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TRITERPENE SAPONINS FROM *CRANIOTOME FURCATA*

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Three saponins, craniosaponin A (**1**) and buddlejasaponins Ia (**2**) and I (**3**) were isolated from the *n*-butanol soluble fraction of *Craniotome furcata* for the first time. Among them, craniosaponin A (**1**) was identified to be a new compound. The structure of craniosaponin A was assigned mainly by spectral methods. A preliminary assay *in vitro* was applied to evaluate their cytotoxicity against several tumor cell-lines.

Keywords: *Craniotome furcata*; Labiatae; Craniosaponin A; Buddlejasaponins I and Ia

INTRODUCTION

Craniotome furcata (Link.) O. Kuntze, the only species of the genus *Craniotome* (Labiatae), is herbaceous plant and is mainly distributed in Yunnan and Shichuan provinces of China [1]. The chemical components of this plant have not been investigated before. In an effort to identify the compounds with chemical diversity and bioactivity, an initial chemical research of the aerial parts of *C. furcata* collected from Lijiang of Yunnan province was carried out. A new triterpene saponin, craniosaponin A (**1**), together with two known saponins, buddlejasaponins Ia (2) [3] and I (**3**) [2], was identified from the *n*-butanol soluble fraction of *C. furcata*. We herein report the isolation and structure elucidation of the triterpene saponins.

RESULTS AND DISCUSSION

Compound **1** was obtained as white amorphous powder. It revealed an $[M + Na]^+$ ion peak at m/z 1129.6 in the positive mode ESIMS. It also exhibited a quasi-molecular ion peak at m/z 1105.6 $[M - H]^-$ in the negative mode ESIMS, together with its significant

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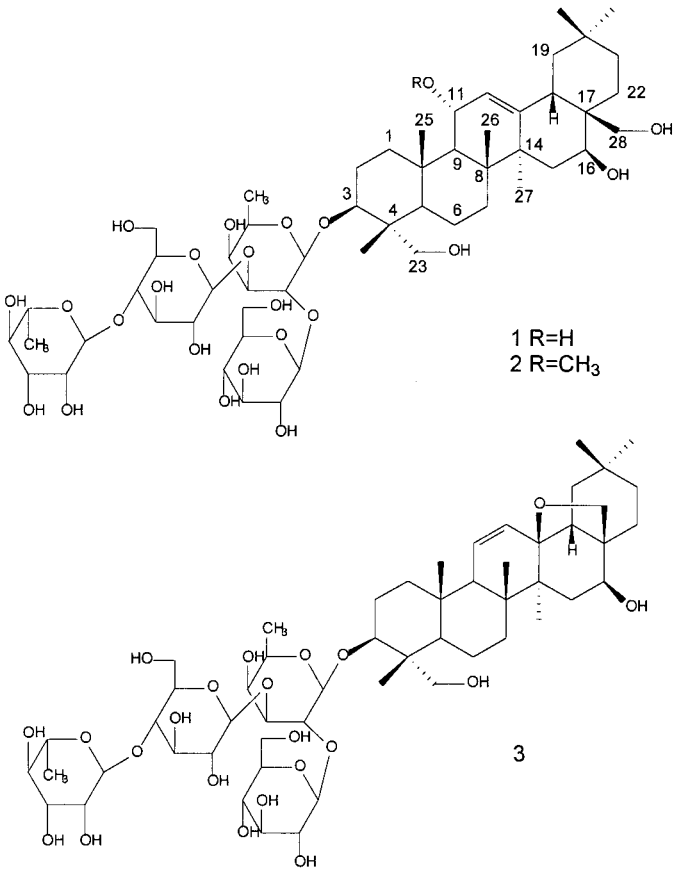

FIGURE 1 Structures of compounds **1**–**3**.

fragments at *m/z* 959.4 $[M-H-146]^-$ 943.5 $[M-H-162]^-$, 797.2 $[M-H-146-162]^-$, 779.3 $[M-H-146-162-H_2O]^-$, 635.3 $[M-H-146-162-162]^-$ and 471.2 $[M-H-146-162-162-146-H_2O]^-$. In the ^{13}C-NMR spectrum (Table II), 54 carbon signals observed are 8 methyls, 12 methylenes (4 of which were oxygenated), 27 methines (23 of which were oxygenated) and 7 quartenary carbons. On the basis of above mentioned MS and NMR spectra data, compound **1** was supposed to be a saponin with the molecular formula of $C_{54}H_{90}O_{23}$, bearing a chain of four sugar moieties.

