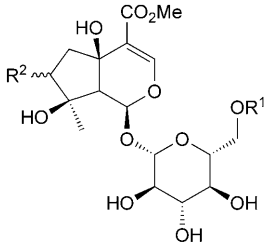
New Iridoid Glycosides from *Lamium eriocephalum* subsp. *eriocephalum*

by **Funda Nuray Yalçın***[a]), **Tayfun Ersöz**[b]), **Kürşat Avcı**[c])[1]), **Charlotte H. Gotfredsen**[d]), **Søren R. Jensen**[d]), and **İhsan Çalış**[b])

[a]) Department of Pharmaceutical Management, Faculty of Pharmacy, Hacettepe University, TR-06100, Ankara (phone: +90-312-3051089; fax: +90-312-3114777; e-mail: funyal@hacettepe.edu.tr)
[b]) Department of Pharmacognosy, Faculty of Pharmacy, Hacettepe University, TR-06100 Ankara
[c]) Department of Pharmaceutical Botany, Faculty of Pharmacy, Hacettepe University, TR-06100 Ankara
[d]) Department of Chemistry, The Technical University of Denmark, Building 201, DK-2800 Lyngby

Two new iridoid glycosides, eriobioside (**1**) and lamerioside (**2**), were isolated from the aerial parts of *Lamium eriocephalum* subsp. *eriocephalum*, along with the two known compounds lamiide (**3**) and ipolamiide (**4**). Their structures were elucidated by spectroscopic methods (UV, 1D- and 2D-NMR) and by mass spectrometry (HR-ESI-MS).

Introduction. – The genus *Lamium* (Lamiaceae) is represented by 27 species in the flora of Turkey [1]. *Lamium album* and *L. maculatum* have been used in Anatolian folk medicine as tonics [2]. As a part of our ongoing phytochemical studies on the secondary metabolites of Turkish *Lamium* species [3], we have studied the iridoid glycosides of *L. eriocephalum* BENTHAM subsp. *eriocephalum*. Herein, we report two new iridoid glycosides, eriobioside (**1**) and lamerioside (**2**), from the title plant, together with two known iridoid glucosides, lamiide (**3**) and ipolamiide (**4**).



	R^1	R^2
1	Glc	H
2	H	α-OH
3	H	β-OH
4	H	H

Glc = β-D-Glucopyranosyl

Results and Discussion. – The H_2O-soluble part of the crude MeOH extract of the aerial parts of *L. eriocephalum* was subjected to medium-pressure liquid chromatography (MPLC) on a C_{18} column, eluting with a H_2O/MeOH gradient to yield five main fractions. Further column-chromatographic separations on silica gel finally afforded the iridoid glycosides **1**–**4**.

[1]) In memory of *Kürşat Avcı* (B. Sc.), 1972–2003.

Compound **1** was obtained as an optically active, amorphous powder. ESI-MS showed the $[M+Na]^+$ peak at *m/z* 591, corresponding to the molecular formula $C_{23}H_{36}O_{16}$. The UV spectrum showed an absorption maximum at 229 nm, indicating an α,β-unsaturated C=O moiety. Analysis of the ^{13}C-NMR (DEPT) spectrum of **1** (*Table 1*) revealed the presence of 23 carbon signals, twelve of which were assigned to two hexose units. The remaining 11 resonances, along with the corresponding ^{1}H-NMR signals, were indicative of a C_{10} iridoid skeleton bearing a MeOOC group at C(4). The ^{1}H-NMR spectrum of **1** (*Table 1*) exhibited signals due to an enol ether conjugated to a MeOOC group (δ(H) 7.43 (*s*, H–C(3)); 3.73 (*s*, MeOOC)), two CH_2 groups (δ(H) 2.26 (*ddd*, J=13.5, 8.2, 5.1 Hz, H_α–C(6)); 2.09 (*ddd*, J=14.3, 9.5, 7.9 Hz, H_β–C(6)); 1.97 (*dd*, J=12.2, 8.6 Hz, H_α–C(7)); 1.56 (*ddd*, J=12.2, 6.9, 5.2 Hz, H_β–C(7))), and a Me group at δ(H) 1.15 (*s*, Me(10)). The resonances at δ(H) 4.61 (*d*, J=7.9 Hz, H–C(1′)) and 4.42 (*d*, J=7.8 Hz, H–C(1″)) were attributed to the two anomeric H-atoms of the hexose units. The corresponding ^{13}C-NMR resonances were observed at δ(C) 99.7 and 105.1, respectively. The chemical shifts and coupling constants of the sugar signals indicated the presence of two β-glucopyranosyl (Glc) moieties.

