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PENTACYCLIC TRITERPENOID GLYCOSYL ESTERS FROM *RUBUS PILEATUS*

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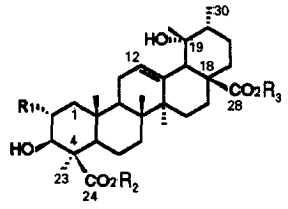
Key Word Index—*Rubus pileatus*; Rosaceae; pentacyclic triterpene; ursane glycosyl esters; methylglucoside.

Abstract—Five pentacyclic triterpenoid glycosyl esters have been isolated from the aerial parts of *Rubus pileatus* and three of them are new compounds. Their structures were elucidated on the basis of spectral data and chemical transformations as 3β,19α-dihydroxyurs-12-en-24,28-dioic acid-28-*O*-(6′-*O*-methyl-β-D-glucopyranosyl) ester, 2α,3β,19α-trihydroxyurs-12-en-24,28-dioic acid-28-*O*-(6′-*O*-methyl-β-D-glucopyranosyl) ester and 2α,3β,19α-trihydroxyurs-12-en-24,28-dioic acid-28-*O*-(3′-*O*-methyl-β-D-glucopyranosyl) ester.

INTRODUCTION

There are more than 700 species of *Rubus* plants represented throughout the world and about 194 species are widespread in China, mainly in the northern regions of China [1]. Fruits of some of the *Rubus* spp. have long been used traditionally as a food and tonic for aged people, also some have been used as anticancer or antibacterial agents in Chinese folk medicine [2]. Previous chemical investigations of this genus have revealed the presence of ursane and oleanane type triterpenoid saponins [3–6], and kaurane and labdane type diterpene glycosides [7–10]. No phytochemical examination on the plant *Rubus pileatus* Focke has been published up to now. We wish to report herein the isolation and structural elucidation of the 24-methyl esters of three new triterpenoid glycosyl esters, 3β,19α-dihydroxyurs-12-en-24,28-dioic acid-28-*O*-(6′-*O*-methyl-β-D-glucopyranosyl) ester (**2**), 2α,3β,19α-trihydroxyurs-12-en-24,28-dioic acid-28-*O*-(6′-*O*-methyl-β-D-glucopyranosyl) ester (**4**) and 2α,3β,19α-trihydroxyurs-12-en-24,28-dioic acid-28-*O*-(3′-*O*-methyl-β-D-glucopyranosyl) ester (**5**), from the ethanol extract of this plant.



	R_1	R_2
2	H	6′-O-methyl-β-D-glucopyranosyl
4	OH	6′-O-methyl-β-D-glucopyranosyl
5	OH	3′-O-methyl-β-D-glucopyranosyl

RESULTS AND DISCUSSION

The ethanol extract of the aerial parts of *Rubus pileatus* was partitioned between petrol and water, and then between *n*-butanol and water. The butanol extract was subjected to column chromatography to afford six fractions (I–VI). Fraction III was further subjected to CC on highly porous resin (SIP 1300) and Sephadex LH-20 to give a crude glycoside fraction, which was then treated with diazomethane in methanol because of the difficulty of separation. The esterified glycoside was further purified by repeated CC on silica gel to yield compounds **1a** and **2a**. Fraction IV was treated in the same way as for fraction III to give **3a** and a mixture of **4a**, and **5a**, the mixture was further separated by preparative reversed phase HPLC to furnish the pure compounds **4a** and **5a**.

The triterpene methylglucosyl esters **1a**–**5a** were indicated by the positive colouration in the Liebermann–Burchard and Molish tests. The known compounds were characterized as ilexsaponin A1 **1** [11] and trachelosperoside A-1 **3** [12] by direct com-

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parison of their spectral data (^{1}H, ^{13}C NMR and DEPT) with those reported, respectively.

