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CERAMIDES FROM MARINE ORGANISMS AND RECENT DEVELOPMENTS ON CERAMIDES

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Abstract

Several ceramides and cerebrosides were reported to have incredible bioactivity. Some of them were claimed to be quite effective in AIDS and HIV related diseases and also for the treatment of disorders (e.g. multiple sclerosis) caused by demyelination while others act as antileukemic, SR Ca$^{2+}$ ATP-ase activators, antihypertensive, coronary vasodialators, histidine carboxylase inhibitors, etc.

In recent years ceramides and cerebrosides were found in the extracts of some marine organisms such as algae (red and green), symbiotic microalgae, sponge, anemone and starfish. Our efforts in search for bioactive components from marine specimens of the Eastern coast of the Bay of Bengal adjoining West Bengal, India provide soft coral, clams, sea worm and holothuroid as new sources for a few interesting ceramides and cerebrosides. The soft coral Nephthea sp. and a sea worm (Neris sp.) afforded (+)-erythro-2-amino-4E,8E-octadecadiene-1,3-diol N-palmitate while the clams Solen brevis and Barnea candida gave 2-amino-4E-octadecaene-1,3-diol N-palmitate. Chemical investigation on the holothuroid Thorsonia investigatoris furnished a pair of glucocerebrosides derived from 2-amino-4-alkene-1,3-diol and α-hydroxy fatty acid components. Further, one of them had an isopropyl moiety on one end of the two fatty tails.

Introduction

Since the advent of human civilization two basic needs for its survival have been food to provide energy to sustain life and drugs to alleviate pain. The requirements mostly came from terrestrial sources and occasionally the sea supplementing with. As the 20th century man continues to search for better ways of satisfying
them, the Oceans have been and are being explored more and more for meeting up their demands.

The dependance on and expectations from the Ocean is elegantly reflected in the Indian legend *Churning of the Sea* in search of the *nectar of immortality*. In the process was also obtained the deadly venom as a bye-product (from the mouth of the snake-king Vasuki) which is now known to contain toxins useful for the treatment of various ailments. Thus man's awareness of the possible biomedical benefits from the sea is not actually new. It is not uncommon, for example, to find prescriptions in the ancient system of medicine which contained pearl\(^1\), shells\(^1\) (e.g. conch and cowri) in powdered form or parts of marine animals (e.g. coral\(^1\)) and plant (sea weed\(^2\)) in the treatment of various ailments. Compared to the vastness of the Oceans and the numerous organisms available therein the number of marine organisms utilized in traditional system of medicine are, however, too few. The lacuna could be due to their inaccessibility, difficulty in preservation without deterioration, i.e. affecting composition, and processing. Consequent upon the awareness of finding new leads from the Ocean intensive investigations on organisms therefrom have started on a global basis.

**Marine Organisms as Sources of Ceramides and Cerebrosides**

Chemical investigations on marine organisms have indeed yielded a wide array of extractives\(^3-6\) having diverse structural patterns and bioactivity. Incidentally ceramides have been reported in more recent years from some marine organisms such as the red algae *Amansa glomerata*\(^7\) and *Laurencia nitida*\(^7\), the green algae *Caulerpa racemosa*\(^8,9\) and *Ulva fasciata*\(^10\), the sea anemone *Anemonea sulcata*\(^11\) and the microalgae *Symbiodinium* sp.\(^12\) while cerebrosides from the starfishes *Acanthaster planci*\(^13-15\) and *Asterias amurensis*\(^16\) and the sponges *Halichondria japonica*\(^17\), *Halichondria panicea*\(^18\) and *Chondropsis* sp.\(^19,20\). It may be recalled that sphingosine (2-amino-4-alkene-1,3-diol or sphingenine), sphinganine (dihydrosphingosine), sphingadienine (2-amino-4,8-alkadiene-1,3-diol), their amides with fatty acids of 12 to 26 carbon atoms (known as ceramides) and closely related compounds cerebrosides (ceramides 1-glycosides) and sphingomyelins are important membrane components in both plant and animal cells, and that they appear to participate in cell regulatory functions and transmembrane signaling.
Ceramides (1 : m = 13,15,17,22,23,24) from the red algae Amansia glomerata and Laurencia nitida were derived from the usual C\textsubscript{18} sphingosine base and were present as intimate mixtures as the acyl moiety varied in the chain lengths. L. nitida also afforded \((+)-25\text{-}N\text{-}acetamidomethyl-3\beta\text{-}acetoxyoctadecanol. However, the green algae Caulerpa racemosa elaborated ceramides 1 \((m = 13, 15, 21, 23)\) derived from sphingosine bases and 2 \((m = 17, 19, 21, 23, 25)\) derived from sphinganine bases.

The sponge Halichondria japonica gave metabolites 3-5 where sphenamine moiety had an isopropyl tail. Halichondria panicea, on the other hand, yielded a pair of galactocerebrosides 6 and 7 derived from different sphenamine bases. Interestingly one of them had also an isopropyl tail but this time on the acyl portion.

The spermatozoa of the starfish Asterias amurensis yielded three groups of cerebrosides - gentiobiosyl, lactosyl and cellobiosyl ceramides. The compositions of the acyl portion \((14h:0, 15h:0, 16h\text{-}0, 18h:0, 24h:1; \text{h}=\text{hydroxylated})\) as well as of the basic unit \((\text{d}18:2, \text{d}19:3, \text{t}22:1, \text{d}=\text{dihydroxy}, \text{t}=\text{trihydroxy})\) of the ceramide part were similar. Studies on the glycosphingolipids from the starfish Acanthaster planci gave six new glucocerebrosides 8-13 and two new ceramide lactosides 14 and 15.

Bioactivity

Studies on ceramides have, however, gained considerable momentum lately (last 5-6 years) as they attracted not only the chemists for their structural diversity but also the biologists and the pharmacologists alike because of their varied and strong bioactivity.

Crude ethanol extract of Ulva fasciata showed in vitro and in vivo antiviral activity against Semeliki forest virus (at 20mg/mouse/7 days) by giving 50% protection. Test made against Encephalomyocarditis Virus with the purified material 16 from U. fasciata exhibited antiviral activity at 1.56 \(\mu\text{g}\) well concentration (in vitro) and at 0.5 mg/mouse/3 administration (in vivo) giving 50% protection.

Symbioramide 17 isolated from the cultured dinoflagellate Symbiodinium sp. (a symbiotic microalgae found in the inside gill cells of Okinawa bivalve Fragum sp.) activated at \(10^{-4}\text{M}\) concentration SR Ca\textsuperscript{2+} ATP-ase activity by 30%. It also exhibited antileukemic
1. \[
\text{NHCO(CH}_2\text{)}_m-\text{H}
\]
\[
\text{H}_0\text{C}_c\text{Hz)}_n-\text{H}
\]
\[
\text{OH}
\]

3. \(m = 19, R = \text{CHOH(CH}_2\text{)}_{11}\text{CHMe}_2\)

4. \(m = 19, X = -(\text{CH}_2\text{)}_{11}^-\)

8. \(m = 21, R = \text{CHOH(CH}_2\text{)}_{11}\text{CH}_3\)

9. \(m = 13, R = \text{CHOH(CH}_2\text{)}_{17}\text{CH}_3\)

10. \(m = 13, R = \text{CHOH(CH}_2\text{)}_{9}\text{CH}_3\text{C}_8\text{H}_17\)

11. \(m = 11, X = \text{C}_2\text{H}_4\text{N}_2, R = \text{CH}_3\)

