ANALYSIS AND COMPOSITION OF OIL OF LEMON BY GAS–LIQUID CHROMATOGRAPHY*

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Prior to the advent of gas–liquid chromatography (GLC), the analysis of essential oils was a laborious and all too frequently unrewarding research problem. Even a cursory examination of POORE'S[1] analyses of orange and lemon oils will illustrate these points. Early attempts to utilize the technique of gas–liquid chromatography gave no notable improvements in this area. BERNHARD[2] was able to obtain only five distinct peaks on examination of lemon oil employing primitive gas–liquid chromatographic apparatus. It appears now that the detectors employed in this early study were insufficiently sensitive to determine components present in minor amounts. Additionally, no thorough search was conducted to determine the best stationary liquid phase for carrying out these separations. After this publication[2], work was initiated to determine what compound or compounds were best suited to serve as a stationary liquid phase for the separation and identification by GLC of the components present in citrus oils. The results of that study[3] indicated that there are three liquids which can serve as suitable stationary liquid phases; they are LAC-2-R446 (the adipate polyester of diethylene glycol partially cross-linked with pentaerythritol), LAC-4-R777 (the succinate polyester of diethylene glycol), and Craig polyester adipate. Although LIPSKY AND LANDOWNE[4,5] had previously reported on the use of LAC-2-R446 and LAC-4-R777 for the separation of unsaturated fatty acid esters, no one has reported their use to separate the components of essential oils[3]. Use of these three stationary phases affords a rather extensive separation of the components present in oil of lemon.

EXPERIMENTAL

The apparatus employed to separate the constituents of the lemon oils was an Aerograph model A-90-C (manufactured by Wilkins Instrument and Research, Inc., Walnut Creek, Calif.). Columns were constructed of stainless steel tubing, ¼ inch O.D. and 10 feet in length. The support material used throughout this study was Sil-O-Cel C-22 diatomaceous earth firebrick (30 to 60 mesh). Fractions of this were sieved to size and further graded by sedimentation in water, after which they were dried intensively. The liquid phase was introduced by deposition from benzene solution,

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the packing then being heated in a stream of helium to a temperature 20–30° higher than any used in the subsequent experiments.

The column was contained in an air thermostat; the carrier gas flow was controlled by a precision reducing valve and measured by a soap bubble flow meter situated at the column outlet. Temperature and flow control were sufficiently good to make possible the use of a four-channel, hot wire katharometer that proved virtually noiseless when connected with a 1 mV recording potentiometer. Sample injection was made by a microsyringe, and the column preheater was maintained at 50° above the temperature at which the column was operated.

Separations were carried out with the aid of two stationary liquid phases: LAC-2-R446[4] and LAC-4-R777[5]. The materials were applied to the solid support in the amount of 25 % w/w.

The parameters of operation were as follows: column temperature 150°; helium flow rate 90 ml/min; sample volume 5 to 20 μl; recorder chart speed 30 in./h. The column outlet was maintained at atmospheric pressure.

The lemon oil samples examined were cold-pressed California oils obtained from the Sunkist Growers, Inc., of Los Angeles, California. They were from last year's crop of fruit (April–May) and were so-called raw oil samples. No antioxidants were added. A typical oil had the following physical properties: specific gravity 25°/25° = 0.852; citral content = 3.40 % (actually measured as total aldehyde content); $\alpha_D^{25°}$ + 55.33°; $n_D^{20°}$ 1.4749.

