

Short communication

# Combination of gas chromatography–mass spectrometry and mass spectral deconvolution for structural elucidation of an unusual C<sub>29</sub>-steroid detected in a complex sedimentary matrix

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Received 10 May 2004; received in revised form 29 July 2004; accepted 2 August 2004

Available online 27 August 2004

## Abstract

A complex sedimentary sample from the Monterey Formation (CA, USA) has been submitted to GC–MS analysis followed by mass spectral deconvolution using Automated Mass Spectral Deconvolution and Identification System (AMDIS). Adjusting the parameters of the software allowed for the extraction of the spectrum of an unusual steroidal hydrocarbon coeluting with the major compound of the chromatogram. Following a careful interpretation of the “extracted” mass spectrum, the structure of the unknown has been postulated to be the 4,14-dimethylcholestane (DMC). Possible origins of this rare steroid are briefly discussed. Thus, application of AMDIS appears to be particularly suitable for the GC–MS analysis of natural complex mixtures characterized by a high number of analytes present in low amounts. © 2004 Elsevier B.V. All rights reserved.

**Keywords:** Deconvolution; AMDIS; 4,14-Dimethylcholestane; Monterey

## 1. Introduction

The Monterey Formation (CA, USA) has been the object of extensive research for the last two decades [1–5]. During a study of the preserved organic matter in sediments from the El Capitan section of this formation, we found that 17 $\alpha$ ,21 $\beta$ -28,30-bisnorhopane (BNH; Fig. 1) constitutes the major compound of the “non aromatic hydrocarbon” fraction (Fig. 2a). This compound was fully identified in a Monterey shale (offshore Santa Barbara) by MS, X-ray crystallography and NMR [6]. Careful examination of the mass spectrum of BNH (Fig. 2b) reveals however the presence of a second component present in lower abundance. Here we present the use of a freely available GC–MS post-processing software for extracting the mass spectrum of this coeluting compound and its mass spectral interpretation.

## 2. Experimental

### 2.1. Sample

The Middle Miocene sample from the Monterey Formation was collected from the El Capitan section (30 km west of Santa Barbara, CA, USA). It corresponds to the 138.6 m level from a 150 m section (10.68–17.74 Ma) whose geological setting and sediment characteristics have been described elsewhere [7].

### 2.2. Sample extraction

All solvents used were previously doubly distilled. Acetone and methylene chloride were supplied from Schweizerhall (Basel, Switzerland, >99% pure) whereas *n*-hexane was obtained from Reactolab (Servion, Switzerland, >99% pure). Laboratory material was sequentially washed with water, acetone and methylene chloride before handling.

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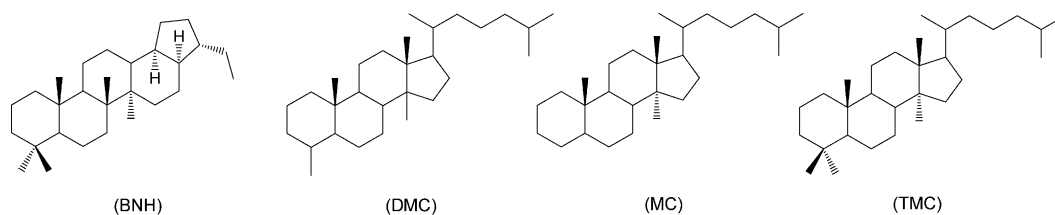


Fig. 1. Structures mentioned in text.

The powdered sediment (2 g) was added to 15 ml of water which was acidified with 6 M HCl (Fluka, Buchs, Switzerland, ACS reagent) to pH = 3 to remove carbonates. The mixture was extracted by ultrasonication with acetone ( $3 \times 150 \text{ ml} \times 10 \text{ min}$ ) and methylene chloride ( $3 \times$ ). Acetone extracts were evaporated and partitioned between water and methylene chloride. The methylene chloride extracts were combined and evaporated to obtain the organic extract (49.1 mg).

