

IRIDOID GLUCOSIDES OF SPECIES OF LAMIUM AND SOME RELATED GENERA

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SUMMARY

Species of *Lamium* and some related genera were investigated paper chromatographically for the presence of iridoid glucosides. Most species investigated were shown to contain such compounds. Two constituents present in several species of *Lamium* and in *Stachys hirta* could be identified with lamiol and acetyllamiol (lamioside) by comparing their properties with those reported by SCARPATI and GUIISO (1967) for these glucosides isolated from *Lamium amplexicaule*.

Some of the more striking results are:

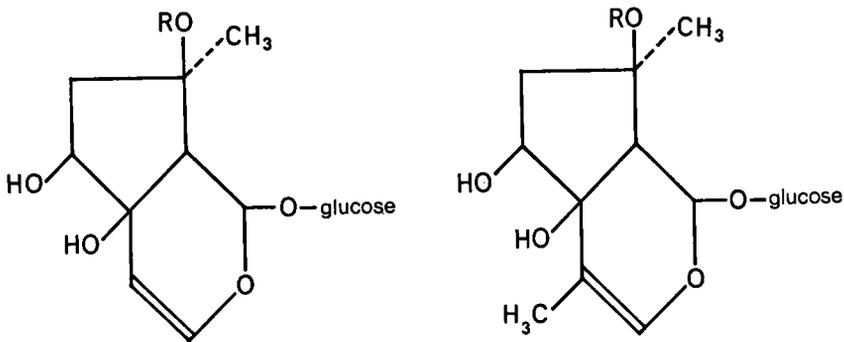
1. The difference between *L. galeobdolon* and the other *Lamium* species. *L. galeobdolon* has not the *Lamium* compounds but harpagid-type iridoid glucosides (the more common type of iridoid glucosides in *Labiatae*).
2. The presence of iridoid glucosides of the *Lamium* type in *Stachys hirta*. The other investigated species of *Stachys* have harpagid-type iridoid glucosides.
3. No traceable amounts of iridoid glucosides could be found in the allotetraploids *L. mollucellifolium* and *L. hybridum*. This is a rather strange result, because the putative parent species do accumulate iridoid glucosides.

1. INTRODUCTION

Aucubin-like glucosides belong to the so called iridoid plant constituents which occur frequently in *Ericales*, *Gentianales*, *Oleales*, *Tubiflorae*, *Dipsacales* and in some polypetalous orders. Present-day knowledge of distribution, chemistry and biochemistry of iridoid compounds suggests that they may be of value to plant systematics (HEGNAUER 1964, 1966, a, b, WIEFFERING 1966). A better knowledge of the distribution of the group as a whole and of its individual members, however, is esteemed essential for a sound judgement of the taxonomic meaning of this character complex. Aucubin is the most-studied member of the iridoid glucosides. It occurs frequently, e.g., in the *Scrophulariaceae*, *Lentibulariaceae*, *Globulariaceae* and *Plantaginaceae*. In the families mentioned it is often accompanied or replaced by catalpin, catalpol, methylcatalpol and related glucosides. In the *Labiatae* the presence of aucubin-like glucosides has been demonstrated conclusively only recently (SCARPATI *c.s.* 1965). The Italian authors isolated harpagide (already known from the *Pedaliaceae*) and its monoacetate from *Melittis melissophyllum* L. Evidence for the frequent occurrence of aucubin-like glucosides in the *Labiatae* has been accumulated in this laboratory during a program of field tests (WIEFFERING 1966). Preliminary paper chromatographic studies had already demonstrated that harpagide and acetylhar-

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pagide are most probably present in members of the genera *Ajuga*, *Galeopsis*, *Stachys*, and *Teucrium* (WIEFFERING 1966). *Lamium* was another genus for which field tests indicated the presence of aucubin-like compounds. Investigations were started which aimed at a chromatographic characterisation of the various constituents of *Lamium*. As a comparison members of some genera related to *Lamium* were investigated at the same time. At the time we made our investigations SCARPATI & GUISO (1967) reported the isolation of lamioside¹ and lamiol from *Lamium amplexicaule*. The properties reported for these two *Lamium*-iridoids enabled us to compare the different iridoid glucosides demonstrated by us to be present in several species of *Lamium* with them. It turned out that lamioside is a rather characteristic constituent of the genus *Lamium*.



