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CONFIGURATIONAL ANALYSIS OF RHYNCHOPHYLLINE-TYPE OXINDOLE ALKALOIDS

THE ABSOLUTE CONFIGURATION OF CILIAPHYLLINE, RHYNCHOCILINE, SPECIONOXEINE, ISOSPECIONOXEINE, ROTUNDIFOLINE AND ISOROTUNDIFOLINE

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Abstract—From a general configurational and conformational analysis of rhynchophyllinoid alkaloids, unique physical criteria are developed that differentiate between the 8 possible configurational types. The criteria are applied to 6 rhynchophylline-type alkaloids of unknown configurations and show that ciliaphylline, specionoxeine and isorotundifoline have the *normal* B configuration while rhynchociline, isospecionoxeine and rotundifoline have the *normal* A configuration. All 6 alkaloids have the C15Hα absolute stereochemistry.

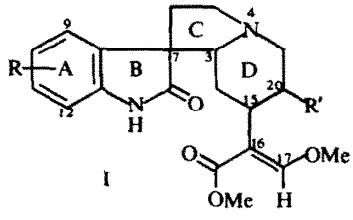
OXINDOLE alkaloids of the rhynchophylline-type[1] (I) have four asymmetric centres (C3, C7, C15 and C20) and hence eight diastereoisomers are possible—each of which can exist as an enantiomorphic pair. If we focus attention on the three asymmetric centres of ring D, C3, C15 and C20, four diastereomeric compounds are possible, the *normal*, *pseudo*, *allo* and *epiallo* configurations (Table 1). Since there are two possible orientations for the oxindole group about the C7 spiro carbon atom, defined by the

TABLE 1. CONFIGURATIONAL TERMINOLOGY FOR OXINDOLE AND INDOLE ALKALOIDS

Configuration	C3H	C15H	C20H	C7 Oxindole+
Normal	α	α	β	A or B
Pseudo	β	α	β	A or B
Allo	α	α	α	A or B
Epiallo	β	α	α	A or B

The same applies for the other enantiomorph (C15Hβ)

+ A = Oxindole CO above C/D plane.
B = below C/D plane.



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lactam CO group being below, A, or above, B, the plane of the C/D ring system,* each of the four configurational types can itself exist in one of two possible configurations, e.g. *normal* A or *normal* B (Table 1).

Configurational and conformational analysis

Conformation preference of each of the configurations. The meaningful allocation of configuration to alkaloids by means of spectral data (e.g. IR, NMR, CD) requires a knowledge of the preferred conformation of each configuration under the conditions employed for measurement, because these spectral parameters are very often conformation dependant.[3,4] Theoretically, four ring D chair conformations are possible for each of the eight configurations due to conformational flipping of one chair form to the other, which inverts each substituent from *axial* to *equatorial* or *vice versa* and inversion of the basic nitrogen N_4 which effectively changes the C/D ring junction from *trans* to *cis* or *vice versa*. However, for each of the eight configurations, two of these ring D chair conformations can be eliminated. One because it requires the sterically impossible vicinal *diaxial* bridging of the C/D ring junction. The other because it requires an *axially* orientated N_4–C5 bond (implying an additional destabilization of about 1·5 kcal/mole[3]) while at the same time *not* relieving any of the nonbonded interactions present in the conformation that has this bond (N_4–C5) *equatorially* orientated. Hence, only two ring D chair conformations need to be *seriously* considered for each of the eight configurations.

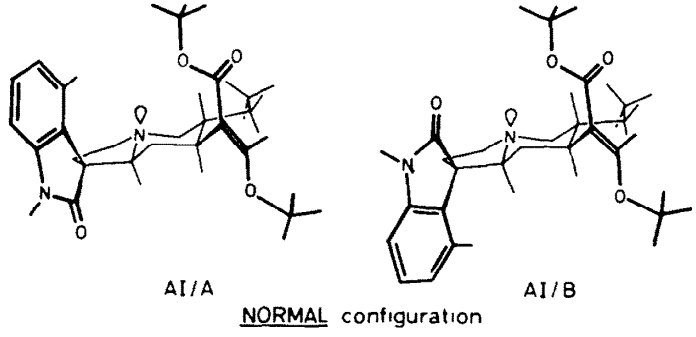

FIG 1.

Normal configuration (C3Hα; C15Hα *and* C20Hβ; Fig. 1). An alkaloid of the rhynchophylline-type (I) possessing the *normal* A spiro configuration will exist predominantly in conformation AI/A while that possessing a *normal* B spiro configuration will exist predominantly in conformation AI/B. A significant contribution by the alternative ring D chair conformation (formed by inversion of N_4 followed by flipping of ring D) is ruled out in each case by the nonbonded interactions arising from an *axial* Et group at C20 and an *axial* orientation for the C3–C7 bond.†

Pseudo configuration (C3Hβ, C15Hα *and* C20Hβ; Fig. 2). In the *pseudo* A oxindole,

* In the original definition of A and B isomeric oxindole alkaloids[2a,b] the stronger base of each pair was designated as B; this is valid only for *normal* and *allo* compounds (see discussion pages 7 and 8). We suggest that the A and B *nomenclature* should refer to the position of the carbonyl group being above or below the plane of the C/D rings (see Ref. 2*c*).

† The alternative conformations have counterparts in the analogous indole alkaloids and a more detailed semi-quantitative analysis has been presented.[3]

the choice of the preferred conformation is between BI/A, and BII/A; in BI/A the C15 and C20 substituents are *equatorial* but the *axial* orientation of the C3–C7 bond forces the lactam CO group under the plane of ring D into close proximity to the *axial* C15 and C21 hydrogens: in BII/A, the C3–C7 bond is now *equatorially* orientated but the C15 and C20 substituents are *axial*. In BI/A the nonbonded interactions arising from the orientation of the lactam CO group can be assigned a destabilization energy of roughly 2–4 kcal/mole (see *epiallo* discussion following)

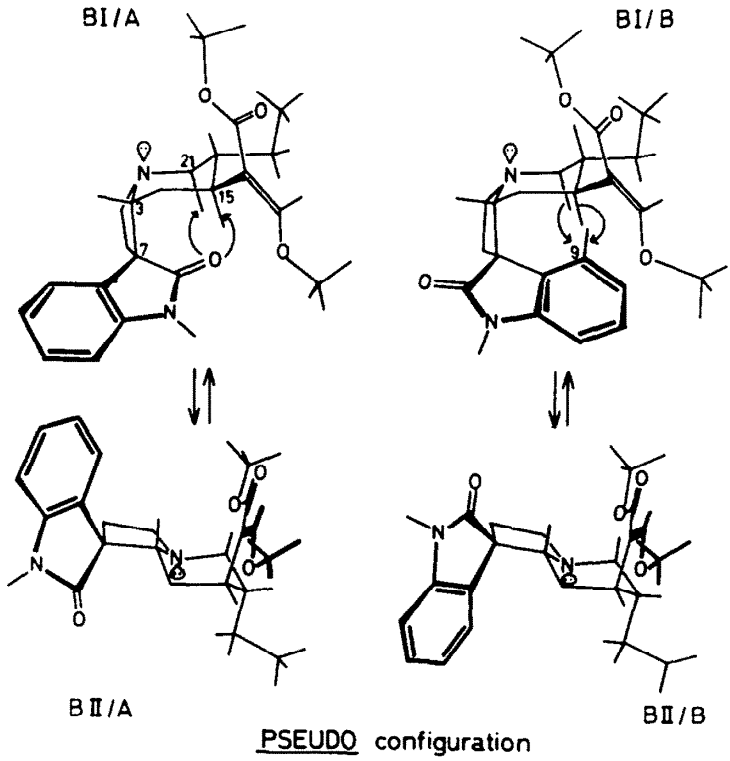

FIG. 2.

while in BII/A the *axial* Et group accounts for a destabilization of about 1·7 kcal/mole.[3] However, the magnitude of the nonbonded interactions arising from an *axial* ester-vinyl-ether grouping orientated perpendicular to a plane drawn through N_4 and C15 (the most stable orientation on the evidence of Dreiding models) is not precisely known, but is probably less than 4 kcal/mole (see *epiallo* discussion following). Hence, an unequivocal decision, between BI/A and BII/A cannot be made, although it is likely that the nonbonded interactions due to the *axial* orientation of the ester-vinyl-ether group in BII/A are greater than 1·3 kcal/mole and thus BI/A is probably the preferred conformation.

