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Triterpenoids from the Roots of *Rubus parvifolius*

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Abstract □ From the roots of *Rubus parvifolius* L., four triterpenoidal sapogenins, ursolic acid **1**, 2α-hydroxyursolic acid **2**, euscapic acid **3**, 2α, 3β, 19α-trihydroxyurs-12-en-23,28-dioic acid **4** and one triterpenoidal glycoside, suavissimoside R_1 **5**, were isolated. The structures were elucidated by spectroscopic methods and chemical transformations. Compound **4** was first isolated as free form.

Keywords □ *Rubus parvifolius*, L. Rosaceae, ursolic acid, 2α-hydroxyursolic acid, euscapic acid, 2α, 3β, 19α-trihydroxyurs-12-en-23,28-dioic acid, suavissimoside R_1

In the preceding paper[1] we reported the isolation and characterization of (−)−epicatechin and a sterol mixture from the roots of this plant. In a continuation of phytochemical work, ursolic acid **1**, 2α-hydroxyursolic acid **2**, euscapic acid **3**, 2α, 3β, 19α-trihydroxyurs-12-en-23,28-dioic acid **4**, and suavissimoside R_1 **5** were isolated as described in the experimental section. The present paper deals with their isolation and structural elucidation.

EXPERIMENTAL METHODS

All m.ps. were determined on Köfler melting point apparatus and are uncorrected. The IR spectra were determined in KBr tablets on a Mattson Polaris FT-IR spectrophotomer. The ^{1}H-NMR (300 MHz) and ^{13}C-NMR (75.5 MHz) were recorded with a Bruker AM 300 using TMS as an internal standard and chemical shifts are given as δ (ppm). The MS spectra were obtained with a Kratos MS 25 RFA GC/MS spectrometer.

Plant material

The plant material was collected near Taegu city (Korea) during the summer season of 1988, and authenticated by Prof. Jong Weon Kim, College of Pharmacy, Hyosung Women's University, Korea. A voucher specimen is deposited in College of Pharmacy, Yeungnam University.

Extraction and isolation

The fresh roots (3.2 kg) of *Rubus parvifolius* were extracted with hot MeOH to give an extract (230 g), partitioned between $CHCl_3$-H_2O and *n*-BuOH-H_2O successively. The $CHCl_3$ soluble part (50 g) was chromatographed on a silica gel column eluted with $CHCl_3$-MeOH solvent system, yielding ursolic acid **1**, 2α-hydroxyursolic acid **2**, euscapic acid **3** as well as 2α, 3β, 19α-trihydroxyurs-12-en-23,28-dioic acid **4**. The *n*-BuOH soluble part (35 g) was also subjected to silica gel column with EtOAc satd. with H_2O-MeOH (gradient) to give suavissimoside R_1 **5**).

Ursolic acid, 1

Colorless needless from MeOH: mp. 286-289℃; IR ν_{max}^{KBr} cm^{-1} 3415 (OH), 1695 (acid), 1650, 815 (trisubstituted double bond); ^{1}H-NMR (pyridine-d_5) δ 0.90, 1.02, 1.04, 1.23 and 1.28 (3H each, s, CH_3), 0.97 and 0.99 (3H each, d, J=5.7 and 7.2 Hz, CH_3), 2.63 (1H, d, J=11.4 Hz, H-18), 3.45 (1H, dd, J=3.0, 7.7 Hz, H-3), 5.49 (1H, t, J=3.3 Hz, H-12); ^{13}C-NMR: see Table I; Anal. Calcd. for $C_{30}H_{48}O_3$: C 78.40, H 10.59; Found: C 78.68, H 10.73.

