

Alkaloids of *Fagraea fragrans* Roxb.

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The major alkaloid of the leaves and fruits of *Fagraea fragrans* Roxb. has been isolated and shown to be 4-(2-hydroxyethyl)-5-vinyl nicotinic acid lactone identical with gentiamine.

THE handsome tree *Fagraea fragrans* Roxb. (*Cyrtophyllum peregrinum* Blume), family Loganiaceae, reaches about 100 ft. high and grows abundantly in Singapore and the south of the Malayan peninsula. A decoction of the leaves and twigs is used in Malay folk medicine for the treatment of dysentery (Burkill & Haniff, 1930) and that of the bark for malaria (Holmes, 1892). The leaves and fruits have an intensely bitter taste. In their phytochemical survey of Malayan plants Douglas & Kiang (1957) found that the leaves and barks of *F. fragrans* gave a strong reaction for alkaloids.

Extraction of the leaves with ether as described in the experimental section has yielded gentianine. Its melting-point was not depressed on admixture with an authentic sample; the infra-red spectra were likewise superimposable.

Gentianine was first isolated from *Gentiana kirilowi* (Proskurnina, 1944) and subsequently from *Enicostema littorale* by Govindachari, Nagarajan & Rajapa (1957) who elucidated its structure. The alkaloid has also been isolated from other genera of the family Gentianaceae (Williaman & Schubert, 1961). This is the first report of its isolation from Loganiaceae and it is interesting to note that taxonomically the two families are closely related.

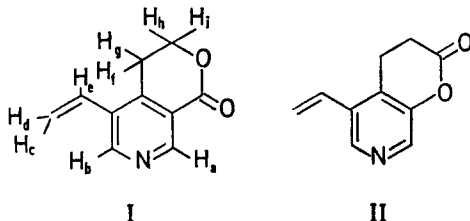
The mass spectrum of this alkaloid shows a very strong peak at m/e 175 due to the molecular ion (M) and in accord with the molecular formula $C_{10}H_9NO_2$; this is also the base peak. The intense peak at m/e 117 (M-58), relative intensity 87%, is attributed to the loss of CH_2-O-CO ; bond cleavage of this sort being common in the fragmentation of alkylated aromatic compounds (McLafferty, 1962). The peak at m/e 147 (M-28) arises from the loss of the vinyl group plus one proton from the molecular ion. Finally, loss of both $CH_2=CH-$ and $CH_2-O-CO-$ accounts for the peak at m/e 90 (M-85).

In the nuclear magnetic resonance spectrum, the two low field singlets (1.29 and 1.04 τ) can only be assigned to protons a and b of the pyridine ring in which both β -positions are substituted. The fact that these protons are shifted to lower fields than is usual for hydrogens in such a position is attributed to diamagnetic shielding by the vinyl and carbonyl groups (Jackman, 1959a). The multiplet between 4.6 - 2.9 τ constitutes an ABX system the pattern of which is similar to that of 4-vinyl pyridine (Varian NMR spectra catalogue No. 155). This arises from protons c, d and e. The peaks centred around 4.38 τ , equivalent to two protons, are assigned to H_c and H_d (the AB part of the system); the multiplet at about

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3.22 τ , equivalent to one proton, is assigned to H_e (X proton). The remaining four protons, H_f - H_j , are accounted for by the two 2-proton triplets indicating two adjacent non-equivalent methylene groups, the splitting for each being 6 c/s. The triplet at 6.92 τ is assigned to H_f and H_g ; that at 5.49 τ to H_h and H_i (structure I).



The alternative structure (II) would be eliminated since a triplet due to the methylene group adjacent to a carbonyl group would then appear at around 7.9 τ instead of at 5.49 τ (Jackman, 1959b).

Experimental

PLANT MATERIAL

Leaves and fruits of *F. fragrans* were collected from plants growing in the grounds of the Medical Faculty, University of Singapore and authenticated with herbarium specimens of *F. fragrans* Roxb. at the Botanic Gardens, Singapore.

The alkaloidal content of the plant showed marked seasonal variation. The yield of alkaloid from the leaves was highest (0.32 %) at the beginning of the flowering season (May-June) and lowest (0.005%) after fruiting had occurred (October-November). The ripe fruits contained about 0.10% of alkaloids.

