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LONG-CHAIN HYDROCARBON PROFILES OF GRAPEFRUIT JUICE SACS

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Abstract—Saturated and mono-unsaturated long-chain hydrocarbons were determined in juice sacs of three grapefruit varieties—Marsh Seedless, Redblush and Foster. In the saturated fraction, the dominant linear hydrocarbon was C_{25} while C_{29} predominated in the monoene fraction. Iso- and anteiso-branched hydrocarbons comprised between 52 and 53% of the saturated fraction but only 9–12% of the monoene. The saturated and mono-unsaturated hydrocarbon profiles of Marsh Seedless, Redblush and Foster were similar. The theoretical relationship of citrus fatty acids to hydrocarbon synthesis was explored and indicated that the primary method of synthesis was via an elongation-decarboxylation pathway. The possibility that some head-to-head condensation was also occurring in hydrocarbon synthesis was indicated by the presence of odd-numbered, anteiso-branched hydrocarbons.

INTRODUCTION

ALTHOUGH citrus fruits are commercially the most important fruit grown in the United States, limited information exists on the lipid composition of these fruits. The available information is summarized in the recently published book on the chemical constituents of citrus fruits by Kefford and Chandler[1] which devotes only four pages to citrus lipids.

As part of a large program being undertaken by the U.S. Citrus and Subtropical Products Laboratory, the authors are conducting studies on the relationship of citrus lipids to juice quality,[2–5] chemotaxonomy[6–9] and citrus juice adulteration. During a study of the long-chain hydrocarbon profiles of orange and tangor (orange–mandarin hybrids) juice sacs,[9] specific hydrocarbon patterns emerged which were characteristic for each variety. Investigation of these profiles suggested that long-chain hydrocarbons might be employed to differentiate citrus species more accurately.

To this end, the present study was undertaken to study the hydrocarbon profiles of grapefruit and relate these profiles to those obtained from oranges and tangors. Three grapefruit cultivars of *C. paradisi*, viz. Marsh Seedless, Redblush and Foster were used in this study.

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RESULTS AND DISCUSSION

Marsh is most important variety and the first seedless grapefruit discovered in Florida.[10] Several pigmented varieties of considerable commercial importance, viz. Thompson and Redblush, trace back to Marsh. Thompson originated as a limb sport of Marsh while Redblush occurred as a bud mutation of Thompson. Foster was discovered near Manatee, Florida, in 1907.[10] It was the first pigmented grapefruit variety in Florida, having preceded Thompson and Redblush by more than ten years.

All three grapefruit varieties were harvested at the same time in mid-March. At this time, all three varieties were at or near an optimum maturity stage subject to commercial processing according to regulations of the Department of Agriculture of Florida and the United States.[11] Hydrocarbon profiles reported in this paper were obtained from mature fruit. The possibility exists that these profiles could depend upon the maturity of the fruit, i.e. either immature or at an advanced stage of senescence.

The lipid content and hydrocarbon concentrations of grapefruit juice sacs are shown in Table 1. The total lipids extracted from 20 g of juice sac powder showed that the percentage

TABLE 1. TOTAL LIPID AND HYDROCARBON CONCENTRATIONS OF GRAPEFRUIT JUICE SACS (mg/20 g dry wt)

Cultivar	Total lipid	Hydrocarbon fraction		
		Saturated	Monoene	Poly-unsaturated
Marsh	204·7 ± 13·0	4·3 ± 0·1	0·5 ± 0·1	0·5 ± 0·1
Redblush	206·2 ± 14·5	4·6 ± 0·3	0·6 ± 0·1	0·8 ± 0·1
Foster	202·2 ± 16·7	3·0 ± 0·1	0·4 ± 0·1	0·3 ± 0·1

of lipid in these three grapefruit were: Foster (1·01), Redblush (1·03) and Marsh (1·03). The total hydrocarbon fraction was separated by argentation TLC into three fractions, viz. saturated, monoene and poly-unsaturated. The poly-unsaturated fraction was obtained by eluting all remaining material between the origin and the monoene band. This fraction was composed of pigments, di-unsaturated hydrocarbons and other undefined hydrocarbons. The saturated hydrocarbons represented 1·5–2·2% of the total lipids while the monoenes showed a range of 0·2–0·3%.

