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J. Nat. Prod., **1992**, 55 (12), 1779-1782• DOI: 10.1021/np50090a012 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 55, No. 12, pp. 1779-1782, December 1992

NEW CEMBRANE-TYPE DITERPENOIDS FROM THE OKINAWAN SOFT CORAL *SINULARIA* SP.

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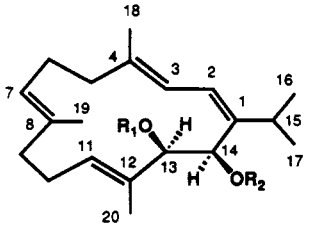
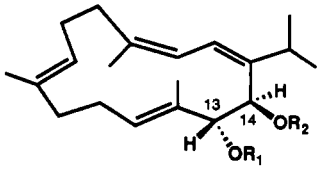
ABSTRACT.—Two new cembrane-type diterpenoids, 14-*O*-acetylsarcophytol B and 14-*O*-acetylsarcophytol J, were isolated from the Okinawan soft coral *Sinularia* sp. Structural assignment was made based on spectroscopic analysis and chemical transformation. The absolute configuration of 14-*O*- acetylsarcophytol B was confirmed by the modified Mosher method.

Sarcophytols A and B [**1**] are cembrane-type diterpenoids isolated from the Okinawan soft coral *Sarcophyton glaucum* (1,2). Their biological activity for inhibiting tumor promotion (3) has made them of particular interest. While investigating chemical substances from Okinawan marine invertebrates, two new sarcophytol-related diterpenoids, **2** and **6**, were isolated from the soft coral *Sinularia* sp. This paper describes the structures of these diterpenoids based on spectroscopic data and chemical transformation. Confirmation of the absolute configuration of **2** was made by the modified Mosher method.

The MeOH extract of specimens (wet wt 2.3 kg) of the soft coral *Sinularia* sp., collected on the coral reef of Ishigaki Island, Okinawa, Japan, was partitioned between EtOAc and H_2O. The EtOAc-soluble portion (8.0 g) was chromatographed on a Si gel column by elution with hexane-EtOAc (10:1 to 1:1), EtOAc, and MeOH, in this order, to give 4 fractions. From fraction 1 [eluted by hexane-EtOAc (10:1)] cembrane C (4) was isolated in 22% yield based on the EtOAc-soluble portion. From fraction 2 [eluted by hexane-EtOAc (1:1)], compounds **2** and **6** were obtained in 0.063% and 0.038% yields, respectively, based on the EtOAc-soluble portion.

Compound **2** was found to have the molecular formula $C_{22}H_{34}O_3$ from the high resolution mass measurement. Its uv and ^{1}H-nmr spectra showed the presence of a conjugated diene group (C=CH-CH=C) [uv 252 nm (ϵ 12000); δ 6.09 (1H, d, J=11.5 Hz), 6.21 (1H, d, J=11.5 Hz)]. The ir and ^{1}H-nmr spectra indicated hydroxy and acetoxy groups to be present [ir 3460 (OH), 1740, 1240 (OAc) cm^{-1}; δ 4.12 (1H, d, J=9.2 Hz, C*H*OH), 2.08 (3H, s, Ac), 5.94 (1H, d, J=9.2 Hz, C*H*OAc)]. The vicinal relationship between these two groups was determined by a decoupling experiment. Irradiation at 4.12 ppm (C*H*OH) changed the doublet at 5.94 ppm (C*H*OAc) into a singlet. The ^{1}H-nmr spectrum showed an isopropyl group, two trisubstituted olefins (MeC=CH), and four allylic methylenes (experimental section). Compound **2** thus





1 $R_1=R_2=H$
2 $R_1=H$, $R_2=Ac$
3 $R_1=MTPA(+)$, $R_2=Ac$
4 $R_1=MTPA(-)$, $R_2=Ac$

5 $R_1=R_2=H$
6 $R_1=H$, $R_2=Ac$
7 $R_1=R_2=Ac$

appeared to be a monoacetate of sarcophytol B [**1**]. The methanolysis of **2** confirmed this speculation. Treatment of **2** with Li_2CO_3 in MeOH gave a diol whose spectral data and sign of optical rotation were identical with those of sarcophytol B [**1**] (1). The position of the acetoxy group at C-14 was shown by a decoupling experiment. Irradiation at 5.47 ppm (H-11) sharpened the signal of 4.12 ppm (C*H*OH, H-13). The structure of **2** was thus assigned as 14-*O*-acetylsarcophytol B.

