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Calicoferols A and B, Two Novel Secosterols Possessing Brine-Shrimp Lethality from the Gorgonian Calicogorgia sp.

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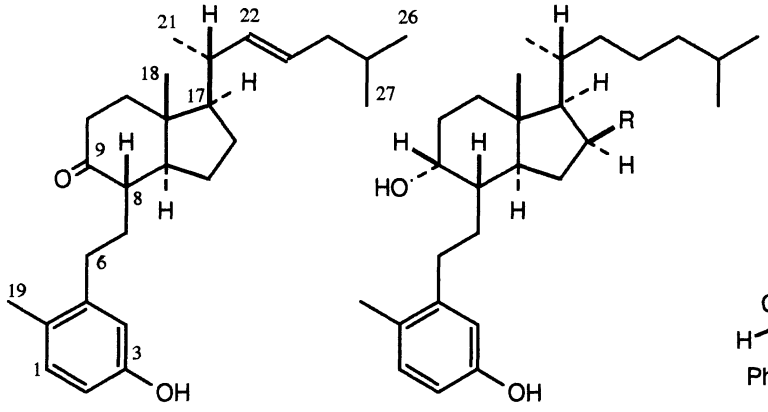
The structures of calicoferols A and B, two new secosterols possessing brine-shrimp lethality isolated from the gorgonian Calicogorgia sp. have been determined on the basis of spectroscopic and chemical evidence.

Marine invertebrates are the richest source of unusual sterols with a wide variety of remarkable variations both in the side chain and nucleus, many of which have no terrestrial counterpart.[1] In our preliminary screening for biological activities of marine organisms, the methanol extract of a gorgonian Calicogorgia sp. showed lethality to brine-shrimp. Bioassay directed fractionation of the extract led to the isolation of two new secosterols responsible to the observed activity.[2] The present paper deals with the structural determination of these new compounds, designated calicoferols A and B.

Specimens of the animal (3.0 kg) were collected using SCUBA at Sukumo Bay in September 1988. Freshly collected organisms were kept frozen until just prior to extraction. Thawed material was homogenized with methanol and left at room temperature for a few hours. After filtration, the methanol solution was concentrated in vacuo to an aqueous suspension and extracted with hexane. Fractionation of the hexane extract (26.3 g) by Sephadex LH-20 (MeOH/CH_2Cl_2 1:1) and silica gel (hexane/EtOAc) column chromatography, followed by reverse phase HPLC (ODS column, MeOH/H_2O 85:15), gave calicoferols A (25 mg) and B (30 mg).

Calicoferol A (**1**) was obtained as a colorless solid: $[\alpha]_D^{15}$ +4.2° (c 0.24, $CHCl_3$); ν_{max} (CCl_4) 3650, 3400, 1705, 1612, 1595, 1505, and 975 cm^{-1}; λ_{max} (EtOH) 218.0 and 280.3 nm (ε 4980 and 1730), and gave a parent ion at m/z 396.3053 in the HRMS appropriate for a molecular formula of $C_{27}H_{40}O_2$ (ΔM +2.5mmu) requiring 8 degrees of unsaturation. The ^1H NMR spectrum[3] of **1** showed the presence of a 1,2,4-trisubstituted benzene ring with the assigned locus of the methyl group at δ 2.29 (3H, s), 6.55 (dd, J=8.2 and 2.4 Hz), 6.73 (d, J=2.4 Hz), and 6.94 (d, J=8.2 Hz), and a trans disubsti-

tuted olefinic double bond at δ 5.20 (dd, J=15.3 and 8.5 Hz) and 5.32 (ddd, J=15.3, 7.2, and 7.2 Hz). In addition, the ^{13}C NMR data[3] for **1** showed the presence of one ketonic carbon atom at δ 211.21 together with 4 methyl groups, 7 methylene groups, 5 methine groups, and one quaternary carbon atom. The presence of a phenolic hydroxyl group was evident from the above spectral data and the positive $FeCl_3$-$K_3Fe(CN)_6$ test which displayed a blue coloration. A combination of the ^1H-^1H and ^1H-^{13}C COSY spectra together with exhaustive spin decoupling studies allowed a complete assignment of all proton and carbon resonances, leading to a planar formula **1** for calicoferol A. The location of a methyl group at the C_{13} position was revealed by the ^1H-^{13}C long-range correlation between the ^1H-signal at δ 0.65 (18-Me) and the ^{13}C-signals at δ 38.41 (C_{12}), 55.24 (C_{14}), and 55.02 (C_{17}). The relative stereochemistry of **1** was established on the basis of the NOESY experiment and the inspection of the J-resolved spectrum. Observation of nOes among 18-Me, 8-H, and 15β-H suggested that the C/D ring junction is trans and that 18-Me and 8-H are faced to the same side. The presence of nOes between 18-Me and 20-H and between 12β-H and 21-Me established the stereochemistry at C_{17} and C_{20} positions as illustlated in Fig. 1. These stereochemical assignments were supported by the coupling constants of the protons at chiral centers estimated by the scrutiny of the J-resolved spectrum as depicted in Fig. 1. Therefore, calicoferol A must be represented by struture **1**. This structure would be reasonable biogenetically if we assume that calicoferol A is derived from a compound with the cholestane skeleton as illustrated in Scheme 1. Such a transformation has been observed in steroid oxidations by microorganisms.[4] The assigned structure is closely related to that of astrogorgiadiol (3) from a gorgonian Astrogorgia sp.[5] Finally, the structure was confirmed by