The complete assignments of the remaining signals of **1** were made by 2D-NMR experiments (^{1}H,^{1}H-COSY, ^{1}H,^{13}C-HMQC, HMBC) as well as by NOESY analysis. The ^{1}H-NMR signal at δ(H) 7.43 (*s*), assigned to H–C(3), showed that C(4) and C(5) were fully substituted. The assigned NMR data of **1** were almost identical to those of ipolamiide (**4**) [4], except for the presence of additional signals arising from a second Glc unit. The resonance for C(6′) at δ(C) 69.9 was considerably shifted downfield ($\Delta\delta$=7 ppm), and C(5′) was slightly shifted upfield (1 ppm) relative to the corresponding signals of ipolamiide (*Table 1*). Therefore, the second Glc unit was attached to the O-atom at C(6′). This was verified by an HMBC cross-peak between CH_2(6′) and C(1″). Thus, the disaccharide moiety was identified as a 6′-*O*-β-glucopyranosyl-β-glucopyranosyl (=gentiobiosyl) unit. From these data, the structure of compound **1** was established as ipolamiide 6′-*O*-β-glucopyranoside, and named *eriobioside*[2]).

Compound **2** was obtained as an optically active, amorphous powder. The molecular formula $C_{17}H_{26}O_{12}$ was determined by LC/HR-ESI-MS, showing the $[M+HCOO]^-$ peak at *m/z* 467, in good agreement with 17 observed resonances in the ^{13}C-NMR spectrum (*Table 2*). The UV spectrum exhibited a maximum at 229 nm, suggesting a conjugated enol ether.

The ^{1}H-NMR spectrum of **2** (*Table 2*) displayed characteristic signals for a C_{10} iridoid, bearing an MeOOC group at C(4) (δ(H) 7.43 (*s*, H–C(3); 3.75 (*s*, MeOOC)), a CH_2 group (δ(H) 2.54 (*dd*, J=14.8, 8.2 Hz, H_α–C(6)); 1.81 (*dd*, J=14.7, 11.2 Hz, H_β–C(6))), an oxymethine (δ(H) 4.18 (*dd*, J=11.1, 8.2 Hz, H–C(7))), and a Me group (δ(H) 1.03 (*s*, Me(10))). The anomeric sugar resonance at δ(H) 4.61 (*d*, J=7.9 Hz) and the signals at δ(H) 3.19–3.90, together with the corresponding ^{13}C-NMR resonances, indicated the presence of a β-Glc unit. Also, the ^{1}H- and ^{13}C-NMR data of **2** were very similar to those of lamiide (**3**) [5] (*Table 2*). However, both the chemical shifts and coupling constants of CH_2(6) and H–C(7) of **2** suggested that the 7-OH

[2]) For systematic names, see *Exper. Part.*

Table 1. *^{1}H- and ^{13}C-NMR Data* of **1** *and* **4**, *and HMBC Correlations for* **1**. In CD_3OD; δ in ppm, J in Hz. Asterisks (*) mark overlapping signals. Arbitrary atom numbering.

