THE APPLICATION OF INFRA-RED SPECTROPHOTOMETRY TO THE EXAMINATION OF ESSENTIAL OILS

PART I. CINEOLE IN LAVENDER OIL

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From Smith Kline and French Laboratories Ltd., London

Received May 15, 1957

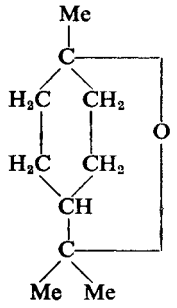
Lavender oils in capillary films, in the range 5000 cm.$^{-1}$ to 650 cm.$^{-1}$ show peaks at 1310, 1220, 1085 and 855 cm.$^{-1}$ of diagnostic value for cineole. A quantitative method based on the cineole peak at 1085 cm.$^{-1}$ in carbon disulphide solution, and using an "Absorbancy Difference" method provides a truer assessment of the cineole content of lavender oils than do the methods currently described. The *o*-cresol method is inapplicable to oils containing cineole in the presence of esters, and alcohols. The cineole contents of English and spike lavender oils are lower than the published figures based on the formation of complexes. The official, French, English and spike lavender oils might be classified in the pharmacopoeias by their cineole content.

LAVENDER oil varies in its composition depending on the botanical source, the environmental conditions during the plant growth, the time of harvesting and the manner in which the oil is isolated. At the present time, the commercial oils are derived mainly from *Lavandula intermedia*, *L. officinalis*, and *L. latifolia*[1,2] and are characterised by their varying amounts of esters, alcohols, and cineole which influence the odour of the oils.

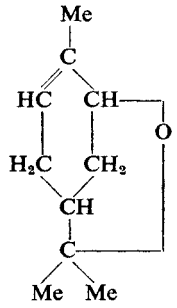
Various methods have been used in the past to determine the cineole contents of lavender oils. Tedesko[3] applied the freezing point method of Kleber and von Rechenburg[4] to spike lavender oil and reported cineole contents of 28·5 to 35·4 per cent but fractional distillation gave only some 10 per cent of cineole. The "cresineol" method of Cocking[5] which gave satisfactory results with eucalyptus oils containing a high proportion of cineole was found inapplicable by Reed[6] to French and spike lavender oils. Others reported that where the cineole content was below 65 per cent, the α-naphthol[7], resorcinol[8] and phosphoric acid[9] complex forming methods did not provide satisfactory answers. Martin and Harrison[10] developed a spectrophotometric method for the estimation of cineole based on a red colour developed with acid *p*-dimethylaminobenzaldehyde, but this was later adversely criticised by Montes[11]. Optical methods, of special value in the detection of adulteration or sophistication were applied by Naves[12] to French and spike lavender oils, but they did not give any useful information on the cineole contents. Presnell[13] and Maennchen[14] published a number of infra-red spectrograms of essential oils as a means of identification, but without quantitative figures.

1:8-Cineole (I) differs from the other constituents of lavender oil in being a cyclic terpene oxide. Two similar compounds pinol (II) and

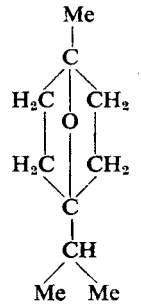
1:4-cineole (III) have been described and while no reference has been found to pinol as a material constituent, the 1:4-cineole has been identified in a few essential oils, notably that from *Piper cubebs*.



1:8-Cineole (I)



Pinol (II)



1:4-Cineole (III)

Since 1:8-cineole is a cyclic ether, its infra-red absorption curve might be expected to show sharp peaks due to the symmetric and asymmetric vibration frequencies of the C–O–C linkage. Barrow and Searles[15] in their study of cyclic ethers showed that the frequencies of the two bands varied with the ring size and with the 6 and 5 membered ring asymmetric vibrations the frequencies approximated to 1100 cm.$^{-1}$ and 1075 cm.$^{-1}$. For the corresponding symmetric vibrations the values were approximately 810 cm.$^{-1}$ and 910 cm.$^{-1}$. From a consideration of the pattern of frequency change with ring size, and the structural formula of cineole, it was expected that its C–O–C bridge would absorb at approximately the above pairs of frequencies. Strong sharp peaks were found at 1085 and 855 cm.$^{-1}$.

Experimental

The instrument was a Hilger H.800 double beam infra-red recording spectrophotometer fitted with sodium chloride optics. The frequency calibration was regularly checked against polystyrene and benzene reference standards and was accurate to ± 3 cm.$^{-1}$. All samples were dried overnight with dried magnesium sulphate.

