatic compounds, which are known to be part of the cross-linked fraction of asphalt (asphaltene) [55-58] are absent in the maltene fraction. The ratio between the aromatic compounds in the asphaltenes, Fig. 2, shows a good match with the relative ratios observed for aromatic compounds in the unfractionated sample. This is in agreement with the information in the literature that aromatic compounds are interconnecting chains in the asphaltenes [56, 58] that are seen here as pyrolysis products.

The marker compounds of asphalt are affected by apolar solvents but the full range of markers present in cross slinked fractions are retained and can be identified using Py-GC/MS. This is reassuring because it means that careful restoration treatment should not negatively affect the analytical tracing of the asphalt in oil paint. It is not entirely clear how boiling or other hot pretreatments affect the asphalt although some loss of the maltenes is to be expected.

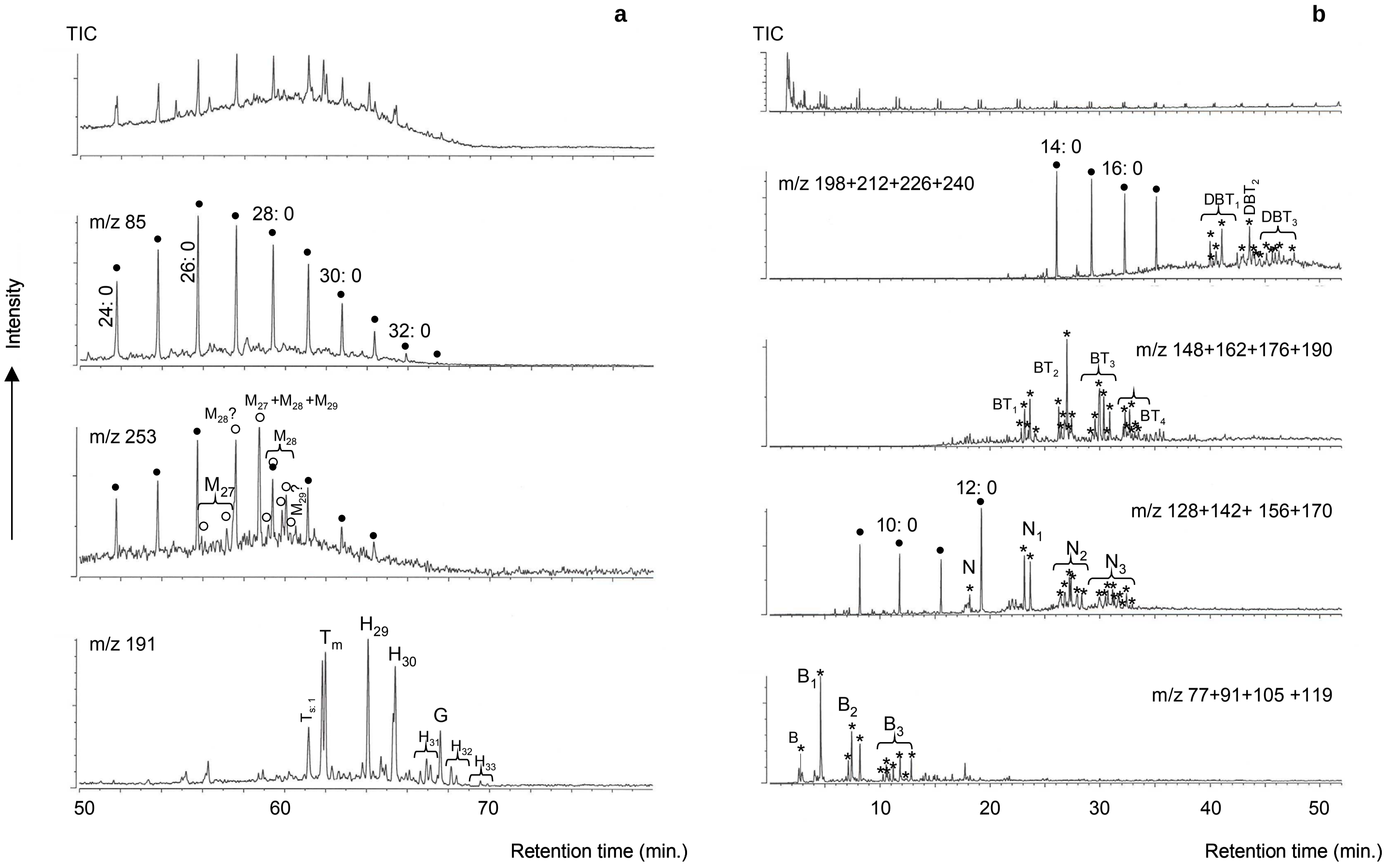

Fig. 4. Biomarkers (a) and markers (b) of the asphaltene fraction of the VEGA asphalt. Biomarkers: hopanoids (m/z 191), monoaromatic steroids (m/z 253) and the C_{23}-C_{35} n-alkane retention time window (m/z 85). Markers: alkylbenzenes (m/z 77+91+105+119), alkylnaphtalenes (m/z 128+142+156+170), alkylbenzothiophenes (m/z 148+162+176+190) and alkyldibenzothiophenes (m/z 198+212 +226+240).

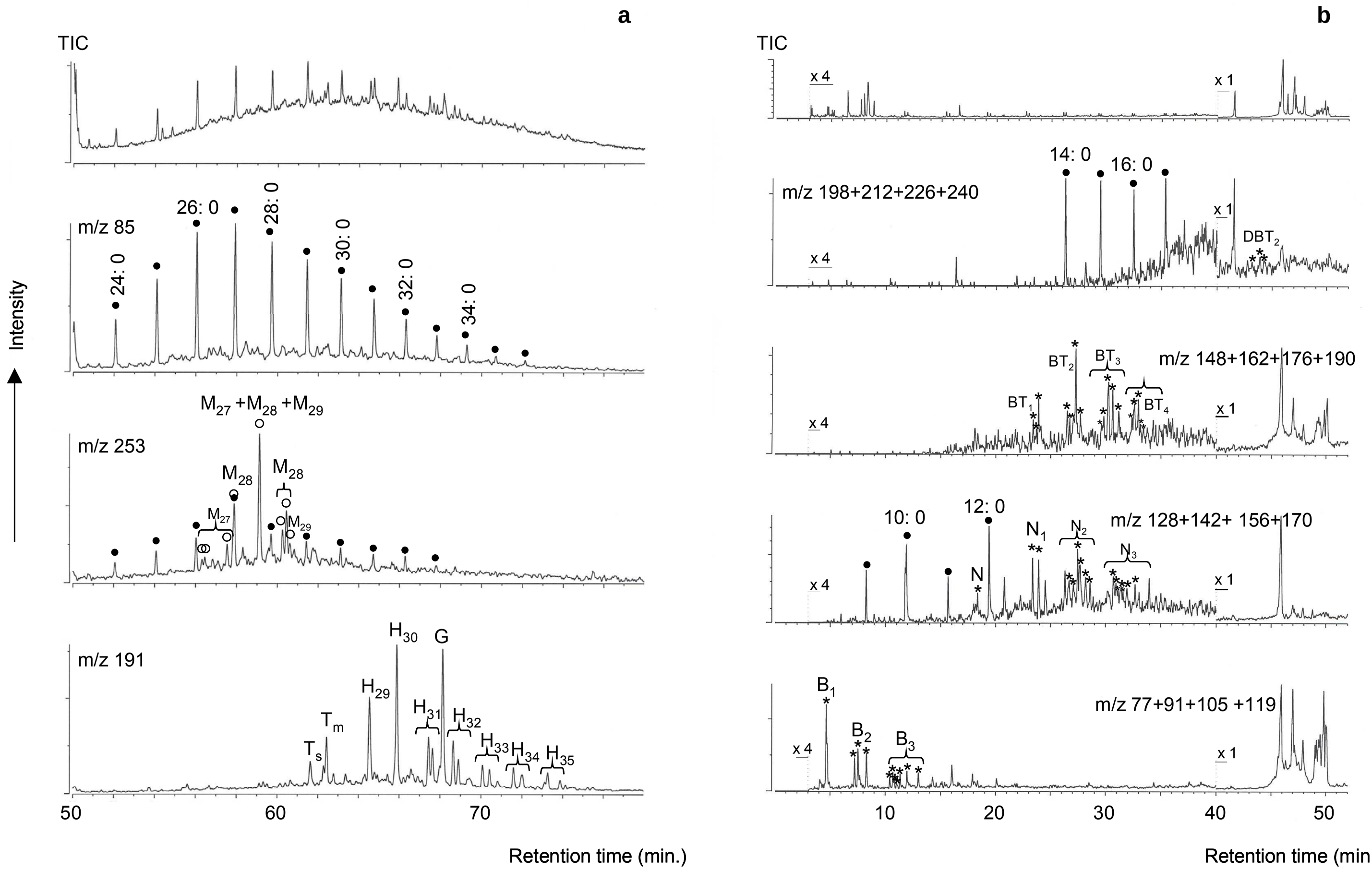

Fig. 5. Biomarkers (a) and markers (b) of the VEGA asphalt-linseed oil control as fresh DCM solution. Biomarkers: hopanoids (m/z 191), monoaromatic steroids (m/z 253) and the C_{23}-C_{35} n-alkane retention time window (m/z 85). Markers: alkylbenzenes (m/z 77+91+105+119), alkylnaphtalenes (m/z 128+142+156+170), alkylbenzothiophenes (m/z 148+162+176+190) and alkyldibenzothiophenes (m/z 198+212 +226+240).

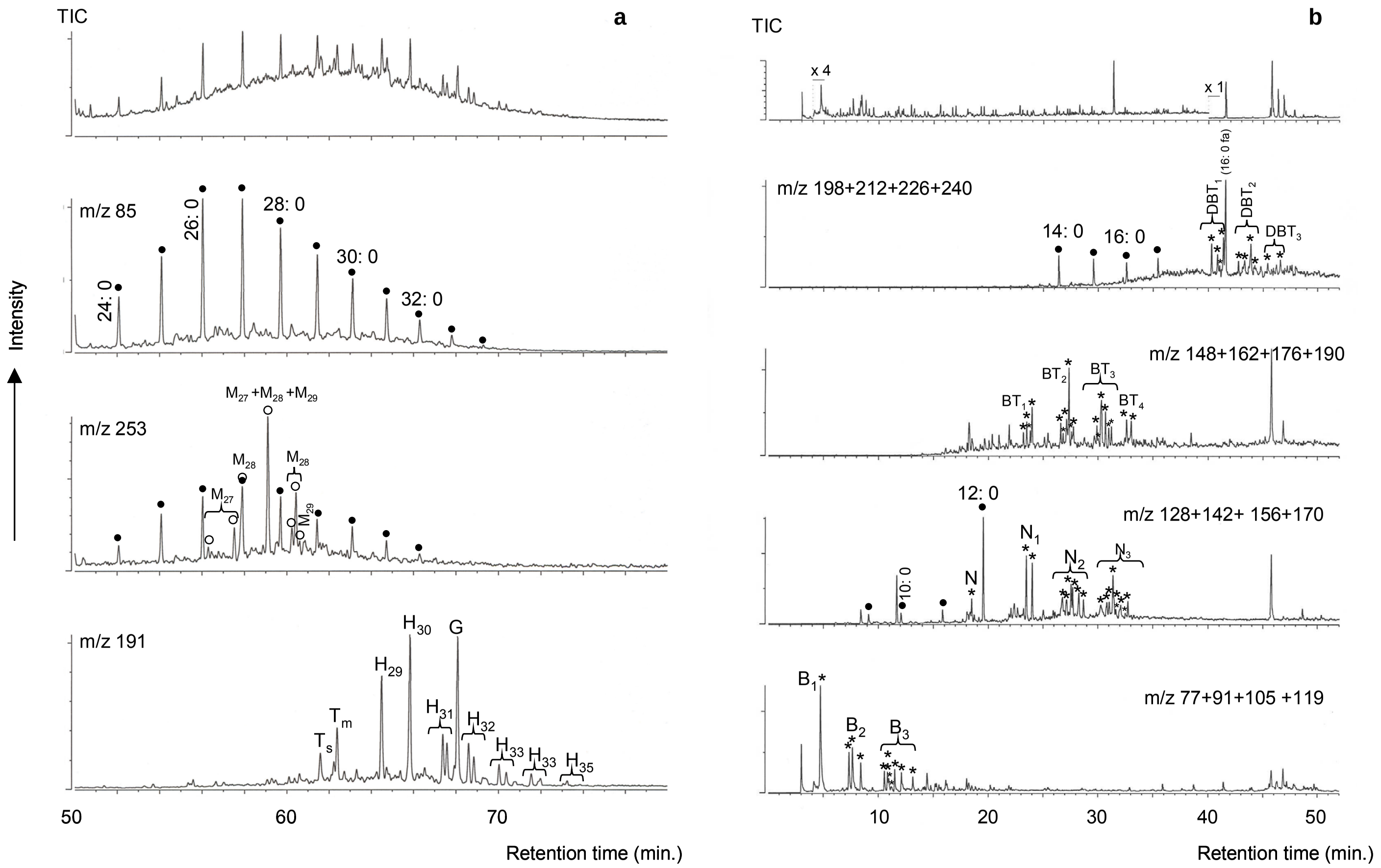

Fig. 6. Biomarkers (a) and markers (b) of the VEGA asphalt-linseed oil DCM solution artificially aged for 12 weeks. Biomarkers: hopanoids (m/z 191), monoaromatic steroids (m/z 253) and the C_{23}-C_{35} n-alkane retention time window (m/z 85). Markers: alkylbenzenes (m/z 77+91+105+119), alkylnaphtalenes (m/z 128+142+156+170), alkylbenzothiophenes (m/z 148+162+176+190) and alkyldibenzothiophenes (m/z 198+212 +226+240).

Accelerated ageing of asphalt in linseed oil-dichloromethane solution

Accelerated ageing in dichloromethane solution has been very effective for the ageing of varnish resins, individual resin components and for study of the degradation of indigo [30, 31, 59]. The accelerated degradation of asphalt and asphalt oil mixtures under the influence of light was therefore studied in this way to simulate the degradation of these compounds under (photo)oxidizing conditions. In this section, the focus is on the effects on the marker signatures. HPSEC results that show that asphalt probably reacts with the oil are reported in section B. Fig. 5a and b shows the markers of the VEGA asphalt pigment after mixing with linseed oil in DCM (control test solution kept at –18 °C in the dark.). Fig 6a and b shows the results after 12 weeks of light exposure at room temperature.

Qualitatively all the hopanes, gammacerane, steroid and aliphatic hydrocarbon compounds observed in the fresh VEGA asphalt are also observed in the fresh asphalt-oil solution. The ratios between the compounds however are not the same. Compared to the fresh asphalt, the asphalt oil solution in DCM shows a difference in the relative amount of the alicyclic hydrocarbons compared to the n-alkanes. The profile of the hopanoids hydrocarbons resembles the m/z 191 profile of the maltene fraction (not shown). The same is true for the monoaromatic steroid hydrocarbons. The aromatic fractions are largely unaffected although some of the dibenzothiophenes (DBT_{3-4}) are not detectable any more. Somehow, the solvent soluble fraction of the asphalt (maltenes) is affected by the dissolution in the oil dichloromethane solution.

Ageing after 12 weeks in the presence of oil leads to complexation of asphalt and drying oil (see section B). The question whether this would affect the marker compounds was studied by Py-GC/MS with tetramethylammoniumhydroxide as transmethylation reagent. The results on the fatty acid methyl esters are reported in the same section B treating the *Effect of asphalt and Kassel earth on the composition of oil paint.* The marker compound signatures in Fig. 6 show that the full range of marker compounds is still detectable. The mass chromatograms of 253 and 191 are very similar to the control sample and look like the distributions observed in the maltene fraction. Gammacerane and the whole series of hopanoids are present, with the same predominance of the H_{30}, H_{29} and H_{31} homologues as observed for the fresh solution. The monoaromatic steroid pattern and the presence of alkane series are well preserved. When comparing the ratios of markers and biomarkers with the control sample, in Fig. 2, the relative amount of aliphatic chains appears diminished by a factor of two, compared to the relative amount of hopanoid compounds. In a similar way the hopanoids seem to have diminished compared to the monoaromatic steroids.

The alkylaromatic hydrocarbons in the Py-GC/MS data also show a high degree of similarity with respect to the fresh asphalt and the unaged solutions. It was evident that dibenzothiophenes were not always completely detectable. In the unaged sample for example, the DBT_3 and DBT_4 were absent although they could be detected in the corresponding sample without addition of oil. The full range of benzothiophenes was again detectable in the sample after 12 weeks of photoxidation. Linseed oil is known to react with sulphur compounds [60]. Whether this reactivity is an issue in these experiments is difficult to judge because there does not seem to be a consistent pattern. When comparing the ratios of aromatics in the aged sample and the control sample, Fig. 2, the alkylbenzenes show a much higher abundance compared to other aromatic compounds. Alkylnaphtalenes and the sulphur containing aromatics show similar ratios compared to the control sample.

Remarkably, the asphalt without the oil (not shown) shows a great resemblance after 12 weeks to the sample with drying oil suggesting that the asphaltic components are relatively resistant to the photoxidative regime that had been imposed. Asphalt can be severely photoxidised under external sunlight conditions leading to increased amounts of aromatic ketones, alcohols, carboxylic acids and sulfuroxides/sulfones [61]. The degradation conditions are much milder although the formation of these compounds can not be excluded. The used protocol may not be able to detect the effect of the oxidation processes expressed by these classes of compounds. Although there is no detectable effect on the asphalt itself, the effect of degradation in dichloromethane solution on the oil is very prominent (see section B).

Asphalt oil paints

Asphalt oil paints were prepared after 19th-century recipes [4, 5, 24] using the VEGA asphalt as pigment. Details about the preparation methods of the William's "Antwerp Brown" paint (OP_1), Merimée's English asphalt paint (OP_2) and another asphalt paint of Merimée (OP_3) are given in Table 1. These paints were painted out on a lead white ground. The samples were artificially aged for 3 months at SRAL to an age that which equals approximately 40 years of light conditions in a museum (see the Experimental section). The asphalt containing oil paints were sampled before (controls) and after ageing. Samples were subjected to Py-TMAH-GC/MS analysis to study the fatty acids from the oil and to asphalt components in one analytical run. The pyrolysis and GC oven conditions are specified in Table 3. The distribution of markers in the samples is shown in Fig. 7$a_{1,2}$, $b_{1,2}$, Fig. 8 $a_{1,2}$, $b_{1,2}$, and in Fig. 2.

Analysis of the control samples before ageing is showing that only some of the hopanoid and aromatic characteristics of the asphalt are preserved after preparation and drying of the paint. The monoaromatic steroid profile could not be detected, which prevents the use of the steroid compounds as asphalt markers in the paint. The aromatic compounds containing sulphur and the long chain n-alkanes could not be detected as well. Due to a flaw in the collection of controls, the actual pigments used in the preparation of the paints were unavailable for analysis. The pretreatment of the asphalt in the form of roasting and melting into resins before incorporation into the paint, however, very likely destroyed a number of the molecular features (see the effect of roasting on the composition of asphalt in section A). The hopanoid compounds were more resilient as was already clear from the analytical data of the roasted asphalt. A small population of hopanoids compounds could be detected. The H_{30} and H_{29} are the highest in intensity in all the OP_i fresh paints. The T_m trisnorhopane, the H_{29}, H_{30} and H_{31} homologues were found in OP_1. Only the H_{29} and H_{30} homologues are detected in OP_2. In the OP_3 sample the detected hopanoids are the T_m, and the H_{29} and H_{30} homologues. The T_s trisnorhopane, or its monounsaturated equivalent and the gammacerane are absent for all three paint samples and the highest hopane homologue, H_{31}, is observed only in OP_1.

The aliphatic chains of asphaltic origin are absent in all the paint samples. Some beeswax derived n-alkanes are detectable in the OP_3 sample. The beeswax provenance is confirmed by the recipe that mentions the use of beeswax and moreover the predominance of the odd alkane homologues C_{25}, C_{27}, C_{29} and C_{31} is specific for beeswax.

Few aromatic compounds survived the making of the oil paint. Benzene is absent in the analytical data of all three samples but toluene and alkylbenzenes up to

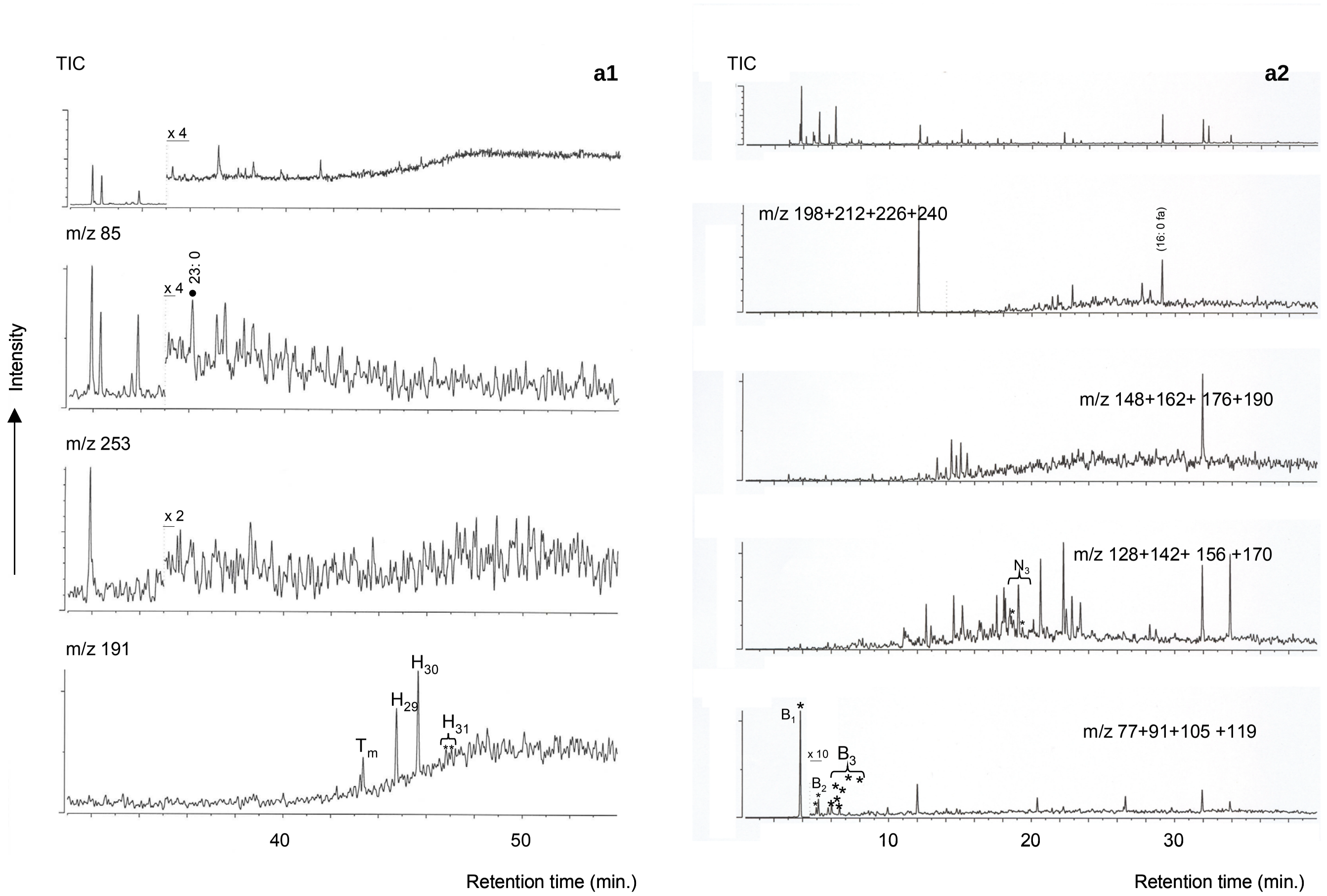

Fig. 7a$_1$, a$_2$. Biomarkers and markers of the fresh VEGA asphalt oil paint reconstructions: William's Antwerp brown method (a$_1$, a$_2$) used as control after curing. Biomarkers: hopanoids (m/z 191), monoaromatic steroids (m/z 253) and the C_{23}-C_{35} n-alkane retention time window (m/z 85). Markers: alkylbenzenes (m/z 77+91+105+119), alkylnaphtalenes (m/z 128+142+156+170), alkylbenzothiophenes (m/z 148+162+176+190) and alkyldibenzothiophenes (m/z 198+212 +226+240).

