Identification of Isotopically Manipulated Cinnamic Aldehyde and Benzaldehyde

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Cinnamic aldehyde and benzaldehyde samples were isolated from botanical sources and compared to labeled isolates from natural origins and those synthetically produced. Products synthesized from petrochemical precursors yielded $\delta^{13}C$ and δD values uniquely different from those of botanical derivation. Upon further comparison with the radiocarbon (^{14}C) activities it was possible to define average $\delta^{13}C$ and δD isotopic values for the naturally derived cinnamic aldehyde (−27.6 ± 0.6 and −116 ± 8, respectively) and benzaldehyde samples (−28.6 ± 0.5 and −105 ± 5, respectively) and the synthetically derived cinnamic aldehyde (−25.4 ± 0.3 and 517 ± 52, respectively, via toluene oxidation) and benzaldehyde samples (−29.2 ± 0.8 and −54 ± 11, respectively, via benzal chloride and −26.1 ± 0.6 and 576 ± 73, respectively, via toluene oxidation). It is also revealed by comparison of isotopic values for certain synthetically derived compounds that ^{14}C manipulation of simulated natural products has occurred.

The essential oils of bitter almond and cassia are important flavors used in the food and beverage industry. Bitter almond oil, chemically similar to benzaldehyde, is the flavor most conspicuous in cherry and almond flavorings. Cassia oil, chemically similar to cinnamic aldehyde, is the principal flavor of cinnamon. Both of these essential oils are or have been produced in quantities of hundreds of tons per year to meet a growing consumer demand. To cope with this demand, many manufacturers have looked to alternate sources for production of these essential oils rather to replace the expensive and time-consuming extractive procedures typically required for botanical extracts. One such source is the petrochemically derived compounds better known as synthetics. Compounds such as cinnamic aldehyde and benzaldehyde can be synthesized inexpensively from fossil fuels and in sufficient purity to be chemically indistinguishable from their botanically grown counterparts. This leads to the potential substitution of the synthetic compounds for their naturally derived equivalent.

The current trend toward natural foods and beverages along with less expensive synthetic routes of manufacturer has increased the potential for economic adulteration of natural flavors with those of synthetic origins. To ensure proper labeling and deter the fraudulent use of synthetic compounds, analytical methods incorporating both stable isotopes and radiogenic isotopes have been developed.

Our research indicates specific ranges for the stable isotope ratios of $^{13}C/^{12}C$ ($\delta^{13}C$) and deuterium/hydrogen (δD) for both cinnamic aldehyde and benzaldehyde from various sources such as those of synthetic origin and those derived from botanical products. On the basis of radiocarbon (^{14}C) activity of these sources and the specific ranges of the stable isotopes, evidence is revealed for the manipulation of the ^{14}C activity.

Radiocarbon. The measurement of ^{14}C activity provides a means to differentiate products derived from fossil fuels from those of modern botanical derivation. This analytical capability has been shown to be of use in determining the addition of petroleum-derived products to flavors and foods (Martin et al., 1984).

^{14}C is formed in the upper atmosphere by the interaction of cosmic radiation with stable nitrogen (Friedlander and Kennedy, 1962). After rapid equilibration of $^{14}CO_2$ and fixation by plants through photosynthetic processes an equivalent activity with respect to the atmospheric, ^{14}C activity is acquired. Because ^{14}C is an unstable radionuclide, the ^{14}C activity decreases on the basis of radioactive decay and a half-life of 5730 years. When a plant carries out photosynthesis, its ^{14}C activity is at steady state with the present atmospheric activity. However, upon its death, the ^{14}C activity decreases by decay alone with no further uptake of present levels of $^{14}CO_2$.

Fossil fuels originally derived from buried plants and animals are devoid of ^{14}C because of their extreme age; i.e., all ^{14}C has decayed away. Therefore, products derived or synthesized entirely from fossil fuels will likewise be devoid of ^{14}C activity.

In contrast to the undetectable amount of ^{14}C in fossil fuel derived products, levels of ^{14}C activity for modern plants have varied considerably over the past 30 years. Prior to 1950 the atmospheric ^{14}C activity was at a steady state of 13.56 disintegrations per minute per gram of carbon (dpm/g of C), which was defined as 100% modern activity. ^{14}C activity increased with the advent of atmospheric nuclear weapons testing and reached a peak of nearly 28 dpm/g of C in 1964 (Nydal and Lovseth, 1983). Following the Limited Test Ban Treaty, the world's ^{14}C activity has been steadily decreasing. Figure 1 portrays the measured and calculated ^{14}C activities for the past 10 years, indicating an average decrease of 0.28 dpm/g of C per year as derived from measurement of ^{14}C activity. The $^{14}CO_2$ decrease is due to the combination of uptake and exchange with the oceanic bicarbonate system, dilution by ^{14}C devoid fossil fuel CO_2, and uptake by the world's biomass.

