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REVIEW

FLAVONOID SULPHATES: A NEW CLASS OF SULPHUR COMPOUNDS IN HIGHER PLANTS

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Abstract—The chemistry of the 36 flavone and flavonol sulphates so far detected in higher plants is summarized. Their distribution in over 160 species from nine dicotyledonous and seven monocotyledonous families is outlined. Methods of detection and identification are described. The possible function of these novel sulphur compounds in relation to their frequent occurrence in water plants is discussed.

INTRODUCTION

The recent discovery that many of the common flavones and flavonols occur relatively widely in plants in a novel conjugated form, covalently linked to inorganic bisulphate,* has opened up a new field in the study of the biochemistry of flavonoid pigments. At the same time, these compounds add significantly to the number of organic sulphur compounds known in higher plants, of which relatively few have so far been described. Indeed, in the case of primary metabolites, only a handful of sulphur compounds are known, i.e. the two protein amino acids, cysteine and methionine, and their derivatives and such compounds as choline sulphate, glutathione and the sulphonated sugar, sulphoquinovose, a component of plant lipids. Sulphated polysaccharides, which are important storage carbohydrates in algae, should also be mentioned. In the case of secondary constituents, there are only three classes where a significant number of structures have been reported: the glucosinolates or mustard oil glycosides, of which over 70 are known [1], the aliphatic sulphides [2] and the thiophenes of the Compositae [3]. There are also a few individual substances containing sulphur among other groups of secondary constituent. Some sulphur based non-protein amino acids have been described [4] and sulphur-containing alkaloids occur in the genus *Nuphar* (Nympheaceae) [5]. In addition, some diterpene sulphoxides have been found in *Podocarpus* (Podocarpaceae) [6] and a methylthionaphthalene has recently been described from *Micrandropsis scleroxylon* (Euphorbiaceae) [7].

To claim flavonoid sulphates as a new class of sulphur compound is an exaggeration in that the first member of the group was reported as long ago as 1937; this was the flavonol persicarin, isorhamnetin 3-sulphate, in *Polygonum hydropiper* [8]. However, previous to the announcement in 1971 of the widespread occurrence of flavone sulphates in the family Palmae [9], there were only three other reports of flavonol sulphates in plants, in *Tamarix laxa* [10], in *Oenanthe stolonifera* [11] and in two *Lasthenia* spp. [12]. Indeed, in reporting patuletin sulphates in *Lasthenia*, Bohm *et al.* [12] wrote "flavonoid bisulphates are amongst

* The term "bisulphate" has been used in the past for flavonols substituted on a hydroxyl with the SO_3^- ion to indicate their formal derivation from hydrogen sulphate HSO_4^- by the splitting out of a molecule of H_2O. Since workers with other types of organic compound similarly substituted tend to use "ethereal sulphate" or simply "sulphate", we propose that the prefix "bi" should be dropped and that the term "flavonoid sulphate" be accepted. A more formal designation would seem to be *O*-monosulphate for monosubstituted compounds and *O*-disulphate, *O*-trisulphate, etc. for polysubstituted compounds.

the rarest of naturally occurring flavonoid". So unregarded has been the occurrence of persicarin that there was no mention of flavonoid sulphates in a recent comprehensive review of sulphur compounds in plants [2].

The discovery of a range of flavone and flavonol sulphates in the Palmae [9, 13, 14] led us to develop an electrophoretic technique (see p. 1153) for surveying plants for sulphates and investigate other related monocotyledonous families for these substances and, as a result, new sources and new derivatives were found, especially in *Saccharum* and some related grasses [15]. In re-examining an earlier report of unidentified flavonoid sulphates in *Zostera*, we examined plants of the Fluviales and again found some new derivatives [16]. We have also examined their occurrences in the dicotyledons, and have found them in several Umbelliferae [17], in many plants of the order Parietales, to which Tamaricaceae belongs [18], and a new source of them in the Rosaceae [19].

