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Microbial Transformation of *sec*-Hydroxyl Group of Polyols into Carbonyl Derivatives by Specific Oxidation Using Methanol Yeast

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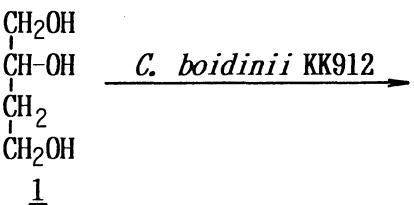
sec-Hydroxyl group of polyol was oxidized to the corresponding carbonyl derivative using methanol yeast, *Candida boidinii* KK912. Thus 1,2,4-butanetriol was oxidized for 2 days to give 1,4-dihydroxy-2-butanone in 78.1% yield. The microbial oxidation for analogous polyols was also discussed.

Application of microbes or enzymes to chemical transformation in the industrial field is mainly dependent on the cost for the preparation of the microbes[1] which are often grown using sugar as a carbon source. On the other hand, methanol produced from natural gas as well as carbon dioxide which causes environmental problems will be one of the most abundant and cheap carbon sources in coming years. Utilization of methanol as a growing substrate for microbes[1,2] will become feasible to introduce a microbial process in the industrial field. This communication reports on the specific oxidation of the secondary hydroxyl group of polyols into the corresponding carbonyl derivaties using methanol yeast, *Candida boidinii* KK912. Specific oxidation of the *sec*-hydroxyl group of polyols by the chemical method is cumbersome and costly. In this report 1,2,4-butanetriol (1) was selected as a substrate for the microbial transformation to give 1,4-dihydroxy-2-butanone (2). 2 can be regarded as a kind of tetrose which has been of interest in both industrial and biological field.



1 2

Candida boidinii KK912 was first isolated by an enrichment culture technique from activated sludge of a municipal sewage plant as a methanol assimilating strain. *C. boidinii* KK912 was grown in an inorganic medium (200 mL, initial pH 5.0)[3] containing 2.0% methanol as a growing substrate in a shaking flask at 30 ℃. The pH of the medium was maintained at 5 with 0.1 mol·dm^{-3} sodium hydroxide throughout the incubation. After 6 days (OD_{660} =3.3), the cells were harvested by centrifugation, washed with distilled water to obtain wet cells (1.5 g wet cells corresponding to 0.3 g dry cells) for the oxidation reaction. The reaction mixture

contained 50 mg of substrate (1), 300 mg of wet cells (corresponding to 60 mg of dry cells) and 200 mg of methanol in 10 mL of 0.07 mol·dm^{-3} phosphate buffer (pH 7). The reaction was performed in a shaking tube with reciprocal shaking at 30 ℃ in the dark. The yield of 2, remaining substrate 1 and methanol were periodically analyzed by HPLC[4] as shown in Fig. 1. It was found that specific oxidation of 1 occurred by *C. boidinii* KK912 in the presence of methanol to give 2 in 78.1% yield after 2 days. No other oxidation product was detected in the reaction mixture during further incubation.

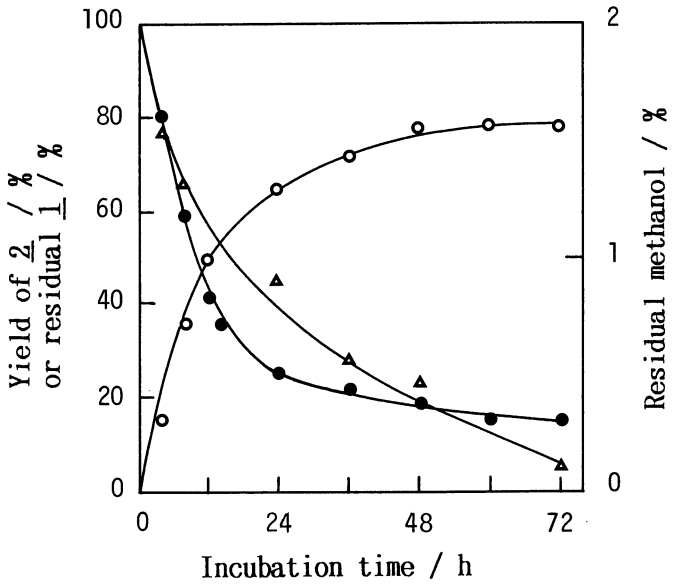


Fig. 1. Microbial transformation of 1 into 2 by *Candida boidinii* KK912. o : Yield of 2, ● : residual 1, ▲ : residual methanol.

After the reaction was over, the cells were separated from the medium by filtration through a 0.2 μm membrane. The filtrate was then evaporated to dryness in vacuo to give a syrup. The crude product was purified by ion-exchange resin column chromatography (Dowex 2x8) to give 2. The isolated product was analyzed by IR, ^{1}H NMR, and ^{13}C NMR spectroscopy.[5] These spectral data agreed completely with those of the authentic compound.

In a similar way, *sec*-hydroxyl groups of 2-butanol, 2,3-butanediol and 1,2,3-butanetriol were oxidized by *C. boidinii* KK912 to give 2-butanone, 3-hydroxy-2-butanone and 3,4-dihydroxy-2-butanone, respectively.

Methanol yeast, *C. boidinii* KK912, was found to be a useful tool for specific oxidation of *sec*-hydroxyl group of polyols into the corresponding carbonyl derivatives. Because of the natural occurrence of *C. boidinii* in the environment, this microbial method has a wide potential applicability in the industrial field.

References

1) Y. Tani, *Baiosaiensu to Indasutori*, 48, 635 (1990).
2) I. Nagai, I. Terao, and T. Urakami, *Bio Ind.*, 4, 97 (1987).
3) S. Matsumura, N. Yoda, and S. Yoshikawa, *Makromol. Chem., Rapid. Commun.*, 10, 63 (1989).
4) HPLC column : TOSOH Co. Ltd., Cation-exchange chromatographic column, TSK-Gel SCX ; Eluent : 0.05 mol·dm^{-3} $HClO_4$; UV detector : JASCO 875UV (208 nm) ; RI detector : SHOWA DENKO Co. Ltd., Shodex RI SE-51.
5) 2 : ^{1}H NMR (D_2O, 90 MHz) : δ 2.66 (2H, t, J=6.39 Hz), 3.81 (2H, t, J=6.12 Hz), 4.33 (2H, s). ^{13}C NMR (D_2O) : δ 41.3 (C-3), 57.2 (C-4), 68.6 (C-1), 212.9 (C-2).

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