prepared by bubbling material separated by gas chromatography (Ucon preparative) through the 2,4-DNPH reagent (*11*). A yellow precipitate was dried, and chromatographed on a Celite–silicic acid column as described above for citronellal. The product, fine yellow needles after crystallization from absolute ethanol, melted at 105.5° to 106° C. The mixed melting point with the 2,4-DNPH derivative of an authentic sample of heptanal was 106° to 107° C. The infrared analysis of the 2,4-DNPH derivative was identical with that of known heptanal.

The amount of hexanal was too small for conversion to derivatives. Consequently, proof of identity must rest on comparison of retention time with that of authentic material as described above.

Acknowledgment

The authors are indebted to Hoffmann-La Roche Inc., Nutley, N. J., for the enriched samples of neral and geranial, members of the U. S. Department of Agriculture, Western Regional Research Laboratory, Albany, Calif.: L. M. White for elementary analyses and G. F. Bailey for infrared analysis of heptanal 2,4-DNPH.

Literature Cited

(1) Bernhard, R. A., *J. Chromatog.* **3,** 471 (1960).
(2) Calvarano, Maria, *Essenze deriv. argrumari* **27,** 173 (1957).
(3) Gordon, B. E., Wopat, F., Jr., Burnham, H. D., Jones, L. C., Jr., *Anal. Chem.* **23,** 1754 (1951).
(4) Guenther, E., "The Essential Oils," Vol. III, Van Nostrand, New York, 1949.
(5) Kirchner, J. G., Miller, J. M., *Ind. Eng. Chem.* **44,** 318 (1952).
(6) Kirchner, J. G., Miller, J. M., Keller, G. J., *Anal. Chem.* **23,** 420 (1951).
(7) Kung, Jo-fen T., Bambara, Paul, Perkins, Frank, Jr., Abstracts, p. 24B, 136th Meeting, ACS, Atlantic City, N. J., September 1959.
(8) Liberti, A., Cartoni, G. P., "Gas Chromatography," ed. D. H. Desty, Butterworths Scientific Publications, London, 1958.
(9) Miller, J. M., Kirchner, J. G., *Anal. Chem.* **24,** 1480 (1952).
(10) *Ibid.*, **25,** 1107 (1953).
(11) Monkman, J. L., *The Perkin-Elmer Instrument News* **8,** 1 (September 1957).
(12) Poore, H. D., U. S. Dept. Agr. Tech. Bull. **241** (1932).
(13) Shriner, R. L., Fuson, R. C., "Identification of Organic Compounds," 3rd ed., Wiley, New York, 1948.
(14) Stanley, W. L., Ikeda, R. M., Vannier, S. H., Rolle, L. A., *J. Food Sci.* **26,** 43 (1961).
(15) Stanley, W. L., Lindwall, R. C., Vannier, S. H., J. AGR. FOOD CHEM. **6,** 858 (1958).
(16) Stanley, W. L., Vannier, S. H., *Food Technol.* **13,** 96 (1959).
(17) Teitelbaum, C. L., *J. Org. Chem.* **23,** 646 (1958).

Received for review October 27, 1960. Accepted July 19, 1961. Fruit and Vegetable Chemistry Laboratory is a laboratory of the Western Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture. Work supported in part by Lemon Products Technical Committee, Los Angeles, Calif. Mention of specific products does not constitute endorsement by the U. S. Department of Agriculture over others of a similar nature not mentioned.

FRUIT FLAVORS AND ODORS

Isolation and Identification of Some Volatile Carbonyl Components from Orange Essence

JOHN A. ATTAWAY and RICHARD W. WOLFORD

Florida Citrus Commission, Lake Alfred, Fla.

GEORGE J. EDWARDS

University of Florida Citrus Experiment Station, Lake Alfred, Fla.

