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An Enzyme-catalyzed Synthesis of Natural α-Tocopherol

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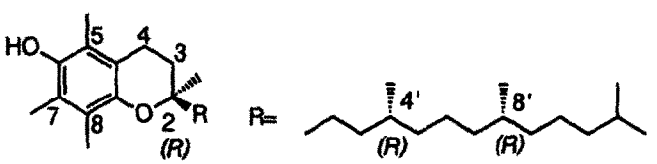
Abstract: The natural α-tocopherol was synthesized by enzyme-catalyzed enantioselective hydrolysis. The unnatural enantiomer as a by-product was also converted to natural α-tocopherol by racemization and repeated enzyme-catalyzed hydrolysis.

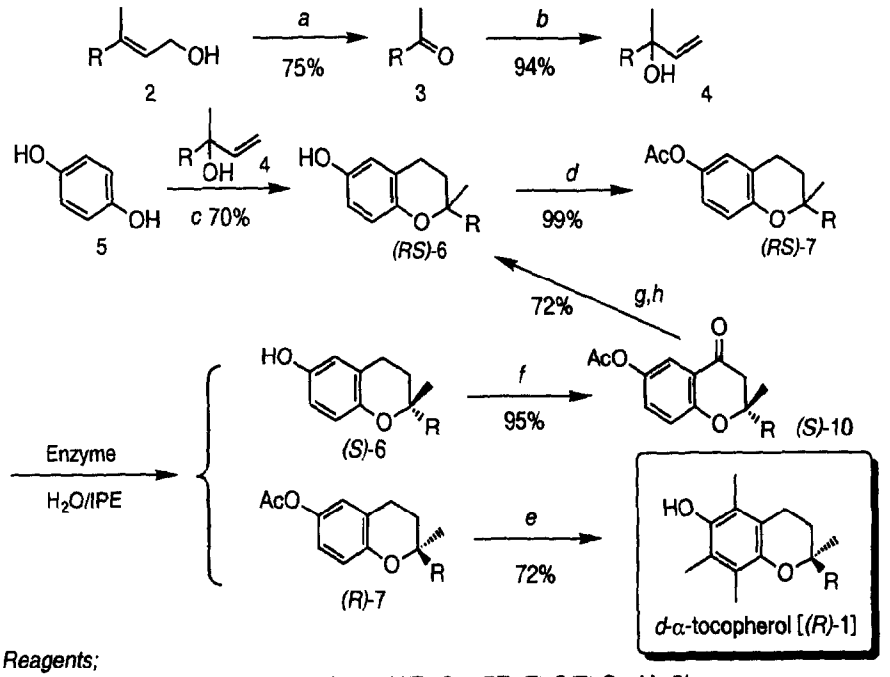
The α-tocopherol (vitamin E) is known as a potent and safe, lipid-soluble antioxidant. Recently, tocopherol and super oxide dismtase(SOD) and other antioxidants are attracting attention as scavengers of super oxide series[1]. As tocopherol is being used for various purposes, in the future, demand will more increase. Presently, the majority of tocopherol is being used as a mixture of its eight isomers, which were reported to show different biological activities. Tocopherol with the *S* configuration at the 2-position of its chroman ring is weak in its biological activities[2]. Previously, *dl*-tocopherol [(2*RS*,4'*R*,8'*R*)-**1**] has been resolved into *d*- and *l*-tocopherol [(*R*) - and (*S*)-**1**] by Karrer[3] and Robeson[4] *et al* . using 3-bromocamphor-8-sulfonate or piperazine, respectively. However, their optical resolution is not efficient and of less utility value[5]. After investigation, we found an effective synthesis of natural α-tocopherol using the enzyme-catalyzed kinetic resolution and the conversion of the unnatural enantiomer to α-tocopherol by its racemization and repeated enzyme-catalyzed hydrolysis.



d-α-tocopherol [(*R*)-**1**]

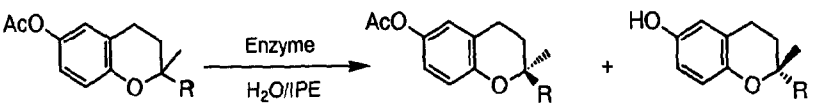
First, enzymatic hydrolysis of *dl*-tocopherol acetate and benzoate were examined, but these reactions were unsuccessful. Next, we chose tocol acetate[(*RS*)-**7**] as less sterically hindered substrate. Its ester site and stereogenic carbon atom of the chroman 2-position were more able to interact with enzymes. The (*RS*)-**7** was synthesized as shown in Scheme 1. Table 1 shows the results of its enzyme-catalyzed hydrolysis. The hydrolysis of (*RS*)-**7** with lipaseAY[6] gave (*R*)-**7**[7] in extremely high optical yield (>99%ee), which was converted to natural α-tocopherol [(*R*)-**1**][8] without racemization by methylation according to Kijima's method[9]. It is an interesting result that (*RS*)-**7** is hydrolyzed with high enantioselectivity by lipaseAY, in spite of the reaction site being fairly remote from the stereogenic carbon atom of its chroman ring. To our knowledge, no enzyme-catalyzed enantioselective hydrolysis of this type of substrate has been reported[10].