R = CO.CH₃: Acetylharpagide, C₁₇H₂₆O₁₁
 R = H: Harpagide, C₁₅H₂₄O₁₀

R = CO.CH₃: Acetylamiol (=Lamioside), C₁₈H₂₈O₁₁
 R = H: Lamiol, C₁₆H₂₆O₁₀

2. THE GENUS LAMIUM

According to BRIQUET (ENGLER-PRANTL, IV, 3a, 1897) *Lamium* is placed in the *Stachyoideae-Stachydeae-Lamiinae*. The subtribe *Lamiinae* comprises 25 genera (e.g. *Galeopsis*, *Wiedemannia*, *Leonurus*, *Lagochilus*, *Ballota*, *Stachys*). *Lamium* comprises approximately 40 species from Europe, extratropical Asia and Northern Africa. Briquet accepted 3 subgenera and subdivided the largest one in two sections.

I *Orvala*: *L. orvala* L. only.

II *Eulamium*:

1. *Pollichia*: within this larger section 4 series or subsections are accepted: *Rhomboidea* (*L. rhomboidea* Benth. only); *Garganica* with *L. garganicum* L., *L. striatum* Sibth. & Sm., *L. longiflorum* Ten.; *Amplexicaulia* with *L. amplexicaule* L., *L. molucellifolium* Fr.; *Purpurea* with *L. purpureum* L. and *L. hybridum* Vill.

¹ As the name lamioside has already been used for a flavonoid compound from *Lamium album* (see HEGNAUER 1966a) we prefer to use the name acetylamiol.

2. *Lamiotypus*: with perennial (e.g. *L. album* L., *L. maculatum* L.) and annual (e.g. *L. galactophyllum* Boiss. et Reut.) species.

III *Galeobdolon*: *L. flexuosum* L., *L. galeobdolon* (L.) Crantz and *L. pubescens* Sibth.

Most authors follow Briquet in the classification of *Lamium*. Some botanists, however (see e.g. Flora U.R.S.S., Fl. of the British Isles), accept generic rank for the subgenus *Galeobdolon* (i.e., *Galeobdolon* Adans. or *Lamiastrum* Heister ex Fabricius according to POLATSCHEK 1966) and *Lamium rhomboideum* Benth. is sometimes placed in the monotypic genus *Erianthera* Benth. (i.e., *E. anomala* Juz. in Flora U.R.S.S.).

Several species of *Lamium* have been studied cytologically. Most members are diploid with a somatic chromosome number 18 ($x = 9$). *L. galeobdolon* however comprises two diploid races (ssp. *galeobdolon*, ssp. *flavidum*) and one tetraploid race (ssp. *montanum*). *L. molucellifolium* Fr. (= *L. intermedium* Fr.) and *L. hybridum* Vill. are believed to be allopolyploids (BERNSTRÖM 1955): *L. hybridum* [$2n = 36$] = *L. purpureum* [$2n = 18$] + *L. bifidum* [$2n = 18$]; *L. molucellifolium* [$2n = 36$] = *L. purpureum* [$2n = 18$] + *L. amplexicaule* [$2n = 18$]). As far as available material allowed, we studied the influence of auto- and allopolyploidy on the patterns of iridoid constituents of *Lamium*.

Many of the species which appear not to have evolved polyploid races are polytypic widespread weeds. Plasticity and genetic differentiation at the diploid level contribute to the variability. It was esteemed desirable to make a preliminary study of the variability of the iridoid pattern of at least one diploid species. *L. maculatum* was chosen for this purpose.

3. EXPERIMENTAL

3.1. Material

Fresh plants cultivated in Leiden and collected around Leiden, as well as herbarium plants were used. Herbarium material: Recent collections of the "Laboratorium van Experimentele Plantensystematiek Leiden" (EPL), sheets from the "Rijksherbarium, Leiden" (L) and from the herbarium Van Ooststroom (vO) were at our disposal.

3.2. Methods

(WIEFFERING 1966; modified)

Extraction: Dry leaves (200 mg) are powdered with sand, extracted for 30 min in 25 ml of boiling water and filtered after cooling. The filtrate is treated with basic lead acetate and the excess of lead is removed by H_2S . The purified aqueous extracts were evaporated on a water-bath after addition of sand and silicagel (100 mg each). The dry residues were extracted for 6 hours with a mixture of acetone and ethanol (9:1) in a mechanical shaker. The filtrate was concentrated to 0,1 ml (corresponding to approximately 200 mg dry plant) and subsequently

used for paper-chromatographic studies. If the extraction was made from fresh plants 1 g of leaves was used.

