THE *MITRAGYNA* SPECIES OF GHANA

THE ALKALOIDS OF THE LEAVES OF *Mitragyna ciliata* Aubr. et Pellegr.

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Seven alkaloids, rotundifoline, isorotundifoline, rhynchophylline, isorhynchophylline, *ciliaphylline*, *rhynchociline* and *mitraciliatine* have been isolated from the leaves of *Mitragyna ciliata* Aubr. et Pellegr. The latter three are hitherto undescribed alkaloids.

IN 1950, Ongley examined the leaves of *Mitragyna ciliata* and isolated the single alkaloid rotundifoline (see also Badger, Cook and Ongley, 1950). Four and sometimes five alkaloids were obtained from the leaves of *M. stipulosa* with evidence of traces of others (Beckett, Shellard and Tackie, 1963). The two species are so closely related taxonomically (Aubreville, 1936) and so similar anatomically (Shellard and Shadan, 1963), that a further examination of the alkaloidal content of the leaves of *M. ciliata* has now been made to enable the alkaloidal content of the two leaves to be compared.

EXPERIMENTAL

Alumina used for column chromatography was Spence-type H, the adsorbent for thin layer chromatography was Alumina (Merck), and the solvent chloroform unless otherwise stated. Recorded R_F values are those obtained using this system. All melting-points are uncorrected. Equivalent weights were determined by non-aqueous titration (Beckett and Tinley, 1952). Elemental analyses were carried out by Mr. G. S. Crouch, School of Pharmacy, University of London and Drs. G. Weiler and F. B. Strauss, Oxford.

Materials

Leaves from trees growing in the savannah and fringing forest zone near Tarkwa in the Western Region of Ghana were collected during various periods from January, 1961, to June, 1962. Details of the authentication of the samples are given by Shellard and Shadan (1963).

Isolation of Alkaloids

An ethanolic extract prepared from 12 kg. of dried coarsely powdered leaves was treated as described by Beckett, Shellard and Tackie (1963), to obtain total crude alkaloid (37 g.). Thin layer chromatography showed at least four alkaloids to be present.

The crude alkaloids were dissolved in dilute sulphuric acid (250 ml.) and after extracting with ether the solution was made alkaline with

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ammonia and re-extracted with ether. The ethereal extract was washed, dried and concentrated to yield prismatic crystals (12·9 g.) of alkaloidal material, m.p. 213–214° (material "A," subsequently shown to be a mixture of two alkaloids). Mixtures of Material "A" and rotundifoline were isolated from the mother liquors and were separated by adsorption and elution through alumina with 10 per cent chloroform in ether followed by chloroform to give rotundifoline (4·9 g.), m.p. 238°, and Material "A" (1·3 g.), m.p. 213° (see below).

The remaining mother liquor was exhaustively extracted with 5 per cent sodium hydroxide. The alkaline extract was acidified with sulphuric acid, made alkaline with ammonia and extracted with ether. Treatment of the ethereal extract by the method described in the previous paper (Beckett and others, 1963) gave colourless prisms of isorotundifoline (2·3 g.), m.p. 131°.

The ethereal mother liquors after this alkaline extraction were washed free from alkali with distilled water and evaporated to give a residue which was dissolved in chloroform and extracted with 5 per cent hydrochloric acid. The chloroform solution yielded a pale yellow residue which on recrystallisation from ether : light petroleum (b.p. 40–60°) (1 : 1), gave fine sandy crystals (1·3 g.), m.p. 141°, of a new alkaloid now named *mitraciliatine.*

The total acid extracts and washings were made alkaline with ammonia and extracted with ether : evaporation gave a residue (10·2 g.) which was shown by thin layer chromatography to contain two alkaloids which differed from those previously described. A solution of the perchlorates in chloroform was passed through a column of alumina (30 × 2 cm.) and the alkaloids eluted with 10 per cent chloroform in ether followed by chloroform. All fractions were monitored by thin layer chromatography. Later fractions contained a single substance isolated as fine colourless needles (5·5 g.), m.p. 178–180°. This is a new alkaloid now named *rhynchociline.*

Earlier fractions containing both alkaloids were evaporated to dryness and the residue, in ether, added to a column of alumina (30 × 2 cm.). Alkaloids were eluted with 1 per cent chloroform in ether. Earlier fractions contained a single substance, faster moving on thin layer chromatography than rhynchociline, and continual treatment in this manner of the bulked fractions containing both alkaloids resulted in the isolation of isorhynchophylline which crystallised with some difficulty from n-hexane-ether (1 : 1) in colourless needles (2·7 g.), m.p. 144°.

Examination of Material "A"

Thin layer chromatography using Kieselgel G and chloroform : acetone (5 : 4) showed Material "A" to be a mixture of two alkaloids. Material "A" (1·2 g.) was treated with 0·6 g. picric acid in methanol (6 ml.). Water was added to incipient crystallisation and after ~18 hr. a yellow picrate (600 mg.), m.p. 130–131° was obtained. This yielded a single alkaloid which recrystallised from acetone in colourless needles (110 mg.), m.p. 222–223°. This is a new alkaloid now named *ciliaphylline.*

The solution from which the ciliaphylline picrate was removed contained small quantities of an alkaloid which corresponds to rhynchophylline (thin layer chromatography using Kieselgel G and chloroform : acetone (5 : 4)).

