Phytochemistry, Vol. 27, No. 8, pp. 2631–2645, 1988.
Printed in Great Britain.

0031-9422/88 $3.00+0.00

DISTRIBUTION OF 6-HYDROXY-, 6-METHOXY- AND 8-HYDROXYFLAVONE GLYCOSIDES IN THE LABIATAE, THE SCROPHULARIACEAE AND RELATED FAMILIES

FRANCISCO A. TOMÁS-BARBERÁN,* RENÉE J. GRAYER-BARKMEIJER, MARIA I. GIL* and JEFFREY B. HARBORNE

Phytochemical Unit, Department of Botany, Plant Science Laboratories, University of Reading, Whiteknights, Reading, RG6 2AS, Berkshire, U.K.

(*Received* 30 *November* 1987)

IN MEMORY OF TONY SWAIN, 1922 1987

Key Word Index—Labiatae; Scrophulariaceae; Verbenaceae; Plantaginaceae; Lentibulariaceae; Buddlejaceae; Callitrichaceae; Globulariaceae; 6-hydroxy-, 6-methoxy- and 8-hydroxyflavone glycosides; chemotaxonomy.

Abstract—A leaf survey of 308 species belonging to the Labiatae, Scrophulariaceae and to five related families has shown that the common flavones, apigenin, luteolin and chrysoeriol are widely present, occurring especially as the 7-glucuronides. In addition 48 species (16% of sample) contain 8-hydroxyflavones and 131 species (43%) 6-hydroxy- or 6-methoxyflavones in glycosidic combination. The frequencies recorded are slightly higher than those found (35% for both groups) in earlier surveys. 8-Hydroxyflavones based on apigenin and luteolin are restricted to the Labiatae (5 genera), Scrophulariaceae (*Gratiola* and *Veronica*) and Lentibulariaceae (*Pinguicula*). In Labiatae, they only occur in the subfamily Lamoideae and not in the Nepetoideae. In *Veronica*, they are restricted to species in the sections *Alsinebe* and *Chamaedrys*. 6-Oxygenated flavones have a more sporadic distribution, but are consistently present in genera such as *Scutellaria*, *Phlomis*, *Micromeria*, *Origanum* and *Thymus* (Labiatae), in *Parahebe* (Scrophulariaceae) and *Globularia* (Globulariaceae). Although uncommon, flavonol glycosides were detected in a few species of the Labiatae, Scrophulariaceae and Globulariaceae, and *C*-glycosylflavones were detected in a few species of the former two families. The chemosystematic significance of these findings is discussed.

INTRODUCTION

An earlier flavonoid survey of a number of highly specialized herbaceous families belonging to the Dicotyledoneae showed that the presence of 6-hydroxyluteolin and scutellarein glycosides is a characteristic feature of the Plantaginaceae, Globulariaceae, Labiatae, Buddlejaceae and Valerianaceae [1]. Glycosides of the same 6-hydroxyflavones (i.e. flavones with a 5,6,7-trihydroxy substituted A-ring) were later detected in some genera belonging to the Scrophulariaceae [2]. Most of these families belong to the order Tubiflorae *sensu* Engler [3], and they are now all considered to be closely related to each other since they also have another chemical feature in common, namely the presence of iridoid glycosides [4, 5].

More recently, 8-hydroxyflavones (i.e. flavones with a 5,7,8-trihydroxy substituted A-ring), occurring as the rare 7-allosylglucosides, have been reported from four genera of two of these families, the Scrophulariaceae (*Veronica* [6, 7]) and Labiatae (*Stachys* [8, 9], *Sideritis* [10] and *Teucrium* [11]). The difficulties in differentiating between 6-hydroxyflavone and 8-hydroxyflavone glycosides have been recently highlighted [12], and a reinvestigation of the flavonoids from *Stachys annua* has revealed that the 6-hydroxyflavone mannosylglucosides reported from this plant [13, 14] are in fact acetylated 8-hydroxyflavone allosylglucosides similar to those found in *Stachys recta* [9]. For this reason, the distribution of 6-hydroxyflavone, 6-methoxyflavone and 8-hydroxyflavone 7-glycosides in the Labiatae, Scrophulariaceae and chemically related families has been re-examined. The chemotaxonomic and phylogenetic implications of the results are discussed. Some of these plants also produce 6-hydroxyflavones in methylated form in the surface wax [e.g. 11], but such excretory flavonoids were not considered during the present investigation.

* Permanent address: Laboratorio de Fitoquimica, Centro de Edafologia y Biologia Aplicada del Segura, CSIC, Apdo. 195, Murcia 30003, Spain.

RESULTS

The leaves of selected species belonging to the Labiatae, Verbenaceae, Scrophulariaceae, Callitrichaceae, Buddlejaceae, Globulariaceae, Lentibulariaceae and Plantaginaceae were investigated for their flavonoid patterns according to standard procedures [15]. Most species contain *O*-glycosides of common flavones such as luteolin, chrysoeriol and apigenin, which are not oxygenated in the 6- and 8-positions. Flavonol-*O*-glycosides and flavone *C*-glycosides were found in a few species. In addition, many species contain 6-hydroxyflavone-*O*-glycosides, whereas others showed 8-hydroxy- or 6-methoxyflavone-*O*-glycosides. Methods of distinguishing between 6- and 8-substitution have already been described [12] and were used throughout in the present work. The presence and absence of the latter three types of flavonoids is presented in Table 1. In this table, species of the Labiatae are arranged into subfamilies according to Erdtman [16], and those of the Scrophulariaceae into subfamilies and tribes according to Engler [3]. The classification of the genus *Veronica* is according to Römpp [17].

Table 1. Distribution of 8-hydroxyflavone, 6-hydroxyflavone and 6-methoxyflavone glycosides in selected species of Labiatae, Schrophulariaceae and related families

Plant species	Place of collection voucher number⁺	8OH	6OH	6OMe
Labiatae				
Subfam. Lamioideae				
Ajuga				
A. chamaepitys (L.) Schreber	Spain	+	−	−
A. iva (L.) Schreber	Spain	+	−	−
A. salicifolia Schreber	Romania	−	−	−
A. genevensis L.	France	+	−	−
A. reptans L.	France	+	−	−
Scutellaria				
S. orientalis L.	Spain	−	+	−
S. galericulata L.	Spain	−	+	−
S. alpina L.	France	−	+	−
S. columnae All.	Italy	−	+	−
S. rubicunda Hornem.	Italy	−	+	−
Stachys				
Sect. *Betonica*				
S. officinalis (L.) Trevisan	Spain	−	−	−
S. alopecuros (L.) Bentham	Spain	−	−	−
S. monieri (Gouan) P.W. Ball	Italy	−	−	−
Sect. *Eriostomum*				
S. germanica L.	Spain	+	−	−
S. alpina L.	France	+	−	−
S. recta L.	Spain	+	−	−
S. heraclea All.	Spain	+	−	−
Sect. *Stachys*				
S. decumbens Pers.	Gece	+	−	−
S. recta L.	Spain	+	−	−
S. palustris L.	Italy	+	−	−
S. sylvatica L.	England	−	−	−
Sect. *Olisia*				
S. ocymastrum (L.) Briq	Spain	+	−	−
S. arvensis (L.) L.	Spain	+	−	−
S. annua (L.) L.	Spain	+	−	−
S. glutinosa L.	Corse	−	−	−
Marrubium				
M. vulgare L.	Spain	−	−	−
M. supinum L.	Spain	−	−	−
M. alysson L.	Spain	−	−	−
M. peregrinum L.	Greece	−	−	−
Prasium				
P. majus L.	Spain	−	t	−
Phlomis				
P. lychnitys L.	Spain	−	+	−
P. herba-venti L.	Spain	−	+	−
P. crinita Cav.	Spain	−	+	−
P. cretica C. Presl.	Greece	−	+	−
P. purpurea L.	Spain	−	−	−
Melittis				
M. mellissophyllum L.	Spain	−	−	+
Galeopsis				
Subgen. *Galeopsis*				

