# THE ISOLATION AND EXAMINATION OF THE ESSENTIAL OIL OF THE KUMQUOT (*F. MARGARITA* (LOUR.) SWINGLE)

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There is a large body of information concerning the botanical and horticultural aspects of the kumquots[1–4], but there is little reported in the literature concerning analyses of the chemical composition of the fruit itself. There are a few reports on the vitamin content of kumquots[5,6], especially vitamin C distribution[6], and some reports on the technology of preserving and utilization of kumquots[7]. Only one group, that of FESTER[8,9] and his co-workers in South America, has reported on the physical properties of the essential oil obtained from the epicarp (flavedo) of the kumquot fruit. FESTER[8] has reported the density, refractive index, optical rotation, acid number, and ester number of the essential oils of a number of South American plants; the physical properties of kumquot oil were included in this publication[8].

Kumquot trees belong to the plant family *Rutaceae* which include two genera, *Citrus* and *Fortunella*. The latter genus includes the kumquot. The kumquot fruit bears a close similarity to citrus fruit. When ripe they are small ovoid or oblong fruits with a bright orange skin that contains large oil sacs, and when split open, present the typical segmented appearance of a citrus fruit. Fruits are generally ½ to ¾ in. in diameter and ¾ to 1–½ in. in length. It is interesting to note the albedo (mesocarp) and flavedo (epicarp) of the kumquot are quite sweet; and the central portion of the fruit, even when ripe, has a rather sour, acid taste. Kumquots are used principally as decorative fruits and in preserves and jelly-making[1,2].

## EXPERIMENTAL

The kumquots used in this study were of the variety *margarita* and grown on the University farm located in Winters (about 7 miles west of Davis), California. They were harvested just after the fruit had reached a rich golden orange color (it is difficult to determine the exact state of maturity of these fruits). Immediately after harvest, the kumquots were brought to the laboratory where they were chilled, thoroughly rinsed in cold water, drained, rinsed in a cool solution of a commercial detergent, rinsed twice with fresh cool water, and dried with clean cloths. Two kilograms of the fruit were selected and the epicarp (flavedo) was carefully stripped from each fruit by means of a stainless steel knife; the mesocarp (albedo) and segment mem-

branes of the fruit were discarded. The epicarp was rinsed in cool distilled water to remove any contamination by the acid present in the juice of the mesocarp, segment membranes, or juice sacs. The weight of the resulting epicarp obtained from 2000 g of whole fruit was 815 g.

The epicarp was added to 500 ml distilled water in a blendor and homogenized for 15 min. The homogenate was transferred to a 1-l round bottom distillation flask and the contents steam-distilled in the conventional manner through a 30 cm bubble-plate column fitted with a Kjeldahl trap and a 50 cm ice water-cooled condenser. The steam distillation was conducted at atmospheric pressure. Distillation was discontinued 15 min after the last drop of oil appeared in the receiver and when the latter contained about 450 ml of total distillate. The aqueous phase was saturated with sodium chloride, and the upper oil phase separated from the water phase in a separatory funnel. A colorless, light oil with a pleasant, fruity aroma resulted. The oil remaining in the aqueous phase was extracted with 3–80 ml portions of *n*-pentane; the extracts and the oil combined, and dried over anhydrous sodium sulfate for 24 h. The combined extracts and oil were filtered, and placed in a rotary flash evaporator where the *n*-pentane was removed under vacuum at 26°. A condenser using dry ice and alcohol (—77°) was used to collect the *n*-pentane and prevent loss of volatile components. The yield of oil above the aqueous phase was 7.57 g and that recovered from the *n*-pentane extractions was 1.13 g, making a total yield of 8.70 g (1.07 % of total peel weight and 0.435 % of the total fresh fruit weight).

A small amount of the oil was placed in a pycnometer and the density at 25° was determined. The density of this steam-distilled kumquot oil was 0.8389. The oil had an optical rotation of $\alpha_D^{25°} + 15.76°$. The refractive index at 20° was 1.4758. An ultraviolet absorption spectrum of the oil was taken, and this curve revealed none of the sharp peaks, or bands generally associated with the coumarin compounds found in typical citrus oils. It is interesting to compare the results obtained from this study with those obtained by FESTER[8] in his study of South American kumquot oil. FESTER found a density at 15° of 0.8505, and a refractive index of 1.4769 (temperature not specified), $\alpha_D^{17.5°} + 93°20'$, an acid number of 2.0, and an ester number of 4.4 (we did not make a determination of acid number or ester number in this study). The differences between these data are quite probably due to differences in species and possibly varietal differences. FESTER[8] did not indicate what species or variety his group examined and reported.

