Secondary metabolites from the cnidarian Cavernularia sp.: structures of the new briaranes cavernulin A and B\textsuperscript{1,2}

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Novel cyclized cembranoids (briaranes) cavernulin A and cavernulin B along with ubiquitous wax esters (derived from saturated fatty acids and some analogous unsaturated fatty acids), cholesterol, ceramides as well as 1-O-alkylglycerols have been isolated from the cnidarian \textit{Cavernularia} sp., the extract of which shows some toxicity to guppy fingerlings and in brine-shrimp assays. The structures of cavernulin A as \((1S^{*},2S^{*},5Z,7S^{*},8R^{*},9S^{*},10S^{*},11R^{*},12R^{*},17R^{*})-2,9\)-diacetoxy-12-butanoyloxy-8-hydroxybriarane-5,13-dien-18-one and cavernulin B as \((1S^{*},2S^{*},5Z,7S^{*},8R^{*},9S^{*},10S^{*},11R^{*},12R^{*},17R^{*})-2,9\)-diacetoxy-8,12-dihydroxybriarane-5,13-dien-18-one are established by appropriate gas chromatographic studies.

Tremendous surge of interests in marine natural product research prevailed throughout the globe during the past couple of decades\textsuperscript{3}. As a part of our search for bioactive components from marine sources a cnidarian, \textit{Cavernularia} sp., was collected from the coastal Bay of Bengal. Preliminary bioactivity studies on the organism indicated that the CH\textsubscript{2}Cl\textsubscript{2}-MeOH extract of raw crushed \textit{Cavernularia} sp. had some toxicity in brine-shrimp assays (LC\textsubscript{50} 386 \(\mu\)g per ml at 24 h) and caused distress of guppy fingerlings (35–40 mm and 0.8–1.0 g) which sank and died in about 45–50 min. This observation further prompted the present investigators to carry out systematic chemical investigations of the aforesaid marine organism. This paper deals with the results of the said chemical investigation including the structural elaboration of interesting and novel marine metabolites cavernulin A (1) and B (2) which incidentally belong to cyclized cnembranoid or briarane (3) group of diterpenoids\textsuperscript{4}. Such briarane diterpenoids have earlier been reported\textsuperscript{3,4} from Gorgonacea (Genus : \textit{Briareum}\textsuperscript{5}, \textit{Solenopodium}\textsuperscript{6}, \textit{Erythropodium}\textsuperscript{7}, \textit{Junceella}\textsuperscript{8}, \textit{Gorgonella}\textsuperscript{9} and \textit{Menella}\textsuperscript{10}), Alcyonacea (soft coral) (Genus : \textit{Minabea}\textsuperscript{11}), Stolonifera (Genus : \textit{Tubipora}\textsuperscript{12}) and Pennatulacea (Genus : \textit{Ptilosarcus}\textsuperscript{13}, \textit{Tochuina}\textsuperscript{14}, \textit{Stylatula}\textsuperscript{15}, \textit{Scytalium}\textsuperscript{16}, \textit{Pteroides}\textsuperscript{17}, \textit{Cavernulina}\textsuperscript{18}, \textit{Veretillum}\textsuperscript{19,20}, \textit{Armina}\textsuperscript{20}, \textit{Renilla}\textsuperscript{21} and \textit{Funiculina}\textsuperscript{22}) and continue to attract the attention of investigators because of the structural complexity and wide range of biological activities, e.g. toxic\textsuperscript{22}, cytotoxic\textsuperscript{19}, antiinflammatory\textsuperscript{23}, antiviral\textsuperscript{24}, antiinsecticidal\textsuperscript{13}, antifouling\textsuperscript{21}, immunomodulatory\textsuperscript{25} and antibacterial\textsuperscript{9}.

