# THE *MITRAGYNA* SPECIES OF GHANA

## THE ALKALOIDS OF THE LEAVES OF *Mitragyna stipulosa* (D.C.) O. KUNTZE

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The alkaloids rotundifoline, rhynchophylline, isorhynchophylline and isorotundifoline have been isolated from the leaves of *Mitragyna stipulosa* (D.C.), O. Kuntze (Rubiaceae). Traces of mitra-phylline were found in a few samples. The approximate concentration in various parts of the tree are recorded.

THE genus *Mitragyna* (Family—Rubiaceae) consists of about 10 species, all of which are trees growing exclusively in humid conditions. Three species occur in Ghana (Irvine, 1961) and also in other parts of West Africa (Aubreville, 1936; Dalziel, 1937). According to Hutchinson and other authorities at the Royal Botanical Gardens, Kew (1963), they are now designated: (i) *Mitragyna stipulosa* (D.C.) O. Kuntze; (ii) *Mitragyna ciliata*, Aubr. et Pellegr. and (iii) *Mitragyna inermis* (Willd.) O. Kuntze.

Various parts of all three species find use in local folk lore medicine for a wide variety of diseases: preparations of the plant are administered both orally and as local applications.

The presence of mitraphylline, rhynchophylline and rotundifoline have been reported; reports of previous investigations being summarised in Table I.

TABLE I

ALKALOIDS AND THEIR SOURCE REPORTED PREVIOUSLY

| Source | Alkaloid | Reference |
|---|---|---|
| *Mitragyna stipulosa* (D.C.) O. Kuntze* | | |
| Bark | unnamed | Michiels and Leroux (1925) |
| Bark | mitraphylline (rubradine) | Denis (1927) |
| Bark | mitraphylline | Michiels (1931), Raymond-Hamet and Millat (1935) |
| Bark | rhynchophylline | Larrieu (1930) |
| Bark | rhynchophylline | Raymond-Hamet and Millat (1934) |
| *M. ciliata* Aubr. et Pellegr. | | |
| Bark | rhynchophylline | Ongley (1950) |
| Leaves | rotundifoline | Ongley (1950) |
| *M. inermis* (Willd.) O. Kuntze | | |
| Bark | unnamed | Larrieu (1930) |
| Bark | rhynchophylline | Ongley (1950) |
| | | Badger, Cook and Ongley (1950) |
| Leaves | mitrinermine† | Raymond-Hamet and Millat (1934) Millat (1946) |

* No investigation of the leaf appears to have been made.
† Shown to be idential with rhynchophylline (Badger, Cook and Ongley, 1950).

* This work forms part of the thesis submitted by A. N. Tackie for the Ph.D. degree of the University of London.

Other alkaloids, reported from various species of *Mitragyna* grown elsewhere than West Africa, are summarised in Table II.

TABLE II
OTHER MITRAGYNA ALKALOIDS AND THEIR SOURCES

| Alkaloid | Source | Reference |
|---|---|---|
| Mitraversine | *Mitragyna diversifolia* (leaves) Havil | Field (1921) |
| Mitragynine | *M. speciosa* (leaves) Korth | Field (1921), Ing and Raison (1939) |
| Mitraspecine | *M. speciosa* (bark, wood) Korth | Denis (1938) |
| Mitragynol | *M. rotundifolia* (Roxb.) O. Kuntze | Ongley (1950), Badger and others (1950) |
| Isorhynchophylline | *M. rubrostipulaceae* (Havil) (*Adina rubrostipulata* (leaves) Schuman) | Marion, Nair Edwards and Seaton (1960) |

## EXPERIMENTAL

Alumina used for column chromatography was Spence-type H, the adsorbent for thin layer chromatography was Alumina (Merck), and the solvent, chloroform.

All melting points are uncorrected. Equivalent weights were determined by non-aqueous titration (Beckett and Tinley, 1962). Elemental analyses were made by Mr. G. S. Crouch, School of Pharmacy, University of London and Drs. G. Weiler and F. B. Strauss, Oxford.

*Materials.* Flowering tops, leaves, stipules, young stem, old stem, stem bark and root bark were obtained from trees growing in the rain forest near the Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, during various periods from January, 1961, to June, 1962. Details of the authentication of the species are reported by Shellard and Shadan (1963).