As a result of these surveys, it now appears that flavonoid sulphates are not rare curiosities but that they occur in a considerable number of plant families in significant amount. Indeed, up to the present, 36 compounds have been detected variously in over 160 species from 16 plant families. No doubt, further surveys will indicate an even wider distribution; it may even be that the ability to conjugate flavonoids as sulphates is a universal feature of sulphur metabolism amongst the angiosperms.

The present review is devoted to the natural distribution of these flavonoid sulphates, their structural variation, methods of detection and characterization. Their possible function, in relation to their predominant occurrences in plants which grow in either fresh or salt water habitats, will also be discussed.

STRUCTURAL VARIATION

The 15 known flavonol sulphates are listed in Table 1 and the 21 known flavone sulphates in Table 2. Seven flavonol aglycones have so far been found as sulphate conjugates. It is interesting that five of these are methylated derivatives, suggesting that there may be an association between *O*-methylation and sulphate formation. The two commonest flavonol sulphates are undoubtedly persicarin or isorhamnetin 3-sulphate,

Table 1. The known flavonol sulphates

Compound	First reported source*	Reference
Quercetin		
3-sulphate	*Oenanthe crocata*	[17]
3,7,3',4'-tetrasulphate	*Flaveria bidentis*	[20]
7-sulphate-3-glucuronide	*Frankenia pulverulenta*	[18]
3-rutinosidesulphate	*Arecastrum romanzoffianum*	[13]
Isorhamnetin		
3-sulphate (persicarin)	*Polygonum hydropiper*	[8]
7-sulphate	*Frankenia pulverulenta*	[18]
7-sulphate-3-glucuronide		
3-rutinosidesulphate	*Arecastrum romanzoffianum*	[13]
Kaempferol		
7-sulphate	*Frankenia pulverulenta*	[18]
7-sulphate-3-glucuronide		
Patuletin		
7-sulphate	*Lasthenia conjugens* and	
7-sulphate-3-glucoside	*L. fremontii*	[12]
Rhamnetin		
3,5,4'-trisulphate-3'-glucuronide	*Tamarix aphylla*	[21]
Rhamnazin		
3-sulphate	*Polygonum hydropiper*	[22]
Tamarixetin		
3-sulphate	*Tamarix laxa*	[10]

* Sulphates have almost invariably been isolated from leaves; for families to which these species belong, see Table 3.

Table 2. The known flavone sulphates

Compound	First Reported Source	Reference
Apigenin		
7-sulphate	*Bixa orellana*	[18]
7-glucosidesulphate	*Phoenix canariensis*	[14]
Luteolin		
7-sulphate	*Bixa orellana*	[18]
4′-sulphate	*Daucus carota*	[17]
7,3′-disulphate	*Zostera marina*	[16]
7-sulphate-3′-glucoside	*Mascarena verschafeltii*	[13]
7-sulphate-3′-rutinoside		
7-glucosidesulphate	*Phoenix roebelenii*	[13]
7-rutinosidesulphate		
Diosmetin		
7-sulphate	*Zostera marina, Z. nana*	[16]
Chrysoeriol		
7-sulphate	*Zostera marina*	[16]
7-glucosidesulphate	*Juncus effusus*	[23]
Tricin		
7-glucosidesulphate	*Saccharum officinale*	[15]
(2 forms)	*Phoenix roebelenii*	[13]
Hypolaetin		
8-sulphate	*Bixa orellana*	[18]
Vitexin		
7-sulphate	*Washingtonia robusta*	[13]
7-glucosidesulphate		
7-rutinosidesulphate		
Isovitexin		
7-sulphate	*Phoenix roebelenii*	[13]
Orientin		
7-sulphate	*Phoenix roebelenii*	[13]
7-glucosidesulphate	*Arecastrum romanzoffianum*	[13]
Iso-orientin		
7-sulphate	*Phoenix roebelenii*	[13]

the first of these compounds to be isolated, and the corresponding quercetin analogue; the pair co-occur in several plants, e.g. in *Oenanthe* species and in *Helianthemum squamatum*.

