Chemoenzymatic enantioselective synthesis of (–)-enterolactone

Robert Chênevert, Ghodsi Mohammadi-Ziarani, Dave Caron, and Mohammed Dasser

Abstract: Enterolactone, a lignan isolated from biological fluids of animals and humans, was synthesized via enzymatic desymmetrization of 2-(3-methoxybenzyl)-1,3-propanediol.

Key words: enterolactone, synthesis, lipase, desymmetrization, lignan.

Résumé : L'entérolactone, une lignane isolée des fluides biologiques des animaux et des humains, a été préparée via une désymétrisation enzymatique du 2-(3-méthoxybenzyl)-1,3-propanediol.

Mots clés : entérolactone, synthèse, lipase, désymétrisation, lignane.

Introduction

Lignans, naturally occurring secondary plant metabolites consisting of two phenylpropanoid units, are widely distributed in the plant kingdom (1). Enterolactone and enterodiol were the first lignans to be found in humans and animals (2, 3). Enterolactone, like other lignans, exhibits a wide range of biological activity (inhibition of cardiac Na^+, K^+-ATPase, antiestrogenic properties, platelet activating factor antagonist, inhibitor of human estrogen aromatase) and has attracted great interest on account of its cancer-protective properties (4). Epidemiological studies reveal a lowered risk of hormone-dependent cancers (breast, prostate) among vegetarians consuming large amount of lignans. Enterolactone and enterodiol are formed in the intestinal tract by the action of bacteria on precursors present in the diet (5).

Many syntheses of racemic enterolactone have been reported (6) but only a few enantioselective syntheses have been achieved (7). We report here a chemoenzymatic enantioselective synthesis of enterolactone via enzymatic desymmetrization of 2-(3-methoxybenzyl)-1,3-propanediol.

Results and discussion

The known diester **1** (8) was reduced to the diol **2** by lithium aluminum hydride in ether (Scheme 1). Diol **2** was subjected to enzyme-catalyzed esterification (for related desymmetrization of propane-1,3-diol derivatives, see ref. 9) by treatment with *Pseudomonas cepacia* lipase using vinyl acetate as acyl donor and solvent to give the optically active monoester (*R*)-(+)-**3** in quantitative yield. The enantiomeric composition of **3** was measured by ^{19}F NMR (282 MHz, in benzene-d_6) analysis of the corresponding (+)-α-methoxy-α-trifluoromethyl-α-phenyl acetate (MTPA, Mosher's ester). The enantiomeric excess was determined to be 94% (10). (The accuracy of this method is ±3%.) The absolute configuration of monoester **3** (*R*; $[\alpha]_D^{25}$ +26.8 (*c* 2.26, $CHCl_3$)) was determined by correlation with enterolactone **8** of known absolute configuration (the absolute configuration of enterolactone was established by X-ray crystal diffraction (11)). The use of *Pseudomonas fluorescens* lipase gave similar results (quantitative yield, ee = 92%, reaction time = 6.5 h).

Monoester (*R*)-(+)-**3** was treated with mesyl chloride in the presence of triethylamine to give mesylate (*S*)-(+)-**4** (Scheme 2). Displacement of the mesylate with cyanide in DMSO afforded nitrile (*R*)-(–)-**5**. Hydrolysis of the nitrile and the acetate groups in acidic medium was followed by ring closure in a one-pot procedure to give lactone (*R*)-(+)-**6**, $[\alpha]_D^{25}$ +6.15 (*c* 1.86, $CHCl_3$) (lit. (7*e*) $[\alpha]_D^{24}$ 6.06 (*c* 7.92, $CHCl_3$).

The anion of lactone **6** was generated by treatment with lithium diisopropylamide and stereoselectively alkylated with *m*-methoxybenzyl bromide (7*c*) to give lactone (*R*,*R*)-(–)-**7**, $[\alpha]_D^{25}$ –39.0 (*c* 2.5, $CHCl_3$) (lit. (7*a*) $[\alpha]_D^{23}$ –39.2 (*c* 0.78, $CHCl_3$)). Demethylation of lactone **7** with boron tribromide provided enterolactone (*R*,*R*)-(–)-**8**. Comparison of the optical rotation values ($[\alpha]_D^{25}$ –38.4 (*c* 0.37, $CHCl_3$)) for the sample obtained with those reported in the literature (7*a*) ($[\alpha]_D^{23}$ –38.4 (*c* 0.25, $CHCl_3$)) confirmed the absolute configuration of (–)-**8** to be (*R*,*R*), which requires that the enzymatic desymmetrization of **2** leads to diol (+)-**3** with *R* configuration at the newly created stereogenic center.

