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INVESTIGATION OF HIGH-MOLECULAR-WEIGHT CARBOXYLIC ACIDS IN PETROLEUM BY DIFFERENT COMBINATIONS OF CHROMATOGRAPHY (GAS AND LIQUID) AND MASS SPECTROMETRY (ELECTRON IMPACT AND CHEMICAL IONIZATION)

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SUMMARY

High-molecular-weight carboxylic acids from various crude oils were analysed using a combination of chromatographic techniques: liquid chromatography on a modified adsorbent, thin-layer chromatography, glass capillary gas chromatography and combined gas chromatography–mass spectrometry. The polycyclic acids of the hopane series were investigated because of their importance as biological markers in correlation problems.

INTRODUCTION

The identification of individual molecules in high-boiling petroleum distillates is of major importance for geochemical and oil recovery correlation studies between oil reservoirs and source rocks or between different crude oils. These correlations can be based on the presence or absence of different biological markers such as polycyclic alkanes or carboxylic acids[1–4]. The isolation and identification of pure substances in geochemical mixtures pose such severe analytical problems that the most modern and sophisticated techniques must be used in order to achieve good results.

An analytical strategy for analysing high-molecular-weight carboxylic acids was developed and applied to crude oils from different origins (Fig. 1).

The acids are extracted from a virgin crude oil by a method originally suggested for biolipids by McCarthy and Duthie[5], using column chromatography with a potassium hydroxide-treated silica. This method has been adapted to crude oils and to large amounts of starting material (up to 150 g) with regard to the very low acid content of crudes by using a recycling solvent system as described in a previous paper[6].

After conversion of the acids into their corresponding methyl esters, a purification step permits their separation from a very polar black material (resins) by recycling liquid chromatography over a second column of potassium hydroxide-treated silica. After separation according to increasing polarity using thin-layer chromatography, the purified methyl esters are separated by steric criteria using urea adduction.

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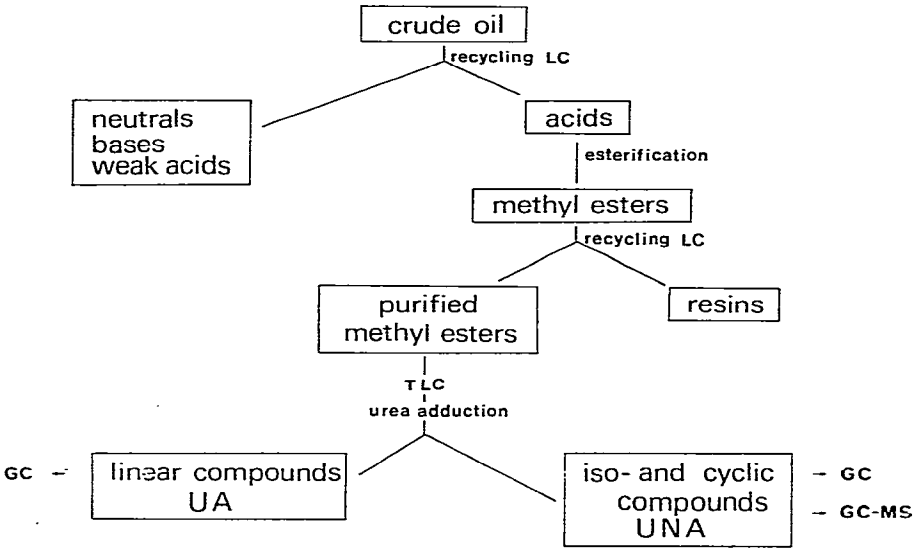


Fig. 1. Analytical scheme for the selective recovery of acids from crude oils.

The analysis of the fractions corresponding to the monocarboxylic acids was performed by glass capillary gas chromatography on high-temperature stable SCOT columns and by gas chromatography–mass spectrometry in the electron impact (EI) and chemical ionization (CI) modes.

EXPERIMENTAL

Great care must be taken to avoid all types of contamination during the analytical procedure. All solvents (Merck, Darmstadt, G.F.R.; p.a. grade) were distilled in an all-glass system before use. The silica (Merck, 63–200 μm) was washed with chloroform in a Soxhlet apparatus for 24 h and reactivated by heating at 120° for 2 h. The vessels were cleaned by ultrasonic treatment in a detergent solution (1% RBS 50 in distilled water) followed by rinsing with water and acetone. All contact of the samples with plastic materials was avoided.