Atom	**1**[a])			**4** [4][b])	
	δ(H)	δ(C)	HMBC (H → C)	δ(H)	δ(C)
H–C(1)	5.80 (*s*)	94.5	C(1′), C(3), C(5), C(8)	5.81 (*s*)	94.1
H–C(3)	7.43 (*s*)	152.7	C(1), C(4), C(5), C(11)	7.44 (*s*)	152.6
C(4)		115.3			115.1
C(5)		71.7			71.6
CH_2(6)	2.26 (*ddd*, *J* = 13.5, 8.2, 5.2), 2.09 (*ddd*, *J* = 14.3, 9.5, 7.9)	38.9	C(5), C(7), C(8)	2.26 (*m*), 1.92 (*m*)	38.8
H–C(7)	1.97 (*dd*, *J* = 12.2, 8.6), 1.56 (*ddd*, *J* = 12.2, 6.9, 5.2)	40.5	C(5), C(6), C(8)	2.10 (*m*), 1.59 (*m*)	40.3
C(8)		79.0			78.9
H–C(9)	2.50 (*s*)	61.8	C(1), C(4), C(5), C(8), C(10)	2.48 (*s*)	61.6
Me(10)	1.15 (*s*)	23.4	C(7), C(8), C(9)	1.15 (*s*)	23.2
C(11)		168.1			168.0
MeO	3.73 (*s*)	51.7	C(11)	3.73 (*s*)	51.7
H–C(1′)	4.61 (*d*, *J* = 7.9)	99.7	C(1)	4.58 (*d*, *J* = 7.9)	99.5
H–C(2′)	3.19 (*t*, *J* = 8.0)	74.4	C(1′)	3.20 (*dd*, *J* = 7.9, 9.5)	74.3
H–C(3′)	3.38*	77.9		3.46 (*t*, *J* = 9.2)	77.3
H–C(4′)	3.31*	71.8		3.42 (*t*, *J* = 9.0)	71.4
H–C(5′)	3.51*	77.4		3.50 (*m*)	78.3
CH_2(6′)	4.20 (*dd*, *J* = 11.8, 1.8), 3.80 (*dd*, *J* = 11.8, 6.2)	69.9	C(1″)	3.90 (*dd*, *J* = 12.0, 1.8), 3.71 (*dd*, *J* = 12.0, 5.8)	62.8
H–C(1″)	4.42 (*d*, *J* = 7.8)	105.1	C(6′)		
H–C(2″)	3.21 (*t*, *J* = 8.0)	74.4	C(1″)		
H–C(3″)	3.36*	77.5			
H–C(4″)	3.31*	71.9			
H–C(5″)	3.28*	78.0			
CH_2(6″)	3.88 (*d*, *J* = 11.8), 3.67 (*dd*, *J* = 11.8, 4.1)	62.8			

[a]) At 400 and 100 MHz, resp. [b]) At 500 and 125 MHz, resp.

group was α-oriented in **2**, as in daunoside [6]. To corroborate the relative configuration of the 7-OH function, a 2D-NOESY experiment was performed. Correlations between H_β–C(6)/H–C(7) and H–C(7)/H–C(9) established the β-orientation of H_β–C(6), H–C(7), and H–C(9). Therefore, the 7-OH group had, indeed, to be in α-position. From these data, the structure of compound **2** was identified as 7-epilamiide, and named lamerioside.

The two known iridoid glucosides, lamiide (**3**) [5] and ipolamiide (**4**) [4], were identified by comparing their 1D- and 2D-NMR spectra as well as their ESI-MS data with those published in the literature.

Iridoid monoglucosides with MeOOC or Me groups in 4-position are considered as chemotaxonomic markers for *Lamium* species [6–9]. Eriobioside (**1**), with a gentiobiosyl moiety, is the first iridoid diglycoside isolated from this genus. Also, 7-epiiridoids show a very restricted distribution in the plant kingdom [6][7], lamerioside (**2**) being the first such representative within the genus *Lamium*.

Table 2. *^{1}H- and ^{13}C-NMR Data* of **2** *and* **3**, *and HMBC Correlations for* **2**. In CD_3OD; δ in ppm, J in Hz. Arbitrary atom numbering.

