Anhydrides as Acylating Agents in Lipase-Catalyzed Stereoselective Esterification of Racemic Alcohols

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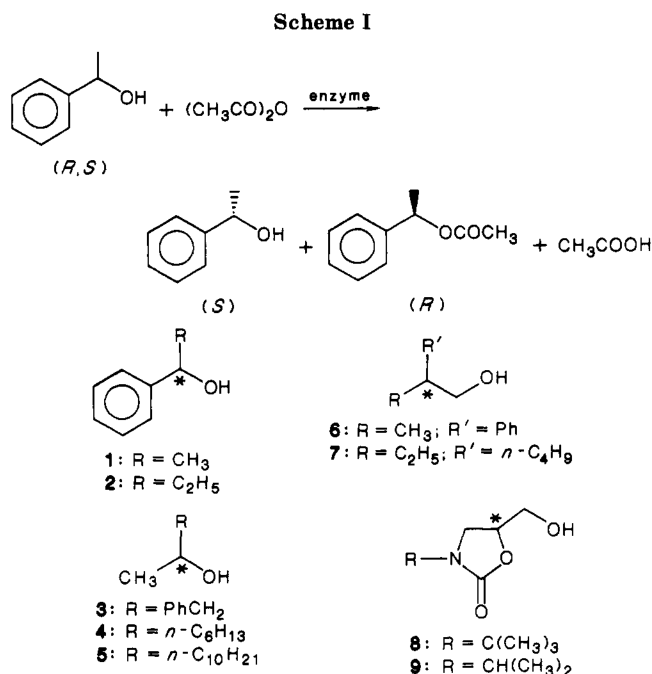
A new enzymatic method for the resolution of optically active alcohols from racemates is reported. It involves lipase-catalyzed esterification in organic solvents, with acetic, propionic, or butyric anhydrides as acylating agents. Lipase Amano P, from *Pseudomonas fluorescens*, adsorbed on Celite 577, was employed as stereoselective catalyst. Under these reaction conditions, the enzyme is not chemically modified by the anhydrides. A number of primary and secondary alcohols have been obtained in high optical purity by this procedure.

The resolution of racemic mixtures of alcohols can be achieved by using the enantioselective properties of hydrolytic enzymes. Optically pure alcohols are obtained either in aqueous solutions by stereoselective hydrolysis of the corresponding racemic esters[1] or in organic solvents by esterification[2] or transesterification[3] of the corresponding racemic alcohols. The choice of the method depends on many factors such as yield, purity, reaction rate, product separation, and enzyme stability. Commercially available hydrolases, such as lipases, are inexpensive and can be used in organic solvents, but other factors limit the application of such methods. The water formed in the esterification processes affects the thermodynamic equilibrium,[4] increasing the reaction time so much that it may not be convenient for industrial application. A similar result occurs in enzyme-catalyzed transesterifications: the alcohol from the reacted ester shifts the equilibrium toward the reactants.[3b]

Recently the use of enol-esters for the transesterification reaction[5] has been proposed as a means for overcoming this difficulty. However the enol-alcohols formed are not stable and decompose to aldehydes, which can give side reactions and can decrease the enzyme activity.

In this paper we would like to report a new method for the resolution of racemic mixtures of primary and secondary alcohols that does not have the disadvantages of the methods previously mentioned. This procedure consists of an enzyme-catalyzed acylation in organic solvents in which anhydrides are used as acylating agents (Scheme I). Since this reaction does not cause the formation of water or alcohol, it is completely shifted toward the products, because the reverse reaction is thermodynamically unfavored. Furthermore the reaction rates are higher than those obtained in the equivalent esterification or transesterification processes.

