Carbon Isotopes in Vanillin and the Detection of Falsified "Natural" Vanillin

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A method has been developed for quantitative removal of the methyl group carbon from vanillin and determination of its isotopic composition. The method is of value for the detection of synthetic vanillins whose isotopic compositions have been adjusted by the addition of [*methyl*-^{13}C]vanillin to resemble natural vanillins.

Vanilla is an important flavor, production of which constitutes a multimillion dollar a year industry. Natural vanilla is extracted from vanilla beans grown primarily in Madagascar, with lesser production from the Comoro Islands, Reunion, Mexico, Java, and Tahiti. Because natural vanilla extracts command a high price, the fraudulent adulteration of vanilla with inexpensive synthetic vanillin, derived from wood pulp lignin, is a serious problem in the commercial market for this product. Considerable effort has been directed toward the development of reliable methods for the detection of such adulteration. Among these is the stable isotope ratio analysis (SIRA) method.

SIRA has found widespread application in food science in recent years (Krueger and Reesman, 1982) and in particular in the analysis of vanilla. Bricout et al. (1974) and Bricout and Koziet (1975) showed that vanillin from vanilla (a crassulacean acid metabolism plant) is slightly enriched in ^{13}C relative to vanillin from wood pulp lignin (derived from Calvin cycle plants). This observation was subsequently made the basis for a test of vanilla authenticity (Hoffman and Salb, 1979). The test established limits for the $^{13}C/^{12}C$ ratios that could be expected from natural vanillins; $^{13}C/^{12}C$ ratios falling outside these limits are considered evidence of fraudulent adulteration.

There has since developed concern that the SIRA test may be circumvented by the addition of highly enriched ^{13}C vanillin to lignin vanillin, thus simulating the SIRA result obtained from natural vanilla. Bricout et al. (1981) published a method to detect simulated natural vanillin, prepared by the addition of methyl-^{13}C-labeled vanillin (the most readily available ^{13}C-labeled vanillin) to lignin vanillin. This method suffers from several drawbacks: it requires relatively large samples of pure vanillin (100 mg), it involves the use of sensitive reagents and tedious sample workups, and the method is incapable of detecting ^{13}C-enriched vanillins other than methyl group enriched vanillin.

Galimov et al. (1975), in a SIRA study of lignin, published a method for the SIRA of methoxyl groups of various aromatic aldehydes, including vanillin. It utilized the Zeisel reaction, reacting a methyl aryl ether with concentrated hydriodic acid to yield methyl iodide, which is combusted to CO_2 for SIRA. It was felt that this might form the basis of a method to detect the substitution of ^{13}C-enriched lignin vanillins for natural vanilla.

A method is presented below for the detection of simulated natural vanillin, a method which overcomes the drawbacks of the Bricout approach.

EXPERIMENTAL SECTION

Vanillins. Natural vanillin was prepared from 20-fold Madagascar vanilla extract. The vanilla was extracted with ether and concentrated, and the concentrate was taken up in methylene chloride. The methylene chloride fraction was dried over Na_2SO_4 and concentrated, and the vanillin was purified by silica gel chromatography using methylene chloride containing 0.5% ethanol. The vanillin fractions were combined, concentrated, and recrystallized from water.

Lignin vanillin was a sample of commercially available vanillin derived from wood pulp lignin.

Simulated natural vanillin was prepared by diluting a small amount of pure [*methyl*-^{13}C]vanillin with a sufficient amount of the above lignin vanillin to obtain a total carbon SIRA value in the range of natural vanilla. The sample was then recrystallized to assure homogeneity.

Vanilla Extracts. Single-fold vanilla extracts of known natural origin were obtained from McCormick and Co., including thirteen from Madagascar, two from Comoro, four from Java, one from Tahiti, and three blends (Madagascar/Java).

Preparation of Pure Vanillin. Vanillin was purified from vanilla extract by a modification of the procedure of Hoffman and Salb (1979). A 100-mL sample of single-strength vanilla extract was distilled to remove ethanol, and sufficient water was added to replace the lost volume. The solution was then extracted twice with 50-mL portions of ether. Centrifugation was often necessary to break the emulsion formed. The organic layers were combined and dried over anhydrous $MgSO_4$. The ether solution was then evaporated to a gum and taken up in about 0.5 mL of ether. Vanillin was isolated by preparatory gas chromatography on a Varian Model 3700 gas chromatograph equipped with a thermal conductivity detector. The injection port temperature was 250 °C and the detector temperature was 250 °C. The column was a 6 ft × $^1/_4$ in. o.d. stainless steel column packed with 5% Carbowax 20 M on Chromasorb WAW. The carrier gas was helium at 40 psi at the head of the column. The column oven temperature was programmed from 70 to 230 °C at 16 °C/min. Samples were collected in Pyrex capillaries cooled with dry ice at the outlet port of the thermal conductivity detector. Typically, four to five 100-μL injections were required to obtain sufficient sample (10–20 mg) for demethylation.

