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TWO PHENYLPROPANOID GLYCOSIDES FROM *SCROPHULARIA SCOPOLII*

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Abstract—Two new phenylpropanoid glycosides, angoroside B and angoroside C, were isolated from the roots of *Scrophularia scopolii* var. *scopolii*. On the basis of chemical and spectral data, their structures were determined to be 3-hydroxy-4-methoxy-β-phenylethoxy-*O*-α-L-arabinopyranosyl-(1→6)-α-L-rhamnopyranosyl-(1→3)-4-*O*-caffeoyl-β-D-glucopyranoside and 3-hydroxy-4-methoxy-β-phenylethoxy-*O*-α-L-arabinopyranosyl-(1→6)-α-L-rhamnopyranosyl-(1→3)-4-*O*-feruloyl-β-D-glucopyranoside, respectively.

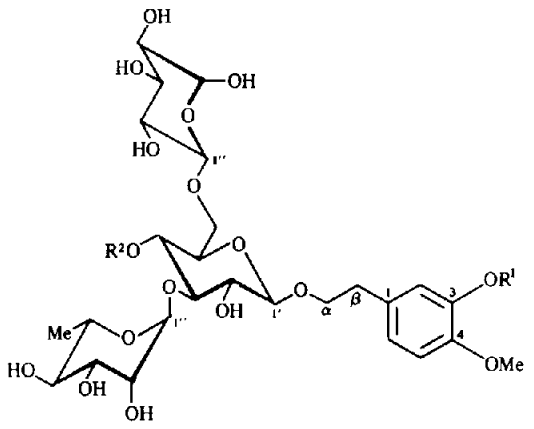
INTRODUCTION

In previous papers, we have reported the isolation and structure elucidation of angoroside A [1] and two triacylated biosidic iridoid glycosides [2] from *Scrophularia scopolii* [Hoppe ex] Pers var. *scopolii*. This paper deals with both the isolation and structure elucidation of two new phenylpropanoid glycosides, angoroside B (**1**) and angoroside C (**2**) from the roots of the same plant.

RESULTS AND DISCUSSION

Angoroside B (**1**) was obtained as an amorphous powder whose M_r was established by FABMS (*m/z* 771 $[M+H]^+$, 793 $[M+Na]^+$. Its ^1H NMR spectrum (Table 1) exhibited six aromatic protons (two ABX systems) belonging to the caffeic acid substituent and the aglycone part, two olefinic protons (*d*, H-α′ and H-β′) which appeared as an AB-system (the coupling constant, $J = 15.9$ Hz, indicated a *trans* geometry), a methoxyl group, a benzylic methylene, a secondary methyl group of rhamnose and three anomeric protons at δ5.19 ($J = 1.7$ Hz), 4.38 ($J = 7.9$ Hz) and 4.24 ($J = 6.6$ Hz) which were consistent with the configurations α for L-rhamnose, β for D-glucose, and α for L-arabinose, respectively. The ^1H NMR spectrum of **1** also suggested that the caffeoyl group occupies the 4-position in D-glucose since H-4′ absorbs at low field (δ4.97, 1H, *t*, $J = 9.5$ Hz). Furthermore, a three proton singlet at δ3.89 was attributed to an aromatic methoxy group on the aglycone moiety. These results were also confirmed by alkaline hydrolysis of **1** to yield caffeic acid and deacyl glycoside **3**.

Deacyl glycoside **3** was isolated as an amorphous powder (negative ion FABMS *m/z* 607 $[M-H]^-$, 1216 $[2M]^-$. Its ^1H NMR spectrum (Table 1) exhibited three aromatic protons resolved as an ABX system belonging to the aglycone moiety, three anomeric protons (δ5.15, 4.31 and 4.29), and an aromatic methoxy group (δ3.81).



	R^1	R^2
1	H	caffeoyl
2	H	feruloyl
3	H	H
4	Me	H

An upfield shift of the signal of H-4′ confirmed that the caffeoyl moiety was attached to the glucose at C-4′. The chemical shift values of three aromatic protons at δ6.81 (*d*, $J = 8$ Hz, H-5), 6.73 (*d*, $J = 2$ Hz, H-2) and 6.66 (*dd*, $J = 8/2$ Hz, H-6) were correlated with those of known compounds, (i.e. deacyl martynoside [3]) indicating that angoroside B contains 3-hydroxy-4-methoxy-β-phenylethanol as the aglycone part. The main fragment peaks recorded in the FABMS spectrum of **3** were at *m/z* 475 $[M-\text{arabinose}]^+$, 461 $[M-\text{rhamnose}]^+$, and 443 $[M-\text{aglycone}]^+$. These results proved that rhamnose, arabinose, and the aglycone were directly attached to the glucose as well as caffeic acid. On the other hand, the