Screening tests on a range of lavender oils in capillary films, covered the frequency ranges 5000 cm.$^{-1}$ to 2000 cm.$^{-1}$ and 2000 cm.$^{-1}$ to 650 cm.$^{-1}$. Pure cineole, examined in a similar manner showed a number of peaks. From a study of the oils available to us we selected four peaks at 1310 cm.$^{-1}$, 1220 cm.$^{-1}$, 1085 cm.$^{-1}$ and 855 cm.$^{-1}$ as suitable for the detection of cineole in lavender oil. (See Fig. 1.) After some preliminary work a standard concentration of 2·5 per cent w/v of dried oil in carbon disulphide was used for quantitative estimations of cineole. This solvent has a low absorbance in the 1085 cm.$^{-1}$ region. In these experiments the sample cell had a path length of 0·500 mm. whereas the reference cell containing only carbon disulphide was adjusted to a path length of 0·485 mm. to compensate for the volume of oil present and thus to maintain the double beam balance. The solutions were examined over the frequency range

1125 cm.$^{-1}$ to 1040 cm.$^{-1}$ at a scanning speed of 18 cm.$^{-1}$ per minute, a slit setting equivalent to a band width of about 2 cm.$^{-1}$ and a recorder chart speed of 0·5 inches per minute. Four replicate tracings were run for each solution and the average used in computing the cineole content by an "Absorbancy Difference" method.

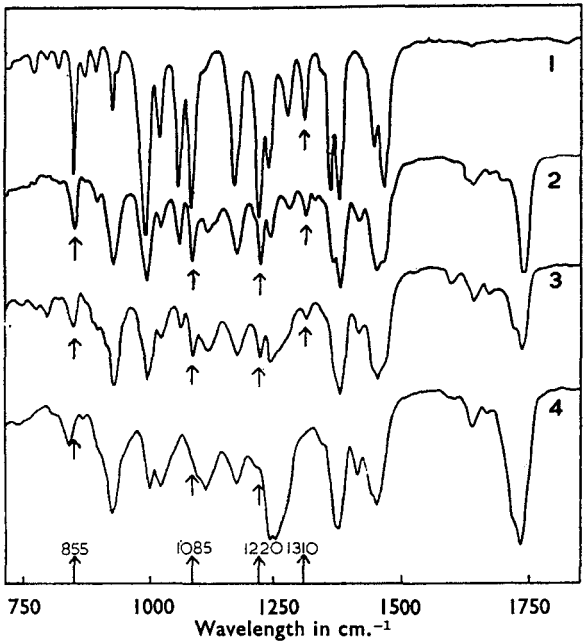


FIG. 1. Infra-red spectrograms, as capillary films from 750 to 1750 cm.$^{-1}$, at approximately 0·02 mm. path length, of cineole and lavender oils.

1. Pure cineole. 2. Spike lavender oil No. 24. 3. English lavender oil No .9. 4. French lavender oil No. 2.

Absorbancy Difference

Since the peak at 1085 cm.$^{-1}$ selected as the basis of a quantitative determination was superimposed on a sloping background absorption due to other components of the oils, the following simplified method was used to correct empirically for this. (See Fig. 2.)

A line BC was drawn across the base of the peak and another line drawn through the peak at A perpendicular to the frequency axis at 1085 cm.$^{-1}$ to cut BC at D. The absorbancy difference used in calculating the cineole content was defined as the optical density at point A minus the optical density at point D. By reference to a standard graph relating cineole to optical density it was possible to estimate the corresponding amount of cineole.

RESULTS

The results obtained with a few oils of known botanical source and history, as well as a range of commercial oils are listed in Tables I and IV.

Standard Cineole Curve, Reproducibility and Recovery Experiments

Solutions of cineole in carbon disulphide were examined over the frequency range 1125 cm.$^{-1}$ to 1040 cm.$^{-1}$ under the operating conditions described above, suitable adjustments being made to the solvent path length in the reference cell to compensate for the amount of cineole in the sample cell.

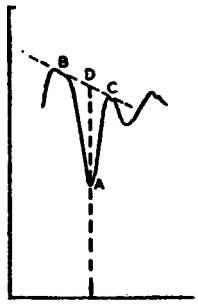


FIG. 2. Base line method for the determination of absorbancy difference at 1085 cm.$^{-1}$.

