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Synthesis of (-)-Methyl Shikimate via Enzymatic Resolution of (1S*, 4R*, 5R*)-4-Hydroxy-6-oxabicyclo[3.2.1]oct-2-en-7-one

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Note

Synthesis of (−)-Methyl Shikimate *via* Enzymatic Resolution of (1*S**, 4*R**, 5*R**)-4-Hydroxy-6-oxabicyclo[3.2.1]oct-2-en-7-one

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The synthesis of methyl (−)-shikimate [(−)-2] was achieved *via* lipase-catalyzed optical resolution of (1*S, 4*R**, 5*R**)-4-hydroxy-6-oxabicyclo[3.2.1]oct-2-en-7-one (3). Transesterification of (±)-3 and vinyl acetate with lipase MY and subsequent hydrolysis gave optically pure (−)-3. This compound was converted to (−)-2 in two steps.**

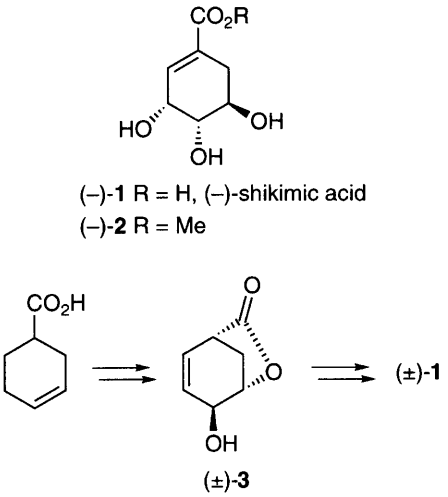
Key words: lipase; (1*S**, 4*R**, 5*R**)-4-hydroxy-6-oxabicyclo[3.2.1]oct-2-en-7-one; shikimic acid; hydrolysis; transesterification

(−)-Shikimic acid [(−)-**1**] is an important key compound for the biosynthesis of aromatic constituents in plants such as aromatic amino acids, coumarins, flavonoids and lignans.[1] The biosynthetic pathway involved is called " the shikimic acid pathway," and shikimic acid and its related compounds are consequently promising candidates for plant-growth regulators and antimicrobial agents. To meet this expectation, a practical preparation of optically active shikimic acid is necessary. Although there have been a number of reports on the synthesis of shikimic acid, the methods were not practical.[2,3] To supply (−)-**1** in a multi-gram quantity, we investigated the preparation by using enzyme-catalyzed optical resolution.[4]

Bartlett *et al.* have reported a practical synthesis of (±)-**1** from commercially available 3-cyclohexenecarboxylic acid (Scheme 1).[5] We identified the possible suitability of (1*S**, 4*R**, 5*R**)-4-hydroxy-6-oxabicyclo[3.2.1]oct-2-en-7-one [(±)-**3**], a key intermediate of this synthesis, as a substrate for resolution by using hydrolytic enzymes. The substrate [(±)-**3**] for the enzyme-catalyzed reaction was synthesized according to Bartlett's procedure. Table 1 shows the results of transesterification with vinyl acylates. Among several enzymes examined, lipase MY (Meito) showed moderate selectivity (E = 9.5).[6] This was not improved by using vinyl chloroacetate as an acyl donor. Enzyme-catalyzed hydrolysis of the corresponding acetate (**4**)[7] was also examined, although all enzymes gave poor results: the E values for lipase MY, pancreatin (Nacalai) and protease D-150 (Nagase) were 7.4, 6.8 and 5.4, respectively. For lipase MY, we tried to improve the selectivity by changing the reaction conditions, the results being summarized in Table 2. The E value was increased to 14 by adding DMSO as a co-solvent.[8] To obtain optically enriched compound, these reactions were combined: the transesterification of (±)-**3** and subsequent hydrolysis of intermediary acetate (−)-**4** (64%ee) gave (−)-**3** (97%ee) (Scheme 2) which was further purified by recrystallization (>99%ee). The enantiomeric purity was determined by an HPLC analysis of the corresponding benzoate. Finally, epoxidation of (−)-**3** gave (−)-**6** and subsequent alkaline hydrolysis afforded enantiomerically pure (−)-**2** in an 80% yield.

Experimental

Melting point (mp) data are uncorrected. Optical rotation values were measured by a Horiba Sepa-300 polarimeter, and mass spectra was recorded with a Jeol JMS − 700 spectrometer. HPLC was performed



Scheme 1. (−)-Shikimic Acid [(−)-**1**] and Bartlett's Synthesis of (±)-**1**.