TABLE III
DISTRIBUTION OF ALKALOIDS IN *M. stipulosa*

| Plant part | Rotundifoline | Rhynchophylline | Mixture of isorotundifoline and isorhynchophylline | Alkaloid $R_F \simeq 0.95$ | Alkaloid $R_F \simeq 0$ |
|---|---|---|---|---|---|
| Entire leaf | 0·15 | 0·04 | 0·3 | <0·001 | 0·001 |
| Midrib | 0·07 | 0·02 | 0·16 | 0 | <0·001 |
| Lamina | 0·25 | 0·065 | 0·4 | <0·001 | <0·001 |
| Stipules | 0·3 | 0·15 | 0·4 | 0 | 0·003 |
| Very young leaves *inside* stipules | 0·8 | 0·13 | 1·12 | 0·045 | 0·005 |
| Flowers | <0·001 | 0·005 | 0·005 | 0 | 0·003 |
| Stem bark | 0·002 | 0·02 | 0·05 | 0 | 0·001 |
| Root bark | 0 | 0 | 0 | 0 | 0 |
| Node runner bark | 0·01 | 0·006 | 0·03 | 0 | <0·001 |

### *Preliminary Investigation and Distribution of Alkaloids*

Various organs of *Mitragyna stipulosa* were examined for total alkaloidal content using a method based on that of Douglas and Kiang (1957). Dried leaves contained 0·3–0·45 per cent, the stem and root bark ~0·1 per cent and the flowering tops practically no alkaloid. The stipules contained ~0·85 per cent and the very young leaves inside the stipules

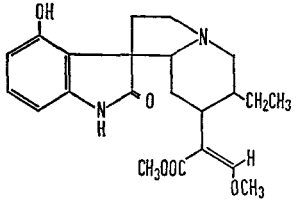
~2·0 per cent, but only very small quantities of these plant organs were available.

The alkaloids rotundifoline, isorotundifoline (hitherto known as mitragynol, Beckett and Tackie, 1963), rhynchophylline and isorhynchophylline were isolated from the leaves, some samples of which also furnished traces of mitraphylline (see later). In addition, traces of two other alkaloids were present.

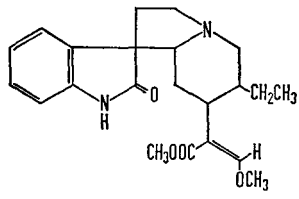
By means of quantitative thin layer chromatography (details to be published elsewhere), the percentage of the different alkaloids in various parts of the plant was determined as reported in Table III.

*Isolation of Alkaloids*

Coarsely powdered leaves (1 kg.) were extracted by refluxing with 96 per cent ethanol (5 litres) 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. This was dissolved in a little ethanol and treated with 5 per cent acetic acid, the acid washings being added to the filtrate which was made alkaline with ammonia and extracted with chloroform. The extract was washed, dried and evaporated to yield the crude alkaloids (4 g.). Bulked crude alkaloids (5 g.) were dissolved in benzene (10 ml.) and added to a column of powdered cellulose (Whatman No. 1, 30 × 2 cm.), the alkaloids being eluted with benzene (~1000 ml.) which on evaporation left a pale brown residue. Recrystallisation four times from absolute ethanol, gave rotundifoline (I) (1·5 g.) as colourless needles, m.p. 237–8° (Barger, Dyer and Sargent, 1939; Beckett and Tackie, 1963).




I




II

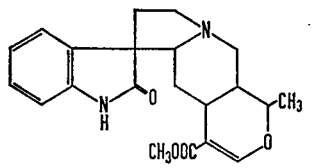
No further crystals could be obtained from the mother liquors and the solvent was removed. The residue (3·0 g.) was dissolved in benzene (10 ml.) and added to a column of alumina (30 × 2 cm.), the alkaloids being eluted with benzene (~1000 ml.) which on evaporation left a pale yellow residue. After decolourisation, recrystallisation from absolute ethanol: methyl ethyl ketone (2:1) yielded rhynchophylline (II) (0·2 g.), m.p. 212–214° (Nozoye, 1957; Seaton and Marion, 1957).

The mother liquors were evaporated to dryness and the residue (2·5 g.) dissolved in ether (10 ml.), the solution added to a column of alumina and the alkaloids eluted as under.

The following fractions were collected: (1) Ether (90 ml.), amorphous alkaloids. (2) Ether (150 ml.), amorphous alkaloids. (3) Ether-chloroform (1 : 1,100 ml.), rotundifoline (90 mg.). (4) Ether-chloroform (1 : 1,100 ml.), amorphous alkaloids. (5) Chloroform (40 ml.), amorphous alkaloids. (6) Chloroform (650 ml.), rhynchophylline (30 mg.).

