Resolution of Bicyclo[3.2.0]hept-2-en-6-ols and Bicyclo[4.2.0]oct-2-en-*endo*-7-ol using Lipases

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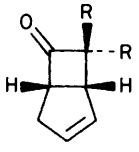
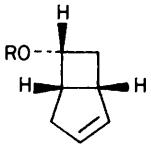
Porcine pancreatic lipase and two fungal lipases effected enantioselective hydrolysis of esters derived from bicyclo[3.2.0]hept-2-en-*endo*-6-ol (**4**) and bicyclo[4.2.0]oct-2-en-*endo*-7-ol (**11**)

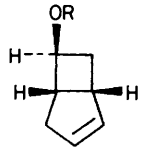
There has been interest in the synthesis of natural products and analogues using bicyclo[4.2.0]octane derivatives and bicyclo-[3.2.0]heptane derivatives as readily obtained starting materials.[1] The availability of these materials in homochiral form is obviously important in connection with these synthetic endeavours. We have explored the methods available for the kinetic resolution of various bicyclo[3.2.0]heptenones using micro-organisms and isolated, partially purified dehydrogenases. For example bicyclo[3.2.0]hept-2-en-6-one (**1**) was resolved using bakers' yeast,[2] *Mortierella ramanniana*,[2] and the dehydrogenase enzyme from *Thermoanaerobium brockii*.[3] 7,7-Dimethylbicyclo[3.2.0]hept-2-en-6-one (**2**) was resolved using *M. ramanniana* and 3α,20β-hydroxysteroid alcohol dehydrogenase from *Streptomyces hydrogenans*.[3] The



(**1**) R = H
(**2**) R = CH_3
(**3**) R = Cl



(**4**) R = H
(**5**) R = $COCH_3$
(**6**) R = $CO(CH_2)_6CH_3$



(**7**) R = H
(**8**) R = $COCH_3$

latter enzyme proved to be an excellent catalyst for the enantioselective reduction of 7,7-dichlorobicyclo[3.2.0]hept-2-en-6-one (**3**).[4]

An alternative strategy for the resolution of ketones such as (**1**) is to reduce the ketone to a secondary alcohol, form an appropriate ester, and effect enantioselective hydrolysis of the ester using an esterase or a lipase as the chiral catalyst.[5] The optically active alcohol and the optically active ester can usually be readily separated by chromatography and both compounds can be converted into the enantiomeric ketones by simple high yielding chemical methods.

Sodium borohydride reduction of the ketone (**1**) gave the *endo*-alcohol (**4**) as the major product; this alcohol could be separated readily from the isomeric *exo*-alcohol (**7**) by chromatography over silica.[6] Acetylation of the alcohol (**4**) gave the ester (**5**). This ester was stirred with porcine pancreatic lipase (ppl) in buffer at 25 °C. After 15 h, the organic material was extracted into ether and, after chromatography, the ester (**5**) (60%) and the alcohol (**4**) (29%) were obtained in optically active form. The alcohol was laevorotatory and thus had the absolute configuration shown.[6] Assessment of the optical purity of the alcohol (**4**) was determined by n.m.r. studies: using tris-[3-(heptafluoropropylhydroxymethylene)-(+)-camphorato] europium(III) as a chiral shift reagent these studies clearly showed an enantiomer ratio >97:<3. Commercially available lipases from *Candida cylindracea* (ccl) and *Mucor miehei* (mml) were equally effective in catalysing enantioselective hydrolysis of the ester (**5**) (Table 1). Increasing the temperature of the hydrolysis of this ester with mml led to poorer enantioselection and much poorer recovery of material due to evaporation of the volatile ester and/or alcohol. Lowering the temperature for this system led to extremely long reaction times. Employment of mml immobilised on Eupergit effected slower, less selective hydrolysis. Finally hydrolysis of the octanoate (**6**) using mml in buffer at room temperature gave an excellent recovery of material but the alcohol obtained had a disappointing optical purity (e.e 78%).

It was disappointing to find that the *exo*-acetate (**8**) was not hydrolysed with a high degree of enantioselectivity using the three hydrolases mentioned above. For example incubation of the ester (**8**) with ccl for 24 h gave laevorotatory *exo*-alcohol (e.e. ~10%), while mml furnished dextrorotatory (1*S*,5*R*)-bicyclo-[3.2.0]hept-2-en-*exo*-6-ol (e.e. 62%).