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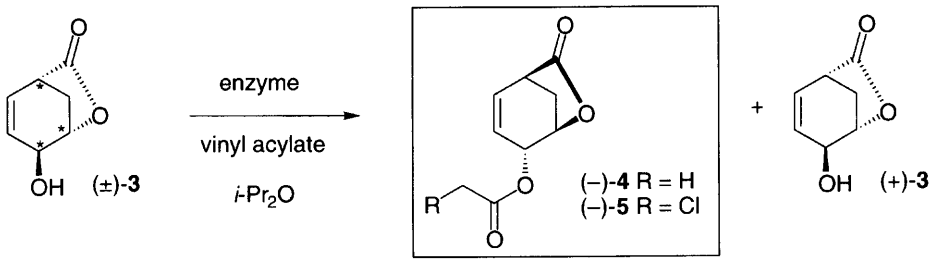
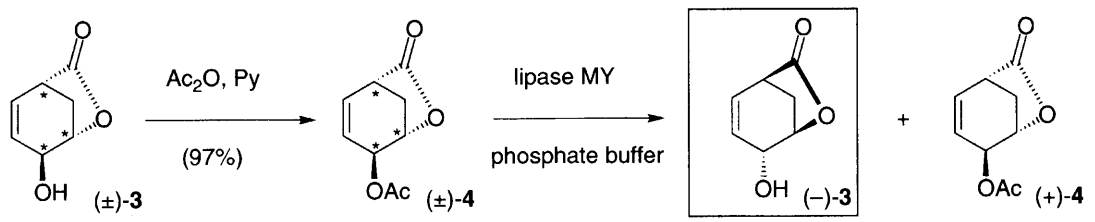
Table 1. Enzyme-catalyzed Transesterification



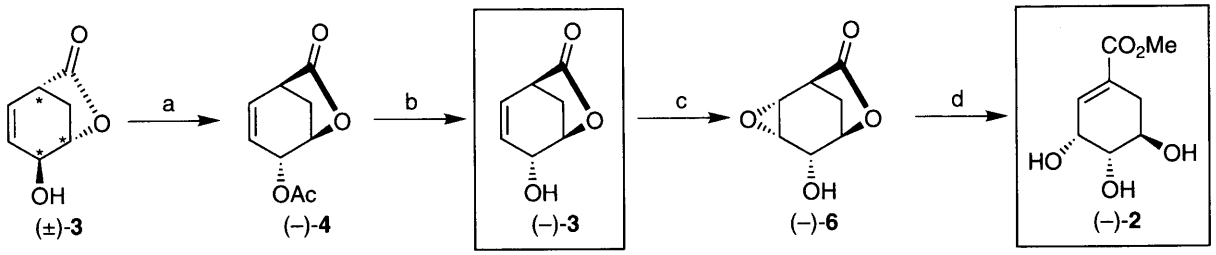
Enzyme	Source	Temp (°C)	Time (h)	Conversion	Product	%ee	E value[a]
Lipase MY (Meito)	*Candida cylindracea*	0		0.30		75	**9.5**
		25	48	0.20	(−)-**4**	62	**7.1**
Crude lipase	*Trichoderma viride*	25		0.10		26	~1
Lipase MY (Meito)	*Candida cylindracea*		5	0.37		34	2.4
Chirazyme[46] L-2, c.-f., C2 (Roche)	*Candida antarctica*	25	8	0.54	(−)-**5**	48	4.9
Chirazyme[46] L-1, c.-f. (Roche)	*Burkholderia cepacia*		72	0.22		4	~1

[a] E values were calculated on the basis of the conversion and ee of the remaining substrates.

Table 2. Enzyme-catalyzed Hydrolysis



Co-solvent	Temp (°C)	pH	Time (h)	Conversion	(−)-**3** (%ee)	E value
	25	5.0	1.5	0.33	57	4.8
i-Pr_2O	25	7.0	2.5	0.32	69	7.4
	15	7.0	3	0.51	50	4.9
Acetone	25	7.0	43	0.41	68	8.3
Hexane	25	7.0	1	0.46	40	3.2
DMSO (40%)			1.5	0.42	61	6.3
(50%)	25	7.0	2	0.31	73	8.8
(55%)			26	0.29	82	**14**
(60%)			168	0.15	84	13
(55%)	0	7.0	24	0.46	72	11



Scheme 2. Synthesis of (−)-Methyl Shikimate.
a) lipase MY, vinyl acetate, *i*-Pr_2O, 0°C (37%, 64%ee). b) i. lipase MY, aq. DMSO (55%v/v), phosphate buffer (pH 7.0), 25°C. ii. recrystalization (54%, >99%ee). c) MCPBA neat (80%). d) K_2CO_3, MeOH (quant).

with Hitachi L-6000 pump and a Hitachi L-4200 UV-VIS detector. Merck silica gel 60 (70 − 230 mesh) was used for column chromatography.

