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IRREGULAR CEMBRANOIDS CONTAINING A 13-MEMBERED CARBOCYCLIC SKELETON ISOLATED FROM A SOFT CORAL, *SARCOPHYTON* SPECIES

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Abstract: The structures and absolute structures of three new 13-membered carbocyclic cembranoids, sarcotol (**1**), sarcotol acetate (**2**), and sarcotal acetate (**3**) from a soft coral, *Sarcophyton* sp. have been determined by spectroscopic and single crystal X-ray analyses, and chemical conversion. Ichthyotoxity tests were performed for **1**, **2**, **3**, and their reduction products.

Many biologically active cembrane diterpenoids have been isolated from soft corals[1]. Recently, unconventional cembranoids having a 12-membered carbon skeleton [2-5] or 13-membered variants[4, 6] have been discovered from Gorgonacea and Alcyonacea. During our investigations of biologically active constituents from marine animals collected around Kagoshima area, Japan, we have isolated three new 13-membered carbocyclic cembranoids, sarcotol **1**, sarcotol acetate **2**, and sarcotal acetate **3** from an unidentified *Sarcophyton* species. Both **2** and **3** displayed ichthyotoxic activity against Japanese killifish, *Oryzia latipes*. Some of the results have been reported in a preliminary paper,[7] and the present paper gives a full account of the isolation, structural elucidation, and ichthyotoxity of the three new compounds and their reduction products.

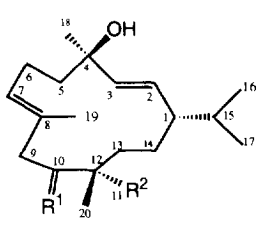
Specimens of *Sarcophyton* sp. were collected at Bonotsu, Kagoshima prefecture. The organism was extracted with methanol and the extract partitioned between water and dichloromethane. The organic layer showed strong ichthyotoxity against killifish, *O. latipes*. Bioassay guided fractionation of the dichloromethane extract by a combination of silica gel, Sephadex LH-20, and finally HPLC gave three new 13-membered carbocyclic cembranoids **1-3**. Their molecular formulas were determined by ^{1}H and ^{13}C-NMR and HREIMS spectroscopy.

Sarcotol **1**, $C_{20}H_{34}O_3$, showed IR absorptions indicative of a hydroxyl group (3293 cm^{-1}), a carbonyl group (1692 cm^{-1}), and a double bond (1655 cm^{-1}). The molecular formula indicated four degrees of unsaturation. Four olefinic resonances [δ_C 137.8 (d), 131.3 (d), 129.9 (d), and 128.6 (s)] and one carbonyl resonance [δ_C 214.3(s)] in the ^{13}C-NMR spectrum accounted for three double equivalents, suggesting that **1** is a monocyclic diterpenoid, most probably based on the cembrane skeleton so often encountered in soft corals. The ^{1}H-NMR and ^{13}C-NMR spectra showed signals corresponding to six methyl groups: an isopropyl [δ_H 0.81 and 0.85 (3H each, d, J=6.6 Hz), 1.53 (1H, m); δ_C 19.8 (q), 20.4 (q), and 32.6 (d)], a tertiary methyl [δ_H 1.16 (s); δ20.4 (s)], a tertiary methyl on a carbon carrying an oxygen function [δ_H 1.31 (s); δ_C 27.6 (s)], an

olefinic methyl [δ_H 1.65 (br s); 15.9 (s)], and a hydroxymethyl group [δ_H 3.48 (1H, dd, *J*=6.2 and 11.4 Hz) and 3.73 (1H, dd, *J*=5.9 and 11.4 Hz); δ_C 66.1 (d)]. These data suggested that **1** was a rearranged cembranoid deivative, since cembranoids usually possess five methyl groups.

The ^{1}H-^{1}H COSY spectrum resolved the partial structures **A**, **B**, and **C** (Figure 1). In **A**, H-3 (δ_H 5.47; d, *J*=15.8 Hz) was coupled to H-2 (δ_H 5.23; dd, J=9.2 Hz and 15.8 Hz), which in turn was coupled to H-1 (*ca* . δ_H 1.6; m). The proton H-1 was also was coupled to H-15 (δ_H 1.53; m) of an isopropyl group as well as to the H-14 (δ_H 0.98; m). In **B**, the methyl groups H-18 were part of an isolated methyl group (δ_H 1.31; s) on a carbon carrying the hydroxyl group. In **C**, H-7 (δ_H 5.29; br t, *J*=6.6 Hz) was coupled to H-6 (δ_H 2.15-2.25; 2H, m) and was also weakly coupled to the H-19 (δ_H 1.65; br s), which in turn was further coupled to one of methylene protons at C-9 (δ_H 2.66; 1H, br d, *J*=12.1 Hz). The chemical shift of this methylene group suggested it was located between olefin and carbonyl groups, leading to partial structure **C**. In addition, the presence of a tertiary methyl group (δ_H 1.16; s) and two methylenes δ_H 1.31 (1H, m, H-13) and 1.72-1.85 (3H, m, H-5 and H-13) as well as an isolated hydroxymethyl group was observed. The gross structure was completed by COLOC experiments as shown in Figure 1. The connectivity of C-3 and C-4 resulted from cross peaks between H-18 and C-4, and between H-18 and C-3. The linkage between C-4 and C-5 was suggested by cross peaks between H-18 and C-5. The H-20 methyl protons displayed correlation with C-10, C-11, and C-12, suggesting bonds between C-12 and C-10, as well as C-11 and C-20. The geometry of the olefinic bonds at C-2 was determined to be *E* by the magnitude of the coupling constant between H-2 and H-3 (*J*=15.8 Hz) and the absence of a NOE between H-2 and H-3. The geometry of the double bond at C-7 was also *E* from the chemical shift of the methyl at C-19 (δ_C 15.9)[8] and the absence of a NOE between H-7 and H-19.

