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ESSENTIAL OILS IN THE SPINY-FRUITED UMBELLIFERAE

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Abstract—The fruits of some 24 species of the tribe Caucalideae of the Umbelliferae were surveyed for essential oils by GLC. The patterns obtained are particularly useful for separating the tribe into genera. *Daucus*, by far the richest taxon in essential oils, is distinguished by the presence of carotol, geranyl acetate and two unidentified components as major constituents. Within *Daucus*, the overall pattern is relatively similar, although the seven species studied can easily be separated on the basis of qualitative and quantitative variations in oil components. Carotol was also found in *Torilis* and *Turgenia* but only in trace amounts. *Daucus* and *Pseudorlaya*, which are morphologically fairly similar, are chemically related in containing four compounds in common, α- and β-pinene, limonene and myristicin. The genus *Torilis* is characterized by the presence of an unidentified sesquiterpene $C_{15}H_{22}$. Furthermore, the five *Torilis* species examined can be distinguished from each other by the presence/absence of biphenyl, carotol and four unidentified components. The genera *Caucalis* and *Turgenia* also have their distinctive oils, while *Artedia* can be separated bv its lack of volatile oil in the fruit. Finally, the essential oil patterns correlate well with other chemical differences which distinguish the genera within the tribe.

INTRODUCTION

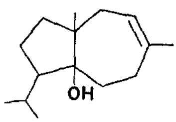
THE TRIBE Caucalideae (14 genera, 80–100 species) as described by Bentham and Hooker[1] and by Boissier[2] contains practically all the umbellifer species which have spines on their fruits. These taxa are redistributed by Drude[3] between the tribe Dauceae and the subtribe Caucalineae of the Scandiceae. The present study of the essential oil patterns in the fruits is part of a multivariate approach to this small but taxonomically controversial group, in which is it hoped to accumulate sufficient morphological and chemical characters to apply numerical methods to systematic classification. Previous publications have included a general survey of the family Umbelliferae in leaf and fruit for flavonoids,[4,5] proteins[4] and for the presence of myristicin[6] and also a preliminary numerical analysis of the Caucalideae incorporating five chemical characters.[7]

Previous work of the essential oils of the Caucalideae has been confined almost entirely to the cultivated carrot *Daucus carota* spp. *sativa*. Buttery *et al.*[8,9] identified some 23

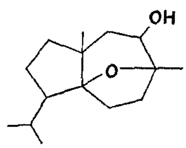
[1] G. BENTHAM and J. D. HOOKER, *Genera Plantarum* **1**, 859 (1867).
[2] E. BOISSIER, *Flora Orientalis* **2**, 819 (1872).
[3] O. DRUDE, in *Die Natürlichen Pflanzenfamilien* (edited by A. ENGLER and K. PRANTL), Vol. 3, p. 63 (1897–8).
[4] R. K. CROWDEN, J. B. HARBORNE and V. H. HEYWOOD, *Phytochem.* **8**, 1963 (1969).
[5] J. B. HARBORNE and C. A. WILLIAMS, *Phytochem.* **11**, 1741 (1972).
[6] J. B. HARBORNE, V. H. HEYWOOD and C. A. WILLIAMS, *Phytochem.* **8**, 1729 (1969).
[7] J. MCNEILL, P. F. PARKER and V. H. HEYWOOD, in *Numerical Taxonomy* (edited by A. J. COLE), pp. 129–145, Academic Press, London (1969).
[8] R. M. SEIFERT, R. G. BUTTERY and L. LING, *J. Sci. Food Agric.* **19**, 383 (1968).
[9] R. G. BUTTERY, R. M. SEIFERT, D. G. GUADAGNI, D. R. BLACK and L. C. LING, *J. Agric. Food Chem.* **16**, 1009 (1968).

components in the seed oil and 28 in the root oil, during a search for the compounds giving the characteristic fresh carrot aroma. Sabinene and myrcene were found to be mainly responsible for the root aroma while the only odour principle definitely identified in the fruit oil was 2-nonenal; a range of mainly C_{15} hydrocarbons and their derivatives were also reported. Other workers[10,11] have shown that carrot seed oil varies qualitatively and quantitatively within different varieties and also that within the fruits of the wild carrot *D. carota* spp. *carota*, there are two chemical races, one with mainly geranyl acetate[12] and the other with carotol (I) and daucol (II) as major constituents.[13,14]