12. \(m = 19, X = \text{CH}_2\text{CH}_2^-, R = \text{H}\)
11 \( m = 17, R = H, R' = -\text{CH}_2\text{CH}_2\text{CH}_3 \)
12 \( m = 18, R = H, R' = -\text{CH} = \text{CH}_2\text{CH}_3 \)
13 \( m = 19, R = H, R' = -\text{CH} = \text{CH}_2\text{CH}_3 \)
26 \( R = H, R' = -(\text{CH}_2)_n\text{CH}_3 \)
27 \( R = \text{CH}_3, R' = -(\text{CH}_2)_n\text{CH}_3 \)
28 \( R = H, R' = -(\text{CH}_2)_n\text{CH}(\text{CH}_3)_2 \)
14 \( R = -(\text{CH}_2)_{21}\text{CH}_3 \)
15 \( R = -(\text{CH}_2)_{13}\text{CH}_3 \)
22 \( R = \text{C}_{22}\text{H}_{45}, R' = -\text{C}_{12}\text{H}_{25} \)
16 \( R = -(\text{CH}_2)_i\text{CH}_3 \)
17 \( R = -(\text{CH}_2)_8\text{CH} = \text{CH}_2\text{CH}_8\text{H}_{17} \)
23 \( R = \text{H} \)
24 \( R = \text{COCH}_3 \)
activity against L1210 murine leukemia cells in vitro with an LC₅₀ value of 9.5 μg/ml.

The galactosyl ceramides 18 (n = 8,9) isolated from the sponge Chondropsis sp. were useful as coronary vasodilators, anti-hypertensive and histidine carboxylase inhibitors. The cerebrosides B₁₈ (19:8E isomer) as well as B₁₉ (19:8Z isomer) from Tetragonaria tetragonoides were found to have antiulcerogenic activity.

Virucides, useful for the treatment of AIDS and HIV-related diseases, contain ceramide as active ingredient. Galactosyl ceramides 2',3,3',4',6'-pentasulphate (from 20 : R² = R³ = SO₃H) at 30 μg/ml inhibited HIV but showed no cytotoxicity. The antibodies against galactosyl ceramide 21 also inhibit the entry of HIV-1 in neural cell lines. Lactosyl ceramide 22 which is non-toxic is useful in the treatment of disorders caused by demyelination. It inhibited at 4 mg/kg encephalomyelitis in rats more strongly than cyclosporin. Ceramide 2 (m = 17) stimulates epidermal growth factor (EGF) receptor phosphorylation in A431 human epidermoid carcinoma cells. There is evidence that ceramide may mediate sphingosine action.

Ceramides and Cerebrosides from New Sources

During our search for bioactive components we investigated some marine organisms of the Eastern coast of the Bay of Bengal adjoining West Bengal, India. Chemical studies of a soft coral (Nephthea sp.) gave several ubiquitous compounds along with a new ceramide 23. The compound 23 showed amide, hydroxyl and trans-1,2-disubstituted olefin bands in the IR spectrum but showed no characteristic UV absorption. Presence of two hydroxyl groups in the molecule was established through acetylation yielding a diacetate. The changes in the ¹H NMR spectrum observed thereupon further established the presence of one -CH₂OH and one -CHOH groups in 23. The carbon-13 spectra of 23 and diacetate 24 were in consonance with the presence of two straight chain systems and also for two -CH=CH- units. The trans orientation of the -CH=CH- units were, however, established from ¹H NMR spectroscopy. Homodecoupling, COSY and ¹³C-¹H correlation experiments on 23 and the diacetate further established the presence of -COCH₂(CH₂)mCH₃ and HOCH₂-CH-CH(OH)CH=CHCH₂CH₂CH=CH-(CH₂)nCH₃ units in 23. Laser NH-CO induced positive as well as negative ion fast atom bombardment (FAB) mass spectrum of 23 established the molecular formula as
C_{34}H_{65}O_{3}N and the fragmentation pattern observed established the length of the acid as C_{16}. The compound 23 has two asymmetric centres and was found to be optically active (dextrorotatory). The relative stereochemical orientation of the molecule was established as erythro through acetonide 25 formation and subsequent $^1H$ NMR spectral study. Compound 23 was also obtained from a sea worm (Neris sp.). The compound 23 isolated by us was isomeric with the ceramide isolated from the sea anemone Anemonia sulcata 21 which had C_{12}-acid part and C_{22}-sphinga-4,8-dienine unit.

A related compound 1 (m = 15) was also found by us in both a razor clam (Solen brevis) and a pholad clam (Barnea candida) and the structure was established by usual spectral techniques like $^1H$ NMR, COSY and homodecoupling experiments and FAB mass spectroscopy.

Chemical investigations of the holothuroid Thorsonia investigatoris resulted in the isolation of a pair of new glucocerebrosides 26 and 27 (or 28) with the ceramide part derived from 2-amino-4-alkene-1, 3-diol and $\alpha$-hydroxyfatty acid/s. The detailed $^1H$ spectral study (including $^1H$-$^1H$ COSY and homodecoupling) of their hexaacetates showed them to possess a $\alpha$-D-glucopyranoside unit, a $\alpha$-OCH$_2$CH$_2$NH-CH(0Ac)-CH=CH-CH$_2^-$ system and a $\alpha$COCH(0Ac)CH$_2^-$ moiety. Interestingly one of them incorporated $\alpha$-CH$_2$CH$_3$ units at the end of the non-polar tails with the methyls appearing at $\delta_e$ 14.3 in the carbon spectrum of the hexaacetate. The other compound had a $\alpha$-CH$_2$CH$_3$ group at the end of one fatty chain (CH$_3$ $\delta_e$ 14.0) and a $\alpha$-CH(CH$_3$)$_2$ group at the end of other tail($\delta_e$ 23.0). The observations were in conformity with structures 26 and 27 (or 28) for the parent compounds. The chain lengths of the tails are yet to be ascertained.

The crude extract of the Nephthea sp. was found to be quite promising as antiviral agent in in vitro (Ranikhet disease virus and Vaccina virus) and in vivo (Vaccina virus) tests. The crude extract of T. investigatoris as well as the fraction containing 26 and 27 showed some toxicity in brine-shrimp assays. The bioactivity of pure 23, 26 and 27 are yet to be evaluated.

Conclusion

As ceramides and cerebrosides are found to be promising bioactive molecules and also quite viable as membrane constituents, the marine organisms providing them may serve us in fighting the
dreadful maladies of mankind. There is also a distinct possibility that the marine biota would act as valuable leads to future traditional system of medicines.

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Chemical Transformations of 6α-Acetoxyazadirone and Two-dimensional NMR Studies on Tetranortriterpenoids†

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Basic hydrolysis of the tetranortriterpenoid 6α-acetoxyazadirone yielded 6α-hydroxydeacetylazadirone which on preferential acetylation in the cold afforded 6α-hydroxydeacetylazadirone. Treatment of 6α-acetoxyazadirone with hydrogen peroxide-selenium dioxide gave 6-O-acetylisonomolide which was a constituent of Chisocheton paniculatus and it underwent acetylation with acetic anhydride-pyridine to 6,21-di-O-acetylisonomolide. Careful hydrogenation of 6α-acetoxyazadirone gave 1,2-dihydro as well as 1,2,20,21,22,23-hexahydro derivatives which were hydrolysed under basic conditions to the corresponding 6,7-diol. 6α-Hydroxy-1,2-dihydrodeacetylazadirone so derived furnished melden, a minor phytoconstituent of Melia azadirachta, on preferential acetylation in the cold and a triol upon NaBH₄ reduction. Acetylation of the latter afforded a triacetate. The structures and nmr assignments of the compounds were settled from two-dimensional homonuclear and heteronuclear correlation (optimized for one-bond and long-range C–H couplings) studies.

