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The alkaloids of the leaves of *Mitragyna inermis* (Willd.) O.Kuntze

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The leaves of *Mitragyna inermis* (Willd.) O. Kuntze have been examined for alkaloids and shown to contain routundifoline, isorotundifoline, rhynchophylline, isorhynchophylline, ciliaphylline, rhynchociline, speciophylline, a small amount of uncarine F, mitraciliatine and traces of a second indole alkaloid which appears to be of the corynanthine type. There is also a polar compound present, Dragendorff positive, which remains on the base line when subjected to thin-layer chromatography with the usual solvent systems used for the mitragyna alkaloids.

The alkaloidal content of the leaves of *Mitragyna stipulosa* (D.C.) O. Kuntze and of *Mitragyna ciliata* Aubr. et Pellagr. has been reported by Beckett, Shellard & Tackie (1963a,b). Tackie (1963) made a preliminary study of the alkaloids of the leaves of *Mitragyna inermis* (Willd.) O. Kuntze and found rotundifoline, isorotundifoline and isorhynchophylline in addition to rhynchophylline previously reported by Raymond-Hamet & Millat (1934).

A detailed examination of the leaves of *M. inermis* now confirms the presence of rotundifoline, isorotundifoline, rhynchophylline, isorhynchophylline, ciliaphylline, rhynchociline, speciophylline, a small amount of uncarine F, mitraciliantine and traces of a second indole alkaloid. The presence of a strongly polar substance which gives an orange colour with Dragendorff's reagents (cf. Tackie, 1963) is confirmed; it is identical with a "base line" alkaloid reported by Shellard & Phillipson (1964) as occurring in the leaves of *Mitragyna rotundifolia* (Roxb.) O. Kuntze from Burma.

EXPERIMENTAL

Column chromatography. The alumina used was Spence-type H and the silica gel was silica gel H (Merck).

The hRf values were obtained using three systems (layer thickness, 250 μm): (*a*) alumina and chloroform, (*b*) silica gel and ether, (*c*) silica gel and chloroform–acetone (5:4).

For preparative thin-layer chromatography, silica gel HF 254 (Merck) 1 mm thick was used, the solvent system being either (i) chloroform–acetone (1:1) or (ii) chloroform–methanol (95:5). All melting points are uncorrected.

Materials

Leaves from trees growing in the Sogakofe district of the Volta Region, Ghana, were collected during various periods up to June, 1966. Details of authentication are given by Pillay (1964) and Shellard & Wade (1969).

Isolation of alkaloids

Coarsely powdered leaves (6·5 kg) were extracted by maceration with 96% ethanol and the extract evaporated under reduced pressure to a thin syrup. After acidifying with glacial acetic acid and diluting with a large volume of water, the precipitated non-alkaloidal matter was filtered off. The filtrate was made alkaline with ammonia and extracted with chloroform. The extract was washed, dried and evaporated to yield crude alkaloidal extract (12·5 g). Thin-layer chromatography showed the presence of at least six alkaloids.

The crude alkaloids were dissolved in dilute sulphuric acid (100 ml) and after extracting with ether, the solution was made alkaline with ammonia and re-extracted with ether. The ethereal extract was washed, dried and evaporated to dryness to yield a purified alkaloidal extract (8·1 g). The alkaline solution was further extracted with chloroform to yield a pale brown residue (0·3 g) which on crystallization from absolute ethanol gave colourless crystals (3 mg) of "base-line alkaloid."

The purified alkaloidal residue was divided into two parts and each treated as follows: approximately 4·0 g was dissolved in chloroform (5 ml) and added to a column of alumina (15 × 2·5 cm) packed in chloroform. The alkaloids were eluted with chloroform (750 ml) and then chloroform–methanol (4:1) (250 ml), 25 ml portions of eluate being collected. Thin-layer monitoring allowed the bulking of like portions to give the following fractions: (1) Chloroform (50 ml), containing a trace of an oxindole alkaloid; the yield was too small to allow characterization. (2) Chloroform (450 ml) containing eight alkaloids—"Fraction A" (5·65 g). (3) Chloroform (150 ml) containing one major alkaloid which crystallized from acetone to give colourless needles of speciophylline, m.p. 183° (223 mg). (4) Chloroform–methanol (250 ml) containing four alkaloids (one of which gave an immediate blue colour with vanillin and hydrochloric acid)—"Fraction B" (162 mg).

