Notes

[Chem. Pharm. Bull. 17(8)1723—1724(1969)]

UDC 581.19 : 547.597.05 : 582.751.9

Studies on the Chemical Components of Rutaceae Plants. III.[1] Components of the Root of *Poncirus trifoliata* RAFINESQUE. (3). Isolation of Limonin

TOSHIAKI TOMIMATSU

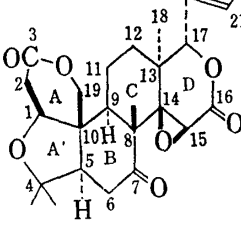
Faculty of Pharmaceutical Sciences, University of Tokushima[2]

(Received September 13, 1968)

In the previous papers of this series, it was reported that marmesin,[3] seselin, and β-sitosterol[1] were obtained from the roots of *Poncirus trifoliata* RAFINESQUE. This paper describes the isolation and identification of limonin as a neutral compound from the roots of *P. trifoliata*. The methanol extract of the air-dried roots when chromatographed on neutral alumina with benzene as eluting agent afforded compound A in a very poor yield, as described in the experimental section.

The molecular formula $C_{26}H_{30}O_8$ of compound A, mp 271—272° (decomp.), was established by elementary analyses. The ultraviolet spectrum in dioxane showed only high-intensity end absorption(226 mμ), while its infrared spectrum had two strong carbonyl bands(1770 and 1710 cm^{-1}) and characteristic bands assigned to a β-substituted furan (3105, 1505, and 890 cm^{-1}). A positive Ehrlich's test[4] on thin-layer chromatography (TLC) and notable color changing by Lieberman-Burchard reaction[5] reveals a further evidence for the presence of a furan ring in the substrate.

The nuclear magnetic resonance spectrum in d_5-pyridine indicated the presence of four C-methyl groups (τ: 8.70, 8.72, 8.72, and 8.73), β-substituted furan ring protons on C-21 and C-23 (2.30) and C-22 (3.43), a furfurylic proton on C-17 (4.21), and an epoxy proton on C-15 (5.38).



I

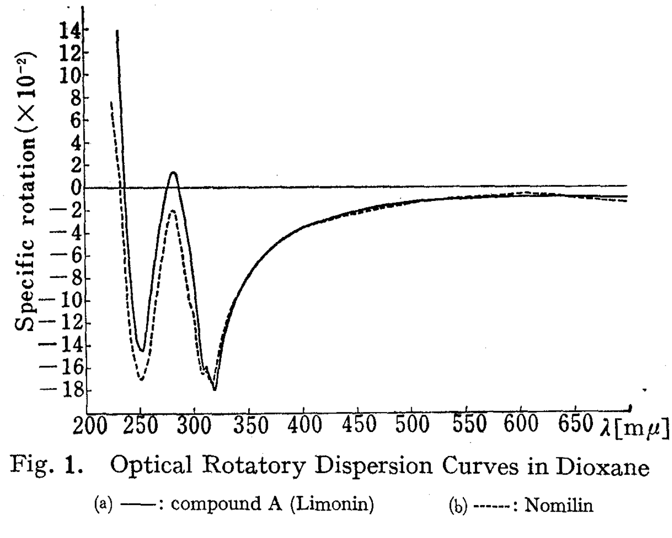


Fig. 1. Optical Rotatory Dispersion Curves in Dioxane

(a) ——: compound A (Limonin) (b) ------: Nomilin

---

[1] Part II: T. Tomimatsu and M. Hashimoto, *Yakugaku Zasshi*, **88**, 1357 (1968).

[2] Location: *Sho-machi, Tokushima.*

[3] T. Tomimatsu, *Yakugaku, Zasshi*, **88**, 643 (1968).

[4] T. Reichstein, *Helv. Chim. Acta*, **15**, 1110 (1932).

[5] V.E. Levine and E. Richmann, *Chem. Abstr.*, **28**, 5781 (1934); T. Nobiyama, *Nippon Kagaku Zasshi*, **79**, 315 (1958).

There was also a complex multiplet centered at about τ 5.2 which integrated for one proton.[6]

The optical rotatory dispersion (ORD) curve of compound A in dioxane exhibits a negative multiple Cotton effect with the first trough occurring at 317, 315, and 314 mμ, as shown in Fig. 1 (curve a). The similarity of the ORD data testifies to the stereochemical homogeneity of limonin, nomilin, and deacetylnomilin[7] about the B-ring.