The relative stereochemistry of sarcophytol B [**1**] was confirmed by X-ray crystallographic analysis using a racemic sarcophytol B synthesized by McMurry *et al.* (5). For the absolute stereochemistry, Kobayashi *et al.* (6) reported 13*R* and 14*R* configurations based on cd data of the bis(4-dimethylamino)-benzoyl ester of **1**. These absolute configurations were confirmed in this study by conducting the modified Mosher method of Ohtani *et al.* (7) on the α-methoxy-α-(trifluoromethyl)phenylacetyl (MTPA) esters **3** and **4**. MTPA esters **3** and **4** were prepared by treating **2** with (*R*)-(+)-MTPA chloride and (*S*)-(−)-MTPA chloride, respectively. The ^{1}H-nmr spectrum for each compound was measured. Figure 1 shows δΔ, i.e., [δ of the (S)-(−)-MTPA ester **4**]−[δ of the (*R*)-(+)-ester **3**]. Their signs are positive due to left-sided protons but negative due to right-sided protons, thus demonstrating the 13*R* configuration based on the modified Mosher method. The relative configurations of the 13 and 14 positions in **1** have already been established, and the present results confirm the 13*R* and 14*R* configurations for sarcophytol B [**1**] and **2**.

The ^{1}H-nmr signals of **6** having the same molecular formula as that of **2**, $C_{22}H_{34}O_3$, were very similar to those of **2** except for signals due to a conjugated diene system [δ 6.19 (1H, d, *J*=11.1 Hz), 6.21 (1H, d, *J*=11.1 Hz)], showing **6** to be a geometrical isomer of **2**. Acetylation of **6** gave a diacetate identical to sarcophytol J diacetate [**7**] (8) obtained from sarcophytol J [**5**] with a 3*Z* configuration. The position of the acetoxy group at C-14 was indicated by a decoupling experiment. Irradiation at 4.15 ppm (H-13) sharpened the signal at 5.20 ppm (H-11). The structure of **6** was thus assigned as 14-*O*-acetyl sarcophytol J.

Assessment is currently being made of the biological activity of **2** and **6**.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURE.—Ir spectra were recorded with a Perkin-Elmer FT-IR 1710 spectrophotometer, and uv spectra with a Hitachi 124 spectrophotometer. ^{1}H-nmr spectra were recorded with a Bruker AM-400 spectrometer (400 MHz). Chemical shifts are given on a δ (ppm) scale with TMS as an internal standard (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad). Ms were taken with a Hitachi M-80 spectrometer. Optical rotations were measured with a JASCO DIP-360 automatic polarimeter. Cc was carried out on Fuji-Davison Si gel BW-820MH (70–200 mesh). Hplc was conducted with an HPLC-8502 (YMC) apparatus using a YMC-Pack A-043 S-5 column (Si gel). Prepara-

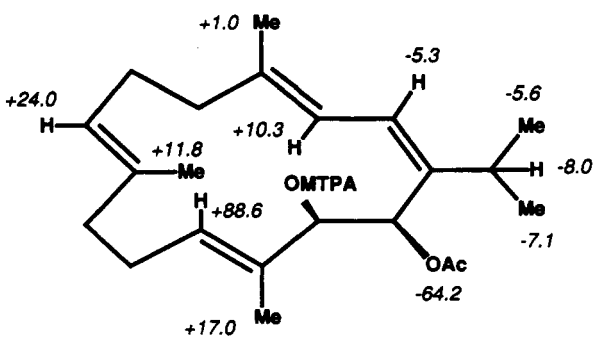


FIGURE 1. δΔ (δ of **4**−δ of **3**) of Hertz.

tive tlc was carried out on Si gel F_{254} tlc plates (Merck).

