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Patents and Literature

Selective Biotransformations

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ABSTRACT

Stereo- and regioselective biotransformations is an area of intense research interest. The ability to take advantage of microorganisms and isolated enzyme systems to perform selective organic syntheses is well known. In recent years, several new methodologies have caused a rapid growth in the area of selective biotransformations. These novel techniques include genetic and protein engineering, biocatalysis in organic media, improved screening procedures, as well as an increased awareness of the value of biotechnology to solve important problems in organic synthesis. Recent US patents and scientific literature on stereo- and regioselective biotransformations are surveyed. Patent abstracts are summarized individually and a list of literature references are given.

INTRODUCTION

The objective of the Patents and Literature Section is to summarize and cite recent developments in industrial and academic research as portrayed within the scope of current patents and literature and to highlight emerging biotechnological research areas. The subject of the first Patent and Literature Section of 1989 is Selective Biotransformations. Additional topics to be covered in this journal include Protein Engineering and Site Directed Mutagenesis, Mammalian Cell Culture, Biodegradable Polymers, and DNA Probes for Clinical Applications.

PATENTS

This section covers the US patents concerning selective biotransformations from the period of January, 1986 to March, 1989. The major search headings were biotransformations, enzyme(s), biocatalysis, and microb(ial)

with the cross terms: *optically active, regioselectivity, stereoselectivity*. Under this search format, over 150 patents were recovered, however, less than 20 were assigned US patent numbers. Most of the patents were Japanese and European. Many of these patents also had US application numbers and will be issued US patent numbers in the near future. The major US patents recovered under this search are described below. Some abstracts have been edited for clarity. Copies of US patents can be obtained from the Commissioner of Patents and Trademarks, Washington, DC 20231.

Takahashi S., Yamada Y., Udea Y., Katayama Y., Shimada Y., and Watanabe K.
PROCESS FOR PREPARING OPTICALLY ACTIVE HYDANTOINS
US 4,812,406, Mar. 14, 1989
Assignee: Kanegafuchi Kagaku Kogyo Kabushiki Kaisha

A process for preparing optically active hydantoins having the general formula $R_1NHCONHCR_2CO$ where R_1 and R_2 are different from each other and are alkyl group, aralkyl group, aryl group, substituted alkyl group substituted aralkyl group, substituted aryl group, or if R_1 and R_2 form an asymmetric cyclic compound, characterized in that one configuration of racemic *N*-carbamoyl-alpha-amino acid having the general formula R_1R_2-$NHCONH_2COOH$ where R_1 and R_2 are as above, is enzymatically converted into the corresponding hydantoins. The present invention provides a highly efficient process for an optical resolution for the synthesis of (S)-6-fluoro-spiro-)chroman-4,4′-imidazolidine)-2′,5′-dione (USAN; Sorbinil), which is an optically active hydratoin attracting public attention as a remedy for the particular chronic symptoms of diabetes such as cataract and neuropathy. The present invention also provides a highly efficient process for an optical resolution (S)-α-methyl-3,4-dihydroxypenylalanine (L-methyldopa), which is an optically active amino acid widely used as an antihypertensive. Further, the present invention provides a novel finding that an *N*-carbamoyl-α-amino acids having no hydrogen atom on its alpha-carbon atom can be biochemically converted into hydantoins by an enzymatic cyclization reaction.

Matson S. L.
METHOD FOR RESOLUTION OF STEREOISOMERS IN MULTIPHASE AND EXTRACTIVE MEMBRANE REACTORS
US 4,800,162, Jan. 24, 1989
Assignee: Sepracor, Inc.

This invention relates to the resolution of racemic mixtures of optically active compounds, including but not limited to the stereochemical purification of chiral organic esters, amides, carboxylic acids, alcohols, and amines. Novel methods utilizing multiphase and extractive enzyme membrane bioreactors are disclosed that selectively produce pure or substantially purified optically active compounds from achiral precursors or mix-

tures of isomers in which one or several of those isomers are biologically inactive or otherwise lack desirable characteristics. There are immiscible solvents on either side of the membrane.

Leuchtenberger, W., Wandrey, C., and Kula, M. R.
PROCESS FOR THE CONTINUOUS ENZYMATIC CONVERSION OF ALPHA-HYDROXYCARBOXYLIC ACIDS INTO THE CORRESPONDING OPTICALLY ACTIVE ALPHA-AMINOCARBOXYLIC ACIDS
US, 4,782,020, Nov. 1, 1988
Assignee: Degussa Aktiengesellschaft

Alpha-hydroxycarboxylic acids are continuously converted into the corresponding optically active α-aminocarboxylic acids. The conversion is carried out in a membrane reactor in the presence of nicotinamide-adenine dinucleotide bound to a water soluble high molecular weight material, a dehydrogenase specific for the α-hydroxycarboxylic acid, a dehydrogenase specific for the corresponding α-aminocarboxylic acid and ammonium ions. An aqueous solution of the α-hydroxycarboxylic acid is continuously added to a substantially lesser amount of the corresponding α-ketocarboxylic acid, and an amount of ammonium ion at least equivalent to the α-hydroxycarboxylic acid to be reacted is added. A difference in pressure between 1 and 15 bar is maintained over the membrane. Behind the membrane, there a filtrate stream containing the alpha-amino-carboxylic acid that is continuously withdrawn.