The relative stereochemisty of all chiral centers except for C-4 was elucidated from the observed NOE cross peaks in C_6D_6 (Figure 2). It was concluded that in the major conformer, H-3, H-7, and H-9β occur on the same face of the ring system, since irradiation of H-3 resulted in a 5.0, 2.1, and 2.3% enhancement of H-1, H-7, and H-9β, respectively. Irradiation of H-20 induced a 1.8% peak enhancement of H-9β (δ_H 2.66). Irradiations of H-9α and H-19 resulted in enhancements of H-19 and H-6α, suggesting that H-6α, H-9α, and H-20 were on the opposite face to H-3.



1 R^1=O R^2=CH_2OH
2 R^1=O R^2=CH_2OAc
3 R^1=β-OAc R^2=CHO
4 R^1=O R^2=*p*-BrPhCO

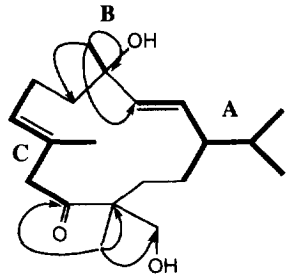


Figure 1. COLOC correlations for **1**.

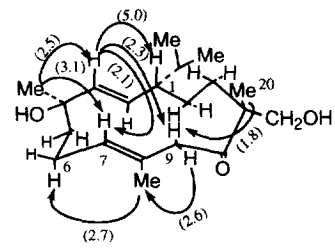


Figure 2. NOE (%) observed for **1**.

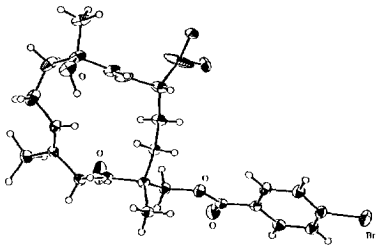


Figure 3. ORTEP representation of **4**

Final confirmation of the structure and stereochemisty was provided by X-ray analysis on sarcotol 11-*O*-*p*-bromobenzoate (**4**), prepared from **1** in a usual manner. A computer-generated perspective drawing of the final X-ray model of **4** is given in Figure 3.

Sarcotol acetate **2**, was isolated as an oil with a molecular formula $C_{22}H_{36}O_4$. The ^{1}H-NMR spectrum was very similar to that of **1**, except for an additional resonance due to acetyl protons at δ_H 2.02 (3H, s). Location of the acetyl group at C-11 was evident from the downfield chemical shift of H-11 (Δ +0.6 ppm) compared to that of **1**, hence **2** is sarcotol 11-*O*-acetate. This was confirmed by acetylation of **1**, which gave the identical monoacetate.

The third compound, sarcotal acetate **3**, $[\alpha]_D^{27}$ -44.0°, $C_{22}H_{36}O_4$, had the same molecular formula as **2**. In the ^{13}C-NMR spectrum, resonances due to an aldehyde (δ_C 204.3) and an acyl group (δ_C 170.1) was identified but the signal due to a ketone group was not observed. The ^{1}H-NMR spectrum of **3** was similar to that of **1**, except for resonances due to acetyl protons at δ_H 1.98 (3H, s), a proton at δ_H 5.30 (1H, dd, *J*=2.2 and 10.3 Hz) on a carbon bearing the acetyl group, and an aldehydic proton at δ_H 9.36 (s). The position of the acetyl group was determined to be at C-10 from the signal patterns of H-9 (δ_H 2.52; 1H, dd, *J*=10.3 and 13.9 Hz and δ_H 2.12; 1H, m) and H-10 (δ_H 5.30; 1H, dd, *J*=2.2 and 10.3 Hz). The relative stereochemistry of the chiral centers was assumed to be identical to that of **1** from the comparison of the NOE experiments of **3** with those of **1** (Figure 4). In particular, enhancement of H-1, and H-3 and H-9β (δ_H 2.52) was observed following irradiation of H-3, H-7, and H-20, respectively. The acetyl group and aldehyde were determined to be in β- and α-configurations, respectively, from the observation of NOEs from H-10 to H-9α at δ_H 2.12, H-11, and H-19.

