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CHEMOSYSTEMATICS OF THE UMBELLIFERAE—A GENERAL SURVEY

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Abstract-Some 300 umbellifer species, representing 52 per cent of the genera of the family, have been surveyed for their leaf phenolics, using both fresh and herbarium tissue. The results show that, with few exceptions, species can be divided into two groups, those with flavone (usually luteolin) and those with flavonol (kaempferol and/or quercetin). These groupings are mainly of interest at the generic level but are also related to tribal divisions and may be of phylogenetic significance in the family. Other classes of flavonoid are rare: leucocyanidin was detected once in ***Apiastrum,*** and the glucoxanthone mangiferin once in ***Heptaptera.*** Furanocoumarins were found in the leaves mainly of ***Angelica, Peucedanum*** and ***Seseli*** species but a survey of seeds of 130 species showed that these compounds were widespread in the family, some correlation with tribal divisions being apparent. Examination of the Umbelliferae for presence of polyacetylenes, simple hydroxycoumarins and the rare sugars, apiose and umbelliferose, has shown that these substances are widespread and consequently of little systematic interest within the family. Soluble proteins and the enzymes peroxidase and esterase present in the seed of selected species from all tribes in the Apioideae were studied by acrylamide gel electrophoresis. Distinct differences in patterns were found to be present at the tribal and generic levels. In some cases, the macromolecular supported the micromolecular data in confirming generic separations. The general value of the various chemical characters in the systematics of the family is discussed.

INTRODUCTION

A MULTIVARIATE approach to the classification of the tribe Caucalideae, family Umbelliferae was initially undertaken at the University of Liverpool and is now being continued at the University of Reading in order to compare chemical with the conventional morphological characters and to test numerical methods in higher plant systematics.[1] In Bentham's[2] classification of the Umbelliferae, the tribe Caucalideae comprises a group of genera with spiny fruits that Drude[3] distributed between the tribes Dauceae and Scandiceae (subtribe Caucalineae) which are widely separated in his system. It was therefore of some interest, before attempting a detailed chemical investigation of the Caucalideae, to carry out a general survey of the whole family in order to see if the plant group in question was in any way chemically distinct. Such a survey might also reveal, more generally, whether there were chemical differences at the tribal and generic level throughout the family.

From the chemotaxonomic view-point, the Umbelliferae is difficult to survey exhaustively, since it is a large family with 240-300 genera and over 3000 species, normally arranged in three subfamilies Hydrocotyloideae, Saniculoideae and Apioideae. It is, however, relatively rich

[1] J. MCNEILL, P. F. PARKER and V. H. HEYWOOD, in ***Numerical Taxonomy*** (edited by A. J. COLE), Academic Press, London and New York (1969).
[2] G. BENTHAM, in Genera ***Plantarum*** (edited by G. BENTHAM and J. D. HOOKER) 1, 859 (1867).
[3] 0. DRUDE, in ***Die natürlichen Pflanzenfamilien*** (edited by A. ENGLER and K. PRANTL) 3 (8), 63 (1897-8).

in secondary constituents and much chemical work has been carried out, especially on the furanocoumarins, terpenoids and polyacetylenes in these plants. In the present survey, the difficulty of obtaining fresh plant material of a representative sample of taxa was circumvented largely by analysing either leaf material from identified herbarium specimens or plant seed. This has limited work mainly to characters which are easily detected using small amounts of plants and to chemicals which are not destroyed during the drying process. However, analyses of dried material have been supported by examination of fresh plant tissue of a reasonable percentage of species and in the case of the polyacetylenes the survey has been limited to roots of living plants. The seeds or fruits were first used exclusively for examination for flavonoids and furanocoumarins but it was then discovered that, although small in size compared to legume seeds, they were relatively rich in protein and enzyme content. Few earlier chemotaxonomic surveys have been devoted to studying such a range of chemical characters and the present examination of the Umbelliferae is one of the first attempts to employ both secondary and macromolecular constituents in such studies.

RESULTS

Leaf Phenolics

The results of surveying 300 umbellifer species for leaf phenolics are given in Table 1. In the case of flavonoids, the results considerably extend earlier chemical studies on some dozen species[4] and the earlier leaf survey of Bate-Smith of ten species.[5] The survey was carried out mainly on herbarium material, some specimens dating from 1840 or earlier. The majority gave a positive result for flavonoids and the few species that were negative (mainly *Angelica* and ***Seseli*** spp.) generally had large amounts of other phenolics, e.g. coumarins, in their leaves. The flavonoids present in most umbellifers are kaempferol and quercetin; glycosides of these two compounds have previously been reported in five species.[4] Other unidentified flavonols are occasionally present (Table 1); one is probably isorhamnetin, already identified in flowers of ***Oenanthe*** *stolonifera* by Matsushita and Iseda.[6] Significantly, neither myricetin nor ellagic acid was detected anywhere; furthermore, a leucoanthocyanidin was only found once, in ***Apiastrum. Hacquetia epipactis,*** reported by Bate-Smith[5] as having leucocyanidin, was negative (two dried specimens examined) in our tests. These results are in line with the predominantly herbaceous character of the family.[4,5] Luteolin was the main flavone detected in many umbellifers; apigenin, although its first isolation was as the 7-apiosyl-glucoside apiin in celery seed ***Apium*** *graveolens*,[7] is in fact rather uncommon. Some unidentified flavones were detected in a few species; in some cases these may well be methylated derivatives. Indeed, the presence of diosmetin (luteolin 4′-methyl ether) was confirmed in ***Cnidium*** *silaifolium* by spectral studies. The occurrence of diosmetin in the leaf of the poisonous hemlock, ***Conium maculatum,*** reported earlier,[8] however, could not be confirmed and only luteolin was found in the specimens available for study.*

From the systematic point of view, the most significant discovery is that nearly all species have ***either*** flavonols ***or*** flavones, but not both, some *Daucus* and ***Laserpitium*** species being

* This is not surprising, since this record is based on the presence of crystals with characteristics attributed to those of "diosmetin".

[4] J. B. HARBORNE, *Comparative Biochemistry* ***of*** *the Flavonoids,* Academic Press, London (1967).
[5] E. C. BATE-SMITH, ***J.*** *Linn. Soc. (Botany) 58,371 (1962).*
[6] A. MATSUSHITA and S. ISEDA, ***Nippon*** *Nogei Kagaku Kaishi 39, 3 17 (1965).*
[7] E. VON GERICHTEN, *Liebig's* Ann. 318, 121 (1901).
[8] 0. A. OESTERLE and G. WANDER, *Helv. Chim. Acta 8, 519 (1925).*

TABLE 1. FLAVONOID AND COUMARIN SURVEY OF LEAVES AND SEED OF THE UMBELLIFERAE GROUPED IN SUBFAMILIES AND TRIBES ACCORDING TO DRUDE IN ENGLER AND PRANTL

Species	Leaf flavone or flavonol	Other leaf phenolics	Seed* furano-coumarins
Subfamily HYDROCOTYLOIDEAE			
Tribe 1. *Hydrocotyleae*			
Actinotus helianthi Labill.	Qu, Km, Ir	−	
Hydrocotyle asiatica L., ***H. americana*** L., ***H. bonariensis*** Lam., ***H. centella*** L., ***H. interrupta*** Muhl, ***H. javanica*** Thunb., ***H. prolifera*** Kellogg, ***H. repanda*** Pers., ***H. ranunculoides*** L. fil., ***H. umbellata*** L., ***H. vulgaris*** L.	Qu (as 3-glucoside), Km	−	
Micropleura renifolia Lag.†	Qu, Km	−	
Platysace ovalis (DC.)	Qu (as 3-rutinoside), Km	−	
Trachymene pilosa Sm.	−	−	
Xanthosia pilosa Rudge	Qu, Km		
Tribe 2. Mulineae			
Asteriscium chilense Cham. & Schlecht†	Qu, Km	−	
Bowlesia incana Ruiz & Pavón‡	−	−	+
Diposis saniculifolia (Lam.) DC.†	Qu, Km	−	
Domeykoa amplexicaulis (Wolff) Mathias & Constance?	Qu	−	
Hermas villosa (L.) Thunb.†	Qu	−	
Huanaca andina Phil.†	Qu, Km	−	
Laretia acaulis (Cav.) Gill & Hooker?	Glycoflavone(?)	−	
Mulinum nivale (Phil.) Constance†	Qu, Km, Ir	Aes	
Spananthepaniculata Jacq.†	Qu, Km	−	
Subfamily SANICULOIDEAE			
Tribe 1. *Saniculeae*			
Alepidea amatymbica Eckl. & Zeyh.	Qu, Km	−	
A. ciliaris La Roche	Qu, Km		
Astrantia bavarica F. W. Schultz	Qu, Km		
A. carniolica Jacq.	Km		
A. major L.	Km		−
A. minor L.	Km		
Eryngium alpinum L.	Qu, Km		
E. amethystinum L.	Qu, Km, Fi	Aes	
E. bourgatii Gouan	Km		
E. campestre L.	Fi	−	
E. campestre (E. virens Link)	Km, Fi	Aes	
E. dilatatum Lam.	Km		
E. planum L.	Qu, Km		
E. glaciale Boiss	Km		
E. ilicifolium Lam.	Km	−	
E. leavenworthii Torrey & Gray	Km		
E. maritimum L.	Km		
E. petiolatum Hooker	Km		
E. spinalba Vill.	Km		
E. tricuspidatum L.	Km		
E, yuccifolium Michx.	Qu, Km		
Hacquetia epipactis (Scop.) DC.	Qu	−	
Sanicula europaea L.	Qu	Ros	
S. laciniata Hooker & Arnott	Qu	−	
S. uradirius Watson	Qu, Km		
S. ***bipinnatifida*** Douglas	*Qu* (as 3-glucoside)		

