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Chromosome numbers, scutellarin and iridoid patterns in the genus *Galeopsis* L. (Labiatae)

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Abstract

J.H. Wieffering 1983. Chromosome numbers, scutellarin and iridoid patterns in the genus *Galeopsis* L. (Labiatae). Bot. Helv. *93:* 239–253. New Chromosome counts for all species of *Galeopsis* are reported (table 4). No deviations from previous counts (table 3) were found. Scutellarin was isolated from *G. pubescens* and *G. tetrahit*. A large number of specimens (table 5; fig. 1) was screened for the presence of this flavone 7-glucuronide. By including other 7-glucuronides (chrysin 7-glucuronide, baicalin) in the microchemical screening a fairly satisfactory specificity of the Molisch-test for scutellarin was demonstrated. The presence or absence of scutellarin proved to be useful for the discrimination between the closely related allotetraploid taxa *G. tetrahit* (+) and *G. bifida* (–). This character is, however, of no use for the phylogenetic interpretation of these taxa. Literature pertaining to iridoid patterns of all species of *Galeopsis* (table 6, fig. 3) is critically evaluated and its bearing on the phylogenetic interpretation of the genus is discussed.

Introduction

Eight years ago we discussed the evolution and relationships within the genus *Galeopsis* L. (Wieffering and Fikenscher 1974b) making use mainly of the extensive biosystematic studies of Müntzing (1927–1943). The need of additional information in order to obtain full understanding of the differentiation within the genus was stressed. The main subject of the 1974 paper (Wieffering and Fikenscher 1974b) was an evaluation of the systematic potentialities of iridoid patterns in the leaves of the various species of *Galeopsis* (classification and nomenclature according to Townsend 1972). By comparative chromatographic investigations of leaf extracts, and on a more limited scale of root and seed extracts, the main iridoid glucosides of all 9 species were traced and tentatively identified with compounds whose structures were known in 1973 (compare table 6 and fig. 3).

Travail dédié au professeur Claude Favarger, à l'occasion de son 70e anniversaire

It was concluded that acetylharpagide, as a leaf constituent, was typical for the species of subgenus *Ladanum* and galiridoside for those of subgenus *Galeopsis.* Moreover two unknown ester glucosides were found in the genus. "Ester Rf 0.65–0.70" (= reptoside, see also note c, table 6) was observed mainly in species of subgenus *Ladanum* and in *G. pubescens.* The so-called "bifida-Stoff" (= ajugoside, see also note d, table 6) seemed to be restricted to the leaves of *G. bifida* and *G. tetrahit* and to accumulate in the roots of all four species of subgenus *Galeopsis.* The two subgenera seemed to be connected by *G. pubescens* which belongs to subgenus *Galeopsis,* but resembles the species of subgenus *Ladanum* in several respects, including the iridoid pattern of the leaves. It was also remarked that the iridoid pattern of the leaves of *Galeopsis* are very similar to those of the leaves of *Lamiastrum* Heister ex Fabr., but are quite distinct from those of the investigated species of *Lamium* L. sensu Ball (1972) (Wieffering and Fikenscher 1974a, b).

The purpose of the present paper is threefold:

1). To report chromosome counts for all species of *Galeopsis,* mainly from localities in the Netherlands, Switzerland, and France and to discuss their systematic meaning.

2). To check the usefulness of the character "presence of scutellarin in leaves" for the taxonomy of *Galeopsis* and for the identification of the taxa within the polyploid aggregate *G. bifida* - *G. tetrahit.*

3). To reevaluate the systematic meaning of iridoid patterns in the genus *Galeopsis.* This became desirable after Sticher and his group (see table 6) had described the isolation and identification of a total of eleven iridoid glucoside from 4 species of *Galeopsis.*

Material and methods

Plants

Plants and/or seeds were collected in nature or received from Botanical Gardens. Seeds were sown and grown to maturity in an experimental garden. Mature specimens from all acquisitions were carefully identified and documented by voucher specimens (voucher numbers in tables 4 and 5; classification and nomenclature according to Townsend 1972). Vouchers are kept in the herbarium of the Laboratorium voor Experimentele Plantensystematiek.

Karyological techniques

Young flower buds were fixed according to Östergren and Heneen (1962) or in Carnoy's fluid ($EtOH$–$CHCl_3$–$AcOH = 6:3:1$ v/v/v). Aceto-carmine staining was used. Satisfactory squash preparations were documented by photographs or by transforming them into permanent mounts according to Zeilinga and Kroon (1965). However, euparal was used for mounting instead of canada balsam. Chromosome counts were accomplished from meiotic meta- or telophases. In the few cases where no satisfactory meiotic divisions could be found, mitotic divisions from actively growing parts of the flower buds were used instead.

Detection of scutellarin

Small fragments of several leaves of each plant were placed in a vial containing a few ml of 5% hydrochloric acid. This transforms the soluble salts of scutellarin into the highly insoluble free acid form (see fig. 2: carboxylic group of glucuronic acid) and thus induces crystallization of the compound. After at least 24 hours the leaf-fragments were transfered to a large drop of aqueous chloralhydrate (7 + 3) on a slide and covered with a coverslip. After clearing by gently warming the preparation is ready for microscopic examination.

