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BIOACTIVE DITERPENOIDS FROM OCTOCORALLIA, 2.[1] DEOXYXENIOLIDE B, A NOVEL ICHTHYOTOXIC DITERPENOID FROM THE SOFT CORAL *XENIA ELONGATA*

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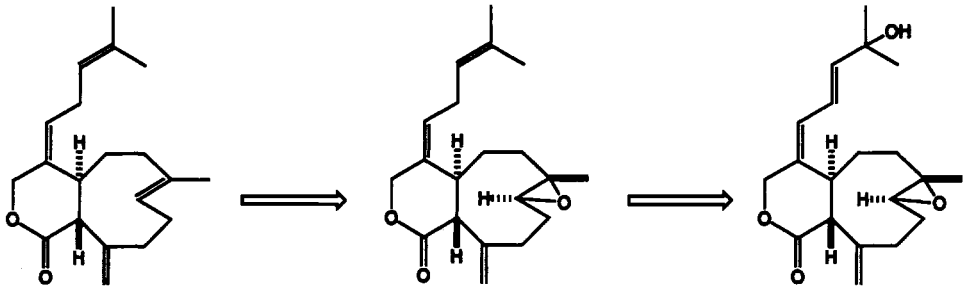
ABSTRACT.—An ichthyotoxic diterpenoid, deoxyxeniolide B [**1**], was isolated from the soft coral *Xenia elongata*. It has also been shown that deoxyxeniolide B [**1**] is gradually oxidized in solution to give the oxidation products **2** and **3**. The structures of these compounds were elucidated on the basis of spectral analysis.

In our continuing search for bioactive marine terpenoids, we have isolated a xenicane-type diterpenoid (2), deoxyxeniolide B [**1**] from the soft coral *Xenia elongata* Dana, 1846 (Octocorallia), collected from the Nichinan Coast in the Miyazaki Prefecture of Japan. This animal is known as "umi-azami" in Japanese. Over 90% of the diterpenoids from *Xenia* spp. belong to the xenicane, caryophyllane (xeniaphyllane), germacrane, and guaiane classes (3), and fully 70% of them are classified as xenicanes, represented by xenicin (2) and xeniolide B (4). In this paper, we report the structure of a new ichthyotoxic xenicane-type diterpenoid, deoxyxeniolide B [**1**]. In addition, we report that **1** is stable in the solid state but is oxidized in solution ($CHCl_3$, Me_2CO, H_2O), to give the oxidation products, oxid-1 [**2**] and oxid-2 [**3**].

The *n*-hexane-soluble part of the MeOH and $CHCl_3$/MeOH extracts obtained from the whole bodies of *Xenia elongata* (3.12 kg) showed ichthyotoxic activity. This *n*-hexane extract (63.74 g) was subjected to Sephadex LH-20 and Si gel cc guided by ichthyotoxicity to give a xenicane-type diterpenoid, deoxyxeniolide B [**1**].

The oxidation products **2** and **3** were obtained from pure **1** in 13% and 6% yields by stirring in $CHCl_3$ solution for two weeks followed by purification with Si gel cc.

A molecular formula of $C_{20}H_{28}O_2$ for **1** was determined from hreims data. The ir spectrum of **1** exhibited absorptions due to an ester functionality (1760 cm^{-1}). The ^1H-, ^{13}C-, and ^1H-^{13}C COSY nmr spectra of **1** suggested the presence of three olefinic methyls (δ_C 17.88, 17.83, 25.63), five methylenes (δ_C 27.27, 27.48, 34.27, 36.66, 39.56), two methines (δ_C



1 **2** **3**

[1]For Part 1, see Miyamoto *et al.* (1).

37.69, 57.08), one oxygen-bearing methylene (δ_C 71.82), one exocyclic methylene (δ_C 116.93, 148.5, in C_6H_6-d_6), three trisubstituted olefins (δ_C 120.09, 125.83, 128.22, 133.21, 134.74, 135.75), and one ester (δ_C 173.55). The eims of **1** showed a molecular ion peak at *m/z* 300 and a fragment peak at *m/z* 231 corresponding to the loss of an isopentenyl group.