Extraction of the acids

The preparation of the alkali-modified silica and the solvent recycling chromatographic apparatus were as described previously[5,6].

The crude oil under investigation (100 g) was deposited on the top of the adsorbent and elution with diethyl ether was started. After 6 h, neutral, basic and weak acidic compounds ($pK_a > 7$) were recovered[6]. The fraction containing the carboxylic acids was eluted with a 20% solution of formic acid in diethyl ether and recycling of ether for 2 h.

After evaporation of the solvents in a rotary evaporator, the methyl esters were obtained by reaction with a methanol–boron trifluoride solution (Alltech, Arlington Heights, Ill., U.S.A.) at room temperature for 12 h; the esters were extracted with chloroform after addition of water.

Purification

Elution with diethyl ether on a second recycling column filled with alkali-modified silica allowed separation from the resins, which remain adsorbed; the methyl esters were eluted in the neutral fraction.

A further purification of the recovered methyl esters was effected by mean of thin-layer chromatography. After deposition of a 1-mm layer of silica gel H (Merck) on glass plates (20 × 20 cm) using a Desaga TLC spreader, drying at 120° was followed by elution with ethyl acetate; the front part (2 cm) of the adsorbent was then discarded and the plates were activated at 140° for 3 h. The methyl esters were eluted with cyclohexane–dichloromethane (4:1).

The fraction corresponding to the monocarboxylic acids was submitted to urea adduction in order to separate linear from branched and cyclic structure molecules[7].

Gas chromatography

Glass capillary columns (0.25 mm I.D.) were prepared using a home-made glass-drawing machine after cleaning of the Pyrex glass tubes with chromic acid solution. The glass was then deactivated by silanization by filling the column with a 10% solution of dimethyldichlorosilane in toluene. After a contact time of 30 min, the tube was emptied, both ends were sealed and the silanization reaction was completed by heating at 140° for 3 h. After cooling, the column was flushed with 1 ml of toluene, followed by 1 ml of methanol.

High-temperature stable SCOT columns were prepared using a two-step dynamic coating procedure. The initial deposition of the silica support (Tullanox 101, Alltech) was made according to the method described by German and Horning[8], except that the use of a surfactant such as benzyltriphenylphosphonium chloride was omitted: a well sonicated suspension of Tullanox plus the stationary phase in chloroform (2:0.5:100) was forced through the capillary tube by applying a pressure of 1.5 bar for a 30-m long column (the speed of the receding meniscus was 1 cm/sec). The second step was effected by the dynamic mercury plug method[9] using solutions of 5–10% of stationary phases (ethylene–propylene copolymer[10], SE-30, Dexsil 300, OV-101) in isooctane with no suspended silica. The speed of coating of the plug was maintained at about 0.5 cm/sec. After coating, the columns were dried under a nitrogen flow of 1 ml/min.

Columns were connected using short (5 cm × 0.4 mm O.D.) glass-to-metal seals and Vespel ferrules[11]: such connections withstand high temperature (350°), pressure (6 bar) and vacuum (10^{-5} torr). Both ends of the capillary columns were enlarged to 0.4 mm I.D. by glass blowing; the presence of the Tullanox layer was not found to affect the fusing of the glass to the metal. All analyses were performed on a Perkin-Elmer 3920B apparatus using an all-glass inlet splitter system and helium as the carrier gas.

Gas chromatography–mass spectrometry

A DuPont Model 21-492-B GC–MS apparatus equipped with a Varian 2700 gas chromatograph (modified for glass capillary work as described elsewhere[11]) and a DuPont Model 094 B-2 disc-based data system was used for spectra acquisition and processing. The columns were connected directly to the mass spectrometer through an "open split" interface of the type described by Henneberg *et al.*[12], working in the

EI or CI mode, and capable of discarding unwanted peaks in the mass spectrometer source[13]. A constant flow of 5 ml/min (NTP) of helium was metered inside the mass spectrometer, independently of the GC flow-rate. In the CI mode, isobutane was used as reactant gas and introduced separately in the mass spectrometer source.

Scanning of masses 600–41 was performed repetitively at 1 sec/decade with a flyback time of 3 sec.

RESULTS AND DISCUSSION

The procedure described here for the selective removal of the acids is rapid and quantitative and can be applied to any crude oils, even those with a low acid content (< 0.1 %). In most instances, 100 mg–1 g of pure monomethyl ester fractions are obtained, which is large enough for further identification by different spectroscopic methods.

