Natural Product Research: Formerly Natural Product Letters

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gnpl20

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Published online: 21 Aug 2006.

To cite this article: U.L.B. Jayasinghe , B.A.I.S. Balasooriya , N. Hara & Y. Fujimoto (2005): Steroidal and Triterpenoidal saponins from the fruits of Diploclisia glaucescens , Natural Product Research: Formerly Natural Product Letters, 19:3, 245-251

To link to this article: http://dx.doi.org/10.1080/14786410410001711824

Natural Product Research, Vol. 19, No. 3, April 2005, pp. 245–251

STEROIDAL AND TRITERPENOIDAL SAPONINS FROM THE FRUITS OF *DIPLOCLISIA GLAUCESCENS*

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(Received 22 August 2003; In final form 10 March 2004)

Chemical investigation of methanol extract of the fruits of *Diploclisia glaucescens* furnished 3-*O*-*β*-D-glucopyranosyl-20-hydroxyecdysone, 3-*O*-[*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranosyl]serjanic acid 28-*O*-*β*-D-glucopyranosyl ester and 3-*O*-[*β*-D-xylopyranosyl(1→2)-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranosyl]serjanic acid 28-*O*-*β*-D-glucopyranosyl ester. The latter saponin was found to be a new natural product while the other two are reported for the first time from the family Menispermaceae.

Keywords: Diploclisia glaucescens; Menispermaceae; Triterpenoidal saponins; Steroidal saponins; Serjanic acid; 20-Hydroxyecdysone

INTRODUCTION

In continuation of our work on high polar secondary metabolites of Sri Lankan flora, the present investigation was carried out in order to study the minor saponins of the fruits of *Diploclisia glaucescens*. *Diploclisia glaucescens*, of the family Menispermaceae, is a liana growing in mid-country regions of India and Sri Lanka. Four triterpenoidal saponins 3-*O*-*β*-D-glucopyranosylphytolaccagenic acid 28-*O*-*β*-D-glucopyranosyl ester (diploclisin) (**1**), 3-*O*-[*β*-D-glucopyranosyl-(1→3)-*β*-D-glucopyranosyl]phytolaccagenic acid 28-*O*-*β*-D-glucopyranosyl ester (**2**), 3-*O*-[*α*-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranosyl]phytolaccagenic acid 28-*O*-*β*-D-glucopyranosyl ester (**3**), 3-*O*-[*α*-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranosyl]serjanic acid 28-*O*-*β*-D-glucopyranosyl ester (**4**) [1]; ecdysteroids 2-deoxy-5*β*, 20-dihydroxyecdysone, 3-deoxy-1*β*,20-dihydroxyecdysone, 2-deoxy-20-hydroxyecdysone, 24-ethyl-20-hydroxyecdysone (makisterone C) and 20-hydroxyecdysone have been reported from the fruits of the plant [2].

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Natural Product Research
ISSN 1478-6419 print: ISSN 1029-2349 online © 2005 Taylor & Francis Ltd
http://www.tandf.co.uk/journals
DOI: 10.1080/14786410410001711824

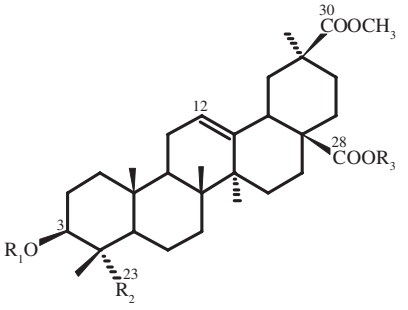
RESULTS AND DISCUSSION

The major component of the EtOAc extract was found to be ecdysteroids [2]. The methanol extract was chromatographed over a column of silica gel with EtOAc and MeOH. The TLC analysis of the column fractions eluted up to 30% MeOH–EtOAc, which indicated the presence of a mixture of ecdysteroids, similar to the ecdysteroids reported from the EtOAc extract [2]. The fractions eluted with 30–50% were combined and re-chromatographed over a column of silica gel with EtOAc–MeOH into two subfractions. Each fraction was further purified by a combination of chromatographic separation over silica gel, reversed phase silica, Sephadex LH-20 and reversed phase HPLC to give compounds **1**–**7** (Fig. 1). The structures of the isolates were established by spectroscopic methods and acid hydrolysis. Among the isolates, compounds **1**–**4** were found to be identical to the previously reported saponins from the fruits of the same plant by us [1]. In this article, we report the isolation and structure elucidation of compounds **5**–**7** and the spectral data for acetate of **2** (**2a**).

