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Resolution of (±)-*anti*-2,3-dioxabicyclo[2.2.2]oct-7-en-5-ol via *Candida cylindracea* lipase: synthesis of (−)- and (+)-*proto*-quercitol

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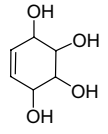
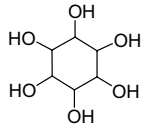
Abstract—Photooxygenation of cyclohexa-1,4-diene afforded *anti*-2,3-dioxabicyclo[2.2.2]oct-7-en-5-yl hydroperoxide. The hydroperoxy endoperoxide was reduced with dimethylsulfide–titanium tetraisopropoxide to produce (±)-*anti*-2,3-dioxabicyclo[2.2.2]oct-7-en-5-ol. The highly efficient enantioselective resolution of the racemic (±)-*anti*-2,3-dioxabicyclo[2.2.2]oct-7-en-5-ol was accomplished with *Candida cylindracea* lipase (CCL) to produce the enantiomerically enriched alcohol and the corresponding acetate: The cleavage of the peroxide linkage by thiourea followed by the oxidation of the double bond with OsO_4 resulted in the formation of (−)-*proto*-quercitol and (+)-*proto*-quercitol, respectively.

1. Introduction

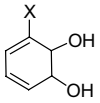
Cyclitols and their derivatives[1] are widespread in nature and have various biological activities. After the discovery of the role of *myo*-inositol phosphates in cell signaling, a dramatic increase in the synthesis of the cyclitol derivatives has been observed.[2] The conduritols (cyclohex-5-ene-1,2,3,4-tetrols) **1** are useful intermediates in organic synthesis as their epoxides can act as irreversible glycosidase inhibitors.[3] Furthermore, conduritols can easily be transferred into various inositol derivatives **2**.[4]



1 conduritol



2 inositol



3 X = CH_3, F, Cl, Br

The developing demand for the synthesis of enantiomerically pure compounds led to the investigation of different approaches for chiral cyclitol derivatives. One of the more promising methods is the use of a microbial enzyme, *Pseudomonas putida*,[5] to convert achiral aromatic compounds to the optically active key compounds, *cis*-cyclohexa-3,5-diene-1,2-diol derivatives **3**. The diol **3** and various conduritol isomers can easily be converted to optically active cyclitol derivatives.[6]

The search for novel methods in enantiomerically pure compound (EPC) syntheses is a major topic in contemporary organic synthesis.[7] The chemo-enzymatic approach for asymmetric synthesis is becoming increasingly accepted in synthetic strategies while the use of biocatalysts as routine chiral catalysts has already found widespread application in preparative organic chemistry over the last decade.[8] Lipases are able to catalyze asymmetric hydrolysis[9] and esterification[10] in a wide range of substrates. This property has attracted a great deal of attention from synthetic chemists since lipases require no added cofactors and are readily available and easily handled. Presently there is an urgent need to investigate substrate specificities of enzymes. During the course of our studies on the biotransformations of (±)-*anti*-5-acetoxy-2,3-dioxabicyclo[2.2.2]-oct-7-en **8b** and (±)-*anti*-2,3-dioxabicyclo[2.2.2]oct-7-en-5-ol **8a**,[11] the screening reactions were first examined with various lipases (i.e., CCL, PLE, HLE, PPL, and

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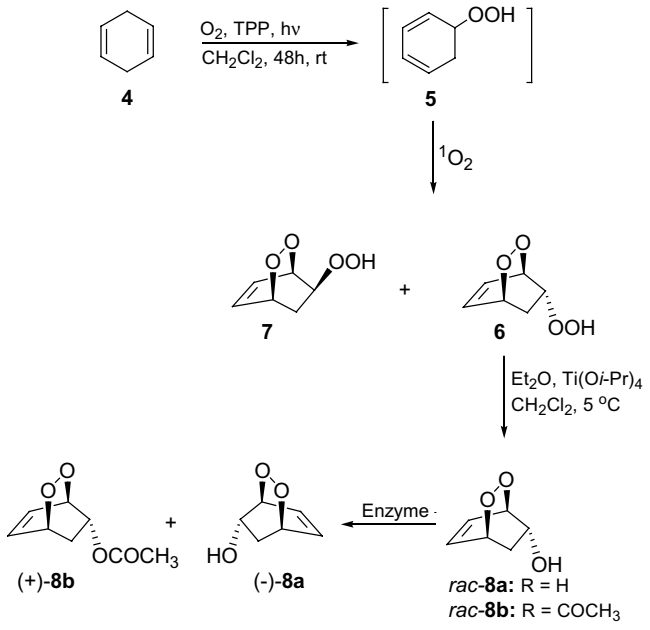
CAL) using a substrate/enzyme ratio, which varied from 1:1 to 1:0.5. Among the lipases studied, CCL proved to be suitable for the enantioselective esterification of substrate **8a**, which showed high enantioselectivity. The observed promising preliminary results directed us toward catalytic studies on this subject. Thus, CCL catalyzed esterification of substrate (±)-**8a** afforded (−)-alcohol **8a** and (+)-ester **8b**.