Scheme I



Results

The resolution of alcohol racemates was accomplished by enzyme-catalyzed acylation, as shown in Scheme I. Racemic alcohols and anhydrides in stoichiometric amount were dissolved in dry benzene. Lipase Amano P adsorbed on Celite was added to the reaction mixture. The suspension was then vigorously shaken at room temperature, and aliquots of 1 μL were periodically withdrawn for gas chromatographic analysis. For enzyme-catalyzed stereoselective reactions, the degree of conversion reached the value of about 50%, and then the reaction was stopped. The immobilized lipase was then readily recovered from the reaction mixture by filtration and reused without significant loss of activity. The liquid phase was treated as indicated in the Experimental Section for isolation of the products. In control experiments under the same conditions without enzyme, no acylation was observed. The enantiomeric excesses (ee) of the optically active alcohols were determined by NMR and GLC analyses. The ee of esters were determined after alkaline hydrolysis to the corresponding alcohols. Tables I and II report the

(1) (a) Oritani, T.; Yamashita, K. *Agric. Biol. Chem.* **1973**, *37*, 1923. (b) Mori, K.; Akao, H. *Tetrahedron Lett.* **1978**, *43*, 4127. (c) Ladner, W. E.; Whitesides, G. M. *J. Am. Chem. Soc.* **1984**, *106*, 7250. (d) Kitsuki, H.; Sawa, I.; Hasegawa, J.; Watanabe, K. *Agric. Biol. Chem.* **1986**, *50*, 2369.
(2) (a) Lagrand, G.; Secchi, M.; Buono, G.; Baratti, J. *Tetrahedron Lett.* **1985**, *26*, 1857. (b) Inagaki, T.; Ueda, H. *Agric. Biol. Chem.* **1987**, *51*, 1345. (c) Sonnet, P. E. *J. Org. Chem.* **1987**, *52*, 3477.
(3) (a) Cambou, B.; Klibanov, A. M. *J. Am. Chem. Soc.* **1984**, *106*, 7250. (b) Kirchner, G.; Scollar, M. P.; Klibanov, A. M. *J. Am. Chem. Soc.* **1985**, *107*, 7072. (c) Ramos Tombo, G. M.; Schar, H. P.; Fernandez Busquets, X.; Ghisalba, O. *Tetrahedron Lett.* **1986**, *47*, 5707. (d) Francalanci, F.; Cesti, P.; Cabri, W.; Bianchi, D.; Martinengo, T.; Foa, M. *J. Org. Chem.* **1987**, *52*, 5079.
(4) Chen, C. S.; Wu, S. H.; Girdaukas, G.; Shi, C. J. *J. Am. Chem. Soc.* **1987**, *109*, 2812.
(5) (a) Sweers, H. M.; Wong, C. H. *J. Am. Chem. Soc.* **1986**, *108*, 6421. (b) Duqueil-Castaign, M.; Jeso, B. D.; Droullard, S.; Maillard, B. *Tetrahedron Lett.* **1987**, *28*, 953.
(6) Dale, J. A.; Dull, D. L.; Mosher, H. S. *J. Org. Chem.* **1969**, *34*, 2534.
(7) *Aldrich Catalog Handbook of Fine Chemicals*; Aldrich Chemical Co.: Milwaukee, WI, 1987; p 1056.
(8) Fluka Catalog, Chemica-Biochemika, 1987, p 1040.
(9) Pickord, R. H.; Kenyon, J. *J. Chem. Soc.* **1914**, 1115.
(10) Pappo, R.; Collins, P.; Jung, C.; *Ann. N.Y. Acad. Sci.* **1971**, *180*, 64-75.

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Table I. Esterification of Secondary Alcohols[a]

				recovered alcohol				produced ester			
substrate	anhydride	time, h	conv, %	yield, %	$[\alpha]^{25}_{D}$, deg	isomer	ee,[b] %	yield, %	$[\alpha]^{25}_{D}$, deg	isomer	ee,[c] %
1	acetic	4	49	43	−41.0[d] (neat)	*S*	>95	39	%106 (*c* 1, ether)	*R*	>95
1	propionic	2	48	46	−38.2 (neat)	*S*	94	40	+101 (*c* 1, ether)	*R*	>95
1	butyric	8	48	42	−37.2 (neat)	*S*	91	37	+97.0 (*c* 1, ether)	*R*	>95
2	propionic	20	47	39	−41.3[e] (*c* 2, hexane)	*S*	87	34	+107 (*c* 1, ether)	*R*	>95
3	acetic	30	45	40	+33.5[f] (*c* 1, benzene)	*S*	81	36	−10.9 (*c* 1, ether)	*R*	92
3	propionic	10	48	43	+38.2 (*c* 1, benzene)	*S*	92	39	−11.5 (*c* 1, ether)	*R*	>95
4	propionic	12	50	35	+7.1[g] (*c* 4, ether)	*S*	54	33	−3.3 (*c* 1, ether)	*R*	61
5	propionic	7	48	35	+4.6[h] (*c* 2, EtOH)	*S*	63	41	−1.2 (*c* 2, EtOH)	*R*	76

