New lipid metabolites from a new *Sinularia* species soft coral

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A new monohydroxysterol, 22,23-dimethyl cholest-5-en-3β-ol (1) has been identified in the monohydroxysterol fraction and two new ceramides (2 and 3) have been isolated from the EtOAc-solubles of a new species of *Sinularia* soft coral, collected from the Andaman and Nicobar Islands. Their structures have been deduced from spectral data, and in the case of 1, by GLC and GC-MS analysis also.

As part of our studies on the isolation and structure elucidation of new polyhydroxysterols1, steroidal glycosides2-4, sphingosine derivatives3-5, cerebroside6, lipid glycosides7,8 and diterpenoids9 from the soft corals of the Andaman and Nicobar Islands, we have examined a new species of *Sinularia* collected at Hori Island, and the results are reported here. The soft coral was extracted with ethanol and the residue from the ethyl acetate soluble portion of the ethanolic extract on extensive chromatography over silica(SiO2)gel with solvents of increasing polarity from n-hexane through EtOAc gave ethyl arachidonate9, 11, 12-epoxyisoneocembrene-A3, a monohydroxysterol mixture, a novel norditerpenoid10, and two new ceramides, compound-B (2) and compound-C (3).

**Results and Discussion**

The monohydroxysterol fraction was found to be homogeneous over silica gel thin layers. It was crystallized from methanol as colourless crystals, m.p. 186–188°. It gave positive Liebermann-Burchard test, characteristic for sterols. Its IR spectrum showed a broad band at 3500 cm⁻¹ for hydroxyl absorption. However, its 1H NMR spectrum showed more secondary methyl signals than expected for a cholestanol or ergostane type steroid, and duplicated carbon chemical shifts found in its 13C NMR spectrum also suggested that it could be a mixture of monohydroxysterols and hence it was acetylated. The sterol mixture formed a monoacetate with Ac₂O/pyridine at room temperature for 24 h and the resultant product upon crystallization from methanol gave colourless plates, m.p. 148–150°. IR spectrum of the acetyl derivative showed the lack of hydroxyl absorption, indicating it to be the acetate of monohydroxysterols. The acetate showed several close running spots on
AgNO₃ impregnated TLC plate. Thereby, it was regarded as a mixture of monohydroxysterols and was subjected to GLC and GC-MS analysis. It was found to be a mixture of seven sterol acetates of cholest-5-en-3β-ol (24%), 24-ethylcholest-5-en-3β-ol (lathosterol) (16%), 24-methylcholest-5,25-dien-3β-ol (25%) and cholest-7-en-3β-ol (11%), 24-methylenecholest-5,23(24)-dien-3β-ol (14%) and unknown sterol, compound-A (35%) (1). Comparison of their individual mass fragmentations and the relative retention times with reported data in the literature, led to the identification of these sterols. These are listed in Table 1 with their characteristic relative retention times (RRₜ) and major mass fragmentation ions.

**Compound-A**, is the major constituent of the sterol mixture (35%). Its molecular formula was found to be C₃₁H₅₂O₂ from the ion at m/z 396 (M⁺-AcOH) in its mass spectrum. The prominent peaks at m/z 255 (M⁺-AcOH-side-chain(C₁₀H₂₁) (70%)) and 120 (25%) were due to cholest-5-ene nucleus. The saturated 22,23-dimethylenecholane side-chain was deduced from the fragment ions at m/z 296 [M⁺-AcOH-C₁₀H₁₃-CH₃(10%)] due to the cleavage of C-22-C-23 bond and ion at m/z 281[M⁺-AcOH-C₈H₁₇-2H(25%)] due to the cleavage of C-20-C-22 bond. Therefore, the structure was assigned as 3β-acetoxy-22,23-dimethylenechol-5-ene (1) to compound-A. It is a new addition to the literature of monohydroxysterols. The monohydroxysterol was a new sterol based on its retention time different from other known sterols and the tentative structure was derived from the mass spectral fragmentation. The mass spectral fragmentation of compound-A is shown in Chart 1; its relative retention time and fragment ions are shown in Table 1.

