Natural Abundance ^{2}H Nuclear Magnetic Resonance Study of the Origin of Raspberry Ketone

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The site-specific natural abundance deuterium distribution of raspberry ketone **3** obtained through a variety of methods has been determined through ^{2}H NMR spectroscopy. This technique provided a means of distinguishing between "natural" raspberry ketones biogenerated from 4-hydroxybenzalacetone (**2**), obtained from 4-hydroxybenzaldehyde of extractive botanical origin and acetone produced by sugar fermentation by reduction using baker's yeast and other microorganisms, and other raspberry ketone samples obtained in different "non-natural" ways. Of natural origin is also a commercial sample obtained in an unspecified manner. A mechanistic interpretation has been proposed to explain the difference of site-specific deuterium content between the examined samples.

Keywords: *Natural abundance ^{2}H NMR; raspberry ketone; natural; adulteration*

There is a current interest in the flavor industry for the generation of substantial quantities of products possessing relevant sensory properties occurring in nature in trace amounts by microbial transformations of precursors accessible by extractive manipulation of plant materials. Relevant examples in this field are the manufacture of (*R*) γ-decanolide by β-oxidation of ricinoleic acid using various microorganisms (Gatfield, 1986), of (*R*) δ-decanolide through the biological saturation of the double bond of Massoi lactone (van der Schaft el al., 1992; Fronza et al., 1992), of (*Z*) 3-hexenol (Müller and Gautier, 1994) from linolenic acid in a multistep enzymic sequence involving, at the latest stage, the use of baker's yeast, and of hexanol by reduction of hexanoic acid in growing cultures of *Colletotrichum gloeosporioides* (Fronza et al., 1995a). Enhanced commercial appeal and value are indeed conferred upon the materials produced in this manner because they can be labeled "natural" products (Stofberg, 1986), thus receiving increased consumer preferences. However, a major problem faced in this area is the occurrence of adulterations of these expensive natural materials with readily available "nature-identical" products of petrochemical origin. The measurement of the ^{13}C and/or ^{14}C content may be a useful tool to verify the nonpetrochemical origin of a substance. However, these methods do not guarantee against the transformation of natural precursors by "non-natural" synthetic methods.

This kind of adulteration might occur, for example, in the production of natural 4-(4-hydroxyphenyl)butan-2-one (raspberry ketone, **3**) from its unsaturated biosynthetic precursor **2**. Indeed, natural raspberry ketone **3**, the key aroma component of raspberry fruit (Schinz and Seidel, 1957), can be obtained in a process mimicking that of nature, by enzymic saturation of the double bond of 4-hydroxybenzalacetone (**2**), prepared upon condensation of 4-hydroxybenzaldehyde of extractive botanical origin with acetone produced by sugars fermentation, using several microorganisms (Fronza et al., 1996b; Fuganti et al., 1996a). In raspberry plant extracts, the C-6−C-4 unsaturated biosynthetic precursor **2** is formed, in turn, by hydrolysis and decarboxylation of the C-6−C-5 product **1** produced by condensation of C-6−C-3 *p*-coumaryl-CoA with malonyl-CoA (Scheme 1) (Borejsza-Wysocki and Hrazdina, 1994). Alternatively, natural raspberry ketone is accessible by enzymic oxidation of the corresponding carbinol, quite widespread in nature (Parmar et al., 1991), obtained by hydrolysis of the glucoside extracted from *Betula alba* (Dumont et al., 1995). Since catalytic hydrogenation of **2** to **3** could be a convenient alternative to the costly microbial process, a means of verifying the authenticity of the biological route followed on going from **2** to **3** as well as the naturalness of the precursor is desirable.

In light of the observations that site-specific deuterium distribution data of food components allowed in many instances (Fronza et al., 1995b, 1996b; Martin and Martin, 1995; Barbeni et al., 1997) a clear definition of their origin, we carried out and present in this paper a ^{2}H NMR study on raspberry ketone samples obtained through a variety of procedures to verify the utility of

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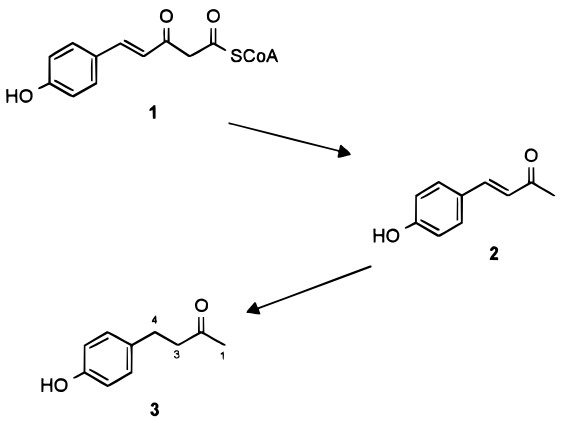
Scheme 1. Steps in the Biosynthesis of the Raspberry Ketone 3



this spectroscopic method in the identification of the pathway followed in its generation.

