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PHENYLPROPANOID GLYCOSIDES ISOLATED FROM *SCROPHULARIA SCOPOLII*

IHSAN CALIS, GIAN-ANDREA GROSS* and OTTO STICHER*

Faculty of Pharmacy, Hacettepe University, 06100 Ankara, Turkey; *Pharmazeutisches Institut, Eidgenössische Technische Hochschule Zürich, CH-8092 Zürich, Switzerland

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Key Word Index—*Scrophularia scopolii* var. *scopolii*; Scrophulariaceae; phenylpropanoid glycosides; acteoside; verbascoside; angoroside A; deacyl angoroside A dimethyl ether; deacyl acteoside dimethyl ether.

Abstract—A new phenylpropanoid glycoside, angoroside A, and a known glycoside, acteoside, were isolated from the roots of *Scrophularia scopolii* var. *scopolii*. On the basis of chemical and spectral evidence, angoroside A was shown to be 3,4-dihydroxy-β-phenylethoxy-*O*-α-L-arabinopyranosyl-(1 → 6)-α-L-rhamnopyranosyl-(1 → 3)-4-*O*-caffeoyl-β-D-glucopyranoside.

INTRODUCTION

As a continuation of our investigations on the glycosidic constituents of plants belonging to the Scrophulariaceae, this paper deals with the isolation and structural elucidation of a new phenylpropanoid glycoside from the roots of *Scrophularia scopolii* [Hoppe ex] Pers. var. *scopolii*, named angoroside A (3). In addition, we describe the isolation of a known phenylpropanoid glycoside, acteoside (= verbascoside) (1) [1, 2].

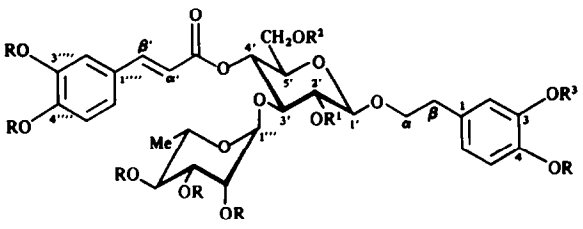
RESULTS AND DISCUSSION

Acteoside (1) was identified by direct comparison with an authentic sample using high pressure liquid chromatography. Analytical data of 1 and its peracetate 2 (IR, UV, MS, NMR) were identical to those published for the authentic compound [1].

Angoroside A (3) was obtained as an air-sensitive amorphous substance, whose M_r was confirmed as 756 by FABMS. The IR spectrum showed absorption bands for hydroxyl groups (3400 cm^{-1}, br), an α,β-unsaturated ester ($\nu_{C=O}$ 1690, $\nu_{C=C}$ 1625 cm^{-1}), and aromatic rings (1600, 1515 cm^{-1}). The ^1H NMR spectrum of 3 (see Table 1) exhibited signals belonging to caffeic acid and 3,4-dihydroxyphenylethanol moieties, respectively: protons of aromatic rings (2 × ABX, δ 6.57–7.05), two *trans* olefinic protons [AB, δ 7.60, 6.27 (2 × 1H, *d*, J = 15.9 Hz)], a triplet at 2.79 ppm (β − CH_2 of aromatic side chain), and two non equivalent protons at δ 4.03 and 3.77, respectively. Three doublets, at δ 4.23 (J = 6.7 Hz)), 4.38 (J = 7.9 Hz) and at δ 5.18 (J = 1.3 Hz), are consistent with the following C-1 configurations: α for L-arabinose, β for D-glucose, and α for L-rhamnose. Due to the close resemblance of molecular rotations of 3 ($[M]_D$ = −554°) and ehrenoside ((7): $[M]_D$ = −548°) the presence of the same forms for glucose (D) and both arabinose and rhamnose (L) could be assumed. The configurations of the sugar moieties of 7 were confirmed by HPLC using polarimetric on-line detection [3]. The acylation at C-4 hydroxyl of glucose was deduced from the downfield shift of H-4 of glucose (δ 4.98, 1H, *t*, J = 9.5 Hz).

