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Abstracts

Selected abstracts from the 7th Japanese Symposium on the Chemistry of Biocatalysis

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Introduction

The symposium on the chemistry of biocatalysis was held on 11 and 12 December 2002 in Hokkaido. Approximately 98 researchers from industry and academic world and 51 students participated to the symposium. We had the following program:

Special lecture: Professor S. Kinoshita (Graduate School of Engineering, Hokkaido University, Japan); "Biological degradation of synthetic polymers," Professor H. Sekizaki (Faculty of Pharmaceutical Science, Health Sciences University of Hokkaido, Japan); "Food waste utilization on drug preparations—biocatalysts investigation in food and industrial waste."

Oral and poster presentation (9 presentations and 49 posters): We enjoyed oral and poster presentations as well as discussions.

Business Booth: Booth-1, Daicel Chemical Industries, Ltd.; Booth-2, Nagase & Co., Ltd.; Booth-3, JASCO Corporation.

Symposium organizer: Harumi Kaga

We thank assistant professor K. Ishihara (Okayama University of Science) for his help in summarizing this abstracts.

Yasuhisa Asano, Editor

Oral Presentations

Lipase-catalyzed reactions at extreme temperatures from −40 to 120 °C

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Burkholderia cepacia lipase immobilized on porous ceramic particles (lipase PS-C II, Amano Enzyme) gave enantiopure ester of 1,1-diphenyl-2-ethanol at high temperatures up to 120 °C, and the same enzyme also showed catalytic activity at −40 °C for a primary alcohol (Fig. 1).

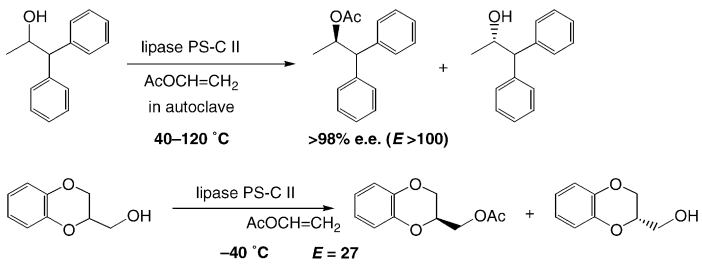

Fig. 1. Lipase-catalyzed reactions at extreme temperatures.

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Biocatalytic reactions in supercritical carbon dioxide

Tomoko Matsuda[a,*], Kazunori Watanabe[a], Ryuzo Kanamaru[a], Tadao Harada[a], Kaoru Nakamura[b]
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Asymmetric reduction of ketones using an alcohol dehydrogenase, kinetic resolution of an alcohol using a lipase, and carboxylation of pyrrole using a decarboxylase in supercritical carbon dioxide were investigated (Fig. 2).

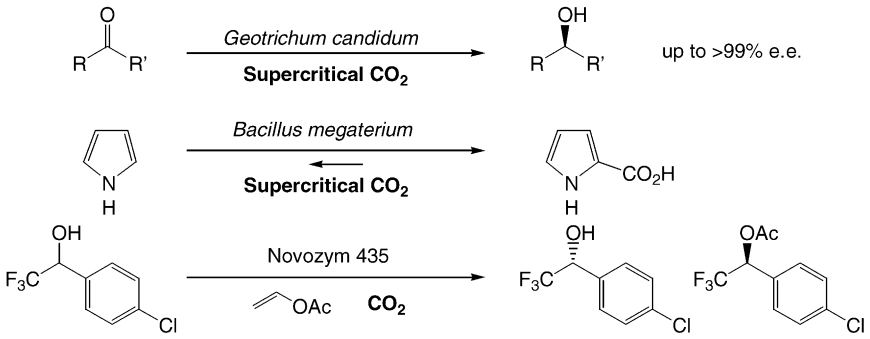

Fig. 2. Biocatalytic reactions in supercritical carbon dioxide.

Poster Presentations

Ability of different biomaterials to enantioselectively catalyze oxidation and reduction reactions

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The pH of reaction medium can determine the direction of $NAD(P)^+$ dependent secondary alcohol dehydrogenase (NAD(P)-E) from immobilized water-soluble biomaterials (e.g. plant leaf, cereal tissues, and vegetables), toward enantioselectively catalyzed oxidation (pH > 7.0) or reduction reaction (pH < 7.0) (Fig. 3).

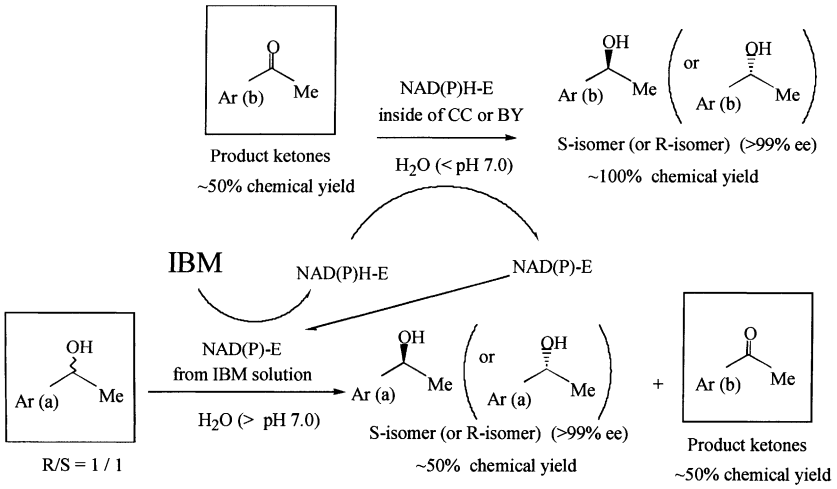

Fig. 3. Enantioselectively catalyzed oxidation or reduction reactions using NAD(P)-E eluted (pH > 7.0) or intracellular NAD(P)-E (pH < 7.0) from different biomaterials.