Measured ^{14}C activities for known natural products can thus be compared to the activity for a certain year to determine the year of harvest. Conversely, the amount or percent of modern carbon of a flavor can be detected to as little as 5% provided the year of harvest or production is accurately known. This includes important flavors and extracts such as benzaldehyde, cinnamic alde-

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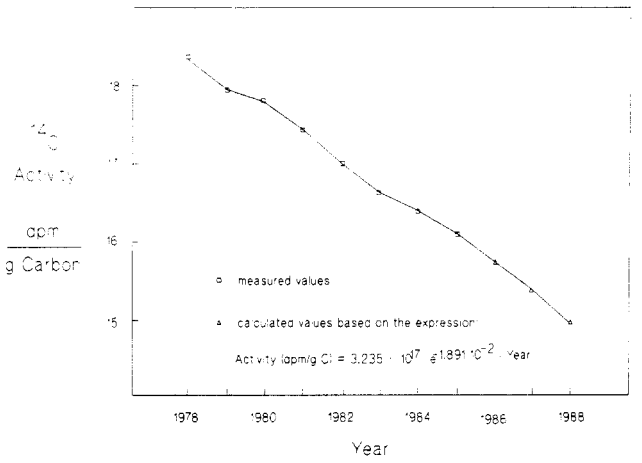

Figure 1. Natural ^{14}C activity 1978–1988.

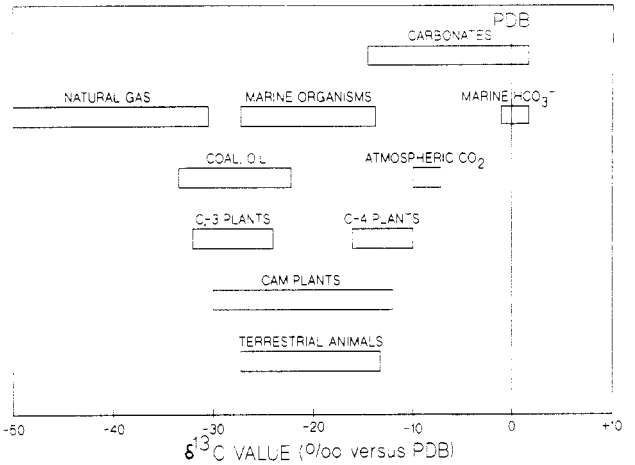

Figure 2. Isotope ranges of carbon pools [source: Winkler (1983)].

hyde, and ethyl butyrate (Krueger, 1987; Hoffman and Salb, 1980; Byrne et al., 1986) where measured ^{14}C activities have been used to determine additions of petroleum-derived components to those of natural origin.

The principal disadvantage of ^{14}C analysis is that one cannot distinguish the source of a modern product on the basis of this test alone. An example would be the inability to distinguish vanilla extracts from various sources. Vanillin derived from vanilla beans and that from lignin, a wood pulp derivative from which vanillin can be synthesized, both possess modern ^{14}C activity and are thus indistinguishable by ^{14}C analysis alone. Another disadvantage is the ability to spike or increase the ^{14}C activity of fossil fuel derived products to a level at or above that considered natural. Since the natural level of ^{14}C in biogenic materials is 1.3×10^{-10} (Allen, 1961), it is possible even at these low levels to simulate a natural ^{14}C activity by the addition of a ^{14}C-labeled compound.

Stable Isotopes. As the name implies, some isotopes of certain elements are stable and do not undergo radioactive decay as does ^{14}C. Isotopes of the same element, such as ^{12}C, ^{13}C, and ^{14}C, differ only in the number of neutrons in the nucleus, and with their respective increase in neutron number an increase in instability is realized as well.

A fundamental property of atoms is that the outer shells of electrons are responsible for chemical behavior, whereas the nucleus, composed of protons and neutrons, accounts for its physical properties. Since the isotopes of the same element differ in the number of neutrons in the nucleus, they are affected differently by the physical processes they undergo. These differences give rise to isotope effects and variations in the relative abundances of the stable isotopes of a specific element. The isotope effects and their specific fractionations between the different isotopes are the basis for authenticating processes and sources of flavors and extracts.

Terminology. Isotope abundances of the lighter elements are typically expressed in delta notation (δ) and written relative to the heavier mass isotope. The δ values are defined as the per mil (‰) or parts per thousand deviation of the sample isotopic ratio versus that of a standard and expressed by

$$‰ = [(R_{sample}/R_{standard}) - 1] \times 10^3$$

where $R = {}^{13}C/{}^{12}C$ or D/H ($^2H/^1H$).

