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Steroids 67 (2002) 555–558

Steroids

# 3-Deoxy-1$\beta$,20-dihydroxyecdysone from the leaves of *Diploclisia glaucescens*

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Received 20 September 2001; accepted 17 October 2001

**Abstract**

Chemical investigation of methanol extract of the leaves of *Diploclisia glaucescens* of the family Menispermaceae furnished a new ecdysteroid, 3-deoxy-1$\beta$,20-dihydroxyecdysone. The structure of the new ecdysteroid was established on detailed analysis of spectral data. The 3-deoxy ecdysteroid showed 40% potency of 20-hydroxyecdysone in the spiracle index assay using the fourth instar larvae of the silkworm *Bombyx mori.* 

*Keywords: Diploclisia glaucescens*; Menispermaceae; 3-Deoxy-1$\beta$,20-dihydroxyecdysone; 20-Hydroxyecdysone; Spiracle index assay; *Bombyx mori*

## 1. Introduction

*Diploclisia glaucescens* of the family Menispermaceae is a creeper growing in the mid-country region of India and Sri Lanka. The leaves of the plant have been used in the treatment of biliousness and venereal diseases [1]. We have previously reported the isolation of stepharine [2], stigmasterol [3], serjanic acid (3$\beta$-hydroxy-30-methoxycarbonylolean-12-en-28-oic acid), phytolaccagenic acid (3$\beta$, 23-dihydroxy-30-methoxycarbonylolean-12-en-28-oic acid) [4], 20-hydroxyecdysone ( $>$ 3%, the highest recorded yield from a plant) [3] and six new triterpenoidal saponins from the stem of the plant [4–7]. In a continuation of our investigation on *D. glaucescens* we now describe the isolation and structural elucidation of a new ecdysteroid, 3-deoxy-1$\beta$,20-dihydroxyecdysone, from the leaves of the plant.

## 2. Experimental

### 2.1. General methods

Mps were determined by a Gallenkamp apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer 241 instrument. $^1$H NMR and $^{13}$C NMR spectra were recorded on a JEOL EX 400 (400 MHz for $^1$H NMR and 100 MHz for $^{13}$C NMR) spectrometer in $CD_3OD$ or $CDCl_3$ solution with tetramethylsilane as an internal reference. Positive ion FABMS were obtained on a JEOL JMS-AX505HA spectrometer with glycerol as matrix. HPLC analysis were carried out on Shimadzu LC-6A apparatus equipped with UV detector under reversed phase C-18 and isocratic solvent condition. IR spectra were recorded on a Shimadzu IR-460 spectrophotometer. UV spectra were recorded on a UV-160 A spectrophotometer.

### 2.2. Plant material

The leaves of *Diploclisia glaucescens* were collected from the Central Province of Sri Lanka in April 2000 and identified by Mr. S.P. Ekanayake, Environmental and Forestry Division, Mahaweli Authority, Polgolla, Sri Lanka. A voucher specimen is deposited at the Institute of Fundamental Studies.

### 2.3. Extraction and isolation

The dry ground leaves of *D. glaucescens* (190 g) were sequentially extracted with hot hexane, dichloromethane and methanol. Evaporation of the methanol gave a dark brown solid (49 g). A portion (45 g) was chromatographed on a column of silica gel (Merck Art 7734) with EtOAc-

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0039-128X/02/$ – see front matter 
PII: S0039-128X(02)00003-X

Table 1
$^{1}H$ and $^{13}C$ NMR data for compound **1** (400 MHz/100 MHz, $CD_3OD$)