Based on the molecular formula and the above-mentioned data, **1** was considered to be a bicyclic diterpenoid with a lactone functionality.

From the COSY nmr spectrum, partial structures **A** and **B** described below could be deduced. Both olefinic methyl signals [δ_H 1.64, 1.72 (each d, J=1.3 Hz)] of the terminal isobutenyl functionality correlated to a methylene signal [δ_H 2.79 (2H, m)] through an olefinic proton [δ_H 5.10 (m)]. This methylene signal also correlated to another olefinic proton [δ_H 5.48 (t, J=7.3 Hz)], which was coupled with one of the oxygen-bearing methylene protons [δ_H 4.81 (br d, J=12.0 Hz)]. The partial structure **A** was thus deduced. Partial structure **B**, which contained a nine-membered ring, was also deduced with the aid of a COSY spectrum. The expected correlations through the nine-membered ring protons [δ_H 2.90 (d, J=7.1 Hz), 2.98 (br d, J=7.1 Hz), 1.6–1.7 (2H, m), 2.18 (2H, dd, J=4.4 and 7.6 Hz), 5.42 (dd, J=3.9 and 11.2 Hz), 2.47 (m), 2.59 (m)], the olefinic methyl protons [δ_H 1.66 (3H, s)], and the exocyclic methylene protons [δ_H 5.03 (each s)] were detected. These partial structures were linked to each other as shown in Figure 1, because HMBC connectivities between the carbonyl at δ_C 173.55 and the methine proton at δ_H 2.90, and the methine carbon at δ_C 37.69 and the olefinic proton at δ_H 5.47, were observed. As a result of these observations, structure **1** was assigned to the new xenicane-type diterpenoid.

The stereostructure of **1** was investigated with the aid of a NOESY nmr spectrum. The *E*-configuration was assigned to the $\Delta^{4(12)}$ double bond based on the observed nOe correlation between H-12 [δ_H 5.47] and H-3_{eq} [δ_H 4.39]. The *E*-configuration of the $\Delta^{7(8)}$ double bond was suggested by the ^{13}C-nmr chemical shift at δ_C 17.88 (C-18 Me) (5). The trans-ring configuration was assigned by the consequent nOe correlations between H-11a [δ_H 4.92] and H-3_{ax} [δ_H 4.81], H-19 [δ_H 4.92], and between H-19 [δ_H 4.92] and Me-18 [δ_H 1.66], and between

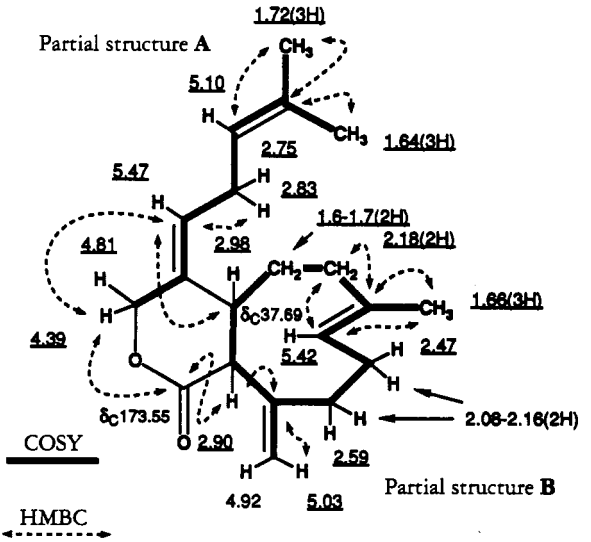


FIGURE 1

H-4a [δ_H 2.98] and H-6 [δ_H 2.18], H-8 [δ_H 5.42], as shown in Figure 2.

The absolute configuration of **1** was investigated by application of the lactone sector rule (6). The ord spectrum of **1** showed a negative Cotton effect with a first extremum at 225 nm ([Φ]-16000). The octant and sector projections predicted the sign of a negative contribution (Figure 3). Thus, the absolute configurations of C-4a and C-11a were *S* and *R*, respectively.

