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FLAVONOID GLYCOSIDES AND SULPHATES FROM THE DILLENIACEAE

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Abstract—In a leaf survey of sixty species from eight genera of the Dilleniaceae, the following flavonoids were characterized: myricetin 3,7,3′,4′-tetramethyl ether, mearnsetin 3-rhamnoside, ombuin 3,3′-disulphate, isorhamnetin 3,7,4′-trisulphate, kaempferol 3,7,4′-trisulphate and apigenin 7-galactosidesulphate.

INTRODUCTION

The Dilleniaceae are a family of tropical trees, shrubs, climbers and herbs. Interest in its taxonomy led to an earlier survey of many species for leaf flavonoid aglycones [1]. Flavonoids previously characterized in the family include rhamnocitrin, the 5-methyl ethers of quercetin and kaempferol [2], and kaempferide [1, 3]. The latter substance has since been shown to be present *in vivo* as the corresponding dihydroflavonol [4]. The two subfamilies Tetraceroideae and Dillenioideae are characterized by the frequent occurrence of flavonoids with methylation at the 7 and 4′ positions [1]. However, in a recent taxonomic treatment of the Dilleniaceae genera (K. Kubitzki, unpublished results), it was shown that the distribution of characters of taxonomic relevance within the family is so reticulate that it does not permit the definition of taxa at tribal or subfamily level. This situation led to a re-investigation of the flavonoids of the Dilleniaceae, extending the analysis to include glycosides which previously had not been studied [5].

RESULTS AND DISCUSSION

A total of 248 samples covering 60 species and 8 genera was investigated. Especially well represented are the neotropical genera *Davilla* and *Doliocarpus* and the pantropical genus *Tetracera* of which a large proportion of species were studied, together with the monotypic New World genera *Curatella* and *Pinzona*. Three species of *Schumacheria*, some *Dillenia* species and one *Acrotrema* species, all from Ceylon, were also included. Material of the predominantly Australian genus *Hibbertia* was available but was not studied because of the absence of a modern and comprehensive taxonomic framework such as is available for the rest of the family.

Here we report only the isolation of some new and interesting substances while a systematic evaluation of the data will be given elsewhere [6]. The compounds include flavonoid glycosides, glucuronides and sulphates and one *O*-methylated aglycone.

Myricetin 3,7,3′,4′-tetramethyl ether, previously identified in *Cistus monspeliensis* [7], was found in *Doliocarpus amazonicus* subsp. *duckeanus*, a woody climber of central Amazonia. Although myricetin occurs frequently in the Dilleniaceae, we were unable to detect the tetramethyl ether in any other species of the family. Another unusual flavonol, mearnsetin 3-rhamnoside, hitherto known from *Acacia mearnsii* [8] and *Dorycnium suffruticosum* [9] was identified in only one species, *Doliocarpus spraguei*, where it co-occurs with quercetin and myricetin 3-rhamnosides. Rhamnocitrin 3-glucuronide was characterized in the two African species *Tetracera rosiflora* and *T. rutenbergii*. It has previously only been recorded from *Verbesina myricephala* [10].

The occurrence in plants of common flavones and flavonols in a conjugated form, covalently linked to inorganic sulphate, is a relatively recent discovery [11] but now well documented [12]. Up to the present, flavonoid sulphates were unknown from the Dilleniaceae, but in the course of this study eleven sulphates have been found in the family. These include the 3-sulphates of quercetin and kaempferol, the 7-sulphates of apigenin and luteolin, myricetin 3-rhamnoside sulphate, ombuin disulphate, isorhamnetin 3,7,4′-trisulphate, kaempferol 3,7-disulphate, kaempferol 3,7,4′-trisulphate, rhamnocitin 3-sulphate and apigenin 7-galactosidesulphate. Details of new or rare compounds are given in Tables 1 and 2. Myricetin rhamnosidesulphate has been found previously in *Davidsonia pruriens* [13], while kaempferol 3,7-disulphate is known from *Reaumuria mucronata* [14] and rhamnocitrin 3-sulphate from *Ammi visnaga* [15]. Ombuin disulphate, isorhamnetin 3,7,4′-trisulphate, kaempferol 3,7,4′-trisulphate and apigenin 7-galactosidesulphate are new substances.