| C No. | $\delta_C$ ppm | $\delta_H$ ppm |
|---|---|---|
| 1 | 73.78 | 3.72 (*d*, *J* = 2.4 Hz) |
| 2 | 68.94 | 3.95 (*m*) |
| 3 | 27.64 | 1.62, 1.73 (*m*) |
| 4 | 25.62 | 1.52, 1.63 (*m*) |
| 5 | 52.12 | 2.29 (*dd*, *J* = 11.6, 5.6 Hz) |
| 6 | 206.14 | – |
| 7 | 121.70 | 5.78 (*d*, *J* = 2.4 Hz) |
| 8 | 167.14 | – |
| 9 | 35.76 | 3.14 (*td*, *J* = 8 Hz, 2 Hz) |
| 10 | 43.65 | – |
| 11 | 21.93 | 1.70 (*m*) |
| 12 | 32.56 | 1.90 (*m*) |
| 13 | 49.00 | – |
| 14 | 85.08 | – |
| 15 | 31.78 | 2.00 (*m*) |
| 16 | 21.39 | 1.70 (*m*) |
| 17 | 50.58 | 2.39 (*t*, *J* = 9.2 Hz) |
| 18 | 18.03 | 0.89 (*s*, Me) |
| 19 | 20.16 | 1.03 (*s*, Me) |
| 20 | 77.91 | – |
| 21 | 21.05 | 1.19 (*s*, Me) |
| 22 | 78.44 | 3.30 (*m*) |
| 23 | 27.37 | 1.26, 1.28 (*m*) |
| 24 | 42.39 | 1.79, 1.44 (*m*) |
| 25 | 71.30 | – |
| 26 | 28.97 | 1.18 (*s*, Me) |
| 27 | 29.69 | 1.18 (*s*, Me) |

MeOH. The column fractions, which contained UV active compounds on TLC, were passed through a column of Sephadex LH-20 with methanol as solvent. Further purification of the UV active fraction by HPLC (STR Prep-ODS 20 × 250 mm column, $H_2O$/MeOH (6:4); 5 ml/min, UV detection 243 nm) yielded compound **1** (68 mg) and **2** (170 mg).

*2.3.1. 3-Deoxy-1β,20-dihydroxyecdysone (**1**)*

mp. 152–155°C (MeOH). $[\alpha]_{25}^{D}$ = +63.3° (*c* = 0.72, MeOH). UV $\lambda_{max}^{EtOH}$ nm: 241. IR $\upsilon_{max}^{CHCl_3}$ $cm^{-1}$: 3450, 1660, 1640. $^{1}H$ NMR and $^{13}C$ NMR ($CD_3OD$), see Table 1. HRFABMS (+) *m/z*: 481.3123 $[MH]^+$. $C_{27}H_{45}O_7$ requires 481.3165. FABMS (+) *m/z*: 481, 463, 445, 427.

*2.3.2. Acetylation of **1***

Compound **1** (20 mg) was allowed to react overnight with $Ac_2O$ (1 ml) and pyridine (1 ml). The reaction mixture was evaporated to dryness with methanol and the products were purified by prep. TLC to give acetates **1a** (12 mg) and **1b** (6 mg).

*2.3.3. 1,2,22-Triacetate (**1a**)*

mp. 109°C; $^{1}H$ NMR ($CDCl_3$): $\delta_H$ 0.86 (3H, *s*, 19-Me), 0.95 (3H, *s*, 18-Me), 1.21 (3H, *s*, 26-Me), 1.23 (3H, *s*, 27-Me), 1.27 (3H, *s*, 21-Me), 1.99, 2.11, 2.12 (each 3H, *s*, -OAc), 3.17 (1H, *m*, H-9), 4.85 (1H, *brd*, *J* = 10.4 Hz, H-22), 5.19 (1H, *m*, H-2), 5.34 (1H, *d*, *J* = 1.6 Hz, H-1), 5.86 (1H, *brd*, *J* = 2.0 Hz, H-7).

*2.3.4. 1,2,22,25-Tetraacetate (**1b**)*

mp. 130°C, $^{1}H$ NMR ($CDCl_3$): $\delta_H$ 0.86 (3H, *s*, 19-Me), 0.96 (3H, *s*, 18-Me), 1.26 (3H, *s*, 21-Me), 1.41 (3H, *s*, 26-Me), 1.44 (3H, *s*, 27-Me), 1.987, 1.995, 2.111, 2.128 (each 3H, *s*, -OAc), 3.17 (1H, *m*, H-9), 4.81 (1H, *brd*, *J* = 10.4 Hz, H-22), 5.19 (1H, *m*, H-2), 5.35 (1H, *d*, *J* = 1.7 Hz, H-1), 5.87 (1H, *brd*, *J* = 2.2 Hz, H-7).

