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A novel triterpenoid carbon skeleton in immature sulphur-rich sediments

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Abstract—A novel S compound, 1,4-bis(2′,5′,5′,8a′-tetramethylhexahydrothiochroman)-butane has been detected in several immature S-rich sediments, of which the desulphurized counterpart was unambiguously identified by synthesis of an authentic standard and coinjection experiments. This C skeleton of the S compound, 1,10-bis(2′,2′,6′-trimethylcyclohexyl)-3,8-dimethyldodecane(I), has not been reported yet in any sediment or organism. We suggest that it may be biosynthesized through an enzymatic cyclization reaction of squalene (II), which shows similarities with the biosynthesis of β,β-carotene (III) from lycopene (IV).

INTRODUCTION

From the time that gas chromatography coupled with mass spectrometry became a routine analytical technique in the field of organic geochemistry numerous compounds have been identified in sediments and crude oil (e.g., Johns, 1986; Philp, 1985; Peters and Moldowan, 1993; references cited therein). One of the ultimate aims of biomarker studies is to relate compounds present in the geosphere with those present in the biosphere. Since the structure of many of these compounds can be severely altered due to diagenetic alterations, this task is not always straightforward. Nevertheless, a large suite of compounds has been identified which provide assistance in the reconstruction of the original biota in the depositional environment (e.g., Peters and Moldowan, 1993). Some compounds, like for instance, highly branched isoprenoids, were even detected in crude oils and sediments first (Yon et al., 1982), before they were detected in organisms (Nichols et al., 1988; Volkman et al., 1994).

Organic S compounds in sediments and crude oil constitute a class of compounds which can reveal unique information about their precursor paleobiochemicals. Since these compounds are formed by a reaction of inorganic S species with functionalized lipids (Sinninghe Damsté et al., 1989; De Graaf et al., 1992) these compounds do not only contain information about the C skeletons of these lipids, but also information on the position and number of functionalities. In this way, a detailed reconstruction of paleobiochemicals is possible. Cyclic triterpenoids, derived from enzymatic cyclization reactions of squalene or its epoxide, are compounds which are often detected in a sulphurized form since they usually contain a number of double bonds suitable for S-incorporation. For example sulphurized hopane, sterane, oleanane, and gammacerane C skeletons are some of the compounds detected in S-rich sediments (e.g., Schmid, 1986; Sinninghe Damsté et al., 1989; Payzant et al., 1986; Kohnen et al., 1991; Adam et al., 1991). Since a number of these compounds are unique for certain organisms, they are very useful as biomarkers.

Here we report the identification of a novel sulphur compound present in several immature S-rich sediments. Its unprecedented triterpenoid C skeleton which was unambiguously identified, points to the existence of a novel biochemical as yet not discovered in Nature.

EXPERIMENTAL

Samples

The Shell Beach sample is from an immature outcrop of the Miocene Monterey Formation (California, USA). The hydrocarbon and organic sulphur compound composition are described by Schouten et al. (1995). The Vena del Gesso sample is from a marl of an evaporitic cycle from the Messinian Vena del Gesso basin in the northern Apennines (Italy). The organic sulphur compounds and other hydrocarbons are described by Kohnen et al. (1992). The JED-sample is from the Cretaceous Jurf ed Darawish oil shale (Jordania). Organic sulphur compounds present in the extract are described by Kohnen et al. (1990).

Extraction and Fractionation

The sediments were extracted with a dichloromethane/methanol (7:1 v/v) mixture. The alkylsulphide fractions were isolated from the extracts by column chromatography and preparative argentation thin layer chromatography as described previously (Kohnen et al., 1992). The alkylsulphide fraction was desulphurized by dissolving it in ca. 4 mL of cyclohexane and adding ca. 0.5 g of Raney nickel. The tube was sealed and heated at 200°C for 3 h. The suspension was centrifuged and the residue was washed with dichloromethane (2×). The combined supernatants were washed with bidistilled water and subsequently dried using $MgSO_4$. The reaction mixture was then hydrogenated by first dissolving it in 99% acetic acid and adding PtO_2. Hydrogen was then bubbled through the solution for 3 h and the mixture was stirred for an additional 72 h. The acetic acid was removed by washing with bidistilled water and following the workup procedure described above for the Raney nickel desulphurization. The product mixture was analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC–MS).

