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GLOCHIDIOSIDE, A TRITERPENE GLYCOSIDE FROM *GLOCHIDION HEYNEANUM*

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Key Word Index—*Glochidion heyneanum*; Euphorbiaceae; triterpenoid glycoside; glochidioside.

Abstract—A new triterpenoid glycoside, glochidioside, has been isolated from *Glochidion heyneanum*. Its structure has been established as 3β[(*O*-β-D-glucopyranosyl-(1 → 3)-*O*-α-L-arabinopyranosyl)oxy]-16β-benzoyloxy-olean-12-ene-21β,23,28-triol by chemical and spectroscopic methods.

INTRODUCTION

The alcoholic extract of the aerial parts of the plant *Glochidion heyneanum* Wall shows anticancer activity in the PS system [1]. So far no chemical studies have been carried out on this plant. It was therefore taken up for detailed chemical investigation. This paper reports the structure elucidation of glochidioside, a new triterpene glycoside isolated from *G. heyneanum*.

RESULTS AND DISCUSSION

Glochidioside, $[\alpha]_D + 42°$ (ethanol), gave a positive Fiegel test for the presence of aglycoside. It was therefore subjected to acid hydrolysis, and the hydrolysate resolved into chloroform- and water-soluble portions. The aqueous portion was found to contain glucose and arabinose (PC), which were separated by chromatography and characterized as D-glucose and L-arabinose.

Chromatography of the chloroform-soluble portion yielded pure aglycone (**1**), $[\alpha]_D + 59$ (EtOH), $C_{37}H_{54}O_6$ (FDMS, $[M]^+$ 594). Its spectral data indicated it to be a pentacyclic triterpenoid of the olean-12-ene or ursan-12-ene series carrying a benzoyloxy and four hydroxyl groups. Thus its IR and UV spectra showed prominent peaks at 1700, 1650, 1270 cm^{-1} and 230, 273 nm respectively for an aromatic ester group (^1H NMR, Table 1), and its EIMS contained a peak at *m/z* 454 $[M-18-122]^+$. On acetylation, the aglycone furnished a tetraacetate which did not show any OH band in the IR spectrum confirming the presence of four OH groups in the molecule.

Treatment of the aglycone with 5% NaOMe gave a debenzoylated product (**3**) (IR, NMR, EIMS), mp 286–288° (FDMS $[M]^+$ 490), which on acetylation gave a pentaacetate (**4**), mp 121–122°. The mps and spectral data of the debenzoylated product of the aglycone and its pentaacetate were identical with those reported for gymnestrogenin and its pentaacetate [2, 3]. Thus the aglycone of glochidioside was a benzoylated derivative of gymnestrogenin. The ^1H NMR signal assignments of the aglycone and its derivatives were made by comparison with the ^1H NMR data of gymnestrogenin pentaacetate. These are given in Table 1.

The position of the benzoyloxy group in the aglycone (**1**) was established as 16β on the grounds that in the ^1H NMR of the aglycone, H-16α resonated at δ5.0 (*q*, *J* = 12, 5 Hz) and was shifted upfield by 0.76 ppm (δ4.24) in the ^1H NMR of the debenzoylated product (**3**) while the other carbinol proton signals showed little change. The aglycone was, thus, characterized as 16-*O*-benzoyl-gymnestrogenin; a new natural compound.

The site of linkage of the sugar moiety to the aglycone was settled by studying the ^1H NMR spectra of the aglycone (**1**) and peracetylated glochidioside (Table 1). It was observed that, in the ^1H NMR of the permethylated glochidioside, the H-3 carbinolic proton resonated at the same frequency (δ3.41) as that of the aglycone while the other carbinolic proton signals were shifted downfield due to acetylation. This clearly established C-3 as the position of the linkage of the sugar moiety to the aglycone.

