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MINOR ALKALOIDS OF *TYLOPHORA ASTHMATICA*
REVISED STRUCTURE OF TYLOPHORINIDINE[a]

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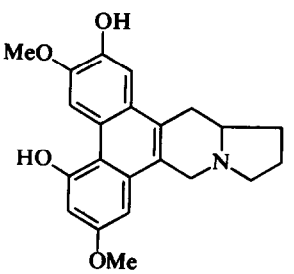
Abstract—The structure of the phenolic alkaloid tylophorinidine, isolated from *Tylophora asthmatica*, has been shown to be **2**. Two other minor alkaloids isolated from the plant have been shown to be *d*-septicine (**11**) and *d*-isotylocrebrine (**14**).

Mulchandani *et al.*, in a recent communication,[1] reported the isolation of a phenolic alkaloid, tylophorinidine, from *Tylophora asthmatica* Wight et Arn syn. *T. indica* (Burm) Merril and suggested structure **1** for it. The evidence presented was not very convincing since this structure fails to explain the presence of two bands at 1757 and 1724 cm^{-1} in the IR spectrum of the diacetate. In the NMR spectrum of the diacetate, the authors assigned C_1-H at δ 8·33 and C_6-H at δ 8·0 whereas the bay proton at C_4 which was assigned at δ 7·9 would normally be expected to be at the lowest field. The inconsistencies in the data presented by Mulchandani *et al.* as well as our interest in the alkaloids of *T. asthmatica* prompted a reinvestigation of the minor alkaloids. This has led in turn to the reisolation of tylophorinidine and revision of its structure as **2**.

[a]Contribution No. 315 from CIBA Research Centre.



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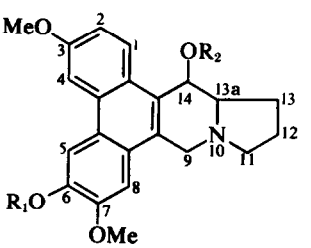
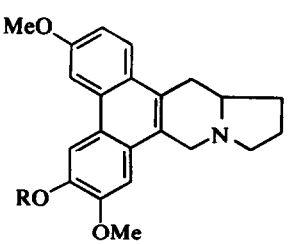
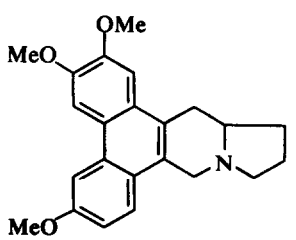


2: $R_1 = R_2 = H$
5: $R_1 = Me$; $R_2 = H$
6: $R_1 = Me$; $R_2 = Ac$
7: $R_1 = R_2 = Ac$



3: R = Me
8: R = H



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The crude alkaloids isolated from fresh leaves of *T. asthmatica* by methods described[2] were subjected to a 30-transfer counter-current distribution using the solvent system $CHCl_3$–3% aq AcOH, a system which had been found by Rao *et al*[3] to be useful in the separation of the alkaloids of *T. crebriflora*. Besides tylophorine[4–7] and tylophorinine,[8,9] we were able to isolate two minor non-phenolic alkaloids and a phenolic alkaloid. The latter isolated in a yield of about 0·001% was identical (in TLC, UV, IR and mixed m.p.) with a sample of tylophorinidine isolated by Mulchandani *et al.*

Tylophorinidine, m.p. 216–218° (d), $[\alpha]_D^{25}$ + 105° ($CHCl_3$, *c* 1·1), $C_{22}H_{23}NO_4$ (M^+ 365), has UV maxima at 260, 287, 313 and 340 nm (log ε 4·64, 4·41, 3·90, 3·17) closely resembling tylophorinine and indicating a 2,3,6-trioxygenated phenanthrene skeleton. The presence of a phenolic OH function was indicated by a green colouration with ferric chloride and by the shift in its UV maxima on addition of sodium hydroxide. The IR spectrum of the base showed an OH band at 3540 cm^{-1}. The NMR spectrum of tylophorinidine (in DMSO-d_6) (Table 1) showed the presence of two OMe groups at δ 3·80 and 3·95, five aromatic protons at δ 6·55–8·00 and a CH-OH function at δ 4·65.

The mass spectrum of tylophorinidine shows the base peak at *m/e* 296 arising by cleavage of the pyrrolidine ring by a retro-Diels–Alder reaction characteristic of phenanthroindolizidine alkaloids. A strong peak at *m/e* 268 arising from 296 by loss of CO was indicative of the presence of an OH at C_{14}.[7]

Methylation of tylophorinidine with diazomethane gave a mono-methyl ether, $C_{23}H_{25}NO_4$ (M^+ 379), whose NMR spectrum (Table 1 for assignments) showed the presence of three OMe groups, a CH-OH function and five aromatic protons. The formation of the monomethyl ether with diazomethane showed the presence of only one phenolic OH group in tylophorinidine. The methyl ether still has an OH group as shown by the formation of an acetate, ν_{max}^{KBr} 1720 cm^{-1}. Its mass spectrum showed a very weak molecular ion at *m/e* 421, the major fragments being represented as shown below.

