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# MURALIOSIDE, AN IRIDOID FROM *CYMBALARIA MURALIS**

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**Key Word Index**—*Cymbalaria muralis*; Scrophulariaceae; iridoid; muralioside; antirrhinoside; linarioside; antirrhide; linaride; macfadienoside; 8-epiloganic acid; 7α-hydroxyharpagide.

**Abstract**—*Cymbalaria muralis* subsp. *pilosa* contains a new iridoid, which we have named muralioside, besides the known antirrhinoside, linarioside, antirrhide, linaride, 8-epiloganic acid and macfadienoside. Muralioside was shown to be 7β-hydroxy-harpagide by spectroscopic means and probably arises from the opening of the epoxide ring of antirrhinoside. Following this biogenetic suggestion, the partial synthesis of muralioside from antirrhinoside was carried out by protic acid catalysis. Copyright © 1997 Elsevier Science Ltd

## INTRODUCTION

*Cymbalaria muralis* Mey. et Sch. [= *Linaria cymbalaria* (L.) Miller] is a small size herb found in several temperate regions of the world and typically growing on moist rocks and walls. The whole plant is used in popular medicine [1]. It is divided into two subspecies, *muralis* and *pilosa* (Vis.) Degen. [2], but this classification is still a matter of debate [3]. In order to verify the characters of differentiation between the two subspecies, we have characterized the iridoid constituents of the subsp. *pilosa*. Previous studies on the iridoids of *C. muralis* led to the isolation of antirrhinoside (**2**), antirrhide, linaride, linarioside, 8-epiloganic acid and 7β-hydroxy-8-epi-iridodial glucoside [4, 5]. Although not reported, it is conceivable that the plants so far studied belonged to the subsp. *muralis*, owing to the great scarcity of the other subspecies. In the case of our previous research [4, 5], we examined *C. muralis* subsp. *muralis*.

*Cymbalaria muralis* subsp. *pilosa* was found to contain antirrhinoside (**2**), linarioside, antirrhide, linaride and 8-epiloganic acid. In addition, we isolated macfadienoside (**3**), previously found in Bignoniaceae, Myoporaceae and Scrophulariaceae [5, 6], and a new iridoid (**1**) for which we propose the name of muralioside.

## RESULTS AND DISCUSSION

Muralioside (**1**) is an iridoid glucoside whose $^1$H NMR data established the following: the absence of C-11 and substitution at C-5 as shown by the signal of H-4 ($\delta$ 5.05) appearing as a sharp doublet ($J$ = 6.3 Hz); the presence of C-10 as a methyl as shown by the singlet at $\delta$ 1.34; the presence of two hydroxyl groups at C-6 and C-7 as shown by the two doublets ($J$ = 5.7 Hz) at $\delta$ 3.82 and 3.77, respectively. The two hydroxyl groups were in the *cis* configuration as shown by a strong NOE between H-6 and H-7. Another evident NOE was observed between H-1 and the C-10 methyl group, thus assigning an α configuration to the latter. The $^{13}$C NMR data are in accordance with previous considerations and indicate hydroxyl groups at C-5 and C-8 (quaternary carbons at $\delta$ 69.6 and 80.4). Finally, the chemical shift of C-1 (94.3 ppm), as well as the small value (0.7 Hz) of the H-1–H-9 coupling constant, are in accord with a β-hydroxyl at C-6 [7]. Therefore, the structure of 7β-hydroxyharpagide could be proposed for **1** on the basis of the above data.

To confirm these assignments and to determine unambiguously the absolute configurations of all the stereogenic centres of **1**, a hydrolytic opening of the epoxide ring of (**2**) was performed by protic acid catalysis. The reaction gave a product identical to **1**, thus confirming the β configuration of hydroxyl groups at C-6 and C-7, assuming the maintenance of the configuration at C-6, which is not involved in the reaction. The β configuration of the hydroxyl at C-8 could be justified by assuming that the reaction follows the classical monomolecular mechanism, i.e. pro-

*Part 15 in the series 'Iridoids in the Flora of Italy'. For part 14 see ref. [11].