Attempts to separate the alkaloids in Fraction A by column chromatography using benzene, ether and chloroform were not successful. However, the last portion of the chloroform eluate gave a pale yellow residue which contained traces of alkaloids, one of which was an indole alkaloid. This was isolated by preparative thin-layer chromatography system (i) and, on recrystallization from ether–light petroleum (b.p. 40–60°) (1:1) gave fine colourless crystals of mitraciliatine, m.p. 140° (85 mg). The remaining combined eluates after concentration were dissolved in 5% acetic acid and extracted with chloroform. The residue, after evaporation to dryness was designated "Fraction C" (4·85 g). The acid solution was made alkaline with ammonia and extracted with chloroform. The residue on recrystallization from dry ether yielded colourless needles of rhynchociline, m.p. 178° (365 mg).

Fraction C was dissolved in ether and added to a silica gel column (12·5 × 2 cm) packed in ether. The alkaloids were eluted with ether (800 ml) and ether–chloroform (4:1) (250 ml), 20 ml portions being collected. Thin-layer monitoring allowed bulking of like portions to give the following fractions: (1) Ether (60 ml) containing one alkaloid which on recrystallization from absolute ethanol gave colourless needles of rotundifoline, m.p. 239° (215 mg). (2) Ether (180 ml) containing chiefly a mixture of rotundifoline and isorhynchophylline—"Fraction D" (2·5 g). (3) Ether (80 ml), which gave a colourless amorphous powder, recrystallization of which from ether–n-hexane gave with some difficulty isorhynchophylline m.p. 144° (650 mg). (4) Ether (200 ml) containing four alkaloids. Recrystallization from absolute ethanol yielded prismatic crystals of rhynchophylline, m.p. 213° (638 mg).

The mother liquors contained one main alkaloid corresponding to that present in fraction 5. (5) Ether (100 ml). This fraction was bulked with the residue from fraction (4) and recrystallized from acetone to give ciliaphylline m.p. 222° (147 mg).

The mother liquors from which rhynchophylline and ciliaphylline had been isolated contained an alkaloid having identical hRf values to isorotundifoline. The dried residue from these mother liquors was therefore dissolved in ether and extracted with 5% sodium hydroxide. This extract was then acidified with hydrochloric acid and made alkaline again with ammonia. Extraction with chloroform yielded a material which was recrystallized three times from acetone to yield colourless rosettes of isorotundifoline, m.p. 132° (97 mg).

The residue from Fraction D was dissolved in chloroform (10 ml) and extracted with 1% citric acid (3 × 10 ml). After washing with water the chloroform was evaporated to dryness and the residue recrystallized from absolute ethanol to yield further crystals of rotundifoline, m.p. 239° (816 mg). The citric acid solution was made alkaline with ammonia, extracted with chloroform and the residue after removal of the solvent was recrystallized from ether–n-hexane to yield crystals of isorhynchophylline, m.p. 145° (1·2 g). The mother liquors from which the isorhynchophylline had crystallized contained a further alkaloid which was isolated by preparative thin-layer chromatography system (ii). Elution of the appropriate zone gave amorphous uncarine F (17 mg).

Fraction B contained traces of oxindole alkaloids previously isolated together with traces of an indole alkaloid (Sp 4). This was isolated by preparative thin-layer chromatography system (i) in a quantity insufficient for identification.

Characterization of alkaloids

The alkaloids were characterized by comparison of the m.p., mixed m.p., ultraviolet and infrared spectra and hRf values in thin-layer systems a, b and c by comparison with authentic specimens of each alkaloid (Table 1).

