Asian Journal of Chemistry; Vol. 26, No. 20 (2014), 6719-6721

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2014.16581

Enzymatic Production of Both Enantiomers of Rhododendrol

MUSA M. MUSA

Department of Chemistry, King Fahd University of Petroleum and Minerals, Dhahran 31261, Kingdom of Saudi Arabia

Corresponding author: Fax: +966 138604277; Tel: +966 138607343; E-mail: musam@kfupm.edu.sa

Received: 25 October 2013; *Accepted*: 4 December 2013; *Published online*: 25 September 2014; AJC-15997

An asymmetric synthetic approach to produce (*R*)- and (*S*)-rhododendrol is described. W110A *Thermoanaerobacter ethanolicus* secondary alcohol dehydrogenase (W110A *Te*SADH), an (*S*)-specific mutant of *Te*SADH, is used in this approach. The enantioselective reduction of 4-(4'-hydroxyphenyl)-2-butanone catalyzed by W110A *Te*SADH yielded (*S*)-rhododendrol, the Prelog product. The *anti*-Prelog product, (*R*)-rhododendrol, is produced from (*rac*)-rhododendrol through enantiospecific kinetic resolution catalyzed by W110A *Te*SADH.

Keywords: Alcohol dehydrogenases, Asymmetric reduction, Biotransformations, Kinetic resolution, Redox reactions, Rhododendrol.

INTRODUCTION

Both enantiomers of 4-(4'-hydroxyphenyl)-2-butanol, known as rhododendrol, are naturally occurring alcohols. (*R*)-Rhododendrol [(*R*)-**2**] has been isolated from *Rhododendron chrysanthum*[1], and (*S*)-rhododendrol [(*S*)-**2**] has been isolated from *Rhododendron maximum*[2]. Rhododendrin, a glycoside derivative of (*R*)-**2**, exhibits analgesic and *anti*-inflammatory properties[3]. Because of the biological importance of rhododendrol, several research groups have reported asymmetric routes for the synthesis of rhododendrol[4]. Lipase-catalyzed resolution was employed in most of the previous attempts, which is subjected to the formation of undesired acetylated by-product(s)[4b,4d]. Optically active alcohols can be produced from their corresponding prochiral ketones through asymmetric reduction or from their racemates through kinetic resolution (KR). Biocatalysis is an attractive approach for preparing various optically active compounds for several reasons[5]. For example, enzymes are highly chemo-, regio- and stereoselective catalysts that minimize the possibility of by-product formation. They are also environmentally benign catalysts. Enzymatic reactions are generally conducted by using water as a reaction medium, which makes them green alternatives to other organic reactions.

Alcohol dehydrogenases (ADHs, EC 1.1.1.X, X = 1 or 2) catalyze the reversible reduction of carbonyl compounds to their corresponding alcohols[6,7]. One notable alcohol dehydrogenase is *Thermoanaerobacter ethanolicus* secondary alcohol dehydrogenase (*Te*SADH, EC 1.1.1.2), a nicotinamide-adenine dinucleotide phosphate (NADPH)-dependent alcohol dehydrogenase[8]. This enzyme is known for its high thermal stability and high tolerance to organic solvents[9,10], which makes it an ideal candidate as a biocatalyst in organic synthesis. It accepts ketones and their corresponding secondary alcohols as substrates. To expand the substrate specificity of *Te*SADH, W110A *Te*SADH was designed to accommodate phenyl-ring-containing substrates, which are not substrates for wild-type *Te*SADH[11]. Both *Te*SADH and W110A *Te*SADH conform to Prelog's rule, according to which the pro-*R* hydride of NADPH is delivered from the *Re* face of a prochiral ketone producing the corresponding (*S*)-configured alcohol in most cases (Fig. 1)[12].

In this study, W110A *Te*SADH is used to produce both enantiomers of rhododendrol. (*S*)-Rhododendrol is produced through the enantioselective reduction of 4-(4'-hydroxyphenyl)-2-butanone, which is known as raspberry ketone. (*R*)-Rhododendrol is produced from (*rac*)-rhododendrol through enantiospecific KR.

