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Note

## Macrocyclic-alkanes: a new class of biomarker

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### Abstract

A new class of cyclic hydrocarbon biomarker, macrocyclic-alkanes and their methylated analogues, have for the first time been unambiguously identified in a *Botryococcus braunii* rich sediment (torbanite). The compounds, consisting of a homologous series of macrocyclic-alkanes (ranging from C<sub>15</sub> to C<sub>34</sub>) and their methylated derivatives (ranging from C<sub>17</sub> to C<sub>26</sub>), were identified by using authentic standards and GC–MS techniques. © 2001 Elsevier Science Ltd. All rights reserved.

**Keywords:** Biomarkers; Macrocyclic-alkanes; Methylated macrocyclic-alkanes; Torbanite; Algae; *Botryococcus braunii*

### 1. Introduction

The majority of hydrocarbon biomarkers reported in sedimentary organic matter and petroleum are acyclic or based on cyclic carbon skeletons readily recognisable as being derived from isoprenoidal natural products such as triterpenoids. While most of these compounds are polycyclic and based commonly on five- and six-carbon membered rings, terpenoids based on larger (i.e.  $n > 10$ ) single carbon ring systems, the macrocycles, are also quite common in natural products. For example, the macrocyclic diterpenoid compounds based on the cembranoid skeleton (1-isopropyl-4,8,12-trimethylcyclotetradecane) are well known and have been reported to occur in both the plant and animal kingdoms (Wahlberg and Eklund, 1992). Barakat and Rulkötter (1993) have identified cembranoid biomarkers in Miocene lacustrine sediments. However, macrocyclic hydrocarbon rings systems not based on isoprene (i.e. non-isoprenoidal) are much less commonly reported. The first report of this type of macrocyclic hydrocarbon in plants was made by Red'kina et al. (1989), with the report of even-carbon-numbered C<sub>24</sub>–C<sub>32</sub> hydrocarbons in the plant *Empetrum nigrum*. Reports of macrocyclic hydrocarbons

in other plants have since appeared in the literature (i.e. Baik et al., 1996; Mimica-Dukic et al., 1998).

To date, the only report of non-isoprenoidal macrocyclic-alkanes in sedimentary material was the tentative identification of cyclic hydrocarbons of the cyclododecane and cyclohexadecane series in the non-aromatic hydrocarbon fractions of the semi-coking oil from an Estonian oil shale (Muurisepp et al., 1994). In this paper we report the first positive identification of two homologous series of macrocyclic hydrocarbons in an extract of a Scottish torbanite deposited in the Late Carboniferous.

### 2. Experimental

#### 2.1. Sample description

The torbanite sample is of Carboniferous age from Torbane Hill in Scotland and has been described by Allan et al. (1980). Briefly the crushed sample was extracted with dichloromethane/methanol (95:5) and the solvent removed by distillation in a Kuderna–Danish apparatus. The extract was separated into aliphatic, aromatic and polar fractions by chromatography on silica gel (Audino et al., 2001). A branched and cyclic fraction was prepared by chromatography on a column of ZSM-5 molecular sieve according to the procedure of

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West et al. (1990) to yield the ZSM-5-excluded (branched/cyclic) fraction.

## 2.2. Reference compounds

Cyclopentadecane was purchased from the Tokyo Kasei Kogyo Company Ltd (Japan). Methylcyclopenta-

decane was prepared from cyclopentadecane via oxidation to cyclopentadecanone by the Gif reaction following the procedure of Barton et al. (1990). The resulting cyclopentadecanone, obtained in low yield (~10%) but high purity (>99%) after chromatography on silica gel was then methylated via the Grignard reaction with methylmagnesium iodide. The resulting

