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RHYNCHOPHYLLINE AND ISORHYNCHOPHYLLINE N-OXIDES FROM SPECIES OF *MITRAGYNA*

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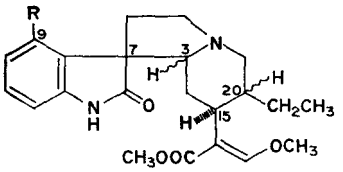
Abstract-The 'base-line' alkaloid previously isolated from leaves of ***Mitragyna rotundifolia*** (Roxb.) 0. Kuntze and ***Mitrugyna inermis*** (Willd.) 0. Kuntze, has been identified as isorhynchophylline N-oxide. Rhynchophylline N-oxide has also been isolated from the leaves of ***M. inermis.***

INTRODUCTION

THE ALKALOIDS obtained from species of ***Mitrugyna*** (***Rubiaceae***) are indolic or oxindolic with closed or open E rings (E *seco*), the structure of the E *seco* oxindole alkaloids being shown in I.



Stereoisomerism of E *seco* oxindole alkaloids occurs with ***allo*** type having $C_3H\alpha$ and $C_{20}H\alpha$, ***normal*** type alkaloids having $C_3H\alpha$ and $C_{20}H\beta$ and ***epiallo*** type alkaloids having $C_3H\beta$ and $C_{20}H\alpha$ ***[pseudo*** type oxindole alkaloids (C_3 and $C_{20}H\beta$) do not exist]. Isomerism also occurs at C_7 to give A and B series oxindoles while substitution may occur at C_9 in the aromatic ring.

The earlier examinations of the leaves of *Mitragyna rotundifolia* (Roxb.) 0 Kuntze, indicated the presence of rhynchophylline (I, ***normal,*** R═H, B series), rotundifoline (I, ***normal,*** R═OH, A series) and mitragynol [1,2] which was later shown to be identical with isorotundifoline (I, ***normal*** R═OH, B series).[3,4] Later investigations showed the absence of rotundifoline and isorotundifoline and the presence of rhynchophylline, isorhynchophylline (I, ***normal*** R═H, A series) and a third alkaloid referred to as the 'base-line' alkaloid because it remained on the base line during TLC with various solvent systems.[5] This alkaloid was similar to an alkaloid isolated from the leaves of ***Mitrugyna inermis*** (Willd.) 0. Kuntze.[6,7]

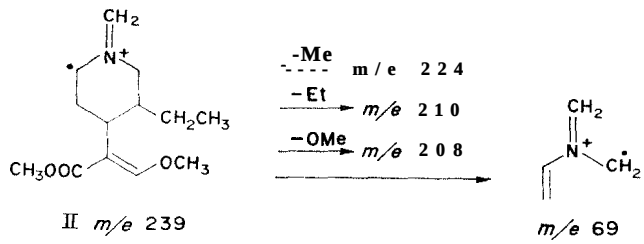
The structure of this alkaloid is now discussed, together with a new isomeric alkaloid obtained from the leaves of M. ***inermis.***

[1] ***G.*** BARGER, E. DYER and L. J. SARGENT, ***J. Org. Chem. 4,418*** (1939).
[2] G. M. BADGER, J. W. COOK and P. A. ONGLEY, ***J. Chem. Soc. 867 (1950).***
[3] A. H. BECKETT and A. N. TACKIE, ***Chem. & Ind.*** 1122 (1963).
[4] G. M. BADGER, L, M. JACKMAN, R. SKLAR and E. WENKERT, ***Proc. Chem. Soc. Lond. 206 (1963).***
[5] **E. J.** SHELLARD and J. D. PHILLIPSON, ***Planta Medica 12, 27 (1964).***
[6] A. N. TACKIE, Ph.D. Thesis. University of London (1963).
[7] E. J. SHELLARD and K. SARPONG, ***J. Pharm. Pharmac. 21,*** (Suppl.), **113S** (1969).