Demethylation of Vanillin. The demethylation of vanillin, and the subsequent collection of the methyl group as methyl iodide, was a modification of the procedure of Galimov et al. (1975). Demethylation was carried out in the apparatus shown in Figure 1. Approximately 10–20 mg of purified vanillin and 3 mL of concentrated hydriodic acid were added to the reaction flask, and the nitrogen flow was adjusted to 15 mL/min. The collection trap was then cooled to −130 °C with pentane slush, and the oil bath temperature was raised to 150 °C. The reaction was run for 30 min, the methyl iodide collecting in the cold trap. (The trap was a length of 10 mm o.d. Pyrex tubing, sealed at one end, and containing 1–2 g of copper oxide (CuO).

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0021-8561/83/1431-1265$01.50/0

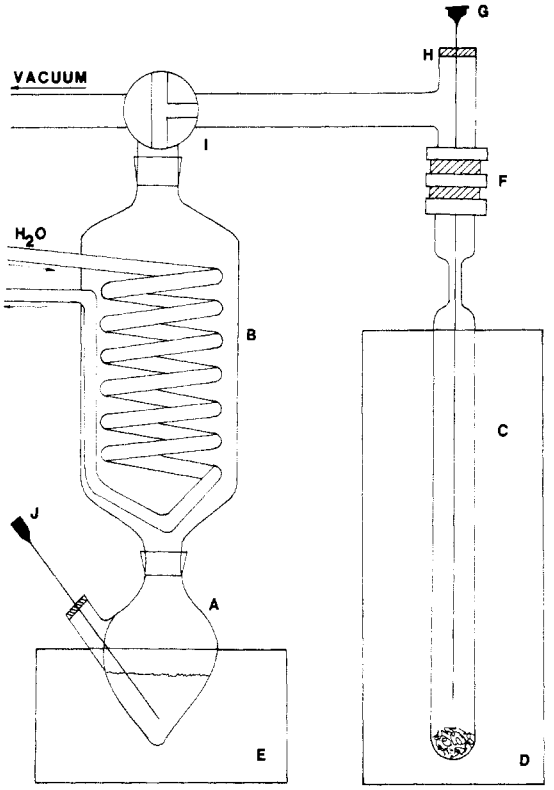

Figure 1. Demethylation apparatus: (A) 10-mL two-neck reaction flask; (B) reflux condenser; (C) methyl iodide collection trap; (D) −130 °C bath (pentane ice); (E) 150 °C oil bath; (F) O-ring tubing coupler; (G) 12-in. syringe needle (N_2 vent); (H) rubber septum; (I) three-way stopcock; (J) Pasteur pipet (N_2 inlet).

Before use, it was heated in air at 500 °C overnight to remove any traces of carbon and constricted near the top to facilitate flame sealing.) When the reaction was completed, the 12-in. syringe needle vent was removed, the pentane slush bath was replaced with liquid nitrogen, and the trap was evacuated and flame sealed.

SIRA of Methyl Iodide. The methyl iodide was combusted by the method of Sofer (1980). The sealed tube containing methyl iodide and CuO was heated to 500 °C overnight to convert the methyl iodide to CO_2. The tube was opened under vacuum, and the CO_2 was purified by passing the gas through a pentane slush trap (−130 °C), followed by repeated sublimation. Yield measurements were made by condensing the CO_2 into a manometer and measuring the mercury displacement. The manometer was calibrated by decomposing known amounts of $CaCO_3$ with HCl and measuring the mercury displacement caused by the CO_2 generated.

SIRA measurements of CO_2 were performed in the standard manner using a Micromass 602D mass spectrometer (Krueger and Reesman, 1982). Results are reported in parts per thousand (‰) difference in the $^{13}C/^{12}C$ ratio from the PeeDee Belemnite standard (PDB), calculated by using the equation

$$\delta\ ^{13}C_{PDB}\ (‰) = \left[\frac{(^{13}C/^{12}C)_{sample}}{(^{13}C/^{12}C)_{PDB\ std}} - 1 \right] \times 1000 \quad (1)$$

SIRA of Whole Vanillin. The total carbon SIRA measurements on vanillin were performed by the method of Hoffman and Salb (1979).