In conclusion, we have completed the enantioselective synthesis (ee = 94%) of (*R*,*R*)-(–)-enterolactone in seven steps and 35% yield from diester **1**. The key step is the enzymatic desymmetrization of diester **2**.

Experimental section

Melting points were recorded on a Thomas–Hoover apparatus, and are uncorrected. Infrared spectra were recorded

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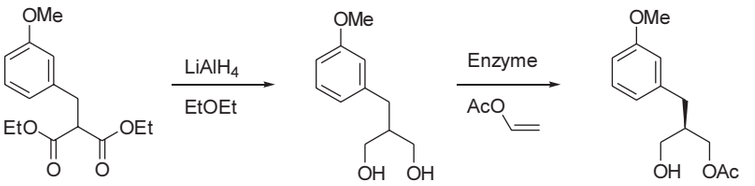
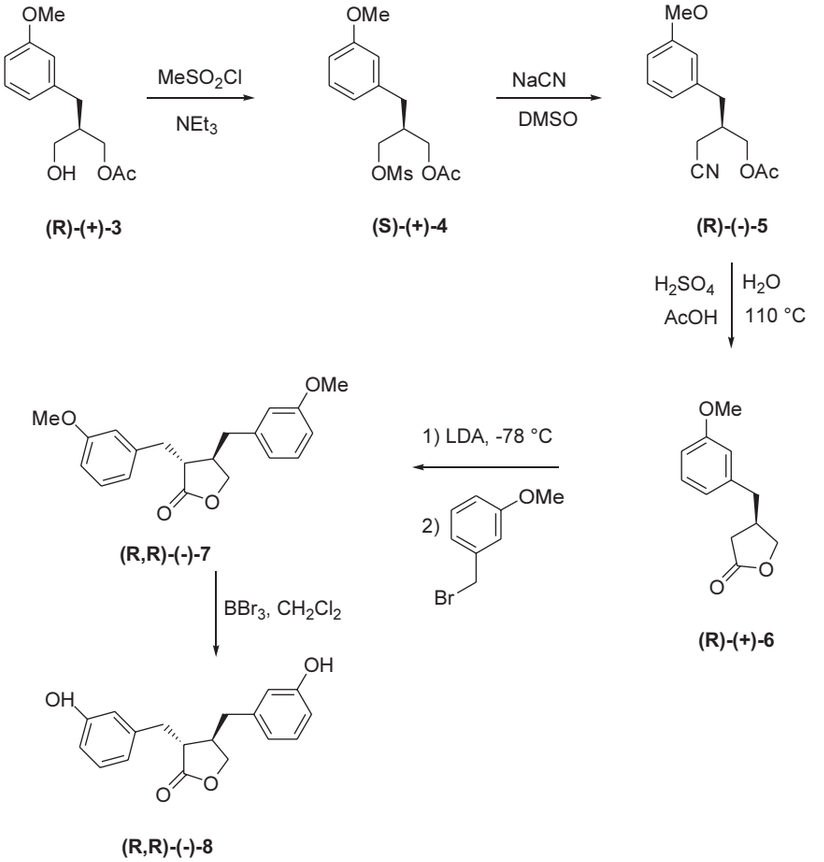
R. Chênevert,[1] G. Mohammadi-Ziarani, D. Caron, and M. Dasser. Département de chimie, Faculté des sciences et de génie, Université Laval, Québec, QC G1K 7P4, Canada.

[1]Author to whom correspondence may be addressed. Telephone: (418) 656-3283. Fax: (418) 656-7916. e-mail: robert.chenevert@chm.ulaval.ca

Scheme 1.



Scheme 2.



using a Perkin Elmer 781 spectrometer. NMR spectra were recorded in $CDCl_3$ solutions at 300 MHz (^1H), 282 MHz (^{19}F), 75 MHz (^{13}C) on a Bruker AC-300 instrument. Optical rotation values were obtained from a JASCO DIP-300 polarimeter. Column purifications were conducted by flash chromatography on silica gel 60 (230–400 mesh). *Pseudomonas cepacia* lipase and *Pseudomonas fluorescens* lipase were purchased from Amano and Aldrich, respectively.

