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LIPASE-CATALYZED ESTER FORMATION IN ORGANIC SOLVENTS

AN EASY PREPARATIVE RESOLUTION OF α-SUBSTITUTED CYCLOHEXANOLS

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Summary : The commercial preparation "lipase My" catalyzes ester formation from an alcohol and a fatty acid in organic solvents. Preparative resolution of α-substituted cyclohexanols, including menthol, was achieved under these conditions.

Resolution of esters by enzymatic enantioselective hydrolysis can be used for the preparation of optically active alcohols[2]. Lipases are a peculiar class of hydrolases, with triglycerides as natural substrates, and with a mode of action related to their interfacial properties in biphasic systems[3]. These enzymes are also known to catalyze ester formation[4].

In our laboratory, we have studied this esterification in organic media, and used the stereoselective potentialities of lipases[5] to resolve alcohols. The enzyme selected is obtained from the yeast Candida cylindracea and is available in large quantities as a crude product named "lipase My" (Meito Sangyo Co., Japan). We have found that this crude preparation catalyzes the synthesis of esters from alcohols and fatty acids in an apolar organic solvent. In this work, we describe the enantioselectivity of the enzyme towards α-substituted cyclohexanols. All the compounds tested have been resolved in a single step with very high optical purities.

"Lipase My" (5g) was added to a solution (100 ml) of the alcohol to be resolved (0.25 M) and of an acid such as lauric acid (0.25 M) in an apolar organic solvent such as hexane or heptane. The reaction was carried out usually at 40°C under continuous stirring. Ester formation was followed by G.L.C. analysis in order to obtain the desired degree of conversion (ca. 40 %).

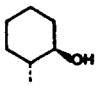
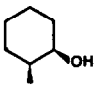
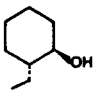
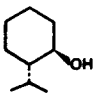
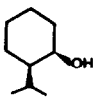
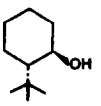
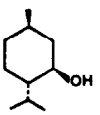
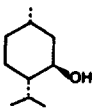
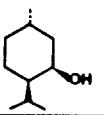
Alcohol	T°C (time)	% reaction	Configuration of the reacting enantiomer		Optical purity of the reacting alcohol	Optical purity of the remaining alcohol
(±) Trans 2-methyl cyclohexanol	40°C (5.5 h)	44 %	(-)		98 %[b]	80 %[b]
(±) Cis 2-methyl cyclohexanol	40°C (10.5 h)	52 %	(-)		80 %[b]	86 %[b]
(±) Trans 2-ethyl cyclohexanol	40° C (8 h)	46 %	(-)		96 %[b]	90 %[b]
(±) Trans 2-isopropyl cyclohexanol	35° C (11 h)	37 %	(-)		≃100 %[a]	68 %[a]
(±) Cis 2-isopropyl cyclohexanol	40° C (192 h)	30 %	(-)		88 %[a]	-
(±) Trans 2-tert-butyl cyclohexanol	40° C (95 h)	34 %	(-)		≃100 %[a]	-
(±) Menthol	45° C (8 h)	45 %	(-)		95 %[a]	-
	40° C (88 h)	57 %			69 %[a]	88 %[a]
(±) Isomenthol	40° C (17 h)	42 %	(-)		87 %[a]	70 %[a]
(±) Neomenthol	40° C (88 h)	34 %	(-)		96 %[a]	55 %[a]

Table 1 : Resolution of α-substituted cyclohexanols with lipase My[10].

a. determined by G.L.C. analysis after isopropyl isocyanate derivatization[6].

b. determined by G.L.C. analysis after derivatization with (+)-(R)-1-phenyl ethyl isocyanate (see text).

The enzyme was then filtered off, the solvent removed under vacuum and the crude products subjected to bulb-to-bulb distillation yielding the unreacted enantiomeric alcohol (ca. 50°C/0.1 mm Hg), lauric acid (100°C/0.1 mm Hg), and finally, the ester (150°C/0.1 mm Hg). Under these conditions, no significant esterification occured during the distillation. Hydrolysis of the ester gave the second enantiomeric alcohol.

This procedure has been used for the resolution of gram amounts of alcohols and was easily applied in scale-up resolution of (±) menthol (1 mole). The recovered enzymatic powder can be reused after being dried under vacuum and no significant loss of activity was observed after repeating the reaction twice under the preceeding conditions.

After reduction of the ester with lithium aluminium hydride and suitable derivatization, optical purities were determined by G.L.C. analysis on the recovered alcohols. In general the alcohols were analyzed as the isopropyl isocyanates on a Chirasil capillary column[6]. However in some cases, they were quantitatively derivatized with (+)-(R)-1-phenylethyl isocyanate and the diastereoisomeric urethanes analyzed on an SE52 capillary column.

The results of the resolution of α-substituted cyclohexanols are described in table 1. The absolute configuration of these compounds is well known[7]. Candida cylindracea lipase is specific to the R-alcohols. Furthermore, the α-trans compounds are esterified more rapidly than the α-cis ones. This resolution is based on a kinetic competition between the two enantiomers. Very good enantioselectivity has been observed for a 35-45 % conversion, and, as shown in table 1 for menthol, with a more significant degree of esterification (ca. 57 %) the unreacted enantiomer can be obtained with high optical purity.

The methodology described in this paper is very simple. The enantioselectivity of the enzyme is very high compared to the hydrolytic reaction catalyzed by the same lipase in emulsion[5b,8]. The mild experimental conditions and the low cost of the enzyme allows the preparation of gram amounts or more, of optically quasi-pure alcohols. Furthermore, the catalyst is easily recovered and can be reused. Conceptually, this reaction is similar to transesterification catalyzed by lipases[9].

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