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Asymmetric biotransformation of phenyl-C4 derivatives in rat liver (S-9) and baker's yeast

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Abstract

When phenylbutyne (**1**) was incubated in rat liver S-9, reduction of triple bond and regioselective hydroxylation occurred to give phenylbutane (**3**) and (2*S*)-4-phenyl-3-butyn-2-ol (**2**), respectively. In the reaction of (**1**) with baker's yeast, reduction of triple bond occurred to give (**3**). In the incubation of 4-phenyl-3-buten-2-one (**6**) in rat liver, (2*S*)-4-phenyl-2-butanol [(2*S*)-**8**] were afforded from control rat liver, while (2*R*)-isomers [(2*R*)-**8**] were isolated from PB treated rat liver. 2,3-Epoxy-1-phenyl-1-butanone (**10**) was enantioselectively reduced to give the corresponding (1*R*,2*S*,3*R*)- and (1*R*,2*R*,3*S*)-epoxy alcohols (**11**, **12**) in high optical yields in rat liver and baker's yeast. However, in the same reaction of 2,3-epoxy-4-phenyl-2-butanone (**13**), reduction of ketone proceeded enantioselectively in baker's yeast to give (1*S*,2*S*,3*R*)- and (1*R*,2*R*,3*R*)-epoxyalcohols (**14**, **15**) in high optical yields (each 98% ee). © 1998 Published by Elsevier Science B.V. All rights reserved.

Keywords: Rat liver (S-9); Baker's yeast; Reduction of triple bond; Chiral epoxyalcohol; Stereoselective redox reaction; Regioselective oxidation

1. Introduction

It is known that, when drugs are therapeutically used as racemic form, each enantiomer often exhibits different pharmacological activities. However, much attention has not been paid for regio- or stereoselective redox reactions in the metabolism of drugs. In a previous paper [1], we reported that regio- and stereoselective oxidation of butylbenzene in rat liver supernatant fraction (S-9) occurred to give (2*S*)- and (1*R*)-butanols in good to high optical yields (78–87% ee) and (1*R*,3*S*)-butanediol in good optical yield (72% ee). As a further extension of this study, we have investigated the regio- and stereoselective redox reaction of unsaturated phenyl-C4 derivatives and phenyl-epoxyketone in rat liver (S-9) and baker's yeast, and searched the utilities of drug metabolic enzyme from rat liver for enzymatic syntheses in organic chemistry.

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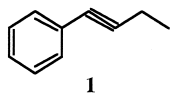
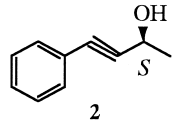
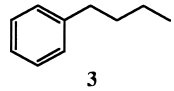
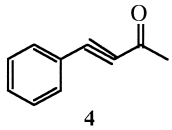
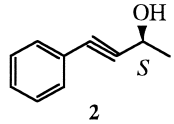
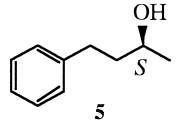
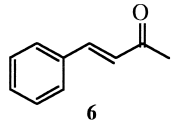
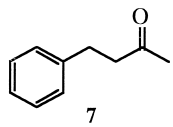
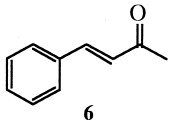
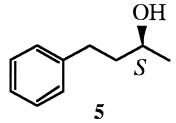
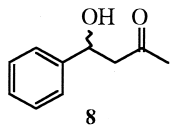
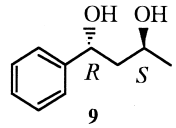
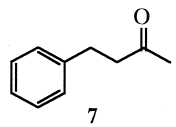
Table 1
Metabolism of unsaturated phenyl-C4 in rat liver and baker's yeast