EXPERIMENTAL PROCEDURES

Deuterium NMR data (61.4 MHz) were recorded at 306 K on a Bruker ARX 400 spectrometer equipped with a process controller, a 10-mm selective deuterium probehead, and a ^{19}F lock channel, under CPD (Waltz 16 sequence) proton decoupling conditions. Samples of **3** were prepared by weighing about 0.7 g of solid sample and solubilized into (a) 3 mL of benzene plus 0.6 mL of dioxane; or (b) 3.6 mL of dioxane. Fifty microliters of hexafluorobenzene (Merck) for the ^{19}F lock and 70 mg of *tert*-butyl sulfide (Aldrich) as internal (D/H) standard were added. We did not use tetramethylurea (TMU) as internal standard since the signal of the NCH_3 groups is overlapped with the methylene peaks of raspberry ketone. On the contrary, the six methyl groups of *tert*-butyl sulfide produce a very intense singlet due to the 18 equivalent positions in a free zone of the spectrum (1.38 ppm in dioxane and 1.34 ppm in the mixture dioxane/benzene), thus allowing the use of a little quantity of material to reach a good S/N ratio. Moreover, *tert*-butyl sulfide is a high-boiling liquid (147−151 °C), inert and stable under the measurement conditions.

The (D/H) value of the internal standard was calibrated against the official standard TMU (Community Bureau of References, BCR) with a certified (D/H) of 136.67 ppm. The measured value of the internal standard resulted in a (D/H) of 130 ppm. Three spectra were run for each sample, collecting 4000 scans and using the following parameters: acquisition time, 6.8 s; spectral width, 1200 Hz; 16K memory size; and pulse length, 18 ms (90°). Each free induction decay (FID) was Fourier transformed with a line broadening of 2 Hz, manually phased and integrated. The signal-to-noise ratio (S/N) was >100 (methyl peak).

The molar fractions f_i were calculated from the integral areas as

$$f_i = S_i/\Sigma_i S_i \quad (1)$$

where S_i is the area of the ith peak.

The (D/H) values were calculated according to the formula

$$(D/H)_i\ (ppm) = I_{sa}M_{sa}W_{st}N_{st}(D/H)_{st}/I_{st}M_{st}W_{sa}N_{sa} \quad (2)$$

where I_{sa} is the integral for the i position of sample, I_{st} is the integral for standard, M_{sa} is the molecular weight of sample (164), M_{st} is the molecular weight of standard (146), W_{sa} is the weight of sample, W_{st} is the weight of standard, N_{sa} is the number of positions integrated for site i of sample (three for methyl and two for methylene and aromatic sites), N_{st} is the number of positions integrated for standard (18), and $(D/H)_{st}$ is the isotope ratio of the standard (130 ppm).

A total of 18 samples were analyzed:

- 6 natural samples [sample 1 obtained (in Milan) from 4-hydroxybenzalacetone **2**, prepared from 4-hydroxybenzaldehyde of natural extractive origin and acetone from sugar fermentation, upon baker's yeast reduction (Fronza et al., 1996); samples 7, 8, 14, and 15 obtained (in Grasse) from the natural precursor **2**, by reduction with different microorganisms; and sample 16 sold as natural in 1995 from one of the retailers that subsequently provided sample 11].
- 2 synthetic commercial samples (sample 4 of unspecified origin and sample 6 from Aldrich).
- 1 synthetic sample (sample 2) obtained from **2**, prepared from commercial synthetic 4-hydroxybenzaldehyde and acetone, upon catalytic hydrogenation at normal pressure and room temperature in ethyl acetate in the presence of 10% Pd/C. The material, separated from a minute amount of the corresponding carbinol upon SiO_2 column chromatography with increasing amounts of ethyl acetate in hexane, was crystallized from cyclohexane.
- 2 semisynthetic samples (sample 3, prepared by catalytic hydrogenation exactly as sample 2 using the natural precursor **2**, and sample 5, prepared exactly as sample 1 upon baker's yeast reduction of the synthetic precursor **2**, which afforded upon catalytic hydrogenation sample 2).
- 3 commercial samples sold as natural (samples 9−11).
- 2 synthetic samples (samples 12 and 13) obtained from sample 4 upon aqueous basic and acid treatment, respectively. Sample 12 has been obtained by submitting the synthetic sample 4 (4 g) to the action of a refluxing solution of NaOH, 10 g, in 80 mL of 1:1 tap water/1,4-dioxane, for 3 h. The cooled reaction mixture was poured under stirring into a two-phase system made up with 20 mL of concentrated sulfuric acid in 200 mL of ice water and 150 mL of methylene chloride. The organic phase was separated, washed with water and $NaHCO_3$ solution, and dried (Na_2SO_4). The residue obtained upon evaporation of the solvent was crystallized from cyclohexane. Sample 13 was obtained from sample 4, 5 g, and kept in concentrated HCl, 50 mL, at 100 °C for 1 h. The reaction mixture was diluted in ice−water, and the precipitate was collected upon filtration, washed with water, and, finally, crystallized from cyclohexane.
- 2 acetyl derivatives (samples 17 and 18) of the precursor **2**. Sample 17 was obtained by reacting synthetic precursor **2**, which provided samples 2 and 5 of raspberry ketone **3**, with 1.2 mol equiv of acetic anhydride and pyridine in methylene chloride for 16 h at room temperature. The reaction mixture was washed, in sequence, with dilute HCl, $NaHCO_3$ solution, and water, dried (Na_2SO_4), and evaporated under vacuum. The residue was crystallized from hexane. Sample 18 was obtained in the same manner starting from the natural modification of precursor **2**, which provided samples 1 and 3 of raspberry ketone **3**.

