Phytochemistry, Vol 27, No 11, pp 3575–3578, 1988
Printed in Great Britain

0031–9422/88 $3.00+0.00
Pergamon Press plc

TRITERPENOIDS FROM *GLOCHIDION HEYNEANUM**

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(*Received* 9 *March* 1988)

Key Word Index *Glochidion heyneanum*, Euphorbiaceae, triterpenoid glycosides, glochidioside N and Q

Abstract—Two new triterpene glycosides, glochidioside N and glochidioside Q were isolated from *G heyneanum*, besides nine known compounds, glochidone, glochidonol, glochidiol, lup-20(29)-en-1β,3β-diol, epimachaerinic acid, β-amyrin, stigmasterol, sitosterol-β-D-glucoside and D-mannitol.

INTRODUCTION

Earlier, we reported the isolation of glochidioside, a new triterpene glycoside, from *Glochidion heyneanum* [1] In the present communication the isolation and structure elucidation of two new triterpenoid glycosides, glochidioside N and Q are reported along with a number of known triterpenes from this plant.

RESULTS AND DISCUSSION

Repeated column chromatography of the *n*-butanol fraction yielded pure glochidioside N as an amorphous colourless solid. A positive Fiegel test revealed the presence of a carbohydrate moiety in glochidioside N The IR spectrum displayed bands for a hydroxyl group (3400, 1100, 1070 cm^{-1}) and an aromatic ester group (1710, 1700, 1690, 1280 cm^{-1}). The presence of an aromatic ester group was also substantiated by UV absorption at 230 and 273 nm

Substance N was subjected to acid hydrolysis In the hydrolysate the sugar moiety was identified as glucose by paper chromatography. The aglycone portion was purified by column chromatography and crystallized from $CHCl_3$–MeOH into colourless crystals (**1**), mp 282–284°, $[\alpha]_D+59°$ (EtOH; *c* 1) Compound **1** had the molecular formula $C_{37}H_{54}O_6$ (FDMS, $[M]^+$ at *m/z* 594). The aglycone was finally characterized as 16-*O*-benzoyl-gymnestrogenin on the basis of mixed mp, IR, NMR and MS [1].

The position of the sugar linkage to the aglycone was fixed on the basis of the ^1H NMR spectral analyses of the aglycone and peracetylated glochidioside N In the ^1H NMR spectrum of the peracetylated glochidioside N, the H-3 carbinolic proton resonated at the same frequency (δ3 56) as that of the aglycone while the other carbinolic proton signals were shifted downfield due to acetylation. Thus C-3 was established as the position of the sugar linkage to the aglycone The ^1H NMR spectrum of the peracetylated glochidioside N had a ^1H doublet at δ4 66 ($J=8$ Hz) for the anomeric proton of the D-glucopyranose The high magnitude of the *J* value suggested that the sugar had a β-linkage and was in $4C_1$ conformation. Thus, the complete structure of glochidioside N was established as 3β-[(*O*-β-D-glucopyranosyl)oxy]-16β-benzoyloxy-olean-12-ene-21β,23,28-triol [**2**]

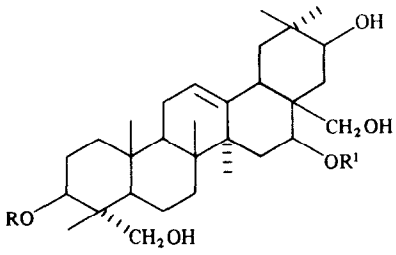
Glochidioside Q was also obtained as an amorphous solid which gave a positive Fiegel test revealing its glycosidic nature The IR spectrum displayed bands at 3400, 1130 and 1080 cm^{-1} for a hydroxy group and 1705, 1650 (C=O) and 1280 cm^{-1} for an aromatic ester group. Acid hydrolysis of substance Q furnished an aglycone and a mixture of sugars

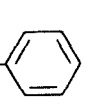
Chromatography of the aglycone portion yielded colourless needles, mp 282°, $[\alpha]_D+59°$ (EtOH) The aglycone was found to be the same as obtained from glochidioside N, viz 16-*O*-benzoyl gymnestrogenin (**1**) on the basis of mixed mp, IR, NMR and MS [1]

