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Three New Xenicane Diterpenoids from Okinawan Soft Coral of the Genus, *Xenia*

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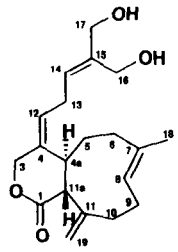
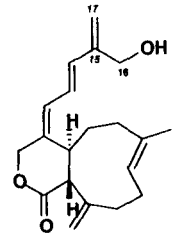
Abstract: Three new xenicane diterpenoids, xeniaol, xeniadiol and xeniatriol, were isolated from the Okinawan soft coral of the genus, *Xenia*. Their structures were elucidated by spectroscopic analysis.

Keywords: marine metabolites; natural products; terpenes and terpenoids; circular dichroism

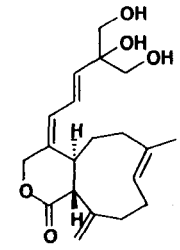
Following the isolation of xenicin[2] from soft coral *Xenia elongata*, various xenicane diterpenoids have been isolated from marine alga and soft coral.[3] Many of these are considerable interest from their unique structural features and biological activity. [3] During studies on the chemical constituents of Okinawan marine invertebrates,[4] three new xenicane diterpenoids xeniadiol (**1**), xeniaol (**2**) and xeniatriol (**3**), were isolated from the soft coral of the genus, *Xenia* and structural determinations were made based on spectroscopic analytical data.



xeniadiol (1)



xeniaol (2)



xeniatriol (3)

Specimens of soft coral (wet wt 1.2 kg), from the coral reef of Ishigaki Island, Okinawa, Japan, in May 1992, were immersed in MeOH and then EtOAc. The MeOH and EtOAc extracts were combined and partitioned between EtOAc and H_2O. The EtOAc-soluble portion (13.7 g) was repeatedly chromatographed on silica gel column to give xeniadiol (**1**)(0.12 % yield based on the EtOAc-soluble portion), xeniaol (**2**)(0.044% yield) and xeniatriol (**3**)(0.058% yield).

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Table 1 NMR data for xeniadiol (**1**), xeniaol (**2**) and xeniatriol (**3**)

	1		2		3	
No.	^{13}C NMR[a]	^{1}H NMR[b]	^{13}C NMR[a]	^{1}H NMR[b]	^{13}C NMR[a]	^{1}H NMR[b]
1	173.6 (C)	-	173.2 (C)	-	173.3 (C)	-
3	71.6 (CH_2)	4.39 (1H, d, 11.8)	71.3 (CH_2)	4.46 (1H, d, 12.0)	71.5 (CH_2)	4.43 (1H, d, 12.0)
		4.81 (1H, d, 11.8)		4.89 (1H, d, 12.0)		4.87 (1H, d, 12.0)
4	136.8 (C)	-	138.5 (C)	-	138.4 (C)	-
4a	37.4 (CH)	2.95 (1H, m)	37.1 (CH)	3.12 (1H, m)	37.5 (CH)	3.12 (1H, m)
5	36.5 (CH_2)	1.69 (2H, m)	37.3 (CH_2)	1.73 (2H, m)	37.2 (CH_2)	1.71 (2H, m)
6	39.5 (CH_2)	2.18 (2H, m)	39.6 (CH_2)	2.20 (2H, m)	39.6 (CH_2)	2.19 (2H, m)
7	134.6 (C)	-	134.5 (C)	-	134.7 (C)	-
8	125.9 (CH)	5.41 (1H, dd, 11.1, 4.2)	126.1 (CH)	5.45 (1H, dd, 11.0, 4.1)	126.2 (CH)	5.46 (1H, dd, 11.0, 4.2)
9	27.4 (CH_2)	2.14 (1H, m)	28.0 (CH_2)	2.14 (1H, m)	27.9 (CH_2)	2.10 (1H, m)
		2.46 (1H, m)		2.46 (1H, m)		2.46 (1H, m)
10	34.1 (CH_2)	2.11 (1H, m)	33.8 (CH_2)	2.05 (1H, m)	34.0 (CH_2)	2.10 (1H, m)
		2.56 (1H, m)		2.62 (1H, m)		2.60 (1H, m)
11	147.8 (C)	-	144.7 (C)	-	146.9 (C)	-
11a	56.9 (CH)	2.90 (1H, br d, 7.1)	57.4 (CH)	2.98 (1H, d, 8.2)	57.3 (CH)	2.96 (1H, br d, 7.8)
12	126.5 (CH)	5.47 (1H, t, 7.4)	127.6 (CH)	6.08 (1H, d, 11.0)	126.6 (CH)	6.06 (1H, d, 11.2)
13	26.5 (CH_2)	2.93 (2H, m)	123.7 (CH)	6.51 (1H, dd, 15.6, 11.0)	126.2 (CH)	6.64 (1H, dd, 15.3, 11.2)
14	126.8 (CH)	5.54 (1H, t, 7.4)	134.8 (CH)	6.36 (1H, d, 15.6)	136.0 (CH)	5.78 (1H, d, 15.3)
15	138.7 (C)	-	144.7 (C)	-	75.3 (C)	-
16	59.4 (CH_2)	4.34 (2H, br s)	62.8 (CH_2)	4.39 (2H, br s)	67.1 (CH_2)	3.62 (1H, d, 11.2)
						3.75 (1H, d, 11.2)
17	66.6 (CH_2)	4.23 (2H, br s)	117.2 (CH_2)	5.24 (1H, s)	67.1 (CH_2)	3.60 (1H, d, 11.2)
				5.36 (1H, s)		3.74 (1H, d, 11.2)
18	17.8 (CH_3)	1.64 (3H, s)	18.1 (CH_3)	1.63 (3H, s)	18.0 (CH_3)	1.63 (3H, s)
19	117.0 (CH_2)	4.90 (1H, s)	118.0 (CH_2)	4.94 (1H, s)	117.8 (CH_2)	4.92 (1H, s)
		5.03 (1H, s)		5.06 (1H, s)		5.05 (1H, s)