Compound **2a** was obtained as an amorphous powder. Its IR spectrum showed the absorption bands for hydroxyl groups (3396 cm^{-1}), ester carbonyl groups (1724 and 1706 cm^{-1}), and a glycosidic linkage (1069 cm^{-1}). The negative FAB mass spectrum displayed a quasi-molecular ion peak at m/z 691 $[M-1]^-$ and a prominent fragment ion peak at m/z 515 $[M-176-1]^-$ due to the loss of sugar moiety. These results were consistent with the molecular formula $C_{38}H_{60}O_{11}$ (9 unsaturations) previously established on the basis of the ^{13}C NMR and DEPT spectra (Table 1). These spectra, together with the ^{1}H NMR spectrum, indicated the presence of 6×-CH_3, 2×-OCH_3, 9×-CH_2-, 1×-OCH_2-, 4×>CH-, 6×-OCH<, 1×-CH=C<, 5×>C<, 1×-O-C< and 2×-CO_2-. These data allowed the identification of **2a** as a pentacyclic triterpene glycosyl ester. The olefinic carbon signals at δ 128.4 (CH, C-12) and 139.2 (C, C-13) in its ^{13}C NMR spectrum suggested that **2a** possesses an urs-12-ene type carbon skeleton [13, 14].

Comparison of the ^{13}C NMR data of **2a** with that of 3β,19α-dihydroxyurs-12-en-24,28-dioic acid-28-*O*-β-D-glucopyranosyl ester (**1**) (ilexsaponin A1) previously isolated from the roots of *Ilex pubescens* [11] showed that their structures were very similar, except that **2a** had two additional methoxy groups, one was the C-24 ester methyl (at δ 50.9, CH_3, and 177.6, C, C-24), and the other was attached in the C-6′ position of the glucosyl moiety which caused the resonance signal of C-6′ to shift from δ 62.2 in **1** to 72.5 in **2a**. Moreover, the seven carbon resonance signals of the sugar moiety observed in its ^{13}C NMR spectrum corresponded almost exactly to those of 6′-*O*-methyl-β-D-glucopyranose [15].

Table 1. ^{13}C NMR and DEPT data for compounds **2a**, **4a** and **5a** (100 MHz; pyridine-d_5)

	C	**2a**	DEPT	**4a**	DEPT	**5a**	DEPT
Aglycone	1	39.5	CH_2	48.4	CH_2	48.4	CH_2
	2	28.6	CH_2	67.8	CH	67.8	CH
	3	77.9	CH	83.6	CH	83.7	CH
	4	49.6	C	50.5	C	50.5	C
	5	56.8	CH	56.6	CH	56.6	CH
	6	20.7	CH_2	20.5	CH_2	20.6	CH_2
	7	33.6	CH_2	33.4	CH_2	33.4	CH_2
	8	40.3	C	40.2	C	40.2	C
	9	47.2	CH	47.1	CH	47.1	CH
	10	37.7	C	38.4	C	38.6	C
	11	24.2	CH_2	24.1	CH_2	24.1	CH_2
	12	128.4	CH	128.1	CH	128.1	CH
	13	139.2	C	139.0	C	139.0	C
	14	42.1	C	41.9	C	42.0	C
	15	29.1	CH_2	29.2	CH_2	29.0	CH_2
	16	26.1*	CH_2	25.9*	CH_2	25.9*	CH_2
	17	48.6	C	48.2	C	48.2	C
	18	54.4	CH	54.2	CH	54.2	CH
	19	72.6	C	72.2	C	72.4	C
	20	42.1	CH	41.9	CH	42.0	CH
	21	26.6*	CH_2	26.5*	CH_2	26.5*	CH_2
	22	37.5	CH_2	37.5	CH_2	37.4	CH_2
	23	24.1	CH_3	24.4	CH_3	24.4	CH_3
	24	177.6	C	176.8‡	C	176.8‡	C
	25	13.4	CH_3	14.6	CH_3	14.6	CH_3
	26	17.2†	CH_3	17.1†	CH_3	17.0†	CH_3
	27	24.3	CH_3	24.2	CH_3	24.2	CH_3
	28	176.9	C	176.7‡	C	176.7‡	C
	29	27.0	CH_3	26.7	CH_3	26.8	CH_3
	30	16.6†	CH_3	16.5†	CH_3	16.5†	CH_3
	24-CO_2Me	50.9	CH_3	50.9	CH_3	50.9	CH_3
Sugar moiety	1′	95.6	CH	95.5	CH	95.3	CH
	2′	73.9	CH	73.7	CH	73.0	CH
	3′	78.2	CH	77.7	CH	88.6	CH
	4′	71.0	CH	70.8	CH	70.1	CH
	5′	78.8	CH	78.6	CH	78.8	CH
	6′	72.5	CH_2	72.4	CH_2	61.8	CH_2
	OMe	59.1	CH_3	59.0	CH_3	60.8	CH_3