All but two of the flavonol sulphates listed are monosulphates, the exceptions being the tetrasulphate of quercetin found in *Flaveria bidentis* [20] and the trisulphate of rhamnetin 3′-glucuronide from *Tamarix aphylla* [21]. However, other flavonol polysulphates have been provisionally detected in plants, especially in the Tamaricaceae, and are being actively investigated at the present time [18]. The position of sulphate attachment in all but the above two examples, is either at the 7- or the 3-hydroxyl. There are, however two sulphates with glycosidic attachment, where the sulphate is linked through the sugar moiety; these are quercetin and isorhamnetin 3-rutinoside-sulphates from *Arecastrum*. Presumably, the sulphate is linked here to the 6-position of the glucose, by analogy with the polysaccharide sulphates of the algae which are predominantly of this type [25], but this has yet to be proved.

Most of the 21 known flavone sulphates (Table 2) are based on apigenin or luteolin or a related compound. The simplest are the 7-sulphates and such derivatives of apigenin, luteolin, diosmetin (luteolin 4′-methyl ether) and chrysoeriol (luteolin 3′-methyl ether) are all known. All four co-occur, for example, in eel-grass, *Zostera marina*. The only flavone disulphate so far described, luteolin 7, 3′-disulphate, also occurs in *Zostera*. Two sulphates with a more unusual position of linkage are hypolaetin (8-hydroxyluteolin) 8-sulphate present in *Bixa* and luteolin 4′-sulphate, which is present in *Daucus carota* along with the 7-sulphate.

Although the first flavone sulphate to be identified was luteolin 7-sulphate-3′-glucoside in *Mascarena* [9], other examples of flavones with separate sugar and sulphate attachments are rare; there is only one so far, the related 7-sulphate-3′-rutino-

Table 3. Natural distribution of flavonoid sulphates

Family	Genera and species	References
Dicotyledoneae		
Rosaceae	*Acaena* (5), *Dendriopoterium menendezii*, *Potentilla* (3), *Sanguisorba minor*, *S. officinalis**	[19]
Polygonaceae	*Polygonum hydropiper* var. *vulgare*, *P. perfoliatum*, *P. thunbergii*	[8, 19, 22]
Bixaceae	*Bixa orellana*	[18]
Frankeniaceae	*Frankenia corymbosa*, *F. ericifolia*, *F. laevis*, *F. pulverulenta*, *F. thymifolia*	[18]
Tamaricaceae	*Tamarix africana*, *T. aphylla*, *T. canariensis*, *T. gallica*, *T. hispida*, *T. pentandra*, *T. laxa*, *T. smyrnensis*, *Myricaria germanica*	[10, 18, 21]
Guttiferae	*Hypericum coadnatum*, *H. elodes*, *H. grandiflorum*	[18]
Cistaceae	*Helianthemum squamatum*	[18]
Umbelliferae	*Ammi visnaga*, *Daucus carota**, *Oenanthe* (6)	[11, 17]
Compositae	*Flaveria tridentis*, *Lasthenia fremontii*, *L. conjugens*	[12, 20]
Monocotyledoneae		
Gramineae	*Bothriochloa* (5), *Chionochloa* (2), *Chloris* (1), *Cynodon* (2), *Cortaderia* (4), *Dimeria* (1), *Eragrostis* (1), *Erianthus* (1), *Gymnopogon* (1), *Gynerium* (1), *Heptachloa* (1), *Notodanthonia* (1), *Panicum* (2), *Pennisetum* (1), *Saccharum* (5), *Schima* (1), *Setaria* (1), *Spartina* (1), *Tripsacum* (1), *Zea mays*.	[23]
Juncaceae	*Juncus* (11), *Marsippospermum grandiflora*, *Rostkovia magellanica*, *Prionium serratum*	[23]
Liliaceae	*Bellevalia flexuosa*, *Lachenalia unifolia*	[19, 23]
Palmae	*Areca* (1), *Arecastrum* (2), *Bactris* (1), *Butia* (5), *Calamus* (1), *Clinostigma* (1), *Cocothrinax* (1), *Copernicia* (1), *Cyrtostachys* (1), *Daemonorops* (1), *Gaussia* (1), *Gigliolia* (1), *Iguanura* (1), *Jubaea* (1), *Kothalsia* (1), *Licuala* (1), *Linospadix* (1), *Livistona* (4), *Mascarena* (3), *Mauritia* (1), *Microcoelum* (1), *Nannorhops* (1), *Neodypsis* (1), *Nypa* (1), *Opsiandra* (1), *Phoenix* (10), *Pritchardia* (2), *Ptychosperma* (2), *Rhapis* (2), *Rhopalostylis* (1), *Rhopaloblaste* (1), *Sabal* (2), *Thrinax* (1), *Trithrinax* (1), *Veitchia* (1) and *Washingtonia* (2).	[13, 14]
Restionaceae	*Hypolaena fastigiata*	[19]
Zannichelliaceae	*Zannichellia palustris*	[16]
Zosteraceae	*Zostera angustifolia*, *Z. marina*, *Z. nana*	[16, 24]