RESULTS AND DISCUSSION

When cold-pressed California lemon oil was examined by means of GLC employing a stationary liquid phase of LAC-2-R446, thirty-two peaks were evident on the chromatogram (Fig. 1); twenty-two of these peaks have been numbered for purposes

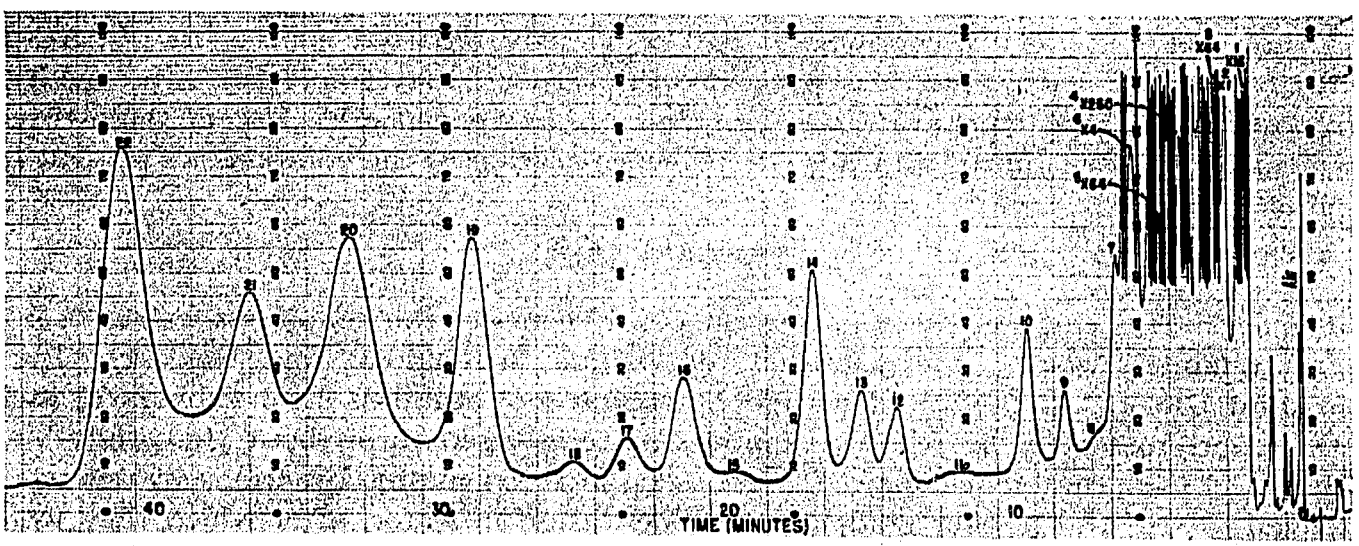

Fig. 1. Gas–liquid chromatogram of the components of a cold-pressed California lemon oil. Sample size, 20 μl; temperature, 150°; helium flow rate, 90 ml/min; stationary phase, LAC-2-R446 on a support of Sil-O-Cel C-22 (30–60 mesh), 25% by weight; stainless steel column 10 ft. by ¼ in. O.D.; 1 mV recording potentiometer; chart speed, 30 in./h. Peak identities are presented in Table I.

of identification. It may be seen that there are ten small peaks near the origin of the chromatogram (between the air peak and peak 1) which are not numbered. These peaks differ in both position, that is, retention distance, and amount, from sample to sample and have not as yet presented any consistent pattern. The peaks numbered from 1 through 22 differ generally only in amount from sample to sample. Thus the principal investigations and identifications were concerned with these peaks or components (work is currently in progress on the identification of the first ten or so peaks in various oil samples). The assignment of various peak identities was made by determination of the corrected retention volumes (V_R°) [6] of known compounds and comparison

TABLE I

RELATIVE RETENTION VOLUMES OF THE COMPONENTS OF COLD-PRESSED CALIFORNIA LEMON OIL

Stationary phase LAC-2-R446; temperature 150°; helium flow rate 90 ml/min. *n*-Decanal = 1.00.

