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# Enantiogenic synthesis of (*R*)-(−)-3-hydroxy-1-penten-4-one

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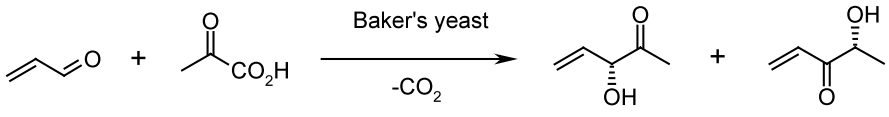
**Abstract**—Condensation of pyruvate and acrolein with whole cells of baker's yeast resulted in a mixture of 3-hydroxy-1-penten-4-one **1** and 4-hydroxy-1-penten-3-one **2**. The absolute configuration (*R*) and the enantiomeric excess (ee 72%) of the compound **1** were determined. 

## 1. Introduction

Asymmetric carbon–carbon bond formation based on enzymatic addition reactions remains one of the most challenging subjects in organic synthesis of bioactive molecules and chiral synthons. The main enzymatic systems belonging to the class of lyases which are capable of forming C–C bonds in a highly stereoselective manner, are aldolases,[1] transketolases,[2] oxynitrilases,[3] and pyruvate decarboxylase (PDC).[4] PDC, which uses thiaminpyrophosphate (TPP) and $Mg^{2+}$ as cofactors, catalyzes the irreversible non-oxidative decarboxylation of pyruvate into acetaldehyde and $CO_2$.[5] Since its first detection in yeast extracts,[6] PDC has been found in many other fungi,[7] plants,[8,9] and bacteria.[10,11] PDC was mainly used for the biogeneration of acyloins which are an interesting class of molecules (particularly functionalized and unsymmetrically substituted ones).[12] (*R*)-(−)-Phenylacetylcarbinol ((*R*)-PAC), for instance, was used as a chiral precursor to produce (1*R*,2*S*)-(−)-ephedrine and (1*S*,2*S*)-(+)-ephedrine, which are congestants and anti-asthmatic drugs.[13] The most commonly used method for the production of (*R*)-PAC has been the acyloin condensation of pyruvate and benzaldehyde using baker's yeast as catalyst.[14] The capability of baker's yeast to synthesize other aromatic acyloins from pyruvate and a wide range of α,β-unsaturated aldehydes has been intensively studied. In fact, Fuganti and Grasselli[15,16] incubated cinnamaldehyde derivatives with baker's yeast and identified the unsaturated diols, extended by a C2 unit, which were derived from reduction of the acyloins. Only recently, numerous aliphatic acyloins were generated from pyruvate and aldehydes using PDC from *Zygosaccharomyces bisporus*.[17] However, acrolein, which is well known as enzyme inhibitor,[18,19] was not investigated as a substrate to produce 3-hydroxy-1-penten-4-one **1**. In the present work, whole cells of baker's yeast were used to accomplish the first asymmetric synthesis of (*R*)-3-hydroxy-1-penten-4-one ((*R*-**1**) from pyruvate and acrolein.

## 2. Results and discussion

Biotransformation of acrolein and pyruvate was carried out with whole cells of baker's yeast under non-fermenting conditions (Scheme 1). After 1 h of incubation, compound **1** and its tautomer, 4-hydroxy-1-penten-3-




**Scheme 1.** Biotransformation of acrolein and pyruvate using baker's yeast.

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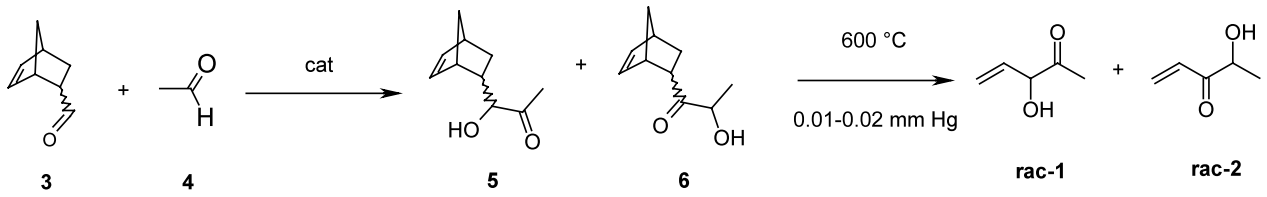



**Scheme 2.** Chemical synthesis of racemic 3-hydroxy-1-penten-4-one ((±)-**1**) and 4-hydroxy-1-penten-3-one ((±)-**2**).

