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Enzymatic Hydrolysis of *Meso*-cyclobutene Esters.

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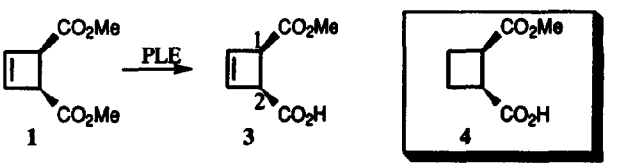
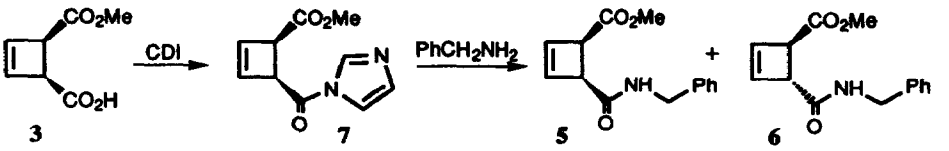
Abstract: Dimethyl *cis*-2-cyclobutene-1,2-dicarboxylate **1** was kinetically resolved by porcine liver esterase to give methyl hydrogen (1*R*, 2*S*)- (+)-*cis*-3-cyclobutene-1,2-dicarboxylate **3**. ((1*R*,4*S*)-4-Ethanoyloxymethyl-2-cyclobutenyl)methyl ethanoate **2** was kinetically resolved using the lipase from *Pseudomonas fluorescens* (Amano P) to give ((1*R*,4*S*)-4-hydroxymethyl-2-cyclobutenyl)methyl ethanoate **8**.

cis-1,2-Disubstituted-3-cyclobutenes are of interest as precursors of chiral substituted dienes. As *meso*-compounds, they are convertible, in principle, into homochiral products in ≤ 100% yield. Below are decribed the asymmetrisations of dimethyl *cis*-3-cyclobutene-1,2-dicarboxylate **1** and ((1*R*,4*S*)-4-ethanoyloxymethyl-2-cyclobutenyl)methyl ethanoate **2**.



Following the rough guide that stereodiscriminating hydrolyses of esters of chiral or *meso*-acids are most likely to succeed using esterases, and comparable hydrolyses of esters of chiral or *meso*-diols with lipases, hydrolysis of the *meso* diester **1** with porcine liver esterase (PLE) was studied. The (1*R*, 2*S*)-monoester **3** was obtained in 96% yield and 86% ee, as determined by ^{1}H n.m.r. analysis of the diastereoisomeric salt formed with (*S*)-(α)-methylbenzylamine, using for this estimation the fully resolved signals attributable to the vinylic protons. The absolute configuration was assignable as the monoester **3** had previously been correlated with the saturated ester **4**. (There is some confusion in the literature over the sign of rotation of the monoester **4**,[1,2] but the assignment of Sabbioni and Jones[2] is consistent with the expected mode of hydrolysis of diester **1** based on the currently favoured model of PLE hydrolysis.[3]) Conversion of monoester **3** into the benzylamide followed different courses depending on how the reaction was carried out. Treatment of monoester **3** with carbonyldi-imidazole (CDI) in $CDCl_3$ with addition of benzylamine immediately following cessation of CO_2 evolution (about 3 minutes) gave the crystalline amide **5** in 68% yield with optical rotation and m.p. unchanged on further recrystallisation.



