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10-DEOXYMELITTOSIDE, AN IRIDOID DIGLUCOSIDE, AND OTHER IRIDOIDS FROM *LAMIASTRUM GALEOBDOLON**

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Key Word Index—*Lamiastrum galeobdolon*; Labiatae; iridoid glucosides; 10-deoxymelittoside; harpagide; acetyl harpagide.

Abstract—From the aerial parts of *Lamiastrum galeobdolon* subsp. *flavidum* two known iridoid glucosides, harpagide and 8-*O*-acetylharpagide, were isolated, together with a new iridoid diglucoside, 10-deoxymelittoside.

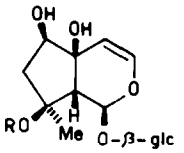
INTRODUCTION

Lamiastrum galeobdolon (L.) Ehrend. et Polatschek [*Lamium galeobdolon* (L.) Cranz] is a medium size herb which grows in shadowy and moist woods [2]. In Italy it is known by the popular name of 'ortica mora' and an infusion of the leaves is used in traditional medicine as an antiphlogistic, an astringent, a cicatrizent, a diuretic and an emocathartic [3]. Two subspecies are present in Italy: *montanum* in Central and Southern provinces of the country and *flavidum* in the Northern provinces. The chemical study of the iridoid components of the latter subsp. is the subject of the present communication.

RESULTS AND DISCUSSION

CC of a charcoal-treated methanol-extract of the whole plant gave compounds **1**–**3**. The first two were identified as harpagide (**1**) and 8-*O*-acetylharpagide (**2**) by their spectral data and by comparison with authentic samples. The third iridoid (**3**) was a new natural substance having the structure of 10-deoxymelittoside. Compound **3**, white amorphous powder, $[\alpha]_D = -60.5$ (*c* 1; MeOH) was an iridoid glucoside, which on acid hydrolysis in the usual way [4] gave 2 moles of D-glucose. It showed no UV absorption, indicating the absence of conjugated systems and its ^{1}H NMR spectrum (400 MHz, CD_3OD, Table 1) presented the following characteristic signals: (1) a doublet at δ5.80 (J = 2.5 Hz) typical of the acetalic H-1 of the dihydropyrane ring in the iridoid structure. (2) A doublet at δ6.39 (J = 6.5 Hz) and a doublet of doublets at δ5.15 (J = 6.5 and 1.0 Hz) attributable to an unsubstituted C-3/C-4 double bond. The 1.0 Hz coupling constant of H-4 was attributed to a coupling with H-9, as already observed in other iridoids [5]. This is in contrast to the previous assignments of coupling between H-4 and H-1, reported for monomelittoside (**4**)[6] and harpagide (**1**) [7]. (3) A doublet of triplets at δ5.61 (J = 2.5 and 1.2 Hz) and a broad singlet at δ1.89 attributed to H-7 and Me-10 respectively, of the isoprenyl sequence CH(7)=C(8)–CH (9). Me(10) (4) A broad doublet at δ4.28 (J = 2.5 Hz) due to a proton located at C-6, bearing also a secondary alcoholic function. Thus the aglucone of **3** is 10-deoxymonomelittoside. The remaining signals belonged to two glucose moieties as shown by the anomeric H-1′ peaks (δ4.75 and 4.65, doublets with J = 8.0 Hz), indicative of a β-configuration for the glucosidic linkages, and H_2-6′ peaks (δ3.96–3.70).

The ^{13}C NMR spectrum of **3** (100 MHz, Table 2) confirmed the proposed structure. The C-5, C-6 and C-1 values were indicative of a β-configuration for OH-6, as demonstrated by a direct comparison between the spectroscopic data of several pairs of C-6 epimers of iridoid glucosides, including monomelittoside (**4**) and 6-epimonomelittoside [8]. However, even by utilizing the data for monomelittoside (**4**) and of melittoside (**5**) (5-*O*-glucosylmonomelittoside), it was not possible to assign



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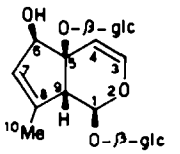
2 R = Ac



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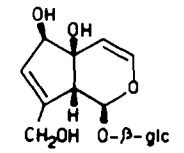

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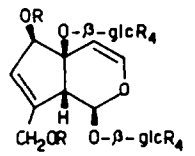

5 R = H
6 R = Ac

*Part 11 in the series "Iridoids in the Flora of Italy". For part 10 see ref. [1].

