

Three novel pregnane glycosides from *Epigynum auritum*

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Abstract—Three novel pregnane glycosides with an unusual aglycone (Epigynumgenane), named Epigynosides A (**1**), B (**2**) and C (**3**), were isolated from the aerial part of *Epigynum auritum*. Their structures were elucidated by spectral means and confirmed by X-ray diffraction analysis.

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1. Introduction

Epigynum Wight (Apocynaceae) is a genus including about 14 species. Among them, there is only one—*E. auritum* distributed in China. Its unique taxonomic position attracted us to investigate its chemical constituent. This paper deals with the isolation and structural elucidation of three novel pregnane glycosides, Epigynosides A (**1**), B (**2**) and C (**3**), with an unusual aglycone (Epigynumgenane).

2. Results and discussion

Epigynoside A (**1**) was isolated as a white powder. Its molecular formula $C_{28}H_{46}O_8$ was established by ^{13}C NMR (Table 1) and negative-ion HRFABMS data. ^{13}C NMR and HMQC-TOCSY exhibited signals for a seven-carbon glycosyl group and an aglycone formed with 21 carbons. The structure of sugar moiety of **1** was deduced by the correlations between H-2' (δ_H 3.72) with H-1' (δ_H 5.46) and H-3' (δ_H 4.64), H-3' with H-4' (δ_H 3.77) and H-2', H-5' (δ_H 4.45) with H-6' (δ_H 1.51) in 1H - 1H COSY spectrum. The HMBC spectrum showing the cross peak of OMe-H (δ_H 3.60) with C-2' (δ_C 81.6), indicated that the methoxyl was attached to C-2'. The stereochemistry of the sugar moiety was deduced by the correlations between H-1' with H-5' in ROESY spectrum and confirmed by the X-ray diffraction analysis. So, the structure of the sugar was deduced to be 2-*O*-methyl-6-deoxy- β -D-idopyranose (Fig. 1).¹ The linkage

of the sugar moiety to the aglycone was decided by the cross peak of H-1' with C-3 in HMBC spectrum. ^{13}C NMR and DEPT spectra of the aglycone showed the presence of one olefinic bond, three methyl groups, eight methylenes (one of them was oxygenated), five methines (two of them were oxygenated) and three quaternary carbons in which one was in very low-field (δ_C 101.1). The chemical shifts of the aglycone carbons were similar to that of pregn-5-ene-3 β ,17 α ,20*S*-triol except for ring-D.² The special form of ring-D was deduced by the obvious correlations between the low-field quaternary carbon (δ_C 101.1) with H-16 (δ_H 3.75), H-20 (δ_H 4.18), H-21 (δ_H 1.48) and H-18 (δ_H 1.15) in HMQC-TOCSY spectrum. And this was supported by the correlations between H-15 (δ_H 1.37) with H-14 (δ_H 1.99) and H-16 (δ_H 4.17), H-21 (δ_H 1.48) with H-20 (δ_H 4.18) in 1H - 1H COSY spectrum. The ROESY spectrum showing crossing signals between H-8 (δ_H 1.37) with H-18 (δ_H 1.15) and H-19 (δ_H 0.95), H-9 (δ_H 1.02) with H-14 (δ_H 1.99), and H-20 (δ_H 4.18) with H-18 (δ_H 1.15), indicated the β , α , and α configurations for H-8, H-14 and OH-17, respectively. Finally, the configuration of position-20, which could not be explained by ROESY spectrum, and all the inference about **1** was determined by X-ray diffraction analysis (Fig. 2). Therefore, the structure of the aglycone of **1** was elucidated as a novel pregnane, named Epigynumgenane. And the structure of **1** was decided to be Epigynumgenane 3-*O*-2'-*O*-methyl-6'-deoxy- β -D-idopyranoside.

