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N-OXIDES OF THE OXINDOLE ALKALOIDS, ISORHYNCHOPHYLLINE, RHYNCHOPHYLLINE, RHYNCHOCILINE AND CILIAPHYLLINE

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Key Word Index—*Mitragyna tubulosa*, Rubiaceae, *N*-oxides, oxindole alkaloids, isorhynchophylline, rhynchophylline, rhynchociline, ciliaphylline

Abstract—The *N*-oxides of isorhynchophylline, rhynchophylline, rhynchociline and ciliaphylline were prepared, for comparison with a new alkaloid from *Mitragyna tubulosa*

INTRODUCTION

OXINDOLE alkaloids are known to occur naturally in species of *Mitragyna*[1] and *Uncaria*[2,3] as tertiary bases and as *N*-oxides During the investigation of alkaloids from the leaves of *Mitragyna tubulosa* Havil ,[1] a polar oxindole alkaloid was identified as ciliaphylline *N*-oxide[4] In order to establish the identity of this new natural *N*-oxide, it was necessary to prepare the *N*-oxides of rhynchociline (I, R = OMe, A configuration at C-7) and ciliaphylline (I, R = OMe, B configuration at C-7) Rhynchociline appears to form two *N*-oxides while ciliaphylline forms only one Since these alkaloids are not readily available, in order to investigate *N*-oxide formation, it was necessary to use the corresponding aromatic unsubstituted alkaloids isorhynchophylline (I, R = H, A configuration at C-7) and rhynchophylline (I, R = H, B configuration at C-7) This publication deals with the characterisation of two *N*-oxides of isorhynchophylline, two *N*-oxides of rhynchociline, one *N*-oxide of rhynchophylline and one *N*-oxide of ciliaphylline

RESULTS AND DISCUSSION

Isorhynchophylline and rhynchophylline treated with *m*-chloroperbenzoic acid yield the corresponding *N*-oxide. TLC shows that only one major spot is present in each case and they correspond in R_fs with the previously described *N*-oxides.[1] Treatment of isorhynchophylline with H_2O_2, however, results in a mixture containing three polar alkaloid spots on TLC These three compounds were separated by preparative TLC and each was shown to have a UV spectrum identical with that of isorhynchophylline—rhynchophylline Their MS are very similar and each one has the molecular-ion peak at m/e 400, indicating the presence of one additional oxygen Upon reduction with H_2SO_3 two of these compounds yield

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[1] SHELLARD, E J , PHILLIPSON, J D and SARPONG, K (1971) *Phytochemistry* **10**, 2505

[2] PHILLIPSON, J. D and HEMINGWAY, S R (1973) *Phytochemistry* **12**, in press

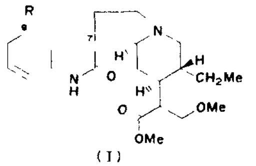
[3] PHILLIPSON, J. D. and HEMINGWAY, S R (1973) *Phytochemistry* **12**, in press

[4] SHELLARD, E J and RUNGSIYAKUL, D (1973) *Planta Med* in press.

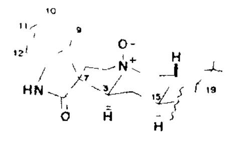
isorhynchophylline and they are therefore considered to be stereoisomeric *N*-oxides of isorhynchophylline Two configurations of the *N*-4 oxygen are possible, since it is known that the related heteroyohimbine alkaloid akuammigine forms two stereoisomeric *N*-oxides differing only by the configuration of the oxygen at *N*-4 [5]

The NMR spectrum, MS and R_fs of one of these isorhynchophylline *N*-oxides are identical with those of the isorhynchophylline *N*-oxide previously described,[1] in particular, the signal for the C-9 proton appears downfield at δ 8 14, indicating its close proximity to the *N*-4 oxygen This signal is absent from the NMR spectrum of the second *N*-oxide of isorhynchophylline and it is concluded that the *N*-4 oxygen is most likely to be on the opposite side of the molecule to the C-9 proton The poorly resolved three-proton triplet for the C-19 methyl group in the spectrum of isorhynchophylline *N*-oxide appears at δ 0 78 and in the spectrum of the second *N*-oxide at δ 0 84, showing that the relative positions of the C-19 methyl group and the N-4 oxygen are different in the two *N*-oxides