2α-Hydroxyursolic acid, 2

White amorphous from MeOH; mp. 255-256℃; IR ν_{max}^{KBr} cm^{-1} 3420 (OH), 1690 (acid), 1640, 810 (trisubstituted double bond); MS *m/z* (rel. int.) 472 (M^+, 4.9), 454 (M-H_2O, 2.8), 426 (M-HCOOH, 7.7),

Table I. ^{13}C-NMR spectral data of triterpenoids isolated form *Rubus parvifolius* and related compounds*

Carbon No.	1[-]	2[-]	3[-]	4[+]	5[+]	6[+,+]	7[-,-]
C-1	39.3	48.2	42.7	48.1[a]	48.0	38.9	41.8
C-2	28.0	68.6	66.1	68.6	68.5	27.2	66.5
C-3	78.5	83.9	79.2	81.1	80.8	79.0	79.0
C-4	39.5[a]	39.9	38.7[a]	54.5	54.6	38.6[a]	38.2[a]
C-5	55.9	56.0	48.6	52.2	52.1	55.2	48.0
C-6	18.9	18.9	18.6	21.4	21.3	18.3	18.1
C-7	33.7	33.6	33.4	33.3	33.1	33.0	32.6
C-8	39.6[a]	39.6[a]	40.5	40.5	40.5	39.5	40.1
C-9	48.2	48.0	47.5	48.3[a]	48.4	47.6	46.9
C-10	37.7	38.5	38.6[a]	38.4	38.4	37.0	38.3[a]
C-11	23.8	23.8	24.0	24.1	24.0	23.3	23.7
C-12	125.7	125.6	128.0	127.8	128.0	125.6	129.0
C-13	139.3	139.4	139.8	140.0	139.1	138.1	138.2
C-14	40.1	40.1	42.1	42.3[b]	41.9	42.0	41.3
C-15	28.8	28.7	29.1	29.2	28.9	28.1	28.2
C-16	25.0	25.0	26.3	26.4	25.8	24.2	25.5
C-17	42.6	42.6	48.6	48.6	48.4	48.1	47.2
C-18	53.7	53.6	54.5	54.5	54.2	52.9	53.2
C-19	39.4[a]	39.5[a]	72.7[a]	72.8	72.5	39.0	73.2
C-20	39.4[a]	39.5[a]	42.3	42.1[b]	41.9	38.7[a]	41.1
C-21	31.3	31.2	26.9	27.0	26.5	30.7	26.0
C-22	37.5	37.5	38.3	38.6	37.5	36.6	37.4
C-23	28.9	29.4	29.3	180.7	180.0	28.0	28.5
C-24	16.5	17.9[b]	22.2	13.5	13.2	15.4[b]	21.8
C-25	15.8	17.0	16.7[b]	17.3[c]	17.3[a]	15.6[b]	16.2[b]
C-26	17.6	17.5[b]	17.2	17.2[c]	17.2[a]	16.9[c]	16.7
C-27	24.1	25.0	24.6	24.7	24.3	23.6	24.7
C-28	180.0	180.0	181.0	180.0	176.8	178.1	178.4
C-29	21.6	21.4	27.1	27.2	26.8	17.0[c]	27.4
C-30	17.5	17.5	16.6[b]	16.7	16.5	21.2	16.1[b]
OCH_3						51.4	51.5
C-1′					95.6		
C-2′					73.8		
C-3′					78.7		
C-4′					71.0		
C-5′					79.0		
C-6′					62.2		

*Chemical shifts were reported in ppm from TMS.
[+]In pyridine-d_5, [-,-] in $CDCl_3$
[a,b,c]Assignments may be reversed in each column.

409 (M-COOH-H_2O, 2.0), 248 (D/E ring, 100), 203 (D/E ring-COOH, 71.6); ^{1}H-NMR (pyridine-d_5) δ 0.99, 1.04, 1.06, 1.21, 1.26 (3H each, s, CH_3), 0.96 and 0.99 (3H each, d, J=5.9 and 8.7 Hz, CH_3), 2.61 (1H, d, J=11.2 Hz, H-18), 3.37 (1H, d, J=9.0 Hz, H-3), 4.06 (1H, ddd, J=4.5, 9.0, 11.2 Hz, H-2), 5.46 (1H, t, J=3.1 Hz, H-12); ^{13}C-NMR: see Table I; Anal. Calcd. for $C_{30}H_{48}O_4$: C 76.27, H 10.17; Found: C 76.20, H 10.20.

