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IRIDOIDS AND ALKANES IN TWELVE SPECIES OF *GALIUM* AND *ASPERULA*

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Key Word Index—*Galium*; *Asperula*; Rubiaceae; iridoids; *n*-alkanes; asperuloside; galium glycoside; chemotaxonomy.

Abstract—The results of an analysis of the iridoid and *n*-alkane patterns in twleve species of *Asperula* and *Galium* revealed similarities rather than differences between and within the genera. The ease with which artefacts can be produced from asperuloside limits the taxonomic usefulness of asperuloside-type iridoids. A sub-division of the genus *Galium* based on the dominant alkanes was not completely in accord with the existing division of the genus into sections. The alkane patterns are largely unaffected by plant age or geographical location. They do support some of the taxonomic views currently held concerning the inter-relationships of different species in the genus *Galium*.

INTRODUCTION

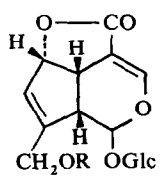
There is considerable taxonomic difficulty in distinguising between taxa of the general *Asperula* and *Galium* and in a recent revision of European members [1, 2], a number of species were moved from one genus to the other. Sectional classification within these genera also presents many problems. With the position of many of the species in doubt, phytochemical evidence might be of assistance in clarifying the situation, although the results so far have not been very encouraging. Numerous workers have reported [3–5] the presence of flavonoids, coumarins and phenolic acids in *Asperula* and *Galium* but no distinctive pattern was discernible either between the genera or within each genus. Anthraquinones have been reported from the roots of many members of the tribe Rubieae [4, 5]. Borisov [6] claims that these constituents may be used to distinguish between the various sections of *Galium*. However his argument is weakened in that is based on evidence that members of the section *Aparine* contain only three anthraquinones, whereas Burnett and Thomson [4] have reported thirteen known anthraquinones from *G. aparine* alone. In the present study, we have examined alkanes and iridoids to see if they have any values as taxonomic markers in these plants.

RESULTS AND DISCUSSION

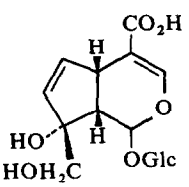
In the present study the iridoid and hydrocarbon profiles of eight species representing seven sections of *Galium*, three species from two sections of *Asperula* and *Cruciata laevipes*, were investigated. Our attention was confined to those species which are indigenous to Ireland and Britain or which were readily available from the National Botanic Gardens, Dublin and the Botanic Gardens of Trinity College Dublin.

Iridoids

The occurrence of asperuloside **1a**, monotropein **2** and related compounds in the whole plants of *Galium* and *Asperula* was examined by TLC using suitable standards. A comparison of the distribution pattern with that reported in the literature [3, 7–10] indicates that no taxonomic conclusions can yet be drawn from the use of asperuloside **1a** type iridoids as markers. All except three of the species tested so far contain asperuloside **1a**. The exceptions are *G. glaucum* [9] (syn *Asperula glauca*), *G. turkestanicum* [7] and *G. uliginosum* [10]. Therefore no distinction can be made between *Asperula* and *Galium* or between the various *Galium* sections, on the basis of the distribution of asperuloside and monotropein **2**.



1a R = Ac
1b R = H



2

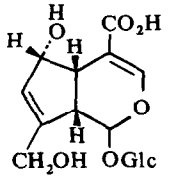


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Table 1. Composition of *n*-alkanes from species of *Galium*, *Asperula* and *Cruciata*

Species	Section*	% Alkane dry weight	Mole %†												
			C_{19}	C_{20}	C_{21}	C_{22}	C_{23}	C_{24}	C_{25}	C_{26}	C_{27}	C_{28}	C_{29}	C_{30}	C_{31}
G. odoratum (L.) Scop.	*Hylaea*	0.024	3	3	4	2	2	+	+	–	55	–	29	+	+
G. boreale L	*Platygalium*	0.022	1	+	1	+	2	+	2	–	8	–	60	3	17
G. rubioides L.	*Platygalium*	0.027	–	–	–	–	–	–	–	–	4	–	90	–	6
G. saxatile L.	*Leptogalium*	0.067	+	+	+	+	2	+	+	+	17	+	57	–	21
G. aparine L.	*Kolgyda*	0.03	+	+	+	+	2	+	2	+	11	+	69	2	8
G. mollugo L.	*Leiogalium*	0.006	5	2	2	3	7	2	3	–	11	+	43	3	17
G. cynanchica L.	*Cyananchicae*	0.04	–	–	–	–	–	–	–	–	–	–	51	–	49
G. palustre L.	*Aparinoides*	0.04	2	+	+	+	+	–	+	–	2	–	32	2	60
G. verum L.	*Galium*	0.078	–	–	–	–	–	–	–	–	+	–	21	–	78
A. tinctoria L.	*Glabella*	0.038	–	–	–	–	–	–	–	–	–	–	22	–	78
A. pontica Boiss.	–	0.053	–	–	–	–	–	–	–	–	–	–	13	–	87
Cr. laevipes Opiz.	–	0.089	–	–	–	–	–	–	–	–	–	–	18	–	82

