Phytochemistry, 1973, Vol. 12, pp. 1481 to 1487. Pergamon Press. Printed in England.

INDOLE AND OXINDOLE ALKALOIDS FROM *UNCARIA BERNAYSIA**

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(*Received* 5 *November* 1972. *Accepted* 28 *November* 1972)

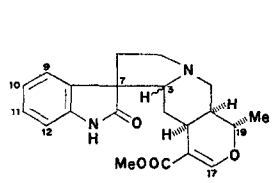
Key Word Index—*Uncaria bernaysii*; Rubiaceae; pteropodine; isopterodine; speciophylline; uncarine; N-oxides; akuammigine; tetrahydroalstonine.

Abstract—Minor alkaloids isolated from the leaves and stems of *Uncaria bernaysii* F.v.M. (Rubiaceae) have been identified as tetrahydroalstonine, akuammigine and the *N*-oxides of the four stereoisomeric oxindole alkaloids isopteropodine, pteropodine, speciophylline and uncarine F.

INTRODUCTION

FOUR interconvertible stereoisomeric pentacyclic oxindole alkaloids (I) have been reported to be present in the leaves of *Uncaria bernaysii* F.v.M.[1–3] The stereochemistry of these four isomers has been established as, isopteropodine (uncarine E, I, *allo* A), pteropodine (uncarine C, I, *allo* B), speciophylline (uncarine D, I, *epiallo* A) and uncarine F (I, *epiallo* B).[3,4]

No further alkaloids have been reported from *U. bernaysii* leaves and other parts of the plant have not been investigated for their alkaloid content. In the course of screening *Uncaria* species for alkaloids, small samples of leaves and stems of *U. bernaysii* were examined. Both leaves and stems were shown to contain the same major alkaloids, namely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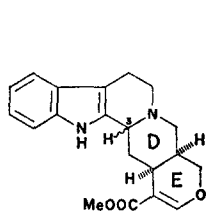
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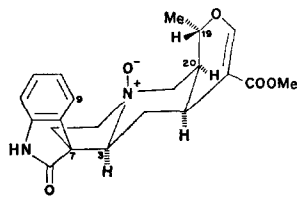
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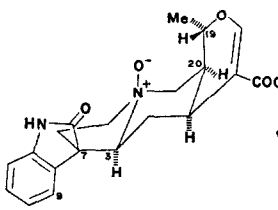
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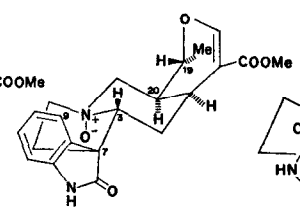
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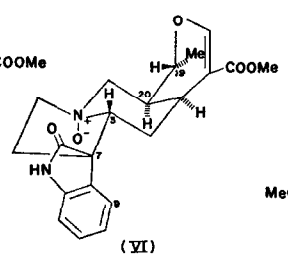
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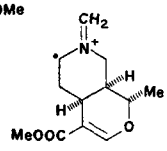
(VII)

* Part I in a projected series "Alkaloids from *Uncaria* species".

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[1] JOHNS, S. R. and LAMBERTON, J. A. (1966) *Tetrahedron Letters* 4883.

[2] HART, N. K., JOHNS, S. R. and LAMBERTON, J. A. (1967) *Chem. Commun.* 87.

[3] BEECHAM, A. F., HART, N. K., JOHNS, S. R. and LAMBERTON, J. A. (1968) *Australian J. Chem.* **21**, 491.

[4] BEECHAM, A. F., HART, N. K., JOHNS, S. R. and LAMBERTON, J. A. (1971) *Tetrahedron Letters* 991.

four isomers of the oxindole type, isopteropodine, pteropodine, speciophylline and uncarine F, although in differing proportions. Also present were six minor alkaloids whose characterization and identification is now discussed.