[a] All the reactions were performed in benzene (40 mL) at room temperature (21 ± 2 °C); substrate, 16 mmol; anhydride, 16 mmol; enzyme, 0.12 g of lipase Amano P adsorbed on 0.48 g of Celite 577 (see the Experimental Section). [b] Estimated by NMR analysis of the derived MTPA esters.[6] [c] Determined on the basis of the optical purity of the alcohol obtained from the ester by alkaline hydrolysis (see the Experimental Section). [d] Lit.[7] $[\alpha]^{25}_{D}$ −41.3° (neat). [e] Lit.[8] $[\alpha]^{20}_{D}$ −47.0° (*c* 2.2, hexane). [f] Lit.[9] $[\alpha]^{25}_{D}$ +41.8° (benzene). [g] Lit.[10] $[\alpha]^{25}_{D}$ +12.9° (*c* 6, ether). [h] Lit.[3b] $[\alpha]^{25}_{D}$ +7.2° (*c* 5, EtOH).

Table II. Esterification of Primary Alcohols[a]

				recovered alcohol				produced ester			
substrate	anhydride	time, h	conv, %	yield, %	$[\alpha]^{25}_{D}$, deg	isomer	ee, %	yield, %	$[\alpha]^{25}_{D}$, deg	isomer	ee,[b] %
6	acetic	1	60	34	+4.5[e] (neat)	*R*	28[c]	50	−0.4 (benzene)	*S*	8
7	acetic	0.5	60	30	−0.57[f] (neat)	*R*	36[c]	40	+0.41 (neat)	*S*	17
8	acetic	0.7	50	42	+46.0[g] ($CHCl_3$)	*S*	>95[d]	40	−36.1 ($CHCl_3$)	*R*	>95
8	propionic	0.5	50	45	+45.9 ($CHCl_3$)	*S*	>95	42	−34.3 ($CHCl_3$)	*R*	>95
9	acetic	0.7	50	44	+55.3[h] ($CHCl_3$)	*S*	>95[d]	43	−43.0 ($CHCl_3$)	*R*	>95
9	propionic	0.5	50	41	+55.4 ($CHCl_3$)	*S*	>95	40	−42.1 ($CHCl_3$)	*R*	>95
9	butyric	2	50	45	+55.3 ($CHCl_3$)	*S*	>95	41	−42.4 ($CHCl_3$)	*R*	>95

[a] All the reactions were performed in benzene (40 mL) at room temperature (21 ± 2 °C); substrate, 24 mmol; anhydride, 24 mmol; enzyme, 0.08 g of lipase Amano P adsorbed on 0.32 g of Celite 577. [b] Determined on the basis of the optical purity of the alcohol obtained from the ester by alkaline hydrolysis (see the Experimental Section). [c] Estimated by GC analysis after oxidation to the acids followed by conversion to the diastereomeric amide with (*S*)-α-methylbenzylamine.[2c] [d] Estimated by GC analysis of the derived MTPA esters.[12] [e] Lit.[13] for *S* $[\alpha]^{24}_{D}$ −17.5° (neat). [f] Lit.[14] $[\alpha]^{25}_{D}$ −1.53° (neat). [g] Lit.[15] $[\alpha]^{16}_{D}$ +47.8° (*c* 1, $CHCl_3$). [h] Lit.[15] $[\alpha]^{21}_{D}$ +57.12° (*c* 1.17, $CHCl_3$).

results obtained with secondary and primary alcohols, respectively.

Discussion

The acylation of secondary alcohols was efficiently accomplished with anhydrides, with benzene as solvent (Table I). We have always found that the enzyme preferentially utilizes the *R* enantiomer; consequently, at the end of the reaction, the remaining alcohol was enriched in the *S* form and the ester produced was enriched in the *R* form. With aromatic substrates, the reaction afforded products in high optical purity (>90%). Although the conversion rates of the aliphatic alcohols 2-octanol and 2-dodecanol were also high, the reactions were not stereoselective.