Table 1. *Continued*

Plant species	Place of collection voucher number⁺	80H	60H	60Me
G. bifida Boenm.	France	–	+	–
G. speciosa Miller	Yugoslavia	–	+	–
G. tetrahit L.	France	–	+	–
G. pubescens Bess.	Poland	–	–	–
Subgen. *Ladanum*				
G. ladanum L.	Spain	+	–	–
G. angustifolia Ehrh. ex Hoffm.	Spain	+	–	–
G. segetum Necker	Belgium	+	–	–
G. pyrenaica Bartl.	Spain	+	–	–
Lamium				
L. hybridum Vill.	Spain	–	–	–
L. orvala L.	Yugoslavia	–	–	–
L. album L.	England	–	–	–
L. purpureum L.	Spain	–	–	–
L. amplexicaule L.	Spain	–	–	–
L. maculatum L.	England	–	–	–
Lamiastrum				
L. galeobdolon (L.) Ehrend. & Polatschek.	Spain	–	–	–
Ballota				
B. hirsuta Bentham	Spain	–	–	–
B. acetabulosa (L.) Bentham	Greece	–	–	–
B. pseudodictamnus (L.) Bentham	Greece	–	–	–
Leonorus				
L. cardiaca L.	Austria	–	–	–
L. marrubiastrum L.	Germany	–	–	–
Molucella				
M. spinosa L.	Turkey	–	–	–
Teucrium				
Sect. *Teucrium*				
T. fruticans L.	Spain	–	–	–
T. pseudochamaepitys L.	Spain	–	–	–
Sect. *Stachybotrys*				
T. arduini L.	Yugoslavia	–	+	+
Sect. *Scorodonia*				
T. scorodonia (Hill) Schreber	Spain	–	+	–
T. massiliense L.	France	–	+	–
Sect. *Scordium*				
T. scordium L.	Spain	–	–	–
T. spinosum L.	Spain	–	–	–
Sect. *Chamaedrys*				
T. chamaedris L.	Switzerland	+	–	–
T. webbianum Boiss.	Spain	+	–	–
T. flavum L.	Italy	–	+	–
T. fragile Boiss	Spain	–	–	–
T. marum L.	Spain	–	–	–
Sect. *Polium*				
T. pyrenaicum L.	Spain	–	–	–
T. rotundifolium Schreber	Spain	–	+	–
T. buxifolium Schreber	Spain	–	+	–
T. montanum L.	France	–	–	–
T. libanitis Schreber	Spain	–	+	–
T. aragonense Loscos & Pardo	Spain	–	t	–
T. pumilum L.	Spain	–	+	–
T. polium L.	Spain	–	t	–

Table 1. *Continued*

Plant species	Place of collection voucher number+	80H	60H	60Me
Sideritis				
Subgen. *Sideritis*				
Sect. *Sideritis*				
S. foetens Clemente ex Lag.	Spain	+	–	–
S. glacialis Boiss.	Spain	+	–	–
S. linearifolia Lam.	Spain	+	–	–
S. angustifolia Lag.	Spain	+	–	–
S. spinulosa Barnades ex Asso	Spain	+	–	–
S. leucantha Cav.	Spain	+	–	–
S. incana L.	Spain	+	–	–
S. hirsuta L.	Spain	+	–	–
S. hyssopifolia L.	Spain	+	–	–
S. stachydioides Willk.	Spain	+	–	–
Sect. *Empedoclia*				
S. syriaca L.	Italy	+	–	–
S. clandestina (Bory & Chanb.) Hayek	Greece	+	–	–
S. scardica Griseb.	Greece	+	–	–
Sect. *Hesiodia*				
S. montana L.	Italy	t	–	–
S. romana L.	Spain	t	–	–
Subgen. *Marrubiastrum*				
Sect. *Marrubiastrum*				
S. argocephalus (Webb & Berth.) Clos.	Canary Islands	–	–	–
S. dasygnaphala (Webb & Berth.) Clos.	Canary Islands	–	–	–
S. candicans Aiton	Canary Islands	–	–	–
S. macrostachya Poir.	Canary Islands	–	–	–
S. dendrochahorra Bolle	Canary Islands	t	–	–
S. canariensis L.	Canary Islands	+	–	–
Sect. *Empedocleopsis*				
S. gomerae De Noé ex Bolle	Canary Islands	–	–	–
Subfam. Nepetoideae				
Micromeria				
M. marginata (Sm.) Chater.	France	–	–	–
M. nervosa (Desf.) Bentham	Crete	–	+	–
M. juliana (L.) Bentham	Italy	–	–	–
M. cristata (Hampe) Griseb.	Yugoslavie	–	+	–
M. parviflora (Vis.) Reichenb.	Spain	–	+	–
M. graeca (L.) Bentham	Spain	–	–	–
M. hispida Boiss & Helar. ex Bentham	Crete	–	+	–
M. foliformis (Aiton) Bentham	Spain	–	+	–
M. fruticosa (L.) Druce	Spain	–	+	–
Salvia				
Sect. *Salvia*				
S. officinalis L.	Spain	–	+	t
S. triloba L.	*	–	+	+
S. lavandulaefolia Vahl.	Spain	–	+	+
Sect. *Aethiopis*				
S. sclarea L.	*	–	–	–
S. argentea L.	*	–	–	–
Sect. *Dryhosphace*				
S. glutinosa L.	*	–	–	–
Sect. *Plethiosphace*				
S. transylvanica (Schur ex Griseb.) Schur	*	–	–	–
S. nemorosa L.	*	–	–	–
S. verbenaca L.	*	–	–	–