The gas–liquid chromatographic equipment used to separate the components of the essential oil was an Aerograph model A-90-C, equipped with a ¼ in. O.D. stainless steel column. An improved four-cell catharometer assembly was employed for detection. The exact experimental parameters of operation, *i.e.*, temperature, flow rate, etc., accompany the figures and tables.

The kumquot oil was deterpenated by a modified KIRCHNER procedure[10] as follows: 15 g of dried (heated at 125° for 3 h immediately before use), 100 mesh silicic acid were added to a solution of 3.0 g of the essential oil in 25 ml of *n*-pentane with shaking, and the mixture was allowed to stand at room temperature with occasional

shaking for 2 h. The silicic acid was filtered off on a sintered glass crucible under reduced pressure, and the pentane solution dried over anhydrous sodium sulfate for 1 h. The terpenoid fraction was eluted from the adsorbent by placing the silicic acid in 25 ml of absolute ethanol and allowing the mixture to stand with occasional swirling at room temperature for 2 h. The adsorbent was filtered off as above and discarded. The ethanol was dried over anhydrous sodium sulfate. When dry, the pentane and ethanol solutions were each filtered, and the respective solvents removed by means of a rotary flash evaporator under moderate vacuum (*ca.* 1.0 mm Hg) at 26°. The terpene and the terpenoid fractions were then stored at −10° until used.

## RESULTS AND DISCUSSION

When kumquot oil was examined by means of gas–liquid chromatography employing a stationary liquid phase consisting of LAC-2-R446 (the adipate polyester of diethylene glycol partially cross-linked with pentaerythritol)[11,12], 14 major peaks were evident on the chromatogram. The assignment of various peak identities was made by determination of the corrected retention volumes ($V_R^\circ$)[13] of known compounds and comparison 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 kumquot oils and re-examined by gas–liquid chromatography. Data are presented in the form of relative retention

TABLE I

CORRECTED RELATIVE RETENTION VOLUMES OF THE COMPONENTS OF THE ESSENTIAL OIL OF KUMQUOT

Stationary phase: LAC-2-R446*
(*n*-Decanal = 1.00)

| *Peak* | $V_R^\circ/V_R^\circ$ | | *Compound* |
|---|---|---|---|
| | *Unknown* | *Known* | |
| 1 | 0.0387 | — | — |
| 2 | 0.0663 | — | — |
| 3 | 0.141 | 0.148 | (α-Pinene) |
| 4 | 0.213 | 0.227 | (Myrcene) |
| 5 | 0.301 | 0.298 | *d*-Limonene |
| 6 | 0.843 | 0.840 | *n*-Octyl acetate |
| 7 | 1.00 | 1.00 | *n*-Decanal |
| 8 | 1.50 | 1.47 | *n*-Undecanal |
| | | 1.50 | Bornyl acetate |
| 9 | 1.75 | — | — |
| 10 | 2.03 | — | — |
| 11 | 2.35 | 2.38 | Citronellol |
| 12 | 2.71 | — | — |
| 13 | 3.26 | 3.25 | Geranyl propionate |
| 14 | 3.78 | 3.63 | (*trans*-Carveol) |

* Temperature 150°; helium flow rate 90 ml/min.

volumes, $(V_{-}/V_R^{\circ})$[13] (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[14,15].

In order to be well within the bounds of experimental error, an arbitrary limit of agreement for corrected relative retention volumes 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 (4.7 % difference); peak (4) myrcene (6.2 % difference); peak (14) *trans*-carveol (4.1 % difference). These differences are still well within the 7 % limit found by BERNHARD[14] and JAMES[15].