Acetylation of 3 resulted in undecaacetate 4, $C_{56}H_{66}O_{30}$ (FABMS: m/z 1219 $[M+H]^+$). The main fragment peaks recorded in the FABMS spectrum were M − 238 (diacetyl aglycone) at m/z 981, m/z 273 (terminal triacetyl-*O*-rhamnose), m/z 259 (terminal triacetyl-*O*-arabinose), m/z 247 (diacetyl caffeoyl), and m/z 205 (monoacetyl-*O*-glucose). The ^1H NMR spectrum of 4 revealed the presence of 11 acetyl signals belonging to four aromatic and seven aliphatic acetyl groups (see Table 1).

Acid hydrolysis of angoroside A yielded glucose, arabinose, and rhamnose. Methylation of 3 [4] followed by acid hydrolysis yielded, 2,3,4-trimethyl-L-rhamnose and 2,3,4-trimethyl-L-arabinose which were identical (TLC) with authentic samples. These results indicate that the rhamnose and arabinose are attached directly to the glucose as terminal sugars. In order to confirm the site of acylation, 3 was also methylated with diazomethane and treated with aqueous potassium hydroxide to give deacyl angoroside A dimethyl ether (5) (FABMS: m/z 623 $[M+H]^+$). The IR spectrum of 5 showed no ester absorption. The ^1H NMR spectrum of 5 (see Table 1) indicated the presence of three aromatic protons (δ 6.90–6.79) as an ABX system, three anomeric protons (δ 5.15, 4.29 and 4.28), and two aromatic methoxy groups (δ 3.82 and 3.78).



1 R = R^1 = R^2 = R^3 = H
2 R = R^1 = R^2 = R^3 = COMe
3 R = R^1 = R^3 = H, R^2 = Ara
4 R = R^1 = R^3 = COMe, R^2 = Ara(ac)$_3$
7 R = R^2 = R^3 = H, R^1 = Ara
8 R = R^1 = H, R^2 = Glc, R^3 = Me

Table 1. ^{1}H NMR spectral data of 3, 4 and 5*

Proton(s) at C-atom	3 (CD_3OD, 300 MHz)		4 ($CDCl_3$, 400 MHz)		5 (CD_3OD, 400 MHz)	
Aglycone						
2	6.72	*d* (2)	7.04	*d* (1.4)	6.9	*d* (1.8)
5	6.68	*d* (8)	7.08	*d* (8)	6.85	*d* (8.2)
6	6.57	*dd* (2/8)	7.12	*dd* (1.4/8)	6.79	*dd* (1.8/8.2)
α	4.03	*m*	4.11	*m*	4.01	*m*
	3.79–3.7	*m*	3.78	*m*	3.77–3.71†	
β	2.79	*t* (7.2)	2.86	*m*	2.86	*t* (7.1)
OCH_3					3.82	*s*
					3.78	*s*
Glucose						
1′	4.38	*d* (7.9)	4.36	*d* (8)	4.29	*d* (8)
2′	3.39	*dd* (7.9/9)	5.06–4.98†		3.51–3.36†	
3′	3.81	*t* (9)	3.87	*t* (9.3)	4.03	*t* (9.8)
4′	4.98	*t* (9.5)	5.06–4.98†		3.51–3.36†	
5′	3.79–3.70†		3.65–3.56†		3.51–3.36†	
$6_{A'}$	3.6–3.44†		3.65–3.56†		3.77–3.71†	
$6_{B'}$	3.86	*dd* (1.4/11.5)	3.85	*d* (11.0)	4.08	*dd* (1.7/11.4)
Arabinose						
1″	4.23	*d* (6.7)	4.44	*d* (6.9)	4.28	*d* (6.9)
2″	3.6–3.44†		5.15	*dd* (6.9/9.4)	3.56	*dd* (6.9/8.8)
3″	3.6–3.44†		5.06–4.98		3.51–3.36†	
4″	3.79–3.7†		5.23		3.77–3.71†	
$5''_{eq}$	3.79–3.7†		3.97	*dd* (3.2/13)	3.84	*dd* (3.1/13)
$5''_{ax}$	3.6–3.44†		3.65–3.56†		3.51–3.36†	
Rhamnose						
1‴	5.18	*d* (1.3)	4.83	*d* (1.9)	5.15	*d* (1.6)
2‴	3.92	*dd* (1.7/3)	5.06–4.98†		3.92	*dd* (1.6/3.3)
3‴	3.6–3.44†		5.07	*dd* (3.3/10)	3.68	*dd* (3.3/9.5)
4‴	3.29	*t* (9.5)	4.94	*t* (10)	3.28	*dd* (8.1/8.8)
5‴	3.6–3.44†		3.65–3.56†		3.51–3.36†	
6‴	1.09	*d* (6.2)	1.03	*d* (6.2)	1.23	*d* (6.2)
Caffeic acid						
2‴′	7.06	*d* (1.9)	7.35	*d* (2)		
5‴′	6.78	*d* (8.2)	7.23	*d* (8.4)		
6‴′	7.0	*dd* (1.9/8.2)	7.4	*dd* (2/8.4)		
α′	6.27	*d* (15.9)	6.33	*d* (16)		
β′	7.6	*d* (15.9)	7.64	*d* (16)		
$OOOCH_3$			2.31, 2.3, 2.28, 2.26 (arom). 2.09, 2.08, 2.02, 2.01, 1.97, 1.93, 1.87 (aliph.)			

