Glucuronides of *Diploclisia glaucescens*

[1]U.L.B. JAYASINGHE*, [1]G.P. WANNIGAMA AND [2]J.K. MACLEOD
[1]*Department of Chemistry, University of Peradeniya, Peradeniya, Sri Lanka*
[2]*Research School of Chemistry, Australian National University, Canberra, A.C.T. 0200, Australia*

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Summary: Spectroscopic evidence is presented for the structures of four new triterpenoid saponins 3-*O*-β-D-glucuronopyranosylserjanic acid, 3-*O*-β-D-glucuronopyranosylphytolaccagenic acid, 3-*O*-β-D-glucuronopyranosyl-28-*O*-β-D-glucopyranosylserjanic acid and 3-*O*-β-D-glucuronopyranosyl-28-*O*-β-D-glucopyranosylphytolaccagenic acid isolated from *Diploclisia glaucescens*. One saponin has shown acceptable molluscicidal activity.

Introduction

Diploclisia glaucescens (Bl.) Diels (Menispermaceae) (*local name: ata thiththa wel*) is a creeper which grows 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]. Chemical investigation of the seeds of the plant gave five phytoecdysteroids, including ecdysterone [2]. Six saponins have been isolated from the stem of the plant. Of these, two have been identified as 3-*O*-β-D-glucopyranosylphytolaccagenic acid [3] and 3,28-di-*O*-β-D-glucopyranosyl phytolaccagenic acid (diploclisin) [4]. A brief account of our structural investigations on the other four saponins has already been reported [5]. The present paper details the isolation and structural analysis of these saponins **1-4**.

Results and Discussion

3-O-β-D-glucuronopyranosylserjanic acid (1)

Colourless microcrystalline needles, mp > 250°C, $[\alpha]_D$ + 18° (MeOH; *c* 0.22); IR ν_{max}^{KBr} cm^{-1}: 3425, 2950, 1730, 1690, 1600, 1380, 1280, 1210, 1150, 1080, 1020, 940; ^{1}H-NMR ($CDCl_3$ + CD_3OD):δ 0.80, 0.86, 0.95, 1.08, 1.15, 1.16 (each 3H, *s*, 6xMe), 1.9 (*m*, H-11), 2.0 (*m*, H-19), 2.7 (1H, *m*, H-18), 3.2 (1H, *m*, H-5), 3.71 (3H, *s*, C-30 OMe), 4.38 (1H, *d*, *J*=9Hz, H-1'), 5.34 (1H, *m*, H-12); ^{13}C-NMR: Table-1; FABMS +ve *m/z* 677 $[M+H]^+$, 483 [aglycone-OH]$^+$, FABMS -ve *m/z* 675 $[M-H]^-$, 499 [aglycone-H]$^-$. High resolution FABMS -ve *m/z* 675 (found $[M-1]^-$ 675.3727. $C_{37}H_{55}O_{11}$ requires 675.3744.

3-O-β-D-glucuronopyranosylphytolaccagenic acid (2)

Light brown microcrystalline needles, mp 238°C, $[\alpha]_D$ + 32.5° (MeOH; *c* 0.4); IR ν_{max}^{KBr} cm^{-1}: 3450, 1730, 1705, 1600, 1390, 1270, 1080; ^{1}H-NMR ($CDCl_3$+CD_3OD): δ 0.69, 0.78, 0.96, 1.13, 1.17 (each 3H, *s*, 5xMe), 1.9 (*m*, H-11), 2.68 (1H, *m*, H-18), 3.25, 3.40 (each 1H, *d*, *J* = 11 Hz, H-23), 3.7 (3H, *s*, C-30 OMe), 4.43 (1H, *d*, *J* = 9 Hz, H-1'), 5.31 (1H, *m*, H-12); ^{13}C-NMR Table-1; FABMS +ve *m/z* 715 $[M+Na]^+$; FABMS -ve *m/z* 691 $[M-H]^-$, 515 [aglycone-H]$^-$; High resolution FABMS -ve *m/z* 691 (found [M-1], 691.3701. $C_{37}H_{55}O_{12}$ requires 691.3694).

