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Xenimanadins A–D, a family of xenicane diterpenoids from the Indonesian soft coral *Xenia* sp.

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Abstract

Four novel xenicane diterpenoids, xenimanadins A–D (**1**–**4**), characterized by the unusual 2,6-dimethoxytetrahydropyran functionality, have been isolated from the Indonesian soft coral *Xenia* sp., together with three known xeniolides. The stereostructure of these metabolites has been established through extensive interpretation of NMR data and application of the modified Mosher method. Xenimanadins were tested against tumor cell lines, revealing details about the cytotoxic potential of xenicane diterpenoids.

Keywords: *Xenia* soft corals; Indonesia; Diterpenes; Structure elucidation; NMR

1. Introduction

Soft corals belonging to genus *Xenia* (order Alcyonacea, family Xeniidae) are prolific producers of diterpenoid metabolites, which are collectively called xenicanes.[1] Among these molecules, a remarkable group, structurally characterized by a pyran ring fused to a nine-membered carbocyclic ring to build a 2-oxabicyclo[7.4.0]tridecane system, represents an interesting class of bioactive metabolites exhibiting antitumor[2,3] and antibacterial[4] activities. These metabolites have been classified according to the structure of the six-membered ring (ring A). In this way, starting from 1977, with the isolation of the first member of this class,[5] four families have been identified (Fig. 1): xenicins,[5] xenialactols,[6] xeniolides A[7] and xeniolides B.[7] It has been hypothesized that these molecules were derived biogenetically from GGPP through a common precursor, namely the nine-membered dialdehyde derivative reported in Figure 1. Some molecules maintaining an opened ring A

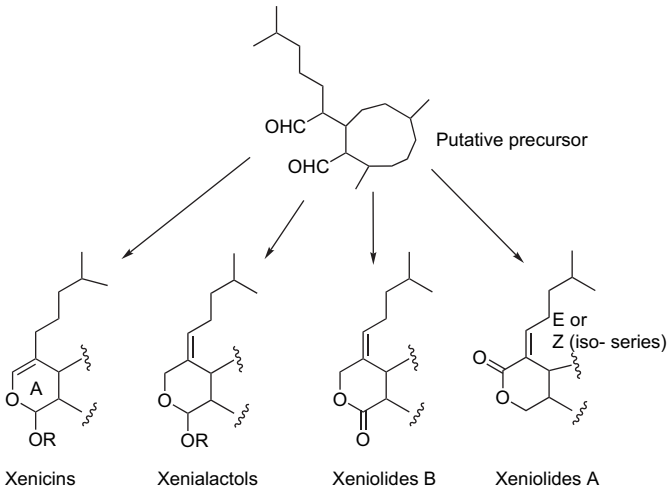

Figure 1. The four classes of pyran-cyclononane xenicane diterpenoids and their postulated precursor.

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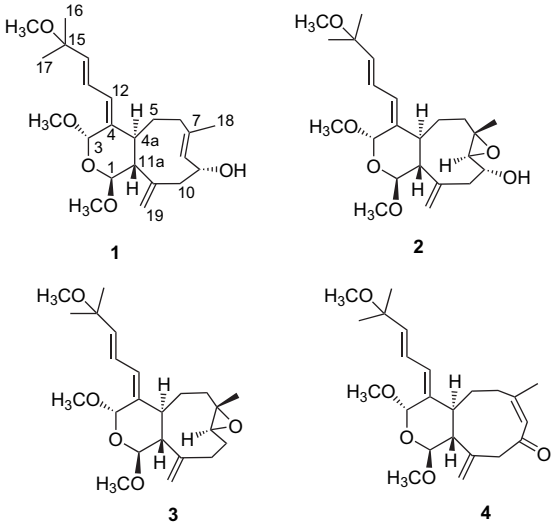

Figure 2. Xenimanadins A–D (**1**–**4**).

