Stable Isotope Characterization of Raspberry Ketone Extracted from *Taxus baccata* and Obtained by Oxidation of the Accompanying Alcohol (Betuligenol)

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The natural abundance ^{2}H NMR characterization of raspberry ketone **1** extracted from himalayan *Taxus baccata* and of the accompanying (*R*) carbinol **2** is performed and compared with that of samples of **1** obtained from **2** by oxidation with *Candida boidinii* and CrO_3, respectively. The determination of the $\delta(^{13}C)$ and/or $\delta(^{18}O)$ values of the above extractive products and of benzoic acid (**6**) and 4-butylphenol (**10**), obtained from natural of synthetic **1**, and of 4-phenylbutan-2-ol (**8**), prepared from extractive **2**, allows a description of the labeling pattern of this set of products. A graph of $(D/H)_3/(D/H)_2$ vs $(D/H)_5/(D/H)_4$ (Figure 3) of the presently examined samples and of those previously characterized in the laboratory, including three commercial samples sold as natural, defines three areas, containing (a) the material of botanical origin and that produced from extractive **2** by biooxidation, (b) those produced by bioreduction of the unsaturated ketone **3**, and (c) the synthetic samples.

Keywords: *Raspberry ketone; betuligenol; extraction; Taxus baccata; oxidation; Candida boidinii; isotopic content*

INTRODUCTION

The current need by the flavor industry for the natural modification of key odorants can be met either by manipulation of natural sources or by biogeneration from natural precursors (Stofberg, 1986). In this context, two biological approaches to the *natural* modification of 4-(4-hydroxyphenyl)butan-2-one (raspberry ketone, **1**), impact flavor of raspberry fruit (Schinz and Seidel, 1957), based on the oxidation of extractive 4-(4-hydroxyphenyl)butan-2-ol (**2**) and on the reduction of the natural unsaturated ketone **3**, respectively, were recently proposed (Dumont et al., 1995; Fronza et al., 1996a) (Scheme 1). In connection with these findings, in a study designed to the determination of the origin of raspberry ketone the ^{2}H natural abundance NMR data of commercial materials sold as natural were compared with those of the samples produced by synthesis or biogenerated from **3** (Fronza et al., 1998). The significance of the latter findings was limited by the lack of a sample of *extractive* raspberry ketone **1** as standard. Now we report on the stable isotope characterization of raspberry ketone **1** *extracted* from a botanical source, together with that of several samples obtained by bio- and chemical oxidation, respectively, of carbinol **2** which accompanies **1** in the examined plant extract.

The natural occurrence of raspberry ketone **1** seemed apparently limited to raspberry fruit. Indeed, the extractive manipulation of over 400 L of raspberry juice provided only a few hundreds of milligrams of material (Schinz and Seidel, 1957). At variance with **1**, carbinol **2**, in the two enantiomeric forms, was reported to be quite widespread in glucosylated (Smite et al., 1993; Pan and Lundgren, 1994; Chu et al., 1994; Fuchino et al., 1996) and/or in the free form (Parmar et al., 1991; Das et al., 1993) in many plants, including *Betula alba* and *Taxus* spp. Surprisingly enough, from the *Taxus baccata* extract received from the himalayan region of India, we isolated, close to carbinol **2**, also raspberry ketone **1** in substantial amount. Accordingly, carbinol **2** was converted by biological and chemical oxidation to raspberry ketone **1** and the isotopic composition of *extractive* raspberry ketone and of the two samples obtained from the *natural* precursor **2** by the two above-mentioned procedures was determined. The comparison of the

Scheme 1. Modes of Generation of Raspberry Ketone 1

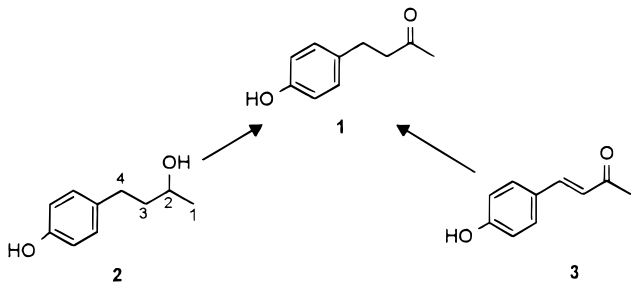

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Figure 1. Numbering of the positions of the raspberry ketone **1** and of the carbinol **2** used in the spectra and in the tables.

