Chemical Constituents of Pericarps of *Rosa davurica* PALL., a Traditional Chinese Medicine

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From pericarps of *Rosa davurica* (Rosaceae), a traditional Chinese medicine, eight known tetracyclic triterpene acids, three known flavonoids, ethyl β-fructopyranoside and methyl 3-*O*-β-glucopyranosyl-gallate were isolated.

Keywords *Rosa davurica*; Rosaceae; traditional Chinese medicine; triterpene acid; flavonoid; ethyl β-fructopyranoside; methyl 3-*O*-β-glucopyranosyl-gallate

The fruits of *Rosa davurica* PALL. (刺玫果, Chinese name: cimeiguo, Rosaceae) have been used as a traditional Chinese medicine for treatment of dyspepsia, gastroenteralgia and menoxenia, and also as a folk medicine (tonic).[1] Isolation of betulinic acid and oleanolic acid from pericarps of this plant has been reported previously.[2] As a part of Chinese–Japanese cooperative studies on Chinese medicinal plants, we have re-investigated constituents of pericarps of this plant collected in Heilongjiang, Northeast district of China.

The dried pericarps was extracted with hot methanol. The methanol extract was suspended in water, then extracted with chloroform and butanol successively. The chloroform and butanol fractions were each separated by repeated reversed-phase and normal-phase chromatography to give compounds **1**—**9** from the chloroform fraction and **10**—**14** from the butanol fraction in yields of 0.007, 0.0014, 0.0032, 0.0028, 0.0048, 0.0032, 0.002, 0.0023, 0.0032, 0.0004, 0.0012, 0.001, 0.004 and 0.0045%, respectively.

The compounds **1**, **3** and **5** were identified as betulinic acid, oleanolic acid and ursolic acid by comparison of the melting points and proton and carbon-13 nuclear magnetic resonance (^{1}H- and ^{13}C-NMR) spectra with those of the authentic samples.

Comparison of the ^{1}H- and ^{13}C-NMR spectra of **2** with those of **1** led to the characterization of **2** as 2α-hydroxybetulinic acid (alphitolic acid).[3]

Based on comparison of the ^{1}H- and ^{13}C-NMR spectra with those of **3**, **4** was identified as 2α-hydroxyoleanolic acid (maslinic acid).[4]

The comparison of the ^{1}H- and ^{13}C-NMR spectra of **7** with those of **5** as well as with those of **2** and **4** showed that **7** is identical with 2α-hydroxyursolic acid.[5]

Based on the inspection of the ^{1}H- and ^{13}C-NMR spectra, **6**, **8** and **9** were identified as the known triterpenes, 19α-hydroxyursolic acid (pomolic acid),[6] 2α,19α-dihydroxyursolic acid (tormentic acid)[7] and 2α,3α,19α-trihydroxyurs-12-en-28-oic acid (euscaphic acid),[8] respectively. It is noteworthy that the 19α-hydroxyursolic acid derivatives are known to be characteristic of rosaceous plants.

Compound **10** afforded fructose on acid hydrolysis. From the ^{1}H- and ^{13}C-NMR spectra, **10** was identified as ethyl β-D-fructopyranoside.

Compounds **11** and **12** were identified as quercetin and hyperin, respectively by direct comparison of the physical and spectral data with those of authentic samples. Compound **13** was identified as tiliroside by comparison of the ^{13}C-NMR spectrum with the reported data.[9]

Compound **14** afforded gallic acid and glucose on hydrolysis. The ^{1}H- and ^{13}C-NMR spectra of **14** indicated the presence of a carbomethoxyl group and a β-glucopyranosyl unit. Further, the signals due to aromatic protons and carbons revealed that one of the three hydroxyl groups of the gallate moiety is unsymmetrically substituted. It follows that **14** can be formulated as methyl 3-*O*-β-glucopyranosyl-gallate. This methyl ester might be formed from the corresponding free acid during the process of extraction with hot methanol.