The ultra-violet spectra were recorded on a Bausch and Lomb Spectronic 505, the infra-red spectra on a Nihon-Bunko IRS-5 and a Perkin-Elmer 137-G, the NMR spectrum on a Varian A60. The mass spectrum was recorded by Dr. Ragnar Ryhage at the Laboratory for Mass Spectrometry, Karolinska Institutet, Stockholm.

Extraction. The air-dried, powdered leaves were moistened with ammonia and extracted by repeatedly shaking with solvent ether. The ether extract was shaken with 2% hydrochloric acid; the acid phase separated and basified with ammonia and the alkaloidal material taken up in chloroform. The chloroform solution, dried over anhydrous sodium sulphate, was evaporated to dryness under reduced pressure to yield a colourless crystalline residue, gentianine (0.32% yield).

Gentianine crystallised from hot water in colourless prisms, m.p. 82-83°, $[\alpha]_D^{20} \pm 0^\circ$ (c, 1.0% in ethanol), λ_{\max} (H_2O) 219 $m\mu$ ($\log \epsilon$ 4.41), 285 $m\mu$ ($\log \epsilon$ 3.14), inflection 240-250 $m\mu$, ν_{\max} (CCl_4) 3015, 1745, 1638 and 928 cm^{-1} . [Govindachari & others (1957) cite m.p. 82-83°, $[\alpha]_D^{30} \pm 0^\circ$ ($CHCl_3$), λ_{\max} 220 $m\mu$ ($\log \epsilon$ 4.38), 280 $m\mu$ ($\log \epsilon$ 3.2).] Found: C, 68.6; H, 5.4; N, 8.2. Calc. for $C_{10}H_9NO_2$: C, 68.6; H, 5.2; N, 8.0%.

The NMR spectrum was recorded in deuteriochloroform:carbon

tetrachloride (1:1) with tetramethylsilane as internal standard. The mass spectrum showed major peaks at m/e 175, 147, 117 and 90.

The *picrate*, crystallised from hot water, m.p. 126–127° [Govindachari & others (1957) cite m.p. 123–124°]. Found: C, 47.6; H, 3.1. Calc. for $C_{10}H_9NO_2 \cdot C_6H_3N_3O_7$: C, 47.5; H, 3.0%.

The alkaloid is moderately soluble in water (1.2% at 25°), completely soluble in dilute acids from which the original compound can be recovered on basifying with ammonia or aqueous sodium carbonate solution. It dissolves in 10% aqueous sodium hydroxide but is not regenerated on neutralisation. Reduction of the alkaloid with zinc and hydrochloric acid gave a secondary ring base demonstrated by the formation of copper dithiocarbamate (Feigl, 1956).

Dihydrogentianine. The alkaloid (132 mg) in ethanol (10 ml) absorbed only one equivalent of hydrogen on hydrogenation over Adam's platinum oxide under normal conditions. The product crystallised from water in colourless crystals (54 mg), m.p. 77–78°, λ_{\max} (H_2O) 227 $m\mu$ ($\log \epsilon$ 3.9), 272 $m\mu$ ($\log \epsilon$ 3.5), ν_{\max} (CCl_4) 2980, 2900, 1745, 1420, 1390 cm^{-1} , ν_{\max} (KBr) 1725, 1580, 1410, 1395 cm^{-1} . Found: C, 67.6; H, 6.4. Calc. for $C_{10}H_{11}NO_2$: C, 67.8; H, 6.3%. [Govindachari & others cite m.p. 74–76°, λ_{\max} 270 $m\mu$ ($\log \epsilon$ 3.4)].

Lithium aluminium hydride reduction. The alkaloid (250 mg) in dry ether was refluxed (8 hr) with $LiAlH_4$ (100 mg). The cooled reaction mixture was decomposed with crushed ice; saturated ammonium chloride solution (20 ml) was added and the mixture extracted continuously with ether (12 hr). The product (150 mg) crystallised from benzene/ethanol m.p. 135–136°, λ_{\max} (H_2O) 238 $m\mu$ ($\log \epsilon$ 4.0), 272 $m\mu$ ($\log \epsilon$ 3.5), ν_{\max} (KBr) 3305, 3125, 1630, 1590, 1485 cm^{-1} . The spectrum in chloroform revealed peaks at 3640 and 3280 cm^{-1} . Found: C, 67.3; H, 7.5; N, 8.2. Calc. for $C_{10}H_{13}NO_2$: C, 67.0; H, 7.3; N, 7.8%.

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