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The alkaloidal pattern in the leaves, stem-bark and root-bark of *Mitragyna* species from Ghana

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The leaves, stem-bark and root-bark of *Mitragyna ciliata*, *M. inermis* and *M. stipulosa* have been examined quantitatively and qualitatively for alkaloids. In addition, the plant parts of *M. stipulosa* collected at 12 regular monthly intervals have been similarly examined. The presence of mitraphylline in the leaves of this plant and the absence of indole alkaloids was confirmed. The predominant leaf alkaloids are oxindoles of the A series, those of the stem- and root-bark, oxindoles of the B series. Whereas the general alkaloidal pattern of *M. ciliata* appears similar to that of *M. inermis* rather than *M. stipulosa*, a more detailed study suggests that *M. ciliata* and *M. inermis* have a distinctly different metabolic pattern in relation to the C(9)-OMe alkaloids.

The nature of the alkaloids present in the leaves of *Mitragyna stipulosa* (D.C.) O. Kuntze, *Mitragyna ciliata* Aubr. et Pellegr. (Beckett, Shellard & Tackie, 1963) and *Mitragyna inermis* (Willd.) O. Kuntze (Shellard & Sarpong, 1969), has already been reported. Whereas *M. ciliata* and *M. stipulosa* are almost identical botanically yet have a different alkaloidal pattern, *M. inermis* which differs very markedly in its botanical character from *M. ciliata* is very similar to it in alkaloidal pattern.

A comparative study has, therefore, been made of the alkaloids present in the leaves, stem-bark and root-bark of these three species using specimens collected from the same plants on the same occasion. In addition an investigation has been made of the alkaloids present in the leaves, stem-bark and root-bark of *M. stipulosa* collected from the same tree at regular monthly intervals during the course of one year.

EXPERIMENTAL

Materials

The leaves, stem-bark and roots were collected from localities in Ghana as follows: *M. ciliata*—Tarkwa district, West Region. *M. inermis*—Sogakofe district, Volta Region. *M. stipulosa*—The University Campus, Kumasi, Ashanti Region.

The materials for the comparative study of the three species were collected in September 1966. The leaves, stem-bark and roots of *M. stipulosa* was collected from December 1968 to November 1969.

The root-bark was cut away from the xylem; leaves, stem-bark and root-bark were separately coarsely powdered.

Thin-layer plates. Silica gel (G). Merck 0·25 mm thick.

Solvent systems. Chloroform–acetone (5:4), chloroform–methanol (95:5) (for separation of rotundifoline and isorhynchophylline).

Spray reagents. 0·2M $FeCl_3$ in 35% $HClO_4$ (Shellard & Alam, 1968).

Colour intensities were determined by reflectance scanning using a Chromoscan Densitometer (Joyce Loebl) with TLC attachment (Filter 465, aperture 1003).

Method

The powdered materials (leaves and root-bark—10 g, stem-bark—25 g) were extracted with 96% ethanol. After removal of the solvent under reduced pressure, glacial acetic acid (5 ml) was added and the mixture poured into distilled water (50 ml). After 12 h the mixture was filtered, the residue being washed with 5% acetic acid. The filtrate was made alkaline with ammonia and the alkaloids extracted with chloroform. After removal of solvent the crude alkaloids were dissolved in 5% sulphuric acid and non-alkaloidal material extracted with ether, the aqueous solution was again made alkaline with ammonia and re-extracted with chloroform. The purified alkaloids were dissolved in chloroform (5 ml) and added to the top of an alumina column (5 × 1 cm) containing chloroform. The total alkaloids were eluted with chloroform and after removal of the solvent were dissolved in methanol (5 ml). Two, 5 and 10 μl quantities were applied to the thin-layer plates, five plates being used for each determination, known quantities of the individual authentic alkaloids being chromatographed on the same plates at the same time.

For the estimation of an alkaloid present only in traces, the plate was loaded sufficiently to give a spot which yielded a reasonably sized peak with the densitometer. The results are given in Table 1 and in Fig. 1A–D.

