THE OCCURRENCE OF ASPERULOSIDIC GLYCOSIDES IN THE RUBIACEAE*

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SUMMARY

Some properties of the new iridoid compounds Galium glucoside and Gardenia glucoside are described.

Galium glucoside and asperuloside occur in many species belonging to the *Rubioideae* (sensu Bremekamp); they were not found in other subfamilies of the *Rubiaceae*.

Gardenia glucoside occurs in several species of the tribe *Gardenieae* (subfamily *Ixoroideae*).

The distribution of the asperulosidic glucosides in the *Rubiaceae* corresponds with the classification proposed by Bremekamp, although there are some exceptions (*Hamelieae*, *Opercularia* and *Pomax*, possibly the *Gaertnereae*). To a somewhat less degree the system proposed by Verdcourt is supported.

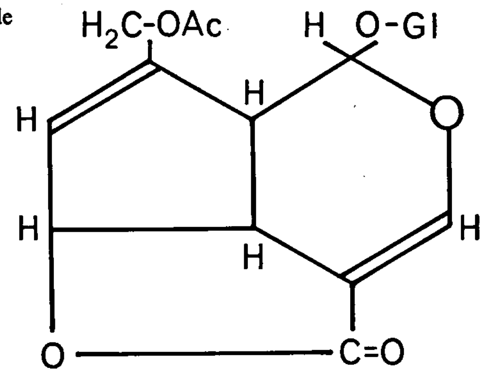
1. INTRODUCTION

Apart from the classification arrived at by BREMEKAMP (1966) the only other modern system of the *Rubiaceae* was proposed by VERDCOURT (1958); both authors considered their classifications as tentative. The systems have several features in common, but deviate in some points. The main differences are in the position of the *Urophylloideae* sensu Bremekamp, which are included in the subfamily *Rubioideae* by Verdcourt, and in the relationship between the *Cinchonoideae* and the *Ixoroideae* (both sensu Bremekamp) which are united in the subfamily *Cinchonoideae* by Verdcourt. Both systems diverge widely and principally from all older classifications which appeared to become more and more unsatisfactory as the number of described species increased.

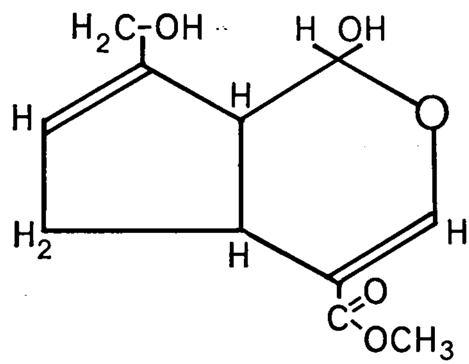
In 1954 BRIGGS & NICHOLLS reported on the presence or absence of the iridoid glucoside asperuloside (1) in most species of *Coprosma* and in many other *Rubiaceae*. The reaction they used for the detection of asperuloside is now known to be not specific for this glucoside; it detects in addition some structurally and most probably biogenetically related glycosides. The value of the results of the test is somewhat limited since the latter was applied to herbarium material; it is known that it depends on the way of preparing and keeping herbarium material whether iridoid glycosides remain intact or degrade. In well-prepared and well-stored dried plants asperuloside and related glycosides

* This paper is dedicated to Professor Dr. Ir. G. van Iterson who contributed so much to my scientific education, not in the last place by his enlightening example in scientific performance. The subject and the contents of this paper honour Professor Dr. C. E. B. Bremekamp whose work on the *Rubiaceae* for the first time created real insight in the natural relationships within this family.

1. Asperuloside



2. Genipin



can survive for long times; Briggs and Nicholls mention that a specimen collected in 1769 showed a positive asperuloside reaction. From 5 *Coprosma* species asperuloside was actually isolated by the authors mentioned.

It occurred to the present author that the wealth of data in the paper by Briggs and Nicholls together with data from other authors might be the basis of a chemotaxonomic study of the *Rubiaceae* using the occurrence of asperuloside or related glycosides as a character. For this purpose seeds and vegetative parts of either fresh or herbarium material were investigated in order to add to the data from the literature, and, to a limited extent, to verify the results obtained with herbarium material.