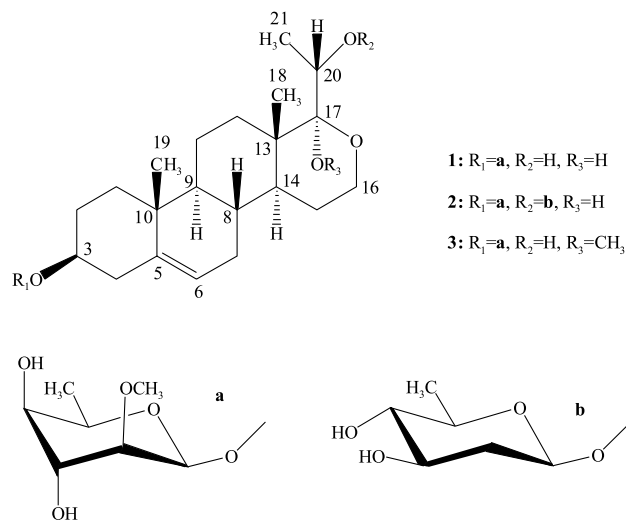
Epigynoside B (**2**) was isolated as a white powder. Its molecular formula $C_{34}H_{56}O_{11}$ was established through ^{13}C NMR and negative-ion HRFABMS data. Comparison of the 1H and ^{13}C NMR spectral data of **2** (Table 1) with that of **1**, revealed that they were similar except for one more sugar in

Keywords: *Epigynum auritum*; Epigynoside; Sugar moiety.

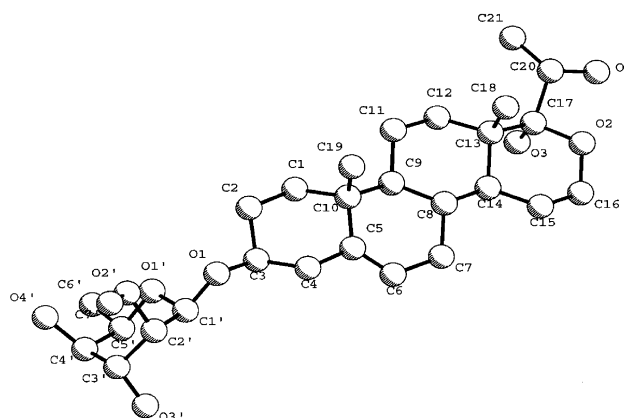
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Table 1. ^{13}C and ^1H NMR data of compounds **1–3** (with SiMe_4 as internal standard; δ in ppm, J in Hz)

