122. Lagotoside: A New Phenylpropanoid Glycoside from *Lagotis stolonifera*

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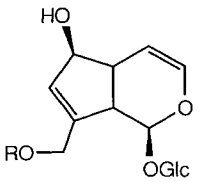
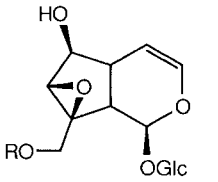
From the aerial parts of *Lagotis stolonifera* (Scrophulariaceae), a new phenylpropanoid glycoside, lagotoside (**8**), and the three known glycosides ehrenoside (**5**), verbascoside (= acteoside; **6**), and plantamajoside (**7**) were isolated, together with the four known iridoid glucosides aucubin (**1**), catalpol (**2**), globularin (**4**), and lythantosalin (**3**). The structure of the new compound **8** was elucidated on the basis of chemical and spectral data as 2-(3-hydroxy-4-methoxyphenyl)ethyl *O*-[α-L-arabinopyranosyl-(1 → 2)]-*O*-[α-L-rhamnopyranosyl-(1 → 3)]-4-*O*-feruloyl-β-D-glucopyranoside.

1. Introduction. – The genus *Lagotis* is represented with only one species in the flora of Turkey. It was originally placed in the Selaginaceae but has a more natural position in the Scrophulariaceae [1]. Therefore, it was of interest to investigate the iridoid as well as phenolic constituents of *Lagotis stolonifera* (C. KOCH) MAXIM.

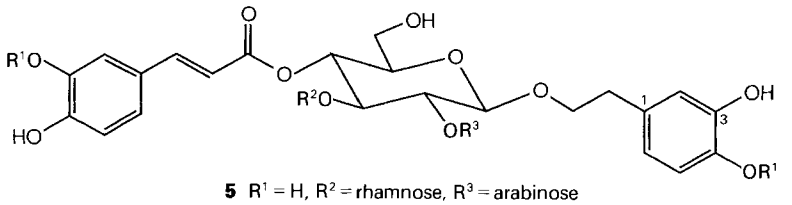
2. Results and Discussion. – The H_2O-soluble part of the MeOH extract of the air-dried aerial parts of the plant was fractionated using polyamide column chromatography. The fractions eluted with H_2O yielded aucubin (**1**), catalpol (**2**), and their (*E*)-cin-



1 R = H
3 R = $C_6H_5CH{=}CHCO$

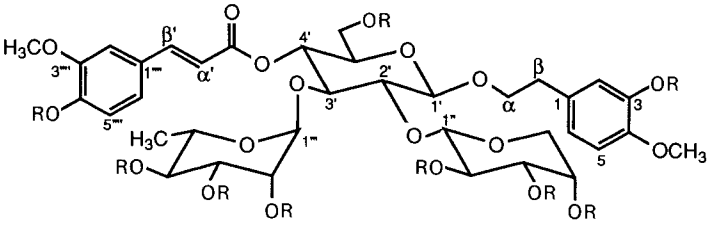


2 R = H
4 R = $C_6H_5CH{=}CHCO$

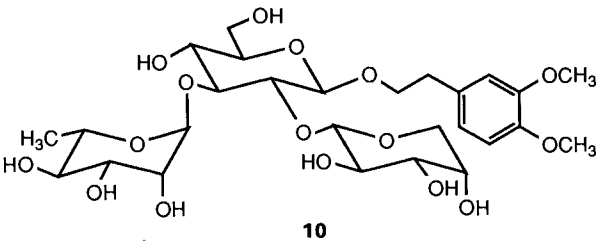


5 R^1 = H, R^2 = rhamnose, R^3 = arabinose
6 R^1 = H, R^2 = rhamnose, R^3 = H
7 R^1 = H, R^2 = glucose, R^3 = H
8 R^1 = Me, R^2 = rhamnose, R^3 = arabinose

namoyl derivatives lythanthosalin [2] [3] (= isoscrophularioside; **3**) and globularin [4] (= scutellarioside; **4**), respectively. The phenolic substances were eluted with 25, 50, and 75% MeOH, respectively, and were further separated by RP-MPLC using a gradient of MeOH in H_2O. Ehrenoside [5] (**5**), verbascoside [6] (= acteoside; **6**), plantamajoside [7] (**7**), and the new amorphous phenylpropanoid glycoside, lagotoside (**8**), were isolated. Compounds **1**–**8** were identified spectroscopically (UV, IR, ^{1}H-NMR, ^{13}C-NMR, and FAB-MS).



