New Xenia Diterpenoids from the Indonesian Soft Coral *Xenia* sp.

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Two new xeniolides, xeniolide-F (**1**) and 9-hydroxyxeniolide-F (**2**), along with isoxeniolide-A (**3**) and 7,8-oxido-isoxeniolide-A (**4**), have been isolated from *Xenia* sp. and their structures established on the basis of extensive NMR and MS studies.

Octocorals (phylum Cnidaria) have been widely studied, as they are responsible for the production of a huge array of skeletal classes of terpenes with unique substitution patterns and functionalities.[1] More specifically, specimens belonging to the *Xenia* genus (order Alcyonacea, family Xeniidae) have proved to be a rich source of a class of diterpenes called xenia diterpenoids or xenicanes.[2] All of these compounds contain a cyclononane skeleton, and they have been structurally divided into five types: xenicins (posessing a 2-oxabicyclo[7.4.0]tridecane ring system),[3] xeniolides (the lactone derivatives of the xenicins),[4] xenia-phyllanes (having a bicyclo[7.2.0]undecane ring system),[5] xeniaethers (bearing a oxabicyclo[7.3.0]undecane ring system),[6] and, more recently, azamilides (with an opened A-ring and the nine-membered monocarbocyclic skeleton acylated with a series of C16–C20 saturated fatty acids).[7] Although biosynthetic studies have not been performed on xenicanes, it is generally believed that they derive from geranylgeraniol diphosphate through a transannular carbocation cyclization.[3] A 1,3-diol of azamilides was proposed as a common precursor for xenicins, xeniolides, and xenia-ethers.[8]

During the course of our search for novel and bioactive marine natural products from Indonesian octocorals,[9] we isolated and identified from *Xenia* sp. two new compounds: xeniolide-F (**1**) and 9-hydroxyxeniolide-F (**2**), along with isoxeniolide-A (**3**) and 7,8-oxido-isoxeniolide-A (**4**). The isolation and structural determination of these compounds are described below.

Specimens of soft corals were collected from the Togian Islands near Sulawesi Island (Indonesia) and immersed in MeOH. The methanol extracts were partitioned between CH_2Cl_2/H_2O to yield a brown organic residue, which was subsequently partitioned between $MeOH/H_2O$ and solvents of increasing polarity. The CH_2Cl_2 extracts were submitted to repeated silica gel flash column chromatography (CH_2Cl_2 polarized with MeOH) and finally purified by reversed-phase HPLC to give compounds **1**–**4**.

Compound **1** was isolated as a colorless solid. The IR spectrum of **1** shows absorptions due to hydroxyl (3419 cm^{-1}) and conjugated diene (1640 cm^{-1}) groups. The presence of the conjugated diene group was also confirmed by the UV spectrum [λ_{max} 267 nm]. The ESMS (positive ion) displays the pseudomolecular ions at m/z 333 ([M + H]$^+$), 355 ([M + Na]$^+$), and 371 ([M + K]$^+$), which along with the fragments at m/z 315 ([M − H_2O + H]$^+$), 297 ([M − 2H_2O + H]$^+$), and 293 ([M − H_2O − CO_2 + Na]$^+$) suggests a molecular formula of $C_{20}H_{28}O_4$. This molecular

R = H, Xeniolide-F , (**1**)
R = OH, 9-Hydroxyxeniolide-F , (**2**)

3

4
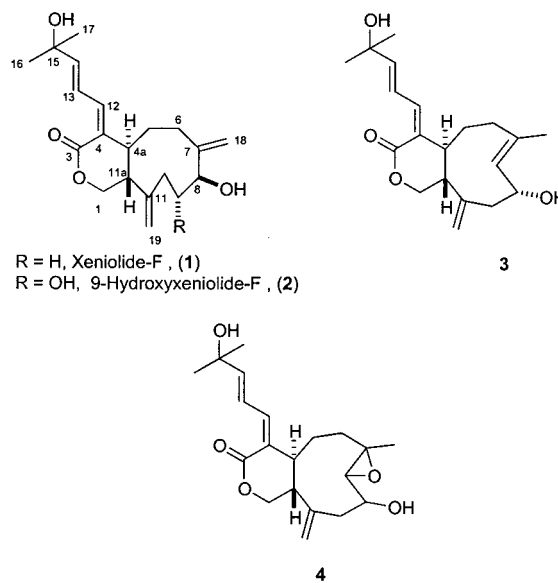
formula was corroborated by HRFABMS, which shows the [M + Na]$^+$ peak at m/z 355.1883.