Production of N^{α}-*Z*-aminoadipate-*δ*-semialdehyde from N^{α}-*Z*-lysine with amine oxidase from *Aspergillus niger*

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More than 95% of N^{α}-*Z*-L-lysine and N^{α}-*Z*-D-lysine were oxidized by amine oxidase from *Aspergillus niger* to N^{α}-*Z*-L-aminoadipate-*δ*-semialdehyde (N^{α}-*Z*-L-AASA) and N^{α}-*Z*-D-AASA, respectively, in the presence of catalase (Fig. 4).

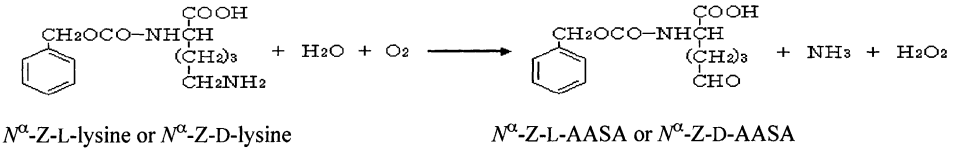

Fig. 4. Oxidation of N^{α}-*Z*-L-lysine and N^{α}-*Z*-D-lysine by amine oxidase from *Aspergillus niger*.

Novel serine protease from earthworm: catalytic functions and application (part IV)

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Novel serine proteases, purified from earthworm, were characterized for application of the catalytic functions. The enzymes, composed of six-isozymes, acted on various proteins, peptides, ester compounds, and so on. They were very stable and strongly resistant to organic solvents and detergents (Fig. 5).

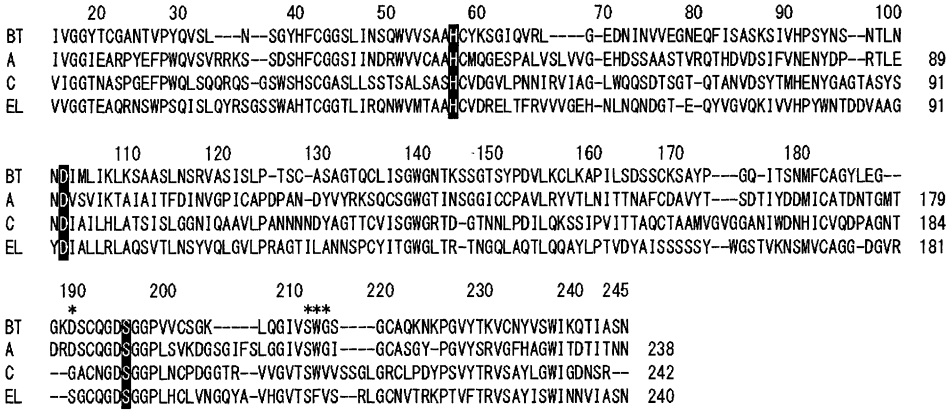

Fig. 5. Sequence alignment of the earthworm proteases, isozyme A (A), isozyme C (C), bovine trypsin (BT), and porcine elastase (EL). The numberings shown above and across the sequence are based on those of the chymotrypsinogen A and the active earthworm proteases, respectively. The amino acid residues of the catalytic triad are represented by reversal letters. The primary substrate specificity determinant and the subsites, S1, S2, and S3 are indicated by asterisks.

Enzymatic conversion of bioactive compounds (part V): stabilization and functionarization of naturally occurring plant pigments

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The regioselective acylation of flavonoid glucosides was achieved by lipase-catalyzed transesterification in dry organic solvent. The participation of the acyl group in flavonoid glucoside molecules resulted in increasing of the physiological function (thermostability and light-resistibility) of the acylated flavonoid glucosides (Fig. 6).

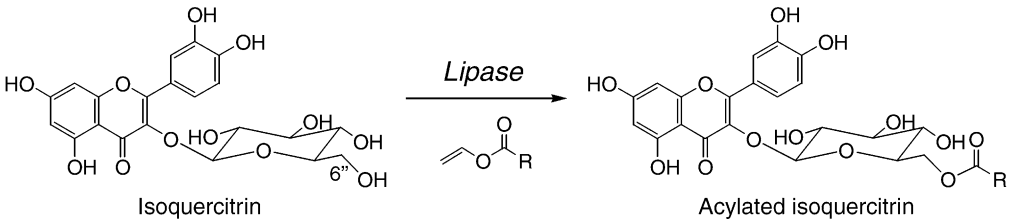

Fig. 6. Lipase-catalyzed esterification of flavonoid glucoside.

Stability of catalytic antibodies immobilized on mesoporous silicates

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Catalytic antibodies (38C2 and 84G3) were immobilized on various mesoporous silicates (FMS, PESO, SBA). These silicates had similar adsorption ratios to two antibodies (Fig. 7).

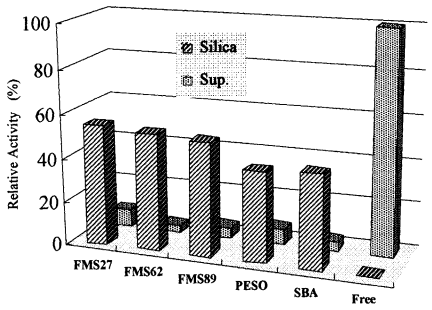

Fig. 7. Immobilization ratios of catalytic antibody 84G3 on mesoporous silicates.

Biocatalytic reduction of ketones by a semi-continuous flow process using supercritical carbon dioxide

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The immobilized resting-cell of *Geotrichum candidum* was used as a catalyst for the reduction of a ketone in a semicontinuous flow process using supercritical carbon dioxide for the first time; it was also applied for the asymmetric reduction of a ketone and resulted in excellent enantioselectivity (>99% e.e.) and a higher space-time yield than that of the corresponding batch process (Fig. 8).

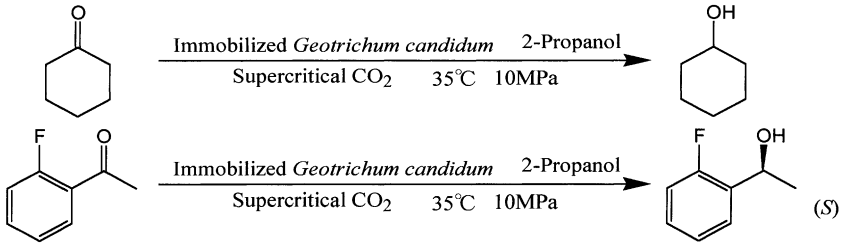

Fig. 8. Biocatalytic reduction of ketones by a semi-continuous flow process using supercritical CO_2.

