

**Registry No.** Sterculic acid, 1089-40-3; methyl steculate, 3220-60-8; methyl malvalate, 5026-66-4; methyl dihydrosterculate, 10152-62-2; cytochrome P-450, 9035-51-2; 1,2-dipentylcyclopropene, 54467-84-4; 1,2-dipropylcyclopropene, 10306-92-0; 1,2-dihexylcyclopropene, 35365-52-7; 1,2-diheptylcyclopropene, 35365-53-8.

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## Carbon Isotope Ratios in Natural and Synthetic Citric Acid as Indicators of Lemon Juice Adulteration

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Citric acid from pure lemon juices possess a mean  $\delta^{13}\text{C}$  value of  $-24.1\text{‰}$  (parts per thousand). Commercial citric acid is produced by fermentation of  $\text{C}_3$  plant,  $\text{C}_4$  plant, and hydrocarbon sources; its  $\delta^{13}\text{C}$  value is related predictably to the source. That from  $\text{C}_4$  sources can be readily detected when added to lemon juice. Only the presence of a high level of the sample of hydrocarbon derived citric acid analyzed in this study could be useful as an indicator of adulteration. Sugars may also be added in the preparation of adulterated juices in order to maintain brix-acid ratios appropriate for pure lemon juice; they can be analyzed for  $\delta^{13}\text{C}$  to test whether  $\text{C}_4$  plant derived sugars such as those in high fructose corn syrup or cane syrup are present.

#### INTRODUCTION

Single-strength lemon juice contains, on the average, 9.3 g of soluble solids/100 g of juice, of which over 60% is citric

acid and over 20% is sugars. Adulteration of lemon juice with citric acid has been a long-standing problem, since lemon juice and concentrates are sold on the basis of titratable acidity, calculated as citric acid (Swisher and Swisher, 1980; Petrus and Vandercook, 1980). Citric acid is relatively inexpensive, being produced by large-scale microbial fermentations of a variety of sources, including beet and cane molasses, corn sugars, and paraffin. Stable

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isotope ratio analysis (SIRA) of  $^{13}\text{C}/^{12}\text{C}$  ratios has been useful for detecting some modern and sophisticated adulterations, where classical chemical analyses are unrevealing. Applications of SIRA to food analysis have been reviewed (Winkler and Schmidt, 1980; Krueger, 1984) and include the detection of cane or corn syrups illegally added to orange juice (Nissenbaum et al., 1974; Doner and Bills, 1982).

The basis of  $^{13}\text{C}/^{12}\text{C}$  ratios for adulteration detection lies with observations that while these ratios in nature are  $\sim 1/90$ , they vary slightly, depending on the source of the carbon analyzed. These variations can be accurately determined by SIRA, and among plants and derived compounds, these ratios depend mainly on the photosynthetic pathway. For example,  $\text{C}_4$  plants, such as corn, sugar cane, and sorghum, fix carbon dioxide to phosphoenolpyruvate (PEP) by using PEP carboxylase.  $\text{C}_3$  plants, such as lemon, orange, and apple trees, fix carbon dioxide to ribulose-1,5-bisphosphate (RuBP) by using RuBP carboxylase. A pronounced kinetic isotope effect is associated with RuBP carboxylase, so  $\text{C}_3$  plant carbon is depleted in  $^{13}\text{C}$  relative to carbon from  $\text{C}_4$  plants, and organic compounds from  $\text{C}_3$  and  $\text{C}_4$  plants can easily be distinguished.

This report examines the applicability of SIRA for detecting undeclared addition of citric acid to lemon juice. Carbon isotope ratios were determined for citric acid isolated from lemons, and from commercial citric acid samples, to gain information regarding fermentation carbon sources used for its production.

#### EXPERIMENTAL SECTION

Lemons, samples of bottled single-strength lemon juices, and frozen concentrated lemonade (sugar added) were purchased from local supermarkets. Juice was expressed from the lemons and then centrifuged at 8000g for 10 min, filtered, and lyophilized to thick syrups. Samples of commercial citric acid were kindly provided by Miles Laboratories, Incorporated (Elkhart, IN), and Pfizer Chemical Division (Groton, CT), the two United States suppliers of bulk citric acid. High fructose corn syrup (HFCS) was obtained from Clinton Corn Processing Company, Cedar Rapids, IA. A lemon juice adulterant was prepared by stirring 6.0 g of anhydrous citric acid and 4.3 g of HFCS (70% solids) into 100 mL of water. Mixtures adulterated to levels of 50 and 75% were prepared by mixing appropriate quantities of the adulterant with lemon juice. Citric acid was isolated from lemon juice and adulterated mixtures by precipitation of its calcium salt. Thus, to juice samples ( $\sim \text{pH } 2.25$ ) solid calcium hydroxide was gradually added with stirring until the pH reached 8.5. Upon heating at 60 °C, calcium citrate precipitated and was isolated by filtration; it was washed with water and ethanol and then dried in a vacuum oven (60 °C, 2 h). TLC analysis established that the precipitate is mainly citric acid and is totally free from contamination with lemon juice sugars. The filtrates, free of calcium citrate, were lyophilized to syrups consisting mainly of the sugars fructose and glucose.

