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METABOLITES OF THE SOFT CORAL *SINULARIA OVISPICULATA* FROM THE INDIAN OCEAN[1]

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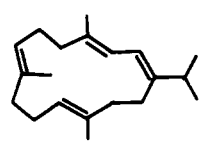
ABSTRACT.—The soft coral *Sinularia ovispiculata* collected on the coasts of the Andaman and Nicobar Islands of the Indian Ocean yielded two new metabolites, (2*E*,7*E*) -4, 11-dihydroxy-1,12-oxidocembra-2,7-diene [**4**] and (2*E*,7*E*)-4,11-dihydroxy-1,12-oxidocembra-2,7,15-triene [**7**], in addition to three known cembrane diterpenes **1–3**, four polyhydroxysterols, (24S)-24-methylcholestane-3β,5α,6β,25-tetraol, (24*S*)-24-methylcholestane-3β,5α,6β,25-tetraol 25-monoacetate, 24-methylenecholest-5-ene-3β,7β,16β-triol-3-*O*-α-L-fucopyranoside, and 24-methylenecholestane-1α,3β,5α,6β-tetraol (numersterol A), and pregnenolone. Structural elucidation of all compounds was carried out through spectral analysis and chemical reactions.

Soft corals of the genus *Sinularia* are a rich source of novel terpenoids and steroids (1–5). The soft coral *Sinularia ovispiculata* Tixier-Durivault (Alcyoniidae) collected on the coasts of the Andaman and Nicobar Islands of the Indian Ocean was examined, and the results are reported in this communication.

Si gel chromatography of the EtOAc-soluble portion of the 95% aqueous EtOH extract of the soft coral afforded two new compounds: (2*E*,7*E*)-4,11-dihydroxy-1,12-oxidocembra-2,7-diene [**4**] and (2*E*,7*E*)-4,11-dihydroxy-1,12-oxidocembra-2,7,15-triene [**7**]. Also isolated were known compounds: batyl alcohol (6), pregnenolone (7), isoneocembrene A [**1**] (8), 11,12-epoxyisoneocembrene A [**2**] (9), nephthenol [**3**] (10), (24S)-24-methylcholestane-3β, 5α, 6β, 25-tetraol 25-monoacetate (11,12), (24S)-24-methylcholestane-3β,5α,6β,25-tetraol (11,12), 24-methylenecholest-5-ene-3β, 7β,16β-triol-3-*O*-α-L-fucopyranoside (13), 24-methylenecholestane-1α,3β, 5α,6β-tetraol (numersterol A) (14), and a mixture of monohydroxysterols.

Compound **4** was obtained as colorless crystals, $[\alpha]^{25}D$ +113.5° (*c*=0.1, $CHCl_3$) and analyzed for $C_{20}H_{34}O_3$ (hrms $[M]^+$ 322.2493). Its ir spectrum has bands for hydroxyl (3500 cm^{-1}) and ether (1060 cm^{-1}) functions. The ^1H-nmr spectrum contained the signals of two secondary methyls at δ 0.82 and 0.86 (3H, d each, 7.0 Hz), two tertiary methyls at δ 1.07 and 1.31 (3H, s each), an olefinic methyl at δ 1.68 (3H, brs), three olefinic protons at δ 5.18 (1H, dd, 9.6, 5.4 Hz), 5.55 and 5.82 (each 1H, d, 16.0 Hz), and a hydroxymethine at δ 3.48 (1H, dd, 1.0, 11.0 Hz). The presence of a *trans*-disubstituted double bond flanked by quaternary carbons was recognized by the multiplicities and coupling constants of the two olefinic proton resonances at δ 5.55 and 5.82 (1H, d each, 15.6 Hz). The compound formed a monoacetate **5** which also showed absorption band for a hydroxyl group in the ir spectrum. The ^{13}C-nmr spectrum of **4** contained four oxy-

[1]Part of the investigation was submitted in May 1989 for presentation at 17th IUPAC International Symposium on the Chemistry of Natural Products, New Delhi, India, Feb. 4–9, 1990 (Abstract p. 234). The organism was noted as *Alcyonium* sp. but was subsequently identified as *Sinularia ovispiculata*.



