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THE OCCURRENCE OF IRIDOID GLYCOSIDES IN THE VERBENACEAE*

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SUMMARY

Many Verbenaceae contain one or more iridoid glycosides, several of which are still unidentified. Some of the latter were characterized by their specific optical rotations and R_F-values on paper chromatograms. Iridoid glycosides were found in the tribes Viticeae and Clerodendreae (Viticoideae), Physopsideae (Chloanthoideae), Citharexyleae, Priveae, Lantaneae and Verbeneae (Verbenoideae) and Caryopterideae (Caryopteridoideae). In some of the subfamilies iridoid glycosides occur which are known from taxa of the Labiatae, viz. lamiide, ipolamiide, harpagide, acetylharpagide, and possibly some other glycosides.

The prevalence and the distribution of iridoid glycosides in the Verbenaceae are in harmony with the propositions of JUNELL and of EL-GAZZAR and WATSON on the delimitation of the Verbenaceae and the Labiatae, based on anatomical and morphological characters. They would, however, as well be in harmony with other delimitations.

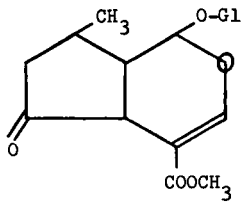
1. INTRODUCTION

Several members of the Verbenaceae are known to contain one or more iridoid glycosides. Verbenaloside (I), first isolated as cornine from a *Cornus* species, occurs in some *Verbena* species (CHEYMOL, 1937), while aucuboside (II) and its p. hydroxybenzoyl derivative, agnuside, have been found in *Vitex agnus-castus* (WINDE, 1959, WINDE & HÄNSEL 1960) and in several other *Vitex* species (HÄNSEL c.s. 1965, RIMPLER 1972 a, b).

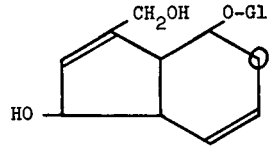
During the last few years a number of other iridoid glycosides have been discovered in this family: unedoside (III) from two *Stilbe* species (RIMPLER 1972c) and stilbericoside (IV) from one *Stilbe* species (RIMPLER & PISTOR 1974); lamiide (V) from *Chascanum cernuum* (RIMPLER 1972d) and, together with several esters of lamiide, the durantosides, from *Duranta repens* (RIMPLER & TIMM 1974); hastatoside (VI) from two *Verbena* species (RIMPLER & SCHÄFER 1973); nyctanthoside (VII) from *Nyctanthes arbor-tristis* (RIMPLER & JUNGHANNS 1975). In addition, a number of Verbenaceae are known to contain "pseudoindicans" which probably will turn out to be iridoid glycosides (GRESHOFF 1898, WINDE 1959).

* This is the sixth publication in the series "Occurrence of iridoid glycosides in the Dicotyledoneae". Previous papers of this series were published as follows:

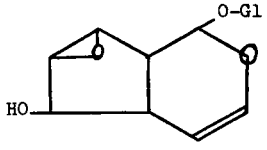
Acta Bot. Neerl. **18** (1969): 124–137 (Rubiaceae); ibid. **19** (1970): 329–340 (Scrophulariaceae); *Oesterr. Bot. Z.* **119** (1971): 395–398 (Theligonaceae); *Acta Bot. Neerl.* **21** (1972): 417–427 (Labiatae); ibid. **23** (1974): 677–679 (Loasaceae).