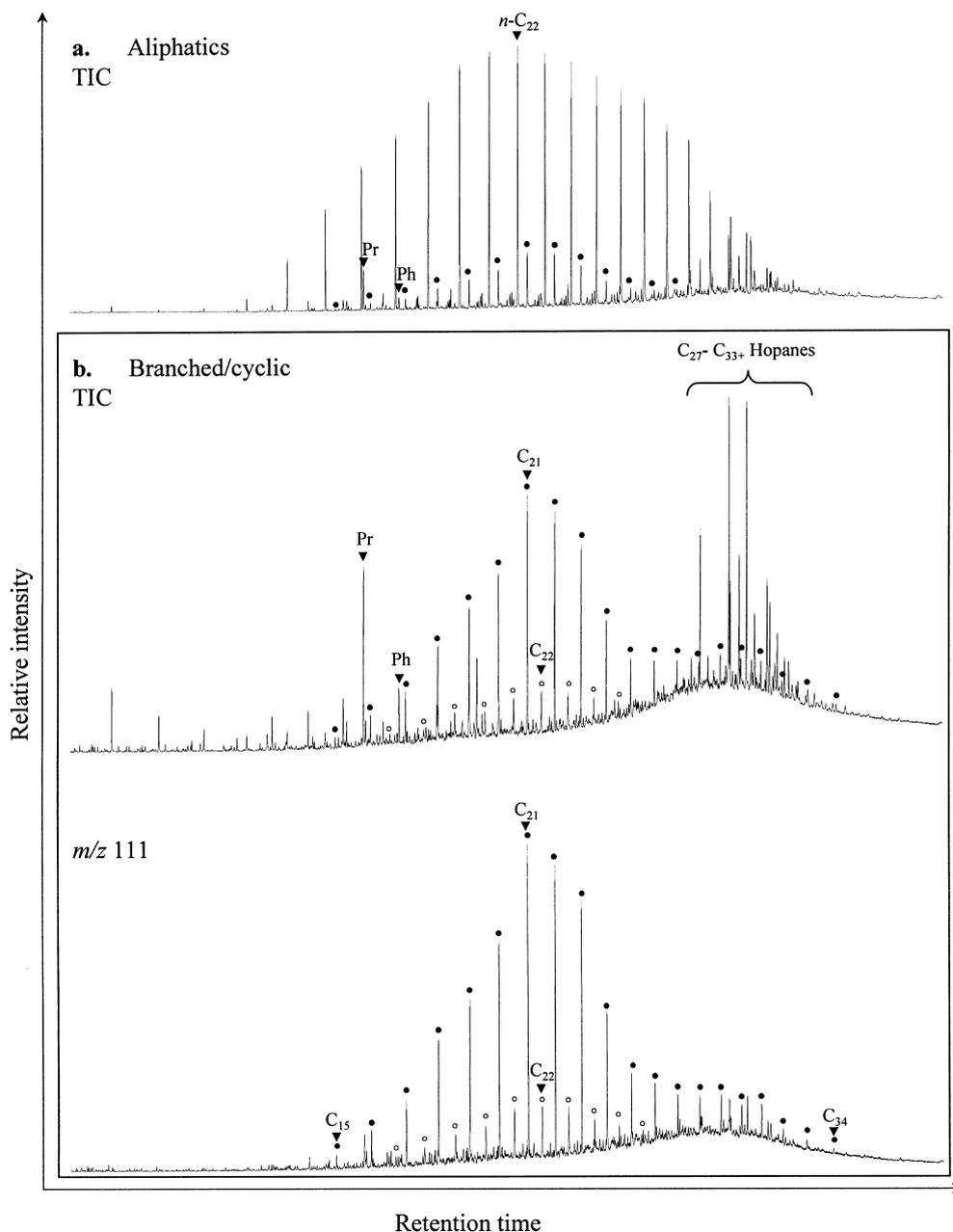


Fig. 1. GC-MS traces of the aliphatic fraction of the torbanite extract: (a) total ion chromatogram of the aliphatic fraction; (b) branched and cyclic fraction of the same sample (top) and the  $m/z$  111 trace of the same fraction (bottom). Filled circles indicate the macrocyclic-alkanes, open circles indicate the methyl substituted macrocyclic-alkanes, Pr and Ph indicate pristane and phytane, respectively, and the numbers above the peaks indicate the total carbon number of each compound.

1-methylcyclopentadecanol was converted to 1-chloro-1-methylcyclopentadecane with thionylchloride, which was hydrogenated under basic conditions (potassium hydroxide in ethanol, hydrogen at 60 psi, 5% Pd/C) to give methylcyclopentadecane (~96% pure).