*To whom all correspondence should be addressed.

Table-1: ^{13}C-NMR data of **1-4** ($CDCl_3$+CD_3OD, 75 MHz) edited by APT.

Carbon No.	1	2	3	4
1	39.5	39.3	39.7	39.3
2	26.5	25.9	26.7	25.8
3	90.8	82.0	90.9	81.9
4	42.4	43.7	42.6	43.6
5	56.6	48.0	56.8	-A
6	19.0	18.7	19.1	18.7
7	33.7	31.2	33.8	31.3
8	39.9	40.3	40.0	40.3
9	-A	48.0	-A	-A
10	37.6	37.5	37.7	37.4
11	24.2	24.3	24.4	24.3
12	124.0	124.0	124.3	124.2
13	144.3	144.6	144.2	144.1
14	40.2	42.6	40.4	42.6
15	30.4	28.6	31.2	28.6
16	24.0	24.1	24.0	23.9
17	46.7	46.8	47.2	47.1
18	43.6	43.7	43.7	43.6
19	43.0	42.4	43.1	43.0
20	44.7	44.8	44.8	44.7
21	31.1	30.5	31.2	31.1
22	34.6	34.8	34.1	34.0
23	15.9	64.8	16.0	64.8
24	16.9[B]	13.3	17.0[B]	13.3
25	17.4[B]	16.4[B]	17.5[B]	16.5[B]
26	28.4	17.6[B]	28.5	17.5[B]
27	26.3	26.4	26.3	26.3
28	181.1	181.3	177.5	177.4
29	28.7	28.7	28.6	28.6
30	178.7	178.8	178.7	178.7
30 OMe	52.3	52.3	52.3	52.3
1'[A]	106.4	104.6	106.5	104.3
2'	75.0	74.7	75.2	74.4
3'	77.4	77.9	77.5	77.6
4'	73.1	73.3	73.5	73.2
5'	76.1	76.1	76.2	75.9
6'	-c	-c	-c	-c
1"			95.6	95.4
2"			73.6	73.4
3"			78.0	78.1
4"			70.8	70.8
5"			76.1	77.9
6"			62.2	62.2

'Sugar moiety at C-3; "Sugar moiety at C-28
[A]Masked by solvent
[B] Values in same column are interchangeable
[C]signal too weak for definitive assignment

3-O-β-D-glucuronopyranosyl-28-O-β-D-glucopyranosylserjanic acid (3)

Light brown needles; mp 253-255°C, $[\alpha]_D$ + 20° (MeOH; *c* 0.1.); IR ν_{max}^{KBr} cm^{-1}; 3400, 2950, 1730, 1600, 1430, 1390, 1260, 1210, 1150, 1070; ^{1}H-NMR ($CDCl_3$+CD_3OD): δ 0.79, 0.86, 0.96, 1.07, 1.15, 1.17 (each 3H, *s*,6 x Me), 2.68 (1H, *m*, H-18), 3.17 (1H, *m*, H-5), 3.67 (3H, *s*, C-30 OMe), 4.35 (1H, *d*, *J* = 7.5 Hz, H-1'), 5.32 (1H, *m*, H-12), 5.35 (1H, *d*, *J* = 9 Hz, H-1") (for single and double prime numbering see footnote in Table-1); ^{13}C-NMR: Table-1; FABMS +ve *m/z* 877 $[M+K]^+$, 861 $[M+Na]^+$, 483 [aglycone-OH]$^+$; FABMS -ve *m/z* 837 $[M-H]^-$, 675 $[M-H-162]^-$, 499 [aglycone-H]$^-$. High resolution FABMS -ve *m/z* 837 (found: $[M-1]^-$, 837.4264, $C_{43}H_{65}O_{16}$ requires 837.4273).