Herein we report on the highly efficient enantioselective resolution of (±)-**8a** and the conversion of the resulting enantiomerically enriched alcohol (−)-**8a** and acetate (+)-**8b** to cyclopentol derivatives, (+)- and (−)-*proto*-quercitols **10**, respectively.[12] To the best of our knowledge this is the first time an enzymatic resolution of a hydroxyl group in the presence of an endoperoxide functionality has been described.

2. Results and discussions

Racemic **8a** was obtained using tetraphenylporphyrin-sensitized photooxygenation of 1,4-cyclohexadiene **4** in methylene chloride at room temperature, which resulted in the formation of the bicyclic endoperoxides **6** and **7** in a ratio of 88:12.[11,13] The reaction mixture was chromatographed on a silica gel column with ether/petroleum ether (1:1) as the eluant to produce (±)-**6** in 63% as the first fraction and (±)-**7** in 7% yield, as the second fraction (Scheme 1).

Subsequent reduction of the hydroperoxide group in (±)-**6** with dimethylsulfide in the presence of a catalytic amount of Ti(O-*i*Pr)$_4$ produced (±)-**8a** in 95% yield. (±)-*anti*-5-Acetoxy-2,3-dioxabicyclo[2.2.2]oct-7-en **8b** was synthesized by the acetylation of (±)-**8a** using acetyl chloride. The first bioconversion was performed by PLE according to the following general procedure. To a stirred solution of 500 mg (±)-**8b** in a 50 mL pH 7.00 phosphate buffer, 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. After 12 h, substrate (±)-**8b** had completely decomposed to produce unidentified compounds. This was presumably due to the side reaction of the sensitive endoperoxide moiety of substrate (±)-**8b** with enzymes present in phosphate buffer. Similar decomposition reactions were observed with the other enzymes, that is, PPL, HLE, and CCL in the phosphate buffer. Due to these uncontrolled side reactions, we changed the direction of our study to the enantioselective esterification of substrate (±)-**8a** with the enzymes in vinyl acetate. The enzyme catalyzed acetylations of (±)-**8a** with PLE, PPL, HLE, and CCL were examined. PLE produced decomposition products as indicated in enzyme catalyzed ester hydrolysis, whereas PPL and HLE did not show any reaction after 72 h (entries 3 and 4, respectively). However, the bioconversion of substrate (±)-**8a** with CCL proved successful. For the enantiomeric separation of the racemic mixture; to a stirred solution of (±)-**8a** (500 mg) in vinyl acetate (5 mL), CCL (10 mg) was added in one portion and the reaction mixture shaken at 20 °C. The conversion was monitored by TLC. After 38 h, 50% conversion was observed. The products were then separated using preparative TLC. Enantiomerically enriched compound (−)-**8a** and compound (+)-**8b** were isolated with 91% ee in 47% yield and with 72% ee in 42% yield, respectively (entry 1 in Table 1). By utilizing threefold more catalyst, (−)-**8a** and (+)-**8b** were isolated with 88% ee in 49% yield and 66% ee in 40% yield, respectively (entry 2 in Table 1).

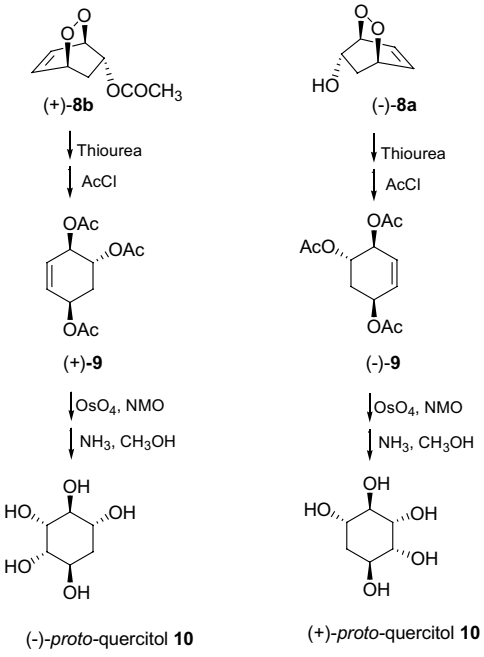


Scheme 1.

Table 1. Enzymatic resolution of (±)-**8a**

Entry	Enzyme	Time (h)	Yield of **8a** (%)[a]	$[\alpha]_D^{20}$	Ee (%)[b]	Yield of **8b** (%)[a]	$[\alpha]_D^{20}$	Ee (%)[c]
1	CCL	38	47	−23.33	91	42	+16.9	72
2	CCL[d]	24	49	−22.54	88	40	+15.47	66
3	PPL	72				—		
4	HLE	72				—		

[a] Yields (%) are shown as the isolated products.
[b] Enantiomeric excess values are determined by the Chiralcel ODH chiral column HPLC analysis.
[c] Enantiomeric excess values are determined by the Phenomenex Chirex (*S*)-Leu and (*R*)-NEA chiral column HPLC analysis.
[d] CCL was used in threefold excess with respect to entry 1.