Results and Discussion

The \textit{Cavernularia} species was thoroughly extracted with CH\textsubscript{2}Cl\textsubscript{2} and subsequently with CH\textsubscript{2}Cl\textsubscript{2}-MeOH (1 : 1). Chromatographic resolution (column and preparative thin layer chromatography) of the CH\textsubscript{2}Cl\textsubscript{2} extracted mass afforded wax esters (fatty esters of saturated fatty acids and of some analogous unsaturated fatty acids) (4), ceramides (5), cholesterol (6), 1-O-alkylglycerol (7) and also a novel cyclized cembranoid (briarane), cavernulin A (1), C\textsubscript{28}H\textsubscript{40}O\textsubscript{9}, \(R_{f} 0.35\) in CHCl\textsubscript{3}-MeOH (97 : 3), \([\alpha]_{D}^{20} 56.4^{\circ} (c 0.42)\). The CH\textsubscript{2}Cl\textsubscript{2}-MeOH (1 : 1) extract yielded another new briarane diterpenoid cavernulin B (2), C\textsubscript{28}H\textsubscript{40}O\textsubscript{9}, \(R_{f} 0.3\) in CHCl\textsubscript{3}-MeOH (95 : 5), \([\alpha]_{D}^{20} 50.5^{\circ} (c 0.20)\) together with small amounts of ceramides (5) as well as 1-O-alkylglycerol (7) as the polar components.

The IR spectrum (KBr) of cavernulin A (1) indicated the presence of hydroxyl (3400–3450 cm\textsuperscript{-1}) and five-membered lactone (1770 and 1220 cm\textsuperscript{-1}) functionalities. The...
detailed 1D (\(^1\)H-\(^{13}\)C-, homodecoupling) and 2D (COSY, NOESY and XHCCOR) NMR experiments of cavernulin A were in consonance with its formulation as 1. The \(^{13}\)C NMR spectrum of 1 showed 28 carbons (7 -t-, 11 -CH-, 10 -CH-, 4 -CH2- and 7 -CH3) signals (Table 1) in conformity with its molecular formula C\(_{28}\)H\(_{40}\)O\(_9\). Of the seven quaternary carbons, four (169.1, 170.2, 172.9 and 176.1) were for the ester and lactone carbonyl carbons and the rest for an olefinic, one oxygenated and one non-oxygenated carbons. Among ten methine carbon signals, four (70.7, 71.6, 77.8 and 81.1) were oxygenated, three olefinic (118.6, 120.4
and 142.3) and the remaining three (35.4, 39.3 and 42.3) non-oxygenated ones. The proton network in I was established by homodecoupling and $^1$H-$^1$H COSY experiments. Further, two-dimensional $^{13}$C-$^1$H correlation experiment on I optimized for one-bond C-H coupling allowed identification of the corresponding protonated carbon resonances correlating with the signal/s of the proton/s attached with that carbon.

The homodecoupling as well as COSY-90 experiments established that a doublet signal at $\delta$ 5.61 ($^1$H, $J$ 10.1 Hz)
was coupled with a double-doublet signal at δ 5.69 (1H, J 10.1 and 5.8 Hz) which in turn was coupled with a doubledoublet signal at δ 4.84 (1H, J 5.8 and 2.5 Hz). The latter signal was again coupled to a multiplet at δ 5.09 (1H) which was further coupled with a doublet at δ 1.03 (3H, J 7.4 Hz) as well as a doublet doublet at δ 2.68 (1H, J 5.5 and 3.7 Hz). The double-doublet at δ 2.68 was also coupled with a doublet at δ 5.25 (1H, J 5.5 Hz). The 1H signals at δ 5.61 and 5.69 correlating with CH resonances at 142.3 and 120.4 were thus for the olefinic proton resonances and 1H-1H coupling constant value (10.1 Hz) indicated the presence of cis-oriented double bond. Thus the aforesaid observations accounted for the presence of fragment-I in I.

\[
\text{-CH=CH(CH(OCOR))-CH(CH_3)}-\text{-CH-CH-OCO-}
\]

fragment-I

\[
\text{-CO-O-CH}_2CH_2H_bCH_2H_d^\text{-CO-O-CH-CH=C(CH_3)_2-CH-CH-OCO-}
\]

fragment-II

\[
\text{-O-CO-CH}-\text{CH}_3\text{-CO-CH}_2CH_2CH_3
\]

fragment-IV

It has further been noted that a broad doublet signal at δ 4.35 (1H, J 5.3 Hz) was coupled to a pair of geminally coupled multiplets at δ 1.62 (1H) and 2.75 (1H), both of which were further coupled with another pair of geminal protons resonating as multiplets at δ 2.03 (1H) and 2.52 (1H). These observations were commensurate with the presence of two adjacent methylene groups in compound I. The 13C-1H correlation experiment identified the carbon resonances associated with fragment-II of the compound at δc 81.1 (d), 32.4 (t) and 29.3 (t), respectively.