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

The structure of compound **2a** was further supported by the ^{1}H NMR spectrum which indicated the presence of five singlet methyl groups at δ 0.86, 1.17, 1.36, 1.52, and 1.64 (each 3H, *s*), one doublet methyl group at δ 1.02 (3H, *d*, $J = 7.0$ Hz), two methoxyl singlets at δ 3.34 and 3.59 (each 3H, *s*), one olefinic proton signal at δ 5.53 (1H, *br s*, H-12), one sharp singlet at δ 2.89 (1H, *s*, H-18) and a broad doublet at δ 3.27 (1H, H-3α). Moreover, the anomeric proton signal at δ 6.17 (1H, *d*, $J = 8.0$ Hz) in the ^{1}H NMR spectrum indicated the β-configuration for the methylglucopyranosyl moiety.

The EI mass spectral fragmentation pattern of **2a** provided useful information which allowed us to decide whether the position of the β-methylglucopyranosyl ester was located at the 24- or 28-COOH. The prominent fragment ion peak appeared at *m/z* 516 [M − methylglucopyranosyl]$^+$ which fragmented to the ions at *m/z* 252 and 264 due to the typical retro-Diels–Alder reaction of ring-C of Δ^{12}-ursene triterpenes indicated that the ester methyl group was located at 24-COOH [11, 16], and that the β-methylglucopyranosyl group must be located at the 28-COOH.

Long-range correlations between ^{1}H and ^{13}C nuclei were established by the HMBC experiment which provided further conclusive evidence to determine the location of the 6′-*O*-methyl-β-D-glucopyranosyl ester. The signal of C-24 in the ^{13}C NMR spectrum at δ 177.6 (C) correlated with the corresponding protons in the ^{1}H NMR spectrum at δ 3.34 (24-ester methyl group) and 3.27 (H-3α). These results suggested that the ester methyl group was connected at C-24 position. Consequently, the 6′-*O*-methyl-β-D-glucopyranosyl group must be linked at C-28 position.

In conclusion, the structure of compound **2a** was clarified as 3β,19α-dihydroxyurs-12-en-24,28-dioic acid-24-methyl ester-28-*O*-(6′-*O*-methyl-β-D-glucopyranosyl) ester. Therefore, the natural compound should be 3β,19α-dihydroxyurs-12-en-24,28-dioic acid-28-*O*-(6′-*O*-methyl-β-D-glucopyranosyl) ester (**2**).