RESULTS AND DISCUSSION

The 'base-line' alkaloid from *M. rotundifolia*, m.p. 242–243°, analysed for $C_{22}H_{28}N_2O_5$ (mol. wt. 400), while the equivalent weight was determined by non-aqueous titration as 399; the indication being that the alkaloid contains one additional oxygen atom to that present in rhynchophylline (mol. wt. 384). The UV spectrum (EtOH), λ_{max} 243 nm (log ϵ, 4·30), 283 nm (log ϵ, 3·18), λ_{min} 225 nm (log ϵ, 4·09), 278 nm (log ϵ, 3·06) is identical with that of rhynchophylline and isorhynchophylline. In the IR spectrum a broad band occurs at 2500 cm-' similar to that given with rotundifoline, suggesting the possible presence of hydrogen bonding. An imino group (3350 cm^{-1}) ester and oxindole carbonyls (1695 and 1685 cm-') and a double bond (1625 cm-') are also indicated to be present. The NMR (60 MHz) shows a three-proton triplet at S 0·86 for the $C_{18}Me$ confirming that the alkaloid is an E *seco* and not closed E ring type. Two three-proton singlets at S 3·63 and 3·67 corresponding to two methoxyl groups and a signal at S 7·29 belonging to an olefinic proton also points to the presence of the ester/vinyl ether grouping, $CH_3COOC(=)CH.OCH_3$. The aromatic protons appear as a four-proton multiplet at S 7·20 confirming that the oxindole moiety is unsubstituted in the aromatic ring. The imino group is revealed by a one-proton singlet at S 8·25, which disappears on deuteration,

A similar 'base-line' alkaloid was obtained recently from the leaves of ***M. inermis'*** as colourless needle crystals (13 mg), m.p. 238–240°, undepressed by admixture with the *M. rotundifolia* 'base-line' alkaloid; the UV and IR spectra and TLC with several systems were identical. The mass spectrum of the alkaloid was also very similar to that of the ***M. rotundifolia*** 'base-line' alkaloid which showed a molecular ion peak (M^+) at ***m/e*** 400 with a prominent peak at ***m/e*** 384 (M^+-16; molecular ion peak for rhynchophylline) and at ***m/e*** 239 (II, also found in the mass spectrum of rhynchophylline). Peaks associated with fragment II were also present at ***m/e*** 224 (loss of Me), 210 (loss of Et), 208 (loss of OMe) and 69; these peaks are characteristic for rhynchophylline-type alkaloids.[8,9] Peaks belonging to the oxindole portion of the molecule were noted at ***m/e*** 130, 144, 145, 146 and 159.[9] The mass spectrum therefore, indicated that the 'base-line' alkaloid has a rhynchophylline-type structure which readily loses 16 mass units. Hence, it is considered to be an N-oxide of one of the rhynchophylline-type alkaloids, i.e. isorhynchophylline ***(normal,*** A), rhynchophylline ***(normal,*** B), corynoxine (*allo*, A), corynoxine B (*allo*, B)



II *m/e* 239 *m/e* 69

When the ***M. inermis*** 'base-line' alkaloid was reduced with sulphurous acid, the reaction mixture made alkaline with ammonia, extracted with chloroform, and examined by TLC, the results clearly indicated that only one alkaloidal spot was present, corresponding to isorhynchophylline, and that rhynchophylline, corynoxine and corynoxine B were absent.

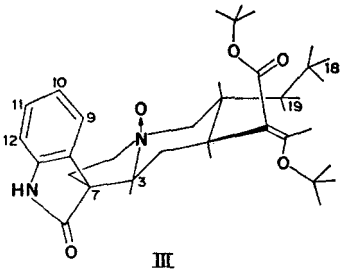
[8] A. H. BECKETT, D. DWUMA-BADU and R. E. HADDOCK, *Tetrahedron* **25, 5961 (1969).**

[9] B. GILBERT, J. A. BRISSOLESE, N. FINCH, W. I. TAYLOR, H. BUDZIKIEWICZ, J. M. WILSON and C. DJERASSI, *J. Am. Chem. Soc.* **85, 1523 (1963).**