TABLE 1-continued

Species	Leaf flavone or flavonol	Other leaf phenolics	Seed* furano-coumarins
Tribe 2. Lagoecicae			
Arctopus echinatus L.†	Qu	–	
Lagoecia cuminoides L.	Qu	–	
Subfamily APIOIDEAE			
Tribe 1. Echinophoreae			
Echinophora tenuifolia L.	Qu		
Pycnocycla ledermannii Wolff†	Qu	–	–
Tribe 2. Scandiceae (Scandicineae)			
Anthriscus sylvestris (L.) Hoffm.	Lu		
Chaerophyllum aromaticum L., C. *aureum* L., C. *coloratum* L., C. *heldreickii* Orph. ex Boiss., C. *hirsutum* L., C. *temulentum* L.	Lu (as 7-glucoside)	–	
Huetiu cynapioides subsp. *macrocarpa* (Boiss. & Spruncr) P. W. Ball (*Freyera parnassica* Boiss. & Heldr.)	Lu	–	–
Molopospermum peloponnesiacum (L.) Koch.:	Qu (as 3-glucoside), Km		–
Myrrhis odorata (L.) Scop.	Lu (as 7-glucoside)	–	–
Osmorhiza claytonii (Michx.) C. B. Clarke	Lu	–	
0. *chilensis* Hooker & Arn.	Lu (as 7-glucoside)	–	
Scandix australis L., S. *balansae* Reuter, S. *pecten-veneris* subsp. *brachycarpa (Cuss.)* Thell., subsp. *marrorhynca* (C. A. Meyer) Rouy & Camus	Lu (as 7-glucoside)	–	+
Tinguarra cervariaefolia (DC). Parl.†	LU	–	
T. montana Bentham†	LU	–	
Tribe 2. Srandiceae (Caucalineae)			
Astrodaucus littoralis (Bieb.) Drude, *A. orientalis* (L.) Drude, *A. persicus* (Boiss.) Drude.	Lu (as 7-glucoside)	Iso	
Caucalis platycarpos L., C. *microcarpa* Hooker & Am.	Lu	–	
Ckaetosciadium trichospermum (L.) Boiss.†‡	Lu (as 5- and 7-glucoside)	–	
Lisaea hetrrocarpa (DC.) Boiss, *L. syriaca* Boiss.†	Lu	–	
Orlaya grandiflora (L.) Hoffm., 0. *kochii* Heywood	Lu (as 7-glucoside)		
Psammogeton canescens (DC.) Vatke	Qu	Coumarins	
P. setifolium (Boiss.) Boiss.	LU		
Torilis arrensis (Hudson) Link, subsp. *arvensis*, subsp. *purpurea* (Ten.) Hayek, (*T. heterophylla* Guss.), subsp. *neglecta* (Schultes) Thell. *T. japonica* (Houtt.) DC., *T. nodosa* (L.) Gaertner, *T. stocksiana* (Boiss.) Drude, *T. ucranica* Sprengel, *T. tenella* (Delile) Reichenb. fil.	Lu (as 7-glucoside)	–	–
Turgenia latifolia (L.) Hoffm.	Lu	–	–
Turgeniopsis foeniculacea (Fenzl) Boiss.†	Qu	–	
Tribe 3. Coriandreae			
Bifora radians Bieb.	Qu, Km		+

TABLE *l-continued*

Species	Leaf flavone or flavonol	Other leaf phenolics	Seed* furano-coumarins
B. testiculata (L.) Roth.	Qu, Km (as 3-rutinoside)	–	+
Coriandrum sativum L.	Qu (Km in flower)	–	–
Tribe 4. Smyrnieae			
Apiastrum angustifolium Nutt.†	Qu	Umb, leucocyanidin	
Arracacia arguta (Rose) Matthias & Constance	Qu	Umb	
Astoma seselifolium DC.†	Lu	–	
Cachrys Iibanotis L.	Qu	Coumarins	
C. ferulacea (L.) Calestani	Qu, Km	Aes	
C. trifida Miller	Qu	Umb, coumarins	
Conium maculatum L.	Lu	–	–
Donnellsmithia hintonii Mathias & Constance†	Qu, Km	–	
Erigenia bulbosa (Michx.) Nutt.	Lu	–	
Grafia golaka (Hacq.) Reichenb.	Qu, Km	–	
Heptaptera triquetra (Vent.) Tutin	Qu, Km	Mangiferin, Coumarins	
Hladnikia pastinacifolia Reichenb.	Qu (as 3-rutinoside)	–	
Magydarispanacifolia (Vahl) Lange	Flavonol	Umb, coumarins	
Musenium divaricatum Nutt.	Qu, Km	Umb	
Oreomyrrhis eriopoda (DC.) Hooker?	Lu	–	
Orogenia linearifolia S. Watson	Qu	–	
Physospermum verticillatum (Waldst. & Kit.) Vis.	Qu, Km	–	
P. cornubiense (L.) DC.	Qu	–	
Pleurospermum austriacum (L.) Hoffm.	Qu, Km	Coumarins	
Prangos uloptera DC.	Qu (as 3-glucoside)	Aes	
Smyrnium olusatrum L. S. ***perfoliatum*** L., S. ***rotundifolium*** Miller	Qu, Km		
Tauschia texana A. Gray†	Km	Umb	
Trachydium depressun Boiss.†	Km	–	
Vicatia millefolia (Kltzsch.) C. B. Clarke?	LU	–	
Tribe 5. Apieae			
Aegopodium podagraria L.	Qu	–	
Aethusa cynapium L.	Qu, Km	–	+
Ammi majus L.	Qu	Umb	+
A. visnaga (L.) Lam.	Qu Km	Umb	
Annesorhiza hirsuta Eckl. & Zeyh.†	Qu'	–	
Apium graveolens L.	Lu	–	–
A. inundatum (L.) Reichenb. fil.	Qu	–	+
A. nodiflorum (L.) Lag.	*Qu* (as 3-glucoside)	–	
Athamanta turbith (L.) Brot. subsp. ***haynaldii*** (Borbás) Tutin	Lu (Lu in flowers)	–	
A. turbith subsp. ***hungarica*** (Borbás) Tutin	Lu (Lu in flowers)	–	
A. macedonica (L.) Sprengel	Qu, Km (Km in flowers)		
Berula erecta (Hudson) Coville	Qu	–	
Bupleurum affine Sadler	*Qu* (as 3-rutinoside)		
B. angulosum L.	Qu (as 3-rutinoside), Km	–	
B. dianthifolium Guss.	Qu (as 3-rutinoside)		
B. falcatum L.	Qu (as 3-rutinoside)	Isoflavone(?)	
B. gibraltaricum Lam.	Km (as 3-rutinoside)		
Carum carvi L.	Qu, Km (also in flowers)	–	
C. ***rigidulum*** (Viv.) Koch ex DC.	Qu, Km (also in flowers)		
C. ***heldreichii*** Boiss.	Qu (as 3-rutinoside)		
C. ***verticillatum*** (L.) Koch	Qu (as 3-rutinoside), Km	Coumarin	

TABLE *1-continued*

Species	Leaf flavone or flavonol	Other leaf phenolics	Seed* furano-coumarins
Cenolophium ***denudatum*** (Hornem.) Tutin	Qu, Km		
Chaemaesciadum *acaule* C. A. Meyer†	Qu		–
Cicuta *bulbifera* L.	Qu (as 3-rutinoside), Km	–	
C. *maculata* L.	Qu		
C. ***virosa*** L.	Qu, Km	–	–
Cnidium *silaifolium* (Jacq.) Simonkai	Qu, Km	Coumarins	+
Conopodium bunioides (Boiss.) Calestani, C. *majus* (Gouan) Loret, C. *thalictrifolium* (Boiss.) Calestani	Diosmetin, Lu, Flavone	–	
Crithmum maritimum L.	Qu, Km	Coumarin	
Cryptotaenia canadensis (L.) DC.†	Glycoflavone	–	+
C. *elegans* Webb ex Bolle	Lu (as 7-glucoside)		
Cuminum cyminum L.	LU	–	
Cymopterus ***terebinthinus*** Torrey & Gray?	Qu	Umb	
Cynosciadium ***digitatum*** DC.	***Qu***	–	
Dethawia *tenuifolia* (Ramond ex DC.) Godron	Lu	–	
Diplolophium *zambesianum* Hiern†	Qu		
Endressia pyrenaica (Gay ex DC.) Gay	Lu	–	
Eulophus bolanderi Coulter & Rose†	Qu, Km	–	
Eurytaenia *texana* Torrey & Gray†	Qu, Km	–	
Falcaria vulgaris Bernh.	Qu, Km	–	
Foeniculum vulgare Miller	Qu, Km	–	
Heteromorpha arborescens Cham. & Schlecht	Qu, Km	Aes	
Hohenackeria polyodon Cosson & Dur.	Qu (as 3-rutinoside)		
Kundmannia sicula (L.) DC.	Qu	–	
Lereschia thomasii (Ten.) Boiss.	***Qu*** (as 3-glucoside)	Coumarin	
Lichtensteinia ***burchellii*** Hooker fil.	Qu	–	
Ligusticum *lucidum* Miller	***Qu*** (as 3-rutinoside)	Coumarins	
L. *mutellina* (L.) Crantz.	Qu, Lu (as 7-glucoside)	–	
L. scoticum L.	Lu		
Lilaeopsis ***carolinensis*** Coulter & Rose†	Qu		
Meum ***athamanticum*** Jacq.	***Qu*** (as 3-rutinoside), Qu (in flower)		
Oenantke *aquatica* (L.) Poiret	Qu, Km	–	
0. ***banatica*** Heuffel	Qu, Km		
0. ***crocata*** L.	Qu		
0. *fistulosa* L.	Lu	–	
0. ***foucaudii*** Tesseron	Qu		
0. ***globulosa*** L.	Qu, Km		
0. ***lachenalii C.C.*** Gmelin	Qu, Ir(?)	–	
0. peucedanifolia Pollich	Qu, Km	–	
0. *pimpinelloides* L.	Qu		
0. *sarmentosa* Presl ex DC.	Qu	–	
0. *silaifolia* Bieb.	Qu	–	
Oliveria *decumbens*†	Qu, Km		
Petroselinum *crispum* (Miller) A. W. Hill	Qu		
P. *segetum* (L.) Koch	Lu		
Pimpinella *anisum* L., ***P. major*** (L.) Hudson, ***P.*** *siifolia* Leresche, ***P. tragium*** Vill.	Qu (as 3-rutinoside)	–	
P. saxifraga L.	Km	–	
P. *procumbens* (Boiss.) H. Wolff	Qu (as 3-rhamnoside)	–	
P. stadensis D. Dietr.	Lu (as 7-glucoside)		
Pleurospermum *austriacum* (L.) Hoffm.	Qu		
Polemannia *grossulariifolia* Eckl. & Zeyh.†	Qu, Km		