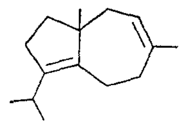
Carotol (I), daucol (II) and daucene (III) are three azulene-based sesquiterpenes which appear to be characteristic of the domestic carrot, although one of them, carotol, has also been reported in the seed oil of an unrelated umbellifer, *Seseli tortuosum*.[15] The only other member of the Caucalideae to have been examined previously for essential oils, namely *Torilis scabra* (syn. *Caucalis scabra*), yielded a related oxygenated sesquiterpene caucalol (IV).[16]



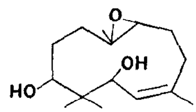
Carotol (I)



Daucol (II)



Daucene (III)



Caucalol (IV)

RESULTS

A preliminary comparison of essential oils in roots and fruits of several wild species of the Caucalideae indicated that fruits were a richer source of oil than roots, so that all subsequent work was carried on mature fruits. The results of essential oil analyses of fruits from some 24 species, for which sufficient seed was available, are presented in Table 1. α- and β-pinene, limonene, geranyl acetate and biphenyl were identified by co-chromatography with authentic samples by GLC on SE30 and carbowax 20M columns. Carotol (I), caryophyllene and myristicin were characterized by both GLC co-chromatography and by MS measurements, following purification by preparative TLC. Geranyl acetate, caryophyllene and myristicin were also identified by TLC on silica gel using benzene and spraying with vanillin–H_2SO_4.[6]

Mass spectral measurements were also made on purified samples of three of the fifteen major unidentified constituents *S*1–*S*15 (Table 1). *S*3, present in all *Daucus* species studied, has MW 204 and may be identical to daucene (III) ($C_{15}H_{24}$, MW 204), which has previously been reported in *D. carota* by Pigulevskii and Kovaleva.[17] *S*9, a trace constituent in *Daucus* but a major component in *Torilis*, has MW 202 ($C_{15}H_{22}$) and a typical sesquiterpene MS fragmentation pattern (see Experimental) and may be α-curcumene. This compound has,

[10] C. V. PIGULEVSKII, V. I. KOVALEVA and D. V. MOTSKUS, *Trudy Vses. Sov. Vilnyus Akad Nank Litovsk, SSR.* 153 (1959).
[11] F. SÖRM and L. URBANEK, *Coll. Czech. Chem. Commun.* **13**, 49 (1948).
[12] E. STAHL, *Arch. Pharm.* **297**, 500 (1964).
[13] Y. P. TALWAR, M. C. NIGAM and K. L. HANDA, *Indian Oil Soap J.* **28**, 249 (1963).
[14] E. STAHL and H. JORK, *Arch. Pharm.* **298**, 273 (1965).
[15] G. CHIURDOGLU, *Tetrahedron* **8**, 271 (1960).
[16] S. SASAKI, Y. ITAGAKI, H. MORIYAMA, K. NAKANISHI, E. WATANABE and T. AOYAMA, *Tetrahedron Letters* 623 (1966).
[17] G. V. PIGULEVSKII and V. I. KOVALEVA, *Dolk. Akad. Nauk URSS*, **141**, 1384 (1961).

in fact, been reported in carrot by Pigulevskii *et al.*[18] although its presence was not confirmed by Buttery *et al.*[8,9] The third compound *S*10 characteristic of *D. carota* subsp. *gummifer*, has the formula $C_{15}H_{24}O$ (Found: 220·1828, MW required 220·1827) and appears to be a sesquiterpene alcohol new to *Daucus*.

All the results in Table 1 are expressed as a percentage of total volatile oil. There is, however, great variation in the actual amount of oil present. Thus, the total oil present in fruits of three representative taxa *D. carota* subsp. *gummifer*, *Torilis arvensis* and *Orlaya grandiflora* were found to be 14, 8 and 5·5%/dry wt respectively, while the ratios of amounts of volatile oils in the three samples were in the same descending order, i.e. 5:3:2.