The standard for carbon isotopes is PDB: the CO_2 gas obtained by reacting the Cretaceous belemnite, *Belemnitilla americana*, from the Peedee Formation of South Carolina with 100% phosphoric acid represents the zero point for the $\delta^{13}C$ scale. Since this standard no longer exists, a working standard is used and calibrated to available standards such as NBS-20, NBS-21, and NBS-22, whose isotopic ratios are known relative to PDB (Hoefs, 1973). The standard for hydrogen isotopes is SMOW (Standard Mean Ocean Water), which represents the zero point for the δD scale. Additional standards exist such as SLAP (Standard Light Antarctic Precipitation), which is used to establish a linear scale and normalize results (Gonfiantini, 1978).

The stable isotopes of carbon, ^{13}C and ^{12}C, exist in nature in the relative proportions of 1.11:98.89 (Faure, 1977). The slight difference in mass results in fractionations by physical and chemical reactions occurring in nature. The abundance of each isotope in plants is slightly different from the atmospheric CO_2 from which it is derived. Through photosynthesis, plants are selectively enriched in ^{12}C and thus depleted in ^{13}C, relative to atmospheric CO_2, but to different amounts depending on the photosynthetic pathway the plant utilizes. For the two possible pathways, Calvin and Hatch–Slack, or a combination of the two referred to as the Crassulecean acid metabolism (CAM), there exist definite and unique ranges for the isotopic ratios as a result of the biosynthesis. The Calvin and Hatch–Slack pathways, better known by C-3 and C-4, respectively, on the basis of their 3- or 4-carbon acid intermediates, are displayed among other major carbon pools and their respective isotopic ranges in Figure 2. The unique $\delta^{13}C$ ranges have been found to be most useful in the analysis and source derivation of many flavors and extracts (Hoffman & Salb, 1979; Lucchesi, 1979). We are primarily interested here in the Calvin or C-3 process. This process is common to flowering plants such as apples, grapes, peaches, plums, cherries, and citrus fruits, as well as sugar beets, rice, wheat, barley, oats, rye, potatoes, and cassia (Bricout, 1982; Hillaire-Marcel, 1986; Hoffman & Salb, 1980). Cinnamic aldehyde and benzaldehyde are examples of major constituents derived from Calvin-type plants. In the Calvin process the CO_2 is fixed by the carboxylation of ribulose 1,5-diphosphate to give 3-phosphoglycerate (Calvin & Bassham, 1962). The resulting isotopic abundance reveals a range for Calvin or C-3 plants of −24 to −32‰. This represents a depletion of ^{13}C between −18 and −25‰ from the atmospheric CO_2 ratio of −7‰ relative to PDB.

The stable isotopes of hydrogen, deuterium and hydrogen, exist in nature in the relative abundance of 0.015 and 99.9% (Faure, 1977). The minor variation in these abundances due to the physical and chemical processes have been found to be useful in the identification and verification of authenticity of flavors and foods. The δD

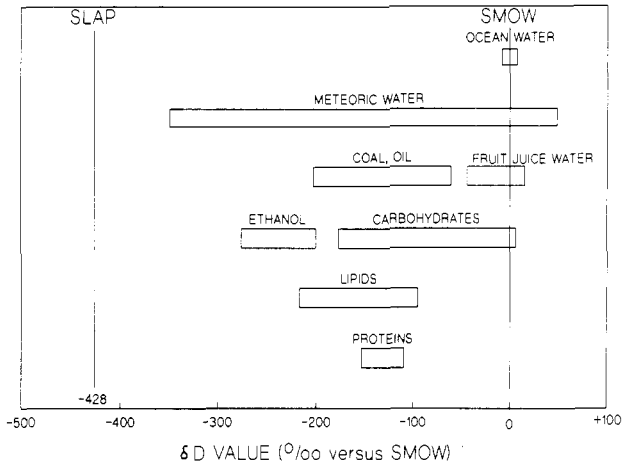

Figure 3. Isotope ranges of hydrogen pools [source: Winkler (1983)].

ratio has already been utilized in the characterization of ethanol in various wines (Bricout et al., 1975), in the identification of synthetic linalool, citral, and menthol (Bricout, 1982), in the detection of beet sugar additives to orange juice (Donor et al., 1987), and in the source assignment of bitter almond oil (Butzenlechner et al., 1989).

The hydrosphere represents the major hydrogen reservoir and exhibits a nearly constant isotopic ratio for δD. It is thus the source of the international standard SMOW to which measured ratios are compared (Craig, 1961a).

Water that evaporates from the oceans and precipitates through meteorological processes is referred to as meteoric water. This water is progressively depleted of the heavier isotope deuterium, with respect to the vapor from which it is derived, as the evaporation/condensation cycle proceeds. This is most evident geographically where water vapor movement to higher altitudes and latitudes with the associated temperature decrease depletes deuterium in both cloud water and rainwater (Craig, 1961b).

In the botanical domain, isotopic fractionations occur within the evapotranspiration cycle within the plant itself as water evaporates through the stomata. Also during CO_2 and H_2O assimilation isotopic fractionation occurs with subsequent depletion of deuterium. However, these are known to be of minor amounts and secondary importance, relative to the deuterium/hydrogen ratio of the source water and of the local environmental conditions (Yapp & Epstein, 1982).