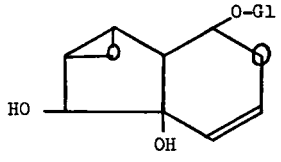
I verbenaloside



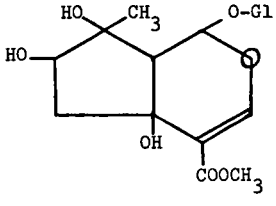
II aucuboside



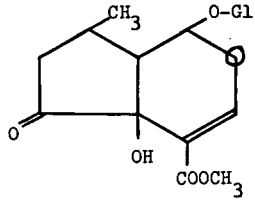
III unedoside



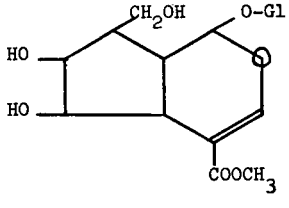
IV stilbericoside



V lamiide



VI hastatoside



VII nyctanthoside

The homogeneity of the Verbenaceae as delimited e.g. by MELCHIOR (1964) has been questioned several times, and inclusion of the larger part of the family into the Labiatae has been advocated (JUNELL 1934, EL GAZZAR & WATSON 1970).

It seemed worth while to obtain more insight in the distribution of iridoid glycosides over the subdivisions of the Verbenaceae. Although this attempt was seriously hampered by the difficulty of obtaining suitable plant material the results allow some cautious conclusions to be drawn.

2. MATERIALS AND METHODS

Vegetative parts used for extraction were taken from specimens cultivated in the Botanical Garden of this laboratory. The seed and fruit samples tested were obtained *via* the seed-lists of horti. To avoid erroneous results caused by misnamed samples either extracts were prepared in duplicate or triplicate from samples of two or three horti; or from the samples used for extraction a part was sown and the resulting plants were identified.

The preparation of extracts, the isolation of glycosides and the technique of paper chromatography were performed as described previously (KOOIMAN 1970) R_F-values were obtained using n.butanol-ethanol-water (40:11:19 v/v) as a solvent.

3. RESULTS AND DISCUSSION

Throughout the present study the subdivision of the Verbenaceae as outlined by MELCHIOR (1964) is maintained. Approximately 30% of the genera and 4% of the species of the family were available for investigation. The species of which samples have been investigated are recorded in *table 1* together with the results of the paper chromatographic inspection of the extracts.

Iridoid glycosides were found in the tribes Viticeae and Clerodendreae of the subfamily Viticoideae, in the Physopsideae (subfamily Chloanthoideae), in most tribes of the Verbenoideae, and in the Caryopterideae (subfamily Caryopteridoideae). In the investigated species of the Callicarpeae and Tectoneae (both Viticoideae), of the Chloantheae and Achariteae (both Chloanthoideae), and of the Petraeae (Verbenoideae) no iridoid glycosides were found. As for the last mentioned three tribes this is the result of studying exclusively seed material, since unfortunately no vegetative parts were available. No material was available for investigation of species belonging to the tribes Monochileae (Verbenoideae) and Teijsmanniodendreae (Caryopteridoideae), and to the subfamilies Nyctanthoideae, Stilboideae, Symphorematoideae and Avicennioideae.

The types of iridoid glycosides differed rather widely and showed a difinite relation with the subdivision of the family. In the Viticeae (subfamily Viticoideae) several species of *Vitex* have aucuboside and agnuside, and some *Clerodendrum* species (tribe Clerodendreae, subfamily Viticoideae) have iridoid glycosides of which the structures are as yet unknown. One of these glycosides has

Table 1. Iridoid glycosides in the Verbenaceae.