* According to Ehrendorfer [1, 2]. † The values are approximated to the nearest 1 % and peaks of relative area < 1 % are denoted by +.

A number of minor iridoids other than asperuloside and monotropein were noted on the chromatograms. An attempt to isolate these from *G. boreale* was unsuccessful. The asperuloside-free extract containing these minor iridoids gave asperuloside tetraacetate on acetylation. The source of the asperuloside tetraacetate may be deacetylasperuloside **1b** or deacetylasperulosidic acid **3**, the latter having been postulated recently as a precursor in the biosynthesis of asperuloside [11]. The possibility that the iridoids **1b** and **3** are artefacts, produced by the hydrolysis or methanolysis [12] of asperuloside prior to, or during, extraction, cannot unequivocally be ruled out but seems unlikely since the plant samples were extracted with acetone, a solvent in which asperuloside is stable.

The apparently facile manner in which asperuloside gives rise to artefacts is an indication of the unsuitability of this type of iridoid as a taxonomic marker. Kooiman [13] utilized an iridoid isolated from an aqueous extract of seeds of *G. aparine* as a taxonomic marker in the Rubiaceae. He named this iridoid 'galium glycoside' and suggested that it might be deacetylasperulosidic acid **3**. Hegnauer [3] however considers that this iridoid may be scandoside (6-epideacetylasperulosidic acid). To further elucidate the structure we have examined a small sample of the 'galium glycoside' kindly sent to us by Dr Kooiman. Acetylation indicated the presence of two components, one of which had an R_f value on TLC similar to that of asperuloside tetraacetate. The second acetate gave an R_f value on TLC different from that of monotrepein pentaacetate. The NMR spectrum in D_2O of the parent glycoside showed the absence of an acetyl group whilst the IR spectrum gave no signal at 1735–1745 cm^{-1} corresponding to the lactone ring of asperuloside and deacetylasperuloside [14]. On the limited evidence available we therefore suggest 'galium glycoside' is a mixture of deacetylasperulosidic acid **3** and an unidentified iridoid.

n-*Alkanes*

The results of the *n*-alkane survey are presented in Table 1. Replicate GLC analyses of the hydrocarbon fractions of the powdered plant of the species *G. aparine* and *G. boreale* were undertaken to ensure species specificity in the hydrocarbon fractions. For the *Galium* and *Asperula* species studied, *n*-nonacosane (C_{29}) and *n*-hentriacontane (C_{31}) are the most frequent major components of the homologous series of *n*-alkanes present. *n*-Heptacosane (C_{27}) is the major constituent in one species, *G. odoratum* (syn. *Asperula odorata*).

Although the alkane patterns at the generic level are not distinctive the species may be divided into three main groups based on the major alkane present. *Cruciata laevipes* (syn. *G. cruciata*) has an alkane profile identical with two *Galium* species, *G. palustre* and *G. verum*. This is a further indication of the chemical similarities in these plants. Attempts to relate the qualitative results for *n*-alkanes with the various sectional classifications of *Galium* [1, 2, 15, 16] in general failed but two points of interest to emerge. The similar alkane patterns obtained for *G. boreale* and *G. rubioides* support Ehrendorfer's [1] decision not to divide section *Platygalium* into two as suggested by Pobedimova [16], and the differences in alkane content support the separation of *G. mollugo* from *G. verum*.

Variation in n-*alkane content*

Variations in the alkane profiles of the *Galium* species due to age, plant part, and geographical location were considered. Sampling over five months (May–September) of *G. aparine* and the analysis of the alkane profile show an initial percentage increase of the dominant alkane (*n*-C_{29}) with subsequent reduction and concurrent appearance of even numbered homologues. Our results and those of Wilkinson and Kasperbauer [17] show that the phenomenon of chain-length elongation is dependent on factors other than plant age. Differences in the dominant alkane from morphologically distinct parts of *G. verum* and *G. aparine* were noted. For example, the leaves of *G. verum* have *n*-C_{31} as the dominant alkane while in the petals *n*-C_{29} is dominant. Finally the variation due to geographical location within and without Ireland was examined. The dominant alkane in five species (*G. boreale*, *G. odoratum*, *G. palustre*, *G. saxatile* and *G. aparine*) remained unaltered irrespective of source.

