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Total Synthesis of (-)-Rosmarinecine by Intramolecular Cycloaddition of (*S*)-Malic derived Pyrroline *N*-Oxide

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SUPPORTING INFORMATION

Experimental section

General Remarks: All operations were carried out under inert gas and with anhydrous solvents where required. R_f values refer to TLC on 0.25-mm silica gel plates (Merck F_{254}) with the same eluent used for separation of the compound by flash column chromatography. Melting points (m.p.) are uncorrected. Optical rotation measurements were carried out with a Jasco DIP-370 polarimeter. ^{1}H and ^{13}C NMR spectra (in $CDCl_3$ solution, unless otherwise stated) were recorded at 200 MHz and 50.3 MHz, respectively, with a Varian Gemini spectrometer or at 500 MHz (^{1}H) with a Bruker DRX 500 spectrometer; the chemical shift for ^{1}H and ^{13}C NMR spectra are given in ppm from TMS. IR spectra were recorded with a Perkin-Elmer 881 spectrophotometer. Mass spectra (EI, 70 eV) were recorded with a QMD 1000 Carlo Erba instrument by GC or direct inlet. Elemental analyses were carried out with a Perkin-Elmer 240 C or a Perkin-Elmer 2400 instrument.

Maleic acid monomethyl ester (8):
A 3.4 M solution of maleic anhydride in MeOH was heated at reflux for 30 minutes. After evaporation of the solvent under reduced pressure a yellow oil was obtained, enough pure to be used for the next step. ^{1}H NMR: δ = 10.80 (br s, 1 H), 6.46 (AB system, 2 H), 3.88 (s, 3 H).

(2a*R*,3*S*,6a*R*,6b*R*)-3-carbomethoxy-2-oxo-1,4-dioxa-4a-aza-hexahydrocyclopenta[*cd*]pentalene (9) *via* process in Scheme 3: DEAD (2.45 mL, 15.6 mmol) was added dropwise at 0 °C to a solution of nitrone **7** (526 mg, 5.20 mmol), triphenylphosphine (4.18 g, 15.6 mmol) and of **8** (811 mg, 6.24 mmol) in dry THF (52 mL). The mixture was stirred for 3 h at 0 °C and then concentrated under reduced pressure. Purification of the crude mixture by flash column chromatography (eluent AcOEt/MeOH, 95:5) afforded **9** as a white solid (R_f = 0.31, 776 mg, 3.64 mmol, 70%). - m.p. 140-141 °C; $[\alpha]_D^{23}$ = -31.3 (*c* = 0.38, $CHCl_3$). ^{1}H NMR: δ = 5.13 (m, 1 H, H-6a), 4.74 (d, *J* = 6.3 Hz, 1 H, H-3), 4.45 (dd, *J* = 7.8, 7.7 Hz, 1 H, H-6b), 3.84 (s, 3 H, Me), 3.80-3.74 (m, 2 H, Ha-5, H-2a), 3.10-3.03 (m, 1 H, Hb-5), 2.45-2.41 (m, 2 H, Ha-6, Hb-6). ^{13}C NMR: δ = 174.1 (s, C=O), 166.3 (s, C=O), 83.2 (d, C-6a), 79.1 (d, C-3), 71.5 (d, C-6b), 54.5 (t, C-5), 52.6 (q, Me), 52.2 (d, C-2a), 32.4 (t, C-6). MS, m/z (%): 213 (M^+, 3), 183 (1), 169 (4), 154 (86), 108 (49), 83 (100). IR (CCl_4): 3007, 1775, 1768, 1169 cm^{-1}. $C_9H_{11}NO_5$ (213.19): calcd. C 50.71, H 5.20, N 6.57; found C 50.70, H 5.34, N 6.73.