*Values in parentheses are coupling constants in Hz.

†Signal pattern unclear due to overlapping.

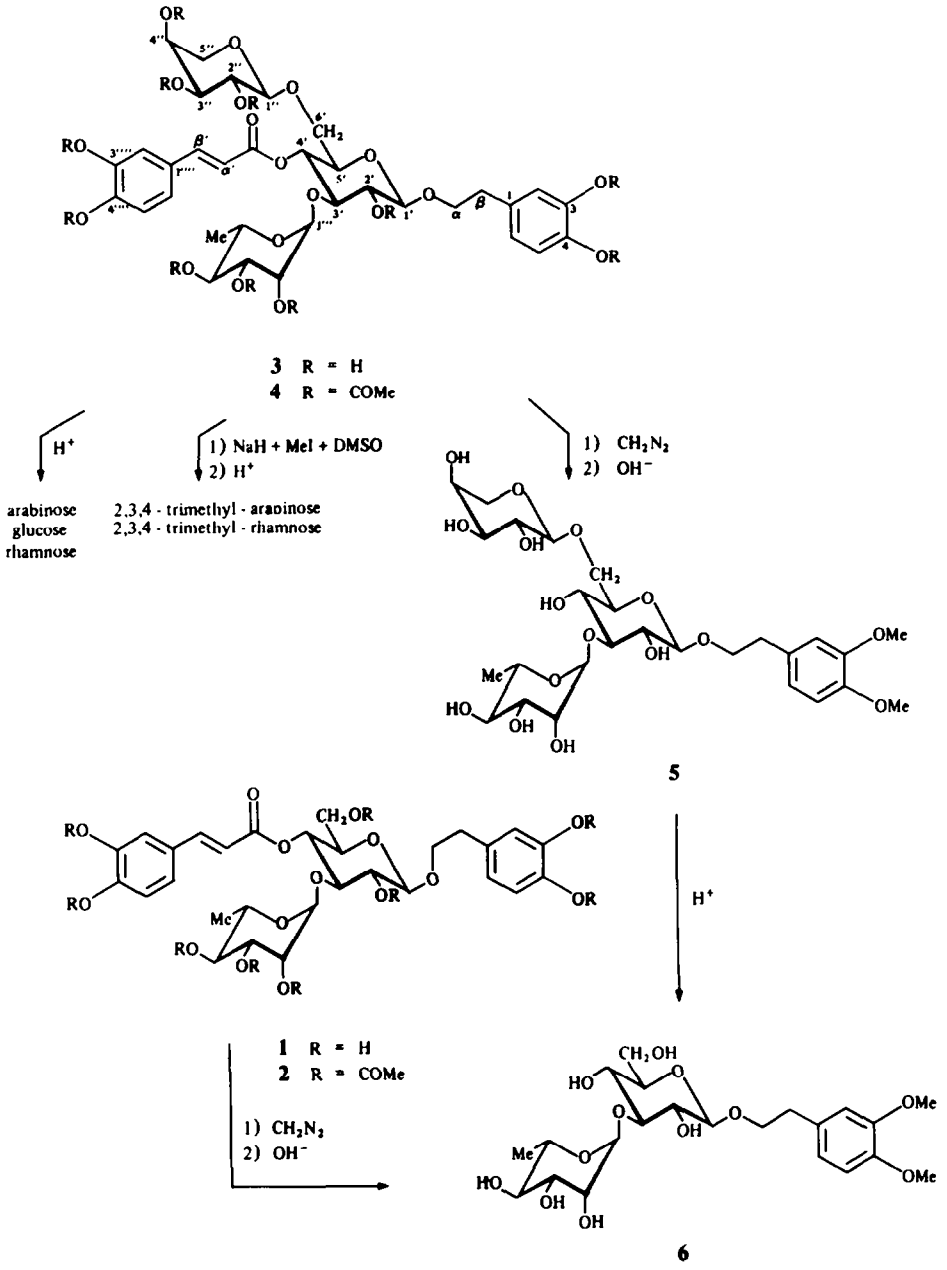
Comparison of the ^{1}H NMR spectra of 3 with 5 supported the attachment of the caffeate moiety at the C-4 carbon of glucose, since the H-4′ signal of 5 is shifted upfield. ^{13}C chemical shifts of 3 and 5 could easily be assigned on the basis of comparison with those of compounds of similar glycosidation patterns, such as ehrenoside (7) [3], and cistanoside A (8) [5] (see Table 2 and Fig. 1).

In order to confirm the location of arabinose and rhamnose on the glucose moiety 5 was subjected to partial hydrolysis. This yielded a partially hydrolysed product 6, which was identical (TLC) with an authentic sample prepared from acteoside. This is in good agreement with the well-known observation that hydrolysis of a primary alcohol function (here, C-6′ of glucose) goes more easily than the corresponding secondary alcohol. The ^{1}H NMR spectrum of 6 showed the presence of the caffeoyl and phenethyl moieties, as well as two sugar components, rhamnose and glucose [anomeric protons at δ5.14 (*d*, J = 1.6 Hz) and δ4.30 (*d*, J = 7.9 Hz), respectively]. The δ values of 6 were identical with the corresponding signals of deacyl acteoside dimethyl ether [6].

Consequently, the structure of 3 has been established as 3,4 -dihydroxy-β-phenylethoxy-*O*-α- L-arabinopyranosyl-(1 → 6)-α-L-rhamnopyranosyl-(1 → 3)-4-*O*-caffeoyl-β-D-glucopyranoside.

EXPERIMENTAL

General procedures. UV spectra were determined in spectroscopic grade MeOH. ^{1}H and ^{13}C NMR spectra were obtained



Scheme 1. Chemical degradation of angoroside A(3) and acteoside (1).

