160. Lavandulifolioside: A New Phenylpropanoid Glycoside from *Stachys lavandulifolia*

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A new phenylpropanoid glycoside has been isolated from the methanolic extract of the aerial parts of *Stachys lavandulifolia* (Lamiaceae), lavandulifolioside (**1**). On the basis of chemical and spectral data the structure of the new compound **1** has been elucidated as β-(3,4-dihydroxyphenyl)ethyl *O*-α-L-arabinopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→3)-4-*O*-caffeoyl-β-D-glucopyranoside.

1. Introduction. – *Stachys lavandulifolia* VAHL *var. lavandulifolia* (Lamiaceae) is known as 'hairy tea' in Turkey. Since it is used as a herbal tea in the vicinities of Antalya (South Anatolia), it was investigated for its essential oil [1].

Caffeic acid esters such as chlorogenic and quinic acids are important substances from the point of view of chemotaxonomic studies, and their distribution in the Lamiaceae Family at the generic level has considerable value [2]. In respect of this consideration, we have further studied the water-soluble constituents of *Stachys lavandulifolia*.

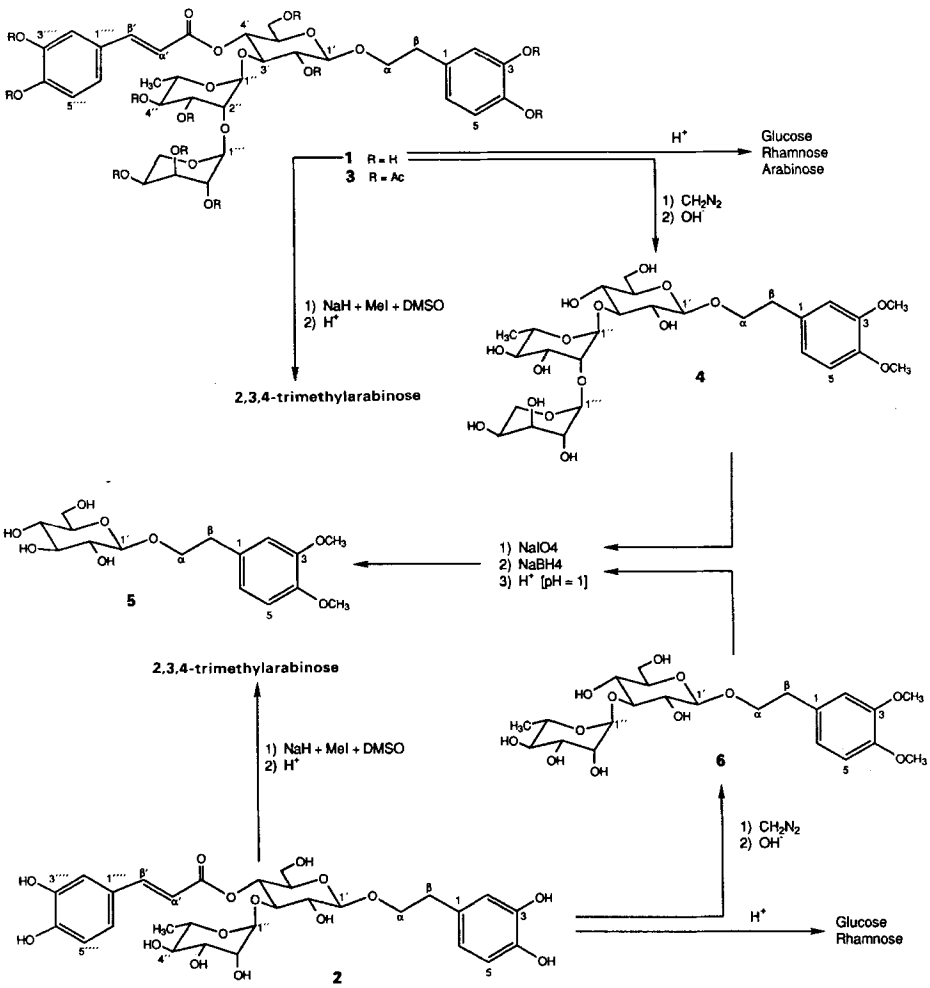
After a series of chromatographic separations of the methanolic extract, we isolated the fractions rich in two phenylpropanoid glycosides (caffeic-acid sugar esters). These compounds were purified by using reversed-phase vacuum liquid chromatography (RP-VLC), resulting in lavandulifolioside (**1**), a new phenylpropanoid glycoside and an already known glycoside, acteoside (**2**). We describe here the structural elucidation of the new substance, lavandulifolioside (**1**).

