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SYNTHESIS AND LIPASE-CATALYZED ACETYLATION OF *N*-[(2-ALKOXYCARBONYL)BENZOYL]-4-HYDROXYISOXAZOLIDINES

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Abstract. Several N-[(2-alkoxycarbonyl)benzoyl]-4-hydroxyisoxazolidines were prepared as the racemic mixtures, and resolved by the lipase-mediated acylation.

In the research on the use of lipases in the preparation of chiral synthons for flavoring, pharmaceutical and pesticide chemistry, our attention was focused on some *O*-subsituted hydroxyphthalimides, well known as precursors of *O*-substituted hydroxylamines. The chiral secondary alcohols with a hydroxylamine function, which were expected to be formed by an alkaline hydrolysis of those phthalimides, might be regarded as very useful and interesting building blocks in the synthetic organic chemistry, in particular in the research on new biologically active compounds[1,2].

By making *N*-hydroxyphthalimide react with epibromohydrin and triethylamine in a DMF solution Leclerc and al.[3] obtained the expected *N*-(2,3-epoxypropoxy)

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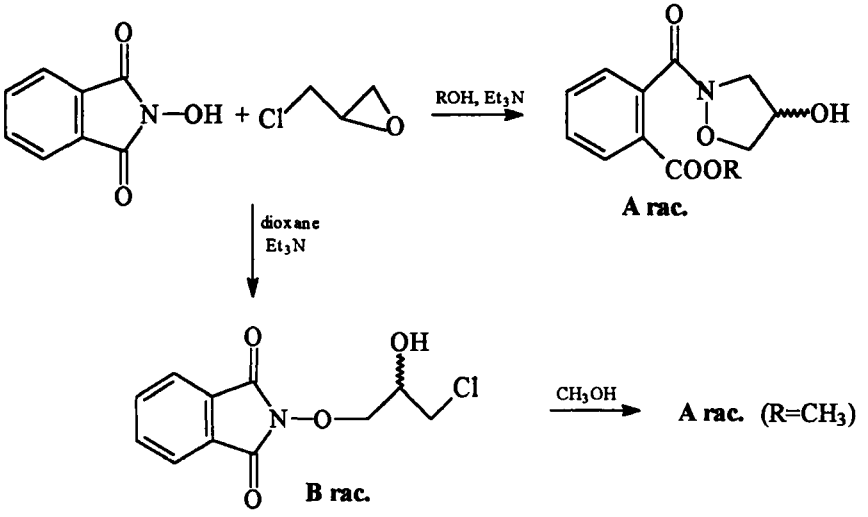
phthalimide which upon fission of the oxirane ring with aqueous hydrochloric or hydrobromic acid yielded the desired *N*-(3-chloro)- or *N*-(3-bromo-2-hydroxypropoxy)phthalimide, respectively. In our attempt to simplify the procedure, an equimolar mixture of *N*-hydroxyphthalimide and epichlorohydrin dissolved in methanol was treated with an excess of triethylamine at 40°C. However, the reaction gave only a quantitative yield of racemic *N*-[2-(methoxycarbonyl) benzoyl]-4-hydroxyisoxazolidine (**A rac.** 1) which was found identical with the compound prepared by Leclerc[3] from *N*-(3-bromo-2-hydroxypropoxy)phthalimide.



When other alcohols were used as the solvents (and as the reactants, in fact) several alkoxycarbonyl derivatives of 2-benzoyl-4-hydroxyisoxazolidine (**A rac.**) were obtained in very high yields as finely crystalline compounds. There was no trace of the formation of the epoxypropoxyphthalimide or *N*-(3-chloro-2-hydroxypropoxy)phthalimide (**B rac.**). The method proved therefore to be quite

Table 1. The yields and properties of *N*-[2-(alkoxycarbonyl)benzoyl]-4-hydroxyisoxazolidines (**A rac.**)

