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CHEMOTAXONOMY OF THE ACANTHACEAE. IRIDOIDS AND QUATERNARY AMINES

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IN MEMORY OF TONY SWAIN, 1922–1987

Key Word Index—Acanthaceae; iridoid glucosides; 8(*S*)-7,8-dihydroaucubin; 6-*epi*-stilbericoside; thunbergioside; quaternary amines; betaine; trigonelline; cyanogenic glucoside; taxiphyllin; chemotaxonomy.

Abstract—Fourteen of the 40 investigated species of Acanthaceae *sensu lato* contain iridoid glucosides. A total of 20 iridoids are now reported from the family; most have been isolated in the present work. Seven of the compounds are so far unique for the family, namely 6-*O*-acetylshanzhiside methyl ester, 6-*O*-acetylbarlerin, 8(*S*)-7,8-dihydroaucubin, eranthemoside, hygrophiloside, 6-*epi*-stilbericoside, and thunbergioside. A plausible biogenetic scheme interrelating the iridoid glucosides is presented. Notable amounts of betaine were isolated or detected by ^{1}H NMR spectroscopy in crude extracts of virtually all (31/32) species belonging to subfamilies Acanthoideae and Ruellioideae. Trigonelline was similarly found in 14 species belonging to all five subfamilies. Other, unidentified quaternary methylammonium compounds were detected in the subfamilies Thunbergioideae, Mendoncioideae and Nelsonioideae. Quaternary methylammonium compounds are not usual in the order Scrophulariales and therefore the common occurrence of such compounds in the five subfamilies confirms their positioning within the Acanthaceae; this contrasts with the opinion of many taxonomists who prefer to raise one or more of these subfamilies to family rank. The common occurrence of the rare iridoid glucoside stilbericoside in *Thunbergia*, Stilbaceae and Retziaceae supports Dahlgren's placing of the two latter families in Scrophulariales.

INTRODUCTION

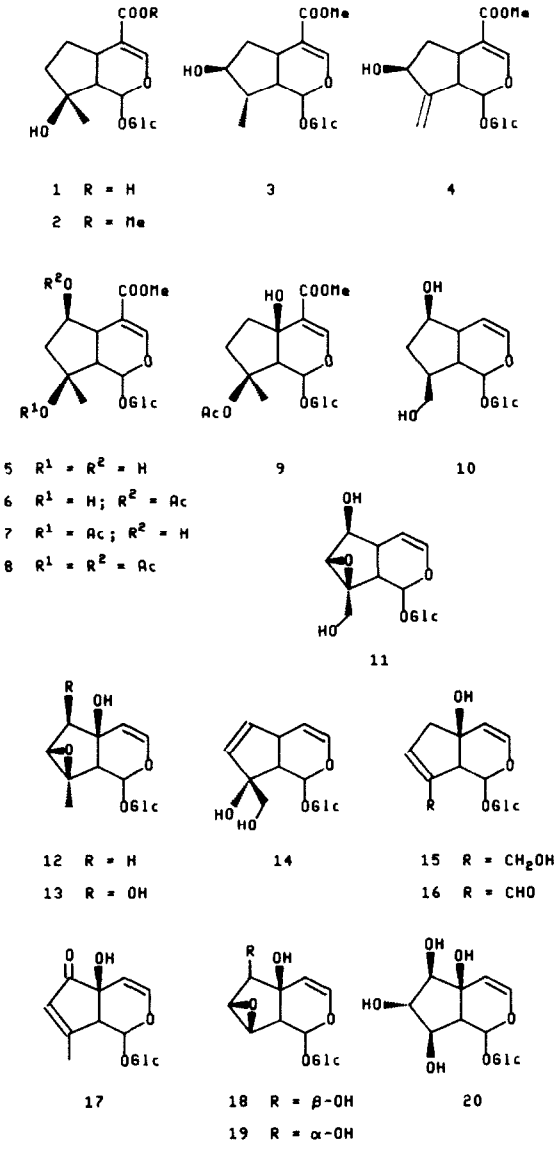
The Acanthaceae is a large family with *ca* 250 genera and 2700 species [1]. The smaller taxa Thunbergiaceae, Mendonciaceae and Nelsoniaceae have variously been assigned family rank [2] or been included as subfamilies within the Acanthaceae [3], largely depending on the taxonomist in question being a 'splitter' or a 'lumper'. The Acanthaceae together with the Scrophulariaceae, Gesneriaceae, Bignoniaceae, Pedaliaceae, Martyniaceae and a few smaller families, form a taxonomically homogeneous order, usually designated as the Scrophulariales.

