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# SATURATED AND MONO-UNSATURATED LONG-CHAIN HYDROCARBONS FROM LEMON JUICE SACS

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**Key Word Index**—*Citrus limon*; Rutoceae; lemon fruit; hydrocarbon profiles; chemotaxonomy.

**Abstract**—Three varieties of lemon—Lisbon, Malta and Kusner—were examined for their content of juice sac saturated and mono-unsaturated long-chain hydrocarbons. The saturated fractions were 20 times the concentrations of the monoene fractions. The dominant linear hydrocarbon in the saturated fraction was $C_{25}$ while $C_{29}$ predominated in the monoene fraction. The saturated hydrocarbon profiles for Lisbon and Kusner were very similar to the profiles previously reported for Eureka lemon and Persian lime. The mono-unsaturated profiles were distinct for each of the three lemon varieties. In addition the lemon mono-unsaturated profiles were quite distinct from the hydrocarbon profiles previously reported for several other citrus species. In general the data support the elongation–decarboxylation mechanism for hydrocarbon synthesis proposed by Kolattukudy.

## INTRODUCTION

IN RECENT studies carried out at this Laboratory, the relationship of citrus lipids to juice quality[1–4] and chemotaxonomy[5–8] have been investigated. Long-chain hydrocarbons were shown to have greater potential as chemotaxonomic indicators than other citrus lipids and thus have been studied in greater detail.[9–13] In a previous investigation[9] only one lemon cultivar, Eureka, was studied. This lemon cultivar appeared to have a hydrocarbon pattern noticeably different from other citrus species, viz. oranges, grapefruit and mandarins.

The present study was undertaken to determine if specific hydrocarbon patterns existed in lemon which could be useful in distinguishing this species from other citrus species. In this study three varieties of *Citrus limon*, namely Lisbon, Malta and Kusner were examined for their saturated and mono-unsaturated long-chain hydrocarbon content.

## RESULTS AND DISCUSSION

Lemons can be grouped into two varietal classes, either acid or acidless. The major or common acid varieties can be further subdivided into three groups, viz. Eureka, Lisbon

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* One of the laboratories of the Southeastern Marketing and Nutrition Research Division, Agricultural Research Service, U.S. Department of Agriculture.

[1] H. E. NORDBY and S. NAGY, *Phytochem.* **8**, 2027 (1969).

[2] S. NAGY and H. E. NORDBY, *J. Agric. Food Chem.* **18**, 593 (1970).

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[9] S. NAGY and H. E. NORDBY, *Lipids* in press.

[10] S. NAGY and H. E. NORDBY, *Paper presented at Conf. on Citrus Chem Util.*, Winter Haven (1971).

[11] S. NAGY and H. E. NORDBY, *Phytochem.* **11**, 2789 (1972).

[12] S. NAGY and H. E. NORDBY, *Phytochem.* **11**, 2865 (1972).

[13] S. NAGY and H. E. NORDBY, *Lipids* in press.

and Villafranca. This subdivision is based on the physical appearance of the tree. The three lemon varieties examined in this study belong to the Lisbon group.[14] Although Kusner and Malta belong to this group, the physical appearance of both the tree and fruit show many distinctive differences from that of Lisbon.

All three varieties were picked when mature in late October. Lisbon is second to Eureka in commercial importance. This variety of Portuguese origin is known for its vigor, hardiness and high productivity. Kusner originated from the breeding program of the Soviet government at Sukhum, Georgia, U.S.S.R., however the fruit has been reported to be indistinguishable from the Spanish variety, Vernia.[14] Malta from northern India is probably the same as the Lebanese variety, Malti, which is of unknown origin.

Examination of Table 1 reveals that all three varieties yield approximately similar quantities of total juice sac lipids, i.e. 258–269 mg/20 g powder. These lemon values are similar to those reported for limes (241–322 mg/20 g).[12] Lemons and limes contain less sugar in their juice sacs than grapefruit, oranges, mandarins and tangelos, and thus, show a higher lipid content per unit weight of juice sac powder.