The foregoing data suggest compound A to be a limonoid. Particularly, it appears very probable that compound A is identical with limonin. The constitution and stereochemistry of limonin have been detailed as represented in (I).[8-11] The infrared (IR) (KBr) spectrum and ORD (in dioxane) curve of compound A were superimposable on those of limonin, respectively. Their identity was further confirmed by comparison of *Rf* values on TLC and by the mixed melting determination.

This is the first example of the isolation of limonin from the roots of *Poncirus trifoliata*, and it is interesting in the biogenetic point of view that the presence of limonin was proved together with marmesin, seselin, and β-sitosterol in the same plant.

Experimental[12]

Extraction of Compound A (Limonin) from *Poncirus trifoliata*——Roots of *P. trifoliata* RAFINESQUE were obtained from the hedge growing in School of Medicine, University of Tokushima. Cut and air-dried roots, 31 kg, were extracted with boiling MeOH for 3 days by a cyclic-percolation method. The MeOH extract was concentrated under reduced pressure to a dark semi-solid concentrate. The syrupy residue remained after decantation of a syrup was further extracted with hot benzene for several days, and the extract was filtered. The total benzene solution was concentrated and then chromatographed on a column of neutral alumina. The last elution gave a fraction containing mostly compound A. The benzene solution was evaporated to dryness to yield 84 mg of colorless crystalline residue. Recrystallization from MeOH or AcOEt yielded colorless pillars or colorless scales, mp 271—272° (decomp.). The material showed a fine spot on TLC with 5% *p*-dimethylaminobenzaldehyde in EtOH and hydrogen chloride vapor. The *Rf* value (0.49) was identical with that of limonin ($CHCl_3$–AcOEt, 1:1 silica gel, Merck). Liebermann–Burchard Reaction: yellow-brown→dark brown→light brown. *Anal.* Calcd. for $C_{26}H_{30}O_8$: C, 66.37; H, 6.43. Found: C, 66.08; H, 6.05. ORD[7] ($c=0.097$, dioxane) $[\alpha]^{29}$ (mμ): −72° (700), −72° (680), −82° (640), −103° (600), −103° (589), −113° (580), −133° (540), −185° (500), −216° (480), −390° (440), −575° (370), −780° (350), −985° (340), −1293° (330), −1857° (318), −1632° (309), −739° (298), ±0° (288), +123° (280), −328° (270), −1129° (260), −1447° (252), −226° (240). The authentic sample of limonin, mp 279—280° (decomp.), afforded colorless pillars, mp 271—272° (decomp.), by recrystallization from MeOH due to the retaining solvent on crystallization.[8]

Acknowledgement The author express his gratitude to Dr. D.L. Dreyer, San Francisco State College for his helpful advice and gift of the authentic samples of limonin, nomilin, and deacetylnomilin. Thank are due to Dr. T. Shingu, Kyoto University, for the measurement of NMR spectrum.

---

6) D.L. Dreyer, *Tetrahedron*, **21**, 75 (1965).
7) D.L. Dreyer, *J. Org. Chem.*, **30**, 749 (1965).
8) A. Melera, K. Schaffner, D. Arigoni, and O. Jeger, *Helv. Chim. Acta*, **40**, 1420 (1957).
9) D. Arigoni, D.H.R. Barton, E.J. Corey, O. Jeger, L. Caglioti, S. Dev, P.G. Ferrini, E.R. Glazier, A. Melera, S.K. Pradhan, K. Schaffner, S. Sternhell, J.F. Templeton, and S. Tobinaga, *Experientia*, **16**, 41 (1960).
10) S. Arnott, A.W. Davie, J.M. Robertson, G.A. Sim, and D.G. Watson, *Experientia*, **16**, 49 (1960).
11) D.H.R. Barton, S.K. Pradhan, S. Sternhell, and J.E. Templeton, *J. Chem. Soc.*, **1961**, 255.
12) Melting points were uncorrected. The NMR spectrum was obtained in d_5-pyridin with TMS as internal standard, using a Varian A-60 Spectrometer. The ORD curves were run in dioxane using a Jasco-ORD/UV-5.