The corn cob contains large quantities of glucose and xylose, 41.7 and 31.2%, respectively, with only 4.6% arabinose and trace amounts of mannose and galactose. The husks surrounding the ear of corn contain almost as much xylose as the cob: 29.6%. The leaves and stalk of the corn plant both contain more than 18% xylose. The entire corn plant contains large quantities of xylose and glucose, between 2 and 7% arabinose, and only small amounts of mannose and galactose.

The corn pith and corn fiber (Table III) are the products of air classification of the center portion of the corn stalk (Jones et al., 1979). This material was scraped from the corn stalk, ground, and then submitted to a gentle air stream. The fiber was the more dense material and remained behind. These materials differed little in composition.

A variety of plant residues have been examined for their neutral carbohydrate composition to determine the possible utility of the residue. Certainly the corn plant residue is the richest in the xylans, with xylose contents ranging from 19% of the stalk and leaves to 30% of the cobs and husks. Also, the 2–3 tons per acre of residue make the corn crop most appealing for the production of xylitol. Another very promising crop would be the kenaf crop; although containing only 15% xylose, the plant does produce between 10 and 20 tons of material per acre annually. These residues are excellent sources for the recovery of xylose, leading to the production of xylitol, a potential noncariogenic sweetener.

LITERATURE CITED

Agricultural Statistics, U.S. Department of Agriculture, Washington, DC, 1977.
Agricultural Statistics, U.S. Department of Agriculture, Washington, DC, 1978.
Aspinall, G. O., Hunt, K., Morrison, I. M., *J. Chem. Soc.*, 1945 (1966).
Bagby, M. O., *TAPPI Press Nonwood Plant Fiber Pulping Prog. Rep.* 8, 75 (1977).
Butterfield, R. O., Rohwedder, W. K., Bitner, E. D., Ernst, J. O., Wolf, D. J., Dutton, H. J., *Prog. Lipid Res.* **17**, 93 (1978).
Coe, W. B., Turk, J., *Symp. Process. Agric. Munic. Wastes*, 29–37 (1972).
Jones, R. W., Krull, L. H., Blessin, C. W., Inglett, G. E., *Cereal Chem.* **56**, 441 (1979).
MaKinen, K. K., *Futurist* **6**, 135 (1976).
Matsuoka, S., British Patent 1 317 003, May 16, 1973.
Pintauro, N., *Food Technol. Rev.* **40**, 335 (1977).
Reese, E. T., Mandel, M., Weiss, A. N., *Adv. Bioeng.*, 181–200 (1972).
Sannella, J. L., Whistler, R. L., *Arch. Biochem. Biophys.* **98**, 116 (1962).
Sawardeker, J., Sloneker, J. H., Jeanes, A., *Anal. Chem.* **37**, 1602 (1965).
Scheinin, A., MaKinen, K. K., *Acta Odontol. Scand., Suppl.* **33**, 70 (1975).
Sloneker, J. H., *Anal. Biochem.* **43**, 539 (1971).
Sloneker, J. H., *Biotechnol. Bioeng. Symp.* **6**, 235 (1976).
Walshuttl, J., *J. Food Sci.* **38**, 1262 (1973).
Whistler, R. L., BeMiller, J. N., *J. Am. Chem. Soc.* **78**, 1163 (1956).
Whistler, R. L., Corbett, W. M., *J. Org. Chem.* **21**, 694 (1956).
Whistler, R. L., Gaillard, B. D. E., *Arch. Biochem. Biophys.* **93**, 332 (1961).
Whistler, R. L., Lauterback, G. E., *J. Am. Chem. Soc.* **80**, 1987 (1958).

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Glass Capillary Gas Chromatography for Quantitative Determination of Volatile Constituents in Cold-Pressed Grapefruit Oil

Charles W. Wilson, III,* and Philip E. Shaw

Thirty-two components of Florida cold-pressed grapefruit oil were separated on a 30-m glass capillary column coated with Carbowax 20M. They were quantitated on the basis of both normalization and internal standard methods by use of a microprocessor-controlled GC terminal. Compounds not previously quantitated for grapefruit oil were β-pinene, *cis*- and *trans*-limonene oxides, citronellyl acetate, octanol, humulene, and carvone. Knowledge of their presence is important, since their individual contributions to the overall flavor profile of grapefruit oil can then be determined.

