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Hydride transfer versus electron transfer in the reduction of 4-phenyl-3-halo-3-buten-2-ones mediated by *Pichia stipitis*

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ABSTRACT

Reductions of (*Z*)-$C_6H_5CH{=}CXC(=O)CH_3$ (X = Cl, Br) mediated by *Pichia stipitis* gave 4-phenylbutan-2-one through dehalogenation of intermediaries 3-halo-4-phenylbutan-2-one by an electron transfer mechanism. The addition of 1,3-dinitrobenzene avoids the dehalogenation and thus the corresponding (2*S*,3*S*)-halohydrins were obtained in excellent enantiomeric excesses by a hydride transfer mechanism. Irganox® 1010 and 1076 were also used to inhibit the electron transfer mechanism. The obtained halohydrins are important chiral building blocks to obtain optically active epoxides and aminoalcohols.

1. Introduction

The enones have been investigated as potential substrates for biocatalytic reductions that can lead to the introduction of 1, 2 or 3 new chiral centers into an achiral structure [1–3]. Therefore, the bioreduction of C=C and C=O conjugated double bonds is an important method for preparing chiral building blocks. The Baker's yeast (*Saccharomyces cerevisiae*) is the most used microorganism to mediate the reduction of enones [2,4–9], although some other microorganisms such as *Beauveria sulfurescens* ATCC 7159 [10], *Pichia stipitis* CCT 2617 [11], *Pyrococcus furiosus* DSM 3638 [12], *Geotrichum candidum, Mortierella isabellina*, *Rhodotorula rubra* [13], have also been used for this purpose.

The enantioselective reductions of C=C and C=O bonds of α-haloenones are very useful to give halohydrins in high ee, that can be converted to enantioenriched epoxides. The later compounds are very useful synthetic intermediates and were used in the preparation of a number of biological active natural products such as aminoalcohols derivatives [14]. There are some papers describing the α-haloenones bioreduction including the reduction of a series of 3-fluor-3-alken-2-ones [15], 3-chloro-3-alken-2-ones [16] and α-bromoenones [6] both mediated by Baker's yeast giving the corresponding halohydrins.

In this work, we found in the preliminary experiments that the reduction of haloenones (*Z*)-3-chloro-4-phenyl-3-buten-2-ones **1a** and (*Z*)-3-bromo-4-phenyl-3-buten-2-ones **1b** mediated by *P. stipitis* gave the dehalogenated product 4-phenylbutan-2-one **2** instead of the corresponding halohydrins (Scheme 1).

The dehalogenation mechanism of 3-chloro-2-methylcinnamaldehyde mediated by Baker's yeast giving 2-methyl-3-phenylpropan-1-ol was proposed in a cascade reaction sequence: reduction of C=C bond to provide 3-chloro-2-methyl-3-phenylpropanaldehyde followed by a spontaneous elimination of HCl, and finally the reduction of C=C and C=O bonds [17]. A similar spontaneous elimination of HCl was suggested for dehalogenation of 3-chloropropiophenone during experiments of Baker's yeast reduction to give propiophenone [18]. Taking into account those cascade reaction sequence, the reduction of haloenones **1a** and **1b** will provide at first the corresponding α-haloketone due to reduction of C=C bond. Since it is known that the α-bromoketone does not spontaneously eliminate HBr [19] as does β-haloaldehyde and β-haloketone, the obtaining dehalogenated product 4-phenylbutan-2-one (Scheme 1) stimulates us to study the mechanism of **1a** and **1b** biotransformation.