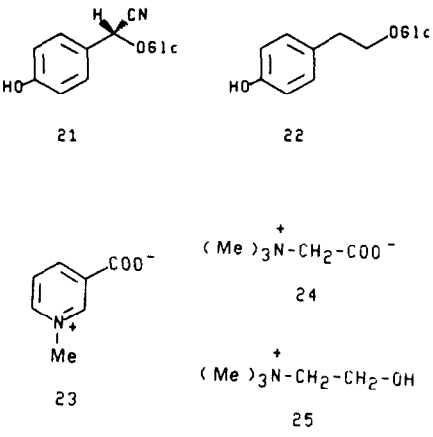
Hegnauer [4] was the first to realize the potential usefulness of the distribution of iridoids in the taxonomy of the sympetalous families and used this character to demonstrate that at least two major phyletic lines exist. This dichotomy was later expressed most radically in the system of Dahlgren [5, 6] and the theme has been discussed in reviews on the distribution and systematic importance of iridoids [3, 7, 8]. Despite the large size of Gesneriaceae (2000 species [1]) and Acanthaceae, no iridoids have been reported from the former family and only a few from the latter. Thus Wieffering [9] in a chromatographic survey showed the probable existence of an iridoid (*Cardanthera*-pseudoindican) in *Cardanthera triflora* (= *Hygrophila difformis*). Later [10] the two iridoids barlerin and acetylbarlerin were reported from *Barleria prionitis*. However, the structures proposed for these compounds were inconsistent with the ^{1}H NMR data given in the paper. Catalpol (**11**) was then isolated from *Asystasia bella* as reported in the review by Hegnauer and Kooiman [3]. Furthermore, Kooiman in his own chromatographic investigation of seed material found indications of iridoids in several species, while the leaf material he examined was negative [3]. Recently we reinvestigated the iridoids in *Barleria prionitis* [11] and corrected the structures for barlerin and acetylbarlerin to 8-*O*-acetyl- and 6,8-di-*O*-acetyl-shanzhiside methyl ester (**7** and **8**), respectively. Additionally, we found the parent glucoside (**5**) in the plant. '*Cardanthera*-pseudoindikan' from *Hygrophila difformis* was isolated and the structure determined to be **16**, and the compound was named hygrophiloside [12]. Two reports on the compounds in *Barleria lupulina* have appeared [13, 14]. This plant also contains **5**, **7** and **8**, and in addition the minor constituents 6-*O*-acetylshanzhiside methyl ester (**6**) and ipolamiidoside (**9**). Finally, we have isolated and characterized the compound eranthemoside (**14**) from *Eranthemum pulchellum* [15].

We have now examined a number of species within the Acanthaceae including members of all the subfamilies, and report here the results of the investigation.

RESULTS

Fresh, frozen or dry plant material was used in the present investigation. Extraction was performed with ethanol and a water-soluble fraction was prepared as described in Experimental. An initial analysis of the extract was performed by recording an ^{1}H NMR spectrum with a high signal to noise ratio, so that very small





(0.005%) concentrations of iridoids could be detected by enhancing the spectrum amplitude in the low field interval 5.5–10 ppm. Since the quaternary amino acids trigonelline (**23**) and betaine (**24**) were encountered in many of the plants, the presence of these was also determined by this method (see Experimental). In the NMR spectrum of iridoids the signal of the proton at C-3 appears at *ca* 7.5 ppm in compounds carrying a carboxyl group at C-4, while at *ca* 6.3 ppm in compounds without such a group. In cases where the presence of an iridoid was thus indicated, the compound(s) was isolated by reversed phase chromatography.

The species investigated are listed in Table 1 together with the quaternary amines detected by NMR and with the compounds isolated from the plants. The listing follows the classification of the family by Bertel Hansen [16]. The compounds in the Table and Figure have been numbered according to presumed biosynthetic complexity. In the following, we will comment on each of the plants in which glycosides were found.

Thunbergia

Four members of this genus were investigated [17] and from all were isolated the iridoid stilbericoside (**18**). In *T. alata* and *T. mysorensis* this compound was accompanied by 6-*epi*-stilbericoside (**19**) and thunbergioside (**20**), respectively. The two latter compounds are novel, but stilbericoside has been reported from *Stilbe ericoides* [18], *Xeroplana gymnopharyngia* [19] (both Stilbaceae) and in *Retzia capensis* (Retziaceae) [19].

Nelsonia canescens

Two iridoids were isolated from this plant, namely shanzhiside methyl ester (**5**) and galiridoside (**12**). The former compound is fairly widespread having been reported from Lamiaceae (2 genera [20, 21]), Scrophulariaceae (4 genera [22–25]) and Rubiaceae (2 genera [26, 27]), while galiridoside has so far only been reported from *Galeopsis* [28] and *Leonurus* [29] (both Lamiaceae).

Hygrophila polysperma

This aquatic plant provided a minute amount of mussaenosidic acid (**1**) and larger amounts of isoaucubin (**15**). The latter compound is known only from *Aeginetia indica* (Orobanchaceae) [30], while **1** is more common with four occurrences, namely in *Melampyrum* (Scrophulariaceae) [31], *Cistanche* (Orobanchaceae) [32], *Utricularia* (Lentibulariaceae) [33] and *Avicennia* (Verbenaceae) [34].

Phaulopsis imbricata

The compound 8(*S*)-7,8-dihydroaucubin (**10**) has not been reported from a plant source so far, but it has been synthesized from aucubin [35]; that natural and synthetic material were identical was proved by comparison of the ^{1}H and ^{13}C NMR spectra.

Ruellia rosea

A cyanogenic glucoside (**21**) was isolated from this plant. The NMR spectra (see Experimental) clearly showed the presence of a *p*-hydroxyphenyl moiety indicating that the compound was either taxiphyllin or the diastereomer dhurrin. Comparison of the ^{1}H and ^{13}C NMR

Table 1. Iridoids and quaternary amines in Acanthaceae.