***Euscapic acid*, 3**

White amorphous from MeOH: mp. 269-270℃; IR ν_{max}^{KBr} cm^{-1} 3429 (OH), 1691 (acid), 1643, 1030, 1000 (sec. OH), 930 (ter. OH), 830, 810 (trisubstituted

double bond): MS *m/z* (rel. int.) 488 (M^+, 7.5), 470 (M-H_2O, 3.8), 442 (M-HCOOH, 23.4), 455 (M-H_2O-CH_3, 4.4), 264 (D/E ring-H_2O, 35.2), 224 (A/B ring, 19.4), 219 (D/E ring-COOH, 26.3), 218 (D/E ring-HCOOH, 28.3), 201 (D/E ring-COOH-H_2O, 30.7); ^{1}H-NMR (pyridine-d_5) δ 0.92, 1.00, 1.14, 1.30, 1.45, 1.67 (3H each, s, CH_3), 1.15 (3H, d, J=6.6 Hz, CH_3), 3.05 (1H, s, H-18), 3.68 (1H, d, J=3.0 Hz, H-3), 4.21 (1H, dt, J=3.0, 7.7 Hz, H-2), 5.44 (1H, br. s, H-12); ^{13}C-NMR: see Table I; Anal. Calcd. for $C_{30}H_{48}O_5$: C 73.77, H 9.84; Found: C 73.69, H 9.88.

2α, 3β, 19α-Trihydroxyurs-12-en-23,28-dioic acid, 4

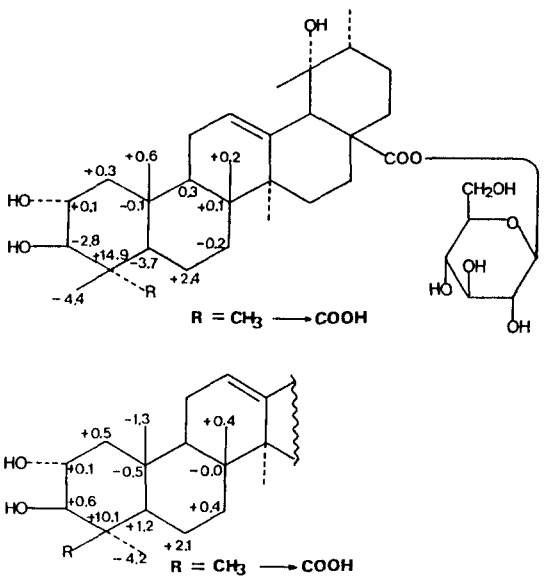
White needless from MeOH: mp. over 300°C; IR ν_{max}^{KBr} cm^{-1} 3410 (OH), 1695 (acid), 1650, 1030, 1010 (sec. OH), 935 (ter. OH), 810 (trisubstituted double bond); ^{1}H-NMR (pyridine-d_5) δ 1.03, 1.07, 1.36, 1.60, 1.63 (3H each, s, CH_3), 1.04 (3H, d, J=6.5 Hz, CH_3), 2.96 (1H, s, H-18), 3.01 (1H, ddd, J=4.6, 12.6, 14.6 Hz, H-16), 4.19 (1H, ddd, J=4.5, 9.4, 11.0 Hz, H-2), 4.54 (1H, d, J=9.4 Hz, H-3), 5.52 (1H, br. s, H-12); ^{13}C-NMR: see Table I; Anal. Calcd. for $C_{30}H_{48}O_4$: C 76.27, H 10.17; Found: C 76.10, H 10.40.

Suavissimoside R_1, 5

Colorless needless from MeOH: mp. 239-241°C; IR ν_{max}^{KBr} cm^{-1} 3409 (OH), 1724 (esteric C=O), 1691, 1073, 1040 (glycosidic C−O), 935 (ter. OH), 815 (trisubstituted double bond); ^{1}H-NMR (pyridine-d_5) δ 1.14, 1.17, 1.36, 1.60, 1.67 (3H each, s, CH_3), 1.06 (3H, d, J=6.4 Hz, CH_3), 2.89 (1H, s, H-18), 5.52 (1H, brs, H-12), 6.22 (1H, d, J=8.0 Hz, anomeric H); ^{13}C-NMR: see Table I; Anal. Calcd. for $C_{36}H_{56}O_{12}$: C 65.53, H 8.24; Found: C 65.38, H 8.37.