To determine the sequence of sugars in glochidioside, it was subjected to permethylation by the Hakomari method [4]. The permethylated glycoside was hydrolysed with acid and the resulting partially methylated sugars were identified as 2,4-di-*O*-methyl arabinose and 2,3,4,6-tetra-*O*-methylglucose by GC-MS analyses of their alditol acetates according to Jansson *et al.* [5]. The patterns of methylation of these sugars clearly indicated that glucopyranose was the terminal sugar joined to the arabinopyranosyl residue through 1 → 3 linkage. The latter was joined by a glycosidic link to C-3 of the aglycone. Further support for this sequence of sugars was forthcoming from the EIMS of the permethylated glochidioside as shown in **5**. The ^1H NMR of the permethylated glochidioside had two 1H doublets at δ4.45 (*J* = 8 Hz) and δ4.13 (*J* = 7 Hz) assignable to the anomeric protons of the D-glucopyranose and L-arabinopyranose moieties. The high magnitude of the $J_{1,2}$ values suggested that both the sugars were in the $^4C_1$ conformation but D-glucopyranose was linked through β-linkage and L-arabinopyranose through an α-linkage. Thus the complete structure of glochidioside is represented by **6**.

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Table 1. ^{1}H NMR data of compounds 1–4 ($CDCl_3$, J in Hz)

H	1	2	3	4
3	3.70 *m*	4.75 *q* (10, 6)	3.64 *m*	4.77 *q* (10, 6)
12	5.27 *br s*	5.34 *br s*	5.24 *br s*	5.31 *br s*
16	5.00 *q* (12, 5)	5.62* *q* (12, 5)	4.24 *q* (12, 5)	5.53* *q* (12, 5)
21	3.70 *m*	4.86 *q* (12, 5)	3.64 *m*	4.59 *q* (12, 5)
23	3.75 *d* (11)	3.87 *d* (12)	3.75†‡ *d* (11)	3.86 *d* (12)
23	3.40 *d* (11)	3.71 *d* (12)	3.45 *d* (11)	3.70 *d* (12)
28	4.11 *d* (12)	4.07 ABq (11)	4.10†‡ *d* (12)	4.05 ABq (11)
28	3.15 *d* (12)		3.21 *d* (12)	
Quaternary Me	0.96, 0.99, 1.00, 1.02, 1.15, 1.27 *s*	0.82, 0.98, 1.00, 1.14, 1.24, 1.30 *s*	0.89 (6H), 0.98 (6H), 1.05, 1.06 *s*	0.84, 0.92, 0.99 (9H), 1.28 s
OAc		2.03, 2.07, 2.08, 2.14 *s*		2.0, 2.02, 2.06, 2.08 *s* (2 × OAc)
Aromatic H	7.45 2H, *t* (8) 7.70 1H *t* (8) 8.06 2H *d* (8)	7.44 2H *t* (8) 7.54 1H *t* (8) 8.02 2H *d* (8)		

*Attention is drawn to the unusual downfield shift as compared to 1. It may be due to anisotropic effect of the neighbouring acetoxyl groups.

†Geminal relationship established by decoupling experiments.

‡Interchangeable.

EXPERIMENTAL

For GC-MS a 3% OV-17 column at 200° was used and the EIMS of the separated products were recorded at 20 eV. CC: silica gel; PLC TLC: silica gel G with the following solvents: (1) EtOAC–MeOH–H_2O (8:1:1), (2) $CHCl_3$–MeOH (47:3), (3) hexane–Me_2CO (4:1), (4) EtOAC–Me_2CO–H_2O (8:8:1). Compounds were visualized by spraying with 1% $Ce(SO_4)_2$ in 1 M H_2SO_4. PC was carried out on Whatman No. 1 paper using *n*-BuOH satd with H_2O as the eluant.

Extraction and isolation. The air dried, aerial part (16 kg) of *G. heyneanum* (voucher specimen deposited in CDRI herbarium) was extracted with EtOH and the alcoholic extract concd under red. pres. at 50°. The *n*-BuOH soluble portion of the extract, on repeated chromatography over silica gel impregnated with 2% boric acid yielded glochidioside (150 mg) as an amorphous, colourless powder, $[\alpha]_D$ + 42 (*c* 1; EtOH); IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 2920, 1710, 1700, 1690, 1290, 1130, 1080.

Acid hydrolysis of glochidioside. Glochidioside (100 mg) was refluxed with 80% ethanolic 2 M HCl (5.0 ml) for 3 hr. After evaporation of EtOH and addition of H_2O (2.5 ml) the hydrolysate was refluxed for a further 1 hr and then extracted with $CHCl_3$ (3 × 1 ml). The aq. acidic phase was neutralized with Amberlite IR 410 (CO_3^{2-}) resin. The organic layer was successively washed with aq. $NaHCO_3$ soln and H_2O, then evaporated to give a residue (60.0 mg) containing the aglycone.