2. Results and Discussion. – Lavandulifolioside (**1**) was obtained as an amorphous substance with the mol.wt. 756.71 g (calc. for $C_{34}H_{44}O_{19}$): Positive-ion FAB-MS: 757 ($[M + H]^+$). Negative-ion FAB-MS: 755 ($[M - H]^-$). $[\alpha]_D^{20} = 40.3$ ($c = 0.595$, H_2O).

Its UV spectrum exhibited peaks at 203, 219 (sh), 242 (sh), 291 (sh), 333 nm, confirming its polyphenolic nature. IR bands (cm^{-1}) for OH groups (3400, br.), an α,β-unsaturated ester ($\tilde{\nu}$(C=O) 1685, $\tilde{\nu}$(C=C) 1620), and aromatic rings (1600, 1510) were observed.

The ^{1}H-NMR spectrum *(Table 1)* exhibited signals belonging to caffeic acid and 3,4-dihydroxyphenylethanol moieties in addition to the protons of three sugars: six

Scheme. *Chemical Degradation of Lavandulifolioside* (**1**) *and of Acteoside* (**2**)



aromatic protons (2 *ABX*, 6.56–7.05 ppm), the coupling constants of which gave the substitution pattern indicated in the formula, two (*Z*)-olefinic protons (6.27, 7.59 (*AB*, $J = 15.9$, 2 H)), and two CH_2 groups ($CH_2(\beta)$, *t*, 2.8; $CH_2(\alpha)$, 2*m*, 3.8–3.24, 4.04 ppm). Three signals of anomeric protons appeared as *d* at 5.47 ($J = 1.07$, H–C(1″)), 4.37 ($J = 7.9$, H–C(1′)), and 4.32 ppm (J=7.2, H–C(1‴)).

The signal of H–C(4′) was shifted about 1 ppm downfield (4.92 ppm, *t*, $J = 9.2$ Hz), indicating acylation at this location.

To elucidate the complete structure of **1**, the following chemical degradation methods were employed *(Scheme)*. Complete acid hydrolysis resulted in glucose, rhamnose, and arabinose. Permethylation by *Hakomori*'s method [3] and subsequent acid hydrolysis yielded only 2,3,4-trimethyl-L-arabinose as a totally methylated sugar, indicating it as a terminal sugar.

Table 1. *^{1}H-NMR Spectral Data of* **1** **3**, **4**, *and* **5**[a])