Position	1 (δ_{C})	1 (δ_{H})	2 (δ_{C})	2 (δ_{H})	3 (δ_{C})	3 (δ_{H})
1	37.2 (t)	0.99–1.02 (m), 1.75–1.77 (m)	37.2 (t)	1.02–1.06 (m), 1.84–1.88 (m)	37.4 (t)	0.93–0.97 (m), 1.72–1.76 (m)
2	30.2 (t)	1.77–1.81 (m), 2.10–2.14 (m)	30.2 (t)	1.73–1.77 (m), 2.10–2.14 (m)	30.4 (t)	1.75–1.79 (m), 2.12–2.16 (m)
3	77.8 (d)	3.82–3.86 (m)	77.8 (d)	3.80–3.84 (m)	78.1 (d)	3.86–3.90 (m)
4	39.1 (t)	2.38 (dd, $J=11.2$, 2.4 Hz); 2.57 (dd, $J=10.5$, 2.4 Hz)	39.1 (t)	2.36 (dd, $J=11.2$, 2.4 Hz); 2.55 (dd, $J=10.5$, 2.4 Hz)	39.3 (t)	2.38 (dd, $J=11.2$, 2.4 Hz); 2.58 (dd, $J=11.2$, 2.9 Hz)
5	140.5 (s)		140.5 (s)		140.8 (s)	
6	121.9 (d)	5.36 (br, s)	121.9 (d)	5.37 (br, s)	122.1 (d)	5.45 (br, s)
7	32.0 (t)	1.48–1.52 (m), 2.03–2.07 (m)	32.0 (t)	1.48–1.52 (m)	32.2 (t)	1.50–1.54 (m), 2.02–2.06 (m)
8	31.7 (d)	1.35–1.39 (m)	31.7 (d)	1.38–1.42 (m)	32.1 (d)	1.21–1.25 (m)
9	49.7 (d)	1.01–1.05 (m)	49.6 (d)	0.97–1.01 (m)	49.6 (d)	0.96–1.00 (m)
10	37.2 (s)		37.2 (s)		37.5 (s)	
11	21.1 (t)	1.30–1.34 (m), 1.47–1.51 (m)	20.5 (t)	1.36–1.4 (m), 1.53–1.57 (m)	20.8 (t)	1.14–1.18 (m), 1.50–1.54 (m)
12	33.4 (t)	1.80–1.84 (m), 1.90–1.94 (m)	33.2 (t)	1.55–1.59 (m), 1.90–1.94 (m)	33.8 (t)	1.72–1.76 (m)
13	40.5 (s)		40.9 (s)		41.6 (s)	
14	43.7 (d)	1.97–2.01 (m)	43.7 (d)	1.99–2.01 (m)	43.3 (d)	1.80–1.84 (m)
15	25.4 (t)	1.35–1.39 (m)	25.2 (t)	1.28–1.32 (m)	25.1 (t)	1.33–1.37 (m)
16	61.1 (t)	3.73–3.77 (m), 4.15–4.19 (m)	60.9 (t)	3.78–3.82 (m), 4.18–4.22 (m)	62.7 (t)	3.79–3.83 (m)
17	100.7 (s)		101.0 (s)		102.1 (s)	
18	15.1 (q)	1.15 (s)	15.3 (q)	1.15 (s)	17.3 (q)	1.02 (s)
19	19.8 (q)	0.95 (s)	20.2 (q)	0.95 (s)	19.5 (q)	0.92 (s)
20	70.5 (d)	4.16–4.20 (m)	79.6 (d)	4.23 (q, $J=6.3$ Hz)	70.5 (d)	4.23 (q, $J=6.3$ Hz)
21	20.6 (q)	1.48 (d, $J=6.3$ Hz)	19.3 (q)	1.48 (d, $J=6.3$ Hz)	20.0 (q)	1.51 (d, $J=6.0$ Hz)
17-OMe					51.1 (q)	3.53 (s)
1'	98.0 (d)	5.46 (br, s)	98.0 (d)	5.45 (br, s)	98.2 (d)	5.48 (br, s)
2'	81.6 (d)	3.70–3.74 (m)	81.5 (d)	3.71 (d, $J=3.2$ Hz)	81.7 (d)	3.73–3.77 (m)
3'	69.5 (d)	4.64 (br, s)	69.4 (d)	4.63 (br, s)	69.5 (d)	4.68 (br, s)
4'	72.8 (d)	3.77 (br, s)	72.7 (d)	3.81 (br, s)	72.9 (d)	3.80 (br, s)
5'	71.3 (d)	4.43–4.47 (m)	71.2 (d)	4.43 (q, $J=8.2$ Hz)	71.5 (d)	4.43–4.47 (m)
6'	17.3 (q)	1.51 (d, $J=6.3$ Hz)	17.2 (q)	1.53 (d, $J=6.8$ Hz)	17.5 (q)	1.52 (d, $J=6.3$ Hz)
2'-OMe	60.0 (q)	3.60 (s)	60.0 (q)	3.60 (s)	60.2 (q)	3.61 (s)
1''			102.6 (d)	5.21 (d, $J=8.8$ Hz)		
2''			40.6 (t)	2.00–2.04 (m), 2. 65–2.69 (m)		
3''			72.1 (d)	4.03–4.07 (m)		
4''			78.6 (d)	3.53 (t, $J=8.8$ Hz)		
5''			72.9 (d)	3.63–3.37 (m)		
6''			18.8 (q)	1.62 (d, $J=6.0$ Hz)		