Note: the figure in brackets indicates the number of positive species in any given genus.
* This indicates that sulphates are variably present in these plants.

side from *Opsiandra maya*. More common are flavones with *O*-glycosidically linked sulphates, e.g. luteolin 7-glucosidesulphate from *Phoenix roebelenii*. Finally, there are a number of glycosylflavone sulphates, including simple 7-sulphates of vitexin, orientin and their 6-isomers. In the case of sulphates of the *O*-glycosides of vitexin and orientin, the location of the inorganic acid residue is not yet known, i.e. it could be on the sugar of either the 7- or 8-substituent, and work is in progress to establish this position of attachment.

So far, sulphates of flavonoid classes other than flavonols and flavones have yet to be described but there seems no reason why such compounds should not be found in the future. Anthocyanins with *O*-sulphate linkages would be of particular interest, since these would be expected to be zwitterionic in character. Sulphates of simpler plant

phenolics almost certainly are present in plants. Thus, in the course of surveying plants for flavonoid sulphates by electrophoresis at pH 2·2, other anionic compounds have been detected, some of which have the characteristic colour reactions of hydroxycinnamic acid derivatives. These are being actively studied at the present time.

NATURAL DISTRIBUTION

Flavonoid sulphates have now been found in over 160 species belonging to nine dicotyledonous and seven monocotyledonous families (Table 3). Since many of these families are widely separated from each other in any accepted taxonomic arrangement of the angiosperms, the presence/absence of sulphates clearly has no overall systematic significance. The only general point is that they have so far only been found in families which are herbaceous and/or advanced morphologically. Even this generalization may be false, since it may only reflect the inadequate sampling of woody plants.

In the dicotyledons, sulphates have been recorded in one group of five related families of the order Parietales (*Sensu* Emberger) and there are also isolated reports of them in the Rosaceae, Polygonaceae, Umbelliferae and Compositae. The discovery of sulphates in the Rosaceae arose by chance during a phytogeographical sampling of the genus *Acaena* from Northern and Southern temperate regions [26]. On extending this observation to other members of the tribe Sanguisorbeae to which *Acaena* belongs, positive records were found in several other taxa, notably in *Sanguisorba* where the character is variable, being confined to about 25% of the plant populations so far screened [19]. Species of all other Rosaceae so far tested have been found to lack these conjugates but further sampling is necessary to confirm or otherwise that the character is restricted to the tribe Sanguisorbeae. It is interesting, with regard to the correlation between flavonoid sulphates and presence of ellagic acid noted in the Parietales (see below), that the Sanguisorbeae is in the Rosoideae, the only subfamily of the Rosaceae in which ellagic acid has been found [27].