RESULTS AND DISCUSSION

Preliminarily, a study was performed designed to identify a suitable solvent enabling a satisfactory separation of the signals in the NMR spectrum of raspberry ketone **3**. After many attempts, we decided to use two different solvents: (a) dioxane, which produces a good separation for the signals of the aromatic hydrogens ($\Delta\delta = 0.32$ ppm) while the methylene groups almost overlap each other, and (b) the mixture benzene/dioxane in the volume ratio 3:0.6. In this mixture the signals of the methylene groups are well separated ($\Delta\delta = 0.37$ ppm), while those of the aromatic hydrogens are very near.

The ^2H spectra were run in both solvents for every sample to obtain precise measurements of the deuterium content for all groups of isotopomers. The spectra have been divided into five regions defining different groups of nuclei. The assignment of the signals of **3** in the two solvent systems is reported in Table 1 and

Table 1. Deuterium Chemical Shift Values of 4-(4-Hydroxyphenyl)butan-2-one (3) (Raspberry Ketone) and of the Acetyl Derivative of Its Unsaturated Precursor 2[a]

peak[b]	**3**	δ^c (ppm)	δ^d (ppm)	**2** (acetyl derivative)	δ^d (ppm)
1	-OH	7.56	7.60		
2	2 H *meta* OH	6.97	6.97	2 H *meta* OAc	7.62
3	2 H *ortho* OH	6.88	6.65	2 H *ortho* OAc	7.13
4	benzylic CH_2	2.71	2.72	=CH β CO	7.48
5	$-CO-CH_2-$	2.34	2.63	=CH α CO	6.70
6	$-CO-CH_3$	1.73	2.02	$-CO-CH_3$[e]	2.28
				$-CO-CH_3$[e]	2.23

[a] The chemical shifts are referred to internal TMS. [b] Numbering of different groups of nuclei used in Tables 2 and 3 for the isotopic parameters. [c] Solvent: dioxane (0.6 mL)/benzene (3.0 mL). [d] Solvent: dioxane. [e] Not assigned.

3 **2**

Figure 1. Numbering of the positions of the raspberry ketone **3** and of the unsaturated precursor **2** used in the ^2H NMR spectra and in the tables.

Figure 1 together with the assignment of the spectrum of the acetyl derivative of the unsaturated precursor **2**. The two resonances due to the methylene groups at positions 3 and 4 of the raspberry ketone framework (peaks 5 and 4, respectively, Table 1) were unambiguously assigned on the basis of studies on [1-$^2H_3$;3-$^2H_2$]-4-(4-hydroxyphenyl)butan-2-one, prepared from **3** upon base-catalyzed exchange with deuterated water (Fuganti et al., 1996b). The $(D/H)_i$ values of the examined samples are reported in Table 2, whereas the molar fraction values f_i, calculated without taking into account the mobile hydrogen atom of the phenolic group, are reported in Table 3. Since the signal of the methyl group of **3** (peak 6, Table 1) falls in a free zone of the spectrum either in dioxane or in benzene/dioxane mixture, the $(D/H)_6$ values could be measured precisely in both solvents. Such values were practically identical within experimental error (see Table 2), showing that the change of the solvent does not introduce any systematic error in the evaluation of the deuterium content.

The analyzed samples include the following: (i) two synthetic commercial samples (samples 4 and 6); (ii) two samples obtained from the synthetic sample 4 upon aqueous basic and acid treatment, respectively (samples 12 and 13); (iii) five natural samples obtained in two laboratories upon microbial reduction of the natural precursor **2** (samples 1, 7, 8, 14, and 15); (iv) one sample obtained upon catalytic hydrogenation of the natural precursor, which provided sample 1 (sample 3); (v) one sample obtained upon catalytic hydrogenation of the synthetic modification of precursor **2** (sample 2); (vi) one sample obtained upon baker's yeast reduction of the synthetic precursor used in the preparation of sample 2 (sample 5); (vii) three commercial samples sold as natural (samples 9−11); (viii) the acetyl derivative of the synthetic and, respectively, natural modification of precursor **2**, used in the production of samples 2 and 5 and, respectively, 1 and 3 (samples 17 and 18). Moreover, we include in the subsequent discussion also a commercial sample of raspberry ketone sold as natural, the deuterium content of which has been analyzed previously by one of the authors (D.J.) in another laboratory (sample 16).

