Triterpenoidal Constituents of *Diploclisia glaucescens*

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Abstract

The pentacyclic triterpenoids serjanic acid and phytolaccagenic acid as well as a new glycoside, 3-*O*-β-D-glucopyranosylphytolaccagenic acid have been isolated from the stem of *Diploclisia glaucescens* (Bl.) Diels (Menispermaceae). New spectroscopic data are furnished for serjanic acid and phytolaccagenic acid.

Key words

Diploclisia glaucescens, Menispermaceae, triterpenoids, glycoside.

Introduction

Diploclisia glaucescens (Bl.) Diels (= *Cocculus macrocarpus* W. & A.) is a creeper growing in the mid-country regions of South India and Sri Lanka. The leaves have been used in the treatment of biliousness and venereal diseases (1). A preliminary survey has indicated the presence of alkaloids in the plant (2). Chemical investigation of the seeds of the plant gave the moulting hormone, ecdysterone and four other phytoecdysteroids (3). Our investigation of the stem of the plant gave ecdysterone in much higher yields (4) as well as a new bidesmosidic triterpenoid saponin, diploclisin (5).

Materials and Methods

Plant material

D. glaucescens was kindly identified and collected in the month of May 1985 from the Central Province of Sri Lanka by Professor S. Balasubramaniam, Department of Botany, University of Peradeniya, Sri Lanka. A voucher specimen is deposited at the National Herbarium, Peradeniya.

Apparatus

Melting points were determined on a Reichert apparatus and are uncorrected. IR spectra were obtained using a Shimadzu 408 instrument; UV spectra were obtained on a Shimadzu 160 instrument; ^{1}H-NMR spectra (60 MHz) were obtained on a Varian T-60 instrument; High resolution ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker WM 250 instrument. Carbon resonances were edited by DEPT analysis. Chemical shifts are given in δ (ppm) with TMS as internal standard. Mass spectra were obtained on a Varian MAT 311A instrument. Optical rotations were measured on a Perkin-Elmer 241 instrument. GC analysis was carried out using a Varian 3300 instrument installed with a DB 225 capillary column. The oven temperature was programmed from 150 °C to 200 °C at 3 °C/min; H_2 was used as carrier gas (6 ml/min). Injector and detector temperatures were both maintained at 250 °C.

Isolation

The dry, ground, mature stem of *D. glaucescens* (500 g) was sequentially extracted with hot light petroleum ether (40–60 °C) and hot MeOH. Evaporation of the MeOH gave a dark brown solid (60 g) a portion of which (7 g) was partitioned between CH_2Cl_2 and 2 N HCl. Evaporation of the CH_2Cl_2 extract gave a light brown gum (1.9 g). Separation of a portion (1.7 g) of the latter on silica gel (30 g, Merck Art. 9385) by medium pressure liquid chromatography with CH_2Cl_2, CH_3OH as solvents gave the three compounds **1a** (80 mg), **2** (120 mg), and **3a** (40 mg) in order of increasing polarity. Compounds **1a** and **2** were further purified by adsorption chromatography over silica gel (Merck Art. 7736) and identified as serjanic acid and phytolaccagenic acid, respectively. Compound **3a** was further purified by preparative layer chromatography on silica gel (Merck Art. 7730) and identified as 3-*O*-β-D-glucopyranosylphytolaccagenic acid.

Serjanic acid (**1a**)

Colourless microcrystalline needles, m.p. 249–251 °C (EtOAc-*n*-hexane); $[\alpha]_D^{20}$: +77° (*c* 0.2, $CHCl_3$); UV (EtOH) λ_{max} 204 nm (log ε 3.84); IR (KBr) ν_{max} 3450 (OH), 2950, 1730 (ester), 1690 (CO_2H), 1455, 1430, 1390, 1270, 1205, 1150, 1040 cm^{-1}; ^{1}H-NMR (60 MHz, C_5D_5N): δ 0.90 (3H, s, Me), 0.97 (6H, s, 2Me), 1.20 (6H, s, 2Me), 1.27 (3H, s, Me), 3.63 (3H, s, C-30-OMe), 5.57 (1H, m, H-12); EIMS: *m/z* (rel. int., %) = 500 (M^+, $C_{31}H_{48}O_5$, 3), 454 ($M - CO_2 - H_2$, 17), 410 (13), 292 (78), 246 (100), 232 (24), 207 (35), 187 (91).

