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STEROIDAL SAPONINS FROM *DIURANTHERA MAJOR*

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Key Word Index—*Diuranthera major*; Anthericaceae; roots; steroidal saponins; neohecogenin; neotigogenin; diuranthoside A, B, C; chloromaloside A.

Abstract—In addition to chloromaloside A, three new steroidal saponins, diuranthosides A–C, were isolated from the fresh roots of *Diuranthera major*. On the basis of chemical and spectroscopic analysis, the structures of diuranthosides A–C were established as neotigogenin 3-*O*-*β*-D-glucopyranosyl(1→2)-[*β*-D-xylopyranosyl(1→3)]-*β*-D-glucopyranosyl (1→4)-*β*-D-galactopyranoside, neohecogenin 3-*O*-*β*-D-glucopyranosyl(1→3)-*β*-D-xylopyranosyl(1→3)-[*β*-D-glucopyranosyl(1→2)]-*β*-D-glucopyranosyl(1→4)-*β*-D-galactopyranoside and neohecogenin 3-*O*-*β*-D-glucopyranosyl (1→3)-*β*-D-glucopyranosyl(1→2)-[*β*-D-glucopyranosyl(1→3)-*β*-D-xylopyranosyl(1→3)]-*β*-D-glucopyranosyl(1→4)-*β*-D-galactopyranoside, respectively.

INTRODUCTION

Diuranthera (Anthericaceae), a monotype genus which is endemic in the southwest of China, is recognized to be morphologically very close to the genus *Chlorophytum*. We have recently studied *C. malayense* Ridley, from which four new steroidal saponins were isolated [1]. In continuation of our chemical studies on Liliiflorae plants, we have investigated *D. major* Hemsl. which is a traditional Chinese medicine used in the treatment of wound bleeding and injuries from falls, fractures, contusions and strains [2]. This paper deals with the isolation and structure elucidation of three new steroidal saponins, diuranthosides A–C, together with chloromaloside A, from the fresh roots of *D. major*.

RESULTS AND DISCUSSION

The crude saponins were subjected to column chromatography on silica gel and reversed phase highly porous polymer, MCI gel CHP20P, yielding saponins **1–4**, all of which were predicted to be spirostanol glycosides by the usual colour test and IR spectra [3].

Saponin **1** was proved to be identical with chloromaloside A which was obtained from *C. malayense* [1] by direct comparison of TLC behaviour, mp, $[\alpha]_D$, ^1H and ^{13}C NMR spectra with an authentic sample.

Saponin **2** was hydrolysed with mineral acid to yield D-galactose, D-glucose and D-xylose as sugar components in a ratio of 1:2:1 and an aglycone. This aglycone was determined as neotigogenin (**5**) by direct comparison of TLC behaviour, mp, IR spectrum with an authentic sample [1]. The FAB mass spectrum of **2** showed molecular ion peaks at *m/z*1057 $[M+Na]^+$ and 1041 $[M+Li]^+$, suggesting a M_r of 1034 ($C_{50}H_{82}O_{22}$). The fragment ion peak at *m/z* 925 $[1057-132$ (pentose)$]^+$ indicated the presence of a terminal xylose unit. In the EI mass spectrum of acetylated **2**, the fragment ion peaks at *m/z* 331 $[\text{hexose}(OAc)_4]^+$ and 259 $[\text{pentose}(OAc)_3]^+$ were observed. The ^{13}C NMR spectrum of **2** revealed that it was a 3-*O*-glycoside of neotigogenin [4, 5]. On comparison of ^1H and ^{13}C NMR spectra of **2** with those of **1**, the signals due to sugar moieties were fully superimposable. The only difference being their aglycones, saponin **2** and **1** showed one spot on HPTLC plate, while on reversed-phase HPTLC plate, they showed two spots. Thus, the structure of **2** was established to be neotigogenin 3-*O*-*β*-D-glucopyranosyl(1→2)-[*β*-D-xylopyranosyl(1→3)]-*β*-D-glucopyranosyl (1→4)-*β*-D-galactopyranoside, and was named diuranthoside A.

