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Phyllaemblic acid, a novel highly oxygenated norbisabolane from the roots of *Phyllanthus emblica*

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Abstract

Phyllaemblic acid, a novel highly oxygenated norbisabolane was isolated from the roots of *Phyllanthus emblica*, and its structure was fully characterized by spectroscopic and chemical means. The absolute stereochemistry was determined by applying modified Mosher's method to an opportune degradation product.

Keywords: Phyllanthus emblica; norbisabolane; phyllaemblic acid; stereochemistry.

Phyllanthus emblica L., an euphorbiaceous plant, is widely distributed in subtropical and tropical areas of China, India, Indonesia, and the Malay Peninsula, and has been used for the anti-inflammatory and antipyretic treatment by many traditional medicinal systems, such as Chinese herbal medicine, Tibetan medicine, and Ayurvedic medicine. The minorities living in the Southwest of China use its root for the treatment of eczema and the Nepali use it as an astringent and hematostatic.[1] As a part of our chemical studies on this plant, we report here the isolation and structure elucidation of a novel highly oxygenated norbisabolane named phyllaemblic acid (**1**), from the root (Fig. 1).

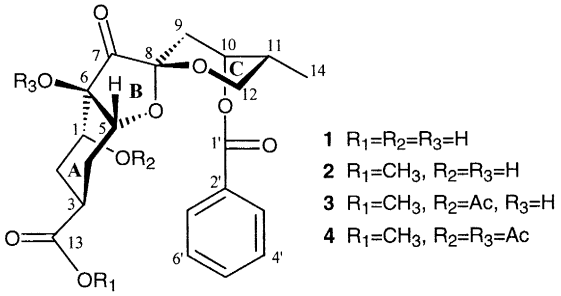

Fig. 1.

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Acetone extract (60% Aq.) of the air-dried roots of *P. emblica* [2] was partitioned between EtOAc and water, and then the organic phase was successively subjected to chromatographies over Sephadex LH-20, Chromatorex ODS and silica gel to afford **1** (0.0421%), as a yellowish amorphous powder.[3] The molecular formula $C_{21}H_{24}O_9$ of **1** was determined by FABMS [*m/z* 421($M+H)^+$] and elementary analysis. [3] The ^{13}C NMR spectrum of **1** showed a set of signals for a benzoyl group and 14 carbon resonances (Table 1). From the ^1H, ^{13}C, ^1H–^1H COSY and HMQC spectra, the two partial structures of C-1 to C-5 (A ring) and C-9 to C-12 (C ring) were deduced. HMBC data (Table 1) of **1** suggested a spiro-acetal structure at C-8, a benzoyl ester at C-10, and the connectivities of C(9)–C(8)–C(7) and C(1)–C(6)–C(5) of which the latter was supported by a W-letter-type coupling between H-1 and H-5. Considering chemical shifts of C-1, C-5 and C-6, the connectivities between the A, B and C rings were deduced as shown in Fig. 1. Proton coupling constants of **1** indicated that H-1, 5 and 10 were equatorially orientated while H-3 and H-11 were axially orientated, and the ring junction at C(5)-C(6) was *cis*.

Methylation of **1** with CH_2N_2 yielded **2**,[4] which was further acetylated with acetic anhydride in

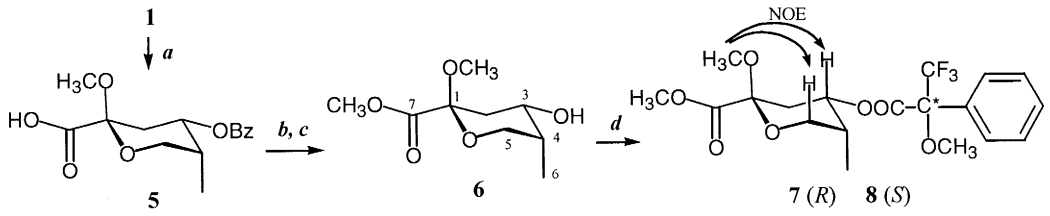
Table 1
^1H, ^{13}C NMR, HMBC and NOESY spectral data for **1** and **4**[a]

