维普资讯 http://www.cqvip.com

Note

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

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

[a] *State Key Laboratory of Phytochemistry and Plant Resouces in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, Yunnan 650204, China*

[b] *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**), 6β-hydroxy-oleanolic acid 3-*O*-[β-*D*-mannopyranosyl (1-4), (2-*O*-3)] β-*D*-glucuronate · methyl · ester, a new trisaccharide derivative (**2**), α-methyl-*L*-bis [α-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 $C_{43}H_{66}O_{15}$ was determined by negative-ion HRFABMS spectrum (calcd 821.4279, found [M − 1]$^-$ 821.4323) and NMR spectrum. The IR spectrum showed absorption bands at 3408, 1741, 1632 and 918 cm^{-1}, 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 Δ^{12} double bond.[1] Two fragments at 661 [M-glc-3H]$^-$ and 471 [M-glc-(6-carbomethoxy)glc-3H]$^-$ in the negative-ion FABMS showed that **1** was a saponin with two glycosyl groups. This was also supported by two anomeric proton singnals (δ 4.97 and 5.22) in ^{1}H NMR spectrum. ^{1}H NMR and ^{13}C NMR spectra of aglycone of **1** were similar to those of 6α-hydroxyl-3-epi-oleanolic acid except for the fact that the chemical shifts of C-3 and C-7 were upfielded to δ 89.6 and 41.0 from δ 76.1 and 36.2, respectively (The value from the literature was recorded in CD_3OD).[2] This was explained by the β 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 δ 4.76 (H-6) and the broad doublet at δ 3.30 (J = 7.2 Hz, H-3) also supported the β configurations. These ^{1}H NMR and ^{13}C NMR spectral data of aglycone were assigned by 2D NMR spectrum as shown in Table 1, which indicated **1** to be 6β-hydroxyl-oleanolic acid with two glycosyl groups. The ^{13}C NMR spectrum of sugar moiety of **1** shows 13 carbon atoms. Distortionless enhancement by polarization transfer (DEPT) spectrum divided these signals as 1 × CH_3, 1 × CH_2, 9 × CH, 2 × C (Table 1). The positions of C and H were assigned by ^{1}H-^{1}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 δ 5.22 (H-1″) with δ 4.99 (H-3′) and δ 4.60 (H-5′). Therefore, the structure **a** was the only correct one. The long-rang coupling of the quaternary carbon signal at δ 93.9 (C-2″) with the proton signal at δ 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 6β-hydroxy-oleanolic acid 3-*O*-[β-*D*-mannopyranosyl (1-4), (2-*O*-3)]β-*D*-glucuronate · methyl · ester (Fig. 1).

* E-mail: luoshide@hotmail.com; Tel.: 86-871-5223097; Fax: 86-871-5223038
Received December 31, 2002; revised and accepted August 7, 2003.

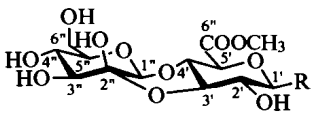
维普资讯 http://www.cqvip.com

Table 1 ^{13}C NMR and ^{1}H NMR data (δ) of compound **1**

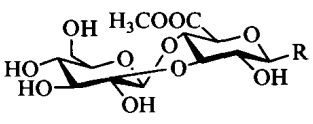
Position	δ_C	δ_H	Position	δ_C	δ_H
1	40.7 (t)	0.93—0.95 (m), 1.46—1.48 (m)	23	28.1 (q)	1.42 (s)
2	26.8 (t)	2.00—2.02 (m), 2.11—2.14(m)	24	18.6 (q)	1.60 (s)
3	89.6 (d)	3.30 (brd, J = 7.2 Hz)	25	17.1 (q)	1.50 (s)
4	41.3 (s)		26	18.7 (q)	1.55 (s)
5	56.3 (d)	0.87 (brs)	27	26.4 (q)	1.31 (s)
6	67.4 (d)	4.76 (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)	$COOCH_3$	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) **a**
22	33.4 (t)	1.81—1.83 (m), 2.03—2.05 (m)			4.50 (d, J = 11.7 Hz) **b**