If the initial reaction mixture was allowed to stand for one hour before addition of benzylamine, the *trans* amide **6** was obtained. Isomerisation of the intermediate imidazolide **7** could be monitored by n.m.r. After approximately thirty minutes the mixture attained equilibrium (11% *cis*: 89% *trans*). The crystalline *trans* amide was recrystallised to constant m.p. and rotation. Evidence that isomerisation at the imidazolide methine centre had occurred was obtained from the circular dichroism curves (Figure) which show the same negative values of $\Delta\varepsilon$ for the $n\rightarrow\pi^*$ ester absorption but opposite $\Delta\varepsilon$ values for the $n\rightarrow\pi^*$ amide band. Deprotonation of a related cyclobutene ester has been observed.[5]

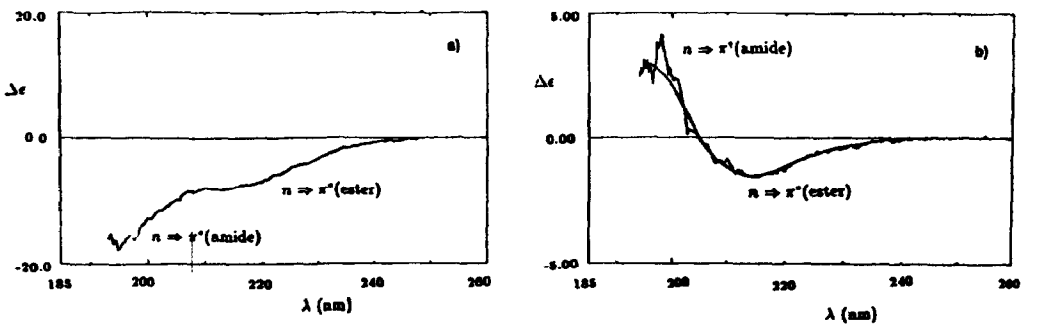
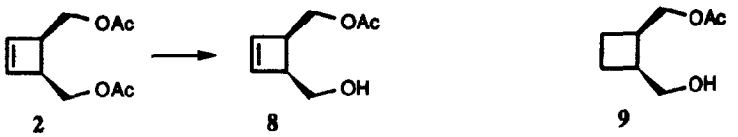


Figure. Circular dichroism curves for a) methyl (1*R*, 2*S*)-(+)-2-benzylcarbamoyl-3-cyclobutenecarboxylate **5**; b) methyl (1*R*, 2*R*)-(-)-2-benzylcarbamoyl-3-cyclobutene carboxylate **6**.

Eleven enzymes were screened for hydrolytic activity towards diester **2**. The lipases from wheat germ, *Candida cylindracea*, *Mucor miehei* (Lipozyme), the esterases from porcine liver and electric eel cholinesterase, rapidly hydrolysed the diester to diol. α-Chymotrypsin and trypsin had no effect. The lipases from porcine pancreas (Steapsin, PPL), *Aspergillus niger* and *Rhizopus javanicus* gave a product of low optical purity, but excellent results were obtained with the lipase from *Pseudomonas fluorescens* (Amano P). When the hydrolysis of diester **2** was controlled by autotitration with pH stat control at pH 7.0, and stopped after one molar equivalent of alkali had been added, the product monoester **8** was obtained with 86% ee as determined by ^{1}H n.m.r. in the presence of (S)-(+)-2 2,2-trifluoro-1-(9-anthryl)ethanol. Repetition of the hydrolysis but halting it after 0.8 molar equivalents of alkali had been added gave monoacetate **8** in 75% yield and >97% ee. The product was fully characterised and converted into the fully characterised *p*-phenylbenzoate.



2 8 9

(The minor enantiomer was not visible in the ^{1}H n.m.r. spectrum. The precision of the measurement was estimated as ±3% by the addition of racemate). Hemmerle and Gais[4] had previously studied the hydrolysis of diester **2** using porcine pancreatic lipase, and had obtained a dextrorotatory product that was correlated with the corresponding laevorotatory saturated analogue **9** of unambiguously determined absolute configuration.[6] In our hands, the maximum optical purity obtainable with PPL (Steapsin) was 16% ee. Although a product of only low optical purity was obtained, it was shown to be dextrorotatory in agreement with the results of Hemmerle and Gais.[4] The reason for the discrepancy became clear when the hydrolysis was studied with different preparations of PPL of increasing purity. For amounts of enzyme of nominally the same activity, it was found that the rate of hydrolysis was inversely proportional to the purity of the enzyme (Table). The hydrolysis of diester **2** by PPL must therefore be attributable to a minor activity, which would account for the difficulty in reproducing results obtained with this enzyme.

