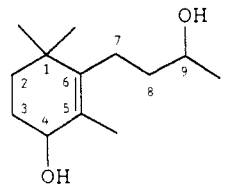
4-Hydroxy-7,8-dihydro-β-ionone and Isomeric Megastigma-6,8-dien-4-ones: New C_{13} Norisoprenoids in Quince (*Cydonia oblonga*, Mill.) Fruit

Peter Winterhalter, Markus Herderich, and Peter Schreier*

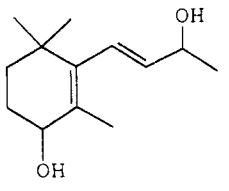
Lehrstuhl für Lebensmittelchemie, Universität Würzburg, Am Hubland, D-8700 Würzburg, West Germany

In a polar fraction of an extract obtained from quince fruit juice (*Cydonia oblonga*, Mill.) by solvent extraction and subsequent liquid chromatographic separation on silica gel, capillary gas chromatography (HRGC) and capillary gas chromatography–mass spectrometry (HRGC–MS) revealed the occurrence of 4-hydroxy-7,8-dihydro-β-ionone. The identification was verified by comparison of HRGC and MS data of the new natural product with those of a synthesized reference compound. In addition, biomimetic studies with another 4-oxygenated ionone derivative from quince fruit, i.e., 4-hydroxy-β-ionol, were carried out. Heat treatment of this diol at pH 3.5 gave a number of volatile C_{13} norisoprenoids, including 2,2,6,7-tetramethylbicyclo[4.3.0]nona-4,7,9(1)-triene, isomeric retro-α-ionones, megastigma-5,8(*E*)-dien-4-one, and megastigma-5,8(*Z*)-dien-4-one. As major degradation products (70%) a mixture of four isomeric ketones was obtained. After preparative HPLC separation ^1H and ^{13}C NMR analyses in combination with NOE experiments revealed the structure of the main isomer as megastigma-6(*E*),8(*E*)-dien-4-one. This component was also detected as trace constituent in quince fruit juice.

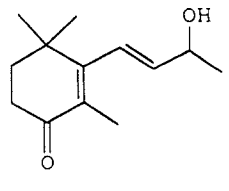
C_{13} norisoprenoids are important aroma constituents, in particular, in tobacco (Enzell et al., 1977) and tea leaves (Schreier, 1988). A considerable number of carotenoid degradation products have been also found in quince (*Cydonia oblonga*, Mill.) fruit (Tsuneya et al., 1983; Ishihara et al., 1986). As precursors of these aroma components, free and bound C_{13} norisoprenoids have been identified (Winterhalter and Schreier, 1988a,b), including 4-hydroxy-7,8-dihydro-β-ionol (**1**), the natural precursor of isomeric theaspiranes in quince. In addition to **1**, the following structurally related compounds have been detected, i.e., 4-hydroxy-β-ionol (**2**), 4-oxo-β-ionol (**3**), and 4-hydroxy-β-ionone (**4**).



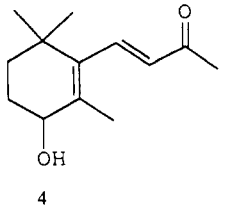
1



2



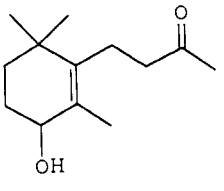
3



4

At present, our knowledge about the natural occurrence of 4-oxygenated ionone derivatives is rather scarce. Thus, free diol **1** has been found exclusively in quince, whereas **2**–**4** have been additionally detected in *Osmanthus fragrans* (Kaiser and Lamparsky, 1978) and **2** also in tobacco (Weeks and Seltmann, 1986). However, the most recent identification of **1**–**3** as glucoconjugates in the juice of the purple passion fruit (Winterhalter, 1990) indicates that 4-oxygenated ionone derivatives are obviously more common in nature as previously expected.

This paper concerns the structural elucidation of an additional representative of 4-oxygenated ionones in quince, i.e., 4-hydroxy-7,8-dihydro-β-ionone (**5**). Furthermore, the potential role of diol **2** as a precursor of aroma components is pointed out, from which under thermal treatment at acidic pH (3.5) a number of conversion products are formed.



