Enzymes and Catalysts. II. 1) Pig Liver Esterase-Catalyzed Asymmetric Synthesis of (-)- and (+)-Cucurbitine and Its (-)-Thio Analogue

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The asymmetric synthesis of (-)- and (+)-cucurbitine was carried out by a method involving 1,3-dipolar cycloaddition of the intermediary azomethine ylide and pig liver esterase (PLE)-catalyzed hydrolysis. The thio analogue, (-)-3-aminotetrahydrothiophene-3-carboxylic acid, was also synthesized.

Keywords—cucurbitine; pig liver esterase; 1,3-dipolar cycloaddition; pyrrolidine; tetrahydrothiophene; half ester; asymmetric synthesis

(-)-Cucurbitine ((-)-1), 2) an unusual amino acid isolated from the seeds of several species of Cucurbitaceae, is known to inhibit the growth of immature Schistosoma japonicum. Asymmetric synthesis of this structurally interesting compound has not been reported. 3) We wish to describe here an effective synthetic route to (-)-cucurbitine ((-)-1) and its thio analogue ((-)-2).

Our method included the 1,3-dipolar cycloaddition of the intermediary azomethine ylide (4), 4) and thiocarbonyl ylide (13), 5) with diethyl methylene malonate (5), 6) asymmetric hydrolysis of the cycloadducts (6, 10 and 14) to the chiral half esters ((+)-7, (-)-11 and (-)-15) with pig liver esterase (PLE), 7) and conversion of the carboxyl group of the half esters to an amino group through Curtius rearrangement. 8)

Diethyl N-benzylpyrrolidine-3,3-dicarboxylate (6), a key starting compound, was synthesized by 1,3-dipolar cycloaddition of the intermediary azomethine ylide (4), derived from N-benzyl-N-(methoxymethyl)trimethylsilylmethylamine (3), 4) and diethyl methylenemalonate (5) in CH₂Cl₂ at 0 °C in 86% yield. Treatment of the 3,3-diester product (6) with PLE in 0.1 M phosphate buffer solution at 25 °C afforded the chiral half ester ((+)-7) in 63% yield. The enantiomeric excess (ee) of (+)-7 was determined to be 10% by proton nuclear magnetic resonance (¹H-NMR) spectroscopic analysis of ethyl methyl N-benzylpyrrolidine-3,3-dicarboxylate (8) using tris-[3-(heptfluoropropylhydroxymethylene)-d-camphorato]europium (III) derivative as a shift reagent. The carboxyl group of (+)-7 was then converted to the amino group with retention of configuration through Curtius rearrangement. Thus, the half ester ((+)-7) was treated with ethyl chloroformate in the presence of triethylamine, and then the reaction mixture was treated with sodium azide. After the usual work-up, the crude product was subjected to thermal rearrangement in benzene and treated with 20% HCl solution to afford (-)-9. The debenzylation of (-)-9 with H₂/Pd–C gave (-)-1, natural cucurbitine, in 40% yield from the half ester. The infrared (IR) spectrum of the product was identical with that reported for an authentic sample. 3)

Diethyl pyrrolidine-3,3-dicarboxylate (10), prepared from 6 by catalytic hydrogenolysis, was subjected to similar asymmetric hydrolysis for 12 h to give the half ester ((-)-11).
Through Curtius rearrangement, \((-\)-11) was converted to unnatural cucurbitine \((+\)-1). The optical yield was determined to be 20% ee based on the optical rotation.