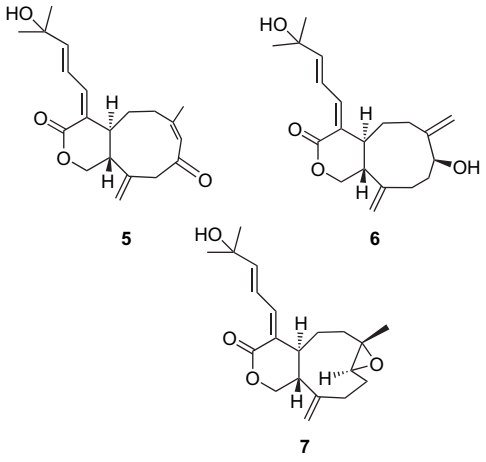

Figure 3. The known blumiolide C (**5**), xeniolide F (**6**), and deoxyoxidoisoxeniolide A (**7**).

(e.g., azamilides[8]) or exhibiting a contracted ring A (e.g., xeniaethers[9] and xeniaphyllanes[6]) have also been found.

We have recently analyzed a specimen of *Xenia* sp. collected along the coasts of Manado (Indonesia), and the present paper describes the isolation and the structural elucidation of seven xenicane diterpenoids from this source. Four of these metabolites are new compounds showing an unusual 2,6-dialkoxypyran system, and we propose to designate this class of xenicanes with the name xenimanadin (xenimanadins A–D, **1**–**4**, Fig. 2). The three additional isolated metabolites are known compounds belonging to the class of xeniolide A (**5**–**7**, Fig. 3).

2. Results

Specimens of *Xenia* sp. were collected in the Bunaken Marine Park of Manado (North Sulawesi, Indonesia) and extracted repeatedly with methanol. The methanol extract was partitioned between water and ethyl acetate to yield a brown organic fraction, which was subsequently chromatographed by MPLC over silica gel column using *n*-hexane/EtOAc mixtures of increasing polarity. The obtained fractions were further purified by HPLC to yield seven pure xenicane diterpenoids: the new xenimanadins A–D (**1**–**4**, Fig. 2) and the known blumiolide C (**5**),[10] xeniolide F (**6**),[11] and deoxyoxidoisoxenioliode A (**7**).[10] Compounds **5**–**7** have been identified by comparison of obtained spectroscopic data with those reported in the literature.[10,11]

The ESI-MS (positive ions) spectrum of xenimanadin A (**1**) exhibited a pseudo-molecular ion peak at *m/z* 415 $[M+Na]^+$, while the molecular formula $C_{23}H_{36}O_5$, implying six unsaturation degrees, was assigned to **1** by means of HREIMS. The ^{13}C NMR spectrum of **1** ($CDCl_3$, Table 2) showed 22 signals (the resonances of two carbons being coincident), eight of which were located in the sp^2 region of the spectrum. The ^1H NMR spectrum of **1** ($CDCl_3$, Table 1), exhibited a series of well resolved signals between δ_H 4.40 and 6.70, three methyl singlets between δ_H 3.10 and 3.60, a series of multiplets between δ_H 1.50 and 2.60 and, finally, three methyl singlets at δ_H 1.70 (3H) and 1.30 (6H). All these proton signals were associated with those of the directly linked carbons by utilizing an HSQC experiment. Obtained information indicated the presence of three methoxy groups, two dioxymethines (δ_H 4.47, δ_C 104.5; δ_H 5.46, δ_C 100.0), and four double bonds