EXTRACTION AND ISOLATION.—The soft coral *Sinularia* sp. was collected on the coral reef of Ishigaki Island (Okinawa, Japan) in November 1990 at a depth of 1–2 m. The present soft coral has fingered processes with green-white tentacles. The surface is brown and the tissue is milk-white. These morphological characteristics strongly suggested that it belongs in the genus *Sinularia* (presumably *Sinularia polydactyla*). A voucher specimen (No. SC-7) is deposited at our laboratory, Tokyo College of Pharmacy (Tokyo, Japan). Wet specimens (2.3 kg) were extracted with MeOH. The MeOH extract was partitioned between EtOAc and H_2O. The EtOAc-soluble portion (8.0 g) was chromatographed on a Si gel column (100 g). Stepwise elution with hexane-EtOAc (10:1 to 1:1), EtOAc, and MeOH gave four fractions. The second fraction [4.45 g eluted with hexane-EtOAc (1:1)] was further subjected to Si gel cc [hexane-EtOAc (5:1) as an eluent] followed by hplc [hexane-EtOAc (5:1) as an eluent] to give 14-*O*-acetylsarcophytol J [**6**] (3 mg, colorless oil) and 14-*O*-acetylsarcophytol B [**2**] (5 mg, colorless oil).

14-O-Acetylsarcophytol B [**2**].—$[\alpha]_D$ +235.2° (c=0.60, $CHCl_3$); uv λ max nm (ε) 252 (12000); ms *m/z* $[M]^+$ 346; hrms calcd for $C_{22}H_{34}O_3$ $[M]^+$ 346.2509, found 346.2552; ir ν max cm^{-1} 3460, 1740, 1240; ^1H nmr ($CDCl_3$) δ 1.04 (3H, d, J=7.8 Hz, H-16 or -17), 1.06 (3H, d, J=8.0 Hz, H-16 or -17), 1.44 (3H, s, H-19), 1.65 (3H, d, J=1.0 Hz, H-20), 1.73 (3H, d, J=1.0 Hz, H-18), 2.08 (3H, s, OAc), 2.47 (1H, septet, J=6.8 Hz, H-15), 4.12 (1H, d, J=9.2 Hz, H-13), 5.02 (1H, t, J=7.2 Hz, H-7), 5.47, (1H, t, J=6.1, 5.7 Hz, H-11), 5.94 (1H, d, J=9.2 Hz, H-14), 6.09 (1H, d, J=11.5 Hz, H-3), 6.21 (1H, d, J=11.5 Hz, H-2).

14-O-Acetylsarcophytol J [**6**].—$[\alpha]_D$ −85.6° (c=0.21, $CHCl_3$); ms *m/z* $[M]^+$ 346; hrms calcd for $C_{20}H_{31}O_2$ $[M-Ac]^+$ 303.2353, found 303.2351; ^1H nmr ($CDCl_3$) δ 1.00 (3H, d, J=6.7 Hz, H-16 or -17), 1.09 (3H, d, J=6.9 Hz, H-16 or -17), 1.55 (3H, s, H-19 or -20), 1.63 (3H, s, H-19 or -20), 1.83 (3H, s, H-18), 2.10 (3H, s, OAc), 2.44 (1H, m, H-15), 2.64 (1H, dt, J=3.5, 12.8 Hz, H-5), 4.15 (1H, d, J=10.0 Hz, H-13), 4.85 (1H, dd, J=9.2, 9.4 Hz, H-7), 5.20 (1H, dd, J=8.7, 10.6, Hz, H-11), 5.91 (1H, d, J=10.0 Hz, H-14), 6.19 (1H, d, J=11.1 Hz, H-2 or -3), 6.21 (1H, d, J=11.1 Hz, H-2 or -3).

METHANOLYSIS OF **2**.—Li_2CO_3 (3 mg) was added to a solution of **2** (5 mg) in MeOH (1 ml), and the mixture was stirred at room temperature for 5 h. After dilution with excess Et_2O, the mixture was filtered through a short Si gel column. The filtrate was concentrated under reduced pressure to give sarcophytol B [**1**] (4 mg): $[\alpha]_D$ +165.5° (c=0.14, $CHCl_3$) [lit. (1) $[\alpha]_D$ +164° ($CHCl_3$)].