No	R	Yield (%)	Mp. (°C)	Analysis Calculated %C	%H	%N	Found %C	%H	%N
1	CH_3	80	112-114	57.36	5.21	5.58	57.12	5.23	5.42
2	CH_3CH_2	75	86-87	58.60	5.70	5.28	58.28	5.51	5.25
3	$CH_3CH_2CH_2$	70	38-40	60.21	6.14	5.02	59.87	5.89	5.02
4	$(CH_3)_2CH$	60	88-89	60.21	6.14	5.02	59.81	5,57	4.89
5	$C_6H_5CH_2$	30	104-106	66.05	5.23	4.28	65.77	5.12	4.20

Table 2. ^{1}HNMR and IR data of *N*-[2-(alkoxycarbonyl)benzoyl]-4-hydroxyisoxazolidines (**A rac.**).

No	^{1}H NMR (δ ppm, $CDCl_3$)	IR in Nujol (cm^{-1})
1	3.87(s, 3H); 3.61-4.20(m, 5H); 4.68-4.82 (m, 1H); 7.44-7.95(m, 4H).	3410 (ν_{OH}); 1740 ($\nu_{C=O\ ester}$); 1640 ($\nu_{C=O\ amide}$)
2	1.38(t,3H,J=7.1Hz); 3.58-4.38(m, 5H); 4.36 (q,2H, J=7.1Hz); 4.67-4.85(m,1H); 7.38-8.04(m,4H).	3380 (ν_{OH}) ; 1725 ($\nu_{C=O\ ester}$); 1630 ($\nu_{C+=O\ amide}$)
3	1.00(t,3H,J=7.6Hz); 1.63-1.86(m,2H); 3.45-4.40(m,5H); 4.25(t,2H,J=6.6Hz); 4.65-4.95 (m,1H); 7.40-8.15(m, 4H).	3305, 3380 (ν_{OH}); 1710 ($\nu_{C=O\ ester}$); 1620 ($\nu_{C=O\ amide}$)
4	1.33(d,6H,J=6.3Hz); 3.34-4.46(m.,5H); 4.57-4.95(m.,1H); 5.18(sep.,1H,J=6.3Hz);7.30-8.10(m.,4H).	3360 (ν_{OH}); 1705 ($\nu_{C=O\ ester}$); 1620 ($\nu_{C=O\ amide}$)
5	1.80(bs,1H); 3.50-4.25(m,5H); 4.61-4.68(m,1H); 5.32 (s,2H); 7.31-8.05(m,9H).	3280 (ν_{OH}); 1710 ($\nu_{C=O\ ester}$); 1605 ($\nu_{C=O\ amide}$)

general. The yields and some properties of the compounds synthesized in that way are presented in Table 1, whereas the ^{1}HNMR and IR spectral data are collected in Table 2.

The expected *N*-(3-chloro-2-hydroxypropoxy)phthalimide (**B rac.**) was however obtained in a good yield when the alcohol solvent was replaced in a similar

reaction with dioxane. A nucleophillic attack of an alkoxy anion, as observed when refluxing **B rac.** in methanol, quantitatively converted the compound into the corresponding isoxazolidine derivative **A rac.** ($R=CH_3$).

The interest in pure enantiomers of the chiral isoxazolidines has been significantly increasing in the last years. The usual method of their preparation consists in an intramolecular 1,3-dipolar cycloaddition of nitrones to olefinic moieties[4-7] but the cycloadducts obtained in this way show a moderate optical purity. The use of chiral catalysts in the intermolecular cycloaddition reactions seems to be more promising. Thus, enantiomeric excesses of prepared polysubstituted isoxazolidines were significantly increased since the early experiment of Seerden[8] and in some cases can be now as high as 90%[9-11]. However, the main problem in the use of synthetic chiral catalysts is their high price and no recycling possibility. The enzyme-catalyzed resolution of racemic mixtures[12] seems to offer a simpler and more economical way, which can be applied to at least some of the chiral isoxazolidines. Several examples of similar resolutions are known in the case of the isoxazole and isoxazoline derivatives[13,14] but to the best of our knowledge the lipase-catalyzed transesterification reactions have been used to resolve the racemates of isoxazolidine alcohols at no time in the past. The *N*-benzoyl-4-hydroxyisoxazolidines with a secondary hydroxy group (**A rac.**) have been considered at present as suitable models for investigating this resolution possibility. The stereoselective esterifications of the 4-hydroxyisoxazolidine derivatives were carried on in a methyl *tert*-butyl ether solution, at room temperature using Amano AK lipase (*Pseudomonas fluorescens)*, and vinyl acetate as the acyl donor.