	1[b]			**4**[c]		
No.	^{13}C	^1H	HMBC (H to C)	^{13}C	^1H	NOESY (cross peaks)
1	71.5	3.91(br s)	3,5,6	70.3	5.18(br s)	2,1-OAc
2	33.2	1.92(dd, 3.0, 14.0) 1.70(ddd, 2.5, 13.0, 14.0)	3,4,13	29.2	2.11(dd, 3.0, 13.0) 1.70(dt, 3.0, 13.0)	1,3
3	31.7	2.81(tt, 3.0, 13.0)	13	30.7	2.43(tt, 3.0, 13.0)	2,4
4	29.0	2.28(dd, 3.0, 15.0) 1.85(ddd, 4.0, 13.0, 15.0)	2,3,13	27.9	2.22(dd, 3.5, 13.0) 1.79(dt, 3.5, 13.0)	3,5
5	76.4	4.26(br s)	1,3,6	70.8	4.89(t, 1.5)	4,12,6-OAc
6	75.6			76.9		
7	213.9			214.0		
8	100.5			99.1		
9	32.6	2.31(dd, 3.0, 15.0) 1.98(dd, 3.0, 15.0)	7,8,10,11	31.7	2.34(dd, 3.5, 14.5) 2.12(dd, 3.5, 14.5)	10,11,1-OAc
10	71.1	5.33(q, 3.0)	8,11,12,14,1'	69.8	5.29(q, 3.5)	9,11
11	34.3	2.18(dddq,3.0,4.5,11.5,7.0)	14	32.7	2.23(dddq,3.5,4.5,11.5,7.0)	10,12,14
12	63.4	4.02(t, 11.5) 3.58(dd, 4.5, 11.5)	8,10,11,14	63.2	4.09(t, 11.5) 3.76(dd, 4.5, 11.5)	5,11,12,14
13	179.5			174.7		
14	13.1	0.90(d, 7.0)	10,11,12	12.9	0.98(d, 7.0)	10,11,12
1'	168.0			166.4		
2'	132.1			130.9		
3',7'	130.7	8.14(dd, 1.5, 7.5)	1',4',5',6'	129.7	8.12(dd, 8.0, 1.5)	3,10,11,12,14,1-OAc,4',5',6'
4',6'	129.5	7.59(dt, 1.5, 7.5)	1',2',3',7'	128.3	7.46(br d, 8.0)	OMe,11,3',4',5',6',7'
5'	134.1	7.63(tt, 1.5, 7.5)	3',4',5',6',7'	132.8	7.57(tt, 1.5, 8.0)	OMe
OMe				51.8	3.60(s)	
1-OAc				168.1[d]		
				20.5	1.99(s)	1,3,9,10,3',7'
6-OAc				168.6[d]		
				21.0	2.09(s)	6

[a] The δ values are in ppm, ^1H NMR data recorded at 500 MHz, ^{13}C NMR data recorded at 125 MHz. [b] Data recorded in CD_3OD. [c] Data recorded in $CDCl_3$. [d] assignments may be interchanged.

pyridine to give 1-*O*-mono (**3**) and 1,6-*O*-di (**4**) acetate.[5] The NOESY spectrum of **4** (Table 1) showed a cross peak between H-5 and H-12, which confirmed the relative configuration of C-8 spiro-acetal carbon. The NOE correlations of benzoyl protons with H-3 and the methoxyl group at C-13 revealed that rings A and C adopted flattened chair conformation, and the benzoyl group was extended over the A ring. In addition, the remaining NOEs listed in Table 1 established the relative configuration of **1**. These structural features were similar to those of phyllanthoside, a glycoside with a bisabolane aglycone and relative stereochemistry being determined by crystal X-ray analysis, from *P. brasiliensis*.[6,7]

The absolute stereochemistry of **1** was determined by applying the modified Mosher's method to an opportune degradation product (Scheme 1). Oxidation of **1** with $KMnO_4/NaIO_4$ in $MeOH/H_2O$ afforded **5**,[8a] which was further hydrolyzed and methylated to give **6**.[8b] Large coupling constants of H-3 ($J_{2ax,3}$=9.6 Hz for **5** and 10.8 Hz for **6**, $J_{3,4}$=4.5 Hz), indicated axial orientation of H-3 in **5** and **6**. Treatment of two aliquots of **6** with (+) and (–)-MTPA in the presence of DCC and DMAP provided mono ester derivatives **7** and **8**,[9] respectively. According to the modified Mosher method[10,11] for secondary alcohols, the $\Delta\delta$ (*S*–*R*) values (Fig. 2) assigned the *S* configuration at C-3 of **6**, corresponding to C-10 of compound **1**. Hence, based on the above evidences, the absolute configuration of phyllaemblic acid was concluded to be as shown in **1**. Although several norbisabolenes with a skeleton lacking one of the terminal dimethyl carbons of bisabolane skeleton have so far been known,[12] phyllaemblic acid, with an highly oxygenated structure, is the first norbisabolane biogenetically synthesized by oxidative removal of the central methyl carbon.[6] The presence of glycosides of **1** and its related compounds has already been confirmed, and studies on structure and biological activity of these compounds are now in progress.