TABLE *1-continued*

Species	Leaf flavone or flavonol	Other leaf phenolics	Seed* furano-coumarins
Portenschlagiella ramosissima (Portenschl.) Tutin†	Qu, Km		+
Ptychotis saxifraga (L.) Loret & Barr.	Qu, Lu (as 3-glucoside)		
Rhyticarpus difformis Bentham†	Qu, Km		
Ridolfia segetum Moris	Qu, Km	–	
Schultzia crinita Sprengel†	Lu	–	
Selinum calidense	Qu	–	
S. carvifolia (L.) L.	Lu (as 7-glucoside)	–	
S. *capitellatum* Bentham ex S. Watson	Qu		
S. *pyrenaeum* (L.) Gouan	Qu	Umb, Aes	–*
Seseligracile Waldst. & Kit.	Qu (as 3-glucoside), Km	14 other spp. all had Umb, Aes and other coumarins	+
S. *hippomarathrum* Jacq.	Qu (as 3-glucoside), Km		
S. *libanotis* (L.) Koch	Qu		
Silaum silaus (L.) Schinz. & Thell.	Qu, Km		
Sison amomum L.	Qu (as 3-glucoside), Km	–	
Sium latifolium L., S. *sisarum* L., S. *thunbergii* DC.	Qu (as 3-rutinoside)		
Thaspium trifoliatum (L.) Gray†	Qu (as 3-rutinoside and 3-glucoside), Km		+
Trinia glauca (L.) Dum.	Qu, Km		+
T. frigida (Boiss. & Heldr.) Drude	Qu (as 3-rutinoside)	–	+
Trochtscanthes nodiflora (Vill.) Koch	Lu (as 7-glucoside)		
Tribe 6. Peucedaneae			
Angelica sylvestris L.	Qu	9 other spp. lacked flavonoids but had coumarins, inc. Umb.	+
Astydamia canariensis DC.†	Qu	–	
Capnophyllum peregrinum (L.) Lange†	Km	Umb	
Choritaenia capensis Bentham†	Km	–	
Conioselinum tataricum Hoffm.	Qu	–	
Dorema aureum Stocks†	Km		
Eriosynaphe longifolia (Fischer ex Sprengel) DC.*	Flavonol		
Ferula sadlerana Ledeb.	Qu	–	
Ferulago campestris (Besser) Grec.	Qu, Km	–	
F. granatensis Boiss.	Qu	Umb	
F. sartorii Boiss.	Qu, Km		
F. sylvatica (Bess.) Reichenb.	Qu (as 3-rutinoside)	Aes	
Heracleum austriacum L. subsp. *austriacum*, *H. austriacum* subsp. *siifolium* (Scop.) Nyman	Qu (as 3-rutinoside)		
H. sphondylium L., *H. minimum* Lam., *H. lanatum* Michx., *H. rigens* Wall.	Km	Umb	+
Johrenia distans (Griseb.) Halácsy	Qu	–	
Levisticum officinale Koch	Qu (as 3-rutinoside)		
Lomatium californicum Nutt.†	Qu	–	
Malabaila aurea (Sibth. & Sm.) Boiss.	Qu (as 3-glucoside), Km		
Opopanax chironium (L.) Koch, 0. *hispidus* (Friv.) Griseb.	Glycoflavones(?)	Coumarins	+
Ormosciadium aucheri Boiss.	Qu (Qu in flowers)		
Ostruthium spp.	Qu	Coumarins	
Pastinaca sativa L. subsp. *sativa*	Qu (as 3-glucoside), Qu & Km in both flowers and seeds		

TABLE *1-continued*

Species	Leaf flavone or flavonol	Other leaf phenolics	Seed* furano-coumarins
Peucedanum alsaticum L., ***P. arenarium*** Waldst. & Kit., ***P. austriacum*** (Jacq.) Koch., ***P. carvifolia*** Vill., ***P. cervaria*** (L.) Lapeyr., ***P. gallicum*** Latourr., ***P. lancifolium*** Lange, ***P. latifolium*** (Bieb.) DC., ***P. leiocarpum*** Nutt., ***P. macrocarpum*** Nutt., ***P. officinale*** L., ***P. oreoselinum*** (L.) Moench, ***P. palustre*** (L.) Moench, ***P. simplex, P. officinale*** subsp. ***stenocarpum*** (Boiss. & Reut.) Font Quer, ***P. terticillare*** (L.) Koch ex DC.	Qu (as 3-rutinoside), Km	Most spp. have coumarins, some with Aes or Umb	+
P. villosum Nutt.	Ap (as 7-glucoside)	–	–
Polytaenia nuttallii DC.†	Qu	Coumarins	–
Tordylium maximum L.	Qu, Km	Umb	–
T. officinale L.	Qu, Km (in flowers)	–	
Zosima orientalis Hoffm.†	Qu, Km	Coumarins	+
Tribe 7. Laserpitieae			
Elaeoselinum foetidum (L.) Boiss.†	Lu		
E. asclepium (L.) Bertol. subsp. ***meoides*** (Desf.) Fiori and subsp. ***asclepium***	Lu	–	
E. gummiferum (Desf.) Tutin	Qu		
E. tenuifolium (Lag.) Lange	Km	–	
Laser trilobum (L.) Borkh.†‡	Lu (as 7-glucoside)	–	–
Laserpitium krapfii Crantz	Lu (as 7-glucoside), Ap	Aes	
L. gallicum L.	Lu, Ap		
L. latifolium L.	Qu		+
L. nestleri Soyer-Willemet	Lu, Km	–	
L. nitidum Zanted.	Lu, Ap	–	
L. peucedanoides L.	Qu (as 3-glucoside), Km	–	+
L. prutenicum L.	Qu, Lu	–	+
L. siler L.	Qu, Lu, Ap	–	–
Thapsia garganica L.	Lu (as 7-glucoside)	–	
T. villosa L.	Lu (as 7-glucoside)	–	
Tribe 8. Dauceae			
Artedia squamata L.	Qu (as 3-glucoside)	–	+
Daucus aureus Desf.	Lu	–	–
D. broteri Ten.	Lu		
D. carota L.	Lu (as 7-glucoside)	Umb	
D. crinitus Desf.	Lu		–
D. durieua Lange	Qu, Km, Lu		
D. glochidiatus (Labill.) Fischer & C. A. Meyer	Qu	–	
D. involucratus Sibth. & Sm.	Qu (as 3-glucoside)		
D. muricatus (L.) L.	Lu		
D. setifolius Desf.	Qu	–	
Pseudorlaya pumila (L.) Grande	Lu		

* Key: Km, Kaempferol; Qu, Quercetin; Ir, Isorhamnetin(?); Lu, Luteolin; My, Myricetin; Ap, Apigenin; flavonol = unidentified flavonol; flavone = unidentified flavone; Aes, aesculetin; Umb, umbelliferone; coumarin(s) = unidentified furanocoumarin(s); Ros, rosmarinic acid; Iso, isochlorogenic acid ; + , present; – not detected (blank = not examined).

† Source of material: Herbarium, Royal Botanic Gardens, Kew.

‡ Source of material: fresh plants grown from spontaneous seed. All other material from the University of Liverpool Herbarium. Most species in the Scandiceae (Caucalineae) and the Dauceae were examined both as fresh and herbarium material.

rare exceptions. Furthermore, flavones were found almost entirely in taxa generally considered to be more specialized or advanced (e.g. ***Daucus, Torilis),*** while flavonols predominated in the less advanced genera (e.g. ***Hydrocotyle***). Thus, the replacement of flavonol

TABLE 2. UMBELLIFER SPECIES KNOWN TO CONTAIN POLYACETYLENES IN THE ROOTS

Tribe, genus and species	Reference*	Tribe, genus and species	Reference*
HYDROCOTYLEAE		*Ligusticum mucronatum* Hort.	2
Trachymene australis	1	*Libanotis transcaucasica* Schisk.	2
SANICULEAE		*Oenanthe crocata, 0. javanica*	1, 3
Eryngium planum L.	1, 2	*0. pimpinelloides, 0. peucedanifolia*	1, 3
SCANDICEAE (Scandicineae)		*Petroselinum sativum*	1
Chaerophyllum tenellum	1	*Pimpinella koreana*	3
Osmorhiza aristata	3	*P. saxifraga* L.	2
Myrrhoides nodosa (L.) Cannon	2	*Seseli ugoense*	3
SCANDICEAE (Caucalineae)		*S. gummiferum* Pall. as ex Sm.	2
Astrodaucus orientalis (L.) Drude	2	S. *dichotomum* Pallas	2.3
Chaetosciadum trichospermum (L.) Boiss.	2	S. *buchtormense* Koch	2
Orlaya daucorlaya Murb.	2	S. *libanotis* (L.) Koch	2
Torilis arvensis (Hudson) Link, *T. nodosa* (L.) Gaertner	2	*Sium suave* Walter, S. *sisarum* L.	1, 2
T. japonica	3	*Thaspium trifoliatum* (L.) Gray	2
Turgenia latifolia (L.) Hoffm.	2	PEUCEDANEAE	
SMYRNIEAE		*"Angelica deculsiva", "A. edulis"*	3
Conium maculatum L.	2	*A. miqueliana, A. polymorpha*	3
APIEAE		*A. sylvestris* L.	2
Aegopodium podagraria	1	*Conioselinum chinense*	3
Aethusa cynapium	1	*Opopanax chironium* (L.) Koch, 0. *hispidus* (Friv.) Griseb.	1, 2
Ammi visnaga	1	*Peucedanum verticillare*	1
Apium graveolens	1	*Pastinaca sativa*	4
Bupleurum rotundifolium	1, 3	*Tordylium maximum* L.	2
B. longeradiatum	3	LASERPITIEAE	
Carum carvi	1	*Thapsia villosa* L.	2
Cicuta virosa	1	DAUCEAE	
Crithmum maritimum	1	*Daucus aureus* Desf. ,*D. carota*† L.	2
Cryptotaenia canadensis	1	*D. crinitus* Desf., *D. pusillus* Michx.	2
Falcaria vulgaris	1	*D. guttatus* Sibth. & Sm.	2
		Pseudorlaya pumila (L.) Grande	2