8 R = H lagotoside
9 R = Ac



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The FAB-MS of lagotoside (**8**) was compatible with the molecular formula $C_{36}H_{48}O_{19}$ (*m/z* 784 (M^+), 807 ($[M + Na]^+$)). UV maxima at 232, 262, and 326 nm confirmed the polyphenolic nature of **8**. The IR spectrum of **8** contained characteristic absorptions for OH (3400 cm^{-1}, br.), α,β-unsaturated ester (1700 (C=O) and 1630 cm^{-1} (C=C)), and aromatic (1595 and 1510 cm^{-1}) functionalities. The ^{1}H-NMR and ^{13}C-NMR data for **8** were extremely similar to that of ehrenoside (**5**; *Tables 1* and *2*), the major differences being the presence of resonances for 2 aromatic MeO groups in **8** (δ(H) 3.89 and 3.82, δ(C) 56.5 and 56.6). The resonances for two *ABX* systems and the chemical-shift values belonging to acyl and aglycone moieties were in good agreement with those of angoroside C, which has ferulic-acid (= (*E*)-3-(4-hydroxy-3-methoxyphenyl)prop-2-enoic acid) and 2-(3-hydroxy-4-methoxyphenyl)ethanol moieties [8]. Three signals for anomeric protons appeared as *d* at δ 4.55 (J = 7.8 Hz, H–C(1) of β-D-glucose), 4.52 (J = 6.7 Hz, H–C(1) of α-L-arabinose), and 5.18 (J = 1.5 Hz, H–C(1) of α-L-rhamnose). The feruloyl group was positioned at C(4) of the glucose on the basis of the strong deshielding of the H–C(4) signal (δ 4.98 (*t*, J = 9.5 Hz)).

Acetylation of **8** gave the corresponding nonaacetate **9**. Both **5** and **8**, upon methylation with diazomethane followed by alkaline hydrolysis yielded **10** (deacylehrenoside bis(methyl ether) = deacyllagotoside methyl ether). This finding further supported the proposition that **5** and **8** had the same glycosidation pattern.

The ^{1}H-NMR spectrum of **9** showed the presence of 2 aromatic and 7 aliphatic AcO groups (*Table 1*). ^{1}H-NMR-data comparisons for **9** and ehrenoside undecaacetate [5] indicated that the two molecules were similar, except for signals arising from their different acyl and aglycone moieties. All chemical-shift values attributed to the sugar moieties of **9** were comparable to those of ehrenoside undecaacetate (*Table 1*). The FAB-MS of **9** exhibited the $[M + H]^+$ peak at *m/z* 1163. Observed fragment ions at *m/z* 273 (2,3,4-tri-*O*-acetylrhamnose oxonium ion) and

m/z 259 (2,3,4-tri-*O*-acetylarabinose oxonium ion) indicated rhamnose and arabinose to be the terminal sugars, as is the case of ehrenoside [5].

The ^{1}H-NMR data of **10** indicated the presence of 3 aromatic protons (*ABX* at δ 6.88, 6.87, and 6.82), 3 anomeric protons (δ 4.46, 4.52, and 5.07), and 2 aromatic MeO groups (δ 3.79 and 3.82), the complete assignment of all resonances (*Table 1*) being based on the results of a ^{1}H,^{1}H COSY experiment. ^{13}C-NMR resonances of **10** (*Table 2*) were assigned on the basis of comparison with those of ehrenoside (**5**) and from the results of a short-range (J = 136 Hz) C,H-correlated 2D-NMR spectrum. Confirmation of the sugar sequence within **10** was possible from the results of 2D-ROESY and 2D-NOESY measurements. Thus, the anomeric proton of rhamnose (δ 5.07) showed NOE to H–C(3′) (δ 3.65) and the anomeric proton of arabinose (δ 4.52) to H–C(2′) (δ 3.54). These results were consistant with the predicted NOE interactions that were made from viewing the *Dreiding* model of **10**, and showed good correlation with data reported for ehrenoside [5], where the interglycosidic linkages were established by selective decoupling experiments (INDOR effects).