2-(3-Methoxybenzyl)-propane-1,3-diol (2)

Lithium aluminum hydride (1.825 g, 48.0 mmol) was suspended in anhydrous ether (100 mL) at 0°C under a dry N_2 atmosphere. Diester **1** (3.37 g, 12.0 mmol) was added dropwise and the reaction mixture was stirred at 25°C for 4 h. The mixture was cooled and acidified with 3 N HCl (65 mL), filtered through a bed of Celite, and then extracted with ethyl acetate (2 × 100 mL). The combined extracts were washed with saturated aqueous $NaHCO_3$ (2 × 50 mL) and brine (2 × 50 mL), dried ($MgSO_4$), and evaporated. The crude product was purified by flash chromatography (ethyl acetate:hexane 2:3, to pure ethyl acetate) to yield diol **2** as a white solid (1.76 g, 75%); mp 82°C (recrystallized from ether) (lit. (6*l*) mp 81–82°C). IR (KBr): 3350, 3020–2800, 1605, 1588, 1491, 1264, 1036, 873, 778, 695 cm^{-1}; ^1H NMR (MeOD): 1.90 (1H, m), 2.61 (2H, d, J = 7.0 Hz), 3.54 (4H, d, J = 5.6 Hz), 3.74 (3H, s), 6.76 (3H, m), 7.15 (1H, m); ^{13}C NMR ($CDCl_3$): 34.17, 43.75, 55.03, 64.49, 111.17, 114.77, 121.55, 129.28, 141.49, 159.49.

Lipase-catalyzed acylation of diol 2. 3-Hydroxy-2-(3-methoxybenzyl)propyl acetate ((*R*)-3)

To a solution of diol **2** (40 mg, 0.167 mmol) in vinyl acetate (3 mL) was added *Pseudomonas cepacia* lipase (35 mg), and the mixture was stirred at room temperature. The prog-

ress of the reaction was monitored by TLC and the reaction was stopped after disappearance of the starting material (26 min). The enzyme was filtered and washed with diethyl ether. The solvent was evaporated and the crude product was purified by flash chromatography (ethyl acetate:hexane, 10:90 to 50:50) to give monoester (*R*)-(+)-**3** as a colorless oil in quantitative yield (ee = 94%). The use of *Pseudomonas fluorescens* lipase gave similar results (quantitative yield, ee = 92%, reaction time 6.5 h). $[\alpha]_D^{25}$ +26.8 (*c* 2.26, $CHCl_3$); IR (neat): 3425, 1738, 1605, 1588, 1491, 1043 cm^{-1}; ^1H NMR ($CDCl_3$): 2.07 (3H, s), 2.10 (2H, m), 2.62 (2H, m), 3.50 (1H, dd, *J* = 11.2 Hz, *J* = 6.2 Hz), 3.60 (1H, dd, *J* = 11.2 Hz, *J* = 4.5 Hz), 3.79 (3H, s), 4.07 (1H, dd, *J* = 11.1 Hz, *J* = 6.5 Hz), 4.18 (1H, dd, *J* = 11.1 Hz, *J* = 4.6 Hz), 6.76 (3H, m), 7.21 (1H, t, *J* = 7.5 Hz); ^{13}C NMR ($CDCl_3$): 20.71, 34.25, 42.22, 55.01, 61.95, 63.92, 111.39, 114.76, 121.31, 129.32, 140.86, 159.63, 171.45; HRMS (EI) calcd. for $C_{13}H_{18}O_4$ (M^+): 238.1205; found: 238.1199.

1-Acetoxy-2-(3-methoxybenzyl)-3-(mesyloxy)propane ((*S*)-4)

A solution of **3** (296.4 mg, 1.244 mmol) in anhydrous THF (10 mL) was prepared under nitrogen. The solution was cooled to 0°C and triethylamine (0.184 mL, 1.32 mmol) was added first, and then methanesulfonyl chloride (0.152 mL, 1.96 mmol) was added dropwise. The solution was stirred for 2 h while the temperature was increased to 25°C. The solvent was evaporated and the residue was taken up in ether. The organic phase was washed with a 1 N HCl solution, with a saturated $NaHCO_3$ solution (three times), dried, and concentrated. The crude product was purified by flash chromatography (silica gel, elution with hexanes:ethyl acetate, 3:1) to yield (*S*)-(+)-**4** (380 mg, 97%) as a colorless oil. $[\alpha]_D^{25}$ 5.40 (*c* 2.34, $CHCl_3$); IR (neat): 3100–3000, 3000–2800, 1738, 1602, 1587, 1490, 1350, 1155 cm^{-1}; ^1H NMR ($CDCl_3$): 1.99 (3H, s), 2.32 (1H, m), 2.63 (2H, d, *J* = 7.4 Hz), 2.91 (3H, s), 3.71 (3H, s), 3.93–4.16 (4H, m), 6.69 (3H, m), 7.14 (1H, t, *J* = 7.8 Hz); ^{13}C NMR ($CDCl_3$): 20.58, 33.78, 36.85, 39.32, 54.96, 62.78, 68.57, 111.83, 114.63, 121.16, 129.52, 139.47, 159.68, 170.54; HRMS (EI) calcd. for $C_{14}H_{20}O_6$ S (M^+): 316.0980; found: 316.0990.

