Supplementary Material for Organic & Biomolecular Chemistry
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Supplementary data

Detoxification pathways of the phytoalexins brassilexin and sinalexin in *Leptosphaeria maculans*: isolation and synthesis of the elusive intermediate 3-formylindolyl-2-sulfonic acid

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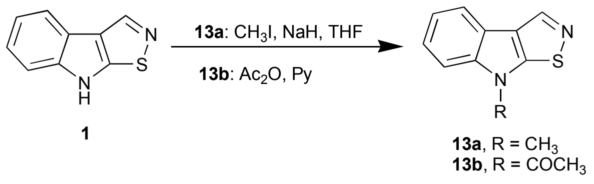
General experimental procedures

All chemicals were purchased from Sigma-Aldrich Canada Ltd., Oakville, ON. All solvents were HPLC grade and used as such, except for CH_2Cl_2 and $CHCl_3$, that were redistilled and THF, which was dried by distillation over Na/benzophenone. Organic extracts were dried over Na_2SO_4 and solvents removed under reduced pressure in a rotary evaporator. HPLC analysis was carried out with a high performance liquid chromatograph equipped with quaternary pump, automatic injector, and diode array detector (wavelength range 190 - 600 nm), degasser, and a Hypersil ODS column (5 µm particle size silica, 4.6 i.d.×200 mm), equipped with an in-line filter. Mobile phase A: 75% H_2O - 25% CH_3CN to 100% CH_3CN, for 35 min, linear gradient, and a flow rate 1.0 ml/min; B: 100% H_2O for 10 minutes, then linear gradient to 50% H_2O-50% CH_3CN for 15 minutes, flow rate 1 ml/min. Fourier transform IR spectra were obtained on Bio-Rad FTS-40 spectrometer in KBr. NMR spectra were recorded on Bruker Avance 500 spectrometers; for ^1H (500 MHz), δ values were referenced as follows $CDCl_3$ ($CHCl_3$, 7.23 ppm), CD_3CN (CHD_2CN, 1.94 ppm), CD_3SOCD_3 (CD_3SOCHD_2, 2.50 ppm); for ^{13}C (125 MHz) $CDCl_3$ (77.23 ppm), CD_3CN (1.39 and 118.69 ppm), CD_3SOCD_3 (39.51 ppm). Mass spectra (MS) were obtained on a VG 70 SE and Q Star XL Applied Biosystems mass spectrometers.

Syntheses



Scheme 6. Synthesis of 8-substituted brassilexins **13a** and **13b**.

Synthesis of 8-methylbrassilexin (**13a**, Scheme 6). NaH (60% suspension in mineral oil, 17 mg, 0.5 mmol) was added to a solution of brassilexin (**1**, 44 mg, 0.25 mmol) in dry THF (2 ml) and the mixture was allowed to stir at room temperature. After 10 minutes, MeI (30 μl, 0.5 mmol) was added and stirring was allowed to continue for further 60 min at room temperature. The mixture was then diluted with 1 M HCl (20 ml) and extracted with EtOAc. The combined organic extract was dried, the solvent was evaporated and the residue was chromatographed (SiO_2, hexane-acetone, 5:1) to afford 8-methylbrassilexin (**13a**) as a brownish solid (47 mg, 100%). Mp. 67-69 °C; HPLC R_t = 17.0 min. ν_{max}(KBr)/cm^{-1} 3048, 1648, 1490, 1464, 1319, 1261, 912 and 743. δ_H(500 MHz; CD_3CN) 3.87 (s, 3H); 7.26 (dd, J = 8, 8 Hz, 1H); 7.40 (dd, J = 8, 8 Hz, 1H); 7.48 (d, J = 8 Hz); 7.93 (d, J = 8 Hz); 8.71 (s, 1H). δ_C(125 MHz; CD_3CN) 34.2 (q), 111.4 (d), 121.4 (s), 121.5 (d), 121.8 (d), 125.0 (d), 127.0 (s), 146.2 (s), 149.4 (d), 163.2 (s). HRMS (EI) calc. for $C_{10}H_8N_2S$ (M^+) 188.0408, found 188.0408. MS-EI, *m/z* 188 (M^+, 100%), 155 (15), 146 (11).

Synthesis of 8-acetylbrassilexin (**13b**, Scheme 6). Ac_2O (0.1 ml, 1 mmol) was added to a solution of brassilexin (**1**, 17 mg, 0.10 mmol) in pyridine (0.2 ml) and the mixture was stirred at room temperature. After 2 hours the reaction mixture was diluted with 1 M HCl and allowed to stand at 3 ºC for 60 min. The precipitate was filtered off, was washed with water and allowed to dry to yield 8-acetylbrassilexin (**13b**) as a white solid (16 mg, 73%). Mp. 179-180 ºC (crystallization from CH_2Cl_2-hexane, lit.[1] 172 – 176 °C). HPLC R_t = 15.4 min. ν_{max}(KBr)/cm^{-1} 2927, 1691, 1480, 1376, 1300, 1200, 1018 and 755. δ_H(500 MHz; DMSO-D_6) 2.90 (s, 3H); 7.45 (dd, J = 8, 8 Hz, 1H); 7.50 (dd, J = 8, 8 Hz, 1H); 8.03 (m, 2H); 8.94 (s, 1H). δ_C(125 MHz; DMSO-D_6) 24.4 (q), 115.4 (d), 120.7 (d), 122.6 (s), 124.0 (d), 125.4 (d), 127.5 (s), 140.1 (s), 147.2 (d), 157.1 (s), 168.1 (s). HRMS (APCI) calc. for $C_{11}H_9N_2OS$ (M+H$^+$) 217.0430, found 217.0430.

