Rare Phenanthroindolizidine Alkaloids and a Substituted Phenanthrene, Tyloindane, from Tylophora indica

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RARE PHENANTHROINDOLIZIDINE ALKALOIDS AND A SUBSTITUTED PHENANTHRENE, TYLOINDANE, FROM *TYLOPHORA INDICA*

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ABSTRACT.—From the aerial parts of *Tylophora indica*, five new dihydrophenanthroindolizidine alkaloids, tyloindicines F [**2**], G [**3**], H [**4**], I [**5**], and J [**6**], and a benzcyclopent-substituted phenanthrene derivative, tyloindane [**1**], have been isolated along with tylophorine. The structures have been determined by analysis of spectral data and chemical reactions.

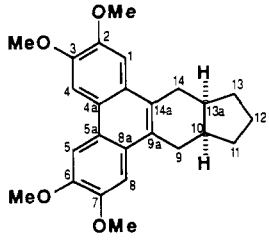
Recently, structural elucidation of five new phenanthroindolizidine alkaloids, tyloindicines A–E, isolated from *Tylophora indica* (Burm. f.) Merr. (syn. *Tylophora asthmatica* W.&A.) (Asclepiadaceae), has been reported (1). The present paper deals with the characterization of another five bases, designated as tyloindicines F [**2**], G [**3**], H [**4**], I [**5**], and J [**6**], and a substituted phenanthrene hydrocarbon, tyloindane [**1**]. In addition, tylophorine was isolated.

RESULTS AND DISCUSSION

The total alkaloidal fraction (0.52%) isolated from the aerial parts of the plant was triturated with EtOAc to give an insoluble amorphous powder of tyloindane [**1**] with the bases retained in the solution. The EtOAc-soluble mixture was chromatographed over basic Al_2O_3 with solvents of increasing polarity. Tyloindicine F [**2**] was obtained in 0.004% yield from C_6H_6-EtOAc (1:1). Further elution of the column with C_6H_6-EtOAc (1:3) afforded tylophorine (0.015%), tyloindicine G [**3**] (0.001%), and tyloindicine H [**4**] (0.0013%). Tyloindicine I [**5**] (0.009%) and tyloindicine J [**6**] (0.006%) were obtained by elution with EtOAc. The physical and spectroscopic data of tylophorine were identical with those of the authentic alkaloid.

Tyloindane [**1**], mp 278–279° (dec), $C_{25}H_{28}O_4$ ($[M]^+$ 392), had different uv and ir characteristics from the *Tylophora* alkaloids. The compound did not react with $FeCl_3$ and oxidizing reagents such as ceric sulfate, HNO_3, bromine H_2O, or chromic acid. The absence of reaction with Ac_2O in pyridine or CH_2N_2 ruled out the presence of a hydroxyl group. The ir spectrum did not show any absorption bands beyond 2905 cm^{-1}. The presence of a phenolic hydroxyl group was also ruled out because of the absence of any shift in the uv spectrum on addition of NaOH. The compound did not show positive results in any nitrogen or alkaloid tests, and it was insoluble in HCl, indicating further the non-alkaloidal nature of the molecule. The base peak at *m/z* 324 in the mass spectrum arose due to expulsion of cyclopentene (mu 68), by a retro-Diels-Alder reaction from $[M]^+$, supporting the absence of any functional group in ring D or E.

The ^{1}H-nmr spectrum of **1** exhibited the presence of a singlet integrating for two protons at δ 7.70 assignable to H-4 and H-5; two one-proton singlets at δ 7.13 and 6.90 attributable to H-1 and H-8, respectively; and a broad singlet at δ 3.56 integrating for twelve protons due to the presence of four methoxy groups. A two-proton broad multiplet at 2.13 ($W\frac{1}{2}$ = 16 Hz) was assigned to axial α-protons at C-10 and C-13a since equatorial-axial and equatorial-equatorial integrations would be expected to have low coupling constants. The spin-spin couplings for the aromatic protons were identical to those detected in ^{1}H-nmr spectra of tylophorine (7) and 13a-hydroxytylophorine (2). On the basis of this evidence, the structure of **1**, which possessed a 9,10,13a,14-tetrahydro-1′,4′-(2,3-dimethoxybenzo)-10,13a-cyclopenta-6,7-dimethoxyphenanthrene skeleton, was established.



