CHROM. 4748

Investigation into the composition of lavender and lavandin oil

Some new components

The oils of lavender, lavandin and spike lavender, belonging to the plant family of the *Labiatae*, show characteristic differences with regard to their chemical composition. These differences are shown clearly by gas chromatographic analysis of the major components *viz.* camphor, 1,8-cineol, linalol and linalyl acetate.

In genuine French lavender oil we found less than 1% 1,8-cineol and camphor and about equal amounts of linalol (31%) and linalyl acetate (38%). Samples of genuine French lavandin oil contained 6% cineol, 7% camphor, 35% linalol and 19% linalyl acetate, whereas samples of genuine Spanish spike lavender oil showed a greater amount of cineol (25%) and camphor (14%) and a negligible percentage of linalyl acetate (0.3%). The percentage of linalol turned out to be 47%. Comparable investigations have been carried out by NAVES[1], HOLNESS[2], KOLSEK AND MATICIC[3], STELTENKAMP AND CASAZZA[4] and ILLE[5].

The relative retention times of the main constituents on diethylene glycol succinate (DEGS), Carbowax 20M, Carbowax monostearate and Castorwax are listed in Table I. Castorwax appeared to be selective for the separation of linalol, camphor and linalyl acetate. This is in agreement with the results, obtained by KOLSEK[3]. On a packed column with Carbowax 20M complete separation of the three compounds could only be obtained by programming the temperature slowly. Camphor elutes from this phase and also from Carbowax monostearate *before* linalol and linalyl acetate, while from DEGS it occurs *after* the latter compounds.

TABLE I

RELATIVE RETENTION TIMES OF SOME MAJOR COMPONENTS OF LAVENDER, LAVANDIN AND SPIKE LAVENDER OIL

Analytical conditions:

[a] Column 3 m, 1/8 in. O.D., loaded with 10% (w/w) diethylene glycol succinate on acid-washed Embacel support. Temperature: programmed from 75–200°, 20 min at a rate of 1.1°/min, followed by 2°/min.

[b] Column 3 m, 1/4 in. O.D., loaded with 10% (w/w) Carbowax monostearate on acid-washed Embacel support. Temperature: programmed from 75–200°, at a rate of 3°/min.

[c] Column 2 m, 1/8 in. O.D., loaded with 10% (w/w) Castorwax on acid-washed Embacel support. Temperature: isothermal 120°.

[d] Column 2 m, 1/8 in. O.D., loaded with 10% (w/w) Carbowax 20M on acid-washed Embacel support. Temperature: programmed from 70–200°, 12 min at a rate of 0.9°/min, followed by 1.1°/min.

Component	*Relative retention times (camphor = 1.00)*			
	DEGS[a]	*CMS*[b]	*Castorwax*[c]	*Carbowax 20M*[d]
1.8-Cineol	0.30	0.28	0.35	0.31
Camphor	1.00	1.00	1.00	1.00
Linalol	0.92	1.05	0.78	1.08
Linalyl acetate	0.95	1.35	1.15	1.13

TABLE II

LIST OF NEW CONSTITUENTS FOUND IN LAVENDER AND LAVANDIN OIL

Lavender oil		*Lavandin oil*	
Constituent	*Method of identification*[a]	*Constituent*	*Method of identification*
β-Phellandrene	RRT	β-Phellandrene	RRT
Terpinolene	RRT, IR	Menthone	RRT, IR
α-Thujene	RRT	*p*-Methyl acetophenone	RRT, IR
α-Phellandrene	RRT		
Perillyl alcohol	RRT		
Perillaaldehyde	RRT		
n-Hexanal	RRT		
n-Heptanal	RRT		
Phellandral	RRT		

[a] RRT = relative retention time; IR = infrared spectrum.

Further investigation into the composition of lavender and lavandin oils led to the identification of some constituents not previously reported to be present in these oils. They are listed in Table II.

