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Novozyme 435 Asymmetric Hydrolysis of Enol Ester with Series Acid Moiety

QUAN LI, WEIMIN JIA*, ZHIJIAN WANG and XIAODAN GUO

School of Perfume and Aroma Technology, Shanghai Institute of Technology, Shanghai, P.R. China

*Corresponding author: Tel: +86 13817705124; E-mail: sying5@126.com

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(R)-2-pentylcyclopentanone can be synthesized by the asymmetric hydrolysis of enol esters, catalyzed by immobilized candida antarctica (novozyme 435) lipase. Different acid moieties influence the stereoselectivity of lipase. Enol esters can be prepared from anhydrides and 2-pentylcyclopentanone. When introducing optical (S)-(+)-2-methyl-butyric acid, the hydrolysis of optical enol ester showed great enhancement of the specific rotation compared to the racemic enol ester, the specific rotation of product raise from $[\alpha]^{25}_{D}$-10° (c 0.1, CH_3OH) to $[\alpha]^{25}_{D}$-72° (c 0.1, CH_3OH). However, when bringing chiral acid moity, the specific rotation still can not catch up with the value compared to the isobutyric moiety. The specific rotation of (R)-2-pentylcyclopentanone is $[\alpha]^{25}_{D}$-102.20° (c 0.1, CH_3OH), the optimum temperature and pH were 30 °C and 6.5, respectively. Then 81.06 % ee of (R)-δ-decalactone was prepared by the Baeyer-Villiger oxidation of (R)-2-pentylcyclopentanone.

Keywords: Asymmetric hydrolysis, Specific rotation, Enol ester, (R)-2-Pentylcyclopentanone, Chiral acid moiety.

INTRODUCTION

Recently, the research of optically active aroma chemicals has been reported[1-3]. Stereoisomers show different physiological activity[4] as the receptor protein can discriminate two enantiomers (R) and (S) of a chiral odorant molecule, therefore optically active aroma chemicals show a powerful chiral odorant whereas the antipode is weak odorless[5]. The perfume δ-decalactone is found naturally in coconut and raspberry and the (R)-enantiomer is main ingredients[6]. It is racemic to synthesis perfume by ordinary chemical methods, chiral catalyst is usually expensive for example the reduction of α-substituted cyclopentanone catalyzed by BINAP-Ru(II) show high optical purity[7,8], but the application is severe restricted to the high price of catalyst.

Applications of biological catalysts are low cost and have been carried out by employing the whole cell or enzyme[9,10]. It prefer to use single purified enzyme as it can avoid side reactions of irrelevant enzymes exist in the cell. (R)-δ-decalactone can be obtained according to the reduction of keto acid and Massoia lactone: Literature[11,12] reported the baker yeast reduce the massioa lactone to δ-decalactone, in the process of reduction, excess concentration of substrate and product poison the baker yeast. Similar status exist in the reduction of carbonyl acid to hydroxy acid which go against to baker yeast[13]. Thus it shows great superiority to employ single purified enzyme in the optimum condition of pH and temperature.

Literature[14-16] has decleared a way to produce chiral α-substituted cyclohexanone through the hydrosis of enol esters. Referring to the former methods, it is easy to prepare (R)-δ-decalactone by the Baeyer-Villiger oxidation[17-19] of (R)-(2)-pentylcyclopentanone which can be obtained by asymmetric hydrolysis of enol esters. The general formula of synthesis and hydrolysis have been shown in Fig. 1. The influence of R_2 has been described in the hydrolysis of enol acetates[15] and 99 % ee 2-substituted cyclohexanone can be obtained. The influence of R_1[16] also can be seen in the enantioselective hydrolysis of 1-acetoxy-2-methylcyclohexene, the highest ee % was 90 %.

However, few papers concern about the effect on the alchol moiety in the hydrolysis of enol ester with chiral carboxylic acid moiety. The objective of the present work is to explore the optimum anhydride in its optimum pH and temperature. And the specific rotation of hydrolyzate changes with the appearance of chiral enol ester. The result was carefully discussed base on the structure of lipase.

EXPERIMENTAL

Conversion was detected by GC-1690 gas chromatograph. The specific rotation determined by Autopal IV polarimeter. The specific rotation of (R)-2-pentylcyclopentanone has not been reported before, but the absolute configuration was assigned based on the lactone synthesized by Baeyer-Villiger oxidation, the specific rotation of (R)-δ-decalactone is reported

to be $[\alpha]^{25}_D + 55.82°$ (c 1.93, CH_3OH)[20]. The ee % was determined by the HPLC (high performance liquid chromatography). Reagents and chemicals were purchased from commercial resource and used without any handling.