Table 1. Hydrolysis of 6-acyloxybicyclo[3.2.0]hept-2-enes using lipase enzymes

Substrate[a]	Enzyme	Reaction time(h)	Reaction temp. (°C)	Yield of recovered material (%)	Yield of alcohol (%)	Optical purity of alcohol (% e.e)
(**5**)	ppl[b]	15	25	89	29	≥94
(**5**)	ccl[c]	48	25	87	23	≥94
(**5**)	mml[d]	24	25	92	25	≥94
(**5**)	mml[e]	430	5	78	16	93
(**5**)	mml[e]	120	38	56	15	64
(**5**)	mml[f]	319	25	71	14	84
(**6**)	mml[e]	51	25	100	17	78
(**8**)	ppl[b]	72	20	62	9	24
(**8**)	ccl[g]	24	25	68	12	~10
(**8**)	mml	191	20	88	7	62

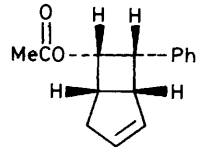
[a] Substrate (0.5 g) was suspended in 0.1M phosphate buffer pH 7 (15 ml). [b] 4 620 Units, 420 mg. [c] 3.5×10^4 Units, 70 mg. [d] 1 000 Units, 1.0 g. [e] 500 Units, 0.5 g. [f] 1.0 g Protein immobilised on Eupergit. [g] 1×10^5 Units, 0.2 g.

Table 2. Hydrolysis of *endo*-7-acetoxybicyclo[4.2.0]oct-2-ene (**12**) using lipase enzymes

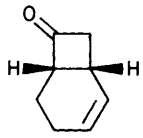
Substrate[a]	Enzyme	Reaction time(h)	Reaction temp. (°C)	Yield of recovered material (%)	Yield of alcohol (**11**) (%)	Optical purity of alcohol (% e.e)
(**12**)[b]	ppl[d]	48	25	94	27	≥96
(**12**)[b]	mml[e]	68	25	94	30	≥96
(**12**)[c]	ccl[f]	46	25	95	24	70

[a] Substrate was suspended in 0.1M phosphate buffer pH 7 (15 ml). [b] 2.0 g Substrate. [c] 0.5 g Substrate. [d] 8 800 Units, 0.8 g. [e] 4 000 Units, 4.0 g. [f] 21 000 Units, 0.042 g.

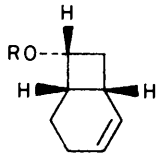
It is noteworthy that the acetate (**9**)* was not hydrolysed to the corresponding alcohol by any of the lipases when suspended, together with the macromolecules, in aqueous solution.



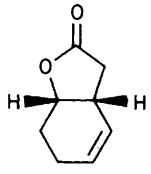
(**9**)



(**10**)



(**11**) R = H
(**12**) R = $COCH_3$



(**13**)

Bicyclo[4.2.0]oct-2-en-7-one (**10**) is easily obtained by [2 + 2]cycloaddition of cyclohexa-1,3-diene and dichloroketene, followed by hydrodechlorination.[7] Somewhat surprisingly, sodium borohydride reduction of this ketone gave the *endo*-7-ol (**11**) as the sole product. The alcohol (**11**) was acetylated to give the ester (**12**). Incubation of the ester with ppl for 48 h was followed by chromatographic separation of the alcohol formed from the recovered ester. Assessment of the optical purity of the re-acetylated alcohol by the n.m.r. method described above showed the material to be essentially one enantiomer (Table 2). The absolute configuration of the optically active alcohol (**11**) was determined by its conversion into the lactone (**13**) which had been prepared previously in optically active form.[8] The enzyme from *Mucor miehei* was equally effective in hydrolysing the ester enantiospecifically but ccl was less selective (Table 2).

Discussion and Conclusions

The use of esterase and lipase enzymes for the preparation of homochiral bicyclo[3.2.0]hept-2-en-*endo*-6-ol and hence homochiral bicyclo[3.2.0]hept-2-en-6-one is unlikely to compete with other methods that are available (particularly the chemical methods)[9] principally because the reduction of the ketone (**1**) to the alcohol (**4**) cannot be accomplished cleanly using the cheaper reducing agents. The impurity in the reduction is the alcohol (**7**) and the corresponding ester (**8**) is not hydrolysed with high enantioselectivity by the enzymes studied.

* The corresponding ketone was kindly provided by Dr. B. J. Wakefield (Salford University).