The reaction rates are significantly higher than those reported up to now for other enzyme-catalyzed resolutions in organic solvents. We carried out the resolution of α-phenethyl alcohol with lipase Amano P in order to compare our approach with the enzymatic transesterification.[3b] Trichloroethyl butyrate was used as the activated ester to enhance the reaction rate. The reaction was completed in 120 h, affording products with ee greater than 90%.[11] Using butyric anhydride in the enzyme-catalyzed acylation, we obtained the same ee, but the reaction time was reduced to 8 h. As shown in the Experimental Section, the yields and the purities of the products obtained by both methods are comparable. However, the reaction rate of asymmetric acylation is much higher than that of transesterification. This confirms the absence of product (alcohol) inhibition in the acylation reaction differently from transesterification.

Primary alcohols were also esterified in organic solvents with anhydrides as acylating agents. The results are shown in Table II. The reaction rates are even higher than those observed with secondary alcohols. However, the resolution of the racemic mixture was fully accomplished only in the case of **8** and **9**. The *R* enantiomers of these alcohols are preferentially esterified by lipase P. In a similar way, in the enzymatic resolution of these compounds by hydrolysis of the corresponding racemic esters, lipase P reacts preferentially with the *R* enantiomer.[16] At the end of the reaction the alcohol produced is in the *R* form, while the ester in the *S* form. This indicates that stereospecificity of enzymatic hydrolysis in aqueous solution is preserved in the organic solvent in the case of the acylation reaction. Therefore, the two procedures can be considered complementary for the final product composition.

As shown in Tables I and II, the fastest rates are observed when propionic anhydride is used as the acylating agent. The stereoselectivity of the reaction does not depend on the anhydrides used.

The resolution of racemic alcohols with anhydrides was also tried with other enzymes. Unlike lipase P, porcine pancreatic lipase (PPL) and lipase from *Candida cylindracea* displayed low activity toward secondary alcohols;[17] yet both enzymes showed high activity toward the primary alcohols but poor enantioselectivity. For example, when

(11) The same reactions have been studied by using porcine pancreatic lipase with trichloroethyl butyrate[3b] and lipase from *Candida cylindracea* with tributyrin.[3a] The reactions reached about 45% conversion after 133 and 150 h, respectively.

(12) Hamaguchi, S.; Asada, M.; Hasegawa, J.; Watanabe, K. *Agric. Biol. Chem.* **1984**, *48*, 2331.

(13) Bernstein, H.; Whitemore, C. *J. Am. Chem. Soc.* **1939**, *61*, 1326.

(14) Kenyon, J.; Platt, B. C. *J. Chem. Soc.* **1939**, 635.

(15) Tsuda, Y.; Yoshimoto, K.; Nishikawa, T. *Chem. Pharm. Bull.* **1976**, *29*, 3593.

(16) Hamaguchi, S.; Hasegawa, J.; Kawaharada, H.; Watanabe, K. *Agric. Biol. Chem.* **1984**, *48*, 2055.

(17) One gram of phenylethanol and 800 mg of acetic anhydride were dissolved in 20 mL of benzene; 80 mg of PPL adsorbed on 300 mg of Celite was added to the solution. After 24 h the conversion was 4%.

2-phenyl-1-propanol and acetic anhydride were allowed to react in benzene in the presence of PPL immobilized on Celite, the ee of unreacted alcohol was 59% at 50% conversion.

Lipase P was also able to catalyze efficiently the acylation reaction under different experimental conditions. The enzymatic acylations could also be carried out in other organic solvents immiscible with water, such as hexane, methylene chloride, toluene, and chloroform. The presence of water in the reaction mixture had a significant inhibitory effect on the enzymatic activity. Therefore, anhydrous organic solvents were employed. The amount of water associated with the immobilized enzyme was the only water present in the reaction mixture. In the standard procedure the water content was about 1% (±0.1). The immobilized enzyme, recovered after acylation reaction, was recycled several times with a small loss of activity. This indicates that under these reaction conditions the enzyme is not chemically modified by acetic or propionic anhydrides, although they are strong acylating agents for the protein amino side chains in aqueous solution.[18] There was no evidence of chemical modification of the enzyme after recovery from the organic solvents as indicated by DISC electroforesis in nondenaturating conditions.