**Compound-B**, m.p. 130–132° was analyzed for C₃₄H₆₅NO₃ and its IR spectrum showed strong bands for hydroxyl (3350, 1040) and secondary amide (1640, 1540), in addition to trans-double bond (970) and an aliphatic chain (2918, 2851, 1453 cm⁻¹), suggesting it to be a fatty acid amide. Furthermore, compound-B was found to possess normal types of side-chains, since the carbon atom signals due to terminal methyl groups were observed at δ14.1 (normal form) in the ¹H NMR spectrum of compound-B (Table 2). The existence of an unbranched fatty acid and an unbranched long-chain base was also suggested by its ¹H NMR spectrum. The ¹H NMR spectrum (90 MHz, pyridine-d₅, TMS standard) of compound-B showed the presence of a very broad singlet at δ 1.25 (aliphatic methyls), signals for two terminal methyl groups belonging to fatty acid and sphingosine components at δ 0.81 (6H) and showed δ 12.12 (2H), two hydroxymethylene protons (δ 3.95 and 4.32) and one hydroxymethine proton (δ 4.36). It also showed the presence of four olefinic protons at δ 5.45 [(dd, J = 15, 7.0 Hz), 5.50 (dt, J = 15, 6.5 Hz)] and 5.70 (2H, m), three multiplets at δ 1.55, 1.98 and 2.05 due to methylene groups adjacent to double bonds and a downfield proton signal at δ 8.20 (amide NH) which was exchangeable with D₂O suggesting the compound to be a fatty acid amide of a sphingosine derivative. The ¹³C NMR spectrum (Table 2) showed a carbonyl carbon signal at δ 175.6 (s) and car-
bon bearing NH functionality at δ 54.5 (d) indicated the presence of a secondary amide group. Further, it displayed four sp² carbon signals at δ 132.1 (d), 131.9 (d), 131.0 (d) and 129.8 (d) and two hydroxyl bearing carbon signals at δ 61.8 (t) and 72.5 (d), revealing the presence of a dihydroxydiene system in 2. The signals of polymethylene fragments were observed at δ 36.6-20.7, while that of terminal methyl groups at δ 14.1 (q). From the above spectral data it was found that compound-S belongs to a ceramide of the N-acylsphingadienine class.

The acyl chain and a sphingadienine part of compound-S were deduced from EI-mass spectroscopy. The EI-MS showed a molecular ion at m/z 535 consistent with the molecular formula C₃₄H₆₅N₃O₃. The prominent mass fragment ion at m/z 298 [M⁺-237(C₁₆H₂₉O) (40%)] due to allylic cleavage¹⁸ and fragment ion as base peak at m/z 281(71%) suggested the presence of C₁₆-acyl side-chain, and the sphingadienine moiety must be a C₁₈-unit. The mass spectral fragmentation of compound-B is shown in Chart 2. The stereochemistry of the C-4/C-5 double bond was assigned as trans by the large vicinal coupling constant (J 15 Hz) displayed between H-4 and H-3 and the other two olefinic protons showed a multiplet which indicates the trans(E)-configuration of the two vinyl groups¹⁹ and also the ¹³C NMR chemical shifts of the methylene groups adjacent to the (C-1₀) trans isomer (δ 32-33)₂⁰. The presence of two double bonds at C-4 and C-1₀ was deduced mainly from its ¹³C NMR data (Table 2) by analogy with compounds found in literature⁵,²¹.

Komori et al.²¹ isolated seven new cerebrosides from
Table 2. \(^1\)H and \(^{13}\)C NMR data (\(\delta\) ppm) for compounds 2 and 3 in C\(_2\)D\(_5\)N

