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COMPARATIVE LONG-CHAIN HYDROCARBON PROFILES OF ORANGE AND TANGOR JUICE SACS

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Abstract—Long-chain hydrocarbons were determined in juice sacs from six varieties of oranges and two varieties of tangors for the region C_{20}–C_{38}. The four most prominent hydrocarbons in all varieties were linear C_{23} and C_{25}, and iso-branched C_{23} and C_{25}. Iso- and anteiso-branched hydrocarbons comprised approximately 50 per cent of the total fraction. The most prominent branched hydrocarbons were the iso-branched, odd-numbered paraffins, while anteiso-branched, even-numbered paraffin were the next most abundant.

INTRODUCTION

THERE exists limited information on the lipid composition of citrus juices. The vast majority of citrus lipid research has been concerned with the volatile flavoring constituents of the essential oil group; however, nonvolatile lipids have received little attention. General lipid compositional studies on orange juice have been conducted by Huskins *et al.*,[1] Swift and Veldhuis,[2] and Nagy and Nordby.[3] Fatty acids,[4-6] sterol glucosides[7] and phospholipids[8] have been the only specific lipids analysed in citrus. Although paraffinic hydrocarbons have been qualitatively determined in cold-pressed orange oil,[9] no information is available on the composition of these hydrocarbons in juice sacs.

The present study was undertaken to further our knowledge of citrus lipids. Information on hydrocarbon profiles is important, not only in the field of citrus chemotaxonomy but also has practical utility in detecting adulteration in processed citrus juice. In this study long-chain hydrocarbon profiles were determined in six orange varieties of *C. sinensis*, viz. Walker Early, Parson Brown, Hamlin, Washington Navel, Pineapple and Valencia, and two tangors, viz. Temple (*C. temple*) and Temple × Kinnow (*C. temple* × *C. reticulata*).

RESULTS AND DISCUSSION

Long-chain hydrocarbons between C_{20} and C_{38} were the dominant paraffins observed in this study and therefore, were the only ones quantified. Paraffins above C_{38} were detected

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Reference to specific products of commercial manufacturer are for illustration only and do not constitute endorsement by the U.S. Department of Agriculture.

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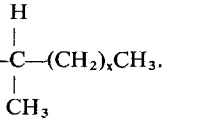
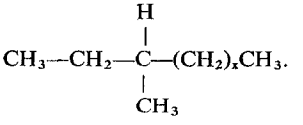
TABLE 1. PARAFFINIC HYDROCARBON PROFILES OF ORANGE AND TANGOR JUICE SACS

Carbon No.	W. Early			P. Brown			Hamlin			W. Navel		
	N[a]	I[b]	AI[c]	N	I	AI	N	I	AI	N	I	AI
20	0·09[d]	—	—	0·11	—	—	0·11	—	—	0·13	—	—
21	0·80	t[e]	t	1·21	t	t	0·88	t	t	1·10	t	t
22	2·12	0·57	t	2·36	0·47	t	2·53	0·20	t	2·56	0·41	t
23	14·45	14·37	t	15·09	14·77	t	14·48	12·26	t	15·54	12·05	t
24	4·58	2·71	9·44	4·77	4·06	8·21	5·23	3·42	7·94	5·12	3·24	7·29
25	13·02	13·93	1·59	11·27	13·70	1·92	13·77	12·32	1·42	13·78	12·64	1·35
26	1·45	1·23	6·17	1·02	1·51	5·60	1·95	1·28	5·76	1·78	1·29	5·63
27	2·29	3·68	0·47	2·20	3·66	0·42	3·44	3·07	0·40	3·23	3·75	0·41
28	0·44	0·15	1·61	0·47	0·16	1·49	0·65	0·16	1·57	0·57	0·18	1·77
29	1·28	0·63	0·03	1·09	0·77	0·04	1·50	0·72	0·04	1·22	0·89	0·05
30	0·29	0·02	0·43	0·33	0·03	0·45	0·67	0·03	0·51	0·41	0·04	0·58
31	1·06	0·29	t	1·03	0·24	t	1·37	0·25	t	1·14	0·30	t
32	0·19	0·02	0·17	0·32	t	0·19	0·52	0·02	0·17	0·38	t	0 32
33	0·18	0·13	0·01	0·47	0·05	t	0·55	0·04	t	0·40	0·01	t
34	0·07	t	t	0·21	t	t	0·32	t	t	0·19	t	t
35	0·04	t	t	0·17	t	—	0·20	t	—	0·18	t	—
36	t	—	—	0·09	—	—	0·15	—	—	0·12	—	—
37	t	—	—	0·05	—	—	0·10	—	—	0·04	—	—
38	t	—	—	t	—	—	t	—	—	t	—	—