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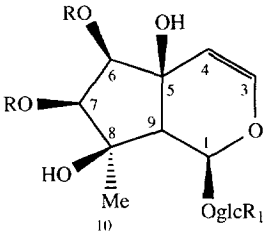
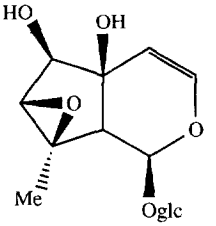
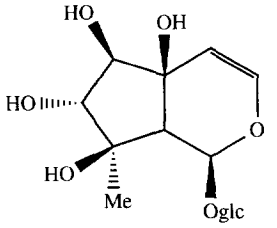



**1** R = H **2** R = Me

**5** R = Ac **3** R = $CH_2OH$




**4**

tonation of the epoxide function followed, as a result of steric hindrance due to the *cis* junction of the bicyclic iridoidic moiety, by the opening of the protonated epoxide in an independent step which generates the more stable C-8 carbocation which is then hydrated from the more accessible $\beta$ side of the molecule.

As further confirmation, the opening of the epoxide ring of **2** with aqueous base gives the 7-hydroxyl epimer of **1**, 7α-hydroxyharpagide (**4**) [8], whose NMR data are in accordance with the above considerations. In fact for **4**, $J_{6,7}$ = 9.0 Hz, i.e. typical of a *trans* H-6–H-7 relationship and in accord with other iridoids with similar substitution (cf. coupling constant values of 9.0–9.7 Hz in the 6,7 *trans* relationship and of 4.2–5.4 Hz in the *cis* case [9, 10]). Preparation of the hexaacetyl derivative (**5**) further confirmed the presence of two secondary and two tertiary hydroxyl functions in the cyclopentane iridoid ring of **1**.

The study of the iridoid pattern of *Cymbalaria* supports the view that the previous classification of *C. muralis* within the *Linaria* genus is unsuitable, since although antirrhinoside/antirrhide-type compounds are predominant in both cases, in *Cymbalaria*, probably due to ring opening of the 7–8 epoxide function, polyhydroxylated compounds occur. These are so far absent in *Linaria*. Furthermore, the pattern of iridoids isolated from subsp. *muralis* and *pilosa* show some remarkable differences, i.e. the presence of muralioside and macfadienoside in the latter subspecies.

## EXPERIMENTAL

*Material and methods.* NMR: 500 MHz ($^1$H) and 125 MHz ($^{13}$C); MS: Kratos-80; TLC: silica gel $SiF_{254}$ (Merck); PC: Schleider & Schull No. 2043b MgI. Spray reagents: 2 N $H_2SO_4$, vanillin (3 g vanillin, 4 ml conc. HCl, 100 ml MeOH) and resorcinol (5 g resorcinol, 4 ml conc. $H_2SO_4$, 300 ml EtOH).

*Plant material. Cymbalaria muralis* Mey. et Sch. subsp. *pilosa* (Vis.) Degen. was collected in Latium in February 1996. Voucher specimens were identified by M.L. Leporatti, Dipartimento di Biologia Vegetale, and are deposited in the Herbarium of the Dipartimento di Biologia Vegetale, Università di Roma 'La Sapienza'.

*Extraction and isolation.* Fresh material (whole plant, 300 g) was exhaustively extracted with EtOH at room temp. and the extract evapd to an aq. suspension. Charcoal (50 g) was added until a negative vanillin test was obtained and the resulting suspension stratified on a Gooch funnel. Elution with $H_2O$ and 5% EtOH removed salts and sugar (resorcinol test), whereas 20, 50, 70, 80 and 95% EtOH eluted iridoid-containing frs. The 20% EtOH fr. (4.1 g) was subjected to CC on silica gel (50 g) in satd aq. BuOH, to give **2** (400 mg) and partially purified iridoid frs containing (**3**) (80 mg) and (**1**) (35 mg). Final purification of **3** was achieved by further chromatography on charcoal, eluting with a gradient of $H_2O$–MeOH: pure **3** was obtained in the 30% MeOH fr. Final purification of **1** was achieved by further CC on silica gel in BuOH–MeOH–$H_2O$ (7:1:3). The 50% EtOH fr. together with the 80% EtOH fr. (1.1 g) were subjected to CC on silica gel (50 g) in satd aq. BuOH, affording **2** (200 mg), linarioside (20 mg), antirrhide (40 mg), linaride (25 mg), **3** (20 mg) and 8-epiloganic acid (50 mg). Final purification of 8-epiloganic acid was achieved by esterification with $CH_2N_2$ in MeOH

