JOURNAL OF NATURAL PRODUCTS

NOTE

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Synthesis of *N*-[(3*S*)-2,6-Dioxo-1-(2-phenylethyl)-3-piperidinyl]-(2*S*)-2-methylbutanamide ((−)-Julocrotine)

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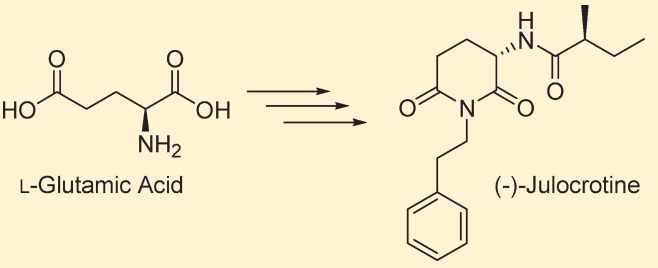
Supporting Information

ABSTRACT: The total synthesis of (−)-julocrotine (**1**) starting from L-glutamic acid in 41% overall yield is described. The methodology utilizes protection, deprotection, and regioselection (carbonyl differentiation via oxazolidinone) protocols, and glutarimide ring formation is the key step.



The glutarimide moiety is present in many natural and non-natural products that are reported to have antibacterial, antitumoral, anti-inflammatory, and other pharmacological activities.[1,2] Julocrotine (**1**), a natural glutarimide alkaloid, was first isolated from *Julocroton montevidensis* Klotzsch in 1925 by Anastasi,[3] and its structure was elucidated in the early 1960s by a sequence of degradation reactions.[4] Later, in 2004, Suarez[5] isolated it along with three additional glutarimide alkaloids from *Croton cuneatus* Klotzsch, a small tree that grows in the Amazonian region of Venezuela. It is used by natives as an anti-inflammatory and analgesic agent and also to treat gastrointestinal diseases.[6,7] The in vitro cytotoxic activity of these glutarimide alkaloids was evaluated against several human tumor cells lines, and julocrotine (**1**) did not show significant cytotoxicity against the cancer cells.[5] Recently, it was found that **1**, isolated from *Croton pullei* var. *glabrior* Lanj. (Euphorbiaceae), is a potent antiproliferative agent when tested in vitro against the promastigote and amastigote forms of *Leishmania* (*L.*) *amazonensis*, with no cytotoxic effects on host cells and with a mechanism that seems to be independent from NO production.[8]

As far as we know, there has been only one report[9] on the synthesis of a lower homologue of julocrotine (**1**), using harsh reaction conditions. In view of the importance of glutarimide alkaloids and the promising findings that **1** inhibits the growth of parasites such as *L. amazonensis*, the need to develop practical methodology for the synthesis of julocrotine and its derivatives was obvious.

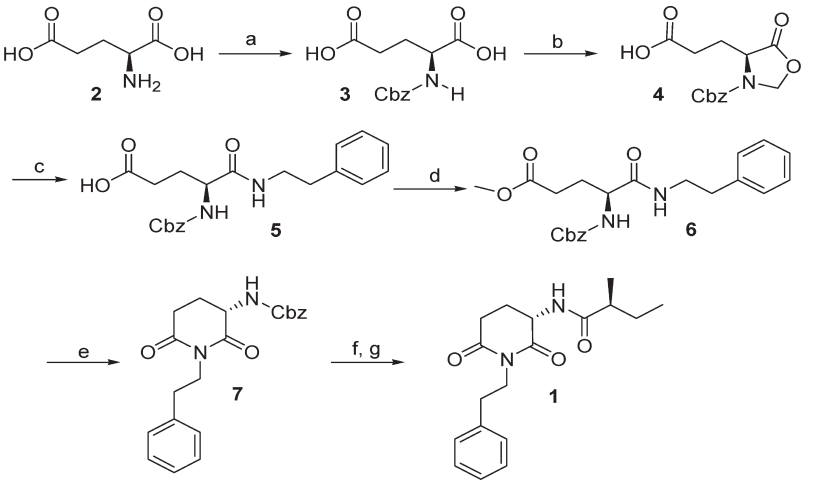
In the present work we report an efficient method for the synthesis of optically active julocrotine (**1**) that utilizes protection, deprotection, and regioselection[10−15] (carbonyl differentiation via oxazolidinone) protocols, with formation of the glutarimide ring as the key step.

