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PHENANTHROINDOLIZIDINE ALKALOIDS FROM *TYLOPHORA TANAKAE*

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Key Word Index—*Tylophora tanakae*; Asclepiadaceae; phenanthroindolizidine alkaloids; 7-demethyltylophorine.

Abstract—From the fresh leaves of *Tylophora tanakae*, ten phenanthroindolizine alkaloids were isolated. Among them, eight were new alkaloids and their structures were determined. Two known alkaloids were identified as isotylocrebrine and tylophorine.

INTRODUCTION

Tylophora is a genus known to contain phenanthroindolizidine alkaloids [1–5]. Although several species are distributed in Japan, no chemical investigations on them have been described. Since *T. tanakae* is indigenous to the Ryukyu Islands and known as a feeding source for caterpillars of *Ideopsis similis*, one of the Danaid butterflies, our studies on the alkaloids in the leaves were attempted in order to investigate the possible relationship between the plant and the butterfly.

RESULTS

A mixture of alkaloids (**1**–**10**) obtained from the fresh leaves was purified by means of column chromatography and preparative TLC. Among these, **1** and **2**, showing the same molecular formula, $C_{24}H_{27}O_4$, were identified as isotylocrebrine (3,4,6,7-tetramethoxyphenanthroindolizidine) [6] and tylophorine (2,3,6,7-tetramethoxyphenanthroindolizidine) [5, 7], respectively, based on the UV and NMR considerations.

FAB mass spectrometry of **3** afforded a $[M + 1]^+$ at *m/z* 380.1862 ($C_{23}H_{25}NO_4$), 14 mu smaller than **1** or **2**, and an intensive peak at *m/z* 310 ($C_{19}H_{18}O_4$), due to the phenanthrene moiety, suggesting the presence of three methoxyl and one hydroxyl groups, instead of four methoxyls as in **1** or **2**. Since **3** showed the same UV maximum at 261 nm and the same coupling patterns in the ^1H signals due to rings A and B as those of **1** (Table 1), the substituents in rings A and B appeared to be at C-3,4,6,7 or C-2,3,5,6; the former was preferred based on the cross-peaks between H-14β/H-1 (*d*, *J* = 9 Hz) and H-9/H-8 (*s*) in the 2D-NOESY spectrum. In the same way, the locations of the methoxyl groups were assigned to C-4,6,7 based on NOE evidence that cross-peaks were observed between H-5/4-OMe, 6-OMe and H-8/7-OMe, but none between H-2 and any methoxyl groups. Therefore, **3** was identified as 3-demethylisotylocrebrine.

FAB mass spectrometry of **4** afforded a $[M + 1]^+$ at *m/z* 396.1811, suggesting a molecular formula, $C_{23}H_{25}NO_5$. Although the substitution pattern in rings A and B seemed to be the same as in **3**, a hydroxyl group was assignable in the indolizidine moiety based on the lower field shift of H-1 (+ 0.40 ppm), as well as a peak at *m/z* 378 ($[M + 1 - H_2O]^+$) and a carbinyl carbon signal at δ64.3. The corresponding carbinyl proton was observed at δ5.05 as a doublet signal, showing NOE to H-1. The location of the hydroxyl group was thus determined to be 14α, retaining a *trans*-configuration to H-13a, based on a small coupling constant (*J* = 2 Hz). The 14α-OH group was further confirmed by the fact that C-13 was shifted to upper field in comparison with that of **3** (− 6.9 ppm). Alkaloid **4** was thus assigned as 3-demethyl-14α-hydroxyisotylocrebrine.

Alkaloid **5** appeared to have a molecular formula of $C_{24}H_{27}NO_5$, one oxygen more than that of **1**, based on a $[M + 1]^+$ at *m/z* 410.1963. Multiplicity of the proton signals due to rings A and B was the same as that of **1**, and the fragment peak at *m/z* 324, due to the phenanthrene moiety with four methoxyl groups, was observed as in **1** and **2**. The extra oxygen atom present in **1** was assignable to the indolizidine ring, of which the coupling pattern of the ^1H signals was similar to that of **1**, while H-9, 11, 13a showed lower field shifts. In the ^{13}C NMR spectrum (Table 2), C-9,11,13a were observed at lower fields in comparison with those of **1** (+ 11.9, + 16.5, + 11.2 ppm, respectively). Therefore, **5** was characterized to be the *N*-oxide of **1**.