The results all show that *Daucus* is by far the richest genus in the group both in variety and quantity of volatile oils; it is characterized by the presence of geranyl acetate and carotol and of two unidentified components *S*4 and *S*5. Carotol, a major constituent in most *Daucus* taxa, does occur in trace amounts in *Torilis* and *Turgenia*, where its identity is based only on retention times. It is of interest that the two subspecies of the wild *Daucus carota* examined both contain geranyl acetate and carotol. Two further samples from Hampshire of one of these subspecies, *carota*, contained only geranyl acetate, confirming the presence of geographical races in this taxon. Daucol (II), reported by other workers[8,9] as a constituent of the cultivated carrot, was not found but this may have been because no authentic marker was available for comparison. It is also of note that the subspecies *gummifer* is easily distinguished by having a large quantity of compounds *S*10 and *S*11, neither of which occur anywhere else in the group.

α- and β-pinene and limonene are present in all but one species of *Daucus* and otherwise only in *Pseudorlaya pumila*, *Pseudorlaya* being a genus which is often closely linked to *Daucus* on morphological grounds.[3] However, it may be noted that in most *Daucus* species β-pinene is present in higher concentration than α-pinene, whereas the reverse is true in *Pseudorlaya*.

Another chemical link between *Daucus* and *Pseudorlaya* is the presence of myristicin. In a previous survey,[6] this phenylpropanoid was detected by TLC in fruits of both known species of *Pseudorlaya*, *P. pumila* and *P. minuscula*, and in *Daucus glochidiatus*. Myristicin also occurs in root of, but not seed of, the cultivated carrot. This link has been strengthened by the discovery in the course of the present work of myristicin in fruit, leaf and root of another *Daucus* species, namely *D. australis*. This distribution of myristicin throughout the organs of the plant as in *D. australis* is unusual but it also happens in both *Pseudorlaya* species. Myristicin thus not only links the two genera, but, within *Daucus*, it links *D. glochidiatus* and *D. australis*, two cytologically related taxa occurring in Australasia,[19] with the cultivated (but not the wild) carrot of Europe. Unfortunately, lack of seed material has so far prevented a detailed study of the other oil constituents of the Australasian *Daucus* species.

Orlaya species are poor in volatile oils and lack any single component which separates them from other genera. However, two of the three species have biphenyl, which is otherwise rare in the group. It is worth remarking that *O. daucorlaya* and *O. grandiflora* which are difficult to distinguish taxonomically by fruit morphology, are clearly different in the oil pattern. Previous chemical studies have shown that the fruit flavonoids of these two *Orlaya* species are generally the same, although there is one substance, quercetin 7-glucoside, which is present in *O. grandiflora* but absent from *O. daucorlaya*.[5]

[18] L. V. Pigulevskii and V. I. Kovaleva, *Drug Cosmet. Ind.* **97**, 749 (1965).
[19] D. M. Moore, unpublished results.

TABLE 1. GLC OF ESSENTIAL

Species	Accession No.	α-Pinene	β-Pinene	Limonene	Geranyl Acetate	Caryophyllene
Daucus aureus Desf.	3402	—	—	—	—	1
D. aureus Desf.	3403	—	—	—	4	2
D. carota ssp. *sativa*		4	10	2	—	2
D. carota L. ssp. *carota*	1692	2	14	1	23	2
D. carota ssp. *gummifer*	2083	1	12	1	6	—
D. crinitus Desf.	3390	1	2	1	10	8
D. littoralis Sibth. & Sm.	2948	7	17	22	—	7
D. muricatus (L.) L.	2458	1	1	—	—	—
D. pusillus Michx.	3395	2	4	1	6	2
D. setifolius Desf.	3394	3	1	5	9	1
Orlaya daucorlaya Murb.	2861	—	—	—	—	10
O. grandiflora (L.) Hoffm.	1763	—	—	—	—	—
O. kochii Heywood	3302	—	1	—	—	—
Pseudorlaya pumila (L.) Grande	3605	11	1	6	—	—
Torilis arvenis (Hudson) Link	1784	—	—	—	—	—
T. japonica (Houtt.) DC.	3194	—	—	—	—	—
T. leptophylla (L.) Reichenb.	2056	—	—	—	—	—
T. nodosa (L.) Gaertner	3123	—	—	—	—	—
T. tenella (Delile) Reichenb.	3344	—	—	—	—	—