The structure of **1** was completely solved by a combination of 1D and 2D NMR methods. The carbon resonance at δ_C 168.9 (s), along with those at δ_C 131.2 (s), 138.6 (d), 122.7 (d), and 148.0 (d), in the ^{13}C NMR and DEPT spectra showed the presence of an α,β,γ-unsaturated ester, while the quaternary carbon signals at δ_C 149.5 (s) and 149.1 (s) along with the methylene olefinic carbon signals at δ_C 113.5 (t) and 115.5 (t) indicate the presence of two disubstituted *exo* methylene double bonds. Furthermore, the presence of three oxygenated carbons was inferred from the carbon signals at δ_C 76.6 (d), 70.9 (s), and 70.4 (t). Four methylene groups (two allylic) were deduced from four triplet signals at δ_C 25.4–35.7 and, finally, two methyl groups from two quartet signals at δ_C 29.4 (q) and 29.3 (q).

The ^1H NMR spectrum confirmed the presence of two *exo* methylene double bonds by the fact that four singlet signals were observed at δ_H 5.23, 5.05, 4.95, and 4.73. In addition, the spectrum contained resonances for one oxygenated methine at δ_H 4.19 and one oxygenated methylene at δ_H 4.05 and 3.80. Two intense singlet signals are also observed at δ_H 1.364 and 1.359 (s, 3H each), and these correspond to two methyl groups. In this manner the seven degrees of unsaturation present in **1** were established.
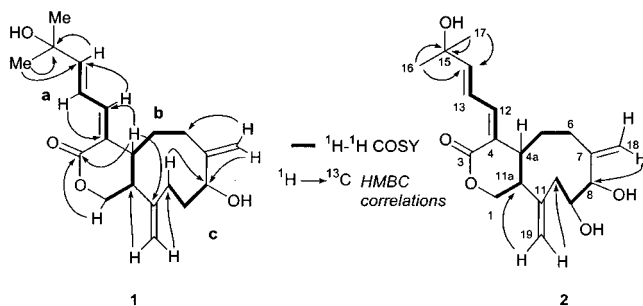
The combined use of ^1H–^1H COSY and gHMQC on **1** allowed us to distinguish three spin systems (see **a**–**c** in Figure 1) and two methyl groups linked to an oxygenated quaternary carbon. A gHMBC experiment was used to assemble the skeletal fragments through quaternary car-

Table 1. ^1H and ^{13}C NMR (500 and 125 MHz) Chemical Shifts (ppm) and HMBC and NOESY Correlations for Xeniolide-F (**1**) and 9-Hydroxyxeniolide-F (**2**) in CDCl$_3$

