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Enzymatic Preparation of (4*R*,6*R*)-4-Hydroxy-1,7-dioxaspiro[5.5]undecane and its Antipode, the Minor Component of the Olive Fruit Fly Pheromone†

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Abstract—(4*S*,6*S*)-4-Hydroxy-1,7-dioxaspiro[5.5]undecane (**2**) and (4*R*,6*R*)-4-acetoxy-1,7-dioxaspiro[5.5]undecane (**3**) were prepared by asymmetric hydrolysis of (±)-**3** with pig liver esterase. Copyright © 1996 Elsevier Science Ltd

Introduction

In 1980 Baker, Francke and their co-workers isolated and identified 1,7-dioxaspiro[5.5]undecane (olean, **1**) as the major component of the female-produced sex pheromone of the olive fruit fly, *Bactrocea oleae* (formerly *Dacus oleae*).[2,3] (4*R**,6*R**)-4-Hydroxy-1,7-dioxaspiro[5.5]undecane (**2**) was also isolated as the minor component.[3,4] Bioassay of the synthetic enantiomers of **1** revealed that (*R*)-**1** is active on male insects, while (*S*)-**1** is active on females.[5]

Our 1985 synthesis of the enantiomers of **1** and **2** employed (*S*)-malic acid as the starting material, and was rather lengthy.[6,7] It occurred to us that the enzymatic resolution of (±)-**2** might simplify the synthesis, because **2** gives **1** by two-step deoxygenation. This paper describes the preparation of both the enantiomers of **2** by employing the asymmetric hydrolysis of the corresponding acetate (±)-**3** with pig liver esterase (PLE, EC 3.1.1.1).

Results and Discussion

Screening of the hydrolytic enzymes

(4*R**,6*R**)-4-Hydroxy-1,7-dioxaspiro[5.5]undecane [(±)-**2**] was prepared from the epoxide **A** (Scheme 1) and dihydropyran **B** by the method of Kocieński and Yeates.[8] Acetylation of (±)-**2** furnished the acetate (±)-**3**. The overall yield of (±)-**3** was 38% based on **A** (three steps).

Table 1 summarizes our screening of hydrolytic enzymes to find out the most suitable one for asymmetric hydrolysis of (±)-**3**. The hydrolysis was carried out in 0.1 M phosphate buffer (pH = 7.5) containing organic solvents.[9] PLE was found to be the most appropriate enzyme when it was employed at a low temperature (−10 to −5 °C) with methanol as the co-solvent. Although both PLE-A (Amano) and PLE (Sigma) gave good results, less expensive PLE (Sigma) was employed. Comparison of the sign of the optical rotation of the resulting alcohol **2** with the known data[6,7] revealed that (4*S*,6*S*)-**3** was preferentially hydrolyzed.