CONVERSION OF **2** TO ITS MTPA ESTERS.—(*R*)-(+)-α-Methoxy-α-(trifluoromethyl)-phenylacetyl chloride (0.02 ml) was added to a solution of **2** (2 mg) in pyridine (0.2 ml) and CCl_4 (0.2 ml). After addition of 4-dimethylaminopyridine (1 mg), the mixture was stirred at room temperature for 15 h. The reaction mixture was diluted with Et_2O; washed with H_2O, saturated $CuSO_4$ solution, H_2O, saturated $NaHCO_3$ solution, H_2O, and saturated NaCl solution; dried over anhydrous Na_2SO_4; and concentrated under reduced pressure. The residue was purified by preparative tlc [hexane-EtOAc (5:1)] to give **3** (3 mg, colorless crystals).

Similar reaction of **2** (2 mg) with (*S*)-(−)-α-methoxy-α- (trifluoromethyl)-phenylacetyl chloride gave **4** (2 mg, colorless crystals).

14-O-Acetyl-13-O-{(R)-α-methoxy-α-(trifluoromethyl)phenylacetyl}sarcophytol B [**3**].—$[\alpha]_D$ +155.4° (c=0.15, $CHCl_3$); ms *m/z* $[M]^+$ 562; hrms calcd for $C_{32}H_{41}F_3O_5$ $[M]^+$ 562.2906, found 562.2919; ^1H nmr ($CDCl_3$) δ 1.04 (3H, d, J=6.7 Hz, H-17), 1.12 (3H, d, J=6.9 Hz, H-16), 1.38 (3H, br s, H-19), 1.66 (3H, br s, H-19), 1.72 (3H, d, J=1.0 Hz, H-18), 1.95 (3H, s, OAc), 2.52 (1H, m, H-15), 3.55 (3H, br s, OMe), 4.95 (1H br t, J=8.1 Hz, H-7), 5.19 (1H, m, H-11), 5.40 (1H, d, J=10.2 Hz, H-13), 6.04 (1H, br d, J=11.5 Hz, H-3), 6.12 (1H, d, J=10.2 Hz, H-14), 6.27 (1H, d, J=11.5 Hz, H-2), 7.40 (3H, m, phenyl), 7.52 (2H, m, phenyl).

14-O-Acetyl-13-O-{(S)-α-methoxy-α-(trifluoromethyl)phenylacetyl}sarcophytol B [**4**].—$[\alpha]_D$ +163.1° (c=0.12, $CHCl_3$); ms *m/z* $[M]^+$ 562; hrms calcd for $C_{32}H_{41}F_3O_5$ $[M]^+$ 562.2906, found 562.2900; ^1H nmr ($CDCl_3$) δ 1.02 (3H, d, J=6.7 Hz, H-17), 1.10 (3H, d, J=6.9 Hz, H-16), 1.41 (3H, br s, H-19), 1.71 (3H, br s, H-20), 1.73 (3H, d, J=0.8 Hz, H-18), 1.79 (3H, s, OAc), 2.50 (1H, m, H-15), 3.51 (3H, br s, OMe), 5.01 (1H, br t, J=7.6 Hz, H-7), 5.41 (1H, m, H-11), 5.42 (1H, d, J=10.2 Hz, H-13), 6.07 (1H, d, J=11.6 Hz, H-2), 6.13 (1H, d, J=10.2 Hz, H-14), 6.25 (1H, d, J=11.6 Hz, H-2), 7.40 (3H, m, phenyl), 7.52 (2H, m, phenyl).

ACETYLATION OF **6**.—Ac_2O (0.1 ml) was added to a solution of **6** (1.4 mg) in pyridine (0.2 ml), and the mixture was left at room temperature overnight. The reaction mixture was diluted with EtOAc, washed with H_2O, saturated $CuSO_4$ solution, H_2O, saturated $NaHCO_3$ solution, H_2O, and saturated NaCl solution, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by Si gel cc [hexane-EtOAc (6:1) as an eluent] to give sarcophytol J

diacetate [7] (1.3 mg, colorless oil): $[\alpha]_D$ −114.3° (c=0.13, $CHCl_3$) [lit. (2) $[\alpha]_D$ −233° ($CHCl_3$)].

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Received 15 June 1992