Chimia 43 (1989) 351–353


# Preparative β-Ketoester Reductions and Ester Hydrolyses by Yeast, Using Free Cells in Organic Media**

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*Abstract:* Free cells of yeast have been used for preparative β-ketoester reductions and ester hydrolyses in a mixture of isopropyl hexadecanoate and soybean phospholipids. The commercially available pressed lager baker's yeast was used without adding water to the organic medium. In this way, ca. 50 g substrate could be easily converted by 500–700 g yeast in a 1.5 L volume, thus surpassing the reported procedures in aqueous media. Switching from aqueous to organic medium led, in a couple of cases, to an increase of the enantioselectivity from 90% (in water) to > 99% *ee* (in the organic medium) for ketone reduction and from 74% to > 99% *ee* for ester hydrolysis as compared to the best reported data for aqueous conditions.

*Introduction and Inspiration*

Biotransformations achieved by baker's yeast in aqueous media are currently attracting considerable attention as a useful method in organic synthesis[1]. Despite the importance of organic solvents in biocatalysis[2], there are very few reports on applications of non-aqueous media for microbial transformations[3]. To the best of our knowledge, the only very recently reported synthetic applications of yeast in the presence of organic solvents are those by *Nakamura* et al.[4] and *Fadnavis* et al.[5]. In the former case, cell immobilization techniques were used, and the reaction was performed in hexane in the presence of 20% water. In the latter case, glutaraldehyde-crosslinked yeast was used in reverse micelles of bis(2-ethylhexyl) sulfosuccinate sodium salt in trichloromethane-isooctane (1:9 volume/volume) in the presence of aqueous phosphate buffer; although the enantioselective hydrolysis of racemic esters was realized, the procedure used was quite complex in this case.

Recently, *Luisi* et al. reported the solubilization of baker's yeast cells in isopropyl hexadecanoate (isopropyl palmitate, IPP)[6] as solvent and 4% soybean phospholipids[7] as surfactant («asolectine»[8]; IPP/asolectine = 25:1 weight/weight)[9]. A significant amount of water (up to $w_0 = 20$)[10] and freshly cultured yeast cells were added together with a nutritive solution. These studies are mainly concerned with the fundamental aspects of solubilization and were performed on a biochemical scale[11] in order to get clear thermodynamically stable solutions (water-in-oil microemulsions). Under these conditions the cells were still alive after several days.

Inspired by these results, we investigated the possibility of using a Luisi-type system in preparative organic synthesis. For practical purposes, we chose to work with large cell concentrations (suspensions) instead of with microemulsions. Also, we used the commercially available non-modified pressed baker's yeast (*Saccharomyces cerevisiæ*) in IPP/asolectine without adding water or nutrients. The resulting mixture can be regarded as a yeast-containing «microaqueous»[12] organic medium.

*Results and Discussion*

The preparative-scale reactions were performed using an IPP/asolectine ratio of about 2 (L/kg). More concentrated media with a v/w ratio of 1 could also be used, although the viscosity of the mixtures become very high. In all cases, vigorous agitation is necessary in order to overcome problems caused by excessively high viscosity of the reaction mixtures. The yeast can readily be finely suspended in this medium if the yeast/asolectine ratio is about 1 (w/w)[13]; for a comparison, the cell concentrations employed are about $10^3$–$10^4$ times larger than those used by *Luisi* et al. When higher yeast/asolectine ratios (i.e. 1.5–2 w/w) were used, the «solubilization» was slow, and very strong agitation was required. Although addition of water increases the «solubility» of the pressed lager yeast in this medium, we restrained from adding it in order to keep the conditions as «lipophilic» as possible[14].

The reactions with various substrates were performed at 30°C. The amount of substrate that can be converted under these conditions depends on its structure and toxicity. A typical average yeast/substrate ratio could be 1700–2000 g/mol (ca. 11 w/w in the case of *tert*-butyl 3-oxobutanoate; see Table 1); in the case of ethyl 3-oxobutanoate this ratio could be lowered to 1000–1300 g/mol (ca. 8–10 w/w). When substrates or products are more toxic this ratio becomes higher (up to 800 g/mol; ca. 47 w/w for ethyl 2-oxocyclohexanecarboxylate). If the ratio is too high, the volume of the reaction mixture and the amount of asolectine needed become so large that the technical and economical limitations of this procedure are reached. Up to ca. 50 g of β-ketoester could be reduced by 500–700 g of baker's yeast in a volume of 1.5 L, and 22 g of ester were submitted to saponification by 220 g of yeast in a volume of 0.5 L (see Table 1). The product alcohols **1**–**4** were thus obtained from the corresponding achiral or racemic ketones, and the acetate and octynol **5**, **6** were prepared by hydrolysis of the corresponding racemic acetate *rac*-**5**. All products were isolated by distillation from the reaction medium followed by a second distillation step; sufficient volatility of the products is a necessary condition for the application of the method. The results are collected in Table 1.