Acetylation of **1a**

Compound **1a** (40 mg) was allowed to react overnight with Ac_2O (1.5 ml) and pyridine (1.5 ml). After work-up, the monoacetate of **1a** (40 mg) was obtained as pale yellow microcrystalline needles, m.p. 196–198 °C (CH_2Cl_2-*n*-hexane); $[\alpha]_D^{22}$: +27° (*c* 0.13, $CHCl_3$); IR (KBr) ν_{max} 3450, 2950, 1730, 1700, 1460, 1370, 1240, 1150, 1020 cm^{-1}; ^{1}H-NMR (60 MHz, $CDCl_3$): δ 0.76, 0.95, 1.15, 1.27 (each 3H, s, Me), 0.87 (6H, s, 2Me), 2.05 (3H, s, C-3-OAc),

5.38 (1H, m, H-12); EIMS: *m/z* (rel. int., %) = 542 (M^+, $C_{33}H_{50}O_6$, 2), 496 (M – CO_2 – H_2, 16), 292 (62), 246 (100), 232 (27), 215 (24), 203 (14), 187 (98); FDMS: *m/z* (rel. int., %) = 542.188 (100) (M^+; $C_{33}H_{50}O_6$ requires 542.360).

Methylation of **1a** *by diazomethane*

Compound **1a** (20 mg) in MeOH was treated with ethereal CH_2N_2, the solvents were evaporated, and the residue was recrystallised from CH_2Cl_2-*n*-hexane giving methyl serjanate (**1b**) as colourless micro-needles, m.p. 173–175 °C; $[\alpha]_D^{22}$: +77.3° (*c* 0.22, $CHCl_3$); IR (KBr) ν_{max} 3450, 2925, 1725, 1455, 1200, 1135 cm^{-1}; ^{1}H-NMR (250 MHz, C_5D_5N): δ 0.87, 0.94, 1.06, 1.22, 1.24, 1.28 (each 3H, s, 6Me), 3.66, 3.67 (each 3H, s, $2CO_2Me$), 5.57 (1H, m, H-12); EIMS: *m/z* (rel. int., %) = 515 (M^+ + 1, $C_{32}H_{51}O_5$, 10), 496 (M – H_2O, 5), 455 (20), 306 (100), 246 (70), 233 (20), 207 (30), 187 (85).

Phytolaccagenic acid (**2**)

Colourless needles, m.p. 285–287 °C (acetone); $[\alpha]_D^{30}$: +98.1° (*c* 0.13, MeOH); UV (EtOH) λ_{max} 203 nm (log ε 3.71); IR (KBr) ν_{max} 3440 (OH), 2930, 1730 (ester), 1700 (CO_2H), 1460, 1385, 1210, 1145, 1040 cm^{-1}; ^{1}H-NMR (250 MHz, $CDCl_3$ + CD_3OD): δ 0.77, 0.85, 0.95, 1.14, 1.15 (each 3H, s, 5Me), 2.68 (1H, dt, *J* = 3.5 Hz, 13.5 Hz, H-18), 3.42, 3.63 (each 1H, d, *J* = 10.7 Hz, H-23), 3.70 (3H, s, C-30-OMe), 5.34 (1H, m, H-12); ^{13}C-NMR ($CDCl_3$ + CD_3OD): δ 39.06, 26.87, 75.43, 42.60, 48.50, 18.98, 33.20, 40.08, 49.68, 37.61, 23.97, 123.40, 144.22, 42.44, 28.48, 23.97, 46.62, 43.50, 43.01, 44.62, 31.12, 34.52, 69.69, 12.10, 16.10, 17.30, 26.29, 180.92, 28.69, 178.55, 52.12 (C-1 to C-30 and C-30-OMe); EIMS: *m/z* (rel. int., %) = 516 (M^+, $C_{31}H_{48}O_6$, 2), 498 (M – H_2O, 2), 470 (M – CO_2 – H_2, 8), 292 (70), 246 (95), 232 (27), 206 (23), 187 (100); FDMS: *m/z* (rel. int., %) = 516 (M^+, $C_{31}H_{48}O_6$, 100); HRMS gave M^+ 516.3459 ($C_{31}H_{48}O_6$ requires 516.3448). Compound **2** was identical (co-TLC, IR) with an authentic sample of phytolaccagenic acid, kindly supplied by Prof. Dr. W. S. Woo, Seoul National University, Korea.