Identification by GC and GC–MS of high-boiling and complex mixtures requires both high-temperature stable columns and a high loading capacity so that individual substances that are present in the 10–50-ng range after injection will give a clear mass spectrum; on the other hand, deactivation of the surface is not critical for methyl ester analysis. These requirements are met by SCOT columns coated with Tullanox as the support and a non-polar stationary phase.

The efficiency in terms of plates per metre measured on n-C_{24} at 250° (k' = 1.0–1.5) was found to be better with gum stationary phases. Columns coated with ethylene–propylene copolymer (EP) showed consistently higher efficiency (1800–2100 plates/m) than those coated with SE-30 (1600–1800 plates/m); on the other hand, OV-101 columns exhibited only 1200–1400 plates/m. Columns coated with EP phase were routinely used up to 315° for both GC and GC–MS analyses. Up to 500 μg of

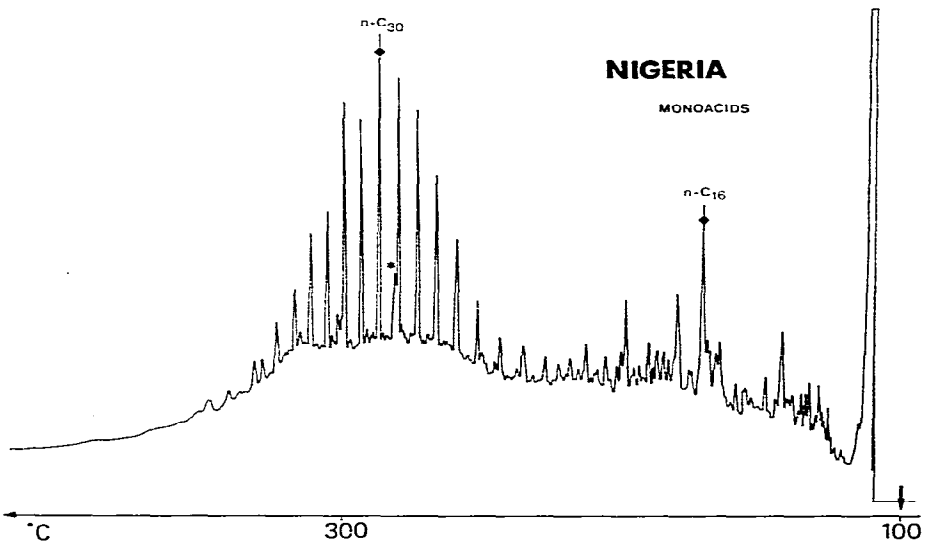
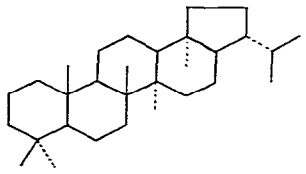


Fig. 2. GC analysis of the methyl esters from a Nigerian crude oil. SCOT column, 18 m × 0.5 mm, coated with OV-101. Temperature: 100–300° at 4°/min, then isothermal. Inlet pressure: 0.5 bar (helium).

sample can be injected by a splitless injection technique[14,15] without deterioration of the resolution; such a large amount is often necessary with very complex ester mixtures.

The results from the analysis of three crude oils of various origins, with acid contents ranging from 0.3 to 5%, served as examples of the method.

Gas chromatography is accurate enough for the identification of normal fatty acids, as shown in Fig. 2. However, the *n*-fatty acid pattern and its comparison with the *n*-alkane pattern from the same crude oil do not give meaningful data for correlation studies. Better information is obtained from polycyclic carboxylic acid distributions, with special emphasis on pentacyclic triterpenoid acids of the hopane series. Saturated alkanes with a hopane-type skeleton occur in significant concentration in the geosphere[16,17] and have been isolated from all known sedimentary organic matter (crude oils, coals, peats, oil shales, recent sediments).



HOPANE

A bacterial origin has been postulated to be the main source of these substances[18]. The corresponding acids with the functional group on the C-21 side chain (Fig. 3) are frequently encountered in sediments and oil shales[19], but their presence in crude oils has not, to our knowledge, been reported yet.