6α-Acetoxyazadirone (1), a major constituent of Chisocheton paniculatus, is an interesting tetranortriterpenoid possessing an α,β-unsaturated ketone moiety, a trisubstituted double bond, a β-substituted furan unit along with two acetoxyl groups at C-6 and C-7 positions. As a result it was found to be susceptible to reagents like selenium dioxide, alkaline hydrogen peroxide, N-bromosuccinimide, sodium borohydride, m-chloroperoxybenzoic acid and osmium tetroxide. The present investigators intended to utilise 6α-acetoxyazadirone (1) further and the results of their studies aimed at chemical correlation of 1 with the insect growth regulator meliacins nimocinolide (2) and isonomolide (3) isolated previously as minor constituents of Azadirachta indica and also with the minor tetranortriterpenoid meldenin (4) found in Melia azadirachta and 6-O-acetylisonomolide (5) isolated from the seeds of Chisocheton paniculatus, are presented here-with.

It has been documented that treatment of 3-isopropylfurane (6) with N-bromosuccinimide generated 4-hydroxy-2-isopropyl-2-enolide (7) but 4-hydroxy-3-isopropyl-2-enolide (8) was produced when a peracid was employed as the oxidant 6α-Hydroxyazadirone (9) is quite likely to be the intermediate for the meliacins 2 and 3 as similar oxidative modification around the furan unit in 9 would generate them, while 9 could, in turn, be derived from 10 by preferential monoacetylation. Basic hydrolysis of 6α-acetoxyazadirone (1) smoothly afforded a compound, C₂₅H₃₄O₄ (M⁺ 410), m.p. 205°, devoid of acetate moieties (ir, pmr and cmr) but had hydroxy group (ir) and a newly generated –CH–CHOH–CHOH– unit (pmr, COSY) and in conformity with the formation of 6α-hydroxydeace-
tylazadirone (10). Treatment of the diol 10 with acetic anhydride-pyridine at about ice-cold temperature for 20 h afforded mainly a monoacetylated product (ir, pmr, COSY) along with some of the diacetylated derivative identical with 6α-acetoxyazadirone (1). A careful analysis of the pmr spectrum of the monoacetylated product II revealed that the proton resonance at δ 4.16 (1H, br d, J 11 6 Hz, H-6) in 10 has suffered significant downfield shift (~ 30 ppm) in consonance with a preferential acetylation of the equatorial hydroxyl group at C-6. Compound 9, the required intermediate for the generation of the plant insect growth regulator meliacins nimocinolide (2) and isonimocinolide (3) through oxidative modification of its furan system, was likely to be formed upon selective acetylation of the C-7 hydroxyl could not be isolated even in traces.

It has been observed that treatment of 6α-acetoxyazadirone (1) with hydrogen peroxide-selenium dioxide in t-BuOH and stirring for about 3 h gave a product which was devoid of β-substituted furan unit present in 1. Instead it showed pmr signals (δpyridine) at δ 6.00 (1H, s, H-22) and 6.40 (1H, s, H-21) and cmr signals at δ 100.3 (d, C-21), 119.6 (d, C-22), 170.5 (s, C-20) and 173.2 (s, C-23) commensurate with the presence of a 3-substituted-4-hydroxy-but-2-enolide moiety. Since all other pmr and cmr signals as in 1 were present, the product was identified as 6-O-acetylisonimocinolide (5). It underwent acetylation to 6,21-di-O-acetylisonimocinolide (12). Compound 5 was reported previously from the seeds of Chiosocheton paniculatus and was characterised as its acetate. Further experiments to correlate 1 with 2 and 3 are in progress.

Careful hydrogenation of 6α-acetoxyazadirone (1) afforded two components which were separated by column chromatography. The less polar fraction identified as compound 13, C30H40O6 (M+ 496), m.p 236° was devoid of conjugated double bond (ir, pmr, cmr) The polar component, C30H44O6, m.p 210°, had no conjugated double bond, as also the β-substituted furan unit. Instead, a tetrahydrofuranyl unit was there and the cmr spectrum of the polar component adduced for the presence of a pair of compounds ((4a,b) epimeric at C-17 in it. 6α-Acetoxy-1,2-dihydroazadirone (13) was hydrolysed under basic condition to 6α-hydroxy-1,2-dihydrodeacetylazadirone (15) which afforded the product 17, C29H26O4 (M+ 414), m.p 191°, which underwent acetylation to the triacetate 18, C32H44O7, m.p. 163°, upon treatment with Ac2O-pyridine.

The proton and carbon chemical shift assignments reported in this paper were arrived at from a combination of homonuclear as well as heteronuclear correlation studies on compounds 4, 5, 10-16 prepared as also on 6α-acetoxyazadirone (1) for use as model. Information about the proton network in all the compounds was secured from two-dimensional 1H-1H correlation experiments optimized for one-bond 13C-1H correlation and was characterised as its acetate. Further experiments to correlate 1 with 2 and 3 are in progress.

Careful catalytic reduction of 11 produced a 1,2-dihydro derivative which was identical with meldenin۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴۴
**Table 1—Carbon-13 Nmr Signals of Some Tetranortriterpenoids**

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<td>125.9</td>
<td>125.9</td>
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<td>32.7</td>
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*ΔTMS = δCDCl3 + 76.9 ppm  **In d2-pyridine, ΔTMS = δDpyridine(C-2) + 149.9 ppm

Nance at δ 40.5 in 1 was linked to C-10 as it showed long-range correlations with proton signals at δ 1.19 (H3-19), 7.16 (d, H-1), 5.97 (d, H-2) and 2.55 (d, H-5) while that at δ 44.6 was associated with C-4 as it had long-range correlation with proton resonances at δ 1.19 (H3-19), 2.55 (d, H-5) and 5.92 (d, H-2). Again the proton resonances at δ 0.88 (3H, s), 1.33 (3H, s), 1.64-1.70 (2H, m) showed LR X-H correlation with the quartemary carbon resonance at δ 806

158.0 (C-14) and were thus identified as for those of H3-18, H3-30 and H2-12 respectively. The former proton resonance was for H3-18 as it showed LR correlation with δ 46.8 (s, C-13) which was further correlating with H-17 (δ 2.81, br d) and H-15 (δ 5.38, m) resonances. A long-range correlation between H-17 resonance (δ 2.81, br d) and C-21 resonance (δ 139.4) in 1 was noted. Using heteronuclear correlations the carbon resonance assignments for 4,5,10-
16 (vide Table 1) were made in analogous fashion and those for 17 and 18 followed therefrom.

**Experimental**

Melting points reported are uncorrected. Column chromatography was carried out with silica gel (60–120 mesh, B.D.H.). TLC was performed on silica gel G (E. Merck) plates using benzene-ethyl acetate (4:1) as the solvent system. Samples were crystallized from chloroform-petrol mixture, unless otherwise stated. IR spectra (KBr) were recorded on a Perkin-Elmer 782 spectrophotometer. Pmr, cmr, 2D-nmr (XHCCOR and LR1JCHCORR) spectra were run on a Bruker AM 300L supercon spectrometer equipped with ASPECT 3000 computer fitted with an array processor using benzene-ethyl acetate (4:1) as the solvent system.