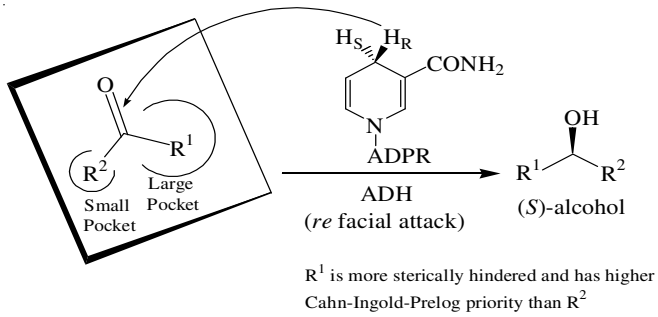


Fig. 1. Prelog's rule for predicting the stereochemical outcome for ADH-catalyzed asymmetric reduction of prochiral ketones. ADPR: adenosine diphosphoribose

EXPERIMENTAL

Capillary gas chromatographic measurements were performed on a GC equipped with a flame ionization detector and HP chiral-20B column (30 m, 0.32 mm [i.d.], 0.25 μm film thickness) using Helium as the carrier gas. Nuclear Magnetic Resonance spectra were recorded on 500 MHz spectrometer at 500 MHz (^{1}H) and at 125 MHz (^{13}C) at room temperature using the solvent peak as an internal standard. Commercial grade solvents were used without further purification. 4-(4'-Hydroxyphenyl)-2-butanone, $NaBH_4$ and $NADP^+$ were used as purchased from commercial suppliers. All buffer solutions were adjusted to pH 8.0 at room temperature.

Gene expression and purification of W110A *Te*SADH: W110A *Te*SADH was expressed in recombinant *Escherichia coli* BL21(DE3) cells and purified as reported[13].

Synthesis of (*S*)-rhododendrol [(*S*)-2]: A mixture of Tris-HCl buffer solution (7 mL, 50 mM, pH 8), 2-propanol (3 mL), $NADP^+$ (1 mg), W110A *Te*SADH [0.7 mg in 100 μL of Tris-HCl buffer (50 mM, pH 8.0)] and **1** (0.365 mmol) was added in the same sequence to a round-bottomed flask equipped with a magnetic stirrer and a condenser. The reaction mixture was stirred for 24 h at 50 °C. It was then extracted with ethyl acetate (3 × 5 mL). The combined organic layers were washed with brine solution (5 mL), dried with sodium sulfate and then concentrated under vacuum. A fraction of the remaining residue was treated with pyridine and acetic anhydride as reported prior to its analysis by a GC equipped with a chiral column[14]. The percent conversion was 61 % and the optical purity was 97.2 %. The remaining underivatized residue was purified with silica gel using hexane/ethyl acetate. $[\alpha]^{20}_D$ +14.1 (*c* 0.667, ethyl acetate), lit.[4b], $[\alpha]^{24}_D$ + 16.9 (*c* 1.06, EtOH) 99% ee. ^{1}H NMR (CD_3OD), δ: 1.18 (d, 3H, *J* = 6.1 Hz), 1.60-1.75 (m, 2H), 2.50-2.59 (m, 1H), 2.60-2.71 (m, 1H), 3.68-3.76 (m, 1H), 6.70 (d, 2H, *J* = 8.25 Hz), 7.01 (d, 2H, *J* = 8.25 Hz); ^{13}C NMR (CD_3OD), δ: 23.5, 32.3, 42.4, 67.9, 116.1, 130.2, 134.4, 156.3.