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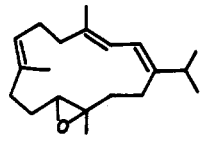

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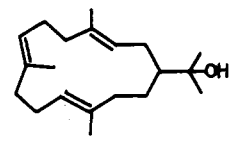

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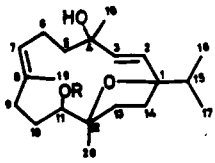
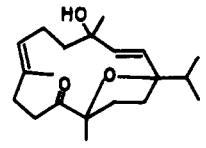


4 R=H
5 R=Ac



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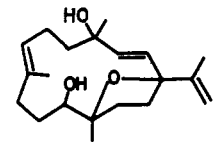

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genated carbon signals at δ 88.2 (s), 84.6 (s), 76.2 (d), and 74.4 (s), two of which must be assigned to an ether ring, possibly five-membered, and this is supported by strong ir absorption at 1060 cm^{-1}. The overall spectral characteristics suggest a cembrane carbon skeleton.

Interpretation of the ^{1}H- and ^{13}C-nmr data (Tables 1 and 2) strongly suggested a five-membered ether ring between C-1 and C-12 with the disubstituted olefin at C-2, a tertiary hydroxyl at C-4, and a trisubstituted olefin. This proposal was supported by the chemical transformation described below.

Oxidation of **4** with Collins reagent gave ketone **6**, which showed no absorption above 210 nm in the uv spectrum

TABLE 1. Selected ^{1}H-nmr Data of Compounds **4**–**7**.[a]

Proton	Compound			
	4	**5**	**6**	**7**
Me-16	0.82 d, 7.0	0.82 d, 7.0	0.81 d, 7.0	—
Me-17	0.86 d, 7.0	0.86 d, 7.0	0.86 d, 7.0	1.72 br s
Me-18	1.31 s	1.32 s	1.30 s	1.28 s
Me-19	1.68 br s	1.68 br s	1.68 br s	1.68 br s
Me-20	1.07 s	1.10 s	1.32 s	1.13 s
H-11	3.48 dd, 1.0, 11.0	4.68 br d, 11.0	—	3.49 dd, 1.0, 11.0
H-2	5.55 d, 15.6	5.60 d, 16.0	5.60 d, 16.0	5.65 d, 15.4
H-3	5.82 d, 15.6	5.82 d, 16.0	5.82 d, 16.0	5.83 d, 15.4
H-7	5.18 dd, 9.6, 5.4	5.20 m	5.20 m	5.20 t, 7.4
H-16	—	—	—	4.75, 4.98 br s
Acetoxyl methyl	—	1.98 s	—	—

[a]Spectra of **4** and **7** in $CDCl_3$ at 360 MHz; spectra of **5** and **6** at 90 MHz in $CDCl_3$.

TABLE 2. ^{13}C-nmr Data of Compounds **4** and **7**.[a]

Carbon	Compound	
	4	**7**
C-1	84.6	87.5
C-2	129.0	130.5
C-3	137.7	136.1
C-4	74.4	74.0
C-5	42.9	43.6
C-6	28.4	24.0
C-7	128.4	127.6
C-8	133.6	133.5
C-9	29.6	29.6
C-10	34.6	35.9
C-11	76.2	75.4
C-12	88.2	85.3
C-13	36.6	34.6
C-14	35.2	34.5
C-15	39.2	149.5
C-16	18.4	109.2
C-17	17.6	19.0
C-18	29.3	28.8
C-19	16.7	17.1
C-20	20.0	19.6

[a]Spectra **4** and **7** are recorded in $CDCl_3$ at 50.4 MHz.

indicating lack of conjugation with the double bonds. However, the resonance of the methyl group on C-12 shifted downfield by 0.23 ppm (δ 1.07 in **4**, δ 1.30 in **6**), suggesting its position as alpha with respect to the carbonyl. The ^{1}H-^{1}H COSY spectrum of **4** showed that the hydroxymethine at δ 3.48 is coupled to an upfield proton at δ 1.81. This observation favors the position of secondary hydroxyl on C-11 rather than on C-5. The stereochemistry of **4** at C-1, C-4, C-11, and C-12 could not, however, be ascertained. Compound **4** may therefore, be described as (2*E*,7*E*)-4,11-dihydroxy-1,12-oxidocembra-2,7-diene [**4**]. This new cembranoid possesses a C-1 to 12 ether linkage which has not been reported before in marine cembranoids.

