Friedelin, the Major Component of Grapefruit Epicuticular Wax

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Marsh grapefruit (*Citrus paradisi* Macf.) fruit epicuticular wax was fractionated into various lipid classes by thin-layer chromatography and quantified by gas chromatography, and structures were verified by mass spectroscopy and ^{1}H and ^{13}C magnetic resonance spectroscopy. Friedelin was shown for the first time to be the major component of grapefruit epicuticular wax. Other triterpenes were also present in lesser quantities. Triterpene structures verified were β-amyrin, β-amyrone, β-amyrin acetate, α-amyrin, α-amyrone, α-amyrin acetate, and 24-methylenecycloartanol. Further analyses revealed friedelin levels to be 5–45% of the wax in 8 of 14 other fruits and vegetables and from 9% to 18% in the leaves of 3 citrus cultivars.

INTRODUCTION

Chilling injury (CI) is a disorder that occurs with a number of fruits and vegetables when they are stored at low but nonfreezing temperatures (≤12 °C) for periods specific to the species. Brown pits or stains can appear on grapefruit peel when the fruit is stored for 3 weeks or more at 5 °C. Several treatments have been reported to reduce CI on grapefruit (Wang, 1990), but temperature conditioning appears to be one of the most successful treatments (Hatton and Cubbedge, 1982, 1983). Temperature conditioning involves holding the fruit at 15 °C for 7 days prior to low-temperature storage (Hatton and Cubbedge, 1983). Nordby et al. (1987) postulated that temperature conditioning changed the composition or level of lipid components in the peel and that these changes were responsible for the decrease in CI symptom development. In that study (Nordby et al., 1987) analysis of the lipids from whole grapefruit peel showed only a very slight change in fatty acid levels. Squalene was shown to be a trace component of the epicuticular wax from freshly harvested grapefruit but increased dramatically with temperature conditioning (Nordby and McDonald, 1990). The increase in levels of squalene, C_{23}–C_{25} alkanes, and C_{24}–C_{26} aldehydes in the epicuticular wax of grapefruit during temperature conditioning compensated for the decreases in levels of squalene, C_{27}–C_{33} alkanes, and C_{28}–C_{34} aldehydes during low-temperature storage (Nordby and McDonald, 1991).

Compositional studies on the total waxes of citrus fruit other than grapefruit report the major components to be linear alkanes, aldehydes, primary fatty alcohols, fatty acids (Baker et al., 1975; Freeman et al., 1979b; Sala et al., 1992), and, in one case, wax esters (Schulman and Monselise, 1970). Minor wax components reported in these studies were acidic triterpenes, secondary alcohols, and ketones. Acidic triterpenes, β-diketones, and aldehydes are major fruit wax components in apple and pear (Silva Fernandes et al., 1964), grape (Radler and Horn, 1965), cranberry (Croteau and Fagerson, 1971), and blueberry (Freeman et al., 1979a). McDonald et al. (1993) reported the major components of grapefruit wax to be terpenoids, aldehydes, alkanes, and alcohols.

To determine the composition of the whole wax from grapefruit and other fruits, a GLC program was devised in which all major components of the wax were quantified from a single GLC injection (McDonald et al., 1993). We have recently observed (unreported data), however, that minor wax components besides squalene (Nordby and McDonald, 1990) may have a direct relationship with CI. These minor components could not be resolved from the major grapefruit wax components under the most optimum GLC parameters with the GLC columns available at the time.

Thus, a TLC–GLC–MS method was devised similar to methods by Gulz et al. (1987) for *Citrus halimii* leaf wax and by Hemmers et al. (1989) for the epicuticular wax from *Euphorbia lathyris*. Preparatory TLC of grapefruit wax using benzene as solvent gave a complex chromatogram with a very strong band at R_f 0.32 and a TLC migration ratio very close to that reported for secondary alcohols in cabbage wax (Kolattukudy, 1968) and for pea wax (Kolattukudy, 1969). Preliminary GLC–MS analyses of this R_f 0.32 grapefruit wax area indicated, however, a singular component of a triterpene ketone structure. Friedelin had been reported as a component of whole grapefruit peel (Weizmann et al., 1955) and *C. halimii* leaf wax (Gulz et al., 1987). In addition to friedelin, whole grapefruit peel is a rich source of C_{29} and C_{30} sterols (Goad et al., 1967; Williams et al., 1967), compounds biosynthetically very close to squalene and to friedelin (Heftmann, 1968).

