Tetrahedron Letters,Vol.28,No.12,pp 1303-1306,1987 0040-4039/87 $3.00 + .00
Printed in Great Britain Pergamon Journals Ltd.

ENZYME-MEDIATED ASYMMETRIC HYDROLYSIS OF α-BENZYLOXYCARBOXYLIC ESTERS

Yasuo Kato, Hiromichi Ohta,* and Gen-ichi Tsuchihashi
Department of Chemistry, Facultly of Science and Technology, Keio University,
Hiyoshi 3-14-1, Kohoku-ku, Yokohama 223, Japan

Summary: Incubation of dl-α-benzyloxycarboxylic esters with grown cells of a bacterium, Corynebacterium equi IFO 3730, afforded the chiral esters of high optical purities via asymmetric hydrolysis. This reaction has been revealed to have a wide applicability to alkane- and arylalkane-carboxylic esters.

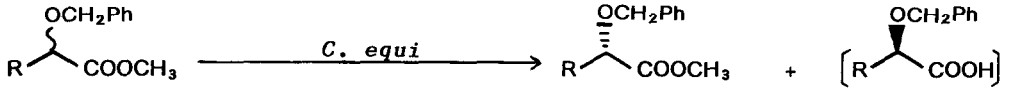
Optically active α-benzyloxycarbonyl compounds are widely used as "chiral synthons" in natural product synthesis.[1] A variety of methodologies have been developed for stereo-controlled addition to these compounds.[2] Hitherto, most of chiral α-alkoxy carbonyl compounds have been synthesized via a few steps staring from naturally occurring compounds, such as mannitol, ascorbic acid, α-hydroxy carboxylic acids (lactic acid, malic acid, tartaric acid) and amino acids.[1,3] In most cases, it is desirable to obtain the chiral molecules in their protected forms for the transformations of the later stage to target compounds. Among many protecting groups for hydroxyl proton, benzyl ether has been widely used because of its stability to both acid and base, and feasibility for deprotection. However, main drawback of this protecting group is that it requires a strong base for its introduction,[4] which sometimes causes the epimerization. Thus, a convenient way to overcome this limitation is to introduce the chiral center *after* protection of the hydroxyl group.

Enzymatic processes have been utilized for the introduction and differentiation of chiral centers.[5] One of the advantages of biochemical methods is that it is possible to distinguish the configuration of the asymmetric carbon in the reaction of functional groups remote from the chiral center,[6] which facilitates the apparent asymmetric introduction of a protected hydroxyl group. A known bacterium, *Corynebacterium equi* IFO 3730 has been demonstrated to have a high ability to hydrolyze various esters enantioselectively.[7] In this study we applied this microorganism to the asymmetric hydrolysis of dl-α-benzyloxy carboxylic esters.

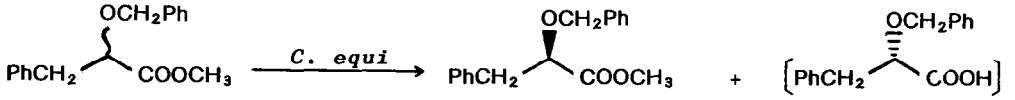
dl-Methyl 2-benzyloxybutanoate (100 mg) and a suspension of grown cells of *C. equi* (5 ml) were added to 45 ml of the medium containing inorganic salts and 4% of hexadecane as the sole carbon source.[8] The mixture was shaken at 30 °C for 24 hr. The broth was extracted with a 200 ml portion of ethyl acetate three times. The combined extract was concentrated and purified by column chromato-

graphy on silica gel. The unreacted substrate was recovered in a yield of 38% (76% of theoretical yield) in its optically active form. The optical purity was revealed to be over 99% e.e. (*S* form) by the following sequence of reactions. Reduction of the ester with lithium aluminium hydride afforded 2-benzyloxybutanol which was esterified with (R)-(+)-MTPA chloride.[9] The diastereomeric excess of the MTPA ester (hence, the enantio excess of the benzyloxybutanoate) was determined by HPLC.[10] Another expected product, the antipodal hydrolyzed acid, was not found in the organic extract, presumably due to the further biological degradation. Absolute configuration of the esters was determined after conversion to the corresponding *gem*-diol (LAH reduction followed by deprotection by hydrogenolysis over Pd-C), whose specific rotations were compared with the reported values[11] (except entry 6 [12]).

The strict substrate specificity of enzymes is, in a sence, one of the disadvantages of the biological method, when it is desired to be applied in organic synthesis. It is preferred that an enzyme system has strict stereospecificity and a broad spectrum of applicability for a variety of compounds at the same time. The compounds cited in Table 1 were subjected to the hydrolysis with *C. equi*. The substrate which has olefinic bond (entry 4) and branched



R=alkyl, allyl (entry 1 - 5)



(entry 6)

Table 1. Asymmetric Hydrolysis of $RCH(OBn)CO_2CH_3$

Entry	R	Incubation (hr)	Recovery (%)	$[\alpha]_D$ [a] (degree)	Opt. purity (% e.e.)	Abs. config.
1	C_2H_5	24	38	+61	>99	S
2	C_3H_7	36	42	+75	>99	S
3	C_4H_9	48	42	+54	>99	S
4	CH_2=$CHCH_2$	24	40	+46	>99	S
5	$(CH_3)_2CH$	65	48	+8.6	90	S
6	$PhCH_2$	36	35	+45	95	R

a) Measured in methanol (c=0.5-1) at room temperature.

alkanoic ester (entry 5) were also fitted to the enzyme and gave the S-form of the starting materials. On the other hand, changing the alkyl or alkenyl moiety of the substrate with a phenylmethyl group, caused the reversal of selectivity in stereochemical course, resulting in the formation of R-form (entry 6). The dramatic reversal of the absolute configration is supposed to be related to the presense of benzyloxy group. In the binding site of the enzyme, O-benzyl group might be displaced by C-benzyl group in this case.

