Ponfolin: A New Coumarin from Trifoliate Orange

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A new coumarin, ponfolin, was isolated from the root of Trifoliate Orange [Poncirus trifoliata
(L.) Raf. (Rutaceae)], and its structure was determined as I.

Keywords—Trifoliate Orange; Poncirus trifoliata; Citrus trifoliata; Rutaceae; coumarin;
ponfolin; nordentatin; clausarin; seselin; xanthyletin

During a current project dealing with the constituents of Citrus plants, an investigation
of the root of Trifoliate Orange [Poncirus trifoliata (L.) Raf.; Citrus trifoliata (Rutaceae)]
demonstrated the presence of a new coumarin named ponfolin (I). Previously the coumarins
poncitrin (dentatin), nordentatin, marmesin, and seselin were isolated from the root of this
plant.1–5) This paper describes the isolation and structure elucidation of a new coumarin,
ponfolin (I).

The acetone extract of the root of Trifoliate Orange was subjected to column chromato-
graphy on silica gel to isolate a new coumarin, ponfolin, along with four known
coumarins, nordentatin (2), clausarin (3), seselin (4), and xanthyletin (5).

Ponfolin (I) was obtained as a colorless oil, and the molecular formula C_{24}H_{26}O_{4} was
determined by high-resolution mass spectrometry. The ultraviolet (UV) absorptions at λ_{max}
206, 230, 273, and 329 nm were similar to those of usual pyranocoumarins such as nordentatin
(2).6) The infrared (IR) bands at 1715, 1640, and 1610 cm⁻¹, and two AB-type signals at δ 6.06
and 7.94 (each doublet, J = 10 Hz), and at δ 5.65 and 6.62 (each doublet, J = 10 Hz)
accompanied with a six-proton singlet at δ 1.44 in the proton nuclear magnetic resonance (¹H-
NMR) spectrum also indicated the presence of a pyranocoumarin nucleus in the molecule.
The ¹H-NMR spectrum of ponfolin showed two ABX pattern signals at δ 4.82 (1H, double-
doublets, J=1, 11 Hz), 4.85 (1H, double-doublets, J=1, 17 Hz), and 6.28 (1H, double-
doublets, J=11, 17 Hz), and at δ 5.08 (1H, double-doublets, J=1, 11 Hz), 5.20 (1H, double-
doublets, J=1, 17 Hz), and 6.06 (1H, double-doublets, J=11, 17 Hz), and four tertiary
methyl signals at δ 1.44 (6H, singlet) and 1.65 (6H, singlet), suggesting the presence of two
1,1-dimethylallyl moieties.

Treatment of ponfolin (I) with diluted hydrochloric acid in methanol at room tempera-
ture for one hour afforded colorless needles, mp 180–182 °C, as a sole product, which was
shown to be identical with authentic nordentatin (2)6) by IR and ¹H-NMR spectral
comparisons, and mixed melting point and co-thin layer chromatography (TLC) determi-
nation. On the basis of these results, ponfolin can be represented by the formula I.

Known coumarins, nordentatin (2),7) clausalin (3),8) seselin (4),9) and xanthyletin (5),10)
were also isolated and characterized.
Experimental

All melting points were measured on a micro melting point hot stage apparatus (Yanagimoto). $^1$H-NMR spectra were recorded on a PS-100 (JEOL) or FX-100 (JEOL) spectrometer in deuteriochloroform except where otherwise stated. Chemical shifts are given in ppm ($\delta$) with tetramethylsilane (TMS) as an internal reference. Mass spectra (MS) were taken with an M-52 (Hitachi) or M-80 (Hitachi) spectrometer with a direct inlet system. UV spectra were determined in MeOH and IR spectra were recorded in CHCl$_3$, silica gel GF$_{254}$ (Merck) and silica gel 60 (70–230 mesh ASTM) (Merck) were used for TLC and column chromatography, respectively. The abbreviations used are as follows: s, singlet; d, doublet; dd, double-doublet.

**Isolation of Ponfolin (1) and Four Known Coumarins from Trifoliolate Orange [Poncirus trifoliata (L.) Raf.]**

The root (95 g) of Trifoliolate Orange (Japanese name “Karakuchi”) cultivated at Okitsu Branch Fruit Tree Research Station, Shizuoka, was extracted with acetone at room temperature. The acetone extract was chromatographed on silica gel column. Elution with hexane-diisopropyl ether (3:2) yielded twenty fractions. Fraction 2 was subjected to preparative TLC developed with hexane-diisopropyl ether (5:2) to obtain ponfolin (1) (60 mg) and clausarin (3) (10 mg). Seselin (4) (861 mg) and xanthyletin (5) (332 mg), and nordenatin (2) (90 mg) were isolated as crystals from fractions 5–8, 9–10, and 15–20, respectively.