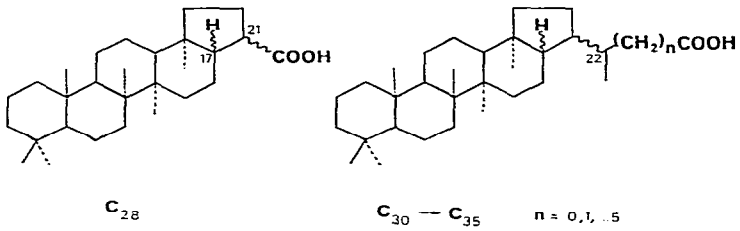
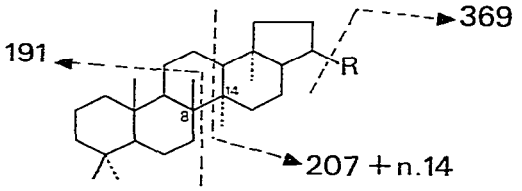


Fig. 3. Structure of the hopanoic acids.

Two series of similar compounds, defined by their stereochemistry at C-17 and C-21 locations as (17β,21β)H and (17β,21α)H hopanes occur in the geosphere and in living organisms. The third type, (17α,21β)H, has been found only in the geosphere. Recognition of each series can be made on the basis of their mass spectra because of highly specific fragmentation patterns; this fragmentation is governed, as for the corresponding alkanes, by the breaking of the C-8–C-14 bond, giving rise to the two major ions (Fig. 4). An empirical rule, valid for the hopane esters and alkanes, assumes that the stereochemistry at the C-17 and C-21 locations may be derived from the ratio of the relative intensities of the two ions at *m/e* 191 and 191 + *n*.14 for the alkanes[20], at *m/e* 191 and 207 + *n*.14 for the esters. The values measured for the ratio of the intensities of the two major ions in the case of the ester series are listed in Table I.



R = $[CH_2]_n COOCH_3$

Fig. 4. Mass fragmentation pattern of the methyl esters of the hopane series.

TABLE I

MASS SPECTROMETRIC DIFFERENTIATION OF THE METHYL ESTERS OF THE HOPANE TYPE

Stereochemistry	*Ratio m/e 191/(207 + n.14)*
(17α,21β)H	2.20–3.30
(17β,21α)H	1.00–1.20
(17β,21β)H	0.65–0.75

Complete separation of the different isomeric hopane homologues cannot be effected by GC alone, even for a urea non-adducted fraction analysed on a capillary column. On the other hand, a mass chromatogram of the ion at *m/e* 191 is specific for the series and it gives the hopane pattern as shown in Fig. 5 for a Nigerian crude oil. Identification was confirmed by coinjection with authentic synthetic standards[21] [(17α,21β)H-C_{31}, (17β,21α)H-C_{31} and (17β,21β)H-C_{32}] and by comparison of the mass spectra of the corresponding compounds. The complete results of the analysis of the hopanoic acids extracted from the three samples studied is given in Fig. 6. The relative abundance of the different homologues with the same stereochemical configuration at C-17 and C-21 is plotted against the carbon number of the acid. The presence of doublets in the figure is due to the occurrence of two epimers at C-22. The relative retentions of the two C-22 epimers of the (17α,21β)H series increase with increasing carbon number of the C-21 side-chain, while the relative retentions of the two epimers of the (17β,21α)H series decrease, making the resolution of the two (17β,21α,22α)- and (17β,21α,22β)-C_{32} isomers impossible with our columns.

This diagram can be used to discuss different geochemical hypotheses, and is of great value for the fingerprinting of crude oils and for correlation studies. For instance, the (17β,21β)H series are the remnant of the natural hopanes: epimerization at the different locations (C-17, C-21, C-22) is believed to occur under geological conditions[22]; thus, it may explain why few of these compounds are still found in crude oils. On the other hand, epimerization at C-22 should increase with increasing maturation: the crude oil from West Germany is a good example of a strongly degraded crude oil; the ratio of the abundance of the two C-22 epimers is close to unity, whereas this ratio differs markedly from unity in the Nigerian sample, which has been less degraded.