	24	Other amines	**23**	Iridoids	Vouchers*
Subfam. I. Thunbergioideae					
Thunbergia alata Sims	tr	tr	—	**18, 19**	4027/1 (BHH)
—*fragrans* Roxb.		tr	+	**18, 20**	4027/2 (C)
—*grandiflora* (Rottl.) Roxb.		+	+	**18**	4027/3 (BHH)
—*mysorensis* (Wight) T. Anders.	tr	+	tr	**18, (22)**	P1981/5464 (BHH)
Subfam. II. Mendoncioideae					
Mendoncia coccinea Vellozo	tr	+	+		Regis de Brito s.n. (C)
Subfam. III. Nelsonioideae					
Nelsonia canescens (Lam.) Spreng.	tr	++	+	**5, 12**	Friis & Vollesen 4.2.1985 (C)
Elytraria virgata Michaux		+	—		4031/1 (BHH)
Staurogyne lasiobotrys (Nees) O. Ktze	tr	+	—		Kerr 11946 (C)
Subfam. IV. Acanthoideae					
Acanthus montanus (Nees) T. Anders.	++	++	+		4071/4 (C)
Crossandra nilotica Oliver	+++	+	+		S1977/752 (C)
Subfam. V. Ruellioideae					
Tribe A. Ruellieae					
Brillantaisia lamium Benth.	+++	+	—		4103B/3 (BHH)
Eranthemum pulchellum Andrews	+++	+	+	**14**	4087/1 (C)
Hygrophila difformis (L.f.) Blume	+	+	—	**16**	IOK 16–83 (C)
—*polysperma* (Roxb.) T. Anders.	+	+	—	**1, 15**	IOK 1–88 (C)†
Phaulopsis imbricata (Forssk.) Sweet	+++	+	—	**10**	4097C/2 (BHH)
Ruellia dipteracanthus Hemsl.	+++	+	—		4047/15 (BHH)
—*graecizans* Backer	+++	++	—		4047/11 (BHH)
—*portellae* Hook.f.	+++	++	—		4047/12 (BHH)
—*rosea* Mart.	++	+	—	**(21)**	4047/8 (BHH)
—*tweedieana* Griseb.	+++	+	—		4047/21 (BHH)
Sanchezia nobilis Hook.f.	+++	+	—		4021/1B, (C)
Strobilanthes dyeriana Mast.	tr	+	—		4053/2 (BHH)
—*isophylla* (Nees) T. Anders.	+++	++	—		4053/5 (BHH)
Tribe C. Andrographideae					
Andrographis laxiflora (Bl.) Lindau	+++	tr	—	**17**	4101/3 (BHH)
Tribe D. Justicieae					
Subtribe a. Barleriinae.					
Asystasia bella (Harv.)Benth. & Hook.f.	+++	+	—	**2, 3, 4, 11**	4057/2 (BHH)
Barleria strigosa Willd.	+++	++	—		S1939/0808
—*lupulina* Lindl.	+++	+	—	**5, 6, 7, 8, 9**	Jensen 5/85‡
—*prionitis* L.	++		—	**5, 7, 8**	see ref. [11]
Chamaeranthemum gaudichaudii Nees	+++	+	—	**13**	4087/2 (C)
Pseuderanthemum carruthersii (Seem.) Guill.	+++	+	—		4087D/5 (C)
Schaueria calycotricha (Link & Otto) Nees	+++	+	—		4082B/1 (BHH)
Subtribe b. Isoglossinae					
Hypoestes antennifera S. Moore	+++	++	—		4097/3
—*phyllostachya* Bak.	+++	++	+		4097/4 (BHH)
Peristrophe speciosa (Roxb.) Nees	+++	+	+		4095/1 (BHH)
Subtribe c. Justiciinae					
Jacobinia mohintli (Nees) Benth. & Hook.f.	+++	++	+		P1907/5555 (C)
—*pauciflora* Benth. & Hook.f.	+++	+	+		4082C/3 (BHH)
—*suberecta* André	+++	++	+		4082C/5 (BHH)
Justicia brandegeana Wassh. & Smith	+++	+	—		4082/1 (BHH)
—*formosa* Willd.	+++	++	—		4089/1 (BHH)
Pachystachys lutea (Schult.) Nees	+++	+	+		4089B/2 (BHH)

*Vouchers are deposited either at the Botanical Museum, Copenhagen (C) or at The Herbarium of the Botanical Garden, Copenhagen (BHH).

†A submerse form is commonly used for aquaristics. This was used in the present work, and the determination is thus with some reservation.

‡The voucher is deposited at Bishop Museum, Honolulu, Hawaii, U.S.A.

data of our compound with published data for the diastereomeric pairs of cyanogenic glycosides [36, 37] proved insufficient to determine the identity despite the use of identical solvents (D_2O) and internal reference (DSS), possibly due to temperature and/or concentration effects. However, comparison with a ^1H NMR spectrum of a mixture of the pair prunasin–sambunigrin showed the same shift for the benzylic proton of prunasin and that of our compound, proving it to be taxiphyllin (**21**). This compound has been reported from many members of the Monocotyledonae but has a scattered distribution in the Dicotyledonae [38].

Andrographis laxiflora

The compound isolated in small amount from this plant, namely teuhircoside (**17**) has previously only been reported from *Teucrium hircanium* [39].

Asystasia bella

Besides the previously known [3] occurrence of catalpol (**11**) which we could confirm, we isolated the compounds mussaenoside (**2**), 8-epiloganin (**3**) and gardoside *methyl ester* (**4**). Catalpol is one of the most widespread iridoids and has been reported from virtually all the iridoid-containing families in Lamianae. Of the remaining compounds mussaenoside is also widespread while **3** and **4** have been recorded only in Scrophulariaceae-Rhinanthoideae. Interestingly, in the four reports concerning gardoside methyl ester, this compound consistently co-occurred with 8-epiloganin, namely in *Melampyrum* [31], *Pedicularis* [25], *Castilleja* [22], and *Parentucellia* [24]. Furthermore, in the three former cases compounds **2**, **3** and **4** were found together, thus apparently constituting a syndrome.

Barleria species

One species was devoid of iridoids while the two other species contained the glucosides **5**–**9**. As mentioned above shanzhiside (**5**) is reported from several families. This is not so with the remaining compounds, as **6** and **8** are so far only found in *Barleria* [11, 13, 14]. Barlerin (**7**) have been reported from *Lamium* [21], *Phlomis* [20] (Lamiaceae) and *Mussaenda* [27] (Rubiaceae) while ipolamiidoside has only been found once before, namely in *Lamium* [40].

