J. Pharm. Pharmac., 1968, **20**, *Suppl.*, 61S–64S Received May 1, 1968

SHORT COMMUNICATION

Examination of fennel fruits by gas chromatography without preliminary distillation

T. J. BETTS

IT has been observed that growing fruits of fennel, *Foeniculum vulgare* Mill., of both the bitter (var. *vulgare* (Mill.) Thelung) and sweet (var. *dulce*) forms grown in the same garden develop approximately the same amount of anethole per 100 fruits during their maturation (Betts, 1968). Thirteen dried fennel fruits of various origins have now been examined by gas-liquid chromatography to see how the content and proportion of the essential oil constituents relate to the values previously recorded. Anethole contents differed widely, and a fennel virtually free of anethole was found. Estragole was present instead of anethole, its presence being confirmed by infrared spectroscopy. This fennel has not been reported in commerce before.

EXPERIMENTAL

Drug material and its extraction. Specimens of dried fennel fruit were obtained from the sources indicated in Table 1, where they are also described. 20 to 50 larger mericarps of each specimen, excluding stunted or abnormal fruits, were crushed under n-hexane (0·5 ml) and the solvent decanted into a 2 ml volumetric flask; the fruit residue was triturated again with further small quantities of n-hexane and the extracts used to make the contents of the flask up to volume. (This n-hexane contained (−)-carvone as an internal standard, together with sufficient dry ethanol to yield a clear solution. Carvone was selected as having a retention time between anethole and estragole.)

Gas chromatography was as previously described (Betts, 1968), three columns being used under the operating conditions detailed in Table 2, with hydrogen supplied to the flame ionization detector at half the column flow rate, thus giving maximum detector sensitivity. 1 μl aliquots of the fennel extracts were injected onto the columns, reference solutions of anethole, estragole, fenchone and limonene being used to identify and evaluate peaks.

Isolation of fennel fruit constituent for infrared spectroscopy. Crushed fennel fruit J (0·8 g) was co-distilled with water (100 ml) and n-hexane (2 ml) for 3 hr in the B.P. apparatus for Determination of Volatile Oil in Drugs. The oil in hexane condensate was dried and passed through a column of silicagel MFC (2·5 g), eluting with n-hexane. After removal of terpene hydrocarbons, the eluate of the next constituent was evaporated on

From the Department of Pharmacognosy, The School of Pharmacy, University of London, Brunswick Square, London, W.C.1, England.
Present address: Department of Pharmacy, Western Australian Institute of Technology, Bentley, Western Australia.

TABLE 1. ORIGIN AND DESCRIPTION OF FENNEL SPECIMENS

Reference letter and source	Size (length × width)	Colour	Form	Av. wt. in mg of 100 cremocarps
A Museum specimen "Roman"	16–8 mm × 3 mm	Pale brown	Mostly obovoid lanceolate cremocarps	1670
B Essen Botanical Garden, Germany. Recent	10–9 mm × 3 mm	Dark green with buff ridges	Mostly flat or recurved mericarps, well ridged	1555
C Museum specimen "Saxon"	9–6 mm × 3 mm	Dark brown with buff ridges	Mixed cremocarps and mericarps	1460
D Museum specimen "Indian" presented 1939	8–6 mm × 2·5 mm	Pale buff	Mostly cremocarps	1190
E Stock drug at School of Pharmacy	9–5 mm × 2·5 mm	Pale buff -greenish	Mixed cremocarps and mericarps	1165
F Myddelton House, 1967. "Sweet" variety grown in School Drug Garden	6 mm × 3 mm	Brownish buff	Mostly ovoid arcuate mericarps, well ridged	1130
G Retail grocer's proprietary spice pack	9–6 mm × 2·5 mm	Pale buff -greenish	Mostly cremocarps	1090
H Museum specimen, un-named	8–6 mm × 3 mm	Pale buff -greenish	Mixed cremocarps and mericarps	785
J Istanbul Botanical Garden, Turkey. Recent	6 mm × 1·5 mm	Grey brown -greenish	Mostly narrow mericarps, some recurved, not well ridged	710
K Nsukka, Nigeria, Africa. Recent	7–5 mm × 1·5 mm	Grey or brown	Mostly narrow cremocarps with very fine wavy ridges	560
L Broadstairs, Kent, England. Wild, sea cliffs, 1965	4–3 mm × 1·5 mm	Dark grey	All ovoid arcuate mericarps	540
M Myddelton House, 1967. "Bitter" variety grown in School Drug Garden	4·5 mm × 1·5 mm	Dark grey -brownish	Mostly ovoid arcuate mericarps	530
N Myddelton House, 1966. "Bitter" variety grown in School Drug Garden	4·5 mm × 1 mm	Brown with lighter ridges	Mostly ovoid arcuate mericarps	475