**Table 1.** Acyloins synthesized by baker's yeast

| Acyloin | Ee (%)[a] | Absolute configuration | $[\alpha]_D^{25}$ | RI[b] |
|---|---|---|---|---|
| 3-Hydroxy-1-penten-4-one **1** | 72 | *R* | −75 | 1372 |
| 4-Hydroxy-1-penten-3-one **2** | 92 | *R* | n.d.[c] | 1381 |

[a] Determined by chiral GC analysis.
[b] Linear retention index on a DB-Wax column.
[c] Not determined.

one **2**, were identified in the reaction mixture by GC–MS. The molecular structure of the latter was confirmed by comparison of its analytical data (GC–MS, $^1$H NMR) with those reported in literature.[20] However, in order to prove the structure of acyloin **1**, a reference compound was chemically synthesized as shown in Scheme 2. The condensation of 5-norbornen-2-carboxaldehyde **3** and acetaldehyde **4** was performed according to Stetter and Daembkes[21] and yielded 1-[bicyclo-[2.2.1]5-hepten-2-yl]-1-hydroxy-2-propanone **5** and 1-[bicyclo[2.2.1]5-hepten-2-yl]-2-hydroxy-1-propanone **6**. Retro-Diels–Alder reaction of the latter compounds led to the formation of racemic acyloins (±)-**1** and (±)-**2** in a yield of 35%.

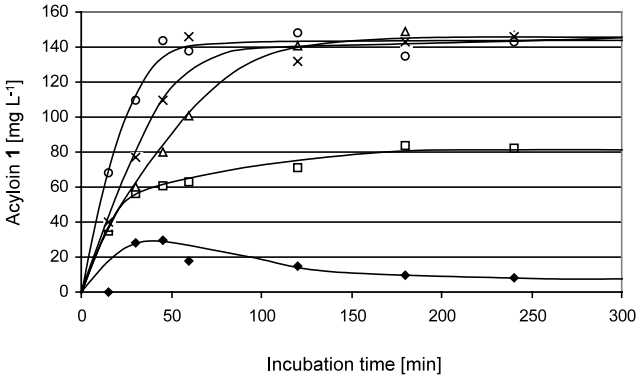
The analytical data (GC–MS, $^1$H NMR, and $^{13}$C NMR) of acyloin **1** obtained by biotransformation were identical to those of the chemically prepared reference compound. The enantiomeric excess of compounds **1** (ee 72%) and **2** (ee 92%) were determined by direct GC analysis on a chiral column, using the racemic compounds from chemical synthesis as references (Table 1). Their stereochemistry was deduced after reduction into 3-hydroxy-2-pentanone and 2-hydroxy-3-pentanone and subsequent chiral GC analysis. The absolute configurations of the latter were assigned as *R* by comparison to chiral reference compounds which were produced as described in the literature.[22,23] The influence of initial substrate concentrations on the production of 3-hydroxy-1-penten-4-one **1** was investigated (Scheme 3). At substrate concentrations in the range 25 to 150 mM, compound **1** was only produced during the first two hours of incubation, reaching a plateau thereafter. The maximal product concentration of compound **1** amounted to 150 mg $L^{-1}$. It is quite remarkable that acrolein, which inactivates several enzymes irreversibly, is transformed into the desired product even with rather modest yields. The additional formation of acyloin (*R*)-**2** with an enantiomeric excess of 92% proved that both tautomers are enzymatically produced, and suggested that chemical isomerization of acyloins is less likely to be involved.

In conclusion, the acyloin 3-hydroxy-1-penten-4-one **1** was synthesized for the first time with baker's yeast (PDC activity). Some experimental conditions were optimized and the absolute configuration (*R*) and enantiomeric excess (ee 72%) were determined. The formation of (*R*)-**1** was accompanied by that of its tautomer (*R*)-4-hydroxy-1-penten-3-one ((*R*)-**2**), which represented a minor component of the mixture and was produced with an ee value of 92%. In the future, other aliphatic aldehydes will be tested in order to fathom the frontiers of baker's yeast catalyzed acyloin formation. Furthermore, other methodologies such as the kinetic resolution of racemic compound **1** using lipases or the reduction of 1-penten-3,4-dione using dehydrogenases might be alternative ways to prepare both enantiomers of compound **1**.