Our synthetic approach to the synthesis of **1** is outlined in Scheme 1 and was based on a sequence of well-known procedures[10−15] starting from L-glutamic acid (**2**). The key step of this methodology is glutarimide ring formation.[14] Thus, **2** was converted into its *N*-Cbz-protected derivative **3**, which, in turn, was treated with paraformaldehyde in the presence of *p*-TsOH to afford the oxazolidinone **4** with 94% yield over two steps. The oxazolidinone ring-opening of **4** with 2-phenethylamine led to the corresponding γ-carboxy-α-amide **5** in 90% yield. At this stage, the obvious next step was to convert the γ-carboxy-α-amide **5** into glutarimide **7**. Several attempts to promote ring closure using standard procedures ($SOCl_2$,[16] *N*,*N*′-dicyclohexylcarbodiimide (DCC)[17]) and alternative methods such as acetyl chloride and acetic anhydride were unsuccessful. However, the cyclization was achieved after converting compound **5** into its methyl ester **6**, which, in the presence of *p*-TsOH in refluxing toluene, afforded **7** in 64% yield. Hydrogenolysis of **7** and acylation of the free amine, generated in situ with (*S*)-2-methylbutanoic acid using DCC as a condensing agent, provided optically active julocrotine (**1**) in 75% yield. The yield of the last step was improved to 89% using 4-(4,6-dimethoxy-1,3,5-triazin-2yl)-4-methylmorpholinium chloride (DMTMM),[18] as the condensing agent, instead of DCC. The identity of **1** was confirmed on the basis of comparison of its NMR, IR, optical rotation, and melting point data with literature values.[4,5,19,20] The NMR description of **1** has been ameliorated in comparison to previous descriptions.[19,20]

In conclusion, the total synthesis of optically active julocrotine (**1**) has been achieved in six steps with good overall yield (41%). The methodology presented here is flexible and is being applied to the synthesis of other natural products containing the 2-aminoglutarimide moiety with potentially interesting pharmacological properties.

Received: March 15, 2011
Published: May 05, 2011

Scheme 1. Synthetic Route Leading to (−)-Julocrotine (1)



(a) NaOH, CbzCl/0 °C; (b) $(CH_2O)_n$, *p*-TsOH·H_2O, benzene, Δ; (c) 2-phenethylamine, MeOH, rt; (d) $SOCl_2$, MeOH, rt; (e) *p*-TsOH·H_2O, toluene, Δ; (f) H_2-Pd/C, MeOH, rt; (g) DCC, CH_2Cl_2, or DMTMM, MeOH, (*S*)-2-methylbutanoic acid, rt.

EXPERIMENTAL SECTION

General Experimental Procedures. Commercially available reagents were used without purification unless otherwise stated. L-Glutamic acid, $SOCl_2$, CbzCl, *p*-TsOH.H_2O, paraformaldehyde, and 2-phenethylamine were purchased from Merck. DCC, 10% Pd/C, and (*S*)-2-methylbutanoic acid were purchased from Sigma-Aldrich. Where required, solvents were dried with suitable drying agents and distilled under argon. Melting points were measured with a Microquímica MQPF-301 apparatus and are uncorrected. FTIR spectra were recorded on a Perkin-Elmer FT-IR 1600 instrument. NMR spectra were recorded on a Varian AS-400 NMR spectrometer (operating at 400 MHz for ^{1}H and at 100 MHz for ^{13}C). Chemical shifts (δ) are quoted in parts per million (ppm) and are referred to residual $CHCl_3$ as the internal standard. Coupling constants, *J*, are reported in Hz with the abbreviations (s) singlet, (d) doublet, (t) triplet, (q) quartet, and (m) multiplet. Elemental analysis was performed on a Carlo Erba CHNS-O EA-1110 CE instrument. Optical rotations were measured using a Perkin-Elmer 343 polarimeter. EIMS were acquired on a Shimadzu CGMS QP-5050A instrument at an ionizing voltage of 70 eV, and ESIMS measurements were recorded employing a 3200 Q TRAP, AB Applied Biosystems/MDS Sciex. Commercial silica 60 GF254 TLC plates were used and visualized under UV light at 254 and/or by chemical staining (solution of phosphomolybdic acid in MeOH/H_2SO_4). Flash column chromatography was carried out using silica gel (60A, 230−400 mesh). Hydrogenolysis was performed using a Parr hydrogenation apparatus.

