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# A New Triterpenoid Saponin and a New Glycoside from *Epigynum aurilum*

CAO, Jian-Xin<sup>a</sup> (曹建新) LAI, Guo-Fang<sup>a</sup> (来国防) WANG, Yi-Fen<sup>a</sup> (王易芬)  
 YANG, Liu-Bing<sup>b</sup> (杨刘兵) LUO, Shi-De<sup>\*</sup> (罗士德)

<sup>a</sup> State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, Yunnan 650204, China

<sup>b</sup> Kunming University of Science and Technology, Kunming, Yunnan 650093, China

A new triterpenoid saponin with novel sugar moiety (**1**), a new trisaccharide derivative (**2**), as well as a known monosaccharide derivative (**3**), were isolated from the dried stem of *Epigynum aurilum*. The structures of compounds **1** and **2** were determined by MS and NMR spectram analyses.

**Keywords** *Epigynum aurilum*, Apocynaceae, triterpenoid saponin, saccharide derivative

## Introduction

*Epigynum* Wight (Apocynaceae) is a genus including about 14 species. In the 14 species, there is only one, *Epigynum aurilum* distributed in China. Its unique taxonomic position attracted us to investigate its chemical constituent. This paper deals with the isolation and structural elucidation of a new triterpenoid saponin (**1**), 6 $\beta$ -hydroxy-oleanolic acid 3-*O*-[ $\beta$ -*D*-mannopyranosyl(1-4)](2-*O*-3)] $\beta$ -*D*-glucuronate·methyl·ester, a new trisaccharide derivative (**2**),  $\alpha$ -methyl-*L*-bis[ $\alpha$ -methyl-*D*-fructopyranosyl(1-2)](1-3)]arabinofuranose, as well as a known monosaccharide derivative (**3**), from the dried stem of *E. aurilum*.

## Results and discussion

The dried stem of *E. aurilum* was extracted with alcohol followed by column chromatographic separation to give compounds **1**—**3**, respectively.

Compound **1** was obtained as a white powder. The molecular formula C<sub>43</sub>H<sub>66</sub>O<sub>15</sub> was determined by negative-ion HRFABMS spectrum (calcd 821.4279, found [M - 1]<sup>-</sup> 821.4323) and NMR spectrum. The IR spectrum showed absorption bands at 3408, 1741, 1632 and 918 cm<sup>-1</sup>, which corresponded to hydroxyl groups, a carboxyl group and olefinic bonds, respectively. In EIMS spectrum, the peak at *m/z* 248 was given from retro-Diels-Alder fragmentation, and it is assumed that **1** was a pentacyclic triterpene with a  $\Delta^{12}$  double bond.<sup>1</sup> Two fragments at

661 [M-glc-3H]<sup>-</sup> and 471 [M-glc-(6-carbomethoxy)glc-3H]<sup>-</sup> in the negative-ion FABMS showed that **1** was a saponin with two glycosyl groups. This was also supported by two anomeric proton signals ( $\delta$  4.97 and 5.22) in <sup>1</sup>H NMR spectrum. <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra of aglycone of **1** were similar to those of 6 $\alpha$ -hydroxyl-3-epi-oleanolic acid except for the fact that the chemical shifts of C-3 and C-7 were upfielded to  $\delta$  89.6 and 41.0 from  $\delta$  76.1 and 36.2, respectively (The value from the literature was recorded in CD<sub>3</sub>OD).<sup>2</sup> This was explained by the  $\beta$  configurations of H-3 and H-6 and supported by the corrections between H-5 with H-3 and H-6 in ROESY spectrum. And the broad singlet at  $\delta$  4.76 (H-6) and the broad doublet at  $\delta$  3.30 (*J* = 7.2 Hz, H-3) also supported the  $\beta$  configurations. These <sup>1</sup>H NMR and <sup>13</sup>C NMR spectral data of aglycone were assigned by 2D NMR spectrum as shown in Table 1, which indicated **1** to be 6 $\beta$ -hydroxyl-oleanolic acid with two glycosyl groups. The <sup>13</sup>C NMR spectrum of sugar moiety of **1** shows 13 carbon atoms. Distortionless enhancement by polarization transfer (DEPT) spectrum divided these signals as 1  $\times$  CH<sub>3</sub>, 1  $\times$  CH<sub>2</sub>, 9  $\times$  CH, 2  $\times$  C (Table 1). The positions of C and H were assigned by <sup>1</sup>H-<sup>1</sup>H COSY and HMBC spectra (Table 2). And all coupling constants of H of sugar moiety (Table 1) explained the configurations unambiguously except for C-1'' and C-2''. The steric positions of C-1'' and C-2'' produced four kinds of possible structures **1a**—**1d** (Fig. 1). But ROESY spectrum (Table 2) showed interactions of  $\delta$  5.22 (H-1'') with  $\delta$  4.99 (H-3') and  $\delta$  4.60 (H-5'). Therefore, the structure **a** was the only correct one. The long-rang coupling of the quaternary carbon signal at  $\delta$  93.9 (C-2'') with the proton signal at  $\delta$  4.99 (H-3') was shown. Thus, there was a glycoside bond between C-2'' and C-3'. This was supported by the unsaturated value of compound **1**. Combined the above analysis, the structure of **1** was elucidated as 6 $\beta$ -hydroxy-oleanolic acid 3-*O*-[ $\beta$ -*D*-mannopyranosyl(1-4)](2-*O*-3)] $\beta$ -*D*-glucuronate·methyl·ester (Fig. 1).