The first discovery of flavonol sulphates in plants in 1937 was in the Polygonaceae, in the leaves of the water-pepper, *Polygonum hydropiper*. However, this family does not appear to be a rich source of these compounds. They are present in two other *Polygonum* species (see Table 3), but a search in our laboratory of 12 other *Polygonum* spp. and of several taxa of related genera failed to reveal any further occurrences. Another early report of flavonol sulphate was in *Oenanthe stolonifera* of the Umbelliferae [11]. In this case, testing other species of the same genus showed that most members of *Oenanthe* contain sulphates. Quercetin and isorhamnetin 3-sulphates were found, for example, in all parts of the water dropwort, *Oenanthe crocata*, and luteolin 7-sulphate was detected in *O. fistulosa* [17]. However, a widespread representative survey of other umbellifers showed that these compounds were generally absent from the family. Two other sources were noted: in *Ammi visnaga* in the same tribe as *Oenanthe* and in *Daucus carota* in the tribe Dauceae. In the case of *Ammi*, it is interesting that the morphologically similar *A. majus* lacks these compounds. In the case of the wild carrot, *D. carota*, two flavone sulphates luteolin 7- and 4′-sulphate are present as variable characters. Their occurrence is correlated with geography in that they are more frequent in carrot populations from Northern Europe than in those from the Mediterranean area.

The only systematically significant occurrence of sulphates in the dicotyledons is their presence in families of the order Parietales. Here, they occur consistently in the Bixaceae (*Bixa orellana* is the only known taxon of this family), Frankeniaceae and Tamaricaceae and are present occasionally in Guttiferae (in 3 of 12 *Hypericum* spp.) and in Cistaceae (in 1 of 22 spp. surveyed). By contrast, they are absent from all species so far studied of the Cochlospermaceae, Flacourtiaceae, Turneraceae and Violaceae. Curiously, the character is positively correlated, with the exception of *Cochlospermum*, with the distribution of ellagic acid in these families. These patterns of distribution correspond well with some classificatory arrangements of the families of this order, notably that of Cronquist [28]. Members of the Tamaricaceae and Frankeniaceae are especially close to each other in their heath-like appearance and their preference for arid or saline habitats, so it is satisfying to find that their flavonoid chemistry is almost identical.

In the monocotyledons, flavonoid sulphates have been found mainly in three families: in the Palmae (in 50% of 125 spp.), in the Juncaceae (in 35% of 38 spp.) and in the Gramineae (in 12% of 206 spp.). There are also isolated occurrences in the Liliaceae, Restionaceae, Zosteraceae and Zannichelliaceae (see Table 3). The Palmae are undoubtedly the most important source of these sulphates so far discovered. Since they were found to be present in leaves of 50% of 125 species representing 70 genera and there are some 3400 palm species, they could well be present in as many as 1700 species in the family. So far, a total of 13 flavone *O*- and *C*-glycoside conjugates and two flavonol conjugates have been identified in palms [13]. Characteristically, the compounds in the Palmae have sulphate attached through a sugar residue rather than directly linked to a phenolic hydroxyl. They occur in most of the eight subfamilies, being especially common in the Phoenicoideae, but are noticeably absent from *Chamaedorea* of the Arecoideae and from the Caryotoideae. They are uniformly present in such important palm genera as *Arecastrum* (2 spp. surveyed) *Butia* (5 spp.), *Mascarena* (3 spp.) and *Washingtonia* (2 spp.). Sulphates have also been found in the flowers of 7 of 9 palm taxa examined [14]. One new conjugate, apigenin 7-glucosidesulphate, not found in palm leaves, is present in female flowers of *Phoenix canariensis*.

In the case of the grasses, sulphates appear to be restricted to three of the six subfamilies, the Phragmitiformes (in 40% of taxa), the Chloroideae (in 22%) and the Panicoideae (in 15%). All grasses which have been found to have sulphates are, with the single exception of the genus *Cortaderia*, tropical or subtropical plants [15, 23]. Sulphates are characteristic at the generic level in *Bothriochloa* (5/5 spp.), *Cortaderia* (4/4), *Panicum* (3/5) and *Saccharum* (5/6). In the latter genus, tricin 7-glucosidesulphate has been identified as the most widespread conjugate. Indeed, its distribution in clones of cultivated *Saccharum officinarum* has been intensively investigated, and the results have been useful in providing supporting evidence regarding the origin of some of the cultivars. The data, for example, support the view derived from morphological studies, that the cultivated sugar cane originated from the wild species *S. robustum*, since, in both groups of plant, sulphates are present with a high frequency (80 and 82% respectively). By contrast, certain other taxa which have been also considered at one time as putative parents, notably *Ripidium* and *Miscanthus* species, completely lack sulphates [15].