In the ^1H and ^{13}C NMR spectra of **6** (Tables 1 and 2), four methoxyl signals were observed with similar chemical shifts and signals due to H- and C- 9, 11, 13a were

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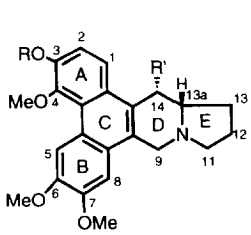
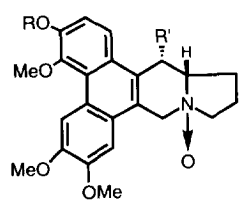
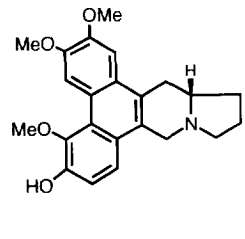


1 : R=Me, R'=H
3 : R=R'=H
4 : R=H, R'=OH



5 : R=Me, R'=H
6 : R=Me, R'=OH
7 : R=H, R'=OH



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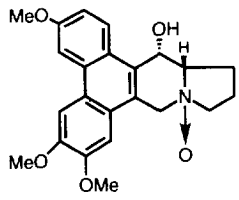

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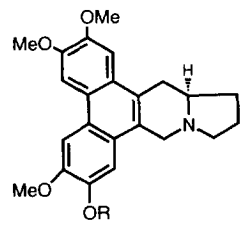

2 : R=Me
10 : R=H

seen at lower fields as found in **5**, indicating **6** to be an *N*-oxide of an isotylocrebrine-type alkaloid. The location of the secondary hydroxyl group was assigned to 14α based on the cross-peak between H-1 (δ8.08 (*d*, *J* = 9 Hz))/H-14β (δ5.23, *br s*). Two of the four methoxyl groups were confirmed to be at C-3 and C-4, since H-1 and H-2 showed *ortho*-coupling with each other (*J* = 9 Hz) and cross-peaks were observed between H-1/C-3 and H-2/C-4 in the HMBC spectrum (Table 3). The location of the methoxyl groups in ring B was assigned to C-6 and C-7 based on the HMBC spectrum, along with *para*-coupling of H-5 and H-8. Alkaloid **6** was thus characterized as 14α-hydroxyisotylocrebrine *N*-oxide.

The molecular formula of **7** was suggested to be $C_{23}H_{25}NO_6$, based on the $[M + 1]^+$ (*m/z* 412.1759) in the FAB mass spectrum. Since **7** showed a similar pattern of ^1H and ^{13}C signals due to the indolizidine moiety as those of **6**, only three methoxyl groups could be present in the phenanthrene moiety. The multiplicity in the ^1H signals due to the phenanthrene moiety was in good agreement with that of **4**, and **7** was thus characterized to be the *N*-oxide of **4**.

Alkaloid **8** showed the same molecular formula, $C_{23}H_{25}NO_4$ and the same NMR pattern due to the indolizidine moiety as **3**. Based on the fact that H-8, showing an NOE cross-peak with H-9, was observed at δ7.53 as a doublet signal (*J* = 9 Hz) as a result of coupling with H-7, and NOE was also observed between H-14β/H-1 (δ7.33, *s*), the substituents in **8** were located at C-2, 3, 5, 6. Since cross-peaks were observed between H-1/2-OMe and H-4/3-OMe, 5-OMe, the methoxyl groups were assigned to C-2, 3 and 5. Alkaloid **8** is, thus, characterized as 6-demethyltylocrebrine.

FAB mass spectrometry of **9** suggested the molecular formula, $C_{23}H_{25}NO_4$. In the UV spectrum, the absorption maxima showed a different pattern from those of **1**–**8**; five aromatic proton signals were observed in the ^1H NMR spectrum (Table 1). By comparison of the NMR spectra with those of **6** and **7**, the presence of 14α-OH and *N*-oxide functions was suggested. Since the ^1H signals due to ring A appeared as an ABX pattern and cross-peaks were observed between H-2, 4/3-OMe, H-5/6-OMe and H-8/7-OMe, the structure of **9** was determined to be an *N*-oxide of the already known alkaloid, tylophorinine (3, 6, 7-trimethoxy-14α-hydroxyphenanthroindolizidine) [8].

