New Sesquiterpenoids from the Soft Coral *Sinularia intacta* of the Indian Ocean

Ammanamanchi S. R. Anjaneyulu*, Ponnapalli M. Gowri, and Moturu V. R. Krishna Murthy

School of Chemistry, Andhra University, Visakhapatnam-530 003, India

Received March 31, 1999

Chemical examination of the soft coral species *Sinularia intacta* collected from the Moyli Island of the Gulf of Mannar of the Indian Ocean resulted in the isolation of three new sesquiterpenoids, **1**, **2**, and **3**, along with the known compounds 8,9-secoafricanane-8,9-dione (**5**), $\Delta^{9(15)}$-africanene (**6**), 1-*O*-hexadecyl-2,3-dihexadecanoylglycerol, batyl alcohol, (24*R*)-24-methylcholesterol, gorgosterol, a mixture of two monohydroxy-4α-methyl sterols, 4α,24-dimethylcholestan-3*β*-ol, 4α,24ξ-dimethyl-23ξ-ethylcholestan-3*β*-ol, a mixture of ceramides, and ergost-24(28)-ene-3*β*,5α,6*β*-triol. The structures of the new sesquiterpenoids were established as (9*R*)-africanane-9,15-diol (**1**), (9*R*)-9-methoxyafricanan-15-ol (**2**), and (9*S*)-africanane-9,15-diol-15-monoacetate (**3**) by a study of their spectral data and partial synthesis.

Soft corals are known to produce a large number of sesqui- and diterpenoids besides a host of steroids and other compounds.[1] Soft corals of the genus *Sinularia* form an important group occurring widely in the different coral reefs of the world. More than 30 species of this genus have been chemically examined.[2] In our continuing interest in the bioactive principles from the marine organisms of the Indian Ocean, we have examined quite a good number of soft corals including those of the *Sinularia* genus.[3−5] We have now examined the species *Sinularia intacta* (Alcyoniidae) collected from the Moyli Island of the Gulf of Mannar of the Indian Ocean, and the results are reported here. This species has not been examined earlier.

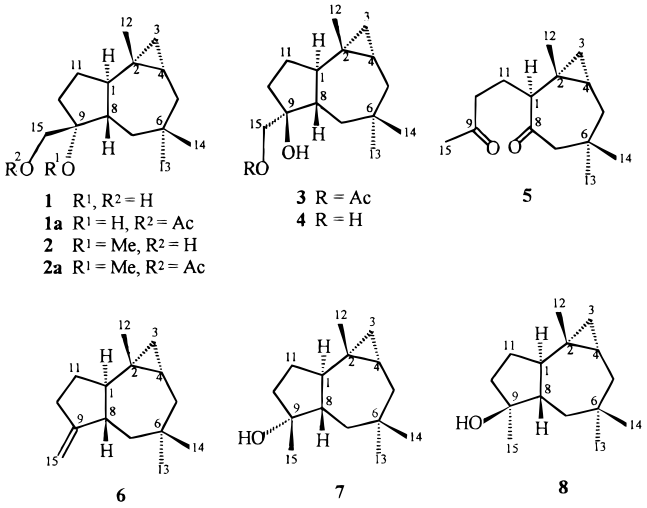
Results and Discussion

The organism was percolated repeatedly with cold methanol. The aqueous methanolic extract, left over after evaporation of the solvent under vacuum, was extracted with ethyl acetate. The ethyl acetate extract after concentration under vacuum left a greenish gummy residue, a part of which when subjected to vacuum liquid chromatography[6] over a column of Si gel using eluants of increasing polarity starting from petroleum ether through ethyl acetate to methanol furnished three new sesquiterpenoids, **1**, **2**, and **3**, in addition to 8,9-secoafricanane-8,9-dione (**5**)[7] and some known steroid and lipid derivatives. (See Chart 1.) The structures of the new sesquiterpenoids were established as (9*R*)-africanane-9,15-diol (**1**), (9*R*)-9-methoxyafricanan-15-ol (**2**), and (9*S*)-africanane-9,15-diol-15-monoacetate (**3**), respectively, by a study of their spectral data and partial synthesis. (9*S*)-Africanane-9,15-diol (**4**), though known by synthesis, has not yet been isolated as such from nature. It was found to be present (through ^{1}H and ^{13}C NMR analysis) in an inseparable mixture along with its epimer (**1**). The other known compounds isolated are $\Delta^{9(15)}$-africanene (**6**),[8a,b] 1-*O*-hexadecyl-2,3-dihexadecanoylglycerol,[9] batyl alcohol,[10] (24*R*)-24-methylcholesterol,[11] gorgosterol,[12a,b] two monohydroxy sterols 4α,24-dimethylcholestan-3*β*-ol[13] and 4α,24ξ-dimethyl-23ξ-ethylcholestan-3*β*-ol,[14] ergost-24(28)-ene-3*β*,5α,6*β*-triol,[15] and a mixture of ceramides.

