

Secondary metabolites from the cnidarian *Cavernularia* sp. : structures of the new briaranes cavernulin A and B^{†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 (1*S**,2*S**,5*Z*,7*S**,8*R**,9*S**,10*S**,11*R**,12*R**,17*R**)-2,9-diacetoxy-12-butanoyloxy-8-hydroxybriara-5,13-dien-18-one and cavernulin B as (1*S**,2*S**,5*Z*,7*S**,8*R**,9*S**,10*S**,11*R**,12*R**,17*R**)-2,9-diacetoxy-8,12-dihydroxybriara-5,13-dien-18-one are established from one-dimensional [¹H and homodecoupling as well as ¹³C (NDC and DEPT-135)] and two-dimensional (COSY-90, X-H correlation optimized for ¹J_{C-H} 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 interests in marine natural product research prevailed throughout the globe during the past couple of decades³. 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 diterpenoids⁴. Such briarane diterpenoids have earlier been reported^{3,4} from Gorgonacea (Genus : *Briareum*⁵, *Solenopodium*⁶, *Erythropodium*⁷, *Junceella*⁸, *Gorgonella*⁹ and *Menella*¹⁰), Alcyonacea (soft coral) (Genus : *Minabea*¹¹), Stolonifera (Genus : *Tubipora*¹²) and Pennatulacea (Genus : *Ptilosarcus*¹³, *Tochuina*¹⁴, *Stylatula*¹⁵, *Scytalium*¹⁶, *Pteroides*¹⁷, *Cavernulina*¹⁸, *Veretillum*^{19,20}, *Armina*²⁰,

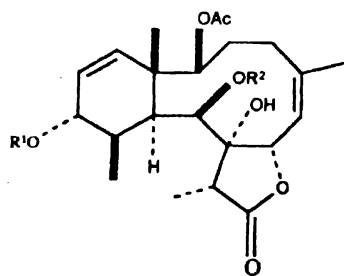
*Renilla*²¹ and *Funiculina*²²) and continue to attract the attention of investigators because of the structural complexity and wide range of biological activities, e.g. toxic²², cytotoxic¹⁹, antiinflammatory²³, antiviral²⁴, antiinsecticidal¹³, antifouling²¹, immunomodulatory²⁵ and antibacterial⁹.

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), C₂₈H₄₀O₉, *R*_f 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), C₂₄H₃₄O₈, *R*_f 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

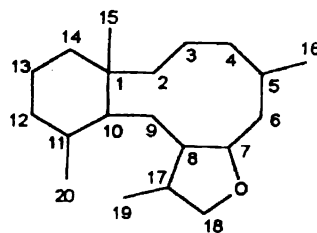
[†]Dedicated to Professor P. J. Scheuer, Department of Chemistry, University of Hawaii at Manoa, Honolulu, Hawaii 96822, U.S.A. on the occasion of his 90th. birthday.



