Differentiation of Natural and Synthetic Benzaldehydes by ^2H Nuclear Magnetic Resonance

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The site distribution of natural abundance deuterium in various sources of benzaldehyde was studied by ^2H NMR. The distribution of deuterium among the aromatic sites provided a means of differentiating petrochemical and botanical sources and of detecting adulteration of costly bitter almond oil. The absolute abundance of each deuterated species was determined by a combination of ^2H NMR and isotope ratio mass spectrometry.

Benzaldehyde has a variety of commercial applications including use as a synthetic intermediate and as a flavor ingredient in the food and beverage industry. The popular trend toward natural foods has increased the demand for costly bitter almond oil and, unfortunately, increased the occurrence of adulteration with inexpensive petrochemical benzaldehyde.

Until recently (Culp and Noakes, 1990), stable isotope and especially radioisotope measurements were thought to provide a good indication of the production route and validation of plant origin. Benzaldehyde prepared from the oxidation of toluene showed no modern ^{14}C activity but had very high deuterium levels (Butzenlechner et al., 1989). Benzaldehyde produced from the chlorination of toluene followed by hydrolysis of intermediate benzal chloride also showed no modern ^{14}C activity but had overall deuterium levels similar to those of botanical materials. However, strong evidence (Culp and Noakes, 1990) has been presented for the manipulation of the total ^{14}C content of petrochemical benzaldehyde to mimic that of botanical material. As benzaldehyde is very important commercially, a new means of verifying authenticity was necessary.

In 1981, ^2H NMR was used to show large differences in the distribution of natural abundance deuterium at individual sites in ethanol derivatives (Martin and Martin, 1981). Since then, site-specific deuterium distribution data have been reported for many botanical and petrochemical materials including anethole (Martin et al., 1982, 1990), camphor (Grant et al., 1982), 3-carene (Leopold et al., 1990), citral, eugenol, and geraniol (Martin et al., 1990), ethanol (Martin et al., 1988), limonene (Leopold et al., 1988), α-pinene (Martin et al., 1986), and vanillin (Toulemonde and Horman, 1983). In this paper, the results of a ^2H NMR study on benzaldehyde from various sources are reported.

EXPERIMENTAL PROCEDURES

Deuterium NMR data (55.28 MHz) were obtained at 302 K on a Bruker AM360 spectrometer with an Aspect 3000 computer and process controller using standard Bruker DISRS7 software and equipped with a 5-mm deuterium probe with proton decoupling and ^{19}F lock. Samples were prepared as 85% solutions in hexafluorobenzene (Aldrich Chemical Co., Inc.) for lock. One to three data sets were acquired for each sample using the following parameters: 5.99-s acquisition time, 996-Hz spectral width with 11.9K data points, 8K scans, 6.5-μs (90°) ^2H pulse, and 3.5-W broadband decoupling. Each free induction decay was processed three times with one order of zero filling to 0.03 Hz/point (64K) digital resolution, Fourier transformation with sensitivity enhancement (0.663-Hz line broadening), and manual peak phasing and integration.

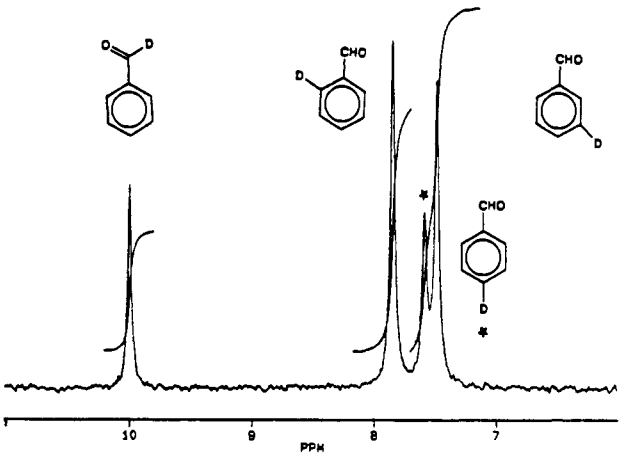

Figure 1. Natural abundance 55.28-MHz ^2H NMR spectrum of benzaldehyde.

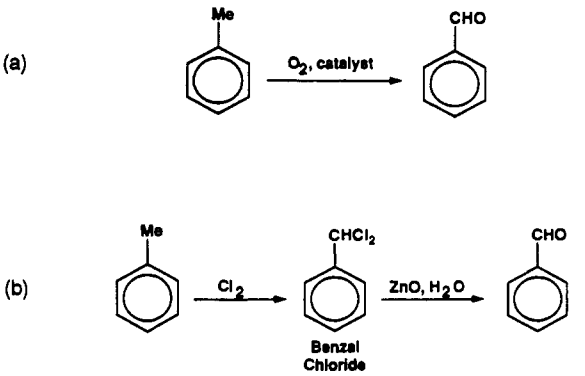

Figure 2. Petrochemical benzaldehyde from catalytic oxidation of toluene (a) or by chlorination of toluene, followed by hydrolysis of the intermediate benzal chloride (b).