The reactions were rather slow at room temperature and the conversions close to 50% resulted in a moderate enantiomeric excess of the prepared acetates (**C**) and unchanged alcohol (**A**) substrates. The reaction conditions, yields and enantiomeric purities of the separated acetates and alcohols are summarized in the Table 3. The

ee (%) of the esters and alcohols were determined by HPLC on a Chiracel ODH chiral column. Optical rotations were measured in a methanol solution. Enantioselectivities of the reactions (E) were calculated from the enantiomeric excess (ee) and the conversion degree (c) of the substrate and the product, respectively, by using generally known equations[15].

According to the accepted standards[15] only the reactions with E>15 may be considered as acceptable from the preparative point of view although their further optimization is certainly necessary.

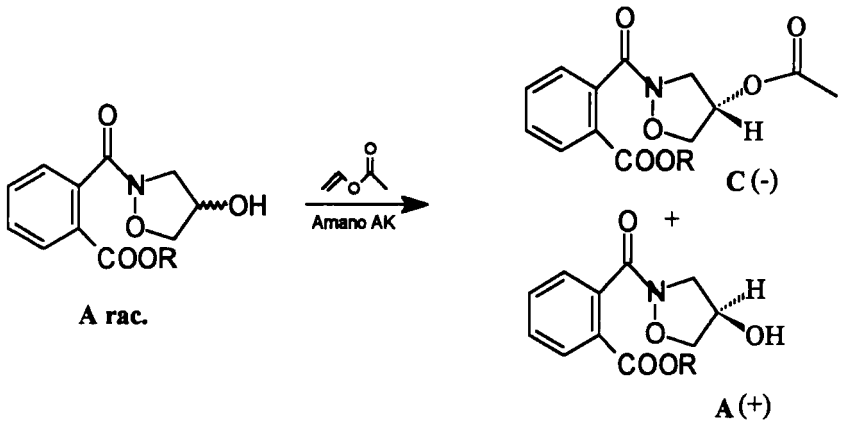


Table 3. The enantiomeric purities of the prepared *N*-benzoylisoxazolidines (**A**) and their acetates (**C**).

No	R	reaction time (hrs)	conversion (%)	acetates			alcohols		
				$[\alpha]_D^{20}$	ee (%)	E	$[\alpha]_D^{20}$	ee (%)	E
1	CH_3	28	43	-35	82	20	+29	62	20
2	CH_3CH_2	19	39	-46	82	17	+30	52	17
3	$CH_3CH_2CH_2$	45	52	-41	52	13	+33	76	13
4	$(CH_3)_2CH$	36	55	-30	64	10)	+52	79	11
5	$C_6H_5CH_2$	36	46	-31	86	23	+21	73	23

Table 4. The ^{1}HNMR and IR data of *N*-[(2-alkoxycarbonyl)benzoyl]-4-acetoxy-isoxazolidines (**C rac.**).