5

EXPERIMENTAL SECTION

Fruits. Fresh ripe quince fruits (*C. oblonga*, Mill.) were available from the local market.

Isolation of Free C_{13} Norisoprenoids. The isolation was carried out as previously described for diol **1** by solvent extraction and liquid chromatographic preseparation on silica gel (Winterhalter and Schreier, 1988a).

Reference Compounds. **3**, **4**, and **7A/B** were donated samples. Compound **5** was prepared according to the method of Pascual et al. (1988) by regioselective reduction of the side-chain double bond of 4-hydroxy-β-ionone (**4**). After liquid chromatographic purification on silica gel (pentane–diethyl ether gradient) **5** was obtained in about 90% yield: R_i 1648; MS, *m/z* (%) 41 (27), 43 (100), 55 (18), 69 (11), 77 (14), 79 (17), 91 (19), 93 (26), 107 (27), 111 (17), 119 (53), 121 (41), 136 (23), 152 (19), 177 (6), 192 $(M - H_2O)^+$ (18); ^1H NMR (δ, TMS) 0.90 and 0.96 (6 H, 2 s, 2 CH_3 C1), 1.65 (3 H, s, CH_3 C5), 2.09 (3 H, s, CH_3 C9), 1.1–1.8 (4 H, m, 2 H, C2; 2 H C3), 2.04 (1 H, br s, OH), 2.1–2.5 (4 H, m, 2 H, C7; 2 H, C8), 3.83 (1 H, t, J = 4.4 Hz, H

0021-8561/90/1438-0796$02.50/0

Table I. Products Formed in Model Reactions after Thermal Treatment (SDE, 2 h) of 4-Hydroxy-β-ionol (2) at pH 3.5

peak no.[a]	compound	%
IS	internal standard	
1	2,2,6,7-tetramethylbicyclo[4.3.0]-nona-4,7,9(1)triene (**6**)	5.1
2	unknown (MW 192)	2.7
3	retro-α-ionone (isomer 1) (**8A**)	4.6
4	megastigma-6,8-dien-4-one (isomer 1) (**10A**)	2.5
5	megastigma-6,8-dien-4-one (isomer 2) (**10B**)	19.5
6	retro-α-ionone (isomer 2) (**8B**)	4.0
7	megastigma-5,8(*E*)-dien-4-one (**7A**)	9.2
8	megastigma-6,8-dien-4-one (isomer 3) (**10C**)	4.4
9	megastigma-6,8-dien-4-one (isomer 4) (**10D**)	44.0
10	megastigma-5,8(*Z*)-dien-4-one (**7B**)	2.3
	unknown trace components	1.7

[a] Cf. peak numbers in Figure 1.

C4). MS and ^1H NMR data were in close agreement with those recently published by Pascual et al. (1988).

Preparation of 4-Hydroxy-β-ionol (2). Diol **2** was prepared by reduction ($LiAlH_4$) of 1.4 g of 4-oxo-β-ionone. Liquid chromatographic purification on silica gel yielded 1.1 g of pure diol **2**, showing the following data: R_i 1628; MS, *m/z* (%) 41 (35), 43 (100), 55 (29), 69 (20), 77 (16), 91 (25), 93 (22), 105 (16), 107 (21), 111 (19), 119 (24), 121 (28), 135 (9), 149 (9), 159 (14), 192 $(M - H_2O)^+$ (11); ^1H NMR (δ, TMS) 0.91 and 0.93 (6 H, 2 s, 2 CH_3 C1), 1.18 (3 H, d, J = 7 Hz, CH_3 C9), 1.65 (3 H, s, CH_3 C5), 3.2–3.8 (2 H, m, H C4; OH), 4.21 (1 H, quintet, J = 7 Hz, H C9), 5.32 (1 H, dd, J = 17 and 7 Hz, H C8), 5.90 (1 H, d, J = 17 Hz, H C7); FTIR (vapor phase; ν, cm^{-1}) 3646, 3040, 1615, 961.