A *pseudo* B oxindole will exist predominantly in conformation BII/B since, in the alternative BI/B, the aromatic ring is forced under the plane of ring D yielding strong nonbonded interactions between the C9 aromatic hydrogen and the *axial* C15 and C21 hydrogen atoms. The distance between the C9 hydrogen in BI/B and the

C15 and C21 *axial* hydrogens is only about 1·3 Å (Dreiding model), which implies such a prohibitive destabilization[5] that this conformation is most unlikely.

Allo configuration (C3Hα, C15Hα *and* C20Hα; Fig. 3). The *allo* A and B oxindoles exist primarily in conformation CI/A and CI/B respectively. The alternative conformation (formed by inversion of N_4 followed by ring D flipping) involves in each case much larger nonbonded interactions.

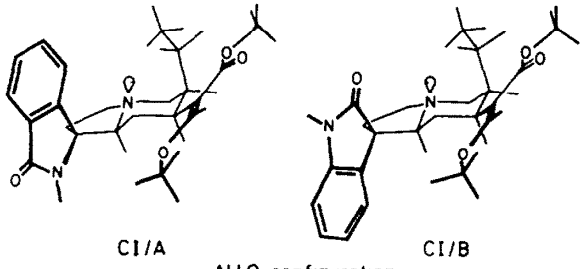

FIG. 3.

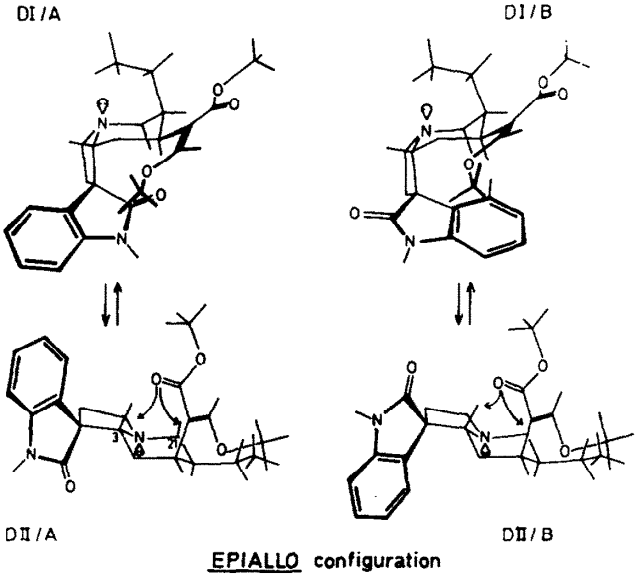

FIG. 4.

Epiallo configuration (C3Hβ, C15Hα *and* C20Hα; Fig. 4). The choice of the preferred conformation for the *epiallo* A oxindole rests between DI/A (*axial* C3–C7 bond forcing the lactam CO under the plane of ring D and an *axial* Et group) and DII/A (*axial* ester-vinyl-ether group). In DI/A, the distance of the lactam CO group from the C15 and C21 *axial* hydrogens is about 1·7 Å whereas in DII/A the distance of the ester CO group from the *axial* C3 and C21 hydrogens is about 1·6 Å in the most stable rotational conformation of the C15 ester-vinyl-ether group (Dreiding models). It therefore seems reasonable to assume that the nonbonded interactions arising from

the CO group orientations in DI/A and DII/A are probably of the same order of magnitude but with that of DI/A being slightly less. It is known that, in the corresponding *epiallo* indole alkaloids, such an orientation as in DII/A for the ester vinyl-ether group accounts for roughly 4 kcal/mole of destabilization energy;* hence the nonbonded interactions induced by the lactam CO in DI/A are probably in the region of 2–4 kcal/mole. Therefore, since DI/A also has an *axial* Et group, DII/A is probably the preferred conformation.

The preferred conformation for the *epiallo* B oxindole will be conformation DII/B, because of the strong nonbonded interactions between the C9 aromatic hydrogen and the *axial* C15 and C21 hydrogens (see *pseudo* B discussion) in conformation DI/B.

Relative stabilities of the normal, pseudo, allo and epiallo configurations. In general, oxindole alkaloids may be isomerized[1] about the C3 and/or C7 centres by treatment with either pyridine or acetic acid or simply by heating. The isomerization involves scission of the C3–C7 bond[6–8] and hence possible inversion of one or both of the centres. Starting with a given isomer, four isomeric compounds should result upon isomerization namely two *normal* (A and B) and two *pseudo* (A and B), *or* the two *allo* (A and B) and two *epiallo* (A and B) depending on the initial configuration. The relative ratios of the isomers so obtained will reflect their relative stabilities in the equilibrating system.

Normal A and B oxindoles, in their preferred conformation AI/A and AI/B respectively will be more stable than their *pseudo* counterparts. In the *pseudo* A oxindole BI/A, the destabilization induced by an *axial* C3–C7 bond relative to an *equatorial* bond in AI/A or AI/B should be of the order of 2–4 kcal/mole. Similarly, the *axial* C15 and C20 substituents of a *pseudo* B oxindole, BII/B should significantly destabilize this configuration relative to the *normal* A and B configurations (AI/A and AI/B) where both these substituents are *equatorial*. Hence the thermal isomerization in pyridine of any of the four configurations discussed above should result, at equilibrium, in a mixture in which the two *normal* configurations predominate almost to the exclusion of the two *pseudo* configurations, assuming that the solvent does not specifically stabilize the *pseudo* configurations.

This conclusion is in fact supported by experiment. Both rhynchophylline (I, R = H, R′ = Et) and isorhynchophylline (I, R = H, R′ = Et), compounds known to possess the *normal* B and A configurations respectively,[2,7,8] on treatment with pyridine yielded a mixture in which rhynchophylline (20%) and isorhynchophylline (80%) are the only isomers detectable.[7,9,10] Even in acetic acid, a solvent which would be expected to stabilize *pseudo* BII/A (by the possibility of formation of an intramolecular hydrogen bond in the cation, relative to *normal* AI/A) isomerization of these two compounds again yields a mixture in which only rhynchophylline (80%) and isorhynchophylline (20%) are found.[7,8–10]

Similarly, in the *allo/epiallo* set, the *allo* configuration CI/A and CI/B can be shown to be more stable than the corresponding *epiallo*, DII/A and DII/B, configurations. Isomerization of any of these four isomers should thus result in a preponderance of the *allo* configurations. Corynoxine† (I, R = H, R′ = Et) is a rhynchophyllinoid

* Calculated from the position of the conformational equilibrium given by the NMR spectrum of the corresponding indole alkaloid.[4]

† Corynoxine was obtained by $Pb(OAc)_4$ treatment of corynantheidine; experimental details will be published separately.

oxindole alkaloid of known *allo* A configuration. Acetic acid treatment of corynoxine yielded a mixture which, at equilibrium, contained only corynoxine (20%) and an isomer of corynoxine (80%) now named *corynoxine* B. Since corynoxine has the *allo* A configuration, the only configurational possibilities left for corynoxine B are *allo* B, *epiallo* B and *epiallo* A. Either *allo* B or *epiallo* A but not *epiallo* B would be expected to predominate after acetic acid isomerization because stabilization of the conjugate base by formation of an intramolecular hydrogen bond (between the protonated lone pair of N_4 and the lactam CO) is possible only in these two configurations. Pyridine isomerization of corynoxine yielded a mixture which, at equilibrium, contained only corynoxine (80%) and the same corynoxine B as discussed above (20%). Pyridine isomerization would be expected to stabilize *epiallo* B at the expense of *allo* B and particularly *epiallo* A (the only difference in stability between *epiallo* A and *epiallo* B must arise from the difference in the C7 spiro configuration) because of destabilization due to electrostatic repulsion[2c] between the lone pair of N_4 and the lactam CO which occurs in the free base form of the latter two configurations. Since either acetic acid or pyridine treatment produce the same isomer, corynoxine B must have the *allo* B configuration.

Hence, isomerization will differentiate the *normal* from *pseudo* and the *allo* from the *epiallo* configuration.