2. METHODS AND MATERIALS

The simple test used by Briggs and Nicholls consisted of heating the sample in dilute hydrochloric acid and observing the blue or green discoloration of the solution brought about by asperuloside or a related glycoside.

In the present contribution extracts of either plants or seeds were examined by paper chromatography in either n.butanol-ethanol-water (40:11:19 v/v) (A) or n.butanol-acetic acid-water (5:1:4 v/v) (B), and spraying with the acidic p.anisidine phosphate reagent, consisting of p.anisidine (0.5 g), concentrated phosphoric acid (3 ml), concentrated hydrochloric acid (1 ml), and 70% ethanol (100 ml). In solvent A asperuloside has an R_F value of approximately 0,47, Gardenia glucoside 0,43, while Galium glucoside forms a rather long streak from near the start line of the chromatogram. In solvent B the R_F values are: asperuloside 0.49, Gardenia glucoside 0.44, and Galium glucoside 0.28. The colours obtained after heating the sprayed chromatogram at 105° for 1 minute are in daylight brownish-grey for asperuloside and Galium glucoside, and greyish-blue for Gardenia glucoside, in ultraviolet light the spots of asperuloside and Galium glucoside are reddish-brown, while that of Gardenia glucoside is green.

In addition to the paper chromatographic examination the plant and seed extracts were tested with the copper reagent of TRIM & HILL (1952) which indicates the same compounds as the simple reagent of Briggs and Nicholls, but which is far more sensitive.

Most of the investigated seeds were present in the Seed Collection of the Laboratory of General and Technical Biology; the seeds of some species were obtained *via* the seed-lists of horti. Dried material was used from the Herbarium of this Laboratory and from the Rijksherbarium at Leyden, while fresh material was obtained from the Botanical Garden of this Laboratory.

3. RESULTS AND DISCUSSION

The Gardenia and Galium glucosides have not yet been mentioned in the literature. Gardenia glucoside was isolated from leaves of *Gardenia jasminoides* by extraction, followed by adsorption on charcoal from the aqueous extract, elution with 50% ethanol and preparative paper chromatography with solvent A. It was not obtained in a crystalline form; it has a specific optical rotation in water $[\alpha]_D - 96°$ and reacts weakly with the silver reagent of TREVELYAN c.s. (1950). The colour developed with the copper reagent is blue. By treatment with dilute alkali a compound is formed which on paper chromatograms is indistinguishable from Galium glucoside and from alkali treated asperuloside; moreover methanol is liberated by the alkali treatment. Probably Gardenia glucoside is the methyl ester of Galium glucoside or a structurally strongly related compound. Galium glucoside was isolated by extracting seeds of *Galium aparine* with water, adsorbing the glucoside on charcoal, eluting with 50% ethanol and separating the glucoside by preparative paper chromatography with solvent B. It has a free carboxyl group but does not contain an acetyl group; it is probably identical with the free acid form of de-acetyl-asperuloside and thus an isomer of monotropeoside. Crystalline Galium glucoside has no sharp melting point (it softens near 138° and blows up near 145°) and has $[\alpha]_D + 33.5°$ (in water); it reacts weakly with the silver reagent and develops a blue colour with the copper reagent.

The data referring to the presence of asperulosidic glycosides from both the literature and the present investigations are recorded in *table 1* in which the species of the *Rubiaceae* are arranged as well as at present possible according to