(9*R*)-Africanane-9,15-diol (**1**), $C_{15}H_{26}O_2$, mp 122−125 °C, $[M]^+$ 238 EI mass, showed broad hydroxylic absorption (3400 cm^{-1}) in its IR spectrum, but no absorption in its UV spectrum. It gave on acetylation with pyridine and acetic anhydride, a monoacetate $C_{17}H_{28}O_3$, mp 115−116 °C, which still showed hydroxylic absorption (3300 cm^{-1}) in addition to the acetate absorption (1735, 1235 cm^{-1}), indicating the presence of two hydroxyls in **9**, one acylable and the other a hindered secondary or a tertiary hydroxyl. In the absence of unsaturation, the molecule should be tricyclic. The ^{1}H NMR spectrum of **1** resembles that of $\Delta^{9(15)}$-africanene (**6**)[8a,b] and related compounds[16,17] (Table 1) in showing the presence of three cyclopropyl protons at δ 0.22 t, J = 3.6 Hz, δ 0.49 m, and δ 0.54 dd, J = 3.6, 6.4 Hz. The acylable hydroxyl was found to be primary by recognizing the α-methylene protons at δ 3.46 and 3.58, each a doublet with J = 10.8 Hz which moved downfield in the spectrum of its monoacetate δ 4.0, 4.18, each d, J = 10.8 Hz. This was also supported by the presence of only three tertiary methyl signals at δ 0.91, 0.95, and 0.99, as for $\Delta^{9(15)}$-africanene, but a hydroxymethyl (15-OH) in the place of one (possibly 15-H_2) methylene group. The unacylable tertiary hydroxyl might be located at C-9, C-1, or C-8.

Dihydroxy africananes have not so far been reported either from terrestrial or from marine sources, while some monohydroxy derivatives are known such as africanol with α-hydroxyl at C-8 from *Lemnalia africana*,[16] isoafricanol

Chart 1



* To whom correspondence should be addressed. Tel.: (0891) 530284. Fax: (0891) 555547/570365. E-mail: pandarth@md3.vsnl.net.in.

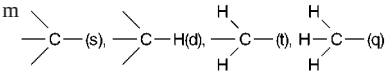
10.1021/np9901480 CCC: $18.00
Published on Web 10/29/1999

Table 1. ^{1}H NMR Data of Compounds (**7**),[17] **1**, **1a**, **2**, **3**, **4**, and (**4**):[8b] Chemical Shifts (δ), *J* in Parentheses (Hz)

position	(**7**)[17] (400 MHz, $CDCl_3$)	**1** (300 MHz, $CDCl_3$)	**1a** (90 MHz, $CDCl_3$)	**2** (300 MHz, $CDCl_3$)	**3** (300 MHz, $CDCl_3$)	**4** (90 MHz, $CDCl_3$)	(**4**)[8b] (60 MHz, $CDCl_3$)
1α-H	1.61 (m)	1.8 (m)		2.16 (m)	2.08 (dt)	1.81 (m)	
3α-H	0.213 (dd, 4.3, 4.8)	0.54 (dd, 3.6, 6.5)	0.55 (m)	0.594 (dd, 3.9, 8.1)	0.55 (m)	0.55 (m)	0.67 (m)
3*β*-H	0.525 (dd, 8.3, 4.8)	0.22 (t, 3.6)	0.2 (m)	0.19 (t, 3.9)	0.2 (m)	0.2 (m)	0.2 (m)
4*β*-H	0.454 (dddd, 4.8, 8.3, 10.9, 6.3)	0.49 (m)		0.486 (m)	0.49 (m)		
5α-H	1.092 (dd, 14.5, 10.9)	1.12 (m)		0.94 (m)	1.08 (m)		
5*β*-H	1.795 (ddd, 2.3, 14.5, 6.3)	1.79 (m)		1.82 (m)	1.81 (m)		
7α-H	1.097 (dd, 12.8, 11.8)	1.15 (m)		1.05 (m)	0.95 (m)		
7*β*-H	1.437 (ddd, 2.3, 12.8, 2.3)	1.39 (dt, 3, 12)		2.1 (m)	1.35 (m)		
8*β*-H	1.65 (m)	1.82 (m)		1.70 (m)		1.82 (m)	
9*β*-H	-						
10α-H		1.1 (m)		1.04 (m)			
10*β*-H		1.4 (m)	1.8−1.6 (m)	1.65 (m)			
11α-H	1.85−1.50 (m)	0.88 (m)		0.85 (m)	1.77−1.5 (m)	1.8−1.5 (m)	
11*β*-H		1.77 (m)	0.95 (s)	2.0 (m)			
12-H_3	0.962 (s)	0.95 (s)	0.90 (s)	1.0 (s)	1.03 (s)	0.95 (s)	1.0 (s)
13-H_3	0.918 (s)	0.91 (s)	0.99 (s)	0.884 (s)	0.89 (s)	0.90 (s)	0.9 (s)
14-H_3	0.987 (s)	0.99 (s)	4.18 (d, 10.8) &	1.01 (s)	0.99 (s)	1.0 (s)	1.05 (s)
15-H_2/H_3	1.250 (s)	3.58 (d, 10.8) & 3.46 (d, 10.8)	4.0 (d, 10.8) 2.1 (s)	3.64 (d, 12) & 3.41 (d, 12)	4.1 (m)	3.55 (m)	3.53 (m) CH_2OH
O*Me*/OAc				3.25 (s)	2.1 (s)		