Carbon No.	Pineapple			Valencia			Temple			Temple × Kinnow		
	N	I	AI	N	I	AI	N	I	AI	N	I	A
20	0·11	—	—	0·19	—	—	0·03	—	—	0·05	—	—
21	1·32	t	t	0·71	t	t	0·31	t	t	0·34	t	t
22	2·41	0·36	t	2·60	0·09	t	1·73	0·04	t	1·00	0·03	t
23	15·35	12·90	t	17·37	10·08	t	17·55	10·37	t	11·38	6·39	t
24	5·69	2·28	8·29	5·92	3·39	7·19	5·49	3·48	5·02	5·56	2·81	4·06
25	13·98	12·46	1·42	14·93	12·76	1·37	16·77	15·79	0·98	18·53	17·41	0·97
26	2·08	1·14	5·56	1·64	1·46	5·56	1·95	0·92	4·37	2·43	2·25	5·09
27	3·23	2·63	0·22	2·77	3·19	0·40	4·13	4·45	0·16	3·76	6·81	0·75
28	0·41	0·09	0·84	0·50	0·15	1·50	0·41	0·18	0·99	0·48	0·43	2·39
29	1·94	0·58	0·03	1·41	0·68	0·04	1·47	0·71	0·03	1·10	1·66	0·07
30	0·38	0·02	0·31	0·50	0·02	0·40	0·24	0·03	0·27	0·41	0·10	0·71
31	1·70	0·18	t	1·05	0·24	0·11	1·19	0·34	0·04	0·77	0·48	0·15
32	0·46	t	0·17	0·48	0·01	0·19	0·20	0·01	0·12	0·40	0·03	0·16
33	·50	0·30	t	0·39	0·12	t	0·07	0·01	t	0·40	0·17	t
34	0·26	t	t	0·23	t	t	0·07	t	t	0·19	t	t
35	0·21	t	—	0·17	t	t	0·05	t	t	0·16	t	t
36	0·12	—	—	0·13	—	—	0·03	—	—	0·04	—	—
37	0·07	—	—	0·06	—	—	t	—	—	0·05	—	—
38	t	—	—	t	—	—	—	—	—	0·03	—	—

[a] Linear chain hydrocarbon.

[b] Iso-branched, general structure 

[c] Anteiso-branched, general structure 

[d] Mean of 3–6 determinations.

[e] Trace, less than 0·01%.

in all citrus but always at trace concentrations (below 0·01%). Quantitative determination of C_{20}–C_{38} paraffins was accomplished on a 3·6 m column; however, this column could not be effectively used to determine paraffins greater than C_{38} because of peak broadening. To detect and differentiate paraffins greater than C_{38} required that separation be conducted on a 1 m column.