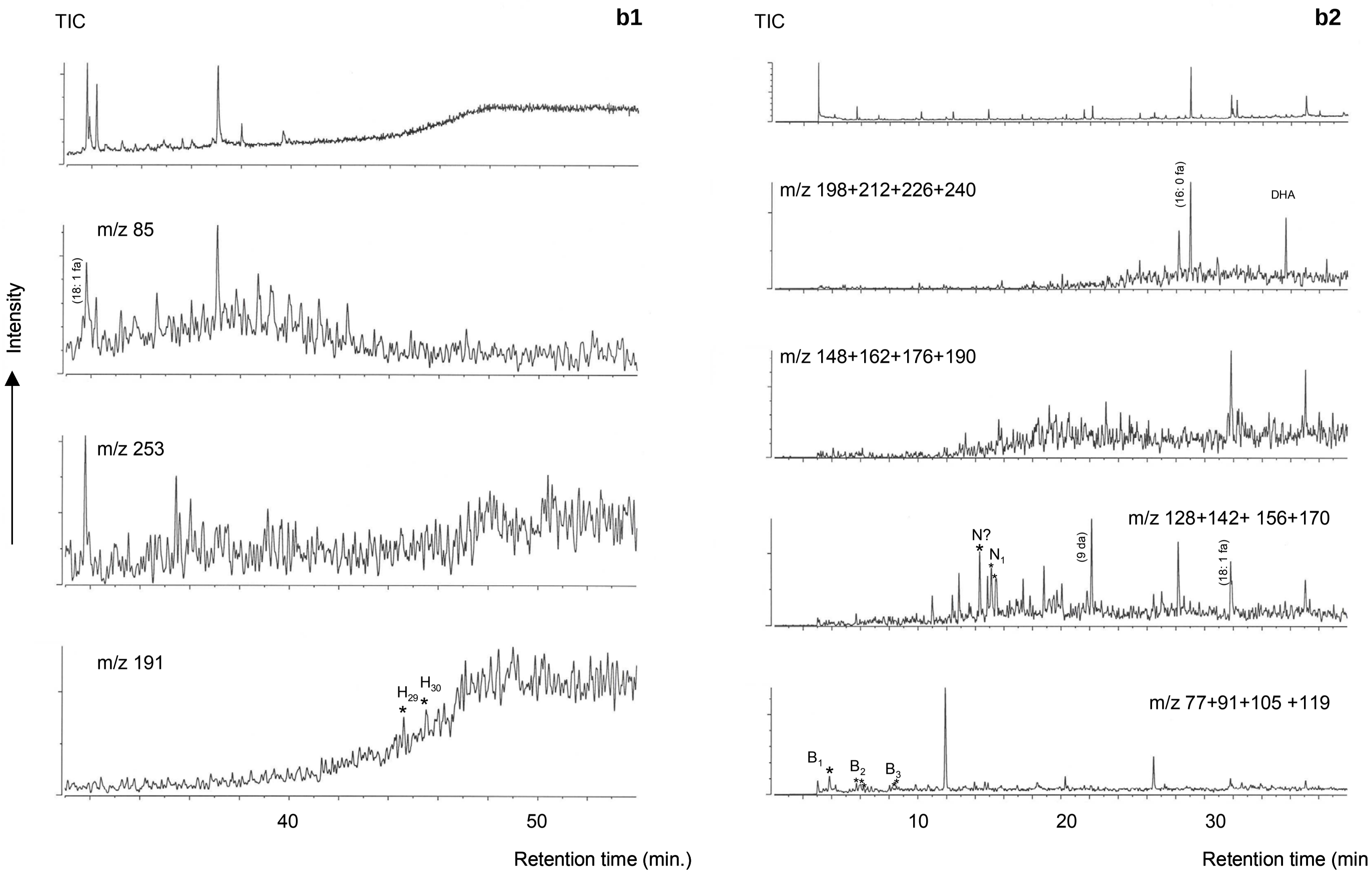

Fig. 7b$_1$, b$_2$. Biomarkers and markers of the fresh VEGA asphalt oil paint reconstructions: Merimée English's method (b$_1$, b$_2$) used as control after curing. Biomarkers: hopanoids (m/z 191), monoaromatic steroids (m/z 253) and the C_{23}-C_{35} n-alkane retention time window (m/z 85). Markers: alkylbenzenes (m/z 77+91+105+119), alkylnaphtalenes (m/z 128+142+156+170), alkylbenzothiophenes (m/z 148+162+176+190) and alkyldibenzothiophenes (m/z 198+212 +226+240).

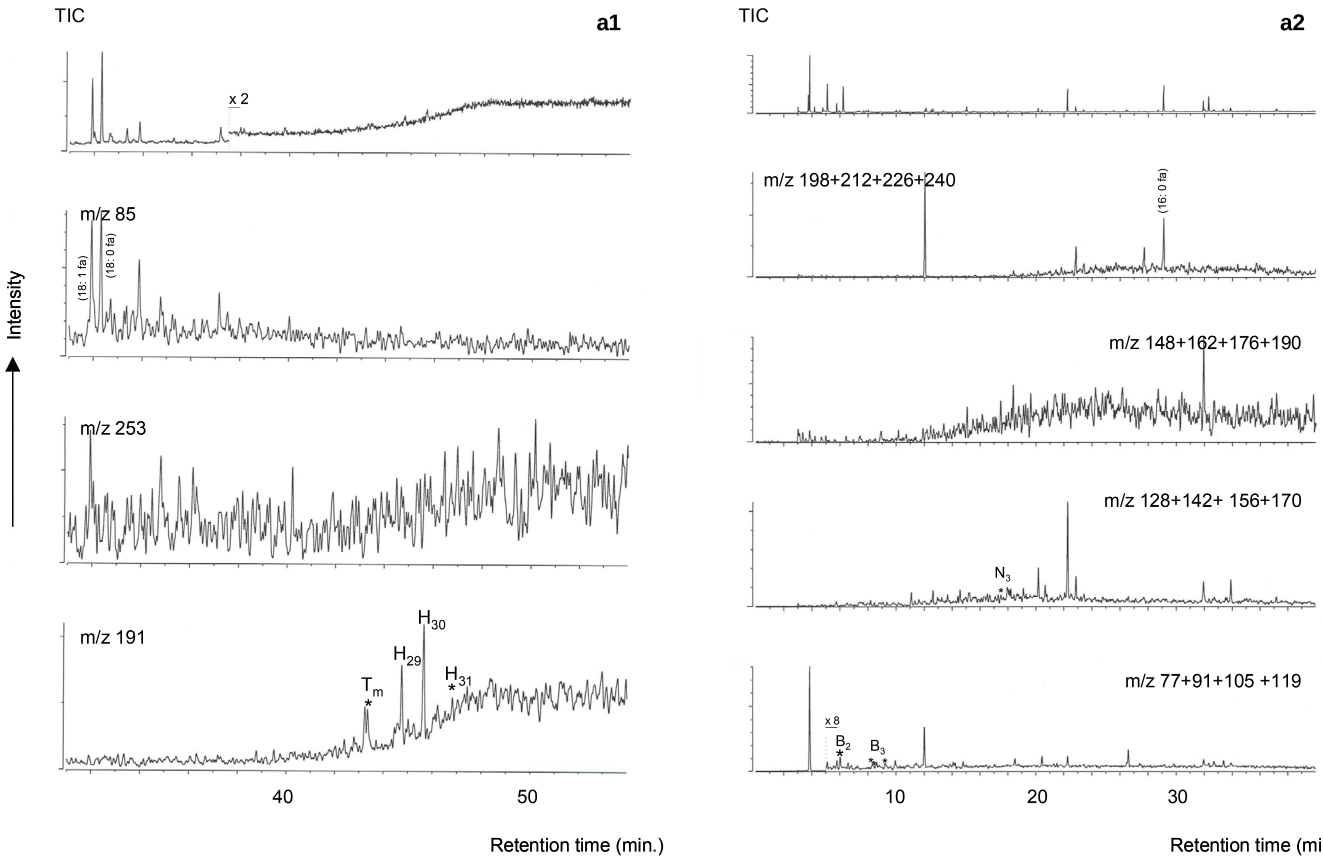

Fig. 8a$_1$, a$_2$. Biomarkers and markers of the VEGA asphalt oil paint reconstructions artificially aged for 3 months: William's Antwerp brown method (a$_1$, a$_2$). Biomarkers: hopanoids (m/z 191), monoaromatic steroids (m/z 253) and the C_{23}-C_{35} n-alkane retention time window (m/z 85). Markers: alkylbenzenes (m/z 77+91+105+119), alkylnaphtalenes (m/z 128+142+156+170), alkylbenzothiophenes (m/z 148+162+176+190) and alkyldibenzothiophenes (m/z 198+212 +226+240).

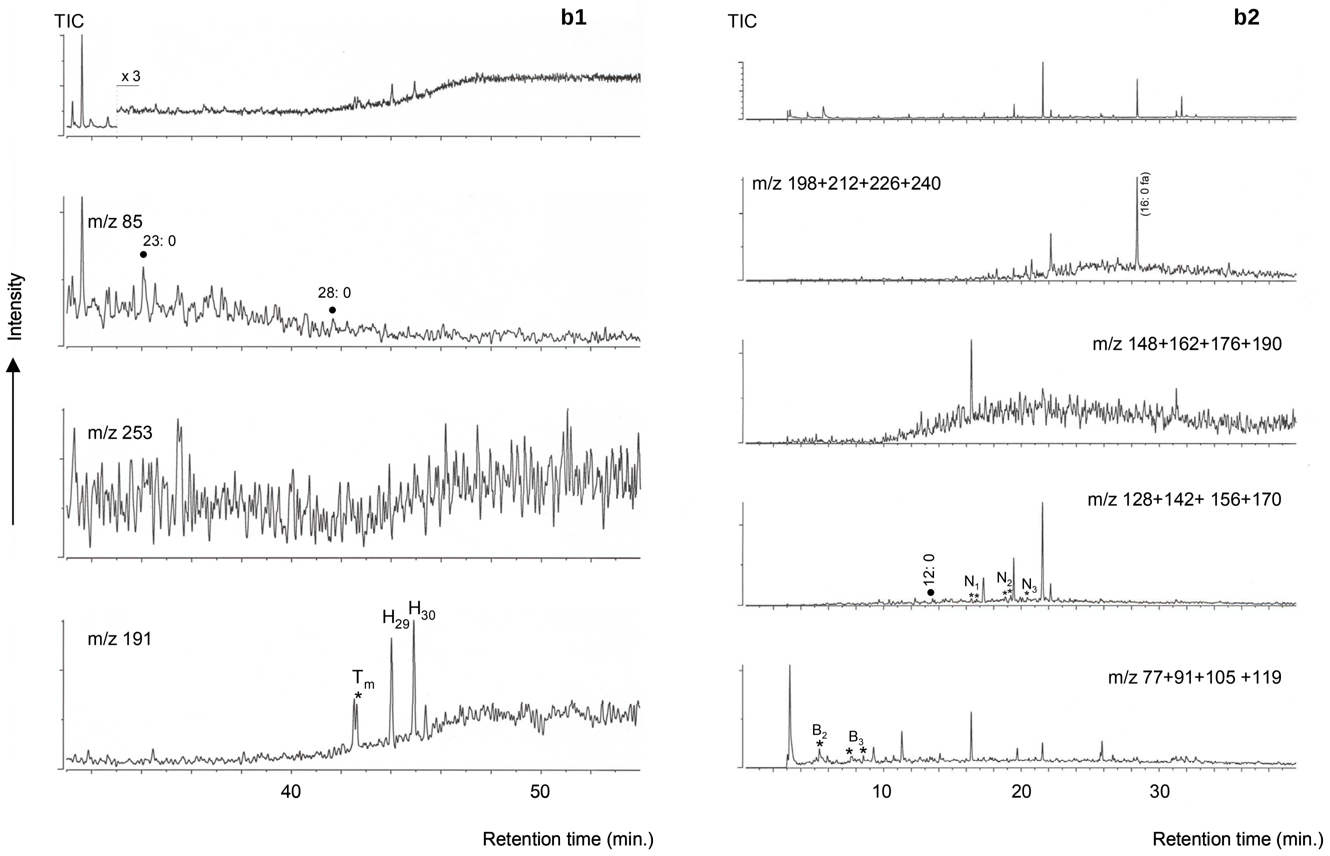

Fig. 8b$_1$, b$_2$. Biomarkers and markers of the VEGA asphalt oil paint reconstructions artificially aged for 3 months: Merimée English's method (b$_1$, b$_2$). Biomarkers: hopanoids (m/z 191), monoaromatic steroids (m/z 253) and the C_{23}-C_{35} n-alkane retention time window (m/z 85). Markers: alkylbenzenes (m/z 77+91+105+119), alkylnaphtalenes (m/z 128+142+156+170), alkylbenzothiophenes (m/z 148+162+176+190) and alkyldibenzothiophenes (m/z 198+212 +226+240).

the C_3- substituted benzene are present. Naphthalene is absent only in OP_1, but the N-N_3 naphthalenes are present in all fresh samples. All the sulphur containing compounds are absent.

The analysis of the samples after artificial ageing is showing similar results as for the non-aged samples. With respect to the hopanes, the T_m, H_{29} and H_{30} homologues are traceable in the aged paint reconstruction (Table 4, Fig. $8a_1$,b_1), H_{30} or H_{29} showing the highest relative intensity. The monoaromatic steroids are absent. Aliphatic chains characteristic for asphalt are not observed but alkanes originating from beeswax (C_{27}, C_{29}, C_{31}) were detected in the aged OP_3. Only some alkylbenzenes, some of the higher homologues, are present in the aged samples (Table 4). Benzene is absent in all three aged samples. Naphthalene is certainly absent in two of them, OP_{1a} and OP_{3a}. Their partial disappearance had been noticed already in the fresh and aged asphalt-oil solutions. Fig. 2 presents semiquantitative relationships calculated on the basis of mass chromatograms of the markers listed in Table 4. The difference between OP_3 control and aged is negligible. In OP_2 a difference is observed in the relative intensity of the hopanoids that are higher after ageing. The same phenomenon is observed in OP_1. Alkylmarkers are strongly affected by ageing in OP_1, but alkylnaphtalenes become more prominent after ageing. The sulphur containing compounds as (di)benzothiophenes are also absent in the aged oil paint reconstructions.

In conclusion, marker compounds can be traced using the Py-TMAH-GCMS but their relative amounts are strongly affected by the preparation method of the pigment in the oil paint production process. Changes due to ageing appear to be of secondary importance. This introduces the possibility of tracing asphalt in complex paint samples with this technique.

Summarising this subsection, it can be said that asphalt (bio)markers in artificially light aged asphalt-linseed oil solutions are rather well preserved, which suggests that the conditions are too mild for extensive degradation of the asphalt molecule. The fate of marker compounds in oil paint that contain asphalt is certainly related to the pretreatments applied to the initial pigment. High temperatures have shown to severely affect the composition and signature of an asphalt. Only hopanoid markers and alkyl aromatics are preserved. This was confirmed when artificially aged asphalt containing oil paints prepared according to 19th-century recipes were analysed. In this case only some of the hopanes and some of the alkyl aromatics are still present. Solvent cleaning is inferred to have similar actions to solvent extraction of an asphalt. Apolar solvents were shown to remove part of the biomarkers, present in the maltene fraction. Only hopanes seem to be reliable for the identification of asphalt in the aged oil paint reconstructions. Unfortunately, the oil paint production process involving asphalt pigment is unknown when samples suspected to contain asphalt components are taken from a painting. As a consequence, the determination of asphalt in painting samples is surely difficult and the data will be hard to interpret.

B) Effect of asphalt and Kassel earth on the composition of oil paint

In the sections above, the emphasis was placed on the chemical nature of asphalt and Kassel earth as pigment materials. The next section will present data on the effect of addition of these pigments on the composition of the drying oil. Data are presented on the kinetics of the drying of linseed oil in the presence of pure asphalt, on the accelerated ageing of oil with and without pure asphalt in solution and on the effect of asphalt and Kassel earth on the composition of drying oil paint.

Fatty acids, dicarboxylic acids and their ratios in ageing oil paint

Linseed oil as drying oil is a mixture of triglycerides (TAGs) containing mostly saturated C_{16} and saturated and unsaturated C_{18} fatty acid chains that will oxidise and cross-link upon exposure to light and oxygen [62-66]. The drying of linseed oil can be summarised as the “consumption” of double bonds. This is observed also in the Py-TMAH-GC/MS and ESI-FTICR-MS measurements of the linseed oil DCM test solutions (see further in section B). The decrease in the relative amount of unsaturated C_{18} fatty acids with the progress of chemical drying, preferentially affects the more unsaturated $C_{18:3}$ and $C_{18:2}$ fatty acids and only at a later stage the $C_{18:1}$ fatty acids. It has been shown before that the monounsaturated C_{18} fatty acids react away about 1000 times slower than the polyunsaturated C_{18} fatty acids [67, 68]. The fact that $C_{18:1}$ survives longer in the paint makes this compound a possible marker for monitoring the drying of (linseed) oil paint if it is compared to the saturated C_{18} fatty acid as an oil internal “standard”. For this reason the ratio, i.e. $C_{18:1}$: C_{18} FA, is used as a tool to monitor the drying of linseed oil. Values higher than one correspond to a still relatively high content in $C_{18:1}$ fatty acid, i.e. a poor drying of the oil. Ratios lower than one are related to a good or normal drying condition of the oil. The lower the values below one, the more ideal the drying of that sample.

Chemical drying of drying oils introduces peroxides that are later on converted into volatile matter and residual matter enriched in oxygen containing functional groups like aldehyde and acids. Introduction of antioxidants like asphalt and related organic rich pigments are likely affecting this process. The relative amounts of dicarboxylic acids are possible markers for the degree of antioxidising activity. The ratio of azelaic acid (C_9 dicarboxylic acid) to stearic acid (C_{18} or $C_{18:0}$ fatty acid), C_9 DA: C_{18} FA, is introduced as a measure of the chemical drying of oil. The unsaturated C_{18} fatty acids are considered the source of the azelaic acid in the advance of the drying process while the stearic acid is the indifferent component taken as an internal standard. Oil paints after a longer period of time change into an ionomeric structure because the glycerol ester bonds hydrolyse to form free acids among which unsaturated C_{18} fatty acids and dicarboxylic acids that react with metals (mostly lead) from pigments and driers [64-66]. The availability of such cations in asphaltic oil paints may be an issue in the overall stability of a century old asphaltic oil paint. I.e. the ionomeric stage is not reached. This is the explanation why a low relative amount of azelaic acid (a C_9 DA: $C_{18:0}$ FA ratio much below one) is indicative for antioxidant activity. A rather high C_9 DA: $C_{18:0}$ FA ratio (close to 1 or higher) is indicative for a normal, respectively a good drying of the oil paint.

Kinetics of drying of an asphalt-oil mixture (FTIR study)

FTIR was used to monitor the induction time for the drying of the linseed oil with and without asphalt following a method introduced by Muizebelt et al. [67-69]. Van der Weerd has described the setup [70]. The induction time is related to the time required for the *cis* double bonds originally present in the fatty acid chains of the oil TAGs to change into their *trans* configuration. The *trans* configuration favours the steric interaction between free radicals after the initiation reaction has taken place with the atmospheric oxygen as well as the reaction of the intact TAGs chains with the peroxi radicals formed (propagation reaction). [66-68, 71].

Results are shown in Fig. 9 for an asphalt-linseed oil mixture (5 % asphalt), pure linseed oil and linseed oil plus 5 % smalt. The y axis gives the intensity of the absorbance characteristic at 3010 cm^{-1} of the *trans* C-C double bond. The x axis shows the induction time. The shortest induction time of 60 hrs is observed for the linseed oil plus smalt. The relative absorbance for 3010 drops very quickly leading to almost zero after 120 hours. Linseed oil alone shows a longer induction time of 80 hrs and shows a much slower drop off. The longest induction time of about 160 hrs is observed for the asphalt-linseed oil mixture. Thus asphalt clearly slows down the chemical drying of oil.

Accelerated ageing of asphalt and drying oil in dichloromethane solution

Comparative studies were performed on the accelerated ageing of linseed oil, asphalt and asphalt-linseed oil solutions in dichloromethane with DTMS, ESI-FTICR-MS, SEC and Py-TMAH-GCMS. Samples were exposed to light for 12 weeks. Samples were taken at once (control) and after one, two, three, four, eight and twelve weeks.