Table 2. ^{13}C NMR Data of Compounds **7**, **8**, **1**, **1a**, **2**, **3**, and **4**

carbon no.	**7**[a,b] ($CDCl_3$, 75.5 MHz)	**8**[a] ($CDCl_3$, 75.5 MHz)	**1**[b] ($CDCl_3$, 22.5 MHz)	**1a** ($CDCl_3$, 22.5 MHz)	**2**[b] ($CDCl_3$, 22.5 MHz)	**3** ($CDCl_3$, 22.5 MHz)	**4** ($CDCl_3$, 22.5 MHz)
1	48.73 (d)	48.87	49.8 (d)	49.5	49.4 (d)	49.6	50.1
2	20.60 (s)	20.75	20.1 (s)	20.8	20.5 (s)	20.9	20.4
3	23.76 (t)	23.70	23.6 (t)	23.7	23.6 (t)	23.6	23.5
4	21.90 (d)	21.90	21.8 (d)	21.8	21.8 (d)	21.7	21.7
5	43.40 (t)	43.67	43.3 (t)	43.3	43.1 (t)	43.5	43.3
6	33.30 (s)	33.46	33.9 (s)	33.9	33.9 (s)	33.9	33.5
7	43.36 (t)	45.08	44.4 (t)	44.5	44.7 (t)	48.3	48.3
8	48.15 (d)	48.74	44.1 (d)	44.0	43.5 (d)	44.1	44.5
9	81.27 (s)	80.34	82.9 (s)	81.6	87.1 (s)	81.2	82.6
10	41.55 (t)	42.07	37.3 (t)	37.8	31.8 (t)	36.9	36.7
11	23.35 (t)	23.07	23.9 (q)	23.8	23.5 (t)	23.9	23.8
12	19.62 (q)	19.80	19.7 (q)	19.7	19.9 (q)	20.5	19.7
13	34.06 (q)	34.07	33.3 (q)	33.3	33.6 (q)	33.3	34.0
14	24.47 (q)	24.17	24.2 (q)	24.3	23.8 (q)	24.3	24.3
15	25.73 (q)	22.47	68.7 (t)	70.2	62.8 (t)	67.6	65.3
16				171.2	50.1 (s)	171.5	
17				20.3		20.7	

[a] Data taken from the ref 17. [b] Substitution pattern derived from the DEPT spectrum 

(C-8 epimer), leptographiol (**7**) and isoleptographiol (**8**), epimeric C-9 hydroxyafricananes from *Leptographium lundbergii*,[17] and a 5-hydroxy derivative from *Senecio oxyriifolius*.[18] The position of the tertiary hydroxyl in **1** was taken to be at C-9 by consideration of the carbon-13 chemical shifts. The ^{13}C NMR spectrum of **1** showed 15 signals whose substitution pattern was derived from its DEPT spectrum. The chemical shifts (Table 2) of the respective carbons were assigned by a comparative study of the related compounds.[8a,b,16,17] The two oxygenated carbons came at δ 68.7 (t) and 82.9 (s). The latter value, assignable to a carbon bearing a tertiary hydroxyl, is closer in its chemical shift to the value δ 81.27 of C-9 bearing hydroxyl in leptographiol (**7**) than to the values of C-8 bearing hydroxyl in africanol[17] (δ 87.3) and isoafricanol[17] (δ 85.81), suggesting that **1** also has the hydroxyl at C-9. The deviation (1.63 ppm) noticed in the chemical shift of C-9 of **1** to that in leptographiol (**7**) might be due to the deshielding influence of the vicinal hydroxyl at C-15. The chemical shifts of C-8 (δ 44.1) and C-10 δ (37.3) in **1** moved upfield (~4.6 ppm) when compared to the values of the same carbons in leptographiol (**7**), consistent with the influence of the 15-OH on the β-carbons (C-8 and C-10).[19] The chemical shifts of the remaining carbons were very close in **7** and **1**. The appearance of methyl carbons C-13 and C-14 with a large difference of (9 ppm) chemical shift as noticed in leptographiol[17] and $\Delta^{9(15)}$-africanene[8a,b] indicated trans configuration between the five- and seven-membered rings.

To provide further proof for the structure of **1**, it was aimed to obtain this diol by partial synthesis from $\Delta^{9(15)}$-africanene (**6**) by oxidation with osmium tetroxide. A literature survey revealed that such a diol[17] was obtained from $\Delta^{9(15)}$-africanene, but its ^{13}C NMR data were not recorded. In the same reaction repeated now, only one (>90%) diol (**4**) could be isolated from the reaction, presumably by the regioselective attack of OsO_4 from the less hindered side of the $\Delta^{9(15)}$ double bond. The ^{13}C NMR spectral characteristics of **4** agreed to a large extent with the corresponding values of **1** (Table 2), except for the chemical shifts of two carbons, C-7 and C-15. The C-7 signal appeared at lower field (~4 ppm), while the C-15 signal appeared at higher field by 3.4 ppm in **1** (δ 44.4, 68.7)

compared to those of **4** (δ 48.3, 65.7). Thus compounds **1** and **4** are C-9 epimers, and since the relative and absolute configuration of the diol **4** was decided by X-ray analysis as (9*S*)-africanane-9,15-diol (**4**), the relative and absolute configuration of **1** could be taken as (9*R*)-africanane-9,15-diol (**1**) with a β-CH_2OH at C-9. It is noteworthy to see a similar effect on the chemical shifts of C-7 and C-15 (δ 43.36, 25.73) in leptographiol (**7**), with a β-methyl at C-9 compared to the values (δ 45.08, 22.47) in its C-9 epimer, isoleptographiol (**8**) (Table 2).

