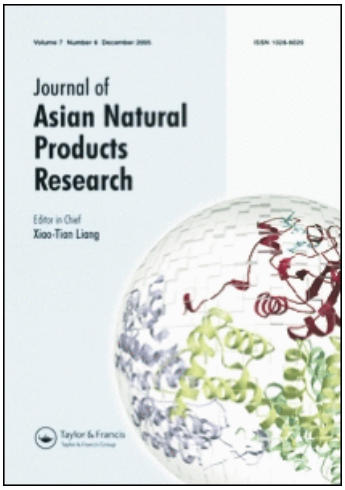

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The absolute stereochemistry of protoxylogranatin A – a new protolimonoid from the seeds of Chinese mangrove *Xylocarpus granatum*

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A new protolimonoid, named protoxylogranatin A, has been isolated from the seeds of a Chinese mangrove *Xylocarpus granatum*. Its structure was elucidated on the basis of modern spectroscopic data. The absolute configuration was determined by the application of the modified Mosher MTPA ester method. The first complete assignments of ^{1}H and ^{13}C NMR spectral data for this protolimonoid were achieved by means of 2D NMR techniques, including ^{1}H–^{1}H COSY, HSQC, HMBC and NOESY spectra.

Keywords: protolimonoid; *Xylocarpus granatum*; Meliaceae; protoxylogranatin A

1. Introduction

The mangrove *Xylocarpus granatum* (Meliaceae) is known to produce antifeedant limonoids, especially phragmalins and mexicanolides. Previous investigations on the seeds of two Meliaceae plants, the mangroves *X. granatum* and *Xylocarpus moluccensis*, uncovered an obacunol, two phragmalins, three andirobins, and 14 mexicanolides, including xyloccensins[1–5] A–K. Recently, 14 phragmalins and 15 mexicanolides, named xyloccensins L–Z and xylogranatins[6–9] A–E, have been reported from a Chinese mangrove *X. granatum*. Two mexicanolides, named xyloccensins[10] X and Y, the structures of which are different from those of the same names as we reported, have been identified in a mixture from the fruit of an Indian mangrove *X. moluccensis*. In this paper, we present the isolation and characterization of a new protolimonoid, protoxylogranatin A, from the seeds of a Chinese mangrove *X. granatum*. Its relative structure was elucidated on the basis of modern spectroscopic data. The absolute configuration was determined using the modified Mosher MTPA ester method. The first complete assignments of ^{1}H and ^{13}C NMR spectral data for this protolimonoid were achieved by means of 2D NMR techniques, including ^{1}H–^{1}H COSY, HSQC, HMBC and NOESY spectra.

2. Results and discussion

The ethanolic extract of the seeds of *X. granatum* was subjected to sequential extraction with petroleum ether and ethyl acetate as described in the experimental section. The resulting ethyl acetate extract was chromatographed using silica gel as

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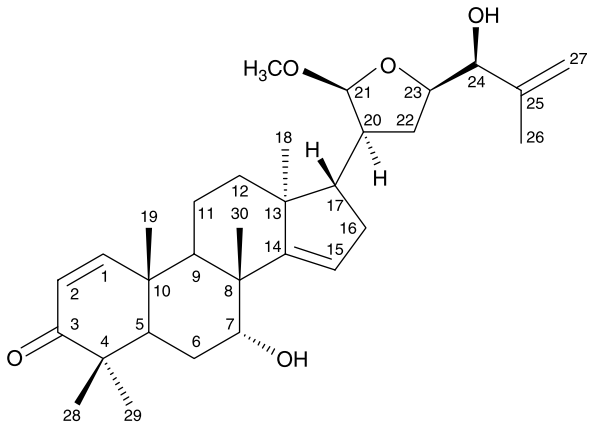

Figure 1. Structure of compound **1**.

the adsorbent followed by preparative reverse-phase C_{18} HPLC to yield protoxylogranatin A (**1**) (Figure 1).