* 1. BOHLMANN *et al.*, Chem. *Ber.* 94,958 (1961).
2. This paper.
3. I. YOSHIOKA *et al., Yakugaku Zasshi* 86, 1216 *(1966).*
4. S. SAFE and V. THALLER, *J. Chem. Soc. c, 1220 (1966).*
† Both wild and cultivated forms were examined.

by flavone appears to have an evolutionary significance within the family, as it probably has among the angiosperms generally.[4,5] In general, flavonoid type is very consistent at the generic level (Table 1). Thus flavonols occur in all species that have been examined of ***Carum*** (4), ***Eryngium*** (15), ***Hydrocotyle (1 I), Peucedanum*** (16), ***Pimpinella (7)*** and ***Sanicula (4).*** Similarly, flavones are present throughout ***Chaerophyllum*** (6), ***Conopodium (3)*** and

Torilis (8). In the few genera where this is not so, differences can be related to some morphological characteristics. For example, in *Oenanthe,* ten of the eleven species studied have leaf flavonols; the exception with flavone, *O. fistulosa*, is a species with a very highly specialized floral morphology. Again, differences in the genera *Daucus* and *Laserpitium* show some correlation with taxonomic heterogeneity. At the sub-family level (Table 2) presence of flavones separates the Apioideae from the other two sub-families. Within the Apioideae, tribes vary according to whether flavones are rare (Peucedaneae, in one of forty-six species), common (Dauceae, in seven of eleven species) or predominant (e.g. Caucalineae, twenty-one of twenty-three species).

The flavonoid pattern in the flowers of umbellifers has not been examined so far extensively because of lack of material but a sample analysis (Table 1) indicates that the pattern is similar to that of the leaves. Anthocyanin is too rare in either flower or leaf to be of any systematic interest; the pigments that have been studied in leaf and Bowers of the Caucalideae are uniformly based on the commonest anthocyanidin cyanidin and it is clear that there has been little selection for an anthocyanin-based flower colour in much of the family.

A study of the flavonoid glycosidic pattern in the Umbelliferae indicates that in the leaf it is generally a simple one (Table 1). Luteolin is commonly present as the 7-glucoside and quercetin as the 3-glucoside and 3-rutinoside. More complex patterns are present in the Caucalideae; luteolin occurs in leaves of some genera, e.g. *Torilis*, as a range of other glycosides, including the 5-glucoside and 7-rutinoside, and a complex mixture of Aavone glycosides, which are under examination, occurs, for example, in the fruits of wild specimens of *Daucus carota*. C-Glycosylflavones are rare or absent and they have not been found with certainty in any plant; the leaf flavones in *Opopanax chironium* (Peucedaneae) appear to be *C*-glycosides of apigenin and luteolin but do not correspond in R_f with known glycoflavones. Glycoflavones are found in a number of unrelated plant groups in close association with glycoxanthones and it is interesting that mangiferin (2-*C*-glucosyl-1,3,6,7-tetrahydroxyxanthone) has been found in a single umbellifer species *Heptaptera triquetra* (*Colladonia triquetra)* (Smyrnieae). This substance is more often associated with flavonols than flavones, as in the present case.

Other leaf phenolics detected during the present survey were the two hydroxycoumarins, umbelliferone and aesculetin, already well known as constituents of the fruits and roots of this family. They were of widespread but sporadic occurrence throughout the tribes. Other coumarins, presumably furanocoumarins, were detected particularly in the leaves of *Angelica*, *Peucedanum* and *Seseli* species, but were not further identified.

Polyacetylene Distribution

Polyacetylenes were first identified in the family as toxic principles of the water dropwort, *Oenanthe crocata,* and in *Cicuta virosa* and *Aethusa cynapium.*[9,10] Bohlmann and his co-workers[11] later surveyed roots of forty-one species from thirty-five genera and found falcarinone or related compounds in fourteen species and unidentified acetylenes in three further species. However, the remainder, including the domestic carrot, *Daucus carota,* were reported as apparently lacking in polyacetylenes. Subsequently, the acetylene carotatoxin was isolated[12] from *D. carota* and shown to be identical to falcarinol, $CH_2{=}CH{-}CHOH{-}$

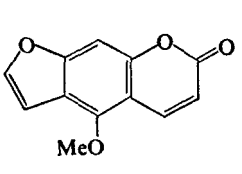
[9] E. F. L. J. ANET, B. LYTHGOE, M. H. SILK and S. TRIPPETT, *J. Chem. Soc.* 309 (1953).
[10] F. BOHLMANN, C. ARNDT, H. BORNOWSKI and P. HERBST, *Chem. Ber.* 93,981 (1960).
[11] F. BOHLMANN, C. ARNDT, H. BORNOWSKI and K. M. KLEINE, *Chem. Ber. 94,958* (1961).
[12] D. G. CROSBY and N. AHARONSON, *Tetrahedron 23,465* (1967).

$[C\equiv C]_2—CH_2—CH=CH—(CH_2)_6—CH_3$.[13,14] Very recently, Yoshioka *et al.*[15] obtained positive tests for polyacetylenes in thirteen umbellifer species from ten genera.

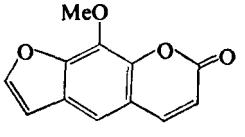
The above surveys were mainly limited to plants of the Apieae and Peucedaneae and the present work was developed to see if the acetylenic character is uniformly distributed throughout the family and in particular to see if there was any structural variation in acetylenic type in the tribe Caucalineae. Ether extracts of fresh roots of thirty-five species, from twenty-five genera, most of which had not been previously surveyed, were analysed for polyacetylenes by TLC and u.v. spectroscopy. Every plant examined (see Table 2) showed the presence of two or more polyacetylenes, one of which corresponded in R_f and u.v. spectral maxima with the major domestic carrot polyacetylene, and is presumably falcarinol. It is clear from these results that polyacetylenes are widespread, if not universal, in the family. The earlier negative findings of Bohlmann *et al.* were probably due to insensitive methods of detection or to quantitative variations; several of the species reported by them as negative have now been shown to be positive. The present survey reports the first record of polyacetylenes in the Laserpiteae (in *Thapsia*). They are clearly widespread in the Caucalineae and the Dauceae, occurring in both wild and domestic forms of *D. carota* and in other *Daucus* such as *D. aureus* and *D. crinitus.* Some variations in polyacetylene pattern were noted among *Daucus* species and these are being further investigated. It must be concluded from this study that, as a general character in the family, polyacetylenes, because of their ubiquitous occurrence, have little systematic interest at the tribal level.

Furanocoumarins and Simple Sugars

Furanocoumarins such as bergaptene (I) and xanthotoxin (II) and their isoprenoid derivatives have been isolated from the roots or fruits of some thirty-five umbellifer species and provide a chemical character linking the Umbelliferae with the Leguminosae and Rutaceae.[16,17] Furanocoumarins often occur as complex mixtures and as many as fifteen such compounds have been isolated from a single umbellifer species. Previous work has been restricted entirely to species of the Smyrnieae (e.g. *Prangos),* the Apieae *(Ammi, Pimpinella)* and the Peucedaneae (e.g. *Peucedanum)* and the present survey was carried out on seed material to see if these substances occur throughout the family.



(I) Bergaptene



(II) Xanthotoxin

Presence of furanocoumarins in the seeds was determined by appearance of mauve, blue or yellow fluorescent spots on thin-layer chromatograms run in appropriate solvent systems.[18] In many cases the simple hydroxycoumarins umbelliferone or aesculetin were detected,

[13] R. K. BENTLEY and V. THALLER, *Chem. Commun. 439* (1967).
[14] R. K. BENTLEY, D. BHATTACHARJEE, E. R. H. JONES and V. THALLER, *J.* Chem. *Soc.* (c) 685 (1969).
[15] I. YOSHIOKA, T. TIMURA, H. IMAGAWA and K. TAKARA, *Yakugaku Zeisshi* **86,** 1216 (1966).
[16] F. M. DEAN, *Naturally Occurring Oxygen Ring Compounds,* Butterworth's, London (1963).
[17] *Chem. Abst.* 1960-1968.
[18] L. HÖRHAMMER, H. WAGNER and D. KRAEMER-HEYDWELLER, *Deutsche Apoth.-Ztg.* 106, 267 (1966).

