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# NOTE

## Alternative biological sources for 1,2,3,4-tetramethylbenzene in flash pyrolysates of kerogen*

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**Abstract**—Pyrolysates of kerogens isolated from Indian Ocean surface sediments and from Black Sea Unit II contain abundant 1,2,3,4-tetramethylbenzene (1,2,3,4-TMB) suggesting the presence of macromolecularly-bound isorenieratane skeletons derived from isorenieratene biosynthesized by green sulphur bacteria (cf. Hartgers *et al.*, 1994c). However, the $^{13}C$ content of 1,2,3,4-TMB is identical to that of algal lipids, excluding an origin from green sulphur bacteria. The presence of 1,2,3,4-TMB in pyrolysates of stable residues of several marine algae indicates that it can also have an algal origin. Therefore, the assessment of photic zone anoxia on the basis of the abundant presence of 1,2,3,4-TMB in the pyrolysate is not possible without determination of its stable carbon isotopic composition and comparison with those of algal lipids.

*Key words*—1,2,3,4-tetramethylbenzene, algae, Arabian Sea sediments, Black Sea sediments, kerogen pyrolysates, diaromatic carotenoids, $^{13}C$ of TMB

### INTRODUCTION

The occurrence of alkylbenzene moieties in sedimentary high molecular weight organic matter has recently been studied in detail (Hartgers *et al.*, 1994a). Analysis of the $C_1$–$C_4$ alkylated benzene compositions of pyrolysates of 47 kerogens, coals and asphaltenes, from various locations and of different geological ages, revealed significant differences. Four end member sample types were discerned on the basis of principal component analysis and are characterized by a relative enhancement of specific alkylbenzenes relative to the average alkylbenzene distribution pattern. These were related to the presence of specific, structurally related, biologically derived precursor moieties in the macromolecular substances. Cleavage of the relatively weak $\beta$-bond of the alkylbenzene moieties in the kerogen is thought to predominantly generate the alkylbenzenes in the pyrolysates. The alkylbenzene distribution of one end member sample type was dominated by 1,2,3,4-tetramethylbenzene (1,2,3,4-TMB). More detailed investigations (Hartgers *et al.*, 1994b, 1994c) of this end member sample type have unambiguously shown that this can be due to a significant input of diaromatic carotenoids (e.g. isorenieratene) from green sulphur bacteria (Chlorobiaceae). This was demonstrated by (i) the carbon isotopic compositions of 1,2,3,4-TMB and *n*-alkanes and *n*-alk-1-enes in the kerogen pyrolysates and (ii) identification of the ethyltrimethylbenzenes, which demonstrated that 1-ethyl-2,3,6-trimethylbenzene (the $\gamma$-cleavage product of macromolecularly-bound isorenieratane) dominated their distribution. Since green sulphur bacteria require both light and hydrogen sulphide, 1,2,3,4-TMB can be considered as a "pyrolytic" marker for photic zone anoxia.

In this note we show that flash pyrolysates of kerogens isolated from surface sediments from the Indian Ocean and a sediment from the Black Sea also contain abundant 1,2,3,4-TMB. However, the stable carbon isotopic composition of 1,2,3,4-TMB and the distribution of $C_5$ alkylated benzenes reveal that 1,2,3,4-TMB does not originate predominantly from isorenieratene biosynthesized by Chlorobiaceae. The presence of 1,2,3,4-TMB in flash pyrolysates of stable residues isolated from a number of marine algae demonstrates that this component can also have an algal origin. These results imply that the relatively high abundance of 1,2,3,4-TMB in kerogen pyrolysates cannot be used as such to assess the occurrence of photic zone anoxia in past depositional systems.

### MATERIALS AND METHODS

*Kerogen isolation.* Surface sediment samples from various locations in the Indian Ocean (Table 1) were recovered during the expedition of R.V. Tyro in the Indian Ocean (Netherlands Indian Ocean Program, 1992). The sediment from the Black Sea Unit 2 is from a core taken at 41° 39′N, 30° 44′E (Boon *et al.*, 1979). Sediment samples were extensively Soxhlet extracted using a mixture of dichloromethane (DCM) and methanol (MeOH) (7.5:1). The residues remaining after extraction were subjected to

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Table 1. Description of Indian Ocean sediment samples studied.