Degradation Products of 2. The degradation of diol **2** (1 g) was carried out at pH 3.5 with simultaneous distillation extraction (SDE) (Winterhalter and Schreier, 1988b). Among the volatile degradation products (0.9 g) (composition, cf. Table I) the four isomeric megastigma-6,8-dien-4-ones **10A–D** showed the following data. **10A**: R_i 1484; MS, *m/z* (%) 41 (85), 55 (98), 67 (56), 79 (56), 93 (68), 107 (49), 121 (100), 135 (44), 149 (5), 163 (7), 177 (37), 192 (M^+) (56). **10B**: R_i 1490; MS, *m/z* (%) 41 (96), 55 (90), 67 (47), 79 (63), 93 (67), 107 (51), 121 (100), 135 (39), 149 (10), 163 (14), 177 (44), 192 (M^+) (60). **10C**: R_i 1516; MS, *m/z* (%) 41 (97), 55 (85), 67 (43), 79 (56), 93 (70), 107 (48), 121 (100), 135 (37), 149 (7), 163 (9), 177 (33), 192 (M^+) (57). **10D**: R_i 1521; MS, *m/z* (%) 41 (80), 55 (82), 67 (44), 79 (54), 93 (70), 107 (50), 121 (100), 135 (38), 149 (10), 163 (14), 177 (36), 192 (M^+) (54). The major isomer **10D** was isolated by preparative HPLC (LiChrospher 100, 5 μm; column, 250 × 16 mm; Knauer, Berlin) at 220 nm with hexane–*tert*-butyl methyl ether (90:10, v/v) as eluent: FTIR (vapor phase; ν, cm^{-1}) 3033, 2971, 2934, 2880, 1731, 961; ^1H NMR (δ, TMS) 1.11 and 1.17 (6 H, 2 s, 2 CH_3 C1), 1.31 (3 H, d, J = 7.8 Hz, CH_3, C5), 1.77 (3 H, dd, J = 6.7 and 1.6 Hz, CH_3 C9), 1.62–1.91 (2 H, m, H_2, C2), 2.32–2.59 (2 H, m, H_2 C3), 3.44 (H, q, J = 7.8 Hz, H C5), 5.71 (H, dq, J = 14.8 and 6.7 Hz, H C9), 5.95 (H, d, J = 11.1 Hz, H C7), 6.16 (H, ddq, J = 14.8, 11.1, and 1.6 Hz, H C8); ^{13}C NMR (δ, TMS) 18.41 (CH_3 C9), 20.44 (CH_3 C5), 29.21 and 29.68 ($(CH_3)_2$ C1), 35.34 (C1), 35.40 (C2), 35.78 (C3), 46.33 (C5), 123.78, 126.63, 129.76 (C7, C8, C9), 145.13 (C6), 214.38 (C4).

Capillary Gas Chromatography (HRGC). A Carlo Erba Fractovap 4160 gas chromatograph with FID equipped with a J&W fused silica DB 5 capillary column (30 m × 0.259 mm (i.d.), df = 0.25 μm) was used. Split injection (1:50) was employed. The temperature program was from 60 to 200 °C at 2.5 °C/min. The flow rates for the carrier gas were 2.5 mL/min of He, for the makeup gas 30 mL/min of N_2, and for the detector gases 30 mL/min of H_2 and 300 mL/min of air, respectively. The detector temperature was kept at 250 °C. Volumes of 1 μL were injected.

Results of qualitative analyses were verified by comparison of HRGC retention, mass spectral, and FTIR vapor-phase data with those of authentic reference substances. Quantitative HRGC determinations were carried out by standard controlled calculations on a Hewlett-Packard 3388 A laboratory data system.

