Phytochemistry, 1972, Vol. 11, pp. 1741 to 1750. Pergamon Press. Printed in England.

FLAVONOID PATTERNS IN THE FRUITS OF THE UMBELLIFERAE*

J. B. HARBORNE and CHRISTINE A. WILLIAMS
Phytochemical Unit, Department of Botany, The University, Reading, RG1 5AQ, Berks.

(*Received* 8 *December* 1971)

Abstract—Fruit flavonoids have been surveyed in some 100 species representing all the major tribes of the Umbelliferae. Of the 25 flavone and flavonol glycosides detected, by far the most common were luteolin 7-glucoside and quercetin 3-rutinoside. Isorhamnetin was found to occur regularly in two tribes, Peucedaneae and Apieae and it was isolated for the first time as the 3-glucuronide from *Anethum sowa*. The distribution of flavones vs. flavonols in the subfamily Apioideae was correlated closely with the results of a previous survey of the same plants for their leaf flavonoids. There was also some correlation with morphology in that species with spines on the fruit (tribe Caucalideae) contained a much richer variety of flavonoids than species in other tribes. In these plants, luteolin is present with *O*-methylation (as chrysoeriol), with complex *O*-glycosylation (e.g. as 7-glucuronosylglucoside and 7-diglucoside) and with alterations in the usual position of sugar attachment (as the 5- and 4′-glucosides). The results add further chemical support to the view that Bentham's circumscription of the tribe Caucalideae is a more natural one than that of Drude and also suggest that the Caucalideae is a highly specialised group within the family. The discovery of apigenin and luteolin 7-glucuronosylglucosides in *Cuminum cyminum* supports its removal from the Apieae and transfer to the Caucalideae.

INTRODUCTION

AS PART of a multivariate approach to the chemosystematics of the Umbelliferae with emphasis on the Caucalideae,[1] we now wish to report the results of a flavonoid survey of the fruits in this family. This complements an earlier survey of flavonoids in the leaf tissue and of proteins and enzymes in the fruits.[2] The fruits have also been previously surveyed by us for the presence of the psychotropic phenylpropanoid myristicin.[3]

Because the fruits of some umbellifers are used in spices and flavourings (e.g. fennel, caraway) or in pharmaceutical preparations (dill), their chemistry has been extensively studied, the most frequent compounds to have been reported being either terpenes or furanocoumarins.[4] However, these substances seem to occur in quantity in the fruits of the members of only a few tribes and thus appear to be of limited use as taxonomic markers within the family as a whole.[2] Attention was therefore, turned to the flavonoids, since in view of the leaf results obtained earlier,[2] one would expect a more useful distribution. Since only a little work has been done on flavonoids in umbellifer fruits, it was also of general interest to examine the flavonoid patterns in a representative sample of the family.

* Part XVII in the series "Comparative Biochemistry of the Flavonoids". For Part XVI see *Phytochem.* **10**, 1569 (1971).

[1] J. MCNEILL, P. F. PARKER and V. H. HEYWOOD, in *Numerical Taxonomy* (edited by A. J. COLE), Academic Press, New York (1969).
[2] R. K. CROWDEN, J. B. HARBORNE and V. H. HEYWOOD, *Phytochem.* **8**, 1963 (1969).
[3] J. B. HARBORNE, V. H. HEYWOOD and C. A. WILLIAMS, *Phytochem.* **8**, 1729 (1969).
[4] J. G. NIELSEN, in *Biology and Chemistry of the Umbelliferae* (edited by V. H. HEYWOOD), Academic Press, New York (1971).

RESULTS

Mature fruits were surveyed for flavonoids by two-dimensional chromatography of powdered de-fatted seed extracts, followed by examination of flavonoid aglycones present in hydrolyzed extracts. These analyses were supported by more detailed spectral and other studies of flavonoids in fruits of individual species where new or uncommon compounds were found to be present. Usually, only a few accessions of a given species have been available for study. Intraspecific variation has, however, been deliberately sought for in some dozen more accessible species without revealing much change in pattern. In the case of cultivated umbellifers, both wild and cultivated seed samples have been compared.

The results of the survey are presented in Table 1. In general, the fruit pattern was found to be remarkably similar to the leaf pattern[2] and correlations with the earlier survey, particularly regarding the aglycones present, are indicated in Table 1. While only 1% of umbellifers lack detectable flavonoids in the leaf, some 17% of the present sample apparently lack flavonoids in the fruit. This figure may be artificially high because the survey was conducted on relatively small seed samples (often only 5–10 fruits) and also, in some fruits, there is a very high concentration of furanocoumarins, which possibly obscured the flavones present. The results obtained in Table 1 are discussed below relative to the tribal distribution patterns. A summary of the results are presented in Table 2.