using a Bruker WM 300 spectrometer [300.13 MHz (^{1}H NMR), 75.47 MHz (^{13}C NMR)] and a Bruker AM 400 [400.13 MHz (^{1}H NMR)] in FT mode. FABMS were recorded with a Kratos AEI-MS 50 spectrometer in *m*-nitrobenzylalcohol (NOBA) or in glycerin at 8.3 keV. An HPLC-system (Waters Associates) equipped with two pumps (Models M 6000 and M 45), a WISP 710 B autosampler, and a Knauer prepacked analytical cartridge (10 cm × 4 mm i.d.) of Spherisorb ODS (3 μm) were employed throughout this study. The system was equipped with a Hewlett–Packard 1040 A high-speed spectrophotometric detector. LPLC was carried out in reversed phase mode using a Pharmacia FPLC system (two pumps P-500 and a gradient controller LCC-500). Columns (Labomatic) (80 cm × 18 mm i.d. and 40 cm × 13 mm i.d.) were filled with Sepralyte C-18 (prep. grade, 40 μm). For on-line UV detection, a LKB Uvicord S detector was used (206 nm). Polyamide (Woelm), silica gel 60 (70–230 mesh, Merck), and Sephadex LH-20 (Pharmacia) were used for CC, and silica gel 60 F_{254} (Merck) TLC plates and Schleicher & Schüll 2043 a MgL paper for PC. Phenylpropanoids were detected by UV fluorescence and/or spraying with vanillin–H_2SO_4 and sugars by aniline phthalate reagent followed by heating at 100° for 5–10 min.

Plant material. Plant material was collected between Istanbul

Table 2. ^{13}C NMR spectral data of 3, 5, 7† and 8‡

Carbon No.	3 (CD_3OD)		5 (CD_3OD)	7 (CD_3OD)	8 (CD_3OD)	
Aglycone						
1	131.6	*s*	133.22	131.87	131.5	
2	116.59	*d*	113.09	117.5	113.9	
3	146.3	*s*	150.32	145.95	148.7	
4	144.7	*s*	148.93	144.62	145.7	
5	117.2	*d*	114.13	116.52	116.1	
6	121.38	*d*	122.35	121.5	122.4	
α	72.43	*t*	72.27	72.05	72.1	
β	36.65	*t*	36.74	36.25	36.6	
OCH_3		*q*	56.54 (×2)		56.6	
Glucose						
1′	105.07	*d*	105.3	104.06	104.1	
2′	72.2	*d*	76.93	82.63	75.9	
3′	81.72	*d*	84.01	81.41	81.5	
4′	70.52	*d*	71.77	70.58	70.3	
5′	75.0	*d*	75.74	75.72	74.6	
6′	69.07	*t*	69.42	62.39	69.3	
Arabinose					*Glucose*	
1″	104.19	*d*	104.3	102.97	104.5	
2″	72.13	*d*	72.35	72.98	74.9	
3″	73.85	*d*	73.98	74.49	77.6	
4″	69.55	*d*	69.6	69.5	71.4	
5″	66.84	*t*	66.83	66.81	77.6	*d*
					62.6	*t*
Rhamnose						
1‴	103.13	*d*	102.74	103.16	102.8	
2‴	72.43	*d*	72.27	71.99	72.1	
3‴	72.43	*d*	72.06	71.99	72.1	
4‴	74.12	*d*	74.21	73.79	73.7	
5‴	70.52	*d*	69.99	70.86	70.6	
6‴	18.56	*q*	17.92	18.49	18.4	
Caffeic acid						
1⁗	127.68	*s*		127.61	127.6	
2⁗	114.69	*d*		115.27	114.7	
3⁗	146.87	*s*		146.7	146.6*	
4⁗	149.88	*s*		149.66	149.5*	
5⁗	116.39	*d*		114.78	116.5	
6⁗	123.34	*d*		123.21	123.1	
α′	115.33	*d*		116.34	115.4	
β′	148.26	*d*		147.93	148.1	
C=O	168.38	*s*		168.32	168.3	

*Corrected values (see [5]).
†At 75 MHz [3].
‡At 22.5 MHz [5].

and Ankara in July 1984. A voucher specimen has been deposited in the Herbarium of the Pharmacognosy Department, Faculty of Pharmacy, Hacettepe University, Ankara, Turkey.

