Phytochemistry, 1975, Vol. 14, pp. 557–563. Pergamon Press. Printed in England.

9-HYDROXYRHYNCHOPHYLLINE-TYPE OXINDOLE ALKALOIDS

SARAH R. HEMINGWAY,* PETER J. HOUGHTON,† J. DAVID PHILLIPSON* and EDWARD J. SHELLARD†

* Department of Pharmacognosy, The School of Pharmacy, University of London, 29–39 Brunswick Square, London WC1N 1AX, England; † Pharmacognosy Research Laboratories, Department of Pharmacy, Chelsea College, University of London, Manresa Road, London SW3 6LX, England

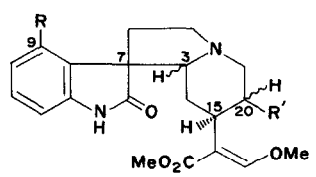
(*Received* 14 *May* 1974)

Key Word Index—*Mitragyna speciosa*; *M. parvifolia*; Rubiaceae; 9-hydroxyrhynchophylline-type oxindole alkaloids; speciofoline; isospeciofoline; mitrafoline; isomitrafoline; rotundifoleine; isorotundifoleine; 3-*epi*-isorotundifoline.

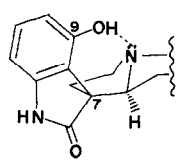
Abstract—Speciofoline has been assigned the *epiallo* B configuration on the basis of isomerization studies, NMR and CD spectra, and three new speciofoline isomers, mitrafoline (*allo* A), isomitrafoline (*allo* B) and isospeciofoline (*epiallo* A) have been isolated from *Mitragyna speciosa* Korth. Two new C-20 vinyl alkaloids, rotundifoleine and isorotundifoleine, have been separated as minor products from crystalline samples of rotundifoline and isorotundifoline respectively, previously isolated from *M. parvifolia* (Roxb.) Korth. A transient product observed during the isomerization of isorotundifoline has been identified as the *pseudo* B isomer, 3-*epi*-isorotundifoline.

INTRODUCTION

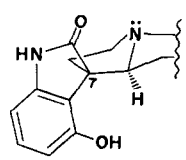
Three 9-hydroxyrhynchophylline-type oxindole alkaloids* (**1**, R = OH, R′ = Et), rotundifoline [1] (*normal* A), isorotundifoline [1] (*normal* B) and speciofoline [2] (unknown stereochemistry), have been isolated previously from species of *Mitragyna*. Rotundifoline and speciofoline are non-phenolic (i.e. are non-acidic phenols) since the C-9 hydroxyl forms a strong intramolecular hydrogen bond with the lone pair of electrons of N-4 (partial **2**) [3,4], while isorotundifoline, which can readily be isomerized to rotundifoline, behaves as a typical phenol (partial **3**). Since the *allo* isomers corynoxine and corynoxine B (**1**, R = H; R′ = Et) are known to be present in species of *Mitragyna* [5], it was anticipated that their 9-hydroxy-derivatives might also be found. Similarly the C-20 vinyl analogues of 9-hydroxyrhyncophylline-type alkaloids (**1**, R = OH; R′ = —CH=CH$_2$) might be expected to occur naturally since such analogues are known for rhynchophylline (corynoxeine) [6], isorhynchophylline (isocorynoxeine) [5,7], and for their 9-MeO counterparts, ciliaphylline (specionoxeine) [8] and rhynchociline (isospecionoxeine) [8].



(**1**)



(**2**)



(**3**)

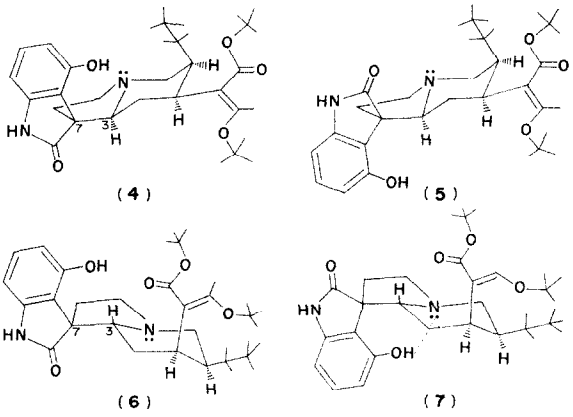
* Eight diastereoisomeric compounds are theoretically possible for oxindole alkaloids of structure **1**. All known compounds of this type possess C-15 Hα configuration and the four possible configurations are defined as *normal* (C-3 Hα, C-20 Hβ), *pseudo* (C-3 Hβ, C-20 Hβ), *allo* (C-3 Hα, C-20 Hα) and *epiallo* (C-3 Hβ, C-20 Hα). Each of these can exist as A or B isomers depending upon the configuration at C-7; in the A isomer the lactam carbonyl is below the plane of the C–D rings and in the B isomer it is above.