Differentiation between normal and allo configurations. The C18 Me triplet signal in the NMR spectrum of corynantheidine-type *indole* alkaloids of the *allo* configuration is more "symmetrical" than that of the corresponding *normal* configuration

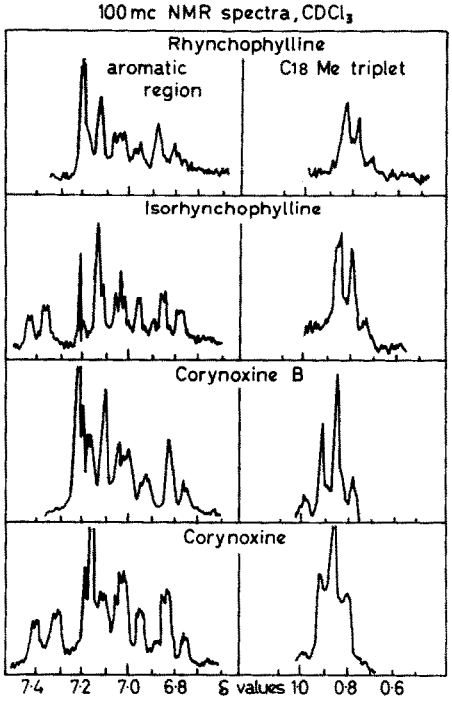

FIG. 5.

because of the closer proximity of the C19 methylene protons to the lone pair of N_4 in the former configuration,[3,4] this feature should also be applicable to the oxindole alkaloids.

The NMR spectra of corynoxine (I, R = H, R = Et, *allo* A) and corynoxine B (I, R = H, R' = Et, *allo* B), was compared with that of isorhynchophylline and rhynchophylline, which possess the *normal* A and B configurations respectively (Fig. 5). The two *allo* configurations (A and B) have a more "symmetrical" C18 Me triplet signal than the two *normal* configurations and indicate the applicability of the above criterion to the oxindole alkaloids.

Differentiation between pseudo and epiallo configurations. The *pseudo* and *epiallo* configurations will be differentiated one from the other readily by determining whether the products obtained upon pyridine isomerization have the *normal* or *allo* configurations. The *pseudo* will yield *normal* compounds while the *epiallo* will yield *allo* compounds.

Differentiation between A *and* B *spiro carbon configurations.* In *normal* and *allo* compounds A and B, configurations have been usually assigned[1] on the basis of p*Ka* and isomerization data and sometimes supported by TLC evidence.[1,2] The stronger base of each pair in *normal* and *allo* compounds has been taken[2a,b] to be B (lactam CO above the plane of the C/D ring) due to stabilization of the cation by means of an intramolecular hydrogen bond between protonated N_4 and the lactam CO because such stabilization is not possible in the A configuration (lactam CO below the plane of the C/D ring, Fig. 6). This bonding is also used to explain the predominance of the B isomer after equilibration using acetic acid. The predominance of the A isomer after pyridine equilibration is regarded as being due to electrostatic repulsion between the lone pair of electrons of N_4 and the lactam CO group in the free base form of the B isomer[2c] (Fig. 6). However, uncritical use of the above criteria

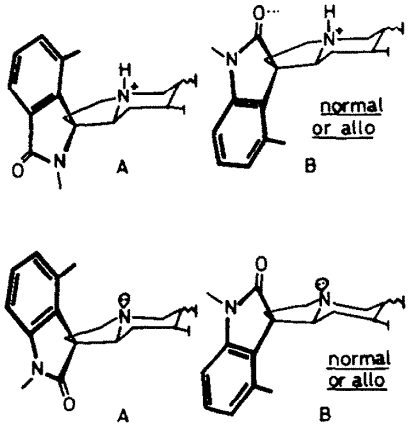

FIG. 6.

may give incorrect assignments. For instance, in the preferred conformations of the two *normal* (AI/A and AI/B) and two *allo* (CI/A and CI/B) configurations, the stronger base of each pair will have the B configuration but of the two *epiallo* (DII/A and DII/B)

and two *pseudo* (BII/A and BII/B) configurations, the stronger base of each pair will have the A configuration.*

An additional and more unequivocal method of assigning A and B configurations to rhynchophylline-type compounds is therefore desirable. To this end, use is now made of the differences in the aromatic region of the 100 mc spectra observed for the pair rhynchophylline and isorhynchophylline (Fig. 5). For isorhynchophylline, a one-proton doublet, which can only be due to the C9 or C12 aromatic hydrogen, occurs at 7·40 δ (Fig. 5) whereas the lowest field aromatic signal in rhynchophylline occurs at 7·20 δ. A change from A to B configuration should not alter significantly the magnetic environment of the C12 hydrogen but, in the *normal* A configuration (Fig. 1) unlike that of the *normal* B configuration, the C9 hydrogen is positioned over the plane of ring C in close proximity to the N_4 deshielding[4] lone pair of electrons; thus the C9 proton signal of the *normal* A oxindole should occur at lower field than that of the *normal* B oxindole. Similar considerations for the preferred conformations of the other configurational A and B pairs, excluding 9-substituted compounds, leads to the generalizations outlined in Table 2.

TABLE 2. RELATIVE CHEMICAL SHIFT POSITION OF C9 AROMATIC HYDROGEN IN OXINDOLE ALKALOIDS

Ring D configuration	A Spiro configuration		B Spiro configuration	
Normal	—	AI/A	N.e.	AI/B
Pseudo	N.e.	BI/A	—	BII/B
Allo	—	CI/A	N.e.	CI/B
Epiallo	N.e.	DII/A	—	DII/B

— indicates deshielding.
N.e. = no effect.

Configurations of ciliaphylline, rhynchociline, specionoxeine, isospecionoxeine, rotundifoline and isorotundifoline

Chemical constitution of ciliaphylline, rhynchociline, specionoxeine, and isospecionoxeine. Rhynchociline and ciliaphylline,[13] two isomeric oxindole alkaloids isolated from *Mitragyna ciliata*, were shown to be of the rhynchophylline-type (I) and contain an aromatic OMe group. In addition to the six oxindole alkaloids: rhynchophylline,[2,7,9] mitraphylline,[14] isomitraphylline,[14] speciophylline,[14] speciofoline,[15] and rotundifoline[16] previously isolated from *M. speciosa* we have now isolated two new isomeric oxindole alkaloids named *specionoxeine* and *isospecionoxeine.* As biogenetic considerations[17] lead to the expectation of specific structural patterns in the alkaloids isolated from the same plant (e.g. reversal of the substituents at C15 and C20 would not be expected) the similarity† of the analytical and spectral data

* In the cation form conformation BII/A is probably preferred over BI/A because of the possibility of stabilization by intramolecular hydrogen bonding. However, if BII/A is not the preferred conformation under these conditions, little difference in bascity would be expected between the two *pseudo* configurations BI/A and BII/B. A similar argument applies to the two *epiallo* configurations.

† The difference between the UV spectra of rhynchophylline isorhynchophylline and those of the other four alkaloids (Table 3) can be accounted for by the presence of the aromatic methoxy group in the latter compounds.

of specionoxeine, isospecionoxeine, rhynchociline, ciliaphylline, rhynchophylline and isorhynchophylline (Table 3) establish beyond reasonable doubt that specioxeine and isospecionoxeine are oxindole alkaloids of the rhynchophylline-type and contain an aromatic OMe group.