	Proton(s)	**1** (300 MHz)		**3**[b]) (600 MHz)	**4** (400 MHz)	**5** (80 MHz)
Aglycone	H–C(2)	6.70	(*d*, *J* = 1.9)	7.01–7.07	6.88	
	H–C(5)	6.68	(*d*, *J* = 8.0)	7.01–7.07	6.84	6.92–6.77[c])
	H–C(6)	6.56	(*dd*, *J* = 8.0, 1.9)	7.01–7.07	6.77	
	$CH_2(\alpha)$	4.04/3.8–3.24		4.10/3.61	4.06/3.75	3.96–2.96[c])
	$CH_2(\beta)$	2.8	(*t*, *J* = 7.2)	2.85	2.86	2.78
	CH_3O		(*s*)		3.81	3.74
	CH_3O		(*s*)		3.78	3.71
β-Glucose	H–C(1′)	4.37	(*d*, *J* = 7.9)	4.37 (*J* = 8.0)	4.29 (*J* = 7.85)	4.08
	H–C(2′)	3.8–3.24[c])		5.05 (*J* = 8.0)	3.27 (*J* = 9.13)	
	H–C(3′)			3.97 (*J* = 9.4)	3.45 (*J* = 9.00)	
	H–C(4′)	4.92	(*t*, *J* = 9.2)	5.20[c])	3.32 (*J* = 9.82)	3.96–2.96[c])
	H–C(5′)	3.8–3.24[c])		3.66 (*m*)	3.27 (*J* = 2.06, 5.50)	
	$H-C(6'_A)$			4.14 (*J* = 12.2, 2.8)	3.66 (*J* = 11.88, 2.06)	
	$H-C(6'_B)$	3.86	(*dd*)	4.18 (*J* = 12.2, 4.6)	3.85 (*J* = 11.88, 5.50)	
α-Rhamnose	H–C(1″)	5.47	(*d*, *J* = 1.07)	4.98 (*J* = 1.6)	5.44 (*J* = 2.04)	
	H–C(2″)	3.95	(*dd*, *J* = 1.07, 3)	3.90 (*J* = 1.6, 3.1)	3.97 (*J* = 3.32)	
	H–C(3″)			4.90 (*J* = 3.1, 10.1)	3.75 (*J* = 9.62)	
	H–C(4″)	3.8–3.24[c])		4.80 (*J* = 10.1)	3.38 (*J* = 9.49)	
	H–C(5″)			3.71 (*m*)	3.95 (*J* = 6.14)	
	$CH_3(6'')$	1.06	(*d*, *J* = 6.2)	1.04 (*J* = 6.2)	1.22	
α-Arabinose	H–C(1‴)	4.32	(*d*, *J* = 7.2)	4.31 (*J* = 6.7)	4.34 (*J* = 7.29)	
	H–C(2‴)			5.16 (*J* = 6.7, 9.0)	3.62 (*J* = 9.24)	
	H–C(3‴)	3.8–3.24[c])		4.99 (*J* = 9.0, 4.5)	3.50 (*J* = 3.60)	
	H–C(4‴)			5.20[c])	3.77 (*J* = 2.50, 1.61)	
	$CH_2(5''')$			3.92 (*J* = 13.0, 3.5)	3.84 (*J* = 12.3, 2.5)	
				3.54 (*J* = 13.0, 1.3)	3.52 (*J* = 12.3, 1.61)	
Caffeic acid	H–C(2⁗)	7.05	(*d*, *J* = 1.8)	7.34 (*J* = 1.9)		
	H–C(5⁗)	6.78	(*d*, *J* = 8.2)	7.21 (*J* = 8.4)		
	H–C(6⁗)	6.95	(*dd*, *J* = 8.2, 1.8)	7.38 (*J* = 8.4, 1.9)		
	H–C(α′)	6.27	(*d*, *J* = 15.9)	6.33 (*J* = 16)		
	H–C(β′)	7.59	(*d*, *J* = 15.9)	7.67 (*J* = 16)		

[a]) The spectra were recorded in CD_3OD (**1**, **4**), $CDCl_3$ (**3**), and $(D_6)DMSO$ (**5**). Chemical shifts in ppm relative to internal TMS.
[b]) Additional signals: 2.29, 2.28, 2.27, 2.26 (4 × CH_3COO, arom.); 2.10, 2.08, 2.07, 2.02, 1.99, 1.90, 1.67 (7 × CH_3COO, aliph.).
[c]) Signal pattern unclear due to overlapping.

Acetylation of **1** gave undecaacetate **3**. The ^{1}H-NMR spectrum of **3** revealed the presence of 11 Ac signals belonging to four aromatic and seven aliphatic Ac groups *(Table 1)*.