The molecular formula of **10**, $C_{23}H_{25}NO_4$, was the same as those of **3** and **8**. An intense fragment ion peak was observed at *m/z* 310, as in **3**, suggesting that there were three methoxyl and one hydroxyl groups in the phenanthrene ring. Based on the fact that **10** showed the same UV absorption maxima as those of **2** and four aromatic protons were all observed as a singlet signal, the substituents in **10** were located at C-2, 3, 6, 7. Cross-peaks were observed between H-1/H-14a and 2-OMe, H-4/3-OMe, and H-5/6-OMe in NOE measurements, and H-8 showed NOE only to H-9a. Therefore, the hydroxyl group was assigned to C-7 and the methoxyl groups were at C-2, 3, 6. The location of the methoxyl groups was confirmed by the HMBC spectrum (Table 3). Methylation of **10** by CH_2N_2 afforded a product which was identical to **2** by TLC and ^1H NMR. Unlike the other alkaloids, the H-8 and C-8 signals were of weak intensity.

DISCUSSION

The configuration at C-13a in naturally occurring tylophorine (**2**), with negative rotation and negative cotton in ORD, was assigned to be *S* and, vice versa, isotylocrebrine (**1**) with positive rotation and ORD, *R*, based on consideration of the degradation products of **2** [9]. However, synthetic (*S*)-(+)-tylophorine had a positive rotation and CD. The stereochemistry at C-13a in the naturally occurring alkaloids therefore needs to be revised [10, 11]. In our study, **1** and **3**–**9** all showed positive rotation values and positive CD, and C-13a was assigned

Table 1. ^{1}H NMR spectral data of alkaloids [δ ppm from TMS in CD_3OD–$CDCl_3$ (400 MHz), J Hz in parentheses]

H	1	3	4	5*	6	7*	8	9	2	10
1	7.81 *d* (9)[a]	7.69 *d* (9)[a]	8.09 *d* (9)[a]	7.83 *d* (9)[a]	8.08 *d* (9)[a]	7.85 *d* (9)[a]	7.33 *s*[a,b]	8.12 *d* (9)[a]	7.32 *s*[a,b]	7.29 *s*[a,b]
2	7.34 *d* (9)[b]	7.23 *d* (9)	7.29 *d* (9)	7.43 *d* (9)[b]	7.43 *d* (9)[b]	7.24 *d* (9)		7.30 *dd* (9,2)[b]		
4							9.23 *s*[c,d]	7.79 *d* (2)[c]	7.86 *s*[c]	7.79 *s*[c]
5	9.33 *s*[c,d]	9.26 *s*[b,c]	9.19 *s*[b,c]	9.29 *s*[c,d]	9.37 *s*[c,d]	9.27 *s*[b,c]		7.73 *s*[d]	7.85 *s*[d]	7.78 *s*[d]
7							7.22 *d* (9)			
8	7.17 *s*[e,f,g]	7.15 *s*[d,e,f]	6.87 *s*[d]	7.00 *s*[e,f]	7.03 *s*[e,f]	6.89 *s*[d,e]	7.53 *d* (9)[e]	6.84 *s*[e,f]	7.16 *s*[e,f]	7.25 *br s*[e]
9	4.60 *d* (15)[f]	4.56 *d* (15)[e]	4.17 *d* (15)[d]	5.00 *d* (15)[f]	5.25 *d* (14)[f]	5.09 *d* (15)[e]	4.62 *d* (15)[e]	5.06 *d* (15)[f]	4.63 *d* (15)[f]	4.59 *d* (15)[e,f]
	3.69 *d* (15)[g]	3.62 *d* (15)[f]	3.41 *d* (15)	4.65 *d* (15)	4.65 *d* (14)	4.41 *d* (15)	3.65 *d* (15)	4.48 *d* (15)	3.70 *d* (15)[g]	3.66 *d* (15)
11	3.46 *td* (9, 3)	3.43 *td* (9, 3)	3.34 *td* (9, 2)	3.86 *td* (9, 2)	3.85 *br t* (9)	3.73 *br t* (9)	3.41 *td* (9,2)	3.75 *br t* (9)	3.47 *td* (9, 2)	3.45 *td* (9, 3)[f]
	2.53 *q* (9)	2.48 *q* (9)	2.41 *q* (9)	3.65 *q* (9)	3.69 *q* (9)	3.67 *q* (9)	2.46 *q* (9)	3.67 *q* (9)	2.54 *q* (9)[g]	2.53 *q* (9)
12	1.94–2.07	1.90–2.05	1.92–2.02	2.34 *m*	2.54 *m*	2.40 *m*	1.92–2.05	2.46 *m*	1.95–2.04	2.04 *m*
	–	–		2.11 *m*	2.24 *m*	2.20 *m*		2.20 *m*		1.97 *m*
13	2.31 *m*	2.28 *m*	2.32 *m*	2.25 *m*	2.93 *m*	2.75 *m*	2.27 *m*	2.81 *m*	2.30 *m*	2.28 *m*
	1.79 *m*	1.76 *m*	1.99 *m*	2.17 *m*	2.27 *m*	2.16 *m*	1.78 *m*	2.18 *m*	1.79 *m*	1.79 *m*[g]
13a	2.56 *m*	2.50 *m*	2.52 *m*	3.41 *m*	3.54 *m*	3.24 *m*	2.52 *m*	3.26 *m*	2.60 *m*	2.61 *m*
14α	2.95 *dd* (16,10)	2.90 *dd* (16,11)		3.23 (2H)[a]			2.89 *dd* (16,10)		3.38 *dd* (15,2)[a]	3.35 *dd* (15,3)[a]
β	3.40 *dd* (16,2)[a]	3.37 *br d* (16)[a]	5.05 *d* (2)[a]		5.23 *br s*[a]	5.06 *d* (2)[a]	3.32 *dd* (16,3)[a]	5.01 *d* (3)[a]	2.94 *dd* (15,12)	2.94 *dd* (15,11)[g]
–OMe										
2							4.05[b]		4.05[b]	4.04[b]
3	4.05[b]			4.03[b]	4.06[b]		4.06[c]	4.03[b,c]	4.11[c]	4.09[c]
4	3.94[c]	3.88[b]	3.89[b]	3.92[c]	3.95[c]	3.86[b]				
5							3.88[d]			
6	4.07[d]	4.06[c]	4.08[c]	4.02[d]	4.08[d]	4.03[c]		4.03[d]	4.11[d]	4.09[d]
7	4.06[e]	4.04[d]	3.97[d]	3.99[e]	4.05[e]	3.96[d]		3.97[e]	4.05[e]	

