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DIPLOCLISIN, A BIDESMOSIDIC TRITERPENOID SAPONIN FROM *DIPLOCLISIA GLAUCESCENS*

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Abstract—The mature stem of *Diploclisia glaucescens* furnished diploclisin, a new bidesmosidic saponin. The structure of diploclisin was established by chemical and spectroscopic methods as β-D-glucopyranosyl 3β-(β-D-glucopyranosyloxy)-23-hydroxy-30-carbomethoxyolean-12-en-28-oate.

INTRODUCTION

The *Diploclisia* genus of the Menispermaceae is found in Sri Lanka, South India and South China. *D. glaucescens* (Bl.) Diels (= *Cocculus macrocarpus* W. & A.), the only species of *Diploclisia* found in Sri Lanka is a creeper whose leaves have been used in the treatment of biliousness and venereal diseases [1]. An ethanol extract of the seeds of the plant collected in India, showed activity as an insect control agent. The active compounds were identified as five phytoecdysteroids [2].

We have reported the isolation of stigmasterol and ecdysterone from a methanol extract of the stem of *D. glaucescens* [3]. Further separation of this extract led to the isolation of a new bidesmosidic saponin (**1**), named diploclisin. The present paper describes the elucidation of the structure of **1** and reports the results of tests carried out on **1** for molluscicidal, spermicidal, antifungal and hemolytic properties.

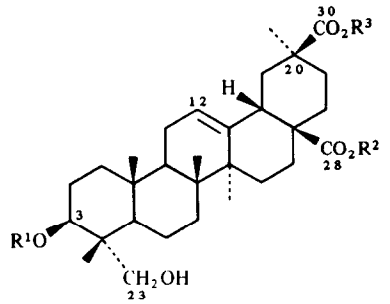
Diploclisin (**1**) is the first example of a bidesmosidic saponin containing phytolaccagenic acid (**2**) as genin. Phytolaccagenic acid was first isolated from the acid hydrolysate of the saponin fraction of *Phytolacca americana* (Phytolaccaceae) [4] and fully characterized as 3β,23-dihydroxy-30-carbomethoxyolean-12-en-28-oic acid [5]. Phytolaccagenic acid was later shown to be the genin of monodesmosidic saponins of *P. americana* [6, 7]. TLC has shown the presence of phytolaccagenic acid in the methanol extract of the stem of *D. glaucescens*. Hence this constitutes the first report of the occurrence of phytolaccagenic acid, both in free and combined forms, from outside the family Phytolaccaceae.

RESULTS AND DISCUSSION

The mature stem of *D. glaucescens* was defatted with hot petrol and then extracted with hot methanol. Separation of the methanol extract by adsorption chromatography over silica gel gave stigmasterol, ecdysterone, and diploclisin (**1**) in yields of 0.03, 3.2 and 0.48%, respectively.

Diploclisin (**1**) showed in its IR spectrum characteristic absorptions of hydroxyl, ester and glycosidic units. The FABMS peaks of **1** at *m/z* 841.6 $[M+H]^+$ and 679.5 $[M-C_6H_{10}O_5+H]^+$ gave evidence for the molecular formula $C_{43}H_{68}O_{16}$ of **1**. Hydrolysis of **1** with 4 M HCl gave phytolaccagenic acid (**2**) and D-glucose, as the only sugar. The presence of two anomeric doublets (δ 5.12 and 6.29, $J=7.8$ Hz) in the ^{1}H NMR spectrum of **1** indicated the presence of two D-glucose residues. Permethylation of **1** by the Hakomori method [8] followed by acid hydrolysis gave 2,3,4,6-tetra-*O*-methyl-D-glucose as the only sugar. The configuration at each anomeric centre in **1** was established as β from the magnitude of the coupling constant of each anomeric doublet in its ^{1}H NMR spectrum. The anomeric doublets observed for α-D-glucopyranosides have much lower coupling constants (3.5 Hz) [9]. Compound **1** is thus a di-β-D-glucopyranoside of phytolaccagenic acid (**2**).