Partial methylation of **1** with CH_2N_2 followed by alkaline hydrolysis yielded deacylavandulifolioside dimethyl ether (**4**) (mol.wt.622 (calc. for $C_{27}H_{42}O_{16}$)). Positive FAB-MS: 623 ($[M + H]^+$). Negative FAB-MS: 621 ($[M - H]^-$). ^{1}H-NMR *(Table 1)*. Permethylation of **4** followed by acid hydrolysis gave 2,3,4-trimethylarabinose as the terminal sugar, which supported the proposed structure. To obtain further information on the structure of the sugar chain, **4** was treated with $NaIO_4$ giving **5** as a final product, which was also obtained from compound **6** in the same way. The action of $NaIO_4$ oxidation on lavandulifolioside affected arabinose which has either 1‴→2″ or 1‴→4″ bonds with rhamnose, and rhamnose which is bonded with glucose as 1″→3′. These results were also supported by the FAB-MS spectrum of **4**. $[M + Na]^+$ ion (*m/z* 645) was observed as the base peak. The M^+ ion at *m/z* 622 was recorded with 33% relative abundance. In addition, the characteristic fragments resulting from the cleavage of the interglycosidic linkages at *m/z* 491, 441, 345, 279, 183, and 133 were obtained. These findings also indicated the sequences of the sugars in the oligoglycosidic chain to be arabinose→rhamnose→glucose→aglycone.

The interglycosidic linkages, especially the site of attachment of arabinose to rhamnose, as well as the assignment of the chemical shifts of the partially overlapping ring protons of the three sugar moieties, were assured using various techniques of 2D-NMR spectroscopy. The assignment of the sugar protons *(Table 1)* was achieved by homonu-

Table 2. *^{13}C-NMR Spectral Data of* **1** (CD_3OD, 75.47 MHz) *and* **4** (CD_3OD, 100 MHz)[a])

C-Atom	**1**		**4**	C-Atom	**1**		**4**
Aglycone				Rhamnose			
C(1)	131.66	*s*	133.13	C(3″)	72.29	*d*	72.15
C(2)	116.62	*d*	114.10	C(4″)	74.36	*d*	74.37
C(3)	146.19	*s*	150.27	C(5″)	70.68	*d*	69.94
C(4)	144.72	*s*	148.91	C(6″)	18.50	*q*	17.90
C(5)	117.23	*d*	113.07				
C(6)	121.34	*d*	122.29	Arabinose			
C(α)	72.08	*t*	71.85	C(1‴)	107.43	*d*	107.43
C(β)	36.65	*t*	36.70	C(2‴)	72.95	*d*	72.85
CH_3O			56.53	C(3‴)	74.47	*d*	74.37
CH_3O			56.44	C(4‴)	69.87	*d*	69.82
				C(5‴)	67.32	*t*	67.29
Glucose				Caffeic acid			
C(1′)	104.30	*d*	104.14	C(1⁗)	127.78	*s*	
C(2′)	76.12	*d*	75.56	C(2⁗)	114.83	*d*	
C(3′)	82.42	*d*	84.76	C(3⁗)	146.87	*s*	
C(4′)	70.42	*d*	70.17	C(4⁗)	149.82	*s*	
C(5′)	76.12	*d*	77.85	C(5⁗)	116.42	*d*	
C(6′)	62.48	*t*	62.66	C(6⁗)	123.23	*d*	
				C(α′)	115.38	*d*	
Rhamnose				C(β′)	148.03	*d*	
C(1″)	102.06	*d*	101.67	C=O	168.34	*s*	
C(2″)	82.74	*d*	82.59				

[a]) Chemical shifts in ppm relative to internal TMS.