It turns out that the yields and the enantio- and diastereoselectivities of these reactions are generally better (compounds **1**, **2**, **4**, **5**) than those obtained in water (see Table 1 and references cited therein). In all cases, the system proved to be applicable on a preparative scale. In the case of the cyclopentane derivative **4**, the specific rotation at room temperature was + 16.0° in trichloromethane (*c* 8.55), and + 25.3° in methanol (*c* 9.52). Careful examination of the literature as well as $^{19}$F-NMR spectroscopy of the (*S*) «Mosher» derivatives of **4** and of *rac*-**4** (*cis* + *trans*) led to an assignment of the enantioselectivity > 99% *ee*, which was ca. 10% higher than the enantiomeric excess previously observed in water. The yeast hydrolysis of racemic 1-octyn-3-yl acetate *rac*-**5** [yeast/substrate = 10:1 (w/w) = 1700 g/mol] gave enantiomerically pure (*R*)-(+)-1-octyn-3-yl acetate (*R*)-**5** in 28% yield after 67% conversion (hydrolysis) (i.e. 85% corrected yield). This constitutes an improvement of 26% *ee* compared with the best data reported for aqueous conditions, and this result was obtained in the absence of buffer or pH control.

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** Part of the master's thesis of T. Arslan, ETH Zürich (1989). – We thank Prof. Dr. P.L. Luisi and N. Pfammatter, Institut für Polymere, ETH Zürich, for helpful discussions and for allowing T. Haag to use special equipment, material and chemicals of the Luisi group.

*Conclusion and Implications*

We have described in this paper a new way of carrying out preparative biotransformations in organic media. By using baker's yeast as the microorganism and enantioselective ketone reduction as well as ester hydrolysis as model reactions, we have demonstrated that this system may be superior to the classical aqueous one, in terms of the enantioselectivity of the reactions and of the preparative scale applicability. The procedure is only applicable when the products are sufficiently volatile.

*A Typical Procedure*

*Preparation of the Hydroxyester* **4**:

Asolectine[7,8] (500 g) was dissolved in isopropyl hexadecanoate[6] (1 L; 1 h, 30°C; 120 rpm shaking) and the resulting solution was evenly distributed to four 2 L Erlenmeyer flasks (with four breaker walls). After addition of the baker's yeast (700 g, Klipfel AG, Rheinfelden, Switzerland; 175 g to each Erlenmeyer flask), ethyl 2-oxocyclopentanecarboxylate was added (50 g, 0.32 mol; 12.5 g or 80 mmol to each flask). The reaction vessels were equipped with 15 cm plastic pipes (∅ 5 cm) and shaken at 190 rpm at 30°C. The reaction was followed by gas chromatography (GC) by taking a 10 mL aliquot from the reaction medium. This aliquot was diluted with acetone and filtered over Celite. The filtrate was cooled at −78°C, filtered again at this temperature, and this procedure was repeated 2–3 times in order to remove all the remaining asolectine and IPP. Acetone was evaporated and the resulting mixture analyzed by GC. In this way the reaction was found to be complete after 19 hours. The entire reaction mixture was centrifuged (7500 rpm, 45 min, at 15–20°C) for separation of the yeast. Product **4** was distilled (160–180°C/0.15 mmHg) from the supernatant and isolated after a second distillation step (120°C/12 mmHg) in 52% yield (26.4 g, 0.167 mol) and in enantiomerically and diastereomerically pure form (> 99% *ee* as determined by the «Mosher» method, and > 99% *cis*). $[\alpha]_D^{24} = +16.0°$ ($c = 2.28$, $CHCl_3$), $[\alpha]_D^{24} = +25.3°$ ($c = 9.52$, MeOH) (lit.[13]: $[\alpha]_D^{23} = +14.4°$ ($c = 1.37$, $CHCl_3$), $[\alpha]_D^{23} = +21.1°$ ($c = 0.36$, MeOH), 89% *ee*).

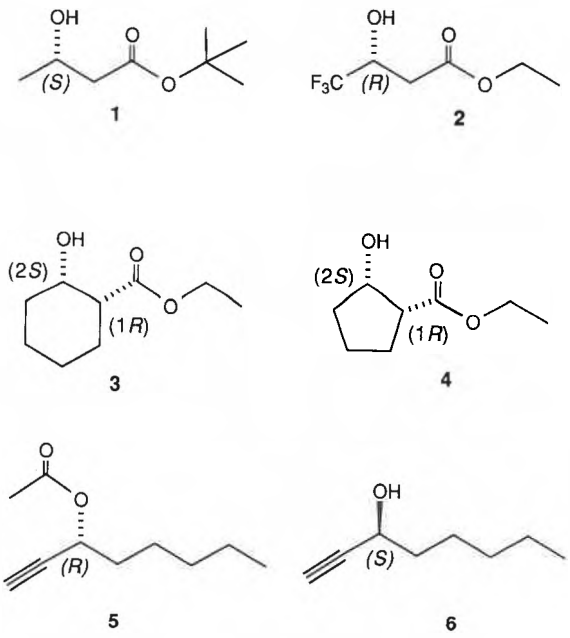


Table 1. Preparative conversions with yeast in the isopropyl hexadecanoate/asolectine medium (IPP:asolectine = 2 L/kg).