TABLE 3. DISTRIBUTION OF CHEMICAL CHARACTERS IN THE UMBELLIFERAE

Subfamily and tribe	No. species with flavonol	No. species with flavone	Generic ascertainment	Furocoumarin distribution	Presence of Umbelliferose	Presence of Polyacetylenes	Patterns of* proteins	Patterns of* enzymes
Hydrocotyloideae								
1. Hydrocotyleae	16	0	13/34	1/2		+		
2. Mulineae	7	0		1/1	–	–		
Saniculoideae								
1. Saniculeae	26	0	7/9	1/3		+		
2. Lagoecieae	2	0		—	–	+		
Apioideae								
1. Echinophoreae	2	0	2/5		–			Esterase III
2. Scandiceae								
(a) Scandicineae	1	16	17/21	6/11	+	+	IV	Esterase III
(b) Caucalineae	2	19		2/10	+	+	II	Esterase I
3. Coriandreae	3	0	2/5	1/3	+	+	III	Esterase II
4. Smyrnieae	21	5	22/29	2/3	+	+	III	Esterase II
5. Apieae	79	20	52/85	14/29	+	+	I	Esterase I (no peroxidase)
6. Peucedaneae	46	1	23/41	15/19	+	+	IV	Esterase IV
7. Laserpitieae	7	11	4/8	3/6	+	+	V	Strong peroxidase
8. Dauceae	5	7	3/4	1/7	+	+	1	Esterase I (medium peroxidase)

* The numbers refer to particularly distinctive, intense protein or enzyme bands of different R_p *value (see* Figs 1-3).

together with the more complex coumarins. Analyses were carried out on comparable amounts of seed material and species that were positive had an average of five components with as many as eight appearing in a few taxa.

The results of surveying some 130 species for seed furanocoumarins are recorded in Table 1 and show that these substances are characteristic of the family, being present in every tribe. The distribution at the tribal level is summarized in Table 3 and it is clear that the sampling is too low to draw any definite systematic conclusion. However, the results confirm that the Peucedaneae and the Smyrnieae are very rich in such constituents. By contrast, both the Caucalineae (2/10 spp.) and the Dauceae (1/7 spp.) are relatively poor in furanocoumarins and there is some indication here supporting the union of these taxa in one tribe, as in the system of Bentham and Hooker.

An unusual oligosaccharide α-D-galactosido-2^{G}-sucrose, called umbelliferose, was reported in roots of thirteen genera of the Umbelliferae by Wickstrom and Boerheim-Svendsen in 1956.[19,20] It was present in five of the eight tribes of the subfamily Apioideae but was not recorded in the Caucalineae, Coriandreae, Laserpiteae or Dauceae. A survey was therefore initiated to see if this trisaccharide, apparently peculiar to the Umbelliferae, was uniformly distributed throughout the family. It was indeed, readily detected in fresh root extracts of ***Torilis nodosa, Turgenia latifolia*** (both Caucalineae), ***Coriandrum sativum*** (Coriandreae), ***Thapsia villosa*** (Laserpiteae) and ***Daucus carota*** (in all of three subspecies) (Dauceae). It was detected in all of eighteen umbellifer taxa examined and seems to be universal in the family and thus valueless as a tribal character.

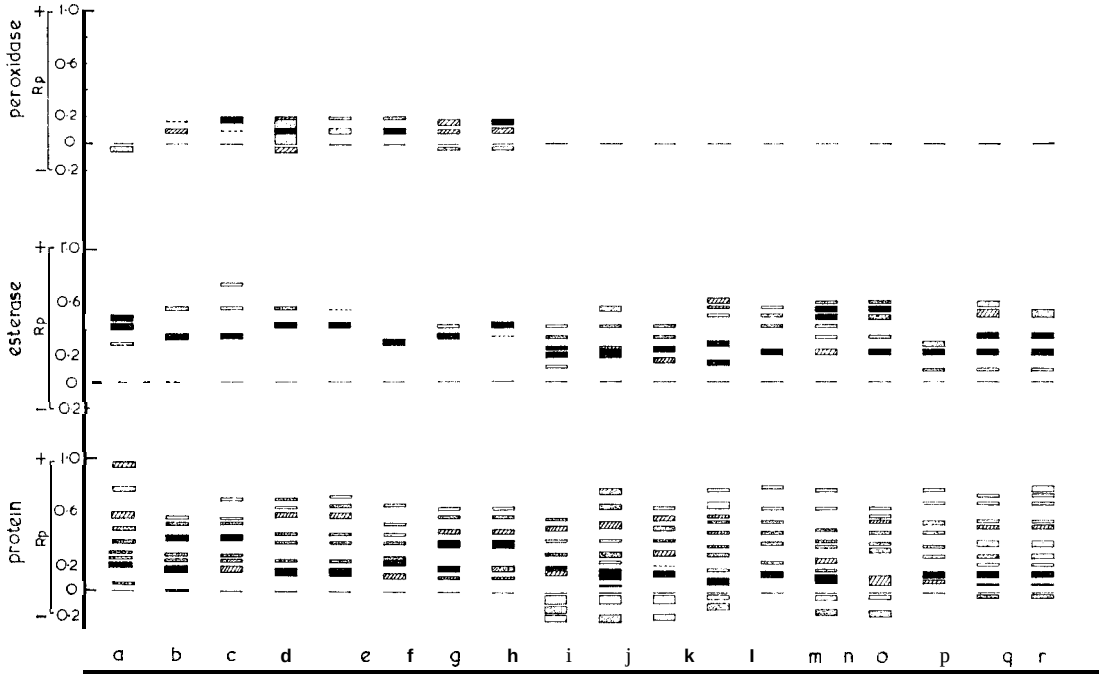
Examination of the free sugars in roots, leaves and fruits of representative taxa of the family also failed to show any other characters of systematic interest. The rare pentose sugar apiose, first reported[7] in combined form as a flavone glycoside in ***Apium graveolens*** seed, was detected free in a number of species but its distribution was quite sporadic. Apiose was also present occasionally in the polysaccharide fraction of umbellifer species but again its distribution was of no taxonomic significance.

Seed Proteins

In recent years, a number of investigators have shown a correlation between protein composition and systematics in higher plants. Using electrophoretic techniques, Johnson and Hall[21] investigated relationships in the Triticineae (Gramineae) and Boulter ***et al.***[22–24] examined separately the systematic relationships of albumin and globulin fractions in seeds of certain legumes, and of two dehydrogenase enzymes within the same family while Vaughan and Denford[25] surveyed the albumin and globulin fractions of the seeds of a number of ***Brassica*** and ***Sinapis*** species, correlating the results with the established taxonomy. Other workers[26,27] have used immunochemical techniques to demonstrate taxonomic affinities between species (see review by Fairbrothers[28]). Rarely, however, has a sufficient number of

[19] A. WICKSTROM and A. BOERHEIM-SVENDSON, *Acta Chem. Scand.* **10**, 1199 (1956).
[20] A. BOERHEIM-SVENDSEN, *Med. Norsk. Farm. Selsk.* **20**, 1 (1958).
[21] B. L. JOHNSON and 0. HALL, *Am. J. Botany 52,506 (1965).*
[22] D. J. Fox, D. A. THURMAN and D. BOULTER, *Phytochem. 3,417 (1964).*
[23] D. BOULTER, D. A. THURMAN and E. DERBYSHIRE, *New Phytol.* **66**, 27 *(1967).*
[24] D. A. THURMAN, D. BOULTER E. DERBYSHIRE and B. L. TURNER, *New Phytol.* **66**, 37 *(1967).*
[25] J. G. VAUGHAN and K. E. DENFORD, *J. Exp. Botany 19,724 (1968).*
[26] P. G. H. GELL. J. G. HAWKES and S. T. C. WRIGHT. *Proc. R. Soc.* B 151.364 (1960).
[27] R. N. LESTER, R. E. ALSTON and B. L. TURNER, *Am. J. Botany 52,165* (1965).
[28] D. E. FAIRBROTHERS, in *Modern Methods in Plant Taxonomy* (edited by V. H. HEYWOOD), p. 141, Academic Press, London and New York (1968).

species been examined to show the true potential of seed proteins as general taxonomic characters. In this present investigation, extracts from 174 samples of seed, covering ninety-



FIGS. l-3. INTERPRETATIVE DIAGRAMS ILLUSTRATING THE ELECTROPHORETIC DISTRIBUTIONS OF PROTEIN, ESTERASE AND PEROXIDASE COMPONENTS IN SEED EXTRACTS OF SPECIES OF UMBELLIFERAE SUBFAM. APIOIDEAE.

FIG. I. Tribe I. Echinophoreae
- a. ***Echinophora sibthorpiana Guss.***

Tribe II. Scandiceae

1. Scandicineae
- b. *Anthriscus nemorosa* (Bieb.) Sprengel
- C. ***A. sylvestris*** (L.) Hoffm.
- d. ***Chaerophyllum aromaticurn*** L.
- e. C. ***hirsutum*** L.
- f. ***Molopospermum peleponnesiacum*** (L.) Koch
- g. ***Scandix iberica*** Bieb.
- h. S. ***pecten-veneris*** L.

2. Caucalineae
- 1. ***Torilis nodosa*** (L.) Gaertner
- j. ***T. japonica*** (Houtth.) DC.
- k. *T. tenella* (Delile) Reichenb. fil.
- 1. *Caucalis* ***platycarpos*** L.
- m. ***Pseudorlayapumila*** **(L.)** Grande
- n. ***Orlaya kochii*** Heyw.
- 0. 0. *grandiflora* (L.) Hoffm.
- p. ***Turgenia latifolia*** **(L.) Hoffm.**
- q. ***Astrodaucus littoralis*** (Bieb.) Drude
- r. ***A. orientalis*** **(L.)** Drude

nine species and thirty-nine genera, with representation from all eight tribes of the subfamily Apioideae, were examined by acrylamide gel electrophoresis. Examination was made of the general protein pattern, using amido black and light green as staining reagents, and also of enzymatic activity with respect to esterase, peroxidase, catalase, and in some instances

amylase. The block technique was used because this method affords a more direct means of comparison between a number of samples under strictly comparable conditions in a single gel preparation. Also, by slicing the gel horizontally, it is possible to compare each sample for a variety of properties, by differential staining of the slices.