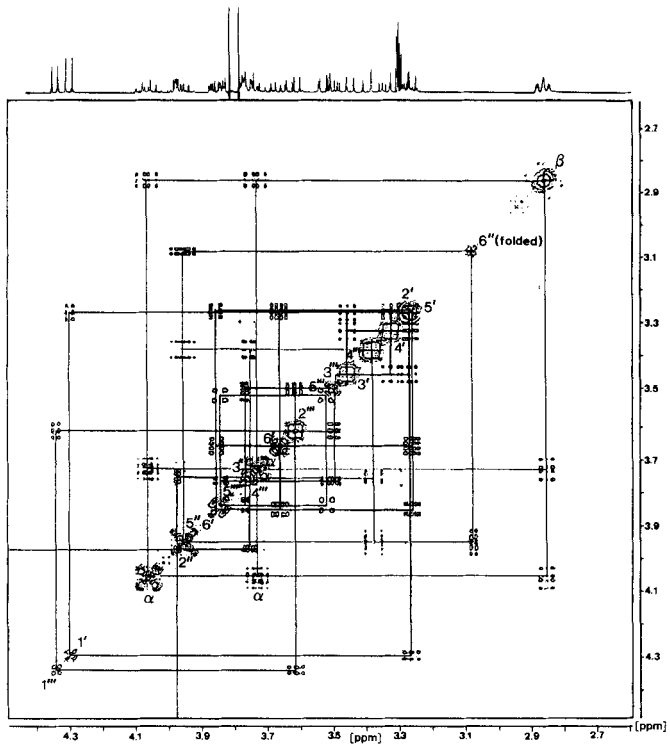


Fig. 1. *2D-^1H,^1H-NMR COSY spectrum of lavandulifolioside undecaacetate* (**3**)

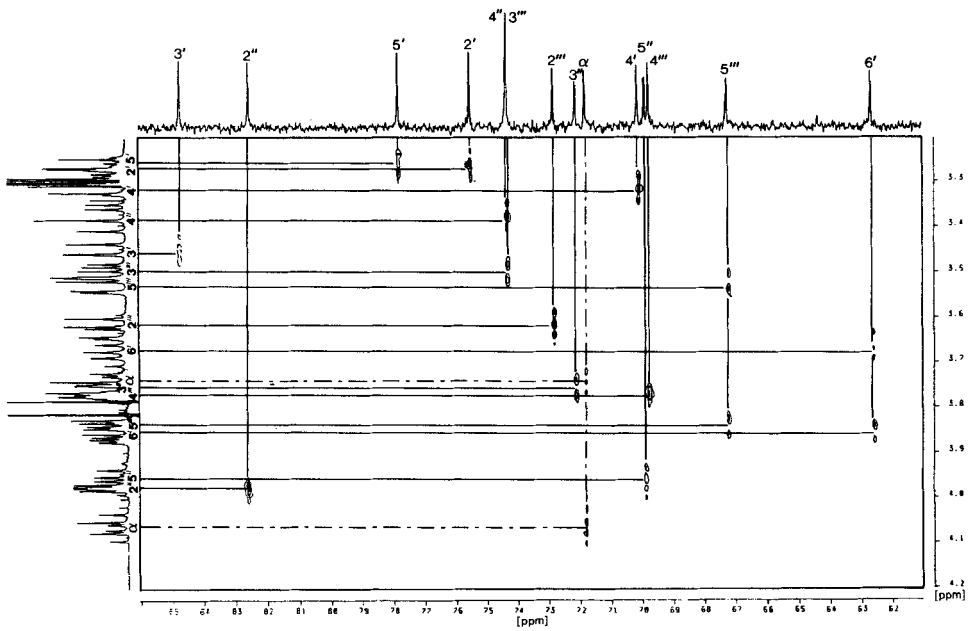


Fig. 2. *C,H-Heteronuclear-correlated 2D-NMR spectrum of deacyllavandulifolioside dimethyl ether* (**4**)

