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VARIATION OF THE LONG-CHAIN HYDROCARBON PATTERN IN DIFFERENT TISSUES OF DUNCAN GRAPEFRUIT

STEVEN NAGY, HAROLD E. NORDBY and JACK C. LASTINGER

Citrus and Subtropical Products Laboratory,* Winter Haven, FL 0000, U.S.A.

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Key Word Index—*Citrus paradisi*; Rutaceae; Duncan grapefruit; long-chain hydrocarbon profiles; tissue variation; chemotaxonomy.

Abstract—Long-chain hydrocarbons were analyzed in peel wax, leaf wax, stem, seeds and juice sacs of Duncan grapefruit. The distribution of alkanes in juice sacs were linear (44·4%), *iso* (35·4%) and *anteiso* (20·2%) while seeds showed linear (53·3%), *iso* (24·6%) and *anteiso* (22·1%). The alkanes in stem, peel wax and leaf wax were linear (>98%) and *iso*-branched (<2%). The major linear alkanes were C_{25} in juice sacs and seeds and C_{31} in stem, peel wax and leaf wax. A definable mono-unsaturated hydrocarbon fraction was detected only in juice sacs and peel wax. The major monoene in juice sacs was C_{29} while C_{30} predominated in peel wax.

INTRODUCTION

During studies on the lipid composition of citrus juice sacs, the authors found that specific long-chain hydrocarbon patterns existed which might be used in species differentiation [1]. The question arose as to whether hydrocarbon patterns in other portions of the same plant were similar to those found in juice sacs of the fruit. To resolve this question, the authors examined the long-chain hydrocarbon profiles of peel wax, leaf wax, stem, seeds and juice sacs of Duncan grapefruit.

RESULTS AND DISCUSSION

The amounts of the mixed saturated hydrocarbons isolated from various parts of the tree and fruit of Duncan grapefruit are given in Table 1. Major differences were observed in the relative percentage distribution of alkanes in juice sacs and seeds when contrasted to stem, peel wax and leaf wax. For juice sacs, the major hydrocarbon was C_{25} followed by C_{23}. The distribution of branched-chain hydrocarbons showed a specific pattern, i.e. *iso*-branched, odd-numbered alkanes were always found at higher percentages than their *anteiso*-branched, odd-numbered homologs. Conversely, *iso*-branched, even-numbered hydrocarbons were found at lower percentages than their *anteiso*-branched, even-numbered homologs. This distribution pattern had been observed for other grapefruit cultivars [2]. The high percentage of branched alkanes in Duncan grapefruit juice sacs (*iso*—35·4%, *anteiso*—20·2%) has also been observed in juice sacs of sweet oranges [3], mandarins [4], lemons [5], limes [6] and other hybrid citrus fruit [7]. Recent evidence indicates that the hydrocarbon component of juice sacs may come from an epicuticular wax layer surrounding the outer surface of the vesicle [8,9].

The alkane distribution of grapefruit seeds showed a pattern similar to juice sacs but the relative percentages were noticeably different. The C_{25} alkane was the most prominent hydrocarbon but it was not as dominant as the C_{25} in juice sacs. The linear alkane pattern of seeds showed a bimodal distribution with peaks at C_{25} and C_{31}. This distribution pattern has been confirmed in grapefruit seeds of fruit taken from different citrus

* One of the laboratories of the Southern Region, Agricultural Research Service, U.S. Department of Agriculture.

Table 1. Saturated long-chain hydrocarbon profiles of juice sac, seed, stem, peel wax and leaf wax of Duncan grapefruit (wt%)

Carbon no.	Juice sac			Seed			Stem*		Peel wax*		Leaf wax*	
	L†	I‡	AI§	L	I	AI	L	I	L	I	L	I
20	0·1‖	—	—	0·6	—	—	0·3	—	0·2	—	0·2	—
21	0·3	tr¶	—	1·0	—	—	0·3	—	0·2	—	0·2	—
22	1·2	tr	0·4	2·6	0·3	0·4	0·7	—	0·4	—	0·4	—
23	11·2	7·2	tr	6·3	4·6	tr	1·1	tr	1·0	0·1	0·4	tr
24	6·2	3·0	6·6	3·8	1·1	3·3	0·9	—	0·7	—	0·4	—
25	15·8	17·5	2·8	8·7	6·8	tr	1·1	tr	4·0	tr	0·4	tr
26	2·6	0·3	6·9	3·5	0·4	3·3	1·3	—	1·7	—	0·3	—
27	4·0	5·4	0·6	5·0	3·8	0·4	2·9	0·3	10·8	0·3	0·5	0·3
28	0·6	0·2	2·1	3·0	0·4	3·2	3·2	—	2·4	tr	0·7	—
29	1·2	1·3	0·1	4·6	3·2	1·2	12·0	tr	21·8	tr	4·1	tr
30	0·4	0·1	0·7	2·4	0·7	3·8	5·9	—	3·8	tr	2·7	—
31	0·6	0·4	tr	5·5	2·6	1·0	46·4	1·1	40·6	0·5	43·5	0·8
32	0·2	tr	—	2·2	tr	3·8	5·7	—	2·7	tr	10·4	—
33	tr	—	—	2·7	0·7	0·7	14·8	—	7·0	tr	33·3	—
34	—	—	—	0·6	tr	1·0	2·0	tr	0·7	tr	0·9	—
35	—	—	—	0·8	tr	tr	tr	—	0·7	0·4	0·3	0·2
Total	44·4	35·4	20·2	53·3	24·6	22·1	98·6	1·4	98·7	1·3	98·7	1·3