The objectives of this study were to (1) determine the components of the major lipid classes in grapefruit epicuticular wax, (2) verify that the structure of the major triterpenone component is friedelin, and (3) determine the relative levels of friedelin in waxes of other fruits and vegetables and leaves of other citrus fruits. Successful completion of these objectives will present more data that wax composition might be related to chilling injury in cold-stored commodities.

EXPERIMENTAL PROCEDURES

Sample Collection. Marsh grapefruits (*Citrus paradisi* Macf.) were harvested on December 15, 1992, from a grove near Babson Park in central Florida. Equal numbers of fruit were collected from the interior and exterior canopies of three trees. These trees had received normal cultural practices. Fruits were washed and dried; waxes and fungicides were not applied.

Micky Lee watermelons, Dasher cucumbers, Tahiti limes, Keitt and Tommy Atkins mangos, Climax rabbiteye blueberries, Sunny tomatoes, Arkin carambolas, Bearss lemons, and Valencia oranges were obtained at mature stages from research stations, commercial packing houses, or groves and plantings around Florida from 1991 to 1993. Cabbage, bananas, and cranberries were from local markets. Hybrid kumquats, hybrid mandarins, and Ambersweet

oranges were obtained from the U.S. Horticultural Research Laboratory, Orlando, FL. Leaves from sour orange on sour orange rootstock as well as Ambersweet orange and Valencia orange both on Cleopatra rootstock were obtained from 6-month-old potted plants in a greenhouse at the U.S. Horticultural Research Laboratory, Orlando, FL. The above fruit had not been subjected to commercial waxing. These fruits and leaves were washed by hand and patted dry with paper towels prior to wax extraction.

Wax Isolation. Samples of Marsh grapefruit epicuticular wax were obtained within 3 h after harvesting. Nine fruits, each 300–450 g, were sequentially placed in a 14-cm porcelain crucible with 100 mL of chloroform. Each fruit was swirled in the solvent for 2 min. An additional 10 mL of chloroform was added before the next fruit was bathed, and the combined wax extract of the first three fruits was poured into a 500-mL round-bottom flask and reduced in volume on a rotoevaporator while the next three fruits were being bathed. After all nine fruits were extracted, the crucible was rinsed with 20 mL of chloroform and the extract reduced to approximately 15 mL on a rotoevaporator at 30 °C under reduced pressure. The wax extract was transferred to a 25-mL volumetric flask, 3 mL of 0.718 mg/mL cholestane internal standard was added, and the mixture was made up to 25 mL. This procedure was replicated three times for fruit from the exterior tree canopy and three times for fruit from the interior tree canopy of the same three trees.

Wax samples from the other fruits and vegetables were obtained by an extraction procedure previously reported (Nordby and McDonald, 1991). Leaf wax extracts were obtained by sequentially dipping five leaves for 2 min in 150 mL of chloroform in a 16-cm crucible. After reduction in volume, the extract was transferred to a 5-mL volumetric flask, 1 mL of 0.144 mg/mL cholestane internal standard was added, and the sample was made up to 5 mL. Triplicate leaf extracts were prepared.

A representative sample of wax coating the juice sacs of grapefruit was prepared from both an exterior canopy and an interior canopy fruit by carefully peeling, sectioning, and removing the sectional membrane covering the clusters of juice sacs. With each fruit, the sacs were placed in a 400-mL beaker and spooned for 2 min with 200 mL of chloroform. The extract was dried over Na_2SO_4 and the volume reduced to 1 mL.