(2a*R*,3*S*,7a*R*,7b*R*)-3-hydroxy-2,4-dioxo-octahydrofuro[2,3,4-*gh*]pyrrolizine (10) *via* process in Scheme 3: 20% $Pd(OH)_2/C$ (372 mg) was added to a solution of **9** (600 mg, 2.81 mmol) in methanol (120 mL). The suspension was stirred under hydrogen atmosphere at room temperature for 24 h. After filtration through Celite and concentration, the crude mixture was purified by flash column chromatography (eluent AcOEt) to give **10** as a white solid (R_f = 0.16, 304 mg, 1.66 mmol, 59%). - m.p. 169-170 °C; $[\alpha]_D^{20}$ = +51.0 (*c* = 0.38, abs EtOH). ^{1}H NMR (D_2O): δ = 5.19 (dd, *J* = 5.5, 4.8 Hz, 1 H, H-7a), 4.90 (dd, *J* = 5.3, 4.8 Hz, 1 H, H-7b), 4.48 (s, 1 H, H-3), 3.79 (ddd, *J* = 11.8, 9.7, 5.7 Hz, 1 H, Ha-6), 3.50 (d, *J* = 5.3 Hz, 1 H, H-2a), 3.24 (ddd, *J* = 11.8, 9.7, 5.7 Hz, 1 H, Hb-6), 2.59 (m, 1 H, Ha-7), 2.43 (m, 1 H, Hb-7). ^{13}C NMR (D_2O): δ = 179.7 (s, C=O), 177.4 (s,

C=O), 85.5 (d, C-6a), 79.9 (d, C-3), 69.1 (d, C-6b), 53.3 (d, C-2a), 44.4 (t, C-6), 34.3 (t, C-7). MS, m/z (%): 183 (M^+, 67), 155 (40), 127 (36), 83 (44), 68 (59), 55 (100). IR (KBr): 3322, 2991, 1767, 1677, 1427, 1199, 1177 cm^{-1}. $C_8H_9NO_4$ (183.19): calcd. C 52.46, H 4.95, N 7.65; found C 52.15, H 5.29, N 7.31.

(-)-Rosmarinecine (4) *via* process in Scheme 3: Red-Al® (3.5 M in toluene, 1 mL, 3.5 mmol) was added to a solution of **10** (52 mg, 0.28 mmol) in dry THF (20 ml). The mixture was heated at reflux for 3 h, then cooled to room temperature and quenched with water (180 μL), 2 N NaOH (160 μL) and water (350 μL), and then it was diluted with THF (15 mL) and stirred for 5 min. The resulting solution was concentrated to ca. 5 mL, and the crude product was purified by flash column chromatography on silica gel (eluent $CHCl_3$/MeOH/NH_4OH, 10:5:1) to give **4** (R_f = 0.23) as a white solid which was recrystallized from acetone/pentane to provide (-)-rosmarinecine (43.6 mg, 0.25 mmol, 90%). The silica gel column was prepared with a large Celite plug (half the height of the silica gel) at the bottom of the column, as suggested in ref. 3b. - m.p. 155-157 °C; $[\alpha]_D^{23}$ = -65.6 (*c* = 0.88, abs EtOH). ^{1}H NMR (CD_3OD, 500 MHz): δ = 4.39 (ddd, *J* = 9.4, 7.7, 7.7 Hz, 1 H, H-6), 4.25 (m, 1 H, H-1), 4.02 (dd, *J* = 11.0, 6.8 Hz, 1 H, Ha-8), 3.91 (dd, *J* = 11.0, 3.7 Hz, 1 H, Hb-8), 3.41 (dd, *J* = 8.1, 2.8 Hz, 1 H, H-7a), 3.23 (ddd, *J* = 9.7, 8.4, 1.3 Hz, 1 H, Ha-3), 3.02 (dd, *J* = 11.1, 8.1 Hz, 1 H, Ha-5), 2.93 (dd, *J* = 11.1, 7.2 Hz, 1 H, Hb-5), 2.81 (ddd, *J* = 11.3, 10.2, 6.8 Hz, 1 H, Hb-3), 2.32-2.27 (m, 1 H, H-7), 1.90-1.78 (m, 2 H, Ha-2, Hb-2). ^{13}C NMR (CD_3OD): δ = 72.8 (d, C-7a), 72.3 (d, C-1), 71.1 (d, C-6), 64.5 (t, C-5), 59.6 (t, C-8), 55.2 (t, C-3), 51.7 (d, C-7), 36.2 (t, C-2). $C_8H_{15}NO_3$ (173.21): calcd. C 55.47, H 8.73, N 8.09; found C 55.04, H 8.61, N 7.79.

Cycloaddition of nitrone 11 to styrene: A solution of nitrone **11** (784 mg, 4.24 mmol) and styrene (975 μL, 8.48 mmol) in toluene (4.2 mL) was allowed to react at 80 °C for 11 h. The crude mixture was purified by flash column chromatography eluting at first with petroleum ether to remove the excess of styrene, then with AcOEt/petroleum ether (1:1) to afford adduct **12** (R_f = 0.45, 881 mg, 3.05 mmol, 72%) as a mixture of two diastereoisomers; successive elution with AcOEt gave **13** (R_f = 0.20, 204 mg, 0.71 mmol, 17%) as a mixture of two diastereoisomers.

