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# Microbial Deoxygenation of *N*-Oxides with Baker's Yeast-NaOH

**Woonphil Baik*, Dong Ik Kim, Sangho Koo, Jong Uk Rhee, Sung Hee Shin and Byeong Hyo Kim[1]**

Department of Chemistry, Myong Ji University, Yong In, Kyung Ki Do, 449-728, **Korea**

[1]Department of Chemistry and Research Institute of Basic Science, Kwangwoon University, **Korea.**

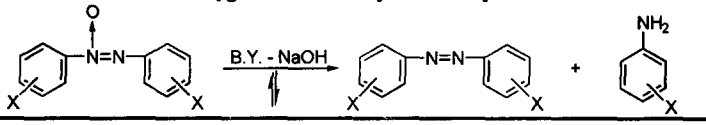
***Abstract:*** The microbial deoxygenation of a series of aromatic and heteroaromatic *N*-oxide compounds, including quinoline *N*-oxides, isoquinoline *N*-oxides, 2-aryl-2*H*-benzotriazole 1-oxides, benzo[*c*]cinnoline *N*-oxide and azoxybenzenes, has been performed with bakers'yeast-NaOH.


The enantioselective reduction of carbonyl groups to their hydroxy groups by bakers' yeast (*Saccharomyces ceravisiae*) is one of the most significant examples of microbial enzymatic biotransformations.[1] Recently, the reduction method of aromatic nitro compounds containing electron withdrawing groups to their corresponding anilines by bakers' yeast- NaOH system has been developed and showed excellent selectivity over carbonyl and other labile substituents.[2] In accord with previous observations,[3] however, in the absence of NaOH the reduction of nitroarenes proceeds very slowly (>3 day) and gives rise to quite low yields, despite use of excess bakers' yeast. Moreover, poor selectivity was reported in the reduction of aromatic nitro compounds which have carbonyl substituents. It is worth mentioning that the use of NaOH is crucial for the selective reduction of nitroarenes. On the other hand, aromatic nitroso compounds are reduced to the corresponding anilines in the absence of NaOH and the rate of reduction is greatly enhanced by the substituents regardless of the electronic nature.[4] It also shows high selectivity over carbonyl and other labile substituents. This finding indicates that the reduction of nitrosoarenes is so fast (<2h) that the biotransformation of carbonyl substituent is not influenced. The deoxygenation of pyridine *N*-oxides with bakers' yeast in the absence of NaOH was reported.[5,6] However, use of excess bakers' yeast (500 g with 0.5 g of substrate), low yield (0-44%) and long reaction time (>160 h) have impeded this reagent to employ as a good reducing agent in synthetic organic chemistry.

As a part of our continuing study on the microbial enzymatic reduction of nitro coumpounds with bakers' yeast and NaOH,[2,4] we decided to carry out the deoxygenation of a series of aromatic and heteroaromatic *N*-oxide compounds varying chemical environments. Azoxybenzene (0.5 g), when treated with bakers'yeast (30 g) and NaOH (4 g) in EtOH (90 mL)-$H_2O$ (40 mL) at reflux, gave azobenzene exclusively (see Table 1). As a control experiment, a suspension containing azoxybenzene (0.5 g) was stirred with varying amounts of NaOH. In the absence of NaOH, no azo product was formed and the unreacted azoxybenzene was recovered. Small amount (25 %) of azobenzene was observed when 1 g of NaOH was employed. But as we increased the amount (4 g) of NaOH, the deoxygenation to azo compound was rapidly completed. In addition,

halogen substituted azoxybenzenes were efficiently reduced to give their corresponding azobenzenes without dehalogenation. The reduction of azoxybenzenes with electron donating groups, such as methoxy or methyl, was not completed under more forcing conditions. In fact, these results which electron donating groups retard the reactivity of azoxybenzene with bakers' yeast-NaOH system, coincide with the case of nitroarenes.[2,3] In most cases, the over reduction of azoxybenzenes to anilines was hardly observed. In a couple of cases were observed a trace amount of aniline by GLC. We separately tried the reduction of isolated azobenzene. This biotransformation was not occurred with bakers' yeast-NaOH and the starting material was recovered. From these observation, unique selectivity of bakers' yeast catalyzed biotransformation of azoxybenzenes to azobenzenes was found in NaOH solution without over reduction nor dehalogenation.

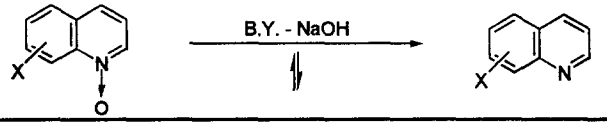
**Table 1.** Reductive Deoxygenation of Azoxybenzenes by Baker's Yeast - NaOH




| entry | X | Conditions | | Product • Yields (%) | | Recovery |
|---|---|---|---|---|---|---|
| | | NaOH (g) | time (h) | azo | aniline | (%) |
| 1 | H | 4 | 20 | 96 | 0 | 0 |
| 2 | H | 0 | 24 | 0 | 0 | 100 |
| 3 | 2.2'-Cl | 4 | 24 | 85 | 3 | 5 |
| 4 | 3,3'-Br | 4 | 12 | 93 | 0 | trace |
| 5 | 3,3'-I | 4 | 20 | 95 | 0 | 0 |
| 6 | 4,4'-$OCH_3$ | 8 | 24 | 71 | 0 | 25 |
| 7 | 4,4'-$CH_3$ | 8 | 48 | 72 | trace | 23 |

In an extension of microbial deoxygenation of *N*-oxides with bakers' yeast, we have also examined the reduction of heteroaromatic *N*-oxide compounds. Quinoline *N*-oxides were well responded to the microbial deoxygenation by bakers' yeast-NaOH, thus providing quinolines in high yields.