To determine that friedelin resides in the epicuticular wax of grapefruit peel, an individual fruit was dipped for 10 s in 80 mL of chloroform in a crucible. The extract was transferred to a flask and the procedure repeated three times on the same fruit for 30, 60, and 120 s. After the last extraction, the fruit and crucible were rinsed into the fourth extract, the four extracts were reduced in volume, 1 mL of 0.718 μg/mL cholestane was added to each extract, and each was made up to 5 mL for GLC analysis. This test was run in quadruplicate.

Standard and Reference Compounds. Friedelin was isolated from a new laboratory cork by refluxing 2 g of finely chopped cork with 80 mL of methanol for 1 h, filtering, and removing the methanol. The residue was transferred to a Florisil Sep-Pak (Waters Associates, Milford, MA) with 3 mL of chloroform. Nonpolar material was eluted from the Sep-Pak with 10 mL of chloroform followed by 15 mL of acetone to remove the crude friedelin, which was then crystallized from ethyl acetate. The final sample was obtained from the R_f 0.32 band by TLC. 24-Methylenecycloartanol was isolated from Florida Spanish moss *Tillandsia usneoides* L. Bromeliaceae obtained from Winter Haven, FL. Extraction and isolation procedures were similar to reported procedures (Atallah and Nicholas, 1971). Commercial friedelin, oleanolic acid, and ursolic acid were purchased from Supelco Inc. (Bellefonte, PA). α-Amyrin and β-amyrin were from K and K Laboratories (Plainview, NY).

Derivatizations. Forty milligrams of friedelin isolated as the R_f 0.32 band from grapefruit wax was placed with 100 mg of $NaBH_4$, 0.2 mL of benzene, and 0.1 mL of methanol in an acetylation tube and left for 60 h at room temperature. After recovery with chloroform and washing with water, the triterpenol derivative was purified as the R_f 0.19 band by TLC. The triterpenone TLC fraction was likewise reduced to triterpenols. The triterpenol fraction was oxidized to triterpenones with CrO_3 (Anjaneyulu et al., 1993) and purified by TLC with solvent 1. The triterpenol acetate fraction was hydrolyzed with 8% methanolic KOH (Anjaneyulu et al., 1993), and the triterpenols were purified by TLC with solvent 2. Free fatty acids in the grapefruit wax were methylated with BCl_3 in methanol (Nordby et al., 1981) and purified by TLC. Linear alcohols and triterpenols in the grapefruit wax and the secondary alcohols in cabbage wax were acetylated with acetic anhydride in pyridine at room temperature for 24 h. Attempts were made to acetylate the R_f 0.32 band from grapefruit by the above procedure.

Chromatography, MS, and NMR. TLC separations, detection, and recovery of components from epicuticular waxes of grapefruit, other fruit, and citrus leaves were obtained on silica gel plates developed in benzene (solvent 1) as previously described for grapefruit epicuticular wax (Nordby and McDonald, 1991). For the TLC–GC–MS analyses of grapefruit, 1.5 mL of both exterior and interior canopy fruit extracts were combined for TLC plating. For recovery of components in the TLC–GC–MS analyses, the less optimally resolved sets of two bands at R_f 0.10 and 0.19, 0.32 and 0.37, as well as 0.51 and 0.56, were eluted as three sets. For optimum MS analyses of the triterpenols, this fraction was further separated by TLC into five fractions with hexane–methanol–ethyl acetate 85:10:5 (solvent 2) (Sharma and Dawra, 1991). For GLC analyses 1-μL chloroform solutions of wax were used in the no-split, isotherm mode.