**Ponfolin (1)** — Colorless oil. High resolution MS: Calcd for C$_{24}$H$_{28}$O$_4$: 380.1986. Found: 380.2015. UV $\lambda_{	ext{max}}$ nm (log ε): 206 (4.29), 230 (4.29), 273 (4.41), 329 (4.05). IR $\nu_{	ext{max}}$ cm$^{-1}$: 1715, 1640, 1610, 1580. MS $m/z$(%): 380 (M$^+$, 6), 365 (16), 312 (57), 297 (100). $^1$H-NMR (CDCl$_3$-acetone-d$_6$): $\delta$: 1.44 (12H, s, 4 × CH$_3$), 1.65 (6H, s, 2 × CH$_2$), 4.82 (1H, dd, $J = 11, 17$ Hz), 4.85 (1H, dd, $J = 1, 11$ Hz), 6.08 (1H, dd, $J = 1, 17$ Hz), 5.20 (1H, dd, $J = 1, 11$ Hz), 6.55 (1H, d, $J = 10$ Hz), 7.94 (1H, d, $J = 10$ Hz).

**Treatment of Ponfolin (1) with Dil. Hydrochloric Acid** — Two drops of 5% HCl were added to a methanolic solution (0.5 ml) of ponfolin (5 mg), and the mixture was left for 1 h at room temp. The solution was diluted with 10 ml of CHCl$_3$, dried over anhyd. K$_2$CO$_3$, filtered, and then evaporated to dryness. The residue was recrystallized from CHCl$_3$ to afford colorless prisms (3 mg), mp 180–182°C. The IR and $^1$H-NMR (CDCl$_3$-acetone-d$_6$ spectra were superimposable on those of authentic nordenatin (2) and co-TLC (diisopropyl ether) gave a single spot.

**Nordenatin (2)** — Colorless prisms from MeOH, mp 181–182°C. IR $\nu_{	ext{max}}$ cm$^{-1}$: 3600, 3300, 1720, 1620, 1600, 1560. $\nu_{	ext{max}}$ (Nujol) cm$^{-1}$: 3200, 1690, 1610, 1590, 1560. $^1$H-NMR (acetone-d$_6$): $\delta$: 1.44 (6H, s), 1.62 (6H, s), 4.77 (1H, dd, $J = 1, 11$ Hz), 4.84 (1H, dd, $J = 1, 17$ Hz), 5.66 (1H, d, $J = 10$ Hz), 6.00 (1H, d, $J = 10$ Hz), 6.20 (1H, dd, $J = 11, 17$ Hz), 6.68 (1H, d, $J = 10$ Hz), 8.02 (1H, d, $J = 10$ Hz). This was shown to be identical with an authentic sample by IR and $^1$H-NMR (acetone-d$_6$) comparisons, and co-TLC (diisopropyl ether).

**Clausarin (3)** — Colorless needles from CHCl$_3$, mp 208–210°C. IR $\nu_{	ext{max}}$ cm$^{-1}$: 3600, 3320 1710, 1620, 1600, 1570. $^1$H-NMR (CDCl$_3$-acetone-d$_6$): $\delta$: 1.42 (6H, s), 1.44 (6H, s), 1.62 (6H, s), 4.80 (1H, dd, $J = 1, 11$ Hz), 4.88 (1H, dd, $J = 1, 17$ Hz), 4.94 (1H, s), 4.98 (1H, dd, $J = 1, 11$ Hz), 5.01 (1H, dd, $J = 1, 17$ Hz), 5.66 (1H, d, $J = 10$ Hz), 6.17 (1H, dd, $J = 11, 17$ Hz), 6.28 (1H, dd, $J = 11, 17$ Hz), 6.72 (1H, d, $J = 10$ Hz), 7.94 (1H, d, $J = 10$ Hz). This was shown to be identical with an authentic sample by IR and $^1$H-NMR comparisons, and co-TLC [diisopropyl ether–hexane (1:1)].

**Seselin (4)** — Colorless prisms from acetone, mp 118–120°C. IR $\nu_{	ext{max}}$ cm$^{-1}$: 1730, 1640, 1600. $^1$H-NMR: $\delta$: 1.44 (6H, s), 5.68 (1H, d, $J = 10$ Hz), 6.18 (1H, dd, $J = 10$ Hz), 6.68 (1H, d, $J = 10$ Hz), 6.84 (1H, d, $J = 10$ Hz), 7.16 (1H, d, $J = 10$ Hz), 7.55 (1H, d, $J = 10$ Hz). This was shown to be identical with an authentic sample by IR and $^1$H-NMR comparisons, and co-TLC [diisopropyl ether–hexane (1:1)].

**Xanthyletin (5)** — Colorless needles from hexane–diisopropyl ether, mp 128–129°C. IR $\nu_{	ext{max}}$ cm$^{-1}$: 1730, 1710, 1625, 1570. $^1$H-NMR: $\delta$: 1.44 (6H, s), 5.66 (1H, d, $J = 10$ Hz), 6.19 (1H, d, $J = 10$ Hz), 6.32 (1H, d, $J = 10$ Hz), 6.68 (1H, s), 7.00 (1H, s), 7.54 (1H, d, $J = 10$ Hz). This was shown to be identical with an authentic sample by IR and $^1$H-NMR comparisons, and co-TLC [diisopropyl ether–hexane (1:1)].

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References