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A pyridine ring-containing ecdysteroid from *Diploclisia glaucescens*

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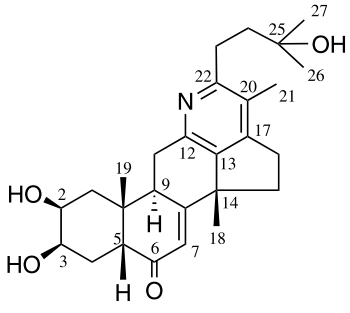
Abstract—A novel pyridine ring-containing ecdysteroid, named diploclidine, was isolated from the methanol extract of the leaves of *Diploclisia glaucescens*, and its structure was determined by spectral means.

Diploclisia glaucescens (Blume) Diels (Menispermaceae) is a creeper growing in the mid-country regions of South India, Sri Lanka and south part of China, and the leaves of the plant have been used in the treatment of biliousness and venereal diseases.[1] Our group reported earlier that this plant is a rich source of 20-hydroxyecdysone (3% content of the dried stem).[2] Previous phytochemical studies of this plant led to the isolation of several ecdysteroids,[2–4] including a unique 3-deoxy-1β,20-dihydroxyecdysone,[5] oleanane saponins,[6] lignan compounds,[7] acetylenic fatty acids,[8] a proaporphine alkaloid.[9] In the present study, we have investigated the alkaloidal constituents of the leaves of the plant to provide a new pyridine ring-containing ecdysteroid **1**, named diploclidine.

The dry ground mature leaves of *D. glaucescens*, collected from the central province of Sri Lanka in April 2001, were extracted with methanol. The resulting extract was dissolved in 2N HCl and the soluble part was washed with dichloromethane. The acidic extract was neutralized with NH_4OH and then re-extracted with dichloromethane. Chromatographic separation of the alkaloid fraction on silica gel and alumina columns, and p-tlc on silica gel plates furnished compound **1** (6 mg, 0.00015% based on dry stem).

Diploclidine, obtained as a sticky-solid, showed $[\alpha]_D^{24}$ +78.9 (*c* 0.8, MeOH) and a quasimolecular ion peak at *m*/*z* 440 $[M+H]^+$ in the positive FABMS. The molecular formula, $C_{27}H_{37}O_4N$, was deduced from positive HRFABMS data (*m*/*z*: 440.2837 $[M+H]^+$, $C_{27}H_{38}O_4N$ requires 440.2801). The UV spectrum (MeOH) of **1** showed three maxima at 215, 240 and 275 nm. The absorption at 240 nm could be due to a 7-en-6-one chromophore of ecdysteroids, while the other at 275 could be due to the pyridine ring.



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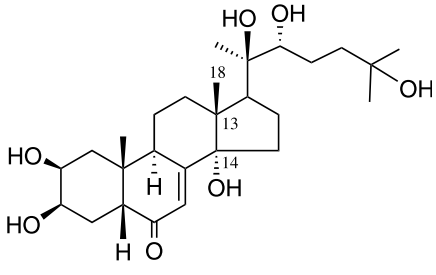

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Keywords: steroids and sterols; *Diploclisia glaucescens*; ecdysteroid; pyridines.
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The ^{1}H NMR of **1** showed the signals of five tertiary methyls at δ 1.07, 1.31, 1.33, 1.37 and 2.57. In addition, the signals of two oxymethine protons at δ 3.73 (ddd, $J=12.0$, 3.8, 2.5 Hz) and 4.04 (q-like, $J=2.5$ Hz) and an olefinic proton at 6.04 (d, $J=2.7$ Hz) were observed. These were reminiscent of the signals of H-2, H-3 and H-7 of ecdysteroids such as 20-hydroxyecdysone **2**. Further, the H–H COSY spectrum of **1** revealed that the H-2 and H-3 are adjacent to each other, and the signals at δ 2.48 and 2.67 are assigned to H-5β and H-9α, respectively, which were observed in their characteristic splitting patterns.[10] Thus, compound **1** was suggested to be an ecdysteroid with a trivial A,B-ring structure (2β,3β-dihydroxy-7-en-6-one with 5β-H), but a much-modified C,D-ring structure wherein a nitrogen atom should be impregnated. In the ^{13}C NMR spectrum of **1**, signals due to five sp^2 carbons at δ 130.2, 136.6, 142.0, 155.2 and 158.6 were characteristic, besides two sp^2 carbons at δ 123.5 and 171.7 ascribable to the C-7,8-double bond.