| Sample Name | Location | Water depth (m) | Depth interval | Core type | Approximate age (yrs) |
|---|---|---|---|---|---|
| NIOP 451T | 23 40'53' N 66 02'97' E | 542 | 0–1 m | trip core | ? |
| NIOP 453T | 23 15'30' N 65 44'50' E | 1556 | 0–1 m | trip core | ? |
| NIOP 455-118b | 23 33'40' N 65 57'30' E | 995 | 34–36 cm | piston core | 7.700* |
| NIOP 455-115b | 23 33'40' N 65 57'30' E | 995 | 74–76 cm | piston core | 10.000* |
| NIOP 455-113b | 23 33'40' N 65 57'30' E | 995 | 89–91 cm | piston core | 12.000* |
| NIOP 455-109b | 23 33'40' N 65 57'30' E | 995 | 139–141 cm | piston core | 13.000* |
| NIOP 921 | 16 04'23' N 52 36'28' E | 455 | 0–9 mm | box core | 30† |
| NIOP 921 | 16 04'23' N 52 36'28' E | 455 | 9–18 mm | box core | 90† |
| NIOP 921 | 16 04'23' N 52 36'28' E | 455 | 18–27 mm | box core | 150† |
| NIOP 921 | 16 04'23' N 52 36'28' E | 455 | 27–36 mm | box core | 210† |
| NIOP 921 | 16 04'23' N 52 36'28' E | 455 | 36–45 mm | box core | 270† |

[a]*Reichart G.J. (1995) *personal communication*, [b]†Helder W. (1995) *personal communication*, ? = not known

Table 2. The occurrence of 1,2,3,4-TMB in the pyrolysates of stable residues of various marine algae

| Name | Class | Origin | Presence of 1,2,3,4-TMB |
|---|---|---|---|
| *Chlorella spaerckii* | Chlorophyceae | culture | - |
| *Brachiomonas submarina* | Chlorophyceae | culture | - |
| *Chlorococcum* sp. | Chlorophyceae | culture | √ |
| *Stichococcus bacillaris* | Chlorophyceae | culture | - |
| *Ulva fenestrata* | Chlorophyceae | field sample | √ |
| *Nannochloropsis salina* | Eustigmatophyceae | culture | - |
| *Nannochloropsis* sp. | Eustigmatophyceae | culture | - |
| *Nereocystis luetkeana* | Phaeophyceae | field sample | √ |
| *Emiliania huxleyii* | Prymnesiophyceae | culture | √ |

saponification (reflux in 1N KOH/MeOH) and acid hydrolysis (reflux in 2N HCl). Subsequently, the kerogens were isolated from the residues by a modification of the method reported by Durand and Nicaise (1980). Briefly, the residues were treated with 6 N HCl for 2 h and a second time for 16 h after washing with distilled water. After centrifugation the residues were washed with distilled water ( × 3) before treatment with a mixture of 6 N HCl and 40% HF (1:3) for 2 h. After washing with distilled water a second digestion for 16 h with a mixture of 6 N HCl and 40% HF (2:3) was performed. Finally, the residues were treated once again with 6 N HCl for 2 h. All isolation procedures were performed in closed PTFE bombs at *c.* 75°C. The isolated kerogens were washed with distilled water until the supernatant liquid, after centrifugation, was neutral and subsequently extracted with MeOH ( × 2) and DCM ( × 2). The kerogens were dried in vacuo at 40°C for 24 h prior to analysis. The residue after base and acid hydrolysis of the Black Sea sediment was used as such.

*Isolation of "stable" residues of algae*. Details concerning the isolation of stable residues from algal cultures or field samples of algae are described elsewhere (Gelin *et al.*, submitted). Briefly, the algae (Table 2) were ultrasonically extracted with mixtures of MeOH and DCM and the residues were treated subsequently with 1N KOH/MeOH (reflux, 1 h), 2N HCl (reflux, 8 h), 6N $H_2SO_4$ (20°C, 2 h) and 0.5 N $H_2SO_4$ (reflux, 16 h). After each treatment the products released were removed by extraction.