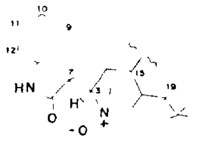
The percentage relative abundance of the molecular ion of pentacyclic oxindole alkaloid *N*-oxides is higher for those in which the oxygen at N-4 is *anti* to the oxindole carbonyl than for the isomers in which the N-4 oxygen is *syn* to the oxindole carbonyl [3] The percentage relative abundance of the molecular ion of isorhynchophylline *N*-oxide is 22 %, contrasting with only 3 % for the second *N*-oxide of isorhynchophylline The two *N*-oxides of isorhynchophylline can therefore be represented as structures II and III The isorhynchophylline *N*-oxide previously described[1] is now designated as *anti*-isorhynchophylline *N*-oxide (partial II) and the second *N*-oxide as *syn*-isorhynchophylline *N*-oxide (partial III) * *anti*-Isorhynchophylline *N*-oxide possesses the 4-*R* absolute configuration and *syn*-isorhynchophylline *N*-oxide the 4-*S* absolute configuration



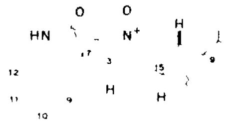
(I)



(II) *anti* - Isorhynchophylline *N* - oxide



(III) *syn* Isorhynchophylline *N* - oxide



(IV) Rhynchophylline *N* oxide

The third *N*-oxide produced in the reaction of isorhynchophylline with H_2O_2 yields rhynchophylline on reduction with H_2SO_3 and is therefore considered to be a *N*-oxide of rhynchophylline The NMR spectrum, MS and R_fs of this third *N*-oxide are in agreement with those published for rhynchophylline *N*-oxide [1] The absence of a downfield aromatic proton signal in the NMR spectrum and the low percentage relative abundance of the molecular ion (11 %) indicate that the C-9 proton is on the opposite side of the molecule to the N-4 oxygen, i e the N-4 oxygen and oxindole carbonyl are *syn* The signal for the C-19 methyl group appears at δ 0 78, i e. it has the same chemical shift as does that of *anti*-isorhynchophylline *N*-oxide, showing that the relative positions of the C-19 methyl group

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* The nomenclature is consistent with that used for the corresponding tertiary alkaloids, *anti* oxindole alkaloids have been defined as those alkaloids in which the pair of electrons on N-4 and the oxindole carbonyl are on opposite sides of the molecule, when they occur on the same side of the molecule the oxindole alkaloids are defined as being *syn* [6]

[5] MERLINI, L , NASINI, G and PHILLIPSON, J D (1972) *Tetrahedron* **28,** 5971

[6] SHAMMA, M , SHINE, R J , KOMPIS, I , STICZAY, T , MORSINGH, F , POISSON, J and POUSSET, J-L (1967) *J Am Chem Soc* **89,** 1739

and the N-4 oxygen are very similar in both rhynchophylline *N*-oxide and *anti*-isorhynchophylline *N*-oxide Hence, rhynchophylline *N*-oxide can be represented by structure IV; it possesses the 4-*R* absolute configuration

When rhynchophylline is treated with H_2O_2, three polar alkaloid spots are observed on TLC Separation by preparative TLC affords three compounds identified as *anti*-isorhynchophylline *N*-oxide, *syn*-isorhynchophylline *N*-oxide and rhynchophylline *N*-oxide (comparison of UV, MS, R_fs and R_fs of the reduction products) These results show that whether isorhynchophylline or rhynchophylline is used, some isomerisation takes place during treatment with H_2O_2 The percentage yields of the *N*-oxides formed with H_2O_2 are shown in Table 1

TABLE 1 YIELDS OF ISORHYNCHOPHYLLINE AND RHYNCHOPHYLLINE *N*-OXIDES WITH H_2O_2

N-oxide formed	% yield of *N*-oxide From isorhynchophylline	 From rhynchophylline
anti-Isorhynchophylline *N*-oxide	39	18
syn-Isorhynchophylline *N*-oxide	8 6	11
Rhynchophylline *N*-oxide	10	24