Compound **5** showed a UV maximum at 242 nm indicating an α,β-unsaturated carbonyl group. The positive ion FABMS of **5** showed peaks at m/z 665 $[M+Na]^+$, 643 $[M+H]^+$, 481 $[M+H-C_6H_{10}O_5]^+$, which gave evidence for the molecular formula $C_{33}H_{54}O_{12}$ indicating the presence of a hexose moiety in **5**. Analysis of the ^{13}C NMR spectrum (d_5-py) of compound **5** showed 33 carbon signals and most of them were superimposable with the reported data for the ^{13}C NMR data of 20-hydroxyecdysone except for the carbons of ring A [3] and the hexose moiety. The ^1H NMR and H-H COSY spectra (d_4-methanol) of compound **5** showed the relevant peaks for 20-hydroxyecdysone and a glucopyranosyl moiety. In ^1H NMR of **5**, the H-3 of 20-hydroxyecdysone moiety appeared at δ 4.13 (brq, $J=2.0$ Hz), low field than that of 20-hydroxyecdysone, which appeared at δ 3.94 [3]. Irradiation of H-3 caused an NOE enhancement of the anomeric signal that appeared at δ 4.48 ($J=8.0$ Hz) indicating the attachment of glucopyranosyl moiety through the 3-O of 20-hydroxyecdysone moiety. Hence, compound **5** was identified as 3-*O*-β-D-glucopyranosyl-20-hydroxyecdysone [4]. This is the first report on the isolation of **5** from the family Menispermaceae.

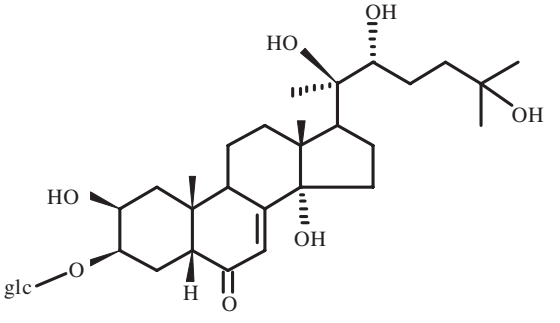
Compound **6** gave serjanic acid (**8**) and D-glucose upon acid hydrolysis. The detailed analysis of positive FABMS, ^1H and ^{13}C NMR, H-H COSY and NOE studies indicated compound **6** to be 3-*O*-[β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl]serjanic acid 28-*O*-β-D-glucopyranosyl ester (**6**), which has been previously reported from *Phytolacca thyrsiflora* [5]. This is the first report on the isolation of **6** from the family Menispermaceae.

Compound **7** yielded serjanic acid (**8**), D-glucose and D-xylose upon acid hydrolysis. The molecular formula of **7** was established as $C_{54}H_{86}O_{24}$ by positive mode FABMS m/z 1141 $[M+Na]^+$ indicating the presence of three glucose and one xylose moieties. An intense peak observed at m/z 979 $[M-162+H]^+$ in the positive FABMS suggested the presence of a 28-COOglc ester. This was supported by the NMR signals of **7** at δ 6.28 (d, $J=8.1$ Hz)/δ 95.7 due to H-1/C-1 of a glucopyranosyl moiety (designated as -glc′′) and at δ 176.0 for C-28 of the aglycone, which are typical values for 28-COOglc. In addition, the NMR spectra showed the presence of three sets of anomeric signals (correlated by HMQC experiments) at δ 4.90 (d, $J=7.7$ Hz)–δ 104.6, δ 5.52 (d, $J=7.7$ Hz)–δ 103.1 and δ 5.38 (d, $J=6.9$ Hz)–δ 106.4. Glycosidation at C-3 of **8** was evident from the chemical shift of C-3 [δ 89.0, correlated with H-3 at δ 3.29



	R_1	R_2	R_3
1	glc-	CH_2OH	-glc
2	glc-^{3}glc-	CH_2OH	-glc
3	rha -^{2}glc-^{2}glc-	CH_2OH	-glc
4	rha -^{2}glc-^{2}glc-	CH_3	-glc
6	glc-^{2}glc-	CH_3	-glc
7	xyl-^{2}glc-^{2}glc-	CH_3	-glc
8	H	CH_3	H

glc = β-D-glucopyranosyl
rha = α-L-rhamnopyranosyl
xyl = β-D-xylopyranosyl



5

FIGURE 1. Structure of compounds **1**–**8**

(dd, $J = 13.6$, 4.2 Hz) by HMQC] [6]. Compound **7** is, therefore, a bidesmoside of **8** having a trisaccharide composing of two units of glucose and one unit of xylose at C-3. The arrangement of these sugars was revealed by the NOE studies. Irradiation of the glc H-1′ (δ 4.90) caused an NOE enhancement of the H-3 signal indicating the attachment of glc C-1′ to C-3 of the aglycone. Irradiation of glc H-1″ (δ 5.52) resulted in the enhancement of glc H-2′ (δ 4.09) indicating the attachment of glc C-1″ through the glc O-2′. Irradiation of xyl H-1 (δ 5.38) resulted in the enhancement of glc H-2″ (δ 4.18) indicating the attachment of xyl C-1 through the glc O-2″. These data clearly indicated the linear nature of the sugar chain at C-3. Anomeric configurations of the