In Table 2 are shown the saturated paraffinic hydrocarbon profiles of the three grapefruit varieties. Saturated hydrocarbons were determined for the region between C_{20} and C_{35}. Hydrocarbons were detected in trace percentages up to the region C_{45}–C_{50}. Identification in this latter region was based on equivalent carbon lengths and comparison with a long-chain hydrocarbon standard. No method was employed to differentiate the various isomeric structures of hydrocarbons exceeding C_{35}. Although the hydrocarbon range investigated was between C_{20} and C_{35}, 95% of grapefruit long-chain hydrocarbons were found between C_{22} and C_{29}.

From Table 2 it may be seen that the two dominant linear hydrocarbons were C_{23} and C_{25}, the dominant iso-branched structures were C_{23} and C_{25}, and the major anteiso-branched structures were generally greater than their iso-branched homologs. Conversely, odd-numbered hydrocarbons showed the opposite relationship, i.e. iso-branched paraffins were

[10] W. REUTHER, H. J. WEBBER and L. D. BATCHELOR (editors), *The Citrus Industry*, Vol. 1, p. 542, University of California Press, California (1967).

[11] *Chemistry and Technology of Citrus, Citrus Products and Byproducts*, Agriculture Handbook No. 98, p. 8, U.S. Dept. of Agriculture, Washington, D.C. (1962).

present at larger relative percentages than anteiso-branched. The total percentages for the three isomeric structures in this fraction were:

Marsh—linear	(47·84), iso	(34·52), anteiso	(17·64)
Redblush—	(46·65),	(34·55),	(18·80)
Foster—	(46·87),	(34·41),	(18·72).

TABLE 2. SATURATED LONG-CHAIN HYDROCARBON PROFILES OF GRAPEFRUIT JUICE SACS (wt %) (mean of 4–6 determinations)

Carbon No.	Marsh			Redblush			Foster		
	N	I	AI	N	I	AI	N	I	AI
20	0·26	t	t	0·17	t	t	0·19	t	t
21	0·39	0·05	t	0·36	0·04	t	0·37	0·03	0·01
22	1·62	0·06	0·05	1·64	0·11	0·04	2·04	0·11	0·06
23	13·54	8·11	0·07	13·59	9·12	0·06	13·55	10·28	0·18
24	6·69	2·43	6·23	6·42	2·62	6·52	6·19	2·71	7·38
25	15·89	15·73	0·97	14·92	15·13	1·80	14·47	14·83	1·35
26	2·55	1·35	6·70	2·38	1·19	6·69	2·48	1·01	6·80
27	3·64	4·33	0·59	3·96	4·13	0·76	4·17	3·61	0·56
28	0·82	0·45	1·98	0·75	0·34	1·98	0·91	0·27	1·71
29	1·26	1·30	0·13	1·36	1·30	0·10	1·52	1·03	0·06
30	0·39	0·23	0·70	0·24	0·17	0·63	0·39	0·17	0·51
31	0·50	0·42	0·04	0·46	0·32	0·09	0·37	0·30	0·02
32	0·14	0·02	0·15	0·10	0·02	0·11	0·10	0·02	0·07
33	0·12	0·04	t	0·19	0·06	t	0·06	0·04	t
34	0·03	t	0·03	0·04	t	0·02	0·02	t	0·01
35	t	t	—	0·07	t	t	0·04	t	—

N Linear chain hydrocarbon.

I Iso-branched, general structure $CH_3-\underset{\displaystyle CH_3}{\overset{\displaystyle H}{\overset{|}{\underset{|}{C}}}}-(CH_2)_xCH_3$.