Scheme 1 *Enzymatic Synthesis of d-α-Tocopherol*



Reagents;
a $KMnO_4$/acetone *b* vinyl bromide,t-BuLi/Et_2O *c* BF_3-Et_2O/Et_2O *d* AcCl, Et_3N/THF *e* CH_2O,H_3BO_4,H_2/Pd-C/$(CH_3O)_3B$ *f* AcCl,Et_3N/THF,$Na_2Cr_2O_7$/AcOH *g* NaOEt/EtOH *h* $NaBH_4$, then H_2/Pd-C/MeOH

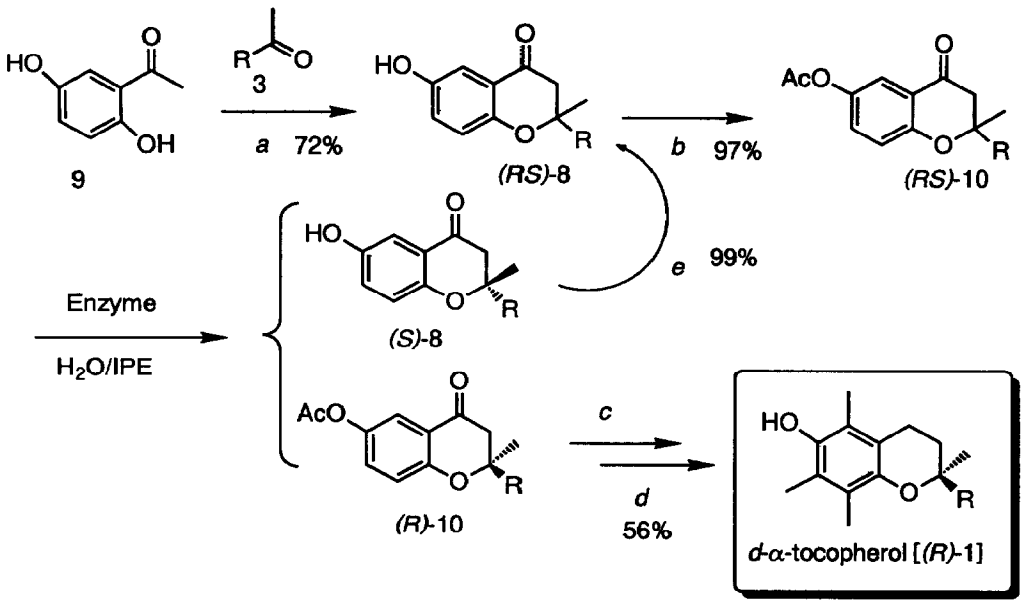
Table 1 *Lipase-catalyzed Kinetic Resolution of Tocol acetate RS-7* [a]



Entry	Enzyme[6]	Time(h)	C.Y. (%) [b]	O.Y. (%ee) [c]	C.Y. (%) [b]	O.Y. (%ee) [c]
1	lipase AY	1	32	>99	60	38
2	CHE	48	42	76	50	55
3	lipase AH	48	78	2.5	16	12
4	lipase PS	48	100	0	0	0
5	lipase M	48	100	0	0	0

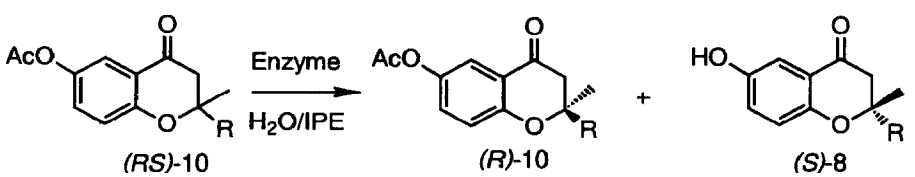
a All reactions were carried out by stirring a mixture of substrate(100mg)lipase(100mg)and IPE saturated with water at 25°C. b Isolated yield c Optical yields of 7 were determined by HPLC analyses using a column packed with Chiralcel OD-H (2-propanol/hexane),and optical yields of 6 were determined after conversion to 7.