Chamaeranthemum gaudichaudii

A small amount of anthirrinoside (**13**) could be isolated from this plant. The compound has been reported from *Galeopsis* (Lamiaceae) [41] as well as from several genera from Scrophulariaceae [42].

The presence of quaternary ammonium compounds in the family has already been established previously as trigonelline has been isolated from *Acanthus ilicifolius* [43] and betaine from *Eranthemum pulchellum* [15]. Furthermore, in an investigation of quaternary ammonium compounds in mangrove plants, *A. ilicifolius* was reported to contain notable amounts of these substances [44].

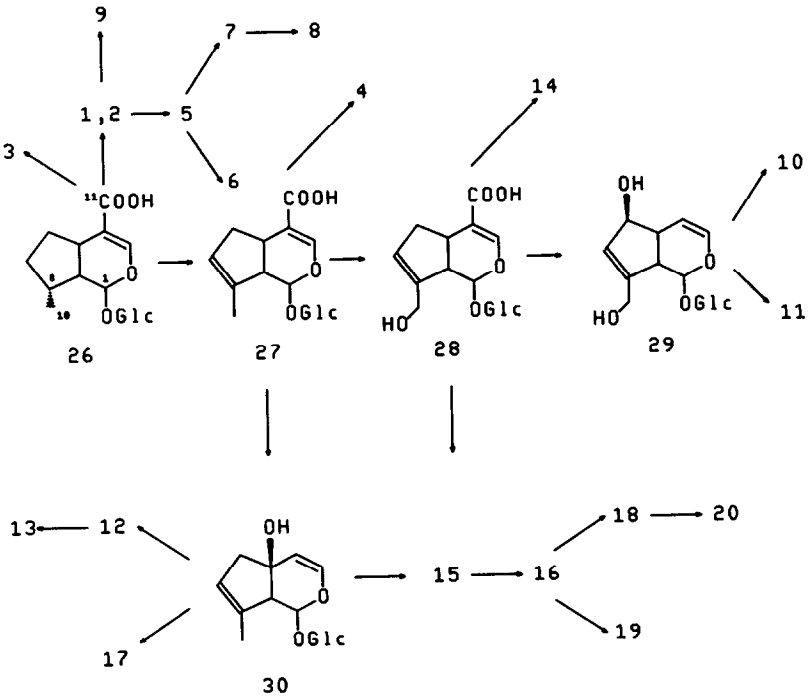
DISCUSSION

Iridoids

From the results of the present investigation we can conclude that iridoid glucosides are not rare in the Acanthaceae as we have isolated such compounds from 14 of the 40 species examined. However, the amounts present in most of the species are generally smaller than usually encountered in, for example, members of the Scrophulariaceae, and this fact may explain why so few reports have been previously published.

Biosynthetically, all the isolated compounds may be interpreted as being derived from 8-*epi*-deoxyloganic acid (**26**, i.e. with an 8-α-methyl group). This is evidently the case with compounds **1**–**3** and **5**–**9** where the stereochemistry at C-8 has been preserved after hydroxylation and possible further derivation [45, 46]. The remaining compounds do not obviously belong to this biosynthetic series, in particular **10**, **14** and **18**–**20**, as the two former actually have the opposite stereochemistry at C-8, and the latter have lost the C-10 carbon atom during biosynthesis. However, using existing knowledge of biosynthetic pathways we have sketched a possible biosynthetic network linking the individual compounds found in the present work. Thus catalpol (**11**) derives from aucubin (**29**) which is biosynthesized from 8-*epi*-deoxyloganic acid (**26**) via **27** and **28** [46–48]. Using this sequence as a main frame, most of the remaining compounds can be accounted for with a limited number of simple oxidation and decarboxylation steps (the intermediacy of **26** in the formation of **13** is also known [47]). Compound **10** can be derived from aucubin in one step by reduction, the result being a net inversion at C-8 when compared with the precursor **26**. Likewise, compound **14** can be derived from geniposidic acid (**28**) in two steps, namely oxidation/allylic rearrangement followed by a decarboxylation step (the former reaction has precedence in the formation of gardenoside [cf. 48], although in this case with the opposite stereochemistry at C-8). The compounds **18**–**20** from *Thunbergia* belong to a rare group of iridoid glucosides lacking both the C-10 and C-11 carbon atoms. No biosynthetic work has been performed on such compounds, but since loss of the C-11 carbon atom is known to occur by decarboxylation of a conjugated 11-carboxyl group (cf. aucubin formation), loss of the C-10 carbon atom is perhaps best imagined to take place in a similar fashion by decarboxylation of a 10-carboxyl group conjugated with a 7,8-double bond. The compounds **15** and **16** in *Hygrophila* with a 7,8-double bond and with a hydroxymethyl or carbaldehyde group, respectively, as C-10, are likely candidates for intermediates in a pathway leading to **18**/**19**. Only a few reaction steps would lead from **16** to these compounds, namely (i) oxidation of the carbaldehyde to carboxylic acid, (ii) decarboxylation, (iii) oxidation at C-6 and (iv) epoxidation of the double bond.

As stated in the Results, the compounds: **6**, **8**, **10**, **14**, **16**, **19** and **20** are so far unique to the Acanthaceae while the remaining iridoid glucosides except **1**–**5** and **11** are each only reported once or a very few times from more or less closely related families. Remarkably, aucubin (**29**), one of the most common iridoids, has not been found in the present investigation. This is a feature shared with Lamiaceae, which also contain compounds derived from aucubin [i.e. catalpol (**11**)]. In conclusion, the uniqueness and highly derived state of the iridoids demonstrates that the



Acanthaceae is an advanced family, in good accordance with accepted views.