Ursolic acid methylester, 6

White needless from MeOH: mp. 169-172°C; IR ν_{max}^{KBr} cm^{-1} 3420 (OH), 1728 (esteric C=O), 1640 (C=C), 1030 (sec. OH), 810 (trisubstituted double bond); MS *m/z* (rel. int.) 470 (M^+, 2.5), 452 (M-H_2O, 0.8), 411 (M-$COOCH_3$, 2.2), 410 (M-CH_3COOH, 2.0), 262 (D/E ring, 45.6), 208 (A/B ring, 6.3), 203 (D/E ring-$COOCH_3$, 100), 190 (A/B ring-H_2O, 12.8); ^{1}H-NMR ($CDCl_3$) δ 0.75, 0.78, 0.92, 0.95, 1.05 (3H each, s, CH_3), 0.85 and 0.94 (3H each, d, J=5.9 and 6.4 Hz, CH_3), 2.34 (1H, d, J=11.9 Hz, H-18), 3.21 (1H, dd, J=3.6, 9.0 Hz, H-3), 3.60 (3H, s, $COOCH_3$), 5.25 (1H, t, J=3.0 Hz, H-12); ^{13}C-NMR: see Table I.



Scheme 1. Carboxylic acid induced shift value for isomeric pairs at C-4 in tormentic acid 28-*O*-glucosyl ester.

Euscapic acid methylester, 7

Colorless needless from MeOH: mp. 125-129°C; IR ν_{max}^{KBr} cm^{-1} 3428 (OH), 1725 (esteric C=O), 1035, 1005 (sec. OH), 930 (ter. OH); MS *m/z* (rel. int.) 502 (M, 8.3), 484 (M-CH_3COOH, 15.7), 278 (D/E ring, 12.4), 224 (A/B ring, 3.2), 219 (D/E ring-$COOCH_3$, 18.1), 218 (D/E ring-CH_3COOH, 15.3); ^{1}H-NMR ($CDCl_3$) δ 0.67, 0.86, 0.96, 1.02, 1.21, 1.26 (3H each, s, CH_3), 0.94 (3H, d, J=6.6 Hz, CH_3), 2.47 (1H, dt, J=4.6, 12.6, 14.6 Hz, H-16α), 2.60 (1H, br. s, H-18), 3.43 (1H, d, J=2.5 Hz, H-3), 3.60 (3H, s, $COOCH_3$), 3.99 (1H, dt, J=2.5, 11.2 Hz, H-2), 5.36 (1H, t, J=3.2 Hz, H-12); ^{13}C-NMR: see Table I.

Euscapic acid methylacetate, 8

White needless from MeOH: mp. 234-236°C; IR ν_{max}^{KBr} cm^{-1} 3400 (OH), 1725, 1250 (acetate); ^{1}H-NMR ($CDCl_3$) δ 0.74, 0.88, 0.98, 1.04, 1.21, 1.31 (3H each, s, CH_3), 0.96 (3H, d, J=6.7 Hz, CH_3), 1.96, 2.11 (3H each, s, OAc), 2.55 (1H, s, H-18), 3.74 (3H, s, $COOCH_3$), 4.98 (1H, d, J=2.4 Hz, H-3), 5.22-5.26 (1 H, m, H-2), 5.35 (1H, br. s, H-12).

2α, 3β, 19α-Trihydroxyurs-12-en-23,28-dioic acid methylester, 9

White needless from MeOH: mp. 103-104℃; IR ν_{max}^{KBr} cm^{-1} 3410 (OH), 1725 (esteric C=O), 1650 (C=C), 1040, 1010 (sec. OH), 850, 810 (trisubstituted double bond); MS *m/z* (rel. int.) 546 (M^+, 9.5), 528 (M-H_2O, 4.6), 486 (M-CH_3COOH, 44.8), 278 (D/E ring, 24.2), 268 (A/B rig, 16.0), 219 (D/E ring-$COOCH_3$, 23.0), 201 (D/E ring-$COOCH_3$-H_2O, 36.8); ^{1}H-NMR ($CDCl_3$) δ 0.67, 1.01, 1.18, 1.21, 1.27 (3H each, s, CH_3), 0.95 (3H, d, *J*=6.7 Hz, CH_3), 2.52 (1H, dt, *J*=4.5, 11.7, 14.6 Hz, H-16), 2.60 (1H, s, H-18), 3.60 (3H, s, $COOCH_3$), 3.61 (1H, d, *J*=8.2 Hz, H-3), 3.73 (3H, s, $COOCH_3$), 3.78 (1H, m, H-2), 5.36 (1H, t, *J*=3.0 Hz, H-12).