*Dissolved in CD_3OD.

[a–f] or [g] A cross-peak was observed between these signals in the 2D-NOESY spectrum.

Table 2. ^{13}C NMR spectra data of alkaloids [δ (ppm) in CD_3OD–$CDCl_3$]

C	1†	3	4†	5*†	6†	7*	8	9†	2†	10†
1	119.5	119.6	121.0	122.1	120.9	123.6	103.2	125.6	103.8	103.0
2	112.0	116.4	116.2	114.7	112.3	119.9	148.8[a]	118.1	148.5[a]	148.4
3	150.5	149.1	148.1	153.6	150.9	153.0	148.2[a]	157.7	148.3[b]	148.4
4	145.8	143.7	143.9	148.2	145.4	146.4	108.6	103.4	103.3[c]	103.4
5	109.1	108.7	108.3	111.5	108.9	111.1	143.9	103.2	103.5[c]	102.9
6	147.5	147.1	147.3	150.3	148.2	150.8	147.3[a]	149.0	148.4[b]	147.1
7	148.4	148.1	148.1	151.2	148.6	151.1	116.4[b]	148.6	148.6[a]	145.5
8	102.5	102.3	102.6	104.9	102.6	105.2	118.7[b]	102.5	103.0	106.7
9	53.4	53.3	53.5	67.3	65.3	67.5	53.6	64.8	53.3	53.4
11	54.5	54.3	54.9	71.0	69.2	71.6	54.3	68.8	54.5	54.5
12	20.9	20.7	21.3	21.4	19.3	21.5	20.8	18.9	20.9	21.1
13	30.4	30.3	23.6	28.8	21.6	24.0	30.4	21.2	30.5	30.6
13a	60.1	59.9	65.0	71.3	70.1	72.5	59.9	69.7	60.1	60.3
14	32.9	32.8	64.3	29.4	63.7	66.2	32.8	63.2	32.7	32.6
ring C	126.6	126.6	128.8	128.1	127.9	131.9	127.3	130.5	125.7	125.6
	126.4	125.7	125.6	128.0	125.1	130.4	125.8	127.1	125.3	125.2
	125.9	125.3	125.5	127.4	124.4	127.0	124.6	123.9	124.8	124.7
	125.0	123.7	123.5	125.8	124.1	126.4	124.0	123.2 (×2)	123.8	124.3
	123.4	123.1	123.4	125.5	124.0	126.0	122.9	118.1	123.6	123.9
	122.9	122.5	121.0	122.5	122.5	119.1	120.5		123.4	123.0
–OMe										
2							55.1		55.5[d]	55.6
3	55.3			57.1	55.7		55.1	54.4	55.7	55.9
4	59.6	58.8	59.2	61.2	59.4	61.0				
5							59.0			
6	56.0	55.0	55.1	57.8	55.0	57.1		54.8	55.7	55.7
7	55.3	55.0	55.1	57.1	55.1	57.1		54.8	55.4[d]	

