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REDUCTION OF PHENYLKETONES BY IMMOBILIZED BAKER'S YEAST

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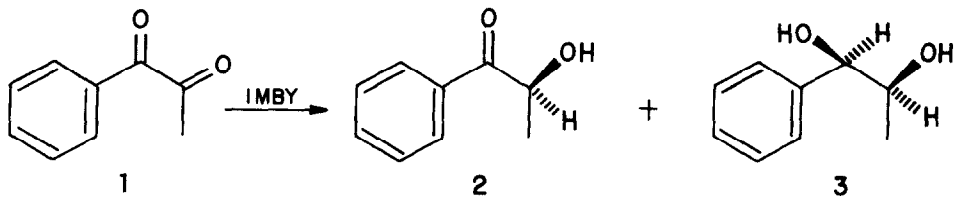
Abstract: Baker's yeast immobilized on chrysotile and montmorillonite stereoselectively reduced 1-phenyl-1,2-propanedione to the corresponding (1R,2S)-diol. The immobilized biocatalyst was also successfully used for asymmetric synthesis of 2-amino-1-phenylethanol and (R)- or (S)-1-phenyl-2-chloroethanol. The catalyst was reusable for more than 8 cycles.

Asymmetric reduction of ketones by baker's yeast (BY) *(Saccharomyces cerevisiae)*, which is readily available, inexpensive and possess high enantioselectivity,[1] has been widely used to obtain chiral alcohols. Nevertheless, the isolation of the products is frequently troublesome because of the complexity of the reaction medium. The use of immobilized cells enhance the operational stability of yeast, simplifies the isolation of the products and permits the reuse of the catalyst. Many systems have been used for immobilization of baker's yeast such as polyurethane prepolymer,[2] carrageenan,[3] and alginates.[4] In this paper, we describe the use of two supports for immobilization of baker's yeast and its activity in reduction of phenylketones. The supports used are montmorillonite K10 (an aluminosilicate)[5] and chrysotile (an magnesium silicate)[6] which are very abundant in the central region of Brazil. Although the immobilization of microorganisms and enzymes on clays have been reported in recent years,[7] their application in synthesis have not been investigated.

The immobilization of the yeast was obtained by adding fresh baker's yeast (20 g) to a suspension of chrysotile or montmorillonite (20 g) in water at 30°C and then gently shaking the resultant suspension for 2 h. After vacuum filtration, the immobilized baker's yeast (IMBY) was kept in a refrigerator. The crude chrysotile was washed under strong water flow on a sieve (0.0062 mm) during 10 minutes, then treated with a buffer of acetic acid - sodium acetate (pH 4.7) and sonicated at 25 kHz during 30 min.[8]

Baker's Yeast Reduction of 1-Phenyl-1,2-propanedione

In the past, we and others have studied the reduction of 1-phenyl-1,2-propanedione 1 with free baker's yeast.[9] We decided to start our study with this compound in order to compare the regioselectivity and the stereochemical control caused by the immobilization of the yeast in the reduction. After chrysotile-IMBY (20 g) in water was mechanically stirred for 30 minutes, the ketone (1 mmol) was added and the temperature maintained at 30°C for 24 h (Method A). The mixture was separated by filtration and the products were extracted with chloroform. After evaporation of the organic layer, the residue was subjected to a preparative thick layer chromatography. The S ketoalcohol 2 was isolated in 35% yield ($[\alpha]_D^{25} = -66^o$; c 1.80, $CHCl_3$; 77% optical yield) and also the diol 3 in 4% yield. The baker's yeast has some saccharides in its cell that can produce NADPH along the pentose phosphate pathway and has reducing power without adding sucrose. The reuse of IMBY without treatment was not able to reduce the ketone 1. However, when the once used IMBY was treated with 2% solution of KCl and sucrose (1:1 ratio of sucrose:IMBY), kept overnight in the refrigerator and then filtrated and washed with water, the IMBY reduced 1 to give 2 and 3 (30% and 5% yield, respectively). The reduction of 1 was repeated using the same procedure above except that a 2% solution of KCl and sucrose (1:1 ratio of sucrose:IMBY) was used instead of pure water (Method B). The isolated product was (-)-(1R,2S)-1-phenylpropane-1,2-diol 3 in 23% yield. The ^1H nmr 100 MHz spectrum of the isolated 3 was in good agreement with that of the eritro isomer as described elsewhere.[10] Using this procedure, we achieved an active IMBY that allowed us to reuse it in further reactions as exemplified in Table 1.