TABLE 1. FLAVONOIDS IN UMBELLIFER FRUITS

Plant species	Seed flavonol or flavone
Subfamily HYDROCOTYLOIDEAE	
Tribe 1. Hydrocotyleae	
Hydrocotyle bonariensis Lam.	Qu 3-glucoside
Trachymene caerulea R. Grah.	Unidentified flavonol glycosides
Tribe 2. Mulineae	
Bowlesia incana Ruiz & Pavón	None detected (none in leaf)
B. tenera Spreng.	None detected
Subfamily SANICULOIDEAE	
Tribe 1. Saniculeae	
Astrantia major L.	Km* and Qu 7-glucosides, eight minor flavonoids
Eryngium planum L.	None detected (Qu, Km in leaf)
Sanicula marilandica L.	None detected
Subfamily APIOIDEAE	
Tribe 2. Scandiceae, Subtribe: Scandicineae	
Anthriscus cerefolium (L.) Hoffm.	Lu 7-glucoside
A. sylvestris (L.) Hoffm.	Lu* 7-glucoside and Lu 7-diglucoside
Chaerophyllum aromaticum L.	Lu* 7-glucoside
C. aureum L.	Lu* 7-glucoside and Lu 7-diglucoside
C. bulbosum L.	Qu 3-rutinoside, Lu 7-glucoside and Lu 7-diglucoside
C. hirsutum L.	Qu 3-glucoside, Lu* 7-glucoside
C. temulentum L.	Qu 3-rutinoside
Molopospermum peloponnesiacum (L.) Koch	Qu* 3-rutinoside
Myrrhis odorata (L.) Scop.	Lu 7-glucoside
Myrrhoides nodosa (L.) Cannon	Qu 3-rutinoside
Osmorhiza claytonii (Michx.) C. B. Clarke	None detected (Lu in leaf)
Scandix pecten-veneris L.	Lu 7-glucoside and Lu 7-diglucoside
S. stellata Banks & Solander	Qu glycoside, Lu 7-glucoside
Tinguarra sicula B. & H.f	Lu 7-glucoside and Lu 7-diglucoside

TABLE 1.—*continued*

Plant species	Seed flavonol or flavone
Subtribe: Caucalineae	
Astrodaucus orientalis (L.) Drude	Qu glucoside
Caucalis platycarpos L.	Lu* 7-glucoside, Lu 7-diglucoside and Lu 7-rutinoside
C. microcarpa Hooker & Arn.	Lu-7-glucoside
Chaetosciadium trichospermum (L.) Boiss.	Lu-7 glucoside,* Ap 7-glucoside, two Lu 7-diglucosides
Lisaea syriaca Boiss.	Lu* 7-rutinoside, Lu 4′-glucoside
Orlaya daucorlaya Murb.	Km and Qu 3-glucuronides, Lu 7-glucuronosylglucoside
O. grandiflora (L.) Hoffm.	Qu 7-glucoside, Km and Qu 3-glucuronides, Lu* 7-glucuronosylglucoside
O. kochii Heywood	Qu 3-glucoside, Qu 3-galactoside, Lu* 7-glucoside and Lu 7-diglucoside
Torilis arvensis (Hudson) Link	Lu 7-glucoside* and Lu 7-rutinoside
T. leptophylla (L.) Reichenb. f.	Lu 7-glucoside,* Lu 7-rutinoside, Lu 7-diglucoside and Lu 7-triglucoside (?)
T. tenella (Delile) Reichenb. f.	Lu 7-glucoside,* Lu 7-rutinoside, Lu 7-diglucoside and Ap glucoside
T. nodosa (L.) Gaertner	Lu 7-glucoside,* Lu 7-rutinoside, Lu 7-diglucoside and Lu 5-glucoside
T. japonica (Houtt.) DC.	Lu 7-glucoside,* Lu 7-rutinoside and Chr. 7-glucoside
Turgenia latifolia (L.) Hoffm.	Lu* 7-glucoside, Lu 7-arabinoside, Lu 7-arabinosylglucoside, Chr. 7-glucoside, Lu 4′-glucoside and Lu 4′-diglucoside
Tribe 3. Coriandreae	
Bifora radians Bieb.	Qu* 3-rutinoside
Coriandrum sativum L.	Qu* glycoside
Tribe 4. Smyrnieae	
Conium maculatum L.	Lu 7-rutinoside* (in 3 accessions)
Physospermum cornubiense (L.) DC.	Qu glycosides (in 2 accessions)
Prangos goniocarpus	Qu glycosides
P. odontalgica (Pallas)	Qu glycosides
Smyrnium olusatrum L.	Qu* glycosides and vicenin derivative (Qu- and Irh-3-glucoside in leaf)
Tribe 5. Apieae	
Aegopodium podagraria L.	Qu* glycosides
Aethusa cynapium L.	Qu 3-rutinoside
Ammi majus L.	Qu and Lu glycosides (charged flavonoids)
Anethum graveolens L.	Km 3-glucuronide and glycoflavone
A. sowa Roxb.	Km, Qu, and Irh 3-glucuronides
Apium graveolens L.	Ap and Lu 7-apiosylglucosides
Bupleurum aciphyllum	Qu 3-rutinoside, Irh and Km glycosides
B. affine Sadler	Qu 3-rutinoside*
Carum carvi L.	Km,* Qu*, and Irh 3-glucosides and 3-galactosides
C. copticum B. & H.	Qu 3-rutinoside
Conopodium capillifolium (Guss.) Boiss.	None detected
Crithmum maritimum L.	None detected (Qu, Km in leaf)
Cuminum cyminum L.	Ap and Lu* 7-glucosides, Ap and Lu 7-glucuronosylglucosides
Foeniculum vulgare Miller	Qu 3-glucuronide*
Oenanthe aquatica (L.) Poiret	None detected
O. crocata L.	charged flavonol glycosides (Potassium bisulphates?)
O. pimpinelloides L.	charged flavonol glycosides (Potassium bisulphates?)
O. silaifolia Bieb.	charged flavonol glycosides (Potassium bisulphates?)
Petroselinum crispum (Miller) A. W. Hill	Ap and Lu 7-apiosylglucosides
Pimpinella peregrina L.	Qu and Irh glycosides
P. saxifraga L.	Qu and Irh glycosides (Km in leaf)
Seseli libanotis (L.) Koch	Qu* 3-rutinoside
S. transcaucasica Schischk.	Qu 3-rutinoside
Trinia kitaibelii Bieb.	None detected