Lipase-catalyzed reaction in an ionic liquid solvent system

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1-Butyl-2,3-dimethylimidazolium tetrafluoroborate ([bdmim]BF_4) was found to be an excellent solvent to realize a lipase-recycling system using vinyl acetate as acyl donor; no accumulation of an acetaldehyde oligomer was observed in this solvent system and it was possible to use the lipase repeatedly ten times while still maintaining perfect enantioselectivity and high reactivity (Fig. 9).

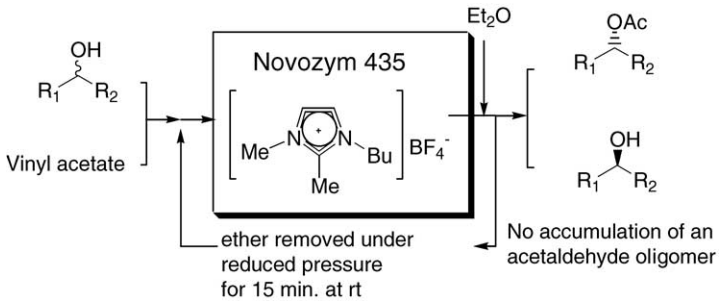

Fig. 9. Perfect recycled use of lipase in an ionic liquid solvent system.

Asymmetric hydrogenation of *N*-substituted maleimides by the cultured plant cells

Asuka Takarada[a], Yuya Sato[a], Toshifumi Hirata[a,*], Akihito Matsushima[b], Yoko Kondo[c], Hiroki Hamada[c]

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Hydrogenation of 2-methyl-*N*-phenylmaleimide by the cultured cells of *Nicotiana tabacum* and *Marchantia polymorpha* was highly enantiospecific to give (*R*)-2-methyl-*N*-phenyl succinimide (Fig. 10).

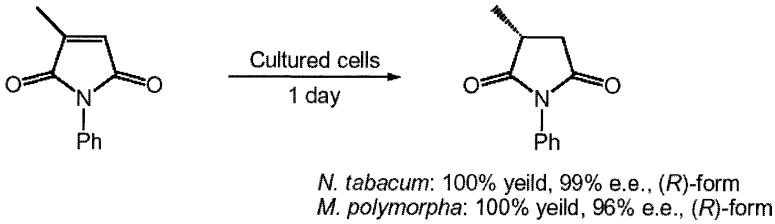

Fig. 10. Hydrogenation of 2-methyl-*N*-phenylmaleimide by the cultured plant cells.

Construction of a functionalized oligosaccharide library by using a transition state analogue as a glycosyl donor

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An efficient enzymatic synthesis of functionalized oligosaccharides (oligo-*N*-acetyl lactosamides) has been achieved by means of chitinase-catalyzed transglycosylation utilizing sugar oxazolines, a transition state analogue for chitinase (Fig. 11).

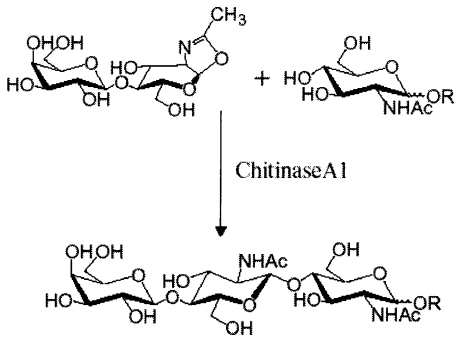

Fig. 11. Transglycosylation catalyzed by chitinase A1.

A correlation between conformational flexibility of enzymes and steric effects of substrates for subtilisin-catalyzed transesterification

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A correlation between optimum conformational flexibility of enzymes induced by the addition of dimethyl sulfoxide and Es parameter (the scale of steric effects of substrates) was found for subtilisin-catalyzed transesterification of the racemic esters in *i*-octane (Fig. 12).

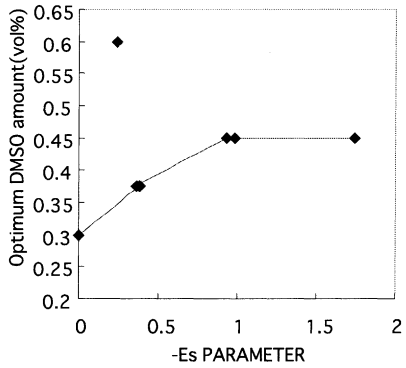

Fig. 12. A correlation between conformational flexibility of enzymes and Es parameter of substrates.

A remarkable improvement of enantioselectivity of lipase in organic solvents by use of lipase coated with phosphates

Hiromi Yumoto[a], Naomi Hiroshima[a], Shuichi Mori[b], Shin-ichi Ueji[a,b,*]
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The enantioselectivity of lipase-catalyzed esterification of 2-(4-ethylphenoxy) propionic acid was found to be dramatically enhanced by using lipase coated with phosphates, which was prepared by lyophilizing lipase in the presence of phosphates (Table 1).

Table 1
Enhancement of the enantioselectivity by using lipase coated with phosphates in diisopropyl ether

Enzyme	Time (h)	Conversion (%)	e.e. (%)	*E* value
Native lipase VII	29	37.6	84.8	20
LipaseVII coated with sodium phophate dibasic	24	38.4	99.2	449
LipaseVII coated with sodium tripolyphophate	24	37.5	97.9	170
LipaseVII coated with DNA sodium salt	117	37.4	91.6	40

Effect of metal cations on the enantioselectivity of crown ether modified lipase in organic media

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For the esterification of 2-(4-substituted phenoxy) propionic acids with 1-butanol catalyzed by the 18-crown-6 ether modified lipase in diisopropyl ether, its enantioselectivity was found to be most significantly enhanced by addition of KCl among the other alkali metal ions used here (Fig. 13).