[a] 125MHz, $CDCl_3$. [b] 500MHz, $CDCl_3$.

The molecular formula, $C_{20}H_{28}O_4$, of xeniadiol (**1**) was established by high resolution FABMS. The IR spectrum of **1** showed absorptions due to a hydroxy group (3412 cm^{-1}) and ester functionality (1724 cm^{-1}). All twenty carbons appeared in the ^{13}C NMR spectrum and the DEPT spectrum indicated the presence of one methyl, eight sp^3 methylenes, two sp^3 methines, one sp^2 methylene, three sp^2 methines and five sp^2 quaternary carbons. ^{1}H and ^{13}C NMR correlations were demonstrated by the HMQC spectrum. ^{1}H and ^{13}C NMR spectra showed the presence of three olefinic methines [δ_H 5.41 (1H, dd, J = 4.2, 11.1 Hz), δ_C 125.9 (CH); δ_H 5.47 (1H, t, J = 7.6 Hz), δ_C 126.5 (CH); δ_H 5.54 (1H, t, J = 7.4 Hz), δ_C 126.8 (CH)], one exo methylene [δ_H 4.90 (s, 1H), 5.03 (s, 1H), δ_C 117.0 (CH_2)], one olefinic methyl [δ_H 1.64 (3H, br s), δ_C 17.8 (CH_3)], one lactone carbonyl [δ_C 173.6 (C)] and oxygenated three methylenes [δ_H 4.23 (2H, br s), δ_C 66.6 (CH_2); δ_H 4.34 (2H, br s), δ_C 59.4 (CH_2); δ_H 4.39 (1H, d, J = 11.8 Hz), 4.81 (1H, d, J = 11.8 Hz), δ_C 71.6 (CH_2)]. The presence of two primary hydroxy groups was confirmed by acetylation. Acetylation of **1** with acetic