Capillary Gas Chromatography–Mass Spectrometry (HRGC–MS). A Varian Aerograph 1440 gas chromatograph equipped with a Carlo Erba water-cooled on-column injection system was combined by direct coupling to a Finnigan MAT 44 mass spectrometer. The same type of column as mentioned above for HRGC analysis was used. The conditions were as follows: temperature, isothermal for 2.5 min at 60 °C and then from 60 to 200 °C at 2.5 °C/min; carrier gas flow rate, 2.5 mL/min of He; temperature of ion source and all connection parts, 200 °C; electron energy, 70 eV; cathodic current, 0.8 mA. Volumes of 0.5 μL were used.

Capillary Gas Chromatography–Fourier Transform Infrared Spectroscopy (HRGC–FTIR). HRGC–FTIR analysis was carried out with a Nicolet 20 SXB system interfaced by a Dani 6500 gas chromatograph equipped with FID. The same type of column as mentioned before was used. Total sample injection mode employing programmed temperature vaporization (PTV) (40–180 °C, 0.1 min) was performed. The temperature program was 50 to 160 °C at 10 °C/min. Light pipe and transfer line were held at 160 °C; He (2.5 mL/min) was employed as carrier gas. Vapor-phase FTIR spectra were recorded from 600 to 4000 cm^{-1} with a resolution of 8 cm^{-1}.

Nuclear Magnetic Resonance Spectroscopy. ^1H NMR and ^{13}C NMR spectra were recorded at 400 MHz on a Bruker WM 400 and at 200 MHz on a Bruker AC 200 instrument, respectively, with $CDCl_3$ as solvent and Me_4Si as reference standard. NOE measurements were carried out at −20 °C.

RESULTS AND DISCUSSION

Identification of 4-Hydroxy-7,8-dihydro-β-ionone (5). The aroma compounds of quince fruit (*C. oblonga*, Mill.) were preseparated by liquid chromatographic fractionation on silica gel (Winterhalter and Schreier, 1988a). In a polar fraction two new C_{13} norisoprenoids were detected, whose mass spectral data have been published previously. Meanwhile, the structure of one of these compounds, i.e., unknown B (Winterhalter and Schreier, 1988b), could be elucidated as 4-hydroxy-7,8-dihydro-β-ionone (**5**) by comparison of its chromatographic and mass spectral data with that of a synthesized reference compound (Pascual et al., 1988). As far as we known, **5** has not been found in nature as yet.

The structural similarity of **5** with compounds **1–4**, also detected in quince fruit for the first time (Winterhalter and Schreier, 1988b), suggests a common biogenetic pathway. Canthaxanthine can be regarded as a carotenoid precursor of these 4-oxygenated ionone derivatives (Demole et al., 1979). However, studies on the oxidative degradation of this carotenoid have not been carried out as yet. Furthermore, additional precursors of **1–5** have to be considered. For example, a number of 4-oxygenated ionones have been described as minor products of the lipoxygenase catalyzed cooxidation of β-carotene (Hohler, 1986).

Degradation of 4-Hydroxy-β-ionol (2) at pH 3.5. Continuing our biomimetic studies about the formation of C_{13} norisoprenoids (Winterhalter and Schreier, 1988a,b), the behavior of **2** in acidic solution was investigated. Thermal treatment (2 h, SDE) at pH 3.5 led to its rapid and complete degradation. The HRGC separation of the products formed is outlined in Figure 1; in Figure 2 their structures are represented.

Among the degradation products shown in Figure 2 only **6** and **7A/B** have been described as natural aroma substances. The bicyclic triene **6** has been found in quince fruit (Ishihara et al., 1986), and the isomeric megastigma-5,8-dien-4-ones **7A/B** have been detected in passion fruit