Again, homodecoupling and COSY spectra of I revealed that a broad doublet signal at δ 5.52 (1H, J 10.0 Hz) was found to couple to a doublet signal at δ 5.23 (1H, J 10.0 Hz) while the former signal was also weakly coupled to a vinyl methyl signal at δ 2.02 (br s). The XH-CORR experiment confirmed that the corresponding protonated carbon signals were at δc 118.6 (d), 77.8 (d) and 28.2 (q), respectively, in conformity with the presence of fragment-III, requiring the presence of a quaternary olefinic carbon atom which incidentally resonated at δc 146.9.

The 1H and 13C spectra also displayed signals assignable to fragment-IV as it was observed that a doublet signal at δ 1.15 (3H, J 7.2 Hz) coupling with a quartet signal at δ 2.42 (1H, J 7.2 Hz) in the 1H spectrum of I were linked to carbon resonances at δc 6.6(q) and 42.3(d), respectively. The upfield carbon resonance at δc 6.6 for the methyl group was an indication that the methyl group was attached to α-carbon of a five-membered lactone moiety.

A triplet at δ 0.93 (3H, J 7.5 Hz) coupled with a multiplet at δ 1.62 (2H) which in turn was further coupled to a triplet at δ 2.24 (2H, J 7.4 Hz). The signal positions indicated the presence of a butanoyl group (fragment-V) in cavernulin A (I). This was further confirmed by the 13C NMR spectrum of the compound I (Table I).

Besides, the compound also exhibited additional 1H signals for a quaternary methyl at δ 1.04 (3H, s) and two acetyl methyls at δ 2.06 (3H, s) and 2.18 (3H, s), and displayed additional carbon signals for a quaternary methyl [δc 16.1 (q)], acetate methyls [δc 21.0 (q), 21.6 (q)], quaternary carbon [δc 43.0 (s)], oxygenated quaternary carbon [δc 82.8 (s)], acetate carbonyls [δc 169.1 (s), 170.2 (s)] and lactone carbonyl [δc 176.1 (s)].

The aforesaid observations on cavernulin A were thus in conformity with the alternative structures I and 8. The gross structure as I and the position of three ester functions, i.e., two acetates and one butyrate group at C-2, C-9 and C-12, respectively, were, however, ascertained by accounting for the ion peaks observed in the mass spectrum of cavernulin A (I) and subsequently by its derivation from the congener diterpenoid cavernulin B (2) by the action of butyric anhydride and pyridine.

An ion peak at m/z 238 (40%) [ca. C13H18O4] (ion fragment a generated by RDA collapse of six-membered ring and simultaneous cleavage of allylic C(3)-C(4) bond) was clearly in conformity with the gross structure I and that the butyrate group could be present at C-2 or C-12. Thus an acetate function was present at C-9. Again an ion peak at m/z 265 (10%) [ca. C15H21O4] (ion fragment b formed by the cleavage of C(1)-C(2) and C(8)-C(9) bonds) further supported the gross structure I with the butyrate group present at C-9 or C-12. Consequently, the presence of butyrate group at C-12 would account for the formation of both the ion fragments a and b, and thus two acetate functions were at
C-2 and C-9, respectively. Similar ion fragments are not achievable from 8.

Reciprocative NOESY correlations of H-2, H-9, H-12, H-13, H-14, H-15, H-17, H-2'-2' and H-3'-3' with H-10, H-20, C-9-OAc as well as H-3', H-14, H-3'-5, C-9-OAc, H-3', H-3'-4', respectively, indicated their close proximity. Consideration of the coupling constants of the various \(^1\)H NMR signals of cavernulin A was helpful further in establishing its relative stereochemistry at the various asymmetric centres and thus it may be represented as \((1S^*,2S^*,5Z,7S^*,8R^*,9S^*,10S^*,11R^*,12R^*,17R^*)-2,9\text{-diacetoxy-12-

butanoyloxy-8-hydroxybriar-5,13-dien-1\text{8-one}}\) (1).

