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Enzymatic resolution of (±)-2-*endo*-hydroxymethyl and acetoxymethyl substituted hexachloronorbornene derivatives

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Abstract—(±)-2-*endo*-Hydroxymethyl-1,4,5,6,7,7-hexachlorobicyclo[2.2.1]hept-5-ene and (±)-2-*endo*-acetoxymethyl-1,4,5,6,7,7-hexachlorobicyclo[2.2.1]hept-5-ene were resolved by using various hydrolases to afford enantiomerically enriched products with ees of 94–98%. The absolute configuration was determined by transforming 2-*endo*-acetoxymethyl-1,4,5,6,7,7-hexachlorobicyclo[2.2.1]hept-5-ene into 2-*endo*-hydroxymethyl-bicyclo[2.2.1]hept-5-ene with known absolute configuration.

1. Introduction

Syntheses of polychlorinated norbornene derivatives have been of growing interest for some years.[1] These are used as starting materials leading to the synthesis of α-diketones[2] and γ-lactone-fused cyclopentanoids,[3] which serve as precursors for natural products.[4] Asymmetric synthesis of these compounds has further importance as they can easily be converted into biologically active precursors[5] and chiral ligands.[6] A hexachloronorbornene carboxylic acid derivative was found to be a suitable resolving agent of some optically active biological molecules in high performance liquid chromatography.[7] Moreover, high *endo* selectivity can be obtained during the Diels–Alder reactions of hexachlorocyclopentadiene because of the steric repulsion of its chlorine atoms at the C-5 position.[8] In a stereoselective manner, some asymmetric Diels–Alder reactions of hexachlorocyclopentadiene with various chiral dienophiles have been investigated.[9,10]

In the literature, however, there have only been a few examples of enzymatic resolution of hexa- and tetrachlorinated norbornene derivatives.[5,6,11] Recently, we reported the enzymatic resolution of (±)-2-hydroxymethyl-1,4,5,6,7,7-hexachlorobicyclo[2.2.1]hepta-2,5-diene, (±)-2-acetoxymethyl-1,4,5,6,7,7-hexachlorobicyclo[2.2.1]-hepta-2,5-diene[12] and enzymatic desymmetrization of *meso*-bis(acetoxymethyl)-1,4,5,6,7,7-hexachlorobicyclo-[2.2.1]hepta-2,5-diene and *meso*-bis(hydroxymethyl)-1,4,5,6,7,7-hexachlorobicyclo[2.2.1]hepta-2,5-diene.[13] In connection to these biotransformations, we investigated the enzymatic resolution of (±)-2-*endo*-hydroxymethyl-1,4,5,6,7,7-hexachlorobicyclo[2.2.1]hept-5-ene (±)-**1** and (±)-2-*endo*-acetoxymethyl-1,4,5,6,7,7-hexachlorobicyclo-[2.2.1]hept-5-ene (±)-**2**. Both hydroxymethyl (±)-**1** and acetoxymethyl (±)-**2** norbornene derivatives possess more flexible structures than corresponding norbornadiene derivatives. In particular, (±)-2-*endo*-hydroxymethyl derivative (±)-**1** has already been used as a precursor in some intramolecular cyclization reactions to afford some polycyclic natural products.[2,14] These studies prompted us towards the development of a practical method as mentioned above to obtain enantiomerically enriched products using the enzymatic resolution approach. Screening reactions were first executed with various hydrolases (i.e., PPL, CCL, PLE, HLE and PL) using substrate:enzyme ratios from 1:1 to 1:0.5. Among the hydrolases studied, CCL, PLE, PPL and HLE proved to be suitable for the resolution of these substrates. The observed promising preliminary results directed us towards a thorough catalytic study. All of the enzymes afforded (1*S*,2*R*,4*R*) configured acetoxy derivative (−)-**2** with high ee values. Herein, we report the highly efficient resolution of the racemic substrates (±)-**1** and (±)-**2** with CCL, PLE, PPL and HLE (Scheme 1).