TABLE 3. SPECTRAL PROPERTIES OF SOME MITRAGYNA OXINDOLE ALKALOIDS

NMR δ from TMS 100 mc	Rhynchophylline	Isorhyncho-phylline	Cilia-phylline	Rhyncho-ciline	Specion-oxeine	Isospecio-noxeine
$C_{18}Me$	0·77 (unres)	0·79 (unres)	0·78 (unres)	0·80 (unres)	—	—
Ring OMe	—	—	3·83	3·86	3·83	3·86
Vinyl/OMe	3·67	3·65	3·67	3·68	3·67	3·68
Ester OMe	3·58	3·55	3·59	3·58	3·58	3·57
$C_{20}CH:CH_2$	—	—	—	—	4·90 (2H) 5·52 (1H)	4·90 (2H) 5·52 (1H)
Aromatic (10, 11, 12)	6·78–7·20	6·78–7·20	6·51–6·53	6·47–6·56	6·52–6·55	6·49–6·55
Vinyl	7·21	7·14	7·23	7·17	7·18	7·13
NH ($CDCl_3$)	8·48	8·42	8·37	7·78	8·54	8·36
IRν (KCl)						
>C=C<	1646, 1623 cm^{-1}	1645, 1625	1640, 1620	1685, 1605	1640, 1619	1600 1634, 1614,
Vinyl	—	—	—	—	995, 918	980, 912
Ester/oxindole carbonyl	1732, 1708	1730, 1705	1728, 1705	1708	1730, 1713	1705
NH	3415	3420	3400, 3270	3400, 3280	3280	3260
UV(EtOH)	280 (3·15)	280 (3·15)	287 (3·46)	286 (3·48)	288 (3·29)	288 (3·52)
λ_{max}	245 (4·24)	245 (4·24)	244 (4·24)	242 (4·24)	245 (4·18)	244 (4·26)
(log ε)			222 (4·44)	225 (4·41)	223 (4·49)	223 (4·46)

The NMR spectra of specionoxeine and isospecionoxeine differ from those of rhynchociline and ciliaphylline in not having a 3-proton triplet, attributable to the Me protons of the C20 Et group, in the 0·8δ region. However, the two new alkaloids have multiplets, integrating for three protons in the olefinic region (4·5–5·8 ppm; Fig. 7) corresponding in chemical shift, multiplicity and integral with the vinyl hydrogen signals[18,10] of paynantheine and corynantheine.* Specionoxeine and isospecionoxeine also show vinyl bands[19] in their IR spectra at 995 and 918 cm^{-1}.

Hydrogenation of specionoxeine yielded ciliaphylline while hydrogenation of isospecionoxeine yielded rhynchociline as shown by examination of the hydrogenation products on two TLC systems and comparison of their R_f values with those of authentic samples of ciliaphylline and rhynchociline, spotted on the same plate (Fig. 8). This conclusion is supported by the similarities in the NMR (Fig. 7) CD (Fig. 11) curves and TLC behaviour (Fig. 8) within each of the two pairs of alkaloids

* Corynantheine is the C20 vinyl indole alkaloid analogue of rhynchophylline while paynantheine is 9-methoxycorynantheine.[18]

100 mc NMR spectra of some NORMAL oxindole alkaloids in $CDCl_3$ ppm (δ values) from TMS.

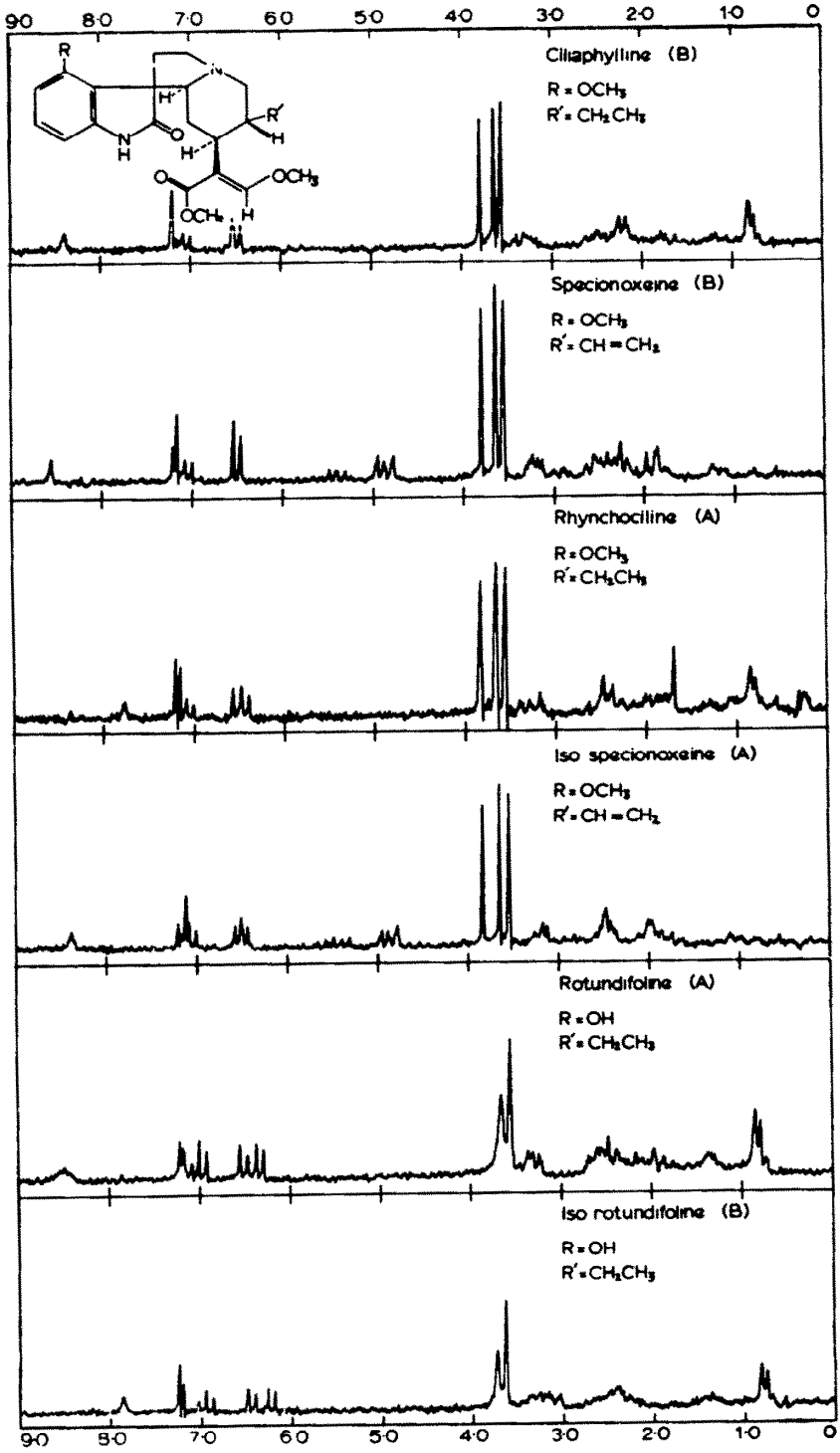

FIG. 7.

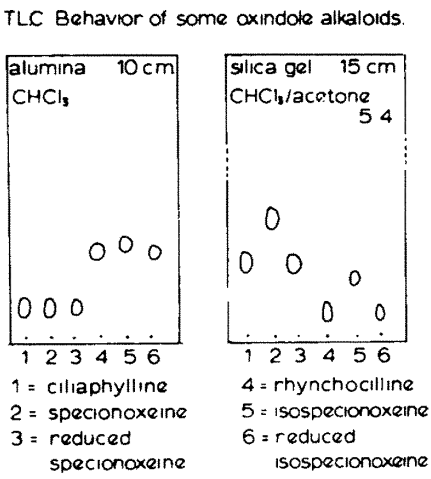

FIG. 8.

specionoxeine/ciliaphylline and isospecionoxeine/rhynchociline. Thus specionoxeine and isospecionoxeine are the vinyl analogues of the corresponding Et containing alkaloids ciliaphylline and rhynchociline respectively.

Specionoxeine on treatment with pyridine yielded, at equilibrium, a mixture of 65% specionoxeine and 35% isospecionoxeine (TLC evidence, see Experimental), while treatment with acetic acid yielded 50% specionoxeine and 50% isospecionoxeine. Similarly, treatment of ciliaphylline with pyridine yielded 65% ciliaphylline and 35% rhynchociline; the latter, isolated by preparative TLC, was identical with natural rhynchociline in its NMR, IR and CD spectra. Treatment of ciliaphylline with acetic acid yielded 50% ciliaphylline and 50% rhynchociline.

Isospecionoxeine and rhynchociline give rise to two *apparent* "triplets" (2H each) at 6·53 δ and 6·52 δ respectively, and a one-proton triplet at 7·11 δ (Fig. 7). The apparent "triplets" result from two overlapping doublets at 6·55 δ and 6·49 δ (J = 7·5 c/s) for isospecionoxeine and at 6·56 (J = 8·1 c/s) and 6·47 δ (J = 7·5 c/s) for rhynchociline. This pattern of two doublets and one triplet is consistent only with a 3-spin system in which the three protons under consideration are adjacent to each other (ABX). Hence, isospecionoxeine and rhynchociline must have the aromatic OMe group in the 9 or 12 position. Specionoxeine and ciliaphylline each give rise to two one-proton doublets coinciding at about 6·52 δ and a one-proton triplet at 7·10 δ (Fig. 7). Again this pattern (A_2X) is only consistent with substitution in either the 9 or 12 position.