### 2.3. Instrumentation

Gas chromatographic–mass spectrometric (GC–MS) analyses were performed using a HP 5973 MSD interfaced to a HP 6890 gas chromatograph with a 60 m × 0.25 mm i.d. WCOT fused silica capillary columns coated with either a 0.25 μm methylsilicone or 5% phenylmethylsilicone stationary phase (DB-1 and DB-5, J&W Scientific). For routine analyses the GC oven was programmed from 40 to 300°C at 3°C/min with initial and final hold times of 1 and 30 min, respectively. For isothermal GC analyses the oven was maintained at 190°C for 180 min then programmed to 300°C at 15°C/min and held for 30 min. The samples were dissolved in hexane and injected on-column for routine analyses, or via a vaporising injector at 280°C in splitless mode for isothermal analyses, using a HP 6890 auto-sampler. Helium was used as the carrier gas at a linear velocity of 28 cm/s with the injector operating at constant flow. Typically, the MS was operated at an ionisation energy of 70 eV, a source temperature of 180°C, with an electron multiplier voltage of 1800 V and a mass range of 35–550 amu.

### 3. Results and discussion

The mass chromatogram of the aliphatic hydrocarbon fraction of the torbanite extract (Fig. 1a) shows abundant *n*-alkanes ranging between C<sub>14</sub> and C<sub>34</sub> and maximising at C<sub>22</sub>. Eluting between the *n*-alkanes is an unusual series of relatively abundant compounds ranging from C<sub>15</sub> to C<sub>34</sub>. Fig. 1b shows the mass chromatogram of the branched/cyclic fraction of the extract, in which these compounds are prominent. The mass spectrum of the most abundant compound (C<sub>21</sub>), shown in Fig. 2a, shows a molecular ion at *m/z* 294 (C<sub>21</sub>H<sub>42</sub>) typical of a cyclic hydrocarbon and shows a characteristic ion at *m/z* 266 representing the loss of ethylene.

GC–MS analysis of the branched and cyclic fraction (Fig. 1b) also shows a second, less abundant series of compounds eluting between members of the first series (both series of compounds are more easily visible in the *m/z* 111 extracted ion trace (Fig. 1b)). The mass spectrum of the most abundant member of this series (C<sub>22</sub>) is shown in Fig. 2b, and shows a molecular ion at *m/z* 308 (C<sub>22</sub>H<sub>44</sub>) typical of a cyclic hydrocarbon and a characteristic ion at *m/z* 280 representing the loss of ethylene, as observed in the first series. This second series also has an ion corresponding to the loss of 15 amu, indicative of a methyl fragment: this suggests that the second series contain the methylated analogues of the first series, and range from C<sub>17</sub> to C<sub>26</sub>.

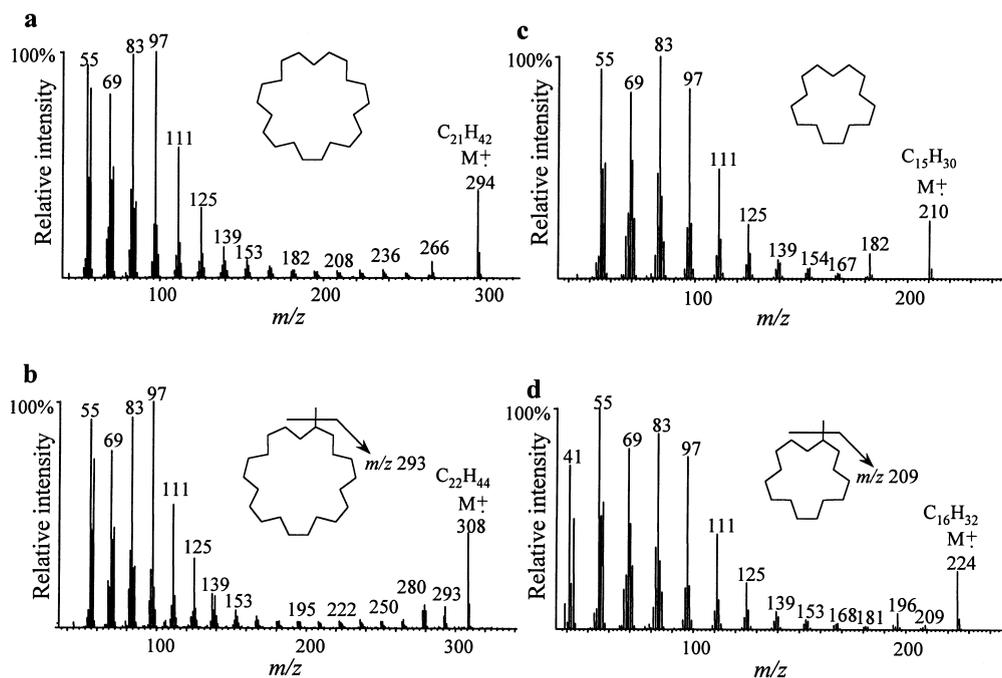


Fig. 2. Mass spectra of (a) C<sub>21</sub> macrocyclic-alkane and (b) C<sub>22</sub> methylmacrocyclic-alkane identified in the sample and (c) cyclopentadecane and (d) methylcyclopentadecane reference compounds.