Table 1. Occurrence of asperulosidic glycosides in the *Rubiaceae*

	Organ [a])	Test [b])	Remarks [c])
Cinchonoideae			
Cinchoneae			
Cinchona officinalis L.	l	–	
C. succirubra Pav.	s	–	log?
Dolicholobium sp.		–	BN
Hintonia latiflora (Sessé et Moc.) Bullock var. *leiantha* Bullock		–	BN
Hymenodictyon floribundum (Hochst. et Stend.) Robins.	s	–	log?
Naucleae			
Cephalanthus occidentalis L.		–	BN
Nauclea purpurascens Korth.	h	–	
N. rhynchophylla Miq.	s	–	
Sarcocephalus cordatus Miq.		–	BN
Condamineae			
Bikkia grandiflora Reinw.		–	BN
Rondeletieae			
Rondeletia odorata Jacq.	l	–	
Schenckia blumenaviana K. Schum.	s	–	
Wendlandia luzoniensis DC.		–	BN
Sipaneae			
Sipanea hispida Benth.	h	–	
Virecta multiflora Sm.	h	–	
Mussaendeae			
Mussaenda frondosa L.		–	BN
M. erythrophylla K. Schum. et Thonn.	h, s	–	
Sabiceae			
Sabicea cinerea Aubl.	h	–	
Urophylloideae			
Urophylleae			
Pleiocarpidia borneensis (Miq.) Brem.	h	–	
Praravinia bullata Brem.	h	–	
Urophyllum macrophyllum (Bl.) Korth.	h	–	
Pauridiantheae			
Pauridiantha afzelii (Hiern.) Brem.	h		
P. canthiifolia Hook. f.	h	–	
Simireae			
Sickingia sampaioana Standl.	h	–	
Ophiorrhizeae			
Ophiorrhiza bracteata Korth.	h	–	log?
O. leptantha A. Gray		–	BN
O. mungos L.	s	–	
Pomazotoideae			
Pomazoteae			
Lerchea bracteata Val.	h	–	
Pomazota pilosa (Miq.) Val.	h	–	
Guettardoideae			
Guettardeae			
Bobea elatior Gand.		–	BN

Table 1. Continued

	Organ [a])	Test [b])	Remarks [c])
Guettarda ambigua DC.		–	BN
G. blodgettii Shuttl.		–	BN
G. speciosa L.		–	BN
Timonius polygamus (Forst.) Robins.		–	BN
Ixoroideae			
Coptosapelteae			
Coptosapelta diffusa (Champ.) Steen.	h	–	
Acranthereae			
Acranthera multiflora Val.	h	+	
Cremasporeae			
Cremaspora coffeoides Hemsl.	h	–	
Gardenieae			
Gardenia brighamii H. Mann		–	BN
G. jasminoides Ellis	l	+	grd
G. grandiflora Lour.		+	LBN
G. remyi H. Mann		+	BN
G. taitensis DC.		–	BN
Genipa americana L.	h	+	gen
Macrosphyra longistyla (DC.) Hook. f.	s	+	grd
Randia aculeata L.	f	–	
R. cumingiana Vid.		–	BN
R. graeffii Rein.		–	BN
R. malabarica Lamk.	h	–	
Rothmannia hispida (K. Schum.) Fagerl.	s	–	
R. longiflora Salisb.	s	–	
Tocoyena bullata Mart.	h	+	
Ixoreae			
Chomelia brasiliana A. Rich.	s	–	
Coffea arabica L.		–	BN
C. arabica L.	f, l	–	
G. oxyloba Janka		–	BN
Ixora bracteata Cheesem.		–	BN
I. coccinea L.		–	BN
I. cumingiana Vid.		–	BN
Pavetta indica L. var. *polyantha* Hook. f.	h	-	
P. lanceolata Eckl.	h	–	
P. zimmermanniana Valet.	s	–	
Tarenna fragrans (Bl.) Kds. et Val.	h	–	
Tricalysia lanceolata (Sond.) Burtt-Davy	h	–	
Chiococceae			
Chiococca racemosa Jacq.		–	BN
Vanguerieae			
Ancylanthos cistifolius Welw.	s	–	
Canthium barbatum (Benth. et Hook) Seem.		–	BN
C. odoratum (Forst) Seem.		–	BN
C. pedunculare Cav.		–	BN
Pygmaeothamnus zeyheri (Sond.) Robijns	h	–	
Vangueria edulis Vahl	s	–	