Diploclisin (**1**) reacted with acetic anhydride and pyridine giving a nonaacetate. The ^{1}H NMR spectrum of the nonaacetate showed two anomeric doublets (δ 4.51 and 5.55, $J=7.8$ Hz) as well as a broad multiplet (δ 5.0–5.25) integrating for six axial protons of two acetylated β-D-glucopyranosyl moieties, providing further evidence for the bidesmosidic nature of **1**. Com-



	R¹	R²	R³
1	glc	glc	Me
2	H	H	Me
3	glc	H	H
4	glc	Me	Me
5	H	H	H

glc = β-D-glucopyranosyl

pound **1** failed to react with diazomethane showing the absence of a free carboxyl group and hence the attachment of one D-glucose unit as a C-28 ester.

Reaction of **1** with 0 5 M potassium hydroxide hydrolysed the C-28 ester as well as the C-30 ester giving compound **3** Removal of one D-glucose residue on alkaline hydrolysis of **1** was accompanied by the appearance of only the higher field anomeric doublet (δ 5 17, J = 7 6 Hz) in the ^{1}H NMR spectrum of **3** The molecular formula $C_{36}H_{56}O_{11}$ for compound **3** was indicated by the FABMS peak at m/z 665 3 $[M+H]^+$. The IR spectrum of **3** did not indicate the presence of ester groups whereas the strong absorption at 1700 cm^{-1} indicated carboxyl Compound **3** was evidently a dicarboxylic acid as it reacted with diazomethane giving a product **4** showing absorption for two carbomethoxyl groups (δ3.62 and 3 66) in its ^{1}H NMR spectrum Five hydroxyl groups are present in **3**, as its acetylation product showed the presence of five acetate groups (δ 2.01, 2 02, 2.10, 2 11and 2 24) in its ^{1}H NMR spectrum Hence compound **3** is the monodesmosidic saponin 3β-(β-D-glucopyranosyloxy)-23-hydroxyolean-12-en-28,30-dioic acid or 3-*O*-β-D-glucopyranosylesculentic acid It is interesting to note that alkaline hydrolysis [5] of phytolaccagenic acid gives esculentic acid (**5**) a triterpene isolated from *P esculenta* [10] Diploclisin (**1**) is then the bidesmosidic saponin, β-D-glucopyranosyl 3β-(β-D-glucopyranosyloxy)-23-hydroxy-30-carbomethoxyolean-12-en-28-oate or 3,28-di-*O*-β-D-glucopyranosylphytolaccagenic acid

The ^{13}C NMR spectrum of **1** and its nonaacetate provided further evidence for the structure assigned to **1**. The signal for C-23 appeared at δ 64 77 for **1** and at 65.20 for the nonaacetate The positions of these signals excluded the possibility of glycosylation through C-23. In the latter case the C-23 signal is known to appear at lower fields (*ca* δ 80) [11]. The signal for C-3 appeared at δ 82.19 for **1** and at 83.55 for the nonaacetate, while the signal for C-28 appeared at 175 97 for **1** and at 175.07 for the nonaacetate. The positions of these signals were in agreement with recorded data for comparable bidesmosidic saponins [12]. The anomeric carbon atom attached to the genin through C-3 and C-28 resonated at δ 105 83 and 95 75, respectively for **1** and at 102 59 and 91.53, respectively, for the nonaacetate These signals, as well as the signals for C-2′, C-3′, C-4′ and C-5′ in each β-D-glucopyranosyl moiety of **1** and the nonaacetate, agree well with recorded data for related compounds [13]

Several triterpenoid saponins have been tested for activity against the snail (mollusc) *Biomphalaria glabrata*, one of the vectors of schistosomiasis None of the bidesmosidic saponins showed any significant molluscicidal activity, while most monodesmosidic saponins showed activity [14]