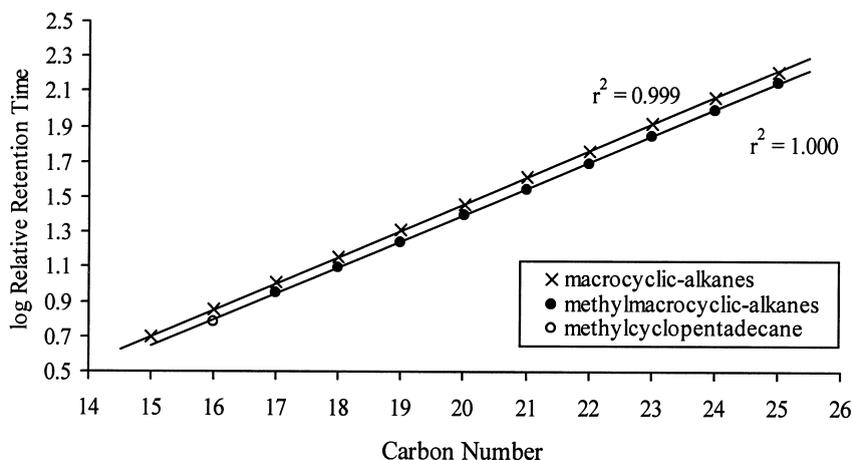


Fig. 3. Plots of the log of relative retention time versus carbon number for the macrocyclic- and methylmacrocyclic-alkanes in the torbanite obtained by isothermal GC–MS analysis (190°C using a J&W DB-5 capillary column). The excellent correlations indicate homologous series.

Based on the mass spectral features of the two series, the compounds were tentatively assigned as macrocyclic-alkanes and methylated-macrocyclic alkanes. In order to confirm the assignments, cyclopentadecane and methylcyclopentadecane were used as reference compounds. The mass spectra of the reference compounds (Fig. 2c and d) compare well to those in the sample, having similar features to those of the two series of unknown compounds. Co-injection of the sample with authentic cyclopentadecane (using two different GC stationary phases) revealed identical retention times and mass spectra to the first compound of the first, more abundant series, confirming the occurrence of cyclopentadecane in the sample. A plot of the log of relative retention times versus carbon numbers for cyclopentadecane and the remaining compounds in the first series was constructed from the results of isothermal GC–MS analysis. As shown in Fig. 3, this plot gives an excellent correlation ( $r^2 = 0.999$ ), confirming the assignment of the first series of compounds as a homologous series of macrocyclic-alkanes. Co-injection of the sample with methylcyclopentadecane showed it not to be present in the sample. However, a plot of the log of relative retention times versus carbon numbers for the authentic methylcyclopentadecane and the compounds in the second, less abundant series, constructed from the results of isothermal GC–MS analysis is shown in Fig. 3. This plot shows an excellent correlation ( $r^2 = 1.000$ ), confirming the assignment of the second series of compounds as a homologous series of methylated macrocyclic-alkanes.

While the hydrocarbon fractions of torbanite extracts (e.g. Allan et al., 1980; Derenne et al., 1988) and other *B. braunii* rich sediments (e.g. Derenne et al., 1997, 2000) have been previously studied, this is the first report of the occurrence of macrocyclic-alkanes in sediments. The

only other reports of this class of compound, is of hydrocarbons in certain angiosperms, which show only a limited distribution of even-carbon-numbered homologues (e.g. Red'kina et al., 1989). This is inconsistent with the distribution found in the torbanite sample and makes them unlikely candidates as a source in a sample of Carboniferous age. While the lipids of the three extant races of *B. braunii* have been well studied (Metzger et al., 1991, and references therein), no macrocyclic-alkanes, or likely precursor for the macrocyclic-alkanes have, as yet, been reported. More samples are currently under investigation to assess the general occurrence and significance of the macrocyclic-alkanes in sediments and crude oils.