Alkaline hydrolysis of suavissimoside R_1 5

Alkaline hydrolysis of **5** was performed by refluxing with 6N KOH in MeOH for 1 hr. The reaction mixture was neutralized with 10% H_2SO_4 and then extracted with EtOAc. The EtOAc layer was washed with water and concentrated to give a product, which was identified as 2α, 3β, 19α-trihydroxyurs-12-en-23,28-dioic acid **4** by direct comparison (mmp, IR, ^{1}H-NMR, ^{13}C-NMR) with an authentic sample.

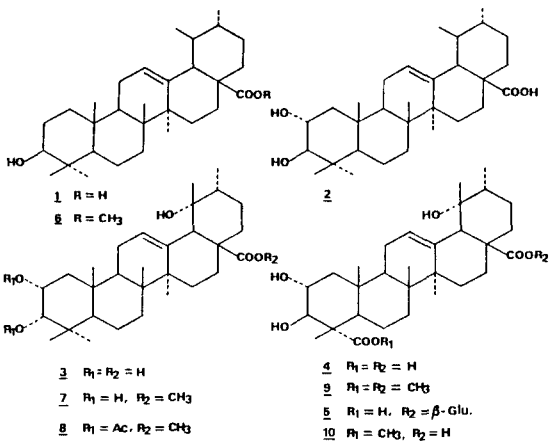
Methylation of suavissimoside R_1 5 and alkaline hydrolysis of suavissimoside R_1 methylester

Compound **5** was methylated with ethereal CH_2N_2 to give a methylester and alkaline hydrolysis of the methylester were carried out in the same manner as described above. The product was subjected to silica gel column chromatography eluting with $CHCl_3$-MeOH (gradient, 10 to 30%) to yield 2α, 3β, 19α-trihydroxyurs-12-en-23,28-dioic acid-23-methylester **10**: IR ν_{max}^{KBr} cm^{-1} 3416 (OH), 1740 (esteric C=O), 1697 (COOH), 1034 (sec. OH), 934 (ter. OH); MS *m/z* (rel. int.) 486 (M^+-HCOOH, 20.6), 414 (17.5), 264 (D/E ring, 31.7), 246 (D/E ring-H_2O, 31.7), 231 (D/E ring-H_2O-CH_3, 34.9), 219 (D/E ring-COOH, 22.2), 205 (17.5), 201 (D/E ring-COOH-H_2O, 71.4), 185 (25.4), 146 (79.4).

RESULTS AND DISCUSSION

Ursolic acid **1**, mp. 286-289℃, gave positive Liebermann-Burchard test and its IR spectrum showed absorption bands of O-H (3400 cm^{-1}), acid group (1695 cm^{-1}), and trisubstituted double bond (1650, 830, 815 cm^{-1}). Compound **1** afforded monomethyl ester **6** upon methylation with ethereal diazomethane. The MS data of **6** showed a molecular ion peak at *m/z* 470 and significant fragment peaks at *m/z* 208 and 262 denoting the retro Diels-Alder cleavage fragments commonly found in the spectra of olean-12-en or urs-12-en derivatives possessing one hydroxyl group in rings A/B and one carboxyl group in rings D/E[2], and two peaks at *m/z* 190 and 203 indicated further successive losses of H_2O and CH_3COO from the above peaks. The ^{1}H-NMR spectrum of **1** showed five tertiary methyl singlets at δ 0.91-1.28, together with two secondary methyl groups at δ 0.97 and 0.99. Furthermore, the doublet originating from H-18 at δ 2.63 and an olefinic proton at δ 5.49 strongly demonstrated the urs-12-en type in its structure. And the 3β-hydroxyl functionality was suggested by the peak at δ 3.45 (1H, dd, *J*=3.0 and 7.7 Hz). The observation of ^{13}C-NMR signals at δ 180.0, 42.6 and 53.7, each due to C-28, C-17 and C-18, confirmed the attachment site of carboxylic group. Consequently, **1** was identified as ursolic acid and the direct comparison with authentic standard supported this conclusion.