**Figure 1.** Structural formula of **1**, **2** and **3**.

2. The structure of this sugar was deduced by the correlations of the anomeric proton signal at δ 5.21 (H-1'') with C-2'' (δ_{C} 40.6), C-3'' (δ_{C} 72.1), C-4'' (δ_{C} 78.6), C-5'' (δ_{C} 72.9) and C-6'' (δ_{C} 18.8) in HMQC-TOCSY spectrum. The ^1H – ^1H COSY spectrum showed the correlations between

**Figure 2.** Crystal structure of **1** (hydrogens deleted).

H-2'' (δ_{H} 2.67) with H-3'' (δ_{H} 4.05) and H-1'', H-4'' (δ_{H} 3.53) with H-3'' and H-5'' (δ_{H} 3.65), H-5'' with H-6'' (δ_{H} 1.62). The relative stereochemistry of the sugar was assigned from the ROESY correlations of H-1'' with H-3'' and H-5'' and supported by the coupling constants in ^1H NMR spectrum (Table 1). Accordingly, the sugar was elucidated as 2,6-dideoxy- β -D-arabo-hexose (olivose).³ The linkage of the sugar moiety to the aglycone was decided by the cross peak of H-1'' with C-20 (δ_{C} 79.6) in HMBC spectrum. Therefore, **2** was decided to be Epigynumgenane 3-*O*-2'-methyl-6'-deoxy- β -D-idopyranoside-20-*O*- β -D-olivopyranoside.

Epigynoside C (**3**) was isolated as a white powder. Its molecular formula $\text{C}_{29}\text{H}_{48}\text{O}_8$ was established through ^{13}C NMR and negative-ion HRFABMS data. Comparison of the ^1H and ^{13}C NMR spectral data of **3** (Table 1) with that of **1**, revealed that they were similar except for one more methoxyl in **3**. The linkage of the methoxyl to C-17 was decided by the cross peak of OMe-H (δ_{H} 3.53) with C-17 (δ_{C} 102.1) in HMBC spectrum. Therefore, **3** was decided to be 17-*O*-methyl-epigynumgenane 3-*O*-2'-methyl-6'-deoxy- β -D-idopyranoside.

3. Experimental

3.1. General experimental procedures

Optical rotations were measured with a Horiba SEAP-300 spectropolarimeter. IR (KBr) spectra were obtained on a Bio-Rad FTS-135 infrared spectropolarimeter. ^1H , ^{13}C NMR and 2D NMR spectra were recorded on a DRX-500 MHz NMR spectrometer with TMS as the internal standard. MS spectral data were obtained on a VG Autospec-3000 spectrometer. Si gel (200–300 mesh) for column chromatography and GF₂₅₄ for TLC were obtained from the Qindao Marine Chemical Factory, Qindao, People's Republic of China. Macroporous resin D1300 was obtained from the Bengbu Liaoyuan Resin Factory, Bengbu, People's Republic of China. Sephadex LH-20 was obtained from Pharmacia Co. FUJI (ODS-Q₃) gel was obtained from Mitsubishi Chemical Co.

3.2. Plant material

The aerial part of *E. aurilum* was collected from Xishuangbanna, Yunnan Province, People's Republic of China, in September 1999. It was identified by Professor Yang, Z. H., Department of Taxonomy, Kunming Institute of Botany, Academia Sinica, Kunming, People's Republic of China.

3.3. Extraction and isolation

The dried aerial part of *E. aurilum* (14 kg) was extracted three times with EtOH under reflux. After removal of the solvent in vacuo, the residue (1.3 kg) was partitioned in H_2O and extracted with petroleum ether, AcOEt, and *n*-BuOH three times respectively. The AcOEt fraction (138 g) was chromatographed over macroporous resin D1300. The column was eluted with EtOH– H_2O (0:100–95:5) to give four fractions. Fraction 3 (50 g) was repeatedly chromatographed over silica gel using CHCl_3 –MeOH and petroleum

ether– Me_2CO as eluent to give 12 fractions. The fraction 1 (114 mg) and 2 (30 mg) were chromatographed on Sephadex LH-20 eluted by MeOH to yield **1** (67 mg) and **3** (10 mg), respectively. The fraction 3 (367 mg) was chromatographed on FUJI (ODS-Q₃) gel using MeOH– H_2O (80:20) to yield **2** (63 mg).