TABLE 1.—*continued*

Plant species	Seed flavonol or flavone
Tribe 6. Peucedaneae	
Angelica archangelica L.	Qu glycoside
A. hendersonii	Qu glycosides
A. genuflexa Nutt. ex Torr. & Gray	Qu glycosides
Ferula communis L.	None detected
F. ferulago L.	
Heracleum sphondylium L.	Qu 3-rutinoside
Levisticum officinale Koch	Qu 3-rutinoside
Lomatium nudicaule Pursh	Qu 3-rutinoside
L. triternatum (Pursh) Coulter & Rose	
Peucedanum cervaria (L.) Lapeyr.	Km, Qu and Irh glycosides (Qu present as 3-rutinoside)
P. officinale L.	
P. oreoselinum (L.) Moench	
P. venetum Koch	
P. verticullare (L.) Koch ex DC.	
Pastinaca sativa L.	Irh glycoside (in wild accessions)
Tordylium maximum L.	Qu* 3-rutinoside and other glycosides
T. syriacum	Qu 3-rutinoside and one other Qu glycoside
Tribe 7. Laserpitieae	
Elaeoselinum foetidum (L.) Boiss.	None detected
Laser trilobum (L). Borkh.	
Laserpitium hispidum Bieb.	Lu 7-glucoside
L latifolium L.	None detected
L. prutenicum L.	
L. siler L.	
Thapsia garganica L.	Lu* and Ap 7-glucosides
T. villosa L.	None detected
Tribe 8. Dauceae	
Artedia squamata L.	Km and Qu* glucosides
Daucus australis Kotov	Lu, Ap and Qu glucosides
D. aureus Desf.	Ap and Lu* 7-glucosides, Lu 5-glucoside and Chr 7-glucoside
D. carota L. ssp. *sativa*	Lu 7-glucoside,* Lu 7-diglucoside, Ap and Lu 7-rutinosides Lu 4′-glucoside and 4′-diglucoside, Qu 3-glucoside
D. carota L. ssp. *carota*	Lu 7-rutinoside, Lu 4′-glucoside, Qu 3-glucoside and unidentified aglycone
D. crinitus Desf.	Lu* 7-glucoside, Lu-7-rutinoside, Qu 3-glucoside, Km and Qu 3-rutinosides
D. glochidiatus (Labill.) Fischer & C. A. Meyer	Qu* 3-monoglycoside
D. littoralis Sibth. & Sm.	Lu 7-glucoside, Qu 3-glucoside and 3-diglucoside
D. muricatus (L.) L.	Lu* 7-glucoside, Lu 4′-glucoside and Lu 4′-diglucoside
D. pusillus Michx.	Ap and Lu 7-glucosides, Lu 7-rutinoside, Km and Qu 3-glucosides
D. setifolius Desf.	Ap and Lu 7-glucosides, Lu 7-rutinoside, Lu 7-glucuronosylglucosides, Qu* 3-glucoside
D. syrticus Murb.	Ap, Lu and Qu glucosides
Pseudorlaya minuscula (Pau.) Lainz	Lu 7-glucoside, Qu 3-glucoside and Qu 3-rutinoside
P. pumila (L.) Grande	Ap 7-glucoside, Ap 7-diglucoside, Ap 7-arabinosylrhamnosylglucoside, Chr 7-glucoside, Chr 7-diglucoside, Qu 3-glucoside and Qu 3-diglucoside (Lu in leaf)