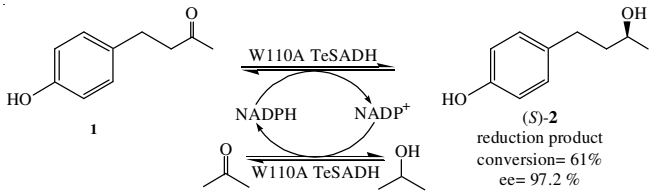
Synthesis of (*rac*)-rhododendrol [(*rac*)-2]: A mixture of **1** (1 g, 6.2 mmol) and methanol (25 mL) was placed in a 100 mL round-bottomed flask equipped with a magnetic stirrer then placed in an ice bath. A solution of $NaBH_4$ (0.26 g, 7 mmol) in distilled water (20 mL) was added slowly to the reaction flask. The mixture was stirred at room temperature for 10 h. It was then filtered and concentrated under vacuum until 20 mL remains. The remaining was extracted with ethyl acetate (3 × 20 mL), dried with sodium sulfate and then concentrated under vacuum to produce oil that soon became white solid in 94 % yield. ^{1}H NMR and ^{13}C NMR are similar to those for (*S*)-**2**.

Synthesis of (*R*)-rhododendrol [(*R*)-2]: A mixture of Tris-HCl buffer solution (4.5 mL, 50 mM, pH 8), acetone (0.5 mL), $NADP^+$ (1 mg), W110A *Te*SADH [0.7 mg in 100 μL of Tris-HCl buffer (50 mM, pH 8)] and (*rac*)-**2** (0.365 mmol) was added in the same sequence to a round-bottomed flask equipped with a magnetic stirrer and a condenser. The reaction mixture was stirred for 24 h at 50 °C. It was then extracted with ethyl acetate (3 × 5 mL). The combined organic layers were washed with brine solution (5 mL), dried with sodium sulfate and then concentrated under vacuum. The remaining residue was treated with pyridine and acetic anhydride then analyzed by a GC equipped with a chiral column. The percent conversion to ketone was 45.8 % and the optical purity of (*R*)-**2** was 87.1 % (*E*-value = 190).

Calculation of *E*-value: *E*-value was calculated from the formula $E = \ln[(1-c)(1-ee_s)]/\ln[(1-c)(1+ee_s)]$, where *c* is the percentage conversion of (*rac*)-**2** to **1** and ee_s is the enantiomeric excess of the slow reacting enantiomer, (*R*)-**2**, in W110A *Te*SADH-catalyzed KR.

RESULTS AND DISCUSSION

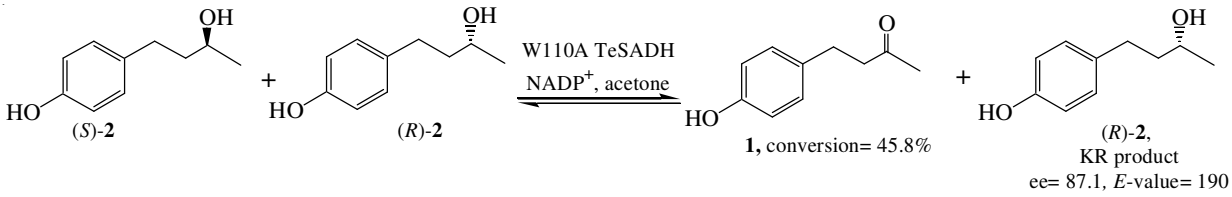
By using W110A *Te*SADH, the enantioselective reduction of raspberry ketone **1** produced (*S*)-**2** in 61 % conversion and 97.2 % ee (**Scheme -I**). The reaction was conducted in a Tris-HCl buffer solution (50 mM, pH 8.0) containing 2-propanol (30 %, v/v). 2-Propanol serves as a cosubstrate by delivering a hydride to the oxidized form of the coenzyme $NADP^+$ and, therefore, regenerates the reduced form of the coenzyme, NADPH. This approach, called "substrate-coupling[15]", has been used successfully in ADH-catalyzed transformations in which ADHs with high tolerance to organic solvents, such as *Te*SADH and *Rhodococcus ruber* DSM 44541, are employed[16]. Using a high percentage of 2-propanol not only enhances the solubility of hydrophobic substrate **1**, but also shifts the equilibrium to the reduction pathway. The produced alcohol has an *S* configuration, which is consistent with Prelog's rule. The same stereochemical results were obtained for the W110A *Te*SADH-catalyzed reduction of a series of phenyl-ring-containing ketones with similar structures to **1**[17].



