New Triterpenoid Saponins from the Roots of Potentilla tormentilla

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NEW TRITERPENOID SAPONINS FROM THE ROOTS OF *POTENTILLA TORMENTILLA*

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ABSTRACT.—The triterpene fractions of the dried roots of *Potentilla tormentilla* were investigated and three new compounds were isolated. The new structures were established by detailed nmr spectral studies as 3α,19α-dihydroxyurs-12-en-28-oic acid-(28→1)-β-D-glucopyranosyl ester [**1**], 2α,3β-dihydroxyurs-12-en-28-oic acid-(28→1)-β-D-glucopyranosyl ester [**2**], and 3β,19α-dihydroxyolean-12-en-28-oic acid-(28→1)-β-D-glucopyranosyl ester [**3**].

As part of our study on the constituents of plants of the Rosaceae (1,2), we report herein the results of a chemical investigation of the dried rhizomes and roots of *Potentilla tormentilla* Stokes (syn. *Potentilla erecta* Rautchel) and the isolation of three new compounds. This plant is used in traditional medicine of Europe for the treatment of diarrhea, leukorrhea, and tuberculosis; anti-inflammatory, diaphoretic, tonic, and haemostatic properties are also known (3,4). Recently, biological investigations of the polar extracts have shown cytotoxic and tumor-inhibitory activity against breast, stomach, lip, and tongue cancers (5), and anti-allergic and immunostimulant activity with release of interferon (6). Previous phytochemical studies have led to the isolation of five catecholic and pyrogallic tannins (7–10) and two triterpenes, namely, ursolic and tormentic acids (11,12).

RESULTS AND DISCUSSION

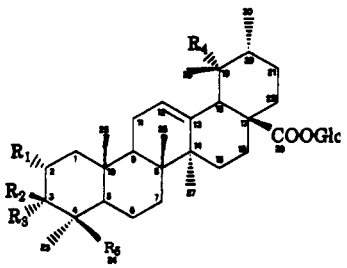
The plant material was powdered, defatted with petroleum ether and exhaustively extracted in a Soxhlet apparatus with $CHCl_3$, with a mixture of $CHCl_3$-MeOH (9:1), and finally with MeOH at room temperature.

The $CHCl_3$ extract yielded ursolic and tormentic acids, α-amyrin, lupeol, and three urs-12-en-28-oic acid derivatives, identified as 2α-hydroxyursolic acid, 2α,3α-dihydroxyurs-12-en-28-oic acid, and euscaphic acid.

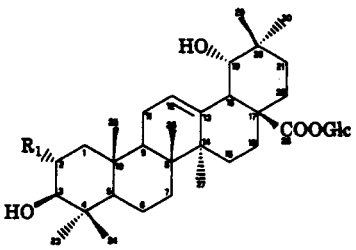
The $CHCl_3$-MeOH extract was fractionated by column chromatography (cc) on Sephadex LH-20 with MeOH. The portion containing the bulk triterpenes, when chromatographed on Si gel with $CHCl_3$/MeOH mixtures followed by low-pressure cc on Lichroprep RP-18 with MeOH/H_2O mixtures, yielded the three new compounds **1–3**, euscaphic acid 28-β-D-glucopyranosyl ester (kaji-ichigoside F-1) [**4**], and tormentic acid 28-β-D-glucopyranosyl ester (rosamultin) [**5**].

The fabms spectrum of **1**, in positive-ion mode, indicated a quasi-molecular peak $[M+H]^+$ at *m/z* 635 corresponding to the formula $C_{36}H_{58}O_9$ (confirmed by ^{13}C-nmr and DEPT analyses) and a prominent fragment peak at *m/z* 473 due to the loss of a hexose unit. The ir spectrum showed bands for tertiary hydroxyl (3500 cm^{-1}), hydroxyl (3330–3450 cm^{-1}), ester carbonyl (1730 cm^{-1}), trisubstituted double bond (1665 and 850 cm^{-1}), and *gem*-dimethyl (1378–1363 cm^{-1}) groups.

