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BIOCATALYTIC SYNTHESIS OF (*S*)-2-TRIDECANYL ACETATE AND (*S*)-2-PENTADECANYL ACETATE, AGGREGATION PHEROMONE COMPONENTS OF *Drosophila mulleri* and *D. busckii*, BY ENANTIOSELECTIVE HYDROLYSIS WITH LIPASE

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Abstract—The two chiral pheromone acetates, (*S*)-2-tridecanyl acetate and (*S*)-2-pentadecanyl acetate, were synthesized with an enantiomeric excess (e.e.) of almost 100% by *Pseudomonas cepacia* lipase-catalyzed hydrolysis of their corresponding racemic acetates in an acetone–water solvent system.

Key Words—*Drosophila mulleri*, *D. busckii*, Diptera, Drosophilidae, aggregation pheromone, (*S*)-2-tridecanyl acetate, (*S*)-2-pentadecanyl acetate, lipase, hydrolysis, enantioselectivity, chirality.

INTRODUCTION

Lipases have been widely utilized over the past five years as a routine reagent for organic synthesis, mainly because of their ability to accept and transform enantioselectively not only natural substrates but also organic xenobiotics (Klibanov, 1990; Halgas, 1992). The enzymatic hydrolysis of the acetates of racemic alkan-2- and -3-ols was carried out by using lipase PS (lipase from *Pseudomonas cepacia*, Amano PS) in the presence of organic media and showed that the

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enantioselectivity could be enhanced in all of the organic–water solvent systems tested, particularly in an acetone–water system (Naoshima et al., 1992, 1993).

(*S*)-2-Tridecanyl acetate **(1)**, an aklan-2-yl acetate, is an aggregation pheromone component of *Drosophila mulleri* (Bartelt et al., 1989) and was previously synthesized by several methods (Gopalan and Jacobs, 1990; Enders and Plant, 1991; Hintze and Hoppe, 1992). The present work is a simplified, short-step synthesis of (*S*)-2-tridecanyl acetate **(1)** with almost 100% e.e. based upon the enzymatic hydrolysis of racemic acetate with lipase PS. The same enzymatic strategy also yielded (*S*)-2-pentadecanyl acetate **(2)** with almost 100% e.e., which is an aggregation pheromone component of *D. busckii* (Schaner et al., 1989).

METHODS AND MATERIALS

IR spectra were determined on a Fourier transform Perkin-Elmer 1720 IR spectrometer. ^{1}H NMR spectra were obtained on a Fourier transform Hitachi R-1500 (60 MHz) spectrometer or a Bruker AMX-R400 spectrometer in $CDCl_3$ solutions, using Me_4Si as an internal standard. Column chromatography was performed with 70–230 mesh silica gel (Merck Kieselgel 60 Art 7734). All solvent systems were expressed in ratios by volume (v/v). Gas chromatography was carried out on a Hitachi G-3000 chromatograph equipped with a SE-30 25-m × 0.25-mm capillary column (GL Sciences, Tokyo, Japan), using He as carrier gas. Optical rotations were measured with a Horiba SEPA-200 high-sensitivity polarimeter. Lipase from *Pseudomonas cepacia* was used (Amano Lipase PS, Amano Pharmaceutical Co., Nagoya, Japan).

Determination of Enantiomeric Purity. The enantiomeric purity of acetates (*S*)-**1** and (*S*)-**2** is based on that of the alcohols prepared by treating the acetates with KOH in methanol. Enantiomeric excesses (e.e.) of the alcohols were determined by a GC analysis of the diastereomeric esters prepared by treating the alcohols with (*S*)-2-acetoxypropionyl chloride (Slessor et al., 1985; Millar et al., 1991). The diastereomers derived from racemic alcohols were each separated into two equal peaks (column temperature, 200° C). For (±)-2-tridecanol: R_t 10.8 and 11.5 min. For (±)-2-pentadecanol: R_t 12.5 and 13.4 min.

(±)-2-Tridecanyl Acetate [(±) − 1]. To a stirred solution of (±)-2-tridecanol (5 g, 25 mmol) and 4-pyrrolidinopyridine (0.8 g) in dry CH_2Cl_2 (70 ml) was added acetic anhydride (5.1 g, 50 mmol) at room temperature. After being stirred for 5 hr, the mixture was poured into ice-water and extracted with CH_2Cl_2. The extract was washed successively with 10% HCl and water, dried over Na_2SO_4, and concentrated. The crude product obtained was purified by column chromatography on silica gel (110 g) with hexane–ether (20 : 1) to give (±)-**1** as a colorless liquid (5.76 g, 95%). The IR and ^{1}H NMR spectra were identical with those reported for (*S*)-**1** (Enders and Plant, 1991).

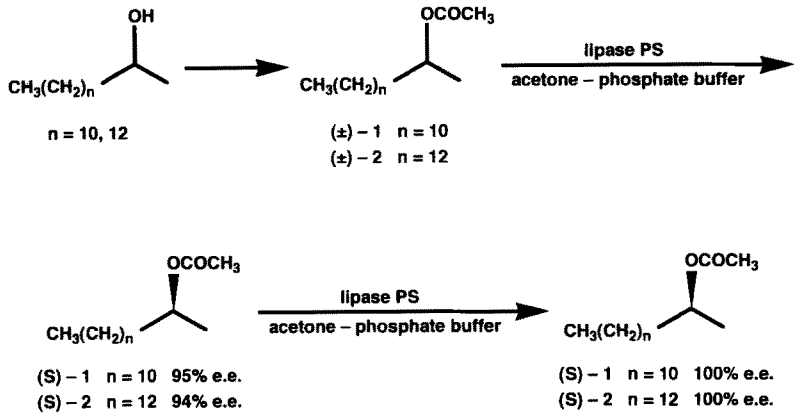
(±)-2-Pentadecanyl Acetate [(±)-2]. (±)-2-Pentadecanol (5 g, 21.9 mmol) was treated with acetic anhydride (4.47 g, 43.8 mmol) in dry CH_2Cl_2 (70 ml) in the presence of 4-pyrrolidinopyridine (0.8 g). The usual workup of the reaction mixture and subsequent purification by column chromatography on silica gel (110 g) with hexane–ether (20 : 1) gave (±)-**2** as a colorless liquid (5.63 g, 95%). Compound **2** was identified by comparison of its IR and ^{1}H NMR spectra with those of (±)-**1.**