Oxidation of 6a-acetoxyazadirone (1) to isonomocinolide-6-acetate (5): A sample of 6a-acetoxyazadirone (1; 50 mg) in t-BuOH (6 ml) was treated with 20% H₂O₂ (2 ml) and selenium dioxide (4 mg). The mixture was stirred for 3 h using magnetic stirrer. White precipitate formed was collected by filtration. Attempted crystallization afforded isonomocinolide-6-acetate (5) as amorphous material (47 mg), C₃₀H₄₀O₉; ν̃max 3260 (OH), 1765 (γ-lactone), 1740 (ester CO), 1650 (conjugated CO), 1225 (C=O-CO) cm⁻¹; pmr δ (d₅-pyridine) 0.79 (3H, s, H₃-18), 0.89 (3H, s, H₃-19), 1.05 (3H, s, H₂-30), 1.14 (3H, s, H₂-29), 1.24 (3H, s, H₂-28), 1.64 (2H, m, H₂-11), 1.85 (2H, m, H₂-12), 1.90 (3H, s, OOCOCH₃), 1.95 (3H, s, OOCOCH₃), 2.12 (3H, m, H-9 and H₂-16), 2.46 (1H, d, J 12.3 Hz, H-2), 2.90 (1H, m, H-17), 5.10 (1H, m, H-15), 5.40 (1H, br d, J 12.3 Hz, H-6), 5.50 (1H, br s, H-7), 5.90 (1H, d, J 10.1 Hz, H-2), 6.00 (1H, s, H-22), 6.40 (1H, s, H-21) and 7.00 (1H, d, J 10.1 Hz, H-1). Compound 5 was also obtained in good yield by simple stirring of 1 (20 mg) in dioxygen (2 ml) with 30% H₂O₂ (2 ml) for a prolonged period (more than 36 h).

Acetylation of isonomocinolide-6-acetate (5): Isonomocinolide-6-acetate (5; 30 mg) was treated with acetic anhydride (0.5 ml) and pyridine (6 drops) and the mixture was kept for 24 h. Cold water was added and the solution was acidified with dil HCl. Extraction with chloroform (3×20 ml), usual work up and removal of chloroform gave a crude mass showing two spots in TLC. Preparative thin layer chromatography of this crude mass on silica gel afforded the monoacetylated compound which on crystallization from chloroform-petrol mixture gave 1,2-dehydromeldemn (11; 20 mg), C₂₈H₃₆O₂, Rₗ 0.5 along with compound 1 (28 mg). Compound 11 showed pmr signals at δ 0.83 (3H, s, H₃-18), 1.17 (3H, s, H₃-19), 1.19 (3H, s, H₂-29), 1.28 (3H, s, H₃-30), 1.32 (3H, s, H₂-28), 1.63 (1H, m, H₂-12), 1.75 (1H, m, H₂-11), 1.94 (2H, m, H₂-11 and H₂-12), 2.18 (3H, s, OOCOCH₃), 2.30 (1H, dd, J 11.7 and 5.7 Hz, H-9), 2.42 (1H, ddd, J 15.5, 7.4 and 2.0 Hz, H₂-16), 2.55 (1H, dd, J 15.5 and 10.6 Hz, H₂-16), 2.75 (1H, d, J 12.3 Hz, H-5), 2.86 (1H, dd, J 10.6 and 7.4 Hz, H-17), 4.08 (1H, br s, H-7), 5.46 (1H, dd, J 12.3 and 2.1 Hz, H-6), 5.56 (1H, d, J 2.0 Hz, H-15), 5.89 (1H, d, J 10.1 Hz, H-2), 6.29 (1H, br s, H-22), 7.11 (1H, d, J 10.1 Hz, H-1), 7.27 (1H, br s, H-21) and 7.39 (1H, br s, H-23).

Acetyl of 6a-hydroxydeacetylazadirone (10) to 1,2-dehydromeldemin (11): 6a-Hydroxydeacetylazadirone (10; 50 mg) in pyridine (0.5 ml) was treated with freshly distilled acetic anhydride (0.5 ml) at about 0°C and the reaction mixture was kept as such for 20 h. It was diluted with ice-cold water (20 ml) and then acidified with dil HCl. Extraction with chloroform (3×20 ml), usual work up and removal of chloroform gave a crude mass which on purification by tlc afforded the monoacetylated compound which on crystallization from chloroform-petrol mixture gave 1,2-dehydromeldemn (11; 20 mg), C₂₈H₃₆O₂, Rₗ 0.5 along with compound 1 (28 mg).
Reduction of 6a-acetoxy-1,2-dihydroazadirone (1) · 6a-Acetoxyazadirone (100 mg) in ethanol (25 ml) was hydrogenated for 20 min in the presence of Pd·C (10%, 20 mg) at about atmospheric pressure. The catalyst was removed by filtration. Solvent removal from the filtrate gave a crude mass showing two spots in tlc which were separated by column chromatography over silica gel. Benzene eluates afforded a light yellow solid crystallizing in white needles of 6a-acetoxy-1,2-dihydroazadirone (13; 18 mg). C_{30}H_{40}O_6, m p 236°, [a]_{D} +135.6°, R_{f} 0.7. Later benzene eluates gave a light yellow solid which yielded 6a-acetoxy-1,2,20,21,22,23-hexahydroazadirone (14a,b) as white crystals (78 mg), C_{30}H_{44}O_6, m p 210°, [a]_{D} +140.5°. Similar hydrogenation experiment with 1 (200 mg) for a shorter period (8 mm) improved the yield of 13 (112 mg) over 14a,b (66 mg). Compound 13 displayed v_{max} at 1737 (ester CO), 1710 (CO), 1380, 1370 (gem dimethyl), 1240 (CO·O·C), 875 (furan) cm^{-1}; δ 0.76 (3H, s, H_{3}-18), 0.86 (3H, s, H_{3}-19), 1.18 (3H, s, H_{3}-29), 1.25 (3H, s, H_{3}-28). 1.27 (3H, s, H_{3}-30), 1.56 (2H, m, H_{3}-11 and H_{3}-12), 1.73 (1H, m, H_{3}-11), 1.83 (2H, m, H_{3}-21), 1.85 (1H, m, H_{3}-12), 1.99 (3H, s, OCOCH_{3}), 2.00 (3H, s, OCOCH_{3}), 2.15 (1H, m, H-15), 2.33 (1H, m, H-2), 2.35 (1H, m, H-2), 2.39 (1H, m, H-16), 2.42 (1H, m, H-16), 2.45 (1H, m, H_{3}-23), 2.50 (2H, m, H-5 and H_{b}-16), 2.70 (1H, m, H-17), 5.02 (1H, d, J 10.2 Hz, H-2), 6.01 (1H, s, H-22), 6.86 (1H, br, H-21) and 7.10 (1H, d, J 10.2 Hz, H-1). 