Table 1 shows the paraffinic hydrocarbon profiles of six orange and two tangor varieties. The four most dominant hydrocarbons are normal (linear) C_{23} and C_{25}, and iso-branched C_{23} and C_{25}. In all cases but one (exception Temple × Kinnow), normal C_{23} is found at the highest concentration. Normal C_{25} is the most dominant paraffin in the Temple × Kinnow tangor. Iso-branched C_{23} and C_{25} are found in the six orange varieties at essentially equivalent concentrations. In the two tangors, however, this relationship is nonexistent. In the Temple tangor the ratio of iso C_{23} to iso C_{25} is *ca.* 2:3 while in the Temple × Kinnow this ratio is *ca.* 1:3. Anteiso-branched C_{23} is found in all varieties at trace concentrations while anteiso C_{25} is found below 2%.

The most dominant even-numbered paraffin is C_{24}. In all six orange varieties the following exists for C_{24}, viz. anteiso > normal > iso. This relationship exists for other even-numbered paraffins as well, viz. C_{26}, C_{28} and C_{30}; however, for even-numbered hydrocarbons below C_{24} and above C_{30} the relationship normal > anteiso > iso is manifest. The two tangors show minor exceptions to these relationships. The C_{24} hydrocarbon is again the most dominant even-numbered paraffin but the relationship normal > anteiso > iso exists for C_{24}. For C_{26}, C_{28} and C_{30} the sequence is similar to oranges, i.e., anteiso > normal > iso. Even-numbered hydrocarbons below C_{24} and above C_{30} show a relationship similar to oranges, i.e., normal > anteiso > iso.

TABLE 2. PERCENTAGE COMPOSITION OF NORMAL, ISO AND ANTEISO PARAFFINIC HYDROCARBONS

Carbon No.	W. Early			P. Brown			Hamlin			W. Navel		
	N	I	AI	N	I	AI	N	I	AI	N	I	AI
Odd-numbered	33·12	33·03	2·10	32·58	33·19	2·38	36·29	28·66	1·86	36·63	29·64	1·81
Even-numbered	9·23	4·70	17·82	9·68	6·23	15·94	12·19	5·11	15·95	11·26	5·16	15·50
Total (Odd and even)	42·35	37·73	19·92	42·26	39·42	18·32	48·42	33·77	17·81	47·89	34·80	17·31
Odd/Total	78·20	87·54	10·54	77·09	84·19	12·99	74·94	84·86	10·44	76·48	85·17	10·45

Carbon No.	Pineapple			Valencia			Temple			Temple × Kinnow		
	N	I	AI	N	I	AI	N	I	AI	N	I	AI
Odd-numbered	38·30	29·05	1·67	38·86	27·07	1·92	41·54	31·67	1·21	36·49	32·92	1·94
Even-numbered	11·92	3·89	15·17	12·19	5·12	14·84	10·15	5·66	10·77	10·59	5·65	12·41
Total (Odd and even)	50·22	32·94	16·84	51·05	32·19	16·76	51·69	36·33	11·98	47·08	38·57	14·35
Odd/Total	76·26	88·19	9·91	76·12	84·09	11·45	80·33	87·17	10·11	77·50	85·35	13·51

Comparative examination of the six orange varieties reveals that these varieties can be categorized into three distinct classes. Valencia is distinct from all other oranges because it possesses a higher normal C_{23} and lower iso C_{23} content. Walker Early and Parson Brown are in another class because they have a lower total normal odd-numbered carbon content and a higher iso-branched, odd-numbered carbon content than the other varieties. Also both possess a higher iso C_{23} content than the others. The third class consists of Hamlin, Washington Navel and Pineapple. These three are quite similar and do not have hydrocarbon profile characteristics useful in differentiating one from the other.

The two tangor varieties can be readily distinguished from the orange varieties by their higher contents of normal C_{25} and iso C_{25}. An even more distinguishing feature of the tangors is observed in the isomeric ratio of C_{24}. For the six orange varieties the isomeric ratio, i.e. normal: iso: anteiso, is *ca.* 2:1:3 while for the tangors the ratio is 2:1:2.

Table 1 reveals that there are hydrocarbons present in some orange and tangor varieties which are absent in others. The reported absence of these paraffins implies only that they were not detected under our experimental conditions and may be present at concentrations below 0·01%.