*Dissolved in CD_3OD.
†Signal assignments were based on ^{13}C–^1H COSY spectra.
[a–d]Interchangeable within the same column.

Table 3. Correlations between ^1H and ^{13}C signals (3 bond) in HMBC spectra of alkaloids **6** and **10** (in CD_3OD–$CDCl_3$)

	6		10
H-1	C-3	H-4	C-2
H-2	C-4	H-5	C-7
H-5	C-7	2-OMe	C-2
H-8	C-6	3,6-OMe	C-3, C-6
3-OMe	C-3	H-9a	C-13a
4-OMe	C-4	H-9b	C-11
6-OMe	C-6	H-11a	C-13, C-13a
7-OMe	C-7	H-11b	C-9
H-9a	C-13a	—	—

the *S*-configuration, but *R* in **2** based on the negative rotation value and CD. The 13a-configuration in **10** is tentatively assigned as *R* based on the negative rotation value although a clear CD curve was not obtained.

Aristolochic acids with similar nitro-phenanthrene structures are the constituents of *Aristolochia* species which are known as feeding sources for *Pachliopta* and *Atrophaneura* butterflies and, recently, aristolochic acids were shown to be defence substances and oviposition stimulants for these butterflies [12–14]. Some of the phenanthroindolizidine alkaloids from *T. tanakae* also showed oviposition stimulant activities for *Ideopsis similis* [15].

EXPERIMENTAL

General. ^1H NMR, 400 or 500 MHz and ^{13}C NMR, 100 MHz in $CDCl_3$ and/or CD_3OD with TMS as int. standard. UV and CD spectra were measured in MeOH. For TLC and silica gel CC (normal phase), the following solvent systems were used. 1: $CHCl_3$–MeOH–H_2O (10:1:2–7:3:1.2, bottom layer), 2: EtOAc–MeOH–H_2O, (5:1:4–4:1:3, upper layer), 3: benzene–Me_2CO, (2:1). Spray reagent for TLC: Dragendorff's reagent.

Plant material. Fr. leaves and stems of *T. tanakae* Maxim. were collected in June 1994 at Hateruma-jima. A part of them was cultivated in the plant gardens in Fukuoka and Hiroshima Universities. Voucher No. of dried plant sample: FUK-941028A.

Extraction and isolation of alkaloids. Fr. leaves and stems (3.0 kg) were extracted with MeOH. The MeOH soln was concd *in vacuo* and H_2O added. The deposit was filtered off and the filtrate extracted with benzene (extract 1.39 g) and then $CHCl_3$ (0.53 g). The benzene extract was dissolved in 10% HOAc and extracted with Et_2O. The aq. layer was then made alkaline with NH_4OH and

extracted with $CHCl_3$ (Fr. A, 150 mg). This extract (0.53 g) was treated with MeOH and the ppt. (Fr. B, 90 mg) filtered off. The MeOH-soluble fr. was concd and treated with acid and alkali as described above (Fr. C, 150 mg). Frs A–C were combined and subjected to successive silica gel CC and prep. TLC to afford **1** (17 mg), **2** (10 mg), **3** (15 mg), **4** (18 mg), **5** (8 mg), **6** (7 mg), **7** (13 mg), **8** (19 mg), **9** (9 mg) and **10** (63 mg).

Isotylocrebrine (3,4,6,7-*tetramethoxyphenanthroindolizidine*) (**1**). Mp 198–203° (dec). $[\alpha]_D^{26}$ + 20.2° ($CHCl_3$; *c* 0.50). FABMS *m/z* 394.2016 calcd for $C_{24}H_{27}NO_4$ + H 394.2019, 324, 307, 154, 70. UV λ_{max} nm (log ε); 245 (4.31), 263 (4.66), 278 (4.27), 285 (4.22), 306 (3.88), 316 (3.81). CD (*c* 6.7×10^{-5}). $[\theta]_{259}$ + 4480. ^1H and ^{13}C NMR: see Tables 1 and 2.