RESULTS

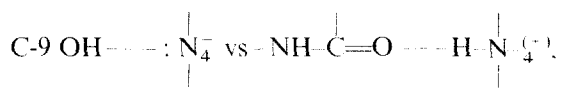
This paper describes the isolation, from *M. speciosa*, of three new interconvertible isomers of speciofoline (**1**, R = OH; R′ = Et); the stereochemistry of these four alkaloids is established for speciofoline as *epiallo* B, for mitrafoline as *allo* A, for isomitrafoline as *allo* B and for isospeciofoline as *epiallo* A. 3-*Epi*-isorotundifoline, the *pseudo* B isomer of rotundifoline has been shown to be formed as a transient product during the isomerization of isorotundifoline, but not identified as a natural product. Two new isomeric alkaloids, rotundifoleine and isorotundifoleine, have also been obtained by separation from crystalline compounds previously identified as rotundifoline and isorotundifoline from *M. parvifolia* [9] and they have been characterized as the respective C-20 vinyl analogues (**1**, R = OH; R′ = —CH=CH$_2$).

DISCUSSION

The new alkaloid mitrafoline, which has identical behaviour with rotundifoline in many TLC systems [10], was characterized as a non-phenolic 9-hydroxyrhynchophylline-type alkaloid (**1**, R = OH; R′ = Et), by means of its UV, IR, MS and NMR spectra. However, on isomerization in both pyridine and acetic acid, four isomers were produced, none of which was identified as isorotundifoline although one did correspond chromatographically with speciofoline. TLC examination of the isomerization products of small samples of speciofoline indicated that they were identical with those of mitrafoline, having spots corresponding to speciofoline and mitrafoline and to the less stable phenolic isomers, isospeciofoline and isomitrafoline. These results indicated that since rotundifoline and isorotundifoline have been assigned the *normal* configuration, the four interconvertible compounds were the *allo/epiallo* isomers. The preferred conformations for each of the four possible configurations, *allo* A, *allo* B, *epiallo* A and *epiallo* B are represented by structures **4**, **5**, **6** and **7** respectively [8]. Theoretically, isomerization of any of these four epimers should yield predominantly the two having the *allo* configuration, as was recently reported for the isomerization of corynoxine and corynoxine B when no evidence for the formation of *epiallo* isomers could be obtained [11]. However, in the case of the 9-hydroxy compounds,



some degree of stabilization could be conferred upon an isomer of the *epiallo* B configuration (**7**), by formation of an intramolecular hydrogen bond between the C-9 hydroxyl and N-4 [8]. Study of Dreiding models indicated that the distance between N-4 and the phenolic hydroxyl group is 1·6 Å for the *allo* A isomer (**4**) and 1·9 Å for the *epiallo* B isomer (**7**). The latter therefore possesses a weaker intramolecular hydrogen bond. Furthermore, the bulky axial substituent at C-15 tends to destabilize the *epiallo* B configuration and hence it would be expected that of the two non-phenolic isomers, the *allo* A would predominate over the *epiallo* B on isomerization. During isomerization in acid conditions, some stabilization of *allo* B and *epiallo* A configurations is expected by intramolecular hydrogen bonding between the lactam carbonyl and the protonated N-4. Whatever the net result of the competition between the two types of intramolecular hydrogen bond, viz.



it is anticipated that the two phenolic isomers with the *allo* B (**5**) and *epiallo* A (**6**) configurations would form on isomerization in acid [8], with the *epiallo* A being less favoured due to the destabilization induced by the axial C-15 substituent.

From TLC examination of the products of a series of isomerizations of the four alkaloids in pyridine or in acetic acid, it appeared that they were interconvertible and that the two non-phenolic isomers, mitrafoline and speciofoline, were the major products in both basic and acidic conditions. The relative proportions of the products of

Table 1. δ Values for some E-*seco* oxindole alkaloids (100 MHz NMR)

