

A New Triterpene Ester from *Eriobotrya japonica*

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A new triterpene ester, 3-*O*-*trans*-feruloyl euscaphic acid, was isolated from the leaves of *Eriobotrya japonica*. The structure of this compound was elucidated by means of chemical and spectroscopic studies.

Key words *Eriobotrya japonica*; triterpene; 3-*O*-*trans*-feruloyl euscaphic acid; Rosaceae

Topical application of the alcoholic extract from the leaves of *Eriobotrya japonica* LINDL has traditionally been used for the treatment of prickly heat, eczema and skin disease.¹⁾ Some triterpenes have been isolated from this plant^{1–3)} and one of them, maslinic acid, was found to show an anti-inflammatory effect on carrageenin-induced edema and an inhibitory effect on histamine-induced ileum contraction.¹⁾ Furthermore, 3,6,19-trihydroxy-urs-12-en-28-oic acid⁴⁾ and tormentic acid,⁵⁾ isolated from the same source, have been reported to have a hypoglycemic effect. Triterpenoid constituents thus seem to be efficiently responsible for the pharmacological activity of this plant. During our search of triterpenoid constituents in the leaves of *E. japonica*, a new triterpene ester (**1**) was isolated. This paper deals with the structural elucidation of **1**.

Results and Discussion

The chloroform soluble fraction of the alcoholic extract from the fresh leaves of *Eriobotrya japonica*, which has been found to have an anti-inflammatory effect by the carrageenin edema test, was subjected to repeated column chromatography to afford a new triterpene ester, **1**, along with several known compounds.

The IR absorptions of **1**, mp 185–190 °C, at 1720, 1700, 1630 and 1265 cm⁻¹, and the UV absorptions at 290 and 320 nm suggested an α,β unsaturated aromatic acid ester and free carboxylic acid moieties. **1** gave a positive color reaction to Lieberman–Burchard and FeCl₃ reagents, respectively. Electron impact-mass spectra (EI-MS) showed peaks at m/z 664 (M⁺). Acetylation of **1** in the usual manner (Ac₂O–pyridine) afforded diacetate (**1a**), mp 240–243 °C, m/z 748 (M⁺). The ¹H-NMR spectrum of **1a** (Table 1) resembled that of the diacetate of euscaphic acid (**2**) or tormentic acid (**3**) except for signals due to a 1,3,4-tri-substituted aromatic ring, *trans* olefinic protons ($J=15.8$ Hz), one methoxyl and one aromatic acetyl group, which suggested the presence of a *trans* feruloyl moiety. (Nuclear Overhauser effect (NOE) was observed between H-2' and 3'-OCH₃). The 2 α ,3 α -*O*-substitution of **1a** was evident from the J value of two protons ascribable to H-3 (5.12 ppm, d, $J=2.4$ Hz) and to H-2 (5.30 ppm, dd, $J=2.4, 10.8$ Hz). The linked position of the ferulic acid moiety was indicated at C-3 by the NMR chemical shifts of H-3 and C-3, as deduced by comparison with analogous compounds (Table 1, 2). The urs-12-ene

structure of the triterpenic moiety was supported by the resonance of carbon at C-12 (129.0 ppm), which is typical of an urs-12-ene-derivative and very different from an olean-12-ene analogue⁶⁾ in the ¹³C-NMR spectrum, and by the resonances of six methyl groups in the region δ 0.77–1.35, one of which was a doublet (3H, H-30), and of a proton at H-18 (2.56 ppm, 1H, s) characteristic of a 19 α -OH ursolic acid derivative⁷⁾ in the ¹H-NMR spectrum. The MS spectrum of **1a** exhibited peaks at m/z 748 (M⁺), 688 (M–AcOH)⁺, 485 (M–264*+H)⁺, 264*, and 219 (acetyl feruloyl). (*Fragment due to D/E ring by *retro* Diels–Alder cleavage characteristic of 12-ene-triterpenoid). Saponification of **1** with an alkali solution gave euscaphic acid and ferulic acid, which were identified by comparison of the spectral data with those of authentic sample, respectively. Therefore, we determined the structure of **1** to be 3-*O*-*trans*-feruloyl euscaphic acid, and report for the first time its existence in nature.

Table 1. Partial Comparison of the ¹H-NMR Spectral Data for **1**-, **2**-, and **3**-Diacetates in CDCl₃

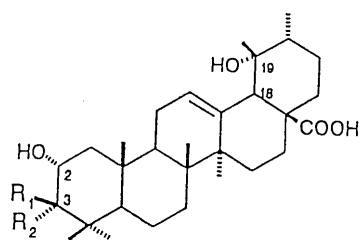
Proton	1a	2a	3a
2	5.30 (dd, $J=10.8, 2.4$)	5.27 (m)	5.05 (m)
3	5.12 (d, $J=2.4$)	5.00 (d, $J=2.5$)	4.69 (d, $J=10.0$)
12	5.36 (br)	5.38 (br)	5.27 (m)
18	2.56 (s)	2.50 (s)	2.51 (s)
α	6.44 (d, $J=15.8$)		
β	7.66 (d, $J=15.8$)		
OAc	1.96, 2.33	1.98, 2.14	1.98, 2.05
OMe	3.88		

¹H-NMR data represent chemical shifts in ppm, multiplicity (J) in Hz. **2a**, euscaphic acid diacetate; **3a**, tormentic acid diacetate.