Table 1. *^{1}H-NMR Data* (300 MHz, CD_3OD) *of Ehrenoside* (**5**), *Lagotoside* (**8**), *Deacyllagotoside Methyl Ether* (**10**), *and Lagotoside Nonaacetate* (**9**). Chemical shifts δ in ppm relative to internal TMS, J in Hz.

	5	**8**	**10**	**9**[a])
Aglycone				
H–C(2)	6.74 (*d*)	6.76 (*d*, *J* = 2)	6.88 (*d*)	7.16–6.92[b])
H–C(5)	6.68 (*d*)	6.84 (*d*, *J* = 8)	6.87 (*d*)	
H–C(6)	6.57 (*d*)	6.70 (*dd*, *J* = 8, 2)	6.82 (*dd*)	
$CH_2(\alpha)$	4.08 (*m*), 3.68 (*m*)	4.05 (*m*), 3.51–3.75 (*m*)	4.09 (*m*), 3.72 (*m*)	3.9–4.16[b])
$CH_2(\beta)$	2.78 (*m*)	2.81 (*m*)	2.87 (*t*)	2.89 (*m*)
CH_3O		3.89 (*s*)	3.82 (*s*), 3.79 (*s*)	3.86 (*s*)
Glucose				
H–C(1′)	4.54 (*d*)	4.55 (*d*, *J* = 7.8)	4.46 (*d*)	4.46 (*d*)
H–C(2′)	3.67 (*dd*)	3.68 (*dd*, *J* = 7.8, 8.3)	3.54 (*dd*)	3.86[b])
H–C(3′)	3.98 (*t*)	3.99 (*t*, *J* = 9.0)	3.65 (*t*)	3.97 (*t*)
H–C(4′)	4.94 (*t*)	4.98 (*t*)	3.78[b])	5.11–5.17[b])
H–C(5′)	3.48–3.72[b])	3.51–3.75[b])	3.28[b])	3.64 (*m*)
CH_2(6′)	3.48–3.72[b])	3.51–3.75[b])	3.67, 3.87[b])	4.12 (*m*)
Arabinose				
H–C(1″)	4.52 (*d*)	4.52 (*d*, *J* = 6.7)	4.52 (*d*)	4.87 (*d*)
H–C(2″)	3.48–3.72[b])	3.51–3.75[b])	3.62[b])	5.11–5.17[b])
H–C(3″)	3.48–3.72[b])	3.51–3.75[b])	3.55[b])	5.11–5.17[b])
H–C(4″)	3.76 (br. *m*)	3.75 (br. *m*)	3.78[b])	5.22[b])
CH_2(5″)	3.81 (*dd*), 3.19 (*dd*)	3.82[b]), 3.21 (*dd*, *J* = 12.3, 1)	3.87 (*dd*), 3.29 (*dd*)	3.51 (*dd*), 3.94[b])
Rhamnose				
H–C(1‴)	5.17 (*d*)	5.18 (*d*, *J* = 1.5)	5.07 (*d*)	5.11 (*d*)
H–C(2‴)	4.04 (*dd*)	4.03 (*dd*, *J* = 1.5, 3.3)	4.03 (*dd*)	5.22 (*dd*)
H–C(3‴)	3.48–3.72[b])	3.51–3.75[b]) (*J* = 3.3, 9.5)	3.66 (*dd*)	5.11–5.17[b])
H–C(4‴)	3.30 (*t*)	3.29 (*t*, *J* = 10.5)	3.42 (*t*)	4.99 (*t*)
H–C(5‴)	3.48–3.72[b])	3.51–3.75[b])	3.95 (*m*)	3.86[b])
CH_3(6‴)	1.10 (*d*)	1.10 (*d*, *J* = 6.2)	1.25 (*d*)	1.10 (*d*)
Acyl moiety				
H–C(2⁗)	7.05 (*d*)	7.19 (*d*, *J* = 2)		7.16–6.92
H–C(5⁗)	6.77 (*d*)	6.81 (*d*, *J* = 8.2)		
H–C(6⁗)	6.95 (*dd*)	7.08 (*dd*, *J* = 8.2, 2)		
H–C(α′)	6.27 (*d*)	6.37 (*d*, *J* = 15.9)		6.34 (*d*)
H–C(β′)	7.58 (*d*)	7.66 (*d*, *J* = 15.9)		7.66 (*d*)
CH_3O		3.82 (*s*)		3.83 (*s*)

[a]) In $CDCl_3$. Additional signals: 2.32, 2.31 (2 arom. AcO); 1.74, 1.98, 1.99, 2.03, 2.06, 2.08, 2.13 (7 aliph. AcO).
[b]) Signal partly merged with other resonances.