RESULTS AND DISCUSSION

Total alkaloid extracts of leaves and stems of *U. bernaysii* were separated on TLC and examined using a ferric chloride–perchloric acid spray reagent which differentiates between pentacyclic oxindole alkaloids and heteroyohimbines. The results suggested that the major alkaloids of both stems and leaves were the known oxindole alkaloids isopteropodine, pteropodine, speciophylline and uncarine F which had been isolated previously from the leaves of *U. bernaysii*.[1–3] The presence of other, minor alkaloids was indicated including two having the colour reaction characteristic of the closed E-ring heteroyohimbines of type II.

These two alkaloids were separated by preparative TLC and identified as the known compounds tetrahydroalstonine (II, C-3 Hα, *allo*) and akuammigine (II, C-3 Hβ, *epiallo*) which correspond to the major oxindole alkaloids in their D/E ring systems. Other minor alkaloids were indicated by the presence of a spot which stayed on the base-line in many TLC systems and which gave an oxindole colour reaction. It was suspected that this might be due to *N*-oxides since rhynchophylline and isorhynchophylline *N*-oxides isolated from a species of the closely related genus *Mitragyna* showed similar TLC behaviour.[5] When more polar solvent systems were used four minor oxindole spots were observed. The *N*-oxides of isopteropodine, pteropodine, speciophylline and uncarine F were prepared and shown to correspond in their TLC behaviour to the four minor alkaloids of *U. bernaysii*.

It is possible that oxidation at N-4 of pentacyclic oxindole alkaloids would result in the formation of two *N*-oxides. Certainly, akuammigine (II, C-3 Hβ) forms two such diastereoisomeric *N*-oxides[6] and further examples are known for other types of alkaloid.[7] During the preparation of the *N*-oxides of isopteropodine, pteropodine, speciophylline and uncarine F only single products were detected on TLC. The configuration of the oxygen at N-4 in these compounds was established from 100 MHz NMR spectra.

The signal for the C-19 Me group appears in the NMR spectrum of isopteropodine *N*-oxide (III, *allo* A) as a doublet at δ 1·55 in contrast to δ 1·42 in the spectrum of the tertiary base.[3] A similar change in chemical shift is noted in the spectrum of pteropodine *N*-oxide (IV, *allo* B) where the C-19 Me doublet appears at δ 1·51 as against δ 1·35 in the tertiary alkaloid.[3] These differences indicate that in both *N*-oxides the C-19 Me group is deshielded by the oxygen at N-4 so that the C/D ring junctions are *trans*. When the δ values of the C-19 Me signals in the spectra of speciophylline *N*-oxide (V, *epiallo* A) and uncarine F *N*-oxide (VI, *epiallo* B) are compared with those of the corresponding tertiary bases no such downfield shifts are noted. Similarly, the signal for the C-19 H appears well downfield at δ 5·1 for isopteropodine *N*-oxide and pteropodine *N*-oxide, a downfield shift of 0·6 and 0·7 ppm respectively, in comparison to the corresponding tertiary bases. Again, no such shifts of this signal are noted in the spectra of speciophylline *N*-oxide and uncarine F *N*-oxide. The C-19 H/C-20 H coupling constants for the four *N*-oxides are the same as for the corresponding tertiary alkaloids (J 11 Hz) suggesting that isopteropodine *N*-oxide and pteropodine *N*-oxide retain the *trans* pseudo diaxial configuration whilst speciophylline

[5] SHELLARD, E. J., PHILLIPSON, J. D. and SARPONG, K. (1971) *Phytochemistry* **10**, 2505
[6] MERLINI, L., NASINI, G. and PHILLIPSON, J. D. (1973) *Tetrahedron* in press.
[7] PHILLIPSON, J. D. (1971) *Xenobiotica* **1**, 419.

