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On the Stereochemistry of the Baeyer-Villiger Degradation of Arylalkyl-ketones Structurally Related to Raspberry Ketone by *Beauveria bassiana*

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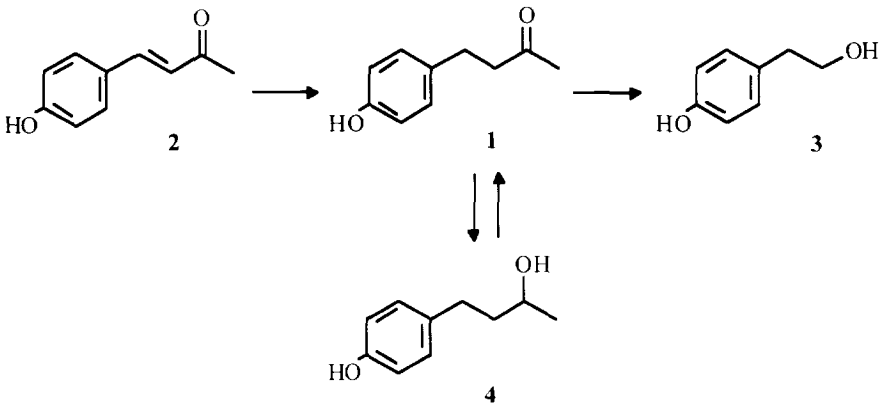
Abstract: The mode of transformation in *Beauveria bassiana* (ATCC 7159) of ketones **5-9** and **16** has been studied and compared with that of C-6--C-4 **2**, which through raspberry ketone **1** gives rise to C-6--C-2 tyrosol **3**. Of the fed materials, only product **5** behaves as **2**, *i.e.*, is a good substrate for a formal Baeyer-Villiger chain-shortening transformation, which provides the secondary carbinol **10** enriched in the (*S*) enantiomer. Stereochemical analysis of the products obtained in the incubation of an authentic sample of (*S*) **5**, obtained with baker's yeast upon reduction of the corresponding unsaturated ketone, indicates that the Baeyer-Villiger degradation leading to **10** occurs with kinetic preference for the (*S*) enantiomer and retention of configuration at the migrating carbon atom. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

4-(4-Hydroxyphenyl)-butan-2-one **1**, referred to as *raspberry ketone*, is the impact flavour of raspberry fruit.[1] It has been recently shown in cell-free extract of raspberry fruits and in tissue cultures that the C-6--C-4 framework of **1** is formed through the intermediacy of a C-6--C-5 β-ketoacid, originated by condensation of *p*-coumaryl-CoA and malonyl-CoA, followed by decarboxylation to the unsaturated ketone **2**. This material is subsequently converted into **1** by a NADPH-dependent reductase.[2] In a study[3] designed to obtain the *natural*[4] modification of raspberry ketone **1** we submitted to the action of several microorganisms the unsaturated ketone **2**, prepared by condensation of 4-hydroxybenzaldehyde of extractive origin with acetone derived from sugar fermentation. In all instances we observed the formation from **2** of **1**, accompanied by the saturated carbinol **4**, generally enriched in the (*S*) enantiomer. In the explored instances, there is no formation of the unsaturated carbinol, at variance with what occurs with structurally related substrates.[5] The microbial screening included also *Beauveria bassiana* (ATCC 7159). This microorganism mediates the conversion of **2** into **1**, accompanied by an amount of **4** which increases with the incubation time. However, continuing in the culture, carbinol **4** is oxidized back to the ketone **1** and the latter, in a subsequent step, is quantitatively degraded to C-6--C-2 tyrosol **3** (Scheme 1). Deuterium labeling experiments[6] indicated the retention at position 1 of **3** of the two deuterium atoms located at position 3 of the C-6--C-4 framework of **1**, suggesting that the fragmentation of the carbon skeleton of **1** thus observed occurs by a Baeyer-Villiger type oxygen insertion into the 2,3 C-C bond and hydrolysis of the acetate ester.[7]

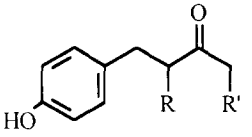


Scheme 1

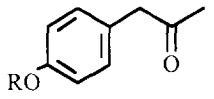
Studies on the steric outcome of microbial Baeyer-Villiger degradation of racemic synthetic ketones have been recently reported, showing the preparative interest of this transformation.[8]

RESULTS AND DISCUSSION

We now present the results of an investigation on the mode of degradation of ketones **5-9**, structurally related to **1**, by growing cultures of *B. bassiana*.