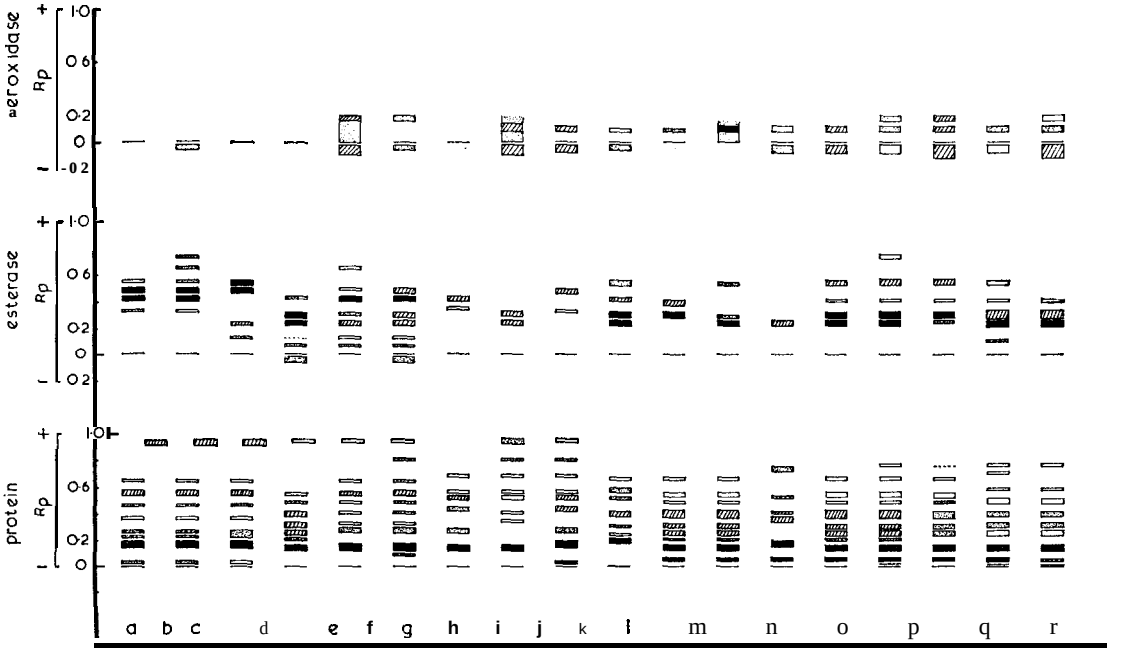


FIG. 2. Tribe III. Coriandreae
a. ***Coriandrum melphitense*** Ten. & Guss.
b. C. ***sativum*** L.
c. ***Bifora radians*** Bieb.

Tribe IV. Smyrnieae
d. ***Conium maculatum*** L.
e. ***Prangos lophoptera*** Boiss.
f. ***P. ferulacea*** Lindley (L.) Calestani
g. ***Smyrnium perfoliatum*** L.
h. ***Cachrys trifida*** Miller

Tribe V. Apieae
j. ***Ammi visnaga*** (L.) Lam.
k. ***Apium graveolens*** L.
l. ***A. inundatum*** (L.) Reichenb. fil.
m. ***Bupleurum prealtum*** L.
n. ***Falcaria vulgaris*** Bernh.

Tribe VIII. Dauceae
o. ***Daucus carota*** L. subsp. ***sativus*** (Hoffm.) Arcangeli
p. ***D. carota*** L. spont.
q. ***D. aureus*** Desf.
r. ***D. carota*** L. subsp. ***gadecaei*** (Rouy & Camus) Heywood

The investigation has revealed a number of differences between the species examined, some of which are undoubtedly of taxonomic significance. Staining with amido black showed that most species of the Caucalineae, except ***Turgenia Iatifolia*** (three accessions), ***Orlaya grandiflora*** (two acc.) and ***Pseudorlaya*** (two sp., five acc.), contained cationic proteins which gave heavily staining bands. These cathodic-migrating bands were not seen with amido-black staining in any of the samples from other tribes. However, the variation is quantitative only, since in many cases cationic peroxidases were detected in other species, the enzymatic

assay being a more sensitive means of protein detection than the amido-black reagent. With regard to the other tribes, the Dauceae and Apieae gave essentially similar protein patterns as did the Coriandreae with the Smyrnieae, and the Scandiceae with the Peucedaneae. The Laserpiteae were distinct in that their electrophoretograms showed a number of strong

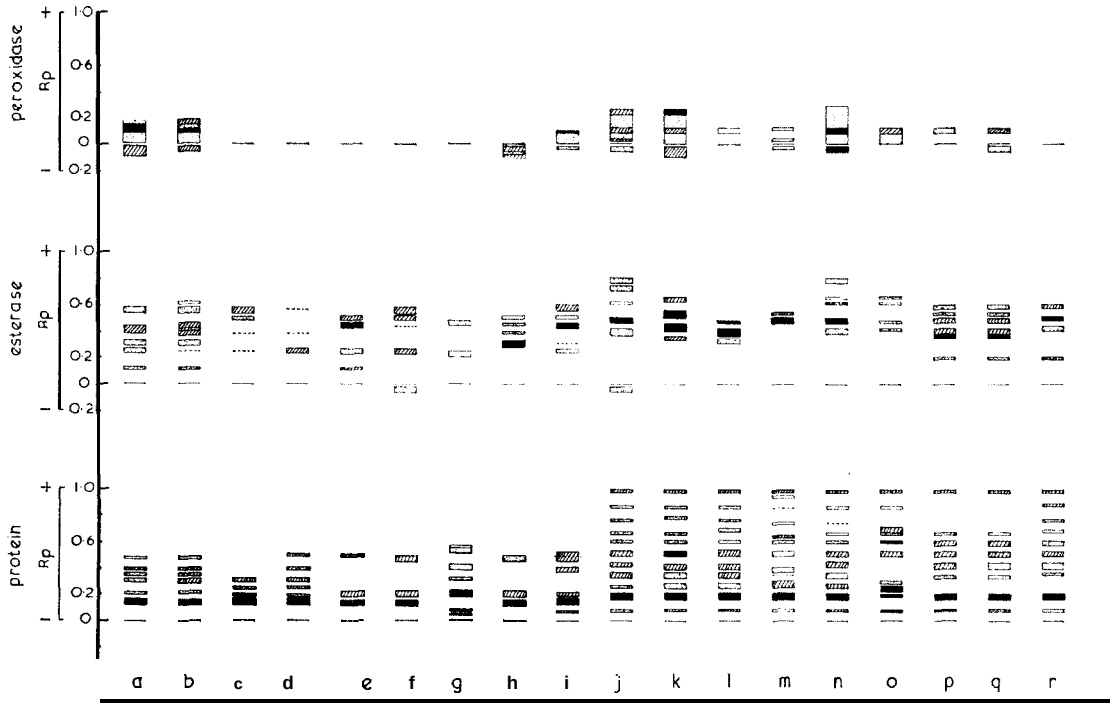


FIG. 3. Tribe VI. Peucedaneae
a. ***Ferula tenuisecta*** Korov.
b. ***Ferula tschimganica*** Lipsky
c. ***Opopanax hispidus*** (Friv.) Griseb.
d. 0. ***chironium*** (L.) Koch
e. ***Heracleum stevenii*** Manden.
f. ***H. ponticum*** (Lipsky) Schischk. ex Grossh.
g. ***Pastinaca sativa*** L.
h. ***Peucedanum lubimenkoanum*** Kotov
i. ***P. oreoselinum*** (L.) Moench

Tribe VII. Laserpitieae
j. ***Laserpitium latifolium*** L.
k. ***L. siler*** L.
l. ***L. halleri*** Crantz
m. ***L. archangelica*** Wulfen
n. ***Laser trilobum*** (L.) Borkh.
0. ***L. trilobum*** (L.) Borkh.
p. ***Elaeoselinum thapsoides*** DC.
q. ***E. gummiferum*** (Desf.) Tutin
r. ***Thapsia villosa*** L.

bands of high electrophoretic mobility (R_p).[22] Some high R_p bands were also seen in several species of the Caucalineae (e.g. *Torilis, Astrodaucus*). Examples of the typical protein patterns for the various tribes are shown in Figs. l-3. Staining for protein with the light-green reagent was much weaker than amido-black, but was useful in that it allowed better resolution of some close-running bands.

All species were shown to contain a variable number of esterase enzymes. However, in only three species, ***Conium maculatum, Cachrys ferulacea*** (Smyrnieae) and ***Pastinaca sativa*** (Peucedaneae) was a cationic esterase observed. In respect of the general esterase patterns, the Apieae and Dauceae were very similar to one another, and to ***Torilis*** and ***Pseudorlaya*** of the Caucalineae. The Coriandreae again bore close resemblance to the Smyrnieae, as did the Scandiceae with the Laserpitieae and Echinophoreae. In each of these groups there was a single major esterase component and a variable number (2-5) of minor bands, the main difference being the R_p of the major component. The Peucedaneae presented a rather mixed esterase pattern, sometimes with more than one major component and of variable R_p, whilst for the most part the Caucalineae, particularly ***Astrodaucus, Caucalis*** and ***Orlaya,*** had distinctive esterase patterns with several very strong bands.

A strong peroxidase reaction was shown by many species of the tribe Laserpitieae (though not ***Laser trilobum***, ***Laserpitium archangelica*** and ***L. gallicum***) and by ***Chaerophyllum*** species (Scandiceae). Other species of the Scandiceae and the Dauceae gave a very modest peroxidase reaction, but in other tribes the reaction was weak (Caucalineae) or absent altogether. In all cases both cationic and anionic peroxidases were observed.

Oddly, most of the species with weak or no evident peroxidase activity gave a strong catalase reaction. Catalase activity was negligible in the Laserpitieae. However, in the whole survey only one catalase band per species was observed, and this always occupied the same region on the electrophoretogram (R_p approx. 0·1). Amylase activity was examined for many species but it appeared to be a very poor taxonomic character, and it did not allow any differentiation between species.

