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NEW CEMBRANOIDS FROM PLEXAURA

WILFRED R. CHAN and WINSTON F. TINTO
Chemistry Department, The University of the West Indies,
St. Augustine, Trinidad
PERCY S. MANCHAND and LOUIS J. TODARO
Chemistry Research Department, Hoffmann-La Roche Inc.,
Nutley, New Jersey 07110
and LEON S. CIERESZKO
Department of Chemistry, The University of Oklahoma,
Norman, Oklahoma 73019

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ABSTRACT - Three new cembranoids, dihydroplexaurolone (**2**) and two dehydroplexaurolones (**3**) and (**4**), have been isolated from the gorgonian *Plexaura A* . The structure of **2** was established by X-ray crystallographic studies. Crystals of **2** belong to space group $P2_1$, with a=10.711 (2) A, b=10.976 (3) A, c=17.148 (4) A, β=99.99 (2)°, and Z=4. The structure was solved by a multiple solution procedure and was refined by block diagonal least-squares to give R=0.046 and wR=0.050.

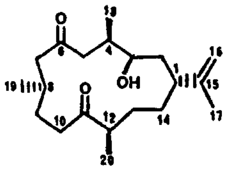
The gorgonian corals (phylum Cnidaria, order Gorgonacea) are widely distributed in most tropical and subtropical marine habitats.[1] Interest in the secondary metabolites[2] of these animals was highlighted with the discovery[3] that the gorgonian *Plexaura homomalla* produced large quantities of a mixture of prostaglandins. Recent investigations on the related *P. flava* have resulted in the isolation of some unusual lipids and compounds of mixed acetate-mevalonate biogenesis.[4]

In the Caribbean there is a *Plexaura* species, referred[5] to as *Plexaura A* , that has been observed off Tobago, Curacao, Puerto Rico, the U.S. Virgin Islands, Bonaire, and the San Blas Islands of Panama. Plexaura A , although not fully characterized, can be distinguished from *P. homomalla* on the basis of its morphology. A previous study of *Plexaura A* has led to the isolation and structure elucidation (by X-ray crystallography) of the cembranoid plexaurolone (**1**), which crystallized from a hexane extract of the organism.[6] A re-examination of *Plexaura A* has now yielded three additional cembranoids: dihydroplexaurolone (**2**), from a hexane extract, and the isomeric dehydroplexaurolones **3** and **4**, from a dichloromethane extract.

Dihydroplexaurolone (**2**), $C_{20}H_{36}O_3$, m.p. 125-126°C, $[\alpha]25$ +42°, had IR bands for hydroxyl (3420 cm^{-1}), carbonyl (1705 cm^{-1}), and olefinic (1645, 880 cm^{-1}) groups. The ^{13}C NMR spectrum (Table 1, $CDCl_3$) of **2** revealed all twenty carbon atoms. These were attributed to four methyls (δ 16.0, 16.1, 20.9, and 21.0), seven sp^3 methylenes, one sp^2 methylene (δ 109.2), six methines, two of which bore hydroxyl groups (δ 70.2 and 75.1), a fully substituted alkene (δ 149.8), and a ketone (δ 211.4). In addition, the ^1H NMR spectrum ($CDCl_3$) showed that three of the methyl groups were secondary (δ 1.00, 0.97, and 0.93, each a doublet, $\underline{J}$=6 Hz) and the fourth was vinylic (δ 1.70). Absorptions assigned to two olefinic protons appeared as broad singlets at δ 4.67 and δ 4.70. Since irradiation of the signal at δ 1.70 sharpened the resonances at δ 4.67 and δ 4.70, we inferred that an isopropenyl group was present in **2**. Two one-proton multiplets at δ 3.35 and δ 3.50 are attributed to methine protons of secondary hydroxyl groups.

The spectral data, coupled with the co-occurrence of **2** and plexaurolone (**1**) in *Plexaura A*, provided strong evidence that **2** was related to **1** by reduction of one of the carbonyl groups in the latter. The relative stereostructure of dihydroplexaurolone (**2**) was established by a single crystal X-ray analysis. An ORTEP perspective drawing of **2** as determined from the X-ray crystallographic analysis is displayed in the Figure. The unit cell was found to contain two independent molecules, that is, two molecules not related by crystallographic symmetry. These two molecules differed in conformation about the C-9 - C-10 bond.

The less polar isomeric triketones, **3** and **4**, were isolated from a dichloromethane extract by preparative-scale thin layer chromatography. One of the triketones, m.p. 93-95°C, was confirmed as **3** since it was produced when plexaurolone was oxidized with pyridinium chlorochromate. The other triketone, m.p. 196-199°C, gave **3** when treated with base: it must therefore be a stereoisomer of **3** at C-4 and/or C-12. Clarification of this stereochemical point was not pursued. Recently,[7] the diacetate **5** has been isolated from the mollusc *Ovula ovum*.