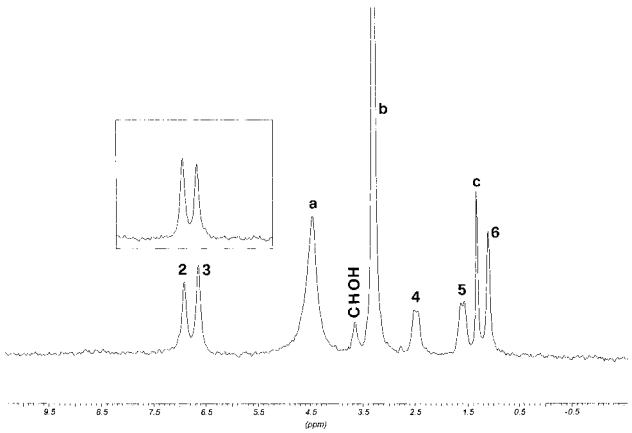


Figure 2. Natural abundance ^2H spectrum in methanol of extractive and synthetic (window) carbinol **2**: (a) signal of water and methanol hydroxyl groups; (b) signal of methanol methyl group; (c) signal of *tert*-butyl sulfide used as internal (D/H) standard. The peaks are numbered according to Table 1 and Figure 1.

present results with those previously acquired (Fronza et al., 1998) allowed a precise distinction of the origin, *i.e.*, natural extractive, natural biogenerated from **3**, and synthetic, of different raspberry ketone samples, as indicated in Figure 3.

EXPERIMENTAL PROCEDURES

Isolation of 1 and 2 from Commercial *T. baccata* Extract from the Hymalayan Region of India. SiO_2 column chromatography of the extract with increasing amounts of ethyl acetate in hexane provided different fractions enriched in **1** and **2**, respectively. The operation was repeated several times to eventually provide raspberry ketone **1**, mp 80 °C (from cyclohexane), undepressed with an authetic sample from Aldrich, and carbinol **2**, thick oil which solidified on standing: $[\alpha]_D^{20}$ −13.6° (*c* 1, EtOH); ^1H NMR (CH_3OH, the same solution used for the deuterium measurements) δ 1.10 (3H, d, J = 6.5 Hz), 1.61 (2H, m), 2.49 (2H, m), 3.66 (1H, qt, J = 6.5 Hz), 6.65 (2H, d, J = 8.6 Hz), 6.93 (2H,d, J = 8.6 Hz), 8.91 (1H, s br).

Oxidation of 2 to 1 by Biological and Chemical Means. *(A) With Candida boidinii Cultures. Candida boidinii* CBS 2428, grown routinely on MA slants (Malt Extract Broth Oxoid 2%, agar 1.5%), was seeded in a 300 mL Erlenmeyer flask containg 50 mL of MPGB medium (malt 2%, peptone 0.5%, glucose 1%, pH = 6.5) and grown in a rotatory incubator (140 rpm) at 28 °C for 24 h. The 24 h old culture was inoculated (10%) into a 7.5 L jar fermentor (Chemap), containing 5 L of MPGB medium at pH = 6.5. The temperature was maintained at 28 °C, and the stirring, at 500 rpm. At 12 and 22 h, respectively, was added 0.1% methanol, and at 24 h, after substitution of the air flow for oxygen, the carbinol **2**, 5 g. The incubation was continued for 24 h. The transformation mixture was treated with ethyl acetate, 1 L, and filtered under vacuum on a Celite pad. The organic phase was separated, and the aqueous phase was extracted with ethyl acetate (2 × 0.7L). The residue obtained upon evaporation of the dried combined organic extract was submitted to SiO_2 (200 g) chromatography to provide raspberry ketone **1**, mp 80 °C (from cyclohexane), 2.8 g (55%), and unreacted carbinol **2**, thick oil, 1.5 g (30%), shown to contain, at the HPLC analysis on Chiracel OD, ca. 65% excess of the *S* enantiomer. Analysis conditions: Merck-Hitachi L-6000 pump with L-4000 UV detector (254 nm), 9:1 hexane−-propanol at 0.6 mL/min; RT, *R* enantiomer = 29 min and *S* enantiomer = 31 min.

(B) With CrO_3 in Sulfuric Acid/Acetone. Carbinol **2** (1.66 g, 10 mmol) in acetone (30 mL) was treated portionwise, at 5−10 °C and under stirring, with an aqueous solution, containing, in 100 mL, CrO_3 (26.72 g) and concentrated sulfuric acid (23 mL), until the color no longer turned to green. After the addition of a few drops of methanol the reaction mixture was partially evaporated under vacuum at low temperature and diluted with ice water. The mixture was extracted with ethyl acetate (2 × 100 mL), and the organic phase was washed with 3% $NaHCO_3$. Evaporation of the dried solution and column chromatography on SiO_2 of the residue provided raspberry ketone **1** (1.1 g, 67%), mp 80 °C (from cyclohexane).