Hydrolysis of 6a-acetoxy-1,2-dihydrazadione (13): Compound 13 (100 mg) was refluxed with 10% methanolic KOH (10 ml) for 50 min on a water-bath. The contents were then cooled and diluted with water (2 ml). After distilling off methanol, water (25 ml) was added and the solution was acidified with dil. HCl. The product was extracted with CHCl_{3} (3×25 ml) usual work-up and removal of chloroform gave a mass which on crystallization afforded 6a-hydroxy-1,2-dihydrazadione (15; 80 mg). C_{26}H_{36}O_{4}, m p 205°, [a]_{D} +93.3°, R_{f} 0.4, v_{max} 3562 (OH), 3555 (OH), 1705 (CO), 1383, 1378 (gem dimethyl), 872 (furan) cm^{-1}; δ 0.82 (6H, s, H_{3}-18 and H_{3}-19), 1.15 (3H, s, H_{3}-30), 1.34 (6H, s, H_{3}-28 and H_{3}-29), 1.49 (1H, m, H_{3}-12), 1.52 (1H, m, H_{3}-11), 1.72 (3H, m, H_{3}-12 and H_{3}-11), 1.82 (1H, m, H_{3}-12), 2.06 (1H, dd, J 12.0 and 0 Hz, H-9), 2.16 (1H, d, J 11.3 Hz, H-5), 2.25 (1H, m, H_{3}-2), 2.36 (1H, m, OH), 2.40 (1H, m, H_{3}-16), 2.46 (1H, dd, J 14.5 and 10.5 Hz, H_{3}-16), 2.67 (1H, m, H_{3}-2), 2.80 (1H, dd, J 10.5 and 7.4 Hz, H-17), 3.30 (1H, br, H-7), 4.01 (1H, br d, J 11.3 Hz, H-6), 5.04 (1H, br, d, J 24 Hz, H-15), 6.25 (1H, br, s, H-22), 7.22 (1H, br, H-21) and 7.35 (1H, br, H-23).

6a-Hydroxy-1,2-dihydrazadione (15a,b): Basic hydrolysis of 14a,b (25 mg) as in the previous case, usual work-up and purification of the reaction product by column chromatography afforded 16a, b, C_{26}H_{36}O_{4}, as amorphous material, v_{max} 1710 (CO), 1378 (gem dimethyl) cm^{-1}; δ 0.82 (3H, s, H_{3}-19), 0.88 (3H, s, H_{3}-18), 1.15 (3H, s, H_{3}-30), 1.34 (6H, s, H_{3}-28 and H_{3}-29), 3.00–3.85 (4H, m, H_{3}-21 and H_{3}-23), 3.94 (1H, br, s, H-7), 4.03 (1H, br d, J 11.6 Hz, H-6) and 5.09 (1H, br, s, H-15).

Preferential acetylation of 6a-hydroxy-1,2-dihydrazadione (15): Compound 15 (40 mg) in pyridine (0.5 ml) was treated with freshly distilled acetic anhydride (0.5 ml) at about 0° and the reaction mixture was kept as such for 20 h. Dilution with ice-cold water (20 ml) was followed by acidification with dil. HCl. Extraction with chloroform (3×20 ml), usual work-up and removal of chloroform gave a crude mass showing two spots in tlc. Preparative tlc of this crude mass over silica gel afforded monoacetylated compound which on crystallization gave meldenin (4.22 mg), C_{28}H_{36}O_{4}, m p 240°–244° (lit 240°–44°). m/z 496 (M^{+} 61%), 481 (2), 436 (6), 393 (3), 376 (87), 361 (74), 348 (5), 213 (18), 146 (15), 145 (53), 81 (100). Compounds 14a,b showed v_{max} at 1740 (ester CO), 1710, 1380, 1370 (gem dimethyl), 1250, 1230 (CO·O·C) cm^{-1}; δ 0.70 (3H, s, H_{3}-19), 0.85 (3H, s, H_{3}-29), 0.88 (3H, s, H_{3}-18), 1.05 (3H, s, H_{3}-30), 1.06 (3H, s, H_{3}-28), 1.25, 1.35 (total 1H, m, H_{3}-12), 1.37 (11H, m, H_{3}-22), 1.40 (1H, m, H-17), 1.50 (2H, m, H_{3}-11), 1.65 (2H, m, H_{3}-11), 1.72 (1H, m, H_{3}-12), 1.82, 1.88 (total 1H, m, H_{3}-11), 1.87 (1H, m, H_{3}-16), 1.86 (3H, s, OCOCH_{3}), 2.07 (3H, s, OCOCH_{3}), 2.00 (1H, m, H_{3}-16), 2.05 (1H, m, H-9), 2.08 (1H, m, H-20), 2.16 (1H, m, H_{3}-2), 2.28 (1H, d, J 12.5 Hz, H-5), 2.62 (1H, m, H_{3}-2), 3.06, 3.08 (total 1H, m, H_{3}-23), 3.58 (1H, m, H-23), 3.66 (1H, m, H_{3}-23), 3.75, 3.81 (total 1H, m, H_{3}-21), 5.08 (1H, br, s, H-15), 5.12 (1H, br d, J 12.3 Hz, H-6) and 5.23 (1H, br, s, H-7). 808
2.65 (1H, d, J 12.0 Hz, H-5), 2.71 (1H, m, H2-2), 2.84 (1H, d, J 10.3 and 7.4 Hz, H-17), 4.05 (1H, br s, H-7), 5.31 (1H, br d, J 12.0 Hz, H-6), 5.58 (1H, br s, H-15), 6.29 (1H, br s, H-22), 7.27 (1H, br s, H-21) and 7.39 (1H, br s, H-23), m/z: 454 (M+, 20%), 439 (4), 410 (11), 394 (37), 379 (16), 359 (41), 214 (16), 213 (36), 146 (21), 145 (62), 136 (10), 121 (55), 81 (100). Compound 4 was also obtained by careful reduction of 6α-acetoxydeacety lazadirone (11) following an analogous procedure.

6α-Hydroxy-1,2-dihydrodeacetyla:adirol (17): Sodium borohydride (15 mg) was added in portions to a stirred ice-cold solution of 17 (20 mg) in methanol (2 ml) and the reaction mixture was kept overnight at room temperature. The solvent was then boiled off, the residue treated with water (20 ml) and extracted with chloroform (3 x 20 ml) The semi-solid mass obtained was purified by column chromatography to afford 17 (16 mg), C30H45O7, m.p 191° (lit. 188-89°), Rf 0.50 (EtOAc); vmax 3400 (OH), 1390 (gem dimethyl), 870 (furan) cm-1; δ 83 (3H, s, H3-18), 0.99 (3H, s, H3-19), 1.03 (3H, s, H2-29), 1.18 (3H, s, H3-30), 1.35 (3H, s, H3-28), 2.71 (1H, d, J 12.0 Hz, H-5), 2.75 (1H, m, H-17), 3.18 (1H, d, J 10.0 and 6.0 Hz, H-3), 3.92 (1H, br s, H-7), 4.13 (1H, br d, J 12.0 Hz, H-6), 5.53 (1H, m, H-15), 6.27 (1H, br s, H-22), 7.26 (1H, br s, H-21) and 7.37 (1H, br s, H-23); m/z 414 (M+, 24%), 396 (21), 381 (32), 363 (45), 319 (100), 213 (35). Acetylation of 17: Trol 17 (10 mg) in pyridine (0.3 ml) was treated with acetic anhydride (0.3 ml) and kept at room temperature overnight. Usual work-up afforded the triacetate 18 (9 mg), C32H44O7, m p 163°; Rf 0.56, νmax 1725 (ester CO), 1378, 1372 (gem dimethyl), 1235 (C=O-CO), 865 (furan) cm-1, 0.76 (3H, s, H3-18), 0.90 (3H, s, H3-19), 0.97 (3H, s, H2-29), 1.06 (3H, s, H3-28), 1.26 (3H, s, H3-28), 1.99 (6H, s, 2×OOCOCH3), 2.04 (3H, s, OOCOCH3), 2.72 (1H, d, J 12.0 Hz, H-5), 4.50 (1H, m, H-3), 5.32 (2H, m, H-7 and H-15), 5.55 (1H, dd, J 12.0 and 2.1 Hz, H-6), 6.24 (1H, m, H-22), 7.21 (1H, m, H-21) and 7.35 (1H, m, H-23).