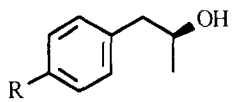


5 R = Me; R' = H
6 R = H; R' = Me
7 R = Et; R' = H

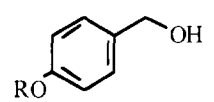


8 R = H
9 R = Me

Under the experimental conditions in which **1** is quantitatively converted into **3,** only product **5** yields in *B. bassiana* in *ca.* 40% yield carbinol **10**, $[\alpha]^{20}_{D}$ + 21.6, shown by GLC analysis on Megadex 5 to possess 0.65 *e.e.*. The homologous materials **6** and **7** were recovered unchanged, whereas the C-6--C-3 methylketones **8** and **9** provided respectively *ca.* 3% and 10% of the C-6--C-1 benzyl alcohols **14** and **15**, close to the carbinols **10** and **13**. Product **10** obtained from (*RS*) **5** was assigned the (*S*) absolute configuration because of its conversion, *via* the 1-phenyl-1H-tetrazolyl derivative **11** and subsequent hydrogenolysis,[9] into the known[10] (*S*) carbinol **12**, 0.65 *e.e.*, $[\alpha]^{20}_{D}$ +26.6 (c = 5.3 in benzene) (lit[10] +41).



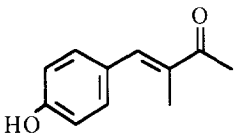
10 R = OH
11 R = Ph-1H-Tetrazole
12 R = H
13 R = OMe



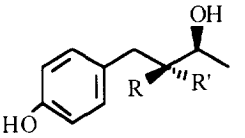
14 R = H
15 R = Me

Information on enantiomeric preference and stereochemistry of the above reported chain-shortening Baeyer-Villiger conversion of **5** into **10** was obtained from transformation experiments of (*S*) **5**. The material,

possessing 0.54 *e.e.*, $[\alpha]^{20}_D$ +27 (c = 1 in $CHCl_3$) was obtained[11] by baker's yeast mediated reduction of the unsaturated ketone **16**. Under our experimental conditions, product **5** was produced from **16** together with a 3:1 mixture of carbinols **17** and **18** (ketone/carbinol ratio: *ca.* 3:1, see experimental) proved to be enantiomerically pure by ^1H NMR with chiral shift reagent [Eu(tfc)$_3$].



16



17 R = Me; R' = H
18 R = H; R' = Me

C-6--C-5, (*S*) **5** (0.54 *e.e.*) in *B. bassiana* provided C-6--C-3 (*S*) **10** possessing 0.78 e.e., whereas the survived methyl ketone shows 0.44 *e.e.* and (*S*) configuration. These results thus show that (i) the enzymatic degradation process occurs with kinetic preference for the (*S*) enantiomer of ketone **5** and (ii) the fragmentation of the carbon skeleton proceeds with retention of configuration at the migrating carbon atom. Similar stereochemical features emerged in *Acinetobacter* sp. during the oxidation of cyclic ketones to the corresponding lactones.[12] Indeed, also in these circumstances, the insertion of oxygen into the C-C bond takes place with retention of configuration and kinetic preference for the (*S*) enantiomer.

The stereochemical analysis of the transformation by *B. bassiana* of the above set of compounds was completed with the assignment of the (*S*) stereochemistry to carbinols **10** and **13** obtained from **8** and **9**, respectively. The *e.e.* values, determined by GLC analysis, resulted 0.83 and 0.94, respectively. The absolute configuration of **13** was determined by correlation with **10**, through methylation of the latter with CH_2N_2. For sake of comparison, **8** and **9** were submitted to the action of fermenting baker's yeast which provided **10** and **13** of (*S*) absolute configuration and 0.91 and 0.98 *e.e.*, respectively.

The unsaturated ketone **16,** precursor in baker's yeast of (*S*) **5**, was recovered unchanged when incubated in growing cultures of *B. bassiana,* which effectively reduced ketone **2** to a mixture of **1** and **4**.

Thus, the microbial enzyme(s) presiding in *B. bassiana* over the Baeyer-Villiger degradation of raspberry ketone **1** into tyrosol **3** show a rather narrow tolerance towards substrate structural modifications, since among ketones **5-9** only **5** is significantly oxidized. Moreover, product **16**, the α-methyl analog of **2**, is not a good substrate for the enzymes mediating the saturation of the double bond α to the carbonyl. In this context, it is worth mentioning that *B. bassiana* effectively saturates the double bond of aliphatic α,β-unsaturated methyl ketones, whereas in the case of α-substituted cyclo hexenones the reduction was hindered by bulky substituents. Moreover the saturated ketones obtained in the first instance hold S absolute configuration.[13] In *B. bassiana* the Baeyer-Villiger degradation of **5** occurs with the same stereochemical features shown in *Acinetobacter*[12] in the ring opening of alkyl-substituted cyclohexanones and cyclopentanones, *i.e.*, kinetic preference for the (*S*) enantiomer and retention of configuration at the migrating carbon atom.