Table 1. *Continued*

Plant species	Place of collection voucher number⁺	80H	60H	60Me
Sect. *Hemisphace*				
S. verticillata L.	*	–	–	–
Thymbra				
T. capitata (L.) Cav. Lag. Griseb.	Spain	–	–	–
Thymus				
Sect. *Mastichina*				
T. mastichina L.	Spain	–	+	–
T. tomentosus Willd.	Spain	–	+	–
Sect. *Micantes*				
T. caespititius Brot.	Portugal	–	+	–
Sect. *Piperella*				
T. piperella L.	Spain	–	t	–
Sect. *Pseudothymbra*				
T. villosus L.	Portugal	–	t	–
T. longiflorus Boiss.	Spain	–	+	–
T. membranaceus Boiss.	Spain	–	t	–
T. antoninae Rouy & Coincy	Spain	–	+	–
Sect. *Thymus*				
T. capitellatus Hoffmanns & Link	Portugal	–	+	–
T. camphoratus Hoffmanns & Link	Portugal	–	+	–
T. carnosus Boiss.	Spain	–	+	–
T. vulgaris L.	Spain	–	+	–
T. zygis L.	Spain	–	+	–
T. serpylloides Bory	Spain	–	+	–
T. hyemalis Lange	Spain	–	+	–
Sect. *Hyphodromi*				
T. leptophyllus Lange	Spain	–	+	–
T. mastigophorus Lacaita	Spain	–	+	–
Sect. *Serpyllum*				
T. nervosus J. Gay	Spain	–	t	–
T. praecox Opiz	France	–	+	–
T. praecox subsp. *articus* E. Durand	England	–	+	–
T. pulegioides L.	Spain	–	+	–
T. serpyllum L.	France	–	+	–
Nepeta				
N. nuda L.	France	–	–	–
N. latifolia DC.	Spain	–	–	–
N. cataria L.	Spain	–	–	–
N. tuberosa L.	Spain	–	–	–
N. nepetella	Spain	–	–	–
N. scordotis L.	Crete	–	–	–
N. multibracteata Desf.	Spain	–	–	–
Glechoma				
G. hederacea L.	England	–	–	–
Prunella				
P. vulgaris (L.) L.	England	–	–	–
P. laciniata L.	England	–	–	–
Melissa				
M. officinalis L.	Spain	–	–	–
Ziziphora				
Z. hispanica L.	Spain	–	–	–

Table 1. *Continued*

Plant species	Place of collection voucher number⁺	80H	60H	60Me
Hyssopus				
H. officinalis L.	England	–	–	–
Lycopus				
L. europaeus L.	England	–	–	–
Horminium				
H. pyrenaicum L.	Spain	–	–	–
Elsholtzia				
E. ciliata (Thunb.) Hylander	Poland	–	–	–
Satureia				
S. obovata Lag.	Spain	–	–	–
S. salzmanii P.W. Ball	Spain	–	–	–
Acinos				
A. alpinus (L.) Moench.	Spain	–	–	–
A. suaveolens (Sm.) G. Don fil.	Greece	–	–	–
A. arvensis (Lam.) Dandy	Spain	–	–	–
A. rotundifolius Pers.	Spain	–	t	–
Calamintha				
C. grandiflora (L.) Moench.	France	–	–	–
C. sylvatica Bromf.	Spain	–	–	–
C. nepeta (L.) Savi.	Italy	–	–	–
Clinopodium				
C. vulgare L.	Spain	–	–	–
Lavandula				
L. stoechas L.	Spain	–	–	–
L. viridis L' Hér.	Spain	–	–	–
L. dentata L.	Spain	–	–	–
L. angustifolia Miller	Spain	–	–	–
L. latifolia Medicus	Spain	–	–	–
L. ianata Boiss.	Spain	–	–	–
L. multifida L.	Spain	–	+	+
Mentha				
M. longifolia (L.) Hudson	Spain	–	–	–
M. aquatica L.	Spain	–	–	–
M. suaveolens Ehrhart	Spain	–	–	–
M. pulegium L.	Spain	–	–	–
M. arvensis L.	Spain	–	–	–
M. spicata L.	Spain	–	–	–
Rosmarinus				
R. officinalis L.	Spain	–	+	+
R. eriocalix Jordan & Fourr.	Spain	–	+	+
Origanum				
O. vulgare L.	Spain	–	–	–
O. virens Hoffmans & Link	Spain	–	t	–
O. majorana L.	Spain	–	+	–
O. onites L.	Crete	–	+	–
O. scabrum Boiss. & Heldr.	Greece	–	t	–
Verbenaceae				
Duranta				
D. plumeri Jacq.	Egypt	–	+	+
Lippia				
L. nodiflora (L.) Michx.	Spain	–	+	+[s]

Table 1. *Continued*

Plant species	Place of collection voucher number+	80H	60H	60Me
L. canescens Kunth	Spain	–	+	+[s]
L. triphylla	*	–	–	–
Verbena				
V. bonariensis L.	England	–	–	–
V. supina L.	Spain	–	–	–
V. officinalis L.	Spain	–	+	–
Lantana				
L. camara L.	Crete	–	–	+
Vitex				
V. agnus-castus L.	Spain	–	–	–
Scrophulariaceae				
Subfam. Scrophularioideae				
Tribe Gratioleae				
Gratiola				
G. linifolia Vahl	Spain	–	t	–
G. neglecta Torrey	France	–	t	–
G. officinalis L.	Italy/LEP 13348	–	+	–
	Greece	+	–	–
	Bot. Garden/LEP 8410	+	–	–
Mimulus				
M. guttatus DC.	LEP 3499	–	–	–
M. ringens L.	LEP 13396	–	–	–
Torenia				
T. fournierii Linden ex Fourn.	LEP 23189	–	–	–
Tribe Verbasceae				
Verbascum				
V. blattaria L.	LEP 13459	–	–	–
V. chaixii L. ssp. *chaixii*	LEP 6602	–	–	–
V. chaixii L. ssp. *austriacum* (Schott) Hayek	LEP 5615	–	–	–
V. lychnitis L.	LEP 8274	–	+	–
V. nigrum L.	LEP 20387	–	–	–
V. phlomoides L.	LEP 20410	–	–	–
V. thapsus L.	LEP 23582	–	–	–
Tribe Scrophularieae				
Penstemon				
P. calycosus Small	LEP 13405	–	+	–
P. spectabilis Thurb. ex Gray	LEP 7101	–	+	–
Phygelius				
P. capensis E. Mey. ex Benth.	LEP 6879	–	–	–
Scrophularia				
S. canina L.	LEP 20985	–	+	–
S. vernalis L.	LEP 12356	–	+	–
Tribe Antirrhineae				
Nemesia				
N. strumosa Benth. var. *compacta* Voss	LEP 19483	–	–	–
Linaria				