Table 1 ^{1}H NMR spectral data of angoroside B (**1**), angoroside C (**2**) and deacyl glycoside **3** (300 13 MHz in CD_3OD Values in parenthesis are coupling constants in Hz)

H	1	2	3
Aglycone			
2	6 71 *d* (2)	6 75 *d* (2)	6 73 *d* (2)
5	6 69 *d* (8)	6 83 *d* (8 2)	6 81 *d* (8)
6	6 57 *dd* (8/2)	6 70 *dd* (8 2/2)	6 66 *dd* (8/2)
α	4 03 *m*	4 05 *m*	4 05–3 95*
	3 78–3 70*	3 80–3 72*	3 80–3 68*
β	2 79 *t* (7 3)	2 83 *t* (7 2)	2 8 *t* (7 3)
OMe	3 89 s	3 89 s	3 81 s
Glucose			
1′	4 38 *d* (7 9)	4.38 *d* (7 9)	4 29 *d* (7 8)
2′	3 39 *dd* (9 1/8)	3 40 *dd* (9/7 9)	3 39 *t* (9 5)
3′	3 9–3 7*	3 90–3 72*	4 05–3 95*
4′	4 97 *t* (9 5)	4 97 *t* (9 5)	3 52–3 34*
5′	3 9–3 7*	3 9–3 72*	3 52–3 34*
6′	3 62–3 53*	3 62–3 53*	3 8–3 68*
6′	3 9–3 7*	3 9–3 72*	4 08 *dd* (11 4/1 6)
Arabinose			
1″	4 24 *d* (6 6)	4 24 *d* (6 6)	4 31 *d* (6 7)
2″	3 62–3 53*	3 62–3 53*	3 58 *dd* (8 8/6 7)
3″	3 48 *dd* (8 7/3 4)	3 48 *dd* (8 7/3 4)	3 52–3 34
4″	3 9–3 7*	3 9–3 7*	3 8–3 68*
$5''_{eq}$	3 9–3 7*	3 9–3 7*	3 85 *dd* (12 4/3 3)
$5''_{ax}$	3 46 *dd* (12 3/1 6)	3 47 *dd* (12 3/1 6)	3 52–3 34*
Rhamnose			
1‴	5 19 *d* (1 7)	5 2 *d* (1 7)	5 15 *d* (1 7)
2‴	3 91 *dd* (3 2/1 7)	3 91 *dd* (3 3/1 7)	3 93 *dd* (3 4/1 8)
3‴	3 62–3 53*	3 62–3 53*	3 69 *dd* (9 5/3 3)
4‴	3 29 *t* (9 5)	3 29 *t* (9 4)	3 28 *t* (9 5)
5‴	3 62–3 53*	3 62–3 53*	3 52–3 34*
6‴	1 1 *d* (6 2)	1 1 *d* (6 2)	1 24 *d* (6)
Acyl moiety			
2⁗	7 19 *d* (1 9)	7 2 *d* (1 8)	
5⁗	6 81 *d* (8 1)	6 81 *d* (8 2)	
6⁗	7 08 *dd* (8 1/1 9)	7 08 *dd* (8 2/1 8)	
α′	6 36 *d* (15 9)	6 37 *d* (15 9)	
β′	7 66 *d* (15 9)	7 66 *d* (15 9)	
OMe		3 82 *s*	

*Signal pattern unclear due to overlapping

^{13}C NMR spectrum of **1** (Table 2) showed almost the same chemical shifts as those of angoroside A [1], except for the signals due to the aglycone moiety which indicated that rhamnose, caffeic acid, and arabinose were linked to C-3′, C-4′ and C-6′ hydroxyl groups, respectively These results were also confirmed by the methylation of deacyl glycoside **3** with diazomethane to yield deacyl angoroside A dimethyl ether as reported previously [1] In addition, the chemical shift values for C-atoms of glucose (Table 2) were also in good agreement with the corresponding signals of echinacoside, cistanoside A and B [4], leucosceptoside B [5], and forsythoside B [6], which exhibit similar glycosidation patterns on the centrally located glucose moiety

These results led us to conclude that the structure of angoroside B (**1**) is 3-hydroxy-4-methoxy-β-phenylethoxy-*O*-α-L-arabinopyranosyl-(1→6)-α-L-rhamnopyranosyl-(1→3)-4-*O*-caffeoyl-β-D-glucopyranoside, while deacyl glycoside **3** is 3-hydroxy-4-methoxy-β-phenylethoxy-*O*-α-L-arabinopyranosyl-(1→6)-α-L-rhamnopyranosyl-(1→3)-β-D-glucopyranoside