3-O-β-D-glucuronopyranosyl-28-O-β-D-glucopyrano-sylphytolaccagenic acid (4)

White microcrystalline needles, mp > 300°C $[\alpha]_D$ + 20° (MeOH; *c* 0.2); IR ν_{max}^{KBr} cm^{-1}: 3425, 2925, 1720, 1640, 1610, 1380, 1260, 1210, 1070; ^{1}H-NMR ($CDCl_3$+CD_3OD): δ 0.71, 0.80, 0.99, 1.15, 1.18 (each 3H, *s*, 5xMe), 1.9 (*m*, H-11), 2.71 (1H, *m*, H-18), 3.63, 3.82 (each 1H, *d*, *J* = 12 Hz, H-23), 3.71 (3H, *s*, C-30 OMe), 4.46 (1H, *d*, *J* = 7.5 Hz, H-1'), 5.35 (1H, *m*, H-12), 5.37 (1H, *d*, *J* = 7.5 Hz, H-1"); ^{13}C-NMR: Table-1; FABMS +ve *m/z* 877 $[M+Na]^+$, 693 $(M+H-162]^+$, 499[aglycone-OH]$^+$; FABMS -ve *m/z* 853 $[M-H]^-$; 691 $[M-H-162]^-$; High resolution FABMS -ve *m/z* 853 (found $[M-1]^-$, 853.4217. $C_{43}H_{65}O_{17}$ requires 853.4222).

Pentamethyl derivative, $C_{42}H_{66}O_{11}$ of 1.

EIMS *m/z* (rel.int.): 744$[M-2H]^+$ (1), 686 $[M-HCO_2Me]^+$ (5), 497 [aglycone-OH]$^+$ (36), 437 (28), 377 (10), 306 $[C_{18}H_{26}O_4]^+$ (68), 246 (78), 233 $[C_{10}H_{17}O_6]^+$ (38), 201 (80), 187 (100) (found: $[M-HCO_2Me]^+$, 686.4393. $C_{40}H_{62}O_9$ requires 686.4394); CIMS (NH_3) *m/z* (rel.int.): 764 $[M+NH_4]^+$ (5), 497 [aglycone-OH]$^+$ (100), 233 (32).

Hexamethyl derivative, $C_{43}H_{68}O_{12}$ of 2

EIMS *m/z* (rel.int.): 774 $[M-2H]^+$ (1), 716 $[M-HCO_2Me]^+$ (3), 527 [aglycone-OH]$^+$ (10), 495 (12), 467(8), 435(8), 306 $[C_{18}H_{26}O_4]^+$ (66), 247 (48), 246 (74), 233 (28), 220 (33), 201 (100); FABMS +ve *m/z* 799 $[M+Na]^+$, 777 $[M+H]^+$, 527[aglycone - OH]$^+$.

3-O-β-D-glucuronopyranosylspergulagenic acid (1a)

Light brown microcrystalline needles, mp 258°C, $[\alpha]_D$ +60° (MeOH; *c* 0.1); IR ν_{max}^{KBr} cm^{-1}: 3450, 2925, 2850, 1690, 1600, 1460, 1390, 1030; ^{1}H-NMR ($CDCl_3$+CD_3OD): δ 0.78, 0.82, 0.88, 0.93, 1.03, 1.16 (each 3H, *s*, 6xMe), 2.78(1H, *m*, H-18),

3.2(1H, *m*, H-5), 4.34 (1H, *d*, *J*=9 Hz, H-1′), 5.32 (1H, *m*, H-12); ^{13}C-NMR ($CDCl_3$+CD_3OD): δ 89.0 (C-3), 54.8 (C-5), 122.2 (C-12), 142.8(C-13), 179.0(C-28), 179.7(C-30), 104.7(C-1′), 73.3 (C-2′), 74.2(C-3′), 71.4(C-4′), 75.8(C-5′), 170.4(C-6′); FABMS +ve *m/z* 685[M+Na]$^+$, 469[aglycone-OH]$^+$, FABMS -ve *m/z* 661 [M-H]$^-$, 485 [aglycone-H]$^-$.