The ^{1}H- and ^{13}C-NMR spectral data of sugar moiety of compound **1** were in good agreement with those of compound **2** [3], suggesting that the compounds **1** and **2** have the same sugar linkage. This was supported by the very similar fragmentation patterns of two compounds in their ESIMS. The fragments at *m/z* 959.4 $[M-H-146]^-$, 943.5 $[M-H-162]^-$ and 797.2 $[M-H-146-162]^-$ showed the same two terminal sugar moieties of rhamnose and glucose, and the same cleavage of sugar sequence of rhamnose/glucose-glucose-fucose was also clearly showed in the ESIMS. The corresponding molecular formulas for the sugar moiety and triterpene aglycone were made up to be $C_{24}H_{42}O_{19}$ and $C_{30}H_{50}O_5$, respectively. The degree of unsaturation for the aglycone was thus determined to be six.

A double bond should be existed in the aglycone of compound **1** according to the olefinic proton at δ_H 5.66 ($d, J = 3.7$ Hz), and two carbons at δ_c 128.21 (methine) and 145.35 (quartenary). The rest five degrees of unsaturation were accounted for the five rings of the triterpene aglycone. Then two hydroxymethylenes and three hydroxymethines in the aglycone were distinguished according to the DEPT experiment and the chemical shifts of carbons at δ_c 65.09, 68.73 (methylenes), 66.47, 66.78 and 83.13 (methines). Analyses of ^{1}H- and ^{13}C-NMR spectra data of compound **1** indicated that the aglycone moiety of compound **1** was very similar to that of compound **2**, the only difference occurred at C-11 position in the C-ring. A hydroxyl group was thus assigned to the C-11 in compound **1** instead of a methoxyl group at the C-11 in compound **2**. The stereochemistry of hydroxyl group was attributable to α-configuration on the basis of the coupling patterns and coupling constants of H-9 at δ_H 2.08 ($d, J = 8.5$ Hz), H-11 at δ_H 4.53 ($dd, J = 3.7, 8.5$ Hz) and H-12 at δ_H 5.66 ($d, J = 3.7$ Hz), which were consistent with those of compound **2** [3].

On the basis of above evidences and the analyses of its HMQC, HMBC and TOCSY spectra, the structure of compound **1** was identified to be ($3\beta, 4\alpha, 11\alpha, 16\beta$)-11, 16, 23, 28-terthydroxy-olean-12-en-3-O-[6-deoxy-α-L-mannopyranosyl-(1→4)-*O*-β-*D*-glucopyransyl-(1 → 3)-*O*-[β-*D*-glucopyranosyl-(1→2)]-6-deoxy-β-*D*-galactopyranoside, namely craniosaponin A.

Compounds **2** and **3** were respectively identified to be buddlejasaponins Ia and I by the analyses of their ^{1}H-, ^{13}C-NMR and ESIMS spectral data [2,3].

Three saponins were tested for their cytotoxicity with microculture tetrazolium assay (MTT) and the data were analyzed with flow cytometry [4]. Only compound **3**, buddlejasaponin I, was showed significant activities of IC_{50} at 8.1×10^{-7} and 1.9×10^{-6} mol/L against P388 and A-549 tumor cell-lines, respectively.

EXPERIMENTAL

General

IR spectra were measured on a Nicolet Magna 750 spectrometer with KBr discus. NMR spectra were obtained on a Brucker AM-400 MHz spectrometer. General ^{1}H-NMR data were run at 400 MHz, and ^{13}C NMR were measured at 100.6 MHz. Chemical shifts are expressed in ppm relative to TMS. ESIMS were recorded with a Finnigan LCQDECA Mass spectrometer.

Plant Material

The whole plant of *C. furcata* (Link.) O. Kuntze was collected at Lijiang of Yunnan province of China, and was identified by Professor Zhong-Wen Lin in Kunming Institute of Botany, The Chinese Academy of Sciences, where a voucher specimen is deposited.

Extraction and Isolation

The air-dried powdered whole herb of *C. furcata* (2.5 kg) was extracted three times with 95% EtOH at room temp. The extract was evaporated to dryness under reduced

pressure and the residue (160 g) was obtained. The crude extract was dissolved in 300 mL methanol and decolorized on a MCI GEL CHP20P column to obtain 147 g of brown syrup. It was then dissolved in water (2000 mL) and the aqueous suspension was extracted with ethyl acetate and *n*-butanol to give fractions *E* (21 g) and *N* (56 g), respectively. The aqueous solution was evaporated to dryness under reduced pressure to afford fraction *W* (71 g).

