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ISOLATION OF *(E)* -3,4′,5-TRIHYDROXY-3′-METHOXYSTILBENE FROM *GNETUM CUSPIDATUM* BL.

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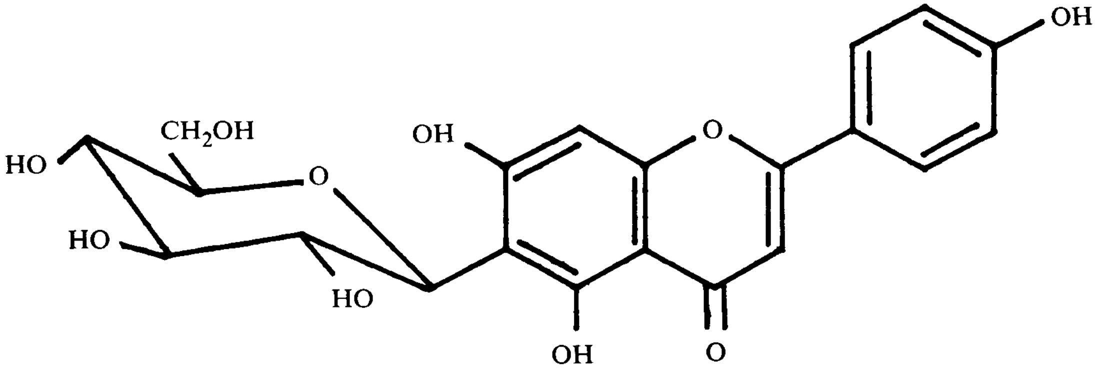
Abstract

The stems of Gnetum cuspidatum *Bl. have yielded (E) -3,4′,5-trihydroxy-3′-methoxystilbene (isorhapontigenin) (4) has been isolated from the stems of* Gnetum cuspidatum *Bl.*

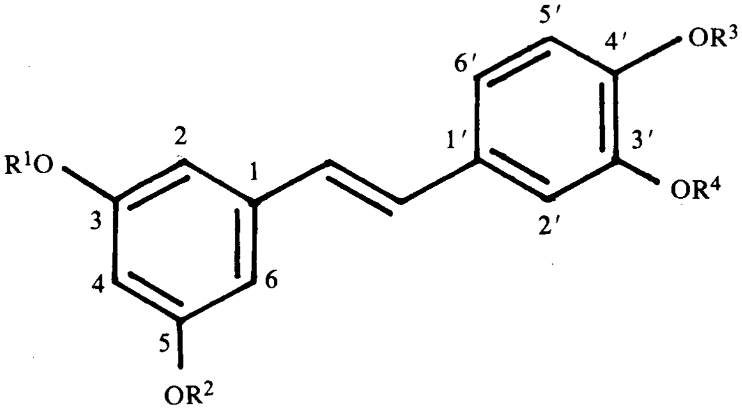
The genus *Gnetum* belongs to the family Gnetaceae and comprises about 30 species[1]. These gymnosperms are usually dioecious shrubs or woody climbers and are distributed throughout the tropics[1]. Eight *Gnetum* species occur in Thailand[2]; some of these yield edible fruits but the plants are not used in traditional Thai medicine, although several have reputations as folk remedies in Malaysia[3].

Apart from the work of Arthur *et al.*[4], who obtained an alkaloid hydrochloride (which was assigned the impossible molecular formula $C_{25}H_{27}ClNO_8$) from *G. indicum,* there had been little interest in the constituents of *Gnetum* species until recently, when Wallace and Morris[5] identified isovitexin (1) and related *C*-glycosylflavones in the leaves of *G. gnemon.* We have now examined the constituents of a species identified as *G. cuspidatum* Bl. (commonly called เมื่อย) which was collected at "Coke" island (เกาะโคก) in Songkhla Lake.

Chromatography of the methanolic extract of the stems readily gave a single constituent as a pale brown crystalline solid, m.p 179°C, the u.v. absorption of which [λ_{max} 234 (log ε 3.34), 303 (3.46), 325 (5.56) nm] suggested that it was a *trans*-stilbene derivative[6].



(1)



(2) $R^1 = R^2 = R^4 = H$, $R^3 = CH_3$
(3) R^1 = B-D-glucopyranosyl, $R^2 = R^4 = H$, $R^3 = CH_3$
(4) $R^1 = R^2 = R^3 = H$, $R^4 = CH_3$
(5) R^1 = B-D-glucopyranosyl, $R^2 = R^3 = H$, $R^4 = CH_3$
(6) $R^1 = R^2 = R^3 = Ac$, $R^4 = CH_3$

The molecular formula $C_{15}H_{14}O_4$ was established by high resolution mass spectrometry and the substance readily formed a triacetate, m.p. 99–100°C.

The n.m.r. spectrum indicated that the substance might be identical with either rhapontigenin[7] (2) (lit[7] m.p. 185°C, triacetate, 114°C) or isorhapontigenin[8,9] (4) (lit[9] m.p. 182–183°C, triacetate, 103–104°C), which are the aglycones of rhapontin[7] (3), and *(E)*-3,4′,5-trihydroxy-3′-methoxystilbene-3-*O*-β-D-glucopyranoside (5)[9], respectively.

Direct comparison of the substance isolated from *G. cuspidatum* with synthetic rhapontigenin[10] (m.p. 190–192°C), kindly provided by Professor E. Reimann, revealed that these two substances are not identical.