Scheme 2



Reagents; *a* pyrrolidine/toluene *b* AcCl,Et_3N/THF *c* $NaBH_4$,thenH_2/Pd-C/MeOH *d* CH_2O, H_3BO_4,H_2/Pd-C/$(CH_3O)_3B$ *e* EtONa/EtOH

Table 2 *Lipase-catalyzed Kinetic Resolution of RS-10* [a]



Entry	Enzyme[6]	Time(h)	C.Y.(%)[b]	O.Y.(%ee)[c]	C.Y.(%)[b]	O.Y.(%ee)[c]
1	lipaseAH	2	13	42	87	31
2	lipaseAK	8	36	18	64	39
3	lipaseM	24	40	74	55	40
4	lipaseAY	0.7	18	9.7	73	22
5	F-AP15	72	53	13	40	41

a All reactions were carried out by stirring a mixture of substrate(100mg)lipase(100mg)and IPE saturated with water at 25°C. *b* Isolated yield. *c* Optical yields of 10 were determined by HPLC analyses using a column packed with Chiralcel OD-H (2-propanol/hexane),and optical yields 8 were determined after conversion to 10.

Acetylation of the other enantiomer [(*S*)-**6**] and subsequent oxidation with $Na_2Cr_2O_7$ gave 4-oxotocol acetate [(*S*)-**10**], which was easily racemized with NaOEt. Reduction of the (*RS*)-**8** thus obtained with $NaBH_4$ and subsequent hydrogenation with Pd-C gave (*RS*)-**6**. This (*RS*)-**6** was then employed as a substrate of enzyme-catalyzed hydrolysis.

Alternatively, (*RS*)-**10** was synthesized directly from 2,5-dihydroxyacetophenone (**9**) as shown in Scheme 2 and similarly submitted to enzyme-catalyzed hydrolysis. Table 2 shows the results. The (*R*)-**10**[11] obtained by enzyme-catalyzed kinetic resolution was reduced to (*R*)-tocol by $NaBH_4$ reduction and Pd-C catalyzed hydrogenation, which was then methylated to give natural α-tocopherol [(*R*)-**1**][8]. The unnatural enantiomer [(*S*)-**8**] was converted to the substrate [(*RS*)-**10**] for the enzyme-catalyzed hydrolysis by racemization and subsequent acetylation.

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6. Enzymes were kindly supplied by Amano Pharmaceutical Co.,Ltd. lipaseAY(*Candida rugosa*) CHE(*Cholesterol esterase*),lipaseAH(*Pseudomonas sp.*),lipasePS(*Pseudomonas sp.*),lipaseM(*Mucor javanicus*) lipaseAK(*Pseudomonas sp.*),F-AP15(*Rhizopus javanicus*).
7. (*R*)-**7**: $[\alpha]_D^{20}$ +4.1 (*c* 0.6 EtOH). ^{1}H-NMR($CDCl_3$) δ: 0.80-0.88(12H,m), 1.05-1.65 (24H,m), 1.65-1.91(2H,m), 2.25(3H,s), 2.70(2H,t,J=4.4Hz), 6.53-7.67(3H,m).
8. Identification was carried out by comparison of HPLC analysis using a column packed with Daicel Chiralcel OD-H(hexane/2-propanol=2000/1) with an authentic *d*-α-tocopherol. (*R*)-**1**:$[\alpha]_D^{20}$ +0.68 (*c* 0.8 EtOH). [lit.[5] +0.75(EtOH), authentic *d*-α-tocopherol +0.70 (*c* 1.0 EtOH)]. ^{1}H-NMR ($CDCl_3$) δ: 0.82-0.88(12H,m), 1.07-1.60(24H,m), 1.72-1.85(2H,m), 2.10-2.18(9H,m), 2.60(2H,t,J=2Hz), 4.18(1H,s). MASS:m/z(M^+)430.
9. Kijima.S.;Nonaka.A.; JP,60-4183(**1985**).
10. Previously,we have reported another type of substrate, of which reaction site a little remote from the stereogenic carbon atom. (a)Murata.M.;Achiwa.K.; *Tetrahedron Lett*,**1991**,32,6763. (b)Ebike.H.;Terao.Y.;Achiwa.K.; *Tetrahedron Lett*,**1991**,32,5805.
11. (*R*)-**10**:$[\alpha]_D^{20}$ +9.02 (*c* 0.6 EtOH) ^{1}H-NMR ($CDCl_3$) δ: 0.83-0.90(12H,m), 1.00-1.80 (24H,m), 2.25(3H.s), 2.70(2H,dd,J=17Hz), 6.91-7.54(3H,m).