Triterpenoid Glycosides from *Rosa rugosa*

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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-$
\text{70}$% 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$^\circ$ 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 la as a genin. Compound 1a, mp 270-2$^\circ$ 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 $\delta$ 0.69-1.31, secondary methyl signal at $\delta$ 0.95 (3H,d,J = 6.3Hz), two acetyl signals at $\delta$ 1.96 (3H) and 2.12(3H), one carbomethoxyl signal at $\delta$ 3.61(3H), a doublet(1H,J = 2.4Hz) centered at $\delta$ 4.98 due to H-3, a multiplet($1H, W_{1/2}$=20Hz) at $\delta$ 5.19-5.29 due to H-2 and a multiplet centerd at $\delta$ 5.36 for an olefinic proton. And also its IH-NMR spectrum showed a broad singlet at $\delta$ 2.61 ascribable to H-18, probably indicating $\beta$-proton at C-18 and also $\alpha$-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 2a, 3a, 19$\alpha$-trihydroxy-urs-12-en-28-oic acid(euscaphic acid), previously known from *Euscaphis japonica*. 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 $\beta$-glucopyranosyl ester moiety and an anumeric carbon signal ($\delta$ = 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 anumeric proton signal also indicated the $\beta$-configuration for glycoside linkage. Accordingly, the structure of 1 was established as A or B$^3$.

Methylation with CH$_2$N$_2$ of 1a and subsequent acetylation with acetic anhydride-pyridine gave a monomethylester, mp 196-8$^\circ$ and a methylester diacetate, mp 148-150$^\circ$, 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 $\delta$ 0.69-1.31, secondary methyl signal at $\delta$ 0.95 (3H,d,J = 6.3Hz), two acetyl signals at $\delta$ 1.96 (3H) and 2.12(3H), one carbomethoxyl signal at $\delta$ 3.61(3H), a doublet(1H,J = 2.4Hz) centered at $\delta$ 4.98 due to H-3, a multiplet($1H, W_{1/2}$=20Hz) at $\delta$ 5.19-5.29 due to H-2 and a multiplet centerd at $\delta$ 5.36 for an olefinic proton. And also its IH-NMR spectrum showed a broad singlet at $\delta$ 2.61 ascribable to H-18, probably indicating $\beta$-proton at C-18 and also $\alpha$-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 2a, 3a, 19$\alpha$-trihydroxy-urs-12-en-28-oic acid(euscaphic acid), previously known from *Euscaphis japonica*. 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.
28-β-D-glucopyranosyl euscaphic acid (Kaji-ichi-goside F1) 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, 1H-NMR and 13C-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 2α, mp 264-6 ° as a genin and the IR, MS, 1H-NMR and 13C-NMR spectra of 2α were similar to those of 1a but showed the two secondary hydroxyl groups at C-2 and C-3 were axially linked [β4.74 (d, J = 10.3Hz, H-3) and β'5.11-5.26 (m, H-2)] in the 1H-NMR spectrum of 2α methylester diacetate. Thus, from the above evidence, 2α was characterized as 2α, 3/β, 19α-trihydroxy-urs-12-en-28-oic acid (tormentic acid).

In the 13C-NMR spectrum of 2, a set of carbon signals due to β-glucopyranosyl ester moiety and anomeric carbon signal (β=95.6ppm) at rather highfield strongly indicated that one mole of glucose was linked to the 28-carboxylic acid of 2α 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 Ac2O / pyridine at room temp. and methylation was carried with diazomethane in the usual manner. Each sapogenin was obtained by alkaline hydrolysis using 6N-NH4OH 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-I(JASCO) chromatography using the following conditions: Column; Develosil ODS-5(Nomura Chem., 4.5 x 259 mm), UV detector; 203 nm, CH3CN: H2O(7:3) as eluting solvent, Flow rate; 1.3 ml/min..

**Extraction and fractionation**

This was carried out as described previously. 1,2)