Scheme-I: Synthesis of (*S*)-**2** *via* W110A TeSADH-catalyzed asymmetric reduction

By taking advantage of the reversibility of ADH-catalyzed asymmetric transformations, *anti*-Prelog alcohols can be produced through enantiospecific oxidation of their racemates through KR. (*rac*)-Rhododendrol was produced by reducing **1** with $NaBH_4$. The enantiospecific oxidation of (*rac*)-**2**, through KR resulted in 45.8 % conversion to **1**, leaving (*R*)-**2**, the slow-reacting enantiomer, in 87.1 % ee (**Scheme- II**). This reaction was conducted in a Tris-HCl buffer solution (50 mM, pH 8) containing acetone (10 %, v/v). Acetone was used as both a cosubstrate to regenerate the oxidized form of the coenzyme, $NADP^+$, and as a cosolvent to enhance the solubility of the substrate. Although the ee of (*R*)-**2** in the W110A *Te*SADH-catalyzed KR is 87.1 % ee, the process is still stereospecific and the *E*-value is 190. This stereospecific KR method can be used to produce (*R*)-**2** from (*rac*)-**2** and generate raspberry ketone **1**, which is a crucial ketone for the flavor industry.

The stereochemical identity of (*S*)-**2** was confirmed by comparing its sign of optical rotation with reported values. The stereochemical identity of (*R*)-**2** was confirmed by co-injecting (*R*)-**2** with (*S*)-**2** in a GC equipped with a chiral



Scheme-II: Synthesis of (*R*)-**2** *via* W110A TeSADH-catalyzed KR

column. The optical purity of both (*S*)-**2** and (*R*)-**2** was determined for their diacetate derivatives as shown in Fig. 2. No by-products were observed in the asymmetric reduction of **1** to produce (*S*)-**2** or in the KR of (*rac*)-**2** to produce (*R*)-**2**. Expanding substrate specificity for *Te*SADH, as well as its high temperature and organic solvent tolerance, makes this enzyme an ideal choice as a biocatalyst in organic synthesis.

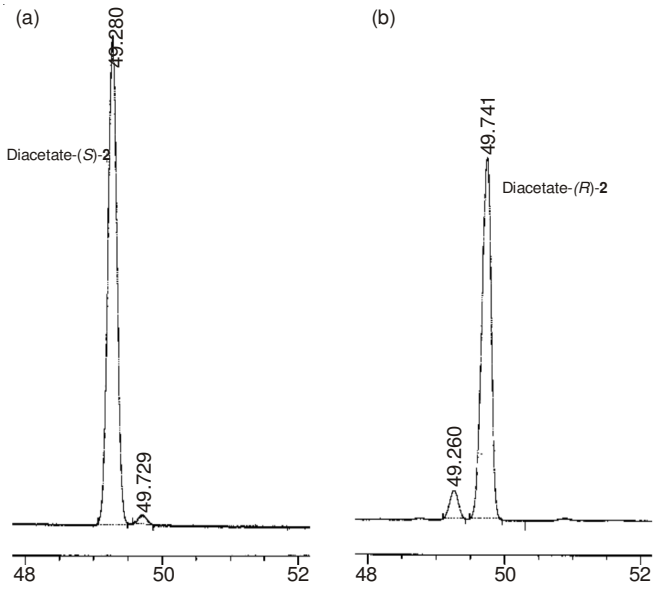


Fig. 2. GC chromatograms for: a) diacetate derivative for (*S*)-**2** produced from W110A TeSADH-catalyzed reduction; (b) diacetate derivative for (*R*)-**2** produced from W110A TeSADH-catalyzed KR

Conclusion

Redox reactions catalyzed by W110A *Te*SADH were used to produce (*S*)- and (*R*)- rhododendrol. (*S*)-Rhododendrol was produced through the W110A *Te*SADH-catalyzed enantioselective reduction of raspberry ketone and (*R*)-rhododendrol was produced through the W110A *Te*SADH-catalyzed enantiospecific KR of (*rac*)-rhododendrol. The protocol is simple and the same enzyme is used to produce both enantiomers of rhododendrol.