Substrate	Products	Isolated yield(%) 0 100	$[\alpha]_D^{22-24}$ ($CHCl_3$)	Optical yield (%ee)
 **1** 1-Phenyl-1-butyne	 **2**	28	-14.9	43
		37	-18.5	54
		0.0	-	-
	 **3**	14	-	-
		15	-	-
		7	-	-
 **4** 4-Phenyl-3-butyn -2-one	 **2**	48	-2.1	8
		51	0.0	-
		5	-15.9	62
	 **5**	0.0	-	-
		0.0	-	-
		31	+6.3	36
	 **6**	1	-	-
		1	-	-
		3	-	-
	 **7**	8	-	-
		8	-	-
		8	-	-
 **6** 4-Phenyl-3-buten -2-one	 **5**	33	+10.0	57
		6	+8.0	46
		43	+15.4	86
	 **8**	0.4	*S* -10.4	23
		10	*R* +12.1	27
		0.0	-	-
	 **9**	3	+68.3	98
		4	+65.0	98
		0.0	-	-
	 **7**	54	-	-
		48	-	-
		15	-	-

Control Treated with PB Baker's yeast

2. Materials and methods

2.1. Preparation of S-9 fraction

Wistar male rats (180–200 g) were purchased from Japan SLC (Hamamatsu, Japan). Control rats, to which any drugs were not given, and sodium phenobarbital(PB) pretreated rats for 3 days (80 mg kg^{-1} day^{-1}, i.p.) were killed by decapitation and S-9 fraction was prepared by centrifugation as described previously [1]. The amount of protein was determined by the method of Lowry et al. [2].

2.2. Incubation conditions and isolation

A mixture (total volume of 200 ml), containing S-9 (1.0 ml, equivalent to 0.33 g of liver), $MgCl_2$ (60 mM), nicotinamide (80 nM), NADP (1.3 mM), Glucose-6-phosphate (25 mM), substrate(10 mM), and 0.1 M sodium phosphate buffer (pH 7.4) was agitated vigorously at 37°C for 1 h [3].

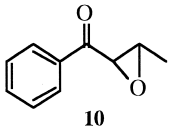
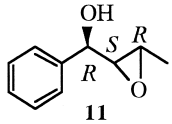
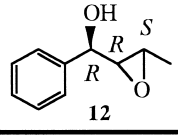
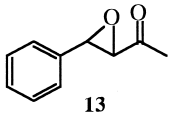
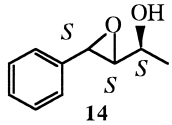
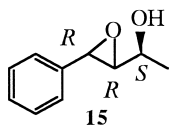
The incubation mixture was next extracted continuously with $CHCl_3$ using a Soxhler apparatus and the extract was dried over Na_2SO_4. The solvent was removed under reduced pressure to give the residue which was purified by silica gel column chromatography (Wakogel C-200, from Wako Pure Chemical Industries).

Incubation with baker's yeast and isolation was studied as described previously [4].

2.3. Instrument

The structure of products was determined by NMR (JEOL PMX-60si, JNM-EX-270, JNM-GMX-400 and JNM-Lambda 600 spectrometers) and Ms (JEOL JMN-DX 303). Optical rotations were recorded on a JASCO DIP-360 polarimeter. Enantiomeric excesses (% ee) were calculated from the ^1H NMR spectrum of (−)- or (+)-MTPA ester, or by HPLC analysis [column: Chiralcel OD (Daicel Chemical Industries), solvent: hexane–propan-2-ol (95:5)].

