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Hydroxylation of α-haloacetophenone derivatives by *Nostoc minutum* NIES-29 and *Spirulina platensis*

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ABSTRACT

Biotrransformation of α-haloacetophenone derivatives was investigated with the cyanobacterium *Nostoc minutum* NIES-29 and *Spirulina platensis*. It was found that biotransformation of α-haloacetophenone derivatives gives the corresponding hydroxyl compounds. In the case of α-bromoacetophenone derivatives (1-4), the substitution of bromine by a hydroxyl group occurred preferentially. For the α-chloroacetophenone derivatives (5, 6), a carbonyl group was reduced to hydroxyl group as major products.

KEYWORDS

Nostoc minutum NIES-29;
Spirulina platensis;
Biotransformation;
Hydroxylation;
α-Bromoacetophenone;
α-Chloroacetophenone.

INTRODUCTION

From the view point of green chemistry, biotransformations play an important role in organic synthesis. In recently, enantiopure chiral secondary alcohols and primary α-hyroxyketones have been used in the pharmaceuticals, agricultural chemicals, flavors and fragrances. In the synthesis of enantiopure chiral secondary alcohols, prochiral ketones or racemic alcohols are commonly used as starting material. For example, they are synthesized by the asymmetric reduction of corresponding prochiral ketones using chemical[1-3] or biocatalytic methods[4, 5].

α-Hydroxyketones are usually prepared by one of the following methods: α-hydroxylation by treatment of their enolate forms with molybdenum peroxide reagent in THF-hexane at -70 °C[6], transformation of the enamine derivatives of ketones to α-hydroxy derivatives by molecular oxygen[7], and α-hydroxylation of silyl enol eters with *m*-chloroperbenzoic acid[8], selective oxidative cleavage of β-cyclodextrin-epoxide complex with IBX (*o*-iodoxybenzoic acid),[9] or with certain other oxidizing agents[10].

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It is known that there has been considerable interest in the development of direct methods for the synthesis of α-hydroxyketones using nontoxic hypervalent iodine reagents, which involve the following methods: reaction of ketone with iodobenzene diacetate in the presence of potassium hydroxide in methanol and then hydrolysis of the dimethylacetates[11]; oxidation of enol silyl ether of acetophenone using the system iodosobenzene/boron trifluoride etherate/water in methylene chloride at -40 °C[12], reaction of ketones with [bis(trifluoroacetoxy)]iodobenzene and trifluoroacetic acid in acetonitrile-water under acidic conditions[13], homogeneous catalytic oxidation of styrene and styrene derivatives with hydrogen peroxide in the presence of transition metal-substituted polyoxotungstate[14].

We have been investigating the organic synthesis from the view point of green chemistry. In our previous paper, we found that a novel reaction of α-halo ketone (α-bromo and α-chloro ketone) with irradiation under microwave gives the corresponding α-hydroxyketone and pyrazine derivative in good yields[15]. However, we tried much more synthetic method as compared with microwave method. We have reported that biotransformation of a synthetic substance into a more useful substance by plant culutured-cells is an important reaction in synthetic chemistry[16-19].

More recently, we reported that convenient simple procedure for the preparation of α-hydroxyketones from α-bromo and α,α'-dibromo alkanone with *Spirulina platensis* under mild conditions[20].

Under these conditions, biotransformation of 2-bromo-4'-chloroacetophenone (2) and 2-bromo- 4'-bromoacetophenone (3) using *S. platensis* gave the corresponding α-hydroxy compounds in 11 and 6% in low yield, respectively. Here, we report the improvements for these compounds.

MATERIAL AND METHODS

Analytical and algae

GC-MS: Shimadzu GCMS-QP5050 (EI-MS 70eV) using DB1 (0.25mm x 30m, 0.25μm) capillary column GC; GC: GC-17A. ^{1}H-NMR; JEOL α-500 spectrometer. $CDCl_3$ with tetramethylsilane as internal standard was used. *S. platensis* NIES-39 and *N. minutum* NIES-29[20] were obtained from the National Institute for Environmental Studies (NIES-Collection).

Cultivation

S. platensis was grown in SOT medium (pH 10.0) and *N. minutum* was grown in MDM medium (pH 8) under continuous illumination provided by fluorescent lamp (2000 lx) with air-bubbling at 25 °C.

General reaction conditions

Substrates (20 mg) were added to suspended culture of *S. platensis* (0.8 g/L as dry weight) or *N. minutum* (0.7 g/L as dry weight) in medium (100 ml). The mixture was treated with a shaker (120 rpm) at 25 °C in the light (2000 lx). The end of the reaction, algae was filtered, and resulting mixture was extracted with Et_2O. All the products were determined by ^{1}HNMR, ^{13}CNMR, GC and GC-MS analyses.