Compound **7** was obtained as colorless crystals, $[\alpha]^{25}D$ +81.0° (*c*=0.59, MeOH) and analyzed for $C_{20}H_{32}O_3$ (hrms $[M]^+$ 320.2359). Its ir spectrum had hydroxyl (3460 cm^{-1}) and ether (1065 cm^{-1}) bands. The ^{1}H-nmr spectrum contained four methyl signals at δ 1.13, 1.20 (each 3H, s), 1.68 and 1.72 (each 3H, brs), five olefinic protons at δ 4.75, 4.94 (each 1H brs), 5.20 (1H, t, 7.4 Hz), 5.65, and 5.85 (each 1H, d, 15.4 Hz) and a hydroxymethine at δ 3.49 (1H, dd, 1.0, 11.0 Hz). The presence of two broad singlets in the ^{1}H-nmr spectrum at δ 4.75 and 4.98 and the carbon signals at δ 109.2 (t), 149.5 (s) in the ^{13}C-nmr spectrum suggest the presence of a terminal methylene group (>C=CH_2) in the molecule. A comparison of the ^{1}H-nmr spectra of **4** and **7** revealed that the signals due to the isopropyl group in **4** had been replaced by signals assigned to an isopropylidene group in **7**; all other signals were almost identical. The structure of compound **7** may thus be proposed as (2*E*,7*E*)-4,11-dihydroxy-1,12-oxidocembra-2,7,15-triene [**7**], a new cembranoid.

EXPERIMENTAL

COLLECTION, EXTRACTION, AND ISOLATION.—The specimens of the soft coral *S. ovispiculata* (dry wt after extraction ca 1.2 kg) were collected off North Bay (11° 43′ N, 92° 25′ E) in Middle Andamans of the Andaman and Nicobar Islands of the Indian Ocean during December 1985. The voucher specimens are on deposit at the Northern Territory Museum of Arts and Sciences, Darwin, N.T., Australia (NTM C 10960) and School of Chemistry, Andhra University, Visakhapatnam, India (MF/CBR/09). The extraction was carried out at room temperature using EtOH by percolation every 48 h (5×6 liters). The 95% aqueous EtOH was concentrated under reduced pressure and extracted with EtOAc, and the EtOAc extract was dried over anhydrous $MgSO_4$. Evaporation of the solvent gave a dark brown gum (40g) which was chromatographed over Si gel using solvent mixtures of increasing polarity from petroleum ether (bp 60-80°) through C_6H_6 to EtOAc. Repeated rechromatography of the selected fractions with suitable solvents yielded five cembrane derivatives, isoneocembrene A [**1**] (200 mg), 11,12-epoxyisoneocembrene A [**2**] (300 mg), nephthenol [**3**] (250 mg), (2*E*,7*E*)-4,11-dihydroxy-1,12-oxidocembra-2,7-diene [**4**] (60 mg), and (2*E*,7*E*)-4,11-dihydroxy-1,12-oxidocembra-2,7,15-triene [**7**] (20 mg); four polyhydroxysterols, (24*S*)-24-methylcholestane-3β,5α,6β,25-tetraol 25-monoacetate (220 mg), (24*S*)-24-methylcholestane-3β,5α,6β,25-tetraol (130 mg), 24-methylenecholest-5-ene-3β,7β,16β-triol-3-*O*-α-L-fucopyranoside (35 mg), and 24-methylene-

cholestane-1α,3β,5α,6β-tetraol (numersterol A) (650 mg); pregnenolone (600 mg); and a mixture of monohydroxysterols (1.26 g).

(2E,7E)-4,11-Dihydroxy-1,12-oxidocembra-2,7-diene [**4**].—Crystalline solid: mp 143–145°; hrms $[M]^+$ 322.2493 ($C_{20}H_{34}O_3$ requires 322.2508); $[\alpha]^{25}D$ +113.5° (*c*=0.1, $CHCl_3$); ir ($CHCl_3$) 3560, 2940, 1630, 1380, 1370, 1060, 875 cm^{-1}; ^1H nmr see Table 1, ^{13}C nmr see Table 2; eims *m/z* (%) $[M]^+$ 322 (8), 304 (31), 299 (100), 201 (70), 243 (48), 193 (39), 137 (76).