Table 1. Continued

	Organ [a])	Test [b])	Remarks [c])
Rubioideae			
Hedyotideae			
Bouvardia capitata Bullock		–	BN
B. triphylla Salisb.	s, h	+	glm
Dentella repens Forst.		–	BN
Gouldia axillaris Wawra		+	BN
G. purpurea (Fosb.) Skottsb.		+	BN
G. st.- johnii Fosb.		+	BN
G. terminalis (Hook. et Arn.) Hillebr.		+	BN
Hedyotis acuminata (Cham. et Schl.) Stend. f. *grayana* Fosb.		+	BN
H. acuminata (Cham. et Schl.) Stend. f. *sherffiana* Fosb.		+	BN
H. angusta Fosb. var. *umbrosa* Fosb.		+	BN
H. centranthoides (Hook. et Arn.) Stend.		+	BN
H. elmeri Merr.		+	BN
H. fluviatilis (Forbes) Fosb. var. *kamapuaana* (Degen.) Fosb. f. *hathewayi* Fosb.		+	BN
H. glaucifolia (A. Gray) Fosb. var. *waimeae* (Wawra) Fosb.		+	BN
H. hispida Retz.		–	BN
H. microphylla Merr.		–	BN
H. schlechtendahliana Stend. var. *cordata* (Cham. et Schl.) Fosb.		+	BN
H. schlechtendahliana Stend. var. *glabrescens* Fosb. f. *kaalensis* Fosb.		+	BN
H. schlechtendahliana Stend. var. *rigida* Fosb.		–	BN
H. uniflora DC.		+	BN
H. vestita R. Br.		+	BN
Houstonia purpurea L.		+	BN
Kadua glomerata Hook. et Arn.		+	BN
K. grandis A. Gray		+	BN
K. longipedunculata Skottsb.		+	BN
Manettia bicolor Paxt.	l	+	LBN
Mycetia lateriflora (Bl.) Reinw. ex Korth.	h	–	
Oldenlandia corymbosa L.	s	+	glm
O. foetida Forst. f.		+	BN
O. umbellata L.	r	+	LBN
Pentas lanceolata (Forsk.) K. Schum.	s	+	glm
Cruckshanksieae			
Anotis hirsuta (L.f.) Miq. et Boerl.	h	–	
A. leschenaultiana (W. et Arn.) Hook. f.	h	–	
Cruckshanksia flava Clos.	h	–	
C. hymenodon Hook. et Arn.		–	BN
Oreopolus citrinus Schl.		+	BN
Argostemmatideae			
Argostemma montanum Bl.	h	+	glm
Coccocypseleae			
Coccocypselum repens Sw.	h	+	glm
Schradereae			
Lucinaea korthalsiana Miq.	h	–	

Table 1. Continued

	Organ [a])	Test [b])	Remarks [c])
Schradera capitata Vahl.	h	+	1 spot
Hamelieae			
Hamelia patens Jacq.		–	BN
H. patens Jacq.	l	–	
Hoffmannia ghiesbregtii Hemsl.	l	–	
Spermacoceae			
Borreria laevis (Lam.) Griseb.	s	+	glm
Diodia teres Walt.		+	BN
D. dasycephala Cham. et Schl.	s	+	asp + glm
D. virginiana L.		+	BN
Hydrophylax carnosa Sond.	h	–	
Richardsonia pilosa H. B. et K.	f, s	+	asp+glm
R. scabra A. St. Hil.		+	BN
Spermacoce glabra Michx.		+	BN
S. tenuior L.	f	+	asp+glm
Anthospermeae			
Anthospermum aethiopicum L.	h	+	asp+glm
Coprosma (73 species)		73+	BN
C. baueri Endl.	f	+	asp+glm + ao
C. baueri Endl.		–	BN
C. benefica W. R. B. Oliv.		–	BN
Mitchella repens L.		+	BN
Nertera cunninghamii Hook. f.		+	BN
N. depressa Gaertn.		+	BN
N. dichondraefolia (A. Cunn.) Hook. f.		–	BN
N. setulosa Hook. f.		–	BN
Normandia neo-caledonica Hook. f.		+	BN
Opercularia varia Hook. f.		+	BN
Phyllis nobla L.	s	+	asp+glm
Plocama pendula Ait.?	h	+	asp+glm +ao
Pomax umbellata Sol.	h	+	asp+glm
Putoria calabrica (L. f.) Pers.	l	+	LBN
Rubieae			
Asperula aristata L.		–	BN
A. capitata Kit.		–	BN
A. ciliata Roch.		–	BN
A. cynanchica L.		–	BN
A. cynanchica L.		+	Pl
A. cynanchica L.	h	+	glm
A. galioides Bieb. (= *glauca* (L.) Bess.)		–	BN
A. galioides Bieb.	h	+	glm
A. hexaphylla All.	h	+	glm
A. odorata L.	l	+	LBN
A. orientalis Boiss. et Hohen.	s	+	glm
A. perpusilla Hook. f.		+	BN
A. taurina L.		–	BN
A. tenella Heuff.		–	BN
A. tinctoria L.	l	+	LBN
Callipeltis cucullaria Stev.	s	+	asp+glm