The isomerization and hydrogenation data established that the four alkaloids must be similarly substituted. Therefore, the differences seen in the aromatic splitting patterns of the two sets; ciliaphylline/specionoxeine and rhynchociline/isospecionoxeine (Fig. 7) cannot be due to different ring substitution but must arise from differences in chemical shift induced by a different configuration at C7. Conclusive evidence that these four alkaloids are substituted in the 9-position rather than in the 12-position is obtained from the NMR spectrum of N-acetyl ciliaphylline (Fig. 9). One of the aromatic hydrogens is shifted significantly downfield, ca. 75 c/s, relative

to the position of the analogous proton in ciliaphylline. This shift[16] arises from the deshielding effect of the CO group of the N_1-acetyl moiety on the immediately adjacent aromatic proton, i.e. a proton in the 12 position. Hence, these four alkaloids can only be substituted in the 9-position.

NMR Spectra of the aromatic region of ciliaphylline and its N-acetyl derivative.

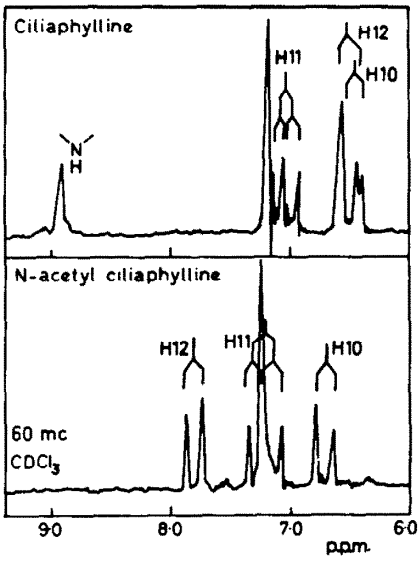

FIG. 9. NMR spectra of the aromatic region of ciliaphylline and its N-acetyl derivative.

The relationship existing between the four alkaloids is summarized schematically in Fig. 10.

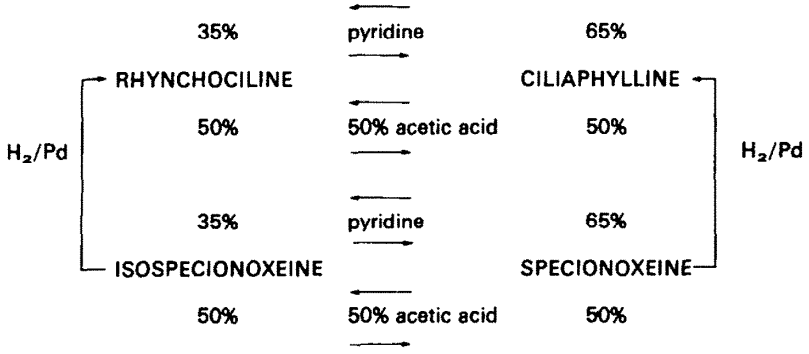

FIG. 10.

Configurational analysis of rhynchociline, ciliaphylline, specionoxeine and isospecionoxeine

Geometry about the C16–C17 *double bond.* The four oxindole alkaloids under discussion (Table 3) have similar δ values (7·18 ± 0·05 ppm) for the NMR signal of the C17 vinyl hydrogen and these values are comparable to those given by rhynchophylline (7·21) isorhynchophylline (7·14) corynoxine (7·16) and corynoxine B (7·21). This indicates identical C16–C17 double bond geometry in all eight alkaloids, since different geometry would lead to significant differences in the chemical shift position of the C17 vinyl hydrogen signal.[4] Rhynchophylline and corynoxine have been obtained by the oxidative rearrangement of the indoles dihydrocorynantheine[2] and corynantheidine[11] respectively, both of known *trans* (methoxy/carbomethoxy) configuration.[4] Thus, a similar *trans* geometry about the C16–C17 double bond is established for rhynchophylline and corynoxine since the reaction conditions[2,11] employed should not lead to *complete* isomerization to a *cis* configuration. Rhynchociline, ciliaphylline, isospecionoxeine, specionoxeine, rhynchophylline, isorhynchophylline, corynoxine and corynoxine B have therefore the *trans* (methoxy/carbomethoxy) configuration about the C16–C17 bond.

Relative configurational stabilities of 9-methoxyrhynchophyllinoid oxindole alkaloids. A conformational analysis similar to that presented for rhynchophyllinoid oxindoles unsubstituted in the 9-position indicates that the preferred conformation for each of the configurations of the 9-methoxylated alkaloids should be analogous to that which occurs in the non-substituted case. The stability arguments are unchanged and as a consequence pyridine isomerization should result in the two *normal* configurations predominating over the *pseudo* and the two *allo* over the *epiallo*.

Ring D *configuration of rhynchociline, ciliaphylline, specionoxeine and isospecionoxeine.* As stated earlier, pyridine isomerization of either ciliaphylline or rhynchociline results in a mixture, at equilibrium, in which only ciliaphylline (65%) and rhynchociline (35%) can be detected. Hence, ciliaphylline and rhynchociline probably have either the *normal* or *allo* configurations. Comparison of the appearance of the C18 Me triplet signal of rhynchociline and ciliaphylline (Fig. 7) with that of rhynchophylline and corynoxine (Fig. 5) indicates that rhynchociline and ciliaphylline and therefore specionoxeine and isospecionoxeine have the *normal* configuration.

Differentiation between A *and* B *configurations in 9-methoxy oxindole alkaloids.* The presence of a 9-MeO group in rhynchophylline-type alkaloids not only influences the interpretation of the pK_a and equilibration data but also precludes the NMR method of assigning A and B configurations delineated earlier. Stabilization by intramolecular hydrogen bonding is possible for the cation of both the A (N_4H^+/9-OMe) and the B(N_4H^+/lactam CO) configurations. Thus it is possible that the A configuration could be the stronger base and hence would predominate under acid conditions. Interpretation of the pyridine equilibration would also be equivocal because the electrostatic repulsion occurring between the N_4 lone pair and the lactam CO in the B configuration would have to be considered relative to that occurring between the N_4 lone pair and the oxygen lone pairs of the 9-OMe group in configuration A. A new criterion is therefore necessary to differentiate 9-OMe A and B *normal* oxindoles and can be found by making use of the difference in proximity of the 9-OMe group to N_4 in the A and B configurations. A protonated amino group not only deshields via the inductive effect but also exhibits a long range deshielding effect[20]

through space. Thus, it may be expected that, on changing from deuterochloroform to glacial acetic acid as solvent, a greater downfield shift would occur in the chemical shift position of the protons of the aromatic OMe group in that configurational isomer (A) which has the 9-OMe group in closest proximity to N_4. The position of the protons of the aromatic OMe group of rhynchociline shift 0·2 ppm downfield on changing from deuterochloroform to acetic acid while those of ciliaphylline only shift 0·08 ppm (Table 4). The small shift seen for ciliaphylline is of the same order of magnitude as that observed for speciogynine[4] a 9-methoxyindole of *normal* configuration in which the 9-OMe group is distant from N_4 (Table 4).

TABLE 4. CHEMICAL SHIFT OF THE AROMATIC METHOXY GROUP IN $CDCl_3$ AND ACETIC ACID IN SOME MITRAGYNA ALKALOIDS

Alkaloid	δ in $CDCl_3$	δ in Acetic acid	δ AcOH– δ $CDCl_3$
Ciliaphylline	3·83 ppm	3·91 ppm	Δ 0·08 ppm
Rhynchociline	3·86	4·06	Δ 0·20
Speciogynine	3·87	3·91	Δ 0·04

The above evidence, along with that of the C18 Me group appearance and hydrogenation and isomerization data establishes rhynchociline and isospecionoxeine as *normal* A 9-methoxyoxindoles and ciliaphylline and specionoxeine as *normal* B 9-methoxyoxindoles.

