Synthesis of (5Z,13S)-5-Tetradecen-13-olide, Aggregation Pheromone of the *Cryptolestes* Grain Beetle, Employing Asymmetric Bioreduction with Immobilized Bakers' Yeast Entrapped in Calcium Alginate Beads

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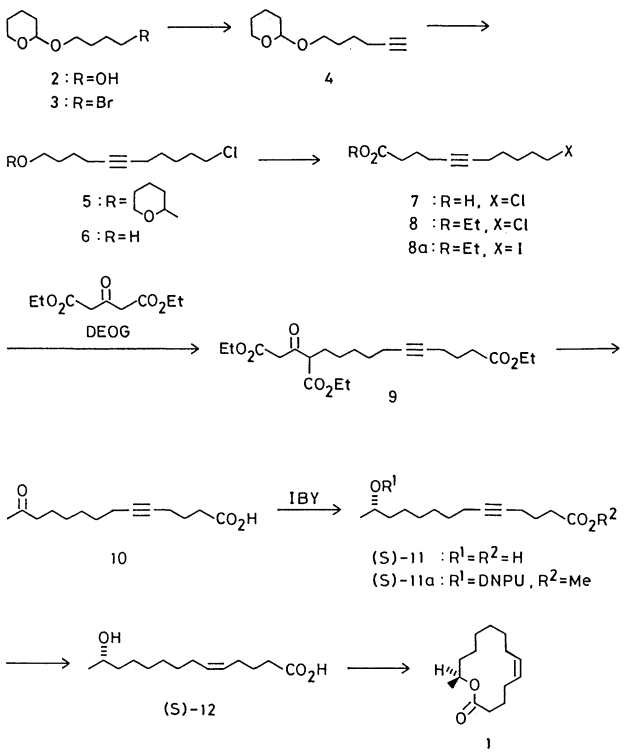
Synopsis. (5Z,13S)-5-Tetradecen-13-olide, a synergist of the aggregation pheromone of the flat grain beetle, was synthesized in an optically pure form by means of an asymmetric bioreduction of the intermediate acetylenic keto acid with immobilized bakers' yeast entrapped in gels of calcium alginate.

We have systematically been employing biocatalysts for several interesting processes, including the asymmetric synthesis of biologically active compounds[1] and stereoselective reductions of keto esters.[2,3] Our strategy for biotransformations in chemical synthesis is the use of immobilized biocatalysts, such as bakers' yeast and lipases entrapped in calcium alginate or carrageenan. The immobilized system is simpler than conventional approaches employing free biocatalysts in aqueous solutions, provides an enhanced stability of the biocatalysts in aqueous solutions and probably in organic solvents as well, and presumably raises the reproducibility of the chemical and optical yields.[1-4]

A continuing concern is the use of immobilized bakers' yeast (IBY) in biochemical processes for pheromone synthesis. Along these lines, we will here describe an asymmetric synthesis of (5Z,13S)-5-tetradecen-13-olide (**1**), which had previous been isolated as a synergist of the aggregation pheromone of the flat grain beetle, *Cryptolestes pusillus* Schönherr,[5] employing IBY entrapped in calcium alginate beads. The key feature of the present synthesis is that the 13S configuration of the pheromone **1** is set up by the bioreduction of the acetylenic keto acid **9** with IBY.[6]

Results and Discussion

First, the monoTHP ether **2** derived from 1,4-butanediol was converted in the usual manner to the bromide **3**, which was then treated with lithium acetylide-ethylenediamine to give the terminal alkyne **4** in an 82% yield. The alkyne **4** was alkylated with *n*-BuLi and 1-bromo-5-chloropentane, followed by treatment with *p*-TsOH to give the chloro alcohol **6** in a 72% yield from **4**. Compound **6** was oxidized with chromium trioxide and subsequently esterified with ethanol, thus giving the chloro ester **8** in a 98% yield; the ester, upon subsequent treatment with sodium iodide, gave the corresponding iodo ester **8a** in an almost quantitative yield. The regioselective alkylation of diethyl 3-oxoglutarate (DEOG)[7] with **8a** successfully proceeded in the presence of $Mg(OEt)_2$, yielding the monoalkylated 3-oxoglutarate **9**, which was subsequently converted by a decarboxylative hydrolysis to the acetylenic acid **10** in a 67% yield from DEOG.



Scheme 1.

