Stereochemistry of the Baeyer−Villiger-Type Conversion of 4-(4-Hydroxyphenyl)butan-2-one (Raspberry Ketone) into Tyrosol Mediated by *Beauveria bassiana*

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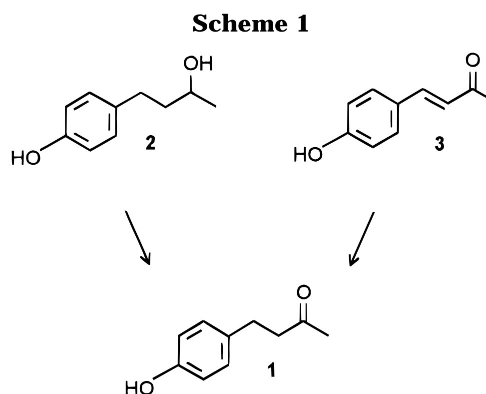
Feeding experiments in *Beauveria bassiana* (ATCC 7159) of (*2R,3S*)-[2,3-$^2H_2$]-4-(4-hydroxyphenyl)-butan-2-ol (**15**) and (*2R,3R*)-[1,3-$^2H_4$]-4-(4-hydroxyphenyl)-butan-2-ol (**16**) afford (*1S*)- and (*1R*)-[1-^{2}H]tyrosol (**17**) and (**18**), respectively, as indicated by NMR studies on the (+)-MTPA esters **21** and **23** and comparison with an authentic sample of the (*S*) enantiomer. These results indicate that the C-2, Baeyer-Villiger-type, chain shortening of the C-6−C-4 framework of the intermediate raspberry ketone **1** to give the C-6−C-2 tyrosol (**4**) occurs with retention of configuration. The asymmetrically labeled substrates **15** and **16** have been obtained by enzymic resolution of derivatives of (*2SR,3RS)*-**6** and (*2SR,3SR*)-**12**, prepared, in turn, by *syn* catalytic reduction with deuterium and with hydrogen gas, respectively, of the (*Z*) enol acetates **5** and **11**.

4-(4-Hydroxyphenyl)butan-2-one (**1**) formally entered the reign of food flavors 70 years ago when it was obtained in Japan[1] in a study designed to the evaluation of the sensory properties of analogs of zingerone (the 3-methoxy analog of **1**), but its aromatic significance was not perceived at that time. Its presence in trace amounts in raspberry fruit was reported 40 years later,[2,3] when it was isolated in minute amounts out of over 400 L of raspberry juice and recognized as impact flavor of raspberry fruit and, accordingly, named *raspberry ketone*. In raspberry, the content of **1** is very small and variable.[4−7] The recent legislative discrimination between chemically identical food flavor components of synthetic origin and those derived from natural sources[8] has stimulated studies designed to obtain substantial amounts of natural flavor ingredients not accessible by extractive manipulation of plant materials through biotechnological means.[9] In this context, two enzymic approaches to raspberry ketone (**1**) have been recently proposed, on the basis of the use of natural precursors (Scheme 1). In the first,[10] product **1** is obtained by enzymic dehydrogenation of the corresponding carbinol **2**, accessible in the (*R*) configuration by hydrolysis of the glycoside betuloside, extracted from *Betula alba* bark, whereas the (*S*) enantiomer is obtained similarly from *epi*-rhododendrin, present in certain *Acer* species.[11] Alternatively, *natural*[8] raspberry ketone (**1**) can be obtained by enzyme-mediated saturation of the double bond of product **3**, prepared by condensing 4-hydroxybenzaldehyde of botanical extractive origin with acetone produced by sugar fermentation.[12] This route mimics the natural biosynthetic process. Indeed, it has been recently shown in raspberry fruit extracts that the late biosynthetic intermediate to C-6−C-4 **1** is the unsaturated ketone **3**, formed, in turn, by hydrolysis and decarboxylation of the C-6−C-5 product **26**, formed by condensation of C-6−C-3 *p*-coumaryl-CoA with malonyl-CoA.[13]

Scheme 1



In a screening for microorganisms[14] able to convert **3** into **1**, the formation from **3** of **1** and **2** was observed in growing cultures of *Beauveria bassiana* (ATCC 7159) at short incubation time. However, continuing in the contact with the cells the carbinol **2** is oxidized to **1**, which