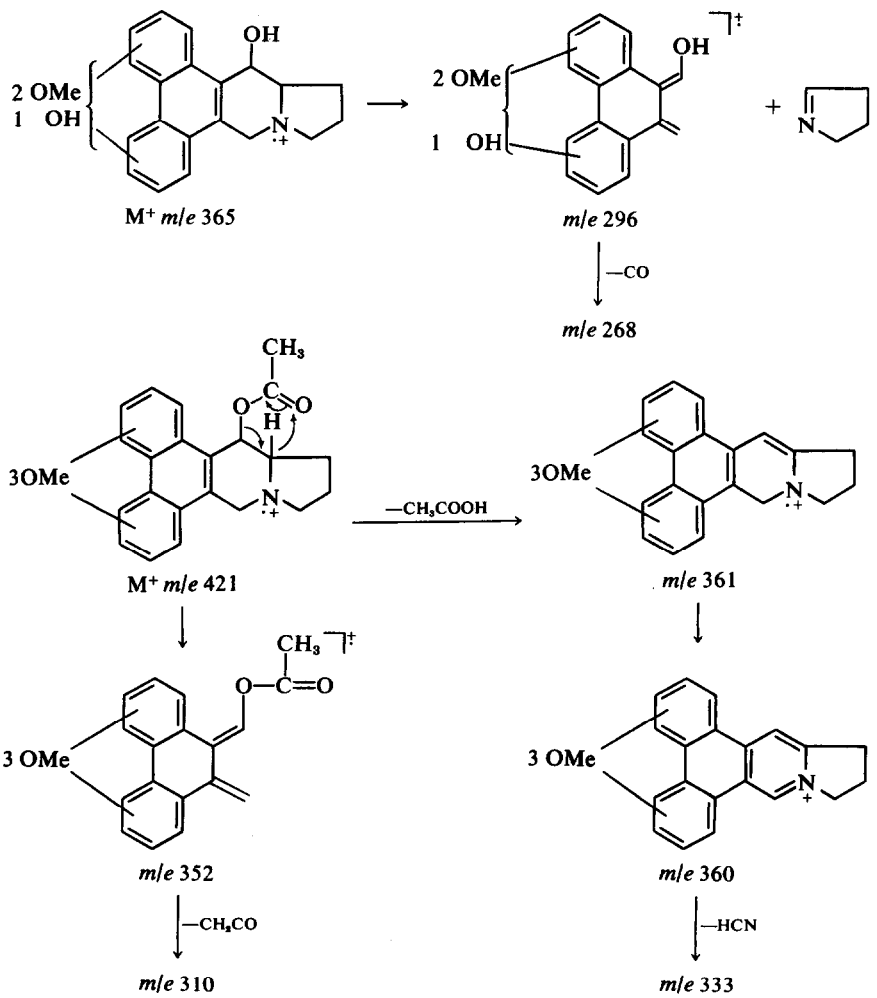


Table 1. NMR spectral data

Proton	Tylophorinidine DMSO-d_6 2	Tylophorinidine DMSO-d_6 + CF_3CO_2H 2	O-Methyl-tylophorinidine $CDCl_3$ 5	O-Methyl-tylophorinidine DMSO-d_6 5	O-Methyl-tylophorinidine DMSO-d_6 + CF_3CO_2H 5
C-1	8·00 (d) (J = 10)	8·05 (d) (J = 10)	8·33 (d) (J = 9)	8·20 (d) (J = 9)	8·10 (d) (J = 9)
C-2	7·10 (dd) (J = 10,2)	7·15 (dd) (J = 10,2)	7·16 (dd) (J = 9,2)	7·18 (dd) (J = 9,2)	7·20 (br)
C-4	7·75 (d) (J = 2)	7·62 (d) (J = 2)	7·55 (d) (J = 2)	7·85 (d) (J = 2)	7·50 (br)
C-5	7·90 (s)	7·86 (s)	7·33 (s)	7·81 (s)	7·30
C-8	6·55 (s)	6·75 (s)	5·70 (s)	6·40 (s)	6·50 (s)
C-14	4·65 (br s)	5·18 (br s)	4·60 (br)	4·68 (br)	4·95 (br s)
O-Me	3·80 (s), 3·95 (s) (3H each)	3·92 (s), 3·95 (s) (3H each)	4·02, 3·98, 3·63 (s, 3H each)	4·00 (6H, s), 3·75 (3H, s)	3·98, 3·95, 3·85 (s, 3H each)
OH	8·20 (disappears with D_2O)		5·90 (disappears with D_2O)		