The ^{13}C-nmr spectrum of **1** revealed thirty-six carbon signals which were assigned using DEPT ^{13}C-nmr (Table 1). The Δ^{12-13} functionality of the triterpenoid skeleton was deduced from the resonance of the sp^2 carbons C-12 (tertiary carbon deduced by DEPT pulse sequence) at 129.3 ppm and C-13 (quaternary carbon) at 139.8 ppm, and their chemical shifts were useful to distinguish between urs-12-ene and olean-12-ene analogs (13,14). The comparison of the ^{13}C-nmr spectrum of **1** with those of the urs-12-en-28-



	R_1	R_2	R_3	R_4	R_5
1	H	H	OH	OH	CH_3
2	OH	OH	H	H	CH_3
4	OH	H	OH	OH	CH_3
5	OH	OH	H	OH	CH_3
7	OH	OH	H	OH	CH_2OH
8	H	OH	H	OH	COOH
9	OH	OH	H	OH	COOH



3 R_1=H
6 R_1=OH

oic acids isolated from the $CHCl_3$ extract showed a weak (ca. −3 ppm) upfield shift of the C-28 signal, and indicated that this carbon bore a sugar moiety. In addition, the shifts observed on the carbons of the sugar unit, particularly the values at 95.8 ppm of the anomeric carbon, were in agreement with a site of glycosylation at the 28-carboxyl group (15).

A detailed analysis of the ^{1}H-nmr spectrum confirmed the characteristic features for an urs-12-en-28-oic acid-(28→1)-β-D-glycosyl ester skeleton bearing one α-OH at C-19 (16,17). This spectrum was characterized by signals for seven methyls [six singlets (δ 0.70–1.25) and one doublet (δ 0.96, 3H, J=6 Hz, CH_3-30)], a one-proton broad singlet at δ 2.59 (H-18), a two-proton multiplet at δ 5.35 (H-12 and H-1′), and a signal at δ 2.62 (ddd with J=13.5, 13.5, and 4.5 Hz) due to the C-16 axial proton (18). COSY nmr experiments suggested the presence of α-OH substitution at C-3 from a signal at δ 3.40 (1H, t, J=3.4 Hz), whose chemical shift and splitting pattern were typical of an equatorial H-3 (16). This substitution was confirmed by the low-field ^{13}C-nmr signal at δ 76.2 (d) of C-3 and by the chemical shifts of C-1, C-2, C-4, C-5 at ppm 34.0 (t), 25.4 (t), 38.2 (s), and 50.2 (d), respectively (19).

The sugar moiety was identified as β-D-glucopyranose by comparison of the ^{13}C-nmr data with those of other urs-28-oic-β-glucopyranosyl esters (17). Alkaline hydrolysis of **1** afforded D-glucose and 3-*epi*-pomolic acid. Therefore **1** was 3α,19α-dihydroxyurs-12-en-28-oic acid-(28→1)-β-D-glucopyranosyl ester [3-*epi*-pomolic acid-(28→1)-β-D-glucopyranosyl ester], a new saponin.

Compound **2**, as **1**, showed a quasi-molecular peak $[M+H]^+$ at *m/z* 635 and a

TABLE 1. ^{13}C-Nmr Resonances of Compounds **1-3**.