*Flash pyrolysis*. Kerogens, and stable residues of algae, were analyzed by means of Curie point pyrolysis–gas chromatography (Py–GC) using a FOM-4LX Curie point pyrolysis unit directly connected to the injector of a Hewlett Packard 5890 Series II gas chromatograph. Samples were pressed on flattened ferromagnetic wires with a Curie temperature of 610°C. Pyrolysis was conducted by inductive heating of the sample coated wires in 0.15 s to the Curie temperature at which it was held for 10 s. A Curie point high frequency generator (Fisher 9425) was used to induce

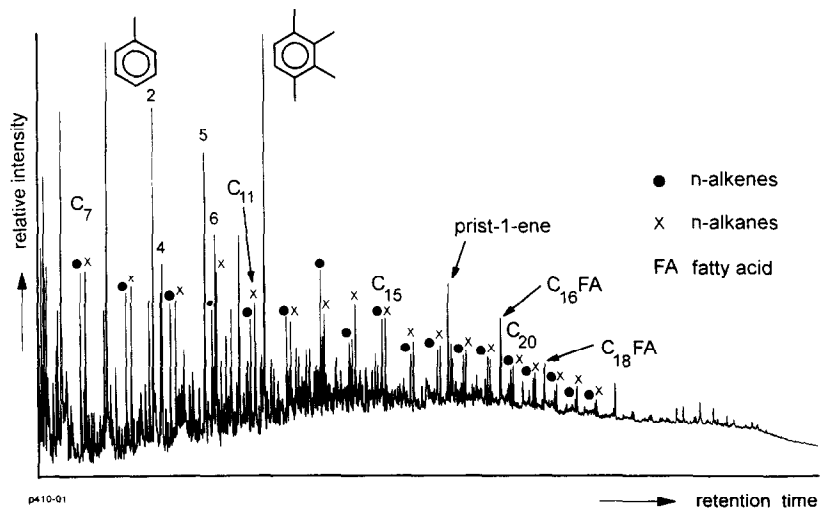


Fig. 1. Curie point (610°C) flash pyrolysates of the kerogen isolated from a surface sediment in the Indian Ocean at Site 451. The identification of the numbered peaks is listed in the caption of Fig. 2.

the magnetic field. Separation of the pyrolysis products was achieved using a fused silica column (25 m × 0.32 mm I.D.) coated with CP-Sil 5 (film thickness 0.45 μm). Helium was used as carrier gas. The gas chromatograph, equipped with a cryogenic unit, was held at 0°C for 5 min before it was programmed at 3°C $min^{-1}$ to 320°C at which it was held for 10 min. Py–GC–mass spectrometry (Py–GC–MS) was performed using the same conditions as above. The column was directly inserted into the EI ion source of a VG Autospec Ultima mass spectrometer operated at 70 eV with a mass range of $m/z$ 40–800 and a cycle time of 1.8 s.

*Off-line pyrolysis*. Off-line pyrolysis was performed with approximately 100 mg isolated kerogen sample (c. 650 mg of residue for the Black Sea sediment) which was heated for 2 h in a glass tube positioned in a cylindrical oven at a temperature of 400°C under a nitrogen flow. The volatile products were trapped in two successive cold traps (the first at room temperature and the latter cooled with solid $CO_2$/acetone) filled with hexane/DCM (7:1, v/v). The off-line pyrolysate was fractionated by column chromatography on activated $Al_2O_3$ (2.5 h at 150°C; 25 × 1 cm; $V_o$ = 42 ml) into an alkane/alkene, an aromatic hydrocarbon, and a polar fraction. The presence of 1,2,3,4-TMB in the off-line pyrolysate was confirmed by coinjection of a 1,2,3,4-TMB standard (Janssen Chimica).

*irm GC–MS*. The saturated hydrocarbon and aromatic hydrocarbon fraction from the off-line pyrolysates were subjected to irm–GC–MS measurements. Isotope-ratio-monitoring gas chromatography–mass spectrometry (irm–GC–MS) was performed on a DELTA-C irm–GC–MS system described in detail by Schouten *et al.* (submitted).