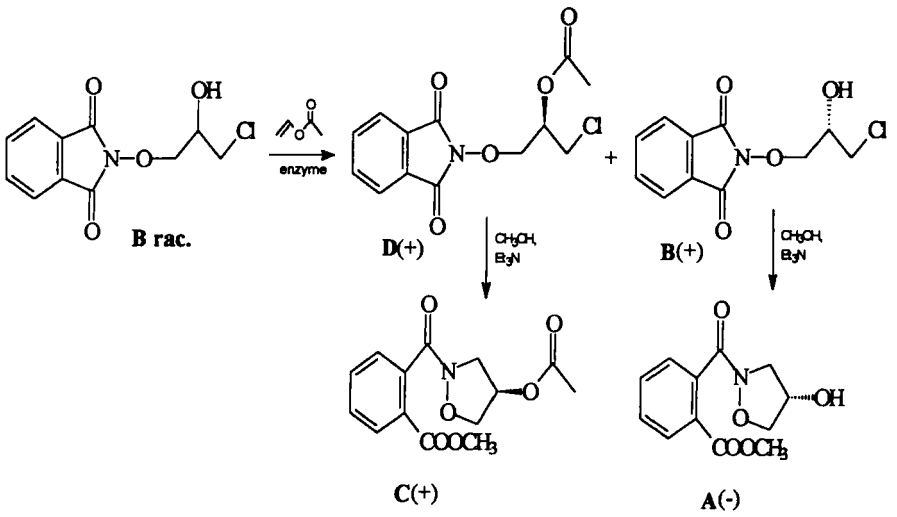
No	R	^{1}HNMR (δ ppm, $CDCl_3$)	IR (film, cm^{-1})
6	CH_3	2.09(s,3H); 3.96(s,3H); 3.55-4.12 (m,3H); 4.37-4.58(m,1H); 5.55-5.65 (m,1H); 7.40-7.99(m.,4H).	1735, 1710 ($\nu_{C=O\ ester}$) 1660 ($\nu_{C=O\ amide}$) 1275, 1240 (ν_{C-O})
7	CH_3CH_2	1.33(t,3H,J=7.2Hz); 2.07(s,3H); 3.60-4.45(m,4H); 4.30(q,2H,J=7.2Hz); 5.55-5.65(m,1H); 7.41-8.0(m,4H).	1735, 1710 ($\nu_{C=O\ ester}$) 1660 ($\nu_{C=O\ amide}$) 1280, 1240 (ν_{C-O})
8	$CH_3CH_2CH_2$	0.99(t,3H,J=7.2Hz); 1.63-1.84(m,2H); 2.09(s,3H); 3.62-4.55(m,4H); 4.22(t,3H,J=7.2Hz); 5.55-5.65(m,1H); 7.40-7.99(m.,4H).	1740, 1710 ($\nu_{C=O\ ester}$) 1660 ($\nu_{C=O\ amide}$) 1275,1235 (ν_{C-O})
9	$(CH_3)_2CH$	1.26(d,6H,J=6.4Hz); 2.01(s,3H); 5.55-4.50(m,4H); 5.12(sep.,1H, J=6.4Hz); 5.45-5.60(m,1H); 7.40-7.99(m,4H).	1735, 1705 ($\nu_{C=O\ ester}$) 1660 ($\nu_{C=O\ amide}$) 1275, 1225 (ν_{C-O})
10	$C_6H_5CH_2$	2.06(s,3H); 3.45-4.40(m,4H); 5.28 (s,2H); 5.25-5.45(m,1H); 7.25-8.15 (m.,9H).	1730, 1710 ($\nu_{C=O\ ester}$) 1645 ($\nu_{C=O\ amide}$) 1280, 1235 (ν_{C-O})

For analytical purposes (determination of optical purities) the racemic mixtures of the acetates (**C rac.**) were prepared from *N*-(2-alkoxycarbonyl)benzoyl-4-hydroxyisoxazolidines (**A rac.**) by acetylation with acetyl chloride in the presence of pyridine. They were separated by column chromatography as viscous oils. The spectroscopic properties of the *N*-[(2-alkoxycarbonyl)benzoyl]-4-acetoxyisoxazolidines are summarized in Table 4.

Looking for a method for the preparation of the desired hydroxyisoxazolidines with a higher optical purity than that presented in Table 3, we have investigated the enzyme-catalyzed esterification of *N*-(3-chloro-2-hydroxypropoxy) phthalimide (**B rac.**). The reaction with vinyl acetate carried out at room temperature in the

presence of Amano AK lipase gave after 2 days a 35% conversion with 53% ee of the alcohol **B** (+), and 99% ee of the ester **D** (+).