Table 2
Metabolism of phenyl epoxyketone in rat liver and baker's yeast

Substrates	Products	Isolated yield(%) 0–100	$[\alpha]^{22\text{-}24}_D$ ($CHCl_3$)	Optical yield (%ee)
 **10**	 **11**	21		-
		27	-51.5	99>
		18	-37.6	77
	 **12**	25	-34.6	99>
		28	-34.6	99>
		15	-31.7	98
 **13**	 **14**	9	-9.1	46
		7	-11.7	59
		22	-19.5	98
	 **15**	5	+7.4	46
		6	+7.9	49
		10	+16.0	98

Control Treated with PB Baker's yeast

3. Results and discussion

When 1-phenyl-1-butyne (**1**) was incubated with rat liver (S-9), as shown in Table 1, regioselective hydroxylation and reduction of triple bond occurred to give (2*S*)-4-phenyl-3-butyn-2-ol (**2**) [5] and phenylbutane (**3**), respectively. The chemical yield of the oxidation product (**2**) was higher in PB treated rat (37%) than in control rat liver (28%), whereas that of (**3**) was 14 and 15% in control and PB treated rat liver, respectively. Noticeably, the reduction of triple bond also occurred in the incubation with baker's yeast giving the phenylbutane (**3**) in low yield (7%), though (**2**) was slightly present in the reaction.

In the incubation of 4-phenyl-3-butyn-2-one (**4**), four metabolites; (**2**), (2*S*)-4-phenyl-2-butanol (**5**) [1,6], 4-phenyl-2-butanone (**6**) and 4-phenyl-2-butanone (**7**) were isolated from baker's yeast. However, from rat liver, only three metabolites (**2**, **6**, **7**) were observed. Phenylalkynol (**2**) was obtained in moderate chemical yield in control (48%) and PB treated rat liver (51%) but almost in racemic form. However, in baker's yeast, optically active (**2**) was obtained in 62% ee but in low chemical yields (5%). Interestingly, (**5**) was isolated from the incubation with baker's yeast, not from rat liver. Considering the metabolic pathway of (**1**), we found that the alcohol (**5**) might be transformed into (**6**) or (**7**), not into yn-ol (**2**).

In the incubation of enone (**6**), four metabolites: (**5**), 4-hydroxy-4-phenyl-2-butanone (**8**), (1*R*,3*S*)-1-phenyl-1,3-butanediol (**9**) [1] and (**7**) were isolated from rat liver. From baker's yeast only two (**5**, **7**) of the above metabolites were observed. Phenylbutanone (**7**) was obtained in moderate chemical yield in both control and PB treated rat liver (48–54%), but (**7**) was low in baker's yeast (15%). The yield of (**5**) was higher in baker's yeast (43%, 86% ee) than in control rat liver (33%, 57% ee) or PB treated rat liver (6%, 46% ee). During the reaction, regioselective and enantioselective oxidation also occurred to give (1*R*,3*S*)-1,3-butanediol (**9**) in 3–4% chemical yield and 98% ee in each case.

Interestingly, it was found that the configuration of 4-hydroxy-4-phenyl-2-butanone (**8**) [7] was different between control and PB-treated rat liver. (4*S*)-Hydroxy-4-phenyl-2-butanone [(*S*)-**8**] was obtained from control rat liver in 23% ee, while from PB treated rat liver, (4*R*)-isomer [(*R*)-**8**] was isolated in 27% ee. It is likely that cytochrome *P*-450 may be responsible for the formation of such metabolites.

In the metabolic study of 2, 3-epoxy-1-phenyl-1-butanone (**10**) [7] and 3, 4-epoxy-4-phenyl-2-butanone (**13**) [8] in rat liver and baker's yeast, it was found that epoxyketone (**10**) was enantioselectively reduced by PB treated rat liver to give (2*R*,3*S*,4*R*)- and (2*S*,3*R*,4*R*)-4-phenyl-2,3-epoxy-4-butanols (**11**,**12**) [7] both with an ee > 99%. In contrast, 3, 4-epoxy-4-phenyl-2-butanone (**13**) was enantioselectively reduced by baker's yeast to give the corresponding (1*S*,2*S*,3*R*)- and (1*R*,2*R*,3*R*)-epoxyalcohols (**14**, **15**) [8] in high optical yields (each 98% ee), while enantioselective reduction was low in control and PB treated rat liver (> 59% ee) (Table 2).

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