N-oxide and uncarine F *N*-oxide (*J* 1·5 Hz) retain the *trans* pseudo diequatorial configuration.[3] Hence the NMR spectra are consistent with the C-19 position being closer to the oxygen at N-4 for isopteropodine *N*-oxide and pteropodine *N*-oxide than for the pair speciophylline *N*-oxide and uncarine F *N*-oxide. The C-9 H signal in the spectrum of isopteropodine *N*-oxide is clearly distinguished as a one-proton double doublet (*ortho* and *meta* coupling) appearing at δ 8·03, downfield from the other three aromatic protons which appear as a multiplet at δ 6·78–7·22. In contrast to this the C-9 proton in the spectrum of pteropodine *N*-oxide is not distinguishable from the other aromatic protons. Uncarine F *N*-oxide also has the C-9 proton signal downfield (δ 8·13) from the other aromatic protons whereas the C-9 H signal in the spectrum of speciophylline *N*-oxide appears with the other aromatic proton signals. Thus isopteropodine *N*-oxide and uncarine F *N*-oxide have their C-9 protons deshielded by the oxygen on N-4, indicating the closeness of the aromatic ring to the N-4 oxygen in this pair of isomers, in contrast to pteropodine *N*-oxide and speciophylline *N*-oxide. The NMR spectra are therefore in agreement with structure III for isopteropodine *N*-oxide, IV for pteropodine *N*-oxide, V for speciophylline *N*-oxide and VI for uncarine F *N*-oxide. III and IV possess the 4-R absolute configuration and V and VI the 4-S absolute configuration.

The MS of the prepared *N*-oxides of isopteropodine, pteropodine, speciophylline and uncarine F are very similar to those of the corresponding tertiary alkaloids with prominent peaks at *m/e* 130, 144, 145, 146 (indole peaks), 69 ($C_4H_7N^+$), 223 ($C_{12}H_{17}NO_3$, VII) and 368 (M^+ of tertiary base).[8] Differences between the MS of some isomeric heteroyohimbine alkaloids have been interpreted in terms of their stereochemistry but this has proved more difficult for the corresponding oxindole alkaloids since they readily undergo thermal isomerization.[9] However, MS can be used to differentiate between the A and B series of unsubstituted E-*seco* pentacyclic oxindole alkaloids.[5] The *N*-oxides of isopteropodine, pteropodine, speciophylline and uncarine F readily lose oxygen by thermal decomposition but if the spectra are determined at 225° immediately the ion current appears, distinct differences can be observed, enabling these four isomers to be differentiated. The relative abundance of the molecular ion at *m/e* 384 can be used to distinguish isopteropodine *N*-oxide (100%) from speciophylline *N*-oxide (3·5%) and from the other two isomers pteropodine *N*-oxide (50%) and uncarine F *N*-oxide (45%). Furthermore, uncarine F *N*-oxide (VI) differs from the other three isomers in having the base peak of its spectrum at *m/e* 367 (M^+-17). Prominent peaks at *m/e* 239 ($C_{12}H_{17}NO_4$ by accurate mass measurement, i.e. the alicyclic portion of the molecule which has retained the N-4 oxygen) appear in the spectra of isopteropodine *N*-oxide (III, 32%) and pteropodine *N*-oxide (IV, 53%) but the other two isomers have *m/e* 239 peaks of very low intensity. The occurrence of the *m/e* 239 peak in the MS of the two *allo* isomers (III and IV) can probably be explained by the close proximity of the C-19 proton to the oxygen at N-4; certainly it can be used to distinguish the two *allo* isomers from the two *epiallo* isomers (V and VI). MS of the natural compounds enabled identification of isopteropodine *N*-oxide, pteropodine *N*-oxide and speciophylline *N*-oxide on the basis of the features described.

The two indole alkaloids tetrahydroalstonine (II, *allo*) and akuammigine (II, *epiallo*) found as minor alkaloids in the *U. bernaysii* leaves and stems extracted, possess D and E ring systems which are identical in structure to the major oxindole alkaloids isolated. A

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

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

similar situation exists for *U. rhynchophylla* Miq. where the E-*seco* indole and oxindole alkaloids isolated also have identical D/E ring systems.[10] Pentacyclic oxindole and indole alkaloids with common D/E ring systems are also found to occur together in several species of the related genus *Mitragyna*. Since heteroyohimbine alkaloids are readily converted chemically into the corresponding oxindole alkaloids it has been postulated that heteroyohimbines are synthesized in the plant and converted into oxindole alkaloids.[11] Recently, pteropodine and isopteropodine have been converted into tetrahydroalstonine and akuammigine[10] and it is possible that oxindole alkaloids may be converted to indole alkaloids within the plant. Furthermore, tetrahydroalstonine and akuammigine are readily interconvertible chemically as are isopteropodine, pteropodine, speciophylline and uncarine F. Hence the following relationships may be possible within *Uncaria bernaysii* and in species of *Mitragyna*:[12]

allo indole	⇌	*allo* oxindoles A and B
⇅		⇅
epiallo indole	⇌	*epiallo* oxindoles A and B.

