Sarcoaldesterols A and B, Two New Polyhydroxylated Sterols from the Soft Coral *Sarcophyton* sp.

Akemi Umeyama,* Noboru Shoji, Mai Ozeki, and Shigenobu Arihara

Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Tokushima 770, Japan

Received February 21, 1996[⊗]

Two new steroids, sarcoaldesterols A (**1**) and B (**2**), have been isolated from the Okinawan soft coral *Sarcophyton* sp. The structures of **1** and **2** were proposed on the basis of extensive NMR experiments.

Marine organisms have been the source of many steroids, and several groups have been involved chemically and pharmacologically.[1,2] In a continuation of our survey of marine organisms for pharmacologically active substances, we have isolated two new steroids, sarcoaldesterols A (**1**) and B (**2**), from the soft coral *Sarcophyton* sp. collected off Okinawa Island. We now report the isolation and structure elucidation of the two compounds.

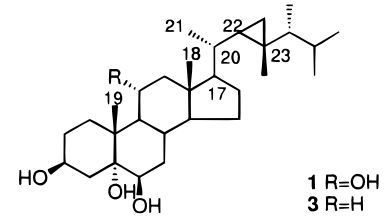
A MeOH/CH_2Cl_2 (1:1) extract of the soft coral was divided into EtOAc-, BuOH-, and H_2O-soluble portions. The EtOAc- and BuOH-soluble portions were combined and chromatographed on Sephadex LH-20 and Si gel columns. Final purification by reversed-phase HPLC afforded two new steroids, sarcoaldesterols A (**1**) and B (**2**).

Sarcoaldesterol A (**1**) was obtained as a white powder (mp 285.5−287.0 °C). The molecular formula, $C_{30}H_{52}O_4$, which was determined by mass measurement (HREIMS *m/z* 476.3881, calcd 476.3866), indicated five degrees of unsaturation. Fragment ions at *m/z* 458 $(M - H_2O)^+$, 440 $(M - 2\ H_2O)^+$, and 422 $(M - 3\ H_2O)^+$ showed the loss of three successive H_2O molecules, characteristic of hydroxyl groups. A broad IR absorption (3300−3500 cm^{-1}) was attributable to hydroxyl groups. The ^{13}C-NMR spectra indicated the presence of four oxygenated carbons [δ 67.4 (d), 68.5 (d), 76.5 (d), 76.8 (s)]. The ^{1}H-NMR spectra contained three methyl singlets (δ 0.81, 0.86, 1.92), four methyl doublets [δ 0.85 (d, J = 6.6 Hz), 0.95 (d, J = 6.6 Hz), 0.95 (d, J = 5.9 Hz), 1.09 (d, J = 5.9 Hz)] and three hydroxy−methine protons [δ 4.20 (m), 4.37 (m), 4.89 (m)]. It also showed signals characteristically ascribable to a cyclopropane-bearing gorgosterol-type side chain [δ −0.14 (dd, 1H, J = 5.1, 4.4 Hz), 0.13 (m, 1H), 0.19 (m, 1H), 0.43 (dd, 1H, J = 8.8, 3.7 Hz)].

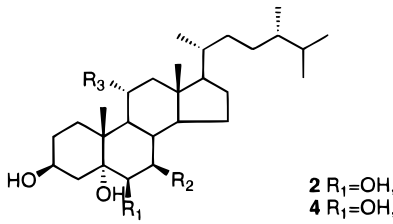
A combination of COSY and HMBC experiments (see Experimental Section) enabled us to construct the structure of **1**. These correlations clearly established the structure of the side chain for **1**. The stereochemistry of the side chain was determined by comparison of NMR data of **1** with those of xeniasterol C (**3**), which was isolated from the soft coral *Xenia* sp.[3] The β-OH group at the C-3 position and the α-OH group at the C-11 position could be assigned from the splitting pattern of the H-3 [δ 4.89 (1H, m)] and H-11 [δ 4.37 (1H, m)] protons. The stereochemistry of the 6β-OH group of **1** was proven by the shape of the H-6 proton (δ 4.20, br s). Sarcoaldesterol A (**1**) can, thus, be designated as gorgosta-3β,5α,6β,11α-tetraol.