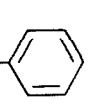
Paper chromatography of the sugar portion along with other sugars revealed the presence of glucose as the sugar moiety, which was finally characterized as D-glucose, $[\alpha]_D$ $+48°$ (H_2O; *c* 1). Again the site of linkage of the sugar to the aglycone was found to be C-3 by comparison of ^1H NMR spectra of aglycone **1** with that of peracetylated glochidioside. It was found that apart from H-3 all the other carbinolic protons were shifted downfield due to acetylation, while H-3 resonated at the same frequency (δ3.52). Therefore, in glochidioside Q the sugar moiety was located at C-3 of the aglycone

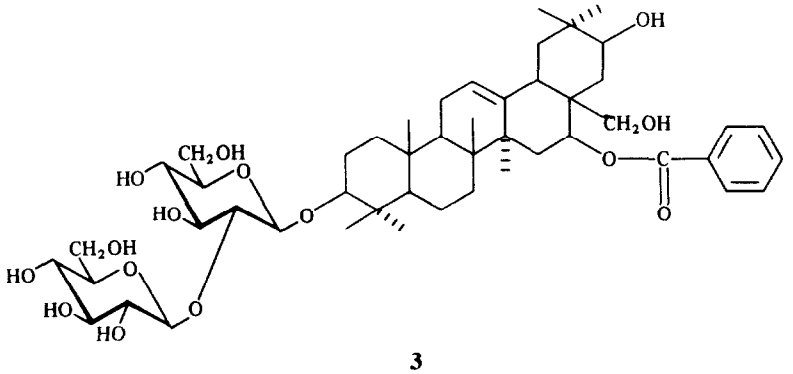
In order to determine the linkage and number of sugar units in glochidioside Q, it was subjected to permethylation by the Hakomori's method [2] The permethylated glycoside was hydrolysed with acid and the resulting partially methylated sugars were identified as 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl glucose and 1,2,5-tri-*O*-acetyl-3,4,6-tri-*O*-methyl glucose by GC/MS analyses of their alditol acetates according to Jansson *et al.* [3]. Evidently in glochidioside Q, the terminal glucopyranosyl unit was linked to the other glucopyranosyl unit through 1→2 linkage. The latter was in glycosidic linkage with the aglycone at C-3. The ^1H NMR spectrum of the peracetylated glochidioside Q had two ^1H doublets at δ4.59 ($J=8$ Hz) and 4.50 ($J=8$ Hz) for the anomeric protons of the two glucose units The high magnitude of $J_{1,2}$ values suggested that the sugars were in $4C_1$ (D) conformation and were linked together through β-linkages Thus the structure of glochidioside Q has been

*CDRI Communication No 4221



1 R = H, R^1 = 

2 R = Glc, R^1 = 



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established as 3β-[(*O*-β-D-glucopyranosyl-(1→2)-*O*-β-D-glucopyranosyl)oxy]-16β-benzoyloxy-olean-12-ene-21β,23,28 triol (**3**)

The chloroform-soluble fraction of the alcoholic extract of the plant on chromatography over silica gel yielded β-amyrin, glochidone, stigmasterol, glochidonol, glochidiol, lup-20(29)-en-1β,3β-diol and epimachaerinic acid, while repeated column chromatography of the *n*-BuOH fraction yielded sitosterol-β-D-glucoside and D-mannitol

Literature survey revealed the presence of glochidone, glochidiol, glochidonol, and lup-20(29)-en-1β,3β-diol in other species of genus *Glochidion* also This observation is significant from the view point of chemotaxonomy

EXPERIMENTAL

Mps uncorr IR spectra in KBr pellets The ^{1}H NMR spectra were recorded at 90 or 400 MHz Mass spectra were recorded with a JEOL JMS-D-300 mass spectrometer Gas liquid chromatography was conducted in a chemito 3800 gas chromatograph CC employed silica gel impregnated with 4% boric acid TLC were performed on silica gel G and Silica gel G impregnated with 2% boric acid using the following solvent systems (1) EtOAc-2% aq Me_2CO (3 2), (2) EtOAc–Me_2CO–H_2O (8 8 1), (3) $CHCl_3$–MeOH (47 3), (4) C_6H_6–MeOH (23 2), (5) C_6H_6–MeOH (24 1), (6) C_6H_6 The 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