Preparation of microbial culture

SOT medium was prepared by mixing $NaHCO_3$ (16.8 g), K_2HPO_4 (0.5 g), $NaNO_3$ (2.5 g), K_2SO_4 (1.0 g), $MgSO_4 \cdot 7H_2O$ (0.2 g), $CaCl_2 \cdot 2H_2O$ (0.04 g), $FeSO_4 \cdot 7H_2O$ (0.01 g), Na_2EDTA (0.08 g) and A5 solution (1 mL) in distilled H_2O (1 L).

A5 solution was H_3BO_3 (286 mg), $MnSO_4 \cdot 7H_2O$ (250 mg), $ZnSO_4 \cdot 7H_2O$ (22.2 mg), $CuSO_4 \cdot 5H_2O$ (7.9 mg) and $Na_2MoO_4 \cdot 2H_2O$ (2.1 mg) dissolved in distilled H_2O (100 mL).

MDM medium was prepared by mixing KNO_3 (1.0 g), $MgSO_4 \cdot 7H_2O$ (0.25 g), K_2HPO_4 (0.25 g), NaCl (0.1 g), $CaCl_2 \cdot 2H_2O$ (0.01 g), A5 solution (1 mL) and Fe solution (1 mL) in distilled H_2O (1 L). The medium was adjusted to pH 8.0.

Fe solution was $FeSO_4 \cdot 7H_2O$ (200 mg) and concH_2SO_4 (0.026 mL) dissolved in distilled H_2O (100 mL).

RESULTS AND DISCUSSION

We have been investigated biotransformation of

α-bromo and α,α'-dibromo alkanone with algae of *Spirulina platensis*. It was found that in the case of α-bromo ketone, the corresponding α-hydroxyketone was obtained in good yields (80-95%).

The versatility of *S. plantensis* hydroxylation system is further exemplified by α-bromo aromatic ketone as substrates. 2-Bromoacetophenone (1) gave 2-hydroxy derivative (1a) in 55% yield. In the case of 2-bromo-4'-methylacetophenone (4), 2-hydroxy compound (4a) was prepared in 35% yield. Furthermore, biotransformation of 2-bromo-4'-chloro (2) and 2-bromo-4'-bromo compound (3) gave the corresponding α-hydroxy derivatives in 11 and 6% yield, respectively. These results summarized in TABLE 1.

From these results, it was found that C2-bromo derivative using bromo or chloro atom at the C4' position is difficult the conversion to hydroxyl group.

Moreover, biotransformation of 2-chloroacetophenone (5) using *S. platensis* yielded 2-chlorophenylethanol (5c) (9%) and styrene oxide (5d) (21%).

From these results, it is considered that the C2-chloro atom is difficult to convert the hydroxyl group. Therefore, it is considered that the carbonyl group was reduced and then converted to the oxide compound in SOT medium (pH 10.0).

On the other hand, in order to examine better results, we tried biotransformation with *Nostoc minutum*[21]. The conversion of 2-bromoacetophenone (1) using *N. minutum* afforded 2-hydroxy compound (1a) and 1-phenylethane-1,2-diol (1b) in 75 and 18 %, respectively. Figure 1 and 2 show the time course of biotransformation of compounds 1 and 5. Moreover, 2-bromo-4'-chloro- (2) and 2-bromo-4'-bromoacetophenone (3) gave the corresponding 2-hydroxy derivative in 81 and 69%, respectively. In the case of 2-chloroacetophenone (5) and 2-chloro-4'-methylacetophenone (6), 2-hydroxy compound was obtained in 74 and 95% yield, respectively. These results summarized in TABLE 1.

On the basis of these results, it was found that *N. minutum* is optimal alga for conversion of 2-hydroxy compound from 2-haloacetophenone derivative.

2-Hydroxy-1-phenylethanone (1a)[22]

Pale-yellow needles; m.p. 85-86 °C; IR (KBr): ν_{max} 3428, 1687 cm^{-1}; ^1H NMR ($CDCl_3$): δ3.53 (1H, *br s*), 4.88 (2H, *s*), 7.49 (2H, *t*, *J* = 7.7 Hz), 7.61 (1H, *t*, *J* = 7.4 Hz), 7.92 (2H, *d*, *J* = 7.6 Hz); ^{13}C NMR ($CDCl_3$): δ 65.4, 127.6, 128.9, 133.3, 134.2, 198.4; EIMS *m/z* 136 $[M]^+$.

1-Phenylethane-1,2-diol (1b)[23]

White needles; m.p. 64-65 °C; IR (KBr): ν_{max} 3410 cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.68 (1H, *dd*, *J* = 11.0, 8.5 Hz), 3.79 (1H, *dd*, *J* = 11.0, 3.0 Hz), 4.84 (1H, *dd*, *J* = 8.0, 3.0 Hz), 7.30-7.38 (5H, *m*); ^{13}C NMR ($CDCl_3$): 68.1, 74.7, 126.1, 128.1, 128.6, 140.5; EIMS *m/z* 138 $[M]^+$.

