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Thermodesorption–gas chromatography–mass spectrometric analysis of biological materials for potential molecular precursors of the constituents of the crude oils

WILHELM PÜTTMANN

Lehrstuhl für Geologie, Geochemie und Lagerstätten des Erdöls und der Kohle, RWTH Aachen, Lochnerstrasse 4-20, W-5100 Aachen (Germany)

ABSTRACT

Thermodesorption technique coupled on-line with gas chromatography (GC) and mass spectrometry (MS) has been developed for the determination of hydrocarbons trapped in coal and rock samples. The method can also be applied to the determination of volatile constituents present in biological materials. The desorption step is carried out in the pre-heated injector of a gas chromatograph. The mobilized compounds are transferred without splitting to the initial part of a cold GC column. After 10 min of desorption the GC–MS analysis is started. The identification of individual compounds is achieved by GC–MS analysis of related molecular ions or significant fragment ions. When micro-samples (0.25–2.0 mg) of dried plant material are heated to 300°C in a helium stream, compounds such as long-chain fatty acids, carboxylic acid esters, aldehydes and ketones as well as steroids and tocopherols are desorbed from the biological matrix without decomposition. In addition, the thermally labile chlorophylls decompose to yield phytadienes as pyrolysis products of the phytyl moiety. The phytadienes can be used to estimate the original amount of chlorophyll present in the sample and to determine the chlorophyll:tocopherol ratio in different algae and terrestrial plant materials. This ratio partly reflects the pristane:phytane concentration ratio determined in extracts of mature sediments rich in related fossilized biological materials. The analysis also provides chemotaxonomic information about the sterol composition of plants.

INTRODUCTION

The isoprenoid alkanes pristane ($C_{19}H_{40}$) and phytane ($C_{20}H_{42}$) are common constituents of ancient rocks, coals and crude oils. Both compounds have been suggested to originate from chlorophyll as the major precursor molecule [1]. Oxidation during the earliest stages of chlorophyll decomposition generates phytanic acid, the precursor of pristane. Under anaerobic conditions the phytyl side-chain of chlorophyll is reduced to phytane after hydrolysis. Low pristane:phytane concentration ratios in crude oils were once thought to indicate a primary marine plant input, whereas land plant material was thought to produce the high pristane:phytane ratios commonly observed in coals [1]. However, organic geochemical research has shown that a wide variety of potential precursor molecules for both compounds exist in nature, including tocopherols [2] and archaebacterial diphytanyl ethers [3,4]. The significance of the pristane:phytane ratio as a palaeo-environmental indicator [5] has

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been questioned based on these finding and by considering the geological constraints of a large set of crude oils and rocks which have been investigated [6]. However, it remains to be clarified whether the pristane:phytane ratio in fossil materials is primarily controlled by palaeo-environmental conditions during sedimentation or by the type of organic matter input. This is unclear largely as a result of insufficient knowledge about the abundance and relative amounts of potential precursor molecules for pristane and phytane in recent plant material.

In this study, thermodesorption (TD) coupled on-line to gas chromatography (GC) and mass spectrometry (MS) is used for the analysis of plant material for pristane and phytane precursors and also for sterols as precursor molecules of steranes in crude oils and coals.

Volatile compounds emitted from plants have recently been analysed using head-space analytical techniques [7,8], overcoming the difficulties in isolating these volatiles by conventional solvent extraction and separation methods [7]. The advantage of the application of head-space techniques is the avoidance of contamination during the extraction and treatment of extracts. Plant constituents which are volatile at higher temperatures can be recovered in a similar way by the thermal treatment of samples in an inert gas atmosphere. However, this can cause not only desorption of the molecules present in the plant, but also the generation of fragments from high-molecular-weight compounds and biopolymers. A useful method to study the nature of biopolymers is pyrolysis combined with GC and MS [9–11]. An alternative approach is the application of pyrolysis combined with field ionization MS. The application of this method to spruce needles provided information about the chemical structure of pyrolysis products generated from macromolecules and at the same time information about the content of thermally stable constituents such as α-tocopherol and β-sitosterol in plants [12,13].