TABLE I NMR data of **6** and **7**

C. No.	***6*** δc	*7* δc	*7* δ_H
1	38.7	38.7	0.84, 1.36
2	26.5	26.5	1.8
3	89.0	89.0	3.29 (dd, 13.6, 4.2)
4	39.8	39.8	–
5	55.8	55.8	0.73 (d, 11.8)
6	18.5	18.5	1.35
7	33.1	33.1	1.43, 1.30
8	39.5	39.5	–
9	48.0	48.0	1.59 (m)
10	36.9	36.9	–
11	23.7	23.7	1.82 (m)
12	124.0	124.0	5.56 (m)
13	143.8	143.8	–
14	42.0	42.0	–
15	28.3	28.3	1.15 (m)
16	23.5	23.5	1.68, 2.04
17	46.5	46.5	–
18	43.2	43.2	3.21 (dd, 13.6, 4.2)
19	42.4	42.4	1.77–2.21
20	43.9	43.9	–
21	30.5	30.5	1.34, 2.04
22	34.0	34.0	1.82, 1.90
23	28.2	28.2	1.27 (s)
24	16.8	16.8	1.09 (s)
25	15.5	15.5	0.81 (s)
26	17.4	17.4	1.07 (s)
27	26.0	26.0	1.25 (s)
28	176.0	176.0	–
29	28.3	28.3	1.19 (s)
30	176.9	176.9	–
31	51.7	51.7	3.58
Glc at C-3			
1′	105.0[a]	104.6	4.90 (d, 7.7)
2′	83.3	82.7	4.09
3′	78.4	77.9	4.24
4′	71.7	71.0	4.07
5′	78.0	77.7	3.82
6′	62.7	62.9	4.43[+]
Glc			
1″	105.9[a]	103.1	5.52 (d, 7.7)
2″	77.1[a]	84.6	4.14
3″	78.0	78.2	3.90
4″	71.6	70.7	4.09
5″	78.2	77.7	4.09
6″	62.8	62.9	4.30[+]
xyl			
1		106.4	5.38 (d, 6.9)
2		75.9	4.06
3		78.7	4.31
4		71.8	4.13
5		67.4	3.67, 4.27
Glc at C-28			
1‴	95.7	95.7	6.28 (d, 8.1)
2‴	74.1	74.1	4.18
3‴	78.9	78.8	4.24
4‴	70.9	70.9	4.31
5‴	79.3	79.3	3.95
6‴	61.9	61.9	4.31

[a]These assignments are based on the H-H COSY and HMQC experiments, although not consistent with those reported [5].
[+]May be interchangeable.

sugars in **7** were determined to be β from the magnitude of the coupling constants of the anomeric protons. Hence, the structure of **7** was established as a new bidesmosidic saponin 3-*O*-[β-D-xylopyranosyl-(1→2)-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl]-serjanic acid 28-*O*-β-D-glucopyranosyl ester. Assignments of ^{1}H and ^{13}C NMR data for **7** are listed in the Table I. The ^{13}C-data for the sugar moiety at C-3 are in good agreement with those of dammarane saponins having the same sugar linkage [7].

EXPERIMENTAL

General

Melting points were determined by a 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 spectrometer. ^{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, 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 was carried out on Shimadzu LC-6A apparatus equipped with UV detector under reversed phase C-18 and isocratic solvent conditions.

Plant Material

The fruits of *D. glaucescens* were collected from the central province of Sri Lanka in May 2001. A voucher specimen is deposited at the Institute of Fundamental Studies.