	organ	
VITICOIDEAE-CALLICARPEAE		
Callicarpa americana L.	f	–
Callicarpa bodinieri Lévl. var. *giraldii* R.	f, l	–
Callicarpa dichotoma K. Koch	f	–
Callicarpa japonica Thunb.	l	–
Callicarpa longifolia Lam.	f	–
VITICOIDEAE-TECTONEAE		
Petitia domingensis Jacq.	f	–
Tectona grandis L.	l, w	–
VITICOIDEAE-VITICEAE		
Cornutia coerulea (Jacq.) Moldenke	s	–
Gmelina arborea Roxb.	s	–
Gmelina asiatica L.	s	–
Gmelina hystrix Schult. ex Kurz	s	–
Gmelina leichhardtii F. Muell	s	–
Premna lignum-vitae (A. Cunn.) Pieper	s	–
Vitex agnus-castus L.	f, l	auc + agn + 2 o
Vitex agnus-castus L. var. *pseudo-negundo* Hausskn.	s	auc + agn
Vitex cannabifolia Sieb. et Zucc.	l	auc (?) + agn (?)
Vitex cannabifolia Sieb. et Zucc.	s	–
Vitex cymosa Bert. ex Spreng.	f	–
Vitex ferruginea Schum. et Thonn.	l	auc (?) + auc ester
Vitex glabrata R. Br.	l, s	auc + agn
Vitex lucens T. Kirk	l	auc + auc ester
Vitex macrophylla R. Br.	s	auc + agn
Vitex negundo L.	l	auc + auc ester
Vitex negundo L. var. *heterophylla* (Franch.) Rehd.	s	–
Vitex trifolia L.	f	auc (?) + agn (?)
VITICOIDEAE-CLERODENDREAE		
Clerodendrum buchanani (Roxb.) Walp.	l	–
Clerodendrum bungei Steud.	s	2 gl
Clerodendrum canescens Wall.	l	1 gl
Clerodendrum canescens Wall.	s	–
Clerodendrum kaempferi (Jacq.) Sieb.	s	–
Clerodendrum philippinum Schau.	l	–
Clerodendrum schweinfurthii Gürke	s	1 gl ?
Clerodendrum speciosissimum Van Geert	s, l	–
Clerodendrum splendens G. Don.	s	1 gl ?
Clerodendrum thomsonae Balf. f.	l	3 gl
Clerodendrum trichotomum Thunb.	l, s	–
Clerodendrum trichotomum Thunb. var. *fargesii* (Dode) Rehd.	l, s	1 gl
Clerodendrum triplinerve Rolfe	s	3 gl
Clerodendrum viscosum Vent.	s	–
Teucridium parvifolium Hook f.	s	–
CHLOANTHOIDEAE-PHYSOPSIDEAE		
Dicrastylis beveridgei Hemsl.	s	–
Dicrastylis verticillata J. M. Black	s	cat ?
Lachnostachys eriobotrya (F. Muell.) Druce	s	2 gl
Physopsis lachnostachya C. A. Gardn.	s	me cat ?

Table 1. continuated

	organ	
CHLOANTHOIDEAE-CHLOANTHEAE		
Cyanostegia angustifolia Turcz.	s	–
CHLOANTHOIDEAE-ACHARITEAE		
Pityrodia atriplicina (F. Muell.) Benth.	s	–
Pityrodia paniculata (F. Muell.) Benth.	s	–
Spartothamnella juncea (A. Cunn.) Briq.	l, s	–
Spartothamnella teucriiflora (F. Muell.) Moldenke	s	–
VERBENOIDEAE-CITHAREXYLEAE		
Citharexylum berlandieri B. L. Robinson	s	–
Citharexylum montevidense (Spreng.) Moldenke	s	–
Duranta repens L.	l, s	lam + dur
Duranta serratifolia (Griseb.) Kuntze	l	lam (?) + dur (?)
Rhaphithamnus spinosus (A. L. Juss.) Moldenke	l, s	1 gl
VERBENOIDEAE-PETRAEEAE		
Petrea volubilis L.	s	–
VERBENOIDEAE-PRIVEAE		
Priva aspera H.B.K.	s	–
Priva aspera H.B.K.	l	1 gl
VERBENOIDEAE-LANTANEAE		
Aloysia chamaedryfolia Cham.	s	–
Aloysia gratissima (Gill. et Hook.) Troncoso	s	–
Aloysia triphylla (L'Hér.) Britton	s	–
Bouchea pseudochascanum	l	2 gl
Lantana camara L.	l	1 gl
Lantana camara L.	f	–
Lantana fucata Lindl.	f	–
Lantana hirta Grah.	l, f	–
Lantana hispida H.B.K.	f	–
Lantana montevidensis (Spreng.) Briq.	f	–
Lantana rugosa Thunb.	f	–
Lantana trifolia L.	s	–
Phyla cuneifolia (Torr.) Greene	s	–
Phyla nodiflora (L.) Greene	l, s	–
Stachytarpheta angustifolia (Mill.) Vahl	l	ipo
Stachytarpheta angustifolia (Mill.) Vahl	s	–
Stachytarpheta cayennensis (L. C. Rich.) Vahl	l, s	ipo
Stachytarpheta indica (L.) Vahl	l	ipo
Stachytarpheta jamaicensis (L.) Vahl	l	ipo
Stachytarpheta purpurea Greenm.	l	ipo
Stachytarpheta purpurea Greenm.	s	–
VERBENOIDEAE-VERBENEAE		
Junellia patagonica (Speg.) Moldenke	s	–
Junellia patagonica (Speg.) Moldenke	l	ver
Stylodon carneus (Medic.) Moldenke	s	–
Verbena bipinnatifida Nutt.	s	lam
Verbena bipinnatifida Nutt.	l	ver + lam
Verbena bonariensis L.	l	ver
Verbena bracteata Lag. et Rodr.	s	–
Verbena canadensis (L.) Britton	s	lam
Verbena corymbosa Ruiz et Pav.	s	–

