Arch. Pharm. Res. 10(4), 219-222 (1987)

Triterpenoid Glycosides from *Rosa rugosa*

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(Received October 5, 1987)

Abstract □ From the underground parts of *Rosa rugosa*(Rosaceae), 28-O-glucosides of euscaphic acid, tormentic acid and arjunic acid were isolated and characterized by spectral data.

Keywords □ *Rosa rugosa,* Rosaceae, euscaphic acid, tormentic acid, arjunic acid, triterpenoid glycoside, ^{13}C-NMR

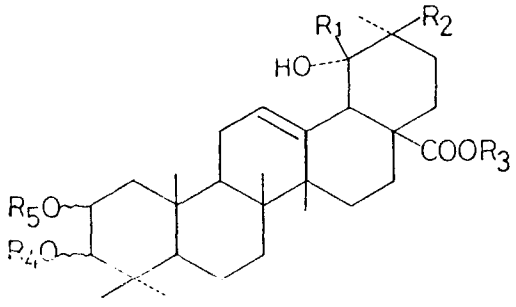
In the course of searching for hypolipemic drugs from Korean folkloric medicines, we found that an ethylacetate soluble fraction of methanol extract from the underground parts of *Rosa rugosa* (Rosaceae) significantly lowered serum cholesterol level in rats[1,2]. And it was also found that (+)-catechin from the ethylacetate soluble fraction could be one of the active principles from this plant[2]. Through the continuous work on the plant, additional three triterpenoid glycosides (**1-3**) were isolated from the same plant part. This paper deals with the isolation and characterization of those triterpenoid glycosides.

Column chromatography on silica gel of the ethylacetate soluble fraction of methanol extract, eluting with $CHCl_3$-MeOH-7% HAc furnished two triterpenoid glycosides with fine needles. But a more polar compound of two showed two peaks when detected on HPLC, even though it showed one homogeneous spot on TLC with several solvent systems. Thus, it was subjected to HPLC with an ODS column to give two compounds as a main one (**2**) from late eluting fractions and a minor one (**3**) from early eluting fractions.

Compound 1, mp 198-202° gave a positive reaction in Liebermann-Burchard and Molisch tests and showed hydroxyl(3420 cm^{-1}), ester(1730 cm^{-1}) and glycoside(1,100-1,000 cm^{-1}) absorption bands in its IR spectrum. Alkaline hydrolysis of **1** gave **1a** as a genin. Compound 1a, mp 270-2° showed absorption bands at 3450(OH) and 1,700(COOH) cm^{-1} in its IR spectrum. The MS spectrum of **1a** showed a molecular ion at *m/z* 488 and *retro*-Diels-Alder fragment ions at *m/z* 264 and 223, suggesting that it is a compound having Δ^{12}-oleanene or Δ^{12}-ursene skeleton with one hydroxyl and one carboxyl groups at ring D or E and two hydroxyl groups at ring A or B[3].

Methylation with CH_2N_2 of **1a** and subsequent acetylation with acetic anhydride-pyridine gave a monomethylester, mp 196-8° and a methylester diacetate, mp 148-150°, respectively. These results indicated the presence of a sterically hindered hydroxyl group in **1a**. The ^{1}H-NMR spectrum of the methylester diacetate showed six tertiary methyl signals at δ 0.69-1.31, secondary methyl signal at δ0.95 (3H,d,J=6.3Hz), two acetyl signals at δ1.96 (3H) and 2.12(3H), one carbomethoxyl signal at δ3.61(3H), a doublet(1H,J=2.4Hz) centered at δ4.98 due to H-3, a multiplet(1H,$W_{1/2}$=20Hz) at δ5.19-5.29 due to H-2 and a multiplet centered at δ5.36 for an olefinic proton. And also its ^{1}H-NMR spectrum showed a broad singlet at δ2.61 ascribable to H-18, probably indicating β-proton at C-18 and also α-amyrin type of a triterpenoid with a methyl group and a sterically hindered hydroxyl group at C-19. The ^{13}C-NMR analysis(Table I) of **1a** confirmed the above suggestion. From the above results, **1a** was characterized as 2α, 3α, 19α-trihydroxy-urs-12-en-28-oic acid(euscaphic acid), previously known from *Euscaphis japonica.*[4] A direct comparison(mmp, co-TLC and MS) with an authentic sample kindly supplied by Dr. M. Takani of University of Kanazawa, Japan confirmed the identity of these two terpenoids.