Configurational analysis of the 9-hydroxyrhynchophyllinoid oxindole alkaloids. An analysis for 9-hydroxyrhynchophylline-type oxindole alkaloids, similar to that discussed earlier for the non 9-substituted alkaloids, indicates that the preferred conformation for each configuration is the one that is directly analogous to that presented earlier for each configuration of the non 9-substituted case. However, different conclusions are reached regarding the relative configurational stabilities (i.e. *normal/pseudo* and *allo/epiallo*) and therefore the results to be expected of the pyridine or acetic acid isomerizations are not necessarily transferable to the 9-hydroxy alkaloids. Although, it is apparent that in the *normal/pseudo* configurational set, the *normal* configuration AI/A is more stable than the *pseudo* configuration BII/B, the latter could be more stable than the *normal* configuration AI/B depending on whether or not the stabilization conferred on *pseudo* configuration BII/B by formation of the intramolecular hydrogen bond[21,22] is sufficient to overcome the destabilization induced by the *axial* C15 and C20 substituents.

A similar situation also occurs in the *allo/epiallo* configurational set in which, for a similar reason, *epiallo* configuration DII/B could be more stable than *allo* configuration CI/B.

However, pyridine isomerization will still differentiate the *normal* from the *pseudo* and the *allo* from the *epiallo* configurations, because the isomer that will predominate at equilibrium will be either *normal* configuration AI/A or *allo* configuration CI/A depending on the configuration of the starting isomer.

Relative configuration of rotundifoline and isorotundifoline. The diastereoisomers rotundifoline[16,21–23] and isorotundifoline[21–23] are known to be 9-hydroxy oxindole alkaloids of the rhynchophylline-type. As the name implies, isorotundifoline is isomeric with rotundifoline (about the C3 and/or C7 centres) but it has the same

configuration at the C15 and C20 centres. Although the behaviour of isorotundifoline is typically phenolic, rotundifoline is non-phenolic in both its reactions and physical properties due to the formation of a strong intramolecular hydrogen bond between the phenolic hydrogen and the N_4 lone pair.[21,22]

The similarity in the chemical shift position of the C17 vinyl hydrogen signal of rotundifoline and isorotundifoline (Fig. 7) to that of each of the eight rhynchophyllinoid alkaloids discussed earlier indicates that these two hydroxy containing alkaloids must also have the *trans* (methoxy/carbomethoxy) C16–C17 double bond geometry.

Pyridine isomerization of either rotundifoline or isorotundifoline yields, at equilibrium, a mixture in which only rotundifoline (90%) and isorotundifoline (10%) are found. Similarly, rotundifoline (60%) and isorotundifoline (40%) are the only products of acetic acid isomerization (see Experimental). These data, coupled with the non phenolic behaviour of rotundifoline, are consistent only with rotundifoline having the *normal* configuration AI/A, or the *allo* configuration CI/A. Isorotundifoline must therefore have the *normal* configuration AI/B or the *allo* configuration CI/B as its phenolic properties eliminate both the *pseudo* configuration BII/B and the *epiallo* configuration DII/B as configurational possibilities. The non-symmetrical appearance

TABLE 5. GENERAL PROCEDURE FOR CONFIGURATIONAL ANALYSIS OF RHYNCHOPHYLLINE-TYPE ALKALOIDS*

I *Determination of ring* D *configuration*

Isomerization data (acidic or basic)

Original alkaloid present in equilibrium mixture		Original alkaloid *not* present in equilibrium mixture	
Normal or	*Allo*	*Pseudo* or	*Epiallo*
Unsymmetrical	Symmetrical	↓ Alkaloids on isomerization yield	
C18 Me triplet	C18 Me triplet		
		Normal	*Allo*

II *Determination of C7* **A** *or* **B** *spiro configuration*

A: 9-*Unsubstituted oxindoles*

(1) Downfield shift of 9 hydrogen

Normal:	**A** Configuration	*Pseudo:*	**B** Configuration
Allo:	**A** Configuration	*Epiallo:*	**B** Configuration

B: 9-*Methoxyindoles*

(1) Downfield shift of 9-OMe in glacial acetic acid compared with $CDCl_3$

Normal:	**A** Configuration	*Epiallo:*	**B** Configuration
Allo:	**A** Configuration	*Pseudo:*	**B** Configuration

(2) *Pseudo:* **A** More basic than *Pseudo* **B**
Epiallo: **A** More basic than *Epiallo* **B**

C: 9-*Hydroxyoxindoles*

(1) *Normal* and *Allo:* **A** non phenolic
B phenolic

(2) *Pseudo* and *Epiallo:* **A** phenolic
B non phenolic

* Based on the preferred conformations.

of the C18 methyl triplet signal in the NMR spectrum of both rotundifoline and isorotundifoline (Fig. 7) indicates that these alkaloids must possess the *normal* rather than the *allo* configuration.

Not knowing the degree of stabilization conferred on a configuration by the presence of an intramolecular hydrogen bond between the aromatic hydroxy hydrogen group and the lone pair of N_4, it is not possible to predict whether *normal* configuration AI/B is more or less stable than *pseudo* configuration BII/B. The rotundifoline isomerizations, however, indicate that, even under basic conditions, (which would be expected to favour *pseudo* configuration BII/B, as opposed to acidic conditions), the *normal* configurations AI/A and AI/B are the only isomers found. Hence the stabilization induced by the intramolecular hydrogen bond on *pseudo* configuration BII/B is not sufficient to overcome the destabilization induced by the *axial* C15 and C20 substituents.

The general procedure for configurational analysis of rhynchophylline-type alkaloids is given in Table 5.

Circular dichroism and absolute configuration. The similarity of the CD curves (Fig. 11) of ciliaphylline, specionoxeine and isorotundifoline, compounds which possess similar *normal* configuration AI/B shows that these compounds have identical absolute configuration about C15. So too, the similarity of the CD curves of rhynchociline, isospecionoxeine and rotundifoline (Fig. 11), compounds which possess similar *normal* configuration, AI/A, shows that these three alkaloids have identical absolute configuration about C15. The isomerization of ciliaphylline to a compound identical with naturally occurring rhynchociline and the isomerization of isorotundifoline to a compound identical with rotundifoline indicates that all six alkaloids must have the same absolute configuration at C15. Because rotundifoline, specionoxeine and isospecionoxeine were isolated from *Mitragyna speciosa*, which has also yielded the oxindole alkaloids, rhynchophylline, mitraphylline and isomitraphylline of known C15Hα absolute configuration, the same stereochemistry about C15 is indicated for all six alkaloids discussed above. It is extremely unlikely that the *same* plant species would produce such closely related alkaloids of the opposite configurational sense.

This conclusion, however, presents a problem in the interpretation of the differences in the CD curves of rotundifoline (and rhynchociline) and isorotundifoline (and ciliaphylline) relative to their non 9-substituted analogues, isorhynchophylline and rhynchophylline (Fig. 11) even after taking into account the UV shifts and differences in $\Delta\varepsilon$ values caused by the presence of the OH (or OMe) group in the former pairs of alkaloids. Instead of displaying similar curves, the sign of the Cotton effect in the 290 mμ region for isorhynchophylline is opposite to that of rotundifoline (and rhynchociline; Fig. 11), while those of rhynchophylline in both the 290 and 250 mμ region are opposite to those of isorotundifoline (and ciliaphylline; Fig. 11).

The possibility that rotundifoline (and rhynchociline) and isorotundifoline (and ciliaphylline) have the C15Hβ, rather than C15Hα absolute configuration as in isorhynchophylline and rhynchophylline, is unacceptable not only on biogenetic grounds[17] but also because the two sets of curves, allowing for UV differences, do not approximate mirror images of each other, particularly in the 290 mμ region, as would be expected if the two sets of alkaloids had opposite absolute stereochemistry.

