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Tronoharine, a novel hexacyclic indole alkaloid from a Malayan *Tabernaemontana*

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Abstract *: A novel hexacylic indole alkaloid, tronoharine, was obtained from the stem-bark extract of Tabernaemontana corymbosa and its structure elucidated by spectral analysis.*

Plants of the genus *Tabernaemontana* are rich in indole alkaloids[1,2] and have provided many new compounds with intriguing molecular skeletons.[1-4] We previously reported the presence of new chippiine-type derivatives as well as vobasinyl-iboga bisindoles from the Malayan species, *Tabernaemontana corymbosa*.[5,6] The bisindole compounds were notable for their ability to reverse multidrug resistance in vincristine-resistant KB cells.[6] We have now obtained minor amounts of a new indole alkaloid, tronoharine **1** from the stem extract, which possesses a novel hexacyclic carbon skeleton.

Tronoharine **1** was obtained in amorphous form, $[\alpha]_D$ +35 ($CHCl_3$, *c* 0.104). The UV spectrum showed absorption maxima at 202, 250, 272, and 296 nm (log ε 4.43, 3.88, 3.83, and 3.58 respectively) typical of an indole chromophore, while the IR spectrum showed bands due to hydroxyl (3353 cm^{-1}) and lactam carbonyl (1684 cm^{-1}) functions. The EIMS of **1** showed a molecular ion at *m/z* 336 with other significant fragment peaks at *m/z* 321 (M - Me), 307 (M - Et) and 279 (M - Et - CH_2=CH_2). HREIMS measurements gave the molecular formula $C_{21}H_{24}N_2O_2$ requiring eleven degrees of unsaturation.[7] The ^1H and ^{13}C NMR spectra (Table 1) showed the presence of an intact indole nucleus, a lactam carbonyl, an oxymethine, and an ethyl side chain. The upfield region of the ^{13}C NMR spectrum showed the signals of one methyl, six methylenes, four methines and only one quaternary carbon. COSY and HMQC experiments revealed the presence of the following partial structures, *viz*., a $CH(OH)CH_2$ unit, a NCH_2CH_2C unit and a $CH_2CH_2CHCH(CH_2CH_3)CH$ fragment. The

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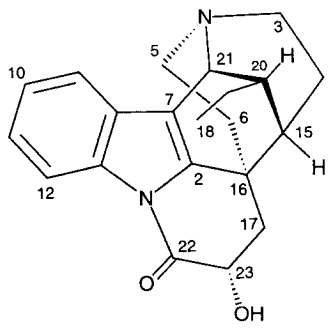
latter fragment, containing the ethyl side chain, is linked end to end by the *N*(4) atom, forming a piperidine ring, as indicated by the observed three-bond correlation from C-3 to H-21 in the HMBC spectrum. Other HMBC correlations (Table 1, Fig. 1) allowed the fragments to be connected as shown in structure **1**.



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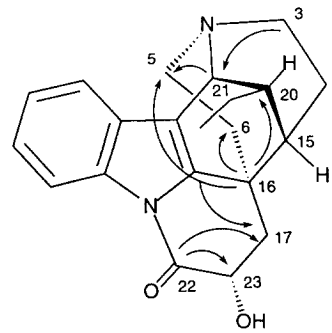
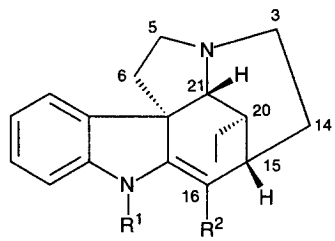


Fig. 1

The two-bond correlation from the lactam carbonyl carbon to the oxymethine hydrogen indicated that the hydroxy substituent is α to the carbonyl function. This observation, coupled with the observed three-bond correlations from C-22 and C-2 to H-17, the similarity of the UV spectrum of **1** with that of eburnamonine[8] and tacamonine,[9] as well as the low field shift of the aromatic H-12,[10] indicates formation of another six-membered lactam ring incorporating the indole nitrogen. The presence of another six-membered ring fused to the indole portion at C-7 and C-2, and incorporating C-16, C-15, C-20 and C-21, is indicated by the following three-bond HMBC correlations: C-7/H-20, C-16/H-20, and C-2/H-21. This leaves the CH_2CH_2 fragment and one remaining ring to be accounted for. This ethylene fragment is deduced to form the bridge from *N*(4) to the quaternary carbon, C-16. The H and C resonances of C-5 are characteristic of a methylene adjacent to a nitrogen atom, and in addition the observed HMBC correlations from C-16 to H-5 (^3J) and H-6 (^2J), and from C-21 to H-5, constitute further support for this proposal. The COSY spectrum indicated long range W-coupling between H-21 and H-15 (1.6 Hz). This is also confirmed by homonuclear decoupling, whereby irradiation of the H-15 multiplet at δ 2.35 causes collapse of the H-21 doublet of doublets (*J* 2.5, 1.6 Hz) to a doublet (*J* 2.5 Hz). This fixes the relative stereochemistry of these two hydrogens as β. Irradiation of H-15 causes NOE enhancement of H-14β (δ 1.90) and H-17β (δ 2.74). Irradiation of H-14β in turn causes NOE enhancement of H-20, which fixes its stereochemistry as shown in **1**. Irradiation of H-17β on the other hand, causes NOE enhancement of H-23, whereas irradiation of H-17α did not have any effect on H-23 (Fig. 2). These observations establish the stereochemistry of H-23 as β, which is also consistent with the observed $J_{23\text{-}17}$ coupling constant of 13 Hz, requiring H-23β and H-17α to be *trans*-diaxial to each other. The stereochemistry of the C(5)-C(6) ethylene bridge determines the absolute configuration of tronoharine. A possible origin of the tronoharine-type carbon skeleton is from a suitable aspidospermatan-type precursor (**2**) *via* cleavage of the C(6)-C(7) bond, followed by formation of a new bond between C(6) and C(16). A stepwise sequence incorporating a series of alkyl and hydride shifts and involving the cationic intermediates **3**, **4** and **5** can be