Table 2. Partial Comparison of the ¹³C-NMR Chemical Shifts (δ ppm) of **1**, **2**, and **3** in Pyridine-*d*₅

Carbon	1	2	3
2	65.1	65.2	68.6
3	81.5	78.3	83.9
12	126.5	127.0	128.0
13	140.0	138.9	140.3
19	72.9	71.8	72.7
20	42.2	41.2	42.2
28	180.5	179.7	180.7
29	26.4	26.2	27.8
30	16.8	15.7	17.3

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	R ₁	R ₂
1	H	
2	H	OH (euscaphic acid)
3	OH	H (tormentic acid)

Fig. 1

Experimental

Melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. ¹H- and ¹³C-NMR were taken on a JEOL-GX 400 spectrometer in pyridine-*d*₅ and CDCl₃ solutions with tetramethylsilane as an internal standard, and chemical shifts are recorded in δ -values. MS were obtained with a JEOL JMS DX-300 spectrometer (70 eV) using a direct inlet system. IR and UV spectra were obtained from a Hitachi 260-10 spectrometer and Hitachi 220S spectrophotometer, respectively. Column chromatography was done with Kieselgel 60 (petroleum benzin-AcOEt) and Cosmosil 75C₁₈-OPN (93% MeOH). Shim-pack PREP-ODS(H) (4.6 \times 250 mm, 20 \times 250 mm, 88% CH₃CN, detect.: 210 nm, flow rate: 1.0 ml/min for analysis, 7.0 ml/min for preparative) was used for HPLC.

Extraction and Isolation The dried leaves of *Eriobotrya japonica* (60 g) were extracted with MeOH at room temp., and the MeOH extract was partitioned between CHCl₃ and H₂O as reported before.³⁾ The CHCl₃ extract (30 g) was chromatographed first on a silica gel column by stepwise elution with petroleum benzin-AcOEt (10:3 \rightarrow 1:1) and then on a reverse phase column (Cosmosil) with 93% MeOH to give Frs. A—C. Tormentic acid and euscaphic acid were isolated from fr. A and maslinic acid, 2 α -hydroxy ursolic acid, oleanolic acid and ursolic acid were isolated from fr. C (identified by HPLC). Fraction B was further purified by repeated chromatography to afford compound **1** (8.3 mg).

3-O-*trans* Feruloyl Euscaphic Acid (1) Amorphous compound, mp 185—190 °C (dec.). UV $\lambda_{\text{max}}^{\text{MeOH}}$: 290, 320 nm. IR $\nu_{\text{max}}^{\text{KBr}}$: 3500 (OH), 1720 (ester), 1700 (COOH), 1630, 1600 (C=C) and 1265 cm⁻¹ (C-O). EIMS *m/z*: 664[M]⁺, 618 [M-HCO₂H]⁺, 602 [M-CO₂-H₂O]⁺, 470 [M-194]⁺, 424 [618-194]⁺, 194 [ferulic acid]⁺. ¹H-NMR (pyridine-*d*₅): indistinct (assigned in acetate **1a**). ¹³C-NMR: Table 2.

1-Diacetate (1a) **1** (10 mg) was acetylated with acetic anhydride (0.5 ml) and pyridine (0.5 ml) at room temp. (15 h) to give diacetate **1a**, colorless needles, mp 240—243 °C. ¹H-NMR (CDCl₃): δ 0.77, 0.93, 1.04, 1.08, 1.21, 1.36 (each 3H, s), 0.95 (3H, d, *J* = 6.72 Hz, Me-20), 1.95 (2-OAc), 2.32 (4'-OAc), 2.56 (1H, s, H-18), 3.88 (3'-OCH₃, showed NOE with H-2'), 5.12 (1H, d, *J* = 2.4 Hz, H-3), 5.30 (1H, dd, *J* = 10.8, 2.4 Hz, H-2), 5.36 (1H, br, H-12), 6.44 (1H, d, *J* = 15.8 Hz, H- α), 7.05 (1H, d,

J = 7.9, H-5'), 7.11 (1H, d, *J* = 1.8, H-2'), 7.15 (1H, q, *J* = 7.9, 1.8 Hz, H-6'), 7.66 (1H, d, *J* = 15.8 Hz, H- β). ¹³C-NMR (CDCl₃): δ 56.0 (3'-OMe), 68.4 (C-2), 73.2 (C-19), 77.3 (C-3), 111.5 (C-2'), 118.4 (C- α), 121.2 (C-6'), 123.3 (C-5'), 129.0 (C-12), 133.3 (C-1'), 138.0 (C-13), 141.5 (C-3'), 144.2 (C- β), 151.4 (C-4'), 166.3 (2-OAc), 168.8 (3-OCO-), 170.5 (4'-OAc), 184.0 (C-28). EI-MS *m/z*: 748[M]⁺, 706 [M-COCH₂]⁺, 688 [M-60]⁺, 485 [M-264*+H]⁺ (fragment containing A/B ring by retro Diels-Alder cleavage), 442 [M-264*-COCH₂]⁺, 424 [M-264*-AcOH]⁺, 265 [484-219**]⁺, 264* (fragment containing D/E ring by retro Diels-Alder cleavage), 246 [264*-H₂O]⁺, 219** [acetyl feruloyl]⁺, 194 [ferulic acid]⁺, 177 [219-COCH₂]⁺.

Saponification of 1 **1** was saponified with 5% KOH in MeOH at room temp. (7 h). The hydrolysate was made acidic with dilute HCl and extracted with Et₂O. The Et₂O extract was subjected to silica gel column with hexane-AcOEt eluants to give euscaphic acid, mp 270—272 °C, and ferulic acid, mp 174 °C, which were identified by comparison (IR, TLC) with an authentic sample, respectively.

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