Characterisation of the Alkaloids

Rotundifoline, isorotundifoline and isorhynchophylline were characterised by comparison of melting-points, mixed melting-points, infra-red spectra and approximate R_F values by thin layer chromatography with the corresponding alkaloids obtained from the leaves of *Mitragyna stipulosa.*

Ciliaphylline was soluble in acetone, chlorobenzene, chloroform, ethanol; slightly soluble in ether; insoluble in sodium hydroxide, m.p. 222–223°. Approx. R_F value 0·12. $[\alpha]_D^{25}$ $-89·5°$ (c, 0·65 in $CHCl_3$). Found: C, 66·6; H, 7·7; N, 7·2; OMe, 21·3; equiv. wt., 390. $C_{23}H_{30}O_5N_2$ requires C, 66·8; H, 7·3; N, 6·8; OMe (3) 22·5; equiv. wt., 414. pK_a 6·75 (electrometric titration in 80 per cent methyl cellosolve), 7·5 (electrometric titration in H_2O). λ_{max} (EtOH) 222 mμ (log ϵ 4·44), 244 mμ (log ϵ 4·24), 287 mμ (log ϵ 3·46). λ_{min} 234 mμ (log ϵ 4·20), 276 mμ (log ϵ 3·40). ν_{max} (Nujol) 1,728, 1,705, 1,640, 1,620, 1,500, 1,380, 1,335, 1,300, 1,402, 1,180–1,300 (a number of fused bands), 1,150, 1,170, 780, 770, 740 cm.$_{-}{}^{1}$.

The *picrate* crystallised in yellow prisms from aqueous methanol, m.p. 130–131° (decomp.). Found: C, 52·8; H, 5·1; N, 9·7. $C_{23}H_{30}O_5N_2$, $C_6H_3O_7N_3$, H_2O requires C, 52·7; H, 5·3; N, 10·6.

Rhynchociline was soluble in acetone, chloroform, ethanol, ether; insoluble in n-hexane, light petroleum, sodium hydroxide, m.p. 178–180°. Approx. R_F value 0·30. $[\alpha]_D^{22}$ $+6·2$ (c, 2 in $CHCl_3$). Found: C, 66·6; H, 7·7; N, 7·3; equiv. wt., 387. $C_{23}H_{30}O_5N_2$ requires C, 66·8; H, 7·3; N, 6·8; equiv. wt., 414. $pK_a = 6·7$ (electrometric titration in 80 per cent methyl cellosolve), 8·3 (electrometric titration in H_2O). λ_{max} (EtOH), 225 mμ (log ϵ 4·41), 242 mμ (log ϵ 4·24), 286 mμ (log ϵ 3·48). λ_{min} 238 mμ (log ϵ 4·22), 277 mμ (log ϵ 3·39). ν_{max} (Nujol) 3,525 (weak), 3,100, 1,685, 1,605, 1,270, 1,240, 970 (weak), 780, 730 cm.$^{-1}$.

The perchlorate crystallised in colourless needles from ethanol and water (1 : 1), m.p. 221–223°. Found: C, 53·5; H, 6·1; N, 5·6; OMe 17·7. $C_{23}H_{30}O_5N_2.HClO_4$ requires C, 53·6; H, 6·0; N, 5·4; OMe (3) 18·1.

Rhynchociline is identical with an isomer obtained by isomerisation of ciliaphylline in 50 per cent acetic acid or pyridine (unpublished observations).

Mitraciliatine. Soluble in acetone, chloroform, ethanol, ether; insoluble in light petroleum, sodium hydroxide, m.p. 140–141°. Approx. R_F value 0·32. Found: C, 68·8; H, 7·5; N, 6·83; OMe, 22·7; equiv. wt., 405. $C_{23}H_{30}O_4N_2$ requires C, 69·3; H, 7·6; N, 7·0; OMe, 23·4; equiv. wt., 398. λ_{max} (EtOH) 228 mμ (log ϵ 4·59), 292·4 mμ (log ϵ 3·91). Shoulders at 248·8 mμ (log ϵ 4·17), 284 mϵ (log ϵ 3·89). λ_{min} 289·5 mμ (log ϵ 3·84). ν_{max} (Nujol) 3,150, 1,690, 1,245, 1,105, 770, 730 cm.$^{-1}$.

The *perchlorate* crystalised in yellow prisms from ether-ethanol, m.p. 229–230°. Found: C, 55·3; H, 6·3; N, 5·6; OMe, 17·3. $C_{23}H_{30}O_4N_2.HClO_4$ requires C, 55·4; H, 6·2; N, 5·6; OMe (3) 18·7. With vanillin and hydrochloric acid it gives an intense blue colour.

DISCUSSION

By using thin layer chromatography seven alkaloids have been identified from the leaves of *M. ciliata.* Four of these, rotundifoline, isorotundifoline, rhynchophylline and isorhynchophylline have been reported previously and also occur in the leaves of *M. stipulosa.* The hitherto unreported alkaloids *ciliaphylline* and *rhynchociline* are interconvertible by heating in acetic acid or in pyridine. These alkaloids are of the rhynchophylline pattern but possess an extra aromatic methoxy group.

The third new alkaloid, *mitraciliatine*, unlike the above is an indole alkaloid as indicated by its colour reaction and ultra-violet and infra-red spectra.

The presence of both oxindole and indole alkaloids in the same plant organs is unusual though it is not uncommon for them to occur in separate organs of the same plant. Mitragynine, which is known to be an indole (Field, 1921; Ing and Raison, 1939; Hendrickson, 1961; Joshi, Raymond-Hamet and Taylor, 1963), was isolated by Field (1921) from the leaves of *Mitragyna speciosa* while mitraspecine, an oxindole alkaloid (Raymond-Hamet, 1950), was isolated from the bark of this plant by Denis (1938). The significance of these results in relation to the biosynthesis of the mitragyna alkaloids is being investigated.

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