Alkaloid	C-18Me	OMe	COOMe	C-10H	C-11H	C-12H	C-17H	NH
Isorhynchophylline	0·79*t*	3·65*s*	3·55*s*		6·78–7·20*m*		7·14*s*	8·42*s*
Rhynchophylline	0·77*t*	3·67*s*	3·58*s*		6·78–7·20*m*		7·21*s*	8·48*s*
Rotundifoline	0·80*t*	3·69*s*	3·58*s*	6·36*d*	7·04*t*	6·57*d*	7·22*s*	8·30*s*
Isorotundifoline	0·78*t*	3·76*s*	3·67*s*	6·28*d*	7·00*t*	6·50*d*	7·29*s*	7·88*s*
Isorotundifoleine	—*	3·76*s*	3·66*s*	6·30*d*	7·00*t*	6·50*d*	7·25*s*	8·23*s*
Mitrafoline	0·88*t*	3·62*s*	3·57*s*	6·40*d*	7·05*t*	6·58*d*	7·30*s*	8·56*s*
Isomitrafoline	0·94*t*	3·78*s*	3·66*s*	6·38*d*	7·01*t*	6·60*d*	7·35*s*	8·35*s*
Isospeciofoline	0·84*t*	3·76*s*	3·63*s*	6·32*d*	6·98*t*	6·57*d*	7·36*s*	7·80*s*
Speciofoline	0·82*t*	3·77*s*	3·64*s*	6·32*d*	7·03*t*	6·51*d*	7·36*s*	8·13*s*

* $-C\underline{H}=CH_2$ δ 5·35*m*; $-CH=C\underline{H}_2$ δ 4·86–5·06*m*.

acetic acid isomerization were, mitrafoline (50%), speciofoline (25%), isomitrafoline (15%) and isospeciofoline (10%). On the basis of the preceding argument they may therefore be assigned the following configurations: mitrafoline, *allo* A (**4**); speciofoline, *epiallo* B (**7**), isomitrafoline, *allo* B (**5**) and isospeciofoline, *epiallo* A (**6**).

Comparison of the NMR spectra (see Table 1) of the four speciofoline isomers with those of the *normal* alkaloids, rotundifoline and isorotundifoline, shows that the C-18 Me signal appears downfield in the former. In the case of mitrafoline, the chemical shift for the C-18 methyl signal at δ 0·88 is in close agreement with the corresponding signal (δ 0·87) in the spectrum of corynoxine (**1**, R = H; R′ = Et, *allo* A). Of the 9-hydroxy E-*seco* oxindole isomers, the one giving the C-18 methyl signal furthest downfield is isomitrafoline (δ 0·94); this may be due to the proximity of this group to the N-4 lone pair electrons and thus indicates that isomitrafoline possesses the *allo* B (**5**) configuration. The similarity in chemical shift of the C-18 methyl signals in the spectra of isospeciofoline and speciofoline (δ 0·84 and 0·82, respectively) indicates that this group is in a similar environment in both isomers and therefore possibly that they both have the *epiallo* configuration. The fact that both the C-18 methyl signals are downfield from those in the spectra of rotundifoline and isorotundifoline is perhaps accounted for by deshielding by the bulky C-15 axial substituents. Since isospeciofoline is phenolic and speciofoline is non-phenolic, the indication is that isospeciofoline possesses the *epiallo* A configuration (**6**) and that speciofoline possesses the *epiallo* B configuration (**7**).

The signals for methoxy, carbomethoxy and C-17 olefinic protons appear further downfield in the NMR spectra of isomitrafoline, isospeciofoline and speciofoline when compared with the corresponding signals for mitrafoline, isorhynchophylline, rhynchophylline and rotundifoline (see Table 1). This deshielding can be attributed to the proximity of the bulky C-15 axial substituent and the C-9 hydroxyl in isospeciofoline or the lactam carbonyl in speciofoline. The chemical shifts of the two methoxyl signals in the spectrum of isomitrafoline are similar to those of isorotundifoline and

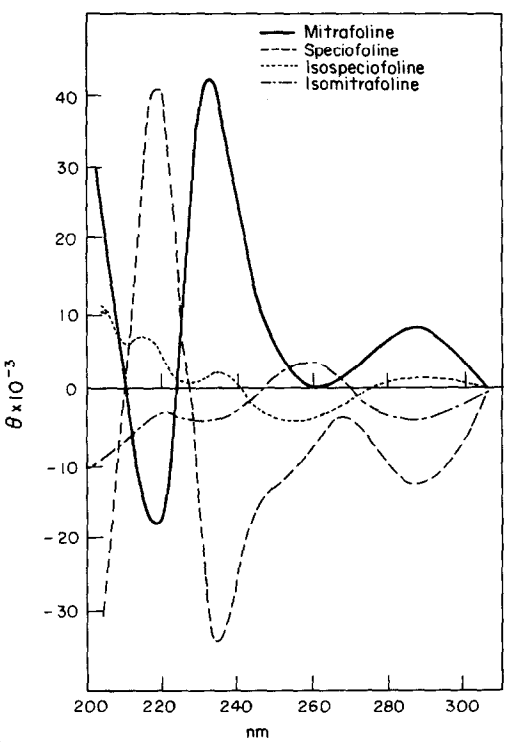


Fig. 1. CD spectra of *allo* and *epiallo* 9-hydroxyrhynchophylline-type oxindole alkaloids.

since they differ from those of rhynchophylline, probably indicate the effect of deshielding by the free phenolic hydroxyl group.