Table 2. *^{13}C-NMR Data* (CD_3OD, 75.5 MHz) *of Ehrenoside* (**5**), *Lagotoside* (**8**), *and Deacyllagotoside Methyl Ether* (**10**). Chemical shifts δ in ppm relative to internal TMS.

		5	**8**	**10**
Aglycone	C(1)	131.95 (*s*)	133.3 (*s*)	133.3 (*s*)
	C(2)	116.57 (*d*)	113.0 (*d*)	114.4 (*d*)
	C(3)	146.13 (*s*)	147.7 (*s*)	150.4 (*s*)
	C(4)	144.80 (*s*)	147.4 (*s*)	149.1 (*s*)
	C(5)	117.54 (*d*)	117.4 (*d*)	113.3 (*d*)
	C(6)	121.54 (*d*)	121.5 (*d*)	122.6 (*d*)
	C(α)	72.05 (*t*)	71.9 (*t*)	71.7 (*t*)
	C(β)	36.75 (*t*)	36.7 (*t*)	36.8 (*t*)
	CH_3O		56.5 (*q*)	56.6 (2*q*)
Glucose	C(1′)	104.18 (*d*)	104.2 (*d*)	103.0 (*d*)
	C(2′)	81.45 (*d*)	81.3 (*d*)	81.4 (*d*)
	C(3′)	82.73 (*d*)	82.7 (*d*)	86.3 (*d*)
	C(4′)	70.68 (*d*)	70.7 (*d*)	70.6 (*d*)
	C(5′)	75.92 (*d*)	75.9 (*d*)	77.6 (*d*)
	C(6′)	62.49 (*t*)	62.5 (*t*)	62.7 (*t*)
Arabinose	C(1″)	103.10 (*d*)	103.1 (*d*)	104.4 (*d*)
	C(2″)	73.06 (*d*)	73.09 (*d*)	72.8 (*d*)
	C(3″)	74.56 (*d*)	74.6 (*d*)	74.3 (*d*)
	C(4″)	69.57 (*d*)	69.5 (*d*)	69.1 (*d*)
	C(5″)	66.88 (*t*)	66.8 (*t*)	66.8 (*t*)
Rhamnose	C(1‴)	103.27 (*d*)	103.2 (*d*)	103.4 (*d*)
	C(2‴)	72.17 (*d*)	72.2 (*d*)	72.3 (*d*)
	C(3‴)	72.13 (*d*)	72.1 (*d*)	72.2 (*d*)
	C(4‴)	73.88 (*d*)	73.0 (*d*)	73.9 (*d*)
	C(5‴)	70.95 (*d*)	71.0 (*d*)	70.5 (*d*)
	C(6‴)	18.51 (*q*)	18.5 (*q*)	17.9 (*q*)
Acyl moiety	C(1⁗)	127.70 (*s*)	127.7 (*s*)	
	C(2⁗)	114.86 (*d*)	111.9 (*d*)	
	C(3⁗)	146.89 (*s*)	149.4 (*s*)	
	C(4⁗)	149.87 (*s*)	150.9 (*s*)	
	C(5⁗)	116.37 (*d*)	116.6 (*d*)	
	C(6⁗)	123.27 (*d*)	124.4 (*d*)	
	C(α′)	115.29 (*d*)	115.3 (*d*)	
	C(β′)	148.02 (*d*)	147.9 (*d*)	
	C=O	168.39 (*s*)	168.3 (*s*)	
	CH_3O		56.6 (*q*)	

The structure of **8** has thus been established as 2-(3-hydroxy-4-methoxyphenyl)ethyl *O*-[α-L-arabinopyranosyl-(1 → 2)]-*O*-[α-L-rhamnopyranosyl-(1 → 3)]-4-*O*-feruloyl-β-D-glucopyranoside. Lagotoside (**8**) is the dimethyl derivative of ehrenoside (**5**), formerly isolated from *Veronica bellidioides* [5].

