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TRITERPENOID GLYCOSIDES FROM LEAVES OF *ILEX CORNUTA*

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Key Word Index—*Ilex cornuta*; Aquifoliaceae; triterpene glycoside; pomolic acid; oleanolic acid.

Abstract—Two new triterpene glycosides have been isolated from the methanol extract of leaves of *Ilex cornuta*. Their structures were established as pomolic acid 3β-*O*-α-L-2-acetoxyarabinopyranosyl-28-*O*-β-D-glucopyranoside and 29-hydroxyoleanolic acid 3β-*O*-α-L-arabinopyranosyl-28-*O*-β-D-glucopyranoside on the basis of spectroscopic data and chemical evidence. Adenosine was identified as the active principle of the methanol extract which is responsible for the increase of coronary blood flow in the isolated blood-perfused dog heart preparation.

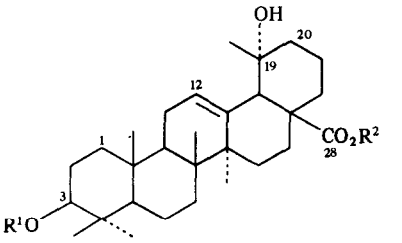
INTRODUCTION

The herb 'Kǔdíngchá' is the dried leaves of *Ilex cornuta* Lindl. (Aquifoliaceae), and it is known in China as a folklore medicinal plant, used for the treatment of dizziness and hypertension. Recently, this herb has been recommended in China for the control of excessive weight and for fertility regulation [1]. Nakanishi *et al.* [2] reported the isolation of two triterpene glycosides, ilexside I methyl ester and ilexside II from the leaves of this plant. A methanol extract of *I. cornuta* has been found to increase coronary blood flow in the isolated blood-perfused dog heart preparation [3]. During the course of our search for the active substances of *I. cornuta*, we have isolated two new triterpene glycosides, **1** and **2**, the structures of which are described in this paper.

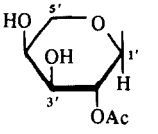
RESULTS AND DISCUSSION

A methanol extract of the leaves of *I. cornuta* was extracted successively with methylene chloride and ethyl acetate. From both extracts, a combination of column chromatography and HPLC led to the isolation of compounds **1** and **2** along with the known pomolic acid glycosides ziyu-glycoside I (**4**) and II (**3**).

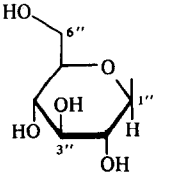
Compound **1**, mp 193–195°, exhibited an intense molecular ion peak $[M+1]^+$ at *m/z* 831 on FABMS. Its IR spectrum showed the presence of hydroxyl (3450 cm^{-1}) and ester (1730 cm^{-1}) groups. The ^{13}C NMR spectrum of **1** (summarized in Table 2) was very similar to that of ziyu-glucoside I (**4**) [4] except for the extra signals at δ 21.2 (*q*) and 170.0 (*s*), whereas ^1H NMR spectrum of **1** contained signals due to an acetoxy group at δ2.09 (3H, *s*) and 5.88 (1H, *dd*, *J* = 9.5, 7.3 Hz) as well as signals corresponding to those of **4**. This spectral evidence suggests that **1** could be a pomolic acid diglycoside, the sugar moieties of which comprise of glucose and arabinose. The sugar components were confirmed by the TLC detection of glucose and arabinose obtained from acid hydrolysis (2 N HCl–MeOH) of **1**. The position of the extra acetoxy group was determined by comparison of the ^1H NMR spectrum of compound **1** and its per-trifluoroacetate (Table 1). On the trifluoroacetylation of **1**, all the signals of the protons due to the glucoside and arabinoside moieties showed



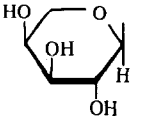
1 R^1 =



R^2 =

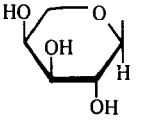


3 R^1 =



R^2 = H

4 R^1 =



R^2 =

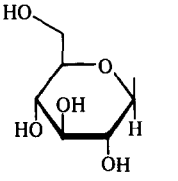


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Table 1. ^{1}H NMR data of compound **1**, its trifluoroacetate and compound **4** (400 MHz, pyridine-d_5, TMS as internal standard)