The injector was at 270 °C, the 0.75 mm × 30 m × 1 μm Supelcowax SPB-1 column (Supelco) was at 250 °C, and the FID was at 300 °C. For mass spectra of standard friedelin and friedelin from grapefruit, cork, cucumber, lemon, orange, and citrus leaves, the standard or sample was isolated as the R_f 0.32 component by TLC and recovered with chloroform. Mass spectra were determined on a Hewlett-Packard 5971 GC–MS (Hewlett-Packard, Avondale, PA) with a 12 m × 0.2 mm × 0.3 μm HP-1 column. The oven temperature was held at 240 °C for 8 min, raised to 250 °C at 2 °C/min, and held there for 40 min. Transfer line and injection port were at 280 °C; mass analyzer was at 70 eV, 180 °C, and 30 Torr with a helium flow of 1.0 mL/min. Mass spectra of filican-3-one, cabbage secondary alcohols, and components of the various lipid classes isolated by TLC from grapefruit epicuticular wax were also obtained under these GC–MS parameters.

Most prominent *m/e* (%) ions in grapefruit triterpenols were C5 428 (5), 349 (10), 335 (6), 274 (12), 205 (11), 191 (10), 167 (17), 149 (100), 135 (22), 123 (45); C6 (β-amyrin) 426 (4), 411 (2), 218 (100), 203 (55), 189 (14), 175 (10), 145 (7), 135 (12), 119 (17), 107 (18); C7 428 (9), 413 (54), 395 (9), 349 (12), 245 (4), 218 (12), 167 (15), 149 (100), 133 (12), 121 (18); C8 426 (4), 411 (26), 259 (81), 247 (73), 229 (94), 205 (88), 191 (55), 149 (55), 135 (71), 119 (100); C9 (α-amyrin) 426 (15), 411 (13), 349 (1), 218 (100), 207 (18), 203 (25), 189 (34), 161 (18), 135 (34), 107 (37); C10 426 (8), 274 (84), 259 (100), 205 (37), 173 (35), 149 (29), 137 (43), 123 (42), 119 (78), 109 (81); C11 M^+ absent, 349 (37), 220 (37), 203 (43), 189 (60), 173 (41), 159 (41), 149 (100), 133 (78), 107 (90); C12 440 (12), 425 (27), 407 (50), 379 (6), 300 (14), 175 (65), 161 (59), 149 (67), 119 (100); C13 (24-methylenecycloartanol) 440 (7), 425 (20), 407 (36), 379 (15), 203 (26), 175 (48), 161 (46), 147 (55), 119 (75), 107 (100).

Most prominent *m/e* (%) ions in 24-methylenecycloartanol standard were 440 (9), 425 (24), 407 (53), 379 (17), 203 (15), 175 (48), 161 (42), 147 (65), 119 (79), 107 (100).

Most prominent *m/e* (%) ions in grapefruit triterpenones were D4 (β-amyrone) 424 (14), 409 (5), 218 (100), 205 (49), 203 (61), 189 (25), 149 (16), 147 (12), 135 (21), 109 (39); D5 (α-amyrone) 424 (18), 409 (12), 245 (14), 218 (100), 205 (38), 161 (25), 149 (28), 135 (33), 119 (41); D6 424 (49), 257 (64), 245 (93), 207 (63), 205 (100), 149 (57), 135 (44), 133 (44), 123 (39), 109 (86); D7 424 (15), 423 (59), 311 (100), 271 (67), 245 (20), 191 (40), 173 (49), 147 (50), 125 (86), 119 (95); D8 438 (33), 423 (20), 395 (12), 355 (11), 217 (11), 201 (26), 175 (36), 149 (51), 121 (85), 107 (100); D10 (friedelin) 426 (17), 411 (9), 302 (9), 273 (23), 231 (26), 218 (26), 205 (36), 163 (45), 123 (91), 109 (100); D11 M^+ absent, 273 (7), 255 (1), 163 (16), 147 (10), 135 (20), 125 (38), 123 (100), 119 (17), 109 (42).