Key. Qu = quercetin; Km = kaempferol; Irh = isorhamnetin; Lu = luteolin; Ap = apigenin; Chr = chrysoeriol. * Indicates a correlation with results of the leaf analysis for flavonoids (see Ref. 2).

Scandiceae

The Scandiceae (subfamily Apioideae, Tribe 2) contain some 21 genera, being divided by Drude[5] into two subtribes, the Scandicineae and the Caucalineae, on the basis of fruit characters. The former have elongated, cylindrical fruits with smooth epicarp, whereas in the latter subtribe, the fruits are subspherical and bristly. The earlier leaf flavonoid survey indicated that the tribe was characterised by the presence of luteolin, 33 species having this flavone and only three having flavonols instead. A survey of fruits of 28 species (Table 1) shows again that luteolin is the most frequent compound in the tribe. Eighteen species have flavones alone, six have both flavones and flavonols and four have flavonols alone. There is, thus, an excellent correlation between leaf and fruit flavonoid patterns.

TABLE 2. FLAVONOID PATTERNS IN TRIBES OF THE SUBFAMILY APIOIDEAE

Tribe	Predominant flavonoid	Minor flavonoids
Flavonol tribes		
Coriandreae	Quercetin (2/2 spp.)	
Smyrnieae	Quercetin (4/5 spp.)	Luteolin only in *Conium*, glycoflavone in *Smyrnium*
Apieae	Quercetin or Kaempferol (17/18 spp.)	Glucuronides in *Anethum*, isorhamnetin in several genera, $KHSO_4$ salts in *Oenanthe*
Peucedaneae	Quercetin (10/10 spp.)	Isorhamnetin in *Pastinaca*
Flavone tribes		
Laserpitieae	Luteolin (2/2 spp.)	
Scandiceae, subtribe Scandicineae	Luteolin (10/13 spp.)	
Scandiceae, subtribe Caucalineae	Luteolin (13/14 spp.)	Chrysoeriol, apigenin, luteolin as 5- or 4′-glucoside
Dauceae	Luteolin (12/14 spp.)	

Differences in fruit morphology at the subtribal level are correlated with chemistry in that members of the subtribe Caucalineae show a much richer variation in flavonoids and contain many constituents not found in the Scandicineae. Chrysoeriol (luteolin 3′-methyl ether) for example is present in *Torilis japonica* and *Turgenia latifolia*. Luteolin 4′-glucoside, a relatively uncommon glycoside not before reported in the Umbelliferae, occurs in *T. latifolia* and *Lisaea syriaca*. In *T. latifolia*, this 4′-glucoside is accompanied by the 4′-diglucoside, which has not previously been found in plants. Luteolin 5-glucoside, previously found in leaves of *Torilis* and *Chaetosciadium*,[6] has been detected in fruits of *Torilis nodosa*.

Other uncommon glycosides found in the subtribe Caucalineae include the 7-glucuronylglucoside and the 7-arabinosylglucoside of luteolin. There also appear to be several isomeric 7-diglucosides of luteolin (see Table 3 and Experimental), e.g. one in *Caucalis platycarpos* and another in *Torilis*. One *Torilis* species, *T. leptophylla*, contains another glucose containing luteolin derivative, which from R_fs relative to the diglucoside, may be the 7-triglucoside. Unfortunately, lack of plant material has prevented the full characterisation on these compounds.