Scheme 1. (a) $NaIO_4/KMnO_4$, $MeOH:H_2O$ (2:3); (b) 10% KOH, MeOH; (c) CH_2N_2/ether, MeOH; (d) (*R*) or (*S*)-MTPA, DCC, DMAP, CH_2Cl_2

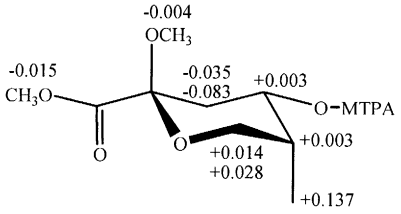


Fig. 2. $\Delta\delta$ (*S*–*R*) values (ppm) for MTPA ester derivatives of compound **6**

Acknowledgements

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2. The roots of *Phyllanthus emblica* L. were collected in the Wenshan county of Yunnan province, China, and air-dried. A voucher specimen is deposited with the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences.
3. Compound **1**: $[\alpha]^{25}_{D}$ +19.3 (*c* 0.28, CH_3OH); FABMS *m/z*: 421 $[M+H]^+$, 299 $[M-C_7H_5O_2+H]^+$, 105 $[C_7H_5O]^+$, 77 $[C_6H_5]^+$; anal. calcd for $C_{21}H_{24}O_9 \cdot 1/2H_2O$: C, 58.74; H, 5.87. Found: C, 58.79; H, 5.79.
4. A solution of **1** (15 mg) in MeOH (2 ml) was treated with CH_2N_2/Et_2O at rt. After concentrated in vacuo, the mixture was purified on a silica gel column to afford **2** (8 mg), as a yellowish powder, $[\alpha]^{25}_{D}$ +19.3 (*c* 0.85, CH_3OH); FABMS *m/z*: 435$[M+H]^+$.
5. Compound **2** (100 mg) was treated with pyridine (1 ml) and Ac_2O (1 ml) at rt overnight and then subjected to a silica gel column to give **3** (15 mg) and **4** (106 mg). Compound **3**: a yellowish powder, $[\alpha]^{24}_{D}$ +11.0 (*c* 0.20, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 4.99 (s, H-1), 2.24 (br d, J=14.5 Hz, H-2a), 1.79 (dt, J=3.0, 13.0 Hz, H-2b), 2.45 (tt, J=3.0, 13.0 Hz, H-3), 2.06 (br d, J=13.0 Hz, H-4a), 1.92 (dt, J=3.5, 13.0 Hz, H-4b), 4.36 (s, H-5), 2.33 (dd, J=3.5, 14.5 Hz, H-9a), 2.08 (dd, J=3.0,14.5 Hz, H-9b), 5.29 (q, J=3.0 Hz, H-10), 2.22 (m, H-11), 4.06 (t, J=11.3 Hz, H-12a), 3.65 (dd, J=4.5,11.3 Hz, H-12b), 0.97 (d, J=7.0 Hz, H-14), 8.14 (dd, J=8.0,1.5 Hz, H-3′,7′), 7.48 (br t, J=8.0 Hz, H-4′,6′), 7.58 (tt, J=1.5, 8.0 Hz, H-5′), 3.61 (s, $COOCH_3$), 1.98 (s, 1-OAc). Compound **4**: a yellowish powder, $[\alpha]^{25}_{D}$ −2.4 (*c* 0.25, $CHCl_3$); ^{1}H and ^{13}C data were shown in Table 1.
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8. (a) A solution of **1** (100 mg) in MeOH:H_2O (2:3, 5 ml) was treated with $KMnO_4$ (25 mg) and $NaIO_4$ (80 mg) at rt for 5 h, and then acidified with 1N HCl to ca. pH3. After removal of MeOH by evaporation, the mixture was successively applied to MCI gel and silica gel column to give **5** (20 mg) as a colorless