Tylophorine (2,3,6,7-*tetramethoxyphenanthroindolizidine* (**2**). Mp 280–290° (dec). $[\alpha]_D^{26}$ − 13.1° ($CHCl_3$; *c* 0.35). FABMS *m/z* 394.2017 calcd for $C_{24}H_{27}NO_4$ + H 394.2019, 324, 307, 154, 136, 70. UV λ_{max} nm (log ε); 222 (4.38), 241 (4.54), 250 (4.71), 257 (4.84), 283 (4.54), 288 (4.58), 302 (4.33), 323 (3.45). CD (*c* 1.5×10^{-5}): $[\theta]_{257}$ − 9150. ^1H and ^{13}C NMR: see Tables 1 and 2.

3-*Demethylisotylocrebrine* (**3**). Mp 193–203° (dec). $[\alpha]_D^{28}$ + 34.5° ($CHCl_3$ + MeOH, (1:1); *c* 0.80). FABMS *m/z* 380.1862 $C_{24}H_{27}NO_4$-H requires 380.1862, 310, 176, 154, 95, 69. UV λ_{max} nm (log ε); 244 (4.09), 261 (4.33), 277 (4.04), 283 (3.99), 303 (3.67), 313 (3.56). CD (*c* 1.7×10^{-4}): $[\theta]_{260}$ + 4120. ^1H and ^{13}C NMR: see Tables 1 and 2.

3-*Demethyl*-14α-*hydroxyisotylocrebrine* (**4**). Mp 210–213° (dec). $[\alpha]_D^{27}$ + 91.2° ($CHCl_3$–MeOH (1:1); *c* 0.35). FABMS *m/z* 396.1813 $C_{23}H_{25}NO_5$ + H requires 396.1811, 378, 329, 307, 289, 178, 154, 136, 107. UV λ_{max} nm (log ε); 243 (4.32), 262 (4.60), 277 (4.28), 285 (3.99), 305 (3.80), 317 (3.78). CD (*c* 6.3×10^{-5}). $[\theta]_{259}$ + 7460. ^1H and ^{13}C NMR: see Tables 1 and 2.

Isotylocrebrine N-*oxide* (**5**). Solid. $[\alpha]_D^{30}$ + 28.9° MeOH; *c* 0.30). FABMS *m/z* 410.1963 $C_{24}H_{27}NO_5$ + H requires 410.1968, 392, 324, 176, 154, 136, 86. UV λ_{max} nm (log ε); 246 (4.21), 265 (4.50), 280 (4.18), 287 (4.10), 308 (3.72), 321 (3.15). CD (*c* 1.5×10^{-4}): $[\theta]_{258}$ + 9330. ^1H and ^{13}C NMR: see Tables 1 and 2.

14-α-*Hydroxyisotylocrebrine* N-*oxide* (**6**). Mp 212–215° (dec). $[\alpha]_D^{26}$ + 8.3° ($CHCl_3$–MeOH (1:1) *c* 0.12). FABMS *m/z* 426.1913 $C_{24}H_{27}NO_6$ + H requires 426.1916, 408, 339, 326, 307, 176, 154, 136. UV λ_{max} nm (log ε); 245 (4.39), 262 (4.43), 278 (4.11), 284 (3.99), 309 (3.65), 321 (3.59). CD (*c* 3.0×10^{-4}): $[\theta]_{260}$ + 20000.

3-*Demethyl*-14α-*hydroxisotylocrebrine* N-*oxide* (**7**). Mp 215–223° (dec). $[\alpha]_D^{30}$ + 5.5° (MeOH; *c* 0.12). FABMS *m/z* 412.1759 $C_{23}H_{25}NO_6$ + H requires 412.1760, 394, 371, 329, 307, 301, 176, 154, 136. UV λ_{max} nm (log ε); 244 (4.24), 260 (4.36), 277 (4.11), 285 (4.02), 308 (3.68), 319 (3.63). CD (*c* 4.3×10^{-5}): $[\theta]_{258}$ + 13020. ^1H and ^{13}C NMR: see Tables 1 and 2.

