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Asymmetric Bioreduction of Ethyl 3-Oxobutanoate by Immobilized Baker's Yeast Entrapped in Calcium Alginate Beads. Application of the Immobilized Biocatalyst to the Synthesis of (5Z,13S)-5-Tetradecen-13-olide, Aggregation Pheromone of Cryptolestes Grain Beetle[1)]

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Immobilized baker's yeast entrapped in calcium alginate beads of 1-1.5 mm diameter was reusable more than 10 times and stereoselectively reduced ethyl 3-oxobutanoate to the corresponding (S)-hydroxy ester in high chemical and optical yields. The immobilized biocatalyst was also successfully used for an asymmetric synthesis of (5Z,13S)-5-tetradecen-13-olide, a synergist of the aggregation pheromone of the flat grain beetle.

Use of biochemical or biological reactions has provided an effective tool of preparing chiral synthons for synthesis of biologically active substances in recent years.[2)] Especially microbial asymmetric reduction of carbonyl compounds with baker's yeast has been widely used as a useful method for obtaining optically active alcohols. For example, ethyl 3-oxobutanoate (1) was successfully transformed to a useful chiral synthon, ethyl (S)-3-hydroxybutanoate (2), by using free[3)] and immobilized[4)] baker's yeast systems. Immobilized baker's yeast (IBY) system, compared with free baker's yeast (FBY) system, has been found to have advantages in terms of the separation of products from the catalyst and of the reuse of the catalyst.[5,6)]

For last two years we have been employing IBY entrapped in carrageenan or calcium alginate beads of 3-4 mm diameter in organic synthesis.[6)] The optical yields of products obtained for the IBY's were similar to or sometimes higher than those observed for FBY, while the chemical yields for the IBY's were always lower than those for FBY. The lowering of chemical yields may be due to the difference in the apparent activity of baker's yeast based presumably on each surface area of FBY and the IBY's. We have now prepared IBY, which have a larger specific surface area compared with the 3-4 mm immobilized beads, by entrapping a certain amount of baker's yeast in calcium alginate beads of 1-1.5 mm diameter and first examined the bioreduction of 1 with the immobilized biocatalyst.

The present IBY (IBY-1A) was prepared by rapidly adding a homogeneous solution

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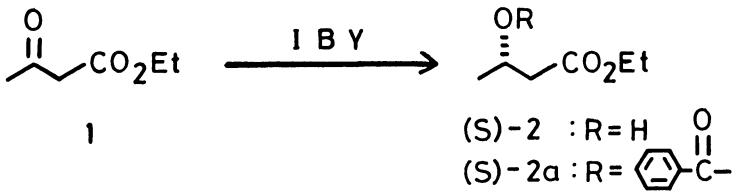
Table 1. Repetitive Use of Immobilized Baker's Yeast in the Asymmetric Bioreduction of Ethyl 3-Oxobutanoate (1)[a)]