* *Anteiso*-branched alkanes were detected in stem, peel wax and leaf wax but at percentages less than 0·01%; therefore omitted from table. † Linear chain. ‡ *Iso*-branched. § *Anteiso*-branched. ‖ Mean of 3–4 determinations. ¶ Trace, less than 0·1%.

groves and thus, appeared intrinsic to the seed. The percentages of branched seed alkanes were more evenly distributed when contrasted to juice sacs. The alkane distributions of stem, peel wax and leaf wax were noticeably different from those of seeds and juice sacs in their levels of linear and branched components. Linear alkanes comprised over 98% of the hydrocarbons in these three grapefruit parts. The low percentages for *iso*-branched alkanes were evident with *iso* C_{31} being dominant. While *anteiso*-branched alkanes were detected in stem, peel wax and leaf wax, they were never observed at percentages greater than 0·01% and therefore, were omitted from Table 1. The major alkane in stem, peel wax and leaf wax was C_{31} but the relative distributions of the major alkanes within the C_{27}–C_{33} region were noticeably different. The 3 major alkanes in the stem were C_{29} (12·0%), C_{31} (46·4%) and C_{33} (14·8%); in peel wax the major alkanes were C_{27} (10·8%), C_{29} (21·8%) and C_{31} (40·6%) while in leaf wax C_{31} (43·5%), C_{32} (10·4%) and C_{33} (33·3%) predominated.

The hydrocarbon fraction from these five plant parts was subjected to argentation TLC. Only within juice sacs and peel wax was there a definable mono-unsaturated hydrocarbon fraction. The monoene components of juice sacs comprised 9·5% of the total hydrocarbon fraction while monoenes in peel wax comprised 5·0%. The distribution of these monoenes is shown in Table 2. In juice sacs, linear structures accounted for greater than 95% of the monoenes; with linear odd-numbered monoenes accounting for over 91% of the total monoene fraction. This high percentage was in agreement with monoene profiles reported for juice sacs of other citrus species [3–7]. In Duncan peel wax, linear structures also accounted for over 95% of the monoenes. In con-

Table 2. Mono-unsaturated long-chain hydrocarbon profiles of juice sac and peel wax of Duncan grapefruit (wt%)

Carbon no.	Juice sac			Peel wax		
	L	I	AI	L	I	AI
20	0·3	—	—	0·2	—	—
21	0·1	—	—	0·1	—	—
22	0·1	—	—	1·1	—	—
23	1·1	0·1	—	0·7	tr	—
24	0·4	0·1	0·1	7·1	0·2	0·1
25	11·4	0·6	0·4	2·5	tr	tr
26	0·9	0·1	1·2	18·0	tr	0·1
27	16·5	0·2	0·1	3·9	1·4	tr
28	1·3	0·2	0·5	18·2	0·1	0·1
29	51·5	0·1	tr	5·7	0·5	tr
30	0·9	0·1	0·3	25·9	tr	tr
31	11·4	tr	tr	6·0	0·2	—
32	tr	—	—	7·5	tr	tr
33	tr	—	—	0·4	—	—
34	—	—	—	—	—	—
35	—	—	—	—	—	—
Total	95·9	1·5	2·6	97·3	2·4	0·3

trast to juice sacs, peel wax monoenes showed an overwhelming dominance of linear, even-numbered structures (78%) over the odd-numbered series (19·3%). Recent unpublished data has shown that even-numbered monoenes predominate in peel waxes of other citrus species as well. The dominance of even-numbered monoenes has not to our knowledge been reported in peel waxes of citrus and non-citrus fruits. The major monoene found in juice sacs was C_{29} with C_{25}, C_{27} and C_{31} showing lesser percentages. In peel wax, the major monoene, C_{30}, was one carbon number greater than the dominant juice sac monoene. In addition to C_{30}, three relatively major peel wax monoenes showing noticeable percentages were C_{26}, C_{28} and C_{32}.

EXPERIMENTAL

Hydrocarbon preparation. Juice sacs—Saturated and monounsaturated hydrocarbons from juice sacs were extracted, purified and quantitated according to methods previously described [2]. *Seeds.* Two batches of seeds (6 g) were pulverized and extracted with $CHCl_3$. The $CHCl_3$ extract was removed from the seed meal by filtration, evaporated to dryness, and saponified with 10% KOH in MeOH. The non-saponifiable fraction was removed after the addition of H_2O and Et_2O to the saponification mixture. This fraction was separated on $AgNo_3$-impregnated TLC plates with 2% ethyl ether in petrol yielding the alkane fraction. *Peel wax.* Whole fruit (*ca* 590 g) was dipped for 10 min in 300 ml $CHCl_3$. Care was taken not to crack or bruise the flavedo portion of the peel. The $CHCl_3$ extract was concentrated and the hydrocarbon fraction separated by TLC as described under *Seeds. Leaves.* Epicuticular leaf wax was obtained by dipping mature leaves for 15 sec in 200 ml $CHCl_3$ so as not to disrupt structural integrity or extract chlorophyll. Hydrocarbons in the leaf extract were separated according to the above procedures. *Stems.* Stems were ground in a micro Wiley mill in 3 batches of *ca* 16 g each. Combined powder was soaked in $CHCl_3$ for 2 hr, filtered and re-extracted. The filtrates were combined, evaporated to dryness and TLC separated.

GC. Long-chain hydrocarbons were analyzed on 3% SP-1000 columns [3] and percentages calculated with the aid of an Autolab System IV computing integrator according to methods previously described [10].

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