Table 1
^1H NMR data of xenimanadins A–D (**1**–**4**) in $CDCl_3$ at 500 MHz

Position	**1**	**2**	**3**	**4**
	δ_H, mult., *J* in Hz	δ_H, mult., *J* in Hz	δ_H, mult., *J* in Hz	δ_H, mult., *J* in Hz
1	4.47, d, 8.8	4.53, d, 8.8	4.48, d, 8.8	4.71, d, 8.5
1-OMe	3.42, s	3.45, s	3.43, s	3.45, s
3	5.46, br s	5.50, br s	5.48, br s	5.51, br s
3-OMe	3.59, s	3.60, s	3.59, s	3.54, s
4a	2.30, br dd, 10.6, 4.5	2.37, br dd, 10.6, 4.5	2.32, br dd, 10.6, 4.5	2.31, br dd, 10.6, 4.5
5	1.88, m 1.53, m	1.94, m 1.67, m	1.92, m 1.61, m	1.71, m 1.71, m
6	2.15[a] 2.13[a]	2.16, m 1.14, m	2.20, m 1.12, m	2.87, m 2.24, m
8	5.17, br d, 7.0	2.88, d, 7.2	2.89, dd, 11.4, 2.7	5.93, s
9	4.74, br t, 7.0	3.78, m	1.50[a] 1.45[a]	
10	2.53, dd, 14.4, 7.0 2.35, br d, 14.4	2.66, dd, 13.5, 7.0 2.46, br d, 13.5	2.43, br t, 10.8 2.26, m	3.32, s 3.32, s
11a	1.69, dd, 10.6, 8.8	2.08, dd, 10.6, 8.8	2.13, dd, 10.6, 8.8	2.51, m
12	6.08, d, 11.3	6.07, d, 11.3	6.04, d, 11.3	5.92[a]
13	6.63, dd, 15.8, 11.3	6.60, dd, 15.8, 11.3	6.58, dd, 15.8, 11.3	6.43, dd, 15.8, 11.3
14	5.61, d, 15.8	5.63, d, 15.8	5.60, d, 15.8	5.66, d, 15.8
15-OMe	3.17, s	3.17, s	3.15, s	3.14, s
16	1.30, s	1.30, s	1.26, s	1.26, s
17	1.30, s	1.30, s	1.30, s	1.30, s
18	1.70, s	1.42, s	1.37, s	1.81, s
19	4.94, br s 4.91, br s	5.12, br s 5.01, br s	4.98, br s 4.91, br s	5.08, br s 5.02, br s

[a] Overlapped with other signals.

(four of the eight sp^2 carbons are monoprotonated, one is diprotonated, and three are unprotonated). Consequently, taking into account the degrees of unsaturation implied by molecular formula, xenimanadin A (**1**) must be a bicyclic compound.

The COSY spectrum of **1** disclosed the presence of three spin systems, (A)–(C), depicted in bold in Figure 4: moiety (A) includes a sequence of three double bond methines; moiety (B) starts with the dioxymethine at δ_H 4.47 and terminates with the relatively deshielded methylene at δ_H 2.15 and 2.13; moiety (C) includes the fourth double bond methine, which is coupled with a carbinolic proton (δ_H 4.47; δ_C 68.0), in turn coupled with an allylic methylene (δ_H 2.35 and 2.53).

A series of key $^{2,3}J_{H,C}$ correlations evidenced by the g-HMBC (gradient-selected heteronuclear multiple bond coherence) spectrum (Fig. 4) allowed us to assemble the above skeletal fragments and, including also the remaining uncoupled moieties, to draw the planar structure of xenimanadin A (**1**). In particular, the three correlation peaks of H_3-18 (δ_H 1.70) allowed us to join moieties (B) and (C); analogously, the three cross-peaks exhibited by the exocyclic double bond protons at δ_H 4.91 and 4.94 (H_2-19) indicated the presence of the cyclononene ring. Going on, valuable information came from the g-HMBC cross-peaks of the two dioxymethine protons: the $^{2,3}J$ cross-peaks of H-3 with C-1, C-4, C-4a, and C-12 indicated the structure of ring A, its junction to the nine-membered ring, and the linkage site of moiety (A). Attachment of this moiety to the dimethyl-bearing C-15 was deduced by the cross-peaks exhibited by the methyl protons H_3-16/H_3-17. Finally, carbons linking the three methoxy groups were identified on the basis of the ^3J cross-peak of the corresponding methyl protons.

Once the gross structure of xenimanadin A (**1**) was in hand, our next task was the definition of the configuration at the five asymmetric carbons and at the three double bonds. For this aim, analysis of coupling constants and information arising from a ROESY experiment (Fig. 5) proved to be particularly valuable. The *Z* geometry of the $\Delta^{4,12}$ double bond was deduced on the basis of the ROESY cross-peaks H-3/H-13 and H-12/H-4a; analogously, the *E* geometry of $\Delta^{7,8}$ double bond was indicated by the ROESY cross-peak H-8/H_2-6. As for the double bond $\Delta^{13,14}$, its *E* geometry was easily deduced by considering the large value of $J_{H\text{-}13/H\text{-}14}$ (15.8 Hz). The relative arrangement of the four asymmetric carbons around the six-membered ring was deduced on the basis of the following scalar and spatial couplings. The trans junction of the two rings was suggested by the ROESY correlation H-11a/H-5β and by the coupling

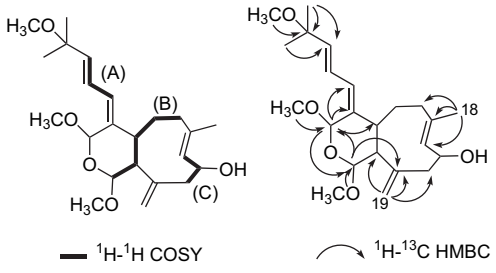

Figure 4. COSY and $^{2,3}J$ H→C HMBC correlations of xenimanadin A (**1**).

