SHORT PAPER

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Enzyme catalysed kinetic resolution of racemic methyl 3-acethylbicyclo [2.2.1] hept-5-ene-2-carboxylate using pig's liver esterase†

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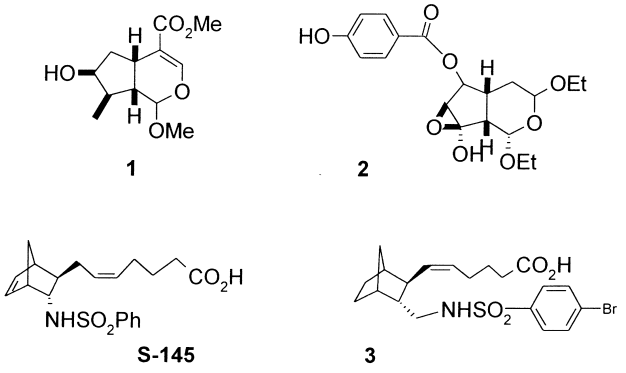
Racemic methyl 3-*endo*-acetylbicyclo[2.2.1]hept-5-ene-2-*exo*-carboxylate and methyl 3-*exo*-acetylbicyclo[2.2.1]hept-5-ene-2-*endo*-carboxylate were prepared (as a 1:1 mixture in 98% yield) from (E)-methyl 4-*oxo*-2-pentenoate, using Diels–Alder methodology. The mixture was separated chromatographically and resolved into the enantiomers by pig liver esterase (PLE) with low to high ee's (98%).

Keywords: kinetic resolution, pig liver esterase

Homochiral compounds belonging to the bicyclo[2.2.1]heptane series are useful synthetic building blocks for terpenoid natural products such as 1-*O*-methyl loganin aglucone **(1)**, and specionin **(2)**[1]. Several pharmaceutical products possess structures related to this unit,[2] among which are the carbocyclic analogues of prostaglandin endoperoxides. These include recently discovered drugs such as S-145 which antagonises the action of thromboxane (TXA_2), and compound **3** as a potent inhibitor of PGE_2. The biological activity of these compounds depends on the absolute and relative configuration of four chiral centres present in these molecules. For example the *d* isomer of S-145 was found to be several times more potent than *l* isomer, exhibiting a higher binding affinity to the receptor.[3] Therefore it would be desirable to obtain new homochiral functionalised bicyclo[2.2.1] heptane intermediates which could be further elaborated to structures analogous to S-145 and **1–3**. In this respect we have exploited the catalytic potential of PLE for the optical resolution of new products with bicyclo[2.2.1]heptane skeleton.

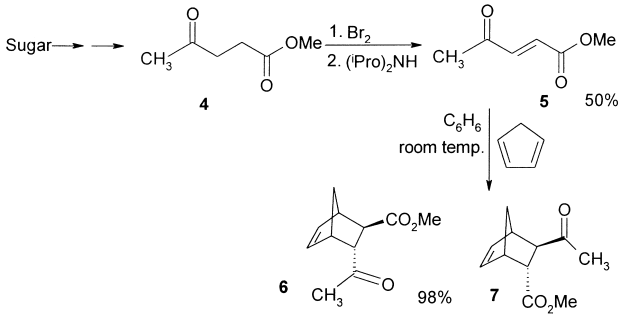
Several chemical and enzymatic syntheses of chiral norbornene derivatives have been reported.[4] As a continuation of our studies in preparation of optically active functionalised bicyclo[2.2.1]heptenes,[5] with the aim of their utilisation as chirons in biologically interesting cyclopentenoids, we describe here the synthesis and kinetic resolution of racemic methyl 3-*endo*-acetylbicyclo[2.2.1]hept-5-ene-2-*exo*-carboxylate **(6)** and methyl 3-*exo*-acetylbicyclo[2.2.1] hept-5-ene-2-*endo*-carboxylate **(7)** using pig liver esterase as a chiral catalyst.