TABLE 1. TOTAL LIPID AND HYDROCARBON CONCENTRATIONS OF LISBON, KUSNER AND MALTA LEMONS (mg/20 g dry wt)

| Variety | Total lipid | Hydrocarbon fraction<br>Saturated | Monoene | Complex |
|---|---|---|---|---|
| Lisbon | 261·0 ± 7·9 | 3·8 ± 0·3 | 0·2 ± 0·0 | 0·4 ± 0·2 |
| Kusner | 269·0 ± 5·2 | 3·9 ± 0·2 | 0·1 ± 0·0 | 0·5 ± 0·1 |
| Malta | 258·5 ± 6·8 | 4·5 ± 0·3 | 0·3 ± 0·1 | 0·4 ± 0·2 |

The average total lemon hydrocarbon concentration is 4·7 mg/20 g powder. This value is nearly the same as reported for other citrus varieties,[9,13] however, the ratio of saturated to mono-unsaturated for the lemon varieties is 20:1. This ratio is far greater than the 3:1–13:1 ratios reported for other citrus species.[9–13]

In the succeeding paper, compositional comparisons will be made among linear, iso- and anteiso-branched structures in both the saturated and mono-unsaturated groups of lemon juice sac lipids. Table 2 shows the saturated hydrocarbon profiles of the three lemons and reveals that, aside from minor percentage differences, all lemons possess similar profiles. Figure 1 depicts graphically the profile of the saturated hydrocarbon group of Lisbon lemon. In Fig. 1(a) odd-numbered linear, iso and anteiso hydrocarbons are plotted. Figure 1(a) shows that these odd-numbered paraffins have progressively greater percentage values over the range $C_{21}$–$C_{25}$ and progressively lower values between $C_{25}$ and $C_{35}$. Similar curves, not shown, were drawn for Kusner and Malta and evinced comparable profiles.

The general shape of the saturated, even-numbered series curves, Fig. 1(b), is very similar to the odd-numbered group. The apex of even-numbered linear and iso paraffin curves is at $C_{24}$ while the anteiso apex is at $C_{26}$ for Lisbon. For the even-numbered, iso-branched group in Malta and Kusner the apex appears to be at $C_{26}$. This, however, is only a minor difference as iso $C_{24}$ and $C_{26}$ are present at relatively similar percentages in these three lemons. One interesting element of Fig. 1 is that the profile of the odd-numbered

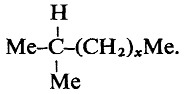
[14] W. REUTHER, H. J. WEBBER and L. D. BATCHELOR (editors), *The Citrus Industry*, Vol. I, University of California Press, California (1967).

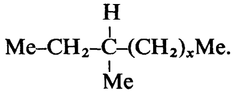
ante*iso* group is nearly identical to that of the even-numbered iso group. In composite form, the total percentages of the three isomeric structures for this saturated fraction is shown in Table 3. Table 3 shows the data from this study and also, data from a previous study on Eureka lemon.[9] The isomeric group with the largest percentage is the linear group which comprises between 47 and 59% of this fraction. The linear odd- to even-numbered ratio for Lisbon, Kusner and Eureka is approximately 3:1 while Malta shows a higher ratio of 4:1. The ratio of odd- to even-numbered iso-branched paraffins also shows consistency for these four lemons, viz. Lisbon (5·2:1), Malta (5·3:1), Kusner (5·1:1) and Eureka (4·5:1). In contrast to the linear and iso-branched groups where odd-numbered paraffins predominate, even-numbered anteiso-branched paraffins are found at percentages 3–4 times that of the anteiso, odd-numbered.