The hrfabms spectrum of oxid-1 [**2**] showed a molecular ion, corresponding to the molecular formula $C_{20}H_{28}O_3$. The ir spectrum of **2** exhibited absorptions due to a δ-lactone (1760 cm^{-1}) functionality. Comparison of the ^{13}C-nmr spectrum of compound **2** with that of **1** indicated signals due to an epoxide moiety at δ_C 59.3 (s) and 64.0 (d), in place of the signals due to the $\Delta^{7(8)}$ olefin of **1**. In the ^1H-nmr spectrum of **2**, the signal due to the epoxymethine proton was observed at δ_H 2.99 as a double doublet (J=3.0 and 11.5 Hz). The *E*-configuration of the C-7(C-8) epoxide was apparent because an nOe correlation between Me-18 [δ_H 1.21 (3H)] and H-8 [δ_H 2.99] was not observed. Accordingly, the structure of **2** was assigned as the 7(8)-*E*-epoxy derivative of deoxyxeniolide B.

The hrfabms spectrum of oxid-2 [**3**] showed a molecular ion, corresponding to the molecular formula $C_{20}H_{27}O_4$, and the ir spectrum indicated the presence of a hydroxyl group. In the uv spectrum of **3**, an absorption due to a conjugated

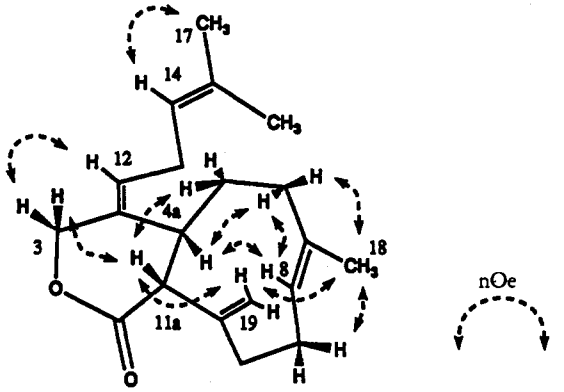


FIGURE 2. NOe correlations of **1**.

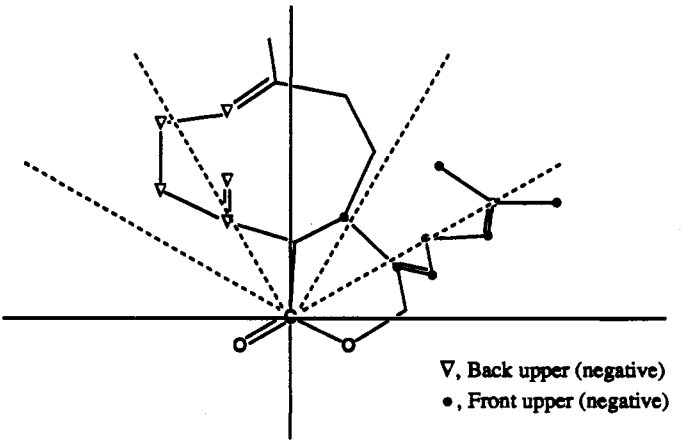


FIGURE 3. Lactone sector projection of **1**.

diene was observed at 242 nm (ε max 23400). The ^{1}H-nmr spectrum of **3** exhibited similar signals for a δ-lactone, an epoxy, and a nine-membered ring, when directly compared with those of **2**. The remaining signals were assignable to conjugated olefinic protons and two tertiary methyls [δ_H 5.93 (d, J=15.5 Hz), 6.27 (dd, J=15.5 and 11.2 Hz), 6.10 (br d, J=11.2 Hz), and 1.36 (6H, s)]. These data suggested the presence of the same side-chain moiety as that of xeniolide B, and indicated the structure of oxid-2 to be **3**. This same compound has also been isolated by Iwagawa *et al.* from the soft coral *Xenia florida* (7).

Deoxyxeniolide B [**1**] showed ichthyotoxicity against the mosquito fish (*Orizias latipes*), and its lethal concentration (LC_{100}) within 1 h was 15 ppm. Its oxidation products [**2** and **3**] were not toxic in this test system.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Details have been reported previously (1). The nmr spectra were measured on a Varian Unity Plus 500 MHz nmr spectrometer. Optical rotations were taken on a Jasco J-720W spectropolarimeter. Keiselgel 60 F_{254} was used for tlc.