Quantitative analyses of cold-pressed citrus oils are becoming increasingly important, particularly in relation to quality control methods, chemotaxonomy of hybrid fruit, and those natural insect attractants or toxicants present in the oils. The need for better techniques to obtain reliable quantitative information on cold-pressed citrus oils is readily apparent from the widely different quantitative values that have been reported (Shaw, 1979). Recent developments in column technology and instrumentation for glass capillary gas chromatography have made it the current method of choice for obtaining reliable quantitative values for individual components of cold-pressed citrus oils.

Considerably less has been reported regarding the quantitative analysis of grapefruit oils than of the other major citrus oils (Shaw, 1979). Early methods for quantitating grapefruit oil [reviewed by Shaw (1979)] usually involved gas chromatography on packed columns. However, in two studies on grapefruit aldehydes, citral was determined colorimetrically (Yokoyama et al., 1961) and

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major aldehydes were separated as their dinitrophenylhydrazone derivatives by thin-layer chromatography (Braddock and Kesterson, 1976). A recent method for quantitating cold-pressed grapefruit oil by packed-column gas chromatography (Wilson and Shaw, 1978) involved correction for the detector response and for the percentage of nonvolatiles present that were not eluted from the column (Shaw et al., 1971; Shaw and Coleman, 1974).

The current report describes the use of glass capillary gas chromatography for quantitation of the volatile flavor constituents of cold-pressed grapefruit oil and compares these results to those obtained by earlier methods. Advantages of an internal standard method to preclude the need for determining and correcting for nonvolatile constituents are presented.

EXPERIMENTAL SECTION

A commercial blend of cold-pressed white grapefruit oil from Duncan and Marsh seedless grapefruit was obtained in November 1977, and a sample was stored in a completely filled vial at 5 °C until used in this study. Authentic compounds previously identified as constituents of grapefruit cold-pressed oil were coinjected with the sample for peak assignment based on peak enrichment. Compounds obtained commercially or isolated from citrus oils by a previously described technique (Wilson and Shaw, 1978) were used in standard solutions for tests to determine detector response factors.

For quantitative analyses a Hewlett Packard Model 5840A gas chromatograph equipped with a Model 5840A GC terminal and a glass-lined capillary inlet splitter was used. Separations were achieved on a 30-m glass capillary column (J & W Scientific Inc., Orangevale, CA) coated with Carbowax 20M. The He flow ($\bar{u}$) was 22 cm/s at 85 °C and 8 psi pressure. Chromatographic analyses were run in triplicate, and the results were calculated on the basis of both an internal standard method and a normalization method by use of the GC microprocessor terminal; heptanol was used as the internal standard. The capillary inlet and flame detector temperatures were 250 and 300 °C, respectively. The capillary inlet was operated in the split mode, and the 1-μL sample of cold-pressed oil was split 100/1. The oven temperature was held at 85 °C for 10 min, raised to 185 °C at 6 °C/min, and held at 185 °C for 100 min. Chart speed was 1 cm/min, and the area reject, slope sensitivity, and attenuation settings were 30, 0.10, and 4, respectively.

RESULTS AND DISCUSSION

We separated and quantitated 32 components of a Florida, white, cold-pressed grapefruit oil (Table I). Also, we were the first to report the quantitation of seven of those components: β-pinene, *cis*- and *trans*-limonene oxides, octanol, citronellyl acetate, humulene, and carvone. The results are listed in Table I, along with those we obtained earlier by a packed column normalization method for the same oil sample (Wilson and Shaw, 1978) and with values reported previously by others (Shaw, 1979). The compounds in this table are listed in increasing order of their gas chromatographic retention times on a polar column.