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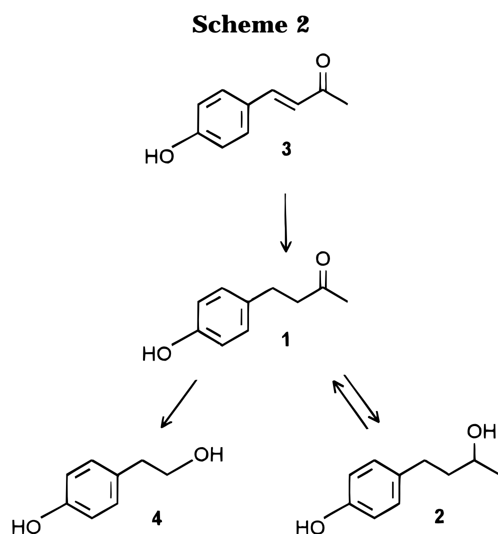
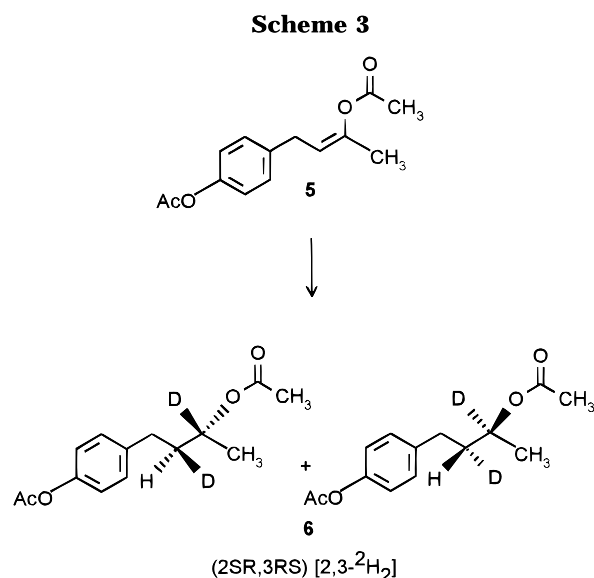
Scheme 2

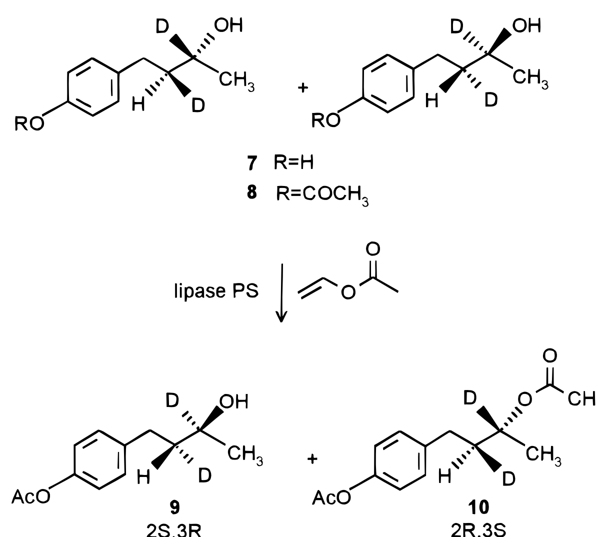


is degraded, in turn, to C-6−C-2 tyrosol (**4**) (Scheme 2).[15] Exploratory experiments with [1,3-$^2H_5$]raspberry ketone **1** indicated that the microbial conversion into **4** takes place with retention at position 1 of the educt **4** of the two deuterium atoms originally present at position 3 of the raspberry ketone framework. These observations thus suggest that the conversion of **1** into **4** is a Baeyer−Villiger-type degradation.[16] In light of the importance of the stereochemical information for the elucidation of enzymic mechanisms and of the interest for knowledge on the mode of biodegradation of flavor materials in relation to the evolution of the sensory properties of food, we have performed a stereochemical study of the fragmentation of the carbon skeleton of **1** during the conversion into **4**, and now we present the results obtained.

The analysis would require feeding experiments in *B. bassiana* of raspberry ketone **1** asymmetrically deuterated at position 3 and the determination of enantiomeric purity and absolute configuration of the resulting 1-deuterated tyrosol (**4**). Preliminary experiments[15] in *B. bassiana* indicated no significant loss of the deuterium located at position 3 of the raspberry ketone framework in the transformation into tyrosol (**4**). However, actual substrates for the experiments in *B. bassiana* were the (*2R*) carbinols **15** and **16**, bearing at position 3 a deuterium atom in the (*S*) and (*R*) configuration, respectively. The use of these carbinols as substrates instead of the labeled ketone **1** seemed more suitable to ensure the stereochemical integrity of the deuterated chiral center at position 3 during the synthesis. Moreover, we preferred to deal with the (*R*) form of the carbinol **2** in these experiments since it is oxidized at lower rate than the (*S*) enantiomer under the selected conditions of growth, thus keeping the actual concentration in the medium of the ketone **1** at a low level. This fact could minimize the danger of loss of enantiomeric purity at position 3 in the intermediate ketone by enolization. The stereochemistry of the deuterated tyrosol samples produced by biotransformations in *B. bassiana* was established by comparison of the NMR data of the (+)-MTPA derivative with the NMR of (+)-MTPA derivative of an authentic sample of (1*S*)-[1-^{2}H]tyrosol obtained from

Scheme 3





oxidative deamination of [1-$^2H_2$] tyramine by *Willia anomala* (Scheme 6).[17]

The synthesis of the required (*2R*) 4-(4-hydroxyphenyl)-butanols asymmetrically labeled at position 3 with deuterium required as intermediates the (*Z*) enol acetates **5** and **11**, respectively. These materials afford by *syn* catalytic reduction using in one instance deuterium and, in the second, hydrogen gas, racemic acetate esters bearing a defined stereochemical relationship between the oxygen-substituted chiral center at position 2 and the adjacent deuterium-labeled methylene group at position 3. Finally, separation from the racemic mixtures of the enantiomerically pure (*2R*) carbinols allows the obtainment of the required precursors, bearing at position 3 deuterium substitution of the (*S*) and (*R*) configurations, respectively.