At present, we do not know if **1** and **5-9** are substrates of a Baeyer-Villiger degradation in the above mentioned microorganism, but we determined that in *B. bassiana* α-*n*-butyl and α-*n*-pentyl cyclopentanones are not converted into the δ-lactones obtained from these substrates in *Acinetobacter*.[12] Finally, from a naturalistic point of view it is worth mentioning that raspberry ketone **1**, produced in 0.02-0.37 ppm[14] in the raspberry fruit at the moment of ripening and possessing an extremely low perception threshold, is degraded

to the primary metabolite tyrosol **3** by the microorganism *B. bassiana*, not producing endogenously raspberry ketone, through a chemical reaction such as a Baeyer-Villiger degradation. Additionally, the operation is performed by means of an enzymatic system showing an extremely narrow substrate specificity.

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EXPERIMENTAL: ^{1}H NMR spectra were recorded with Bruker CXP-300 or ARX-400 instruments in the FT mode. GLC analyses were performed on a DANI 8610 equipped with PTV injector and FID detector. Two different chiral capillary column (MEGA, Legnano, Italy) were utilised. For the separation of compounds **10** a 25 mt x 0,25 mm Megadex 5, film thickness 0.25 μm, was adopted, while for compounds **5** and **13**, which are not separable into their enantiomers on this column, a *t*BDA-β-cdx, of the same size was used. Temperature programs for Megadex 5: 40 °C, 20 °C/min, 130 °C, 2 min, 1 °C/min; carrier gas H_2, 0.8 bar; retention times for compounds **10**, **S** 31.00, **R** 31.25. Temperature programs for *t*BDA-β-cdx: 40 °C, 20 °C/min, 140 °C, 2 min, 1 °C/min; carrier gas H_2, 0.8 bar; retention times (min) for compounds **5** and **13** respectively: **5**, **R** 39.05, **S** 39.36; **13**, **S** 18,59, **R** 18.99.

Preparation of Substrates: Products **5-7** were obtained upon catalytic hydrogenation (10% Pd/C, EtOH/AcOEt) of the corresponding unsaturated ketones.[11] Product **5**, ^{1}H NMR ($CDCl_3$) δ 1.08 (3H, CH_3, d), 2.09 (3H, CH_3, s), 2.53 (1H, CH_2, q), 2.74-2.97 (3H, CH_2, CH, m), 5.85 (1H, OH, broad), 6.74 (2H, Ph, d) and 7.00 (2H, Ph, d). Product **6**, ^{1}H NMR ($CDCl_3$) δ 1.03 (3H, CH_3, t) 2.41 (2H, CH_2, q), 2.69 (2H, CH_2, dd), 2.73 (2H, CH_2, dd), 5.15 (1H, OH, s), 6.74 (2H, Ph, d) and 7.02 (2H, Ph, d). Product **7**, ^{1}H NMR ($CDCl_3$) δ 0.89 (3H, CH_3, t) 1.58 (2H, CH_2, m), 2.01 (3H, CH_3, s), 2.55-2.87 (3H, CH_2, CH, broad signal), 5.25 (1H, OH, s), 6.74 (2H, Ph, d) and 7.02 (2H, Ph, d). Methyl ketones **8** and **9** were obtained according to a reported procedure from the corresponding phenylacetic acids.[15]

Microbial Transformations in B. bassiana: 5 ml of T1 medium were 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 were inoculated in 50 ml of the same medium and shaken at 180 rpm for 24 h at 30 °C. 5 ml of this culture were inoculated in 50 ml of fresh T3 medium and incubated for 3 days in the same conditions. 3 ml of the content of the flask were inoculated in 50 ml of MPGB medium and shaken at 180 rpm at 30 °C for 24 h. At this point 50 mg of substrates **5-9** and **16** dissolved in 0.5 mL of EtOH were added and the mixture stirred at 180 rpm for 48/120 h at 30 °C. The incubation mixture was extracted with 2x25 ml of ethyl acetate. The separated organic phase, once dried, was evaporated under vacuum to give a crude extract which was used directly for GLC analysis. Composition of the media: T1, corn step atomised 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, $FeSO_4 \cdot 7H_2O$ 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.