TABLE 1 : Biotransformation of 2-haloacetophenone derivatives by *Nostoc minutum* and *Spirulina platensis*[20]

Entry	SubstrateProduct	(Yield %)	*N. minutum*	*S. platensis*	
1	1	1a (75)	1b (18)	○	
2	1	1a (55)			○
3	2	2a (81)		○	
4	2	2a (11)			○
5	3	3a (69)	3b (25)	○	
6	3	3a (6)			○
7	4	4a (79)		○	
8	4	4a (35)			○
9	5	5c (74)	1a (4) 1b (4)	○	
10	5	5c (10)	5d (21)		○
11	6	6c (95)		○	

Reaction conditions: substrate (20 mg), algae (dry weight 0.5 g/L), and medium (150 ml) were employed; Yields were determined by GLC

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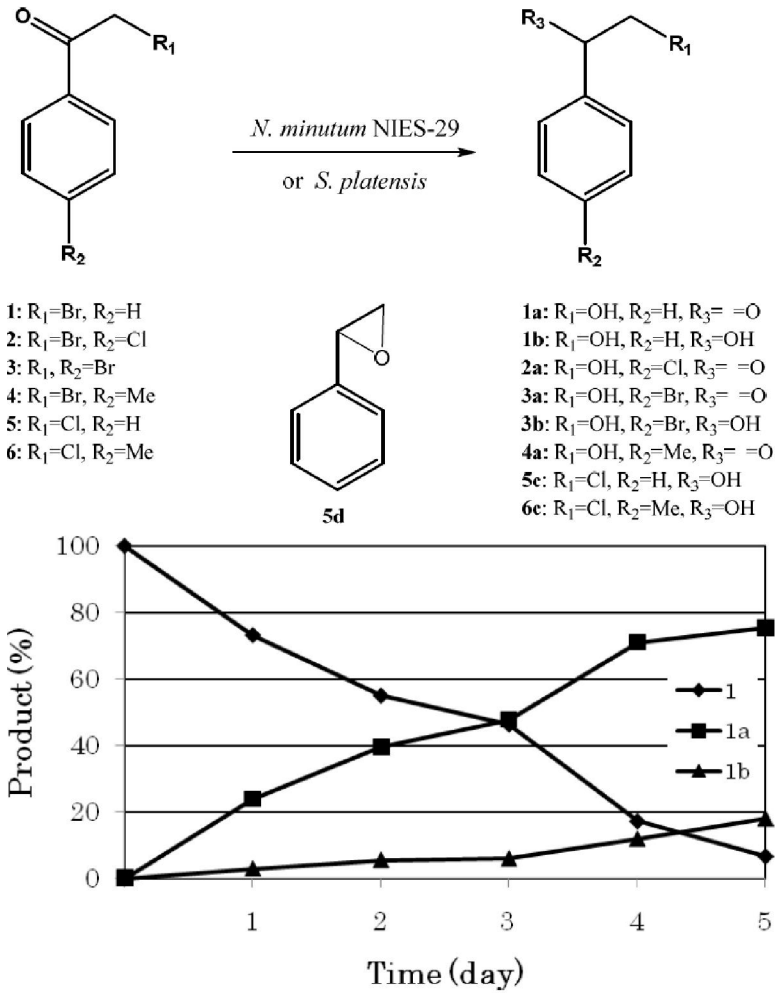

Figure 1 : Biotransformation of 2-bromoacetophenone (1) by *N. minutum* NIES-29

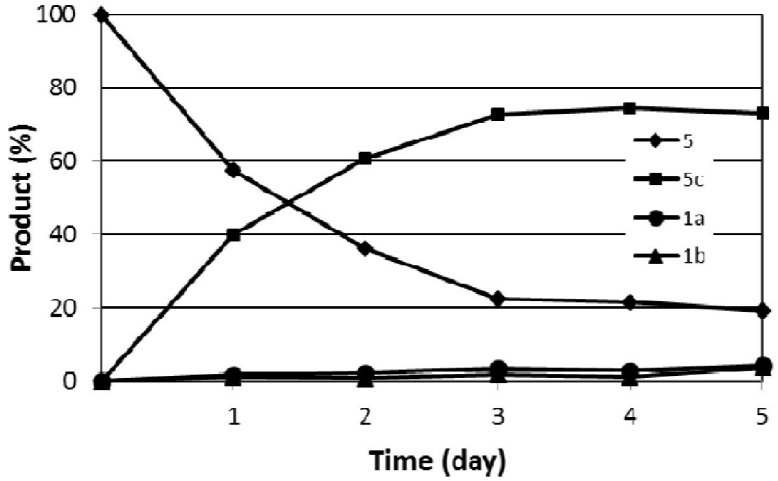

Figure 2 : Conversion of 2-chloroacetophenone (5) by *N. minutum* NIES-29

1-(4-Chlorophenyl)-2-hydroxyethanone (2a)[22]