As a further check on identity, relative retention volumes were evaluated on a

TABLE II

CORRECTED RELATIVE RETENTION VOLUMES OF THE COMPONENTS OF THE ESSENTIAL OIL OF KUMQUOT

Stationary phase: LAC-4-R777*
(*n*-Decanal = 1.00)

| *Peak* | $V_R^{\circ}/V_R^{\circ}$ | | *Compound* |
|---|---|---|---|
| | *Unknown* | *Known* | |
| 1 | 0.0572 | — | — |
| 2 | 0.0960 | — | — |
| 3 | 0.142 | 0.136 | (α-Pinene) |
| 4 | 0.221 | 0.217 | Myrcene |
| 5 | 0.301 | 0.294 | (*d*-Limonene) |
| 6 | 0.618 | — | — |
| 7 | 0.725 | 0.715 | Methyl heptenone |
| 8 | 0.789 | 0.791 | 3-Hepten-1-ol |
| 9 | 0.846 | 0.849 | *n*-Octyl acetate |
| 10 | 0.902 | — | — |
| 11 | 1.00 | 1.00 | *n*-Decanal |
| 12 | 1.15 | 1.15 | Linalool |
| | | 1.18 | (Linalyl acetate) |
| 13 | 1.27 | — | — |
| 14 | 1.41 | 1.41 | *n*-Undecanal; Linalyl propionate |
| 15 | 1.75 | 1.71 | (Decyl acetate) |
| 16 | 1.85 | 1.80 | (Citronellyl acetate) |
| 17 | 2.08 | 2.10 | *n*-Decanol |
| 18 | 2.35 | 2.31 | Terpinyl acetate |
| 19 | 2.45 | 2.41 | Geraniol; α-Terpineol |
| | | 2.46 | Citronellol |
| 20 | 2.76 | 2.74 | Geranyl acetate |
| 21 | 2.86 | — | — |
| 22 | 3.23 | 3.22 | Citral |
| 23 | 4.08 | 4.01 | *trans*-Carveol |
| | | 4.05 | Linalyl butyrate |
| | | 4.06 | Geranyl butyrate |
| 24 | 4.51 | — | — |

* Temperature 150°; helium flow rate 90 ml/min.

second stationary liquid phase LAC-4-R777 (the succinate polyester of diethylene glycol)[12] (Table II). The data supplied by the use of another stationary liquid phase lend credence to the tentative identification of the compounds present in the oil. Employing a liquid phase of LAC-4-R777, kumquot oil showed 24 major peaks on the chromatogram. The identities were assigned on the basis of agreement of corrected relative retention volumes for the unknown peaks with those values for known compounds. Those peaks that differed by more than the 2 % limit of agreement are: peak (3) α-pinene (4.4 % difference); peak (5) *d*-limonene (2.4 % difference); peak (12) linalyl acetate (2.5 % difference); peak (15) decyl acetate (2.3 % difference); peak (16) citronellyl acetate (2.8 % difference). Once again these differences are well within the 7 % limit[14,15].

The choice of the two LAC liquid stationary phases was most certainly not an arbitrary one. While it was possible to demonstrate the presence of only 5 distinct peaks in whole lemon oil[16] using non-polar stationary liquid phases, *e.g.*, D.C. Silicone Fluids, the use of these polyester stationary liquid phases increased this resolution to some 20–30 compounds[17]. Thus it appeared advisable to use these materials to separate the components of kumquot oil. Reference to Fig. 1 shows a typical chro-