Another possibility, that rotundifoline (and rhynchociline) and isorotundifoline

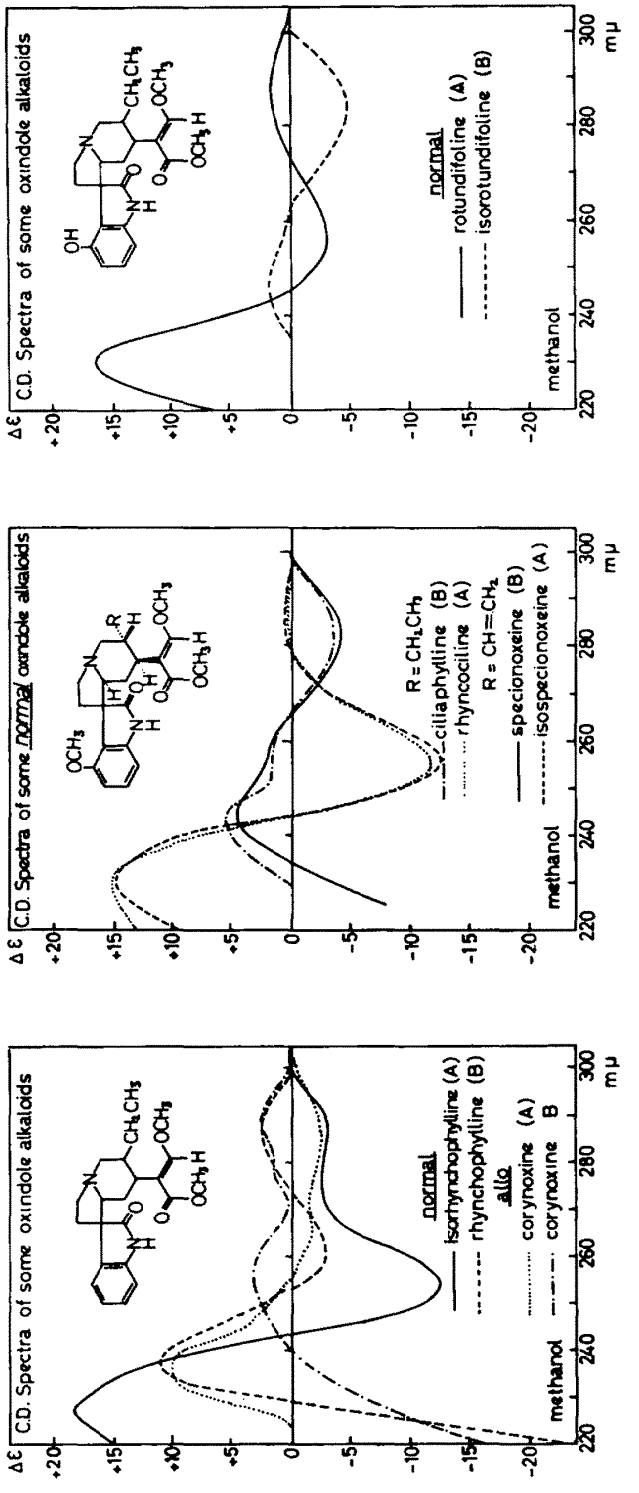
C.D. Spectra of some oxindole alkaloids
normal
isorhynchophylline (A)
rhynchophylline (B)
allo
corynoxine (A)
corynoxine B
methanol
C.D. Spectra of some normal oxindole alkaloids
R = CH₂CH₃
ciliaphylline (B)
rhyncociline (A)
R = CH=CH₂
specionoxeine (B)
isospecionoxeine (A)
methanol
C.D. Spectra of some oxindole alkaloids
normal
rotundifoline (A)
isorotundifoline (B)
methanol
Δε
mμ

FIG. 11.

(and ciliaphylline) do not have the *normal* A and B configuration respectively, is rejected because of the wealth of evidence supporting these assignments accumulated in the present work. Furthermore, the inapplicability of assigning configuration based on proposals[11] founded on the signs of Cotton effects given by the CD of certain non 9-substituted and 10,11-dimethoxy substituted oxindole alkaloids may be demonstrated as follows. The sign of the bands in the 290 and 215 mμ region have been related to the A and B configuration of the oxindoles as (−) and (+) for the A and (+) and (−) for the B, respectively, while a (+) sign in the 250 mμ region (or 2nd effect) indicates a β configuration for the C3H and a (−) sign indicates an α configuration for the C3H. On this basis isorotundifoline (and ciliaphylline) would be allocated a *pseudo* A or *epiallo* A configuration and rotundifoline (and rhynchociline) a *normal* B or *allo* B configuration. This conclusion is incorrect since rotundifoline can be prepared from isorotundifoline (and rhynchociline from ciliaphylline) by isomerization with pyridine.*

Thus the 9-hydroxy and 9-methoxyoxindole alkaloids must be considered in a class different from other oxindole alkaloids when making configurational assignments based on CD data. Moreover, it is important to note that the second Cotton effect of corynoxine B is positive in sign (Fig. 11) rather than negative as would be expected for an oxindole alkaloid of the *allo* β configuration if the published CD proposals[11] are applied.

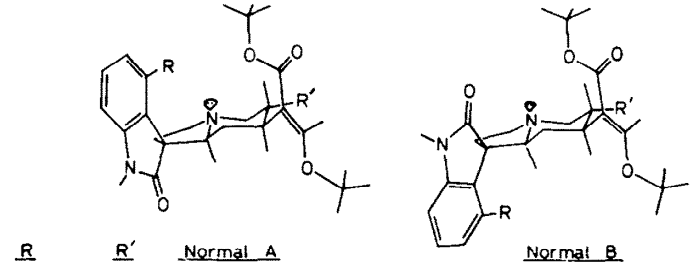

FIG. 12.

In conclusion, rhynchociline and its vinyl analogue isospecionoxeine are 9-methoxy-*normal* A oxindole alkaloids while rotundifoline is a 9-hydroxy *normal* A oxindole alkaloid; ciliaphylline and its C20 vinyl analogue specionoxeine are the 9-methoxy-*normal* B oxindole alkaloids while isorotundifoline is the 9-hydroxy-*normal* B oxindole alkaloid (Fig. 12).

EXPERIMENTAL

M.ps are uncorrected. IR spectra: Unicam SP 100 (0·5% KCl discs); UV spectra: either a Hilger Uvispek Spectrophotometer or a Beckman DK-2, EtOH; NMR spectra: 60 Mc: either a Perkin–Elmer R-10 or a Varian A-60; 100 Mc: Varian HR 100, 5–10% w/v $CDCl_3$ solns (TMS internal ref) or glacial AcOH solns (DSS internal ref); CD curves: Roussel–Jouan Dichrograph (2·0, 1·0, 0·5 and 0·2 cm cells). Due to the unfavourable ratio between UV and CD extinction, the experimentally measured intensities of the curves varied from only 1–30 mm at maximum sensitivity 1·5. This leads to uncertainty in the absolute magnitude of the Δε values, particularly in the region below 250 mμ but repeated measurements leave no

* See discussion page 14.

uncertainty in sign. The solns for CD measurements were prepared using approximately 1 mg of sample accurately weighed in an aluminum boat on a Cahn Electrobalance; the sample and boat were transferred to a 5 ml volumetric flask and the soln made up to volume from a newly opened bottle of spectroscopic grade MeOH (20°). Column chromatography: Aluminium oxide, Spence, type H; TLC—Silica gel G, Merck or Aluminium Oxide G, Merck. All R_f values quoted are based on two determinations. Equiv wts: non aqueous titration N/50 perchloric acid in glacial AcOH, Oracet blue as indicator.

Isolation of specionoxeine and isospecionoxeine. The crude alkaloidal base (200 g), obtained by the neutralization of the evaporated mother liquor residues of the total alkaloidal picrate from *M speciosa* (New Guinea) leaves (87 Kg), was dissolved in $CHCl_3$ (200 ml) and added to an aluminium oxide column (diameter: 3 cm, length: 100 cm) holding ether. The column was eluted successively with ether (12·5 l), $CHCl_3$ (10 l.) and MeOH (5 l.) and the resultant solns evaporated under reduced press to yield 3 main fractions (1: ether, 42·4 g; 2: $CHCl_3$, 38·7 g; 3: MeOH, 21 g). TLC (alumina: $CHCl_3$–benzene, 1/1, or $CHCl_3$) indicated that fraction 1 contained corynantheidine, mitragynine, ajmalicine, paynantheine, speciogynine, speciociliatine and isomitraphylline, fraction 2 contained speciociliatine and isomitraphylline and fraction 3 contained rhynchophylline, ciliaphylline and mitraphylline. Fractions 2 and 3 also contained two new alkaloids now named *specionoxeine* and *isospecionoxeine*. Specionoxeine was isolated (60 mg) from fraction 2 using a Quickfit 120 tube Steady State instrument (lower phase pH 4·85 PO_4 buffer, upper phase: AcOEt).

