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Bidesmosidic saponins from the fruits of *Diploclisia glaucescens*

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Abstract

Chemical investigation of methanol extract of the fruits of *Diploclisia glaucescens* (Menispermaceae) furnished two new bidesmosidic saponins 3-*O*-[β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl]phytolaccagenic acid 28-*O*-β-D-glucopyranosyl ester, 3-*O*-[α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl]phytolaccagenic acid 28-*O*-β-D-glucopyranosyl ester, together with known 3-*O*-β-D-glucopyranosylphytolaccagenic acid 28-*O*-β-D-glucopyranosyl ester and 3-*O*-[α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl]serjanic acid 28-*O*-β-D-glucopyranosyl ester. The last saponin is reported for the first time from the family Menispermaceae.

Keywords: *Diploclisia glaucescens*; Menispermaceae; Phytolaccagenic acid; Serjanic acid; Triterpenoid glycosides; Saponins

1. Introduction

Diploclisia glaucescens (Bl.) Diels (=*Cocculus macrocarpus* W. & A.) (Menispermaceae) is a liana growing in India and Sri Lanka. *D. glaucescens* is the only species of *Diploclisia* found in Sri Lanka. The leaves of the plant have been used in the treatment of biliousness and venereal diseases (Chopra et al., 1956). We have previously reported the isolation of stepharine (Jayasinghe et al., 1992), stigmasterol (Bandara et al., 1989a), serjanic acid (3β-hydroxyolean-12-ene-28,30-dioic acid 30-methyl ester), phytolaccagenic acid (3β, 23-dihydroxyolean-12-ene-28,30-dioic acid 30-methyl ester) (Bandara et al., 1990), 20-hydroxyecdysone (Bandara et al., 1989a) and six new phytolaccagenic acid and serjanic acid saponins from the stem of the plant (Bandara et al., 1989b, 1990; Jayasinghe et al., 1993, 1998). In this paper we report the isolation and structure elucidation of four bidesmosidic saponins 3-*O*-β-D-glucopyranosylphytolaccagenic acid 28-*O*-β-D-glucopyranosyl ester (**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**) and 3-*O*-[α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl] serjanic acid 28-*O*-β-D-glucopyranosyl ester (**4**) from the fruits of *D. glaucescens*. Compounds **2** and **3** are new triterpenoid saponins while compound **4** is isolated for the first time from the family Menispermaceae.

2. Results and discussion

The dry ground fruits of *D. glaucescens* were defatted with *n*-hexane and extracted with methanol. Chromatographic separations of the methanol extract over silica gel, sephadex LH-20 and reversed phase HPLC afforded compounds **1**, **2, 3** and **4**. The structures of these four saponins were established by detailed analysis of spectral data and their acetates **3a** and **4a**, including NOE, H–H COSY, HMQC and HMBC experiments, positive FAB and ESI mass spectrometry (MS) and the analysis of acid hydrolysis products.

Acid hydrolysis of **1**–**3** with 4 N HCl afforded phytolaccagenic acid (**5**) and the similar treatment of **4** afforded serjanic acid (**6**) as the aglycones previously described (Jayasinghe et al., 1993). TLC analysis of the acid hydrolysates of **1**–**4** indicated that the presence of D-glucose in **1** and **2,** and D-glucose and L-rhamnose in **3** and **4**.

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Compound **1** was identified as 3-*O*-β-D-glucopyranosylphytolaccagenic acid 28-*O*-β-D-glucopyranosyl ester (diploclisin) by the direct comparison with an authentic sample (Bandara et al., 1989b).

The molecular formula of **2** was established as $C_{49}H_{78}O_{21}$ by positive mode ESI-MS (*m*/*z* 1003 $[M+H]^+$), indicating the presence of three glucose units. The ^{1}H NMR spectrum confirmed this {δ 5.06 (*d*, *J* = 7.3 Hz), 5.23 (*d*, *J* = 7.9 Hz) and 6.31 (*d*, *J* = 8.1 Hz)} and indicated that the anomeric configurations were all β. An intense peak at *m*/*z* 839 in negative mode ESI-MS suggested that there is a 28-COOglu ester present (Domon and Hostettmann, 1984). This is associated with typical anomeric shifts for 28-COOglu ester: H-1‴ (δ 6.31) which correlated with C-1‴ (δ 95.7) in the HMQC spectrum. Signals in the ^{13}C NMR spectrum at δ 176.0 (C-28) and δ 81.9 (C-3) confirmed this and indicated that the other two glucose units are attached to C-3 of the aglycone (Jayasinghe et al., 1998). NOE interactions between H-3 and H-1′ (δ 5.06) and between H-1″ (δ 5.23) and H-3′ (δ 4.06) established the attachment of the sugars as in **2**. Hence compound **2** was determined to be a new saponin 3-*O*-[β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl]phytolaccagenic acid 28-*O*-β-D-glucopyranosyl ester. Complete ^{1}H and ^{13}C NMR assignments for **2** are given in the Tables 1 and 2.