As compared with packed and metal capillary columns, glass capillary columns are better for separating volatile flavor components of food products because they afford better resolution, more reproducible retention times, and in many instances base line separation of components that are marginally separated by packed columns (Jennings, 1979). Thus, because of increased column efficiency, the probability of a single peak representing more than one compound is greatly reduced. Peaks at longer retention times are sharper; therefore, integration by the microprocessor is more accurate, especially for relatively smaller peaks (<0.1% of the total peak area).

Table I. Quantitative Analyses (wt %) of Cold-Pressed Grapefruit Oil

component	capillary column		packed column	
	int std	norm	norm[a]	lit.[b]
α-pinene	0.38	0.39		0.2–1.6
β-pinene	0.05	0.04		
sabinene	1.08	1.04		0.7
myrcene	3.67	3.41	2.12	1.4–2.1
limonene	84.84	83.66	85.60	86–95
γ-terpinene	0.12	0.12		0.5–0.8
octanal	0.79	0.81	0.71	0.3–0.7
nonanal	0.12	0.14	0.04	0.04–0.1
cis-limonene oxide	0.09	0.09		
trans-limonene oxide	0.04	0.05		
octyl acetate	0.05	0.05	0.09	
citronellal	0.13	0.13	0.14	
decanal	0.46	0.49	0.60	0.3–0.6
α-copaene	0.06	0.07	0.06	
linalool	0.14	0.13	0.30	0.0–0.4
octanol	0.04	0.04		
β-copaene	0.04	0.02	0.01	
β-elemene	0.02	0.06	0.06	
caryophyllene	0.25	0.31	0.25	
citronellyl acetate	0.06	0.04		
neral	0.11	0.07		0.03
α-terpineol	0.05	0.04		0.2
humulene	0.07	0.07		
dodecanal	0.22	0.21		0.1–0.21
neryl acetate	0.02	0.02	0.22	0.1–0.2
geranial	0.11	0.08	0.11	
carvone	0.02	0.02		
geranyl acetate	0.04	0.04		0.1–0.2
Δ-cadinene	0.12	0.07	0.11	
perillaldehyde	0.07	0.04	0.20	
elemol	0.04	0.04	0.04	
nootkatone	0.02	0.03		0.065–0.81

[a] Values reported by Wilson and Shaw (1978). [b] Values summarized by Shaw (1979).

When the normalization method is used (Table I), the nonvolatiles present in the oil must be both determined by an independent method (usually distillation) and corrected for if the capillary column is to afford reasonably accurate results. This correction is especially critical for the analysis of grapefruit oil, because it contains up to 7.5% of material that is too nonvolatile to be eluted from a gas chromatographic column during a normal run (Wilson and Shaw, 1978). Distillation of cold-pressed oils does not completely separate GC-volatile compounds from nonvolatiles, and so there is some inherent error in this method. Use of an internal standard precludes such an error because a known percentage of the total weight of oil injected is represented by the internal standard regardless of how much of the nonvolatile portion either fails to vaporize in the capillary inlet or fails to be eluted from the column by the end of the run. For all components the values we obtained by the internal standard and normalization methods agreed well. The largest peak, corresponding to limonene, should have been affected the most by errors due to any independent method of determining nonvolatiles. Such was the case as indicated by the difference of 1.18% between the assays made by the two methods. However, the error relative to the amount of compound was greatest for compounds present in low amounts, e.g., β-copaene, β-elemene, and Δ-cadinene. The reason is that the accuracy of electronically integrating peaks representing less than 0.1% of the total peak area is relatively low (Shaw, 1979). Thus, for small peaks, errors

Table II. Comparison of Quantitative Aldehyde Values from Cold-Pressed Grapefruit Oil

source of values	% of total aldehyde fraction						total aldehyde, %[a]
	C_8	C_9	C_{10}	C_{12}	neral	geranial	
Braddock and Kesterson (1976)	30		18	12.7	2.1	7.1	1.63
current study	39	6.0	23	11	5.5	5.5	1.5

[a] Total aldehydes were determined by the standard USP (1965) procedure.

due to the effect of nonvolatiles on the normalization method seem less important than those due to integration, even on capillary columns when those small peaks are sharp and relatively easy to integrate.