Caucalis platycarpos 3405 contained β-pinene (9) and *S*14 (25) and *Turgenia latifolia* 2016 contained carotol (9) and *S*15 (17). The results are expressed as percentage of volatile oil and the retention times on S.E. 30 and carbowax 20 M columns are as follows: α-pinene 4·2/4·1; β-pinene 4·8/5·5; limonene 5·8/7·0; geranyl acetate 11·8, 12·1/18·0; 18·6, caryophyllene 13·2/15·0; carotol 17/22·6; *S*1 13·9/15·5; *S*2 20/26·4;

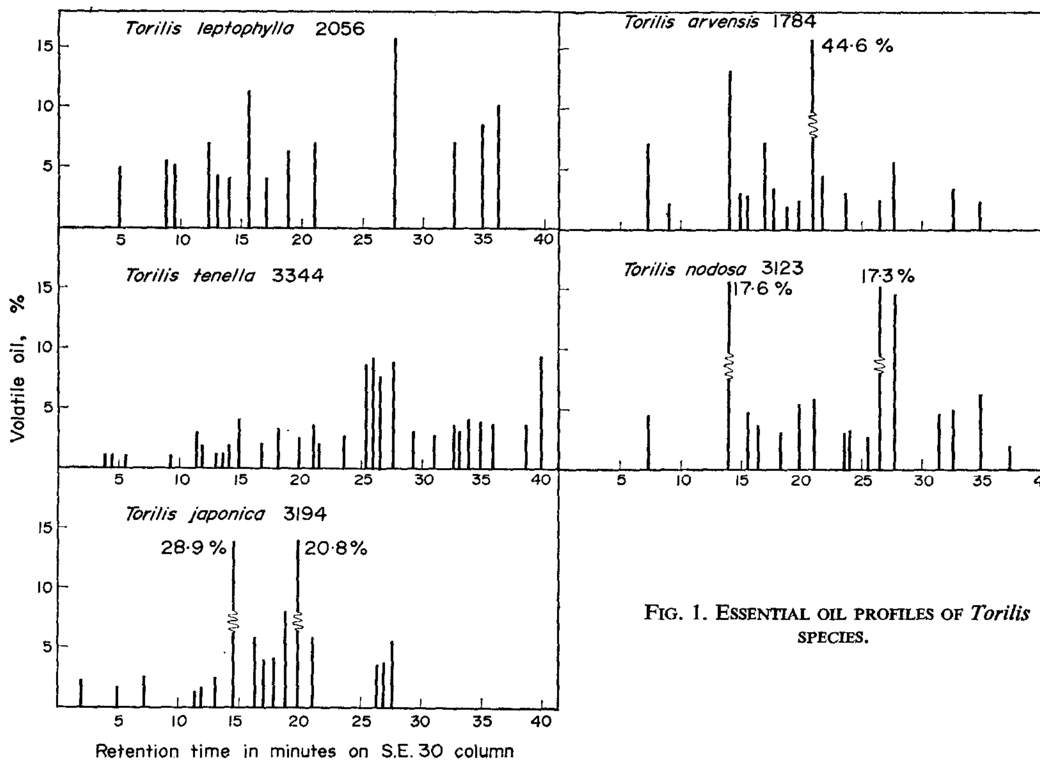


FIG. 1. ESSENTIAL OIL PROFILES OF *Torilis* SPECIES.

OILS IN CAUCALIDEAE FRUITS

Carotol	*S*1	*S*2	*S*3	*S*4	*S*5	*S*6	*S*7	*S*8	*S*9	Other components
4	—	4	1	29	13	—	—	—	2	
5	2	3	6	8	5	—	5	—	—	
4	1	7	3	6	—	—	4	—	3	
20	4	2	12	2	2	—	—	—	—	
12	—	2	9	6	2	—	—	—	1	*S*10–7 *S*11–9
17	—	2	5	1	1	6	18	—	1	
3	2	1	2	4	3	1	1	—	—	
—	—	2	1	11	11	—	—	1	2	
38	4	4	5	4	4	6	4	—	—	
32	3	1	5	2	4	—	—	—	—	
—	—	5	—	—	—	5	—	1	1	Biphenyl–2
—	—	—	11	—	—	15	—	—	—	
—	—	2	4	—	—	4	—	9	3	Biphenyl–1
—	—	1	—	—	—	1	—	—	1	*S*12–47 Myristicin–9
7	—	—	13	—	—	—	—	—	45	
3	—	—	29	—	—	—	4	—	6	Biphenyl–2 *S*13–21
4	4	—	—	—	—	4	—	—	7	
—	—	—	—	—	—	18	—	—	6	
1	1	—	4	—	—	—	—	—	4	Biphenyl–1