The molecular formula of **3** was established as $C_{55}H_{88}O_{25}$ by positive and negative mode ESI-MS (*m*/*z* 1149 $[M+H]^+$ and *m*/*z* 1147 $[M-H]^-$), indicating the presence of three glucose and one rhamnose units. An intense peak at *m*/*z* 985 $[M-H-162]^-$ in the negative

Table 1
^{1}HNMR spectral data for compounds **2**–**4** (400 MHz in pyridine-d_6)

H no	**2**	**3**	**4**
3	4.28 (*dd*, 11.8, 4.3)	4.15 (*dd*, 11.5, 4.2)	3.32 (*dd*, 11.6, 4.0)
12	5.58 (*m*)	5.57 (*m*)	5.58 (*m*)
18	3.20 (*dd*, 13.7, 3.8)	3.22 (*dd*, 13.8, 3.8)	3.23 (*dd*, 13.8, 3.8)
23	3.71 (*d*, 10.5) 4.32 (*d*, 10.5)	3.69 (*d*, 7.7)	1.37 (*s*)
24	0.96 (*s*)	1.07 (*s*)	1.10 (*s*)
25	0.93 (*s*)	0.90 (*s*)	0.82 (*s*)
26	1.13 (*s*)	1.11 (*s*)	1.13 (*s*)
27	1.22 (*s*)	1.21 (*s*)	1.27 (*s*)
29	1.18 (*s*)	1.20 (*s*)	1.22 (*s*)
OMe	3.60 (*s*)	3.60 (*s*)	3.60 (*s*)
	Glc A at C-3		
1′	5.06 (*d*, 7.3)	5.16 (*d*, 7.6)	4.95 (*d*, 7.6)
2′	4.04	4.42	4.42
3′	4.06	4.54 (*t*, 8.9)	4.54 (*t*, 9.8)
4′	4.12	4.15	4.09 (*t*, 9.8)
5′	3.81 (*m*)	3.89 (*m*)	3.93 (*m*)
6′	4.30 4.43	#	#
	Glc B		
1″	5.23 (*d*, 7.9)	5.94 (*d*, 7.6)	5.86 (*d*, 7.6)
2″	4.06	4.31	4.27
3″	4.25 (*t*, 8.8)	4.22	4.24
4″	4.19	4.15	4.07 (*t*, 9.8)
5″	4.03 (*m*)	3.84 (*m*)	3.85 (*m*)
6″	4.30 (*m*) 4.54$^+$	#	#
		Rha	Rha
1		6.32 (*s*)	6.41 (*s*)
2		4.81 (*bs*)	4.76 (*bs*)
3		4.68 (*dd*, 9.4, 2.9)	4.71 (*dd*, 9.4, 2.8)
4		4.32	4.34
5		5.01 (*m*)	5.04 (*m*)
6		1.84 (*d*, 6.4)	1.81 (*d*, 6.4)
	Glc C at C-28		
1‴	6.31 (*d*, 8.1)	6.31 (*d*, 8.3)	6.30 (*d*, 8.0)
2‴	4.18	4.20	4.20 (*t*, 8.0)
3‴	4.26 (*t*, 8.8)	4.29	4.28 (*t*)
4‴	4.38	4.40	4.41
5‴	3.97 (*dt*, 9.6, 2.9)	3.97	3.99 (*bd*, 7.8)
6‴	4.39 (*bs*) 4.39 (*bs*)	#	#

Determined by H–H and C–H COSY spectra, coupling constants could not be measured accurately due to the overlay of the signals; # δ 4.21–4.56.