Starting material for the synthesis of precursor **15** (Scheme 3) was the (*Z*) enolacetate **5**, prepared in admixture with the (*E*) isomer from **1** by the action of $Ac_2O/HClO_4$.[18] The two isomers cannot be completely

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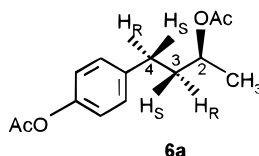
separated, and after column chromatography, the mixture was enriched up to 90% of compound **5**. The stereochemistry of **5** was assigned from the comparison of the ^{13}C resonances of the methyl groups of (*E*) and (*Z*) isomers. The methyl and methylene groups are oriented trans in the (*Z*) and cis in the (*E*) isomers. Thus, going from the (*Z*) to the (*E*) structure, the ^{13}C methyl signal is shifted upfield from 19.7 to 15.4 ppm due to the steric γ-effect induced by the methylene group.

Saturation of the double bond of **5** with deuterium gas in presence of PtO_2 affords the (*2SR,3RS*)-[2,3-$^2H_2$] diacetate **6**. In ethyl acetate there is also the formation of the acetate of 4-*n*-butylphenol, produced by hydrogenolytic cleavage of the carbon−oxygen bond. The use of $PdCl_2/D_2$ as reducing system does not cause the hydrogenolytic cleavage of the carbon−oxygen bond, but produces saturation of the double bond with large scrambling of the labels. Some scrambling of the labels also occurs using PtO_2 as catalyst; in fact, the deuterium NMR spectra show that the positions 1 and 4 of the aliphatic chain possess about 10% of deuterium atoms.

In previous work,[12] we assigned the diastereotopic methylene hydrogens linked to C-3 and C-4 carbons on the basis of a detailed ^1H and ^{13}C NMR study carried out on the nondeuterated diacetate **6a**. Using benzene



as solvent, we have shown that hydrogens H_R-3 and H_S-3 resonate at 1.46 and 1.68 ppm, respectively. The deuterium spectrum of **6** in C_6H_6 displays two strong signals (Figure 1) at 4.90 and 1.43 ppm, corresponding to the deuterium atoms D-2 and D_R-3 originated from the *syn* addition of D_2 to the double bond of **5**. A signal also occurs at 1.64 ppm, indicating that about 15% of D_S-3 nuclei is present. These deuterium atoms derive from the presence of ca. 10% of (*E*) isomer in compound **5** and from some scrambling occurring during the reduction; in fact, other deuterium signals are present in the spectrum at 1.01 ppm (ca. 5%, CH_3), 2.32 ppm (ca. 10%, H_R-4), and 2.40 ppm (ca. 10%, H_S-4).

The deuterium spectrum of the diacetate **12** obtained from the catalytic reduction of the [1,3-$^2H_5$]enol acetate **11** is complementary to that of **6** (Figure 1). This spectrum carried out in benzene shows two main signals at 0.98 ppm (CD_3) and 1.65 ppm (D_S-3). A minor signal occurring at 1.43 ppm indicates that some amount (ca. 15%) of D_R-3 atoms, originating from the residual (*E*) isomer in the (*Z*) enol acetate **11**, is also present.

From the racemic diacetyl derivative **6** the desired product **15** would eventually become accessible by separation of the enantiomers. This goal was achieved by enzymic kinetic resolution.

The enzymic esterification process of racemic **2** was first explored using vinyl acetate as acyl donor in the presence of *Candida cylindracea* lipase (CCL) type VII, Sigma, and porcine pancreas lipase (PPL) type II, Sigma, but the results were unsatisfactory both in terms of regio- and enantioselectivity. Good results were obtained using lipase PS Amano (lipase from *Pseudomonas cepacia*),