anhydride in pyridine gave the diacetate [δ_H 2.07 (3H, s), 2.08 (3H,s), 4.59 (2H, br s), 4.69 (2H, br s)]. Partial structure **A** was demonstrated by the following ^{1}H-decoupling experiments and ^{1}H-^{1}H COSY spectrum (Figure 1). The two oxygenated methylene proton signals, [δ_H 4.23 (2H, br s), 4.34 (2H, br s)], arising from primary hydroxy groups, were correlated to the olefin proton, [δ_H 5.54 (1H, t, J = 7.4 Hz)], which itself was correlated to another olefin proton, [δ_H 5.47 (1H, t, J = 7.4 Hz)], through methylene proton [δ_H 2.93 (2H, m)]. This olefin proton was correlated to an oxygenated methylene, [δ_H 4.39 (1H, d, J = 11.8 Hz), 4.81 (1H, d, J = 11.8 Hz)]. Partial structure **B**, present in a nine-membered ring, was correlatied to the nine-membered protons [δ_H 5.41 (1H, d, J = 4.2, 11.1 Hz), 2.14 (1H, m), 2.46 (1H, m), 2.11 (1H, m), 2.56 (1H, m), 2.90 (1H, br d, J = 7.1 Hz), 2.95 (1H, m), 1.69 (2H, m), 2.18 (2H, m)], olefinic methyl protons [δ_H 1.64 (3H, br s)] and exo methylene [δ_H 4.90 (1H, br s), 5.03 (1H, br s)] (Figure 2). The *E* configuration of the carbon - carbon double bond (C-7) was indicated by the ^{13}C chemical shift (δ_C 17.8, CH_3)[5] of the olefinic methyl group (C-18).

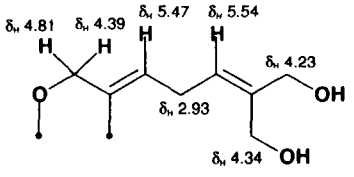


Figure 1. Partial Structure A

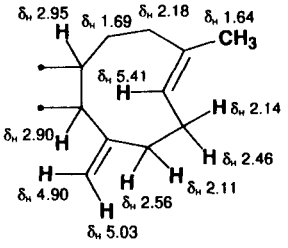


Figure 2. Partial Structure B

HMBC of **1** indicated the two partial structures **A** and **B** to be coupled to produce a gross structure (Figure 3). The stereostructure of **1** was determined by the NOESY spectrum (Figure 4). The *E* configuration of the carbon – carbon double bond (C-4, 12) was determined by NOESY correlation between

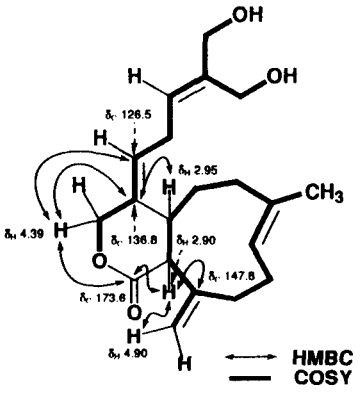


Figure 3. Planar Structure of **1**

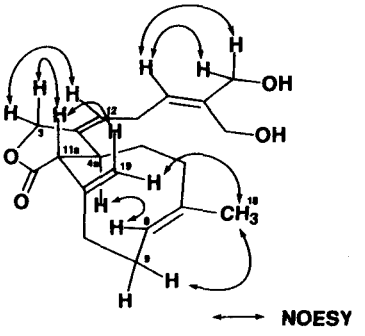


Figure 4. NOESY Correlations of **1**

H-3_{eq} (δ_H 4.39) and H-12 (δ_H 5.47). The *trans*-ring junction was assigned according to NOESY correlations between H-4a (δ_H 2.95) and H-8 (δ_H 5.41) and between H-18 (δ_H 1.64) and H-19 (δ_H 5.03) and between H-11a (δ_H 2.90) and H-19 (δ_H 4.90) and H-11a (δ_H 2.90) and H-3_{ax} (δ_H 4.81).

The absolute configuration of xeniodiol (**1**) was determined by application of the lactone sector rule.[6] The lactone sector projection of **1** predicted the negative Cotton effect (Figure 5). The CD spectrum of **1** showed negative Cotton effect at 214 nm ($\Delta\varepsilon$ -7.3).[7] Thus, the absolute configuration of **1** was determined to be 4a*S* and 11a*R*.