\* E-mail: luoshide@hotmail.com; Tel.: 86-871-5223097; Fax: 86-871-5223038

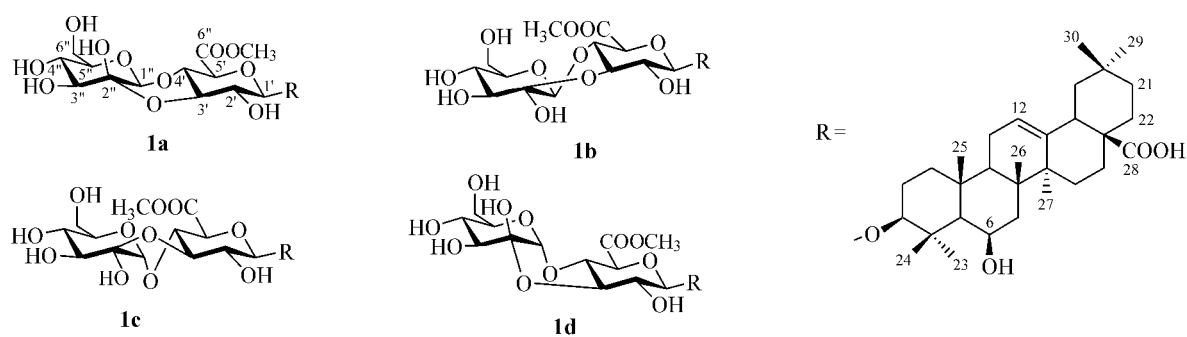
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**Table 1**  $^{13}\text{C}$  NMR and  $^1\text{H}$  NMR data ( $\delta$ ) of compound **1**