RESULTS AND DISCUSSION

Bricout et al. (1981) reasoned that the addition of methyl-^{13}C-labeled vanillin to lignin vanillin would concentrate the added ^{13}C in the methyl group of simulated natural vanillin. If the $^{13}C/^{12}C$ ratio of all eight carbon atoms of lignin vanillin were the same, or very similar, then removal of the methyl group would yield a residual molecule with a SIRA result similar to that of lignin vanillin. They reacted lignin vanillin and methyl-^{13}C-enriched lignin vanillin with boron tribromide–dimethyl sulfide complex, obtaining the demethylated product 3,4-dihydroxybenzaldehyde (Figure 2). SIRA of the two products obtained gave similar results (~−27‰), values typical of lignin vanillin, whereas the 3,4-dihydroxybenzaldehyde derived from natural vanillin gave SIRA results typical of natural vanillin (~−20‰).

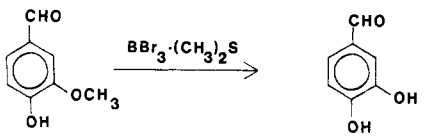

Figure 2.

Figure 3.

Table I. Carbon SIRA on Natural, Synthetic, and Simulated Natural Vanillins

sample type	$\delta\ ^{13}C_{PDB}$ (total carbon)	$\delta\ ^{13}C_{PDB}$ (methyl carbon)	methyl carbon minus total carbon
vanilla vanillin (Madagascar)	−21.4	−24.2	
		−23.3	
		−24.2	
		−24.3	
		mean: −24.0	−2.6
lignin vanillin (synthetic)	−27.3	−28.8	
		−28.5	
		−28.3	
		−28.2	
		mean: −28.4	−1.1
simulated natural vanillin (methyl-^{13}C-enriched lignin vanillin)	−20.6	+26.4	
		+25.9	
		+25.2	
		+25.5	
		mean: +25.8	+46.4

Following similar logic, it was reasoned that if the added ^{13}C was concentrated in the methyl group, then SIRA of the methyl carbon, rather than the rest of the molecule, would be a very sensitive test for simulated natural vanillin of this type. The methyl group of vanillin could be removed as methyl iodide by reaction with concentrated hydriodic acid (Figure 3). Since the average SIRA value of eight carbon atoms would be increased from −27‰ to −20‰ by ^{13}C addition in the methyl group, the SIRA value of the methyl group itself should be increased about 50‰ from its initial value. This is an enormous change and should be easily detected. Further, the value should be well into the positive range (+20 to +30‰), which is outside the natural range for plant-derived carbon (Krueger & Reesman, 1982), thus providing definitive evidence of falsification.

The results obtained from SIRA of three vanillins are shown in Table I. The first sample is a vanillin from natural vanilla extract. The total carbon SIRA value of −21.4‰ is typical of natural vanilla. The methyl carbon SIRA value, four analyses averaging −24.0‰, is somewhat

Table II. Carbon SIRA on Various Natural Vanillins

extract source	δ $^{13}C_{PDB}$ (total carbon)	δ $^{13}C_{PDB}$ (methyl carbon)
Madagascar	−21.4	−24.3
	−20.3	−25.7
	−20.6	−24.5
	−21.5	−24.6
	−21.3	−26.1
	−21.2	−23.3
	−21.1	−25.7
	−21.3	−26.0
	−20.5	−25.9
	−21.6	−24.7
	−20.8	−24.4
	−21.2	−24.9
	−21.1	−24.6
mean:	−21.1	−25.0
Comoro Islands	−19.9	−23.6
	−20.2	−27.4
mean:	−20.1	−25.5
Java	−19.3	−25.2
	−20.7	−25.1
	−19.6	−25.7
	−19.5	−25.1
mean:	−19.8	−25.3
blends (Madagascar/ Java)	−21.3	−24.9
	−21.0	−24.4
	−21.1	−24.7
mean:	−21.1	−24.7
Tahiti	−18.5	−11.1