Synthesis of Standard (Fig. 1)

To a stirred suspension of 20 mL dry diethylether and 0.3 g of magnesium curls, 1.8 mL (1.1 g) of 1,4-dibromobutane was slowly added under a nitrogen atmosphere. After several minutes of stirring at room temperature, 1.8 mL (1.9 g) of β-ionone was slowly added. The solution was then refluxed for 3 h. The reaction mixture was extracted with diethylether. Subsequently, an aliquot of the reaction products was dehydrated by dissolving it in 85% H_3PO_4 and stirring at room temperature for 1 h. The products were extracted with diethylether. Finally, an aliquot of the dehydrated reaction products was hydrogenated with PtO_2 in acetic acid (99%). Hydrogen was bubbled through for 2 h and the solution was stirred for an additional 24 h at

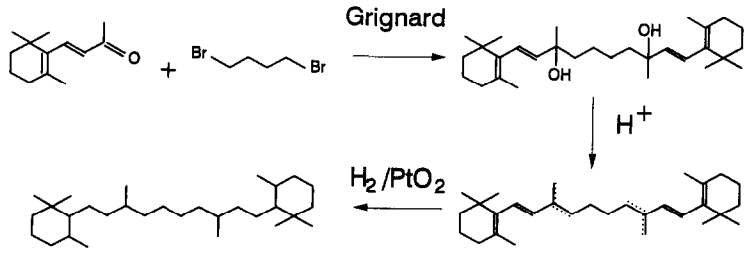

FIG. 1. Synthetic route for synthesis of 1,10-bis(2',2',6'-trimethylcyclohexyl)-3,8-dimethyldodecane (I).

room temperature. The desired product, 1,10-bis(2',2',6'-trimethylcyclohexyl)-3,8-dimethyldodecane, was isolated from the reaction mixture, using high pressure liquid chromatography (HPLC). The sample (ca. 35 mg) was separated using a Porosil C_{18} semipreparative column (Waters) and ethylacetate/methanol (20:80 v/v) as eluent. Appropriate subfractions were combined and separated further using a Novapak C_{18} analytical column (Waters) with ethylacetate/methanol (20:80 v/v) as eluent. Finally, a fraction of 3.9 mg of 1,10-bis(2',2',6'-trimethylcyclohexyl)-3,8-dimethyldodecane was obtained with a GC-purity higher than 95%. The compound was characterized by ^{1}H-NMR (400 MHz in $CDCl_3$); δ (ppm): 0.84 (doublet, 6H, J = 7.2 Hz, methylgroups on C3- and C-8), 0.85 (doublet, 6H, J = 6.4 Hz, methylgroups on C2' and C-2"), 0.86 (singlet, 6H, axial methylgroups C6' and C6"), 0.93 (singlet, 6H, equatorial methylgroups C6' and C6"), 1.00–1.50 (multiplets, 32 H), and 1.84–1.94 (multiplet, 2H, H's of C1' and C1").

Gas Chromatography

GC was performed using a Hewlett Packard 5890 equipped with an on-column injector. A fused silica capillary column (25 m × 0.32 mm) coated with CP Sil-5 (film thickness 0.12 μm) was used with helium as carrier gas. For the coinjection experiments, a capillary column (50 m × 0.33 mm) coated with Ultra-1 (thickness 0.2 μm) was also used. Both a flame ionization detector (FID) and a sulphur-selective flame photometric detector (FPD) were used, requiring a stream-splitter at the end of the column (split ratio FID:FPD = 1:4). The samples were dissolved in ethyl acetate and injected at 70°C. Subsequently, the oven was programmed to 130°C at 20°C/min and then at 4°C/min to 320°C at which it was held for 15 min.