In conclusion, in this study we report a new approach for the use of enzymes in organic solvents. The method represents a significant improvement in the use of enzymes for the preparative production of optically active alcohols. The described procedure is fast, simple and can be readily scaled up.

Experimental Section

The optical rotation was measured with a Perkin-Elmer 241 polarimeter. ^{1}H NMR and ^{19}F NMR spectra were recorded in $CDCl_3$ solution [$(CH_3)_4Si$ and TFA as internal standard, respectively] on a Bruker AM-300 instrument. GC analyses were carried out on a 2 mm × 4 m SP 2100 3% column at 100–250 °C and with a flame ionization detector. The optical purity of compounds **6** and **7** was determined with a 0.32 mm × 30 m SPB-1 capillary column at 220 °C of the diasteromeric amide[2c] and for **8** and **9** with a 0.5 m × 4 mm DCQF-1 10% on Chromosorb AW DMCS column at 220 °C of the corresponding MTPA esters.[12] Lipase Amano P (30 units/mg) was purchased from Amano Chemical Co. Porcine pancreatic lipase (13 units/mg) and lipase from *Candida cylindracea* (500 units/mg) were purchased from Sigma Chemical Co. All racemic alcohols were obtained commercially. All organic solvents used in this work were of analytical grade and had been previously dehydrated by shaking with 4-Å molecular sieves for 24 h at room temperature.

Adsorption of Enzymes on Celite. Celite 577 (2 g) was washed with water and 0.1 N phosphate buffer and then added to a solution of 500 mg of lipase Amano P in 10 mL of a 0.1 N phosphate buffer. The mixture was spread on a watch glass and left to dry at room temperature with occasional mixing until visibly dry. The water content was about 1% as determined by the Fisher method.

Lipase-Catalyzed Acylation of 1–5. The following procedure is representative. To a magnetically stirred solution of (*R*,*S*)-1-phenylethanol (2 g, 16.3 mmol) and acetic anhydride (1.66 g, 16.3 mmol) in benzene (40 mL) was added lipase Amano P (0.12 g, 3600 units) supported on Celite 577 (0.48 g), and the reaction mixture was stirred at room temperature. Periodically 1-μL aliquots of the liquid phase were withdrawn and analyzed by GC. After 24 h, approximately 50% conversion was reached, and the reaction was stopped. The solid enzyme preparation was filtered off, and the filtrate was washed with 5% aqueous Na_2CO_3 (40 mL), dried over sodium sulfate, and evaporated to dryness. Chromatography on silica gel with 95:5 *n*-hexane/ether as eluant afforded 0.86 g (43%) of (*S*)-(−)-1-phenylethanol [$[\alpha]^{25}_D$ −41.0° (neat) ee > 95%; ^{1}H NMR ($CDCl_3$) δ 1.31–1.48 (3 H, d), 2.38 (1 H, s), 4.61–4.95 (1 H, q), 7.30 (5 H, s). Anal. Calcd for $C_8H_{10}O$: C, 78.65; H, 8.25. Found: C, 78.61; H, 8.27] and 1.02 g (39%) of (*R*)-(+)-1-phenylethanol acetate [$[\alpha]^{25}_D$ +106° (*c* 1, ether); ^{1}H NMR ($CDCl_3$) δ 1.45–1.60 (3 H, d), 2.06 (3 H, s), 5.71–6.08 (1 H, q), 7.32 (5 H, s). Anal. Calcd for $C_{10}H_{12}O_2$: C, 72.56; H, 8.10. Found: C, 72.52; H, 8.12]. Optically active alcohols **1–5** and the corresponding esters have been prepared using the procedure indicated above (Table I). ^{1}H NMR analysis of each compound was in good agreement with the literature data and elemental analysis showed a purity ≥95%.