Table 1. *Continued*

Plant species	Place of collection voucher number[+]	80H	60H	60Me
L. alpina (L.) Miller	LEP 20311	–	–	+
L. arvensis (L.) Desf.	LEP 20922	–	–	+
Maurandia				
M. semperflorens Jacq.	LEP 13381	–	–	–
Subfamily Rhinanthoideae				
Tribe Digitaleae				
Erinus				
E. alpinus L.	Switzerland/LEP 10824	–	+	–
Rehmannia				
R. angulata	LEP 3969	–	–	–
Sibthorpia				
S. africana L.	LEP 22293	–	–	–
Tribe Veroniceae				
Chionohebe (= *Pygmea*)				
Ch. densifolia (F. Muell.) Briggs & Ehrend.	New Zealand/LEP 25004	–	+	–
Ch. pulvinaris (Hook. f.) Briggs & Ehrend.	New Zealand/LEP 25005	–	+	–
Hebe				
ca 50 cultivars studied	-	–	+	–
Paederota				
P. bonarota (L.) L.	Italy/LEP 11036	–	+	–
P. lutea Scop.	Austria/LEP 5626	–	+	–
Parahebe				
P.birleyi (N. E. Br.) Oliver	New Zealand/LEP 25020	–	+	–
P. canescens Oliver	New Zealand/LEP 24899	–	–	–
P. catarractae (Forst. f.) Oliver ssp. *diffusa* (Hook. f.) Garnock-Jones	NewZealand/LEP 24891	–	–	–
ssp. *lanceolata* (Benth.) Garnock-Jones	New Zealand/LEP 24892	–	t	–
ssp. *martinii* Garnock-Jones	New Zealand/LEP 26210	–	+	–
ssp. *catarractae*	New Zealand/LEP 26212	–	+	–
P. decora Ashwin	New Zealand/LEP 26216	–	+	–
P. derwentiana (Andr.) Briggs & Ehrend.	Australia/LEP 12341	–	+	–
P. hookeriana (Walp.) Oliver	New Zealand/LEP 24894	–	–	–
P. linifolia (Hook. f.) Oliver ssp. *linifolia*	New Zealand/LEP 26220	–	+	–
P. lyallii (Hook. f.) Oliver	New Zealand/LEP 24900	–	+	–
P. olsenii (Col.) Oliver	New Zealand/LEP 19213	–	+	–

Table 1. *Continued*

Plant species	Place of collection voucher number⁺	80H	60H	60Me
P. perfoliata (R. Br.) Briggs & Ehrend.	Australia/LEP 25001	–	+	–
Veronica				
Sect. *Pseudolysimachia*				
V. longifolia L.	Cultivated/LEP 20391	–	+	–
Sect. *Veronicastrum*				
V. gentianoides Vahl	Cultivated/LEP 12255	–	+	–
V. alpina L.	Switzerland/LEP 20290	–	+	–
V. bellidioides L.	Italy/LEP 22930	–	+	–
V. fruticans Jacq.	Switzerland/LEP 20157	–	+	–
V. fruticulosa L.	Switzerland/LEP 10522	–	+	–
V. satureioides Vis.	Austria/LEP 25012	–	+	–
V. ponae Gouan	Austria/LEP 25011	–	+	–
Sect. *Alsinebe*				
V. serpyllifolia L.	England/LEP 26228	–	+	–
V. arvensis L.	England/LEP 26222	–	–	–
V. peregrina L.	Netherlands/LEP 11625	–	+	–
V. grisebachii Walter	Turkey/LEP 21359	–	+	–
V. polita Fries	England/LEP 20789	–	+	–
V. agrestis L.	England/LEP 24902	+	–	–
V. persica Poiret	England/LEP 26227	+	+	–
V. filiformis Sm.	England/LEP 21668	+	–	–
V. ceratocarpa C. A. Meyer	Iran	+	–	–
V. opaca Fries	Austria	+	–	–
V. hederifolia L. ssp. *hederifolia*	Austria/LEP 22582	–	+	–
ssp. *lucorum* (Klett & Richter) Hartl	England/LEP 20797	–	–	–
V. cymbalaria Bodard	Spain/LEP 20537	–	–	–
Sect. Chamaedrys				
V. austriaca L. ssp. *austriaca*	Croatia/LEP 25007	–	+	–
	Cultivated/LEP 12260	–	+	–
V. austriaca L. ssp. *teucrium* (L.) D. Webb	Czechoslovakia/LEP 21415	+	–	–
V. prostrata L. ssp. *scheereri* J. P. Brandt	France/LEP 6186	+	–	–
V. filifolia Lipsky	Austria/LEP 24888	+	–	–
V. officinalis L.	Netherlands/LEP 21671	–	+	–
V. aphylla L.	Switzerland/LEP 17802	–	+	–
V. chamaedrys L. ssp. *chamaedrys*	England/LEP 24901	–	–	–
V. chamaedrys L. ssp. *vindobonensis* M. Fischer	Austria/LEP 24890	–	–	–
V. urticifolia Jacq.	Switzerland/LEP 20045	–	–	–
V. peduncularis Bieb.	LEP 24899	+	–	–
V. montana L.	Netherlands/LEP 19548	–	+	–
V. scutellata L.	Austria/LEP 19548	–	–	–

Table 1. *Continued*

Plant species	Place of collection voucher number+	80H	60H	60Me
Sect. *Beccabunga*				
V. beccabunga L.	Switzerland/LEP 20038	–	+	–
V. anagallis-aquatica L.	Netherlands/LEP 20794	–	+	–
V. catenata Pennell	Netherlands/LEP 8522	–	+	–
Tribe Rhinantheae				
Bellardia				
B. trixago (L.) All. = *Bartsia trixago* L.	LEP 21672	–	–	–
Euphrasia				
E. arctica Lange ssp. *borealis* (Townsend) Yeo	LEP 8513	–	–	–
E. officianalis L.	LEP 21462	–	–	–
Odontites				
O. verna (Bellardi) Dumort	LEP 21426	–	–	–
Melampyrum				
M. arvense L.	LEP 13384	–	–	–
M. cristatum L.	LEP 6819	–	–	–
M. nemorosum L.	LEP 17831	–	–	–
M. pratense L.	LEP 27533	–	–	–
Pedicularis				
P. comosa L.	LEP 17365	–	–	–
P. foliosa L.	LEP 10750	–	–	–
Rhinantus				
R. angustifoluis C.C. Gmelin = *R. glaber* Lam.	LEP 14856	–	–	–
Callitrichaceae				
Callitriche				
C. palustris L.	Belgium	–	–	–
C. obtusangula Le Gall.	Spain	–	–	–
C. platycarpa Kütz	France	–	–	–
C. stagnalis Scop.	Spain	–	–	–
Buddlejaceae				
Buddleja				
B. caryopterifolia W.W.Sm.	Cultivated	–	+	–
B. albifolia Hemsley	Crete	–	+	–
B. davidii Franchet	England	–	+	–
Globulariaceae				
Globularia				
G. nudicaulis L.	Spain	–	+	–
G. repens Lam.	Spain	–	+	–
G. meridionalis (Podp.) O. Schwarz	Italy	–	+	–
G. cordifolia L.	Spain	–	+	–
G. alypum L.	Spain	–	+	–
G. spinosa L.	Spain	–	+	–

Table 1. *Continued*

Plant species	Place of collection voucher number⁺	8OH	6OH	6OMe
G. vulgaris L.	Spain	−	+	−
G. punctata Lapeyr.	Spain	−	+	−
Plantaginaceae				
Plantago				
P. nivalis Boiss.	Spain	−	−	−
P. maritima L.	Spain	−	+	−
P. coronopus L.	Spain	−	−	−
P. serraria L.	Spain	−	−	−
P. crassifolia Forskal.	Spain	−	+	−
P. subulata L.	Spain	−	+	−
P. alpina L.	Spain	−	+	−
P. atrata Hoppe	Switzerland	−	−	−
P. lanceolata L.	Spain	−	−	−
P. media L.	Spain	−	−	−
P. major L.	Spain	−	−	+
Lentibulariaceae				
Pinguicula				
P. vulgaris L.	Italy	+	−	−

⁺ Vouchers of the plant samples used for this study are present either in the herbarium of Reading University (unnumbered specimens), or in the herbarium of the Laboratorium voor Experimentele Plantensystematiek, University of Leiden, Holland (LEP numbers). * Plants cultivated in the Plant Science Laboratories glass-house (Reading).[5] Sulphates: 8-hydroxyflavone glycosides (8OH), 6-hydroxyflavone glycosides (6OH), 6-methoxyflavone glycosides (6OMe).