(1R, 4S*, 5S*)-4-Acetoxy-6-oxabicyclo[3.2.1]oct-2-en-7-one [(±)-4].* A solution of (±)-**3** (1.00 g, 7.14 mmol) and acetic anhydride (1.46 g, 14.3 mmol) in pyridine (2.26 g, 28.6 mmol) was stirred for 4 h at 20°C. The reaction mixture was diluted with Et_2O, successively washed with water, 2 M HCl aq. (twice) and a sat. aq. $NaHCO_3$ soln., dried ($MgSO_4$) and concentrated *in vacuo*. The residue was chromatographed on silica gel. Elution with hexane/EtOAc (3:2) gave (±)-**4** (1.26 g, 6.92 mmol, 97%) as colorless powder, mp 91–92°C (hexane/EtOAc). The spectral data (^1H NMR and IR) were identical with those reported.[7]

Optical resolution. A suspension of (±)-**3** (1.00 g, 7.14 mmol), vinyl acetate (922 mg, 10.7 mmol) and lipase MY (500 mg) in *i*-Pr_2O (50 ml) was stirred with a magnetic bar at 0°C for 5 d. The reaction mixture was filtered through a Celite pad and concentrated *in vacuo*. The resulting residue was chromatographed on silica gel, eluting with hexane/EtOAc to give (+)-**3** (599 mg, 4.28 mmol, 59.9%, 37%ee) as colorless needles, mp 93.0–93.5°C (hexane/*i*-PrOH), $[\alpha]_D^{20}$ +194° (*c*=1.01, $CHCl_3$) and (−)-**4** (472 mg, 2.59 mmol, 36.3%, 64%ee) as a colorless oil, $[\alpha]_D^{20}$ −322° (*c*=1.05, $CHCl_3$).

A suspension of (−)-**4** (471 mg, 2.59 mmol) and lipase MY (200 mg) in a phosphate buffer (pH 7.0, 4 ml) and aq. DMSO (55%v/v, 4.9 ml) was stirred at 25°C for 20 hr. The reaction mixture was filtered through a Celite pad, and the organic layer was separated. The resulting aqueous layer was extracted with EtOAc, and the combined organic layer was washed with brine and concentrated *in vacuo*. The residue was chromatographed on silica gel, eluting with hexane/EtOAc to give (−)-**3** (170 mg, 1.21 mmol, 46.7%, 97%ee) as colorless needles, mp 93.0–93.5°C (hexane/*i*-PrOH), $[\alpha]_D^{20}$ −460° (*c*=1.05, $CHCl_3$) and (−)-**4** (213 mg, 1.17 mmol, 45.2%, 27% ee) as a colorless oil. (−)-**3** was further purified by recrystallization with hexane/*i*-PrOH {99.1%ee, $[\alpha]_D^{20}$ −478° (*c*=1.00, $CHCl_3$)}.

Determination of the ee value for 3 by an HPLC analysis. Alcohol **3** produced by each enzymatic reaction was treated with benzoyl chloride (BzCl) in pyridine. After the reaction had been completed, the solution was diluted with Et_2O, successively washed three times with 2 M HCl aq., H_2O and brine, dried with $MgSO_4$ and concentrated *in vacuo*. The remaining pyridine was azeotropically removed with toluene. The ee value of the benzoyl ester was analyzed by HPLC (column, Daicel Chiralcel® OD, 4.6× 250 mm; temperature, 20°C; eluent, hexane/*i*-PrOH =7:1 at 0.5 ml/min; t_R, 22.1 min [benzoate of (−)-**3**] and 25.0 min [benzoate of (+)-**3**]; detection at 254 nm.

(1S, 2R, 3S, 4R, 5R)-2,3-Epoxy-4-hydroxy-6-oxabicyclo[3.2.1]octan-7-one [(−)-6]. (−)-**3** (139 mg, 1.00 mmol) and MCPBA (80%, 688 mg, 3.2 mmol) were mixed in a mortar. The mixture was dissolved in EtOAc, successively washed with a sat. aq. $NaHCO_3$ soln. and brine, dried with $MgSO_4$ and concentrated *in vacuo*. The residue was chromatographed on silica gel (hexane/EtOAc=3:2) to give (−)-**6** (124 mg, 0.795 mmol, 79.5%) as white powder, mp 127–127.5°C (hexane/*i*-PrOH) (lit.,[5] a white solid, mp 122.5–123°C; lit.,[7] mp 123–125°C), $[\alpha]_D^{21}$ −203° (*c*=1.02, $CHCl_3$). The spectral data (^1H NMR and IR) were identical with those reported.[5]

Methyl shikimate [(−)-2]. A suspension of (−)-**6** (40.2 mg, 0.256 mmol) and K_2CO_3 (10 mg, 0.07 mmol) in dry MeOH (0.5 ml) was stirred at 20°C for 40 min. The reaction mixture was neutralized with NH_4Cl and concentrated *in vacuo*. The residue was purified by PTLC ($CHCl_3$/EtOH=9:1) to give (−)-**2** (48.0 mg, 0.255 mmol, quant.) as colorless powder, mp 110–111°C (EtOAc), $[\alpha]_D^{21}$ −131° (*c*=0.125, EtOH) {lit.,[3] colorless crystals, mp 112–113°C, $[\alpha]_D^{29}$ −130° (*c*=0.91, EtOH)}. The spectral data (^1H NMR and IR) were identical with those reported.[3]

Acknowledgments

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