Thin layer chromatography on alumina with chloroform as the running solvent was used to monitor all fractions collected in this analysis.

After removal of these crystallised alkaloids, the fractions were bulked, evaporated to give "uncrystallisable bases" (2·2 g.) which were dissolved in ether (10 ml.) added to an alumina column (30 × 2 cm.) and eluted with ether-chloroform (95 : 5); rotundifoline was found in the first few fractions, later fractions yielded a mixture of other alkaloids, while rhynchophylline remained on the column. After removal of the rotundifoline from the eluate the bulked fractions were evaporated to give an amorphous white residue (2·0 g.) which judged by thin layer chromatography, was free from rotundifoline and rhynchophylline. This residue in ether was extracted with 5 per cent sodium hydroxide and the resultant alkaline solution saturated with carbon dioxide to give a precipitate which was extracted with ether. Evaporation of the solvent left a residue which gave prismatic crystals from acetone-light petroleum (b.p. 40–60°) (1 : 1) (1·2 g.) m.p. 130–132°; prolonged drying under vacuum at 100° was needed to remove the acetone of crystallisation. This substance was identified as *isorotundifoline* (Beckett and Tackie, 1963).




III

The original ethereal solution from which the isorotundifoline had been removed by alkaline extraction, gave on evaporation, a white residue from which white needle crystals of isorhynchophylline (700 mg.) m.p. 144° (Seaton, Nair, Edwards and Marion, 1960) were obtained with some difficulty from n-hexane ether (1 : 1). Dried leaves (5 kg.) were collected in April, 1962, at the beginning of the rainy season, and a concentrated alcoholic extract prepared. After removal of rotundifoline, rhynchophylline, isorotundifoline and isorhynchophylline by the methods described above, the alkaline liquors still contained alkaloid which was only very sparingly soluble in ether. A chloroform extract, on evaporation, gave a residue which yielded white silky needles (0·24 g.) of mitraphylline (III), m.p. 267–268° (Seaton, Tondeur and Marion, 1958) from absolute ethanol. A further 0·11 g. of mitraphylline was obtained during the recrystallisation of the crude rotundifoline when a small fraction of this material, sparingly soluble in absolute ethanol, was recrystallised from large volumes of this solvent.

*Characterisation of the Alkaloids*

*Rotundifoline.* Soluble in acetone, chlorobenzene, chloroform, ethanol, sparingly soluble in ether, insoluble in sodium hydroxide, m.p. 239–40°. *Approximate $R_F$ value, 0·54. $[\alpha]_D^{20}$ + 124·7° (*c*, 2 in $CHCl_3$); + 115·9 (*c*, 2 in EtOH). Found: C, 65·9; H, 6·9; N, 6·9; OMe, 15·3; equiv. wt., 400, 402. Calc. for $C_{22}H_{28}O_5N_2$†: C, 66·0; H, 7·0; N, 7·0; OMe, 15·5; equiv. wt., 400. $pK_a$ 4·85 (electrometric titration in 80 per cent methyl cellosolve), 5·3 (electrometric titration in $H_2O$). $\lambda_{max}$(EtOH) 221 mμ (log ε 4·39), 289 mμ (log ε 3·42). Shoulder 242·5 mμ (log ε 4·14). $\lambda_{min}$ 272 mμ (log ε 3·21). $\nu_{max}$ (Nujol) 3,240, 2,450 (broad) 1,700, 1,625, 1,275, 1,250, 1,107, 847, 780, 750, 730 $cm.^{-1}$. $\nu_{max}$ ($CHCl_3$) 3,440 $cm.^{-1}$.

The *perchlorate* crystallised from glacial acetic acid, m.p. 280–281°. Found: C, 52·9; H, 5·5; N, 5·6. $C_{22}H_{28}O_5N_2.HClO_4$ requires C, 52·7; H, 5·8; N, 5·6.

The *methiodide* crystallised from absolute methanol, m.p. 243·4°. Found: C, 51·5; H, 5·3; N, 5·2; equiv. wt. 540, 543. $C_{22}H_{28}O_5N_2$. MeI requires C, 50·9; H, 5·7; N, 5·2; equiv. wt., 542.