### 2.3. Sample fractionation

Neutral compounds were separated from acidic ones using solid phase extraction (SPE). Acetone (25 ml) was used for conditioning the cartridge (Isolute Amino 2g, IST Ltd., Hengoed Mid Glamorgan, UK). Methylene chloride (5 ml) was used for depositing the sample and 25 ml of acetone were necessary for eluting neutral compounds. The latter were further fractionated by flash chromatography using a 20 cm  $\times$  0.6 cm column filled with 3 g of 230–400 mesh silica gel (Fluka, Buchs, Switzerland). The neutral extract was deposited on

the top of the column with 200  $\mu\text{l}$  of methylene chloride and 25 ml of hexane were used for eluting the “non aromatic hydrocarbon” fraction.

### 2.4. GC–MS analyses

GC–MS analyses were performed on a HP 5890 chromatograph Series II Gas Chromatograph (Hewlett-Packard, Avondale, PA, USA) coupled to a VG Trio 2 mass spectrometer (VG Instruments, Danvers, MA, USA) using a DB-5 fused silica capillary column, 30 m  $\times$  0.25 mm i.d., 0.25  $\mu\text{m}$  film thickness (J&W Scientific, Folsom, CA, USA). Injections were made in the splitless mode with a helium head pressure of 0.85 MPa (velocity: 0.35  $\text{m s}^{-1}$ ). The injector, the transfer line and the source temperatures were set to 315, 300 and 220  $^{\circ}\text{C}$  respectively. The temperature programme was: 80  $^{\circ}\text{C}$  for 1 min followed by a gradient at 4  $^{\circ}\text{C min}^{-1}$  to 300  $^{\circ}\text{C}$ , final temperature held for 30 min. Scan rate was adjusted to 1 s per scan from 50 to 700 amu. The “non aromatic hydrocarbon” fraction was dissolved in 50  $\mu\text{l}$  of hexane before injection of a 1  $\mu\text{l}$  aliquot.

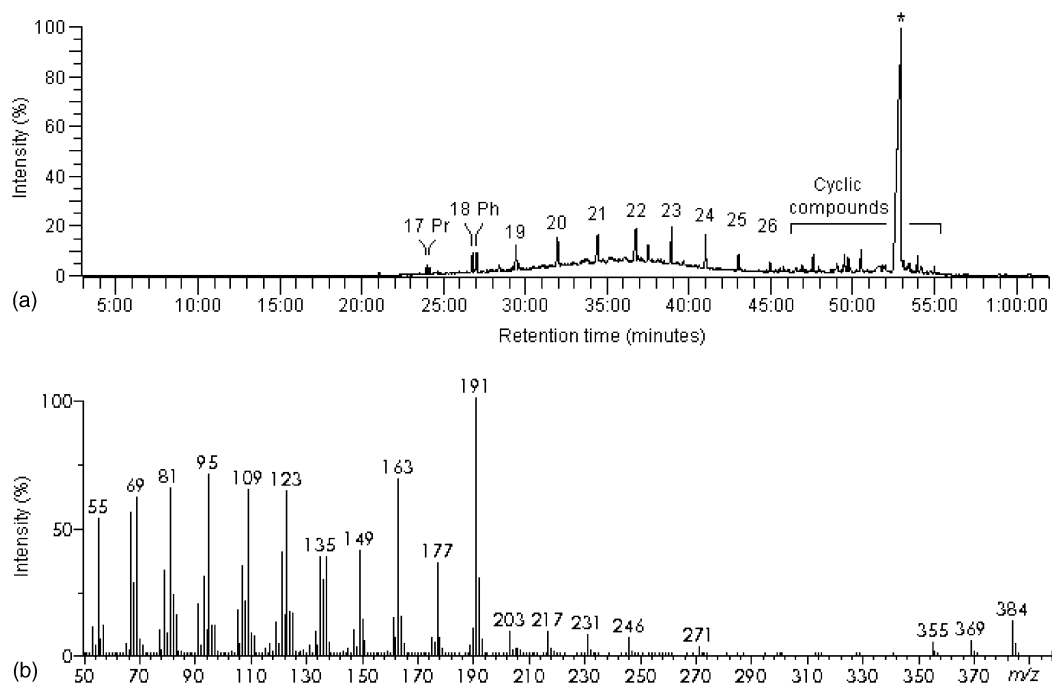


Fig. 2. TIC chromatogram of the “non aromatic hydrocarbon” fraction (a) and the mass spectrum of 17 $\alpha$ ,21 $\beta$ -28,30-bisnorhopane (BNH) (b) corresponding to the major peak (\*) in the chromatogram.

