Alternative Route to Carbacyclin using Lipase-Catalysed Enantioselective Hydrolysis of Methyl 8-Acetoxybicyclo[4.3.0]non-3-ene-7-carboxylate

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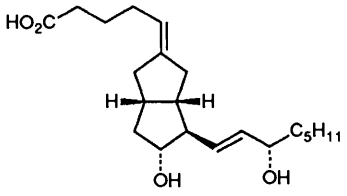
Pseudomonas fluorescens lipase was found to catalyse the hydrolysis of the racemic acetate methyl 8-acetoxybicyclo[4.3.0]non-3-ene-7-carboxylate **4** in a highly enantioselective fashion to provide the optically active (−)-methyl 8-hydroxybicyclo[4.3.0]non-3-ene-7-carboxylate (−)-**3**. This compound was easily converted into a bicyclo[3.3.0]octane derivative, methyl 3-acetoxy-7-oxobicyclo[3.3.0]octane-2-carboxylate **8**, an intermediate in the synthesis of carbacyclin **1**.

Enantioselective synthesis of carbacyclin **1**,[1] a potent inhibitor of platelet aggregation as well as a powerful vasodilator, has aroused much synthetic interest. At the heart of its synthesis is the enantioselective construction of bicyclo[3.3.0]octane intermediates such as **8**.[2] Our initial approach to optically active



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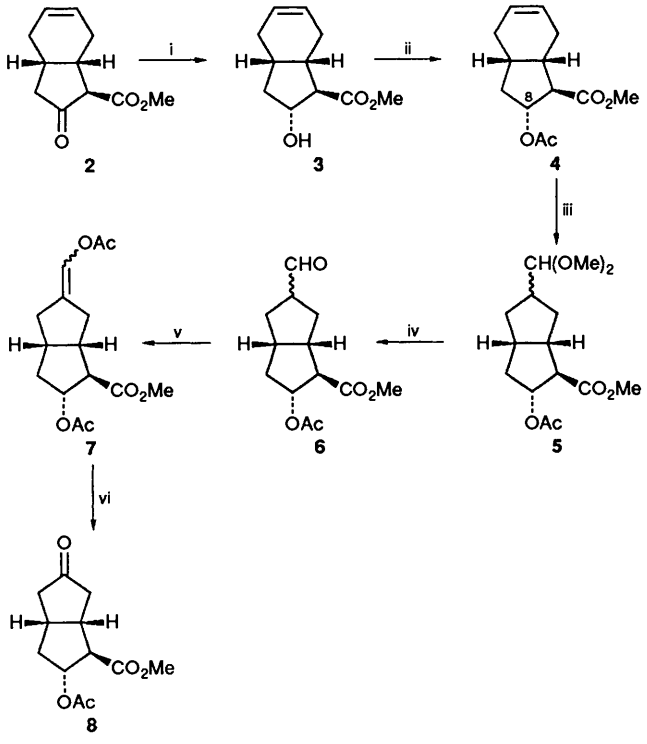
carbacyclin has included a microbial reduction of methyl 8-oxobicyclo[4.3.0]non-3-ene-7-carboxylate.[3] However, tedious work-up and poor yields render this approach less than practical. Herein, we describe an efficient solution to these problems by using lipase-catalysed enantioselective hydrolysis. This new route demonstrates the usefulness and applicability of the recently developed lipase-catalysed enantioselective hydrolysis[4] to the synthesis of optically active bicyclo[4.3.0]nonene as well as bicyclo[3.3.0]octane skeletons.

Results and Discussion

The substrate **4** was prepared by stereoselective reduction of keto ester **2** with $NaBH_4$ followed by subsequent treatment with acetic anhydride (see Scheme 1). In order to identify lipases that might effect the desired transformation with respect to yield and enantioselectivity, screening of several commercially available lipases was undertaken. The hydrolysis was performed in a 0.1 mol dm^{-3} phosphate buffer solution (pH 7.0) at 33 °C and was terminated after 24 h. The enantiomeric excess was determined by comparison of the ^1H NMR spectrum of the (+)-methoxy(phenyl)trifluoromethylacetyl (MTPA) ester[5] of the hydrolysed alcohol with that of the MTPA ester of the racemic alcohol in which a good separation of two methyl signals of the methyl esters centred at δ 3.73 and 3.71 was observed. The absolute configuration was assigned by oxidation of the hydrolysed alcohol into the known compound.[3] The results are summarized in Table 1. Except for entry 4, the acetate hydrolysed with lipases was always of the *R*-configuration, which is required for the synthesis of carbacyclin. Among the