more negative than the total carbon SIRA. This may represent a small natural fractionation of isotopes between the methyl group carbon and the other carbon in the molecule. The second sample is a lignin vanillin. The total carbon SIRA value of −27.3‰ is typical of lignin vanillins. The methyl carbon SIRA value of −28.2‰ is again slightly more negative than the total carbon SIRA, which is consistent with the observation of Galimov et al. (1975) regarding lignin vanillin. The third sample is the same lignin vanillin that has been adjusted by the addition of a sufficient amount of methyl-^{13}C-labeled vanillin to simulate the total carbon SIRA value of a natural vanillin. The total carbon SIRA value of −20.6‰ is almost identical with the average result for natural Madagascar vanillins reported by Hoffman and Salb (1979). The methyl carbon SIRA result, however, was +25.8‰. This was 49.8‰ more positive than the natural vanilla result. The expected methyl carbon SIRA value of the simulated natural vanillin can be calculated from the total carbon SIRA results and the lignin methyl carbon SIRA results:

$$\delta\ ^{13}C(\text{simulated natural methyl}) = 8[\delta\ ^{13}C(\text{simulated natural total}) - \delta\ ^{13}C(\text{lignin total})] + \delta\ ^{13}C(\text{lignin methyl}) \quad (2)$$

$$\delta\ ^{13}C(\text{simulated natural methyl}) = +25.2‰ \quad (3)$$

This calculated result is within experimental error of the average measured value of +25.8‰.

Yields of the demethylation reaction, based on the amount of vanillin reacted, were generally 95–100%. The analytical precision of methyl carbon SIRA on vanillin, as judged from replicate analyses in Table I, is approximately ±0.5‰ (standard deviation).

The SIRA results for vanillin from 23 natural vanillin extracts are shown in Table II. The total carbon SIRA values are typical of results on similar samples previously reported by Hoffman and Salb (1979). Madagascar vanillins are somewhat more negative than those from Java, while Tahitian vanillin is the most positive. The average values for Madagascar, Java, and Tahiti vanillin are somewhat more negative than the averages reported by Hoffman and Salb in 1979. A similar shift toward more negative total carbon SIRA in vanillins was noted by Guarino (1982).

These variations may be due to climatic or environmental influences. Vanilla is a crassulacean acid metabolism (CAM) plant and utilizes both Calvin (C_3) and Hatch-Slack (C_4) photosynthetic pathways. It has been experimentally demonstrated that light intensity, humidity, CO_2 content of air, and other environmental factors will influence the degree to which a CAM plant will favor one photosynthetic pathway over the other. This variability will be reflected in the isotopic composition of the plant tissues (Crews et al., 1976).

The methyl carbon SIRA results on natural vanillins have a narrow distribution, averaging −25.0‰ with a standard deviation of ±0.9‰ (excepting the Tahitian sample discussed below). The isotopic fractionation between methyl carbon and total carbon in natural vanilla is similar to that seen by Galimov et al. (1975) in lignin-derived vanillin. The methyl carbon is 3–5‰ more negative in all samples except the Tahitian sample. The geographical groupings seen in the total carbon SIRA by Hoffman and Salb (1979), and confirmed by our analyses, are not reflected in the methyl carbon SIRA analyses.

The single Tahitian sample gave a very different methyl carbon SIRA value of −11.1‰. Tahitian vanilla is a different species (*Vanilla tahitensis* Moore) from other vanillas (*Vanilla planifolia* Andrews) and thus may have different metabolic characteristics. The high methyl carbon SIRA value measured for this Tahitian sample may account for the relatively high total carbon SIRA values observed for Tahitian vanillas.

It is of interest to note that the methyl carbon in the Tahitian sample has the isotopic composition expected from a Hatch-Slack (C_4) pathway (approximately −10‰), while all the other vanillins have the isotopic composition expected from a Calvin (C_3) pathway (approximately −25‰) (Smith and Epstein, 1971). Since both pathways are operative in CAM plants, it is possible that vanillin in *V. tahitensis* derives its methyl carbon from a C_4 pathway while vanillin from *V. planifolia* derives its methyl group from a C_3 pathway.

Since the methyl carbon SIRA values of natural vanillin form a narrow distribution and the methyl carbon SIRA values of simulated natural vanillin are approximately 50‰ greater than the natural vanillin range, the reported technique will reliably distinguish between them. In fact, although we have not examined mixtures of natural and simulated natural vanilla, the precision and sensitivity of the method are high enough that the addition of as little as 10% methyl-^{13}C-enriched lignin vanillin to natural vanillin should be readily detectable.

It may also be possible to use methyl carbon SIRA to detect synthetic vanillins enriched with ^{13}C in the ring or aldehyde positions. If synthetic vanillins were enriched in these positions to resemble natural vanillin, the methyl carbon SIRA value should still be typical of lignin vanillin (−28 to −30‰). Methods for the detection of aldehyde or ring-labeled vanillin are currently being examined.