		1					**2**		
C	δ_C mult	δ_H mult (J in Hz)	HMBC	NOESY	δ_C mult	δ_H mult (J in Hz)	HMBC	NOESY	
1	70.4 t	α: 3.80 t (11.2)	C: 4a, 11a	H-4a, H-19α	70.2 t	α: 3.83 t (11.2)		H-19α	
		β: 4.05 dd (11.2, 4.7)	C: 3, 4a, 11a	H-11a		β: 4.11 dd (11.2, 5.1)			
3	168.9 s				169.0 s				
4	131.2 s				131.1 s				
4a	46.4 d	2.83 m	C: 3, 4, 5, 11, 11a, 12	H-1α, H-8, H-5α H-19α, H-12	45.6 d	2.84 m		H-10α, H-12, H-19α	
5	35.7 t	α: 1.94 m		H-4a, H-8	36.6 t	α: 1.98 m			
		β: 1.76 m	C: 4a, 7			β: 1.74 m			
6	25.4 t	α: 2.10 m	C: 4a, 8		27.8 t	α: 2.26 m			
		β: 2.46 m	C: 4a, 5, 7			β: 2.46 m			
7	149.1 s				145.5 s				
8	76.6 d	4.19 brd (7.6)		H-4a, H-5α, H-18α	81.1 d	3.96 d (8.6)		H-18α	
9	31.9 t	α: 2.12 m			71.0 d	4.05 brdt (8.6, 8.6, 4.0)		H-11a	
		β: 1.94 m							
10	32.9 t	α: 2.15 m	C: 8, 11		42.2 t	α: 2.48 m		H-4a	
		β: 2.29 m	C: 8, 11, 11a, 19			β: 2.60 dd (17.6, 4.0)			
11	149.5 s				147.0 s				
11a	40.9 d	2.59 m	C: 1, 4a, 5, 11, 19	H-1β	42.5 d	2.48 m		H-5β, H-9, H-19β	
12	138.6 d	6.38 d (11.0)	C: 4a, 14	H-4a, H-14	139.1 d	6.40 d (11.1)		H-4a, H-14	
13	122.7 d	6.99 dd (15.4, 11.0)	C: 4, 12, 15	H-16, H-17	122.8 d	6.99 dd (15.4, 11.1)		H-16, H-17	
14	148.0 d	6.08 d (15.4)	C: 12, 15, 16, 17	H-12, H-16, H-17	148.7 d	6.09 d (15.4)		H-16, H-17	
15	70.9 s				70.9 s				
16	29.3 q	1.36 s	C: 14, 15, 17	H-13, H-14	29.4 q	1.37 s	C: 14, 15	H-13, H-14	
17	29.4 q	1.36 s	C: 14, 15, 16	H-13, H-14	29.3 q	1.37 s	C: 14, 15	H-13, H-14	
18	115.5 t	α: 5.23 s	C: 6, 7, 8	H-8	118.8 t	α: 5.29 s	C: 8	H-8	
		β: 5.05 s	C: 6			β: 5.11 s			
19	113.5 t	α: 4.73 s	C: 10, 11, 11a	H-1α, H-4a	115.4 t	α: 4.81 s	C: 11a	H-1α, H-4a	
		β: 4.95 s	C: 10, 11a			β: 5.06 s	C: 10	H-11a	

Figure 1. Selected COSY and HMBC correlations observed for **1** and **2**.

bons and heteroatoms. Thus, these substructures were connected through ^{13}C–^1H long-range coupling constant correlations between the protons H-1β (δ_H 4.05) and H-4a (δ_H 2.83) and the carbonyl lactone C-3 (δ_C 168.9), between the *exo* methylene protons H-18 (δ_H 5.23 and 5.06) and carbons C-6 (δ_C 25.4) and C-8 (δ_C 76.6), and between the *exo* methylene protons H-19 (δ_H 4.95 and 4.73) and carbons C-11a (δ_C 40.9) and C-10 (δ_C 32.9). These relationships are represented in Figure 1. All these data allowed us to identify compound **1** as a new xeniolide diterpenoid.

With the gross structure of **1** in hand, the relative stereochemistry of compound **1** was deduced from the magnitude of $^3J_{H,H}$ coupling constants obtained from the ^1H NMR spectrum, by information from NOESY correlations, and by comparison of its spectroscopic data to those of xeniolide analogues. The Z geometry was assigned to the $\Delta^{4,12}$ double bond on the basis of the observation of a NOESY correlation between H-4a and H-12 and on the chemical shift of H-13 at δ_H 6.99, which is downfield in relation to the E isomer ($\delta_H \sim$6.5)[10] due to its proximity to the carbonyl group. The E geometry of the Δ^{13} double bond was established by the large coupling constant observed between H-13 and H-14 ($J = 15.4$ Hz). An NOE correlation from H-4a to axial H-1α at δ_H 3.80 (t, $J = 11.2$ Hz) and an NOE correlation from H-11a to equatorial H-1β at δ_H 4.05

(dd, $J = 11.2$ and 4.7 Hz) were observed. This suggests that H-4a and H-1α are on the same face of the ring—assigned arbitrarily as the α face—and that H-11a and H-1β are on the opposite face, thus indicating a *trans*-juncture of the two rings. Finally, the β orientation of OH at C-8 in **1** was easily deduced from the NOESY correlation between H-4a at δ_H 2.83 and the oxymethine proton H-8 at δ_H 4.19 (brd, $J = 7.6$ Hz), which is in turn correlated by NOESY with H-18α at δ_H 5.23. Therefore, we have named compound **1** as xeniolide-F.