Although all the major alkaloids are present in the leaves and stems collected from the same plants at the same time, the proportions differ (Table 1) and the following observations can be made from the results of quantitative TLC experiments: (1) Speciophylline (*epiallo* A) is the major alkaloid in the leaves, whereas in the stems about equal proportions of speciophylline and the thermodynamically more stable isomer pteropodine are the major alkaloids. In the hooks it is isopteropodine which predominates. (2) The ratio of the two *allo* to the two *epiallo* alkaloids is 0·5:1 in the leaves, 1·2:1 in the stems and 1·5:1 in the hooks. (3) The ratio of the two isomers with the A configuration to the two with the B configuration is approximately 1:1 in all three plant parts, being 1·2:1 in the leaves, 0·8:1 in the stems and 1:1 in the hooks. (4) The major *N*-oxide in a particular plant part does not always correspond to the major tertiary base.

TABLE 1. THE PENTACYCLIC OXINDOLE ALKALOIDS AND THEIR *N*-OXIDES IN *Uncaria bernaysii*

Alkaloid content	Stems	Leaves	Hooks	Alkaloid content	Stems	Leaves	Hooks
Total alkaloid in plant part %	0 7	2 7	1 2				
Oxindoles as % total alkaloid				*N*-oxides as % total alkaloid			
Isopteropodine (*allo* A)	24	14	29	Isopteropodine *N*-oxide	1·5	0·6	1 9
Pteropodine (*allo* B)	28	17	25	Pteropodine *N*-oxide	1 0	0·6	1 1
Speciophylline (*epiallo* A)	28	37	23	Speciophylline *N*-oxide	0·8	2 0	1·5
Uncarine F (*epiallo* B)	14	25	15	Uncarine F *N*-oxide	0 5	0·5	2 1

Differences in alkaloid proportions of this type have been noted in *Mitragyna* species and a study of the alkaloid content of different plant parts of *M. parvifolia* from India, Ceylon and Burma has shown that there is considerable variation in alkaloid proportions

[10] AIMI, N., YAMANAKA, E., ENDO, J., SAKAI, S. and HAGINAWA, J. (1972) *Tetrahedron Letters* 1081.
[11] SHELLARD, E. J. and PHILLIPSON, J. D. (1964) *Planta Med.* **12**, 160.
[12] SHELLARD, E. J. and HOUGHTON, P. J. (1972) personal communication.

from month to month.[13,14] This indicates that these alkaloids are taking part in metabolic processes, although at present their function in the plant is not understood.

Since isopteropodine, pteropodine, speciophylline and uncarine F are easily interconverted and the proportions present in equilibrated mixtures depend on whether they have been treated with acid or base, the significance of the proportions of the individual alkaloids present in the crude alkaloid extracts previously isolated from *U. bernaysii* leaves has been questioned.[3] Doubts have also been expressed about the natural occurrence of some alkaloid *N*-oxides since they could conceivably be formed during the extraction and isolation procedures.[7] Therefore pure tertiary alkaloids were dissolved in ethyl acetate saturated with 10% ammonia solution and allowed to stand for several days, then extracted with 2% H_2SO_4 followed by basification with ammonia and extraction with chloroform. TLC examination indicated that no isomerization had occurred and there was no evidence for *N*-oxide formation. It is therefore believed that the proportions of tertiary alkaloids quoted represent the proportions within the plant and that the *N*-oxides isolated are natural products and not artifacts. In further control experiments, tertiary alkaloids were eluted from alumina columns using benzene, chloroform and methanol. When examined by TLC the eluted alkaloids were almost unchanged; only traces of isomers had been formed in some cases. There was no evidence of *N*-oxide formation by the oxindole alkaloids during extraction or chromatography on alumina.