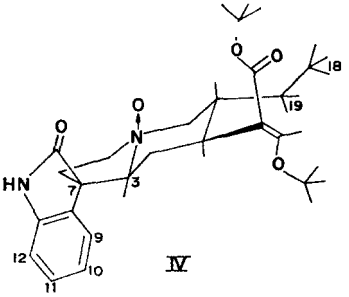
When isorhynchophylline was treated with 15 % hydrogen peroxide at room temperature, crystals separated out of the reaction mixture, which were identified as isorhynchophylline N-oxide and had m.p., mixed m.p., UV, IR, NMR and mass spectra identical with those of the 'base-line' alkaloid. Rhynchophylline N-oxide was similarly prepared from rhynchophylline, isolated as an amorphous solid and shown to differ from isorhynchophylline N-oxide by TLC. TLC comparison of rhynchophylline N-oxide with the mother-liquors from which the 'base-line' alkaloid of *M. inermis* was obtained, showed that the major spot in the mother-liquors corresponded to rhynchophylline N-oxide. This major component was isolated from the 'base-line' alkaloid mother-liquors by preparative TLC to yield an amorphous solid (2 mg), identical with rhynchophylline N-oxide (by mass spectrum and TLC behaviour in three systems). Reduction of a trace of the new alkaloid with sulphurous acid, followed by extraction and TLC examination, showed only one alkaloid to be present, namely, rhynchophylline, and that isorhynchophylline, corynoxine and corynoxine B were absent.

In order to check whether the N-oxides were reduced to the corresponding bases without isomerization at C_7 samples of the prepared rhynchophylline and isorhynchophylline N-oxides were similarly treated with sulphurous acid, the reaction mixtures made alkaline with ammonia and extracted with chloroform. TLC examination confirmed that the N-oxides had been reduced to the corresponding alkaloids and that no isomerization had taken place.

Further evidence for the structures and, more particularly, the conformations of isorhynchophylline N-oxide (III) and rhynchophylline N-oxide (IV) was obtained from a study of their NMR spectra. The 100 MHz spectrum of prepared isorhynchophylline N-oxide showed a poorly resolved three-proton triplet at δ 0·78 (C_{18}Me) which established that the C_{19} methylene protons are not affected by the oxygen at Nb.[10] This suggests that the conformation of the N-oxide is the same as that of the parent base. Support for this is also provided by the signals for the aromatic protons which appear as a three-proton multiplet at δ 6·82–7·20 (C_{10}, C_{11} and C_{12}) and as a one-proton doublet at δ 8·22 (C_9). Compared with the NMR spectrum of isorhynchophylline, the signal for the C_9 proton is shifted downfield by 0·8 ppm and it is evident that this shift must be due to deshielding by the oxygen on Nb.



III

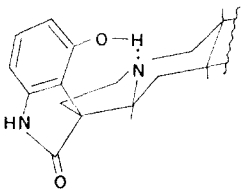


IV

The 100 MHz NMR spectrum of rhynchophylline N-oxide (IV) is similar to that of isorhynchophylline N-oxide (III) but differs in that the signal for the C_9 proton appears with the other aromatic protons in the four-proton multiplet at δ 6·90–7·22. Hence, in rhynchophylline N-oxide the C_9 proton is not affected by the oxygen at Nb and this is further evidence for no conformational changes.

[10] W. F. TRAGER, CALVIN M. LEE, J. D. PHILLIPSON, R. E. HADDOCK, D. DWUMA-BADU and A. H. BECKETT, *Tetrahedron 24, 523 (1968).*