1 2 3

Since chrysotile swells extensively, it is necessary to use a large volume of water, which complicates extraction of the reaction products. We decide to change the support to the commercially available clay montmorillonite K 10. The same method described for chrysotile was used to produce montmorillonite-IMBY. The yeast has a good stability on this support and it is easier to extract the reaction products than with chrysotile since a

smaller volume of water is necessary. After 8 cycles the clay-baker's yeast was in good conditions for further use in chemical reactions. In Table 1 we show the results of the reduction of 1 with this catalyst. The yields were better than with chrysotile-IMBY. After the first reaction the chemical yield decreased, but increased with further reuse, stabilizing at 30-40% after the 5th reuse. Compared with the free baker's yeast[9a], the clay-IMBY gave nearly the same optical yield but the chemical yields are much poor. The reason for the decrease in the chemical yield could be related to the strong interaction of the diol 3 and the support. The montmorillonite-IMBY always gave better chemical yields than the chrysotile-IMBY.

Table 1. Reduction of 1-phenyl-1,2-propanedione by baker's yeast immobilized on chrysotile (chrys) and montmorillonite K 10 (Method B).

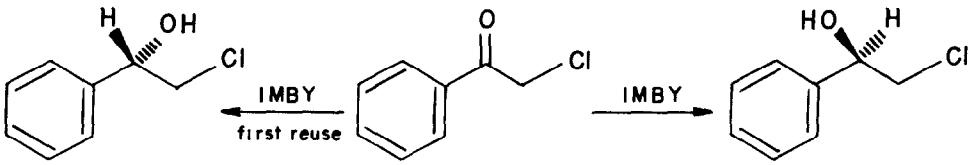
Reuse number	yield of 3 (%)[a]		$[\alpha]_D^{25}$,b,c		optical yield (%)	
	chrys	K 10	chrys	K 10	chrys	K 10
1	23	47	-38^o	-37^o	94	93
2	15	22	-37^o	-34^o	91	85
3	25	25	-38^o	-33^o	96	83
4	18	33	-37^o	-33^o	92	83
5	17[d]	23	-30^o	-29^o	75	72
6		30		-38^o		96
7		39		-30^o		75
8		35		-29^o		73

[a]In all reactions about 4% of monoalcohol 2 was isolated. [b]Lit.[11] $[\alpha]_D^{25} = -40^o$; [c]The specific rotation were measured in chloroform with c = 1.2; [d]The reaction was carried out with the IMBY in a tubular reactor using a peristaltic pump to recycle the reaction medium.

Baker's Yeast Reduction of α-Chloroacetophenone

Recently, we have studied the reductions of α-haloketones by free baker's yeast[12] and we found that α-chloroacetophenone gives (-)-(R)-2-chloro-1-phenylethanol 5 in 37-84% chemical yield and 44-90% optical yield. We have repeated that reaction using the chrysotile-IMBY and the Method A gave a 67% chemical yield of 5, $[\alpha]_D = -30.6^o$, c 1.78, C_6H_{12} (64% optical yield). Using Method B we have isolated the same alcohol 5 in 62% yield,