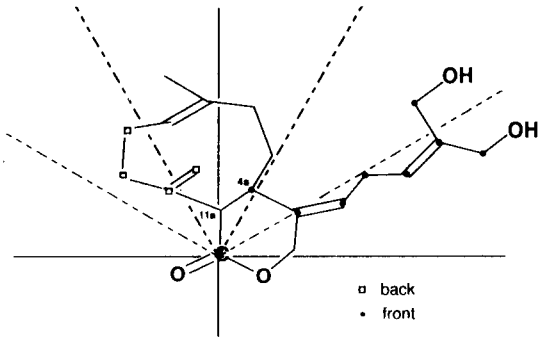


Figure 5. Lactone Sector Projection of **1**

The molecular formula, $C_{20}H_{26}O_3$, of xeniaol (**2**) was found based on the high resolution EIMS spectrum. The IR spectrum of **2** showed absorptions due to a hydroxy group (3390 cm^{-1}) and ester functionality (1739 cm^{-1}). The conjugated triene group (CH_2=C-CH=CH-CH=C-) was shown present by the UV spectrum [λ_{max} 275 nm (ε 15700)] and ^{1}H NMR [δ_H 5.24 (1H, s), 5.36 (1H, s), 6.36 (1H, d, *J*=15.6 Hz), 6.51 (1H, dd, *J* = 15.6, 11.0 Hz), 6.08 (1H, d, *J* = 11.0 Hz)]. ^{1}H and ^{13}C NMR spectra of **2** were closely related to those of xeniadiol (**1**) except for signals of the CH_2=C-CH=CH-CH=C- group instead of the HO-CH_2-C=CH-CH_2-CH=C- group in **1** (Figure 6), suggesting xeniaol to possibly have the structure of **2**. Structural confirmation was made based on ^{13}C-^{1}H COSY, COLOC and NOESY spectra.

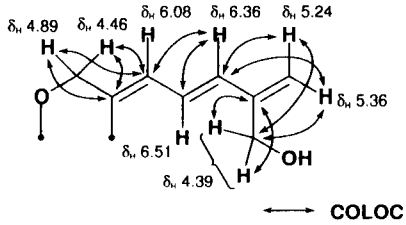


Figure 6. Partial Structure of **2**

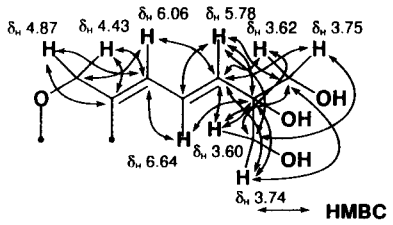


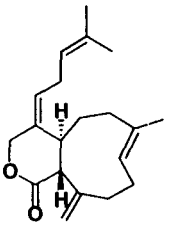
Figure 7. Partial Structure of **3**

The molecular formula, $C_{20}H_{28}O_5$, of xeniatriol (**3**) was indicated by the high resolution FABMS spectrum. The IR spectrum of **3** showed absorptions due to a hydroxy group (3452 cm^{-1}) and ester functionality (1725 cm^{-1}). The conjugated diene group (-CH=CH-CH=C-) was shown by the UV spectrum

[λ_{max} 244 nm (ε 9600)] and ^{1}H NMR [δ_H 5.78 (1H, d, J = 15.3 Hz), 6.64 (1H, dd, J = 15.3, 11.2 Hz), 6.06 (1H, d, J = 11.2 Hz)]. ^{1}H and ^{13}C NMR spectra of **3** were closely related to those of xeniadiol (**1**) except for signals of the HO-C-CH=CH- group instead of the -C=CH-CH_2- group in **1** (Figure 7). The structure of xeniatriol thus appeared to be **3**, as was confirmed by the HMQC, HMBC, and NOESY spectral data.

The absolute configurations of xeniaol (**2**) and xeniatriol (**3**) were apparently 4a*S* and 11a*R*, as also for **1**, in that **2** and **3** were present together with **1** in the same soft coral.