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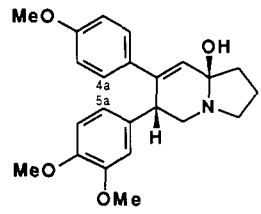
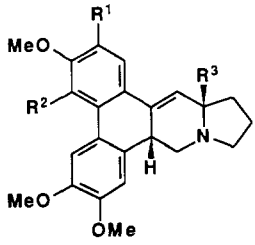
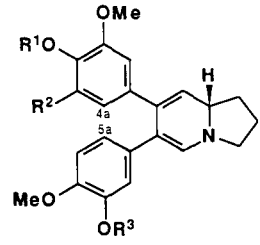


2



3 R_1=OMe, R_2=H, R_3=OH
4 R_1=R_3=H, R_2=OH
7 R_1=R_3=H, R_2=OMe
8 R_1=R_3=H, R_2=OAc



5 R_1=R_3=Me, R_2=OH
6 R_1=R_2=H, R_3=Ac
9 R_1=R_3=Me, R_2=OAc
10 R_1=R_3=Me, R_2=OMe
11 R_1=R_3=Ac, R_2=H
12 R_1=Me, R_2=H, R_3=Ac
13 R_1=Me, R_2=R_3=H

Tyloindicine F [**2**], mp 245–247° (dec), $C_{23}H_{27}NO_4$ ([M]$^+$ 381), showed a hydroxyl band at 3350 cm^{-1} in the ir spectrum. It was found to be non-phenolic due to absence of any shift in its uv maxima on addition of NaOH and resistance to $FeCl_3$ and to CH_2N_2. It also resisted acetylation with Ac_2O/pyridine, suggesting the tertiary nature of the hydroxyl group. The mass spectrum showed a peak [M − H_2O]$^+$ at *m/z* 363. A peak at *m/z* 296, due to loss of hydroxypyrrolidine from [M]$^+$, indicated the presence of a phenanthroindolizidine skeleton as observed in 13a-substituted *Tylophora* alkaloids (6,8). In the ^{1}H-nmr spectrum, the presence of seven aromatic protons suggested **2** to be a cleaved phenanthrene analogue similar to (+)-septicine (4), 13a-hydroxyseptic*i*ne (5), or tylohirsuticine (6). However, the position of the aromatic signals in **2** differed considerably from these alkaloids, indicating a different substitution pattern of the three methoxy and one hydroxy functions. In the ^{1}H-nmr spectrum, two downfield multiplets at δ 8.00 and δ 7.70, integrating for one and two protons, were assigned to H-4 and H-1, H-5 protons, respectively. A two-proton multiplet at δ 7.10 was attributed to H-2 and H-4a. A signal at δ 6.96 was assigned to H-5a. A doublet at δ 6.73 (J = 3.0 Hz) was assignable to H-8. A one-proton singlet at δ 5.60 indicated the presence of a 14-olefinic proton in a six-membered ring. These protons showed a substitution pattern characteristic of cleaved phenanthroindolizidine alkaloids. The three methoxy groups appeared at δ 3.73 (2 × MeO) and δ 3.66 (1 × MeO). A D_2O exchangeable proton appeared at δ 5.00 (angular OH). A one-proton triplet at δ 2.83 (J = 4 Hz) was assigned to the angular β-H-9a (equatorial). Further, tyloindicine F with a (−)-rotation possessed an α-13a-OH since dextrorotatory phenanthroindolizidine alkaloids contain a β-arrangement at C-13a (4,9). The structure of the cleaved phenanthrene alkaloid, tyloindicine F, was determined as **2** which possessed a 9,9a,11,12,13,13a,14a-hexahydro-3,6,7-trimethoxybenzo[*f,h*]pyrrolo[1,3-*b*]isoquinoline skeleton.

