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TRITERPENES AND TRITERPENE GLYCOSYL ESTER FROM *RUBUS PUNGENS* CAMB. VAR *OLDHAMII*

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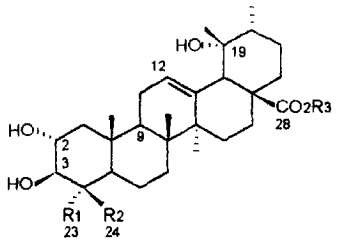
Key Word Index—*Rubus pungens*; Rosaceae; pentacyclic triterpene; ursane glycosyl esters; methylglucoside.

Abstract—A new triterpene glycoside, 2α,3β,19α-trihydroxyurs-12-ene-23,28-dioic acid-28-*O*-(6′-*O*-methyl-β-D-glucopyranosyl) ester and a new triterpene derivative, 24-*O*-butyl-2α,3β,19α,24-tetrahydroxyurs-12-en-28-oic acid, together with six known compounds, have been isolated from the aerial parts of *Rubus pungens* Camb. var *oldhamii*. The structures of these compounds were established mainly by spectroscopic methods.

INTRODUCTION

In a previous paper, we reported the isolation of three new A-ring oxygenated 19α-hydroxyursane-type triterpene methylglucosides from the aerial parts of *Rubus pileatus* Focke [1]. In this paper, we report the isolation and structural elucidation of a new triterpenoid glycosyl ester, 2α,3β,19α-trihydroxyurs-12-ene-23,28-dioic acid-28-*O*-(6′-*O*-methyl-β-D-glucopyranosyl) ester **1**, and a new triterpene, 24-*O*-butyl-2α,3β,19α,24-tetrahydroxyurs-12-en-28-oic acid **2**, together with six known compounds, from the aerial parts of *Rubus pungens* Camb. var *oldhamii*.



	R_1	R_2	R_3
1	COOH	CH_3	6′-O-methyl-β-D-glucopyranosyl
1a	$COOCH_3$	CH_3	6′-O-methyl-β-D-glucopyranosyl
2	CH_3	$CH_2OButyl$	H

RESULTS AND DISCUSSION

The ethanol extract of the aerial parts of *Rubus pungens* Camb. var *oldhamii* was suspended in hot water, and then extracted successively with petrol (60–90°), ethyl acetate, and *n*-butanol. The butanol-soluble fraction was subjected to CC on highly porous resin and the glycosidic fraction was treated with diazomethane because of the difficulty of separation. The esterified glycoside was further purified by repeated CC on Sephadex LH-20 and silica gel to give **1a**. Compounds **2–8** were obtained from the ethyl acetate extract by repeated silica gel column chromatography.

Compounds **3–8** were identified by means of ^1H NMR, ^{13}C NMR, MS, and IR as five known triterpenes and one known flavonoid, i.e. **3**: tormentic acid, isolated from the leaves of *R. moluccanus* [2] and several other plants, *Vochysia vismiaefolia* [3], *Rosa davurica* Pall [4], **4**: 2α,3β,19α,23-tetrahydroxyurs-12-en-28-oic acid (19α-hydroxyasiatic acid), the aglycone of niga-ichigosides F1 isolated from the leaves of *R. microphyllus* [5], **5**: β-daucosterol [6] and **6**: kampferol-3-*O*-α-L-rhamnopyranoside, isolated from the whole plants of *Euphorbia aleppica* [6], **7**: 2α,3β,19α-trihydroxyurs-12-ene-23,28-dioic acid, the aglycone of suavissimoside R1, obtained from the roots of *R. suavissimus* S. Lee [7], **8**: 2α,3β,19α-trihydroxyolean-12-ene-23,28-dioic acid, isolated from *Barringtonia acutangula* [8]. The ^{13}C NMR spectral data for compound **8** are reported for the first time in this paper.

Compound **1a** was obtained as an amorphous

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Table 1. ^{13}C NMR and DEPT data for compounds **1a**, **2** and **8** (100 MHz; pyridine-d_5)