The presence of scutellarin (the 7-glucuronide of scutellarein) is indicated by large, yellow spherocrystals (fig. 1). This test was also applied to *Scutellaria altissima* (known to contain scutellarin), *S. columnae* (containing baicalin (the 7-glucuronide of baicalein), fig. 2), and *S. galericulata* (containing the 7-glucuronide of chrysin, fig. 2). Since spherocrystals were formed only in *S. altissima*, the HCl-test already used by Molisch and Goldschmiedt (1901) and by Strecker (1909) must be rather specific for scutellarin. The presence of scutellarin, baicalin, and chrysin 7-glucuronide in the above mentioned species of *Scutellaria* was proven by isolation (tables 1 and 2).

Isolation and identification of flavone 7-glucuronides (reference substances)

Glucuronides (present in plants as salts) were extracted from fresh plants by boiling water and subsequently precipitated from the aqueous extracts by hydrochloric acid. Purification was performed by crystallization from methanol (98%) or from methyl cellosolve, which is a much better solvent for scutellarin than methanol. The compounds were identified by their melting points and U.V. spectroscopy (Marsh 1955; Ruygrok unpublished). Moreover scutellarin was hydrolyzid and characterized by its aglycon, scutellarein. The relevant facts are reported in tables 1 and 2.

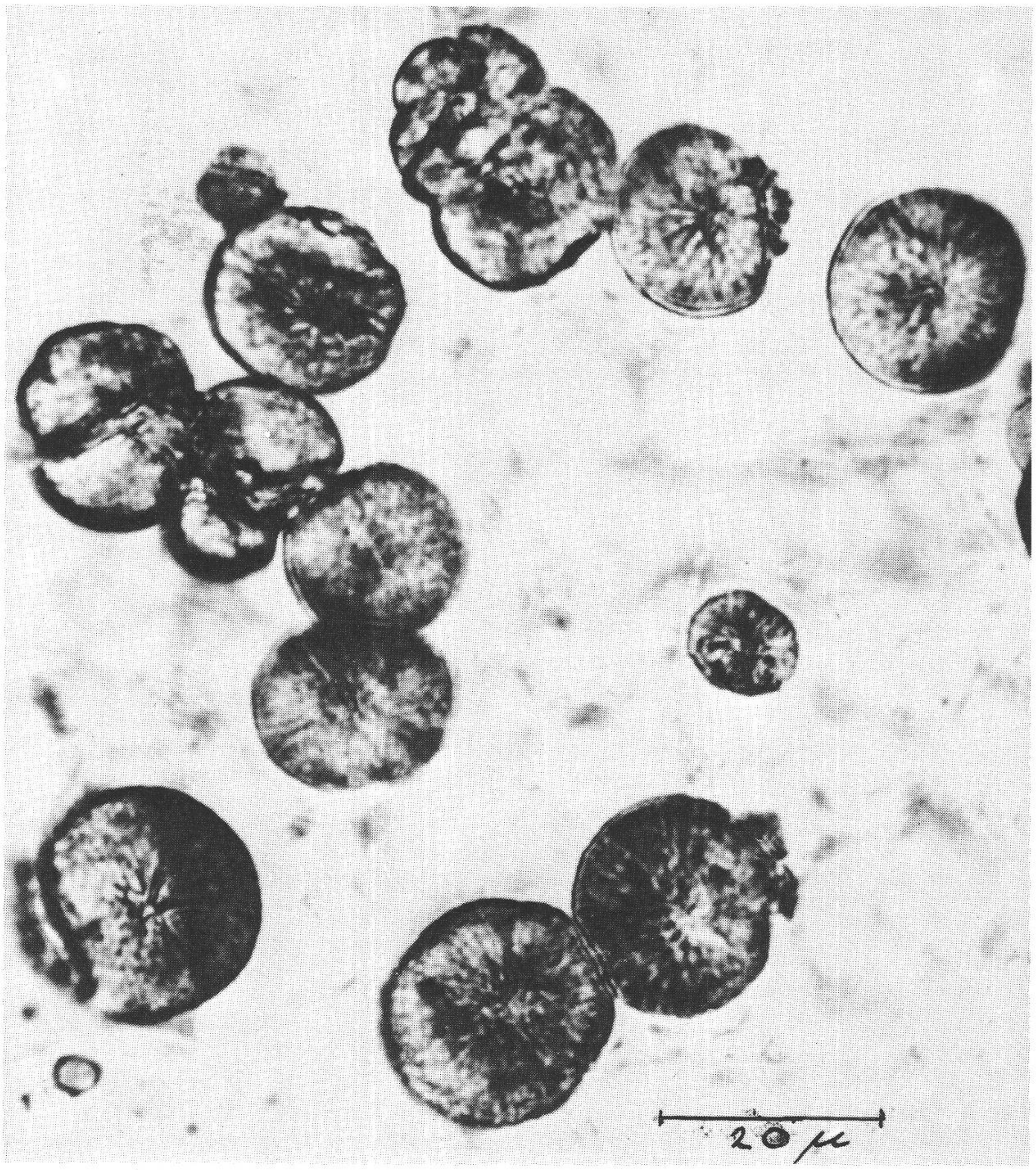


Fig. 1. Spherocrystals of scutellarin in the leaf-epidermis of *Galeopsis tetrahit* L.