While sulphates have been found infrequently in the grasses, they have been found to be completely absent from the related sedges. A search of 40 species from 10 genera from the Cyperaceae failed to yield any source of the conjugates. The only related family where they do occur is in the rushes, the Juncaceae. Here the two main compounds are the 7-glucosidesulphates of luteolin and chrysoeriol [23]. They are present in *Juncus* itself (in 12 of 18 spp.) and three other genera, but have not been detected in *Luzula* (14 spp.) or *Oxychloe* (2 spp.).

Too little is yet known about the biosynthesis and metabolism of flavonoid sulphates and of the effect of environment on their production to be able to assess their reliability and constancy as taxonomic markers. However, they do have the advantage that they are quite stable in dried leaf tissue and can be detected in small samples of herbarium tissue of some antiquity. As with other chemical characters used for systematic purposes, they show variation in concentration, in distribution in different tissues and at the populational level. It has been found, for example, that in some plants, the whole of the flavonoid compliment appears to be bound as sulphate (e.g. *Zostera*), in others about 50% is bound (e.g. *Oenanthe*, *Bixa*) and in yet others sulphates are only minor constituents (e.g. *Daucus*). In addition, the relative concentrations of bound to unbound flavonoid in the leaf is probably also subject to seasonal variation.

With regard to the distribution of sulphates in different tissues, they have been found so far mainly in leaves and most surveys have been restricted to leaves because of their greater accessibility over other organs. However, our studies have shown that in some plants they may be found more widely in leaf, stem, flower and fruit (e.g. *Oenanthe*) whereas in others they are quite strictly confined to the leaf (e.g. *Daucus*). Finally with regard to variation at the populational level, this has been already noted in several plants, notably in *Sanguisorba officinalis* (Rosaceae) and in *Daucus carota* (Umbelliferae). In the latter

plant, different populations have been sampled and the frequency of sulphate has been found to vary with geography. Thus, in British samples, the frequency was 72% (in 23 of 32 samples) whereas in France, the frequency was 38% (3/8), in Spain and Portugal 20% (2/8) and in central and Eastern Europe 10% (2/18). This situation is complicated by the fact that there are also smaller intrapopulational variations in sulphate frequency. Further studies of populational variation in *Daucus* and in several other plants are in progress.

DETECTION AND IDENTIFICATION

Flavone and flavonol sulphates can usually be distinguished from other flavonoids on two-dimensional paper chromatograms or on cellulose TLC plates by their relatively low mobilities in alcoholic solvents (e.g. *n*–BuOH–AcOH–H_2O) and by their high mobilities in aqueous solvents (e.g. 5% acetic acid). Their appearance is also distinctive since the spots are generally arrow-shaped instead of being round. Their presence in a plant extract can be confirmed by low voltage paper electrophoresis at pH 2·2 for 2–3 hr when sulphates move towards the anode, leaving all other flavonoids (except anthocyanins which are cathodic) at the origin. Other acidic flavonoids, such as the malonated flavone and flavonol glycosides recently found in parsley [29] and flavonoid glucuronides, are also immobile at this pH. If the pH of the buffer is increased to 4, these latter two classes of flavonoid become mobile, while neutral flavones and flavonols still remain at the origin [19].

Sulphates are generally more water-soluble than the corresponding glycosides. When obtained crystalline, flavonol sulphates are white and salt-like in appearance, not pale yellow in colour like the glycosides. The melting point is usually higher than the corresponding sugar derivatives; e.g. isorhamnetin melts at 305–307°, the 3-sulphate at 280°, the 3-glucoside at 240° and the 3-rutinoside at 180°. Sulphates show the same colour reactions on paper and the same UV spectral characteristics as the corresponding methyl ethers or glycosides, i.e. quercetin 3-sulphate is similar to rutin, quercetin 7-sulphate to quercimeritrin, luteolin 7-sulphate to the 7-glucoside, etc. In the mass spectrometer, sulphate residues are lost more readily than sugar groups and parent ion peaks are not usually detectable.