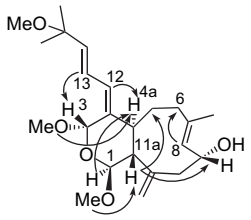

Figure 5. Diagnostic ROESY correlations detected for xenimanadin A (**1**).

constant $J_{H\text{-}11a/H\text{-}4a}$=10.6 Hz, supporting a pseudo-diaxial orientation. ROESY cross-peaks H-1/H-4a and 1-OMe/H-11a supported the trans ring junction and pointed also to the trans orientation of H-1 and H-11a; accordingly, the coupling constant $J_{H\text{-}1/H\text{-}11a}$=8.8 Hz is in agreement with a pseudo-diaxial orientation. The relative orientation of H-3 was inferred by the ROESY cross-peak of 3-OMe with H-4a, indicative of the trans relationship between H-3 and H-4a. The relative configuration at the remaining chiral carbon of the molecule, located at C-9, was suggested by a cross-peak between H-11a and H-9, indicative of the cis orientation of these two protons on the nine-membered ring.

The presence of a secondary alcohol group in the structure of xenimanadin A (**1**), offered us the possibility of upgrading the above determined relative stereochemistry to the absolute one, through application of the modified Mosher's method.[12] For this aim, two aliquots of **1** were treated with (*R*)- and (*S*)-MTPA chloride in dry pyridine to give the corresponding (*S*)-**1a** and (*R*)-**1b** MTPA esters. The pattern of $\Delta\delta$ ($S-R$) values (see Fig. 6) allowed us to establish the *R* configuration at C-9 and, consequently, to deduce the complete stereostructure of xenimanadin A (**1**) as reported in Figure 2.

Stereostructural determination of the remaining xenimanadins B–D (**2**–**4**) followed the same approach above detailed for xenimanadin A (**1**). Accurate inspection of COSY, HSQC, and g-HMBC spectra was instrumental to deduce their planar structures and the signal assignments reported in Tables 1 and 2, while, as above, stereochemical details were deduced on the basis of scalar coupling constants and spatial couplings. For these molecules, the absolute configuration has not been determined, however, their structures have been drawn in Figure 2 by extending the absolute configuration above deduced for xenimanadin A (**1**). For brevity, here we will discuss only the significant differences between each of these molecules and

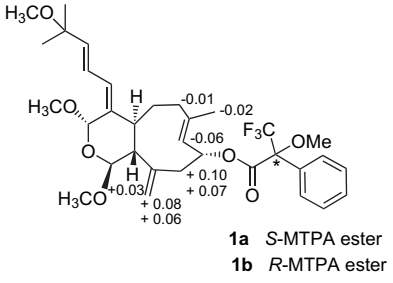

Figure 6. Application of the modified Mosher's method for secondary alcohols on the MTPA esters of xenimanadin A (**1a** and **1b**). $\Delta\delta$ ($\delta_S-\delta_R$) are given in parts per million.

Table 2
^{13}C NMR data of xenimanadins A–D (**1**–**4**) in $CDCl_3$ (125 MHz)