Seventeen compounds were common to both our 1978 and present studies (Table I). In the current study, lower values were obtained for limonene, octyl acetate, decanal, linalool, neryl acetate, and perillaldehyde than those in the previous study with a packed column (norm). The superior resolution of the capillary column probably accounts for the lower values of all these compounds, except for linalool, since single peaks obtained with a packed column are more likely to represent mixtures of more than one component. For linalool, quantitative information in grapefruit oil is quite variable (Kesterson and Hendrickson, 1964; Attaway et al., 1967). Kesterson and Hendrickson (1967) reported that measurable amounts of linalool were initially present in cold-pressed oil but gradually declined during 1 year of storage. Their findings may explain the difference in linalool values we obtained for the same oil in our two studies conducted a year apart. Our identification of measurable amounts of *cis*- and *trans*-linalool oxides (not shown in Table I) in the oil might account for the decreased linalool content reported in this study.

Quantitative data obtained with the packed and capillary columns were in good agreement for octanal, citronellal, most sesquiterpene hydrocarbons, and elemol. The fact that these compounds were well resolved on the packed column explains why the quantitative results were comparable to those obtained with the capillary column.

Of the compounds listed in Table I, 15 had been quantitated by others, and our values for many of these compounds are within or close to the reported range of values. However, we found almost twice as much myrcene as had been reported earlier. Most likely this difference was due to the superior resolving power of the capillary column. It separated myrcene completely from the most abundant component, limonene, so that myrcene could be integrated accurately for the first time. We found much less γ-terpinene and neryl and geranyl acetates than had been found previously, probably because they were more cleanly separated from other compounds of similar retention times. The concentration of α-terpineol was much lower than that previously reported. However, the concentration of this known degradation product of *d*-limonene varies in citrus oils depending on the processing and storage history of the oil sample (Shaw, 1977).

Volatile aldehydes are among the compounds believed important to grapefruit flavor, and total aldehyde content is often used as a quality index for citrus oils (Kesterson et al., 1971). Individual aldehyde values determined in this study are within or very close to reported literature values, where the latter exist. Braddock and Kesterson (1976) determined the percentage of each major aldehyde in the total aldehyde fraction of Duncan grapefruit oil by a procedure involving thin-layer chromatography as the only means of separating the aldehydes, which had been converted to their dinitrophenylhydrazone derivatives. Their results are compared with our current results in Table II. For the major aldehydes, octanal, decanal, and dodecanal, the two results are in good agreement. For total neral plus geranial (citral) the results are similar, but the ratios of neral to geranial are considerably different. Braddock and Kesterson (1976) noted an absence of nonanal and stated that this aldehyde was probably present at a level not detectable by their method. Total aldehyde content was 2.0% when calculated as the sum of the individual aldehyde values in Table I, but 1.5% when determined by a standard total aldehyde test (United States Pharmacopeia, 1965). By that test, total aldehydes are measured as decanal, since it is often the main aldehyde in grapefruit and other cold-pressed oils.

Nootkatone has been suggested as a major flavor-impact compound in grapefruit (MacLeod and Buigues, 1964). The presence of only a small amount of nootkatone in the oil (Table I) is in agreement with earlier reported results by Kesterson et al. (1971), who found a relatively low level (0.065%) of nootkatone in a sample of oil obtained in November, the same month the oil we examined had been processed. Nootkatone content increases significantly during the processing season, so the oils processed late in the season (May–June) contain the highest nootkatone levels (0.75–0.81%).

Other minor components that may be important to the flavor and aroma of grapefruit oils include several acetate esters. Two of the four esters quantitated in this study, octyl acetate and citronellyl acetate, had not been quantitated previously for grapefruit oils. Their importance, as well as that of neryl and geranyl acetates (Table I), to grapefruit flavor has not been definitely established. However, Moshonas (1971) noted that these acetate esters are among the major oxygenated components of grapefruit oil, although not of orange oil. He suggested that the carbonyl-containing constitutents of grapefruit, as well as other citrus oils, are the primary compounds determining the characteristic flavor. Our quantitative results should permit a more accurate assessment of the importance of these esters to grapefruit flavor.