Extraction and prepurification. The air dried roots of *S. scopolii* var. *scopolii* (180 g) were extracted in MeOH at 40° (3 × 1 l). After concn of the combined extracts to 500 ml, Et_2O (500 ml) was added. After removal of the ppt. the MeOH–Et_2O (1:1) soluble fraction was evap. to dryness (17 g, 9.44%). The residue was fractionated by CC on silica gel (150 g) eluting with $CHCl_3$–MeOH–H_2O 40:10:1. The fractions were monitored by TLC using $CHCl_3$–MeOH–H_2O 40:10:1 and EtOAc–MeOH–H_2O (100:16.5:13.5). Five fractions were collected (A–E).

Isolation of acteoside (1). Fraction D (1.6 g) was rechromatographed on a silica gel column (60 g) EtOAc–MeOH–H_2O, 100:16.5:13.5). The faster eluting zone was concd to yield crude 1. Repeated separations on Sephadex LH-20 gave 190 mg of 1. The identity of 1 was confirmed by HPLC coupled with UV/Vis spectroscopy by diode array detection using 15% THF + 0.2% *o*-H_3PO_4 as solvent followed by comparison with an authentic compound.

Isolation of angoroside A (3). The slower running zone of fraction D yielded a crude preparation of 3. Purification was carried out by LPLC using a linear H_2O–MeOH gradient (10 → 40% MeOH in 24 hr) at a flow rate of 2 ml/min. Fractions of 18 ml each were collected. Fractions containing pure 3 (TLC

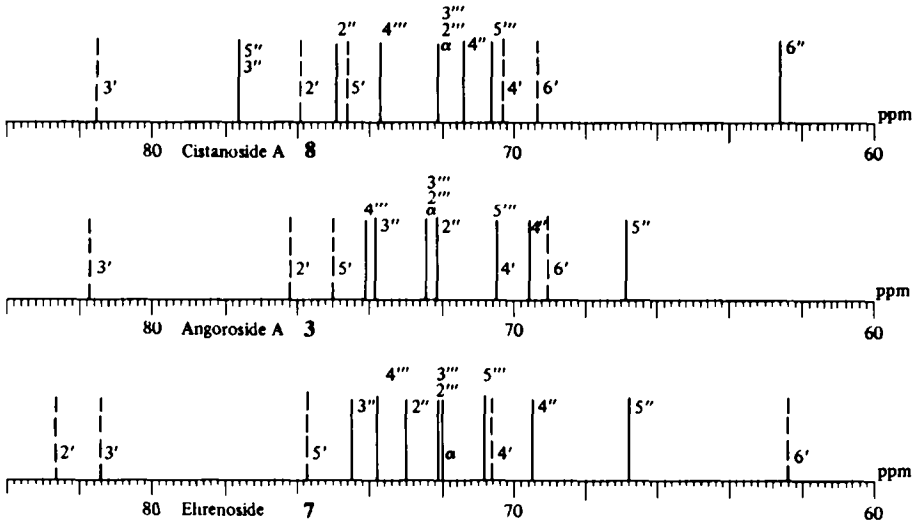


Fig. 1. Schematic representation of the ^{13}C NMR spectra (60–84 ppm) of **8**, **3** and **7**. Signals assigned to the central glucose moieties are plotted in dotted lines. The strong similarity of the C 6′ and C 2′ signal positions in **8** and **3** confirm the proposed 6-substitution for **3**. In **7** these signals are shifted about 6 ppm, C 6′ to higher field and C 2′ to lower field, respectively.

monitoring) were concd and lyophilized to give 30 mg of **3**, $[\alpha]_D^{20} = -68.75°$ (MeOH; *c* 0.32). FABMS (glycerin): *m/z* 757 ([M + H]$^+$, calc. for $C_{34}H_{44}O_{19}$; 756.71), 779 [M + Na]$^+$. UV λ_{max}^{MeOH} nm: 332, 288, 248, 218, 206. IR ν_{max}^{KBr} cm^{-1}: 3400, 1690, 1625, 1600, 1515. ^1H NMR (CD_3OD) see Table 1. For ^{13}C NMR (CD_3OD) see Table 2.