H	1	Trifluoroacetate	4
Arabinose			
1′	4.70 *d* (7.3)*	5.11 *d* (7.4)	4.75 *d* (6.8)
2′	5.88 *dd* (9.5, 7.3)	5.79 *dd* (9.4, 7.4)	—
3′	4.15 *dd* (9.5, 3.0)	6.24 *dd* (9.4, 3.5)	4.17 *dd* (8.1, 3.4)
4′	—	6.15 *dd* (3.5, 2.5)	—
5′α	3.78 *d* (11.6)	4.41 *d* (13.8)	3.81 *d* (12.2)
5′β	—	4.51 *dd* (13.8, 2.5)	—
Glucose			
1″	6.31 *d* (7.7)	6.75 *d* (8.4)	6.31 *d* (8.1)
2″	—	6.40 *dd* (8.4, 8.4)	4.23 *dd* (8.1, 8.1)
3″	—	6.92 *dd* (8.4, 8.4)	—
4″	—	6.38 *dd* (8.4, 8.4)	—
5″	—	5.22 *ddd* (8.4, 3.9, 2.5)	—
6″	—	5.03 *dd* (12.3, 2.5)	4.48 *dd* (12.2, 2.7)
		5.08 *dd* (12.3, 3.9)	—
Aglycone			
3	3.18 *dd* (11.6, 4.3)	3.28 *dd* (11.8, 4.4)	3.22 *dd* (11.2, 4.1)
12	5.54 *t* (3.4)	5.55 t (3.5)	5.55 *t* (3.1)
29	1.69 *s*	1.68 *s*	1.69 *s*
30	1.06 *d* (6.5)	1.10 *d* (6.9)	1.06 *d* (6.4)
18	2.93 *s*	2.77 *s*	2.93 *s*
Me × 5	0.88, 0.89, 1.07, 1.18, 1.38 (each *s*)	0.89, 0.92, 1.08, 0.93, 1.40 (each *s*)	0.91, 0.96, 1.19, 1.26, 1.39 (each *s*)
Ac	2.09 *s*	2.13 *s*	

*Coupling constants (J in Hz) are given in parentheses.

large downfield shifts except for the anomeric protons (H-1′ and H-1″) and H-2″ on the arabinopyranoside moiety, suggesting that a hydroxyl group is acetylated at C-2 on the arabinoside. The anomeric proton signals of **1** at δ4.70 (d, J = 7.3 Hz) and 6.31 (d, J = 7.7 Hz) indicated the anomeric configuration of its arabinoside and glucoside linkages to be α and β, and their linkage positions to be at C-3 and C-28, respectively. The β equatorial orientation of 3-*O*-arabinoside was evident from the J value (dd, J = 11.6 Hz). Thus, the structure was assigned as pomolic acid 3β-*O*-α-L-2-acetoxyarabinopyranosyl-28-*O*-β-D-glucopyranoside.

Compound **2**, mp 222–223°, showed a molecular ion peak $[M+1]^+$ at m/z 789 on FABMS. The ^{1}H NMR spectrum of **2** exhibited the presence of the following groups: six tertiary methyl groups [δ0.85, 0.94, 1.09, 1.11, 1.24 and 1.26 (each *s*)], $-CH_2OH$ [δ3.55 (2H, *d*, J = 6.0 Hz) and 6.15 (1H, *t*, J = 6.0 Hz, OH)], an olefinic proton [δ5.47 (1H, *t*, J = 3.4 Hz)] and two sugar moieties, the anomeric protons of which appeared at δ4.75 (d, J = 6.9 Hz), 6.35 (d, J = 7.7 Hz). The ^{13}C NMR spectrum of **1** (Table 2) indicated that one of the seven tertiary methyl groups in oleanolic acid [5] was replaced with a hydroxymethyl group (δ 72.8, *t*) and the sugar units comprised of arabinose and glucose. On acid hydrolysis, compound **2** yielded arabinose and glucose. Although the molecular ion peak of **2** did not occur on EIMS, the characteristic fragment ions were observed at m/z 264, derived from m/z 472 (aglycone) via a retro-Diels–Alder cleavage, and at m/z 233 and 219 (Figure 1), disclosing that a C-29 or C-30 methyl group must be oxidized to the hydroxymethyl group. In the case of 30-hydroxyoleanolic acid methyl ester, the ^{13}C NMR signal for the C-29 methyl

