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A FUROSTANOL GLYCOSIDE FROM RHIZOMES OF *DIOSCOREA COLLETTII* VAR. *HYPOGLAUCA*

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Key Word Index—*Dioscorea collettii* var. *hypoglauca*; Dioscoreaceae; furostanol glycoside; hypoglaucin F.

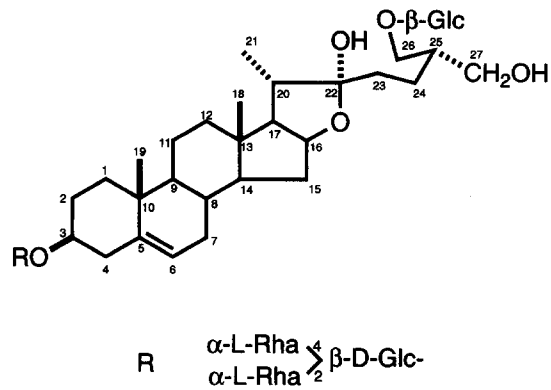
Abstract—A new furostanol saponin, named hypoglaucin F, was isolated from the rhizomes of *Dioscorea collettii* var. *hypoglauca*, along with seven known compounds. On the basis of detailed chemical and spectroscopic evidence, the structure of hypoglaucin F was determined as (25*S*)-26-*O*-β-D-glucopyranosyl 22-hydroxy-5-en-furostane-3β, 26, 27-triol 3-*O*-α-L-rhamnopyranosyl-(1 → 2)-[α-L-rhamnopyranosyl-(1 → 4)]-β-D-glucopyranoside.

INTRODUCTION

The rhizomes of *Dioscorea collettii* Hook. f. var *hyplauca* Palibin Pei et Ting are used as a traditional Chinese medicine 'Fenbei Bixie' for the treatment of cervical carcinoma, carcinoma of the bladder and renal tumour. It is widely distributed in southeast China and was included in the Pharmacopoeia of the People's Republic of China (1990). Tang and co-workers reported the isolation of four steroidal saponins [1, 2]. Ten steroidal sapogenins, such as diosgenin, yamogenin and isonarthogenin, have been isolated from the acid-treated rhizomes of the plant [3]. This paper reports the isolation of one new furostanol glycoside, named hypoglaucin F, together with seven known steroidal saponins, from the rhizomes of this plant.

RESULTS AND DISCUSSION

The crude saponin fraction of *D. collettii* var. *hypoglauca* was fractionated by a combination of silica gel chromatography and HPLC on silica gel RP-18 to afford compounds **1**–**12**. Compounds **1**–**11** were identified as the known prosapogenin A of dioscin (**1**) [4], dioscin (**2**) [5], gracillin (**3**) [6], protoneodioscin (**4**) [7], protodioscin (**5**) [8], protoneogracillin (**6**) [9], protogracillin (**7**) [8], methyl protoneodioscin (**8**) [8],



methyl protodioscin (**9**) [8], methyl protoneogracillin (**10**) [10] and methyl protogracillin (**11**) [8], respectively, based on their spectral data and by a comparison of their physical properties with those reported in the literature for these saponins. It seems that the 22-hydroxy compounds **4**–**7** existed in nature and that they were methylated to form the corresponding 22-methoxyfurostanol glycosides (**8**–**11**, respectively) when treated with methanol during the process of isolation [11].

Hypoglaucin F (**12**) was obtained as a white amorphous powder and showed a purple coloration with Ehrlich reagent. On acid hydrolysis, GC analysis of the pertrimethylsilylated sugars in the hydrolysate of **12** showed rhamnose and glucose to be present in a ratio of 1:1. The aglycone of **12** was identified as isonarthogenin (25*S*) with the authentic sample. On enzymic hydrolysis with β-glucosidase, compound **12**

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gave glucose and the corresponding spirostanol saponin **12a**, which was identified as isonarthogenin 3-*O*-α-L-rhamnopyranosyl-(1 → 2)-[α-L-rhamnopyranosyl-(1 → 4)]-β-D-glucopyranoside [12].

The locations of the glycosidic linkages were elucidated by analysis of the two-dimensional NMR spectra, especially the HMBC spectrum. Complete assignments of the ^1H and ^{13}C NMR signals of four sugars and the aglycone were achieved with the aid of the ^1H–^1H COSY and HMQC spectra (Table 1).

