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Double Enantioselective Enzymic Synthesis of Carbonates and Urethanes.

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Abstract: Double enantioselective lipase-catalyzed alkoxycarbonylation of racemic alcohols and amines with racemic vinyl carbonates to yield carbonates and carbamates with two stereogenic centers was carried out using *Candida antarctica* lipase.

INTRODUCTION

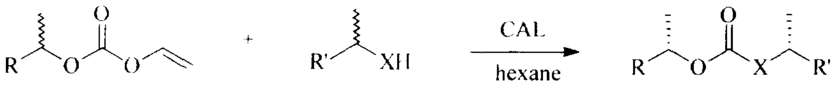
Enzymes have been widely employed as chiral catalysts in organic synthesis for kinetic resolutions of racemates and for the asymmetrizations of prochiral compounds.[1,2] Usually, these processes have been carried out between a racemic or prochiral hydroxy or amino compound and an achiral acylating agent or *vice versa* in order to achieve a resolution of the amine or alcohol in the former case or the acylating agent in the latter case. As Chen and Sih[3] remarked' the matching of appropriate racemic acids with racemic alcohols in a double kinetic resolution experiment is an exciting area that warrants systematic exploration in the future.' However, until now, only a few papers describing lipase-catalyzed reactions of this kind have been put forward.[4]

Recently, we have developed some methodologies for the synthesis of asymmetric chiral carbonates and urethanes through enzymatic alkoxycarbonylation of racemic alcohols[5] and amines[6] and by the kinetic resolution of the starting racemic vinyl carbonates with different alcohols and amines.[7] Herein, we simultaneously use racemic vinyl carbonates and racemic amines or alcohols for the double enatioselection in alkoxycarbonylation catalyzed by lipase from *Candida antarctica* to yield carbonates and carbamates with two stereogenic centers.

RESULTS AND DISCUSSION.

We knew that CAL (*Candida antarctica* lipase SP 435 lipase B) was able to catalyze reactions between vinyl carbonates and alcohols or amines, yielding chiral carbonates and urethanes. When resolving alcohols[5] or amines,[6] the enzyme follows the Kazlauskas rule[8] and the *R* enantiomers are obtained. But if the enzyme is used with racemic vinyl carbonates, a striking feature is observed, the enantioselectivity of the lipase is changed, thus the *S* enantiomers of the vinyl carbonates are resolved and *S* carbonates and carbamates are produced.[7] In order to apply the previously developed methodology in a study of double enantioselectivity, racemic vinyl carbonates **1a** and **1b**, were prepared from the appropriate racemic alcohol and vinyl chloroformate. These compounds were subjected to a series of alcohols **2**, and amines **3** in an alkoxycarbonylation reaction (Scheme 1) and the results are summarized in the Table 1.

In agreement with our earlier investigations, the lipase was selective towards the *S* enantiomer of the vinyl carbonate and *R* enantiomer of the nucleophile. The best d.e.'s were obtained when employing vinyl carbonate **1a** as the alkoxycarbonylating agent. From these latter data and Table 1 one fact could be drawn, the presence of an aromatic moiety in the vinyl carbonate plays an important role in the diastereoselectivity of the process. Least successful results were obtained with carbonate **1b** (see Table 1).



1a R= Ph
1b R= Hexyl

2 X= O
3 X= NH

4

Scheme 1.

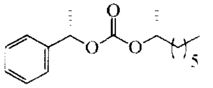
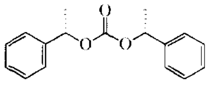
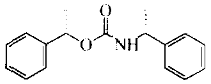
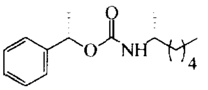
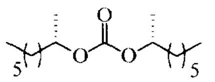
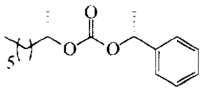
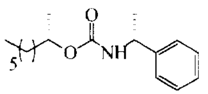
Reactions were followed by G.C. employing camphor as ISTD. A previous calibration of starting carbonates **1a** and **1b** was used to calculate conversions. Diastereoisomers of products presented separated peaks in G.C. and thus d.e.'s could be measured under the same conditions used to control reactions. In order to calculate e.e.'s a chiral G.C. column was tested but enantiomers could not be resolved, and so we decided to employ HPLC using a Chiralcel OD column.