DISCUSSION

1. *Taxonomic History of the Umbelliferae*

As an introduction to the discussion of the chemical data presented in this paper we have felt it useful to give a brief survey of the taxonomic background of the Umbelliferae. The Umbelliferae have been recognized as a natural group since the earliest studies of plants dating back to Theophrastus (see Rodriguez[29]). So striking are the features in which the members agree, such as the typical unbellate inflorescence, pentamerous perianth and androecium, and characteristic fruits, that the group occupies a special role in the development of the systematist's concept of natural groups and affinity (cf. Sachs[30]). In practice this is mainly true as regards the Apioid group of Umbelliferae to which most North Temperate members belong.[31] This very uniformity in structural features makes for considerable difficulties in subdividing the family and there has been disagreement between authors from the time of Linnaeus until the present day as to the number and circumscription of the tribes to be recognized. In this respect it is similar to other "natural" families such as the Compositae and Cruciferae where recognition of tribes and genera is often highly unsatisfactory or frankly artificial.

At the subfamily level, morphological divisions are fairly clear-cut and most people nowadays follow Drude (in Engler and Prantl[3]) in recognizing three subfamilies based largely on anatomical features : Hydrocotyloideae, Apioideae and Saniculoideae. While the

[29] R. L. Rodriguez, *Univ. Calif. Publ. Bot. 29, 145* (1946).
[30] J. von Sachs, *History of Botany* (transl. H. E. F. Garnsey and I. B. Balfour), Clarendon Press, Oxford (1890).
[31] M. E. Mathias and L. Constance, *Univ. Calif. Publ. Bot. 33, No. 2 (1962).*

first of these is sometimes recognized as a separate family, the Hydrocotylaceae, Calestani [32] did not agree that the main feature separating the Hydrocotyloideae from the Saniculoideae, the woody endocarp, is sufficient for even subfamilial recognition and united the two groups. On the other hand, he considered ***Lagoecia*** (of the subfamily Saniculoideae) to be sufficiently different to merit treatment as a subfamily of its own, the Lagoecineae.

At tribal and subtribal level there is much greater diversity of treatment, depending on which characters (usually of the fruit) are given greater importance (cf. Ref. 1). Thus Boissier, following Bentham (in Bentham and Hooker*), recognized eleven tribes, Drude (in Engler and Prantl[3]) twelve which were often considerably different in circumscription from those of Boissier, Calestani[32] ten, again often very different from those of the other authors, and Cerceau-Larrival[33] twenty-seven based largely on fruit seedling and pollen characters, later modified[34] to thirty-one following a detailed study of the pollen-types *Torilis–Caucalis–Daucus* group of genera.

2. *Relationships with the Araliaceae*

Despite its naturalness, systematists have long recognized a close relationship between the Umbelliferae and the Araliaceae (and to a lesser extent the Cornaceae) : the three families are frequently placed in a single higher group (e.g. the cohort Umbellales, Bentham in Bentham and Hooker[2]) and indeed the Umbelliferae and Araliaceae are united as one family by some workers such as Baillon,[35] Calestani[32] and recently by Thorne.[36] Calestani discusses and tabulates the differences between the two families and concludes that although several characters separate them none of them is without exceptions and none has "real systematic importance". If lack of exceptions is a criterion, then both must be united as one family which he calls the Apiaceae. Anatomical studies have stressed the similarities between the families and an important role is played by such genera as ***Myodocarpus*** of the Araliaceae, which although sometimes placed in the Umbelliferae, is similar in its fruit structure to the schizocarpous condition of the Umbelliferae. A special study of this genus was made by Baumann,[37] who showed that while its fruits showed certain features such as primary ribs and resin canals which are characteristic of, although more strongly developed in, the Apioid group of the Umbelliferae, in vegetative features, inflorescence and flower structure the genus agreed more closely with the Araliaceae. Baumann concluded that the Umbelliferae were derived from a pre-Araliaceous stock which had become more highly evolved than the Araliaceae.

Rodriguez,[29] following a study of the woody genus ***Myrrhidodendron*** and the histology of secondary xylem throughout the Umbelliferae which he compared with a number of Araliaceae and other Umbellales, agreed with Baumann's concept of a number of divergent lines arising from a theoretical pre-Araliaceae. As regards wood anatomy and other morphological considerations, he considered the Araliaceae as forming an uninterrupted series with the Umbelliferae but standing at a more primitive level, although exhibiting at the same time a greater versatility of characters. Accordingly he felt it to be immaterial whether they were labelled as separate families or as a single one. On the other hand he found the relationship of these two families to the others placed in the same order, i.e. the Cornaceae, Nyssaceae

[32] V. CALESTANI, *Webbia* **1**, 89 **(1905)**.
[33] M. T. CERCEAU-LARRIVAL, *Mém. Mus. Nat.* **Hist.** *ser. B*, **14**, 1 **(1962).**
[34] M. T. CERCEAU-LARRIVAL, ***Pollen et Spores*** **7** (1), **35 (1965).**
[35] **H. E. BAILLON,** ***Histoire des Plantes 7, 84-256*** **(1879-1880).**
[36] **R. F. THORNE,** *Aliso* **6**, 57 *(1968).*
[37] M. *G.* **BAUMANN,** *Ber. Schw. Bot. Ges.* **56, 13 (1946).**

and Garryaceae less obvious, so agreeing with most other workers (cf. Cronquist [38]) although Tamamschian (reported in Cronquist [38]) considers there to be a close relationship between the Cornales and Umbellales. It is clear, therefore, that a full assessment of the phytochemical characteristics of the Umbelliferae would have to take into account the Araliaceae as well. The survey reported here has, however, been restricted so far to the former family. It is evident from what is already known of the chemical constituents of the Araliaceae[39] that they are similar to the Umbelliferae. In terms of phenolic constituents the data agree in broad terms with the evidence from wood anatomy (see Rodriguez,[29] Fig. 69) as regards the relative advancement of the two families but this is largely to be expected due to the degree of necessary correlation between these two features.

This then is the taxonomic background against which the chemical data have to be considered. To summarize, the family shows considerable uniformity in several conspicuous morphological features which lead to its instant recognition, and superimposed on this uniformity there is a reticulate distribution of characters which makes tribal and generic delimitation difficult and which leads to the conclusion that no one subfamily is overall more advanced in evolutionary terms than any other, although in respect of individual features character trends can be detected. This is well illustrated by Rodriguez[29] who pointed out that in order to list the species in a continuous sequence according to mean vessel-length, the systematic division into subfamilies and tribes had to be ignored. In other words the family illustrates in a very clear fashion the concept of evolution of characters (semophyleses) as opposed to overall evolutionary advancement which is usually an average condition since individual characters evolve at different rates.[40]

3. *Chemotaxonomic Relationships*

Much of the chemical data obtained in the present survey, summarized in Table 3, support the above view of the family; thus, surveys for acetylenes, free sugars, simple and furanocoumarins show rather clearly the chemical homogeneity of these plants. How far these characters distinguish umbellifers from related families is not at present completely known, except that polyacetylenes have also been reported in species of the Araliaceae.

Once again, the only secondary constituents showing significant variation within the family are the flavonoids. Sufficient numbers of species and genera have been surveyed to show that presence of flavone versus flavonol is correlated with systematics. Tribes can be divided (Table 3) into two broad groups: the majority of nine in which flavones are rare or lacking and four in which flavones are common or predominant (Apieae, Dauceae, Laserpitieae and Scandiceae). The significance of this division, which may have some systematic and evolutionary significance, is difficult to assess. Certainly, the subfamily Hydrocotyloideae which is completely lacking in flavones in less typically umbelliferous than, say, the Apioideae, and closer to the Araliaceae in possessing a woody endocarp, no separate carpophoer and frequent absence of vittae, but cannot be regarded as in any way ancestral to present-day Apioideae or Saniculoideae but rather as one of several separate independently developed lines from some pro-Araliaceous-Umbelliferous stock. Again, the Saniculoideae, which in our sample completely lacks flavones, is not to be regarded as primitive in view of the many specializations of features it shows but has probably evolved separately from the other groups

[38] A. CRONQUIST, *The Evolution and Classification of Flowering Plants*, Nelson, London and Edinburgh (1968).
[39] R. HEGNAUER, *Chemotaxonomie der Pflanzen*, Vol. 2. (1964).
[40] P. H. DAVIS and V. H. HEYWOOD, *Principles of Angiosperm Taxonomy*, Oliver and Boyd, Edinburgh and London (1963).

of the family for a long period of time. The Apioideae in respect of presence or absence of flavones is very mixed : although most tribes possess them, in some they are well represented while in others they are rare or absent altogether in our sample.

At the tribal level the most distinctive differences observed during this survey are in the protein and enzyme patterns. This is largely because these patterns contain many items of information which can be assessed visually, although it is at present difficult to analyse and interpret this information in terms of individual characters, even with the use of computers (cf. Ref. 25). For example, it is difficult to know the degree of homology which can be accorded to bands appearing in the same position but in different samples. This difficulty has been largely overcome by the use of enzyme stains, the results of which confirm the cruder protein patterns obtained with amido-black. It is generally possible by the combined use of protein and enzyme patterns to distinguish all tribes within the subfamily Apioideae, with the exception of the Smyrnieae and Coriandreae. This ties in with preliminary results reported by Pickering and Fairbrothers[42] who indicate that by using serological and disc electrophoretic methods the three subfamilies can be separated on the basis of their protein chemistry. The sample of species examined in the protein survey has, however, been limited by the seed material available and differences observed may possibly reflect differences at the generic rather than at the tribal level.