Scheme 1 *Reagents and conditions:* i, $NaBH_4$; ii, Ac_2O, pyridine; iii, $Tl(NO_3)_3$, TMOF; iv, 88% HCO_2H; v, Ac_2O, THF, DMAP; vi, O_3, CH_2Cl_2

tested lipases, *Pseudomonas fluorescens* lipase (PFL) was found to be the best catalyst, with which the alcohol (−)-**3** was obtained in 96% enantiomeric excess (ee) (48% chemical yield). It should be noted that this screening process has not been optimized; nevertheless, it did disclose another facet of an enzyme's specificity with the substrate.

The ring contraction of the bicyclo[4.3.0]nonene (−)-**4**, which was prepared by acetylation of the alcohol (−)-**3**, into a bicyclo[3.3.0]octane **5** was efficiently effected by treatment with thallium(III) nitrate in the presence of trimethyl orthoformate (TMOF)[6] as solvent. The bicyclo[3.3.0]octane acetal **5** was isolated in 73% yield with diastereoselectivity 80:20 based on ^1H NMR spectral analysis of the product (two types of doublets, appearing at δ 4.18 and 4.16). This mixture was then treated with 88% formic acid to produce a mixture of aldehyde **6** in the ratio 5:1. The aldehyde proton produced two types of doublets, at δ 9.67 and 9.65 (the major one). Treatment of aldehyde **6** with acetic anhydride in the presence of 4-(dimethylamino)pyridine (DMAP) in tetrahydrofuran (THF) gave enol

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Table 1 Lipase-catalysed hydrolysis of compound (±)-**4**

Entry[a]	Lipase	Enantio-selectivity	Product **3** Yield (%)	Product **3** ee (%)	Recovered acetate **4** Yield (%)	Recovered acetate **4** ee (%)
1	My-30	*R*	24	53	75	8
2	Amano P	*R*	51	96	47	87
3	C.C. Sigma	*R*	24	60	75	19
4	OF-360	*S*	24	8	33	2
5	Lipase Tokyo	*R*	33	93	66	19
6	Amano A-6	*R*	49	12	49	7
7	Amano A	*R*	21	22	76	2

[a] Entry: 1, *Candida cylindracea* MY-30; 2, *Pseudomonas fluorescens*; 3, *Candida cylindracea* (Sigma); 4, *Candida cylindracea* OF-360; 5, *Chromobacterium viscosum*; 6, *Aspergillus niger*; 7, *Aspergillus niger*.

acetate **7** in 65% yield. The olefinic proton of its ^{1}H NMR spectrum showed two singlet peaks, at δ 7.02 and 7.00, in the ratio 1:1. Ozonolysis of compound **7** proceeded smoothly to provide the bicyclo[3.3.0]octan-3-one **8**, a desired intermediate for the synthesis of carbacyclin.

It is noteworthy that lipase-catalysed enantioselective hydrolysis is simple and practical and the results described here not only provide a practical route to carbacyclin but also shed light on the extensive application of PFL to the synthesis of a variety of designed chirons.[7]

Experimental

General Details.—For general details, see ref. 6. The lipases from *Pseudomonas fluorescens* (Amano P), *Aspergillus niger* (Amano A-6) and (Amano A) were obtained from Amano Pharmaceutical Co., Japan. *Candida cylindracea* (MY-30) and (OF-360) were obtained from Meito Sangyo Co., Ltd, Japan.

Methyl 8-Hydroxybicyclo[4.3.0]*non*-3-ene-7-*carboxylate* **3**.—Sodium borohydride (380 mg, 10 mmol) was added portionwise to a solution of compound **2** (2 g, 10 mmol) in methanol (20 cm^3) at −40 °C. The mixture was stirred for 4 h. Acetone (1 cm^3) was then added to quench the reaction. The reaction mixture was diluted with brine and then extracted with ethyl acetate. The extract was washed with brine and dried ($MgSO_4$). After concentration under reduced pressure, the crude product was chromatographed on silica gel and eluted with 10% ethyl acetate in hexane to afford the *title compound* **3** (1.86 g, 93%) as an oil (Found: M$^+$, 196.251. $C_{11}H_{16}O_3$ requires M, 196.248); ν_{max}(neat)/cm^{-1} 3450 and 1730; δ_H(100 MHz) 1.40–2.40 (9 H, m), 2.54 (1 H, dd, *J* 9.2, 7.1, 7-H), 3.71 (3 H, s, CO_2Me), 4.55 (1 H, m, 8-H) and 5.76 (2 H, br, olefinic H); *m/z* (EI) 196 (M$^+$), 178, 164 and 119.

