A New Acylated Triterpene from the Roots of *Chaenomeles japonica*

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A new acylated triterpene together with prunasin, (−)-epicatechin, daucosterol, and three triterpenes, ursolic, oleanolic, and pomolic acids was isolated from the root of *Chaenomeles japonica* (Thunb.) Lindl. (Rosaceae) and determined to be 3-*O*-(*E*)-3,5-dihydroxycinnamoylursolic acid on the basis of NMR and FAB-MS experiments.

Key words *Chaenomeles japonica*; Rosaceae; acylated triterpene; 3-*O*-(*E*)-3,5-dihydroxycinnamoylursolic acid

Chaenomeles japonica (Thunb.) Lindl. (Rosaceae) is a small shrub distributed in the southern parts of the Korean peninsula and is commonly grown as a garden plant. The fruits of this plant have been used as a stomachic and astringent agent in traditional medicine.[1)] Previous chemical studies of *C. japonica* have yielded roseoside,[2)] monoterpene glucosides,[3)] epicatechin, and leucoanthocyanin[4)] from fruits, flavonol glycosides and epicatechin[5)] from leaves, and α-tocopherol and fatty acids[6)] from seed oils. To our knowledge, no chemical and biological investigations have been carried out on the roots of the plant. In our search for biologically active natural products, we investigated the constituents of the roots of *C. japonica*, from which a new acylated triterpene (**1**) together with prunasin, (−)-epicatechin, daucosterol, and the three triterpenes ursolic, oleanolic, and pomolic acids was isolated. This paper describes the isolation and structure elucidation of these compounds.

Six known compounds were isolated from the CH_2Cl_2 and EtOAc extracts of the roots of *C. japonica*, as described in the Experimental section, and were identified as daucosterol,[7)] the three triterpenes ursolic, oleanolic, and pomolic acids[7—10)] from the CH_2Cl_2 extract, and prunasin[10,11)] and (−)-epicatechin[7,12,13)] from the EtOAc extract, respectively, by comparison of their physical and spectral data with reported values. Among these isolates, five, excluding (−)-epicatechin, were isolated from this plant for the first time.

Fraction 21 from the CH_2Cl_2-soluble fraction was purified by recycling preparative HPLC with EtOAc to afford compound **1**, which was deduced to be a triterpenoid based on a positive Liebermann–Burchard test and a molecular formula of $C_{39}H_{54}O_6$ suggested by its high-resolution FAB-MS. This compound exhibited UV maxima at 243, 299, and 329 nm, suggesting the presence of considerable conjugation in the molecule. In the IR spectrum, absorption bands at 3391 (OH), 1701 (ester C=O), 1605, 1514 (aromatic C=C), 1610, and 976 (*trans*-disubstituted double bond) were apparent. The ^{1}H-NMR spectrum exhibited five singlet methyl groups, two doublet methyl groups, an oxymethine proton in proximity to an ester group at δ 4.86 (dd, J=4.8, 11.1 Hz), a typical ursene H-18 (br d, J=11.4 Hz) and H-12 (br s) protons, and 3,5-dihydroxycinnamoyl moiety protons. This ester group was placed at C-3 as deduced from the downfield shift observed for H-3 in the ^{1}H-NMR spectrum. Compound **1** was considered to be an ursolic acid derivative with an extra (*E*)-3,5-dihydroxycinnamoyl moiety by comparison of its ^{13}C-NMR data with those of ursolic acid.[14,15)] Thus compound **1** was assigned to be 3-*O*-(*E*)-3,5-dihydroxycinnamoylursolic acid. A number of reports on the isolation of acylated triterpenes from plants have appeared, and some have been demonstrated a variety of biological properties including anti-inflammatory,[16)] cytotoxic,[9,17,18)] and antitumor[19—21)] activities. To our knowledge, the compound reported here is the second example of an acylated triterpene possessing pentacyclic triterpenes with an unusual phenylpropanoid, (*E*)-3,5-dihydroxycinnamic acid moiety.[22)] The only other example of this type of congener is described as betulinic acid 3,5-dihydroxycinnamate[23)] from *Spiraea mongolica* Maxim.

Experimental

General Procedures Melting points were measured on a Mitamura-Riken apparatus and are uncorrected. The optical rotations were determined on a JASCO P-1020 polarimeter. The IR spectra were obtained on a JASCO FT/IR-5300 spectrometer. EI mass spectra were obtained on a Hewlett-Packard 5989B spectrometer. The FAB mass spectrum was obtained in a 3-nitrobenzyl alcohol matrix in a positive-ion mode on a VG-VSEQ spectrometer. The NMR spectra were measured on a Varian Gemmi 2000 instrument (300 MHz), and the chemical shifts are referenced to tetramethylsilane (TMS). Recycling preparative HPLC was performed on a JAIKOREA-LC-918 (Japan Analytical Industry Co., Japan) equipped with a JAI refractive index (RI) detector and a JAI UV detector operating at 294 nm. TLC was performed on silica gel 60F_{254} (Merck).

Plant Material The roots of *C. japonica* were collected in April 1998 at the Economic Plants Research Station of our institute in Kyeonggi Province, Korea, and authenticated by emeritus professor H. J. Chi of the Natural Products Research Institute, Seoul National University.