Trace amounts of β-phellandrene have been identified by gas chromatography on different liquid phases[6]. The hydrocarbon fraction was isolated by percolation of the oil over silica gel with pentane. In addition, the pentane eluate of lavender oil showed the presence of α-thujene, α-phellandrene and terpinolene. Of these, terpinolene has also been found in lavandin oil[4], its presence being confirmed by us. In the carbonyl fraction of lavandin oil, isolated by treating the oil with Girard-P reagent in the presence of methanol and acetic acid, menthone and *p*-methyl acetophenone were identified by IR spectroscopy. The latter has hitherto only been found in a few essential oils. It also appeared to be a constituent of spike lavender oil[7,8]. Up to now we have not detected it in lavender oil. The carbonyl fraction of lavender oil, isolated by means of sodium bisulfite contained perilla-aldehyde, *n*-hexanal, *n*-heptanal and

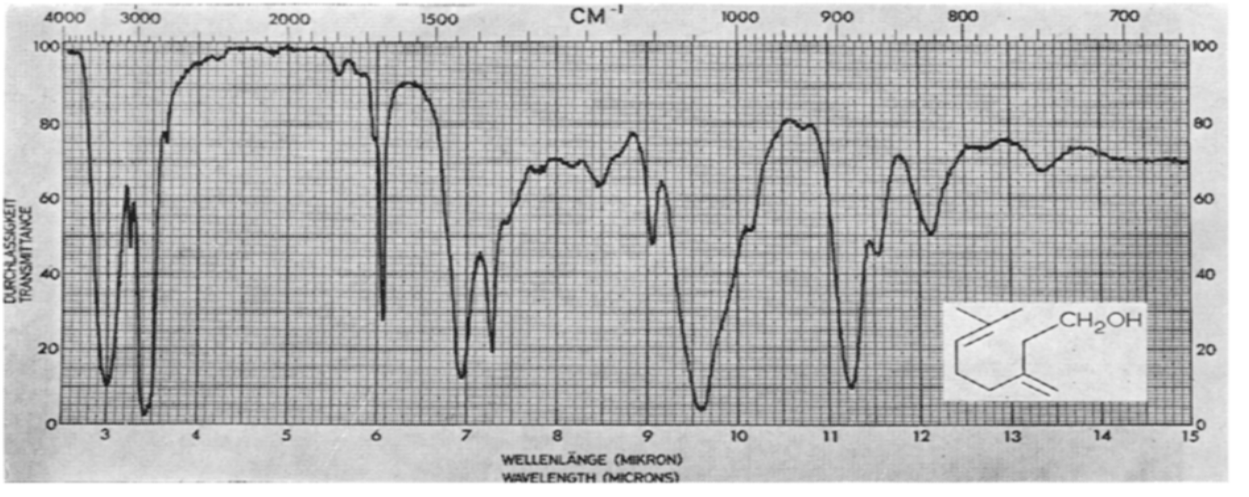

Fig. 1. IR spectrum of isogeraniol. Pure liquid, cell thickness 0.023 mm.

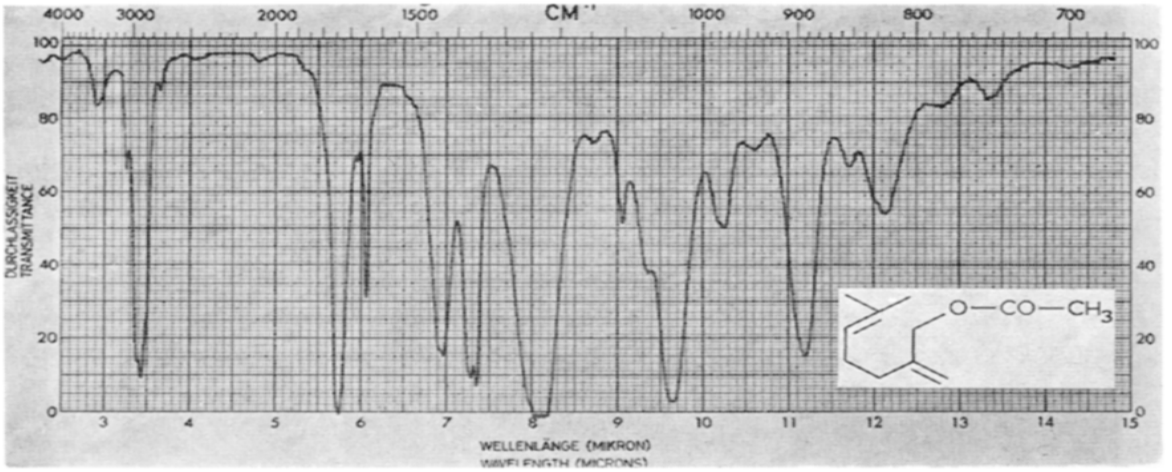

Fig. 2. IR spectrum of isogeranyl acetate. Pure liquid, cell thickness 0.021 mm.

