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Hydrolase-catalyzed preparation of (*R*)- and (*S*)-4-hydroxy-2,6,6-trimethyl-2-cyclohexen-1-ones (phorenols), the key synthetic intermediates for abscisic acid

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Abstract

Preparation of both the enantiomers of 4-hydroxy-2,6,6-trimethyl-2-cyclohexen-1-one (phorenol), which are versatile synthetic intermediates for abscisic acid and carotenoids, was achieved by hydrolase-catalyzed hydrolysis of the corresponding chloroacetate. The hydrolysis with esterase SNSM-87 (Nagase) enriched the (*S*)-ester, while lipase P (Amano) afforded the (*R*)-ester.

1. Introduction

(+)-Abscisic acid [(+)-ABA] is a plant hormone with particular activity for environmental stress response as well as senescence promotion and growth inhibition.[1] In particular, ABA acts antagonistically against other plant hormones. Among plant hormones, only ABA is not supplied practically because of its high cost, though several syntheses of optically active ABA were reported.[2] Thus, development of a practical synthesis of (+)-ABA is desired for agrochemical use.

4-Hydroxy-2,6,6-trimethyl-2-cyclohexen-1-ones (*S*)- and (*R*)-**1** (phorenols) are versatile synthetic intermediates for the synthesis of optically active natural carotenoids[3] and degraded carotenoids,[3] especially for the synthesis of (+)-ABA.[2b] Kienzle et al. prepared (*S*)- and (*R*)-**1** by chemical modification of the baker's yeast reduction product **2** (Scheme 1).[4] Their procedure was somewhat lengthy and used manufacturally impractical baker's yeast. Recently, our group reported the preparation of (*S*)- and (*R*)-**1** through enzymatic resolution of diol (±)-**3**;[5] however, multi-steps were necessary and the yields and selectivity were low. The Rh metal-catalyzed asymmetric hydrogenation of enol ester **4** has been reported.[6] For the practical preparation of (+)-ABA as well as carotenoids and degraded carotenoids, we

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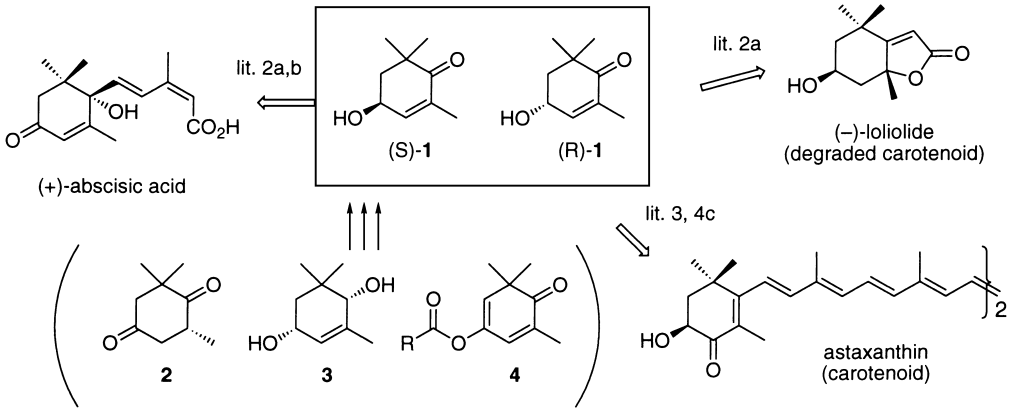
have investigated direct enzymatic resolutions[7] of (±)-**1** and its ester (±)-**5**, which were easily prepared from commercially available 4-oxoisophorone.

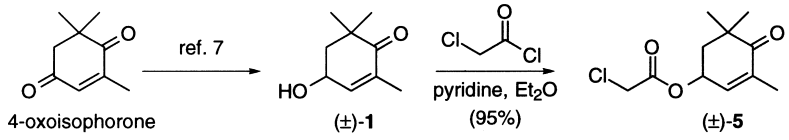


Scheme 1.