Position	**1**	**2**	**3**	**4**
	δ_C, mult.	δ_C, mult.	δ_C, mult.	δ_C, mult.
1	104.5, d	104.4, d	104.4, d	100.0, d
1-OMe	57.2, q	57.0, q	57.1, q	57.7, q
3	100.0, d	99.7, d	99.7, d	97.9, d
3-OMe	55.5, q	55.3, q	55.2, q	54.8, q
4	139.5, s	135.9, s	135.5, s	138.7, s
4a	51.3, d	51.6, d	51.7, d	50.6, d
5	36.0, t	36.1, t	35.4, t	32.6, t
6	40.5, t	40.6, t	40.6, t	30.3, t
7	133.5, s	60.5, s	59.9, s	146.6, s
8	131.5, d	67.0, d	62.4, d	130.0, d
9	68.0, d	69.0, d	27.8, t	203.3, s
10	47.2, t	44.9, t	33.9, t	51.6, t
11	150.5, s	150.0, s	139.0, s	143.4, s
11a	56.1, d	54.8, d	54.3, d	53.3, d
12	128.7, d	129.0, d	128.8, d	128.7, d
13	126.2, d	126.1, d	126.1, d	126.8, d
14	140.6, d	141.3, d	141.1, d	141.2, d
15	75.2, s	75.8, s	75.7, s	74.9, s
15-OMe	50.9, q	50.6, q	50.6, q	50.5, q
16	26.1, q	26.0, q	26.2, q	26.1, q
17	26.1, q	26.0, q	30.1, q	26.1, q
18	18.0, q	17.6, q	18.3, q	27.4, q
19	112.7, t	114.4, t	105.5, t	116.0, t

xenimanadin A. These differences are restricted to the functionalities of the nine-membered ring, and, accordingly, H/C resonances of the six-membered ring and of the di-unsaturated side chain resulted to be almost coincident (see Tables 1 and 2).

Xenimanadin B (**2**) is closely related to xenimanadin A, indeed its molecular formula exhibits only one oxygen atom more ($C_{23}H_{36}O_6$). Preliminary inspection of ^{1}H and ^{13}C NMR spectra of **2** (Tables 1 and 2) revealed the absence of the double bond $\Delta^{7,8}$, while the unsaturation degree was guaranteed by an epoxide group ($\delta_{C\text{-}7}$ 60.5; $\delta_{H\text{-}8}$ 2.88, $\delta_{C\text{-}8}$ 67.0). The COSY spectrum of **2** showed the coupling of the epoxide doublet (δ_H 2.88) with a carbinolic proton at δ_H 3.78, which, in turn, was coupled with H_2-10 protons (δ_H 2.66 and 2.46). g-HMBC correlations (H_3-18 with C-6, C-7 and C-8; H_2-19 with C-10, C-11, and C-11a) allowed us to attach this segment to moiety (B), thus unveiling the planar structure of this additional xenimanadin. The ROESY cross-peak H-8/H-6α revealed the geometry of the epoxide ring, while the spatial coupling H-8/H-4a connected this geometry with that of the asymmetric centers belonging to the six-membered ring. Finally, the spatial coupling H-11a/H-9 indicated the cis orientation of these two protons.

Xenimanadin C (**3**) showed the same molecular formula of xenimanadin A ($C_{23}H_{36}O_5$). COSY and HSQC spectra of **3** revealed that, also in this case, the subunit C-7/C-10, including an epoxide group followed by two methylenes in sequence (δ_H 1.50 and 1.45, δ_H 2.43 and 2.26), accounted for all the structural differences of **3** with **1**. The g-HMBC couplings H_2-19/C-10, H_3-18/C-6, H_3-18/C-7, and H_3-18/C-8 allowed the attachment of this subunit to the remaining parts of the molecule, thus building up the xenimanadin C (**3**) planar structure. ROESY spectrum revealed that compound **3** possessed the same epoxide geometry as **2** (cross-peaks H_3-18/H_2-9 and H-8/H-4a).

The molecular formula of xenimanadin D (**4**), $C_{23}H_{34}O_5$ by HREIMS, includes two hydrogen atoms less than that of xenimanadin A (**1**). Analysis of the ^{13}C NMR spectrum of **4** ($CDCl_3$, Table 2) suggested that a conjugated ketone group (signal at δ_C 203.3) could account for the additional unsaturation degree. Combined inspection of COSY, HSQC, and g-HMBC spectra suggested that all the differences between compounds **3** and **1** consisted in the replacement of the secondary alcohol with the ketone group, locating it at C-9. Accordingly, the sp^2 methine H-8 and H_2-10 resonated, both downfield shifted, as singlets at δ_H 5.93 (1H) and δ_H 3.32 (2H), respectively. The g-HMBC correlations H-8/C-9, H-8/C-10, and H_2-19/C-10 proved to be particularly helpful to define the structure of this moiety, while the ROESY cross-peak of H_3-18 with H-8 indicated the *Z* geometry of the double bond.