3-O-β-D-glucuronopyranosylesculentic acid (2a)

Light yellow microcrystals, mp>275°C, $[\alpha]_D$ + 29°(MeOH; *c* 0.35); IRν_{max}^{KBr} cm^{-1}: 3425, 2925, 1700, 1455, 1385, 1120, 1035; ^{1}H-NMR ($CDCl_3$+CD_3OD): δ 0.69, 0.78, 0.96, 1.15, 1.16 (each 3H, *s*, 5xMe), 2.78(1H, *m*, H-18), 3.58, 3.75 (each 1H, *d*, *J*=10.4Hz, H-23), 4.43(1H, *d*, *J*=8Hz, H-1′), 5.31 (1H, *m*, H-12); ^{13}C-NMR ($CDCl_3$+CD_3OD): δ 83.3 (C-3), 123.9(C-12), 144.6 (C-13), 181.2(C-28), 180.5(C-30), 105.3(C-1′), 74.7(C-2′), 76.1(C-3′); 72.8(C-4′), 77.5(C-5′), 171.8(C-6′); FABMS +ve *m/z* 679 [M+H]$^+$, 485[aglycone-OH]$^+$; FABMS -ve *m/z* 677 [M-H]$^-$, 501[aglycone-H]$^-$.

Tri-O-acetyl-3-O-methyl-β-D-glucuronatopyranosyl-serjanic acid (1b)

Colourless microcrystals, mp 238°C, $[\alpha]_D$ +25° ($CHCl_3$; *c* 0.2); IR ν_{max}^{KBr} cm^{-1}: 2925, 1745, 1700, 1370, 1220, 1030; ^{1}H-NMR($CDCl_3$): δ 0.71, 0.72, 0.89, 0.90, 1.11, 1.14 (each 3H, *s*, 6xMe), 2.67 (1H, *m*, H-18), 3.11 (1H, *m*, H-5), 3.69, 3.75 (each 3H, *s*, 2 x CO_2Me), 4.59 (1H, *d*, *J* = 7.9Hz, H-1′), 5.34 (1H, *m*, H-12); ^{13}C-NMR ($CDCl_3$): δ 123.5 (C-12), 142.8(C-13), 183.0(C-28), 177.1(C-30), 52.3, 52.8(C-30 Me/C-6′ OMe), 103.0 (C-1); FABMS +ve *m/z* 839 [M+Na]$^+$, 949[M+Cs]$^+$, 483[aglycone-OH]$^+$; FABMS-ve *m/z* 815 [M-H].

Hepta-O-acetyl-3-O-methyl-β-D-glucuronato-pyranosyl-28-O-β-D-glucopyranosylserjanic acid (3b)

Colourless microcrystals, mp 104-106°C, $[\alpha]_D$ + 13.3° ($CHCl_3$; *c* 0.6); IRν_{max}^{KBr} cm^{-1}: 2900, 1740, 1460, 1370, 1210, 1030, 970, 880; ^{1}H-NMR($CDCl_3$): δ 0.71, 0.73, 0.89, 0.90, 1.11, 1.14 (each 3H, *s*, 6xMe), 2.65 (1H, *m*, H-18), 3.10 (1H, *m*, H-5), 3.73, 3.76 (each 3H, *s*, 2xCO_2Me), 4.6 (1H, *d*, *J*=7.8Hz, H-1′), 5.38(1H, *m*, H-12), 5.56 (1H, *d*, *J*=7.8 Hz, H-1″); ^{13}C-NMR ($CDCl_3$): δ 123.7 (C-12), 142.3(C-13), 175.0(C-28), 177.2(C-30), 51.8, 52.8(C-30 Me/C-6′ OMe), 103.0 (C-1′), 91.5 (C-1″); FABMS +ve *m/z* 1169 [M+Na]$^+$.