The HMQC and HMBC spectra of **1** confirmed the above formulation and provided further evidence on the structure. As can be seen in Figure 1, long-range correlations were observed between 19-H_3 (δ 1.07) and C-10, C-1, C-5 and C-9; 26-H_3/27-H_3 (δ 1.33 and 1.37) and C-25 and C-24; 18-H_3 (δ 1.31) and C-14, C-8, C-15 and C-13, and between 21-H_3 (δ 2.57) and two olefinic carbons at δ 130.2 and 155.2. These HMBC data, together with additional correlations given in Figure 1, indicated the presence of a pyridine ring as shown in the formula. The sp^2 carbon signals were assigned as δ

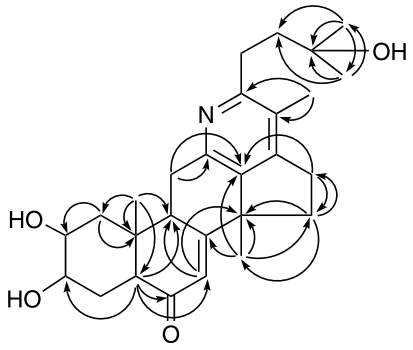

Figure 1. HMBC correlations (H→C) of **1**.

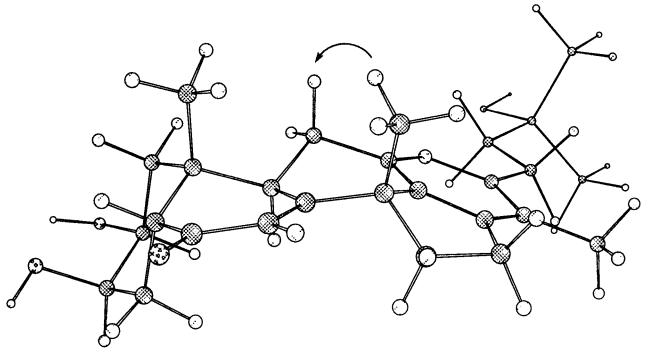

Figure 2. A pertinent NOE correlation and the most stable conformation (deduced from MM2 calculation) of **1**.

Table 1. NMR data of **1** ($CDCl_3$, 500 MHz/125 MHz)

C	δ_C	δ_H[a]
1	36.9	1.66 (dd, 13.7, 12.0)
		1.94 (dd, 13.7, 3.8)
2	67.6	3.73 (ddd, 12.0, 3.8, 2.5)
3	67.4	4.04 (ddd, 2.5)
4	31.1	1.59 (ddd, 14.2, 13.4, 2.5)
		1.89 (dt, 14.2, 4.5, 2.5)
5	49.3	2.48 (dd, 13.4, 4.5)
6	202.0	–
7	123.5	6.04 (d, 2.7)
8	171.7	–
9	40.8	2.67 (m)
10	40.3	–
11	21.4	2.42 (dd, 14.4, 13.0)
		3.24 (dd, 14.4, 4.0)
12	158.6	–
13	136.6	–
14	47.1	–
15	40.1	2.12 (ddd, 12.4, 11.0, 8.6)
		2.40 (dd, 12.4, 7.0)
16	31.9	2.86 (dd, 16.5, 8.6)
		3.20 (ddd, 16.5, 11.0. 7.0)
17	142.0	–
18	26.3	1.31 (s)
19	22.6	1.07 (s)
20	130.2	–
21	22.1	2.57 (s)
22	155.2	–
23	22.9	2.72 (td, 12.5, 5.9)
		2.92 (ddd, 13.0, 12.5, 2.8)
24	44.4	1.35 (m)
		1.77 (td, 13.0, 5.9)
25	70.9	–
26	27.6	1.37 (s)
27	30.4	1.33 (s)

[a] Coupling constants are expressed in Hz.

158.6 (C-12), 136.6 (C-13), 142.0 (C-17[11]), 130.2 (C-20[11]), 155.2 (C-22[11]). It should be noted that the 18-methyl group is not at the C-13 position but attached to the C-14 position.

Finally, the orientation of the methyl group at C-14 was determined to be β based on the NOE study in which the 11-Hβ axial proton (δ 2.42, dd, $J=14.4$, 13.0 Hz) was enhanced upon irradiation of the methyl group (Fig. 2). No NOE between the methyl group and H-9 was observed. Hence, the structure of **1** was established as shown in the formula. Complete ^{1}H and ^{13}C NMR assignments are listed in Table 1.

Diploclidine is a novel ecdysteroid with a pyridine-ring as the fifth ring system, and the occurrence of the 18-methyl group at C-14 is unprecedented. From the biosynthetic point of view, the position of the methyl group can be accounted for by two ways. One is that a protosterol-type intermediate having a methyl group at the 14β-position[12] is metabolized to compound **1** without migration of the methyl group. The other is that an ecdysteroid precursor having a 14α-hydroxyl group and a methyl group at the 13β-position undergoes migration of the methyl group from C-13 to C-14 with concomi-

tant elimination of the 14α-hydroxyl group during the pyridine ring formation. Large-scale extraction of diploclidine and attempted isolation of related compounds are under way for the screening of their possible biological activities.

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