(2E,7E)-11-Acetoxy-4-hydroxy-1,12-oxidocembra-2,7-diene [**5**].—A solution of **4** (10 mg) in Ac_2O (200 μl) and pyridine (200μl) was kept at room temperature overnight. Usual workup followed by Si gel chromatography yielded the acetate **5** (8 mg): crystalline solid; mp 109–110°; found C 72.41, H 9.85 ($C_{22}H_{36}O_4$ required C 72.41, H 9.95%); ir ($CHCl_3$) 3510, 1725, 1375, 1060, 985 cm^{-1}; ^1H nmr see Table 1.

OXIDATION OF **4**.—A mixture of **4** (15 mg.) dry Et_2O (4 ml), and Collins reagent (60 mg) was stirred at 10° and the progress of the reaction was monitored by tlc. In 2 h, all the starting material was reacted and the excess reagent was filtered off. Evaporation of the filtrate followed by Si gel chromatography yielded the ketone **6** (8 mg): colorless crystals, mp 126–128°; found C 75.04, H 10.01 ($C_{20}H_{32}O_3$ requires C 74.95, H 10.06%); ir ($CHCl_3$) 3500, 1720, 1375, 1065, 985 cm^{-1}; ^1H nmr see Table 1.

(2E,7E)-4,11-Dihydroxy-1,12 oxidocembra-2,7,15,-triene [**7**].—Crystalline solid: mp 156–158° hrms $[M]^+$ 320.2359 ($C_{20}H_{32}O_3$ requires 320.2351); $[\alpha]^{25}D$ +81.0° (*c*=0.59, MeOH); ir ($CHCl_3$) 3460, 1620, 1065, 870 cm^{-1}; ^1H nmr see Table 1; ^{13}C nmr see Table 2; eims *m/z* (%) $[M]^+$ 320 (9), 302 (30), 191 (25), 159 (26), 133 (100), 93 (55).

24-Methylenecholestane-1a,3β,5α,6β-tetraol (numersterol A).—Crystalline solid: mp 280–282°; hrms $[M-H_2O]^+$ 430.3470 ($C_{28}H_{46}O_3$ requires 430.3462); $[\alpha]^{25}D$ +5.0° (*c*=0.82, EtOH); ir (KBr) 3500, 1380, 1370, 1080 cm^{-1}; ^1H nmr (DMSO-d_6, 360 MHz) δ 0.68 (3H, s, Me-18), 0.95 (3H, d, 7.0 Hz, Me-21), 0.99 (3H, s, Me-19), 1.04 (6H, d, 6.5 Hz, Me-26, -27), 2.20 (1H, m, H-25), 3.21 (1H, brs, H-1), 3.58 (1H, brs, H-6), 4.05 (1H, m, H-3), 4.66 and 4.70 (each 1H, brs, H-28), 4.36, 4.48, and 5.20 (each 1H, -OH); ^{13}C nmr (pyridine-d_5, 50.4 MHz) δ 76.0 (C-1), 40.8 (C-2), 63.5 (C-3), 40.5 (C-4), 78.8 (C-5), 76.5 (C-6), 35.7 (C-7), 31.9 (C-8), 43.9 (C-9), 43.6 (C-10), 21.8 (C-11), 41.5 (C-12), 42.0 (C-13), 56.8 (C-14), 24.9 (C-15), 28.0 (C-16), 57.0 (C-17), 12.8 (C-18), 19.2 (C-19), 34.0 (C-20), 19.2 (C-21), 36.3 (C-22), 32.1 (C-23), 157.4 (C-24), 36.0 (C-25), 23.4 (C-26), 23.3 (C-27), 107.0 (C-28); eims *m/z* (%) $[M]^+$ 448 (16), 433 (25), 430 (20), 412 (55), 397 (26), 346 (100), 328 (100), 285 (28), 262 (35), 244 (17), 208 (14).

ACETYLATION OF NUMERSTEROL A.—A mixture of numersterol A (50 mg), Ac_2O (750 μl), and pyridine (750 μl) was kept at room temperature for overnight and after usual workup followed by Si gel chromatography gave diacetate (14 mg) and triacetate (20 mg) as colorless liquids. These derivatives were not reported earlier (14).

Numersterol A 3,6-diacetate.—Colorless liquid: found C 72.06, H 9.72 ($C_{32}H_{56}O_6$ requires C 72.14, H 9.83%); ir ($CHCl_3$) 3400, 1730, 1380, 1070 cm^{-1}; ^1H nmr ($CDCl_3$, 360 MHz) δ 0.69 (3H, s, Me-18), 0.94 (3H, d, 7.0 Hz, Me-21), 1.06 (6H, d, 6.5 Hz, Me-26, -27), 1.20 (3H, s, Me-19), 2.03 and 2.07 (each 3H, s, OAc), 4.60 (1H, brs, H-6), 4.70 and 4.75 (each 1H, brs, H-28), 5.40 (1H, brm, H-3); eims *m/z* (%) 532 (4), 517 (8), 514 (14), 472 (22), 394 (48), 370 (80), 352 (100).