We also synthesized the thio analogue, 3-aminotetrahydrothiophene-3-carboxylic acid \((2)\) from diethyl tetrahydrothiophene-3,3-dicarboxylate \((14)\). The diester \((14)\) was synthesized by 1,3-dipolar cycloaddition of the intermediary thiocarbonyl ylide \((13)\), derived from bis(trimethylsilyl)methyl)sulfoxide \((12)\),\(^5\) with diethyl methylenemalonate \((5)\) in hexamethytriamide phosphate (HMPA) at 100 °C, followed by hydrolysis with PLE at 25 °C in pH 8.0 phosphate buffer solution. A pH value of 8 was maintained by addition of 1 N NaOH. The half ester \((-\)-15), obtained in 83% yield, was then treated successively with ethyl chloroformate at 0 °C in acetone and with sodium azide. The resulting azide derivative was subjected to thermal rearrangement in benzene followed by refluxing in 20% HCl solution to afford the thio analogue \((-\)-2) in 85% yield. By direct desulfurization of \((-\)-2) to isovaline \((+\)-16))\(^9\) with Raney-Ni, the absolute configuration and optical yield of \((-\)-2) were determined to be 3-(S) and 6% ee, respectively.

Interestingly, in the asymmetric hydrolysis of the pyrrolidine \((10)\) and the tetrahydrothiophene \((14)\), the pro-R ester group was hydrolyzed, whereas in the case of the N-benzylpyrrolidine derivative \((6)\), the pro-S ester group was cleaved. Although the optical yields, 6-20% ee, were not as high as expected,\(^10\) we have established short routes for asymmetric syntheses of chiral cucurbitine and its thio analogue.

Experimental

All melting points are uncorrected. IR spectra were recorded with a JASCO A 202 infrared spectrophotometer. \(^1\)H-NMR spectra were measured with a JEOL-90Q (90 MHz) FT-NMR spectrometer. \(^13\)C-NMR spectra were measured with a JEOL-90Q (22.5 MHz) FT-NMR spectrometer. Chemical shifts are reported relative to internal tetramethylsilane or 3-(trimethylsilyl)-1-propanesulfonic acid sodium salt hydrate and given as \(\delta\)-values. Coupling constants are given in Hertz and splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. Optical rotations were measured with a JASCO DIP-140 digital polarimeter. High-resolution mass spectra (MS) were recorded on a JEOL JMS-DX303 mass spectrometer.

**Diethyl N-Benzylpyrrolidine-3,3-dicarboxylate (6)**—A 1 mol/l solution of trifluoroacetic acid in \(\text{CH}_2\text{Cl}_2\) (1 ml) was added to a mixture of \(\text{N-benzyl-N-}(\text{methoxymethyl})\text{trimethylsilylmethylamine (3)}\)\(^4\) (2.87 g, 12 mmol) and diethyl methylenemalonate \((5)\) (1.72 g, 10 mmol) in \(\text{CH}_2\text{Cl}_2\) (6 ml) in an ice-water bath. The reaction mixture was stirred for 2 h, washed with saturated NaHCO\(_3\) and saturated NaCl, dried over MgSO\(_4\), and concentrated under reduced pressure. The residue was purified by silica gel column chromatography with benzene-tetrahydrofuran (THF) (30:1) to give an oil, 2.63 g (86%), 1739 (CO). \(^1\)H-NMR (CDCl\(_3\)) \(\delta: 1.22\) (6H, \(t\), \(J = 6.9\) Hz, \(2\times \text{CH}_3\)), 2.43-2.66 (4H, \(m\), \(N\text{CH}_2\text{CH}_2\)), 3.04 (2H, \(s\), \(N\text{CH}_2\text{C}\)), 3.62 (2H, \(s\), \(N\text{CH}_2\text{Ph}\)), 4.18 (4H, \(q\), \(J = 6.9\) Hz, \(2\times \text{OCH}_2\)), 7.29 (5H, \(s\), \(\text{C}_6\text{H}_5\)). \(^13\)C-NMR (CDCl\(_3\)) \(\delta: 13.37\) (q), 32.62 (t), 53.37 (t), 59.44 (t), 60.03 (t), 61.44 (s), 61.55 (t), 127.00 (d), 128.24 (d), 128.52 (d), 138.76 (s), 171.32 (s). Anal. Calcd for \(\text{C}_{17}\text{H}_{23}\text{N}_2\text{O}_4\): C, 66.86; H, 7.59; N, 4.59. Found: C, 66.44; H, 7.53; N, 4.46.