Naturally occurring alkaloid *N*-oxides are known for several types of indole, pyrrolizidine, quinolizidine and piperidine bases. Their role in plant metabolism is not known although several theories have been advanced for some specific alkaloids.[7] *N*-oxides of akuammigine and tetrahydroalstonine have been reported to be present in an *Uncaria* species[6] and *N*-oxides of the oxindole alkaloids rhynchophylline and isorhynchophylline have been found in a species of *Mitragyna*.[5] The discovery of the *N*-oxides of the four isomeric oxindole alkaloids isopteropodine, pteropodine, speciophylline and uncarine F may be an indication that the oxindole alkaloids are oxidized further to non-basic products (e.g. hydroxylamines, oximes). Other possibilities are that the *N*-oxides may play some part in the interconversion of the isomers or that the reversible oxidation of the tertiary base to the *N*-oxides is essential to some metabolic process in the plant.

EXPERIMENTAL

Plant material obtained from Markham Bridge, Lae, New Guinea, in 1968 was collected and identified by C. E. Ridsdale of the Rijksherbarium, Leiden.

IR spectra were in Nujol; 100 MHz NMR spectra in $CDCl_3$ with TMS internal reference; MS were determined on AEI MS 902 high resolution mass spectrometer at 70 eV, inlet temp., 225° for oxindole alkaloids and their *N*-oxides, 220° for heteroyohimbines.

For TLC data see Table 2.

Alkaloids were detected by means of Dragendorff's reagent and with 0 2 M $FeCl_3$ in 35% $HClO_4$, followed by heating the plates at 90° for 1 hr; closed E-ring oxindole alkaloids gave pink spots and indole alkaloids grey spots. GLC, 0·5 m, 5% SE 52 on Varoport 30, 230°, R_t: tetrahydroalstonine 11·5 min, akuammigine 9·0 min, oxindole alkaloids 9·7 min.

Extraction and separation of alkaloids. Dried plant material (leaves 35 g, stems 102 g, hooks 5·3 g) was moistened with 10% NH_4OH and double macerated with EtOAc. Concentrated EtOAc extracts were shaken with 2% H_2SO_4, made alkaline with NH_4OH and extracted into $CHCl_3$ which was washed, dried and concentrated to give total crude alkaloid; yield, leaves 940 mg (2·7%), stems 697 mg (0·7%), hooks 65 mg (1·2%). The total crude alkaloids were separated into three major fractions using alumina (type H, Laporte) columns and eluting successively with C_6H_6, C_6H_6–$CHCl_3$ (3:1), MeOH. Fractions containing pteropodine

[13] SHELLARD, E. J. and HOUGHTON, P. J. (1971) *Planta Med.* **20**, 82.
[14] SHELLARD, E. J. and HOUGHTON, P. J. (1972) *Planta Med.* **21**, 263.

and isopteropodine were combined and partitioned between C_6H_6 (isopteropodine, major) and 0·2 N MeCOOH (pteropodine, major).[15] Other alkaloids were separated by prep. TLC, speciophylline and uncarine F using system B, indole alkaloids by system C and *N*-oxides systems D and E. The yields of minor alkaloids are given in Table 3.

TABLE 2. THE hR_f OF *Uncaria bernaysii* ALKALOIDS

Alkaloid	A	B	C	D	E	Alkaloid	A	B	C	D	E
Isopteropodine	73	52	26	—	—	Akuammigine	55	30	48	—	—
Pteropodine	71	47	26	—	—	Isopteropodine *N*-oxide	0	0	0	28	37
Uncarine F	67	36	25	—	—	Pteropodine *N*-oxide	0	0	0	8	8
Speciophylline	36	9	11	—	—	Uncarine F *N*-oxide	0	0	0	26	30
Tetrahydroalstonine	78	73	72	—	—	Speciophylline *N*-oxide	0	0	0	5	3

Solvent key: TLC silica gel G/GF_{254} (Merck) (2:1) with (A) $CHCl_3$–Me_2CO (5:4); Et_2O–EtOAc (1:1); (C) Et_2O–Et_2NH (19:1), (D) $CHCl_3$–MeOH (6:1); (E) EtOAc–*iso* PrOH–conc. NH_4OH (12:7:1).