Next, the bioreduction of the keto acid **10**, after treatment with an aqueous solution of KOH, was attempted employing IBY entrapped in 3—4 mm-diameter carrageenan beads as has been described previously.[1] Unfortunately, this reduction did not proceed satisfactory, as only a poor yield of the desired alcohol was obtained. Therefore, a new type of IBY, which had a larger specific surface than the previous one and which could provide a reasonable yield of product, was prepared by entrapping an adequate amount of bakers' yeast in calcium alginate beads ca 1.5 mm in diameter.[2] As was expected, the bioreduction of **10** with the new IBY proceeded easily, producing the chiral alcohol (*S*)-**11** in a 40% chemical yield and with 95% ee.

Finally, the hydrogenation of (*S*)-**11** using P-2 nickel as the catalyst and the lactonization of the

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resulting olefinic keto acid (*S*)-**12** with 2-chloro-1-methylpyridinium iodide[8] gave the pheromone **1** in a 48.5% yield. The identity of the synthetic pheromone with the natural one was confirmed by a comparison of their 400 MHz ^{1}H NMR spectra.[5]

Experimental

General. The IR spectra were determined on a Fourier transform Perkin Elmer 1720 spectrometer. The ^{1}H and ^{13}C NMR spectra were obtained on Fourier transform Hitachi R-1500 and Hitachi R-90H spectrometers respectively in $CDCl_3$ solutions, using tetramethylsilane as the internal standard. The EI and CI mass spectra were recorded on a JEOL JMS-D300 mass spectrometer at 70 eV and 200 eV (isobutane) respectively, using a direct insertion probe. The optical rotations were measured on a Horiba SEPA-200 high sensitivity polarimeter. Column chromatography was carried out with 70—230 mesh silica gel (Merck Kieselgel 60 Art. No. 7734). The homogeneity of the reaction products was always checked on TLC by using silica-gel precoated plates (Merck Kieselgel 60F 254 Art. No. 5735) and various solvent systems.

Determination of the Optical Purity of (*S*)-11. HPLC analysis for determining the optical purity of (*S*)-**11** was carried out on a Gasukuro Kogyo model 576 liquid chromatograph equipped with a UV detector (254 nm). A Sumipax OA 2100 4×250 mm column was used, with hexane: 1,2-dichloroethane: ethanol=100:20:1 (flow rate, 1 mL min^{-1}) as the eluent. Under these conditions, a 3,5-dinitrophenylurethane (DNPU) derivative (*S*)-**11a** prepared from (*S*)-**11**[1b] showed two peaks, whose retention times were 10.8 min (97.5%) and 11.8 min (2.5%) respectively. The optical purity of (*S*)-11, 95% ee, was deduced after the separation of the DNPU derivative of (±)-**11** into two equal peaks.

Preparation of Immobilized Bakers' Yeast Entrapped in Calcium Alginate. A mixture of sodium alginate (7.5 g) and distilled water (250 mL) was sterilized at 120 °C for 20 min. To the resulting clear solution, cooled to room temperature, was added a mixture of bakers' yeast (10 g, Oriental Yeast Co., Ltd., Tokyo) and distilled water (50 mL); the new mixture was then stirred for 10 min until it became homogeneous. This homogeneous solution was rapidly added to a 2% aqueous solution of $CaCl_2$ with a syringe, immobilized bakers' yeast entrapped in calcium alginate beads ca 1.5 mm in diameter was thus obtained and subsequently stored at 0—5 °C in an aqueous solution of $CaCl_2$.

6-Tetrahydropyranyloxy-1-hexyne (4). A solution of lithium acetylide-ethylenediamine (18.4 g, 0.29 mol) in dry DMSO (100 mL) was added to a stirred solution of 4-bromo-1-butanol THP ether **3** (51 g, 0.22 mol) in dry DMSO (250 mL) under nitrogen at 5 °C. The mixture was stirred for 24 h at room temperature and then water (180 mL) was slowly added. The resulting mixture was diluted with water and extracted with ether. The work-up of the extract gave a yellow viscous liquid, which was purified by fractional distillation to give a colorless liquid of **4** (32.2 g, 82%): bp 61—62 °C/0.6 mmHg (1 mmHg=133.32 Pa); IR (neat) 3280, 2120 cm^{-1}; ^{1}H NMR δ=1.20—1.85 (10H, m), 1.95 (1H, t, J=2.5 Hz), 2.05—2.40 (2H, m), 3.20—3.95 (4H, m), 4.55 (1H, br s); ^{13}C NMR δ=98.63, 84.12, 68.39, 66.77, 62.05.