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Secondary metabolites from the cnidarian *Cavernularia* sp.: Structures of the new briaranes cavernulin A and Bsup†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 *Cavernularia* sp., the extract of which shows some toxicity to guppy fingerlings and in brine-shrimp assays. The structures of cavernulin A as (15S*,25S*,5Z,7S*,8R*,9S*,10S*,11R*,12R*,17R*)-2,9-diacetoxy-12-hydroxybriara-5,13-dien-18-one and cavernulin B as (15S*,25S*,5Z,7S*,8R*,9S*,10S*,11R*,12R*,17R*)-2,9-diacetoxy-12-diethylbriara-5,13-dien-18-one are established from one-dimensional 1H and homodecoupling as well as two-dimensional (COSY-90, X-H correlation optimized for 1JCH and NOESY) NMR and other (IR and Mass) spectral and some chemical studies. The lengths of the alkyl chains in wax esters, ceramides and 1-O-alkylglycerols are established by appropriate gas chromatographic studies.

Tremendous surge of interest in marine natural product research prevailed throughout the globe during the past couple of decades. As a part of our search for bioactive components from marine sources a cnidarian, *Cavernularia* sp., was collected from the coastal Bay of Bengal. Preliminary bioactivity studies on the organism indicated that the CH2Cl2-MeOH extract of raw crushed *Cavernularia* sp had some toxicity in brine-shrimp assays (LC50 386 μg 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 cembranoid or briarane (3) group of diterpenoids. Such briarane diterpenoids have earlier been reported from Gorgonacea (Genus: *Briareum*, Solenopodium*†3,4, Erythropodium*5, Junceello*6, Gorgonella*7, Menella*8), Alcyonacea (soft coral) (Genus: *Minabea*9), Stolonifera (Genus: *Tubipora*10) and Pennatulacea (Genus: *Stylatula*11, *Scyttium*12, *Cavernula*13, *Verrillium*14, 15, *Armina*16, *Renilla*17, *Funiculina*18, 19), and continue to attract the attention of investigators because of the structural complexity and wide range of biological activities, e.g., toxic22, cytotoxic19, antifouling21, immunomodulatory25 and antibacterial9.

**Results and Discussion**

The *Cavernularia* species was thoroughly extracted with CH2Cl2 and subsequently with CH2Cl2-MeOH (1:1). Chromatographic resolution (column and preparative thin layer chromatography) of the CH2Cl2 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), C24H34O9, Rf 0.35 in CHCl3-MeOH (97:3), [α]D-56.4° (c 0.42). The CH2Cl2-MeOH (1:1) extract yielded another new briarane diterpenoid cavernulin B (2), C24H34O9, Rf 0.3 in CHCl3-MeOH (95:5), [α]D-50.3° (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⁻¹) and five-membered lactone (1770 and 1220 cm⁻¹) functionalities. The
detailed 1D (H-, C-, homodecoupling) and 2D (COSY, NOESY and XHCO) NMR experiments of cavernulin A were in consonance with its formulation as 1. The 13C NMR spectrum of 1 showed 28 carbons (7 -CH-, 10 -CH-, 4 -CH2- and 7 -CH3) signals (Table 1) in conformity with its molecular formula C28H40O9. 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, 120.6) and three non-oxygenated (77.4, 79.8 and 80.8).
Table 1 ¹H and ¹³C NMR Chemical shifts for cavemulin A (1), cavemulin B (2) and cavemulin B diacetate (9) and two-dimensional (¹H-¹H COSY and ¹³C-¹H) correlation data

<table>
<thead>
<tr>
<th>Position</th>
<th>Cavemulin A (1)</th>
<th>Cavemulin B (2)</th>
<th>Diacetate (9)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>¹H&lt;sup&gt;α&lt;/sup&gt;</td>
<td>¹³C&lt;sub&gt;α&lt;/sub&gt;&lt;sup&gt;α&lt;/sup&gt;</td>
<td>¹H&lt;sup&gt;α&lt;/sup&gt;</td>
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<td>1</td>
<td>4.35 br d (5.3)</td>
<td>146 9 (s)</td>
<td>4.39 br d (5.3)</td>
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<tr>
<td>2</td>
<td>5.25 d (5.5)</td>
<td>71 6 (d)</td>
<td>H-10</td>
</tr>
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<td>3</td>
<td>2.75 m (H-2)</td>
<td>32 4 (s)</td>
<td>H-3, H-4</td>
</tr>
<tr>
<td>4</td>
<td>2.52 m (H-3)</td>
<td>29 3 (s)</td>
<td>H-2, H-3, H-4</td>
</tr>
<tr>
<td>5</td>
<td>2.03 m (H-4)</td>
<td>146 9 (s)</td>
<td>146 3 (s)</td>
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<tr>
<td>6</td>
<td>5.23 d (10)</td>
<td>77 8 (d)</td>
<td>H-6</td>
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<tr>
<td>7</td>
<td>82 8 (s)</td>
<td>82 6 (s)</td>
<td>5.27 d (5.5)</td>
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<tr>
<td>8</td>
<td>71 6 (d)</td>
<td>72 2 (d)</td>
<td>H-10</td>
</tr>
<tr>
<td>9</td>
<td>2.66 dd (5.5, 3.7)</td>
<td>34 5 (d)</td>
<td>35 4 (d)</td>
</tr>
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<td>10</td>
<td>2.09 m</td>
<td>39 3 (d)</td>
<td>H-10, H-12, H-20</td>
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<td>11</td>
<td>4.84 dd (5.8, 2.5)</td>
<td>70 7 (d)</td>
<td>H-11, H-13</td>
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<td>12</td>
<td>5.69 dd (10 1.5 8)</td>
<td>120 4 (d)</td>
<td>H-12, H-14</td>
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<tr>
<td>13</td>
<td>5.61 d (10 1)</td>
<td>142 3 (d)</td>
<td>H-13</td>
</tr>
<tr>
<td>14</td>
<td>16 1 (q)</td>
<td>10 2 (q)</td>
<td>10 1 (q)</td>
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<td>10 1 (q)</td>
<td>10 1 (q)</td>
<td>10 1 (q)</td>
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<tr>
<td>17</td>
<td>2.42 q (7 2)</td>
<td>42 3 (d)</td>
<td>H-19</td>
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<tr>
<td>18</td>
<td>176 1 (s)</td>
<td>176 7 (s)</td>
<td>2.18 s</td>
</tr>
<tr>
<td>19</td>
<td>11 5 d (7 2)</td>
<td>6 6 (d)</td>
<td>H-17</td>
</tr>
<tr>
<td>20</td>
<td>10 3 d (7 4)</td>
<td>13 7 (q)</td>
<td>H-11</td>
</tr>
</tbody>
</table>

¹H values (Hz) are given in parentheses. ¹³C Multiplicities were determined by DEPT experiment and indicated by usual symbols. ¹Protonated carbon signal showed ¹³C-¹H correlation optimized for ¹H-C with the corresponding ¹H signal on the previous column.

and 142 3) and the remaining three (35 4, 39 3 and 42 3) non-oxygenated ones. The proton network in 1 was established by homodecoupling and ¹H-¹H COSY experiments. Further, two-dimensional ¹³C-¹H correlation experiment on 1 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 δ 5.61 (1H, J 10.1 Hz)
was coupled with a double-doublet signal at $\delta$ 5.69 (1H, J 10 1 and 5 8 Hz) which in turn was coupled with a double-doublet signal at $\delta$ 4.84 (1H, J 5 8 and 2.5 Hz). The latter signal was again coupled to a multiplet at $\delta$ 2.09 (1H, J 5 5 and 3.7 Hz). The double-doublet at $\delta$ 2.68 was also coupled with a double at $\delta$ 5.25 (1H, J 5.5 Hz). The $^1$H signals at $\delta$ 5.61 and 5.69 correlating with CH resonances at 14.27 and 12.04 were thus for the olefinic proton resonances and $^1$H-$^1$H 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 1.