The IR spectrum (KBr) of cavernulin B (2) indicated the presence of hydroxyl (3400–3450 cm\(^{-1}\)) and five-membered lactone (1770 and 1225 cm\(^{-1}\)) functionalities. The \(^1\)H NMR spectrum of compound 2 clearly accounted for the various structural fragments present in the molecule. The presence of a \(\text{cis}-\text{double bond as part of the structural unit }-\text{CH=CH-CHOH-CH(CH\(_3\))CH-CH(OCOR)}, \text{-, similar to fragment-I in 1, was clearly discernible since a double doublet signal at } \delta 5.73 \text{ (1H, } J 10.1 \text{ and } 5.9 \text{ Hz) was found to couple with a doublet resonance at } \delta 5.42 \text{ (1H, } J 10.1 \text{ Hz) as well as with a double-doublet appearing at } \delta 3.85 \text{ (1H, } J 5.9 \text{ and } 1.7 \text{ Hz). Further, a multiplet at } \delta 2.10 \text{ (1H) was coupled to three signals resonating at } \delta 3.85 \text{ (1H, dd), } 0.96 \text{ (3H, d) and } 2.64 \text{ (1H, dd). The latter signal was also coupled to an oxygenated methine proton signal at } \delta 5.27 \text{ (1H, d). Thus this oxygenated carbon was attached with a quaternary system. The spectrum of compound 2 also showed a broad doublet at } \delta 4.39 \text{ (1H) and four multiplets at } \delta 2.74, 1.65, 2.52 \text{ and } 2.02 \text{ (1H each). The } \text{H}-\text{H} \text{ correlation study of compound 2 further indicated them to be for two adjacent methylene units. Further, the multiplets at } \delta 2.74 \text{ and } 1.65 \text{ for a set of geminal protons were coupled to the oxygen bearing methine proton resonating at } \delta 4.39 \text{ (br d). The } \text{C}-\text{H} \text{ correlation experiment identified the carbon resonances associated with the fragment-II of the compound 2 at } \delta c 81.2 \text{ (d), } 32.0 \text{ (t) and } 29.0 \text{ (t), respectively.}

Again, homodecoupling and COSY spectra of 2 revealed that a broad doublet signal at } \delta 5.52 \text{ (1H, } J 10.1 \text{ Hz) was found to couple to a doublet signal at } \delta 5.21 \text{ (1H, } J 10.1 \text{ Hz) while the former signal was also weakly coupled to a vinyl methyl signal at } \delta 1.97 \text{ (br s). The XHICORR experiment confirmed that the corresponding protonated carbon signals in 2 were at } \delta c 118.7 \text{ (d), } 77.9 \text{ (d) and } 28.4 \text{ (q), in conformity with the presence of fragment-III, requiring the presence of a quaternary olefinic carbon atom for which the singlet resonance at } \delta 1.146 \text{ is ascribable.}

The \(^1\)H and \(^{13}\)C spectra of 2 also displayed signals asignable to fragment-IV as it was observed that a doublet signal at } \delta 1.18 \text{ (3H, } J 7.2 \text{ Hz) coupling with a quartet signal at } \delta 2.37 \text{ (1H, } J 7.2 \text{ Hz) in the } \text{H} \text{ spectrum of 2 were linked to carbon resonances at } \delta 6.7 \text{ (q) and } 42.5 \text{ (d), respectively. The upfield carbon resonance at } \delta 6.7 \text{ for the methyl group was an indication that the methyl group was attached to a } \alpha\text{-carbon of a five-membered lactone moiety.}

Besides, the compound also displayed additional \(^1\)H signals for a quaternary methyl at } \delta 1.02 \text{ (3H, s) and two acetoxy methyls at } \delta 2.10 \text{ (3H, s) and } 2.18 \text{ (3H, s), and exhibited additional carbon signals for a quaternary methyl \([\delta c 15.9 \text{ (q)}, \text{ acetate methyls } [\delta c 21.2 \text{ (q), } 21.6 \text{ (q)}], \text{ quaternary carbon } [\delta c 43.0 \text{ (s)}, \text{ oxygenated quaternary carbon } [\delta c 82.6 \text{ (s)}], \text{ acetate carbons } [\delta c 169.1 \text{ (s), } 170.9 \text{ (s)}] \text{ and lactone carbonyl } [\delta c 176.7 \text{ (s)}].