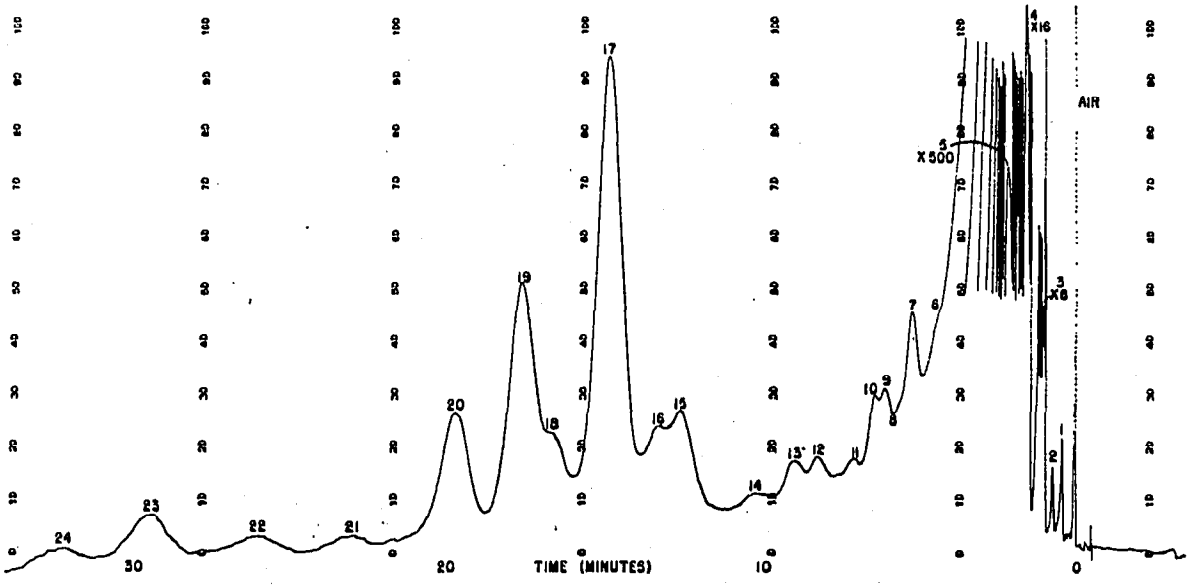


Fig. 1. Gas–liquid chromatogram of the components of the essential oil of kumquot. Sample size: 40 μl; temperature: 150°; helium flow rate: 90 ml/min; stationary phase: LAC-4-R777 on a support of Sil-O-Cel C-22 (42–60 mesh), 20 % by weight; stainless steel column 10 ft. by ¼ in. O.D.; 1 mV recording potentiometer; chart speed: 30 in./h. Peak identities are presented in Table II.

matogram for whole kumquot oil employing a LAC-4-R777 column. The curve indicates a wide divergence in the boiling points of the components present, and this makes efficient separation of these constituents by gas–liquid chromatography at a fixed temperature inconvenient. A sure sign of this wide boiling point range is indicated by the crowding of the peaks at the initial or starting point of the curve and the distention of the peaks near the terminal portion of the curve. A possible solution

to this problem would be to resort to temperature programming or multi-stage chromatographic columns[18]. Since this equipment was unavailable to us, a preliminary separation of the components present was deemed desirable. Such a separation must be conducted under the mildest of conditions, and it was decided to employ the chromatographic deterpenation procedure of KIRCHNER AND MILLER[10] for this purpose. By this technique the kumquot oil was separated into two major fractions: one, the hydrocarbon fraction (terpenes) with a boiling range of some 60°, and the other the terpenoid fraction (the compounds bearing oxygen functions, *e.g.*, esters, aldehydes, ketones, etc.) with a boiling range of some 80°. This now permitted the chromatographic examination of the two fractions at different column temperatures. The first temperature selected would be the optimum for the separation of the terpenes, and the second temperature selected would be the optimum column temperature for the separation of the terpenoid compounds.

Examination of the terpene fraction from kumquot oil revealed that there are six distinct peaks in the chromatograms when the oil is separated on a LAC-2-R446 column. The data for these separations are presented in Table III. Once again two peaks differed by more than the 2 % limit of agreement; these were peak (3) α-pinene (4.0 % difference) and peak (4) camphene (4.2 % difference).

TABLE III

CORRECTED RELATIVE RETENTION VOLUMES OF THE COMPONENTS OF THE TERPENE FRACTION OF THE ESSENTIAL OIL OF KUMQUOT

Stationary phase: LAC-2-R446*
(*d*-Limonene = 1.00)

| *Peak* | $V_R^\circ/V_R^\circ$ | | *Compound* |
|---|---|---|---|
| | *Unknown* | *Known* | |
| 1 | 0.148 | — | — |
| 2 | 0.213 | — | — |
| 3 | 0.365 | 0.379 | (α-Pinene) |
| 4 | 0.497 | 0.519 | (Camphene) |
| 5 | 0.745 | 0.742 | Myrcene |
| 6 | 1.00 | 1.00 | *d*-Limonene |

* Temperature 100°; helium flow rate 145 ml/min.

When the terpene fraction was examined by means of a stationary liquid phase consisting of LAC-4-R777, 9 distinct peaks were detected. Reference to Table IV shows that the corrected relative retention volumes for the unknowns agree well with the corrected relative retention volumes of the knowns. Only two peaks, peak (5) camphene (4.9 % difference) and peak (8) γ-terpinene (2.4 % difference) exceeded the arbitrary 2 % limit of agreement. The chromatogram for this separation employing LAC-4-R777 is presented in Fig. 2. The capital S near the origin of the chromatogram indicates some residual solvent, from the deterpenation step, that remained in the terpene fraction.