The reaction enantioselectivity E was higher than 100. The esterification catalyzed by Amano PS lipase (*Pseudomonas cepacia*) gave a similar result: after two days at room temperature the conversion was 36%, the enantiomeric excess of the prepared acetate **D** (+) as high as 99%, the substrate alcohol **B** (+) as low as 57%, and the reaction enantioselectivity E>100. In order to increase the optical purity of the alcohol, Novozym 435 was used as the catalyst. The reaction carried out for 3 days at room temperature resulted in a 43% conversion. Its enantioselectivity E was >100, the enantiomeric excess of the acetate **D** (+) was 99% and the ee of the separated alcohol substrate **B** (+) was 77%. The latter results, which are much better than those obtained in the resolutions of the isoxazolidine derivatives **A rac.** show that the method is quite useful for practical purposes. They also indicate that the efficiency of the kinetic resolution of chiral secondary alcohols is substantially improved when the substituents adjacent to the hydroxy group markedly differ in



size. The prepared optically active alcohol **B** (+) and the acetate **D** (+) of its enantiomer were reacted with methanol to quantitatively yield the appropriate levorotatory hydroxyisoxazolidine **A** (-) (R=CH_3) and the dextrorotatory acetate **C** (+) (R=CH_3), respectively.

The optical rotation of *N*-[2-(methoxycarbonyl)benzoyl]-4-hydroxyisoxazolidine (**A**) obtained in this cyclization reaction was of an opposite sign to that of the slower reacting enantiomer in the Amano AK lipase catalyzed resolution of **A rac.**. The chiral centers in both the low and fast reacting enantiomers retain the same absolute configuration irrespective of the resolution method.

General remarks and procedures

Melting points were determined on a Franz Kustner melting point apparatus and are not corrected. ^{1}H NMR spectra were recorded on Varian VXR-300 spectrometer in $CDCl_3$ with TMS as the internal standard. IR spectra were taken on a Carl Zeiss Specord M80 instrument as neat or Nujol suspension. Optical rotation measurements were recorded on a P20 Bellingham and Stanley polarimeter. The optical purity was determined by HPLC on Thermo-Separation Products P-100 instrument. The determination of the products purity, and reaction monitoring were accomplished by TLC on Silica gel 60 F_{254} plates and the column chromatography on Silica Gel 60 (less than 230 mesh). Novozym 435 was generously provided by Novo-Nordisk Co. and the Amano AK and Amano PS lipases by Amano Co.

General method for the preparation of *N*-[2-(alkoxycarbonyl)benzoyl]-4-hydroxyisoxazolidines (A rac.).

Epichlorohydrin (9.2 g; 8 ml; 0.1 mol) was added dropwise to a stirred solution of *N*-hydroxyphthalimide (16.3 g; 0.1 mol) and triethylamine (0.36 g; 0.5 ml; 0.035 mol) in 30 ml. of the appropriate alcohol. The mixture was stirred at 40° C for 6 hours*, the solvent was removed under reduced pressure, and the crystalline residue was recrystallized from ethyl acetate to give colorless crystals. The yields and properties of the prepared compounds are summarized in Tables 1 and 2.

* In the synthesis of the carbobenzyloxy derivative (5A) the reagents were stirred for 6 hours at 70°.

General method for the preparation of *N*-[2-(alkoxycarbonyl)benzoyl]-4-acetoxyisoxazolidine racemates (C rac.).

Freshly distilled acetyl chloride (0.021mol, 1.5ml) was added slowly to a stirred solution of *N*-[2-(alkoxycarbonyl)benzoyl]-4-hydroxyisoxazolidine (0.02mol) in10

ml of anhydrous THF and pyridine (0.021 mol, 1.6ml) at 0°C. Upon addition of molecular sieves (2g) the mixture was refluxed for 8 h. Addition of conc. hydrochloric acid precipitated pyridine-HCl which was filtered off. The filtrate was treated with $NaHCO_3$ soln. and then extracted with $CHCl_3$. Evaporation of the solvent left an oily residue which was purified on a silica gel column with an ethyl acetate/hexane 1:1 mixture. The properties of the prepared acetates **C rac.** are summarized in Table 4.