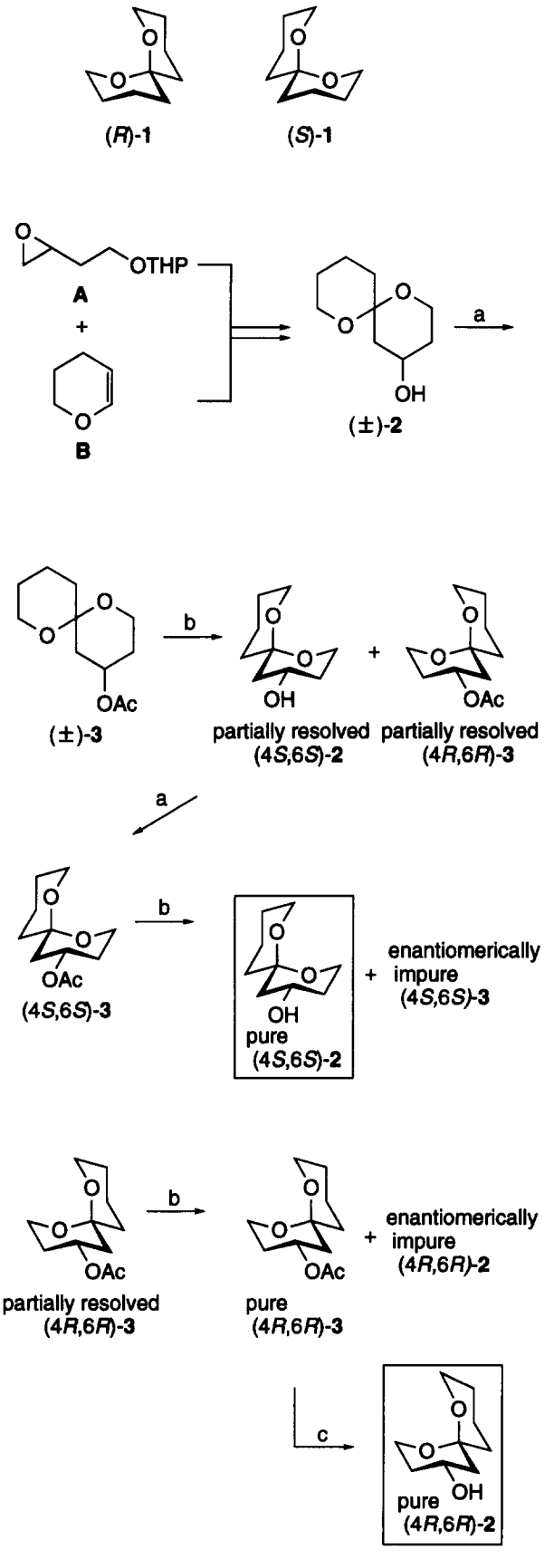
Preparative-scale hydrolysis of (±)-3 and the synthesis of the enantiomers of 2

Scheme 1 summarizes the synthesis of the pure enantiomers of **2**. Asymmetric hydrolysis of (±)-**3** gave a mixture of the almost pure acetate (4*R*,6*R*)-**3** and the partially resolved alcohol (4*S*,6*R*)-**2**. The mixture was readily separable by silica gel chromatography. Acetylation of the impure alcohol (4*S*,6*S*)-**2** yielded enantiomerically impure (4*S*,6*S*)-**3**, which was again treated with PLE to give enantiomerically pure (4*S*,6*S*)-**2**, $[\alpha]_D^{25}$ +122° (*n*-pentane), and unhydrolyzed and enantiomerically impure (4*S*,6*S*)-**3**.

In order to secure highly enantiomerically pure (4*R*,6*R*)-**2**, the almost pure acetate (4*R*,6*R*)-**3** was further treated with PLE to give enantiomerically pure (4*R*,6*R*)-**3** and the hydrolyzed and enantiomerically impure alcohol (4*R*,6*R*)-**2**. Treatment of the pure acetate (4*R*,6*R*)-**3** with potassium carbonate in methanol furnished enantiomerically pure (4*R*,6*R*)-**2**, $[\alpha]_D^{26}$ −126° (*n*-pentane).

The present enzymatic method of synthesis is simple and efficient enough to allow us to provide gram quantities of both (4*R*,6*R*)- and (4*S*,6*S*)-4-hydroxy-1,7-dioxaspiro[5.5]undecane (**2**).

†Pheromone Synthesis, Part 173. For Part 172, see ref 1.



Scheme 1. Synthesis of the enantiomers of **2**. Reagents: (a) Ac_2O,C_5H_5N; (b) PLE, 0.1 M phosphate buffer, (pH 7.5) containing 20% (v/v) of MeOH; (c) K_2CO_3, MeOH.

Experimental

All bps are uncorrected. IR spectra were measured as films for oils on a Hitachi Perkin–Elmer 1600 spectrometer. ^{1}H NMR spectra were recorded in $CDCl_3$ with $CHCl_3$ or with TMS as an internal standard at 60 MHz on a Hitachi R-24B spectrometer or at 270 MHz on a Jeol JMN-EX 270L spectrometer. ^{13}C NMR spectra were recorded in $CDCl_3$ with $CHCl_3$ as an internal standard at 67.8 MHz on a Jeol JMN-EX 270L spectrometer. Optical rotations were measured on a Jasco DIP-1000 spectropolarimeter. GLC analyses were performed on a Shimadzu GC-14A. Column chromatography was carried out on columns packed with Merck Kieselgel 60, Art. no. 7734. PLE (EC 3.1.1.1 200 units/mg protein) were purchased from Sigma.