This study focusses on the qualitative analysis of thermally stable plant constituents of molecular mass less than 500. The compounds are thermally extracted from plant material in a helium gas flow using a desorption temperature of 300°C. As the recovery rates for individual compounds have not yet been determined, the method cannot provide quantitative data at this stage. The method has previously been used to study the hydrocarbon content of coals and rock samples [14,15] and the uptake of atmospheric pollution by plant waxes [16].

EXPERIMENTAL

Samples

The sample material analysed and amount of sample used for the analysis are listed in Table I. The *Chlorococcales* algae were cultured in the laboratory; algae samples 4–8 originate from the North Sea. The spruce and pine needles were taken from trees in a remote area of the Eifel mountains of western Germany.

The samples were stored in a deep freezer and then dried under a nitrogen atmosphere before carrying out the analysis. The amount of sample required for the analysis depended on the lipid content of each sample and on the sensitivity of the mass spectrometer.

TABLE I

SAMPLES ANALYSED USING TD–GC–MS

Sample no.	Sample	Amount analysed (mg)
1	*Tetraedron minimum (Chlorococcales)*	0.830
2	*Chlorella fusca (Chlorococcales)*	0.638
3	*Pediastrum duplex (Chlorococcales)*	0.250
4	*Scenedesmus obliquus (Chlorococcales)*	0.322
5	*Fucus* sp. *(Phaeophyceae)*	0.830
6	*Halidrys* sp. *(Phaeophyceae)*	0.670
7	*Cladophora (Chlorophyceae)*	0.520
8	*Plocamium (Rhodophyceae)*	0.984
9	Spruce needles	1.851
10	Pine needles	1.815

Thermodesorption

Crushed sample material is placed in small glass tubes (10 × *ca.* 1 mm I.D.) with one end sealed. The glass tubes are pre-heated in a gas flame to remove contamination before the sample is introduced. The exact sample weight is determined using a micro-scale autobalance (Perkin-Elmer).

Thermodesorption is carried out with an autosampler commonly used with elemental analysers (Carlo Erba), which is mounted on top of a split–splitless injection system for GC (Varian). The autosampler drops the glass tubes into the liner of the heated injection port under a helium atmosphere. Helium is also used as the carrier gas for GC. The injection port is operated at a temperature of 300°C in the splitless mode. Volatile compounds present in the sample are transferred to the inlet end of the GC column where they are trapped. Test measurements revealed that a desorption time of 10 min is sufficient for a complete release of the desorbable compounds. The glass tubes are removed from the GC liner after each run.

Gas chromatography

Trapping of the desorbed compounds is achieved by maintaining the oven temperature of the gas chromatograph at 40°C during thermodesorption. The GC separation is carried out using a Varian 3700 gas chromatograph equipped with a 25 m × 0.25 mm I.D. fused-silica column coated with chemically bonded SE-54 silicone as the stationary phase (film thickness 0.25 μm). After the completion of desorption the oven temperature is increased from 40 to 300°C at a rate of 4°C/min and held at the final temperature for 20 min.

Mass spectrometry

The GC capillary column is directly routed into the ion source of a Finnigan MAT 8200 mass spectrometer, which is operated with an electron energy of 70 eV, an emission current of 1.0 mA and an ion source temperature of 240°C. The mass spectra are recorded from 50 to 700 mass units with an scan time of 1.1 s. The data are processed with an INCOS data system. Mass chromatography is used for the selective registration of individual ions.

Determination of the relative intensities has been carried out by comparing the peak integration data of phytadienes (for chlorophyll) and of α-tocopherol and the steroids using the reconstructed ion chromatogram (RIC) intensities. When superimposition with other compounds occurred, the data were obtained from mass chromatograms of fragments or molecular ions using a correction factor determined by comparing the peak intensity in the mass chromatogram and the RIC of a sample where superimposition of the compound is not visible.