Tyloindicine G [**3**], mp 237–238° (dec), $C_{24}H_{27}NO_5$ ([M]$^+$ at 409), showed one tertiary hydroxy group at 3450 cm^{-1} in the ir spectrum. Compound **3** did not react with $FeCl_3$, CH_2N_2, or Ac_2O/pyridine. The ms exhibited ion fragments at *m/z* 393 [M − Me]$^+$, 379 [393 − Me]$^+$, and 361 [379 − H_2O]$^+$. The base peak at *m/z* 324 arose due to loss of 85 mu of hydroxypyrrolidine. The ^{1}H-nmr spectrum of **3** showed a broad signal integrating for 12 protons at δ 3.90 assignable to four methoxy groups: one two-proton singlet at δ 7.83 attributable to H-4 and H-5, two one-proton singlets at δ 7.20 and 6.97 assigned to H-1 and H-8, respectively, H-14 olefinic proton as a singlet at δ 5.37, one D_2O exchangeable proton at δ 4.76, and a triplet at δ 2.90 (J = 2.5 Hz) due to the α proton attached to C-9a. The substitution pattern for the four methoxy groups was identical to that observed for tyloindane [**1**]. Negative rotation of **3** suggested a β position of the hydroxyl group at C-13a (4,9). On the basis of this evidence the structure of tyloindicine G, which possessed a 9,9a,11,12,13,13a,14a-hexahydro-2,3,6,7-tetramethoxybenzo[*f,h*]pyrrolo[1,2-*b*]-13a-hydroxyisoquinoline skeleton, was established as **3**.

Tyloindicine H [**4**], mp 225–226° (dec), $C_{23}H_{25}NO_4$ ([M]$^+$ 379), closely resembled α-4-desmethylisotylocrebrine in its uv and ir spectral characteristics, indicating a a 3,6,7-trimethoxy-substituted phenanthrene skeleton. The extra oxygen was in the form of a hydroxyl group which was phenolic in nature as indicated by violet coloration with $FeCl_3$, a shift in uv maxima on addition of alkali, and the formation of a monomethyl derivative **7** with CH_2N_2. The ir spectrum of **4** showed a broad band at ca. 3450 cm^{-1} typical of a hindered hydroxyl group. In the mass spectrum, the base peak arose at *m/z* 310 due to loss of a pyrrolidine ring characteristic of phenanthroindolizidine alkaloids. Other prominent peaks appeared at *m/z* 365 [379 − Me]$^+$, 296 [310 − Me]$^+$, and 281 [296 − Me]$^+$. The ^{1}H-nmr spectrum of **4** displayed two one-proton singlets at δ 8.06 and 6.96 assignable to H-5 and H-8, respectively, two ortho-coupled doublets integrating for one proton each at δ 7.86 and δ 7.20 attributable to H-1 and H-2, one broad singlet at δ 5.20 due to H-14 in a six-membered ring, one D_2O exchangeable proton at δ 4.76 (OH), and a broad singlet for three methoxy groups at δ 3.83 (9H). One multiplet at δ 3.26 ($W_{1/2}$ = 4.5 Hz) and a triplet at δ 2.83 (J = 4.0 Hz), integrating one proton each, were assigned to the angular equatorial β protons at the C-13a and C-9a, respectively. Negative rotation of the compound also suggested a β position of H-13a (4,9).

Acetylation of **4** yielded a monoacetyl product **8**. The monomethyl derivative **7** could not be further acetylated, confirming the presence of one phenolic hydroxyl function. The site of demethylation in **4** was decided by comparison of ^{1}H nmr spectra of **4**, its monoacetyl product **8**, and its monomethyl ether **7**. There was a large deshielding (δ 1.30) of the signal due to H-5 in the monomethyl ether. The monoacetylated product showed no such effect, thus excluding the possibility of a phenolic hydroxyl at C-6. This effect had already been established previously in the isotylocrebrine type of alkaloids (3,5). Further proof of the phenolic hydroxyl at C-4 was obtained from the positive Gibb's test for the free para position to the hydroxyl. These data substantiated structure **4** for the alkaloid named tyloindicine H which possessed a 9,9a,11,12,13,13a,14a-hexahydro-3,6,7-trimethoxy-4-hydroxybenzo[*f,h*]pyrrolo[1,2-*b*]isoquinoline skeleton.