The dehalogenation of α-haloketones mediated by microorganisms is an interesting reaction that generally is observed in bioreductions of α-iodoacetophenone [20], ethyl 2-chloroacetoacetate [21,22], 2-chloro-3-oxoesters [23,24] and ethyl 4-chloroacetoacetate [25]. The dehalogenation of α-haloketones by microorganisms might be associated with glutathione [22] or by a mechanism that involve a radical NAD species [20]. The α-

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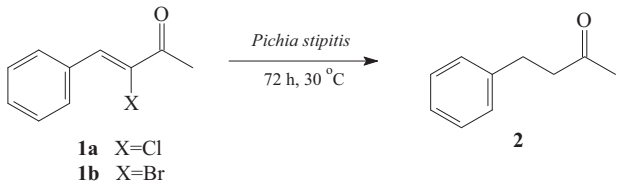


Scheme 1.

haloketones have been used as mechanistic probe in the reduction reactions of NADH-dependent horse liver alcohol dehydrogenase (NADH/HLADH) [26], in reductions mediated by Baker's yeast [20], in reductions using 1,4-dihydronicotinamides [27–29] and even for identification of reductants in sediments [30]. Dehalogenated ketone is the product obtained by a multistep electron transfer (e^-, $H^\bullet$, or e^-, H^+, e^- as has been suggested) [31,32] while halohydrin is obtained by hydride transfer process in which the mechanism is discussed elsewhere [33]. Generally, a small quantity of 1,3-dinitrobenzene (DNB), an efficient radical ion scavenger [34], is added in order to inhibit the free radical process [20,26–29]. If an enzyme mediates a hydride transfer process, the product is an optically active halohydrin since the DNB did not hinder the enzyme controlled formation of halohydrin [26]. Therefore, in this work, the reduction reaction of the haloenones **1a** and **1b** mediated by *P. stipitis* is investigated and the results are discussed in the light of the above mentioned concepts.

2. Experimental

2.1. Materials and methods

^{1}H NMR spectra were determined at 250 MHz (Varian Gemini 250) or 500 MHz (INOVA 500). ^{13}C NMR spectra were determined at 62.5 MHz (Varian Gemini 250) or 125.7 MHz (INOVA 500). Chemical shifts are reported in ppm relative to tetramethylsilane (TMS, $\delta = 0.00$ for ^{1}H) in $CDCl_3$. IR spectra were recorded on a FT-IR BOMEM MB-100 from Hartmann and Braun and absorptions are reported in cm^{-1}. GC/MS(A) analyses were obtained on a QP 5000-Shimadzu (to provide data presented in Figs. 1 and 2) using a silica capillary column DB1 from J&W Scientific (30 m × 0.25 m ID × 0.25 μm film thickness) and helium as a carrier gas (1.0 mL/min). The split ratio was 1:30. The injector temperature was kept 270 °C and detector was kept at 280 °C. The column temperature was held at 80 °C for 3 min and then increase to 280 °C at a rate of 10 °C/min. 1 μL of a compound solution (1 mg/mL) in ethyl acetate was injected and the retention times in min are reported for each compound; GC/MS(B) analyses were obtained on an Agilent 6890 Series GC System and mass spectra were recorded with a Hewlett-Packard 5973 Mass Selective Detector (70 eV) using a HP-5MS fused silica capillary column (crosslinked 5% phenyl ethyl siloxane, 30 m × 0.25 m ID × 0.25 μm film thickness) and helium as a carrier gas (1.2 mL/min). The split ratio was 1:30. The injector temperature was kept 270 °C and detector was kept at 280 °C. The column temperature was held at 80 °C for 3 min and then increase to 180 °C at a rate of 20 °C/min and from 180 °C to 290 °C at a rate of 30 °C/min and then kept constant for 3 min. 1 μL of a compound solution (1 mg/mL) in ethyl acetate was injected and the retention times in min are reported for each compound. Chiral GC/FID analyses were obtained on an Agilent 6850 Series GC System, using a Hydrodex chiral capillary column (30 m × 0.25 mm × 0.25 μm). Hydrogen was used as a carrier gas (1 mL/min), the injector temperature was 200 °C and the detector temperature was 220 °C. The column temperature was held at 80 °C and then increase to 180 °C at a rate of 1 °C/min, and then kept constant for 5 min. 1 μL of a compound solution (1 mg/mL) in ethyl acetate was injected and the retention times in min are reported for each compound. Thin-layer chromatography (TLC) analyses were performed with precoated aluminum sheets (silica gel 60 Merck), and flash column chromatography was carried out on silica (200–400 mesh, Merck). *trans*-4-Phenyl-3-buten-2-one, Oxone® and triethylamine were purchased from Aldrich. Sodium chloride, sodium bromide and dichloromethane were purchased from Synth. 4-Phenylbutan-2-one was obtained by reduction of *trans*-4-phenyl-3-buten-2-one with H_2/Pd-C in ethyl acetate, and 4-phenylbutan-2-ol was obtained by reduction of 4-phenylbutan-2-one with $NaBH_4$ in EtOH. All reagents were used as received. The yeast strain *P. stipitis* CCT 2617 is stored at André Tosello Research Foundation (Campinas-SP, Brazil) [35].