TABLE 2. SATURATED LONG-CHAIN HYDROCARBON PROFILES OF LEMON JUICE SACS (wt%)

| Carbon No. | Lisbon L* | Lisbon I† | Lisbon AI‡ | Malta L | Malta I | Malta AI | Kusner L | Kusner I | Kusner AI |
|---|---|---|---|---|---|---|---|---|---|
| 20 | 0·14§ | t‖ | t | 0·10 | t | t | 0·16 | t | t |
| 21 | 0·33 | t | t | 0·20 | t | 0·01 | 0·29 | t | t |
| 22 | 1·17 | 0·20 | 0·16 | 0·81 | 0·23 | 0·17 | 1·14 | 0·19 | 0·15 |
| 23 | 7·38 | 5·27 | t | 4·24 | 5·19 | t | 6·99 | 4·52 | t |
| 24 | 5·06 | 1·76 | 3·77 | 3·28 | 1·67 | 3·34 | 4·65 | 1·26 | 3·26 |
| 25 | 16·06 | 10·72 | 1·67 | 14·22 | 11·98 | 1·19 | 16·49 | 7·81 | 1·78 |
| 26 | 3·49 | 1·60 | 6·30 | 2·90 | 2·18 | 8·27 | 4·02 | 1·35 | 6·06 |
| 27 | 9·07 | 4·31 | 1·41 | 9·81 | 5·09 | 1·86 | 9·88 | 3·53 | 1·55 |
| 28 | 1·61 | 0·56 | 2·54 | 1·71 | 0·60 | 3·43 | 2·20 | 0·49 | 2·12 |
| 29 | 4·70 | 3·09 | 0·71 | 5·28 | 3·72 | 0·83 | 6·74 | 2·93 | 0·58 |
| 30 | 1·06 | 0·48 | 1·39 | 0·84 | 0·37 | 1·48 | 1·53 | 0·53 | 1·36 |
| 31 | 2·14 | 0·63 | 0·18 | 2·94 | 0·69 | 0·21 | 4·10 | 0·71 | 0·18 |
| 32 | 0·26 | t | 0·25 | 0·27 | t | 0·23 | 0·42 | t | 0·36 |
| 33 | 0·33 | t | t | 0·41 | t | t | 0·49 | t | t |
| 34 | 0·08 | — | — | 0·11 | — | — | 0·07 | — | — |
| 35 | 0·12 | — | — | 0·14 | — | — | 0·11 | — | — |

* Linear chain hydrocarbon.

† Iso-branched,

$Me\text{-}\overset{H}{\underset{Me}{C}}\text{-}(CH_2)_xMe$.

‡ Anteiso-branched,

$Me\text{-}CH_2\text{-}\overset{H}{\underset{Me}{C}}\text{-}(CH_2)_xMe$.

§ All values are the mean of 5–7 determinations.

‖ t = Trace, less than 0·01%.

Analysis of Table 4 reveals that monoenes are present mainly in the linear form with odd-numbered monoenes in greater abundance than even-numbered. Odd-numbered iso- and anteiso-branched monoenes are at insignificant percentages when compared to their odd-numbered linear homolog. Even-numbered linear and iso monoenes have $C_{24}$ as the major hydrocarbon while $C_{26}$ is dominant for the even-numbered anteiso series. A profile curve of the even-numbered linear, iso and anteiso monoene series (not shown) was found to be quite similar to the profile curve of the even-numbered saturated series, Fig. 1(b).

Table 5 shows in composite form the percentage composition of the linear, iso- and anteiso-branched monoenes. Examination of Table 5 reveals that Lisbon, Kusner and Eureka have very similar monoene compositions. While the total percentage of iso-branched structures was greater than anteiso-branched in the saturated fraction, the opposite is shown for the monoene fraction, i.e. anteiso > iso. The isomer found at the lowest percentage in this group is the even-numbered, iso-branched monoene.