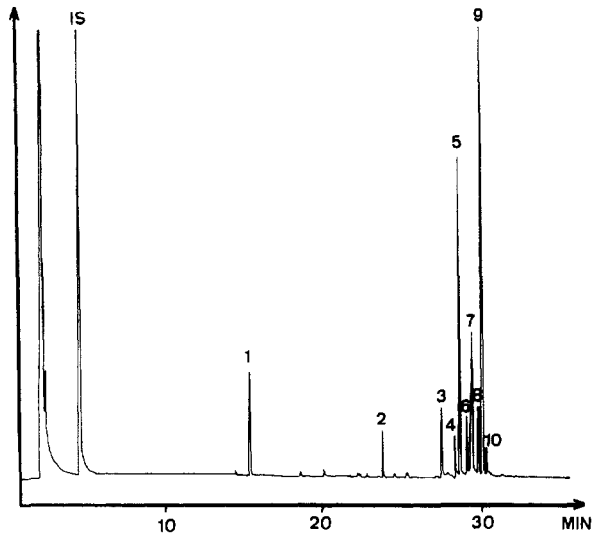


Figure 1. HRGC separation (J&W; 30 m × 0.25 mm (i.d.); fused silica WCOT DB 5 capillary column, df = 0.25 μm) of thermal degradation products (SDE; pH 3.5) of 4-hydroxy-β-ionol (**2**).

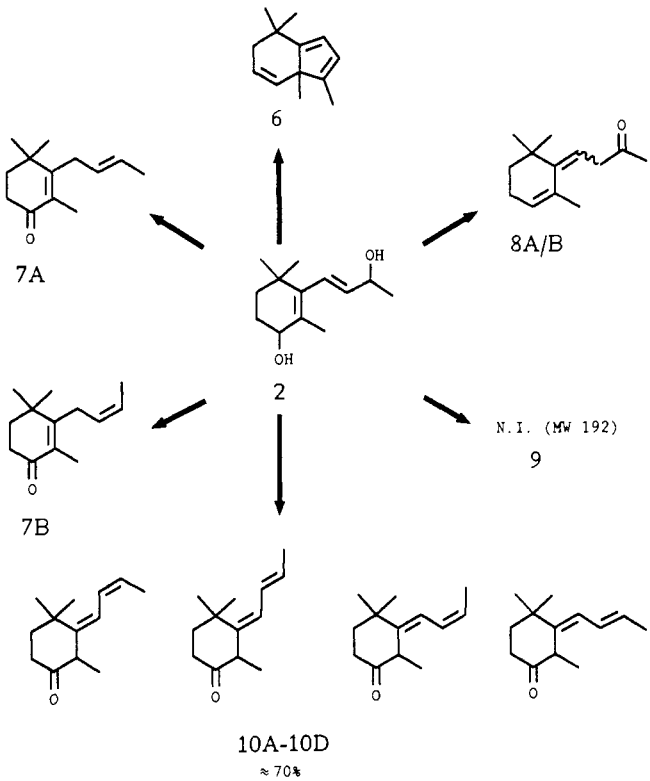
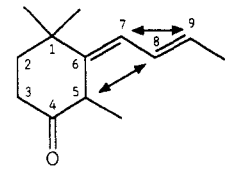


Figure 2. Structures of thermal degradation products of 4-hydroxy-β-ionol (**2**) under SDE conditions (100 °C, pH 3.5).

(Demole et al., 1979) and rum (Ter Heide et al., 1981). In addition, the *E* isomer **7A**, described to exhibit a "fruity-floral and woody" flavor, has been also identified in *Osmanthus* (Kaiser and Lamparsky, 1978) and tobacco (Demole et al., 1979). In this last-mentioned paper, **7A** has been described as chemical degradation product of **2**; 76% **7A** and 12% β-ionone have been recovered, and no additional products have been found. As shown in Figure 2, under the reaction conditions used in the present investigation, a higher number of degradation products was observed, including the retro-α-ionones **8A/B**. Their structures were elucidated by comparison of their chromatographic and mass spectral data with those of synthesized reference compounds (Winterhalter and Schreier, 1988a). Furthermore, ketone **9** was detected (R_i 1388) that could not be identified. Mass spectral analysis delivered the following data: *m/z* (%) 41 (16), 53 (8), 77 (15), 79 (12), 91 (17), 93 (100), 108 (37), 121 (4), 163 (5), 192 (M^+) (10).