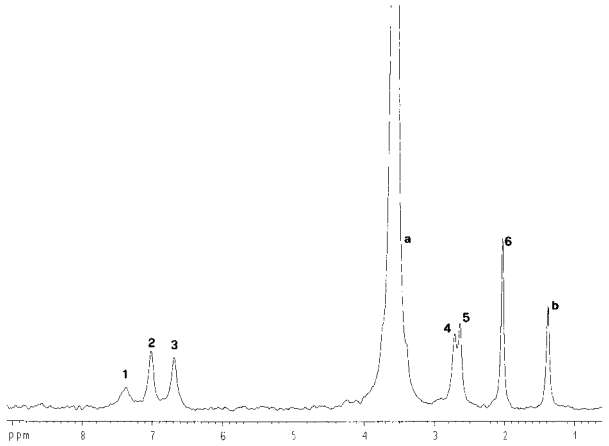


Figure 2. Deuterium spectrum in dioxane of synthetic raspberry ketone (sample 6): (a) signal of the solvent; (b) signal of *tert*-butyl sulfide used as internal (D/H) standard. The peaks are numbered according to Table 1.

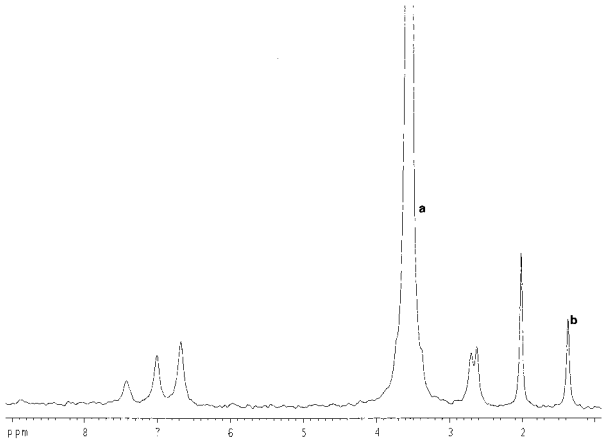


Figure 3. Deuterium spectrum in dioxane of natural raspberry ketone (sample 7): (a) signal of the solvent; (b) signal of *tert*-butyl sulfide used as internal (D/H) standard.

The deuterium content values $(D/H)_2$ and $(D/H)_3$ (Table 2) relative to the aromatic portion of the molecule allow a clear-cut differentiation between the samples derived from the synthetic precursor **2** and those obtained from the natural one. The samples obtained from the synthetic **2**, irrespective of the method followed in the conversion, show $(D/H)_3 < (D/H)_2$, whereas the reverse is true for samples 1, 3, 7, 8, 14, and 15, derived from the natural material, and for the commercial sample 16 of unspecified origin. The spectra in dioxane of the synthetic sample 6 and of the natural sample 7 are reported in Figures 2 and 3, respectively, clearly showing the different behaviors of the aromatic signals (peaks 2 and 3). The deuterium spectrum of sample 7 obtained in the mixture benzene/dioxane is reported in Figure 4 for the purpose of comparison with the spectrum obtained in pure dioxane. The difference of deuterium content observed between natural and synthetic samples of **3** well reflects the isotopic distribution present in samples 17 and 18, corresponding to the acetyl derivative of the synthetic and natural modifications, respectively, of the unsaturated precursor **2**. The spectra in dioxane of these samples are reported in

Table 2. $(D/H)_i$ Isotopic Ratios for Raspberry Ketone 3 and for the Acetyl Derivative of the Precursor 2[a]