Isolation of glochidioside N Repeated chromatography of *n*-BuOH soluble portion over silica gel impregnated with 4% boric acid using EtOAc (satd with H_2O)–MeOH (45 8) as the solvent system yielded glochidioside N (50 mg) as an amorphous powder IR ν_{max}^{KBr} cm^{-1} 3400 (OH), 2950, 1710 1700, 1690, 1280, 1100, 1070

Acid hydrolysis of glochidioside N Glochidioside N (35 mg) was refluxed with 2 N HCl in 80% aq EtOH for 3 hr After addition of H_2O followed by evapn of EtOH the hydrolysate was refluxed for a further 1 hr and then extracted with $CHCl_3$ (3 × 1 ml) The aqueous acidic phase was neutralised with Amberlite IR 410 (CO_3^{2-}) resin The organic layer was successively washed with aq $NaHCO_3$ soln and H_2O, then evapd to give a residue (20 mg) containing the aglycone

Aglycone The crude aglycone (20 mg) was purified by CC using $CHCl_3$–MeOH (47 3) eluate and crystallized from the same solvent system into colourless needles, mp 282–284 °, $[\alpha]_D$ +59° (EtOH, *c* 1), IR ν_{max}^{KBr} cm^{-1}, 3450 (OH), 2950, 1700, 1650, 1320, 1280, 1070, 730, UV λ_{max}^{MeOH} nm 230, 273, FDMS *m/z* 594 $[M]^+$, EIMS *m/z* 576 $[M-18]^+$ ^{1}H NMR (400 MHz, $CDCl_3$) 3 70 (2H, *m*, H-3, H-21), 5 00 (1H, *q*, *J* = 12 5 Hz, H-16)

Isolation and identification of sugar The neutralized aq fraction of the hydrolysate was evapd to a syrup (8 mg) which was chromatographed over silica gel (1 0 g) and the fractions monitored by TLC in solvent system (2) Pure sugar was obtained in EtOAc (satd with H_2O) + 30% Me_2CO This was identified as D-glucose, $[\alpha]_D$ 48° (H_2O, *c* 1) by paper chromatographic comparison with the authentic sample

Acetylation of glochidioside N Glochidioside N (10 mg) was refluxed at 100 with Ac_2O–pyridine (1 ml each) for 2 hr, then

evapd to dryness The residue was dissolved in $CHCl_3$ and washed with H_2O (2 × 1 ml), then evapd to dryness The residue thus obtained was purified by CC over silica gel using *n*-hexane–Me_2CO (9 1) as eluant. It did not show any OH bands in the IR spectrum ^{1}H NMR (400 MHz, $CDCl_3$) δ0.78, 0 88, 0 96, 0 99 (6H), 1 14 (3H, *s*, Me), 1 99, 2 01, 2.05, 2 09, 2 10, 2 22, 2 13, (3H each, *s*, 7 × OAc), 3 56 (1H, *q*, J = 10, 5 Hz, 3α-H), 4 66 (1H, *d*, J = 8 Hz, anomeric H), 4 87 (1H, *q*, J = 12, 6 Hz, 21α-H), 5 34 (1H, *m*, 12-H), 5 62 (1H, *q*, J = 12, 5Hz, 16α-H), 7.43 (2H, *t*, J = 8 Hz), 7 55 (1H, *t*, J = 8 Hz), 8.03 (2H, *d*, J = 8 Hz)

Glochidioside Q (**3**) Repeated CC over silica gel impregnated with 4% boric acid using EtOAc–2% Aq Me_2CO (7 3) as eluant yielded glochidioside Q as an amorphous powder (80 mg), IR ν_{max}^{KBr} cm^{-1} 3400(OH), 1130, 1080, 1705, 1650 (C=O), 1280.