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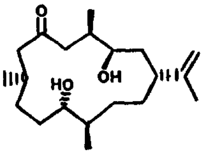

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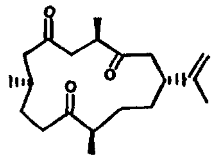

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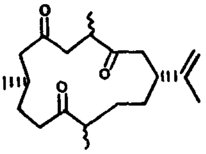

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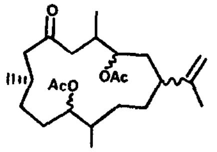

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Table 1. ^{13}C-NMR Data[a]

C-atom	2	3	4
1	41.8	47.2	47.3
2	37.9	46.4	46.8
3	70.2	215.6	215.3
4	36.1	36.6	38.3
5	49.2	47.4	47.4
6	211.4	210.4	210.8
7	47.6	49.5	50.9
8	38.1	29.5	27.1
9	30.5	32.1	32.2
10	29.1	41.4	41.2
11	75.1	214.4	214.5
12	28.2	41.5	42.6
13	29.0	31.6	31.3
14	27.5	29.9	30.7
15	149.8	147.6	147.5
16	109.2	110.6	110.6
17	21.0	20.1	20.4
18	13.6[b]	16.4[b]	16.5[b]
19	20.9	21.4	22.1
20	16.1[b]	17.3[b]	17.3[b]

[a] Spectra were recorded at 50.4 MHz in $CDCl_3$ and chemical shifts are in ppm from TMS. Multiplicities were obtained by "off resonance" decoupling. Assignments were based on comparison with model compounds and DEPT spectra (on **2**).

[b] Values with identical superscripts in each column may be interchanged.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES. - Melting points were determined on a Kofler hot plate and are uncorrected. Ir spectra were recorded on a Pye-Unicam SP3-200 spectrometer. ^1H and ^{13}C nmr spectra were determined in $CDCl_3$ on Bruker WP 80 SU and a Varian XL-200 spectrometers with TMS as the internal standard. Optical rotations were measured on a Schmidt-Haensch Polartronic-D polarimeter. Light petroleum refers to the fraction b.p. 60-80°C.

ISOLATION OF DIHYDROPLEXAUROLONE (2). - The hexane extract of *Plexaura A*, after removal of plexaurolone (120 mg) by crystallization, was subjected to preparative-scale thin layer chromatography (SiO_2) with light petroleum - acetone (3:1) as eluent. A less polar component (27 mg), which crystallized from light petroleum - acetone, was identified as plexaurolone (**1**) (m.p., mixed m.p., spectral data). A more polar component, dihydroplexaurolone (**2**, 22 mg), crystallized from light petroleum - acetone, as prisms, m.p. 125-126°C; $[\alpha]_D$ +42° (*c* 0.12, $CHCl_3$); ir (Nujol) cm^{-1} 3420, 3080, 1705, 1645, 1040, 880; ^1H nmr ($CDCl_3$) δ 4.70 (1H, b s), 4.67 (1H, b s), 3.42 (2H, m), 1.70 (3H, d, *J*=1 Hz), 1.00 (3H, d, *J*=6 Hz), 0.97 (3H, d, *J*=6 Hz), and 0.93 (3H, d, *J*=6 Hz); eims *m/z* (%) 324 (M^+, 1), 306 (21), 288 (9), 273 (3), 235 (9), 223 (10), 192 (8), 175 (18), 163 (17), 149 (24), 123 (49), 107 (50), 93 (52), 69 (100).

ISOLATION OF THE TRIKETONES 3 AND 4. - Specimens of *Plexaura A* were collected at Speyside, Tobago at a depth of 6-8 m and identified at the Institute of Marine Affairs, Chaguaramas. A voucher specimen was kept at the Institute.

The dried, minced animals (80 g) were percolated with CH_2Cl_2 (500 mL). Evaporation of the solvent gave a viscous, brown oil (3.7 g). This, in light petroleum, was chromatographed over tlc grade SiO_2 (5 cm diam. column). The fraction eluted with $CHCl_3$-EtOAc (2:1) was subjected to preparative-scale tlc with light petroleum - Me_2CO (3:1) as eluent. This gave, in order of increasing R_F, plexaurolone (**1**, 83 mg), m.p. 110-111° (from light petroleum-Me_2CO), identified by comparison with an authentic sample, dehydroplexaurolone (**3**, 64 mg), and the stereoisomer **4** (16 mg).