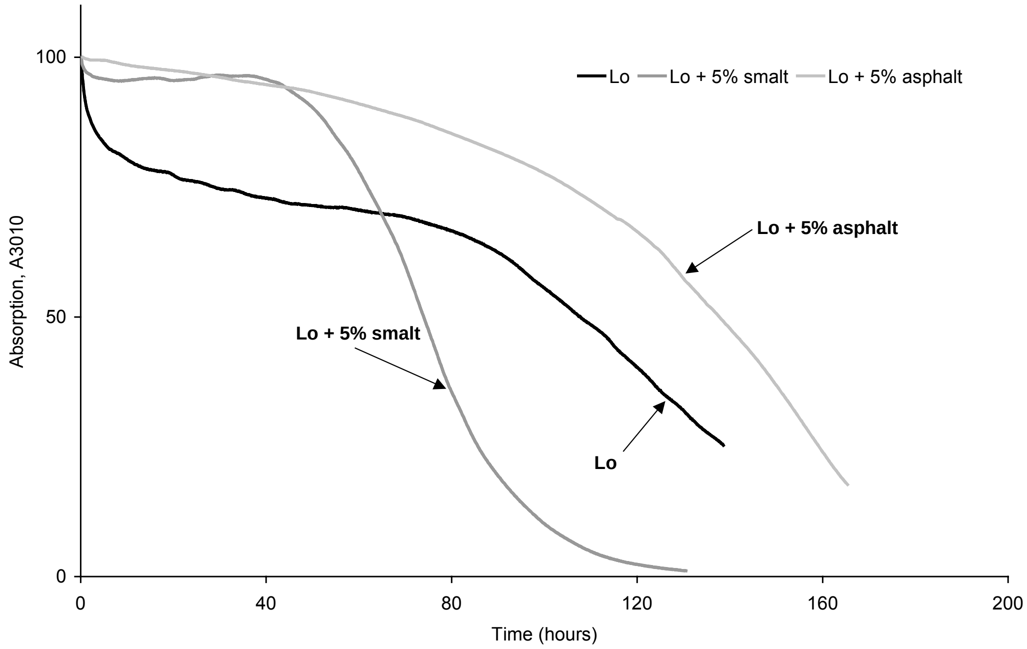

Fig. 9. Induction time of the oxidation of linseed oil, a linseed oil with 5 % smalt drier and a linseed oil with 5 % asphalt.

The chemical composition of the linseed oil over the 12 weeks of exposure analysed by DTMS changes substantially leading to a rapid decrease of the polyunsaturated moieties and the appearance of cross linked fractions relatively enriched in saturated fatty acid moieties. The appearance of the asphalt DTMS spectra remains rather similar. The DTMS data obtained for the artificially light aged asphalt-linseed oil solutions and the linseed oil solutions for 1-12 weeks are compared by discriminant analysis (DA). The control samples of linseed oil or asphalt-linseed oil solutions are included in the discriminant analysis. The scatter plot of the first two discriminant functions (DF1 and DF2) is shown in Fig. 10a for linseed oil and asphalt-linseed oil solutions. Linseed oil solutions are coded as “Lo” followed by a number indicative for the weeks of artificial light ageing (1, 2, 3, 4, 8, 12) or the letter “c” for the control samples. E.g. Lo1 is a linseed oil solution artificially aged for 1 week.

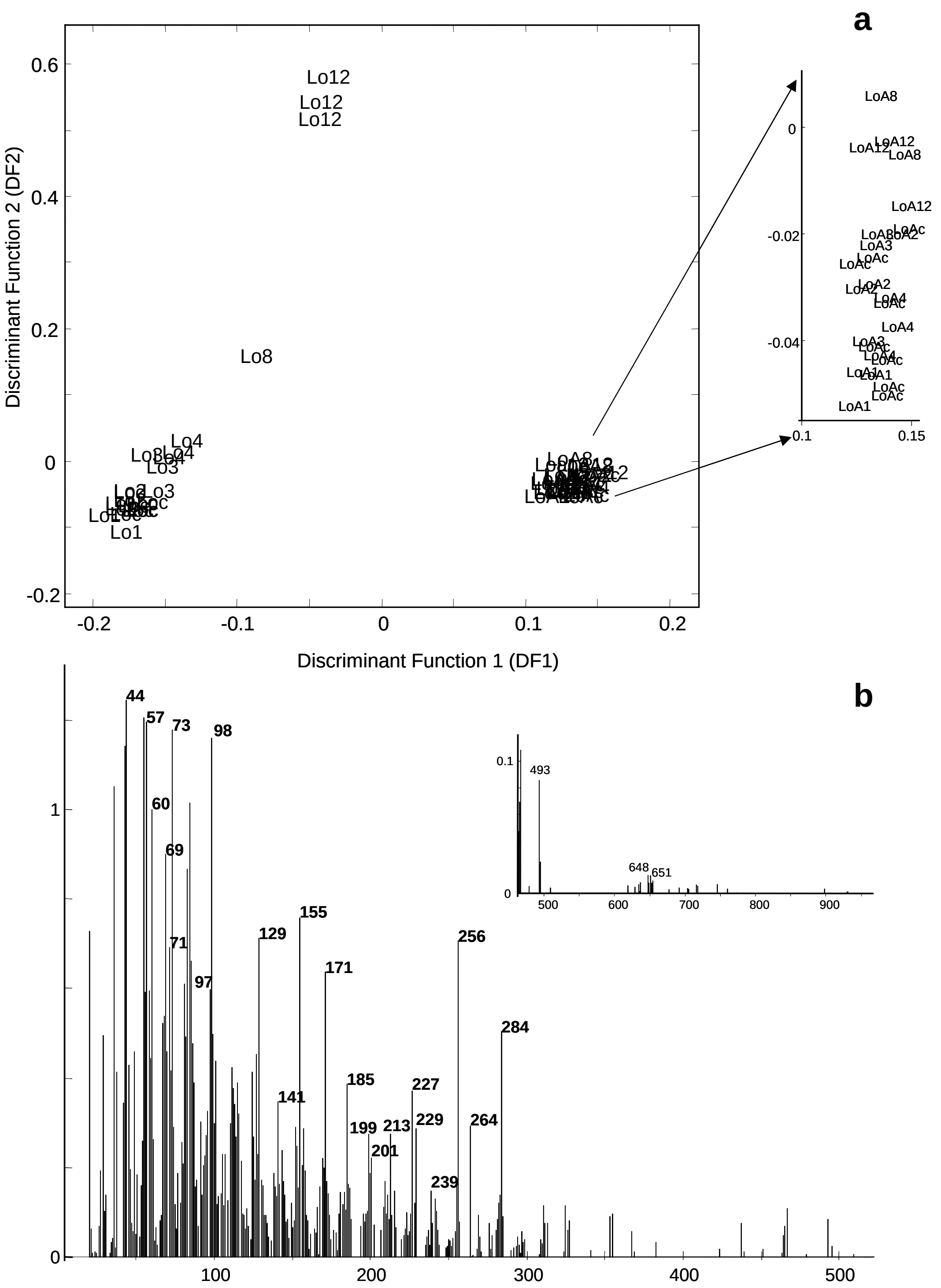

Fig. 10. Discriminant analytical map of the chemical composition (DTMS data) of VEGA asphalt-linseed oil DCM test solutions (right) in comparison with linseed oil DCM test solutions comparing initial state (control) and solutions artificially aged for 1, 2, 3, 4, 8 and 12 weeks (a). Mass spectrum of the DF2 (b).

Asphalt-linseed oil solutions are coded as “LoA” followed as well by a number (as above) or the letter “c”, having the same significance as for the linseed oil solutions. The distribution of samples along DF1 indicates the different composition of the samples, linseed oil solution, Lo, in the negative side of DF1 and linseed oil solutions containing asphalt, LoA, in the positive side of DF1 (see also the enlarged detail for LoA solutions). Another distribution of samples is observed along the DF2 axis. The control Lo solutions and the solutions aged for 1-4 weeks are very much grouped in the same area in Fig. 10a. The Lo solution aged for 8 weeks, Lo8, is further away on the DF2 axis and the Lo solution aged for 12 weeks even further away, on the positive side of DF2 axis.

These observations indicate that the DF2 axis represents the time axis and the distribution along this axis coincides with the changes in composition of the linseed oil solution from control to after 12 weeks of artificial light ageing. Similar assignments can be made also for the LoA solutions. It is clear that the composition of asphalt-linseed oil solutions (DF1+) along DF2 axis is almost constant in time compared to the composition of linseed oil solutions along the same axis. The enlargement shown on the right side of the figure points out that the composition of solutions of linseed oil in the presence of asphalt, LoA, also changes in time but much slower! So drying linseed oil changes qualitatively much more than when mixed with asphalt. Asphalt alone does not change too much on this time scale. It is believed that the access of oxygen to the oil triglycerides is hindered by physical interactions but also chemical interactions between asphalt and linseed oil. Indications for the chemical interactions between asphalt and oil are observed for example in the DTMS measurement of the 12 weeks LoA solution. Peaks characteristic for asphalt and linseed oil are present in the mass spectrum corresponding to the pyrolysis event in the TIC. The pyrolysis event in the TIC of a DTMS measurement corresponds to material chemically bound in a network. Linseed oil is represented by peaks corresponding to C_{16}, $C_{18:\ 1}$ and C_{18} fatty acids (25% relative abundance) at m/z 256, m/z 264 and m/z 284, respectively, as well as peaks corresponding to C_{55} and C_{57} TAGs (3% relative abundance). These are symmetrically distributed clusters around m/z 854 (probably $C_{55:\ 4}$ TAG) and m/z 878 (probably $C_{57:\ 5}$ TAG). In the same LoA12 solution asphalt peaks are represented by m/z 191, 370, 398, 412, 426, 440. 454, 468 and 482 for hopanoids (30% relative abundance of the molecular ions), m/z 231 and 245 for triaromatic steroids, m/z 267 for methylmonoaromatic steroids, m/z 217 (218), 372, 386, 414, 428 for 14α(β) steranes (30% relative abundance of the molecular ions).

As suggested above the presence of both asphalt and linseed oil peaks in the chemically bound fraction of the LoA12 solution points to chemical interactions between the two. The oil component appears to have been drying (i.e. cross-linked and oxidised) to some extent, which is supported by the low relative abundance of TAGs and the much higher relative abundance of fatty acid moieties. Oxidation and especially cross-linking of the two are suggested and addressed again later on inthe text. The spectrum for the positive side of DF2 (Fig. 10b) as already suggested by the DF1-DF2 plot, gives determining features in the mass spectrum of the 12 weeks aged linseed oil solution. The higher intensity peaks are observed in the fragment ion range below m/z 190. Fatty acids as such or released by elimination reactions are indicated by m/z 284 ($C_{18:\ 0}$), 264 (M-H_2O of $C_{18:\ 1}$) and 256 ($C_{16:\ 0}$) and less specific fragment ions at m/z 185, 129, 98, 84, 73, 60 and 57. The ions at m/z 155 and 171 are assigned to mid-chain hydroxystearic acids after observed in GC/MS data of dried linseed oil. Azelaic acid shows a specific fragment ion at m/z 152, which is inconspicuous in the spectrum in Fig. 10b. Very few peaks and of very low relative intensity are observed in the mass range above m/z 300. The absence of peaks typical for diglycerides and triglycerides in the fresh linseed oil (or the low content in double bonds) [66] together with the presence of peaks indicative of dicarboxylic acids as oxidative products of oil confirm the advanced state of oxidation of the oil after 12 weeks of artificial light ageing. As mentioned above when asphalt is present in the linseed oil solution less oxidation of the oil is observed (peaks of diglycerides and triglycerides are still present). To summarise, the presence of asphalt in the linseed oil solution is interpreted as impeding a normal drying of the oil (see the changes in chemical composition for the linseed oil solutions).

More information about the drying of the asphalt-linseed oil solutions was derived from ESI-FTICR-MS and HPSEC data by comparison with data obtained for linseed oil test solutions.

The oxidation of the oil was monitored as the oxygen uptake in the TAGs using ESI-FTICR-MS. The main triglycerides present in linseed oil are the TAGs containing C_{57} and C_{55} carbon atoms (the C_{55} TAGs and C_{57} TAGs), i.e. $C_{18:\,n}$- $C_{18:\,n}$-$C_{18:\,n}$ and C_{16}-$C_{18:\,n}$- $C_{18:\,n}$, where n has values between 0 and 3. Van den Berg has assigned the compounds in the ESI-FTICR-MS of linseed oil in an earlier study [66, 72]. Fig. 11 shows ESI-FTICR-MS data of the C_{55} and C_{57} TAGs area (m/z 850-980) for the control linseed oil solution (Loc), linseed oil solution after four (Lo4) and twelve weeks (Lo12) of artificial light ageing, and asphalt-linseed oil solutions after four (LoA4) and twelve weeks (LoA12) of artificial light ageing. The inserts in Fig. 11 display the compounds in the mass range up to m/z 1200. The clusters of TAGs are coded as C_{55} and C_{57}, corresponding to the number of carbon atoms in their structure. The numbers above each peak in the mass spectra in Fig. 11 correspond to the sum of the double bonds present in the fatty acid moieties of the triglycerides (Table 5). The clusters at higher m/z values in the same figure and inserts are oxidation products of the C_{57} TAGs. The number of the supposedly incorporated oxygen atoms after comparing the measured mass with the exact value is marked above as “+1”, “+2”, etc. The measured molecular masses for the compounds observed with ESI-FTICR-MS, which are triglycerides and their oxidised compounds, were compared with the exact (calculated) molecular mass. The exact and measured values of the C_{55} and C_{57} triglycerides are given in Table 5. The measured values in Table 5 originate from the ESI-FTICR-MS measurement done for the 4 weeks aged linseed oil test solution. A slight mass difference (error)[4] from the exact mass is observed for the oil TAGs in the test solutions. Surprisingly, it was observed that the error was lower when the solutions were less aged[5].

The fresh oil very quickly incorporates two atoms of oxygen during preparation of the control solution [66] and this is shown in Fig. 11 (see Loc). The C_{55} TAGs with 6, 5, 4 double bonds and the C_{57} TAGs with 9 double bonds are the main compounds of the Loc solution. At least 18 atoms of oxygen were incorporated in the C_{57} TAGs after 4 weeks of artificial light ageing (see Lo4 and insert, in Fig. 11).

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[4] The error, ε, was calculated as the modulus of the difference between the exact mass of the compound and the measured value ($\varepsilon = |MW_{exact} - MW_{measured}|$, in amu).

[5] For example values of 10^{-3} were calculated for the control linseed oil solution and the 4 weeks aged linseed oil solution with a few values in the 10^{-2} or 10^{-1} range for the 4 weeks aged linseed oil solution. The 4 weeks aged asphalt-linseed oil solution gives mass errors of 10^{-2}. The values of ε for the 12 weeks aged solutions are in both cases in the range of 10^{-1}.

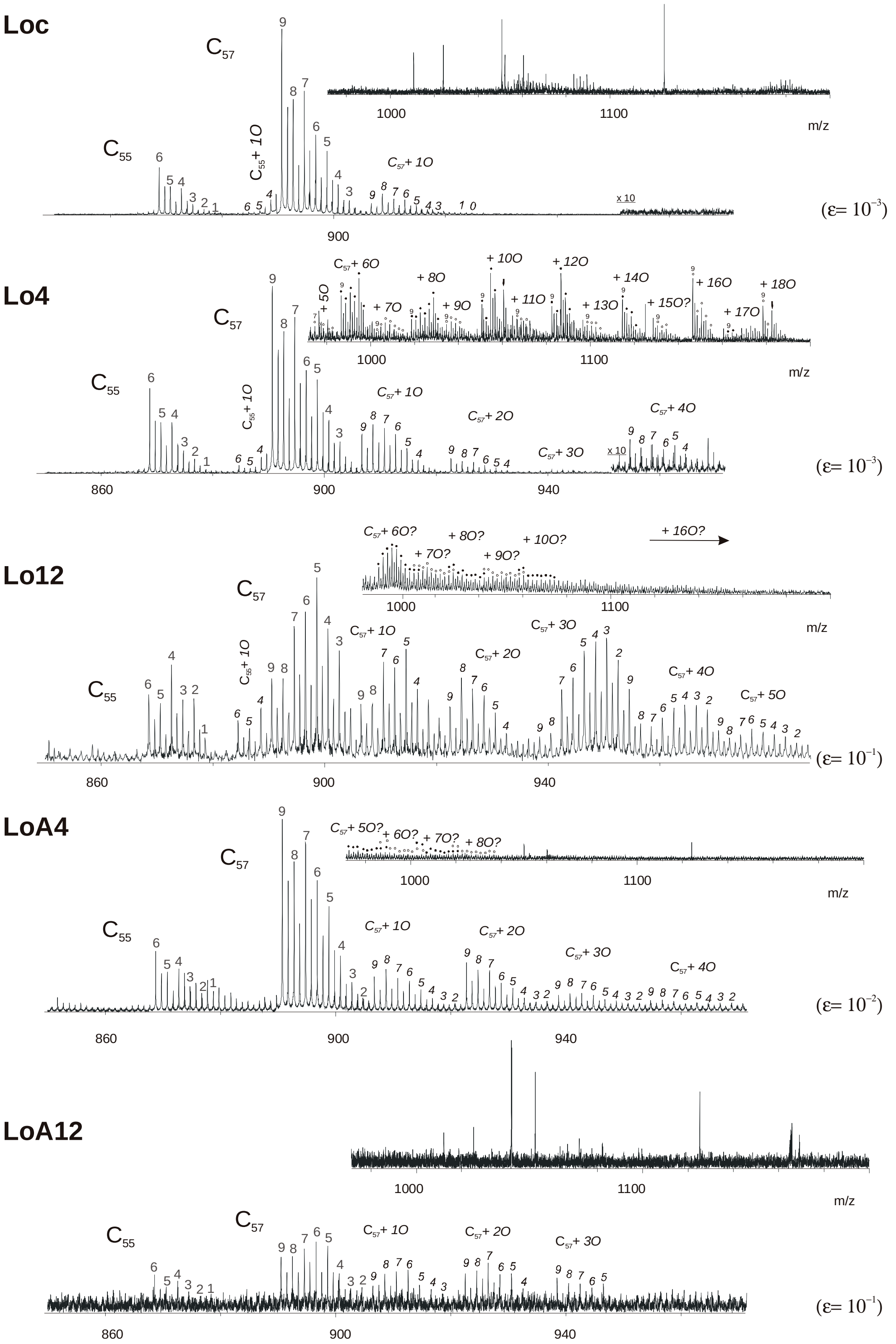

Fig. 11. ESI-FTICR-MS data showing compositional changes of ageing linseed oil triglycerides in linseed oil DCM test solutions: linseed oil control solution (a), linseed oil solution after 4 weeks of light ageing (b), linseed oil solution after 12 weeks of light ageing (c), VEGA asphalt-linseed oil solution after 4 weeks of light ageing (d) and VEGA asphalt-linseed oil solution after 12 weeks of light ageing (e).

The C_{55} and C_{57} TAGs with up to 6 and 9 double bonds remain the main compounds after 4 weeks but the relative amount of the C_{55} TAGs is higher compared to the original oil suggesting a preferential oxidation of the linolenic moieties in the C_{57} TAGs. After 12 weeks of artificial light ageing C_{57} TAGs with about ten atoms of oxygen are still observed in linseed oil (see Lo12 and insert in Fig. 11). After 12 weeks the relative intensity of the more unsaturated moieties in the unoxidised TAGs is decreased while the triglycerides with one to four oxygen incorporated have increased intensities.

Table 5. Possible combinations of fatty acid chains in the linseed oil triglycerides [62, 63, 66], the number of double bonds present in the correspondent triglycerides and their measured and exact molecular weight (MW). The position of the fatty acid chains given in the first column of the table is interchangeable.

TAG	**Double bonds no.**	**Measured[6] MW (FT-ICR-MS)**	**Exact MW (calculated)**	**ε (x 10^{-3} amu)**
C_{55} ($C_{55}H_{94}O_6$)				
$C_{16:0}$-$C_{18:3}$- $C_{18:3}$	6	850.708	850.705	3
$C_{16:0}$-$C_{18:3}$- $C_{18:2}$	5	852.721	852.721	0
$C_{16:0}$-$C_{18:3,2}$- $C_{18:1,2}$	4	854.737	854.736	1
$C_{16:0}$-$C_{18:3,2}$- $C_{18:0,1}$	3	856.752	856.752	0
$C_{16:0}$-$C_{18:2,1}$- $C_{18:0,1}$	2	858.770	858.768	2
$C_{16:0}$-$C_{18:1}$- $C_{18:0}$	1	860.783	860.783	0
$C_{16:0}$-$C_{18:0}$- $C_{18:0}$	0	low signal	862.799	-
C_{57} ($C_{57}H_{92}O_6$)				
$C_{18:3}$-$C_{18:3}$- $C_{18:3}$	9	872.687	872.689	2
$C_{18:2}$-$C_{18:3}$- $C_{18:3}$	8	874.706	874.705	1
$C_{18:1,2}$-$C_{18:3,2}$- $C_{18:3,3}$	7	876.718	876.721	3
$C_{18:0,1,2}$-$C_{18:3,2,2}$- $C_{18:3,3,2}$	6	878.733	878.736	3
$C_{18:0,1,1}$-$C_{18:2,2,1}$- $C_{18:3,2,3}$	5	880.751	880.752	1
$C_{18:0,0,1}$-$C_{18:1,2,1}$- $C_{18:3,2,2}$	4	882.769	882.768	1
$C_{18:0,0,1}$-$C_{18:0,1,1}$- $C_{18:3,2,1}$	3	884.785	884.783	2
$C_{18:0,0}$-$C_{18:0,1}$- $C_{18:2,1}$	2	low signal	886.799	-
$C_{18:0}$-$C_{18:0}$- $C_{18:1}$	1	overlap $C_{57:9}$ + 1O	888.815	-
$C_{18:0}$-$C_{18:0}$- $C_{18:0}$	0	overlap $C_{57:8}$ + 1O	890.830	-
$C_{57:9}$ + 1O	9	906.719	906.719	-
$C_{57:9}$ + 2O	9	922.713	922.713	-
$C_{57:9}$ + 3O	9	938.709	938.709	-
$C_{57:9}$ + 4O	9	954.704	954.703	1

The C_{55} TAGs with 4 double bonds (with symmetrically distributed peaks for 6, 3 and 2 double bonds) and the C_{57} TAGs with 5 (with 6, 7 and 4 double bond peaks symmetrically distributed) are the main compounds of the linseed oil solution. Other

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[6] as example, values from the FT-ICR-MS measurement for the test linseed oil solution aged for 4 weeks are shown

reactions than oxidation alone for example cross-linking reactions or formation of smaller oxidation products could possibly explain the lower relative abundance of the peaks of oxidised TAGs in the ESI-FTICR-MS measurement and the absence of the more oxidised TAGs.