(10 ml) and further CC on silica gel in $CHCl_3$–MeOH 8:2. All known compounds were identified by comparison with authentic samples.

*Muralioside* (**1**). Amorphous powder, $[\alpha]_D^{20} = -43$ (MeOH, *c* 0.2). IR [$\nu_{max}^{KBr}$: 3500, 1650, 1240 $cm^{-1}$; $^1H$ NMR ($D_2O$): $\delta$ 6.32 (1 H, *d*, *J* = 6.3 Hz, H-3), 5.61 (1 H, *d*, *J* = 0.7 Hz, H-1), 5.05 (1 H, *d*, *J* = 6.3 Hz, H-4), 4.70 (1 H, *d*, *J* = 7.5 Hz, H-1′), 3.86 (1 H, *dd*, *J* = 13.0, 2.2 Hz, H-6′a), 3.82 (1 H, *d*, *J* = 5.7 Hz, H-6), 3.77 (1 H, *t*, *J* = 5.7 Hz, H-7), 3.66 (1 H, *dd*, *J* = 13.0, 5.8 Hz, H-6′b), 3.43 (1 H, *t*, *J* = 9.2 Hz, H-3′), 3.40 (1 H, *ddd*, *J* = 9.2, 5.8, 2.2 Hz, H-5′), 3.34 (1 H, *t*, *J* = 9.2 Hz, H-4′), 3.24 (1 H, *dd*, *J* = 9.2, 7.5 Hz, H-2′), 2.42 (1 H, *brs*, H-9), 1.34 (3H, *s*, $H_3$-10); $^{13}C$ NMR ($D_2O$): $\delta$ 25.8, C-10; 56.3, C-9; 62.6, C-6′; 69.6, C-5; 71.6, C-4′; 74.4, C-2′; 77.3*, C-5′; 78.1*, C-3′; 78.9*, C-7; 80.4, C-8; 80.7, C-6; 94.3, C-1; 100.0, C-1′; 108.3, C-4; 143.3, C-3.* Indicates interchangeable assignments. FAB-MS *m/z*: 403 $[M+Na]^+$, 419 $[M+K]^+$.

*Alkaline hydrolysis of* **2**. Antirrhinoside (**2**) (100 mg) was dissolved in a satd soln of $Ba(OH)_2$ (5 ml) and refluxed for 4 hr. The soln was then neutralized by bubbling with $CO_2$ and, after removal by filtration of the $Ba(CO_3)_2$ residue, $H_2O$ was removed *in vacuo*. The organic residue (120 mg) was subjected to CC on silica gel in BuOH–MeOH–$H_2O$ (7:1:3) affording 50 mg 7α-hydroxyharpagide (**4**) and 20 mg unreacted **2**.