Methyl 8-Acetoxybicyclo[4.3.0]*non*-3-*ene*-7-*carboxylate* **4**.—Acetic anhydride (1 g, 10 mmol) was added to a stirred solution of hydroxy ester **3** (1.5 g, 7.7 mmol) and DMAP (10 mg) in pyridine (10 cm^3) at room temperature. After being stirred for 3 h the mixture was poured onto the cold 10% hydrochloric acid, then extracted with ethyl acetate (50 cm^3 × 2). The extract was washed successively with 5% aq. $NaHCO_3$ and brine and was then dried over $MgSO_4$. After removal of the solvent, the residue was purified by chromatography. The fraction eluted with 5% ethyl acetate in hexane afforded the *title compound* **4** (1.82 g, 92%) as a liquid (Found: M$^+$, 238.286. $C_{13}H_{18}O_4$ requires M, 238.281); ν_{max}(neat)/cm^{-1} 1740 and 1735; δ_H 1.40–2.52 (8 H, m), 2.03 (3 H, s, OAc), 2.67 (1 H, dd, *J* 8.5, 6.2, 7-H), 3.71 (3 H, s, CO_2Me), 5.36 (1 H, m, 8-H) and 5.71 (2 H, br, olefinic H); *m/z* (EI) 238 (M$^+$), 207, 178, 165 and 118.

Typical Procedure for Lipase-catalysed Hydrolysis.—PFL (500 mg) was added to a stirred mixture of compound **4** (1 g, 4.2 mmol) in 0.1 mol dm^{-3} phosphate buffer (200 cm^3; pH 7). The mixture was stirred for 24 h at 33 °C. Hydrolysis was terminated by extraction with diethyl ether. The extract was washed with brine and dried over $MgSO_4$, then concentrated under reduced pressure to give a residue, which was subjected to column chromatography. The fraction eluted with 5% ethyl acetate in hexane afforded a starting material diastereoisomer, compound (+)-**4** (490 mg, 48%), $[\alpha]_D$ +18.1° (*c* 0.82, $CHCl_3$). The second fraction, eluted with 30% ethyl acetate in hexane, afforded the alcohol (−)-**3** (430 mg, 51%), $[\alpha]_D$ −13.3° (*c* 0.6, $CHCl_3$).

Methyl (1S,6S,7R,8R)-8-*Acetoxybicyclo*[4.3.0]*non*-3-*ene*-7-*carboxylate* (−)-**4**.—Title compound (−)-**4** was prepared from the optically active (−)-**3** in a manner similar to that used for the preparation of racemic **4**; the product had $[\alpha]_D$ −19.5° (*c* 0.8, $CHCl_3$).

Methyl (1S,2R,3R,5S,7RS)-3-*Acetoxy*-7-*dimethoxymethylbicyclo*[3.3.0]*octane*-2-*carboxylate* **5**.—A solution of thallium(III) nitrate trihydrate (4.2 g, 9.5 mmol) in TMOF (20 cm^3) was added dropwise to a stirred solution of the bicyclononene (−)-**4** (1.5 g, 6.3 mmol) in TMOF (10 cm^3) at 25 °C. The mixture was stirred for 20 min, then diluted with methylene dichloride. After filtration, the filtrate was washed successively with 5% aq. $NaHCO_3$ and brine, and was then dried ($MgSO_4$). The solvent was removed to leave a residue, which was purified by flash column chromatography. The fraction eluted with 5% ethyl acetate in hexane gave the *title compound* **5** (1.38 g, 73%) as an oil (Found: M$^+$, 300.359. $C_{15}H_{24}O_6$ requires M, 300.355); ν_{max}(neat)/cm^{-1} 1742 and 1735; δ_H 1.40–2.64 (10 H, m), 2.01 (3 H, s, OAc), 3.34 [6 H, s, $(OMe)_2$], 3.68 (3 H, s, CO_2Me), 4.14 [$\frac{4}{5}$ H, d, *J* 7.3, C*H*$(OMe)_2$], 4.16 [$\frac{1}{5}$ H, d, *J* 6.8, C*H*$(OMe)_2$] and 5.24 (1 H, m, 3-H); *m/z* (EI) 300 (M$^+$), 268, 225 and 208.