In the ^{13}C-NMR spectrum of **1**, a set of carbon signals due to β-glucopyranosyl ester moiety and an anomeric carbon signal (δ=95.7ppm) at rather highfield strongly indicated that one mole of glucose was linked to the 28-carboxylic acid of **1a** in the ester form. And the relative large coupling constant (J=7.8Hz) of the anomeric proton signal also indicated the β-configuration for glucoside linkage. Accordingly, the structure of **1** was established as



	R_1	R_2	R_3	R_4	R_5
1a	CH_3	H	H	α-OH	α-OH
1	CH_3	H	β-Glc	α-OH	α-OH
2a	CH_3	H	H	β-OH	α-OH
2	CH_3	H	β-Glc	β-OH	α-OH
3a	H	CH_3	H	β-OH	α-OH
3	H	CH_3	β-Glc	β-OH	α-OH

28-β-D-glucopyranosyl euscaphic acid (Kaji-ichigoside F_1) which was previously isolated from *Rubus trifidus.*[5]

Compound 2, mp 206-210° gave a positive reaction in Liebermann-Burchard and Molisch tests. Its IR, ^{1}H-NMR and ^{13}C-NMR spectra were similar to those of **1** but showed the somewhat different linkage of the two secondary hydroxyl groups in rings A and B rather than 2α and 3α-like **1**. Alkaline hydrolysis of **2** gave **2a**, mp 264-6° as a genin and the IR, MS, ^{1}H-NMR and ^{13}C-NMR spectra of **2a** were similar to those of **1a** but showed the two secondary hydroxyl groups at C-2 and C-3 were axially linked [δ4.74 (d, J = 10.3Hz, H-3) and δ5.11-5.26(m, H-2)] in the ^{1}H-NMR spectrum of **2a** methylester diacetate. Thus, from the above evidence, **2a** was characterized as 2α, 3β, 19α-trihydroxy-urs-12-en-28-oic acid(tormentic acid).

In the ^{13}C-NMR spectrum of **2**, a set of carbon signals due to β-glucopyranosyl ester moiety and an anomeric carbon signal (δ = 95.6ppm) at rather highfield strongly indicated that one mole of glucose was linked to the 28-carboxylic acid of **2a** in the ester form. The relative large coupling constant (J = 7.6Hz) of anomeric proton signal also indicated the β-configuration for glucoside linkage. Accordingly, the structure of **2** was established as 28-β-D-glucopyranosyl tormentic acid (Rosamultin) which was previously isolated from *Rosa multiflora.*[6]

Compound 3, mp 235-8° was also positive Liebermann-Burchard and Molisch tests. Its IR, ^{1}H-NMR and ^{13}C-NMR spectra were similar to those of **2** but showed the oleanane type triterpenoid glycoside rather than **1** and **2**. Alkaline hydrolysis of compound 3 gave a **3a**, mp >300° and its IR and MS spectra were similar to those of **2a**. The ^{1}H-NMR spectrum of **3a** methylester diacetate showed the signals at δ 3.12(1H), 3.31(1H, d, J = 3.5Hz) ascribable to H-18 and H-19, respectively, indicating the β-amyrin type of a triterpenoid with a methyl group and a sterically hindered hydroxyl group at C-19. Further, a comparison of the ^{13}C-spectrum of **3** with that of **2** revealed that the signals due to C-2 and C-3 were similar, suggesting two secondary hydroxyl groups were present at C-2α and C-3β. Thus, from the above evidence, **3a** was characterized as 2α, 3β, 19α-trihydroxy olean-12-en-28-oic acid (arjunic acid).

In the ^{13}C-spectrum of **3**, a set of carbon signals due to β-glucopyranosyl ester moiety and anomeric carbon signal (δ = 95.8ppm) at rather highfield strongly indicated that one mole of glucose was linked to the 28-carboxylic acid of **3a** in the ester form. The relative large coupling constant (J = 7.8 Hz) of anomeric proton signal also indicated the β-configuration for glucoside linkage. Accordingly, the structure of **3** was established as 28-β-D-glucopyranosyl arjunic acid (Arjunetin) which was previously isolated from *Terminalia arjuna.*[7]

Studies on the hypolipemic activities of these compounds and further chemical examination of this plant are under way.