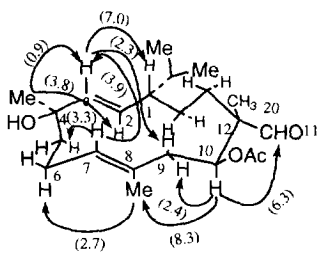


Figure 4. NOE (%) observed for **3**

Final proof of the stereochemistry of **3** was established by comparing the sodium borohydride reduction products of **1**, **2** and **3** (Figure 5). Reduction of sarcotal acetate **3** afforded an alcohol (**5**) ($C_{22}H_{34}O_4$; $[\alpha]_D^{27}$ -16.2°) and its isomer (**6**) ($[\alpha]_D^{27}$ -48.0°) in 40% and 50% yields, respectively. The ^{1}H-NMR spectrum of **5** was similar to that of **3**, except that resonances assigned to hydroxymethyl protons at δ_H 3.12 (1H, dd, *J*=5.4 and 11.7 Hz) and 3.28 (1H, dd, *J*=8.6 and 11.7 Hz) were observed in place of those due to the aldehydic proton in **3**. On the other hand, the ^{1}H-NMR spectrum of **6** was similar to that of **5**, except that resonance for H-10 (δ_H 3.66; 1H, ddd, *J*=1.8, 4.0, and 8.8 Hz) was shifted upfield (Δ δ +1.4) and that for H-11 (δ_H 4.00; 2H, AB, *J*=11.3 Hz) was shifted downfield (Δ δ –0.8). The above results showed that compound **6**, the 10-deacetyl-11-acetyl derivative of **5**, was formed from **5** by an ester exchange. Reduction of **2** afforded the expected alcohols **6** (13%) and **7** (21%), together with **5** (12%), **8** (7%), and **9** (22%). The resonance for C-20 in compound **7** (δ_C 20.7) was deshielded compared with the value in compound **6** (δ_C 15.1), suggesting that **7** was the C-10 epimer of **6**. Further proof was obtained by the observation of a NOE between H-10 and H-20. Compounds **8** and **9**, $C_{20}H_{36}O_3$ were 11-deacetyl derivatives of **6** and **7**, respectively. The only major difference in the ^{1}H-NMR spectra between **6**

and **8** was that the chemical shifts of H-11 in **6** was shifted downfield ($\Delta\ \delta$ –0.48) compared to that of **8**, while the chemical shift of H-11 in **7** was also shifted downfield ($\Delta\ \delta$ –0.52) compared to that of **9**. As further proof it was found that both **8** (7%) and **9** (47%) was also obtained by reduction of **1** with sodium borohydride. The optical rotations of **5** and **6** prepared from either **2** or **3** were similar (see experimental), suggesting the same absolute stereochemistry, and implying that the absolute structure of **3** as depicted.

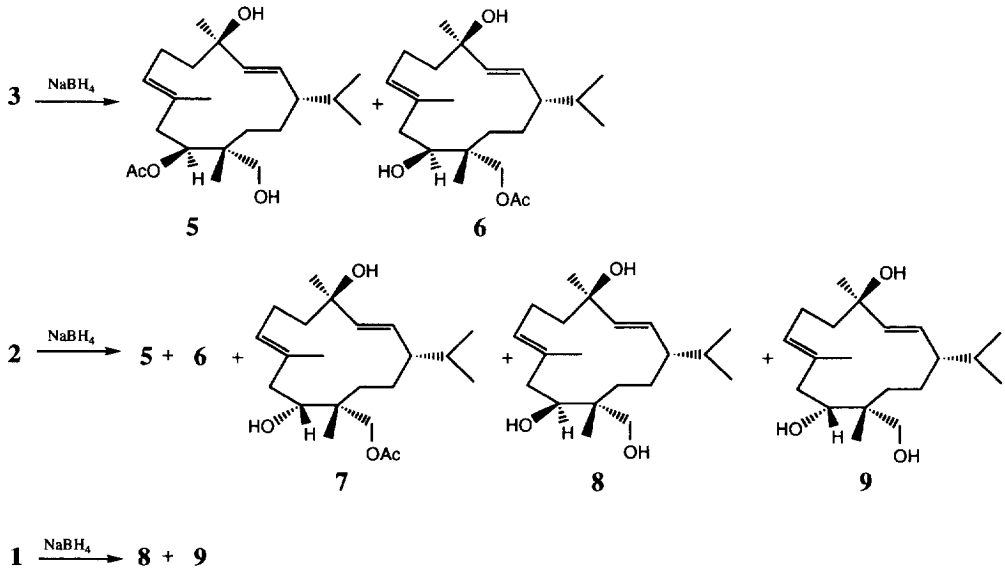


Figure 5. Reduction products of **3**, **2**, and **1**.

The biogenesis of these unusual diterpenoids is interesting, and sarcotol, sarcotol acetate, and sarcotal acetate could be formed by a ring contraction mechanism of a 14-membered cembrane shown in Figure 6, in which an C-11, C-12 epoxide rearranges to give an aldehyde group at C-12.