Numersterol A 1,3,6-triacetate.—Colorless liquid: found C 71.20, H 9.38 ($C_{34}H_{58}O_7$ requires C 71.04, H 9.47%), ir ($CHCl_3$) 3520, 1730, 1395, 1070 cm^{-1}; ^1H nmr ($CDCl_3$, 360 MHz) δ 0.68 (3H, s, Me-18), 0.92 (3H, d, 7.0 Hz, Me-21), 1.06 (6H, d, 6.5 Hz, Me-26, -27), 1.20 (3H, s, Me-19), 2.02, 2.06, 2.12 (each 3H, s, -OCOC*H*$_3$), 4.68 (1H, brs, H-6), 4.70 and 4.75 (each 1H brs, H-28), 5.10 (1H, brs, H-1), 5.40 (1H, brm, H-3); eims *m/z* (%) 574 (8), 556 (10), 538 (14), 514 (40), 496 (32), 430 (60), 412 (48), 398 (42), 370 (12), 352 (11).

GAS CHROMATOGRAPHIC ANALYSIS OF MONOHYDROXYSTEROL MIXTURE.—The sterol mixture was acetylated using Ac_2O/pyridine and the acetyl derivative was analyzed on 3% OV-17 glass capillary column: oven temperature 260°, injection port temperature 350°. The following eight steryl acetates were identified by comparison of relative retention times (15) using cholesteryl acetate as reference: cholesterol (0.61%), cholestanol (5.95%), 7-cholestenol (7.43%), 24-methylenecholesterol (60.6%), fucosterol (2.48%), 22-stigmasterol (1.00%), 5,7-ergostadienol (7.43%), 8(14)-stigmastenol (0.50%). One component could not be identified.

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LITERATURE CITED

1. D.J. Faulkner, *Nat. Prod. Rep.*, **9**, 323 (1991) and references cited therein.
2. P. Ciminello and W. Fenical, *Experientia*, **46**, 980 (1990).
3. T. Kusumi, K. Yamada, M.D. Ishitsuka, Y. Fujita, and H. Kakisawa, *Chem. Lett.*, 1315 (1990).
4. K. Iguchi, K. Nishimura, K. Yamazaki, M. Iwashima, and Y. Yamada, *Chem. Lett.*, 127 (1992).
5. T. Yamada, T. Kusumi, M.D. Ishitsuka, and H. Kakisawa, *Chem. Lett.*, 33 (1992).
6. C.G. Naik, S.Y. Kamat, and R.S. Ropgi, *Indian J. Chem.*, **21B**, 76 (1982).
7. Ch. Bheemasamkara Rao, D. Venkata Rao, and T. Sudhakara Rao, *Indian J. Chem.*, **29B**, 683 (1990).
8. B.F. Bowden, J.C. Coll, and D.M. Tapiolas, *Aust. J. Chem.*, **36**, 2289 (1983).
9. B.F. Bowden, J.C. Coll, W. Hicks, R. Kazlauskas, and S. J. Mitchell, *Aust. J. Chem.*, **31**, 2707 (1978).
10. S. Carmley, A. Groweiss, and Y. Kashman, *J. Org. Chem.*, **46**, 4279 (1981).
11. J.M. Moldowan, B.M. Tursch, and C. Djerassi, *Steroids*, **24**, 387 (1974).
12. M. Kobayashi, T. Hayashi, P. Nakajima, and H. Mitsuhashi, *Steroids*, **34**, 285 (1981).
13. M. Kobayashi, F. Kanda, D.S. Rao, D.V. Rao, and Ch. Bheemasankara Rao, *Chem. Pharm. Bull.*, **38**, 2400 (1990).
14. O.S. Jingu, Y. Xiaoqiang, Z. Longmei, and M.C.W. Thomas, *J. Nat. Prod.*, **52**, 934 (1989).
15. T. Itoh, H. Kanti, K. Fukushima, T. Tomura, and T. Matsumoto, *J. Chromatogr.*, **234**, 65 (1982).

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