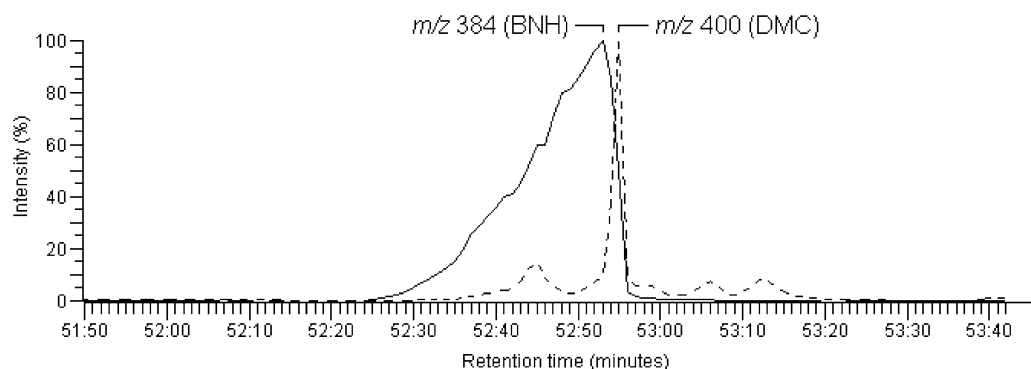


Fig. 3. Mass chromatograms of  $m/z$  384 (solid line) and  $m/z$  400 (dotted line) showing elution of the unknown compound at the second part of the peak of  $17\alpha,21\beta$ -28,30-bisnorhopane (BNH).

### 2.5. Mass spectral deconvolution using AMDIS software

Automated Mass Spectral Deconvolution and Identification System software (AMDIS), version 2.1, provided by the National Institute of Standards and Technology (NIST, USA, web address: <http://chemdata.nist.gov/mass-spc/amdis/index.html>) has been used for post-processing the MS data files.

## 3. Results and discussion

### 3.1. GC–MS of the saturated hydrocarbon fraction of Monterey sample and mass spectral deconvolution

The GC–MS chromatogram (Fig. 2a) shows that the “non aromatic hydrocarbon” fraction contains, besides linear hydrocarbons, pristane and phytane, a complex mixture of steroidal and triterpenoidal compounds. The latter are largely dominated by  $17\alpha,21\beta$ -28,30-bisnorhopane (Fig. 2b) previously identified as a major component of the hydrocarbon fraction in other Monterey Formation samples [6,8]. However, Single Ion Monitoring (SIM) shows that BNH elutes with another compound ( $M_r$  400) present in lower amounts. This can be shown on Fig. 3 by the mass chromatograms of  $m/z$  384 and 400, corresponding to molecular ion of BNH and the closely eluting unknown compound.

Mass spectral deconvolution using AMDIS package [9,10] allowed us to obtain a “clean” spectrum of the unknown (Fig. 4). The deconvolution parameters of AMDIS were systematically tested for the best deconvolution of closely eluting components in the particular area of the chromatogram. Note that these parameters need to be adjusted for different areas of the chromatogram since the peak width increases with retention time. The best result has been obtained with the following parameters: *Component width* = 20; *Adjacent peaks subtraction* = 2; *Resolution* = High; *Sensitivity* = Medium and *Shape requirements* = Low. The estimated amount of the unknown was approximately 10% of the BNH from AMDIS’s total ion current determination for BNH and the unknown.

### 3.2. Identification of 4,14-dimethylcholestane

The extracted mass spectrum of the unknown shows strong analogies with those of  $14\alpha$ -methylcholestane (MC) and 4,4,14-trimethylcholestane (lanostane; TMC) published by Muccino and Djerassi [11] who studied the fragmentation mechanisms of these two compounds through mass spectra of deuterium labelled analogues. Characteristic MS ions of these two compounds (Table 1) are induced by the presence of the methyl group on C-14 and are also present in the spectrum of the unknown, indicating the presence of a C-14 methyl group. The molecular weight and the mass shifts are indicative of a supplementary methyl group on ring A.