CONCLUSION

Since analytical values for citrus oils have not been obtained by unambiguous methods, the best method for quantitative analysis cannot be determined with absolute certainty. However, capillary column gas chromatography by a chromatograph with microprocessor or computer calculation capabilities would seem to be the current method of choice. An internal standard method gives the most accurate analytical results for oils, such as citrus oils, containing nonvolatiles that are not eluted from a gas chromatographic column.

LITERATURE CITED

Attaway, J. A.; Pieringer, A. P.; Barabas, L. J. *Phytochemistry* **1967**, *6*, 25.
Braddock, R. J.; Kesterson, J. W. *J. Food Sci.* **1976**, *41*, 1007.
Jennings, W. *J. Chromatogr. Sci.* **1979**, *17*, 636.
Kesterson, J. W.; Hendrickson, R. *Am. Perfum. Cosmet.* **1964**, *79*, 34.
Kesterson, J. W.; Hendrickson, R. *Am. Perfum. Cosmet.* **1967**, *82*, 37.
Kesterson, J. W.; Hendrickson, R.; Braddock, R. J. *Univ. Fla., Inst. Food Agric. Sci., Bull.* **1971**, No. 749.
MacLeod, W. D.; Buigues, N. M. *J. Food Sci.* **1964**, *29*, 565.
Moshonas, M. G. *J. Agric. Food Chem.* **1971**, *19*, 769.
Shaw, P. E. "Citrus Science and Technology"; Nagy, S., Shaw, P. E., Veldhuis, M. K., Eds.; Avi Publishing Co.: Westport, CT, 1977; Vol. 1, Chapter 11.
Shaw, P. E. *J. Agric. Food Chem.* **1979**, *27*, 246.

Shaw, P. E.; Coleman, R. L. *J. Agric. Food Chem.* **1974**, *22*, 785.
Shaw, P. E.; Coleman, R. L.; Moshonas, M. G. *Proc. Fla. State Hortic. Soc.* **1971**, *84*, 187.
United States Pharmacopeia, 17th rev.; Mack Publishing Co.: Easton, PA, 1965.
Wilson, C. W., III; Shaw, P. E. *J. Agric. Food Chem.* **1978**, *26*, 1432.
Yokoyama, F.; Levi, L.; Laughton, P. M.; Stanley, W. L. *J. Assoc. Off. Agric. Chem.* **1961**, *44*, 535.