4-Acetoxy-3-(3-methoxybenzyl)butyronitrile ((*R*)-(–)-5)

To a solution of mesylate **4** (152.8 mg, 0.483 mmol) in anhydrous DMSO (5 mL) was added finely pulverized sodium cyanide (31.9 mg, 0.651 mmol). The solution was stirred at 45°C for 12 h. Ethyl acetate (200 mL) was added and the organic layer was washed with water (8 × 50 mL) dried ($MgSO_4$), and evaporated. The crude product was purified by flash chromatography (silica gel, hexane:ethyl acetate, 3:1) to give nitrile (*R*)-(–)-**5** (118.4 mg, 99%) as a colorless oil. $[\alpha]_D^{25}$–10.77 (*c* 1.96, $CHCl_3$); IR (neat): 3100–3000, 3000–2800, 2250, 1742, 1604, 1589, 1493, 1250–1040 cm^{-1}; ^1H NMR ($CDCl_3$): 2.07 (3H, s), 2.13–2.42 (2H, m), 2.38 (1H, m), 2.66 (1H, dd, *J* = 13.7 Hz, *J* = 7.4 Hz), 2.76 (1H, dd, *J* = 13.7 Hz, *J* = 6.3 Hz), 3.77 (3H, s), 3.98 (1H, dd, *J* = 11.5 Hz, *J* = 6.7 Hz), 4.17 (1H, dd, *J* = 11.5 Hz, *J* = 4.4 Hz), 6.72 (3H, m), 7.22 (1H, m); ^{13}C NMR ($CDCl_3$): 18.94, 20.62, 36.44, 36.61, 55.05, 64.99, 112.06, 114.62, 117.73, 121.13, 129.71, 138.94, 159.80, 170.51; HRMS (EI) calcd. for $C_{14}H_{17}O_3N$ (M^+): 247.1208; found: 247.1200.

4-[(3-Methoxyphenyl)methyl]dihydro-2(3*H*)-furanone ((*R*)-(+)-6)

Nitrile **5** (114 mg, 0.461 mmol) was dissolved in water (5 mL), acetic acid (10 mL), and concentrated sulfuric acid (1 mL). The solution was stirred at 110°C for 2 h. Ethyl acetate (300 mL) and 2 M NaOH (60 mL) were added to the solution and the layers were decanted. The organic layer was washed with water (3 × 50 mL), dried ($MgSO_4$), and evaporated. The crude product was purified by flash chromatography (silica gel, ethyl acetate:hexane, 1:4) to afford lactone (*R*)-(+)-**6** (89.4 mg, 94%) as a colorless oil. $[\alpha]_D^{25}$ +6.15 (*c* 1.86, $CHCl_3$) (lit. (7*e*) $[\alpha]_D^{24}$ 6.06 (*c* 7.92, $CHCl_3$) (lit. (7*d*) $[\alpha]_D^{20}$ +6.41 (*c* 2.08, $CHCl_3$)). IR (film): 3100–3000, 3000–2800, 1775, 1601, 1584, 1492, 1300–1000 cm^{-1}; ^1H NMR ($CDCl_3$): 2.24 (1H, dd, *J* = 17.5 Hz, *J* = 7.2 Hz), 2.57 (1H, dd, *J* = 17.2 Hz, *J* = 7.6 Hz), 2.71 (2H, dd, *J* = 9.3 Hz, *J* = 3.2 Hz), 2.81 (1H, m), 3.76 (3H, s), 4.00 (1H, dd, *J* = 9.1 Hz, *J* = 6.3 Hz), 4.31 (1H, d, *J* = 8.7 Hz, *J* = 6.9 Hz), 6.74 (3H, m), 7.22 (1H, m); ^{13}C NMR ($CDCl_3$): 34.10, 36.92, 38.78, 55.06, 72.53, 111.73, 114.47, 120.85, 129.68, 139.76, 159.75, 176.77.