Saponin **3** gave neohecogenin (**6**) as aglycone and D-galactose, D-glucose and D-xylose as sugar residues in a ratio of 1:3:1 on acid hydrolysis. The molecular formula ($C_{56}H_{90}O_{28}$) was deduced from the peaks at *m/z* 1233 $[M+Na]^+$ and 1217 $[M+Li]^+$ in the FAB mass spectrum. The sugar sequence of **3** was established as follows. The EI mass spectrum of acetylated **3** exhibited a fragment ion peak at *m/z* 331 $[\text{hexose}(OAc)_4]^+$ with a strong relative intensity, indicating the presence of at least one terminal hexose unit. It has been noticed that there was no fragment ion corresponding to a terminal pentose (D-xylose) (*m/z*: 259), suggesting that the xylose should locate in an inner position in the sugar chain. On comparison of the whole ^{13}C NMR spectrum of **3** with that of **1**, a set of additional signals, corresponding to a *β*-D-glucopyranosyl unit appeared, and the signals due to D-xylose moiety varied, while all the other signals remained almost unaffected. It was observed that the signal of C-3 of D-xylose was markedly displaced downfield at *δ*86.7 and the remaining carbon signals were shifted upfield to various degrees (Table 1). Thus, C-3 of the D-xylose was the glycosylated position to which the additional D-glucose was linked. Based on the above evidence, **3** was considered to be neohecogenin 3-*O*-*β*-D-glucopyranosyl(1→3)-*β*-D-xylopyranosyl(1→3)-[*β*-D-glucopyranosyl(1→2)-*β*-D-glucopyranosyl(1→4)-*β*-D-galactopyranoside, which was named diuranthoside B.

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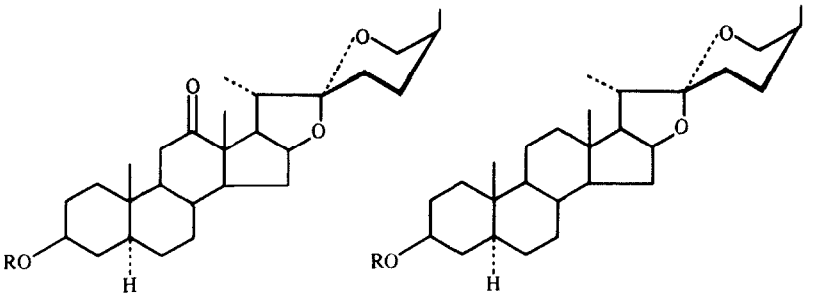

1 R = —Gal(4)—Glc(2)—Glc; Glc(3)—Xyl

2 R = —Gal(4)—Glc(2)—Glc; Glc(3)—Xyl

3 R = —Gal(4)—Glc(2)—Glc; Glc(3)—Xyl(3)—Glc

4 R = —Gal(4)—Glc(2)—Glc(3)—Glc; Glc(3)—Xyl(3)—Glc

5 R = H

6 R = H

Saponin **4** afforded D-galactose, D-glucose and D-xylose in a ratio of 1:4:1 on acid hydrolysis on TLC [6]. On comparison of ^{13}C NMR spectrum with **3**, **4** only showed a set of additional signals of a terminal β-D-glucopyranosyl unit which was deduced to be attached to the hydroxy group at C-3 (δ87.9) of a terminal D-glucose of **3**. In the FAB mass spectrum of **4**, besides molecular ion peaks at *m/z* 1395 $[M(C_{62}H_{100}O_{33})+Na]^+$ and 1379 $[M+Li]^+$, the fragment ion peak at *m/z* 1101 $[M+Na-(xylose+glucose)]^+$ was present. This suggested that the additional glucose should be linked to the glucose which was attached to C-2 of the inner glucose. Consequently, the structure of **4** was established as neohecogenin 3-*O*-β-D-glucopyranosyl(1→3)-β-D-glucopyranosyl-(1→2)-[β-D-glucopyranosyl(1→3)-β-D-xylopyranosyl-(1→3)]-β-D-glucopyranosyl(1→4)-β-D-galactopyranoside, and was named diuranthoside C.

Of particular significance is the close resemblance of the steroidal saponins from *D. major* and *C. malayense* [1]. This could provide some evidence regarding their chemotaxonomy.

EXPERIMENTAL

Mps: uncorr. NMR spectra were measured in pyridine-d_5 and were recorded at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR (DEPT) using TMS as int. standard. EIMS were measured at 20 eV accelerating voltage.

Plant material. The roots of *D. major* Hemsl. were collected in the Botanical Garden of Kunming Institute of Botany, Chinese Academy of Sciences, and identified by Prof. H. Li and Mr Y. P. Yang.

Extraction and isolation. The fresh roots (1.25 kg) were extracted with hot MeOH. The combined extracts were concd *in vacuo*. The residue (67 g) was suspended in H_2O, defatted with petrol and then extracted with *n*-BuOH. The *n*-BuOH fraction (5.4 g) was subjected to CC on silica gel eluting with $CHCl_3$–MeOH–H_2O (50:10:1 to 10:10:1) to afford saponin **3** (50 mg), **4** (40 mg) and a mixture (226 mg) which showed one spot on HPTLC plate. The mixture was further chromatographed on a MCI gel CHP20P column, and elution with 70% aq. EtOH gave **1** (70 mg) and **2** (45 mg).