Both the natural bidesmosidic saponin **1** and the monodesmosidic saponin **3** showed no activity against *B glabrata* even at concentrations of 50 ppm Compound **1** showed no activity against human spermatozoa even at a concentration of 33 mg/ml Compound **1** also showed no activity against the fungus *Cladosporium cladosporioides* and gave a negative hemolysis test

EXPERIMENTAL

Mps uncorr Chemical shifts are given in δ (ppm) with TMS as int standard Assignment of chemical shifts in ^{13}C NMR spectra were made with the aid of DEPT analysis FABMS were obtained in the positive ion mode GC analyses were carried out using a DB 225 capillary column The oven temperature was programmed from 150° to 220° at 3°/min, H_2 was used as carrier gas (6 ml/min) Injector and detector temp were both maintained at 250°

Plant material D. glaucescens was identified and collected in May from the Central Province of Sri Lanka by Prof S Balasubramaniam (Department of Botany, University of Peradeniya, Sri Lanka)

Extraction and isolation The dry, ground, mature stem of *D glaucescens* (500 g) was sequentially extracted with hot petrol (40–60°) and hot MeOH. Evapn of the MeOH gave a dark brown solid (60 g) A portion (15 g) was separated on a column of 200 g of silica gel (Merck, Art 9385) by MPLC with petrol, EtOAc and MeOH as eluants Following the elution of stigmasterol (30 mg) and ecdysterone (4 g), diploclisin (**1**) was obtained as a fine white powder (600 mg) on elution with 15% EtOAc–MeOH Further purification of **1** was effected by adsorption chromatography over silica gel

Diploclisin (**1**) Colourless microcrystalline needles, mp 171–173°, $[\alpha]_D^{22}$ + 25° (MeOH, *c* 0 12), IR ν_{max}^{KBr} cm^{-1} 3350 2950, 1730, 1640, 1450, 1390, 1080, ^{1}H NMR (C_5D_5N) δ0 90, 0 96, 1.10, 1 15, 1 18 (each 3H, *s*, H-24, H-25, H-26, H-27, H-29), 3 3 (1H, *m*, H-3), 3 56 (3H, *s*, C-30 OMe), 3 65 and 4 02 (each 1H, *d*, J = 11 5 Hz, H-23),5 12 and 6 29 (each 1H, *d*, J = 7 8 Hz, anomeric protons), 5 60 (1H, *m*, H-12), ^{13}C NMR (C_5D_5N) aglycone δ 38 75 (C-1), 25.94 ((C-2), 82 19 (C-3), 43 49 (C-4), 47.69 (C-5), 18 33 (C-6), 32 88 (C-7), 39 99 (C-8), 48 18 (C-9), 36 99 (C-10), 23 88 (C-11), 143 82 (C-13), 42 09 (C-14), 28 38 (C-15), 23 60 (C-16), 46 56 (C-17), 43 23 (C-18), 42 43 (C-19), 44 02 (C-20), 30 62 (C-21), 34 08 (C-22), 64 77 (C-23), 13 75 (C-24), 16 28 (C-25), 17 59 (C-26), 26 17 (C-27), 175 97 (C-28), 28 18 (C-29), 176 83 (C-30), 51 75 (C-30 OMe) sugar moieties δ 105 83, 75 86, 78 37, 71 62, 78 71 (C-1′, C-2′, C-3′, C-4′, C-5′ of D-glucopyranosyl moiety at C-3), 95 75, 74 14, 78 89, 70 91, 79 37 (C-1′, C-2′, C-3′, C-4′, C-5′ of D-glucopyranosyl moiety at C-28), 61.63 and 61 97 (C-6′ of D-glucopyranosyl moiety at C-3/C-28), FABMS m/z 841 6 $[C_{43}H_{68}O_{16}(M)+H]^+$, 679 5 $[C_{37}H_{58}O_{11}(M-C_6H_{10}O_5)+H]^+$