Part *N* (55 g) was chromatographed on a column of silica gel (400 g, 200–300 mesh) eluting with chloroform–methanol (4 : 1, 3 : 1, 2 : 1, 1 : 1 and methanol) to give fractions 1–13. The fraction 2 was extensively chromatographed on a C-18 silica gel column to yield craniosaponin A (**1**) (40 mg). The 9–11 fractions were combined and rechromatographed on C-18 silica gel column eluting with MeOH–H_2O (6 : 4) to give a major fraction, which was then recrystallized in ethanol to obtain compound **3** (1.7 g). The fractions 5–7 were combined and chromatographed on a column of C–18 silica gel eluting with MeOH–H_2O (6 : 4) to yield a major fraction, which was then rechromatographed on a silica gel column to give compound **2** (640 mg).

Craniosaponin A (1)

White amorphous powder. $[\alpha]_D^{20} - 4.3^\circ$ (MeOH; *c* 0.49); IR (KBr disc) ν_{max} 3386, 2941, 1643, 1452, 1385 and 1066 cm^{-1}; No UV absorption between 210–400 nm; Positive ion mode ESIMS *m/z* 1129.6 $[M + Na]^+$, negative ion mode ESIMS *m/z* 1105.6 $[M - H]^-$,

TABLE I ^{1}H-NMR data of compounds 1 and 2 [δ, *J*(Hz)] (400 MHz, pyridine-d_5)

Comp.	**1**	**2**	*Comp.*	**1**	**2**
Aglycone			Fucose		
1	2.75	2.10, 1.57	1	5.01 (*d*, 7.7)	5.00 (*d*, 8.0)
2	2.37 (*dd*), 2.16 (*d*)	2.35 (*dd*), 2.12 (*d*)	2	4.74	4.74
3	4.27	4.25	3	4.13 (*dd*, 3.1, 9.6)	4.14 (*dd*, 3.1, 9.6)
5	1.79	1.75	4	4.23	4.25
6	1.78, 1.51	1.87, 1.46	5	3.68 (*q* like, 6.6)	3.70
7	1.74, 1.37	1.71, 1.32	6	1.46 (*d*, 6.6)	1.45 (*d*, 6.6)
9	2.08 (*d*, 8.5)	2.02 (*d*, 8.5)	Glucose (at C-2 of fuc)		
11	4.53 (*dd*, 3.7, 8.5)	3.87 (*dd*, 3.6, 8.5)	1	5.68 (*d*, 7.5)	5.68 (*d*, 7.7)
12	5.66 (*d*, 3.7)	5.60 (*d*, 3.6)	2	4.19	4.18
15	2.22, 1.78	2.20, 1.77	3	4.30	4.30
16	4.68 (*dd*, 12.0, 5.0)	4.72	4	4.28	4.28
18	2.52 (*dd*, 14.0, 4.7)	2.60 (*dd*, 13.5, 4.3)	5	3.75	3.75
19	1.82, 1.22	1.92, 1.36	6	4.38, 4.42	4.38, 4.42
21	1.70, 1.35	1.73, 1.37	Glucose (at C-3 of fuc)		
22	2.87, 1.88	2.88, 1.90	1	5.34 (*d*, 7.7)	5.35 (*d*, 7.8)
23	3.83, 4.44	4.44, 3.80	2	4.03 (*t* like, 8.3)	4.03 (*t* like, 8.3)
24	1.20 (s)	1.20 (s)	3	4.24	4.23
25	1.26 (s)	1.17 (s)	4	4.48	4.48
26	1.20 (s)	1.15 (s)	5	3.81	3.82
27	1.50 (s)	1.46 (s)	6	4.24, 4.18	4.25, 4.18
28	3.78, 4.47	4.44, 3.81	Rhamnose		
29	0.92 (s)	0.96 (s)	1	5.93 (br *s*)	5.94 (br *s*)
30	1.02 (s)	1.08 (s)	2	4.76	4.77
OMe		3.29 (s)	3	4.63 (*dd*, 3.1, 9.3	4.63 (*dd*, 3.2, 9.3)
			4	4.43	4.43
			5	5.05	5.05
			6	1.82 (*d*, 6.2)	1.83 (*d*, 6.2)

959.4 $[M-N-146]^-$, 943.5 $[M-H-162]^-$, 797.2 $[M-H-146-162]^-$, 779.3 $[M-H-146-162-H_2O]^-$, 635.3 $[M-H-146-162-162]^-$ and 471.2 $[M-H-146-162-162-146-H_2O]^-$; ^{1}H- and ^{13}C-NMR spectra data: see Tables I and II.