Acid hydrolysis of glochidioside Q Glochidioside Q (35 mg) was refluxed with 2 N HCl in 80% aq EtOH for 3 hr, H_2O (1 5 ml) was added, EtOH was evapd and the reaction mixture was refluxed again for 1 hr The reaction mixture was worked-up in the same manner as in the case of glochidioside N

Paper chromatography of the aqueous portion (15 mg) as well as its $[\alpha]_D$ +48° (H_2O, *c* 1) at equilibrium revealed the presence of D-glucose as the sugar moiety while the aglycone, mp 282°, $[\alpha]_D$ +59° (C_2H_5OH, *c* 1) was found to be the same as obtained from glochidioside N on the basis of mmp, IR, NMR and MS

Permethylation of glochidioside Q Glochidioside Q (30 mg) was dissolved in dry DMSO (20 0 ml) in a 25 ml flask sealed with a rubber septum N_2 was passed in the flask with the help of two injection needles and dimsyl sodium (2M, 5 0 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 (8 ml) was added and the resultant mixture stirred at room temperature for 1 hr Excess MeI was evapd 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 evapd to dryness The permethylated product (21.0 mg) was chromatographed over silica gel (2 g) using *n*-C_6H_{14}–Me_2CO as the solvent system The *n*-C_6H_{14}–Me_2CO (9 1) eluate contained pure permethylated product Its IR spectrum was devoid of hydroxyl bonds

Preparation of alditol acetates Permethylated glochidioside Q (10 mg) was subjected to hydrolysis with HCO_2H at 100° for 1 hr and with 2 N H_2SO_4 at 100° for 2 hr. The hydrolysate was then 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) added and stirred at room temp After neutralization with Amberlite IR-120 (H^+) resin, H_3BO_3 formed in the reaction was removed by repeated evapn with MeOH, followed by acetylation with Ac_2O–pyridine (1 1, 2 ml) at 100° for 1 hr The resulting mixture of alditol acetates was analysed by GC/MS on a column of 3% OV-225 at 170° The GC showed two peaks, A and B (ratio 1 1 R_t 13 0 and 7 2 min)

Alditol acetate A EIMS *m/z* 189, 179, 161, 145, 101, 99, 87 for 1,2,5-tri-*O*-acetyl-3,4,6-tri-*O*-methyl glucitol

Alditol acetate B EIMS *m/z* 205, 161, 145, 129, 117, 101 for 1,5-di-*O*-acetyl 2,3,4,6-tetra-*O*-methyl glucitol

Peracetylated glochidioside Q Glochidioside Q (10 mg) was acetylated in with Ac_2O–pyridine (1 1, 2 ml) in the usual manner ^{1}H NMR (400 MHz, $CDCl_3$) δ0.78, 0 88, 0.96, 1.0, 1 0 (6H), 1 14 (3H each, *s*, Me), 1 96, 1 97, 1 99, 2 01, 2 05, 2 06, 2 09, 2 10 (3H, each *s*, 8 × CO_2Me), 3 52 (1H, *q*, J = 10, 5 Hz, 3α-H), 4.50 (1H, *d*, J = 8 Hz, anomeric H), 4 59 (1 H, *d*, J = 8 Hz, anomeric H), 4 86 (1H, *q*, J = 12, 6 Hz, 21α-H), 5 35 (1H, *m*, 12-H), 5 61 (1H, *q*, J = 12, 5 Hz, 16α-H)

Glochidone (**4**) This was crystallized from $CHCl_3$–MeOH as colourless needles, mp 163–164°, TLC in system (6), $[\alpha]_D$ + 70 2° ($CHCl_3$, *c* 1) [lit mp 164–165°, $[\alpha]_D$ + 73 41°], UV λ_{max}^{MeOH} nm 228, IR ν_{max}^{KBr} cm^{-1}, 1660 (α,β unsaturated ketone), 885 (=CH_2) It was characterized as **4** on the basis of IR, ^{1}H NMR and MS [4], ^{1}H NMR; Table 1

Glochidonol (**5**) This was obtained as an amorphous solid from the repeated CC of the $CHCl_3$ soluble portion over silica gel TLC in system (5), $[\alpha]_D$ + 46° ($CHCl_3$, *c* 1) lit. $[\alpha]_D$ + 49 4°, IR ν_{max}^{KBr} cm^{-1} 3400 (OH), 1700 (C=O), 1630 (α,β unsaturated ketone), 880 (=CH_2) cm^{-1} Its structure was established as [5]. ^{1}H NMR Table 1