	C	1a	DEPT		2	DEPT	8	DEPT
Aglycone	1	48.0	CH_2		47.9	CH_2	47.3	CH_2
	2	68.3	CH		68.7	CH	68.4	CH
	3	80.6	CH		85.8	CH	80.8	CH
	4	55.1	C		44.0	C	54.2	C
	5	52.3	CH		56.6	CH	51.7	CH
	6	21.3	CH_2		19.4	CH_2	20.9	CH_2
	7	33.0	CH_2		33.9	CH_2	32.7	CH_2
	8	40.5	C		40.4	C	39.7	C
	9	47.9	CH		47.8	CH	48.2	CH
	10	37.6	C		38.3	C	38.3	C
	11	24.0	CH_2		24.4	CH_2	23.7	CH_2
	12	127.9	CH		127.9	CH	122.7	CH
	13	139.1	C		140.4	C	144.5	C
	14	41.9	C		42.1	C	41.7	C
	15	29.0	CH_2		29.3	CH_2	28.2	CH_2
	16	25.9*	CH_2		27.0*	CH_2	23.8	CH_2
	17	48.5	C		48.3	C	45.9	C
	18	54.2	CH		54.6	CH	44.4	CH
	19	72.3	C		72.7	C	80.8	CH
	20	41.9	CH		42.4	CH	35.3	C
	21	26.5*	CH_2		26.4*	CH_2	28.6	CH_2
	22	38.4	CH_2		38.5	CH_2	33.3	CH_2
	23	178.1	C		24.2	CH_3	181.1*	C
	24	13.0	CH_3		71.9	CH_2	13.3	CH_3
	25	17.3**	CH_3		17.3**	CH_3	16.6**	CH_3
	26	17.1**	CH_3		17.1**	CH_3	17.5**	CH_3
	27	24.4	CH_3		24.7	CH_3	24.5	CH_3
	28	176.9	C		180.7	C	180.6*	C
	29	26.8	CH_3		27.1	CH_3	28.4	CH_3
	30	16.5**	CH_3		16.8**	CH_3	24.5	CH_3
	23-CO_2Me	51.7	CH_3	Butyl 1′	65.7	CH_2		
Sugar moiety	1′	95.5	CH	2′	30.8	CH_2		
	2′	73.8	CH	3′	19.4	CH_2		
	3′	77.8	CH	4′	13.8	CH_3		
	4′	71.0	CH					
	5′	78.7	CH					
	6′	72.5	CH_2					
	OMe	59.1	CH_3					

*, **, Interchangeable values in the same column.

powder. It gave a positive colouration in the Liebermann-Burchard and Molish tests for triterpene glycoside. The IR spectrum showed hydroxyl (3377 cm^{-1}), ester carbonyl (1732 cm^{-1}), and glycosidic linkage (1060 cm^{-1}) absorption bands. The FAB-mass spectrum displayed a molecular ion peak at m/z 708 $[M]^+$ and two prominent fragment ion peaks at m/z 690 $[M-H_2O]^+$ and 533 $[M-\text{methylglu}+1]^+$ due to the loss of water and a methylglucosyl moiety. These results, in consideration with its ^1H NMR, ^{13}C NMR and DEPT spectra (Table 1), suggested the molecular formula $C_{38}H_{60}O_{12}$ for **1a**.

The ^{13}C NMR and DEPT spectra of **1a** revealed the presence of eight methyl, nine methylene, twelve methine, and nine quaternary carbon atoms in the molecule. The chemical shifts of these signals suggested that **1a** is a pentacyclic triterpenoid saponin. The signals at δ 127.9 and 139.1, methine and quaternary, indicated that **1a** had an urs-12-ene type framework [9, 10]. The signals of the methylglucosyl which appeared at δ 95.5 (CH), 73.8 (CH), 77.8 (CH), 71.0 (CH), 78.7 (CH), 72.5 (CH_2), and 59.1 (CH_3) were in good agreement with that of 6-*O*-β-D-methylglucopyranose [11]. The anomeric carbon resonance at δ 95.5 indicated that the saccharide moiety was linked with the aglycone through an ester bond [12]. The presence of two carboxyl groups was also suggested on the basis of the molecular formula of **1a**, and these two carboxyl carbon signals appeared at δ 178.1 and 176.9 in the ^{13}C NMR spectrum.

The ^1H NMR spectrum of **1a** indicated the presence of one secondary methyl for H-30 at δ 1.01 (3H, *d*, $J = 7.6$ Hz), five singlet methyls for H-24, H-25, H-26, H-27, and H-29 at δ 1.05, 1.11, 1.31, 1.53, and 1.57

(each 3H, *s*), one olefinic proton resonance as a broad singlet for H-12 at δ 5.46, and a sharp singlet signal for H-18 at δ 3.02. These data further confirmed that **1a** had a 19α-hydroxyl substituted urs-12-ene type carbon skeleton [14]. The signals at δ 4.37 (1H, *br t*, $J = 9.6$ Hz, H-2β) and 3.59 (1H, *d*, $J = 7.2$ Hz, H-3α) suggested that the hydroxy groups in rings A/B were assignable to 2α and 3β. The ^{1}H NMR spectrum also showed the presence of two methoxyl groups at δ 3.34 and 3.57 (each 3H, *s*), and the anomeric proton signal at δ 6.15 (1H, *d*, $J = 8.1$ Hz), which indicated that the methylglucopyranosyl group was linked with the aglycone in the β-configuration.

