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Structure, biological activity, and a biomimetic partial synthesis of the lirofolines, novel pentacylic indole alkaloids from *Tabernaemontana*

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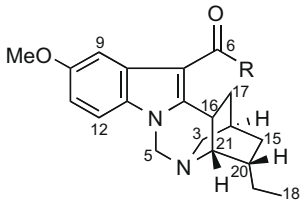
ABSTRACT

Two new pentacyclic indole alkaloids, lirofolines A and B, possessing a novel rearranged ibogan ring system, are obtained from two Malayan *Tabernaemontana* species (*Tabernaemontana corymbosa* and *Tabernaemontana divaricata*) and the structures are established by analysis of the spectroscopic data. A biomimetic partial synthesis of lirofoline A from ibogaine via the Polonovski reaction is carried out. Lirofolines A and B showed significant activity in reversing multidrug resistance in vincristine-resistant KB cells (IC_{50} 3.4 and 7.5 μg/ml, respectively).

Plants of the genus *Tabernaemontana* are rich sources of structurally novel, as well as biologically active, indole and bisindole alkaloids.[1–4] We recently reported the structures of three minor alkaloids isolated from the stem-bark extract of *Tabernaemontana corymbosa*, viz., conolutinine,[5] conoliferine, and isoconoliferine.[6] Conolutinine is characterized by a novel ring system incorporating a diazaspiro center and fused oxadiazepine–tetrahydrofuran rings, while conoliferine and isoconoliferine represent the first examples of alkaloid–lignan conjugates derived from the union of an iboga alkaloid and a lignan moiety. Other recent examples of unusual alkaloids from the Malayan *Tabernaemontana* which are notable for possessing novel ring systems, and which were postulated to be derived from known monoterpenoid indole precursors through pathways involving deep-seated rearrangements include, inter alia, the hexacyclic alkaloid, tronoharine,[7] the pentacyclic indole, tronocarpine,[8] and the indole-derived quinolinic alkaloids, voastrictine[9] and voaharine.[10] The Malayan representatives of this genus are also notable for producing new indole and bisindole alkaloids, including a number which exhibited important biological activities.[11–26] We now report the isolation and structure elucidation of two new pentacyclic indole alkaloids, lirofolines A (**1**) and B (**2**), from the stem-bark extracts of *T. corymbosa* and *T. divaricata*, respectively.[27]



1 R = H
2 R = CH_2OH

Lirofoline A (**1**) was obtained as a colorless oil (yield, ca. 0.4 mg kg^{-1}) from the stem-bark extract of *T. corymbosa* with $[\alpha]_D$ −41 (*c* 1.36, $CHCl_3$). The UV spectrum (EtOH, λ_{max} 216, 258, 280, and 309 nm) indicated the presence of a conjugated indole chromophore, while the IR spectrum showed a band at 1651 cm^{-1} due to a conjugated carbonyl function. The presence of the methine signals at δ_H 10.1 and δ_C 182.9 revealed that the conjugated carbonyl observed in the IR spectrum is due to an aldehyde function. The EIMS of **1** showed a molecular ion at *m*/*z* 324 as the base peak with a significant fragment ion peak at *m*/*z* 295 due to M−CHO or M−CH_2CH_3.[28] The ^1H NMR spectrum (Table 1) showed, in addition

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to the CHO function mentioned previously, the presence of a 10-methoxy-substituted indole ring (from the coupling pattern of the three aromatic hydrogens as well as from the HMBC data), an aromatic methoxy group, an ethyl side chain, and an isolated methylene, observed as a pair of downfield AB doublets at δ 4.88 and δ 4.94 (J = 12 Hz). The ^{13}C NMR spectrum (Table 1) gave a total of 20 carbon resonances (two methyls, five methylenes, eight methines, and five quaternary carbons) in agreement with the molecular formula ($C_{20}H_{24}N_2O_2$, 10 degrees of unsaturation) obtained from HREIMS measurements.