2D NMR spectral ($^1H-^1H$, $^1H-^{13}C$ COSY, and $^1H-^1H$ NOESY) data obtained for **1** on a 90 MHz instrument revealed some partial correlations (experimental) in support of the structure of the molecule. The structure was also supported by its mass fragmentation. The molecular ion $[M]^+$ 238, though of low intensity, is distinctly seen followed by the ion *m/z* 220 $[M - 18]^+$ and *m/z* 207 $[M - CH_2OH]^+$, the latter forming the base peak. This was followed by the ion *m/z* 189 $[M - CH_2OH - H_2O]^+$.

(9*R*)-9-Methoxyafricanan-15-ol (**2**), mp 118−120 °C, was analyzed for $C_{16}H_{28}O_2$, and this was supported by the mass ion *m/z* 220 $[M - MeOH]^+$ (100%) in its EI mass. Its spectral characteristics closely resemble those of **1**. It showed hydroxylic (3300 cm^{-1}) and methoxyl (2880 cm^{-1}) absorptions in its IR spectrum, suggesting that it might be a monomethyl ether of **1**, which was also supported by the presence of methoxy protons at δ 3.25 in its ^1H NMR spectrum (Table 1). The tertiary hydroxyl at C-9 or the primary hydroxyl at C-15 of **1** might be in the form of methyl ether in **2**. On acetylation with pyridine, acetic anhydride **2** gave a monoacetate, oil, $C_{18}H_{30}O_3$, which showed the acetate (1735 and 1225 cm^{-1}) and methoxyl (2880 cm^{-1}) absorptions, suggesting that **2** has a free hydroxymethyl and a tertiary hydroxyl as a methyl ether, as otherwise it would not have been acetylated. Consistently, in the ^1H NMR spectrum of the acetate (**2a**), α-acetoxymethylene protons moved downfield to δ 4.15 from 3.58, 3.46 in **1**.

The ^{13}C NMR spectrum of **2** showed 16 carbon signals whose substitution pattern was derived from the DEPT spectrum, and the chemical shift assignments were made by comparison with related compounds[8a,b,16,17] (Table 2). The ^{13}C values of all the carbons of **2** agreed closely with those of **1** except for the difference in the values of three carbons, C-9, C-10, and C-15. The C-9 in **1** (δ 82.9) moved downfield in **2** (δ 87.1), while the C-15 in **1** (δ 68.7) moved upfield in **2** (δ 62.8). A similar upfield shift of 5.5 ppm was also noticed on C-10 in **2**. These differences between **1** and **2** could be expected because of the different functional groups present in them at C-9, a methoxyl in **2** in place of a tertiary hydroxyl in **1**, and the consequent downfield and upfield shifts on the α- and β-carbons, respectively.[19] Except for the differences in the chemical shifts of the above three carbons, the shifts of the remaining carbons in **1** and **2** are very close and suggest that they have same relative stereochemistry, and hence **2** is (9*R*)-9-methoxyafrican-15-ol. The structure was supported by its mass fragmentation. Although it did not exhibit the molecular ion, it showed the ion at *m/z* 220 $[M - MeOH]^+$, which formed the base peak, followed by the ion at *m/z* 188 $[M - 2\ MeOH]^+$, next in intensity (57%).

(9*S*)-Africanane-9,15-diol-15-monoacetate (**3**), colorless oil, was analyzed for $C_{17}H_{28}O_3$, and this was supported by its EI mass spectrum, $[M]^+$ 280. It exhibited hydroxylic (3300 cm^{-1}) and acetate absorptions (1740 and 1230 cm^{-1}) in its IR spectrum. Its ^1H NMR spectrum (Table 1) resembled those of compounds **1** and **2**, suggesting that it might be an acetate of **1**. The signals at δ 4.0, d, J = 10.8 Hz, 4.3 d, J = 10.8 Hz, each integrating for one proton, account for the α-acetoxymethylene protons, suggesting that **3** might be the 15-acetate of **1** with the tertiary hydroxyl free at C-9. But **3** differed in its ^{13}C NMR chemical shifts (Table 2) of C-7 (δ 48.3) and C-15 (δ 67.6) carbons from the corresponding carbons of the acetate (**1a**) of **1**, C-7 (δ 44.5) and C-15 (δ 70.2). On the other hand, the former values in **3** agreed with those (δ 48.4, 67.7) of the acetate of **4**, the synthetic diol, suggesting that **3** is not the acetate of **1**, but the acetate of its epimeric diol **4**. The structure of **3** could thus be deduced as (9*S*)-africanane-9,15-diol-15-monoacetate. The structure was also supported by its mass fragmentation. Its molecular ion $[M]^+$ 280, though of low intensity, could be noticed, followed by the ions at *m/z* 263 $[M - OH]^+$, *m/z* 220 $[M - 60]^+$, and *m/z* 207 $[M - CH_2OAc]^+$, the last one forming the base peak.