(2*R*,3a*R*,4*S*)-4-hydroxy-2-phenyl-2,3,3a,4,5,6-hexahydropyrrolo[1,2-*b*]isoxazole (14): A mixture of **12** (103 mg, 0.356 mmol) and PPTS (107 mg, 0.424 mmol) in dry ethanol (4.2 mL) was heated at reflux for 3 h. After concentration under reduced pressure the crude mixture was stirred twice with Ambersep 900 OH (200 mg) in methanol (10 mL). Filtration through Celite and concentration afforded **14** (64.4 mg, 0.314 mmol, 89%) as a colorless solid whose spectral data were identical to those reported in the supporting information of ref. 15.

(2*R*,3a*R*,4*R*)-3-carbomethoxy-prop-2-enoic acid 2-phenyl-2,3,3a,4,5,6-hexahydro-pyrrolo[1,2-*b*]isoxazole-4-yl-ester (15): DEAD (142 μL, 0.9 mmol) was added dropwise at 0 °C to a solution of adduct **14** (62 mg, 0.3 mmol), triphenylphosphine (237 mg, 0.9 mmol) and of **8** (98 mg, 0.75 mmol) in dry THF (3 mL). The mixture was stirred for 2 days at room temperature and then concentrated under reduced pressure. Purification of the crude mixture by flash column chromatography (eluent AcOEt/petroleum ether, 1:1) afforded **15** as a white solid (R_f = 0.14, 65.6 mg, 0.207 mmol, 69%). - m.p. 55-56 °C; $[\alpha]_D^{22}$ = -11.1 (*c* = 0.34, $CHCl_3$). ^{1}H NMR: δ = 7.41-7.25 (m, 5 H, Ph), 6.33 (s, 2 H, sp^2 carbon hydrogens), 5.41 (dt, *J* = 6.1, 4.2 Hz, 1 H, H-4), 4.99 (dd, *J* = 9.2, 6.0 Hz, 1 H, H-2), 4.06 (ddd, *J* = 8.0, 6.2, 1.8 Hz, 1 H, H-3a), 3.79 (s, 3 H, Me), 3.39-3.30 (m, 2 H, Ha-6, Hb-6), 2.65 (ddd, *J* = 12.6, 6.0, 1.8 Hz, 1 H, Ha-3), 2.38-2.14 (m, 3 H, Hb-3, Ha-5, Hb-5). ^{13}C NMR: δ = 165.0 (s, C=O), 164.2 (s, C=O), 139.7 (s, Ph), 129.6 (d, sp^2 carbon), 129.4 (d, sp^2 carbon), 128.2 (d, 2 C, Ph), 127.5 (d, Ph), 126.0 (d, 2 C, Ph), 78.8 (d, C-2), 75.2 (d, C-4), 67.6 (d, C-3a), 53.6 (t, C-6), 52.0 (q, Me), 38.9 (t, C-3), 30.9 (t, C-5). MS, m/z (%): 317 (M^+, 5), 204 (16), 187 (23), 170 (13), 144 (20),

113 (97), 104 (73), 91 (38), 85 (67), 77 (100), 59 (53). IR ($CHCl_3$): 3030, 2980, 1728, 1440, 1390, 1160 cm^{-1}. $C_{17}H_{19}NO_5$ (317.34): calcd. C 64.34, H 6.03, N 4.41; found C 64.45, H 6.17, N 4.44.

9 *via* process in Scheme 4: A solution of **15** (175 mg, 0.55 mmol) in *o*-dichlorobenzene (10 mL) was heated at reflux for 14 h. The crude mixture was purified by flash column chromatography eluting at first with pentane to remove the excess of *o*-dichlorobenzene, then with AcOEt to afford **9** (R_f = 0.19, 82 mg, 0.38 mmol, 70%) as a brown solid, which was recrystallized from pentane to obtain an analytically pure white solid, whose spectral data were identical to those reported for **9** obtained *via* the process in Scheme 3. - m.p. 138-139 °C; $[\alpha]_D^{27}$ = -53.5 (*c* = 0.39, $CHCl_3$). $C_9H_{11}NO_5$ (213.19): calcd. C 50.71, H 5.20, N 6.57; found C 50.69, H 5.22, N 6.51.