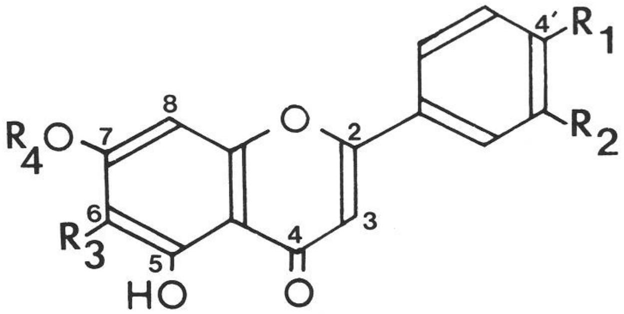


Fig. 2. Scutellarin and related glucuronides occuring in *Galeopsis* and *Scutellaria*.
Scutellarin: $R_1 = R_3 = OH$; $R_2 = H$; $R_4 = GlA$
Baicalin: $R_3 = OH$; $R_1 = R_2 = H$; $R_4 = GlA$
Chrysin glucuronide: $R_1 = R_2 = R_3 = H$; $R_4 = GlA$
Scutellarein (= 6-hydroxyapigenin): $R_1 = R_3 = OH$; $R_2 = R_4 = H$
Baicalein: $R_1 = R_2 = R_4 = H$; $R_3 = OH$
Chrysin: R_1-$R_4 = H$
6-Hydroxyluteolin: R_1-$R_3 = OH$; $R_4 = H$
GlA = glucuronic acid

Table 1. Yields and properties of glucuronides isolated from fresh aerial parts of some species of *Scutellaria* and *Galeopsis*

Taxon and voucher no	Amount of extracted material	mg crude compound	mg purified compound	M.P. (°C) (uncorr.)	Rf-values[b]		
					A	B	C
S. altissima 12099	400 g	5200	c. 3000 (S)[a]	300	.40–.45	.32–.38	.42
S. columnae 17840	50 g	1016	400 (B)	218	.54	.56	.61
S. galericulata 18319	70 g	1188	437 (CH)	221	.52	.54	.61
G. tetrahit 14820	10 g	–	4 (S)	300	.43	.30	.42
G. pubescens 12013	65 g	874	75 (S)	300	.45	.33	.42

[a] (S) = scutellarin (m.p. in lit. 300 °C; "fusion instantanée" of hydrated form according to Charaux 235–240 °C).
(B) = baicalin (m.p. in lit. 223 °C).
(CH) = chrysin 7-glucuronide (m.p. in lit. 225–226 °C):
[b] (A) = phenol, water-saturated; Whatman 1 paper, ascending.
(B) = borax – borid acid buffer, pH 8,6 (Tabellenboekje Kon. Ned. Chem. Ver., 18[e] ed. 1962); Whatman 1 paper, ascending.
(C) = pentanol – AcOH – water = 20:12:10 (Bose and Fröst 1967); Cellulose – Fertigplatten (Merck), ascending.

Table 2. UV spectra of glucuronides isolated from *Scutellaria* and *Galeopsis:* absorption peaks[a]

Source	Scutellarin	Baicalin	Chrysin 7-glucuronide
Marsh 1955	285; *335*	246; *279;* 314	*270;* 306 (shoulder)
present paper:			
S. altissima	286; *336*	–	–
S. columnae	–	247; *280;* 316	–
S. galericulata	–	–	*272;* 310 (shoulder)
G. tetrahit	286; *336*	–	–
G. pubescens	287; *337*	–	–

[a] major peak (λ_{max}) *underlined.*

Table 4. Chromosome counts in the genus *Galeopsis*. Personal counts

Sub-genus	Taxon	2n[a]	Origin[b]	Voucher number[c]
Ladanum	*G. angustifolia*[d]	16	Switzerland, Ticino	12396
			Bot. Garden	8637
	G. ladanum[d]	16	Bot. Garden	8606, 8635, 8636, 12343
	G. pyrenaica[d]	16	France, Pyrénées Orientales	17076
		16	Bot. Garden	8599, 8607, 12019, 12379, 15592, 18012
	G. reuteri[d]	16	France, Alpes Maritimes	17217 (see Table 3)
	G. segetum[d]	16	Netherlands, N-Brabant	18020
		16	Bot. Garden	8605, 8608, 8638, 8639, 8640, 12012, 12017, 12022, 12380, 12381
Galeopsis	*G. bifida*[d]	32	Netherlands, N-Brabant	19492
		32	Netherlands, Overijssel	8758
		32	Bot. Garden	8603, 8609, 14862
	G. pubescens[e]	16	Netherlands, Gelderland	8755
		16	Switzerland, Ticino	8304, 12395, 12397
		16	Bot. Garden	8759, 15581, 15583
	G. speciosa[d]	16	Eastern Germany, Sachsen-Anhalt	17593
		16	Netherlands, Groningen	20766
		16	Netherlands, Overijssel	22872
		16	Bot. Garden	8760, 11533, 12344
	G. tetrahit[e]	32	Italy, Pavia	15852
		32	Netherlands, Drenthe	12365, 12368, 14810, 14817, 14818, 14820
		32	Netherlands, Gelderland	16684
		32	Netherlands, Groningen	12370, 12372, 21454
		32	Netherlands, N-Brabant	19494
		32	Netherlands, N-Holland	19491
		32	Netherlands, Utrecht	18005
		32	Netherlands, Zeeland	12340
		32	Netherlands, Z-Holland	18011, 23589 (see Table 3)
		32	Switzerland, Glarus	6466, 10566, 10581
		32	Switzerland, Graubünden	10663, 20326
		32	Switzerland, Neuchâtel	8105, 8299, 20455
		32	Switzerland, St. Gallen	19858
		32	Switzerland, Ticino	12399, 12400, 15843, 18010