$^{13}\text{C}/^{12}\text{C}$  ratios were determined by Coastal Science Laboratories, Incorporated (Austin, TX) with a Micromass 625D mass spectrometer. After direct combustion of sample to  $\text{CO}_2$ ,  $^{13}\text{C}/^{12}\text{C}$  ratios were determined by comparison of  $^{13}\text{C}^{16}\text{O}_2$  and  $^{12}\text{C}^{16}\text{O}_2$  ion beams and were expressed as  $\delta^{13}\text{C}$  values (units of ‰), defined as parts per thousand differences in the  $^{13}\text{C}/^{12}\text{C}$  ratio of a sample from that in standard PeeDee Belemnite (PDB), and are calculated by using the equation

$$\delta^{13}\text{C} (\text{‰}) = [({}^{13}\text{C}/{}^{12}\text{C})_{\text{sample}} / ({}^{13}\text{C}/{}^{12}\text{C})_{\text{standard}} - 1]10^3$$

Reproducibility for  $\delta^{13}\text{C}$  determinations is 0.2‰ or better.

**Table I.  $\delta^{13}\text{C}$  Values for Citric Acid from Lemons, Commercial Lemon Juices, Commercial Citric Acid Samples, and Citric Acid and Sugar-Enriched Fractions from Adulterated<sup>a</sup> Lemon Juice Samples**

sample	source	$\delta^{13}\text{C}$ , ‰
citric acid	lemons	-23.6, -23.7, -24.2
citric acid	single-strength lemon juice	-24.1, -25.1
citric acid	corn sugar fermentation	-9.7, -10.1
citric acid	paraffin fermentation	-27.2
citric acid	presumably beet sugar fermentation	-25.2, -25.2
citric acid	adulterant-lemon juice, 1:1	-17.6
sugars	adulterant-lemon juice, 1:1	-15.7
citric acid	adulterant-lemon juice, 1:3	-20.3
sugars	adulterant-lemon juice, 1:3	-20.0

<sup>a</sup> See Experimental Section for Details on preparation of adulterated mixtures; they were prepared by mixing pure lemon juice (containing citric acid of  $\delta^{13}\text{C} = -24.2\text{‰}$ ) with citric acid of  $\delta^{13}\text{C} = -9.7\text{‰}$  (from corn sugar fermentation), and HFCS with  $\delta^{13}\text{C} = -9.8\text{‰}$ .

#### RESULTS AND DISCUSSION

Citric acid isolated as calcium salts from three lemons gave  $\delta^{13}\text{C}$  values in the range expected for  $\text{C}_3$  plant derived compounds, -23.6, -23.7, and -24.2‰. A larger sampling of lemons would be expected to give a range of values only slightly greater than this, perhaps similar to orange juice (42 samples), which gave a range in  $\delta^{13}\text{C}$  values from -23.4 to -25.6‰ (Doner and Bills, 1982). Commercial citric acid samples (2) produced by fermentation of corn syrups gave values of -9.7 and -10.1‰, the range expected from products of  $\text{C}_4$  plant photosynthesis. Similar values would be obtained for citric acid produced by fermentation of cane ( $\text{C}_4$  plant) molasses. If cane and corn were the only feedstocks for fermentative production of citric acid, SIRA could readily detect it when added to lemon juice, since intermediate  $\delta^{13}\text{C}$  values would result. But other materials, including beet molasses and paraffin, are also used. Relative costs of the various feedstocks often determine which is used at a given time; information regarding relative proportions of each used for citric acid production is proprietary.

The results of SIRA analysis for  $\delta^{13}\text{C}$  are presented in Table I. While it is possible to classify the citric acid samples according to  $\text{C}_3$ ,  $\text{C}_4$ , or paraffin source on the basis of  $\delta^{13}\text{C}$  values, it is apparent that only  $\text{C}_4$  sources will be useful markers for detection of adulteration. Recent fermentation technology enables citric acid production from hydrocarbons (*n*-alkanes). This citric acid would be expected to give  $\delta^{13}\text{C}$  values in the range of about -20 to -30‰, since it is known that petroleum possesses values from -21.5 to -33.5‰ (Silverman, 1967). The citric acid sample of petroleum origin gave a value of -27.2‰. A high level of adulteration with such citric acid would be required to allow its detection, in which case a  $\delta^{13}\text{C}$  value somewhat more negative than the -24.1‰ mean value for the pure lemon juice citric acids (5 samples) would be obtained. Commercial citric acid samples (2), possessing values of -25.2‰, are probably prepared by beet ( $\text{C}_3$  plant) molasses fermentation and so cannot be detected.