Sarcoaldesterol B (**2**) was isolated as a semisolid. The molecular formula of **2** was determined to be $C_{28}H_{50}O_4$ (HREIMS *m/z* 450.3707, calcd 450.3709), differing from the molecular formula of **1** by lacking C_2H_2. The IR spectrum suggested that **2** possessed hydroxyl groups (3300−3500 cm^{-1}). The ^{13}C-NMR spectra indicated the presence of four oxygenated carbons [δ 67.5 (d), 68.6 (d), 76.5 (d), 76.9 (s)]. The ^{1}H-NMR spectra showed two methyl singlets (δ 0.79, 1.89), four methyl doublets [δ 0.81 (d, J = 6.6 Hz) × 2, 0.88 (d, J = 6.6 Hz), 0.98 (d, J = 5.9 Hz)], and three hydroxy−methine protons [δ 4.19 (m), 4.35 (m), 4.88 (m)]. Examination of the ^{13}C-NMR and ^{1}H-NMR data obtained for **2** revealed that it differed from **1** only by having no cyclopropane ring and no methyl group on C-23 in the side chain.

The combinations of COSY and HMBC experiments (see Experimental Section) enabled us to construct the structure of **2**. The stereochemistry of the side chain was determined by comparison of NMR data of **2** with those of xeniasterol B (**4**), which was isolated from the soft coral *Xenia* sp.[3] The stereochemistry of the hydroxy groups at C-3, C-5, C-6, and C-11 positions was the same as those of **1**. Thus, sarcoaldesterol B (**2**) can be designated as ergosta-3β,5α,6β,11α-tetraol.



1 R=OH
3 R=H



2 R_1=OH, R_2=H, R_3=OH
4 R_1=OH, R_2=OAc, R_3=H

Experimental Section

General Experimental Procedures. The following instruments were used: JASCO FT/IR-5300 (IR), JASCO DIP-360 polarimeter (optical rotation), JEOL JMS-HX-100 mass spectrometer (HRMS), JEOL JNM-GX-400FT NMR spectrometer (^{1}H and ^{13}C NMR).

* To whom correspondence should be addressed. Phone: 0886-22-9611. FAX: 0886-55-3051.

[⊗] Abstract published in *Advance ACS Abstracts,* August 15, 1996.

S0163-3864(96)00255-8 CCC: $12.00

Soft Coral Material. The soft coral *Sarcophyton* sp. (1.3 kg, wet wt) was collected at a depth of 2−3 m off Okinawa Island and was kept frozen (−20 °C) until used. The soft coral was identified by Dr. P. Alderslade of the Northern Territory Museum of Arts and Sciences. The voucher sample of the organism under consideration is deposited at the Museum and Art Gallery of the Northern Territory, Darwin, Australia, under registration number NTM C12146. It consists of a small fragment together with two 12−20-mm thick vertical slices taken through the parent colony. Although the material does not give a clear indication of the colonial form, it is clear they are from a species of the soft coral genus *Sarcophyton*, family Alcyoniidae. The surface layer of the polypary and the base contain many skeletal sclerites of an extremely distinctive form, being oval to somewhat egg-shaped. The only nominal species with sclerites that come anywhere near this architectural design are *S. cinerum* Tixier-Durivault, 1946, and *S. solidum* Tixier-Durivault, 1958. However, not only are the oval sclerites in these species of a different form from those in our specimen, but all of the other sclerites from comparable regions of the colonies bear little resemblance. Correspondingly, it is evident that the samples represent a new species of *Sarcophyton*.

Extraction and Isolation of Metabolites. The frozen sample (1.3 kg) was lyophilized and exhaustively extracted with MeOH/CH_2Cl_2 (1:1) (2 L × 4) at room temperature for 1 day. The extract was concentrated, and the resulting residue was extracted with EtOAc and BuOH (500 mL × 3). The EtOAc- and BuOH-soluble portion (220 g) was repeatedly subjected to Si gel flash column chromatography (using increasing concentrations of MeOH in $CHCl_3$ as eluent), followed by Sephadex LH-20 column chromatography ($CHCl_3$/MeOH, 1:1) and reversed-phase HPLC (75−80% MeOH) to give **1** (40 mg, 0.0031% wet wt) and **2** (41 mg, 0.0032%).

