Natural Abundance ^2H Nuclear Magnetic Resonance Study of the Origin of (*R*)-δ-Decanolide

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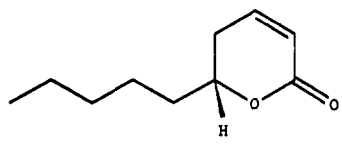
The site-specific natural abundance deuterium distribution of (*R*)-δ-decanolide obtained through a variety of synthetic methods has been determined by ^2H NMR spectroscopy. This technique provided a means of distinguishing between "natural" δ-decanolide biogenerated from δ-2-decenolide isolated from *Cryptocaria massoia* (Massoi lactone) with bakers' yeast and other δ-decanolides obtained in different "non-natural" ways. A mechanistic interpretation has been proposed to explain the difference of site-specific deuterium content between the δ-decanolides obtained by reduction of Massoi lactone with bakers' yeast or by catalytic hydrogenation.

The generation of substantial quantities of products of relevant sensory properties occurring in nature in trace amounts by microbial degradation of abundant extractive materials is of current interest in the flavor industry, as shown by the manufacture of (*R*)-γ-decanolide from ricinoleic acid using various microorganisms (Gatfield, 1986). 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. Accordingly, several criteria, including the determination of the enantiomeric composition of chiral molecules (Werkhoff et al., 1990), have been proposed for the determination of the "naturalness" of the aroma components. The measurement of the ^{13}C and/or ^{14}C content is also a useful tool to verify the nonpetrochemical origin of a substance. However, it does not guarantee against the transformation of natural precursors by "non-natural" synthetic methods.

This kind of adulteration might occur in the production of natural (*R*)-δ-decanolide (2) from the unsaturated analog 1. Indeed, natural (*R*)-δ-decanolide (2), a key aroma component not accessible by extraction, can be prepared by biohydrogenation of 1 (Massoi lactone), isolated from the bark of the Massoi tree (*Cryptocaria massoia*) (van der Schaft et al., 1992). Since catalytic hydrogenation of 1 to 2 could be a convenient alternative to the costly microbial process, a means of verifying the authenticity of the biological route followed on going from 1 to 2 is desirable.



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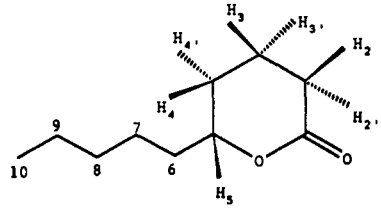

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In the light of the observations that site-specific deuterium distribution data of food components allowed in many instances (Martin et al., 1982, 1983, 1986; Grant et al., 1982; Toulemonde and Horman, 1983; Hagedorn, 1992; Hanneguelle et al., 1992; Carle et al., 1992) a clear definition of their origin, we carried out and present in this paper a ^2H NMR study on δ-decanolide samples obtained through a variety of procedures to verify the potential utility of this spectroscopic method in the identification of the pathway followed in its generation.

EXPERIMENTAL PROCEDURES

Deuterium NMR data (46.076 MHz) were recorded at 302 K on a Bruker AC 300 spectrometer equipped with process controller, a 10-mm selective deuterium probehead, and a ^{19}F lock channel, under broad-band proton decoupling conditions. Samples were prepared by carefully weighing nearly pure (95% GC) δ-decalactone (about 3 g), hexafluorobenzene for ^{19}F lock (150 mg, Merck), and dioxane as internal (D/H) standard (500 mg, Fluka). Dioxane was previously tested by isotope ratio mass spectrometry (IRMS) and showed an averaged (D/H) value of 150.5 ppm [standard mean ocean water (SMOW)]. The NMR measurement of a sample composed by dioxane and official TMU (tetramethylurea) [Community Bureau of Reference (BCR EC010) (D/H) = 136.67 ppm] gave a (D/H) value for dioxane consistent with the IRMS value. Five spectra were run for each sample, collecting 1600 scans and using the following parameters: 6.8-s acquisition time, 0.05-s relaxation delay, 1200-Hz spectral width, 16K memory size, 15-μs (90°) pulse length. Each free induction decay was Fourier transformed with no zero filling (0.15 Hz/point digital resolution) and a line broadening of 0.5 Hz, manually phased and integrated. S/N was > 130. The molar fractions f_i were calculated from the integrated areas