RESULTS AND DISCUSSION

Lipids with long carbon chains

The RIC in the scan range 1200–4100 obtained from the GC–MS analysis of *Tetraedron minimum* (sample 1) is shown in Fig. 1. Constituents with lower boiling points (<200°C) were not detected in this sample. Two major compounds are the *n*-C_{16} fatty acid (3) and the *n*-C_{18} monounsaturated fatty acid (5). The dominance of the C_{18} unsaturated fatty acid over the C_{16} fatty acid has been reported previously [17] to be a typical feature of green algae. Owing to their high polarity, both compounds elute from the SE-54 column as broadened peaks. This is unavoidable because the total lipid content of the algae, which ranges from non-polar to highly polar compounds, is transferred to the column. Except for fatty acids, other types of compounds are sufficiently resolved by the SE-54 capillary column. *n*-Alkanes are minor constituents of the desorbed material with the exception of *n*-heptadecane (1) and *n*-tricosane (6). The dominance of these two *n*-alkanes is very rare in plant materials

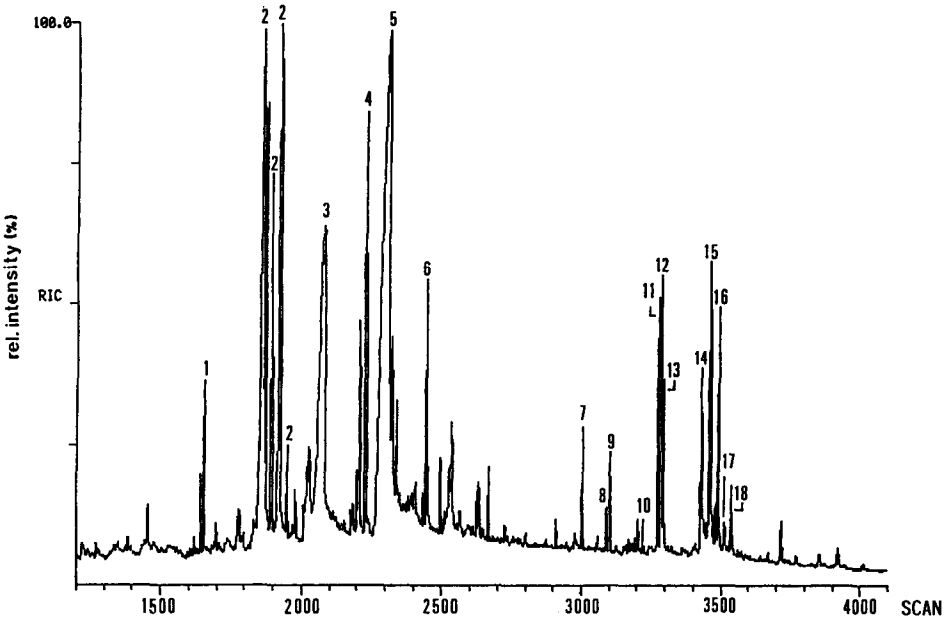


Fig. 1. RIC in the scan range 1200–4100 obtained by GC–MS analysis of the volatile material thermally desorbed from the algae *Tetraedron minimum* at 300°C. For identification of compounds see Table II.

and in sedimentary extracts. Twenty years ago the predominance of n-C_{17} and n-C_{23} within the n-alkane pattern of the Eocene Green River oil shale was ascribed to the abundance of *Tetraedron* sp. in this shale [18]. Recently, combined morphological and organic geochemical investigations have shown this abundance to also exist in the Eocene Messel lake sediment deposits near Darmstadt, Germany. *Tetraedron* algae, identified by scanning electron microscopy as *Tetraedron minimum*, make up the major part of the organic matter in the sediment [10,19].

In the lower boiling point range of the thermal extract of *Tetraedron minimum* phytadienes [2] are present at a relatively high abundance, in addition to fatty acids and n-alkanes. The elution order of the five possible isomers has been partly clarified [20]. It has been proposed that phytadienes are short-lived geochemical intermediates which are easily incorporated into the macromolecular network in sediments [20]. Nevertheless, phytadienes have been detected in the paraffinic fractions of extracts of green and brown leaves recently deposited on soil [21]. Attempts to find the compounds in the solvent extracts of the samplses analysed in this study failed. Their absence in fresh biological materials indicates that the formation of phytadienes in living plants is not an important process.