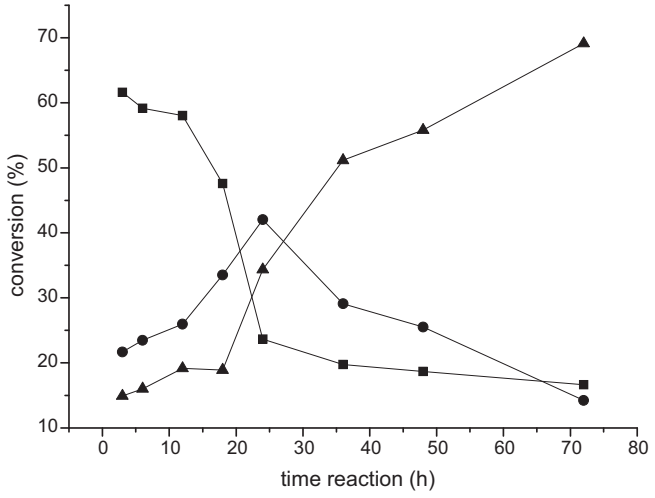


Fig. 1. Bioreduction of (*Z*)-3-chloro-4-phenyl-3-buten-2-one (■) by *P. stipitis* in aqueous medium giving 3-chloro-4-phenyl-2-butanone (●) as transient and 4-phenyl-2-butanone (▲) as final product.

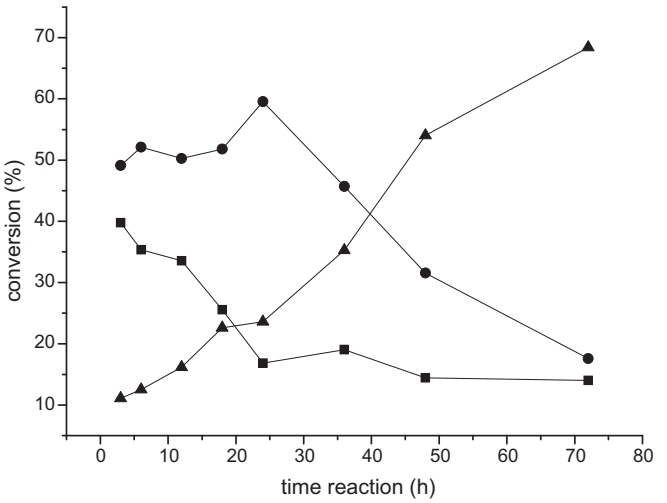


Fig. 2. Bioreduction of (*Z*)-3-bromo-4-phenyl-3-buten-2-one (■) by *P. stipitis* in aqueous medium giving 3-bromo-4-phenyl-2-butanone (●) as transient and 4-phenyl-2-butanone (▲) as final product.

2.2. Growth conditions of P. stipitis

P. stipitis CCT 2617 was cultivated in YM (yeast-malt extract) nutrient broth (1000 mL) under aseptic conditions, incubation at 30 °C on an orbital shaker (180 rpm) for 1 day just before use. All

materials and medium were sterilized in an autoclave at 121 °C before use and the yeast was manipulated in a laminar flow cabinet.