Gas Chromatography-Mass Spectrometry

GC–MS was performed using a Hewlett-Packard 5890 gas chromatograph interfaced to a VG Autospec Ultima mass spectrometer operated at 70 eV with a mass range of m/z 40–800 and a cycle time of 1.7 s (resolution 1000). The gas chromatograph was equipped with a fused silica capillary column (25 m × 0.32 mm) coated with CP Sil-5 (film thickness = 0.2 μm). The carrier gas was helium. The samples were on column injected at 60°C and subsequently the oven was programmed to 130°C at 20°C/min and then at 4°C/min to 300°C at which it was held for 10 min.

Isotope-Ratio-Monitoring Gas Chromatography-Mass Spectrometry

The DELTA-C irm-GC-MS-system is in principal similar to the DELTA-S system as has been described previously (Hayes et al., 1990). The gas chromatograph was equipped with a fused silica capillary column (25 m × 0.32 mm) coated with CP Sil-5 (film thickness = 0.2 μm) with helium as carrier gas. The samples (dissolved in hexane or ethylacetate) were on-column injected at 70°C and subsequently, the oven was programmed to 130°C at 20°C/min, and then at 4°C/min to 320°C/min at which it was held for 20 min. The isotopic values were calculated by integrating the mass 44, 45, and 46 ion currents of the peaks produced by combustion of the chromatographically separated compounds and that of CO_2-spikes produced by admitting CO_2 with a known ^{13}C-content at regular intervals into the mass spectrometer. Values reported were determined by at least two analyses and the results were averaged to obtain a mean value and to calculate the standard deviation. The stable carbon isotope compositions are reported in the delta notation against the PDB ^{13}C standard.

RESULTS AND DISCUSSION

Identification of 1,10-bis(2',2',6'-trimethylcyclohexyl)-3,8-dimethyldodecane [I]

GC-analysis of several alkylsulphide fractions of immature sulphur-rich sediments (Cretaceous Jurf ed Darawish Oil Shale, Miocene Vena del Gesso Basin, and Miocene Monterey Formation) revealed a novel compound present in relatively high amounts. In the sample from the Monterey Formation this compound is dominating this fraction (Fig. 2b). Based on the response of the sulphur-selective flame-photometric-detector, it contains two sulphur atoms (Fig. 2c). Its mass spectrum (Fig. 2a) is dominated by the molecular ion at m/z 478 and possesses some smaller fragments at m/z 168, 211, 308, and 409. An unidentified compound with an identical mass spectrum has been reported in Monterey samples from Point Arena and in a sample from the Tertiary Ozouri Formation (Gabon; de Lemos Scofield, 1990). The mass spectrum shows many similarities with that of a thiane possessing a β,β-carotane carbon skeleton (V), previously tentatively identified by Trifilieff (1987). This sulphur compound has mass fragments of m/z 69, 211, and 518 and a molecular ion at m/z 588. The difference of 110 daltons of the molecular weight compared to that of the novel sulphur compound may be explained by an additional sulphur-atom (as determined by the FPD-response) and ten carbon atoms less. This led us to propose the structure as shown in Fig. 2 for the novel sulphur compound (VI). The broadness of the chromatographic peak suggests that the sedimentary compound is composed of several steroeisomers consistent with its proposed structure. To confirm this hypothetical structure, the alkylsulphide fraction of Shell Beach was desulphurized. The usual Raney nickel and nickel boride desulphurization techniques (Schouten et al., 1993) were, however, not effective on this sulphur compound, probably due to the severe steric protection of the methyl-groups and the cyclohexyl-rings. Desulphurization under "forcing conditions" (Raney nickel, cyclohexane, sealed tube, 200°C, 3h; Payzant et al., 1986) yielded a mixture of unsaturated compounds. This mixture was first hydrogenated using standard techniques (H_2, PtO_2, ethyl acetate, room temperature; Sinninghe Damsté et al., 1989), but these failed to hydrogenate the unsaturated compounds, probably due to steric hinderance of the cyclohexyl-rings. By using 99% acetic acid as solvent and pro-