Hamaguchi S., Ohashi T., and Watanabe K.
METHOD FOR PRODUCING OPTICALLY ACTIVE GLYCOL DERIVATIVES
US 4,745,066, May 17, 1988
Assignee: Kanegafuchi Kagaku Kogyo Kabushiki Kaisha

A method for producing optically active glycol derivatives by biochemical resolution that comprises contacting a racemic ester of the general formula (RS) $R_1OSO_2R_3OCOR_2$ (where R_1 is an aliphatic hydrocarbon group of 1 to 16 carbon atoms, R_2 is an aliphatic hydrocarbon group of 1 to 8 carbon atoms, and R_3 is an aromatic hydrocarbon group such as phenyl, tolyl, or naphtyl) with a microorganism or animal organ-derived enzyme having stereoselective hydrolytic activity to asymmetrically hydrolyze the racemic ester is provided. This method produces an optically active alcohol of general formula $R_1OSO_2R_3OH$ (where R_1 and R_3 have the same meaning as defined above) and an unreacted ester of the general formula $R_1OSO_2R_3OCOR_2$ (where R_1, R_2, and R_3 have the same meanings as defined above). The optically active compounds are separated from each other by hydrolyzing the ester to give an optically active glycol derivative which is antipodal to the above alcohol and isolating the same optically active glycol derivative. The invention provides a method for producing optically active glycol

derivatives, which is expedient, does not require costly reagents, and is suited to commercial scale production.

Sih C. J.
PROCESS FOR PREPARING L-CARNITINE AND CHEMICAL INTERMEDIATES EMPLOYED THEREIN
US 4,710,468, Dec. 1, 1987
Assignee: Sigma-Tau Industrie Pharmaceutiche Riunite S.p.A

A process for preparing L-carnitine that comprises exposing γ-substituted acetoacetic acid esters or amides to the fermentative enzymatic action of a microorganism that contains L-β-hydroxyacyl CoA dehydrogenase (EC 1.1.1.35), recovering the resulting, optically active corresponding γ-substituted-β-hydroxybutyric acid derivative and converting said derivative to L-carnitine. An improvement in the process is also disclosed that comprises reacting a 4-chloro-3(R)-hydroxybutyrate with sodium iodide or bromide to produce the corresponding 4-iodo- or 4-bromo-3(R)-hydroxybutyrate, converting the 4-iodo- or 4-bromo-3(R)-hydroxybutyrate to the trimethylamino-3(R)-hydroxybutyrate salt, then converting the trimethylamino-3(R)-hydroxybutyrate salt into L-carnitine. Novel chemical intermediates prepared in the processes are also disclosed.

Sih C. J.
PROCESS FOR PREPARING A COMPOUND FOR USE IN THE PRODUCTION OF L-CARNITINE
US 4,642,290, Feb. 10, 1987
Assignee: Sigma-Tau Industrie Pharmaceutiche Riunite S.p.A

A process for preparing L-carnitine that comprises exposing γ-substituted acetoacetic acid esters or amides to the fermentative enzymatic action of a microorganism that contains oxidoreductase enzymes, recovering the resulting, optically active, corresponding γ-substituted-β-hydroxybutyric acid derivative and converting said derivative to L-carnitine.

Grabley, S.
PROCESS FOR THE RACEMIZATION OF OPTICALLY ACTIVE AMINO ACIDS
US 4,638,086, Jan. 20, 1987
Assignee: Hoechst Aktiengesellschaft

The racemization of optically active aminoacids (including their *N*-acyl derivatives) by heating with carboxylic acids takes place particularly readily with carboxylic acids of low volatility. Only catalytic quantities of acid are needed; it is advantageous to use equimolar quantities or an excess, which serves as a diluent. A reaction mixture from an enzymatic racemate reso-

lution can be heated directly for the purpose of racemization, after separation of the L-amino acid and the water.