It has been proposed,[11] on the basis of the CD curves of rhynchophylline/isorhynchophylline and of mitraphylline/isomitraphylline (the corresponding ***normal*** B and ***normal*** A closed E-ring oxindole alkaloids), that a negative Cotton effect in the 280-290 nm region can be attributed to the A oxindole configuration whereas alkaloids with the B configuration show in the same region a positive Cotton effect. A negative Cotton effect in the 250-270 nm region has been attributed to a $C_3H\alpha$-configuration. Similar arguments have been used for establishing the structures of the four closed E-ring oxindole alkaloids, uncarines C, D, E and F (i.e. *allo* A and B, ***epiallo*** A and B).[12,13] However, this is not always the case, because the *allo* B alkaloid, corynoxine B, shows a positive Cotton effect between 250 and 270 nm, suggesting a $C_3H\beta$-configuration,[10] when it does, in fact, possess a $C_3H\alpha$-configuration. Apparently anomalous behaviour is shown by rotundifoline (9-hydroxyisorhynchophylline) which has **a** positive Cotton effect and by isorotundifoline (9-hydroxyrhynchophylline) which



V

has a negative Cotton effect, in the 280-290 nm region. Although the structure of rotundifoline (partial V) is similar to that of isorhynchophylline N-oxide (III), in that an oxygen atom is present between Nb and C_9, there is no indication of anomalous behaviour and the CD curves of isorhynchophylline N-oxide and isorhynchophylline both show a negative Cotton effect between 280 and 290 nm; rhynchophylline N-oxide and rhynchophylline curves have a positive Cotton effect in the same region (Fig. 1). The fact that the isorhynchophylline N-oxide prepared from isorhynchophylline and the naturally-occurring isorhynchophylline N-oxide have identical CD curves demonstrates that they both possess the same absolute configuration.

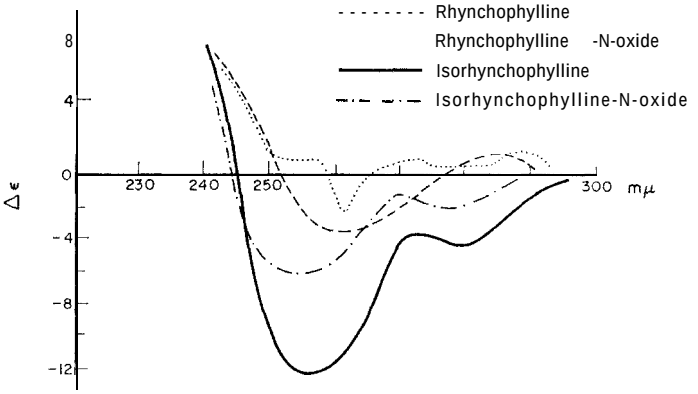


FIG. 1. C.D. CURVES OF RHYNCHOPHYLLINE, ISORHYNCHOPHYLLINE AND THEIR N-OXIDES.

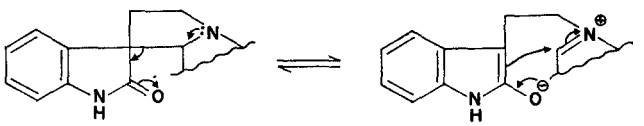
[11] J. L. POUSSET, J. POISSON and M. LEGRAND, ***Tetrahedron Letters 6283 (1966).***
[12] A. F. BEECHAM, N. K. HART, S. R. JOHNS and J. A. LAMBERTON, ***Tetrahedron Letters 991 (1967).***
[13] A. F. BEECHAM, N. K. HART, S. R. JOHNS and J. A. LAMBERTON, *Austral. J. Chem.* **21,491 (1968).**

Mass spectrometry has been shown to reflect stereochemical differences in the heteroyohimbine alkaloids.* However, it has been stated that the abundance of the m/e 239 fragment in the E-*seco* oxindole alkaloids seems to be independent of the stereochemistry at C_7, perhaps due to thermal isomerizations at the high temperature of the mass spectrometer inlet.[8] Nevertheless, if the relative abundances reported for the ***m/e 239*** peak are compared for the four unsubstituted ***normal*** and ***allo*** A and B, E-seco oxindole alkaloids, it can be seen that the relative abundance is higher for the B isomers than for the corresponding A isomers, i.e. ***m/e 239*** relative abundance for isorhynchophylline (***normal*** A) 54%, rhynchophylline (***normal*** B) 82%; corynoxine (***allo***, A) 46%, corynoxine B (***allo*** B) 71%.