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

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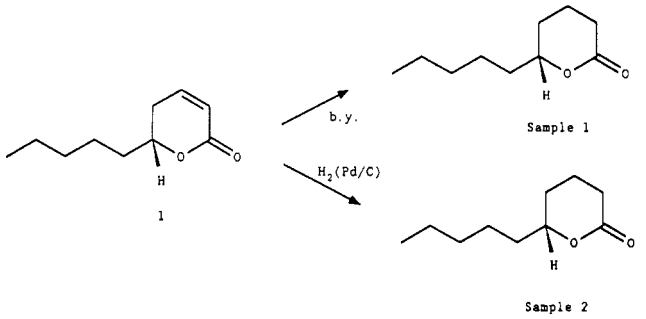
Scheme I



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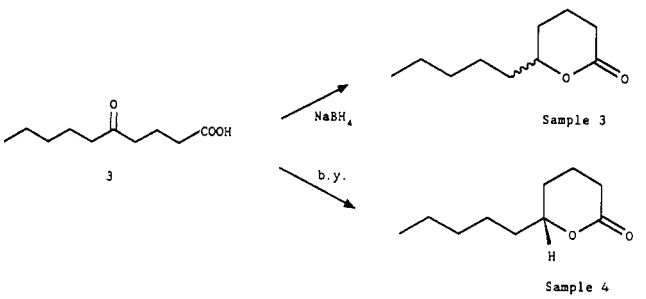
Scheme II



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where S_i is the area of the ith peak. The corresponding statistical molar fractions are

$$F_i = n_i / \sum_i n_i \quad (2)$$

where n_i is the number of equivalent or isochronous deuterium nuclei of the ith peak. The absolute values of the site-specific (D/H) ratios were calculated according to the formula (Martin et al., 1985)

$$(D/H)_i = n_{WS} G_{WS}(MW_L)\, S_i (D/H)_{WS} / n_i g_L (MW_{WS})\, S_{WS} P_L \quad (3)$$

where WS stands for the working standard (dioxane) with a known isotope ratio $(D/H)_{WS}$ and L for the product under examination; n_{WS} and n_i are the number of equivalent hydrogens of dioxane and of the ith peak; g_{WS} and g_L are the weights of dioxane and lactone; MW_L and M_{WS} are the corresponding molecular weights; S_i and S_{WS} are the areas of ith peak and of dioxane, respectively; P_L is the purity of the lactone. $(D/H)_{WS}$ is the working standard isotope ratio as determined by isotope-ratio mass spectrometry on the SMOW scale (Gonfiantini, 1978).

Sample 1 of (*R*)-δ-decanolide was obtained upon bakers' yeast reduction of Massoi lactone 1 (Robertet, Grasse, France) as reported in the literature (van der Schaft et al., 1992), whereas sample 2 was prepared from the same substrate by catalytic hydrogenation in ethyl acetate at room temperature in the presence of 10% Pd/C using hydrogen gas from methane cracking (Scheme I).

Samples 3 and 4 were prepared from the keto acid 3 obtained from glutaric acid and 1-bromopentane (Aldrich) according to a reported general procedure (Fryisawa and Sato, 1988). This material in one instance was treated with bakers' yeast (Franke, 1965; Utaka et al., 1987) to give sample 4 of δ-decanolide in the *R* enantiomeric form. Alternatively, the keto acid was treated with $NaBH_4$ in ethanol, affording, after acidification and usual workup, sample 3 of (*R,S*)-δ-decanolide (Scheme II).

Finally, the commercial samples 5, 6, 7, and 8, all of synthetic origin, were gifts of Grinsted, Charabot, Van Neck, and Treatt's, respectively.