[a] With the exception of two counts published earlier (see Table 3 – v.d. Brand et al.; Kliphuis and Wieffering) and a few specimens where no meiotic metaphases could be found, only meiotic metaphases from PMC's were studied. For the sake of convenience the haploid numbers were doubled.
[b] Only country and provinces (cantons, départements) are given. Plants grown from seeds procured by Botanical Gardens were carefully identified.
[c] Each voucher number represents a separate sample (acquisition, collection).
[d] All samples, *G. bifida* 19492 excepted, gave a negative reaction for scutellarin (see also Table 5).
[e] All samples, *G. tetrahit* 23589 excepted, gave a positive reaction for scutellarin (see also Table 5).

Results

New chromosome counts in Galeopsis

According to Müntzing (1930a) the genus *Galeopsis* comprises three coenospecies; *Ladanum* (all 5 ecospecies of subgenus *Ladanum)*, the diploid pair *G. pubescens* and *G. speciosa* (2n = 16), and the tetraploid pair *G. bifida* and *G. tetrahit* (2n = 32). The latter pair is to be interpreted as an allopolyploid complex consisting of two ecospecies, each made up of a rather large number of genoecodemes (= ecotypes sensu Turesson) (for terminology see e.g. Stace 1980).

Müntzing (1930a, b) found a highly sterile triploid plant among the F_2 of a hybridization experiment with *G. pubescens* and *G. speciosa.* On backcrossing with *G. pubescens* this plant produced only one viable seed. From this seed a tetraploid self-fertile plant was grown. By selfing and selecting in the descendant generations Müntzing obtained plants indistinguishable from, and interfertile with, naturally occurring *G. tetrahit.* As early as 1932 Müntzing formulated his conviction that natural polyploids in most cases arise via unreduced gametes, especially egg cells (Müntzing 1932b p. 136 ff.). In recent years this view is rapidly gaining ground (e.g. Harlan and deWet 1975; deWet 1980; Lewis 1980b).

There remain, however, still some questions to be answered before the tetraploid complex within the genus *Galeopsis* is fully understood (Wieffering and Fikenscher 1974b). A very intriguing question concerns the possibility of a polytopic origin of both species, *G. bifida* and *G. tetrahit.* This pattern of evolution becomes still more likely if tetraploid cytodemes do exist within the diploid parent species. Intraspecific polyploidy is known from many plant species (e.g. Lewis 1980a) and seems to be restricted in some species to marginal populations (Sieber and Murray 1980). In any case it seemed highly desirable to extend considerably the karyological investigations of *Galeopsis* and to cover, as far as possible, new localities of all species of the genus. My results are reported in table 4 and compared with chromosome counts of other scientists which are summarized in table 3.

Tables 3 and 4 demonstrate clearly that chromosome numbers are constant in each species of *Galeopsis.* All new counts reported in table 4 confirm former counts. This makes it highly probable that there is only one way to tetraploidy in *Galeopsis,* viz. hybridization of diploid species and polyploidization by production of non-reduced gametes in F_1- and F_2-plants, i.e. the process already described by Müntzing. Two questions, however, remain unsolved: (1) Did *G. tetrahit* arise only once? (2) Did *G. bifida* arise independently from *G. tetrahit* or did it arise by ecological specialization within allopolyploid *G. tetrahit?* Much work is still needed to procure an unambiguous answer to both questions.

Scutellarin as a genetic and taxonomic marker in Galeopsis

Molisch and Goldschmiedt (1901) described scutellarin as a new flavonoid constituent of several species of *Scutellaria.* Scutellarin is slowly hydrolized by strong acids to scutellarein and a sugar-like compound. The latter was later shown to be glucuronic acid (Goldschmiedt and Zerner 1910). Marsh (1955) showed scutellarin to be the 7-glucuronide of 5:6:7:4′-tetrahydroxyflavone (= scutellarein = 6-hydroxyapigenin) (fig. 2). Molisch (Molisch and Goldschmiedt 1901) described also some microchemical reactions which are highly characteristic of scutellarin (and perhaps closely related glucuronides). If leaf fragments are placed in a cold aqueous solution of a strong acid