The diol **4**, epimeric to **1**, though known earlier by synthesis, has not yet been isolated from any natural source. It could now be detected, though not isolated as such, in an inseparable mixture along with **1** (as revealed from the ^{13} C NMR spectrum of the mixture, which showed the presence of C-7 and C-15 carbons of both the compounds: of **1** at δ 44.4 and 68.7 and of **4** at δ 48.3 and 65.3.

A solid that separated from the EtOAc/MeOH (9.5:0.5) fraction of the main column showed a single spot on TLC, but was later found to be a mixture of ceramides from its diagnostic spectral values.[20] The IR spectrum showed the hydroxylic (3349 cm^{-1}), amide (3224, 1610 cm^{-1}), and olefinic absorptions (980 cm^{-1}). Its ^1H NMR spectrum showed the NH proton doublet at δ 8.60 (J = 9 Hz), CH_2-OH δ 3.5 (m), and >*CH*OH δ 4.25 to 4.75, 4H (m). Its ^{13}C NMR spectrum showed signals for the amide carbonyl (δ 174.2), olefinic carbons (δ 130.5 and 130.7), and four oxygenated carbons at (δ 61.7, 72.8, 72.3, and 76.4). Its mass spectrum, however, gave inconsistent mass fragmentation by EI, CI modes. The structural elucidation of the individual ceramides will be undertaken after separation of the pure compounds.

Experimental Section

General Experimental Procedures. Elemental analyses were determined on a Carlo Erba-1108 instrument. UV spectra were recorded on a Milton Roy 1201 spectrophotometer. IR spectra were recorded on a Perkin-Elmer 840 spectrophotometer. ^1H NMR spectra were measured on a Bruker 400 MHz, a Gemini 200 MHz, or a JEOL JNM EX-90 spectrometer using $CDCl_3$ as solvent and tetramethylsilane as internal reference. ^{13}C NMR spectra were measured on a JEOL JNM EX-90 spectrometer at 22.5 MHz using $CDCl_3$ as solvent and TMS as internal reference. Mass spectra were obtained on a JEOL JMS-300 spectrometer. Melting points were determined on a VEB-analytic Dreder HMK hot plate and are uncorrected. Optical rotations were determined on a Rudolph Autopol III polarimeter.

Animal Material. The soft coral *S. intacta* is abundantly available at the Moyli Island of the Gulf of Mannar (9° 16′ N, 79° 12′ E) of the Indian Ocean, from where it was collected in June 1993. It was described as *S. intacta* contaminated with a little of *Sinularia maxima* by Dr. Phil Alderslade, Curator, Museum and Art Gallery of the Northern Territory, and the specimens were preserved at the museum as NTMC-12479 and NTMC-12483 and at the Museums of NIO, Goa, and School of Chemistry, Andhra University, as AU1-080.

Extraction and Isolation. The soft coral was sliced into small pieces, and the specimens were percolated with methanol (5 × 5 L). The aqueous methanolic concentrate was extracted with ethyl acetate (8 × 500 mL), and the combined ethyl acetate extract was dried over anhydrous $MgSO_4$ and concen-

trated in vacuo to yield a dark greenish gummy residue (60 g) The dry weight of the organism after extraction was 3.5 kg. A part of the above residue (40 g) was subjected to vacuum liquid chromatography[6] over a column of Si gel (450 g, 230−400 mesh Sigma) using eluants of increasing polarity starting from petroleum ether through ethyl acetate to methanol. Elution of the column with petroleum ether afforded $\Delta^{9(15)}$-africanene (**6**): colorless oil; 60 mg; $[\alpha]_D^{25}$ +86° (*c* 1.1, $CHCl_3$), identified by comparison of its physical and spectral (IR, ^1H and ^{13}C NMR) data.[8a,b] Elution with a mixture of petroleum ether/ethyl acetate (9.8:0.2) gave the fatty glyceride 1-*O*-hexadecyl-2,3-dihexadecanoylglycerol: colorless prisms (acetone); 20 mg; mp 60−61 °C; $[\alpha]_D^{25}$ +7.5° (*c* 1.0, $CHCl_3$); identified by comparison of its physical and spectral (IR, ^1H and ^{13}C NMR, and mass) data.[9] Elution with a mixture (9:1) of petroleum ether/ethyl acetate gave a mixture of monohydroxy sterols: colorless needles (chloroform-methanol); 200 mg; mp 145−146 °C. Its positive Liebermann-Burchard test with a play of colors (pink, blue, green) and ^1H NMR spectrum suggested its steroid nature. Its acetyl derivative (Ac_2O−Py) showed a single but not sharp spot on 20% silver nitrate−Si gel TLC plate, indicating it to be a mixture of monohydroxy sterols. The acetate mixture by GC/MS analysis was found to consist of the acetates of 4α,24-dimethylcholestan-3β-ol,[13] R_t (min) 3.46, major fragmentation peaks 398(20), 382(20), 314(50), 271 (22), 255(5), 229(4), 161(15), 159(20), 119 (20), 69(52), 55 (100), 43-(86), and 4α,24ξ-dimethyl-23ξ-ethylcholestan-3β-ol,[14] R_t (min) 4.70, major fragmentation peaks 426(27), 355(8), 314(45), 299 (25), 271(75), 255(35), 299(28), 213(35), 161(20), 159(50), 83 (40), 55(85),43(100).