Further support for these assignments is obtained from the CD spectra (Fig. 1). The CD spectrum of mitrafoline closely resembles that of rotundifoline [8], showing strong positive C.E.s in the 290 and 235 nm regions. This indicates that the asymmetric centres at C-3 and C-7, which are close to the oxindole chromophore, possess the same relative stereochemistry and thus supports the *allo* A configuration (**4**) assigned to mitrafoline on the basis of the isomerization and NMR data.

When the CD spectra of the two non-phenolic isomers, mitrafoline and speciofoline, are compared (Fig. 1), they are seen to have C.E.s of opposite sign in the 290, 235 and 220 nm regions, an indication that, since they are interconvertible, they must differ at both the C-3 and C-7 centres. Hence speciofoline must possess the *epiallo* B configuration (**7**).

For rhynchophylline-type oxindole alkaloids (**1**, R = H; R′ = Et), a negative C.E. in the 290 nm region is correlated to the A configuration and a positive C.E. in the same region to the B configuration [12–14]. The signs for the C.E.s at 290 nm are reversed for the 9-hydroxy alkaloids since rotundifoline (**1**, R = OH; R′ = Et) exhibits a positive C.E. in this region and isorotundifoline (B configuration) exhibits a negative C.E. [8] Therefore the rules relating the sign of the C.E.s [12–14], which have been applied to the rhynchophylline-type alkaloids, cannot be applied to their C-9 OH analogues [8]. If the presence of the C.E. at 290 nm in the CD spectra of the 9-hydroxy substituted alkaloids is dependent on the configuration at C-7, then mitrafoline and isospeciofoline which have positive C.E.s similar to rotundifoline, will possess the A configuration. Since isospeciofoline is phenolic it would possess the *epiallo* A configuration (**6**). The other phenolic isomer, isomitrafoline, shows opposite C.E.s to isospeciofoline in three regions of the CD spectrum and since the isomers are interconvertible they must differ at C-3 and C-7. Isomitrafoline must therefore be the *allo* B isomer (**5**).

It appears, therefore, that not only must the C-9 hydroxy alkaloids be considered as a separate case from the 9-unsubstituted oxindole alkaloids when deductions are made from CD spectra, but also that the phenolic and non-phenolic alkaloids must be considered separately (see Fig. 1).

During these investigations further isomerizations of rotundifoline and isorotundifoline were undertaken. It was noted that when isorotundifoline was isomerized in pyridine, and the resulting mixture examined by TLC, an alkaloid of R_f intermediate to rotundifoline and isorotundifoline was produced, which disappeared when equilibrium was reached. This compound was isolated by preparative TLC and its UV, MS and isomerization data revealed that it was a rotundifoline isomer (**1**, R = OH; R′ = Et). The absence of a bathochromic shift with alkali and the similarity of its CD spectrum with speciofoline, from which it could not be separated on TLC, indicated that this transient compound must be the *pseudo* B isomer. Since this compound differs from isorotundifoline only in its configuration at C-3 it was named 3-*epi*-isorotundifoline. Rhynchophylline-type oxindole alkaloids (**1**, R = H) possessing the *pseudo* configuration are considered too unstable to exist under ordinary conditions, but it has been postulated that stability might be conferred on the *pseudo* B configuration of the 9-hydroxy analogues by formation of an intra-molecular hydrogen bond between the C-9 OH and N-4 [8]. There is no evidence to suggest that it exists as a natural product.

Chemical confirmation of the assignment of *normal* configuration to rotundifoline and isorotundifoline has been presented previously since gambirine (9-hydroxydihydrocorynantheine) was converted to rotundifoline [16]. However, the results presented in this paper show that the *allo* A isomer, mitrafoline, has similar TLC behaviour to rotundifoline and also that the CD spectra of these two alkaloids are very similar and it cannot therefore be considered that the chemical confirmation has been rigorously established. However, the results do provide further evidence for the *normal* configuration of rotundifoline and isorotundifoline in that the *allo*/*epiallo* isomers are now known. Nevertheless, chemical confirmation of the absolute configuration of the 9-hydroxyrhynchophylline-type alkaloids is needed since the C-15 Hα absolute configuration has previously been assumed only on the basis of biosynthetic considerations [8].