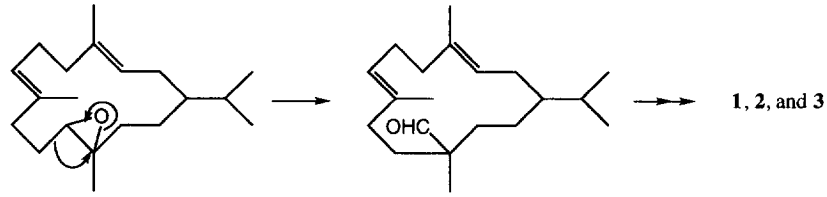


Figure 6. Possible biogenetic pathway for **1**, **2**, and **3**

Ichthyotoxity tests were performed for sarcotol **1**, sarcotol acetate **2**, and sarcotal acetate **3**, and their reduction products **5**-**9**. Compounds **2**, **3**, **5**, and **7** were ichthyotoxic to Japanese killifish, *O. latipes* at 20 ppm which died within 24 hr, while **6** was not toxic at 20 ppm. Compound **8** and **9** showed toxicity at 20 ppm over 24 hr.

EXPERIMENTAL SECTION

Melting points were uncorrected. Optical rotations were measured on a JASCO DIP-370S spectropolarimeter. IR spectra were recorded on a JASCO FT/IR-5300 spectrophotometer. NMR spectra were recorded with a JEOL JNM-GX 400 spectrometer. Mass spectra were obtained with a JEOL JNM D300 spectrometer at 70 eV. Rigaku AFC5R diffractometer was used in the X-ray work.

Extract and isolation. Specimens of *Sarcophyton* sp. were collected at depths of -8 m at Bonotsu, Kagoshima prefecture. The reference sample was deposited in Wakayama Prefectural Museum of Natural History and identified by Dr. Y. Imahara. The organisms (wet weight: 2.4 kg) was chopped into small pieces and extracted with CH_3OH immediately after collection. The CH_3OH extract (44.5g) was suspended into H_2O and extracted with CH_2Cl_2. The CH_2Cl_2 layer was dried over Na_2SO_4, filtered, and evaporated to dryness. A portion (5 g) of the CH_2Cl_2 extract (13.5g), which showed strong ichthyotoxic activity against killifish *O. latipes*, was chromatographed on silica gel using CH_2Cl_2-CH_3OH increasing proportion of CH_3OH to elute the column. The fraction eluted with CH_3OH-CH_2Cl_2 (3:97) were applied to a column of Sephadex LH-20 and eluted with CH_3OH. The toxic fraction was further purified by silica gel chromatography with CH_3OH-CH_2Cl_2 (0.8:99.2) and then EtOAc-CH_2Cl_2 (1:4) to give sarcotol **1** (35 mg) as needles. Sarcotol acetate **2** (20 mg) was obtained from the mother liquors by reversed-phase C_{18} chromatography with CH_3OH-H_2O (16:9). The remaining CH_2Cl_2 (8 g) extract was subjected to silica gel chromatography with CH_2Cl_2-hexane (4:1) and then EtOAc-hexane (3:7) to give an ichthyotoxic fraction (78 mg) that was purified by reversed-phase C_{18} chromatography with CH_3OH-H_2O (13:7) to give sarcotal acetate **3** (8 mg).

Sarcotol 1. Needles, mp 113°C, $[\alpha]_D^{27}$ -189.7° (*c* 0.01, CH_3OH); UV λ_{max}^{MeOH} 208 nm (ε 3500); IR (Nujol) 3293, 1692, and 1655 cm^{-1}; MS *m/z* (relative intensity) 322 (M^+; 3), 304 (29), and 123 (100); ^{1}H-NMR ($CDCl_3$) δ 0.81 and 0.85 (3H each, d, *J*=6.6 Hz, H-16, 17), 0.98 (1H, m, H-14endo), 1.16 (3H, s, H-20), 1.31 (1H, m, H-13exo), 1.31 (3H, s, H-18), 1.40 (H-14exo), 1.53 (1H, m, H-15), 1.60 (1H, m, H-1), 1.65 (3H, s, H-19), 1.72-1.85 (3H, m, H-5 x 2 and H-13endo), 2.18 (2H, m, H-6), 2.66 (1H, br d, *J*=12.1 Hz, H-9α), 3.48 (1H, dd, *J*=6.2 and 11.4 Hz, H-11), 3.53 (1H, d, *J*=12.1 Hz, H-9β), 3.73 (1H, dd, *J*=5.9 and 11.4 Hz, H-11), 5.23 (1H, dd, *J*=9.2 and 15.8 Hz, H-2), 5.29 (1H, br t, *J*=6.6 Hz, H-7), and 5.47 (1H, d, *J*=15.8 Hz, H-3); ^{1}H-NMR (C_6D_6): δ 0.81 and 0.85 (3H each, d, *J*=6.6 Hz), 0.98 (3H, s, H-20), 1.02 (1H, m, H-14endo), 1.13 (3H, s, H-20), 1.13 (1H, m, H-13exo), 1.29 (1H, m, H-14), 1.42 (1H, oct, *J*=6.6 Hz, H-15), 1.45 (1H, m, H-1), 1.57-1.68 (3H, m, H-5 x 2 and H-13endo), 1.70 (3H, s, H-19), 1.97-2.03 (1H, m, H-6), 2.08 (1H, m, H-6), 2.56 (1H, br d, *J*=12.1 Hz, H-9α), 3.27 (1H, d, *J*=12.1 Hz, H-9β), 3.50 and 3.64 (AB, br d, *J*=11.0 Hz, H-11), 5.08 (1H, m, H-7), 5.11 (1H, dd, *J*=8.6 and 16.1 Hz, H-2), and 5.37 (1H, d, *J*=16.1 Hz, H-3); ^{13}C-NMR ($CDCl_3$) δ 15.9 (C-19), 19.8 and 16.7 (C-16, and 17), 20.4 (C-20), 23.4 (C-6), 25.8 (C-14), 27.6 (C-18), 32.6 (C-15), 34.9 (C-13), 43.1 (C-5), 49.0 (C-9), 50.2 (C-1), 53.7 (C-11), 66.1 (C-11), 73.1 (C-4), 128.6 (C-8), 129.9 (C-2), 131.3 (C-7), 137.8 (C-3), and 214.3 (C-10). HREIMS *m/z* 322.2524 (M^+, calcd for $C_{20}H_{34}O_3$, 322.2508).