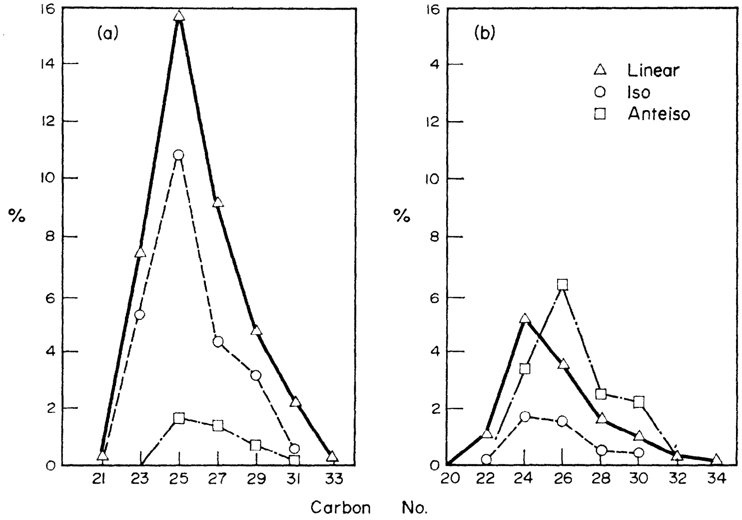


Fig. 1. Percentage composition of odd-numbered (a), and even-numbered (b) hydrocarbons in Lisbon lemons.

A significant varietal difference is observed in the ratio of total anteiso to total iso-branched monoenes. Malta has 10·0% iso and 23·3% anteiso-branched monoenes. The other three varieties have 8·1–9·6% iso and 13·3–14·2% anteiso. This greater percentage of anteiso in Malta is accounted for in lower percentages of linear $C_{23}$, $C_{25}$, $C_{27}$ and $C_{31}$ relative to those found in the other varieties.

Table 3. Percentage composition of saturated linear, iso and anteiso hydrocarbons in four lemons

| Carbon | Lisbon | | | Malta | | | Kusner | | | Eureka* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| No. | L | I | AI | L | I | AI | L | I | AI | L | I | A |
| Odd-numbered | 40·1 | 24·0 | 4·0 | 37·3 | 26·7 | 4·1 | 45·1 | 19·5 | 4·1 | 40·1 | 24·0 | 3·1 |
| Even-numbered | 12·9 | 4·6 | 14·4 | 10·0 | 5·0 | 16·9 | 14·2 | 3·8 | 13·3 | 13·6 | 5·3 | 13·9 |
| Total | 53·0 | 28·6 | 18·4 | 47·3 | 31·7 | 21·0 | 59·3 | 23·3 | 17·4 | 53·7 | 29·3 | 17·0 |

* Values calculated from Ref. 9.

Comparative examination of lemon long-chain hydrocarbon profiles to other citrus species reveals some interesting similarities and differences. The saturated profiles of lemons resemble the saturated profile of Persian lime (*C. latifolia* Tanaka).[12] The mono-unsaturated profiles of lemons and lime, however, are noticeably different; lime shows a much higher percentage of linear $C_{29}$ monoene. The monoene fraction is also useful in distinguishing lemon varieties from orange varieties.[9,10] Oranges show $C_{25}$ as the major linear monoene while lemons show $C_{29}$. In grapefruit as in lemons, the major linear monoene is $C_{29}$,[9,11] however, the relative percentage of this hydrocarbon in grapefruit is twice that of lemons. The mandarin, Dancy (*C. reticulata*), is readily differentiated from the lemons by showing $C_{31}$ as the major linear monoene.[9]

TABLE 4. MONO-UNSATURATED LONG-CHAIN HYDROCARBON PROFILES OF LEMON JUICE SACS (wt %)

