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ALKALOIDS FROM *TYLOPHORA INDICA**

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Key Word Index—*Tylophora indica*; Asclepiadaceae; rare phenanthroindolizidine alkaloids; tyloindicines A–E; (+)-14-hydroxyisotylocerebrine; 4,6-desdimethylisotylocrebrine; structural determination.

Abstract—Seven rare and four known alkaloids have been isolated from two varieties of *Tylophora indica*. The new alkaloids include tyloindicines A–E, (+)-14-hydroxyisotylocrebrine and 4,6-desdimethylisotylocrebrine. Tylophorine, 6-desmethyltylophorine, tylophorinidine and 5-hydroxy-*O*-methyltylophorinidine were the known alkaloids. Structural studies indicate that apart from tyloindicine B all alkaloids possess the dibenzo-[*f*, *h*] pyrrolo [1, 2*b*] isoquinoline skeleton but differ in the number, nature and distribution of the oxygen-bearing substituents, in the presence or absence of C-13a or benzylic hydroxyls and an angular methyl function. Tyloindicine B possesses a cleaved substituted phenanthrene moiety nucleus and an angular methyl group on the indolizidine portion.

INTRODUCTION

In a recent communication [2], we have ascribed antiamoebic activity to various phenanthroindolizidine alkaloids isolated by us earlier [3–5] from *Tylophora* and deduced many interesting structure-activity relationships [2]. In addition to amoebicidal properties, these alkaloids have been found to possess anticandidal activity [6]. The demonstration of these pharmacological properties further prompted us to extend our isolation work from *T. hirsuta* to *T. indica* species. The aerial parts of the latter have been extensively studied for their alkaloids by many workers [7–12]. However, during our bulk isolations of two varieties of *T. indica*, one cultivated in our sub-tropical campus, the other collected from tropical region of Madras, we encountered seven new alkaloids named as tyloindicines A–E, (+)-14-hydroxyisotylocrebrine and 4,6-desdimethylisotylocrebrine in addition to the four known alkaloids tylophorine, 6-desmethyltylophorine, tylophorinidine and 5-hydroxy-*O*-methyltylophorinidine. The structural elucidations of these alkaloids are described herein.

RESULTS AND DISCUSSION

The total alkaloidal mixture (0.17% yield) isolated from the aerial parts of the sub-tropical plant was chromatographed on basic alumina with solvents of increasing polarity. Tyloindicine A (**1**) and 6-desmethyltylophorine (**2**) were obtained from benzene eluant fractions in 0.013 and 0.003% yields, respectively. Further elution of the column with benzene–ethyl acetate (9:1) afforded tylophorine (**3**, 0.05% yield) and tylophorinidine (**4**, 0.003% yield). 5-Hydroxy-*O*-methyltylophorinidine (**5**, 0.006% yield) and tyloindicine B (**6**, 0.017% yield) were obtained from the benzene–ethyl acetate (3:1) and (1:1) eluates, respectively.

The total alkaloidal fraction (0.1% yield) isolated from the aerial parts of the tropical plant was also subjected to CC over basic alumina with solvents of increasing polarity. (+)-14-Hydroxyisotylocrebrine (**7**, 0.002% yield) and tylophorine (**3**, 0.03% yield) were obtained from the benzene–ethyl acetate (9:1) and (3:1) eluates, respectively. 4,6-Desdimethylisotylocrebrine (**8**) and tyloindicine C (**9**) were obtained as a mixture from the benzene–ethyl acetate (1:1) eluants. Alkaloid **8** (0.002% yield) was separated from **9** by fractional crystallization from methanol–acetone (1:1). The mother liquor upon further purification afforded **9** (0.001% yield). Further elution of the main column with ethyl acetate afforded tyloindicine D (**10**, 0.005% yield). Tyloindicine E (**11**, 0.0002% yield) was obtained in ethyl acetate–methanol (9:1) eluates.

Compound **1** possessed a different substitution pattern of the four methoxy groups from that observed in tylophorine [9, 12] tylocrebrine [13, 14] and isotylocrebrine [10] containing the same number of methoxyls. Alkaloid **6** had a cleaved phenanthroindolizidine nucleus as encountered earlier in the characterization of (+)-septicine [10] C-13a-hydroxysepticine [3] and tylohirsuticine [5], with an angular methyl function on the indolizidine moeity. The base **6** contained one methoxy and one hydroxyl function on C-3 and C-4, respectively, as detected in tylohirsuticine [5], but differed from this alkaloid in the attachment of an acetoxy group at C-7 whose presence was characterized for the first time in *Tylophora* alkaloids. Compound **7** resembled the alkaloid A of Rao [15] in the substitution pattern of four methoxyls and one benzylic hydroxyl but was of opposite specific rotation. Alkaloids **8** and **9** had isotylocrebrine type substituents arrangement on a phenanthrene nucleus with the exception of an extra angular methyl group. Compound **10** resembled closely 5-hydroxy-*O*-methyltylophorinidine (**5**) [5] reported earlier by us from *T. hirsuta* but differed from it in the absence of a 14-hydroxyl group.

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The physical and spectroscopic data for 6-desmethyltylophorine (**2**) [8], tylophorine (**3**) [9, 12] tylophorinidine (**4**) [10, 12] and 5-hydroxy-*O*-methyltylophorinidine (**5**) [5] were in agreement with those previously reported in the literature.