Sarcotol acetate 2. Oil, $[\alpha]_D^{27}$ -182.4° (*c* 0.187, CH_3OH); UV λ_{max}^{MeOH} 208 nm (ε 2130); IR (film) 3466, 1744, 1699, 1661, and 1240 cm^{-1}; MS *m/z* (relative intensity) 364 (M^+; 2), 123 (98), and 81 (100); ^{1}H-NMR ($CDCl_3$) δ 0.79 (3H, d, *J*=7.0 Hz, H-16 or H-17), 0.83 (3H, d, *J*=6.6 Hz, H-17 or H-16), 0.92 (1H, m, H-14exo), 1.18 (3H, s, H-20), 1.31 (3H, s, H-18), *ca.* 1.30 (1H, m, H-14endo), 1.35 (1H, m, H-14endo), 1.52 (1H, oct, *J*=6.6 Hz, H-15), 1.60 (1H, m, H-1), 1.68 (3H, s, H-19), 1.79 (2H, m, H-5), 1.89 (1H, dt, *J*=3.7 and

14.0 Hz, H-13endo), 2.02 (3H, s, OAc), 2.18 (2H, m, H-6), 2.75 (1H, br d, *J*=12.5 Hz, H-9α), 3.44 (1H, d, *J*=12.5 Hz, H-9β), 4.11 and 4.22 (AB, *J*=11.0 Hz, H-11), 5.24 (1H, dd, *J*=8.4 and 15.8 Hz, H-2), *ca* . 5.24 (1H, obscured, H-7), and 5.43 (1H, d, *J*=15.8 Hz, H-3); ^{13}C-NMR ($CDCl_3$) δ 16.2 (C-19), 19.7 (C-16 or 17), 20.2 (C-17 or 16), 20.8 (C-20), 20.9 (OCO<u>C</u>H$_3$), 23.6 (C-6), 25.5 (C-14), 28.1 (C-18), 32.5 (C-15), 34.3 (C-13), 42.8 (C-5), 48.6 (C-9), 49.1 (C-10, 51.6 (C-11), 67.0 (C-12), 73.2 (C-4), 128.8 (C-8), 129.5 (C-2), 131, 2 (C-7), 137.6 (C-3), and 170.9 (O<u>C</u>OCH$_3$). HREIMS *m/z* 364.2622 (M^+, calcd for $C_{22}H_{36}O_4$, 364.2614).

Sarcotal acetate 3. Oil, $[\alpha]_D^{27}$ -44.0° (*c* 0.1, CH_3OH); UV λ_{max}^{MeOH} 209nm (ε 3780); IR (film) 3427 and 1736 cm^{-1}; MS *m/z* (relative intensity) 364 (M^+; 2) and 95 (100); ^{1}H-NMR ($CDCl_3$) δ 0.80 and 0.83(3H each, d, *J*=5.6 Hz, H-16, 17), *ca*.. 1.05 (1H, m, H-14endo), 1.14 (3H, s, H-20), *ca* . 1.30 (1H, m, H-14exo), 1.34 (3H, s, H-18), *ca* . 1.40 (1H, m, H-13), 1.48 (1H, m, H-15), 1.64 (1H, m, H-1), 1.71 (3H, br s, H-19), *ca* . 1.78 (2H, m, H-5), 1.98 (3H, s, OAc), 2.12 (3H, m, H-6 & H-9α), 2.52 (1H, dd, *J*=10.3 & 13.9 Hz, H-9β), 5.18 (1H, dd, *J*=10.2 & 15.8 Hz, H-2), 5.26 (1H, br t, *J*=6.6 Hz, H-7), 5.30 (1H, dd, *J*=2.2 & 10.3 Hz, H-10), 5.50 (1H, d, *J*=15.8 Hz, H-3), and 9.36 (1H, s, H-11); ^{13}C-NMR ($CDCl_3$) δ 14.0 (C-20), 15.8 (C-19), 19.7, 20.5 (C-16, 17), 21.1 (OCO<u>C</u>H$_3$), 23.5 (C-6), 26.0 (C-14), 26.9 (C-18), 30.6 (C-13), 32.4 (C-15), 42.1 (C-9), 43.2 (C-5), 50.7 (C-1), 54.3 (C-12), 72.4 (C-10), 73.0 (C-4), 129.9 (C-2), 130.6 (C-7), 130.8 (C-8), 138.9 (C-3), 170.1 (O<u>C</u>OCH$_3$), and 204.3 (C-11). HREIMS *m/z* 364.2567 (M^+, calcd for $C_{22}H_{36}O_4$, 364.2572).