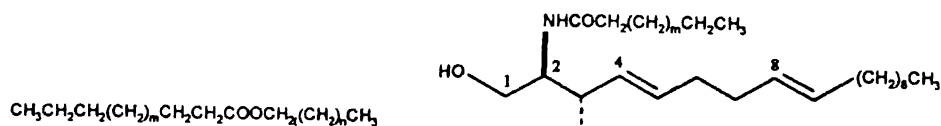
1 $R^1 = -COCH_2CH_2CH_3$, $R^2 = Ac$

2 $R^1 = H$, $R^2 = Ac$

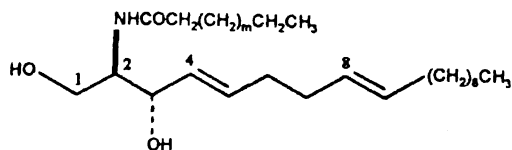
11 $R^1 = Ac$, $R^2 = H$



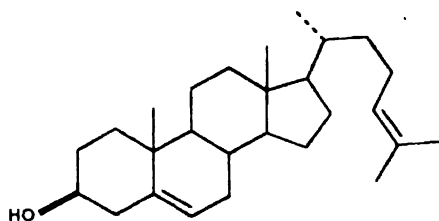
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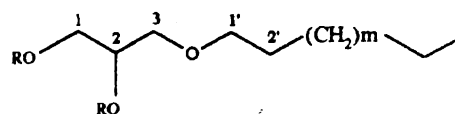
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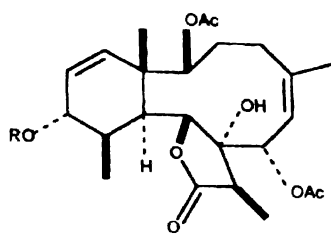


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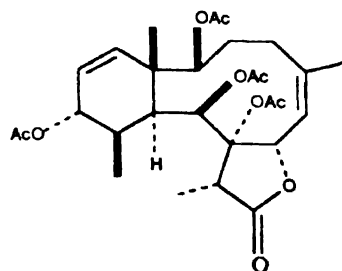
7 $R = H$

12 $R = TMS$



8 $R = -COCH_2CH_2CH_3$

10 $R = H$



9

detailed 1D (1H -, ^{13}C -, homodecoupling) and 2D (COSY, NOESY and XHCORR) NMR experiments of cavernulin A were in consonance with its formulation as 1. The ^{13}C NMR spectrum of 1 showed 28 carbons (7 $-C-$, 10 $-CH-$, 4 $-CH_2-$ and 7 $-CH_3$) signals (Table 1) in confor-

mity 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

Table 1. ^1H and ^{13}C NMR Chemical shifts and two-dimensional (^1H - ^1H COSY and ^{13}C - ^1H) correlation data for cavernulin A (**1**), cavernulin B (**2**) and cavernulin B diacetate (**9**)