Tyloindicine A (**1**), mp 199–201°, $[M]^+$ *m/z* 393 ($C_{24}H_{27}NO_4$), $[\alpha]_D^{35}$ +7.2 (MeOH; *c* 2.1) had a different UV and IR spectral characteristics from the other alkaloids indicating a different substitution pattern of methoxy groups. The compound did not respond to ferric chloride, oxidizing reagents such as ceric sulphate, nitric acid, bromine water or chromic acid, acetic anhydride in pyridine and to diazomethane showing the absence of an hydroxyl group. Its IR spectrum did not show any band beyond 2900 cm^{-1}. The presence of a phenolic hydroxyl was also ruled out due to the absence of any shift in its UV spectrum on addition of sodium hydroxide. In its mass spectrum the base peak at *m/z* 323, arising from the cleavage of the characteristic pyrrolidine fragment by a retro-Diels–Alder reaction from the $[M]^+$ peak, supported the absence of any functional group in ring D or E. The ^1H NMR spectrum of **1** showed the presence of four aromatic protons at δ7.94 (*d*, *J* = 9 Hz), 7.70 (*d*, *J* = 9 Hz) 7.36 (*d*, *J* = 9 Hz) and 7.10 (*d*, *J* = 9 Hz) assigned to C-1, C-7, C-2 and C-8, respectively. The *ortho* value of the same coupling constants (*J* = 9 Hz) for all the four aromatic protons suggested the presence of two pairs of methoxy groups on adjacent positions in ring A and C. Out of the four possible combinations, three were ruled out on the basis of known spin–spin interactions for the aromatic protons in tylophorine [11] (C-1, C-4, C-5 and C-8 as *s*), tylocrebrine [14] (C-1, C-4, C-7 and C-8) as two *s* and two *d*) and isotylocrebrine [10] (C-1, C-2, C-5 and C-8 as two *s* and two *d*). Thus structure **1** has been assigned to tyloindicine A.

Tyloindicine B (**6**), mp 183–185°, $[M]^+$ at *m/z* 393 ($C_{24}H_{27}NO_4$), $[\alpha]_D^{35}$ +14.30° (MeOH; *c* 1.05) had UV maxima at 220, 230, 260, 310 and 355 nm. Its IR spectrum showed hydroxyl and carbonyl stretchings at 3370 and 1710 cm^{-1}, respectively. The hydroxyl group is phenolic in nature as indicated by a green coloration with ferric chloride and by the presence of a shift in its UV maxima at 230, 280, and 320 nm on addition of sodium hydroxide. Acetylation of **6** gave a monoacetylated product further confirming the presence of one hydroxyl group. A monomethyl ether was formed on treatment of **6** with diazomethane which could not be further acetylated proving the existence of one phenolic hydroxyl group. Deacetylation of the monomethyl ether afforded 7-deacetyltyloindicine B. The hydroxyl function of this product was shown to be phenolic in nature by a green coloration with ferric chloride, a shift in its UV spectrum from 228, 275 and 335 nm to 235 and 280 nm on addition of sodium hydroxide and by remethylation with diazomethane. The mass spectrum of **6** showed a $[M-Me]^+$ ion at *m/z* 378 (4.4%) and a $[M-Ac]^+$ ion at *m/z* 349 (1.3%) indicating the presence of an angular methyl function as well as acetyl group, respectively. The fragement at *m/z* 310 (10.6%) arose due to loss of 83 mass units in the form of methylpyrrolidine from $[M]^+$ by retro-Diels–Alder reaction as encountered earlier in phenanthroindolizine alkaloids [16]. An intense peak at *m/z* 295 (67%) resulted from the fragment at *m/z* 310 due to loss of 15 mass units. The base peak at *m/z* 252 arising from the loss of an acetyl group from *m/z* 295 was further indicative of the presence of an acetyl group. In the ^1H NMR spectrum the presence of seven aromatic protons indicated **6** to be a cleaved phenanthrene analogue like *d*-septicine [10], 13a-hydroxysepticine [3] and tylohirsuticine [5]. However, the position of the aromatic signals in **6** differed considerably from these alkaloids indicating a different substitution pattern. The seven aromatic protons appearing at δ8.14 (*d*, 1 H, *J* = 3 Hz), 7.90 (*dd*, 2H, *J* = 3 Hz and 9 Hz), 7.70 (*m* 1H), 7.16 (*m*, 2H) and 6.97 (*d*, 1H, *J* = 3 Hz) fitted well with the substitution arrangement shown in the cleaved form. In the light of earlier findings [3, 10] the shielded signal at δ6.97 has been assigned to C-8 in **6**. Because this proton appeared as doublet with a *m*-coupling constant (*J* = 3 Hz), the acetoxyl function could therefore be placed at C-7. The deshielded signal at δ8.14 could be assigned to the proton adjacent to the phenolic hydroxyl at C-4a. The doublets at δ7.90 and 7.16 integrating for two protons each have been assigned to the C-1, C-5a and C-2, C-5 protons, respectively. The methoxy protons appeared at δ3.80 along with a D_2O exchangeable (phenolic OH) proton at δ6.30. The acetoxy group, encountered for the first time in phenanthroindolizidine alkaloids, appeared as a singlet at δ2.52. Another singlet at δ1.60 integrating for three protons could be assigned to an angular methyl function as described in earlier work [5, 16]. The cleaved phenanthrene alkaloid **6**, for which the structure was deduced from the above data, has been named as tyloindicine B.