A similar relationship exists for the two N-oxides i.e. ***m/e 239*** relative abundance for isorhynchophylline N-oxide (***normal*** A) 43%, rhynchophylline N-oxide (***normal*** B) 78%. Hence, it would appear that if unsubstituted alkaloids are compared, the relative abundance of the ***m/e 239*** peak could well be a reflection of their stereochemistry. This, however, is not the case with the C_9 substituted oxindole alkaloids.

It is tempting to ascribe the broad band at 2500 cm-' in the IR spectrum of isorhynchophylline N-oxide to the hydrogen bonded C_9H since a Dreiding model shows the close proximity of this hydrogen to the oxygen at Nb. However, this seems unlikely since there is no evidence that aromatic C-H takes part in such bonding. We are not therefore in a position to assign the band at 2500 cm^{-1}.

The naturally-occurring oxindole alkaloids can be readily isomerized about the C_3 and/or C_7 centres by heating with either pyridine or acetic acid. It has been suggested that such isomerization involves scission and reformation of the C_3—C_7 bond (VIa⇌VIb).[14–16]



VI a VI b

In order to establish what would happen to oxindole N-oxides under such conditions, rhynchophylline N-oxide and isorhynchophylline N-oxide were each refluxed for 48 hr with both pyridine and 50% aqueous acetic acid. TLC examinations of the reaction mixtures showed that with pyridine the oxygen at Nb was lost and the parent oxindole alkaloid produced, whether rhynchophylline or isorhynchophylline, isomerized in the expected manner to give a mixture of isorhynchophylline (major) and rhynchophylline (minor). With acetic acid it was also noted that both N-oxides readily lost the oxygen at Nb.

There is a possibility that the N-oxides are formed during the isolation procedure, particularly as the N-oxides were isolated after the tertiary bases had been obtained. In order to establish whether the N-oxides are natural products, a small quantity of ***M. inermis*** leaves was extracted by maceration with 96% ethanol. The concentrated alcoholic extract proved too crude for TLC examination, but after shaking with chloroform: 5% ammonia the chloroform layer could be examined by TLC, and showed that rhynchophylline N-oxide and isorhynchophylline N-oxide were present in the extract together with rhynchophylline and isorhynchophylline. As similar treatment with rhynchophylline and isorhynchophylline did

[14] E. WENKERT, J. H. UDELHOFEN and N. K. BHATTACHARYYA, *J. Am. Chem. Soc.* **81**, 3763 (1959).
[15] J. C. SEATON, M. D. NAIR, 0. E. EDWARDS and L. MARION, *Can. J. Chem.* **38**, 1035 (1960).
[16] J. L. POUSSET and J. POISSON, *C.R. Acad. Sci., Paris 259, 597 (1964).*

not give the N-oxides nor the other isomer, it is considered that rhynchophylline and isorhynchophylline and their N-oxides are natural products and not artefacts.

From the biosynthetic point of view it might be envisaged that A and B oxindole tertiary alkaloids are first formed and that the N-oxides are formed subsequently. This would be supported by the fact that the N-oxides of both A and B isomers were isolated. Once formed, it may be that the parent alkaloids and corresponding N-oxides are interconvertible.

The isolation of isorhynchophylline N-oxide and rhynchophylline N-oxide from two species of *Mitragyna* would suggest that these alkaloids may also be present in other species of *Mitragyna* and it could be anticipated that other N-oxides of oxindole and heteroyohimbine E-*seco* and E-closed alkaloids will also be isolated.

EXPERIMENTAL

M.ps are uncorrected; IR spectra were in (Nujol); NMR spectra in $CDCl_3$ with TMS internal reference; CD curves, Roussel-Jouan Dichrograph, methanol solutions, 1 mg/5 ml; mass spectra were determined on AEI MS902 high resolution mass spectrometer at 70 eV, inlet temperature, 285"; equivalent weight by titration of 5 mg samples with N/50 perchloric acid in HOAc using Oracet blue indicator; TLC, silica gel G (Merck) with A, $CHCl_3$–acetone (5 : 4); B, MeOH; C, EtOAc–*iso*PrOH–conc. NH_4OH (60: 35 : 5).