Compound 2 had two acetate groups but its treatment with pyridine and acetic anhydride afforded compound 9 containing four acetates in conformity with the presence of two acetylable hydroxyl groups in 2. Upon acetylation the doublet of doublet signal at } \delta 3.85 \text{ in 2 underwent downfield shift of about } 1 \text{ ppm and appeared at } \delta 4.84 \text{ (1H, dd, } J 5.9 \text{ and } 2.6 \text{ Hz) in 9. Thus, one of the two hydroxy groups was attached with a methine carbon. The signal at } \delta 5.42 \text{ of compound 2 also shifted to } \delta 5.64 \text{ (d) on acetylation for the diamagnetic anisotropic deshielding effect of the newly introduced acetate carbonyl group. Other proton signals of compound 2 and its acetate 9 appeared almost at the similar positions. So, the second hydroxy group was attached with a quaternary carbon.}

Two different structures, viz. 2 and 10 would thus account for the various spectral characteristics described above for cavernulin B. Conclusive structural assignment was made through assignment the ion fragments observed in the mass spectrum of cavernulin B. The presence of ion peaks with \(m/z\) 281 \(\text{[C}_{15}\text{H}_{22}\text{O}_{3}]\), 280 \(\text{[C}_{14}\text{H}_{20}\text{O}_{3}]\), 222 \(\text{[C}_{13}\text{H}_{18}\text{O}_{3}]\), 196 \(\text{[C}_{11}\text{H}_{16}\text{O}_{3}]\), 169 \(\text{[C}_{9}\text{H}_{13}\text{O}_{3}]\), 167 \(\text{[C}_{9}\text{H}_{11}\text{O}_{3}]\) and 107 \(\text{[C}_{8}\text{H}_{11}]\) were commensurate with the structure 2 for cavernulin B.

The relative stereochemistry at the various asymmetric centres of cavernulin B was established with the help of a NOESY experiment and consideration of coupling constants of various \(^1\)H signals and construction of molecular model. Similar reciprocative NOESY correlations for cavernulin B as in cavernulin A (1) were observed except those for butyrate moiety present in the latter and in accordance with the stereostructure \((1S^*,2S^*,5Z,7S^*,8R^*,9S^*,10S^*,11R^*,12R^*,17R^*)-2,9\text{-diacetoxy-8,12-dihydroxybriar-5,13-dien-18-one}\) (2). A closely related briarane solenolide F (11) has previously been reported from \textit{Solenopodium} sp.
9, H-12, H-17 and H2-19 resonated at δ 3.64 (t, J 8.5 Hz), 4.86 (dd, J 5.8 and 1.4 Hz), 3.39 (q, J 7.2 Hz) and 1.18 (d, J 7.2 Hz) respectively.26

To our knowledge this is the first report of the occurrence of briaranes in the genus Cavernularia belonging to the order Pennatulacea.

Experimental

Column chromatography was carried out with silica gel (60–120 mesh) and TLC was performed on silica gel G plates. IR spectra (KBr) were recorded on a Perkin-Elmer 782 spectrophotometer. PMR, CMR, 2D-NMR (1H-1H COSY and XHCOcorr) spectra were recorded on a Bruker AM 300L supercon spectrometer equipped with ASPECT 3000 computer fitted with an array processor using programme version DISR87.1 or DISR94.1 in CDCl3 as solvent at 300.13 MHz for proton and at 75.47 MHz for carbon. The chemical shifts values are in δ (ppm) downfield from TMS. Standard procedures were used for two-dimensional NMR experiments. Optical rotations were measured with a Perkin-Elmer M5890, Series DISR87.1, or DISR94.1 in pyridine as solvent at 25°. Gas chromatographic experiments were done with a Hewlett-Packard M3394A using appropriate fractions afforded a novel cyclized cembranoid (1). The raw organism (6 kg) was treated with butyric anhydride (0.2 ml). The reaction mixture was warmed on a water-bath for a brief period and kept at room temperature for 24 h. It was then treated with CH3OH (1 ml) and after about 2 h the solvents were removed under reduced pressure. The product was purified by chromatography to afford cavernulin B diacetate (9; 2 mg) as colourless amorphous mass, Rr 0.35 in CHCl3-MeOH (97 : 3).