The C_{32} αβ-isomers are more abundant in the three examples, although the maturity of the crude oils investigated differs widely. This agrees with the observation

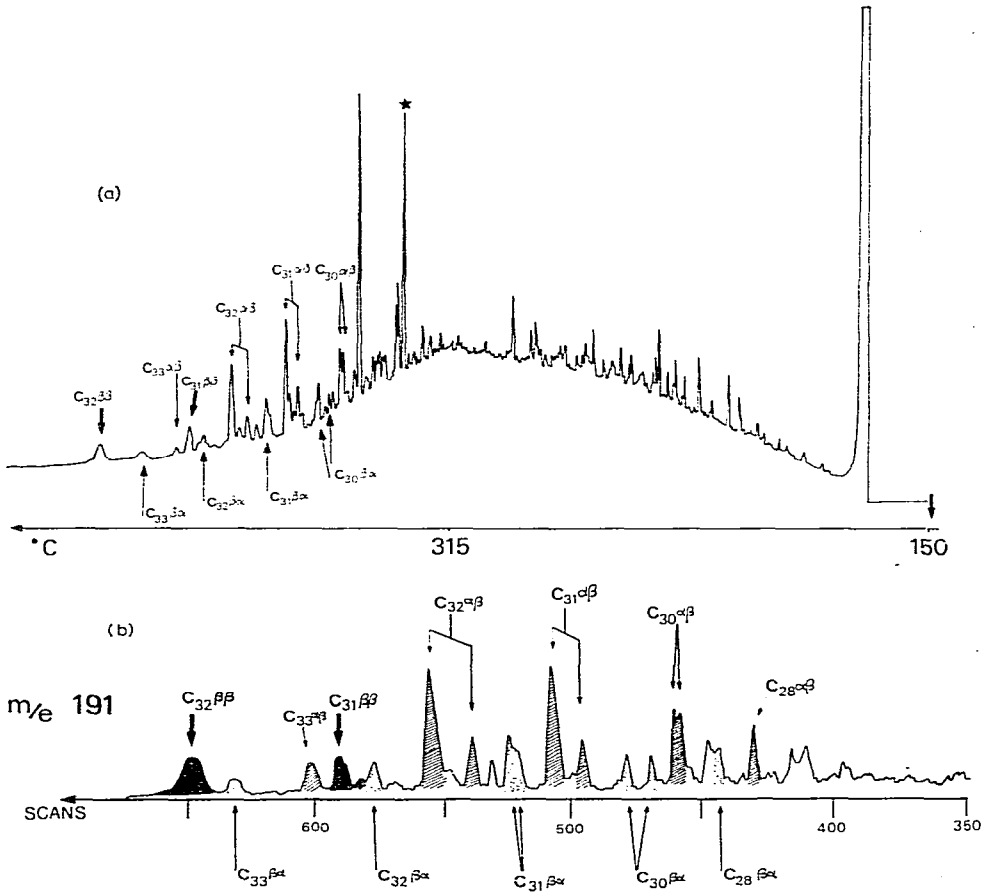


Fig. 5. (a) GC analysis of the urea non-adducted fraction from Nigerian crude oil. SCOT column, 45 m × 0.25 mm, coated with EP. Temperature: 150–315° at 4°/min, then isothermal. Inlet pressure: 0.7 bar (helium). (b) GC–MS analysis of the same fraction. SCOT column, 47 m × 0.25 mm, coated with EP. Temperature: 130–310° at 4°/min, then isothermal. Inlet pressure: 1 bar (helium).

of the decrease of the abundance of the $\beta\beta$- and $\beta\alpha$-isomers together with the increase in the abundance of the $\alpha\beta$-isomers when studying sediments of increasing maturity[22,23]. The absence of any of the C_{29} hopane isomers among the acids is noteworthy; it parallels the absence or the low abundance of the C_{28} hopane isomers among the alkane fractions of most crude oils[24]; the two facts probably arise from a common origin.

Similarly, a plot of the relative abundance of the saturated hopane series against their carbon number gives another set of characteristic patterns that can be correlated with the hopanoic acid fingerprint[25,26].

In the CI mode, using isobutane as the reactant gas, the methyl ester derivatives of linear, aliphatic and polycyclic monocarboxylic acids exhibit only the quasi-molecular ion, so that no structural information can be derived from CI data. However, other relevant information is obtained: for example, using the solid probe inlet, the CI spectrum of the total mixture gives an evaluation of the average mass range of the