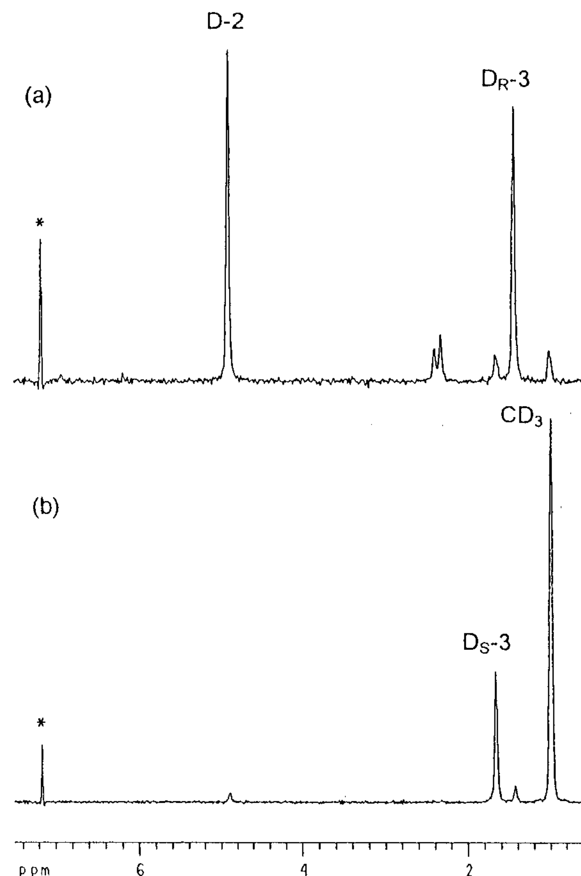


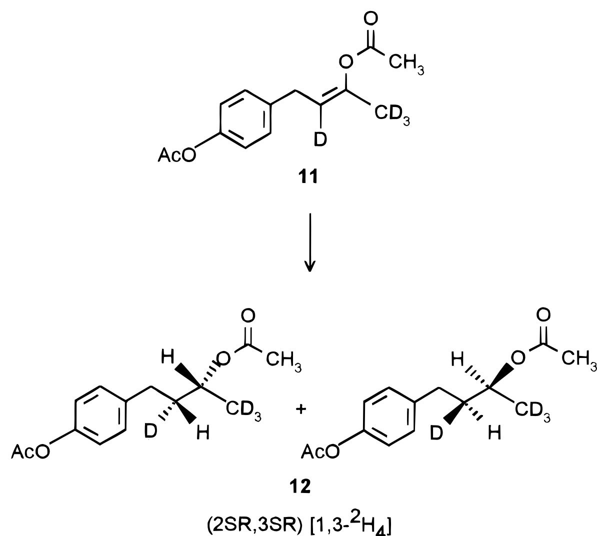
Figure 1. Deuterium NMR spectra in benzene of (a) compound **6** and (b) compound **12**. The asterisks denote the natural abundant deuterium signal of the solvent.

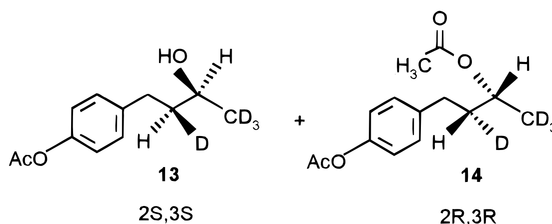
$(CH_3)_3COCH_3$ as solvent, and vinyl acetate. Under these conditions, carbinol **2** afforded the two isomeric monoacetates, the diacetate, and some residual carbinol **2** (*ca.* 25%) showing 0.96 ee and (*S*) configuration. When the phenolic acetate was used as substrate, *ca.* 45−50% of the (*R*) diacetate possessing 0.99 ee formed after 40 h. Accordingly, these conditions were applied in the preparation of (*2R,3S*)-**10** from the phenolic monoacetate obtained from **6**. The conversion involved basic hydrolysis and selective monoacetylation. Finally, product **10** was hydrolyzed to the desired stereospecifically deuterated precursor **15** upon treatment with ethanolic NaOH.

The diastereoisomeric product **16**, bearing at position 3 a deuterium substituent in the (*R*) reconfiguration, was obtained in a complementary way (Scheme 4). In this instance, starting material is the deuterated enol acetate **11**, prepared, as above, with $Ac_2O/HClO_4$ from [1,3-$^2H_5$]-**1**. The deuterium labeling in positions adjacent to the carbonyl group of **1** has been introduced by means of base-catalyzed exchange with deuterated water in 1,4-dioxane. In the formation of the enol acetate **11** there is some loss of deuterium, particularly at position 3. From **11**, the desired carbinol **16** has been obtained, as above, through the intermediacy of products **12**−**14**.

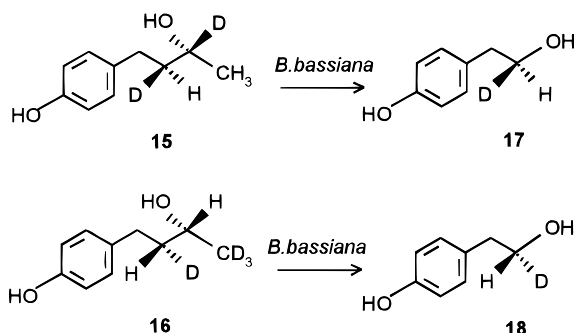
Feeding experiments in *B. bassiana* of carbinols **15** and **16** afforded tyrosol samples **17** and **18** (Scheme 5), shown by NMR studies to contain at position 1 the same amount of label present in position 3 of the precursors. For the stereochemical analysis the phenolic monoacetates **20** and **22** that gave the esters **21** and **23,** respectively, with

Scheme 4

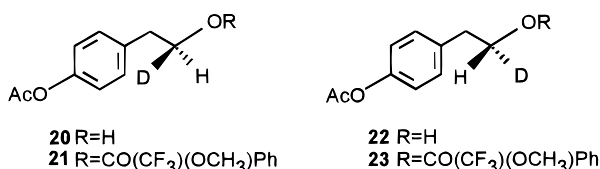




Scheme 5

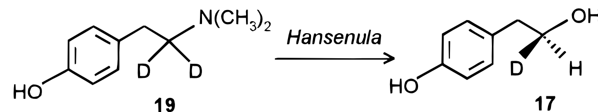


the chloride of (+)-α-methoxy-α-(trifluoromethyl)phenylacetic acid [(+)-MTPA][19] were used. NMR studies on the