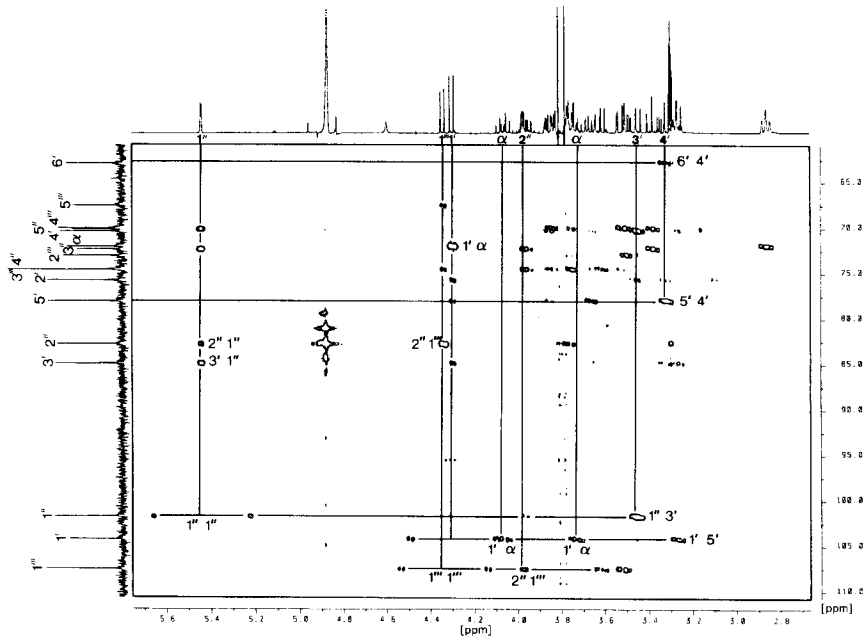


Fig. 3. *Long-range inverse-correlated spectrum of deacyllavandulifolioside dimethyl ether* (**4**)

clear-correlated 2D-NMR spectra of **3** *(Fig. 1)* and **4**. The ^{13}C-NMR signals of **4** *(Table 2)* were assigned on the basis of comparison with those of similar compounds, such as teucrioside [4], ehrenoside [5], and angoroside A [6] and with the help of C,H heteronuclear-correlated 2D-NMR spectra, one for establishing direct C–H bonding *(Fig. 2)*, and another for long-range coupling relations *(Fig. 3)*. The long-range inverse-correlated spectrum of deacyllavandulifolioside dimethyl ether (**4**) *(Fig. 3)* made clear that the sugar sequences must be as α-L-arabinose (1‴→2″)-α-L-rhamnose (1″→3′)-β-D-glucose [7].

The 2D-^{1}H, ^{1}H-NMR-COSY spectrum of **3** *(Fig. 1, Table 1)* confirmed these results by obtaining no downfield shifts upon acetylation for H–C(2″) (3.9 (*dd*, $J(2'',1'') = 1.6$, $J(2'', 3'') = 3.1$, 1 H)) or for H–C(3′) (3.97 (*dd*, $J(3',2') = 8$, $J(3',4') = 9.4$, 1 H)).

Based on the results mentioned above, the structure of lavandulifolioside (**1**) was established as β-(3,4-dihydroxyphenyl)ethyl *O*-α-L-arabinopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→3)-4-*O*-caffeoyl-β-D-glucopyranoside. Acteoside (**2**) was identified by direct comparison with an authentic sample using TLC. Spectral data of **2** (UV, IR, ^{1}H-NMR, and ^{13}C-NMR) were identical to those published for the authentic compound [8].