Labiatae

Flavonoids from subfamily Lamioideae: the species included in this subfamily are normally rich in the usual 7-glycosides (including 7-glucuronides) of luteolin, apigenin and chrysoeriol. Some genera produce almost exclusively flavonol 3-glycosides (mainly glycosides of quercetin but also of kaempferol and isorhamnetin): *Lamium*, *Lamiastrum*, *Leonorus* and *Teucrium* (section *Teucrium* only). Other individual species produce flavonols in addition to flavone glycosides, namely *Stachys officianalis*, *S. sylvatica* and *S. glutinosa*, *Marrubium peregrinum*, *Phlomis purpurea* and *Ballota hirsuta*, and several species of *Teucrium* in sections other than *Teucrium* [11]. Flavone *C*-glycosides (in particular, vicenin-2) have been reported in some members of the subfamily Nepetoideae [18]. In this study of members of the subfamily Lamioideae, apigenin *C*-glycosides were found to be generally absent from seven of the 14 genera surveyed (*Marrubium*, *Phlomis*, *Melittis*, *Lamium*, *Lamiastrum*, *Moluccella* and *Sideritis*). In *Stachys* only *S. officinalis* produced *C*-glycosides. On the other hand, several species from the genera *Ajuga*, *Scutellaria*, *Galeopsis*, *Ballota*, *Prasium*, *Leonorus* and the section *Scordium* of *Teucrium* accumulate flavone *C*-glycosides.

The distribution of 6-hydroxy- and 8-hydroxyflavone glycosides within this subfamily is quite different, since these compounds never co-occur. 8-Hydroxyflavone 7-allosylglycosides, in acetylated form, were detected in most *Stachys* species. Only the species from section *Betonica* and *S. sylvatica* and *S. glutinosa* were devoid of these compounds, and it is noteworthy that these species produced flavonol glycosides instead (see above). Acetylated 8-hydroxyflavone allosylglucosides were also detected in two species of *Teucrium* belonging to section *Chamaedrys*, namely *T. chamaedrys* and *T. webbianum* [11]. In *Galeopsis* these compounds were detected in subgenus *Ladanum*, but were absent from the species included in subgenus *Galeopsis* which accumulate 6-hydroxyflavone glycosides instead. *Sideritis* species belonging to subgenus *Sideritis* generally accumulated these compounds (sections *Sideritis* and *Empedoclia* in considerable amounts and section *Hesiodia* in trace amounts or absent). *Sideritis* species from subgenus *Marrubiastrum*, including all the species endemic to the Canary Islands, generally do not produce this type of compound, with the exception of *S. canariensis* and *S. dendrochahorra*. In all these cases, acetylated compounds were detected together with unacetylated compounds. The various *Ajuga* species analysed also contain 8-hydroxyflavone 7-glycosides, but in this case they are different from the 7-allosylglucosides described above, since glucose and glucuronic acid were the sugars detected. Their chromatographic behaviour suggest that these compounds are 7-monoglycosides of hypolaetin, but lack of plant material prevented their full characterization. 6-Hydroxyflavone glycosides are also represented in this subfamily, and these compounds are universally present in all the species studied of the genus *Scutellaria*. In this genus, 7-glucosides and 7-glucuronides of 6-hydroxyluteolin, scutellarein and baicalein were detected. In several species chrysin (5,7-dihydroxyflavone) 7-glycosides were also present. This is the only genus in all the families studied in the present survey, which produce flavonoids with an unsubstituted B-ring. 6-Hydroxyflavone 7-glycosides were also detected in the majority of species of *Phlomis* and *Galeopsis* (section *Galeopsis*) and in several sections of genus *Teucrium* (*Stachybotrys*, *Scorodonia* and *Polium*).

Glycosides of 6-methoxyflavones are nearly completely absent from this subfamily. Only in *Teucrium arduini* the rare compound cirsimaritin 4'-glucoside was detected as well as nepetin and hispidulin glycosides in *Melittis melyssophyllum*.

The presence of *p*-coumaroylglycosides of apigenin, chrysoeriol, luteolin and kaempferol in this subfamily of the Labiatae deserves special comment. These Labiatae compounds are readily detected by their typical UV spectra in methanol with a broad maximum around 315 nm corresponding to the *p*-coumaroyl residue [19], chromatographic behaviour similar to that of flavone aglycones and saponification to produce the glycosides. These compounds have been detected in *Stachys* species (only in section *Betonica* in *S. officinalis* and *S. monieri*), in all the species studied of *Marrubium*, in *Phlomis* and *Ballota* where they have been described before [19, 20], in the species of subgenus *Ladanum* in the genus *Galeopsis*, in *Sideritis* species from subgenus *Marrubiastrum* (*S. macrostachya*, *S. dendrochahorra*, *S. dasygnaphala*, *S. candicans*, *S. argoscephalus* and *S. canariensis*) and in *Sideritis stachydioides*, a primitive member of section *Sideritis*.

Flavonoids in subfamily Nepetoideae: in this subfamily glycosides of the common flavones luteolin, apigenin and chrysoeriol are frequently found. More unusual glycosides of these flavones were found in several genera. Thus, luteolin 5-glycosides were especially frequent in *Thymus* species, and apigenin 4'-glycosides in *Ziziphora hispanica*. *Prunella* species only accumulate flavonol glycosides, but flavonol and flavone glycosides appear to cooccur regularly in *Thymus* species, *Glechoma hederacea*, *Melissa officinalis*, *Horminium pyrenaicum*, *Acinos* species, *Calamintha* species, *Clinopodium vulgare*, *Lavandula latifolia*, *Nepeta nepetella*, *Mentha aquatica*, *Micromeria graeca* and *M. fruticosa* and *Salvia verbenaca* and *S. transylvanica*. The distribution of flavone *C*-glycosides (usually vicenin-2) within the subfamily reveals that these compounds are present universally in *Micromeria* species (with the exception of *M. parviflora*), *Thymus* species, in the species included in section *Salvia* of genus *Salvia* and in section *Majorana* of genus *Origanum*, in *Thymbra capitata*, *Ziziphora hispanica* and *Hyssopus officinalis*. Our results confirm the earlier report of vicenin-2 in most of these species [18]. *Elsholtzia ciliata* was exceptional in generally lacking any of the common flavonoids, and having chalcones present instead.

In this subfamily, no 8-hydroxyflavone glycosides have been detected. By contrast, 6-hydroxyflavone glycosides (mainly 6-hydroxyluteolin glycosides) were universally present in *Thymus* species. These compounds were also detected in 70% of the *Micromeria* species studied, in all the species included in section *Salvia* of genus *Salvia*, in four of the five *Origanum* species studied (with the exception of *O. vulgare*), in *Lavandula multifida* and in *Rosmarinus officinalis* and *R. eriocalix*. The absence of 6-hydroxyflavone glycosides from *Thymbra* capitata, a problematic taxon that has also been classified as *Thymus capitatus* and *Coridothymus capitatus*, supports its inclusion in genus *Thymbra* as has been recently proposed on the basis of its external flavonoid aglycones [21], and coincides with chemical data previously reported [22].