| Compound[a] | Yield% [b] (Conv. %) | *ee* % (*cis*:*trans* [c]) | $[\alpha]_D^{24}$ (*c*, $CHCl_3$) | Time [h] | Amount of substrate used [g] | Concentration [g/L] | Yeast/Substrate w/w [g/mol] |
|---|---|---|---|---|---|---|---|
| **1** | 72 | 91[d] | + 33.0 (3.33) | > 96[e] | 47.7 | 45 | 10.6 (1700) |
| **2** | 50 | 58[f] | + 11.7 (10.22) | 120 | 30.0 | 39 | 11.8 (2200) |
| **3** | 82 | ≥ 99 (> 99)[g] | + 28.4 (5.09) | 24 | 25.0 | 10 | 46.6 (7900) |
| **4** | 52 | ≈ 99 (> 99)[h] | + 16.0 (2.28)[i] | 19 | 50.0 | 33 | 14.0 (2200) |
| **5** (from *rac*-**5**) | 28(67) | > 99[j] | + 82.5 (4.29) | 139 | 22.2 | 45 | 9.85 (1700) |
| **6** (from *rac*-**5**) | 42(67) | 41[k] | − 2.72 (3.81) | 139 | 22.2 | 45 | 9.85 (1700) |
| **5** (from *rac*-**5**) | 30(*46*) | 68[j] | + 55.8 (4.29) | 49 | 22.2 | 45 | 9.85 (1700) |
| **6** (from *rac*-**5**) | 34(*46*) | 79[k] | − 5.2 (5.08) | 49 | 22.2 | 45 | 9.85 (1700) |

[a] *tert*-Butyl (3*S*)-(+)-3-hydroxybutanoate (**1**), ethyl (3*R*)-(+)-4,4,4-trifluoro-3-hydroxybutanoate (**2**), ethyl (1*R*,3*S*)-(+)-3-hydroxycyclohexanecarboxylate (**3**), ethyl (1*R*,3*S*)-(+)-3-hydroxycyclopentanecarboxylate (**4**), (3*R*)-(+)-1-octyn-3-yl acetate (**5**), (3*S*)-(−)-1-octyn-3-ol (**6**). All compounds were characterized by $^1$H- and $^{13}$C-NMR, MS, IR, and all the C, H analyses were correct. – [b] All the products were distilled from the centrifugated reaction medium and isolated after a second distillation step. Compounds **5** and **6** were separated by distillation using a Fischer separation column apparatus (high-performance distillation apparatus). – [c] For both **3** and **4**, only one diastereoisomer was detected by $^{13}$C-NMR (100 MHz) and $^1$H-NMR (400 MHz). NOE experiments confirmed that both **3** and **4** were *cis*-forms. – [d] By comparison with Ref.[15]. – [e] Not optimized, 90 rpm shaking. – [f] By comparison with Ref.[16]; the reaction was performed in the presence of ethanol (5 mL; yeast/EtOH = 71 g/mL; [EtOH] = 0.6%). The yeast was starved for four days in this medium before the substrate was added. – [g] By comparison with Ref.[17]. – [h] Determined by $^{19}$F-NMR analysis of the (*S*)-MTPA ester («Mosher» derivative) of **4** and *rac*-**4** (*cis* + *trans*). This result is consistent with the data reported by *Sato* et al.[18]. – [i] $[\alpha]_D^{24} = +25.3°$ ($c = 9.52$, MeOH) (lit.[18]: $[\alpha]_D^{23} = +21.1°$ ($c = 0.36$!, MeOH), 89% *ee*). – [j] By comparison with Ref.[19]. – [k] Determined by $^{19}$F-NMR analysis of the (*S*)-MTPA ester of **6** («Mosher» derivative).

Received: September 8, 1989 [FC 176]

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[10] $w_o$ = (moles of $H_2O$)/(moles of surfactant).

[11] I.e. $10^6$ cells/mL ≈ 400 μg fresh cells/mL; yeast/asolectine = $2 \cdot 10^8$ cells/g; asolectine/fresh yeast ≈ 14 w/w.

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[13] I.e. IPP/yeast = 2 L/kg; [yeast] = yeast/(volume IPP + asolectine) ≈ 350 mg/mL ≈ $10^9$–$10^{10}$ cells/mL.

[14] The only water which is present in the system comes from the pressed industrial lager yeast itself, which is a compact paste containing only intracellular and extracellular bound water.

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