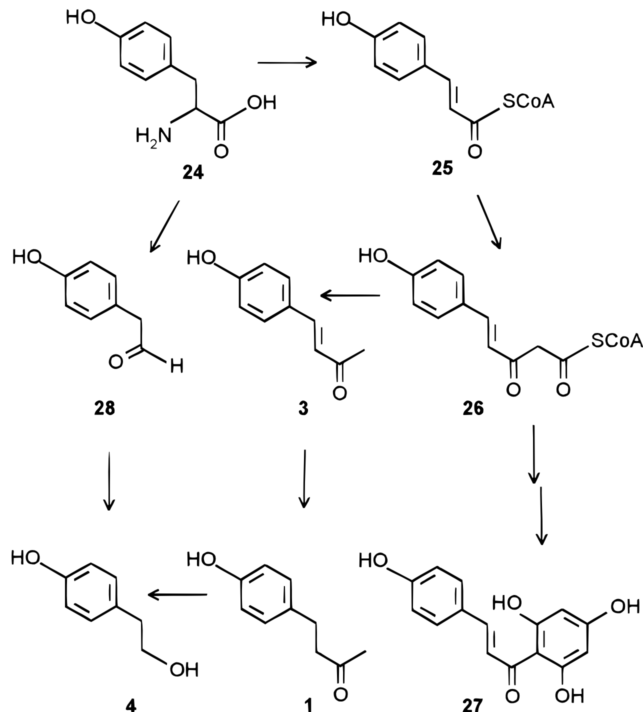


non deuterated material indicated distinct signals for the diastereoisomeric protons at position 1. The chemical shift difference between these hydrogens was determined under decoupling of the methylene protons at position 2. Under these conditions the diastereotopic hydrogens at C-1 gave rise to two doublets (AB system) at 4.54 and 4.51 ppm. The spectra of the (+)-MTPA esters **21** and **23** carried out in the same conditions showed two broad singlets at 4.52 and 4.49 ppm, respectively. The broadening of the signals is due to the unresolved deuterium–

Scheme 6



Scheme 7



hydrogen coupling constants. The AB system at 4.54 and 4.51 ppm was also present in the spectra since the starting materials were not completely deuterated. The upfield shift by 0.02 ppm observed for the signals of the labeled compounds **21** and **23** with respect to the corresponding signals of the protio compound is due to the isotope effect exerted by the geminal deuterium atom. The NMR analysis clearly showed that products **21** and **23** are diastereoisomeric. In addition, product **21** was identical to the derivative of authentic (*S*) [1-^{2}H]tyrosol (**17**), prepared from [1-$^2H_2$]tyramine in *Willia anomala* Hansen (Scheme 6).[17] Thus, the oxidative fragmentation at the 2,3-positions, adjacent to the carbonyl carbon, of the skeleton of C-6–C-4 **1** to provide C-6–C-2 tyrosol (**4**) proceeds with retention of configuration at the migrating center. The same course is shown by both the enzymic and the chemical Baeyer–Villiger fragmentation of cyclohexanone.[20,21]

The microbial conversion of raspberry ketone (**1**) into tyrosol (**4**) discussed herein might hold some biosynthetic relevance. Indeed, C-6–C-4 raspberry ketone **1** can be considered as a product of mixed biosynthetic origin, with subunits supplied by both the acetate and shikimate pathways, respectively (Scheme 7). Chain elongation of the C-6–C-3 *p*-coumaryl-CoA **25** derived from phenylalanine/tyrosine **24** with three malonyl-CoA units eventually provides the C-15 framework **27** present in flavonoids.[22] In the raspberry plant,[13] deviation from the main operation of flavonoid biosynthesis, at the beginning

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of the sequence, at the level of the C-6−C-5 intermediate **26**, provides upon hydrolysis and loss of a C-1 unit as carbon dioxide, the C-6−C-4 unsaturated ketone **3**, from which raspberry ketone (**1**) is produced. In *B. bassiana* Baeyer−Villiger chain shortening of **1** provides C-6−C-2 tyrosol (**4**), the ubiquitous primary metabolite of shikimate-derived tyrosine, formed from the latter by oxidative decarboxylation to **28** and reduction. This metabolic operation brings back a product of the shikimate primary metabolism, *i.e.*, **4**, a secondary metabolite of mixed biosynthetic origin, *i.e.* **1**. Very little is known on the mechanism that regulates in nature the generation of flavor materials, apart from the consideration that these products are formed at the moment of ripening, when catabolic processes prevail and nothing is known on the biodegradation of plant-specific odorants. Raspberry ketone (**1**), possessing a very low odor threshold and present in raspberry fruit at ppm level, likely plays some role in the ecosystem. It is formed through a plant-specific deviation from the ubiquitous flavonoid-producing system. However, the present observations indicate the presence in nature of an enzymic capacity designed to the biodegradation of **1** to the primary metabolite tyrosol **4**. The enzymic shunt between secondary metabolism (raspberry ketone) and primary metabolism (tyrosol) relys on a rather sophisticated reaction such as a Baeyer-Villiger oxidation. This capacity has now been demonstrated to be present in *B. bassiana*, but future experiments might possibly demonstrate that the flavor-control mechanism in raspberry fruit consists of a Baeyer−Villiger degradation of highly odorant raspberry ketone (**1**) to tyrosol (**4**), devoid of sensory properties. Experiments in raspberry plants designed to identify this enzymic activity are in progress.

Experimental Section

The proton NMR spectra were recorded at 400 MHz at room temperature, and the deuterium spectra were run at 61.6 MHz in the gated proton broadband decoupling mode to eliminate the proton−deuterium coupling constants that cause broadening of the signals. *J* values are given in Hz. TLC analyses were performed on Merck Kieselgel 60 F_{254} plates. All chromatographic separations were carried out on silica gel columns. Due to the variable content of deuterium in the labeled compounds the elemental analyses supporting the purity and authenticity of the products were performed on the corresponding nondeuterated species obtained in the exploratory experiments.