TABLE 2. RETENTION TIMES RELATIVE TO (—)-CARVONE OF FENNEL FRUIT CONSTITUENTS ON THE GAS CHROMATOGRAPHIC COLUMNS

Column		10% polyethylene glycol adipate on 100–120 mesh Celite, purged at 215°	10% silicone elastomer E 301 on 100–120 mesh Chromosorb W, purged at 325°
Operating temperature		140°	125°
Theoretical plates (approx.):	fenchone	1200	550
	estragole	2000	1000
	anethole	2350	1600
t_r rel.:	limonene	0·08	0·32
	fenchone	0·25	0·42
	estragole	0·69	0·72
(standard)	carvone	1·00	1·00
	anethole	1·33	1·16
	anisaldehyde	2·8	1·6

Mobile phase nitrogen, with flow rate at column exit 40 ml/min. The Carbowax column previously used (Betts, 1968) gave very similar results to those above with polyethylene glycol adipate.

sodium chloride discs for infrared spectroscopy. The spectrum obtained (Perkin-Elmer 237) was that of estragole, not anethole, and the constituent gave a gas chromatographic peak corresponding to estragole.

RESULTS AND DISCUSSION

Wide variations were noted in the content of the constituents of the essential oils of different fennel fruits (Table 3). This bore no relation to the weight of the fruits. In Museum specimens A, C, D (Table 1), as well as specimen G, the anethole had partly decomposed to anisaldehyde on prolonged storage. Calculations from the peak area of the latter substance were made, based on the relative molecular hydrocarbon content of the two molecules, to deduce the amount of anethole that had decomposed, these values being added to the anethole figures in Table 3. In the Indian fennel D, most of the anethole had decomposed.

TABLE 3. ESSENTIAL OIL CONSTITUENTS OF FENNEL SPECIMENS

Reference (see Table 1 for details)	mg per 100 cremocarps (and % of total)					Oil yield % w/w
	Anethole + *anisal*[1]	Estragole	Fenchone	Limonene etc.[2]	Total	
Bitter (approx. 60% anethole, 30% fenchone)						
N	19·9 (63)	0·7	10·2 (32)	0·8	31·6	6·66
M	19·1 (64)	0·7	9·1 (31)	0·9	29·8	5·62
C	13·9 + *3·3* (55)	1·9	10·5 (34)	1·5	31·1	2·13
A	1·3 + *1·4* (59)	0·5	1·1 (24)	0·3	4·6	0·28
D	0·1 + *0·9* (56)	0·2	0·5 (28)	0·1	1·8	0·15
Sweet (approx. 80% anethole, 10% fenchone)						
B	42·6 (81)	1·8	5·7 (11)	2·3	52·4	3·37
F	19·5 (82)	0·8	3·2 (13)	0·3	23·8	2·11
K	6·3 (79)	0·3	0·9 (11)	0·5	8·0	1·43
G	5·8 + *0·6* (80)	0·3	0·8 (10)	0·5	8·0	0·73
Anethole-free (approx. 80% estragole, 15% fenchone)						
L	nil	14·1 (86)	2·4 (14)	trace	16·5	3·06
J	trace	13·9 (84)	2·7 (16)	trace	16·6	2·34
H	trace	11·8 (79)	2·2 (15)	0·9	14·9	1·90
Anomalous						
E	4·1 (46)	3·4 (38)	0·7 (8)	0·7	8·9	0·76

[1] *Anisaldehyde figures have been converted to anethole equivalents and added to the anethole to give the* percentage of this in the total oil, as anisaldehyde is a decomposition product.
[2] Limonene figures include total terpene hydrocarbons.