Table 2. ^{13}C NMR data of **1**, **2** and **4** (50.28 MHz, pyridine-d_5, TMS as internal standard)

Carbon	1	2	4
1	38.9	38.9	39.0
2	26.7	26.5	26.5
3	89.1	88.8	89.9
4	38.8	39.5	39.4
5	55.9	56.0	56.0
6	18.7	18.7	18.7
7	33.6	33.2	33.6
8	40.6	40.0	40.6
9	47.8	48.1	48.6
10	37.0	37.1	37.0
11	24.1	23.8	24.4
12	128.6	128.8	128.6
13	139.3	148.8	139.1
14	42.1	42.2	42.1
15	29.2	28.3	29.2
16	26.2	23.5	24.0
17	48.7	47.5	47.8
18	54.5	41.0	54.3
19	72.8	41.2	72.7
20	42.1	36.3	54.3
21	26.4	28.3	26.4
22	37.6	32.0	37.4
23	28.0	28.8	29.1
24	17.4	17.5	17.4
25	15.5	15.6	15.4
26	16.7	16.8	16.7
27	27.1	26.1	27.1
28	176.9	176.5	176.7
29	25.5	72.8	27.0
30	16.6	19.7	16.3
Arabinose			
1	104.4	107.0	106.6
2	72.4	73.7	72.9
3	74.3	74.4	74.2
4	69.7	69.1	68.8
5	66.9	66.2	65.8
Glucose			
1	95.8	95.7	95.6
2	74.1	74.1	74.0
3	79.0	78.8	78.7
4	71.5	71.4	71.6
5	78.9	79.0	78.7
6	62.6	62.4	62.7
MeCO	21.2		
MeCO	170.0		

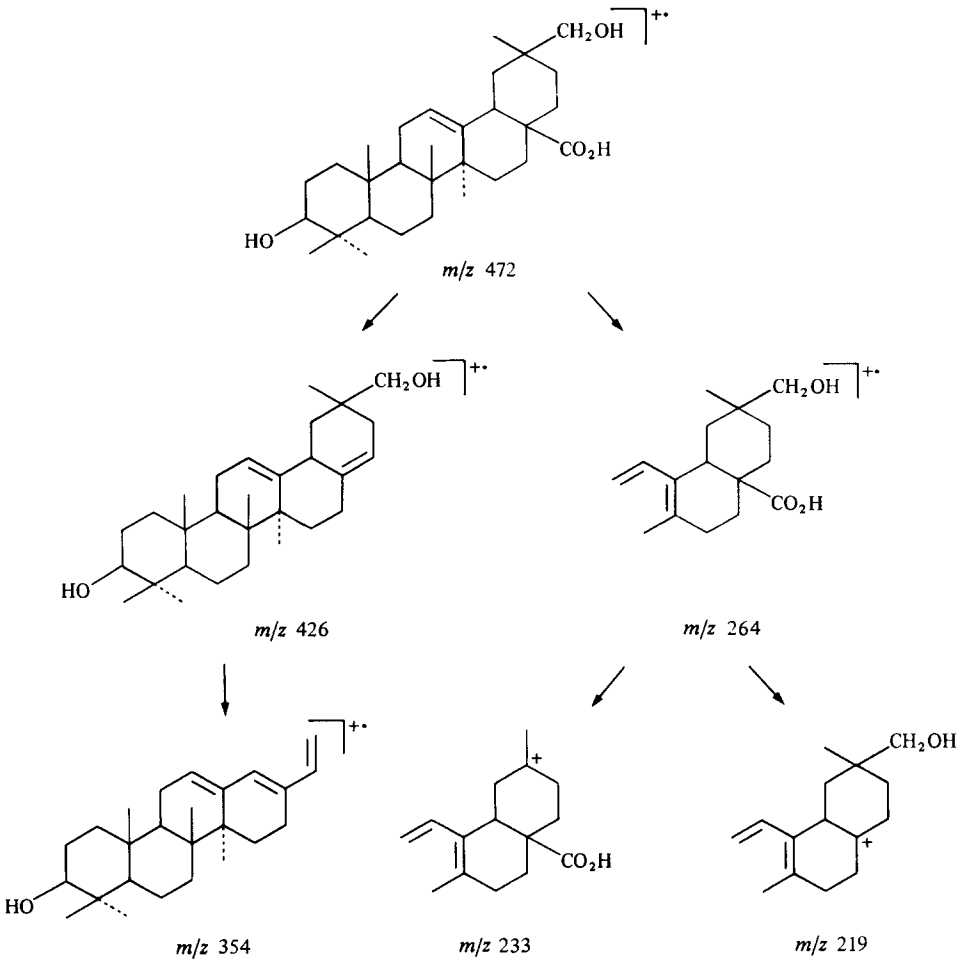
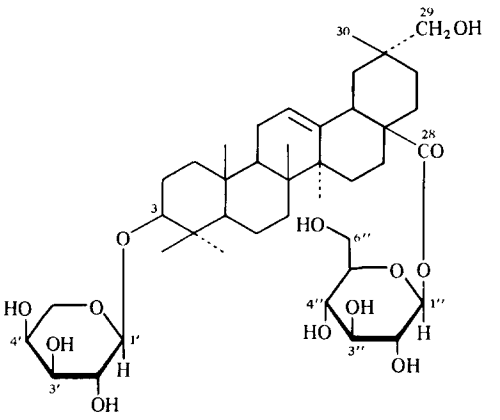