## RESULTS AND DISCUSSION

Flash pyrolysates of kerogens isolated from several surface sediment samples from the Arabian Sea (Table 1) and a kerogen from the Black Sea Unit II contain, in addition to *n*-alkanes, *n*-alk-1-enes, isoprenoid alkenes and fatty acids, high amounts of alkylbenzenes, in particular 1,2,3,4-TMB (e.g. Fig. 1). This suggests that the kerogen contains a significant amount of macromolecularly-bound aromatic carotenoids derived from green sulphur bacteria (cf. Hartgers *et al.*, 1994b, 1994c). However, stable carbon isotopic analysis of 1,2,3,4-TMB generated by off-line pyrolysis of the kerogen ($\delta_{TOC} = -21.5 \pm 0.1‰$) isolated from the Arabian Sea surface sediment at site 451 revealed that the $\delta^{13}C$ value of 1,2,3,4-TMB ($\delta = -25.1 + 0.5‰$) was indistinguishable from the average $\delta^{13}C$ values of the algal derived $C_{11}$–$C_{19}$ *n*-alkanes ($\delta = -24.8‰$) and prist-1-ene ($\delta = -24.8 \pm 0.4‰$) in the off-line pyrolysate. For the off-line pyrolysate of the Black Sea sediment these comparisons were to some extent complicated by partial coelution of 1,2,3,4-TMB with a methylindene, which contributed c. 30% to the peak integrated for determination

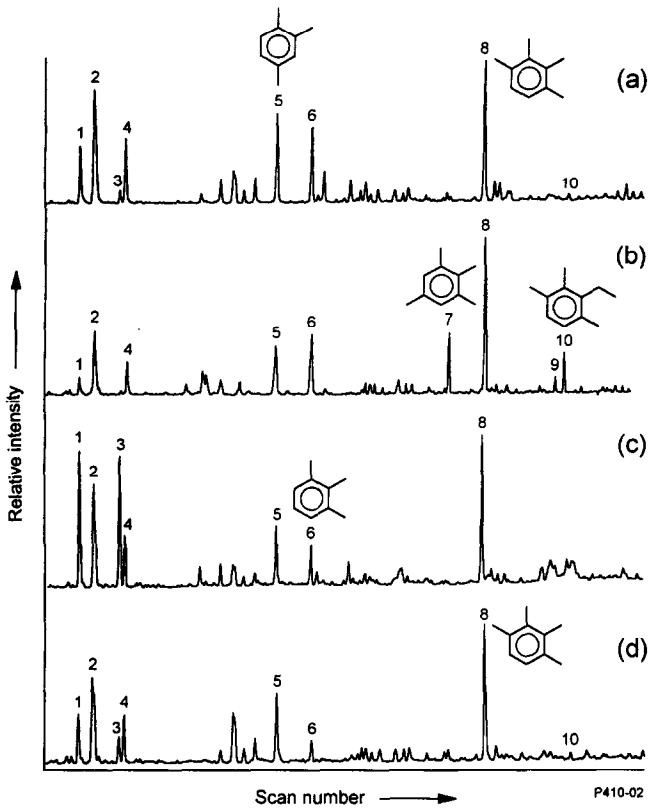


Fig. 2. Summed, partial, accurate mass chromatograms ($m/z$ 78.05 + 91.08 + 92.08 + 105.09 + 106.09 + 119.10 + 120.10 + 133.12 + 134.13 + 147.14 + 148.14) of pyrolysates of (a) the isolated kerogen of the NIOP 451 surface sediment, (b) the isolated kerogen of the Duvernay formation (Redwater well, 1159 m; data from Hartgers *et al.*, 1994c) and the stable residues of (c) the marine green alga *Chlorococcum sp.* and (d) the coccolithophorid *Emiliania huxleyii*. The stable residue of *E. huxleyii* is in fact the residue after an additional HI treatment (to cleave ether bonds). The dominance of 1,2,3,4-TMB was, however, similar in the residue after HCl treatment. Key: 1 = ethylbenzene, 2 = *m*- and *p*-xylene, 3 = styrene, 4 = *o*-xylene, 5 = 1,2,4-trimethylbenzene, 6 = 1,2,3-trimethylbenzene, 7 = 1,2,3,5-tetramethylbenzene, 8 = 1,2,3,4-tetramethylbenzene, 9 = 1-ethyl-3,4,5-trimethylbenzene, 10 = 1-ethyl-2,3,6-trimethylbenzene. Identifications of these alkylbenzenes are based on the data reported by Hartgers *et al.* (1992).