Alkaloid **7**, mp 240–242°, $[M]^+$ at *m/z* 409 ($C_{24}H_{27}NO_5$), $[\alpha]_D^{35}$ +30° (MeOH; *c* 0.3) showed one broad hydroxyl band 3450 cm^{-1} in its IR spectrum. The hydroxyl function was not phenolic, the compound giving no reaction with ferric chloride and diazomethane and no bathochromic shift in the UV spectrum with alkali. Its mass spectrum showed a weak peak at *m/z* 391 (3.5% due to loss of H_2O from $[M]^+$). A peak at *m/z* 339 (4.7%) arose due to loss of a pyrrolidine ring at *m/z* 70 (67.6%) from $[M]^+$ by a retro-Diels–Alder reaction. The base peak at *m/z* 310, which resulted from expulsion of CHO from *m/z* 339, indicated the hydroxyl function to be on C-14 [3, 13]. The ^1H NMR spectrum of **7** showed the presence of four methoxy groups at δ4.00, 3.92, 3.90 and 3.80; four aromatic protons at δ8.24 (*s*, 1H), 8.0 (*d*, *J* = 9 Hz), 7.28 (*d*, *J* = 9 Hz) and 7.10 (*s*, 1H) assigned to C-5, C-1, C-2, and C-8, respectively, one D_2O exchangeable proton at δ4.60 and signals at δ6.04 (*br s*) and δ3.40 (*br s*) due to protons attached to the hydroxyl carbon at C-14 and C-13a, respectively. The deshielding (by 0.21 ppm) of the aromatic proton at C-1 in **7** from the similar structure of isotylocrebrine [10] could be easily postulated due to the presence of a benzylic hydroxyl at C-14.

Acetylation of **7** yielded a monoacetylated product (ν_{max}^{KBr} 1735 cm^{-1}). The placement of the hydroxyl at C-14 instead of C-9 in isotylocrebrine alkaloids has been established conclusively by Rao [15]. These data led to structure **7** for the base which was identical to alkaloid A of Rao isolated from *T. crebriflora* [15]. However, alkaloid A possessed a (−)-rotation. It was concluded that the base **7** and Rao's alkaloid A were related to each other as diastereoisomers. As this new stereoisomer with (+)-rotation has been isolated from a natural source, it has been named (+)-hydroxyisotylocrebrine (**7**) in which OH-14 and H-13a are *trans* diaxially disposed. The hydroxyl is below the plane of the molecule and H-13a above it as observed for (+)-*O*-methyltylophorinidine [5].

Alkaloid (**8**), mp 177–179°, $[M]^+$ at *m/z* 365

($C_{22}H_{23}NO_4$), $[\alpha]_D^{35}+7.3°$ (MeOH; *c* 1.75) had UV maxima at 215, 228, 270, 310, 335 and 355 nm. The IR spectrum showed two hydroxyl bands at 3515 and 3405 cm^{-1}. The hydroxyl functions were shown to be phenolic by a green coloration with ferric chloride and the presence of a shift in its UV maxima at 230, 280 and 315 nm on addition of sodium hydroxide. Its mass spectrum showed an intense $[M]^+$ peak at *m/z* 365 (51.8%) with a base peak at *m/z* 295 which arose due to cleavage of the pyrrolidine fragment of *m/z* 70 (99%) from $[M]^+$ by a retro-Diels–Alder reaction. A prominent peak at *m/z* 280 (28.9%) was due to the loss of 15 mass units (Me) from the base peak. The ^1H NMR spectrum of **8** showed the presence of two methoxy groups at δ4.00 and 3.92; four aromatic protons at δ8.33 (*s*, 1H), 8.04 (*d*, 1H, *J* = 9 Hz), 7.26 (*d*, 1H, *J* = 9 Hz) and 7.10 (*s*, 1H), assigned to C-5, C-1, C-2 and C-8, respectively, two D_2O-exchangeable phenolic hydroxyls at δ7.83 and 6.96 and a broad signal at δ4.70 due to a proton attached to C-13a. The deshielded singlet at δ8.33 could be conveniently assigned to the proton on C-5 placed in between two phenolic hydroxyls, whereas the shielded singlet at δ7.10 was assigned to C-8. The shielded (δ7.26) and the deshielded (δ8.04) doublets integrating for one proton each have been assigned to C-2 and C-1 protons, respectively.

Acetylation of **8** gave a diacetylated product. Methylation of **8** with diazomethane formed isotylocrebrine. The ^1H NMR spectra of **8**, its diacetylated product, its dimethyl ether and isotylocrebrine were compared. There was a large deshielding (by δ1.00) of the signal due to the proton at C-5 in isotylocrebrine. The diacetylated product showed no such effect. The effect appears to be due to the increased electron as a result of its close proximity to the phenolic hydroxyls. Base **8** also responded to the Gibb's test. From these data structure **8** for the alkaloid named 4,6-desdimethylisotylocrebrine is suggested.