Many of the samples and related isotope ratio mass spectrometry data were gifts of the Flavor and Extract Manufacturers' Association (FEMA)—Isotope Studies Committee, the Center for Applied Isotope Studies (University of Georgia), and Krueger Food Laboratories, Inc., Cambridge, MA. The botanicals were prepared from cassia bark (Culp and Noakes, 1990) or apricot, cherry, and peach kernels (Culp and Noakes, 1990; Butzenlechner et al., 1989). Additional petrochemical benzaldehyde samples were purchased from Aldrich and Fisher Scientific, Inc.

Sample purities were >94% as determined by capillary GC analyses on a Hewlett-Packard Model 5890 gas chromatograph using a 50 m × 0.32 mm bonded methyl silicone capillary column and a 50 m × 0.32 mm fused silica Carbowax 20M column with the following conditions: injector temperature, 250 °C; detector temperature, 250 °C; oven temperature programmed from 75 to 225 °C at 2 °C/min followed by a 30-min hold at 225 °C with

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Table I. Site-Specific Deuterium Distribution in Benzaldehyde by Source

source, sample no./n[a]	mole fraction of deuterium: f [formyl] (SD)[b]	f [ortho] (SD)	f [meta + para] (SD)	ADR (SD)[c]	ppm (±2)[d]
synthetic/PhMe + O_2					
1/3	0.5078 (26)	0.1996 (15)	0.2927 (10)	0.6818 (29)	237
2/4	0.5200 (183)	0.1959 (76)	0.2840 (111)	0.6900 (119)	261
3/1	0.5801	0.1711	0.2487	0.6882	253
4/1	0.5717	0.1731	0.2552	0.6733	259
synthetic/PhMe + Cl_2					
5/3	0.1696 (30)	0.3326 (47)	0.4976 (35)	0.6681 (129)	151
botanical/cassia PhCHO					
6/6	0.1478 (74)	0.3690 (66)	0.4837 (70)	0.7632 (20)	145
7/1	0.1380	0.3825	0.4795	0.7977	
8/2	0.1481	0.3643	0.4875	0.7477	
9/2	0.1879 (138)	0.3498 (65)	0.4623 (20)	0.7577 (47)	142
10/3	0.1477 (36)	0.3544 (40)	0.4979 (20)	0.7119 (103)	139
11/2	0.1446 (44)	0.3666 (157)	0.4888 (114)	0.7509 (24)	139
12/5	0.1627 (70)	0.3542 (71)	0.4832 (110)	0.7336 (30)	142
botanical/bitter almond oil					
13/3	0.1532 (90)	0.3160 (124)	0.5300 (36)	0.5951 (278)	141
14/5	0.1545 (30)	0.3173 (68)	0.5282 (85)	0.6012 (223)	142
15/2	0.1798	0.2988	0.5213	0.5732	
16/2	0.1414	0.3210	0.5376	0.5971	
17/2	0.1505	0.3075	0.5421	0.5672	
mean values					
synthetic/PhMe + O_2	0.5449 (363)	0.1849 (149)	0.2702 (214)	0.6813	252
synthetic/PhMe + Cl_2	0.1696	0.3326	0.4976	0.6681	151
cassia PhCHO	0.1538 (168)	0.3630 (112)	0.4833 (109)	0.7518 (264)	142
bitter almond oil	0.1543 (159)	0.3117 (87)	0.5332 (81)	0.5906 (178)	140

[a] n = number of determinations. [b] Values f [i] are the mole fractions of site-deuterated molecular species i averaged over n determinations with SD = standard deviation × 10^4. [c] ADR (aromatic distribution ratio) = f [ortho]/f [meta + para]. [d] Total parts per million of deuterium as determined by standard combustion and isotope ratio mass spectrometry.

