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Umbelliferous fruit identification by thin-layer chromatography

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Unknown umbelliferous fruits, including powders, may be identified by thin-layer chromatography of petroleum extracts on silicic acid containing fluorescein. The constituents of the essential oils thus extracted are usually diagnostic. The method is particularly useful in distinguishing Indian dill (*Anethum sowa* Roxb.) from dill (*A. graveolens* L.), and will detect an admixture of one part of the former in four parts of the latter.

WHILST whole umbelliferous fruits can be distinguished more or less readily, one from another, powdered fruits present a much more difficult problem, and in the case of the two dills, *Anethum graveolens* L. and *A. sowa* Roxb., they appear to be indistinguishable. As a method of examining quickly the constituents of such fruits, thin-layer chromatography seemed a suitable method. Silicic acid—starch "chromatostrips" (Kirchner, Miller & Keller, 1951) provided one of the earliest forms of thin-layer chromatograms; these were devised to study terpenes including limonene and carvone, and were subsequently used for essential oils. By such means the oils distilled from the following umbelliferous fruits have been examined: anise (Paris & Godon 1961, Schrantz, Lopmeri, Strömmer, Salonen & Brunni 1962, Wellendorff 1963); caraway (El-deeb, Karawya & Wahba 1962); coriander (Pertsev & Pivnenko 1962, Wellendorff 1963); and fennel (Schrantz & others 1962; Wellendorff, 1963). The method has been applied here to petroleum extracts of these and other umbelliferous fruits.

Experimental

Silicic acid ("Kieselgel G" Macherey, Nagel) was spread 0·25 mm thick after mixing with 0·05% aqueous fluorescein sodium (25 g to 50 ml). The plates were heated at 105° for 30 min, then stored over a desiccant. The running solvent, chloroform: benzene, 1 : 1 by volume was used in a tank having the walls lined with absorbent paper wetted with the solvent.

The umbelliferous fruits (500 mg) were extracted with light petroleum* (5 ml) at room temperature. Powdered fruits were shaken with the solvent and used as soon as the suspension had settled. Whole fruits were broken in a mortar, the tougher ones being triturated with a little washed sand, before extraction. Approximately 0·03 ml of the extract was applied to the thin-layer plate by ten successive applications with a glass rod to produce a spot which was about 1 cm in diameter. The chromatographic solvent was allowed to rise about 15 cm from the starting line; this took about 40 min at 20°.

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* Where light petroleum is used this refers to the fraction b.p. 60–80°.

Spot detection. The plate was first examined in ultra-violet light ("Hanovia" lamp with 366 mμ filter) and the presence of any dark, fluorescence-quenching spots noted against the bright yellow fluorescein background. After brief treatment with bromine vapour to convert the fluorescein to eosin, the plate was re-examined in ultra-violet light to note any persistent fluorescein fluorescence, indicating spots of unsaturated substances against the dull background. The plate was then sprayed with a saturated solution of 2,4-dinitrophenylhydrazine in N hydrochloric acid, which revealed some ketones and aldehydes as orange spots. When the plate had air-dried, it was finally sprayed with sulphuric acid containing 1% vanillin. With this, some substances gave coloured spots immediately or in a few minutes (e.g. khellin, thymol), but others (e.g. fenchone) were only properly visible in up to 12 hr at room temperature.

Results and discussion

Table 1 records observations made on reference substances known to be present in certain umbelliferous fruits. Table 2 lists the results obtained with several different samples of various umbelliferous fruits, extracted and chromatographed by the method above. Table 3 is in the form of a key derived from Table 2, and provides an aid to identifying an unknown umbelliferous fruit.

TABLE 1. RESULTS OBTAINED WITH REFERENCE SUBSTANCES

Substance (and source)	Average Rf value	Fluoresc.-quenching	See after bromination	Dinitro ph. hydrazine	Vanillin/ sulph. acid
(+)-Limonene (B)	0·79	−	+	−	yellow then brown
Anethole "pure 21/22°" (B)[1] ..	0·72	+	+	(+)	−
	0·36	−	−	+	−
p-Cuminaldehyde (L)	0·59	−	−	+	−
Thymol (M & B)	0·39	+	+	−	red-mauve
Anisaldehyde (P & S)	0·38	−	−	+	−
(+)-Carvone (B)	(0·36–0·47)	−	+	+	−
Linalöl (P & S)	0·26	−	+	−	mauve-brown
Fenchone[2]	0·19	−	−	−	slow mauve-brown
Khellin[3]	0·02	+	−	−	bright yellow

[1] Anethole gave two spots, the faster moving one being the principal constituent, and the slower moving one corresponding to anisaldehyde, presumably being a decomposition product. The reaction of the faster moving spot to 2,4-dinitrophenylhydrazine is anomalous, and is probably due to other decomposition products appearing at this position. The extracts of umbelliferous fruits known to contain anethole gave similar results.

[2] Fenchone was prepared from (+)-fenchyl alcohol by chromic acid oxidation. A light petroleum extract of the crude reaction mixture was used; this was chromatographically distinct from fenchyl alcohol. This saturated ketone is unreactive, and did not form a hydrazone on spraying with dinitrophenylhydrazine.