The COSY and HMQC spectra revealed, in addition to the aromatic and the isolated methylene mentioned previously, the presence of two additional spin systems, namely, an $NCHCHCH_2$ and an $NCH_2CHCH_2CHCH_2CH_3$. These partial structures are characteristic of the ethyl-substituted isoquinuclidine unit of an iboga skeleton. This was further substantiated by the characteristic long-range W-couplings (ca. 2.5 Hz) observed between H(17α)/H(15α), H(17β)/H(3β), H(15β)/H(21), and H(15β)/H(3α), which are characteristic of the 2-aza-bicyclo[2.2.2]octane unit associated with an iboga ring system. The major fragments revealed, which are the 10-methoxy-indole and the 2-aza-6-ethylbicyclo[2.2.2]octane units, are also found in ibogaine (**3**, Scheme 1), indicating a close similarity between the two structures. Careful study of the NMR data, however, revealed a major departure when compared to those of ibogaine (**3**). Thus, signals associated with the C(5)–C(6) tryptamine bridge of an iboga structure were absent, which were replaced instead by signals from a conjugated aldehyde function (δ_H 10.1, δ_C 182.9).

The observed two- and three-bond correlations from the aldehyde H(6) to C(7) and C(8), respectively, in the HMBC spectrum, indicated the attachment of the aldehyde group at the aromatic C(7) (Fig. 1). The attachment of the 2-aza-6-ethylbicyclo[2.2.2]octane fragment was deduced to be from C(2) to C(16), which is unchanged from that in an iboga skeleton. This was clearly indicated by the observed correlations from H(16) and H(17) to C(2) in the HMBC spectrum (Fig. 1). Since the indolic NH signal is conspicuously absent (usually observed in most iboga derivatives), and there was no indication of any *N*-methyl or carbamate functions in the ^{1}H NMR spectrum of **1**, the remaining isolated methylene function, $C(5)H_2$, must be linked to both the indole as well as the isoquinuclidine nitrogens, which would be consistent with its observed carbon shift value (δ 67.1). This is also in excellent agreement with the observed long-range correlations from H(5) to C(2), C(3), C(13), and C(21) in the HMBC spectrum of **1**.

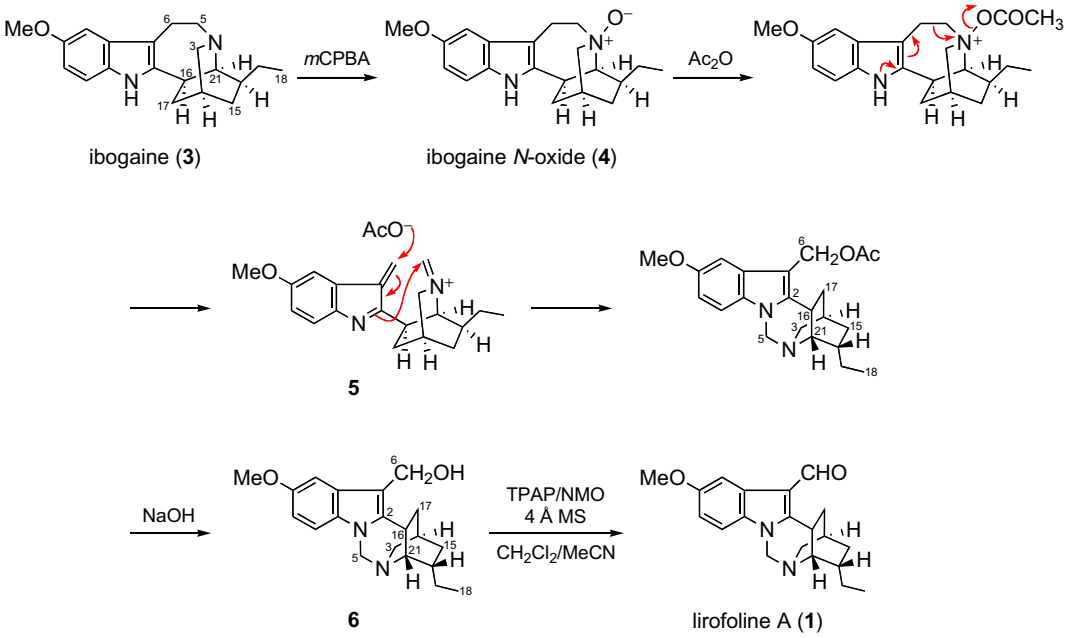
The structure is also consistent with the observed NOE interactions for **1** (Fig. 2). Thus, irradiation of H(9) caused enhancement of H(6), while irradiation of H(6) in turn caused enhancement of H(9) and H(16). In addition, reciprocal NOEs observed between H(20)/H(16) confirmed the orientation of H(20) as β, while other NOE interactions (Fig. 2) were in complete agreement with the proposed structure of **1**.