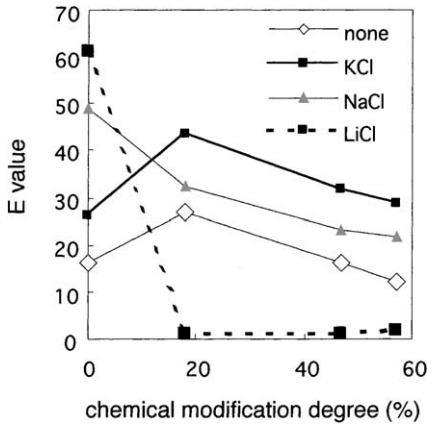

Fig. 13. The variation of the enantioselectivity of the crown ether modified lipases with various modification degrees by addition of the metal ions.

A new method to improve the functions of lipase, based on the combination of chemical modification and refolding of lipase

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A new method was tried to improve the functions of lipase, based on the combined effects of the chemical modification and the refolding of lipase, thus leading to the enhancement of the enantioselectivity of the lipase-catalyzed hydrolysis of butyl 2-(4-substituted phenoxy) propionates, the enantioselectivity enhancement of which can be attributed to the conformational change of the lipase estimated from the result of the CD and FT-IR spectra (Fig. 14).

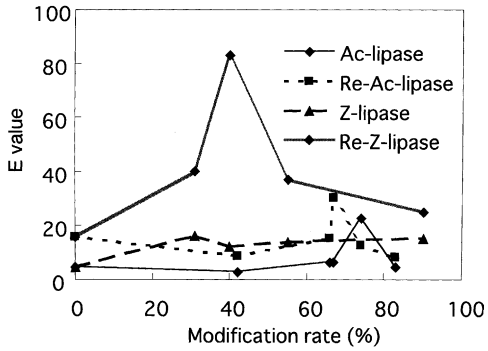

Fig. 14. The effects of chemical modification and refolding of lipase on the enantioselectivity of lipase-catalyzed hydrolysis of butyl 2-(4-ethylphenoxy) propionate. Ac-, Z-, Re-lipases indicate acetylated, benzyloxycarbonylated, and refolded lipase, respectively.

Microbial deracemization of α-substituted carboxylic acids—optimization of reaction conditions and mechanistic investigations using cell-free extract

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Through the investigation of whole cell system and cell-free system including the study of inhibitors and the detection of intermediates, we could obtain the supporting evidences that deracemization is a competitive reaction against the fatty acid metabolism and proceeds via a part of β-oxidation pathway (Fig. 15).

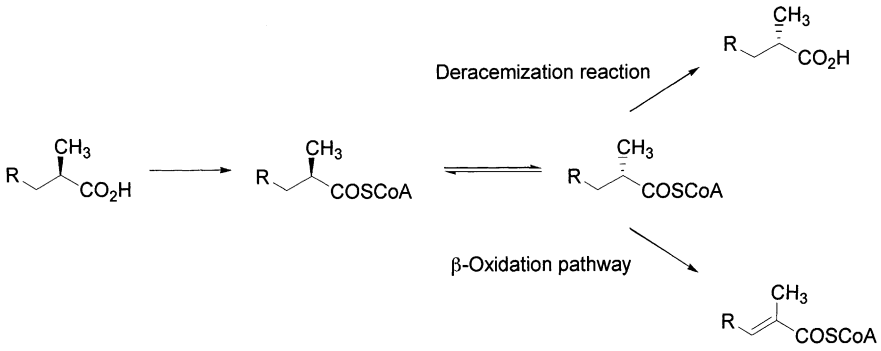

Fig. 15. A crossroads of the deracemization and β-oxidation pathway.

Enzymatic decarboxylation of phenylmalonates with a hydrophilic α-substituent

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Arylmalonate decarboxylase (AMDase) catalyzed asymmetric decarboxylation of α-phenylmalonates with a hydrophilic substituent (OH, NH_2) at α-position, and these substrates exhibited characteristic "pH-rate of reaction" profiles compared to that of phenylmalonate (Table 2).

Table 2
AMDase-catalyzed reaction profiles of phenylmalonates with an α-substituent (=R)

R	HPLC yield (%)	Isolated yield (%)	e.e. (%)	Configuration	Optimum pH	K_m (mM)	k_{cat} (s^{-1})	Relative activity (%)
H	–	99	–	–	8.5	13.9	353	100
Me	–	99	99	R	NT	25.5	29.8	4.6
OH	98	92	96	R	7.0	1.88	2.45	5.1
NH_2	97	71	96	R	9.5	31.2	1.73	0.22

Production of chiral alcohols by enantioselective reduction with phenyl trifluoromethyl ketone reductase (PTKR) from Leifsonia sp. S749

Kousuke Inoue*, Yoshihide Makino, Nobuya Itoh

Biotechnology Research Center, Faculty of Engineering, Toyama Prefectural University, Kurokawa 5180, Kosugi, Toyama 939-0398, Japan. E-mail: itoh@pu-toyama.ac.jp

Chiral alcohols were synthesized with high enantioselectivity and molar yield by using *E. coli* cells efficiently expressing the *ptkr* gene from *Leifsonia* sp. S749, and the reaction proceeded without the NADH-regeneration system because PTKR could reproduce NADH in the presence of 2-propanol (Fig. 16).

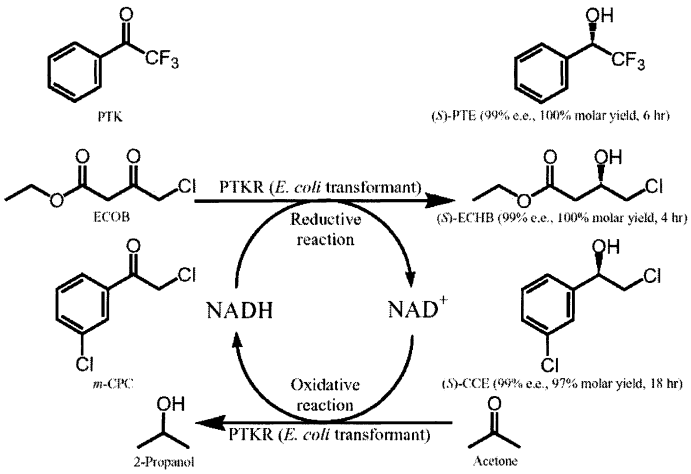

Fig. 16. Synthesis of chiral alcohols by the recombinant PTKR system.