Range of hR_f values:

	A	B	C
isorhynchophylline	70-72	68-75	84-89
rhynchophylline	24-33	60-64	69-72
isorhynchophylline N-oxide	0	42–44	40–41
rhynchophylline N-oxide	0	11–13	5-6

Isolation of isorhynchophylline N-oxide. The alkaloid was isolated from *M. rotundifolia* leaves[5] and from *M, inermis* leaves as previously described.[6,7] ***M. rotundifolia alkaloid.*** Colourless needle crystals from light petroleum-ethanol, m.p. 242-243". Found: C, 65·28; H, 7.37; N, 7.47; eq. wt. 399 $C_{22}H_{28}N_2O_5$ required, C, 65.99; H, 7·0; N, 7·0; eq. wt. 400. UV (EtOH) λ_{max} 243 nm (log ϵ, 4·30), 283 nm (log ϵ, 3·18), λ_{min} 225 nm (log ϵ, 4.09). IR ν 3350 cm^{-1} (NH, weak), 2500 cm^{-1} (broad), 1700 cm^{-1} (broad CO and 1675 infl, ester and oxindole carbonyl), 950 cm^{-1} (N-O) NMR signals ($CDCl_3$) 60 MHz, δ 0·86 (3H, t, C_{18}Me), 3.63 (3H, s, ester MeO), 3.67 (3H, s, vinyl MeO), 7·20 (4H, m, aromatic), 7.29 (1H, s, olefinic), 8.25 (1H, s, NH, disappears on deuteration). Mass spectrum *m/e* 400 (M^+, 8 %), 384 (78 %), 239 (43 %), 224 (37 %), 210 (13 %), 208 (14 %), 159 (15 %), 146 (34 %), 145 (21 %), 144 (35 %), 130 (100%) 69 (61%).

Identical m.p., mixed m.p., UV, IR, TLC (systems B and C) with synthetic isorhynchophylline N-oxide.

M. inermis alkaloid. m.p., mixed m.p., UV, IR, TLC (systems B and C) identical with *M. rotundfolia* 'base-line' alkaloid and synthetic isorhynchophylline N-oxide, Mass spectrum, *m/e* 400 (M^+), 384,239, 224, 210, 208, 159, 146, 145, 144, 130, 69.

Isolation of rhynchophylline N-oxide. The mother liquor of isorhynchophylline N-oxide, isolated from *M. inermis,* was subjected to preparative TLC (silica gel/methanol), the major alkaloidal band extracted with methanol, concentrated to dryness and extracted with $CHCl_3$. Concentration to dryness yielded 2 mg of an amorphous residue which was found to be identical in UV, IR, and TLC (systems A, B and C) with synthetic rhynchophylline N-oxide. Mass spectrum, *m/e* 400 (M^+,11 %), 384 (66 %), 239 (100 %), 224 (56 %), 210 (42 %) 208 (50 %), 159 (29 %), 146 (18 %), 145 (19 %), 144 (36 %), 130 (63 %), 69 (96%).