Paper chromatography (ascending technique; paper Schleicher – Schüll 2043b Mgl.): We used 3 solvents and 5 spray reagents for detection of spots. For comparison aucubin, agnuside and harpagide were co-chromatographed with our extracts. *Solvents*: *A* butanol/acetic acid/water = 4/1/5; *B* isopentanol/acetic acid/water/hexane = 3/3/3/1; *C* butanol/acetic acid/water = 63/10/27 (SCARPATI & GUIISO 1967). *Spray reagents*: *a.* $SbCl_3$ in chloroform; *b.* benzidine-trichloroacetic acid; *c.* ureum – HCl; *d.* 1N H_2SO_4 in methanol; *e.* vanillin (1g) and conc. HCl (3 ml) dissolved in methanol (150 ml) (SCARPATI & GUIISO 1967). After heating chromatograms treated with reagent *e* for 2–3 min at 100° the following colours were observed: Acetylharpagide pink lilac (dark red in UV, 254 nm); acetylamiol pink lilac (orange brown); agnuside pink lilac (dark red); asperulin blue violet; aucubin pink lilac (dark orange); catalpol yellow brown (yellow brown); harpagide pink lilac (dark red); lamiol pink lilac (orange brown); loganin no colour (purple); monotropein blue violet (brown red).

In our experience reagents *c* and *e* are most useful for the detection of iridoid glucosides of *Labiatae* (i.e., harpagide and lamiol and their esters). On keeping chromatograms treated with reagent *e* the colours gradually change to brown in the case of lamiol and acetylamiol and to bluish grey in the case of harpagide and acetylharpagide.

4. RESULTS

4.1. Compounds of presumed iridoid nature

We started our investigation with several of the most common species of the subgenus *Eulamium* and with *Lamium orvala*. In most species presumably iridoid glucosides which were not identical with known compounds were detected. Such constituents are symbolized by plant names and R-values in solvent A as indicated in *table 1*. Some properties of all presumably iridoid constituents encountered during this investigation are summarized in *table 1*.

Guided by the report of SCARPATI & GUIISO (1967) and using extracts of *Lamium amplexicaule* we were able to identify our compound L. amp. 49 with acetylamiol (= lamioside). L. amp. 49 is easily hydrolysed to lamiol. Our compound L. amp. 41 is probably identical with lamiol. In *table 2* colour reactions and R_f -values observed by us for L. amp. 49 (acetylamiol) and L. amp. 41 (probably lamiol) are summarized.

4.2. Iridoid glucosides detected in species of *Lamium*

Lamium orvala L.: Field test (WIEFFERING 1966) blue with fresh and dry leaves. Detected: Acetylamiol, L. orv. 41 and 62. Investigated: EPL 5493, EPL 8824, vO 18778 (here L. alb. 83 also).

Lamium garganicum L.: Field test greenish blue with fresh and dry leaves.

Table 1. Some properties of constituents of presumed iridoid nature encountered during this investigation.

Constituent	Average R _F -values in solvent			Colours with spray reagent				
	A	B	C	a	b ¹	c	d	e ²
L. amp. 41 ³	0.41	0.31	0.37	PBr	R→B→Br	Or	PBr	PkL→Br
L. amp. 49 ⁴	0.49	0.41	0.45	PBr	R→B→Br	Or	PBr	PkL→Br
L. amp. 56	0.56	0.50	0.57	Pk	Br	o	Pk	PkL→YBr
L. alb. 63	0.63	-	-	YBr	YBr	YOr	o	o
L. alb. 77	0.77	-	-	Pk	o	o	Pk	o
L. alb. 83	0.83	-	-	YBr	Y	YOr	o	o
L. gal. 36 ⁵	0.36	-	0.33	PBr	B→BrY	Y	PBr	PkL→BG
L. gal. 50 ⁶	0.50	-	0.48	PBr	B→BrY	Y	PBr	PkL→BG
L. gar. 39	0.39	0.28	-	B	Y	YOr	P	PB
L. gar. 67	0.67	0.46	0.64	P	YBr	Or	P	PkL→Y
L. gar. 90	0.90	-	0.89	P	o	Or	o	PkL→Y
L. mac. 70	0.70	-	-	RBr	Y	o	Pk	o
L. orv. 41	0.41	0.35	-	Gr	Y	o	BG	o
L. orv. 62	0.62	0.52	0.64	P	Y	Or	P	PkL→YBr
St. hirta 69	0.69	-	-	P	o	Or	o	PkL→Br
St. pal. 43	0.43	-	-	o	o	Y	o	PkL
Mel. mel. 18 ⁷	0.18	-	-	RBr	Br	Y	G	o
Bal. nigra 56	0.56	-	-	o	o	o	o	GB
Gal. bif.	0.56	-	-	o	o	o	o	PkL
Gal. tetr. 47 ⁸	0.47	-	-	P	P→Br	Y	PBr	PkL

-: Solvent not used. o: no colour. B = blue, Br = brown, G = grey, Gr = green, L = lilac, Or = orange, P = purple, Pk = pink, R = red, Y = yellow.