Such is the variation in flavonoids in the fruits of the Caucalineae that these pigments

[5] O. DRUDE, in *Die Natürlichen Pflanzenfamilien* (edited by A. ENGLER and K. PRANTL), Vol. 3 (8), p. 63 (1897–8).
[6] J. B. HARBORNE, *Phytochem.* 6, 1569 (1967).

TABLE 3. CHROMATOGRAPHIC DATA FOR FLAVONE GLYCOSIDES FROM UMBELLIFER FRUITS

Flavone glycoside*	R_f (× 100) in BAW	BEW	PhOH	H_2O	15% HOAc
Apigenin					
7-Glucoside	52	45	84	01	21
7-Rutinoside	34	48	73	09	40
7-Diglucoside	48	31	38	—	25
7-Arabinosylrhamnosylglucoside (?)	41	50	75	—	39
7-Glucuronosylglucoside	23	19	33	15	38
Luteolin					
7-Glucoside	33	28	56	01	11
7-Diglucoside (from *Daucus carota*)	20	28	46	23	56
7-Diglucoside (from *Torilis leptophylla*)	26	23	28	05	30
7-Diglucoside (from *T. tenella*)	20	29	70	08	41
7-Rutinoside	23	38	56	05	25
7-Glucuronosylglucoside	17	09	24	08	24
7-Arabinosylglucoside	39	28	62	05	22
7-Triglucoside (?)	14	41	43	06	58
4′-Glucoside	52	57	68	02	15
4′-Diglucoside	47	41	39	03	18
5-Glucoside	26	34	43	00	14

can be used to separate genera and species (see Table 1). Thus, *Orlaya* can be separated from *Torilis* and *Caucalis* since it contains flavonols as well as flavones. Further, some compounds are diagnostic at the species level. *O. grandiflora* (8 accessions) can be distinguised from the very morphologically similar *O. daucorlaya* (4 accessions) by the presence of quercetin 7-glucoside, while *O. kochii* is distinctive in lacking the 3-glucuronides of kaempferol and quercetin present in the other two species.

Smyrnieae

Only a small sample of the Smyrnieae (subfamily Apioideae, Tribe 4), a large tribe with over 29 genera, has been available for study and the results (Table 1) are not, therefore, truly representative. As in the leaves, the most common flavonoids of the fruits are quercetin derivatives. The only exception is hemlock *Conium maculatum*, the fruits of which (3 accessions) contain luteolin 7-rutinoside, previously found in the leaf.[2] It is perhaps not surprising that hemlock is distinctive in its flavonoid pattern, since it is the only umbellifer known to contain alkaloids.[7]

Our flavonoid results with alexanders, *Smyrnium olusatrum*, a member of the type genus, requires some comment. Ulobelen and Oksuz[8] recently reported in the leaves of this plant the presence of kaempferol and quercetin 3,7-diglucoside, a glucosidic type new to the family. However, the experimental evidence presented by these authors was equivocal. For example, partial acid hydrolyses of 3,7-diglucosides normally yield 7-glucosides[9] whereas these authors claim to have obtained 3-glucosides from their materials. We, therefore, investigated leaf flavonoids in 15 accessions of this plant, from a range of geographical

[7] J. W. FAIRBAIRN, in *Biology and Chemistry of the Umbelliferae* (edited by V. H. HEYWOOD), Academic Press, New York (1971).
[8] A. ULUBELEN and S. OKSUZ, *Lloydia* **33**, 397 (1970).
[9] J. B. HARBORNE, *Phytochem.* **4**, 107 (1965).

sites and could find no evidence at all for 3,7-diglucosides, although authentic markers were available for comparison. Instead, we found a mixture of quercetin and isorhamnetin 3-glucosides to be uniformly present, as major constituents; two other 3-monosides were there in minor amount. Fruits of the plant contained, as expected, some quercetin glucoside, but again there was no evidence for quercetin 3,7-diglucoside being present. A major compound of the fruit was a di-*C*-glycosylapigenin; it was identical in R_f to a 6,8-di-*C*-rhamnosylapigenin recently found in *Senecio*[10] and may have a similar structure.

Apieae

Fruits have been examined of 23 taxa in what is the largest tribe (Tribe 5, 85 genera) of the Apioideae and the one with the greatest number of familiar cultivated plants. As expected from the leaf analyses, most fruits contain flavonol glycosides, with quercetin 3-rutinoside being common. There are, however, three special features of the fruit flavonoids which appear to distinguish certain members of the tribe. (1) The presence of flavonol 3-glucuronides in both *Anethum* and *Foeniculum* is exceptional. Kaempferol and quercetin 3-glucuronides have only been noted in the family before in the leaf of fennel *Foeniculum vulgare*.[11] (2) The relatively uncommon isorhamnetin is present in a number of taxa (*Anethum*, *Bupleurum*, *Carum*, *Oenanthe*). (3) Charged flavonols may be present in several taxa. Thus, electrophoretically mobile flavonols were noted in 3 of 4 species of *Oenanthe* and in *Ammi majus*. Since there is an earlier report of persicarin, isorhamnetin 3-potassium sulphate, in flowers of *Oenanthe stolonifera*,[12] it is likely that related compounds do occur in the fruits. A more detailed study of these compounds in species of *Oenanthe* is now in progress.