Tyloindicine C (**9**), mp 223–225°, $[M]^+$ at *m/z* 379 ($C_{23}H_{25}NO_4$), $[\alpha]_D^{35}+16°$ (MeOH; *c* 0.25), had UV maxima at 210, 230, 270, 310, 335 and 355 nm. The IR spectrum showed two hydroxyl bands at 3515 and 3400 cm^{-1}. The presence of phenolic hydroxyls was confirmed by a green coloration with ferric chloride and a shift in its UV maxima at 225, 278 and 317 nm on addition of sodium hydroxide. Its mass spectrum showed a peak at *m/z* 364 (14.9%) due to loss of 15 mass units from the $[M]^+$ which indicated the presence of an angular methyl group. The peaks at *m/z* 310 (8.0%) and 296 (93.8%) originated from $[M-69]^+$ and $[M-83]^+$ ions, respectively by retro-Diels–Alder reactions characteristic of phenanthroindolizidine alkaloids. The loss of methylpyrrolidine at *m/z* 83 (6.6%) along with a pyrrolidine fragment at *m/z* 69 (100%) further confirmed the presence of an angular methyl function. The ^1H NMR spectrum of **9** was almost identical to that of **8** in the aromatic region. It showed the presence of two methoxy groups at δ3.80 and 3.70, four aromatic protons at δ8.03 (*s*, 1H), 7.90 (*d*, 1H, *J* = 9 Hz), 7.00 (*d*, 1H, *J* = 9 Hz) and 6.79 (*s*, 1H), assigned to C-5, C-1, C-2 and C-8, respectively, two D_2O exchangeable phenolic protons at δ7.66 and 6.60 and a methyl singlet at δ1.60 indicative of an angular methyl function. The arguments given for the structure elucidation of **8** also holds good for **9** with an addition of an angular methyl function. Acetylation of **9** yielded a diacetylated product. Because methylation of **9** with diazomethane afforded a dimethyl ether identical to 13a-methyltylohirsutine, the structure **9** for the alkaloid named tyloindicine C was assigned.

Tyloindicine D (**10**), mp 229–231° (decomp), $[M]^+$ at *m/z* 409 ($C_{24}H_{27}NO_5$), $[\alpha]_D^{35}+1.6°$ (MeOH; *c* 0.06) closely resembled the isotylocrebrine-type (**7**, **8** and **9**) and tylophorinidine-type (**5**), alkaloids in UV and IR spectral behaviour indicating the same substitution pattern of the methoxy and hydroxyl groups. The presence of a phenolic hydroxyl was indicated by the shift in its UV maxima at 222, 230, 272, 318, 332 and 350 nm to 230, 280, 320 and 350 nm on addition of sodium hydroxide. The IR spectrum showed a strong band at *ca* 3250 cm^{-1}. Its mass spectrum showed a peak at *m/z* 339 (7.5%) arising from the expulsion of a neutral pyrrolizidine ring at *m/z* 70 (100%) by a characteristic Diels–Alder reaction. The peaks at *m/z* 324 (11.1%) 309 (14.7%) and 294 (13.5%) arose by cleavage of methyl fragments in a stepwise manner from *m/z* 339. The ^1H NMR spectrum of **10** showed the presence of one methoxy group at δ4.10, three methoxy groups at δ3.92, three aromatic protons at δ8.00 (*d*, 1H, *J* = 9 Hz), 7.20 (*d*, 1H, *J* = 9 Hz) and 7.16 (*s*, 1H) assigned to C-1, C-2 and C-8, respectively, and one D_2O-exchangeable proton at δ7.80 (phenolic OH). The ^1H NMR values of the aromatic protons at C-1, C-2 and C-8 in **10** were in agreement with corresponding values of 5-hydroxy-*O*-methyltylophorinidine [5] isolated from wild population of *T. hirsuta*. Compound **10** on acetylation gave a monoacetylated product. A monomethyl ether was formed on treatment with diazomethane which in turn could not be acetylated proving the presence of only one phenolic hydroxy (further proof of the phenolic hydroxyl at C-4 came from the positive Gibb's test for a free-hydroxyl group). These data led to structure **10** for the alkaloid named tyloindicine D.

Tyloindicine E (**11**), mp 350° (decomp), $[M]^+$ at *m/z* 349 ($C_{22}H_{23}NO_3$), $[\alpha]_D^{35}-12°$ (MeOH; *c* 0.25) had UV maxima at 210, 232, 277, 310 sh, 338 and 357 nm. The presence of a phenolic hydroxyl was indicated by the shift in its UV maxima at 226, 284 and 322 nm on addition of sodium hydroxide. The IR spectrum showed a band at 3450 cm^{-1}. Acetylation of **11** yielded a monoacetyl derivative. Methylation of **11** with diazomethane formed a monomethyl ether, desoxypergularinin [9], which could not be acetylated further. Its mass spectrum showed a weak $[M]^+$ at *m/z* 349 (4.6%) with a more pronounced peak at *m/z* 279 (16.4%) arising from elimination of the characteristic pyrrolidine nucleus at *m/z* 70 (15%). The base peak at *m/z* 265 resulted from expulsion of 14 mass units from the fragment at *m/z* 279. The ^1H NMR spectrum of **11** showed the presence of two methoxy functions at 3.94 and 3.42; five aromatic protons at δ7.96 (*d*, *J* = 9 Hz), 7.72 (*s*), 7.62 (*d*, *J* = 3 Hz), 7.20 (*d*, *J* = 9 Hz) and 7.04 (*s*) assigned to C-1, C-5, C-4, C-2 and C-8, respectively and one D_2O exchangable proton, at δ8.14 (phenolic OH). These data led to structure **11** for the alkaloid tyloindicine E.