Table 2 summarizes in composite form the percentage composition of odd- and even-numbered hydrocarbons in the three structures, i.e., normal, iso and anteiso. Perusal of this table reveals that odd-numbered paraffins are mainly found as normal and iso-branched structures while even-numbered paraffins are found primarily as normal and anteiso-branched. For all eight varieties (oranges and tangors), the ratio percentage of odd-numbered paraffins to total paraffins (even plus odd) is 75–80% in the normal structure, 84–88% in the iso structure and 10–14% in the anteiso structure.

While this study was concerned with the saturated long-chain hydrocarbons, unsaturated long-chain hydrocarbons are also synthesized by citrus fruit. These unsaturated compounds comprise *ca.* 3% of the total hydrocarbon fraction. Separation of monounsaturated hydrocarbons by argentation TLC and then followed by hydrogenation of this fraction revealed GLC profiles distinctly different from the saturated paraffins. Each long-chain linear monoene was found accompanied by its iso and anteiso homolog but at concentrations much lower than observed for the saturated branched hydrocarbons.

Biosynthesis of long-chain hydrocarbons in higher plants appears to proceed via elongation—decarboxylation pathway[10,11] which employs fatty acids as priming units. Citrus synthesizes a multitude of normal, iso- and anteiso-branched fatty acids.[4,5] Branched acids are formed in plants by employing isobutyrate, isovalerate and 2-methylbutyrate as initial primers. These priming units are derived from the amino acids valine, leucine and isoleucine.[10–13] Valine and leucine act as primers in the formation of iso-branched even-numbered and odd-numbered fatty acids, respectively. Isoleucine functions as the priming unit for anteiso-branched, odd-numbered fatty acids. Formation of anteiso-branched, even numbered fatty acids would require 3-methylvalerate as the priming unit. To date no anteiso-branched, even-numbered fatty acid has been detected in citrus.[5] According to the elongation–decarboxylation theory, iso even-numbered fatty acids would give rise to iso odd-numbered hydrocarbons and anteiso odd-numbered fatty acids would produce

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[11] P. E. KOLATTUKUDY, *Phytochem.* **6**, 963 (1967).
[12] P. E. KOLATTUKUDY, *Biochem.* **5**, 2265 (1966).
[13] F. D. GUNSTONE, *An Introduction to the Chemistry and Biochemistry of Fatty Acids and Their Glycerides*, p. 183, Chapman & Hall, England (1967).

anteiso even-numbered hydrocarbons. Since no anteiso even-numbered fatty acids have ever been reported in citrus, then theoretically, no anteiso odd-numbered hydrocarbon should be found. Perusal of Table 1 shows, however, the occurrence of anteiso odd-numbered paraffins, albeit at very low concentrations. Hunter and Brogden,[9] from this Laboratory, could not detect any anteiso odd-numbered paraffins in orange oil. From their data we assumed that citrus was incapable of forming this structural-type paraffin.[5] Through improvement in gas chromatographic techniques, as reported in this paper, the occurrence of this paraffin was unmistakeable. The question remains as to how this type of paraffin is synthesized in citrus and what precursors are employed.

In oranges and tangors, iso-branched fatty acids comprise between 0·4 and 0·8 per cent and anteiso-branched between 0·06 and 0·18 per cent of the total fatty acids.[5] The branched hydrocarbons, on the other hand, show a range between 32 and 39 per cent for iso structures and between 12 and 20 per cent for anteiso components. It appears, *a priori*, that citrus does not effectively utilize branched chain fatty acids and therefore, metabolizes these compounds to branched hydrocarbons. In support of this premise is the fact that branched fatty acids are found only in appreciable concentrations in monoesterified lipids, *viz.* steryl esters.[6] Branching imparts steric hinderance and therefore, it would be expected that these compounds would be present only in small amounts in multiesterified lipids, e.g. triglycerides and phospholipids.