¹ Colour changes during warming.

² Colour changes on treated chromatograms appear within a few hours.

³ Probably identical with lamioi.

⁴ Identical with acetylamiol (lamioside).

⁵ Identical with harpagide.

⁶ Identical with acetylharpagide.

⁷ Unknown << Pseudoindikan >> of *Melittis melissophyllum* (WIEFFERING 1966).

⁸ Tetrahit - Pseudoindikan (WIEFFERING 1966.)

Detected: Acetylamiol and L. gar. 39 and 67. Investigated: EPL 8835; vO 21274 (ssp. *grandiflorum*; L. gar 67 and 90).

Lamium longiflorum Ten.: Field test negative. Detected: Traces of acetylamiol and L. gar. 67. Investigated: L (Boom 24339).

Lamium striatum Sibth. et Sm.: Field test blue. Detected: Acetylamiol, L. gar. 39 and L. alb. 83. Investigated: L (Flor. Turc. As. 709, 1846).

Lamium amplexicaule L.: Field test generally blue with fresh leaves. Detected: Acetylamiol, L. amp. 41 (probably lamioi) and L. amp. 56. Investigated: Fresh plants and EPL 8833; in EPL 10981 (collected 10.10.1967) no iridoid compounds could be detected.

Lamium molucellifolium Fr.: Field test negative. No iridoid constituents detectable. Investigated: L (Pl. Suec., F. Hard).

Table 2. Some properties of acetylamiol (L. amp. 49) and lamiol (L. amp. 41).

Compound	Average R_f -values with solvent			Colours with spray reagent				
	A	B	C	a	b ¹	c	d	e ²
Lamiol (L. amp. 41)	0.41	0.31	0.37	PBr	R → B → Br	Or	PBr	PkL → Br
Acetylamiol (= lamio- side = L. amp. 49)	0.49	0.41	0.45	PBr	R → B → Br	Or	PBr	PKL-Br

¹ Colour change during warming

² Colour changes appear on treated chromatograms within a few hours.

Lamium purpureum L.: Field test usually greyish purple with fresh and dry leaves. Detected: Acetylamiol and L. mac. 70. Investigated: EPL 8823 and EPL 10299.

Lamium hybridum Vill.: Field test negative. No iridoid constituents detectable. Investigated EPL 8657 and EPL 10988.

Lamium maculatum L.: Field test variable; blue, bluish green or negative with fresh and dry leaves. Detected: Acetylamiol, L. amp. 41 (probably lamiol), L. amp. 56, L. mac. 70, L. alb. 83. Investigated: EPL 3044, 4105, 5905, 6388, 6892, 8107, 8819, 8820, 8821, 10028, 10213, 10254, 10447, 10448, 10514, 10521, 10539, 10583, 10657, 10698, 10989, 10990, 10991.

Lamium album L.: Field test negative. Detected: Small amounts of acetylamiol and Lam. alb. 63, 77 and 83. Investigated EPL 8827; no iridoid constituents detectable in EPL 8826 and 8828.

Lamium galactophyllum Boiss. et Reut.: Field test negative. No detectable iridoid constituents. Investigated: L (Flor. Turc. As. 1685).

Lamium galeobdolon (L.) Crantz: Field test variable; blue, bluish green, green or rarely negative with fresh and dry leaves. Detected: Harpagide, acetylharpagide and Mel. mel. 18. Investigated: EPL 2974, 4118, 4125, 6312, 6590, 8830, 8831, 8832, 10360, 10369, 10446, 10536, 10553, 10986.

4.3. Diploid and tetraploid *Lamium galeobdolon* and the allotetraploid species of *Lamium*

Fourteen sheets of *L. galeobdolon* were examined for iridoid glucosides. According to morphological characters the diploid subsp. *galeobdolon* was represented by 4 plants (EPL 4118, 4125, 8830, 8832) and the tetraploid subsp. *montanum* (Pers.) Hyl. by 10 specimens (EPL 2974, 6312, 6590, 8831, 10360, 10369, 10446, 10536, 10553, 10986). No chemical differences between diploid and tetraploid plants were observed. In both taxa plants growing in full sun appear to be richer in iridoid glucosides, than shadow plants.