(*Z*)-Enol Acetate (5). A solution of 4-(4-hydroxyphenyl)-butan-2-one (**1**) (25 g, 152 mmol) in CCl_4 (475 mL) was treated with Ac_2O (288 mL), containing 0.75 mL of 70% perchloric acid. The reaction mixture was kept overnight at rt, concentrated to remove the majority of Ac_2O, diluted with CH_2Cl_2 , and washed several times with 3% $NaHCO_3$. The dried organic phase was chromatographed on SiO_2 with hexane/AcOEt (8:2) to provide the (*Z*)-enol acetate **5**, 10 g (40%), oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.93 (3H, q, J = 1.1), 2.17 (3H, s), 2.25 (3H, s), 3.26 (2H, d, J = 7.5), 5.17 (1H, tq, J = 7.5, 1.1), 6.97 (2H, m), 7.18 (2H, m); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 19.7 (CH_3), 20.8 (CH_3), 21.2 (CH_3), 31.3 (CH_2), 115.5 (CH), 121.6 (2CH), 129.4 (2CH), 137.8 (C), 146.0 (C), 149.2 (C), 169.1 (C), 169.6 (C). Sample **5** contains about 10% of the (*E*)-isomer: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.94 (3H, q, J = 1.0), 2.10 (3H, s), 2.26 (3H, s), 3.37 (2H, d, J = 7.7), 5.31 (1H, tq, J = 7.7, 1.0), 7.00 (2H, m), 7.21 (2H, m); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 15.4 (CH_3), 21.1 (CH_3), 21.2 (CH_3), 32.2 (CH_2), 116.0 (CH), 121.7 (2CH), 129.3 (2CH), 137.6 (C), 146.8 (C), 149.3 (C), 169.1 (C), 169.6 (C). Anal. Calcd for $C_{14}H_{16}O_4$: C, 67.73; H, 6.50. Found: C, 67.64; H, 6.48.

(2*SR*,3*RS*)-[2,3-$^2H_2$]Diacetate (6). The (*Z*)-enol acetate **5** (10 g, 40.3 mmol) in AcOEt (100 mL) was stirred at rt in deuterium atmosphere in the presence of 0.9 g of PtO_2 at normal pressure. At the end of the absorption, the filtered reaction mixture was evaporated and chromatographed on SiO_2 with hexane/AcOEt (7:3) to provide the acetate ester of 4-butylphenol, 4.64 g (60%) [^{1}H NMR (400 MHz, $CDCl_3$) δ 0.92 (3H, s), 1.34 (0.5H, m), 1.58 (1.3H, m), 2.29 (3H, s), 2.60 (2H, m), 6.99 (2H, m), 7.18 (2H, m); ^{2}H NMR (61.4 MHz, $CHCl_3$) δ 1.33 (1.5 D), 1.56 (0.7 D)] and subsequently, product **6**, 4 g (40%), oil: ^{1}H NMR (400 MHz, C_6D_6) δ 1.03 (3H, s), 1.65 (1H, m broad), 1.71 (3H, s), 1.77 (3H, s), 2.34 (1H, dd, J = 14.0, 9.9), 2.43 (1H, dd, J = 14.0, 6.6), 6.91 (2H, m), 7.00 (2H, m); ^{2}H NMR (61.4 MHz, C_6H_6) δ main signals 1.43 (0.9 D), 4.90 (1D); minor signals 1.00 (0.1 d), 1.64 (0.1 D), 2.31 (0.13 D), 2.39 (0.1 D) (see also Figure 1). Anal. Calcd for $C_{14}H_{18}O_4$: C, 67.18; H, 7.25. Found: C, 67.25; H, 7.21.