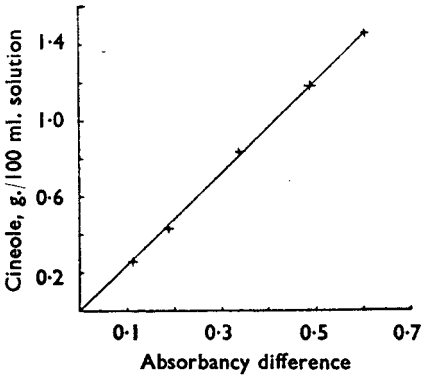


FIG. 3. Standard cineole curve.

When the "Absorbancy Difference" (AD) was plotted against the concentration of cineole (as g./100 ml. of solution), a straight line passing through the origin was obtained indicating that Beer's Law applied over the concentration range tested. The maximum concentration was greater than that likely to be present in genuine lavender oil. (See Fig. 3.)

TABLE I

PHYSICAL CONSTANTS AND SAMPLE CODING OF THE OILS EXAMINED

Sample Number	Supplier	Type of Oil	at n20°	$[\alpha]_D^{20}$ (l = 1dm.)	Wt./ml. at 20°
1	A	French	1·4503	−3·60	0·883
2	B	*French (Basse-Alpes) 1953	1·4633	—	0·877
3	C	French—(extra)	1·4618	−6·40	0·885
4	C	French—(cultivated)	1·4618	−7·40	0·887
5	C	French—(Barreme)	1·4613	−6·80	0·885
6	D	French—(Grasse)	1·4623:	—	—
7	D	French—1956	1·4638	−5·20	0·901
8	D	French (Hautes-Alpes) 1956	1·4633	−6·00	0·893
9	E	*English—1955	1·4689	−10·80	0·880
10	E	*English—1956	1·4706	−12·10	0·880
11	F	*English—1954	1·4744	−10·80	0·885
12	F	*English—1956	1·4733	−9·20	0·876
13	L	English	—	—	—
14		Spike—1954	1·4688	−3·20	0·911
15	G	†Spike—(Cuenca)	1·4673	nil	0·902
16	G	†Spike—(Murcia)	1·4683	+1·00	0·906
17	H	†Spike—(Spanish, extra)	1·4668	−2·20	0·899
18	H	†Spike—(Spanish)	1·4691	+1·50	0·904
19	J	†Spike—(Spanish, extra)	1·4668	−2·50	0·898
20	K	†Spike—(Spanish)	1·4683	—	—
21	H	†Spike—(Spanish)	1·4663	−1·20	0·905
22	J	†Spike—(Spanish, prime)	1·4677	+1·30	0·907
23	J	†Spike—(Spanish, extra)	1·4664	−1·60	0·904
24	G	†Spike—(Cuenca)	1·4654	−1·90	0·900
25	E	*English—Special Variety 1956	1·4608	−6·90	0·893

* Authentic oils. † Samples under 20/-/lb.

A sample of English lavender oil (No. 13) was assayed a total of six times to check the reproducibility of the method. The results obtained varied from 11·2 per cent w/w to 11·6 per cent w/w with an average cineole content of 11·4 per cent w/w.

TABLE II

RECOVERY OF CINEOLE ADDED TO A SAMPLE OF FRENCH LAVENDER OIL

Mix No.	Oil g./100 ml. solution	Cineole g. added/100ml. solution	Total cineole g./100ml. solution	AD* at 1087 cm.$^{-1}$	Cineole g./100ml. solution found	Recovery per cent
1	2·500	nil	0·025	—	—	—
1	2·267	0·224	0·247	0·102	0·245	99·3
2	2·380	0·146	0·170	0·072	0·157	103·0
3	2·145	0·373	0·395	0·164	0·400	101·2
4	1·717	0·815	0·832	0·342	0·825	99·1
5	2·120	0·389	0·410	0·174	0·420	102·4
6	1·669	0·835	0·852	0·346	0·835	98·0

* AD = Absorbancy difference, see text.

A series of determinations based on mixtures prepared from a French lavender oil of a known, one per cent, cineole content and pure cineole gave recoveries ranging from 98 per cent to 103 per cent when assayed as 2·5 per cent w/v solutions in carbon disulphide by the technique described above. (See Table II.)