Quaternary methylammonium compounds

Betaine and trigonelline are found only sporadically in the plant kingdom and only in the Chenopodiaceae do they occur in a large number of species [49]. They have to our knowledge not been used for taxonomic purposes. The presence of such compounds in plants have been connected with salt-stress conditions [49] and some mangrove plants have been found to accumulate large amounts of betaines [44] (i.e. *Acanthus ilicifolius*).

Delimitations of the family

As noted in the introduction the subfamilies Thunbergioideae, Mendoncioideae and Nelsonioideae have variously been raised to family rank. Of the modern taxonomists Thorne [50] includes the whole taxon in Acanthaceae, while Cronquist [52] and Takhtajan [51] consider Mendoncioideae a separate family. Dahlgren [8, 6], Willis [1] and Bremekamp [2, 53], the latter being a specialist of the family, all raise Thunbergioideae and Mendoncioideae to family rank and consider that Nelsonioideae belongs to the Scrophulariaceae (Willis not being too specific on this point). Bremekamp even claims the position of Nelsonioideae as being close to Rhinantheae.

The results of the present investigation are highly relevant in this context. The data in Table 1 show clearly that Acanthaceae *sensu stricto* (i.e. subfamilies IV and V) is a virtually homogeneous entity with regard to the presence of betaine in large quantities. (*Strobilanthes dyeriana* is the only exception, containing only traces of betaine). The species of the remaining subfamilies only contain trace amounts of this compound but on the other hand they do contain notable amounts of other, unidentified quaternary methylammonium compounds. Trigonelline (also belonging to this group) has a more fortuitous distribution but still occurs in all the subfamilies. Taking only this information into account, it would seem logical to include only subfamilies IV and V (Acanthoideae and Ruellioideae) in Acanthaceae, leaving the remaining subfamilies as more or less closely related entities.

However, we have also collected data on the general occurrence of quaternary methylammonium compounds in most of the remaining families of Scrophulariales. The results are listed in Table 2. It can be seen that such compounds are not usually accumulated in large amounts in other families of the order. Therefore, not the particular compound, but the mere presence of methylammonium compounds would seem to constitute a character–the conclusion being that the five subfamilies are more closely related to each other than to any of the remaining families in the order.

The iridoids are not very helpful in this context as they have been found consistently only in *Thunbergia*. They are present in most of the subfamilies except II and IV, but only one and two species, respectively, were examined in these taxa. The presence in *Hygrophila* (subfamily V) of compounds **15** and **16** which are probable precursors of **18**–**20** in *Thunbergia* (subfamily I) indicate a further link between the two subfamilies.

Relationships on the family and higher level

With regard to the widespread occurrence of iridoids in members of families in the order Scrophulariales (see Table 2), Acanthaceae fits well in the order. The lack of these compounds in many species appears to be a secondary development already fulfilled in Gesneriaceae. The finding of the iridoid glucosides lacking the C-10 and C-11 carbon atoms in *Thunbergia* (**18**–**20**) is of particular interest, as these compounds have a very limited distribution. As noted above stilbericoside (**18**) has earlier been found in Stilbaceae and Retziaceae here accompanied by

Table 2. Content of quaternary methylammonium compounds* and iridoids in the families of Scrophulariales (*sensu* Dahlgren [6])

Family	Species	Me–N^+	Iridoids	Ref.
Bignoniaceae	*Catalpa bignonioides* Walt.	—	+	Unpublished
Myoporaceae	*Myoporum laetum* Forst.fil.	—	—†	[7]
Gesneriaceae	*Saintpaulia ionantha* Wendt.	+		Unpublished
Buddlejaceae	*Buddleja davidii* Franch	—	+	[46]
Scrophulariaceae	*Pedicularis* (3 species)	tr	+	[25]
	Melampyrum (2 species)	—	+	[31]
	Linaria vulgaris L.	tr	+	[47]
Globulariaceae	Not available		+†	
Selaginaceae	*Hebenstreitia dentata* L.	tr	+	[45]
Stilbaceae	*Xeroplana gymnopharyngia* Rourke	—	+	[19]
Retziaceae	*Retzia capensis* Thunb.	—	+	[19]
Plantaginaceae	*Plantago major* L.	—	+	[47]
	Litorella uniflora (L.) Asch.	—	+	Unpublished
Lentibulariaceae	*Utricularia australis* R.Br.	++	+	[33]
	Pinguicula vulgaris L.	—	+	[33]
Pedaliaceae	*Harpagophytum procumbens* DC.‡	—	+	Unpublished
Trapellaceae	Not available			
Martyniaceae	Not available		+†	

*Data on methylammonium compounds were mainly obtained by reinspection of spectra of crude extracts in works earlier published by us.

†Iridoids are known from the family [3].