***Conversion of* (S) 5 *into* (S) 10**. Incubation of (**S**) **5**, 0.54 *e.e.*, 250 mg, obtained by baker's yeast reduction of **16**,[11] in *B. bassiana* cultures, 250 ml, affords after 72 h upon CH_2Cl_2 extraction (3 x 100 ml) and column chromatography of the residue products **5**, 180 mg (71%), 0.44 *e.e.*, and the carbinol **10**, 80 mg (38%), $[\alpha]^{20}_D$ + 25.9 (c = 1 in $CHCl_3$), shown by GLC to possess 0.78 *e.e.*.

Conversion of 10 into 12. Carbinol **10**, 1.2g (7.9 mmol) in acetone (50 ml) was stirred 48h with chlorophenyltetrazole, 1.4 g (8 mmol), in the presence of finely powdered K_2CO_3. The filtered reaction

mixture was taken to dryness and the residue upon column chromatography provided the 5-phenyltetrazolyl derivative **11**, 1.3 g (57 %). ^{1}H NMR ($CDCl_3$) δ 1.15 (3H, CH_3, m), 1.8 (1H, OH, br s), 2.68 (2H, CH_2, t), 3.95 (1H, CH, m), 7.25 (2H, Ph, m), 7.48 (5H, Ph, m) and 7.75 (2H, Ph, m). The latter product, 0.5 g (1.7mmol) dissolved in EtOH was hydrogenated at r.t. in the presence of 10% Pd/C, 50 mg, in a Parr apparatus. After 48 h the filtered reaction mixture was evaporated and chromatographed to provide carbinol **12** in 95% yield, with 0.65 *e.e.*, $[\alpha]^{20}_D$ +26.6 (c = 5.3 in benzene) (lit.[10] +41). ^{1}H NMR ($CDCl_3$) δ 1.21 (3H, CH_3, d) 2.13 (1H, OH, broad), 2.71 (2H, CH_2, m), 3.99 (1H, CH, m) and 7.12-7.31 (5H, Ph, m).

***Conversion of* 8 *and* 9 *into* 10 (14) *and* 13 (15).** Incubation of **8-9**, 250 mg in *B. bassiana* cultures, 250 ml, affords, after 72 h upon CH_2Cl_2 extraction (3 x 100 ml) and column chromatography of the residue products, **10, 13, 14** and **15. 10**, 99 mg (39%), 0.83 e.e.., $[\alpha]^{20}_D$ +26.6 (c = 1 in $CHCl_3$), ^{1}H NMR ($CDCl_3$) δ 1.23 (3H, CH_3, d), 1.98 (1H, OH, s), 2.57 (1H, CH_2, dd), 2.73 (1H, CH_2, dd), 3.99 (1H, CH, m), 6.28 (1H, OH, s), 6.75 (2H, Ph, d) and 7.03 (2H, Ph, d). **13**, 127 mg (50%), 0.94 e.e.., $[\alpha]^{20}_D$ +30.9 (c = 1 in $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.22 (3H, CH_3, d), 1.54 (1H, OH, s), 2.61 (1H, CH_2, dd), 2.73 (1H, CH_2, dd), 3.79 (3H, CH_3, s), 3.99 (1H, CH, m), 6.84 (2H, Ph, d) and 7.13 (2H, Ph, d). Compounds **14** and **15,** isolated respectively in 3 and 10% yield, resulted identical by GC/MS, with samples obtained upon $NaBH_4$ reduction of the corresponding aldehydes.

Baker's Yeast Reduction of 16. In an open beaker containing 2 l of tap water at 38 °C were mixed 1 kg of commercially available baker's yeast (Eridania, Italy,), 200 g of **D**-glucose and 5 g (28 mmol) of **16**. The fermentation mixture was stirred for 48 h at 25 °C. The crude reaction mixture was filtered through a pad of celite and extracted with ethyl acetate (3 x 250 mL). The residue upon evaporation of the solvent was purified by chromatography on silica so as to obtain 3.2 g (18 mmol) of **5** (S) $[\alpha]^{20}_D$ + 27 (c =1, $CHCl_3$) and 1.1 g (6 mmol) of a 3:1 mixture of **17** and **18**. Using this type of conditions (presence of glucose) we obtain better transformation yields (85%) without depriving too much the optical purity (e.e. 0.54 versus 0.58).[11]

***Baker's yeast reduction of* 8 *and* 9**. The same conditions adopted for the B. Y. reduction of **16** were followed for the preparation of substrates **10** $[\alpha]^{20}_D$ + 29.2, e.e. 91% and **13** $[\alpha]^{20}_D$ +32.2, e.e. 98%.

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