

leading to 11-13 in 10-15% yield. This aldol-like condensation of the activated methylene group of 1-pyrroline with the furancarboxaldehydes is in analogy to the formation of 3-substituted tetrahydropyridines from piperidine and furancarboxaldehydes as demonstrated by Miller et al. (1984) for the lysine/glucose system. As will be shown in a future paper, reactions of arginine and different sugars also lead to compounds 11-13 as well as to different substituted cyclopent[b]azepinones. These results will demonstrate a closely related pool of reactive intermediates in both the proline and the arginine experiments. Reductive media such as proline/ascorbic acid easily transform 1-pyrroline 11 into the corresponding pyrrolidine 14, a main reaction product of this experiment. Compounds 15 and 16 derive from compounds 11 and 12, respectively, at elevated temperatures by isomerization to the more stable pyrrole systems.

Registry No. 1, 104704-36-1; 2, 104704-35-0; 3, 97073-24-0; 4, 97073-18-2; 5 (diastereomer 1), 118248-22-9; 5 (diastereomer 2), 118248-23-0; 6 (diastereomer 1), 118248-24-1; 6 (diastereomer 2), 118248-25-2; 7, 104704-31-6; 8, 118248-26-3; 9, 90086-89-8; 10, 118248-27-4; (E)-11, 118248-28-5; (Z)-11, 118248-29-6; (E)-12, 118248-30-9; (Z)-12, 118248-31-0; (E)-13, 118248-32-1; (Z)-13, 118248-33-2; (E)-14, 118248-34-3; (Z)-14, 118248-35-4.

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Assignment of Bitter Almond Oil to Natural and Synthetic Sources by Stable Isotope Ratio Analysis

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Benzaldehyde from natural sources showed a mean $\delta(^{13}\text{C})$ value $-29.7 \pm 0.5\%$ and a mean $\delta(^2\text{H})$ value $-125 \pm 14\%$. Synthetic benzaldehyde had $\delta(^{13}\text{C})$ and $\delta(^2\text{H})$ values that depended on the manufacturing process. Products synthesized from benzal chloride had a mean $\delta(^{13}\text{C})$ value $-28.7 \pm 1.5\%$ and a mean $\delta(^2\text{H})$ value $-40 \pm 21\%$ and those derived from catalytic oxidation of toluene $-26.8 \pm 0.4\%$ and $+777 \pm 20\%$, respectively. Taking into account that also degradation of benzaldehyde by air exposure can proceed with ^2H enrichment, the $\delta(^2\text{H})$ determination is a nonadulterable method for the origin assignment of benzaldehyde.

Benzaldehyde is the main component of the essential oils from the seeds and kernels of bitter almonds, apricots, peaches, plums, cherries, and cherry laurel. It is an important flavor additive for many food products. Because of the limited supply and high price of the natural product, synthetic benzaldehyde is often (legally) used as a substitute. However, the application of the less expensive artificial flavor has to be indicated, and it is suspected that this is not always done. Thus, analytical methods for determining the origin of this flavoring compound are needed.

The distinction of natural and synthetic flavoring compounds is commonly only possible by the determination

of their ^{14}C content (which can be adulterated) or by stable isotope ratio analysis. A number of corresponding applications have been reported in the last few years. Well-known examples are the distinction of natural vanillin (from vanilla sp., a CAM plant) from semisynthetic vanillin (base lignin, eugenol, or guajacol from C_3 plants) (Hoffman and Salb, 1979) and of lemon grass (C_4 plant) citral from synthetic citral (Bricout and Koziat, 1976).

While it is generally possible to identify the source of aromatic compounds originating from C_4 plants, CAM plants, or some synthetic sources on the basis of $\delta(^{13}\text{C})$ values, a corresponding distinction among products from different C_3 plants or between C_3 plants and many synthetic products is not possible. More conclusive results are obtained from the D/H ratios. Thus, the $\delta(^2\text{H})$ values of natural isoprenoids like citral, anethol, linalool, menthol, and carvone are significantly more negative than those of their synthetic counterparts. In general, most natural