**(+)-N-Benzyl-3-ethoxycarbonylpyrrolidine-3-carboxylic Acid ((+)-7)**—A mixture of PLE (300 unit) and 6 (1.155 g, 3.79 mmol) in 0.1 M phosphate buffer of pH 8.0 (30 ml) was stirred vigorously at 25 °C for 4 h. The pH value was adjusted to 10, then the solution was washed with \(\text{CH}_2\text{Cl}_2\), neutralized with 3 N HCl, and concentrated under reduced pressure. CHCl\(_3\) was added to the residue. Insoluble material was filtered off and the filtrate was concentrated under reduced pressure to give an oil, 660 mg (63%). \([\alpha]_{D}^{25} + 5.03^\circ\) (\(c = 12.00\), CHCl\(_3\)). \(\nu_{\text{max}}^\text{cm}^{-1}: 1637, 1729\) (CO). \(^1\)H-NMR (CDCl\(_3\)) \(\delta: 1.25\) (3H, \(t\), \(J = 7.1\) Hz, \(\text{CH}_3\)), 2.22-2.75 (2H, \(m\), \(\text{NCH}_2\text{CH}_2\)), 3.05 (2H, \(s\), \(\text{NCH}_2\text{C}\)), 4.19 (2H, \(q\), \(J = 7.1\) Hz, \(\text{OCH}_2\)), 4.23 (2H, \(s\), \(\text{NCH}_2\text{Ph}\)), 7.26-7.64 (5H, \(m\), \(\text{C}_6\text{H}_5\)). \(^13\)C-NMR (CDCl\(_3\)) \(\delta: 13.87\) (q), 31.75 (t), 53.48 (t), 56.57 (t), 58.62 (t), 61.55 (s), 62.70 (t), 129.28 (d), 129.82 (d), 130.74 (d), 137.41 (s), 169.20 (s), 171.53 (s). High-resolution MS (m/z): Calcd for \(\text{C}_{14}\text{H}_{17}\text{NO}_4\): 233.1416. Found: 233.1408.

**Ethyl Methyl N-Benzylpyrrolidine-3,3-dicarboxylate (8)**—An ethereal solution of diazomethane was added in large excess to a solution of (+)-7 (60 mg, 0.22 mmol) in ether (5 ml). After concentration under reduced pressure, the residue was purified by silica gel column chromatography with CHCl\(_3\)-THF (20:1) to give an oil, 45 mg (70%). \([\alpha]_{D}^{15} + 155^\circ\) (\(c = 1.0\), CHCl\(_3\)). IR \(\nu_{\text{max}}^\text{cm}^{-1}: 1734\) (CO). \(^1\)H-NMR (CDCl\(_3\)) \(\delta: 1.22\) (3H, \(t\), \(J = 7.1\) Hz, \(\text{CH}_3\)), 2.34-2.50 (2H, \(m\), \(\text{NCH}_2\text{CH}_2\)), 2.60-2.77 (2H, \(m\), \(\text{NCH}_2\text{CH}_3\)), 3.05 (2H, \(s\), \(\text{NCH}_2\text{C}\)), 3.63 (2H, \(s\), \(\text{NCH}_2\text{Ph}\)), 3.72 (3H, \(s\), \(\text{OCH}_3\)), 4.19 (2H, \(q\), \(J = 7.1\) Hz, \(\text{OCH}_2\)), 7.29 (5H, \(s\), \(\text{C}_6\text{H}_5\)). \(^13\)C-NMR (CDCl\(_3\)) \(\delta: 13.98\) (q), 32.62 (t), 52.60 (q), 53.26 (t), 59.38 (t), 60.03 (t), 61.60 (t), 61.82 (s), 126.94 (d), 128.19 (d), 128.52 (d), 138.65 (s), 170.29 (s), 171.86 (s).
High-resolution MS (m/z): Calcd for C₇H₁₉NO₄ (M⁺): 291.1471. Found: 291.1534.