The EI mass spectrum of **1a** showed two peaks at *m/z* 269 and 264, the characteristic retro-Diels-Alder cleavage peaks of an urs-12-en type derivative which has two hydroxy groups and one ester methyl group in rings A/B, and one hydroxy group and one carboxyl group in rings D/E [7, 13]. This means the ester methyl was located in 23-COOH and that the methylglucosyl moiety must be attached in 28-COOH.

The ^{13}C NMR spectral data of **1a** were compared with 2α,3β,19α-trihydroxyurs-12-ene-23,28-dioic acid-28-*O*-β-D-glucopyranosyl ester (suavissimoside F1) [7]. The data of **1a** showed two more methoxyl groups than that of suavissimoside R1, one was the 23-ester methyl at δ 51.7 and another was the 6′-*O*-methyl in the sugar residue at δ 59.1. Because of the etherification, the C-6′ signal was shifted 10.2 ppm (from δ 62.3 to 72.5) to the lower fields. The remaining signals of **1a** were observed at almost the same position to those of suavissimoside R1.

Based on the above evidence, the structure of compound **1a** was established as 2α,3β,19α-trihydroxyurs-12-ene-23,28-dioic acid-23-methyl ester-28-*O*-(6′-*O*-methyl-β-D-glucopyranosyl) ester, and that the correlated naturally occurring compound **1** should be 2α,3β,19α-trihydroxyurs-12-ene-23,28-dioic acid-28-*O*-(6′-*O*-β-D-glucopyranosyl)ester.

Compound **2**, another amorphous powder, gave the positive Liebermann-Burchard colouration test for triterpene. The IR spectrum indicated the presence of hydroxyl (3456 cm^{-1}), carboxylic (1692 cm^{-1}), trisubstituted double bond (3015, 1640, 864 cm^{-1}) and C—O—C (1078 cm^{-1}) in the molecule. Its ^{13}C NMR and DEPT spectra revealed thirty-four carbon signals including seven methyls, twelve methylenes, seven methines, and eight quaternary carbon atoms in **2**. These data, in combination with the data in the mass spectrum, suggested the molecular formula $C_{34}H_{56}O_6$ for **2**. The EI mass spectrum displayed the molecular ion peak at *m/z* 560 and a fragment peak at *m/z* 504 $[M-\text{butyl}+1]^+$, which derived a series of peaks at *m/z* 458 $[504-\text{HCOOH}]^+$, 264, 246, 219, 201, 187, and 146. The peak at *m/z* 264 is the characteristic retro-Diels-Alder cleavage peak of an olean-12-en or urs-12-en-28-oic acid derivative which possess a hydroxy group on either the D or E ring [7, 13]. The ^{1}H NMR spectrum showed the signal at δ 3.03 (1H, *s*, H-18), which suggested the presence of the 19α-hydroxyl substituted urs-12-ene skeleton [14].

The ^{13}C NMR and DEPT spectra of **2** revealed almost the same signals as those reported for 2α,3β,19α,24-tetrahydroxyurs-12-en-28-oic acid (hyptatic acid B) [15, 16], except the peaks due to the butyl signals at δ 13.8 (CH_3), 19.4 (CH_2), 30.8 (CH_2), and 65.7 (CH_2). Since the C-24 signal showed significant downfield shift (6.2 ppm) comparison to hyptatic acid B, the position of butyl group was proved to be attached on the C-24 oxygen.

From the above results, the structure of compound **2** was determined to be 24-butoxy-2α,3β,19α,24-tetrahydroxyurs-12-en-28-oic acid.

EXPERIMENTAL

General

Mps: uncorr.; IR (film, MeOH) and optical rotations were determined on a Nicolet FT-170 SX and a J-20C instrument, respectively. ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz) and DEPT spectra were recorded on a Bruker AM-400 NMR spectrometer in pyridine-d_5 with TMS as int. standard. FABMS and EIMS data were obtained on a VG-ZAB-HS spectrometer.

Plant material

Aerial parts of *R. pungens* Camb. var *oldhamii* were collected in September 1994 at Zhang county, Gansu Province, People's Republic of China. It was identified by Prof. Zexiang Peng, Department of Biology, Lanzhou University. A voucher specimen was deposited in the Institute of Organic Chemistry, Lanzhou University.