EXPERIMENTAL

Mps: uncorr. ^1H NMR δvalues are given in ppm downfield from TMS. TLC was carried out on silica gel in C_6H_6–EtOAc–Et_2NH (6:3:1), spots were detected with Dragendroff's reagent.

Isolation of alkaloids from sub-tropical plants. Air-dried aerial parts of *T. indica* (7.0 kg), grown at RRL campus, were collected during October 1984 and extd with MeOH. The EtOAc-sol. brown coloured crude total alkaloids (12 g, 0.17%) were scpd as

described in refs [3–5]. On TLC these showed seven major and two minor components. The EtOAc–sol. portion was concd *in vacuo* and subjected to CC over basic Al_2O_3 after the formation of a slurry. The column was eluted with mixts of C_6H_6, EtOAc and MeOH of increasing polarity.

Tyloindicine A. (**1**). Fr. 1 eluted with C_6H_6 gave **1** (0.9 g, 0.013% yield; from Me_2CO), mp 199–201°, $[\alpha]_D^{35}+7.2$ (MeOH; *c* 2.1); UV λ_{max}^{MeOH} nm: 228, 280, 335, 355 and 375 (log ε 4.09, 4.85, 1.72, 1.69 and 1.66). IR ν_{max}^{KBr} cm^{-1}: 2900, 1600, 1510, 1460,1410, 1290, 1205, 1160, 1110, 1025 and 780. ^1H NMR (DMSO-d_6 + 1drop of TFA): δ7.94 (*d*, *J* = 9 Hz, *o*-coupled H, C-1), 7.70 (*d*, *J* = 9 Hz, *o*-coupled H, C-7), 7.36 (*d*, *J* = 9 Hz, *o*-coupled H, C-2), 7.10 (*d*, *J* = 9 Hz, *o*-coupled H, C-8), 5.40 (*m*, C-13a H), 4.00 (*br s*, 12 H, 4 OMe); MS *m/z* (rel.int.): 393 $[M]^+$ ($C_{24}H_{27}NO_4$, 32.1), 377 (0.2), 363 (7.1), 323 (100), 308 (16.9), 293 (26.8), 85 (24.2), 83 (72.7), 70 (7.9).

6-Desmethyltylophorine (**2**). Frs 2–3 from the main column eluted with C_6H_6 gave **2** (0.2 g, 0.003% yield; from $CHCl_3$–MeOH (1:1), mp 234–236° (decomp.), (lit. mp. 235–237° decomp), $[\alpha]_D^{35}-166.6°$ (MeOH; *c* 0.12); UV λ_{max}^{MeOH} nm; 210, 225, 270, 305, 330 and 350 (log ε 6.06, 7.58, 6.61, 2.87, 3.1 and 2.04); λ_{max}^{MeOH} (on addition of NaOH) nm: 230, 280 and 320 (log ε 9.52, 9.29 and 4.20). MS *m/z* (rel. int.): 379 $[M]^+$ ($C_{23}H_{25}NO_4$, 30.8), 364 (11.8), 348 (28.1), 309 (26.5), 295 (76.0), 279 (100) and 70 (88.1). Acetylation of **2** with Ac_2O–pyridine at room temp for 24 hr and usual work-up gave a monoacetylated product, mp 212–213°, IR λ_{max}^{KBr} 1725 and 1225 cm^{-1}. Methylation of **2** with CH_2N_2 gave tylophorine, mp and mmp 290–292° (decomp.).

Tylophorine (**3**). Frs 4–5 from the main column eluted with C_6H_6–EtOAc (9:1) afforded **3** (3.6 g, 0.05% yield, from $CHCl_3$–MeOH), mp and mmp 290–292° (decomp.) [lit mp 290–292° (decomp)]. $[\alpha]_D^{35}-22.60°$ (MeOH; *c* 1.2). MS, *m/z* (rel. int.) 393 $[M]^+$ ($C_{24}H_{27}NO_4$, 82.6).

Tylophorinidine (**4**). Fr. 6 from the main column eluted with C_6H_6–EtOAc (9:1) gave **4** (0.22 g, 0.003% yield, from Me_2CO); mp 215–217° (lit. mp 213–214°), $[\alpha]_D^{35}+122°$ (MeOH; *c* 0.48), (lit. $[\alpha]_D+125°$ (MeOH; *c* 0.1). MS *m/z* (rel. int.); 365 $[M]^+$ ($C_{22}H_{23}NO_4$, 15.5).

5-Hydroxy-O-methyltylophorinidine (**5**). Fr 7 from the main column eluted with C_6H_6–EtOAc (3:1) gave **5** (0.4 g, 0.006% yield, from Me_2CO), mp and mmp 243–245° (decomp) [lit. mp 245–247°] $[\alpha]_D^{38}+55.85°$ (MeOH; *c* 0.65), (lit $[\alpha]_D^{18}+58.97°$ (MeOH; *c* 0.8), MS *m/z* (rel. int.): 395 $[M]^+$ ($C_{23}H_{25}NO_5$, 5.2).