(−)-N-Benzyl-3-aminopyrrolidine-3-carboxylic Acid ((−)-9) — A solution of triethylamine (238 mg, 2.35 mmol) in acetonitrile (4 ml) was added to a solution of (−)-7 (500 mg, 1.80 mmol) in acetonitrile (4 ml). While maintaining the temperature at 0 °C, a solution of ethyl chloroforamide (275 mg, 2.53 mmol) in acetonitrile (1 ml) was added slowly. The mixture was stirred for 1 h at 0 °C and sodium azide (177 mg, 2.72 mmol) in water (1 ml) was added dropwise. The mixture was stirred at 0 °C for 1 h, then poured into ice-water (30 ml) and the whole was extracted with ether. The organic layer was dried over MgSO₄ and concentrated under reduced pressure to leave an oil, which was dissolved in benzene (15 ml). The benzene solution was refluxed for 3 h and removal of the benzene under reduced pressure afforded an oil, which was dissolved in 20% HCl solution. This solution was refluxed for 2 h and concentrated under reduced pressure. The residue was purified with Amberlite IR-120B (H⁺) ion-exchange resin column chromatography (5% aqueous ammonia) to give a white solid, 183 mg (44%), mp 200 °C (dec.). [α]D²⁰ = −6.60° (c = 1.00, MeOH). IR ν,ν,N = 1679 (CO), 3456 (N–H). ¹H-NMR (CDCl₃): δ: 1.70–3.89 (2H, m, NCH₂CH₂), 2.82–3.84 (4H, m, NCH₂CH₂), 3.62 (2H, s, SCH₂), 3.80 (2H, s, SCH₂C), 4.18 (4H, q, J = 7.2 Hz, 2 x OCH₂). High-resolution MS (m/z): Calcd for C₁₆H₂₁N₂O₄ (M⁺): 291.1471. Found: 291.1534.

−)-3-Ethoxycarbonyltetrahydrothiophene-3-carboxylic Acid ((−)-15) — A mixture of PLE (200 unit) and 14 (6.67 g, 30 mmol) and 5 (3.44 g, 20 mmol) in HMPA (4 ml) was added to HMPA (4 ml) at 100 °C and the mixture was refluxed for 2 h, then poured into ice-water (30 ml) and the mixture was extracted with ether. The organic layer was dried over MgSO₄ and concentrated under reduced pressure to leave an oil, which was dissolved in benzene (10 ml). The benzene solution was refluxed for 2 h and removal of the benzene under reduced pressure afforded an oil, which was dissolved in 20% HCl solution (5 ml). The solution was refluxed for 2 h and concentrated under reduced pressure to give an oil. This was purified by Amberlite IR-120B (H⁺) ion-exchange resin column chromatography (5% aqueous ammonia) to give a white solid, which was dissolved in CH₂CN (4 ml). Trimethylsilyl iodide (152 mg, 0.76 mmol) was added to the 30° C, [α]D³⁰ = 3.94° (c = 0.16, H₂O). The IR, ¹H-NMR, and ¹³C-NMR spectral data were in agreement with those of (−)-1.
(232 mg, 1 mmol) in 0.1 M phosphate buffer of pH 8.0 (20 ml) was stirred vigorously at 25 °C for 4 h. The pH was adjusted to 10, and the mixture was washed with CH₂Cl₂. The pH was adjusted to 2 with 10% HCl solution and the solution was extracted with CHCl₃. The organic layer was dried over MgSO₄ and concentrated under reduced pressure to give an oil, 170 mg (83%). \([\lambda]_22D -0.26 (c = 3.40, CHCl₃). IR νmax cm⁻¹: 1737 (CO), 3216 (COOH). 1H-NMR (CDCl₃) δ: 1.29 (3H, t, J = 7.1 Hz, CH₃), 2.57 (2H, t, J = 6.1 Hz, SCH₂CH₂), 2.98 (2H, t, J = 6.1 Hz, SCH₂C), 3.39 (2H, s, SCH₂C), 4.26 (2H, q, J = 7.1 Hz, OCH₂), 9.28-9.52 (1H, br, COOH). 13C-NMR (CDCl₃) δ: 13.92 (q), 29.75 (t), 36.73 (t), 37.66 (t), 62.39 (t), 64.26 (s), 169.31 (s), 175.38 (s). High-resolution MS (m/z): Calcd for C₈H₁₂O₄S (M +): 204.0456. Found: 204.0435.