Most prominent *m/e* (%) ions in grapefruit triterpenol acetates were E1 (β-amyrin acetate) 468 (3), 393 (1), 281 (1), 218 (100), 203 (54), 189 (16), 175 (7), 149 (5), 135 (9), 119 (13), 105 (15); E2 468 (30), 453 (6), 301 (30), 241 (52), 229 (59), 205 (100), 191 (37), 159 (32), 119 (73), 109 (93); E3 (α-amyrin acetate) 468 (9), 249 (3), 218 (100), 203 (24), 189 (25), 161 (13), 147 (13), 133 (20), 119 (21), 107 (24).

Most prominent m/e (%) ions in ficicane-3-one standard were 426 (41), 411 (37), 341 (38), 191 (95), 179 (56), 163 (26), 149 (30), 121 (86), 119 (25), 109 (100).

With friedelin isolated from grapefruit wax, the ^1H NMR was run at 300 MHz and the ^{13}C NMR at 74.5 MHz in $CDCl_3$ with TMS as internal standard. The ^{13}C NMR of synthetic friedelin was run at 50 MHz. ^1H NMR methyl shifts ($CDCl_3$): 1.19, 1.05, 1.01, 0.97, 0.92, 0.87, 0.85, 0.84, 0.73; ^{13}C NMR (δ $CDCl_3$) 6.8 (C23), 14.6 (C24), 17.9 (C25), 18.2 (C7), 18.6 (C26), 20.2 (C27), 22.2 (C1), 28.1 (C20), 30.0 (C17), 30.5 (C12), 31.8 (C30), 32.1 (C28), 32.4 (C15), 32.8 (C21), 35.0 (C29), 35.3 (C19), 35.6 (C11), 36.0 (C16), 37.4 (C9), 38.3 (C14), 39.2 (C22), 39.7 (C13), 41.3 (C6), 41.5 (C2), 42.1 (C5), 42.8 (C18), 53.1 (C8), 58.2 (C4), 59.5 (C10), 213.0 (C3).

RESULTS AND DISCUSSION

With benzene as the TLC solvent, grapefruit epicuticular wax separated into seven lipid classes given the labels A–G in Table 1. Class A (R_f 0.00) was a faint absorbing band at the origin of the TLC plate. GLC analyses of the chloroform extract of this band did not reveal major peaks which could be assigned to either oleanolic or ursolic acid. The peaks, however, corresponded to free fatty acids in a series to C_{32} in length. The structures were confirmed by GLC analyses of their FAME derivatives along with standards. These acids accounted for less than 4% of the wax, much less than that reported for other citrus (Baker et al., 1975; Freeman et al., 1979b). Class B lipids (R_f 0.10) consisting of linear alcohols accounted for 1.5% of the total epicuticular wax with carbon numbers ranging from C_{24} to C_{30}. Ratio of odd- to even-numbered-carbon alcohols was 2:1. Lipid class C (R_f 0.19) accounted for over 11% of the wax with four components each over 1%. Triterpenol T6 (RRT 2.73) was determined to be β-amyrin, T9 (RRT 2.99) α-amyrin, and T13 (RRT 3.83) 24-methylenecycloartanol by their mass spectra, RRT, and R_f values as well as their acetate derivatives' mass spectra, RRT, and R_f values matching those of the respective standards. Friedelinol was not detected in grapefruit wax when the triterpenol lipid class was analyzed by GC–MS. Triterpenol T7 could only be resolved from T6 when the triterpenol wax fraction was further separated using TLC solvent system 2. At the present time structures for T7 and the other triterpenols are being investigated. Sterols may account for some of these components since sterols isolated from grapefruit peel (Williams et al., 1967; Goad et al., 1967) have structures very similar to those of triterpenols. Only three triterpenol acetates were observed in the R_f 0.40 area of the plate (lipid class E). E1 RRT 3.48 was shown to be β-amyrin acetate and E3 (RRT 3.83) α-amyrin acetate by their mass spectra and RRTs being the same as with acetates prepared from standard amyrins. E2 upon hydrolysis gave a mass spectrum nearly identical to that obtained from triterpenol T8. Long-chain aldehydes (R_f 0.56, lipid class F) accounted for 39% of grapefruit wax. This value falls in line with 37.5%, the average value for the aldehydes reported for four other citrus species (Baker et al., 1975). This new TLC–GLC–MS method of analyzing grapefruit wax brings out the fact that what seemed to be pure aldehydes by the symmetrical shape of their peaks on the chromatograms were in reality mixtures of three or four compounds. The C_{30} aldehyde peak at RRT 2.57, for example, contains 36% other compounds. Although alkanes (R_f 0.80, lipid class G) were twice as abundant as fatty acids and 4 times the amount of alcohols, the 7% alkane level is one-third to one-sixth that of the levels reported for alkanes in waxes of other citrus (Baker et al., 1975). The profile fell in line with the profile reported for mature grapefruit (Nordby and Nagy, 1977) with C_{31} being the major alkane. With three exceptions the alkanes were resolved from the other wax components by GLC, these being C_{29}, C_{30}, and C_{33}.