Scheme 1

The adducts **6** and **7** were prepared from easily available (*E*)-methyl 4-oxopentenoate **(5)** (prepared by applying the method used for the preparation of (*E*)-ethyl levulinate[6] on methyl levulinate **4**) and cyclopentadiene by Diels–Alder methodology in 98% yield (*endo*/*exo* ratio: ~1:1 by GC and ^{1}H NMR) (Scheme 2). Conventional separation of the adducts by chemical method using iodolactonisation,[5a] appeared impossible because of a haloform reaction of the acetyl group at both isomers. Therefore separation was carried out chromatographically using preparative thin layer chromatography (TLC) (ether/ petroleum ether: 1/3) which furnished the desired adducts pure.

Incubation of racemic *exo*-ester **6** with PLE (0.1 M phosphate buffer solution, pH=8, room temperature) immediately initiated the hydrolysis process. However, after 1.83 hours (50% conversion) the rate of the reaction considerably decreased. Work up led to the isolation of unreacted enantiomer (–)**-6**, $[\alpha]_D^{25}$=–84.7° (c 1.42, MeOH), ee=75% and *endo*-3-acetylbicyclo [2.2.1] hept-5-ene-2-*exo*-carboxylic acid (+)**-8** which showed optical rotation of $[\alpha]_D^{20}$=+106.2° (c=1.1, MeOH) corresponding to ee=98%, *E*=460. A sample of (+)**-8** was transformed into its methyl ester by treatment with ethereal solution of diazomethane, $[\alpha]_D^{2\ 0}$=+110.5° (c 0.80, MeOH), ee=98%.



Scheme 2

Incubation of racemic *endo*-ester **7** with PLE under our standard condition led to a relatively much slower reaction in contrast to *exo*-ester **6**, reaching to 50% conversion after 11.5 hours. Conventional work up provided the unreacted enantiomer of the *endo*-ester (+)**-7**, $[\alpha]_D^{20}$=+15°, ee=13.5% and *endo*-acid (–)**-9**, $[\alpha]_D^{20}$=–75°, ee=70%, *E*=12. A sample of this acid was also methylated by ethereal solution of diazomethane to give the related methyl ester, $[\alpha]_D^{20}$=–78°, ee=70%. Enantiomeric excesses of all optically active products were established with ^{1}H NMR spectroscopy (400 MHz) using $Eu(hfc)_3$.

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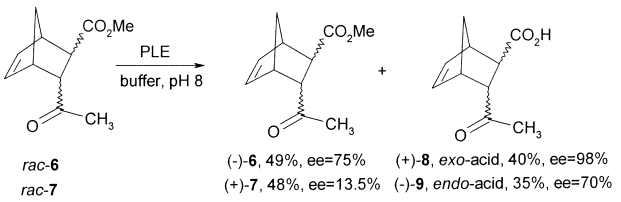
† This is a Short Paper, there is therefore no corresponding material in *J Chem. Research (M).*



Scheme 3

In this study it was found that, the *exo*-carboxylate function is hydrolysed considerably faster than *endo*-ester function. These results conform to the results obtained for corresponding norbornane esters[4c] and also fit Tamm's PLE-substrate model.[7] We further conclude that the high enantioselectivity (ee=75-98%) observed in the resolution of *exo*-ester adduct **(6)**, makes this substrate a valuable candidate for further elaboration to analogous structures presented in Scheme 1.

Experimental

General: Chemicals were purchased from Merck and Fluka. Melting points were measured with ElectroThermal melting point apparatus and are uncorrected. IR spectra were determined on a Shimadiazo IR-470 spectrometer. ^{1}H NMR spectra were recorded on a Bruker AC, FT-NMR (80 MHZ) in deuteriochloroform ($CDCl_3$) with tetramethylsilane (TMS). Exact molecular weight was measured on a Bruker double focusing VG 7070 E mass spectrometer. Preparative thin layer chromatography (TLC) was carried out on plates prepared from Merck Kieselgel 60 H, F_{254}, Art No 7730. GC was carried out using Buck Scientific 910 (capillary column, MXT-5, 15 m). Optical rotations was measured by Atago (Polax) polarimeter. All solvents used were dried and distilled according to standard procedures.

