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Conolutinine, a hexacyclic indole alkaloid with a novel ring system incorporating a diazaspiro center and fused oxadiazepine–tetrahydrofuran rings

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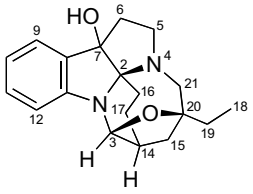
ABSTRACT

A hexacyclic indole alkaloid possessing an unprecedented ring system incorporating a diazaspiro center and fused oxadiazepine–tetrahydrofuran rings has been isolated from the Malayan *Tabernaemontana corymbosa*. The structure was established by analysis of the spectroscopic data and a possible biogenetic pathway from a cleavamine-type precursor is presented.

Plants of the genus *Tabernaemontana* are well known as prodigious sources of biologically active indole and bisindole alkaloids.[1–4] In recent years, a number of alkaloids of unusual structures have been reported from plants of this genus. The Malayan *Tabernaemontana corymbosa* for instance, has provided several new alkaloids which are characterized by novel molecular skeletons such as the hexacyclic alkaloid, tronoharine,[5] the pentacyclic indole, tronocarpine,[6] and the quinolinic alkaloid, voastrictine.[7] The same plant also yielded a number of new indole and bisindole alkaloids,[8–17] including several vobasinyl-iboga bisindoles which reverse multidrug-resistance in vincristine-resistant KB cells.[8] In continuation of our studies of biologically active alkaloids from Malaysian *Tabernaemontana*,[4–15,18,19] we now report the isolation of a new indole alkaloid, conolutinine (**1**), characterized by an unusual ring system, which was isolated from the stem-bark extract of the same plant collected from a different location.

Conolutinine (**1**) was isolated in trace amount after repeated chromatographic fractionation of the basic fraction from the EtOH extract as a colorless oil, $[\alpha]_D$ −25 ($CHCl_3$, *c* 0.18). The UV spectrum (EtOH, 202, 249, and 305 nm) indicated the presence of a dihydroindole chromophore, while the IR spectrum showed a band at 3392 cm^{-1}, indicating the presence of an OH functionality. The EI mass spectrum of 1 showed an M^+ peak at *m*/*z* 312, which was analyzed for $C_{19}H_{24}N_2O_2$, with a prominent fragment peak due to loss of H_2O at *m*/*z* 294.[20] The ^{13}C NMR spectrum (Table 1) comprising one methyl, seven methylenes, six methines, and five quaternary carbons, accounted for all 19 carbon resonances. In addition to the resonances which can be readily assigned to the carbons of the aromatic ring, the spectrum was characterized by the presence of four low-field signals from δ 84 to 87. The ^1H NMR spectrum (Table 1) showed signals due to four aromatic hydrogens, corresponding to an unsubstituted indole ring (δ 6.80–7.30), and an ethyl side chain (δ_H 0.90, δ_C 9.0; δ_H 1.59, δ_C 33.0). The COSY and HMQC data revealed, in addition to the presence of an unsubstituted indole moiety and an ethyl group, an isolated aminomethylene, an aminoethylene, one $-CH_2CH_2-$ fragment, and a $-CHCHCH_2-$ unit.



1 7α-OH
2 7β-OH

The aromatic carbon resonances can be readily assigned based on the coupling behavior of the attached hydrogens as well as

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

Position	δ_C	δ_H
2	86.6	–
3	84.3	5.59 (d, 7.6 Hz)
5	52.9	2.29 (m)
5′		2.93 (m)
6	34.4	2.29 (m)
6′		2.52 (m)
7	86.0	–
8	132.1	–
9	124.1	7.30 (d, 7.5 Hz)
10	119.4	6.82 (td, 7.5, 1 Hz)
11	130.2	7.21 (td, 7.5, 1 Hz)
12	108.8	6.80 (d, 7.5 Hz)
13	148.4	–
14	36.4	2.78 (m)
15β	35.9	1.91 (t, 3 Hz)
15α		2.19 (dd, 13, 4.4 Hz)
16	23.7	1.86 (m)
16′		2.52 (m)
17	22.6	1.54 (m)
17′		1.86 (m)
18	9.0	0.90 (t, 7.3 Hz)
19	33.0	1.59 (m)
19′		1.59 (m)
20	84.1	–
21β	65.2	2.48 (d, 13 Hz)
21α		2.84 (d, 13 Hz)

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

the characteristic ^{13}C shifts of dihydroindoles. This was further confirmed by the recognition of H(9) from its NOE interaction with one of the H(6) protons, which in turn allowed the assignment of the $-NCH_2CH_2-$ fragment as that corresponding to the C(5)–C(6) unit. The three-bond H(6) to C(8) correlation in the HMBC spectrum provided additional support for the assignment, while the three-bond correlation from H(9) to the downfield quaternary signal at δ 86.0 indicated that this signal was due to C(7). The three-bond correlation from the isolated methylene hydrogens (H(21)) to C(5) confirmed its attachment to N(4), while correlations from H(18) to C(20) and H(19) to C(21) suggested attachment of the ethyl side chain and the isolated methylene to the quaternary C(20) carbon, which was linked to an oxygen atom as was evident from its observed shift at δ 84.1. This leaves two other centers associated with the remaining two low-field resonances, one, a quaternary center at δ 86.6, and the other, an oxymethine at δ 84.3. The former resonance was assigned to the doubly spirocyclic, diazaspiro center, C(2), while the latter is due to a methine carbon C(3) which is linked to both a nitrogen (N(1)) and an oxygen atom. The three-bond correlations from this oxymethine hydrogen (H(3)) to C(2) indicated the attachment of the $-CHCHCH_2$ fragment (corresponding to C(3)–C(14)–C(15)) to N(1). The correlations from H(19) and H(21) to C(15) indicated connection of C(15) to C(20). This leaves the remaining ethylene fragment (corresponding to C(16)–C(17)), which must be branched from C(2), the correlation from H(16) to C(14) indicating attachment of C(17) to C(14). Insertion of an ether bridge linking the quaternary C(20) and the oxymethine C(3), and placement of an OH substituent at C(7), complete the assembly of the ring system of conolutinine, which is also in accord with the full HMBC data (Fig. 1).