3. Discussion

Xenimanadins A–D build an interesting group of diterpenoids and they can be considered as a fifth family of pyrancyclononane derivatives in the xenicane class (see Fig. 1). Their 2,6-dimethoxytetrahydropyran functionality appears to be strictly reminiscent of the structure of the postulated dialdehyde precursor, which, notably, has never been isolated to date. The presence of this functionality could raise the question whether compounds **1**–**4** are actually artifacts produced during the extraction process with methanol. Unfortunately, due to the lack of material, we cannot unambiguously shed light on this issue by repeating the extraction with a different solvent. However, the absence in our extract of stereoisomers at C-1 and/or C-3 makes the formation of **1**–**4** unlikely during the extraction procedure. In addition, the following literature evidence gives strong support to the genuine natural origin of xenimanadins A–D. (i) A xenicane dialdehyde (**8**, Fig. 7) derivative possessing a rearranged nine-membered ring has been recently isolated from *Xenia umbellata*.[13] Interestingly,

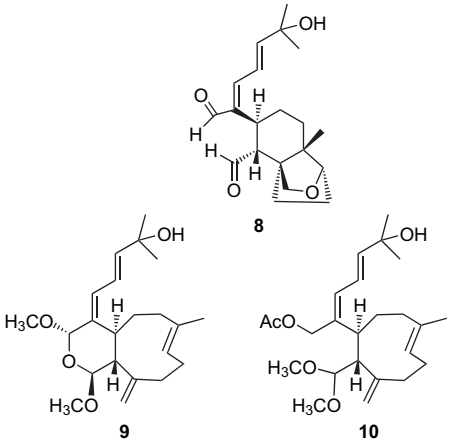

Figure 7. A rearranged xenicane dialdehyde derivative (**8**) and two known methoxylated xenicanes (**9** and **10**).

this molecule was shown to be unreactive toward MeOH, which was used during the purification procedure. (ii) A member of the xenimanadin family had been found before the present work, namely compound **9** (Fig. 7),[14] absent in our sample. Notably, this compound was obtained by using a completely methanol-free procedure (extraction in CH_2Cl_2; chromatographies in hexane/EtOAc mixtures),[14] thus demonstrating that *Xenia* soft corals are able to biosynthesize these methoxylated derivatives. (iii) This ability is confirmed by the recent isolation of compound **10** (Fig. 7) from *Xenia elongata*,[2] also in this case obtained through a methanol-free procedure.

Compounds **5** and **7** are cytotoxic against P-388 (mouse lymphocytic leukemia) tumor cell line with IC_{50} of 0.2 and 4.7 μg/mL, respectively.[10] On the contrary, xenimanadins A–D (**1**–**4**) proved to be non-cytotoxic (IC_{50}>20 μg/mL) against several cell lines (including P-388). Comparison of structures and activities of xenimanadin D (**4**) and blumiolide C (**5**) clearly indicates that ring A must exert a key role to determine the cytotoxic potentialities of these compounds.

4. Experimental

4.1. General experimental procedures

Optical rotations ($CHCl_3$) were measured at 589 nm on a Perkin–Elmer 192 polarimeter. ^1H (500 MHz) and ^{13}C (125 MHz) NMR spectra were measured on a Varian INOVA spectrometer. Chemical shifts were referenced to the residual solvent signal ($CDCl_3$: δ_H 7.26, δ_C 77.0). Homonuclear ^1H connectivities were determined by the COSY experiment. One-bond heteronuclear ^1H–^{13}C connectivities were determined with the HSQC experiment. Two- and three-bond ^1H–^{13}C connectivities were determined by gradient-HMBC experiments optimized for a $^{2,3}J$ of 9 Hz. Through-space ^1H connectivities were evidenced by using a ROESY experiment with a mixing time of 500 ms. ESI-MS spectra were performed on a LCQ Finnigan MAT mass spectrometer. Medium pressure liquid chromatography was performed on a Büchi apparatus using a silica gel (230–400 mesh) column; HPLC were achieved on a Knauer apparatus equipped with a refractive index detector and analytical LUNA (Phenomenex) SI60 (250×4 mm) columns.