Angoroside C (**2**) was isolated as an amorphous powder (FABMS *m/z* 785 $[M+H]^+$) Its ^{1}H NMR spectrum (Table 1) showed similar signals to **1**, additionally, a signal of an aromatic methoxyl group was visible Alkaline hydrolysis of **2** yielded ferulic acid and deacyl glycoside **3** which was identical with the deacyl glycoside **3** obtained upon alkaline treatment of **1** The ^{13}C NMR data of **2** (Table 2) showed similar chemical shift values of glycosidic C-atoms as those of **1** and angoroside A [1], hence, **1** and **2** have the same sugar moieties as angoroside A (glucose rhamnose and arabinose) From the

Table 2. ^{13}C NMR spectral data of angoroside B (1) and angoroside C (2) (75.47 MHz in CD_3OD)

C	1	2
Aglycone		
1	131.51 *s*	133.00 *s*
2	111.76 *d*	112.90 *d*
3	146.14 *s*	147.60 *s*
4	144.65 *s*	147.41 *s*
5	117.13 *d*	117.20 *d*
6	121.31 *d*	121.31 *d*
α	72.43 *t*	72.27 *t*
β	36.54 *t*	36.67 *t*
OMe	56.42 q	56.54 q
Glucose		
1′	105.01 *d*	105.16 *d*
2′	76.10 *d*	76.22 *d*
3′	81.47 *d*	81.60 *d*
4′	70.45 *d*	70.56 *d*
5′	74.89 *d*	74.98 *d*
6′	68.97 *t*	69.13 *t*
Arabinose		
1″	104.11 *d*	104.19 *d*
2″	72.39 *d*	72.47 *d*
3″	73.76 *d*	73.86 *d*
4″	69.47 *d*	69.56 *d*
5″	66.79 *t*	66.87 *t*
Rhamnose		
1‴	103.00 *d*	103.08 *d*
2‴	72.39 *d*	72.37 *d*
3‴	72.03 *d*	72.11 *d*
4‴	74.03 *d*	74.12 *d*
5‴	70.45 *d*	70.56 *d*
6‴	18.41 *q*	18.54 *q*
Acyl moiety		
1⁗	127.65 *s*	127.72 *s*
2⁗	115.06 *d*	111.86 *d*
3⁗	149.41 *s*	149.48 *s*
4⁗	150.90 *s*	150.97 *s*
5⁗	116.55 *d*	116.59 *d*
6⁗	124.42 *d*	124.49 *d*
α′	116.29 *d*	115.10 *d*
β′	148.14 *d*	148.18 *d*
C=O	168.14 *s*	168.38 *s*
OMe	—	56.54 *q*

similarity of these spectral data and the formation of **3** by alkaline hydrolysis, the linkage positions of the sugars and ferulic acid were also concluded to be C-3′, C-6′ and C-4′ in the glucose moieties of **2**.

In addition to the molecular ion peak *m/z* 785 $[M+H]^+$, FABMS of **2** revealed other peaks at *m/z* 653 $[M-arabinose]^+$, 639 $[M-rhamnose]^+$, 617 $[M-aglycone]^+$, 507 $[M-rhamnose-arabinose]^+$, 485 $[M-arabinose-aglycone]^+$, 471 $[M-rhamnose-aglycone]^+$, 339 $[M-rhamnose-arabinose-aglycone]^+$, and 177 $[feruloyl]^+$ supporting the results mentioned above. Based on these data, the structure of angoroside C (**2**) was established as 3-hydroxy-4-methoxy-β-phenylethoxy-*O*-α-L-arabinopyranosyl-(1→6)-α-L-rhamnopyranosyl-(1→3)-4-*O*-feruloyl-β-D-glucopyranoside.

EXPERIMENTAL

General procedures. The experimental procedures and plant material were the same as reported in [1]. For the semi-prep. HPLC work, a Lichrosorb RP 18 prepacked column (Knauer) was used (25 cm × 16 mm i.d.)

Isolation of angoroside B (**1**). Fraction C (3.0 g) [1] was chromatographed on a silica gel column (60 g) using EtOAc–MeOH–H_2O (100:16.5:13.5) and yielded two fractions. Fraction C1 (main substance, identified as acteoside by comparison with an authentic sample on TLC using $CHCl_3$–MeOH–H_2O, 61 32:7, R_f 0.43) and fraction C2 (200 mg). An aliquot (80 mg) of fraction C2 was rechromatographed (LPLC) on a Sepralyte C18 reversed phase column (1.8 × 80 cm) (H_2O–MeOH 10→40%, gradient elution) to yield angoroside B (**1**, 47 mg, R_f 0.37).