After 4 weeks of artificial ageing oxygenated triglyceride ions with at least 4 incorporated oxygens are present in the asphalt-linseed oil test solution data (see Loa4 in Fig. 11). The ratio between C_{55} and C_{57} TAGs more closely resembles the original oil suggesting an antioxidant activity due to the asphalt. This clearly points to a slower oxidation of the linseed oil in the presence of asphalt. The way of oxidation of the oil in the presence of asphalt also seems to be different. An even number of oxygen atoms is preferentially incorporated in the fatty acid moieties of the oil in the asphalt-linseed oil test solution, while for the linseed oil test solution an odd number of oxygen atoms is preferentially taken up as seen in the data. Peroxidation of the oil moieties can lead to very high number of incorporated oxygen atoms [73]. This appears to be the case for the linseed oil experiment where higher relative intensities are observed with increments of 32 Dalton. At longer exposure time, the relative amount of these highly oxygenated species decreases. It was concluded that peroxides and other highly oxygenated triglycerides are converted into smaller compounds at this stage [74].

The range of oxygenated TAGs is much more limited when asphalt is present. The distribution of oxygenated triglycerides in the asphalt containing sample suggest that only one oxygen atom is reacting to the most reactive double bonds or that peroxides may not form. Longer exposure of the asphalt containing oil sample leads to a rapid decline of all triglycerides. After 12 weeks almost no free TAGs (i.e. unreacted by cross-linking or oxidation) can be detected (LoA12 in Fig. 11). In the same LoA12 sample, C_{55} TAGs with a very low relative abundance and with a distribution that resembles the distribution of unoxidised C_{55} TAGs in the LoA4 solution could be identified. The distribution of C_{57} unoxidised TAGs has a maximum at $C_{57:\ 6}$ and symmetry around this peak is also of low relative abundance and resembles the distribution of the homologues observed for the Lo12 solution. No more than 3 atoms of oxygen are detected as incorporated in TAGs in the LoA12 solution. That suggests that double bonds from TAGs are reacting away in a different way than by oxidation, for example via cross-linking reactions.

A few aspects can be summarised from ESI-FTICR-MS data. Less oxygen is incorporated in the asphalt-oil TAGs after 4 weeks compared to the oil alone. The ESI-FTICR-MS data point out that the incorporation of oxygen in TAGs of the asphalt-oil solution is about 60 % less. Oxidation of TAGs is perceived not only as formation of new clusters of oxidised TAGs but also as a decrease in relative abundance of the unoxidised unsaturated TAGs with ageing. A preferential oxidation of the more unsaturated moieties of the C_{55} and C_{57} TAGs is observed and which resulted in symmetry of the TAG clusters around a middle peak as a maximum. In other words the presence of asphalt in linseed oil solutions in DCM slows down the oxidation of the oil TAGs. The consumption of the unsaturations of the TAGs in such a mixture can take place by cross-linking reactions. Indications for cross-linking reactions have been discussed above, when DTMS results were discussed and other indications derived from SEC measurements are discussed further on.

SEC was used to give information on the cross-linking of the linseed oil or formation of asphalt-linseed oil networks upon the light ageing. The HPSEC results for the linseed oil and the VEGA asphalt-linseed oil DCM test solutions are shown in Fig. 12a and b. Polystyrene standards (PS) were used for the calibration, which implies that the observed molecular weight (MW) values of linseed oil and asphalt in the SEC measurements are in fact apparent MW values. The MW values of the standards as a function of the retardation time are shown on the MW bar in Fig. 12.

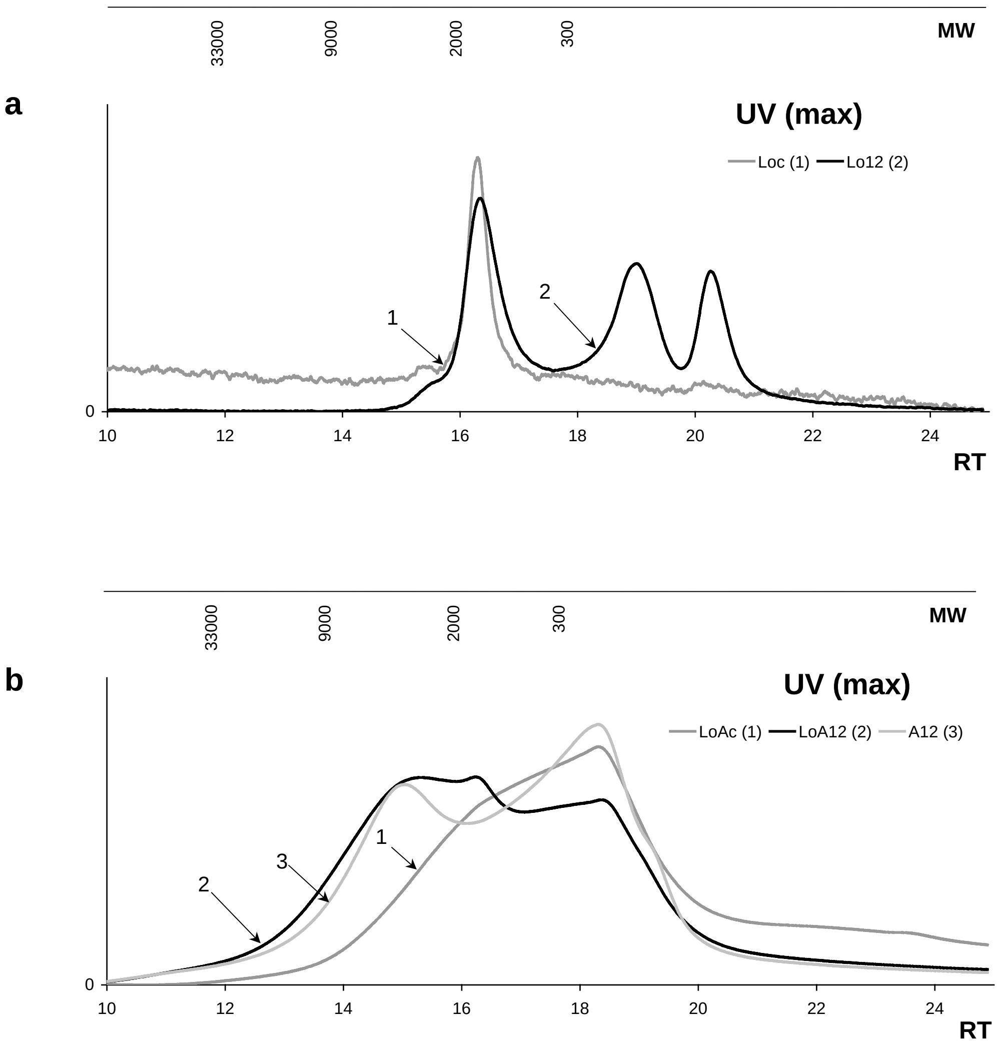

Fig. 12. HPSEC data showing changes in molecular weight of linseed oil DCM test solutions: (a) linseed oil control solution (1), grey solid line "—", and linseed oil solution after 12 weeks of light ageing (2), black solid line "—", (b) VEGA asphalt-linseed oil control solution (1), grey solid line "—",VEGA asphalt-linseed solution oil after 12 weeks of light ageing (2), black solid line "—" and asphalt solution after 12 weeks of light ageing (3), light grey solid line "—".

When the linseed oil solution is analysed by SEC, there is a small difference between the MW curve of the linseed oil in the control test solution, Loc, marked as "1" (grey solid line "—") in Fig. 12a, and the linseed oil in the 12 weeks aged linseed oil solution (Lo12) marked as "2" (black solid line "—") in the same figure. A major peak (at RT~16 min) is observed for the fresh linseed oil solution (1) and is interpreted as the peak corresponding to the TAGs in the fresh linseed oil. As seen from the MW bar in Fig. 12a, the corresponding MW values of PS are around 1600 Dalton. This implies that SEC "sees" TAGs with molecular weights of about 900 Dalton as compounds with an apparent molecular weight of about 1600 Dalton.

A similar major peak is observed for the aged linseed oil solution (2), but a small shift is observed between the fresh and aged and fresh solution. Possibly this has to do with the increased relative abundance of C_{55} TAGs and their small oxidation products, compared to the C_{57} TAGs after 12 weeks of artificial light ageing as observed with ESI-FTICR-MS (for MW values and relative abundances see Lo12 in Fig. 11). Very small amounts of high MW (HMW) material with the apparent MW of ca 2900 Dalton are observed at retention time 15.5 min for Lo12. Two peaks of substances are observed in the aged linseed oil solution between 18-22 min RT that are interpreted as degradation products with a lower MW[7] (LMW). DTMS and Py-TMAH-GC/MS confirm the presence of these smaller degradation products in the form of dicarboxylic acids and various diglycerides.

The chromatogram of the control asphalt-linseed oil solution, LoAc, the chromatogram of the asphalt-linseed oil solution aged for 12 weeks, LoA12, and the chromatogram of the asphalt solution aged for 12 weeks, A12, are compared in Fig. 12b. The chromatograms are marked as “1” (dark grey solid line “—“) for LoAc, “2” (black solid line “—“) for LoA12, and “3” (light grey solid line “—“) for A12. An unresolved envelope (ca 16-18 min RT) is observed in Fig. 12b for the LoAc *solution* (1). A peak corresponding to linseed oil is expected at RT 16 min in the LoAc solution. It is most probably hidden under the broad range of MW of asphalt, mixture of maltenes and asphaltenes that covers the apparent MW ranging from 1900 to values smaller than 500 Dalton.

For the A12 solution (3) a peak is also observed at ca 4000 Dalton (RT~15 min), which probably corresponds to aggregates formed by aged asphaltenes [56, 57]. The LoA12 solution (2) shows a common pattern with the control asphalt-linseed oil solution, i.e. the unresolved peak seen for LoAc. In the chromatogram of the same LoA12 solution another peak is observed at around RT 16 min with an apparent MW of ca 1600 Dalton. This peak has the same RT with the TAG peak observed in the linseed oil solutions (Fig. 12a) and it is interpreted as an oil peak. The LoA12 solution also shows a common pattern with the A12 solution, i.e. the HMW around 4000 Dalton (RT~15 min). This unresolved peak is somewhat broader than the peak observed for the aged asphalt solution, suggesting the formation of HMW other than cross-linked asphalt or cross-linked linseed oil. It is proposed that this HMW material is due to formation of cross-links between asphalt units and TAGs of the oil. It is proposed that asphalt in oil acts as a chemical trap for unsaturated TAGs creating a relatively large network molecule. A clear oxidation path for the ageing of the linseed oil in solution was seen in ESI-FTICR-MS results. From the same data it was clear that less oxidation occurs in the triglycerides of the asphalt-linseed oil solution. However only a very small amount of TAGs are remaining in the latter solution after 12 weeks of light exposure.

The consumption of double bonds (TAGs) in the LoA12 may be explained as suggested by SEC, i.e. the ageing of asphalt-linseed oil solutions take place differently (more cross-linking) compared to the linseed oil solutions (very little cross-linking). SEC of the oil itself demonstrates that a network of oil molecules is not formed under these conditions. Instead, the peroxidsed TAGs seen by ESI-FTICR-MS are converted into smaller degradation products by further oxidation while some of the oxy-TAGs remain. The idea of cross-link formation between asphalt and linseed oil after 12 weeks of ageing is also supported by DTMS

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[7] The corresponding apparent MW values, below 500 Dalton, are not relevant for the interpretation of the data as they are out of the calibration range (for more details see the Experimental section).

observations that show the presence of peaks of both oil and asphalt in the pyrolysis event corresponding to network information.

Mono- and dicarboxylic fatty acids of the aged oils and oils plus asphalt were analysed by Py-TMAH-GCMS. The two ratios derived earlier using the stearic acid as the conservative property of the TAGs of the oils, C_9 DA: $C_{18:\,1}$ FA and $C_{18:\,1}$: $C_{18:\,0}$ FA, are used to estimate the chemical drying of linseed oil solutions with or without asphalt. The ratio of azelaic acid to stearic acid shows the progression of the oxidation of the C_9 position in the unsaturated fatty acids. The ratio of the monounsaturated fatty acids (cis and trans $C_{18:1}$) to stearic acid shows how much unsaturation of the oil is remaining. Light ageing of the oil in dichloromethane solution immediately leads to a decrease of the unsaturated fatty acid moieties and an increase in azelaic acid. Fig. 13 shows that the $C_{18:1}$: $C_{18:0}$ ratio decreases more slowly when asphalt is present in the oil mixture. After week 4 and 8, the ratio starts to decrease more quickly suggesting that a certain lag time has passed and that the antioxidative potential of the asphalt is decreasing. With initial values between 4.5 for the linseed oil control solution and almost 5 for the control asphalt-linseed oil solution, the $C_{18:\,1}$: $C_{18:\,0}$ FA ratio is still much higher than one (ca 2.5) after 12 weeks of artificial light ageing, when asphalt is present. For linseed oil solution aged 12 weeks the value is lower (ca 2). Azelaic acid increases relatively fast in the oil while its formation is slowed down when asphalt is present.

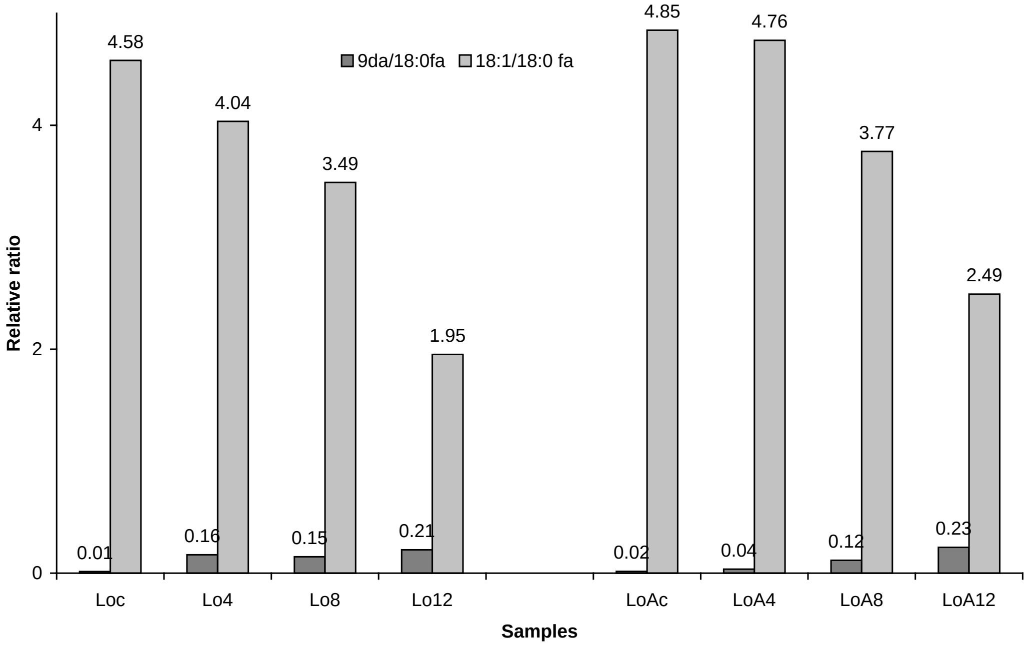

Fig. 13. Ratios of 9da/18fa and 18:1fa/18:0fa fatty acids obtained by GC/MS analysis of asphalt-linseed oil and linseed oil solutions in DCM, light aged for 0-12 weeks.

Summarising, the effect of asphalt on the oxidation of linseed oil could be observed easily when the linseed oil was photoxidised in dichloromethane solution. The results are considered to be relevant for the fate of oil in oil paints. Earlier studies on varnish and indigo in DCM test solutions have shown that similar compounds are being produced in solution as well as in the solid phase of paintings. Asphalt clearly affects the composition of the drying oil. The effect of asphalt on the oxidation of the linseed oil is a general slowing down of the rate of oxidation but the same kind of oil end products are being formed. The electrospray FTICR-MS data suggest that there are different kinds of intermediate compounds formed when asphalt is present. There appears to be a high relative amount of peroxides that form in the oil compared to oil with asphalt. Further studies will have to corroborate this. Asphalt and linseed oil also react with each other as was deduced from the SEC and DTMS data. It is presently unknown what the reaction centres could be, although sulphur compounds in the asphalt are likely candidates. The reaction of asphalt with the oil starts immediately and may explain part of the antioxidant effect. The analysis of the TAGs by ESI-FTICR-MS suggests that a large proportion of the oil TAGs are indeed involved in the cross linking leaving only a small amount of free TAGs in solution after 12 weeks. The ratio of azaleic acid to stearic acid points out that oxidation of the double bonds keeps taking place, which suggests that not all double bonds are sequestered by the asphalt.

Asphalt and Kassel earth containing oil paints

Several sets of oil paint samples were available for analysis with Py-TMAH-GCMS. In Fig. 14 TICs of oil paint samples relevant for the drying of asphalt containing oil paints are shown. The $C_{18:\,1}$: C_{18} FA and C_9 DA: C_{18} FA ratios of these samples are given in Fig. 15 and Fig. 16.

Samples obtained from Von Imhoff (VI) test panels (at CCI) allow the comparison between a natural aged asphalt oil paint (A-VI) and oil paints with ivory black (IvB-VI), Kassel earth (Ke-VI) or plain lead white (PbW-VI). It is important to mention that these samples were prepared without addition of driers that could have promoted the chemical drying process of the oil in the paint. The asphalt used by von Imhoff in preparing the paint was analysed and found to be rather similar to the VEGA asphalt with the exception of the aromatic steroid hydrocarbons, which a have different distribution. This difference is not considered to be of any significance for the effect of the asphalt on the drying of the oil paint. Fig. 14a shows the TIC of the asphalt oil paint after 25 years of natural ageing. The chromatogram shows a lot of peaks[8] from asphalt as minor constituents and several major peaks from methyl ester of fatty acids (C_7-C_{18}, C_{20}-C_{24}) and dicarboxylic acids (C_6-C_{10}). A striking feature is the large peak from the $C_{18:1}$ fatty acid. The presence of the dicarboxylic acids with the quite high peak for the C_9 homologue indicates however that oxidation of the oil takes place but this does not go fast enough to decrease the amount of $C_{18:\,1}$ fatty acid [66]. The ratios of the $C_{18:\,1}$: C_{18} FA and C_9 DA: C_{18} FA derived from this chromatogram are presented as bar graphs in Fig. 15. It is clear by comparison with the lead white paint that the chemical drying of asphalt paint is very strongly slowed down and incomplete. Interestingly, the ivory black oil paint has a relatively large C_9 DA: C_{18} FA pointing to strong oxidation conditions but the $C_{18:\,1}$: C_{18} FA is still rather high. Kassel earth on the other hand has a low $C_{18:\,1}$: C_{18} FA although the geological origin and the organic nature of the Kassel earth might have suggested a potential antioxidant effect. The carbon in the ivory black has however a much stronger antioxidant effect. The other oil paints (OP_1, OP_2 and OP_3) were made with the VEGA asphalt pigment prepared according to 19th-century recipes by Boitelle[9].

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[8] The following notation was used: "●" and "9" for the nonanoic fatty acid methyl ester, "*" and "9da" for the nonadioic dicarboxylic acid dimethyl ester, "αMe9 da" for the α methyl nonadioic dicarboxylic acid dimethyl ester, "○" and "18: 1" for monounsaturated C_{18} fatty acid methyl ester and "◊" and "♦"for alkenes or alkanes and "▼" for hopanoid compounds from the asphalt conponent.

[9] For details regarding the exact composition of the samples see Table 1 and the Experimental section.

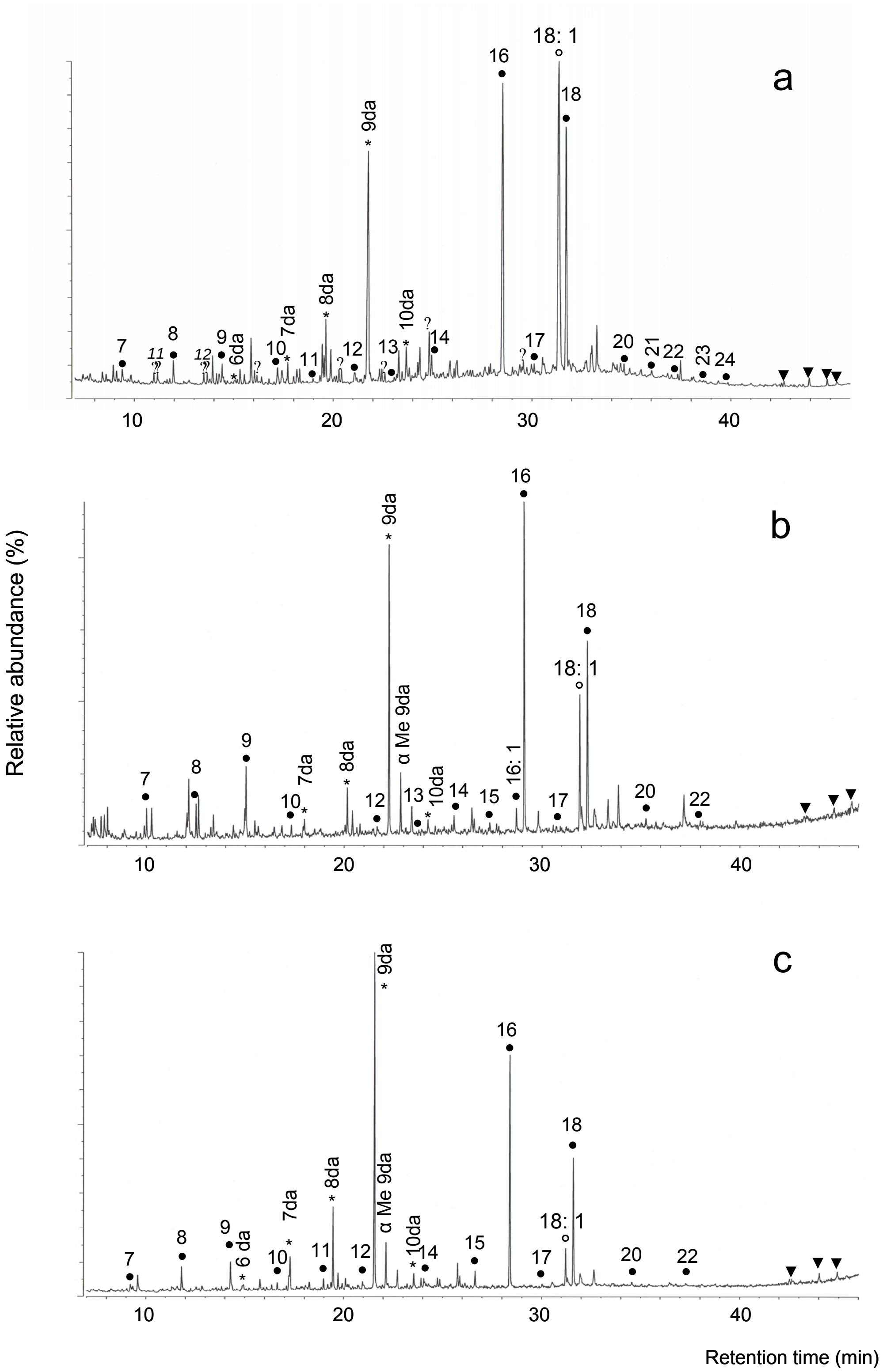

Fig. 14. Py-TMAH-GC/MS Total Ion Current of asphalt aged oil paints without drier: CCI asphalt oil paint (a) and with drier: Wiliam's Antwerp brown (b) and Merimée's English method (c).