Analysis of polymerization and phosphorolysis of α-glucans by glycogen phosphorylases using quartz crystal microbalances

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We kinetically analyzed the reaction catalyzed by glycogen phosphorylases (GP), that is, the binding of GP to the substrates such as amylopectins and the resulting both polymerization and/or phosphorolysis, using quartz crystal microbalances (Fig. 17).

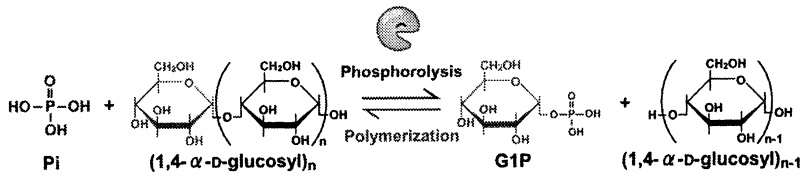

Fig. 17. Reaction scheme of glycogen phosphorylase.

Asymmetric synthesis of 2-substituted 4-chromanones: synthesis of chiral intermediates by lipase-catalyzed reactions

Masashi Kawasaki[a,*], Yuka Asano[a], Kanako Katayama[a], Akira Tanaka[a], Hiroko Kakuda[b], Michimasa Goto[c], Tadashi Kometani[c]
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[b]Toyama Medical and Pharmaceutical University, 2630 Sugitani, Toyama 930-0194, Japan
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(*R*)- and (*S*)-2,6-dimethyl-4-chromanone were prepared from the chiral intermediates which were obtained by lipase-catalyzed reactions (Fig. 18).

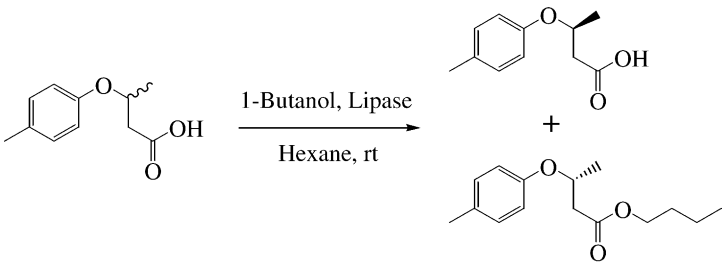

Fig. 18. Kinetic resolution of 3-(4-methylphenoxy)butanoic acid.

Degradation of bisphenol A by plants and cyanobacteria

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[b]Institute for Chemical Research, Kyoto University, Uji, Kyoto 611-0011, Japan
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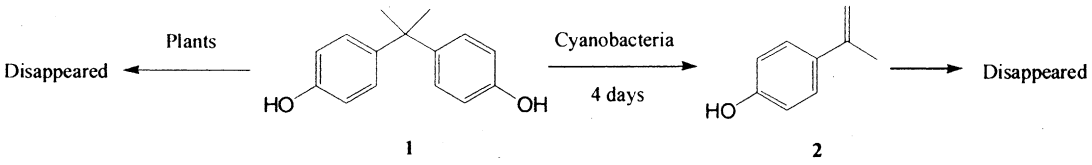
The degradation of 2,2-bis(4-hydroxyphenol)propane (BPA, **1**), a representative endocrine disruptor, was studied with cyanobacteria and plants as a biocatalyst (Table 3).

Table 3
Screening of biocatalysts on the degradation of BPA



Biocatalysts		Time (days)	Bisphenol A	
			Concentration (ppm)	Degradation (%)
Cyanobacteria	*Synechococcus elongatus*	7	10	91
Cyanobacteria	*Synechosystis* sp.	7	10	90
Plants	*Alpinia zerumbet*	14	10	>99
Plants	*Canavaria gladiata*	14	40	>99
Plants	*Rhizophora slylosa*	14	10	31

The degradation of di(2-ethylhexyl) phthalate by cultured plant cells and cyanobacterium

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The di(2-ethylhexyl) phthalate (DEHP) disappeared in the solution after several days of incubation, but a few amount of DEHP remained in the cells of *Caragana chamlagu* and cyanobacterium (Fig. 19).

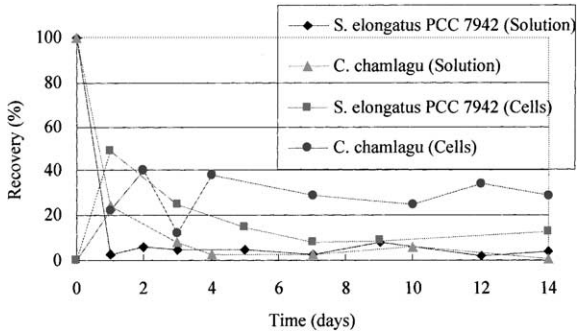

Fig. 19. Degradation of DEHP by *C. chamlagu* and *Synechococcus elongatus* PCC 7942.

Biotransformation of diketones by plant cultured-cells (part 4)

Wen Chai[a], Yoshitomo Matsuura[a], Hiroshi Sakamaki[b], C. Akira Horiuchi[a,*]
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The oxidation of hydroxythymobenzoquinones using some kinds of plant cultured-cells was investigated, and it was found that the biotransformation of 3-hydroxythymo-1,4-benzoquinone (**1**) by *M. polymorpha* gave the γ-hydroxybutenolide (**1a**, 60%) as major products (Fig. 20).

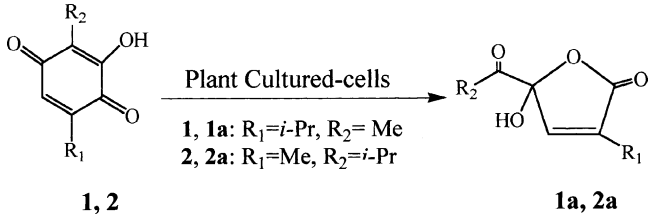

Fig. 20. Biotransformation of hydroxythymobenzoquinones by plant cultured-cells.