**Table 2.** Reductive Deoxygenation of Quinoline *N*-oxides by Bakers' Yeast-NaOH

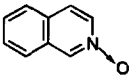
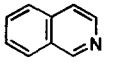
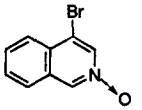
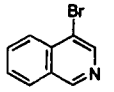
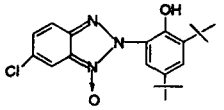
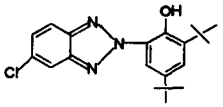
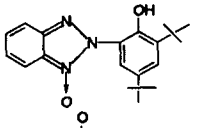
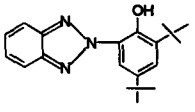
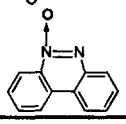
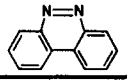



| entry | X | Conditions | | Product Yield (%) |
|---|---|---|---|---|
| | | NaOH (g) | time (h) | |
| 1 | H | 4 | 8 | 91 |
| 2 | H | 8 | 3 | 95 |
| 3 | 3-Br | 8 | 1 | 96 |
| 4 | 2-$CH_3$ | 8 | 2 | 87 |
| 5 | 6-$CH_3$ | 8 | 2 | 93 |
| 6 | 6-$OCH_3$ | 8 | 2 | 90 |

As shown in Table 2, 4 g of NaOH was necessary to exclusively reduce quinoline *N*-oxides (0.5 g) to quinolines. Additional amounts of NaOH enhanced the reaction rate. The reduction of quinoline *N*-oxides containing substituents was shown to be efficient under bakers' yeast-NaOH system independent of the position and electronic nature of substituent.

We next examined the microbial deoxygenation of a series of heteroaromatic *N*-oxide compounds, the results of which are presented in Table 3.

**Table 3.** Deoxygenation of N-oxides by Bakers' Yeast-NaOH

| entry | Substrate | conditions | | Product, | Yield (%) |
|---|---|---|---|---|---|
| | | NaOH (g) | time (h) | | |
| 1 |  | 4 | 6 |  | 90 |
| 2 |  | 4 | 3 |  | 86 |
| 3 |  | 4 | 24 |  | 89 |
| 4 |  | 4 | 24 |  | 91 |
| 5 |  | 8 | 24 |  | 90 |

For example, isoquinoline *N*-oxides (0.5 g) were rapidly and quantitatively reduced to give isoquinolines with bakers' yeast (30 g) and NaOH (4 g) under reflux for several hours. Thus, bakers' yeast mediated biotransformation was easily achieved by controlling of the amount of NaOH and reaction time, as exemplified by the deoxygenation of other heteroaromatic *N*-oxides, 2-aryl-2*H*-benzotriazole 1-oxide (entries 3 and 4) and benzo[*c*]cinnoline *N*-oxide (entry 5). In order to examine the relative reactivites of a series of *N*-oxides, a competition reaction study of an equimolar mixture of isoquinoline *N*-oxide and quinoline *N*-oxide with excess bakers' yeast (60 g) and NaOH (8 g) was conducted. In this case, after the reduction of isoquinoline *N*-oxide was selectively completed to give isoquinoline, then quinoline *N*-oxide was slowly started to be reduced. By controlling the amount of bakers' yeast and NaOH, the reduction of isoquinoline *N*-oxide can be selectively achieved in the presence of the other *N*-oxides tried in this paper. For example, the selective reduction of a mixture of *N*-oxides containing isoquinoline *N*-oxide (0.5 g), quinoline *N*-oxide (0.5 g), azoxybenzene (0.5 g) and 2-aryl-2*H*-benzotriazole 1-oxide (0.5 g) was conducted to give isoquinoline with bakers' yeast (30 g) and NaOH (4 g) at reflux for 4 h, and the other *N*-oxides were all recovered.

The bakers' yeast mediated deoxygenation of *N*-oxide compounds was carried out by preparing a

suspension of bakers' yeast in tap water containing ethanol at reflux. The other solvent systems showed unsatisfactory results. In comparison with the reduction of carbonyl group[1] that require a large amount of bakers' yeast, long reaction time and nutrient, our reduction method of *N*-oxides using a small amount of bakers' yeast affords an easy work-up process. Furthermore, the reactions were completed within 24 h. During our studies on the bakers' yeast mediated biotransformation of *N*-oxide on the aromatic or heteroaromatic compounds, we have found that the reducing power and unique selectivity were achieved by employing NaOH. In the absence of NaOH, no deoxygenation of *N*-oxides was observed under any variation of chemical environments. In addition, the pH of the reaction mixture does not change noticeably during the microbial deoxygenation. In the case of organic bases ($Et_3N$, DABCO or pyridine), no reduction took place. Thus, reducing system of bakers' yeast-NaOH in EtOH-$H_2O$ at reflux is effective for the rapid and selective reduction of *N*-oxide compounds. In addition, the use of nonmetallic reducing agent has a crucial advantage as far as the environmental problem is concerned.

**General Procedure**

To a suspension of bakers' yeast (30 g) in tap water (110 mL) NaOH (4 g) was added. The suspension was stirred at 60 °C for the appropriate time. A substrate (0.5 g) dissolved in EtOH (40 mL) was added to a mixture. The resulting mixture was refluxed for the time period described in Tables. After the reaction was completed, dilute hydrochloride acid and $CH_2Cl_2$ were poured into the flask. The separated organic layer was filtered though a Celite pad, washed with brine solution, and dried over magnesium sulfate. Their GLC chromatograms and mass spectra were obtained and identified with those authentic samples.

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