Isolation of angoroside C (**2**). Fraction B (3.5 g) [1] was also fractionated by silica gel (60 g) CC (EtOAc–MeOH–H_2O, 30:3.2) to yield 2 fractions. Fraction B1 (main substance, identified as acteoside) and fraction B2 (280 mg). Fraction B2 was rechromatographed (LPLC) on a Sepralyte C18 reversed phase column (1.8 × 80 cm) (H_2O–*iso*PrOH, 5→25%, gradient elution) to yield angoroside C (**2**, 95 mg, R_f 0.4).

Angoroside B (**1**). $[\alpha]_D^{20} = -65.7°$ (MeOH, *c* 0.38). FABMS (glycerine): *m/z* 771 ($[M+H]^+$, calc. for $C_{35}H_{46}O_{19}$, 770.74), 793 $[M+Na]^+$, 493 $[M-rhamnose-arabinose]^+$, 485 $[M-aglycone-arabinose]^+$, 441 $[M-aglycone-caffeic\ acid]^+$. UV λ_{max}^{MeOH} nm: 329, 288 (sh), 229 (sh), 218 and 205. IR ν_{max}^{KBr} cm^{-1} 3400, 1680, 1620, 1590 and 1505. ^1H NMR (CD_3OD): see Table 1. ^{13}C NMR (CD_3OD): see Table 2.

Angoroside C (**2**). $[\alpha]_D^{20} = -80.4°$ (MeOH, c 0.38). FABMS (glycerine): *m/z* 785 ($[M+H]^+$, calc. for $C_{36}H_{48}O_{19}$, 784.76), 807 $[M+Na]^+$, 823 $[M+K]^+$, 877 $[M+glycerine]^+$, 653 $[M-arabinose]^+$, 639 $[M-rhamnose]^+$, 617 $[M-aglycone]^+$, 507 $[M-rhamnose-arabinose]^+$, 485 $[M-aglycone-arabinose]^+$, 471 $[M-aglycone-rhamnose]^+$, 339 [4′-*O*-feruloyl-β-D-glucose]$^+$ and 177 $[feruloyl]^+$. UV λ_{max}^{MeOH} nm 329, 288 (sh), 229 (sh), 218 and 204. IR ν_{max}^{KBr} cm^{-1}. 3400, 1685, 1620, 1590 and 1505. ^1H NMR (CD_3OD) see Table 1. ^{13}C NMR (CD_3OD): see Table 2.

Total acid hydrolysis of angoroside B (**1**) *and C* (**2**). The solns of the glycosides (about 5 mg each) in 5% HCl (2 ml) were heated in a boiling water bath for 2 hr, then cooled and filtered. Each filtrate was neutralized with Ag_2CO_3, and the ppt. was removed. The filtrate was evapd to dryness. The residue was shown by PC (descending method using *n*-BuOH–pyridine–H_2O, 9:5:4) to contain arabinose, glucose, and rhamnose.

Partial acid hydrolysis of angoroside A [1] *and B* (**1**). Hydrolysis of angoroside A and 1 (about 2 mg each) in refluxing 2 M trifluoro acetic acid and filtration of the neutral aq. phase through an alumina cartridge afforded arabinose and rhamnose which were identified by TLC (silica gel, Me_2CO–H_2O, 9:1).

Alkaline hydrolysis of angoroside B (**1**) *and C* (**2**). Separate solns of **1** and **2** (15 mg each) in 5% methanolic KOH (2 ml) were kept at room temp. for 2 hr. Each reaction mixture was neutralized with 1 M HCl and filtered. The filtrate was concentrated to dryness *in vacuo*, and the residue was subjected to semi-prep. HPLC (MeOH–H_2O, 2:3), flow rate 10 ml/min). From the alkaline hydrolysis products of **1** and **2**, the same deacyl glycoside **3** was obtained while **1** gave caffeic acid, **2** gave ferulic acid as acyl moieties which were identified by comparison with authentic samples (HPLC).

Deacyl glycoside **3** $[\alpha]_D^{20} = -53.8°$ (MeOH, *c* 0.24). Neg.

FABMS (glycerine). *m/z* 607 ([M−H]$^-$, calc for $C_{26}H_{40}O_{16}$, 608.59) For further fragments, see results and discussion UV λ_{max}^{MeOH} nm 281, 221 and 204 IR ν_{max}^{KBr} cm^{-1} 3400, 1585 and 1500 ^{1}H NMR (CD_3OD) see Table 1

Methylation of deacyl glycoside **3** Methylation of deacyl glycoside **3** with CH_2N_2 in the usual way afforded **4** which was identified by direct comparison as deacyl angoroside A dimethyl ether as already reported [1]

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