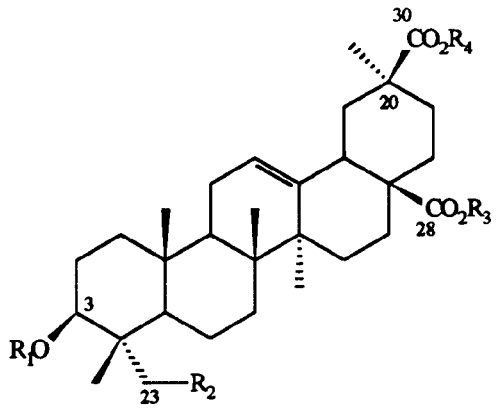
Octa-O-acetyl-3-O-methyl-β-D-glucuronatopyrano-syl-28-O-β-D-glucopyranosylphytolaccagenic acid (4 b)

Colourless microcrystals, mp 124°C, $[\alpha]_D$ + 19.2° ($CHCl_3$; *c* 0.3); IRν_{max}^{KBr} cm^{-1}; 2925, 1745, 1370, 1215, 1025; ^{1}H-NMR ($CDCl_3$+CD_3OD): δ 0.71 (6H, *s*, 2xMe), 0.93, 1.09, 1.14 (each 3H, *s*, 3xMe), 2.64 (1H, *m*, H-18), 3.61, 4.08 (each 1H, *d*, *J*=11.5Hz, H-23), 3.73, 3.76(each 3H, *s*, CO_2Me), 4.57 (1H, *d*, *J* = 7.8Hz, H-1′), 5.38 (1H, *m*, H-12), 5.55 (1H, *d*, *J*=7.8Hz, H-1″); ^{13}C-NMR($CDCl_3$): δ 123.6(C-12), 142.4(C-13), 175.3(C-28), 177.0(C-30), 51.8, 52.8 (C-30 Me/C-6′ OMe), 102.5(C-1′), 91.6 (C-1″); FABMS +ve *m/z* 1227 [M+Na]$^+$.

The defatted stem of *D. glaucescens* was extracted with hot MeOH. Chromatography of the MeOH extract gave stigmasterol, ecdysterone [6] and diploclisin [4]. The residue on the column was extracted with MeOH and the MeOH extract concentrated when *vibo*-quercitol separated out. The residual MeOH extract was partitioned between *n*-BuOH and H_2O. Chromatography of the *n*-BuOH extract over silica gel gave the saponins **1-4** in yields of 0.04%, 0.024%, 0.064% and 0.264% respectively.

High resolution negative ion FABMS provided evidence for the molecular formula of compounds **1-4**. The molecular formulae $C_{37}H_{56}O_{11}$ for **1** and $C_{37}H_{56}O_{12}$ for **2** were determined by the accurate mass measurement of their respective [M-H]$^-$ ions. Compounds **1** and **2** showed characteristic infrared absorptions for hydroxyl, ester, carboxyl and glycosidic units. Hydrolysis of **1** with 4M HCl gave serjanic acid (**5**) $C_{31}H_{48}O_5$, whereas hydrolysis of **2** with the same reagent gave phytolaccagenic acid (**6**), $C_{31}H_{48}O_6$. The acids **5** and **6** were identified by comparison of physical data with those of authentic samples obtained from the same plant.

Examination of the acid hydrolysates of **1** and **2** by paper chromatography indicated that D-glucuronic acid was the sugar component in both compounds. Further confirmation was available from a study of the mass spectra of the products of

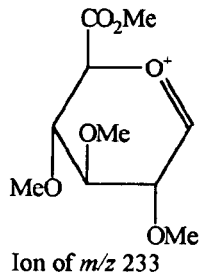


	R_1	R_2	R_3	R_4
1	Glur	H	H	Me
1a	Glur	H	H	H
2	Glur	OH	H	Me
2a	Glur	OH	H	H
3	Glur	H	Glc	Me
4	Glur	OH	Glc	Me
5	H	H	H	Me
6	H	OH	H	Me

Glc = β-D-glucopyranosyl
Glur = β-D-glucuronopyranosyl

Structure I

permethylation of **1** and **2** in DMSO with dry NaOH and MeI [7]. Compound **1** gave a pentamethyl derivative, $C_{42}H_{66}O_{11}$, consistent with its formulation as a glucuronide of serjanic acid (**5**). The EIMS of the pentamethyl derivative showed only a weak molecular ion at *m/z* 746 with a more intense $[M-2H]^+$ ion at *m/z* 744. Accurate mass measurement of the ion at *m/z* 686 indicated a formula $C_{40}H_{62}O_9$, arising from loss of HCO_2Me from the molecular ion. The CIMS of the pentamethyl derivative showed an $[M+NH_4]^+$ ion at *m/z* 764 confirming its molecular weight of 746. Both spectra contained an ion at *m/z* 233, indicating a glucuronic acid moiety in **1**.