Synthesis of enol esters: The synthesis of enol esters catalyzed by toluene-4-sulfonic acid monohydrate was chemically synthesized following the method[16] reported, anhydride can be obtained referring patent[21]. Tables 1 and 2 show the preparation of series enol esters with different R_1 and R_2 group and the general formula of synthesis and hydrolysis have been shown in Fig. 1.

TABLE 2
HYDROLYSIS OF SERIES ENOL ESTERS

Substrate	React time H	Conversion yield[a] %	Specific rotation[b]
(**1**)	2	100	-82.03
(**2**)	24	100	-102.20
(**3**)	150	61	-80.65
(**4**)	150	67	-12.28
(**5**)	150	73	-11.64
(**6**)	2	100	-78
(**7**)	24	100	-10

[a]A conversion was determined by gas chromatography
[b]$[\alpha]_D^{25}$ was determined by Autopal IV polarimeter set at 589 nm wavelength, sample resolved in methanol was kept at 25 °C, and the configuration can be determined to be R after the baeyer-villiger oxidation and determined the specific rotation, the value is plus

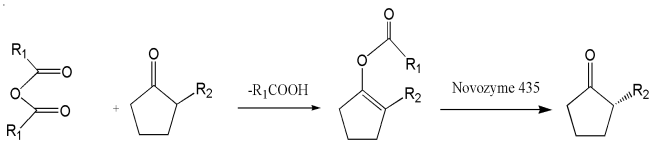

Fig. 1. Synthesis of enol ester and preparation of (R)-2-alkylcyclopentanone

Asymmetric hydrolysis of enol esters: Enol ester hydrolyzed with Novozyme 435 lipase (10 % w/w substrate). The reaction was conducted at a constant temperature and the pH of solution was maintained to constant according to the auto temperature control and auto dropwise 5 % $NaHCO_3$. Gas chromatographic track analyze to ensure the hydrolysis complete or cease.

Baeyer-Villiger oxidation of (R)-2-pentylcyclopentanone: The reaction formula can be seen from Fig. 2. Baeyer-Villiger oxidation is known for the retention of configuration, the configuration of 2-pentylcyclopentanone could be detected after the Baeyer-Villiger oxidation[17-19], which was performed following the method[8] and the retention of configuration is about 90 %.

Fig. 2. Preparation of (R)-δ-decalactone by the Baeyer-Villiger oxidation of (R)-2-alkylcyclopentanone with *m*-CPBA at room temperature

RESULTS AND DISCUSSION

Table-2 shows the effect on conversion yield and specific rotation in the pH of 6.5 and the temperature is 30 °C. The enol esters were obtained by various anhydrides reacted with 2-substituted cyclopentanone, then the enol ester were hydrolyzed, thus the optimum substrate was determined by type of anhydride. The conversion yield of (**1**) and (**2**) were 100 %, but the reaction time increase rapidly, which indicate that the reaction rate decreases rapidly. The product was confirmed to have R configuration by the sign of $[\alpha]^{25}_D$-82° (c 0.1, CH_3OH) and $[\alpha]^{25}_D$-102° (c 0.1, CH_3OH) corresponding to acetic anhydride and isobutyric anhydride, respectively. While the specific rotation of product with 2-methylbutyric anhydride and isovaleric anhydride decrease to -12 and -11°. And it took longer time in the hydrolysis of (**4**) and (**5**), the comparation indicated that the hydrolysis of enol ester is limited to the substrate, especially the acid moiety.

Besides, when (S)-(+)-2-methylbutyric acid was introduced in the enol ester, the specific rotation of product exhibited enormous enhancement from $[\alpha]^{25}_D$-12° (c 0.1, CH_3OH) to $[\alpha]^{25}_D$-80° (c 0.1, CH_3OH) compared to the corresponding racemic enol ester. The result proved that Novozyme 435 prefer to show a better asymmetric hydrolysis to the (S)-enol ester.

In addition, comparing to 2-pentylcyclopentanone, 2-heptylcyclopentanone moiety shows a greater effect in the hydrolysis of enol ester. (**1**) and (**6**), (**2**) and (**7**) are prepared from same anhydride respectively, the hydrolysis of (**7**) shows quite low specific rotation compared to (**2**), while (**1**) and (**6**) have a relative approximate result.

Table-2 indicates the influence of acid moiety in the asymmetric hydrolysis, the highest specific roation were obtained with isobutyric anhydride, the former two iterms have a 100 % conversion yield while the esters with longer chain acid moiety can not hydrolyze completely and the specific rotation of product 2-pentylcyclopentanone decrease.