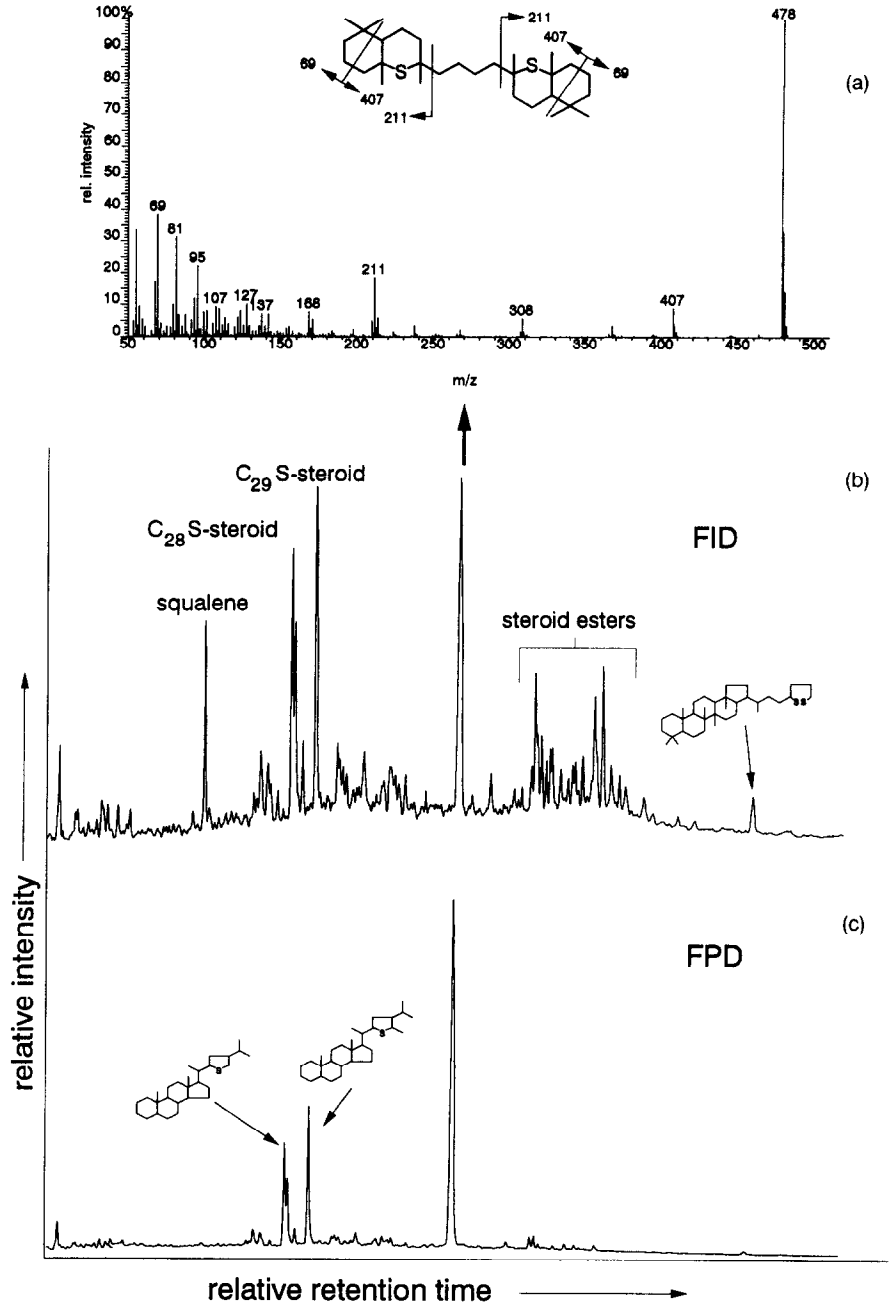

FIG. 2. Gas chromatogram of alkylsulphide-fraction of Shell Beach (Monterey Formation). (a) Mass spectrum of dominant sulphur compound, (b) FID-trace, and (c) FPD-trace.