| Position | \(^1\)H (mult. \(J\), Hz) | \(^{13}\)C (mult.) \(^{23}\) | \(^1\)H (mult. \(J\), Hz) | \(^{13}\)C (mult.) \(^{23}\) |
|----------|-----------------|-----------------|-----------------|-----------------|
| Long chain base (LCB) | | | | |
| 1a       | 3.95 (dd, 12, 3) | 61.8 (t) | 3.98 (dd, 12,3) | 61.9 (t) |
| 1b       | 4.32 (dd, 12, 6) | 54.5 (d) | 4.28 (dd, 12, 4) | 52.9 (d) |
| 2        | 4.05 (m) | 72.5 (d) | 4.60 (m) | 76.5 (d) |
| 3        | 4.36 (m) | 132.1 (d) | 4.70 (m) | 72.4 (d) |
| 4        | 5.45 (dd, 15, 7) | 131.9 (d) | 5.85 (m) | 130.7 (d) |
| 5        | 5.50 (dt, 15, 6.5) | 36.6 (t) | 5.85 (m) | 130.6 (d) |
| 6        | 2.05 (m) | 29.9 (t) | 2.10 (m) | 33.9 (t) |
| 7        | 1.98 (m) | 29.8 (t) | 1.94 (m) | 29.9 (t) |
| 8        | 32.0 (t) | 32.0 (t) | 29.5 (t) | 22.8 (t) |
| 10       | 5.70 (m) | 131.0 (d) | 131.1 and 129.9 for 10,11-double bond. Naturally occurring sphingosine-type long-chain bases possess, 2\(S\),3\(R\)(d-erythro) configurations. Based on the above spectral data, the structure \(N\)-hexacosanoyl-1,3-dihydroxy-2-amino-4,10-octadecadiene (2) is assigned to compound-B, which to the best of our knowledge is a new addition to the literature of ceramides.

\textbf{Compound-C}, had m.p. 144–145\(^\circ\) and analyzed for C\(_{34}\)H\(_{67}\)O\(_3\)N by a combination of EIMS and \(^{13}\)C NMR data. It showed IR bands at 3350 (OH) and 1640 (amide) in addition to trans-double bond (970 cm\(^{-1}\)). Compound-C was found to possess normal\(^{17}\) types of side-chains since the carbon signals due to terminal methyl groups were observed at \(\delta\) 14.1 (normal form) in the \(^{13}\)C NMR spectrum of compound-C (Table 2). The existence of an unbranched fatty acid and an unbranched long-chain base was also suggested by its \(^1\)H NMR spectrum. A carbonyl carbon signal at \(\delta\) 175.5 (s) in the \(^{13}\)C NMR spectrum, a downfield proton signal at \(\delta\) 8.55 (d, J 9.0 Hz) in the \(^1\)H NMR spectrum and a strong IR band at 1640 cm\(^{-1}\) indicated the presence of a secondary amide group. A very strong signal at \(\delta\) 1.25 in
the $^1$H NMR spectrum and lack of upfield methyl signals in the $^{13}$C NMR spectrum revealed that compound-C must be derived from a long-chain fatty acid precursor.

The $^1$H NMR spectrum (90 MHz, pyridine-$d_5$, TMS standard) of compound-C (Table 2) showed the presence of terminal methyl groups belonging to fatty acid and sphingosine components at $\delta$ 0.90 (6H, t, $J$ 6.0 Hz). It also exhibited the presence of characteristic signal at $\delta$ 1.25 (br s) for aliphatic methylenes, two oxymethylene protons at $\delta$ 3.98 (dd, $J$ 12.0, 3.0 Hz), 4.28 (dd, $J$ 12.0, 4.0 Hz), a complex pattern of multiplets at $\delta$ 3.48-4.70 integrating for four protons, three multiplets at $\delta$ 2.10, 1.94 and 1.72 due to methylene groups adjacent to double bond and hydroxyl group and an amide NH signal at $\delta$ 8.55 (1H, d, $J$ 9.0 Hz) and two olefinic protons appearing as multiplet at $\delta$ 5.85. The above data suggested that compound-C was a fatty acid amide of a monounsaturated sphingosine derivative.

The $^{13}$C NMR spectrum of compound-C (Table 2) showed the presence of an amide functionality at $\delta$ 175.5 (s) and 52.9 (d), two olefinic carbon signals at $\delta$ 130.7 (d), 130.6 (d), four oxygenated carbon signals at $\delta$ 61.9 (t), 76.5 (d), 72.4 (d) and 72.9 (d), the signals of polymethylene fragments at $\delta$ 22.8 (t) -33.9 (t), and the signals of two terminal methyl carbons at $\delta$ 14.1 (q). The methylene carbon signal at $\delta$ 35.5 (t) suggested the presence of a 2'-hydroxyacyl side-chain. The above spectral data, a monounsaturated 1,3,4-trihydroxyphosphogine moiety in compound-C was deduced. The olefinic carbon signals at $\delta$ 130.7 (d), 130.6 (d) suggested the presence of 5,6-double bond by analogy of similar carbon signals [$\delta$ 130.3 (d), 130.1 (d)] in astrocerbrosiche-A isolated from *Astropecten latepsinosus*.