Acetylation of **3**. Treatment of **3** (50 mg) with Ac_2O (1 ml) and pyridine (1 ml) at room temp. overnight, followed by the usual work up yielded the undecaacetate **4**, $[\alpha]_D^{20} = -35.1°$ ($CHCl_3$; *c* 0.61). FABMS (NOBA) *m/z*: 1219 ([M + H]$^+$, calc. for $C_{56}H_{66}O_{30}$; 1219.1194), 1176 [M–$COCH_3$]$^+$, 1134 [M–(2 × $COCH_3$)]$^+$, 981 [M – diacetyl aglycone]$^+$, 273 [terminal triacetyl rhamnosyl], 259 [terminal triacetyl arabinosyl], 247 [diacetyl caffeoyl]. ^1H NMR ($CDCl_3$) see Table 1.

Acid hydrolysis of **3**. Compound **3** (20 mg) was dissolved in 5% HCl and heated at 100° for 2 hr, cooled and filtered. The filtrate was neutralized with Ag_2CO_3 the ppt. removed and the filtrate evap. to dryness. The residue was examined for sugars by PC (descending method) using *n*-BuOH–pyridine–water, 9:5:4.

Methylation of **3**. Methylation of **3** (10 mg) by the Hakomori method [4] followed by acid hydrolysis yielded 2,3,4-tri-*O*-methyl-arabinopyranoside and 2,3,4-tri-*O*-methyl-rhamnopyranoside which were identified by comparison with authentic samples (TLC, benzene–acetone, 2:1 and benzene–EtOH, 4:1). Methylation of **3** (40 mg) with diazomethane followed by alkaline hydrolysis resulted in 3,4-dimethoxy cinnamic acid and deacyl angoroside A dimethyl ether (**5**), which were separated on Sephadex LH-20 with MeOH to give a colourless powder (**5**), $[\alpha]_D^{20}$ −48.9° (MeOH; *c* 0.47). FABMS (glycerin): *m/z* 623 [M + H]$^+$, 645 [M + Na]$^+$, 491 [M – arabinosyl]$^+$, 477 [M – rhamnosyl]$^+$, 441 [M – 3,4-dimethoxy-β-phenylethylalcohol]$^+$. UV λ_{max}^{MeOH} nm: 277, 225. IR ν_{max}^{KBr} cm^{-1}: 3400, 1630, 1590, 1510. For ^1H NMR (CD_3OD) see Table 2.

Partial acid hydrolysis of **5**. Compound **5** (5 mg) dissolved in 0.2 N aq. HCl was heated at 100° for 20 min. The reaction mixture was neutralized with $NaHCO_3$ and filtered. The filtrate was concd and the residue chromatographed on TLC plates ($CHCl_3$–MeOH–H_2O, 61:32:7) to give three compounds [R_f 0.7, 0.56 (**6**) and 0.37 (**5**)]. The mixture was purified by silica gel CC ($CHCl_3$–MeOH–H_2O, 12:6:1) to give the main product **6** (1 mg). ^1H NMR (CD_3OD) δ: 6.85–6.66 (3H, aromatic H), 5.14 (1H, *d*, *J* = 1.6 Hz, H-1 of rhamnose), 4.3 (1H, *d*, *J* = 7.9 Hz, H-1 of glucose), 3.84, 3.81 (2 × 3 H, s, 2 × CH_3O), 2.83 (2H, *m*, β-H_2) and 1.24 (3H, *d*, *J* = 6.3 Hz, CH_3 of rhamnose). By direct comparison with an authentic compound (TLC), **6** was found to be deacyl acteoside dimethyl ether. Deacyl acteoside dimethyl ether was prepared from acteoside (**1**) in the same way as described for **3**.

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