Table 1. ^{1}H NMR data of compounds 3–5*

H	3 (CD_3OD)	4† (D_2O)	5‡ (D_2O)
1	5.80 *d*	5.70 *d*	5.50 *d*
	$J_{1,9}$ = 2.5	$J_{1,9}$ = 2.5	$J_{1,9}$ = 5.0
3	6.39 *d*	6.37 *d*	6.58 *d*
	$J_{3,4}$ = 6.5	$J_{3,4}$ = 6.4	$J_{3,4}$ = 6.4
4	5.15 *dd*	5.06 *dd*	5.22 *d*
	$J_{4,9}$ = 1.0	$J_{9,4}$ = 1.2	
6	4.28 *d* (*br*)	4.25 *m*	4.67 *m*
	$J_{6,7}$ = 2.5		
7	5.61 *dt*	5.87 *m*	5.91 *m*
	$J_{7,9}$ = 1.2		
9	3.25 *m*	3.22 *m*	ov.
10	1.89 *s* (*br*)	4.25 *m*	4.30 *m*
1′	4.75 *d*		
	$J_{1',2'}$ = 8.0		
1″	4.65, *d*		
	$J_{1'',2''}$ = 8.0		
6′, 6″	3.96 and 3.85 *dd*		
	J = 2.1 and 12.0		
6′, 6″	3.81 and 3.70 *dd*		
	J = 4.2 and 12.0		

*Coupling constant values in Hz.
†Data taken from ref. [6].
‡Data taken from ref. [14].

unambiguously the position of the second sugar moiety. Although the differences in C-4, C-5 and C-6 in 3 compared to those in 4 and 5 were indicative of a substitution at C-5 or C-6. To resolve this problem, we synthesized compound 3. Melittoside acetate (6) was hydrogenolysed ($Pd(OH)_2$/C–cyclohexene) in conditions selective for the elimination of the primary alcoholic function [9]. The resultant acetate was hydrolysed in alkaline medium affording a product which was identical to 3. Thus the structure and absolute configuration of 10-deoxymonomelittoside was demonstrated for 3.

The genus *Lamiastrum* is considered by many authors to be synonymous with *Lamium*. All the iridoid glucosides isolated so far from *Lamium amplexicaule* [10] and *L. album* [11] have a ten carbon atom skeleton, with C-11 as a carboxylic or a methylenic function, and thus differing from our findings in *L. galeobdolon*. However, as early as 1968 Adema [12] presented chromatographic evidence of the presence of harpagide-type iridoids in this plant and later Wieffering and Fikenscher [13] reported the presence in the subsp. *montanum* of 1, 2 and an aucubin-type compound, which was absent in the other subspecies (*flavidum* and *galeobdolon*)

Structure 3 is quite uncommon, in fact to our knowledge only linaride from *Linaria vulgaris*, teucardosid and teuhircosid from *Teucrium* contain an unsaturated Me-10 [15].

EXPERIMENTAL

PC: Schleicher & Schull No. 2043 b Mgl paper; TLC: silica gel 60 F_{254} and cellulose pre-coated plates (Merck); CC: silica gel 70–230 mesh (Merck), charcoal powder (C. Erba), Li-chromprep. Spray reagents: H_2SO_4; vanillin (vanillin 2 g, conc. HCl 4 ml, MeOH 10 ml); resorcine (resorcine 5 g, conc. H_2SO_4 4 ml, EtOH 300 ml). Evaporation of volatile solvents was performed under red. pres. CHO microanalysis of described compounds gave satisfactory results.

Isolation of the iridoid-containing fraction. Lamiastrum galeobdolon (0.5 kg) was collected in Spring 1985 in Lazio. Voucher specimens of the plant were identified in the Herbarium of Dipartimento di Biologia Vegetale of Universita 'La Sapienza', Roma.

Whole plant (500 g) was extracted with EtOH at room temp.

Table 2. ^{13}C NMR chemical shift values of compounds 3–5*

C	3 (CD_3OD)	3 (D_2O)	4 (CD_3OD)	5 (D_2O)
1	93.2	94.2	93.6	95.7
3	142.8	142.9	142.4	143.9
4	105.0	105.4	108.4	105.6
5	79.1	79.5	72.8	81.0
6	78.6	79.0	80.5	80.4
7	128.7	127.7	127.7	128.4
8	144.5	144.5	148.3	145.5
9	53.5	53.1	53.6	51.4
10	15.8	15.7	60.8	60.1
1′, 1″	99.8, 97.7	98.7, 98.4	99.4	98.8, 98.5
2′, 2″	75.2, 74.9	74.0, 73.7	74.4	73.9, 73.6
3′, 3″	78.5†, 78.3†	76.6†, 76.1†	78.2†	76.5†, 76.3†
4′, 4″	71.7, 70.6	70.2, 70.0	71.6	70.2, 70.1
5′, 5″	78.2†, 77.0†	77.0†	77.4†	77.0†
6′, 6″	62.8, 62.1	61.3	62.4	61.4