EXPERIMENTAL

Isolation and purification of juice sac lipids. Valencia, Hamlin, Parson Brown and Walker Early oranges were obtained from local groves. Pineapple and Washington Navel oranges and the Temple × Kinnow tangor were obtained from Whitmore Experimental Farm (Crops Research Division, U.S.D.A., Orlando, Florida). The Temple tangor was obtained from a local market. All samples were collected at the time of their respective peak maturities. The eight citrus 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 per cent and stored at 5° until lipid extractions were carried out. Lipids were extracted and purified from 15 g of juice sac powder by a method previously described for total orange juice powder.[3] Triplicate extractions were run on each variety.

Column and TLC. The total purified lipid (*ca.* 150–200 mg) was dissolved in $CHCl_3$ and percolated onto an 0·9 × 30 cm column containing 9 g Merck, 70 to 325 mesh silica gel (Brinkmann Instruments, Westbury, N.Y.). Neutral lipids were separated from polar lipids by elution of the column with 150 ml $CHCl_3$. The total neutral lipid fraction was concentrated to a small volume and streaked on precoated silica gel G plates (20 × 20 cm, 250 μ, Analtech, Inc., Wilmington, Del.). These non-activated plates were developed at room temp. in chambers lined with filter paper in hexane–Et_2O (9:1). The band corresponding to the hydrocarbon fraction was scraped from the plate and hydrocarbons eluted from the gel with Et_2O. The hydrocarbon eluent was concentrated to dryness, taken up in hexane and streaked on a $AgNO_3$ impregnated silica gel G plate.[5] Saturated hydrocarbons were separated from unsaturates by development in light petroleum.

GLC. Gas chromatographic analyses were run on an F&M Model 5750 gas chromatograph equipped with flame ionization detectors. Hydrocarbons were determined on a glass column (3·66 m in length and 4 mm i.d.) coated with 3% polymetaphenoxylene (Applied Science Labs., State College, Pa.) on 100/120 mesh, Gas Chrom Q. The injection port and detector were at 300° and the helium flow rate was 80 ml/min. The sample was injected on-column at 210° and programmed at 2° min to 300° and held isothermally at this upper limit until the C_{38} hydrocarbon eluted from the column.

Hydrocarbon standards and quantitation. A linear hydrocarbon standard from C_{10} to C_{36} was purchased from Applied Science. Another hydrocarbon standard containing linear, iso- and anteiso-branched structures from C_{16} to C_{36} was prepared from a Temple tangor total fatty acid fraction in the following manner. The total fatty acid methyl esters prepared from Temple tangors was separated by argentation TLC.[5] The saturated ester band obtained from this plate was reduced to an alcohol with $LiAlH_4$ in Et_2O and the alcohol fraction purified on silica gel G plates in hexane–Et_2O (4:1). The alcohol mixture was then tosylated with *p*-toluene sulfonyl chloride in dry pyridine, extracted into hexane and reduced a

second time with $LiAlH_4$ in Et_2O to the hydrocarbon.[14] The hydrocarbon preparation was purified on silica gel G plates with hexane. By GLC, this standard agreed with the standard purchased from Applied Science with the addition that it also contained branched hydrocarbons. Unsaturated hydrocarbon standards were also prepared from methyl oleate, linoleate and linolenate in a similar manner. The standards were run along with the natural citrus hydrocarbon mixture on $AgNO_3$ plates to confirm the presence of unsaturated citrus hydrocarbons.

Hydrocarbons were determined by comparative GLC retention times against standards and plots of retention time versus equivalent carbon number. Mass spectra of citrus branched hydrocarbons were previously determined by Hunter and Brogden.[9] Quantitative results were obtained by triangulation measurement techniques and by measurement of peak areas with the aid of a disc integrator.

[14] H. E. NORDBY, B. W. HEYWANG, H. W. KIRCHER and A. R. KEMMERER, *J. Am. Oil. Chemists' Soc.* **39**, 183 (1962).