2.3. Procedure for the P. stipitis mediated bioreduction of trans-4-phenyl-3-buten-2-one

trans-4-Phenyl-3-buten-2-one (20 mg) dissolved in 0.5 mL of ethanol was added to an Erlenmeyer containing 20 mL of slurry of growing *P. stipitis* cells. The reaction mixture was incubated in an orbital shaker (200 rpm, 30 °C) for 72 h. Then, the product was extracted with dichloromethane. The solvent was dried with sodium sulfate, evaporated, and the products were analyzed by GC/MS giving 24.3% of 4-phenylbutan-2-one (retention time = 5.9 min), 1.6% of 4-phenylbutan-2-ol (retention time = 6.1 min) and 73.9% of *trans*-4-phenyl-3-buten-2-one (retention time = 7.2 min).

2.4. General procedure for the preparation of α-haloenones **1a** and **1b** [36]

Oxone® (25.2 g, 40 mmol) and NaCl (2.4 g, 40 mmol) or NaBr (4.1 g, 40 mmol) were added to an ice bath cooled mixture of dichloromethane (100 mL) and water (20 mL) in a 250 mL in a round-bottom flask. The mixture was stirred for 40 minutes, and after that *trans*-4-phenyl-3-buten-2-one (3.0 g, 20 mmol) was added and the mixture was stirred for 4 h. After that, triethylamine (8.6 mL, 30 mmol) was added with a dropping funnel, and the reaction mixture was stirred for 1 h. After that, the reaction mixture was neutralized with a 1 M HCl solution, the organic layer was separated, and the aqueous phase was extracted with dichloromethane. The combined organic fractions were dried with sodium sulfate and the solvent was evaporated. The product **1a** was purified by preparative TLC (silica gel containing 10% $AgNO_3$ in mass; elution solvent as hexane/ethyl acetate 9:1) and the **1b** product was purified by preparative TLC (silica gel and hexane/ethyl acetate 9:1).

2.5. General procedure for the P. stipitis mediated bioreduction of α-haloenones **1a** and **1b**

Procedure A (to collect data shown in Figs. 1 and 2): compound **1a** or **1b** (10 mg) dissolved in 1 mL of ethanol was added in each of 8 Erlenmeyer flasks containing 10 mL of slurry of growing *P. stipitis* cells. The reaction mixtures were incubated in an orbital shaker (200 rpm at 30 °C). After the appropriate intervals, one Erlenmeyer was collected and the products were extracted with ethyl acetate, dried with sodium sulfate, the solvent was evaporated and the products analyzed by GC/MS. The results are shown in Fig. 1 for **1a** and Fig. 2 for **1b**.

Procedure B (for isolation the reaction product): compound **1a** or **1b** (100 mg) dissolved in 1 mL of ethanol was added in an Erlenmeyer flask containing 200 mL of slurry of growing *P. stipitis* cells. The reaction mixture was incubated in an orbital shaker (200 rpm at 30 °C) for 72 h. Then, the product was extracted in a continuous extractor with dichloromethane for 2 days. The solvent was dried with sodium sulfate, evaporated, and the products were purified by column chromatography (hexane/ethyl acetate 9:1).

2.6. General procedure for the P. stipitis mediated bioreduction of α-haloenones **1a** and **1b** with addition of m-dinitrobenzene

DNB (1 mg) was added to an Erlenmeyer flask containing 200 mL of slurry of growing *P. stipitis* cells and the mixture was stirred for 40 min in an orbital shaker (200 rpm, 30 °C). Thus, 100 mg **1a** or **1b** dissolved in 1 mL of ethanol were added. The stirring was continued for 24 h. Then, the products were extracted in a continuous extractor with dichloromethane for 2 days. The organic phase was dried with sodium sulfate, the solvent evaporated, and the products were purified by column chromatography (hexane/ethyl acetate 9:1).