3.3.1. Compound 1. White powder; $[\alpha]_{\text{D}}^{22} -105.0$ (*c* 0.3, $\text{C}_5\text{H}_5\text{N}$), IR (KBr) ν_{max} : 3459, 2939, 1630, 1446, 1326, 1268, 1176, 1107, 1075, 1047 cm^{-1} ; ^1H and ^{13}C NMR spectral data, see Table 1; FABMS m/z : 509 ($\text{M}-\text{H}$)[−] (100); HRFABMS m/z 509.3081 ($\text{M}-\text{H}$)[−] (Calcd for $\text{C}_{28}\text{H}_{45}\text{O}_8$ 509.3114).

3.3.2. Compound 2. White powder; $[\alpha]_{\text{D}}^{25} -124.0$ (*c* 0.5, $\text{C}_5\text{H}_5\text{N}$), IR (KBr) ν_{max} : 3436, 2938, 1630, 1446, 1326, 1268, 1176, 1067, 1047 cm^{-1} ; ^1H and ^{13}C NMR spectral data, see Table 1; FABMS m/z : 639 ($\text{M}-\text{H}$)[−] (100), 509 (20); HRFABMS m/z 639.3751 ($\text{M}-\text{H}$)[−] (Calcd for $\text{C}_{34}\text{H}_{55}\text{O}_{11}$ 639.3744).

3.3.3. Compound 3. White powder; $[\alpha]_{\text{D}}^{23} -56.0$ (*c* 0.3, $\text{C}_5\text{H}_5\text{N}$), IR (KBr) ν_{max} : 3443, 2933, 1733, 1669, 1457, 1176, 1067, 1047 cm^{-1} ; ^1H and ^{13}C NMR spectral data, see Table 1; FABMS m/z : 523 ($\text{M}-\text{H}$)[−] (45), 509 (90), 615 ($[\text{M}-\text{H}+\text{Gly}]$)[−] (100); HRFABMS m/z 523.3282 ($\text{M}-\text{H}$)[−] (Calcd for $\text{C}_{29}\text{H}_{47}\text{O}_8$ 523.3271).

3.4. X-ray crystal structure analysis of **1**

A colorless block crystal of dimension $0.10 \times 0.20 \times 0.30 \text{ mm}^3$ was used for data collection. Crystallographic data: $\text{C}_{28}\text{H}_{46}\text{O}_8 \cdot \text{CH}_3\text{COOC}_2\text{H}_5 \cdot (\text{H}_2\text{O})_2$, $M = 665.73$, orthorhombic, $P2_12_12$, $a = 43.798(2)$, $b = 11.221(1)$, $c = 6.985(1) \text{ \AA}$, $V = 3432.8(6) \text{ \AA}^3$, $Z = 4$, $d = 1.233 \text{ g cm}^{-3}$. Intensity data were collected on MAC Science DIP-2030K diffractometer with a graphite monochromator, Mo $K\alpha$ radiation, ω -2 θ scans and $2\theta_{\text{max}} = 50.0^\circ$. The total number of independent reflections measured was 3709, of which 2591 were observed ($|F|^2 \geq 8|F|^2$). The crystal structure was solved by direct method (SHELXS-86) and expanded using difference Fourier techniques, refined by the program NOMCSDP and full-matrix least-squares calculations.⁴ Hydrogen atoms were fixed at calculated positions. The final indices were $R_{\text{f}} = 0.075$ and $R_{\text{w}} = 0.071$ ($w = 1/\sigma|F|^2$). Crystallographic data (excluding structure factors) for the structures in this paper, have been deposited with the Cambridge Crystallographic Data Center as supplementary publication number CCDC 267158. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).

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