Two isomeric phytadienes are generated as main products of pyrolysis of authentic chlorophyll [22]. Thus, under these TD conditions phytadienes can be expected to be the degradation products of chlorophyll present in algae and spruce and pine needles. The amount of phytadienes in the thermal extracts allows the calculation of the amount of chlorophyll in the samples analysed, assuming that complete decomposition of the chlorophyll occurs. Phytol (compound 4 in Table II) survives the thermal

TABLE II

COMPOUNDS IDENTIFIED BY GC–MS ANALYSIS OF THE THERMAL EXTRACT OF *TETRAEDRON MINIMUM*

Compound No.	Compound name	Molecular weight
1	n-Heptadecane	240
2	Phytadienes	278
3	n-Hexadecanoic acid	256
4	Phytol	296
5	n-Octadecenoic acid	282
6	n-Tricosane	324
7	Squalene	410
8	2-Heptacosanone	394
9	n-Hexacosanoic acid methyl ester	410
10	γ-Tocopherol	416
11	2-Nonacosanone	422
12	n-Octacosanoic acid methyl ester	438
13	α-Tocopherol	430
14	Ergost-7-en-3-ol	400
15	Stigmast-7,22-dien-3-ol	412
16	Octacosadienal	404
17	Stigmast-7-en-3-ol	414
18	α-Tocopherolquinol	448

treatment at 300°C as it is present in most of the thermal extracts investigated. Consequently, the phytadienes must originate primarily from chlorophyll which has not yet been hydrolysed.

In the higher boiling point range of the thermal extracts, apart from the olefinic hydrocarbon squalene (7), a set of polar compounds can be detected (Fig. 1 and Table II). These compounds are methyl ketones (8,11), methyl esters of long-chain carboxylic acids (9,12), an unsaturated aldehyde (16) and alcohols such as tocopherols (10, 13, 18) and sterols (14, 15, 17). Despite the polar nature of the compounds they appear as relatively sharp peaks, which indicates that the method allows a simultaneous recognition of volatile plant constituents over a wide polarity range.

Tocopherols

Tocopherols have recently attracted the interest of organic geochemists. α-Tocopherol has been reported to be present in soil extracts [21] and in a wide variety of sedimentary extract [23]. The thermal degradation of α-tocopherol at 350°C yields duroquinone and degradation products of pristene [24]. Tocopherols are remarkably stable under thermal treatment, although the compounds are known to be highly reactive chemically. Under flash pyrolysis conditions at 610°C tocopherol partly survives [2]. During the thermal treatment used in this study, degradation products of tocopherols such as prist-1-ene have not been detected. Thus TD at 300°C appears to be a suitable method for the mobilization of tocopherols from a biological matrix without decomposition.

Fig. 2 shows the presence of tocopherols in the thermal extract of the algae *Tetraedron minimum* using mass chromatography of the base peaks ions. γ-Tocopherol is recognized in the mass chromatogram of *m/z* 151 (Fig. 2a) and α-tocopherol using *m/z* 165 (Fig. 2b) The base peak integration data show that γ-tocopherol contributes only approximately 5% to the total tocopherol content. An additional compound tentatively identified from mass spectral data is α-tocopherolquinol (18), the hydrolysis product of α-tocopherol. This compound has previously been reported to occur mainly in microorganisms [25]. The other *Chlorococcales* algae investigated here differ from *Tetraedron minimum* in so far as the α-tocopherolquinol was only detected in *Tetraedron minimum*. The role of tocopherols in nature is not fully understood. It has been postulated that the compounds are intermediates in the electron transport associated with both photosynthesis and respiration [26]. The ratio of chlorophyll to tocopherols has therefore been examined in leaves and subcellular fractions from leaves. Chlorophyll:tocopherol concentration ratios ranging from 8.3 to 100 have been determined in various plant materials [27]. The chlorophyll:tocopherol content has been calculated in the samples analysed based on the assumption that the amount of phytadienes generated during thermal extraction reflects the original chlorophyll content of the biological material investigated. Only the peak integration data of α-tocopherol have been used for the calculation. Table III shows that this ratio varies in the algae from 8.4 to 127, which is in a similar range to that described by Bucke *et al.* [27]. Remarkably, the spruce and pine needles have much lower ratios, with values of 3.0 and 1.9, respectively. On average, the *Chlorococcales* algae tend to have higher chlorophyll:tocopherol ratios than the various marine algae.