Table 1. Continued

	Organ [a])	Test [b])	Remarks [c])
Crucianella angustifolia L.		+	LBN
C. maritima L.		+	LBN
G. anisophyllum Vill.	s	+	asp
Galium aparine L.	l	+	LBN
G. arenarium Loisel.		+	Pl
G. boreale L.	s	+	asp+glm
G. broterianum Boiss. et Reut.	s	+	asp+glm
G. cruciata (L.) Scop.	l	+	LBN
G. hercynicum Weig.	s	+	asp+glm
G. mollugo L.	l	+	LBN
G. parisiense L.	s	+	asp+glm
G. saccharatum All.		+	Pl
G. spurium L.	s	+	asp+glm
G. tenuicaule A. Cunn.		+	BN
G. tricorne Stokes	f	+	asp+glm
G. umbrosum Soland. ex Forst.		–	BN
G. valantia Weber	f	+	glm
G. verum L.	l	+	LBN
Phuopsis stylosa (Trin.) Benth. et Hook.	l	+	LBN
P. stylosa (Trin.) Benth. et Hook.	s	+	asp+glm
Relbunium hypocarpium Hemsl.	s	+	asp+glm
Rubia peregrina L.	r, l	+	LBN
R. tinctorum L.	r, l	+	LBN
R. tinctorum L.	l	+	asp+glm
Sherardia arvensis L	l	+	LBN
Vaillantia hispida L.	f	+	asp+glm
V. muralis L.	s	+	asp+glm
Perameae			
Perama hirsuta Aubl.	h	+	glm
Psychotrieae			
Allaeophania rugosa Boerl.	h	+	asp+glm
Chasalia curviflora Thw.	h	–	
Geophila herbacea (L.) K. Schum.	h	–	
Grumilea capensis Sond.	h	–	
Hydnophytum formicarum Jack.	l, f	+	asp+glm
Lasianthus stercorarius Bl.	h, s	+	asp+glm
Myrmecodia armata DC.	l, s	–	
Psychotria bacteriophila Valet.	h	+	glm
P. bullata Seem.		–	BN
P. confertiloba A.C.Sm.		–	BN
P. emetica L.	l	+	
P. forsteriana A. Gray		–	BN
P. grandiflora H. Mann		–	BN
P. hexandra H. Mann		–	BN
P. hirtella Oliv.	f	+	glm
P. hirtula Skottsb.		–	BN
P. insularum A. Gray		–	BN
P. kirkii Hiern	s	+	glm
P. loniceroides Sieber ex DC.		–	BN
P. maingayi Hook. f.	s	+	glm
P. montana Bl.	h	–	