Methyl (1S,2R,3R,5S,7RS)-3-*Acetoxy*-7-*formylbicyclo*[3.3.0]*octane*-2-*carboxylate* **6**.—A mixture of the acetal **5** (1.3 g, 4.3 mmol) and 88% formic acid (10 cm^3) was stirred for 4 h at 25 °C. The reaction mixture was poured into cold, 5% aq. $NaHCO_3$ and extracted with ethyl acetate. The extract was washed successively with 5% aq. $NaHCO_3$ and brine and, after being dried ($MgSO_4$) and concentrated, the crude product was subjected to short flash column chromatography. Elution with 10% ethyl acetate in hexane afforded the *title compound* **6** (0.98 g, 89%) as a yellow oil (Found: M$^+$, 254.291. $C_{13}H_{18}O_5$ requires M, 254.285); ν_{max}(neat)/cm^{-1} 2720 and 1730–1710; δ_H 1.40–2.94 (10 H, m), 2.02 (3 H, s, OAc), 3.70 (3 H, s, CO_2Me), 5.19 (1 H, m, 3-H), 9.60 ($\frac{5}{6}$ H, d, *J* 2.7, CHO) and 9.65 ($\frac{1}{6}$ H, d, *J* 2.4, CHO); *m/z* (EI) 254 (M$^+$), 225, 211, 194 and 163.

Methyl (1S,2R,3R,5S,7EZ)-3-*Acetoxy*-7-*acetoxymethylenebicyclo*[3.3.0]*octane*-2-*carboxylate* **7**.—A mixture of aldehyde **6** (480 mg, 1.9 mmol), acetic anhydride (576 mg, 5.6 mmol), triethylamine (384 mg) and DMAP (22 mg) in THF (6 cm^3) was stirred at 25 °C for 20 h. Removal of the solvent gave a crude product, which was purified by flash column chromatography. The fraction eluted with 5% ethyl acetate in hexane afforded *enol acetate* **7** (380 mg, 73%) as an oil (Found: M$^+$, 296.329. $C_{15}H_{20}O_6$ requires M, 296.323); ν_{max}/cm^{-1} 1750, 1740, 1725 and 1440; δ_H 1.40–2.85 (10 H, m), 2.12 (3 H, s, OAc), 3.68 (3 H, s, CO_2Me), 5.22 (1 H, m, 3-H), 7.00 (0.5 H, s, olefinic H) and 7.02 (0.5 H, s, olefinic H); *m/z* (EI) 296 (M$^+$), 265, 204 and 176.

Methyl (1S,2R,3R,5R)-3-*Acetoxy*-7-*oxobicyclo*[3.3.0]*octane*-2-*carboxylate* **8**.—Ozone was passed into a solution of compound **7** (360 mg, 1.2 mmol) in anhydrous methylene dichloride (12 cm^3) for 8 min at −78 °C. The reaction mixture was then swept with nitrogen, and then acetic acid (1 cm^3) and zinc (750 mg) were added. The suspension was then stirred for 5 h at room temperature. After filtration of the precipitate, the filtrate was evaporated to leave a residue, which was subjected to flash column chromatography. Elution with 5% ethyl acetate in hexane afforded *keto ester* **8** (120 mg, 45%) as an oil (Found: M^+, 240.251. $C_{12}H_{16}O_5$ requires M, 240.258); $[\alpha]_D$ −16.4° (*c* 0.55, $CHCl_3$); ν_{max}/cm^{-1} 1740 and 1735; δ_H 1.35–1.70 (2 H, m), 2.03 (3 H, s, OAc), 2.25–2.95 (6 H, m), 2.72 (1 H, q, *J* 6.6, 2-H), 3.73 (3 H, s, CO_2Me) and 5.40 (1 H, ddd, *J* 12.0, 12.0, 6.6, 3-H); *m/z* (EI) 240 (M^+), 209, 180 and 149.

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