#### 4. Conclusions

New classes of sedimentary biomarkers, namely macrocyclic-alkanes (ranging from C<sub>15</sub> to C<sub>34</sub>) and methylmacrocyclic-alkanes (ranging from C<sub>17</sub> to C<sub>26</sub>) have, for the first time, been unambiguously identified in a *B. braunii* rich sediment (torbanite) of Late Carboniferous age.

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## References

- Allan, J., BJORØY, M., DOUGLAS, A.G., 1979. A geochemical study of the exinite group maceral alginite, selected from three Permo-Carboniferous torbanites. In: Douglas, A.G., Maxwell, J.R. (Eds.), *Advances in Organic Geochemistry 1979*. Pergamon Press, Oxford, pp. 599–618.
- Audino, M., Grice, K., Alexander, R., Boreham, C.J., Kagi, R.I., 2001. An unusual distribution of monomethylalkanes in *Botryococcus braunii*-rich samples: origins and significance. *Geochimica et Cosmochimica Acta*, in press.
- Baik, S.O., Bock, J.Y., Han, S.B., Cho, K.S., Bang, G.P., Kim, I.K., 1996. Analysis of volatile flavour constituents in green tea flower. *Analytical Science and Technology* 9, 331–335.
- Barakat, A.O., Rullkotter, J., 1993. Gas chromatographic/mass spectrometric analysis of cembrenoid diterpenes in kerogen from a lacustrine sediment. *Organic Mass Spectrometry* 28, 157–162.
- Barton, D.H.R., Csuhai, E., Ozbalik, N., 1990. Functionalisation of saturated hydrocarbons. Part XIV. Chemoselective oxidation. *Tetrahedron* 46 (11), 3743–3752.
- Derenne, S., Largeau, C., Casadevall, E., Connan, J., 1988. Comparison of torbanites of various origins and evolutionary stages. *Organic Geochemistry* 12, 43–59.
- Derenne, S., Largeau, C., Hetényi, M., Brukner-Wein, A., Connan, J., Lugardon, B., 1997. Chemical structure of the organic matter in a Pliocene maar-type shale: Implicated *Botryococcus* race strains and formation pathways. *Geochimica et Cosmochimica Acta* 61, 1879–1889.
- Derenne, S., Largeau, C., Brukner-Wein, A., Hetényi, M., Bardoux, G., Mariotti, A., 2000. Origin of variations in organic matter abundance and composition in a lithologically homogeneous maar-type oil shale deposit (Gérce, Pliocene, Hungary). *Organic Geochemistry* 31, 787–798.
- Metzger, P., Largeau, C., Casadevall, E., 1991. Lipids and macromolecular lipids of the hydrocarbon-rich microalga *Botryococcus braunii*. Chemical structure and biosynthesis. Geochemical and biotechnical importance. In: Herz, W., Grisenbach, H., Kirby, G.W., Tamm, C. (Eds.), *Progress in the Chemistry of Organic Natural Products*, Vol. 57. Springer, Wein, pp. 1–70.
- Mimica-Dukic, N., Ivancev-Tumbas, I., Igic, R., Popovic, M., Gasic, O., 1998. The content and composition of essential oil of *Hypericum perforatum* from Serbia. *Pharmaceutical and Pharmacological Letters* 8, 26–28.
- Muurisep, A.M., Urov, K., Liiv, M., Sumberg, A., 1994. A comparative study of non-aromatic hydrocarbons from kukersite and dictyonema shale semicoking oils. *Oil Shale* 11, 211–216.
- Red'kina, N.N., Bryanskii, O.V., Krasnov, E.A., Semenov, A.A., Ermilova, E.V., 1989. Constituents of *Empetraceae*. III. Cycloalkanes from *Empetrum nigrum*. *Khimiyn Prirodnykh Soedinenii* 5, 719–720.
- Wahlberg, I., Eklund, A.M., 1992. Cembranoids, psuedopteranoids, and cubitanoids of natural occurrence. In: Hertz, W., Kirby, G.W., Moore, R.E., Steglich, W., Tamm, C. (Eds.), *Progress in the Chemistry of Organic Natural Products*, Vol. 59. Springer-Verlag, New York, pp. 142–294.
- West, N., Alexander, R., Kagi, R.I., 1990. The use of silicalite for the rapid isolation of branched and cyclic alkane fractions of petroleum. *Organic Geochemistry* 15, 499–501.