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A new ecdysteroid, 2-deoxy-5β,20-dihydroxyecdysone from the fruits of *Diploclisia glaucescens*

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Abstract

Chemical investigation of ethyl acetate extract of the fruits of *Diploclisia glaucescens* of the family Menispermaceae furnished a new ecdysteroid 2-deoxy-5β,20-dihydroxyecdysone, together with 20-hydroxyecdysone, 3-deoxy-1β,20-dihydroxyecdysone, 2-deoxy-20-hydroxyecdysone, 24-ethyl-20-hydroxyecdysone (makisterone C). Latter two ecdysteroids are reported first time from the family Menispermaceae.

Keywords: *Diploclisia glaucescens*; Menispermaceae; Ecdysteroids; 2-Deoxy-5β,20-dihydroxyecdysone; 20-Hydroxyecdysone; 3-Deoxy-1β,20-dihydroxyecdysone; 2-Deoxy-20-hydroxyecdysone; 24-Ethyl-20-hydroxyecdysone

1. Introduction

Diploclisia glaucescens (Bl.) Diels (=*Cocculus macrocarpus* W. & A.) is a liana of the family Menispermaceae growing in India and Sri Lanka. The leaves of the plant have been used in the treatment of biliousness and venereal diseases [1]. Five phytoecdysteroids, 20-hydroxyecdysone (**1**), makisterone A, 24(28)-dehydromakisterone A, 24-epimakisterone A and pterosterone have been reported from the seeds of the plant [2]. We have previously reported the isolation of **1** (>3%, the highest recorded yield from a plant) from the stem [3] and 3-deoxy-1β,20-dihydroxyecdysone (**2**) from the leaves of the plant [4]. In this paper, we report the isolation and structure elucidation of a new ecdysteroid, 2-deoxy-5β,20-dihydroxyecdysone (**3**) together with **1**,**2**,2-deoxy-20-hydroxyecdysone (**4**) and makisterone C (24-ethyl-20-hydroxyecdysone, lemmasterone, podecdysone) (**5**) from the fruits of the plant. Ecdysteroids **4** and **5** are reported first time from the family Menispermaceae.

2. Experimental

2.1. General methods

Melting points (mps) were determined by Gallenkamp apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-360 polarimeter at 25 °C. UV spectra were recorded on a UV-160 A spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Brucker DRX500 (500 MHz for ^{1}H and 125 MHz for ^{13}C) spectrometer in C_5D_5N or CD_3OD solution. Tetramethylsilane was used as an internal standard for ^{1}H shifts and d_5-pyridine ($\delta = 149.8$) or CD_3OD signal ($\delta = 49.0$) was used as a reference for ^{13}C-chemical shifts. Positive ion FABMS were obtained on a JEOL JMS-AX505HA spectrometer with glycerol as matrix. HPLC analyses were carried out on Shimadzu LC-6A apparatus equipped with UV detector under reversed phase C_{18} and isocratic solvent condition.

2.2. Plant material

The unripe fruits of *D. glaucescens* were collected from the central province of Sri Lanka in April 2001 and identified by Mr. S.P. Ekanayake (Environmental and Forestry Division, Mahaweli Authority, Polgolla, Sri Lanka). A voucher

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specimen is deposited at the Institute of Fundamental Studies.

2.3. Extraction and isolation

The dried unripe, ground fruits of *D. glaucescens* (800 g) were defatted with cold *n*-hexane and extracted with ethyl acetate. Evaporation of the ethyl acetate gave brown colored solid (10.8 g). The ethyl acetate extract (10 g) was chromatographed over a column of silica gel (Merck Art 7734) with EtOAc–MeOH. The column fractions, which contained UV active spots on TLC, were combined and passed through a column of Sephadex LH-20 with methanol as eluent. Further purification of the UV active fractions by HPLC (STR Prep-ODS 20 mm × 250 mm column, 60% H_2O–MeOH: 5 ml/min, UV detection: 243 nm) yielded **1** (300 mg), **2** (120 mg), **3** (12 mg), **4** (40 mg) and **5** (30 mg). All these compounds were identified by the detailed analysis of spectral data and identification of **1**, **2**, **4** and **5** were further confirmed by the direct comparison with authentic samples.

2.3.1. 20-Hydroxyecdysone (1)

Mp: 242–244 °C; UV λ_{max}(EtOH): 243 nm; ^{1}H and ^{13}C NMR identical with reported data [3].

2.3.2. 3-Deoxy-1β,20-dihydroxyecdysone (2)

Mp: 152–155 °C; $[\alpha]_D^{25} = +63.3°$ (c = 0.72, MeOH); UV λ_{max}(EtOH): 241 nm; ^{1}H and ^{13}C NMR identical with reported data [4].