Tyloindicine B (**6**). Frs 8–9 eluted with C_6H_6–EtOAc (1:1) gave beads of **6** (0.75 g, 0.017% yield, from $CHCl_3$), mp 183–185°, $[\alpha]_D^{35}+14.3°$ (MeOH; *c* 1.05), UV λ_{max}^{MeOH} nm; 220, 230, 270, 310 and 355 (log ε 1.96, 1.72, 0.32, 5.61, 21.81 and 20.63), λ_{max}^{MeOH} (on addition of NaOH) nm: 230, 288 and 320 (log ε 0.59, 0.35 and 7.03); IR ν_{max}^{KBr} cm^{-1}: 3370, 2950, 1710, 1615, 1520, 1465, 1420, 1230, 1195, 1160, 1105, 1035 and 815. ^1H NMR (DMSO-d_6): δ8.14 (*d*, *J* = 3 Hz, *m*-coupled H, C-4a), 7.90 (*dd*, *J* = 9 and 3 Hz, 2H, *o*-, *m*-coupled H, C-1, C-5a), 7.70 (*m*, 1H C-6), 7.16 (*m*, 2H, C-2, C-5), 6.97 (*d*, *J* = 3 Hz, *m*-coupled H, C-8), 6.30 (*br s*, D_2O exchangable, OH), 3.80 (*s*, 3H, OMe), 2.52 (*s*, 3H, OAc), 1.60 (*s*, 3H, C-13a Me). MS *m/z* (rel. int.): 393 $[M]^+$ ($C_{24}H_{27}NO_4$, 0.2), 378 (4.4), 365 (10.5), 349 (1.3), 329 (0.2), 311 (10.8), 310 (10.6), 295 (67), 252 (100), 83 (6.2), 69 (60.3), 43 (79.7). Acetylation of **6** with Ac_2O–pyridine at room temp gave a monoacetylated product, mp 165–167°, IR ν_{max}^{KBr} 1745 and 1715 cm^{-1}, MS *m/z* (rel. int.): 435 $[M]^+$ ($C_{26}H_{29}NO_5$, 12). Methylation of **6** with CH_2N_2 gave a mono Me ether, mp 206–209°, $C_{25}H_{29}NO_4$ ($[M]^+$ 407). Deacetylation of the mono Me ether with dil HCl (10%) yielded 7-deacetyltyloindicine B 4-Me ether, mp 218–219°, UV λ_{max}^{MeOH} nm: 228, 275 and 335 (log ε 3.65, 4.38 and 2.26), UV λ_{max}^{MeOH} (on addition of NaOH) nm: 235 and 280 (log ε 1.2 and 1.09), IR ν_{max}^{KBr} 3430 cm^{-1}, $C_{23}H_{27}NO_3$ ($[M]^+$ 365). Methylation of the deacetylated product gave a mono Me ether (confirmed by TLC comparison).

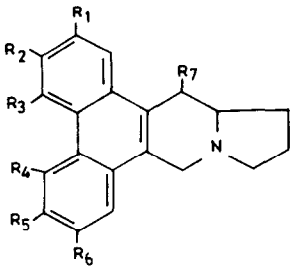
Isolation of alkaloids from tropical plants. Air-dried aerial parts *T. indica* (25 kg) supplied by M/s Pharmaproducts, Madras, India were extd exhaustively by hot percolation with EtOH and the EtOAc-sol. brown coloured alkaloids (24 g, 0.096% yield) sepd as described in refs [3–5]. On TLC it showed six major and one minor components. The EtOAc–sol. portion was concd *in vacuo* and subjected to CC over basic Al_2O_3 after the formation of a slurry. The column was eluted with mixts of C_6H_6, EtOAc and MeOH of increasing polarity.

14-Hydroxyisotylocrebrine (**7**). Fr 1, eluted with C_6H_6–EtOAc (9:1) on crystallization from Me_2CO gave a light yellow powder of **7** (0.5 g, 0.002% yield), mp 240–242°, $[\alpha]_D^{35}+30°$ (MeOH; *c* 0.3); UV λ_{max}^{MeOH} nm: 210, 275, 335 and 350 (log ε 4.66, 5.42, 2.50 and 2.45); IR ν_{max}^{KBr} cm^{-1}: 3450, 2910, 1615, 1505, 1475, 1420, 1255, 1205, 1160, 1115, 1035 and 830. ^1H NMR (DMSO-d_6): δ8.24 (*s*, *p*-coupled H, C-5), 8.00 (*d*, *J* = 9 Hz, *o*-coupled H, C-1), 7.28 (*d*, *J* = 9 Hz, *o*-coupled H, C-2), 7.10 (*s*, *p*-coupled H, C-8), 6.04 (*br s*, 1H, C-14), 4.60 (*br s*, D_2O exchangeable, OH), 4.0 (*s*, 3H, OMe), 3.92 (*s*, 3H, OMe), 3.90 (*s*, 3H, OMe), 3.80 (*s*, 3H, OMe), 3.40 (*m*, 1H, C-13a), 2.60 (*m*, CH_2), 2.40 (*m*, CH_2), 1.80 (*m*, CH_2). MS *m/z* (rel. int.): 409 $[M]^+$ ($C_{24}H_{27}NO_5$, 17.0), 391, (3.5), 377 (13.8), 339 (4.7), 310 (100), 295 (5.6), 280 (5.0), 265 (3.6), 70 (67.6), 29 (6.7) and 18 (99). Acetylation of **7** with Ac_2O–pyridine at room temp. for 24 hr and usual work-up gave a monoacetylated product, mp 211–212°, IR ν_{max}^{KBr} cm^{-1}: 1735.