Sulphates are normally isolated from plants as the potassium salts, presumably because potassium is the major cation in plant cells, and they can be characterized by determination of hydrogen sulphate and/or potassium after acid hydrolysis. These ions can be detected qualitatively by TLC in 20% 0·1 M HCl in EtOH on cellulose plates. After spraying with sodium cobaltous hexanitrite, K^+ appears as a yellow–green spot R_f 0·16 while HSO_4^- appears as a white spot on a pale yellow background at R_f 0·72 [13]. K^+ can be determined quantitatively by flame photometry and sulphate as the barium salt gravimetrically, using standard techniques. Other methods available for the determination of aryl sulphates

Table 4. Electrophoretic mobilities of some flavone and flavonol sulphates

Flavone sulphate	Mobility*	Flavonol sulphate	Mobility*
8-Hydroxyluteolin 8-sulphate	0·52	Quercetin 7-sulphate	0·18
Luteolin 7-sulphate	0·56	Isorhamnetin 7-sulphate	0·25
Apigenin 7-sulphate	0·70	Kaempferol 7-sulphate	0·34
Luteolin 7-rutinosidesulphate	0·72	Quercetin 3′-sulphate	0·66
Luteolin 4′-sulphate	0·75	Quercetin 3-sulphate	1·00
Apigenin 4′-sulphate	0·80	Isorhamnetin 3-sulphate	1·15
Apigenin 7,4′-disulphate†	2·20	Quercetin 7-sulphate-3-glucuronide	1·22
Luteolin 7-sulphate-3′-glucoside	2·50	Isorhamnetin 7-sulphate-3-glucuronide	1·28
Luteolin 7,3′-disulphate	3·0	Kaempferol 7-sulphate-3-glucuronide	1·44
Luteolin 7,4′-disulphate†	3·5		

* Mobility relative to quercetin 3-sulphate, run on Whatman no 3 paper in formate-acetate buffer, pH 2·2 at 400 V/cm for 2·5 hr.

† These are synthetic compounds; all other compounds have been obtained naturally and most also by synthesis.

in animal tissues, e.g. formation of the methylene blue salts [30], could no doubt be applied to flavonoid sulphates if needed.

The flavonoid-sulphate link is normally more susceptible to acid hydrolysis than the corresponding glycosidic link, so that it is usually possible to distinguish between these linkages by measuring relative rates of hydrolysis. As with the corresponding glycosides [31], sulphate is lost from the 3-hydroxyl of flavonols more easily than from the 7-hydroxyl of flavonols and flavones. Sulphatase can also be used for hydrolysing flavonoid-sulphate linkages and the commercially available aryl sulphatase from the Roman snail, *Helix pomatia*, can be used for this purpose [18]. Such sulphatases contain some glycosidase activity and may need to be purified before being used to specifically hydrolyse sulphate bonds in conjugates containing both sulphate and sugar residues.

Measurement of electrophoretic mobility is an important criterion in the structural identification of these flavonoid conjugates. Disulphates are very clearly separated from monosulphates by this means (Table 4). Increasing sulphation on the flavonoid also has a distinct effect on paper chromatographic behaviour; it drastically reduces mobility in phenol as in butanolic solvents, similarly increasing mobility in aqueous solvents. On electrophoresis, at pH 2·2 flavonol sulphates generally move further than flavone sulphates. Also, with flavonol sulphates, substitution in the 3-position increases the mobility over substitution in the 7- or 3′- positions (Table 4.). Finally, flavonoid sulphates containing sugar residues are usually more mobile on electrophoretograms than the corresponding conjugates lacking sugar substitution.