sample	$(D/H)_2$[b]	$(D/H)_3$[b]	$(D/H)_4$[c]	$(D/H)_5$[c]	$(D/H)_6$[c]	$(D/H)_6$[b]	$(D/H)_3/(D/H)_2$	$(D/H)_5/(D/H)_4$
1	130.5 (2.3)	147.9 (2.0)	203.0 (3.6)	176.2 (2.8)	128.6 (2.5)	127.5 (1.8)	1.13	0.87
2	128.5 (2.9)	124.8 (3.1)	202.5 (3.4)	109.0 (2.4)	134.0 (2.7)	135.0 (2.3)	0.97	0.54
3	131.0 (3.1)	182.0 (2.7)	92.8 (2.2)	112.5 (1.9)	127.6 (2.0)	128.0 (2.6)	1.39	1.21
4	130.9 (2.8)	124.4 (1.8)	151.3 (1.7)	128.7 (2.5)	130.9 (2.8)	132.5 (2.4)	0.95	0.85
5	121.3 (3.4)	119.5 (2.6)	202.6 (1.4)	120.6 (2.0)	124.6 (2.1)	123.5 (1.5)	0.99	0.60
6	142.0 (2.9)	132.9 (1.9)	129.4 (2.4)	115.5 (3.2)	122.1 (2.8)	123.0 (2.8)	0.94	0.89
7	149.2 (2.5)	180.4 (2.5)	100.7 (3.0)	101.7 (2.6)	125.8 (2.9)	127.5 (3.2)	1.21	1.01
8	118.2 (3.0)	156.0 (2.6)	112.7 (3.2)	100.2 (2.6)	121.9 (3.2)	123.6 (2.7)	1.32	0.89
9	113.7 (2.8)	105.2 (2.8)	147.8 (3.2)	116.8 (2.8)	122.5 (2.5)	121.5 (2.0)	0.93	0.79
10	113.2 (2.7)	109.0 (3.1)	161.3 (1.8)	114.4 (2.4)	120.6 (2.3)	119.5 (1.7)	0.96	0.71
11	127.9 (2.9)	114.8 (2.8)	121.7 (2.0)	115.9 (2.7)	124.5 (1.9)	124.9 (2.1)	0.90	0.95
12	148.8 (2.1)	125.0 (2.1)	141.3 (3.1)	126.6 (3.1)	125.0 (2.7)	124.0 (2.3)	0.84	0.90
13	128.7 (2.5)	123.3 (2.4)	141.5 (3.1)	124.6 (2.5)	118.9 (3.0)	120.5 (2.8)	0.96	0.88
14	143.3 (2.5)	178.7 (2.7)	99.0 (2.7)	92.3 (2.4)	124.6 (2.3)	124.1 (1.9)	1.25	0.93
15	140.0 (2.5)	174.7 (2.1)	119.7 (2.2)	102.6 (2.3)	123.9 (2.3)	123.0 (2.3)	1.25	0.86
16[b]	112.6	131.1	103.7	73.1		88.8	1.16	0.70
17[b]	115.9 (8.0)	107 (4.5)	156 (6.5)	53.5 (4.5)			0,92	0.34
18[b]	119.7 (7.3)	132.3 (4.8)	67.6 (8.5)	55.7 (5.1)			1.11	0.82

[a] The $(D/H)_i$ values are expressed in ppm and are averaged over three determinations; the standard deviations are reported in parentheses. [b] Solvent: dioxane. [c] Solvent: dioxane (0.6 mL)/benzene (3.0 mL).

Table 3. Deuterium Molar Fractions for Raspberry Ketone 3 and for the Acetyl Derivative of the Precursor 2[a]

sample	f_2[b]	f_3[b]	f_4[c]	f_5[c]	f_6[b]	f_3/f_2	f_5/f_4
1	0.151 (3)	0.171 (4)	0.246 (4)	0.207 (3)	0.223 (3)	1.13	0.84
2	0.170 (4)	0.164 (3)	0.255 (4)	0.143 (3)	0.268 (4)	0.96	0.56
3	0.218 (4)	0.293 (4)	0.120 (3)	0.142 (5)	0.233 (3)	1.34	1.18
4	0.181 (5)	0.168 (4)	0.208 (5)	0.177 (4)	0.266 (3)	0.93	0.85
5	0.166 (3)	0.162 (3)	0.275 (4)	0.149 (4)	0.249 (4)	0.98	0.54
6	0.207 (5)	0.202 (5)	0.176 (4)	0.168 (3)	0.248 (5)	0.98	0.95
7	0.216 (5)	0.263 (5)	0.139 (6)	0.141 (6)	0.240 (5)	1.22	1.01
8	0.185 (4)	0.239 (5)	0.17 (4)	0.152 (3)	0.254 (6)	1.29	0.89
9	0.182 (5)	0.171 (4)	0.212 (5)	0.176 (5)	0.258 (4)	0.94	0.83
10	0.170 (4)	0.166 (6)	0.256 (5)	0.190 (5)	0.220 (4)	0.98	0.74
11	0.207 (5)	0.188 (3)	0.182 (5)	0.172 (4)	0.250 (3)	0.91	0.95
12	0.212 (4)	0.185 (2)	0.196 (3)	0.171 (3)	0.236 (4)	0.87	0.87
13	0.189 (3)	0.185 (4)	0.204 (3)	0.180 (3)	0.242 (5)	0.98	0.88
14	0.221 (4)	0.281 (4)	0.137 (4)	0.127 (5)	0.234 (3)	1.27	0.93
15	0.187 (3)	0.231 (3)	0.178 (5)	0.150 (4)	0.254 (3)	1.24	0.84
16	0.203	0.237	0.187	0.32	0.241	1.17	0.71
17	0.268 (10)	0.247 (5)	0.361 (11)	0.124 (8)		0.92	0.34
18	0.307 (9)	0.352 (5)	0.187 (10)	0.154 (6)		1.14	0.82

[a] The molar fraction values are averaged over three determinations: the standard deviations $\times 10^3$ are reported in parentheses. [b] Solvent: dioxane. [c] Solvent: dioxane (0.6 mL)/benzene (3.0) mL.