Table 2. NMR spectral data

Proton	Acetyl-tylophorinine $CDCl_3$ 6	Acetyl-tylophorinine DMSO-d_6 6	Acetyl O-methyl tylophorinidine $CDCl_3$ 6	Acetyl-O-methyl-tylophorinidine DMSO-d_6 6	Diacetyl-tylophorinidine $CDCl_3$ 7
C-1	7·84 (d) (J = 9)	7·68 (d) (J = 9)	7·84 (d) (J = 9)	7·71 (d) (J = 9)	7·80 (d) (J = 9)
C-2	7·18 (dd) (J = 9,2)	7·21 (dd) (J = 9,2)	7·20 (dd) (J = 9,2)	7·21 (dd) (J = 9,2)	7·18 (dd) (J = 9,2)
C-4	7·85 (d) (J = 2)	8·05 (d) (J = 2)	7·85 (d) (J = 2)	8·06 (d) (J = 2)	7·77 (d) (J = 2)
C-5	7·86 (s)	8·05 (s)	7·88 (s)	8·07 (s)	8·18 (s)
C-8	7·13 (s)	7·20 (s)	7·16 (s)	7·25 (s)	7·20 (s)
C-14	6·64 (d) (J = 2)	6·44 (d) (J = 2)	6·66 (d) (J = 2)	6·48 (d) (J = 2)	6·62 (d) (J = 2)
C-9	4·72, 3·52 (AB q J = 16)	4·53, 3·44 (AB q J = 16)	4·75, 3·57 (AB q J = 16)	4·57, 3·50 (AB q J = 16)	4·70, 3·52 (AB q J = 16)
O-Me	4·08, 4·02, 3·96	4·03, 3·97, 3·93	4·09, 4·04, 3·97	4·04, 3·98, 3·95	3·97 (6H, s)
O-Ac	2·12 (3H, s)	2·03 (3H, s)	2·13 (3H, s)	2·04 (3H, s)	2·39 (3H, s) 2·12 (3H, s)

The NMR spectrum of the acetate (Table 2) was virtually identical with that of acetyltylophorinine (6) and clearly showed the presence of an acetate group at C_{14}.

Acetylation of tylophorinidine yielded a diacetate whose IR spectrum, ν_{max}^{KBr} 1760, 1730 cm^{-1}, showed the presence of a phenolic acetate and an alcoholic acetate group. Its NMR spectrum (Table 2) also showed the presence of two acetate groups at δ 2·39 and 2·12, the former being due to the phenolic acetate group.

Catalytic hydrogenolysis of tylophorinidine with Pd-C in acetic acid in the presence of perchloric acid gave a desoxy base, m.p. 214–218°, $C_{22}H_{23}NO_3$ (M^+ at m/e 349). O-Methyltylophorinidine on similar hydrogenolysis gave a recemic desoxybase, m.p. 215–217° (d), $C_{23}H_{25}NO_3$ (M^+ at m/e 363), identical in all respects (TLC, mixed m.p., UV, IR, NMR and mass spectra) with synthetic *dl*-desoxy-tylophorinine[8] (3). The desoxybase was different from *dl*-antofine[10–12] (4). Treatment of O-methyltylophorinidine with perchloric acid followed by reduction with sodium borohydride also afforded *dl*-desoxytylophorinine (3). This leads to structure 5 for O-methyltylophorinidine and 6 for acetyl O-methyltylophorinidine.

Of the three positions – 3, 6 and 7 – for the location of the phenolic OH in tylophorinidine, position 6 is preferred from a consideration of the NMR spectra of diacetyltylophorinidine (7) and acetyl O-methyltylophorinidine (6) (Table 2). The protons at C_1, C_2, C_4 and C_8 are found at approximately the

Table 3. NMR spectral data

Proton	Tylophorinine DMSO-d_6 10	Tylophorinine DMSO-d_6 + CF_3CO_2H 10	Desoxytylophorinine $CDCl_3$ 3	Desoxytylophorinine DMSO-d_6 3
C-1	8·15 (d) (J = 9)	8·17 (d) (J = 9)	7·90 (d) (J = 9)	7·90 (d) (J = 8)
C-2	7·12 (dd) (J = 9, 2)	7·24 (dd) (J = 9,2)	7·18 (dd) (J = 9,2)	7·20 (dd) (J = 8,2)
C-4	7·86 (d) (J = 2)	7·86 (d) (J = 2)	7·83 (d) (J = 2)	8·02 (d) (J = 2)
C-5	7·88 (s)	7·85 (s)	7·85 (s)	8·04 (s)
C-8	6·77 (s)	7·00 (s)	7·08 (s)	7·15 (s)
C-14	4·70 (br, s)			
O-Me	3·98 (6H, s) 3·83 (3H, s)	4·00 (9H, s)	4·06, 4·04, 3·96	4·04, 4·03, 3·95