Table. Hydrolysis of (1*R*, 4*S*)-4-hydroxymethyl-2-cyclobutenylmethanol **2** with PPL (2000 U).

Lipase source	Activity (U mg^{-1})	Rate of hydrolysis (% h^{-1})
Sigma type II	13	5.0
Boehringer	600	1.6
Sigma type VI-S	16,400	0.5

Experimental

General.

^{1}H n.m.r. spectra were determined at 400 MHz on a Bruker WH400 spectrometer or at 220 MHz on a Perkin Elmer R34 spectrometer. Coupling constants are recorded in Hz. ^{13}C n.m.r. spectra (proton-decoupled) were determined at 100.62 MHz on a Bruker WH400 n.m.r. spectrometer. I.r. spectra were determined on a Perkin Elmer 580-B spectrometer. Mass spectra were determined on a Kratos MS80 mass spectrometer. Optical rotations were determined on an AA-1000 polarimeter (Optical Activity Ltd.) using a 2 dm cell. Autotitrations were carried out using an RTS 882 recording titration system (Radiometer Ltd.). Flash chromatography was carried out using Kieselgel 60 (230-400 mesh) (Merck). Lipases from porcine pancreas and*Candida cylindracea* and Stepasin, electric eel cholinesterase, porcine liver esterase, α-chymotrypsin and trypsin were obtained from

the Sigma Chemical Company Ltd. Purified porcine pancreatic lipase was obtained from Boehringer Mannheim. The lipases from *Aspergillus niger* and *Rhizopus japonicus* were obtained from Biocatalysts Ltd. The lipase from *Mucor miehei* (Lipozyme) was obtained from Novo Industri S/A.

Methyl hydrogen (1*R*, 2*S*)- (+)-3-cyclobutene-1,2-dicarboxylate **3**.

Dimethyl *meso*-3-cyclobutene-1,2-dicarboxylate **3**[7,8] (0.85 g, 5.0 mmol) was dissolved in phosphate buffer (pH 7.0, 67 mM, 10 ml) with stirring at 32°. Porcine liver esterase (260 U) was added. The mixture was stirred and titrated with NaOH (0.5 M) using the autotitrator and pH stat. After the addition of 1 mol equiv. of alkali (9 h), the reaction was stopped by acidification to pH <2 (HCl). The mixture was extracted with dichloromethane (4 x 50 ml). The combined extracts were dried ($MgSO_4$) and evaporated under reduced pressure to give the monoester **3** (0.75 g, 96%). The optical purity (86% ee) was determined by formation of the salt with (*S*)-(-)-α-methylbenzylamine (2 mol/ mol acid **3**). Optical purity was determined conveniently from the vinyl proton signals which were base-line resolved. (Found M^+ 156.0422; $C_7H_8O_4$ requires 156.0422); $[\alpha]_D^{20}$ 6.92 (c 2.5 in $CHCl_3$); ν_{max} (neat) /cm^{-1} 3100 (OH), 1730 (COOMe),1700 (COOH); λ_{max} (EtOH)/nm 202 (ε/dm^3 mol^{-1} cm^{-1} 330), 260 (500); δ_H(220 MHz; $CDCl_3$) 3.66 (3 H, s, CH_3O), 3.92 (2 H, s, 2 x CHCO), 6.25 (2 H, d, J 1.7, 2 x CH=); δ_C (100 MHz; $CDCl_3$) 48.61 ($CHCO_2Me$), 48.86 ($CHCO_2H$), 51.94 (CH_3O), 136.21 and 136.97 (C=C), 170.90 (CO_2Me), 176.03 (CO_2H); *m/z* (EI) 156 (M^+, 2%), 139 (6), 125 (31), 111 (89), 97 (96), 80 (32), 69 (100).