RESULTS AND DISCUSSION

The assignment of the ^2H spectrum of δ-decalactone **2** is based on the analysis of the proton spectrum of a concentrated solution of **2** in $CDCl_3$ performed by homonuclear correlation experiments (COSY). The chemical shifts reported in Table I are almost identical to that of the pure liquid used for the natural abundance ^2H measurements. The analyzed samples of **2** obtained with different synthetic methods included the following: (i) the *R* product formed by bakers' yeast reduction of Massoi lactone 1 (sample 1); (ii) the *R* product formed by catalytic hydrogenation of 1 using 10% Pd/C and hydrogen gas arising from methane cracking (sample 2); (iii) the racemic product formed by $NaBH_4$ reduction of 5-oxodecanoic acid (**3**) (sample 3); (iv) the *R* material formed from the same precursor by bakers' yeast reduction in tap water in the presence of D-glucose (sample 4); and (v) four commercial racemic samples of unspecified synthesis obtained from different suppliers (samples 5–8).

Table I. Assignment of the Deuterium Spectrum of δ-Decanolide 2

group[a]	nucleus[b]	δ,[c] ppm
1	5	4.5
2	2, 2′	2.5–2.7
3	3, 3′, 4	2.1
4	4′, 6, 7, 8, 9	1.5–1.8
5	10	1.1

[a] Numbering of the different groups of nuclei used in Table II for the isotopic parameters. [b] Nuclei numbering according to structure **2**. [c] Chemical shifts referred to the dioxane signal taken at 3.8 ppm.

The ^2H spectra of samples 1, 2, and 5 obtained at 46 MHz are shown in parts A, B, and C, respectively, of Figure 1. The sharp peak at 3.8 ppm is the reference peak (dioxane) used for the determination of the absolute $(D/H)_i$ ratios (see Experimental Procedures). Integration of the five different regions of the spectrum allowed us to calculate the molar fractions f_i which define the intramolecular distribution of deuterium atoms of the five groups of isotopomers. In addition, we have also determined the absolute site-specific isotope ratios $(D/H)_i$ to compare the ^2H content of a given site in different samples (Martin et al., 1985). All data are reported in Table II.

We have limited our investigation to one sample for every specimen since presently our aim is essentially to explore the possibilities of differentiating δ-decalactones obtained via different synthetic methods in the light of the potential interest of biogenerated **2** in the flavor industry. Furthermore, the spectra of **2** are rather complicated, and some signals or group of signals are not very well resolved. For these reasons we decided to take in account and discuss differences of the site-specific deuterium content exceeding 20% of the molar fractions or of the absolute D/H values. Such variations are generally much larger than the standard deviations of the measurements reported in Table II.

The data of samples 1 and 2 (Table II) concern the δ-decalactone obtained from the δ-2-decenolide of Massoi bark oil by reduction with bakers' yeast and by catalytic hydrogenation, respectively (Scheme I). Their ^2H spectra are reported in parts A and B, respectively, of Figure 1. From the comparison of the site-specific deuterium distribution, significant variations can be observed both for mole fractions f_2 and f_3 and for the absolute values $(D/H)_2$ and $(D/H)_3$. Detailed examination of the spectrum of sample 2 shows that this δ-decalactone is largely deuterium depleted at position 2 (signal at 2.7 ppm), while the deuterium proportion at position 2′ (signal at 2.54 ppm) remains practically unchanged compared with that of sample 1. Sample 2 is the only example among the examined samples 1–8 showing a remarkably different deuterium content between positions 2 and 2′ (ratio 2/2′ = 0.6). For all other samples the intensities of the two peaks are nearly equal. The distinction between the positions 2 and 2′ was made in a diluted chloroform solution of **2** by irradiation of proton H-5. In this experiment an