6-*Demethyltylocrebrine* (**8**). Mp 203–213° (dec). $[\alpha]_D^{28}$ + 8.0° (MeOH; *c* 0.05). FABMS *m/z* 380.1860 $C_{23}H_{25}NO_4$ + H requires 380.1862, 310, 178, 154, 136, 70. UV λ_{max} nm (log ε); 261 (4.46), 276 (4.21), 284 (4.15), 303 (3.79), 314 (3.74). CD (*c* 2.9×10^{-4}): $[\theta]_{258}$ + 10340. ^1H and ^{13}C NMR: see Tables 1 and 2.

Tylophorinine N-*oxide* (3,6,7-*trimethoxyphenanthroindolizidine* N-*oxide*) (**9**). Solid. $[\alpha]_D^{29}$ + 15.1° ($CHCl_3$–MeOH (1:1); *c* 0.35). FABMS *m/z* 396.1812 $C_{23}H_{25}NO_5$ + H requires 396.1811, 378, 360, 309, 176, 154, 136. UV λ_{max} nm (log ε); 232 (4.02), 246 (4.21), 263 (4.40), 283 (4.13), 289 (4.14), 315 (3.64). CD (*c* 5.5×10^{-5}): $[\theta]_{257}$ + 11820. ^1H and ^{13}C NMR: see Tables 1 and 2.

7-*Demethyltylophorine* (**10**). Mp 250–260° (dec). $[\alpha]_D^{27}$ − 49.3° ($CHCl_3$; *c* 0.25). FABMS *m/z* 380.1865 $C_{23}H_{25}NO_4$ + H requires 380.1862, 310, 307, 164, 136, 70. UV λ_{max} nm (log ε); 220 (4.49), 240 (4.58), 249 (4.76), 256 (4.91), 282 (4.58), 288 (4.63), 303 (4.41), 323 (3.51). ^1H and ^{13}C NMR: see Tables 1 and 2.

Compound **10** (3 mg) was dissolved in MeOH and CH_2N_2–Et_2O added. The reaction mixt. was concd *in vacuo* after standing at room temp. for 2 hr. The residue showed the same R_f value as **2** on TLC (solvents 1, 2, 3). ^1H NMR ($CDCl_3$) δ: 7.90, 7.89 (1H each, *s*, H-4, 5), 7.35 (1H, *s*, H-1), 7.17 (1H, *s*, H-8), 4.13 (6H, *s*, 3,6-OMe), 4.07 (6H, *s*, 2,7-OMe).

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REFERENCES

1. Ali, M., Ansari, S. H. and Qadry, J. S. (1991) *J. Nat. Prod.* **54**, 1271.
2. Ali, M. and Bhutani, K. K. (1989) *Phytochemistry* **28**, 3513.
3. Gellert, E. (1982) *J. Nat. Prod.* **45**, 50.
4. Govindachari, T. R. and Viswanathan, N. (1978) *Heterocycles* **11**, 587.
5. Phillipson, J. D., Tezcan, I. and Hylands, P. J. (1974) *Planta Med.* **25**, 301.
6. Govindachari, T. R., Viswanathan, N., Radhakrishnan, J., Pai, B. R., Natarajan, S. and Subramaniam, P. S. (1973) *Tetrahedron* **29**, 891.
7. Rao, K. V., Wilson, R. A. and Cummings, B. (1971) *J. Pharm. Sci.* **60**, 1725.
8. Govindachari, T. R., Viswanathan, N. and Pai, B. R. (1974) *Ind. J. Chem.* **12**, 886.
9. Govindachari, T. R., Rajagopalan, T. G. J. and Viswanathan, N. J. (1974) *J. Chem. Soc., Perkin Trans I* 1161.
10. Buckley III, T. F. and Rapoport, H. (1983) *J. Org. Chem.* **48**, 4222.
11. Nordlander, J. E. and Njoroge, F. G. (1987) *J. Org. Chem.* **52**, 1627.
12. Harborne, J. B. (1988) in *Introduction to Ecological Biochemistry*, pp. 265–266, Academic Press, London.
13. Nishida, R. and Fukami, H. (1989) *J. Chem. Ecol.* **15**, 2565.
14. Nishida, R. and Fukami, H. (1989) *J. Chem. Ecol.* **15**, 2549.
15. Honda, K., Tada, A., Hayashi, N., Abe, F. and Yamauchi, T. (1995) *Experientia*, **51**.