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Microbial Transformation of 2-Butyn-1,4-diol into 4-Hydroxy-2-butynoic Acid by Specific Oxidation of the Hydroxymethyl Group

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2-Butyn-1,4-diol was oxidized quantitatively by the liophilized cell of *Rhinocladiella atrovirens* KY801 to afford the monocarboxylated product, 4-hydroxy-2-butynoic acid. Neither aldehydes nor degrading compounds were detected in the reaction mixture.

4-Hydroxy-2-butynoic acid, which contains hydroxyl, carboxyl and acetylenic groups, is attractive as a monomer for production of acetylenic functionl polymers and as a starting material in various fields, such as a raw material for production of acetylenic germicides. The oxidation of 2-butyn-1,4-diol to 4-hydroxy-2-butynoic acid by chromium trioxide [1] and the formation of γ-hydroxytetrolic acid (4-hydroxy-2-butynoic acid) [2] with dibutylphthalate [3] from 2-butyn-1,4-diol by soil bacterium capable of utilizing 2-butyn-1,4-diol as a carbon source but incapable of utilizing D-glucose [2] were only reported. Conventional catalytic oxidation of the diol containing an acetylenic group was, however, not successful, and furthermore, selective mono-oxidation was difficult. Microbial specific oxidation of such compounds will have potential feasibility for industrial production.

Rhinocladiella atrovirens KY801 was first isolated from activated sludge of a municipal sewage plant as a diethylene glycol oxidizing strain.[4] *R. atrovirens* KY801 grows well on D-glucose, glycerol and 1,4-butanediol as the carbon sources. This fungal strain was applied to the specific mono-oxidation of 2-butyn-1,4-diol (1) to 4-hydroxy-2-butynoic acid (2).

$$\underset{1}{HOCH_2\text{-}C{\equiv}C\text{-}CH_2OH} \xrightarrow{\textit{R. atrovirens}\ \text{KY801}} \underset{2}{HOCH_2\text{-}C{\equiv}C\text{-}COOH}$$

R. atrovirens KY801 was grown in an inorganic medium (100 mL, initial pH 6.2) containing 0.2% D-glucose as a growing substrate in a shaking flask at 30 ℃. After 5 days (OD_{660} = 2.0), the cells were harvested by filtration through a 0.2 μm membrane, washed with distilled water and liophilized. Thus, the obtained dry cells (0.1 g) were used for the oxidation of 1. Dry cell of *R. atrovirens* KY801 was stored in a frozen desiccator. Prior to the oxidation reaction with the dry cell, the lyophilized dry cell

was activated by incubating with aqueous D-glucose at 30 ℃. Activation of the dry cell by D-glucose and the subsequent oxidation of 1 were carried out in a three-necked flask equipped with a magnetic stirring bar, a bubbling tube for aeration, a reflux condenser and a pH electrode. For activation of the dry cell, a mixture consisting of 0.1% D-glucose, 0.1% dry cell, 15 mL distilled water and a drop of antifoamer was stirred with aeration at 30 ℃. After 2 - 3 days, when the D-glucose had disappeared from the incubation medium as analyzed by HPLC, 50 mg of 1 in 5 mL of distilled water was added to the incubation medium in the three-necked flask. The pH of the medium was maintained at 6 - 7 with calcium carbonate through the reaction. The yield of 2 and the remaining substrate 1 were periodically analyzed by HPLC[5] as shown in Fig. 1. It was found that using the dry cells of *R. atrovirens* KY801, the yield of 2 reached 99.8% after 3 days incubation, and after 5 days the yield of 2 reached 100%. Furthermore, no assimilation of the oxidation product, 2, was detected during further incubation. After the reaction was over, the cells were separated from the incubation medium by filtration. The filtrate was then evaporated to dryness in vacuo to exclusively give 2. The isolated products were analyzed by elemental analysis, IR, ^{1}H NMR, and ^{13}C NMR spectroscopy.[6] These spectral data agreed completely with those of the authentic compound.

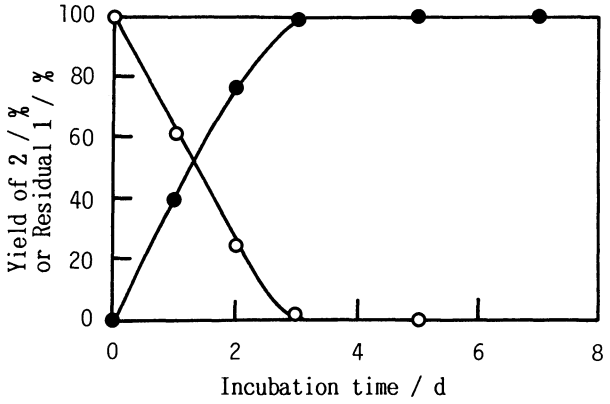

Fig. 1. Oxidation of 1 to 2 by liphilized cell of *Rhinocladiella atrovirens* KY801. ○ : 1, ● : 2.

The dry cell method of *R. atrovirens* KY801 was found to be a useful tool for oxidizing a diol containing an acetylenic bond to the corresponding monocarboxylated acetylenic compound. This method will be useful for industrial applications.

References

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3) T. Miyoshi and T. Harada, Biochim. Biophys. Acta, 222, 684 (1970).
4) S. Matsumura, N. Yoda, and S. Yoshikawa, Makromol. Chem., Rapid Commun., 10, 63 (1989).
5) HPLC column : TOSOH Co. Ltd., Cation-exchange chromatographic column, TSK-Gel SCX ; Eluant : 0.05 mol·dm^{-1} $HClO_4$; UV detector : JASCO 875UV (208 nm) ; RI detector : SHOWA DENKO Co. Ltd., Shodex RI SE-51.
6) 2 : IR ν_{max} (NaCl) cm^{-1} : 3230 (OH), 2240 (C≡C), 1700 (COOH)
^{13}C NMR (D_2O) : δ 51 (CH_2), 78 (C≡C-CH_2), 87 (C≡C-COOH), 159 (COOH) ;
Anal. Found : C, 47.68 ; H, 4.35%. Calcd for $C_4H_4O_3$: C, 48.01 ; H, 4.03%.

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