Table 1. Continued

	Organ [a])	Test [b])	Remarks [c])
P. pinnatinervia Elm.		—	BN
Saprosma arboreum Bl.	h	+	asp+glm
Straussia hawaiiensis A. Gray		+	BN
S. hillebrandii Rock		—	BN
S. kaduana A. Gray		—	BN
S. mariniana A. Gray		—	BN
S. psychotrioides Heller		—	BN
Triainolepideae			
Triainolepis hildebrandtii Vatke.	h	+	glm
Gaertnereae			
Gaertnera vaginata Lam.	h	—	
Pagamea thyrsiflora Spruce	h	—	
Coussareae			
Coussarea albescens Muell.	h	+	glm
Faramea glandulosa Poepp. et Endl.	h	—	
Paederieae			
Leptodermis lanceolata Wall.	l	+	LBN
Paederia foetida L.	l	+	LBN
P. pringlei Greenm.		+	BN
P. scandens (Lour.) Merrill var. *mairei* (Léveillé) Hara	s	+	asp+glm
Serissa foetida Comm.	l	+	LBN
Morindeae			
Damnacanthus major Sieb. et Zucc.	f	+	glm
Morinda bracteata Roxb.		—	BN
M. bucidaefolia A. Gray		+	BN
M. citrifolia L.		+	BN
M. citrifolia L.	f	+	glm
M. forsteri Seem.		+	BN
M. lucida A. Gray	s	+	glm
M. royoc L.		+	BN
M. trimera Hillebr. var. *sanwicense* (Deg.) Skottsb.		+	BN
M. umbellata L.	r	+	LBN
Knoxieae			
Pentanisia variabilis Harv.	h	+	glm
Craterispermeae			
Craterispermum gracile A. Chev.	h	—	
Hillioideae			
Hillieae			
Hillia brasiliensis Cham. et Schl.	h	—	

a) l = leaves, s = seeds, h = herbarium material, f = fruits, r = roots
b) + = asperulosidic glycosides present; — = asperulosidic glycosides absent.
c) BN = Briggs & Nicholls (1954); LBN = literature mentioned by Briggs & Nicholls; Pl = Plouvier (1964); asp = asperuloside; gen = genipin; glm = Galium glucoside; grd = Gardenia glucoside; log = loganoside; ao = and some others.

Table 2. Distribution of asperulosidic glycosides in tribes of the *Rubiaceae*

Subfamily	Tribe	Test +	Test –	Subfamily	Tribe	Test +	Test –
Cinchonoideae	Cinchoneae	–	5	Rubioideae	Hedyotideae	25	6
	Naucleae	–	4		Cruckshanksieae	1	4
	Condamineae	–	1		Argostemmatideae	1	–
	Rondeletieae	–	3		Coccocypseleae	1	–
	Sipaneae	–	2		Schradereae	1	1
	Mussaendeae	–	2		Hamelieae	–	2
	Sabiceae	–	1		Spermacoceae	8	1
Urophylloideae	Urophylleae	–	3		Anthospermeae	84	4
	Pauridiantheae	–	2		Rubieae	32	8
	Simireae	–	1		Perameae	1	–
	Ophiorrhizeae	–	3		Psychotrieae	10	18
Pomazotoideae	Pomazoteae	–	2		Triainolepideae	1	–
Guettardoideae	Guettardeae	–	5		Gaertnereae	–	2
Ixoroideae	Coptosapelteae	–	1		Coussareae	1	1
	Acranthereae	1	–		Paederieae	5	–
	Cremasporeae	–	1		Morindeae	8	1
	Gardenieae	6	8		Knoxieae	1	–
	Ixoreae	–	11		Craterispermeae	–	1
	Chiococceae	–	1	Hillioideae	Hillieae	–	1
	Vanguerieae	–	6				

the classification of Bremekamp. These data are summarized in *table 2.*

Iridoid glycosides which are detected by the p. anisidine and the copper reagents are absent from the subfamilies *Cinchonoideae, Urophylloideae, Pomazotoideae, Guettardoideae* and *Hillioideae;* of one subfamily, the *Gleasonioideae,* no representative was available during the present study. The *Pomazotoideae* were represented in the investigated material by two, and the *Hillioideae* by only one species, all of them being herbarium material; it is therefore too speculative to state that these subfamilies are devoid of asperulosidic glycosides. Verdcourt did not recognize the group of genera together forming Bremekamp's *Pomazotoideae* as a subfamily and kept them, provisionally, in his *Hedyotideae,* remarking that the group forms a link with the *Cinchonoideae.*