2.3.3. 2-Deoxy-5β,20-dihydroxyecdysone (3)

Mp: 128–130 °C; $[\alpha]_D^{25} = +62.4°$ (c = 0.34, MeOH); UV λ_{max}(EtOH): 241 nm; ^{1}H & ^{13}C NMR (C_5D_5N): see Table 1; ^{1}H-NMR data (CD_3OD, 500 MHz): δ 0.89 (3H, *s*, 18-Me), 1.18 (3H, *s*, 19-Me), 1.18 (3H, *s*, 21-Me), 1.18, 1.19 (3H each, *s*, 26-Me, 27-Me), 2.39 (1H, *m*, 17-H), 5.79 (1H, *d*, J = 2.2 Hz, 7-H), 3.28–3.33 (2H, *m*, 9-H, 22-H, overlapped with the solvent signal), 4.06 (1H, *m*, 3-H), 5.84 (1H, *m*, 7-H); ^{13}C NMR (CD_3OD): see Table 1; HRFABMS(+) *m/z*: 481.3124 $[M + H]^+$, $C_{27}H_{45}O_7$ requires 481.3165.

2.3.4. 2-Deoxy-20-hydroxyecdysone (4)

Mp: 215–218 °C; UV λ_{max}(EtOH): 241 nm; ^{1}H NMR identical with reported data [5]; FABMS(+) *m/z*: 465 $[M + H]^+$, 447, 429, 411.

2.3.5. Makisterone C (5)

Mp: 268–270 °C; $[\alpha]_D^{25} = +54.8°$ (c = 1.3, MeOH); UV λ_{max}(EtOH): 243.4 nm; ^{1}H NMR identical with reported data [6]; ^{13}CNMR (CD_3OD, 125 MHz): δ 37.36 (C-1), 68.69 (C-2), 68.50 (C-3), 32.84 (C-4), 51.78 (C-5), 206.43 (C-6), 122.10 (C-7), 168.01 (C-8), 35.09 (C-9), 39.25 (C-10), 21.53 (C-11), 32.50 (C-12), 49.50 (C-13), 85.17 (C-14), 31.79 (C-15), 21.62 (C-16), 50.41 (C-17), 18.07 (C-18), 24.43 (C-19), 78.01 (C-20), 20.96 (C-21), 77.20 (C-22), 25.97 (C-23), 50.28 (C-24), 74.11 (C-25), 25.60 (C-26), 29.10 (C-27), 33.02 (C-28), 14.37 (C-29); FABMS(+) *m/z*: 509 $[M + H]^+$, 491, 473, 455.

Table 1
^{1}H and ^{13}C NMR data for compound **3** (500 MHz/125 MHz, C_5D_5N)

C No.	δ_C (ppm)	δ_H (ppm)
1	24.94 (26.23)[a]	1.51 (*brd*, J = 14.1), 2.17 (*m*)
2	29.11 (29.92)	1.87 (*m*)
3	65.81 (67.89)	4.02 (*m*, $W_{1/2}$ = 14.2 Hz)
4	36.48 (37.50)	1.75, 1.97 (each *brd*, J = 14.4 Hz)
5	80.23 (81.68)	–
6	209.31[b]	–
7	119.66 (121.35)	6.27 (*d*, J = 2.5 Hz)
8	167.33 (168.85)	–
9	37.03 (38.71)	3.60 (*m*)
10	42.37 (43.20)	–
11	21.76 (23.09)	1.79, 1.88 (each *m*)
12	32.02 (33.45)	2.07, 2.61 (each *m*)
13	48.09[b]	–
14	84.01 (85.94)	–
15	31.60 (32.54)	1.90, 2.17 (each *m*)
16	21.32 (22.27)	2.07, 2.47 (each *m*)
17	49.95 (52.06)	3.00 (*t*, J = 9.0 Hz)
18	17.80 (18.84)	1.22 (*s*, Me)
19	17.24 (17.92)	1.11 (*s*, Me)
20	76.76 (78.70)	–
21	21.62 (21.83)	1.60 (*s*, Me)
22	77.50 (79.24)	3.88 (*brd*, J = 8.5 Hz)
23	27.41 (23.16)	1.87, 2.17 (*m*)
24	42.57 (44.04)	1.82, 2.28 (*m*)
25	69.47 (72.10)	–
26	29.92 (29.76)	1.37 (*s*, Me)
27	30.07 (30.50)	1.37 (*s*, Me)

[a] The shifts in parentheses are in CD_3OD.
[b] The shifts were not clear due to overlap of the solvent signal or poor signal to noise ratio.