When measuring e.e.'s in HPLC, all compounds except urethane **4c** had to be chromatographed at 5°C to achieve the separation of the enantiomers. Racemic mixtures of all compounds were prepared by chemical methods, as well as pure diastereoisomers and enantiomers in order to verify the separation of all enantiomers and the correct identification of the major product, which was, as predicted, the *S,R* alkoxycarbonylated compound.

Double enantioselective lipase-catalyzed synthesis of these products should include two consecutive enantiodifferentiation steps. In a first one, there is an enantioselection between two enantiomers of starting racemic vinyl carbonate to yield two diastereoisomeric alkoxycarbonyl-enzyme complexes. The second one would be the transfer of the alkoxycarbonyl moiety from this latter complex to one enantiomer of the racemic nucleophile present in the media. If the reaction is carried out in presence of a matched pair of vinyl carbonate and nucleophile, one of the four possible stereoisomers should be formed in highly selective way. Matched pair means , as set out by Theil,[4] highly enantioselective separation of the alkoxycarbonylating agent to yield a main complex with the enzyme which would react with high selectivity with one enantiomer of the nucleophile. From Table 1, can be deduced that in our case the matched pair is 1-phenylethan-1-yl vinyl carbonate and 2-octanol or octan-2 yl vinyl carbonate and 1-phenylethanol. as well as case **4b** where d.e. was the highest.

From Table 1, one can deduce that the lipase showed higher selectivity when a phenyl group is present in the vinyl carbonate or in the nucleophile. Thus one of the best results was obtained employing carbonate **1a** and 1-phenylethyl alcohol (d.e. was 93%, see Table 1). It seems to be a key feature that the phenyl group is located in the vinyl carbonate, since lower d.e.'s were attained when using carbonate **1b**.

Table 1 Carbonates and Carbamates with Two Stereogenic Centers from **1a** and **1b**.

Entry	Product	Time h.	% Conversion[a]	% d.e.[b]	% e.e (SR/RS)[c]
4a		32	41	88	98
4b		26	43	93	meso
4c		8	53	68	69
4d		24	42	88	76
4e		66	49	47	meso
4f		96	45	62	>98
4g		30	44	65	81

[a] Calculated by G.C. [b]Calculated by G.C. and HPLC, major diastereoisomer is formed by *S,R* and its enantiomeric partner.
[c] Calculated by HPLC, using a Chiralcel OD column.

When utilizing carbonate **1b** to alkoxycarbonylate the alcohols, the reactions were slower than when using carbonate **1a**, and 100 additional mg of lipase had to be added after 48 h. of reaction in order to complete it. For urethane **4g** the reaction time was longer as well, see Table 1. Since the first step of the reaction has to be the alkoxycarbonylation of the Ser105 residue of the CAL and the only difference between these reactions is the vinyl carbonate employed, it seems to be clear that this first step is the slow one and it determines the reaction rate.

From the results described here and in previous reports[5, 6,7] it is certain that the enzymatic alkoxycarbonylation of alcohols and amines is a useful and mild procedure to attain carbonates and urethanes. This is especially important when obtaining asymmetric carbonates, which synthesis by chemical means is generally difficult and normally mixtures are obtained.[9] On the other hand, because of use of lipases, the method could be used to resolve alcohols, amines or starting vinyl carbonates to obtain products with different e.e.'s, and even as shown throughout this report racemic vinyl carbonates and nucleophiles can be resolved in one step in a double enantioselective process to yield products with two stereogenic centers.

CONCLUSIONS

In this work, we show that the use of enzymes in combination with an adequate matching between vinyl carbonate and nucleophile yields high e.e.'s in the synthesis of carbonates and urethanes with two stereogenic centers in a one step procedure.

EXPERIMENTAL

We used an immobilized lipase from *Candida antarctica* SP 435 (CAL) (gifted by Novo Nordisk). All reagents were of commercial quality and were purchased from Aldrich Chemie. Solvents were distilled over an adequate desiccant and stored under argon. For column chromatography, Merck silica gel 60/230-400 mesh was used.. IR spectra were recorded on a Mattson 3000 Infrared Fourier Transform spectrophotometer. Gas chromatography was carried out with a Hewlett-Packard Model 5890 Series II gas chromatograph with flame ionization detection (FID) and a 25 m HP-1 capillary column coated with methylsilicone gum using N_2 as carrier gas. ^{1}H and ^{13}C-NMR were obtained with TMS (tetramethylsilane) as internal standard; using a Brucker AC-300 (^{1}H-300 MHz and ^{13}C-75.5 MHz) spectrometer. Mass spectra were recorded on a Hewlett-Packard 5897 A spectrometer. Microanalyses were performed on a Perkin-Elmer 240B elemental analyzer. HPLC was carried out with a Shimadzu LC10 with a diode array detector, employing a Chiralcel OD column.