*S*3 15/17·8; *S*4 27/21; *S*5 28·8/23; *S*6 13·8/17·2; *S*7 17·6/23·9; *S*8 14·7/27·2; *S*9 21/27·9; *S*10 17·7/25·4; *S*11 15/28·9; *S*12 15·3/25·9; *S*13 20/23·8; *S*14 26·5/20·8; *S*15 26·8/22·2; myristicin 14·2/14·4; biphenyl 13·1/21·9 (for column details see Experimental).

Torilis species commonly contain the unknown sesquiterpene *S*9 and, together with most other taxa in the group, the unknown *S*3. *Torilis* fruits, although not containing a great quantity of oil, do show considerable variations from species to species. This variation is illustrated by Fig. 1, which shows GLC patterns and includes a number of minor oil components not listed in Table 1. This figure indicates the potential usefulness of oil patterns for studying relationships at the species level. Thus, in terms of overall chemical similarity, *T. arvenis*, *T. nodosa* and *T. leptophylla* seem to be related. On the other hand, *T. tenella*, which has a characteristic elongated fruit, is different in having most of its oil with R_t between 25 and 30 min. Again, *T. japonica*, the only species which is biennial instead of annual, is different from the others in lacking oils above a R_t of 28 min.

Essential oils were also recorded (Table 1, footnote) in fruits of species of two other genera, *Caucalis platycarpos* and *Turgenia latifolia*. Since *Caucalis* and *Turgenia* were once included together in the same genus, it is satisfying to find that their separation into different genera is supported by significant differences in their oil content. Finally, it may be mentioned that *Artedia squamata*, another monotypic species like *Turgenia*, is distinctive in completely lacking any volatile oil in the fruits.

DISCUSSION

The results of this essential oil survey show that the most significant differences in terpenoid constituents occur at the generic level within the tribe Caucalideae (Table 2). The various genera can clearly be separated, usually on the basis of several oil components, and the fact that some of these are still unidentified does not detract from their value as generic markers. It is, moreover, significant that these oil results correlate well with those of earlier surveys in the tribe for leaf and fruit flavonoids and for protein and enzyme patterns.[4,5,20] The different kinds of chemical data reinforce each other in supporting the present generic limits, some of which have been much disputed in the past.

TABLE 2. DISTRIBUTION OF MONOTERPENES AND SESQUITERPENES IN THE CAUCALIDEAE FRUITS

Terpenoid	Genus*					
	Torilis	*Orlaya*	*Pseudorlaya*	*Daucus*	*Caucalis*	*Turgenia*
α-Pinene	−	−	+	+	−	−
β-Pinene	−	−	+	+	+	−
Limonene	−	−	+	+	−	−
Biphenyl	+	+	−	−	−	−
Geranyl acetate	−	−	−	+	−	−
Caryophyllene	−	+	−	+	−	−
Carotol	+	−	−	+	−	+
Unknown *S*1	+	−	−	+	−	−
Unknown *S*2	−	+	−	+	−	−
Unknown *S*3	+	+	−	+	+	+
Unknown *S*4	−	−	−	+	−	−
Unknown *S*5	−	−	−	+	−	−

* Based on an analysis of fruit essential oils from five *Torilis* spp., eleven *Daucus* spp., and three *Orlaya* spp.; remaining genera analysed from a single accession. Only major components are included. For details of Unknowns *S*1–*S*5 see Results and for column details see Experimental.