Extraction and Isolation

The unripe, dry ground, mature fruits of *D. glaucescens* (800 g) were defatted with cold *n*-hexane and sequentially extracted with ethyl acetate and methanol in room temperature using a laboratory shaker. Evaporation of ethyl acetate gave a brown solid (10.8 g). Evaporation of methanol gave a dark brown solid (110 g). A portion of methanol extract (10 g) was chromatographed over a column of silica gel with EtOAc–MeOH. The major component of the fractions eluted up to 30% MeOH–EtOAc were found to be a mixture of ecdysteroids (~2 g) and triterpenoidal saponins. Fractions eluted with 30–50% were combined and re-chromatographed over a column of silica gel with EtOAc–MeOH into two subfractions (F_1 and F_2). TLC analysis of the F_1 indicated the presence of both UV (254 nm) active and inactive compounds. Further purification of F_1 and F_2 by a combination of chromatographic separation over silica gel, reversed phase silica, Sephadex LH-20 and reversed phase HPLC afforded compounds **1** (113 mg), **2** (271 mg) and **5** (29 mg) from the fraction F_1 and **3** (110 mg), **4** (743 mg), **6** (90 mg), **7** (23 mg) from the fraction F_2. Final purifications of **1** and **2** were achieved by HPLC [STR Prep ODS 20 × 250 mm column, 5 mL/min, 30% H_2O–MeOH, UV detection 224 nm] and the final purification of **5** (29 mg) was achieved by HPLC [STR Prep ODS 20 × 250 mm column 5 mL/min, 30% H_2O–MeOH, UV detection 254 nm]. Final purifications of **3**, **4**, **6** and **7** were achieved similarly by HPLC with 40% H_2O–MeOH as an eluant.

*Compounds **1**, **2**, **3** and **4*** Spectral data are identical with the reported data [1].

Acetylation of 2

Compound **2** (20 mg) was allowed to react overnight with Ac_2O (0.5 mL) and pyridine (1 mL) in room temperature. The mixture was evaporated to dryness with methanol and the product was purified by prep. TLC to give acetate of **2** (**2a**).

Acetate of 2 (2a)

Colorless amorphous powder; ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.69, 0.71, 0.92, 1.10, 1.14 (each 3H, s, 5 × −Me), 1.975, 2.002, 2.004, 2.008, 2.016, 2.021, 2.035, 2.062, 2.070, 2.079, 2.112, 2.156 (12 × −OAc), 2.64 (1H, dd, J = 13.5, 3.3 Hz, H-18), 3.38 (1H, dd, J = 11.7, 4.8 Hz, H-3), 3.63 (1H, m, glc H-5′), 3.68 (1H, m, glc H-5″), 3.74 (1H, d, J = 9.9 Hz, H_a-23), 3.77 (1H, m, glc H-5‴), 3.88 (1H, J =9.9 Hz, H_b-23), 3.88 (1H, t, J = 9.3 Hz, glc H-3′), 4.01 (1H, dd, J = 12.5, 4.3 Hz, glc H_a-6‴), 4.05 (1H, dd, J = 12.4, 2.4 Hz, glc H_a-6″), 4.16 (2H, m, glc $H_{a,b}$-6′), 4.27 (1H, dd, J = 12.5, 4.3 Hz, glc H_b-6‴), 4.34 (1H, d, J = 8.0 Hz, glc H-1′), 4.36 (1H, dd, J = 12.5, 4.3 Hz, glc H_b-6″), 4.59 (1H, d, J = 8.1 Hz, glc H-1″), 4.88 (1H, t, J = 9.7 Hz, glc H-2″), 4.89 (1H, t, J = 9.3 Hz, glc H-4′), 4.99 (1H, dd, J = 8.1, 9.6 Hz, glc H-2′), 5.06 (1H, t, J = 9.5 Hz, glc H-4″), 5.10 (1H, t, J = 9.5 Hz, glc H-4‴), 5.12 (1H, t, J = 9.5 Hz, glc H-3″), 5.15 (1H, t, J = 8.3 Hz, glc H-2‴), 5.22 (1H, t, J = 9.4 Hz, glc H-3‴), 5.38 (1H, t, J = 3.4 Hz, H-12), 5.55 (1H, d, J = 8.2 Hz, glc H-1‴); FABMS(+): m/z 1529 $[M + Na]^+$.

3-*O*-*β*-D-Glucopyranosyl-20-hydroxyecdysone (5)

