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# CONSTITUENTS OF THE STEM BARK OF *ZANTHOXYLUM LIMONELLA*[*]

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## ABSTRACT

*The bark of* Zanthoxylum limonella *yielded five compounds: lupeol, rutaecarpine, xanthoxyletin, osthol and scopoletin.*

*Zanthoxylum* Linn., in the tribe Zanthoxyleae, subfamily Rutoideae, family Rutaceae, is a large genus of aromatic, prickly dioecious (or rarely monoecious) trees or shrubs, which is distributed mainly in the pantropics but also in the subtropics. The genus dealt with in this article has substantial synonymy with the genus *Fagara*. Only one species has been reported in Thailand.[2] The bark of *Z. limonella* Alston (syn. *Z. budranga* Wall. ex DC., *Z. rhetza* DC.) is noted for its febrifugal, sudorific and diuretic properties.[3] Several alkaloids have been isolated from the stem bark,[4, 5] vitamin E has been detected in the seed oil,[6] and aromatic components have been isolated from the essential oil.[7] Herein we report the isolation and structural elucidation of five components from the bark of *Z. limonella:* the major component lupeol and four other compounds not previously reported to be present in this species.

The dried bark of *Z. limonella* was extracted with 95% ethanol and the components were separated by column and thin-layer chromatography as described in the Experimental Section. The compounds will be discussed in the order in which they were eluted from the column. The first and major component was shown to be lupeol, (1), by comparison of its m.p. and spectral data with values reported in the literature. Lupeol is one of the most widely distributed of all the triterpenoids.

The second component, isolated in very limited quantity, was the quinazolinocarboline alkaloid[8] rutaecarpine, (2), which displayed the expected amide carbonyl and N-H stretching frequencies in its IR spectrum. We report in the Experimental the 400 MHz $^1$H NMR spectra of (2) in two different solvents. In acetone-$d_6$ all eight aromatic protons are resolved and assigned on the basis of multiplicities and homonuclear decoupling experiments. Of particular interest is the downfield resonance ($\delta$8.32 ppm in $CDCl_3$) for H-4, which is located in the deshielding region of the C-6 carbonyl group. Rutaecarpine has been isolated previously from *Evodia rutaecarpa*[9] and has been reported to increase arterial pressure[8] and to be active against silkworm larvae.[9]

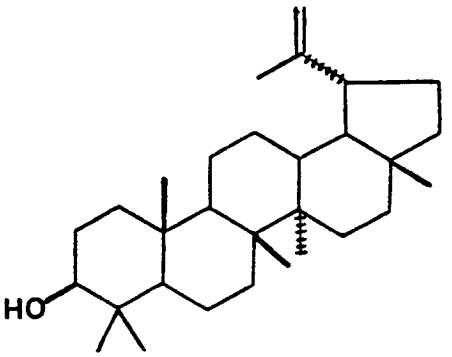
The remaining three compounds all belong to the coumarin family of natural products. The first of these was a cream colored solid which was shown to be xanthoxyletin, (3), by comparison of its m.p.[10] and 400 MHz $^1$H NMR with an authentic sample.[1] Xanthoxyletin is found in a wide range of plant species including *Murraya siamensis*[1] and *Clausena harmandiana*.[11] The next component was a white solid that was established to be osthol, (4), by comparison of its m.p. and $^1$H NMR spectrum with that reported previously.[12] The final component was shown to be scopoletin, (5), by comparison of its m.p. and spectral data with previous reports.[12, 13]

In conclusion, this study reports the isolation from the bark of *Zanthoxylum limonella* the following five components: the ubiquitous lupeol (1), the alkaloid rutaecarpine (2), which exhibits some interesting biological activity and the three coumarins xanthoxyletin (3), osthol (4), and scopoletin (5), Coumarins (3) and (4) were tested for activity in the brine shrimp lethality assay.[14] At a level of 10 $\mu$g/ml, (3) was not very active (one shrimp out of 30 dead) but (4) at this concentration exhibited strong activity (all 30 shrimps were dead after 24 hrs.). Osthol (4) will be screened using more sophisticated assays when additional compound is available.