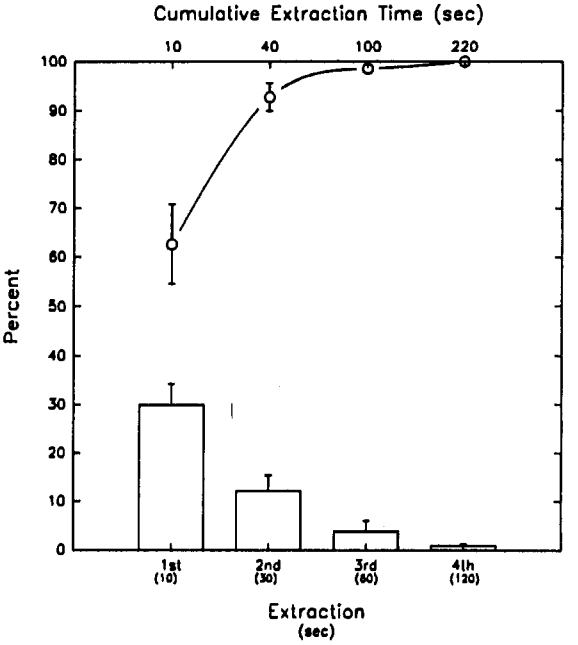

Figure 1. Percent friedelin of total grapefruit epicuticular wax (open circles) and within sequential extractions (open bars) of the fruit in chloroform.

Lipid class D consisted of an intense band at R_f 0.32 and a weak band at R_f 0.37. The major component of lipid class D (D10), exhibited ^1H and ^{13}C NMR signals and a GC–MS fragmentation pattern which identified it as friedelin. Nine ^1H methyl signals (two as a doublet) correspond to the signals reported for the eight methyl groups on the friedelin molecule (Crawford et al. 1975). The ^{13}C spectral features of D10 were identical to a specimen prepared by Dr. N. Fisher from epifriedelinol. All 30 of the signals of D10 were also within 0.1 ppm of the signals of friedelin reported by Patra et al. (1981). To confirm all assignments for these 30 signals, reported reference spectra from closely related triterpenes were also studied. Assignments for C-12 and C-15 were based on signals reported for friedelinol acetate (Anjaneyulu et al., 1993), while the carbonyl being on C-3 was based on signals for 26-nor-D:A-friedoolean-14-3-one and friedelin (Patra et al., 1981; Giner et al., 1993). Filican-3-one, another $C_{30}H_{50}O$ triterpenone, was analyzed as a possible structure for D10. Although filican-3-one's R_f value was 0.32, its RRT was 4.64 vs 3.94 for D10 (Table 1). The MS of filican-3-one was also quite different from that obtained on D10 on the same GC–MS instrument. To determine if finding friedelin in a commercial fruit wax was unique to grapefruit, a number of fruits and vegetables as well as citrus leaves were examined for presence of friedelin (Table 2). GC–MS analyses on the major component in the R_f 0.32 fraction of these samples gave spectra and RRTs identical to those of commercial friedelin and friedelin isolated from cork.