Sarcoaldesterol A (1): white amorphous powder; mp 285.5−287.0 °C; $[\alpha]^{25}_D$ −40.5° (*c* 0.97, pyridine); ^{1}H NMR (C_5D_5N) δ −0.14 (dd, 1H, J = 5.1, 4.4 Hz, H-30), 0.13 (m, 1H, H-22), 0.19 (m, 1H, H-24), 0.43 (dd, 1H, J = 8.8, 3.7 Hz, H-30), 0.81 (s, 3H, Me-18), 0.85 (d, 3H, J = 6.6 Hz, Me-26), 0.86 (s, 3H, Me-29), 0.95 (d, 3H, J = 6.6 Hz, Me-27), 0.95 (d, 3H, J = 5.9 Hz, Me-28), 1.02 (m, 1H, H-20), 1.09 (d, 3H, J = 5.9 Hz, Me-21), 1.20 (m, 1H, H-15), 1.34 (m, 1H, H-17), 1.36 (m, 1H, H-16), 1.38 (m, 1H, H-14), 1.50 (m, 1H, H-25), 1.68 (m, 1H, H-15), 1.69 (m, 1H, H-12), 1.92 (s, 3H, Me-19), 2.01 (m, 1H, H-7), 2.07 (m, 1H, H-16), 2.20 (m, 1H, H-2), 2.33 (m, 1H, H-8), 2.33 (m, 1H, H-9), 2.34 (m, 1H, H-2), 2.38 (m, 1H, H-4), 2.41 (m, 1H, H-7), 2.73 (m, 1H, H-12), 2.75 (m, 1H, H-1), 2.95 (m, 1H, H-1), 3.04 (m, 1H, H-4), 4.20 (br s, 1H, H-6), 4.37 (m, 1H, H-11), 4.89 (m, 1H, H-3); ^{13}C NMR (C_5D_5N) δ 13.4 (q, C-18), 14.4 (q, C-29), 15.7 (q, C-28), 17.8 (q, C-19), 21.4 (q, C-26), 21.4 (t, C-30), 21.7 (q, C-27), 22.4 (q, C-21), 25.0 (t, C-15), 25.9 (s, C-23), 28.8 (t, C-16), 30.1 (d, C-8), 32.3 (d, C-22), 32.3 (d, C-25), 35.4 (t, C-2), 35.6 (t, C-1), 35.6 (d, C-20), 35.8 (t, C-7), 41.1 (s, C-10), 43.5 (t, C-4), 44.0 (s, C-13), 50.9 (d, C-24), 53.0 (t, C-12), 53.1 (d, C-9), 55.9 (d, C-14), 58.0 (d, C-17), 67.4 (d, C-3), 68.5 (d, C-11), 76.5 (d, C-6), 76.8 (s, C-5); EIMS m/z 476 (M^+), 448 ($M - H_2O)^+$, 440 ($M - 2 H_2O)^+$, 422 ($M - 3 H_2O)^+$; HREIMS obsd m/z 476.3881, $C_{30}H_{52}O_4$ calcd m/z 476.3866; COSY (H/H) 1/2, 2/3, 3/4, 6/7, 7/8, 8/14, 9/11, 11/12, 14/15, 15/16, 16/17, 17/20, 20/22, 22/30, 24/25, 24/28, 25/26, 25/27; HMBC (H/C) 4/2, 7/5, 9/8, 12/9, 12/11, 12/13, 12/14, 12/18, 18/12, 18/13, 18/14, 18/17, 19/1, 19/5, 19/9, 19/10, 21/17, 21/20, 21/22, 22/24, 24/23, 24/25, 24/26, 24/27, 24/28, 24/29, 25/24, 25/26, 25/27, 26/24, 26/25, 26/27, 27/24, 27/25, 27/26, 28/23, 28/24, 28/25, 29/22, 29/23, 29/30, 30/17, 30/24, 30/29.