Experimental Part

General. UV spectra (λ_{max}) were determined in spectroscopic-grade MeOH *(Merck)* on a *Hitachi 220S* spectrophotometer. IR spectra (cm^{-1}) were determined on a *Perkin-Elmer 257* instrument in KBr pellets. Optical rotations were measured with a *Perkin-Elmer 141* polarimeter. ^{1}H- and ^{13}C-NMR spectra (δ [ppm], J [Hz]) were obtained at 80, 300.13, 400, and 600 MHz (^{1}H-NMR) and at 75.47 and 100 MHz (^{13}C-NMR) in FT mode using *Bruker WM 80* and *Bruker WM 300* (1D), and *AM 400, AM 600* (2D) instruments with TMS as an internal standard. For the ^{1}H, ^{1}H-correlated spectra (COSY), [9] 512 increments of $2k$ data points and 8 transients were measured. Prior to *Fourier* transformation, the data were multiplied with a cosine function in both dimensions.

In the $^1H,^{13}C$-correlated spectra, the following parameters were used: the standard sequence of *Bax* and *Morris* [10] was used with 256 increments of $2k$ data points and 32 transients each. Zero filling in f_1, multiplication with shifted sine bell functions in both dimensions, and two dimensional *Fourier* transformation resulted in the 256 words × $1k$ data matrix shown in *Fig. 2*.

The inverse detection, long-range $^1H,^{13}C$ correlation was acquired with a pulse sequence described by *Bax* and *Summers* [7]. 720 increments of $4k$ points each were measured with the delays set for a 5 Hz C,H coupling. Shifted sine bell functions were used prior to *Fourier* transformation. The resulting $2k$ × $1k$ data matrix is shown in *Fig. 3*. The 2D spectra were evaluated as contour plots.

FAB-MS were recorded with a *Kratos AEI-MS 50* and *JEOL JMS-D 300* mass spectrometers in glycerin, *m*-nitrobenzyl alcohol (NOBA), or thioglycerol at 8.3 keV. Polyamide *(Woelm)*, silica gel *60* (70–230 mesh, *Merck*), and *Sephadex LH-20 (Pharmacia)* were used for CC. The reversed-phase material of prep. *PAK (500/C18)* cartridges was used for the RP-VLC (reversed phase-vacuum liquid chromatography). Silica-gel-*60-F_{254} (Merck)* TLC plates and *Schleicher Schüll 2043 a Mgl* paper for PC were used. Phenylpropanoids were detected by UV fluorescence and/or spraying with 1% vanillin-H_2SO_4 and 5% ferrichloride-H_2O, and sugars by aniline phthalate reagent followed by heating at 100° for 5–10 min.

Extraction and Purification. The plant material of *Stachys lavandulifolia* VAHL *var. lavandulifolia* was collected from the vicinity of Gündoğmuş, Antalya, Turkey. The air-dried aerial parts of the plant (450 g) were cut into small pieces and extracted with MeOH at 40° (2 × 2 l). After concentration of the combined extracts under vacuum, 0.5 l of H_2O was added, and the H_2O-insoluble material was removed by filtration. The filtrate was exhaustively extracted with petroleum ether to remove lipoid substances. The aqueous phase was then extracted twice with BuOH, and the combined org. phase evaporated under vacuum to dryness yielding 17 g (3.78%). This crude extract was chromatographed over polyamide (100 g) with H_2O, MeOH/H_2O 5–50%. Three fractions *A–C* were collected.

Isolation of Lavandulifolioside (**1**). *Fraction B* was rechromatographed over *Sephadex LH-20* with MeOH, and three fractions *B1–B3* were collected. *Fraction B1* (crude **1**, 850 mg) was subjected to RP-VLC, applying 50 mg of crude **1** in each case to a column prepared with 20 g of reversed-phase material. The system used was a stepwise H_2O/MeOH (80:20, 75:25, 70:30 and 65:35) gradient at a flow rate of 2 ml/min under vacuum. *Data of* **1**: $[\alpha]_D^{20} = -40.3$ ($c = 0.595$, H_2O). UV (MeOH): 203 (4.60), 219 (4.32), 242 (sh), 291 (sh), 333 (4.30). IR (KBr): 3400 (br., OH), 1685 (C=O), α,β-unsaturated ester), 1620 (C=C), 1600, 1510 (arom. ring). ^1H-NMR and ^{13}C-NMR: *Tables 1* and *2*. Negative-ion FAB-MS (glycerin): 755 ($[M - H]^-$), 623 ($[M$ – arabinosyl$]^-$), 593 ($[M$ – caffeoyl$]^-$), 477 ($[M$ – arabinosyl – rhamnosyl$]^-$), 460 ($[M$ – caffeoyl – arabinosyl$]^-$). Positive-ion FAB-MS (glycerin): 757 ($[M + H]^+$). Positive-ion FAB-MS (thioglycerol): 774 ($[M + NH_4]^+$).