EXPERIMENTAL

General procedures

Acetylation was performed with Ac_2O/pyridine at room temp. and methylation was carried with diazomethane in the usual manner. Each sapogenin was obtained by alkaline hydrolysis using 6N-NH_4OH as a reacting solvent. Melting points were determined on a Thomas Hoover 6404-H apparatus and are uncorrected. IR absorption spectra were obtained in KBr pellets on a Shimazu IR-400 spectrophotometer and optical rotations were obtained on a Mitamura Riken Polarimeter. NMR spectra were taken at 25° using TMS as an internal standard on a Jeol GX-270, Jeol FX-90Q or Bruker AM-200 spectrometer. EIMS spectra were obtained on a Jeol 01-SG-2 spectrometer. HPLC was performed by a Tri-Rotar SR-1(JASCO) chromatography using the following conditions: Column; Develosil ODS-5(Nomura Chem., 4.5 × 259 mm), UV detector; 203 nm, CH_3CN: H_2O(7:3) as eluting solvent, Flow rate; 1.3 ml/min..

Extraction and fractionation

This was carried out as described previously.[1,2]

Table 1. ^{13}C-NMR spectral data of compounds 1a, 1, 2a, 2 and 3 (pyridine-d_5, TMS as internal standard)*

carbon No.	1a	1	2a	2	3
1	42.4	42.7	48.2	48.5	47.6
2	66.3	66.0	68.6	68.5	68.6
3	79.0	79.1	83.8	83.7	83.9
4	38.7	38.6	39.8	38.4	38.6
5	48.7	48.5	55.9	56.1	56.1
6	18.6	18.6	18.9	18.9	19.1
7	33.4	33.4	33.5	33.4	33.2
8	40.5	40.6	40.4	40.8	40.3
9	47.5	47.5	47.8	47.7	48.4
10	38.6	38.5	38.4	39.7	39.8
11	24.0	24.0	24.0	24.0	24.6
12	128.1	128.3	127.9	128.2	123.5
13	139.7	139.1	139.9	139.1	144.3
14	42.1	42.0	42.1	42.0	42.1
15	29.4	29.3	29.9	29.2	29.0
16	26.3[a]	26.0[a]	26.9	26.0	28.0
17	48.3	48.5	48.2	48.5	46.5
18	54.5	54.2	54.6	54.2	44.6
19	72.7	72.6	72.7	72.6	81.1
20	42.3	42.0	42.3	42.0	35.5
21	26.8[a]	26.9[a]	26.3	26.6	29.0
22	38.4	37.6	38.4	37.6	33.2
23	29.2	29.3	29.3	29.2	29.3
24	22.2	22.2	17.2[a]	17.5[a]	17.7
25	16.8[b]	16.6[b]	16.8[b]	16.5[b]	16.8
26	17.3[b]	17.3[b]	17.6[b]	17.3[b]	17.5
27	24.7	24.4	24.6	24.4	24.7
28	181.1	176.9	180.6	176.8	177.2
29	27.0	26.6	27.0	26.9	28.7
30	16.6	16.6	16.8[a]	16.8[a]	24.9
1′		95.7		95.6	95.8
2′		73.8		73.9	74.1
3′		78.7		78.7	78.9
4′		71.1		71.1	71.2
5′		79.0		78.9	79.2
6′		62.3		62.3	62.2

a,b; Values with the same symbol may be interchanged in the vertical column.

*The number of protons attached to each carbon atom has been ascertained by means of INEPT experiments performed for all these substances.

Isolation

The EtOAc soluble portion was chromatographed on a Si gel column with $CHCl_3$-MeOH-7% HAc(25:8:5, lower phase) to afford 10 fractions. Fractions 1,2 and 3 were combined and rechromatographed on a Si gel column with $CHCl_3$-MeOH-7% HAc(5:1:1, lower phase) to afford compound 1. Further elution yielded a complex mixture (2 and 3) as white needles. Final separations were achieved by HPLC using the conditions described in the Experimental to give main(2) and minor (3) compounds.