3,4-Bis[(3-methoxyphenyl)methyl]-dihydro-2(3*H*)-furanone (3*R*,4*R*)-(–)-7

To lactone **6** (40.2 mg, 0.195 mmol) in anhydrous THF (1.5 mL) at –78°C was added LDA (2 M solution in CH_2Cl_2, 0.176 mL, 0.351 mmol) and HMPA (0.105 mL, 0.585 mmol). The solution was stirred for 0.5 h at –78°C and 3-methoxybenzyl bromide (70.6 mg, 0.351 mmol) in anhydrous THF (0.5 mL) was added in one portion. The mixture was stirred overnight (15 h) at –78°C and then warmed slowly to 0°C. The excess of base was neutralized at 0°C with a saturated aqueous NH_4Cl solution (3 mL) and the mixture was extracted with ether (5 mL) and ethyl acetate (2 × 10 mL). The combined organic layer was washed with water (3 × 20 mL) and brine (10 mL), dried ($MgSO_4$), and evaporated under reduced pressure. The crude product was purified by flash chromatography (pure hexane, to ether:hexane, 15:85) to give lactone **7** (38 mg, 60%) as a colorless oil. $[\alpha]_D^{25}$ –39.0 (*c* 2.5, $CHCl_3$) (lit.(7*a*) $[\alpha]_D^{23}$ –39.2 (*c* 0.78, $CHCl_3$)). IR(neat): 3100–3000, 3000–2800, 1770, 1600, 1585, 1490, 1300–1100, 870, 782, 696 cm^{-1}; ^1H NMR ($CDCl_3$): 2.43–2.64 (4H, m), 2.91 (1H, dd, *J* = 13.9 Hz, *J* = 7.0 Hz), 3.06 (1H, dd, *J* = 13.9 Hz, *J* = 5.0 Hz), 3.76 (3H, s), 3.78 (3H, s), 3.78 (1H, dd, *J* = 15.0 Hz, *J* = 6.2 Hz), 4.10 (1H, dd. *J* = 9.0 Hz, *J* = 6.8 Hz), 6.76 (4H, m), 6.56 (2H, m), 7.20 (2H, m). ^{13}C NMR ($CDCl_3$): 35.01, 38.43, 41.14, 46.22, 55.00, 55.03, 71.05, 111.74, 112.24, 114.39, 114.72, 120.77, 121.47, 129.53, 129.59, 139.42, 139.17, 159.70, 178.32.

3,4-Bis[(3-hydroxyphenyl)methyl]dihydro-2(3*H*)-furanone (enterolactone, 8)

A solution of BBr_3 in CH_2Cl_2 (1 M, 0.421 mL, 0.421 mmol) was added dropwise to a solution of lactone **7** (34.3 mg, 0.105 mmol) in anhydrous CH_2Cl_2 at 0°C. The solution was stirred at 0°C for 1 h and then at –18°C for 12 h. Water (1 mL) was added to the solution and the aqueous

layer was extracted with CH_2Cl_2 (3 × 5 mL). The combined organic layers were washed with brine, dried ($MgSO_4$), and evaporated. The crude product was purified by flash chromatography (ethyl acetate:hexane, 3:7) to yield enterolactone **8** (26.9 mg, 86%) as a white solid; mp 140–142°C (lit. (6*l*) mp 141–143°C). $[\alpha]_D^{25}$ –38.4 (*c* 0.37, $CHCl_3$) (lit. (7*a*) $[\alpha]_D^{23}$ –38.4 (*c* 0.25, $CHCl_3$)). IR (KBr): 3350, 3100–3000, 3000–2800, 1760, 1595, 1485, 1460, 1350, 1230, 875, 785, 698 cm^{-1}; ^1H NMR (acetone-d_6): 2.46–3.01 (6H, m), 3.85–4.08 (2H, m), 6.5–7.2 (8H, m), 8.34 (2H, s); ^{13}C NMR (acetone-d_6): 35.20, 38.57, 42.04, 46.69, 71.38, 114.15, 114.36, 116.34, 117.01, 120.51, 121.36, 130.19, 130.28, 140.72, 141.21, 158.32, 178.65.

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