same chemical shifts in both the compounds. The proton at C_5, however, occurs at δ 7·88 as a sharp singlet in **6** and is shifted downfield to δ 8·18 in the diacetate **7**. In comparison to a OMe group, the acetoxy group has a deshielding influence of approximately 0·22 ppm on the *ortho*- proton[13] and this leads to position 6 for the acetoxy group in the diacetate. Tylophorinidine hence has the gross structure **2**, desoxytylophorinidine being **8**.

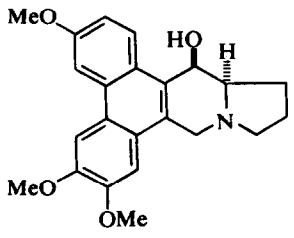
The gross structure **5** assigned to O-methyltylophorinidine also represents tylophorinine. That the two are not identical or antipodal is shown by their physical constants and those of their acetyl derivatives (Table 4).

These differences show that the two pairs must be related to each other as diastereoisomers.

A comparative study of the NMR spectra of O-methyltylophorinidine (**5**), its acetate (**6**) and its desoxy base (**3**) provided a clue to the possible stereochemistry of the alkaloid. An inspection of Tables 1–3 show that the chemical shift of C_1–*H* in **5** undergoes an upfield shift of 0·13 ppm in going from $CDCl_3$ to DMSO-d_6 and an upfield shift of 0·49 ppm upon acetylation. The protons at C_4, C_5 and C_8 in **5** suffer downfield shifts of 0·30, 0·48 and 0·70 ppm respectively when the solvent is changed from $CDCl_3$ to DMSO-d_6. Likewise, these protons are shifted downfield by 0·30, 0·55 and 1·46 ppm respectively in going from **5** to its acetate **6**, both spectra being run in $CDCl_3$. The chemical shifts of the aromatic protons in **6** are close to those of the desoxy base **3**. The chemical shifts of protons in aromatic polycyclics are sensitive to solvents.[14] For instance, as seen from Table 3 in going from $CDCl_3$ to DMSO-d_6, the protons at C_4 and C_5 in **3** are shifted lowfield by 0·19 ppm and the one at C_8 by 0·07 ppm. The changes encountered for O-methyltylophorinidine (**5**), especially for the C_8-H, are, however, of a much higher order and there must be a different factor producing these changes. Dreiding models show that neither the C_{14}-OH nor the lone pair of electrons on the nitrogen can influence the chemical shifts of the C_8-H in all possible conformations. The changes in the chemical shifts may be explained if O-methyltylophorinidine (**5**) exists as a dimer wherein two molecules are held together by H-bonding between the C_{14}-OH and the nitrogen of the other in the manner suggested for norargemonine.[15] An axial disposition of the OH group would facilitate the dimerisation. In the NMR spectrum of the acetate **6**, the C_{14}-H is coupled with the C_{13a}-H to the extent of 2 Hz. A low coupling is possible for both equatorial and axial conformations of the C_{13a}-H. Hydrogenolysis of O-methyltylophorinidine gives only racemic desoxytylophorinine (**8**) whereas tylophorinine leads to the laevo-rotatory desoxy base.[8] If it is assumed that the racemic desoxy base is formed by a facile elimination of the OH in **5** followed by reduction, it becomes reasonable to postulate that the C_{13a}-H in **5** is *trans*-diaxially disposed to the C_{14}-OH. It is thus possible to write the tentative structure **9** for O-methyltylophorinidine. Dreiding models indicate that it is easy to build up the postulated H-bonded dimer for this stereostructure and that in the dimer, the protons at C_4,

Table 4.

	m.p.	$[\alpha]_D^{25}$ ($CHCl_3$)
Tylophorinine	248–249°	−14·2°
O-Methyltylophorinidine	229–231°	+161°
Acetylophorinine	222–223°	−4·64°
Acetyl O-methyl-tylophorinidine	176–177°	+158°



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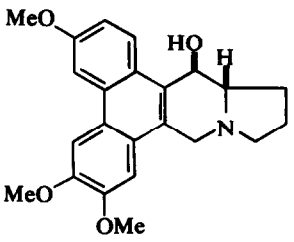

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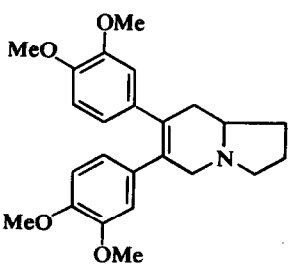
C_5 and C_8 are mutually shielded by the two molecules, as also the OMe at C_7, whereas the proton at C_1 may be slightly deshielded. The effect of a polar solvent like DMSO would be to reduce the H-bonding, and thus in this solvent the protons would have more 'normal' chemical shifts. In the acetate **6** and the desoxybase **3**, H-bonding is totally abolished and the protons assume 'normal' values and exhibit diminished sensitivity to solvents.