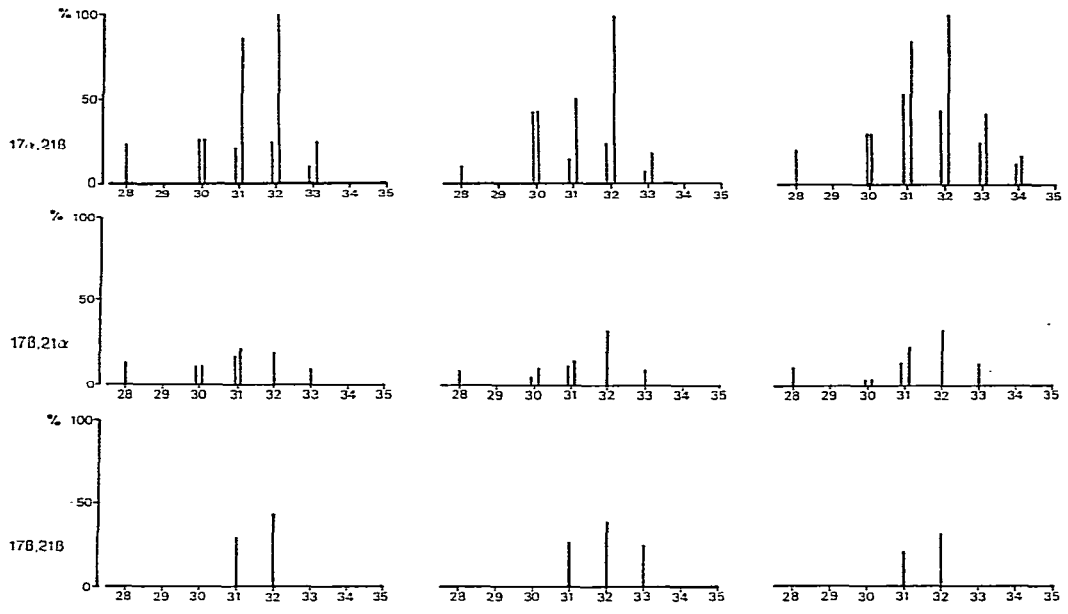


Fig. 6. Histogram of the hopane acid isomers. The abundance of the different homologues, relative to the abundance of the C_{32} $\alpha\beta$-isomer chosen as 100, is plotted against the total carbon number of the acid. A plot for each series ($\alpha\beta$, $\beta\alpha$ and $\beta\beta$) is presented separately.

extract. As the investigated esters are devoid of olefinic bonds, the unsaturation leve arises from the occurrence of cycles; thus the comparison of the ions at *m/e* $C_nH_{2n}O_2,H^+$ with the ions at *m/e* $C_nH_{2n-m}O_2,H^+$ ($m = 2,4,6$) is an indication of the relative abundance of aliphatic, mono-, di- and tricyclic acids.

CI in GC–MS also yields complementary data on the molecular weight of the acids and may help to solve problems posed by complex mixtures from very altered crudes. Such is the case for the crude from West Germany: Fig. 7a shows a plot of the reconstructed chromatogram obtained from the EI/GC–MS data; if one excepts the last eluted peaks corresponding to hopanoic acids, no peaks are observed, but there is a "hump" due to the presence of a large number of acids. However, if CI/GC–MS is used, it is possible to generate mass chromatograms for the possible carbon numbers of the different homologous series: Fig. 7b shows such a plot for the masses at *m/e* 257 + 271 + 285 + 299 (ions $C_{16}H_{32}O_2,H^+ + C_{17}H_{34}O_2,H^+ + C_{18}H_{36}O_2,H^+ + C_{19}H_{38}O_2,H^+$) which correspond to the esters of the acids with 15–18 carbon atoms and gives a selective chromatogram indicative of their distribution pattern. The mass spectrum in Fig. 8a, taken at the maximum of one of the abundant peaks on this mass chromatogram, shows the presence of at least one substance with elemental composition $C_{17}H_{34}O_2$. The mass chromatogram shown in Fig. 7c corresponds to the plot of the intensity of the ion at *m/e* 499 ($C_{34}H_{58}O_2,H^+$); it confirms the presence of C_{33} hopanoic acids. The mass spectrum of the major peak in Fig. 8b exhibits only the quasi-molecular ion; this illustrates how accurately a single substance may be identified from a very complex mixture.