syrup; FABMS *m/z*: 293 $[M-H]^-$; ^{1}H NMR (300 MHz, CD_3OD) δ: 2.42 (dd, J=9.6, 13.2 Hz, Ha-2), 2.01 (dd, J=4.5, 13.2 Hz, Ha-2), 5.47 (dt, J=9.6, 4.5Hz, H-3), 2.21 (m, H-4), 4.15 (dd, J=3.3, 11.7 Hz, Ha-5), 3.77 (dd, J=3.6, 11.7 Hz, Ha-5), 1.10 (d, J=6.9 Hz, CH_3-6), 3.23 (s, 1-OCH_3); ^{13}C NMR (300 MHz, CD_3OD) δ: 99.9 (C1), 33.4 (C2), 71.1 (C3), 34.3 (C4), 66.4 (C5), 11.0 (CH_3-6), 171.7 ($\underline{CO}OCH_3$), 51.2 (1-OCH_3), 167.0 (C1′), 131.4 (C2′), 130.5 (C3′,7′), 129.6 (C4′,6′), 134.3 (C5′). (b) Compound **5** (20 mg) was treated with 10% KOH (0.5 ml) for 6 h and then neutralized with Amberlite IR-120B (H^+ form) resin. After concentration, the residue was treated with CH_2N_2/Et_2O and subjected to a silica gel column to afforded **6** (3.3 mg). ^{1}H NMR (300 MHz, CD_3OD) δ: 1.73 (dd, J=10.8, 13.2 Hz, Ha-2), 1.92 (dd, J=4.5, 13.2 Hz, Hb-2), 4.11 (dt, J=10.8, 4.5 Hz, H-3), 1.89 (m, H-4), 3.79 (dd, J=2.4, 10.8 Hz, Ha-5), 3.63 (dd, J=3.0, 10.8 Hz, Hb-5), 1.01 (d, J=7.2 Hz, CH_3-6), 3.17 (s, 1-OCH_3), 3.78 (s, $COOCH_3$).
9. A solution of **6** (1.5 mg), dicyclohexylcardiimide (2 mg), 4-dimethylaminopyridine (1 mg) and (*R*)-(+)-MTPA (4 mg) in CH_2Cl_2 (0.5 ml) was allowed to stand at rt for 12 h. The reaction mixture was purified by silica gel column to afford (*R*)-MPTA ester **7** (1.4 mg): FABMS *m/z*: 389 $[M-OCH_3]^-$; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 2.181 (dd, J=5.0, 13.0 Hz, He-2), 2.010 (dd, J=11.5, 13.0 Hz, Ha-2), 5.559 (dt, J=11.5, 5.0 Hz, H-3), 2.222 (m, H-4), 3.893 (dd, J=2.5, 12.0 Hz, He-5), 3.697 (dd, J=2.0, 12.0 Hz, Ha-5), 0.943 (d, J=7.0 Hz, 6-CH_3), 3.262 (s, 1-OCH_3), 3.809 (s, $COOCH_3$), 3.535 (d, J=1.5, MTPA–OCH_3), 7.496 and 7.404 (MTPA phenyl protons, m). Axial orientation of OCH_3 groups in **5**, **6**, **7** and **8** was confirmed by DIF-NOE experiment of **7** as shown in Scheme 1. Using (*S*)-(−)-MTPA, the same procedure afforded (*S*)-MTPA ester **8** (1.5 mg): FABMS *m/z*: 389 $[M-OCH_3]^-$; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 2.146 (dd, J=5.0, 13.0 Hz, He-2), 1.927 (dd, J=11.5, 13.0 Hz, Ha-2), 5.562 (dt, J=11.5, 5.0 Hz, H-3), 2.225 (m, H-4), 3.907 (dd, J=2.5, 12.0 Hz, He-5), 3.725 (dd, J=2.0, 12.0 Hz, Ha-5), 1.080 (d, J=7.0 Hz, 6-CH_3), 3.258 (s, 1-OCH_3), 3.794 (s, $COOCH_3$), 3.518 (d, J=1.5, MTPA–OCH_3), 7.488 and 7.401 (MTPA phenyl protons, m).
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