In the *Ixoroideae* exclusively the tribes *Gardenieae* and *Acranthereae* contain species with asperulosidic glycosides; asperuloside itself and Galium glucoside were, however, absent from the *Gardenieae,* the only glucoside found being Gardenia glucoside. Probably this is the same glucoside as that isolated as a syrup by ORTH (1855) from *Gardenia grandiflora* and designated as rubichloric acid (rubichloric acid from other rubiaceous sources was later shown to be identical with asperuloside). It is known that a different iridoid compound, genipin (see p. 125) (DJERASSI *c.s.* 1960), occurs in *Genipa* which also belongs to the *Gardenieae.* Among the species of this tribe in which no asperulosidic glycosides were found are some *Gardenia, Randia* and *Rothmannia* species; the latter two genera were formerly considered to be strongly related with *Gardenia.* Evidently the tribe *Gardenieae* and also the genus *Gardenia* are heterogeneous in respect of the occurrence of these glycosides; this is not altogether surprising since at least the genus *Gardenia* is admittedly artificial (VERDCOURT 1958, p. 223). The members of the tribe *Gardenieae* have many characters in common with the *Ixoreae, e.g.* the taxonomically important pollination mechanism; nevertheless the *Ixoreae* seem to be devoid of asperulosidic glycosides.

The *Rubioideae,* the largest of the subfamilies contains only three tribes in which no asperulosidic glycosides were found, *viz.* the *Hamelieae, Gaertnereae* and *Craterispermeae,* while of one tribe, the *Lathraeocarpeae,* no material was available. Of the *Gaertnereae* and *Craterispermeae* only few species were investigated, the materials of which were herbarium samples; therefore these negative data are not very informative and should be controlled by experiments with fresh material. The *Gaertnereae* have been referred to the *Loganiaceae* by several authors on account of their superior ovaries. Bremekamp, however, is of the opinion that they should be kept in the *Rubiaceae-Rubioideae* because they have raphides and on account of some other characters in which they agree with the *Rubioideae-Psychotrieae;* Verdcourt is convinced of their place within the *Psychotrieae.* The *Hamelieae* have raphides and were therefore placed in the *Rubioideae* by Bremekamp, although they are somewhat aberrant in this subfamily because of the imbricate aestivation of the corolla lobes which is valvate in practically all other *Rubioideae.* The finding that the *Hamelieae* do not contain asperulosidic glycosides might strengthen the arguments for removal of this tribe from the *Rubioideae.*

In the *Psychotrieae* more species without asperulosidic glycosides were found than species containing them. However, in this tribe nearly all the negative records are from herbarium samples; it is likely that the glycosides are present in fresh material of at least part of these species, since nearly though not all fresh material investigated gave a positive reaction. Moreover, some examples, though not within the *Psychotrieae* are listed in *table 1* where herbarium material of a species did not contain asperulosidic glycosides whereas either fresh material or a herbarium sample from a different source was found to give a positive record *(Coprosma baueri, Asperula cynanchica, A. galioides).*

Apart from asperuloside and Galium glucoside still other iridoid glycosides occur in the *Rubioideae.* From *Paederia scandens (Paederieae)* asperuloside and four structurally strongly related glycosides have been isolated (INOUYE *c.s.* 1968), one of which is responsible for the foetid odour of this and related species. Probably the same compound is also responsible for the odour of *Coprosma* species *(Anthospermeae)* and of *Pentanisia foetida (Knoxieae).* Another new glucoside, ipecoside, was isolated by BELLET (1954) from *Cephaëlis ipecacuanha (Psychotrieae);* although this glucoside belongs to the iridoid group it is structurally somewhat less narrowly related with the asperulosidic compounds since it does not have a pentacyclic ring (BATTERSBY *c.s.* 1967); glycosides of this type do not react with the copper reagent nor with p. anisidine to give coloured products.

During the present study chromatographic evidence was obtained for the presence of loganoside or a related compound in some species belonging to subfamilies in which never asperulosidic glycosides have been found. This was indicated on paper chromatograms by red spots which developed with p. anisidine in the course of a few weeks. Two species containing this compound belong to the *Cinchoneae*, the third one to the *Ophiorrhizeae*.

Non-glycosidic iridoids are present in the *Rubiaceae* as monoterpenoid alkaloids, *viz.* the isoquinoline alkaloids; in *table 3* their distribution in this family, as far as known at present, is summarized. Most of these alkaloids occur in the *Cinchonoideae*, although in three other subfamilies these compounds have also been found.