When *m*-chloroperbenzoic acid is used, isorhynchophylline forms *anti*-isorhynchophylline *N*-oxide as the major product with only trace amounts of the other two *N*-oxides; rhynchophylline appears to form only rhynchophylline *N*-oxide The major product from isorhynchophylline and H_2O_2 is *anti*-isorhynchophylline *N*-oxide (39 %) and from rhynchophylline, rhynchophylline *N*-oxide (24 %) The percentage yields given in Table 1 suggest that *anti*-isorhynchophylline *N*-oxide is more stable than rhynchophylline *N*-oxide which, in turn, is more stable than *syn*-isorhynchophylline *N*-oxide A study of Dreiding models reveals that in the two isomers in which the oxindole carbonyl is *syn* to the N-4 oxygen, the distance separating these two groups is 2 7 Å in rhynchophylline *N*-oxide (IV) and 3 1 Å in *syn*-isorhynchophylline *N*-oxide (III) Since these distances are sufficiently large not to give rise to any strong non-bonded interactions, the differences in stability of the two compounds is more likely to be due to the close proximity (1·5 Å) of the C-9 proton and the C-14 axial proton in *syn*-isorhynchophylline *N*-oxide. The distance between the C-9 proton and the N-4 oxygen in *anti*-isorhynchophylline *N*-oxide is estimated to be 2 4 Å, and if rhynchophylline were to form an *anti* N-oxide, the estimated distance between the C-9 proton and N-4 oxygen would be 1 4 Å, the resulting interactions would probably be sufficiently strong for the compound not to be formed or only with great difficulty

A new polar alkaloid isolated from *M tubulosa* was identified as a 9-methoxyrhynchophylline-type *N*-oxide by means of its UV, NMR and MS characteristics[4] In order to identify this alkaloid, the N-oxides of rhynchociline (I, R = OMe, A configuration at C-7) and ciliaphylline (I, R = OMe, B configuration at C-7) have been prepared for comparison purposes As only small amounts of these alkaloids were available *m*-chloroperbenzoic acid was used for *N*-oxide formation, since the yields are higher than with H_2O_2 TLC indicates that rhynchociline forms two *N*-oxides (separated by preparative TLC) and ciliaphylline, only one The prepared *N*-oxides were characterized by means of their UV and mass spectra, R_fs, and reduction to the corresponding tertiary alkaloid As the natural alkaloid has R_fs corresponding to those of ciliaphylline *N*-oxide and not rhynchociline *N*-oxides and since it reduced to ciliaphylline, it is identified as ciliaphylline *N*-oxide[4] The C-9 position of ciliaphylline has a methoxyl substituent, so that it is not possible to use the presence or absence of a downfield aromatic proton signal to ascertain the configuration of the N-4 oxygen The C-19 methyl signal in the NMR spectrum of natural ciliaphylline *N*-oxide occurs at δ 0 80 in close agreement with the value of δ 0 78 for ciliaphylline[7] This suggests that the C-19 methyl is not greatly affected by the N-4 oxygen and that the C-19 methyl group conformation is very similar in both ciliaphylline and its *N*-oxide The low percentage relative abundance of the molecular ion at *m/e* 430 (10%) is identical with that of rhynchophylline *N*-oxide indicating that the N-4 oxygen and the oxindole carbonyl are *syn* The arguments for the production of only one *N*-oxide are the same as those used for rhynchophylline *N*-oxide, hence the natural ciliaphylline *N*-oxide is C-9 methoxy substituted rhynchophylline *N*-oxide (IV) having the same 4-*R* configuration

The formation with *m*-chloroperbenzoic acid of two *N*-oxides of rhynchociline but only one of isorhynchophylline demonstrates that although the C-9 MeO and N-4 lone pair are in close proximity, 9-methoxy-*anti*-isorhynchophylline *N*-oxide and 9-methoxy-*syn*-isorhynchophylline *N*-oxide are formed The two *N*-oxides of rhynchociline are formed in the ratio of 7 3 Insufficient material was available for determining their NMR spectra A study of Dreiding models shows that the isomer corresponding to 9-methoxy-*anti*-isorhynchophylline *N*-oxide will be more stable than 9-methoxy-*syn*-isorhynchophylline *N*-oxide, since in the latter compound strong interactions will take place between the C-9 methoxyl group and the C-14 axial proton and the C-5 axial proton, the estimated distance from the C-9 methoxyl oxygen to the C-14 axial proton is 1 5 Å and to the C-5 axial proton is 1 9 Å The relative abundance of the molecular ion at *m/e* 430 (9%) is higher for the 70% product than for the 30% product (2 5%) It is suggested, therefore, that the rhynchociline *N*-oxide formed in the 70% ratio is 9-methoxy-*anti*-isorhynchophylline *N*-oxide and that the 30% compound is 9-methoxy-*syn*-isorhynchophylline *N*-oxide