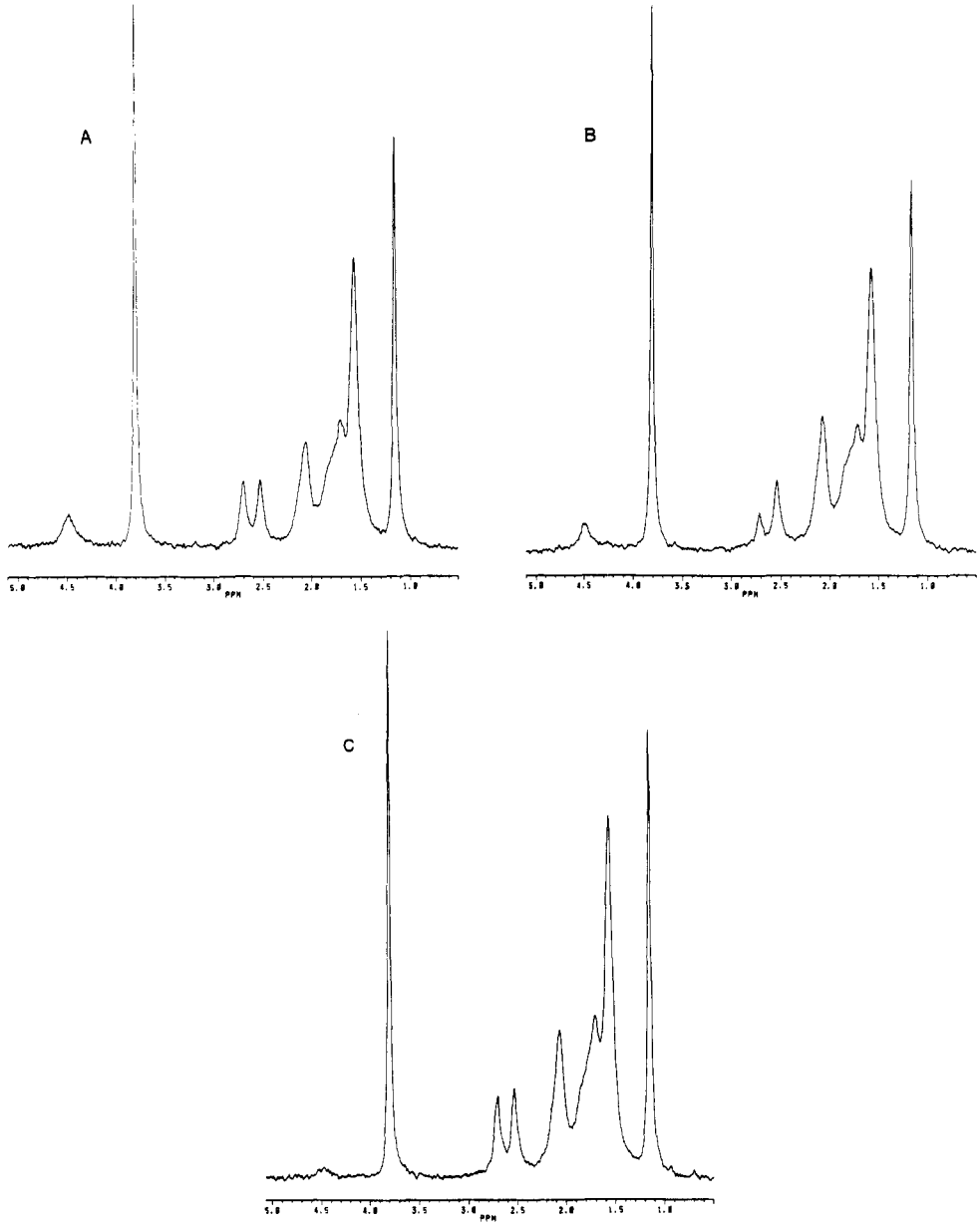

Figure 1. Natural abundance ^{2}H NMR spectra of δ-decanolides obtained from (A) Massoi lactone with bakers' yeast (sample 1), (B) Massoi lactone by catalytic reduction (sample 2), and (C) commercial δ-decanolide (sample 5).