Elution of the column with a mixture of petroleum ether/ethyl acetate (8.5:1.5) gave a fraction consisting of some close running compounds, which on rechromatography over a column of Si gel furnished (**1**−**5**) in addition to gorgosterol, (22*R*,23*R*,24*R*)-22,23-methylene-23,24-dimethylcholest-5-en-3β-ol, colorless needles (methanol); 20 mg; mp 186.5−188 °C; $[\alpha]_D^{25}$ −45° (*c* 1.0, $CHCl_3$); identified by comparison of its physical and spectral (IR, ^1H NMR and mass) data,[11] and (24*R*)-24-methylcholesterol, colorless needles (chloroform/methanol); 20 mg; mp 151−152 °C; $[\alpha]_D^{25}$ −33° (*c* 1.0, $CHCl_3$); identified by comparison of its physical and spectral (IR, ^1H and ^{13}C NMR) data.[12]

Elution with a mixture (7:3) of petroleum ether/ethyl acetate gave 3-octadecyloxy-1,2-propanediol colorless flakes (methanol): 200 mg; mp 69−71 °C; $[\alpha]_D^{25}$+2.6° (*c* 1.0, $CHCl_3$), identified by comparison of its physical and spectral (IR, ^1H and ^{13}C NMR) data.[13] Further elution of the main column with a mixture of ethyl acetate/methanol (9.5:0.5) furnished ergost-24(28)-ene-3β,5α,6β-triol: colorless needles (chloroform/methanol); 20 mg; mp 241−242 °C; $[\alpha]_D^{25}$ −4° (*c* 0.26,C_5H_5N), identified by comparison of its physical and spectral (IR, ^1H and ^{13}C NMR) data,[14] and a mixture of ceramides.

Mixture of Ceramides. A solid separated from the ethyl acetate/methanol (9.5:0.5) fraction, although it showed a single spot on TLC, was found to be a mixture of ceramides from its spectral characteristics: colorless flakes (chloroform/methanol) 25 mg; mp 145−146 °C; IR (Nujol) ν_{max} 3349, 3224, 1610, 1526, 1451, 1366, 1015, 980 cm^{-1}; ^1H NMR (C_5D_5N, 300 MHz) δ 8.60 (d, 9), 5.55 (d, 5.4), 4.25 to 4.75 (m, 4H), 3.5 (m, 2H), 3.4 to 3.89 (m, 4H), 0.88 (t, 6.6), 5.11 (m), and 1.28 (brs); ^{13}C NMR (C_5D_5N, 22.5 MHz) 174.2, 130.5, 130.7, 76.3, 72.8, 72.3, 61.7, 52.8, 35.4, 33.0, 32.7, 31.9, 30.1, 29.8, 29.4, 29.3, 26.4, 25.5, 22.7,14.1.

(9*R*)-Africanane-9,15-diol (1): colorless needles (hexane/acetone); 35 mg; mp 122−125 °C; $[\alpha]_D^{25}$ +15.3° (*c* 1.2, $CHCl_3$); IR (Nujol) ν_{max} 3400, 3080, 2950, 2850, 1235 cm^{-1}; UV ($CHCl_3$) λ_{max} no characteristic absorption above 200 nm;^1H and ^{13}C NMR data (see Tables 1 and 2); partial correlations noticed in its $^1H-^1H$ COSY spectrum, 3β-H (0.22) with 3α-H (0.54); 4β-H (0.49) with 5β-H (1.79) and 5α-H (1.12); NOESY correlations 3β-H with 4β-H; 3β-H and 4β-H with 12-H_3 (0.95); 4β-H with 5β-H (1.79); and $^1H-^{13}C$ COSY spectrum, 3-H (0.22) with C-3 (23.6), 10-H (1.6) with C-10 (37.3), 12-H_3 (0.95) with C-12 (19.7), 13-H_3 (0.91) with C-13 (33.3), 14-H_3 (0.99) with C-14 (24.2) and 15-H_2 (3.5 and 3.4) with C-15 (68.7); EI mass spectrum *m/z* 238 $[M]^+$ (9.1), 220 $[M - H_2O, 43]^+$, 208 (20.1), 207 $[M - CH_2OH]^+$, 100), 189 (22.1), 137 (10.7), 109 (22.1), 95 (32), 83 (20), 69 (18), 55 (4); *anal*. C 75.3%, H 10.6%, calcd for $C_{15}H_{26}O_2$, C 75.6%, H 10.9%.

Acetylation of (1). To **1** (15 mg) in dry pyridine (0.5 mL) was added acetic anhydride (0.5 mL), and the mixture was allowed to stand at room temperature for about 12 h. After the usual workup it yielded (9*R*)-africanane-9,15-diol-15-monoacetate (**1a**), colorless needles (hexane-acetone): 10 mg; mp 115−116 °C; $[\alpha]_D^{25}$ 23.4° (*c* 1.0, $CHCl_3$); IR ν_{max} (Nujol) 3300 (hydroxyl), 3070, 2952, 2890, 1735, 1235,1020 cm^{-1}; EIMS, 280 $[M]^+$ (11.2), 220 $[M - AcOH]^+$ (11.8), 207 (98.0), 203 (67.7), 189 (21.3), 159 (23.0), 133 (32.3), 131 (16.5), 95 (70), 69 (50), 56 (95); *anal*. C 72.6%, H 9.9%, calcd for $C_{17}H_{28}O_3$, C 72.8%, H, 10.2%.