Conversion of Raspberry Ketone 1 into Benzoic Acid (6). Raspberry ketone **1** (1.64 g, 10 mmol) in acetone (60 mL) was treated at reflux under stirring with 1-chloro-5-phenyltetrazole (1.85 g, 11 mmol) in the presence of 4 g of finely powdered potassium carbonate for 8 h (Musliner and Gates, 1971). The reaction mixture was filtered and evaporated to dryness. The crude residue was partitioned between water (100 mL) and ethyl acetate (2 × 200 mL). The residue obtained upon evaporation of the dried organic phase was purified by SiO_2 column chromatography with increasing amounts of ethyl acetate in hexane. The 4′-(5-phenyltetrazolyl) derivative **4** was isolated as amorphous solid (2.6 g, 80%): ^1H NMR δ 2.15 (3H, s), 2.78 (2H, m), 2.93 (2H, m), 7.29 (5H, m), 7.57 (2H, m), 7.80 (2H, m); EI-MS *m*/*z* 309 (M^+ + 1, 25), 265 (M^+ − MeCO, 2), 223 (11), 180 (10), 117 (64), 91 (82), 77 (100), 65 (60); FT-IR (Nujol) ν (cm^{-1}) 1500, 1543, 1595, 1709, 3448. Anal. Calcd for $C_{17}H_{16}N_4O_2$: C, 66.22; H, 5.19; N, 18.16. Found: C, 66.34; H, 5.17; N, 18.21.

The latter product **4** (2.5 g, 8.4 mmol) in ethyl acetate (50 mL) was hydrogenated at normal pressure and room temperature in the presence of 10% Pd/C (0.5 g). At the end of the adsorption, the filtered reaction mixture was evaporated and 4-phenylbutan-2-one **5** (1 g, 80%) was obtained after separation from 5-phenyltetrazolone by SiO_2 chromatography: ^1H NMR δ 2.13 (3H, s), 2.76 (2H, m), 2.90 (2H, m), 7.25 (5H, m); EI-MS *m*/*z* 149 (M^+ + 1, 28), 148 (M^+, 100), 133 (M^+ − Me, 18), 105 (M^+ − MeCO, 58), 91 (42); FT-IR (neat) ν (cm^{-1}) 1718. Anal. Calcd for $C_{10}H_{12}O$: C, 81.04; H, 8.10. Found: C, 81.11; H, 8.08.

Ketone **5** (1 g, 6.7 mmol) in 50 mL of tap water, containing 5 g of K_2CO_3, was treated at 60−70 °C with a 5% $KMnO_4$ solution portionwise, until the reaction mixture was no longer discolored. After cooling, the reaction mixture was filtered and acidified with 5% hydrochloric acid and extracted twice with ethyl acetate. The dried organic phase was concentrated under vacuum, to give the crude benzoic acid **6.** The latter was crystallized twice from water, mp 122−123 °C, undepressed with an authetic sample (0.3 g, 36%).

Conversion of 2 into 4-Phenylbutan-2-ol (8). The sequence leading from **1** to **5** reported above was repeated on **2** to provide, in an analogous manner, carbinol **8**, oil, purified by bulb-to-bulb vacuum distillation: ^1H NMR (250 MHz, $CDCl_3$) δ 1.21 (3H, d, J = 6.5 Hz), 1.70−1.83 (2H, m), 2.57−2.88 (2H, m), 3.82 (1H, m), 4.83 (1H, s, OH), 7.15−7.33 (5H, m).

Conversion of Raspberry Ketone 1 into 4-Butylphenol (10). Raspberry ketone **1** (1.64 g, 10 mmol) in methanol (50 mL) was treated at reflux for 4 h with toluene-4-sulfonic acid hydrazide (2 g, 11 mmol). $NaBH_4$ (0.76 g, 20 mmol) was added under stirring to the cooled reaction mixture. The reaction mixture was boiled for 4 h and then concentrated to a small volume under vacuum. The residue was partitioned between ice water and ethyl acetate (300 mL). The residue obtained upon evaporation of the organic phase was chromatographed on SiO_2 to provide, from increasing amounts of ethyl acetate in hexane, 4-butylphenol (**10**), purified by bulb-to-bulb vacuum distillation, 0.75 g (50%): ^1H NMR (250 MHz, $CDCl_3$), δ 0.91 (3H, t, J = 7.4 Hz), 1.33 (2H, m), 1.55 (2H, m), 2.53 (2H, t, J = 7.7 Hz), 4.75 (1H, s br), 6.74(2H, d, J = 8.5 Hz), 7.03 (2H, d, J = 8.5 Hz).