Sarcoaldesterol B (2): semisolid; $[\alpha]^{25}_D$ −23.6° (*c* 1.5, MeOH); ^{1}H NMR (C_5D_5N) δ 0.79 (s, 3H, Me-18), 0.81 (d, 3H, J = 6.6 Hz, Me-26), 0.81 (d, 3H, J = 6.6 Hz, Me-28), 0.88 (d, 3H, J = 6.6 Hz, Me-27), 0.94 (m, 1H, H-22), 0.98 (d, 3H, J = 5.9 Hz, Me-21), 0.98 (m, 1H, H-23), 1.12 (m, 1H, H-15), 1.19 (m, 1H, H-17), 1.21 (m, 1H, H-24), 1.25 (m, 1H, H-16), 1.35 (m, 1H, H-14), 1.35 (m, 1H, H-20), 1.38 (m, 1H, H-23), 1.42 (m, 1H, H-22), 1.58 (m, 1H, H-25), 1.63 (m, 1H, H-12), 1.67 (m, 1H, H-15), 1.83 (m, 1H, H-16), 1.89 (s, 3H, Me-19), 1.97 (m, 1H, H-7), 2.19 (m, 1H, H-2), 2.28 (m, 1H, H-8), 2.31 (m, 1H, H-9), 2.33 (m, 1H, H-2), 2.37 (m, 1H, H-4), 2.39 (m, 1H, H-7), 2.65 (m, 1H, H-12), 2.72 (m, 1H, H-1), 2.92 (m, 1H, H-1), 3.02 (m, 1H, H-4), 4.19 (br s, 1H, H-6), 4.35 (m, 1H, H-11), 4.88 (m, 1H, H-3); ^{13}C NMR (C_5D_5N) δ 13.5 (q, C-18), 15.7 (q, C-28), 17.8 (q, C-19), 17.8 (q, C-26), 19.1 (q, C-21), 20.8 (q, C-27), 24.8 (t, C-15), 28.7 (t, C-16), 30.1 (d, C-8), 31.0 (t, C-23), 31.8 (d, C-25), 33.0 (t, C-2), 34.0 (t, C-22), 35.5 (t, C-1), 35.8 (t, C-7), 36.6 (d, C-20), 39.4 (d, C-24), 41.1 (s, C-10), 43.3 (t, C-4), 43.6 (s, C-13), 52.9 (t, C-12), 53.0 (d, C-9), 56.0 (d, C-14), 56.5 (d, C-17), 67.5 (d, C-3), 68.6 (d, C-11), 76.5 (d, C-6), 76.9 (s, C-5); EIMS m/z 450 (M^+), 432 ($M - H_2O)^+$, 414 ($M - 2 H_2O)^+$, 396 ($M - 3 H_2O)^+$, 378 ($M - 4 H_2O)^+$; HREIMS obsd m/z 450.3707, $C_{28}H_{50}O_4$ calcd m/z 450.3709; COSY (H/H) 1/2, 2/3, 3/4, 6/7, 7/8, 8/14, 9/11, 11/12, 14/15, 15/16, 16/17, 17/20, 20/21, 20/22, 22/23, 23/24, 24/28, 25/26, 25/27; HMBC (H/C) 1/19, 2/4, 3/2, 3/4, 5/1, 5/4, 5/6, 5/19, 9/7, 9/12, 9/19, 10/1, 10/4, 10/19, 11/12, 12/18, 13/12, 13/15, 13/18, 14/12, 14/18, 17/18, 17/22, 18/12, 19/1, 20/22, 21/22, 23/28, 24/26, 24/27, 25/26, 25/27, 26/27, 27/26.

Acknowledgment. We are grateful to Dr. P. Alderslade of the Northern Territory Museum of Arts and Sciences for his kind identification and the taxonomic description of the soft coral. We thank Prof. J. Kobayashi of Hokkaido University and Mr. Z. Nagahama for help with collections. We are indebted to Ms. I. Okamoto for measurements of mass spectra.

References and Notes

(1) Faulkner, D. J. *Nat. Prod. Rep.* **1995**, *12*, 223−269, and earlier reviews cited therein.
(2) Kerr, R. G.; Baker, B. J. *Nat. Prod. Rep.* **1991**, *8*, 465−497.
(3) Kitagawa, I.; Kobayashi, M.; Zheng, C.; Kiyota, Y.; Ohnishi, M. *Chem. Pharm. Bull.* **1986**, *34*, 4590−4596.

NP960255J