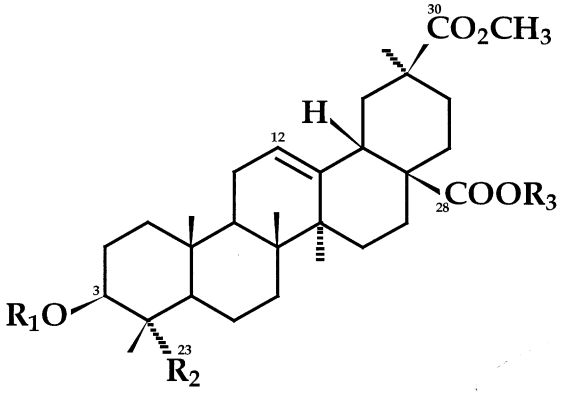
Table 2
^{13}C NMR data for compounds **2**, **3** and **4** (100 MHz in pyridine-d_6)

C no	**2** δ_C	**3** δ_C	**4** δ_C	C no	**2** δ_C	**3** δ_C	**4** δ_C
					Glc A at C-3		
1	38.6	38.6	38.7	1′	105.4	103.7	105.1
2	25.9	25.5	26.4	2′	74.3	79.4[a]	78.9
3	81.9	84.8	89.7	3′	88.8	79.5[a]	79.4
4	43.4	43.2	39.9	4′	69.6	72.5	72.8
5	47.4	48.5	55.9	5′	77.9	78.0	77.9
6	18.1	18.3	18.5	6′	62.4[a]	62.7	63.4
7	32.8	32.9	33.1		Glc B		
8	39.9	39.9	39.6	1″	105.9	101.9	101.9
9	48.1	48.1	48.0	2″	75.5	78.9	78.4
10	36.8	36.9	36.9	3″	78.2	79.3[a]	79.4
11	23.7	23.8	23.7	4″	71.6	71.6	72.6
12	123.8	123.5	124.0	5″	78.7	77.7	77.6
13	143.8	143.8	143.8	6″	62.5[a]	62.6	62.7
14	42.0	42.0	42.0			Rha	Rha
15	28.2	28.3	28.3	1		102.6	102.0
16	23.5	23.5	23.5	2		71.9	72.4
17	46.5	46.5	46.5	3		71.6	71.9
18	43.1	43.3	42.4	4		74.2	74.3
19	42.3	42.4	43.2	5		69.9	69.5
20	43.9	43.9	44.0	6		18.9	19.0
21	30.5	30.5	30.5		Glc C at C-28		
22	34.0	34.0	34.0	1‴	95.7	95.8	95.7
23	64.2	66.0	28.3	2‴	74.1	74.1	74.1
24	13.6	13.3	16.8	3‴	78.8	78.9	78.9
25	16.1	16.1	15.5	4‴	70.9	70.9	70.9
26	17.4	17.5	17.4	5‴	79.3	79.3[a]	79.3
27	26.0	26.0	26.0	6‴	61.9	61.9	61.8
28	176.0	176.0	176.0				
29	28.2	28.3	28.4				
30	176.8	176.9	176.9				
31	51.6	51.7	51.7				

[a] Assignments may be interchanged.

mode ESI-MS spectrum suggested that there is a 28-COOglu ester present. Fragment ions at *m/z* 987 $[M+H\text{-}162]^+$, 841 $[M+H\text{-}162\text{-}146]^+$, 679 $[M+H\text{-}162\text{-}146\text{-}162]^+$ and 499 $[M\text{-}162\text{-}146\text{-}162\text{-}162\text{-}OH]^+$ in the positive mode ESI-MS spectrum suggested a linear nature of the sugars at C-3 of phytolaccagenic acid moiety with rhamnose as a terminal sugar. The peak observed at *m/z* 499 $[\text{aglycone} - OH]^+$ is common in the positive mode of mass spectra of phytolaccagenic acid saponins (Jayasinghe et al., 1998). Signals in the ^{13}C NMR spectrum at δ 176.0 (C-28) and 84.8 (C-3) confirmed a bidesmosidic nature at C-3 and C-28 of **3**. Four sets of anomeric proton and carbon (correlated by the HMQC spectrum) signals were assigned as δ 6.31 (*d*, $J=8.3$ Hz, H-1‴)–δ 95.8 (C-1‴) for 28-COOglu, δ 5.94 (*d*, $J=7.6$ Hz, H-1″)–δ 101.9 (C-1″), δ 5.16 (*d*, $J=7.6$ Hz, H-1′)–δ 103.7 (C-1′) for the other two glucose units, and δ 6.32 (*bs*, H-1)–δ 102.6 (C-1) for rhamnose. Anomeric configurations of the glucose units were all β, whereas that of rhamnose was deduced as α from the negligible coupling of anomeric proton and ^{13}C NMR chemical shifts of the rhamnose moiety. NOE interactions between H-1′ (δ 5.16) and H-3 (δ 4.15), between H-1″ (δ 5.94) and H-2′ (δ 4.42) and between H-1 (δ 6.32) of rhamnose and H-2″ (δ 4.31) indicated the attachment of the sugars at C-3 as in **3**. This sugar linkage was further confirmed by NOE experiments for per-acetate derivative **3a** prepared from **3**. Hence, compound **3** was established to be a new saponin 3-*O*-[α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl]phytolaccagenic acid 28-*O*-β-D-glucopyranosyl ester. Assignments of ^1H and ^{13}C NMR data for **3** are listed in Tables 1 and 2.