Tyloindicine I [**5**], mp 215–217° (dec), $C_{24}H_{27}NO_5$ ([M]$^+$ 409), showed the presence of a phenolic hydroxyl group at 3400 cm^{-1} in the ir spectrum. The ms of **5** displayed the existence of ion fragments at *m/z* 393 [M − Me]$^+$, 379 [393 − Me]$^+$, 365 [379 − Me]$^+$, and 350 [365 − Me]$^+$. The base peak at *m/z* 339 arose due to loss of the pyrrolidine nucleus from [M]$^+$. The peaks at *m/z* 324, 310, 296, and 281 were formed due to subsequent displacement of methyl groups from the base peak. In the ^{1}H-nmr

spectrum the presence of five aromatic protons indicated **5** to be a cleaved phenanthrene analogue. A broad singlet at δ 7.60, integrating for three protons, was assigned to H-1, H-4a, and H-5. A one-proton ortho, meta-coupled doublet at δ 7.00 having coupling constants of 2.5 and 10.5 Hz was attributed to H-5a. In the light of earlier findings (3–5), the signal at δ 6.73 (d, $J = 2.5$ Hz) has been assigned to the meta-coupled H-8. Another shielded singlet at δ 6.43 was due to an olefinic proton in a six-membered ring adjacent to nitrogen at C-9. The vinylic proton at C-14 was observed as a broad doublet at δ 5.46 ($W½ = 6.5$ Hz). A broad signal at δ 3.60, integrating for twelve protons, was attributed to four methoxy groups. A multiplet at δ 3.30 with $W½ = 4.0$ Hz was assigned to the angular C-13a equatorial proton adjacent to nitrogen, and its α arrangement was further confirmed by its negative rotation (4,9). Acetylation of **5** afforded a monoacetylated product **9**. A monomethyl ether **10**, which was formed by treatment with CH_2N_2, could not be acetylated, showing that the base contained only one phenolic hydroxyl function. The cleaved phenanthrene alkaloid **5**, the structure of which was deduced from the above data, has been named tyloindicine I and identified as 9,11,12,13,13a-hexahydro-2,3,6,7-tetramethoxy-4-hydroxy[*f,h*]pyrrolo[1,2-*b*]isoquinoline.

Tyloindicine J [**6**] mp 180–181° (dec), $C_{24}H_{25}NO_5$ ($[M]^+$ 407), showed the presence of a phenolic hydroxyl (3300 cm^{-1}) and a carbonyl (1715 cm^{-1}) in the ir spectrum. Acetylation of **6** yielded a monoacetylated product **11**. Treatment of **6** with CH_2N_2 afforded a monomethyl ether **12**, which could not be acetylated, confirming the existence of only one phenolic hydroxyl group. Deacetylation of the monomethyl ether **12** yielded 7-deacetyltyloindicine J [**13**]. The 7-OH function was found to be phenolic by a green coloration with $FeCl_3$, a shift in uv spectrum on addition of alkali, and remethylation with CH_2N_2. The mass spectrum of **6** showed an ion fragment at *m/z* 364 due to expulsion of an acetyl group from $[M]^+$. A weak peak at *m/z* 338 arose due to loss of the pyrrolidine ring. An intense peak at *m/z* 295 (73.5%) resulted from the fragment at *m/z* 338 due to loss of an acetyl group. In the ^{1}H-nmr spectrum the presence of six aromatic protons indicated **6** to be a cleaved phenanthrene analogue (1, 4–6). The shifts of the aromatic proton signals in **6** were identical to (+)-septicine (4) and 13a-hydroxysepticine (5), indicating the similar substitution pattern of oxygen-bearing groups. The six aromatic protons, appearing at δ 7.76 (1H, d, $J = 2.5$ Hz), 7.67 (2H, dd, $J = 3.0$ and 8.5 Hz), 7.33 (1H, dd, $J = 2.5$ and 9.0 Hz), 7.06 (1H, dd, $J = 2.5$ and 8.5 Hz), and 6.70 (1H, d, $J = 3$ Hz), fit well with the substitution pattern of the cleaved form of phenanthrene analogues. In the light of earlier findings (1–7), the shielded signal at δ 6.70 has been assigned to H-8 in **6**. Because this proton appeared as a doublet with a meta coupling constant ($J = 3$ Hz), the acetoxy function could be placed at C-7. The deshielded signal at δ 7.76 could be assigned to H-1 adjacent to the methoxy group. A double doublet at δ 7.67 integrating for two protons was attributable to H-4 and H-5. Another two doublets at δ 7.33 and δ 7.06 were assigned to H-4a and H-5a, respectively. The olefinic protons appeared as singlets at δ 6.46 (H-9) and 5.53 (H-14). Two signals at δ 3.70 and δ 3.63, integrating for six and three protons respectively, were attributed to three methoxy groups at C-2, C-3, and C-6. A multiplet at δ 3.30 with $W½ = 4.0$ Hz was assigned to the angular α-H-13a (equatorial), and negative rotation of the alkaloid further confirmed the α-arrangement of the proton (4,9). A three-proton singlet appearing at δ 1.90 confirmed the presence of an acetyl group. On the basis of these data the structure of tyloindicine J, which has a 9,11,12,13,13a-hexahydro-2,6-dimethoxy-3-hydroxy-7-acetoxy-secobenzo[*f,h*]pyrrolo[1,2-*b*]isoquinoline skeleton, has been characterized as **6**.