2.7. General procedure for the epoxide ring closure from **4a** and **4b**

An aqueous solution of NaOH (2 M, 1.2 mL) was added drop wise to a round-bottom flask containing a solution of **4a** or **4b** (4 mmol) in diethyl ether (5 mL). The reaction mixture was maintained under magnetic stirring for 6 h. After that, the organic layer was separated, the aqueous layer was extracted with dichloromethane and the combined organic layers were dried under sodium sulfate. The solvent was evaporated and the product was purified by column chromatography (hexane/ethyl acetate 9:1).

2.8. (Z)-3-Chloro-4-phenyl-3-buten-2-one (**1a**)

Following the general procedure for preparation of α-haloenones, compound **1a** was obtained in 20% yield as pale yellow oil; retention time on GC/MS(A): 7.68 min, GC/MS(B): 8.57 min EI-MS *m*/*z* (relative intensity): 51 (14), 63 (6), 75 (15), 102 (55), 115 (5), 129 (20), 137 (24), 145 (28), 165 (25), 179 (100), 180 (M^+, 81), 181 (42), 182 (27); IR (film) 3353, 3055, 2928, 1686, 1596, 1573, 1491, 1447, 1359, 756, 690; ^{1}H NMR (250 MHz, $CDCl_3$): δ 2.56 (s, 3H), 7.41–7.43 (m, 3H), 7.75 (s, 1H), 7.83–7.87 (m, 2H); ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 26.81, 128.65, 130.19, 130.39, 130.86, 132.74, 135.62; 193.37.

2.9. (Z)-3-Bromo-4-phenyl-3-buten-2-one (**1b**)

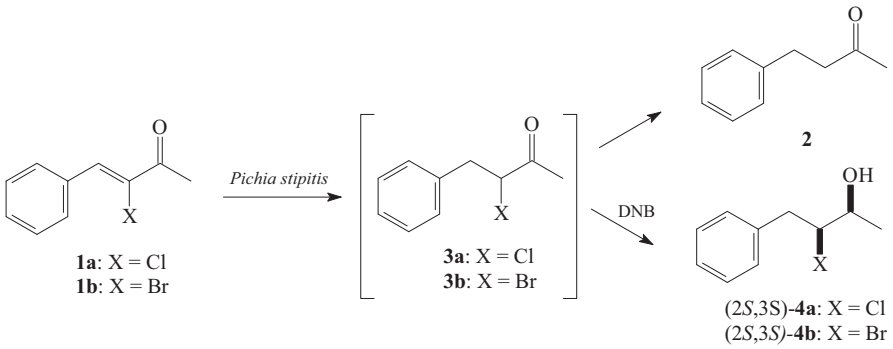
Following the general procedure for preparation of α-haloenones, compound **1a** was obtained in 78% yield as yellow oil; retention time on GC/MS(A): 8.21 min, GC/MS(B): 9.19 min, EI-MS *m*/*z* (relative intensity): 51 (23), 63 (13), 77 (23), 102 (98), 145 (100), 181 (10), 183 (9), 210 (9), 212 (8), 224 (M^+, 53), 226 (52); IR (film): 3102, 30,29, 2922, 2855, 1685, 1602, 1480, 1446, 1356, 1219, 1171, 1073; ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.60 (s, 3H), 7.43–7.45 (m, 3H), 7.85–7.87 (m, 2H), 8.03 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 27.02, 123.25, 128.50, 130.42, 130.47, 133.71, 139.92, 193.13.