The starting materials were readily synthesized from the corresponding carboxylic acid by the sequence of α-bromination by the aid of Br_2/PCl_3, benzyloxylation with sodium benzyloxide, and esterification with methanol in moderate to high yields (70-80%).

The structures of the alcoholic part of the esters have been demonstrated to have sometimes great effects on the optical and chemical yields of the enzymatic reaction.[13] The variation of alkyl groups was examined using 2-benzyloxybutanoate esters, to find that methyl and benzyl esters are the best among entries summarized in Table 2. The fact that the benzyl ester gives a good result makes it possible to shortcut the preparation of the staring material. When 2-hydroxy acids are easily obtained, simultaneous benzylation of both α- and carboxylic hydroxy groups gives the corresponding α-benzyloxy benzyl esters, that can be readily subjected to the microbial transformation.

Table 2. Asymmetric Hydrolysis of $C_2H_5CH(OBn)CO_2R$

Entry	R	Incubation (hr)	Recovery (%)	$[\alpha]_D$ [a] (degree)	Opt. purity (% e.e.)
1	CH_3	24	38	+61	>99
2	C_2H_5	24	37	+69	77
3	$(CH_3)_2CH$	24	32	+65	84
4	C_4H_9	24	36	+56	90
5	$PhCH_2$	24	41	+46	>99

a) Measured in methanol (c=0.5-1) at room temperature.

Resulting α-benzyloxy carboxylic esters[14] were easily transformed to a number of derivatives, such as α-benzyloxy aldehydes, which play an important role in the stereo-controlled synthesis of acyclic compounds. Thus, present biological asymmetric hydrolysis will be expected to add a new entry for the preparation of chiral building blocks.

References

1) J. D. Morrison, "Asymmetric Synthesis," Vol. 4, Academic Press, Inc., New York, 1984.

2) M. T. Reetz and K. Kesseler, J. Org. Chem., 50, 5434 (1985); M. Asami and R. Kimura, Chem. Lett., 1985, 1221; W. C. Still, J. H. McDonald, and J. A. Schneider, Tetrahedron Lett., 21, 1031, 1035 (1980).

3) J. Jurczak, S. Pikul, and T. Bauer, Tetrahedron, 41, 447 (1985).

4) T. W. Green, "Protecting Group in Organic Synthesis," John Wiley & Sons, New York, p. 29, 1900.

5) J. B. Jones, C. J. Sih, and D. Perlman, ed, "Application of Biochemical Systems in Organic Chemistry", Vol. 1 and 2, John Wiley & Sons, New York ; "Enzymes in Organic Synthesis," Ciba Foundation Symposium III, Pitman, London, 1985.

6) F. C. Huang, L. F. H. Lee, R. S. D. Mittal, P. R. Ravikumar, and C. J. Sih, J. Am. Chem. Soc., 97, 4144 (1975); Y. F. Wang, T. Izawa, S. Kobayashi, and M. Ohno, *ibid.*, 104, 6465 (1982); I. J. Jacovac, H. B. Goodbrand K. P. Lock, and J. B. Jones, *ibid.*, 104, 4659 (1982); H. Ohta, H. Tetsukawa, and N. Noto, J. Org. Chem., 47, 2400 (1982).

7) A. S. Gopalan, and C. J. Sih, Tetrahedron Lett, 25, 5235 (1984); H. Ohta, Y. Kato, and G. Tsuchihashi, Chem. Lett., 1986, 217.

8) The medium consists of $(NH_4)_2HPO_4$ 10 g, K_2HPO_4 2 g, $MgSO_4$ $7H_2O$ 0.3 g, $FeSO_4$ $7H_2O$ 10 mg, $MnSO_4$ $4H_2O$ 8 mg, $ZnSO_4$ $7H_2O$ 8 mg, yeast ext. 0.2 g in 1000 ml of distilled water, pH 7.2.

9) J. A. Dale, D. L. Dull, and H. S. Mosher, J. Org. Chem., 34, 2543 (1969).

10) The conditions for HPLC analysis were as follows: Colomn; Zorbaxsil, Solvent; hexane/ethyl acetate=200/1, Flow rate; 0.5-0.7 ml/min, Retention time; Ester from racemate, 50.9 and 53.6 min; Product, 53.0 min.

11) P. A. Levene and H. L. Haller, J. Biol. Chem., 74, 348 (1927); R. U. Lemiuex, Can. J. Chem., 29, 678 (1951); P. A. Levene, J. Biol. Chem., 79, 475 (1928); D. Bartlett, M. Kuna, and P. A. Levene, J. Biol. Chem., 118, 303 (1937).

12) This substrate was deprotected by hydrogenation over Pd-C to give methyl 2-hydroxy-3-phenyl propionate, and their specific rotation was compared to the reported value; K. Inoue, and J. S. Fruton, Biochemistry, 6, 1765 (1967).

13) B. Zhou, A. S. Gopalan, F. VanMiddlesworth, W. R. Shieh, and C. J. Sih, J. Am. Chem. Soc., 105, 5925 (1983); M. Hirama, M. Shimizu, and M. Iwashita, J. Chem. Soc. Chem. Commun., 1983, 599.

14) K. F. Bernady, M. B. Floyd, J. F. Poletto, and M. J. Weiss, J. Org. Chem., 44, 1438 (1979).

(Received in Japan 13 December 1986)