Compound **4** was identified as 3-*O*-[α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl]serjanic acid 28-*O*-β-D-glucopyranosyl ester based on spectral data including 2D-NMR. The NMR data for compound **4** are included in Tables 1 and 2. The sugar sequence and linkage at C-3 was confirmed by the H–H COSY spectrum and NOE experiments for per-acetate derivative **4a** obtained from **4**. This saponin was previously isolated from the family of Phytolaccaceae, *Phytolacca rivinoides*, *P. bogotensis* (Nielsen et al., 1995) and *P. icosandra* (Treyvaud et al., 2000). This is the first report of the isolation of **4** from the family Menispermaceae.



	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
5	H	CH_2OH	H
6	H	CH_3	H

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

3. Experimental

3.1. General

Mps were determined by Gallenkamp apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-360 polarimeter at 25 °C. ^1H NMR and ^{13}C NMR spectra were recorded on a Brucker DRX500 (500 MHz for ^1H and 125 MHz for ^{13}C) or Jeol LAMBDA400 (400 MHz for ^1H and 100 MHz for ^{13}C) spectrometer in C_5D_5N or $CDCl_3$ solution with tetramethylsilane as an internal reference. ESI-MS was conducted using a Finnigan-MAT LCQ ion trap mass spectrometer in the positive or negative ion mode. HPLC analyses were carried out on Shimadzu LC-6A apparatus equipped with UV detector under reversed phase C_{18} and isocratic solvent condition.

3.2. Plant material

The fruits of *D. 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 (IFS/2000/DGF1) is deposited at the Institute of Fundamental Studies.

3.3. Extraction and isolation

The unripe, dry ground mature fruits of *D. glaucescens* (90 g) were defatted with cold *n*-hexane and

extracted with methanol. Evaporation of methanol gave a dark brown solid (3.2 g). A portion (3 g) was chromatographed over a column of silica gel (Merck Art. 7734). The fraction eluted with 20–40% MeOH in EtOAc was further purified by repeated column chromatography over silica gel to give compound **1** (250 mg) and compound **2** (35 mg). The fraction eluted with 50–60% of MeOH in EtOAc was further purified by combination of chromatographic separation over silica gel, sephadex LH-20 and reversed phase HPLC (STR Prep-ODS 20 × 250 mm column, 30% H_2O–MeOH, 5 ml/min; UV detection 220 nm) yielded compound **3** (43 mg) and **4** (132 mg).

*3.3.1. Acid hydrolysis of **1–4***

Each compound **1** – **4** (5 mg) was refluxed with 4 N HCl for 2 h. The product was extracted with EtOAc and the solvent evaporated. Compounds **1–3** gave phytolaccagenic acid (**5**) and the similar treatment of compound **4** gave serjanic acid (**6**). These acids were identified by the direct comparison with authentic samples (Jayasinghe et al., 1993). The aqueous layers of the acid hydrolysis of **1–4** were adjusted to pH 6 with $NaHCO_3$ and freeze-dried. TLC analysis (developing solvent, $CHCl_3$:MeOH:H_2O—7:3:1 × 2) of the pyridine soluble part of the residue indicated the presence of D-glucose in compounds **1** and **2**, and D-glucose and L-rhamnose in compounds **3** and **4**.

*3.3.2. 3-O-β-D-Glucopyranosylphytolaccagenic acid 28-O-β-D-glucopyranosyl ester (**1**)*

Mp 194–198 °C; ^1H and ^{13}C NMR identical with the reported data (Bandara et al., 1989b); ESI-MS(+): *m/z* 863(M + Na), 701; ESI-MS(-): *m/z* 839.