In Table 1, the ^1H and ^{13}C NMR spectral data of **12** are listed for comparison with those of **5**. The ^1H NMR spectrum of **12** showed C-18, C-19 and C-21 methyl groups at δ 0.81 (*s*), 1.04 (*s*) and 1.09 (*d*), respectively. However, the C-27 methyl doublet observed in the spectrum of **5** at δ 0.98 (*d*) was absent in that of **12**, and was replaced by a two-proton signal at δ 3.71 and 4.01, which revealed a typical CH_2OH group next to a chiral centre. The substituent hydroxyl group attached to C-27 led to the chemical shift downfield at C-25 (β-C) and upfield at C-24 and C-26 (γ-C) in the ^{13}C NMR spectrum of **12** when compared with the signals in the spectrum of **5**. Signals for four anomeric protons at δ 6.34 (*d*, J = 0.9 Hz), 5.80 (*d*, J = 0.9 Hz), 4.92 (*d*, J = 7.2 Hz) and 4.74 (*d*, J = 7.8 Hz) and an olefinic proton at δ 5.32 (*br d*) were also observed in the ^1H NMR spectrum of **12**, which were the same as those of **5**. Similarly, the ^{13}C NMR spectral data for the four sugars in **12** were also in agreement with those for **5**. Furthermore, in the HMBC spectrum of **12**, the anomeric proton signals at δ 6.34 (H-1″ of the terminal rhamnose attached to C-2′ of the inner glucose), 5.80 (H-1‴ of the terminal rhamnose attached to C-4′ of the inner glucose), 4.92 (H-1′ of the 2′, 4′-substituted inner glucose attached to C-3 of the aglycone) and 4.74 (H-1⁗ of the terminal glucose attached to C-26 of the aglycone) showed cross-peaks with the carbon signals at δ 78.0 (C-2′ of the 2′, 4′-substituted inner glucose), 78.9 (C-4′ of the 2′, 4′-substituted inner glucose), 78.2 (C-3 of the aglycone) and 72.0 (C-26 of the aglycone). These signals were sufficient to determine the linkages by which the sugars were connected. Based on all the data mentioned above, **12** was determined to be (25*S*)-26-*O*-β-D-glucopyranosyl 22-hydroxy-5-en-furostane-3β, 26, 27-triol 3-*O*-α-L-rhamnopyranosyl-(1 → 2)-[α-L-rhamnopyranosyl-(1 → 4)]-β-D-glucopyranoside.

EXPERIMENTAL

General. Mps: Yanaco MP-S_3 micro-melting point apparatus, uncorr. Optical rotations: Perkin-Elmer 241 polarimeter at 13°. IR: Jasco A-102 (KBr). ^1H and ^{13}C NMR: JNM Alpha-500 (^1H 500 MHz, ^{13}C 125 MHz) spectrometer with TMS as int. standard. Positive-ion FAB-MS: JEOL JMS-DX302. GC: HP-5890 Series II. Prep. HPLC: Liquid Chromatograph LC-10 (Japan Analytical Industry Co.) using an ODS column (Waters, 20 × 250 mm, 5 μm) with flow rate of mobile phase 3.0 ml min^{-1}. CC: silica gel H (10–40 μm, Qindao Haiyang Chemical Factory). TLC and VLC: silica gel G (10–40 μm, Qindao Haiyang Chemical Factory); spots were visualized by spraying with 10% H_2SO_4 followed by heating.

Plant material. Rhizomes of *D. collettii* var. *hypoglauca* were collected in 1994 from Zhejiang Province (China) and were identified by Professor Zherong Jiang (Division of Pharmacognosy, Shenyang Pharmaceutical University). A voucher specimen is deposited at the Herbarium of Shenyang Pharmaceutical University (No. 104), Liaoning Province.

Extraction and isolation. Air-dried powdered rhizomes (1500 g) of *D. collettii* var. *hypoglauca* were refluxed with 75% EtOH (15 l). The combined EtOH solns were concd *in vacuo* to give 133.5 g extract. A suspension of the resulting extract in H_2O was partitioned successively with $CHCl_3$, EtOAc and *n*-BuOH to afford 4 frs, i.e. DC (4.5 g), DE (4.2 g), DB (13.5 g) and DH (102.1 g) residues.

Fr. DC (4.5 g) was subjected to VLC [13] on silica gel G (250 g) and eluted stepwise with $CHCl_3$ (2700 ml), $CHCl_3$–EtOAc (19:1, 1820 ml; 9:1, 900 ml; 17:3, 1920 ml; 7:3, 1450 ml; 13:7, 2120 ml; 2:3; 1800 ml), EtOAc (1 l) and Me_2CO (1 l) to afford 9 frs (DC-1–DC-9). Fr. DC-9 (115 mg) was further chromatographed on silica gel H (30 g) with $CHCl_3$–MeOH (6:1) as eluent to give **1** (18.2 mg) and **2** (20.3 mg).