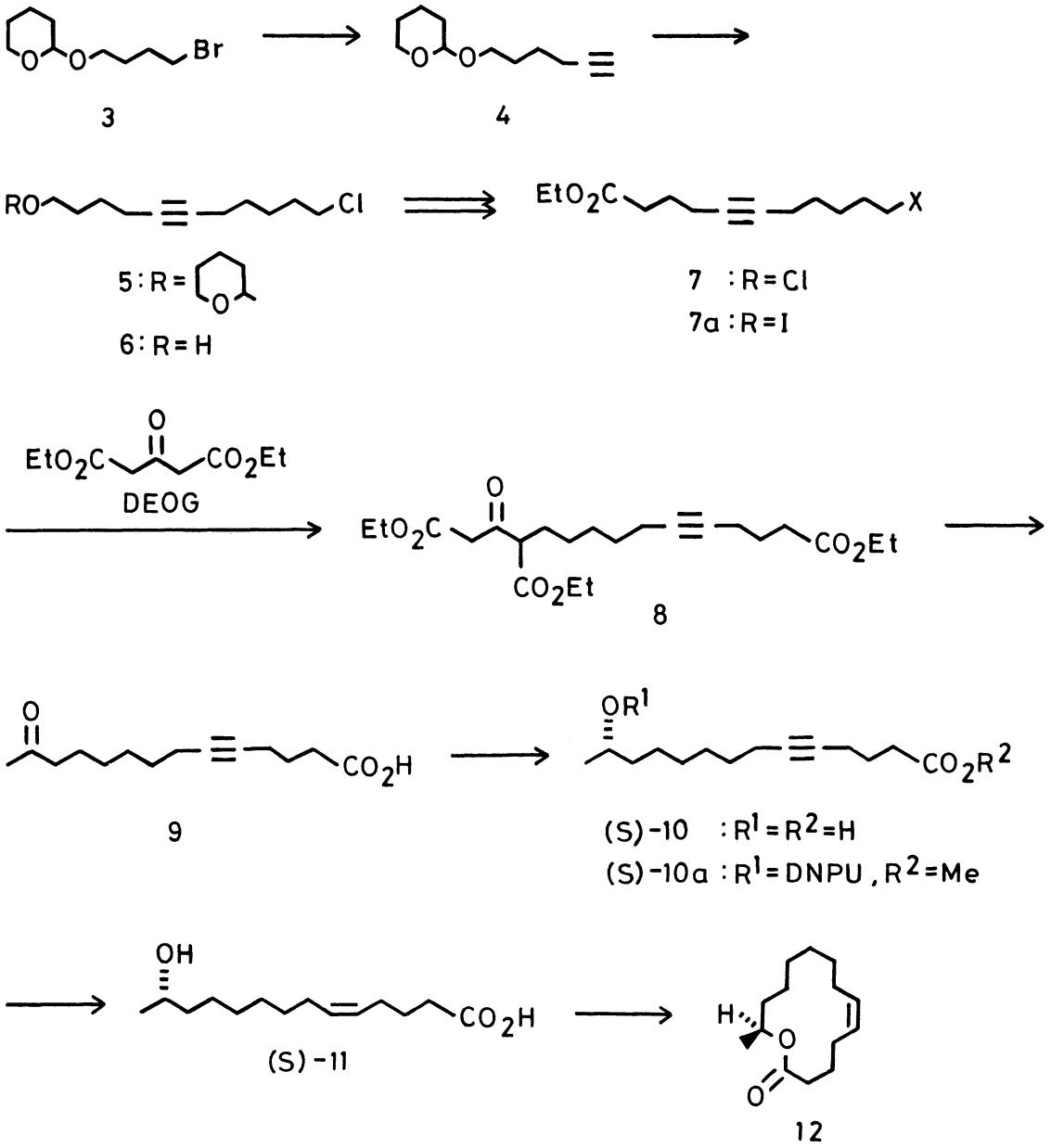


1

Reuse number	IBY-1A[b)]		IBY-3A[c)]		IBY-3C[d)]	
	% yield[e)]	% ee[f)]	% yield[e)]	% ee[f)]	% yield[e)]	% ee[f)]
1	47	98	18	98	11	98
2	46	97	20	98	17	98
3	55	98	25	99	28	99
4	52	99	41	96	39	99
5	64	95	12	98	15	98
6	63	99				
7	75	98				
8	48	97				
9	48	96				
10	18	98				
11	6	98				
12	5	97				

a) All experiments were carried out in water (1000 mL) using 1 (1 g) and IBY's prepared from baker's yeast (50 g)[6)] with the exception of the use of 2 g of 1 for IBY-1A. b) IBY entrapped in calcium alginate beads of 1-1.5 mm diameter. c) IBY entrapped in calcium alginate beads of 3-4 mm diameter. d) IBY entrapped in carrageenan beads of 3-4 mm diameter. e) Chemical yield of 2. Purified by a combination of column chromatography and micro-vacuum distillation. f) Optical yield of (S)-2. Determined by HPLC analysis of each benzoate ester (S)-2a (Daicel Chiralcel OB, hexane: 2-propanol=9:1, 1.0 mL/min, 220 nm). Each (S)-2 showed specific rotations $[\alpha]_D^{22}$ ($CHCl_3$) ranging from +37.63° (c 0.0276) to +42.25° (c 0.0237).

derived from baker's yeast (50 g) and a sterilized solution of sodium alginate (38 g) to a 2% aqueous solution of $CaCl_2$ at room temperature. Table 1 exemplifies the bioreduction of 1 through the reuse of the present IBY and the previous IBY's (IBY-3A, IBY-3C). The first use of IBY-1A gave a 47% yield of 2, but after the 3rd use the chemical yield was raised to 55%, a maximum yield of 75% being obtained after the 7th use. Further, after the 12th use the biocatalyst still maintained the activity showing a 5% chemical yield. It is noteworthy that each biotransformation with the present and the previous IBY catalysts gave (S)-2 in similar



optical yields, but the chemical yields for IBY-1A were higher than those for the previous biocatalysts. These results reflect that the activity of the present IBY is higher than that of the previous IBY's and probably comparable with that of FBY.[7]

Next, we applied the present IBY system to an asymmetric synthesis of (5Z, 13S)-5-tetradecen-13-olide (12), a synergist of the aggragation pheromone of the flat grain beetle, Cryptolestes pusillus Schönherr.[8] Thus, bromide 3, which was prepared from a monoTHP ether of 1,4-butanediol in the usual manner, was treated with lithium acetylide-ethylenediamine to give the terminal alkyne 4 in a 74% yield. Compound 4 was alkylated with BuLi and 1-bromo-5-chloropentane, followed by treatment with p-TsOH to give the chloro alcohol 6 in a 72% yield, which, after

oxidation with chromic acid and esterification gave the chloro ester 7 in a 98% yield. Regioselective alkylation of diethyl 3-oxoglutarate (DEOG) with the iodo ester 7a derived from 7 was performed in the presence of $Mg(OEt)_2$ to give the monoalkylated 3-oxoglutarate 8, which was converted by a decarboxylative hydrolysis to the acetylenic keto acid 9 in a 67% yield from DEOG. Compound 9 (1 g), after treatment with an aqueous solution of KOH, was subjected to a bioreduction with IBY-1A in water to yield a chiral alcohol (S)-10 in a 40% yield[9] and an optical yield of 95% ee,[10] $[\alpha]_D^{23.5}$ +6.84° (c 0.029, $CHCl_3$)[lit,[11] $[\alpha]_D^{23.3}$ +6.0° (c 1.06, $CHCl_3$)]. Hydrogenation of (S)-10 with P-2 nickle and lactonization of the resulting olefinic hydroxy acid (S)-11 with 2-chloro-1-methylpyridinium iodide gave 12 in a 48.5% yield, $[\alpha]_D^{23}$ +52.6° (c 0.012, $CHCl_3$)[lit,[8b] $[\alpha]_D^{32}$ +49.6° (c 4.62, $CHCl_3$); lit,[11] $[\alpha]_D^{23}$ +54.4° (c 1.275, $CHCl_3$)]. The 400 MHz ^1H NMR spectrum of the synthetic 12 completely coincided with that of the natural one.

References

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9) Similar reductions of 9 with IBY-3A and IBY-3C proceeded in poor chemical yields.

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12) All the synthetic compounds containing 12 were fully characterized on the basis of their spectral data.

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