The importance of all these factors is evident in Figure 3, illustrating the range of isotopic abundance for various hydrogen containing pools. Since δD variations occur depending on the photosynthetic pathways, biochemical subgroups, and various botanical types, the possibility of using δD to authenticate natural cinnamic aldehyde and benzaldehyde was investigated.

METHODOLOGY

Samples used in the following study were supplied by the Flavor and Extract Manufacturers' Association (FEMA)—Isotopic Studies Committee (ISC) and from the sample archives of the Center for Applied Isotope Studies (CAIS). Samples were either of botanical origin—cassia bark for cinnamic aldehyde and apricot and cherry pits for benzaldehyde—or they were previously manufactured or extracted compounds and labeled as natural or synthetic by the supplier.

Cinnamic aldehyde was isolated from pulverized cassia bark by first soaking approximately 0.5 kg of bark in 0.2 L of water at 50 °C for 24 h. Steam distillation was then carried out on the resulting slurry. The distillate was extracted three times with 50-mL aliquots of methylene chloride which, upon evaporation, yielded a pure enough quantity of cinnamic aldehyde for isotopic analyses.

Benzaldehyde was isolated from apricot and cherry kernels by first grinding the kernels to a fine powder. This powder was pressed to remove the fixed oil associated with the kernels. Approximately 0.5 kg of the dried pressed cake was then macerated in 2 L of 50 °C water for 24 h to hydrolyze the amygdalin glucoside. This suspension was steam distilled (CAUTION: HCN generated!) and the distillate extracted three times with 50-mL portions of methylene chloride. Upon evaporation of the solvent, benzaldehyde of sufficient purity and quantity was recovered for analysis. All botanical extracts, once isolated, were stored under nitrogen in Teflon and glass vials until subsequent isotopic analysis.

The ^{14}C isotopic analysis was carried out by combusting up to 6 g of cinnamic aldehyde or benzaldehyde in a 3.5-L combustion bomb purged and then sealed under 2000 kPa of pure oxygen. Once cooled, the CO_2 gas was transferred and collected in multiple traps at liquid nitrogen temperature.

Water is removed by an initial trap using a dry ice–propanol mixture. Oxygen is not retained at liquid nitrogen temperatures provided a vacuum is maintained less than 500 mmHg during the transfer.

The cryogenically pure CO_2 is converted to Li_2C_2 by reaction with molten lithium. After cooling, hydrolysis of the Li_2C_2 produces acetylene gas which can be cryogenically purified with liquid nitrogen. The resultant acetylene is then catalytically trimerized to benzene (Noakes et al., 1965). The benzene, with a ^{14}C content equivalent to the original carbonaceous material, is mixed with a scintillation cocktail composed of 2,5-diphenyloxazole and 2,2′-*p*-phenylenebis[4-methyl-5-phenyloxazole]. In our method a sealed standard is run before and after each sample to compensate for instrument drift. Background activity is measured by using a known synthetic material and counted each week. The counters are calibrated by using NBS oxalic acid, which is corrected for fractionation by using $\delta^{13}C$ correction. The samples are counted on a Packard 1050 low-level liquid scintillation counter for a minimum of 1300 and an average of 2700 min. The ^{14}C activities are reported in units of dpm/g of C and exhibit typical precision of ±0.5 dpm/g of C.

Stable isotopic analysis of carbon and hydrogen is carried out on the same sample after isothermal combustion and cryogenic purification. Approximately 2–5 mg of the same material as prepared for ^{14}C measurement is loaded into a Pyrex or quartz ampule with suitable oxidizer such as cupric oxide wire and burned in a programmed cycle to either 575 or 800 °C, respectively (Stuermer et al., 1978).

For this research, using volatile aromatic compounds, the low-temperature combustion proved equivalent to the higher temperature combustion in isotopic abundance and quantitative recovery of CO_2. This is in agreement with earlier work by Bricout and Koziet (1978). The sealed ampule, containing the combustion products, is then connected to a preparation line where the CO_2 can be transferred and purified by using liquid nitrogen and readied for analysis. The water from combustion, separated cryogenically from the other gases, is transferred to another ampule containing purified zinc metal. Once transfer is complete, the ampule is sealed and isothermally reacted at high temperature to form hydrogen gas (Kendall and

Coplen, 1985). Both the CO_2 and H_2 gases are transferred without isotopic fractionation and thus represent the isotopic ratio of the starting organic compound. The purified gases are analyzed for their respective stable isotope ratios by using a Finnigan MAT 251 mass spectrometer with a dual inlet system whereby a known reference gas is alternately analyzed with the unknown sample. By this method, results are relative to a standard and yield optimum precision for isotopic analysis.

The mass spectrometer is calibrated by using National Bureau of Standards NBS-20, Solenhofen limestone, and NBS-22, hydrocarbon oil, for $\delta^{13}C$ and SMOW and SLAP for δD ratios.