Electrospray ionization (ESI)-MS (positive ion mode) of **1** showed pseudo-molecular ion peaks at *m/z* 499 $[M + H]^+$and 521 $[M + Na]^+$, which proposed that **1** has a molecular weight of 498. The molecular formula was determined as $C_{31}H_{46}O_5$ (nine unsaturations) from the HR-ESI-MS at *m/z* 521.3251. The ^1H and ^{13}C NMR spectral data (Table 1) indicated that four out of the nine unsaturations came from a ketone and three carbon–carbon double bonds. Therefore, the other five units of unsaturations stemmed from five rings. The UV maximum at 230 nm and IR (KBr) absorption bands at 3600–3240, 2978 and 1710 cm^{-1} indicated the existence of hydroxyls, carbon–carbon double bonds, and a carbonyl group in **1**. The ^1H and ^{13}C NMR spectral data (Table 1) showed the presence of an acetal carbon (δ_C 104.4, d), a conjugated ketone (δ_C 205.1, s), a terminal double bond (δ_H 4.95, br s, 5.06, br s; δ_C 144.7, 112.7), and three oxygenated methines (δ_C 71.5, 78.0, 80.5).

The above NMR spectral data together with six tertiary methyls in **1** suggested that it was a triterpene. Considering the four rings in the nucleus and one ring in the side chain, it was suggested that **1** might be a protolimonoid. The ^1H and ^{13}C NMR spectral data (Table 1) of **1** were similar to those of

Table 1. ^1H (HSQC) and ^{13}C NMR spectral data for compound **1** (500 and 125 MHz, $CDCl_3$).

No.	^1H NMR δ_H; mult.; *J*(Hz)	^{13}C NMR δ_C; mult.
1	7.14; d; 10.0	158.2; d
2	5.85; d; 10.0	125.6; d
3		205.1; s
4		44.3; s
5	2.40; dd; 7.5, 3.0	44.5; d
6α, 6β	1.88; m, 1.92; m	24.3; t
7	3.99; brs	71.5; d
8		44.8; s
9	2.22; m	36.7; d
10		40.2; s
11α, β	2.00; m, 1.88; m	16.4; t
12α, β	1.68; m, 1.62; m	32.9; t
13		46.6; s
14		161.4; s
15	5.52; s	120.1; d
16α, β	2.15; m, 2.19; m	35.0; t
17	2.05; s	52.8; d
18	1.03; s	20.1; q
19	1.18; s	18.9; q
20	2.25; m	45.3; d
21	4.79; d; 4.5	104.4; d
22α, β	1.74; m, 1.89; m	30.8; t
23	4.24; dd; 7.0, 5.0	80.5; d
24	3.84; t; 4.5	78.0; d
25		144.7; s
26	1.79; s	18.5; q
27a, b	4.95; br s, 5.06; br s	112.7; t
28	1.18; s	27.2; q
29	1.10; s	21.5; q
30	1.14; s	27.6; q
21-OMe	3.39; s	54.7; q
7-OH	2.15; brs	
24-OH	2.83; d; 5.0	

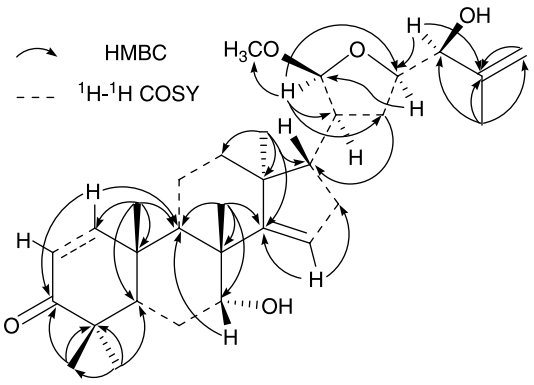

Figure 2. Selected ^{1}H–^{1}H COSY and HMBC correlations for compound **1**.

holstinone[11] B, suggesting **1** might have had a structure similar to that of holstinone B. However, 25-OH and 27-methyl in holstinone[11] B was substituted by an additional terminal double bond (δ_H 4.95, br s, 5.06, br s; δ_C 144.7, s, 112.7, t) in **1**. HMBC correlations from H-24 and H_3-26 to the quaternary carbon C-25 (Figure 2) confirmed the above replacement. Thus, the planar structure of **1** was characterized as shown in Figure 1.