longed stirring, hydrogenation of the unsaturated compounds was finally achieved. An abundant compound eluting just before the C_{28} *n*-alkane (Fig. 3) in the desulphurized and hydrogenated alkylsulphide fraction represents the desulphurized counterpart of the novel sulphur compound. This was confirmed by comparison of the carbon isotopic composition of the sulphur compound ($-32.6 \pm 0.2‰$) with that of its desulphurized counterpart ($-32.8‰$), which are unique values in the fractions analyzed. The mass spectrum of the desulphurized counterpart indicates a C_{30} alkane with two rings. To prove its structure 1,10-bis(2',2',6'-trimethylcyclohexyl)-3,8-dimethyldodecane (I) was synthesized (Fig. 1). The mass spectrum of the synthetic compound is identical to that of the sedimentary compound (Fig. 3) and coinjection experiments on two different GC-columns (CP-Sil 5 and Ultra-1) revealed that they have identical retention times, thus proving the structure of the desulphurized sedimentary compound to be 1,10-bis(2',2',6'-trimethylcyclohexyl)-3,8-dimethyldodecane [I].

Origin of 1,10-bis(2',2',6'-trimethylcyclohexyl)-3,8-dimethyldodecane [I]

To the best of our knowledge, this unambiguously identified C_{30} bicyclic triterpenoid carbon skeleton has not been

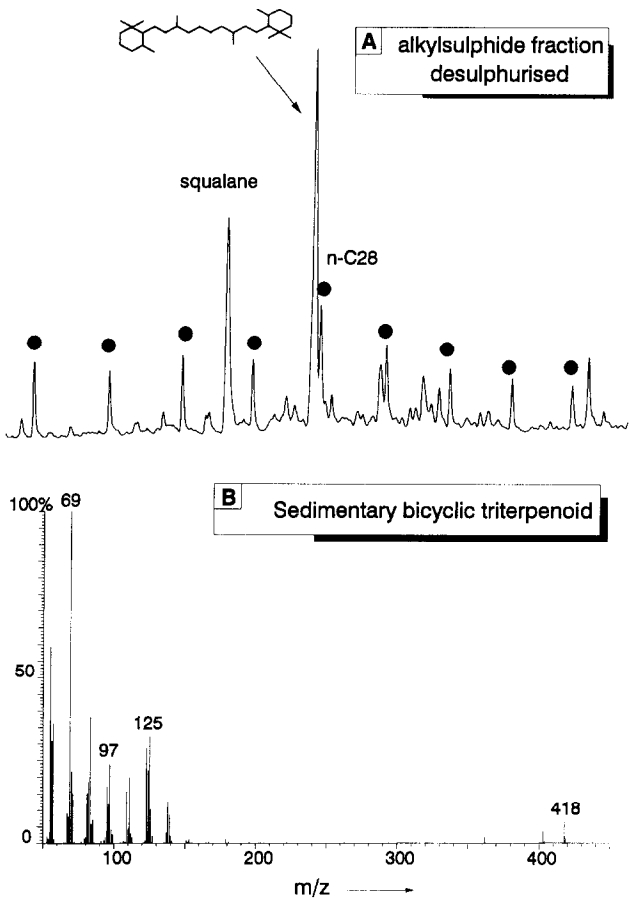

FIG. 3. (A) Gas chromatogram of desulphurized alkylsulphide-fraction and (B) mass spectrum of the dominant compound 1,10-bis(2',2',6'-trimethylcyclohexyl)-3,8-dimethyldodecane (I). ● = *n*-alkanes.

reported before in Nature. This carbon skeleton shows many structural similarities with that of β,β-carotene (III). β,β-Carotene is a carotenoid, which occurs widespread in plants and organisms and is a metabolite derived from cyclization of lycopene (IV; Nes and McKean, 1977; Ratledge and Wilkinson 1989; Fig. 4). It may thus be suggested that the C_{30} bicyclic triterpenoid is derived from a C_{30} carotenoid (VII). Indeed, in some bacteria acyclic C_{30} carotenoids do occur (Taylor, 1984), but cyclization of these compounds has never been reported. This process is deemed unlikely, since all double bonds are conjugated, thereby inhibiting enzymes to cyclisize C_{30} carotenoids in contrast to lycopene (Taylor, 1984).