TABLE IV

CORRECTED RELATIVE RETENTION VOLUMES OF THE COMPONENTS OF THE TERPENE FRACTION OF THE ESSENTIAL OIL OF KUMQUOT

Stationary phase: LAC-4-R777*
(*d*-Limonene = 1.00)

| *Peak* | $V_R^\circ/V_R^\circ$ | | *Compound* |
|---|---|---|---|
| | *Unknown* | *Known* | |
| 1 | 0.214 | — | — |
| 2 | 0.285 | — | — |
| 3 | 0.374 | 0.372 | α-Pinene |
| 4 | 0.382 | — | — |
| 5 | 0.553 | 0.527 | (Camphene) |
| 6 | 0.767 | 0.776 | Myrcene |
| 7 | 1.00 | 1.00 | *d*-Limonene |
| 8 | 1.28 | 1.25 | (γ-Terpinene) |
| 9 | 1.45 | — | — |

* Temperature 100°; helium flow rate 145 ml/min.

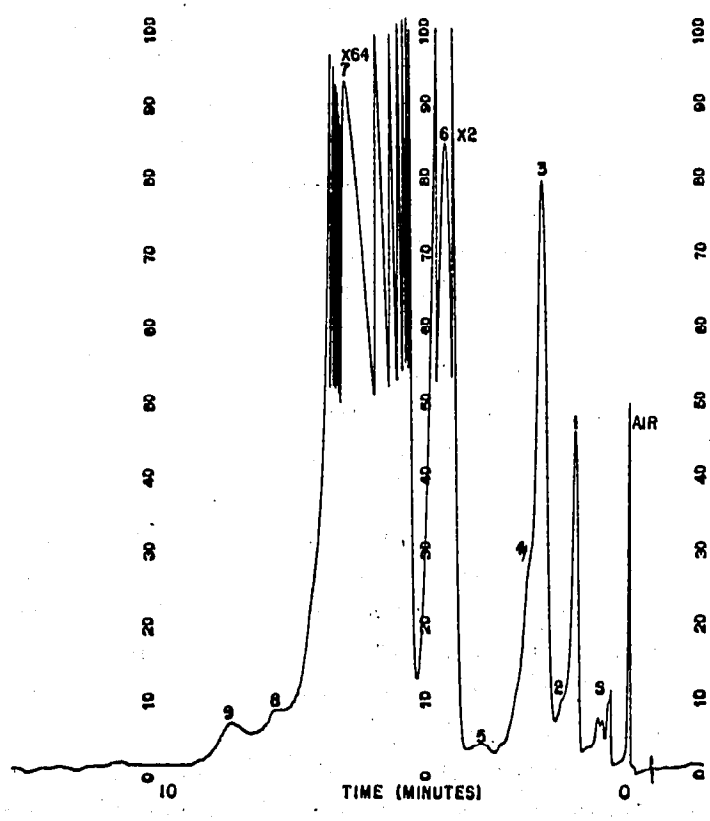


Fig. 2. Gas–liquid chromatogram of the components of the terpene hydrocarbon fraction of the essential oil of kumquot. Sample size: 20 μl; temperature: 100°; helium flow rate: 145 ml/min; stationary phase: LAC-4-R777 on a support of Sil-O-Cel C-22 (42–60 mesh), 20% by weight; stainless steel column 10 ft. by ¼ in. O.D.; 1 mV recording potentiometer; chart speed: 30 in./h. Peak identities are presented in Table IV.

The terpenoid fraction was examined by means of a LAC-2-R446 column and the chromatogram revealed 11 distinct peaks. Only one peak, peak (6), geranyl formate (3.1 % difference) exceeded the arbitrary 2 % limit of agreement. These data are presented in Table V. The terpenoid fraction was next examined employing a LAC-4-R777 column and the chromatogram revealed 21 distinct peaks (Table VI). Those peaks that differ by more than the 2 % limit of agreement are: peak (2) *d*-limonene (2.7 % difference); peak (6) citronellal (2.8 % difference); peak (8) linalyl