Isotopic analysis is typically reported in delta notation (δ) in parts per mil (‰) to give the isotopic composition relative to the international standards PDB or SMOW.

RESULTS AND DISCUSSION

Table I lists the isotopic analyses ^{14}C, $\delta^{13}C$, and δD for 60 cinnamic aldehyde or cassia oil samples. Table II lists the isotopic analyses for 51 benzaldehyde or bitter almond oil samples. Each table is separated into groups of botanical extracts, natural oils, and synthetics. The botanical extracts were samples extracted at CAIS. The natural oils were samples labeled natural by the manufacturer and on the basis of this and their isotopic similarity to the botanical extracts were assumed to be of natural origin. Synthetic samples were delineated by any or all of their isotopic abundances being unambiguously different from the botanical extracts.

Tables I and II indicate ^{14}C activities ranging from low activities of −0.1 to 0.3 dpm/g of C to high activities of 219.8 dpm/g of C, which well exceed present natural activities of 15–16 dpm/g of C. The low activity is not unusual or unexpected since synthetically derived products are lawfully distributed when properly labeled. However, the high activity of 219.8 dpm/g of C for one particular benzaldehyde sample and the excessive activity for two other benzaldehyde samples indicate the addition of ^{14}C to these samples or their precursors. Other explanations may exist for the need of such high activities in a synthetic sample such as that required for tracer studies or as a result from neutron activation. However, as will be made apparent by the $\delta^{13}C$ and δD stable isotope ratios, the more plausible explanation of isotopic manipulation for simulating a natural product will be amply supported.

As shown in Table I, the $\delta^{13}C$ values for all of the groups, botanical extracts, natural oils, and synthetics, fall between −24.0 and −30.4‰. This is in agreement with the narrower range suggested by Smith and Epstein (1971) for Calvin plants of −24 to −30‰. Comparison of the first two groups, botanical extracts and the natural oils, indicate nearly equivalent $\delta^{13}C$ values of −27.6 and −27.5‰, respectively. This would suggest similar processing and extraction techniques and probably a similar source material. The equivalency of ^{14}C activities for each group is supportive of this conclusion and the presumption that the natural oils are labeled accurately.

The range of $\delta^{13}C$ data in Table I extends from −24.0 to −30.4‰, just slightly larger than the −24.0 to −30.0‰ range previously defined for Calvin plants. Since this includes the synthetics group, the overlap in $\delta^{13}C$ values for synthetic cinnamic aldehydes would appear indistinguishable from the natural oils. Upon further data reduction of each individual group, it is evident that the range of values for both the botanical extracts and natural oils

Table I. Isotopic Analysis of Cinnamic Aldehyde

sample	^{14}C, dpm/g of C	$\delta^{13}C$, ‰ PDB	δ D, ‰ SMOW
	Botanical Extracts		
1	16.4	−27.5	−109
2	16.9	−27.9	−124
3	16.3	−26.8	−142
4	17.9	−27.8	−124
5	15.8	−27.4	−124
6	15.5	−27.8	−132
7	15.8	−28.2	−127
8	18.3	−27.2	−129
9	16.8	−27.7	−120
10	15.9	−27.5	−134
mean	16.6	−27.6	−127
SD	0.9	0.4	9
	Natural Oils[a]		
11	16.0	−27.5	−118
12	16.7	−27.7	−115
13	16.5	−27.2	−111
14	16.5	−30.4	−101
15	16.4	−27.4	−125
16	16.9	−29.3	−109
17	16.2	−27.1	−117
18	15.9	−27.6	−127
19	15.8	−27.1	−116
20	15.6	−27.4	−110
21	16.2	−27.3	−106
22	16.1	−27.0	−104
23	15.2	−27.3	−106
24	15.1	−26.8	−105
25	15.0	−27.7	−124
26	15.3	−27.4	−123
27	16.3	−27.2	−110
28	16.5	−26.8	−117
29	15.3	−27.3	−115
30	16.4	−27.5	−102
31	17.0	−27.2	−158
32	15.7	−27.3	−116
33	16.5	−27.5	−121
34	15.5	−26.9	−105
35	16.5	−28.0	−119
36	15.5	−27.0	−109
37	15.7	−27.0	−111
38	16.3	−27.3	−114
39	16.6	−27.7	−117
40	17.3	−27.5	−120
41	17.3	−27.5	−110
42	15.7	−27.7	−117
43	16.0	−28.6	−127
44	16.5	−28.4	−118
45	16.9	−27.5	−113
46	16.7	−28.0	−112
47	15.8	−27.0	−107
48	16.1	−26.9	−108
49	16.3	−27.9	−104
mean	16.2	−27.5	−115
SD	0.6	0.7	10
	Synthetics[b] via Toluene Oxidation		
50	0.1	−25.0	464
51	0.2	−25.7	569
mean	0.2	−25.4	517
SD	0.1	0.3	52
	Synthetics[b] via Various Synthetic Routes		
52	2.6	−24.2	−52
53	2.7	−24.9	−54
54	2.8	−24.0	−60
55	4.9	−27.5	−79
56	6.5	−26.2	213
57	9.7	−26.4	−96
58	11.0	−26.6	−96
59	11.3	−27.2	34
60	14.0	−27.1	72

[a] Labeled natural or isotopically equivalent to botanical extracts. [b] Based on ^{14}C activities and stable isotope values.