Acetylation of **1** Compound **1** (25 mg) was allowed to react overnight with Ac_2O (1 5 ml) and pyridine (1 5 ml) After the usual work-up, the nonaacetate was obtained as a light brown amorphous solid Further purification by prep TLC gave the nonaacetate as colourless needles (20 mg), mp 54°, $[\alpha]_D^{22}$ + 29 1° (MeOH, *c* 0 55), IR ν_{max}^{KBr} cm^{-1} 2950, 1735, 1430, 1365, 1215, 1025, 910, ^{1}H NMR ($CDCl_3$) δ 0 71(6H, *s*, 2 × Me), 0 93, 1 09, 1 14 (each 3H, *s*, 3 × Me), 2 0–2 10 (9 × OAc), 3 50 (1H, *m*, H-3), 3.65 (1H, *d*, J = 11 Hz, 1 × H-23), 3 73 (3H, *s*, C-30 OMe), 4.0–4 3 (5H, *m*, CH_2OAc of two D-glucopyranosyl moieties and 1 × H-23), 4 51 and 5 55 (each 1H, *d*, J = 7 8 Hz, anomeric protons), 5 0–5 25 (6H, *m*, CHOAc of two D-glucopyranosyl moieties), 5 38 (1H, *m*, H-12), ^{13}C NMR ($CDCl_3$) aglycone. δ 38 09 (C-1), 25 16 (C-2), 83 55 (C-3), 47 78 and 47 67 (C-5/C-9), 123 49 (C-12), 142 33 (C-13), 65 20 (C-23), 175 07 (C-28), 176 77 (C-30), 51 92 (C-30 OMe) sugar moieties δ 102 59, 91 53 (C-1′ of D-glucopyranosyl moieties at C-3, C-28), 61 47 and 62 17 (C-6′ of D-glucopyranosyl moiety at C-3/C-28), 67 89, 68 68, 69 77, 71 49, 71 55, 72 44 and 72 83 (two overlapping signals) (C-2′/C-3′/C-4′/C-5′ of two acetylated D-glucopyranosyl moieties)

Acid hydrolysis of **1** Compound **1** (100 mg) was refluxed with 4 M HCl (100 ml) for 2 hr The mixture was extracted with EtOAc The organic layer was evapd to dryness and crystallized from Me_2CO to give phytolaccagenic acid (60 mg), mp 285–287°, $[\alpha]_D^{30}$ + 98 1° (MeOH, *c* 0 13), whose identity was confirmed by comparison with reported IR, MS [5], ^{13}C NMR [7] data and by direct comparison with an authentic sample

The aq phase was adjusted to pH 6 with $NaHCO_3$. After freeze-drying, extraction with pyridine gave the sugar which was identified by TLC and GC. (i) TLC: Both ascending and descending modes were used with Whatman No 1 paper and *n*-BuOH–EtOH–H_2O (40:11.19). Anilinehydrogen phthalate was used as developer. D-Glucose was identified as the only sugar by comparison with a standard (ii) GC: A portion of the sugar was refluxed with $NaBH_4$ in 1 M NH_4OH for 1 hr at room temp., then neutralized with glacial HOAc and evapd Drying was completed by the addition of 10% HOAc–MeOH, evapn and by a final evapn with Analar MeOH. Acetylation was carried out by heating with Ac_2O for 1 hr at 100°, evapn to dryness with MeOH and extraction with $CHCl_3$ The $CHCl_3$ extract was injected into the gas chromatograph and D-glucitol hexaacetate identified as the only alditol acetate, showing the presence of only D-glucose in the acid hydrolysate.