AI Anteiso-branched, general structure $CH_3-CH_2-\underset{\displaystyle CH_3}{\overset{\displaystyle H}{\overset{|}{\underset{|}{C}}}}-(CH_2)_xCH_3$.

t Trace, less than 0·01%.

The relative hydrocarbon percentages for Marsh and Redblush were similar. Foster showed some minor differences in relative percentages from the other two varieties. Marsh and Redblush both showed more anteiso C_{26} than anteiso C_{24}, however, Foster showed a reverse relationship. Also, Marsh and Redblush showed more iso C_{27} than linear C_{27}; the opposite was observed for Foster.

In Table 3 the percentage composition of the mono-unsaturated hydrocarbon fraction is shown. Scrutiny of this table revealed rather low percentages for branched-chain structures which was in contrast to that found for the saturated fraction. A breakdown of the total percentages of the three isomeric structures showed the following:

Marsh—linear	(90·54), iso	(3·94), anteiso	(5·62)
Redblush—	(87·60)	(5·42),	(6·98)
Foster—	(87·62),	(4·47),	(7·91).

The major hydrocarbon in this fraction was linear C_{29}. In contrast to the saturated fraction, which comprised mainly C_{23} and C_{25} hydrocarbons, the mono-unsaturated fraction contained a higher relative percentage of longer chain monoenes, i.e. C_{25}–C_{31}. While the total percentage of iso-branched structures was greater than anteiso-branched in the saturated fraction, the opposite was true for the monoenes, i.e. anteiso > iso. One particular pattern which emerged from the monoene fraction was that even-numbered, anteiso-branched monoenes were always greater than their iso-branched counterparts. With one exception (C_{26}), linear monoenes were found at higher percentages than either of their iso or anteiso counterparts. The three most prominent branched structures in this fraction were iso C_{25}, anteiso C_{24} and anteiso C_{26}.

TABLE 3. MONO-UNSATURATED LONG-CHAIN HYDROCARBON PROFILES OF GRAPEFRUIT JUICE SACS (wt. %)

Carbon No.	Marsh			Redblush			Foster		
	N	I	AI	N	I	AI	N	I	AI
20	0·40	t	0·14	0·63	t	0·20	1·52	t	0·55
21	0·23	t	0·18	0·48	0·15	0·25	0·19	0·08	0·34
22	0·34	0·01	0·23	0·70	0·15	0·36	0·94	0·10	0·47
23	3·35	0·53	0·28	4·41	1·06	0·52	2·64	0·57	0·30
24	1·70	0·05	0·83	2·48	0·17	1·19	2·18	0·20	0·87
25	16·10	2·31	0·37	14·27	2·55	0·45	12·91	2·04	0·85
26	1·18	0·07	2·13	1·10	0·17	2·03	1·38	0·32	2·92
27	17·39	0·74	0·55	15·25	1·06	0·80	15·54	0·58	0·43
28	1·81	0·06	0·67	1·91	0·02	1·09	1·64	0·06	0·53
29	40·81	0·17	0·08	39·75	0·05	0·05	41·52	0·23	0·22
30	0·76	t	0·06	0·92	0·04	0·04	0·59	t	0·18
31	6·05	t	t	5·35	t	t	6·23	0·29	0·25
32	0·06	—	—	0·05	—	—	0·05	—	—
33	0·27	—	—	0·24	—	—	0·22	—	—
34	t	—	—	t	—	—	t	—	—
35	0·09	—	—	0·06	—	—	0·07	—	—

As was the case for the saturated fractions, Marsh and Redblush possess similar monoene profiles. Foster differed slightly from the other two varieties in this fraction. For the linear hydrocarbon group, Foster showed lower percentages of C_{23} and C_{25} but a noticeably higher percentage for C_{20}.

Tables 2 and 3 reveal the absence of some branched structures. The reported absence of these branched isomers implies only that they were not detected under our experimental conditions and may be present at relative percentages below 0·01.