After the successful isolation and characterization of the bicyclic endoperoxides (+)-**8b** and (−)-**8a**, they were submitted to the selective reduction of the peroxide linkages with thiourea under very mild conditions. Since it is only the oxygen–oxygen bond that breaks, the configuration at all three carbon atoms is preserved. For further characterization, the formed diols were converted into the corresponding acetates.[14] OsO_4 oxidation of the double bonds in (+)-**9** and (−)-**9** followed by the ammonolysis of acetate groups resulted in the formation of (−)-*proto*-quercitol and (+)-*proto*-quercitol **10**, respectively.[11,12,15]

3. Experimental

3.1. General

Melting points are uncorrected. Infrared spectra were obtained from KBr pellets on a Mattson 1000 FT-IR spectrophotometer. The ^1H and ^{13}C NMR spectra were recorded on Brucker 400 and 250 MHz spectrometers. Apparent splittings are given in all cases. Optical rotations were measured in a 1 dm cell using a Bellingham and Stanley P20 polarimeter at 20 °C. PLE (pig liver esterase) was purchased from Sigma as a suspension in ammonium sulfate solution (3.2 mol/L). CCL (lipase, typeVII, from *Candida cylindracea*), PPL (lipase, type II, from porcine pancreas), and HLE (horse liver esterase) were purchased from Aldrich. 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 aluminum plates.

3.2. 2,3-Dioxabicyclo[2.2.2]oct-7-en-5-yl hydroperoxide 6

This was synthesized as described in the literature.[13]

3.3. CCL hydrolysis of (±)-*anti*-2,3-dioxabicyclo-[2.2.2]oct-7-en-5-ol 8a

To a stirred solution of 500 mg (±)-**8a** in 5 mL vinyl acetate, 10 mg of CCL was added in one portion and the reaction mixture stirred at 20 °C (TLC monitoring). The reaction mixture was filtered and the vinyl acetate evaporated under reduced pressure. The products (−)-**8a** and (+)-**8b** were purified by preparative TLC (EtOAc/hexane 1:1).

3.3.1. (1*S*,4*S*,5*S*)-2,3-dioxabicyclo[2.2.2]oct-7-en-5-yl acetate 8b. Colorless liquid (279 mg, 42% yield); 72% ee $[\alpha]_D^{20} = +16.9$ (*c* 0.1, MeOH); ν_{max} (liquid film) 2953, 1727, 1450, 1385, 1250, 1094, 962 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 6.75 (1H, dt, $J = 8.2$, 1.3 Hz), 5.95 (1H, br t, $J = 8.2$ Hz); 5.20 (1H, ddd, $J = 7.8$, 4.5, 2.3 Hz), 4.70 (1H, m), 4.60 (1H, m), 2.65 (1H, ddd, $J = 14.2$, 7.8, 3.8 Hz), 1.95 (3H, 3), 1.40 (1H, dm, $J = 14.2$ Hz); δ_C (63 MHz, $CDCl_3$) 171.2, 135.1, 129.8, 71.1, 65.8, 32.8, 22.0).

3.3.2. (1*R*,4*R*,5*R*)-2,3-dioxabicyclo[2.2.2]oct-7-en-5-ol 8a. Colorless prisms (235 mg, 47% yield), mp 105–106 °C (lit. 106–107 °C[11]); 91% ee $[\alpha]_D^{20} = -23.3$ (*c* 0.2, MeOH); δ_H (250 MHz, $CDCl_3$) 6.71 (1H, ddd, $J = 8.2$, 6.1, 1.8 Hz), 6.48–6.42 (1H, m), 4.64–4.55 (2H, m), 4.28–4.20 (1H, m), 2.50 (1H, ddd, $J = 14.0$, 7.9, 3.6 Hz), 1.75 (1H, br d), 1.24 (1H, dm, $J = 14.0$ Hz); δ_C (63 MHz, $CDCl_3$) 134.8, 129.0, 73.0, 70.8, 62.5, 35.0.

3.4. (−)-*proto*-Quercitol 10 and (+)-*proto*-quercitol 10

These were synthesized starting from the triacetates (+)-**9** and (−)-**9**, respectively, as described in the literature.[11,13,15]

(1*R*,2*R*,5*R*)-(−)-2,5-bis(acetyloxy)cyclohex-3-en-1-yl acetate **9** $[\alpha]_D^{20} = -4.8$ (*c* 0.3, MeOH).
(1*S*,2*S*,5*S*)-(+)-2,5-bis(acetyloxy)cyclohex-3-en-1-yl acetate **9** $[\alpha]_D^{20} = +3.8$ (*c* 0.3, MeOH).
(−)-proto-quercitol **10** $[\alpha]_D^{20} = -18.0$ (*c* 0.2, H_2O), lit.[12] $[\alpha]_D^{20} = -25.1$ (*c* 1, H_2O).
(+)-proto-quercitol **11** $[\alpha]_D^{20} = +23.2$ (*c* 0.2, H_2O), lit.[12] $[\alpha]_D^{20} = +25.3$ (*c* 2, H_2O).

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