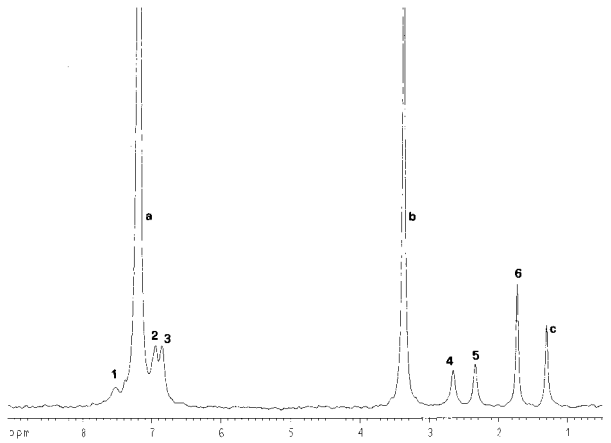


Figure 4. Deuterium spectrum in dioxane (0.6 mL)/benzene (3 mL) mixture of natural raspberry ketone (sample 7): (a) signal of benzene; (b) signal of dioxane; (c) signal of *tert*-butyl sulfide used as internal (D/H) standard. The peaks are numbered according to Table 1.

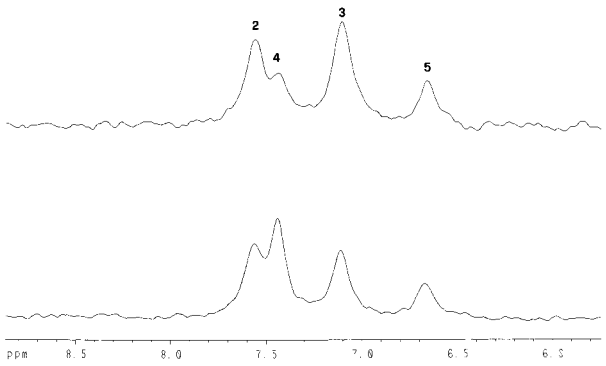


Figure 5. Expanded aromatic region of the deuterium spectra of the acetyl derivative of the precursor **2** of raspberry ketone in the synthetic (bottom trace, sample 17) and natural (top trace, sample 18) modifications. The peaks are numbered according to Table 1.

Figure 5 showing in pictorial form the significant difference in the deuterium content of the aromatic sites.

Very recently, during the writing of the present paper, the results of a SNIF NMR (a trademark of Eurofins Laboratories, Nantes) study carried out on vanillin and *p*-hydroxybenzaldehyde have been published (Remaud et al., 1997). The authors showed that the deuterium content for the position ortho to the hydroxyl group of *p*-hydroxybenzaldehyde is higher for natural samples than for samples of synthetic origin, a trend very similar to that of raspberry ketone outlined here.

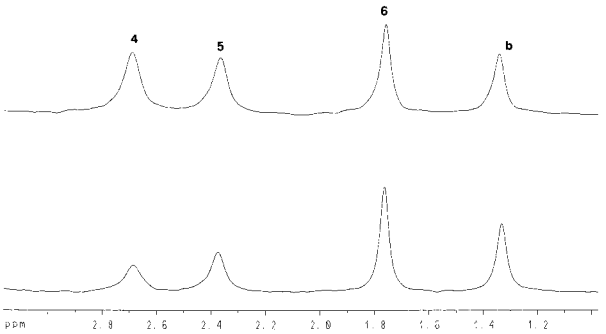
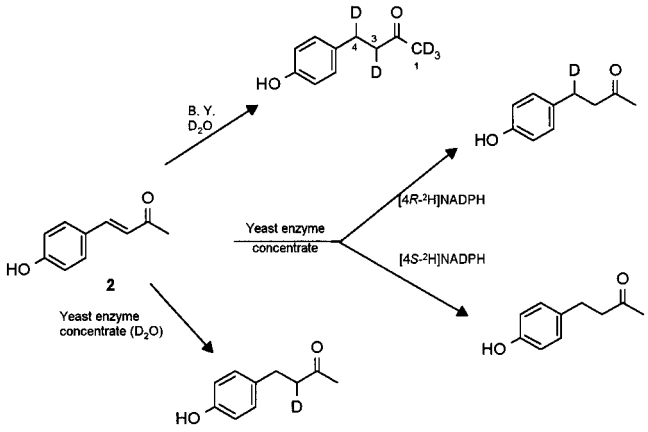


Figure 6. Expanded aliphatic region of the deuterium spectra of raspberry ketones obtained from the natural precursor **2** by reduction with baker's yeast (top trace, sample 1) and by catalytic hydrogenation (bottom trace, sample 3): (b) signal of *tert*-butyl sulfide used as internal (D/H) standard. The peaks are numbered according to Table 1.