Experimental

All melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. Optical rotations were measured with a Union PM-101 automatic digital polarimeter. ^{1}H- and ^{13}C-NMR spectra were recorded on a JEOL FX-100 spectrometer in C_5D_5N solution (flavonoids: in DMSO-d_6) using tetramethylsilane (TMS) as an internal standard. For column chromatography, Kieselgel 60 (Merck, 70—230 mesh) and Diaion HP-20 (Mitsubishi Chem. Ind. Co., Ltd.) were used. All solvent systems for chromatography were homogeneous.

Acid hydrolysis of glycosides and identification of the resulting monosaccharides were done as reported previously.[10]

Extraction and Separation The dried pericarps of fruit of *Rosa davurica* PALL. (4 kg), collected in Heilongjiang, China, were extracted with

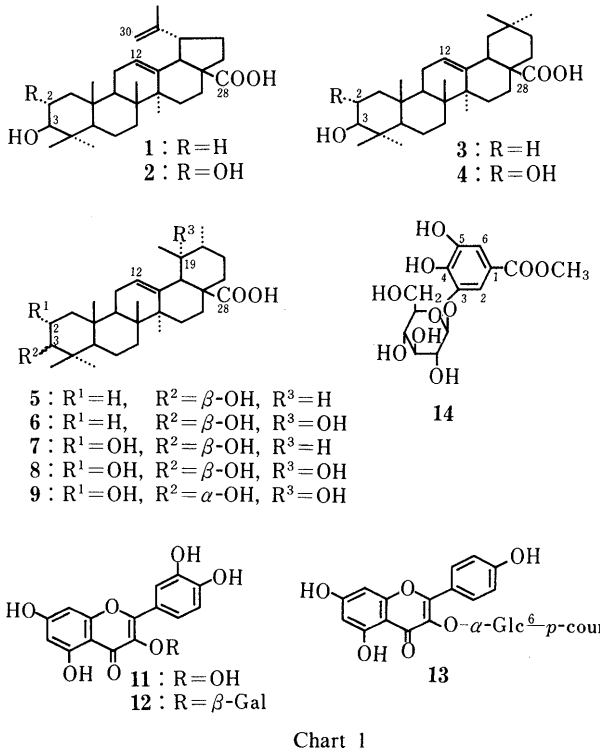

Chart 1

MeOH at 80 °C for 12 h and the solvent was evaporated off under reduced pressure. The MeOH extract (1.8 kg) was suspended in H_2O and the aqueous suspension was extracted with $CHCl_3$ and *n*-BuOH, successively. The $CHCl_3$ extract (55 g) was separated into six fractions, fr. I—VI, by silica gel column chromatography with benzene–acetone (10 : 1, 6 : 1 and then 4 : 1). Fraction III was crystallized from MeOH to give **1**. Fractions IV—VI were further chromatographed on a column of silica gel with benzene–acetone (4 : 1) and then purified by preparative reversed-phase high performance liquid chromatography (column, TSK-GEL ODS-120T, 21.5 mm × 30 cm; solvent, 88% or 90% MeOH; flow rate, 6 ml/min; detection, refraction index to give **3**, **5** and **6** from fr. IV, **2** from fr. V, and **4**, **7**, **8** and **9** from fr. VI. The BuOH extract (140 g) was chromatographed on a column of highly porous polymer (Diaion HP-20) and eluted with H_2O, 30% MeOH, 60% MeOH and MeOH, successively. The fractions eluted with 30% MeOH and 60% MeOH were further subjected to rechromatography on silica gel with $CHCl_3$–MeOH–H_2O (3 : 1 : 0.1 or 6 : 4 : 1) and $CHCl_3$–MeOH–AcOEt–H_2O (6 : 4 : 4 : 1) to give **10** from the 30% MeOH fraction, and **11**, **12**, **13** and **14** from the 60% MeOH fraction. Total yields of **1**—**14** were 0.007, 0.0014, 0.032, 0.0028, 0.0048, 0.0032, 0.002, 0.0023, 0.0032, 0.0004, 0.0012, 0.001, 0.004 and 0.0045%, respectively.