However the resolution of bicyclo[4.2.0]hept-2-en-7-one (**10**) using this strategy *can* be recommended. The required ester (**12**) is obtainable from the ketone without recourse to chromatography and the enzyme-catalysed enantioselective hydrolysis is simple to operate, quick, and relatively cheap. The alcohol obtained was of very high optical purity and was converted into the optically active ketone in almost quantitative yield. Optically pure ester was obtained by continuing the enzyme-catalysed hydrolysis until ≥50% of the racemic starting material had been consumed. The optically active ester was then separated and de-esterified using lithium aluminium hydride to give dextrorotatory alcohol of excellent optical purity (≥94% e.e). The enzyme-catalysed hydrolysis process can be conducted on a multi-gram scale and has already proved useful in providing large quantities of material for synthesis.[10]

Experimental

Where necessary solvents were dried and purified according to recommended procedures.[11] Light petroleum refers to the fraction boiling in the range 40—60 °C; ether is diethyl ether. Organic solvents were dried over magnesium sulphate and evaporation refers to solvent removal on a rotary evaporator under reduced pressure. T.l.c. was performed on precoated plates (Merck silica gel 60F 254). Flash chromatography refers to the method of Still *et al.*[12] using MN Kieselgel 60/230—400 mesh. ^{1}H N.m.r. spectra were recorded on a Bruker AM 250 MHz spectrophotometer and a Perkin-Elmer R-24B 60 MHz spectrophotometer. The optical purity of the alcohols was assessed by ^{1}H n.m.r. using the chiral shift reagent tris-[3-(heptafluoropropylhydroxymethylene)-(+)-camphorato]-europium(III) derivative.

The enzymic resolution of the alcohols was carried out using a Radiometer (Copenhagen) consisting of a PHM 82 standard pH meter, TTT 80 titrator, ABU 80 autoburette, TTA 80 titration assembly, and REC 80 servograph. Optical rotations were determined with a Thorn NPL type 243 automatic polarimeter. Bicyclo[3.2.0]hept-2-en-6-one was supplied by Glaxo Group Research (Ware). Eupergit C was purchased from Dumas Chemicals Ltd. and all enzymes were purchased from the Sigma Chemical Company Ltd. apart from *Mucor miehei* (mml) which was purchased from Novo Enzyme Products Ltd. The latter enzyme was immobilized as follows. The lipase (1.0 g) was dissolved in 1M phosphate buffer (20 ml) and Eupergit C (1.0 g) was added. The mixture was stirred, allowed to stand at room temperature for 16—72 h, and washed on a sintered-glass filter with distilled water. The washings were monitored with a u.v. spectrophotometer until there was no absorbance at 280 nm. Buffer was made up using AnalaR reagents and water purified by a Milli-Q reagent grade water system.

(±)-endo-6-*Octanoyloxybicyclo*[3.2.0]*hept-2-ene* (**6**).—Bicyclo[3.2.0]hept-2-en-*endo*-6-ol (**4**) (0.232 g, 2.11 mmol) was dissolved in dry dichloromethane (25 ml) containing a few

crystals of dimethylaminopyridine (DMAP). Pyridine (0.4 ml) and octanoyl chloride (0.8 ml) were added with stirring. After 2 h under nitrogen at room temperature the reaction mixture was washed with distilled water, saturated sodium hydrogencarbonate, 1M hydrochloric acid, distilled water, and saturated sodium chloride (50 ml of each), dried, and the solvent was removed by evaporation. The residue (0.802 g) was distilled under reduced pressure (170 °C/0.5 mmHg) to yield *endo*-6-octanoyloxybicyclo[3.2.0]hept-2-ene as a clear oil (0.388 g, 78%), R_F 0.66 (ether–light petroleum; 1:1 v/v); δ($CDCl_3$) 5.75 (s, 2 H, 2- and 3-H). 5.20 (m, 1 H, 6-H), and 3.60—0.70 (m, 21 H, remaining H) (Found: M^+, 236.1768. $C_{15}H_{24}O_2$ requires *M*, 236.1776).

(±)-*Bicyclo*[4.2.0]*oct*-2-*en*-7-*ol* (**11**).—The ketone (**10**) (12.5 g, 102 mmol) was dissolved in AnalaR ethanol (15 ml) and added to a stirred solution of sodium borohydride (3.82 g, 102 mmol) in AnalaR ethanol at 0 °C over a one hour period. The excess of solvent was evaporated and the residue was treated with 2M hydrochloric acid (75 ml); the organic fraction was extracted with ether (3 × 200 ml). The combined extracts were washed with saturated sodium hydrogencarbonate (3 × 200 ml), saturated aqueous sodium chloride (3 × 200 ml), dried and the solvent evaporated to give a light brown oil. Chromatography (ether–light petroleum, 1:1 v/v) gave only the *endo*-alcohol (**11**) as a clear oil (10.8 g, 85%), R_F 0.33 (ether–light petroleum, 1:1 v/v); δ($CDCl_3$) 5.7 (2 H, m, 2- and 3-H), 4.35 (1 H, q, 7-H), and 2.8—1.7 (9 H, remaining H).