6-Methoxyflavone glycosides (nepetin and hispidulin glycosides) were detected in the three species included in section *Salvia* of the genus *Salvia*, in the two species of *Rosmarinus* analysed and in *Lavandula multifida*.

Verbenaceae

The flavonoid patterns observed in the nine members of the Verbanaceae surveyed show that only *Verbena bonariensis* produces flavonol glycosides in addition to flavone glycosides which are the most common compounds within the family. While 8-hydroxyflavone glycosides were not detected in the family, 6-hydroxyflavone glycosides were present in *Duranta plumeri* and in *Verbena officinalis* (6-hydroxyluteolin and scutellarein) and in *Lippia nodiflora* and *L. canescens* (in these two species as 6-hydroxyluteolin and 6-hydroxychrysoeriol mono- and disulphates [23]). 6-Methoxyflavone glycosides were detected in *Duranta plumeri* and *Lantana camara*, and 6-methoxyflavone sulphates in *Lippia nodiflora* and *L. canescens*.

Scrophulariaceae

The flavonoids found most commonly in species of the Scrophulariaceae were luteolin, chrysoeriol and apigenin 7-glucuronides. In the genera *Verbascum* and *Scrophularia*, glycosides of the 4'-methoxyflavones diosmetin and acacetin appeared to be common as well. 6-Hydroxyflavone (mainly 6-hydroxyluteolin and sometimes scutellarein) glycosides were found in one third of the genera investigated; they were especially common in the tribes Scrophularieae and Veroniceae. They were not detected or rare in the Verbasceae, Antirrhineae and Rhinantheae. 6-Methoxyflavone glycosides were only found in the genus *Linaria*, but according to the literature they also occur in *Digitalis* [24]. 8-Hydroxyflavone glycosides, as acetylated 7-allosylglucosides, had been found so far in this family in 6 species of *Veronica*: *V. agrestis*, *V. opaca*, *V. ceratocarpa*, *V. persica*, *V. filiformis* and *V. austriaca* ssp. *teucrium* [6]. In the present study they were also detected in *V. postrata* ssp. *scheereri*, *V. filifolia* and *V. peduncularis*. Thus within *Veronica* this type of compound seems to be restricted to the sections *Alsinebe* and *Chamaedrys* (see Table 1). With the exception of *Gratiola*, 8-hydroxyflavone glycosides could not be detected in any other genera of the Scrophulariaceae. Four 8-hydroxyflavone glycosides were found in *G. officinalis*, but they were different from those present in *Veronica*. However, *G. officinalis* appears to contain two different flavonoid races. In plants from natural habitats in France, Italy and Switzerland, 8-hydroxyflavones could not be detected, but they contain instead 7-glucuronides of 6-hydroxyluteolin and/or 6-hydroxychrysoeriol, luteolin and chrysoeriol, a flavonoid pattern very similar to that found in the related species, *Gratiola linifolia*. Plants from Greece (from the wild) and from three different botanic gardens contained 8-hydroxyflavone glycosides. Both races of *G.*

officinalis, however, contain flavone *C*-glycosides (probably apigenin 6,8-di-*C*-glycosides) as well.

The phenomenon of two very closely related taxa containing 8-hydroxyflavone glycosides in one instance and 6-hydroxyflavone glycosides in the other, as in *G. officinalis*, was encountered several times. For instance 8-hydroxyflavones were found in *Veronica agrestis*, but only 6-hydroxyflavones in the closely related *V. polita*; 8-hydroxyflavones in *V. austriaca* ssp. *teucrium*, but 6-hydroxyflavones in ssp. *austriaca*. The same applies to presence or absence of 6-hydroxyflavone glycosides: they are present in *V. hederifolia* ssp. *hederifolia*, but absent from ssp. *lucorum*; present in *Parahebe catarractae* ssp. *cataractae* and ssp. *martinii*, but absent from ssp.*diffusa* and the closely related species *P. hookeriana*. In only one species, *V. persica*, both 6-hydroxy- and 8-hydroxyflavone glycosides were found together. The reason for the co-occurrence of these two types of compounds in *V. persica* may be the fact that this is an allopolyploid species. One of the parent species of *V. persica* may have contained only 8-hydroxyflavone glycosides whereas the other parent only 6-hydroxy compounds.

Flavonols (usually quercetin 3-glycosides) were detected in the leaves of *Torenia fournierii*, *Verbascum blattaria*, *Nemesia strumosa*, *Linaria arvensis*, several cultivars of *Hebe*, *Bellardia trixago*, *Euphrasia arctica* and *E. officinalis*.

Callitrichaceae

The flavonoid patterns of the species included in this family were quite simple, and they were constituted mostly of 7-glucuronides of luteolin, apigenin and chrysoeriol. The four species studied showed a very similar flavonoid pattern (Table 1).

Buddlejaceae

The three species studied in this family contain 6-hydroxyflavone glycosides, in addition of apigenin and luteolin glycosides. No 8-hydroxyflavone nor 6-methoxyflavone glycosides were detected.

Globulariaceae

6-Hydroxyflavone glycosides (6-hydroxyluteolin and scutellarein and in some species their methyl ethers) were universally found in this family, in addition to glycosides of luteolin, apigenin and chrysoeriol. In *Globularia repens*, the presence of flavonol glycosides (quercetin and kaempferol) was also detected.

Plantaginaceae

In this family the presence of 6-hydroxyflavone glycosides was not universal; we found these substances in less than 40% of the species studied. The occurrence of glycosides of luteolin, apigenin and chrysoeriol was evidenced in all the different species analysed. *Plantago major* was the only species which contains 6-methoxyflavone glycosides rather than 6-hydroxyflavone glycosides.

Lentibulariaceae

Pinguicula vulgaris contains a complex mixture of glycosides of hypolaetin, isoscutellarein and flavones not oxygenated in the 8-position. The presence of isoscutellarein in *P. vulgaris* has been reported before [25]. Lack of sufficient plant material prevented us from identifying the glycosides completely. The 8-hydroxyflavone glycosides do not seem to be acylated, since they have low R_f-values on PC with 15% acetic acid.

DISCUSSION

The basic flavonoid pattern of the eight families studied appears to be very similar, namely a mixture of luteolin, apigenin and chrysoeriol glycosides (often including 7-*O*-glucuronides). In addition, 6-hydroxyflavone glycosides are present in at least 30% of the genera in each family, except the Callitrichaceae, and the similarity in flavonoid profiles between the families studied reinforces the hypothesis based on the presence of iridoid glycosides that these taxa are phylogenetically closely related [4, 5]. The basic pattern is augmented or replaced in some taxa by a range of more unusual flavonoids, such as glycosides of the common flavones in which the sugars are attached to positions other than the 7-hydroxyl (e.g. 5- and 4'-*O*-glycosides); glycosides of rarer flavones, e.g. diosmetin, acacetin, chrysin, 6-methoxyflavones and 8-hydroxyflavones; acylated glycosides; flavone *C*-glycosides and flavonol *O*-glycosides. Some of these compounds provide useful taxonomic characters below the family level, especially in the Labiatae and Scrophulariaceae (see below).