Alternatively, the bicyclic triterpenoid carbon skeleton may have been derived from a novel cyclization reaction of squalene. Squalene or its epoxide can be cyclisized through catalysis of different enzymes to amongst others steroids, hopanoids and tetrahymanol. In rare cases, cyclization of the terminal isoprene-units of squalene has been reported leading to the formation of a tricyclic compound ambrein (VIII; Nes and McKean, 1977) and a tetracyclic compound, onocerin (IX; Nes and McKean, 1977). Theoretically, however, the possibility of an enzymatic cyclization of squalene leading to a bicyclic compound, similar to lycopene leading to β,β-carotene, is not excluded since the double bonds are in the appropriate positions and are not conjugated (Nes and McKean, 1977). Additional evidence for this hypothesis is provided by the mode of occurrence of the carbon skeleton in the sediments. Cyclization of squalene could lead to a C_{30} bicyclic triterpenoid possessing four double bonds. Since there are less than four sp^3-hybridized carbon atoms between the double bonds, it can be expected that intramolecular sulphur-incorporation is favoured, leaving no double bonds for the formation of intermolecular sulphur-linkages (Schouten et al., 1994; de Graaf et al., 1992). Indeed, no macromolecularly sulphur-bound compounds possessing the C_{30} bicyclic triterpenoid carbon skeleton were detected in the sediments investigated (Schouten et al., 1995). Furthermore, the tentative structure of the sulphur compound proposed on basis of its mass spectrum would be in full agreement with intramolecular incorporation of sulphur at the double bond positions proposed. In contrast, sulphur-bound β,β-carotane carbon skeletons have only been released from macromolecular fractions (Trifilieff, 1987; Schouten et al., 1994). This is attributed to the multiple double bonds in the precursor biochemical β,β-carotene making it statistically likely that at least one of the many formed sulphur-linkages is intermolecular (Fig. 4).

The question remains which organism biosynthesize (d) the C_{30} bicyclic triterpenoid carbon skeleton from squalene and for what purpose. From our limited sample set, we can infer that this compound was biosynthesized, at least in coastal marine and hypersaline lagoonal depositional environments in the Miocene and in a shallow marine basin during the Cretaceous. This suggests that this compound is not unique to certain depositional settings and/or time spans. Compound specific isotope analysis of both the sulphur compound and the desulphurized counterpart revealed a $\delta\ ^{13}C$-value of $-32.7 \pm 0.2‰$. This value is several per mill lighter than that of the algal-derived steroids ($\delta\ ^{13}C = -25$ to $-27‰$; Schouten et al., 1995) and of the cyanobacterial-derived C_{35} hopane ($\delta\ ^{13}C = -27.7‰$; Schouten et al., 1995). Thus, the ^{13}C-content falls outside the range of other squalene-derived biomarkers from organisms living in the photic zone. The relatively depleted $\delta\ ^{13}C$-value for [VI] may suggest that its bicyclic precursor (Fig. 4) is derived from organisms living deeper in the water column, possibly chemoautotrophic bacteria.

The reason why this compound has not been reported in contemporary organisms may be twofold: either it still has to be detected in cultures or field samples or the organism biosynthesizing this squalene derivative has disappeared during evolution. Since part of the structure of ambrein, derived from intestinal bacteria (Nes and McKean, 1977), is similar to the structure of the bicyclic triterpenoid a bacterial source may tentatively be suggested. The biological purpose of this compound is also not clear. Since the precursor biochemical did not possess conjugated double bonds like carotenoids a biological purpose as pigment can be excluded. Another possibility would be that this compound acts as a membrane rigidifier similar to steroids (Ratledge and Wilkinson, 1989) and hopanoids (Ourisson et al., 1987; Ratledge and Wilkinson, 1989) in eukaryotes and prokaryotes, respectively. However, the molecular dimensions of this molecule do not favour this hypothesis. Until this compound is detected in contemporary organisms suggestions concerning its origin and biological purpose will remain speculative.