2α-Hydroxyursolic acid **2**, mp. 255-256℃, was positive in the Liebermann-Burchard test and showed IR absorption band at 1690 cm^{-1} due to carboxylic acid group along with hydroxyl and trisubstituted double bond as indicated in **1**. The mass spectrum of **2** showed a molecular ion peak at *m/z* 472 in addition to typical retro Diels-Alder fragmentation peaks at 248 (D/E rings) and 223 (A/B rings), and the ion peak corresponding to the release of COOH moiety from rings D/E at *m/z* 203. These information presumed that the carboxyl group of **2** located at rings D/E and the two hydroxyl groups could be present in the rings A/B portion. The position of one secondary hydroxyl at C-3 is highly probable on a biogenetic consideration[3]. The ^{1}H-NMR spectrum of **2** revealed significant signals assignable to the H-18 at δ 2.61 (d, *J*=11.2 Hz) and the olefinic proton at δ 5.46 (t, *J*=3.1 Hz), five methyl singlets (δ 0.99-1.26), two methyl doublets at δ 0.96 (*J*=5.9 Hz) and 0.99 (*J*=8.7 Hz), together with two oxygenated methine protons at δ 3.37 (d, *J*=9.2 Hz) and 4.06 (ddd, *J*=4.5, 9.2, 11.2 Hz), suggesting that **2** was Δ^{12}-ursene with two hydroxyl groups at 2α and 3β[4]. This was further supported by the inspection of the ^{13}C-NMR spectrum of **2** compared with **1** (Table I): The significant differences in the chemical shifts for C-24 (+1.4 ppm) and C-5 (+0.1 ppm) were ob-



served[5]. Accordingly, **2** could be assigned as 2α, 3β-dihydroxyurs-12-en-28-oic acid (2α-hydroxy ursolic acid) and the comparison with literature data[6] established its identity.

Euscapic acid **3**, mp. 269-270℃, showed the similar pattern with **2** in Liebermann-Burchard test and IR spectrum. Treatment of **3** with ethereal CH_2N_2 afforded the monomethyl ester **7**, showing that **3** was a triterpene carboxylic acid. Prolonged treatment of **7** with Ac_2O-pyridine gave the diacetyl derivative **8**. IR spectrum of **8** showed the absorption bands of 3400 cm^{-1} (OH) and 930 cm^{-1} (tertiary OH), indicating that **3** has one sterically hindered OH group. In the ^{1}H-NMR spectrum of **3**, the pattern of the signals of the angular methyl groups at δ 0.92-1.15, a characteristic broad singlet of H-18 at δ 3.05 and an olefinic proton signal at δ 5.44 suggested that **3** was Δ^{12}-ursene type with tertiary hydroxyl at C-19 in α configuration[7]. The MS spectrum of **3** showed a molecular ion peak at *m/z* 488 and fragment ion peak at *m/z* 264 (D/E rings) and 224 (A/B rings) by the RDA cleavage and also *m/z* 264 (D/E rings-H_2O), 219 (D/E rings-COOH) and 146 (RDA fragment of *m/z* 218 ion). The MS spectrum of **7** revealed the base peak at *m/z* 179 due to fragmentation of *m/z* 278 ion peak (RDA fragment of D/E rings). This evidence suggested that two secondary hydroxyl groups were located at either rings A or B. The presence of 2α, 3β-hydroxyl functionality was suggested by its chemical shifts and coupling constants of the ^{1}H-NMR spectrum of **7**: δ 3.43 (d, J=2.5 Hz) and 3.99 (dt, J=2.5, 11.2 Hz). Futhermore, in the ^{13}C-NMR spectrum of **3** compared with **2** (Table I), the upfield shifts showed for C-1 (+5.5 ppm) and C-5 (+7.4 ppm) represented typical γ-gauche shielding effects due to 3α-hydroxyl group[5]. Based on the above evidences, the structure of **3** was determined to be 2α, 3α, 19α-trihydroxyurs-12-en-28-oic acid (euscapic acid). And ^{13}C-NMR spectral data of **3** were superimposable on those of euscapic acid[8].