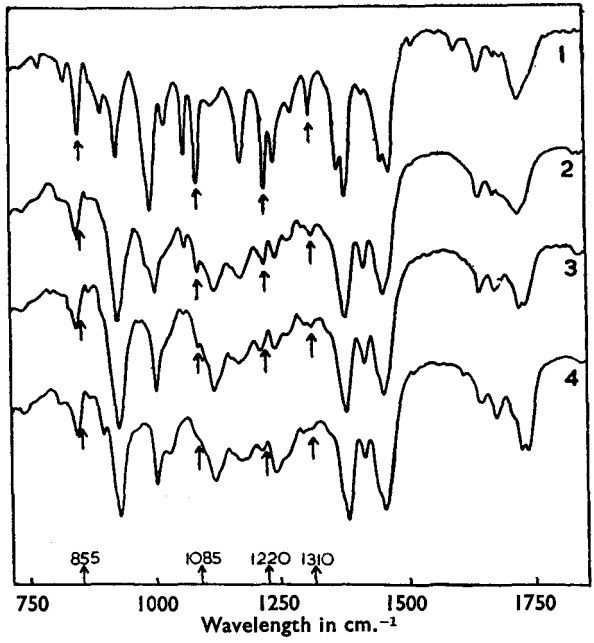


FIG. 4. Infra-red spectrograms, as capillary films from 750 to 1750 cm.$^{-1}$, at approximately 0·02 mm. path length, of fractions from a distilled English lavender oil No. 13.

1. Fraction 3, distilling 170–175°. 2. Fraction 5, distilling 180–190°. 3. Fraction 6, distilling 190–200°. 4. Undistilled residue.

Examination of a Fractionated Oil

One of the methods which had been used for the estimation of the cineole content of lavender oils was to fractionally distil a quantity of the oil and to collect the fraction boiling between 173° and 190°[16]. Subsequent formation of a cineole complex, removal of the liquid non-cineole part of the fraction and recovery of the cineole provided information on the approximate cineole concentration in the original oil.

TABLE III

THE DISTRIBUTION OF CINEOLE DETERMINED BY THE INFRA-RED AND "CRESINEOL" METHODS IN THE FRACTIONS OBTAINED FROM AN ENGLISH LAVENDER OIL (NO. 13) BY DISTILLATION

Fract. No.	Boiling range °C	Per cent w/w of sample	n20°	$[\alpha]_D^{20}$ (l = 1dm.)	Per cent w/w cineole in fraction	
					by infra-red	by "cresineol"
1	95–105°	1	—	—	—	—
2	105–170°	8	1·4598	−1·54°	28·0	27·0
3	170–175°	18	1·4567	−7·04°	40·0	42·5
4	175–180°	8	1·4676	−8·00°	37·5	46·5
5	180–190°	8	1·4687	−11·64°	6·5	26·0
6	190–200°	20	1·4688	−11·64°	1·0	20·5
residue	undistilled	37	—	—	nil	18·5
Original sample		—	1·4680	−9·58°	11·4	27·0

We distilled 100 g. of an English lavender oil (No. 13) at atmospheric pressure through a 6 inch column packed with Dixon Gauze rings and obtained six fractions and an undistilled residue. The first small fraction which consisted almost entirely of water was discarded and the subsequent fractions and the undistilled residue were examined in the form of capillary films from 5000 cm.$^{-1}$ to 650 cm.$^{-1}$, by the "cresineol" method and by the present method.

The capillary film traces conclusively showed that the 2nd, 3rd, 4th and 5th fractions contained varying amounts of cineole whereas the quantities in fraction 6 and in the undistilled residue were extremely small since the four major cineole diagnostic peaks at 1310, 1220, 1085 and 855 cm.$^{-1}$ were barely detectable in fraction 6 and undectable in the undistilled residue. (See Fig. 4.)

The cresineol method used was that described in the British Pharmacopoeia 1953 p. 762 for the determination of cineole in rosemary oil. The apparent cineole contents are shown in Table III. By this method, the recovery was equivalent to about 27 per cent w/w of apparent cineole in the original sample, a value that was almost identical with that obtained from the examination of the unfractionated oil.

By the use of the present infra-red method, an estimation was obtained of the cineole in each fraction and the results are included in Table III. The unfractionated oil was found to contain 11·4 per cent w/w of cineole compared with 13 per cent w/w by calculation.

Estimation of Cineole Content at 1085 *cm.*$^{-1}$ *Peak*

A series of 25 specimens of lavender oils of French, English and spike origin and of different qualities were assayed for cineole by the infra-red

method and the resulting cineole contents are shown in Table IV together with the "apparent cineole" contents where these were estimated by the "cresineol" method.