Compound **4a**, another amorphous powder, revealed the presence of hydroxyl groups (3396 cm^{-1}), ester carbonyl groups (1730 and 1719 cm^{-1}), trisubstituted double bond (1648 cm^{-1}) and glycosidic linkage (1068 cm^{-1}) in its IR spectrum. The positive FAB mass spectrum showed a molecular ion peak at *m/z* 708 [M]$^+$ which suggested the molecular formula $C_{38}H_{60}O_{12}$ for **4a**, and this suggestion was further confirmed by the ^{13}C NMR and DEPT spectral data (Table 1). The signal at δ 95.5 (CH) and its ^{13}C NMR spectrum revealed an ester-linked glycosyl moiety [17]. Comparison of the ^{13}C NMR and DEPT spectral data with those of 2α, 3β, 19α-trihydroxyurs-12-en-24,28-dioic acid-28-*O*-β-D-glucopyranosyl ester (**3**) (trachelosperoside A-1), previously identified from the whole plant of *Trachelospermum asiaticum* [12], showed that their structures were very similar, except that the C-24 free carboxyl group (at δ 180.1 in **3**) was changed to CO_2CH_3 (at δ 176.8 and 50.9 in **4a**), and an additional methoxyl group in the C-6′ position of the glucosyl moiety which caused the resonance of C-6′ shifted downfield by 10 ppm (from δ 62.4 in **3** to 72.4 in **4a**) [15]. Furthermore, the ^{1}H NMR spectrum also showed that the methylglucopyranosyl group was linked with the aglycone in the β-configuration (at δ 6.16, 1H, *d*, $J = 7.2$ Hz). The EI mass spectrum of **4a** showed peaks at *m/z* 532 [M − methylglucosyl]$^+$, 514, 486, 414, 268, 264, 250, 246 and 201. The characteristic retro-Diels–Alder fragment peaks at *m/z* 268 (A/B-ring) and 264 (D/E-ring) indicated that the ester methyl group of **4a** was located on the C-24 carboxyl [11, 16]. Therefore, the position of the sugar moiety of **4a** must be on the C-28 carboxyl group. It follows that the complete structure of compound **4a** would be 2α, 3β, 19α-trihydroxyurs-12-en-24,28-dioic acid-24-methyl ester-28-*O*-(6′-*O*-methyl-β-D-glucopyranosyl) ester, and that the related naturally occurring compound should be 2α,3β,19α-trihydroxyurs-12-en-24,28-dioic acid-28-*O*-(6′-*O*-methyl-β-D-glucopyranosyl) ester (**4**).

Compound **5a** was obtained also as an amorphous powder. The IR spectrum indicated the presence of hydroxyl groups (3429 cm^{-1}), ester carbonyl groups (1727 and 1717 cm^{-1}), trisubstituted double bond (1640 cm^{-1}) and glycosidic linkage (1064 cm^{-1}). The positive FAB mass spectrum also showed a molecular ion peak at *m/z* 708 [M]$^+$ which, in consideration with the ^{13}C NMR and DEPT spectral data (Table 1), suggested the molecular formula $C_{38}H_{60}O_{12}$ for **5a**. The ^{13}C NMR and DEPT spectra of **5a** displayed almost the same signals as those recorded for 2α,3β,19α-trihydroxyurs-12-en-24,28-dioic acid-28-*O*-β-D-glucopyranosyl ester (**3**) (trachelosperoside A-1) [12], except the peak at δ 88.6 (CH) corresponding to the C-3′ carbon of the sugar residue, which was shifted 9.7 ppm (from δ 78.9 in **3** to 88.6 in **5a**) to lower fields because of its etherification. Moreover, the seven carbon signals of the sugar moiety observed in this spectrum corresponded almost exactly to those of 3′-*O*-β-D-methylglucopyranose [15]. In the ^{1}H NMR spectrum of **5a**, the signal at δ 6.21 (1H, *d*, $J = 8.2$ Hz) supported the β-configuration of the methylglucopyranosyl group. The characteristic retro-Diels–Alder fragment ion peaks at *m/z* 268 (A/B-ring) and 264 (D/E-ring) in the EI mass spectrum also indicated that the ester methyl group of **5a** was located on the C-24 carboxyl [11, 16], then the position of the methylglucopyranosyl group must be on the C-28 carboxyl group. From the above evidence, the structure of compound **5a** was proved to be 2α, 3β, 19α-trihydroxyurs-12-en-24,28-dioic acid-24-methyl ester-28-*O*-(3′-*O*-methyl-β-D-glucopyranosyl) ester, and the naturally occurring compound to be 2α,3β,19α-trihydroxyurs-12-en-24,28-dioic acid-28-*O*-(3′-*O*-methyl-β-D-glucopyranosyl) ester (**5**).