| Carbon No. | Lisbon L | Lisbon I | Lisbon AI | Malta L | Malta I | Malta AI | Kusner L | Kusner I | Kusner AI |
|---|---|---|---|---|---|---|---|---|---|
| 20 | 1·60 | t | 0·35 | 1·77 | t | 0·63 | 2·16 | t | 0·47 |
| 21 | 2·00 | 0·09 | 0·35 | 1·96 | 0·10 | 0·63 | 1·86 | 0·04 | 0·39 |
| 22 | 3·07 | 0·08 | 0·39 | 2·85 | 0·09 | 0·66 | 2·64 | 0·03 | 0·45 |
| 23 | 5·79 | 0·70 | 0·32 | 3·80 | 0·99 | 0·58 | 5·07 | 1·66 | 0·42 |
| 24 | 3·68 | 0·52 | 2·24 | 5·75 | 0·43 | 3·88 | 3·15 | 0·42 | 2·14 |
| 25 | 12·20 | 2·46 | 0·95 | 9·51 | 2·39 | 0·98 | 11·67 | 1·88 | 0·56 |
| 26 | 1·44 | 0·21 | 4·18 | 2·17 | 0·40 | 6·06 | 1·90 | 0·17 | 3·39 |
| 27 | 15·19 | 2·28 | 1·03 | 9·70 | 3·15 | 1·50 | 12·97 | 2·45 | 1·05 |
| 28 | 1·16 | t | 2·15 | 1·34 | t | 4·78 | 1·41 | t | 2·25 |
| 29 | 19·98 | 0·82 | 0·05 | 18·38 | 1·02 | 0·68 | 19·67 | 0·96 | t |
| 30 | 0·92 | 0·04 | 0·88 | 1·74 | t | 1·63 | 1·83 | 0·12 | 0·82 |
| 31 | 9·48 | 1·01 | 0·11 | 6·96 | 0·99 | 0·23 | 11·55 | 1·22 | t |
| 32 | 0·21 | t | 0·57 | 0·31 | 0·33 | 1·01 | 0·41 | 0·29 | 0·29 |
| 33 | 0·62 | 0·34 | 0·54 | 0·50 | 0·12 | t | 0·86 | 0·33 | 1·05 |
| 34 | t | — | — | t | — | — | t | t | — |
| 35 | t | — | — | t | — | — | t | — | — |

The tables presented in this paper reveal that, in general, the saturated and mono-unsaturated hydrocarbon profiles follow certain patterns previously reported for other citrus species.[9–13] The major linear, saturated hydrocarbons are generally $C_{23}$, $C_{25}$, $C_{27}$ and $C_{29}$ while major linear monoenes fall in the region $C_{25}$ to $C_{31}$. Even-numbered, iso-branched monoenes are never present at percentages greater than 1 %. In other citrus the saturated anteiso-branched $C_{25}$ is the only odd-numbered anteiso hydrocarbon over 1 %, however, in lemons both anteiso $C_{25}$ as well as anteiso $C_{27}$ are over 1 %. These patterns tend to reinforce the previous hypothesis[11] that in citrus the major biosynthetic mechanism for long-chain hydrocarbon formation is the elongation–decarboxylation pathway postulated by Kolattukudy.[15,16]

TABLE 5. PERCENTAGE COMPOSITION OF MONO-UNSATURATED LINEAR, ISO AND ANTEISO HYDROCARBONS IN FOUR LEMONS

| Carbon No. | Lisbon L | Lisbon I | Lisbon AI | Malta L | Malta I | Malta AI | Kusner L | Kusner I | Kusner AI | Eureka* L | Eureka* I | Eureka* AI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Odd-numbered | 65·2 | 7·7 | 3·3 | 50·8 | 8·8 | 4·6 | 63·6 | 8·6 | 3·5 | 63·9 | 7·3 | 2·4 |
| Even-numbered | 12·1 | 0·9 | 10·8 | 15·9 | 1·2 | 18·7 | 13·5 | 1·0 | 9·8 | 13·8 | 0·8 | 11·8 |
| Total | 77·3 | 8·6 | 14·1 | 66·7 | 10·0 | 23·3 | 77·1 | 9·6 | 13·3 | 77·7 | 8·1 | 14·2 |

* Values calculated from Ref. 9.

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[16] P. E. KOLATTUKUDY, *Phytochem.* **6**, 693 (1967).