The major products (70%) of the degradation of diol **2** consisted of four isomers **10A–D** (peaks 4, 5, 8, and 9 in Figure 1) that showed nearly identical mass spectra (M^+ 192). HRGC–FTIR analysis revealed the structures of unsaturated ketones; the major isomers (peaks 5 and 9 in Figure 1) exhibited trans double bonds (957 and 959 cm^{-1}, respectively). Attempts to fractionate isomers **10A–D** by preparative MPLC led to a partial separation of the major product **10D**. From ^1H NMR data,



10D

its structure as megastigma-6,8-dien-4-one was proposed. Since various attempts to synthesize megastigma-6,8-dien-4-one as a reference compound failed—in all cases **7A** was obtained—the mixture of isomers **10A–D** was further separated by preparative HPLC in order to perform additional structural elucidation. In spite of partial rearrangement to **7A** during the separation on silica gel, a sufficient amount of pure **10D** was obtained to carry out additional spectroscopic studies. ^1H and ^{13}C NMR analyses confirmed its structure as megastigma-6,8-dien-4-one. Six nuclear Overhauser effect (NOE) experiments were carried out for assignment of protons closely located in space. Thus, irradiation of the protons at C7 and C8 resulted in an NOE effect at the protons at C9 and C5, respectively, revealing the structure of **10D** outlined below. Ketone **10D** exhibited a pleasant weak tobacco note with a cooling effect. Finally, it has to be pointed out that **10D** has also been detected as a trace constituent of quince fruit juice (Winterhalter, 1988).

ACKNOWLEDGMENT

Dr. Scheutzow's and E. Ruckdeschel's (Institut für Organische Chemie) NMR analyses are gratefully acknowledged. We thank Dr. E. J. Brunke, Dragoco, Holzminden, and Dr. D. Lamparsky, Givaudan, Dübendorf, for providing several reference compounds as well as the Deutsche Forschungsgemeinschaft, Bonn, for financial support.

LITERATURE CITED

Demole, E.; Enggist, P.; Winter, M.; Furrer, A.; Schulte-Elte, K. H.; Egger, B.; Ohloff, G. Megastigma-5,8-dien-4-on, ein Aromastoff der gelben Passionsfrucht und des Virginia-Tabaks. *Helv. Chim. Acta* **1979**, *62*, 67–75.

Enzell, C. R.; Wahlberg, I.; Aasen, A. J. Isoprenoids and alkaloids of tobacco. *Prog. Chem. Org. Nat. Prod.* **1977**, *34*, 1–79.

Hohler, A. Untersuchung der Bildung und Eigenschaften von flüchtigen Nor-Carotinoiden. Doctoral Thesis, Technical University of Munich, Freising-Weihenstephan, 1986.

Ishihara, M.; Tsuneya, T.; Shiota, H.; Shiga, M.; Nakatsu, K. Identification of new constituents of quince fruit flavor (*Cydonia oblonga*, Mill.). *J. Org. Chem.* **1986**, *51*, 491–495.

Kaiser, R.; Lamparsky, D. Inhaltsstoffe des Osmanthus Absolues. 4. Mitteilung: Megastigma-5,7(E),9-trien-4-on und Megastigma-5,8(E)-dien-4-on. *Helv. Chim. Acta* **1978**, *61*, 2328–2335.

Pascual, A.; Bischofberger, N.; Frei, B.; Jeger, O. Photochemistry of 7,8-dihydro-4-hydroxy-β-ionone and derivatives. *Helv. Chim. Acta* **1988**, *71*, 374–388.

Schreier, P. Analysis of black tea volatiles. In *Modern methods of plant analysis. New Series, Analysis of nonalcoholic beverages*; Linskens, H. F., Jackson, J. F., Eds.; Springer: Berlin, Heidelberg, New York, 1988; Vol. 8, pp 296–320.

Ter Heide, R.; Schaap, H.; Wobben, H. J.; de Valois, P. J.; Timmer, R. Flavor constituents in rum. In *The quality of foods and beverages*; Charalambous, G., Inglett, G., Eds.; Academic Press: New York, 1981; pp 183–200.

Tsuneya, T.; Ishihara, M.; Shiota, H.; Shiga, M. Volatile components of quince fruit (*Cydonia oblonga*, Mill.). *Agric. Biol. Chem.* **1983**, *47*, 2495–2502.