Carbon	Aglycone			Carbon	Sugar Moiety		
	1	**2**	**3**		**1**	**2**	**3**
C-1	34.0 (CH_2)	46.8 (CH_2)	38.8 (CH_2)		Glucose	Glucose	Glucose
C-2	25.4 (CH_2)	69.0 (CH)	28.2 (CH)	C-1′	95.8	95.8	95.2
C-3	76.5 (CH)	83.7 (CH)	78.3 (CH)	C-2′	74.0	74.0	74.1
C-4	38.2 (C)	39.3 (C)	39.4 (C)	C-3′	78.5	78.5	78.0
C-5	50.2 (CH)	55.4 (CH)	56.0 (CH)	C-4′	71.4	71.4	71.3
C-6	19.9 (CH_2)	18.4 (CH_2)	18.9 (CH_2)	C-5′	78.4	78.4	77.7
C-7	34.2 (CH_2)	33.1 (CH_2)	33.5 (CH_2)	C-6′	62.7	62.7	62.4
C-8	38.9 (C)	39.9 (C)	40.0 (C)				
C-9	47.7 (CH)	47.8 (CH)	48.4 (CH)				
C-10	39.4 (C)	38.3 (C)	37.7 (C)				
C-11	24.9 (CH_2)	17.0 (CH_2)	24.2 (CH_2)				
C-12	129.3 (CH)	127.3 (CH)	123.4 (CH)				
C-13	139.8 (C)	138.8 (C)	144.2 (C)				
C-14	41.5 (C)	42.4 (C)	42.0 (C)				
C-15	29.7 (CH_2)	28.7 (CH_2)	29.0 (CH_2)[a]				
C-16	26.6 (CH_2)	24.5 (CH_2)	28.1 (CH_2)				
C-17	47.7 (C)	48.4 (C)	46.6 (C)				
C-18	55.9 (CH)	52.6 (CH)	44.7 (CH)				
C-19	73.7 (C)	39.6 (CH)	81.1 (CH)				
C-20	42.9 (C)	38.9 (C)	35.7 (C)				
C-21	26.6 (CH_2)	30.9 (CH_2)	33.5 (CH_2)				
C-22	38.2 (CH_2)	37.6 (CH_2)	34.9 (CH_2)[a]				
C-23	28.3 (CH_3)	28.8 (CH_3)	28.8 (CH_2)				
C-24	22.3 (CH_3)	17.4 (CH_3)	16.5 (CH_3)				
C-25	16.3 (CH_3)	17.9 (CH_3)	15.6 (CH_3)				
C-26	17.2 (CH_3)	17.9 (CH_3)	17.3 (CH_3)				
C-27	24.6 (CH_3)	24.6 (CH_3)	24.7 (CH_3)				
C-28	178.5 (C)	176.3 (C)	176.0 (C)				
C-29	27.2 (CH_3)	24.0 (CH_3)	28.7 (CH_3)				
C-30	17.9 (CH_3)	21.5 (CH_3)	24.9 (CH_3)				

[a]Assignments interchangeable in the same column.

prominent fragment peak at *m/z* 473 in the fabms spectrum as well as the same absorption bands in the ir spectrum. The ^{13}C-nmr spectrum of **2** revealed thirty-six carbon signals which were sorted out by DEPT ^{13}C-nmr (Table 1). The 12–13 double bond of an urs-12 ene skeleton was indicated from the resonances of the sp^2 carbons C-12 at 127.3 ppm and C-13 (quaternary carbon) at 138.8 ppm (13,14). The signals of the quaternary carbons C-28 and C-1′ at 176.3 and 95.8 ppm indicated, also for this compound, a 28-carboxyl group esterified with a glycosyl moiety (15).

A detailed analysis of the ^{1}H-nmr spectrum confirmed the characteristic features for an urs-12-en-glycosyl-ester skeleton bearing one α-hydroxyl at C-2 (20). This spectrum showed five tertiary (δ 0.72–1.04) and two secondary methyl groups (δ 0.89 and 1.02 with J=6.0 and 6.2 Hz, respectively), with a multiplet at δ 5.30 due to H-12 and H-1′ found also in **1**. In addition, a single proton doublet (J=10 Hz) at δ 2.22 was present whose splitting and chemical shift were characteristic of a H-18 belonging to an ursane-type triterpenoid (21). Finally, the two signals correlating in the ^{1}H-^{1}H COSY nmr spectrum at δ 3.80 (1H, ddd, J=11.0, 9.8, and 4.5 Hz) and at δ 2.99 (1H, d, J=9.8 Hz) of H-2 and H-3, respectively, suggested diequatorial substitution of the OH groups at the C-2 and C-3 positions (22). The sugar moiety was again identified as β-D-glucopyranose as described for **1**. Therefore, **2** was the new saponin 2α,3β-dihydroxyurs-12-en-28-oic acid-(28→1)-β-D-glucopyranosyl ester.