Cavernulin B (2) to cavernulin A (1): Cavernulin B (2; 3 mg) in pyridine (0.2 ml) was treated with acetic anhydride (0.2 ml). The reaction mixture was warmed on a water-bath for a brief period and kept at room temperature for 24 h. It was then treated with CH3OH (1 ml) and after about 2 h the solvents were removed under reduced pressure. The product was purified by chromatography to afford cavernulin A (1; 2 mg) identified by TLC and PMR spectral comparison with the natural specimen.

Acetylation of cavernulin B (2): Cavernulin B (2; 3 mg) in pyridine (0.2 ml) was treated with butyric anhydride (0.2 ml) at about 0° and the reaction mixture was kept as such for 20 h. It was then treated with methanol (1 ml) and after ~2 h the solvents were removed under reduced pressure. The product was purified by chromatography to afford cavernulin A (1; 2 mg) identified by TLC and PMR spectral comparison with the natural specimen.

Wax esters (fatty esters of fatty acids) (4): Colourless semisolid mass (40 mg). Rf 0.8 in light petrol-chloroform (20 : 80); δ 5.35 (0.3H, m, =CH), 4.05 (2H, t, J 6.9 Hz, H2-1’), 2.28 (2H, t, J 7.4 Hz, H2-2’), 2.01 (4H, m, =CH2CH2CH2). 1.60 (2H, m, H2-3’), 1.25 (br s, x CH2) and 0.90 (6H, t, J 6.6 Hz, 2 x CH2CH3); 13C NMR δ 14.0 (2 x CH3), 22.6 (2 x CH2CH3), 25.0 (C-3), 29.7 (x CH3), 32.0 (CH2CH2CH3), 34.4 (C-2), 64.4 (C-1’), 129.9 (=CH) and 173.9 (C-1).

Basic hydrolysis of wax ester fraction and extraction with ether gave the alcohol fraction which was converted into trimethylsilyl ether and analyzed by gas chromatography in
gradient mode (180–320°) over a column of SP 2100 (1.8 m × 2 mm glass column) with an increase of temperature at the rate of 10° per min and employing inlet temperature at 350°, outlet 380° and a flow rate of N₂ at 30 ml per min whereby the major trimethylsilylated alcohol components 16 : 0 (36.2), 17 : 0 (4.1), 18 : 0 (33.3) and 21 : a (10.5%) eluted out successively.

Acidification of the aqueous layer from the above hydrolysis and subsequent extraction with ether afforded fatty acid portion which was converted to its methyl ester (FAME) by treatment with diazomethane. Gas chromatography of FAME over a glass column (1.8 m × 2 mm) of 10% DEGS in liquid phase supported on 80–100 mesh chromosorb W (HP) isothermally at 196° employing inlet temperature at 250°, FID detector at 250° and nitrogen flow rate at 30 ml per min indicated the FAME composition mainly as 16 : 0 (25.7%), 18 : 0 (9.9) and 14 : 0 (11.7) for major components along with some minor ones including unsaturated components (<3% each) in 12.