Stereoselective oxidation of the sulfur compounds by rat liver S-9 fraction

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The stereoselective oxidation of sulfide (**1**) and β-hydroxy sulfur compounds (**3** and **5**) by rat liver S-9 fraction were investigated (Fig. 21).

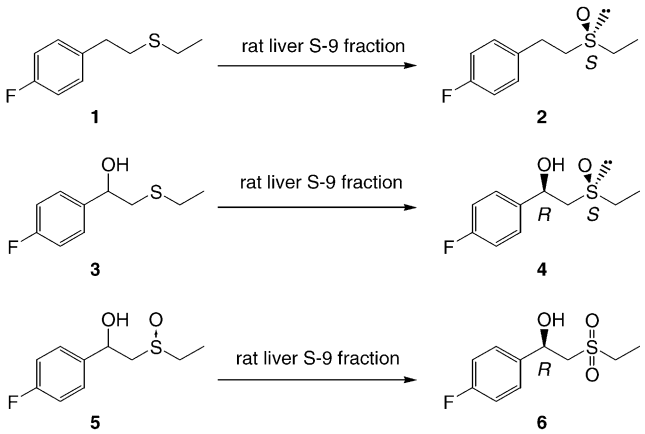

Fig. 21. Enantioselective oxidation of sulfur compounds by rat liver S-9 fraction. Each step was started with racemic material.

Syntheses of biologically active substances such as insect pheromones by use of a FPP synthase

Nagaki Masahiko[a,*], Nakada Minori[a], Maki Yuji[b], Nishino Tokuzo[c], Koyama Tanetoshi[d]
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[b]Department of Materials and Biol. Chem., Yamagata University, Japan
[c]Department of Biochemistry and Engineering, Tohoku University, Japan
[d]IMRAM, Tohoku University, Japan. E-mail: nagaki@cc.hirosaki-u.ac.jp

In order to develop the synthetic methods of such kinds of isoprenoid pheromones, we examined substrate specificity of FPP synthase from *Bacillus stearothermophilus* with respect to allylic substrate homologs with a hydrophilic group at ω-position (Fig. 22).

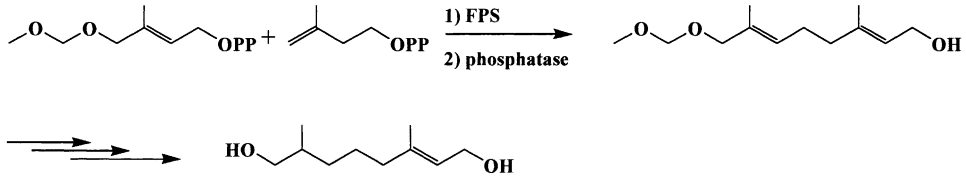

Fig. 22. A thermostable FPP synthase reaction and *Danaus chrysippus* sex insect pheromone.

***Pseudomonas cepacia* lipase (PCL)-catalyzed hydrolysis of acetates of single enantiomers of secondary alcohols: origin of high enantioselectivity in rate-determining step**

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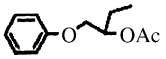
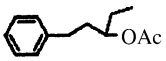
Thermodynamic analysis of PCL-catalyzed hydrolysis of acetates of single enantiomers of secondary alcohols indicated a substrate having high *E* value showed no enthalpy–entropy compensation in the transition state (Table 4).

Table 4
Thermodynamic parameters for *E* value of PCL-catalyzed hydrolysis

	$\Delta\Delta H_{R-S}$	$T\Delta\Delta S_{R-S}$	$\Delta\Delta G_{R-S}$	*E* value
	−4.9	0.4	−5.2	High
	−6.5	−4.1	−2.4	Low

***Pseudomonas cepacia* lipase (PCL)-catalyzed hydrolysis of acetates of single enantiomers of primary alcohols: enantioselectivity of primary alcohols in the acylation step**

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From the kinetics and thermodynamics of the acylation step of *Pseudomonas cepacia* lipase (PCL)-catalyzed hydrolysis of acetates of single enantiomers of primary alcohols, we discussed the difference of the mechanism of the enantioselectivity between the primary and the secondary alcohols (Fig. 23).

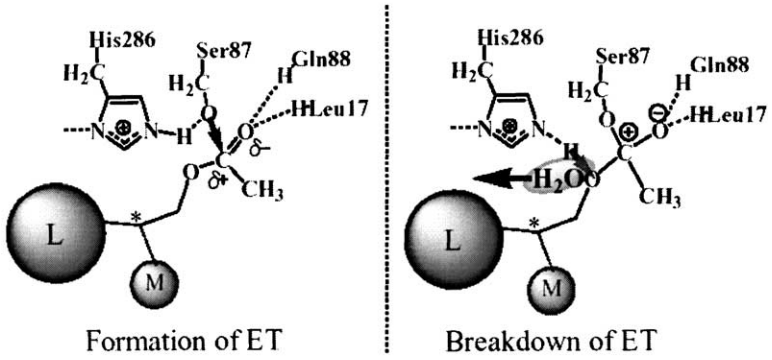

Fig. 23. Transition state of acylation step.

Mechanism of *Candida antarctica* lipase B (CALB)-catalyzed hydrolysis of acetates of primary alcohols: change of the rate-determining step by substituents

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We have examined kinetics and thermodynamics of *Candida antarctica* lipase B (CALB)-catalyzed hydrolysis of the acetates of single enantiomers of primary alcohols to discuss the mechanism of action of the enzyme (Fig. 24).