Peak	V_R°/V_R°		*Compound*
	Unknown	*Known*	
1	0.144	0.148	α-pinene
2	0.189	—	—
3	0.217	0.227	(β-pinene; myrcene)
4	0.299	0.298	*d*-limonene
		0.302	*n*-heptanal
5	0.352	0.351	1,8-cineole
		0.358	γ-terpinene
6	0.420	—	—
7	0.454	0.444	(*n*-hexanol)
		0.455	*n*-octanal
8	0.501	—	—
9	0.583	0.571	(methyl heptenone)
10	0.679	0.676	*n*-nonanal
11	0.842	0.840	octyl acetate
12	1.00	1.00	*n*-decanal
13	1.08	1.06	linalool
14	1.21	1.18	citronellyl acetate
15	1.41	1.43	citronellal
		1.47	(*n*-undecanal)
16	1.53	1.54	tetrahydrogeraniol
17	1.67	—	—
18	1.81	1.83	decyl acetate
19	2.06	—	—
20	2.36	2.30	(α-terpineol)
		2.38	citronellol and/or neral
21	2.61	2.61	geranyl acetate
22	2.93	2.90	citral

with those for the unknown peaks. In this manner, a tentative identification of a large number of the components present was achieved. Confirmation of these results was obtained by an enrichment procedure in which known compounds were added, one at a time, to fresh portions of the lemon oil and re-examined by GLC. Data are presented in the form of relative retention volumes (V_R°/V_R°) [6] (Table I). It should be noted that it is experimentally impossible to distinguish between two compounds

whose relative retention volumes differ by 7 % or less since they will appear on the chromatogram as a single, united peak; differences of 10 to 15 % in relative retention volumes will show peaks that are united, *e.g.*, shoulders, or doublets; and differences of 20 % or more are necessary for complete separation of zones or peaks[7,8].

In order to be well within the bounds of experimental error, an arbitrary limit of agreement not to exceed 2 % was established. Compounds with values for corrected relative retention volumes not agreeing to within 2 % of each other are enclosed in parentheses. In Table I, those peaks that differ by more than the 2 % limit are: peak (3) α-pinene, myrcene (4.4 % difference); peak (7) *n*-hexanol (2.3 % difference); peak (9) methyl heptenone (2.1 % difference); peak (15) citronellal (2.8 % difference);

TABLE II

RELATIVE RETENTION VOLUMES OF THE COMPONENTS OF COLD-PRESSED CALIFORNIA LEMON OIL

Stationary phase LAC-4-R777; temperature 150°; helium flow rate 90 ml/min. *n*-Decanal = 1.00.

Peak	$V_R°/V_R°$		*Compound*
	Unknown	*Known*	
1	0.144	0.136	(α-pinene)
2	0.187	—	—
3	0.219	0.217	β-pinene; myrcene
4	0.299	0.294	*d*-limonene
5	0.358	0.343	(γ-terpinene)
		0.363	1,8-cineole
6	0.417	—	—
7	0.492	0.495	*n*-octanal
8	0.561	—	—
9	0.701	0.701	*n*-nonanal
10	0.824	—	—
11	1.00	1.00	*n*-decanal
12	1.07	1.07	citronellal
13	1.14	1.15	linalool
		1.18	linalyl acetate
14	1.26	—	—
15	1.40	1.41	*n*-undecanal
16	1.85	1.80	citronellyl acetate
17	2.09	2.01	(*n*-dodecanal)
18	2.48	2.41	(α-terpineol and/or geraniol)
		2.46	citronellol
19	2.73	2.74	geranyl acetate
20	3.21	3.22	citral
21	3.59	3.68	(*d*-carvone)

n-undecanal (4.1 % difference); and peak (20) α-terpineol (2.5 % difference). These differences are well within the 7 % limit found by JAMES[7] and BERNHARD[8].