Table 1. *Percentage of alkaloids* ($\times 10^{-2}$) *present in the leaves, stem-bark and root-bark of Ghanaian species of mitragyna collected in September* 1966

	Leaves	Stem-bark	Root-bark
Mitragyna ciliata			
Isorhynchophylline	3·1	1·2	4·8
Rhynchophylline	5·7	2·4	7·2
Rotundifoline	9·1	0·7	0·9
Isorotundifoline	3·5	0·2	0·05
Rhynchociline	4·2	1·6	6·6
Ciliaphylline	1·8	0·5	6·0
Mitraciliatine	2·1	0·0	0·0
Mitragyna inermis			
Isorhynchophylline	13·8	1·5	10·8
Rhynchophylline	6·4	4·6	19·6
Rotundifoline	9·4	0·01	0·8
Isorotundifoline	0·4	1·8	4·4
Rhynchociline	2·6	0·05	6·0
Ciliaphylline	1·0	0·01	2·4
Speciophylline	2·1	0·01	0·05
Uncarine F	0·3	0·01	3·5
Mitraciliatine	0·05	0·0	0·0
Speciogynine	0·01	0·0	0·0
Mitragyna stipulosa			
Isorhynchophylline	2·2	2·5	6·4
Rhynchophylline	3·3	6·4	11·2
Rotundifoline	5·8	0·25	0·5
Isorotundifoline	1·8	0·10	0·05
Mitraphylline	0·0	0·0	0·0

