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Chemosystematic studies in *Daucus* and allied genera

V. H. HEYWOOD

Introduction

The results summarized in this paper form part of a much wider series of studies on the Umbelliferae undertaken during the past six years by the author and his collaborators initially at Liverpool and later at Reading. The main objectives of this research have been to assess the role of chemical data in classifications, the feasibility of producing genuine phenetic classifications by numerical means using mixed data from a wide range of sources and the value of "micro-characters" of fruits in generic and tribal classification. The main approaches have been chemosystematic (CROWDEN, HARBORNE & HEYWOOD 1969), taximetric (MCNEILL, PARKER & HEYWOOD 1969) and morphological-anatomical-cytological, including scanning electron microscopy (HEYWOOD 1968, 1969, 1971; HEYWOOD & DAKSHINI 1971). Recently the work has been extended to cover floral biology and breeding systems. The aim of this paper is to discuss some of the principal results of our phytochemical studies and consider their taxonomic significance.

The Caucalideae

Although we have made a general phytochemical survey of the *Umbelliferae* with a view to establishing its "chemosystematic profile" (cf. HARBORNE 1967: 180-183), our work has been concentrated on the part of the family which contains spiny-fruited species with primary and secondary ridges. This group was treated by BENTHAM (in BENTHAM & HOOKER 1867) as the tribe *Caucalideae*, followed by BOISSIER (1872). It comprises 18 genera and about 80 species (table 1), mainly distributed in Europe, the Mediterranean region and S. W. Asia with only a few species occurring elsewhere, as in *Caucalis*, *Torilis*, and *Daucus*. The latter two genera include several weed species, some of which have spread to the new world.

In contrast with BENTHAM's and BOISSIER's treatment, DRUDE (1897-1898) placed these plants in two groups which were widely separated in his tribal arrangement of

<i>Agrocharis</i> Hochst. (1)	<i>Daucus</i> L. sect. <i>Ctenolophus</i> Pomel (2)
<i>Albertia</i> Regel & Schmal. (1)	<i>Exoacantha</i> Labill. (2)
<i>Ammiopsis</i> Boiss. (1-2)	<i>Lisaea</i> Boiss. (4-5)
<i>Ammodaucus</i> Coss. & Durieu (1)	<i>Orlaya</i> Hoffm. (4)
<i>Artemia</i> L. (1)	<i>Psammogeton</i> Edgew. (5)
<i>Astrodaucus</i> Drude (3)	<i>Pseudorlaya</i> Murb. (2)
<i>Caucalis</i> L. (1-2)	<i>Torilis</i> Adanson
<i>Chaetosciadium</i> Boiss. (1)	subgenus <i>Torilis</i> (3)
<i>Cuminum</i> L.	sect. <i>Torilis</i> (3)
<i>Daucus</i> L.	sect. <i>Lappularia</i> (Pomel) Thell. (6-9)
sect. <i>Daucus</i> (14)	subgenus <i>Pseudocaucalis</i> Drude (6-7)
sect. <i>Durieua</i> (Boiss. & Reuter)	<i>Turgenia</i> Hoffm. (1)
Drude (7)	<i>Turgeniopsis</i> Boiss. (1)

Table 1. — Genera of the Caecalideae (and their number of species).

the family — the tribe (12) *Duceae* containing *Daucus*, *Artemia*, *Ammodaucus* and *Exoacantha* and the tribe (6) *Scandiceae* sub-tribe *Caucalidinae* containing *Torilis*, *Chaetosciadium*, *Ammiopsis*, *Pseudorlaya*, *Astrodaucus*, *Turgeniopsis*, *Caucalis*, *Lisaea* and *Orlaya*. The fruit differences separating the two groups are far from clear as is witnessed by the inclusion in the *Caucalidinae* by DRUDE of two genera, *Orlaya* and *Astrodaucus*, which BENTHAM incorporated in *Daucus*. Apart from the problem of tribal boundaries, the circumscription of genera is difficult and the number of genera recognised varies from 8 to 20.

Chemosystematic studies

1. Phenolic compounds.

As a first step in our phytochemical studies a general survey was made of the distribution of phenolic substances in the family as a whole. Leaf phenolics were surveyed in 300 species representing some 147 genera (CROWDEN, HARBORNE & HEYWOOD 1969). Due to the difficulties of obtaining and cultivating the large numbers of species that a representative sample like this would involve, the sampling was largely done on herbarium specimens, although fresh material was also used whenever possible. Some of the herbarium specimens sampled were well over 100 years old but they still proved satisfactory, a point that is worth stressing in this kind of work. It is, in fact, preferable to use correctly identified herbarium specimens to fresh material raised from botanic garden or even wild seed of known origin if the latter is not later adequately verified.