4.2. Collection, extraction, and isolation

Specimens of *Xenia* sp. (40.0 g, dry weight) were collected in March 2006 in the Bunaken Marine Park of Manado along the coasts of the small island of Siladen (North Sulawesi, Indonesia) at a depth of 2–5 m. A small voucher sample is deposited at the Dipartimento per lo Studio del Territorio e delle sue Risorse, Università di Genova. *Xenia* sp. was extracted (3×3 L) with methanol at room temperature to obtain a brown material, which was partitioned between water and EtOAc to yield a brown organic fraction (1.16 g). This was subsequently chromatographed by MPLC over silica gel (230–400 mesh) column (750×25 mm), using a linear gradient system from *n*-hexane/EtOAc 8:2 to EtOAc. The first fraction (*n*-hexane/EtOAc, 8:2) was purified by analytical HPLC using *n*-hexane/EtOAc 85:15 as eluent, flow rate 0.8 mL/min, affording xenimanadin C (**3**, 1.5 mg, t_R 11 min). A second fraction (*n*-hexane/EtOAc, 7:3) was purified by analytical HPLC using *n*-hexane/EtOAc 7:3 as eluent, flow rate 0.8 mL/min, affording xenimanadins B (**2**, 3.8 mg, t_R 18 min) and D (**4**, 3.9 mg, t_R 10 min). The fraction obtained by eluting with *n*-hexane/EtOAc 6:4 was purified by analytical HPLC using *n*-hexane/EtOAc 65:35 as eluent, flow rate 0.8 mL/min, yielding xenimanadin A (**1**, 6.0 mg, t_R 9 min). The fraction obtained by eluting with *n*-hexane/EtOAc 1:1 was purified by analytical HPLC using *n*-hexane/EtOAc 1:1 as eluent, flow rate 0.8 mL/min, affording deoxyoxidoisoxeniolide A (**7**, 1.0 mg, t_R 10 min). The fraction obtained by eluting with *n*-hexane/EtOAc 4:6 was purified by analytical HPLC using *n*-hexane/EtOAc 1:1 as eluent, flow rate 0.8 mL/min, to afford blumiolide C (**5**, 2.1 mg, t_R 15 min). Finally, the fraction obtained by eluting with EtOAc was purified by analytical HPLC using *n*-hexane/EtOAc 1:9 as eluent, flow rate 0.8 mL/min, to afford xeniolide F (**6**, 1.9 mg, t_R 11 min).

4.2.1. Xenimanadin A (*1*)

Colorless amorphous solid; $[\alpha]_D^{22}$ −4 (*c* 0.11, $CHCl_3$); IR (KBr) ν_{max} 3415, 1608, 1240 cm^{-1}; For ^1H and ^{13}C NMR ($CDCl_3$) see Tables 1 and 2, respectively; ESI-MS (positive ions) *m/z* 415 $[M+Na]^+$, 431 $[M+K]^+$; HREIMS *m/z* 374.2468 $[M-H_2O]$ (calcd for $C_{23}H_{34}O_4$ 374.2452).

4.2.2. Xenimanadin B (*2*)

Colorless amorphous solid; $[\alpha]_D^{22}$ +8 (*c* 0.15, $CHCl_3$); IR (KBr) ν_{max} 3425, 1612, 1236 cm^{-1}; For ^1H and ^{13}C NMR ($CDCl_3$) see Tables 1 and 2, respectively; ESI-MS (positive ions) *m/z* 431 $[M+Na]^+$, 447 $[M+K]^+$; HREIMS *m/z* 390.2418 $[M-H_2O]$ (calcd for $C_{23}H_{34}O_5$ 390.2406).

4.2.3. Xenimanadin C (*3*)

Colorless amorphous solid; $[\alpha]_D^{22}$ +24 (*c* 0.12, $CHCl_3$); IR (KBr) ν_{max} 1632, 1230 cm^{-1}; For ^1H and ^{13}C NMR ($CDCl_3$) see Tables 1 and 2, respectively; ESI-MS (positive ions) *m/z* 415 $[M+Na]^+$, 807 $[2M+Na]^+$; HREIMS *m/z* 361.2390 $[M-OCH_3]$ (calcd for $C_{22}H_{33}O_4$ 361.2379).