Position	Cavernulin A (1)			Cavernulin B (2)			Diacetate (9)	
	$^1\text{H}^a$	$^{13}\text{C}^{b,c}$	^1H - ^1H COSY	$^1\text{H}^a$	$^{13}\text{C}^{b,c}$	^1H - ^1H COSY	$^1\text{H}^a$	^1H - ^1H COSY
1		43.0 (s)			43.0 (s)			
2	4.35 br d (5.3)	81.1 (d)	H _B -3	4.39 br d (5.7)	81.2 (d)	H _B -3	4.34 br d (5.3)	H ₂ -3
3	2.75 m(H _A) 1.62 m(H _B)	32.4 (t)	H _B -3, H ₂ -4 H-2, H _A -3, H ₂ -4	2.74 m(H _A) 1.65 m(H _B)	32.0 (t)	H _B -3, H ₂ -4 H-2, H _A -3, H ₂ -4	2.79 m(H _A) 1.62 m(H _B)	H-2, H _B -3, H ₂ -4 H-2, H _A -3, H ₂ -4
4	2.52 m(H _A) 2.03 m(H _B)	29.3 (t)	H ₂ -3, H _B -4 H ₂ -3, H _A -4	2.52 m(H _A) 2.02 m(H _B)	29.0 (t)	H ₂ -3, H _B -4 H ₂ -3, H _A -4	2.53 m(H _A) 2.02 m(H _B)	H ₂ -3, H _B -4 H ₂ -3, H _A -4
5		146.9 (s)			146.3 (s)			
6	5.52 br d (10.0)	118.6 (d)	H-7, H ₃ -16	5.52 br d (10.1)	118.7 (d)	H-7, H ₃ -16	5.52 br d (10.1)	H-7, H ₃ -16
7	5.23 d (10.0)	77.8 (d)	H-6	5.21 d (10.1)	77.9 (d)	H-6	5.23 d (10.1)	H-6
8		82.8 (s)			82.6 (s)			
9	5.25 d (5.5)	71.6 (d)	H-10	5.27 d (5.6)	72.2 (d)	H-10	5.27 d (5.9)	H-10
10	2.68 dd (5.5, 3.7)	35.4 (d)	H-9, H-11	2.64 dd (5.6, 3.9)	34.5 (d)	H-9, H-11	2.67 dd (5.8, 3.8)	H-9, H-11
11	2.09 m	39.3 (d)	H-10, H-12, H ₃ -20	2.10 m	42.0 (d)	H-10, H-12, H ₃ -20	2.10 m	H-10, H-12, H ₃ -20
12	4.84 dd (5.8, 2.5)	70.7 (d)	H-11, H-13	3.85 dd (5.9, 1.7)	68.3 (d)	H-11, H-13	4.84 dd (5.9, 2.6)	H-11, H-13
13	5.69 dd (10.1, 5.8)	120.4 (d)	H-12, H-14	5.73 dd (10.1, 5.9)	123.8 (d)	H-12, H-14	5.73 dd (10.1, 5.9)	H-12, H-14
14	5.61 d (10.1)	142.3 (d)	H-13	5.42 d (10.1)	139.9 (d)	H-13	5.64 d (10.1)	H-13
15	1.04 s	16.1 (q)		1.02 s	15.9 (q)		1.06 s	
16	2.02 br s	28.2 (q)	H-6	1.97 br s	28.4 (q)		2.05 s	H-6
17	2.42 q (7.2)	42.3 (d),	H ₃ -19	2.37 q (7.2)	42.5 (d),	H ₃ -19	2.43 q (7.2)	H ₃ -19
18		176.1 (s)			176.7 (s)			
19	1.15 d (7.2)	6.6 (q)	H-17	1.18 d (7.2)	6.7 (q)	H-17	1.18 d (7.2)	H-17
20	1.03 d (7.4)	13.7 (q)	H-11	0.96 d (7.2)	13.3 (q)	H-11	1.04 d (7.2)	H-11
2-OCOCH ₃	2.06 s	21.0 (q)		2.10 s	21.2 (q)		2.08 s	
9-OCOCH ₃	2.18 s	21.6 (q)		2.18 s	21.6 (q)		2.19 s	
8-OCOCH ₃							2.04 s	
12-OCOCH ₃							2.23 s	
OCOCH ₃		169.1 (s)			169.1 (s)			
OCOCH ₃		170.2 (s)			170.9 (s)			
OCOCH ₂ -		172.9 (s)						
OCOCH ₂ -	2.24 t (7.4)	36.6 (t)	H ₂ -3'					
-CH ₂ CH ₃	1.62 m	18.4 (t)	H ₂ -2', H ₃ -4'					
-CH ₂ CH ₃	0.93 t (7.5)	12.9 (q)	H ₂ -3'					

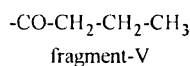
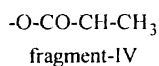
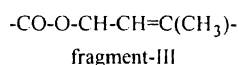
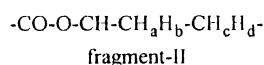
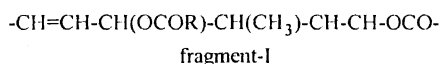
^a*J* values (Hz) are given in parentheses. ^bCarbon multiplicities were determined by DEPT experiment and indicated by usual symbols. ^cProtonated carbon signal showed ^{13}C - ^1H correlation optimized for $^1J_{\text{C-H}}$ with the corresponding ^1H signal on the previous column.

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 ^{13}C - ^1H correlation experiment on **1** optimized for one-bond C-H coupling allowed identifica-

tion 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 δ 5.69 (1H, J 10.1 and 5.8 Hz) which in turn was coupled with a double-doublet signal at δ 4.84 (1H, J 5.8 and 2.5 Hz). The latter signal was again coupled to a multiplet at δ 2.09 (1H) which was further coupled with a doublet at δ 1.03 (3H, J 7.4 Hz) as well as a double 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 **1**.