It was found that most species had either flavonols or flavones, not both. *Daucus durieua* Lange was one of the rare exceptions; it contained the flavonols quercetin and kaempferol and the flavone luteolin (see table 2). This is interesting in that

	<i>Flavonol</i>		<i>Flavone</i>
	Qu	Km	Lu
<i>Caucalidinae</i>			
<i>Astrodaucus</i> (3)			+ ¹
<i>Caucalis</i> (1)			+
<i>Chaetosciadium</i> (1)			+ ²
<i>Lisaea</i> (1)			+
<i>Orlaya</i> (2)			+ ¹
<i>Psammogeton canescens</i>			
<i>Psammogeton setifolium</i>			+
<i>Torilis</i> (6)			+ ¹
<i>Turgenia</i>			+
<i>Turgeniopsis</i>	+		
<i>Dauceae</i>			
<i>Artemia</i> (1)		+	
<i>Daucus aureus</i>			+
<i>D. broteri</i>			+
<i>D. carota</i>			+ ¹
<i>D. crinitus</i>			+
<i>D. durieua</i>	+	+	+
<i>D. glochidiatus</i>	+		
<i>D. muricatus</i>			+
<i>D. setifolius</i>	+		
<i>Pseudorlaya</i> (1)			+

¹As 7-glucoside.

²As 5- and 7-glucoside.

Table 2. — Leaf phenolics. Qu = quercetin, Km = kaempferol, Lu = luteoline. Numbers in brackets refers to the number of species sampled.

there are grounds for regarding this species and the section to which it belongs (sect. *Durieua* (Boiss. & Reuter) Drude) as generically distinct from the rest of *Daucus*. On the other hand, *D. glochidiatus*, also in sect. *Durieua*, was found to contain only quercetin.

The pattern of flavonoids in the fruits is much richer than that in the leaves and on the basis of seed flavonoids tentative groupings can be made (table 3).

Although not too much should be read into these groupings it is interesting to note that the monotypic genus *Turgenia* stands on its own and is quite distinct at the same time from *Caucalis* in which it has frequently been placed. Protein-band characteristics also separate these two groups (see below). The chromosome number of *Turgenia* is also distinctive as is the microstructure of the mericarps (HEYWOOD 1968, 1969).

It is also noteworthy that the genus *Chaetosciadium* is similar to *Torilis* in its flavonoid pattern — both have Luteolin 5-glucoside in the leaves and there are other

similarities. Their protein band patterns too are similar (see below). Again this confirms their close relationship on other grounds despite the very characteristic mericarp ornamentation of *Chaetosciadium* (HEYWOOD 1971).

Variation is found between the different species of *Daucus* and between different subspecies of *Daucus carota* but further work is needed before useful conclusions can be drawn.

2. Polyacetylenes.

In a survey of polyacetylenes in the Umbelliferae we found that they are widespread in both the *Scandiceae-Caucalidinae* (occurring in *Astrodaucus*, *Chaetosciadium*, *Orlaya*, *Torilis* 3 spp., and *Turgenia*) and in the *Dauceae* (*Daucus* 5 spp., and *Pseudorlaya*). This confirms the similarity of these two groups although it may be found that polyacetylenes are universally present in the family. Again variation in polyacetylene pattern within the genus *Daucus* has been noted but further study is needed.

3. Seed proteins.

An intensive survey of seed proteins in the *Umbelliferae* using acrylamide gel electrophoresis gave valuable results. 174 seed samples, representing 99 species and 39 genera were examined. The protein patterns were studied by using amido black and light green as staining reagents and enzymatic activity was investigated with regard to esterase, peroxidase and catalase.

The slab or block technique was employed rather than tubes, as it allows a more direct comparison to be made between the various samples being studied. It also

Group	Tribe	Genera	Compounds
I.	D	<i>Artedia</i> ($x = 8$)	quercetin, kaempferol
	S	<i>Astrodaucus</i> ($x = 10$)	quercetin,
II.	D	<i>Daucus</i> ($x = 9, 10, 11$)	quercetin, kaempferol, luteolin,
	S	<i>Orlaya</i> ($x = 9$)	apigenin (chrysoeriol in
	D	<i>Pseudorlaya</i> ($x = 8$)	<i>Daucus aureus</i>)
III.	S	<i>Caucalis</i> ($x = 10$)	luteolin (apigenin in <i>Torilis tenella</i>
	S	<i>Torilis</i> ($x = 6, 12$)	and <i>Chaetosciadium</i> , both of which
	S	<i>Chaetosciadium</i> ($x = 6$)	genera have Lu 5-glucoside in leaf)
IV.	S	<i>Turgenia</i> ($x = 16$)	luteolin as 4'-glucoside, chrysoeriol as 7-glucoside

Table 3. — Tentative grouping of genera, based on fruit flavonoids. D = *Dauceae*; S = *Scandiceae-Caucalidinae*; x = haploid chromosome number.

has the advantage that the gel block can be cut horizontally into slices which can then be stained differentially for study of different enzymic activities.