*N-[(Phenylmethoxy)carbonyl]-L-glutamic Acid (**3**).* An aqueous solution of NaOH (4 M, 20 mL) and benzyl chloroformate (11.7 mL) were added dropwise simultaneously over 30 min to a mechanically stirred (600 rpm) and cooled (0 °C) solution of L-glutamic acid (11.8 g, 80.0 mmol) in aqueous NaOH (4 M, 20 mL). The cooling bath was removed, and stirring was continued for a further 6 h at room temperature. Then the reaction mixture was extracted with ether (2 × 30 mL). The aqueous phase was adjusted to pH ≈ 3 with HCl (3 M) and extracted with CH_2Cl_2 (4 × 30 mL). The combined extracts were washed with H_2O (2 × 50 mL), dried (Na_2SO_4), and evaporated to afford **3** as a white solid in 98% yield; mp 119−120 °C (lit.[21] 121 °C).

*(4S)-5-Oxo-3-[(phenylmethoxy)carbonyl]-4-oxazolidinepropanoic Acid (**4**).* A suspension of **3** (3.00 g, 10.7 mmol), paraformaldehyde (0.48 g, 16.0 mmol), and *p*-TsOH.H_2O (10.0 mg) in benzene (40 mL) was heated at reflux in a Dean−Stark apparatus for a period of 3 h (TLC control, silica, 40% ethyl acetate/hexane). The solvent was evaporated, and the residue was neutralized with aqueous K_2CO_3 (0.5 M). The aqueous phase was extracted with ethyl acetate (3 × 40 mL), and the combined extracts were washed with H_2O (3 × 30 mL), dried (Na_2SO_4), and evaporated. Flash chromatography of the oily residue over silica gel (4 × 15 cm) with 2% MeOH in $CHCl_3$ afforded the oxazolidinone **4** as a colorless, viscous oil in 96% yield (lit.[13] 94%): IR (KBr) ν_{max} 3300, 1800, 1740, 1510, 1470, 1405, 1120 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.10−2.30 (2H, m, H-3), 2.44 (2H, m, H-4), 4.36 (1H, m, H-2), 5.07 (2H, s, H-10), 5.15 (1H, d, *J* = 6.0 Hz, H-6b), 5.48 (1H, br s, H-6a), 7.40 (5H, m, H-Ar), 9.90 (1H, br s, COOH).

*(4S)-5-Oxo-4-[[(phenylmethoxy)carbonyl]amino]-5-[(2-phenylethyl)amino]pentanoic Acid (**5**).* 2-Phenylethylamine (4.21 g, 34.8 mmol) was added in one portion to a stirred solution of oxazolidinone **4** (2.05 g, 6.97 mmol) in MeOH (25 mL). Stirring at room temperature under argon was continued for 18 h (TLC control, silica, 70% ethyl acetate/hexane). The solvent was evaporated, the residue was suspended in H_2O (20 mL), and HCl (3 M) was added to pH ≈ 2. The aqueous phase was extracted with ethyl acetate (3 × 30 mL), and the combined organic extracts were washed with H_2O (2 × 20 mL) and brine (30 mL), dried (Na_2SO_4), and evaporated. Flash chromatography of the residue over silica gel (4 × 20 cm) with 2% THF in CH_2Cl_2 afforded a white solid, which contained trace impurities (TLC) and was recrystallized from $CHCl_3$/hexane to provide **5** in 90% yield: mp 121−122 °C; $[\alpha]^{20}_D$ −15.6 (*c* 1.090, $CHCl_3$); IR (KBr) ν_{max} 3348, 3298, 1728, 1701, 1641, 1539, 1442, 1266, 1171 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.90 (1H, m, H-3b), 2.00 (1H, m, H-3a), 2.30 (1H, m, H-4b), 2.41 (1H, m, H-4a), 2.77 (2H, t, *J* = 7.0 Hz, H-6), 3.48 (2H, m, H-7), 4.30 (1H, ddd, *J* = 7.2, 7.2, 8.6 Hz, H-2), 5.06 (2H, s, H-12), 6.04 (1H, d, *J* = 8.6 Hz, H-8), 6.92 (1H, t, *J* = 5.08 Hz, H-9), 7.15−7.35 (10H, m, H-Ar); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 28.1 (C-3), 29.9 (C-4), 35.7 (C-6), 41.0 (C-7), 54.1 (C-2), 67.6 (C-12), 126.7 (2C-Ar), 128.2 (2C-Ar), 128.5 (2C-Ar), 128.8 (2C-Ar), 129.0 (2C-Ar), 136.1 (C-Ar), 138.8 (C-Ar), 156.8 (C-10), 171.7 (C-1), 176.3 (C-2); EIMS *m/z* 384 [M$^+$] (10), 105 (98), 92 (84), 84 (100); *anal.* C 65.73%, H 6.21%, N 7.34%, calcd for $C_{21}H_{24}N_2O_5$, C 65.61%, H 6.29%, N 7.29%.