Preparation of isorhynchophylline N-oxide. Isorhynchophylline (150 mg) was dissolved in 96 % ethanol (0·4 ml) and 15 % H_2O_2 added (10 ml). The mixture was allowed to stand at room temp. overnight and then heated on a boiling water bath for 30 min. On cooling colourless needle crystals were obtained, dissolved in water (3 ml) and boiled with Pt wire for 5 min. Two recrystallizations from 50 % ethanol yielded colourless needle crystals (110 mg, 70%) m.p. 242". UV (EtOH) λ_{max} 245 nm (log ϵ, 4·31), 286 nm (log ϵ, 3·23), λ_{min} 226 nm (log ϵ, 4.07). IR ν 3400 cm-' (NH, weak), 2500 cm-' (broad), 1700 cm-' (broad CO with inflexion at 1675 cm-'), 950 cm^{-1} (N-O). NMR ($CDCl_3$) 100 MHz, δ 0·78 (3H, t, poorly resolved, C_{18}Me) 3·60 (3H, s, ester MeO), 3.68 (3H, s, vinyl MeO), 6·82–7·20 (3H, m, aromatic protons C_{10}, C_{11}, C_{12}), 7.23 (1H, s, vinyl), 8.22 (1H, d, C_9H), 11.43 (1H, broad, s, NH, disappears with D_2O). Mass spectrum *m/e* 400 (M^+, 24 %), 384 (67 %), 239 (41 %), 224 (27 %), 210 (16 %), 208 (17 %), 159 (20 %), 146 (23 %), 145 (18 %), 144 (24 %), 130 (47 %), 69 (57 %).

Preparation of rhynchophylline N-oxide. Rhynchophylline (150 mg) was treated with H_2O_2 as described above. Synthetic rhynchophylline N-oxide could not be obtained crystalline but only as an amorphous powder (61 mg, 38%). UV (EtOH) λ_{max} 245 nm (log ϵ, 4·19), 287 nm (log ϵ, 3·41), λ_{min} 226 nm (log ϵ, 3.85). IR ν 3400 cm^{-1} (NH, weak), 1700 and 1710 cm^{-1} (CO, broad doublet). NMR ($CDCl_3$) 100 MHz, δ 0·78

(3H, t, poorly resolved, C_{18}Me), 3·60 (3H, s, ester MeO), 3.75 (3H, s, vinyl MeO), 6·90–7·22 (4H, m, aromatic protons), 7.22 (1H, s, vinyl), 7·60 (1H, s, NH disappears with D_2O). Mass spectrum m/e 400 (M^+, 16%), 384 (54%), 239 (78%), 224 (36%), 210 (28%), 208 (30%), 159 (33%), 146 (18%), 14.5 (15%), 144 (31%), 130 (57%), 69 (180%).

Reduction of N-oxides. Natural and synthetic isorhynchophylline N-oxide and synthetic rhynchophylline N-oxide were reduced as follows. Sulphurous acid (0·5 ml, 5 % w/v SO_2) was added to the N-oxide (1 mg), allowed to stand overnight, made alkaline with ammonia and extracted with $CHCl_3$ (3 x 1 ml). The combined extracts were concentrated and examined by TLC. Natural rhynchophylline N-oxide was reduced by the same procedure using only a trace of material and 1 drop of sulphurous acid. TLC examination (systems A, B and C) showed that isorhynchophylline N-oxide (natural and synthetic) was completely reduced to isorhynchophylline only whilst rhynchophylline N-oxide (natural and synthetic) was partially reduced (estimated 50%) to rhynchophylline only.

Aclcnowledgements-We thank Mr. E. Allman, (then) Head of the Department of Pharmacy, University of Science & Technology, Kumasi, Ghana, and Professor A. N. Tackie, present Dean of the Faculty of Pharmacy at Kumasi, for providing the plant material, Mr. G. McDonough for the 60 MHz NMR spectra, Mr. A. Saunders for C.D. determinations and Mr. R. Brown for the figures. 100 MHz NMR spectra were determined at the Physico-Chemical Measurements Unit, Harwell, Didcot, Berks. for which we thank the Director, and the mass spectra were determined at the School of Pharmacy, University of London, and we acknowledge the help of Dr. B. J. Millard and Mr. D. Carter. We are also grateful to Mr. R. F. Branch for discussions of IR spectra and Dr. N. G. Bisset (both of this College) for helpful comments during the preparation of the manuscript. One of us (KS) wishes to thank the Ghanaian Government and the University of Science & Technology, Kumasi, for a scholarship enabling him to undertake research work in the Pharmacognosy Research Laboratories at Chelsea College, University of London.