There are at present two theories for the biosynthesis of long-chain hydrocarbons in plants. The first theory proposed simultaneously by Clenshaw and Smedley-Maclean[12] and Channon and Chibnall[13] suggests that long-chain hydrocarbons are formed by the condensation of the carboxyl group of a fatty acid with the α-carbon of another fatty acid yielding a β-keto acid which, in turn, would undergo reductive decarboxylation. This mechanism has been termed a head-to-head condensation. As an example of this theory, even-numbered, anteiso hydrocarbons (anteiso C_{30}, C_{32}, C_{34}) would be formed by condensation of anteiso C_{15}, C_{17} or C_{19} fatty acids with either palmitic or stearic acid and this condensation, in turn,

[12] E. CLENSHAW and I. SMEDLEY-MACLEAN, *Biochem. J.* **23**, 107 (1929).
[13] H. T. CHANNON and A. C. CHIBNALL, *Biochem. J.* **23**, 168 (1929).

followed by decarboxylation. Bi-terminally branched-chain hydrocarbons can be formed by this mechanism.

The second theory proposed by Kolattukudy[14–16] is that long-chain hydrocarbons are formed via an elongation-decarboxylation pathway employing fatty acids as priming units. Branched-chain hydrocargons would be derived from branched-chain fatty acids. For example, an even-numbered, iso-branched fatty acid would function as a priming unit for the formation of odd-numbered, iso-branched hydrocarbons. If this pathway is solely operative in plants, no bi-terminally-branched hydrocarbon should be formed.

The mode of synthesis of long-chain hydrocarbons in citrus has not been adequately investigated. Citrus synthesizes a multitude of saturated and unsaturated branched-chain fatty acids.[2,7] Intensive investigation of the fatty acid profiles of organe and tangor juice sacs[6] reveals the presence of two classes of branched fatty acids, viz. odd-numbered, iso- and anteiso-branched acids and even-numbered, iso-branched acids. No even-numbered, anteiso-branched acid has been found in citrus.

If elongation-decarboxylation is the only pathway operative in citrus, no odd-numbered, anteiso-branched hydrocarbon should be formed because this hydrocarbon could only be formed if an even-numbered, anteiso-branched fatty acid is present. Examination of the hydrocarbon profiles reported for oranges and tangors[9] and the grapefruit profiles reported in this paper reveal the presence of odd-numbered, anteiso-branched hydrocarbons albeit at low relative percentages. No bi-terminally branched hydrocarbon has so far been definitively demonstrated in any citrus.

It would appear, *a posteriori*, that the primary mechanism for synthesis of long-chain hydrocarbons in citrus is via the elongation-decarboxylation pathway. In support of this pathway is the fact that the major saturated hydrocarbons are found in the region C_{23}–C_{25}. If the primary mode of synthesis were head-to-head condensations, long chain hydrocarbons should predominate,[17,18] i.e. C_{27}–C_{33}. In support of limited head-to-head condensations in citrus is the inexplicable occurrence of odd-numbered, anteiso-branched hydrocarbons. One possible way in which this hydrocarbon could be formed is through a head-to-head condensation of an odd-numbered, anteiso-branched fatty acid (commonly found in citrus) with an odd-numbered linear acid. This reaction would be followed by reductive decarboxylation to yield the odd-numbered, anteiso-branched hydrocarbons.

In the field of citrus chemotaxonomy, long-chain hydrocarbon profiles can readily be used to differentiate oranges from grapefruit. The major linear, saturated long-chain hydrocarbon in every variety of sweet orange investigated by the authors was C_{23}.[9] In the three grapefruit varieties reported in this paper and three other grapefruit cultivars, viz. Burgundy, Duncan and Thompson,[19] the major linear hydrocarbon was C_{25}. To the best of the authors' knowledge, this is the first distinct difference useful in differentiating oranges from grapefruit based on lipid profiles. Investigations currently being conducted by this Laboratory on lipid profiles indicate that citrus species and closely related cultivars within a species possess their own intrinsic lipid composition. These intrinsic compositions are useful not only in the field of citrus chemotaxonomy but may have potential in setting standards and detecting adulteration in commercial citrus juice.