	2[a])			**3** [5][b])	
	δ(H)	δ(C)	HMBC (H → C)	δ(H)	δ(C)
H–C(1)	5.83 (*s*)	93.5	C(3), C(5), C(1′)	5.82 (*s*)	94.6
H–C(3)	7.43 (*s*)	152.1	C(1), C(4), C(5), C(11)	7.43 (*s*)	152.5
C(4)		116.0			115.5
C(5)		66.3			69.3
CH_2(6)	2.54 (*dd*, J=14.8, 8.2),	47.0	C(5), C(7), C(8)	2.36 (*dd*, J=14.9, 5.2),	46.8
	1.81 (*dd*, J=14.8, 11.2)			2.25 (*dd*, J=14.9, 3.4)	
H–C(7)	4.18 (*dd*, J=11.1, 8.2)	78.4	C(8), C(10)	3.52 (*dd*, J=4.9, 3.4)	77.9
C(8)		79.8			79.2
H–C(9)	2.50 (*s*)	59.0	C(1), C(4), C(10)	2.78 (*s*)	58.2
Me(10)	1.03 (*s*)	15.9	C(7), C(8), C(9)	1.09 (*s*)	21.3
C(11)		168.0			168.1
MeO	3.75 (*s*)	51.7	C(11)	3.73 (*s*)	51.7
H–C(1′)	4.61 (*d*, J=7.9)	99.6	C(1)	4.59 (*d*, J=7.9)	99.7
H–C(2′)	3.19 (*t*, J=9.1)	74.5	C(1′)	3.18 (*dd*, J=9.2, 7.9)	74.5
H–C(3′)	3.38 (*t*, J=8.9)	77.5		3.38 (*t*, J=8.5)	77.5
H–C(4′)	3.33 (*t*, J=8.9)	71.8		3.27 (*dd*, J=9.5, 8.8)	71.7
H–C(5′)	3.36 (*ddd*, J=8.9, 5.8, 2.0)	77.9		3.33 (*m*)	78.5
CH_2(6′)	3.90 (*dd*, J=11.9, 2.0),	62.9		3.89 (*dd*, J=11.9, 2.1),	62.8
	3.67 (*dd*, J=11.9, 5.8)			3.67 (*dd*, J=11.9, 6.0)	

[a]) At 400 and 100 MHz, resp. [b]) At 500 and 125 MHz, resp.

We wish to thank Dr. *Özgür Özsar* (Department of Chemistry, Faculty of Science, Hacettepe University, Ankara) for recording NMR spectra and Dr. *Kristian Fog Nielsen* (BioCentrum, Technical University of Denmark) for recording the LC/HR-ESI mass spectrum of **2**. This study was supported by Hacettepe University (0302301011).

Experimental Part

General. Medium-pressure liquid chromatography (MPLC): *Büchi* glass column (i.d. 3×24 cm) packed with *LiChroprep RP-18* (40–63 μm; *Merck*), with *Büchi-681* chromatography pump. Column chromatography (CC): silica gel *60* (0.063–0.200 mm; *Merck*). TLC: precoated *Kieselgel 60 F_{254}* (*Merck*) aluminum plates, elution with $CHCl_3$/MeOH/H_2O mixtures; visualization by spraying with 1% vanillin in conc. H_2SO_4, followed by heating at 105° for 1–2 min. UV Spectra: *M-Quant Biomolecular* spectrophotometer; λ_{max} (log ε) in nm. Optical rotations: *Rudolph Autopol-IV Automatic* polarimeter. NMR Spectra: *Bruker Avance-400* spectrometer; at 400 (^{1}H) and 100 MHz (^{13}C); δ in ppm rel. to Me_4Si, J in Hz. ESI-MS: *Waters ZQ* mass spectrometer. LC/HR-ESI-MS: *Agilent HP-1100* liquid chromatograph equipped with a *BDS-C-18* reverse-phase column coupled to a *Micromass TOF* mass spectrometer; in m/z.

Plant Material. The aerial parts of *Lamium eriocephalum* BENTHAM subsp. *eriocephalum* were collected from Niğde, Aladağlar, Southeast Anatolia, in June 2002, and identified by Prof. Dr. *Hayri Duman* (Department of Biology, Faculty of Science, Gazi University, Ankara). A voucher specimen (HUEF 02046) was deposited at the Herbarium of the Faculty of Pharmacy, Hacettepe University, Ankara, Turkey.