The following samples were examined: one sample of raspberry ketone **1** extracted from *T. baccata* (sample 1); one sample of raspberry ketone **1** obtained by *Candida boidinii* oxidation of the extractive carbinol **2** (sample 2); one sample of raspberry ketone **1** obtained by CrO_3 oxidation of the extractive carbinol **2** (sample 3); one sample of raspberry ketone **1** obtained by *Candida boidinii* oxidation of synthetic carbinol **2** (sample 4); one sample of raspberry ketone **1** obtained by CrO_3 oxidation of the synthetic carbinol **2** (sample 5); one sample of extractive carbinol **2**, used for the bio- and chemical oxidation to raspberry ketone **1** (sample 6); one sample of synthetic carbinol **2** used for the bio- and chemical oxidation to raspberry ketone **1** (sample 7); one sample of benzoic acid **6** obtained by chemical oxidation of extractive raspberry ketone **1** (sample 8); one sample of 4-phenylbutan-2-ol obtained by deoxygenation of extractive **2** (sample 9); two samples of synthetic raspberry ketone **1** from an unspecified producer and from Aldrich (samples 10 and 11); two samples of raspberry ketone obtained from sample 10 by base and acid treatment, respectively, as reported (Fronza et al., 1998) (samples 12 and 13); one sample of synthetic raspberry ketone **1** obtained from sample 11 by $NaBH_4$ reduction, followed by CrO_3 oxidation (sample 14); two samples of 4-butylphenol (**10**) obtained from synthetic raspberry ketone analyzed as samples 10 and 11 (samples 15 and 16).

The deuterium NMR spectra of raspberry ketone **1** were recorded on a Bruker ARX 400 instrument following exactly the experimental procedure described previously (Fronza et al., 1998). The spectra were run in two different solvents: (a) dioxane producing a good separation for the signals of the aromatic hydrogens and (b) the mixture dioxane/benzene in the volume ratio 0.6/3.0 in which the signals of the methylene groups are well separated.

The NMR spectra of the alcohol **2** were obtained in CH_3OH at 306 K using hexafluorobenzene for the ^{19}F lock and *tert*-butyl sulfide as internal standard. The spectral conditions, the solution concentrations, and the calculation of the molar fractions f_i and of the $(D/H)_i$ values were the same used in the previous work (Fronza et al., 1998).

Stable isotope ratios were determined with a Finnigan MAT (Bremen, Germany) Delta S mass spectrometer, interfaced on-line with a Carlo Erba (ThermoQuest, Milan, Italy) CHN 1108 elemental analyzer for sample combustion. The analytical procedures followed for measuring the $^{13}C/^{12}C$ ratios are the same described elsewhere (Angerosa et al., 1997) as well as those for measuring the $^{18}O/^{16}O$ ratios (Breas et al., 1998).

The measurements are expressed in per mill (‰) versus an international standard:

$$\delta(X)\text{ ‰} = (R_{sample}/R_{standard} - 1) \times 10^3 \qquad (1)$$

where X = ^{13}C or ^{18}O and R = $^{13}C/^{12}C$ or $^{18}O/^{16}O$.

The international standard for ^{13}C is pee dee belemnite (PDB) and the international standard for ^{18}O is standard mean ocean water (SMOW).

RESULTS AND DISCUSSION

The serendipitous discovery of the occurrence in a himalayan *T. baccata* extract of fairly large amounts of raspberry ketone **1** and of carbinol **2** thus allows for the first time the full stable isotope characterization of the natural *extractive* modification of this rare flavor material and of the specimens accessible from *extractive* alcohol **2** by biological (Dumont et al.,1995) and chemical oxidation, respectively. The comparison of the mode of labeling at corresponding positions of **1** and **2** also allows a definition of the biosynthetic relationships linking these two secondary metabolites of *T. baccata*.