Position	$\delta_{\text{C}}$	$\delta_{\text{H}}$	Position	$\delta_{\text{C}}$	$\delta_{\text{H}}$
1	40.7 (t)	0.93—0.95 (m), 1.46—1.48 (m)	23	28.1 (q)	1.42 (s)
2	26.8 (t)	2.00—2.02 (m), 2.11—2.14 (m)	24	18.6 (q)	1.60 (s)
3	89.6 (d)	3.30 (brd, $J = 7.2$ Hz)	25	17.1 (q)	1.50 (s)
4	41.3 (s)		26	18.7 (q)	1.55 (s)
5	56.3 (d)	0.87 (brs)	27	26.4 (q)	1.31 (s)
6	67.4 (d)	4.76 (brs)	28	180.6 (s)	
7	41.0 (t)	1.77—1.78 (m)	29	23.9 (q)	1.00 (s)
8	39.3 (s)		30	33.4 (q)	0.97 (s)
9	48.7 (d)	1.75—1.76 (m)	1'	107.3 (d)	4.97 (d, $J = 8.4$ Hz)
10	36.9 (s)		2'	72.2 (d)	4.10 (t, $J = 8.1$ Hz)
11	24.0 (t)	1.94—1.96 (m), 2.00—2.02 (m)	3'	73.4 (d)	4.99 (t, overlap by H-1')
12	123.0 (d)	5.56 (brs)	4'	69.7 (d)	4.92 (t, $J = 9.5, 10.0$ Hz)
13	144.3 (s)		5'	74.2 (d)	4.60 (d, $J = 9.4$ Hz)
14	42.8 (s)		6'	169.6 (s)	
15	28.4 (t)	2.28—2.32 (m)	COOCH <sub>3</sub>	52.4 (q)	
16	33.4 (t)	0.64—0.66 (m), 1.07—1.09 (m)	1''	96.7 (d)	5.22 (s)
17	46.6 (s)		2''	93.9 (s)	
18	42.2 (d)	3.32—3.34 (m)	3''	79.6 (d)	4.23 (d, $J = 9.5$ Hz)
19	46.8 (t)	1.30—1.33 (m), 1.82—1.84 (m)	4''	69.6 (d)	4.37 (t, $J = 9.5$ Hz)
20	31.1 (s)		5''	79.3 (d)	3.92—3.95 (m)
21	34.3 (t)	1.20—1.24 (m), 1.44—1.46 (m)	6''	62.7 (t)	4.29 (dd, $J = 12.0, 5.9$ Hz) <b>a</b>
22	33.4 (t)	1.81—1.83 (m), 2.03—2.05 (m)			4.50 (d, $J = 11.7$ Hz) <b>b</b>

**Table 2**  $^1\text{H}$ - $^1\text{H}$  COSY, HMBC and ROESY spectra of sugar moiety of compound **1**

H	$^1\text{H}$ - $^1\text{H}$ COSY	HMBC	ROESY
1'	H-2'	C-3, C-2', C-5'	H-3', H-5', H-23
2'	H-1'	C-1', C-3'	H-4'
3'	H-2'	C-1', C-2', C-4', C-2''	H-1', H-5', H-1''
4'	H-3', H-5'	C-3', C-5', C-1''	H-2'
5'	H-4'	C-1', C-3', C-4', C-6'	H-1', H-3', H-1''
COOCH <sub>3</sub>		C-6'	
1''		C-4', C-2'', C-5''	H-3'', H-5'', H-3', H-5'
3''	H-4''	C-2'', C-4''	H-1'', H-5''
4''	H-3'', H-5''	C-3'', C-5'', C-6''	
5''	H-4'', H-6''	C-3'', C-4'', C-6''	H-1'', H-3''
6'' <b>a</b>	H-5'', H-6'' <b>b</b>	C-5''	
6'' <b>b</b>	H-5'', H-6'' <b>a</b>	C-4''	

**Fig. 1** Structure of compound **1** (Sugar = **1a**).

Compound **2** was obtained as a transparent oil. The molecular formula  $C_{20}H_{36}O_{15}$  was determined by negative-ion HRFABMS spectrum (calcd 515.1976, found  $[M - 1]^-$  515.1959) and NMR spectrum. Band at  $3474.6\text{ cm}^{-1}$  in the IR spectrum was indicative of hydroxyl groups. The  $^{13}\text{C}$  NMR spectrum of **2** showed the presence of 20 carbon atoms. DEPT spectrum divided these signals as  $3 \times \text{CH}_3$ ,  $5 \times \text{CH}_2$ ,  $10 \times \text{CH}$  and  $2 \times \text{C}$  (Table 3). Because seven of these carbon signals were doubled, combined with the molecular formula, it is shown that the compound included two similar fragments. The positions of C and H were assigned by  $^1\text{H}$ - $^1\text{H}$  COSY, HMBC and ROESY spectra (Table 4). The position of the glycoside bond was also established by using HMBC experiment. Compared the  $^{13}\text{C}$  spectral data of **2** with the values of  $\alpha$  and  $\beta$  *D*-fructose<sup>3</sup> from the fructose of **2**, it was suggested to be  $\alpha$  configuration. This configuration was confirmed by the obvious interaction between  $\delta$  4.31 (H-1') and  $\delta$  4.88 (H-5') in ROESY. The  $\beta$  configuration of arabinose moiety of **2** was confirmed by comparing its  $^{13}\text{C}$  spectral data with those of *L*-methyl-arabinofuranose<sup>4</sup> and by analyzing the coupling constant values (Table 2). These coupling constant values also decided  $\alpha$  or  $\beta$  configurations of other protons attached to sugars. Therefore, the structure of **2** was established as  $\alpha$ -methyl-*L*-bis[ $\alpha$ -methyl-*D*-fructopyranosyl(1-2)(1-3)]-arabinofuranose (Fig. 2).