Identification of isopteropodine, pteropodine, uncarine F, speciophylline, tetrahydroalstonine and akuammigine. UV (EtOH) spectra, IR spectra (oxindole alkaloids), MS, R_t, R_f in TLC systems A, B and C and colour with $FeCl_3$–$HClO_4$, identical with reference alkaloids.

Characterization of natural N-*oxides.* The naturally occurring *N*-oxides of isopteropodine, pteropodine, uncarine F and speciophylline had R_f and colour with $FeCl_3$–$HClO_4$ in TLC systems D and E, identical with the *N*-oxides prepared from the corresponding bases (see below). The R_t and UV spectra of the prepared *N*-oxides, the natural *N*-oxides and the corresponding tertiary bases were all the same. The MS of the natural *N*-oxides of isopteropodine, pteropodine and speciophylline were consistent with those of the prepared compounds. Reduction of traces of the 4 natural *N*-oxides with 5% H_2SO_3[5] yielded single spots on TLC having identical colour with $FeCl_3$–$HClO_4$ and R_f in TLC system A with the corresponding tertiary alkaloid.

TABLE 3. YIELDS OF *Uncaria bernaysii* MINOR ALKALOIDS

Alkaloid	Leaves (mg)	Leaves (%)	Stems (mg)	Stems (%)
Tetrahydroalstonine	3·0	0·009	1·1	0·001
Akuammigine	18·0	0·05	2·0	0·002
Isopteropodine *N*-oxide	1·0	0·003	1·2	0·001
Pteropodine *N*-oxide	0·9	0·003	1·6	0·002
Uncarine F *N*-oxide	0·5	0·001	4·5*	0·004
Speciophylline *N*-oxide	4·9	0·01	2·3	0·002

* Contained some isopteropodine *N*-oxide.

Preparation of oxindole alkaloid N-*oxides.* Equimolar amounts of alkaloid and *m*-chloroperbenzoic acid stirred in $CHCl_3$ at 0° for 3 hr.[16] Resulting *N*-oxide separated by prep. TLC using system E, eluted with MeOH and the concentrated MeOH residue extracted with $CHCl_3$ and evaporated to dryness.

Isopteropodine N-*oxide.* Isopteropodine (20·9 mg) yielded isopteropodine *N*-oxide (14·7 mg, 67%) UV (EtOH) and R_t identical with isopteropodine, R_f as above; NMR ($CDCl_3$) 100 MHz, δ 1·55 (3H, *d*, *J* 6·5 Hz, C-19 Me), *c.* 5·1 (1H, *m*, *J* 6·5, 11 Hz, C-19 H), 3·62 (3H, *s*, MeO), 6·78–7·22 (3H, *m*, C-10 H, C-11 H, C-12 H), 7·56 (1H, *s*, C-17 H), 8·03 (1H, *dd*, *J* 2, 7 Hz, C-9 H). MS *m/e* 384 (M^+, 100%), 368 (M^+-16, 41%), 367 (M^+-17, 37%), 239 (32%, accurate mass found 239·1174, $C_{12}H_{17}NO_4$ requires 239·1157), 223 (73%), 208 (26%), 202 (69%), 180 (39%), 159 (58%), 146 (34%), 145 (35%), 144 (61%), 130 (95%), 69 (>100%).

[15] SHELLARD, E. J., PHILLIPSON, J. D. and GUPTA, D. (1968) *Planta Med.* **16**, 20.
[16] CYMERMAN CRAIG, J and PURUSHOTHAMAN, K. K. (1970) *J. Org. Chem.* **35**, 1721.

Pteropodine N-*oxide*. Pteropodine (26·2 mg) yielded pteropodine *N*-oxide (15·3 mg, 57%). UV (EtOH) and R_t identical with pteropodine, R_f as above; NMR ($CDCl_3$) 100 MHz, δ 1·51 (3H, *d*, *J* 6·5 Hz, C-19 Me) *c.* 5·1 (1H, *m*, *J* 6·5, 11 Hz, C-19 H), 3·59 (3H, *s*, OMe), 6·80–7·26 (4H, *m*, C-9H, C-10 H, C-11 H, C-12 H), 7·56 (1H, *s*, C-17 H); MS *m/e* 384 (M^+, 50%), 368 (M^+-16, 80%), 239 (53%), 223 (100%), 208 (36%), 180 (36%), 159 (45%), 146 (23%), 145 (23%), 144 (40%), 130 (70%), 69 (>100%).