Table 1 shows that H-8 in **2**, **5**, and **6** undergo upfield chemical shifts of about 0.17 ppm in going from DMSO-d_6 to TFA. Dreiding models show that neither the hydroxyl

TABLE 1. ^{1}H-nmr Chemical Shifts of Compounds **1–8**.

Proton	Compound[a]							
	1 (DMSO-d_6 + TFA)	**2** (TFA)	**3** (DMSO-d_6 + TFA)	**4** (DMSO-d_6 + TFA)	**8** (DMSO-d_6)	**7** (DMSO-d_6)	**5** (TFA)	**6** (TFA)
H-1	7.13 s	7.70 m[b]	7.20 s	7.86 d (9)	7.73 d (9)	7.83 d (9)	7.60 brs[b]	7.76 d (2.5)
H-2	—	7.10 m[b]	—	7.20 d (9)	7.17 d (9)	7.26 d (9)	—	—
H-4	7.70 s[b]	8.00 m	7.83 s[b]	—	—	—	—	7.67 dd (3, 8.5)[b]
H-4a	—	7.10 m[b]	—	—	—	—	7.60 brs[b]	7.33 dd (2.5, 9)
H-5	7.70 s[b]	7.70 m[b]	7.83 s[b]	8.06 s	8.00 s	9.36 s	7.60 brs[b]	7.67 dd (3, 8.5)[b]
H-5a	—	6.96 m	—	—	—	—	7.00 dd (2.5, 10.5)	7.06 dd (2.5, 9)
H-8	6.90 s	6.73 d (3)	6.97 s	6.96 s	6.93 s	7.00 s	6.73 d (2.5)	6.70 d (3)
H-9	—	—	—	—	—	—	6.43 s	6.46 (s)
H-9a	—	2.83 t (4)	2.90 t (2.5)	2.83 t (4)	2.90 m	2.86 m	—	—
H-10	2.13 brm (W½ = 16)	—	—	—	—	—	—	—
H-13a . . .	2.13 brm (W½ = 16)	—	—	3.26 m (W½ = 4.5)	3.30 m (W½ = 4)	3.20 m (W½ = 4)	3.30 m (W½ = 4)	3.30 m (W½ = 4)
H-14	1.43 s	5.60 s	5.37 s	5.20 brs	5.26 brs	5.26 brs	5.46 brs	5.53 s
OMe	3.56 brs[c]	3.73 s[d]	3.90 brs[c]	3.83 brs[e]	3.86 s[e]	3.96 s[c]	3.60 s[c]	3.70 s[d]
	—	3.66 s[f]	—	—	—	—	—	3.63 s[f]
OAc	—	—	—	—	2.10 s	—	—	1.90 s[f]
OH	—	5.00	4.76 brs	4.76 brs	—	—	—	4.60 brs

[a]The numbering is the same in all skeletons.
[b]Overlapping signals.
[c]Intensity 12H.
[d]Intensity 6H.
[e]Intensity 9H.
[f]Intensity 3H.