The relative stereochemistry of **1** was established on the basis of NOE correlations as shown in Figure 3. The significant NOE interactions from H-7 to H_3-30, H-15 and H-6β, but not from H-7 to H-5 and H_3-18, helped to establish this 7β-H and the corresponding 7α-OH. Similarly, those between H-24/H-27b, and H-24/H_3-21-OMe, indicated that H-24 was close to H-27b and H_3-21-OMe in space (Figure 3). Moreover, NOE interactions between H-20/H-21, H-20/H-23, H-21/H_3-18, H-23/H_3-26, H-17/H-15, H_3-18/7-OH, H_3-19/H_3-30, H_3-28/H_3-19, H-5/H_3-29, H-5/H-9 (Figure 3), indicated a *cis* orientation between these respective protons. Based on the above results, the relative structure of **1** was elucidated as shown in Figure 3.

The modified Mosher ester method [12] was employed to determine the absolute configuration of C-24 in **1** (Figure 4). The $\Delta\delta$ values ($\delta_S - \delta_R$) of H-15 (+0.23), H_3-18 (+0.04), H-21 (+0.07), H-23 (+0.06) and H_3-30 (+0.06) were positive, while those of H_2-26 (−0.05, −0.05) and H_3-27 (−0.15) were negative, thus suggesting a 24*S*-configuration of compound **1**. Therefore, the absolute

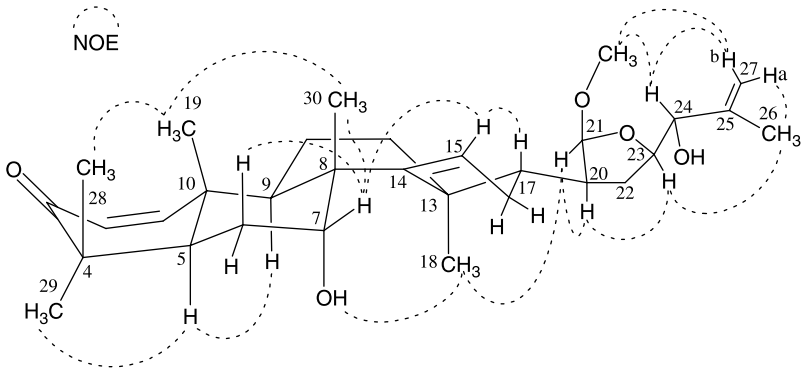

Figure 3. Significant NOE correlations for compound **1**.

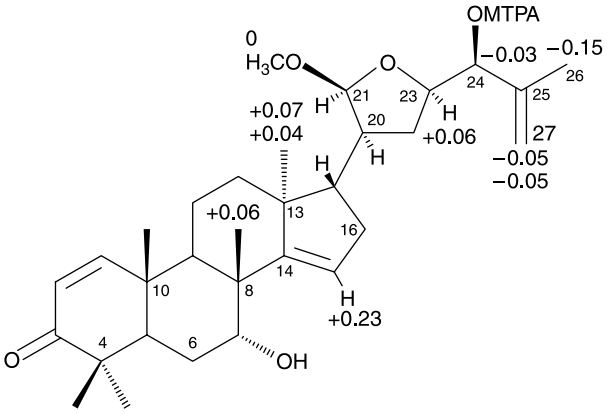

Figure 4. $\Delta\delta$ values [$\Delta\delta$ (ppm) = ($\delta_S - \delta_R$)] obtained for the 24-(*S*)- and 24-(*R*)-MTPA esters (**1s** and **1r**, respectively) of compound **1**.

stereochemistry of compound **1** was identified as shown in Figure 1.

3. Experimental

3.1 General experimental procedures

UV spectra were obtained on a Beckman DU-640 UV spectrophotometer and IR spectra recorded on a Perkin–Elmer FT-IR 1760X spectrophotometer. ESI-MS spectra were measured on a Bruker APEX II spectrometer in the positive ion mode. Optical rotations were recorded on a POLAPTRONIC HNQW5 automatic high-resolution polarimeter (Schmidt and Haensch Co., Ltd., Berlin). NMR experiments were carried out on a Bruker AV-500 spectrometer operating at 500 and 125 MHz for ^{1}H and ^{13}C, respectively, and equipped with an inverse-detection 5 mm probe (TBI probe, ^{1}H 90° pulse width = 6.1 μs, ^{13}C 90° pulse width = 12.3 μs) and operating at room temperature with tetramethylsilane as the internal standard. Approximately 5 mg samples were dissolved in $CDCl_3$ (0.5 ml) to record the NMR spectra.