ANIMAL MATERIAL.—The soft coral was collected in May 1992, from the rocky coast of Nango-cho, Miyazaki Prefecture, Japan, and identified as *Xenia elongata* Dana, 1846, by Mr. Yukimitsu Imahara. A voucher specimen is deposited in the Wakayama Prefecture Museum of National History (Catalog No. 1994-INV-0007).

EXTRACTION AND ISOLATION.—The soft coral was extracted with MeOH (6 liters). The residue was extracted with $CHCl_3$-MeOH (1:1, 3 liters). These concentrated extracts were combined and partitioned between *n*-hexane and H_2O. The *n*-hexane-soluble fraction (63.74 g) showed ichthyotoxicity against *Oryzias latipes* at 100 ppm within 1 h. This active fraction was chromatographed on a Sephadex LH-20 column with $CHCl_3$-MeOH (1:1) as eluent to yield three fractions: 1 (43.17 g), 2 (11.25 g), and 3 (0.34 g). Part of the ichthyotoxic fraction 2 (1.103 g) was chromatographed on Si gel (BW-300) using a *n*-hexane/EtOAc step gradient. An active fraction (80.9 mg, 30 ppm, 2 h) was chromatographed over Si gel with CH_2Cl_2 to give deoxyxeniolide B [**1**] (18.8 mg) as the active component.

Deoxyxeniolide B [**1**].—Amorphous solid: mp 40–42°; $[\alpha]^{27}D$ −22.4° (c=0.96, $CHCl_3$); ir (CCl_4) ν max 2800–3000 (CH, aliphatic), 1760 (C=O, ester), 1140 cm^{-1}; ^{1}H nmr ($CDCl_3$, 500 MHz) δ 1.6–1.7 (2H, m, H-5), 1.64 (3H, d, J=1.1 Hz, Me-16), 1.66 (3H, s, Me-18), 1.72 (3H, d, J=1.1 Hz, Me-17), 2.08–2.16 (2H, m, H-9, H-10), 2.18 (2H, dd, J=4.4 and 7.6 Hz, H-6), 2.47 (1H, m, H-9), 2.59 (1H, m, H-10), 2.75 (1H, dt, J=7.0 and 15.9 Hz, H-13), 2.83 (1H, dt, J=7.0 and 15.9 Hz, H-13), 2.90 (1H, d, J=7.1 Hz, H-11a), 2.98 (1H, br d, J=7.0 Hz, H-4a), 4.39 (1H, d, J=11.7 Hz, H-3eq), 4.81 (1H, d, J=11.7 Hz, H-3ax), 4.92 (1H, s, H-19), 5.03 (1H, s, H-19), 5.10 (1H, br t, J=7.0 Hz, H-14), 5.42 (1H, dd, J=3.9 and 11.2 Hz, H-8), 5.47 (1H, t, J=7.0 Hz, H-12); ^{13}C nmr ($CDCl_3$, 125 MHz) δ 173.55 (s, C-1), 71.82 (t, C-3), 135.75 (s, C-4), 37.69 (d, C-4a), 36.66 (t, C-5), 39.56 (t, C-6), 134.74 (s, C-7), 125.83 (d, C-8), 27.48 (t, C-9), 34.27 (t, C-10), 148.5 (br s, C-11, in C_6H_6-d_6), 57.08 (d, C-11a), 128.22 (d, C-12), 27.27 (t, C-13), 120.09 (d, C-14), 133.21 (s, C-15), 17.83 (q, C-16), 25.63 (q, C-17), 17.88 (q, C-18), 116.93 (t, C-19); ord (c=0.049, MeOH), $[\Phi]^{27}225$ −16000°; eims (30 eV) m/z 300 $[M]^+$ (21), 231 (18), 135 (100); hreims m/z found $[M]^+$ 300.2085 ($C_{20}H_{28}O_2$ requires 300.2088).