(±)-endo-7-*Acetoxybicyclo*[4.2.0]*oct*-2-*ene* (**12**).—The *endo*-alcohol (**11**) (10.8 g, 87 mmol) was dissolved in dry, distilled dichloromethane (150 ml). To the stirred solution was added a few crystals of dimethylaminopyridine followed by 1.5 equiv. of pyridine (10.35 g, 131 mmol) and 1.5 equiv. of acetic anhydride (15.46 g, 131 mmol). The reaction was left overnight under an atmosphere of nitrogen. The reaction mixture was washed with water (2 × 150 ml), saturated sodium hydrogen carbonate (2 × 150 ml), hydrochloric acid (1M, 2 × 150 ml), water (2 × 150 ml), and saturated aqueous sodium chloride (2 × 150 ml). The aqueous washes were back-extracted with dichloromethane (2 × 200 ml) and the combined organic fractions were dried and evaporated to give a clear oil. The oil was purified by distillation using a Kugelrohr oven (b.p. 80 °C, 15 mmHg) to give the title compound (13.5 g, 92%), R_F 0.64 (ether–light petroleum, 1:1 v/v); δ($CDCl_3$) 5.7 (2 H, br s, 2- and 3-H), 5.1 (1 H, m, 7-H), 2.05 (3 H, s, CH_3), and 3.1—1.1 (8 H, remaining H) (Found: M^+, 184.1346. [$C_{10}H_{14}O_2 + NH_4^+$] requires *M*, 184.1338).

Resolution of 6-*Acyloxybicyclo*[3.2.0]*hept*-2-*enes and* endo-7-*Acetoxybicyclo*[4.2.0]*oct*-2-*ene*.—The general procedure was to suspend the ester in 0.1M phosphate buffer, pH 7.0 at 25 °C. The enzyme was added to the stirred suspension and the ensuing hydrolysis reaction monitored by continuous addition of 0.5M sodium hydroxide, to maintain a constant pH.

The reaction was terminated before 50% of the ester had been hydrolysed by extracting the organic phase with ether (3 × 50 ml). The ether solution was dried and evaporated. Chromatography using ether in light petroleum (2:3 v/v) gave the ester and the alcohol. Details of the experimental conditions are recorded in Tables 1 and 2.

(+)-*Bicyclo*[4.2.0]*oct*-2-*en*-7-*one* (**10**).—Under an atmosphere of nitrogen, oxalyl chloride (1.75 ml, 20 mmol) was dissolved in dry distilled dichloromethane (30 ml). To this solution at −60 °C was added distilled dimethyl sulphoxide (2.13 ml) in dichloromethane (10 ml). The reaction mixture was stirred for 2 min and the alcohol (**11**) (1.77 g) in dichloromethane (10 ml) was added over 5 min. After 15 min triethylamine (10.45 ml) was added; the reaction mixture was stirred for 5 min and allowed to warm to room temperature. Water (75 ml) was added and the aqueous layer re-extracted with dichloromethane (75 ml). The organic layers were combined, washed with saturated sodium chloride (150 ml), dried, and evaporated to afford a light yellow oil. Chromatography over silica using ether as eluant yielded a clear oil (1.52 g, 86%), R_F 0.52 (ether–light petroleum, 1.1 v/v); δ($CDCl_3$) 5.8 (2 H, br s, 2- and 3-H), and 3.7—1.2 (10 H, remaining H); $[\alpha]_D^{25}$ +94° (*c* 40.8, ether).

7-*Oxabicyclo*[4.3.0]*non*-2-*en*-8-*one* (**13**).—The ketone (+)-(**10**) (127 mg, 1.04 mmol) was dissolved in glacial acetic acid (2 ml) at 0 °C. To this solution was added an ice-cold mixture of 30% hydrogen peroxide (1 ml) in glacial acetic acid (1 ml). The reaction mixture was stirred for 0.5 h and left to stand for 48 h at 4 °C. Dichloromethane (15 ml) was added and the mixture was washed with water (3 × 15 ml). The combined aqueous layers were extracted with dichloromethane (20 ml) and the combined organic fractions were dried and evaporated. Chromatography (ether–light petroleum, 2:3 v/v) gave a white solid (80 mg, 62%), R_F 0.17 (ether–light petroleum, 1:1 v/v); δ($CDCl_3$) 5.6 (2 H, m, 2- and 3-H), 4.7 (1 H, m, 6-H), and 3.2—1.5 (7 H, remaining H); $[\alpha]_D^{25}$ −23° (*c* 6.0, ether) [lit],[8] $[\alpha]_D^{25}$ −25° (*c* 6.0, ether).

Acknowledgements

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