Tylophorinine was too insoluble in $CDCl_3$ to permit comparative solvent-induced shift studies. Nevertheless, the C_8-H appears at δ 6·77, still about 0·4 ppm downfield from its position in the acetate or 'normal' values. The p*Ka* of tylophorinine showed no detectable difference from that of O-methyltylophorinidine. It appears, therefore, that the OH may be axial in tylophorinine also. The NMR spectrum of acetyltylophorinine (Table 2) shows a C_{14}-H–C_{13a}-H coupling of 2 Hz. The C_{13a}-H must therefore be equatorial leading to structure **10** for tylophorinine.

When this work was completed, Rao *et al*[16] reported the isolation of three minor phenolic alkaloids from *T. asthmatica*. These were called alkaloids A, B and C. Alkaloid A, m.p. 250–252° (d) was assigned the formula $C_{22}H_{23}NO_5$. Alkaloid B, m.p. 235–237° (d), $C_{23}H_{24}NO_4$, was found to be nortylophorine since it gave tylophorine on methylation with diazomethane. Alkaloid C, m.p. 253–255 (d), $C_{22}H_{23}NO_4$, isometric with tylophorinidine gave 'tylophorinine' with diazomethane. The phenolic OH in alkaloid C was assigned to either position 3 or 6. The optical rotations of alkaloid C and its methyl ether were not reported but it is possible that alkaloid C is a solvated form of tylophorinidine. What Rao *et al.* regard as tylophorinine appears from their reported NMR spectrum[17] to be the diastereo-isomeric O-methylthylophorinidine. The alkaloid C was also isolated from *T. dalzellii*. The alkaloid was found to have significant activity in murine leukemia (L-1210 system).[16] Mulchandani *et al.*[18] have reported the isolation of tylophorinidine from *Pergularia pallida* (Asclepiadaceae).

In the present investigation, the presence of two more phenolic alkaloids besides tylophorinidine could be recognised by TLC but these could not be obtained pure. The mother liquor from the crystallisation of tylophorinine also showed the presence of a base with M^+ at *m/e* 409 but this again could not be obtained free from tylophorinine. Of the two hitherto unreported minor non-phenolic bases which were obtained pure, the one with m.p. 136°, $[\alpha]_D^{25}$ +38·8° (MeOH, *c* 1) had the formula $C_{24}H_{29}NO_4$ (M^+ 395). A strong peak at *m/e* 326 (M-69) indicated the presence of an indolizidine skeleton. Its NMR spectrum showed the presence of six aromatic protons. The base was identified as *d*-septicine (**11**) by comparison with a sample of authentic *l*-septicine[3,17] isolated from *T. crebriflora*. The two samples were identical in TLC, UV, IR in CH_2Cl_2 and NMR spectra. *l*-Septicine isolated from *Ficus septica*[19,20] has been reported to have $[\alpha]_D$ −16·2° whereas that from *T. crebriflora*[3] has been reported to have $[\alpha]_D$ −42·5°. The structure of septicine has been confirmed earlier by synthesis.[20,21]

The second minor alkaloid, m.p. 212–214°, $C_{24}H_{27}NO_4$, $[\alpha]_D$ +22·43° ($CHCl_3$, *c* 1·1) has a UV spectrum strikingly similar to that of tylocrebrine (**12**).[22] The mass spectrum showed the molecular ion peak at *m/e* 393 and the base peak at *m/e* 324 arising by loss of the pyrrolidine ring. The NMR spectrum (Experimental) differs from the reported NMR spectrum of tylocrebrine but is very similar to that of Rao's 'alkaloid B'[17] obtained from *T. crebriflora* for which structure **13** was suggested. This favours structure **14** for the alkaloid. The racemic form of **14**, isotylocrebrine, has been synthesized earlier.[22] The present alkaloid was identical with synthetic isotylocrebrine (**14**) in TLC, UV and IR in CH_2Cl_2. Hofmann degradation of the methiodide obtained from the alkaloid yielded the optically inactive methine (**15**), m.p. 136–137°, $C_{25}H_{29}NO_4$ (M^+ 407), identical (mixed m.p., TLC, mass spectra) with the methine obtained syntheti-