Certain of our results do not agree exactly with results of other workers and some discussion is necessary in the case of dill, *Anethum graveolens* and cumin *Cuminum cyminum*.

Seed of dill was recently examined by Dronik,[13] who identified one of five flavonoids present as 6,8-di-*C*-glucosylapigenin or vicenin. This report was of unusual interest, since glycosylflavones are rare in the family.[2] The accession of dill examined by us contained kaempferol 3-glucuronide as a major component. There was a minor glycoflavone constituent, but it was not identical in R_f value to vicenin. There was no evidence for glycoflavone in the related Indian dill, *Anethum sowa*; this contained the 3-glucuronides of kaempferol, quercetin and isorhamnetin in about equal amounts. Although the 3-glucuronides of kaempferol and quercetin are well known plant constituents, this appears to be the first report in plants of the isorhamnetin derivative.

According to Sachindrak and Chakraborti,[14] fruit of cumin contains apigenin 7-apiosylglucoside or apiin, a compound first isolated from celery seed *Apium graveolens*[15] and which also occurs in parsley, *Petroselinum crispum*.[16] We have found instead that cumin contains four flavonoids, the 7-glucosides and 7-glucuronosylglucosides of apigenin and luteolin. It is possible that the Indian workers examined misidentified material, since many botanic garden samples of cumin are incorrectly labelled. We checked our own material by germinating the seed and growing the plants to maturity.

The discovery of 7-glucuronosylglucosides instead of 7-apiosylglucosides in cumin is of

[10] C. W. Glennie, J. B. Harborne, G. D. Rowley and C. J. Marchant, *Phytochem.* **10**, 2413 (1971).
[11] J. B. Harborne and N. A. M. Saleh, *Phytochem.* **10**, 397 (1971).
[12] A. Matsushita and S. Iseda, *Nippon Nogei Kagaku Kaiski* **39**, 317 (1965).
[13] L. Dronik, *Khim. Prir. Soedin.* **6**, 268 (1970).
[14] D. S. Sachindrak and T. B. Chakraborti, *Trans. Bose Res. Inst.* **21**, 61 (1958).
[15] E. Von Gerichten, *Liebig's Ann.* **318**, 121 (1901).
[16] C. G. Nordström, T. Swain and A. J. Hamblin, *Chemy Ind.* 85 (1953).

some taxonomic significance, since the placing of cumin in the Apieae by Drude has been questioned. It has been suggested that cumin should be transferred to the Caucalineae.[1] Our results support its removal from the Apieae, since several Caucalineae contain flavone 7-glucuronosylglucosides, whereas this type of compound is unknown in other members of the Apieae.

Peucedaneae

Flavonols, which were previously reported in leaves of 48 species from 21 genera, were also found to occur regularly in fruits of 13 species from 9 genera (Table 1). The tribe (Apioideae, Tribe 6), characterised by having winged fruit, is thus flavonol- and not flavone-producing.

The only unusual glycoside in the tribe is isorhamnetin 3-glucoside 4′-rhamnoside, pasternoside, reported in the fruit of parsnip, *Pastinaca sativa*, together with quercetin 3-glucoside and 3-rutinoside by Maksyutina and Litvinenko.[17] We have surveyed parsnip fruits for isorhamnetin and find that it is a variable character. Cultivated forms available in this country contain only traces in the fruits. Among wild collections, only one from Chesil Beach of several examined contained appreciable quantities of this flavonol. This variability in *Pastinaca* is very surprising, since fruit flavonoid patterns are otherwise very consistent.

Laserpitieae

The only point of note from a brief survey of 8 species of 4 genera is that this tribe (Apioideae, Tribe 7) generally lacks flavonoids in the fruits. Only two of the eight species, namely *Laserpitium hispidum* and *Thapsia garganica*, proved positive. As expected from the earlier leaf survey, flavones were detected, not flavonols.