Extraction and Isolation. The air-dried, powdered aerial parts of *L. eriocephalum* (100 g) were extracted with MeOH (4×1.0 l, 5 h each) at 40°, and then filtered. The combined MeOH extracts

were evaporated to dryness under reduced pressure. The crude extract (12 g) was taken up in H_2O (100 ml), and the water-soluble portion was successively extracted with CH_2Cl_2 (4 × 100 ml) and BuOH (4 × 100 ml). The remaining aq. phase was evaporated to afford 5.2 g of crude remainder. An aliquot of the aq. extract (2 g) was subjected to RP-MPLC (*LiChroprep RP-18*; MeOH/H_2O 0 → 100% in 25% steps, 250 ml each): five main fractions (*Fr. A–Fr. E*): *Fr. B* afforded **2** (18 mg). *Fr. C* yielded **3** (7 mg). *Fr. D* (200 mg) was subjected to CC (20 g SiO_2; AcOEt/MeOH/H_2O 100 : 5 : 2, 100 : 10 : 5, and 100 : 17 : 13, 300 ml each) to afford **1** (10 mg) and **4** (21 mg).

Eriobioside (= *Methyl (1S*,4aR*,7S*)-1-[(6-O-β-D-Glucopyranosyl-β-D-glucopyranosyl)oxy]-1,4a,5,6,7,7a-hexahydro-4a,7-dihydroxy-7-methylcyclopenta[c]pyran-4-carboxylate*; **1**). Amorphous, colorless powder. $[\alpha]_D^{20} = -70$ ($c = 0.1$, MeOH). UV (MeOH): 229 (3.30). ^{1}H- and ^{13}C-NMR: see *Table 1*. ESI-MS: 591 ($[M+Na]^+$). HR-ESI-MS: 591.1923 ($[M+Na]^+$, $C_{23}H_{36}NaO_{16}^+$; calc. 591.1901).

Lamerioside (= *Methyl (1S*,4aR*,6R*,7R*)-1-(β-D-Glucopyranosyloxy)-1,4a,5,6,7,7a-hexahydro-4a,6,7-trihydroxy-7-methylcyclopenta[c]pyran-4-carboxylate*; **2**). Amorphous, colorless powder. $[\alpha]_D^{20} = -170$ ($c = 0.1$, MeOH). UV (MeOH): 229 (3.50). ^{1}H- and ^{13}C-NMR: see *Table 2*. ESI-MS (pos.): 445 ($[M+Na]^+$, $C_{17}H_{26}NaO_{12}^+$). HR-ESI-MS (neg.): 467.1395 ($[M+HCOO]^-$, $C_{18}H_{27}O_{14}^-$; 467.1401).

REFERENCES

[1] R. R. Mill, in 'Flora of Turkey and the East Aegean Islands', Ed. P. H. Davis, University Press, Edinburgh, 1982, Vol. 7, pp. 126–148.
[2] T. Baytop, 'Therapy with Medicinal Plants in Turkey: Past and Present', Nobel Tıp Kitabevleri, İstanbul, 1999, p. 163.
[3] T. Ersöz, D. Kaya, F. N. Yalçın, C. Kazaz, E. Palaska, C. H. Gotfredsen, S. R. Jensen, İ. Çalış, *Turk. J. Chem.*, submitted.
[4] T. Ersöz, Ü. Ş. Harput, İ. Çalış, *Turk. J. Chem.* **2002**, *26*, 1.
[5] A. M. Assaad, N. El-Sebakhy, M. F. I. Lahloub, S. A. A. El-Khayat, *Alexandria J. Pharm. Sci.* **1992**, *6*, 183.
[6] C. A. Boros, F. R. Stermitz, *J. Nat. Prod.* **1990**, *53*, 1055.
[7] L. J. El-Naggar, J. L. Beal, *J. Nat. Prod.* **1980**, *43*, 649.
[8] M. Wink, M. Kaufmann, *Bot. Acta* **1996**, *109*, 139.
[9] K. Alipieva, R. Taskova, S. R. Jensen, N. Handjieva, *Biochem. Syst. Ecol.* **2006**, *34*, 88; K. Alipieva, T. Kokubun, R. Taskova, L. Evstatieva, N. Handjieva, *Biochem. Syst. Ecol.* **2007**, *35*, 17.

Received October 25, 2006