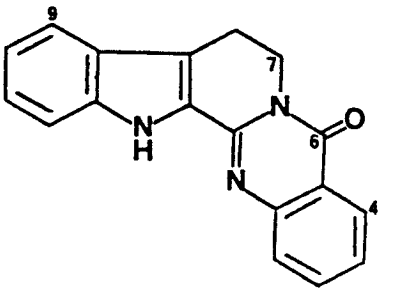
**Instrumentation:** Melting points were determined on a Mel-Temp apparatus and are uncorrected. IR spectra were obtained on a Nicolet Model 20 SX/C FT-IR spectrometer, UV spectra on a Perkin Elmer Lambda 3 spectrophotometer and mass spectra on a VG Micromass 7070F spectrometer. $^1$H and $^{13}$C NMR spectra were recorded on a Bruker WH-400 spectrometer.

**Plant Material:** Stem bark of *Z. limonella* Alston was collected at Ramkhamhaeng National Park, Sukhothai Province, Thailand, during April, 1988. The plant material was identified by comparison with a live specimen from the medicinal plant garden of the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok. A voucher specimen of the plant material has been deposited in the Herbarium of the Faculty of Pharmaceutical Sciences, Chulalongkorn University.

**Isolation of 1-5:** The dried and powdered bark of *Z. limonella* (3 kg) was extracted exhaustively with 95% ethanol in a percolator for 3 weeks. The extract was concentrated *in vacuo* to give a dark brown syrupy mass (450 g), which was suspended in water (200 ml), extracted with chloroform (5×200 ml), dried over anhydrous sodium sulfate and




1

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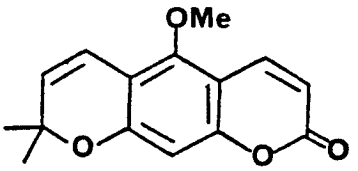


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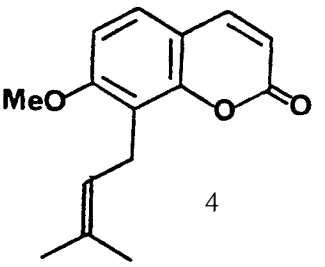


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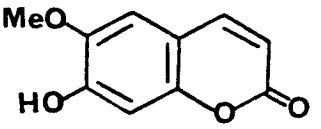


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evaporated to yield 5.6 g of crude extract. The extract was chromatographed on a silica gel column with chloroform and 50 ml fractions were collected. Further purification using silica gel column and thin-layer chromatography gave lupeol (2.96 g), rutaecarpine (2 mg), xanthoxyletin (23 mg), osthol (16 mg) and scopoletin (21 mg).

**Lupeol, 1.** M.p. 212-213°C [lit.[15] 208-213°C]; MS EI, see ref. (15, 16); $^1$H NMR, see ref. (16); $^{13}$C NMR, see ref. (17).

**Rutaecarpine, 2.** M.p. 250-252°C [lit.[9] 263-264°C]; IR ($CCl_4$) 3333, 1648 cm$^{-1}$; $^1$H NMR ($CDCl_3$) $\delta$9.08 (1H, br.s, N-H), 8.32 (1H, d, J = 7.0 Hz, H-4), 7.72 (1H, t, J = 7.0 Hz, H-2), 7.65 (1H, d, J = 7.0 Hz, H-1), 7.65 (1H, d, J = 7.0 Hz, H-9), 7.45 (2H, m, H-3 and H-12), 7.36 (1H, t, J = 7.8 Hz, H-11), 7.20 (1H, t, J = 7.0 Hz, H-10), 4.59 (2H, t, J = 7.0 Hz, H-7$\alpha$ and $\beta$), 3.24 (2H, t, J = 7.0 Hz, H-8$\alpha$ and $\beta$); $^1$H NMR ($CD_3COCD_3$) $\delta$10.83 (1H, br.s, N-H), 8.08 (1H, dd, J = 7.7 and 1.4 Hz, H-4), 8.00 (2H, dd, J = 7.8 and 1.4 Hz, H-1 and H-12), 7.63 (1H, td, J = 7.7 and 1.4 Hz, H-2), 7.57 (1H, d, J = 8.0 Hz, H-9), 7.32 (1H, td, J = 7.7 and 1.4 Hz, H-3), 7.19 (1H, td, J = 8.0 and 1.4 Hz, H-11), 7.01 (1H, t, J = 8.0 Hz, H-10), 4.42 (2H, t, J = 7.0 Hz, H-7$\alpha$ and $\beta$), 3.14 (2H, t, J = 7.0 Hz, H-8$\alpha$ and $\beta$); $^1$H NMR ($CD_3SOCD_3$), see ref. (9).