Lipase-Catalyzed Acylation of 6–9. The following procedure is representative. To a magnetically stirred solution of (*R*,*S*)-5-(hydroxymethyl)-3-*tert*-butyloxazolidin-2-one[16] (4 g, 24 mmol) and propionic anhydride (3.1 g, 24 mmol) in benzene (40 mL) was added lipase Amano P (0.08 g, 2400 units) supported on Celite 577 (0.32 g), and the reaction mixture was stirred at room temperature. Periodically 1-μL aliquots of the liquid phase were withdrawn and analyzed by GC. After 30 min, approximately 50% of conversion was reached and the reaction was stopped. The solid enzyme preparation was filtered off, and the solution washed with 5% aqueous Na_2CO_3 (40 mL), dried over sodium sulfate, and evaporated to dryness. Chromatography on silica gel with 1:1 *n*-hexane/ether as eluant afforded 1.80 g (45%) of (*S*)-(+)-5-(hydroxymethyl)-3-*tert*-butyloxazolidin-2-one [$[\alpha]^{25}_D$ +45.9° (*c* 1, $CHCl_3$) ee >95%; ^{1}H NMR ($CDCl_3$) δ 1.4 (9 H, s), 3.4–3.95 (5 H, m), 4.3–4.6 (1 H, m); Anal. Calcd for $C_8H_{15}NO_3$: C, 55.47; H, 8.77; N, 8.07. Found: C, 55.41; H, 8.71; N, 8.02] and 2.32 g (42%) of (*R*)-(−)-[(propionyloxy)methyl]-3-*tert*-butyloxazolidin-2-one [$[\alpha]^{25}_D$ −34.2° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.05–1.28 (3 H, t), 1.42 (9 H, s), 2.20–2.28 (2 H, q), 3.35–3.85 (2 H, m), 4.1–4.25 (2 H, m), 4.4–4.75 (1 H, m). Anal. Calcd for $C_{11}H_{19}NO_4$: C, 57.62; N, 8.35; N, 6.11. Found: C, 57.60; H, 8.38; N, 6.11.

Optically active alcohols **6–9** and the corresponding esters were prepared by using the above procedure (Table II). ^{1}H NMR analysis of each compound was in good agreement with the literature data, and elemental analysis showed a purity ≥95%.

Alkaline Hydrolysis of Esters 1–9. The following procedure is representative. (*R*)-(+)-1-Phenylethanol acetate, $[\alpha]^{25}_D$ +106° (*c* 1, ether) (1 g) was dissolved in a 1 M solution of NaOH in absolute ethanol (20 mL). The solution was stirred for 4 h at 25 °C and followed by GC analysis. The solvent was then evaporated under vacuum. Chromatography on silica gel with 95:5 hexane/ether as eluent afforded 0.65 g (88%) of (*R*)-(+)-1-phenylethanol, $[\alpha]^{25}_D$ +41.1 (neat); ee ≥95%.

Optically active alcohols **1–9** were prepared from the corresponding enzymatically produced esters by using the above procedure.

Lipase-Catalyzed Transesterification of 1. To a magnetically stirred solution of (*R*,*S*)-1-phenylethanol (2 g, 16.3 mmol) and trichloroethyl butyrate (3.6 g, 16.7 mmol) in benzene (40 mL) was added lipase Amano P (0.12 g, 3600 units) supported on Celite 577 (0.48 g), and the reaction mixture was stirred at 25 °C. Periodically 1-μL aliquots of the liquid phase were withdrawn and analyzed by GC. After 120 h, approximately 48% conversion was reached. The solid enzyme preparation was filtered off, and the solvent was evaporated. Chromatography on silica gel with 95:5 hexane–ether as eluant afforded 0.8 g (40%) of (*S*)-(−)-phenylethanol, $[\alpha]^{25}_D$ −39.1° (neat) ee 95%, and 1.4 g (38%) of (*R*)-(+)-1-phenylethanol butyrate, $[\alpha]^{25}_D$ +97.5° (*c* 1, ether).

MTPA Esters. The following procedure is representative. To a magnetically stirred solution of (*S*)-1-phenylethanol (0.2 g, 1.63 mmol) and dry pyridine (0.4 mL) in carbon tetrachloride (0.5 mL) was added (+)-MTPA chloride[6] (0.043 g, 1.7 mmol). The reaction mixture was then shaken at room temperature for 15 h, diluted with ether (20 mL), washed successively with dilute HCl, saturated sodium carbonate solution, and water, and dried ($MgSO_4$). The filtered ether solution was evaporated, and the residue was dissolved in $CDCl_3$ for NMR analysis. The ee was determined by integration of the CF_3 signals: 1-phenylethanol *S* form δ 5.78, *R* form δ 5.25 (lit.[6] 5.81, 5.30). Compounds **1–5**, **8**, and **9** have been transformed in MTPA esters by using the procedure described above. Enantiomeric excess of **1–5** have been estimated by ^{19}F NMR analysis and of **8** and **9** by GLC analysis.[12]

***α*-Methylbenzylamine Derivatives.** The following procedure is representative. (*R*)-2-Phenyl-1-propanol was oxidized to

(18) Fieser, L. F.; Fieser, M. *Reagents for organic synthesis*; Wiley: New York, 1967; p 142.