Based on these findings, higher pristane:phytane concentration ratios should be predicted in fossil material derived from higher plants than in material derived from

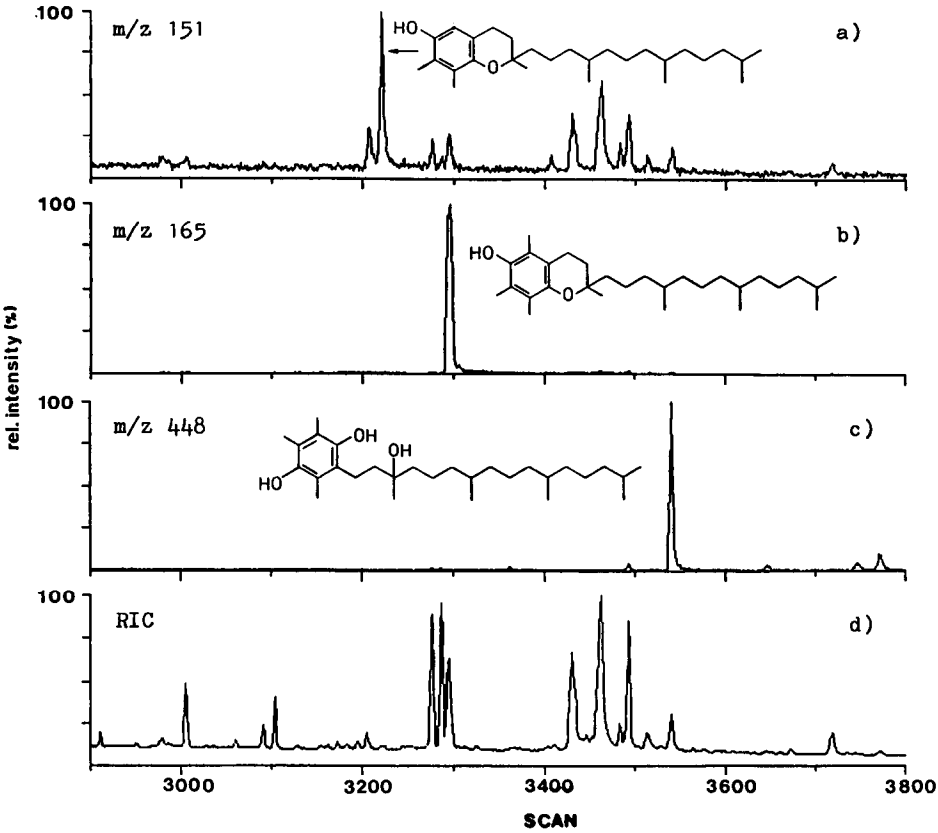


Fig. 2. Identification of tocopherol compounds within the desorbed material of the algae *Tetraedron minimum* (sample 1. in Table I) using GC–MS analysis. Within the scan range 2900-3800 γ-tocopherol is recognized by mass chromatography of *m/z* 151 (a), α-tocopherol by mass chromatography of *m/z* 165 (b) and α-tocopherolquinol by mass chromatography of *m/z* 448 (c). In addition to the mass chromatograms, the RIC is shown in (d).

TABLE III

RELATIVE INTENSITIES OF CHLOROPHYLL *VERSUS* α-TOCOPHEROL AND OF CHLOROPHYLL *VERSUS* THE SUM OF DETECTABLE STEROIDS IN THE ANALYSED SAMPLES

Chlorophyll content estimated from peak integration data of phytadienes in GC–MS analysis.

Sample material	Chlorophyll: α-Tocopherol	Chlorophyll: Steroids
Tetraedron minimum	28.8	3.8
Chlorella fusca	46.3	4.3
Pediastrum duplex	12.7	8.0
Scenedesmus obliquus	37.6	10.7
Fucus sp.	16.0	2.6
Halidrys sp.	8.4	2.1
Cladophora	40.9	4.1
Plocamium	18.6	11.4
Spruce needles	3.0	1.9
Pine needles	1.9	0.8