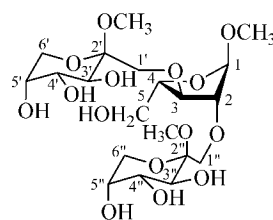


Fig. 2 Structure of compound **2**.

Compound **3** was identified to be 5-hydroxymethyl-2-furancarboxaldehyde by comparing its  $^{13}\text{C}$  NMR spectral data with previously published values.<sup>5</sup> Its spectral data were listed in the Experimental section.

## Experimental

Optical rotations were recorded in pyridine and methanol on an Horiba SEAP-300 spectropolarimeter. IR spectra were taken in KBr pellets on a Bio-Rad FTS-IR spectrophotometer.  $^1\text{H}$  NMR (500 MHz),  $^{13}\text{C}$  NMR (125 MHz) and 2D NMR spectra were recorded in pyridine-*d*<sub>5</sub> on a Bruker DRX-500 NMR spectrometer with TMS as an internal standard. MS data were measured by a VG Autospec 3000 mass spectrometer under negative-ion FAB and EI models. Column chromatography and TLC were carried on silica gel (Qingdao, China), MCI CHP-20P

Table 3  $^{13}\text{C}$  NMR and  $^1\text{H}$  NMR spectral data ( $\delta$ ) of compound **2**

Position	$\delta_{\text{C}}$	$\delta_{\text{H}}$	Position	$\delta_{\text{C}}$	$\delta_{\text{H}}$
1	111.2 (d)	5.33 (brs)	1', 1''	63.7 (t)	4.16 (d, $J = 11.3$ Hz) <b>a</b> ; 4.31 (d, $J = 11.9$ Hz) <b>b</b>
2	82.1 (d)	4.95 (brs)	2', 2''	101.2 (s)	
3	82.9 (d)	4.80 (brs)	3', 3''	72.2 (d)	4.88 (d, $J = 9.7$ Hz)
4	77.9 (d)	4.97 (brs)	4', 4''	71.3 (d)	4.46 (dd, $J = 9.8, 3.2$ Hz)
5	65.4 (t)		5', 5''	70.6 (d)	4.31—4.33 (m)
OCH <sub>3</sub>	55.3 (q)	3.41 (s)	6', 6''	65.0 (t)	3.89 (d, $J = 11.8$ Hz) <b>a</b> ; 4.04 (d, $J = 11.9$ Hz) <b>b</b>
			OCH <sub>3</sub> '	48.7 (q)	3.40 (s)

Table 4  $^1\text{H}$ - $^1\text{H}$  COSY, HMBC and ROESY spectral data of compound **2**

H	$^1\text{H}$ - $^1\text{H}$ COSY	HMBC	ROESY
1		C-2, C-4, OCH <sub>3</sub> (55.3)	OCH <sub>3</sub> , H-2
2		C-1, C-3	H-1
3		C-2, C-4	
4		C-1, C-2, C-3, C-5	
OCH <sub>3</sub>		C-1	H-1
H-1' <b>a</b>		C-2', C-3'	OCH <sub>3</sub> ', H-1' <b>b</b> , H-3'
H-1' <b>b</b>		C-2', C-3', C-3, (H-1'' <b>b</b> with C-2)	OCH <sub>3</sub> ', H-1' <b>a</b> , H-3'
H-3'	H-4'	C-1', C-2', C-4', OCH <sub>3</sub> ' (48.7)	H-1' <b>a</b> and <b>b</b> ,
H-4'	H-3', H-5'	C-3', C-5'	H-6' <b>a</b> , H-5'
H-5'	H-4'	C-4', C-6'	H-6' <b>a</b> , H-4'
H-6' <b>a</b>	H-6' <b>b</b>	C-2', C-5'	OCH <sub>3</sub> ', H-6' <b>b</b> , H-5'
H-6' <b>b</b>	H-6' <b>a</b>	C-2', C-5'	H-4', H-6' <b>a</b> , H-5'
OCH <sub>3</sub> '		C-2'	H-1' <b>a</b> and <b>b</b> , H-6' <b>a</b>

gel, FUJI ( ODS-Q3 ) gel ( Mitsubishi Chemical Co. ) and Lobar RP-C18 gel ( Merck ) using the following solvent systems : CHCl<sub>3</sub>-MeOH-H<sub>2</sub>O and MeOH-H<sub>2</sub>O , respectively.