**Xanthoxyletin, 3.** M.p. 131.5-132.5°C [lit.[10] 132°C]; $^1$H NMR, see ref. (1).

**Osthol, 4.** M.p. 83.5-84.0°C [lit.[12] 83-84°C]; $^1$H NMR, see ref. (12).

**Scopoletin, 5.** M.p. 203.5-204.0°C [lit.[13] 205°C]; MS EI, see ref. (18); $^1$H NMR, see ref. (12, 13).

**Brine Shrimp Assay:** The procedure followed for the brine shrimp lethality assay was that reported by Meyer *et al.*[14] A total of 30 shrimps in three test tubes were used for each assay at a concentration of 10 $\mu$g/ml for (3) and (4). With 3 only one of the shrimps was dead after 24 hrs while with (4) all 30 were dead. We thank Linda Adam for performing this assay.

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## REFERENCES


1. Ruangrungsi, N., Ariyaprayoon, J., Lange, G.L. and Organ, M.G. (1990). *J. Nat. Prod.,* **53,** 946.
2. Smitinand, T. (1980). *Thai Plant Names* (Botanical Names - *Vernacular Names),* Funny Publishing Ltd., Bangkok, p. 354.
3. Pongboonrod, S. (1979). *Mai-tet-muang-thai,* Kasem Bannakich Press, Bangkok, p. 54.
4. Khastagir, H. (1974). *Curr. Sci.,* **16,** 185.
5. Willaman, J.J. and Li, H.L. (1970). *Lloydia,* **33S,** 1.
6. Fish, F., Gray, A.I. and Waterman, P.G. (1975). *Phytochemistry,* **14,** 841.
7. Thappa, R.K., Dhar, K.L. and Atal, C.K. (1976). *Phytochemistry,* **15,** 1568.
8. Bergman, J. (1983). In *The Alkaloids,* Vol. XXI, Brossi, A., Ed., Academic Press, New York, p. 29.
9. Kamikado, T., Murakoshi, S. and Tamura, S. (1978). *Agric. Biol. Chem.,* **42,** 1515.
10. Murray, R.D.H. (1978). In *Progress in the Chemistry of Organic Natural Products,* Vol. 35, Herz, W., Grisebach, H., and Kirby, G.W., Eds., Springer-Verlag, Vienna, p. 312.
11. Wangboonskul, J.D., Pummangura, S., and Chaichantipyuth, C. (1984). *J. Nat. Prod.,* **47,** 1058.
12. Steck, W. and Mazurek, M. (1972). *Lloydia,* **35,** 418.
13. Razdan, T.K., Qadri, B., Harkar, S., and Waight, E.S. (1987). *Phytochemistry,* **26,** 2063.
14. Meyer, B.N., Ferrigni, N.R., Putnam, J.E., Jacobsen, L.B., Nichols, D.E., and McLaughlin, J.L. (1982). *Planta Medica,* **45,** 31.
15. Garcia, B., Marco, J.A., Seoane, E., and Tortajada, A. (1981). *J. Nat. Prod.,* **44,** 111.
16. Gunasekera, S.P., Cordell, G.A., and Farnsworth, N.R. (1982). *J. Nat. Prod.,* **45,** 651.
17. Wenkert, E., Baddeley, G.V., Burfitt, L.R. and Moreno, L.N. (1978). *Org. Magn. Reson.,* **11,** 337.
18. Yamamoto, K., Kato, S., Shimomura, H. (1986). *J. Chromatogr.,* **362,** 274.


## บทคัดย่อ

จากการศึกษาเปลือกของกำจัดต้น *(Zanthoxylum limonella)* พบสารประกอบเคมี 5 ชนิดด้วยกันคือ lupeol, rutaecarpine, xanthoxyletin, osthol และ scopoletin.