Fr. DE (4.2 g) was also subjected to CC on silica gel H (330 g) and was eluted with $CHCl_3$–MeOH (5:1) to afford **3** (46.8 mg).

Fr. DB (13.5 g) was subjected to CC on silica gel H (300 g) and eluted stepwise by $CHCl_3$–MeOH–H_2O (80:5:0.1, 80:20:1, 80:25:1 and 14:6:1; each elution vol. 3 l) to give 4 corresponding frs (DB-1–DB-4). Fr. DB-4 (4718 mg) was further sepd by prep. HPLC (column, ODS, 5 μm, 20 × 250 mm; solvent, 50% aq. MeOH; flow rate, 3.0 ml min^{-1}) to give methyl **8** (366 mg), **9** (2042 mg), **10** (76.7 mg), **11** (441 mg) and **12** (22.1 mg). Compounds **8**–**11** were refluxed with 50% aq. Me_2CO at 90° for 24 hr to give **4** (340 mg), **5** (1810 mg), **6** (60.6 mg) and **7** (394 mg), respectively. Compounds **4**–**7** were easily converted into the corresponding compounds **8**–**11** when refluxed with dry MeOH at 95° for 36 hr. This suggested that **4**–**7** were 22-hydroxyfurostanol saponins, whereas **8**–**11** were their corresponding artifacts, i.e. 22-methoxyl derivatives [14].

Hypoglaucin F (**12**). Amorphous powder, mp > 300° (dec.); $[\alpha]_D$ −94.3° (pyridine; *c* 0.01). IR υ_{max} cm^{-1}: 3400 (OH), 1000–1100 (glycosyl C–O). Anal. calc. for $C_{51}H_{84}O_{23}$. $2H_2O$: C, 55.64; H, 8.00; found, C, 55.54; H, 8.05%. FAB-MS (positive) *m/z*: 1047 $[M+H-H_2O]^+$, 885 $[M+H-H_2O-Glc]^+$, 739 $[M+H-H_2O-Glc-Rha]^+$, 721 $[M+H-H_2O\times2-Glc-Rha]^+$, 575 $[M+H-H_2O\times2-Glc-Rha\times2]^+$, 413 $[M+H-H_2O\times2-Glc\times2-Rha\times2]^+$. ^1H NMR: δ 0.81 (3H, *s*, 18-H_3), 1.04 (3H, *s*, 19-H_3), 1.09 (3H, *d*, J = 6.5 Hz, 21-H_3), 1.60 (3H, *d*, J = 6.5 Hz, Rha 6‴-H_3), 1.74 (3H, *d*, J = 6.5

Table 1. ^{1}H and ^{13}C NMR data* for compounds **12** and **5** in pyridine-d_5 (δ values)†