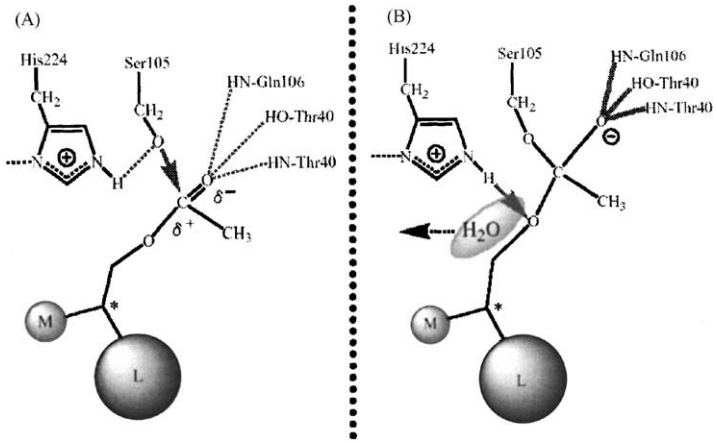

Fig. 24. (A) Formation of ET. (B) Breakdown of ET.

Fungal chitinases coproduced with insect chitinase inhibitors

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A fungal strain TNPT116-Cz producing a chitinase inhibitor FPS-1 was found to express chitinase simultaneously, which could not be inhibited by FPS-1 (Fig. 25).

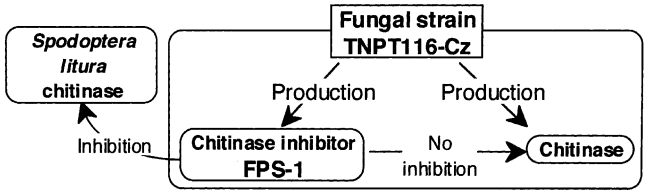

Fig. 25. The relationship between FPS-1 and chitinase coproduced by TNPT116-Cz.

Characterization of cyclo (Leu–Phe) dehydrogenase from an actinomycete *Streptomyces albulus* KO23

Atsushi Morimoto, Chikahiro Takeda, Banri Ikeda, Teruhiko Nitoda, Hiroshi Kanzaki*
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Purified cyclo (Leu–Phe) dehydrogenase from *Streptomyces albulus* KO23 was characterized in detail, and was found to catalyze all reactions involved in the conversion of cyclo (Leu–Phe) to albonoursin (Fig. 26).

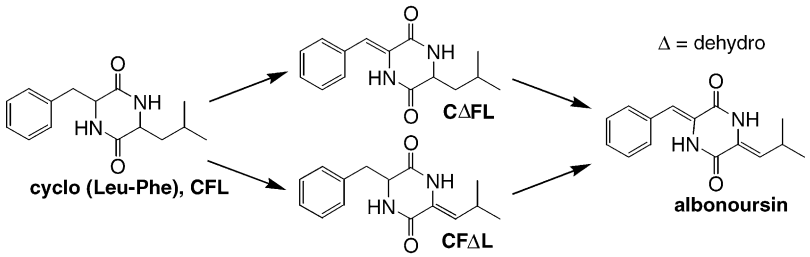

Fig. 26. Dehydrogenation by *Streptomyces albulus* KO23.

Lipase-catalyzed domino dynamic-kinetic-resolution of racemic alcohols/intramolecular Diels–Alder reaction

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A combined use of a lipase and a ruthenium catalyst (**2a** or **b**) achieved the first domino process that involved the dynamic kinetic resolution of (±)−**1** with **3** and the intramolecular Diels–Alder reaction of the resultant **4** to directly provide polysubstituted decalines **5** with up to 95% e.e. in 81% isolated yield (Fig. 27).

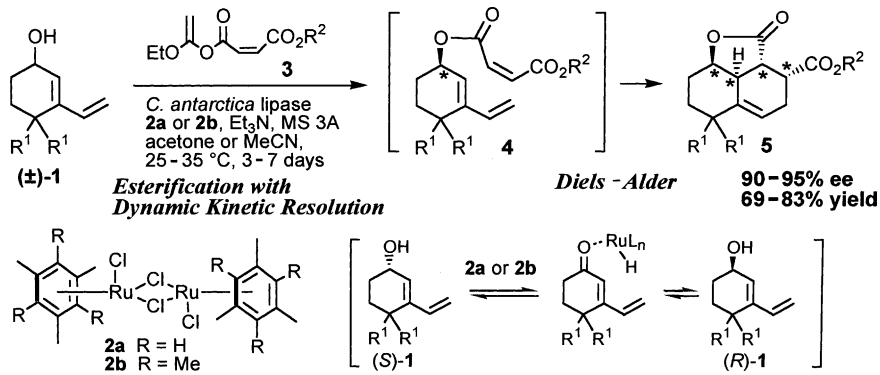

Fig. 27. Lipase-catalyzed domino dynamic-kinetic-resolution of racemic alcohols/intramolecular Diels–Alder reaction.

Enzymatic hydrolysis of cyclic carbonates bearing a methyl group

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Pseudomonas diminuta efficiently catalyzed the hydrolysis of the cyclic carbonates bearing a methyl group to obtain optically active diols (Fig. 28).

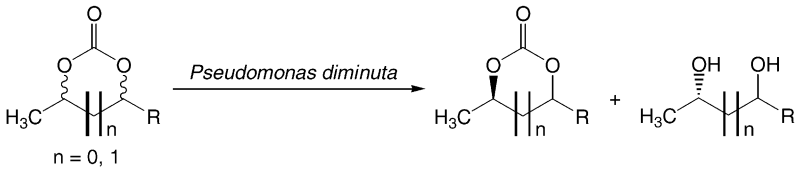

Fig. 28. Enantioselective hydrolysis of cyclic carbonates by a bacterium.

Phytoremediation of endocrine disturbing of plant cultured cells—biotransformation of BPA and BZP

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We have investigated the biotransformation of organic compound by plant suspension cells. In this study we study the biotransformation of bisphenol A and benzophenone by plant suspension cells and it was found that plant suspension cells glycosylate the hydroxyl group of bisphenol A and benzophenone (Fig. 29).