It can be explained by the stereo specificity theory, whose model described the structure of lipase containing active centre[22]. There are two grooves present on the surface of the active site in the structure of lipase detected by the X-ray diffraction[23], one accept acid moiety, the other one accept the alchol

TABLE-1
PREPARATION OF ENOL ESTERS WITH DIFFERENT ANHYDRIDE AND 2-ALKYLCYCLOPENTANONE

	Enol esters with 2-pentylcyclopentanone	Enol esters with 2-heptylcyclopentanone
Acetyl anhydride	1-Acetoxy-2-pentylcyclopentene (**1**)	1-Acetoxy-2-heptylcyclopentene (6)
Isobutyl anhydride	1-Isobutyryloxy-2-pentylcyclopentene (**2**)	1-Isobutyryloxy-2-heptylcyclopentene (7)
(S)-(+)-2-methyl butyl anhydride	1-[(S)-(+)-2-methyl]-butyryloxy-2-pentylcyclopentene (**3**)	-[a]
2-Methyl butyl anhydride	1-[(±)-2-methyl]-butyryloxy-2-pentylcyclopentene (**4**)	-[a]
3-Methyl-butyl anhydride	Isovaleryloxy-2-pentylcyclopentene (**5**)	-[a]

[a]It was difficult to obtain enol esters with corresponding anhydride and 2-pentylcyclopentanone.

moiety, the groove of acid moiety is broader than another[24], which means that the impact of alchol moiety is greater than acid moiety, thus the hydrolyzed result of (**7**) can be explained. The (S)-enantisomer of ester react faster than the (R)-enantisomer as the larger moiety is placed into the stereo specificity pocket. Thus a lower level of asymmetric hydrolysis may be contributed to the acid moiety is too small, while the results of (**4**) and (**5**) may be caused by the large acid moiety.

The different specific rotation of the hydrolyzate of (**3**) and (**4**) were obviously caused by the chiral acid moiety in the enol ester, the introduction of chiral acid moiety affect the stereoselectivity of product, as has been described that the groove of the active centre combine with acid moiety was affected by the chiral acid moiety. Kazlauskas *et al.*[25] declared that in the condition of α-substituted of carboxylic acid, lipases prefer to react with (S)-enantiomers. The chiral acid moiety result in severe steric crowding based on the size of the substi-tuents at the stereocenter. Besides Kazlauskas predicted that enantiomer of chiral carboxylic acid esters react faster than racemic carboxylic acid esters through the X-ray crystallography. The chiral centre of 2-methylbutyric acid is positioned at the a-carbon with regard to the carboxyl moiety changes.

Temperature, as well as pH, could has significant effects on the reaction rate, this is due to the fact that enzymes are highly sensitive towards any slight temperature and pH changes and will denature when it beyond the optimum temperature or pH.

Fig. 3 shows the effect of pH against the specific rotation of (R)-2-pentylcyclopentanone prepared by the hydrolysis of (**2**) in 30 °C. In this investigation, the specific rotation of product change with the increasing of pH from 5.5 to 6.5, the product exhibited the highest specific rotation when pH is 6.5. However, it occurs a sudden change within the addition of 0.5 pH, the peak demonstrated that the Novozyme 435 is sensitive to the pH, especially the pH exceed to the 6.5.

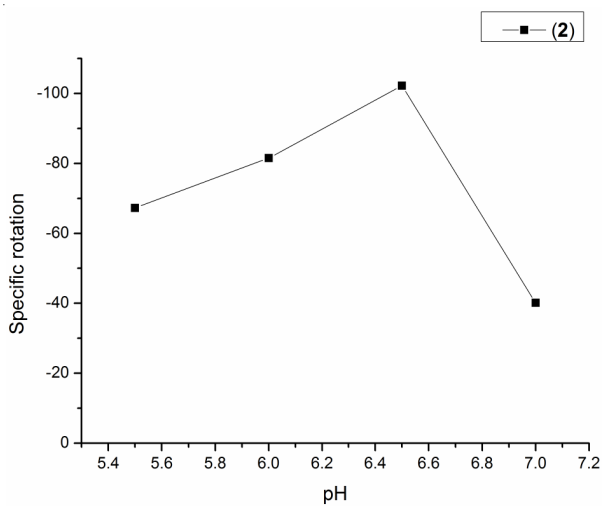

Fig. 3. Effect of pH against the specific rotation of (R)-2-pentylcyclopentanone at 30 °C

Fig. 4 shows that the temperature affect the specific rotation of product at the pH of 6.5. It changes small from 20 °C to 30 °C, however, decrease rapidly when the temperature exceed to 30 °C.