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Table I. Isotope Content (‰ toward Standard) of Natural and Synthetic Benzaldehyde and Its Oxidation Products^a

| sample origin | benzaldehyde | | $\delta(^2\text{H})$ (SMOW) | |
|-----------------------------|-------------------------------|-----------------------------|-----------------------------|-------|
| | $\delta(^{13}\text{C})$ (PDB) | $\delta(^2\text{H})$ (SMOW) | H_5C_6 | CHO |
| bitter almond | -29.9 | -118 | -99 | -213 |
| bitter almond | -29.7 | -113 | -120 | -78 |
| plum | -30.4 | -146 | nd | nd |
| persipan I | -29.9 | -106 | -76 | -256 |
| persipan II | -30.1 | -138 | nd | nd |
| marzipan | -29.3 | nd | -80 | nd |
| commercial | -28.8 | -129 | -142 | -63 |
| natural mean | -29.7 ± 0.5 | -125 ± 14 | | |
| benzal chloride, hydrolysis | | | | |
| I | -28.3 | -11 | -64 | +258 |
| II | -30.4 | -32 | -85 | +233 |
| III | -26.4 | -68 | -87 | +27 |
| IV | -29.6 | -48 | -49 | -42 |
| synthetic A, mean | -28.7 ± 1.5 | -40 ± 21 | | |
| toluene catalytic oxidn | | | | |
| I | -27.3 | +777 | -98 | +5152 |
| II | -26.7 | +753 | -80 | +4808 |
| III | -26.3 | +802 | -74 | +5182 |
| synthetic B, mean | -26.8 ± 0.4 | +777 ± 20 | | |
| ice cream raw material | -26.5 | +764 | -81 | +4937 |

^aThe $\delta(^2\text{H})$ value of the H_5C_6 group was measured by combustion of sodium benzoate, obtained from benzaldehyde oxidation; the $\delta(^2\text{H})$ value of the CHO group was calculated from the difference by the formula $\delta(^2\text{H}_{\text{CHO}}) = 6[\delta(^2\text{H}_{\text{C}_6\text{H}_5\text{CHO}})] - 5[\delta(^2\text{H}_{\text{C}_6\text{H}_5})]$. The large variations in this case are due to the fact that the experimental error enters with a factor 5. nd = not determined.

monoterpenes have $\delta(^2\text{H})$ values between -300‰ and -250‰, the corresponding synthetic substances range near -150‰, and for *l*-menthol and its acetates $\delta(^2\text{H})$ values <-350‰ are reported (synthetic compounds -196 to -242‰). Natural phenylpropanoid derivatives have $\delta(^2\text{H})$ values >-100‰ and their synthetic counterparts >-50‰ (Bricout and Koziet, 1978).

Very recently Krueger (1987) reported the distinction of natural and synthetic benzaldehyde on the basis of the ^{14}C content. For natural bitter almond oil the author found a $\delta(^{13}\text{C})$ value of -28.5‰. Schmid et al. (1981), however, found -22‰ for natural benzaldehyde, a rather unusual value for a secondary plant product from C_3 plants. These results led us to examine systematically the $^{13}\text{C}/^{12}\text{C}$ and D/H ratios, which are not indicated so far in the literature, of natural and synthetic benzaldehyde.

EXPERIMENTAL SECTION

Isolation of Benzaldehyde from Natural Sources [Modified after Hanssen and Sturm (1967)]. Kernels or seeds (140 g) from bitter almonds, apricots, peaches, or plums were ground to a greasy paste by means of a coffee mill. The lipids were separated by a 5-h extraction with pentane in a Soxhlet apparatus and discarded. The remainder was digested in 500 mL of $1/15$ M phosphate buffer (pH 5.8) for 24 h at 20 °C. Then, the suspension was steam-distilled (*caution*, HCN!) and the distillate (200 mL) was extracted four times with 80 mL of pentane. The combined organic phase was dried with Na_2SO_4 , and then the solvent was evaporated under reduced pressure. The remaining oil was distilled in vacuo, and the distillate (pure benzaldehyde) was stored under N_2 atmosphere in brown glass vials (yield 0.2–0.7% of dry material).

Sugar containing almond or fruit kernel products (marzipan, persipan, ice cream raw material) were immediately digested in the above-mentioned buffer (500 g/L, incubation 24 h) and then extracted as described before. The benzaldehyde from the ice cream raw material needed further purification: In the raw extract the hydrogen sulfite adduct of the benzaldehyde was formed by addition of 1 mL of saturated sodium hydrogen sulfite solution.

The precipitate was collected by suction and rinsed with diethyl ether. After resuspension in 30 mL of 1 N HCl the benzaldehyde was collected by means of a second steam distillation and purified as before.

The reliability of these methods was controlled by the determination of the recovery and isotope recovery of a given amount of benzaldehyde submitted to the same procedure. Reference samples were purchased or provided by E. Merck (Darmstadt), C. Roth GmbH u. Co., Chemische Fabrik (Karlsruhe), Aldrich-Chemie GmbH u. Co. KG (Steinheim), Janssen Chimica (Breese, B.), BASF (Ludwigshafen), and H. Bahlsen, Keksfabrik KG (Hannover). These companies indicated the origin and the manufacturing process of the materials.

