Novel Norhumulene and Xeniaphyllane-Derived Terpenoids from a Formosan Soft Coral Sinularia gibberosa

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A novel skeletal norhumulene (1) and six xeniaphyllane-derived compounds, including nonditerpenoids (2, 3) and diterpenoids (4—7), were isolated from the EtOAc extract of the Formosan soft coral Sinularia gibberosa. Their structures were elucidated by spectroscopic analysis. In vitro cytotoxic evaluation of the above metabolites revealed that 2 and 4 showed weak cytotoxicity toward a few cancer cell lines.

Key words diterpenoid; xeniaphyllane; Sinularia gibberosa; cytotoxic

Our previous study on the secondary metabolites of a Formosan soft coral Sinularia gibberosa Tixier-Durvaull (Alcyonidae) has resulted in the isolation of a series of β-caryophyllene-related metabolites and xeniaphyllane-related metabolites.1—3) Some of these metabolites have been shown to exhibit cytotoxicity toward several caner cell lines.1,3) Our continuing investigation on the chemical constituents of this soft coral has again led to the isolation of one novel norhumulene 1, two new norxeniaphyllanes 2 and 3, three new xeniaphyllanes 4—6, and a new xeniaphyllane-derived diterpenoid 7 possessing a rare cyclic peroxyhemiketal (3,6-dihydroxy-1,2-dioxin-3-ol).2,4) We describe herein the isolation, structure elucidation and biological activity of these compounds.

The minced bodies of S. gibberosa were extracted exhaustively with EtOAc. The combined extract was concentrated under reduced pressure, and the residue was fractionated by open column chromatography on silica gel. The terpenoid-containing fractions (characterized by 1H-NMR measurement in CDCl3) were selected for further purification, using open column chromatography on silica gel and normal phase HPLC to afford terpenoids 1—7.

Gibberosin N (1) displayed fourteen carbon signals in the 13C-NMR spectrum (Table 1), attributable to two methyls, five sp2 methylenes, one sp3 methane, one sp2 methylene, two sp2 methines, and three sp2 quaternary carbons. The IR spectrum displayed absorptions at 3423 and 1653 cm−1 for hydroxy group and carbon-carbon double bond, respectively. The NMR signals at δH 4.14 (dd, J=10.4, 4.4 Hz) and δC 74.8 (CH) revealed the presence of a secondary hydroxy group in the molecule. From NMR data (Tables 1, 2) and the IR spectrum displayed absorptions at 3423 and 1653 cm−1 for hydroxy group and carbon-carbon double bond, respectively. The NMR signals at δH 4.14 (dd, J=10.4, 4.4 Hz) and δC 74.8 (CH) revealed the presence of a secondary hydroxy group in the molecule. From NMR data (Tables 1, 2) and the HR-ESI-MS, correspondingly, the gross structure of 1 was established as 9-hydroxy-13-norhumulene-5,7-dione. Furthermore, the olefinic methyls (δH 1.49, s and 1.59, s) attached at C-4 and C-11 were confirmed by HMBC correlations from H-13 to C-3, C-4, and C-5 and H-12 to C-1, C-10, and C-11, respectively. The hydroxy group was determined to be located at C-9 due to the HMBC correlations observed from H-12 (δ 5.18, dd, J=8.4, 7.2 Hz and 4.84, dd, J=10.0, 4.4 Hz) and one exocyclic double bond (δC 153.2, C and 109.5, CH2 and δH 5.29, s and 5.09, s) were positioned. The structure of 1 was thus established as a norhumulene. On the basis of above results, the structure of 1 was established as 9-hydroxy-13-norhumulene-1E,4E,8(14)-triene.

Gibberosin O (2) was found to possess a molecular formula C19H23O3 from the pseudomolecular ion peak appearing at m/z 371.2197 [M+Na]⁺ in the HR-ESI-MS, corresponding to six degrees of unsaturation. The IR spectrum of 2...
2 showed the presence of ester (1734 cm⁻¹) and ketone (1716 cm⁻¹) functionalities. This was further supported by the ¹³C-NMR signals appearing at δ 170.8 (C), 213.3 (C), 216.8 (C), respectively. In the ¹H-NMR spectrum, the signals at δ 1.98 (1H, m), 2.41 (1H, m), 4.91 (1H, s) and 4.92 (1H, s) were found to be HMBC correlated with the carbon signals at δ 47.0 (CH), 41.7 (CH) and 112.5 (CH), respectively, indicating that 2 might be a β-caryophyllene-derived compound.¹,²,⁵ The gross structure of 2 was successively established by the assistance of ¹H–¹H COSY and HMBC correlations as shown in Fig. 1. Accordingly, the C-5 and C-12 positions of two keto-carbonyls 2 were interpreted by the HMBC
correlations observed from H-4 (δ 2.52, m), H2-6 (δ 2.50, m) and H1-20 (δ 1.05, d, J=7.0 Hz) to C-5 (δ 216.8, C), and from H2-13 (δ 2.35, m) and H2-18 (δ 1.18, s) to C-12 (δ 213.3, C). The acetoxy group was also determined to be located at C-15 due to the HMBC correlations observed from H2-14 (1.77, m) and H2-17 (δ 1.23, d, J=6.0 Hz) to C-15 (δ 70.3, CH). Thus, the norxeniaphyllane framework of metabolite 2 and the lack of one methyl group at C-15 relative to other xeniaphyllane diterpenoids1,2,5) were suggested.