With the exception of specimen E, all fennel fruits examined fell into three groups as indicated in Table 3. The classification has been based on the percentage of anethole and fenchone present in the essential oil. The first and second groups probably represent the bitter and sweet varieties respectively, whilst the third group may be *Foeniculum piperitum* Presl., in which estragole replaces anethole as chief essential oil component (Pellini, 1923). Figures for the anethole and fenchone content of the dried Myddelton House grown fruits F, M, N corresponded fairly well with those obtained from fresh, ripe fruits (Betts, 1968). The slightly lower anethole content observed may represent some oil loss on drying and

storing the fruits (in thick polythene bags), for Tóth (1967b) observed a decrease during two years' storage in brown paper bags. Specimen K, for which Osisiogu (1967) found 2·2% oil, only yielded 1·4% here. Such losses may account for the low oil content of some Museum fennels, although specimen C contained as much oil as recent collection N.

Results here confirm the previously observed disagreement (Betts, 1968) with the analyses of Tóth (1967a). Of the thirteen fennels examined, only one contained (slightly) less than 10% fenchone. Either two forms of bitter fennel were examined here, or Tóth suffered preferential loss of fenchone during steam distillation to isolate the oils. Osisiogu (1967) could not detect fenchone in his distilled oil although it is present in fennel K.

The highest proportion of estragole previously recorded for fennel fruit oil is 20% by Naves & Tucakov (1959). Estragole (allyl-*p*-methoxybenzene) shows distinctive infrared spectral peaks at wavenumbers greater than 3020 cm^{-1} and in the region of 1640, 990 and 910 cm^{-1}, these not being shown by its propenyl isomer anethole. These and other matching features could even be detected in the spectrum of the unfractionated oil obtained by steam distillation from anethole-free fennel. Its presence in a Museum specimen and in drug stock E (see below) indicates that this non-B.P.C. fennel occurs in commerce. Such fruits have no distinctive morphological or sensory character, although estragole has a less intensely spicy odour than anethole. The two isomers have the same Rf value and give similar response to detection methods on thin-layer chromatograms (Tóth, 1967b; Betts, 1968). Gas chromatographic examination by direct solvent extraction of the fruits, as used here, is the best method for detecting the anethole-free fennel. The technique is sensitive enough to work with single cremocarps using a concentrated extract. Atypical fennel E was thus found to be a mixed specimen; the larger, narrower fruits being a sweet fennel contributing the anethole and fenchone content of the bulk, whilst the smaller, wider fruits were an estragole fennel, unusually almost devoid of fenchone as well as anethole. On cultivation, the anethole-free fruits grow into plants bearing leaves devoid of anethole and rich in estragole, so the distinction is maintained at other stages of plant growth.

Acknowledgements are due, as in the previous paper (Betts, 1968) to Mr. L. Guglielmi, Mr. M. Humphrey and Dr. I. U. W. Osisiogu for providing some fruits or materials. Thanks also to Mr. J. W. Adcock for help with spectroscopy.

References

Betts, T. J. (1968). *J. Pharm. Pharmac.*, **20**, 469–472.
Naves, Y. R. & Tucakov, J. (1959). *C. r. Séanc. Acad. Sci., Paris*, **248**, 843–845.
Osisiogu, I. U. W. (1967). *Planta Med.*, **15**, 30–31.
Pellini, G. (1923). *J. Soc. chem. Ind., Lond.*, **42**, 858A.
Tóth, L. (1967a). *Planta Med.*, **15**, 157–172.
Tóth, L. (1967b). *Ibid.*, **15**, 371–389.