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

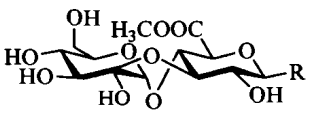
H	^{1}H-^{1}H COSY	HMBC	ROESY
1′	H-2′	C-3, C-2′, C-5′	H-3′, H-5′, H-23
2′	H-1′	C-1′, C-3′	H-4′
3′	H-2′	C-1′, C-2′, C-4′, C-2″	H-1′, H-5′, H-1″
4′	H-3′, H-5′	C-3′, C-5′, C-1″	H-2′
5′	H-4′	C-1′, C-3′, C-4′, C-6′	H-1′, H-3′, H-1″
$COOCH_3$		C-6′	
1″		C-4′, C-2″, C-5″	H-3″, H-5″, H-3′, H-5′
3″	H-4″	C-2″, 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″ **a**	H-5″, H-6″ **b**	C-5″	
6″ **b**	H-5″, H-6″ **a**	C-4″	



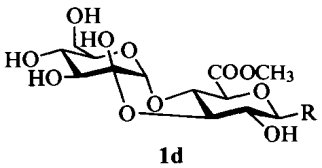
1a



1b



1c



1d

R=

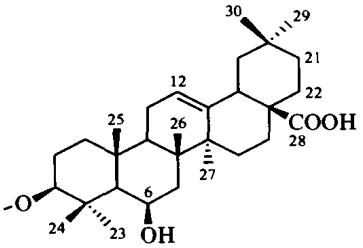


Fig. 1 Structure of compound **1** (Sugar = **1a**).

维普资讯 http://www.cqvip.com

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 cm^{-1} in the IR spectrum was indicative of hydroxyl groups. The ^{13}C NMR spectrum of **2** showed the presence of 20 carbon atoms. DEPT spectrum divided these signals as $3\times CH_3$, $5\times CH_2$, $10\times CH$ and $2\times C$ (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 ^1H-^1H COSY, HMBC and ROESY spectra (Table 4). The position of the glycoside bond was also established by using HMBC experiment. Compared the ^{13}C spectral data of **2** with the values of α and β *D*-fructose,[3] from the fructose of **2**, it was suggested to be α configuration. This configuration was confirmed by the obvious interaction between δ 4.31 (H-1′) and δ 4.88 (H-5′) in ROESY. The β configuration of arabinose moiety of **2** was confirmed by comparing its ^{13}C spectral data with those of *L*-methyl-arabinofuranose[4] and by analyzing the coupling constant values (Table 2). These coupling constant values also decided α or β configurations of other protons attached to sugars. Therefore, the structure of **2** was established as α-methyl-*L*-bis[α-methyl-*D*-fructopyranosyl(1-2),(1-3)]-arabinofuranose (Fig. 2).

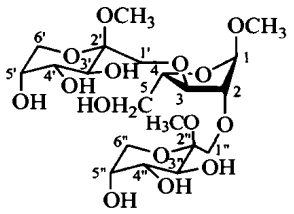


Fig. 2 Structure of compound **2**.

Compound **3** was identified to be 5-hydroxymethy-2-furancarboxaldehyde by comparing its ^{13}C NMR spectral data with previously published values.[5] 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. ^1H NMR (500 MHz), ^{13}C NMR (125 MHz) and 2D NMR spectra were recorded in pyridine-d_5 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}C NMR and ^1H NMR spectral data (δ) of compound **2**

Position	δ_C	δ_H	Position	δ_C	δ_H
1	111.2 (d)	5.33 (brs)	1′, 1″	63.7 (t)	4.16 (d, J = 11.3 Hz) **a**; 4.31 (d, J = 11.9 Hz) **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_3	55.3 (q)	3.41 (s)	6′, 6″	65.0 (t)	3.89 (d, J = 11.8 Hz) **a**; 4.04 (d, J = 11.9 Hz) **b**
			OCH_3′	48.7 (q)	3.40 (s)