It has been reported that the compounds of β-glycosyl esters of A-ring oxygenated 19α-hydroxyursolic acids have the chemotaxonomic significance for the

genus *Rubus* [4]. Thus, the isolation of **2**, **4** and **5** provides interesting support for this point. It also should be noted that this is the first example of the isolation of 19α-hydroxyursolic dioic acids saponins possessing a 24-carboxyl group from the genus *Rubus*.

EXPERIMENTAL

General. Mps: uncorr.; IR (film, MeOH) and optical rotations were recorded 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 performed 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. pileatus* 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. piletatus* (2.5 kg) were extracted successively with 95% EtOH (3 × 5 l each time) at room temp. and the EtOH extracts were taken to dryness *in vacuo* to give the EtOH extract (210 g). A suspension of the resulting extract in H_2O was washed × 3 with petrol (2.0 l each time) and then extracted with *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 fr. (45 g) which was chromatographed on silica gel (160–200 mesh) using a step gradient of $CHCl_3$–CH_3OH–H_2O (60:10:1 to 10:10:1) and finally CH_3OH to give six frs. (I–VI). Fr. III (eluent of $CHCl_3$–CH_3OH–H_2O, 25:5:1) (6 g) was then subjected to CC over highly porous resin (SIP 1300) eluting with H_2O–EtOH (1:0 to 0:1). The 60% EtOH eluent (3.8 g) was further passed through a Sephadex LH-20 column eluted with H_2O–CH_3OH (1:0 to 0:1) and the eluent containing glycosides (2.0 g), which showed a shuttle-like spot in the silica gel TLC plate (R_f = 0.20, $CHCl_3$–CH_3OH–H_2O, 25:5:1), was treated with an ethereal soln of CH_2N_2 to yield methyl esters. The methyl esters were chromatographed repeatedly on silica gel using a solvent system of $CHCl_3$–CH_3OH–H_2O (40:5:1) to afford compounds **1a** (250 mg, R_f = 0.48, $CHCl_3$–CH_3OH–H_2O, 25:5:1) and **2a** (100 mg, R_f = 0.60, $CHCl_3$–CH_3OH–H_2O, 25:5:1). Fr. IV (eluent of $CHCl_3$–CH_3OH–H_2O, 20:5:1) (5.2 g) was isolated with the same procedures as those for fr. III to give **3a** (320 mg, R_f = 0.35, $CHCl_3$–CH_3OH–H_2O, 25:5:1) and a mixt. of **4a** and **5a**. The mixt. was then sepd by prep. reversed phase HPLC (ODS column, eluted with CH_3OH–H_2O, 13:7) to yield compounds **4a** (40 mg, R_f = 0.52, $CHCl_3$–CH_3OH–H_2O, 25:5:1) and **5a** (35 mg, R_f = 0.51, $CHCl_3$–CH_3OH–H_2O, 25:5:1).