Uncarine F N-*oxide*. Uncarine F (31·3 mg) yielded uncarine F *N*-oxide (30·6 mg, 98%). UV (EtOH) and R_t identical with uncarine F, R_f as above; NMR ($CDCl_3$) 100 MHz, δ 1·27 (3H, *d*, *J* 6·5 Hz, C19–Me), *c.* 4·24 (1H, *m*, *J* 1·5, 6·5 Hz, C-19), 4·28 (1H, *m*, C-3 H), 3·50 (1H, *s*, OMe), 6·78–7·23 (3H, *m*, C-10 H, C-11 H, C-12 H), 7·52 (1H, *s*, C-17 H), 8·13 (1H, *dd*, *J* 2, 7 Hz, C-9 H); MS, *m/e* 384 (M^+, 45%), 368 (M^+-16, 47%), 367 (M^+-17, 100%), 223 (50%), 213 (80%), 208 (28%), 180 (13%), 159 (31%), 146 (20%), 145 (19%), 144 (32%), 130 (50%), 69 (45%).

Speciophylline N-*oxide*. Speciophylline (31·7 mg) yielded speciophylline *N*-oxide (21·3 mg, 67%). UV (EtOH) and R_t identical with speciophylline, R_f as above; NMR($CDCl_3$) 100 MHz, δ 1·24 (3H, *d*, *J* 6·5 Hz, C-19 Me), *c.* 4·28 (1H, *m*, *J* 1·5, 6·5 Hz, C-19 H), *c.* 3·85 (1H, *m*, C-3 H), 3·22 (1H, *s*, OMe), 6·84–7·24 (4H, *m*, C-9 H, C-10 H, C-11 H, C-12 H), 7·43 (1H, *s*, C-17 H); MS *m/e* 384 (M^+, 3·5%), 368 (M^+-16, 95%), 223 (100%), 213 (16%), 208 (32%), 159 (16%), 146 (12%), 145 (13%), 144 (16%), 130 (28%), 69 (62%).

Reduction of N-*oxides*. Reduction of <1 mg with 5% H_2SO_3 as previously described.[5] TLC showed that each *N*-oxide was completely reduced yielding the corresponding tertiary alkaloid and no isomerisation occurred except for traces of isopteropodine and pteropodine from speciophylline *N*-oxide.

Quantitative TLC of total alkaloids from leaves, stems and hooks. The alkaloids were extracted as above. For the tertiary alkaloids 4 mg of extract, accurately weighed, was dissolved in $CHCl_3$ (1 ml); for the *N*-oxides 10 mg/ml. 30 μl of the solutions were applied to the plates, together with 10, 30 and 50 μl of solutions of reference alkaloids (1 mg/ml.). The method used has been described previously.[17] TLC systems B and E were used and the plates were sprayed with $FeCl_3$–$HClO_4$. The colour intensities were determined using a Chromoscan densitometer (Joyce Loebl) with TLC attachment (filter 490, aperture 0503).

Acknowledgements—The authors thank Chelsea College, University of London, for a Research Studentship awarded to S. R. Hemingway. We are grateful to Mr. C. E. Ridsdale of the Rijksherbarium, Leiden, who supplied the plant material, and to Professor E. J. Shellard and Mr. P. J. Houghton for gifts of isopteropodine, pteropodine, speciophylline and uncarine F. The mass spectra was determined at the School of Pharmacy, University of London, by Mr. D. Carter and we thank him and Dr. B. J. Millard for their co-operation and helpful discussion. Our thanks are also due to Mr. R. Brown for the production of the diagrams and P. C. M. U. Harwell, for the NMR spectra.

[17] SHELLARD, E. J. and ALAM, Z. (1968) *J. Chromatog.* **33**, 347.