Table 5. Occurrence of scutellarin in leaves of *Galeopsis*

Taxon	Origin	Number of tested samples (voucher number[a])	Scutellarin test[b]
Subgenus *Ladanum*			
G. angustifolia	France	3	0
	Italy	1	0
	Switzerland	5	0
	Western Germany	1	0
	Bot. Garden	2	0
G. ladanum	France	2	0
	Switzerland	6	0
	Bot. Garden	4	0
G. pyrenaica	France	2	0
	Bot. Garden	8	0
G. reuteri	France	1	0
G. segetum	France	5	0
	Luxemburg	1	0
	Netherlands	2	0
	Bot. Garden	11	0
Subgenus *Galeopsis*			
G. bifida	Belgium[c]	2	0
	Eastern Germany	3	0
	France	1	0
	Netherlands	8	0
	Sweden	2	0
	Switzerland	2	0
	U.S.S.R.[c]	1	0
	Western Germany	2	0
	Bot. Garden	3	0
	Netherlands	1 (19492)	+ and 0
	Switzerland	1 (22315)	+ and 0
G. pubescens	Eastern Germany	1	+
	Italy	1	+
	Netherlands	1	+
	Switzerland	14	+
	Bot. Garden	6	+
	France	1 (237)	0
	Italy	1 (234)	0
	Switzerland	1 (12394)	0
G. speciosa	Austria	1	0
	Eastern Germany	2	0
	Finland	1	0
	France	1	0
	Liechtenstein	1	0
	Netherlands	9	0
	Poland	2	0
	Western Germany[c]	1	0
	Bot. Garden	5	0
G. tetrahit	France	3	+
	Great Britain[c]	1	+
	Italy	2	+
	Netherlands	45	+
	Norway	1	+
	Poland	1	+
	Switzerland	53	+
	Western Germany	1	+
	Netherlands	1 (18003)	+ and 0
		3 (14732, 14812, 23589)	0
	Switzerland	1 (20850)	+ and 0
	Switzerland	3 (4841, 8022, 8026)	0

[a] Voucher numbers given only if results were aberrant.
[b] Scutellarin-test according to Molisch (1901) and Strecker (1909) performed with leaf fragments of herbarium specimens: 0 = no spherocrystals; + and 0 = some leaves positive and some negative; + = many spherocrystals in all fragments examined.
[c] Investigated specimens loaned from Rijksherbarium (L).

(preferably HCl, 1–5%) large, yellow spherocrystals slowly appear, mainly in the lower epidermis (fig. 1). On moistening with a solution of barium hydroxide their colour changes to rust-red, and subsequent treating with iodine turns them green.

By applying these reactions, Molisch could demonstrate the presence of scutellarin in 6 species of *Scutellaria,* in *Teucrium chamaedrys* L., and in *G. tetrahit* L. but he could not detect the compound in twelve other species of Labiatae. Strecker (1909) continued the search for scutellarin. He investigated 350 plant species, including many Labiatae, but found scutellarin only in representatives of four genera of Labiatae, *Galeopsis, Scutellaria, Teucrium,* and *Thymus.* Charaux and Rabaté (1940) isolated scutellarin from leaves of *Centaurea scabiosa* L. (Compositae). Accumulation of scutellarin by *G. tetrahit, Scutellaria,* and some species of Compositae was confirmed by Plouvier (1963); he described its isolation from four species of *Scutellaria, G. tetrahit,* and from the three composites *Erigeron canadensis* L. (= *Conyza canadensis* (L.) Cronq.), *Erigeron ramosus* Britton, Stern et Pogg. (= *E. annuus* (L.) Pers. subsp. *strigosus* (Mühl. ex Willd.) Wagenitz), and *Centaurea calcitrapa* L.

Strecker (1909) observed scutellarin in *G. ladanum* L., *G. versicolor* Curtis, and *G. tetrahit* L., but not in *G. bifida* Boenn., *G. pubescens* Besser, *G. walteriana* Schlecht., and *G. neglecta* Schultes. According to Briquet (1893), Porsch (1903), and Townsend (1972) *G. versicolor* Curtis (non Spenner) is a synonym of *G. speciosa* Mill., and *G. walteriana* Schlecht. (written *walterina* by Briquet and *walterana* in Flora Europaea, vol. 3 (1972), p. 351) a synonym of *G. pubescens* Bess. *G. neglecta* Schultes probably belongs to *G. tetrahit* var. *bifida* Lejeune et Courtois (= subsp. *bifida* Fries = *G. bifida* Boenn.) (Briquet 1893) or to *G. tetrahit* L. s.s. (Porsch 1903).

The results of Molisch and Goldschmiedt and of Strecker induced me to investigate more closely the character "presence of scutellarin in leaves" for the whole genus *Galeopsis.* The results of this investigation are summarized in table 5.