In order to obtain pure isorotundifoline for isomerization studies, a crystalline sample of crude

isorotundifoline, previously isolated from *M. parvifolia* [9], was purified by preparative TLC. Isorotundifoleine, which has a slightly higher R_f value than isorotundifoline [10], was isolated as a minor component. The UV spectrum was identical to that of isorotundifoline and its phenolic nature was demonstrated by a bathochromic shift with alkali. The MS showed M^+ at *m/e* 398 (contrasting with *m/e* 400 for rotundifoline isomers) together with ions exhibiting peaks at *m/e* 237, 236, 222 and 206 (cf. MS of corynoxeine) [6], indicating the presence of a second double bond in the alicyclic portion of the molecule. Fragment ions at *m/e* 146, 160, 161 and 162 indicated a hydroxy substituted oxindole moiety [17]. The NMR spectrum was generally very similar to that of isorotundifoline (Table 1) but showed that the signal attributable to the C-18 Me was absent and that signals due to a vinyl substituent at C-20 (δ 5·35, 1 H *m*; C-19 H. δ 4·86–5·06, 2 H *m*; C-18 H's) were present. Comparable NMR spectra have been reported for the C-20 vinyl alkaloids corynoxeine [6] and specionoxeine [8].

The CD spectrum of isorotundifoleine is identical to that of isorotundifoline indicating that it has the same configuration at C-3 and C-7. Chemical confirmation of the structure was obtained by hydrogenation of isorotundifoleine to yield isorotundifoline. These results clearly indicate that isorotundifoleine possesses the same absolute configuration as isorotundifoline and is its C-20 vinyl analogue (**I**, R = OH; R′ = –CH=CH_2, *normal* B).

The *normal* A isomer, rotundifoleine, was detected in a crystalline sample of rotundifoline previously isolated from *M. parviflora* [9], because the MS showed the presence of an ion of appreciable intensity at *m/e* 398, in addition to the M^+ at *m/e* 400. Initially no TLC evidence could be obtained for the presence of a C-20 vinyl compound despite the use of a wide range of TLC systems. When silica gel impregnated with silver nitrate was used a minor component of lower R_f value than rotundifoline was observed and separation was effected by preparative TLC. Isomerization of isorotundifoleine in pyridine resulted in a compound with identical R_f values to rotundifoline on plain silica gel but which behaved as rotundifoleine on silver nitrate impregnated plates. Rotundifoleine, having a CD spectrum identical with that of rotundifoline and being interconvertible with isorotundifoleine, must therefore possess the same absolute configuration as rotundifoline being its C-20 vinyl analogue (**I**, R = OH; R′ = –CH=CH_2, *normal* A).

EXPERIMENTAL

Leaves of *M. speciosa* were collected in the Bangkok area, Thailand, between October 1969 and March 1970 by Mr. B. Tantisewie, Department of Pharmacy, Medical University, Bangkok. Leaves of *M. parvifolia* were obtained from Cochin, India, by Dr. S. B. Rao, Navaratna Pharmaceutical Laboratories, Cochin, India. Specimen samples are retained in the Museum of the Pharmacognosy Research Laboratories, Department of Pharmacy, Chelsea College. The MS were determined on an AEI MS 902 high resolution mass spectrometer at 70 eV with inlet temperatures between 180 and 230°. The 100 MHz NMR spectra were determined in $CDCl_3$ using TMS as internal reference. The TLC systems used were silica gel G/GF_{254} (Merck) with (A), $CHCl_3$–Me_2CO (5:4); (B) $CHCl_3$–EtOH (95:5); (C) Et_2O–EtOAc (1:1); (D) Et_2O; (E) EtOAc; (F) $CHCl_3$–EtOH (6:1).