Compound **3** gave the quasi-molecular peak $[M+H]^+$ at *m/z* 635 in the fabms spectrum corresponding to the formula $C_{36}H_{58}O_9$ (confirmed by ^{13}C-nmr and DEPT

analyses) and the same fragment peak at *m/z* 473 previously found in **1** and **2**. The ir spectrum was similar to **1** and **2**, without any bands attributed to tertiary hydroxyl groups.

The ^{13}C-nmr spectrum of **3** revealed thirty-six carbon signals which were interpreted by DEPT ^{13}C nmr (Table 1). The 12–13 double bond of an olean-12-ene skeleton was assigned from the resonances of the sp^2 C-12 at 123.4 ppm and C-13 (quaternary carbon) at 144.2 ppm (13,14). The signals of the quaternary carbons C-28 and C-1′ at 176.0 and 95.2 ppm indicated the presence of a 28-carboxyl group esterified with a glycosyl moiety (15) as in **1** and **2**.

A detailed analysis of the ^{1}H-nmr spectrum confirmed the characteristic features for an olean-12-en-glycosyl-ester skeleton bearing one α-hydroxyl at C-19 (21). The spectrum showed seven tertiary methyl groups (0.77–1.23 ppm) and a multiplet at δ 5.30 due to H-12 and H-1′. The carbinolic region (3.08–3.72 ppm), in addition to the signals of the sugar protons, revealed a double doublet at δ 3.38 (J=4.5 and 11.5 Hz), a broad doublet at δ 3.45 and a multiplet at δ 3.57 which correlated in HETCOR experiments with the ^{13}C-nmr resonances at 78.3, 81.1, and 44.7 ppm, respectively. These ^{1}H- and ^{13}C-nmr chemical shifts were in agreement with a 3β,19α-dihydroxy substitution pattern, where the α-orientation of the OH at C-19 was derived by the H-18/H-19 coupling constant $J_{ax/eq}$=3.5 Hz (21). Also for this compound, the sugar moiety was identified as β-D-glucopyranose (17) and alkaline hydrolysis afforded D-glucose and 3β,19α-dihydroxyolean-12-en-28-oic acid. Therefore **3** was the new saponin 3β,19α-dihydroxyolean-12-en-28-oic-(28→1)-β-D-glucopyranosyl ester.

The *n*-BuOH-soluble part of the MeOH extract was subjected to Sephadex LH-20 cc to provide a crude glycosidic fraction. This mixture was purified by reversed-phase cc on Lobar to give four triterpenoid esters: 2α,3β,19α-trihydroxyolean-12-en-28-oic acid-(28→1)-β-D-glucopyranosyl ester (arjunetin) [**6**], 24-hydroxytormentic acid-(28→1)-β-D-glucopyranosyl ester [**7**], 3β,19α-dihydroxyurs-12-en-24,28-dioic acid-(28→1)-β-D-glucopyranosyl ester [**8**], and 2α,3β,19α-trihydroxyurs-12-en-24,28-dioic acid-(28→1)-β-D-glucopyranosyl ester (trachelosperoside A-1) [**9**].

Derivatives of α- and β-amyrin are common constituents of the plants of the Rosaceae family: euscaphic acid was isolated from *Pygeum acuminatum* and from the genus *Rosa* (23,24); 2α-hydroxyursolic acid and 2α,3α-dihydroxyurs-12-en-28-oic acid from some species of the genus *Prunus* (23); kaji-ichigoside F-1 [**4**] and rosamultin [**5**] from plants of the genera *Rosa, Tormentilla, Rubus,* and *Sanguisorba* (23–29). Recently, arjunetin [**6**] was found in *Rubus ellipticus* and 24-hydroxytormentic acid-(28→1)-*O*-β-D-glucopyranosyl ester [**7**] in *Rubus accuminatus* (29). Compounds **8** and **9** were previously isolated from species of the Apocynaceae and the Vochysiaceae (30–32).