With regard to *G. tetrahit* my results are in complete agreement with those of Molisch (Molisch and Goldschmiedt 1901) and Strecker (1909). This species accumulates large amounts of scutellarin. I also agree with Strecker with regard to the absence of scutellarin from *G. bifida.* Presence or absence of scutellarin, as detected by the very simple hydrochloric acid test, discriminates nicely between these two closely related taxa. All my other observations, however, are at variance with Strecker's. The only other species of *Galeopsis* which accumulates scutellarin is *G. pubescens.* I did not find scutellarin in any leaf sample neither of subgenus *Ladanum,* nor of *G. speciosa.* Perhaps Strecker confused *G. pubescens* and *G. ladanum* on one side and *G. versicolor* Curtis and *G. versicolor* Spenner on the other side. *G. versicolor* Spenner (non Curtis) is synonymous with *G. tetrahit.*

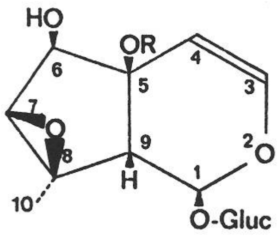
Accumulation of scutellarin in the leaves of the allopolyploid species *G. tetrahit* must derive from *G. pubescens.* The presence of scutellarin in the latter species was confirmed by isolation and spectroscopic and chromatographic characterization (see Material and methods and tables 1 and 2). The absence of scutellarin from the foliage of *G. bifida* can be explained in several ways which are for the time being equally plausible.

The few aberrant observations included in table 5 need some comment. *G. bifida* 19492 grew at the bank of a brooklet, facing a population of *G. tetrahit. G. bifida* 22315 was collected in the transition-zone between a wet and swampy nature-reserve and the neighbouring agricultural fields. Under such circumstances some hybridization may well occur and be responsible for the varying amount of scutellarin in the foliage of *G. bifida.*

G. pubescens 234 and 237 were collected in the southwestern alps at the italian and french side of the border respectively. The two specimens are very much alike. They are not only chemically but also morphologically atypical. The stems and branches are very thin, with the nodes hardly swollen. The general shape of the leaves is about normal but the leaves are very small (less than 3 cm long) and strikingly hairy. These plants from the southwestern edge of the range of the species possibly represent a distinct taxon.

G. tetrahit 8022, 8026, and 18003 were collected in habitats where some hybridization with *G. bifida* does not seem improbable (8022 and 8026 in Switzerland along the river Areuse; 18003 in the Netherlands, at the slope to a pool near the river Rhine).

For *G. pubescens* 12394 (Ticino, Switzerland), *G. tetrahit* 14732, 14812 (Netherlands), 4841 (Bern, Switzerland), and 20850 (Ticino, Switzerland) no explanation can be offered for the deviating behaviour because their habitats were not examined by me. All these plants are typical representatives of the respective species.



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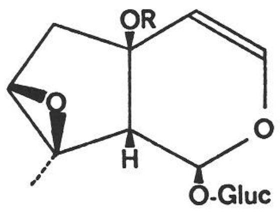

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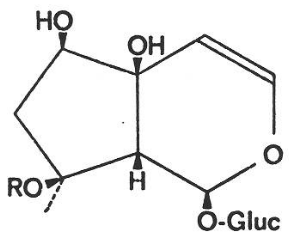

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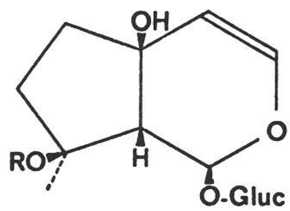

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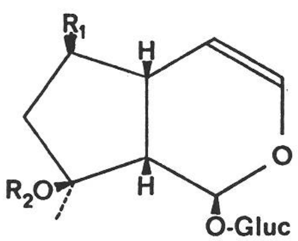

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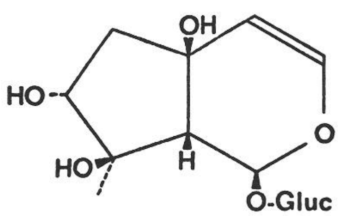

VI

Fig. 3. Iridoid glucosides (= aucubinoids) of species of *Galeopsis* (compare table 4). I. R = H: antirrhinoside **(1)**, R = glucosyl: 5-glucosyl antirrhinoside **(2)**; II. R = H: galiridoside **(3)**, R = glucosyl: 5-glucoxyl galiridoside **(4)**; III. R = H: harpagide **(5)**, R = $COCH_3$: acetylharpagide **(6)**; IV. R = H: 6-deoxyharpagide **(7)**, R = $COCH_3$: reptoside **(8)**; V. R_1 = OH and R_2 = H: ajugol **(9)**, R_1 = OH and R_2 = $COCH_3$: ajugoside **(10)** (Damtoft et al. 1981); R_1 = R_2 = H: gluroside **(11)** (= 6 deoxyajugol); VI. daunoside **(12)**.