A close association between *O*-methylation and sulphate formation has been noted previously [11], and it is therefore not surprising to find among the flavonoid sulphates of the Dilleniaceae three derivatives of *O*-methylated aglycones. Moreover, when considering the occurrence of ellagic acid, flavonoid methyl ethers and

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Table 1. Chromatographic and electrophoretic values and botanical source of flavonoid sulphates

Compound No.	R_f (×100) in BAW	HOAc (15%)	H_2O	Mobility*	Botanical source	Flavonoid sulphate
1	31	62	89	0.66	*Davilla macrocarpa, D. flexuosa*	Myricetin 3-rhamnosidesulphate
2	37	50	78	1.91	*Acrotrema uniflorum*	Ombuin disulphate
3	2	80	94	2.34	*Acrotrema uniflorum*	Isorhamnetin 3,7,4′-trisulphate
4	11	72	94	2.17	*Schumacheria castaneifolia, Dillenia bracteata*	Kaempferol 3,7-disulphate
5	2	84	94	2.38	*Acrotrema uniflorum*	Kaempferol 3,7,4′-trisulphate
6	42	60	82	1.26	*Tetracera poggei, T. rosiflora, T. rutenbergii, T. alnifolia*	Rhamnocitrin 3-sulphate
7	33	23	37	Not measured	*Tetracera stuhlmanniana*	Apigenin 7-galactosidesulphate

* Mobility relative to quercetin 3-sulphate, run on paper Schleicher & Schüll No. 2316 in formate–acetate buffer, pH 2.2 at 40 V/cm for 1 hr.

Table 2. UV spectra of flavonoid sulphates

Compound No.*	MeOH		NaOMe		NaOAc		$NaOAc/H_3BO_3$		$AlCl_3$		$AlCl_3$/HCl	
	Band II	Band I	Band II	Band I	Band II	Band I	Band II	Band I	Band II	Band I	Band II	Band I
1	263	365	dec	dec	270	400	270	368	274	426	278	400
2	255	353	266	398	259	356	260	356	268	400	268	400
3	254	350	268	376	260	355	254	352	275	400	274	396
4	266	350	276	394	270	352	270	353	277	356	277	350
5	250 270	344	280	394	270	344	270	344	278	400	278	400
6	266	365	275	380	274	375	270	356	275	400	275	400
7	266	330	270	384	266	390	265	336	276	380	275	380

* Numbering of substances as in Table 1.

flavonoid sulphates there are some similarities between the Dilleniaceae, Cistaceae, Guttiferae, Bixaceae and other families [16] which may be of some systematic significance.

EXPERIMENTAL

Plant material. This study is based mainly on air-dried material collected by the third author in South America and Ceylon. All determinations were checked and vouchers were deposited at HBG and M. The study was supplemented by the investigation of herbarium material obtained from the herbaria at BR, M, NY, U, US. A complete list of the plant material studied is available on request from the third author. In six cases, extracts from leaves preserved in EtOH were compared with those from air-dried leaves but no difference was noticed.

Flavonoid identifications. Plant material was extracted with boiling H_2O, evapd under red. pres., dissolved in MeOH and the extracts were analysed using standard procedures.

Myricetin 3,7,3',4'-*tetramethyl ether* has λ_{max}^{MeOH} nm: 265, 343; +NaOMe 270, 370 (stable): +NaOAc 267, 344; +$AlCl_3$ 277, 398; R_f in BAW 0.93; in $CHCl_3$–HOAc (2:1, H_2O-saturated) 0.99 and in Forestal 0.90. MS showed a molecular ion *m/z* 374 corresponding to a tetramethylated myricetin derivative. UV data showed that only the 5- and 5'-hydroxyls were free.