TABLE V

CORRECTED RELATIVE RETENTION VOLUMES OF THE COMPONENTS OF THE TERPENOID FRACTION OF THE ESSENTIAL OIL OF KUMQUOT

Stationary phase: LAC-2-R446*
(*n*-Decanal = 1.00)

| *Peak* | $V_R^\circ/V_R^\circ$ | | *Compound* |
|---|---|---|---|
| | *Unknown* | *Known* | |
| 1 | 0.150 | 0.148 | α-Pinene |
| 2 | 0.301 | 0.298 | *d*-Limonene |
| 3 | 0.668 | 0.676 | *n*-Nonanal |
| 4 | 0.835 | 0.840 | *n*-Octyl acetate |
| 5 | 1.00 | 1.00 | *n*-Decanal |
| 6 | 1.66 | 1.61 | (Geranyl formate) |
| 7 | 1.93 | 1.95 | Borneol |
| 8 | 2.28 | 2.28 | α-Terpineol |
| 9 | 2.61 | 2.61 | Geranyl acetate |
| 10 | 3.10 | 3.10 | *d*-Carvone |
| 11 | 3.60 | 3.63 | *trans*-Carveol |

* Temperature 150°; helium flow rate 90 ml/min.

propionate and undecanal (2.1 % difference); and peak (14) terpinyl acetate (2.1 % difference). A chromatogram for this separation using a LAC-4-R777 column is presented in Fig. 3.

Examination of Fig. 1 shows a very large amount of *d*-limonene (peak 5) to be present in the whole oil, and there are also relatively large amounts of *n*-decanol (peak 17), citronellol and/or α-terpineol–geraniol (peak 19), and geranyl acetate (peak 20).

Fig. 2 indicates that α-pinene (peak 3), myrcene (peak 6), and *d*-limonene (peak 7) are the principal terpene hydrocarbons found in kumquot oil. It is interesting to note that no β-pinene was detected in kumquot oil. This appears to be an analogous situation to that of navel orange oil, for here, little evidence has been found for the presence of this compound[19]. However, β-pinene is present in lemon oil[20], grapefruit, lime, mandarin, and bergamot oils[19].

The terpenoid fraction (Fig. 3) reveals relatively large amounts of *n*-decanal and citronellal (peaks 5 and 6), bornyl acetate (peak 9), decyl acetate (peak 10), geraniol and/or α-terpineol (peak 15), geranyl acetate (peak 16) and *trans*-carveol (and quite possibly the butyrates of geraniol and linalool) (peak 20).

In contrast to the citrus oils, kumquot oil appears to contain less of the lower

TABLE VI

CORRECTED RELATIVE RETENTION VOLUMES OF THE COMPONENTS OF THE TERPENOID FRACTION OF THE ESSENTIAL OIL OF KUMQUOT

Stationary phase: LAC-4-R777* (*n*-Decanal = 1.00)

| Peak | $V_R^\circ/V_R^\circ$ | | Compound |
|---|---|---|---|
| | Unknown | Known | |
| 1 | 0.201 | — | — |
| 2 | 0.302 | 0.294 | (*d*-Limonene) |
| 3 | 0.832 | 0.849 | *n*-Octyl acetate |
| 4 | 0.879 | — | — |
| 5 | 1.00 | 1.00 | *n*-Decanal |
| 6 | 1.10 | 1.07 | (Citronellal) |
| 7 | 1.20 | 1.18 | Linalyl acetate |
| 8 | 1.38 | 1.41 | (Linalyl propionate; *n*-Undecanal) |
| 9 | 1.59 | 1.57 | Bornyl acetate |
| 10 | 1.73 | 1.71 | Decyl acetate |
| 11 | 1.80 | 1.80 | Citronellyl acetate |
| 12 | 1.94 | — | — |
| 13 | 2.09 | 2.10 | *n*-Decanol |
| 14 | 2.26 | 2.31 | (Terpinyl acetate) |
| 15 | 2.42 | 2.41 | Geraniol; α-Terpineol |
| 16 | 2.73 | 2.74 | Geranyl acetate |
| 17 | 3.18 | 3.22 | Citral |
| 18 | 3.54 | — | — |
| 19 | 3.68 | 3.68 | *d*-Carvone |
| 20 | 4.04 | 4.01 | *trans*-Carveol |
| | | 4.05 | Linalyl butyrate |
| | | 4.06 | Geranyl butyrate |
| 21 | 4.45 | — | — |

* Temperature 150°; helium flow rate 90 ml/min.