The two olefinic protons showed a multiplet which indicates the trans(E)-configuration of the two vinyl groups.

The $^1$H NMR and $^{13}$C NMR (Table 2) data of compound-C was almost identical with that of N-(2'-hydroxyicosanoyl)-1,3,4-trihydroxy-2-amino-7-ene (4) isolated from *Sinularia gravis*. The molecular ion was observed at $m/z$ 569 in EI-MS spectrum and in the latter, the [M + H] ion at $m/z$ 612 suggested the presence of a 18 carbon unit and the fatty acid chain possesses a 16 carbon unit. Therefore, the structure of compound-C was established as N-(2'-hydroxyhexacosanoyl)-1,3,4-trihydroxy-2-amino-octadeca-5-ene (3) and is also a new addition to the literature of ceramides. The mass spectral fragmentation of compound-C is given in Chart 3.

**Experimental**

M.ps. were determined on a VEB-analytic Dreader HMK hot plate and are uncorrected. IR spectra (KBr/CHCl$_3$) were recorded on a Perkin-Elmer 841 spectrophotometer, UV spectra (MeOH) on a Shimadzu 160A spectrophotometer in methanol, $^1$H NMR spectra on a Jeol JNM EX-90 (90 MHz) and $^{13}$C NMR spectra (pyridine-$d_5$/CDCl$_3$) on Jeol JNM EX-90 spectrometer (22.5 MHz) using TMS as internal standard. GC-MS analysis was provided by R.S.I.C., Lucknow. The analysis was done on a Shimadzu QP-2000 instrument at 70 eV using ULBON HR-I equivalent to OV-17, fused silica capillary column (0.25 mm x 50 M) with film thickness 0.25 micron under programmed conditions of 222-10$^6$/min-250°C, with carrier gas nitrogen flowing at 18 ml/min. The detector was FID. The GLC analyses were done with and without the addition of cholesteryl acetate. Silica gel (100-200 mesh) was used for column chromatography and silica gel-G (Acme) for TLC. All the spots were visualized by spraying 5% sulfuric acid in methanol.

**Collection, extraction and isolation:** The soft coral was collected at Hori Island, Diglipur (93°02' E, 13°30' N) of the Andaman and Nicobar Islands by hand-picking in the intertidal rocky region during March 1993, and identified as *Sinularia* species by Dr. B. Grebnev, Biologist, PIBOC, Russia. The voucher specimen was deposited at PIBOC, Russia and at Department of Organic Chemistry, Andhra.
water, soaked in ethanol. The extraction was carried out using dark coloured residue was extracted with ethyl acetate several times. The ethyl acetate soluble portion was washed with distilled water and dried over anhyd. MgSO₄ and concentrated under vacuum. The residue (40 g) was chromatographed over a column of silica gel with distilled water and dried over anhyd. MgSO₄ using eluants with increasing polarities of solvent mixtures starting from pet. ether, ethyl acetate to methanol (9:1) and ethyl arachidonate mixture of inseparable monohydroxysterols. The ethyl acetate mixture was subjected to GLC-MS analysis. The authors thank Dr. B. Grebnev, Biologist, PIBOC, Russia, for the identification of the coral. The authors thanks are also due to the following: C.S.I.R., New Delhi, for the award of a Research Associateship to one of them (P.R.); U.G.C., New Delhi for the COSIST and DRS programme facilities at Andhra University, Visakhapatnam; Head, R.S.I.C., Lucknow; Chief Wildlife Warden of the Andaman and Nicobar Islands for his cooperation and assistance in collection of the soft coral; Prof. C. Subrahmanyan, Research Supervisor of P.R. and Prof. V. Anjaneyulu for his helpful suggestions.

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