Compound **2** was also isolated as a colorless solid, and its LRFABMS shows the pseudomolecular ions at m/z 349 ([M + H]$^+$) and m/z 371 ([M + Na]$^+$), indicating a molecular formula of C$_{20}$H$_{28}$O$_5$. The formula of **2** differs from that of compound **1** by a single oxygen atom, suggesting the presence of an additional hydroxyl group.

The absence of a signal for one of the methylene carbons in the range δ_C 42.2–27.8 and the appearance of a second oxymethine carbon resonance in the ^{13}C NMR and DEPT-135 spectra of **2**, in relation to those of **1**, confirmed the existence of an additional secondary hydroxyl group in **2**. The location of this extra OH group at C-9 in **2** was deduced by 2D NMR experiments. The ^1H–^1H COSY experiment showed correlations between two oxymethine protons at δ_H 3.96 (H-8) and at δ_H 4.05 (H-9), and this in turn was correlated to the methylene protons δ_H 2.60 and 2.48 (H-10). Further evidence for this was found when an HMBC correlation was observed between the *exo* methylene proton H-18α at δ_H 5.29 (s) and the oxymethine carbon at δ_C 81.1. The remaining spectral data of **2** are very similar to those of compound **1**.

The relative stereochemistry of compound **2** was determined next. The Z and E geometries for the $\Delta^{4,12}$ and Δ^{13} double bonds, respectively, and the *trans*-juncture of two rings in **2** were deduced by direct comparison of the spectroscopic data of **2** with those of **1** as well as the similar NOESY correlations found in both compounds. The α disposition of the OH group at C-9 and the β disposition of
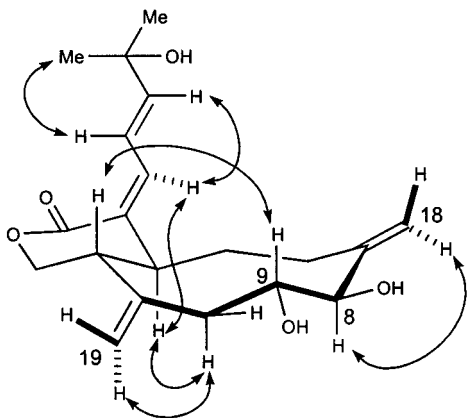
Figure 2. Significant NOE correlations observed in **2**.

the OH group at C-8 were inferred on the basis of the proton–proton coupling constants and by NOESY correlations. Thus, the signal for H-9 is observed at δ_H 4.05 as a broadened doublet of triplets having two coupling constants of nearly 8.6 Hz with H-8 at δ_H 3.96 (d, $J = 8.6$ Hz) and one of the H-10 protons at δ_H 2.48. In addition, there is also a small coupling of 4.0 Hz with the other H-10 proton at δ_H 2.60 (dd, $J = 17.6, 4.0$ Hz). These data suggest that H-9 must have a pseudoaxial orientation on the β face of the molecule. This was confirmed by NOESY correlations between H-11a at δ_H 2.48 in the β face of the molecule and H-9 and also between H-8 at 3.96 and the *exo* methylene proton H-18α at δ_H 5.29 (s) (this correlation is also present in the NOESY of **1**). On the basis of these data, compound **2** was named as 9-hydroxyxeniolide-F. The spectral data of compounds **3** and **4** matched those reported by Braekman et al. for isoxeniolide-A and 7,8-oxido-isoxeniolide-A, respectively, previously isolated from *Xenia novae-britanniae*.[11]

Compounds **1**–**4** showed an IC$_{50}$ >1 μg/mL against mouse (P-388) and human (A-549, HT-29, MEL-28) tumor cell lines.

Experimental Section

General Experimental Procedures. Optical rotations were measured in CH$_2$Cl$_2$ using a JASCO DIP-1000 polarimeter with a sodium lamp operating at 598 nm. NMR spectra were recorded at 500/125 MHz (^{1}H/^{13}C) (AMX-Bruker spectrometer) and 200/50 MHz (^{1}H/^{13}C) (Bruker AC-200 NMR spectrometer) using CDCl$_3$ as solvent and internal standard. Carbon multiplicities were determined using DEPT-135. Atom connectivities were determined using gHMQC, gHMBC, and gCOSY data. NOESY experiments were carried out using a mixing time of 0.8 s. ESMS and AP$_C$IMS were obtained using thermoquest Navigator. (+)-LRFABMS were measured on a VG-Quattro spectrometer, while (+)-HRFABMS were measured on a Trisector EBE spectrometer from Micromass Instruments using thioglycerol with 1% NaI as matrix. Semipreparative HPLC was performed using μ-Bondapak C$_{18}$ columns (250 × 10 mm) with IR detection.