Example of notation: "●" and "9" for the nonanoic fatty acid methyl ester, "" and "9da" for the nonadioic dicarboxylic acid dimethyl ester, "αMe9 da" for the α methyl nonadioic dicarboxylic acid dimethyl ester, "○" and "18: 1" for the monounsaturated C_{18} fatty acid methyl ester, "◊" or "♦" for alkenes or alkanes and "▼" for hopanes from the asphalt component.*

These paints were prepared with addition of litharge as a drying catalyst added to the linseed oil. The TICs of the Py-TMAH-GC/MS measurements are shown in Fig. 14 for samples OP_{1a} (b) and OP_{2a} (c). As observed for the A-VI sample a full range of fatty acids (C_7-C_{18}, C_{20}, C_{22}) and dicarboxylic acids[10] (C_6/C_7-C_{10}) methyl- or dimethyl esters are present. It is interesting to observe that the C_9 dicarboxylic acid is the second or the highest peak in the TICs of the OP_{1a} or OP_{2a} samples and moreover that the $C_{18:1}$ fatty acid peak is rather low. This indicates two possible influences on the drying of linseed oil in OP_1, i.e. the presence of driers and/or the deactivation of asphalt due to the roasting process.

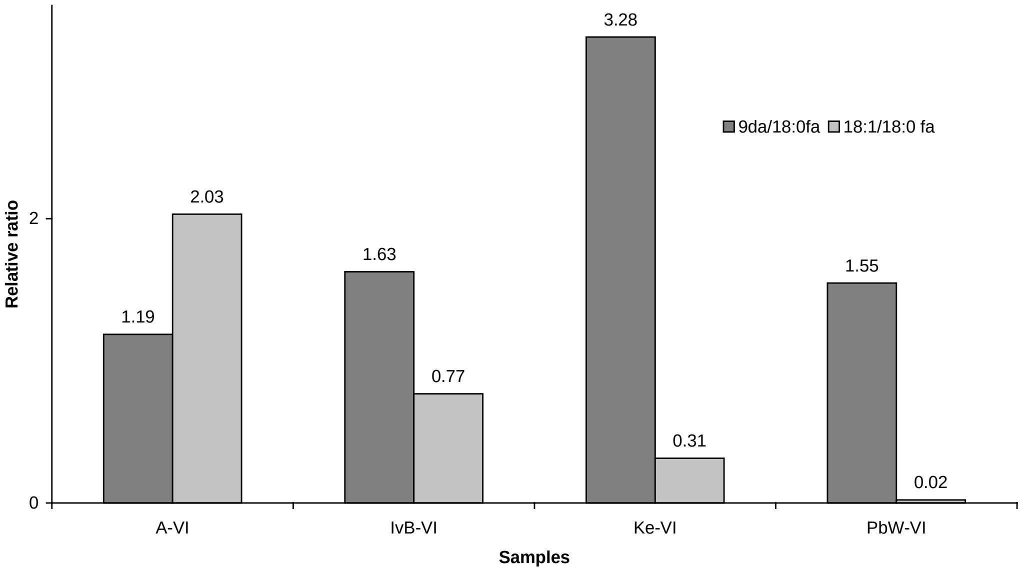

Fig. 15. Degree of chemical drying expressed as ratio of 9da/18:0fa and 18:1 /18:0 fa of naturally aged Von Imhoff asphalt and Kassel earth oil paints in comparison with an ivory black and lead white oil paint.

In a similar way there are indications for three possible influences on the drying of linseed oil in OP_2, i.e. the presence of driers, the melting of the asphalt and/or the presence of resins. Surprisingly it seems that asphalt containing paint prepared according to the three selected 19th-century recipes dries rather well after about 40 years of museum ageing (see Experimental section)! The oil characterising ratios of these oil paints are shown as bar graphs in Fig. 16. The fresh oil paints (OP_{1c}, OP_{2c}, OP_{3c}) already show a relatively high abundance of azelaic acid, which is probably due to a relatively long period of curing (8-10 months [24]) and the lead oxide present as a drying catalyst before exposure to light ageing. There is still a large relative amount of unsaturated fatty acids remaining however that decreases after ageing (OP_{1a}, OP_{2a}, OP_{3a}). The ratios of the three paints before ageing do not differ very much.

After ageing (equivalent to about 40 museum years) paint OP_1 is less oxidised compared to OP_2 and OP_3. In all three aged paints the azelaic acid is a dominant component and the $C_{18:1}$ is smaller than the $C_{18:0}$. The composition of the “asphalt pigment” itself seems to be the determining factor. The pigment in OP_1 was “boiled to cinder”, which substantially altered the properties of the molecular composition of the asphalt as seen above. The fatty acid and dicarboxylic acids of this oil paint

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[10] The α methyl C_9 dicarboxylic acid is a result of unwanted alkylation during the pyrolysis transmethylation process [50].

demonstrate that there is hardly any antioxdising effect left after this method of pigment preparation compared to the asphalt paint prepared by von Imhoff. However, the pigment is largely carbonised so it can still have an effect like the ivory black in the IvB-VI paint. The “asphalt pigment” in OP_2 and OP_3 does not seem to affect the oxidation of the paint but the beeswax might considering the almost identical composition and preparation method for the two oil paints and the less oxidised oil in OP_3 (that contains beeswax). (The oil marker ratios are comparable to Kassel earth oil paint without drier.)

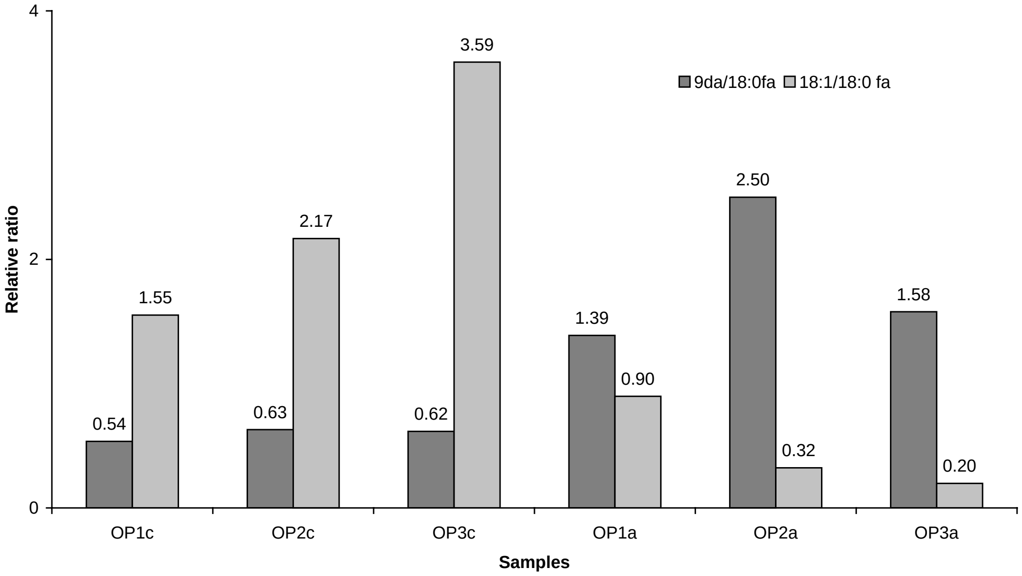

Fig 16. Ratios of 9da/18fa and 18:1/18:0 fa in asphalt paint reconstructions, before and after ageing.

The pigment in these oil paints consists of “prepared” asphalt in a large amount of resin (mastic, gum turpentine and shellac). Since these resins are full of unsaturated hydrocarbons that could interact with the asphalt in similar ways as the unsaturated triglycerides in the oil-asphalt test solutions, it is possible that the potential effect of the asphalt itself on the oil paints neutralised to a large extent in the pigment preparation stage. This implies that the main effects on the drying of the oil are to be expected from other nondrying components in the oil paint such as the added resins and waxes. Earlier studies with resins and oil in DCM test solutions have shown that resins can strongly accelerate the oxidation of oils [32, 33].

Summarising this subsection, it can be said that several techniques have shown that the presence of asphalt in linseed oil paint or linseed oil DCM solutions hinders the drying of the oil. The induction time before oxidation and cross-linking of oil starts, is much longer in an asphalt-oil mixture compared to linseed oil alone. The chemical composition of the ageing oil seems to change much less in the presence of asphalt as observed by DTMS. A higher abundance of monounsaturated C_{18} fatty acid is still observed (by Py-TMAH-GC/MS) in such an asphalt-linseed oil mixture compared to its linseed oil homologue solution. Examination of the linseed oil TAGs before and after ageing in the presence of asphalt have shown that less oxidation takes place even though the amount of TAGs diminishes. HPSEC experiments (supported by DTMS results) have explained this by the occurrence of chemical interactions between asphalt and oil units. The chemical drying of the naturally aged Von Imhoff paint reconstructions containing no drier shows similarities between asphalt and carbon black pigments (like ivory black) in retarding the drying of oil but also the opposite effect of Kassel earth pigments on the drying of oil. The oil in the artificially aged asphalt-oil paints prepared after 19th-century recipes (i.e. with pretreated pigments, driers, resins and wax) is found to dry much better than the oil in the presence of only asphalt (Von Imhoff asphalt-oil sample).

C) Analytical data on selected 19th-century paintings

The paint samples from 19th-century paintings were taken on the supposition that the paint originally contained asphalt or other substances that caused premature cracking and darkening [7, 9, 21, 26-28, 43]. The samples were taken from brown-black darkened areas of several paintings: '*Death of Dido*' by Sir Joshua Reynolds (1723-1792), sample Re_1, '*La descente des vaches*' by Theodore Rousseau, samples Ro_1-Ro_3, and the painting '*Alone*' by Josef Israëls (1824-1911), samples I_1 and I_2. In the case of 'La descente des vaches' samples were available from both the beeswax/resin relined oil sketch (Ro3) and an unlined final painting (Ro1, Ro2), which are in the possession of the Mesdag museum. The research question addressed here is the composition of the oil paint, e.g. the presence or absence of asphalt or Kassel earth pigments, and the additional organic material present that could influence the drying and darkening. Py-TMAH-GC/MS could be performed on most of the samples to determine the fatty acid and dicarboxylic acid distribution. Py-GC/MS and DTMS were used for molecular characterisation of the organic pigments and other organic materials in the paint. The results obtained by mass spectrometry are summarised in Table 6 and discussed further.

From the palmitic to stearic acid ratio (labelled as "p/s" in Table 6) it is suggested that the initial oil paint was prepared with linseed oil (L), as seen for samples Re_1, I_2, Ro_1, Ro_3 or poppy seed oil (P) like Ro_2 [62]. The high p/s ratio derived from the Py-TMAH-GC/MS data obtained for the I_1 sample could be possibly due to the presence of traces of beeswax in the paint. The marking features of the oil paint of these samples (C_9 DA:$C_{18:0}$ FA and the $C_{18:1}$: $C_{18:0}$ FA ratio) are shown as bar graphs in Fig. 17. Surprisingly, in all cases $C_{18:1}$ fatty acids were found to be preserved. In the case of the Rousseau samples Ro_2 and Ro_3, and sample from the Israëls painting I_2, the relative intensity of the $C_{18:1}$ fatty acid (the $C_{18:1}/C_{18:0}$ ratio) is equal or larger than one, which points to poor drying of the oil paint even after 150 years. The low values for the C_9 DA: $C_{18:0}$ FA ratio for all the samples except Ro_1 support the conclusion that the oil paint experienced a poor drying regime. The Ro_1 sample seems to have experienced more oxidising conditions considering the C_9 DA: $C_{18:0}$ FA ratio of about 2. The poor drying of most of these paints could have been caused by the presence of antioxidising materials or by a lack of chemical driers. The latter supposition can be rejected because lead was found in all the sample by DTMS analysis, although it is possible that there is insufficient drier present considering the high abundance of non-drying organic components of the paints (except maybe in Ro_1) as shown in Table 6. All samples contain CO_2 evolved by pyrolysis of a carbonate from an inorganic source. Considering that all the samples are dark the most logical explanation is that lead carbonate was used as a drier. DTMS and Py-GC/MS were used to determine the nature of the materials that could have interfered with the drying process.

Table 6. DTMS, Py-GC/MS and Py-TMAH-GC/MS results of 19th century paint samples.

Legend: "p/s" is the ratio between palmitic and stearic acid indicating the type of oil [62], L stands for linseed oil, P for poppy seed oil, Pb, Hg and S are the atomic symbols for lead, mercury, respectively sulphur. B stands for benzene, B_i stands for alkyl substituted benzenes, N stands for naphthalene, N_i for alkyl substituted naphthalenes, Ph stands for phenol, Phi stands for alkyl substituted phenols, C_x for n-alkanes (x= number of carbon atoms in the alkane chain and i= number of carbon atom in the alkyl substitute group).

Sample's symbol	**DTMS**	**Py-TMAH-GC/MS** **Py-GC/MS**
Ro1 (286/1b)	p/s= 3 → L? beeswax Pb (carbonate?)	p/s= 3 → L? alkanes: C_{14}, C_{15}, C_{27}, C_{29} alkylbenzenes: B_1?, B_2
Ro2 (286/5)	p/s= 4,7 → P Pb carbonate triterpenoids lignite (phenols) polysaccharides HgS (vermillion)	p/s= 4 → P alkanes: C_9-C_{14}, C_{16} alkylbenzenes: B_1?, B_2
Ro3 (287/2a)	p/s= 1,1 → L beeswax Pb carbonate triterpenoids (dammar)	p/s= 3 → L? asphalt/paraffin (alkanes: C_6-C_{32})? alkylbenzenes: B, B_1, B_2 alkylnaphtalenes: N, N_1, N_2 alkylphenols: Ph
Re1 (1029/20)	*p/s= 0,9 → L?* dicarboxylic acids (beeswax?) Pb triterpenoids HgS (vermillion)	p/s= 1,4→ L lignite (phenols: Ph, Ph_1, Ph_2)? alkanes: C_7-C_{10}, C_{12} alkylbenzenes: B, B_1, B_2 alkylnaphtalenes: N, N_1, N_2
I1 (154/5)	p/s= 2,5 → L (beeswax) Pb (carbonate) triterpenoids diterpenoids	p/s= 5,4 → P lignite (phenols: Ph, Ph_1)? asphalt/paraffin (alkanes: C_7-C_{17}, C_{21}-C_{39})? alkylbenzenes: B, B_1-B_3 alkylnaphtalenes: N?, N_1-N_3
I2 (154/2)	p/s= 2,5 → L dicarboxylic acids beeswax Pb carbonate (triterpenoids), (diterpenoids?) asphalt($C_{23\text{-}29,31}$ alkanes)? HgS (vermillion)	p/s= 2,6 → L lignite (phenols: Ph, Ph_1)? alkanes: C_7, C_8, C_{11}, C_{13}-C_{17}, C_{21}, C_{23}-C_{35} alkylbenzenes: B, B_1, B_2 alkylnaphtalenes: N?, N_1

No hopanoids nor steroid compounds characteristic for asphalt were found in the samples. Considering the fact that the hopanoid compounds are surviving well in the oil paint model studies (see above), it is concluded that asphalt as such can not have been the main antioxidising constituent. Small amounts of long chain n-alkanes that are potential indicator of sedimentary organic materials were observed by Py-GC/MS in the Rousseau Ro_3 sample of the oil sketch (C_6-C_{32} alkanes) and Ro_1 sample of the oil painting (C_{27} and C_{29} alkanes), the Israëls I_2 sample (C_{21}, C_{23}-C_{35} alkanes), and the I_1 sample (C_{21}-C_{39} alkanes). Some of the n-alkanes are from the beeswax relining material like for the Rousseau sketch that was wax-relined [19]. The Reynolds painting was glue-lined [28] and the other have not been relined [21]. That suggest beeswax could have been used as a paint component in the other three paintings. And indeed beeswax esters could be demonstrated by DTMS to be part of the sample's composition in Ro$_1$, Ro$_3$, I$_2$ and as trace in Re$_1$ and I$_1$ samples. Beeswax shows a strong odd predominance with C_{25}, C_{27}, C_{29} and C_{31} as main n-alkanes. Samples Ro$_3$ and I$_1$ contain a series of even n-alkanes as well. The distribution in I$_1$ is gaussian (C_{20}-C_{39}) indicative of a petroleum origin. The distribution of the n-alkanes in Ro$_3$ is rather flat and reminiscent of the VEGA n-alkane profile after roasting. If the observed aliphatic chains would be of asphaltic origin it is not clear why the asphalt biomarkers are absent. Alkyl aromatics specific for example to geomaterials/materials of organic provenance like are present as alkylbenzenes and as alkylnaphtalenes in the Ro$_3$, Re$_1$, I$_1$ and I$_2$ samples. Alkyl aromatic compounds and long chain aliphatic compounds as one would expect in non-degraded asphalt are present only in the Ro$_3$, I$_1$ and I$_2$ samples.

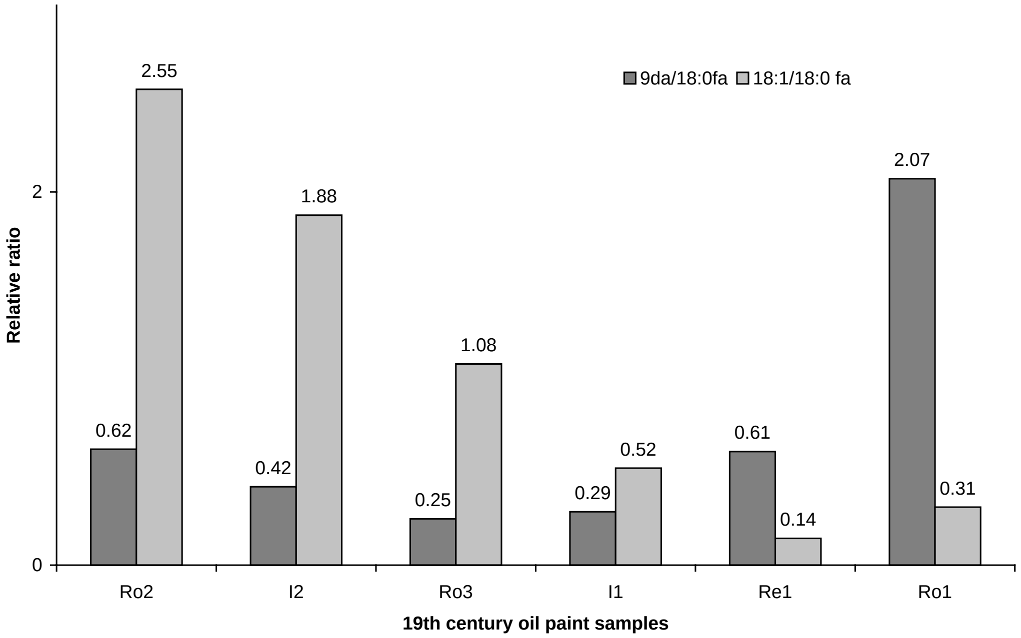

Fig. 17. Degree of chemical drying of brown-black 19th-century oil paint sample expressed as ratios of 9da/18fa and 18:1/18:0 fa.

Lignites like Kassel earth are potentially another source of paraffins (see chapter 4). The presence of phenol and some alkylphenols in some of the analysed paint samples suggests that Kassel earth like lignite was more probably used by Reynolds and Israëls and less likely by Rousseau. The partial mass chromatograms of phenol and higher homologues in sample Re$_1$ from the 'Death of Dido' by Sir J. Reynolds, is shown in Fig. 18. In the uppermost window the correspondent TIC is shown.

A complete search for Kassel earth (bio)markers in DTMS and Py-GC/MS data revealed the absence of montan wax, phyllocladane and vanillic acid. Earlier studies on the fate of Kassel earth in oil paint demonstrated that montan wax, phyllocladane, vanillic acid, alkyl phenols and alkylmethoxyphenols survive the early stages of ageing [53]. However, montan wax and phyllocladane are sensitive to solvent extraction with apolar and polar solvents (chapter 4), so these features can be lost in the pigment preparation process (dewaxing) or due to restoration. Future work on reconstructions should prove if the presence of phenolic compounds in a paint sample is enough evidence for the use of a Kassel earth-like pigment. Alkylphenols characteristic for Kassel earth are present in four of the samples, i.e. Ro_2, I_2, I_1 and Re_1, which represent poorly drying paint.