The e.e.'s of carbonates and carbamates were determined by HPLC using the device and column stated above. For compounds **4c** and **4d** was employed *n*-hexane: isopropanol (90:10, v/v) and for compounds **4a**, **4f** and **4g** (97:3, v/v), as mobile phase, flow rate: 0.5 ml/min., detected at UV 254 nm. Racemic mixtures of all compounds were prepared by chemical methods, as well as pure diastereoisomers and pure enantiomers in order to verify separation of all enantiomers and correct identification of the major product. In all cases, except with compound **4c**, experiments were carried out at 5°C in order to separate all enantiomers.

Racemic and pure diastereoisomer and enantiomer of symmetric carbonates were synthesized from triphosgene following the methodology described by Burk[10]. Asymmetric carbonate **4a** was prepared according to reaction described by Yamada *et al.*[11] Racemic and pure enantiomers and diastereoisomers of the different carbamates were obtained with the appropiate alcohols and amines following the methodology developed by Ghosh.[12] All compounds gave satisfactory spectroscopy data.

Synthesis of racemic vinyl carbonates and their data have been presented in a previous paper.[7]

Synthesis of products 4. General procedure: To a solution of racemic vinyl carbonates **1a** or **1b** (1 mmol) and nucleophile **2** or **3** (1 mmol) in hexane 10mL, CAL (100 mg, when using carbonate **1b** 100 additional mg of lipase were added after 48h of reaction) was added. The reaction was monitored by G.C. and when the conversion was about to reach 50% was terminated by filtering off the enzyme. The organic solvent was evaporated under reduced pressure and chromatographic separation on silica gel of resulting residue gave the carbonate or carbamate.

(*R*)-Octan-2-yl (*S*)-1-phenylethan-1-yl carbonate (4a): oil; Rf= 0.47 (Hexane:ethyl ether 9:1); IR (neat): $\upsilon_{c=o}$= 1745 cm^{-1}; (Found: C, 73.51; H, 9.45. $C_{17}H_{26}O_3$ requires C, 73.35; H, 9.41); ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.35 (m, 5H, ar.), 5,70 (q, 1H, 6.6 Hz), 4.70 (q,1H), 1.60 (d, 3H, 6.6 Hz), 1.45 (m, 2H, CH_2), 1.25 (m, 11H, 8 CH_2 1 CH_3), 0.85 (t, 3H, CH_3); ^{13}C-NMR ($CDCl_3$) δ (ppm): 154.02 (C=O), 141.17 (C), 128.29 (2CH), 127.77 (CH), 125.69 (2CH), 75.73 (CH), 75.08 (CH), 35.67 (CH_2), 31.48 (CH_2), 28.82 (CH_2), 25.01 (CH_2), 22.31 (CH_2), 22.25 (CH_3), 15.06 (CH_3), 13.83 (CH_3). MS (EI, 70 eV), m/z: 278 (M^+), 122 (32.05), 105 (100.00), 77 (21.17).

(*R*)-1-Phenylethan-1-yl (*S*)-1-phenylethan-1-yl carbonate (4b): oil; Rf= 0.75 (Hexane:ethyl ether 95:5); IR (neat): υ_{c-o}= 1746 cm^{-1}; (Found: C, 75.45; H, 6.76. $C_{17}H_{18}O_3$ requires C, 75.52; H, 6.72); ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.45 (m, 10H, ar.), 5,75 (q, 2H 2 CH, 6.6 Hz), 1.65 (d, 6H 2 CH_3, 6.6 Hz); ^{13}C-NMR ($CDCl_3$) δ (ppm): 153.85(C=O), 140.93 (C), 128.40 (2CH), 127.94 (CH), 125.86 (2CH), 76.27 (CH), 22.27 (CH_3). MS (EI, 70 eV), m/z: 270 (M^+), 121 (34.17), 105 (100.00), 77 (23.43).