Aglycone 1. The crude aglycone (60 mg) was purified by CC using $CHCl_3$–MeOH as the solvent system. The $CHCl_3$–MeOH (49:1) eluate furnished pure 1 (50.0 mg) which crystallized from the same solvent into colourless needles, mp 282–284°, $[\alpha]_D$ + 59 (*c* 1; EtOH); IR ν_{max}^{KBr} cm^{-1}: 3450 (OH), 2950, 1700, 1650, 1320, 1280, 1070, 730; UV λ_{max}^{MeOH} nm: 230, 273; ^{1}H NMR ($CDCl_3$): Table 1; FDMS *m/z*: 594 $[M]^+$; EIMS *m/z*: 576, 558, 544, 517, 505, 454, 436, 423, 352, 266, 248, 230, 223, 217, 205, 200, 199 [base peak], 187, 185, 175, 157, 105.

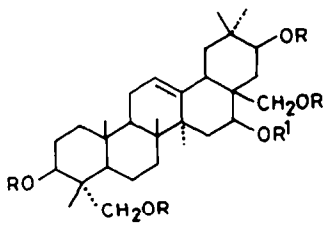
Aglycone tetraacetate (2). The aglycone (10 mg) was acetylated overnight with Ac_2O (0.5 ml) and C_5H_5N (0.5 ml). After evaporation of the solvent, the residue (12 mg) was purified by prep. TLC using *n*-hexane–Me_2CO (4:1). Pure 2 was obtained as a colourless amorphous powder, IR ν_{max}^{KBr} cm^{-1}: 2930, 1720, 1700, 1450, 1375, 1280, 1050; ^{1}H NMR ($CDCl_3$): Table 1; FDMS *m/z*: 762 $[M]^+$.

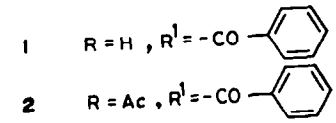
Debenzoylation of 1. The aglycone (1, 30 mg) was refluxed with 5% NaOMe (1 ml) for 3 hr and then the reaction mixture was diluted with H_2O and extracted with $CHCl_3$. The $CHCl_3$ extract was washed free of alkali with H_2O and evaporated to give a residue from which 3 was crystallized ($CHCl_3$–MeOH) as colourless needless (15 mg) mp 286–288° (lit. [2] 288–289°); TLC in solvent (2); IR ν_{max}^{KBr} cm^{-1}; 3420, 2940, 2880, 1460, 1390, 1090, 1070; ^{1}H NMR ($CDCl_3$): Table 1; FDMS *m/z*: 490 $[M]^+$; EIMS *m/z*: 472 $[M-18]^+$, 454, 440, 436, 422, 248, 230, 223, 217, 205, 199, 175.

Pentaacetate of debenzoylated aglycone (4). Debenzoylated aglycone (4, 10.0 mg) was acetylated with Ac_2O and C_5H_5N as usual. The crude product was purified by CC and crystallized (*n*-hexane–$CHCl_3$) as fine colourless needles of 4 (5 mg), mp 121–122° (lit. [3] 121–123°); TLC in solvent (3); IR ν_{max}^{KBr} cm^{-1}: 2930, 1710, 1460, 1370, 1250, 1040; ^{1}H NMR ($CDCl_3$): Table 1.

Isolation and identification of sugars. The neutralized aq. fraction of the hydrolysate was evaporated to a syrup (40 mg) which was subjected to PC whereupon glucose and arabinose were identified by comparison with authentic samples. The mixture was chromatographed over silica gel (4.0 g) and the fractions monitored by TLC in solvent (4). Arabinose (10 mg) and glucose (12 mg) were obtained in the EtOAc (satd with H_2O)–10% Me_2CO and EtOAc (satd with H_2O)–30% Me_2CO eluates respectively. Arabinose $[\alpha]_D$ + 99° (*c* 1; H_2O), glucose, $[\alpha]_D$ + 48° (*c* 1; H_2O).