Table 6. Iridoids in *Galeopsis*

Taxon (number of leaf samples investigated by W–F[a])	References	Iridoid compounds[b]						Plant part
		galiridoside **3**	harpagide **5**	acetylharpagide **6**	reptoside[c] **8**	ajugoside[d] **10**	other compounds	
Subgenus *Ladanum*								
G. angustifolia (7)	W–F[a]	–	+	+ +	+	–	–	leaves
G. ladanum (12)	W–F	?	+	+ +	(+)	–	–	leaves
G. pyrenaica (4)	W–F	+	+	+ +	traces	–	–	leaves
G. reuteri (1)[a]	W–F	–	+	–	–	–	–	leaves
G. segetum (5)	W–F	+ +	+ +	+ +	(+)	–	–	leaves
	Junod–Busch 1976[e]	+	+ +	+ +	+	–	**1,2**	whole plant
Subgenus *Galeopsis* (= subgen. *Tetrahit*)								
G. pubescens (5)	W–F	+ +	+	+	+	–[f]	–	leaves
	Sticher, Rogenmoser, Weisflog 1975	+ +	+	+ +	+ +	–	**12**	aerial parts
	Rogenmoser 1975[g]	+ +	+	+ +	+ +	–	**12**	aerial parts
G. speciosa (5)	W–F	+ +	+	–[h]	–	–[h]	–	leaves
G. bifida (8)	W–F	+ +	+	–[i]	–	traces[i]	–	leaves
	Junod-Busch 1976[k]	+ +	+	+ +	+	+	**4, 7, 9, 12 H, O**	aerial parts
G. tetrahit (11)	W–F	+ +	+	–[l]	–	traces[l]	–	leaves
	Sticher 1970a, 1970b	+ +	+	–	–	–	**7, 11, 12**	leaves
	Sticher, Rogenmoser, Weisflog 1975	+ +	+	–	–	–	**7, 11, 12**	leaves
	Sticher, Weisflog 1975[m]	+ +	+	–	–	–	**7, 11, 12**	leaves
	Weisflog 1975[m]	+ +	+	–	–	–	**7, 11, 12**	leaves

[a] Wieffering and Fikenscher 1974b. 5–10g fresh or dried leaves were extracted; in the case of *G. reuteri* only a few leaves from an herbarium specimen were available.
[b] + + = main constituent; + = present in appreciable amounts; (+) = detected in some samples only; – = not detected. Compounds with known structure indicated by numbers (**1**–**12**); Compounds with as yet unknown structure indicated by letters (**H**, **O**) (fig. 3; Junod-Busch 1976).
[c] Identical with "Ester R_f 0.65–0.70" of W–F (Sticher and Weisflog 1975; Rogenmoser 1975).
[d] Identical with "Bifida-Stoff" of W–F (Sticher and Weisflog 1975; Junod-Busch 1976).
[e] 2.4 Kg of fresh, flowering plants, including roots, were extracted.
[f] In roots (3 samples): (+).
[g] 18.16 Kg of fresh aerial parts of flowering plants were extracted.
[h] In roots (1 sample): **6** + ; **10** + + .
[i] In roots (2 samples): **6** and **10** both + + .
[k] 5.6 Kg of fresh aerial parts of flowering plants from a mixed population containing c. 60% *G. bifida* and 40% *G. tetrahit* were extracted. In roots the same pattern of iridoids was found.
[l] In roots (2 samples): **6** + + : **10** + to + + : in seeds (1 sample): **6** + + : **8** + .

Concluding it may be stated that the production of spherocrystalline masses of scutellarin by immersion of leaf fragments in cold hydrochloric acid is a useful feature to characterize *G. tetrahit* and *G. pubescens.* As far as is known at present, only the 7-glucuronide of 6-hydroxyapigenin does react precisely in the same way as was described by Molisch and by Strecker for scutellarin. Scutellarein glycosides (lacking the carboxylic group of glucuronic acid) and methyl ethers of scutellarein behave otherwise. *G. segetum* (= *G. ochroleuca*) contains scutellarein 7-glucoside and 6-hydroxyluteolin 7-glucoside (Trotin and Pinkas 1979) and *G. ladanum* contains ladanein (7,4′-dimethyl ether of scutellarein) and ladanetin (7-methyl ether of scutellarein) and several glycosides of ladanein (Gritsenko and Litvinenko 1969; Gritsenko, Litvinenko, and Kovalev 1969). Both taxa give a negative scutellarin test. 6-Hydroxyapigenin (= scutellarein) and 6-hydroxyluteolin are rather frequent flavonoids in families of Tubiflorae (Harborne and Williams 1971) but they rarely occur in the form of unmethylated 7-glucuronides. Though it was shown by me that baicalin and chrysin 7-glucuronide (see fig. 2 and Material and methods) do not give the scutellarin-reaction, it may well be that 6- and 8-hydroxyluteolin, when present in a plant as the 7-glucuronide, would produce the same microchemical reactions as does scutellarin.

Iridoid patterns of Galeopsis species

Sticher and his group performed very accurate phytochemical investigations with four species of *Galeopsis:* viz. *G. segetum*, *G. pubescens*, *G. tetrahit*, and *G. bifida.* Their *G. bifida* material was probably influenced by hybridization with *G. tetrahit.* Hybrid populations between these two species are by no means rare (Müntzing 1930a, this paper p...). The results of Sticher's group are compared with our results in table 6 and illustrated by fig. 3.

In most instances our comparative studies agree well with the iridoid glucosides as isolated from four species of *Galeopsis* by Sticher's group. Of course all minor components are not detectable by the analytical procedure applied by us (table 6, note a). Thus compounds **4, 7, 9, 11,** and **12** (fig. 3) which were encountered as minor components in one or more species were not trace in our study.