Ursolic acid and its derivatives have shown a significant activity against P-388 and L-1210 lymphocytic leukemia cells as well as the human lung carcinoma cell line, A-549. Activities against KB, human colon (HCTH-8), and mammary (MCF-7) tumor cells have also been reported. Further investigations proved that esterification of the -OH group at C-3 and/or of the -COOH group at C-17 decreases the cytotoxicity in human tumor cell lines, but increases activity against the growth of L-1210 and P-388 leukemic cells (33). These biological studies indicate that the antitumor activity of the plant could be due to the presence of the triterpenes previously described.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The following instruments were used: nmr, Bruker AC-200 Spectrospin spectrometer; fabms spectra in positive-ion mode in a glycerol matrix, VG ZAB instrument; optical rotation, Perkin Elmer 241 polarimeter at 20°; low-pressure cc, Duramat pump using a Lichroprep RP-18 column. Ir spectra were determined with a Perkin-Elmer spectrophotometer model 684. Tlc was carried out on Si gel 60 F 254 coated Al sheets and RP-18 hptlc plates (Merck). Compounds were visualized

by spraying with cerium sulphate/H_2SO_4 and Godin reagents. One- and two-dimensional nmr spectra were measured as described previously (1).

PLANT MATERIAL AND EXTRACTION.—The dried commercial roots and rhizomes of *P. tormentilla* (800 g), kindly supplied by D.^{CO} Ulrich S.p.A. (Nichelino, TO, Italy), were powdered, defatted with petroleum ether, and exhaustively extracted in a Soxhlet apparatus with $CHCl_3$, $CHCl_3$-MeOH (9:1), and then extracted at room temperature with MeOH.

ISOLATION AND CHARACTERIZATION.—Cc of the $CHCl_3$ extract (5.6 g) over SiO_2 (Si gel 60, Merck) gave, on elution with $CHCl_3$ containing increasing amounts of MeOH (0 to 15%), fractions of impure compounds. Further cc on SiO_2 or Al_2O_3 (neutral alumina, Merck) of these mixtures yielded α-amyrin (15.9 mg), lupeol (12.3 mg), and the following acids: ursolic (38 mg), euscaphic (59 mg), tormentic (63 mg), 2α-hydroxyursolic (13 mg), and 2α,3α-dihydroxyurs-12-en-28-oic (19 mg).

The $CHCl_3$-MeOH extract (6.7 g) was filtered with MeOH over Sephadex LH-20 cc to obtain fractions containing triterpenes. These were subjected to cc on Si gel with $CHCl_3$/MeOH mixtures of increasing polarity (from 90 to 70%), followed by low-pressure cc on RP-8 or RP-18 with MeOH/H_2O mixtures, to yield compound **1** (35 mg), **2** (33 mg), **3** (22 mg), kaji-ichigoside F-1 [**4**] (58 mg), and rosamultin [**5**] (86 mg).

The MeOH extract (25 g) was evaporated *in vacuo* and the residue partitioned between H_2O (400 ml) and *n*-BuOH (600 ml). The organic portion was evaporated *in vacuo* to give a residue (3.95 g), which was subjected to gel filtration over Sephadex LH-20 to obtain three fractions of bulk saponins. These were purified by flash Si gel cc or Lobar Lichroprep RP-18 cc to yield the following compounds: arjunetin [**6**] (22 mg), 24-hydroxytormentic acid-(28→1)-β-D-glucopyranosyl ester [**7**] (14 mg), 3β,19α-dihydroxyurs-12-en-24,28-dioic acid-(28→1)-β-D-glucopyranosyl ester [**8**] (11 mg), and trachelosperoside A-1 [**9**] (18 mg).

HYDROLYSES OF THE SAPONINS.—The glucosyl esters (10 mg) were dissolved in *n*-BuOH (0.74 ml) and added to NaOH (145 mg). The reaction mixtures were quenched by addition of H_2O (2 ml), neutralized with aqueous HCl, and extracted with $CHCl_3$. The $CHCl_3$ layers were evaporated *in vacuo* to yield extracts which were purified by chromatography on Si gel using $CHCl_3$ as eluent to yield the aglycones that were identified by ^{1}H-nmr data by comparison with those of authentic samples. The aqueous layers obtained from the hydrolysis were neutralized with Amberlite IRA 400 (OH^- type) and evaporated to dryness. The sugar residues were directly analyzed by co-tlc on Si gel 60 F_{254} (Merck) with authentic samples using EtOAc-H_2O-MeOH-HOAc (13:3:3:4) as eluent; the detections were performed with *p*-anisidine phthalate and naphthoresorcinol reagents. In all cases only glucose was detected and identified by chromatographic comparisons with an authentic sample.