or acetoxy group nor the lone pair of electrons on the nitrogen can influence the chemical shifts of H-8 in all possible conformations. Govindachari *et al.* (4) reported the upfield change of this proton due to existence of two molecules together in the dimer form by hydrogen bonding between the hydroxyl groups of one molecule and the nitrogen of the other in a manner suggested for norargemonine (10). The effect of a polar solvent like DMSO-d_6 or TFA would be to reduce the hydrogen bonding, and, thus, in these solvents, the protons are expected to have normal chemical shifts. The upfield shifts of C-8 protons in TFA are due to solvent effects, since the chemical shifts of protons in aromatic polycyclics are sensitive to solvents (11).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Decomposition points were determined on a Perfit mp apparatus and are uncorrected. ^{1}H-nmr spectra were determined on a 60 MHz Varian T-60 spectrometer. Chemical shifts are expressed in δ ppm with respect to internal TMS, and coupling constants are in Hz (s = singlet, d = doublet, t = triplet, m = multiplet, br = unresolved broad signal). Eims was recorded by direct inlet with 70 eV ionization in a JEOL JMS-300 mass spectrometer. Tlc was carried out on Si gel plates using C_6H_6-EtOAc-Et_2NH (6:3:1); spots were revealed by uv and by Dragendorff's reagent.

PLANT MATERIAL.—Aerial parts of *T. indica*, collected from the western Himalayan region, were identified by Dr. M.P. Sharma, Lecturer, Faculty of Science, Jamia Hamdard, New Delhi. A voucher specimen has been deposited at the Herbarium of the Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Jamia Hamdard, New Delhi.

ISOLATION OF ALKALOIDS.—Air-dried aerial parts of *T. indica* (2 kg) were extracted exhaustively by hot percolation with MeOH. The extract was dried under reduced pressure, and the 2M HCl-soluble portion was extracted with EtOAc (3 × 1 liter), to remove chlorophyll. The aqueous acidic solution was further acidified (pH 2) with 2 M HCl and washed with EtOAc (3 × 1 liter) to remove neutral components. The aqueous layer was then made alkaline (pH 9) with NH_4OH (30%) solution and repeatedly extracted with EtOAc. The combined extracts were washed with H_2O, dried, and evaporated in vacuo to yield the crude total alkaloid (10.5 g, 0.52%) as a solid brown residue.

SEPARATION OF ALKALOIDS.—The residue containing total bases was triturated with dry EtOAc, and the insoluble compound **1**, tyloindane, was removed by filtration. The EtOAc-soluble portion was concentrated in vacuo and subjected to cc on basic slurry-packed Al_2O_3. The column was eluted with mixtures of C_6H_6, EtOAc, and MeOH of increasing polarities.

TYOINDANE [**1**].—Compound **1** was crystallized from $CHCl_3$-MeOH (1:1) as a pale yellow amorphous powder: (0.16 g, 0.008%), mp 278–279° (dec); $[\alpha]^{30}D$ −0.5° (*c* = 0.20, HOAc); uv λ max (MeOH) (log ε) 205 (11.2), 222 (13.7), 255 (19.8), 288 (17.7), 302 sh (13.8), 322 sh (2.1), 338 (2.1), 355 nm (1.4); ir ν max (KBr) 2905, 1615, 1510, 1465, 1245, 1135, 1025, 1005, 965, 820, 765 cm^{-1}; ^{1}H nmr see Table 1; eims *m/z* (rel. int.) $[M]^+$ 392 (0.2), 324 (100), 309 (29.2), 294 (4.8), 280 (16.3), 265 (9.8), 68 (6.3).

TYLOPHORINE.—Fractions 1–2 eluted with C_6H_6-EtOAc (1:3) gave tylophorine, 0.20 g (0.015%), identified by comparison with an authentic sample by ^{1}H nmr, tlc, mp, and mmp.