Ion of *m/z* 233

Structure II

Compound **2** gave a hexamethyl derivative, $C_{43}H_{68}O_{12}$ in accordance with its formulation as a glucuronide of phytolaccagenic acid (**6**). The EIMS of the hexamethyl derivative contained a $[M-2H]^+$ ion at *m/z* 774, a $[M-HCO_2Me]^+$ ion at *m/z* 716 and an ion at *m/z* 233 $[C_{10}H_{17}O_6]$, while its FABMS showed $[M+H]^+$ at *m/z* 777. Examination of the ^{1}H-NMR and ^{13}C-NMR spectra (Table-1) of **1** and **2** in comparison with the recorded data [8] gave evidence for the β-D-glucuronopyranoside nature of the sugar as well as the position of its attachment at C-3 to the terpenoid moiety. Hence **1** and **2** are 3-*O*-β-D-glucuronopyranoylserjanic acid and 3-*O*-β-D-glucuronopyranosylphytolaccagenic acid respectively. Both positive and negative ion FABMS were in conformity with the structures assigned to **1** and **2**.

The glycosidic bonds of **1** and **2** were stable to alkali. Treatment with alkali merely hydrolysed the C-20 carbomethoxyl group in each compound giving 3-*O*-β-D-glucuronopyranosylspergulagenic acid (**1a**), $C_{36}H_{54}O_{11}$ and 3-*O*-β-D-glucuronopyranosylesculentic acid (**2a**), $C_{36}H_{54}O_{12}$ respectively. Acetylation of **1** with Ac_2O-pyridine followed by a work up procedure involving the use of MeOH resulted in esterification of the carboxyl group of the glucuronide along with acetylation giving **1b**, $C_{44}H_{64}O_{14}$. A study of the ^{1}H-NMR, ^{13}C-NMR and FABMS of **1b** supported the structure assigned to **1**.

Compounds **3** and **4** showed characteristic infrared absorptions for hydroxyl, ester and glycosidic units. The ^{1}H-NMR spectra of each compound showed two pairs of doublets for protons in the anomeric region, indicating the presence of two sugar moieties in each compound. Hydrolysis of **3** with 4M HCl gave serjanic acid (**5**), whereas hydrolysis of **4** gave phytolaccagenic acid (**6**). The acid hydrolysates of **3** and **4** were shown to contain D-glucose, establishing the presence of a D-glucose moiety as well in each compound. The identity of D-glucose was established by conversion into monodeuterio-D-glucitolhexaacetate and confirmed by GC and GCMS studies on the latter [9].

The molecular formula $C_{43}H_{66}O_{16}$ for **3**, consistent with its formulation as a D-glucoside of **1**, was established from the $[M-H]^-$ ion at *m/z* 837.4264. The ion at *m/z* 853.4217 $[M-H]^-$ confirmed the molecular formula $C_{43}H_{66}O_{17}$ for **4**, consistent with its formulation as a D-glucoside of **2**.

The D-glucose residues in **3** and **4** were shown to be pyranosides. Acid hydrolysis of the products of permethylation of **3** and **4** gave 2,3,4,6-tetra-*O*-methyl-D-glucose which was converted into 1-deuterio-1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol identified by GC and GCMS [9]. The β-configuration of the pyranoside groups in **3** and **4** was obtained, as in an earlier investigation [3], from their ^{1}H-NMR spectra. Chemical and spectroscopic evidence indicated that β-D-glucopyranosyl group in **3** and **4** was attached through C-28. Both **3** and **4** did not react with CH_2N_2 and did not show carboxyl absorption in their infrared spectra. The ^{13}C-NMR spectra of **3** and **4** showed that C-28 as well as C-30 gave signals characteristic of esters [8]. Confirmation was available from a study of the alkaline hydrolysis of **3** and **4**. The products obtained were **1a** and **2a** respectively. Thus **3** and **4** are 3-*O*-β-D-glucuronopyranosyl-28-O-β-D-glucopyranosyl-serjanic acid and 3-*O*-β-D-glucuronopyranosyl-28-O-β-D-glucopyranosylphytolaccagenic acid respectively. The ^{1}H-NMR, ^{13}C-NMR (Table-1) and FABMS data for **3** and **4** confirm their structural identification.