Betulinic Acid (1) Colorless needles, mp 300 °C, $[\alpha]_D^{24}$ +12.0° (c=0.60, MeOH).

Alphitolic Acid (2) Colorless needles (from MeOH), mp 275—277 °C, $[\alpha]_D^{24}$ −4.2° (c=0.45, MeOH). ^{1}H-NMR δ: 0.90 (3H, s, CH_3), 1.06 (9H, s, CH_3), 1.25 (3H, s, CH_3), 1.79 (3H, s, CH_3), 3.40 (1H, d, J=10.0 Hz, H-3α), 4.10 (1H, m, $W_{1/2}$=18 Hz, H-2β), 4.77, 4.92 (each 1H, d, J=2 Hz, H-30). ^{13}C-NMR δ: 48.1 (C-1), 68.9 (C-2), 83.7 (C-3), 38.7 (C-10), 17.7 (C-24 or 25), 17.4 (C-24 or 25).

Oleanolic Acid (3) Colorless needles (from MeOH), mp 300 °C, $[\alpha]_D^{25}$ +82.3° (c=0.96, $CHCl_3$).

Maslinic Acid (4) A white powder, $[\alpha]_D^{24}$ +47.5° (c=0.59, MeOH). ^{1}H-NMR δ: 0.94 (3H), 0.98 (3H), 1.01 (6H), 1.08 (3H), 1.27 (3H) (each s, CH_3), 3.25 (1H, d, J=9.5 Hz, H-3α), 3.96 (1H, ddd, J=4.0, 9.5, 11.0 Hz, H-2β), 5.41 (1H, br s, H-12). ^{13}C-NMR δ: 47.7 (C-1), 68.6 (C-2), 83.8 (C-3), 38.6 (C-10), 17.5 (C-24 or 25), 16.8 (C-24 or 25).

Ursolic Acid (5) Colorless needles (from MeOH), mp 292 °C, $[\alpha]_D^{24}$ +71.5° (c=0.82, $CHCl_3$).

Pomolic Acid (6) A white powder, $[\alpha]_D^{25}$ +54.0° (c=0.58, MeOH). ^{1}H-NMR δ: 0.93, 1.04, 1.12 (each 3H, s, CH_3), 1.15 (3H, d, J=3.0 Hz, H-30), 1.25, 1.47, 1.74 (each 3H, s, CH_3), 3.07 (1H, s, H-18), 3.46 (1H, t, J=8.0 Hz, H-3α), 5.62 (1H, br s, H-12). ^{13}C-NMR δ: 128.1 (C-12), 54.6 (C-18), 72.7 (C-19), 42.4 (C-20), 27.0 (C-21), 38.5 (C-22), 27.1 (C-29), 16.8 (C-30).

2α-Hydroxyursolic Acid (7) A white powder, $[\alpha]_D^{24}$ +55.0° (c=0.69, MeOH). ^{1}H-NMR δ: 0.98—1.28 (21H, CH_3), 2.61 (1H, d, J=11.0 Hz, H-18), 3.38 (1H, d, J=9.3 Hz, H-3α), 4.08 (1H, ddd, J=4.2, 9.3, 11.2 Hz, H-2β), 5.44 (1H, br s, H-12). ^{13}C-NMR δ: 48.0 (C-1), 68.6 (C-2), 83.8 (C-3), 38.4 (C-10), 17.5 (C-24 or 25), 17.0 (C-24 or 25).