Table 1. *hRf values of alkaloids in system a, b and c*

	System a	System b	System c
Rotundifoline[1]	52	35	70
Isorotundifoline[1]	43	10	44
Rhynchophylline[2]	13	4	27
Ciliaphylline[3]	12	3	26
Rhynchociline[3]	26	4	8
Speciophylline[4,5]	8	0–1	22
Uncarine F[4]	43	23	61
Mitraciliatine[6]	28	2	6
Indole alkaloid Sp4*	23	0	4
"Base line" alkaloid ..	0	0	0

Authenticated by comparison with sample from:
[1] *M. stipulosa* (Beckett, Shellard & Tackie, 1963a). [2] *M. rotundifolia* (Shellard & Phillipson, 1964). [3] *M. ciliata* (Beckett & others, 1963b). [4] *M. parvifolia* (Shellard, Phillipson & Gupta, 1968) and [5] with picrate from *M. speciosa* (Beckett, Shellard & others, 1966). [6] Synthetic material (Trager, Phillipson & Beckett, 1968).
* λ_{max} (nm) 226, 291, 278 shoulder; λ_{min} (nm) 249, 288. ν_{max} (Nujol), 3300, 1700, 1580 (weak), 1460, 1380, 1330, 1250, 1100, 760, 740 cm^{-1}. Absence of band between 1600 and 1650 (double band).

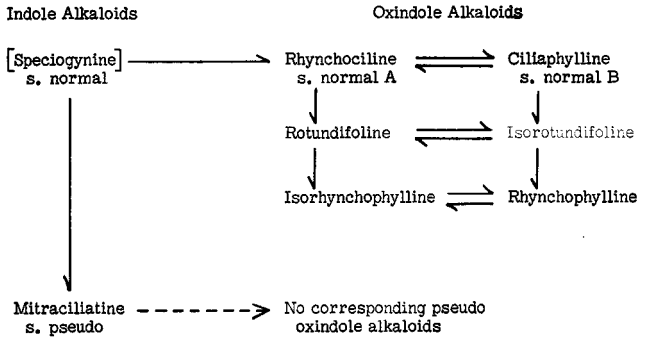
DISCUSSION

Seven of the alkaloids present (six oxindole and one indole alkaloid) are the same as those present in the leaves of *M. ciliata*; all are E *seco* type alkaloids. However, whereas the oxindole alkaloids have the normal configuration [C(3)-Hα, C(20)-Hβ],

the indole alkaloid, mitraciliatine has the pseudo configuration [C(3)-Hβ, C(20)-Hβ]. On the basis of the hypothesis for oxindole alkaloid biogenesis suggested by Shellard, Phillipson & Gupta (1969) it might have been anticipated that the other indole alkaloid (Sp 4) present in traces would have been speciogynine since this is the corresponding alkaloid in the normal series. The hRf values are not consistent with this and the limited spectral data suggest an alkaloid of the corynanthine type. Should this be confirmed it would be the first alkaloid having a carbocyclic E ring to be isolated from species of *Mitragyna*, though they occur in other Rubiaceous plants.

Failure to detect speciogynine may be due to several reasons, similar to those suggested for the absence of tetrahydroalstonine in the leaves of *M. parvifolia* (Shellard, Phillipson & Gupta, 1969).

The suggested biogenetic route for the oxindole alkaloids would be:



Demethylation of the oxindole alkaloids, rhynchociline and ciliaphylline would give rotundifoline and isorotundifoline while removal of the C(9)-OH from these alkaloids would give isorhynchophylline and rhynchophylline respectively. This would be just the opposite of the route envisaged in *M. parvifolia* where the same oxindole alkaloids occur but the corresponding indole alkaloids are dihydrocorynantheine (s. normal) and hirsutine (s. pseudo), both of which are unsubstituted in the C(9) position. It is interesting to note that a (C)9-OH indole alkaloid has not been isolated from any *Mitragyna* species. The two remaining oxindole alkaloids, speciophylline and uncarine F are *closed* E ring alkaloids with epiallo configuration. Unlike *M. parvifolia* there seem to be no corresponding indole alkaloids or *closed* E ring oxindole alkaloids of other configurations present in the leaves of *M. inermis*. However, investigation of the alkaloidal pattern in the leaves, roots and bark of the plant collected at regular intervals throughout the year may reveal the presence of corresponding alkaloids or their precursors, and this is being currently undertaken.

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