Xeniatriol (**3**) is recognized as the xenicane diterpenoid with the most oxidized side chain. These compounds are of interest for their biogenetic pathways. Xeniadiol (**1**) appeared to be derived from deoxyxeniolide B,[8] which was isolated from the soft coral *Xenia elongata*, by the oxidation of the side chain. Xeniaol (**2**) is derived from **1** by dehydration and xeniatriol (**3**), by epoxidation and cleavage of the epoxide.



deoxyxeniolide B

Experimental

General Experimental Procedures. Optical rotations were measured with a JASCO DIP-360 automatic polarimeter. IR spectra were recorded with a Perkin-Elmer FT-IR 1710 spectrometer or JASCO A-302 spectrometer, UV spectra with a Hitachi 124 spectrophotometer, circular dichroism (CD) spectra with a JASCO J-720 spectropolarimeter and ^{1}H and ^{13}C NMR spectra with a Varian Gemini-300, a Bruker AM-400, or a Bruker AM-500. Chemical shifts are given on a δ (ppm) scale with tetramethylsilane (TMS) as the internal standard (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad). EIMS and FABMS spectra and high resolution EIMS (HREIMS) and FABMS (HRFABMS) spectra were obtained with a VG Auto Spec spectrometer. Column chromatography and flash column chromatography were carried out on Merck silica gel 60 (70-230 mesh) and Merck silica gel 60 (230-400 mesh), respectively. Preparative TLC was conducted on a Merck silica gel 60 F_{254} plate.

Animal Material, Extraction and Isolation. The soft coral *Xenia* sp. was collected from the coral reef of Ishigaki Island (Okinawa, Japan) in May 1992 at a depth of 1-3 m. A voucher specimen (SC-II-5) is deposited at this laboratory, School of Pharmacy, Tokyo University of Pharmacy and Life Science (Tokyo, Japan). Wet specimens (1.2 kg) were immersed in MeOH (1.5 L x 2) and then EtOAc (1.5 L x 2) and the extracts were combined and partitioned between AcOEt (1.0 L x 3) and H_2O (1.0 L). The EtOAc-soluble portion (13.7 g) was chromatographed on a silica gel column to give four fractions; fraction 1 (5.0 g) eluted with hexane-EtOAc = 10 : 1 (1 L), fraction 2 (4.1 g) eluted with hexane-EtOAc = 1 : 1 (1 L), fraction 3 (1.6 g) eluted with EtOAc (1 L) and fraction 4 (2.8 g) eluted with MeOH (1 L). A partition (2.1 g) of fraction 2 was subjected to repeated flash silica gel column chromatography (eluted with hexane-EtOAc = 3 : 1 for the first chromatography, hexane-acetone = 7 : 1 for the second chromatography, and chloroform-EtOAc = 100 : 1 for the third chromatography) to give xeniaol (**2**) (6.0 mg). Fraction 4 was subjected to repeated flash silica gel column chromatography (eluted with hexane-EtOAc = 1 : 9 for the first chromatography and hexane-EtOAc = 1 : 3 for the second chromatography) to give fraction 4-1 (262 mg), fraction 4-2 (106 mg), fraction 4-3 (75 mg) and fraction 4-4 (69 mg). Fraction 4-2 was subjected to flash silica gel column chromatography (eluted with hexane-acetone = 3 : 2) to give xeniadiol (**1**) (17 mg). Fraction 4-3 was subjected to flash silica gel column chromatography (eluted with hexane-acetone = 6 : 5) to give xeniatriol (**3**) (8.0 mg).