The relative stereochemistry of the four chiral centers at C-1, C-4, C-9, and C-11 in 2 was established on the basis of the NOE correlations (Fig. 2) observed in the NOESY spectrum. Assuming the β orientation of H-9,1,2) it was found that H-9 exhibited NOE correlations with H-4, H-10β (δ 1.68, m) and H2-18, but not with H-1 (δ 1.98, m), and H2-20, while H-3α (δ 1.65, m) displayed NOE interaction with H-1 and H-20. These observations suggested that H-1, and H-20 should be α-oriented. On the basis of the above findings and other detailed NOE interactions (Fig. 2), the relative structure of compound 2 was unambiguously established as (1S*,4S*,9R*,11S*)-15-acetoxy-16-norxeniaphylla-8(19)-en-5,12-dione.

Gibberosin P (3) had a molecular formula C19H30O3 as established from its HR-ESI-MS (m/z 329.2093, [M+Na]+). The IR spectrum indicated the presence of hydroxy (3420 cm⁻¹) and carbonyl (1716 cm⁻¹) groups. The ¹H- and ¹³C-NMR spectroscopic data of 3 were quite similar to those of 2 except that the oxymethine carbon signal at δ 70.3 (C-15) in 2 was found to be upfield shifted to 67.6 ppm in compound 3. This was associated by the disappearance of the NMR signals of acetae and the IR absorption band of the ester function. Comparison of the ¹H-NMR spectroscopic data of compound 4 with those of compound 2 (Table 1) indicated that compound 4 has the same carbon skeleton from C-1 to C-14, while two tertiary methyl groups (δ C 25.7, CH3, 2C) were found to be attached at C-15 in 4 by showing HMBC correlations from protons of these two methyls to C-14 and C-15. Thus, gibberosin Q (4) was identified as (1S*,4S*,9R*,11S*)-15-acetoxy-xeniaphylla-8(19)-en-5,12-dione.

Gibberosin R (5) exhibited a pseudomolecular ion peak in the HR-ESI-MS at m/z 327.2301, appropriate for a molecular formula of C23H36O5. It differs from 4 in the absence of an acetoxy group as revealed from the disappearance of IR absorption band of ester function and NMR signals of acetate group. The carbon of the side chain carbonyl carbon (δ 215.1, C) was found to be C-14 due to the HMBC correlations observed from H2-13 (δ 2.31, m), H-15 (δ 2.63, septet, J=7.0 Hz), and H2-16 and H2-17 (δ 1.10, d, J=7.0 Hz) to the carbon signal at δ 215.1. On the basis of the above findings, the molecular framework of compound 5 was determined as shown in Fig. 1. Furthermore, the analysis of the NOESY spectrum of 5 revealed 5 possesses the same relative configurations at C-1, C-4, C-5, C-9, and C-11 as those in 2—4. Thus, the relative structure of metabolite 5 was determined as (1S*,4S*,9R*,11S*)-xeniaphylla-8(19)-en-5,14-dione.