Scheme 2. Deuterium Labeling Experiments in the Bioconversion of the Unsaturated Precursor 2 to the Raspberry Ketone 3 Using Yeast Enzymic Systems



An indication of the method followed in the conversion of the precursor **2** into raspberry ketone **3** arises from the $(D/H)_4$ and $(D/H)_5$ values, relative to the methylene groups in positions 4 and 3, respectively, of the raspberry ketone framework. Indeed, these figures fall from 203 and 176.2 ppm to 92.8 and 112.5 ppm, respectively, on going from sample 1 to sample 3 (Figure 6), obtained from the same natural precursor **2** (sample 18), using fermenting baker's yeast and catalytic hydrogenation, respectively. The lower deuterium content exhibited by the side-chain methylene groups of sample 3 with respect to sample 1 is due to the isotopic composition of the employed hydrogen gas, produced from methane of petrochemical origin, depleted of deuterium. On the contrary, sample 2, which is produced from the synthetic precursor **2** using as for sample 3 the catalytic hydrogenation, shows a much higher value of $(D/H)_4$ (202.5 ppm) than does sample 3 (92.8 ppm), reflecting in this case the high deuterium content in this position (156 ppm) of the starting material (sample 17).

More complex is the interpretation of the $(D/H)_4$ and $(D/H)_5$ data relative to the raspberry ketone samples obtained from the unsaturated precursors upon enzymic reduction. In the baker's yeast conversion of **2** into **3**, there should be a formal delivery at position 4 of the unsaturated framework of **2** of hydrogen enriched in deuterium species. In fact, the $(D/H)_4$ value changes from 67.6 to 203 ppm, respectively, on going from the unsaturated sample 18 to sample 1 of raspberry ketone. On the contrary, samples 7, 8, 14, and 15 obtained with different microbial systems show $(D/H)_4$ ratios much lower than that of sample 1. According to the proposed mechanism of enzymic reduction of carbonyl-activated double bonds (Sedgwick and Morris, 1980), the hydrogen atom incorporated α to the carbonyl group originates directly from water, while that at the β position is delivered with the intermediacy of reduced nicotine cofactor(s). This was indeed the case when the unsaturated ketone **2** was reduced using a yeast enzyme concentrate (Fronza et al., 1996b). Moreover, under these circumstances the hydrogen atom stereospecifically delivered in the β position of the framework of **2** arises from [4*R*-^{2}H]NADPH and not from the 4*S* diastereoisomer (Scheme 2).

However, baker's yeast reduction of **2** in deuterated water leads to **3** labeled with deuterium in positions 1, 3, and 4 (Scheme 2). The incorporation of deuterium in position 4 of **3** in the whole-cell system is explained through the intervention of a diaphorase-mediated exchange of the hydrogen atom at position 4 of the reduced nicotinamide cofactor to be delivered in the reduction process with the solvent hydrogen atoms, a phenomenon repeatedly observed in baker's yeast-mediated reductions of carbonyl-activated double bonds (Fronza et al., 1992, 1993; Fogliato et al., 1995). The latter exchange, effective in baker's yeast, and, not necessarily, also in the other microbial systems used in the present work, is conceivably submitted to a deuterium kinetic isotope effect and can vary from one experiment to another. These circumstances could explain, together with the different precursor, the variations of the $(D/H)_4$ values observed on going from entry 1 to entries 7, 8, 14, and 15.

Similarly, the $(D/H)_5$ values of the *natural* raspberry ketone change from the value of 176.2 ppm of sample 1, produced in baker's yeast, to 92.3−102.6 ppm for the set of samples 7, 8, 14, and 15, produced with different microbial systems. The values 103.7 and 73.1 ppm for $(D/H)_4$ and $(D/H)_5$ measured for natural sample 16, although obtained under spectroscopic experimental conditions different from those used here, indicate a similarity of the latter compound with the natural specimens 7, 8, 14, and 15.

The sets of samples 4 and 6 and 9−11, respectively, possess $(D/H)_5$ values in the range 114.4−128.7 ppm. When commercial sample 4 was submitted to acid and base-catalyzed enolization, under conditions that caused almost complete exchange of hydrogen for deuterium when using deuterated water as solvent, the $(D/H)_5$ did not change significantly, as shown by the values of 126.6 and 124.6 ppm, respectively, of samples 14 and 15 so obtained. This observation suggests a similar story for samples 4, 6, and 9−11. This view is further supported by the $(D/H)_3$ and $(D/H)_2$ values of samples 4, 6, and 9−11, quite similar along the whole set, and remarkably different from those of samples 1, 7, 8, and 14−16 of natural origin.