OsO_4 Oxidation of $\Delta^{9(15)}$-Africanene, Isolation of (9*S*)-Africanane-9,15-diol (4). A solution of $\Delta^{9(15)}$-africanene (50 mg, 0.0002 mol) and osmium tetroxide (50 mg, 0.00019 mol) in pyridine (6 mL) was stirred at 50 °C using a magnetic stirrer for 12 h and then cooled. To this was added a solution of sodium bisulfite (1.0 g) in water (10 mL) and pyridine (10 mL) with continued stirring to hydrolyze the osmate ester. The mixture was extracted with ethyl acetate (3 × 30 mL), and the ethyl acetate solution was washed with dilute HCl (2 × 5 mL) to remove the excess of pyridine and then neutralized with $NaHCO_3$ solution. Evaporation of the organic layer showed two spots on TLC. The mixture on chromatography over a small column of Si gel afforded (9*S*)-africanane-9,15-diol (**4**), 25 mg, and unreacted $\Delta^{9(15)}$-africanene (**6**), 20 mg.

Diol (4): colorless needles (hexane/acetone); mp 112−114 °C; $[\alpha]_D^{25}$ +53.4° (*c* 0.05, $CHCl_3$); IR (Nujol) ν_{max} 3400, 3070, 2990, 2875, 1015 cm^{-1}; UV ($CHCl_3$) λ_{max} no absorption above 200 nm; ^1H and ^{13}C NMR data (see Tables 1 and 2); *anal*. C 75.3%, H 10.5%, calcd for $C_{15}H_{26}O_2$, C 75.6%, H 10.9%.

Acetylation of (4). To the diol **4** (20 mg) in dry pyridine (0.6 mL) was added acetic anhydride (0.6 mL) and the solution left overnight. After the usual workup, it yielded a monoacetyl derivative (**3**): oil, 15 mg; $[\alpha]_D^{25}$ +60.2° (*c* 0.05, $CHCl_3$); ^1H and ^{13}C NMR data (see Tables 1 and 2); EIMS *m/z* 280 $[M]^+$ (7.5), 220 $[M - AcOH]^+$ (12.6), 207 $[M - AcOH - Me]^+$ (100), 189 $[M - AcOH - Me - H_2O]^+$, (21.1), 159 (28.5), 95 (40), 83 (60), 44 (40). The physical and spectral characteristics of this acetate were found to be identical with those of the natural acetate (**3**) described below.

(9*R*)-9-Methoxyafricanan-15-ol (2): colorless needles (hexane/acetone); 30 mg; mp 118−120 °C; $[\alpha]_D^{25}$ −22°(*c* 1.2, $CHCl_3$); IR (Nujol) ν_{max} 3300, 3070, 2950, 2880, 1100, 1020 cm^{-1}; UV ($CHCl_3$) λ_{max} no absorption above 200 nm; ^1H and ^{13}C NMR data (see Tables 1 and 2); EIMS *m/z* 220 $[M - MeOH]^+$ (100%), 188 $[M - 2\ MeOH]^+$ (56.9), 146 (19.1), 132 (24.3), 95 (34), 81 (25), 69 (26), 55 (28), 42 (35); *anal*. C 75.9%, H 10.9%, calcd for $C_{16}H_{28}O_2$, C 76.2%, H 11.1%.

Acetylation of (2). To **2** (18 mg) in dry pyridine (0.5 mL) was added acetic anhydride (0.5 mL) at room temperature and the solution left overnight. After the usual workup, it yielded (9*R*)-9-methoxyafricanane-15-monoacetate (**2a**) as a colorless oil: 15 mg; IR (Nujol) ν_{max} 3070, 2880, 1735, 1225 cm^{-1}; ^1H NMR ($CDCl_3$, 90 MHz) 0.55 (m, 2H, 3α-H and 4β-H)), 0.21 (m, 3β-H), 1.0 (s, 12-H_3), 0.89 (s, 13-H_3), 1.1 (s, 14-H_3), 4.15 2H, Abq J = 12 Hz, 3.25 (s, 15-H_3), and 2.1 (s, OCOCH_3); *anal*. C 73.3%, H 9.8%, calcd for $C_{18}H_{30}O_3$ C 73.4%, H 10.2%.

(9*S*)-Africanane-9,15-diol-15-monoacetate (3): colorless oil; 25 mg; $[\alpha]_D^{25}$ −26° (c 1.5, $CHCl_3$); IR (Nujol) ν_{max} 3300, 3070, 2952, 2890, 1740, 1230, 1030 cm^{-1}; ^1H and ^{13}C NMR data (see Tables 1 and 2), EIMS *m/z* 280 $[M]^+$ (11.2), 220 $[M - AcOH]^+$ (11.8), 207 (98), 203 (67.7), 189 $[M - AcOH - Me]^+$ (21.3), 95 (67), 69 (50), 56 (96); *anal*. C 72.5%, H 9.8%, calcd for $C_{17}H_{28}O_3$ C 72.8%, H 10.2%.