Acetylation of 1. Sarcotol **1** (5.4 mg) was treated with Ac_2O in pyridine to give a monoacetate (2.8 mg), $[\alpha]_D^{27}$ -187.5° (*c* 0.08, CH_3OH), MS *m/z* 364 (M^+). The spectral data were identical with those of **2**.

***p*-Bromobenzoylation of 1**. A mixture of **1** (17 mg), *p*-BrBzCl (34 mg), DMAP (2 mg) , and pyridine (2 ml) was stirred at 45°C for 2 days. The reaction mixture was poured into ice-water and extracted with EtOAc. The extract was washed successively with saturated $CuSO_4$, H_2O, saturated $NaHCO_3$, H_2O, and brine and dried over $MgSO_4$. The residue obtained after evaporation of the solvent was purified by reversed-phase C_{18} chromatography with CH_3OH-H_2O (9:1) to afford the mono-*p*-bromobenzoate **4** (10 mg) that was crystallized from MeOH-benzene mixture as prisms, mp 119-120°C; IR (KBr) 3449, 1717, 1686, 1591, and 847 cm^{-1}; ^{1}H-NMR (CD_3OD): δ 0.81 (3H, d, *J*=7.0 Hz, H-16 or 17) and 0.84 (3H, d, *J*=6.6 Hz, H-17 or 16), 0.98 (1H, m, H-14exo), 1.24 (3H, s, H-18), 1.27 (3H, s, H-20), 1.42 (1H, m, H-14endo), 1.48-1.57 (2H, m, H-13 and H-15), 1.64 (3H, s, H-19), 1.64 (1H, m, H-1), 1.71 (1H, m, H-5exo), 1.80 (1H, dt, *J*=3.1 and 9.0 Hz, H-5endo), 2.03 (1H, dt, *J*=4.2 and 13.8 Hz, H-13endo), 2.19 (1H, m, H-6β), 2.74 (1H, br d, *J*=12.1 Hz, H-9α), 3.63 (1H, d, *J*=12.1 Hz, H-9β), 4.33 and 4.65 (AB, *J*=11.0 Hz, H-11), 5.18 (1H, dd, *J*=8.8 and 15.6 Hz, H-2), 5.40 (1H, br t, *J*=6.2 Hz, H-7), 5.48 (1H, d, *J*=16.2 Hz, H-3), 7.65, and 7.83 (2H each, *J*=8.8 Hz, arom-H); ^{13}C-NMR (CD_3OD) δ 16.3 (C-19), 20.3, 20.7, and 21.2 (C-16, 17 or 20), 24.5 (C-6), 27.0 (C-14), 27.9 (C-18), 33.8 (C-15), 35.5 (C-13), 44.4 (C-5), 49.7 (C-9), 51.2 (C-1), 53.2 (C-11), 68.4 (C-11), 73.8 (C-4), 129.2 (C'-1 or C'-4), 129.5 (C'-4 or C'-1), 130.5 (C-8), 130.7 (C-2), 132.1 (C'-2 and 6), 133.1 (C'-3 and 5), 133.2 (C-7), 139.3 (C-3), 166.9 (-<u>C</u>OO-), and 212.3 (C-10). HREIMS *m/z* 486.1783 [$(M-H_2O)^+$, calcd for $C_{27}H_{35}O_3Br$, 486.1787].

X-ray analysis of 3. Crystal data: $C_{27}H_{37}O_4Br$, Mr=$C_{27}H_{37}O_4Br$, Mr=505.49, monoclinic, P21, a=5.854(2), b=10.816(2), c=20.088(2)Å, β=92.97(2)°, V=1270.1(4)Å^3, Z=2, Dx=1.322 g/cm^3, F(000)=532, μ(Cu Kα)=24.38 cm^{-1}. Intensity data were collected at 200K on a Rigaku AFC5R diffractometer using graphite monochromatized Cu Kα radiation (λ=1.54178 Å) up to 2θ=120°. Of the total 2011 unique reflections, 1878 were observed [I>3σ(I)]. The structure was solved by a heavy atom method and refined by full-matrix least-squares techniques to R=0.035 and Rw=0.054. The non-hydrogen atoms were refined

anisotropically, but the hydrogen atoms not refined. The isopropyl group is disordered at the two locations (C16, C17) and (C16', C17'), with an occupancy ratio of 0.583 : 0.417. The hydrogen atoms of the group were not located. The absolute configuration of the molecule was determined based on the Bijvoet inequality relationships mainly due to the anomalous dispersion of the bromine atom ($\Delta f'$=-0.676, $\Delta f''$=1.281): Bijvoet differences of Fo and Fc were compared for 36 pairs with $|\Delta Fcl/\sigma(Fo)|>1.0$, those of 33 pairs exhibiting the same inequality signs. Detailed atomic coordinates, bond distances and angles have been deposited at the Cambridge Crystallographic Data Center.

