Phytoalexins from the crucifer rutabaga: structures, syntheses, biosyntheses and antifungal activity

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Supplementary Information

Table of Contents:

Experimental

General. All solvents were HPLC grade and used as such, except for CH_2Cl_2 and $CHCl_3$ which were redistilled and THF which was dried over Na and benzophenone. Flash CC: C-18 reversed phase silica gel 40 μm. Analytical HPLC analysis was carried out with a high performance liquid chromatograph equipped with quaternary pump, automatic injector, and photodiode array detector (wavelength range 190-600 nm), degasser, and an ODS column (5 μm particle size silica, 4.6 i.d. ×200 mm), equipped with an in-line filter. Mobile phase: H_2O-CH_3CN, 75:25 to 100% CH_3CN, for 35 min, linear gradient, and flow rate 1.0 mL/min. Other conditions as previously reported.[19]

1-Boc-2-chloroindole-3-carboxylic acid (17). 1-Boc-2-chloroindole-3-carboxaldehyde[13] (**16,** 0.56 g, 2 mmol) was dissolved in the mixture of *t*-butanol (10 mL) and 2-methylbut-2-ene (10 mL). A solution of $NaClO_2$ (1.81 g, 20 mmol) and KH_2PO_4 (2.04 g, 15 mmol) in water (10 mL) was then added and the mixture was vigorously stirred at rt. After 2 h the organic layer was separated, the aq layer was extracted with CH_2Cl_2-MeOH (95:5), the combined organic extracts were dried (Na_2SO_4) and the solvent evaporated under reduced pressure. Crystallization (CH_2Cl_2-hexane) afforded 0.567 g (96%) of colorless powder. Mp 175 – 177 ˚C; HPLC t_R = 19.8 min; ^{1}H NMR (500 MHz, $CDCl_3$): 12.68 (br s, 1H, D_2O exchangeable), 8.25 (m, 1H), 8.04 (m, 1H), 7.38 (m, 2H), 1.74 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$): 168.9 (s), 148.3 (s), 135.2 (s), 132.7 (s), 126.2 (s), 125.5 (d), 124.6 (d), 121.8 (d), 114.6 (d), 109.5 (s), 86.8 (s), 28.3 (q); HREIMS *m/z* (% relative abundance) measured: 295.0607 (295.0611 calcd for $C_{14}H_{14}ClNO_4$); EIMS *m/z* (% relative abundance): 295 $[M]^+$ (6), 221 (5), 195 (37), 130 (7). FTIR ν_{max}: 2979, 1745, 1683, 1532, 1445, 1308, 1206, 1150, 745 cm^{-1}.

Methyl 1-Boc-2-chloroindole-3-carboxylate (18). 1-Boc-2-chloroindole-3-carboxylic acid (**17**, 74 mg, 0.25 mmol) was dissolved in ethereal diazomethane solution (3 mL) and

stirred for 5 minutes at rt. The excess of diazomethane was quenched with acetic acid and the solvent was evaporated under reduced pressure. The colorless oil (77 mg, 100%) was obtained, sufficiently pure to use in the next step. HPLC t_R = 35.1 min; ^{1}H NMR (500 MHz, $CDCl_3$): 8.12 (d, J = 8 Hz, 1H), 8.04 (d, J = 8 Hz, 1H), 7.35 (m, 2H), 4.00 (s, 3H), 1.73 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$): 164.0 (s), 148.5 (s), 135.2 (s), 131.1 (s), 126.1 (s), 125.3 (d), 124.3 (d), 121.5 (d), 114.7 (d), 110.5 (s), 86.5 (s), 51.8 (q), 28.3 (q); HREIMS *m/z* (% relative abundance) measured: 309.0765 (309.0768 calcd for $C_{15}H_{16}ClNO_4$); EIMS *m/z* (% relative abundance): 309 $[M]^+$ (20), 252 (6), 236 (13), 209 (100), 178 (23), 148 (5), 114 (22). FTIR ν_{max}: 2980, 1749, 1722, 1532, 1446, 1309, 1147, 747 cm^{-1}.