‡A crude commercial extract obtained from Indena SpA, Italy was used.

the last known member of this class of compounds, namely unedoside, the latter also found in *Arbutus* [54] and *Arctostaphylos* [55], both Ericaceae. The relationship between Ericales and Scrophulariales is uncertain as Dahlgren [6] place these orders in neighbouring superorders while other taxonomists [50–52] support the traditional position of Ericales in the neighbourhood of Theales. Traditionally, Retziaceae and Buddlejaceae have been included in Loganiaceae and/or Gentianales (depending on the level of classification), while Stilbaceae often has been a part of Verbenaceae, a view still held by Thorne [50]. In recent times, however, perhaps with the growing appreciation of chemical characters, we see a tendency to move these (small) families into Scrophulariales. Thus Cronquist [52] has placed Buddlejaceae in Scrophulariales but retains Retziaceae in his Gentianales, after detailed consideration (but see below). In the classification of Takhtajan [51] both of these families are included in Scrophulariales, but both he and Cronquist place Stilbaceae in Lamiales. Dahlgren's first system of classification [5] contained the three taxa in their traditional positions, but after re-consideration he placed them in Scrophulariales [6, 19]. The position of Retziaceae and Buddlejaceae is of particular interest for the use of iridoids in systematics, as these were the only families in Gentianales containing iridoids with no C-11 carbon atom (examples are aucubin (**29**) and stilbericoside (**18**)). With such a revision, iridoids lacking C-11 are only found in *Aucuba* (Cornaceae), Ericaceae, *Eucommia* (Eucommiaceae) and the two orders Scrophulariales and Lamiales. The much smaller group of compounds lacking both the C-10 and C-11 carbon atoms have an even more limited distribution, and the new finding of these compounds in *Thunbergia* fits nicely with the placement of Retziaceae and Stilbaceae in the Scrophulariales. However, with regard to the distribution of iridoids, the separation of Scrophulariales and Lamiales seems artificial.

In an earlier publication [19] the compounds from *Retzia* and *Stilbe* were compared to another group of compounds themselves biogenetically related, namely the iridoid glucosides found solely in *Mentzelia* (Loasaceae) and *Deutzia* (Hydrangeaceae). This group of compounds have lost the C-10 but not the C-11 carbon atom and are thus of an independent origin. Loasaceae and Hydrangeaceae also both contain seco-iridoids, a group of compounds never encountered in Scrophulariales–Lamiales. Furthermore, the compounds found in *Deutzia* have been shown to be formed biosynthetically from iridodial, while stilbericoside (**18**) and congeners presumably belong to the *epi*-series and thus derives biosynthetically from 8-*epi*-iridodial as shown in the biosynthic scheme above. Cronquist in his discussion of the position of Retziaceae has apparently misunderstood the above comparison and refutes the taxonomic significance of the iridoids in *Retzia* and *Stilbe* on the ground that the latter is systematically far removed from Loasaceae and Hydrangeaceae.

EXPERIMENTAL

Microanalyses were performed at NOVO Microanalytical Laboratory, Bagsværd, Denmark. Mps:.

Plant material was obtained from The Botanical Garden, Copenhagen, except for *Staurogyne lasiobotrys* of which four leaves were taken from a herbarium sheet. *Hygrophila difformis* and *H. polysperma* were cultivated in a tropical fresh-water-aquarium by one of us and *Mendoncia coccinea* was collected in

its natural habitat near Rio de Janeiro, Brazil.

Extraction of fresh or frozen plant material (× g) was performed by blending with EtOH (4 × ml) twice. The filtrate was taken to dryness and partitioned in H_2O–Et_2O, after which the aq fraction was passed through a column of Al_2O_3 (× g) and the column washed with H_2O (2 × ml). The residue after evapn was triturated with hot MeOH and passed through a thin layer of act C on a glass filter, in all cases a considerable amount of an inorganic salt only little soluble in MeOH could be isolated. Concentration of the solution provided a clear colourless to red (anthocyanin) syrup or foam. At this point an ^{1}H NMR spectrum (90 MHz, D_2O: δ4.80) was recorded and analysed.

Trigonelline (**23**). The presence of this compound was seen by the following signals: δ9.05 (*br s*, H-2); 8.77 (*dd*, J = 1 and 7 Hz, H-4 and H-6); 8.00 (*t*, J = 7 Hz, H-5); 4.37 (*s*, N-Me).

Betaine (**24**). This compound was characterized by singlets at δ3.91 (2H) and 3.28 (9H). The amounts given in Table 1 are not absolute, but were determined relative to the highest peak of the carbohydrate signals at *ca* 3.9 ppm; signatures + + + means that the peak at δ3.28 was more than 3 times that of the height at 3.9; + + means between 1 and 3 times; + means less the height. *Other quaternary N-methyl amines* giving signals within the interval 3.05 to 3.21 were not identified, but their presence recorded (see Table 1).

Iridoids. By enhancing the spectrum amplitude in the interval δ6 to 8 the presence of signals arising from H-3 of iridoids were determined. In one case an iridoid present in an amount of less than 0.01% was detected and isolated (see below) and thus we judge that the limit of detection is below this content.

Most plant extracts were not further separated. In cases, however, where the presence of iridoid glucosides seemed possible, chromatography on reversed phase columns was performed. Depending on the amount of extract and on the concentration of iridoids, Merck Lobar columns size B or C were used, eluting with mixtures of H_2O and MeOH, with increasing percentage of MeOH, and fractions were monitored by UV (206 and 254 nm). Conditions for the individual separations are described below.

Thunbergia species. The isolation and characterization of the compounds from this genus will be described elsewhere.

Mendonica coccinea. Dry stems and leaves (50 g) were ground in a blender with 80% EtOH and left to stand for 5 days giving 1.1 g of extract. The ^{1}H NMR spectrum showed signals from trigonelline (**23**) and in addition singlets at δ3.28, 3.22 and 3.15, indicating the presence of betaine (**24**, trace amount) and two other quaternary methyl-ammonium compounds. Also a doublet at 6.2 indicated that an iridoid could be present. Chromatography on the C-column provided a number of fractions of which one contained the presumed iridoid (18 mg) in a not quite pure state. Attempts to purify the compound completely did not succeed, but NMR spectroscopy led us to the conclusion that the compound was not of iridoid origin. ^{1}H NMR (500 MHz, D_2O): δ6.98 (*dd*, J = 10 and 1.5 Hz), 6.11 (*d*, J = 10 Hz), 4.33 (*ddd*, J = 1.5, 4.5 and 5.5 Hz), 3.97 and 4.09 (AB-part of ABXY-system), 2.90 (*dd*, J = 4.5 and 17.5 Hz), 2.69 (*dd*, J = 5.5 and 17.5 Hz), 2.35 (2H, XY-part of ABXY-system). ^{13}C NMR spectrum (125 MHz, D_2O): δ201.7, 151.0, 128.6, 81.7, 67.3, 40.1 and 38.8.