3. Results and discussion

The dry ground mature fruits of *D. glaucescens* were defatted with cold *n*-hexane and extracted with ethyl acetate. TLC analysis of ethyl acetate extract showed UV absorbing spots in a R_f range of ecdysteroids. Chromatographic separation of the ethyl acetate extract over silica gel, Sephadex LH-20, and reversed phase HPLC resulted in the isolation of ecdysteroids **1**–**5** (Fig. 1).

Compounds **1**, **2**, **4** and **5** were identified as 20-hydroxyecdysone, 3-deoxy-1β,20-dihydroxyecdysone, 2-deoxy-20-hydroxyecdysone and makisterone C, respectively, by detailed analysis of spectral data [3–6] as well as direct comparison with authentic samples.

The UV spectrum of **3** showed a maximum at λ = 243 nm for an α, β-unsaturated carbonyl group characteristic to ecdysteroids. The FABMS of **3** gave a pseudomolecular ion peak at *m/z* 481 $[M + H]^+$, which is consistent with the molecular formula $C_{27}H_{44}O_7$. The ^{1}H NMR (d_5-pyridine) spectrum of **1** showed five methyl singlets at δ 1.11, 1.22,

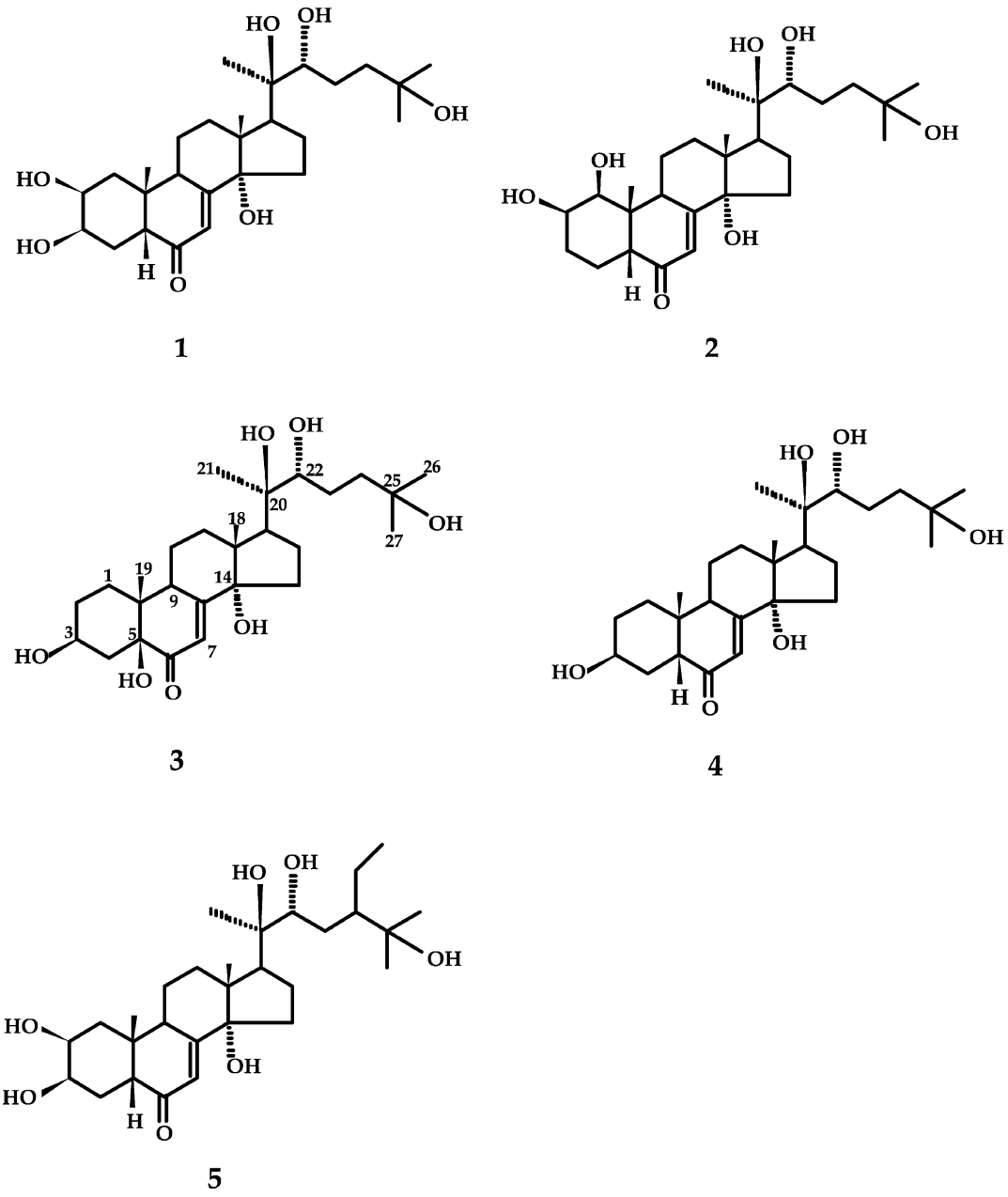

Fig. 1. Structures of compounds **1**–**5**.