#### Extraction and isolation procedure

The stem of *E. aurilum* ( Apocynaceae ) was collected in Xishuangbanna , Yunnan , China in Sept. 1999. The dried aerial part of plant material ( 5 kg ) was extracted three times with EtOH under reflux. After removal of the solvent *in vacuo* , the residue ( 530 g ) was subjected to column chromatography on silica gel ( 200—300 mesh , 5.26 kg ) , eluting with gradient mixtures of CHCl<sub>3</sub>-MeOH-H<sub>2</sub>O [ from CHCl<sub>3</sub> to CHCl<sub>3</sub>-MeOH-H<sub>2</sub>O ( 7:3:0.3 , V:V:V ) ] to give 11 fractions. Then , fraction 9 was repeatedly chromatographed on MCI CHP-20P gel , FUJI ( ODS-Q3 ) gel ( Mitsubishi Chemical Co. ) and Lobar RP-C18 gel ( Merck ) with the solvent system [ MeOH-H<sub>2</sub>O ( 60:40 , V:V ) ] to give compounds **1** ( 21 mg ) , **2** ( 81 mg ) and **3** ( 26 mg ).

**1** White powder , [  $\alpha$  ]<sub>D</sub><sup>25</sup> + 21.67 ( *c* 0.65 , C<sub>5</sub>H<sub>5</sub>N ) ; IR ( KBr )  $\nu$  : 3409 , 2942 , 1742 , 1632 , 1461 , 1305 , 1226 , 1082 , 1044 , 1014 , 913 , 854 , 801 cm<sup>-1</sup> ; Negative-ion FABMS *m/z* : 471 , 499 , 661 , 821 ; Negative-ion HRFABMS *m/z* : 821.4279 [ M - H ]<sup>-</sup> ( calcd for C<sub>43</sub>H<sub>65</sub>O<sub>15</sub> , 821.4323 ). EIMS *m/z* : 203 , 248 , 370 , 437 ; <sup>1</sup>H NMR , <sup>13</sup>C NMR and 2D data see Table 1 and Table 3.

**2** Transparent oil , [  $\alpha$  ]<sub>D</sub><sup>25</sup> - 79.35 ( *c* 0.95 , MeOH ) ; IR ( KBr )  $\nu$  : 3474 , 2941 , 1680 , 1342 , 1262 ,

1169 , 1082 , 1050 , 976 , 863 , 773 cm<sup>-1</sup> ; Negative-ion FABMS *m/z* : 193 , 285 , 321 , 339 , 387 , 515 ; Negative-ion HRFABMS *m/z* : 515.1959 [ M - H ]<sup>-</sup> ( calcd for C<sub>20</sub>-H<sub>35</sub>O<sub>15</sub> , 515.1976 ) ; <sup>1</sup>H NMR , <sup>13</sup>C NMR and 2D data see Table 2 and Table 3.

**3** Transparent oil , <sup>1</sup>H NMR ( C<sub>5</sub>D<sub>5</sub>N-*d*<sub>6</sub> , 400 MHz )  $\delta$  : 4.86 ( s , 2H , CH<sub>2</sub>O ) , 6.62 ( d , *J* = 3.60 Hz , 1H , H-3 ) , 7.32 ( d , *J* = 3.44 Hz , 1H , H-4 ) , 9.70 ( s , 1H , -CHO ) ; <sup>13</sup>C NMR ( C<sub>5</sub>D<sub>5</sub>N-*d*<sub>5</sub> , 100 MHz , TMS )  $\delta$  : 57.3 ( t ) , 109.8 ( d ) , 124.0 ( s ) , 152.9 ( s ) , 163.3 ( s ) , 177.8 ( d ) ; IR ( KBr )  $\nu$  : 3420 , 3214 , 1678 , 1592 , 1520 cm<sup>-1</sup> ; EI-MS : 126 ( M<sup>+</sup> ).

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