Saponin **1**. Needles from MeOH, mp 280–283°, $[\alpha]_D^{12}$ −65.8° (pyridine; *c* 0.038); IR ν_{max}^{KBr} cm^{-1}: 3400 (br), 1710 (C=O), 988, 920, 900, 855 (intensity 920 > 900, 25(S) spirostanol); ^{1}H NMR: δ0.643 (3H, *s*, 19-Me), 1.069 (3H, *d*, 27-Me), 1.080 (3H, *s*, 18-Me), 1.368 (3H, *d*, *J* = 6.5 Hz, 21-Me), 4.876 (1H, *d*, *J* = 7.2 Hz, Gal H-1), 5.195 (1H, *d*, *J* = 8.0 Hz, Glc H-1), 5.240 (1H, *d*, *J* = 8.0 Hz, Glc, H-1), 5.576 (1H, *d*, *J* = 6.7 Hz, Xyl H-1).

Saponin **2**. Needles from MeOH, mp 289–293°, $[\alpha]_D^{21}$ −71.4° (pyridine; *c* 0.518); IR ν_{max}^{KBr} cm^{-1}: 3400 (br), 986, 920, 890, 850 (intensity 920 > 890, 25(S) spirostanol); FABMS *m/z*: 1057 $[M+Na]^+$, 1041 $[M+Li]^+$, 925 $[1057-132$ (pentose)$]^+$; EIMS (acetate) *m/z*: 259 [terminal pentose $(OAc)_3]^+$, 331 [terminal hexose$(OAc)_4]^+$; ^{1}H NMR: δ0.630 (3H, *s*, 18-Me), 0.817 (3H, *s*, 19-Me), 1.082 (3H, *d*, *J* = 6.8 Hz, 27-Me), 1.155 (3H, *d*, *J* = 6.8 Hz, 21-Me), 4.902 (1H, *d*, *J* = 7.6 Hz, Gal H-1), 5.212 (1H, *d*, *J* = 7.8 Hz, Glc H-1), 5.256 (1H, *d*, *J* = 7.7 Hz, Glc, H-1), 5.590 (1H, *d*, *J* = 6.9 Hz, Xyl H-1).

Saponin **3**. Powder, mp 210–213°, $[\alpha]_D^{12}$ −36.7° (pyridine; *c* 0.03); IR ν_{max}^{KBr} cm^{-1}: 3500 (br), 1700 (C=O), 985, 920, 890, 850 (intensity 920 > 890, 25(*S*) spirostanol); FABMS *m/z*: 1233 $[M+Na]^+$, 1217 $[M+Li]^+$; EIMS (acetate) *m/z*: 331 [terminal hexose$(OAc)_4]^+$; ^{1}H NMR: δ0.661 (3H, *s*, 19-Me), 1.026 (3H, *s*, 18-Me), 1.065 (3H, *d*, 27-Me), 1.338 (3H, *d*, *J* = 6.8 Hz, 21-Me), 4.810 (1H, *d*, *J* = 7.4 Hz, Gal H-1), 5.095 (1H, *d*, *J* = 7.6 Hz, Glc′ H-1), 5.109 (1H, *d*, *J* = 7.5 Hz, Glc′ H-1), 5.192 (1H, *d*, *J* = 7.5 Hz, Glc″ H-1), 5.451 (1H, *d*, *J* = 7.2 Hz, Xyl H-1).

Saponin **4**. A pale yellow powder, $[\alpha]_D^{28}$ −29.0° (pyridine; *c* 0.031); FABMS *m/z*: 1395 $[M+Na]^+$, 1379 $[M+Li]^+$, 1101

Table 1. ^{13}C NMR chemical shifts of sugar moieties (in pyridine-d_5, ppm)

		1	2	3	4
Gal	1	102.5	102.5	102.5	102.5
	2	73.1	73.2	73.1	73.1
	3	75.6[a]	75.7[a]	75.6[a]	75.5[a]
	4	79.8	79.9	79.8	79.8
	5	76.1[a]	76.2[a]	76.0[a]	76.0[a]
	6	60.7	60.7	60.7	60.7
Glc (inner)	1	104.9[b]	105.0[b]	104.9[b]	104.9[b]
	2	81.3	81.3	81.1	81.1
	3	87.0	87.0	86.9	87.0
	4	70.5[c]	70.5[c]	70.4	70.4
	5	77.6[d]	77.6[d]	77.5[c]	77.5[c]
	6	62.5	62.6	62.5[d]	62.5[d]
Glc	1	105.0[b]	105.1[b]	104.7[b]	104.3[b]
	2	75.4	75.4	75.3[e]	73.8
	3	78.6	78.7	78.5[f]	87.9
	4	71.1	71.2	71.1	69.8
	5	77.8[d]	77.9[d]	77.9[c]	77.6[c]
	6	63.0	63.0	63.0[d]	62.9[d]
Xyl	1	104.7	104.8	104.5	104.5
	2	75.1	75.1	73.7	73.7
	3	78.6	78.7	86.7	86.8
	4	70.7[c]	70.8[c]	69.2	69.2
	5	67.3	67.3	66.4	66.3
Glc (terminal)	1			104.3[b]	104.3[b]
	2			74.9[e]	75.4[e]
	3			78.4[f]	78.5
	4			71.5	71.7[f]
	5			77.5[c]	77.9[g]
	6			62.4[d]	62.5[d]
Glc (terminal)	1				105.5[b]
	2				75.5[e]
	3				78.5
	4				71.1[f]
	5				78.1[g]
	6				62.4[d]