(E)-methyl 4-oxo-pentenoate **(5):** A solution of bromine in $CHCl_3$ (26 ml) was added at room temperature for 1 hour to a magnetically stirred solution of methyl levulinate (26.1 g, 0.20 mol) (prepared from levulinic acid obtained from sugar [8]) in $CHCl_3$ (148 ml) under an argon atmosphere. Coagulation of the mixture occurred. The mixture was cooled in an ice bath and diisopropyl amine (65 g, 0.64 mol) was added through a dropping funnel in 1 hour. Stirring was continued for another 1 hour at this temperature. The mixture was washed with HCl (1N) and brine. The organic phase was dried ($MgSO_4$), filtered and evaporated in vacuum. The residue was distilled under vacuum (97–100°C/30 mmHg) to provide (*E*)-methyl 4-oxopentenoate (12.9 g, 0.10 mol) in 50% yield, m.p. 58-59°C (after recrystalisation from diethyl ether) (lit.[9] m.p. 54–56°C). The structure of the product was confirmed by spectroscopic analysis. ^{1}H NMR ($CDCl_3$): δ 6.87 (d, *J*=16.1 Hz, 1 H), 6.47 (d, *J*=16.1 Hz, 1 H), 3.64 (s, 3 H), 2.18 (s, 3H) ppm., IR (KBr), 3050, 1720, 1670, 1640, 1320, 1270, 1200, 1180 cm^{-1}.

Preparation of methyl 3-endo-acetylbicyclo[2.2.1]hept-5-ene-2-exo-carboxylate **(6)** *and methyl 3-exo-acetylbicyclo[2.2.1]hept-5-ene-2-endo-carboxylate* **(7):** A solution of freshly distilled cyclopentadiene (1.6 g, 24.2 mmol) in benzene (7 ml) was added to a magnetically stirred solution of *(E)*-methyl 4-oxo-2-pentenoate (2 g, 15.6 mmol) in benzene (5 ml) at room temperature in 15 min. Stirring was continued at room temperature for 2 hours. The solvent was evaporated in a rotary evaporator to give a mixture of methyl 3-*endo*-acetylbicyclo[2.2.1]hept-5-ene-2-*exo*-carboxylate and methyl 3-*exo*-acetylbicyclo [2.2.1]hept-5-ene-2-*endo*-carboxylate adducts (2.97 g, 15.3 mmol) (*endo*/*exo* ~1:1, by GC, ^{1}H NMR) in 98% yield. The mixture was separated by preparative TLC (ether/ petroleum ether, 1/3) to furnish pure methyl 3-*endo*-acetylbicyclo[2.2.1]hept-5-ene-2-*exo*-carboxylate **(6)** (1.2 g, 6.2 mmol), ^{1}H NMR ($CDCl_3$): δ 5.95 (dd, *J*=5.5, 3.1 Hz, 1H), 5.69 (dd, *J*=5.5, 2.5 Hz), 3.5 (s, 3 H), 3.1 (m, 2 H), 2.9 (s, 1 H), 2.6 (d, *J*=4.0 Hz, 1H), 1.96 (s, 3 H), 1–1.6 (m, 2 H) ppm., IR (neat): 3050, 1730, 1720, 1690, 1650, 1310, 1270, 1240, 1210 cm^{-1}, EI/HRMS: M^+, 194.09460 ±0.00075 [calc. for $C_{11}H_{14}O_3$: 194.09429] and methyl 3-*exo*-acetylbicyclo [2.2.1]hept-5-ene-2-*endo*-carboxylate **(7)** (1.1 g, 5.7 mmol), ^{1}H NMR ($CDCl_3$): δ 6.0 (dd, *J*=5.3, 3.2 Hz, 1 H), 5.75 (dd, *J*=5.3, 2.7 Hz), 3.3 (s, 3 H), 3.1 (t, *J*=4.4 Hz, 1 H), 2.93 (br. s, 1 H), 2.77 (br. s, 1 H), 2.55 (d, *J*=4.0 Hz, 1 H), 1.97 (s, 3 H), 0.8–1.2 (m, 2 H) ppm., IR (neat): 3050, 2950, 1720, 1700, 1640, 1300, 1260, 1210, 1180 cm^{-1}, EI/HRMS: M^+, 194.09470 ±0.00070 [calc. for $C_{11}H_{14}O_3$: 194.09429].