*2.4. Spiracle index assay*

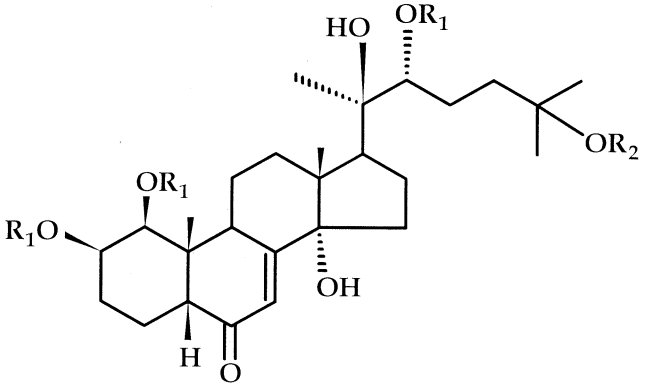
The assay was carried out using fourth instar larvae of *Bombyx mori* in a similar manner as reported previously [8]. The concentrations to induce 50% response were determined to be 0.29 μg and 0.71 μg for **2** and **1**, respectively.

## 3. Results and discussion

The dry ground mature leaves of *D. glaucescens* were defatted with *n*-hexane and extracted with dichloromethane and methanol. A combination of chromatographic separation over silica gel, Sephadex LH-20 and reversed phase HPLC of the methanol extract resulted in the isolation of a new ecdysteroid **1**, along with 20-hydroxyecdysone (**2**) which was identified by direct comparison with an authentic sample.

The highly positive response in the UV absorption and the polarity on a silica gel TLC plate (**1** showed a slightly larger $R_f$ value than **2**) readily identified compound **1** as an ecdysteroid. The UV spectrum of **1** showed a maximum at 242 nm, indicating an α, β-unsaturated carbonyl group. The IR spectrum of **1** revealed a broad hydroxyl absorption (3450 $cm^{-1}$) and strong conjugated carbonyl absorptions (1660 & 1640 $cm^{-1}$) characteristic of an α, β-unsaturated keto group and also corresponding to the 7-en-6-one of ecdysteroids. FABMS of **1** gave a peak at *m/z* 481 for $[M+H]^+$, which is consistent with the molecular formula $C_{27}H_{44}O_7$. The other prominent peaks observed are *m/z* 463 $[M+H\text{-}H_2O]^+$, 445 $[M+H\text{-}2H_2O]^+$, 427 $[M+H\text{-}3H_2O]^+$.

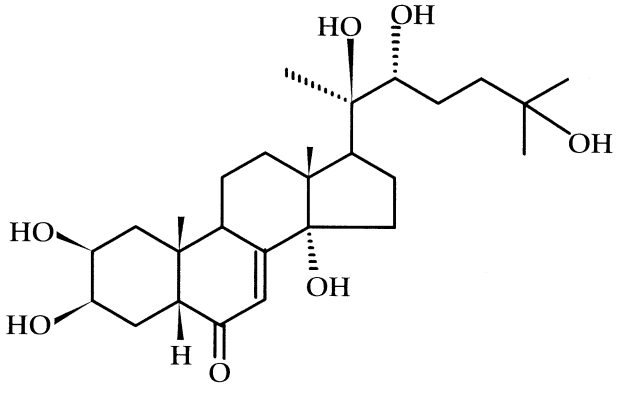
The $^{13}C$ NMR spectrum of **1** gave evidence for the presence of 27 carbons in the compound. Analysis of the $^{1}H$ and $^{13}C$ NMR, and DEPT spectra provided evidence that **1** possessed five methyl groups, one α, β-unsaturated ketone, three oxygenated quaternary carbons, three oxymethines and eight methylenes in the compound **1**. The $^{1}H$ NMR spectrum of **1** showed five methyl singlets at δ 0.89, 1.03, 1.18, 1.18 and 1.19. The chemical shifts of these methyls were in good agreement with the reported data except for the 19-Me singlet observed at δ 1.03, which appeared significantly down field than that (δ 0.96) of 20-hydroxyecdysone [9]. This observation gave the first clue regarding the structural changes of the A ring of **1** in comparison with 20-hydroxyecdysone. Strong HMBC correlations were ob-