Fig. 1. Mass spectral fragmentation of compound **2**.

group was reported to appear at δ28.9 [5], whereas its corresponding signal in **2** was at δ19.7. This difference of the chemical shifts led to the conclusion that the C-29 methyl was replaced with the hydroxymethyl group since the ^{13}C NMR signal of the C-30 methyl group in most oleanane triterpenes has been assigned to a higher field than that of the C-29 methyl group [6]. On trifluoroacetylation, compound **2** gave the per-trifluoroacetate and its ^{1}H NMR spectrum allowed the assignment of all the protons for the sugar units. This confirmed the presence of an arabinopyranoside and a glucopyranoside moiety as deduced from the ^{13}C NMR data (Table 3). The anomeric proton signals at δ4.75 (*d*, J = 6.9 Hz) and 6.35 (*d*, J = 7.7 Hz) led to the assignments of the anomeric configuration and linkage position of the arabinoside unit as α and at C-3, and those of the glucoside unit as β and at C-28. These assignments were supported by the ^{1}H NMR proton signals of the trifluoroacetate of **2** and the ^{13}C NMR signals (Tables 2 and 3). These results led to the elucidation of structure of **2** as 29-hydroxyoleanolic acid 3β-*O*-α-L-arabinopyranosyl-28-*O*-β-D-glucopyranoside.

In addition to compounds **1** and **2**, the known pomolic acid glycoside ziyu-glucoside **I** (**4**) and **II** (**3**) were isolated from *I. cornuta*. The triterpenoid glycosides isolated from this plant were examined to test their effects on changes in



2

coronary blood flow, contractile force and sinus rate using the isolated dog heart preparation but they showed no significant effects. On the other hand, application of this bioassay to a systematic analysis of other fractions of the methanol extract led to the isolation of adenosine [7], which exhibited strong enhancement (1.0 ml at 0.1 μg,

Table 3. ^{1}H NMR data of **2** and its trifluoroacetate (400 MHz, pyridine-d_5, TMS as internal standard)