Other non-drying components discovered in the paint samples are beeswax esters, diterpenoid resins (in I_1, possibly also in I_2) and the triterpenoid resins of

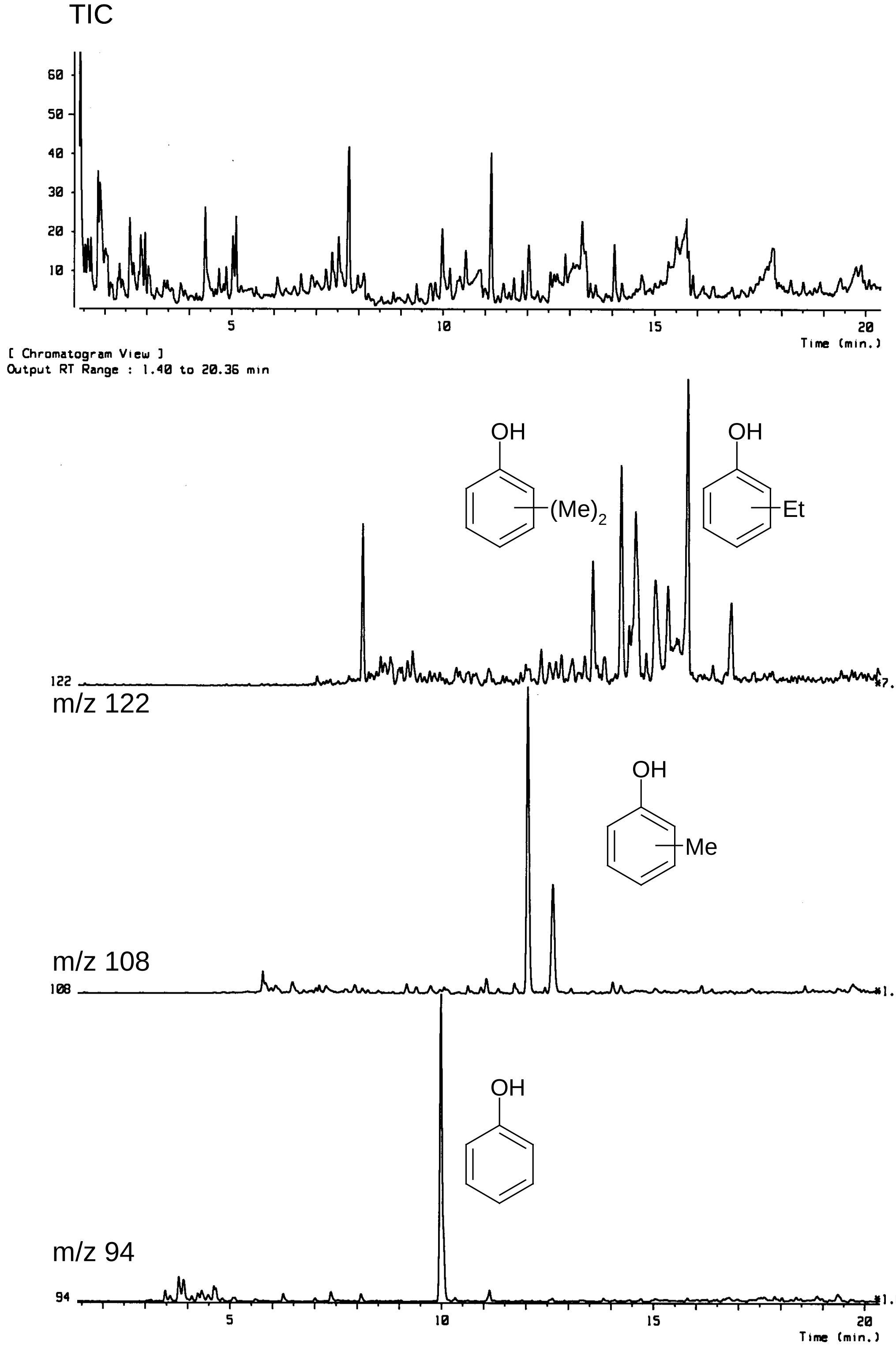

Fig. 18. Phenolic compounds in an oil paint sample (Re_1) suspected of asphalt addition from 'Death of Dido' by Sir Joshua Reynolds.

dammar and probably mastic (Ro_2, Re_1, I_1, possibly also in I_2) [30, 45, 76]. In most of the cases beeswax is an acquired residue from the lining process. The same is probably true for the colophonium derived diterpenoids. The triterpenoids are probably from mastic in the case of the painting by Rousseau (Mesdag museum cat. no. 286) who is known to have used megilps (a mastic oil gel medium [4, 5, 77]) [17, 19, 20]. The dammar resin on the oil sketch from Rousseau is most likely from the varnish.

Three of the samples, Ro_2, Re_1 and I_2, contain mercury and sulphur, which is interpreted as pointing to vermilion known to be a non-drying pigment.

Peaks characteristic of polysaccharides are present in sample Ro_2. Polysaccharides in forms of gums were used for example as paint ingredients in the 19th century [4, 5].

All these compounds detected do not amount to a large abundance of antioxidising materials in the paint that can explain the poor drying according to the indices used here. An attempt to correlate the poor drying of the oil in the 19th-century analysed samples and their composition is made in Table 7. Carbon blacks however can not be detected by the analytical methods used for this research. In the model experiments, the ivory black and the roasted asphalt showed antioxidant properties. It is postulated that such materials could have been introduced by the painters. Unfortunately, it is very difficult to detect these substances analytically. There is not enough evidence about the presence of these other "alike" pigments like ivory black, wine black, bone black, etc. [4, 5]) available to draw any conclusions about their role.

Table 7. Constituents and supposed causes for poor or good drying in the analysed 19th-century paint samples.

Legend: P stands for poppy seed oil, L for linseed oil, Pb carb. for lead white, ttp for triterpenoid resins, dtp for diterpenoid resins, Ph for phenols, sugars for polysaccharides, HgS for vermilion, C_nH_{2n+2} *for alkanes.*

Sample	**Composition**								**Supposed cause poor drying**	**Supposed cause good drying**
	Oil	**Pb carb.**	**Resins**	**Ph**	**Sugars**	**HgS**	**Wax**	**C_nH_{2n+2}**		
Ro_2 very poor drying	P	+	ttp	+	+	+	-	-	HgS, sugars?	Pb carb., ttp, Ph
I_2	L	+	(ttp, dtp?)	+	-	+	B	+	HgS, B, C_nH_{2n+2}	Pb carb., (ttp, dtp), Ph
Ro_3	L	+	ttp	-	-	-	B	+	B, C_nH_{2n+2}	Pb carb., ttp
I_1	L/P	+	ttp, dtp	+	-	-	(B)	+	(B), C_nH_{2n+2}	Pb carb., ttp, dtp, Ph
Re_1	L	+(Pb)	ttp	+	-	+	(B)	-	(B), HgS	Pb, ttp, Ph
Ro_1 best drying	L?	+	-	-	-	-	B	-	B	**Pb carb.**

In conclusion, all the analysed paint samples are characterised by a high abundance of organic materials in a complex matrix. The combination between such a composition, and a complicated painting technique seem to be the cause of the poor (physical and chemical) drying and darkening of these paintings and not the presence of a pigment like asphalt or Kassel earth. The restoration procedures like solvent cleaning and relining might be considered to be an additional cause in the deterioration of these paintings.

Discussion

As a starting point the prevailing viewpoint has been that pigments like asphalt, mummy containing asphalt and Kassel earth are the causes of poor drying in black-brown oil paintings from the 19th century. This belief was based on the information available in the literature about the materials and pigments used in the 19th century. Recipes from this period of paints containing asphalt [4, 5] in combination with remarks from the same period suggested that asphalt was included in paints, mainly after a heat pretreatment and that its use resulted in the formation of premature cracks in the paints. In a similar way it was supposed that mummy pigment is a pigment that should contain asphalt, at least this was one of the reasons why it was used. Another reason for the use of mummy pigments was their higher transparency than the transparency of asphalt paint and often a better behaviour in drying [4, 5]. Kassel earth pigment was used in the oil paint in the 19th century and preliminary results suggested it had an antioxidant activity in the drying of oil paint, rather similar to the asphalt pigment.

However when summarizing this research on this complex subject, other or new explanations have been found. Untreated asphalt indeed results in the poor drying of oil, which is clear from the DCM solution experiments. However analysis of heat pretreated asphalt has shown chemical modifications in the composition of asphalt. High temperatures applied to asphalt can be considered to resemble pyrolysis processes that result in a loss of aliphatic crosslinks and lead to an increase in the relative amount of aromatic compounds. A high amount of aromatic compounds is found also in carbon black pigments. The similarity of the roasted asphalt to carbon black pigments is possible and that would explain the slow oxidation of the oil component when either of these pigments are used. However such similarities were not investigated in this thesis. Nevertheless MS analysis of oil paints prepared with asphalt according to 19th-century recipes [4, 5, 24] and artificially light aged (ca 40 years of museum conditions) have unexpectedly indicated that the oil has dried to a great extent albeit with only small deformation, and wrinkles of the asphalt layers as reported by Boitelle [24]! The $C_{18:1}$: $C_{18:0}$ FA ratio of oil from the aged artificially aged reconstruction samples is below one, compared with the ratio observed for the naturally aged asphalt oil paint (much higher than one). Nevertheless the time factor should not be totally neglected (the equivalent of 40 years museum ageing versus only 25 years of natural ageing.)

Oil paint containing the Kassel earth pigment without drier has shown a drying of the oil similar to lead white paint from the same series. From this it can be inferred that Kassel earth itself has no special influence on the drying of oil paint.

Analysis of 19th-century paintings supposed to contain asphalt or Kassel earth pigments have shown the presence of many other materials known to be the cause of poor drying of oil like megilp, vermilion, beeswax and other waxes, and polysaccharides. It can be inferred that the presence of the other non-drying constituents in the oil paint and the techniques used by the painters [7, 17, 20, 24] are more likely the causes for the disastrous poor chemical drying of oil paints in the 19th-century paintings and the physical behaviour and mobility of paint layers.

Conclusions

Artificial light ageing experiments and to some extent also natural ageing experiments on asphalt containing oil paints have shown the preservation of asphalt markers and biomarkers to some extent. Although the relative ratio between these compounds is modified in time or due to pretreatments of the pigment for example, hopanes, alkylbenzenes and alkylnaphtalenes can survive for ca 40 years of museum conditions. However after 150 years no trace of hopanoids could be found in the analysed 19th-century samples. There are two possibilities: the absence of asphalt or its presence in such a form (degraded and cross-linked to the paint matrix) that makes it "invisible" for the mass spectrometer.

The effects of asphalt and of Kassel earth on the chemical drying of linseed oil paint were shown to be opposite. Asphalt has a strong antioxidant activity and retards the oxidation of oil paint. The formation of a network via cross-linking reactions is shown. Such a preference for cross-linking of triglycerides instead of oxidation is opposed to the situation observed for normal (linseed) oil paint. Interestingly but not totally unexpected, the overall drying effect of the oil paint is dependent on the composition of the paint including chemical modification due to heating of the pigments or solvent cleaning procedures.

Other ingredients than oil and asphalt like driers, resins or wax, when present completely change the drying behaviour of an "asphaltic paint".

The investigations of samples from 19th-century paintings by Sir J. Reynolds, Th. Rousseau and J. Israëls very much agree with the observations made for artificially aged asphalt oil paints. Very little proof for the presence of geomaterials was found (few alkylphenols as lignite markers and long chain alkanes from a possible degraded asphalt although hopanes are absent; alkyl aromatics common for both Kassel earth and asphalt-like pigments were found). The chemical and physical behaviour of these paints is highly related to their complex composition rich in other non-drying components like vermilion, beeswax or megilps. The pretreatments of the pigments (roasting, melting, dewaxing) and restoration procedures (solvent cleaning and relining) certainly have an effect on the chemistry of the paint and the mobility of the paint layers.

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Annex A. Mass Spectrometry as a micro-analytical method to study Kassel earth (Vandyke brown) pigments in oil paint

Abstract

Kassel earth or Vandyke brown pigments have been used in paintings since the 16th century and appear to be a cause of defects (e.g. slow drying) in 19th century paintings. This behaviour is thought to be related to the organic "bituminous" nature of Kassel earth and could be aggravated by the frequent substitutions and adulterations of the pigment with various other materials. Mass spectrometric techniques facilitated the investigation of a 25 years natural aged Kassel earth oil paint (CCI), the original pigment and a 19th-century Kassel earth sample (Hafkenscheid Collection). Unexpectedly, the Kassel earth pigment, thought to slow down the drying of oil shows a totally opposite activity. Specific markers for the pigment itself were identified in all three samples, the two pigments and the oil paint.

Introduction

Mass spectrometry is a micro-analytical technique requiring a few micrograms of sample for analysis. In this study Direct Temperature resolved Mass Spectrometry, DTMS, and (off line transmethylation) Pyrolysis Gas Chromatography Mass Spectrometry, Py-(TMAH)-GC/MS, are used for characterisation of the Kassel earth itself and oil paint made with the same pigment.

Kassel earth is a complex organic material of fossil plant origin [1]. It was used as a pigment since the 16th century, for shadows of flesh and in underpaint. In the 19th century, Kassel earth is mentioned to have caused defects in paintings, *e.g.* fading [2, 3]. It was substituted with other materials and is occasionally found to be adulterated. However, for this presentation we have chosen to focus on the natural variety of Kassel earth pigment. A Kassel earth pigment from the 19th century Hafkenscheid Collection [4, 5] is compared with a Kassel earth from modern sources. An oil paint made with this modern Kassel earth pigment was investigated after 25 years of natural ageing at CCI in Ottawa [6, 7, 8].

Experimental

DTMS is a fast fingerprint method that is well suited for recognition of many classes of compounds. The sample preparation only requires 5-10 µg of sample to be ground in a solvent and applied on a resistively heated filament. By gradually raising the temperature of the filament probe DTMS achieves a physical separation between lower molecular weight compounds (by evaporation) and cross-linked fractions of a sample (by pyrolysis), which is observed in the TIC as two separate events. The overall mass spectrum gives a multitude of peaks that correspond to m/z values of fragment and molecular ions of the compounds present in the whole sample (both free and chemically bound fractions).

For Py-(TMAH)-GC/MS, the sample is ground in the methylation agent, TetraMethylAmmoniumHydroxide (2.5 % solution in water), when the behaviour of the oil network is investigated. Py-GC/MS was also applied to investigate the particular features of the Kassel earth material. Aliquots of sample suspension are applied on a Curie point filament (770 0C for Py-GC/MS, 358 0C for Py-TMAH-GC/MS of the pigments and 610 0C for Py-TMAH-GC/MS of the paint sample). Under Py-GC/MS conditions in our Curie point pyrolysis system both the evaporating and pyrolysing fractions are injected directly into the GC capillary column. The results are displayed as TIC of peaks, i.e. GC separated compounds on base of different elution times. The identity of each peak is supported by the MS information under the peak.

Results and discussion

The DTMS TIC of the Kassel earth oil paint (Ke-VI paint), displays only one event at high temperature indicative for a mature network of the paint, which is in agreement with the degree of oxidation of the oil as observed later with Py-TMAH-GCMS. The two pigments Ke-VI (I_1 in Chapter 4) and Ke-Haf (H_1 in Chapter 4) show the presence of both chemically free and cross-linked fractions, corresponding to evaporation and pyrolysis events respectively.

The DTMS mass spectra of the pigments and Ke-VI paint sample show high peaks at m/z 28 and 44 indicative for decarboxylation of acid moieties in Kassel earth organic matter and some inorganic carbonates. Mass markers of rather low relative intensity present at a low m/z range in the pigment samples are fragment ions of alkylphenols, alkylmethoxyphenols and alkyldihydroxybenzenes indicative of modified and slightly coalified lignin remains. Other markers of similar relative intensity are fragment ions for aliphatic network systems (alkadienes, alkenes, alkanes) and aromatic compounds (alkylbenzenes, alkylnaphthalenes, etc.) pointing to coalified plant fractions [9]. Both sets of characteristics support the idea that Kassel earth is a lignite *i.e.* a slightly coalified fossil plant material. In the range of m/z 300-450 fragment and molecular ions of steroid, hopanes, hopenes, and other triterpenoid compounds are perceived for the pigments (in the noise range for the Ke-VI oil paint sample). At even higher mass ranges, m/z 600-900, peaks characteristic for montan wax esters (C_{46} at m/z 676 and C_{48}-C_{64}) are observed in the pigments (see Fig 1).

The Ke-VI paint is characterised by palmitic ($C_{16:0}$ fatty acid) and stearic ($C_{18:0}$ fatty acid) acids as markers for the oil. No $C_{18:1}$ unsaturated fatty acids (m/z 264) are present suggesting that the chemical drying of the oil is complete. Other Kassel earth pigment marker peaks are of lower intensity in the DTMS spectra of the Ke-VI paint compared to the spectra of the pigments.

The Py-TMAH-GC/MS data of the Ke-VI oil paint shows mostly peaks representing oil paint characteristics, and to a lesser extent markers for the Kassel earth. A series of n-fatty acids (C_6-C_{22}) is observed. The relative amount of diacids (C_9, azelaic acid- major peak in the TIC, C_8, C_7) is extremely high suggesting an advanced degree of chemical drying of the oil [10]. Much smaller peaks, representative for the palmitic and stearic acids are present in the TIC (azelaic/ palmitic acid= 2,8; palmitic/ stearic acid= 1,21). A very tiny peak (more in the noise range) indicative for a trace of remaining $C_{18:1}$ fatty acid is observed. The complete drying of oil paint made with true lignitic Kassel earth points out that the true pigment does not have strong antioxidant properties.

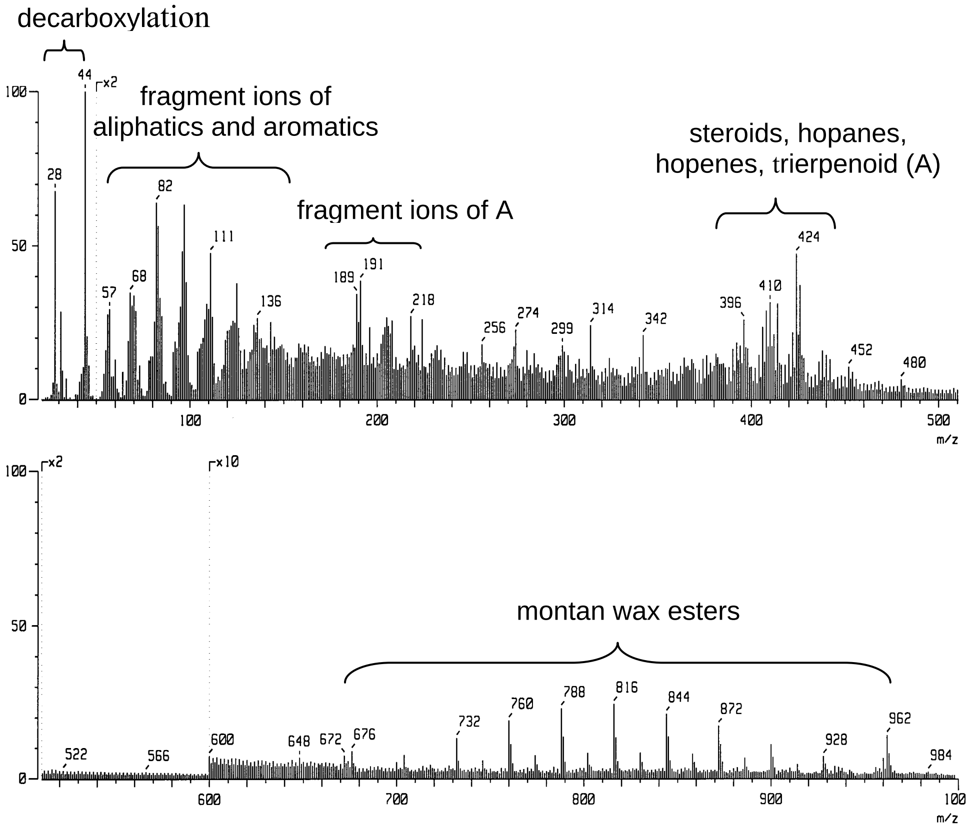

Fig 1. DTMS of a modern Kassel earth pigment of lignitic nature (Ke-VI pigment).

Some of the Kassel earth marker compounds are also present in the Ke-VI paint Py-TMAH-GC/MS data. These are represented by wax derived compounds, such as long chain alcohols (n-C_{24}, C_{26}, C_{28}), alkanes (n-C_{26}-C_{29}) and long chain fatty acids (n-C_{22}-C_{28}). These very long chain alcohols and fatty acids are transesterification products of the montan wax esters seen in the DTMS data. At lower retention times smaller intensity peaks for phenol, alkyl-phenols and vanilic acid are observed. Many of these very long chain compounds are more prominent in the pigment data. Vanilic acid is the main compound in the Kassel earth sample from the Hafkenscheid collection. It has been found as a main compound in soils derived from conifer leaves [11]. The two Kassel earth pigments have similar compositions but are not the same. They are a part of a study comparing 17 different Kassel earth preparations, which are found to differ substantially in aliphatic and aromatic character (see Chapter 4).

Conclusions

DTMS reveals the complex organic nature of the Kassel earth pigment, which consists of aliphatic chains and aromatic (*e.g.* phenols, methoxy- phenols, dihydroxy-benzenes) compounds derived from the slightly coalified woody or leaf material. Very long chain fatty acids and alcohols from montan waxes but not other biomarkers are preserved in the Kassel earth pigmented oil paint and can be used as marker compounds to trace the Kassel earth pigment using micro-analytical MS techniques.

Unexpectedly the distribution of the fatty acids and diacids from the oil paint pigmented with Kassel earth, shows an advanced degree of drying (oxidation) of the oil instead of indications for the assumed retardant effect.

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Annex B. Monitoring the effects of traditional 19th-century additives on the chemical drying of oil paint by Mass Spectrometry

Introduction

Many 19th-century paintings are showing physical evidence for failure of the painting materials. Oil paints in that period were prepared by independent colormen and artists would modify these paint formulations according to their own ideas. This implied the addition of terpenoids as balsams, resins or turpentine, plant or animal derived waxes, various geo-materials such as asphalt, bitumen or coal tar, and prepolymerised plant oils. As these materials tended to retard drying of the paint, copious amounts of drying agent were introduced as well.

Results and Discussion

Apart from developing analytical strategies to trace these various materials in the paintings, we have developed a test system based on the oxidation and cross-linking of linseed oil, a common ingredient of oil paint. The oil is dissolved in dichloromethane and exposed to moderate light conditions for an extended period of time. The dichloromethane act as solvent and free radical producing agent. Additives such as Copaiba balsam, mastic and dammar resin, and asphalt are dissolved in the DCM-oil solutions to investigate how they interfere with the oxidation and cross-linking processes of the oil. Changes in the molecular weight distribution are analysed with Size Exclusion Chromatography (RI and UV detection). Direct Temperature resolved Mass Spectrometry (DTMS) is applied to determine the chemical changes in the triglycerides of the oil due to oxygenation and to trace the formation of condensates. More detailed HRMS and HRMSMS research of the triglycerides, oxygenated triglycerides, their dimers and trimers in the exposed oils is performed with ESI-FTMS using a modified 7T Bruker Apex system.