**Cholesterol** (6) : White crystalline solid (80 mg), Rᵢ 0.5 in CHCl₃-MeOH (95 : 5); δHH 6.33 (1H, d, J 7.0 Hz, NH), 5.78 (1H, dt, J 15.4 and 6.4 Hz, H-5), 5.54 (1H, dd, J 15.4 and 6.1 Hz, H-4), 5.43 (1H, dt, J 15.2 and 5.9 Hz, H-9), 5.37 (1H, dt, J 15.2 and 5.1 Hz, H-8), 4.30 (1H, m, H-3), 3.95 (1H, m, H-2), 3.92 (1H, br d, J 9.9 Hz, H₆-1), 3.69 (1H, br d, J 9.8 Hz, H₆-3), 2.22 (2H, t, J 7.4 Hz, H₂-2', 2.09 (4H, m, H₂-6 and H₂-7), 1.96 (2H, m, H₂-10), 1.63 (1H, m, H₂-3'), 1.25 (huge, br s, x CH₂) and 0.88 (6H, t, J 6.8 Hz, 2 × –CH₂CH₃); δC 14.0 (2 × CH₃), 22.6 (2 × CH₂CH₃), 25.8 (C-3'), 29.5 and 29.7 (x CH₂), 31.9 (CH₃CH₂CH₃), 32.1 (C-7), 32.3 (C-6), 32.5 (C-10), 36.6 (C-2'), 54.9 (C-2), 63.5 (C-1), 74.4 (C-3), 129.0 (C-9), 129.4 (C-4), 131.4 (C-8), 133.5 (C-5) and 173.9 (C-1'). Methanolation of ceramide by formation in boiling in methanol-sulfuric acid and subsequent extraction of the diluted reaction mixture with ether afforded a FAME fraction with the composition m = 10 : 0 (2.6%), 12 : 0 (64.9), 12 : 1ω9 (2.3), 14 : 0 (9.9) and 14 : 1ω9 (5.3) (cf. 5) for major components along with some minor ones.

**Cholesterol** (6) : White crystalline solid (80 mg), m.p. 148°, Rᵢ 0.5 in CHCl₃-MeOH (98 : 2); [α]D + 39°; δHH 5.34 (1H, br d, J 4.6 Hz, H-6), 3.52 (1H, m, H-3), 1.03 (6H, s, H₂-18 and H₂-19), 0.97 (3H, d, J 3.7 Hz, H₆-2') and 0.84 (3H, d, H₆-26) and 0.68 (3H, d, H₆-21); δC 11.7 (C-18), 18.7 (C-21), 19.5 (C-19), 21.0 (C-11), 22.6 (C-26), 22.7 (C-27), 23.8 (C-23), 24.4 (C-15), 28.1 (C-16), 28.2 (C-25), 31.6 (C-2), 31.9 (C-7 and C-8), 35.8 (C-20), 36.2 (C-22), 36.4 (C-10), 37.2 (C-1), 39.4 (C-24), 39.7 (C-12), 42.2 (C-4 and C-13), 50.5 (C-9), 56.1 (C-17), 56.8 (C-14), 71.8 (C-3), 121.5 (C-6) and 140.7 (C-5).

**1-O-Alkylglycerol** (7) : Colourless semisolid mass (20 mg); Rᵢ 0.4 in CHCl₃-MeOH (95 : 5); δHH 5.60 (m), 3.84 (m, H-2), 3.69 (1H, dd, J 11.4 and 3.0 Hz, H₆-3), 3.61 (1H, dd, J 11.3 and 5.5 Hz, H₆-3), 3.52 (1H, dd, J 9.1 and 4.1 Hz, H₆-1), 3.49 (1H, dd, J 9.7 and 5.8 Hz, H₆-3), 3.45 (2H, t, J 6.2 Hz, H₂-1'), 2.14 (m, 2 × OH), 1.54 (m, H₂-2'), 1.25 (br s, x CH₂) and 0.87 (3H, t, J 6.4 Hz, CH₂CH₃); δC 35.4 (CH₃), 22.6 (CH₂CH₂CH₃), 24.1 (C-2'), 29.6 and 29.7 (x CH₂), 31.9 (CH₃CH₂CH₃), 64.2 (C-3), 70.6 (C-1), 71.9 (C-1') and 72.4 (C-2'). The material 7 was converted to ditrimethylsilyl ether 12 and resolved over a glass column (1.8 m × 2 mm) of 3% OV 17 in liquid phase supported on 80–100 mesh chromosorb W (HP) in gradient fashion (180–330°) with increase of temperature at the rate of 10° per min, employing inlet temperature 350°, detector at 380° and flow rate of N₂ at 30 ml per min, whereby the presence of major components with m = 8 : 0 (3.0%), 10 : 0 (7.5), 12 : 0 (41.4), 13 : 0 (8.8), 14 : 0 (25.3) and 15 : 0 (3.0) was noted along with some minor ones including unsaturated components (<3% each) in 12.

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