Methyl 1-Boc-2-chloroindole-3-carboxamide (19). Thionyl chloride (30 μL, 0.45 mmol) was added to a solution of acid **17** (90 mg, 0.3 mmol) in dry THF (3 mL) under Ar atmosphere and the mixture was stirred at rt. After 3 h the reaction mixture was cooled to 0 ˚C, a solution of $MeNH_2$ in THF (2 M, 4 mL, 8 mmol) was added and the mixture was stirred for further 20 min at 0 ˚C. Then, the reaction mixture was diluted with brine (30 mL), extracted with EtOAc, the combined organic extract was dried (Na_2SO_4), the solvent evaporated under reduced pressure and the residue chromatographed on silica gel (hexane-acetone, 2:1). Evaporation of the solvent afforded a colorless oil (79 mg, 85%). An analytical sample was obtained by crystallization from CH_2Cl_2-hexane. Mp 92 – 94 ˚C; HPLC t_R = 20.9 min; ^{1}H NMR (500 MHz, $CDCl_3$): 8.18 (d, J = 8 Hz, 1H), 8.02 (d, J = 8 Hz, 1H), 7.34 (m, 2H), 6.50 (br s , 1H, D_2O exchangeable), 3.08 (d, J = 5 Hz, 3H), 1.72 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$): 163.6 (s), 148.7 (s), 135.2 (s), 126.8 (s), 125.5 (d), 124.2 (d), 123.8 (s), 121.5 (d), 114.8 (s), 114.7 (d), 86.2 (s), 28.3 (q), 26.6 (q); HREIMS *m/z* (% relative abundance) measured: 308.0923 (309.0928 calcd for $C_{15}H_{17}ClN_2O_3$); EIMS *m/z* (% relative abundance): 308 $[M]^+$ (6), 208 (37), 178 (29), 149 (15). FTIR ν_{max}: 3291, 2977, 1743, 1643, 1546, 1447, 1309, 1154, 747 cm^{-1}.

[2,4,5,6,7-D_5]-Tryptamine (24a). [2,4,5,6,7-D_5]-Tryptamine was synthesized from [2,4,5,6,7-D_5]-L-tryptophan (50.9 mg, 0.24 mmol) as previously reported[i] for the non-labeled compound to afford [2,4,5,6,7-D_5]-tryptamine (25.2 mg, 0.15 mmol, 62%). ^{1}H NMR (500 MHz, CD_3OD): δ 2.90 (m, 4H). HRMS-EI *m/z* (% relative abundance): measured 165.1315 (20), calculated for $[M]^+$ ($C_{10}H_7D_5N_2$) 135.0967 (100). FTIR ν_{max}: 3302, 1670 cm^{-1}.

Isalexin (9). HPLC t_R = 3.7 min; ^{1}H NMR (500 MHz, CD_3CN): 8.82 (br s, 1H, D_2O exchangeable), 7.52 (dd, J = 8, 8 Hz, 1H), 6.67 (d, J = 8 Hz, 1H), 6.50 (d, J = 8 Hz, 1H), 3.91 (s, 3H); ^{13}C NMR (125 MHz, CD_3CN): 181.7 (s), 160.8 (s), 160.0 (s), 152.3 (s), 142.0 (d), 108.4 (d), 107.8 (s), 105.6 (d), 57.2 (q); HREIMS *m/z* (% relative abundance) measured: 177.0422 (177.0426 calcd for $C_9H_7NO_3$); EIMS *m/z* (% relative abundance): 177 $[M]^+$ (83), 149 (100), 122 (35), 107 (42), 63 (9). FTIR ν_{max}: 3321, 1748, 1717, 1620, 1601, 1493, 1244, 1101, 775 cm^{-1}. UV λ_{max}(log ε) 199 (4.2), 234 (3.9), 335 (3.4).

([i]) Hashimoto, M.; Eda, Y. Osanai, Y.; Iwai, T.; Aoki, S. *Chem. Lett.* **1986**, *6*, 893-896.