Extraction and separation of alkaloids. (a) *M. speciosa.* The dried powdered leaves (500 g) were moistened with 10% NH_4OH and macerated for 24 hr with three successive portions of EtOAc. The combined, filtered EtOAc extracts were conc to low vol. and extracted with 2% H_2SO_4 which was made alkaline with NH_4OH and extracted into $CHCl_3$ which was washed with H_2O, dried over Na_2SO_4 and conc to dryness yielding total crude alkaloid, 2·13 g (0·42%). An aliquot (1·2 g) dissolved in Et_2O was eluted successively with 200 ml quantities of (i) Et_2O, (ii) $CHCl_3$ and (iii) EtOH from an alumina column (Spence type H, 3 × 15 cm). Evaporation to dryness of (i), (ii) and (iii), followed by TLC examination gave the following results: (i) 686 mg containing mitragynine, payantheine, speciogynine and speciociliatine; (ii) 302 mg and (iii) 60 mg containing the same major oxindole alkaloids. Multiple prep. TLC using systems A, C, D and F resulted in the separation of 4 oxindole alkaloids from (ii) and (iii), speciofoline, 70 mg (0·025%), and 3 new alkaloids now named mitrafoline, 42 mg (0·015%), isomitrafoline, 15 mg (0·005%) and isospeciofoline, 3 mg (0·001%). (b) *M. parvifolia.* The isolation of rotundifoline and isorotundifoline from this material has been described previously [9]. 61 mg of crude crystalline isorotundifoline yielded 6·3 mg of a new alkaloid, named isorotundifoleine, by prep. TLC using system B; 65 mg of crystalline rotundifoline yielded 1·8 mg of a new alkaloid, named rotundifoleine, using system E with plates prepared with 10% $AgNO_3$.

Characterization of the alkaloids. (a) *Speciofoline.* The hR_fs for systems A, B and C were 58, 62 and 50 resp., being identical with reference speciofoline [18]. UV (EtOH) λ_{max} 224, 242 sh., 290 nm; λ_{min} 274 nm; absence of a bathochromic shift in 0·01 N NaOH. CD (MeOH) $[\theta]_{219}$ + 41·9 × 10^3, $[\theta]_{233}$ − 34·0 × 10^3, $[\theta]_{286}$ − 12·4 × 10^3 (see Fig. 1). NMR, see Table 1. MS, *m/e* 400 (M^+, 100), 384 (10), 240 (8), 239 (47), 238 (16), 224 (19), 210 (7), 208 (12). (b) *Mitrafoline.* The hR_fs for systems A, B and C were 62, 70 and 57 resp. UV (EtOH) λ_{max} 224, 243 sh., 292 nm; λ_{min} 274; absence of a bathochromic shift in 0·01 N NaOH. CD (MeOH) $[\theta]_{218}$ − 17·7 × 10^3, $[\theta]_{233}$ + 43·2 × 10^3, $[\theta]_{286}$ + 9·1 × 10^3 (see Fig. 1). NMR, see Table 1. MS, *m/e* 400 (M^+, 100), 239 (74), 238 (15), 224 (17), 210 (5), 208 (10), 184 (2), 146 (10), 110 (10). (c) *Isomitrafoline.* The hR_fs for systems A, B and C were 26, 28 and 27 resp. UV (EtOH) λ_{max} 225, 237 sh., 290 nm;