The band patterns obtained, although inherently difficult to interpret fully, showed great taxonomic interest. The general protein profiles of the *Caucalidinae* and the *Douceae* were similar although when stained with amido black most of the former showed cationic proteins with heavily staining bands, except *Turgenia latifolia*, *Orlaya grandiflora* (in 2 out of 3 accessions) and *Pseudorlaya*. None of the other tribes, including the *Douceae*, showed these cathodic-migrating bands using amido-black staining. Thus *Daucus* shares this negative feature with *Orlaya* and *Pseudorlaya*, which is in agreement with the close association of these genera in other respects. *Turgenia* is very much an isolated genus and its protein profile is quite distinctive apart from the absence of back-migrating bands. *Torilis nodosa* which is somewhat anomalous in the genus in respect of fruit position and structure also has a fairly distinctive band pattern. *Torilis* and *Chaetosciadium* show close similarities in their protein patterns.

On the other hand enzymatic assay proved to be a more sensitive means than amido-black staining of detecting protein differences. In the *Caucalidinae*, *Caucalis*, *Astrodaucus* and *Orlaya* showed characteristic esterase patterns with two strong bands unlike other members of the group and unlike the other tribes including the *Douceae*, although *Daucus carota* (spontaneous) showed two strong bands. In the case of peroxidase reaction, there was little or none in the *Caucalidinae* and modest reactions in the *Douceae*, *Daucus* showing some back-migrating bands.

Further detailed studies in the *Caucalidinae* and *Douceae* protein patterns have been made and these will be published separately (CROWDEN, HARBORNE & HEYWOOD, in prep.). They suggest that the acrylamide gel technique is one of the more useful analytical methods available in this complex for taxonomic purposes. Each genus can be quite well characterised by its protein patterns and discrepancies tend to coincide with areas of taxonomic doubt.

4. Essential oils.

Recently we have studied the essential oils present in the fruits of the *Caucalidinae* and *Douceae* and results will be published separately. They tend to confirm the conclusions that can be drawn from the use of other chemical and taxonomic features. Thus, sesquiterpene patterns show links between *Daucus* and *Torilis*, although *Daucus* species tend as a whole to be much richer in components than the other taxa.

Discussion and Conclusions

Preliminary phytochemical studies have indicated that the various classes of chemical constituents so far surveyed in the *Umbelliferae* are of considerable potential value for taxonomic purposes, especially at the tribal and generic levels. The range of phenolic compounds found is, however, not sufficiently wide to be of much value

in characterising genera although there are a few exceptions, as in the case of *Turgenia latifolia*, which is readily separable from *Caucalis* in which it is sometimes placed, and the example of *Torilis* and *Chaetosciadium* whose chemical similarity confirms their relationship despite the anomalous structure of the mericarp spines. Macromolecular data, even though expressed in the form of unidentified protein bands, show considerable promise and may prove to be of more value than the phenolic compounds in resolving problems of generic and specific status. On the other hand, at the specific level two-dimensional chromatograms of phenolic compounds are probably the most useful chemical means for characterising different taxa (HARBORNE, unpublished).

A major problem involved in the utilization of phytochemical data in classification is how to process them in terms of characters or units of information (cf. RUNEMARK 1968; HEYWOOD 1968a). Spots on chromatograms can be handled as normal presence or absence characters in a numerical taxonomic programme¹ if chemically identified, but no quantitative indication is given by such a method. At the species level, the actual pattern of spots may be more valuable than the simple listing of the compounds represented. In the case of protein bands similar problems are found — there are various elements in an electrophoretogram that should be taken into account: the position of the bands, the thickness of the bands, their intensity and their overall pattern. Simple numerical techniques have been employed for their comparison (VAUGHAN & DENFORD 1968) but these fall far short of a complete description of the data.

The chemical data referred to in this paper are being used along with information from several other sources in an attempt to assess the overall relationships of the *Caucalideae*. Only then will it be possible to assess their value in classification.

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¹ Five chemical characters were used in the pilot taximetric classification of the *Caucalideae* by MCNEILL, PARKER & HEYWOOD (1969).

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