(4*R,6*R**)-4-Hydroxy-1,7-dioxaspiro[5.5]undecane [(±)-2].** To a stirred solution of 2.5 g (30 mmol) of dihydropyran in dry THF (6 ml) was added dropwise 22.5 mL (36 mmol) of 1.6 M solution of *t*-BuLi in pentane at −78 °C. To the resultant solution were successively added dry THF (15 mL) and CuI (2.9 g, 15 mmol) under Ar atmosphere at −78 °C and the mixture was stirred for 45 min. A solution of **A** (1.79 g, 10.4 mmol) in dry THF (5 mL) was added and the mixture was allowed to warm slowly to room temperature. After 2 h at room temperature the mixture was quenched with saturated aqueous ammonium chloride solution and extracted several times with ether. The ether solution was dried over anhydrous sodium sulfate and concentrated in vacuo. The resulting crude oil was dissolved in a mixture of concd hydrochloric acid, water and THF (1:5:20) and allowed to stand at room temperature overnight. The mixture was then neutralized with saturated aqueous sodium hydrogen carbonate solution and extracted several times with ether. The ether solution was dried with anhydrous sodium sulfate and concentrated in vacuo. The residue was chromatographed over silica gel (50 g). Elution with *n*-hexane:ether (1:1) gave 1.00 g (56%) of (±)-**2** as an oil. IR (film): ν 3382 (m, O—H), 1062 (m, C—O), 1047 cm^{-1} (m, C—O). ^{1}H NMR (270 MHz, C_6D_6): δ 0.89 (1H, br, OH), 1.08–1.44 (6H, m, 9,10,11-H), 1.48–1.61 (2H, m, 3-H), 1.73–1.88 (1H, m, 5-H_{ax}), 1.94 (1H, ddd, J = 12.5, 4.5, 2.0 Hz, 5-H_{eq}), 3.33–3.53 (4H, m, 2,8-H), 3.96 (1H, tt, J = 11.0, 5.5 Hz, 4-H).

(4*R,6*R**)-4-Acetoxy-1,7-dioxaspiro[5.5]undecane[(±)-3].** To a stirred solution of (±)-**2** (1.00 g, 58.7 mmol) in dry pyridine (10 ml) was added acetic anhydride (0.89 g, 5.87 mmol) in small portions. The stirring was continued overnight at room temperature and for another 1 h after the addition of water. The resulting mixutre was then poured into water and extracted several times with ether. The combined ethereal extracts were successively washed with saturated aqueous copper(II) sulfate solution, water, saturated aqueous sodium hydrogen carbonate solution and

Table 1. Screening of the enzymes for the asymmetric hydrolysis of (±)-**3**

Entry	Enzyme	Reaction conditions[a]			Amount of the substrate (±)-**3** (mg)	Amount of the hydrolyzed **2** (mg, yield)	$[\alpha]_D$ of **2** in *n*-pentane (°)	Amount of the recovered **3** (mg, yield)	$[\alpha]_D$ of **3** in *n*-pentane (°)
		Temperature (°C)	Solvent	Time					
1	Lipase A (Amano)	rt	20% MeOH in H_2O	13.5 h	105	6.7 (7.9%)	+13.5 (*c* 0.23)	96 (91%)	−0.4 (*c* 1.70)
2	Lipase MY (Meito)	rt	20% MeOH in H_2O	6.5 h	126	9.5 (9.4%)	+9.5 (*c* 0.30)	100 (79%)	−0.8 (*c* 2.56)
3	Lipase AK (Amano)	rt	20% MeOH in H_2O	4 d	119	31 (32%)	−3.2 (*c* 1.12)	49 (41%)	+1.52 (*c* 1.80)
4	PPL[b]	rt	20% MeOH in H_2O	3.5 d	132	34 (33%)	−8.2 (*c* 1.50)	62 (47%)	+4.9 (*c*1.78)
5	PLE-A[c]	rt	20% MeOH in H_2O	15 min	103	43 (52%)	+80.7 (*c* 0.56)	43 (42%)	−62.6 (*c* 1.00)
6	Lipase P (Amano)	rt	20% MeOH in H_2O	3 d	106	60 (70%)	−0.5 (*c* 0.44)	33 (31%)	+4.0 (*c* 0.45)
7	PLE[d]	rt	20% MeOH in H_2O	15 min	121	39 (40%)	+97.3 (*c* 0.56)	72 (60%)	−56.8 (*c* 2.55)
8	PLE	−10 to −5	20% EtOH in H_2O	10 h	110	57 (64%)	+51.3 (*c* 1.59)	30 (33%)	−94.1 (*c* 1.09)
9	PLE	0	20% $(^iPr)_2O$ in H_2O	3 h	101	17 (21%)	+70.6 (*c* 0.79)	67 (67%)	−22.0 (*c* 2.15)
10	PLE	−10 to −5	20% MeOH in H_2O	7 h	110	38 (44%)	+94.4 (*c* 1.76)	43 (39%)	−103 (*c* 1.15)