Lirofoline B (**2**) was obtained as a colorless oil (yield, ca. 3.2 mg kg^{-1}) from the stem-bark extract of *T. divaricata* (single flower variety) with $[\alpha]_D$ −17 (*c* 0.08, $CHCl_3$). The UV spectrum (EtOH, λ_{max} 218, 256, 278, and 308 nm) was similar to that of **1**, while the IR spectrum showed bands due to OH (3407 cm^{-1}) and conjugated carbonyl (1627 cm^{-1}) functions. The ESIMS and FABMS of **2** showed an MH^+ ion peak at m/z 355, while HRFABMS measurements yielded the molecular formula $C_{21}H_{26}N_2O_3$.[29] This showed that **2** differs from lirofoline A (**1**) by the addition of 30 mass units, thereby suggesting the replacement of a H-atom in **1** with a CH_2OH group in **2**. This is in agreement with the ^{1}H and ^{13}C NMR spectra of **2** (Table 1) which showed the presence of a conjugated $COCH_2OH$ group, replacing the conjugated aldehyde group present in **1**. Apart from this, the NMR data of **2** were essentially similar to those of **1**. This proposal was further supported by the observed two- and three-bond correlations from the oxymethylene H(22) to the ketonic carbon, C(6), and the aromatic C(7), respectively, as well as the two-bond correlation from 22-OH to C(6), in the HMBC spectrum, which indicated attachment of the

Table 1
^{1}H (400 MHz) and ^{13}C (100 MHz) NMR data of lirofolines A (**1**) and B (**2**) in $CDCl_3$[a]

Position	1		2	
	δ_H	δ_C	δ_H	δ_C
2	—	153.7	—	152.6
3α	2.72 (dt, 10, 2 Hz)	53.5	2.67 (dt, 10, 2.5 Hz)	53.5
3β	3.27 (dt, 10, 3 Hz)		3.27 (dt, 10, 2.5 Hz)	
5α	4.88 (d, 12 Hz)	67.1	4.92 (d, 12 Hz)	67.2
5β	4.94 (d, 12 Hz)		4.98 (d, 12 Hz)	
6	10.1 (s)	182.9	—	192.5
7	—	111.8	—	108.0
8	—	127.0	—	126.8
9	7.74 (br d, 2.4 Hz)	103.1	7.36 (d, 2 Hz)	103.9
10	—	156.8	—	156.6
11	6.88 (dd, 8.8, 2.4 Hz)	112.8	6.90 (dd, 8.5, 2 Hz)	111.6
12	7.12 (d, 8.8 Hz)	109.8	7.17 (d, 8.5 Hz)	110.0
13	—	129.9	—	129.8
14	1.81 (m)	25.5	1.81 (m)	25.4
15α	1.90 (dddd, 12.5, 10, 4, 2 Hz)	31.1	1.91 (dddd, 13, 10, 4, 2.5 Hz)	31.2
15β	1.20 (ddt, 12.5, 6.8, 2 Hz)		1.18 (ddt, 13, 7.5, 2 Hz)	
16	3.55 (br dt, 12, 2 Hz)	29.7	3.67 (dt, 12, 2 Hz)	31.8
17α	1.70 (m)	33.4	1.58 (m)	32.4
17β	2.20 (br t, 12 Hz)		2.29 (tdd, 12, 2.5, 2 Hz)	
18	0.96 (t, 7.3 Hz)	11.7	0.96 (t, 7.5 Hz)	11.7
19a	1.59 (m)	27.4	1.60 (m)	27.3
19b	1.59 (m)		1.60 (m)	
20	1.72 (m)	37.8	1.73 (dq, 10, 7.5 Hz)	37.7
21	2.85 (br s)	51.8	2.82 (br s)	51.9
22a	—	—	4.73 (dd, 17, 4 Hz)	67.0
22b			4.78 (dd, 17, 4 Hz)	
10-OMe	3.89 (s)	55.8	3.90 (s)	55.9
OH	—	—	4.15 (br s)	—