Mearnsetin 3-*rhamnoside* has λ_{max}^{MeOH} nm: 264, 352; +NaOMe 280, 398 (stable): +NaOAc 276, 380: R_f in BAW 0.90, in HOAc 15% 0.68, in H_2O 0.28. Acid hydrolysis yielded mearnsetin and rhamnose. Mearnsetin had λ_{max}^{MeOH} nm: 264, 366; +NaOMe 280, 414 (stable); +NaOAc 276, 380; R_f in BAW 0.74, in $CHCl_3$–HOAc 0.23 and in Forestal 0.40. This chromatographic behaviour is indicative of a derivative of myricetin which was confirmed by MS, *m/z* (rel. int.): M 332 (100), M − Me 317 (97), M − Me − CO 289 (25), ion B 167 (4), ion A 153 (11). UV shows that only the 4' hydroxyl is blocked.

Rhamnocitrin 3-*glucuronide* has λ_{max}^{MeOH} nm: 266, 352; +NaOMe 270, 394 (stable); +NaOAc 267, 363. R_f in BAW 0.37, in HOAc 15% 0.55 and in H_2O 0.80. Hydrolysis yielded rhamnocitrin and glucuronic acid. Rhamnocitrin had λ_{max}^{MeOH} nm: 266, 366; +NaOMe 267, 425 (decomposing); +NaOAc 266, 385. R_f in BAW 0.74, in $CHCl_3$–HOAc 0.73 and in Forestal 0.53. MS of the aglycone, *m/z* (rel. int.): M 300 (100), M − H 299 (54), M − Me 285 (2), M − Me − CO 257 (38), ion A 167 (9), ion B 121 (66) confirmed the presence of kaempferol 7-*O*-methyl ether. UV of the glucuronide indicated an additional blocking of the hydroxyl in the 3-position.

Flavonoid sulphates. These compounds showed characteristic arrow-shaped spots on the chromatograms, and hydrolysis gave aglycone, sulphate and potassium ions plus a sugar in the case of sulphated glycosides. The presence of sulphate was confirmed by precipitation with $BaCl_2$. Known sulphates were identified by acid hydrolysis to aglycone and sulphate, UV analysis and by comparison with an authentic sample when available.

Myricetin 3-*rhamnosidesulphate.* UV showed that only the 3-hydroxyl was blocked. Hydrolysis yielded myricetin, rhamnose and sulphate. Since only the 3-hydroxyl is blocked, the sulphate must be linked to the sugar moiety.

Ombuin disulphate on acid hydrolysis gave ombuin, which had λ_{max}^{MeOH} nm: 252, 370; +NaOMe 252, 432 (stable); +NaOAc 252, 354. R_f in BAW 0.94, in $CHCl_3$–HOAc 0.98 and in Forestal 0.87. MS of the aglycone, *m/z* (rel. int.): M 330 (100), M − H 329 (16), M − Me 315 (6), M − Me − CO 287 (13), ion A 167 (7), ion B 151 (12). In comparison with the aglycone, UV of the sulphate indicated that the 3-position was blocked and since the electrophoretic mobility of this substance is higher than that of a monosulphate, a second sulphate is probably present attached either to the first or at the 3'-position.

Isorhamnetin 3,7,4'-*trisulphate* gave isorhamnetin on acid hydrolysis. The UV spectrum of the sulphate showed blocking of hydroxyls in the 3-, 7- and 4'-positions.

Kaempferol 3,7,4'-*trisulphate* also yielded kaempferol and sulphate ions on acid hydrolysis but the UV spectrum showed blocking of hydroxyls in the 3-, 7- and 4'- positions.

Apigenin 7-*galactosidesulphate.* After hydrolysis, apigenin, galactose and sulphate ions were detected. UV showed that only the 7-hydroxyl was blocked indicating that the sulphate is attached to the sugar moiety at this position.

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