Pale-yellow needles; m.p. 115-116 °C; IR (KBr): ν_{max} 3423, 1681 cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.42 (1H, *br s*), 4.85 (2H, *s*), 7.48 (2H, *d*, *J* = 8.4 Hz), 7.85 (2H, *d*, *J* = 8.4 Hz); ^{13}C NMR ($CDCl_3$): δ 65.3, 129.0, 129.4, 131.6, 140.8, 197.2; EIMS *m/z* 172 $[M]^+$.

1-(4-Bromophenyl)-2-hydroxyethanone (3a)[22, 24]

Pale-yellow needles; m.p. 133-134 °C; IR (KBr): ν_{max} 3411, 1681 cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.48 (1H, *br s*), 4.85 (2H, *s*), 7.65 (2H, *d*, *J* = 8.8 Hz), 7.78 (2H, *d*, *J* = 8.8 Hz); ^{13}C NMR ($CDCl_3$): δ 65.4, 129.1, 129.6, 132.0, 132.4, 197.5; EIMS *m/z* 216 $[M]^+$.

1-(4-Bromophenyl)ethane-1,2-diol (3b)[25]

White needles; m.p. 83-84 °C; IR (KBr): ν_{max} 3420 cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.62 (1H, *t*, *J* = 9.0 Hz), 3.76 (1H, *br d*, *J* = 11.0 Hz), 4.80 (1H, *br d*, *J* = 6.0 Hz), 7.26 (2H, *d*, *J* = 8.0 Hz), 7.49 (2H, *d*, *J* = 8.0 Hz); ^{13}C NMR ($CDCl_3$): δ 67.9, 74.0, 121.9, 127.8, 131.7, 139.5; EIMS *m/z* 217 $[M]^+$.

1-(4-Methylphenyl)-2-hydroxyethanone (4a)[22]

Pale-yellow needles; m.p. 82-83 °C; IR (KBr): ν_{max} 3428, 1681 cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.48 (3H, *s*), 3.59 (1H, *br s*), 4.85 (2H, *s*), 7.27 (2H, *m*), 7.82 (2H, *m*); ^{13}C NMR ($CDCl_3$): δ 21.8, 65.2, 127.7, 129.6, 130.8, 145.3, 197.9; EIMS *m/z* 150 $[M]^+$.

2-Chloro-1-phenylethanol (5c)[26]

Colorless oil; IR (ZnSe): ν_{max} 3423 cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.66 (1H, *dd*, *J* = 11.0, 9.0 Hz), 3.75 (1H, *dd*, *J* = 11.0, 3.5 Hz), 4.19 (1H, *dd*, *J* = 8.5, 3.5 Hz), 7.32-7.40 (5H, *m*); ^{13}C NMR ($CDCl_3$) δ 51.0, 74.1, 126.0, 128.5, 128.7, 139.9; EIMS *m/z* 156 $[M]^+$.

2-Chloro-1-(4-methylphenyl)ethanol (6c)[27]

Colorless oil; IR (ZnSe): ν_{max} 3425 cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.65 (3H, *s*), 3.64 (1H, *dd*, *J* = 11.0, 8.5 Hz), 3.72 (1H, *dd*, *J* = 11.0, 3.5 Hz), 4.86 (1H, *d*, *J* = 8.5 Hz), 7.18 (2H, *d*, *J* = 8.0 Hz), 7.27 (2H, *d*, *J* = 8.0); ^{13}C NMR ($CDCl_3$): δ 21.2, 50.9, 73.9, 126.0, 129.3, 136.9, 138.3; EIMS *m/z* 170 $[M]^+$.

CONCLUSION

This is the first time for bioconversion of α-haloacetophenone derivative using *Nostoc minutum* NIES-29. The biotransformation of 2-haloacetophenone derivatives (1-6) using *Nostoc minutum* NIES-29 gave the corresponding hydroxyl compounds (1a-6c). For the 2-bromoacetophenone derivatives (1-4), the hydroxyketone was preferentially provided with good yield. On the other hand, the 2-chloroacetophenone derivatives (5, 6) were reduced to chlorohydrins as the major product. From these results, it was found that the chloro atom is difficult to convert hydroxyl group for α-chloroacetophenone derivative. Biotransformation for α-hydroxy ketone from α-bromoacetophenone derivertive is no doubt attributable to the special properties of *N. minutum* system.

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