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 **1**. The ^{13}C - ^1H correlation experiment identified the carbon resonances associated with fragment-II of the compound at δ_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 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 XHCORR experiment confirmed that the corresponding protonated carbon signals were at δ_c 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 δ_c 146.9.

The ^1H and ^{13}C 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 **1** were linked to carbon resonances at δ_c 66.6(q) and 42.3(d), respectively. The upfield carbon resonance at δ_c 66.6 for the methyl group was an indication that the methyl group was attached to α -carbon of a five-membered lactone moiety.

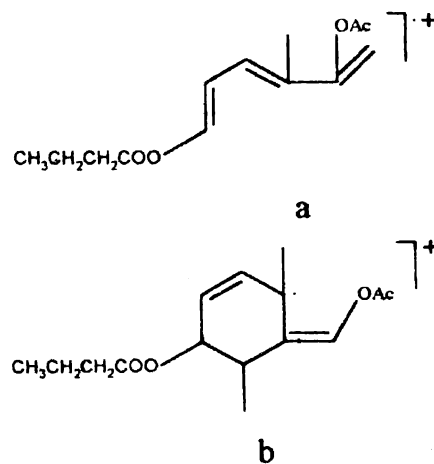
A triplet at δ 0.93 (3H, J 7.5 Hz) coupled with a multip-

let 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 (**1**). This was further confirmed by the ^{13}C NMR spectrum of the compound **1** (Table 1).

Besides, the compound also exhibited additional ^1H signals for a quaternary methyl at δ 1.04 (3H, s) and two acetate methyls at δ 2.06 (3H, s) and 2.18 (3H, s), and displayed additional carbon signals for a quaternary methyl [δ_c 16.1 (q)], acetate methyls [δ_c 21.0 (q), 21.6 (q)], quaternary carbon [δ_c 43.0 (s)], oxygenated quaternary carbon [δ_c 82.8 (s)], acetate carbonyls [δ_c 169.1 (s), 170.2 (s)] and lactone carbonyl [δ_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 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.* $\text{C}_{13}\text{H}_{18}\text{O}_4$] (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.* $\text{C}_{15}\text{H}_{21}\text{O}_4$] (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, H₃-15, H-17, H₂-2' and H₃-3' with H-10, H₃-20, C₉-OAc as well as H₃-20, H-14, H₃-15, C₂-OAc, H₃-20, H₂-3' and H₃-4', respectively, indicated their close proximity. Consideration of the coupling constants of the various ¹H 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 (1*S**,2*S**,5*Z*,7*S**,8*R**,9*S**,10*S**,11*R**,12*R**,17*R**)-2,9-diacetoxy-12-butanoyloxy-8-hydroxybriara-5,13-dien-18-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 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 with 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 δ_c 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 XHCORR experiment confirmed that the corresponding protonated carbon signals in **2** were at δ_c 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 δ_c 146.3 is ascribable.

The ¹H and ¹³C spectra of **2** also displayed signals as-

signable 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 acetate methyls at δ 2.10 (3H, s) and 2.18 (3H, s), and exhibited additional carbon signals for a quaternary methyl [δ_c 15.9 (q)], acetate methyls [δ_c 21.2 (q), 21.6 (q)], quaternary carbon [δ_c 43.0 (s)], oxygenated quaternary carbon [δ_c 82.6 (s)], acetate carbonyls [δ_c 169.1 (s), 170.9 (s)] and lactone carbonyl [δ_c 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 acetylatable 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 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 [*ca.* C₁₅H₂₁O₅], 280 [C₁₅H₂₀O₅], 222 [C₁₃H₁₈O₃], 196 [C₁₁H₁₆O₃], 169 [C₉H₁₃O₃], 167 [C₉H₁₁O₃] and 107 [C₈H₁₁] 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 ¹H signals and construction of molecular model. Similar reciprocal 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 (1*S**,2*S**,5*Z*,7*S**,8*R**,9*S**,10*S**,11*R**,12*R**,17*R**)-2,9-diacetoxy-8,12-dihydroxybriara-5,13-dien-18-one (**2**). A closely related briarane solenolide F (**11**) has previously been reported from *Solenopodium* sp. where H-

9, H-12, H-17 and H₃-19 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) respectively²⁶.