3-epi-*Pomolic acid-(28→1)-β-D-glucopyranosyl ester* [**1**].—[α]D +7° (*c*=0.7, MeOH); ir ν max 3500, 3450–3330, 3029, 2933, 1731, 1665, 1458, 1378, 1365, 1149, 1048, 1030, 970, 900 cm^{-1}; fabms *m/z* 635 $[M+H]^+$, 473 [*m/z* 635-glucose]$^+$; ^{1}H nmr (200 MHz, MeOH-d_4) δ 0.70 (3H, s, Me-26), 0.83 (3H, s, Me-23), 0.91 (3H, s, Me-24), 0.96 (3H, d, *J*=6.0 Hz, Me-30), 0.97 (3H, s, Me-25), 1.22 (3H, s, 27-Me), 1.25 (3H, s, 29-Me), 2.59 (1H, br s, H-18), 2.62 (1H, ddd, *J*=13.5, 13.5 and 4.5 Hz, H-16α), 3.40 (1H, t, *J*=3.5Hz, H-3), 5.30 (1H, t, *J*=3.8 Hz, H-12), 5.35 (1H, d, *J*=7.8 Hz, H-1′); ^{13}C nmr (50 MHz, MeOH-d_4) see Table 1.

2α,3β-Dihydroxyurs-12-en-28-oic acid-(28→1)-β-D-glucopyranosyl ester [**2**].—[α]D +12° (*c*=0.4, MeOH); ir ν max 3450–3330, 3029, 2933, 1731, 1665, 1458, 1378, 1365, 1149, 1048, 1030, 970, 900 cm^{-1}; fabms *m/z* 635 $[M+H]^+$, 473 [*m/z* 635-glucose]$^+$; ^{1}H nmr (200 MHz, MeOH-d_4) δ 0.72 (3H, s, Me-26), 0.85 (3H, s, Me-23), 0.89 (3H, d, *J*=6.0 Hz, Me-30), 0.94 (3H, s, Me-24), 1.02 (3H, d, *J*=6.0 Hz, Me-29), 1.04 (3H, s, 27-Me), 1.09 (3H, s, Me-25), 2.22 (1H, d, *J*=10 Hz, H-18), 2.99 (1H, d, *J*=9.8 Hz, H-3), 3.80 (1H, ddd, *J*=11.0, 9.8, and 4.5 Hz, H-2), 5.27 (1H, t, *J*=3.5 Hz, H-12), 5.30 (1H, d, *J*=7.6 Hz, H-1′); ^{13}C nmr (50 MHz, MeOH-d_4) see Table 1.

3β,19α-Dihydroxyolean-12-en-28-oic acid-(28→1)-β-D-glucopyranosyl ester [**3**].—[α]D +18° (*c*=1.1, pyridine); ir ν max 3400–3300, 1725, 1665, 1615, 1378, 1160, 1060, 850 cm^{-1}; fabms *m/z* 635 $[M+H]^+$, 473 [*m/z* 635-glucose]$^+$; ^{1}H nmr (200 MHz, pyridine-d_5) δ 0.88 (3H, s, Me-26), 0.92 (3H, s, Me-24), 1.02 (3H, s, Me-25), 1.05 (3H, s, Me-30), 1.18 (3H, s, Me-23), 1.33 (3H, s, Me-27), 1.65 (3H, s, Me-29), 3.38 (1H, dd, *J*=4.5 and 11.5 Hz, H-3), 3.45 (1H, d, *J*=3.3 Hz, H-18), 3.57 (1H, m, H-19), 5.47 (1H, t, *J*=3.4 Hz, H-12), 6.11 (1H, d, *J*=6.3 Hz, OH-19), 6.38 (1H, d, *J*=8.1 Hz, H-1′); ^{13}C nmr (50 MHz, pyridine-d_5) see Table 1.

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