Table II. Detection of Adulteration in Natural Benzaldehyde

mixture	mole fraction of deuterium: f [formyl][a]	f [ortho]	f [meta + para]	ADR[b]
75% bitter almond oil/25% cassia PhCHO				
exptl	0.1566	0.4425	0.5519	0.6479
calcd	0.1520	0.3297	0.5183	0.6360
85% bitter almond oil/15% cassia PhCHO				
exptl	0.1533	0.3237	0.5241	0.6192
calcd	0.1542	0.3247	0.5222	0.6218
90% bitter almond oil/10% synthetic/PhMe + Cl_2				
exptl	0.1592	0.3206	0.5201	0.6074
calcd	0.1560	0.3188	0.5231	0.6095
100% bitter almond oil	0.1543	0.3117	0.5320	0.5906
SD[c]	159	87	81	178

[a] Values f [i] are the mole fractions of site-deuterated molecular species i. [b] ADR (aromatic distribution ratio) = f [ortho]/f [meta + para]. [c] SD, standard deviation × 10^4.

helium as carrier gas with 27 cm/s linear velocity and a 0.2-μL injection with 100:1 split.

RESULTS AND DISCUSSION

Deuterium NMR spectra were obtained on benzaldehyde samples from each of the two major petrochemical and two major botanical sources. The petrochemical materials were produced either from the oxidation of toluene or from the chlorination of toluene. The botanical samples were prepared from either cinnamaldehyde from cassia bark (termed cassia benzaldehyde) or amygdalin from fruit kernels (termed bitter almond oil). Since, at natural abundance levels, only 1 in approximately 1600 molecules of benzaldehyde would contain one deuterium, the integrals of the deuterium NMR signals indicate the relative proportions of each of the chemically distinct monodeuterated benzaldehyde species (Figure 1). Following

Table III. Benzaldehyde Site-Specific Deuterium Levels

source	ppm of deuterium (±2): formyl-^{2}H	ortho-^{2}H	[meta + para-^{2}H]	ADR[a]
synthetic/PhMe + O_2	138	46	68	0.6813
synthetic/PhMe + Cl_2	26	50	75	0.6681
cassia PhCHO	22	52	68	0.7518
bitter almond oil	21	44	75	0.5906

[a] ADR (aromatic distribution ratio) = f [ortho]/f [meta + para].

normalization of the sum of the signal areas to 1.000, the individual areas then provide the mole fraction f [i] of each of the deuterated species and thus the site-specific distribution of deuterium.

Table I summarizes the site-specific distribution results for samples from the four different processes. Given the incomplete resolution of the signals from m-[^{2}H]benzaldehyde and p-[^{2}H]benzaldehyde, only the sum of these was quantified. Also shown in Table I are the values for the aromatic distribution ratio (ADR, ratio of f [ortho] to f [meta + para]). Differences in the distribution of deuterium among the aromatic sites were found to be emphasized in the ADR value and useful for comparisons of the processes. For reference, the total deuterium content as determined by combustion and isotope ratio mass spectrometry is shown in the last column. Below the data for the individual samples are shown the average values for each of the processes.

The NMR data for the samples prepared by the catalytic oxidation of toluene (Figure 2a) show a very high proportion of deuterium in the formyl position (f [formyl] = 0.5548), consistent with a large primary kinetic isotope effect expected for the reaction. This is in contrast to the data for benzaldehyde from the benzal chloride process (Figure 2b). In the latter case, the level of deuterium in the formyl position was near-statistical (f [formyl] = 0.1695; statistical = 1/6 or 0.1667), consistent with the

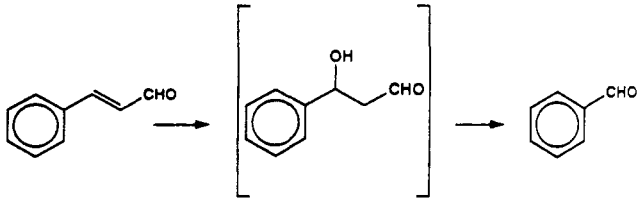

Figure 3. Hydration of cinnamaldehyde and retroaldol to produce benzaldehyde.

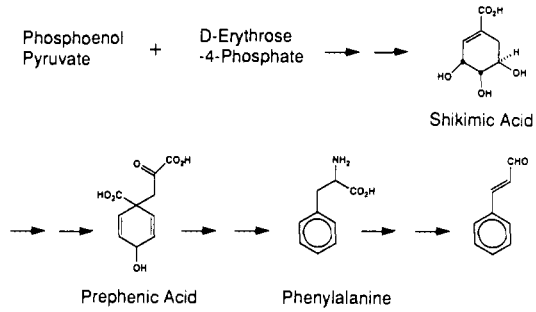

Figure 4. Biosynthesis of cinnamaldehyde.