Enzymatic esterification of *N*-[2-(alkoxycarbonyl)benzoyl]-4-hydroxyisoxazolidine with vinyl acetate.

Vinyl acetate (0.93g; 1ml; 0.1mol) was added to the stirred mixture of the appropriate racemic alcohol (1g; 0.004 mol) and the enzyme (lipase Amano AK, 1 g) in methyl *tert*-butyl ether (30 ml). The mixture was stirred at room temperature for 19-45 h, the enzyme was removed by filtration and the solvent was evaporated under reduced pressure. The remaining mixture of the substrate (**A**) and the corresponding acetate (**B**) was separated by silica gel column chromatography with hexane:ethyl acetate (1:1) as the eluent.

***N*-(3-chloro-2-hydroxypropoxy)phthalimide (B rac.).**

Epichlorhydrin (5.31g; 4.5ml; 0.057mol) was added dropwise to a stirred solution of *N*-hydroxyphthalimide (8.51g; 0.052mol) and triethylamine (0.36g; 0.5ml; 0.035mol) in 10 ml of dioxane. Stirring at 40° was continued for 3 days, 150ml of ethyl acetate were next added, and the solution was extracted with a saturated $NaHCO_3$ solution until the aqueous phase became colourless. The organic layer was washed with water and dried over $MgSO_4$. After evaporation of the solvent, the oily residue (12.13g; 0.047mol., 90%) was purified by recrystallization from ethyl acetate to give 4.38g (33%) of colorless crystals mp. 100-102°.

^{1}HNMR: 3.68(d,2H,J=5.2Hz); 3.87((s,1H); 4.15-4.39(m,3H); 7.71-7.92(m,4H).

Enzymatic esterification of *N*-(3-chloro-2-hydroxypropoxy)phthalimide.

Novozym 435 (0.33 g) and 0.93 g (1 ml, 0.1 mol) of vinyl acetate were added to the solution of 1 g (0.004 mol) of the alcohol (**B rac.**) in 30 ml of *tert*-butyl methyl ether and the mixture was stirred for 3 days at room temperature. The enzyme was filtered off and the residue was concentrated under reduced pressure and passed through a silica gel packed column with ethyl acetate/*n*-hexane 1:3 mixture as the eluent. Two fractions obtained upon evaporation of the sovent gave:

the acetate: 0.42g of yellow oil; conversion c=43%, $[\alpha]_D^{20}$=+5.22 (in acetone), ee = 99%, E>100;

the alcohol: 0.38g of colourless crestals; mp. 100-101° C, $[\alpha]_D^{20}$= +5,7 (in acetone), ee = 77%, E>100.

Cyclization of (+) *N*-(3-chloro-2-hydroxypropoxy)phthalimide to (-) *N*-[2-(methoxycarbonyl) benzoyl]-4-hydroxyisoxazolidine.

The solution of 0.5 g (1.9 mmol) of *N*-(3-chloro-2-hydroxypropoxy)phthalimide and 3 drops of triethylamine in 4 ml of methanol was stirred 1 hr. at room temperature, and the solvent was removed under reduced pressure. To the residue 5 ml. of saturated aqueous $NaHCO_3$ was added, and the mixture was extracted with chloroform. The chloroform solution was dried over magnesium sulfate and evaporated yielding 0.5 g of colourless crystals. Recrystallization from ethyl acetate gave the pure compound mp.112-114° , ee = 91%, $[\alpha]_D^{20}$ = -34° (in methanol).

In the same manner the enantiomeric acetate was obtained from N-(3-chloro-2-acetoxypropoxy)phthalimide as a viscous liqud. The yield was theoretical, and after the solvents evaporation the optical rotation measured in methanol was $[\alpha]_D^{20}$ = +48°, and ee = 99%.

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