Experimental Part

General. See [9].

Plant Material. Lagotis stolonifera was collected in the vicinity of Karahamza village, Selim, Kars (East Anatolia), during May 1989. A voucher specimen has been deposited in the Herbarium of Pharmacognosy Department, Faculty of Pharmacy, Hacettepe University, Ankara, Turkey.

Extraction and Purification. The air-dried aerial parts of the plant (480 g) were extracted with MeOH (2 × 2 l). After evaporation of the combined MeOH extracts, the residue was partitioned between H_2O and petroleum ether to remove lipophilic substances. The aq. extract (50 g) was dissolved in H_2O and chromatographed over polyamide (250 g) eluting with H_2O, followed by H_2O with increasing MeOH content, to yield *Fractions A* (H_2O, 43.9 g), *B* (25% MeOH, 1.92 g), *C* (50% MeOH, 0.99 g), *D* (75% MeOH, 0.25 g), and *E* (MeOH, 0.63 g).

Isolation of Iridoids **1–4**. A portion of *Fraction A* (25 g) which was rich in iridoids was further chromatographed over silica gel (300 g) with $CHCl_3$/MeOH/H_2O 80:20:2 to 55:45:5 to give *Fractions A1* (177 mg), *A2* (622 mg), *A3* (8.55 g), *A4* (1.372 g), and *A5* (7.130 g). Further purification was performed by MPLC (352 × 18.5 mm, packed with *Sepralyte C18*) using a H_2O/MeOH gradient at a flow rate of 4.0 ml/min. *Fraction A2* yielded pure **4** (24 mg) and **3** (357 mg) with H_2O/MeOH (40–50% MeOH, stepwise gradient), while *Fraction A4* yielded **2** (154 mg) and **1** (165 mg) with H_2O/MeOH (0–40% MeOH, stepwise gradient).

Isolation of Phenylpropanoid Glycosides **5–8**. *Fraction B* (1.92 g) was rich in **5**. *Fraction C* (0.99 g) was chromatographed over silica gel (50 g) using $CHCl_3$/MeOH/H_2O 61:32:5 to give *Fractions C1* (274 mg) and *C2* (320 mg). They were separately subjected to MPLC using the same conditions with H_2O/MeOH (20–30% MeOH, stepwise gradient) to give **8** (36 mg) and **5** (41 mg). *Fraction D* (0.25 g) was also rich in phenylpropanoid glycosides. It was subjected to a series of chromatographic separations (silica gel CC and MPLC, respectively, as reported above for **5** and **8**) to yield **7** (31 mg) and **6** (10 mg).

Lagotoside (**8**). Amorphous, colourless powder. $[\alpha]_D^{20} = -33.8$ (H_2O, $c = 0.334$). UV (MeOH): 232, 262, 326. IR (KBr): 3400, 1700, 1630, 1595, 1510. ^{1}H-NMR, ^{13}C-NMR: *Tables 1* and *2*. FAB-MS (NOBA): 807 ($[M + Na]^+$), 784 (M^+).

Acetylation of **8**. Treatment of **8** (10 mg) with Ac_2O (0.5 ml) and pyridine (0.5 ml) at r.t. overnight, followed by column chromatography over silica gel using benzene/acetone 4:1 gave nonaacetate **9** (10.5 mg). ^{1}H-NMR: *Table 1*.

Methylation of **8**. Methylation of **8** (20 mg) with CH_2N_2 followed by alkaline hydrolysis (5% KOH in MeOH) resulted in the isolation of 3,4-dimethoxycinnamic acid and *2-(3,4-dimethoxyphenyl)ethyl* O-*[α-*L*-arabinopyranosyl-(1 → 2)]*-O-*[α-*L*-rhamnopyranosyl-(1 → 3)]-β-*D*-glucopyranoside* (**10**) which were separated over silica gel with $CHCl_3$/MeOH/H_2O 80:20:2. ^{1}H-NMR, ^{13}C-NMR: *Tables 1* and *2*.

Methylation of **5**. Methylation of **5** (20 mg) with CH_2N_2 followed by alkaline hydrolysis (5% KOH in MeOH) resulted in the isolation of 3,4-dimethoxycinnamic acid and deacylehrenoside bis(methyl ether), the latter being identified as **10** by TLC comparison with an authentic sample.

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