(R)-2-phenylpropionic acid by using Jones Reagent[19] and converted to the acid halide with $SOCl_2$/DMF in ether. To a magnetically stirred solution of (R)-α-methylbenzylamine (0.48 g, 4.0 mmol) in toluene (20 mL) was added (R)-phenylpropionic acid chloride (0.3 g, 1.8 mmol). The reaction mixture was then shaken at room temperature for 1 h and washed with dilute HCl and successively with water. The organic layer was dried ($MgSO_4$) and evaporated to dryness, and the residue was crystallized from ethyl acetate/hexane to give desired product in 62% yield. Anal. Calcd for $C_{17}H_{19}NO$: C, 80.57; H, 7.56; N, 5.53. Found: C, 80.50; H, 7.52; N, 5.58. Enantiomeric excess was estimated by GLC analysis of diastereomeric amides: R,R form 3.10 min, R,S form 3.40 min (lit.[2c] 3.14, 3.48).

The procedure above described has been used for determination of ee of 2-ethyl-1-hexanol (6).

Electrophoresis. Polyacrylamide discontinuous gel electrophoresis in nondenaturating conditions was performed according to the method developed by Ornstein[20] and by Davis.[21] The concentrations of acrylamide were 8% in the resolving gel and 4% in the stacking gel. The electrophoretic separations were run under constant current output (25 mA). The gels were stained with Coomassie blue G-250. Three lipase P samples were recovered by filtration from the reactions in benzene in the presence of acetic, propionic, and butyric anhydrides, respectively. The enzymes were then extracted from the solid support with an aqueous buffer at pH 7. No differences in the electrophoretic mobility were observed between the three protein samples and a freshly prepared aqueous solution of lipase P. Conversely, acetylation of lipase P with acetic anhydride in aqueous solution[18] was complete after 1 h at pH 7.

(19) Means, G. E.; Feeney, R. E. *Chemical Modification of Proteins*; Holden-Day: San Francisco, 1971.

(20) Ornstein, L. *Ann. N.Y. Acad. Sci.* **1964**, *121*, 321.

(21) Davis, B. J. *Ann. N.Y. Acad. Sci.* **1964**, *121*, 404.

Notes

A Simple Way to (3a*R*,4*S*,7a*S*)-(*Z*)-1-Ethylideneoctahydro-7a-methyl-1*H*-4-indenol, a Synthon for Total Synthesis of Vitamins D

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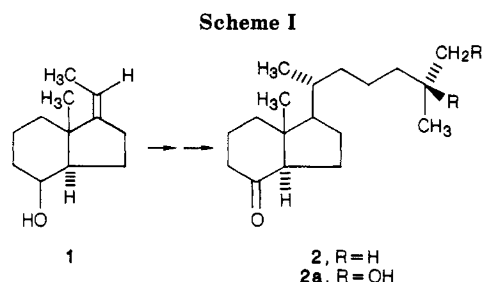
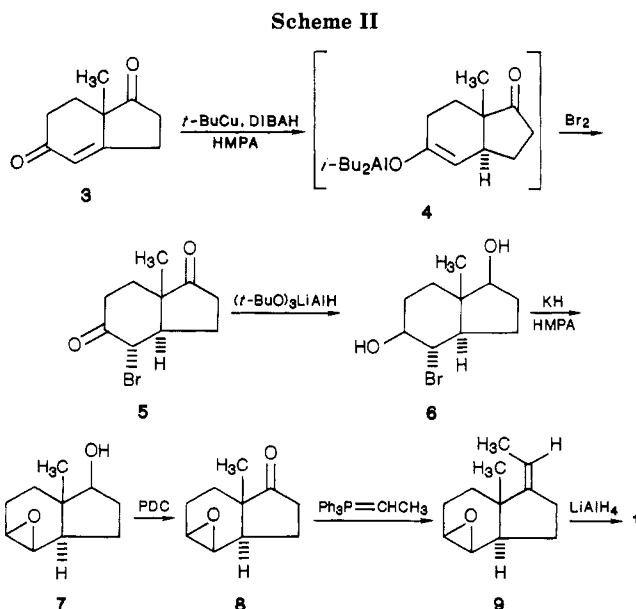
The discovery of hydroxylated metabolites[1] of vitamin D_3, which are more active than commonly used vitamin D_3, has induced interest in the synthesis of these compounds. Several reviews[2] and papers[3] have recently been published on this subject. Uskokovic[4] et al. have reported the synthesis of very useful synthon 1 which was transformed into Grundmann ketone 2 and into its hydroxylated derivatives 2a (Scheme I).