*Methyl (4S)-5-Oxo-4-[[(phenylmethoxy)carbonyl]amino]-5-[(2-phenylethyl)amino]pentanoate (**6**).* To a stirred solution of acid amide **5** (1.04 g, 2.70 mmol) in dry MeOH (20 mL), under argon, was added dropwise $SOCl_2$ (0.42 g, 2.70 mmol). After addition, stirring was continued at room temperature for 3 h (TLC control, silica, 50% ethyl

acetate/hexane) and the solvent was then evaporated. The residue was taken up in CH_2Cl_2 (30 mL) and washed with saturated aqueous $NaHCO_3$ (3 × 20 mL) and brine (30 mL). The organic phase was dried (Na_2SO_4) and evaporated to afford a white solid. The solid was recrystallized from a mixture of $CHCl_3$/hexane to provide **6** in 84% yield: mp 124−125 °C; $[\alpha]^{22}_D$ −10.8 (*c* 1.002, $CHCl_3$); IR (KBr) ν_{max} 3293, 1732, 1692, 1648, 1534, 1444, 1253 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.90 (1H, m, H-3b), 2.07 (1H, m, H-3a), 2.31 (1H, m, H-4b), 2.44 (1H, m, H-4a), 2.79 (2H, t, *J* = 6.8 Hz, H-6), 3.52 (2H, m, H-7), 3.65 (3H, s, H-13), 4.16 (1H, ddd, *J* = 6.8, 6.8, 7.4 Hz, H-2), 5.07 (2H, s, H-12), 5.75 (1H, d, *J* = 7.4 Hz, H-8), 6.38 (1H, br s, H-9), 7.15−7.35 (10H, m, H-Ar); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 28.2 (C-3), 30.3 (C-4), 35.8 (C-6), 40.9 (C-7), 52.1 (C-13), 54.4 (C-2), 67.3 (C-12), 126.7 (2C-Ar), 128.3 (2C-Ar), 128.5 (2C-Ar), 128.8 (2C-Ar), 129.0 (2C-Ar), 136.4 (C-Ar), 138.8 (C-Ar), 156.5 (C-10), 171.3 (C-1), 174.0 (C-5); EIMS *m*/*z* 398 [M^+] (17), 105 (90), 92 (85), 84 (100); *anal.* C 66.41%, H 6.56%, N 7.06%, calcd for $C_{22}H_{26}N_2O_5$ C 66.32%, H 6.58%, N 7.03%.

*Phenylmethyl (3S)-[2,6-Dioxo-1-(2-phenylethyl)-3-piperidinyl]carbamate (**7**).* A mixture of compound **6** (1.0 g, 2.5 mmol) and *p*-TsOH.H_2O (0.24 g, 1.25 mmol) in toluene (20 mL), under argon, was refluxed for 8 h (TLC control, silica, 2% THF/CH_2Cl_2). The solvent was evaporated, the crude was taken up in ether (50 mL) and washed with saturated aqueous $NaHCO_3$ (2 × 20 mL), and the organic layer was dried (Na_2SO_4). Evaporation of the solvent and flash chromatography of the residue over silica gel (4 × 25 cm) with CH_2Cl_2 and then 30% MeOH/CH_2Cl_2 afforded a white solid. The solid was recrystallized from a mixture of $CHCl_3$/hexane to provide **7** as white needles in 64% yield: mp 122−123 °C; $[\alpha]^{20}_D$ −30.6 (*c* 1.12, $CHCl_3$); IR (KBr) ν_{max} 3335, 2954, 1686, 1546, 1425, 1303, 1008 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.74 (1H, dq, *J* = 4.8,13.2 Hz, H-4b), 2.48 (1H, m, H-4a), 2.67 (1H, m, H-5b), 2.81 (3H, m, H-8, H-5a), 4.02 (2H, m, H-7), 4.27 (1H, m, H-3), 5.15 (2H, s, H-12), 5.64 (1H, br, H-9), 7.20 - 7.33 (10H, m, H-Ar); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 25.5 (C-4), 32.1 (C-5), 35.4 (C-8), 41.4 (C-7), 52.6 (C-3), 69.3 (C-12), 126.3 (2C-Ar), 128.1 (2C-Ar), 128.3 (2C-Ar), 128.5 (2C-Ar), 128.9 (2C-Ar), 136.0 (C-Ar), 138.9 (C-Ar), 158.5 (C-10), 171.4 (C-6), 172.9 (C-2); EIMS *m*/*z* 366 [M^+] (10), 105 (100), 91 (95), 84 (80); *anal.* C 68.92%, H 6.18%, N 7.68%, $C_{21}H_{22}N_2O_4$ calcd for C 68.84%, H 6.05%, N 7.65%.