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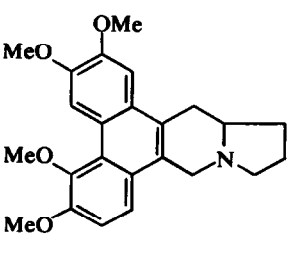

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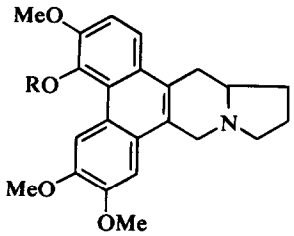
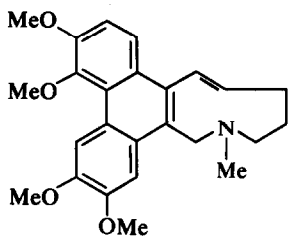


13: R = H
14: R = Me

cally from racemic **14**. Its m.p. was depressed to 120–130° on admixture with tylocrebrine methine. The new alkaloid is hence *d*-isotylocrebrine (**14**).

From *T. crebriflora*, Rao *et al*[3] have isolated three alkaloids closely related to isotylocrebrine—alkaloids 'A' (**16**), 'B' (**13**) and 'C' (**17**). Hydrogenolysis of 'A' gave a base, m.p. 219–220°, whose $[\alpha]_D$ was not reported. It was also obtained from 'B' by methylation. The product was not compared with synthetic **14** but is in most probability isotylocrebrine **14**. Isotylocrebrine has hitherto not been isolated from any plant.



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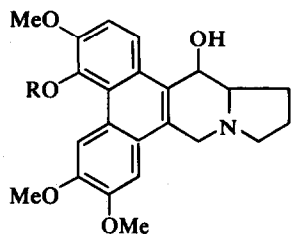
EXPERIMENTAL

M.ps are uncorrected. UV spectra were determined in 95% EtOH using a Beckman DK 2A spectrophotometer. Optical rotations were determined at 25°. NMR spectra were run at 60 or 100 MHz.

Isolation of the total alkaloids. The fresh leaves of *T. asthmatica* (5 kg) collected around Madras were extracted with EtOH in the cold thrice by percolation. The combined extracts were concentrated on the water-bath to about 2 l., allowed to settle overnight and filtered from tarry material. The clear filtrate was further concentrated to 1 l. *in vacuo*, diluted with 0·5 N HCl and extracted with ether (2 × 500 ml) to remove chlorophyll. The aq soln was basified with NH_4OH and extracted with CH_2Cl_2. The CH_2Cl_2 extract was washed with H_2O, dried (Na_2SO_4) and evaporated to yield the crude total alkaloids (8 g) as a light brown solid.

Separation of the alkaloids. The crude alkaloid (8 g) was subjected to a 30-transfer counter-current distribution (tubes of capacity 300 ml) using the solvent system, $CHCl_3$-aq AcOH (3%). Samples from individual tubes were examined by TLC on SiO_2 using $CHCl_3$-MeOH (93:7). Tube 1 contained mostly resinous material and was discarded. The other tubes were pooled together appropriately, basified with NH_4OH and extracted with more $CHCl_3$. The $CHCl_3$ extracts were washed with H_2O, dried (Na_2SO_4) and evaporated.

Tubes 2–10 gave a solid which was crystallised from $CHCl_3$-MeOH to yield tylophorine (3 g). The mother liquor from the crystallisation was evaporated and the residue chromatographed over Al_2O_3 in C_6H_6. The column was eluted successively with C_6H_6, C_6H_6–$CHCl_3$ (1:1) and $CHCl_3$. The C_6H_6 eluates gave *d*-septicine (**11**) as a colourless solid which crystallised from ether as feathery needles (40 mg), m.p. 136°, undepressed by admixture with authentic *l*-septicine. It had $[\alpha]_D$ +38·8° (MeOH, *c* 1), λ_{max} 238 (sh), 287 nm (log ε 4·20, 3·98); NMR: δ 6·65 (s, 4H), 6·52 (s, 2H), 3·77 (s, 6H), 3·56 (s, 6H) (Found: C, 73·0; H, 7·7; N, 3·6. $C_{24}H_{29}NO_4$ requires: C, 72·9; H, 7·4; N, 3·5%). Mass spectrum: *m/e* 395 (M^+, 6), 326 (30), 295 (85), 264 (78), 175 (38), 165 (36), 163 (30), 151 (100). Its TLC, UV, IR in CH_2Cl_2 and NMR spectra were identical with those of authentic *l*-septicine. The C_6H_6-$CHCl_3$ eluates gave **14** (100 mg) as a light yellow solid which, crystallised from CH_2Cl_2-MeOH, had m.p. 212–214°, $[\alpha]_D$ +22·4° ($CHCl_3$, *c* 1·1), λ_{max} 262, 285 (sh), 310 (sh), 344, 361 nm (log ε 4·73, 4·31, 3·89, 3·22, 3·07); NMR: δ 9·33 (s, 1 H, C_5-H), 7·77 (d, J = 9 Hz, 1 H, C_1-H), 7·2 (d, J = 9 Hz, 1 H, C_2-H), 7·12 (s, 1 H, C_8-H), 4·07 (s, 3 H, OMe), 4·03 (s, 3H, OMe), 4·0 (s, 3H, OMe), 3·93 (s, 3H, OMe) (Found: C, 73·5; H, 7·3; N, 3·6. $C_{24}H_{27}NO_4$ requires: C, 73·3; H, 6·9; N, 3·6%). Mass spectrum: *m/e* 393 (M^+, 16), 324 (100), 309 (21), 294 (38), 279 (9), 278 (10), 251 (8). It was identical (TLC, UV, IR in CH_2Cl_2) with synthetic isotylocrebrine.[22] Further elution of the column with C_6H_6-$CHCl_3$ (1:1) and $CHCl_3$ yielded more of tylophorine (300 mg).