As a further check on identity, relative retention volumes were evaluated on a second stationary liquid phase, LAC-4-R777 (Table II). The data supplied by the use of another liquid phase lend credence to the tentative identification of the compounds present in the oil. Employing a liquid phase of LAC-4-R777, lemon oil showed twenty-one major peaks on the chromatogram. Peak identities were assigned on the basis of agreement of relative retention volumes for the unknown peaks with those

values for known compounds. Those peaks that differ by more than the 2 % limit of agreement are: peak (1) α-pinene (5.8 % difference); peak (5) γ-terpinene (4.4 % difference); peak (17) *n*-dodecanal (4.0 % difference); peak (18) α-terpineol and/or geraniol (2.9 % difference); and peak (21) *d*-carvone (2.4 % difference). Once again these differences are well within the 7 % limit[7,8].

The chemistry of cold-pressed California lemon oils was investigated by POORE[1] who identified the following compounds: α-pinene, β-pinene, *d*-limonene, γ-terpinene, citral, *n*-octanal, *n*-nonanal, geraniol, linalool, a tertiary alcohol (?), a crystalline aldehyde, acetic, caprylic, caproic acids, bisabolene, and cadinene. POORE[1] could not find any methyl heptenone in California lemon oils, although this compound was reported to occur in Italian lemon oils by workers at Schimmel & Co.[9]. The results of the investigation described herein agree well with most of the findings of POORE. In addition to those compounds reported by POORE, a number of esters were detected, and the presence of methyl heptenone appears likely. It may well be that POORE's methods of isolation, *i.e.*, steam distillation, hydrolysis, etc., did not permit the recovery of intact ester components present in the oils.

A proximate composition of a typical cold-pressed California lemon oil was determined by integrating the areas under the appropriate peaks (Fig. 1) and is presented in Table III. It should be noted that these values do not represent either the

TABLE III

PROXIMATE COMPOSITION OF A TYPICAL COLD-PRESSED CALIFORNIA LEMON OIL

Peaks	*Compound**	*Per cent composition***
First 10 unnumbered peaks	—	0.06
1	α-pinene	2.65
2	—	0.19
3	β-pinene; myrcene	12.69
4	*d*-limonene; *n*-heptanal	72.35
5	γ-terpinene; cineole	8.50
6	—	0.74
7	*n*-octanal; (*n*-hexanol)	0.15
8	—	0.09
9	(methyl heptenone)	0.06
10	*n*-nonanal	0.09
11	octyl acetate	0.04
12	*n*-decanal	0.06
13	linalool	0.08
14	citronellyl acetate	0.17
15	citronellal; (*n*-undecanal)	0.03
16	tetrahydrogeraniol	0.11
17	—	0.06
18	decyl acetate	0.05
19	—	0.32
20	citronellol; neral	0.51
21	geranyl acetate	0.40
22	citral	0.61
Total		100.01

* Peak assignments based upon data from Table I.
** Values based upon integration of areas under appropriate peaks (Fig. 1).

precise weight per cent or mole per cent of the components in question, but some intermediate value that differs from either of these by a small but significant factor (usually from 1 to 5 %)[10]. The actual katharometer response may vary considerably with the nature of the components, and thus the output signal may not be linear with concentration in a highly varied, multicomponent system[11].

The per cent citral indicated in Table III differs from that reported in the experimental section above. The value reported in the experimental account was determined by reaction with hydroxylamine (the common method employed in the citrus industry) and actually reflects the total aldehyde and ketone content of the oil. The percentage reported in Table III more nearly represents the citral content exclusive of other aldehydes and ketones that are present in the oil.

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SUMMARY

Cold-pressed California lemon oil was examined by means of gas–liquid chromatography and a number of the constituents present was tentatively identified by means of corrected retention volume correlation employing two stationary liquid phases. The components identified by these procedures were: α-pinene, β-pinene, myrcene, *d*-limonene, *n*-heptanal, 1,8-cineole, γ-terpinene, *n*-hexanol, *n*-octanal, methyl heptenone, *n*-nonanal, linalool, *n*-decanal, citronellyl acetate, citronellal, *n*-undecanal, tetrahydrogeraniol, decyl acetate, geraniol, α-terpineol, citronellol, neral, geranyl acetate, citral, and *d*-carvone.

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