Table 1. continuated

	organ	
Verbena elegans H.B.K.	s	–
Verbena hastata L.	l	–
Verbena hispida Ruiz et Pav.	s	–
Verbena hispida Ruiz et Pav.	l	ver
× *Verbena hybrida* Voss	l	ver
Verbena laciniata (L.) Briq.	s	–
Verbena lasiostachys Link	l	ver (+ lam ?)
Verbena litoralis H.B.K.	s	–
Verbena litoralis H.B.K.	l	ver
Verbena macdougalii Heller	l	ver
Verbena officinalis L.	l	ver
Verbena peruviana (L.) Britton	s	–
Verbena rigida Spreng.	l	ver
Verbena rigida Spreng.	s	–
Verbena supina L.	l	ver
Verbena tenera Spreng.	s	lam ?
Verbena tenuisecta Briq.	l	ver + lam
Verbena urticifolia L.	s	–
Verbena urticifolia L.	l	ver
CARYOPTERIDOIDEAE-CARYOPTERIDEAE		
× *Caryopteris clandonensis* Simmonds	l	ac harp
Caryopteris divaricata (Sieb. et Zucc.) Maxim.	s	harp + ac harp
Caryopteris incana (Thunb.) Miq.	l, s	harp + ac harp

auc	= aucuboside	f	= fruits
auc ester	= ester of aucuboside	l	= vegetative parts
agn	= agnuside	w	= wood
cat	= catalpol	s	= seeds
me cat	= methoxycatalpol	2 gl	= two glycosides
lam	= lamiide	+ 2 o	= and two other spots
dur	= durantoside	(?)	= indistinct paper chromatographic evidence
ipo	= ipolamiide		
ver	= verbenaloside	—	= no spots on paper chromatogram
harp	= harpagide		
ac harp	= acetylharpagide		

R_F=0.35 (n.butanol-ethanol-water, 40:11:19 v/v) and is stained blackish-blue (purple in ultraviolet light) by the acidic anisidine reagent, the second has R_F=0.14 and gives a brown spot (orange-brown in u.v.). The latter glycoside was obtained as crystals ($[\alpha]_D = -32°$ (H_2O); m.p. 166–169°). A third compound may be an iridoid with R_F=0.63, giving a pink spot (pink also in ultraviolet light).

Of the Chloanthoideae seeds of *Dicrastylis verticillata* contain a compound which, because of the few seeds available, could not be identified, but which might be identical with catalpol. *Physopsis lachnostachya* contains an iridoid glycoside which possibly is methoxycatalpol (known to occur in *Buddleja*), while *Lachnostachys eriobotrya* has two iridoid glycosides which are chromatograph-

ically identical with the unidentified components of *Microcorys exserta* (Labiatae – Prostantheroideae).