The substance was finally identified as *(E)*-3,4′,5-trihydroxy-3′-methoxystilbene (4) : the orientation of the methoxy group was supported by an N.O.E. experiment-- irradiation of the OCH_3 region increased significantly (15%) the integral of $H_{2'}$ which resonated at δ 7.26.

This conclusion was confirmed by direct comparision of the triacetate (6) with authentic specimens provided by both Professor E. Reimann, who has also synthesized this compound (personal communication) and Professor E. P. Swan[9]. The two substances proved to be identical on t.l.c. in three solvent systems, and the mixed m.p. was not depressed.

Melting points were determined on an Electrothermal melting point apparatus. Ultraviolet spectra were recorded with a Beckmann DK-2A instrument. Low-resolution mass spectra were measured with a Varian MAT-CH7 spectrometer. High resolution mass spectra were obtained on a Varian MAT 311 instrument. Nuclear magnetic resonance (n.m.r.) spectra were determined with a Bruker WP 80 spectrometer operating at 80 MHz for Fourier transform ^1H spectra; the N.O.E. experiment was carried out with a Bruker HX 90 instrument operating at 90 MHz.

A voucher specimen of the plant material (VP 9) has been deposited in the Herbarium of the Biology Department at the Prince of Songkla University, Hat Yai, Thailand.

Isolation of (E)-*3,4′,5-trihydroxy-3′-methoxystilbene (isorhapontigenin) (4) from* G. cuspidatum.

Fresh stems (3kg) of *G. cuspidatum,* collected at Coke Island in Lake Songkla, Southern Thailand, were cut into small pieces and extracted with methanol at room temperature. The extract was evaporated under reduced pressure and the resulting brown tar (250 g) was poured into distilled water (300 ml) then extracted with ether (3 × 200 ml).

Evaporation of the combined ether extracts gave a brown semi-solid (21 g). This crude product was pre-adsorbed on silicic acid and chromatographed on the same adsorbent (200 g). The column was eluted with $CHCl_3$ and $CHCl_3$/MeOH in varying proportions. and 200 ml. fractions were collected. The 5th to 7th fractions eluted with

$CHCl_3$/MeOH (97:3) showed one spot on t.l.c. These fractions were combined and evaporated to give a brown solid (1.2 g) which crystallized from benzene/light perroleum to give *(E)*-3,4′,5-trihydroxy-3′-methoxystilbene (isorhapontigenin) (4) as pale brown prisms, m.p. 179°C (lit[9] 182–183°). Found : M^+, 258.0888. Calculated for $C_{15}H_{14}O_4$: 258.0892. λ_{max} (EtOH) : 234(log ε 3.34), 303 (3.46), 325 (5.56) nm. N.m.r. (CD_3COCD_3) δ : 3.89 (s, 3H, OCH_3), 6.26 (t, 1H, H_4, J = 2.5), 6.54 (d, 2H, H_2 and H_6, J = 2.5), 6.85 (d, 1H, $H_{5'}$, J = 8.5), 6.96 (s, 2H, $C\underline{H} = C\underline{H}$), 6.99 (dd, one wing of which overlaps with the signal from the olefinic protons, 1H, $H_{6'}$; J = 8.5, 2.0), 7.26 (d, 1H, $H_{2'}$, J = 2.0), 7.67 (s, 1H, ArOH exchanged with D_2O), 8.14 (s, 2H, 2ArOH exchanged with D_2O). Mass spectrum (80°/70 eV)m/e : 258 (100%), 197 (30%), 169 (9%).

(E)-3,4′,5-triacetoxy-3′,-methoxystilbene (6)

The stilbene (4) (100 mg) was dissolved in pyridine (3 ml) and acetic anhydride (2 ml); the reaction mixture was left at room temperature overnight, then worked up as usual. The product crystallized from aqueous methanol to give the triactate (6) (55 mg) as colourless needles, m.p. 99–100°C (lit[9] 103–104°C), not depressed on admixture with authentic specimens, N.m.r. ($CDCl_3$) δ : 2.31 (s, 9H, $COCH_3$), 3.87 (s, 3H, OCH_3), 6.82 (t, 1H, H_4, J = 2.5), 7.01 (m, 5H, $C\underline{H} = C\underline{H}$, $H_{2'}$, $H_{5'}$ and $H_{6'}$), 7.09 (d, 2H, H_2 and H_6, J = 2.5). Mass spectrum (80°/70 eV) m/e : 384 (13%), 342 (100), 300 (70), 258 (98).

Acknowledgements

We are grateful to Miss Puangpen Sirirugsa and Mr. Gordon Congdon for identifying the plant material; to Professor Eberhard Reiman for samples of rhapontigenin (2) and its n.m.r. spectrum, and the triacetate (6), and to Professor E.P. Swan who also supplied the same acetate (6). We are also grateful to Dr. Lindsay Byrne for n.m.r. spectra, and to Dr. M. Stringer and Dr. S. Wilkinson for mass spectra. We thank the International Foundation for Science for financial support.

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บทคัดย่อ

ส่วนประกอบทางเคมีที่สำคัญของลำต้นของต้นเมื่อย *(Gnetum cuspidatum* Bl.*)* คือ *(E)*-3,4′,5-trihydroxy-3′-methoxystilbene (isorhapontigenin) (4).