TABLE IV

PERCENTAGE W/W CINEOLE FOUND IN SAMPLES OF LAVENDER OIL, COMPARED WITH SOME RESULTS USING THE "CRESINEOL" METHOD

Sample No.	Type	Cineole per cent w/w	
		by infra-red	by "cresineol"
1	French	1·0	—
2	French*	less than 1	23
3	French	2·7	—
4	French	less than 1	—
5	French	less than 1	—
6	French	3·4	—
7	French	2·7	—
8	French	2·5	21
9	English*	11·8	28
10	English*	13·0	—
11	English*	9·2	—
12	English*	11·4	—
13	English	11·4	27
14	Spike	23·7	38
15	Spike	25·9	—
16	Spike	31·5	—
17	Spike	22·8	—
18	Spike	22·0	—
19	Spike	26·0	—
20	Spike	24·1	32
21	Spike	29·6	—
22	Spike	22·4	—
23	Spike	24·2	—
24	Spike	28·5	—
25	English-special*	less than 1	—

* Authentic oils.

DISCUSSION

It has long been recognised by the expert that a "good nose" is a useful analytical tool in the grading and identification of lavender oils, but it is of little value for quantitative measurements.

The monograph on lavender oil in the British Pharmacopoeia 1953 specifies certain constants for the English, Commonwealth and French varieties, but makes no reference to the presence or absence of cineole. The British Pharmaceutical Codex 1954 on the other hand, includes the following statement . . . "Cineole occurs in some quantity in English oil, but only in traces in French oil."

The Essential Oil Sub-committee of the Society of Public Analysts[17] studied the *o*-cresol method for the determination of cineole and reported that where the oils contained appreciable amounts of alcohols, esters, aldehydes and ketones the method yielded high results and they recommended that such determinations should be reported as "apparent cineole" content by *o*-cresol. The results in Table III and IV demonstrate the unsatisfactory character of the "cresineol" method when applied to lavender oils and suggest that it is the higher boiling fractions that are mainly responsible for the errors arising from the use of this method. From the fractionation of the English oil reported above, fraction 6 and the undistilled residue, which together represented some 57 per cent of the original oil, contained little or no cineole when examined by the

infra-red method and yet appeared to contain substantial amounts of cineole when tested with *o*-cresol.

In the past the quality of lavender oils has often been based on the ester content, but the results in Table IV show that it is possible to identify also the commercial oils by their cineole content. Thus French oils contain little or no cineole, English oils contain up to 13 per cent and the spike lavender oils from 22 to 31 per cent of cineole. Some doubt has been expressed whether genuine French lavender oil contains any cineole and considerable care needs to be exercised in collecting from genuine plants, free from weeds or other contaminants before this question can be answered. Reports suggest that some French oils offered for sale have been blended with lavender oil obtained from *L. hybrida*, a hybrid derived from *L. officinalis* and *L. latifolia*, and if this were the case then one should expect to identify cineole in the blend. From a consideration of the data in Tables I and IV and the price per lb., it would appear that the cheaper grades of French oil available commercially have been blended to sell at a low price.

We have included in Tables I and IV the results of our examination of a lavender oil (No. 25) specially cultivated in England, which is similar in many respects to a French type oil. This oil, which has some 45 per cent esters appears to be akin to the Old English or Giant Blue variety described by Seager.[18]

Before any general classification on a basis of the cineole content of the official lavender oils can be attempted it will be necessary to examine a larger range of genuine oils.

Reference has already been made to the fact that a peak at 855 cm.$^{-1}$ might serve as a useful diagnostic characteristic for the presence of cineole in lavender oil, but quantitative measurements similar to those used at the 1085 cm.$^{-1}$ peak were found to be unreliable.

Acknowledgements. The authors would like to thank Messrs. Givaudan, Stafford Allen and Yardley who kindly provided the authentic samples used in this investigation.

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DISCUSSION

The paper was presented by MR. S. G. E. STEVENS.

DR. J. W. FAIRBAIRN (London) said as far as was known the French oil was derived from *Lavandula officinalis*, the spike oil from *Lavandula latifolia* and the English oil from *Lavandula intermedia*, which was a hybrid of the other two. If authentic oils behaved similarly it would be an interesting example of taxonomy supporting the analytical results.

MR. A. R. ROGERS (Brighton). Each assay was based on four replicates; what was the variation within those replicates?

DR. J. B. STENLAKE (Glasgow). How reliable did the authors regard their measurements? It seemed from the tracings that the 855 peak appeared to be free from any interference from other peaks and might give a better lead.

MR. S. G. E. STEVENS replied. He would be glad to examine authentic samples. Whether four or six replicates were taken the results, in the case of an English oil, were of the order shown in the text, page 845 where the values were between 11·2 and 11·6 per cent cineole. It was true that as the amount of cineole increased, accuracy in terms of cineole assessment was greater. The peak at 855 cm.$^{-1}$ was not applicable to oils having a low cineole content (Fig. 4) being swamped by extraneous absorption in this area by other components of the lavender oils of French type.