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 XHCORR) 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 in a Perkin-Elmer M241 electronic polarimeter in CHCl₃ at 25°. Gas chromatographic experiments were done with 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 *Cavernularia* 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 dm³) and then with CH₂Cl₂-MeOH (1 : 1; 5 dm³) while the liquid expelled by the raw uncrushed organism was extracted with EtOAc (2 dm³) 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), cavernulin 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**).

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

Cavernulin A (1) : Colourless semisolid mass (20 mg), R_f 0.35 in CHCl₃-MeOH (97 : 3), $[\alpha]_D$ -56.4° (c 0.42); ν_{max} 3450–3400, 2920, 1770, 1735, 1455, 1370, 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), 26.5 (10) and 238 (40).

Cavernulin B (2) : Colourless semisolid mass (11 mg), R_f 0.3 in CHCl₃-MeOH (95 : 5); $[\alpha]_D$ -50.3° (c 0.20); ν_{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 (14), 315 (10), 312 (12), 281 (5), 280 (19), 222 (9), 196 (16), 169 (20), 167 (49) and 107 (100%).

Acetylation of cavernulin B (2) : 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 B diacetate (**9**; 2 mg) as colourless amorphous mass, R_f 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 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 ester of fatty acids) (4) : Colourless semisolid mass (40 mg), R_f 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, =CHCH₂CH₂), 1.60 (2H, m, H₂-3), 1.25 (br s, x CH₂) and 0.90 (6H, t, J 6.6 Hz, 2 × CH₂CH₃); ¹³C NMR δ 14.0 (2 × CH₃), 22.6 (2 × CH₂CH₃), 25.0 (C-3), 29.7 (x CH₂), 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 (11.7), 22 : 0 (2.8), 25 : 0 (7.5), 16 : 1 (13.5), 18 : 1ω9 (17.8), 18 : 3ω3 (3.2) and 20 : 4ω6 (4.3).

*Ceramide or N-acyl-2-aminosphinga-4,8-diene-1,3-diol*²⁷ (**5**) : Colourless semisolid mass (30 mg), *R_f* 0.5 in CHCl₃-MeOH (95 : 5); δ_H 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, H_A-1), 3.69 (1H, br d, *J* 9.8 Hz, H_B-1), 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); δ_C 14.0 (2 × CH₃), 22.6 (2 × CH₂CH₃), 25.8 (C-3'), 29.5 and 29.7 (*x* CH₂), 31.9 (CH₂CH₂CH₃), 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'). Methanolysis of ceramide fraction by 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°, *R_f* 0.5 in CHCl₃-MeOH (98 : 2); [α]_D + 39°; δ_H 5.34 (1H, br d, *J* 4.6 Hz, H-6), 3.52 (1H, m, H-3), 1.03 (6H, s, H₃-18 and H₃-19), 0.97 (3H, d, H₃-27), 0.84 (3H, d, H₃-26) and 0.68 (3H, d, H₃-21); δ_C 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); *R_f* 0.4 in CHCl₃-MeOH (95 : 5); δ_H 5.60 (m), 3.84 (m, H-2), 3.69 (1H, dd, *J* 11.4 and 3.0 Hz, H_A-3), 3.61 (1H, dd, *J* 11.3 and 5.5 Hz, H_B-3), 3.52 (1H, dd, *J* 9.1 and 4.1 Hz, H_A-1), 3.49 (1H, dd, *J* 9.7 and 5.8 Hz, H_B-1), 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); δ_C 14.0 (CH₃), 22.6 (CH₂CH₂CH₃), 26.1 (C-2'), 29.6 and 29.7 (*x* CH₂), 31.9 (CH₂CH₂CH₃), 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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