2.10. 4-Phenyl-2-butanone (**2**)

Following the general procedure for bioreduction of haloenones **1a** and **1b** mediated by *P. stipitis* (Procedure B), after 72 h at 30 °C compound 2 was obtained as colorless oil; retention time on GC/MS(A): 7.30 min, GC/MS(B): 6.79 min, EI-MS *m*/*z* (relative intensity): 51 (12), 65 (9), 77 (23), 91 (64), 105, (92), 133 (19), 148 (M^+, 100); IV (film): 3085, 3062, 3027, 3002, 2922, 1718, 1602, 1583, 1496, 1453, 1408, 1357, 1283, 1228, 1161, 1080, 749, 699; ^{1}H NMR (250 MHz, $CDCl_3$): δ 2.14 (s, 3H), 2.77 (t, 2H, J = 8 Hz), 2.91 (t, 2H, J = 8 Hz), 7.16–7.31 (m, 5H); ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 29.72, 30.07, 45.16, 126.10, 128.49, 148.29, 140.97, 207.95.

2.11. 3-Chloro-4-phenyl-2-butanone (**3a**)

Following the general procedure for bioreduction of haloenone **1a** mediated by *P. stipitis* (Procedure A), compounds **1a**, **2** and **3a** were detected by GC/MS in each Erlenmeyer flasks (data shown in Fig. 1). Retention time of compound **3a** on GC/MS(A): 7.78 min, GC/MS(B): 8.63 min, EI-MS *m*/*z* (relative intensity): 43 (100), 63 (14), 77 (25), 91 (4), 103 (22), 115 (5), 129 (28), 145 (21), 182 (5), 184 (2).



Scheme 2.

2.12. 3-Bromo-4-phenyl-2-butanone (**3b**)

Following the general procedure for bioreduction of haloenone **1b** mediated by *P. stipitis* (Procedure A), compounds **1b**, **2** and **3b** were detected by GC/MS in each Erlenmeyer flasks (data shown in Fig. 1). Retention time of compound **3b** on GC/MS(A): 7.93 min, GC/MS(B): 8.27 min, EI-MS *m*/*z* (relative intensity): 43 (100), 63 (56), 77 (95), 91 (27), 103 (80), 115 (29), 129 (98), 145 (96), 193 (6), 196 (6), 211 (3), 213 (2), 226 (8), 228 (8).

2.13. (2S, 3S)-4-phenyl-3-chloro-2-butanol (**4a**)

Following the general procedure for bioreduction of haloenones mediated by *P. stipitis* with addition of DNB, after 24 h at 30 °C compound **4a** was obtained in 38% yield as pale yellow oil; $[\alpha]_D^{25}$ −20 (c 1.0, $CHCl_3$); Chiral GC/FID analyses show 97% ee, retention time on GC/FID: 24.71 min for (2*S*,3*S*) and 24.85 min for (2*R*,3*R*). Retention time on GC/MS(B): 8.22 min EI-MS *m*/*z* (relative intensity): 51 (11), 65 (14), 78 (67), 91 (100), 105 (37), 131 (59), 148 (67), 184 (M^+, 5), 186 (2); IR (film): 3399, 3025, 2978, 2930, 1603, 1494, 1447, 1365, 1260, 1131, 750, 693; ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.30 (d, 3H, J = 6 Hz), 3.05 (dd, 1H, J_1 = 14 Hz, J_2 = 8 Hz), 3.23 (dd, 1H, J_1 = 14 Hz, J_2 = 6.5 Hz), 3.89–3.96 (m, 1H), 4.05 (ddd, 1H, J_1 = 8.3 Hz, J_2 = 6.5 Hz, J_3 = 3.3 Hz), 7.22–7.35 (m, 5H); ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 20.87, 41.14, 68.46, 69.87, 126.83, 128.50, 129.33, 137.62.