[a] 0.1 M phosphate buffer (pH 7.5).
[b] PPL = pig pancreatic lipase (Sigma).
[c] PLE-A = pig liver esterase-A (Amano).
[d] PLE = pig liver esterase (Sigma).

brine, dried with anhydrous sodium sulfate, and concentrated in vacuo. The residue was chromatographed over silica gel (30 g). Elution with *n*-hexane:ether (10:1) gave 0.85 g (68%) of (±)-**3** as an oil. IR (film): ν 1741 (s, C=O), 1064 (m, C—O), 1042 cm^{-1} (s, C—O). ^1H NMR (270 MHz, $CDCl_3$): δ 1.18–1.76 (9H, m, $3,5_{ax},9,10,11$-H), 1.76–1.81 (1H, m, 5-H_{eq}), 2.02 (3H, s, $—CH_3$), 3.58–3.63 (2H, m, 8-H), 3.70–3.75 (2H, m, 2-H), 5.16 (1H, dt, $J = 11.3$, 4.95, Hz, 4-H).

PLE-catalyzed hydrolysis of the acetate (±)-3

To a mechanically stirred mixture of acetate (±)-**3** (6.65 g, 31.1 mmol) in 0.1 M phosphate buffer (pH = 7.5, 750 mL containing 20% of methanol) was added pig liver esterase (25.3 mg protein, 5060 units) at −12 to −13 °C. The mixture was vigorously stirred for 30 h, keeping the reaction temperature at −12 to −13 °C. Then the mixture was saturated with sodium chloride, filtered through Celite and extracted several times with ether. The combined ethereal extracts were washed with saturated aqueous sodium hydrogen carbonate solution and brine, dried with anhydrous sodium sulfate, and concentrated in vacuo. The residue (6.80 g) was chromatographed over silica gel (150 g). Elution with *n*-hexane:ether (10:1→1:1) gave 2.89 g (43%) of acetate (4*R*,6*R*)-**3** as an oil. $[\alpha]_D^{21}$ −112° (*c* 3.60, *n*-pentane). Its IR and ^1H NMR spectra were identical to those of (±)-**3**. Further elution gave 2.43 g (46%) of alcohol (4*S*,6*S*)-**2** as an oil. $[\alpha]_D^{23}$ +100° (*c* 1.14, *n*-pentane). Its IR and ^1H NMR spectra were identical to those of (±)-**2**.

Second hydrolysis to enrich enantiomeric purity of the partially resolved alcohol