An analytical procedure is described for the detection and identification of carbonyl components in trace amounts from a commercial orange essence. Volatile organic components were separated as an oil by liquid-liquid extraction of the essence and fractionated by gas-liquid chromatography. Carbonyl peaks were detected by bubbling the effluent gas through an ethanolic solution of 2,4-dinitrophenylhydrazine sulfate and identified by their dinitrophenylhydrazones. Carbonyl components identified include acetaldehyde, hexanal, hexenal (two isomers), octanal, octenal (?), furfural (?), neral, geranial, and carvone.

THE IMPORTANCE of oxygenated compounds to the flavor of citrus juices and citrus oils has been described by many investigators. Stanley (*9*) states that although the terpene hydrocarbon, *d*-limonene, is the major component of citrus oils, the oxygenated terpenes representing only about 5% of the oil provide the aroma typical of the individual fruit. Nelson and Mottern (*6*) identified *n*-decyl aldehyde and citral as components of Florida orange oil. More recently, Kirchner and Miller (*5*) reported the presence of acetaldehyde, acetone (trace), furfural (trace), hexanal, octanal, decanal, 2-dodecenal (?), citronellal, three C_{15} carbonyls, and carvone in fresh California Valencia orange juice. The volatiles were removed from the orange juice by low temperature distillation. The lower the pressure and the lower the subsequent distillation temperature, the less was the alteration in the typical aroma of the juice.

Workers in many fields have found the 2,4-dinitrophenylhydrazones to be useful derivatives in the identification of aldehydes and ketones. Ellis, Gaddis, and Currie (*2*) reported a rapid paper chromatographic method for the separation and tentative identification of saturated aldehyde dinitrophenylhydrazones. Gaddis and Ellis (*3*) extended this method to include the unsaturated aldehyde derivatives. Ross (*7*) and Jones, Holmes, and Seligman (*4*) studied the infrared spectra of dinitrophenylhydrazones and found them very useful for the identification of the parent carbonyl compounds. The latter also found that the position of the N—H stretching band, usually found near 3.05 microns, could be used to determine whether the parent compound was an aldehyde or a ketone.

Apparatus and Reagents

Gas chromatographic separations were carried out on an F & M Model 202 programmed temperature gas chromatograph equipped with a thermistor-type detector. A 6-foot column, 0.25-inch o.d., packed with 30 weight %

Carbowax 20 M on Chromosorb regular was used.

Infrared spectra were determined using a Beckman IR-4 recording infrared spectrophotometer.

The natural orange essence used is a concentrated mixture of recovered volatile flavor components. As obtained for analysis, it is predominantly a water solution.

Procedure

Two liters of aqueous Valencia orange essence were saturated with anhydrous sodium sulfate and extracted with two 500-ml. portions of diethyl ether. The ether extracts were concentrated on a steam bath until the temperature reached 40° C. and then re-extracted with isopentane to remove the water. The isopentane was removed by evaporation, leaving 5 to 6 ml. of an aromatic oil.

Gas chromatograms were developed from 3- to 40-μl. quantities of the extracted oil. The injection port of the gas chromatograph was held at 180° C. and the detector at 230° C. The column was programmed from 50° to 250° C. at a rate of 4.5° C. per minute. The helium flow rate was approximately 50 ml. per minute.

For dinitrophenylhydrazone preparation, short lengths of glass tubing were attached to the exit port by rubber sleeves. The tube was immersed in a 1-ml. aliquot of dinitrophenylhydrazine sulfate (*8*) in a 10-ml. beaker. The precipitation of the dinitrophenylhydrazones was almost simultaneous with the appearance of the corresponding carbonyl peak on the recorder. Between twenty and fifty 40-μl. runs were necessary to accumulate the 2 to 5 mg. of derivative required for further study. The derivatives were recrystallized from 95% ethyl alcohol and dried at room temperature in a desiccator. Drying under vacuum or with heat accelerated the tendency of many of the derivatives to become amorphous.