(2*R*,3*S*)-[2,3-$^2H_2$] Diacetate 10. The racemic diacetate **6** (4 g, 15.9 mmol) was refluxed with 26 mL of a solution of 10% aqueous NaOH−EtOH (1:1) for 2 h. Once the hydrolysis was complete (TLC), the reaction mixture was concentrated to a small volume, diluted with water, acidified with dilute HCl, and extracted with Et_2O (3 × 70 mL) to provide **7**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.21 (3H, s), 1.79 (1H, s broad), 1.74 (1.3H, m), 2.59 (1H, dd, J = 9.3, 14.0), 2.66 (1H, dd, J = 6.3, 14.0), 3.81 (0.3 H, m), 5.28 (1H, s broad), 6.74 (2H, m), 7.05 (2H, m); ^{2}H NMR (61.4 MHz, $CHCl_3$) δ 1.71 (1D), 3.81 (1D), deuteration extent ca. 70%. The dried organic phase was evaporated, and the residue, diluted with CH_2Cl_2 (100 mL), was treated with Ac_2O (1.6 mL, 16.95 mmol) and pyridine (1.36 mL). After 12 h the reaction mixture was washed with dilute HCl, 3% $NaHCO_3$, and water. The residue obtained upon evaporation of the organic phase was chromatographed with hexane/AcOEt (8:2) to provide **8**, 2.5 g (75%), oil. The latter product (2.5 g, 11.9 mmol) in $(CH_3)_3COCH_3$ (300 mL) was mechanically stirred at rt for 50 h with vinyl acetate (48 mL, 519.64 mmol) and lipase PS Amano (5 g). The course of the reaction was followed by HPLC analysis. Merck-Hitachi L-6200 apparatus equipped with UV detector L-4200 with D-2500 integrator. Chiral stationary phase: Chiracel OD, Daicel, Japan. The elution conditions were the following: *n*-hexane/*i*-PrOH 9/1; flow 0.6 mL/min, the detector was set at 220 nm. The retention times were 9.9 and 11.2 min for the (*R*) and (*S*) enantiomers, respectively. The filtered reaction mixture was evaporated and chromatographed to afford **10**, 1.4 g (46.7%): $[\alpha]^{21}_D = +11.5°$ (*c* 0.5, $CHCl_3$); 0.98 ee upon HPLC analysis; ^{1}H NMR (400 MHz, C_6D_6) δ 1.03 (3H.s), 1.66 (1H, m broad), 1.71 (3H, s), 1.77 (3H, s), 2.34 (1H, dd, J = 14.0, 9.9), 2.43 (1H, dd, J = 14.0, 6.6), 6.90 (2H, m), 7.00 (2H, m); ^{2}H NMR (61.4 MHz, C_6H_6) δ main signals 1.42 (0.85 D), 4.90 (1D); minor signals 0.99 (0.1 d), 1.65 (0.1 D), 2.31 (0.14 D), 2.39 (0.1 D). Subsequently, the (*2S*) product **9** was eluted, 1.1 g (44%). Anal. Calcd for $C_{14}H_{18}O_4$: C, 67.18; H, 7.25. Found: C, 67.24; H, 7.23.

(*Z*)-[1,3-$^2H_4$]Enol Acetate 11. Product **1** (8.2 g, 0.05 mol) in 1,4-dioxane (20 mL) was treated with D_2O (30 mL) containing KOH (2.8 g) under stirring for 48 h. The reaction mixture was poured into an excess of HCl in crushed ice and Et_2O (200 mL). The residue obtained by evaporation of the solvent was crystallized from cyclohexane to provide [1,3-$^2H_5$]-**1**, mp 81 °C, 8.45 g (100%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.80 (2H, s broad), 6.40 (1H, s broad), 5.75 (2H, m), 7.02 (2H, m); ^{2}H NMR (61.4 MHz, $CHCl_3$) δ 2.11 (3D), 2.70 (2D), deuteration extent ca. 90%. The latter material was converted into the (*Z*)-enolacetate (**11**) as reported for **5** in 30% yield. (Z)-isomer: ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.17 (3H, s), 2.28 (3H, s), 3.26 (2H, s broad), 5.16 (0.3H, t, J = 7.4), 6.98 (2H, m), 7.18 (2H, m); ^{2}H NMR (61.4 MHz, $CHCl_3$) δ 1.91 (3D), 5.19 (1D), deuteration extent ca. 70%. (*E*)-Isomer (ca. 10% in mixture with the (*Z*)-isomer): ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.12 (3H, s), 2.28 (3H, sr, 3.37 (2H, s broad), 5.30 (ca. 0.3H, t, J = 7.8), 7.0 (2H, m), 7.22 (2H, m). ^{2}H NMR (61.4 MHz, $CHCl_3$) δ 1.91 (3D), 5.33 (2D), deuteration extent ca. 70%.

(2*SR*,3*SR*)-[1,3-$^2H_4$]-Diacetate 12. The conversion of **11** into **12** was performed exactly as reported above for **5**, but using hydrogen gas instead of deuterium. Product **12**: ^{1}H NMR (400 MHz, C_6D_6) δ 1.45 (1H, m broad), 1.71 (3H, s), 1.77 (3H, s), 2.34 (1H, dd, J = 14.0, 6.6), 2.43 (1H, dd, J = 14.0,

9.9), 4.91 (1H, m broad), 6.90 (2H, m), 7.00 (2H, m); ^{2}H NMR (61.4 MHz, C_6H_6) δ 0.98 (3D), 1.65 (0.85D), 1.42 (0.15D), deuteration extent ca. 70%.

(2*R*,3*R*)-[1,3-$^2H_4$]Diacetate 14. The conversion of the racemic material **12** into (*2R,3R*)-**14** proceeded as reported for **6**. Product **14**: $[\alpha]^{21}_D = +11.6°$ (*c* 0.5, $CHCl_3$); 0.99 ee by HPLC; ^{1}H NMR (400 MHz, C_6D_6) δ 1.46 (1H, m broad), 1.71 (3H, s), 1.77 (3H, s), 2.35 (1H, dd, $J = 14.0, 6.6$), 2.43 (1H, dd, $J = 14.0, 9.9$), 4.92 (1H, m broad), 6.90 (2H, m), 7.00 (2H, m); ^{2}H NMR (61.4 MHz, C_6H_6) δ 0.98 (3D), 1.65 (0.85D), 1.42 (0.15D), deuteration extent ca. 70%.