Light exposure of the native oil in the test system leads to formation of oxidised triglycerides and high molecular weight cross-linked fractions enriched in saturated and monounsaturated fatty acids. The latter not unlike what is observed in prepolymerised oils such as stand oil (cross-linked under limitation of oxygen). Introduction of the additives changes the rate of chemical change in the oil. 12 weeks of light exposure show the following. Asphalt is a very potent inhibitor of oxidation and cross-linking in the oil. No chemical change in the oil nor the asphalt is evident after 8 weeks. ESI-FTMS shows incorporation of oxygen in oil triglycerides after 8 weeks. DTMS of the oils after 12 weeks points to formation of cross-linked triglycerides. However the data after 8 weeks point out that the oxidised terpenoids are acting as accelerators of oil oxidation. Copaiba balsam being the strongest accelerator leads to almost complete consumption of the unsaturated C18 fatty acid moieties.

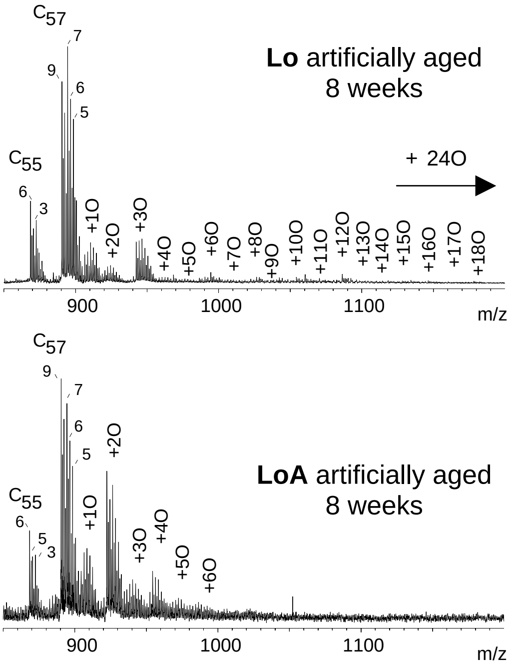

Fig. 1. Partial ESI-FTMS showing autoxidation (i.e. photo-oxidation) of linseed oil artificially aged for 8 weeks as such (incorporation of up to 24 atoms of O) and in the presence of asphalt (only 6 fold oxygenation).

Rezumat

Asfaltul, mumia şi pământul de Kassel sunt pigmenţi bruni sau negri de natură organică folosiţi sau presupus folosiţi de către pictorii secolului XIX, se arată la începutul primului capitol. Multe din picturile atribuite acestui secol sunt cunoscute pentru defectele stratului pictural asociate acestui tip de pigmenţi, defecte agravate în decursul timpului. Putem astfel vorbi de uscarea incompletă a uleiului în culorile preparate cu aceşti pigmenţi, fenomen observat în formă de cracluri premature, migrări sau scufundări ale straturilor respective de culoare. Astfel de fenomene sunt frecvent observate şi au fost raportate de restauratori contemporani, cu ocazia investigării picturilor de secol XIX şi au fost descrise cu mult înainte în literatura de epocă. În ciuda proastei reclame a utilizării pigmenţilor de tip asfalt, mumie şi pământ de Kassel reflectată în calitatea estetică a unei picturi, extrem de puţin este cunoscut şi publicat despre evidenţa acestora la nivel molecular în literatura de specialitate. Ce sunt aceşti pigmenţi de fapt? Este posibilă identificarea lor în probe luate de pe suprafaţa picturilor? Există o legătură directă între prezenţa acestor pigmenţi în straturile de culoare şi uscarea incompletă a uleiului din aceste culori? Aceste întrebări constituie fundamentul prezentei lucrări de doctorat.

Pornind de la asemenea consideraţii, scopul iniţial al acestei lucrări este de a caracteriza la nivel molecular probe de pigmenţi de tip asfalt, mumie şi pământ de Kassel din secolul XIX. Compoziţia chimică a acestor pigmenţi este investigată şi comparată cu probe de referinţă. Odată ce natura şi compoziţia acestor pigmenţi a fost elucidată, următorul scop a fost acela de a încerca identificarea lor în probe prelevate de pe picturi de secol XIX. Rezultatele neconcludente, incerte obţinute prin acest mod de abordare au direcţionat focarul cercetării de la caracterizarea pigmenţilor la investigarea efectului acestor pigmenţi asupra compoziţiei liantului pe bază de ulei. Această nouă direcţie de investigare a fost limitată la cercetarea efectului generat doar de către asfalt şi pământul de Kassel asupra uscării chimice a culorilor tradiţionale pe bază de ulei. Sisteme model îmbătrânite artificial sau natural constând în amestecuri de ulei cu aceşti pigmenţi, sunt folosite pentru a observa şi raporta modificările moleculare care apar cu trecerea timpului în aceşti pigmenţi şi în liantul uleios în sine. Este vizată încercarea de a identifica cauzele uscării incomplete a uleiului în probe selectate de pe picturi de secol XIX, cauze raportate la compoziţia probelor şi efectul acesteia asupra uscării liantului uleios. Capitolul 1 constituie o introducere a pigmenţilor de tip asfalt, mumie şi pământ de Kassel şi a problemelor de conservare legate de folosirea acestor pigmenţi, cu alte cuvinte raţiunea de a exista a acestei lucrări.

Identificarea şi caracterizarea pigmentului asfalt de secol XIX prezentată în capitolul 2 a fost dedusă în primul rând, prin aplicarea spectrometriei de masă bazată

pe creşterea directă a temperaturii (Direct Temperature resolved Mass Spectrometry, DTMS). Tandem DTMS (tandem DTMS, DTMS/MS) a fost folosit pentru confirmarea structurii unor compuşi prezenţi în proba de asfalt. Piroliza probei cuplată cu cromatografie de gaze şi spectrometrie de masă (Pyrolysis Gas Chromatography Mass Spectrometry, Py-GC/MS) a fost aplicată probei investigate pentru a pune în evidenţă compuşii caracteristici asfaltului („markers” sau „biomarkers”) şi pentru a obţine un protocol analitic menit indentificării asfaltului ca tip de pigment. Analiza izotopică a atomilor de carbon cu masa atomică 13, ($^{13}C/^{12}C$) a fost folosită pentru a investiga posibila provenienţă a asfaltului de secol XIX dintr-un depozit de asfalt de la Marea Moartă. Rezultatele au aratat că acest pigment este un asfalt natural, cu o compoziţie caracteristică asfalturilor din Orientul Mijlociu, în acest caz foarte asemănătoare celei a asfalturilor de la Marea Moartă.

Proba de (pigment) mumie de secol XIX a fost caracterizată cu succes folosind spectrometria de masă, după cum este arătat în capitolul 3. DTMS a demonstrat o compoziţie complexă a probei indicând existenţa uleiului şi/sau a grăsimilor, a răşinii mastic îmbătrânite şi a cerii de albine. Adiţional, mai multe tehnici de cromatografie de gaze cuplate cu spectrometrie de masă (GC/MS) au fost folosite pentru caracterizarea şi identificarea compuşilor moleculari specifici în proba de pigment mumie de secol XIX. Protocolul analitic pentru identificarea asfaltului (derivat din Py-GC/MS) a fost folosit cu succes în cazul acestei probe. Din cromatografia de gaze a probei transesterificate cuplată cu spectrometria de masă (Pyrolysis Transesterification GC/MS, Py-TMAH-GC/MS), acizii graşi ai probei de mumie au fost identificaţi ca fiind de origine bactericidă/fungicidă şi/sau umană. Prin aceeaşi tehnică a fost scoasă în evidenţă prezenţa unei raşini de pin neîmbatrânită. GC/MS cu injecţie directă în coloană (on-column GC/MS) a confirmat că răşina de tip terpenoidic din probă este răşină mastic. Prezenţa unui asfalt degradat, a cerii de albină şi a grăsimilor de origine umană nu exclude folosirea de porţiuni provenite dintr-o mumie adevarată la prepararea acestui pigment. Prezenţa componentei uleioase şi a răşinii de mastic sugerează folosirea unui liant de tip „megilp” pentru a obţine o culoare gata de aplicare.

În capitolul 4 este prezentată o serie de pigmenţi tip pământ de Kassel, pământ de Cologne şi brun de Vandyke, identificaţi şi caracterizaţi prin tehnici de DTMS şi GC/MS. Compuşi reprezentativi pentru lignina fosilizată, provenită din lemn, ca şi lipide şi cutină din resturi de frunze au fost identificaţi indicând compoziţia pigmenţilor analizaţi ca fiind asemănătoare lignitului sau cărbunilor bruni. Tehnicile de analiză multivariată folosind analiza componenţilor principali (Principal Component Analysis, PCA), aplicate datelor obţinute după DTMS, au clasificat probele analizate în două grupe: probe cu un conţinut relativ ridicat de componente fosile lemnoase şi probe cu un conţinut relativ ridicat de componente fosile provenite din frunze. DTMS/MS a fost utilizat pentru a confirma structura cerii montane (ceară prezentă pe suprafaţa frunzelor ce au contribuit la formarea depozitului de cărbune) prezentă în probele analizate. Tehnici de extracţie cu solvenţi au arătat că, componentele fosile lemnoase fac parte din reţeaua polimerică a probei. În contrast, componentele fosile provenite din frunze sunt slab ataşate acestei reţele şi ca urmare aproape în întregime extrase cu solvenţi. Py-TMAH-GC/MS a fost folosită cu succes în caracterizarea fracţiilor de lipide (grăsimi), respectiv lignină din probele de pământ de Kassel, pământ de Cologne şi brun de Vandyke. Cu aceeaşi tehnică a fost pusă în evidenţă contribuţia microorganismelor la formarea depozitului de cărbune (degradarea lemnului) precum şi detalii privind taxonomia plantelor din depozitele sursă pentru probele analizate. Studii efectuate cu Py-GC/MS au atestat prezenţa compuşilor tipici pentru răşini naturale (vegetale) şi a compuşilor proveniţi din bacterii. Pe baza rezultatelor obţinute a fost dedus un protocol pentru identificarea pigmenţilor de tip pământ de Kassel, pământ de Cologne şi brun de Vandyke. Rezultatele sugerează o abundenţă ridicată a materialului provenit din frunze pentru pigmenţii de tip pământ de Kassel şi pământ de Cologne, şi o abundenţă ridicată a materialului lemnos pentru pigmenţii de tip brun de Vandyke.

Din punct de vedere analitic DTMS s-a dovedit a fi o tehnică foarte valoroasă ce permite diferenţierea între pigmenţi precum asfalt, mumie şi pământ de Kassel. Pe de altă parte, tehnicile GC/MS folosite au evidenţiat prezenţa compuşilor moleculari prezenţi şi/sau specifici acestor pigmenţi, fapt menit să înlesnească analiza acestui tip de pigmenţi în viitor. Dacă compuşii moleculari pot indica prezenţa unuia din pigmenţii mai sus consideraţi, compuşii moleculari specifici (biomarkers) sunt necesari pentru confirmare.

Capitolul 5 aduce în centrul atenţiei uscarea incompletă a uleiului în culorile pe bază de ulei, preparate cu asfalt sau pământ de Kassel. Cei doi pigmenţi au fost identificaţi cu succes (folosind protocoalele mai sus menţionate) în probe de culoare preparate cu asfalt, respectiv pământ de Kassel, îmbătrânite fie natural, fie artificial. Din păcate, astfel de rezultate se aplică numai parţial probelor provenite din picturi de secol XIX, presupuse a conţine aceşti pigmenţi. Tehnicile de îmbătrânire folosite în cadrul acestor investigaţii au creat o legatură între materialul iniţial –pigmentul şi culoarea de ulei proaspăt preparată– şi produsul final, culoarea de ulei îmbatrânită. După îmbătrânire probele au fost analizate prin spectrometrie de masă, spectroscopie de infraroşu cu transformată Fourier (Fourier Transform Infrared Spectroscopy, FTIR) şi cromatografie de excluziune sterică de înaltă presiune (High Presure Size Exclusion Chromatography, HPSEC). Au fost incluse în această lucrare probe având o compoziţie cunoscută şi îmbătrânite prin diferite tehnici de îmbătrânire. Au fost analizate astfel culori de ulei preparate cu asfalt sau pământ de Kassel de către H.C. Von Imhoff şi îmbătrânite natural în Institutul Canadian de Conservare (Canadian Conservation Institute, CCI). În mod asemănător au fost analizate probe prelevate de pe picturi model conţinând asfalt, preparate de către R. Boitelle in cadrul proiectului MOLART după reţete din secolul XIX şi îmbătrânite artficial la lumină în Atelierul de Conservare din Limburg (Stichting Restauratie Atelier Limburg, SRAL). Soluţii model de asfalt şi ulei de in în diclorometan au fost îmbătrânite la lumină pentru a investiga procesele de oxidare şi reticulare în aceste sisteme. Aceste probe model au fost efectiv caracterizate folosind tehnica de DTMS. Tehnicile de GC/MS (Py-GC/MS, on-column GC/MS, Py-TMAH-GC/MS) aplicate aceloraşi probe au pus în evidenţă prezenţa compuşilor specifici asfaltului în aceste amestecuri (soluţii) de pigment şi ulei de in. A fost arătat că abundenţa acestor compuşi este direct legată de tratamentele iniţiale suferite de pigment cum ar fi prăjirea, încălzirea (până la topire) sau extragerea cu solvenţi, precum şi de metoda de preparare folosită în pregatirea culorii de ulei. Analiza probelor de culoare de ulei indică conservarea unora dintre compuşii specifici ai asfaltului, chiar dacă după îmbătrânire naturală sau artificială la lumină abundenţa lor este mult scăzută! Un mod de abordare asemănător şi cu rezultate similare este descris în anexa A a capitolului 5 unde, pentru o probă de culoare de ulei ce conţine pământ de Kassel este demonstrată conservarea compuşilor moleculari specifici sau mai puţin specifici pământului de Kassel.

Analiza discriminantă (Discriminant Analysis, DA) aplicată rezultatelor de la DTMS a soluţiilor model de asfalt cu ulei de in şi ale soluţiilor model de ulei de in au arătat o variaţie a compoziţiei uleiului, dependentă de prezenţa sau absenţa asfaltului în soluţia analizată. Această dependenţă poate fi explicată ca o încetinire a procesului de uscare a uleiului în prezența asfaltului. Analiza FTIR aplicată unui amestec de asfalt şi ulei de in a confirmat ipoteza că prezența asfaltului încetineşte uscarea uleiului. Se ştie că uscarea uleiului presupune reacții de oxidare şi reticulare a trigliceridelor (TAGs) nesaturate din ulei. Proporțiile dintre acizii graşi monocarboxilici şi acizii dicarboxilici prezenți în ulei, derivate din tehnici de GC/MS, au fost folosite pentru estimarea uscării uleiului în toate probele conținând ulei. Astfel, rapoartele dintre acidul azelaic/acid stearic şi dintre acidul oleic(+elaidic)/acid stearic au fost calculate şi folosite pentru cuantificarea uscării chimice a uleiului. Spectrometria de masă cu transformată Fourier si rezonanță ciclotronică de ioni (Electro Spray Ionisation Fourier Transform Ion Cyclotron Resonance Mass Spectrometry, ESI-FTICR-MS) a arătat că, cantitatea de oxigen încorporată de ulei în prezența asfaltului este mult scăzută, indicând un mecanism diferit de oxidare al uleiului. HPSEC aplicată soluțiilor îmbătrânite de asfalt cu ulei de in a pus în evidență formarea unor substanțe cu mase moleculare ridicate, altele decât cele observate pentru soluțiile individuale de asfalt sau ulei de in. Acest rezultat, combinat cu observația că fracția polimerică a aceleiaşi soluții conține atât ulei cât şi asfalt (rezultat al analizei DTMS), a fost interpretat ca o reticulare între compuşii uleiului şi cei ai asfaltului. Parțial, aceste interpretări sunt discutate şi în anexa B a capitolului 5. Aşadar, atât identificarea compuşilor caracteristici asfaltului sau pământului de Kassel, cât şi obținerea de informații privitoare la uscarea uleiului folosind diferite rapoarte între acizii graşi sunt posibile pentru culori de ulei relativ noi (puțin îmbătrânite). Aceasta sugerează o corelare chimică directă între prezența pigmenților organici bruni sau negri în culorile de ulei şi compoziția uleiului în aceleaşi probe.

În cazul probelor de culoare din secolul XIX nu au putut fi obținute rezultate la fel de detaliate ca în cazul sistemelor model. Analiza DTMS a pus în evidență o compoziție mult mai complexă a acestor probe comparativ compoziției culorilor de ulei preparate după rețete din secolul XIX. Această observație accentuează importanța tehnicii picturale folosite în raport cu compoziția culorii de ulei. Tehnicile de Py-GC/MS şi Py-TMAH-GC/MS au arătat că în unele din probele de secol XIX uleiul s-a uscat mai bine – sub influența sicativilor, a răşinilor şi, posibil, a pământului de Kassel –, iar în alte situații uscarea chimică a uleiului apare încetinită – datorită prezenței pigmentului de vermilion, cerii de albine şi altor ceruri (parafinice) ori datorită posibilei prezențe a unui asfalt degradat.

În concluzie, rezultatele acestei cercetări pun într-o lumină nouă investigarea compoziției chimice a probelor de culoare, presupuse să conțină cel puțin unul din pigmenții adresați aici, probe, provenite din picturi de secol XIX. Numai prin îmbinarea căutării de compuşi specifici pigmentului cu investigarea stării de uscare a uleiului pot fi elucidate întrebările privitoare la cauzele ce au produs uscarea incompletă a liantului uleios în prezența pigmenților de tip asfalt, pământ de Kassel sau mumie.

Summary

The organic black and brown pigments, asphalt, mummy and Kassel earth, are often used or claimed to have been used by painters in the 19th century. In particular paintings from the 19th century show paint defects that can be associated with these pigments. Examples of poor drying of the oil are observed as formation of premature cracks and phenomena, such as migration or sinking-in of layers. These phenomena are already reported in the contemporary literature, but they are also reported nowadays by conservators and restorers investigating 19th-century paintings. With all the indications about the negative influence of asphalt, Kassel earth and mummy pigments on the condition and aesthetic quality of the paintings, surprisingly little or no molecular evidence is so far available. What exactly are these pigments? Is it possible to identify them in painting samples? Are they the reason why the oil binding media dries so poorly? These are questions addressed in this dissertation. An introduction to the asphalt, mummy and Kassel earth pigments and the conservation problems related to the use of these pigments as well as the aims of this dissertation are discussed in Chapter 1.

With such considerations in mind, the primary goal of this thesis is to characterise 19th-century asphalt, mummy and Kassel earth pigments at a molecular level. The chemical composition of these pigments was investigated and compared to relevant pigment reference samples (Chapter 2, 3 and 4). Once the nature and composition of the 19th-century pigments was established, the next challenge was to try and identify them in original 19th-century paint samples. Because of the inconclusive and puzzling data resulting from this approach, the focus of the investigation was shifted from the identification of the pigments in the oil paint samples to the effect of the pigments on the composition of the oil binding medium. This study was limited to research on the effect of asphalt and to some extent Kassel earth on the chemical drying of traditional oil paint. Molecular changes that occur in reference pigments and in the oil binding medium itself during ageing of pigment-oil model systems (paints and solutions) are reported in the last chapter of the thesis and in annexes to that chapter. An attempt to identify the cause of poor drying of the oil in selected 19th-century paint samples is made in the same chapter.

The identification and characterisation of the 19th-century asphalt pigment (from the Hafkenscheid collection, in the Teylers Museum, Haarlem, The Netherlands) is presented in Chapter 2. The analytical work was in the first instance performed with Direct Temperature resolved Mass Spectrometry (DTMS). DTMS tandem MS (DTMS/MS) was to used to confirm the structure of some of the compounds present in the 19th-century sample. Then Pyrolysis Gas Chromatography Mass Spectrometry (Py-GC/MS) was applied to determine the distribution of molecular markers and so-called biomarkers. The results were used to develop an analytical protocol for the identification of asphalt in paint samples. Furthermore the analysis of $^{13}C/^{12}C$ isotopic ratio was carried out to investigate the possible Dead Sea provenance of the 19th-century asphalt sample. These data together demonstrate that the 19th-century asphalt pigment from the Hafkenscheid collection is indeed a natural asphalt of a Middle Eastern provenance. Moreover a rather good similarity to Dead Sea asphalt is suggested.

The results of investigation of the 19th-century mummy pigment (from the same Hafkenscheid collection) by mass spectrometric techniques are reported in Chapter 3. First DTMS revealed a complex bulk composition that consists of oil and/or fat constituents, medium aged mastic resin and beeswax. In addition to DTMS, various GC/MS techniques were applied for the characterisation and identification of molecular markers in the 19th-century mummy sample. For example Py-GC/MS was effectively used to search for the presence of asphalt in the sample. The fatty acids of the mummy pigment sample were investigated by an on-line transesterification method (Py-TMAH-GC/MS). The results pointed to bacterial, fungal and/or human origins of the fat component. Additionally the same technique revealed the presence of a rather fresh (unaged) pine resin in the sample. Then on-column GC/MS was applied for a better characterisation of the triterpenoid resin fraction and the results demonstrated the presence of resin of mastic in the sample. The combination oil and mastic resin point to the use of a megilp medium as a means to bind the pigment mummy pigment powder. The presence of degraded asphalt, beeswax and possibly human fat does not exclude the use of a real mummy parts as constituents of the preparation of the 19th-century Hafkenscheid mummy pigment.

Chapter 4 presents the DTMS and GC/MS investigation of a series of Kassel earth, Cologne earth and Vandyke brown pigments from the 18th- and 19th-century (Vigani, Winsor & Newton and Hafkenscheid collections) as well as reference pigments[1]. Compounds representative for fossil lignin from wood and for lipids and cutin from leaf remains were found, indicating that the pigment samples have a composition reminiscent of the composition of lignite or brown coal formed from tropical forests. Then multivariate analysis techniques using Principal Component Analysis (PCA) applied to the DTMS data, classified the samples in two main groups: samples with a relatively high abundance of fossil wood components and samples with a high abundance of fossil leaf compounds. Moreover DTMS/MS confirmed the structure of the montan waxes originating from the leaf surface wax layers. Next, solvent extraction techniques were applied and revealed that fossil wood components are more likely bound to the polymeric network of the samples, while the fossil leaf components are weakly bound and mostly removable by solvents. The lipid and lignin derived fractions in the Kassel earth, Cologne earth and Vandyke brown pigments were further characterised by Py-TMAH-GC/MS. Therefore fatty acid and alcohol moieties of the montan waxes were identified. Indications for the contribution of lipids (from microorganisms involved in the degradation of wood) and evidence for the type of wood that contributed to the lignite/brown coal deposits were also obtained. Py-GC/MS studies gave information about the presence of plant resins and bacterial biomarkers. As a result a protocol for the identification of Kassel earth, Cologne earth and Vandyke brown pigments was derived. It is proposed to attribute a

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[1] including the Kassel earth pigments described in the *Artists' Pigments, A Handbook of Their History and Characteristics*, vol. 3 in 1997 by Feller and Feller.

higher abundance in fossil leaf material to Kassel and Cologne earth type of pigments and a higher abundance in fossil wood material to the Vandyke brown type of pigments.