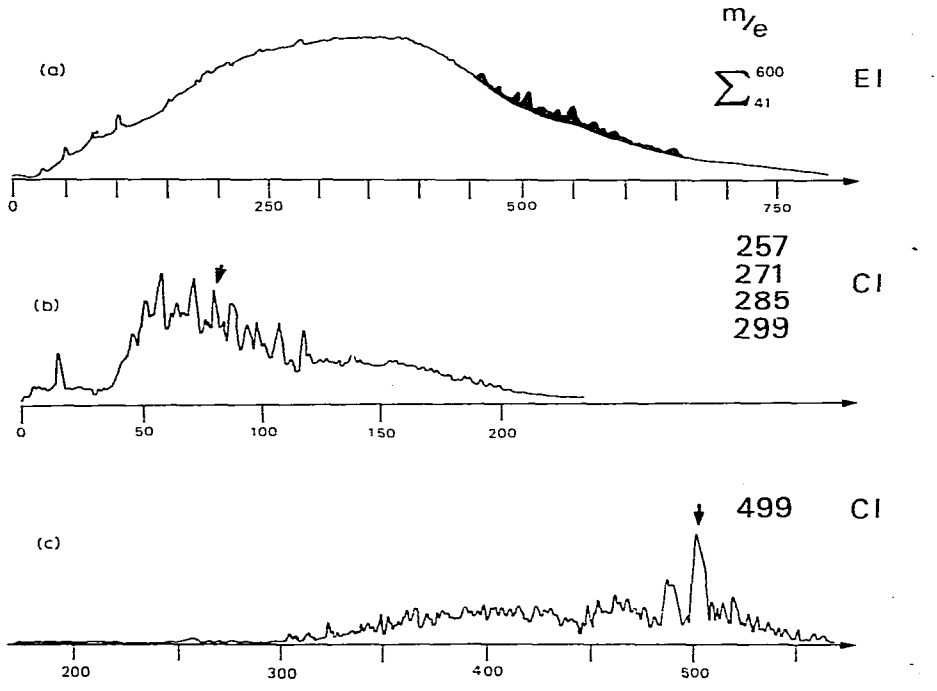


Fig. 7. GC–MS analysis of the monomethyl ester fraction from the crude oil from West Germany (a) EI/GC–MS, reconstructed chromatogram. SCOT column, 45 m × 0.25 mm, coated with EP. Temperature: 130–310° at 4°/min. Inlet pressure: 1 bar (helium). (b) CI/GC–MS, mass chromatogram of the methyl esters corresponding to the acids with 15–18 carbon atoms. SCOT column, 30 m × 0.5 mm, coated with OV-101. Temperature: 180–300° at 4°/min. Inlet pressure: 1 bar (helium). (c) CI/GC–MS, mass chromatogram at *m/e* 499, characteristic of the C_{33} hopanoic acids. Conditions as in (b).

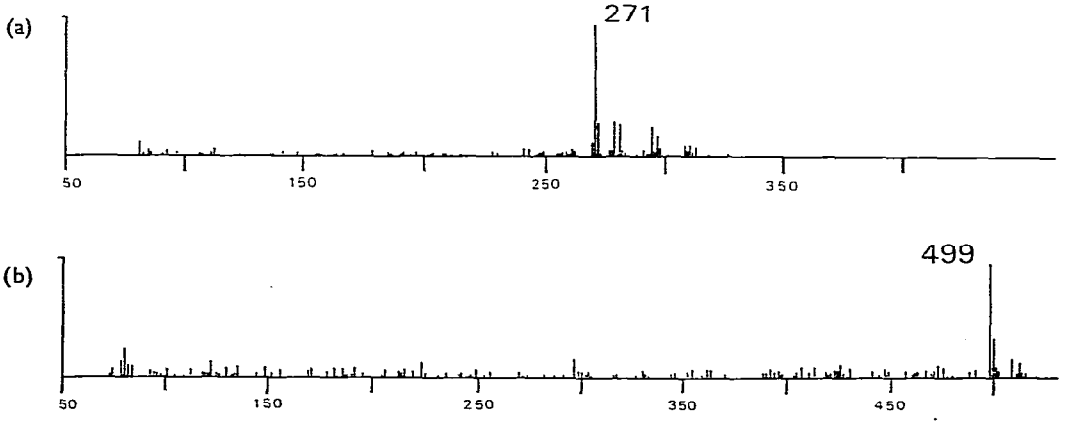


Fig. 8. CI mass spectra. (a) spectrum taken during the CI/GC–MS run shown in Fig. 7b; (b) spectrum taken during the CI/GC–MS run shown in Fig. 7c.