λ_{min} 275 nm; UV (EtOH, 0·01 N NaOH) λ_{max} 233, 245, 310 nm, reversible by addition of HCl. CD (MeOH) $[\theta]_{232} - 5{\cdot}3 \times 10^3$, $[\theta]_{257} + 4{\cdot}2 \times 10^3$, $[\theta]_{285} - 3{\cdot}4 \times 10^3$ (see Fig. 1). NMR, see Table 1. MS, *m/e* 400 (M^+, 100), 384 (10), 240 (14), 239 (75), 238 (22), 224 (33), 210 (15), 208 (20). (d) *Isospeciofoline*. The hR_fs for systems A, B and C were 16, 12 and 8 resp. UV (EtOH) λ_{max} 220, 245 sh., 292 nm: λ_{min} 275 nm; UV (EtOH, 0·01 N NaOH) λ_{max} 233, 310 nm; reversible by addition of HCl. CD (MeOH) $[\theta]_{235} + 2{\cdot}9 \times 10^3$, $[\theta]_{255} - 4{\cdot}0 \times 10^3$, $[\theta]_{286} + 1{\cdot}5 \times 10^3$ (see Fig. 1). NMR, see Table 1. MS, *m/e* 400 (M^+ 100), 384 (12), 240 (20), 239 (65), 238 (18), 224 (29), 210 (13), 208 (19). (e) *Isorotundifoleine*. The hR_fs for systems A, B and C were 52, 50 and 44 resp. The corresponding values for isorotundifoline were 47, 43 and 37. UV (EtOH) λ_{max} 222 nm, 224 sh., 278, 294 nm; UV (EtOH, 0·01 N NaOH) λ_{max} 298, 309 nm reversible by addition of HCl. MS *m/e* 398 (M^+, 100), 383 (6), 381 (4), 367 (13), 237 (27), 236 (32), 222 (35), 206 (30), 192 (256), 178 (17), 175 (13), 162 (14), 161 (13), 160 (25), 152 (11), 146 (25), 108 (148). Prepared isorotundifoleine (see isomerization section), CD (MeOH) $[\theta]_{207} - 25{\cdot}1 \times 10^3$, $[\theta]_{242} + 11{\cdot}3 \times 10^3$, $[\theta]_{285} - 12{\cdot}6 \times 10^3$; NMR, see Table 1. Microhydrogenation: prepared isorotundifoleine (2 mg) dissolved in MeOH (5 ml) and hydrogenated over Pd–C (24 mg, 10%) for 1 hr. The reaction mixture was filtered and TLC indicated the presence of isorotundifoline only, using systems B and C and with system B using plates prepared with 10% $AgNO_3$. (f) *Rotundifoleine*. The hR_fs for systems B and C were 70 and 57 resp. (identical with rotundifoline); on plates containing 10% $AgNO_3$ with systems B and E, 37 and 14 resp. (rotundifoline 49 and 35 resp.). UV (EtOH) λ_{max} 223, 243 sh., 286, 295 nm; λ_{min} 277 nm; absence of a bathochromic shift in 0·01 N NaOH. MS, *m/e* 398 (M^+, 100), 367 (14), 237 (10), 236 (17), 222 (28), 206 (17), 192 (21), 178 (16), 175 (24), 162 (45), 161 (31), 160 (45), 152 (19), 146 (79), 108 (69). Prepared rotundifoleine (see isomerization section), CD (MeOH) $[\theta]_{205} + 21{\cdot}0 \times 10^3$, $[\theta]_{217} - 6{\cdot}6 \times 10^3$, $[\theta]_{232} + 28{\cdot}9 \times 10^3$, $[\theta]_{256} - 7{\cdot}2 \times 10^3$, $[\theta]_{287} + 3{\cdot}2 \times 10^3$. (g) 3-Epi-*isorotundifoline*. The hR_fs for the prepared alkaloid (see isomerization section) for systems A and C were 58 and 50 resp. UV (EtOH) 223, 243 sh., 286, 296 nm; absence of a bathochromic shift in 0·01 N NaOH. CD (MeOH) $[\theta]_{220} + 16{\cdot}7 \times 10^3$, $[\theta]_{234} - 12{\cdot}3 \times 10^3$, $[\theta]_{285} - 3{\cdot}7 \times 10^3$. MS *m/e* 400 ($M^+$, 100), 385 (3), 369 (10), 240 (12), 239 (68), 238 (20), 224 (19), 210 (10), 208 (12), 162 (3), 161 (3), 160 (4), 146 (7).

Isomerization of mitrafoline and speciofoline. (a) *Pyridine*. Each alkaloid (10 mg) was heated separately in pyridine (5 ml) for 36 hr at 150°. The pyridine was evaporated to dryness under reduced pressure and the residue dissolved in $CHCl_3$. TLC indicated that in each case 4 isomers were present in the approx proportions, mitrafoline (40%), speciofoline (40%), isomitrafoline (10%) and isospeciofoline (10%). (b) *Acetic acid*. Each alkaloid (10 mg) was heated separately in 50% HOAc (5 ml) for 36 hrs at 150°, cooled, made alkaline with NH_4OH and extracted with $CHCl_3$. TLC indicated that in each case the same 4 isomers were present in the approx proportions, mitrafoline (50%), speciofoline (25%), isomitrafoline (15%) and isospeciofoline (10%). The products of the mitrafoline and speciofoline isomerizations were separated by prep. TLC using system D and the alkaloids eluted from the bands had identical hR_fs, UV, CD and MS with the natural alkaloids.

Isomerization of isomitrafoline and isospeciofoline. Each alkaloid (2 mg) was heated with pyridine (5 ml) for 24 hr at 130°, extracted and examined as described in (a) above. TLC indicated that in each case mitrafoline and speciofoline were the major products and that isomitrafoline and isospeciofoline were minor products.

Isomerization of rotundifoline containing rotundifoleine. The alkaloid (1 g) was heated in 50% HOAc at 125° for 24 hr, extracted and examined as described in (b) above. The residue obtained from evaporation of the $CHCl_3$ extract was dissolved in Et_2O and extracted with 2·5% NaOH which was made acidic with conc HCl and then alkaline with NH_4OH. Extraction with $CHCl_3$ yielded a mixture of the phenolic alkaloids isorotundifoline and isorotundifoleine. The non-phenolic alkaloids which remained in the Et_2O were further isomerized with 50% HOAc. By repeating this whole procedure a further 8 times and separating isorotundifoleine by prep. TLC using system B, a total of 44·5 mg was obtained. Prepared isorotundifoleine was identical to the natural compound in its hR_fs (systems A, B and C), UV, MS and CD spectra.