The *hydriodide* crystallised from ether-ethanol (1 : 1), m.p. 221–222°. Found: C, 50·1; H, 5·5; N, 5·1; equiv. wt., 530. $C_{22}H_{28}O_5N_2.HI$ requires C, 49·3; H, 5·5; N, 5·3; equiv. wt., 528.

The *hydrobromide* crystallised from ether-ethanol (1 : 1), m.p. 215–217°. Found: C, 54·2; H, 6·4; N, 5·4; equiv. wt., 483. $C_{22}H_{28}O_5N_2.HBr$ requires C, 54·9; H, 6·0; N, 5·8; equiv. wt., 481.

The *trinitrobenzene derivative* crystallised from absolute ethanol, m.p. 195–197°. Found: C, 54·2; H, 5·0; N, 11·5; OMe, 10·1; equiv. wt., 611·5; $C_{22}H_{28}O_5N_2.C_6H_3(NO_2)_3$ requires C, 54·8; H, 5·1; N, 11·4; OMe, 10·1; equiv. wt., 613.

This alkaloid is identical in melting-point, mixed melting-point, optical rotation, ultra-violet and infra-red spectra and $R_F$ value (thin layer chromatography) with an authentic sample of rotundifoline from *Mitragyna rotundifolia* (Badger, Cook and Ongley, 1950) kindly supplied by Dr. J. D. Loudon.

*Rhynchophylline.* Soluble in acetone, chlorobenzene, chloroform, ethanol, slightly soluble in ether, insoluble in sodium hydroxide, m.p. 212–214°. Approximate $R_F$ value, 0·15. $[\alpha]_D^{21}$ — 14·4° (c, 2, $CHCl_3$). Found: C, 68·7; H, 7·1; N, 7·4; OMe, 17·7; equiv. wt., 380. Calc. for $C_{22}H_{28}O_4N_2$, C, 68·8; H, 7·3; N, 7·3; OMe, 16·1; equiv. wt., 384. $pK_a$ 6·4 (electrometric titration in 80 per cent methyl cellosolve), 6·8 (electrometric titration in $H_2O$). $\lambda_{max}$ (EtOH) 208·3 mμ (log ε 4·45), 243·3mμ (log ε 421), 282·0 mμ (log ε 2·93). $\lambda_{min}$ 222·2 mμ (log ε 3·76), 277·8 mμ (log ε 2·89). $\nu_{max}$ (Nujol) 1,725, 1,700, 1,640, 1,280, 1,250, 1,180, 1,125, 1,100, 780, 745 $cm^{-1}$.

This alkaloid is identical in melting-point, mixed melting-point, optical rotation, ultra-violet and infra-red spectra and $R_F$ value by thin layer

* Where $R_F$ values are quoted, these refer to thin layer chromatography on alumina with chloroform as solvent.

† Amended by Beckett and Tackie (1963) from $C_{22}H_{26}O_5N_2$ (Barger, Dyer and Sargent, 1939; Ongley, 1950).

chromatography with an authentic sample of rhynchophylline from *Mitragyna rubrostipulaceae* (Seaton and Marion, 1957; Nozoye, 1958) kindly supplied by Dr. L. Marion.

*Mitraphylline.* Soluble in chloroform, sparingly soluble in acetone, ethanol, ether, insoluble in sodium hydroxide, m.p. 267–268°. Approximate $R_F$ value 0·19. Found: C, 68·5; H, 6·6; N, 7·7; equiv. wt., 370, 372. Calc. for $C_{21}H_{24}O_4N_2$, C, 68·5; H, 6·6; N, 7·6, equiv. wt., 368. $\lambda_{max}$ (EtOH) 208 mμ (log ε 4·67), 241·5 mμ (log ε 4·19), 281 mμ (log ε 3·03). $\lambda_{min}$ 222 mμ (log ε 4·0), 274 mμ (log ε 2·99). $\nu_{max}$ (Nujol) 3,600, 3,250, 1,715, 1,700, 1,620, 1,290, 1,270, 1,190, 1,170, 1,105, 775, 760 cm.$^{-1}$. The *perchlorate* crystallised in silky needles, m.p. 235–237°. Found: C, 53·4; H, 5·3; calc. for $C_{21}H_{24}O_4N_2.HClO_4$, C, 53·7; H, 5·4.

This alkaloid is identical in melting-point, mixed melting-point, ultra-violet and infra-red spectra and $R_F$ value (thin layer chromatography) with an authentic sample of mitraphylline from *Mitragyna rubrostipulaceae* (Seaton and Marion, 1957) kindly supplied by Dr. L. Marion.