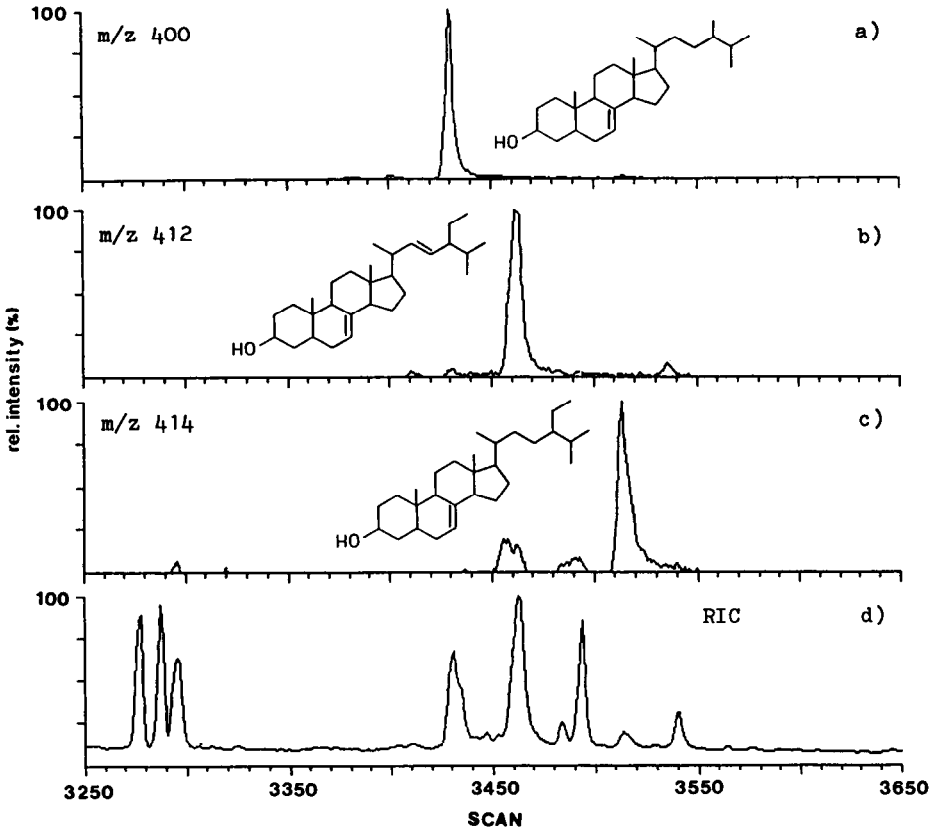


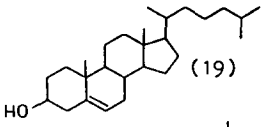
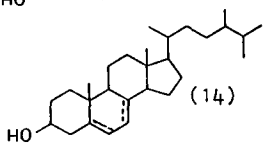
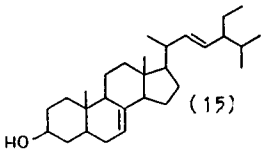
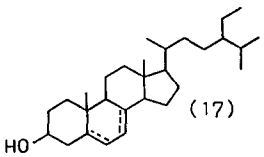
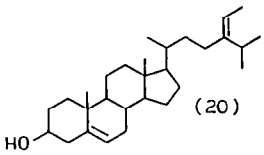
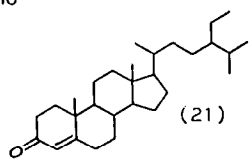
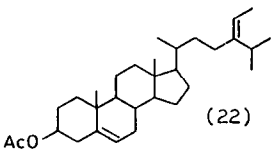
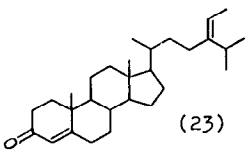
Fig. 3. GC–MS analysis of sterols present in the desorbed material of the algae *Tetraedron minimum* (sample 1 in Table I). Mass chromatography is used for the identification of three individual sterols: (a) *m/z* 400 for ergost-7-en-3-ol; (b) *m/z* 412 for stigmast-7,22-dien-3-ol; and (c) *m/z* 414 for stigmast-7-en-3-ol. The RIC in the scan range 3200–3650 is shown in trace (d). The estimated relative intensities of the sterols are given in Table IV.

algae, assuming that the phytol generated by the hydrolysis of chlorophyll is completely converted to phytane and that the tocopherol is degraded to pristane after burial and heating of the biological material in sediments. In fact, high-volatile bituminous A coals are known to provide extracts with very high pristane:phytane ratios of up to fifteen [28,29], whereas in crude oils generated in hypersaline environments the pristane:phytane ratios are often less than 0.5 [5]. Thus the tendency observed in the biological material investigated here is partly reflected by what is already known from fossil material. However, there are many other potential precursor molecules present in nature. For example, in coals, which are in general deposited and coalified under oxidizing conditions, the proposed pathway [1,5] of pristane generation from phytol via oxidation to phytanic acid and the subsequent decarboxylation may enhance the pristane:phytane ratios.