Final proof of identification for simple flavonoid sulphates can often be achieved by direct comparison with synthetic samples. A general procedure for sulphation using sulphamic acid in pyridine was first applied to a range of phenols and several flavonols by Yamaguchi [32]. According to this author, quercetin gives a single product, the corresponding 3′-sulphate. On repeating this reaction and subjecting the crude product to paper chromatography, we obtained a range of compounds, of which the 3′-sulphate was the major component. Small amounts of both the 7- and the 3-sulphate and of several disulphates were also obtained, so that it is clear that it is possible to obtain several differently substituted sulphates using this procedure. The only position where substitution has not yet been detected is on the 5-hydroxyl, which is presumably unreactive because of hydrogen bonding with the adjacent 4-carbonyl [17].

By carrying out this sulphation reaction in the flavone series, a range of sulphates have been obtained and several of them have been used for direct comparison with natural conjugates [18]. For example, apigenin has given mainly the 4′-sulphate, with some of the 7-sulphate and the 7,4′-disulphate. Luteolin yielded a mixture of 3′- and 4′-sulphate as the main products, together with the 7-sulphate, 7,3′-disulphate and 7,4′-disulphate. Diosmetin similarly gave a mixture of the 3′- and 7-sulphates, together with the 7,3′-disulphate [16].

FUNCTION

Since conjugation with sulphate represents one of the major detoxification mechanisms of foreign phenols in animal tissues [33], it could be argued that sulphation of flavonoids similarly represents a mode of inactivating harmful waste products in plant tissues. Sulphation undoubtedly represents a very effective way of providing water solubility to otherwise insoluble plant substances. It might be considered as an alternative excretory mechanism to the more usual glycosylation reactions in which otherwise reactive flavonoids are forced into the cell vacuole to exclude them from interfering with essential enzymic processes. However, the possibility of flavonoid sulphates having a more dynamic function in salt uptake and metabolism is suggested by their almost exclusive occurrence in plants which have an ecological association with aquatic habitats and from the results of a key feeding experiment in *Zostera*.

The feeding experiment in question was carried out by Nissen and Benson [24], who supplied $^{35}SO_4^{2-}$ to *Zostera* tissues and found after 36 hr that 50% of the radioactivity was present in the flavonoid fraction. These authors did not identify the compounds present, but subsequent work [16] has shown them to be a mixture of the 7-sulphates of luteolin, chrysoeriol, diosmetin and apigenin and the 7,3′-disulphate of luteolin.

All the flavonoid of *Zostera* is in the conjugated form. The results of the feeding experiment do suggest that the flavonoids play an active role in ion balance, in the incorporation of inorganic sulphate or in the transfer of sulphate to other organic substances. Possibly, in plants growing in a particular aquatic habitat, flavonoids are synthesized to provide a storage depot of excess sulphate ion. Alternatively, flavonoids could be universally involved in the transfer of sulphur from the inorganic to organic state, in such a way that sulphate conjugates only accumulated in plants where high concentrations of sulphate ion were present in the environment.

The association between plants which contain these sulphates and a particular type of ecological habitat is by no means complete but it is remarkable how many of the plants listed in Table 3 have some preference for either fresh or salt water conditions. This is clear in the common names of some of the plants: water-pepper, *Polygonum hydropiper* and water dropworts, *Oenanthe crocata*, *O. fistulosa*, etc. As regards saline habitats, it is interesting that the only higher plant of the British Flora which grows in a marine habitat, *Zostera*, has sulphates. Furthermore, *Daucus carota* and *Frankenia* and *Tamarix* species are notable as occurring in saline habitats. Finally, many of the palms (e.g. *Nypa fruticans*) and the rushes (e.g. *Juncus articulatus*, *J. maritimus*) listed in Table 3 grow in wet places either inland or near the sea.

Not all water plants that have been examined contain sulphates. The waterweeds *Ceratophyllum demersum* and *Myriophyllum spicatum*, for example, were tested and were found to lack them. Thus, some particular ecological factor may have to be sought to explain the differential production of flavonoid sulphates in some water plants and not others. Nevertheless, the widespread occurrence of these conjugates in a considerable range of different water plants does require some explanation and indicates that, in the future, research is needed into the possible role of these substances in inorganic sulphur metabolism in plants.

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