Empie, M. W.
RESOLUTION OF RACEMATES OF AMINO ACIDS
US 4,636,470, Jan. 13, 1987
Assignee: Stauffer Chemical Company

Racemates of optically active amino acids substituted at the carbonyl position where the α amino acid nitrogen is underivatized can be resolved using a two-phase solvent system. The racemate is dissolved in a substantially water immiscible organic material that is a solvent for said amino acid racemate but not for the corresponding amino acids. The racemate is also dissolved in water (aqueous phase) and is in equilibrium with the racemate in the organic phase. One of the optical isomers of the amino acid racemate in the aqueous phase is enzymatically hydrolyzed to the corresponding amino acid and is recovered. The preferred product is L-phenylalanine.

Sakimae, A., Kagawa, Y., Numazawa, R., and Onishi, H.
PROCESS FOR PREPARING OPTICALLY ACTIVE CARBOXYLIC ACIDS AND ANTIPODE ESTERS THEREOF
US 4,629,701, Dec. 16, 1986
Assignee: Mitsubishi Rayon Company, Ltd.

Organic carboxylic acid ester represented by the formula $R_1COS(CH_2)_nR_2$-$CHCOOR_3$ where R_1 is an alkyl, an aralkyl or an aryl group; R_2 is an alkyl group; R_3 is an alkyl group; n is 1 or 2, are synthesized in racemic form. The acid ester is then treated with a source containing enzyme capable of rearranging with or without asymmetrically hydrolyzing the ester bond until either a d- or 1-optically active carboxylic acid ester with or without the antipode acid is produced.

Hamaguchi, S., Yamamura, H., Hasegawa, J., Kawaharada, H., and Watanabe, K.
PROCESS FOR PRODUCTION OF OPTICALLY ACTIVE OXAZOLIDINONE DERIVATIVE
US 4,588,694, May 13, 1986
Assignee: Kanegafuchi Kaku Kogyo Kabushini Kaisha

A process for preparing an optically active oxazolidinone derivative by utilizing microorganisms or enzymes having a stereoselective esterase activity capable of asymmetrically hydrolyzing the racemates of the acyloxy-oxazolidinone derivative is described. The compounds are useful as intermediates for preparing optically active β-adrenergic blocking agents.

Sih, C. J.
PROCESS FOR PREPARING OPTICALLY ACTIVE 4-AMINO-3-HYDROXYBUTYRIC ACID
US 4,584,270, Apr. 22, 1986
Assignee: Wisconsin Alumni Research Foundation

The present invention relates to processes for preparing optically-active 4-amino-3-hydroxybutyric acid by asymmetrically cleaving one of the enantiotopic ester groupings of 3-hydroxyglutaric diester by the action of microbial enzymes to obtain a chiral monoacid that is readily converted into optically-active 4-amino-3-hydroxybutyric acid by chemical means.

Bewick, D. W.
PROCESS FOR PRODUCING OPTICALLY ACTIVE ARYLOXYPROPIONIC ACIDS AND DERIVATIVES THEREOF
US 4,568,641, Feb. 4, 1986
Assignee: Imperial Chemical Industries PLC.

A process for the stereospecific inversion of the (S) enantiomer of an alpha-aryloxypropionic acid of formula GUVOCH$_3$CHR, where G is OR$_1$ or R$_2$NH, R$_1$ is hydrogen or a protecting group, and R$_2$ is hydrogen or methyl, U and V each independently represent hydrogen or halogen, and R is a carboxyl group, or an enzymic equivalent thereof. The process comprises contacting the (S) enantiomer with a microorganism having a stereospecific inverting enzyme system, or with an extract of the microorganism contacting such an enzyme system, to convert the (S) enantiomer to the corresponding (R) enantiomer.

Bewick, D. W.
PROCESS FOR PRODUCING OPTICALLY ACTIVE ARYLOXYPROPIONIC ACIDS AND DERIVATIVES THEREOF USEFUL AS HERBICIDES
US 4,565,782, Jan. 21, 1986
Assignee: Imperial Chemical Industries PLC.

A process for the stereospecific inversion of the (S) enantiomer of an alpha-aryloxypropionic acid of formula EUVOCH$_3$CHR, where E is OR$_1$ or R$_2$NH, R$_1$ is an unsubstituted or substituted aryl or heterocyclic ring system, and R$_2$ is hydrogen or methyl, U and V each independently represent hydrogen or halogen and R is a carboxyl group, or an enzymic and herbicidal equivalent thereof. The process comprises contacting the (S) enantiomer with a microorganism having a stereospecific inverting enzyme system, or with an extract of the microorganism containing said enzyme system, to convert the (S) enantiomer to the corresponding (R) enantiomer.

LITERATURE

This section surveys the literature in the area of stereo- and regioselective biotransformations from the period of January, 1987 to December, 1988. The major headings and cross-terms are the same as listed in the patent search. This section is not intended to be all encompassing and lists both review articles and research publications that highlight the varied nature of research in this field during the specified time period.

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