4.2.4. Xenimanadin D (*3*)

Colorless amorphous solid; $[\alpha]_D^{22}$ +6 (*c* 0.20, $CHCl_3$); IR (KBr) ν_{max} 1720, 1640, 1215 cm^{-1}; For ^1H and ^{13}C NMR ($CDCl_3$) see Tables 1 and 2, respectively; ESI-MS (positive ions) *m/z* 413 $[M+Na]^+$, 429 $[M+K]^+$; HREIMS *m/z* 390.2397 (calcd for $C_{23}H_{34}O_5$ 390.2406).

4.3. Preparation of MTPA esters of xenimanadin A

To a solution of xenimanadin A (**1**) (1.0 mg) in 0.3 mL of dry pyridine an excess of (*R*)- or (*S*)-MTPA chloride was added and the mixture left at room temperature for 12 h under stirring. The reaction mixture was then diluted with ether and washed with H_2O and saturated NaCl aqueous solution. The organic layer was dried over Na_2SO_4, concentrated under

reduced pressure, and purified by HPLC (eluent *n*-hexane/EtOAc 9:1) to give (*S*)-MTPA ester **1a** (0.8 mg, 52% yield) and *R*-MTPA ester **1b** (0.7 mg, 45% yield), respectively.

4.3.1. (S)-MTPA ester of xenimanadin A

Colorless oil. ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H 7.55–7.51 (3H, MTPA phenyl, m), 7.43–7.41 (2H, MTPA phenyl, m), 6.60 (1H, H-13, dd, J=15.8, 11.3 Hz), 6.09 (1H, H-12, d, J=11.3 Hz), 5.91 (1H, H-9, t, J=7.0 Hz), 5.60 (1H, H-14, d, J=15.8 Hz), 5.43 (1H, H-3, s), 5.17 (1H, H-8, d, J=7.0 Hz), 4.89 (1H, H-19, s), 4.87 (1H, H-19, s), 4.43 (1H, H-1, d, J=8.8 Hz), 3.61 (3H, MTPA-OMe, s), 3.59 (3H, 3-OMe, s), 3.42 (3H, 1-OMe, s), 3.17 (3H, 5-OMe, s), 2.52 (1H, H-10, dd, J=14.0, 7.0 Hz), 2.32 (1H, H-4a, overlapped), 2.30 (1H, H-10, overlapped), 2.13–2.09 (2H, H-6, m), 1.90–1.88 (1H, H-5, m), 1.79 (3H, H-18, s), 1.71 (1H, H-11a, dd, J=10.6, 8.8 Hz), 1.56–1.54 (1H, H-5, m), 1.30 (6H, H-16 and H-17, s). ESI-MS (positive ions) *m/z* 631 $[M+Na]^+$.

4.3.2. (R)-MTPA ester of xenimanadin A

Colorless oil. ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H 7.58–7.54 (3H, MTPA phenyl, m), 7.43–7.41 (2H, MTPA phenyl, m), 6.60 (1H, H-13, dd, J=15.8, 11.3 Hz), 6.10 (1H, H-12, d, J=11.3 Hz), 5.92 (1H, H-9, t, J=7.0 Hz), 5.60 (1H, H-14, d, J=15.8 Hz), 5.43 (1H, H-3, s), 5.23 (1H, H-8, d, J=7.0 Hz), 4.81 (2H, H-19, s), 4.41 (1H, H-1, d, J=8.8 Hz), 3.59 (3H, 3-OMe, s), 3.56 (3H, MTPA-OMe, s), 3.40 (3H, 1-OMe, s), 3.17 (3H, 5-OMe, s), 2.42 (1H, H-10, dd, J=14.0, 7.0 Hz), 2.32 (1H, H-4a, dd, J=10.6, 4.5 Hz), 2.23 (1H, H-10, d, J=14.0 Hz), 2.13–2.10 (2H, H-6, m), 1.90–1.88 (1H, H-5, m), 1.81 (3H, H-18, s), 1.68 (1H, H-11a, dd, J=10.6, 8.8 Hz), 1.56–1.54 (1H, H-5, m), 1.30 (6H, H-16 and H-17, s). ESI-MS (positive ions) *m/z* 631 $[M+Na]^+$.

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