2.14. (2S, 3S)-4-phenyl-3-bromo-2-butanol (**4b**)

Following the general procedure for bioreduction of haloenones mediated by *P. stipitis* with addition of DNB, after 24 h at 30 °C compound **4b** was obtained in 87% yield as pale yellow oil; $[\alpha]_D^{25}$ −21 (c 1.0, $CHCl_3$) [lit. −28°, *c* 0.03, $CHCl_3$ for (2*S*,3*S*)-isomer, >95% ee] [19]; chiral GC/FID analyses show 98% ee, retention time on GC/FID: 23.10 min for (2*S*,3*S*) and 23.28 min for (2*R*,3*R*). Retention time on GC/MS(A): 7.82 min GC/MS(B): 7.94 min EI-MS *m*/*z* (relative intensity): 51 (8), 65 (9), 78 (26), 91 (86), 105 (56), 131 (100), 148 (34), 228 (M^+, 2), 230 (1); IR (film): 3402, 3026, 2978, 2929, 1603, 1492, 1445, 725, 694, 672; ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.29 (d, 3H), 3.17 (dd, 1H, J_1 = 14.3 Hz, J_2 = 8 Hz), 3.37 (dd, 1H, J_1 = 14 Hz, J_2 = 7 Hz), 3.69–3.79 (m, 1H), 4.21 (ddd, 1H, J_1 = 8 Hz, J_2 = 7 Hz, J_3 = 3 Hz), 7.22–7.35 (m, 5H); ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 22.24, 42.09, 65.56, 68.13, 126.91, 128.57, 129.26, 138.29.

2.15. (2S, 3R)-4-Phenyl-2,3-epoxybutane (**5**)

Following the general procedure for epoxy ring closure from **4a**, compound **5** was obtained as colorless oil showing $[\alpha]_D^{25}$ −25 (c 1.0, $CHCl_3$) [lit. −21°, *c* 0.04, $CHCl_3$ for (2*S*,3*R*)-isomer, >99% ee] [19]. Following the same procedure from **4b**, compound **5** was obtained as a colorless oil showing $[\infty]_D^{25}$ −22 (c 1.0, $CHCl_3$). Both products show the same retention time and spectra data: retention time on GC/MS(A): 6.10 min EI-MS *m*/*z* (relative intensity): 51 (12), 65 (13), 78 (58), 91 (100), 105 (37), 131 (92), 148 (M^+, 3); ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.41 (d, 3H, J = 6 Hz), 2.81 (dd, 1H, J_1 = 14 Hz, J_2 = 6 Hz), 2.95 (dd, 1H, J_1 = 14 Hz, J_2 = 6 Hz), 3.13–3.18 (m, 2H), 7.08–7.36 (m, 5H); ^{13}C NMR (62.5 MHz, $CDCl_3$): δ 13.43, 34.01, 52.90 (C-2), 57.31 (C-3), 126.54, 128.62, 129.20, 137.80.

3. Results and discussion

The enones **1a** and **1b** were prepared by reaction of *trans*-4-phenyl-3-buten-2-one with Oxone® and the salts NaCl and NaBr respectively. Subsequently, triethylamine was added to the resulting mixture following the procedure published elsewhere [36]. In both cases, the major isomers obtained have (*Z*)-structure and were isolated by column chromatography. The assign of the stereoisomers structure are supported by the appearance of vinylic hydrogen singlet in their NMR spectra that is down field for (*Z*) relatively to (*E*)-isomers [37].

Both reduction of **1a** and **1b** mediated by *P. stipitis* yielded 4-phenyl-2-butanone **2** as the only product after 72 h in an orbital shaker at 200 rpm and 30 °C (Scheme 1). In order to investigate those biotransformations, the reactions were monitorated by GC/MS giving the reaction profiles presented in Figs. 1 and 2, that are typical for a consecutive reaction, showing the transient 3-halo-4-phenyl-2-butanones **3a** and **3b** as intermediaries in the production of **2**. Generally, the C=C bond of enones is reduced by an enoate reductase [3]. The microorganism *P. stipitis* used in this work has enoate reductases since *trans*-4-phenyl-3-buten-2-one was reduced by this microorganism to give 4-phenylbutan-2-one as the major product after 72 h at 30 °C. An electronegative group bound to the α-carbon of ketones **3a**–**b** like bromo and chloro should activate the C=O bond to be reduced producing the corresponding halohydrins, as has been observed in reduction of **1b** mediated by Baker's yeast [6]. But, it is clear in this work that the dehalogenation reaction of α-haloketones **3a**–**b** prevail upon the reduction of C=O bond.