TYLOINDICINE F [**2**].—Fractions 3–5 eluted with C_6H_6-EtOAc (1:1) gave **2** as colorless crystals from Me_2CO-MeOH (1:1): 0.08 g (0.004%); mp 245–247° (dec), $[\alpha]^{30}D$ −0.5° (*c* = 0.05, HOAc); uv λ max (MeOH) (log ε) 206 (9.2), 255 (17.3), 285 (13.0), 310 sh (5.5), 340 nm (0.6); ir ν max (KBr) 3350, 1618, 1512, 1470, 1422, 1260, 1235, 1165, 1035, 985 cm^{-1}; ^{1}H nmr see Table 1; eims *m/z* (rel. int.) $[M]^+$ 381 (0.7), 363 (0.1), 311 (6.6), 296 (0.6), 281 (0.7), 266 (0.5), 251 (0.3), 69 (6.5).

TYLOINDICINE G [**3**].—Fractions 6–11 eluted with C_6H_6-EtOAc (1:3) upon crystallization from MeOH-Me_2CO (1:3) afforded **3** as colorless crystals (0.20 g, 0.001%), mp 237–238° (dec), $[\alpha]^{30}D$ −2.35° (*c* = 0.47, HOAc); uv λ max (MeOH) (log ε) 210 (11.8), 255 (19.6), 290 (15.1), 338 (1.3), 355 nm (0.8); ir ν max (KBr) 3450, 2900, 1615, 1515, 1470, 1425, 1250, 1200, 1150, 1030, 840 cm^{-1}; ^{1}H nmr see Table 1; eims *m/z* (rel. int.) $[M]^+$ 409 (8.2), 393 (25.5), 379 (45.5), 361 (11.2), 340 (55.7), 324 (100), 310 (99.2), 295 (30.5), 281 (33.5), 266 (27.8), 251 (16.6), 239 (26.9), 167 (28.7), 151 (16.0), 85 (4.5), 69 (92.3), 32 (99.2), 28 (97.3).

TYLOINDICINE H [**4**].—Repeated cc over basic Al_2O_3 of the mother liquor from **3** gave colorless crystals of **4** (0.26 g, 0.0013%); mp 225–226° (dec); $[\alpha]^{30}D$ −5.5° (*c* = 0.55, HOAc); uv λ max (MeOH)

(log ε) 206 (14.9), 258 (19.8), 286 (17.4), 310 sh (8.7), 342 (1.4), 355 nm (0.7); (MeOH + NaOH) 220 (16.3), 255 (20.1), 285 (17.6), 295 (8.3), 330 sh nm (1.2); ir ν max (KBr), 3400, 2850, 1615, 1510, 1465, 1420, 1245, 1200, 1150, 1105, 1045, 835 cm^{-1}; ^1H nmr see Table 1; eims (rel. int.) $[M]^+$ 379 (21.9), 365 (15.8), 340 (9.8), 324 (22.0), 310 (100), 296 (99.9), 281 (21.0), 267 (19.9), 252 (10.3), 70 (91.3), 32 (91.2), 28 (99.5).

ACETYLATION OF TYLOINDICINE H [**4**].—Tyloindicine H [**4**] (30 mg) in dry pyridine (0.25 ml) and Ac_2O (0.25 ml) was kept overnight at ambient temperature. After workup, it provided a monoacetyl product **8**, which was crystallized from Me_2CO, mp 201–203° (dec); ir ν max (KBr) 1745, 1615 cm^{-1}; ^1H nmr see Table 1.

METHYLATION OF TYLOINDICINE H [**4**].—Tyloindicine H [**4**] (30 mg) in Et_2O (10 ml) was treated with CH_2N_2. After removal of the solvent the product **7** (26 mg) was crystallized from Me_2CO, mp 215–216° (dec), ir ν max (KBr) 2905, 1615 cm^{-1}; ^1H nmr see Table 1.