1-Tetrahydropyranyloxy-11-chloro-5-undecyne (5). A solution of **4** (27.5 g, 0.15 mol) and catalytic amounts of triphenylmethane in dry THF (400 mL) was cooled to −40 °C under nitrogen. To this cooled solution was dropwise added 100 mL of 1.6 M *n*-BuLi in hexane with stirring, and the mixture was allowed gradually to warm to 0 °C while being stirred. After the resulting solution had been cooled again to −40 °C, dry HMPA (120 mL) was added, followed by dropwise addition of 1-bromo-5-chloropentane (28 g, 0.15 mol). The mixture was then allowed to warm to room temperature over a period of 6 h while being stirred; stirring was continued overnight, after which it was poured into ice water and extracted with ether. A work-up of the ethereal solution gave a yellow viscous liquid, which was subsequently purified by fractional distillation to give a colorless liquid of **5** (34.2 g, 79%): bp 134—136 °C/0.35 mmHg; IR (neat) 1140, 1120, 1080, 1030 cm^{-1}; ^{1}H NMR δ=1.34—1.88 (16H, m), 1.98—2.38 (4H, m), 3.30—4.08 (6H, m), 4.53 (1H, broad s); ^{13}C NMR δ=98.69, 80.19, 79.76, 66.99, 62.17, 44.76.

11-Chloro-5-undecyn-1-ol (6). Compound **5** (28.7 g, 0.10 mol) was dissolved in dry methanol containing *p*-TsOH (0.34 g) and the mixture was heated at 60—70 °C for 5 h with stirring. The subsequent work-up of the reaction mixture gave crude **6** as a pale yellow liquid (18.6 g, 91.6%): IR (neat) 3350, 1250, 1058, 727 cm^{-1}; ^{1}H NMR δ=1.42—1.87 (10H, m), 2.05—2.38 (5H, m), 3.44—3.88 (4H, m); ^{13}C NMR δ=80.22, 79.91, 62.05, 44.88. This was used in the next step without further purification.

Ethyl 11-Chloro-5-undecynoate (8). Jones CrO_3 (80 mL) was added dropwise to a stirred and cooled (0—5 °C) solution of **6** (18.6 g, 0.09 mol) in dry acetone (700 mL); stirring was then continued for 15 min at 0—5 °C. The excess CrO_3 was destroyed by adding isopropyl alcohol (100 mL). The mixture was then worked up to give crude acid **7**, which, without any further purification, was esterified with ethanol in the usual manner. The work-up of the reaction mixture gave a yellow viscous liquid, which was purified by column chromatography on silica gel (200 g, hexane: ethyl acetate=3:1) to give a colorless liquid of **8** (22 g, 98% from 6): IR (neat) 1735, 1447, 1375, 1160, 1060, 1030, 765 cm^{-1}; ^{1}H NMR δ=1.27 (3H, t, J=7 Hz), 1.50—1.98 (8H, m), 2.12—2.67 (6H, m), 3.55 (2H, t, J=6 Hz), 4.14 (2H, q, J=7 Hz).

Triethyl 2-Oxo-9-tridecyne-1,3,13-tricarboxylate (9). Compound **9** was synthesized by alkylating diethyl 3-oxoglutarate (DEOG, 16.2 g, 0.08 mol) with ethyl 11-iodo-5-undecynoate (**8a**), which was prepared from **8** in a quantitative yield, in the presence of $Mg(OEt)_2$ according to the procedures described previously.[7] Purification by column chromatography on silica gel (80 g, hexan: ethyl acetate=3:1) gave **9** as a pale yellow liquid (31 g, 94%): IR (neat) 1735, 1240, 1030 cm^{-1}; ^{1}H NMR δ=1.06—1.79 (19H, m), 3.60 (2H, s), 3.66 (1H, t, J=6.8 Hz), 4.05—4.40 (6H,m); ^{13}C NMR δ=197.33, 172.99, 168.97, 166.44, 80.68, 78.97, 61.44, 61.34, 60.16, 58.88, 48.05.

13-Oxo-5-tetradecynoic Acid (10). Monoalkylated 3-oxoglutarate **9** (24.6 g, 0.06 mol) was heated in a 10% aqueous solution of KOH (1000 mL) containing catalytic amounts of hexadecyltrimethylammonium bromide[9] at 80 °C for 1 h. A subsequent work-up of the hydrolyzed mixture gave crude keto acid **10**, which was then purified by a combination of column chromatography on silica gel (100 g, hexane: ethyl acetate=3:1) and recrystallization from hexane to give **10** as needles (10.3 g, 71.5%): mp 31.5—33 °C; IR (KBr) 3150, 1710 cm^{-1}; ^{1}H NMR δ=2.16 (3H, s), 8.47 (1H, s); ^{13}C NMR δ=209.31, 178.85, 81.13, 78.69, 43.63. Found: C, 70.91; H, 9.44%. Calcd for $C_{14}H_{22}O_3$: C, 70.55; H, 9.31%.