In the first instance, alcohol **2**, isolated close to **1** from the extract, was oxidized to raspberry ketone **2**. The biological and purely chemical methods outlined in Scheme 2 were adopted. The choice of the microbial system suitable for the oxidation of **2** to **1** depended upon the enantiomeric composition of the substrate. Indeed, the enantioselectivity in the oxidation changed from *S* to *R* on going from *Beauveria bassiana* to *Candida boidinii* (Fuganti et al., 1996; Dumont et al., 1995). To this end, carbinol **2**, extracted from *T. baccata*, was shown, on the basis of optical measurements, HPLC analysis on a chiral column, and comparison with an authentic sample (Fuganti et al., 1996), to contain ca. 70−75% excess of the *R* enantiomer (=betuligenol), thus confirming the previous data relative to the materials extracted from *Taxus* spp. (Das et al., 1993; Parmar et al., 1991). The biooxidation of extractive (*R*) **2** to ketone **1** (Scheme 2, path a) was thus performed by incubation with cultures of *C. boidinii*. HPLC analysis of samples withdrawn from the fermentation mixture while the reaction proceeds indicated that the (*R*) alcohol was oxidized and that at the end of incubation the *R*/*S* composition of the survived alcohol reached the ratio of ca. 1:2, respectively.

Scheme 2. Modes of Oxidation of 2 to 1

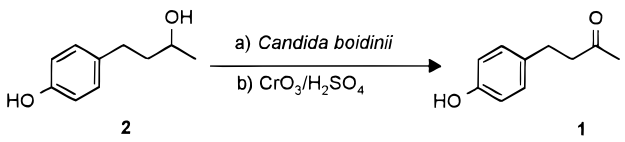


Table 1. Deuterium Chemical Shift Values of the 4-(4-Hydroxyphenyl)butan-2-ol 2[a]

peak[b]	assgnt	δ (ppm)
1	−OH phenolic	8.91
2	2H *meta* aromatic H	6.93
3	2H *ortho* aromatic H	6.65
	−*CH*OH	3.66
4	benzylic CH_2	2.45 and 2.54 (diastereotopic deuterium atoms)
5	CH_2CH−	1.57 and 1.54 (diastereotopic deuterium atoms)
6	CH_3	1.10

[a] The chemical shifts are referred to internal TMS. Solvent: methanol. [b] Numbering of different groups of nuclei used in Tables 2 and 3 for the isotopic parameters. For sake of comparison, the numbering is the same used previously for raspberry ketone (**1**) (Fronza et al., 1998).

The chemical oxidation of extractive **2** to raspberry ketone **1** was performed with CrO_3 in acetone/sulfuric acid solution, obtaining ca. 70% **1** (Scheme 2, path b). Subsequently, raspberry ketone **1** directly extracted from *T. baccata* (sample 1) and the two materials produced from extractive carbinol **2** by biochemical and chemical oxidation, respectively (samples 2 and 3), were submitted to SNIF-NMR (a trademark of Eurofins Laboratories, Nantes, France) (Martin and Martin, 1995) studies. For sake of comparison, a sample of synthetic **2** was oxidized to **1** along the two paths of Scheme 2, and the products were analyzed as samples 4 and 5, respectively.

The $(D/H)_i$ and the f_i values of the five raspberry ketone samples examined are reported in Tables 2 and 3, respectively. The measured values indicate a strict similarity between the extractive material (entry 1) and the two samples produced from extractive carbinol **2** (entries 2 and 3) by the enzymic and chemical methods outlined in Scheme 2. The major noticeable difference between natural extractive **1** (entry 1) and the two samples produced from **2** (entries 2 and 3) is in the $(D/H)_6$ values, ranging from 76.6 and 80.3 ppm, respec-

Table 2. $(D/H)_i$ Isotopic Ratios of 1 (Raspberry Ketone Extracted from *T. baccata* and Produced from Extractive and Synthetic 2 by Biological and Chemical Oxidation) and of Carbinol 2 [4-(4-Hydroxyphenyl)butan-2-ol][a]

	raspberry ketone **1**				
sample	$(D/H)_2$[b]	$(D/H)_3$[b]	$(D/H)_4$[c]	$(D/H)_5$[c]	$(D/H)_6$[b]
1	135.0(2.1)	183.0(2.2)	91.8(3.1)	125.2(2.8)	116.8(2.3)
2	130.1(2.8)	167.9(2.7)	91.0(3.0)	127.3(2.6)	80.3(2.5)
3	126.4(2.7)	166.2(2.7)	91.7(2.8)	121.4(2.4)	76.6(2.3)
4	141.3(2.5)	128.0(2.7)	161.0(2.7)	138.5(2.6)	125.0(2.6)
5	144.3(2.4)	126.3(2.3)	161.8(3.1)	141.7(2.5)	125.1(2.3)

	carbinol **2**					
sample[d]	$(D/H)_2$	$(D/H)_3$	$(D/H)_4$	$(D/H)_5$	$(D/H)_6$	$(D/H)_{CHOH}$
6	127.6(2.4)	171.6(2.5)	91.8(2.8)	128.0(2.79)	88.8(3.3)	100.3(5.0)
7	144.3(2.5)	127.2(2.6)	158.2(3.0)	140.4(2.7)	120.0(3.6)	142.3(5.8)

[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). [d] Solvent: methanol.