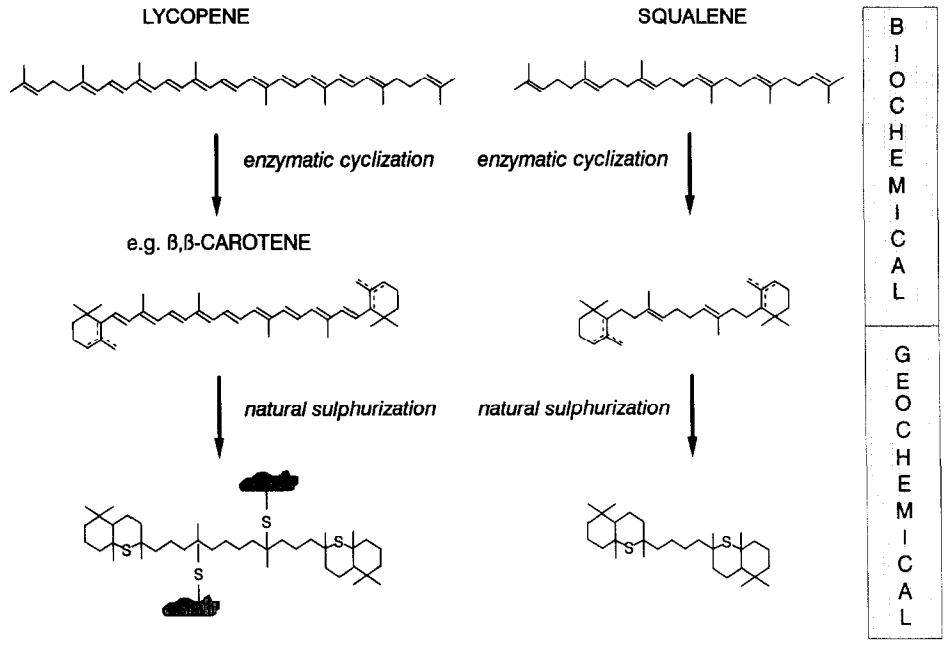

FIG. 4. Graphic representation of the proposed biochemical reactions and geochemical reactions with lycopene and squalene.

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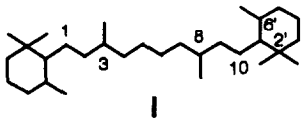
APPENDIX



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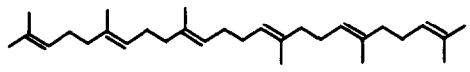

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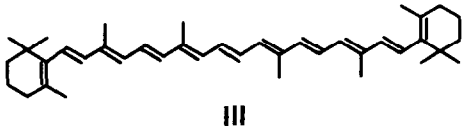

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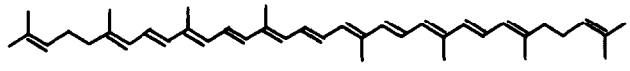

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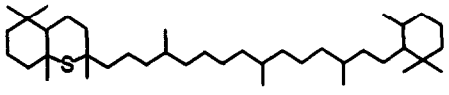

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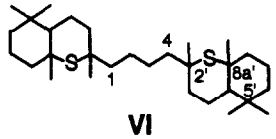

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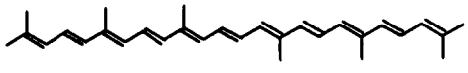

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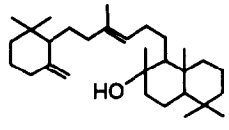
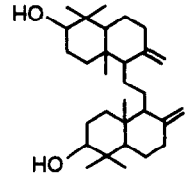


VIII



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