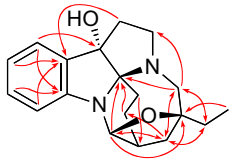


Figure 1. Selected HMBCs of **1**.

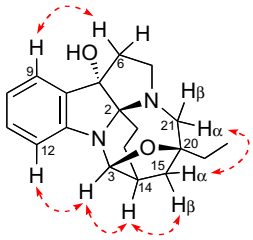
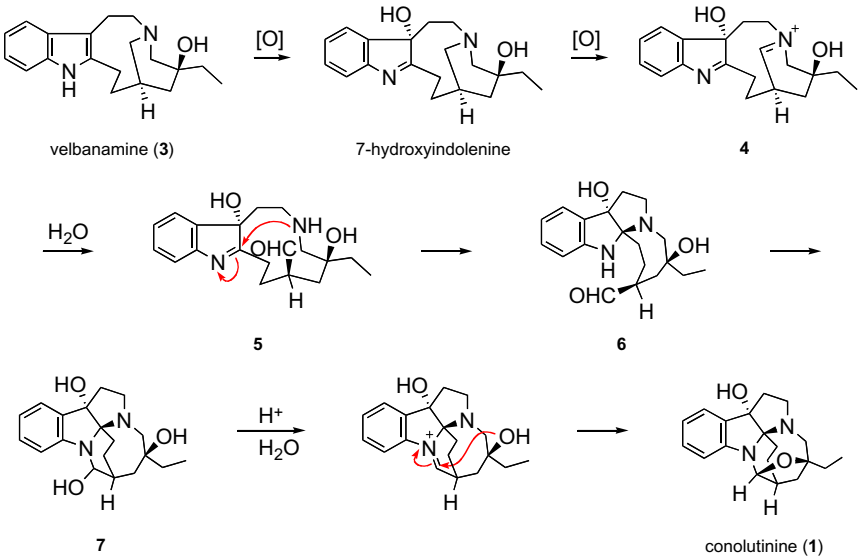


Figure 2. Selected NOEs of **1**.

The proposed structure of conolutinine is also in accord with the NOE data (DNOE, NOESY; Fig. 2). Geometrical constraints associated with the rigid architecture of the molecule require that the ether bridge linking C(3) and C(20), and the ethylene bridge linking



Scheme 1. Possible biogenetic pathway to **1**.

C(2) and C(14), be located on opposite sides with respect to the 1,3-diazocine ring system. This leaves only two possible structures (**1** and **2**) to be considered which differ only in the configuration at C(7). Structure **1** which has a *cis*-fused B/C ring should be more stable compared to **2** with a *trans*-fused B/C ring, an inference supported by examination of models, as well as by the results of molecular modeling calculations.[21] Based on energetic considerations, the structure of conolutinine corresponds with the relative configuration shown in **1**.

A likely pathway to **1** is presented in Scheme 1 from the cleavamine-type alkaloid velbanamine (**3**), which was also isolated from the stem-bark extract. Oxidation of velbanamine to the 7-hydroxyindolenine, followed in succession by another oxidation, gives the iminium ion **4**. Hydrolytic cleavage of **4** leads to the amino aldehyde **5**, which on cyclization via attack of N(4) provides the diazaspiro intermediate **6**. A further cyclization via an intramolecular amine-aldehyde reaction leads to the hemiaminal **7**, which on subsequent dehydration leads to the iminium ion intermediate. Finally, intramolecular attack by the C(20)–OH yields the ring system of conolutinine (**1**).

Conolutinine represents a novel indole alkaloid with an unprecedented hexacyclic ring system incorporating a diazaspiro center and fused oxadiazepine–tetrahydrofuran rings. Conolutinine (**1**) did not show any appreciable effect when tested for synergism with TRAIL, inhibition of NO generation, and inhibition of melanin synthesis. It also showed no appreciable cytotoxicity against both drug-sensitive and vincristine-resistant KB cells, but showed moderate ability to reverse multidrug resistance in vincristine-resistant KB (VJ300) cells (IC_{50} 16 μg/ml in the presence of 0.1 μg/ml of vincristine).

Acknowledgments

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20. HREIMS found *m*/*z* 312.1834 (calcd for $C_{19}H_{24}N_2O_2$, 312.1838).
21. MM2 force field calculations (ChemBio3D Ultra version 11.0) indicate a difference of about 20 kcal/mol favoring structure **1** over **2**.