H	2	Trifluoroacetate
Arabinose		
1′	4.75 *d* (6.9)*	5.37 *d* (7.3)
2′	—	5.90 *dd* (9.5, 7.3)
3′	—	6.60 *dd* (9.5, 3.4)
4′	—	6.29 *dd* (3.4, 2.2)
5′α	—	4.55 *d* (14.6)
5′β	—	4.59 *dd* (14.6, 2.2)
Glucose		
1″	6.35 *d* (7.7)	6.81 *d* (8.2)
2″	—	6.46 *dd* (9.0, 8.2)
3″	—	6.92 *dd* (9.0, 9.0)
4″	—	6.40 *dd* (9.0, 9.0)
5″	—	5.24 *ddd* (9.0, 4.3, 2.6)
6″	—	5.00 *dd* (12.9, 2.6)
		5.09 *dd* (12.9, 4.3)
Aglycone		
3	3.32 *dd* (10.7, 4.3)	3.38 *dd* (11.6, 4.3)
12	5.47 *t* (3.4)	5.47 *t* (3.4)
18	3.33	3.12 *dd* (12.9, 3.9)
29	3.55 *d* (6.0)	4.18 *d* (10.8)
		4.25 *d* (10.8)
Me × 6	0.85, 0.94, 1.09, 1.11, 1.24, 1.26 (each *s*)	0.86, 0.89, 0.90, 1.04, 1.05, 1.22 (each *s*)
29-OH	6.15 *t* (6.0)	

*Coupling constants (*J* in Hz) are given in parentheses.

3.0 ml at 1 μg) of coronary blood flow without serious effects on sinus rate and contractile force. Therefore, we have concluded that adenosine must be the active principle of the methanol extract of *I. cornuta* which is responsible for an increase of coronary blood flow in the isolated blood-perfused dog heart preparation.

EXPERIMENTAL

Mps: uncorr; ^{1}H NMR (400 MHz) and ^{13}C NMR (50.28 MHz): pyridine-d_5, TMS as internal standard; CC: silica gel (Wakogel C-300); TLC: precoated silica gel plates F_{254} and RP-8 plates F_{254} (Merck, 0.25 mm). Spots were visualized by UV (254 nm) and 40% $CeSO_4$–H_2SO_4.

Plant material. Ilex cornuta was identified by Qin Wenjuan. A voucher specimen has been deposited at Beijing Institute of Pharmaceutical Industries.

Extraction and isolation. Air dried powdered leaves (5 kg) of *I. cornuta* collected in China were extracted × 3 with MeOH (20 l.) under reflux for 2 hr. The combined MeOH extract was evaporated *in vacuo* to leave a crude extract, which was dissolved in 60% aq. MeOH (5 l.). The soln obtained was defatted with *n*-hexane, and then extracted successively with CH_2Cl_2, EtOAc and *n*-BuOH. The CH_2Cl_2 fraction (50 g) was chromatographed on silica gel using CH_2Cl_2–EtOAc–MeOH gradient and divided into 10 fractions: fr. 1 (CH_2Cl_2); fr. 2 (CH_2Cl_2–EtOAc, 4 : 1); fr. 3 (CH_2Cl_2–EtOAc, 3 : 2); fr. 4 (CH_2Cl_2–EtOAc, 2 : 3); fr. 5 (CH_2Cl_2–EtOAc, 1 : 4); fr. 6 (EtOAc); fr. 7 (EtOAc–MeOH, 9.5 : 0.5); fr. 8 (EtOAc–MeOH, 9 : 1); fr. 9 (EtOAc–MeOH, 4 : 1); fr. 10 (EtOAc–MeOH, 7 : 3). Fr. 7 (10 g) was purified by CC on silica gel ($CHCl_3$–MeOH, 9.5 : 0.5) to give ziyu-glycoside **II** (**3**, 800 mg). Fr. 8 (7 g) was rechromatographed on silica gel ($CHCl_3$–MeOH, 9 : 1) to yield ziyu-glycoside **I** (**4**, 1.85 g). The EtOAc fraction (60 g) was chromatographed on silica gel using a $CHCl_3$–MeOH gradient and divided into four fractions: fr. 1 ($CHCl_3$); fr. 2 ($CHCl_3$–MeOH, 9 : 1); fr. 3 ($CHCl_3$–MeOH, 9 : 1); fr. 4 ($CHCl_3$–MeOH, 4 : 1). Fr. 2 (2.5 g) was rechromatographed on silica gel ($CHCl_3$–MeOH, 9 : 1), and then the fourth fraction obtained was purified by HPLC [column: Cosmosil C_{18} (20 × 300 mm), solvent: H_2O–MeOH (35 : 65), 8 ml/min, det: UV 215 nm] to afford **1** (34 mg) as a colourless powder, mp 193–195°, $[\alpha]_D^{19}$ 20.5 (*c* 0.5; EtOH). IR ν_{max}^{KBr} cm^{-1}: 3450 (OH), 1730 (ester C=O), 1630 (C=C); FABMS *m/z*: 831 $[M+1]^+$; ^{1}H NMR: Table 1; ^{13}C NMR: Table 2. Fr. 4 (8 g) was chromatographed on silica gel ($CHCl_3$–MeOH, 8.5 : 1.5) followed by Sephadex LH-20 (MeOH) and the crude sample then subjected to HPLC [column: Cosmosil C_{18} (20 × 300 mm), solvent: MeOH–H_2O (3 : 2), 8 ml/min, det: 254 nm] to afford **2** (23 mg) as a colourless powder, mp 222–223°, $[\alpha]_D^{19}$ 7.3 (*c* 0.25; EtOH). IR ν_{max}^{KBr} cm^{-1}: 3440 (OH), 1740 (ester C=O); FABMS *m/z*: 789 $[M+1]^+$; ^{1}H NMR: Table 3; ^{13}C NMR: Table 2.