Dauceae

Fruits of 14 representative taxa from the tribe (Apioideae, Tribe 8) were examined for flavonoids and some 20 compounds were variously identified (Table 1). These identifications only represent major constituents since a feature of the Dauceae fruits is the presence of many minor constituents. Wild accessions of *D. carota*, for example, alone contain from 12 to 20 detectable flavonoid-like compounds in the seeds.

One new aglycone was detected in *D. carota* and *D. aureus* which had colour properties of an aurone; further work is being carried out on its identification. Apart from this compound, the flavonoid pattern in the Dauceae seeds bears a very remarkable resemblance to that in the Caucalineae. Most of the rare flavonoids detected in the Caucalineae also appear in the Dauceae, e.g. chrysoeriol (in *Pseudorlaya*), luteolin 4′-glucoside (*D. muricatus*) luteolin 7-glucuronosylglucoside (*D. setifolius*) and luteolin 5-glucoside (*D. aureus*).

Within *Daucus*, there are considerable differences in fruit flavonoids between species, much more than was apparent in the study of the leaf flavonoids. These differences clearly reflect the known morphological variation in the genus and the fact that the genus is divided by taxonomists into several distinct sections. Within *Daucus carota* alone, there is also variation (see the results for the cultivated and wild carrot seed in Table 1); this will be discussed in more detail elsewhere.

[17] N. P. Maksyutina and V. I. Litvinenko, *Chem. Abs.* **65**, 788 (1966).

DISCUSSION

The results of the present survey of flavonoids in the fruits of the Umbelliferae are summarized in Table 2. Three main conclusions can be drawn from these studies.

(1) Fruit tissue can usefully be employed to provide chemical characters for taxonomic purposes. This is important since in a number of plant families, fruits or seeds are the most accessible materials for chemical studies. In the case of the Umbelliferae, chemical analyses of the fruits are of especial significance, since identification keys are normally based on differences in fruit anatomy or morphology. For flavonoid surveys, it has been shown that only small samples of seed (5–10 fruits) are required and the high percentage of positive results (83% of sample studied) indicates that flavonoids are very evenly distributed throughout the family, more so than other chemicals.

(2) This survey indicates that there is a major division in the subfamily Apioideae between tribes with flavonol containing fruits and those with flavones in the fruits (Table 2). Also this result correlated closely with data obtained earlier from a survey of leaf flavonoids. This latter result is of general importance in chemosystematics, since it indicates that in cases where, for example, only fruit or leaf are available, analysis of either would give a good indication of the flavonoid pattern in that species.

(3) With regard to the classification of the spiny fruited members of the Umbelliferae, the fruit flavonoid data (Table 2) strongly support the Bentham system,[18] which places these taxa together in the tribe Caucalideae, rather than the system of Drude,[5] which separates them into the subtribe Caucalineae of the tribe Scandiceae and the tribe Dauceae (see Table 1). Furthermore, the complexity of the flavonoids present in just these taxa suggests that they are evolutionarily more developed than plants in the other tribes. Finally, it is apparent that the fruit flavonoid data are of great value for separating species and genera within the Caucalideae; this will be analysed in detail in a later publication.

EXPERIMENTAL

Plant Material

Seed samples were mainly of spontaneous origin, supplied by various botanic gardens and by collectors. The Scandiceae and Dauceae material was from the Phytochemical Unit collection, which has been taxonomically verified[2] and of which voucher specimens of the plants are deposited in the University of Reading Herbarium. Seed of cultivated umbelliferae was obtained mainly from Messrs. Suttons of Reading.

Flavonoid Identifications

Flavonoid aglycones. Powdered fruit tissue, which had been pre-extracted with Et_2O, was hydrolysed for 40 min at 100° in 2 N HCl and the aglycones extracted into EtOAc. The aglycones were identified by comparison with authentic samples using standard chromatographic and spectral procedures. Isorhamnetin when present with kaempferol and/or quercetin is not readily distinguished using 1-dimensional PC, and identification was always confirmed by 2-dimensional TLC on cellulose, using 50% HOAc in both directions.

Flavonoid glycosides. Powdered fruit tissue, pre-extracted with Et_2O, was extracted with hot 70% EtOH for 2–3 hr. The glycosides present in the extract were separated and purified by PC on No. 3 paper using the standard flavonoid solvents. In the case of complex mixtures present in most Caucalineae and Dauceae seed, considerable difficulty was experienced and most constituents required 4–5 separations before they were free from other flavonoids and from other impurities. Known glycosides were identified as the basis of R_f, UV spectral analysis, hydrolysis to aglycone and sugar and of direct comparison with authentic samples. R_fs of new or rare glycosides are given in Table 3, and their identification is described in more detail below.