Base hydrolysis of **1**. Compound **1** (150 mg) was refluxed with 0.5 M KOH (150 ml) for 2 hr. The mixture was adjusted to pH 4 with aq HCl and extracted with EtOAc and BuOH The organic layer was evapd to dryness giving compound **3** as a light brown powder (120 mg) Recrystallization from EtOAc–MeOH gave **3** as light brown microcrystalline needles, mp 262°, $[\alpha]_D^{22}$ + 33° (MeOH; *c* 0.15), IR ν_{max}^{KBr} cm^{-1} 3400, 2950, 1700, 1465, 1385, 1070, 1030, ^1H NMR (C_5D_5N) δ0.90, 0 98, 1 04, 1.32, 1.42 (each 3H, *s*, 5 × Me), 5 17 (1H, *d*, J = 7 6 Hz, anomeric proton), 5.73 (1H, *m*, H-12); FABMS *m/z*· 665 3 $[C_{36}H_{56}O_{11}$ (M) + H$]^+$.

Acetylation of **3**. Compound **3** (40 mg) was allowed to react on a steam bath with Ac_2O (2 ml) and pyridine (2 ml) for 2 hr and then overnight at room temp. After usual work-up the acetate of **3** was obtained as a light brown solid (45 mg), which was recrystallized from *n*-hexane–CH_2Cl_2 giving colourless needles, mp 145–147°, $[\alpha]_D^{22}$ + 25° (EtOH; *c* 0.4); IR ν_{max}^{KBr} cm^{-1} 3450, 2925, 1740, 1365, 1220, 1040; ^1H NMR (C_5D_5N)· δ 0 80, 0.81, 1.01, 1 36, 1 43 (each 3H, *s*, 5 × Me), 2.01, 2 02, 2 10, 2 11, 2.24 (each 3H, *s*, 5 × OAc), 4 06 and 4 26 (each 1H, *d*, J = 11 4 Hz, H-23), 5 12 (1H, *d*, J = 7.9 Hz, anomeric proton), 5 73 (1H, *m*, H-12)

Methylation of **3**. Compound **3** (40 mg) in MeOH was treated with ethereal CH_2N_2, the solvents evapd and the residue recrystallized from EtOAc–MeOH giving colourless needles of the diester **4**, mp 155–157°, $[\delta]_D^{22}$ +17 5° (EtOH, *c* 0.4), IR ν_{max}^{KBr} cm^{-1}· 3400, 2950, 1730, 1560, 1405, 1265, 1210, 1115, 1070; ^1H NMR (C_5D_5N). δ 0.85, 0 95, 1 00, 1 18, 1 19 (each 3H, *s*, 5 × Me), 3 62 and 3.66 (each 3H, *s*, C-30 OMe/C-28 OMe), 5.16 (1H, *d*, J = 7 6 Hz, anomeric proton), 5 54 (1H, *m*, H-12).

Permethylation of **1** Compound **1** (200 mg) in DMSO (4 ml) was treated with dimsyl carbanion solution (4 ml), allowed to stand at room temp for 1 hr, and treated with MeI (5 ml). After standing overnight, the reaction mixture was poured onto crushed ice and extracted with Et_2O. The Et_2O extract was evapd to dryness and the residue purified by prep. TLC giving per-*O*-methyldiploclisin as a colourless sticky solid (60 mg), $[\alpha]_D^{22}$ + 33 3° ($CHCl_3$, *c* 0 45), IR ν_{max}^{KBr} cm^{-1} 2950, 1730, 1460, 1380, 1140, 1090, ^1H NMR ($CDCl_3$) δ0 7, 1.15 (each 6H, *s*, 4 × Me), 0.93 (3H, *s*, Me), 3.32–3.63 (27H, overlapping singlets, 9 x OMe), 3.72(3H, *s*, C-30 OMe), 4.13 and 4.28 (each 1H, *m*, anomeric protons), 5.38 (1H, *m*, H-12).

Hydrolysis of per-O-methyldiploclisin. Per-*O*-methyldiploclisin (2 mg) was refluxed with 2 M TFA (2 ml) for 1 hr. The reaction mixture was poured onto crushed ice and filtered. The filtrate was extracted with $CHCl_3$ and analysed by GC. The only sugar present was identified, through the corresponding alditol acetate, as 2,3,4,6-tetra-*O*-methyl-D-glucose.

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