[14] P. E. Kolattukudy, *Science* **159,** 498 (1968).
[15] P. E. Kolattukudy, *Phytochem.* **6,** 693 (1967).
[16] P. E. Kolattukudy, *Biochem.* **5,** 2265 (1966).
[17] P. W. Albro, T. D. Mefhan and J. C. Dittmer, *Biochem.* **9,** 1893 (1970).
[18] P. W. Albro and J. C. Dittmer, *Biochem.* **8,** 1913 (1969).
[19] S. Nagy and H. E. Nordby, unpublished results.

EXPERIMENTAL

Isolation and purification of grapefruit juice sac lipids. Marsh Seedless, Redblush and Foster grapefruit were obtained from Whitmore Experimental Farm (Crops Research Division, U.S.D.A., Orlando, Florida). The three grapefruit were cut in half and the juice sacs separated from core, peel, seeds and carpellary membrane. The juice sacs were freeze-dried to a powder and stored at −18°. Lipids were extracted and purified from 20 g of juice sac powder by a method previously described.[3,9] Quadruplicate extractions were run on each grapefruit variety.

Column and TLC. The total purified lipid (*ca.* 200–250 mg) was dissolved in $CHCl_3$ and applied to a 0·9 × 30 cm column containing 10 g Merck, 70–325 mesh silica gel (Brinkmann Instruments, Westbury, N.Y.). The neutral lipids, which contained the long-chain hydrocarbons, were eluted with 200 ml $CHCl_3$. Glycolipids and phospholipids were eluted with 200 ml acetone and 200 ml MeOH, respectively. The glycolipid and phospholipid fractions were not subjected to any further analysis during this study. The neutral lipid fraction was concentrated to a small volume and streaked on precoated silica gel G plates. These non-activated plates were developed at room temp. in hexane–ethyl ether (9:1). The band corresponding to the long-chain hydrocarbon fraction was removed from the plate and eluted with ethyl ether. This hydrocarbon fraction was streaked on a silver nitrate–impregnated silica gel G plate[6] and developed in 2% ethyl ether in light petroleum. This system separated saturated, mono-unsaturated and poly-unsaturated hydrocarbons. The bands corresponding to the saturated and mono-unsaturated hydrocarbons were eluted with ethyl ether and dry wts taken. The poly-unsaturated fraction (everything between the origin and the mono-unsaturated band) was also eluted and dry wts taken. This latter fraction was not subjected to any further analysis. The mono-unsaturated fraction was dissolved in 1 ml hexane and hydrogenated under 60 lb/in^2 at room temp. for 1 hr with 10 mg of 10% Pd–C catalyst in a Parr apparatus.

GLC and quantitation. The hydrocarbons were determined with an F&M Model 5750 gas chromatograph with flame ionization detectors, separated on a glass column (3·05 m length and 4 mm i.d.) coated with 3% SP-1000 (Supelco, Inc., Bellefonte, Penn.) on 100/120 mesh, Gas Chrom Q. The injection port and detector were at 275° and the helium flow rate was 80 ml/min. The sample was injected on column at 165° and programmed for 5 min at 4°/min and then 2°/min up to 270° and finally held isothermally at this upper limit until the C_{38} hydrocarbon eluted from the column. A hydrocarbon standard containing linear, iso- and anteiso-branched structures from C_{16} to C_{36} was prepared as previously described.[9] The long-chain hydrocarbons of grapefruit were determined by comparative GLC retention times against the above standard and also by plots of retention times vs. equivalent carbon numbers. Mass spectra of citrus long-chain, branched hydrocarbons were previously determined by Hunter and Brogden.[20] Quantitive results were obtained by triangulation and also with a disc integrator.

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