Nelsonia canescens. Frozen plant (56 g) worked-up as described above gave 360 mg extract. In the ^{1}H NMR spectrum signals were seen at δ6.36 (*d*) and 7.45 (*br s*), indicating the presence of iridoids. Signals identical to those of trigonellin (**23**) were seen at low field while a singlet corresponding to a quaternary methylammonium compound was visible at δ3.15. Application to a B-column and elution with H_2O–MeOH (5:1 to 3:1) gave 3 fractions. The first fraction contained carbohydrates, trigonelline and another quaternary methylammonium compound. The second fraction consisted of galiridoside (**12**, 13 mg; 0.02%) characterized only by the ^{1}H NMR spectrum (90 MHz, D_2O): δ6.43 (*d*, J = 6 Hz, H-3); 5.49 (*d*, J = 6.5 Hz, H-1); 5.06 (*d*, J = 6 Hz, H-4), 3.55 (*br s*, H-7); 2.42 (*d*, J = 6 Hz, H-9); 2.17 (AB-system, J = 16 Hz; 6-CH_2); 1.51 (*s*, 10-Me), identical to the published [28] data, except for the different standard used. The third fraction consisted of shanzhiside methyl ester (**5**, 15 mg 0.03%) giving an ^{1}H NMR spectrum identical to that of an authentic sample [11].

Staurogyne lasiobotrys. Four dry leaves from a herbarium sheet (345 mg) was ground with sand and EtOH (80%) and worked-up as above to give 22 mg of extract. The ^{1}H NMR spectrum was mainly that of carbohydrates but small peaks could be seen at δ3.28 and 3.21.

Acanthus montanus. Frozen plant (60 g) gave 460 mg of extract. The ^{1}H NMR spectrum showed signals for trigonelline (**23**) and betaine (**24**–in fact the signal at 3.28 was by far the largest peak in the spectrum). In order to separate the compounds, the extract was applied to the B-column and eluted with H_2O–MeOH (10:1 to 3:1). Only the fraction forming the front had appreciable mass–no iridoids could be detected. The first fraction was evapd onto silica gel (20 g) and in a column placed on top of a further amount of silica gel (30 g). Elution with EtOH (200 ml) removed the carbohydrates (monitored by NMR) followed by MeOH (200 ml) to give almost pure betaine (**24**, 75 mg) and finally MeOH–H_2O (9:1; 200 ml) gave a mixture of **24**, and trigonelline (1:2, 40 mg). Total amount of betaine: 95 mg (0.15%), and of trigonelline: 20 mg (0.03%).

Eranthemum pulchellum (See ref. [15]). From this species 140 g material gave 2.64 g of extract which on a C-column was separated into a polar fraction (1.16 g) followed by eranthemoside (**14**, 97 mg, 0.07%). The polar fraction was further separated as above into (i) carbohydrates (570 mg), (ii) (230 mg) (iii) betaine (400 mg) and (iv) a mixture of betaine and trigonelline (20 mg). The ^{1}H NMR spectrum of fraction (ii) we have now found to be virtually identical to that of choline (**25**): (90 MHz, D_2O) δ4.09 and 3.58 (*m*'s, 2H each); 3.21 (*s*, 9H). However, when inspecting the spectrum of the complete polar fraction, we found that the amount of choline was only 5% of that of betaine, i.e. *ca* 20 mg, the remaining mass of fraction (ii) must thus be inorganic salt giving no signals in the NMR spectrum.

Hygrophila difformis. Frozen plant (335 g) gave 2.18 g of extract (see ref. [12]) from which was isolated hygrophiloside (**16**, 180 mg, 0.05%). Reinspection of the ^{1}H NMR spectrum of the above extract revealed that no **23** was present, while signals could be seen at δ3.28 (**24**), 3.21 and 3.17.

Hygrophila polysperma. Fresh plant (175 g) provided an extract (725 mg). The ^{1}H NMR spectrum showed the presence of an iridoid at δ6.3 and in addition signals at 3.28 and 3.21. Application to a B-column resulted in the isolation of mussaenosidic acid (**1**, 9 mg, 0.005%), with an ^{1}H NMR spectrum identical to that of an authentic sample [33], and isoaucubin (**15**, 149 mg, 0.09%), characterized by the ^{1}H NMR spectrum (500 MHz, D_2O): δ6.37 (*d*, J = 6.1 Hz, H-3), 5.75 (*br s*, H-7), 5.59 (*d*, J = 3.7 Hz, H-1), 5.13 (*d*, J = 6.1 Hz, H-4), 4.21 and 4.17 (*br* AB-system, J = 14 Hz, 10-CH_2), 3.12 (*br s*, H-9), 2.68 and 2.55 (*br* AB-system, J = 16.5 Hz, 6-CH_2), virtually identical to that reported [30]. ^{13}C NMR spectrum (125 MHz, D_2O): 141.0 (C-3), 140.7 (C-8), 127.4 (C-7), 110.0 (C-4), 94.7 (C-1), 74.7 (C-5), 60.2 (C-10), 55.2 (C-9), 46.8 (C-6) and for the carbohydrate moiety 99.1, 73.4, 76.3, 70.5, 77.1, 61.5.