Acetylation of **2**

Compound **2** was acetylated with Ac_2O-pyridine at 60 °C and the diacetate of **2** obtained as colourless crystals, m.p. 147–149 °C (MeOH-ether); ^{1}H-NMR (250 MHz, $CDCl_3$: δ 0.73, 0.83, 0.97, 1.12, 1.15 (each 3H, s, 5Me), 2.02 (3H, s, OAc), 2.07 (3H, s, OAc), 2.69 (1H, dt, *J* = 3.5 Hz, 13.5 Hz, H-18), 3.69, 3.87 (each 1H, d, *J* = 11.6 Hz, H-23), 3.70 (3H, s, C-30-OMe), 5.36 (1H, m, H-12); EIMS: *m/z* (rel. int., %) = 600 (M^+, $C_{35}H_{52}O_8$, 4), 554 (18), (M – CO_2 – H_2, 18), 480 (7), 292 (78), 246 (89), 187 (100).

3-O-β-D-Glucopyranosylphytolaccagenic acid (**3a**)

Pale yellow needles, m.p. 195–197 °C (MeOH-EtOAc); $[\alpha]_D^{22}$: +45.8° (*c* 0.12, MeOH); IR (KBr) ν_{max} 3400 (OH), 2900, 1730 (ester), 1690 (CO_2H), 1450, 1375, 1210, 1075 (glycoside), 1025 cm^{-1}; ^{1}H-NMR (250 MHz, $CDCl_3$ + CD_3OD): δ 0.60, 0.69, 0.87, 1.02, 1.07 (each 3H, s, 5Me), 2.60 (1H, m, H-18), 3.53, 3.74 (each 1H, d, *J* = 12 Hz, H-23), 3.57 (3H, s, C-30-OMe), 4.28 (1H, d, *J* = 7.5 Hz, anomeric proton), 5.19 (1H, m, H-12); ^{13}C-NMR ($CDCl_3$ + CD_3OD): δ (triterpenoid moiety) 65.5 (C-23), 84.5 (C-3), 123.5 (C-12), 144 (C-13), 178.9 (C-30), 180.4 (C-28): δ (sugar moiety) 62.0 (C-6'), 104.5 (C-1').

Acid hydrolysis of **3a**

Compound **3a** (5 mg) was refluxed with 4 N HCl (5 ml) for 2 h. The mixture was extracted with EtOAc. The organic layer was evaporated to dryness and crystallised from Me_2CO to give phytolaccagenic acid, m.p. 285–287 °C, identical with our sample obtained above.

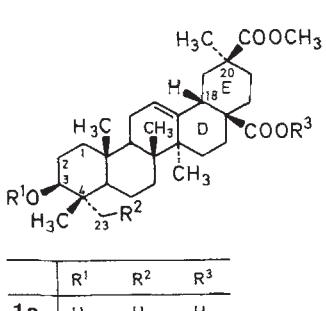
The aqueous phase was adjusted to pH 6 with $NaHCO_3$. After freeze drying and extraction with pyridine, the only sugar present was identified by GC, through D-glucitol hexaacetate, as D-glucose.

Methylation of **3a** *by diazomethane*

Compound **3a** (5 mg) in MeOH was treated with ethereal CH_2N_2, the solvents were evaporated, and the residue was recrystallised from MeOH-EtOAc to give colourless needles of the diester **3b**, m.p. 155–157 °C, identical (m.m.p., co-TLC, IR) with a sample of dimethyl 3-*O*-β-D-glucopyranosylesculentate available with us (5).

Results and Discussion

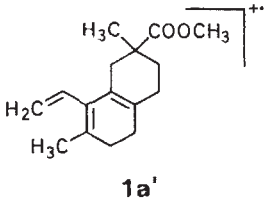
The defatted stem of *D. glaucescens* was extracted with methanol. The basic matter in the methanol extract was removed with 2 N HCl and the water-insoluble, non-basic matter taken up in dichloromethane. Separation of the dichloromethane extract gave serjanic acid (**1a**), phytolaccagenic acid (**2**), and 3-*O*-β-D-glucopyranosylphytolaccagenic acid (**3a**) in yields of 0.15 %, 0.23 %, and 0.08 %, respectively.