Seen together, the data allow a distinction of the examined raspberry ketone samples in two sets. The former, possessing $(D/H)_3/(D/H)_2 > 1$ (Table 2), include all of the samples derived from the precursor **2** obtained by condensing extractive 4-hydroxybenzaldehyde with acetone from sugar fermentation and sample 16 of unknown derivation. In the second set are included the commercial synthetic materials and samples 9−11 sold as natural. However, there is a further distinction among the products of the first set, derived from the

Table 4. Mean Values of $(D/H)_i$ and f_i of Raspberry Ketone 3

	$(D/H)_2$[a]	$(D/H)_3$[a]	$(D/H)_4$[b]	$(D/H)_5$[b]	$(D/H)_6$[b]	$(D/H)_6$[a]	$(D/H)_3/(D/H)_2$	$(D/H)_5/(D/H)_4$
				Natural Samples (Six Samples)				
mean	135.4	170.0	121.3	114.3	125.4	125.6	1.26	0.96
(SD)	(11.1)	(14.4)	(41.2)	(31.0)	(2.5)	(2.3)	(0.09)	(0.13)
				Synthetic Samples (Nine Samples)				
mean	128.3	119.9	155.5	119.1	124.8	124.9	0.94	0.79
(SD)	(11.8)	(8.7)	(29.1)	(6.5)	(4.8)	(5.3)	(0.04)	(0.14)

	f_2[a]	f_3[a]	f_4[b]	f_5[b]	f_6[a]	f_3/f_2	f_5/f_4
			Natural Samples (Six Samples)				
mean	0.192	0.243	0.170	0.156	0.240	1.26	0.94
(SD)	(0.029)	(0.048)	(0.049)	(0.03)	(0.014)	(0.08)	(0.14)
			Synthetic Samples (Nine Samples)				
mean	0.190	0.185	0.210	0.167	0.248	0.97	0.82
(SD)	(0.019)	(0.030)	(0.042)	(0.017)	(0.014)	(0.09)	(0.09)

[a] Solvent: dioxane. [b] Solvent: dioxane (0.6 mL)/benzene (3.0) mL.

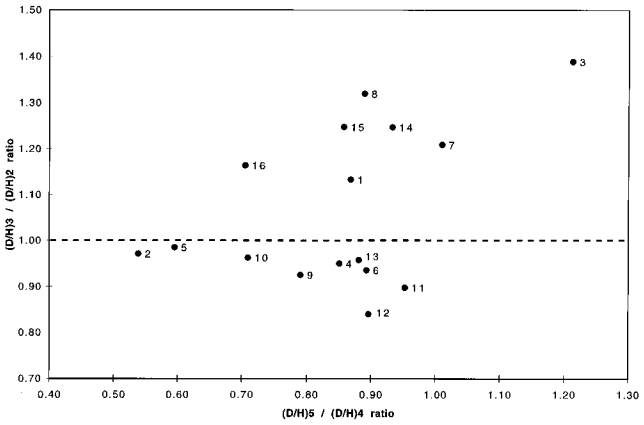


Figure 7. Graphical representation of the ratios $(D/H)_3/(D/H)_2$ vs $(D/H)_5/(D/H)_4$ showing two distinct regions for the natural samples (1, 7, 8, and 14−16) and the synthetic samples (4, 6, 9−11, and 13). Sample 3 prepared from the natural precursor **2** by catalytic reduction appears in a well-separated position. Also, the samples 2 and 5 prepared from the synthetic precursor **2** by catalytic and baker's yeast reduction, respectively, are separated from the other synthetic samples.

natural precursor. Indeed, sample 3 obtained upon catalytic hydrogenation shows a $(D/H)_5/(D/H)_4$ value much higher that those of samples 1, 7, 8, 14, and 15 produced upon bioreduction. Thus, the plot of $(D/H)_3/(D/H)_2$ vs $(D/H)_5/(D/H)_4$ (Figure 7) of the examined samples (entries 1−16) allows the graphical identification of two regions. One includes the natural products formed by reduction of the unsaturated precursor, whereas the other contains the commercial synthetic materials and samples 9−11. In separated positions appear synthetic samples 2 and 5, prepared in the present study through the procedures outlined above, and natural sample 16, claimed as natural, as well as sample 3, originated from the natural precursor by catalytic hydrogenation. In addition, the mean values of f_i and $(D/H)_i$ values are reported in Table 4 for the samples of natural origin (six samples) and of synthetic origin (nine samples). These values confirm the trends outlined above: (i) the mean value of $(D/H)_3$ for natural samples is much higher than that for synthetic samples; (ii) the mean $(D/H)_2$ values are practically indistinguishable for the natural and synthetic raspberry ketones; and (iii) the $(D/H)_4$ mean value is smaller for natural than for synthetic samples.

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