Gibberosin S (6) was found to possess a molecular formula C22H34O4, as indicated from its HR-ESI-MS at m/z 427.2462 [M+Na]+. It also exhibited IR absorptions at 1736 and 1717 cm⁻¹, suggesting the presence of ester and ketone moieties. Comparison of the ¹H- and ¹³C-NMR data of compound 6 with those of 2—5 revealed that 6 is also a 5-oxoxeniaphyllane with a trisubstituted double bond and two acetoxy groups in the side chain. The HMBC correlations (Fig. 1) observed from H2-18 (δ 1.01, s) to C-1 (δ 44.5, CH), C-10 (δ 33.4, CH2), C-11 (δ 40.5, C) and an oxymethine carbon at δ 77.6 (CH) assigned this oxymethine carbon as C-12. This finding together with the upfield shift of C-11 relative to
those of metabolites 2–4 (δC 48.1–48.2), and the downfield shift of C-11 relative to that of 5 (δC 36.6), indicated the C-12 attachment of an acetoxy group. Furthermore, the protons of an oxymethylene group in 6 (δH 4.51 and 4.60, each 1H, d, J=12.0 Hz) were found to exhibit HMBC correlations with the carbonyl carbon of another acetoxy group (δC 170.8, C) and an olefinic carbon (δC 132.2, C, C-15), while this olefinic carbon was found to be correlated with the protons of an olefinic methyl (δH 1.73, s). Therefore, the trisubstituted double bond was positioned between C-14 and C-15 where an acetoxy methyl and a methyl were the substituents at C-15. The 1H–1H COSY correlations found from H-12 (δ 4.68, dd, J=9.2, 3.6 Hz) to H-13 (δ 2.16, m) and from H-13 to the olefinic proton (δ 5.32, dd, J=7.2, 6.8 Hz, H-14) further supported the C-14/C-15 position of the double bond. This double bond was determined to have a Z geometry on the basis of the NOE interaction found between H-14 and H-17. Furthermore, the analysis of the NOESY spectrum of 6 revealed the similar stereochemistries at C-1, C-9, C-10, and C-11 as those in 2–5. Therefore, the relative structure of compound 6 was established as (1S*,4S*,9R*,11S*,14Z)-12,16-diacetoxyxeniaphylla-8(19),14-dien-5-one.

IR spectra were recorded on a JASCO FT-5300 infrared spectrophotometer. Those of metabolites 1–7 were performed using the MTT [3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric method.6,7) Cytotoxicity assays of the test compounds 1–7 were performed using the Hep G2 (human hepatocellular carcinoma) and A549 (human lung carcinoma) cell lines with IC50’s of 18.7 and 19.5 μg/ml, respectively. Also, metabolite 4 showed weak cytotoxicity (IC50 11.0 μg/ml) toward A549 cells. Other metabolites were found to be inactive against the growth of the above three cancer cell lines.

Experimental

Optical rotations were measured on a Jasco DIP-1000 digital polarimeter. IR spectra were recorded on a JASCO FT-5300 infrared spectrophotometer. NMR spectra were recorded on a Bruker AVANCE DPX 300 FT-NMR at 300 MHz for 1H and 75 MHz for 13C, or on a Varian Mercury Plus 400 FT-NMR at 400 MHz for 1H and 100 MHz for 13C, or on a Varian Unity INOVA 500 FT-NMR at 500 MHz for 1H and 125 MHz for 13C, in CDC13, using TMS as an internal standard. LR-MS and HR-MS were obtained by ESI on a Bruker APEX II mass spectrometer. Silica gel (Merck, 230–400 mesh) was used for column chromatography. Precoated silica gel plates (Merck, Kieselgel 60 F-254, 0.2 mm) were used for analytical TLC. High-performance liquid chromatography (HPLC) was performed on a Hitachi L-7100 apparatus equipped with a Bischoff refractive index detector and a Merck Hibar Si-60 column (250 mm×21 mm, 7 μm).

Animal Material

The soft coral Sinularia gibberosa was collected by hand using scuba off the coast of northeastern Taiwan, in May 2004, at a depth of 15–20 m, and was stored in a freezer until extraction. A voucher specimen was deposited in the Department of Marine Biotechnology and Resources, National Sun Yat-sen University (voucher no. SC-20040621-5).

Extraction and Isolation

The bodies of Sinularia gibberosa (1.3 kg fresh weight) were minced and extracted exhaustively with EtOAc, and the extract was concentrated under reduced pressure to give a dark brown viscous residue (15.4 g). The residue was fractionated by open column chromatography on silica gel using n-hexane and n-hexane–EtOAc mixture of increasing polarity to yield 32 fractions. Fraction 15, eluting with n-hexane/EtOAc (5 : 1), was further purified by silica gel column using n-hexane/EtOAc (5 : 1) to give compound 5 (1.2 mg). A combined mixture of fractions 17–19, eluting with n-hexane/EtOAc (5 : 1→4 : 1), was purified by normal phase HPLC using n-hexane/EtOAc (4 : 1) to give compounds 2 (1.5 mg) and 4 (12.4 mg), and then using n-hexane/acetonitrile (5 : 1) to give compounds 1 (1.0 mg) and 6 (1.1 mg). Fraction 20, eluting with n-hexane/EtOAc (3 : 1), was further purified by normal phase HPLC using n-hexane/acetonitrile (3 : 1→3 : 1) to give compound 3 (3.8 mg). Fraction 22, eluting with n-hexane/EtOAc (1 : 1), was further purified by normal phase HPLC using n-hexane/acetonitrile (2 : 1), to give compound 7 (1.9 mg).

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References