No iridoid compounds could be detected in *L. hybridum* (fresh leaves; EPL 8657, 10988) and *L. moleculifolium* (L, Plant. Suec., F. Hard). These results are rather strange because the parental diploid species of these allo-tetraploids do contain acetylamiol and related glucosides. Further studies with fresh plants of these species are highly desirable.

4.4. Chemical variation within *Lamium maculatum*

The observations reported for the *Lamium galeobdolon* complex had already indicated that a relation exists between habitat and the amount of iridoid compounds present in a plant. Plants growing in full sun tend to accumulate more iridoid glucosides, than plants growing in the shade. Twenty three sheets of *Lamium maculatum* were examined and a large quantitative variation was found. Again plants from sunny places, as a rule, contained much more iridoid compounds. The same observation was made with *L. album*. The only specimen (EPL 8827) with detectable amounts of lamiol-type glucosides was collected from plants growing in full sun.

4.5. Some other factors which may affect the amount of iridoid glucosides

Other factors affecting the amount of iridoid glucosides in a given plant sample are the time of collection, the technique of drying and the conditions and time of preservation. Samples collected late in the season, e.g. *L. amplexicaule* EPL 10981, tend to be very poor in iridoid glucosides. Preliminary experiments demonstrated that careful drying of recently collected plants avoids appreciable losses of iridoid compounds. Drying without precautions and long preservation of dried plants, however, may result in an ultimate total loss of iridoid constituents. Some of our negative results (*L. molucellifolium*, *L. galactophyllum*, *L. hybridum* EPL 10988) might have been caused by such conditions.

4.6. Iridoid glucosides of some taxa related to *Lamium*

We examined some members of *Lamiinae* for the presence of lamiol-type glucosides with the following results.

Stachys sylvatica L. (EPL 8840): Harpagide, acetylharpagide and St. pal. 43.

Stachys × *ambigua* Sm. (= *S. palustris* × *S. sylvatica*; EPL 8029): Harpagide, acetylharpagide and Stach. pal. 43.

Stachys palustris L. (EPL 8838): Harpagide, acetylharpagide and St. pal. 43.

Stachys alpina L. (EPL 10513): Harpagide, acetylharpagide and St. pal 43.

Stachus hirta L. (EPL 6774): Traces of L. amp. 41 (probably lamiol), acetyl-lamiol and St. hirta 69 (a lamioltype iridoid).

Stachys annua L. (EPL 8583): Traces of harpagide, acetylharpagide.

Ballota nigra L. (EPL 8841): Ball. nigra 56.

Mollucella laevis L. (L, W. et W. 1182): Four presumably iridoid constituents.

Galeopsis tetrahit L. (EPL 8006): Harpagide and Gal. tetr. 47 (= Tetrahit-Pseudoindikan of WIEFFERING 1966).

Galeopsis bifida Boenningh. (EPL 10997): Harpagide, acetylharpagide and Gal. bif. 56.

5. DISCUSSION

The present investigation has shown that *Lamium* is another genus of the *Labiatae* in which iridoid glucosides are common. Lamiol-type glucosides appear to be characteristic of the subgenera *Orvala* and *Eulamium*. In the subgenus *Galeobdolon* (at least in its most common species, *L. galeobdolon*) the latter are replaced by harpagide-type substances. Furthermore there were indications that the iridoid pattern of *L. orvala* is distinctly different from the patterns of members of the subgenus *Eulamium* and that within *Eulamium* itself *L. garganicum*, *L. longiflorum* and *L. striatum* on the one side and *L. amplexicaule*, *L. maculatum* and *L. purpureum* on the other side have similar iridoid patterns. For a sound taxonomic interpretation of our findings much more research will be needed. As long as the structures of most compounds detected by us remain unknown and as long as details concerning the biogenetical pathways resulting in lamiol-type and in harpagide-type constituents are not unravelled it will be wise not to overrate the taxonomic significance of differences in iridoid patterns.

It is interesting to note that most species of *Stachys*, hitherto investigated, contain harpagide-type iridoid glucosides. *Stachys hirta*, however, accumulates lamiol-type compounds and resembles species of *Eulamium* in this respect.

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