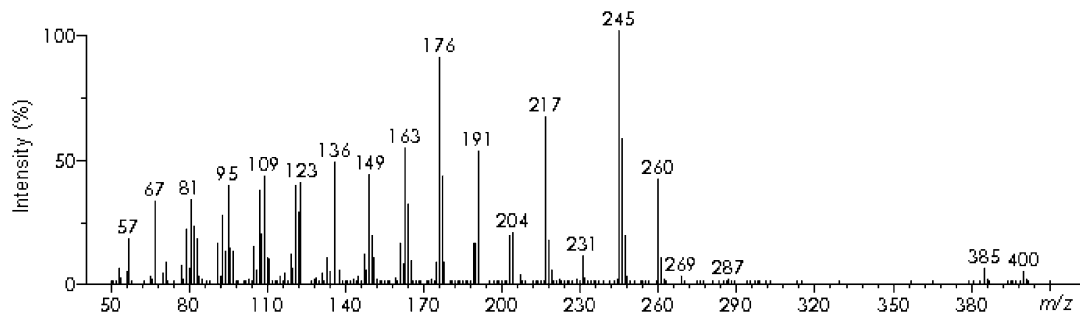


Fig. 4. Mass spectrum after deconvolution of the unknown compound closely eluting with  $17\alpha,21\beta$ -28,30-bisnorhopane (BNH).

Table 1  
Main characteristic ions and relative intensities from the mass spectra of 14 $\alpha$ -methylcholestane (MC) and 4,4,14 $\alpha$ -trimethylcholestane (TMC) (after [11]) and corresponding ions in the spectrum of the unknown

Ion	$M^{+\bullet}$	$a^{\dagger}$ (partial ring D cleavage)	$b^{\ddagger}$ (partial ring D cleavage)	$c^{\S}$ (ring D and partial ring C cleavage)	$d^{\P}$ (ring D and partial ring C cleavage)
Mass loss	–	140	155	183	224
14 $\alpha$ -Methylcholestane (MC)	386(21)	246(43)	231(100)	203(29)	162(70)
4,4,14 $\alpha$ -Trimethylcholestane (TMC)	414(14)	274(50)	259(100)	231(31)	190(93)
Unknown compound (DMC)	400(5)	260(41)	245(100)	217(66)	176(89)

Relative intensities in percentage.

<sup>a</sup> Formation of these ions is induced by the presence of the methyl substitution on C-14. Ions  $a$  and  $b$  retain methyl groups on C-4 and on C-14; ions  $c$  and  $d$  retain methyl groups on C-4 but lose the methyl group on C-14. Full details of ions structures can be found in [11].

Although a methyl substitution at C-3 cannot be excluded on the basis of the mass spectrum alone, biological evidence points to the 4,14-dimethylcholestane (DMC) structure. This is supported by the detection of traces of 24-methyl and 24-ethyl substituted homologues of 4,14-dimethylcholestane in the  $m/z$  260, 245, 217 and 176 chromatograms. Kovats' indices (KIs) of the homologous 4,14-dimethylsteranes are 3046 (DMC), 3144 (24-methyl) and 3232 (24-ethyl) respectively. Moreover, 4,4,14 $\alpha$ -trimethylcholestane is also present in trace amounts at KI = 3127, as evidenced by comparison of its mass spectrum with the published spectrum [11]. On the other hand, 4-methylsteranes or 4,4-dimethylsteranes (lacking the 14-methyl group) are undetectable and regular steranes are barely detectable using  $m/z$  217 chromatogram. This suggests that most of the organic matter of the sample originates from a very peculiar source.

All attempts to determine the full stereochemistry by isolation of the postulated 4,14-dimethylcholestane using chromatography over silica gel or alumina remained unsuccessful. However, new attempts are underway.