**1** $R_1 = R_2 = H$

**1a** $R_1 = Ac$, $R_2 = H$

**1b** $R_1 = R_2 = Ac$




**2**

served from the 19-Me (δ 1.03) to carbons at δ 73.78 (C-1) and 43.65 (C-10), 35.76 (C-9) and 52.15 (C-5). Further in the HMQC spectrum, δ 73.78 carbon was correlated to δ 3.72 proton (*d*, $J = 2.4$ Hz, equatorial proton). The 2.4 Hz coupling is due to the proton at δ 3.95 (*m*, axial proton, attached to δ 68.94 carbon), as revealed by the H-H COSY spectrum. Hence the carbon signal observed at δ 68.94 was unambiguously assigned to C-2. Decoupling of the δ 3.72 proton simplified the signal shape of δ 3.95 proton into a doublet of a doublets ($J = 8.8$, 5.2 Hz), thus forcing to place six membered axial and equatorial protons at the adjacent C-3 position.

The δ 3.95 proton (H-2) was coupled to hydrogens at ca. δ 1.62–1.73. The HMQC spectrum revealed that an unidentified $CH_2$ carbon (δ 27.64) showed connectivities to the methylene protons, thus establishing the assignments of C-3 and H2–3. The characteristic H-5 axial proton at δ 2.29 (*dd*, 11.6, 5.6 Hz attached to δ 52.15) was coupled only with

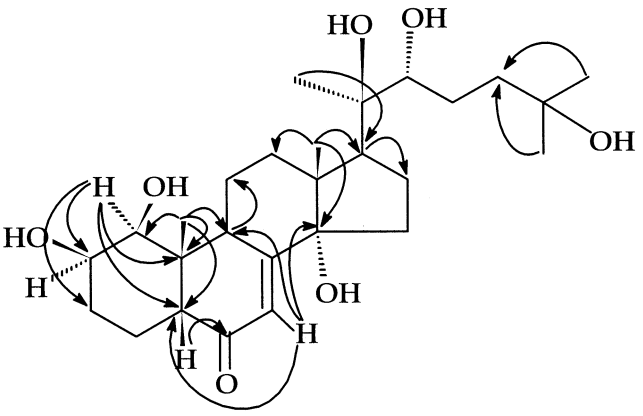



Fig. 1.

upper field protons at δ 1.52–1.63, to which another unassigned $CH_2$ carbon at δ 25.62 (C-4) was correlated as revealed by HMQC. The HMBC spectrum showed correlations from H-1 to C-2, C-3, C-5 and C-10. Stereochemistries at the chiral centers in the A-ring were confirmed as follows. Irradiation of 19-Me caused an NOE enhancement to the H-5 and H-1 signals. Irradiation of H-1 caused an NOE enhancement to the H-2 and 19-Me signals. Irradiation of H-2 caused an NOE enhancement to H-9 signal which appeared at δ 3.14 (attached to C-9) as a typical pattern of H-9 of ecdysteroids. This is reasonably explained by assuming that *cis*-A/B ring junction and β-orientation of the C-2 hydroxyl group (the distance of the two protons was calculated as 2.27 Å by MM2). The occurrence of 1β-OH would cause a down field shift of 19-Me. The NMR spectral data corresponding to the B, C, D rings and the side chain were almost superimposable over those of 20-hydroxyecdysone [9]. The HMBC correlations are shown in Fig. 1. The complete $^1H$ and $^{13}C$ NMR assignments are given in Table 1. Thus the structure of **1** was established to be 3-deoxy-1β,20-dihydroxyecdysone. The $^1H$ NMR spectral data (see Experimental) of 1, 2, 22-tri-acetate (**1a**) and 1, 2, 22, 25-tetra-acetate (**1b**) prepared from **1** further supported the proposed structure of **1**.

Compound **1** retained a significant amount of activity (40% of 20-hydroxyecdysone) when tested in a spiracle index assay using the fourth instar larvae of the silkworm *Bombyx mori*. It is of note that the new 3-deoxyecdysone showed such an activity, since it is generally accepted that the presence of 2β-OH in addition to 3β-OH is required for high hormone activity. In this unique molecule, 1β,2β-glycol might play the role of the popular 2β,3β-glycol in binding to the receptor.

## Acknowledgments

We thank Prof. Sho Sakurai, Kanazawa University, for the spiracle index assay, and Mr. Noriyuki Hara, Tokyo Institute of Technology, for the measurements of MS spectra.

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