Infrared spectra were obtained from a solution prepared by mixing approximately 1 mg. of the derivative with 200 mg. of KBr in CCl_4. The CCl_4 was evaporated and the resulting powder pressed into a flat disk using a Carver hydraulic laboratory press. A disk of KBr alone was used in the reference beam.

Melting points were taken by the capillary tube method.

Paper chromatograms were developed using the method of Ellis, Gaddis, and Currie (*2*), with certain minor changes. Eastman White Label heptane and Fisher USP petrolatum were used instead of Skellysolve C and vaseline, respectively. Some secondary separations were made using unimpregnated paper to avoid contamination when derivatives were eluted for infrared analysis.

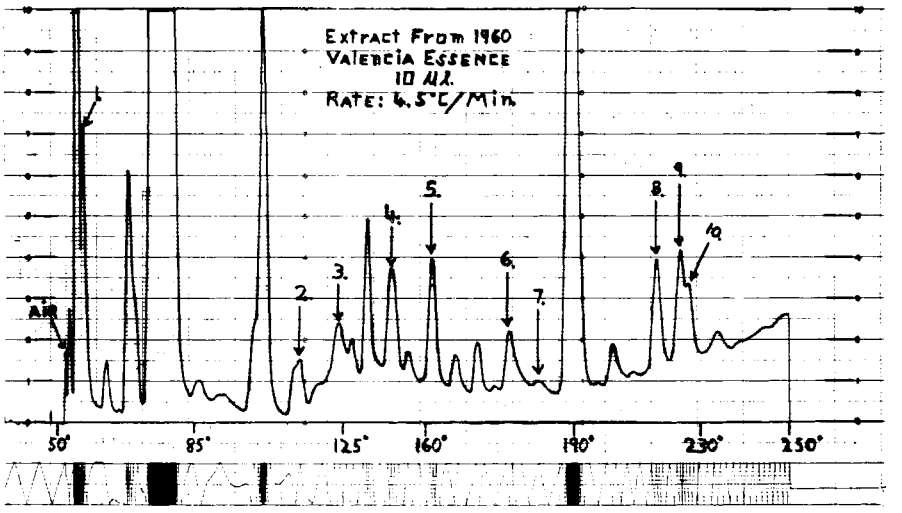

Figure 1. Typical gas chromatogram

1. Acetaldehyde
2. Hexanal
3. Hexenal
4. 2-Hexen-1-al
5. Octanal
6. Octenal (?)
7. Furfural
8. Neral
9. Geranial
10. Carvone

Results

The following carbonyl compounds were identified and are listed in the order of their gas chromatographic retention time: acetaldehyde, hexanal, hexenal (two isomers), octanal, octenal (?) (*7*), furfural (?), neral, geranial, and carvone.

Acetaldehyde. The gas chromatographic retention volume was identical with known acetaldehyde (see Table I) and addition of acetaldehyde increased the amplitude of the unknown peak; such information is hereafter referred to as retention volume data. The dinitrophenylhydrazone of the unknown had the same melting point and an infrared spectrum identical to that of known acetaldehyde dinitrophenylhydrazone. It also had the same chromatographic R_f values in two different solvent systems (see Table II) and was inseparable from the known in these systems; hereafter, such results are referred to as paper chromatographic data.

Hexanal. Retention volume data agreed. Canary yellow dinitrophenylhydrazone, which is characteristic of saturated aliphatic aldehyde derivatives, had the same melting point and infrared spectrum as the known derivative. Paper chromatographic data checked.