Oxidation of Benzaldehyde to Benzoic Acid. To a suspension of 200 mg of benzaldehyde in 5 mL of H_2O in a two-necked flask (reflux condenser, dropping funnel) at 75 °C was gradually added a small excess of a saturated KMnO_4 solution, and heating was continued for 1 h. The MnO_2 formed was separated by filtration and washed several times with a few milliliters of hot water. The solution was acidified with concentrated HCl and cooled to 0 °C. The precipitate was isolated by suction and washed with cold water. From the filtrate was obtained more benzoic acid after evaporation. The combined precipitates were recrystallized from water. The benzoic acid was dissolved in an equivalent amount of NaOH; sodium benzoate was obtained by evaporation and thoroughly dried in vacuo.

Combustion and isotope ratio determination were performed according to Dunbar and Schmidt (1984) and Winkler and Schmidt (1980). $\delta(^{13}\text{C})$ values relative to the PDB standard and $\delta(^2\text{H})$ values relative to the SMOW standard are given automatically by the isotope ratio mass spectrometer (SIRA 24, VG Isogas, Middlewich, GB).

RESULTS AND DISCUSSION

Benzaldehyde from authentic natural sources showed $\delta(^{13}\text{C})$ values between -28.8 and -30.4‰ as expected for shikimic acid derived products from C_3 plants (Benner et al., 1987). Synthetic benzaldehyde samples could be as-

signed on the basis of the $\delta(^{13}\text{C})$ values to two distinct groups with $\delta(^{13}\text{C})$ values between -26.4 and -30.4% (benzaldehyde synthesized from benzal chloride) and -26.3 and -27.3% (benzaldehyde obtained by catalytic oxidation of toluene). As the $\delta(^{13}\text{C})$ values of both synthetic groups are similar to those of the natural benzaldehyde samples, adulteration cannot be detected on this basis. However, a clear distinction is possible with D/H ratios. The natural products showed $\delta(^2\text{H})$ values between -100 and -150% whereas all the $\delta(^2\text{H})$ values of the synthetic samples were more positive and could be assigned to the two groups again, representing the origin from benzal chloride ($\delta(^2\text{H})$ between -68 and -11%) and from catalytic oxidation of toluene ($+753$ to $+802\%$).

Industrial benzaldehyde is prepared either by catalytic oxidation of toluene or by hydrolysis of benzal chloride, a chlorination product of toluene. Although even when toluene is the starting material in both cases, the chlorination must, as a radical reaction, proceed without a large kinetic isotope effect, while the catalytic oxidation of toluene must be accompanied by high intermolecular and intramolecular kinetic isotope effects, having as a consequence a significant ^2H enrichment in the formyl group of the product. Therefore, our results should be due to the isotope effects implied in the industrial processes of benzaldehyde preparation.

Proof arises from the oxidation of the benzaldehyde samples to benzoic acid, combusted as sodium benzoate. The difference in $\delta(^2\text{H})$ values of the benzaldehyde and its oxidation product permitted the calculation of the $\delta(^2\text{H})$ value of the formyl group: $\delta(^2\text{H}_{\text{CHO}}) = 6[\delta(^2\text{H}_{\text{C}_6\text{H}_5\text{CHO}})] - 5[\delta(^2\text{H}_{\text{C}_6\text{H}_5})]$. As demonstrated in Table I, the high ^2H enrichment disappeared after oxidation and the difference proved to be only due to an enrichment in the formyl group. Further proof for our interpretation is the fact that toluene, obtained from one of the industrial benzaldehyde producers, had a $\delta(^{13}\text{C})$ value of -29.4% and a $\delta(^2\text{H})$ value of -95% , which coincides absolutely with the "corrected" values for the synthetic benzaldehyde.

It has to be mentioned that enrichment of ^2H in the formyl group is also observed during benzaldehyde degradation. An exposure of two samples to air for 2 days had a 50% loss of benzaldehyde and a rise of the $\delta(^2\text{H})$ value of the remaining material from $+777$ to $+868\%$, respectively, from $+764$ to $+939\%$. Therefore, largely deteriorated samples of natural origin may have a ^2H enrichment

similar to that of synthetic benzaldehyde from benzal chloride.

However, the sample of ice cream raw material of unknown origin (Table I) was undoubtedly synthetic and prepared by catalytic oxidation of toluene. Therefore, the ^2H analysis of benzaldehyde from fresh unspoiled food material provides a secure means for the assignment of origin or treatment. As artificially ^2H -depleted benzaldehyde is not available, adulterations are largely impossible.

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Registry No. $\text{H}_5\text{C}_6\text{CHO}$, 100-52-7; ^{13}C , 14762-74-4; $^2\text{H}_2$, 7782-39-0.

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