(*R*)-*N*-1-Phenylethyl (*S*)-*O*-1-phenylethyl carbamate (4c): oil; Rf= 0.2 (Hexane:ethyl ether 7:3); IR (neat): $\upsilon_{c=o}$= 1692 cm^{-1}; (Found: C, 75.88; H, 7.09; N, 5.17 . $C_{17}H_{19}NO_2$ requires C, 75.80; H, 7.11; N, 5.20); ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.30 (m, 10H, ar.), 5,75 (q, 1H, 6.6 Hz), 5.05 (bs, NH), 4.85 (q, 1H), 1.55 (d, 3H, CH_3), 1.50 (d, 6H 2 CH_3); ^{13}C-NMR ($CDCl_3$) δ (ppm): 155.01 (C=O), 145.77 (C), 141.92 (C), 128.45 (2CH), 128.30 (2CH), 127.58 (CH), 127.13 (2CH), 125.83 (2CH), 125.26 (CH), 72.76 (CH), 70.20 (CH), 25.08 (CH_3) 22.33 (CH_3). MS (EI, 70 eV), m/z: 269 (M^+), 122 (32.78), 105 (100.00), 77 (23.00).

(*R*)-2-*N*-Heptyl (*S*)-*O*-1-phenylethyl carbamate (4d): oil; Rf= 0.51 (Hexane:ethyl ether 7:3); IR (neat): $\upsilon_{c=o}$= 1694 cm^{-1}; (Found: C, 73.00; H, 9.56; N, 5.34 . $C_{16}H_{25}NO_2$ requires C, 72.95; H, 9.57; N, 5.32); ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.35 (m, 5H, ar.), 5,80 (q, 1H, 6.6 Hz), 4.50 (d, NH), 3.65 (m, 1H), 1.55 (d, 3H, CH_3), 1.3-1.4 (m, 8H 4 CH_2), 1.15 (d, 3H, CH_3), 0.85 (t, 3H, CH_3); ^{13}C-NMR ($CDCl_3$) δ (ppm): 154.90 (C=O), 142.01(C), 128.09 (2CH), 127.30 (CH), 125.58 (2CH), 72.04 (CH), 46.68 (CH), 36.83 (CH_2), 31.32 (CH_2), 25.25 (CH_2), 22.24 (CH_2), 22.23 (CH_3), 20.92 (CH_3), 13.70 (CH_3). MS (EI, 70 eV), m/z: 263 (M^+), 122 (41.00), 105 (100.00).

(*R*)-Octan-2-yl (*S*)-octan-2-yl carbonate (4e): oil; Rf= 0.69 (Hexane:ethyl ether 9:1); IR (neat): $\upsilon_{c=o}$= 1745 cm^{-1}; (Found: C, 71.30; H, 11.96. $C_{17}H_{34}O_3$ requires C, 71.27; H, 11.97); ^{1}H-NMR ($CDCl_3$) δ (ppm): 4.75 (m,2H 2 CH_2), 1.7-1.5 (m, 4H), 1.30 (m, 22H), 0.85 (t, 6H, 2 CH_3), 0.85 (t, 3H, CH_3); ^{13}C-NMR ($CDCl_3$) δ (ppm): 154.36 (C=O), 74.87 (CH), 35.87 (CH_2), 31.66 (CH_2), 29.02 (CH_2), 25.23 (CH_2), 22.49 (CH_2), 19.91 (CH_3), 14.00 (CH_3). MS (EI, 70 eV), m/z: 113 (49.09), 71 (100.00), 57 (90.67), 43 (54.55).

(*S*)-Octan-2-yl (*R*)-1-phenylethan-1-yl carbonate (4f): oil; Rf= 0.47 (Hexane:ethyl ether 9:1); IR (neat): $\upsilon_{c=o}$= 1745 cm^{-1}; (Found: C, 73.51; H, 9.45. $C_{17}H_{26}O_3$ requires C, 73.35: H, 9.41); ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.35 (m, 5H, ar.), 5.70 (q, 1H, 6.6 Hz), 4.70 (q,1H), 1.60 (d, 3H, 6.6 Hz), 1.45 (m, 2H, CH_2), 1.25 (m, 11H, 8 CH_2 1 CH_3), 0.85 (t, 3H, CH_3); ^{13}C-NMR ($CDCl_3$) δ (ppm): 154.02 (C=O), 141.17 (C), 128.29 (2CH), 127.77 (CH), 125.69 (2CH), 75.73 (CH), 75.08 (CH), 35.67 (CH_2), 31.48 (CH_2), 28.82 (CH_2), 25.01 (CH_2), 22.31 (CH_2), 22.25 (CH_3), 15.06 (CH_3), 13.83 (CH_3). MS (EI, 70 eV), m/z: 278 (M^+), 122 (32.05), 105 (100.00), 77 (21.17).