*TMS as internal reference for spectra in CD_3OD and MeOH, 49.6 ppm for those in D_2O.
†These assignments may be interchanged.

for three days. The combined EtOH extracts were concd to an aq. suspension which was treated with charcoal powder (100 g) until negative to the vanillin test. The resulting suspension was stratified on a Gooch funnel (14 cm ϕ) which was eluted with H_2O (5 l.), 5% EtOH (2 l.) and 10% EtOH (3 l.) to eliminate salts, mono and oligosaccarides (negative resorcine test). Elution with 30% EtOH (3 l.) and with 50% and 80% EtOH (2 l. each) afforded an iridoid-containing fraction (1.5 g) which was purified by chromatography on silica gel (80 g) in *n*-BuOH satd with H_2O. The crude iridoids were successively purified by chromatography on silica gel in $CHCl_3$–MeOH (4:1) affording harpagide (**1**, 100 mg), 8-*O*-acetylharpagide(**2**, 80 mg) and 10-deoxymelittoside (**3**, 20 mg). Compound **3**: $[\alpha]_D^{20} = -60.5$ (MeOH; *c* 1.0). IR ν_{max}^{KBr} cm^{-1}: 3500, 1620.

Hydrogenolysis of **6**. Compound **6** (600 mg) was dissolved in 10 ml EtOH, 100 mg $Pd(OH)_2/C$ 50% and 3 ml cyclohexene were added and the reaction was left under reflux for 3 hr. The catalyst was removed by filtration and the mixture (380 mg) of unreacted **6** and its 10-deoxy derivative was separated by chromatography on silica gel (20 g) in $CHCl_3$–t-Bu-Me-ether (4:1) affording 230 mg 10-deoxy derivative and 150 mg **6**.

10-Deoxy derivative (230 mg) was hydrolysed with 10 ml 2 M NaOH in 50% aq. EtOH overnight. The soln was neutralized by bubbling CO_2, the alcohol was eliminated by evaporation under red. pres. and 5 g charcoal were added. The resulting suspension was stratified on a Gooch funnel (1 cm), the salts eliminated with water and the product eluted with MeOH; ^1H and ^{13}C NMR spectra superimposable to those of **3**.

REFERENCES

1. Bianco, A., Passacantilli, P., Righi, G. and Nicoletti, M. (1985) *Phytochemistry* **24**, 1843.
2. Pignatti, S. (1982) *Flora d'Italia*, Vol. II, p. 459. Ed. Agricola, Bologna.
3. Castaldo, P. (1975) *Fitoterapia* **46**, 262.
4. Bianco, A., Bolli, D. and Passacantilli, P. (1981) *Gazz. Chim. Ital.* **111**, 9.
5. Ruhdorfer, J. and Rimpler, H. (1981) *Z. Naturforsch.* **36C**, 697.
6. Scarpati, M. L. and Esposito, P. (1967) *Ric. Sci.* **37**, 840.
7. Lichti, H. and von Wartburg, A. (1966) *Helv. Chim. Acta* **49**, 1552.
8. Bianco, A., Passacantilli, P., Polidori, G., Nicoletti, M. and Messana, I. (1983) *Gazz. Chim. Ital.* **113**, 829.
9. Bianco, A., Passacantilli, P. and Righi, G. (1985) *XV Convegno Nazionale della Divisione di Chimica Organica, Sirmione* 22–27/9. Simposium paper pg. 196.
10. Guiso, M. and Martino, C. (1983) *J. Nat. Prod.* (*Lloydia*) **46**, 157.
11. Eigtved, P., Jensen, S. R. and Nielsen, B. J. (1974) *Helv. Chim. Scand.* **B28**, 85.
12. Adema, F. (1968) *Acta Bot. Neerl.* **17**, 423.
13. Wieffering, J. H. and Fikenscher, L. H. (1974) *Biochem. Syst. Ecol.* **2**, 31.
14. Scarpati, M. L. and Esposito, P. (1967) *Gazz. Chim. Ital.* **97**, 1209.
15. El-Naggar, L. J. and Beal, J. L. (1967) *J. Nat. Prod.* **43**, 649.