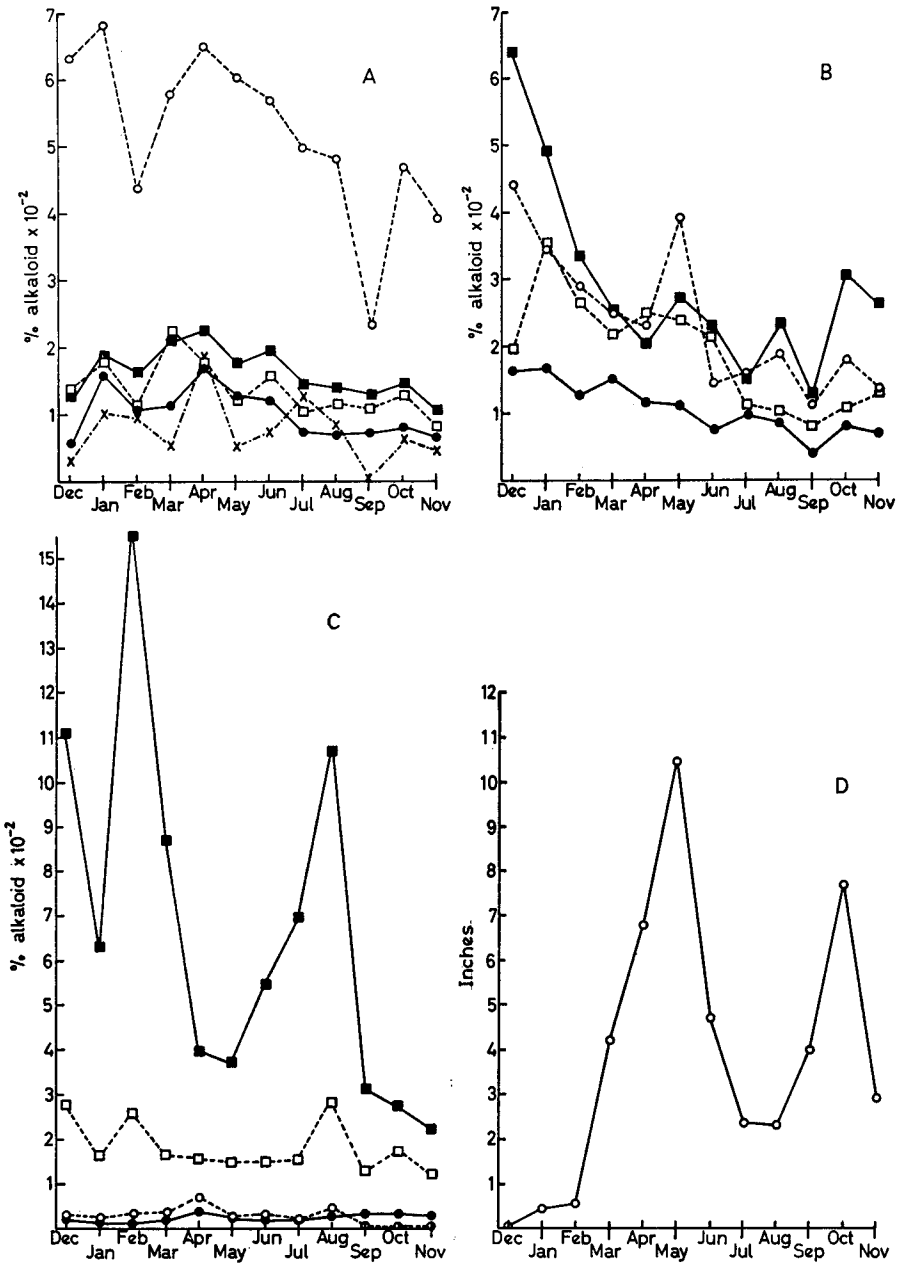


FIG. 1. Variation in the alkaloidal content of the leaves (A), root-bark (B), stem-bark (C) of *M. stipulosa* collected at monthly intervals from December 1968 to November 1969. D. Rainfall of the collecting area (Kumasi) over the same period. - - ○ - - Rotundifoline. —●— Isorotundifoline. - - □ - - Isorhynchophylline. —■— Rhynchophylline. —X— Mitraphylline.

DISCUSSION

The percentage of total alkaloids in the stem-bark is lower than that in the leaves or root-bark (Table 1) which indicates that the alkaloids in the stem-bark are in the translocation stream and that no biogenesis, or storage occurs there. In *M. inermis* and *M. stipulosa* there is a higher alkaloidal content in the root-bark than in the leaves but the opposite obtains in the sample of *M. ciliata* examined. Thus there is some doubt as to whether the site of biogenesis is the leaf or the root although the fact that indole alkaloids occur in the leaf but not in the stem-bark or root-bark suggests that the alkaloids are synthesized in the leaves.

The dominant alkaloid is the same for stem and root-bark and differs from that found in the leaf. In all three species the dominant bark alkaloid is rhynchophylline (E *seco*, normal, B series, C(9)–H); for *M. ciliata* and *M. stipulosa* the main leaf alkaloid is rotundifoline (E *seco*, normal, A series, C(9)–OH); for *M. inermis* it is isorhynchophylline (E *seco*, normal, A series C(9)–H). Whereas the conversion of isorhynchophylline to rhynchophylline in *M. inermis* involves only a change in configuration from the A to the B series of oxindole alkaloids, the conversion of rotundifoline to rhynchophylline involves, in addition, the removal of the C(9)–OH.

In the leaves the dominant alkaloids are the A series oxindoles whereas in the root-bark they are the B series. With the dominant alkaloids the same for stem and root-bark it would appear that conversion takes place in the leaf before translocation to the root. Such dominance of the B series of alkaloids in the stem and root-bark is only true, however, if the total alkaloid is considered (Table 2) since with the C(9)–OMe substituted alkaloids the A series alkaloid (rhynchociline) is found in larger quantities than the B series alkaloid (ciliaphylline) in the barks as well as in the leaves (Table 1).

Table 2. *Percentage of A and B series E seco oxindole alkaloids* ($\times 10^{-2}$) *present in the leaves, stem-bark and root-bark of Ghanaian species of mitragyna collected in September* 1966

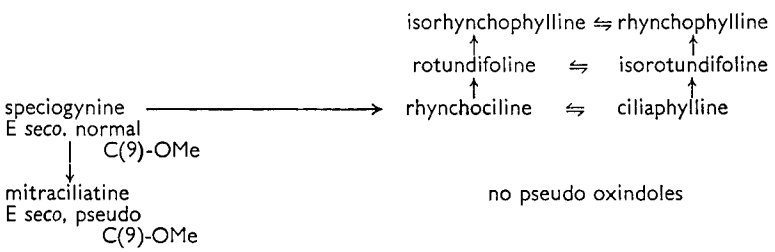
	Leaves		Stem-bark		Root-bark	
	A	B	A	B	A	B
M. ciliata	16·4	11·0	3·5	3·1	11·5	13·2
M. inermis	25·8	7·8	1·55	6·41	17·6	26·7
M. stipulosa	8·0	5·1	2·5	6·4	8·4	11·2

Table 3 shows that in *M. ciliata* the percentage of C(9)–OH alkaloid being translocated and stored in the root-bark falls considerably from the 46% present in the leaves but at the same time there are marked increases in the percentages of C(9)–H and C(9)–OMe alkaloids in the translocation stream and in the root-bark. In *M. inermis*, however, the percentage of C(9)–OMe alkaloids in the stem-bark is lower than in the leaves while the percentage of C(9)–OH alkaloid is not markedly different from that in the leaves. Thus it appears that in *M. ciliata* the C(9)–OH alkaloid is converted into the C(9)–OMe alkaloid and possibly the C(9)–H alkaloid, while in *M. inermis* the C(9)–OMe alkaloid is converted into the C(9)–OH alkaloid before translocation. The absence of C(9)–OMe alkaloids from *M. stipulosa* makes it impossible to compare this plant in the same way but it is clear that for translocation