2. Results and discussion

2.1. Hydrolysis

(±)-4-Hydroxy-2,6,6-trimethyl-2-cyclohexen-1-one (±)-**1** was prepared by regioselective reduction of 4-oxoisophorone with $NaBH_4$–$CeCl_3$ according to Ishihara's procedure.[8] As a trial of the enzyme-catalyzed acetylation of (±)-**1** or hydrolysis of its acetate ester gave poor results (each *E* value[9] ≈2), we tried to use the corresponding chloroacetate ester **5**.[10] The alcohol (±)-**1** was esterified with chloroacetyl chloride in the presence of pyridine in 95% yield (Scheme 2). The result of hydrolysis of chloroacetate (±)-**5** is shown in Table 1. Screening of enzymes was done with the guidance of an *E* value of each reaction. Among several hydrolases examined, esterase SNSM-87[11] (*Klebsiella oxytoca*, Nagase) and lipase P (*Pseudomonas cepacia*, Amano) were adopted for our purpose. Esterase SNSM-87 (Nagase) preferentially hydrolyzed (*R*)-**5** and (*S*)-**5** remained unchanged, while lipase P (Amano) hydrolyzed the (*S*)-enantiomer faster. Further examinations under various conditions of these two enzymes were investigated. As shown in Table 2, the desired pH for esterase SNSM-87 was around 5.0–6.0 and diisopropyl ether was the best solvent (Scheme 3). The fact that the reaction temperature did not effect the selectivity much was supported by the high heat-resistance of this enzyme.



Scheme 2.

The results for lipase P (Amano) are summarized in Table 3. In this case, the initial condition (pH 7.0, 20°C) was the best choice. Finally, (*S*)- and (*R*)-**5** were hydrolyzed to give desired (*S*)- and (*R*)-**1**, respectively (Scheme 3).

Table 1
Enzyme-catalyzed hydrolysis of chloroacetate (±)-**5**[a]

enzymes	source	pH	time	convn[b]	**5**	(%ee)	E value[c]
lipase P (Amano)	*Pseudomonas cepacia* [d]	7.0	24 h	0.51	*R*	84.2	**25**
lipase PS30 (Amano)	*Pseudomonas cepacia*	7.0	6 h	0.70	*R*	45.9	2
CHIRAZYME® L-2, c.-f.,C2 (Roche)	*Candida antarctica*	6.0	24 h	0.96	*R*	7.4	-
CHIRAZYME® L-9, c.-f.,C2 (Roche)	*Mucor miehei*	7.0	24 h	0.94	*R*	4.1	-
lipase (Roche)	*Rhizopus arrhizus*	6.0	20 h	0.12	*R*	2.4	1
lipase, immobilized (Nacalai)	*Pseudomonas fluorescens*	7.0	24 h	0.13	*S*	1.8	1
lipase 2G (Nagase)	*Pseudomonas* sp.	8.0	14 h	0.76	*S*	78.2	4
lipase P (Nagase)	*Pseudomonas* sp.	7.0	6 h	0.35	*S*	18.0	2
immobilized lipase (Toyobo)	*Pseudomonas* sp.	7.0	6 h	0.65	*S*	12.4	1
esterase SNSM-87 (Nagase)	*Klebsiella oxytoca*	7.0	6 h	0.24	*S*	22.6	**8**
pancreatin (Nacalai)	porcine pancreas	6.0	14 h	0.46	*S*	10.2	1
PPL (Sigma Type II)	porcine pancreas	7.0	14 h	0.54	*S*	17.5	1
Rhilipase (Nagase)	*Rhizopus japonicus*	7.0	14 h	0.56	*S*	18.0	2
CHIRAZYME® L-1, c.-f. (Roche)	*Burkholderia cepacia* [e]	7.0	24 h	0.59	*S*	33.0	2
CHIRAZYME® E-1 (esterase, Roche)	pig liver	6.0	2 h	0.26	*S*	12.6	2
lipase, crude	*Trichoderma viride*	6.0	24 h	0.86	*S*	8.7	-
lipase (Sigma Type I)	wheat germ	7.0	72 h	0.72	*S*	31.6	2
lipase MY (Meito)	*Candida cylindracea*	7.0	1 h	1	-	-	-

a) All reactions were performed at 20°C in *i*-Pr_2O-0.1 M phosphate buffer. b) The conversion and ee were measured by HPLC analysis: the ratio of peak area (relative intensity of **5**/**1** = 1.05±0.035) was calculated as referred to ^{1}H-NMR analysis of the same sample. c) E values were calculated on the basis of conversion and ee of the substrate **5**. d) Formerly called *Pseudomonas fluorescens*. e) Formerly called *Pseudomonas cepacia*.