Xeniadiol (1): Colorless oil; $[\alpha]_D^{27}$ -5.0° (*c* 0.6, $CHCl_3$); FABMS *m/z*: $[M+H]^+$ 333; HRFABMS calcd for $C_{20}H_{29}O_4$ $[M+H]^+$ 333.2066: found 333.2049; IR (film) ν_{max} 3391, 1724, 1640 cm^{-1}; CD (MeOH) λ_{ext} ($\Delta\varepsilon$) 214 nm (-7.3); ^1H and ^{13}C NMR see Table 1; HMBC correlation (H/C) 3/1, 3/4, 3/4a, 3/12, 4a/3, 4a/4, 4a/5, 4a/6, 4a/11a, 4a/12, 6/4a, 6/5, 6/7, 6/8, 6/18, 8/6, 8/9, 8/18, 9/7, 9/8, 9/10, 10/8, 10/9, 10/11, 10/11a, 10/19, 11a/1, 11a/4, 11a/4a, 11a/10, 11a/11, 11a/19, 12/3, 12/4a, 12/13, 12/14, 13/4, 13/12, 13/14, 13/15, 14/13, 14/15, 14/15, 14/16, 16/14, 16/15, 16/17, 17/14, 17/15, 17/16, 18/6, 18/8, 19/10, 18/11a; NOESY correlation (H/H) 3/11a, 3/12, 4a/8, 9/18, 11a/19, 14/17, 18/19,.

Xeniaol (2): Colorless oil; $[\alpha]_D^{26}$ -3.9° (*c* 3.9, $CHCl_3$); EIMS *m/z*: $[M]^+$ 314; HREIMS calcd for $C_{20}H_{26}O_3$ $[M]^+$ 314.1882: found 314.1887; IR (film) ν_{max} 3390, 1739, 1640 cm^{-1}; UV (EtOH) λ_{max} 275 nm (ε 15700); ^1H and ^{13}C NMR see Table 1; COLOC correlation (C/H) 1/3, 1/11a, 4/3, 7/5, 7/6, 7/18, 8/9, 8/18, 12/3, 12/14, 13/14, 14/17, 15/16, 16/17; NOESY correlation (H/H) 3/11a, 4a/8, 4a/13, 9/18, 11a/18, 11a/19, 13/16, 18/19.

Xeniatriol (3): Colorless oil; $[\alpha]_D^{28}$ -14.0° (*c* 0.8, $CHCl_3$); FABMS *m/z*: $[M+H]^+$ 349; HRFABMS calcd for $C_{20}H_{29}O_5$ $[M+H]^+$ 349.2015: found 349.2000; IR (film) ν_{max} 3452, 1725, 1640 cm^{-1}; UV (EtOH) λ_{max} 244 nm (ε 9600); ^1H and ^{13}C NMR see Table 1; HMBC correlation (H/C) 3/1, 3/4, 3/12, 4a/11a, 5/6, 6/5, 6/7, 6/8, 8/6, 8/18, 9/10, 10/9, 11a/1, 11a/5, 11a/10, 11a/11, 11a/19, 12/3, 12/4a, 12/14, 13/12, 13/15, 14/13, 14/15, 14/16, 14/17, 16/14, 16/17, 17/14, 17/15, 17/16, 18/7, 18/8, 19/10, 19/11a; NOESY correlation (H/H) 3/11a, 3/12, 4a/8, 4a/12, 5/11a, 11a/18, 11a/19.

Acetylation of xeniadiol (1). Pyridine (0.1 mL) and Ac_2O (0.1 mL) were added to a solution of **1** (5.2 mg) in $CHCl_3$ (0.1 mL) and the mixture was left at room temperature for 16 h. The reaction mixture was concentrated under reduced pressure. The residue was purified by preparative TLC [hexane-acetone (2:1)] to give 16,17-*O*-diacetylxeniadiol (3.2 mg).

16,17-*O*-Diacetylxeniadiol. Colorless oil; $[\alpha]_D^{28}$ -8.1° (*c* 0.32, $CHCl_3$); EIMS *m/z*: $[M-Ac]^+$ 373; IR (film) ν_{max} 1739, 1642 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.65 (3H, s), 1.67 (2H, m), 2.05 - 2.2 (4H, m), 2.07 (3H, s), 2.08 (3H, s), 2.47 (1H, m), 2.60 (1H, m), 2.9 - 3.1 (4H, m), 4.40 (1H, d, J = 11.9 Hz), 4.59 (2H, br s), 4.68 (1H, d, J = 12.4 Hz), 4.69 (1H, d, J = 12.4 Hz), 4.82 (1H, d, J = 11.9 Hz), 4.91 (1H, s), 5.03 (1H, s), 5.46 (2H, m), 5.74 (1H, t, J = 7.3 Hz).

References and Notes

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