1 **2 R = OH** **3 R = H**

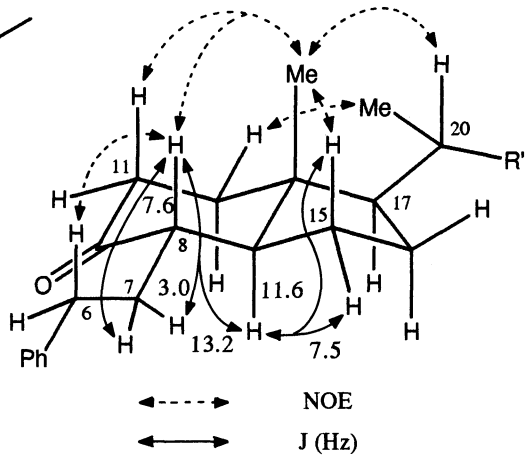
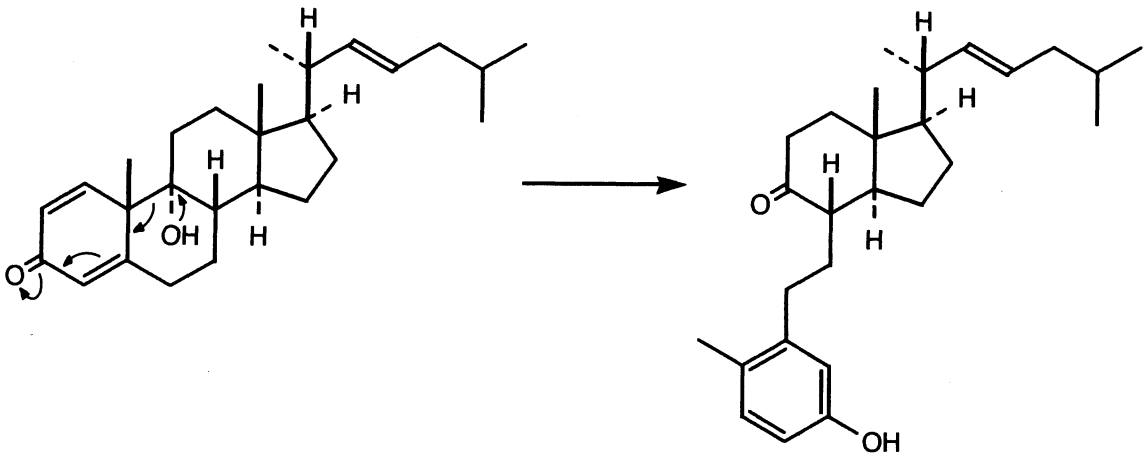


Fig. 1.



Scheme 1. **1**

chemical correlation of **1** with **3**. Thus, hydrogenation of **1** with H_2/Pd-C, followed by reduction with $NaBH_4$, gave a diol, the spectral data of which are identical with those of **3**.

The absolute configuration of **1** was established by studying its CD spectrum which showed a positive Cotton effect at 292 nm ($\Delta\varepsilon$ +1.6). By applying the octant rule to cyclohexanones, the stereochemistry of 8S, 13R, 14S, 17R, 20R, as depicted in structure **1**, can be deduced for **1**. In this case, the C_6 carbon atom must lie in the front-upper-right octant or nearly in the carbonyl plane judging from the ^1H NMR data as shown in Fig. 1.