NOE of ca. 1% was observed for the low-field proton, which thus must be assigned cis to H-5, while no NOE was detected for the high-field proton (trans to H-5). In a previous work we elucidated the steric course of the catalytic reduction of 1 using deuterium gas (Fronza et al., 1992). The reduction proceeds in a syn fashion and occurs trans to the alkyl substituent at C-5; i.e., the deuterium atoms replace the hydrogens H-2 and H-3 of the (*R*)-δ-decalactone (2). In the light of these observations the very small proportion of deuterium at position 2 of sample 2 must be due either to a small content of deuterium in the hydrogen gas used for the reduction or to a kinetic control exerted by the Pd/C catalyst.

On the contrary, the mole fraction f_3 and the $(D/H)_3$ value representing the deuterium content at positions 3, 3′, and 4 of δ-decalactone 2 are significantly lower for sample 1 than for sample 2. This behavior can be rationalized in the light of our previous study (Fronza et al., 1992) where (*R*)-δ-2-decenolide (1) was reduced by bakers' yeast in deuterated water. In this experiment we observed, using a mixture 80:20 of D_2O/H_2O as solvent, an anti addition to the double bond of deuterium atoms being found at positions 2′ and 3 of the final δ-decalactone. Interestingly, the deuterium incorporation is high (ca. 70%) at position 2′ and much smaller (ca. 45%) at position 3. Clearly in this experiment both deuterium atoms must originate from deuterated water. According to the proposed mechanism of microbial reduction of carbonyl-activated double bonds (Sedgwick and Morris, 1980), the deuterium in position 2′ originates directly from water, while the deuterium at position 3 is delivered with the intermediacy of reduced nicotine cofactor(s) which can exchange the hydrogen atom with water under the catalysis of diaphorase. Reasonably, the observed reduced content of deuterium in position 3 is due to a large kinetic isotope effect controlling the deuterium transfer rate from the reduced cofactor to carbon C-3 of 2-δ-decenolide. These observations explain nicely the reduced intensity of the signal at 2.1 ppm, grouping the resonances of 3, 3′, and 4 deuterium atoms, observed in the natural abundance ^{2}H spectrum of δ-decalactone obtained in bakers' yeast compared with that obtained by catalytic reduction.

It would be interesting at this point to compare the absolute deuterium content of sample 1 with that of the

Table II. Site-Specific Deuterium Distribution in δ-Decanolide 2

	mole fraction of deuterium[a] and absolute D/H value[b]				
sample[c]	f_1 $(D/H)_1$	f_2 $(D/H)_2$	f_3 $(D/H)_3$	f_4 $(D/H)_4$	f_5 $(D/H)_5$
statistics[d]	0.056	0.111	0.167	0.500	0.167
1	0.056 (2)	0.130 (2)	0.077 (2)	0.515 (2)	0.222 (4)
	77.4 (3.5)	88.8 (2.2)	35.0 (0.9)	78.4 (2.5)	101.6 (2.5)
2	0.047 (3)	0.094 (2)	0.125 (1)	0.535 (5)	0.199 (3)
	67.1 (5.0)	67.3 (1.5)	59.7 (1.2)	85.0 (0.5)	95.1 (2.5)
3	0.048 (3)	0.141 (2)	0.103 (2)	0.510 (2)	0.198 (3)
	78.4 (6.4)	115.3 (2.9)	55.8 (2.3)	92.4 (1.8)	107.3 (2.5)
4	0.036 (6)	0.143 (4)	0.094 (3)	0.522 (7)	0.205 (2)
	58.5 (10.2)	115.7 (2.8)	50.4 (2.7)	93.5 (2.8)	110.3 (2.5)
5	0.010 (5)	0.154 (6)	0.106 (4)	0.526 (4)	0.204 (5)
	15.6 (8.4)	121.4 (6.7)	55.4 (3.1)	92.1 (1.9)	107.0 (2.2)
6	0.012 (3)	0.134 (5)	0.107 (1)	0.543 (3)	0.203 (6)
	20.1 (4.0)	108.5 (3.9)	57.8 (1.3)	97.6 (1.9)	109.3 (4.5)
7	0.018 (2)	0.120 (5)	0.084 (1)	0.550 (6)	0.228 (2)
	23.8 (2.7)	77.4 (5.0)	35.9 (0.6)	78.7 (0.9)	97.7 (2.1)
8	0.015 (2)	0.147 (3)	0.116 (2)	0.514 (5)	0.208 (1)
	23.3 (3.0)	115.2 (4.7)	60.7 (2.1)	89.4 (1.6)	108.5 (2.1)