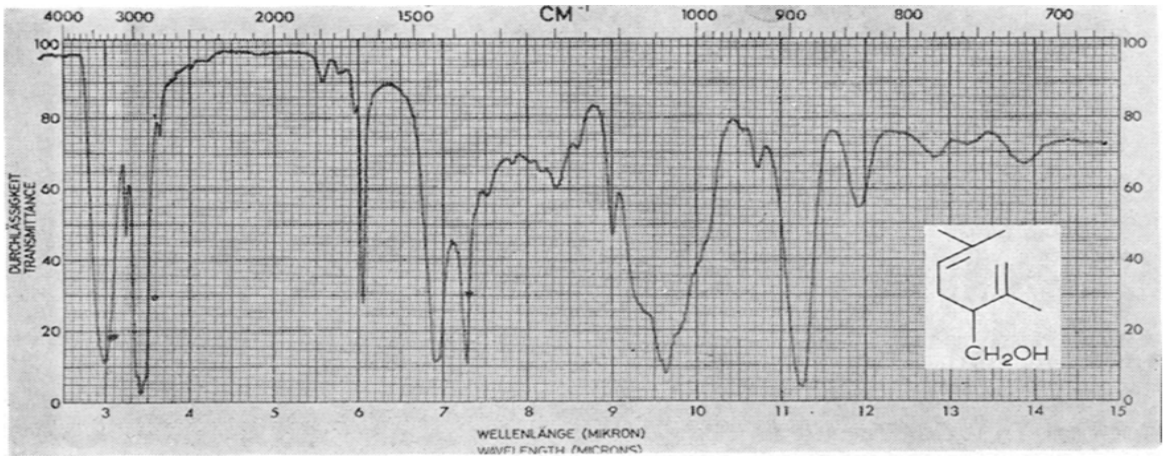

Fig. 3. IR spectrum of lavandulol. Pure liquid, cell thickness 0.021 mm.

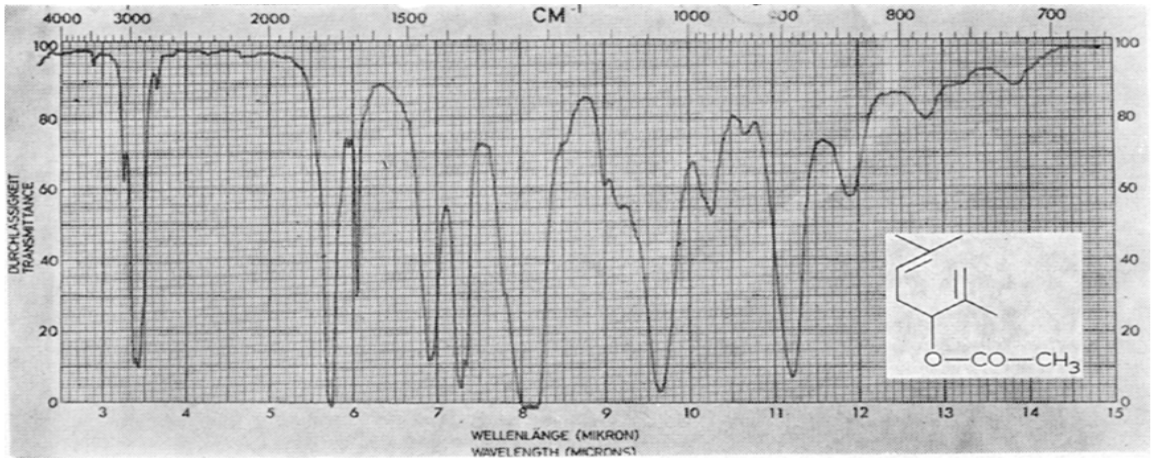

Fig. 4. IR spectrum of lavandulyl acetate. Pure liquid, cell thickness 0.021 mm.

phellandral as new constituents. They were identified by gas chromatography on packed and capillary columns. We also detected a trace of perillyl alcohol in the same fraction. Authentic samples were available of all the components identified. The methods of identification are also shown in Table II.