Weeks, W. W.; Seltmann, H. Effects of sucker control on the volatile compounds in flue-cured tobacco. *J. Agric. Food Chem.* **1986**, *34*, 899–904.

Winterhalter, P. Untersuchungen über Aromavorstufen in Papaya-(*Carica papaya*, L.) und Quittenfrüchten (*Cydonia oblonga*, Mill.). Doctoral Thesis, University of Würzburg, 1988.

Winterhalter, P. Bound terpenoids in the juice of the purple passion fruit (*Passiflora edulis*, Sims). *J. Agric. Food Chem.* **1990**, in press.

Winterhalter, P.; Schreier, P. 4-Hydroxy-7,8-dihydro-β-ionol. The natural precursor of theaspiranes in quince fruit (*Cydonia oblonga*, Mill.). *J. Agric. Food Chem.* **1988a**, *36*, 560–562.

Winterhalter, P.; Schreier, P. Free and bound C13 norisoprenoids in quince (*Cydonia oblonga*, Mill.) fruit. *J. Agric. Food Chem.* **1988b** *36*, 1251–1256.

Received for review August 17, 1989. Accepted December 4, 1989.

Flavor and Compositional Comparison of Orange Essences and Essence Oils Produced in the United States and in Brazil

Manuel G. Moshonas* and Philip E. Shaw

U.S. Citrus & Subtropical Products Laboratory, South Atlantic Area, U.S. Department of Agriculture—Agricultural Research Service, P.O. Box 1909, Winter Haven, Florida 33883-1909

One commercial sample of each of aqueous orange essence and orange essence oil from Brazil was compared with three samples of each product produced in Florida. Brazilian and U.S. products were qualitatively identical, but minor quantitative differences were found. Sensory panels noted aroma differences between aqueous essences and essence oils produced in the United States and Brazil; similar aroma differences were found between these products produced by two different U.S. suppliers. When the products were used to flavor frozen concentrated orange juice (evaporator pumpout), however, no flavor differences were noted between any of the aqueous essences or essence oils.

The most widely used natural flavoring fractions for enhancing the fresh flavor and aroma of processed orange juice, particularly frozen concentrate, are aqueous orange essence and essence oil. Compositional flavor and aroma studies have shown that these fractions contribute fresh flavor top notes to processed juice products (Moshonas and Shaw, 1983). These two important byproducts are collected as the distillate from the second stage of an evaporator during concentration of freshly expressed orange juice (Johnson and Vora, 1983). Aqueous essence is separated from essence oil to produce a flavor fraction that is predominately a water–ethanol solution containing most of the volatile flavor constituents of fresh juice. Essence oil also contains some of the volatile components found in the juice but is largely made up of volatile components found in peel and juice oils. It differs greatly from the peel oil, however, in that it lacks the higher boiling compounds present in peel oil. Worldwide demand for orange juice products continues to increase, resulting in the need by the U.S. citrus industry to import large quantities of Brazilian frozen concentrate, aqueous essence, and essence oil in order to meet the demand of their domestic and foreign markets. Brazil is expecting a record harvest of 250 million boxes (10 200 000 MT) of oranges for the 1989–1990 season while Florida's crop is expected to be 140 million boxes (5 700 000 MT), down from the high of 212 million boxes (8 700 000 MT) for the 1979–1980 season. (Florida Citrus Processors Assn., 1989).

The essence fractions used for flavoring have direct bearing on the quality of orange products to which they are added. This report compares flavor quality, aroma, and compositional profiles of aqueous essences and essence oils produced in the United States with those imported from Brazil.

EXPERIMENTAL SECTION

Commercial aqueous orange essences and essence oils were obtained from citrus processing plants in the United States and in Brazil. Although typical commercial orange essences and essence oils are obtained from processing more than one cultivar, the major source of aqueous essence and essence oil in the United States is the Valencia cultivar and in Brazil it is the Pera cultivar. Since processors blend many samples to produce a uniform product, these are typical samples produced by each of the four processors.