## 3. Experimental

### 3.1. Materials

5-Norbornen-2-carboxaldehyde **3** was purchased from Lancaster Synthesis (Strasbourg, France). All other




**Scheme 3.** Generation of acyloin **1** as function of time at different substrate concentrations. (◆) 10 mM, (□) 25 mM, (△) 50 mM, (×) 100 mM, (○) 150 mM.

chemicals were from Sigma Aldrich Chemical Co (Buchs, Switzerland). Dried baker's yeast was purchased from Hefe Schweiz (Stettfurt, Switzerland).

### 3.2. Analytical methods

NMR spectra were acquired on a Bruker DPX-360 spectrometer (360.13 MHz proton frequency), equipped with a selective 5 mm 1H probehead or a 5 mm quadrinuclear (QNP) probehead. GC–MS analyses were performed on a Finnigan MAT-8430 mass spectrometer combined with an HP 5890 gas chromatograph equipped with a DB-WAX capillary column (30 m×0.25 mm, film thickness 0.25 μm, J & W Scientific), a splitless injector, and using helium as carrier gas (1.5 mL $min^{-1}$). The MS-EI spectra were generated at 70 eV and MS-CI spectra at 150 eV with ammonia as reagent gas. Chiral gas chromatography was performed using a Sichromat double oven gas chromatograph (Siemens, Germany) with hydrogen as carrier gas (1.5 mL $min^{-1}$). The device was equipped with a Carbowax 20M capillary column (30 m×0.32 mm, film thickness 0.38 μm, CS Chromatographie Service) in oven 1 and a Life T-switching device to cut into a chiral β-cyclodextrin capillary column (Cyclosil-B, J&W Scientific), which was situated in oven 2. Preparative GC was performed on a Agilent 5890 Series GC equipped with a KAS-3 cold injection system (Gerstel) and Multi Column Switching System (MCS, Gerstel). Optical rotation was measured at 589 nm on a Perkin–Elmer 241 polarimeter.

### 3.3. Chemical synthesis of racemic 3-hydroxy-1-penten-4-one, (±)-1 and 4-hydroxy-1-penten-3-one, (±)-2

The acyloin condensation of 5-norbornen-2-carboxaldehyde **3** and acetaldehyde **4** was realized according to the protocol described by Stetter and Dämbkes.[21] The procedure was slightly modified in order to increase the yields, notably by the addition of excesses of acetaldehyde at several time points. To 75.3 g of compound **3** (0.616 mol) in EtOH (158 mL), were added 104 mL of **4** (1.85 mol) and 37.8 g of 3,4-dimethyl-5-(2-hydroxyethyl)-thiazolium iodide (0.616 mol). Under strong stirring in an argon atmosphere, 49.3 mL of triethylamine (0.355 mol) were added dropwise. The reaction mixture was then shaken and heated to 65°C. After 4 and 8 h, respectively, 51.8 mL of compound **4** (0.927 mol) were added and heating was continued for a total of 24 h. The mixture was cooled to rt, poured into ice (250 mL), and extracted with $CH_2Cl_2$ (3×75 mL). The organic phase was washed with an aqueous solution of 1 M HCl (100 mL) and with a saturated aqueous solution of $NaHCO_3$ (100 mL). The extract was dried over $MgSO_4$, the solvent was evaporated, and the residue was distilled under reduced pressure (76-84°C/6 Pa). Retro-Diels–Alder reaction of the crude mixture of 1-[bicyclo[2.2.1]5-hepten-2-yl]-1-hydroxy-2-propanone **5** and 1-[bicylo[2.2.1]-5-hepten-2-yl-2-hydroxy-1-propanone **6** was performed by gas phase pyrolysis at 600°C and 1–2 Pa, according to Kramme et al.[24] The compounds were vaporized by heat treatment to 80°C. The vapors penetrated to a quartz tube that had been heated to 600°C. After pyrolysis, the products were collected in traps which were connected to the quartz tube. The first trap, cooled with solid carbon dioxide, contained the products, while in the second trap, which was cooled with liquid nitrogen, cyclopentadien condensed. One isolated 2.14 g of a mixture of acyloins (±)-**1** and (±)-**2** (35%). For analytical purposes, the compounds were purified by preparative gas chromatography.