Colorless amorphous powder; UV λ_{max}^{EtOH}: 241 nm; ^{1}H NMR (500 MHz, CD_3OD): δ 0.88 (3H, s, 18-Me), 0.96 (3H, s, 19-Me), 1.18 (3H, s, 21-Me), 1.19 (6H, s, 26-Me, 27-Me), 2.38 (2H, m, H-5, H-17), 3.30 (2H, m, H-22, glc H-5′), 3.15 (1H, m, H-9), 3.20 (t, J = 8.5 Hz, glc H-2′), 3.33 (1H, t, J = 9.8 Hz, glc H-4′), 3.36 (t, J = 9.0 Hz, glc H-3′), 3.69 (1H, dd, J = 11.8, 5.2 Hz, glc H_a-6′), 3.87 (1H, d, J = 10.0 Hz, glc H_b-6′), 4.02 (1H, dt, J = 11.5, 5.0 Hz, H-2), 4.13 (1H, brq, J = 2.0 Hz, H-3), 4.48 (1H, d, J = 8.0 Hz, glc H-1′), 5.80 (1H, d, 2.5 Hz, H-7); ^{13}C NMR (125 MHz, CD_3OD): δ 36.1 (C-1), 66.0 (C-2), 76.4 (C-3), 32.5 (C-4), 51.9 (C-5), 206.2 (C-6), 122.1 (C-7), 168.1 (C-8), 35.0 (C-9), 39.5 (C-10), 21.5 (C-11), 32.1 (C-12), 48.4 (C-13), 85.3 (C-14), 31.7 (C-15), 21.4 (C-16), 50.5 (C-17), 18.1 (C-18), 24.2 (C-19), 77.9 (C-20), 21.1 (C-21), 78.4 (C-22), 27.4 (C-23), 42.4 (C-24), 71.7 (C-25), 29.0 (C-26), 29.7 (C-27), 102.7 (glc C-1′), 75.2 (glc C-2′), 77.9 (glc C-3′), 71.3 (glc C-4′), 78.0 (glc C-5′), 62.7 (glc C-6′); FABMS(+): m/z 665 $[M + Na]^+$, 643 $[M + H]^+$, 625 $[M + H - H_2O]^+$, 607 $[M + H - 2H_2O]^+$, 589 $[M + H - 3H_2O]^+$, 481 $[M + H - C_6H_{10}O_5]^+$, 463 $[M + H - C_6H_{10}O_5 - H_2O]^+$, 445 $[M + H - C_6H_{10}O_5 - 2H_2O]^+$, 427 $[M + H - C_6H_{10}O_5 - 3H_2O]^+$, 409 $[M + H - C_6H_{10}O_5 - 4H_2O]^+$.

3-*O*-[*β*-D-Glucopyranosyl-(1→2)-*β*-D-glucopyranosyl]serjanic acid 28-*O*-*β*-D-glucopyranosyl ester (6)

M.p. 213–215°C; ^{1}H NMR (500 MHz, CD_3OD): δ 0.84 (3H, s, 25-Me), 1.08 (3H, s, 24-Me), 1.09 (3H, s, 26-Me), 1.19 (3H, s, 23-Me), 1.26 (3H, s, 29-Me), 1.28 (3H, s, 27-Me), 3.20 (1H, dd, J = 13.8, 4 Hz, H-18), 3.26 (1H, dd, J = 11.6, 4.0 Hz, H-3), 3.59

(3H, s, $-CO_2CH_3$), 4.88 (1H, d, $J = 7.7$ Hz, glc H-1′), 5.35 (1H, d, $J = 7.7$ Hz, glc H-1″), 5.56 (1H, m, H-12), 6.26 (1H, d, $J = 8.1$ Hz, glc H-1‴); ^{13}C NMR: see Table I; FABMS(+): m/z 1009 $[M + Na]^+$, 825 $[M - C_6H_{10}O_5 + H]^+$, 483 [aglycone − OH]$^+$.

3-*O*-[*β*-D-Xylopyranosyl-(1→2)-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranosyl]serjanic acid 28-*O*-*β*-D-glucopyranosyl ester (7)

Colorless amorphous powder; $[\alpha]_D^{25} + 23.3$ (c, 1.13, MeOH); ^{1}H and ^{13}C NMR: see Table I; FABMS(+): m/z 1141 $[M + Na]^+$, 979 $[M - C_6H_{10}O_5 + Na]^+$, 483 [aglycone − OH]$^+$.

Acid Hydrolysis of 6 and 7

Both compounds **6** and **7** (5 mg) were refluxed with 4 N HCl (2 mL) for 2 h. The product was extracted with EtOAc and the solvent evaporated. Both compounds **6** and **7** gave serjanic acid (**8**) as aglycone and identified by the direct comparison with authentic sample isolated from the same plant [6]. The aqueous layers of the acid hydrolysis of **6** and **7** were adjusted to pH 6 with $NaHCO_3$ and evaporated to dryness. TLC analysis of the pyridine soluble part (developing solvent $CHCl_3 : MeOH : H_2O$ – 7 : 3 : 1 × 2) of the residue indicated the presence of only D-glucose in compounds **6** and both D-glucose and D-xylose in **7**.

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