*Isorhynchophylline.* Soluble in chloroform, ethanol, ether, insoluble in sodium hydroxide, m.p. 144°. Approximate $R_F$ value 0·50. $[\alpha]_D^{20}$ + 8·6° (*c*, 2 in $CHCl_3$). $pK_a$ 5·2 (electrometric titration in 80 per cent methyl cellusolve), 6·25 (electrometric titration in $H_2O$). $\lambda_{max}$ (EtOH) 204 mμ (log ε 4·46), 239·8 (log ε 4·24). Shoulder 277·8 mμ (log ε 3·25). $\lambda_{min}$ 220·8 mμ (log ε 4·02). $\nu_{max}$ (Nujol) 3,200, 1,695, 1,610, 1,600, 1,105, 750 cm.$^{-1}$.

The *perchlorate* colourless needles from ether-ethanol, m.p. 163–165°. Found: C, 52·8; H, 6·1; N, 5·0; OMe, 12·1. Calc. for $C_{22}H_{28}O_4N_2.HClO_4.H_2O$, C, 52·5; H, 6·2; N, 5·6; OMe, 12·3 per cent.

This alkaloid is identical in melting-point, mixed melting-point, optical rotation, ultra-violet and infra-red spectra and $R_F$ value (thin layer chromatography) with an authentic sample of isorhynchophylline from *Mitragyna rubrostipulaceae* (Seaton, Nair Edwards and Marion, 1960) kindly supplied by Dr. L. Marion.

*Isorotundifoline.* Soluble in acetone, chloroform, ethanol, ether, sodium hydroxide, slightly soluble in water, insoluble in light petroleum, m.p. 130–132°. Approximate $R_F$ value 0·44. $[\alpha]_D^{24}$ — 7·7° (*c*, 2 in $CHCl_3$. Found: C, 66·2; H, 6·9; N, 6·9; equiv. wt., 396, 398. $C_{22}H_{28}O_5N_2$ requires C, 66·0; H, 7·0; N, 7·0; equiv. wt., 400. $pK_a$ 6·7 (electrometric titration in 80 per cent methyl cellusolve), 7·4 (electrometric titration in $H_2O$). $\lambda_{max}$ (EtOH), 218 mμ (log ε 4·43), 289 mμ (log ε 3·49), shoulder 242 mμ (log ε 4·13), $\lambda_{min}$ 270 mμ (log ε 3·31). $\lambda_{max}$ (0·001N NaOH in 70 per cent EtOH), 238 mμ (log ε 4·38), 306·7 mμ (log ε 3·54), shoulder 222 mμ (log ε 4·26). $\lambda_{min}$ 281·7 mμ (log ε 3·36). $\nu_{max}$ (Nujol) 3,250, 1,695, 1,685, 1,625, 1,605, 1,230, 1,140, 1,100, 1,095, 915, 900, 790, 770, 740 cm.$^{-1}$.

The *hydrochloride* crystallised in silky needles, m.p. 216°, from ether-ethanol (1 : 1). $[\alpha]_D^{25}$ — 40·8° (*c*, 2 in $H_2O$). Found: C, 58·9; H, 6·8; N, 6·0; $C_{22}H_{28}O_5N_2.HCl$, $H_2O$ requires C, 58·1; H, 6·7; N, 6·2.

This alkaloid was named *isorotundifoline* because it is identical in

melting-point, mixed melting-point, optical rotation, ultra-violet and infra-red spectra and $R_F$ value (thin layer chromatography) with the isomeric base obtained by isomerisation of rotundifoline (Beckett and Tackie, 1963).

*Other alkaloids.* The presence of two other alkaloids of $R_F = 0$ and 0·95 was indicated by thin layer chromatography (alumina/chloroform).

## Discussion

The isolation of five alkaloids from the leaves of *Mitragyna stipulosa* was facilitated by the use of thin layer chromatography as an aid to separation and identification. The presence of mitraphylline at the advent of the rainy season is an interesting biosynthetic problem and is being investigated further. Another interesting feature is the high alkaloidal content of the very immature leaves, inside the stipules, i.e. before they have been involved in the photosynthetic process.

The isolation of pure alkaloids from the plant permits pharmacological evaluation of chemical entities rather than crude screening of extracts of uncertain composition; pharmacological studies of these alkaloids are in progress.

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