Isomerization of isorotundifoleine. Prepared isorotundifoleine (6 mg) was heated in pyridine at 130° for 40 hr, yielding a mixture of rotundifoleine (60%) and isorotundifoleine (40%) which was sep. by prep. TLC using system C. The rotundifoleine obtained (2 mg) had identical MS and hR_fs in systems B and E using silica gel G plates prepared with 10% aq. $AgNO_3$, to those of natural rotundifoleine.

Isomerization of isorotundifoline. (a) Isorotundifoline (2 mg) was heated in pyridine at 135° for 23 hr, extracted and examined as described in (a) above. TLC indicated that isorotundifoline (80%) was still the major component and that rotundifoleine (10%) and 3-*epi*-isorotundifoline were present. Heating was continued for a further 17 hr and TLC indicated that rotundifoline (90%) and isorotundifoline (10%) were present but that 3-*epi*-isorotundifoline was not present. (b) Isorotundifoline (11 mg) was heated in pyridine at 135° for 23 hr and the mixture separated by prep. TLC using system C. 3-*Epi*-isorotundifoline (1·5 mg) was obtained.

Isomerization of 3-epi-*isorotundifoline*. 3-*Epi*-isorotundifoline (< 1 mg) was heated in 50% HOAc at 135° for 16 hr. TLC indicated that 3-*epi*-rotundifoline was no longer present and that rotundifoline (60%) and isorotundifoline (40%) had been formed.

Acknowledgements—We are grateful to Mr. B. Tantisewie, Department of Pharmacy, Medical University of Bangkok and Dr. S. B. Rao, Navaratna Pharmaceutical Laboratories, Cochin, India, for obtaining the plant material. We thank Mr. D. Carter, The School of Pharmacy, University of London, for MS determinations, Dr. P. M. Scopes, Chemistry Department, Westfield College, Hampstead, for CD determinations and PCMU, Harwell, for the NMR spectra. One of us (SRH) thanks the Pharmaceutical Society of Great Britain for the award of The Burroughs Scholarship.

REFERENCES

1. Saxton, J. E. (1965) in *The Alkaloids* Mánske, R. H. F. Vol. VIII, p. 59, Academic Press, New York.
2. Beckett, A. H., Shellard, E. J. and Tackie, A. N. (1965) *Planta Med.* **13**, 241.
3. Beckett, A. H. and Tackie, A. N. (1963) *Chem. Ind. London* 1122.
4. Beckett, A. H., Lee, C. M. and Tackie, A. N. (1963) *Tetrahedron Letters* 1709.
5. Shellard, E. J. and Houghton, P. J. unpublished observations.
6. Shellard, E. J. and Houghton, P. J. (1972) *Planta Med.* **21**, 382.
7. Haginiwa, J., Sakai, S., Aimi, N., Yamanaka, E. and Shinma, N. (1973) *J. Pharm. Soc., Japan* **93**, 448.
8. Trager, W. F., Lee C. M., Phillipson, J. D., Haddock, R. E., Dwuma-Badu, D. and Beckett, A. H. (1968) *Tetrahedron* **24**, 523.

9. Shellard, E. J. and Phillipson, J. D. (1964) *Planta Med.* **12,** 160.
10. Phillipson, J. D. and Shellard, E. J. (1968) *J. Chromatog.* **32,** 692.
11. Phillipson, J. D. and Hemingway, S. R. (1973) *Phytochemistry* **12,** 2795.
12. Pousset, J. -L., Poisson, J. and Legrand, J. (1966) *Tetrahedron Letters* 6283.
13. Pousset, J. -L., Poisson, J., Shine, R. J. and Shamma, M. (1967) *Bull. Soc. Chim. Fr.* 2766.
14. Poisson, J. and Pousset, J. -L. (1967) *Tetrahedron Letters* 1919.
15. Beecham, A. F., Hart, N. K., Johns, S. R. and Lamberton, J. A. (1967) *Tetrahedron Letters* 99.
16. Merlini, L., Nasini, G. and Haddock, R. E. (1972) *Phytochemistry* **11,** 1525.
17. Beckett, A. H., Dwuma-Badu, D. and Haddock, R. E. (1969) *Tetrahedron* **25,** 5961.
18. Phillipson, J. D. (1965) *The alkaloidal content of some Asian species of* Mitragyna, Ph.D. Thesis, University of London.