Methyl (1R, 2S)-(+)-2-benzylcarbamoyl-3-cyclobutene carboxylate **5**.

To a solution of methyl hydrogen (1*R*, 2*S*)- (+)-*cis*-3-cyclobutene-1,2-dicarboxylate **3** (312 mg, 2.0 mmol) in anhydrous $CDCL_3$ (2.5 ml) was added 1,1'-carbonyldi-imidazole (490 mg), 3.0 mmol). After the evolution of CO_2 had stopped (approximately 3 min), benzylamine (324 mg, 3.0 mmol) was added. The solvent was removed under reduced pressure and the product was purified by flash chromatography with diethyl ether as eluent to give methyl (1*R*, 2*S*)-(+)-2-benzylcarbamoyl-3-cyclobutene carboxylate **5** (333 mg, 68%) m.p. 107-108°C, unchanged on recrystallisation from CH_2Cl_2-light petroleum. (Found M^+ 245.1052. $C_{14}H_{15}NO_3$ requires 245.1052); $[\alpha]_D^{20}$ 11.7 (c 1.0 in $CHCl_3$); ν_{max} (neat) /cm^{-1} 3300 (s, NH), 3080 (w, NH),1720 (s, CH),1645 (s, CONH); λ_{max} (MeOH)/nm 215 (ε/dm^3 mol^{-1} cm^{-1} 150), 254 (25), 258 (33), 262 (39), 267 (30), 270 (19); δ_H(400 MHz; $CDCl_3$) 3.59 (3 H, s, CH_3O), 3.91 and 3.96 (each 1 H, J 5.0, CHCO), 4.39 (1 H, dd, J 14.6, 5.6, CHNH), 4.46 (1 H, dd, J 14.6, 5.9, CHNH), 5.94 (1 H, br s, NH), 6.26 and 6.40 (each, 1 H, d, J 2.9, CH=), 7.33 5H (m, Ar); δ_C (100 MHz; $CDCl_3$) 43.67 (CH_2N), 49.01 (CHCO), 51.46 (CHCO), 51.84 (Me), 127.44 (127.94, 128.58, Ar), 136.35 (CH=), 138.37 (CH=), 169.55 (CONH), 171.40 (CO_2); *m/z* (EI) 245 (M^+, 1%), 186 (4), 140 (7), 106 (100), 91 (67), 80 (8).

Methyl (1*R*, 2*R*)-(-)-2-benzylcarbamoyl-3-cyclobutene carboxylate **6**.

To a solution of methyl hydrogen (1*R*, 2*S*)- (+)-*cis*-3-cyclobutene-1,2-dicarboxylate **3** (136 mg, 0.87 mmol) in

