Geochimica et Cosmochimica Acta Vol. 53, pp. 3073–3079

0016-7037/89/$3.00 + .00

LETTER

Tetrahymanol, the most likely precursor of gammacerane, occurs ubiquitously in marine sediments

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(*Originally submitted March* 6, 1989; *resubmitted in revised form August* 18, 1989; *accepted September* 28, 1989)

Abstract—Tetrahymanol has been identified in several sediment samples from different depositional environments by gas chromatography–mass spectrometry and by coinjections with an authentic standard. Together with literature data this shows that tetrahymanol is likely to be widespread, which is in accordance with the ubiquitous occurrence of its presumed diagenetic product, gammacerane, in more mature sediments and crude oils. The diagenetic conversion of tetrahymanol to gammacerane most likely proceeds via dehydration and subsequent hydrogenation. The intermediate in this conversion, gammacer-2-ene, has been synthesized, and its presence in one sample confirmed by coinjections. The identification of tetrahymanol in marine sediments indicates either that protozoa of the genus *Tetrahymena* are widely distributed or that tetrahymanol is also a natural product of organisms other than *Tetrahymena*.

INTRODUCTION

ACCORDING TO THE BIOLOGICAL marker concept, certain organic compounds occurring in the geosphere can be traced back to a precursor compound in the biosphere, because the basic carbon skeleton of the geochemical product has preserved an unambiguous link with its biogenic precursor. Some precursors have been found only after the identification of alleged diagenetic products in sediments and crude oils, bacteriohopanepolyols and related compounds being classical examples (OURISSON et al., 1982). Other compounds, like the C_{19}–C_{30} tricyclic terpanes, have been related to a postulated natural product, tricyclohexaprenol, predicted to be found in some procaryotes (OURISSON et al., 1982). The recently discovered 3β- and 2α-methylsteranes have, as yet, also no known precursors in the biosphere, and perhaps such precursors will never be found because the biogenic source is extinct (SUMMONS and CAPON, 1988).

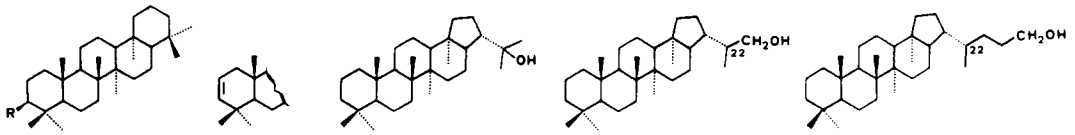
Gammacerane (Ia, R = H; Fig. 1) is a saturated triterpenoid hydrocarbon, which was definitively identified for the first time by HILLS et al. (1966) in Green River Shale extracts and subsequently found in numerous sediments and crude oils. Its oldest occurrence is in late Proterozoic sediments with an estimated age of approximately 850 Ma (SUMMONS et al., 1988). It has been noted that its relative concentration is particularly high in sediments from evaporitic environments (MOLDOWAN et al., 1985; TEN HAVEN et al., 1985, 1988). The only known potential biological precursor of gammacerane is tetrahymanol (gammaceran-3β-ol; Ia, R = OH), which has been found in several species of *Tetrahymena* (MALLORY et al., 1963; HOLZ and CONNER, 1973), in cultures of the anaerobic rumen fungus *Piromonas communis* (KEMP et al., 1984), and, in one case, in a fern (ZANDER et al., 1969).

Reports on the occurrence of tetrahymanol in geological samples are few. It has been found in the Eocene Green River Shale (HENDERSON and STEEL, 1971) and in Pleistocene sediments of Mono Lake (HENDERSON et al., 1972; TOSTE, 1976; REED, 1977). In the latter location, tetrahymanol in one sample occurs as the most abundant component of the alcohol fraction (TOSTE, 1976). Here we report on its occurrence in sediments from different depositional environments. Furthermore, we suggest that a C_{30} pentacyclic alcohol, often encountered in marine sediments, has been misidentified as a hopanol, but may be tetrahymanol.

SAMPLES

The alcohol fractions of 45 extracts, obtained from sediments of different ages representing different palaeoenvironments were checked for the presence of tetrahymanol. These include 10 samples from ODP Leg 105 (TEN HAVEN and RULLKÖTTER, 1988), 23 from ODP leg 112 (TEN HAVEN et al., 1989), 4 from the Gavish Sabkha (DE LEEUW et al., 1985), 1 from Solar Lake (BOON et al., 1983), 1 from the Vena del Gesso basin (VAI and RICCI LUCCHI, 1977), 1 from the Perticara basin (TEN HAVEN et al., 1985), 1 from the Nördlinger Ries impact crater (RULLKÖTTER et al., 1989), 1 from the Monterey formation, 2 from the Jurf ed Darawish formation, and 1 from the Phosphoria formation (SINNINGHE DAMSTÉ et al., 1989).