Compound 1

mp198-202°, colorless needles from MeOH, $[\alpha]_D^{25} = +6.5°$(c 0.5, MeOH), IR ν_{max}^{KBr}(cm^{-1}); 3420(OH), 1730(ester), 1100-1000(glycoside), ^{1}H-NMR (270 MHz, pyridine-d_5) δ; 0.90, 1.02, 1.18, 1.24, 1.37, 1.59(each 3H,s, 6 x -CH_3), 1.06 (3H,d,J = 6.6 Hz,-CH_3), 2.09(1H,s,H-18), 3.76 (1H,brs,H-3), 4.29-4.32(1H,m,H-2), 5.53 (1H,brs, H-12), 6.26 (1H,d,J = 7.8Hz,anomeric).

Compound 1a(euscaphic acid)

mp 270-2°, colorless prisms from MeOH, $[\alpha]_D^{25} = +22°$ (c 0.5, MeOH), IR ν_{max}^{KBr}(cm^{-1}); 3450 (OH), 1700 (COOH), MS (*m/z*, %); 488 (M^+, 1.1), 470 (M^+-H_2O, 0.7), 455 (M^+-H_2O-CH_3, 0.9), 442 (M^+-HCOOH, 5.1), 264 (D/E ring, 4.8), 246 (D/E ring-H_2O, 10.9), 223 (A/B ring-H, 8.7), 219 (D/E ring-COOH, 7.4), 205 (D/E ring-CO_2-CH_3, 13.9), 201 (D/E ring-H_2-COOH, 22.0), ^{1}H-NMR (200 MHz, pyridine-d_5) δ; 0.76, 0.84, 0.96, 0.99, 1.12.1.50 (each 3H, s, 6 x-CH_3), 1.26(3H, d,J = 7.8) Hz, -CH_3), 2.89 (1H, brs, H-18), 3.62 (1H, brs, H-3), 4.36-4.40 (1H, m,H-2), 5.44 (1H, brs, H-12)

Compound 1a methylester

mp 196-8°, colorless needles from MeOH, IR ν_{max}^{KBr}(cm^{-1}); 3400 (OH), 1725 (ester)

Compound 1a methylacetate

mp 148-50°, colorless needles from MeOH, IR ν_{max}^{KBr}(cm^{-1}); 3400 (OH), 1725, 1250 (acetate), ^{1}H-NMR (200 MHz, $CDCl_3$) δ; 0.69, 0.88, 0.99, 1.03, 1.21, 1.31 (each 3H, 6 x -CH_3), 0.95 (3H, d,J = 6.3 Hz,-CH_3), 1.96, 2.12 (each 3H,s, -OAc), 2.61 (1H,s, H-18), 3.61 (3H,s,-OCH_3), 4.98 (1H,d, J = 2.4 Hz, H-3), 5.19-5.29 (1H, m, H-2), 5.35-5.37 (1H,m, H-12), ^{13}C-NMR (20 MHz, $CDCl_3$) δ; 68.2 (C-2), 73.1 (C-19), 80.9 (C-3), 128.7 (C-12), 138.3 (C-13), 178.1 (C-28), 21.5, 20.8 (acetyl methyl), 51.4 (carbomethoxyl), 170.1, 170.4 (acetyl carbonyl)

Compound 2

mp 206-210°, colorless needles from MeOH, $[\alpha]_D^{25}$ = + 12.2° (c 0.6, MeOH), IR ν_{max}^{KBr} (cm^{-1}); 3400 (OH), 1725 (ester), 1080-1000 (glycoside), ^{1}H-NMR (270 MHz, pyridine-d_5) δ ; 1.02, 1.03, 1.12, 1.21, 1.35, 1.60 (each 3H,s,6 x -CH_3), 1.05 (3H, d,J = 5.5 Hz,-CH_3), 2.84 (1H, s,H-18), 3.31 (1H,d, J = 9.5 Hz, H-3), 4.00-4.10 (1H, m,H-2), 5.49 (1H, brs, H-12), 6.14 (1H,d, J = 7.6 Hz, anomeric)