(-)-3-Aminotetrahydrothiophene-3-carboxylic Acid ((-)-2) A solution of triethylamine (101 mg, 1.00 mmol) in acetonitrile (2 ml) was added to a solution of (-)-15 (170 mg, 0.83 mmol) in acetonitrile (3 ml). While maintaining the temperature at 0 °C, a solution of ethyl chloroformate (117 mg, 1.08 mmol) in acetonitrile (1 ml) was added slowly. The mixture was stirred for 30 min at 0 °C and then sodium azide (81 mg, 1.25 mmol) in water (1 ml) was added dropwise. The whole was stirred for 3 h, then poured into ice-water (20 ml) and this mixture was extracted with ether. The organic layer was dried over MgSO₄ and concentrated under reduced pressure to leave an oil, which was dissolved in benzene (10 ml). The benzene solution was refluxed for 1 h and removal of the benzene under reduced pressure afforded an oil, which was dissolved in 20% HCl solution (5 ml). This solution was refluxed for 2 h and concentrated under reduced pressure. The residue was purified by Amberlite IR-120B (H⁺) ion-exchange resin column chromatography (5% aqueous ammonia) to give a white solid, 104 mg, (76%), mp 207-209 °C \([\lambda]_25D -6.67 (c = 1.32, H₂O). IR νKBr max cm⁻¹: 1680 (CO), 3072, 3500 (N-H₃). 1H-NMR (D₂O) (δ): 2.36-2.54 (2H, m, SCH₂CH₂), 2.70-3.26 (2H, m, SCH₂CH₂), 3.03 (1H, d, J = 12.4 Hz, SCHAHC), 3.40 (1H, d, J = 12.4 Hz, SCHBHC). 13C-NMR (D₂O) δ: 31.59 (t), 41.67 (t), 41.83 (t), 72.49 (s), 176.63 (s). Anal. Calcd for C₅H₉NO₂S. H₂O: C, 36.35; H, 6.71; N, 8.48. Found: C, 36.16; H, 6.57; N, 8.40.

(+)-Isovaline ((+)-16) A mixture of (-)-2 (92 mg, 0.56 mmol) and Raney-Ni W6 (1 g) in water (4 ml) was stirred overnight at room temperature. After removal of the catalyst by filtration, the filtrate was concentrated under reduced pressure to afford a solid, which was purified by Amberlite IR-120B (H⁺) ion-exchange resin column chromatography (5% aqueous ammonia) to give a white solid, 25 mg (38%). \([\lambda]_24D + 1.60 (c = 0.50, AcOH). [lit,9) (S)-16, \([\lambda]_5 + 26.3 (c = 2, AcOH)]. IR νmax cm⁻¹: 1585 (CO), 3450 (N-H₃). 1H-NMR (D₂O) δ: 0.91 (3H, t, J = 7.5 Hz, CH₃CH₂), 1.45 (3H, s, CH₃), 1.58-1.89 (2H, m, CH₂CH₂).

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References and Notes

10) Several attempts to improve the asymmetric hydrolysis of the diesters using commercially available enzymes (esterase and lipase) were unsuccessful.