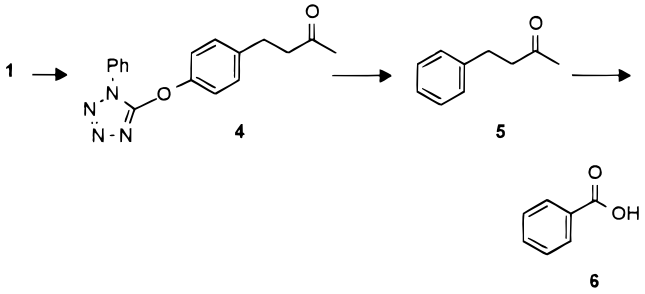
Table 3. Deuterium Molar Fractions of 1 (Raspberry Ketone Extracted from *T. baccata* and Produced from Extractive and Synthetic 2 by Biological and Chemical Oxidation) and of Carbinol 2 [4-(4-Hydroxyphenyl)butan-2-ol][a]

	raspberry ketone **1**				
sample	f_2b	f_3b	f_4c	f_5c	f_6b
1	0.170(4)	0.231(5)	0.157(4)	0.214(5)	0.228(5)
2	0.188(5)	0.248(4)	0.169(6)	0.224(5)	1.172(5)
3	0.200(4)	0.165(5)	0.209(4)	0.180(5)	0.247(3)
4	0.218(5)	0.171(5)	0.175(4)	0.176(4)	0.260(5)
5	0.185(4)	0.170(4)	0.213(5)	0.185(5)	0.252(4)

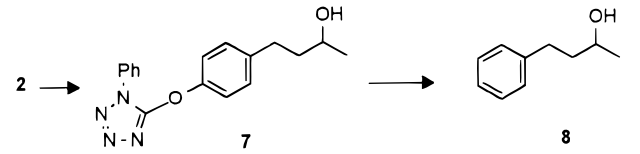
	carbinol **2**					
sample[d]	f_2	f_3	f_4	f_5	f_6	f_{CHOH}
6	0.174(5)	0.237(4)	0.133(5)	0.185(4)	0.193(7)	0.078(9)
7	0.175(4)	0.150(5)	0.183(5)	0.169(5)	0.256(6)	0.066(10)

[a] The molar fractions 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). [d] Solvent: methanol.

Scheme 3. Steps in the Conversion of Raspberry Ketone 1 into Benzoic Acid (6)



Scheme 4. Steps in the Conversion of Carbinol 2 into 4-Phenylbutan-2-ol (8)



Scheme 5. Steps in the Conversion of Raspberry Ketone 1 into 4-Butylphenol (10)

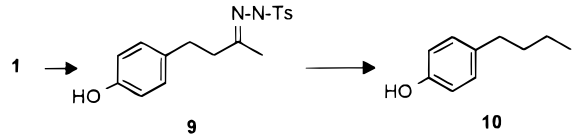


Table 4. $\delta(^{13}C)$ and $\delta(^{18}O)$ Values of Extractive 1 and of Related Products

sample[a]	$\delta(^{13}C)$ (‰)[b]	$\delta(^{18}O)$ (‰)[c]	sample[a]	$\delta(^{13}C)$ (‰)[b]	$\delta(^{18}O)$ (‰)[c]
1	−29.6	13.3	9		19.2
2	−29.6	13.0	10	−27.1	29.9
3	−28.6	16.5	11	−27.7	9.1
4		21.1	12		22.6
5		23.5	13		23.0
6	−29.3	9.2	14	−26.8	14.2
7		21.7	15		17.1
8	−29.0		16		−2.6

[a] The samples are the following: (1) **1** by extraction from *T. baccata*; (2) **1** by oxidation with *Candida boidinii* of the extractive **2**; (3) **1** by oxidation with CrO_3 of extractive **2**; (4) **1** by oxidation with *C. boidinii* of synthetic **2**; (5) **1** by oxidation with CrO_3 of synthetic **2**; (6) **2** by extraction from *T. baccata*; (7) **2** of synthetic origin; (8) benzoic acid (**6**) by oxidation of extractive **1**; (9) 4-phenylbutan-2-ol (**8**) by deoxygenation of extractive **2**; (10, 11) **1** of synthetic origin; (12, 13) **1** of synthetic origin submitted to basic and acid treatment, respectively; (14) **1** of synthetic origin reduced with $NaBH_4$ and oxidated with CrO_3; (15, 16) 4-butylphenol (**10**) obtained from synthetic **1** (samples 10 and 11, respectively). [b] The variations are within 0.2‰. [c] The variations are within 0.3‰.

tively, of the latter to 116.8 ppm of the extractive material. Thus, microbial and chemical oxidations of **2** to **1** induce the same (if any) change in the deuterium content at the enolizable positions adjacent to the carbonyl group and cannot be distinguished by this method.