General procedure for enzyme catalysed kinetic resolution of the ester adducts: A suspension of ester adducts in a 0.1 M phosphate buffer solution (10ml/mmol substrate) at pH=8, was incubated with PLE (60 µl/mmol) at 28°C. The pH was maintained by continuous addition of 0.1 M NaOH using a burette. After reaching an appropriate reaction conversion the reaction was stopped by adding aqueous sodium carbonate (20%) until pH=10 was reached. The aqueous reaction mixture was extracted with chloroform and the combined organic extracts were dried ($MgSO_4$) and evaporated under vacuum to give the unreacted ester product.

The remaining aqueous layer was acidified by dilute HCl (10%) to pH=3 and extracted with chloroform. The combined chloroform extracts were dried ($MgSO_4$) and evaporated under vacuum to give the related acid a product.

Kinetic resolution of racemic methyl 3-endo-acetylbicyclo[2.2.1]hept-5-ene-2-exo-carboxylate **(6):** The adduct (±)**-6** (0.53 g, 2.73 mmol) was subjected to PLE catalysed resolution using the general procedure. The reaction was stopped at 50% conversion, after a reaction time of 1.83 hours, to give the unreacted (–)-methyl 3-*endo*-acetylbicyclo[2.2.1]hept-5-ene-2-*exo*-carboxylate (–)**-6** (0.26 g, 1.34 mmol) (recrystallised from *n*-hexane), yield 49%, m.p. 47–50°C, $[\alpha]_D^{25}$ =–84.7° (c 1.42, MeOH), ee=75% (the ^{1}H NMR and IR of this product was the same as starting racemic ester) and (+)-3-*endo*-acetylbicyclo[2.2.1]hept-5-ene-2-*exo*-carboxylic acid (+)**-8** (0.19 g, 1.1 mmol), yield 40%, m.p. 126–128°C, $[\alpha]_D^{25}$=+106.2° (c 1.1, MeOH), ee=98%, E=460, ^{1}H NMR ($CDCl_3$): δ 11.2 (s, 1 H), 6.0 (dd, *J*=5.6, 3.1 Hz, 1H), 5.8 (dd, *J*=5.6, 2.5 Hz, 1 H), 3.0–3.2 (m, 3 H), 2.63 (dd, *J*=4.0, 1.3 Hz, 1H), 1.96 (s, 3 H), 1.1–1.6 (m, 2 H) ppm., IR (KBr): 3500-2500, 1685, 1645, 1410, 1360, 1260, 1200, 1160, 740 cm^{-1}.

Kinetic resolution of racemic methyl 3-exo-acetylbicyclo [2.2.1]hept-5-ene-2-endo-carboxylate **(7):** The adduct (±)**-7** (0.27 g, 1.40 mmol) was incubated with PLE using the general procedure. The reaction was stopped at 50% conversion, reaction time 11.5 hours, to provide the unreacted (+)-methyl 3-*exo*-acetylbicyclo[2.2.1]hept-5-ene-2-*endo*-carboxylate (+)**-7** (0.13 g, 0.67 mmol), yield 48%, as a colourless liquid, $[\alpha]_D^{25}$ =+15° (c=1.67, MeOH), ee=13.5% (the ^{1}H NMR and IR of this product was the same as starting racemic ester) and (-)-3-*exo*-acetylbicyclo[2.2.1]hept-5-ene-2-*endo*-carboxylic acid (–)**-9** (0.09 g, 0.49 mmol), yield 35%, m.p. 89–92°C, $[\alpha]_D^{25}$ = –75°(c=0.13, MeOH), ee=70%, *E*=12, ^{1}H NMR ($CDCl_3$): δ 9.95 (s, br., 1H), 6.12 (dd, *J*=5.8, 3.0 Hz, 1H), 6.03 (dd, *J*=5.8, 2.4 Hz, 1H), 3.28 (t, *J*=3.7 Hz, 1H), 3.1 (m, 1H), 2.9 (s, 1H), 2.62 (d, *J*=4.5 Hz, 1H), 2.1 (s, 3H), 1.28 (m, 2H) ppm, IR (KBr), 3500–2500, 1680, 1420, 1360, 1300, 1275, 1180, 720 cm^{-1}.

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