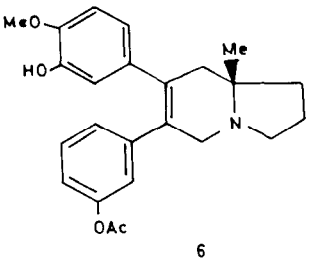
Tylophorine (**3**). Frs 2–3 from the main column eluted with C_6H_6–EtOAc (3:1) afforded light yellow crystals of **3** (7.5 g, 0.03% yield, from $CHCl_3$–MeOH), mp and mmp 291–292° (decomp), $[\alpha]_D^{35}-21.05°$ (MeOH; *c* 0.7); MS *m/z* (rel. int): 393 $[M]^+$ ($C_{24}H_{27}NO_4$, 82.4).

4,6-Desmethylisotylocrebrine (**8**). Frs 4–5 from the main column, eluted with C_6H_6–EtOAc (1:1) on crystallization from Me_2CO (1:1), gave colourless beads of **9** (0.5 g, 0.002% yield), mp 177–179°, $[\alpha]_D^{35}+7.3°$ (MeOH; *c* 1.75), UV λ_{max}^{MeOH} nm: 215, 228, 270, 310, 335 and 355 (log ε 11.68, 13.0, 13.87, 4.0, 2.0 and 1.80), λ_{max}^{MeOH} (on addition of NaOH) nm; 230, 280, and 315 (log ε 18.25, 19.15 and 5.87), IR ν_{max}^{KBr} cm^{-1}: 3515, 3405, 2910, 1615, 1525, 1510, 1465, 1420, 1260, 1230, 1200, 1160, 1100, 1035 and 820. ^1H NMR (DMSO-d_6): δ8.33 (*s*, *p*-coupled H, C-5), 8.04 (*d*, *J* = 9 Hz, *o*-coupled H, C-1), 7.83 (*br s*, D_2O exchangeable, OH), 7.26 (*d*, *J* = 9 Hz, *o*-coupled H, C-2), 7.10 (*s*, *p*-coupled H, C-8), 6.96 (*s*, D_2O exchangeable, OH), 4.70 (*br s*, 1H, C-13a), 4.00 (*s*, 3H, OMe), 3.92 (*s*, 3H, OMe), 2.52 (*m*, CH_2), 2.20 (*m*, CH_2), 1.80 (*m*, CH_2): MS *m/z* (rel. int.): 365 $[M]^+$ ($C_{22}H_{23}NO_4$, 51.8), 295 (100), 281 (22.5), 280 (28.9), 267 (19.6), 265 (9.0), 253 (27.9), 252 (15.0), 225 (25.1), 224 (12.5), 70 (99.0). Acetylation of **8** with Ac_2O–pyridine at room temp. for 24 hr and usual work-up gave a diacetylated product, mp 165–167°, IR ν_{max}^{KBr} 1760 and 1755 cm^{-1}. MS *m/z* (rel. int.): 449 $[M]^+$ ($C_{26}H_{27}NO_6$, 15.0). Methylation of **8** with CH_2N_2 gave isotylocrebrine, mp and mmp 211–213° (lit mp 212–214°).

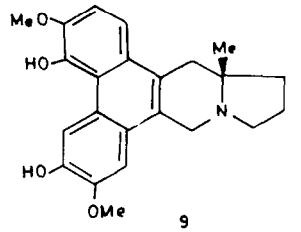
Tyloindicine C (**9**). The dried mother liquor of **8** was chromatographed over basic Al_2O_3 in EtOAc to obtain **9** (0.25 g, 0.001% yield) as brown beads after crystallization from Me_2CO, mp 223–225°; $[\alpha]_D^{35}+16°$ (MeOH; *c* 0.25); UV λ_{max}^{MeOH} nm: 210, 230, 270, 310, 335 and 355 (log ε 13.15, 15.62, 18.25, 3.95, 2.12 and 2.04), λ_{max}^{MeOH} (On additon of NaOH) nm: 225, 278 and 317 (log ε 18.65, 20.15 and 6.12). IR ν_{max}^{KBr} cm^{-1}: 3510, 3405, 2925, 1615, 1525, 1465, 1425, 1260, 1235, 1195, 1160 and 812. ^1H NMR (DMSO-d_6); δ8.03 (*s*, *p*-coupled H, C-5), 7.90 (*d*, *J* = 9 Hz, *o*-coupled H, C-1),; 7.66 (*br s*, D_2O exchangeable, OH), 7.00 (*d*, *J* = 9 Hz, *o*-coupled H, C-2), 6.78 (*s*, *p*-coupled H, C-8), 6.60 (*br s* D_2O-exchangeable, OH), 3.80 (*s*, 3H, OMe), 3.70 (*s*, 3H, OMe), and 1.60 (*s*, 3H, C-Me). MS *m/z* (rel. int.): 379 $[M]^+$



1 $R_1=R_6=R_7=H$; $R_2=R_3=R_4=R_5=OMe$
2 $R_3=R_4=R_7=H$; $R_1=R_2=R_6=OMe$; $R_5=OH$
3 $R_3=R_4=R_7=H$; $R_1=R_2=R_5=R_6=OMe$
4 $R_1=R_3=R_4=H$; $R_2=R_6=OMe$; $R_5=R_7=OH$
5 $R_1=R_3=H$; $R_2=R_5=R_6=OMe$; $R_4=R_7=OH$
7 $R_1=R_4=H$; $R_2=R_3=R_5=R_6=OMe$; $R_7=OH$
8 $R_1=R_4=R_7=H$; $R_2=R_6=OMe$; $R_3=R_5=OH$
10 $R_1=R_7=H$; $R_2=R_3=R_5=R_6=OMe$; $R_4=OH$
11 $R_1=R_3=R_4=R_7=H$; $R_2=R_6=OMe$; $R_5=OH$