Tormentic Acid (8) A white powder, $[\alpha]_D^{25}$ +31.5° (c=0.80, MeOH). ^{1}H-NMR δ: 1.01, 1.08, 1.10 (each 3H, s, CH_3), 1.14 (3H, d, J=3.0 Hz, H-30), 1.28, 1.44, 1.72 (each 3H, s, CH_3), 3.04 (1H, s, H-18), 3.40 (1H, d, J=9.0 Hz, H-3α), 4.10 (1H, m, $W_{1/2}$=20 Hz, H-2β), 5.57 (1H, br s, H-12). ^{13}C-NMR δ: 47.9 (C-1), 68.6 (C-2), 83.8 (C-3), 38.5 (C-10), 127.9 (C-12), 54.6 (C-18), 72.7 (C-19), 42.4 (C-20), 27.0 (C-21), 38.5 (C-22), 17.7 (C-24 or 25), 17.3 (C-24 or 25), 27.1 (C-29), 16.8 (C-30).

Euscaphic Acid (9) A white powder, $[\alpha]_D^{25}$ +12.0° (c=0.65, MeOH). ^{1}H-NMR δ: 0.90, 0.99 (each 3H, s, CH_3), 1.13 (3H, d, J=4.0 Hz, H-30), 1.27 (6H, s, CH_3), 1.42, 1.64 (each 3H, s, CH_3), 3.04 (1H, s, H-18), 3.77 (1H, d, J=3.5 Hz, H-3β), 4.33 (1H, m, $W_{1/2}$=20 Hz, H-2β), 5.59 (1H, br s, H-12). ^{13}C-NMR δ: 42.8 (C-1), 66.1 (C-2), 79.3 (C-3), 38.6 (C-10), 128.0 (C-12), 54.6 (C-18), 72.7 (C-19), 42.4 (C-20), 27.0 (C-21), 38.5 (C-22), 22.3 (C-24), 17.3 (C-25), 27.0 (C-29), 16.6 (C-30).

Ethyl β-Fructopyranoside (10) Colorless needles (from MeOH), mp 157—157.9 °C, $[\alpha]_D^{25}$ −134° (c=1.00, MeOH). ^{1}H-NMR δ: 1.15 (3H, t, J=7.0 Hz, CH_3), 3.72 (2H, q, J=7.0 Hz, –OCH_2–), ^{13}C-NMR δ: 15.7 (CH_3), 56.5 (CH_2), 64.3 (C-6), 64.9 (C-1), 70.5 (C-3), 71.3 (C-5), 72.3 (C-4), 101.2 (C-2). Compound **10** yielded fructose on mineral acid hydrolysis (see ref. 10).

Quercetin (11) Pale yellow needles (from MeOH), mp 300 °C.

Hyperin (12) Pale yellow needles (from MeOH), mp 233—235 °C.

Tiliroside (13) Yellow needles (from MeOH), mp 251—253 °C, $[\alpha]_D^{25}$ −52.5° (c=0.70, MeOH).

Methyl 3-*O*-β-D-Glucopyranosyl-gallate (14) A pale yellow powder, $[\alpha]_D^{24}$ −61.0° (c=1.00, MeOH). *Anal.* Calcd for $C_{14}H_{18}O_{10}$: C, 48.56; H, 5.24. Found: C, 48.26; H, 5.47. ^{1}H-NMR δ: 3.72 (3H, s, CH_3), 5.65 (1H, d, J=6.5 Hz, anomeric H), 7.92, 8.05 (each 1H, d, J=2.0 Hz, aromatic H). ^{13}C-NMR δ: β-glucopyranosyl moiety: 104.6 (C-1′), 75.0 (C-2′), 78.2 (C-3′ or 5′), 70.9 (C-4′), 78.9 (C-3′ or 5′), 62.0 (C-6′), methyl gallate moiety: 121.0 (C-1), 113.6 (C-2), 148.0 (C-3), 143.1 (C-4), 146.8 (C-5), 112.1 (C-6), 167.1 (–CO–O–), 51.7 (–OCH_3). On mineral acid hydrolysis, **14** yielded glucose and an aglycone (see ref. 10). The aglycone was identified as gallic acid by direct comparison of the ^{13}C-NMR spectrum with that of an authentic sample.

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