On TLC with silica gel and neutral solvent systems the R_f of the 70% product is lower than that of the 30% product, this behaviour is reversed if alkaline solvents are used and also if alumina plates are used Rhynchociline shows the same behaviour pattern on TLC when compared with ciliaphylline Since the two *N*-oxides of rhynchociline, in which conformational changes at N-4 are ruled out, exhibit this behaviour, the stronger binding of rhynchociline on silica gel can perhaps be attributed to the proximity of the C-9 methoxyl and the N-4 electron lone pair rather than to a conformational change at N-4 as suggested previously[8]

EXPERIMENTAL

The 100 MHz NMR spectra were determined in $CDCl_3$ using TMS as internal reference, the MS were determined in an AEI MS 902 high resolution mass spectrometer at 70 eV with inlet temp between 220° and 240° The TLC systems used were silica gel G (Merck) with (*A*) MeOH, (*B*) EtOAc–isoPrOH–5 5% NH_4OH (60 35 5), (*C*) EtOAC–isoPrOH–5 5% NH_4OH (45 35 20) and alumina G (Merck) with (*D*) $CHCl_3$–MeOH (1 1), (*E*) EtOAc–isoPrOH–5 5% NH_4OH (60 35 5) The hR_fs are given in Table 2

Preparation of alkaloid N-*oxides Method* 1 The alkaloid was dissolved in a few drops of EtOH, 15% H_2O_2 (1 ml per 15 mg alkaloid) added and the mixture heated on a boiling H_2O bath for 30 min and then with Pt wire for 5 min On cooling, the solution was made alkaline with NH_4OH, extracted with $CHCl_3$ and evaporated to dryness The *N*-oxides were separated by preparative TLC using system *A*, the bands were

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

[8] PHILLIPSON, J D and SHELLARD, E J (1968) *J Chromatog* **32**, 692

extracted with MeOH which was taken to dryness and the residue extracted into $CHCl_3$ Isorhynchophylline (150 mg) yielded *anti*-isorhynchophylline *N*-oxide 60 mg (39%), *syn*-isorhynchophylline *N*-oxide 13 mg (8 6%) and rhynchophylline *N*-oxide 15 mg (10%) Rhynchophylline (50 mg) yielded *anti*-isorhynchophylline *N*-oxide 9 2 mg (18%), *syn*-isorhynchophylline *N*-oxide 5 6 mg (11%) and rhynchophylline *N*-oxide 12 7 mg (24%) *Method* 2 Equimolar proportions of alkaloid and *m*-chloroperbenzoic acid were stirred in $CHCl_3$ at 0° for 3 hr [9] The *N*-oxides were separated by preparative TLC as described for method 1 Isorhynchophylline (10 mg) yielded *anti*-isorhynchophylline *N*-oxide 8 mg (77%) Rhynchophylline (10 mg) yielded rhynchophylline *N*-oxide 6 9 mg (66%) Rhynchociline (5 mg) yielded *anti*-rhynchociline *N*-oxide 3 6 mg (70%), and *syn*-rhynchociline *N*-oxide 1 6 mg (30%) Cilipahylline (5 mg) yielded ciliaphylline *N*-oxide 4 1 mg (79%)

TABLE 2 hR_fs OF E *seco* OXINDOLE ALKALOID *N*-OXIDES

Compound	*A*	*B*	*C*	*D*	*E*
anti-Isorhynchophylline *N*-oxide	62	36	72	—	—
syn-Isorhynchophylline *N*-oxide	48	11	54	16	52
Rhynchophylline *N*-oxide	19	4	49	13	44
anti-Rhynchociline *N*-oxide	34	15	62	90	73
syn-Rhynchociline *N*-oxide	46	10	53	52	16
Ciliaphylline *N*-oxide	18	4	49	13	44