6



9

(C23H25NO4, 12.2), 364 (14.9), 349 (5.9), 334 (11.6), 310 (8.0), 296 (93.8), 281 (6.4), 267 (5.8), 83 (6.6), 69 (100). Acetylation of **9** with Ac_2O–pyridine at room temp for 24 hr and usual work-up gave a diacetylated product, mp 176–177°; IR ν cm^{-1}: 1760 and 1755, MS *m/z* (rel. int.): 463 $[M]^+$ ($C_{27}H_{29}NO_6$, 35.5). Methylation of **9** with CH_2N_2 gave a diMe ether, mp 198–200°. This product was comparable (mmp, TLC, co-TLC, UV and IR) with 13a-methyltylohirsutine [7].

Tyloindicine D (**10**). Further elution of the main column with EtOAc gave a light yellow powder of **10** (0.125 g, 0.005% yield) in frs 6 and 7 after crystallization from Me_2CO; mp 229–231° (decomp.), $[\alpha]_D^{35}+1.6°$ (MeOH; *c* 0.6); UV λ_{max}^{MeOH} nm: 222, 230, 272, 318, 332 and 350 (log ε 8.2, 10.2, 10.5, 4.1, 2.4 and 2.2): λ_{max}^{MeOH} (on addition of NaOH) nm: 230, 280, 320 and 350 (log ε 20.5, 21.2, 6.76 and 4.3) IR ν_{max}^{KBr} cm^{-1}; 3250, 2930, 1615, 1510, 1462, 1420, 1260, 1225, 1190, 1145, 1040 and 815. ^{1}H NMR (DMSO-d_6): δ8.00 (*d*, $J=9$ Hz, *o*-coupled H, C-1), 7.80 (*br s*, D_2O-exchangeable, OH), 7.20 (*d*, $J=9$ Hz, *o*-coupled H, C-2), 7.16 (*s*, C-8), 4.60 (*br s*, 1H, C-13a), 4.10 (*s*, 3H, OMe), 3.96 (*s*, 9H, 3 OMe). MS *m/z* (rel. int.): 409 $[M]^+$ ($C_{24}H_{27}NO_5$, 3.2), 394 (7.1), 379 (10.5), 364 (13.8), 339 (7.5), 324 (11.1), 309 (14.7), 296 (17.6), 295 (86.3), 294 (13.5), 280 (5.6), 266 (5.3), 252 (5.8) and 70 (100). Acetylation of **10** with Ac_2O–pyridine at room temp. for 24 hr and usual work-up gave a monoacetylated product, mp 193–194°; IR ν_{max}^{KBr} cm^{-1}: 1755. Methylation of **10** with CH_2N_2 gave a mono Me ether, mp 217–219°; $C_{25}H_{29}NO_5$ ($[M]^+$ 423).

Tyloindicine E (**11**). Frs 8–10 eluted with EtOAc–MeOH (9:1) gave a brown coloured powder of **11** (0.05 g, 0.0002% yield) on crystallization from MeOH, mp 350° (decomp.), $[\alpha]_D^{35}-12°$ (MeOH; *c* 0.25), UV λ_{max}^{MeOH} nm: 210, 232, 277, 310 *sh*, 338 and 357 (log ε 2.44, 2.83, 2.40, 0.68, 0.23 and 0.22), λ_{max}^{MeOH} on addition of NaOH) nm: 226, 284 and 322 (log ε 3.4, 3.2 and 1.0); IR ν_{max}^{KBr} cm^{-1}: 3450, 2925, 1610, 1520, 1460, 1405, 1265, 1240, 1190, 1155 and 815. ^{1}H NMR (DMSO-d_6): δ8.14 (*br s*, D_2O exchangeable, OH), 7.96 (*d*, $J=9$ Hz, *o*-coupled H, C-1), 7.72 (*s*, *p*-coupled H, C-5), 7.62 (*d*, $J=3$ Hz, *m*-coupled H, C-4), 7.20 (*d*, $J=9$ Hz, *o*-coupled H, C-2), 7.04 (*s p*-coupled H, C-8), 4.23 (*br s*, C-13a), 3.94 (*s*, 3 H, OMe) and 3.42 (*s*, 3H, OMe). MS *m/z* (rel. int.): 349 ($C_{22}H_{23}NO_3$, 4.6), 320 (2.6), 279 (16.4), 265 (100), 250 (10.9), and 70 (15.0). Acetylation of **11** with Ac_2O–pyridine at room temp gave a monoacetylated product, mp 280–282°, IR ν_{max}^{KBr} 1735 cm^{-1}, $C_{24}H_{25}NO_4$ ($[M]^+$ 391). Methylation of **11** with CH_2N_2 gave desoxypergularinin, mp 209–210° (decomp.) (lit. mp 208–209°), $[\alpha]_D^{35}-12.8°$ (MeOH, *c* 0.2) (lit $[\alpha]_D^{25}-13.6$, $CHCl_3$; *c* 0.25), $C_{23}H_{25}NO_3$ ($[M]^+$ 363, 90%).

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