[3] Khellin was prepared from *Ammi visnaga* L. by extraction with ether, and crystallisation from water, m.p. 155°.

With the exception of carvone the Rf values quoted above are constant to ±0·02 if the solvent mixture is allowed to rise exactly 15 cm from the starting line. For carvone the range of values observed is given in brackets.

Compounds such as anethole and thymol, which exhibit fluorescence-quenching, are visible after bromination of the plate as a fluorescing halo.

The reference substances were obtained from (B) W. J. Bush & Co. Ltd., and (P & S) Polak & Schwarz Ltd. (International Flavors & Fragrances Ltd.) and are here acknowledged. Commercial samples were from (L) L. Light & Co. Ltd. and (M & B) May & Baker Ltd.

The essential oils of umbelliferous fruits contain true terpenes such as limonene, α-phellandrene and α-pinene which are visible as a bright fluorescent spot after bromination of the plate. They also give a slight

dark purple, or a brief yellow-brown response to vanillin in sulphuric acid. With the solvent system used here they appear together as the fastest moving spot (Rf $\sim$ 0·80). They appear to have been largely lost from powdered samples. As these components appear to have no diagnostic value, they are not included in Tables 2 and 3. The light petroleum extracts in addition contained fixed oil from the seed endosperm. This was as readily extracted from the broken whole fruits as from the powder. Like arachis and cottonseed oils, this provided a spot of Rf approximately 0·60 to 0·70, visible as a bright fluorescence after bromination of the plate. Only with parsley fruits is this spot superimposed on a constituent of the essential oil, and as the latter can still be seen it is not necessary to eliminate the fixed oil by examining a steam distillate. Again, fixed oil spots have no diagnostic value and are not

TABLE 2. RESULTS OF DIAGNOSTIC SIGNIFICANCE OBTAINED WITH UMBELLIFEROUS FRUIT EXTRACTS

Umbelliferous fruit (and source of samples)	Average spot Rf	Reaction and comments (see footnotes)
Ajowan, *Trachyspermum ammi* (L.) Sprague. (P/S Mus, S/P Mus)	0·41	As thymol; q, f, v red-mauve.
Angelica, *Angelica archangelica* L. (B & S)	0·45	?; intense blue fluorescence; others, less intense, follow.
	0·28	?; q, v brown.
Anise, *Pimpinella anisum* L. (B & S, Sc, S/P st)	0·71	As anethole; q, f, (h). With fixed oil tail.
	0·39	As anisaldehyde; h.
Caraway, *Carum carvi* L. (B & S, S/P st)	0·43	As carvone: f, h.
Coriander, *Coriandrum sativum* L. (B & S, Sc, S/P st)	0·26	As linalol; f, v mauve.
Cumin, *Cuminum cyminum* L. (B.D.H., B & S, Sc, S/P st)	0·58	As cuminaldehyde; h.
	0·55	?; f.
Dill, *Anethum graveolens* L. (S/P Mus, S/P st)	0·42	As carvone; f, h.
Fennel, *Foeniculum vulgare* Mill. (Sc, S/P st)	0·72	As anethole; q, f, (h). With fixed oil tail.
	0·38	As anisaldehyde; h.
Indian dill, *Anethum sowa* Roxb. (P/S Mus, S/P Mus, S/P st)	0·57	Dillapiole?; q, f, v mauve-brown.[1]
	0·46	?; f.
	0·41	As carvone; f, h.
Parsley, *Petroselinum crispum* (Mill.) Airy-Shaw. (B & S, C "Double Curled")	0·64	Apiole?; q, f, v brown. Horse-shoe shape, superimposed on fixed oil.
	0·47	?; f.
	0·33	?; q, f.
Visnaga, *Ammi visnaga* L. (B & S, S/P st)	0·04	As khellin; q, v bright yellow.

[1] The tentative identification of the spot from Indian dill with Rf 0·57 as dillapiole is based on its fluorescence-quenching property and other reactions. These are similar to a constituent of parsley, which is known to contain apiole. They are also identical to the reactions of a constituent of the fruits of *Ligusticum scoticum* L. (also umbelliferous, kindly supplied by Chelsea Physic Garden) which contain dillapiole (Kariyone & Teramoto, 1939).

f = visible as yellow fluorescent spot after bromination of the plate.
h = orange spot produced by spraying with dinitrophenylhydrazine solution.
q = visible initially in ultra-violet light as a dark spot against the fluorescein background.
v = response indicated to spraying with vanillin 1% in sulphuric acid.

The sources of the samples used is indicated above as (B & S) Brome & Schimmer Ltd. and (P/S Mus) Museum of the Pharmaceutical Society of Great Britain, to whom acknowledgements are here made for specimens. Fruits were purchased, packed by (C) Carters Tested Seeds Ltd., and (Sc) W. H. Schwartz & Sons Ltd. Other samples were taken from the School of Pharmacy, University of London stock (S/P st) or Museum (S/P Mus) and were of unknown origin apart from one marked British Drug Houses Ltd., (B.D.H.)