-CH=CH(OCOR)-CH(CH$_3$)-CH-CH-OCOO-

fragment-I

$-CO-O-CH\_2CH\_2CH\_2H$-

fragment-II

$-CO-O-CH=CH(OCOR)$-

fragment-III

$-CH\_2CH\_2CH\_2CH\_2H$-

fragment-IV

$-O-CH\_2CH\_2-CH\_2$-

fragment-V

It has further been noted that a broad doublet signal at $\delta$ 4.35 (1H, J 5 3 Hz) was coupled to a pair of geminally coupled multiplets at $\delta$ 1.62 (1H) and 2.75 (1H), both of which were further coupled with another pair of geminal protons resonating as multiplets at $\delta$ 2.03 (1H) and 2.52 (1H). These observations were commensurate with the presence of two adjacent methylene groups in compound 1. The $^{13}$C-$^1$H correlation experiment identified the carbon resonances associated with fragment-II of the compound at $\delta_C$ 81.1 (d), 32.4 (t) and 29.3 (t), respectively. Again, homodecoupling and COSY spectra of 1 revealed that a broad doublet signal at $\delta$ 1.62 (1H, J 10 0 Hz) was found to couple to a doublet signal at $\delta$ 5.23 (1H, J 10 0 Hz) while the former signal was also weakly coupled to a vinyl methyl signal at $\delta$ 2.02 (br s). The XHCCORR experiment confirmed that the corresponding protonated carbon signals were at $\delta_C$ 118.6 (d), 77.8 (d) and 28.2 (q), respectively, in conformity with the presence of a quaternary olefinic carbon atom which incidentally resonated at $\delta_C$ 146.9.

The $^1$H and $^{13}$C spectra also displayed signals assignable to fragment-IV as it was observed that a doublet signal at $\delta$ 1.15 (3H, J 7 2 Hz) coupling with a quartet signal at $\delta$ 2.42 (1H, J 7.2 Hz) in the $^1$H spectrum of 1 were linked to methine carbon resonances at $\delta_C$ 66 (q) and 42.3 (d), respectively. The upfield carbon resonance at $\delta_C$ 66 for the methyl group was an indication that the methyl group was attached to a-carbon of a five-membered lactone moiety.

A triplet at $\delta$ 0.93 (3H, J 7 5 Hz) coupled with a multiplet at $\delta$ 1.62 (2H) which in turn was further coupled to a triplet at $\delta$ 2.24 (2H, J 7 4 Hz) The signal positions indicated the presence of a butanoyl group (fragment-V) in cavernulin A (1). This was further confirmed by the $^{13}$C NMR spectrum of the compound 1 (Table 1).

Besides, the compound also exhibited additional $^1$H signals for a quaternary methyl at $\delta$ 1.04 (3H, s) and two acetate methyls at $\delta$ 2.06 (3H, s) and 2.18 (3H, s), and displayed additional carbon signals for a quaternary methyl ($\delta_C$ 16.1 (q)), acetate methyls ($\delta_C$ 21.0 (q), 21.6 (q)), quaternary carbon ($\delta_C$ 43.0 (s)), oxygenated quaternary carbon ($\delta_C$ 82.8 (s)), acetate carboxyls ($\delta_C$ 169.1 (s), 170.2 (s)) and lactone carboxyl ($\delta_C$ 176.1 (s)). The aforesaid observations on cavernulin A were thus in conformity with the alternative structures 1 and 8. The gross structure as 1 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 (1) and subsequently by its derivatization from the congener diterpenoid cavernulin B (2) by the action of butyric anhydride and pyridine.

An ion peak at $m/z$ 238 (40%) [ca C$_{13}$H$_{21}$O$_4$] (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 1 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 C$_{13}$H$_{21}$O$_4$] (ion fragment b formed by the cleavage of C(1)-C(2) and C(8)-C(9) bonds) further supported the gross structure 1 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.

Reciprocal NOESY correlations of H-2, H-9, H-12, H-13, H-14, H-15, H-17, H-2’ and H-3’ with H-10, H-20, C-9-OAc as well as H-20, H-14, H-15, C-2-OAc, H-20, H-3’ and H-4’, respectively, indicated their close proximity. Consideration of the coupling constants of the various 1H NMR signals of cavemulin A was helpful further in establishing its relative stereochemistry at the various asymmetric centers and thus it may be represented as (1S*,2S*,3S*,4R*,5Z*,6S*,7S*,8R*,9S*,10S*,11R*,12R*,17R*)-2,9-diaceetoxy-12-butanoyloxy-8-hydroxybranara-5,13-dien-18-one (1).

The IR spectrum (KBr) of cavemulin B (2) indicated the presence of hydroxyl (3400–3450 cm⁻¹) and five-membered lactone (1770 and 1225 cm⁻¹) functionalities. The 1H NMR spectrum of compound 2 clearly accounted for the various structural fragments present in the molecule. The presence of a cis-double bond as a part of the structural unit -CH=CH-CHOH-CH(CH₃)CH-CH(OCOR)-, similar to fragment-I in 1, was clearly discernible since a double doublet signal at δ 5.73 (1H, J 10.1 and 5.9 Hz) was found to couple with a doublet resonance at δ 5.42 (1H, J 10.1 Hz) as well as with a double-doublet appearing at δ 3.85 (1H, J 5.9 and 1.7 Hz). Further, a multiplet at δ 2.10 (1H) was coupled to three signals resonating at δ 3.85 (1H, dd), 0.96 (3H, d) and 2.64 (1H, dd). The latter signal was also coupled to an oxygenated methine proton signal at δ 5.27 (1H, d). Thus this oxygenated carbon was attached to a quaternary system. The spectrum of compound 2 also showed a broad doublet at δ 4.39 (1H) and four multiplets at δ 2.74, 1.65, 2.52 and 2.02 (1H each). The 1H-1H correlation study of compound 2 further indicated them to be for two adjacent methylene units further. The multiplets at δ 2.74 and 1.65 for a set of geminal protons were coupled to the oxygen bearing methine proton resonating at δ 4.39 (br d). The 13C-1H correlation experiment identified the carbon resonances associated with the fragment-II of the compound 2 at δC 81.2 (d), 32.0 (t) and 29.0 (t), respectively.

Again, homodecoupling and COSY spectra of 2 revealed that a broad doublet signal at δ 5.52 (1H, J 10.1 Hz) was found to couple to a doublet signal at δ 5.21 (1H, J 10.1 Hz) while the former signal was also weakly coupled to a vinyl methyl signal at δ 1.97 (br s). The XHICORR experiment confirmed that the corresponding protonated carbon signals in 2 were at δC 118.7 (d), 77.9 (d) and 28.4 (q), in conformity with the presence of fragment-III, requiring the presence of a quaternary olefinic carbon atom for which the singlet resonance at δC 146.3 is ascribable.