[a] The values f_i of the mole fractions are averaged over five determinations; the standard deviations × 10^3 are reported in parentheses. [b] The absolute (D/H) values are averaged over five determinations; the standard deviations are reported in parentheses. [c] The deuterium chemical shifts and the absolute deuterium contents for 2-δ-decenolide (1) are as follows: D-2 [6.1 ppm, $(D/H)_2$ = 101]; D-3 [7.2 ppm, $(D/H)_3$ = 82]; D-4,4′ [2.5–2.6 ppm, $(D/H)_{4,4'}$ = 104]; D-5 (4.6 ppm, $(D/H)_5$ = 75]; D-(6-9) [1.5–1.9 ppm, $(D/H)_{6-9}$ = 101]; D-10 [1.1 ppm, $(D/H)_{10}$ = 102]. [d] Statistical value of the mole fractions (see Experimental Procedures).

starting Massoi lactone 1 (the ^2H data of 1 are reported as a footnote in Table II). The deuterium content of 1 is approximately comparable to that of sample 1 for all positions except for position 3, which shows a deuterium proportion more than 50% lower for the δ-decanolide [$(D/H)_3$ = 35] than for the 2-δ-decenolide [$(D/H)_3$ = 82]. This observation substantiates the hypothesis of a large kinetic isotope effect which controls the deuterium transfer to position 3 in the bakers' yeast reduction of the double bond of 2-δ-decenolide.

Samples 3 and 4 were prepared from 5-oxodecanoic acid by reduction with $NaBH_4$ and bakers' yeast, respectively (Scheme II). Not very remarkable differences can be detected between them. Possibly only the variation of the mole fraction f_1 may be significant in distinguishing the two samples. Still, since the reduction of the carbonyl in bakers' yeast is mediated by reduced nicotine cofactor, the reduced quantity of deuterium at position 5 of sample 4 with respect to that of sample 3 should be due to a kinetic isotope effect similar to that observed above. However, most important for our purposes, they can be clearly distinguished from the natural sample 1, as this latter shows significantly smaller values of the mole fraction f_3 and of the absolute values $(D/H)_2$ and $(D/H)_3$ than that of samples 3 and 4.

Finally, four commercial samples of racemic δ-decanolide have been examined. We have no certain information about the procedure followed in the synthesis. All of these samples are characterized by very low values of the mole fraction f_1 and of the $(D/H)_1$ ratio (Figure 1C) with respect to samples 1–4, making them easy to recognize with this technique. Among them, sample 7 displays significantly lower values of the absolute ratios $(D/H)_2$, $(D/H)_3$, and $(D/H)_4$ and of the mole fraction f_3 which can provide a clear distinction of its origin. Except for $(D/H)_1$, which is about 3 times lower, this sample, strangely enough, exhibits an absolute isotope distribution similar to that of sample 1 obtained from Massoi lactone in bakers' yeast.

In summary, the site-specific deuterium distribution provides a means of distinguishing among a variety of different δ-decalactones obtained in different ways. Most importantly, it is possible to distinguish between the two modes of conversion of Massoi lactone 1 into δ-decanolide 2. Sample 1, biogenerated from 1, is characterized by a low deuterium proportion for the mole fraction f_3 and a statistical distribution for f_1. Sample 2, obtained from 1 by chemical means, can be differentiated by the low deuterium content of position 2. The synthetic commercial samples examined by us are unique in having the lower observed content of deuterium at position 5.

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