The synthesis of synthon 1 described by Uskokovic[4] et al. started from easily synthesized[5] enedione 3 and required 13 steps. We present a simpler six-step synthesis of synthon 1, starting from enedione 3. Recently we reported[6] the stereoselective reductive addition of electrophiles to enedione 3, which led to *trans*-7a-methyloctahydro-1*H*-indene-1,5-dione derivatives. This reductive additions involve hydride transfer from the complex of *tert*-butyl-

Scheme I



Scheme II



(1) Holik, M. F.; DeLuca, H. F. *Annu. Rev. Med.* **1974**, *25*, 349. DeLuca, H. F.; Schoes, H. K. *Annu. Rev. Biochem.* **1976**, *45*, 631. DeLuca, H. F.; Paaren, H. E.; Schos, H. K. *Top. Curr. Chem.* **1979**, *83*, 3.

(2) Georghiou, P. E. *Chem. Soc. Rev.* **1977**, *6*, 83. Lythgoe, B. *Chem. Soc. Rev.* **1980**, *84*, 449. Yakhimovich, R. I. *Russ. Chem. Rev.* **1980**, *49*, 371. Jones, M.; Rasmusson, G. U. *Fortschr. Chem. Org. Naturst.* **1980**, *39*, 64. Kametani, T.; Furuyama, H. *Med. Res. Rev.* **1987**, *7*, 147.

(3) Sardina, F. J.; Mourino, A.; Castedo, L. *J. Org. Chem.* **1986**, *51*, 1264. Mascarenas, J. L.; Mourino, A.; Castedo, L. *J. Org. Chem.* **1986**, *51*, 1269. Andrews, D. R.; Barton, D. H. R.; Hesse, R. H.; Pechet, M. M. *J. Org. Chem.* **1986**, *51*, 4819. Baggiolini, E. G.; Iacobelli, J. A.; Hennessy, B. M.; Batcho, A. D.; Sereno, J. F.; Uskokovic, M. R. *J. Org. Chem.* **1986**, *51*, 3098. Nemoto, H.; Kusobe, H.; Fukumoto, K.; Kametani, T. *J. Org. Chem.* **1986**, *51*, 5311. Castedo, L.; Mourino, A.; Sarandeses, L. A. *Tetrahedron Lett.* **1986**, *27*, 1523. Baggiolini, E. G.; Hennessy, B. M.; Iacobelli, J. A.; Uskokovic, M. R. *Tetrahedron Lett.* **1987**, *28*, 2095. Castedo, L.; Mascarenas, J. L.; Mourino, A. *Tetrahedron Lett.* **1987**, *28*, 2099. Sterling, J.; Slovin, E.; Barasch, D. *Tetrahedron Lett.* **1987**, *28*, 1685. Solladie, G.; Hutt, J. *J. Org. Chem.* **1987**, *52*, 35.

(4) Wovkulich, P. M.; Barcelos, F.; Batcho, A. D.; Sereno, J. F.; Baggiolini, E. G.; Hennessy, B. M.; Uskokovic, M. R. *Tetrahedron* **1984**, *40*, 2283.

(5) Hajos, Z. G.; Parrish, D. R. *J. Org. Chem.* **1974**, *39*, 1615.

(6) Daniewski, A. R.; Kiegiel, J.; Piotrowska, E.; Warchoł, T.; Wojciechowska, W. *Liebigs Ann. Chem.* **1988**, 593.

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