2. Results and discussion

(±)-**1** was synthesized in 89% chemical yield in its pure *endo* form, through a Diels–Alder reaction by heating

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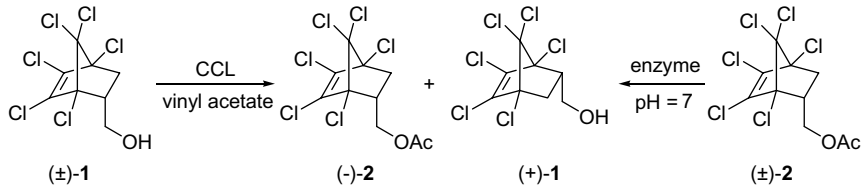


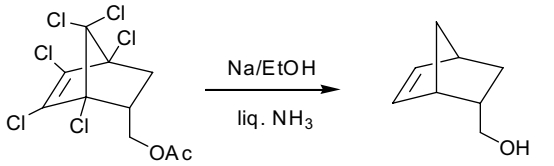
Scheme 1.

a mixture of hexachlorocyclopentadiene and allyl alcohol in a sealed tube. (±)-**2** was subsequently obtained by acetylation of (±)-**1** with acetyl chloride in the presence of pyridine in 81% chemical yield.

The first bioconversion was performed by the enzyme catalyzed acetyl transfer to (±)-**1** in the presence of vinyl acetate as acetyl source and catalytic amount of CCL (substrate:enzyme = 1:0.02). The conversion was monitored by TLC and 50% conversion was obtained after 168 h. The products were separated by flash column chromatography and (−)-**2** was isolated with 97% ee in 39% yield (Table 1, entry 1). All ee determinations were done over acetylated derivative **2**, since it was effectively resolved by HPLC.

Alternatively, acetoxymethyl derivative (±)-**2** was subjected to the enzyme catalyzed hydrolysis. The hydrolysis reactions of (±)-**2** were carried out in pH = 7 buffer at 20 °C using catalytic amounts of PLE, HLE and PPL (Table 1, entries 2, 3 and 4, respectively). The biotransformations were monitored by TLC until 50% conversions were obtained. Among the enzymes, PLE (entry 2) and HLE (entry 3) appeared to be the most efficient in terms of enantioselectivity and the duration of reactions. In entry 4, PPL showed the lowest enantioselectivity among all and longer duration than entries 2 and 3. All of the enzymes afforded the same configured acetoxymethyl derivative (−)-**2**.

For the absolute configuration determination of (−)-**2**, it was transformed into the corresponding 2-*endo*-hydroxymethyl-bicyclo[2.2.1]hept-5-ene **3** derivative via dechlorination with Na in liquid NH_3 (Scheme 2).[15] During the reductive dechlorination, the acetyl group was hydrolyzed to the corresponding alcohol. The absolute configuration of compound (−)-**2** was assigned as (1*S*,2*R*,4*R*) by comparison of its specific rotation with the previously determined value for (1*R*,2*S*,4*S*)-(−)-2-*endo*-hydroxymethyl-bicyclo[2.2.1]hept-5-ene **3**.[16]



Scheme 2.

3. Conclusion

Hexachlorocyclopentadiene afforded only *endo*-adduct **1** in the Diels–Alder reaction with allyl alcohol. The resultant hydroxymethyl and acetoxymethyl *endo*-compounds **1** and **2**, respectively, were subjected to enzymatic resolution with various commercially available hydrolases in catalytic amounts. All enzymes gave the same configured compound (1*S*,2*R*,4*R*)-(−)-**2** with high enantioselectivities between 94% and 98% ee. Among the enzymes used, PLE and HLE revealed the best enantioselectivity with a short reaction duration. The absolute configuration of compound (−)-**2** was determined by transforming it into the corresponding non-chlorinated norbornene derivative by reductive dechlorination. PLE and HLE, used in catalytic levels, renders the process very attractive for large scale preparations.

4. Experimental

The ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ on a Bruker Spectrospin Avance DPX 400 spectrometer. Chemical shifts are given in parts per million downfield from tetramethylsilane. Apparent splittings are given in all cases. Infrared spectra were obtained from KBr pellets on a Mattson 1000 FT-IR spectrophotometer and are reported in cm^{-1}. Mass spectra were recorded on a Varian MAT 212. Optical rotations were measured in

Table 1. Results of the enzyme catalyzed acetylation of (±)-**1** and hydrolysis of (±)-**2**