3β,19α-*Dihydroxyurs*-12-*en*-24,28-*dioic acid*-24-*methyl ester*-28-O-(6′-O-*methyl*-β-D-*glucopyranosyl*) *ester* (**2a**). Amorphous powder, mp. 171–173°. $[\alpha]_D^{20}$ +29° (MeOH, *c* 0.65). FABMS (negative), *m/z*: 691 $[M-1]^-$, 515 [M-methylglucosyl-1]$^-$. EIMS, *m/z* (rel. int.): 516 [M-methylglucosyl]$^+$ (9), 498 (9), 470 (63), 452 (5), 398 (31), 264 (37), 252 (56), 246 (46), 234 (100), 218 (26), 201 (70), 187 (42), 175 (40), 146 (49). ^{1}H NMR: δ 0.86, 1.17, 1.36, 1.52, 1.64 (3H each, *s*, Me-23, 25, 26, 27, 29), 1.02 (3H, *d*, *J* = 7.0 Hz, Me-30), 2.89 (1H, *s*, H-18), 3.27 (1H, *br d*, *J* = 12.5 Hz, H-3α), 3.34, 3.59 (3H each, 24-CO_2Me, 6′-OMe), 3.79–4.33 (*m*, H-2′, 3′, 4′, 5′), 5.14 (1H, *s*, OH-19), 5.53 (1H, *br s*, H-12), 6.17 (1H, *d*, *J* = 8.0 Hz, H-1′). ^{13}C NMR and DEPT: see Table 1).

2α,3β,19α-*Trihydroxyurs*-12-*en*-24,28-*dioic acid*-24-*methyl ester*-28-O-(6′-O-*methyl*-β-D-*glucopyranosyl*) *ester* (**4a**). Amorphous powder, mp 193–195°. $[\alpha]_D^{20}$ +24° (MeOH, *c* 0.32). FABMS (positive), *m/z*: 708 $[M]^+$, 690 $[M-H_2O]^+$, 533 [M − methyl − glucosyl + 1]$^+$. EIMS, *m/z* (rel. int.): 532 [M − methyl − glucosyl]$^+$ (3), 514 (13), 486 (44), 414 (25), 268 (4), 264 (32), 250 (100), 246 (43), 201 (79). ^{1}H NMR: δ 0.96, 1.15, 1.33, 1.60, 1.62 (3H each, *s*, Me-23, 25, 26, 27, 29), 1.01 (3H, *d*, *J* = 6.0 Hz, Me-30), 2.87 (1H, *s*, H-18), 3.05 (1H, *d*, *J* = 9.5 Hz, H-3α), 3.33, 3.59 (3H each, 24-CO_2Me, 6′-OMe), 3.94–4.50 (*m*, H-2′, 3′, 4′, 5′), 4.73 (1H, *br t*, H-2β), 5.16 (1H, *s*, OH-19), 5.49 (1H, *br s*, H-12), 6.16 (1H, *d*, *J* = 7.2 Hz, H-1′). ^{13}C NMR and DEPT: see Table 1.

2α,3β,19α-*Trihydroxyurs*-12-*en*-24,28-*dioic acid*-24-*methyl ester*-28-O-(3′-O-*methyl*-β-D-*glucopyranosyl*) *ester* (**5a**). Amorphous powder, mp. 180–182°. $[\alpha]_D^{20}$ +13° (MeOH, *c* 0.30). FABMS (positive), *m/z*: 708 $[M]^+$, 690 $[M-H_2O]^+$, 533 [M − methyl − glucosyl + 1]$^+$. EIMS, *m/z* (rel. int.): 532 [M − methyl − glucosyl]$^+$ (8), 514 (17), 486 (92), 414 (51), 268 (6), 264 (56), 250 (100), 246 (57), 201 (55). ^{1}H NMR: δ 0.94, 1.21, 1.36, 1.59, 1.63 (3H each, *s*, Me-23, 25, 26, 27, 29), 1.04 (3H, *d*, *J* = 6.0 Hz, Me-30), 2.87 (1H, *s*, H-18), 3.34 (1H, *d*, *J* = 9.2 Hz, H-3α), 3.57, 3.67 (3H each, 24-CO_2Me, 3′-OMe), 3.80–4.40 (*m*, H-2′, 3′, 4′, 5′), 4.72 (1H, *br t*, H-2β), 5.20 (1H, *s*, OH-19), 5.50 (1H, *br s*, H-12), 6.21 (1H, *d*, *J* = 8.2 Hz, H-1′). ^{13}C NMR and DEPT: see Table 1.

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