Since there are incidental reports of the occurrence of acetylated 8-hydroxyflavone allosylglucosides in the Labiatae and Scrophulariaceae [6–11], we expected that this type of compound might be a phyletic marker in this group of families. It seems very unlikely that flavonoids with three unusual features, 8-hydroxylation, presence of an acetyl group and presence of the rare sugar allose, would have evolved separately in two closely related families. Yet this type of substance was only found in subfamily Lamioideae of the Labiatae and in the genera *Gratiola* and *Veronica* of the Scrophulariaceae. 8-Hydroxyflavone glycosides also occur in the Lentibulariaceae, but these are of a different type. They could not be detected in the Verbenaceae, Plantaginaceae, Buddlejaceae, Globulariaceae and Callitrichaceae.

Labiatae

This family has been divided, on the basis of pollen morphology, into two subfamilies namely Lamioideae, mostly with tetracolpate pollen, and Nepetoideae, mainly with hexacolpate pollen [16]. Chemical differences have been reported which support this subfamiliar classification. Thus, subfamily Lamioideae produces iridoid glycosides but does not produce rosmarinic acid, and it is poor in volatile terpenoids, while subfamily Nepetoideae produces rosmarinic acid, does not produce iridoid glycosides and it is generally rich in volatile terpenoids [26 and references therein]. No differences in the flavonoid patterns between these two subfamilies have been described so far.

This study has confirmed that Labiatae species are rich in flavone and 6-hydroxyflavone glycosides [27, 28] which are present in members of both subfamilies. The most important difference in flavonoids we found between the two subfamilies is the fact that 8-hydroxyflavone glycosides and *p*-coumaroylglycosides only occur in subfamily Lamioideae and not in subfamily Nepetoideae.

The flavonoid patterns observed in the Labiatae coincide with subgeneric and sectional classifications as a general rule, as was described previously for *Teucrium* [11]. Thus, species belonging to subgenus *Ladanum* of genus *Galeopsis* accumulate 8-hydroxyflavone glycosides and flavone *p*-coumaroylglycosides, while those belonging to subgenus *Galeopsis* generally accumulate 6-hydroxyflavone glycosides; subgenus *Marrubistrum* of genus *Sideritis* generally contains *p*-coumaroylglycosides while subgenus *Sideritis* contains 8-hydroxyflavone glycosides; in genus *Stachys*, section *Betonica* does not produce 8-hydroxyflavone glycosides which are common in the remainder of the sections, and this suggests that *S. sylvatica* and *S. glutinosa* which are devoid of these compounds are misplaced in sections *Stachys* and *Olisia* respectively. It is interesting that the presence of flavone *p*-coumaroylglycosides brings together the related genera *Sideritis* (subgen. *Marrubiastrum*), *Stachys* (section Betonica), *Marrubium*, *Phlomis*, *Ballota* and *Galeopsis* (Subgen. *Ladanum*). In subfamily Nepetoideae similar correlations have been found. Thus, the presence of 6-hydroxy- and 6-methoxyflavone glycosides clearly differentiates section *Salvia* from the rest of species analysed of this genus, and the absence of 6-hydroxyflavone glycosides, which are universally present in *Thymus* capitata, supports the separation of this taxon from the genus *Thymus* and its inclusion in the genus *Thymbra*, in accordance with previous studies [2].

Verbenaceae

This is a family which is supposed to be closely related to subfamily Lamioideae of the Labiatae having the same type of pollen and containing iridoids, and in one taxonomic treatment the Verbenaceae have been included in a single group together with the Lamioideae [29]. Thus we expected this family to contain 8-hydroxyflavone glycosides as well. However, they could not be detected in the species investigated, but the general flavonoid pattern was very similar to that of the Labiatae. Most species showed the common flavone glycosides, and 44% of the species investigated contained 6-hydroxyflavone glycosides. 6-Methoxyflavone glycosides were more common than in the Labiatae, however, and noteworthy is the presence of 6-hydroxy and 6-methoxyflavone sulphates in two species of *Lippia*.

Scrophulariaceae

The division of the family into the subfamilies Scrophularioideae and Rhinanthoideae is based on one single key character, the aestivation of the corolla lobes, and dates back to the 19th century [30]. Thus they are not necessarily natural groups. This is reflected in their chemistry; the subfamilies are chemically very heterogeneous, both as regards to their iridoid constituents [31], as to their flavonoid patterns (present results). On the other hand, the two tribes making up subfamily Rhinanthoideae, the Digitaleae and the Rhinantheae, can clearly be distinguished on their flavonoid profiles. The Rhinantheae completely lacks 6-hydroxyflavone glycosides, whereas they are very common in the Digitaleae. This distinction agrees with the classification proposed by Bellini [32] who classified the Rhinantheae as a separate family, the Rhinanthaceae, on the basis that all its species are parasitic or semiparasitic and because of the special developmental characteristics of the nectaries. All other Scrophulariaceae are non-parasitic and their nectaries develop in a different way.

The distribution of flavonoids in many species of the genera *Veronica* and *Parahebe* were reported before [2,6]. However, several species of *Veronica* were investigated for the first time in the present work, and the results give us a clearer insight in the occurrence of 8-hydroxyflavone glycosides in the genus. It was confirmed that this type of compound only occurs in subsection *Agrestis* of section *Alsinebe*, and in only those subsections of section *Chamaedrys* which have a basic chromosome number $x = 8$ (in the remaining subsections the number is $x = 9$, and the species in these subsections also have different iridoid patterns and seed morphology than the 8-hydroxyflavone-containing species). This means that presence of 8-hydroxyflavone glycosides provides further evidence that section *Chamaedrys* should be split up into two separate groups. Thus this type of glycoside provides an interesting chemotaxonomic character in *Veronica*.

It is strange that these compounds could only be found in one more genus of the Scrophulariaceae, *Gratiola*, and then in only certain populations of one species. Morphologically, *Gratiola* and *Veronica* are not very closely related and they have always been placed in different tribes and subfamilies of the Scrophulariaceae. *Gratiola* is supposed to be a primitive member of the family and *Veronica* an advanced one. Their other chemical constituents are completely different, *Veronica* being rich in iridoid glycosides [33], whereas *Gratiola* lacks these [31] but contains triterpene glycosides instead [34]. Moreover, the 8-hydroxyflavone glycosides in *Gratiola* only contain glucose as a sugar and no allose, in contrast to those in *Veronica*, so the occurrence of 8-hydroxyflavones in these two genera may be a case of parallel chemical evolution.

In both chemical races of *Gratiola officinalis* we found flavone *C*-glycosides, and this confirms the report by Russian authors [35, 36] that this type of flavonoid is present in the species. The same authors probably also detected the 8-hydroflavone glycosides in *G. officinalis*, but they reported them as scutellarein derivatives, lignoside and isolignoside [37, 38].