*3.3.3. 3-O-[β-D-Glucopyranosyl-(1→3)-β-D-glucopyranosyl]phytolaccagenic acid 28-O-β-D-glucopyranosyl ester (**2**)*

Mp 214–218 °C; $[\alpha]_D^{25}$ + 48.3° (MeOH, *c* 0.65,); ^1H NMR (400 MHz, C_5D_5N): See Table 1; ^{13}C NMR (100 MHz, C_5D_5N): see Table 2; ESI-MS(+): *m/z* 1003, 841, 679; ESI-MS(-): *m/z* 1001, 839.

*3.3.4. 3-O-[α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl] phytolaccagenic acid 28-O-β-D-glucopyranosyl ester (**3**)*

Mp 227–230 °C; $[\alpha]_D^{25}$ + 5.3° (MeOH, *c* 0.7); ^1H NMR (400 MHz, C_5D_5N): see Table 1; ^{13}C NMR (100 MHz, C_5D_5N): see Table 2; ESI-MS(+): *m/z* 1149, 987, 841, 679, 499; ESI-MS(-): *m/z* 1147, 985 (intense peak).

*3.3.5. 3-O-[α-L-Rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl] serjanic acid 28-O-β-D-glucopyranosyl ester (**4**)*

Mp 224–228 °C; ^1H NMR (400 MHz, C_5D_5N): see Table 1; ^{13}C NMR (100 MHz, C_5D_5N): see Table 2.

*3.3.6. Acetylation of **3** and **4***

Each compound (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 products were purified by prep. TLC. Compounds **3** and **4** gave the acetates **3a** and **4a,** respectively.

*3.3.7. Acetate of **3** (**3a**)*

Mp 143–146 °C; ^1H NMR (500 MHz, $CDCl_3$): 0.72, 0.78, 0.94, 1.10, 1.14 (each 3H, *s*, 5x-Me), 1.18 (3H, *d*, *J* = 6.3 Hz, rha H-5′), 1.98–2.14 (14 x-OAc), 2.64 (1H, *m*, H-18), 3.51 (1H, *dd*, *J* = 11.9, 4.9 Hz, H-3), 3.64 (1H, *m*, glc H-5″), 3.68 (1H, *dd*, *J* = 9.5, 7.9 Hz, H-2″), 3.72 (3H, *s*, –$COOCH_3$), 3.76 (1H, *m*, glc H-5′), 3.78 (1H, *m*, glc H-5‴), 3.93 (1H, *dd*, *J* = 9.6, 7.7 Hz, glc H-2′), 4.37 (1H, *d*, *J* = 7.8 Hz, glc H-1′), 4.61 (1H, *d*, *J* = 7.9 Hz, glc H-1″), 4.83 (1H, *d*, *J* = 1.5Hz, rha H-1), 5.02 (1H, *brd*, rha H-2), 5.11 (1H, *t*, *J* = 9.9 Hz, glc H-3′), 5.12 (1H, *t*,*J* = 10 Hz, glc H-3″), 5.16 (1H, *dd*, *J* = 9.3, 8.4 Hz, glc H-2‴), 5.23 (1H, *t*, *J* = 9.4 Hz, glc H-3‴), 5.38 (1H, *brs*, H-12), 5.54 (1H, *d*, *J* = 8.5 Hz, glc H-1‴).

*3.3.8. Acetate of **4** (**4a**)*

Mp 167 °C; ^1H NMR (500 MHz, $CDCl_3$): δ 0.72, 0.82, 0.90, 1.04, 1.12, 1.14 (each 3H, *s*, 5x-Me), 1.20 (3H, *d*, *J* = 6.1 Hz, rha H-6), 1.98–2.13 (13 x-OAc), 2.64 (1H, *m*, H-18), 3.05 (1H, *dd*, *J* = 11.4, 4.4 Hz, H-3), 3.61 (1H, *t*, *J* = 8.6 Hz, glc H-2″), 3.72 (3H, *s*,–$COOC\underline{H}_3$), 4.02 (1H, *dd*, *J* = 8.5, 7.9 Hz, glc H-2′), 4.39 (1H, *d*, *J* = 7.6 Hz, glc H-1′), 4.64 (1H, *d*, *J* = 7.9 Hz, glc H-1″), 4.90 (1H, *brs*, rha H-1), 5.02 (1H, *m*, rha H-2′), 5.15 (1H, *dd*, *J* = 9.3, 8.2 Hz, glc H-2‴), 5.22 (1H, *t*, *J* = 9.3 Hz, glc H-3‴), 5.38 (1H, *brs*, H-12), 5.56 (1H, *d*, *J* = 8.2 Hz, glc H-1‴).

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