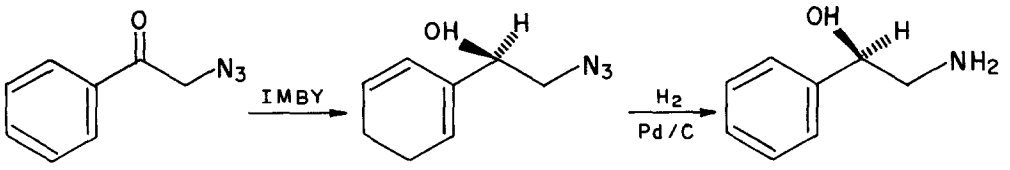
$[\alpha]_D$ = -33.2°, c 1.78, C_6H_{12} (69% optical yield). When we submitted the same substrate to the first reuse with the IMBY, we observed a shift in the stereochemistry of the reduction since we have isolated the S alcohol 6 in 78% yield, $[\alpha]_D$ = +46.6°, c 1.73, C_6H_{12} (97% optical yield). The explanation for this change in the stereochemistry of the alcohol may be the same as that given by Nakamura for the effect of ethyl chloroacetate on the reduction of β-keto esters with BY.[13] During the first reaction the substrate, while reduced, also promotes the inhibition of the "R-enzymes" which afford the (R)-chloroalcohol. In the subsequent reuse of the IMBY, the "S-enzymes" are more active in the reduction giving selectively the (+)-(S)-2-chloro-1-phenylethanol.[14] By this procedure it is possible to efficiently control the stereochemistry of the reduction of the ketones.



6 4 5

Baker's Yeast Reduction of α-Azidoacetophenone

The system montmorillonite-IMBY was used to reduce α-azidoacetophenone 7. Using method B, we have isolated after 24 h (-)-(R)-2-azido-1-phenylethanol 8 ($[\alpha]_D$ = -62.7°, c 3.09, $CHCl_3$) in 36% yield. In the first reuse of the IMBY we have isolated the alcohol 8 in 45% yield ($[\alpha]_D$ = -68.3°, c 2.99, $CHCl_3$). To compare the effect of the immobilization of the yeast, we repeated the same reaction using the free baker's yeast with sucrose and zinc sulfate as additives[15] and we isolated the alcohol 8 in only 12% yield ($[\alpha]_D$ = -69.6°, c 3.01, $CHCl_3$). Using the free BY without additives the yield of 8 increased to 24% ($[\alpha]_D$ = -61.5°, c 2.99, $CHCl_3$). The montmorillonite-IMBY have promoted an significant increase in the chemical yield when compared with the free BY. The stereochemistry of 8 was confirmed by hydrogenation (H_2/Pd-C) of the azide to (-)-(R)-2-amino-1-phenylethanol 9 in 96% yield and 97% optical purity.[16]



7 8 9

Conclusions

We developed a practical and inexpensive method for immobilization of baker's yeast on chrysotile and montmorillonite. With both clays, it is possible to reuse the biocatalyst in the reduction of ketones at least 8 times in water with reasonable optical and chemical yield. Using α-chloroacetophenone as the substrate it is possible to control the enantioselectivity of the product to obtain both the (R)- or the (S)-1-phenyl-2-chloroethanol. To our knowledge this is the first reported example using clays as support for active baker's yeast in chemical reactions. Further studies are in progress in our laboratory.

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References

[1]a. Servi, S. *Synthesis*, **1990**, 1. b. Csuk, R.; Glanzer, B.I. *Chem. Rev.*, **1991**, *91*, 49.

[2]Nakamura, K.; Inoue, K.; Ushio K.; Oka, S.; Ohmo, A. *J. Org. Chem.*, **1988**, *53*, 2589.

[3]a. Tosa, T.; Sato, T.; Mori, T.; Yamamoto, K.; Takata, I.; Nishida, Y.; Chibata, I. *Biotechnol. Bioeng.* **1979**, *21*, 1697. b. Naoshima, Y.; Hasegawa, H.; Saeki, T. *Agric. Biol. Chem.* **1987**, *51*, 3417. c. Naoshima, Y.; Hasegawa, H. *Chem. Lett.* **1987**, 2379.