Mixture of (9*R*)-Africanane-9,15-diol (1) and (9*S*)-Africanane-9,15-diol (4). Although this fraction showed a single spot on TLC, its ^{13}C NMR spectrum showed it to be a mixture of (9*R*)-africanane-9,15-diol (**1**) and (9*S*)-africanane-9,15-diol (**4**), by the presence of the diagnostic C-7 and C-15

NMR signals of both, δ 44.4, 68.7 of the former and δ 48.1, 65.3 of the latter. For ^{1}H and ^{13}C NMR spectral data, see Tables 1 and 2.

8,9-Secoafricanane-8,9-dione (5):[10] colorless oil; 60 mg; $[\alpha]_D^{25}$ +120° (*c* 2.0, $CHCl_3$); IR (Nujol) ν_{max} 3070, 2980, 1687, 1456, 1375, 1456, 1156, 1018, 973, 751, 606 cm^{-1}; UV (hexane) λ_{max} 209.4 nm, UV ($CHCl_3$) λ_{max} 241.6 nm (for full details see ref 10).

Acknowledgment. We are grateful to the Department of Ocean Development, New Delhi, for financial support; the Head, RSIC, Lucknow, for providing spectral data; and the CSIR for sanctioning Emeritus Scientist Scheme to the senior author (A.S.R.A.).

References and Notes

(1) Faulkner, D. J. *Nat. Prod. Rep.* **1999**, *16*, 155−198, and the earlier reviews in this series and the references therein.
(2) Anjaneyulu, A. S. R.; Venkateswara Rao, G. *J. Sci. Ind. Res.* **1995**, *54*, 637−664.
(3) Anjaneyulu, A. S. R.; Sagar, K. S.; Venugopal, M. J. R. V. *Tetrahedron* **1995**, *51*, 10997−11010.
(4) Anjaneyulu, A. S. R.; Venugopal, M. J. R. V.; Sarada, P.; Rao, G. V.; Clardy, J.; Lobkovsky, E. *Tetrahedron Lett.* **1998**, *39*, 135−138.
(5) Anjaneyulu, A. S. R.; Venugopal, M. J. R. V.; Sarada, P.; Clardy, J.; Lobkovsky, E. *Tetrahedron Lett.* **1998**, *39*, 139−142.
(6) Targett, N. M.; Kilcoyne, J. P.; Green, B. *J. Org. Chem.* **1979**, *44*, 4962−4964.
(7) Anjaneyulu, A. S. R.; Mangala Gowri, P. *Indian J. Chem. Sect. B* **1999**, *38*, 4−7.
(8) (a) Kashman, Y.; Bodner, M.; Finer-Moore, Janet S.; Clardy, J. *Experientia* **1980**, *36*, 891−892. (b) Braekman, J. C.; Daloze, D.; Tursch, B.; Hull, S. E.; Declercq, J. P.; Germain, G.; Van Meerssche, M. *Experientia* **1980**, *36*, 893.
(9) Subrahmanyam, C.; Venkateswara Rao, C.; Anjaneyulu, V.; Satyanarayana, P.; Subba Rao, P. V.; Ward, R. S.; Pelter, A. *Tetrahedron* **1992**, *48*, 3111−3120.
(10) Anjaneyulu, A. S. R.; Krishna Murthy, M. V. R.; Rao, G. V. *Tetrahedron* **1997**, *53*, 9301−9312.
(11) Rubinstein, I.; Good, L. J.; Clauge, A. D. N.; Mulherin, J. *Phytochemistry* **1976**, *15*, 195.
(12) (a) Hale, R. L.; Leclercq, J.; Tursch, B.; Djerassi, C.; Gross, R. A.; Weinheimer, A. J.; Gupta K.; Scheuer, P. J. *J. Am. Chem. Soc.* **1970**, *92*, 2179−2180. (b) Ling, N. C.; Hale, R. L.; Djerassi, C. *J. Am. Chem. Soc.* **1970**, *92*, 5281−5282.
(13) Kokke, W. C. M. C.; Bohlin, L.; Fenical, W.; Djerassi, C. *Phytochemistry* **1982**, *21*, 881−887.
(14) Anjaneyulu, A. S. R.; Sarada, P. *J. Chem. Res., Miniprint*, in press.
(15) Kobayashi, M.; Krishna, M. M.; Haribabu, B.; Anjaneyulu, V. *Chem. Pharm. Bull.* **1993**, *41*, 87−89.
(16) Tursch, B.; Braekman, J. C.; Daloze, D.; Fritz, P.; Kelecon, A.; Karlsson, R.; Losman, D. *Tetrahedron Lett.* **1974**, *9*, 747−750.
(17) Abraham, W. R.; Ernst, L.; Witte, L.; Hanssen, H. P.; Sprecher, E. *Tetrahedron* **1986**, *42*, 4475−4480.
(18) Bohlmann, F.; Zdero, C. *Phytochemistry* **1978**, *17*, 1669−1671.
(19) Silverstein, R. M.; Bassler, G. C.; Morrill, T. C. *Spectroscopic identification of organic compounds*, IVth ed.; John Wiley & Sons: New York, 1981; p 266.
(20) Shin, J.; Seo, Y. *J. Nat. Prod.* **1995**, *55*, 948−953.

NP9901480