With regard to the question of whether the Caucalideae are a homogenous group or not, too little is known of essential oil patterns in other tribes of the Umbelliferae for a certain answer to be given. However, the results obtained here, particularly the regular distribution of carotol, caryophyllene and the unknown component *S*3 in *Daucus*, *Torilis* and *Orlaya* do suggest that the tribe, as constituted by Bentham and Hooker,[1] has a characteristic essential oil pattern, which may differ from that in other tribes. At least, an essential oil survey of the Laserpitieae, a tribe placed close to the Dauceae by Drude,[3] has revealed a different range of terpenoids.[21]

Although *Daucus* is the largest and most variable genus within the group, nevertheless it is still remarkable in the quantity and variety of volatile oils in the fruits. The fact that a range of oils occur in the wild *Daucus* species means that there is a reservoir of variability in oils which could be used in breeding experiments to introduce new flavours into the domestic carrot.

Attention has already been drawn (see Results) to the remarkable occurrence of myristicin in the essential oil fraction, not only of fruits but of stems, leaves and roots in a few members of the Caucalideae, notably *Daucus australis*. This discovery may be of significance in insect-plant interactions, since Berüter and Städler[22] have recently shown that a closely related phenylpropanoid, methylisoeugenol, is an oviposition stimulant in carrot leaves for the carrot rust fly.

[20] J. B. HARBORNE, in *Biology and Chemistry of the Umbelliferae* (edited by V. H. HEYWOOD), pp. 293–314, Academic Press, London (1971).
[21] J. W. ADCOCK, Ph.D. Thesis, University of London (1971).
[22] J. BERÜTER and E. STÄDLER, *Z. Naturf.* **266**, 339 (1971).

The results of this survey also suggest that oil patterns may be of considerable taxonomic value for studying relationships at the species level. For example, *Torilis*, a genus of some 10–15 species, shows relatively little morphological variation between species. Chemically, the five species here examined (Fig. 1) do show many differences in terpenoid constituents, which could be used in systematic comparisons.

Our results are mainly based on analysis of single accessions of each species and may, therefore, have to be slightly modified when further accessions become available for study, since intraspecific variation in oil components is well known in some umbellifer fruits. Such variation occurs, for example, in *Daucus carota*, as has been confirmed in these studies. Such chemical variation is, however, almost certainly the exception rather than the rule. Thus, in an analysis of fruit essential oils in the neighbouring Laserpitieae (a tribe of some 30–40 species) several accessions of each species were examined and, with very few exceptions, remarkably consistent species patterns were recorded.[21]

EXPERIMENTAL

Plant material. Mature fruits were collected from plants of known origin, grown under glass in the University of Reading, voucher specimens being deposited in the Department of Botany Herbarium.

Chemicals. A sample of carotol was kindly provided by B. M. Lawrence (Stange Canada Ltd.), the remaining authentic markers being obtained commercially.

GLC. Fruits, ground in a Molineux spice grinder, were then repeatedly extracted with Et_2O, the extract being evaporated to an oily residue. A known volume of this oil was redissolved in an equal quantity of Et_2O and 1 μl injected into the column of a flame ionization chromatograph programmed at 5°/min from 60–260° on 5% SE 30 on Chromport 80–90, 122 cm × 6·7 mm, and 60–160° on 10% Carbowax 20 M on Celite (A.B.S.), 183 cm × 6·7 mm. Carrier gas was N_2 at 35 ml/min and attenuation *ca.* 10 × 64. The oils were purified by TLC on silica gel in benzene and located with vanillin and ethanolic H_2SO_4. Myristicin was identified by previously described TLC procedures.[6]

MS measurements. These were kindly carried out by the SRC Physical Measurements Unit, Harwell. Samples of carotol, caryophyllene and myristicin isolated from the fruits (see Table 1) gave parent ions and fragmentation patterns which agreed closely with published data.[8,9] MS data for the three unidentified sesquiterpenes were as follows (parent ions, followed by major ions in order of intensity): *S*3, 204, 93, 147, 161, 133, 79; *S*9, 202, 134, 93, 119, 107, 79, 67, 105, 91, 55, 159, 187; *S*10, 220, 109, 82, 135, 43, 41, 59, 45, 177.

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Key Word Index—Umbelliferae; Caucalideae; chemotaxonomy; essential oils; carotol; geranyl acetate; myristicin.