Since friedelin had been reported to be present in whole grapefruit peel as early as 1955 (Weizmann et al., 1955), the question arose whether friedelin was really a component of the epicuticular wax or if it might have been extracted by the chloroform from peel structures more similar to the source in cork bark, e.g., suberin. GLC analysis of chloroform extracts of grapefruit wax taken at 10-, 30-, 60-, and 120-s intervals showed 63% of the friedelin in the total wax was extracted within 10 s (Figure 1). This first extract was composed of 30% friedelin, which

Table 1. Composition of Grapefruit Epicuticular Wax in Seven Lipid Classes As Determined by TLC–GLC (Relative Percent in Total Wax)

GLC time, min	RRT[a] chol	lipid class[b] A (TLC R_f 0.00)	B (TLC R_f 0.10)	C (TLC R_f 0.19)	D (TLC R_f 0.32 + 0.37)	E (TLC R_f 0.51)	F (TLC R_f 0.56)	G (TLC R_f 0.80)
2.74	0.25							0.08 (C22)
3.19	0.30							0.21 (C23)
3.70	0.37							0.43 (C24)
3.86	0.38						0.19 (C22)	
4.82	0.46							0.76 (C25)
4.97	0.47						0.16 (C23)	
5.74	0.54		0.13 (C24)					
5.93	0.56							0.39 (C26)
6.24	0.59	0.07 (C24)					2.70 (C24)	
7.20	0.68		0.17 (C25)					
7.57	0.72							0.74 (C27)
7.87	0.75						0.90 (C25)	
9.14	0.89		0.29 (C26)					
9.39	0.93							0.43 (C28)
10.01	0.95	0.40 (C26)					9.04 (C26)	
11.63	1.10		0.51 (C27)					
12.19	1.16							0.76 (C29)
12.75	1.21	0.13 (C27)					0.93 (C27)	
14.88	1.41		0.14 (C28)					
15.30	1.45							0.57 (C30)
16.34	1.55	0.46 (C28)		0.31 (C1)			8.16 (C28)	
17.80	1.69				0.27 (D1)			
19.14	1.81		0.21 (C29)		0.16 (D2)			
19.96	1.89	0.18						1.76 (C31)
20.94	1.98	0.04 (C29)			0.15 (D3)		0.48 (C29)	
22.87	2.16			0.06 (C2)				
24.60	2.33		0.06 (C30)					
25.27	2.39			0.30 (C3)				0.30 (C32)
26.98	2.57	0.38 (C30)		0.18 (C4)	1.82 (D4)		4.25 (C30)	
28.15	2.67			0.40 (C5)				
28.80	2.73			1.72 (C6 + C7)				
29.69	2.83				1.38 (D5)			
30.50	2.89			0.80 (C8)	0.24 (D6)			
31.51	2.99			3.40 (C9 + C10)				
32.88	3.11							0.37 (C33)
33.65	3.20				1.40 (D7)			
34.71	3.29	0.05 (C31)		0.12 (C11)			0.29 (C31)	
35.44	3.38				1.57 (D8)			
36.95	3.48			1.37 (C12)	0.49 (D9)	0.57 (E1)		
40.56	3.83	0.20		1.45 (C13)		0.37 (E2)		
41.78	3.94				27.91 (D10)	0.50 (E3)		0.14 (C34)
42.77	4.05	0.44						
44.73	4.24	0.63 (C32)		0.78 (C14)	0.77 (D11)		8.18 (C32)	
45.72	4.33			0.50 (C15)				
52.43	4.94			0.12 (C16)				0.02 (C35)
57.53	5.45						0.74 (C33)	
65.63	6.20	0.10		0.18 (C17)				
74.06	7.02						3.15 (C34)	
class totals		3.08	1.52	11.67	36.17	1.44	39.15	6.98

[a] GLC retention time relative to cholestane. [b] Lipid classes: A, free fatty acids; B, linear alcohols; C, triterpenols; D, triterpenones; E, triterpenol acetates; F, aldehydes; G, alkanes.

decreased to less than half this amount in the succeeding sequential extractions. This is evidence that friedelin resides in the epicuticular wax and not in deeper layers where more time for chloroform penetration is needed for extraction. The results from this extraction time study were very similar to the results reported for squalene (the precursor of friedelin) in temperature-conditioned grapefruit (Nordby and McDonald, 1990).