### 3.3. Origins of 4,14-dimethylcholestane

Sterols are well-known "biological markers" due to their skeleton preservation in sedimentary environments for a long geological record and to their structural specificity or distribution reflecting their sources. Sterols bearing a 14 $\alpha$ -methyl group and one methyl group at C-4 have actually been identified in some algae and higher plants but they are generally minor or trace components [12–18]. Only some echinoderms (Holothurians) contain significant amounts of these sterols [19,20]. For example, in *Holothuria scabra*, 4 $\alpha$ ,14 $\alpha$ -dimethyl-5 $\alpha$ -cholest-9(11)-en-3 $\beta$ -ol and 4 $\alpha$ ,14 $\alpha$ -dimethyl-5 $\alpha$ -cholest-7-en-3 $\beta$ -ol were identified as major components of the sterol fraction [21]. However, at this stage of investigation, it would be premature to conclude that 4,14-dimethylcholestane is a specific biomarker for echinoderms.

### Acknowledgements

This work was supported by the Fonds National Suisse de la Recherche Scientifique (Grant Nos. 20-63779.00 and 20-68091.02).

### References

- [1] M.A. Kruege, Org. Geochem. 10 (1986) 517.
- [2] S. Schouten, M. Schoell, W.I.C. Rijpstra, J. Sinninghe Damsté, J.W. De Leeuw, Geochim. Cosmochim. Acta 61 (1997) 2065.
- [3] C.M. Isaacs, K.A. Pisciotto, R.E. Garrison, Dev. Sed. 36 (1983) 247.
- [4] J.A. Curiale, J.R. Odermatt, Org. Geochem. 14 (1989) 1.
- [5] C.M. Isaacs, J. Rullkoetter, The Monterey Formation — From Rocks to Molecules, Columbia University Press, New York, 2000.
- [6] W.K. Seifert, J.M. Moldowan, G.W. Smith, E.V. Whitehead, Nature 271 (1978) 436.

- [7] C. Pellaton, Distribution of sedimentary organic matter (palynofacies) with respect to palaeoenvironmental conditions: two case histories from the Miocene of the USA (Ph.D. dissertation), University of Geneva, Switzerland, *Terre & Environment* 43, 2003.
- [8] J.A. Curiale, D. Cameron, D.V. Davis, *Geochim. Cosmochim. Acta* 49 (1985) 271.
- [9] S. Dagan, *J. Chromatogr. A* 868 (2000) 229.
- [10] J.M. Halket, A. Przyborowska, S.E. Stein, W.G. Mallard, S. Down, R.A. Chalmers, *Rapid Commun. Mass Spectrom.* 13 (1999) 279.
- [11] R.R. Muccino, C. Djerassi, *J. Am. Chem. Soc.* 96 (1974) 556.
- [12] S.P. Vishnoi, A. Shoeb, R.S. Kapil, *Planta Med.* 54 (1988) 40.
- [13] D.D. Sawaikar, E.D. Pandhare, *Ind. J. Chem., Sect. B* 31 (1992) 639.
- [14] S. Rangaswami, K.N.N. Ayengar, *J. Res. Ind. Med.* 3 (1968) 1.
- [15] M. Masaoud, J. Schmidt, G. Adam, *Phytochemistry* 38 (1995) 795.
- [16] T. Itoh, T. Ishii, T. Tamura, T. Matsumoto, *Phytochemistry* 17 (1978) 971.
- [17] S. Afaq-Husain, M. Shameel, K. Usmanghani, M. Ahmad, V.U. Ahmad, *Bot. Mar.* 34 (1991) 215.
- [18] T. Itoh, H. Tani, K. Fukushima, T. Tamura, T. Matsumoto, *J. Chromatogr.* 234 (1982) 65.
- [19] L.P. Ponomarenko, A.I. Kalinovsky, O.P. Moiseenko, V.A. Stonik, *Comp. Biochem. Phys. B* 128 (2001) 53.
- [20] M.L. Cordeiro, C. Djerassi, *J. Org. Chem.* 55 (1990) 2806.
- [21] V.A. Stonik, L.P. Ponomarenko, T.N. Makarieva, V.M. Boguslavsky, A.S. Dmitrenok, S.N. Fedorov, S.A. Strobikin, *Comp. Biochem. Phys. B* 120 (1998) 337.