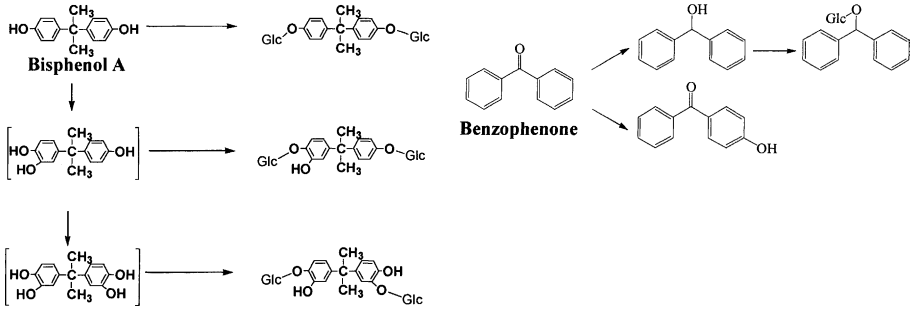

Fig. 29. Biotransformation of BPA and BZP by the cultured cells of *Eucalyptus perriniana*.

Development of functional foods by plant cultured cells: biotransformation of vanillin

Jun-ichi Takimura, Sumiko Tanaka, Tsutomu Furuya, Hiroki Hamada*
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We have investigated the biotransformation of organic compound by plant cultured cells. In this study we study the biotransformation of vanillin by plant cultured cells. In the paper we report that the plant cultured cells have glycosylation and hydroxylation ability in the biotransformation of vanillin (Fig. 30).

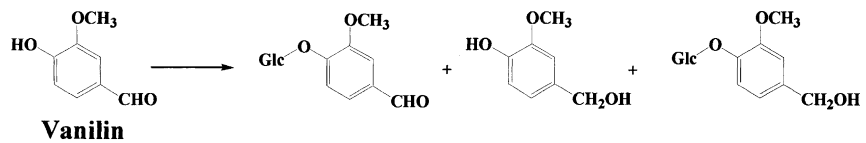

Fig. 30. Biotransformation of vanillin by *Eucalyptus perriniana*.

Development of functional foods by cultured cells of Catharanthus roseus: biotransformation of curcumin

Takafumi Hara, Mori Shuhei, Tsutomu Furuya, Hiroki Hamada*

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We have investigated the biotransformation of organic compound by plant cultured cells. In this study we study the biotransformation of curcumin by the cultured cells of *C. roseus* and it was found that the cultured cells of *C. roseus* glycosylate the hydroxyl group of curcumin (Fig. 31).

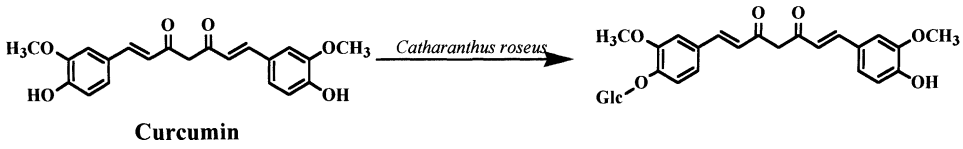

Fig. 31. Biotransformation of curcumin by *C. roseus*.

Simple preparation of optically pure trifluoromethylalkanol through lipase catalyzed reaction

Yumiko Takagi[a,*], Fumi Yamana[a], Toshiyuki Itoh[b]

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[b]Department of Materials Science, Faculty of Engineering, Tottori University, 4-101 Koyama-cho Minami, Tottori 680-8552, Japan. E-mail: ytakagi@ed.kagawa-u.ac.jp

We report the results of lipase-catalyzed hydrolysis reaction of diacetates of bis(trifluoromethyl)alkanediols and synthesis of novel liquid crystalmolecules which possesses chiral bis(trifluoromethyl)alkanol moieties and aromatic core structure at the center of the molecular flame (Fig. 32).

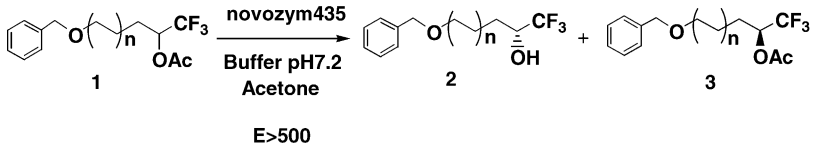

Fig. 32. Enzymatic hydrolysis of PEG-tagged carbonates.

Enzyme-mediated enantioselective hydrolysis of PEG-tagged carbonates

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PEG-tagged substrates, which had some unique properties, were hydrolyzed by PPL with enantioselectivity. The resulting optically active *sec*-alcohols were easily separated from the substrates (Fig. 33).

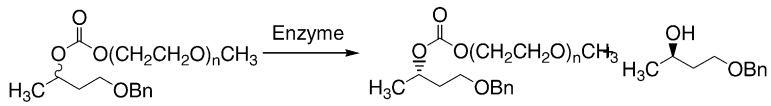

Fig. 33. Optical resolution of 1,1,1-trifluoromethyl-2-alkanol by CAL-catalyzed enantioselective hydrolysis.

A process for producing L-arabinose-containing syrup by treating the beet pulp and the effect on the elevation of blood glucose in rats

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L-Arabinose-containing-syrup could be economically produced by treating the beet pulp directly with an enzyme and the elevation of blood glucose after sucrose ingestion in rats was significantly suppressed by adding the syrup (Fig. 34).

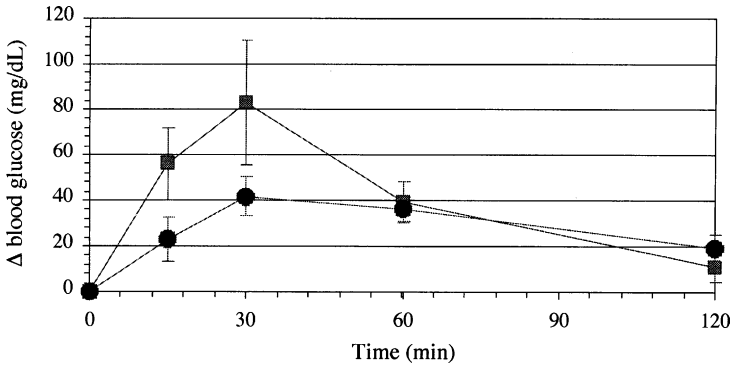

Fig. 34. Blood glucose after sucrose ingestion in rats. (■) Sucrose; (●) sucrose + L-arabinose-containing-syrup.