Reduction of 3. A solution of **3** (4 mg) in CH_3OH (1 ml) was stirred with $NaBH_4$ (5 mg) for 3 hr. The solution was added to ice-water, extracted with EtOAc. The EtOAc extract was washed with H_2O and dried over Na_2SO_4, filtered, and evaporated to give a residue. The residue was subjected to silica gel column chromatography with acetone-CH_2Cl_2 (1:19) and then purified by a C_{18} reversed-phase column with CH_3OH-H_2O (7:3), yielding **5** (1.2 mg) and **6** (2.0 mg). **5**: oil, $[\alpha]_D^{27}$ -16.2° (*c* 0.06, CH_3OH); IR (film) 3785, 1717, 1660, and 1250 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 0.80 (3H, s, H-20), 0.83, 0.88 (3H each, d, *J*=6.6 Hz, H-16, 17), 1.33 (3H, s, H-18), *ca* . 1.52 (1H, m, H-15), 1.67 (3H, br s, H-19), 1.83 (1H, dt, *J*=3.5, 9.1 Hz, H-5exo), 1.99 (1H, br d, *J*=15.4 Hz, H-9α), 2.08 (3H, s, CH_3CO), 2.13 (2H, m, H-6), 2.32 (1H, dd, *J*=5.3, 8.6 Hz, HO), 2.61 (1H, dd, *J*=9.4, 14.5 Hz, H-9β), 3.12 (1H, br dd, *J*=5.4, 11.7 Hz, H-11), 3.28 (1H, dd, *J*=8.6, 11.7 Hz, H-11), 5.06 (1H, dd, *J*=1.1, 9.2 Hz, H-10), 5.20 (1H, dd, *J*=9.9, 15.8 Hz, H-2), 5.21 (1H, obscured, H-7), and 5.49 (1H, d, *J*=15.8 Hz, H-3); ^{13}C-NMR ($CDCl_3$) δ 15.2 (q), 15.8 (q), 20.2 (q), 20.7 (q), 21.3 (q), 23.3 (t), 25.3 (t), 26.8 (d), 32.5 (d), 33.3 (t), 42.4 (t), 43.0 (s), 43.6 (t), 52.0 (d), 65.2 (d), 72.8 (s), 73.3 (d), 129.2 (d), 130.9 (d), 131.8 (s), 138.3 (d), and 171.5 (s). HREIMS *m/z* 348.2659 [$(M-H_2O)^+$, calcd for $C_{22}H_{36}O_3$, 348.2662]. **6**: oil, $[\alpha]_D^{27}$ -48.0° (*c* 0.01, CH_3OH), ^{1}H-NMR ($CDCl_3$) δ 0.82, 0.86 (3H each, d, J=7.0, H-16, 17), 0.88 (3H, s, H-20), 1.32 (3H, s. H-18), 1.50 (1H, m, H-15), 1.64 (3H, br s, *J*=19), 1.80 (1H, dt, *J*=3.7, 8.8 Hz, H-5exo), 2.04 (1H, br d, *J*=14.3 Hz, H-9α), 2.08 (3H, s, CH_3CO), 2.12 (2H, m, H-6), 2.50 (1H, dd, *J*=8.8, 14.3 Hz, H-9β), 3.66 (1H, ddd, *J*=1.8, 4.0, 8.8 Hz, H-10), 3.82 (1H, d, *J*=11.0, H-12), 4.18 (1H, d, *J*=11.4 Hz, H-12), 5.15 (1H, obscured, H-7), 5.17 (1H, dd, *J*=10.2, 15.8 Hz, H-2), and 5.47 (1H, d, *J*=15.8 Hz, H-3); ^{13}C-NMR ($CDCl_3$) δ15.1 (q), 15.9 (q), 20.0 (q), 20.5 (q), 21.0 (q), 23.4 (q), 25.4 (t), 26.9 (d), 32.5 (q), 33.4 (t), 42.2 (s), 43.4 (t), 45.1 (t), 51.2 (d), 68.9 (t), 71.6 (d), 72.9 (s), 128.6 (d), 130.4 (d), 132.3 (s), 138.3 (d), and 171.5 (s). HREIMS *m/z* 366.2762 (M^+), calcd for $C_{22}H_{38}O_4$, *m/z* 366.2768).