3.2 Plant material

The seeds of *X. granatum* were collected in January 2006 from Hainan Island, southern China. The identification of the plant was performed by Professor Yongshui Lin, Laboratory of Marine Biology, South China Sea Institute of Oceanology, Chinese Academy of Sciences. A voucher sample (No. GKLMMM-002-6) is being maintained in the Herbarium of South China Sea Institute of Oceanology.

3.3 Extraction and isolation

The dried seeds (6 kg) of *X. granatum* were crushed and extracted three times with 95% ethanol at room temperature. After removal of the solvent by evaporation, the residue (200 g) was suspended in water and defatted with petroleum ether. Then, the aqueous layer was further extracted with ethyl acetate and concentrated to give a brown gum (86 g), which was subjected to silica gel column chromatography (chloroform–methanol, 100:0–2:1). The fractions eluted with chloroform–methanol (25:1–15:1) were combined and purified by preparative HPLC (YMC-Pack ODS-5-A, 250 × 20 mm i.d.) with acetonitrile–water (30:70–45:55) to yield **1** (6 mg).

*3.3.1 Protoxylogranatin A (**1**)*

Amorphous powder, $[\alpha]_D^{25}$ − 25 (*c* 0.6, acetonitrile). UV (MeCN) λ_{max} 214, 230 nm; IR (KBr) ν_{max} 3600–3240, 2978, 1710, 1634,

870 cm^{-1}. ^1H NMR and ^{13}C NMR spectral data ($CDCl_3$), see Table 1. HR-ESI-MS *m/z* 521.3251 $[M + Na]^+$ (calcd for $C_{31}H_{46}O_5Na$, 521.3243).

3.4 Mosher's MTPA esters 1s and 1r

A half portion of **1** (2 mg) was treated with (*R*)-α-methoxy-α-(trifluoromethyl)-phenylacetyl chloride (MTPACl) (10 μl) and (dimethylamino)pyridine (1 mg) in dried pyridine (0.5 ml) at room temperature for 5 h. The reaction mixture was concentrated and purified by RP-HPLC (YMC-Pack ODS S-5, 250 × 10 mm i.d.) with aqueous acetonitrile (88%) to afford the 24-*O*-(*S*)-MTPA ester **1s**. The 24-*O*-(*R*)-MTPA ester **1r** was prepared in the same way. As a result of our experiment, 7-OH in **1** was not reacted with MTPACl in the above condition.

3.4.1 Compound *1s*

Amorphous powder; ^1H NMR ($CDCl_3$): δ 7.15 (d, $J = 10$ Hz, H-1), 5.85 (d, $J = 10$ Hz, H-2), 5.51 (br s, H-15), 5.34 (d, $J = 9$ Hz, H-24), 5.12 (br s, H-27b), 5.02 (br s, H-27a), 4.84 (d, $J = 4$ Hz, H-21), 4.29 (dd, $J = 16$, 90 Hz, H-23), 3.99 (br s, H-7), 3.31 (s, 3H, 21-OMe), 1.59 (s, 3H, H-26), 1.18 (s, 3H, H-19), 1.18 (s, 3H, H-28), 1.14 (s, 3H, H-30), 1.10 (s, 3H, H-29), 1.02 (s, 3H, H-18); ESI-MS *m/z* 737 $[M + Na]^+$.

3.4.2 Compound *1r*

Amorphous powder; ^1H NMR ($CDCl_3$): δ 7.18 (d, $J = 10$ Hz, H-1), 5.87 (d, $J = 10$ Hz, H-2), 5.28 (br s, H-15), 5.37 (d, $J = 9$ Hz, H-24), 5.17 (br s, H-27b), 5.07 (br s, H-27a), 4.77 (d, $J = 4$ Hz, H-21), 4.23 (dd, $J = 16$ Hz, 9 Hz, H-23), 3.98 (br s, H-7), 3.31 (s, 3H, 21-OMe), 1.74 (s, 3H, H-26), 1.19 (s, 3H, H-19), 1.18 (s, 3H, H-28), 1.08 (s, 3H, H-30), 1.08 (s, 3H, H-29), 0.98 (s, 3H, H-18); ESI-MS *m/z* 737 $[M + Na]^+$.

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