The samples range in age from very recent (Solar Lake, Gavish Sabkha) to Permian (Phospharia Retort shale) and are all immature; the highest vitrinite reflectance measured in one of the Monterey samples is 0.40%. The ODP drilling locations represent two largely different depositional environments, as reflected by the organofacies of the sediments, *viz* Baffin Bay (ODP Leg 105) with a dominance of terrigenous organic matter and the Peruvian upwelling area (ODP Leg 112) with a dominance of marine organic matter. The Monterey sample can be regarded as a Miocene equivalent of the Peruvian samples. The Gavish Sabkha and Solar Lake samples represent Recent hypersaline environments, and the Vena del Gesso and Perticara samples are bituminous shales interbedded between massive gypsum deposits from Messinian evaporitic basins. The Miocene bituminous



Ia Ib II III 22R IV 22S V 22R

FIG. 1. Structures of selected compounds discussed in text: Ia. Tetrahymanol (R = OH), gammacer-3-one (R = O), or gammacerane (R = H); Ib. Gammacer-2-ene; II. 17β(H),21β(H)-Hopan-22-ol (diplopterol); III. (22*R*)-17β(H),21β(H)-Hopan-29-ol (neriifoliol); IV. (22*S*)-17β(H),21β(H)-Hopan-29-ol (dryocrassol); V. (22*R*)-17β(H),21β(H)-Bishomohopan-32-ol. Because there is no satisfactory way to simultaneously represent the configuration at C-21 and C-22, only the former is depicted.

laminite from the Nördlinger Ries has been deposited in a slightly evaporitic, stagnant lake. The Jurf ed Darawish oil shale samples of Cretaceous age are thought to have been deposited in small basins with high productivity. The Permian Phosphoria retort shale is a phosphatic mudstone and dolomitic marlstone deposited in a presumably shallow water environment.

The aliphatic hydrocarbon fractions of the extracts from the Nördlinger Ries, the Monterey formation, and the Phosphoria formation samples were checked for the presence of gammacerane and gammacer-2-ene.

EXPERIMENTAL

A detailed description of extraction procedure, chromatographic separation of the extracts, gas chromatography (GC), and gas chromatography–mass spectrometry (GC-MS) measurements has been published previously (TEN HAVEN and RULLKÖTTER, 1988). The ketone plus ester fractions from the Peruvian samples were subjected to saponification, and the liberated alcohols isolated. Prior to analysis, the alcohol fractions were silylated with BSTFA + 1% TMCS in the presence of an equal volume of pyridine at 50°C for two hours. Experiments with diplopterol (II), as a tertiary alcohol standard, showed that a less rigorous procedure (absence of pyridine) afforded the corresponding trimethylsilyl (TMS) ether in only 5% yield, even after 24 hours; whereas, in the presence of pyridine, quantitative conversion was achieved almost instantly. It must be noted here that the assignment of a mass spectrum as silylated diplopterol is presumably wrong when no pyridine has been used in the derivatization process. Also there is no reliable method for a satisfactory acetylation of diplopterol.

Coinjections on the GC-MS system (both in the full scan mode as well as in the selective ion recording mode) were carried out with authentic tetrahymanol (Ia; R = H), diplopterol (II; 17β(H),21β(H)-hopan-22-ol), (22*R*)-17β(H),21β(H)-hopan-29-ol (III), (22*S*)-17β(H),21β(H)-hopan-29-ol (IV), (22*R*)-17β(H),21β(H)-bishomohopan-32-ol (V), and gammacer-2-ene (Ib). Tetrahymanol was isolated from *Tetrahymena pyriformis;* the three C_{30}-hopanols have been synthesized from 22-hydroxyhopan-3-one (DUNSTAN et al., 1957; ZUNDEL and ROHMER, 1985a); the C_{32}-hopanol has been obtained by $H_5IO_6/NaBH_4$ treatment of bacteriohopanetetrol derivatives (ROHMER et al., 1984). Gammacer-3-one (Ia; R = O) was obtained by oxidation of tetrahymanol with Collins' reagent. Gammacer-2-ene (Ib) was synthesized by converting gammacer-3-one into the tosylhydrazone derivative and subsequent treatment with butyllithium in the presence of TMEDA, as described for the synthesis of Δ^2-diplopterol (BISSERET et al., 1985). The NMR spectra, obtained on a Bruker W200 spectrometer, were in accordance with its structure: ^{1}H-NMR (200 MHz, $CDCl_3$) δ (ppm): 0.800 (3H, s), 0.829 (3H, s), 0.855 (6H, s), 0.878 (3H, s), 0.948 (3H, s), 0.977 (3H, s), 0.999 (3H, s), 5.39 (2H, m, 2-H and 3-H).