The Verbenoideae-Citharexyleae have two types of glycosides. *Duranta* species have lamiide (R_F=0.44; $[\alpha]_D$=–100°(H_2O)) and one or more lamiide esters (durantosides) (lamiide ester from *D. repens* R_F=0.81; $[\alpha]_D$=–34° (H_2O)), while in *Raphithamnus spinosus* a compound with R_F=0.26, staining pink (also pink in u.v. light) occurs which is not identical with catalpol nor with Asarina glucoside. In the tribe Priveae of this subfamily *Priva aspera* has a compound which is possibly identical with the fourth type of Clerodendrum glycosides. The Lantaneae have several types of iridoid glycosides. *Bouchea pseudochascanum* contains two compounds (R_F=0.32 and 0.41) which stain brown (reddish-brown in u.v. light), one of which may be identical with ipolamiide. *Lantana camara* has an iridoid glycoside in the leaves (not in seeds) with R_F=0.08, staining blackish (dark with a purple pink border in u.v. light). *Stachytarpheta* species have ipolamiide (R_F=0.61; $[\alpha]_D$=–138° (H_2O); m.p. = 142° for the compound of *S. jamaicensis*; $[\alpha]_D$–143° (H_2O) for *S. cayennensis*). In the Verbeneae several *Verbena* species contain verbenaloside ($[\alpha]_D$=–176° (H_2O); m.p. 181–182°), while some species have an iridoid glycoside which is paper chromatographically identical with lamiide (R_F=0.44; colour with acidic anisidine initially greenish-grey, later grey; in u.v. light greyish-purple with a blue border).

Species of *Caryopteris* (Caryopteridoideae) have harpagide and acetylharpagide. Acetylharpagide was identified by comparison with authentic acetylharpagide on paper chromatograms (both R_F=0.59; same colours with the anisidine reagents), and by its optical rotations ($[\alpha]_D$=–126° (H_2O) and –122° (methanol)).

Table 1 shows that, apart from the many Verbenaceae containing one or more iridoid glycosides, many other Verbenaceae seem to be devoid of such glycosides. Often one type of glycoside or a set of glycosides occurs in several species of a genus, while for other species of the same genus no indications for the presence of iridoid glycosides were obtained with the methods used in the present study. It can not be stated that certain glycosides are characteristic for a subfamily of even a tribe although there are trends in this direction; if more material becomes available it is quite possible that more characteristic glycoside patterns will show up. Anyway, the family contains a rich variety of iridoid glycosides.

In the Verbenoideae, the Caryopteridoideae and possibly in the Chloanthoideae iridoid glycosides occur, which also occur in some parts of the Labiatae. Several Verbenoideae have lamiide and (or) ipolamiide in common with *Lamium* species (Stachydoideae-Stachydeae). *Caryopteris* shares harpagide and acetylharpagide with the Ajugoideae and the tribes Marrubieae and Stachydeae of the Stachydoideae. There are indications that in the *Chloanthoideae* catalpol and methoxycatalpol occur, catalpol being a constituent of several Scutellarioideae and Prostantheroideae, and the as yet unknown compounds of *Lachnostachys* may be identical with those of *Microcorys* (Prostantheroideae). Now it is most interesting that the taxa of Labiatae just mentioned belong phylogenetically to the most primitive part of the family (HILLSON 1959, WUNDERLICH 1967), and

that the Ajugoideae and Prostantheroideae are so similar to certain taxa of the Verbenaceae that they are generally called the "verbenoid" Labiatae (BRIQUET 1897b).