[4]a. Kierstan, M.; Bucke, C. *Biotechnol. Bioeng.* **1977**, *19*, 387. b. Kawai, M.; Tajima, K.; Mizuno, S.; Niimi, K.; Sugiobka, H.; Butsugan, Y.; Kozawa, A.; Asano, T.; Imai, Y. *Bull. Chem. Soc. Jpn.* **1988**, *61*, 3014. c. Nakamura, K.; Kawai, Y.; Oka, S.; Ohmo, A. *Tetrahedron Lett.* **1989**, *30*, 2245.

[5]Thorez, J. in *Preparative Chemistry Using Supported Reagents*; Laszlo, P.; Academic Press: San Diego, 1987; p 177.

[6]Streib, W.C. in *Kirk-Othmer Encyclopedia of Chemical Technology*, 3rd ed.; John Wiley: N. York, 1978; V 3; p 267.

[7]a. Lavie, S.; Stotzky, G. *Appl. Environ. Microbiol.*, **1986**, *51*, 65. b. Kocoshis, S.A.; Ghent, C.N.; Gryboski, J.D. *Dig. Dis. Sci.*, **1984**, *29*, 1148. c. Welsh, F.W.; Willisans, R.E.; Veliky, I.A. *Enzyme Microb. Technol.*, **1987**, *9*, 500. d. Yoshii, Y.; Takechi, T.; Tanji, Y.; Tsubone, T. *Jpn. Kokai Tokkyo Koho JP 63,137,681*. e. Tarafdar, J.C.; Agnihori, N.P.; Chhonkar, P.K. *J. Indian Soc. Soil Sci.*, **1988**, *36*, 553.

[8]Parizotto Jr., O.; Comerlato, M.H.; Pedroso, P.R., Moran, P.J.S.; Rodrigues, J.A.R.; Carvalho, M.; Joekes, I. *Pat. BR*, **1989**, 8.903.849.

[9]a. Brenelli, E.C.S.; Moran, P.J.S.; Rodrigues, J.A.R. *Synth. Commun.*, **1990**, *20*, 261. b. Chênevert, R.; Thiboutot, S. *Chem. Lett.*, **1988**, 1191. c. Takeshita, M.; Sato, T. *Chem. Pharm. Bull.*, **1989**, *37*, 1085. d. Kometani, T.; Kitatsuji, E.; Matsuno, R. *Chem. Lett.*, **1989**, 1465.

[10]a. Ohata, H.; Ozaki, K.; Konishi, J.; Tsuchihashi, G., *Agric. Biol. Chem.*, **1986**, *50*, 1261. b. Bowlus, S.B.; Katzenellenbogen, J.A. *J. Org. Chem.*, **1974**, *39*, 3309. c. Kingsbury, C.A.; Cowles, C.R. *J. Org. Chem.*, **1975**, *40*, 1302.

[11]Imuta, K.; Kawai, K.; Ziffer, H. *J. Org. Chem.*, **1980**, *45*, 3352.

[12]Carvalho, M. de; Okamoto, M.T.; Moran P.J.S.; Rodrigues, J.A.R. *Tetrahedron*, **1991**, *47*, 2073.

[13]Nakamura, K.; Kawai, Y.; Ohno, A. *Tetrahedron Lett.*, **1990**, *31*, 267.

[14]Further use of the IMBY was not achieved since no reduction occurred after the first reuse.

[15]Zinc sulfate has been used by some authors on the reduction of ketones, see MacLeod, R.; Prosser, H.; Fikentscher, L.; Lanyi, J.; Mosser, H.S. *Biochemistry*, **1964**, *3*, 838.

[16]a. Meyers, A.I.; Slade, J. *J. Org. Chem.*, **1980**, *45*, 2785. b. Pratessi, R.; Grasci, M. *Farmaco, Ed. Sci.*, **1953**, *8*, 86; *Chem. Abstr.*, **1954**, *48*, 1955.