Extraction and purification

Powdered, dried aerial parts of *R. pungens* Camb. var. *oldhamii* (5.0 kg) were extracted successively with 95% EtOH (3 × 12 l each time) at room temp. and the EtOH extracts were taken to dryness under vacuum to give an extract (350 g). A suspension of the resulting extract in hot H_2O (1200 ml) was washed with petrol (3 × 800 ml each time), and then extracted with EtOAc and *n*-BuOH saturated with H_2O (3 × 1.5 l each time). The *n*-BuOH extract was taken to dryness to give the crude glycosidic fraction (40 g) which was chromatographed on highly porous resin (SIP 1400) eluting successively with the solvent system H_2O–EtOH (1:0–0:1). The glycosidic fraction was treated with CH_2N_2 in MeOH and then passed through Sephadex LH-20 CC and finally purified by repeated CC on silica gel (200–300 mesh) using a step-gradient of $CHCl_3$–CH_3OH–H_2O (40:10:1) to afford **1a** (25 mg).

The EtOAc fr. (65 g) was also subjected to CC on silica gel (200–300 mesh) eluting with $CHCl_3$–CH_3OH (15:1–6:1) to give three crude frs A, B, and C. Frac-

tion A was further separated by repeated silica gel column chromatography (200–300 mesh, $CHCl_3$:CH_3OH, 15:1) to yield **2** (20 mg), **3** (25 mg), and **4** (20 mg). Fraction B was treated in the same procedure as those for fr. A, eluting with $CHCl_3$–CH_3OH (10:1) to give compounds **5** (30 mg) and **6** (30 mg). compounds **7** (15 mg) and **8** (15 mg) were obtained from fr. C by repeated silica gel CC (200–300 mesh) eluting with $CHCl_3$:CH_3OH (8:1).

2α,3β,19α-trihydroxyurs-12-ene-23,28-dioic acid-23-methyl ester-28-O-(6′-O-methyl-β-D-glucopyranosyl) ester (**1a**). Amorphous powder, mp 216–218°. $[\alpha]_D^{20}$ +88.5° (MeOH, *c* 0.65). IR ν_{max}^{film} cm^{-1}: 3377, 3020, 2981, 1732, 1648, 1575, 1454, 1060, 826, 773. FABMS (positive), *m/z*: 708 $[M]^+$, 690 $[M-H_2O]^+$, 533 $[M-$methylglucosyl$+1]^+$. EIMS, *m/z* (rel. int.): 533 $[M-$methylglucosyl$+1]^+$ (12), 514 $[M-H_2O]^+$ (29), 487 (31), 486 (35), 269 (44), 264 (61), 250 (100), 246 (55), 201 (46), 187 (14), 146 (21). ^1H NMR: δ 1.05, 1.11, 1.31, 1.53, 1.57 (3H each, *s*, Me-24, 25, 26, 27, 29), 1.01 (3H, *d*, *J* = 7.6 Hz, Me-30), 3.02 (1H, *s*, H-18), 3.34, 3.57 (3H each, 23-CO_2Me, 6′-OMe), 3.59 (1H, *d*, *J* = 7.2 Hz, H-3α), 3.65–4.35 (*m*, H-2′, 3′, 4′, 5′, 6′), 4.37 (1H, *br t*, *J* = 9.6 Hz, H-2β), 5.15 (1H, *s*, OH-19), 5.46 (1H, *br s*, H-12), 6.15 (1H, *d*, *J* = 8.1 Hz, H-1′). ^{13}C NMR and DEPT: see Table 1.

24-O-butyl-2α,3β,19α,24-tetrahydroxyurs-12-ene-28-oic acid (**2**). Amorphous powder, mp 177–179°. $[\alpha]_D^{20}$ +53.2° (MeOH, *c* 0.40). IR ν_{max}^{film} cm^{-1}: 3456, 3015, 2985, 1692, 1640, 1455, 1078, 1040, 967, 938, 864, 744. EIMS *m/z* (rel. int.): 560 $[M]^+$ (18), 504 $[M-$butyl$+1]^+$ (6), 486 (8), 458 (12), 386 (8), 264 (15), 246 (13), 219 (15), 201 (29), 187 (25), 146 (100). ^1H NMR: δ 0.81 (3H, *t*, *J* = 7.4 Hz, H-4′), 0.88 (3H, *d*, *J* = 6.8 Hz, H-30), 0.97, 1.05, 1.41, 1.56, 1.70 (3H each, *s*, Me-23, 25, 26, 27, 29), 1.29 (2H, *m*, H-3′), 1.59 (2H, *m*, H-2′), 3.03 (1H, *s*, H-18), 3.54 (1H, *d*, *J* = 9.4 Hz, H-3α), 3.70 (1H, *d*, *J* = 11.0 Hz, H-24a), 4.30 (1H, *br t*, *J* = 9.5 Hz, H-2β), 4.35 (2H, *t*, *J* = 6.6 Hz, H-1′), 4.45 (1H, *d*, *J* = 11.0 Hz, H-24b), 5.14 (1H, *s*, OH-19), 5.56 (1H, *br s*, H-12). ^{13}C NMR and DEPT: see Table 1.

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