Reduction of 2. A solution of **2** (12.2 mg) in CH_3OH (2 ml) was treated with $NaBH_4$ (12 mg). After usual work-up, the mixture was subjected to chromatography on silica gel with acetone-CH_2Cl_2 (3:97 to 1:19) and on an ODS with CH_3OH-H_2O (3:2 to 13:7) to give **5** (1.5 mg), **6** (2.8 mg), **7** (2.6 mg), **8** (0.8 mg), and **9** (2.7 mg). The spectral data of **5** and **6** were identical with those of **5** and **6** prepared from **3**, respectively. The spectral data of **8** and **9** were also in good agreement with those of **8** and **9** obtained from **1**, respectively. **5**: $[\alpha]_D^{27}$ -11.4° (*c* 0.035, CH_3OH); **6**: $[\alpha]_D^{27}$ -77.0° (*c* 0.08, CH_3OH); **7**: $[\alpha]_D^{27}$ -21.3° (*c* 0.13, CH_3OH); **8**: $[\alpha]_D^{27}$ -62.1° (*c* 0.04, CH_3OH); **9**: $[\alpha]_D^{27}$ +48.0° (*c* 0.10, CH_3OH)

Reduction of 1. A solution of **1** (20 mg) in CH_3OH (1 ml) was treated with $NaBH_4$ (20 mg) in the same way as described above, yielding **8** (1.4 mg) and **9** (9.5 mg) **8**: $[\alpha]_D^{27}$ -45.7° (*c* 0.07, CH_3OH), IR (film) 3385 and 1645 cm^{-1}; ^{1}H-NMR ($CDCl_3$-CD_3OD) δ 0.84, 0.87 (3H each, d, *J*=6.6 Hz, H-16, 17), 0.93 (3H, s, H-20), 1.31 (3H, s, H-18), 1.51 (1H, m, H-15), 1.67 (3H, br s, H-19), 1.80 (1H, dt, *J*=6.3, 14.7 Hz, H-5exo), 1.94 (1H, br d, *J*=14.7 Hz, H-9α), 2.12 (2H, m, H-6), 2.52 (1H, dd, *J*=9.0, 15.0 Hz, H-9β), 3.48, 3.55 (1H each, d, *J*=10.6 Hz, H-12), 3.85 (1H, d, *J*=8.1 Hz, H-10), 5.14 (1H, dd, *J*=9.5, 15.8 Hz, H-2), 5.18 (1H,

obscured, H-7), and 5.46 (1H, d, *J*=15.8 Hz); ^{13}C-NMR ($CDCl_3$-CD_3OD) δ14.9 (q), 15.9 (q), 20.0 (q), 20.7 (q), 23.6 (t), 25.8 (t), 26.5 (q), 33.1 (d), 35.3 (t), 42.6 (s), 43.9 (t), 45.8 (t), 52.0 (d), 70.4 (t), 73.0 (s), 74.9 (d), 129.0 (d), 130.4 (d), 137.2 (s), and 138.5 (d). HREIMS *m/z* 306.2562 [$(M-H_2O)^+$, calcd for $C_{20}H_{34}O_2$, 306.2559]. **9**: $[\alpha]_D^{27}$ +55.8° (*c* 0.33, CH_3OH), IR (film) 3352 and 1660 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ0.73 (1H, dt, *J*=5.1, 12.8 Hz, H-13endo), 0.82, 0.84 (3H each, d, *J*=6.6 Hz), 0.98 (1H, ddd, *J*=4.0, 12.5, 23.5 Hz, H-14endo), 1.14 (3H, s, H-20), 1.32 (3H, s, H-18), *ca* . 1.36 (1H, m, H-14exo), 1.58 (1H, m, H-15), 1.65 (3H, br s, H-19), 1.81 (1H, dt, *J*=3.9, 13.0, H-13exo), *ca* . 1.92 (1H, m, H-5exo), *ca* . 1.96 (1H, m, H-6endo), 2.26-2.33 (1H, m, H-6exo), 2.38 (1H, br d, *J*=15.0 Hz, H-9α), 2.59 (1H, br dd, *J*=10.1, 14.1 Hz, H-9), 2.72 (1H, br d, H-6.2 Hz, OH), 3.49 (1H, br d, *J*=10.3 Hz, H-11), 3.59 (1H, m, H-10), 3.88 (1H, d, *J*=10.3 Hz, H-11), 5.12 (1H, br d, *J*=9.2 Hz, H-7), and 5.30-5.39 (2H, m, H-2 and 3); ^{13}C-NMR ($CDCl_3$) δ 16.4 (q), 19.7 (q), 20.3 (q), 20.4 (q), 22.1 (t), 24.7 (t), 30.2 (q), 32.0 (t), 32.2 (d), 42.0 (s), 43.4 (t), 46.5 (t), 49.1 (d), 68.1 (t), 73.0 (s), 77.7 (d), 129.7 (d), 129.9 (d), 132.7 (s), and 137.3 (d). HREIMS *m/z* 306.2583 [$(M-H_2O)^+$, calcd for $C_{20}H_{34}O_2$, 306.2559].

Bioassay. The bioassay was performed on compounds **1-9**. In this test two Japanese killifish, *O. latipes*, were put in a beaker (50 ml) containing H_2O (50 ml), and a solution of each test compound (dissolved in <125 μl of MeOH) was added. In a control experiment only MeOH (125 μl) was added, which showed no observable effect on the killifish.

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