16: R = Me
17: R = H

Tubes 11–15 in the counter-current distribution gave tylophorinine contaminated with tylophorine and another base with M^+ at *m/e* 409. Purification of tylophorinine was effected by chromatography over Al_2O_3 in C_6H_6-$CHCl_3$ (1:1). The later fractions were converted to the hydrochloride and crystallised from EtOH to yield tylophorinine hydrochloride (0·3 g), m.p. 255–256° (d), pure by paper chromatography in n-BuOH-H_2O-AcOH (20:19:1). Basification with NH_4OH and crystallisation from $CHCl_3$-MeOH gave tylophorinine, m.p. 247–248° (d). The base with M^+ at *m/e* 409 was present in the mother liquor of the crystallisation of the hydrochloride but could not be obtained pure.

Tubes 16–30 gave a mixture of phenolic alkaloids. The base on keeping in CH_2Cl_2-ether at 0–5° overnight deposited **2** (200 mg) as a light yellow solid. Repeated crystallisation from CH_2Cl_2-MeOH gave pure tylophorinidine, m.p. 216–218° (d). Chromatography of the mother liquor on SiO_2 in CH_2Cl_2 and elution with CH_2Cl_2-MeOH (49:1) gave more tylophorinidine (100 mg). Tylophorinidine has $[\alpha]_D$ +105° ($CHCl_3$, *c* 1·1), λ_{max} 260, 287, 313, 340 nm (log ε 4·64, 4·41, 3·90, 3·17), shifted to λ_{max} 256, 298, 335 nm (log ε 4·73, 4·53, 4·0) on addition of NaOH, ν_{max}^{Nujol} 3540 cm^{-1} (OH). NMR: Table 1 (Found: C, 72·2; H, 6·7; N, 3·5. $C_{22}H_{23}NO_4$ requires: C, 72·3; H, 6·3; N, 3·8%); Mass spectrum: *m/e* 365 (M^+, 11), 296 (100), 281

(20), 268 (19), 267 (25), 253 (40), 225 (45), 210 (12), 209 (12), 181 (15), 165 (16), 163 (16), 146 (30).

Hofmann degradation of d-*isotylocrebrine*. A soln of **14** (200 mg) in CH_2Cl_2 (25 ml) was refluxed for 2 hr with MeI (3 ml) and left overnight at 30°. The soln was evaporated *in vacuo* and the residue shaken with Ag_2O (from 1·5 g of $AgNO_3$) and H_2O (20 ml) for 5 hr. The soln was filtered, evaporated *in vacuo* to dryness and the residue heated at 100° for ½ hr at 0·005 mm. Extraction with C_6H_6 and chromatography of the product over Al_2O_3 in C_6H_6 gave **15** (30 mg), m.p. 136–137° (from C_6H_6-hexane), identical (TLC, mixed m.p., mass spectra) with **15** from synthetic isotylocrebrine.[22] (Found: C, 73·9; H, 7·5. Calc. for $C_{25}H_{29}NO_4$: C, 73·7; H, 7·2%); Mass spectrum: *m/e* 407 (M^+, 100), 392 (56), 377 (23), 376 (15), 362 (17), 349 (16), 325 (20), 324 (40), 281 (12), 280 (30), 203·5 (23), 189 (13), 162 (69).