2α, 3β, 19α-trihydroxyurs-12-en-23,28-dioic acid **4**, mp. over 300℃, showed positive Liebermann-Burchard test and similar absorption pattern to **2** in the IR spectrum. Methylation of **4** with ethereal diazomethane gave a dimethylester derivative **9** and the ^{1}H-NMR spectrum of **9** showed two carbomethoxyl groups at δ 3.60 and 3.73. The MS spectrum of **9** revealed a molecular ion peak at *m/z* 546 with a base peak at *m/z* 175 and abundant fragment at *m/z* 278 (D/E rings) and 267 (A/B rings) suggesting that one hydroxyl and one carboxylic acid functionalities were located at D/E rings, and another two hydroxyl and one carboxylic acid groups at rings A/B. The structure of rings D/E residue was identical with that of **3** by the observation of mass fragmentation pattern. The ^{1}H-NMR spectrum of **4** showed oxygenated methine protons at δ 4.19 (1H, ddd, J=4.5, 9.4, 11.0 Hz) and 4.54 (d, J=9.4 Hz), indicating that hydroxyl configurations of C-2 and C-3 were proposed to be 2α and 3β, respectively. In the ^{13}C-NMR spectrum of **4** compared with **2** (Table I), the significant differences in the chemical shifts for C-3 (−2.8 ppm), C-4 (+14.6 ppm) and C-5 (−3.5 ppm) were observed. Such differences clearly demonstrated that carboxylic acid residue located at C-4. In calculation of carboxylic acid induced shift value for isomeric pairs at C-4 in tormentic acid 28-*O*-glucosyl ester, ^{13}C-NMR chemical shifts of rings A/B residue were identical with those of 2α, 3β, 19α-trihydrolxyurs-12-en-23,28-dioic acid 28-*O*-glucosyl ester as shown in Sheme 1[9]. From the above results, the structure of **4** was assigned as 2α, 3β, 19α-trihydroxyurs-12-en-23,28-dioic acid. This compound was isolated from *Rubus suavissimus* as 28-*O*-glucosyl ester form[10] but never has been reported in nature as free form.

Suavissimoside R_1 **5**, mp. 239-241℃, gave positive reaction in Liebermann-Burchard and Molisch tests and showed hydroxyl (3429 cm^{-1}), ester (1724 cm^{-1}) and glycoside (1073-1040 cm^{-1}) absorption bands in its IR spectrum. Alkaline hydrolysis of **5** gave an aglycone, which was identified as 2α, 3β, 19α-trihyd-

roxyurs-12-en-23,28-dioic acid **4** by direct comparison (mmp, IR, ^{1}H-NMR, ^{13}C-NMR) with an authentic sample. In the ^{13}C-NMR spectrum of **5**, a set of carbon signals due to β-glucopyranosyl ester moiety and an anomeric carbon signal (δ 95.6 ppm) at rather highfield strongly indicated that one mole of glucose was linked to one carboxylic acid of **5** in the ester form. And the relative large coupling constrant (J=8.0 Hz) of the anomeric proton signal at δ 6.22 also indicated the β configuration for glucoside linkage. Methylation with CH_2N_2 of **5** and subsequent alkaline hydrolysis gave a monomethylester **10**. MS spectrum of **10** showed fragment ion peaks at *m/z* 486 corresponding to the release of (COOH+H) from a molecular ion and at *m/z* 414 due to RDA cleavage of ring C from *m/z* 486 ion, and the significant fragment ion peak at *m/z* 264 (D/E rings) ascribable to RDA cleavage of ring C. Accordingly, β-glucopyranosyl moiety was linked to the C-28-carboxylic acid of **5** in the ester form. In the light of these evidences, the structure of **5** was established as 2α, 3β, 19α-trihydroxyurs-12-en-23,28-dioic acid 28-*O*-β-D-glucopyranosyl ester, suavissimoside R_1, which had been isolated from *Rubus suavissimus*[10].

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