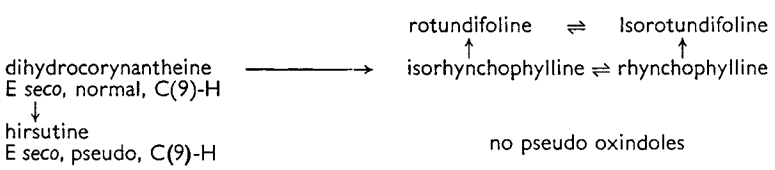
Table 3. *Percentage of total alkaloid present as C(9)–H, C(9)–OH and C(9)–OCH_3 alkaloids in the leaves, stem-bark and root-bark of Ghanaian species of mitragyna collected in September* 1966

	Leaves	Stem-bark	Root-bark
Mitragyna ciliata			
C(9)–H	32	54·5	47
C(9)–OH	46	13·0	4·0
C(9)–OMe	22	32·5	44
Mitragyna inermis			
C(9)–H	60	76	70
C(9)–OH	29	26·5	11·8
C(9)–OMe	11	7·5	18·2
Mitragyna stipulosa			
C(9)–H	42	98·7	90
C(9)–OH	58	1·3	10
C(9)–OMe	0	0	0

the C(9)–OH alkaloids are converted to the C(9)–H series. Whereas the initial work suggested that the alkaloidal pattern of *M. ciliata* and *M. inermis* are similar, this more detailed study suggests a different metabolic pattern for the C(9)–OMe alkaloids. In *M. stipulosa* the enzyme system controlling the methylation of the C(9)–OH alkaloids appears to have been lost. This plant also differs from the others by the absence of even traces of recognizable indole alkaloids which throws some doubt upon the validity of the hypothesis for oxindole biogenesis suggested by Shellard, Phillipson & Gupta (1969) and supported by the alkaloids present in *M. inermis* and *M. ciliata* for which the suggested pathway would be:



In *M. stipulosa* the pathway would have to be:



The close affinity of *M. stipulosa* to *M. ciliata* would not support this reversal in the mode of formation of the C(9)–OH although it does represent one of the pathways in *M. parvifolia* (Roxb.) Korth (Shellard & others, 1969).

The presence of the *epiallo* closed E ring oxindole alkaloids, speciophylline and uncarine F in the leaves, stem and root bark of *M. inermis* with no trace of the related

indole or oxindole alkaloids needs explanation though it is possible that an investigation similar to that made on *M. stipulosa* might reveal them at some times during the year. The absence of mitraphylline in the leaves of *M. stipulosa* collected in September might also be for this reason but no corresponding indole alkaloids, ajmalicine and 3-isoajmalicine, nor isomitraphylline, were detected at any time.

There appears to be some relation between the amount of rotundifoline in the leaf of *M. stipulosa* and the heavy rainfall while the movement of the rhynchophylline and isorhynchophylline corresponds to the dry seasons which follow the heavy rains. There must be some metabolism of the rhynchophylline and isorhynchophylline in the root bark since there is no accumulation of these alkaloids in the root-bark after the considerable translocation through the stem-bark, until the dry period at the turn of the year (Dec.–Jan.). The isorotundifoline content in the root-bark is also high at this time of the year but it also increases during the rainy period of May. The periods of minimum content of rotundifoline in the leaf correspond roughly to the periods of maximum content of rhynchophylline in the stem-bark and this supports the contention that rotundifoline is dehydroxylated to rhynchophylline for purposes of translocation.

It is possible that the isolation of immediate precursors of the indole alkaloids could lead to a modification of the hypothesis since it depends upon the identity of the D/E rings and the stereochemistry about C(3) in the indole and oxindole alkaloids. In this respect the root bark does contain indolic polar substances.

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REFERENCES

Beckett, A. H., Shellard, E. J. & Tackie, A. N. (1963.) *J. Pharm. Pharmac.*, **15**, *Suppl.*, 158T–165T; 166T–169T.

Shellard, E. J. & Alam, M. Z. (1968). *J. Chromat.*, **33**, 347–369.

Shellard, E. J. & Sarpong, K. (1969). *J. Pharm. Pharmac.*, **21**, *Suppl.*, 113S–117S.

Shellard, E. J., Phillipson, J. D. & Gupta, D. (1969). *Planta Medica*, **17**, 51–58.