O-*Methyltylophorinidine* (**5**). A soln of **2** (250 mg) in CH_2Cl_2 (20 ml) and MeOH (5 ml) was treated with excess ethereal diazomethane. The soln was evaporated and residue crystallised from CH_2Cl_2-ether to yield **5** (150 mg), m.p. 229–231° (d), $[\alpha]_D$ + 161° ($CHCl_3$, *c* 1·5), λ_{max} 259, 286, 312, 340 nm (log ϵ 4·70, 4·42, 3·88, 3·08); NMR: Table 1 (Found: C, 73·1; H, 6·9; $C_{23}H_{25}NO_4$ requires: C, 72·8; H, 6·6%); Mass spectrum: *m/e* 379 (M^+, 13), 310 (100), 295 (8), 281 (7), 267 (6), 251 (2), 239 (5), 237 (6), 224 (9), 208 (3), 196 (4), 181 (3), 177 (3), 165 (6), 152 (4), 70 (12).

Acetyl O-*methyltylophorinidine* (**6**). O-Methyltylophorinidine (50 mg) was heated at 45–50° for 2 hr with Py (0·1 ml) and Ac_2O (0·2 ml) and then left overnight at 30°. H_2O was added and the soln basified with NH_4OH and extracted with CH_2Cl_2. The CH_2Cl_2 extract was washed with H_2O, dried (Na_2SO_4) and evaporated. The residue, crystallised from CH_2Cl_2-hexane, had m.p. 176–177°, $[\alpha]_D$ + 158° ($CHCl_3$, *c* 1·5), ν_{max}^{KBr} 1720 cm^{-1}, NMR: Table 2 (Found: C, 71·0; H, 6·8. $C_{25}H_{27}NO_5$ requires: C, 71·2; H, 6·5%); Mass spectrum: *m/e* 421 (M^+, 1), 361 (38), 360 (42), 357 (100), 352 (14), 344 (11), 342 (15), 340 (13), 333 (32), 314 (25), 313 (22), 310 (41), 299 (17), 294 (17), 281 (10), 271 (10), 251 (4), 241 (10), 237 (7), 228 (12), 181 (5), 165 (6), 152 (3), 114 (9).

Diacetyltylophorinidine (**7**). Tylophorinidine (120 mg) was heated with Py (1·5 ml) and Ac_2O (2 ml) at 40–45° for 24 hr. The soln was evaporated *in vacuo*, diluted with H_2O and worked up as above to yield the diacetate (60 mg), m.p. 193–195° (d) (from CH_2Cl_2-MeOH), ν_{max}^{KBr} 1760, 1730 cm^{-1}; NMR: Table 2 (Found: C, 69·4; H, 6·4; N, 2·9. $C_{26}H_{27}NO_6$ requires: C, 69·5; H, 6·1; N, 3·1%).

Desoxytylophorinidine (**8**). A soln of tylophorinidine (200 mg) in AcOH (30 ml) containing $HClO_4$ (60–70%; 0·2 ml) was reduced with H_2 at 45 lbs/in² in a Parr apparatus at 50–60° in pr of Pd-C (10%; 200 mg) for 3 hr. The soln was filtered, evaporated *in vacuo*, basified with NH_4OH and extracted with CH_2Cl_2. The product crystallised from CH_2Cl_2-MeOH to yield the desoxybase (20 mg), m.p. 214–218° (d); Mass spectrum: *m/e* 349 (M^+, 36), 281 (41), 280 (100), 265 (28), 254 (11), 237 (18), 194 (7), 174·5 (9), 165 (8).

Hydrogenolysis of O-*methyltylophorinidine*. (a) A soln of **5** (220 mg) in AcOH (40 ml) containing $HClO_4$ (60–70%; 0·3 ml) was shaken with H_2 at 40 lbs/in² in pr of Pd-C (10%; 400 mg) and worked up as above. Chromatography of the product over Al_2O_3 in CH_2Cl_2 yielded **3** (100 mg), m.p. 215–217° (d) (from CH_2Cl_2-MeOH) identical (TLC, mixed m.p., UV, IR, NMR and mass spectra) with the synthetic racemic base; $[\alpha]_D$ 0° ($CHCl_3$, *c* 0·9), λ_{max} 259, 287, 311 (sh), 341 nm (log ϵ 4·71, 4·45, 3·87, 3·02); NMR: Table 3; Mass spectrum: *m/e* 363 (M^+, 80), 294 (100), 279 (40), 251 (38), 236 (20), 220 (14), 208 (26), 189 (18), 181·5 (24), 165 (26).

(b) A soln of **5** (100 mg) in AcOH (10 ml) was heated at 110° for 2 hr with $HClO_4$ (60–70%; 0·3 ml) and evaporated *in vacuo*. The residue was dissolved in MeOH (10 ml) and treated with $NaBH_4$ (500 mg). After 3 hr at 30°, H_2O was added and the soln extracted with CH_2Cl_2 to yield **3** (60 mg), identical with the above product. Tylophorinine (200 mg) on similar treatment also gave *dl*-desoxytylophorinine (130 mg).

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