A New *ent*-Kaurane Glycoside from the Stems of *Acanthopanax gracilistylus*

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Abstract: **Objective** To study the chemical constituents from the stems of *Acanthopanax gracilistylus*. **Methods** The chemical constituents of the plant were isolated and puried by column chromatography and their structures were elucidated on the basis of physicochemical properties and spectral data. **Results** A new *ent*-kaurane glycoside, named kaurane acid glycoside A｛16α,17-dihydroxy-*ent*-kauran-19-oic 19-[β-*D*-glucopyranosyl-(1→2)-β-*D*-glucopyranosyl] ester｝(**1**), was isolated from the *n*-butanol part. **Conclusion** Compound **1** is a new one.

Key words: *Acanthopanax gracilistylus*; Araliaceae; kaurane acid glycoside A
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Introduction

The plant *Acanthopanax gracilistylus* W. W. Smith belongs to Araliaceae, which is widely distributed in China and its dried roots are listed officially in the *Chinese Pharmacopoeia* as *Acanthopanacis Cortex* (named as *Wujiapi*) which has been used for the treatment of paralysis, arthritis, rheumatism, lameness, and liver disease (Pharmacopoeia Committee of P. R. China, 2005). Some diterpene derivatives from the root bark of *A. gracilistylus* have been isolated and identified (Liu Yook, and Chang, 2004). And some potential activities of these components have been studied (Liu *et al*, 2010). Sixteen known constituents have been isolated from the stems in previous reports (Xian, Qian, and Li, 2010). In connection with our interest in this plant, chemical study on this part was undertaken in our laboratory and a new *ent*-kaurane glycoside was isolated from the *n*-butanol part.

Materials and methods

Mass spectra were recorded on Waters Synapt Q-TOF spectrometer. ^{1}H-NMR (DMSO-d_6) was taken on Bruker ACF-500 MHz and ^{13}C-NMR (DMSO-d_6) spectra were taken on Bruker ACF-125 MHz, using TMS as internal standard. All solvents used were of analytical grade. Sephadex LH-20 (Pharmacia Biotech) and silica gel (200–300 mesh) were used for column chromatography, and precoated silica gel GF_{254} plates used for TLC (Qingdao Marine Chemical Company).

The seeds and stems of *A. gracilistylus* were collected in Xuyi Prefecture, Jiangsu Province in 2008 and identified by Prof. QIAN Shi-hui, Department of Resource of Traditional Chinese Medical Materials, Jiangsu Province Institute of Traditional Chinese Medicine.

The 90% and 60% ethanol extracts of the stems (4.0 kg) were concentrated and suspended in H_2O, then partitioned by petroleum ether, EtOAc, and *n*-BuOH. The *n*-BuOH fraction (55.0 g) was subjected to silica gel column chromatography, eluted with $CHCl_3$-MeOH gradiently, to yield 28 fraction. Fr 11 was applied to silica gel and Sephadex LH-20 column chromatography to afford compound **1** (75 mg).

Results

The compound was obtained as white powder. ESI-MS (*m/z* 659 $[M-H]^-$). The molecular formula $C_{32}H_{52}O_{14}$ was deduced by the consistent of ESI-MS, ^{1}H-NMR (300 MHz), and ^{13}C-NMR (75 MHz) spectrum data.

Signals of anomeric protons at δ 5.52 (J = 7.7 Hz) and 6.26 (J = 7.9 Hz) typical of an ester-linked β-hexose suggested that two different types of glycosidic linkage were presented (Harinantenaina, Kasai, and Yamasaki, 2002a). The ^{13}C-NMR and distortionless enhancement by polarization transfer (DEPT) spectra exhibited 32 carbon signals, 12 of which could be assigned to those of sugar

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units and the remaining 20 (five quaternaries, three methines, ten methylenes, and two methyl carbons) to a diterpenoid aglycone. ^{1}H-NMR and ^{13}C-NMR spectral data were in close agreement with those reported for 16α-hydroxy-*ent*-kauran-19-oic acid (Xiang and Xu, 1983) except for the chemical shift of the carboxyl group which was shifted upfield due to substitution.