anhydrous $CDCl_3$ (1 ml) was added 1,1'-carbonyldi-imidazole (230 mg, 1.42 mmol). There was a rapid evolution of CO_2. After 1 h the *cis:trans* ratio of imidazolide was 10:90 (by integration of the signals due to the methyl groups). Benzylamine (160 mg, 1.5 mmol) was added. The solvent was removed under reduced pressure and the product was purified by flash chromatography using ether:light petroleum (2:1) as eluent to give methyl (1*R*, 2*R*)-(-)-2-benzylcarbamoyl-3-cyclobutene carboxylate **6** as colourless platelets, 139 mg (65%).m.p. 79-80°C ($CHCl_3$-light petroleum) (Found: C, 68.8; H, 6.0; N, 5.8. $C_{14}H_{15}NO_3$ requires C, 68.5; H, 6.2; N, 5.7); $[\alpha]_D^{20}$ -255 (c 0.88 in $CHCl_3$); ν_{max} (neat) /cm^{-1} 3270 (m, NH), 3100 (w, NH),2950 (w, CH),1740 (m, COOMe),1640 (s, CONH); λ_{max} (MeOH)/nm 229 (ε/dm^3 mol^{-1} cm^{-1} 180), 249 (116), 254 (139), 260 (158), 265 (144), 269 (118); δ_H(400 MHz; $CDCl_3$) 3.66 (1 H, s, OMe), 3.71 (1 H, br s, CHON), 3.76 (1 H, br s, CHCO2), 4.37 (1 H, dd, J 514.7, 5.6, CHNH), 4.42 (1 H, dd, J 14.7, 5.7), 6.21 (1 H, J 2.8, CH=), 6.24 (1 H, J 2.8, CH=), 6.27 (1 H, br s, NH), 7.27 (5 H, m, Ar); δ_C (100 MHz; $CDCl_3$) 43.44 (CH_2N), 49.15 (CHCON), 50.50 ($CHCO_2$), 51.85 (CH_3O), 127.34 (127.55, 128.51, Ar), 137.33 (C=), 137.42 (C=), 170.21 (CON), 171.70 (CO2); *m/z* (EI) 245 (M^+, 8%), 186 (8), 140 (9), 106 (100), 91 (32), 80 (12).

((1*R*,4*S*)-4-Hydroxymethyl-2-cyclobutenyl)methyl ethanoate **8**.

Method (a), using porcine pancreatic lipase.

To ((1*R*,4*S*)-4-ethanoyloxymethyl-2-cyclobutenyl)methyl ethanoate **2**[7] (199 mg, 1.0 mmol) in phosphate buffer (pH 7.0, 67 mM, 5 ml) containing NaCl (0.4 M) was added with stirring porcine pancreatic lipase (Steapsin, 260 U). The pH was maintained by adding NaOH (0.1 M) containing NaCl (0.4 M) using the autotitrator and pH stat. After the addition of one mole equivalent of alkali (43 h), the reaction mixture was extracted with ethyl acetate (3 x 25 ml). The extracts were combined, dried ($MgSO_4$) and the solvent was removed under reduced pressure to give ((1*R*,4*S*)-4-hydroxymethyl-2-cyclobutenyl)methyl ethanoate **8** 110 mg (as a mixture with starting diacetate and diol (85:7:8). The monoacetate **8** was isolated by flash chromatography using ethyl acetate as eluent to give 80 mg (51%), 16% ee as determined by ^1H n.m.r. in the presence of (*S*)-(+)-2,2,2-trifluoro-1-(9-anthryl)ethanol. Spectroscopic data as for the material prepaed usibg the lipase from *Pseudomonas fluorescens* , below. $[\alpha]_D^{20}$ 1.26 (c 2.0 in $CHCl_3$ (16% ee)).

((1*R*,4*S*)-4-Hydroxymethyl-2-cyclobutenyl)methyl ethanoate **8**.

Method (b), using the lipase from *Pseudomonas fluorescens*. To the diacetate **2** (495 mg, 2.5 mmol) in phosphate buffer (pH 7.0, 67 mM, 10 ml) was added with stirring lipase from *Pseudomonas fluorescens* (10 mg, 300 U). The pH was maintained by the addition of NaOH (0.5 M) using an automatic titrator and pH stat. After the addition of 0.8 mol equiv. of alkali (18.5 h), the mixture was extracted with CH_2Cl_2 (3 x 50 ml). The aqueous residue was saturated with NaCl and again extracted with CH_2Cl_2 (2 x 50 ml). The combined extracts were dried ($MgSO_4$) and evaporated under reduced pressure. The product was purified by flash chromatography using CH_2Cl_2: diethyl ether (4:1) as eluent to give the monoacetate **8**, 293 mg (75%), >97% ee