Table II. Isotopic Analysis of Benzaldehyde

sample	^{14}C, dpm/g of C	$\delta\ ^{13}C$, ‰ PDB	δ D, ‰ SMOW
	Botanical Extracts		
61	15.4	-28.0	-84
62	15.7	-27.5	-86
63		-28.2	-125
mean	15.6	-27.9	-98
SD	0.2	0.4	23
	Natural Oils[a]		
64	16.1	-28.4	-114
65	16.2	-29.2	-122
66	15.6	-27.1	-93
67	15.7	-29.8	-105
68	16.2	-28.4	-119
69	15.2	-28.9	-109
70	16.1	-30.0	-117
71	16.7	-28.5	-121
72	16.6	-28.0	-114
73	16.7	-28.6	-92
74	16.6	-29.2	-91
75	16.7	-29.6	-88
76	16.0	-28.2	-103
77	16.5	-28.6	-118
78	16.0	-28.4	-119
79	16.1	-29.0	-122
80	16.3	-29.1	-125
81	16.1	-29.0	-152
82	16.2	-27.9	-117
83	16.3	-28.1	-120
84	16.2	-27.1	-94
85	16.0	-28.8	-96
86	16.5	-28.5	-86
87	16.6	-28.7	-85
88	16.1	-28.6	-127
89	16.7	-28.2	-96
90	16.4	-28.4	-90
91	16.6	-28.5	-105
92	16.3	-29.3	-102
93	16.5	-28.6	-82
mean	16.3	-28.6	-108
SD	0.4	0.7	16
	Synthetics[b] via Benzal Chloride		
94	-0.1	-30.0	-48
95	0.0	-29.1	-47
96	0.0	-28.5	-67
mean	0.0	-29.2	-54
SD	0.1	0.8	11
	Synthetics[b] via Toluene Oxidation		
97	-0.1	-26.5	641
98	0.0	-25.5	623
99	0.0	-26.6	603
100	0.0	-25.8	663
101	0.0	-26.9	604
102	0.1	-26.0	587
103	0.3	-25.8	669
mean	0.0	-26.2	627
SD	0.1	0.5	32
104	15.2	-25.9	594
105	15.2	-25.4	576
106	16.2	-25.7	605
107	16.4	-27.0	421
108	16.8	-26.3	378
109	36.0	-27.0	490
110	109.4	-25.6	496
111	219.8	-25.7	493
mean		-26.1	507
SD		0.6	82

[a] Labeled natural or isotopically equivalent to botanical extracts. [b] Based on ^{14}C activities and stable isotope values.

is much narrower than that of Calvin type plants in general. By close examination of Table I the differentiating of natural cinnamic aldehyde extracts from some synthetically derived samples, based solely on their $\delta^{13}C$ values, may be possible but is best substantiated by including the other isotopic analyses.

In contrast to the natural oils and extracts, the cinnamic aldehyde synthetics group has a greater variation in $\delta^{13}C$ values and a mean value significantly lower at -26.0‰. It is difficult to make conclusions on the basis of the $\delta^{13}C$ values alone for the synthetics especially since the ^{14}C exhibit such variation within this group. However, it can be concluded, with good certainty, that some synthetic cinnamic aldehydes with less than 3 dpm/g of C ^{14}C activity, less than 20% natural, have $\delta^{13}C$ values enriched in ^{13}C by 2–3‰ relative to natural oils and extracts. This means the $\delta^{13}C$ values can be used in some cases to differentiate an extract via a particular synthetic pathway from that derived via a botanical pathway. More evidence to the derivation of the synthetic cinnamic aldehydes listed in Table I will be brought to light with the inclusion of δD values for these samples and will be discussed in succeeding paragraphs.

The $\delta^{13}C$ values for benzaldehyde are listed in Table II according to the groups botanical extracts, natural oils, and synthetics. Again, the values for both the botanical extracts and natural oils fall within a narrower range, -27.1 to -30.0‰, than that described for most Calvin-type plants. Also, as in Table I, the similarity in the mean $\delta^{13}C$ values between the botanical extracts and the natural oils suggests a similar process or botanical precursor. Unlike the natural extracts, which can be treated as one single group, the $\delta^{13}C$ values for the synthetics portray a definite bimodal distribution to the data. Samples 94–96 are depleted in ^{13}C relative to the natural samples with a mean value of -29.2‰. The remaining samples 97–111, are enriched in ^{13}C by nearly 2‰ with respect to the natural extracts. These data indicate two unique sources for these synthetic samples.