Filtrates after removal of citric acid from juices were also analyzed by SIRA. These filtrates consist mainly of the sugars fructose and glucose and their  $\delta^{13}\text{C}$  values are close to those of the citric acid from the same juice, as would be expected. The filtrate from a purchased sample of lemonade concentrate (added sugar declared on label) had a value of -10.3‰, so HFCS had probably been added. The citric acid from this sample had a  $\delta^{13}\text{C}$  of -24.8‰. Since adulteration of lemon juice would probably involve the addition of both sugars and citric acid so that proper

brix-acid ratio be maintained, it is suggested that both be tested for  $\delta^{13}\text{C}$ . For example, in Table I are included results of SIRA for citric acid and sugars in lemon juice adulterated to different levels with corn sugar fermentation-derived citric acid and HFCS. The higher the degree of adulteration, the less negative are the  $\delta^{13}\text{C}$  values.

$\delta^{13}\text{C}$  values of citric acid from lemon juices may be especially useful for imported samples. Citric acid from an Argentinean sample of allegedly pure lemon juice gave a value of  $-17.7\%$ . This suggests adulteration with citric acid from a  $\text{C}_4$  fermentation source.

In summary, SIRA should be included in the series of analytical strategies aimed at detecting lemon juice adulteration.  $\text{C}_4$  plant derived citric acid can be detected, and in some cases, possibly so can paraffin derived citric acid. Sugars from corn or cane syrups also can be detected on the basis of  $\delta^{13}\text{C}$  values of this fraction.

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**Registry No.** Citric acid, 77-92-9; carbon-12, 7440-44-0; carbon-13, 14762-74-4.

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## Volatile Components of Corn Roots: Possible Insect Attractants

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The volatiles of corn roots have been studied by using Tenax adsorbent trapping followed by capillary GLC-MS analysis. Sesquiterpene hydrocarbons were the principal components of the low concentration (ca. 10 ppb) of volatiles found. The major sesquiterpene hydrocarbon could not be identified. Others that were identified included  $\beta$ -caryophyllene, longifolene, bazzanene (tentative), cyclosativene, and  $\alpha$ -ylangene.

#### INTRODUCTION

Corn roots are attacked by a number of insects such as the corn root worm (*Diabrotica* species). It seems possible that these insects locate the corn roots by orienting toward some characteristic volatile odor compound associated with the roots. The authors had previously identified volatile compounds associated with the corn leaves (Buttery and Ling, 1984) with some preliminary observations on the corn roots. The present study was carried out to give a more complete identification of corn root volatiles.

#### EXPERIMENTAL SECTION

**Materials.** Corn roots were obtained from corn plants grown in two different areas during the summer of 1983 and 1984. These were Stylepak and Jubilee varieties grown on an experimental field at Berkeley and Bonanza variety grown on an experimental field at Davis, CA. The plants were pulled out of the ground and the roots cut from the stalk with a sharp knife. Roots from relatively young plants (ca. 60 cm high) and mature plants (ca. 2 m high) were used for separate studies.

**Isolation of Volatiles.** The method used was essentially the same as that described previously by the authors for corn leaves (Buttery and Ling, 1984). The roots (800 g) were cut into pieces (ca.  $4 \times 4 \times 6$  cm) and placed in a 12-L flask within about 2 h of harvesting. Most of the

soil on the roots was dislodged in the process of cutting but was not washed off because of the possibility of removing volatiles. The Tenax trap consisted of a Pyrex tube packed with 10 g of Tenax (14 cm long  $\times$  2.2 cm diameter). Air drawn from outside the laboratory (purified passing through activated charcoal) was led into the flask through a Teflon tube and passed over the roots and out through the Tenax trap. The flow of air was 1 L per min and was continued for 24 h. The trapped volatiles were eluted from the trap with freshly distilled diethyl ether containing a trace (less than 0.001%) of Ethyl antioxidant 330. The extracts from two collections were combined and then concentrated to a small volume (5  $\mu\text{L}$ ) by using a warm water bath and low hold up fractional distillation columns.

**Capillary Gas Liquid Chromatography-Mass Spectrometry Analysis (GLC-MS).** The capillary GLC column used for the main part of the work was a 150 m long by 0.66 mm i.d. Pyrex capillary wall coated with Silicone OV-3. For some of the work a Pyrex capillary of the same dimensions but wall coated with Carbowax 20-M was used. The silicone capillary was temperature programmed from 20 to 170  $^{\circ}\text{C}$  at 1  $^{\circ}\text{C}$  per min and held at 170  $^{\circ}\text{C}$  for 2 more h. The carbowax capillary was held at 60  $^{\circ}\text{C}$  for the first 40 min and then programmed to 170  $^{\circ}\text{C}$  at 1  $^{\circ}\text{C}$  per min and held at 170  $^{\circ}\text{C}$  for 2 h. The column inlet pressure was 15 p.s.i. He. The column was coupled to the mass spectrometer with a Llewellyn-Littlejohn type single stage silicone rubber membrane molecular separator. The mass spectrometer was a modified Consolidated 21-620 cycloidal type instrument with 70 eV ionization volt-

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