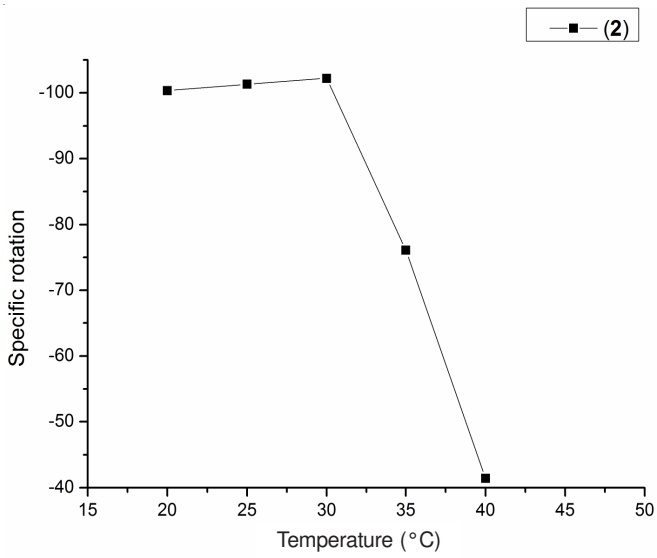

Fig. 4. Effect of temperature against the specific rotation of (R)-2-pentylcyclopentanone at the pH of 6.5

Generally, the enantioselectivity of lipase is controlled by the stereo recognition of the active site. Figs. 3 and 4 have demonstrated that the Novozyme 435 is rather sensitive to pH and temperature. The effect of pH and temperature may due to the deformation of Novozyme 435.

Fig. 5 shows that the hydrolyzing process of (**3**) and (**4**) in the condition of pH = 6.5 and 30 °C in the whole reaction time. The rate of hydrolysis react decreases with the reaction conduct and the hydrolysis ceased at about 100 h. Apparently the hydrolyzing degree of (**4**) is greater than (**3**). However, the most important result is that the yield and the specific rotation of hydrolyzate show enormous difference, both results demonstrate that the influence to alcohol moiety of (S)-2-methylbutyric moiety in enol ester is positive. The introduction of chiral acid moiety fortifies the stereoselective in the asymmetric hydrolysis.

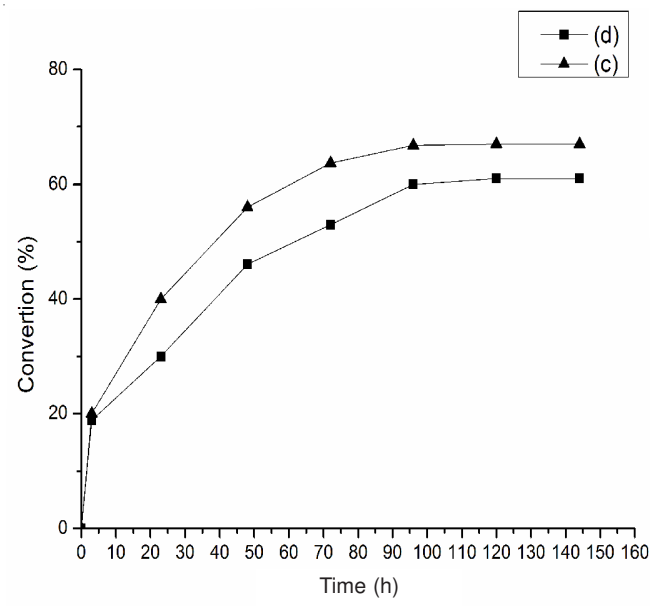

Fig. 5. Conversion yield of novozyme 435 catalyze of (3) and (4) at the pH = 6.5 and the temperature 30 °C

However, the introducing of chiral acid still can not reach at the top specific rotation. Therefore, the optimum anhydride is isobutyric anhydride. The Baeyer-Villiger oxidation was performed with the hydrolyzate of (**2**) and the ee % of (R)-δ-decalactone is 81.06 %.

Conclusion

The performance of Novozyme 435 hydrolyzing enol ester was improved enormously by bringing in a chiral (S)-(+)-2-methylbutyric acid moiety compared to racemic 2-methylbutyric acid, while further increase depends on variety of anhydride. The optimum anhydride is isobutyric anhydride and enantioselectivity was strongly affected by the changes in pH and temperature in the hydrolysis of (**2**). From the structural point of view, the enantioselectivity enhancement was probably due to the acid moiety filled in the stereo specificity pocket affect the alchol moiety filled the groove at the site of active centre in the process of hydrolysis. The method may be applied in the synthesis of chiral 2-substituted ketone or alcohol.

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