6-Methoxyflavone glycosides seem to occur only very sporadically in the Scrophulariaceae. They were only found in species of *Linaria* in the present survey. They had been found before in this genus, and in species of *Digitalis* [34]. Flavonol glycosides occur more frequently in the leaves of of Scrophulariaceae, although they are far less common than flavone glycosides. They seem to be especially characteristic of the genera *Euphrasia* and *Hebe*, and they may provide useful taxonomic characters in some of the genera.

Other families

Within each of the families Callitrichaceae, Buddlejaceae, Globulariaceae and Plantaginaceae, the flavonoid patterns seem to be very homogeneous, and they are very similar to those of certain groups within the Scrophulariaceae, Labiatae or Verbenaceae. This shows the close phylogenetic relationship between these families. However, there are certain differences between them as well to distinguish the smaller families from each other, such as lack of 6-hydroxyflavone glycosides in the Callitrichaceae. The one species investigated for the Lentibulariaceae, *Pinguicula vulgaris*, shows an aberrant flavonoid pattern. It accumulates various 8-hydroxyflavone glycosides of a different type from those found in the Labiatae and Scrophulariaceae. However, glycosides of the common flavones are also present in this species, which links it to the other families. It would be interesting to find out whether other species in this family also accumulate glycosides of 8-hydroxyflavones, or those of 6-hydroxyflavones instead.

EXPERIMENTAL

Plant material. Vouchers of the plant samples used for this study are present either in the herbarium of Reading University, England, or in the herbarium of the Laboratorium voor Experimentele Plantensystematiek (LEP), University of Leiden, Holland.

Extraction and identification of flavonoids. Dried leaves were extracted with MeOH–H_2O (4:1) and the extracts 2D paper-chromatographed with *n*-BuOH–HOAc–H_2O (4:1:5, upper phase) and 15% HOAc. The different spots were visualized under UV light (366 nm; 8-hydroxyflavone glycosides give strong yellow spots in daylight), eluted with MeOH–H_2O (4:1) and their UV spectra recorded using a microcell (*ca* 1 ml). Identification of the different compounds was achieved as reported previously [6–11] and by chromatographic comparisons against authentic markers.

Acknowledgements—The authors are grateful to the University of Reading, U.K. for use of laboratory facilities, and to the herbaria of Reading University and of the Lab. voor Experimentele Plantensystematiek, University of Leiden, for providing the plant material. FTB is indebted to the Spanish CSIC for a fellowship.

REFERENCES

1. Harborne, J. B. and Williams, C. A. (1971) *Phytochemistry* **10**, 367.
2. Grayer-Barkmeijer, R. J. (1978) *Biochem. Syst. Ecol.* **6**, 131.
3. Engler, A. (1964) *Syllabus der Pflanzenfamilien* Vol. II, 12th Edn. (H. Melchior, ed.). Borntrager, Berlin.
4. Jensen, S. R., Nielsen, B. J. and Dahlgren, R. (1975) *Bot. Notiser* **128**, 148.
5. Hegnauer, R. and Kooiman, P. (1978) *Planta Med.* **33**, 1.
6. Grayer-Barkmeijer, R. J. (1979) *Chemosystematic investigations in Veronica L.* (Scrophulariaceae) *and related genera.* Diss. Univ. Leiden.
7. Chari, V. M., Grayer-Barmeijer, R. J., Harborne, J. B. and Österdahl, B. G. (1981) *Phytochemistry* **20**, 1977.
8. Lenherr, A. (1983) *Biosystematische und Chemotaxonomische Untersuchungen in der Artengruppe Stachys recta L.* Diss. ETH Zürich nr. 7453.
9. Lenherr, A., Lahloub, M. F. and Sticher, O. (1984) *Phytochemistry* **23**, 2343.
10. Barberán, F. A. T., Tomás, F. and Ferreres, F. (1984) *Phytochemistry* **23**, 2112.
11. Harborne, J. B., Tomás-Barberán, F. A., Williams, C. A. and Gil, M. I. (1986) *Phytochemistry* **25**, 2811.
12. Barberán, F. A. T., Tomás, F. and Ferreres, F. (1985) *Tetrahedron* **41**, 5733.
13. Sheremet, I. P. and Kommissarenko, N. F. (1971) *Khim. Prir. Soedin.* **7**, 583.
14. Sheremet, I. P. and Kommissarenko, N. F. (1971) *Khim. Prir. Soedin.* **7**, 721.
15. Harborne, J. B. (1977) *Phytochemical Methods.* Chapman & Hall, London.
16. Erdtman, G. (1945) *Svensk. Bot. Tidskr.* **39**, 279.
17. Römpr, H. (1928) *Repert. Spec. Nov. Regn. Veg.* Beih. **50**, 1.
18. Husain, S. Z. and Markham, K. R. (1981) *Phytochemistry* **20**, 1171.
19. Tomás, F., Nieto, J. L., Barberán, F. A. T. and Ferreres, F. (1986) *Phytochemistry* **25**, 1253.
20. Ferreres, F., Tomás-Barberán, F. A. and Tomás-Lorente, F. (1986) *J. Nat. Prod.* **49**, 554.
21. Barberán, F. A. T., Hernández, L. and Tomás, F. (1986) *Phytochemistry* **25**, 561.
22. Adzet, T. and Martinez, F. (1981) *Biochem. Syst. Ecol.* **9**, 293.
23. Tomás-Barberán, F. A., Harborne, J. B. and Self, R. (1987) *Phytochemistry* **26**, 2281.
24. Doherty, G. O. P., Haynes, N. B. and Whalley, W. B. (1963) *J. Chem. Soc.* 5577.
25. Jay, M. and Gonnet, J. F. (1973) *Phytochemistry* **12**, 953.
26. Cantino, P. D. and Sanders, R. W. (1986) *Syst. Bot.* **11**, 163.
27. Harborne, J. B. (1967) *Comparative Biochemistry of Flavonoids.* Academic Press, London.
28. Hegnauer, R. (1966) *Chemotaxonomie der Pflanzen* Vol. IV. Birkhauser, Basel.
29. El-Gazzer, A. and Watson, L. (1970) *New Phytol.* **69**, 451.
30. Thieret. J. W. (1967) *Sida* **3**, 87.
31. Kooiman, P. (1970) *Acta Botanica Neerlandica* **19**, 329.
32. Bellini, R. (1907) *Anali di Botanica* **6**, 131.
33. Grayer-Barkmeijer, R. J. (1973) *Biochem. Syst.* **1**, 101.
34. Hegnauer, R. (1973) *Chemotaxonomie der Pflanzen* Vol. VI. Birkhauser, Basel.
35. Litvinenko, V. I., Borodin, L. I. and Kurinnaya, N. V. (1969) *Khim. Prir. Soedin.* **5**, 328.
36. Borodin, L. I., Litvinenko, V. I. and Kurinnaya, N. V. (1970) *Khim. Prir. Soedin*, **6**, 19.
37. Borodin, L. I., Litvinenko, V. I. and Kurinnaya, N. V. (1969) *Khim. Prir. Soedin.* **5**, 437.
38. Borodin, L. I., Litvinenko, V. I. and Kurinnaya, N. V. (1970) *Farm. Zh. (Kiev)* **25**, 62.