The following three different capillary columns were applied: a 50 m Hewlett Packard column coated with Ultra 2 (i.d. = 0.32 mm; f.t. = 0.53 μm), a 50 m Chrompack column coated with CPSil5 (i.d. = 0.25 mm; f.t. = 0.44 μm), and a 25 m Chrompack column coated with CPSil 19 (i.d. = 0.23 mm; f.t. = 0.18 μm).

MASS SPECTRA AND GC RETENTION OF STANDARDS

Direct probe mass spectra of three alcohol standards, measured as their TMS-ether derivatives, and of gammacer-2-ene and gammacer-3-one are shown in Fig. 2. Tetrahymanol and both hopan-29-ols are characterized by major fragment ions at *m/z* 191 and 279 (ring C cleavage) and 189 (ring C cleavage-TMSOH), and, less important, fragments at *m/z* 395 (M^+-TMSOH-CH_3), 410 (M^+-TMSOH), 485(M^+-CH_3), and the parent ion (M^+) at *m/z* 500. The mass spectrum of (22*R*)-hopan-29-ol TMS-ether (mass spectrum of acetate derivative has been published by DASTILLUNG et al., 1980) shows *m/z* 191 as base peak, and the fragment at *m/z* 189 has an intensity of approximately 90%. In addition, the hopan-29-ols, as well as extended hopanols (cf., DASTILLUNG et al., 1980), exhibit a small but characteristic fragment at *m/z* 369 (M^+-side chain) which is not present in the mass spectrum of tetrahymanol. Diplopterol has the same principal fragmentation pattern as the hopan-29-ols, with the major difference that charge retention in a side chain fragment gives rise to the base peak at *m/z* 131 (cf., BISSERET et al., 1985; ZUNDEL and ROHMER, 1985b; TEN HAVEN et al., 1987). In addition, a fragment ion at *m/z* 367 can be noted, which is due to secondary fragmentation of the side chain after initial loss of TMSOH as confirmed by metastable ion recording measurements (500 → 369, 500 → 367, and 410 → 367). The fragments due to ring C cleavage, *m/z* 189 and 191, are of lower importance. The mass spectrum of non-derivatized diplopterol is almost identical to that of diploptene (hop-22(29)-ene), *viz.* *m/z* 189 (81%), 191 (100%), 341 (8%), 367 (9%), 369 (5%), 395 (7%), 410 (M^+-18; 17%) with additional fragment ions at *m/z* 413 (3%) and 428 (M^+; 6%).

Ring C cleavage also dominates the fragmentation of gammacer-2-ene and gammacer-3-one giving rise to fragment ions *m/z* 189, 191 and 191, 205, respectively. A minor but characteristic ion at *m/z* 328 in the mass spectrum of gammacer-2-ene is caused by loss of 4-methylpenta-1,3-diene due to a retro-Diels-Alder reaction.

A partial total ion current chromatogram (selective ion recording mode), showing the relative retention times of the five alcohol standards, is given in Fig. 3 together with mass chromatograms of *m/z* 191 and 369. The four C_{30} triterpenoid alcohols can almost be baseline separated on an apolar capillary column (50 m CPSil5). On a slightly more polar column (Ultra 2: 5% phenyl) (22*R*)-hopan-29-ol and diplopterol coelute. The mass chromatogram of *m/z* 369 (Fig. 3) clearly shows that this fragment is highly characteristic of hopanoid-type triterpenoids.

RESULTS AND DISCUSSION

Of the numerous samples investigated two will be discussed in detail.

A partial total ion current chromatogram (full scan mode) of the alcohol fraction of a sample from Site 679 (ODP Leg 112; Peru upwelling) is shown in Fig. 4. It is representative of a suite of 23 samples from 3 different sites in the same area (TEN HAVEN et al., 1989). The samples cover a depth range of 0.35 to 86.75 m and are of Quaternary/Pliocene