Dehydroplexaurolone (**3**), crystallized as needles from light petroleum-Me_2CO, m.p. 93-95°C, $[\alpha]_D$ +48° (*c* 0.27, $CHCl_3$); ir (Nujol) cm^{-1} 1705, 1645, 890: ^1H nmr ($CDCl_3$) δ 4.72 (2H, bs), 1.68 (3H, b s), 1.04 (3H, d, *J*=6 Hz), 1.03 (3H, d, *J*=6 Hz), 1.00 (3H, d, *J*=6 Hz); ^{13}C nmr, see Table 1; eims *m/z* (%) 320 (M^+, 30), 302 (6), 263 (20), 245 (8), 217 (15), 179 (8), 165 (32), 152 (100), 123 (73), 109 (32), 95 (42), 69 (89); hrms: found, 320.2361; $C_{20}H_{32}O_3$ requires 320.2351.

The isomeric triketone **4** crystallized as cubes from light petroleum - Me_2CO, m.p. 196-199°C, $[\alpha]_D$ -11° (*c* 0.09, $CHCl_3$), ir (Nujol) cm^{-1} 1705, 1640, 890; ^1H nmr δ 4.72 (2H, b s), 1.68 (3H, b s), 1.04 (3H, d, *J*=6 Hz), 0.99 (3H, d, *J*=6 Hz), 0.93 (3H, d, *J*=6 Hz); ^{13}C nmr, see Table 1; eims m/z (%): 320 (M^+, 32), 302 (7), 263 (32), 245 (9), 217 (15), 179 (9), 165 (34), 152 (86), 123 (62), 109 (34), 95 (97), 69 (100); hrms: found, 320.2364; $C_{20}H_{32}O_3$ requires 320.2351.

OXIDATION OF PLEXAUROLONE (1). - A mixture of plexaurolone (**1**, 90 mg) and pyridinium chlorochromate (96 mg) in CH_2Cl_2 (2 mL) was stirred at room temperature for 2 hr. Work up in the usual manner afforded dehydroplexaurolone (**3**), which was identified by m.p., $[\alpha]_D$, ir, and ^1H nmr.

BASE TREATMENT OF 4. - Compound **4** (16 mg) was treated with KOH in MeOH (2%) under reflux for 1 hr. The product was shown to be a mixture of (**3**) and (**4**) by tlc, ir, and ^1H nmr.

X-RAY CRYSTALLOGRAPHIC ANALYSIS OF DIHYDROPLEXAUROLONE (2). - A crystal of dimensions 0.20 x 0.5 x 0.9 mm was used for data collection on a Hilger-Watts diffractometer (Ni-filtered Cu Kα radiation, θ-2θ scans, pulse height discrimination). Of 4148 accessible reflections for $\theta < 75°$, 3568 were considered to be observed $[I > 2.5\sigma(I)]$. Crystal data: $C_{20}H_{36}O_3$, MW=324.50, monoclinic space group $P2_1$, a = 10.711(2)A, b = 10.976(3)A, c = 17.148(4)A, β = 99.99(2)°, Z = 4, d_{calcd} = 1.086 g cm^{-3}, μ (CuKα) = 5.6 cm^{-1}.

The unit cell contained two independent molecules, that is, two molecules not related by crystallographic symmetry. The two molecules differed in conformation about the C-9 and C-10 bond. The structure was solved by a multiple-solution procedure[8] and was refined by block-diagonal least squares in which the matrix was partitioned into two blocks. Three reflections which were strongly affected by extinction were excluded from the final refinement and difference map. In the final refinement, the non-hydrogen atoms were refined anisotropically, except for the disordered carbon atoms C-9 and C-10. The occupancies of these atoms were adjusted to 0.75 in one conformer and 0.25 in the other so as to obtain approximately equal isotropic thermal factors between these two pairs of carbon atoms. The hydrogen atoms were included in the structure factor calculations but their parameters were not refined. The final discrepancy indices are R=0.046 and wR=0.050 for the remaining 3565 observed reflections. The final difference map had no peaks greater than $\pm 0.2 eA^{-3}$.

SUPPLEMENTARY MATERIAL. - Listings of bond lengths, bond angles, selected torsion angles, final atomic parameters, final thermal parameters, and final structure factors (Fo and Fc) for dihydroplexaurolone **(2)**.

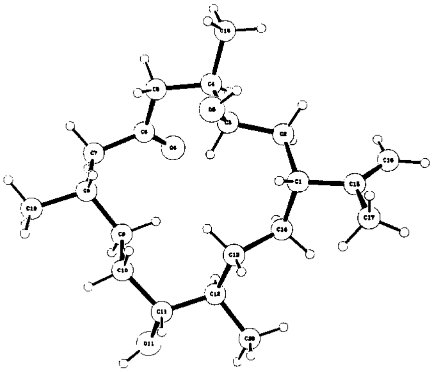

Figure. An ORTEP Prespective Drawing of **2**.

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