And ^{1}H-NMR and ^{13}C-NMR data revealed the occurrence of two β-glucopyranoses as sugar residues. One β-glucose was directly bound to the carboxyl group at C-19 proven by the correlation of C-19 (δ 175.9) with the anomeric proton (H-1′, δ 6.26) in the HMBC spectrum. Moreover, long-range correlations were shown between C-2′ of glucose (δ 80.74) and the anomeric proton of another β-glucose (δ 5.52). Assignment of all sugar signals followed from HSQC, HMBC, and 1DTOCSY spectra (Table 1) and was in accordance with *Chemical Analysis Handbook* (Yu and Yang, 1999). The stereochemistry at C-16 was determined to be in an α-orientation as regards the hydroxyl group by comparison of the chemical shift values for C-16 and C-17 with those reported in the literature (Harinantenaina, Kasai, and Yamasaki, 2002b). For chemical shift values of C-16 hydroxylated *ent*-kauranes, Harinantenaina, Kasai, and Yamasaki (2002b) reported that the signals of C-16 with a β-hydroxyl group and C-17 appeared at δ 79.7 and 70.4 while those of C-16 with an α-hydroxyl group appeared at δ 81.6 and 66.4, respectively. The chemical shift values for C-16 and C-17 of this compound were δ 81.64 and 66.47, respectively, suggesting the α-orientation of the hydroxyl group at C-16. From the above data, the structure of the compound was unambiguously concluded to be 16α,17-dihydroxy-*ent*-kauran-19-oic 19-[β-*D*-glucopyranosyl-(1→2)-β-*D*-glucopyranosyl] ester (Fig. 1) which is described here for the first time.

Table 1 ^{1}H-NMR (300 MHz) and ^{13}C-NMR (75 MHz) spectroscopic data for compound 1 in pyridine-d_5

C		δ_C	δ_H	HMBC
1	α	40.66	0.75(1H,m)	H-20
	β		1.72(1H,m)	
2	α	19.97	1.41(1H,m)	
	β		2.15(1H,m)	
3	α	37.86	1.10(1H,m)	18
	β		2.75(1H,m)	
4		44.38		3α,5,18
5		57.49	1.04(1H,m)	18,20
6	α	22.38	1.95(1H,m)	7α
	β		2.16(1H,m)	
7	α	42.68	1.42(1H,m)	15β
	β		1.72(1H,m)	
8		44.84		9,14α,15α, β
9		56.09	0.97(1H,br d,8.1)	15α, β,20
10		39.92		9,11α, β,20
11	α	18.90	1.46(1H,m)	9
	β		1.60(1H,m)	
12	α	26.64	1.38(1H,m)	9,14α
	β		1.77(1H,m)	
13		45.80	2.38(1H,m)	14α, 15α, β,17α, β
14	α	37.86	2.0(1H,m)	15α
	β		2.70(1H,m)	
15	α	53.81	1.71(1H,d,14.0)	14α
	β		1.78(1H,d,14.0)	
16		81.64		14α,15β,17α, β
17	α	66.47	4.04(1H,d,10.8)	15α
	β		4.10(1H,d,10.8)	
18		29.28	1.41(3H,s)	
19		175.90		5,18, 1′
20		16.71	1.04(3H,s)	5,9
1′		93.49	6.26(1H,d,7.9)	H-2′,5′α, β
2′		80.74	4.41(1H,m)	H-1″
3′		78.48		H-2′
4′		70.87		H-2′, 3′
5′		79.12	3.94(2H,m)	
6′α, β		62.18	4.34-4.40(2H,m)	
1″		105.50	5.52(1H,d,7.7)	H-2′,2″,5″α, β
2″		76.26	4.02(1H,m)	
3″		78.52		
4″		71.97		
5″		78.18	4.22(2H,m)	
6″α, β		63.03	4.54-4.56(2H,m)	

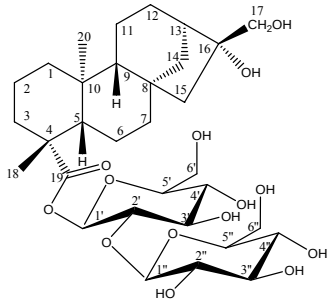

Fig. 1 Structure of compound 1

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