In almost the same manner as above, the partially resolved (4*S*,6*S*)-**2** (2.31 g, 13.4 mmol) was acetylated to give 2.77 g (12.9 mmol. 96%) of (4*S*,6*S*)-**3**. Then, in almost the same manner as above, the resulting acetate (4*S*,6*S*)-**3** was treated with PLE (5.15 mg protein, 1030 units) in 0.1 M phosphate buffer (same conditions as above, 350 mL) for 11 h at −12 to −13 °C to give 1.23 g (44%) of impure (4*S*,6*S*)-**3** {$[\alpha]_D^{23}$ +60.8° (*c* 2.41, *n*-pentane); its IR and ^1H NMR spectra were identical to those of (±)-**3**} and 1.07 g (48%) of (4*S*,6*S*)-**2**. A portion of (4*S*,6*S*)-**2** was distilled to give an analytical sample as a colorless oil; bp 80–82 °C/0.75 Torr; n_D^{26} 1.4797; $[\alpha]_D^{25}$ +122° (*c* 2.25, *n*-pentane [cf. ref 7 $[\alpha]_D^{20}$ +120° (*c* 2.61, *n*-pentane)]. The enantiomeric purity of (4*S*,6*S*)-**2** was shown to be 99.1% ee by GLC analysis: GLC [Column, DMPGCD-TH {octakis(2,6-di-*O*-methyl-3-*O*-*n*-pentyl)-γ-cyclodextrin}, 0.32 mm × 50 m at 70 °C + 1.0 °C/min; Carrier gas, He, 1.0 kg/cm²], $t_R = 77.5$ [(4*S*,6*S*)-**2**, 99.55%] and 74.3 min [(4*R*,6*R*)-**6**, 0.45%]. Its IR and ^1H NMR spectra were identical to those of (±)-**2**. ^{13}C NMR (C_6D_6, 67.8 MHz): δ 18.9, 25.5, 35.6, 35.8, 45.8, 58.8, 60.2, 64.2, 97.3. HRMS: Calcd for $C_9H_{16}O_{13}$: 172.1100. Found: 172.1100. Acetylation of (4*S*,6*S*)-**2** yielded (4*S*,6*S*)-**3** as an oil, bp 79 °C/1.0 Torr; n_D^{27}1.4643; $[\alpha]_D^{25}$ +117° (*c* 1.05, *n*-pentane).

Second hydrolysis to enrich enantiomeric purity of the partially resolved acetate

In almost the same manner as above, the almost resolved (4*R*,6*R*)-**3** (2.83 g, 13.2 mmol) was treated with PLE (12.7 mg protein, 2530 units) in 0.1 M phosphate buffer (same conditions as above, 350 mL) for 68 h at −12 to −13 °C to give 1.97 g (70%) of (4*R*,6*R*)-**3** as an oil {bp 70 °C/0.9 Torr; n_D^{28} 1.4643; $[\alpha]_D^{25}-116°$ (*c* 1.09, *n*-pentane); its IR and ^{1}H NMR spectra were identical to those of (±)-**3**} and 0.51 g (22%) of impure (4*R*,6*R*)-**2** $[\alpha]_D^{26}-76.2°$ (*c* 2.11, *n*-pentane); its IR and ^{1}H NMR spectra were identical with those of (±)-**2**.

Hydrolysis of the acetate (4*R*,6*R*)-3

To a stirred solution of the acetate (4*R*,6*R*)-**3** (1.90 g, 8.89 mmol) in methanol (10 mL) was added potassium carbonate (2.48 g, 18.0 mmol) in one portion. The mixture was vigorously stirred for 3 h at room temperature and methanol was removed in vacuo. Then the resulting mixture was diluted with water and extracted several times with ether. The combined ethereal extracts were washed with saturated aqueous sodium hydrogen carbonate solution and brine, dried with anhydrous sodium sulfate and concentrated in vacuo to give 1.43 g (94%) of (4*R*,6*R*)-**2** as an oil. A portion of it was distilled to give an analytical sample as a colorless oil; bp 76–78 °C/0.6 Torr; n_D^{26} 1.4794; $[\alpha]_D^{25}$ −126° (*c* 21.3 *n*-pentane) {cf. ref 7 $[\alpha]_D^{20}-116°$ (*c* 2.41, *n*-pentane)}. Its IR and ^{1}H NMR spectra were identical to those of (±)-**2**. ^{13}C NMR (C_6D_6, 67.8 MHz): δ 18.9, 25.5, 35.6, 35.8, 45.8, 58.9, 60.2, 64.2, 97.4. The enantiomeric purity of (4*R*,6*R*)-**2** was shown to be 99.8% by GLC analysis: GLC [Column, DMPGCD-TH {octakis(2,6-di-*O*-metyl-3-*O*-*n*-pentyl)-γ-cyclodextrin}, 0.32 mm × 50 m at 70 °C + 1.0 °C/min; carrier gas, He, 1.0 kg/cm^2], t_R 73.5 [(4*R*,6*R*)-**2**, 99.9%] and 77.3 min [(4*S*,6*S*)-**2**, 0.10%]; HRMS: Calcd for $C_9H_{16}O_3$: 172.1100. Found: 172.1108.

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