[a] Assignments based on COSY, HMQC, HMBC, and NOE.



Scheme 1.

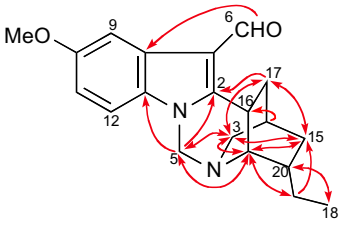


Figure 1. Selected HMBCs of **1**.

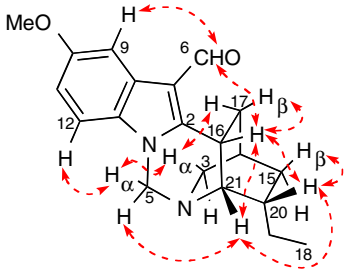


Figure 2. Selected NOEs of **1**.

$COCH_2OH$ side chain at C(7). The structure of **2** was also consistent with the NOE data, which showed reciprocal NOEs between H(9)/H(22) and H(16)/H(22). Other NOEs observed were essentially similar to those observed for **1**.

Although lirofolines A and B (**1** and **2**) were obtained as interesting novel natural products from two different Malayan *Tabernaemontana* species, a search of the literature revealed that the basic ring system has been encountered previously as side products in reactions in the ibogaine and catharanthine series (chemical transformations of ibogaine to voacangine[30] and coupling of catharanthine and its derivatives with vindoline[31–38]). It follows that the ring system of the lirofolines in all probability arises from similar precursors and in like fashion, via scission of the C(5)–C(6) bond of an oxidized derivative to give the intermediate **5**, followed by the intramolecular bond formation between C(5) and *N*(1). Based on this supposition, a biomimetic conversion of ibogaine (**3**) into lirofoline A (**1**) under Polonovski conditions was carried out (Scheme 1). Thus, ibogaine *N*-oxide (**4**)[39] [prepared from **3** and *m*CPBA (1 equiv); CH_2Cl_2, −30 °C] on treatment with acetic anhydride in CH_2Cl_2 (50 ml) at −10 °C, followed by hydrolysis (NaOH) gave a single major product **6**[40] in 70% yield. Subsequent oxidation of alcohol **6** with a catalytic amount of tetra-*n*-propylammonium perruthenate (TPAP, 5 mol %) in the presence of excess *N*-methylmorpholine *N*-oxide (NMO, 20 equiv) and 4 Å molecular sieves, gave lirofoline A (**1**) in 30% yield. The structure was confirmed by comparison of its spectroscopic data, $[\alpha]_D$ and TLC with those of the authentic material.