Hexenals. Two peaks gave orange dinitrophenylhydrazones which are indicative of either unsaturated aldehydes or of ketones. These derivatives gave the same infrared spectrum, which was also identical to the spectrum of known 2-hexenal dinitrophenylhydrazone. The spectrum of the known 2-hexenal indicated that it was *cis*-2-hexen-1-al. One peak had the same retention volume as the major peak of the known aldehyde, while the other was identical to one of the smaller peaks produced by the known. The derivatives melted almost identically and were inseparable by paper chromatography, leading to the conclusion that the second of the two

Table I. Relative Corrected Retention Volumes of Carbonyl Components Found in Orange Essence

Stationary phase. Carbowax 20M, 30% w./w.
Octanal = 1.00

Compound	V_{ro}
Acetaldehyde	0.045
Hexanal	0.64
Hexenal	0.74
2-Hexenal-1	0.89
Octanal	1.00
Octenal (?)	1.20
Furfural (?)	1.29
Neral	1.61
Geranial	1.68
Carvone	1.70

Helium inlet flow 50 ml./min. at 25° C.
Starting temperature 50° C.
Program rate approx. 4.5° C./minute

Table II. Comparative R_f's of Known and Experimental Dinitrophenylhydrazones

Compound	Known Deriv.	Exptl. Deriv.	Mixed
Propylene Glycol System			
Acetaldehyde	0.30	0.30	0.30
Hexanal	0.93	0.93	0.92
Hexenal[a]	0.89	0.89	0.87
Petrolatum System			
Acetaldehyde	0.70	0.69	0.69
Hexanal	0.55	0.55	0.54
Hexenal[a]	0.59	0.58	0.59
Octanal	0.38	0.39	0.38
Octenal	0.40	0.41	0.40
Geranial[b]			
Neral	0.43	0.43	0.42
Carvone	0.28	0.28	0.28

[a] Data for isomer identified as 2-hexen-1-al.
[b] Data identical for isomer geranial and neral.

hexenals was *cis*-2-hexen-1-al, while the first was another isomer.

Octanal. Retention volume data corresponded. Canary yellow derivative had an infrared spectrum identical to that of octanal-dinitrophenylhydrazone. Paper chromatographic data agreed.

Octenal (?). Retention volume data checked. Melting point and paper chromatographic data agreed. However, infrared spectra compared poorly. Elemental analysis of the unknown derivative indicated an eight- or nine-carbon, unsaturated compound as the parent compound.

Furfural (?). Retention volume data agreed. Characteristic color of derivative checked. This was a trace component and not detected in all samples.

Neral (Citral b). The retention volume was the same as that of neral obtained from gas chromatographic fractionation of commercial citral. The infrared spectra and paper chromatographic data were essentially the same for the derivatives of both geranial and neral, but the geranial derivative melted at a much lower temperature.

Geranial (Citral a). Retention volume data, infrared spectra, melting point, and paper chromatographic data corresponded.

Carvone. Retention volume data agreed. Bright red color, infrared spectrum, and paper chromatographic data of dinitrophenylhydrazone checked. This peak formed as a shoulder on the peak for geranial. The combined materials were condensed and reacted with dinitrophenylhydrazine, and the mixed derivatives separated paper chromatographically on unimpregnated Whatman No. 3 paper, using heptane as the developing solvent.

Discussion

This procedure gives good identification of small amounts of aldehydes and ketones in gas chromatographic effluents. In some cases the melting points tend to be slightly lower than those reported in the literature, largely because of the small quantities involved, which make repeated recrystallizations impractical. However, good infrared spectra and paper chromatographic data can be obtained even when traces of impurities are present. The infrared spectra are sufficiently characteristic to permit even octanal and nonanal-dinitrophenylhydrazones to be distinguished.

Two carbonyl compounds encountered in this study—geranial and neral—reacted more slowly with dinitrophenylhydrazine than did the others and could not be directly precipitated by bubbling the effluent gas through the reagent solution. Their derivatives were formed by condensing the effluent from the suspected peaks and reacting it with dinitrophenylhydrazine sulfate according to the normal procedures. Some other carbonyl compounds will probably behave in this manner.

Hexenal and octenal have not previously been reported as constituents of orange juice. Their detection may have been the result of the large degree of concentration of all the components in the essence, or they may be artifacts.