Permethylation of glochidioside. Glochidioside (30 mg) was dissolved in dry DMSO (15.0 ml) in a 25 ml R.B. flask sealed with a rubber septum. N_2 was passed in the flask with the help of two injection needles and Dimsyl sodium (2 M, 7.5 ml) was added by means of a syringe. The reaction-mixture was stirred for 30 min and left at room temp. overnight. After cooling the reaction mixture, MeI (10.0 ml) was added and the resultant mixture stirred at room temp. for 1 hr. Excess MeI was evaporated under vacuum, and the reaction mixture poured into H_2O (20 ml) and extracted with $CHCl_3$ (4 × 15 ml). The combined $CHCl_3$ phase was washed with H_2O (4 × 10 ml) and evaporated to dryness. The permethylated product (31.0 mg) was chromatographed over





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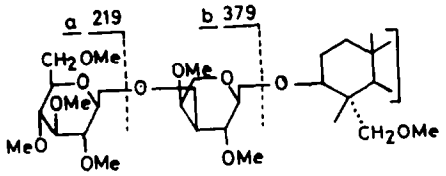
2 R = Ac, R^1 = -CO-

3 R = R^1 = H

4 R = R^1 = Ac



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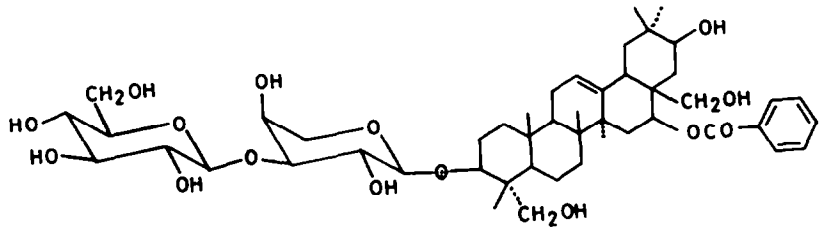

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silica gel (3 g) using $CHCl_3$–Me_2CO as the solvent system. The $CHCl_3$–Me_2CO (3:1) eluate contained pure permethylated product (10.0 mg); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2900, 1660, 1440, 1240; ^1H NMR ($CDCl_3$): δ4.13 (1H, *d*, *J* = 7 Hz, anomeric H), 4.45 (1H, *d*, *J* = 8 Hz, anomeric H); EIMS *m/z*: 862 $[M-31-31]^+$, 497 [M – sugar – 32]$^+$, 379 [*b*]$^+$, 276 [RDA fragment – 32]$^+$, 219 [*a*]$^+$, 199 $[276-32-45]^+$.

Preparation of alditol acetate from permethylated glochidioside. Permethylated glochidioside (7.0 mg) was treated with 90% aq. HCOOH (1 ml) at 100° for 1 hr. The acid was then evaporated *in vacuo* and the residue heated with 0.1 M aq. H_2SO_4 (1.5 ml) at 100° for 16 hr. The mixture was extracted with $CHCl_3$ and the aq. acidic phase was neutralized with Amberlite IR 410 (CO_3^{2-}) resin. The aq. portion was concd to 2 ml, $NaBH_4$ (50 mg) was added and stirred at room temp. for 2 hr. Amberlite IR-120 (H^+) resin was then added until the pH was 3.5. It was filtered and evaporated. H_3BO_3 formed in the reaction was removed by repeated evaporation with MeOH. The resulting alditols were treated with Ac_2O and C_5H_5N (1:1, 2 ml) at 100° for 1 hr, then evaporated and the residue containing the alditol-acetate was analysed by GC-MS. The GC showed two peaks A and B (ratio 1:1, R_t 6.5 and 7.15 min respectively). Alditol acetate A, EIMS *m/z*: 233, 201, 173, 159, 127, 117, 101, 90; alditol acetate B, EIMS *m/z*: 205, 161, 145, 129, 117, 113, 101, 87, 75, 71.

Peracetylated glochidioside. Glochidioside (20.0 mg) was acetylated by heating with Ac_2O and C_5H_5N at 100° and worked up as usual. The product, purified by CC, was obtained as an amorphous powder; IR ν_{max}^{KBr} cm^{-1}: 2970, 1730, 1370, 1240, 1080; ^1H NMR ($CDCl_3$): δ3.42 (1H, *q*, *J* = 10, 5 Hz, H-3), 4.27 (1H, *d*, *J* = 8 Hz, anomeric H), 4.63 (1H, *d*, *J* = 8 Hz, anomeric H), 4.90 (1H, *d*, *J* = 12, 5 Hz, H-21), 5.36 (1H, *s* (*br*), C=CH), 5.61 (1H, *d*, *J* = 12, 5 Hz, H-16).

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