*N-[(3S)-2,6-Dioxo-1-(2-phenylethyl)-3-piperidinyl]-(2S)-2-methylbutanamide, (−)-Julocrotine (**1**).* Method 1: A suspension of compound **7** (3.67 g, 10.0 mmol) and 10% Pd/C (0.40 g) in MeOH (100 mL) was hydrogenated at 40 psi during 2 h (TLC control, silica, 2% THF/CH_2Cl_2). The catalyst was removed by filtration through a pad of Celite (2 × 3 cm), and the filtrate was evaporated. To a stirred and cooled (0 °C) solution of the resulting residue in dry CH_2Cl_2 (100 mL) was added DCC (0.25 g, 12.0 mmol), and then (*S*)-2-methylbutanoic acid (0.122 g, 12.00 mmol) was added dropwise. After addition, the bath was removed, stirring was continued for 3 h (TLC control) at room temperature, and the solvent was evaporated. The crude was suspended in ether (200 mL) and filtered, and the filtrate was evaporated. Flash chromatography of the residue over silica gel (3 × 20 cm) with 2% THF/CH_2Cl_2 afforded a white solid. The solid was recrystallized from a mixture of ether/hexane to give **1** in 75% yield.

Method 2: A suspension of compound **7** (0.24 g, 1.00 mmol) and 10% Pd/C (24.0 mg) in MeOH (2 mL) was hydrogenated at 20 psi during 2 h (TLC control, silica, 2% THF/CH_2Cl_2). The catalyst was removed by filtration through a pad of Celite (1 × 2 cm), and to the resulting filtrate with stirring was added DMTMM (0.33 g, 1.20 mmol) followed by addition of (*S*)-2-methylbutanoic acid (153 mg, 1.50 mmol). Stirring was continued for 12 h at room temperature. The solvent was evaporated, and flash chromatography of the residue over silica gel (3 × 6 cm) with 70% ethyl acetate/hexane gave compound **1** in 89% yield: mp 106−107 °C (lit.[4] 108−109 °C); $[\alpha]^{20}_D$ −46.6 (*c* 0.88, MeOH) (lit.[4] $[\alpha]_D$ −50.1 (*c* 1.19, MeOH)); IR (KBr) ν_{max} 3279, 2965, 1730, 1687, 1644, 1545, 1150 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.93 (3H, t, *J* = 7.4 Hz, H-13), 1.16 (3H, d, *J* = 6.8 Hz, H-14), 1.47 (1H, m, H-12b), 1.68 (2H, m, H-4b, H-12a), 2.22 (1H, m, H-11), 2.50 (1H, m, H-4a), 2.68 and 2.76 (2H, d of AB system, *J* = 5.2, *J* = 13.2 Hz, H-5b, H-5a), 2.80 (2H, t, *J* = 7.6 Hz, H-8), 4.00 (2H, m, H-7), 4.49 (1H, ddd, *J* = 5.2, 5.2, 13.2 Hz, H-3), 6.30 (1H, d, *J* = 5.2 Hz, H-9), 7.25 (5H, m, H-Ar); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 12.1 (C-13), 17.6 (C-14), 24.6 (C-4), 27.5 (C-12), 31.9 (C-5), 34.2 (C-8), 41.9 (C-7), 43.2 (C-11), 51.4 (C-3), 126.8 (C-Ar), 128.7 (2C-Ar), 129.2 (2C-Ar), 138.4 (C-Ar), 171.3 (C-6), 172.1 (C-2), 177.1 (C-10); ESIMS *m*/*z* 317.2 [$M + H]^+$; *anal.* C 68.69%, H 7.33%, N 8.89%, calcd for $C_{18}H_{24}N_2O_3$ C 68.33%, H 7.65%, N 8.85%.

■ ASSOCIATED CONTENT

Supporting Information. NMR spectra and IR spectra of **1**, **5**, **6**, and **7**; COSY spectra of **1** and **7**. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ACKNOWLEDGMENT

The authors thank the Central de Análises (Departamento de Química, UFSC, Florianópolis) for spectroscopic analyses. A.C.J. and L.L.S. are grateful to CNPq and CAPES for research fellowships. Financial support from MCT/CNPq (Universal 473919/07-9) is also gratefully acknowledged.

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