Extraction and Isolation The dried root parts of *C. japonica* (2.2 kg)

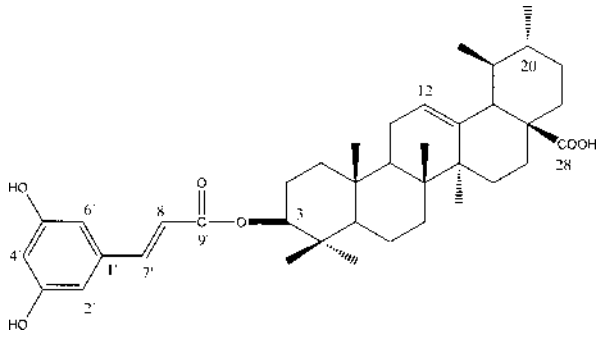

Fig. 1

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were extracted 3 times with MeOH at room temperature. The MeOH extract was evaporated under reduced pressure to dryness and then partitioned in succession between H_2O and *n*-hexane, CH_2Cl_2, EtOAc, and then *n*-BuOH and yielded 7.0, 3.4, 25.4, and 42.9 g of the respective extracts. The CH_2Cl_2 fraction (3.4 g) was chromatographed on a silica gel column eluted with *n*-hexane–EtOAc mixtures of increasing polarity to give 38 fractions. Fractions 14 and 30 were crystallized from MeOH to give a mixture of ursolic acid and oleanolic acid[7,8] (105 mg) and daucosterol[7] (44 mg), respectively. Fraction 19 was subjected to chromatography on silica gel 60 using $CHCl_3$–MeOH–H_2O (100 : 2 : 1) to afford pomolic acid[9,10] (17 mg). Fraction 21 (70 mg) was further purified by recycling HPLC using a gel-permeation chromatography column (JAIGEL GS-310, 21.5 mm×300 mm×2) with EtOAc as the mobile phase at a flow rate of 5 ml/min to yield compound **1** (22 mg). A portion of the EtOAc fraction (19.9 g) was chromatographed in a similar manner on silica gel 60 using CH_2Cl_2–MeOH–H_2O (14 : 4 : 1) as an eluent to yield 10 fractions. Fractions 3—5 were combined, concentrated, and then crystallized from $CHCl_3$–MeOH to yield prunasin[10,11] (6.32 g). Fraction 8 (1.01 g) was rechromatographed on silica gel using *n*-hexane–EtOAc (1 : 1) as an eluent to yield (−)-epicatechin[7,12,13] (62 mg). All the isolates were identified by comparison of spectroscopic and physical data with the values reported in the literature.

Compound (**1**) was obtained as amorphous powder (MeOH). mp 205—206 °C. $[\alpha]_D^{21}$ +55.85° (c=0.173, MeOH). Positive-ion FAB-MS *m/z*: 641.3818 (Calcd for $C_{39}H_{54}O_6$+Na: 641.3798), 439 $[(M-C_9H_8O_4)+H]^+$. IR (KBr) cm^{-1}: 3391, 2948, 1701, 1610, 1605, 1514, 1447, 1273, 1184, 1113, 1019, 976, 856, 808. UV λ_{max} (MeOH) nm (log ε) 243 (3.90), 299 (3.99), 329 (4.10). ^{1}H-NMR (pyridine-d_5, 300 MHz) δ: 0.83 (3H, s, 25-CH_3), 0.94 (3H, s, 24-CH_3), 0.95 (3H, s, 23-CH_3), 0.96 (3H, d, J=5.4 Hz, 30-CH_3), 1.00 (3H, d, J=6.9 Hz, 29-CH_3), 1.01 (3H, s, 26-CH_3), 1.23 (3H, s, 27-CH_3), 2.12 (1H, dt, J=4.2, 12.9 Hz, H-16α), 2.30 (1H, dt, J=4.2, 13.2 Hz, H-15β), 2.62 (1H, br d, J=11.4 Hz, H-18), 4.86 (1H, dd, J=4.8, 11.1 Hz, H-3), 5.47 (1H, br s, H-12), 6.69 (1H, d, J=15.9 Hz, H-8′), 7.23 (2H, s, H-2′,6′), 7.66 (1H, s, H-4′), 8.03 (1H, d, J=15.9 Hz, H-7′). ^{13}C-NMR (pyridine-d_5, 75.5 MHz) δ: 38.36 (C-1), 24.10 (C-2), 80.45 (C-3), 38.10 (C-4), 55.62 (C-5), 18.48 (C-6), 33.35 (C-7), 39.90 (C-8), 47.82 (C-9), 37.05 (C-10), 23.55 (C-11), 125.45 (C-12), 139.25 (C-13), 42.48 (C-14), 28.66 (C-15), 24.89 (C-16), 48.03 (C-17), 53.51 (C-18), 39.48 (C-19), 39.40 (C-20), 31.08 (C-21), 37.43 (C-22), 28.26 (C-23), 17.13 (C-24), 15.54 (C-25), 17.37 (C-26), 23.91 (C-27), 179.86 (C-28), 17.49 (C-29), 21.41 (C-30), 126.98 (C-1′), 116.72 (C-2′), 147.73 (C-3′), 122.02 (C-4′), 150.42 (C-5′), 115.62 (C-6′), 145.64 (C-7′), 115.86 (C-8′), 167.33 (C-9′).

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