TABLE IV

DISTRIBUTION OF STEROLS AND STEROIDAL KETONES ANALYSED IN THE MATERIAL DESORBED FROM EIGHT ALGAE AND TWO NEEDLES FROM HIGHER PLANTS

The sample numbers reflect the analysed specimen listed in Table I. Abundance: X = low, XX = moderate and XXX = high. Ac = Acetyl.

Structures of Compounds	Sample numbers									
	1	2	3	4	5	6	7	8	9	10
 (19)	–	–	–	–	–	–	–	XX	–	–
 (14)	Δ^7 XX	Δ^7 XX	Δ^7 XX	Δ^7 XX	–	–	Δ^5 X	–	Δ^5 XX	–
 (15)	Δ^7 XXX	Δ^7 XXX	Δ^7 XXX	Δ^7 XXX	–	–	–	–	–	–
 (17)	Δ^7 X	Δ^7 X	Δ^7 X	Δ^7 X	–	–	Δ^5 XXX	–	Δ^5 XX	Δ^5 XX
 (20)	–	–	–	–	XXX	X	–	–	–	–
 (21)	–	–	–	–	–	–	X	–	XX	X
 (22)	–	–	–	–	–	X	–	–	–	–
 (23)	–	–	–	–	–	XX	–	–	–	–

Steroids

More than 70 years ago it was recognized that sterols can be converted into petroleum-like substances by thermal or chemical treatment [30]. A major interest in organic geochemical research is to reconstruct the reaction pathways of steroids found in the sedimentary record back to their precursor biological material. This requires a knowledge of the steroid composition of recent and ancient biological input materials and the possible transformation processes which affect the steroids in sediments. A summary of the current state of knowledge of the biological occurrence of steroids with different side-chains and their geological fate has been presented by Mackenzie *et al.* [31]. The lack of information concerning the steroid composition of biological materials is mainly due to time-consuming preparative procedures, including the derivatization of sterols prior to GC analysis. This work shows that sterols survive thermal desorption in an atmosphere of inert gas. The compounds are recovered by on-line GC–MS analysis as relatively sharp peaks, which provide rapid information about the steroid composition of biological material without any pretreatment of the samples except for drying.