Rf values given above are less constant than for the reference substances, varying in some cases by $\pm$0·06. The constituents of an extract, however, are all advanced or retarded together. Only obvious constantly appearing spots are recorded as various minor spots are apparent from time to time.

All the above fruits give a terpene spot Rf approximately 0·80, a fixed oil spot Rf approximately 0·65, and a fatty acid streak from the starting line.

included in Table 2 and 3. The extracts also contained varying amounts of free fatty acids, which formed a streak from the starting line to a position about Rf 0·30. These were visible initially in ultra-violet light as a brighter yellow streak against the fluorescein background, and also as a bright fluorescent streak after bromination. Like the fixed oil spots, these streaks can be stained with a spray of 0·05% aqueous rhodamine 6G. Most whole fruits other than ajowan gave only short streaks of fatty acid, but powdered fruits, unless freshly powdered, gave longer streaks which interfered with essential oil components of Rf value less than 0·30, such as linalöl in coriander. Most of the fatty acid streak could be eliminated by steam distilling the powdered umbelliferous fruit and chromatographing a light petroleum extract of the condensate. The increased amount of

TABLE 3. IDENTIFICATION OF UNKNOWN UMBELLIFEROUS FRUIT

Fluorescence-quenching		Rf 0·70. Anise or Fennel. Confirmed by spot Rf 0·40 (−f, + h). 0·65. Parsley. Distinguished by absence of spot Rf 0·40 (+f, +h). 0·60. Indian dill. Confirmed by spot Rf 0·40 (+f, +h). 0·40. Ajowan. (Red-mauve with v). 0·25. Angelica. Confirmed by spot Rf 0·45 (intense blue fluorescence). 0·05. Visnaga. (Yellow with v).
No fluorescence-quenching	Visible after bromine	Rf 0·55. Cumin. Confirmed by spot Rf 0·60 (−f, +h). 0·45–0·40. Caraway or Dill. (+h). 0·25. Coriander. (Mauve-brown with v).
	Not visible after bromine	Other common umbelliferous fruit, e.g., carrot, celery, hemlock, parsnip.

f = following bromination of the plate, the spot does (+) or does not (−) appear as a yellow fluorescent area.
h = spraying with dinitrophenylhydrazine solution does (+) or does not (−) give the spot an orange colour.
v = response indicated to spraying with vanillin 1% in sulphuric acid.
The above Rf values are averages, and given in round figures.
Terpene, fixed oil and fatty acid spots are present in all the extracts, but are ignored here, as are minor spots.

fatty acid in powders compared with whole fruits suggests that powdering facilitates the action of an esterase on the fixed oil. The two samples of ajowan examined were distinct in containing as much free fatty acid in the whole as other umbelliferous fruits did in powdered form. Both came from museums, and during prolonged storage an esterase may have taken effect.

Light petroleum was selected as the extracting solvent because it is reasonably selective in dissolving essential oils but not many other constituents. Some principles other than oils are occasionally obtained; khellin is extracted from visnaga and thus serves to identify this fruit. Some pigments are extracted, but remain on the starting line. With a few fruit extracts, often those obtained from new supplies of material, substances which gave bright blue fluorescing spots in ultra-violet light were noted: this was observed with visnaga and parsley. The brilliant blue fluorescent streak from angelica fruits was distinctive; it was probably due to coumarin and umbelliferone derivatives which, Guenther (1950a) records, have been found in the extract.

Guenther (1950b) and Schrantz & others (1962) have recorded anisaldehyde as being a constituent of fennel oil. A spot corresponding chromatographically to this compound, which is likely to be a decomposition product of anethole, was observed in the extracts of both anise and fennel.

The fluorescence-quenching of substances such as thymol and anethole is readily observed and of diagnostic value, especially in distinguishing Indian dill from dill. A sample of "dill" from a drug broker, and one from a retail source were both shown to be Indian dill by the method described here, the identification being supported by the macroscopical characters of the fruit. It is interesting to note the claim that Indian dill contains a toxic principle dillapiole and that it should not be used in place of European dill (Wallis 1960). The chromatographic method will detect an admixture of one part Indian dill with four parts dill.

According to Brockmann & Volpers (1947) the fluorescence-quenching of aromatic compounds is due to their absorbing part of the ultra-violet illumination. Eugenol exhibits fluorescence-quenching against fluorescein, and since dillapiole and apiole are chemically similar they too may be expected to exhibit fluorescence-quenching. The relevant spots from Indian dill and parsley fruits (Table 2) are assigned on this basis.

Although thin-layer chromatography serves to identify many umbelliferous fruits, other methods are still required to distinguish caraway from dill, and anise from fennel. The anethole spot from anise is normally more intense than that from fennel, and this may be a guide. In theory, medicinal fennel, which is *F. vulgare* var. *vulgare* (Mill.) Thelung, should be distinguished from anise (and from sweet fennel) by its content of fenchone. This very unreactive ketone, however, was not noted in any sample of fennel examined.

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