## EXPERIMENTAL

*Isolation and purification of lemon juice sac lipids.* Mature Kusner, Lisbon and Malta lemon samples were obtained from Whitmore Experimental Farm (Plant Science Research Division, U.S.D.A., Orlando, Florida). The fruit were cut in half and the intact juice sacs (vesicles) carefully separated from core, peel, seeds and carpellary membrane with the aid of a citrus spoon. The juice sacs were freeze-dried to a powder possessing a moisture content no greater than 4% and stored at −18° until lipid extractions were carried out. Lipids were extracted from 20 g of juice sac powder with distilled $CHCl_3$ and MeOH by a method previously described for total juice sac powder.[2] Quadruplicate extractions run on single batches of fruit from each variety were purified on Sephadex columns.[2]

*Column and TLC.* Columns, 0·9 × 30 cm, containing 10 g Merck, 70–325 mesh silica gel (Brinkman Instruments, Westbury, N.Y.) were washed by elution with 100 ml distilled $CHCl_3$. The total purified lipid (*ca.* 150–200 mg) was dissolved in distilled $CHCl_3$ and percolated into this pre-washed column. The neutral lipids were eluted with 200 ml distilled $CHCl_3$. Pre-coated silica gel G plates (20 × 20 cm, 500 $\mu$, Analtech, Inc., Wilmington, Del.) were washed free of hydrocarbon impurities by development with $CHCl_3$–MeOH–HAC (14:6:1) in a TLC chamber for 4 hr. The plates were dried at room temp. for 1 hr and then activated for 1 hr at 105°. The neutral lipid fraction was concentrated to a small vol. and streaked on these plates. The plates were developed at room temp. in hexane–ethyl ether (23:2). The band corresponding to the long-chain hydrocarbon fraction was scraped from the plate and eluted with ethyl ether. This fraction was in turn re-streaked on a silver nitrate-impregnated silica gel G plate[1] and developed in 2% ethyl ether in light petrol. (30–60° boiling range). This solvent system separated the saturated and mono-unsaturated hydrocarbons. These two hydrocarbon fractions along with a complex fraction (everything between the origin and the monoene band) were eluted with ethyl ether and dry wts taken. The monoene fraction was dissolved in 1 ml hexane and hydrogenated in a Parr apparatus with 10 mg 10% Pd–C catalyst under 60 $lbs/in^2$ at room temp. for 1 hr.

*Quantitation analyses.* The hydrocarbons were analyzed by GLC with an F & M Model 7610 A FID gas chromatograph. The analyses were determined on dual glass columns (3·05 m in length and 4 mm i.d.) coated with 3% SP–1000 (Supelco, Inc., Bellefonte, Pennsylvania) on 100–120 mesh Gas Chrom Q. Asbestos gaskets coated with Dexsil 300[17] were used with the U-shaped columns for more stable column operation. The injection ports were at 190° and the detectors at 275°. He flow in the analytical column was at 80 ml/min at 40 $lbs/in^2$. The sample (1·5–4·5$\lambda$ representing 1–10% hydrocarbon in heptane) was injected on-column at 165° and programmed for 5 min at 4°/min, 2°/min for 20 min, then 3°/min up to 270° and finally held isothermally at this upper limit until the $C_{35}$ hydrocarbon eluted from the column. A hydrocarbon standard containing linear, iso- and anteiso-branched structures from $C_{16}$ to $C_{36}$ was prepared from a long-chain fatty acid mixture as previously described.[5] The long-chain, saturated and hydrogenated monoene hydrocarbons from three varieties of lemon juice sac lipids were determined by comparing their GLC $R_t$s against the above standard as well as by plots of retention times vs. equivalent carbon numbers. MS of citrus long-chain, branched hydrocarbons were previously determined by Hunter and Brogden[18] from this Laboratory. Quantitative measurements were obtained with the aid of a disc integrator and by triangulation.

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[17] M. BEROZA and M. C. BOWMAN, *Analyt. Chem.* **43,** 808 (1971).

[18] G. L. K. HUNTER and W. B. BROGDEN, *Phytochem.* **5,** 807 (1966).