Entry	Substrate	Enzyme	Substrate:enzyme ratio	Time (h)	Ester	Yield[a] (%)	$[\alpha]_D^{20}$	ee[b] (%)
1	(±)-**1**	CCL	1:0.02	168	(−)-**2**	39	−1.5	97
2	(±)-**2**	PLE	500 mg:100 μL	23	(−)-**2**	49	−1.5	98
3	(±)-**2**	HLE	1:0.08	20	(−)-**2**	40	−1.5	98
4	(±)-**2**	PPL	1:0.08	100	(−)-**2**	47	−1.4	94

[a] Yields (%) are given as the isolated esters.
[b] Enantiomeric excess values are determined by the Chiralcel OD-H chiral column HPLC-analysis.

a 1 dm cell using a Bellingham and Stanley P20 polarimeter at 20 °C. HPLC measurements were performed with ThermoFinnigan spectra system instrument. Separations were carried out on Chiralcel OD-H analytical column (250 × 4.60 mm) with hexane–2-propanol as eluent. Column chromatography was performed on silica gel (60-mesh, Merck). TLC was carried out on Merck 0.2 mm silica gel 60 F_{254} analytical aluminium plates. PLE (pig liver esterase) was purchased from Sigma as a suspension in ammonium sulfate solution (3.2 mol/L). CCL (lipase, type VII, from *Candida rugosa*), HLE (horse liver acetone powder) and PPL (lipase, type II, from porcine pancreas) were purchased from Aldrich.

4.1. Synthesis of (±)-2-*endo*-hydroxymethyl-1,4,5,6,7,7-hexachlorobicyclo[2.2.1]hept-5-ene, (±)-1

A mixture of allyl alcohol (1.74 g, 30 mmol) and hexachlorocyclopentadiene (2.73 g, 10 mmol) containing a few crystals of hydroquinone was sealed under vacuum in a thick-walled Pyrex tube. The mixture was heated at 145 °C for 4 h. The crude product was purified by flash column chromatography to afford (±)-**1** (EtOAc–hexane, 1:2) (2.94 g, 89% yield). Mp: 159–160 °C. ^{1}H NMR: δ 1.43 (t, 1H, OH, J = 5.0 Hz), 1.86 (dd, 1H, *endo* CH_2, J = 4.1 and 12.6 Hz), 2.59 (dd, 1H, *exo* CH_2, J = 8.8 Hz and 12.6 Hz), 2.96–3.03 (m, 1H, CH), 3.39–3.45 (m, 1H, CH_2O), 3.73–3.79 (m, 1H, CH_2O). ^{13}C NMR: δ 38.7, 49.4, 62.5, 79.1, 81.4, 103.0, 130.2, 132.4 IR (neat): 1599, 3345 cm^{-1}. HRMS: calcd for $C_8H_6Cl_6O$ $(M+H)^+$: 328.8628. Found: 328.8645.

4.2. Acetylation of (±)-2-*endo*-hydroxymethyl-1,4,5,6,7,7-hexachlorobicyclo[2.2.1]-5-heptene, (±)-1

To a stirred solution of (±)-**1** (1.50 g, 4.5 mmol) in CH_2Cl_2 (25 mL), dry pyridine (0.72 g, 9.1 mmol) was added at 0 °C and the mixture stirred for 30 min under an inert atmosphere. Acetyl chloride (0.54 g, 6.8 mmol) was added dropwise. The resultant mixture was stirred for 17 h at rt. The organic phase was extracted with 0.1 M HCl (3 × 50 mL), saturated $NaHCO_3$ (3 × 50 mL) and brine (2 × 50 mL), dried over $MgSO_4$ and solvent evaporated under reduced pressure. The crude product was purified by flash column chromatography to afford (±)-**2** (EtOAc–hexane, 1:2) (1.38 g, 81%). ^{1}H NMR: δ 1.86 (dd, 1H, *endo* CH_2, J = 4.2 and 12.6 Hz), 1.99 (s, 3H, CH_3), 2.60 (dd, 1H, *exo* CH_2, J = 8.9 and 12.6 Hz), 3.00–3.13 (m, 1H, CH), 3.92 (dd, 1H, CH_2O, J = 6.7 and 11.7 Hz), 4.08 (dd, 1H, CH_2O, J = 5.8 and 11.7 Hz). ^{13}C NMR: δ 21.1, 38.6, 46.4, 62.7, 79.0, 81.3, 102.9, 130.5, 132.3, 170.8. IR(neat): 1745, 1585 cm^{-1}. HRMS: calcd for $C_{10}H_8Cl_6O_2$ $(M+H)^+$: 370.8734. Found: 370.8717.