10 *via* process in Scheme 4: 20% $Pd(OH)_2/C$ (174 mg) was added to a solution of **9** (132 mg, 0.62 mmol) in methanol (25 mL). The suspension was stirred under hydrogen atmosphere at room temperature for 15 h. After filtration through Celite and concentration, the crude mixture was purified by flash column chromatography (eluent AcOEt/MeOH, 10:1) to give **10** (R_f = 0.4, 63.5 mg, 0.35 mmol, 56%), as a white solid, whose spectral data were identical to those reported for **10** obtained *via* the process in Scheme 3. - m.p. 183-185 °C; $[\alpha]_D^{27}$ = +94.2 (*c* = 0.31, abs EtOH). $C_8H_9NO_4$ (183.19): calcd. C 52.46, H 4.95, N 7.65; found C 52.90, H 4.79, N 7.65.

(-)-Rosmarinecine (4) *via* process in Scheme 4: Red-Al® (3.5 M in toluene, 830 μL, 2.77 mmol) was added to a solution of **10** (42 mg, 0.23 mmol) in dry THF (15 ml). The mixture was heated at reflux for 3 h, then cooled to room temperature and quenched with water (260 μL), 2 N NaOH (230 μL) and water (520 μL), and then it was diluted with THF (11 mL) and stirred for 30 min. The resulting solution was concentrated to ca. 2 mL, and the crude product was purified by flash column chromatography on silica gel (eluent $CHCl_3$/MeOH/NH_4OH, 10:5:1) to give **4** (R_f = 0.2) as a white solid which was recrystallized from acetone/pentane to provide (-)-rosmarinecine (34 mg, 0.2 mmol, 85%). - m.p. 166-168 °C; $[\alpha]_D^{20}$ = -122.3 (*c* = 0.61, abs EtOH). ^{1}H NMR (CD_3OD, 200 MHz): δ = 4.39 (ddd, *J* = 9.5, 7.7, 7.5 Hz, 1 H, H-6), 4.26-4.02 (m, 1 H, H-1), 4.03 (dd, *J* = 11.0, 6.6 Hz, 1 H, Ha-8), 3.91 (dd, *J* = 11.0, 3.9 Hz, 1 H, Hb-8), 3.38 (dd, *J* = 8.2, 2.8 Hz, 1 H, H-7a), 3.21 (m, 1 H, Ha-3), 3.01 (dd, *J* = 11.2, 7.9 Hz, 1 H, Ha-5), 2.90 (dd, *J* = 11.2, 7.2 Hz, 1 H, Hb-5), 2.78 (m, 1 H, Hb-3), 2.36-2.22 (m, 1 H, H-7), 1.93-1.78 (m, 2 H, Ha-2, Hb-2). The spectral data recorded at 500 MHz are identical to those reported for **4** obtained *via* the process in Scheme 3. $C_8H_{15}NO_3$ (173.21): calcd. C 55.47, H 8.73, N 8.09; found C 55.43, H 8.53, N 7.81.

Mosher ester of 10 obtained *via* process in Scheme 4: (+)-MTPA-Cl (12 μL, 63.7 μmol) was added dropwise at 0 °C under nitrogen atmosphere to a solution of **10** (10.6 mg, 57.9 μmol) in dry pyridine (100 μL). The reaction was monitored by TLC until no more starting material was detected. The mixture was quenched with water, extracted with diethyl ether and washed with 10% HCl and with a saturated aqueous solution of $NaHCO_3$, dried over Na_2SO_4, filtered and concentrated. The ^{1}H NMR spectrum showed a single set of signals. - ^{1}H NMR: δ = 7.55-7.41 (m, 5 H, Ph), 5.59 (s, 1 H), 5.08-5.02 (m, 1 H), 4.72 (dd, *J* = 5.1, 4.4 Hz, 1 H), 3.96 (ddd, *J* = 12.5, 8.1, 7.3 Hz, 1 H), 3.52 (s, 3 H, Me), 3.43 (d, *J* = 5.1 Hz, 1 H), 3.26 (ddd, *J* = 12.5, 8.1, 7.7 Hz, 1 H), 2.53-2.44 (m, 2 H).

Mosher ester of 10 obtained *via* process in Scheme 3: the procedure is identical to that reported above. The ^{1}H NMR spectrum showed, apart from the signals of the major diastereoisomer reported above, a second set of signals of a minor diastereoisomer. - ^{1}H NMR (detected signals): δ = 5.79 (s, 1 H), 4.58 (dd, *J* = 5.1, 4.4 Hz, 1 H).