Phaulopsis imbricata. From 50 g plant was obtained 415 mg of extract. ^{1}H NMR indicated an iridoid (δ6.3). No. **23** could be seen, but a large signal from **24**. Chromatography on a B-column (10:1) gave 8(*S*)-7,8-dihydroaucubin (**10**, 4 mg, 0.008%), solely identified by NMR. ^{1}H NMR spectrum (500 MHz, D_2O):

δ 6.30 (*dd*, J = 6.2 and 2.2 Hz, H-3), 5.37 (*d*, J = 3.1 Hz, H-1), 4.89 (*dd*, J = 6.2 and 2.5 Hz, H-4), 4.07 (*m*, H-6), 3.67 and 3.63 (*dd*'s, J = 10.5 and 6.0, 10-CH_2), 2.63 (*m*, H-5), 2.20–2.27 (3H, H-7, H-8 and H-9), 1.38 (*m*, H-7), essentially as that reported for a synthetic specimen [35]. ^{13}C NMR spectrum (125 MHz, D_2O): δ140.6 (C-3), 104.9 (C-4), 96.4 (C-1), 78.0 (C-6), 66.5 (C-10), 42.8 (C-9), 41.5 (C-5), 41.0 (C-8), 36.1 (C-7), 99.2 (C-1'), 77.0 (C-5), 76.5 (C-3), 73.5 (C-2), 70.4 (C-4), 61.5 (C-6), identical to that reported [35].

Ruellia rosea. Frozen plant (128 g) gave 540 mg of extract. The ^{1}H NMR spectrum showed several peaks in the aromatic region and that **24** was present and **23** not present. Chromatography on a B-column (10:1 to 3:1) gave taxiphyllin (**21**, 40 mg, 0.03%). ^{1}H NMR spectrum (90 MHz, D_2O DSS): δ7.49 and 6.95 (4H, AA'BB'-system, J = 9 Hz, *p*-subst. arom), 5.93 (*s*, benzylic H), 4.73 (*d*, J = 7 Hz, H-1'), DHO-peak: 4.78. ^{13}C NMR (22.6 MHz, D_2O): δ158.2 (C-4), 130.8 (C-2 and C-6), 124.9 (C-1), 118.9 (CN), 116.9 (C-3 and C-5), 68.9 (α-C), 101.0 (C-1'), 77.1 (C-5'), 76.4 (C-3') 73.6 (C-2'), 70.3 (C-4'), 61.5 (C-6'). The presence of the diastereomeric compound dhurrin could not be detected in the NMR spectra.

Andrographis laxiflora. Fresh plant (29 g) gave 790 mg of extract. Betaine (**24**) showed large signals in the ^{1}H NMR spectrum, while **23** could not be seen. A doublet at δ6.4 indicated the presence of an iridoid. Chromatography on the B-column (10:1 and 5:1) provided teuhircoside (**17**, 5 mg, 0.02%), characterized solely by NMR. ^{1}H NMR spectrum (500 MHz, D_2O): δ6.31 (*d*, J = 6.4 Hz, H-3), 6.01 (*m*, H-7), 5.99 (*d*, J = 1.4 Hz, H-1), 4.92 (*dd*, J = 6.4 and 1.1 Hz, H-4), 4.69 (*d*, J = 8.0 Hz, H-1'), 3.33 (*m*, H-9), 2.21 (*m*, 10-Me), as reported [39], except for an interchange of H-1 and H-7. ^{13}C NMR (125 MHz, D_2O): δ208.8 (C-6), 177.1, (C-8), 142.4 (C-3), 128.1 (C-7), 104.1 (C-4), 92.1 (C-1), 72.5 (C-5), 56.9 (C-9), 18.2 (C-10), 98.7 (C-1'), 77.1 (C-5'), 76.1 (C-3'), 73.3 (C-2'), 70.4 (C-4'), 61.5 (C-6'), identical to that reported [39], except for the different standards used.

Asystasia bella. An extract of 3.9 g was obtained from fresh plant (250 g). The ^{1}H NMR spectrum showed large signals for betaine and peaks arising from iridoids were visible. By chromatography on a C-column (10:1 to 1:1) we succeeded in isolating catalpol (**11**, 550 mg; 0.2%), gardoside methyl ester (**4**, 56 mg; 0.02%), mussaenoside (**2**, 150 mg; 0.06%) and an intermediate fraction (200 mg). Prep. TLC (silica gel; $CHCl_3$–MeOH, 3:1) of the latter provided 8-epiloganin (**3**, 24 mg, 0.01%) as the faster moving band. The slower moving band contained a mixture of minor components which we are working on at present. The compounds **2**–**4** and **10** were identified by comparison of the ^{1}H NMR spectra with those of known compounds [31, 33].

Chamaeranthemum gaudichaudii. Frozen plant (42 g) was worked-up to give 0.59 g of extract. The ^{1}H NMR spectrum showed large amounts of **24** and signals at *ca* 6.5 indicated an iridoid. Chromatography on the B-column (10:1 and 5:1) gave anthirrinoside (**13**, 5 mg; 0.01%) solely characterized by the ^{1}H NMR spectrum (90 MHz, D_2O): δ6.47 (*d*, J = 6 Hz, H-3), 5.51 (*d*, J = 6.5 Hz, H-1), 4.98 (*d*, J = 6 Hz, H-4), 4.09 (*d*, J = 2 Hz, H-6), 3.60 (*d*, J = 2 Hz, H-7), 2.50 (*d*, J = 6.5 Hz, H-9), 1.47 (*s*, 10-Me), essentially as reported [56].

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