4.3. CCL Acetylation of (±)-2-*endo*-hydroxymethyl-1,4,5,6,7,7-hexachlorobicyclo[2.2.1]hept-5-ene, (±)-1

To a stirred solution of 500 mg (±)-**1** in 5 mL vinyl acetate, CCL (10 mg) was added in one portion and the reaction mixture stirred at 20 °C (TLC monitoring). The reaction mixture was filtered and vinyl acetate evaporated under reduced pressure. The products (1*R*,2*S*,4*S*)-(+)-**1** and (1*S*,2*R*,4*R*)-(−)-**2** were purified by flash column chromatography (EtOAc–hexane, 1:2). (1*R*,2*S*,4*S*)-(+)-**1**: (0.15 g, 30% yield).[9] (1*S*,2*R*,4*R*)-(−)-**2**: (0.22 g, 39% yield). HPLC-analysis of (−)-**2**: Chiralcel OD-H at room temperature, *n*-hexane–2-propanol = 98:2, 1.0 mL/min, 254 nm, t_1 = 6.3 min (minor), t_2 = 6.8 min (major), $[\alpha]_D^{20} = -1.5$ (*c* 1.53, MeOH).

4.4. General procedure for enzymatic hydrolysis of (±)-2-*endo*-acetoxymethyl-1,4,5,6,7,7-hexachlorobicyclo[2.2.1]hept-5-ene, (±)-2

To a stirred solution of 500 mg (±)-**2** in 50 mL pH 7.00 phosphate buffer, 40 mg of HLE (PPL or 100 μL PLE) was added in one portion and the reaction mixture stirred at 20 °C in a pH stat unit. The conversion was monitored by TLC. The reaction mixture was extracted with ethyl acetate, dried over $MgSO_4$ and concentrated under reduced pressure. The products (1*R*,2*S*,4*S*)-(+)-**1** and (1*S*,2*R*,4*R*)-(−)-**2** were purified by flash column chromatography (EtOAc–hexane, 1:2).

PLE hydrolysis products:
(1*R*,2*S*,4*S*)-(+)-**1** (98 mg, 22% yield). (1*S*,2*R*,4*R*)-(−)-**2**: (0.25 g, 49% yield). $[\alpha]_D^{20} = 1.5$ (*c* 2.39, MeOH).
HLE hydrolysis products:
(1*R*,2*S*,4*S*)-(+)-**1** (110 mg, 25% yield). (1*S*,2*R*,4*R*)-(−)-**2:** (0.20 g, 40% yield). $[\alpha]_D^{20} = -1.5$ (*c* 0.97, MeOH).
PPL hydrolysis products:
(1*R*,2*S*,4*S*)-(+)-**1** (44 mg, 10% yield). (1*S*,2*R*,4*R*)-(−)-**2:** (0.24 g, 47% yield). $[\alpha]_D^{20} = -1.4$ (*c* 1.44, MeOH).

4.5. Dechlorination of (1*S*,2*R*,4*R*)-(−)-2-*endo*-acetoxymethyl-1,4,5,6,7,7-hexachlorobicyclo[2.2.1]hept-5-ene (1*S*,2*R*,4*R*)-(−)-2

To a stirred solution of metallic sodium (0.6 g, 26 mmol) in liquid NH_3 (30 mL), (1*S*,2*R*,4*R*)-(−)-**2** (0.51 g, 1.37 mmol) in absolute EtOH–ether (12 mL, 1:1 ratio) was added dropwise under argon atmosphere over 20 min. The resultant mixture was stirred for an additional 20 min and then solid NH_4Cl was added in small portions until the solution became colourless. NH_3 was removed by passing N_2 through the mixture and ice-water was added. The resultant mixture was acidified with 2 M HCl and extracted with ether (3 × 50 mL). Organic phase was washed with saturated $NaHCO_3$ (3 × 50 mL), brine (2 × 50 mL), dried over $MgSO_4$ and evaporated under reduced pressure. The crude product was purified by flash column chromatography to afford (1*R*,2*S*,4*S*)-(−)-**3**. (EtOAc–hexane, 1:2). (0.12 g, 70%). $[\alpha]_D^{20} = -72.0$ (*c* 1.05, 95% EtOH). All spectroscopic data and optical rotations are in accordance with the literature.[16]

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