Table 3. Distribution of monoterpenoid alkaloids in the *Rubiaceae* taken from Manske & Holmes (1950–)

Subfamily	Tribe	Genus
Cinchonoideae	Cinchoneae	Capirona
		Cinchona
		Corynanthe
		Mitragyna
		Pausinystalia
		Pseudocinchona
		Remijia
		Uncaria
Ixoroideae	Gardenieae	Bothriospora
		Tocoyena
Rubioideae	Spermacoceae	Richardsonia
	Psychotrieae	Psychotria
Hillioideae	Hillieae	Hillia

Although a considerable number of species of the *Rubiaceae* have been investigated for the presence of asperulosidic glycosides the former constitute only 5% of the number of species of this family; 24% of the genera are represented in in this study. The search for monoterpenoid alkaloids has covered an even much more limited number of species, and about other iridoid compounds in this family nearly nothing is known at present. Therefore the results of this study should be handled with much caution.

Still, it is obvious from the data presented that iridoid compounds occur frequently in the *Rubiaceae*. It appears that in the subfamily *Rubioideae* predominantly asperuloside and Galium glucoside are present; these glucosides seem to be absent from all other investigated subfamilies. Of the *Ixoroideae* only the *Gardenieae* and the *Acranthereae* contain asperulosidic substances; so far Gardenia glucoside and genipin have been recognized.

The available data support both Bremekamp's and Verdcourt's systems; they do not support any of the older classifications. This indicates that the presence or absence of raphides, a character introduced by Bremekanp for the subdivision of the family, is a useful criterion for a natural classification. In the system proposed by Bremekamp the *Ophiorrhizeae* are a tribe of the subfamily *Urophylloideae*, while Verdcourt considers this tribe as one belonging to his *Rubioideae.* The results of the present study speak for Bremekamp's view rather than for Verdcourt's. Also the incorporation of *Pauridiantha* and related genera in the *Urophylloideae* by Bremekamp rather than near the *Rubioideae-Psychotrieae* (the latter suggested by Verdourt) is supported by the results of the present study.

On the other hand Bremekamp's surmise that *Opercularia*, *Pomax* and a related genus after a more detailed study would be placed in a new family *Operculariaceae* (which would find a natural place in the *Dipsacales*) does not gain in probability by this study: *Opercularia varia* and *Pomax umbellata* gave positive reactions for asperulosidic glycosides which is quite normal for species belonging to the *Rubioideae-Anthospermeae* (in which tribe they are usually included), however quite unusual for members of the *Dipsacales* (sensu WAGENITZ 1959).

In connexion with the phylogenetic considerations by Verdcourt it is interesting to note that this author thinks the "*Ixoreae* group of tribes in the *Cinchonoideae*" (it is not clear what Verdcourt means: the tribes with ixoroid pollination mechanism?) are the least divergent from the hypothetical primitive *Rubiaceae*. The present author suggests that this might as well be thought of the *Gardenieae* as of the *Ixoreae* because of the close resemblance of both tribes. The heterogeneity of the *Gardenieae*, also in respect of the prevalence of asperulosidic glycosides, might reflect the central position of this tribe in the family, its predecessors possibly having given rise to the subfamilies devoid of asperulosidic glycosides at one side, and to the *Rubioideae* which predominantly contain these glycosides at the other side. The type of asperulosidic glycoside would have shifted from Gardenia glucoside to asperuloside and Galium glucoside.

Concerning the position of the *Rubiaceae* it is noteworthy that so far only one plant species outside this family has been found which contains Gardenia glucoside. It is *Thevetia neriifolia* Juss. *(Apocynaceae)* in which PARIS & ETCHEPARE (1966) incorrectly claimed to have demonstrated the presence of aucuboside (KOOIMAN, in preparation). The occurrence of Gardenia glucoside in both *Thevetia* and *Gardenia* is of some support to the inclusion of the *Rubiaceae* in the *Gentianales* by Wagenitz and therefore indirectly another argument for the narrow relationship of this family with the *Loganiaceae* (recognized by Bremekamp, Verdcourt, Wagenitz and other authors) which are known to contain several iridoid compounds.

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