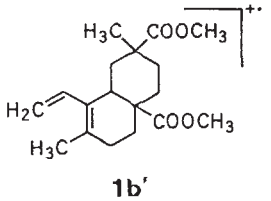
	R^1	R^2	R^3
1a	H	H	H
1b	H	H	CH_3
2	H	OH	H
3a	glc	OH	H
3b	glc	OH	CH_3

glc = β-D-glucopyranosyl

Serjanic acid (**1a**) was first isolated as an amorphous solid by the acid hydrolysis of the saponins of a *Serjania* species (Sapindaceae) (6). A partial description of the ^{1}H-NMR spectrum of **1a**, as well as the UV, IR, ^{1}H-NMR, and mass spectra of methyl serjanate (**1b**) are included in this report. Serjanic acid (**1a**) has been subsequently isolated by the hydrolysis of the saponins of *Phytolacca octandra* (Phytolaccaceae) (7). Serjanic acid (**1a**) has now been obtained crystalline by us and spectroscopic data are reported for the acid, its monoacetate, and methyl serjanate (**1b**). These data support the characterisation of serjanic acid (**1a**) as 3β-hydroxy-30-methoxycarbonylolean-12-en-28-oic acid (6). The base peak in the mass spectra of **1a** and



1a'



1b'

its monoacetate at $m/z = 246$ is ascribed to the fragment ion **1a'** while the base peak in the mass spectrum of **1b** at $m/z = 306$ is ascribed to the fragment ion **1b'** Serjanic acid (**1a**) is reported for the first time from the Menispermaceae.

Phytolaccagenic acid (**2**) was first isolated as a product of acid hydrolysis of the saponin fraction of *Phytolacca americana* L. (Phytolaccaceae) (8). The structure and stereochemistry of the acid have been established as 3β,23-dihydroxy-30-methoxycarbonylolean-12-en-28-oic acid (9). ^{13}C-NMR data for the acid have also been reported (10). More extensive spectral data have now been obtained by us for phytolaccagenic acid (**2**) and its diacetate. These data support the assigned structure and stereochemistry of phytolaccagenic acid (**2**), especially in the E ring.

A hetero COSY spectrum of **2** optimized for $^1J_{CH} = 130$ Hz gave the assignments of the carbon atoms bearing protons. The signals at $\delta = 44.62$ and 28.69 in the ^{13}C-NMR spectrum of **2** were assigned to C-20 and C-29, respectively, in analogy with recorded data for olean-12-enes with *cis*-D/E ring fusion (11). The hetero COSY spectrum of **2** optimized for $J = 20$ Hz showed $^3J_{CH}$ coupling of C-30 with the ester methyl protons as well as with H-29. A cross peak due to $^2J_{CH}$ coupling of H-29 with C-20 was also observed. The results show unambiguously the attachment of the methoxycarbonyl group to C-20. The axial (β) configuration of the methoxycarbonyl group at C-20 was evident from the observation of a strong NOE between the ester methyl protons and H-18 (β). This observation also furnishes evidence for the chair-chair conformation of rings D and E in **2**.

Compound **3a** showed strong absorptions in its IR spectrum at 3400 (broad), 1730, 1690, and 1075 cm^{-1} characteristic of hydroxyl, ester, carboxyl, and glycosidic units, respectively. Hydrolysis of **3a** with 4 N HCl gave phytolaccagenic acid and D-glucose. The ^{13}C-NMR spectrum of **3a** showed signals at $\delta = 84.5$, 180.4, and 104.5 for C-3, C-28, and C-1' (anomeric carbon), respectively. These signals indicated a D-glucopyranosyl moiety linked to the triterpenoid through C-3 (12). The magnitude of the coupling constant (7.5 Hz) of the anomeric doublet ($\delta = 4.28$) in the ^{1}H-NMR spectrum of **3a** indicated a β-D-glucopyranosyl moiety (13). Hence **3a** is 3-(β-D-glucopyranosyloxy)-23-hydroxy-30-methoxycarbonylolean-12-en-28-oic acid or 3-*O*-β-D-glucopyranosylphytolaccagenic acid. The structure was confirmed by the diazomethane methylation of **3a**. The product, dimethyl 3-*O*-β-D-glucopyranosylesculentate (**3b**) was identical with a sample obtained by diazomethane methylation of the product of alkaline hydrolysis of 3,28-di-*O*-β-D-glucopyranosylphytolaccagenic acid (diploclisin) (5).

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