2.2. Transesterification

On the other hand, in the case of enzymatic transesterification, esterase SNSM-87 (Nagase) and lipase P (Amano) did not catalyze the reaction. Among enzymes examined, CHIRAZYME® L-1, c.-f. (*Burkholderia cepacia*, Roche), L-2, c.-f., C2 (*Candida antarctica*) and L-9, c.-f., C2 (*Mucor miehei*) worked to give the (*S*)-chloroacetate (Scheme 4), while crude lipase from *Trichoderma viride*, lipase MY (*Candida cylindracea*, Meito), lipase 2G (*Pseudomonas* sp., Nagase) and bioprase AL-45 (*Bacillus subtilis*, Nagase) afforded (*R*)-ester (Table 4). However, the *E* values of each reaction were low.

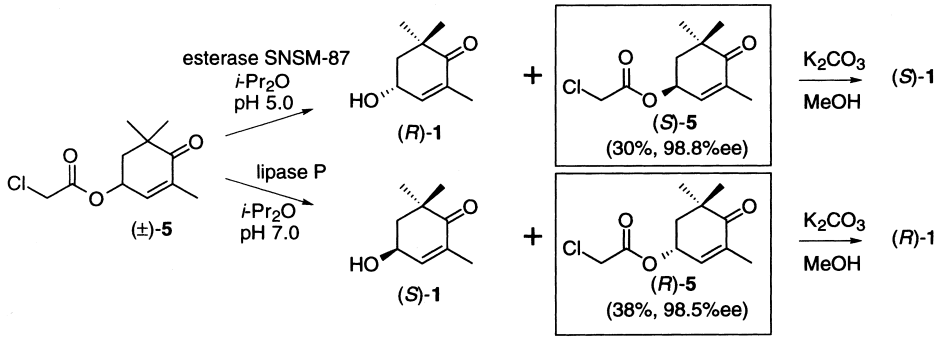
3. Conclusion

Both enantiomers of 4-hydroxy-2,6,6-trimethyl-2-cyclohexen-1-one (phorenol, **1**), the key intermediates for the synthesis of (+)-abscisic acid and other degraded carotenoids and carotenoids, were prepared. These were afforded by hydrolysis of the corresponding chloroacetate with different enzymes (esterase SNSM-87 for *S* and lipase P for *R*); however, the transesterification gave poor results. Since this method could supply (*R*)- and (*S*)-**1** in four steps from commercially available 4-oxoisophorone, it is applicable to practical production of (+)-abscisic acid and other carotenoids.

Table 2
Survey of reaction conditions for esterase SNSM-87[a]

solvent	temp (℃)	pH	time	conversion	ee of (*S*)-**5** (%ee)	E value
i-Pr_2O	0	7.0	10 h	0.21	20.0	9
	20	4.5	8 h	0.40	53.4	18±3[b]
		5.0		0.47	75.7	27±1[b]
		5.5		0.38	49.7	18±2[b]
		6.0		0.25	30.0	22±3[b]
		7.0		0.24	22.6	7±1[b]
		8.0		0.29	28.6	11±3[b]
	40	5.0	46 h	0.70	**98.8**	10
acetone	0	5.5	108 h	~0	-	-
	20		108 h	0.40	44.5	8
toluene	0	5.5	108 h	~0	-	-
	20		108 h	0.20	14.5	5
	40		24 h	0.15	11.4	5

a) Acetate buffer (0.1 M) was used at pH 4.5-5.5, while phosphate buffer (0.1 M) was used at pH 6.0-8.0.
b) Average values of duplicate or triplicate experiments.

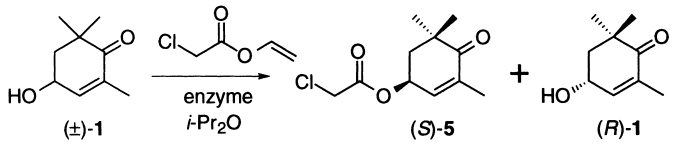


Scheme 3.

Table 3
Survey of reaction conditions for lipase P (Amano)[a]

solvent	temp	pH	time	conversion	ee of (*R*)-**5** (%ee)	E value
i-Pr_2O	20℃	6.0	24 h	0.64	32.1	2
		7.0		0.51	84.2	22±2[b]
		8.0		0.45	44.0	5
	40℃	7.0	48 h	0.62	**98.5**	20

a) All reactions were performed using phosphate buffer (0.1 M). b) Average value of triplicate experiments.



Scheme 4.