From a microanalytical point of view, DTMS was proved a valuable tool that allows the discrimination between asphalt, mummy, Kassel earth, Cologne earth and Vandyke brown pigments. The GC/MS techniques, on the other hand, reveal common and specific marker molecules, including the biomarkers, in these pigments, which should make the recognition of such materials much easier in the future. The less specific markers can give only indications for the presence of one of these pigments, but the presence of biomarkers is required for confirmation.

Chapter 5 focuses on the poor drying of oil paint with asphalt and Kassel earth pigment investigating the fate of pigment biomarkers, the influence of the pigment on the drying of oil and case studies of 19th-century paint samples. As a start, the protocols for identification of these pigments were tested on natural or artificially light aged reference pigments and paint model systems. The ageing techniques presented in this thesis created a link between the initial material, (pretreated) pigment and freshly prepared pigmented oil paint, and the aged pigmented paint as “final” product. Paint samples of known composition and ageing history were used for research and provided valuable information on the fate of marker molecules in paint. Therefore asphalt and Kassel earth oil paints prepared by H.C. von Imhoff and naturally aged at the Canadian Conservation Institute in Ottawa, and the 19th-century asphalt-linseed oil paint reconstructions made by R. Boitelle for the MOLART project and aged at the artificial light-ageing facility of the Limburg Conservation Studios (SRAL) gave a good insight into the influence of pigment preparation and ageing on the chemical drying of oil. Asphalt specific markers were traced in the pretreated pigments and in the oil paints with the various available MS techniques (DTMS, Py-GC/MS, on-column GC/MS, Py-TMAH-GC/MS). It was shown that the presence and abundance of these compounds is directly related to the pretreatment of the pigment, for example roasting, heating or solvent extraction and also to the preparation method of the oil paint. Thus, the analysis of the natural and light aged paint samples shows that even though some markers are lost or diminished in abundance, some of the asphalt biomarkers are preserved! A similar approach is described in the Annex A of Chapter 5 for a naturally aged, Kassel earth containing oil paint. This also shows that the (bio)markers of the pigment can be retrieved from the oil paint. So a number of these pigment-specific molecules or biomarkers remains identifiable in oil paint made with asphalt or Kassel earth pigment although there is a strong dilution in the paint preparation process, which will make it difficult to retrieve all those markers in original paint samples.

Simpler pigment-oil model systems prepared with asphalt and linseed oil in dichloromethane (DCM) and exposed to light, provide a more detailed chemical insight into the processes of oxidation and cross-linking that play a role in the drying of asphalt-oil paint. First, the changes in linseed oil and asphalt-linseed oil DCM solutions were traced with DTMS. Next, the discriminant analysis was applied to the DTMS data of the model asphalt-linseed oil and linseed oil solutions and revealed a different composition of the oxidising oil in the presence of asphalt. This was translated as a lower speed of drying of the oil. Additionally results of Fourier Transform Infrared Spectroscopy (FTIR) applied to an asphalt-oil mixture in comparison to oil alone supported the supposition that asphalt retards the drying of the oil. Drying of oil is known to involve oxidation and cross-linking reactions of the unsaturated triglycerides (TAGs) in oil. These mechanisms were proven with

Electrospray-Fourier Transform-Ion Cyclotron Resonance MS (ESI-FTICR-MS) and High Performance Size Exclusion Chromatography (HPSEC) techniques. On the molecular level, the technique of ESI-FTICR-MS showed that less oxygen was incorporated in the aged TAGs of the linseed oil in the presence of asphalt, which points to a different mechanism of the oxidation of the oil compared to linseed oil alone. HPSEC data of linseed oil DCM solutions (no asphalt) show a very limited formation of high molecular weight material. The presence of asphalt in an oil DCM solution increases the molecular weight of the asphalt fraction. In combination with DTMS results, this was interpreted as an indication that oil and asphalt components form a network macromolecule as a result of cross-linking reactions between the asphalt and TAGs. The oxidation and cross-linking reactions also influence the fatty acid and dicarboxylic acid distributions. The changing ratio between azelaic acid and stearic acid and the ratio between oleic (+ elaidic) acid and stearic acids were determined by GC/MS. They have been identified to be a measure for the condition of the chemical drying of the oil. Paint made with natural asphalt has still a comparatively high abundance of remaining oleic and elaidic acid and a relatively low abundance of azelaic acid as compared to the non-reactive stearic acid after 25 years of exposure. The preparation methods of asphalt pigments and asphalt paint according to 19th-century recipes change the antioxidising power of the asphalt, which can be deduced from the ratios of fatty acids and dicarboxylic acids. Unexpectedly, the same ratios determined for oil paint made with Kassel earth pigment demonstrated that Kassel earth apparently does not affect the chemical drying of oil paint. It is, therefore, possible to trace compounds specific to asphalt or Kassel earth in rather fresh oil paints as well as to get information about the drying of the oil paint using ratios of the fatty components. This points to a direct chemical correlation between the presence of the organic black and brown asphalt and Kassel earth pigments and the composition of the drying oil.

Detailed results similar to those acquired from the model systems could not be obtained from the original 19th-century paint samples. DTMS showed that the composition of painting samples is in reality more complex than the composition of asphalt oil paint model samples prepared according to 19th-century recipes. This is an indication that the painting technique itself plays an important role in determining the composition of the oil paint on the painting, too. Py-CG/MS and Py-TMAH-GC/MS indicate that in some samples the oil has dried better under the influence of driers, resins and possibly Kassel earth pigments, while in other samples the chemical drying of oil seems retarded due to the presence of vermilion, and components such as beeswax and paraffin waxes and/or possibly degraded asphalt. Nevertheless not enough evidence in the form of biomarkers was found for the presence of asphalt or Kassel earth pigments in these samples.

One can learn from these investigations that a study of the paint composition requires a search for specific pigment markers as well as an investigation of the drying state of the oil. These will offer answers to the questions regarding the cause of the poor drying of the oil binding medium in connection to asphalt, Kassel earth or mummy pigments.

Samenvatting

Organische zwarte of bruine pigmenten asfalt, mummie pigment, en Kasselse aarde zijn vaak gebruikt of althans er wordt gezegd dat ze vaak zijn gebruikt door schilders in de 19^e eeuw. Vooral schilderijen uit de 19^e eeuw vertonen verfdefecten, die geassocieerd worden met deze pigmenten. Als voorbeelden van slechte droging van de verf noemen wij de vorming van jeugdbarsten en andere verschijnselen zoals het migreren en verzinken van verflagen. Deze verschijnselen worden reeds genoemd in de literatuur uit die tijd, maar worden ook hedendaags genoemd door conservatoren en restoratoren, die 19^e eeuwse schilderijen onderzoeken. Ondanks alle geruchten en verhalen over de negatieve invloed van de donkergekleurde organische pigmenten op de toestand en esthetische kwaliteit van schilderijen, is er verbazingwekkend weinig tot geen moleculaire informatie beschikbaar. Wat zijn dit voor pigmenten? Is het mogelijk om ze te identificeren in schilderijen? Zijn zij de oorzaak van het slechte drogen van olieverf? Dit zijn vragen die in dit proefschrift aan de orde komen. Een inleiding in de pigmenten gemaakt met asfalt, mummie pigment, en Kasselse aarde tezamen met de conserveringsproblemen, die te maken hebben met het gebruik van deze pigmenten, worden gegeven in hoofdstuk 1.

Met deze overwegingen in het achterhoofd, is het voornaamste doel van dit proefschrift om 19^e eeuwse pigment monsters van asfalt, mummie pigment, Kasselse aarde te karakteriseren. De chemische samenstelling van deze pigmenten is onderzocht en vergeleken met relevante referentiemonsters. Toen de aard en samenstelling van deze pigmenten waren vastgesteld, was de volgende uitdaging om ze te trachten te identificeren in de verouderde olieverf van schilderijen. Omdat deze benadering tot niet éénduidige en verwarrende resultaten leidde, is het kernthema van het onderzoek vervolgens gericht op de effecten, die dit soort pigmenten hebben op de samenstelling van het oliebindmiddeldeel van de verf. De studie beperkt zich tot het effect van asfalt en in zekere mate Kasselse aarde op het chemisch drogen van traditionele olieverf. De moleculaire veranderingen in deze pigmenten en in het oliebindmiddel, die werden onderzocht in olieverfmodelsystemen samengesteld met deze pigmenten, zijn gerapporteerd in het laatste hoofdstuk en aanhangsels aan dat hoofdstuk. Een poging om de oorzaak van het slechte drogen van olieverf in enkele 19^e eeuwse schilderijen vast te stellen, komt ook in dat hoofdstuk aan de orde.

Hoofdstuk 2 gaat over de identificatie en karakterisering van een 19^e eeuws asfalt pigment uit de Hafkenscheid collectie, die in Het Teylersmuseum wordt bewaard. In eerste instantie zijn de analyses uitgevoerd met directe temperatuursopgeloste massaspectrometrie (DTMS). DTMS tandem massa-spectrometrie (DTMS/MS) is gebruikt om de structuur vast te stellen van sommige verbindingen in het asfaltmonster. Pyrolyse-gaschromatografie-massaspectrometrie (Py-GCMS) werd toegepast om de verdeling van kenmerkende moleculen en zogenaamde biomarkers vast te stellen teneinde een analytisch protocol voor typering van asfalt in verfmonsters te ontwikkelen. De analyse van de $^{13}C/^{12}C$ isotopenverdeling leverde informatie over een mogelijke relatie en herkomst van het 19^{e} eeuwse asfaltmonster uit het Dode Zee gebied. Alle gegevens bij elkaar tonen aan dat het asfaltmonster uit de Hafkenscheid collectie inderdaad een asfalt is, die veel lijkt op het asfalt uit de Dode Zee. In ieder geval is het afkomstig uit het Midden Oosten.

De resultaten van het onderzoek van het 19^{e} eeuwse mummie pigment uit de Hafkenscheid collectie, dat met succes kon worden gekarakteriseerd met massaspectrometrische technieken, zijn gerapporteerd in hoofdstuk 3. DTMS wees op een bulksamenstelling bestaande uit olie en/of vet componenten, enigszins verouderde mastiek en bijenwas. Naast DTMS werden verscheidene GC/MS technieken toegepast voor het karakteriseren en identificeren van kenmerkende moleculen in het mummie pigmentmonster. Het Py-GCMS protocol werd gebruikt om asfalt op te sporen met positief resultaat. De vetzuren in het mummie pigmentmonster, die onderzocht werden met een on-line omesterings GCMS-techniek (Py-TMAH-GC/MS) vertonen kenmerken van bacteriën en schimmels, maar deze vetzuren zijn ook niet onbekend in menselijke vetten. Met deze techniek werden ook moleculen aangetroffen die overeenkomen met relatief verse dennenhars. On-column GC/MS ingezet voor een preciezere identificatie van de triterpenoid harsmoleculen bevestigde mastiekhars als bestanddeel van het monster. De aanwezigheid van asfaltresten, bijenwas en menselijke vetten zijn voldoende bewijs voor het gebruik van echte mummie bij de bereiding van dit pigment. De olie en mastiek wijzen op het gebruik van megilp medium als middel om mummie pigmentpoeder te binden.

Hoofdstuk 4 gaat over DTMS en GC/MS onderzoek aan een serie monsters van Kasselse aarde, Keulse aarde en Vandyke (Vandijk) bruin pigment. Moleculen representatief voor fossiel lignine uit houtresten en voor lipiden en cutine uit resten van bladeren zijn gevonden hetgeen er op wijst dat de pigmenten een samenstelling hebben die overeenkomt met ligniet of bruinkool gevormd uit tropische bossen. De DTMS spectra van de pigmenten konden met de multivariaat analyse techniek van principale componentenanalyse (PCA) worden geclassificeerd in twee hoofdgroepen: monsters met een relatief hoog gehalte aan fossiele houtresten en monsters met nogal veel bladresten. DTMS/MS bevestigde de structuur van de montaanwassen, die afkomstig zijn van de waslaag van bladeren. De vetstoffen van bladeren bleken veelal oplosbaar met organische oplosmiddelen, terwijl de fossiele hout componenten meer gebonden bleken aan een polymeernetwerk. De lipide en lignine fracties in de onderzochte Kasselse aarde, Keulse aarde en Vandyke bruin pigmenten werden met succes gekarakteriseerd met Py-TMAH-GCMS. Op die manier werden deelstrukturen van de montaanwassen geïdentificeerd, werden vetzuurresten gevonden voor microorganismen, die betrokken zijn bij de afbraak van het hout, en kwamen er aanwijzingen naar voren over het type hout dat heeft bijgedragen aan het bulk organisch materiaal in de ligniet afzettingen. Py-GC/MS studies verschaften informatie over de aanwezigheid van harsresten en bacteriële biomarkers. Op basis van de diverse marker moleculen wordt een protocol voorgesteld voor de identificatie van Kasselse aarde, Keulse aarde en Vandyke bruin pigment. Een hoger gehalte aan fossiele bladresten lijkt een kenmerkende eigenschap van Kasselse aarde en Keulse aarde, terwijl Vandyke bruin pigmenten meer houtresten lijken te bevatten.

Vanuit een algemeen micro-analytisch gezichtspunt, heeft DTMS zijn waarde bewezen bij het onderscheid van asfalt, mummiepigment en de lignietpigmenten Kasselse aarde, Keulse aarde en Vandyke bruin. GC/MS anderzijds toont veel gewone en bijzondere kenmerkende moleculen aan, inclusief de biomarkers, die het veel makkelijker zullen maken om deze pigmentgroepen in de toekomst te herkennen. De minder specifieke marker moleculen geven wel aanwijzingen voor de aanwezigheid van een van deze pigmenten, maar voor bevestiging van een toekenning zijn de biomarkers onontbeerlijk.

Hoofdstuk 5 richt zich op het slecht doordrogen van olieverf met asfalt of Kasselse aarde. De protocollen voor het herkennen van asfalt en Kasselse aarde werden getest op natuurlijke en kunstmatig licht verouderde pigment en verfmodelsystemen. De versnelde verouderingsmethoden, die werden toegepast bij het onderzoek, vormden de schakel tussen de uitgangsmaterialen, de vers gemengde gepigmenteerde olieverf, en de licht verouderde verf als eindproduct. Monsters met een bekende samenstelling en verouderingsgeschiedenis, die beschikbaar waren gesteld voor onderzoek, gaven waardevolle informatie over wat er met marker moleculen gebeurt in verf. Verf bereid met asfalt en Kasselse aarde door H.C. von Imhof en natuurlijk verouderd bij het Canadian Conservation Institute in Ottawa en 19^{e} eeuwse asfalt-olieverfmodellen gemaakt door R. Boitelle voor MOLART en verouderd bij de Stichting Restauratie Atelier Limburg gaven een goed inzicht in de effecten van pigment voorbereiding, verfdroging en veroudering. Met behulp van de verschillende beschikbare MS technieken (DTMS, Py-GC/MS, on-column GC/MS en Py-TMAH-GC/MS) zijn de asfalt specifieke marker moleculen in het pigment en de gepigmenteerde olieverfmonsters gevolgd. Het is duidelijk dat de voorbewerking van het asfaltpigment door roosteren, verwarming of oplosmiddel extractie een beduidende invloed heeft op de aanwezigheid en relatieve hoeveelheid van deze markers, maar ook de bereidingswijze van de olieverf heeft een merkbare invloed. Bij de analyse van de natuurlijke en kunstmatig lichtverouderde pigment-olieverfmonsters kwam aan het licht dat, hoewel sommige marker moleculen niet stabiel waren of verminderden in relatieve hoeveelheid, een aantal asfalt biomarkers verouderingsresistent bleken. Vergelijkbare resultaten werden verkregen bij de Kasselse aarde olieverf modelsystemen beschreven in de Annex van hoofdstuk 5. Zowel voor asfaltolieverf als voor verf gemaakt met Kasselse aarde geldt dus dat een aantal pigment specifieke moleculen herkenbaar blijven, alhoewel er natuurlijk wel een verdunningseffect optreedt door de verfbereiding, waardoor het toch moeilijk kan zijn ze in zeer kleine verfmonsters van schilderijen terug te vinden.

Toch geven eenvoudiger modelsystemen, bereid met asfalt en lijnzaadolie in dichloormethaan (DCM) oplossingen, die aan licht zijn blootgesteld, een gedetailleerder chemisch inzicht in de processen van oxidatie en cross-linking, die ook bij de verfdroging in combinatie met asfalt pigment een rol spelen. De veranderingen in lijnzaadolie en asfalt-lijnzaadolie DCM oplossingen werden gevolgd met DTMS, waarbij de discriminant analyse toegepast op de massaspectra een kwantitatief beeld verschafte. Het asfalt in de lijnzaadolie vertraagde de chemische verandering van de olie zeer sterk hetgeen vertaald kan worden als het sterk remmen van de chemische droging in de olieverf door aanwezigheid van asfalt. Dat laatste werd ook ondersteund door Fourier Transform Infrarood (FTIR) metingen. De droging van de olie draait om de oxidatie en cross-linkingsreacties van de onderverzadigde triglyceriden. Met Electrospray-Fourier Transform-Ion Cyclotron Resonantie MS werd op moleculair niveau aangetoond dat er in aanwezigheid van asfalt minder zuurstof werd ingebouwd in de triglyceriden van de lijnzaadolie. Er zijn tegelijkertijd ook aanwijzingen dat asfalt de reactiviteit van de zuurstof beïnvloed. De lijnzaadolie geanalyseerd met size exclusie chromatografie (HPSEC) vertoond een zeer beperkte mate van cross-linking in de DCM oplossing. In aanwezigheid van asfalt daarentegen wordt het moleculair gewicht van het asfalt groter. In combinatie met DTMS metingen werd duidelijk dat componenten van de olie en het asfalt samen een netwerkmacromolecuul vormen als gevolg van cross-linkingsreacties. Deze reacties hebben ook gevolgen voor de vetzuur- en dizuurverdeling. Met GC/MS zijn de veranderende azeleïne /stearine zuur verhouding en de verhouding van oliezuur (+ elaidine zuur)/ stearine zuur bepaald als maat voor de chemische droogtoestand van de olie en de olieverf. In verf gemaakt met natuurlijk asfalt, is de relatieve hoeveelheid resterend olie- en elaidinezuur betrekkelijk hoog en het azeleïne zuur gehalte betrekkelijk laag vergeleken met het niet-reactieve stearine zuur. De voorbewerkingen van het asfalt en asfaltverf volgens 19^{e} eeuwse recepten veranderen de anti-oxyderende kracht van het natuurlijk asfalt vrij sterk wat is af te lezen uit de verhoudingen van dit soort vetzuren. Bij olieverf met Kasselse aarde bleek onverwachts dat dit pigment weinig invloed uitoefende op de samenstelling van de olie. Het is dus mogelijk om door een combinatie van voornamelijk MS metingen inzicht te krijgen in de sporencomponenten, die specifiek zijn voor asfalt en Kasselse aarde, en tegelijkertijd de moleculaire signatuur van het oliedeel te bevragen. Het is duidelijk dat er een directe relatie is tussen de samenstelling van de bestanddelen van de olie en olieverf enerzijds en anderzijds met de aard en reactiviteit van de organische zwarte en bruine pigmenten.

Zulke detailleerde resultaten als verkregen met de modelsystemen, kwamen niet uit de metingen van verfmonsters uit 19^{e} eeuwse schilderijen. Deze monsters waren overigens gekozen op grond van voornamelijk fysische verschijnselen. Uit de DTMS gegevens blijkt dat de samenstelling van de schilderijmonsters in werkelijkheid veel complexer is dan de samenstelling van asfalt-olieverf, die gemaakt is volgens 19^{e} eeuwse methoden. Dit is een aanwijzing dat de werkwijze van de schilder ook een belangrijke rol speelt met betrekking tot de samenstelling van de verf op het schilderij. Py-GC/MS en Py-TMAH-GC/MS tonen aan dat in sommige monsters de olieverf chemisch veel droger is onder invloed van chemische drogers, harsen en mogelijk Kasselse aarde pigmenten, dan in andere schilderijen waarin de oliedroging vertraagd lijkt door aanwezigheid van pigmenten als vermiljoen en componenten als bijenwas, paraffine wassen en mogelijk partieel afgebroken asfalt.

Wij concluderen uit dit onderzoek dat als we meer te weten willen komen over wat het slechte drogen van het oliebindmiddel in relatie met asfalt, mummiepigment en Kasselse aarde veroorzaakt, een studie naar specifieke marker moleculen in de pigmenten gecombineerd met onderzoek naar de toestand van droging van de verf, zijn vruchten afwerpt.

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About the author

On 22 April 1970 I was born in Bucharest, Romania. In 1988 I graduated the Industrial High Scool no. 27 in Bucharest with the diploma subject *Studies upon ferrites and superaluminous pottery*. There after I followed the courses of the Faculty of Industrial Chemistry of The Polytechnic University of Bucharest and I specialised in The Technology of Macromolecular Compounds. In 1993 I graduated with the diploma paper *The study of the Polyvinyl Acetate in methanolic solution using the Brabender plastograf*. Following graduation I worked as a researcher in the Laboratory of Polymeric Materials of the National Building Research Institute (INCERC) in Bucharest, Romania, on polymeric sheets, paints and adhesives used in construction. In 1995 I started to work as a chemist on the restoration of paintings at the Panel Painting Studio of the National Museum of Art of Romania (Muzeul Național de Artă al României), in Bucharest. This period gave me the opportunity to get an insight into art history, restoration practice and the chemistry of Romanian icons on wood and panel paintings in general. During this stage, that lasted untill 1998, I spent six months as an intern at the Central Research Laboratory for Objects of Art and Science (now Institute of Netherlands Institute for Cultural Heritage), in Amsterdam. Here I investigated paint samples from a series of 17th, 18th and 19th-century Romanian icons and pigments and binders from *The Massacre of the Innocents*, painting attributed to Pieter Bruegel the Elder's studio, all from the National Museum of Art of Romania. In August 1997 I started my PhD at FOM-AMOLF in Amsterdam on the characterisation and identification of the asphalt, mummy and Kassel earth pigments and their effect on the chemical drying of traditional oil paint. The results of this research are the subject of my thesis.