The $\delta(^{13}C)$ and/or $\delta(^{18}O)$ values of samples 1−5 of raspberry ketone **1** are reported in Table 4. The three samples possessing the same *natural* skeleton (samples 1−3) show, as expected, nearly identical $\delta(^{13}C)$ values (−29.6, −29.60, and −28.6‰, respectively). Similarly, the $\delta(^{18}O)$ values of extractive **1** and of the material produced from **2** by biooxidation are in the same range (13.3 and 13.0‰, respectively). The $\delta(^{18}O)$ value of the ketone produced from the extractive alcohol by CrO_3 oxidation is slightly higher (16.5‰) than that obtained by biooxidation. A similar difference in the $\delta(^{18}O)$ values, *i.e.*, 21.1 vs 23.5‰, is observed in samples 4 and 5, obtained from the identical synthetic carbinol (sample 7) by biological and chemical oxidation, respectively.

In a subsequent experiment designed to define the ^{13}C labeling pattern of the C-6−C-4 skeleton of raspberry ketone **1**, the extractive material was converted through deoxygenation of derivative **4** (Musliner and Gates, 1971) to **5** and then via alkaline $KMnO_4$ oxidation to benzoic acid (**6**), which accounts for the aromatic C-6−C-1 part of the molecule (Scheme 3). This material

(sample 8) shows $\delta(^{13}C)$ of −29.0‰ very similar to that of sample 1 (−29.6‰), thus indicating a rather uniform mode of labeling of the carbon framework of **1**.

The isotopic characterization of extractive carbinol **2** (sample 6) was then performed in comparison with that of the synthetic material (sample 7) used for the preparation of samples 4 and 5. Figure 2 shows the whole natural abundance ^2H NMR spectrum of sample 6 and, in the window, the part of the spectrum of the synthetic carbinol (sample 7) which differs most significantly from that of sample 6. The $(D/H)_i$ and the f_i values relative to these two samples are reported in Tables 2 and 3. Again, differences appear in the mode of labeling of the aromatic moiety (see Figure 2) and in the values of $(D/H)_4$ and $(D/H)_5$ ranging from 91.8 to 158.2 and from 128.0 to 140.4 ppm, respectively, on going from sample 6 to 7. Moreover, the methyl group adjacent to the carbinol moiety (position 6 of the spectrum) of the *extractive* carbinol **2** (sample 6) is particularly depleted in deuterium content. As seen above, this characteristic is reflected in samples 2 and 3 of the ketone **1** derived from 6 through the two oxidative paths of Scheme 2.

As far as the $\delta(^{13}C)$ and $\delta(^{18}O)$ values are concerned (Table 4), *extractive* alcohol **2** (sample 6) shows a $\delta(^{13}C)$ value of −29.3‰, as the extractive ketone **1**, while its $\delta(^{18}O)$ value is 9.22‰, significantly lower than the values of raspberry ketone **1** (13.0 and 16.5‰ of samples 2 and 3, respectively) to which it was converted through the oxidation of Scheme 2. Interestingly enough, synthetic carbinol **2** (sample 7) shows a $\delta(^{18}O)$ value of 21.7‰, while the derived ketone **1** (samples 4, from biooxidation, and sample 5, from CrO_3 treatment) display the values 21.1 and 23.5‰. Thus, in the oxidation of **2** to **1** the $\delta(^{18}O)$ values remain roughly constant in the biological conversion, whereas they increase slightly when using chemical reagents.