Table 4
Enzyme-catalyzed transesterification[a,b]

enzyme	source	time	convn	5	(%ee)	E value
CHIRAZYME® L-1, c.-f. (Roche)	*Burkholderia cepacia*	36 h	0.66	*S*	37.0	4
CHIRAZYME® L-2, c.-f., C2 (Roche)	*Candida antarctica*	36 h	-[c]	*S*	16.9	-[c]
CHIRAZYME® L-9, c.-f., C2 (Roche)	*Mucor miehei*	36 h	0.65	*S*	35.4	4
bioprase AL-45 (protease, Nagase)	*Bacillus subtilis*	72 h	0.14	*R*	54.6	4
lipase, crude	*Trichoderma viride*	48 h	-[d]	*R*	43.5	-[d]
lipase MY (Meito)	*Candida cylindracea*	48 h	0.59	*R*	31.0	3
lipase 2G (Nagase)	*Pseudomonas* sp.	48 h	0.13	*R*	48.3	3

a) All reactions were performed in *i*-Pr_2O at 20°C using vinyl chloroacetate as acyl donor. b) The reaction did not proceed with enzymes listed below: Rhilipase (*Rhizopus japonicus*, Nagase); XP-415 (*Rhizopus delemar*, Nagase), esterase SNSM-87 (Nagase), lipase P (Nagase), lipase P (Amano), lipase PS-30 (Amano), pancreatin (Nacalai), CHIRAZYME® E-1 (Roche). c) Conversion and E value could not be calculated because the oxidation product (4-oxoisophorone) was formed about 12% yield. d) 4-Oxoisophorone was formed in about 7% yield.

4. Experimental

4.1. General

Optical rotations were measured on a Horiba Sepa-300 polarimeter. UV spectrum was measured on a Shimadzu UV-1600 spectrometer. IR spectrum was measured on a Jasco Report-100 infrared spectrometer. ^{1}H NMR spectrum was measured on a Varian Gemini 2000 spectrophotometer (300 MHz) using tetramethylsilane as an internal standard. Mass spectra were recorded on a Jeol JMS-700 mass spectrometer. HPLC was performed on a Hitachi L-6000 pump and a Hitachi L-4200 UV–vis detector. Merck silica gel 60 (70–230 mesh) was used for column chromatography.

4.2. (±)-4-Oxo-3,5,5-trimethyl-2-cyclohexenyl chloroacetate (±)-5

To an ice-cooled mixture of 4-hydroxy-2,6,6-trimethyl-2-cyclohexen-1-one (±)-**1** (1.50 g, 9.73 mmol) and pyridine (1.24 g, 14.8 mmol) in dry ether (30 ml) was added chloroacetyl chloride (1.32 g, 11.7 mmol) and the resulting mixture was stirred for 5 h at 20°C. The reaction mixture was diluted with ether, washed with water, 2 M HCl aq. solution, satd aq. sodium hydrogen carbonate solution and brine, dried with anhydrous magnesium sulfate and concentrated in vacuo. The residue was purified with silica gel column chromatography (hexane/ethyl acetate) to give (±)-**5** (2.13 g, 9.23 mmol, 94.9%) as a colorless oil.

UV (c=8.7×10^{-5} mol/l in EtOH) ε=9900 (λ_{max} 228 nm). IR ν_{max} (film): 2960 cm^{-1} (s), 2930 (s), 2860 (s), 1755 (s, C=O), 1740 (s, C=O), 1680 (s, C=C), 1470 (m), 1450 (m), 1410 (m), 1380 (m), 1350 (m),

1315 (s), 1280 (s), 1260 (s), 1160 (br. s, C–O), 1105 (m), 1045 (m), 1000 (s), 980 (s), 960 (s), 860 (m), 790 (m), 700 (w). ^{1}H NMR (300 MHz, $CDCl_3$): δ=1.182 (s, 3H, 5-Me), 1.187 (s, 3H, 5-Me), 1.82 (t, 3H, *J*=1.7 Hz, 3-Me), 2.00 (dd, 1H, *J*=12.9, 9.3 Hz, 6-H), 2.21 (ddd, 1H, J=12.9, 5.5, 1.9 Hz, 6-H), 4.11 (s, 2H, Cl-CH_2), 5.71 (1H, m, 1-H), 6.53 (dq, 1H, *J*=4.1, 1.4 Hz, 2-H). EIMS m/z: 43, 61, 98, 174, 230 (M^+). HREIMS m/z (M^+): calcd for $C_{11}H_{15}O_3Cl$: 230.0710; found: 230.0711.