Preparation of the per-trifluoroacetate of **1** *and* **2**. A mixture of **1** (5 mg), pyridine-d_5 (0.5 ml) and anhydrous trifluoroacetic anhydride (20 μl) was allowed to stand at room temp. in the NMR tube. After the reaction was completed (giving one product as checked by TLC), the reaction mixture was subjected directly to ^{1}H NMR analysis (Table 1). The product, judged by the data, corresponds to the hexatrifluoroacetate, in which the 19-tert-methyl group remained unreacted. The per-trifluoroacetate of **2** was also prepared in a similar way and its ^{1}H NMR data are summarized in Table 3.

Acid hydrolysis of **1**. A mixture of **1** (3 mg), 2 N HCl (1 ml) and MeOH (2 ml) was refluxed for 3 hr. MeOH was removed *in vacuo* to leave an aq. residue, which was extracted with Et_2O. The aq. layer was neutralized with Amberlite MB-3, concd and subjected to the TLC analysis on silica gel, solvent: $CHCl_3$–MeOH–H_2O (6 : 4 : 0.5). R_f = 0.33 for arabinose, 0.20 for glucose.

Acid hydrolysis of **2**. A mixture of **2** (2 mg), 2 N HCl (1.5 ml), and MeOH (2 ml) was refluxed for 3 hr. MeOH was removed *in vacuo* to leave an aq. residue, which was extracted with Et_2O. The aq. layer was neutralized with Amberlite MB-3, concd and subjected to the TLC analysis on silica gel, solvent: $CHCl_3$–MeOH–H_2O (6 : 4 : 0.5). R_f = 0.33 for arabinose, 0.20 for glucose.

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REFERENCES

1. But, P. H., Ng, K. H. and Kong, Y. C. (1985) *Shoyakugaku Zasshi* **39**, 46.
2. Nakanishi, T., Terai, H., Nasu, M., Miura, I. and Yoneda, K. (1982) *Phytochemistry* **21**, 1373.
3. Hashimoto, K. and Kubota, K. (1973) *Nauyn-Schmiedeberg's Arch. Pharmacol.* **278**, 135.
4. Yoshioka, I., Sugawara, T., Ohsuka, A. and Kitagawa, I. (1971) *Chem. Pharm. Bull. Tokyo* **19**, 1700.
5. Whehrli, F. W. and Nishida, T. (1979) *Progress in the Chemistry of Organic Natural Products*, Vol. 36, p. 38. Springer, New York.
6. Nakanishi, K., Goto, T., Ito, S., Natori, S. and Nozoe, S. (1984) *Natural Products Chemistry*, Vol. 3, p. 179. Kodansha Ltd., Tokyo.
7. Pushan, W., Xuanling, G., Yixiong, W., Fukuyama, Y., Miura, I. and Sugawara, M. (1984) *Phytochemistry* **23**, 2033.