ACKNOWLEDGMENT

We thank Dr. Patrick G. Hoffman and Dr. Robert Esmond of McCormick and Co., Inc., who generously provided the vanilla samples used in this work, and Stephen A. Morin, who performed the isotopic analyses.

Registry No. Vanillin, 121-33-5; [*methyl*-^{13}C]vanillin, 86884-84-6; ^{13}C, 14762-74-4; lignin, 9005-53-2.

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Received for review February 14, 1983. Accepted July 11, 1983.

Pyrolysis of Tropical Vegetable Oils

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Pyrolysis products of babassu (*Orbignya martiana*), piqui (*Caryocar coriaceum*), and palm oils (*Elaeis guineensis*) were analyzed by GC/MS using library search programs. The chief products of pyrolysis were straight-chain alkanes and 1-alkenes. Small amounts of cyclic hydrocarbons were detected in triglycerides constituted by oleic acid as the major moiety. Pyrolysis of oleic acid was also studied. A pathway for the cracking reactions involved with the decomposition of the saturated fatty acids is proposed.

Edible oils are essential for the human diet and therefore their use for other purposes has thus far not recieved much attention. However, during and after World War II several studies on the pyrolysis of vegetable oils were carried out for using the products thus obtained as fuel (Otto, 1945; Chang and Wan, 1947; Hsu et al., 1950). The studies concerning the chemical composition of the volatile compounds produced were not complete due to instrumental limitation and the analytical methods thus used (Hsu et al., 1950). Reports on the thermal cracking of behenic acid by Jurg and Eisma (1964) showed that C_3–C_6 hydrocarbons were chiefly produced when betonite was used as the catalyst. However, Traitler and Kratzl (1980) obtained *n*-alkylbenzenes from pyrolysis of fatty acids using lignin as the catalyst. Craveiro et al. (1981a,b) obtained long-chain methyl ketones when babassu oil was pyrolyzed in the presence of CaO.

On the basis of these studies it is clear that the pyrolysis products from fatty acids or glycerides are highly dependent on the nature and amount of catalyst used. For this reason we decided to investigate cracking reactions, carried out in the absence of catalyst, of three vegetable oils extracted from native or acclimated plants largely dispersed in northern and northeastern Brazil: *Orbygnya martiana* (babassu), *Caryocar coriaceum* (piqui), and *Elaeis guineensis* (palm).

EXPERIMENTAL SECTION

Samples. Crude oil samples of babassu and piqui oils were obtained from industries in the state of Ceará, and the palm oil was a commercial sample originated from Bahia State.

Pyrolysis. Cracking reactions were carried out at 300–500 °C (atomospheric pressure) in a glass apparatus with internal and external temperatures being measured by a thermometer and pyrometer. The percent yields of volatiles so obtained were in the range 60–80 (v/v). Hydrogenation reactions were carried out on a Parr hydrogenation apparatus at 60–70 psi by using Pt as the catalyst over a period of 3 h. CO_2 evolution was detected as $CaCO_3$.

Table I. Fatty Acid Composition of Babassu, Palm, and Piqui Oils

	yields, %		
fatty acid	babassu[a]	piqui[b]	palm[c]
capric	5.0		
lauric	48.5		2.8
myristic	20.0		1.5
palmitic	11.0	48.0	45.6
oleic	10.0	49.0	34.4
stearic	3.5	2.7	8.8
linoleic			5.5

[a] *O. martiana*; 72% of oil in the seed (Ruskin, 1975). [b] *C. coriaceum*; 47.4% (yield determined as w/w) in the mesocarp. [c] *E. guianensis*; 30–70% of oil in the fruits (Heiduschaka and Agsten, 1930).

Gas Chromatography and Gas Chromatography/Mass Spectrometry. Analytical chromatograms were obtained on a FID Varian instrument equipped with fused silica capillary column (30 m × 0.20 mm i.d.) by using methyl silicone as the stationary phase, N_2 as the carrier gas (1 mL/min) at 40–250 °C, and 4 °C/min programmed temperature conditions. The separation and analysis of the cracked samples were made on a HP 5995 mass spectrometer coupled to GC equipped with a glass open tubular column (SP 2100, 30 m × 0.25 mm i.d.) using He as the carrier gas (1 mL/min) and the temperature programmed as above. Chromatographic retention times and peak areas were used to calculate Kovat's indexes and compound percentages, respectively.

Compound Identifications. Compound identifications involved library search programs (Craveiro et al., 1981a,b) and visual comparison with published mass spectra (Stenhagen et al., 1974; Heller and Milne, 1978). Com-

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0021-8561/83/1431-1268$01.50/0