There is one striking discrepancy, however, in the results of the two groups. It concerns acetylharpagide which is lacking in the leaves of *G. bifida* according to our observations and present in large amounts according to Junod-Busch (1976). This affects our former conclusion that acetylharpagide as a leaf constituent is typical of species of subgenus *Ladanum* and of *G. pubescens* but lacks in the other three species of subgenus *Galeopsis.* In this respect two facts should not be forgotten. Firstly there are appreciable differences between the different parts of a plant (Wieffering and Fikenscher 1974a). The material extracted by Junod-Busch (1976) did contain besides leaves also stems, and all parts of flowers. Secondly, hybridization may affect the iridoid patterns of a given population. Three of the *Galeopsis* iridoids are acetylated compounds, i.e. acetylharpagide **(6)**, reptoside **(8)**, and ajugoside **(10).** Compounds **(6)** and **(8)** are more characteristic of the species of subgenus *Ladanum* and of *G. pubescens* if only leaves are examined, and **(10)** occurs in roots and leaves of species of subgenus *Galeopsis.* It is not impossible that acetylation of glucosides is affected by hybridization. Junod-Busch assumed that the glucosides **(4), (6), (8), (9),** and **(10)** are typical for *G. bifida* because they were not encountered by Weisflog in *G. tetrahit.* On the other hand she could not trace gluroside **(11)** in her *G. bifida* material; the latter compound is therefore assumed to show intraspecific variation (this conclusion is based on the presence of c. 40% *G. tetrahit* plants in the extracted sample of *G. bifida*). In this respect it is interesting to retain that Weisflog (1975) extracted leaves only in his study of the iridoid compounds of *G. tetrahit*. In agreement with us he did not find acetylharpagide in the leaves of this taxon. It is not impossible that most of the acetylharpagide isolated by Junod-Busch from *G. bifida* stemmed from stems and flowers not from leaves.

Another discrepancy which deserves to be mentioned is the fact that antirrhinoside **(1)** and glucosylantirrhinoside **(2)**, which were isolated by Junod-Busch (1976) from *G. segetum* in amounts equalling those of galiridoside **(3)** and surpassing those of reptoside **(8)**, were not detected by us. Most probably **1** was masked on our chromatograms by **6**, and **2** was overlooked because it does scarcely react with Godin's reagent. Hitherto **1** and **2**, two iridoids formerly known only from Scrophulariaceae, have been traced only in one species of subgenus *Ladanum;* possibly they represent a biochemical character of this subgenus.

I think it is safe to maintain that iridoid patterns represent characters worth of further study. One should realize, however, that the patterns may vary with plant parts and that they may be affected by hybridization. Moreover it is more than likely that there is some variation within each species, especially in the highly variable taxa belonging to subgenus *Galeopsis* (Briquet 1893; Porsch 1903; Henrard 1919). Therefore much more research has to be performed before these patterns can be safely used as taxonomic and biosystematic markers in *Galeopsis*.

Conclusions

The caryological and chemical characters treated in this paper contribute to the understanding of *Galeopsis* in an evolutionary sense.

Absence of infraspecific polyploidy suggests that there is only one way to polyploidy in *Galeopsis;* repeated hybridization and production of unreduced gametes by hybridogenic plants.

Presence of scutellarin in *G. tetrahit* and *G. pubescens* and its absence in all other species shows clearly that the character was introduced in *G. tetrahit* by *G. pubescens*.

It seems that a more detailed study of iridoid compounds of all species of *Galeopsis* would be rewarding in the context of efforts undertaken to disclose the phylogenetic history of the genus in all details.

Two essential questions remain still unanswered: (1) Did *G. tetrahit* have a monotopic or a polytopic origin? (2) Is *G. bifida* an ecodeme of *G. tetrahit* or does it have an independent origin from the two diploid species of subgenus *Galeopsis?*

Zusammenfassung

Chromosomenzahlen wurden für Herkünfte aller bekannten *Galeopsis*-Arten ermittelt (Tabelle 4); es wurden keine von den bereits bekannten (Tabelle 3) abweichende Zahlen gefunden.

Scutellarin wurde aus *G. pubescens* und *G. tetrahit* isoliert. Alle *Galeopsis*-Arten und viele Herkünfte wurden mit Hilfe eines Schnelltests auf Vorkommen dieses 7-Glucuronids geprüft (Fig. 1 und 2; Tabelle 5). Da auch andere 7-Glucuronide (Fig. 2; Tabellen 1 und 2) berücksichtigt wurden, durfte die mineralsäureinduzierte Auskristallisation in

den Blattzellen (Fig. 1) als ziemlich spezifisch für Scutellarin betrachtet werden. Scutellarin läßt sich zur Unterscheidung von *G. bifida* (fehlt) und *G. tetrahit* (vorhanden) heranziehen.

Die Literatur über Iridoiglycoside der Gattung und deren mögliche taxonomische Bedeutung werden kritisch besprochen.

Auf eine mögliche Bedeutung der chemischen Merkmale für das Verständnis der phylogenetischen Zusammenhänge in der Gattung *Galeopsis* wird hingewiesen.

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