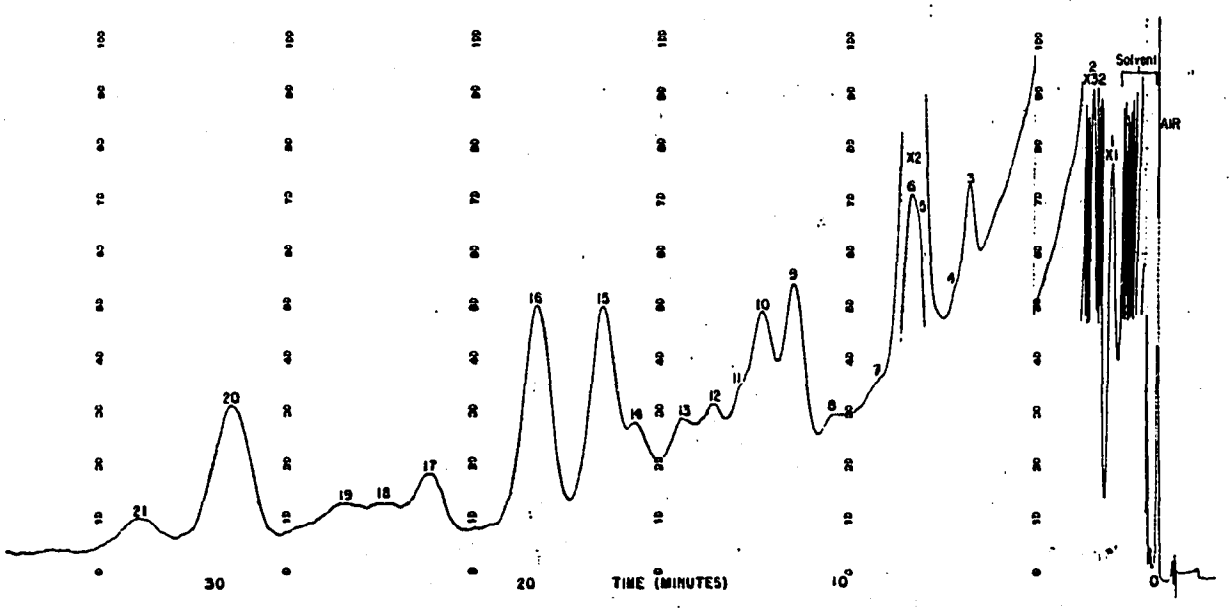


Fig. 3. Gas–liquid chromatogram of the components of the terpenoid fraction of the essential oil of kumquot. Sample size: 20 μl; temperature: 150°; helium flow rate: 90 ml/min; stationary phase: LAC-4-R777 on a support of Sil-O-Cel C-22 (42–60 mesh), 20% by weight; stainless steel column 10 ft. by ¼ in. O.D.; 1 mV recording potentiometer; chart speed: 30 in./h. Peak identities are presented in Table VI.

straight-chain aldehydes from $C_8$–$C_{12}$. No octanal was detected in this study. The citral content of the oil is low and is generally comparable to the citral level found in orange oils. It is noticeably lower than that level found in lemon oils. The absence of large amounts of these aldehydes may account, at least in part, for the milder, less penetrating aroma of kumquot oil.

We were unable to detect any sesquiterpenes[20] in the oil. It may well be that the method of isolation precludes their occurrence in the fractions examined.

The complex nature of this oil will require further studies and it is hoped in the future, we will be able to report the results of a more detailed examination of the oil.

## SUMMARY

1. The essential oil of the kumquot was isolated from the epicarp of the ripe fruit by steam distillation.

2. The whole oil was examined by means of gas–liquid chromatography and a number of the components present were tentatively identified.

3. The whole oil was deterpenated and the terpene and terpenoid fractions were examined individually by gas–liquid chromatography.

4. The data for gas–liquid chromatographic examinations are presented in the form of corrected relative retention volumes ($V_R^\circ/V_R^\circ$) employing two stationary liquid phases, LAC-2-R446 and LAC-4-R777.

5. The oil was found to contain large amounts of *d*-limonene, some α-pinene, myrcene, terpene esters, aldehydes, a ketone, and some free alcohols.

6. The physical constants for the oil, *i.e.*, refractive index, optical rotation, etc., are presented.

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