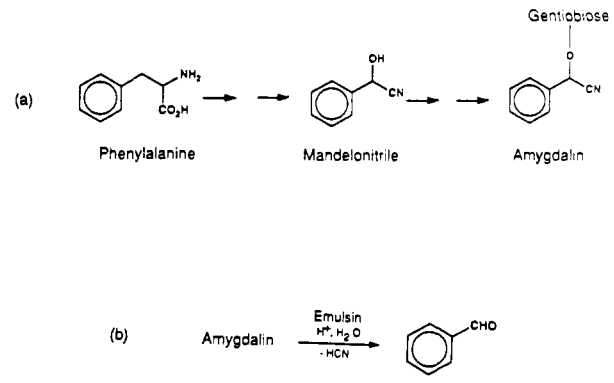

Figure 5. Biosynthesis of amygdalin (a) and hydrolysis to produce bitter almond oil (b).

minimal isotope effect expected for this free-radical reaction (Butzenlechner et al., 1989).

For all of the petrochemical samples, the aromatic distribution should remain unchanged from that of toluene itself. Since toluene is produced by catalytic reforming of paraffins in refinery streams, the manufacturing process would be expected to provide a nearly random distribution of deuterium in the ring. From the ^{2}H NMR analyses, the relative distribution of deuterium among the aromatic sites is nearly statistical (ADR = 0.6813 ± 0.0087 for the oxidation process; ADR = 0.6681 ± 0.0129 for chlorination/hydrolysis; statistical ADR = 2/3 or 0.6667).

The deuterium levels and distribution for cassia benzaldehyde would remain unchanged from those of the original cinnamaldehyde backbone as the hydration and retroaldol process (Figure 3) do not involve abstraction of a hydrogen isotope at the site α to the aromatic ring or on the ring itself. Since the biosynthesis of cinnamaldehyde (Figure 4) involves many different bond-forming and bond-breaking reactions before complete aromatization, the sequence would be expected to result in differentiation of the isotopes. In fact, the NMR data show a dramatically nonrandom distribution of deuterium among the aromatic sites (f [ortho] = 0.3630, ADR = 0.7518).

Bitter almond oil is obtained by steam distillation of the benzaldehyde released from the cyanogenic glycoside amygdalin in fruit kernels by the action of the native enzyme emulsin under acidic conditions (Figure 5). Since there is no exchange of hydrogen at the α position, the process does not change the isotopic distribution. As amygdalin is formed from phenylalanine via mandelonitrile, a nonstatistical aromatic distribution of deuterium would also be expected. Differences in the isotopic outcome between the two botanical sources then would be dependent on differences in plant enzymes, transpiration mechanisms, or local water supplies. The ^{2}H NMR data show distinct aromatic distribution patterns. Bitter almond oil is unique in having the lowest proportion of deuterium at the ortho site (f [ortho] = 0.3117, ADR = 0.5906).

As autoxidation of benzaldehyde is often encountered in stored materials, it was important to determine the effect this might have on the isotope distributions. A sample of cassia benzaldehyde was placed under a slow stream of air for 2 weeks, during which time 41% of the material had oxidized to benzoic acid. The benzoic acid was removed by base extraction and the recovered benzaldehyde reanalyzed. The site-specific distribution data indicate a significant fractionation of isotopes in the formyl position (original f [formyl] = 0.1538 ± 0.0167; oxidized f [formyl] = 0.3003) consistent with an earlier benzaldehyde degradation and mass spectrometry study (Butzenlechner et al., 1989). There is, however, no significant change in the ADR (original ADR = 0.7518 ± 0.0265; oxidized ADR = 0.7652). Thus, the ADR value provides a clear indication of the actual source, independent of the age of the sample.

The effectiveness of the technique in detecting adulteration of bitter almond oil was demonstrated on known mixtures. These results are shown in Table II. It can be seen from these data that an ADR value greater than 0.6084 for an unknown sample could indicate 10% or higher adulteration of a bitter almond oil with the other commercial sources of benzaldehyde. The presence of benzaldehyde from the benzal chloride process can also be confirmed by mass spectrometric detection of the characteristic ring-chlorinated toluene byproduct.

Table III shows a comparison of absolute deuterium levels for each of the monodeuterated species for each source. These data were obtained by factoring the NMR distribution data with the total abundance level for each process. Bitter almond oil shows the lowest absolute level as well as the lowest proportion of deuterium at the ortho site.

In summary, deuterium NMR provides a practical means of determining the site-specific distribution of deuterium in benzaldehyde as well as differentiating petrochemical, cassia, and bitter almond oil benzaldehydes. The ADR value (f [ortho]/f [meta + para]) can be readily calculated from the ^{2}H NMR spectrum and provides an indication of the authenticity of bitter almond oil. The absolute level of o-[^{2}H]benzaldehyde is a unique discriminant for bitter almond oil.

ABBREVIATIONS USED

ADR, aromatic distribution ratio; SD, standard deviation × 10^4.

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