Acknowledgment

The authors express appreciation to the Libby, McNeill & Libby Co., Ocala, Fla., and E. J. Kelley & Associates, Winter Haven, Fla., for the aqueous orange essence. A sample of 2-hexenal was obtained through the courtesy of Union Carbide Chemicals Co., South Charleston, W. Va.

Literature Cited

(1) Bedoukian, P. Z., *J. Am. Chem. Soc.* **79,** 889 (1957)
(2) Ellis, R., Gaddis, A. M., Currie, G. T., *Anal. Chem.* **30,** 475 (1958).
(3) Gaddis, A. M., Ellis, R., *Ibid.*, **31,** 870 (1959).
(4) Jones, L. A., Holmes, J. C., Seligman, R. B., *Ibid.*, **28,** 191 (1956).
(5) Kirchner, J. G., Miller, J. M., J. AGR. FOOD CHEM. **5,** 283 (1957).
(6) Nelson, E. K., Mottern, H. H., *J. Am. Chem. Soc.* **56,** 1238 (1934).
(7) Ross, J. H., *Anal. Chem.* **25,** 1288 (1953).
(8) Shriner, R. L., Fuson, R. C., "Systematic Identification of Organic Compounds," 3rd ed., p. 171, Wiley, New York, 1948.
(9) Stanley, W. L., "Citrus Flavors. Flavor Research and Food Acceptance," p. 344, Reinhold, New York, 1958.

Received for review February 2, 1961. Accepted July 13, 1961. Division of Agricultural and Food Chemistry, 139th Meeting, ACS, St. Louis, Mo., March 1961. Cooperative research by the Florida Citrus Commission and the Florida Citrus Experiment Station. Florida Agricultural Experiment Station Journal Series, No. 1236.

FOOD PLANT POLYSACCHARIDES

Structure of the Polysaccharide of the Japanese Water Plant, *Brasenia schreberi* J. F. Gmel.

AKIRA MISAKI and FRED SMITH

Department of Agricultural Biochemistry, University of Minnesota, St. Paul 1, Minn.

Composition of the Polysaccharide and Isolation of 2-O-(β-D-Glucopyranosyluronic Acid)-D-Mannose

DURING investigations on the relationship between structure and physical properties of polysaccharides attention had been drawn to the polysaccharide which forms the main component of the mucilaginous coating of the Japanese water plant, Junsai (*Brasenia schreberi* Gmel.). This plant, which belongs to the lotus family (*Nymphaeceae*) (*15*), grows in swampy areas, possesses branching roots, and develops fine long stems under water with elliptical leaves that protrude through the surface. In summer, dark reddish violet flowers appear on the surface of the water. The transparent gelatinous mucilage which covers the leaves and stems of the plant is commonly used as a food in Japan.

The polysaccharide was conveniently isolated from plant stems and leaves by treating them with hot water or with dilute sodium hydroxide at room temperature (*16*) followed by addition of ethanol to the extract to precipitate the required polysaccharide. Purification was effected by use of the fact that the polysaccharide forms an insoluble copper hydroxide complex when treated with Fehling solution (*16*). The polysaccharide, recovered from the copper complex as a white amorphous powder, constitutes 0.4 to 0.5% of the weight of the wet plant or about 40% of the dry weight of the plant. The polysaccharide, which is acidic in character (equivalent weight, 1040), after further purification via its acetate ($[\alpha]_D^{25}$ − 6° in acetone and in chloroform), showed $[\alpha]_D^{25}$ + 9° in water, in contrast to the product isolated previously (*16*) in 1940, which had $[\alpha]_D^{18}$ + 28° in water. The substance, which revealed no evidence of heterogeneity by electrophoresis on glass paper (*14*), did not reduce boiling Fehling solution. It gave a negative test with iodine solution, but a strong positive reaction for uronic acid by the carbazole test (*4*).