Microbial Conversion of 15 and 16 into Tyrosol (17) and (18). The diacetate esters **10** and **14** were quantitatively converted into **15** and **16** by NaOH-catalyzed hydrolysis, as reported above. Product **15**: $[\alpha]^{21}_D = -15.5°$ (*c* 0.5, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.21 (3H, s), 1.70 (1H, s very broad), 1.73 (1.2H, m), 2.59 (1H, dd, $J = 9.3, 14.0$), 2.66 (1H, dd, $J = 6.3, 14.0$), 3.82 (0.3 H, m), 5.20 (1H, s very broad), 6.74 (2H, m), 7.05 (2H, m); ^{2}H NMR (61.4 MHz, $CHCl_3$) δ 1.71 (1D), 3.80 (1D), deuteration extent ca. 70%. **16**: $[\alpha]^{21}_D = -16°$ (*c* 0.5, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.72 (1.3H, m), 2.59 (1H, dd, $J = 6.5, 14.0$), 2.67 (1H, dd, $J = 9.1, 14.0$), 3.82 (1H, m broad), 6.74 (2H, m), 7.05 (2H, m); ^{2}H NMR (61.4 MHz, $CHCl_3$) δ 1.20 (3D), 1.76 (1D), deuteration extent ca. 70%. The microbial incubation of **15** and **16** was made as follows. Five mL of T1[23] medium was seeded with the microorganism and incubated for 4 days at 30 °C. The biomass was suspended in 4 mL of T3, medium and 2 mL of this suspension was inoculated in 50 mL of the same medium and shaken at 180 rpm for 24 h at 30 °C. Five mL of this culture was inoculated in 50 mL of fresh T3 medium and incubated for 1 day in the same conditions. Three mL of the content of the flask was inoculated in 50 mL of MPGB medium and shaken at 180 rpm at 30 °C for 24 h. At this point, 50 mg of substrate was added to each conical flask and the mixture stirred at 180 rpm for 120 h at 30 °C. The incubation mixture was extracted with AcOEt (3 × 150 mL for 400 mL of aqueous phase). Column chromatography of 400 mg of the residue of substrates **15** and **16** gave, upon evaporation, the organic extracts tyrosol **17** and **18** in 93 and 65 mg yield, respectively. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.60 (1H, very broad), 2.80 (2H, d, $J = 6.4$), 3.81 (0.7H, tt, $J_{H,H} = 6.3$, $J_{H,D} = 1.7$) and 3.83 (0.6H, t, $J_{H,H} = 6.3$), 4.95 (1H, very broad), 6.78 (2H, m), 7.09 (2H, m); ^{2}H NMR (61.4 MHz, $CHCl_3$) δ 3.83(1D), deuteration extent ca. 70%. Anal. Calcd for $C_8H_{10}O_2$: C, 69.54; H, 7.30. Found: C, 69.62; H, 7.21.

(+)-MTPA Esters 21 and 23. A stirred solution of **17** (**18**) (100 mg, 0.552 mmol) in CH_2Cl_2 was treated with Ac_2O (0.058 mL, 0.58 mmol) and pyridine (0.05 mL, 0.608 mmol). After 12 h, the reaction mixture was washed with dilute HCl, 3% $NaHCO_3$, and water. The residue obtained upon evaporation of the organic phase was chromatographed by PLC with hexane/AcOEt (7:3) to provide **20** (**22**), 75 mg (75%). A stirred solution of **20** (75 mg, 0.414 mmol) in CH_2Cl_2 was treated with pyridine (0.04 mL, 0.5 mmol) and with the chloride of (+)-α-methoxy-α-(trifluoromethyl)phenylacetic acid (0.1 mL, 0.535 mmol) for 2 h at rt. The residue obtained upon evaporation of the organic phase was chromatographed by PLC with hexane/AcOEt (7:3) to provide **21**, 200 mg (91%), oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.29 (3H, s), 2.99 (2H, m), 3.46 (3H, q, $J = 1.2$), 4.47−4.57 [(1.3H, m); with irradiation of the multiplet at δ 2.99: 4.54 and 4.51 (0.6H, AB system $J = 11$), 4.52 (0.7H, s broad)], 6.99 (2H, m), 7.16 (2H, m), 7.33−7.45 (5H, m); ^{2}H NMR (61.4 MHz, $CHCl_3$) δ 4.51 (1D), 2.97 (0.3D), deuteration extent ca. 70%. The same procedure carried out on product **22** provided **23**, 200 mg (91%), oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.29 (3H, s), 2.99 (2H, m), 3.46 (3H, q, $J = 1.2$), 4.47−4.57 [(1.3H, m); with irradiation of the multiplet at δ 2.99: 4.54 and 4.51 (0.6H, AB system $J = 11$), 4.49 (0.7H, s broad)], 6.99 (2H, m), 7.16 (2H, m), 7.33−7.45 (5H, m); ^{2}H NMR (61.4 MHz, $CHCl_3$) δ 4.54 (1D), deuteration extent ca. 70%.

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(23) T1: corn step atomized 12 g/L, D-glucose 10 g/L, agar 30 g/L, pH 5.5. T3, bacto-triptone 10 g/L, K_2HPO_4 1 g/L, *D*-glucose 30 g/L, $FeSO_4{\cdot}7HO$ 0.01 g/L, $MgSO_4{\cdot}7H_2O$ 0.5 g/L, $ZnSO_4{\cdot}7H_2O$ 0.3 g/L, KCl 0.5 g/L, pH 7.2. MPGB, D-glucose 20 g/L, peptone 5 g/L, malt 20 g/L.