Table 4 ^1H-^1H COSY, HMBC and ROESY spectral data of compound **2**

H	^1H-^1H COSY	HMBC	ROESY
1		C-2, C-4, OCH_3 (55.3)	OCH_3, H-2
2		C-1, C-3	H-1
3		C-2, C-4	
4		C-1, C-2, C-3, C-5	
OCH_3		C-1	H-1
H-1′ **a**		C-2′, C-3′	OCH_3′, H-1′ **b**, H-3′
H-1′ **b**		C-2′, C-3′, C-3, (H-1″ **b** with C-2)	OCH_3′, H-1′ **a**, H-3′
H-3′	H-4′	C-1′, C-2′, C-4′, OCH_3(48.7)	H-1′ **a** and **b**,
H-4′	H-3′, H-5′	C-3′, C-5′	H-6′ **a**, H-5′
H-5′	H-4′	C-4′, C-6′	H-6′ **a**, H-4′
H-6′ **a**	H-6′ **b**	C-2′, C-5′	OCH_3′, H-6′ **b**, H-5′
H-6′ **b**	H-6′ **a**	C-2′, C-5′	H-4′, H-6′ **a**, H-5′
OCH_3′		C-2′	H-1′ **a** and **b**, H-6′ **a**

维普资讯 http://www.cqvip.com

gel, FUJI (ODS-Q3) gel (Mitsubishi Chemical Co.) and Lobar RP-C18 gel (Merck) using the following solvent systems: $CHCl_3$-MeOH-H_2O and MeOH-H_2O, 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 $CHCl_3$-MeOH-H_2O [from $CHCl_3$ to $CHCl_3$-MeOH-H_2O (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_2O (60:40, *V*:*V*)] to give compounds **1** (21 mg), **2** (81 mg) and **3** (26 mg).

1 White powder, $[\alpha]_D^{25.2}$ + 21.67 (*c* 0.65, C_5H_5N); IR (KBr) ν: 3409, 2942, 1742, 1632, 1461, 1305, 1226, 1082, 1044, 1014, 913, 854, 801 cm^{-1}; Negative-ion FABMS *m/z*: 471, 499, 661, 821; Negative-ion HRFABMS *m/z*: 821.4279 $[M-H]^-$ (calcd for $C_{43}H_{65}O_{15}$, 821.4323). EIMS *m/z*: 203, 248, 370, 437; ^1H NMR, ^{13}C NMR and 2D data see Table 1 and Table 3.

2 Transparent oil, $[\alpha]_D^{25.2}$ − 79.35 (*c* 0.95, MeOH); IR (KBr) ν: 3474, 2941, 1680, 1342, 1262, 1169, 1082, 1050, 976, 863, 773 cm^{-1}; Negative-ion FABMS *m/z*: 193, 285, 321, 339, 387, 515; Negative-ion HRFABMS *m/z*: 515.1959 $[M-H]^-$ (calcd for $C_{20}H_{35}O_{15}$, 515.1976); ^1H NMR, ^{13}CNMR and 2D data see Table 2 and Table 3.

3 Transparent oil, ^1H NMR (C_5D_5N-d_6, 400 MHz) δ: 4.86 (s, 2H, CH_2O), 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); ^{13}C NMR (C_5D_5N-d_5, 100 MHz, TMS) δ: 57.3 (t), 109.8 (d), 124.0 (s), 152.9 (s), 163.3 (s), 177.8 (d); IR (KBr) ν: 3420, 3214, 1678, 1592, 1520 cm^{-1}; EI-MS: 126 (M^+).

Acknowledgements

The authers are grateful to the members of analytical group of State Key Laboratory of Phytochemistry and Plant Resouces in West China, Kunming Institute of Botany, for the spectral measurements.

References

1 Djerassi, C.; Budzikiewicz, H.; Wilson, J. M. *J. Am. Chem. Soc.* **1985**, 3688.
2 Akhtar, N.; Malik, A. *Phytochemistry* **1993**, *32*, 1523.
3 Stephen, J.; Angyal, S.; Geoffrey, S. *Aust. J. Chem.* **1976**, *29*, 1249.
4 Yu, D. Q.; Yang, J. S. *The Handbook of Analisis Chemistry*, 2 edn., **1999**, *7*, 902.
5 Shen, Y. M.; Mu, Q. Z. *Planta Med.* **1990**, *56*, 472.

(E0212312 SONG, J. P.; LU, Z. S.)