Apigenin glycosides. Apigenin 7-rutinoside, isolated, e.g. from cultivated carrot seed, was identified as the rutinoside from H_2O_2 oxidation, which gave rutinose and from R_f comparison with a synthetic sample of rhoifolin kindly supplied by Prof. H. Wagner; it was different from a sample of the 7-neohesperidoside run at the same time. Apigenin 7-diglucoside, isolated from *Pseudorlaya pumila* was identified chiefly on the basis

[18] G. BENTHAM, in *Genera Plantarum* (edited by G. BENTHAM and J. D. HOOKER), Vol. 1, p. 859, Spottiswoode London (1867).

of R_f comparison and the nature of the glucose–glucose linkage is not known. The 7-arabinosylrhamnosylglucoside (?) also obtained from *P. pumila* was similarly identified on the basis of R_f, spectrum and hydrolysis products and the order of attachment of sugars is not known.

Luteolin glycosides. For identification of luteolin 5-glucoside, see Ref. 9; the 4'-glucoside was identified on the basis of its distinctive colour reactions and spectral properties, and it was confirmed by comparison with samples isolated from flowers of *Spartium junceum*[19] and leaves of *Pyrus ussuriensis*.[20] The 4'-diglucoside had exactly the same colour and spectral properties as the 4'-glucoside but R_fs were lower in BAW and higher in aqueous solvents (see Table 3). On acid hydrolysis, it gave glucose and traces of a diglucose.

The 7-rutinoside of luteolin was identified, as in the case of the apigenin derivative, by detection of rutinose after H_2O_2 oxidation. The 7-glucuronosylglucoside, present in leaves as well as fruits of several Caucalineae, was so identified in the usual way; in addition, partial acid hydrolysis yielded the 7-glucoside and not the 7-glucuronide, indicating that the order of the two sugars is as shown. The 7-arabinoside of luteolin, from fruit of *Turgenia latifolia*, was inseparably mixed with some 7-glucoside; the mixture had similar R_fs to pure 7-glucoside in BAW and BEW, but higher values in aqueous solvents. The 7-arabinosylglucoside, also from *Turgenia*, gave glucose and arabinose in approx. 1:1 ratio, had R_fs of a diglycoside and the spectral properties of a 7-glycoside. A number of luteolin 7-glycosides were encountered which gave glucose as the only sugar and which R_f data indicated were 7-diglucosides (see Table 3). None was present in sufficient quantity for further identification. One present in *T. leptophylla* had an exceptionally low R_f in BAW and appeared, therefore, to be a 7-triglucoside.

Flavonol glycosides. The only novel glycoside encountered was isorhamnetin 3-glucuronide, from Indian dill, *Anethum sowa*. It was identified on the basis of spectral analysis and acid and enzymic hydrolysis to isorhamnetin and glucuronic acid. The R_f data fitted in well with comparable data for the related quercetin and kaempferol 3-glucuronides. Values ($\times$ 100) were as follows, in the order kaempferol, isorhamnetin and quercetin: 58, 53, 40 in BAW; 32, 27, 22 in BEW; 17, 10, 14 in H_2O; 52, 42, 44 in 15% HOAc; and 22, 33, 15 in PhOH.

Glycoflavones. At least three disubstituted glycosylapigenins differing from the 6,8-diglycoside vicenin were variously detected. R_fs ($\times$ 100) in BAW and 15% HOAc were: from *Anethum graveolens* 25/44; from many Caucalideae and Dauceae as a minor constituent 18/46; in *Smyrnium olusatrum* 24/53 (cf. vicenin 25/50). That in *Smyrnium* was examined in more detail. It clearly separated from added vicenin when chromatographed in PhOH (*Smyrnium* compd 57, vicenin 79) but it co-chromatographed with 6,8-di-*C*-rhamnosylapigenin from *Senecio* in all solvents.

Acknowledgements—The authors are grateful to Professor V. H. Heywood for his interest and encouragement and to the Science Research Council for financial support. They thank A. O. Chater for provision of herbarium leaf samples of *Smyrnium olusatrum* and Miss M. Moglia for wild collections of Umbellifer seed from the Italian flora.

[19] A. SPADA and R. CAMERONI, *Gazz. Chim. Ital.* **88**, 204 (1958).
[20] J. S. CHALLICE and A. H. WILLIAMS, *Phytochem.* **7**, 1781 (1968).

Key Word Index—Umbelliferae; chemotaxonomy; fruits; flavonoids; luteolin-7-glucoside; quercetin-3-rutinoside.