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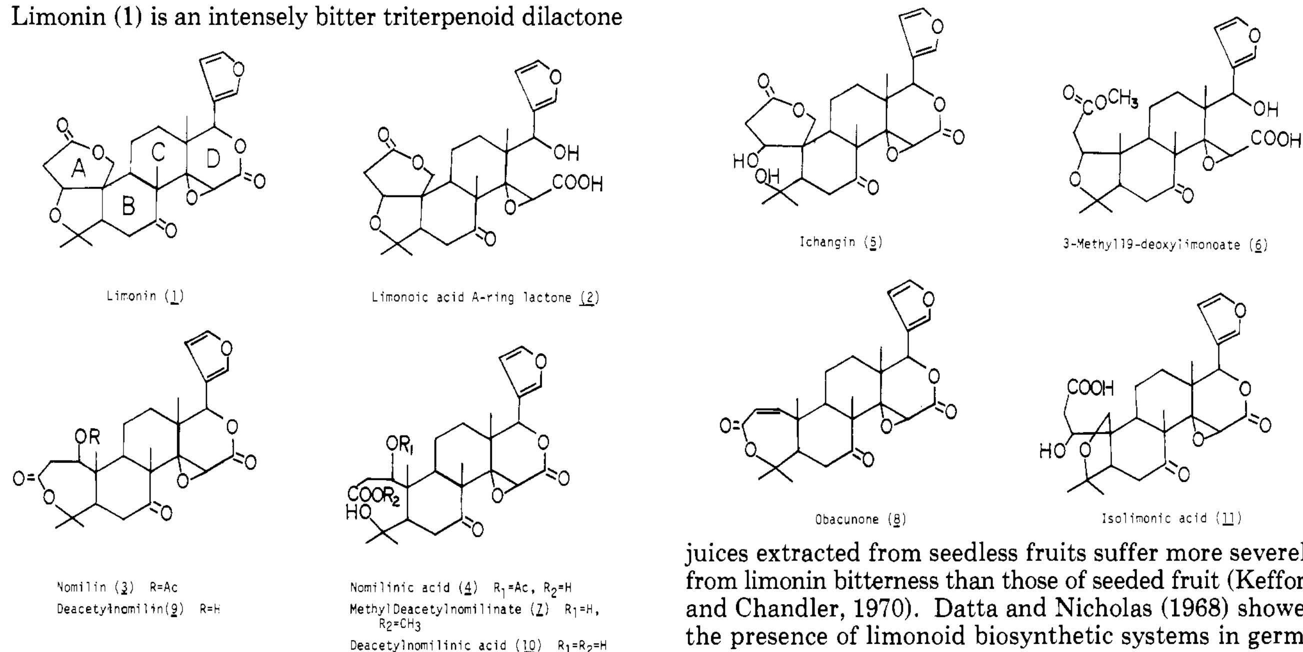
Limonoids in Citrus Seeds: Origin and Relative Concentration

Shin Hasegawa,* Raymond D. Bennett, and Carl P. Verdon

Radioactive tracer experiments showed that citrus seeds accumulate limonoids as they are translocated from fruit tissue during growth of the fruit. De novo synthesis of limonoids from ^{14}C-labeled acetate in the seeds could not be demonstrated. The major neutral and acidic limonoids in seeds of various citrus species and hybrids were quantitatively analyzed.

Limonin (1) is an intensely bitter triterpenoid dilactone



present in citrus seeds, and it is largely responsible for the delayed development of bitterness in citrus juices. The intact fruits do not normally contain 1 but rather a nonbitter precursor, limonoic acid A-ring lactone (2), which is converted gradually to 1 in the juice after extraction from the fruit (Maier and Beverly, 1968). This delayed bitterness is a very serious economic problem in the citrus industry.

A group of triterpene derivatives chemically related to 1 found in Rutaceae and Meliaceae families have been named limonoids. Other limonoids such as nomilin (3), nomilinic acid (4), and ichangin (5) are also bitter, but they are insignificant in juice technology because of their low levels in juice.

Citrus seeds contain large amounts of limonoids. It has been suggested that limonoids present in the seeds are translocated from the fruit tissue and that consequently juices extracted from seedless fruits suffer more severely from limonin bitterness than those of seeded fruit (Kefford and Chandler, 1970). Datta and Nicholas (1968) showed the presence of limonoid biosynthetic systems in germinated Valencia orange seeds by demonstrating the incorporation of ^{14}C-labeled mevalonate into 1. However, it is uncertain whether dormant seeds are capable of synthesizing limonoids, and, if they are, how significant those synthesized are in relation to the total limonoids accumulated in the seeds.

Limonoid research has been centered mainly on the isolation, structure determination, and, more recently, biochemistry of individual limonoids. Practically no data are available on the relative amounts of individual limonoids in citrus seeds. Dreyer (1966a) reported the occurrence of limonoids in citrus and their hybrids in respect to taxonomy, but only the major neutral limonoids known at that time were reported and they were not reported on a quantitative basis.

Therefore, we studied the mechanism of limonoid accumulation in citrus seeds and also made quantitative analyses of neutral and acidic limonoids in various citrus seeds.

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EXPERIMENTAL SECTION

Materials. Calamin, retrocalamin, cyclocalamin, methyl isoobacunoate diosphenol, calaminic acid, retrocalaminic

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