Calicoferol B (**2**) was isolated as a colorless oil: $[\alpha]_D^{21}$ -16.2° (c 0.09, $CHCl_3$); ν_{max} (CCl_4) 3640, 3380, 1620, 1590, and 1510 cm^{-1}; m/z 416.3300 ($C_{27}H_{44}O_3$ requires 416.3290). Inspection of ^1H and ^{13}C NMR spectra[3,5] of **2** and **3** established the close structural similarity of these two compounds. The difference between **2** and **3** resided solely in the presence of an additional secondary hydroxyl group in **2** (δ_H 4.09; δ_C 71.79). The location of the second hydroxyl group was proved from the scrutiny of the ^1H NMR data. Thus, examination of the proton connectivity by the same techniques described above elucidated the location of the hydroxyl group at C_{16}. The ^1H NMR signals due to 13-Me and 20-H in **2** appeared in lower field (+0.28 and +0.60 ppm respectively) compared with those of **3**. The deshielding of these protons would be reasonably interpreted through the consideration of the paramagnetic anisotropy by the 16β-OH disposed just as in 1,3-diaxial relationship. This is supported by the coupling pattern of 16-H (ddd, J=7.6, 7.6, and 4.5 Hz). From the evidence outlined above, we assigned the structure **2** for calicoferol B.

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2) The LD_{50}-values of calicoferols A and B against brine shrimp lavae were 1.8 ppm and 2.3 ppm, respectively. The procedure for the bioassay was the same as that reported by T. Miyauchi: T. Miyauchi, Seitaikagaku, **8**, 41 (1986) and references cited therein.

3) **1**: ^{1}H NMR (C_6D_6) δ 0.65 (3H, s, 18-H), 0.92 and 0.93 (3H each, d, J=6.7 Hz, 26- and/or 27-H), 0.94 (3H, d, J=6.7 Hz, 21-H), 1.03 (1H, m, 15β-H), 1.40 (1H, ddd, J=13.2, 11.3, and 7.5 Hz, 14-H),# 1.46 (1H, m, 15α-H), 1.74 (1H, m, 12β-H), 1.96 (1H, m, 20-H), 2.12 (1H, ddd, J=13.2, 7.6, 3.0 Hz, 8-H),# 2.14 (1H, m, 11β-H), 2.29 (3H, s, 19-H), 2.56 (1H, ddd, J= 12.8, 11.6, 5.5 Hz, 6-Ha), 2.78 (1H, ddd, J=12.8, 11.8, and 4.8 Hz, 6-Hb), 5.20, (1H, dd, J=15.3 and 8.5 Hz, 22-H), 5.32 (1H, ddd, J=15.3, 7.2, and 7.2 Hz, 23-H), 6.55 (1H, dd, J=8.2 and 2.4 Hz, 2-H), 6.73 (1H, d, J=2.4 HZ, 4-H), and 6.94 (1H, d, J=8.2 Hz, 1-H). ^{13}C NMR (C_6D_6) δ 11.56 (18), 18.52 (19), 20.97 (21), 22.43 (26 and 27), 25.25 (15), 28.30 (7), 28.86 (25), 29.63 (16), 31.51 (6), 38.15 (11), 38.41 (12), 40.21 (20), 42.28 (24), 42.71 (13), 50.44 (8), 55.02 (17), 55.24 (14), 113.17 (2), 116.35 (4), 127.05 (23), 127.59 (10), 131.43 (1), 137.88 (22), 142.64 (5), 154.97 (3), and 211.21 (9).
2: ^{1}H NMR (C_6D_6) δ 0.88 (3H, s, 18-H), 0.936 and 0.942 (3H each, d, J= 6.7 Hz, 26- and/or 27-H), 1.00 (3H, d, J=6.7 Hz, 21-H), 1.33 (1H, ddd, J=12.9, 11.9, and 7.4 Hz, 14-H),# 1.41 (1H, dddd, J=11.9, 7.0, 7.0, and 2.2 Hz, 8-H),# 2.23 (3H, s, 19-H), 3.79 (1H, brs, 9-H), 4.09 (1H, ddd, J=7.6, 7.6, and 4.5 Hz, 16-H), 6.57 (1H, dd, J=8.2 and 2.5 Hz, 2-H), 6.74 (1H, d, J=2.5 Hz, 4-H), and 6.97 (1H, d, J=8.2 Hz, 1-H). ^{13}C NMR (C_6D_6) δ 12.44 (18), 18.45 (19), 18.51 (21), 22.77 and 23.00 (26 and/or 27), 24.68 (23), 28.40 (25), 30.14 (20), 30.26 (11), 30.80 (7), 31.22 (6), 34.68 (12), 36.59 (22), 37.35 (15), 39.92 (24), 40.82 (8), 43.07 (13), 45.62 (14), 61.75 (17), 67.12 (9), 71.79 (16), 113.13 (2), 116.16 (4), 127.40 (10), 131.39 (1), 142.83 (5), and 155.02 (3). # These J values were estimated on the basis of the J-resolved spectra.

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