On the basis of gynoeceum morphology JUNELL (1934) proposed a number of transfers of verbenaceous taxa of whith the most relevant for the present study are the following. *Caryopteris* would belong to the Ajugeae, which in its turn would be a tribe of the Viticoideae; this subfamily would belong to the Labiatae. The Chloanthoideae would also belong to the Labiatae. The subfamily Stilboideae would become a separate family *Stilbinaceae*. Junell proposed to keep the Verbenoideae as a family Verbenaceae, characterized by a racemose inflorescence and ovules inserted on the borders of the carpels, in contradistinction to the cymose Chloanthoideae and Viticoideae which have the ovules on the inner sides of the carpels as in the verbenoid Labiatae. Because of their central placentations the Symphorematoideae and Avicennioideae should be excluded from the Verbenaceae though they can be derived from the Viticoideae.

By computational analysis of 54 anatomical and morphological characters applied to 400 species of Labiatae and Verbenaceae, EL-GAZZAR & WATSON (1970) obtained classifications which show a close resemblance with Junell's propositions. All verbenaceous species studied (68 species belonging to 22 genera) join with the group A Labiatae which comprise all iridoid glycoside containing species (but not exclusively these: compare KOOIMAN 1972). Within group A of El-Gazzar and Watson three (sub-) groups I-III are recognizable. The first of these contains the subfamilies Prostantheroideae, Ajugoideae, Prasioideae and part of the Stachydoideae (viz. the subtribe Melittinae) of the Labiatae, and the subfamilies Viticoideae, Stilboideae and Chloanthoideae (Dicrastylidaceae in terms used by El-Gazzar and Watson) of the Verbenaceae. The second (sub-) group contains exclusively the Verbenoideae; and the third does not contain any Verbenaceae.

The principal difference with Junell's propositions is the position of the Stilboideae, which according to El-Gazzar and Watson do not deserve a family status, and should even not be kept as a subfamily.

The occurrence of the iridoid glycoside nyctanthoside in a *Nyctanthes* species (RIMPLER & JUNGHANNS 1975) is fitting with the position of the Nyctanthoideae as a subfamily of the Verbenaceae. However, it is fitting as well with the conception that *Nyctanthes* belongs to the Oleaceae in which iridoid glycosides occur as well or with the opinion that *Nyctanthes* should be placed into a separate family (see for arguments on the position of *Nyctanthes* in the system: KUNDU & DE 1968; KSHETRAPAL & TIAGI 1970). The positions of the Symphorematoideae and Avicennioideae must remain out of consideration as no material of these subfamilies has been studied by El-Gazzar and Watson nor by the present author. Each of these subfamilies has repeatedly been claimed to be sufficiently different from the rest of the Verbenaceae to justify either a family status of its own or incorporation into another family (MOLDENKE 1971). However, it should be borne in mind that also the Chloanthoideae have been transferred to a separate family Chloanthaceae (HUTCHINSON 1959) or Dicrastylidaceae (MOL-

DENKE 1971), as well as the Stilboideae to a family Stilbinaceae (JUNELL 1934) or Stilbaceae (HUTCHINSON 1959, MOLDENKE 1971). In addition, all these small families are to be found in several nineteenth century systems (for references see BRIQUET 1897a, 1900).

The iridoid patterns in the Verbenaceae and Labiatae as found in the present study and in a previous one (KOOIMAN 1972) are in harmony with the conceptions of Junell and of El-Gazzar and Watson, although it can not be said that they support these conceptions. These patterns would also be in harmony with some more differentiation on suprageneric levels; for instance, it would be well conceivable to have a subfamily Prostantheroideae into which the Chloanthoideae have been merged (this was suggested as a possibility by Junell), along with a subfamily Ajugoideae, into which *Caryopteris* has been incorporated. Although for the present study no material of the Stilboideae was available for investigation, the occurrence of iridoid glycosides in this taxon is known (RIMPLER 1972c, RIMPLER & PISTOR 1974); it is curious to note that two species of the ericoid genus *Stilbe* have unedoside as an iridoid constituent, which also occurs in the non-ericoid, but ericaceous, *Arbutus unedo*.

Chemotaxonomical indications for narrow relations of (parts of) the Verbenaceae with other families of the Scrophulariales, among these indications the iridoid patterns, are available. They are discussed by HEGNAUER (1973).

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