To better understand the possible diagnostic significance of the $\delta(^{18}O)$ variations for the mode of conversion of **2** into **1**, two more samples of synthetic raspberry ketone were analyzed (samples 10 and 11). Moreover, sample 10 was submitted, in separate experiments, to basic and acid treatment, respectively, in tap water to provide samples 12 and 13. The $\delta(^{18}O)$ values (Table 4) of samples 10−13 are the following: 29.9, 9.1, 22.6, and 23.0‰. This means that the $\delta(^{18}O)$ values can vary significantly from one synthetic sample to the other and that, on equilibration with water, the product with high value (29.9‰, sample 10) decreases to 22.6 and 23.0‰ (samples 12 and 13, respectively). The tendency of raspberry ketone samples equilibrated with water to reach $\delta(^{18}O)$ values around 15−20‰ under acidic conditions is supported from the observation that sample 11 (with a value of 9.1‰), on $NaBH_4$ reduction, followed by CrO_3/H_2SO_4 oxidation, provides sample 14 showing a value of 14.2‰. The possible different contribution to the mean $\delta(^{18}O)$ values of the two oxygen atoms present in the molecules of **1** and **2** was subsequently determined, through selective degradations to monooxygenated derivatives. Thus, the phenolic oxygen present in the extractive carbinol **2** (sample 6) was regioselectively removed by hydrogenolysis (Musliner and Gates, 1971) of derivative **7** which provides 4-phenylbutan-2-ol (**8**) (Scheme 4). The latter material (sample 9) (Table 4) shows a value of 19.2‰, suggesting, when compared with the value 9.2‰ of precursor **2** (sample 6), that the phenolic oxygen removed in the conversion possessed a negative $\delta(^{18}O)$ value. Biosynthetic considerations are outside the scope of the present study. However, it is worth mentioning that **1** and **2** are metabolites arising from *p*-coumaryl-CoA and malonyl-CoA respectively (Borejsza-Wysocki and Hrazdina, 1994; Fronza et al., 1996b). The present result thus indirectly defines the $\delta(^{18}O)$ of the oxygen atom introduced in the *Taxus baccata* plant in the para position of cinnamic acid during its conversion into *p*-coumaric acid (Haslam, 1974).

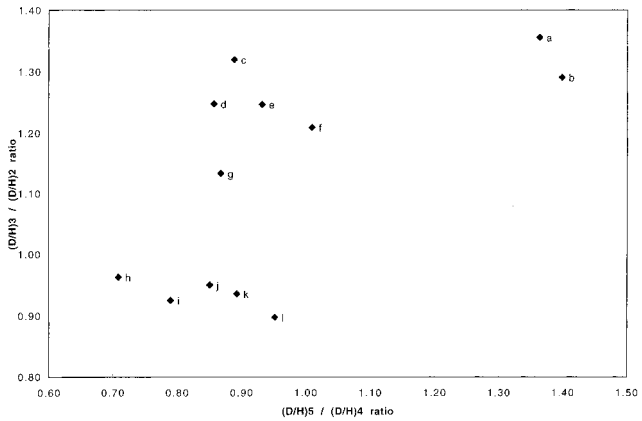


Figure 3. Graphical representation of the ratios $(D/H)_3/(D/H)_2$ vs $(D/H)_5/(D/H)_4$ showing three distinct regions for samples of raspberry ketone of different origin: a region containing the samples a extracted from *T. baccata* and b obtained by microbial oxidation of the extractive carbinol **2**; a region containing the samples c−g biogenerated with different microorganism from the natural unsaturated ketone **3**; and a region containing the samples h−l of synthetic commercial origin including three samples sold as natural.

The synthetic raspberry ketone samples mentioned above (samples 10 and 11) were converted into 4-butylphenol (**10**), via $NaBH_4$ in situ reduction of the intermediate **9** (Scheme 5). The latter monooxygenated products (samples 15 and 16) (Table 4), which retained the phenolic oxygen, show $\delta(^{18}O)$ values of 17.1 and −2.6‰ vs 29.9 and 9.1‰, respectively, of the starting dioxygenated materials (samples 11 and 12), thus confirming the significant difference of $\delta(^{18}O)$ of the two oxygen functions present in **1**.

Finally, the present SNIF-NMR studies on samples of **1** directly extracted from *T. baccata* and produced by biooxidation of **2** (samples 1 and 2) were considered together with those previously reported (Fronza et al., 1998), which included (a) two synthetic products (samples 10 and 11 of the present study), (b) three commercial samples sold as natural, and (c) five natural materials obtained by bioreduction of **3**. The graphical representation of Figure 3 is derived plotting the ratio $(D/H)_3/(D/H)_2$ of the above-mentioned products originated in different ways vs the ratio $(D/H)_5/(D/H)_4$. Three well-cut regions can be identified, which allow us to distinguish the origin of the following materials: (i) extractive from *Taxus* and biogenerated by oxidation of **2**; (ii) biogenerated upon enzymic saturation of **3**; and (iii) synthetic. In the latter group fall also three commercial samples sold as natural.

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