of the isotopic composition. However, in this case also, the $\delta^{13}C$ value of the 70/30 mixture of 1,2,3,4-TMB and methylindene ($\delta = -28.4 \pm 0.4‰$) was close to that of the average $\delta^{13}C$ values of the algal derived $C_{11}$–$C_{19}$ *n*-alkanes ($\delta = -28.0‰$) and prist-1-ene ($\delta = -30.9 \pm 0.1‰$) in the off-line pyrolysate. These data are essentially different from those reported by Hartgers *et al.* (1994b) for the pyrolysate of the isolated kerogen from the Duvernay formation. In that case, 1,2,3,4-TMB ($\delta = -19.4 \pm 0.1‰$) was significantly enriched in $^{13}C$ relative to the $C_{11}$–$C_{17}$ *n*-alkanes ($\delta_{av.} = -30.8‰$), prist-1-ene ($\delta = -31.7 \pm 0.6‰$) and total kerogen ($\delta_{TOC} = -28.5 \pm 0.1‰$). This was attributed to an origin of 1,2,3,4-TMB from $\beta$-cleavage of macromolecularly-bound isorenieratane skeletons derived from isorenieratene from green sulphur bacteria. These obligate anaerobic photosynthetic bacteria fix carbon through the reversed tricarboxylic acid (TCA) cycle leading to biomass anomalously enriched in $^{13}C$ (Quandt *et al.*, 1977; Sirevag *et al.*, 1977). This generally leads to a difference in carbon isotopic composition of sedimentary lipids derived from algae and green sulphur bacteria of 13–16‰ (Koopmans *et al.*, in press). Clearly, a source other than green sulphur bacteria has to be invoked for 1,2,3,4-TMB in pyrolysates of kerogen of Indian Ocean surface sediments. In the case of the Black Sea kerogen a contribution from macromolecularly-bound isorenieratane was anticipated, since free and S-bound isorenieratene have been reported to occur in both Unit I and Unit II sediments (Sinninghe Damsté *et al.*, 1993; Repeta, 1993). However, in this case an extremely light isotopic composition of the coeluting methylindene ($\delta = -57‰$) has to be assumed to give 1,2,3,4-TMB a value of $\delta = -16‰$, which could be anticipated from the $\delta^{13}C$ value of the isorenieratane skeleton previously determined (Sinninghe Damsté *et al.*, 1993). Therefore, in this case an additional alternative source for 1,2,3,4-TMB seems also likely.

Apart from the large differences in $\Delta\delta$ of 1,2,3,4-TMB and algal lipids of the Duvernay and the Indian Ocean kerogen pyrolysates, significant differences in their alkylbenzene distributions (Fig. 2a,b) can also be noted. These are (i) the absence of 1,2,3,5-tetramethylbenzene and 1-ethyl-3,4,5-trimethylbenzene and (ii) the much reduced abundance of 1-ethyl-2,3,6-trimethylbenzene in the pyrolysates of the kerogens from Indian Ocean sediments. The first difference is explained by the fact that in the Duvernay kerogen an unusual diaromatic carotenoid with a 3,4,5-/2,3,6-trimethyl aromatic substitution pattern is incorporated, which gives rise to the generation of these two alkylbenzenes on pyrolysis (Hartgers *et al.*, 1993, 1994c). The low abundance of 1-ethyl-2,3,6-trimethylbenzene (the $\gamma$-cleavage product of macromolecularly-bound isorenieratane; Hartgers *et al.*, 1994c) also indicates that 1,2,3,4-TMB is probably derived from a moiety other than diaromatic carotenoids from green sulphur bacteria.

In the course of our investigations concerning the presence of aliphatic and phenolic biopolymers in a suite of marine algae (Gelin *et al.*, submitted; van Heemst *et al.*, submitted) it was found that the flash pyrolysates of some of the stable residues of the alga, obtained after extraction and base and acid hydrolyses contain abundant 1,2,3,4-TMB (Table 2; e.g. Fig. 2 c,d). This clearly shows that 1,2,3,4-TMB may also have an algal origin. It is noteworthy that in the pyrolysates of the stable residues of the algae as in those of the Indian Ocean kerogens 1-ethyl-2,3,6-trimethylbenzene is also a very minor component (0–5% of the 1,2,3,4-TMB concentration).

## CONCLUSIONS

These results clearly indicate that 1,2,3,4-TMB in kerogen pyrolysates can be derived from both green sulphur bacteria and marine algae. This means that the assessment of photic zone anoxia using the "pyrolytic" biomarker 1,2,3,4-TMB is not possible. An origin of 1,2,3,4-TMB from Chlorobiaceae can only be established by (i) a significant enrichment (c. 15‰) in its $^{13}C$ content relative to that of algal lipids and (ii) the presence of significant quantities in the pyrolysate of its pseudohomologue 1-ethyl-2,3,6-trimethylbenzene.

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