(S)-*2-Tridecanyl Acetate [(S)-1].* A mixture of (±)-**1** (4 g, 16.5 mmol), lipase PS (1.6 g), acetone (48 ml), and 0.1 M phosphate buffer (pH 8.0, 72 ml) was stirred for 145 hr at 30°C. GC analysis showed that conversion was about 52%. After filtration through Celite, the filtrate was extracted with ether and the extract was washed with brine, dried, and concentrated. Purification by column chromatography on silica gel (70 g) with hexane–ether (25 : 1) gave (*S*)-**1** (1.76 g, 44%) with 95% e.e., $[\alpha]_D^{20} = +4.48°$ (c3.58, pentane). This acetate (1 g, 4.13 mmol) was added to a mixture of lipase PS (0.4 g), acetone (12 ml), and 0.1 M phosphate buffer (pH 8.0, 18 ml). The mixture was stirred for 115 hr at room temperature; GC showed a conversion of 17%. Column chromatography as already described gave (*S*)-**1** (0.77 g, 77%) with almost 100% e.e., $[\alpha]_D^{20} = +4.75°$ (c2.46, pentane) {literature value: $[\alpha]_D^{23} = +4.6°$ (c0.57, hexane), Bartelt et al., 1989}. The IR and ^{1}H NMR spectra were identical with those of racemic **1.**

(S)-*2-Pentadecanyl Acetate [(S)-2].* A mixture of (±)-**2** (4 g, 14.8 mmol), lipase PS (1.6 g), acetone (48 ml), and 0.1 M phosphate buffer (pH 8.0, 72 ml) was stirred for 155 hr at 30°C. GC showed a conversion of 55%. Column chromatography on silica gel (70 g) with hexane–ether (25 : 1) gave (*S*)-**2** (1.64 g, 41%) with 94% e.e., $[\alpha]_D^{20} = +4.61°$ (c3.57, pentane). This acetate (1 g, 3.7 mmol) was submitted to a second hydrolysis with lipase PS (0.4 g) in a mixture of acetone (12 ml) and 0.1 M phosphate buffer (pH 8.0, 18 ml). After being stirred for 150 hr (20% conversion), the mixture was worked up in the usual way. Column chromatography gave (*S*)-**2** (0.72 g, 72%) with almost 100% e.e., $[\alpha]_D^{20} = +4.99°$ (c2.55, pentane). The IR and ^{1}H NMR spectra were identical with those of racemic 2.

RESULTS AND DISCUSSION

As shown in Scheme 1, the key feature of the present synthesis is that the *S* configuration of the chiral pheromones **1** and **2** is established by the enantioselective hydrolysis of (±)-acetates **1** and **2** with lipase PS in an acetone–water solvent system. (±)-2-Tridecanol was converted by treatment with acetic anhydride in the presence of 4-pyrrolidinopyridine into acetate **1,** and the latter was hydrolyzed with lipase PS in an acetone–phosphate buffer. The enzymatic



SCHEME 1.

hydrolysis resulted in 52% conversion, and the unreacted acetate (95% e.e.) was resubmitted to lipase hydrolysis. (*S*)-Pheromone acetate **1** thus obtained showed an enantiomeric purity of almost 100%. Similarly, the acetate **2** derived from (±)-2-pentadecanol was initially hydrolyzed with lipase PS (55% conversion), and (*S*)-acetate **2** of 94% e.e. was obtained. The acetate was submitted to the second hydrolysis with lipase PS to give (*S*)-pheromone acetate **2** of almost 100% e.e.

Recently, Gopalan and Jacobs (1990) reported a four-step synthesis of (*S*)-**1** with 98% e.e. via the baker's yeast reduction of a ketosulfone (21% overall yield). Enders and Plant (1991) synthesized (*S*)-**1** with 93.5% e.e. in six steps from propiophenone (48% overall yield). More recently, the pheromone **1** with 98% e.e. was prepared in six steps with a 29% overall yield by the enantioselective lithiation and methylation of dodecyl carbamate (Hintze and Hoppe, 1992). A six-step synthesis of (*S*)-**1** in ca. 50% overall yield was also described starting from ethyl (*S*)-lactate (Bartelt et al., 1989). (*S*)-Pheromone **2** was prepared from ethyl (*S*)-lactate in the same manner as reported for (*S*)-**1** (Bartelt et al., 1989; Mori, 1992); there was no description of the optical rotation of (*S*)-**2** (Schaner et al., 1989).

In conclusion, we have prepared the two chiral pheromone acetates (*S*)-**1** and (*S*)-**2** with high enantiomeric excesses in three steps from (±)-2-tridecanol (32% overall yield) and from (±)-2-pentadecanol (28% overall yield), respectively, by biochemical transformation with lipase. The present method would be expected to facilitate the synthesis of optically active natural products such as chiral pheromone esters and alcohols.

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