Fraction 3 was treated with ethanolic picric acid to yield yellow prismatic crystals (14·1 g). Liberation of the free base (7 g) from the above picrate followed by repeated recrystallization from ether and then acetone yielded colourless crystals (700 mg) shown by TLC to be a mixture rich in specionoxeine. Isolation of a pure sample of specionoxeine was accomplished by preparative TLC (silica gel, $CHCl_3$–acetone, 5/4) to yield 133 mg, m.p. 225°C when recrystallized from acetone. (Found: C, 67·05; H, 6·9; N, 6·6; OMe 23·3; Eq. wt. 414·5. $C_{23}H_{28}N_2O_5$ requires: C, 67·0; H, 6·85; N, 6·8; OMe 22·6%; Eq. wt. 412·5). The ether and acetone mother liquors, above, were combined, concentrated to dryness, mixed with alumina (10 g), added to a dry alumina column (diam 2·5 cm; length 20 cm) and eluted with ether (1 l.). The ethereal eluate was concentrated to dryness under reduced press and the residue recrystallized repeatedly from ether–hexane to yield 58 mg, m.p. 179° of pure isospecionoxeine. (Found: C, 66·7; H, 7·1; N, 6·7; OMe, 19·0%; Eq. wt. 410. $C_{23}H_{28}N_2O_5$ requires: C, 67·0; H, 6·85; N, 6·8; OMe, 22·6%; Eq. wt. 412·5). Isospecionoxeine formed a perchlorate, m.p. 230–231° from EtOH–ether. (Found: C, 53·3; H, 5·7; N, 5·3. $C_{23}H_{28}N_2O_5$ $HClO_4$ requires: C, 53·9; H, 5·46; N, 5·66%.)

Hydrogenation of specionoxeine and isospecionoxeine (Fig. 8). Specionoxeine (5 mg) was dissolved in MeOH (2 ml) and hydrogenated on a microhydrogenation apparatus in the presence of 10% Pd–C (25 mg). After 1 hr, the soln was filtered and spotted directly on freshly prepared TLC plates (alumina: $CHCl_3$ and silica gel: $CHCl_3$–acetone 5/4) with reference samples of ciliaphylline, rhynchociline, specionoxeine and isospecionoxeine. Isospecionoxeine was treated in a similar fashion. The alumina: $CHCl_3$ system showed the following R_f values: ciliaphylline, specionoxeine and reduced specionoxeine (0·11), rhynchociline, and reduced isospecionoxeine (0·30) and isospecionoxeine (0·34). The silica gel: $CHCl_3$–acetone (5/4) system showed the following R_f values: ciliaphylline and reduced specionoxeine (0·26), specionoxeine (0·41), rhynchociline and reduced isospecionoxeine (0·09) and isospecionoxeine (0·21).

Isomerization of rhynchociline and ciliaphylline. Rhynchociline (5 mg) was refluxed for 48 hr with pyridine (2 ml), concentrated to dryness under reduced press and the residue dissolved in $CHCl_3$ (1 ml). Another sample of rhynchociline (5 mg) was refluxed for 48 hr in 50% glacial AcOH (2 ml), made alkaline with conc NH_4OH then extracted with $CHCl_3$ (3 × 2 ml). The combined $CHCl_3$ extracts were washed with water, dried over Na_2SO_4, filtered and then concentrated to 1 ml. Ciliaphylline was treated in a similar fashion and the final solns of the 4 reactions were examined on TLC with reference samples of authentic rhynchociline and ciliaphylline.

TLC using the systems described below indicates that pyridine isomerization yields 65% ciliaphylline and 35% rhynchociline while the acid isomerization yields 50% of each.*

System	Rhynchociline R_f	Ciliaphylline R_f
Alumina:chloroform	0·30	0·11
Alumina:cyclohexane–chloroform (3/7)	0·10	0·03
Silica gel:chloroform–acetone (5/4)	0·09	0·26

*Estimation by two independent observers based on TLC spot size.

Isomerization of specionoxeine and isospecionoxeine. Specionoxeine and specionoxeine were subjected to the pyridine and AcOH isomerizations (48 hr) as described above. TLC using the systems described below indicates that pyridine yields 65% specionoxeine and 35% isospecionoxeine while acid treatment yields 50% of each.

System	Isospecionoxeine R_f	Specionoxeine R_f
Alumina:$CHCl_3$	0·34	0·11
Silica gel:$CHCl_3$–acetone (5/4)	0·21	0·41

Isomerization of rhynchophylline and isorhynchophylline. Rhynchophylline and isorhynchophylline were subjected to the pyridine and AcOH isomerizations (48 hr) as described above. TLC using the systems described below, indicates that pyridine yields 20% rhynchophylline and 80% isorhynchophylline while acid treatment yields 70% rhynchophylline and 30% isorhynchophylline.

System	Isorhynchophylline R_f	Rhynchophylline R_f
Alumina:$CHCl_3$	0·42	0·09
Alumina:cyclohexane–$CHCl_3$ (3/7)	0·18	0·04
Silica gel: $CHCl_3$–acetone (5/4)	0·60	0·25

Preparative isomerization of ciliaphylline. Ciliaphylline (125 mg) was refluxed for 30 hr in pyridine (2 ml), evaporated to dryness under reduced press and the residue separated by preparative TLC (silica gel: $CHCl_3$–acetone 5/4) yielding amorphous solid I (29 mg, 35%) and amorphous solid II (50 mg, 65%). Crystallization of solid I from ether–hexane yielded white sandy crystals m.p. 179° (5 mg) undepressed by admixture and identical to (NMR, CD, TLC) authentic rhynchociline. Crystallization of solid II from acetone yielded colourless prismatic crystals (26 mg), m.p. 223° undepressed by admixture with and identical to (NMR, CD, TLC) authentic ciliaphylline.

Isomerization of rotundifoline and isorotundifoline. Rotundifoline (50 mg) was refluxed in pyridine (5 ml) for 46 hr; TLC (silica gel: $CHCl_3$–EtOH 20/1) thus indicated a mixture comprised of about 95% rotundifoline and 5% isorotundifoline. To ensure that the major alkaloid present was in fact rotundifoline, 4 ml of the pyridine solutions was evaporated under reduced press and the residue washed with ether then recrystallized twice from absolute EtOH. This material was identical with rotundifoline (IR, NMR, CD). The remaining 1 ml of pyridine soln was allowed to reflux for another 20 hr; TLC then indicated a mixture comprised of 90% rotundifoline and 10% isorotundifoline. The attainment of equilibrium was established by refluxing isorotundifoline (10 mg) in pyridine (1 ml) for either 48 or 66 hr to give a mixture which had the same composition as that given by rotundifoline after 66 hr.

Rotundifoline (50 mg) was refluxed in 50% glacial AcOH (5 ml) for 20 hr at which time TLC indicated a mixture comprised of 60% rotundifoline and 40% isorotundifoline. Part of this soln (4 mls) was made alkaline with conc NH_4OH then extracted with ether. This ethereal soln was extracted with 5% sodium hydroxide, washed with water, dried over Na_2SO_4 then evaporated to dryness. The residue was recrystallized from abs EtOH and the product (22 mg) was identical with rotundifoline (IR, NMR, CD). The 5% NaOH extract was neutralized with dil HCl and extracted with ether. This ethereal extract was washed with water, dried over Na_2SO_4 then evaporated to dryness. The residue was recrystallized from a mixture of acetone–light pet ether and the product (14 mg) was identical with isorotundifoline containing acetone of crystallization (IR, NMR, mixed mps). The remaining 1 ml of AcOH soln was refluxed for another 28 hr; TLC then indicated that the alkaloidal mixture composition was unchanged (i.e. 60% rotundifoline and 40% isorotundifoline). Refluxing isorotundifoline (10 mg) in 50% glacial AcOH for 48 hrs gave a mixture of identical composition with that discussed above (TLC evidence).

To establish the attainment of equilibrium, the pyridine was evaporated from both the 1 ml isomerized rotundifoline and isorotundifoline sols, 50% glacial AcOH (1 ml) added to each and then both allowed to reflux for 48 hr. The composition of the mixture so obtained (TLC evidence) was identical to that obtained by direct acetic acid isomerization of either rotundifoline or isorotundifoline. Similarly, the products of the 1 ml AcOH soln of isomerized rotundifoline and isorotundifoline was refluxed with pyridine (1 ml) for 66 hr, after isolation by neutralization and extraction with ether. The composition of the resulting mixture was identical with that obtained by direct pyridine isomerization of either rotundifoline or isorotundifoline.

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