determined as above. (The minor enantiomer could not be detected until the sample was spiked with racemic material). (Found: C, 61.3; H, 7.7; M^+, 96.0580. $C_8H_{12}O_3$ requires C, 61.5; H, 7.75; M^+, 96.0575); $[\alpha]_D^{20}$ 6.2 (c 2.0 in $CHCl_3$); ν_{max} (neat) /cm^{-1} 3450 (OH), 3050 (w, C=CH),2900 (m, CH),1740 (s, COO); λ_{max} (MeOH)/nm 206 (ε/dm^3 mol^{-1} cm^{-1} 303), 264 (93); δ_H(400 MHz; CCl_4) 1.91 (3 H, s CH_3CO), 3.06 (2 H, m, =CHCHCHCH=), 3.55 (2 H, d, J 7.0, CH_2OH), 4.00 (1 H, dd, J 9.0, 11.4, CHCH$_2$OH), 4.21 (1 H, dd, J 5.90, 11.4, CHCH$_2$OAc), 5.97 (2 H, app s, HC=CH); δ_C (100 MHz; $CDCl_3$) 20.84 (CH_3CO), 44.31 (CH_2OH), 47.91 (CH_2OAc), 62.04 (CHCH$_2$OH), 64.00 (CHCH$_2$OAc), 137.37 (C=), 138.04 (C=), 170.59 (CO); *m/z* (CI (NH_3)) 157 ($(M+1)^+$, 32%),96 (100), 67 (91), 53 (8), 43 (39).

((1R, 4S)-4-(4-Phenylphenyl)methanoyloxymethyl-2-cyclobutenyl)methyl ethanoate.

To a stirred solution of ((1*R*,4*S*)-4-hydroxymethyl-2-cyclobutenyl)methyl ethanoate **8** (242 mg, 1.55 mmol) in anhydrous pyridine (2.5 ml) was added 4-phenylbenzoyl chloride (408 mg, 1.87 mmol). The mixture was stirred overnight and concentrated under reduced pressure. The residue was dissolved in diethyl ether, filtered, and the filtrate was evaporated under reduced pressure. The residue was purified by flash chromatography with diethyl ether: light petroleum (1:2) as eluent to give ((1*R*, 4*S*)-4-(4-phenylphenyl)methanoyloxymethyl-2-cyclobutenyl)methyl ethanoate (521 mg, 99%, >97% pure by ^1H n.m.r.). After recrystallisation (light petroleum), 445 mg (85%), m.p. 61-62°C. (Found: C, 74.7; H, 6.1; M^+, 336.1334. $C_{21}H_{20}O_4$ requires C, 75.0; H, 6.0; M^+, 336.1362); $[\alpha]_D^{20}$ -11.1 (c 1.0 in $CHCl_3$); ν_{max} (neat) /cm^{-1} 3050 (w, C=C), 2950 (w, CH),1745 (s, MeCO),1720 (s, ArCO); λ_{max} (MeOH)/nm 204 (ε/dm^3 mol^{-1} cm^{-1} 33 100), 272 (30 500); δ_H(220 MHz; $CDCl_3$) 1.95 (3 H, s, CH_3CO), 3.28 (1 H, m, CHCH$_2$), 3.39 (1 H, m, CHCH$_2$), 4.27 (2 H, d, J 7, CH_2O), 4.47 (2 H, d, J 7, CH_2O), 6.17 (1 H, m, CH=), 6.20 (1 H, m CH=), 7.47 (3 H, m, Ar), 7.70 (4 H, m, Ar), 8.17 (2 H, d, Ar); δ_C (100 MHz; $CDCl_3$) 20.80 (Me), 44.37 (CH_2OAc), 44.43 (CH_2OCOAr), 63.82 (CH), 64.17 (CH), 126.94 (127.14, 128.02, 128.80, 129.99, Ar), 137.73 (C=C), 137.80 (C=C), 166.13 (CO), 170.81 (CO); *m/z* (EI) 336 (M^+, 4%), 276 (3), 198 (100), 182 (16), 152 (38), 96 (8), 78 (12).

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