ACKNOWLEDGEMENTS

The author acknowledges the support provided by King Abdulaziz City for Science and Technology (KACST) through the Science and Technology Unit at King Fahd University of Petroleum and Minerals (KFUPM) for funding this work through project No. 11-BIO1666-04 as part of the National Science, Technology and Innovation Plan. The author thanks Prof. Claire Vieille, from Department of Microbiology and Molecular Genetics at Michigan State University, for providing the plasmid of W110A *Te*SADH; and Prof. Samir M. Hamdan, from the Division of Biological and Environmental Sciences and Engineering at King Abdullah University of Science and Technology, for providing purified W110A *Te*SADH.

REFERENCES

1. K. Archangelsky, *Arch. Exp. Path. Pharm*, **46**, 313 (1901).
2. W.H. Tallent, *J. Org. Chem*., **29**, 988 (1964).
3. M.H. Kim, A. Nugroho, J. Choi, J.H. Park and H.J. Park, *Arch. Pharm. Res*., **34**, 971 (2011).
4. (a) G. Sabitha, B. Thirupathaiah and J. Yadav, *Synth. Commun*., **37**, 1683 (2007).; (b) Y. Yuasa, S. Shibuya and Y. Yuasa, *Synth. Commun*., **33**, 1469 (2003).; (c) B. Das, S. Padma Rao, K.V.N.S. Srinivas and J.S. Yadav, *Phytochemistry*, **33**, 1529 (1993).; (d) T. Kitayama, S. Isomori and K. Nakamura, *Tetrahedron Asymmetry*, **24**, 621 (2013).
5. U.T. Bornscheuer, G.W. Huisman, R.J. Kazlauskas, S. Lutz, J.C. Moore and K. Robins, *Nature*, **485**, 185 (2012).
6. F. Hollmann, I.W.C.E. Arends and D. Holtmann, *Green Chem*., **13**, 2285 (2011).
7. M. Hall and A.S. Bommarius, *Chem. Rev*., **111**, 4088 (2011).
8. D.S. Burdette and J.G. Zeikus, *Biochem. J*., **302**, 163 (1994).
9. D.S. Burdette, V.V. Tchernajencko and J.G. Zeikus, *Enzyme Microb. Technol*., **27**, 11 (2000).
10. M.M. Musa, K.I. Ziegelmann-Fjeld, C. Vieille and R.S. Phillips, *Org. Biomol. Chem*., **6**, 887 (2008).
11. K.I. Ziegelmann-Fjeld, M.M. Musa, R.S. Phillips, J.G. Zeikus and C. Vieille, *Protein Eng. Des. Sel*., **20**, 47 (2007).
12. V. Prelog, *Pure Appl. Chem*., **9**, 119 (1964).
13. M.M. Musa, R.S. Phillips, M. Laivenieks, C. Vieille, M. Takahashi and S.M. Hamdan, *Org. Biomol. Chem*., **11**, 2911 (2013).
14. A. Ghanem and V. Schurig, *Tetrahedron Asymmetry*, **14**, 57 (2003).
15. W. Kroutil, H. Mang, K. Edegger and K. Faber, *Curr. Opin. Chem. Biol*., **8**, 120 (2004).
16. M.M. Musa and R.S. Phillips, *Catal. Sci. Technol*., **1**, 1311 (2011).
17. M.M. Musa, K.I. Ziegelmann-Fjeld, C. Vieille, J.G. Zeikus and R.S. Phillips, *J. Org. Chem*., **72**, 30 (2007).