Further, **3** and **4** were acetylated and the product worked up as for **1** and **2**, giving **3b**, $C_{58}H_{82}O_{23}$ and **4b**, $C_{60}H_{84}O_{25}$ respectively. The structures assigned to **3** and **4** were fully supported by the ^{1}H-NMR, ^{13}C-NMR and FABMS spectra of **3b** and **4b**.

Since saponins are among the best known natural molluscicides, each of the saponins isolated in this investigation was tested for activity against the snail, *Biomphalaria glabrata*. Among the criteria for preliminary acceptance of a compound as a molluscicide is the ability to cause 100% lethality to *B. glabrata* snails at minimum concentrations up to 20 ppm. In this regard, only the compound **1** was acceptable as it showed 100% lethality to the above snails at a minimum concentration of 12 ppm.

Experimental

Mps (uncorr.) were determined on a Kofler hot-stage apparatus. IR spectra were obtained using a Shimadzu 408 spectrometer or JASCO IRA-1 spectrometer. Optical rotations were measured using a Perkin Elmer 241 polarimeter at 22°. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Varian VXR 300 instrument. Proton-proton and proton-carbon connectivities were esablished from COSY (correlation spectroscopy) and HETCOR ((heteronuclear chemical shift correlation spectroscopy). EI (electron impact) and CI (chemical ionization) mass spectra were recorded at 70eV on a VG 7070F mass spectrometer while FABMS (fast atom bombardment mass spectra) were recorded at 30kV(Cs+) on a VG ZAB-SEQ instrument with glycerol as matrix. For high resolution FABMS the internal reference used was tetrameric trifluoroethyl phosphonitrilate.

GLC (gas liquid chromatography) was carried out using a Varian 3300 instrument installed with a DB 225 capillary column. The oven temperature was programmed from 150°C to 220°C at 3°C min^{-1}. H_2 was used as carrier gas (6 ml min^{-1}). Injector and detector temperatures were maintained at 250°C. GLCMS (gas liquid chromatography mass spectra) were recorded using a Hewelett Packard 5970B instrument with a 12m HP-1 bonded methyl silicone column. The oven temperature was programmed from 100°C to 250°C at 10°C min^{-1}. HPLC (high performance liquid chromatography) was performed with a Water Associates 6000A solvent supply unit connected to a Waters U6K injector. Eluates were detected with a Waters R401 differential refractometer.

Extraction and isolation

The dry, ground, mature stem of *D. glaucescens* (500 g) was extracted with hot MeOH. Evaporation of the MeOH gave a dark brown solid (60 g). A portion (15 g) was separated by chromatography over silica gel and stigmasterol (30 mg), ecdysterone (4g) and diploclisin (600 mg) eluted. After eluting diploclisin, the column was washed with MeOH. Concentration of MeOH extract resulted in the separation of *vibo*-quercitol (82 mg), which was recrystallised from MeOH-EtOAc as colourless needles, mp 186°, $[\alpha]_D$ + 164° [MeOH; *c* 0.11]. The identity of *vibo*-quercitol was established from its ^{1}H-NMR and ^{13}C-NMR data [10] as well as by comparison with an authentic sample.

After removal of *vibo*-quercitol, the MeOH was evaporated to give a brown solid (7g) which was partitioned between *n*-BuOH and H_2O. The *n*-BuOH layer was washed three times with H_2O and

evaporated to give the crude saponins as a brown solid (5.5 g). Separation of this mixture by chromatography over silica gel employing, $CHCl_3$-MeOH-H_2O (7:3:1) as the eluent gave saponins **1** (50 mg), **2** (30 mg), **3** (80 mg) and **4** (330 mg). All four compounds were further purified by chromatography over silica gel. The purity of each compound was checked by HPLC [μ Bondapak Tm/C_{18}, HPLC grade CH_3CN-H_2O (3:7), 1.5 ml/min].