Acetylation of **1**. Treatment of **1** (20 mg) with Ac_2O (1 ml) and pyridine (1 ml) at r.t. overnight followed by the usual workup yielded the undecaacetate **3**. $[\alpha]_D^{20} = -53$ ($c = 0.420$, $CHCl_3$). IR (KBr): 1750 (C=O), 1635 (C=C), 1500 (arom. ring). ^1H-NMR: *Table 1* and *Fig. 1*.

Acid Hydrolysis of **1**. Compound **1** (10 mg) was dissolved in 5% HCl and heated at 100° for 2 h, cooled and filtered. The filtrate was neutralized passing through *Dowex* (Cl^- form) and evaporated to dryness. The residue was examined for sugars by PC (descending method) using BuOH/pyridine/H_2O 9:5:4.

Methylation of **1**. Methylation of **1** (30 mg) by *Hakomori*'s method [3] followed by acid hydrolysis yielded 2,3,4-tri-*O*-methylarabinopyranoside which was identified by comparison with an authentic sample (TLC, benzene/acetone 2:1 and benzene/EtOH 4:1). Methylation of **1** (40 mg) with CH_2N_2 followed by alkaline hydrolysis resulted in 3,4-dimethoxycinnamic acid and deacyllavandulifolioside dimethyl ether (**4**), which were separated on *Sephadex LH-20* with MeOH to give a colourless powder (**5**). UV (MeOH): 204 (4.38), 228 (3.87), 279 (3.41). IR (KBr): 3400, 1630, 1590, 1510. ^1H-NMR and ^{13}C-NMR: *Tables 1* and *2*. Negative-ion FAB-MS (glycerin): 621 ($[M - H]^-$). Positive-ion FAB-MS (glycerin): 622 (M^+), 623 ($[M + H]^+$), 645 ($[M + Na]^+$), 491 ($[M$ – arabinosyl$]^+$), 441 ($[M$ – (β-(3,4-dihydroxyphenyl)ethyl alcohol)$]^+$), 345 ($[M$ – arabinosyl – rhamnosyl$]^+$), 279, 183, 181, 133.

$NaIO_4$ Oxidation of **1** *and* **2**. Solns. of 15 mg of each glycoside **1** and **2** in 10 ml of H_2O were treated with 0.1 g of $NaIO_4$ separately; the mixtures were left at r.t. for 12 h in the dark. The unchanged periodate was destroyed by addition of a few drops of ethylene glycol. The mixture was evaporated, and the residue was dissolved in 20 ml of H_2O and extracted with BuOH (5 × 10 ml). The BuOH extracts were washed with 10 ml of H_2O and 0.1 g of $NaBH_4$; the mixture was kept stirring during the process. Then, it was neutralized with dil. HCl and evaporated with the addition of MeOH (3 × 10 ml) to remove boric acid. The product was dissolved in H_2O and extracted with BuOH (3 ×). The BuOH extracts were combined and evaporated to dryness. The residue was dissolved in H_2O and adjusted to pH 1 with dil. HCl and kept at 50°. After 2 h, the hydrolisate was extracted with BuOH. The BuOH extracts were combined and evaporated to dryness. The resulting residue was purified by silica gel CC using $CHCl_3$/MeOH/H_2O 80:20:2 to give pure **5**. ^1H-NMR: *Table 1*.

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