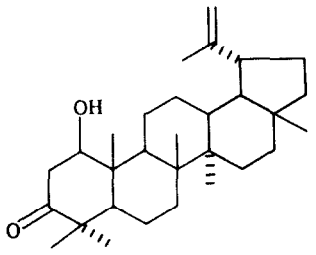
Glochidiol (**6**) This crystallized from MeOH–$CHCl_3$, mp 256° TLC in solvent (4), M^+ at *m/z* 442 Its IR, MS and ^{1}H NMR data were in agreement with those of **6** [4–6] ^{1}H NMR Table 1

Lup-20(29)-en-1β,3β-diol (**7**) It was crystallized from $CHCl_3$–MeOH, mp 230-232° literature mp 235° TLC in solvent system (4) M^+ at *m/z* 442 IR ν_{max}^{KBr} cm^{-1} 3360 (OH), 2970, 1630, 1450, 1380, 1050, 890 (=CH_2) It was characterized by comparing the ^{1}H NMR spectral data of its dihydrodiacetate with that reported in the lit [7] ^{1}H NMR Table 1

Epimacherinic acid (**8**) This was crystallized from EtOH as colourless needles, mp 258–260, IR ν_{max}^{KBr} cm^{-1}, 3490 (OH), 2999

Table 1 ^{1}H NMR chemical shifts of compounds **4, 5, 6** and **7** in $CDCl_3$ (J values are given in parentheses)

	4	**5**	**6**	**7**
Quaternary methyls	0.76, 0 90 (6H), 1.03, 1 06, 1 43, *s*	0 78, 0 84, 0 98 (6H), 1 04, 1 06, *s*	0 78, 0 82, 0 90 (6H), 0 97, 1 04, *s*	0 70, 0 73, 0 85 0 90 (6H), 0 99
Vinylic methyl	1 60, 3H, *s*	1 68, 3H, *s*	1 67, 3H, *s*	1 62
–C=CH_2	4 50, 1H, 1H, *br s* 4 60, 1H, *br s*	4 56, 1H, *br s* 4 69, 1H, *br s*	4 51, 1H, *br s* 4 61, 1H, *br s*	4 46, 1H, *br s* 4 56, 1H, *br s*
CHOH		3 91, 1H, *q* (8, 4)	3 48, 1H, *m* 3 78, 1H, *q* (12, 6)	3 18, 1H, *q* (12, 6) 3.35, 1H, *q* (10, 4)
CH_2–C=O	5 73, 1H, *d*, (10) 7.02, 1H, *d*, (10)	2 22, 1H, *q* (14, 4) 3 01, 1H, *q* (14, 8)		



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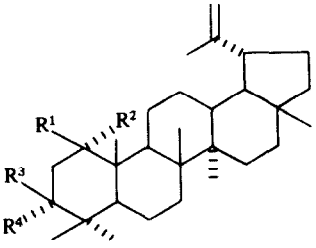

6 R^1 = OH, R^2 = H, R^3 = H, R^4 = OH
7 R^1 = OH, R^2 = H, R^3 = OH, R^4 = H

(Me), 1700 (C=O), 1460, 1390, 1060, 833, ^{1}H NMR (90 MHz, $CDCl_3$ + DMSO-d_6 (2 drops) δ0 70, 0 72, 0 82 (6H), 0 92 (6H), 1 06 (3H each, *s*, 7 × Me), 2 55 (1H, *d*, J = 2 5 Hz, H-18), 3 02 (1H, *q*, J = 12, 6, CHOH), 3 35 (1H, *q*, J = 9, 4 5 Hz, CHOH), 5 20 (1H, *m*, H-12) It was characterized as epimachaerinic acid [8] by comparison with the data of the diacetate methyl ester reported in the literature

β-Amyrin, stigmasterol, sitosterol-β-D-glucoside and D-mannitol were indentified on the basis of mmp, superimposable IR spectrum and co-TLC with the respective authentic samples

Acknowledgements—The authors thank the staff of the Regional Sophisticated Instruments Centre, CDRI for spectral and analytical data, and Dr B N Mehrotra for identifying and supplying the plant and Mr J P Chaturvedi for technical assistance Thanks are also due to the Division of Cancer Treatment, National Cancer Institute, Bethesda, U S A for anticancer screening and for a Senior Research fellowship to one of us (R S)

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