(*S*)-(+)-13-Hydroxy-5-tetradecynoic Acid [(*S*)-11]. A mixture of D-glucose (30 g) and bakers' yeast (50 g) immobilized in calcium alginate beads 1.5 mm in diameter in water (1000 mL) was shaken for 5 h at 35 °C. After the addition of D-glucose (30 g) and of the potassium salt derived from **10** (1 g) to the fermenting mixture, the mixture was shaken for an additional 48 h at 35 °C. The reaction mixture was filtered and then worked up to give a yellow liquid, which was

purified by column chromatography on silica gel (20 g, hexane:ethyl acetate:acetic acid=:100:50:1), followed by preparative TLC (hexane:ethyl acetate:acetic acid=50:50:0.1), to give (*S*)-**11** (0.4 g, 40%): $[\alpha]_D^{23.5}$ +6.84° (*c* 2.92, $CHCl_3$) [lit,[10] $[\alpha]_D^{23.3}$ +6.0° (*c* 1.06, $CHCl_3$)]; ^{13}C NMR δ=177.94, 81.25, 78.72, 68.17. The IR and ^1H NMR spectra were identical with those reported.[10] Found: C, 69.77; H, 10.01%. Calcd for $C_{14}H_{24}O_3$: C, 69.96; H, 10.07%.

(5*Z*,13*S*)-13-Hydroxy-5-tetradecenoic Acid [(*S*)-12]. The hydrogenation of (*S*)-**11** (0.36 g, 1.5 mmol) in ethanol (5 mL) was carried out in the presence of a P-2 nickel catalyst, which was prepared as described previously.[5b,11] After hydrogenation had been completed, charcoal was added to the reaction mixture and the suspension was filtered. A subsequent work-up of the filtrate gave a yellow liquid, which was purified by column chromatography on silica gel (10 g, hexane:ethyl acetate:acetic acid=75:25:1) to give (*S*)-**12** as a colorless liquid (0.31 g, 87.8%): $[\alpha]_D^{23.5}$ +6.13° (*c* 1.85, $CHCl_3$) [lit,[10] $[\alpha]_D^{22.6}$ +6.4° (*c* 1.225, $CHCl_3$), lit,[5b] $[\alpha]_D^{32}$ +4.9° (*c* 4.06, $CHCl_3$)]; ^{13}C NMR δ=178.52, 131.04, 128.24, 68.24. The IR and ^1H NMR spectra were identical with those previously reported.[5b,10] Found: C, 69.04; H, 10.89%. Calcd for $C_{14}H_{26}O_3$: C, 69.38; H, 10.81%.

(5*Z*,13*S*)-5-Tetradecen-13-olide (1). A solution of (*S*)-**12** (0.25 g, 1.03 mmol) and triethylamine (0.85 g) in dry acetonitrile (60 mL) was added dropwise to a solution of 2-chloro-1-methylpyridinium iodide (1.1 g) in refluxing acetonitrile (60 mL) under argon over a period of 6 h by the use of a motor-drive syringe (Micro Feeder JP-V, Furue Science, Tokyo). After the addition had been completed, the mixture was refluxed a further 2 h and then cooled to room temperature. The subsequent work-up of the reaction mixture gave crude **1**, which was purified by column chromatography on silica gel (2 g, hexane:[α]$_D^{23}$ +52.6° (*c* 1.20, $CHCl_3$) [lit,[10] $[\alpha]_D^{23}$ +54.6° (*c* 1.275, $CHCl_3$), lit,[5b] $[\alpha]_D^{23}$ +49.6° (*c* 4.62, $CHCl_3$)]; ^{13}C NMR δ=173.12, 130.71, 128.69, 69.21, 34.67, 33.75, 26.98, 26.59, 26.22, 25.18, 25.03, 23.23, 20.64; EIMS *m/z* (%) 225 (M^++1, 2.5), 224 (M^+, 7), 206 (2), 181 (5), 164 (6), 140 (5), 126 (22), 110 (18), 95 (26), 81 (73), 67 (86), 55 (100), 41 (99); CIMS *m/z* (%) 225 [$(M+H)^+$, 100]. The IR and ^1H NMR spectra were identical with those previously reported.[5b,10] Found: C, 75.06; H, 10.72%. Calcd for $C_{14}H_{24}O_2$: C, 74.95; H, 10.78%.

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