Since friedelin was present at such a high concentration in grapefruit epicuticular wax, we wondered whether the fruit's juice sac wax also contained friedelin. A GLC analysis of this wax from both exterior and interior canopy fruit failed to show evidence of friedelin; however, traces of both amyrins were present.

Lipid class D contains, besides friedelin at 77%, a number of triterpenones with MS parent ions of 424. Their presence in the minor band at R_f 0.37 and their M + 424 suggest that their structures are similar to that of friedelin with one double bond (Hemmers et al., 1989; Gulz et al., 1987; Koops et al., 1991). Although these compounds had the same R_f 0.37 value as the secondary alcohols in cabbage, their MS spectra and GLC retention times were quite different.

Some common fruits such as pear, apple (Silva et al., 1964), and grape (Radler and Horn, 1965) contain a considerable amount of triterpene material, these being mainly the two acidic triterpenes, oleanolic acid, and ursolic acid. Cranberry wax, which also contains a large percentage of these triterpenes, additionally contains sterols, triterpene alcohols, triterpenyl acetates, and traces of triterpene hydrocarbons (Croteau and Fagerson, 1971). Triterpenones, however, have not been reported before in fruit epicuticular waxes, although they are present in leaf waxes (Gulz et al., 1987; Hemmers et al., 1989). Triterpenone D4 (RRT 2.57) was shown to be β-amyrone and triterpenone D5 (RRT 2.83) α-amyrone by their MS,

Table 2. Friedelin Component of Epicuticular Wax of Selected Fruits and Leaves

fruit	rel % (total wax)
cucumber Dasher 2	44.5
grapefruit Marsh	27.9
lime Tahiti	18.9
kumquat (hybrid)	12.2
orange Ambersweet	10.8
lemon Bearss	9.0
mandarin (hybrid)	7.4
orange Valencia	5.3
mango Keitt, Tommy Atkins	nd[a]
cranberry	nd
banana	nd
carambola Arkin	nd
blueberry Climax	nd
watermelon Mickey Lee	nd
tomato Sunny	nd
orange leaves	
sour orange	17.7
Ambersweet	12.7
Valencia	9.1

[a] nd, not detected.

RRTs, and R_fs matching the respective spectra and retention times of ketone derivatives of the standard and isolated triterpenols. Likewise, triterpenones D6, D7, and D8 had mass spectrums and RRTs which indicated that they were structurally related to the respective triterpenols C8, C10, and C12. Further studies are being run to confirm these latter three and the other minor triterpenone structures. The present paper on grapefruit is the first report of a triterpenone such as friedelin being the major component of the epicuticular wax from fruit.

Our finding friedelin to be present in substantial percentages in the waxes of three citrus leaves agrees with the finding of this compound in *C. halimii* leaves (Gulz et al., 1987) and tends to show that *C. halimii* may not be unique within its genus. Friedelin as well as other triterpenones and triterpenols has been reported (Baas, 1985) to be sensitive to UV light, being transformed into secotriterpenes. Grapefruits from the exterior canopy of a tree are more susceptible to chilling injury than fruit from the interior canopy (Purvis, 1980; McDonald et al., 1993). We are presently investigating the possibility that differences in the triterpene area of chromatograms from exterior and interior canopy grapefruit waxes may be due to degraded friedelin or similar compounds. The finding of friedelin in epicuticular waxes of other citrus raises the question of whether triterpenones play a role in light-induced disorders such as rind-staining in oranges (Arpaia et al., 1991; Sala et al., 1992).

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