Position	12		5	
	H	C	H	C
1	0.98, 1.74 (o)‡	37.5	0.96, 1.74 (o)	37.5
2	1.86, 2.07 (o)	30.2	1.82, 2.07 (o)	30.2
3	3.86 (*m*)	78.2	3.86 (*m*)	78.2
4	2.70, 2.79 (*m*)	39.0	2.71, 2.78 (*m*)	39.0
5		140.9		140.9
6	5.32 (*br d*)	121.8	5.31 (*br d*)	121.8
7	1.50, 1.87 (o)	32.3	1.48, 1.87 (o)	32.4
8	1.55 (o)	31.7	1.56 (o)	31.7
9	0.89 (o)	50.4	0.88 (o)	50.4
10		37.2		37.2
11	1.41 (o)	21.1	1.45 (o)	21.1
12	1.06, 1.66 (o)	39.9	1.10, 1.74 (o)	40.0
13		40.5		40.8
14	1.07 (o)	56.7	1.07 (o)	56.6
15	1.43, 2.02 (o)	32.2	1.44, 2.02 (o)	32.5
16	4.49 (o)	81.5	4.92 (o)	81.1
17	1.76 (o)	62.9	1.92 (o)	63.9
18	0.81 (*s*)	16.3	0.89 (*s*)	16.5
19	1.04 (*s*)	19.4	1.04 (*s*)	19.4
20	1.92 (o)	42.0	2.23 (o)	40.7
21	1.09 (*d*, 6.9)§	14.9	1.32 (*d*, 6.5)	16.5
22		109.5		110.7
23	1.65 (o)	31.3	2.01, 2.03 (o)	37.2
24	1.65 (o)	24.0	1.66, 2.03 (o)	28.3
25	2.04 (o)	36.7	1.91 (o)	34.3
26	3.45, 3.92 (o)	72.0	3.62, 3.92 (o)	75.2
27	3.71, 4.01 (o)	63.7	0.98 (*d*, 6.8)	17.5
C-3 sugar part				
Glc 1′	4.92 (*d*, 7.2)	100.3	4.92 (*d*, 7.2)	100.3
(inner) 2′	4.19 (o)	78.0	4.19 (o)	77.9
3′	4.19 (o)	77.9	4.19 (o)	78.0
4′	4.34 (o)	78.9	4.34 (o)	78.8
5′	3.63 (*m*)	76.9	3.62 (*m*)	76.9
6′	4.07, 4.19 (o)	61.4	4.07, 4.19 (o)	61.4
Rha 1″	6.34 (*d*, 0.9)	102.0	6.34 (*d*, 0.9)	102.0
(1 → 2) 2″	4.79 (*dd*, 0.9, 3.5)	72.5	4.80 (*dd*, 0.9, 3.5)	72.5
3″	4.59 (*dd*, 3.5, 9.0)	72.8	4.59 (*dd*, 3.5, 9.0)	72.8
4″	4.31 (o)	74.2	4.32 (o)	74.2
5″	4.92 (o)	69.5	4.92 (o)	69.5
6″	1.74 (*d*, 6.5)	18.6	1.74 (*d*, 6.5)	18.5
Rha 1‴	5.80 (*d*, 0.9)	103.0	5.80 (*d*, 0.9)	102.9
(1 → 2) 2‴	4.64 (*dd*, 0.9, 3.5)	72.5	4.64 (*dd*, 0.9, 3.5)	72.5
3‴	4.50 (o)	72.7	4.50 (o)	72.7
4‴	4.29 (o)	73.9	4.29 (o)	73.9
5‴	4.85 (o)	70.5	4.86 (o)	70.4
6‴	1.60 (*d*, 6.5)	18.5	1.60 (*d*, 6.5)	18.6
C-26 sugar part				
Glc 1⁗	4.74 (*d*, 7.8)	105.0	4.75 (*d*, 7.8)	104.9
2⁗	4.00 (o)	75.2	3.99 (o)	75.2
3⁗	4.19 (o)	78.6	4.19 (o)	78.4
4⁗	4.20 (o)	71.8	4.20 (o)	71.8
5⁗	3.92 (o)	78.6	3.91 (o)	78.6
6⁗	4.37, 4.54 (o)	62.9	4.34, 4.50 (o)	62.9

*Recorded on a JNM Alpha-500 (^{1}H 500 MHz, ^{13}C 125 MHz) spectrometer in C_5D_5N.
†All of the signals were assigned by ^{1}H–^{1}H COSY, HMQC and HMBC spectra.
‡Overlapped signals are indicated by '(o)'.
§*J* values (in parentheses) are reported in Hz.

Hz, Rha 6″-H_3), 4.74 (1H, *d*, J = 7.8 Hz, Glc-1‴), 4.92 (1H, *d*, J = 7.2 Hz, Glc-1′), 5.32 (1H, *br d*, 6-H), 5.80 (1H, *d*, J = 0.9 Hz, Rha-1‴), 6.34 (1H, *d*, J = 0.9 Hz, Rha-1″). ^{13}C NMR: Table 1.

Acid hydrolysis of **12**. Compound **12** (a few mg) was heated with 2 N HCl–dioxane (1:1, 2 ml) in a sealed tube at 100° for 4 hr. The aglycone was identified as isonarthogenin when compared with the corresponding authentic sample. The reaction mixt. was concd to dryness under N_2 at room temp. For GC analysis, the residue was trimethylsilylated with hexamethyldisilazane–trimethylchlorosilane (2:1) [15] at room temp. GC: SE30 capillary column (12 m × 0.22 mm i.d.); detector: FID (270°); column temp. 170–210°, rate 5° min^{-1}; carrier gas: N_2 (30 ml min^{-1}). R_t: rhamnose (3.72 min) and glucose (7.12 min).

Enzymic hydrolysis of **12**. An acetate buffer soln (pH 4.2, 5 ml) of **12** (15 mg) and β-glucosidase (12 mg) was incubated at 37° for 24 hr [16]. After dilution with H_2O, the reaction mixt. was applied to a column of ODS 1220T (12 g) and eluted with H_2O and then with MeOH–H_2O (7:3). The eluate of MeOH–H_2O (7:3) gave isonarthogenin 3-*O*-α-L-rhamnopyranosyl-(1 → 2)-[α-L-rhamnopyranosyl-(1 → 4)]-β-D-glucopyranoside.

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