Acid hydrolysis of ***1-4***

Each compound (5 mg) was refluxed with 4M HCl (5 ml) for 2h. The product was extracted with EtOAc and the solvent evaporated. Compounds **1** and **3** gave serjanic acid (**5**), which was recrystallised from *n*-hexane-EtOAc as colourless microcrystalline needles, mp 249-251°C, $[\alpha]_D$ + 74°($CHCl_3$, *c* 0.2) identical with an authentic sample from the same plant [3]. Compounds **2** and **4** gave phytolaccagenic acid (**6**), which was recrystallised from Me_2CO as colourless needles, mp 285-287°, $[\alpha]_D$ + 98° (MeOH, *c* 0.13), identical with an authentic sample [3].

The aq. acid hydrolysates of **1** and **2** were adjusted to pH 6 with $NaHCO_3$. After freeze-drying and extraction with pyridine gave D-glucuronic acid which was identified by paper chromatography (Whatman No. 1 paper, ascending and descending modes in *n*-BuOH-EtOH-H_2O (40:11;19), with anilinehydrogen phthalate as developer).

The aq. acid hydrolysates of **3** and **4** also subjected to the above procedure and the sugars obtained by extracting with pyrdine. A portion was reduced with $NaBD_4$ in 1M NH_4OH for 1 hr at room temperature, neutralised with glacial HOAc and evaporated. Drying was completed by the addition of HOAc-MeOH (1:9) and evaporation to dryness. Acetylation was effected by heating with Ac_2O for 1 h at 100°C, evaporation to dryness with MeOH and extracting the product with $CHCl_3$. GLC and GLCMS showed that the $CHCl_3$ extract contained monodeuterio-D-glucitol hexaacetate, $C_{18}H_{25}DO_{12}$; EIMS *m/z* (rel.int.): 362(55), 361(5), 290(5), 289(5), 218(15), 217(15), 146(25), 145(20), 115(100); CIMS (NH_3) *m/z* (rel.int.): 453 $[M+NH_4]^+$ (100).

Permethylation of ***1-4***

Each compound (5 mg) in DMSO (2 ml) was treated with dry NaOH (25 mg), stirred at room temperature for 10 min and CH_3I (2 ml) added. After standing the mixture for 20 min, the excess CH_3I was removed by evaporation and the permethylated product extracted with CH_2Cl_2.

Hydrolysis of permethylated 3 and 4

Each permethylated product (2 mg) was refluxed with 2M trifluoroacetic acid (2 ml) for 1h. The reaction mixture was poured into crushed ice and filtered. The filtrate was extracted with $CHCl_3$ to give 2,3,4,6-tetra-*O*-methyl-D-glucose. The methylated sugar was reduced with $NaBD_4$, as described for the acid hydrolysates of **3** and **4**. GC and GC-MS showed that the product was 1-deuterio-1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol, $C_{14}H_{25}DO_8$. EIMS *m/z* (rel.int.): 162(52), 161(50), 118(65), 102(100), 101(40).

Alkaline hydrolysis of ***1-4***

Each compound was refluxed with 0.5 M KOH (1 ml per mg) for 2 h. The reaction mixture was adjusted to pH 4 with dil. HCl and extracted with EtOAc and *n*-BuOH. The organic layer was evaporated to dryness. Compounds **1** and **3** gave compound **1a** whereas compounds **2** and **4** gave **2a**.

Acetylation of ***1,3 and 4***

Each compound was allowed to react overnight with Ac_2O (1 ml per 20 mg) and pyridine (1 ml per 20 mg). The mixture was evaporated to dryness with C_6H_6, $C_6H_5CH_3$ and MeOH and the product purified by prep. TLC. Compounds **1,3** and **4** gave **1b, 3b** and **4b** mixture respectively.

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