Biological Material. Specimens of *Xenia* sp. were collected in October 1996 in the Togian Islands near Sulawesi Island (Indonesia) at depth range of 27–33 m. These specimens have four very regular rows on either side of the tentacles, with 15–18 pinnules in the aboral rows. Sclerite diameters in the base and in capitulum reached 20–22 × 14–16 μm at maximum. Voucher samples are deposited at the Departamento de Química Fundamental, Universidade de A Coruña, under reference UDC 96007. A picture can be obtained from the authors.

Extraction and Isolation. Specimens of the gorgonian (0.3 kg) were homogenized in MeOH (3 × 2.5 L), and the solvent was evaporated under reduced pressure. The crude extract was

partitioned between CH$_2$Cl$_2$ and H$_2$O (1:1). The fraction soluble in CH$_2$Cl$_2$ was evaporated under reduced pressure and partitioned between 10% aqueous MeOH (400 mL) and hexane (2 × 400 mL). Water was added to the polar fraction until the mixture became 50% aqueous MeOH, and this was then extracted with CH$_2$Cl$_2$ (3 × 400 mL). After evaporation of the solvent, the combined organic layers yielded 0.25 g (hexane) and 0.41 g (CH$_2$Cl$_2$) of residue. The viscous oil (0.41 g) obtained from the CH$_2$Cl$_2$ fraction was purified by flash column chromatography (eluting with CH$_2$Cl$_2$/MeOH mixtures of increasing polarity) to give a fraction rich in diterpenes. This mixture was separated by reversed-phase HPLC eluting with MeOH/H$_2$O (1:1) to give compounds **1** (4.7 mg, 1.6 × 10^{-4} % wet weight), **3** (2.1 mg, 0.7 × 10^{-4} % wet weight), and **4** (7.6 mg, 2.5 × 10^{-4} % wet weight). Compound **2** (1.1 mg, 0.4 × 10^{-4} % wet weight) was further purified by reversed-phase HPLC eluting with MeOH/H$_2$O (45:55).

Xeniolide-F (1): amorphous colorless solid; $[\alpha]^{25}_D$ +91.6° (c 0.23, CH$_2$Cl$_2$); IR (neat) ν_{max} 3419, 1711, 1640 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 267 (4.35) and 207 (4.23) nm; (+)-ESMS m/z 333 [M + H]$^+$, 355 [M + Na]$^+$, 371 [M + K]$^+$, 315 ([M − H$_2$O + H]$^+$), 297 ([M − 2H$_2$O + H]$^+$), 293 ([M − H$_2$O − CO$_2$ + Na]$^+$); (+)-HRFABMS m/z 355.1883 [M + Na]$^+$ (calcd for C$_{20}$H$_{28}$O$_4$Na, Δ 0.7 mmu); ^{1}H and ^{13}C NMR see Table 1.

9-Hydroxyxeniolide-F (2): amorphous colorless solid; $[\alpha]^{25}_D$ +38.3° (c 0.05, CH$_2$Cl$_2$); IR (neat) ν_{max} 3715, 1734, 1640 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 271 (3.49) and 223 (3.55) nm; (+) LRFABMS m/z 371 [M + Na]$^+$, 349 [M + H]$^+$; ^{1}H and ^{13}C NMR, see Table 1.

Isoxeniolide-A (3): amorphous colorless solid; (+)-APcIMS m/z 333 [M + H]$^+$, 355 [M + Na]$^+$, 371 [M + K]$^+$, 315 [M + H − H$_2$O]$^+$, 297 [M + H − 2H$_2$O]$^+$, 271 [M + H − H$_2$O − CO$_2$]$^+$, 253 [M + H − 2H$_2$O − CO$_2$]$^+$.

7,8-Oxido-isoxeniolide-A (4): amorphous colorless solid; (+)-AP$_C$IMS m/z 349 [M + H]$^+$, 331 [M + H − H$_2$O]$^+$, 313 [M + H − 2H$_2$O]$^+$, 285 [M + H − H$_2$O− CO]$^+$.

References and Notes

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