OGNYANOV and co-workers[9,10] have reported isogeranyl acetate as a new component in lavender oil. The identification was based upon the similarity of the IR spectrum of the reduced ester (*i.e.* isogeraniol) with that of published spectra[11,12]. However, careful comparison of the IR spectra (published by the Bulgarian investigators[9]) with those of authentic and pure samples of isogeraniol and isogeranyl acetate (Figs. 1 and 2) revealed that they were not identical. The spectra of OGNYANOV and co-workers appeared to be those of lavandulol and lavandulyl acetate. (Figs. 3 and 4). In spite of the close resemblance between the spectra of isogeraniol and lavandulol, they can be easily distinguished by:

(a) the shape of the C–OH stretching band at 9.6 μ (1042 cm^{-1});

(b) the positions and pattern of the bands in the region of 7.4–9.1 μ (1351–1099 cm^{-1});

(c) the position of the CH out of plane deformation band of the tri-substituted ethylenic bond, which in isogeraniol is positioned at 12.13 μ (824 cm^{-1}), in lavandulol at 11.92 μ (839 cm^{-1}) and in the spectrum of OGNYANOV at 11.9 μ (840 cm^{-1});

(d) the position of the bands in the 12.7–14 μ (787–714 cm^{-1}) region.

The IR spectra of the acetates show characteristic differences with respect to the position of the out of plane deformation band of the tri-substituted double bond, which occurs at 12.14 μ (824 cm^{-1}) in isogeranyl acetate, at 11.90 μ (840 cm^{-1}) in lavandulyl acetate and at 11.88 μ (842 cm^{-1}) in the spectrum of OGNYANOV. Conformity of the bands in the region of 12.5–14 μ (800–714 cm^{-1}) in the spectra of lavandulyl acetate and the spectrum under discussion is easily established. In our sample of lavender oil we found 5.0% of lavandulyl acetate, but only 0.4% in lavandin oil. The identification was also confirmed by NMR studies.

Another compound of importance in the oils of the genus lavandula appeared to be terpinen-4-ol. OGNYANOV and coworkers[9] found 12% of this alcohol in Bulgarian lavender oil, while NAVES AND TULLEN[13] found 1.5–3.5% in French lavender oil. SFIRAS AND VANDERSTREEK[14] detected the alcohol in lavandin oil, but its presence in this oil has not since been confirmed.

Our investigations also led to the identification of terpinen-4-ol by IR techniques. The highest percentage (5.6%) was found in French lavender oil; lavandin oil contained 2.4% of this alcohol and Spanish spike lavender oil 0.8%.

The authors wish to thank the management of the N.V. Chemische Fabriek "Naarden" for permission to publish these results.

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Received March 26th, 1970

J. Chromatog., 50 (1970) 127–131

CHROM. 4771

Gas chromatographic determination of some maleimides produced by the oxidation of heme and chlorophyll *a*

Our studies of chlorophyll biosynthesis (*e.g.*, see ref. 1), which involve the feeding of specifically labeled ^{14}C compounds to plants and the determination of the ^{14}C incorporation into the various portions of the chlorophyll molecule, have been hindered because of the lack of a method for the determination of microgram quantities of maleimides produced upon chromic acid oxidation of milligram quantities (usually available for these studies) of chlorophyll*. Only very recently have we established that the technique of chromate oxidation can be used with less than 10 mg quantities of porphyrin[2].

MORLEY AND HOLT[3] have shown that gas phase chromatography is an excellent method for non-destructively purifying milligram quantities of maleimides. Based on their report, a study was made of the feasibility of gas phase chromatography for the quantitative estimation of microgram quantities of maleimides. This report describes a gas chromatographic procedure found to be suitable for the determination of some maleimides, derived from the oxidation of heme and chlorophyll, in concentrations as low as 0.050 $\mu g/\mu l$ to within 3% experimental error.

Methods and materials

Preparation of maleimides. Maleimide, succinimide, citraconimide, methylethylmaleimide, hematinic acid methyl ester, and dihydrohematinic acid methyl ester were used for the sensitivity and reproducibility tests described herein. These compounds were prepared and characterized during an earlier study[2].

Gas phase chromatography. Maleimides were separated and estimated by gas phase chromatography on a 10 ft. × $\frac{1}{8}$ in. stainless-steel column packed with 20%

* The maleimides obtainable from the chromic acid oxidation of chlorophyll *a*, which are demonstrated in Fig. 1, include methylvinylmaleimide (ring I) which has been isolated only recently[4], methylethylmaleimide (ring II), methylmaleimide (ring III—citraconimide) when the oxidation is performed after degradation of pheophorbide *a* to pyrroporphyrin, and dihydrohematinic acid (ring IV).