OXIDATION OF **1**.—Deoxyxeniolide B [**1**] (34.1 mg) was dissolved in $CHCl_3$ (3.4 ml), and the solution was stirred for 14 days at room temperature. This solution was evaporated *in vacuo* to yield the oxidative products **2** and **3**, which were treated by chromatography on Si gel (FL-60D, Fuji) using *n*-hexane-EtOAc (3:1→1:1) as eluent to afford unreacted **1** (12.4 mg), as well as oxid-1 [**2**] (4.5 mg) and oxid-2 [**3**] (2.1 mg); R_f values by tlc with *n*-hexane-EtOAc (3:1): **1** (0.68), **2** (0.50), **3** (0.19).

Oxid-1 [**2**].—Amorphous solid: $[\alpha]^{27}D$ +161.4° (c=0.38, $CHCl_3$); ir (CCl_4) ν max 2800–3000 (CH, aliphatic), 1760 (C=O, ester), 1240, 1140 cm^{-1}; ^{1}H nmr ($CDCl_3$, 270 MHz) δ 1.21 (3H, s, Me-18), 1.61 (3H, br s, Me-16), 1.70 (3H, br s, Me-17), 1.40–1.55 (2H, m, H-5), 1.79 (1H, ddd, J=2.6, 5.0, and 13.2 Hz, H-6), 1.89 (1H, m, H-6), 2.17 (2H, m, H-9), 2.22 (1H, m, H-10), 2.70 (2H, m, H-13), 2.81 (1H, m, H-10), 2.99 (1H, dd, J=2.9 and 11.5 Hz, H-8), 2.9–3.0 (1H, H-4a), 3.19 (1H, d, J=9.9 Hz, H-11a), 4.38 (1H, d, J=11.7 Hz, H-3eq), 4.83 (1H, d, J=11.7 Hz, H-3ax), 5.05 (1H, m, H-14), 5.14 (1H, s, H-11), 5.18 (1H, s, H-11), 5.51 (1H, br t, J=7.0 Hz, H-12); ^{13}C nmr ($CDCl_3$, 67.5 MHz) δ 172.7 (s, C-1), 71.6 (t, C-3), 136.3 (s, C-4), 36.0 (d, C-4a), 35.5 (t, C-5), 38.6 (t, C-6), 59.3 (s, C-7), 64.0 (d, C-8), 27.6 (t, C-9), 29.7 (t, C-10), 57.5 (d, C-11a), 129.9 (d, C-12), 27.3 (t, C-13), 120.4 (d, C-14), 134.0 (s, C-15), 18.6 (q, C-16), 25.6 (q, C-17), 18.0 (q, C-18), 119.6 (t, C-19); fabms (positive) m/z 317 $[M+H]^+$ (100), 299 $[M-17]^+$ (36), 147

(79); hrfabms *m/z* found $[M]^+$ 317.2101 ($C_{20}H_{29}O_3$ requires 317.2115).

Oxid-2 [**3**].—Amorphous solid: ir (CCl_4) ν max 3540, 3200–3400 (OH), 2800–3000 (CH, aliphatic), 1740 (C=O, ester) cm^{-1}; uv (EtOH) (ϵ max) 242 (23,400); ^1H nmr ($CDCl_3$, 270 MHz) δ 1.21 (3H, s, Me-18), 1.36 (6H, s, Me-16, Me-17), 3.01 (dd, J=2.8 and 11.3 Hz, H-8), 3.12 (1H, m, H-4a), 3.24 (1H, d, J=10.2 Hz, H-11a), 4.45 (1H, d, J=12.2 Hz, H-3eq), 4.91 (1H, d, J=12.2 Hz, H-3ax), 5.16 (1H, br s, H-19), 5.20 (1H, br s, H-19), 5.93 (1H, d, J=15.5 Hz, H-14), 6.10 (1H, d, J=11.2 Hz, H-12), 6.27 (1H, dd, J=11.2 and 15.5 Hz, H-13); fabms (positive) *m/z* 333 $[M+H]^+$ (12), 332 $[M]^+$ (19), 331 $[M-H]^+$ (32), 315 $[M-17]^+$ (76), 154 (100); hrfabms *m/z* found $[M-H]^+$ 331.1900 ($C_{20}H_{27}O_4$ requires 331.1908).

ICHTHYOTOXICITY.—This assay was carried out as described previously (1).

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