1.37, 1.37 and 1.60. The chemical shifts of the four signals out of five were in accord with those reported for **1** [3], thus permitting us to assign the signals at δ 1.22, 1.37, 1.37 and 1.60 to 18-Me, 26-Me, 27-Me and 21-Me, respectively. In contrast, the signal at δ 1.11, which could be assignable to 19-Me, was downfield shifted by 0.05 ppm compared to that of **1**. The spectrum showed a signal of an oxymethine proton (δ 4.02) that was not flanked by another oxymethine group, as opposed to the signals due to a common C-2 and C-3 glycol moiety. Further, a signal for 5-H seemed lacking in the spectrum, while 7-H olefinic and 22-H oxymethine protons appeared at δ 6.27 and 3.88, respectively. The ^{13}C NMR spectrum of **1** showed 27 peaks including a carbonyl (C-6, δ 209.31) and two olefinic carbons (C-7 at δ 119.66 and C-8 at δ 167.33). In the region of oxygen bearing carbons, signals of two methine carbons at δ 77.50 (C-22) and 65.81, and four quaternary carbons at δ 69.47 (C-25), 76.76 (C-20), 80.23, 84.01 (C-14) were observed. The signals at δ 65.81 and 80.23 were unambiguously assigned to C-3 and C-5, respectively. The HMBC spectrum showed the correlation from 19-Me to the carbon signals at δ 24.94 (C-1, CH_2), 42.37 (C-10, quaternary C), 37.03 (C-9, CH) and 80.23. A HMBC correlation from 7-H to the last carbon signal was also observed. Thus, it became evident that the C-5 position was hydroxylated. The 5β-OH substitution was evidenced by the chemical shift of C-19 (δ 17.24) which is significantly

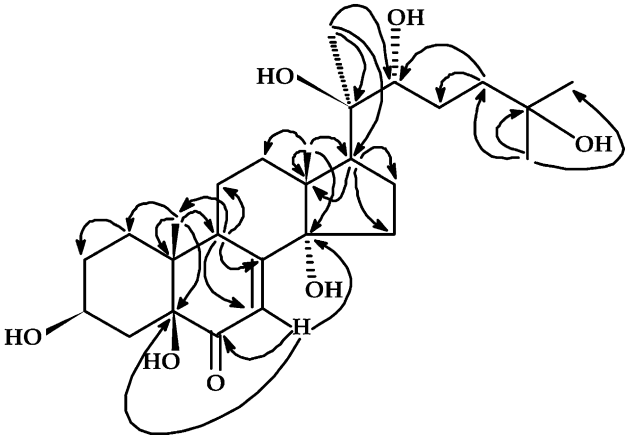

Fig. 2. The HMBC correlations of **3**.

upfield shifted (7 ppm) compared to **1** [7] and the downfield shift of 19-Me (vide supra) [8] which can be explained by pyridine-induced deshielding effect of 5β-OH group. The HMQC spectrum allowed to assign C-1 methylene protons to the signals at δ 1.51 and 2.17, which were correlated only to signals at δ 1.87 as revealed by the ^{1}H–^{1}H COSY spectrum. It is of note that C-1 is upfield shifted (5 ppm) than those of ecdysteroids with 2,3-dihydroxy group. Taken together, it is obvious that C-2 is not hydroxylated but a methylene carbon which was successfully assigned to the signal at δ 29.11 by HMQC spectrum. Another methylene carbon resonating at δ 36.48, linked to protons at δ 1.75 (*brd*) and δ 1.97 (*brd*), was assigned to C-4, since decoupling experiments irradiating 3-H at δ 4.02 collapsed the methylene protons into sharp AB doublet (J = 14.4 Hz). The experiments clearly indicated that the spin-spin coupling constants between 3-H and 4α-H, and between 3-H and 4β-H are less than a few Hz, therefore indicating equatorial orientation of 3-H (axial orientation for 3-OH). The NMR signals due to protons and carbons of ring-C and D and side-chain are in excellent agreement with the reported data for **1** [9]. On the basis of the whole data described above, the structure of **3** was established as 2-deoxy-5β,20-hydroxyecdysone. The complete ^{1}H and ^{13}C NMR assignments of **3** are listed in Table 1. The HMBC correlations are shown in Fig. 2. The ^{13}C NMR data obtained in CD_3OD are also included in the Table (for ^{1}H data, see Section 2). In addition, the ^{13}C NMR data for makisterone C (**5**) are reported for the first time (see Section 2).

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