Compound 2a (tormentic acid)

mp 264-6°, colorless needles from MeOH, IR ν_{max}^{KBr} (cm^{-1}); 3400 (OH), 1695 (COOH), MS (*m/z*, %); 488 (M^+, 0.7), 470 (M^+-H_2O, 0.6), 455 (M^+-H_2-CH_3, 0.4), 442 (M^+-HCOOH, 3.6), 264 (D/E ring, 34.3), 246 (D/E ring-H_2O, 32.4), 223 (A/B ring-H, 6.7), 219 (D/E ring-COOH, 11.9), 205 (D/E ring-CO_2-CH_3, 15.6), 201 (D/E ring-H_2O-COOH, 67.7), ^{1}H-NMR (270 MHz, pyridine-d_5) δ; 1.00, 1.07, 1.09, 1.26, 1.43, 1.70 (each 3H,s,6 x -CH_3), 1.10 (3H,d, J = 6.3 Hz,-CH_3), 3.03 (1H,s, H-18), 3.38 (1H,d, J = 9.3 Hz, H-3), 4.07 (1H,dd, J = 14.4 and 7.1 Hz, H-2), 5.57 (1H, brs, H-12)

Compound 2a methylester

mp 200-2°, colorless needles from MeOH, IR ν_{max}^{KBr} (cm^{-1}); 3400(OH), 1720(ester).

Compound 2a methylacetate

mp 162-4°, colorless prisms from MeOH, IR ν_{max}^{KBr} (cm^{-1}); 3400(OH), 1730,1240(acetate), ^{1}H-NMR (80MHz,$CDCl_3$)δ; 0.69, 0.87, 1.03, 1.20, 1.25, 1.30 (each 3H,s,6 x -CH_3), 0.94 (3H,d, J = 7.2Hz,-CH_3), 1.94, 2.10 (each, 3H,s,-OAc), 2.59(1H,s,H-18), 3.59 (3H,s,-OCH_3), 4.74 (1H,d, J = 10.3 Hz,H-3), 5.11-5.26 (1H,m, $W_{1/2}$ = 14 Hz, H-2), 5.36 (1H,brs, H-12).

Compound 3

mp 235-8°, colorless needles from MeOH, $[\alpha]_D^{25}$ = + 25° (c 0.7,MeOH), IR ν_{max}^{KBr} (cm^{-1}); 3400 (OH), 1725 (ester), 1080-1000 (glycoside), ^{1}H-NMR (270 MHz, pyridine-d_5)δ; 0.98, 1.05, 1.08, 1.14, 1.15, 1.26, 1.60 (each 3H,s,7 x -CH_3), 3.37 (1H,d, J = 8.9 Hz, H-3), 4.40 (1H,d, J = 5.9 Hz, H-19), 5.50 (1H, brs, H-12), 6.34 (1H,d, J = 7.8 Hz, anomeric).

Compound 3a(arjunic acid)

mp 300°, colorless needles from acetone, IR ν_{max}^{KBr} (cm^{-1}); 3400(OH), 1700(COOH), MS(*m/z*,%); 488 (M^+,1.2), 470(M^+-H_2O, 1.7), 455(M^+-H_2O-CH_3, 1.0), 442(M^+-HCOOH,5.9), 264 (D/E ring, 5.3), 246 (D/E ring-H_2, 12.6), 223 (A/B ring-H, 11.3), 219(D/E ring-COOH,10.6), 205 (D/E ring-CO_2-CH_3,19.9), 201(D/E ring-H_2O-COOH, 30.4).

Compound 3a methylester

mp 196-8°, colorless needles from MeOH, IR ν_{max}^{KBr} (cm^{-1}); 3400(OH), 1715(ester).

Compound 3a methylacetate

mp 233-5°, colorless needles from MeOH, IR ν_{max}^{KBr} (cm^{-1}); 3400(OH), 1710,1250(acetate), ^{1}H-NMR (80MHz,$CDCl_3$)δ; 0.66 (3H,s,-CH_3), 0.88 (6H, s,2 x -CH_3), 0.94 (6H, s,2 x -CH_3), 1.02 (3H, s,-CH_3), 1.23(3H,s,-CH_3), 1.95,2.02(each 3H, s,-OAc), 3.12 (1H,brs,H-18), 3.31 (1H,d, J = 3.5 Hz, H-19), 3.60 (3H,s,-OCH_3), 4.71 (1H,d, J = 10.4 Hz, H-3), 5.03 (1H,dd, J = 10.8 and 4.3 Hz, H-2), 5.34-5.39 (1H, m,H-12).

ACKNOWLEDGEMENT

We wish to thank Dr. M. Hattori (Research Institute for WAKAN-YAKU, Toyama Medical and Pharmaceutical University, Japan) for NMR and MS measurements.

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