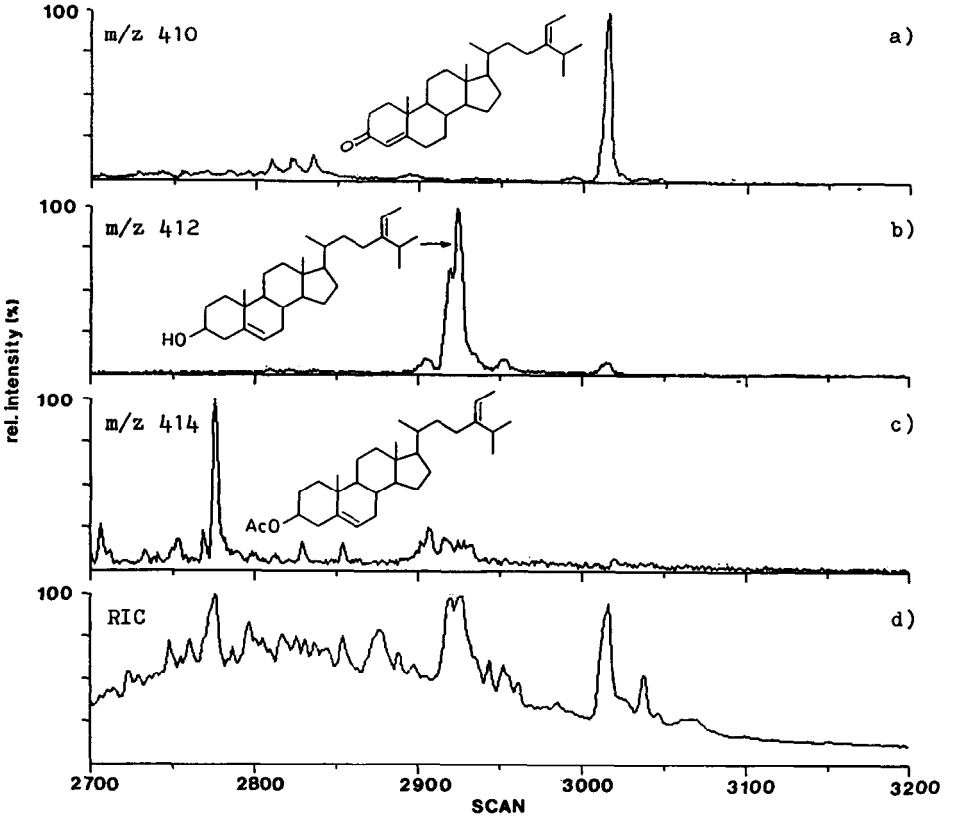


Fig. 4. GC–MS analysis of sterols present in the desorbed material of the algae *Halidrys* sp. (sample 6 in Table I). Mass chromatography of *m/z* 410 (a), 412 (b) and 414 (c) is used to recognize the three individual sterols. The RIC is shown in (d).

In the material desorbed from *Tetraedron minimum* three sterols are present at relatively high abundances, as shown by mass chromatography of the molecular ions of individual sterols. The compounds are identified based on published mass spectral data [32]. Ergost-7-en-3-ol (14) is recovered by mass chromatography of *m/z* 400 (Fig. 3a). The mass chromatogram of *m/z* 412 (Fig. 3b) shows one major peak which represents stigmast-7,22-dien-3-ol (15) as the highest intensity sterol. Stigmast-7-en-3-ol (17) contributes with only minor intensity, as seen by a comparison of the *m/z* 414 trace (Fig. 3c) and the RIC (Fig. 3d). All the *Chlorococcales* algae investigated here provided a similar sterol distribution, with the Δ^7-components as the dominant constituents. It is remarkable that the related Δ^5-sterols, which are usually dominant in biological materials, cannot be detected in the *Chlorococcales* algae.

Table IV lists the sterols and steroid ketones identified in the ten samples analysed, with an indication of their relative abundance. The list shows that in the analysed samples the occurrence of Δ^7-sterols is restricted to the *Chlorococcales* algae. *Cladophora* and spruce and pine needles contain the related Δ^5-C_{28} and C_{29} sterols (14,17), accompanied by stigmast-4-en-3-one (21 in Table IV). The other marine algae, *Fucus* sp., *Halidrys* sp. and *Plocamium* each have very specific steroid compositions. *Plocamium* shows a complete lack of C_{28} and C_{29} steroids (Table IV). The only steroid detected in high abundance in this alga is Δ^5-cholestenol (19). Similarly, the steroid composition of *Fucus* sp. is strongly dominated by only one compound, which has been identified as stigmast-5,24(28)-dien-3-ol (20). This is confirmed by the analysis of the algae *Halidrys*, sp. the steroid composition of which is also dominated by $\Delta^{24(28)}$-steroids. As shown by GC–MS analysis of the thermal extract of these algae, the steroids are dominated by stigmast-4,24(28)-dien-3-one (Fig. 4a). In addition, the stigmast-5,24(28)-dien-3-ol (Fig. 4b) and the related acetate (Fig. 4c) are recovered by mass chromatography of *m/z* 412 and *m/z* 414, respectively. The compounds are shown in Table IV as compounds 20, 22 and 23.

Peak integration data have been compared to assess the amount of steroids in relation to the chlorophyll content of the samples analysed. The results (Table III) show that the ratio of chlorophyll to the sum of steroids varies between 0.8 and 11.4. Like the chlorophyll:tocopherol ratio, the lowest values are observed in the terrestrial plant material. In the algae the ratio varies significantly without any dominance in the algae from the North Sea or the *Chlorococcales* algae.

These results indicate that the well known problems of the correlation of the sterols detected in sediments with those in potential biological precursors could probably be partly solved by the use of the type of analysis described in this paper. This requires a microscopic analysis of well defined micro-samples in combination with micro-TD and GC–MS analysis of the lipid content.

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