[a–g] Signals may be interchangeable within each column.

Table 2. ^{13}C NMR chemical shifts of aglycone moieties (in pyridine-d_5, ppm)

C	1	2	3	4
1	36.7	37.3	36.7	36.7
2	29.7	30.0	29.6	29.6
3	77.3	77.6	77.3	77.3
4	34.7	34.9	34.6	34.7
5	44.5	44.8	44.5	44.5
6	28.6	29.0	28.6	28.6
7	31.4	32.5	31.4	31.7
8	34.4	35.3	34.4	34.4
9	55.6	54.5	55.6	55.6
10	36.3	35.9	36.3	36.3
11	38.0	21.3	38.0	38.0
12	212.7	40.2	212.7	212.8
13	55.4	40.8	55.3	55.4
14	56.0	56.5	55.9	56.0
15	31.7	32.1	31.7	32.1
16	79.8	81.3	79.7	79.8
17	54.2	62.9	54.1	54.2
18	16.3	16.6	16.3	16.3
19	11.8	12.4	11.7	11.8
20	43.1	42.5	43.1	43.1
21	13.7	14.8	13.7	13.7
22	109.8	109.7	109.7	109.8
23	26.4	26.4	26.4	26.4
24	26.2	26.2	26.1	26.1
25	27.5	27.6	27.5	27.5
26	65.2	65.2	65.2	65.2
27	16.1	16.3	16.1	16.1

$[M+Na-Xyl-Glc]^+$, ^{1}H NMR: δ0.654 (3H, *s*, 19-Me), 1.070 (3H, *d*, 27-Me), 1.082 (3H, *s*, 18-Me), 1.371 (3H, *d*, J=6.4 Hz, 21-Me), 4.870 (1H, *d*, J=7.0 Hz, Gal H-1), 5.178 (1H, *d*, J=7.6 Hz), 5.235 (1H, *d*, J=7.1 Hz), 5.250 (1H, *d*, J=7.0 Hz), 5.527 (1H, *d*, J=7.4 Hz), 5.730 (1H).

Acid hydrolysis of **2** *and* **3**. A soln of **2** (20 mg) or 3 (20 mg) in 1 M H_2SO_4 in 50% EtOH (4 ml) was heated at 100° for 5 hr. The usual work-up gave aglycones **5** (2.6 mg) and **6** (3.5 mg), respectively. Compound **5**: needles from MeOH, mp 190–192°; IR ν_{max}^{KBr} cm^{-1}: 3520, 980, 920, 890, 850 (intensity 920>890, 25(*S*) spirostanol). Compound **6**: needles from MeOH, mp 237–240°; IR ν_{max}^{KBr} cm^{-1}: 3530, 1700 (C=O), 980, 920, 890, 850 (intensity 920>890, 25(*S*) spirostanol). Identification of compounds **5** and **6** was based on the direct comparison of TLC behaviour, mp and IR spectra with authentic samples which were obtained from *C. malayense* [1].

The aqueous layer was neutralized with $BaCO_3$, filtered and concd *in vacuo*. The residue was examined with TLC, using $CHCl_3$–MeOH–H_2O [(7:3:1, lower layer) (9 ml)]+HOAc (1 ml) or *iso*-PrOH–M/30 H_3BO_3 (17:3) as solvent systems and aniline/phthalate as detection. D-Galactose, D-glucose and D-xylose were detected in a ratio of 1:2:1 for saponin **3** and 1:3:1 for saponin **4**.

Acid hydrolysis on TLC plate [6] *and identification of resulting monosaccharides*. Saponin **4** was hydrolysed with HCl vapour on a TLC plate (90° for 30–50 min), followed with the same process described above for identifying the sugars.

Acetylation of saponins **2**–**4**. To each compound (1–2 mg) was added Ac_2O–pyridine (1:1) (0.1 ml) in a micro-tube. After standing at room temp. for 48 hr, the soln was evapd to dryness with N_2 and then subjected to EIMS analysis.

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