envisaged leading to the tronoharine ring system. The carbocation **3** is formed by an initial protonation of C-16. On the assumption that tronoharine is biosynthetically related to the aspidospermatan-type alkaloids, structure **1** is preferred over the enantiomer **6**. Tronoharine **1** represents a previously unencountered indole alkaloid having a novel hexacyclic carbon skeleton.



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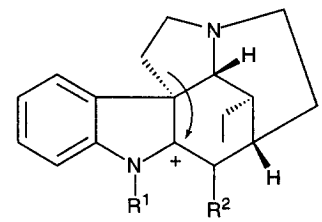

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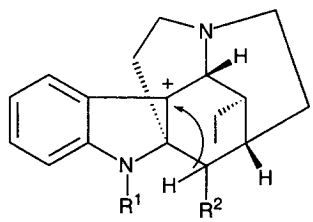

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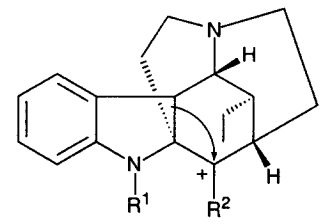

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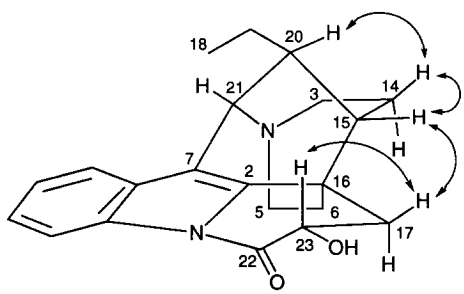

Fig. 2

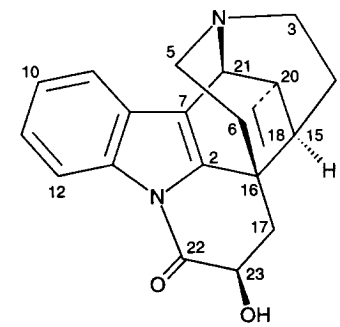

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Table 1 ^{1}H and ^{13}C NMR spectral data of tronoharine **1**[a]

Position	δ_C	δ_H	HMBC ^2J	HMBC ^3J	NOE[b]
2	141.8	-	-	6, 17, 21	
3α	45.6	2.47 td (12, 4.5)	-	5, 15, 21	
3β	-	3.04 ddd (12, 5, 2)			21
5a	53.2	2.87 ddd (12, 7.5, 1.2)	6	3, 21	
5b		3.08 td (12, 7.5)			
6a	42.4	1.75 ddd (13.5, 7.5, 1.2)	5	15	
6b	-	2.72 ddd (13.5, 12, 7.5)			
7	113.2	-	-	20	
8	136.9	-	-	10, 12, 21	
9	120.4	7.22 ddd (7.5, 1, 0.6)	-	11	10, 21
10	125.3	7.14 td (7.5, 1)	-	12	11
11	127.7	7.26 td (7.5, 1)	-	9	12
12	115.5	8.07 ddd (7.5, 1, 0.6)	-	10	11
13	138.6	-	-	9, 11	
14α	26.7	1.80 m	3	-	
14β	-	1.90 tdd (12, 5, 3.5)			15, 20
15	34.7	2.35 m	-	3, 6, 17, 21	14β, 17β, 20
16	49.8	-	6	5, 20	
17α	34.4	2.19 dd (17, 13)	23	15	
17β	-	2.74 dd (17, 7.9)			15, 23
18	11.5	0.71 t (7.3)	19	20	
19a	23.4	0.82 m	18, 20	-	
19b	-	0.82 m			
20	41.9	2.02 tdd (7, 2.5, 2)	-	18	14β, 15, 21
21	65.4	3.81 dd (2.5, 1.6)	-	3, 5, 15	3β, 9, 20
22	168.9	-	23	17	
23	67.6	4.50 dd (13, 7.9)	17	-	17β

[a]$CDCl_3$, 400 MHz; assignments based on COSY, HMQC, HMBC and NOE. [b]NOE's of geminal hydrogens not indicated.

References and Notes

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