Buddlejasaponin Ia (2)

White amorphous powder. Positive ion mode ESIMS *m/z* 1143.7 $(M+Na]^+$, negative ion mode ESIMS *m/z* 1119.8 $[M-H]^-$, 973.3 $(M-H-146]^-$, 957.4 $[M-H-162]^-$, 811.5 $[M-H-146-162]^-$, 793.3 $[M-H-146-162-H_2O]^-$, 649.3 $[M-H-146-162-162]^-$ and 471.4 $[M-H-146-162-162-146-CH_3OH]^-$; ^{1}H- and ^{13}C-NMR spectra data: see Tables I and II.

Buddlejasaponin I (3)

White amorphous powder. Positive ion mode ESIMS *m/z* 1111.7 $[M+Na]^+$, negative ion mode ESIMS *m/z* 1087.5 $[M-H]^-$, 941.4 $[M-H-146]^-$, 925.5 $[M-H-162]^-$, 779.3 $[M-H-146-162]^-$, 761.3 $[M-H-146-162-H_2O]^-$ and 617.3 $(M-H-146-162-162]^-$; ^{1}H-NMR spectrum data (pyridine-d_5, 400 MHz): 0.85 (3H, *s*, Me-30), 0.89 (3H, *s*, Me-29), 0.92 (3H, *s*, Me-25), 1.03 (3H, *s*, Me-24), 1.05 (3H, *s*, Me-27), 1.35 (3H, *s*, Me-26), 5.62 (1H, *dd*, $J=10.2, 2.8$ Hz, H-12), 5.95 (1H, *br d*, $J=10.2$ Hz,

TABLE II ^{13}C-NMR data of compounds **1** and **2** (100 MHz, pyridine-d_5)

Comp.	**1**	**2**	*Comp.*	**1**	**2**
Aglycone			30	24.10	24.18
1	41.55	40.23	11-OMe		54.21
2	26.17	26.58	Fucose		
3	83.13	82.86	1	104.15	104.26
4	44.00	44.05	2	77.28	77.22
5	48.53	48.23	3	84.84	84.93
6	18.59	18.52	4	72.13	72.19
7	33.62	33.33	5	70.52	70.65
8	41.09	41.16	6	17.30	17.35
9	55.72	52.28	Glucose (at C-2 of fuc)		
10	38.30	38.23	1	104.05	104.07
11	66.78	76.12	2	76.29	76.42
12	128.21	122.66	3	78.84	78.99
13	145.35	148.41	4	72.14	72.35
14	44.11	44.11	5	77.69	77.78
15	36.97	37.00	6	63.12	63.32
16	66.47	66.41	Glucose (at C-3 of fuc)		
17	43.92	43.19	1	105.06	105.14
18	44.08	44.15	2	75.56	75.72
19	46.64	47.14	3	76.42	76.60
20	31.12	31.26	4	78.43	78.46
21	34.35	34.40	5	77.34	77.37
22	26.17	26.09	6	61.33	61.46
23	65.09	64.92	Rhamnose		
24	13.46	13.42	1	102.92	102.94
25	17.83	18.03	2	72.63	72.75
26	18.44	18.52	3	72.82	72.94
27	26.92	26.43	4	73.99	74.11
28	68.73	68.73	5	70.56	70.56
29	33.41	33.44	6	18.68	18.71

H-11); ^{13}C-NMR spectrum data (pyridine-d_5, 100 MHz): aglycone moiety: 38.50 (C-1), 25.66 (C-2), 82.39 (C-3), 43.75 (C-4), 47.56 (C-5), 17.52 (C-6), 31.49 (C-7), 42.07 (C-8), 52.95 (C-9), 36.11 (C-10), 132.16 (C-11), 131.02 (C-12), 83.90 (C-13), 45.53 (C-14), 35.98 (C-15), 63.98 (C-16), 46.88 (C-17), 52.04 (C-18), 37.62 (C-19), 31.49 (C-20), 34.57 (C-21), 25.94 (C-22), 64.23 (C-23), 12.59 (C-24), 18.46 (C-25), 19.94 (C-26), 20.74 (C-27), 72.91 (C-28), 33.54 (C-29), 23.72 (C-30), fucose: 104.07, 76.92, 84.68, 71.94, 70.41, 17.12, glucose at C-2 of fucose: 103.80, 76.18, 78.75, 72.11, 77.62, 63.04, glucose at C-3 of fucose: 104.92, 75.46, 76.32, 78.15, 77.09, 61.16, rhamnose: 102.66, 72.50, 72.67, 73.83, 70.30, 18.55.

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