TYLOINDICINE I [**5**].—Fractions 12–20 of the main column eluted with EtOAc on crystallization from Me_2CO-MeOH (3:1) afforded 0.18 g (0.009%) dark yellow colored beads of **5**: mp 215–217° (dec); $[\alpha]^{30}D$ −2.2° (*c* = 0.44, HOAc); uv λ max (MeOH) (log ε) 205 (12.0), 225 sh (10.8), 258 (17.8), 285 (13.2), 310 sh (5.6), 342 (0.9), 365 nm (0.4); (MeOH + NaOH) 218 (20.0), 255 (17.9), 288 (14.1), 295 (14.6), 335 sh nm (6.7); ir ν max (KBr) 3400, 2900, 1615, 1515, 1465, 1420, 1240, 1195, 1145, 1100, 1040, 920, 820 cm^{-1}; ^1H nmr see Table 1; eims *m/z* (rel. int.) $[M]^+$ 409 (2.5), 393 (9.7), 379 (12.0), 365 (15.9), 350 (6.3), 339 (18.8), 324 (38.9), 310 (75.5), 296 (100), 281 (19.1), 70 (99.9), 32 (99.5), 28 (99.6).

ACETYLATION OF TYLOINDICINE I [**5**].—Tyloindicine I [**5**] (20 mg), in pyridine (0.5 ml) and Ac_2O (1 ml) was kept overnight at room temperature. Excess Ac_2O was decomposed by adding H_2O (5 ml), and the solution was extracted with $CHCl_3$ (3 × 2 ml). The $CHCl_3$ layer was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated to yield the monoacetyl product **9**, which was crystallized from Me_2CO, mp 182–183° (dec), ir ν max (KBr) 2900, 1725 cm^{-1}.

METHYLATION OF TYLOINDICINE I [**5**].—Tyloindicine I [**5**] (20 mg) in Et_2O (5 ml) was treated with CH_2N_2. After removal of the solvent, the monomethyl derivative **10** was crystallized from $CHCl_3$-MeOH (1:1): mp 226–227° (dec); ir ν max (KBr) 2900, 1610 cm^{-1}.

TYLOINDICINE J [**6**].—The mother liquor from **5**, on purification with cc, gave light yellow crystals of **6** (0.12 g, 0.006%): mp 180–182° (dec); $[\alpha]^{30}D$ −2.85 (*c* = 0.57, HOAc); uv λ max (MeOH) (log ε) 205 (14.0), 222 sh (17.1), 255 (19.2), 288 (16.4), 315 sh (7.6), 342 (1.4), 355 nm (0.6); (MeOH + NaOH) 210 (20.1), 253 (18.0), 285 (14.2), 295 sh (13.9), 335 sh nm (5.0); ir ν max (KBr) 3300, 2900, 1715, 1615, 1520, 1470, 1420, 1230, 1200, 1155, 1100, 1040, 1005, 970, 820 cm^{-1}; ^1H nmr see Table 1; eims *m/z* (rel. int.) $[M]^+$ 407 (0.5), 392 (2.6), 378 (4.4), 364 (8.8), 338 (0.13), 324 (14.0), 309 (37.7), 295 (73.5), 69 (100), 42 (99.9).

ACETYLATION OF TYLOINDICINE J [**6**].—Tyloindicine J [**6**] (15 mg) was treated with pyridine (0.5 ml) and Ac_2O (2 ml) at room teperature. Unreacted Ac_2O was decomposed with H_2O (5 ml), and the solution was extracted with $CHCl_3$ (3 × 2 ml). The $CHCl_3$ layer was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated to obtain the monoacetyl product **11**. It was crystallized from Me_2CO; mp 150–151° (dec), ir ν max (KBr) 2905, 1740, 1720, 1605 cm^{-1}.

METHYLATION OF TYLOINDICINE J [**6**].—Tyloindicine J [**6**] (20 mg) was methylated with CH_2N_2 in Et_2O (5 ml). After removal of the solvent the monomethyl product **12** was obtained, mp 201–202° (dec); ir ν max (KBr) 2910, 1725, 1610 cm^{-1}.

DEACETYLATION OF **12**.—Methylated tylohirsuticine J [**12**] (10 mg) was added to 0.5 N HCl (2 ml) and heated on a steam bath for 15 min. The reaction mixture was made alkaline with 30% NH_4OH to pH 9, extracted with $CHCl_3$ (3 × 5 ml), and the extract was dried to yield the deacetylated product **13**: mp 247–249° (dec); uv λ max (MeOH) 220, 258, 285, 355 nm; (MeOH + NaOH) 230, 280, 335; ir ν max (KBr) 3380, 2910, 1610 cm^{-1}.

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