CONCLUSION

Investigation of high-molecular-weight carboxylic acids extracted from crude oils poses various analytical problems which can be solved by choosing an appropriate technique. The combination of a rapid (8 h) and quantitative extraction method with GC–MS is well adapted to the examination of a collection of different crude oils. SCOT columns, which may be rapidly and reproducibly produced, are suited for this kind of work, with regard to their stability and high loading capacity.

The distribution of the hopanoic acids affords valuable information for correlation studies and could be interesting as an indication of the degree of maturation of the crude oils. However, other acids, probably having a steroid structure but different from the steroid acids previously isolated from petroleum by other works[27], are under investigation and could afford other series of interesting biological markers.

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REFERENCES

1 J. G. Pym, J. E. Ray, G. W. Smith and E. V. Whitehead, *Anal. Chem.*, 47 (1975) 1617.
2 W. K. Seifert, in R. Campos and J. Goñi (Editors), *Advances in Organic Geochemistry 1975*, ENADIMSA, Madrid, 1975, p. 21
3 D. Leythaeuser, A. Hollerbach and H. Hagemann, in R. Campos and J. Goñi (Editors), *Advances in Organic Geochemistry 1975*, ENADIMSA Madrid, 1975, p. 3.
4 A. Van Dorsselaer, J. M. Schmitter, P. Albrecht, J. Claret and J. Connan, in *Advances in Organic Geochemistry 1977*, Moscow, in press.
5 R. D. McCarthy and A. H. Duthie, *J. Lipid. Res.*, 3 (1962) 117.
6 Z. Ramljak, A. Šolc, P. Arpino, J. M. Schmitter and G. Guiochon, *Anal. Chem.*, 49 (1977) 1222.
7 R. W. Schiessler and D. Flitter, *J. Amer. Chem. Soc.*, 74 (1952) 1720.
8 A. L. German and E. C. Horning, *J. Chromatogr. Sci.*, 11 (1973) 76.
9 G. Schomburg and H. Husmann, *Chromatographia*, 8 (1975) 517.
10 M. Thizon, C. Eon, P. Valentin and G. Guiochon, *Anal. Chem.*, 48 (1976) 1861.
11 P. Arpino, C. Vidal-Madjar, G. Guiochon and S. Békássy, *J. Chromatogr.*, 138 (1977) 173.
12 D. Henneberg, U. Heinrichs and G. Schomburg, *Chromatographia*, 8 (1975) 449.
13 P. Arpino, unpublished results.
14 K. Grob and G. Grob, *Chromatographia*, 5 (1972) 3.
15 K. Grob and K. Grob, Jr., *J. Chromatogr.*, 94 (1974) 53.
16 A. Van Dorsselaer, A. Ensminger, C. Spyckerelle, M. Dastillung, O. Sieskind, P. Arpino, P. Albrecht, G. Ourisson, P. W. Brooks, S. J. Gaskell, B. J. Kimble, R. P. Philp, J. R. Maxwell and G. Eglinton, *Tetrahedron Lett.*, (1974) 1349.
17 A. van Dorsselaer, P. Albrecht and G. Ourisson, *Bull. Soc. Chim. Fr.*, (1977) 165.
18 M. Rohmer and G. Ourisson, *Tetrahedron Lett.*, (1976) 3637.
19 P. Arpino, in *Sciences Géologiques*, Geological Institute of Strasbourg, France, 1973, No. 39.
20 A. van Dorsselaer, *Thesis*, Université Louis Pasteur, Strasbourg, France, 1975.
21 S. Hasan, A. van Dorsselaer and P. Albrecht, in preparation.
22 A. Ensminger, P. Albrecht, G. Ourisson and B. Tissot, in R. Campos and J. Goñi (Editors), *Advances in Organic Geochemistry 1975*, ENADIMSA, Madrid, 1975, p. 45.
23 A. Ensminger, *Thesis*, Université Louis Pasteur, Strasbourg, France, 1977.
24 E. V. Whitehead, in B. Tissot and F. Bienner (Editors), *Advances in Organic Geochemistry 1973*, TECHNIP, Paris, 1973, p. 225.
25 J. M. Schmitter, *Thesis*, Université Louis Pasteur, Strasbourg, France, 1978.
26 J. M. Schmitter and P. Arpino, in preparation.
27 W. K. Seifert, E. J. Gallegos and R. M. Teeter, *J. Amer. Chem. Soc.*, 94 (1972) 5880.