The separate and unique $\delta^{13}C$ values for the synthetic benzaldehyde samples are of interest for determining synthetic mechanisms and precursors used in the manufacture of benzaldehyde. An unexpected finding was two separate and unique ^{14}C activities for the one set of $\delta^{13}C$ values. This is indicated by the data from two sets of data, samples 97–103 and samples 104–111. The realization that manipulation of the radiocarbon content, 0 dpm/g of C in the case of synthetic benzaldehyde, had probably occurred led us to investigate the deuterium/hydrogen isotope ratio as collaboration and verification of this theory.

Referring to Tables I and II, the δD values of the botanical extracts and natural oils for both cinnamic aldehyde and benzaldehyde fall within the range of carbohydrates illustrated in Figure 3. The aromatic hydrogen atoms of these extracts are those originally present in the sugar molecules at the time of biosynthesis (Smith and Jacobson, 1976).

The minor variation between the botanical extracts and natural oils for each extract is most likely a consequence of variation in extraction methodology and the various sites from which the natural oils were produced. As will be shown, this variation is of minor consequence in the use of δD in authenticating the origin of these extracts.

The δD values of the synthetics, indicated in Table I, show a varied and complex array of values. When these δD values are combined with their associated $\delta^{13}C$ and ^{14}C results, a more discernible picture is revealed. The data indicate five values with positive or enriched deuterium abundance far beyond what has been measured, and published scientifically, for a Calvin plant or any botanical product.

The extremely positive δD values were associated with a synthetic route of manufacture because of their unique deuterium abundance. Our preliminary work in the identification of this unique synthetic pathway (Przybyla, 1987) has since been substantiated as synthetic in origin (Butzenlechner et al., 1989).

The two well-known synthetic routes of benzaldehyde production, from which cinnamic aldehyde can readily be made, are via benzal chloride from the oxidative chlorination of toluene and by the direct catalytic oxidation of toluene. These two synthetic routes represent uniquely different chemical kinetics with subsequently unique isotopic abundance of ^{13}C and deuterium. They will, however, have little effect on the ^{14}C abundance since this is nonexistent or, at least, at undetectable levels as a result of the petrochemical origin.

As was indicated in earlier data and corroborated here, the two synthetic routes are illustrated by δD values in excess of 400‰ and $\delta^{13}C$ values of −26‰ or more positive for the toluene oxidation method and δD values more positive than −75‰ and $\delta^{13}C$ values ranging from −24 to −30‰ for the benzal chloride synthetic route. This latter $\delta^{13}C$ range is difficult to substantiate because of the overlap with $\delta^{13}C$ ranges of many C-3 plants including the isolates, cinnamic aldehyde and benzaldehyde. This is evident by the $\delta^{13}C$ values of samples 94–96 of Table II. The δD value resulting from the toluene oxidation method is unique enough to be useful for the study of ^{14}C manipulation.

As can be seen from samples 50 and 51 in Table I and samples 97–103 in Table II, the extremely enriched deuterium value and moderately enriched ^{13}C value are accompanied by undetectable ^{14}C activity as would be expected from a petrochemically derived precursor. Other samples in Table I, such as sample 56, indicate the possible dilution of a naturally derived product with that of a petrochemically derived product by the aforementioned method. This is indicated by, principally, the ^{14}C activity level at or about the 50% level of 6.5 dpm/g of C. The $\delta^{13}C$ and δD values corroborate such a dilution when the isotopic abundances of the end members—synthetic cinnamic aldehyde from toluene and natural botanically produced cinnamic aldehyde—are considered.

Sample 55 is likewise a diluted natural sample by virtue of the 4.9 dpm/g of C ^{14}C activity. It exhibits the stable isotope ratios for $\delta^{13}C$ and δD equivalent to a sample diluted with cinnamic aldehyde derived via the benzal chloride route. The δD value lies somewhat higher, less negative, than that of a botanically derived product to nearly the same extent as its ^{14}C activity lies toward that of a natural ^{14}C activity.

Samples 52–54 indicate the addition of approximately 80% petroleum-derived material to a naturally derived product on the basis of the ^{14}C activities of 2.6–2.8 dpm/g of C. The similarity to a synthetic precursor for the cinnamic aldehyde samples is further substantiated by the average $\delta^{13}C$ and δD values of −24.4 and −55‰, respectively. The isotopic abundances in these samples indicate a synthetic route used in the commercial production of cinnamic aldehyde by condensing benzaldehyde with acetaldehyde (Martin et al., 1984). In this case, the condensation of a naturally derived acetaldehyde with synthetic benzaldehyde forms cinnamic aldehyde with a 2/9 natural to total carbon ^{14}C ratio. This is indicated by the carbon ratio of its precursors or a ^{14}C activity of about 3 dpm/g of C. The $\delta^{13}C$ ratio is slightly less negative and the δD ratio is more negative than that of synthetic benzaldehyde, which substantiates the use of a modern acetaldehyde in manufacture.