Certainly, the macromolecular data obtained are most immediately applicable to problems at the generic level, where in fact most difficulties are experienced by taxonomists. In this connexion, it is satisfying that the protein and the flavonoid data support each other in drawing generic limits. Two examples may be mentioned. Thus the monotypic genus ***Turgenia*** *latifolia*, which has often in the past been classified with *Caucalis*, is readily separated from the latter genus on the basis of the specific occurrence of luteolin 4'-glucoside and chrysoeriol 7-glucoside in the seed and of the absence of peroxidase enzymes and of back-migrating bands in its seed protein pattern. The recognition of ***Turgenia*** as a separate genus is also supported by details of the microstructure of the fruit, as revealed by scanning electron microscopy (Heywood[43] and unpublished) and in a pilot multivariate numerical assessment. Again, *Daucus* and ***Astrodaucus,*** two genera which as their names imply are closely allied morphologically, can be separated on a leaf phenolic character, the complete replacement of chlorogenic acid as the principle caffeic ester by isochlorogenic acid in the latter genus, and also on their protein patterns, only ***Astrodaucus*** showing the presence of a cation migrating band.

Finally, turning to the taxonomic problem of whether the spiny-fruited umbellifers should be separated into two groups as Drude would have it, or combined in a single tribe according to Bentham, the present chemical survey does provide a partial answer. On balance, the data obtained, particularly regarding the flavone and furanocoumarin characters, are in favour of treating the taxa as a single unit. Furthermore, the Caucalineae and Dauceae have very similar protein patterns and are united in a common esterase pattern. By contrast, protein and enzyme patterns clearly distinguish the Caucalineae from the Scandicineae and if one wished to maintain the tribe Scandiceae as in the classification of Drude, one would have to accept considerable chemical heterogeneity within its bounds.

Although there is little chemical diversity of pigmentation in the flowers, schizogenous

[41] V. H. HEYWOOD, ***Modern Methods in Plant Taxonomy,*** 1, Academic Press, London and New York (1968).
[42] J. L. PICKERING and D. E. FAIRBROTHERS, ***Am. J. Botany 55,737*** (1968).
[43] V. H. HEYWOOD, ***Proc. Linn. Soc. Lund. 179,287*** (1968).

oil-canals are found in the root, stem and usually in the pericarp of the fruits (where they are termed vittae). The various oils and resins secreted are largely responsible for the characteristic odour and taste of many of the species which are used for medicinal and culinary purposes, e.g. Betts.[44] These oils and resins show considerable diversity and clearly have a high selective value. They have not yet been fully surveyed during this study and results of work in progress [45] will be reported later.

EXPERIMENTAL

Plant Material

Living material, largely belonging to the Caucalideae, was grown from seed of known origin at the University of Liverpool Botanic Gardens, Ness, Cheshire. Voucher specimens are housed at the Herbarium, Department of Botany, University of Reading, and details of seed sources are available on request. Herbarium material was sampled from Umbelliferae specimens made available by the courtesy of the curators of the University of Liverpool Herbarium and the Keeper of the Herbarium, Royal Botanic Garden, Kew. In the case of specimens in the Liverpool Herbarium, sampling labels have been affixed to the sheets of material examined.

Flavonoids

These were extracted and identified by standard procedures. Both direct and acid-hydrolysed leaf extracts were chromatographed in four solvents against authentic markers. In addition, co-chromatography and spectral confirmation was carried out in representative cases. Details of the identification of individual flavonoids in the Umbelliferae, and of mangiferin in *Heptaptera*, will be given in a later paper.

Furanocoumarins

The presence of furanocoumarins was recorded in leaf extracts when spots with intense mauve, light green of light yellow fluorescence in u.v. light and of usually high R_f values in most of the solvent systems used for flavonoids were noted on chromatograms. For the seed survey, seeds were ground into a powder with sand, extracted twice with netrol. ether and the residue hydrolysed for 30 min with 2 N HCl and the coumarins extracted into ethyl -acetate. The concentrated petrol. ether and ethyl acetate extracts were separately chromatographed in ether-benzene-10 % HOAc (1: 1: 1) on silica gel plates. Species containing furanocoumarins showed the presence of several (from three to twelve) blue, mauve, green or yellow fluorescent spots with R_f between 0·1 and 0·9 in one or both extracts. Spectral measurements on representative species confirmed the coumarin nature of the substances. Species already known to be rich in furanocoumarin content responded in the same way. The simple hydroxycoumarins, umbelliferone and aesculetin, were run as markers on all plates and their occurrence (when present) confirmed by co-chromatography.

Polyacetylenes

Fresh roots were thoroughly washed, dried and then macerated in ether and left for a week at 4" in the dark. The ether layer was poured off, dried and concentrated at room temperature. The extracts were screened for polyacetylenes by TLC on silica gel in benzene–$CHCl_3$ (9: 1), pentane–ether (9: 1) and $CHCl_3$–MeOH (9: 1). Polyacetylenes appeared as brown spots or bands of approx. R_fs 0.12, 0·28 and 0·49 after the plates had been sprayed with 0·4 % isatin in conc. H_2SO_4 and heated for 10 min at 110". Other constituents with blue or red colours occasionally appeared but further spectral examination showed them not to be acetylenes. The polyacetylene bands, on elution into ether, each gave five or six intense peaks in the short u.v., in the region 230-310 nm characteristic of known polyacetylenes. A domestic carrot extract, which contained falcarinol, was run as a marker on all chromatograms.

Sugars

Fresh roots were macerated in MeOH, and the filtered extract concentrated and examined for the presence of umbelliferose by chromatography on paper in the usual sugar solvents (over-developed for oligosaccharides) and sprays. Umbelliferose appeared in the species examined (seep. 1975) as a brown spot with aniline hydrogen phthalate of R_G 0·64 in butanol-acetic acid-water (4:1:5), 0·62 in butanol-benzene-pyridine-water (4: 1: 1: 3) and 0·70 in butanol-ethanol-water (4 : 1: 2 : 2) (lit. value $R_{Sucrose}$ 0·70 in isopentanol-pyridine-water (7:7:6)). An unknown oligosaccharide was detected in the parsnip, *Pastinaca sativa*, with R_G 0·44 in butanol-acetic acid and water. The free sugars present in the alcohol-soluble and alcohol-insoluble (after acid hydrolysis)

[44] T. J. BETTS, *J. Pharm. Pharmac. 20,469 (1968).*
[45] J.B. HARBORNE, V. H. HEYWOOD and C. A. WILLIAMS, *Phytochem.* 8, 1729 (1969).

fractions of seed, leaf and fruit of three to seven representative species from the various umbelliferae tribes were examined by paper chromatography. All the usual monosaccharides were detected and, in addition, apiose was found occasionally to be present, mainly in the polysaccharide fraction; its distribution was unrelated to taxonomy. Authentic apiose marker was obtained by hydrolysing apiin, from celery seed.

Preparation of Samples for *Electrophoresis*

Whole seeds (about 25 mg) were ground to a *fine* powder with an equal weight of sand, and the grinding continued with 6-8 drops of extracting buffer to produce a thick slurry. This slurry was allowed to stand for 0·5 to 1 hr before centrifuging. The supernatant (40 μl) was used for electrophoresis. Protein content of the solutions prepared in this way varied usually between 2-3 per cent (Lowry estimation).[46] Concentrations were not adjusted if they fell within this range. The extracting buffer contained hydroxymethylamine methane (35 μM), citric acid (2.5 μM), ascorbic acid (6 μM), cysteine-HCl(6 μM) and sucrose (0·5 M).

Electrophoresis

Horizontal electrophoresis was conducted in a Shandon apparatus (Kohn type) using a 7·5% acrylamide gel, essentially after the method of Lund.[47] The gel was polymerized in a flat perspex mould (150 x 100 x 6 mm) covered with a plate-glass lid from which projected a row of thirteen celluloid slot formers (5·0 x 1·5 x 5·5 mm) approx. 25 mm from the cathode side of the gel. Each slot accepted 40 μl of sample. A constant current of 20 mA was maintained throughout the electrophoresis, during which time the potential rose from 90 to about 170 V. The sample slots were filled with extracting buffer, and pre-electrophoresis conducted for approx. 1·5 hr, when the buffer was replaced by the seed extract samples. Electrophoresis of the samples was continued for a further 2–2·5 hr, or until the borate-ion boundary had migrated 50 mm towards the anode from the sample slots.

After electrophoresis the sample slots were cleared of liquid and the gel cut horizontally, using a taut-wire slicer (Shandon), into four slices (150 x 100 x 1·5 mm). Each slice was then stained separately to reveal the various protein and enzyme fractions.

Staining Reactions

Protein. One slice was submerged for 1 hr in a solution of 0·7% amido black in 7% acetic acid. The residual dye was subsequently removed by repeated washing with 7% acetic acid. In some cases light green (0·4% in 7% acetic acid) was used to stain-protein. ***Esterusewas*** demonstrated by immersing a slice in 100 ml of phosphate buffer (0·1 M, pH 6.3) containing or-naohthylacetate, 100 mg, and diazo blue B (Michrome 250-E. Gurr), 50 mg, for 5–10 min.' The background was clarified by washing with water. ***Peroxidase*** was demonstrated by immersing a slice in 100 ml of acetate buffer (0·1 M, pH 4.4) containing o-dianisidine, 100 mg, and H_2O_2 (30 vols.), 0·5 ml. Other reagents, e.g. benzidine, guaiacol, catechol, may also be used, but *O*-dianisidine gives the most intense and stable reaction product. ***Catalase*** activity was observed as areas of oxygen evolution during evaluation of peroxidase activity. ***Amylase.*** To observe amylase, 0·6% soluble starch is added to the gel during preparation. A slice is incubated for 1 hr in phosphate buffer (0·1 M, pH 6·4) and then transferred to a solution of I_2 (0.005 %) in 1·5 KI.

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[46] 0. H. LOWRY, N. J. ROSEBOROUGH, A. L. FARR and R. J. RANDALL, *J. Biol. Chem.* 193,265 (1951).
[47] B. M. LUND, *J. Gen. Microbiol.* ***40,413 (1965).***