The 1H and 13C spectra of 2 also displayed signals assignable to fragment-IV as it was observed that a doublet signal at δ 1.18 (3H, J 7.2 Hz) coupling with a quartet signal at δ 2.37 (1H, J 7.2 Hz) in the 1H spectrum of 2 were linked to carbon resonances at δ 6.7 (q) and 42.5 (d), respectively. The upfield carbon resonance at δ 6.7 for the methyl group was an indication that the methyl group was attached to α-carbon of a five-membered lactone moiety. Besides, the compound also displayed additional 1H signals for a quaternary methyl at δ 1.02 (3H, s) and two acetyl methyls at δ 2.10 (3H, s) and 2.18 (3H, s), and exhibited additional carbon signals for a quaternary methyl [δC 15.9 (q)], acetate methyls [δC 21.2 (q), 21.6 (q)], quaternary carbon [δC 43.0 (s)], oxygenated quaternary carbon [δC 82.6 (s)], acetate carbonyls [δC 169.1 (s), 170.9 (s)] and lactone carbonyl [δC 176.7 (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 δ 3.85 in 2 underwent downfield shift of about 1 ppm and appeared at δ 4.84 (1H, dd, J 5.9 and 2.6 Hz) in 9. Thus, one of the two hydroxyl groups was attached with a methine carbon. The signal at δ 5.42 of compound 2 also shifted to δ 5.64 (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 hydroxyl 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 cavemulin B. Conclusive structural assignment was made through assignment the ion fragments observed in the mass spectrum of cavemulin B. The presence of ion peaks with m/z 281 [ca. C₁₃H₂₁O₅], 280 [C₁₃H₂₉O₅], 222 [C₁₃H₁₆O₅], 196 [C₁₁H₁₆O₅] 169 [C₉H₁₃O₃], 167 [C₉H₁₁O₄] and 107 [C₈H₁₁] were commensurate with the structure 2 for cavemulin B.

The relative stereochemistry at the various asymmetric centres of cavemulin B was established with the help of a NOESY experiment and consideration of coupling constants of various 1H signals and construction of molecular model. Similar reciprocal NOESY correlations for cavemulin B as in cavemulin A (1) were observed except those for butyrate moiety present in the latter and in accordance with the stereostructure (1S*,2S*,3S*,4R*,5Z*,6S*,7S*,8R*,9S*,10S*,11R*,12R*,17R*)-2,9-diaceetoxy-8,13-dihydroxybranara-5,13-dien-18-one (2). A closely related briarane solenolid F (11) has previously been reported from Solenopodium sp where H-
9. H-12 H-17 and H-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.

To our knowledge this is the first report of the occurrence of briaranes in the genus *Cavemularia* 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 XH-CORR) spectra were recorded on a Bruker AM 300L supercon spectrometer equipped with ASPECT 3000 computer fitted with an array processor using programme version DISR871 or DISR941 m CDC13 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 Hitachi RMU 6L spectrometer operating at 70 eV. Mass spectra were taken with a Hewlett-Packard M5890, Series II gas chromatograph fitted with a Hewlett-Packard integrator M3394A using appropriate experimental conditions. Mass spectra were taken in a Hitachi RMU 6L spectrometer operating at 70 eV.

**Animal material**

The marine organism *Cavemularia* sp (Phylum Cnidaria, Class Anthozoa, Order *Pennatulacea*, Family: Veretillidae) was collected from the Eastern Coast of Bay of Bengal near Digha (latitude 21°37'N, longitude 87°31'30" E, which is about 180 km west of Kolkata), West Bengal, and was stored in a freezer until extraction. The organism was identified at the Z S I, Kolkata.

**Extraction and isolation**

The raw organism (6 kg) was crushed mechanically and extracted with CH2Cl2 (5 l) and then with CH2Cl2-MeOH (1, 1, 5 l) while the liquid expelled by the raw uncruushed organism was extracted with EtOAc (2 l) after saturation with NaCl. Solvents were removed under reduced pressure to afford the respective extracts (≈5, 4 and 1.5 g, respectively). Both CH2Cl2-MeOH and EtOAc extracts showed similar TLC behavior and were mixed together. Chromatographic resolution of the CH2Cl2 extract and subsequent preparative TLC of the appropriate fractions afforded a novel cyclized cembranoid (briarane), cavemulin A (1) together with a few ubiquitous compounds such as wax esters (fatty esters of saturated fatty acids and also of some analogous unsaturated fatty acids) (4), ceramides (5), cholesterol (6), 1-O-alkylglycerol (7). Simi-

lar chromatographic resolutions of the mixed CH2Cl2-MeOH (1 : 1) and EtOAc extracts afforded another new briarane diterpenoid cavemulin B (2) together with small amounts of ceramides (5) as well as 1-O-alkylglycerol (7) as the polar components.

**Cavemulin A (1)**: Colourless semisolid mass (20 mg), Rf 0.35 in CHCl3-MeOH (97 : 3), [α]D -56.4° (c 0.42), $\nu_{max}$ 3450–3400, 2920, 1770, 1735, 1455, 1355, 1220, 970 cm⁻¹. EIMS m/z 460 (M⁺-HOAc, 5%), 448 (5), 447 (7.5), 434 (10), 433 (15), 407 (5), 406 (7.5), 405 (10), 392 (15), 391 (32.5), 374 (12.5), 373 (22.5), 345 (17.5), 331 (100), 330 (15), 313 (75), 285 (25), 266 (12.5), 265 (10) and 238 (40).

**Cavemulin B (2)**: Colourless semisolid mass (11 mg), Rf 0.3 in CHCl3-MeOH (95 : 5), [α]D -50.3° (c 0.20), $\nu_{max}$ 3450–3400, 2920, 1770, 1740, 1460, 1385, 1225, 975 cm⁻¹. EIMS m/z 432 (M⁺-H2O, 4%), 390 (5), 372 (5), 344 (4), 330 (4), 315 (10), 312 (2), 281 (5), 280 (4), 222 (9), 196 (16), 169 (20), 167 (49), 107 and 106 (100%).

**Acetylation of cavemulin B (2)**: Cavemulin 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 cavemulin B diacetate (9, 2 mg) as colourless amorphous mass, Rf 0.35 in CHCl3-MeOH (97 : 3).

**Cavemulin A (1)**: Cavemulin A (1; 2 mg) was purified by chromatography to afford cavemulin A (1; 2 mg) identified by TLC and PMR spectral comparison with the natural specimen.

**Wax esters (fatty ester 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, H-2'), 2.28 (2H, t, J 7.4 Hz, H-2'), 2.01 (4H, m, =CHCH2CH2), 1.60 (2H, m, H-3'), 1.25 (br s, $\times$ CH3) and 0.90 (6H, t, J 6.6 Hz, 2 $\times$ CH2CH3). 13C NMR δ 14.0 (2 $\times$ CH3), 22.6 (2 $\times$ CH2CH3), 25.0 (C-3), 29.7 ($\times$ CH3), 32.0 (CH2CH3CH2), 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 x 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 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 x 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 C₁₀ (8.0%), C₁₂ (7.5), C₁₄ (4.1), C₁₆ (3.0%), C₁₈ (2.0%), C₂₀ (1.0%) and C₂₂ (0.5%) with a 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), 14 : 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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