Samples 57 and 58 appear to be diluted natural samples to the extent of 35% with benzal chloride derived material, and samples 59 and 60 appear to be some combination of a toluene-derived synthetic and natural botanical extract due to their enriched or high δD values.

The δD values help to clarify the process or source of these essential oils in most cases, as is the case for cinnamic aldehyde and benzaldehyde derived from the oxidation of toluene. Because this is not a biosynthetic process found in nature and the resulting δD values are outside the range typically found in biosynthesized compounds, it is safe to assume these δD values would be accompanied by and indicate ^{14}C activities equivalent to those of petrochemically derived compounds. In Table II, samples 97–103 support just such a conclusion. Samples 104–111, however, indicate ^{14}C activities at or above modern ^{14}C levels found in nature. Since the ^{14}C of the biosphere is the controlling factor for natural ^{14}C content and since the kinetics that affect the abundance of deuterium and ^{13}C in a molecule have little or not effect on the abundance of ^{14}C at its infinitesimal level (even for processes such as catalytic oxidation or chlorination), it is difficult to suggest biochemical or naturally occurring physical mechanisms to derive such enrichments of ^{14}C. Likewise, the extreme enrichments of deuterium have not been associated with any natural biosynthetic process. Even deuterium enrichments due to the oxidation of natural benzaldehyde as previously reported (Butzenlechner et al., 1989) were not encountered in the scope of this work.

Samples 109–111, exhibiting similar δD and $\delta^{13}C$ values, but ^{14}C activities higher than those ever found in equilibrium with the atmosphere, must be rationalized as ^{14}C-labeled samples. The most likely explanation for a 10 times natural ^{14}C activity level in sample 111 would be the manipulation of synthetic benzaldehyde in an attempt to simulate that derived from apricots, peaches, cherries, or the like.

CONCLUSION

This study, as well as others, has shown the utility of stable isotope ratio analyses for authenticating foods and flavors of natural origin. Previous CAIS work with FEMA had first indicated the possible manipulation of ^{14}C in benzaldehyde (Pryzbyla, 1988). The research presented here confirms and supports evidence of ^{14}C manipulation to simulate natural ^{14}C levels of activity.

The results presented here did not awaken researchers assigned with the task of uncovering methods of adulteration to the possibility of ^{14}C labeling, but rather substantiated its existence. As each method of adulteration is circumvented by a more creative and elaborate method of detection, the economic incentive and potential for distributing such adulterated products will certainly be reduced.

The incorporation of the three isotopic abundances, ^{14}C, $\delta^{13}C$, and δD, has enhanced the capability to determine the authenticity of two essential oils: cassia and bitter almond. When modern levels of ^{14}C activities are substantiated by isotopic ratios for $\delta^{13}C$ and δD within the ranges −27.6 ± 0.6 and −116 ± 8, respectively, for cinnamic aldehyde and −28.6 ± 0.5 and −105 ± 5, respectively, for benzaldehyde, a clearer indication of their natural origin can be obtained. Conversely, for samples with isotopic ratios for $\delta^{13}C$ and δD of −25.5 ± 0.3 and 517 ± 52, respectively, for cinnamic aldehyde and either −29.2 ± 0.8 and −54 ± 11 or −26.1 ± 0.6 and 576 ± 73, respectively, for benzaldehyde, a synthetic derivation is certainly indicated.

Where it was considered of little utility previously, $\delta^{13}C$ has the potential to suggest manipulation when samples fall outside a fairly narrow range. The ^{14}C analysis has certain application in substantiating a synthetic source when levels below modern concentrations are encountered. However, due to the potential for ^{14}C additions as we have uncovered here, natural ^{14}C levels supported with the corresponding natural stable isotopic ratios provide a better indicator of natural origin.

Deuterium/hydrogen isotopic ratios have proven to be the latest and most useful of the isotopic analytical methods with which to detect adulteration of foods and flavors. The mass difference between these two isotopes corresponds to a greater isotope effect and a more sensitive measurement for classifying different flavoring substances. Even though the δD measurement is more complex and suffers from exchange reactions, it has found utility in numerous cases on its own and in support of other methods.

As present methods become more precise and additional isotopic and analytical methods are made available, the implementation of these methods will be valuable in protecting the consumer and industry from unknowingly buying falsely labeled products.

ACKNOWLEDGMENT

We thank Donald F. Smith and Jeff M. Legato of the Center for Applied Isotope Studies for their assistance in sample preparation, Patrick G. Hoffman of McCormick & Co. for his critical review of the manuscript, and the Flavor Extract Manufacturers' Association of The United States for their technical and financial support of this research.

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Received for review October 13, 1989. Accepted February 15, 1990.