Taking into account that the production of ketone is due to dehalogenation of α-haloketone that may be proceed by a free radical chain process, a small quantity of 1,3-dinitrobenzene (DNB) was added in order to inhibit the free radical process. It is worth to mention that the addition of small quantity of DNB was effective to avoid dehalogenation of α-haloacetophenones in reduction reaction using isolated enzyme NADH/HLADH [26] and dehalogenation of α-iodoacetophenone in the reduction reaction mediated by Baker's yeast [20].

The experiments were carried out in duplicate with haloenones **1a** and **1b** varying quantities of DNB in the reaction mixture from 0.1 mg to 8.0 mg of DNB added to 200 mL of growing *P. stipitis* slurry. The best result in terms of conversion and inhibition of the

radical reaction was obtained with 1.0 mg of DNB according to GC/MS analyses of the reaction products. Interestingly, the corresponding halohydrins **4a** and **4b** were formed in 38 and 87% yield in high diastereoisomer ratio syn:anti (>99:1) and in high ee, 97 and 98%, respectively, together with some quantity of haloketones **3a** and **3b** after 24 h at 30 °C. No ketone 2 was produced after 24 h of reaction at 30 °C. The halohydrins **4a** and **4b** were isolated and the configuration was assigned as (2*S*,3*S*) based on ^{13}C NMR shifts of C2 and C3 and optical rotation of the corresponding epoxide derivative **5** [19].

Summarizing the results, a general competition reaction system may be proposed for the *P. stipitis* reduction of α-haloenones **1a** and **1b** (Scheme 2) through the intermediates **3a** and **3b** respectively. From those intermediates there is a competition reaction between the electron transfer process and the hydride transfer process. In both cases, the electron transfer process prevails over the hydride transfer process. However, the electron transfer process is inhibited with addition of DNB and thus the C=O bond of intermediates **3a** and **3b** are reduced mediated by an oxidoreductase producing the corresponding halohydrins (2*S*,3S)-**4a** and (2*S*,3S)-**4b** in high diastereoselectivity and high ee.

In order to validate the proposed inhibition of electron transfer process, other inhibitors of radical reaction were used. Thus, the procedure stated in 2.6 of the experimental section was followed with addition of 1.0 mg of Irganox® 1010 (pentaerythritol tetrakis(3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate) or 1.0 mg of Irganox® 1076 (octadecyl 3,5-di-(tert-butyl)-4-hydroxyhydrocinnamate) dissolved in 0.5 mL of ethanol, in place of the addition of DNB, in parallel experiments. In both cases, halohydrins **4a** (60–77%) and **4b** (42–69%) were formed jointly with **3a** (20–30%) and **3b** (15–28%) and no formation of ketone 2 from **1a** and only 2% of **2** was formed from **1b**, after 24 h of reaction at 30 °C. Also, we found the Irganox 1010 to be slightly better than Irganox 1076 as inhibitor of radical process in this media.

4. Conclusion

Reductions of (*Z*)-3-chloro-4-phenyl-3-buten-2-one and (*Z*)-3-bromo-4-phenyl-3-buten-2-one mediated by *P. stipitis* gave 4-phenylbutan-2-one through dehalogenation of the transient 3-halo-4-phenylbutan-2-one. The addition of 1,3-dinitrobenzene avoids the dehalogenation and thus the corresponding (2*S*,3*S*)-halohydrins were obtained in excellent enantiomeric excesses. A general competition reaction system is proposed between a hydride transfer and an electron transfer mechanisms for reaction of the transient 3-halo-4-phenylbutan-2-one. The electron transfer mechanism was efficiently inhibited by DNB, and thus, the hydride transfer mechanism mediated by an oxidoreductase gave halohydrin in high ee. The electron transfer process was also efficiently inhibited by Irganox® 1010 and 1076.

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