(*S*)-*O*-Octan-2-yl- (*R*)-*N*-1-phenylethyl carbamate (4g): oil; Rf= 0.52 (Hexane:ethyl ether 7:3); IR (neat): $\upsilon_{c=o}$= 1691 cm^{-1}; (Found: C, 73.53; H, 9.82; N, 5.00 . $C_{17}H_{27}NO_2$ requires C, 73.59; H, 9.82; N, 5.05); ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.30 (m, 5H, ar.), 5.00 (d, NH), 4.80 (m, 2H 2 CH), 1.45 (d, 3H, CH_3), 1.45-1.10 (m, 13H 5 CH_2 1 CH_3); 0.90 (t, 3H, CH_3). ^{13}C-NMR ($CDCl_3$) δ (ppm): 155.59 (C=O), 143.70 (C), 128.46 (2CH), 127.07 (2CH), 125.78 (CH), 71.43 (CH), 50.38 (CH), 36.17 (CH_2), 31.67 (CH_2), 29.09 (CH_2), 25.26 (CH_2), 22.51 (CH_2), 22.50 (CH_3) 20.23 (CH_3), 14.01 (CH_3). MS (EI, 70 eV), m/z: 277 (M^+), 164 (73.76), 150 (100), 120 (26.55), 105 (89.45), 77 (21.62).

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REFERENCES

1. Santaniello, E.; Ferraboschi, P.; Grisenti, P.; Manzocchi, A., *Chem. Rev.*, **1992**, *92*, 1071.
2. a) Wong, C.-H.; Whitesides, G.M. *Enzymes in Synthetic Organic Chemistry*; Eds. Elsevier Science Ltd., Oxford, 1994.
   b) Faber, K. *Biotransformations in Organic chemistry*, Springer-Verlag. **1995**.
3. Chen, C.-S. and Sih, C.J., *Angew. Chem. Int. Ed. Engl.*, **1989**, *28*, 695.
4. a) Brieva, R.; Rebolledo, F. and Gotor, V., *J. Chem. Soc. Chem. Commun.*, **1990**, 1386.
   b) Fowler, P.W.; MacFarlane, E.L.A. and Roberts. S.M., *J. Chem. Soc., Chem. Commun*, **1991**, 453.
   c) Holmberg, E. and Hult, K., *Biocatalysis*, **1992**, *5*. 289.
   d) Chen, P.-Y.; Wu, S.-H. and Wang, K.-T., *Biotechnol. Lett.*, **1993**, *15*, 181.
   e) MacFarlane, E.L.A.; Roberts, S.M.; Steukers, V.G.R. and Taylor, P.L., *J. Chem. Soc., Perkin Trans 1*, **1993**, 2287.
   f) Theil, F. and Björkling F., *Biotechnol. Lett.*, **1993**. *15*, 605.
   g) Theil, F.; Kunath, A.; Ramm, M.; Reither, T. and Schick, H., *J. Chem. Soc. Perkin Trans 1*, **1994**, , 1509.
5. Pozo, M.; Pulido, R. and Gotor, V., *Tetrahedron*, **1992**, *48*, 6477.
6. Pozo, M. and Gotor, V., *Tetrahedron*, **1993**, *49*, 4321.
7. Pozo, M. and Gotor, V., *Tetrahedron*, **1993**, *49*, 10725.
8. Cygler, M.; Grochulski, P.; Kazlauskas, R.J.; Schrag, J.D.; Bouthillier, F.; Rubin, B.; Serreqi, A.N. and Gupta, A.K., *J. Am. Chem. Soc.*, **1994**, *116*, 3180.
9. Cella, J.A. and Bacon, S.W., *J. Org. Chem.*, **1984**, *49*. 1122-1125.
10. Burk, R.R. and Roof, M.B., *Tetrahedron Lett.*, **1993**. *34*, 395-398.
11. Teranishi, K.; Kayakiri, T.; Mizutani, M.; Hisamatsu, M. and Yamada, T., *Biosci. Biotech. Biochem.*, **1994**, *58*, 1537-1539.
12. Ghosh, A.K.; Duong, T.T.; McKee, S.P. and Thompson, W.J., *Tetrahedron Lett.*, **1992**, *33*, 2781.

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