TABLE 3 NMR DATA ON SOME E *seco* PENTACYCLIC OXINDOLE ALKALOID *N*-OXIDES

Protons	*anti*-Isorhynchophylline *N*-oxide	*syn*-Isorhynchophylline *N*-oxide	Rhynchophylline *N*-oxide	Ciliaphylline *N*-oxide
C-19 Me	0 78 (3H, *t*, *J*, 7 Hz)	0 84 (3H, *t*, *J*, 7 Hz)	0 78 (3H, *t*, *J*, 7 Hz)	0 80 (3H, *t*, *J* 7 Hz)
-OMe (ester)	3 62 (3H, *s*)	3 64 (3H, *s*)	3 61 (3H, *s*)	3 60 (3H, *s*)
-OMe (vinyl)	3 70 (3H, *s*)	3 78 (3H, *s*)	3 75 (3H, *s*)	3 78 (3H, *s*)
-OMe (aromatic)	—	—	—	3 92 (3H, *s*)
C-9 H	8 14 (1H, *dd*, *J* 2, 7 Hz)	6 84–7 32 (4H, *m*)	6 85–7 50 (4H, *m*)	—
C-10 H	7 18 (1H, *t*, *J* 2, 7 Hz)			6 56 (1H, *dd*, *J* 1 5, 8 Hz)
C-11 H	7 02 (1H, *t*, *J* 2, 7 Hz)			7 16 (1H, *t*, *J* 1 5, 8 Hz)
C-12 H	6 92 (1H, *dd*, *J* 2, 7 Hz)			6 73 (1H, *dd*, *J* 1 5, 8 Hz)
C-17 H	7 24 (1H, *s*)	7 25 (1H, *s*)	7 29 (1H, *s*)	7 32 (1H, *s*)

Characterization of prepared N-*oxides* The UV spectra (EtOH) of the *N*-oxides were identical with those of the corresponding tertiary bases, isorhynchophylline and rhynchophylline, λ_{max} 245, 286 nm, λ_{min} 226 nm, rhynchociline and ciliaphylline, λ_{max} 221, 243, 297 nm, λ_{min} 234, 276 nm The hR_fs are given in Table 2 and the NMR data in Table 3 Reduction of 1 mg or less of each *N*-oxide with 5% H_2SO_3, as previously described,[1] yielded one spot on TLC having R_fs identical with those of the corresponding tertiary base The

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[9] CYMERMAN CRAIG, J and PURUSHOTHAMAN, K K (1971) *J Org Chem* **35**, 1721

MS data are as follows *anti*-Isorhynchophylline *N*-oxide, *m/e* 400 (M^+ 22%), 384 (M^+-16, 60%), 382 (33%), 239 (88%), 224 (48%), 210 (31%), 208 (35%), 159 (50%), 146 (28%), 145 (27%), 144 (52%), 130 (100%), 69 (100%) *syn*-Isorhynchophylline *N*-oxide, *m/e* 400 (M^+, 2 5%), 384 (M^+-16, 100%), 382 (31%), 239 (79%), 224 (43%), 210 (17%), 208 (22%), 159 (14%), 146 (12%), 145 (12%), 144 (22%), 130 (57%), 69 (93%) Rhynchophylline *N*-oxide, *m/e* 400 (M^+, 10%), 384 (M^+-16, 68%), 382 (21%), 239 (71%), 224 (53%), 210 (33%), 208 (35%), 159 (36%), 146 (33%), 145 (25%), 144 (54%), 130 (100%), 69 (100%) *anti*-Rhynchociline *N*-oxide, *m/e* 430 (M^+, 9%), 414 (M^+-16, 79%), 412 (50%), 239 (100%), 224 (48%), 210 (25%), 208 (35%), 189 (24%), 176 (26%), 175 (20%), 174 (32%), 160 (24%), 69 (100%) *syn*-Rhynchociline *N*-oxide, *m/e* 430 (M^+, 2 5%), 414 (M^+-16, 100%), 412 (20%), 239 (83%), 224 (23%), 210 (20%), 208 (24%), 189 (14%), 176 (14%), 175 (12%), 174 (20%), 160 (11%), 69 (80%), Ciliaphylline *N*-oxide, *m/e* 430 (M^+, 10%), 414 (M^+-16, 100%), 412 (45%), 239 (85%), 224 (33%), 210 (20%), 208 (26%), 189 (7%), 176 (10%), 175 (10%), 174 (16%), 160 (10%), 69 (55%)

Characterization of natural ciliaphylline N-*oxide* The UV spectrum (EtOH) was identical with that of ciliaphylline and rhynchociline The R_fs were identical with Table 2 and the MS consistent with prepared ciliaphylline *N*-oxide The NMR data are given in Table 3 Reduction of 1 mg with 5% H_2SO_3 yielded one spot on TLC having R_fs identical with those of ciliaphylline

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