*4.3. (S)-4-Oxo-3,5,5-trimethyl-2-cyclohexenyl chloroacetate (S)-**5**: hydrolysis catalyzed by esterase SNSM-87*

A mixture of (±)-**5** (60.0 mg, 260 μmol) and esterase SNSM-87 (60 mg) in diisopropyl ether (1.5 ml) and 0.1 M acetate buffer (pH 5.0, 1.5 ml) was stirred for 2 d at 40°C. The reaction mixture was filtered through a Celite pad and the organic layer was separated. The aq. layer was extracted with ether and the combined organic layer was washed with satd aq. sodium hydrogen carbonate solution and brine, dried with anhydrous magnesium sulfate and concentrated in vacuo. The residue was purified with silica gel column chromatography (hexane/ethyl acetate) to give (*R*)-**1** (27.5 mg, 158 μmol, 60.7%) and (*S*)-**5** (12.2 mg, 52.9 μmol, 20.3%, 98.8% ee) as colorless oils. (*S*)-**5**: $[\alpha]_D^{24}$ −73.7 (*c*=0.455, EtOH). The enantiomeric purity of (*S*)-**5** was determined by HPLC detected at 230 nm at 20°C; column, Daicel Chiralcel® OB-H, 4.6×250 mm; solvent, hexane:2-propanol (7:1), 1.0 ml/min; t_R=11.4 min [99.4%, (*S*)-**5**] and 18.0 min [0.6%, (*R*)-**5**].

*4.4. (R)-4-Oxo-3,5,5-trimethyl-2-cyclohexenyl chloroacetate (R)-**5**: hydrolysis catalyzed by lipase P (Amano)*

A mixture of (±)-**5** (60.0 mg, 260 μmol) and lipase P (Amano) (60 mg) in diisopropyl ether (1.5 ml) and 0.1 M phosphate buffer (pH 7.0, 1.5 ml) was stirred for 2 d at 40°C. The reaction mixture was filtered through a Celite pad and the organic layer was separated. The aqueous layer was extracted with ether and the combined organic layer was washed with satd aq. sodium hydrogen carbonate solution and brine, dried with anhydrous magnesium sulfate and concentrated in vacuo. The residue was purified with silica gel column chromatography (hexane/ethyl acetate) to give (*S*)-**1** (28.9 mg, 187 μmol, 71.9%) and (*R*)-**5** (16.0 mg, 69.0 μmol, 26.7%, 98.5% ee) as colorless oils. (*R*)-**5**: $[\alpha]_D^{22}$ +70.0 (*c*=0.800, EtOH).

*4.5. (S)-4-Oxo-3,5,5-trimethyl-2-cyclohexenyl chloroacetate (S)-**5**: acylation catalyzed by CHIRAZYME® L-1*

A mixture of (±)-**1** (20.0 mg, 130 μmol), vinyl chloroacetate (23.5 mg, 195 μmol) and CHIRAZYME® L-1, c.-f., lyo. (20 mg) in diisopropyl ether (1.0 ml) was stirred for 4 h at 20°C. The reaction mixture was filtered through a Celite pad and concentrated in vacuo. The residue was purified with silica gel column chromatography (hexane/ethyl acetate) to give (*S*)-**5** (19.8 mg, 85.8 μmol, 66%, 37% ee) and (*R*)-**1** (6.82 mg, 44.2 μmol, 34%) as colorless oils. (*S*)-**5**: $[\alpha]_D^{23}$ −22.0 (*c*=1.57, EtOH).

*4.6. 4-Hydroxy-2,6,6-trimethyl-2-cyclohexen-1-one **1**: alkaline hydrolysis*

A mixture of (±)-**5** (300 mg, 1.30 mmol) and potassium carbonate (100 mg, 0.72 mmol) in methanol (50 ml) was stirred for 4 h at 20°C. The mixture was concentrated in vacuo to remove most of the methanol. The residue was diluted with water and extracted with ether. The combined organic layer was washed with satd aq. sodium hydrogen carbonate solution and brine, dried with anhydrous magnesium

sulfate and concentrated in vacuo. The residue was purified with silica gel column chromatography (hexane/ethyl acetate) to give (±)-**1** (209 mg, 1.19 mmol, 92.3%) as a colorless oil.

(*S*)-**1** was obtained in the same manner; $[\alpha]_D^{24}$ −50.0 (*c*=0.100, EtOH) {lit.[4b] $[\alpha]_D^{20}$ −49.0 (*c*=1, EtOH)}.

(*R*)-**1** was obtained in the same manner; $[\alpha]_D^{24}$ +52.7 (*c*=0.445, EtOH) {lit.[4d] $[\alpha]_D^{20}$ +47.7 (*c*=1, EtOH)}.

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11. Esterase SNSM-87 is available for laboratory use on request. Please contact the Research and Development Center, Nagase and Co. Ltd.; fax +81-78-992-1050.