**3.3.1. (±)-3-Hydroxy-1-penten-4-one, (±)-1**. $^1H$ NMR ($CDCl_3$) $\delta$ 5.84 (m, H-2), 5.56 (dt, $J_1 = 17.0$ Hz/$J_2 = 10.2$ Hz/$J_3 = 6.7$ Hz, vinyl $H_{trans}$), 5.37 (dt, $J_1 = 17.0$ Hz/$J_3 =$ 1.4 Hz, vinyl $H_{cis}$), 4.62 (d, $J_4 = 6.3$ Hz, H-3), 3.74 (s, OH), 2.24 (s, H-1). $^{13}C$ NMR ($CDCl_3$) $\delta$ 119.4 (C-1), 134 (C-2), 79.2 (C-3), 206.9 (C-4), 25.2 (C-5). MS-EI, $m/z$ (relative intensity): 100 (8) $[M]^+$, 57 (60), 43 (100). Spectral data of (±)-**1** are reported for the first time.

**3.3.2. (±)-4-Hydroxy-1-penten-3-one, (±)-2**. $^1H$ NMR ($CDCl_3$) $\delta$ 6.48 (m, vinyl $H_{trans}$, vinyl $H_{cis}$), 5.92 (m, H-2), 4.51 (m, H-4), 3.50 (OH), 1.39 (d, H-5). MS-EI, $m/z$ (relative intensity): 100 (5) $[M]^+$, 56 (61), 55 (50), 45 (100). Spectral data of (±)-**2** agreed with those described in the literature.[20]

### 3.4. Biogeneration of acyloins 1 and 2 using whole cells of baker's yeast

To 0.1 M citrate buffer (pH 6.0, 500 mL), containing baker's yeast (100 g), 2 mM thiamine pyrophosphate, and 20 mM $MgSO_4$, were added glucose (10 g, 56 mmol), sodium pyruvate (2.8 g, 25 mmol), and acrolein (1556 mg, 25 mmol). The mixture was stirred at 23°C and after 1 h of incubation, the reaction mixture was centrifuged, and the supernatant was extracted continuously with pentane/$CH_2Cl_2$ (2:1, 800 mL) overnight. The organic phase was dried over $Na_2SO_4$ and concentrated using a Vigreux column at 40°C. Acyloins **1** and **2** were obtained in yields of 3 and 0.6%, respectively, using the conditions described above. The purification of compounds **1** and **2** was done by preparative gas chromatography. MS and NMR data agreed with those obtained from chemical synthesis.

**3.4.1. Determination of enantiomeric excess and stereochemistry**. The enantiomeric excess of compounds **1** and **2** were determined by direct GC analysis on a chiral column, using the racemic compounds from chemical synthesis as references. Their stereochemistry, was deduced after reduction into 3-hydroxy-2-pentanone and 2-hydroxy-3-pentanone, which was carried out as follows: a crude mixture of **1** and **2** (50 mg) was dissolved in methanol (10 mL), and after 10 min stirring, palladium catalyst (30 wt.% on activated carbon, 1 mg) was added. The reaction vessel was evacuated and refilled with hydrogen three times in order to completely remove the oxygen. The solution was stirred again and the reaction was run until the hydrogen consumption was complete (1 h). The vessel was evacuated, the catalyst filtered, and the methanol solution was directly analyzed by GC–MS and chiral gas chromatography. The analysis revealed a mixture of (*R*)-3-hydroxy-2-pentanone and (*R*)-2-hydroxy-3-pentanone by comparison with chiral reference compounds which were produced as described elsewhere.[22,23]

**3.4.2. (*R*)-3-Hydroxy-1-penten-4-one**. Ee = 72%. $[\alpha]_D^{25} = -75$ (*c*, 0.06, $CHCl_3$).

**3.4.3. (*R*)-4-Hydroxy-1-penten-3-one**. Ee = 92%.

### 3.5. Influence of acrolein concentration on the formation of acyloin, (*R*)-1

Biotransformation studies were carried out with commercially-available dried baker's yeast in shaking flasks. To citrate buffer (0.1 M, pH 6.0, 50 mL), containing baker's yeast (10 g), 2 mM thiamine pyrophosphate and 20 mM $MgSO_4$, was added 1 g of glucose (5.6 mmol). Sodium pyruvate and acrolein were then added to give 10, 25, 50, 100 and 150 mM initial concentrations. The mixtures were incubated at 23°C and after 15 min, 30 min, 45 min, 60 min, 2 h, 3 h, 4 h, and 24 h, 5 mL samples were withdrawn, centrifuged, and 4-hydroxy-4-methyl-2-pentanone was added to the supernatant as internal standard for relative quantification. The aqueous phases were extracted twice with diethyl ether. The combined etheral solutions were dried over $Na_2SO_4$ and concentrated using a Vigreux column at 40°C. The extracts were analyzed by GC.

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