Oleanolic acid was isolated from an ethereal extract of *G. boreale* and characterized as its acetate [18]. Because of its widespread occurrence in the Rubiaceae [3], this triterpene is of little taxonomic value in the family.

EXPERIMENTAL

Plant material. Most samples were derived from plants growing in their natural habitat, but some cultivated plants were used (Trinity College Botanical Gardens; National Botanic Gardens, Dublin 9). Voucher specimens were deposited in the National Herbarium of the last mentioned Gardens (DBN).

Preparation of extracts. Powdered air-dried samples of flowering plants (30–40 g) were extracted with *n*-hexane and subsequently with Et_2O and hot Me_2CO. The *n*-alkanes were obtained from vacuum-evapd residues of *n*-hexane extracts and purified by preparative TLC on Si gel and subsequently on $AgNO_3$ (10%) impregnated plates. Rhodamine-B was the spray reagent. The residue from the Me_2CO extract (1 g) was used directly in the chromatographic analysis of iridoids (TLC Developers (i) Et_2O–MeOH (4:1); (ii) $CHCl_3$–isopropanol (3:2); (iii) $CHCl_3$–MeOH (7:2, 4:1); (iv) $CHCl_3$–C_6H_6–MeOH (3:1:1); (v) CH_2Cl_2–Me_2CO (4.5:5.5)). Standards: asperuloside, monotropein. The iridoid material was detected by spraying with 50% EtOH/H_2SO_4.

GLC analysis of the n-*alkane fraction.* The *n*-alkanes were analyzed under standard conditions (FID; 3% SE-30 on chromsorb AW, DMCS, N_2 flow 20 ml/min). The column was held at 215° for 20 min (*n*-C_{19} to *n*-C_{26}) and programmed to 250° (*n*-C_{26} to *n*-C_{35}). The alkanes were identified by comparison with standard references. Relative % of each component (*n*-C_{19}–*n*-C_{32}) was determined from peak area measurements obtained from a disc integrator.

Isolation of oleanolic acid and iridoids from G. boreale. The powdered air-dried plant (800 g) was exhaustively extracted with petrol (bp 40–60°), Et_2O and Me_2CO. The residue from the Et_2O extract gave oleanolic acid which on acetylation (C_5H_5N–Ac_2O method) afforded needles (116 mg) of 3-acetylolean-12-en-28-oic, acid, mp 253–254° (mmp; IR; $[\alpha]_D$) [18]. The residue (20.2 g) from the Me_2CO extract was fractionated by column chromatography (Si gel), 700 g); eluents: CH_2Cl_2, CH_2Cl_2–MeOH (9:1; 4:1, 7:3) and MeOH. Fractions (i) (5.0 g) and (ii) (4.0 g) were discarded as iridoids were absent. Fraction (iii) a green solid (3.08 g) was washed at 0° with anhydrous C_6H_6. The residue was crystallized from MeOH/EtOAc to yield needles of asperuloside (552 mg) mp and mmp 131–132° (IR, NMR); tetraacetate, mp, mmp 148–150°; (lit. [12], [19] 150–151°; 154.5–155°); $[\alpha]_D^{21°}$ −110.8° ($CHCl_3$). Fraction (iv) (6.53 g) showed the presence of 3 minor iridoids R_f 0.75, 0.42, 0.27; 'galium glycoside' [13] R_f 0.15. Separation by prep TLC was unsuccessful. Acetylation (Ac_2O (14 ml)–C_5H_5N (7 ml)) at room temp gave a solid (11.1 g). Analysis by TLC showed three iridoid positive components (R_f 0.6; 0.1 and 0.0 in $CHCl_3$–EtOAc, 1:2). Crude fractionation (Si gel: 350 g) and appropriate combination of iridoid containing residues gave two fractions. The first fraction (4.12 g) X_1 was chromatographed on 1 mm Si gel (P_{254}) plates. ($CHCl_3$–EtOAc, 19:1). Elution with $CHCl_3$ of the iridoid band (R_f 0.38) gave a syrup which on treatment with $CHCl_3$–*n*-C_5H_{12}–Et_2O afforded crystals of asperuloside tetraacetate; mp 148–150°. Insufficient material was available for the purifiation of the second fraction (X_2).

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