Despite the above transformation, the possibility that the lirofolines in the present case could be artifacts was rendered unlikely by the observation that repeated extractions of fresh material of *T. divaricata* consistently provided **2**, while **1** was isolated from an entirely different *Tabernaemontana* species. Furthermore, subjecting the putative precursor of the lirofolines, ibogaine (**3**), or its *N*-oxide **4**, to reaction under the conditions of the extraction, resulted only in recovery of intact starting material, with no evidence of any transformation into either **1** or **2**. In any case, the present isolations represent the first examples in which such a ring system is encountered in a naturally occurring alkaloid.

Lirofolines A (**1**) and B (**2**) showed no appreciable cytotoxicity against both drug-sensitive and vincristine-resistant KB cells, but showed significant activity in reversing multidrug resistance in vincristine-resistant KB (VJ300) cells (IC_{50} 3.4 and 7.5 μg/ml, respectively, in the presence of 0.1 μg/ml of vincristine).

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39. *Compound* **4**: Light yellowish oil; $[\alpha]_D$ −76 (*c* 0.16, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.71 (1H, br s, NH), 7.19 (1H, d, *J* = 7.19 Hz, H-12), 6.82 (1H, d, *J* = 2.3 Hz, H-9), 6.78 (1H, dd, *J* = 8.7, 2.3 Hz, H-11), 3.93 (1H, m, H-5), 3.83 (3H, s, 10-OMe), 3.79 (1H, br d, *J* = 11.5 Hz, H-3), 3.71 (1H, br d, *J* = 11.5 Hz, H-3), 3.54 (1H, br d, *J* = 12 Hz, H-5), 3.49 (1H, br s, H-21), 3.24 (1H, m, H-16), 3.08 (1H, m, H-6), 2.95 (1H, m, H-6), 2.26 (1H, m, H-15), 2.17 (1H, m, H-14), 2.10 (2H, m, H-19), 1.97 (1H, m, H-17), 1.82 (1H, m, H-17), 1.69 (1H, m, H-20), 1.49 (1H, m, H-15), 0.93 (3H, t, *J* = 7.8 Hz, H-18); ^{13}C NMR (100 MHz, $CDCl_3$) δ 153.9 (C-10), 139.8 (C-2), 130.3 (C-13), 128.1 (C-8), 111.8 (C-11), 111.5 (C-12), 107.2 (C-7), 100.0 (C-9), 76.9 (C-5), 72.4 (C-21), 70.3 (C-3), 56.1 (10-OMe), 43.1 (C-20), 37.0 (C-16), 31.6 (C-15), 31.2 (C-19), 30.4 (C-17), 26.1 (C-14), 21.1 (C-6), 13.0 (C-18); IR (film) ν_{max} 3149 cm^{-1}; UV (EtOH) λ_{max} (log ε) 210 (4.00), 224 (3.98), 280 (3.58), 297 (3.50), 307 (3.32); ESIMS *m*/*z* 327 $[MH]^+$; HRESIMS *m*/*z* 327.2080 (calcd for $C_{20}H_{27}N_2O_2$, 327.2073).
40. *Compound* **6**: Light yellowish oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.10 (1H, d, *J* = 8.9 Hz), 7.09 (1H, d, *J* = 2.3 Hz), 6.80 (1H, dd, *J* = 8.9, 2.3 Hz), 4.88 (1H, d, *J* = 11.4), 4.78 (1H, d, *J* = 11.4 Hz), 3.86 (3H, s), 3.94 (2H, s), 3.19 (1H, dt, *J* = 10, 3 Hz), 3.15 (1H, br dt, *J* = 12, 2.2 Hz), 2.79 (1H, dt, *J* = 10, 2 Hz), 2.74 (1H, br s), 2.07 (1H, br t, *J* = 12 Hz), 1.86 (1H, m), 1.76 (1H, m), 1.69 (1H, m), 1.58 (2H, m), 1.56 (1H, m), 1.52 (1H, m), 0.94 (3H, t, *J* = 7.3 Hz); ESIMS *m*/*z* 327 $[MH]^+$; HRESIMS *m*/*z* 327.2072 (calcd for $C_{20}H_{27}N_2O_2$, 327.2067).