*Compound* **4**. Amorphous powder. $^1H$ NMR ($D_2O$): $\delta$ 6.30 (1H, *d*, *J* = 6.2 Hz, H-3), 5.70 (1H, *s*, H-1), 5.20 (1H, *d*, *J* = 6.2 Hz, H-4), 4.75 (1H, *d*, *J* = 7.5 Hz, H-1′), 3.87 (1H, *dd*, *J* = 13.0, 2.2 Hz, H-6′a), 3.80 (1H, *d*, *J* = 9.0 Hz, H-6), 3.67 (1H, *dd*, *J* = 13.0, 5.8 Hz, H-6′b), 3.45 (1H, *d*, *J* = 9.0 Hz, H-7), 3.43 (1H, *t*, *J* = 9.2 Hz, H-3′), 3.41 (1H, *t*, *J* = 9.2 Hz, H-4′), 3.35 (1H, *ddd*, *J* = 9.2, 5.8, 2.2 Hz, H-5′), 3.28 (1H, *dd*, *J* = 9.2, 7.5 Hz, H-2′), 2.42 (1H, *brs*, H-9), 1.11 (3H, *s*, $H_3$-10); $^{13}C$ NMR ($D_2O$): $\delta$ 21.6, C-10; 55.1, C-9; 62.3, C-6′; 65.0, C-5; 71.1, C-4′; 74.1, C-2′; 75.7, C-8; 77.5*, C-5′; 77.8*, C-3′; 79.6, C-7; 83.3, C-6; 92.7, C-1; 100.0, C-1′; 110.2, C-4; 140.5, C-3. *Indicates interchangeable assignments.

*Acid hydrolysis of* **2**. Antirrhinoside (**2**) (200 mg) was dissolved in 0.25 M $H_2SO_4$ (5 ml) and left to stand for 3 days at 5°. The soln was then neutralized with satd $NaHCO_3$ soln and treated with charcoal until negative to the vanillin test. The resulting suspension was stratified on a Gooch funnel and salts removed by elution with $H_2O$. The iridoid fr. was then eluted with MeOH. The organic residue (130 mg) was subjected to CC on silica gel in BuOH–MeOH–$H_2O$ (7:1:3) affording 50 mg of unreacted **2** and 70 mg **1**.

*Acetyl derivative* (**5**). Muralioside (**1**) (20 mg) was dissolved in 0.1 ml $Ac_2O$ and 0.2 ml pyridine and left at room temp. for 2 hr. MeOH was then added and the volatile materials removed *in vacuo*. The residue (25 mg) was composed mainly of **5** and was suitably pure for analyt. purposes.

*Compound* **5**. $^1H$ NMR ($CDCl_3$): $\delta$ 6.33 (1H, *d*, *J* = 6.3 Hz, H-3), 5.64 (1H, *d*, *J* = 0.7 Hz, H-1), 5.24 (1H, *d*, *J* = 9.2 Hz, H-3′), 5.16 (1H, *d*, *J* = 4.4 Hz, H-6), 5.08 (1H, *dd*, *J* = 6.3, 1.4 Hz, H-4), 5.02 (1H, *t*, *J* = 9.2 Hz, H-4′), 4.97 (1H, *d*, *J* = 4.4 Hz, H-7), 4.92 (1H, *dd*, *J* = 9.2, 7.5 Hz, H-2′), 4.79 (1H, *d*, *J* = 7.5 Hz, H-1′), 4.25 (1H, *dd*, *J* = 13.0, 5.8 Hz, H-6′a), 4.10 (1H, *dd*, *J* = 13.0, 2.2 Hz, H-6′b), 3.66 (1H, *ddd*, *J* = 13.0, 5.8, 2.2 Hz, H-5′), 2.69 (1H, *brs*, H-9), 2.10, 2.07, 2.00, 1.98 (3H, 6H, 6H, 3H, 4*s*, 6xAc), 1.48 (3H, *s*, $H_3$-10); $^{13}C$ NMR ($CDCl_3$): $\delta$ 20.5, 20.7, 21.1, 21.0, $COCH_3$; 26.7, C-10; 54.3, C-9; 61.6, C-6′; 66.6, C-5; 68.2, C-4′; 71.1*, C-2′; 71.9*, C-3′; 72.1, C-5′; 76.5**, C-6; 77.7**, C-7; 81.2, C-8; 92.3, C-1; 96.0, C-1′; 142.1, C-3; 106.3, C-4; 169.4, 169.9, 170.0, 170.6, 170.8, 172.0, $CO$Me$_3$. *,**Indicate interchangeable assignments.

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