Spectral data for brassilexin (**1**). Mp. 172-173 °C (lit.[1] 164-167 °C); HPLC R_t = 13.0 min; ν_{max}(KBr)/cm^{-1} 3389, 3072, 1647, 1505, 1464, 1369, 1239 and 740. δ_H (500 MHz; CD_3CN) 7.23 (dd, J = 8, 8 Hz, 1H); 7.33 (dd, J = 8, 8 Hz, 1H); 7.56 (d, J = 8 Hz); 7.91 (d, J = 8 Hz); 8.69 (s, 1H); 9.85 (br s, D_2O exch., 1H). δ_C(125 MHz; CD_3CN) 112.5 (d), 120.2 (d), 120.4 (s), 120.9 (d), 124.1 (d), 127.7 (s), 144.6 (s), 147.8 (d), 159.5 (s). HRMS (EI) calc. for $C_9H_6N_2S$ (M$^+$) 174.0252, found 174.0252. MS-EI, *m*/*z* 174 (M$^+$, 100%), 142 (16), 120 (11).

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[1] M. Devys, M. Barbier, I. Loiselet, T. Rouxel, A. Sarniguet, A. Kollmann and J. F. Bousquet, *Tetrahedron Lett*., 1988, **29**, 6447.

Spectral data for sinalexin (2). HPLC R_t = 20.1 min; ν_{max} (KBr)/cm^{-1} 2935, 1660, 1500, 1472, 1436, 1251 and 751. δ_H(500 MHz; CD_3CN) 4.17 (s, 3H); 7.32 (dd, J = 8, 8 Hz, 1H); 7.45 (dd, J = 8, 8 Hz, 1H); 7.59 (d, J = 8 Hz); 7.94 (d, J = 8 Hz); 8.73 (s, 1H). δ_C(125 MHz; CD_3CN) 65.8 (q), 110.8 (d), 121.9 (d), 123.0 (d), 125.3 (s), 125.8 (s), 142.8 (s), 149.6 (d), 157.3 (s). HRMS (EI) calc. for $C_{10}H_8N_2OS$ (M^+) 204.0356, found 204.0355. MS-EI, m/z 204 (M^+, 87%), 173 (100), 149 (43), 129 (24), 102 (13).

Spectral data for 3-aminomethyleneindole-2-thione (8). Mp. 197-200 °C; HPLC R_t = 8.4 min; ν_{max}(KBr)/cm^{-1} 3238, 3128, 1641, 1446, 1398, 1210 and 739. δ_H (500 MHz; CD_3CN) 7.10 (m, 3H); 7.33 (br s, D_2O exch., 1H); 7.49 (d, J = 8 Hz, 1H); 8.28 (m, 1H); 9.85 (br s, D_2O exch., 1H); 11.04 (br s, D_2O exch., 1H). δ_C(125 MHz; CD_3CN) 108.2 (s), 110.4 (d), 115.9 (d), 122.4 (d), 124.3 (d), 129.4 (s), 139.0 (s), 151.2 (d), 179.0 (s). HRMS (EI) calc. for $C_9H_8N_2S$ (M^+) 176.0408, found 176.0412. MS-EI, m/z 176 (M^+, 100%), 149 (46), 117 (11).

Spectral data for 3-aminomethylene-1-methoxyindole-2-thione (14). Mp. 104-106 °C; HPLC R_t = 13.4 min. ν_{max}(KBr)/cm^{-1} 3263, 3112, 1641, 1341, 1214 and 740. δ_H(500 MHz; $CDCl_3$) 4.19 (s, 3H); 6.18 (br s, D_2O exch., 1H); 7.16 (m, 1H); 7.23 (m, 2H); 7.41 (d, J = 8 Hz, 1H); 8.10 (m, 1H); 11.15 (br s, D_2O exch., 1H). δ_H(500 MHz; CD_3CN) 4.11 (s, 3H); 7.15 (dd, J = 8, 8 Hz, 1H); 7.23 (m, 2H); 7.37 (br s, D_2O exch., 1H); 7.56 (d, J = 8 Hz, 1H); 8.28 (m, 1H); 10.99 (br s, D_2O exch., 1H). δ_C(125 MHz; $CDCl_3$) 63.3 (q), 105.2 (s), 107.8 (d), 115.2 (d), 122.9 (d), 124.0 (s), 124.2 (d), 135.5 (s), 147.9 (d), 172.1 (s). HRMS (EI) calc. for $C_{10}H_{10}N_2OS$ (M^+) 206.0512, found 206.0514. MS-EI, *m/z* 206 (M^+, 100%), 174 (27), 148 (40).

Spectral data for 3-aminomethylene-1-methylindole-2-thione (**15**). Mp. 126-128 °C; HPLC R_t = 15.0 min. ν_{max}(KBr)/cm^{-1} 3248, 3093, 1643, 1427, 1321, 1232, 1088 and 743. δ_H(500 MHz; $CDCl_3$) 3.75 (s, 3H); 6.03 (br s, D_2O exch., 1H); 7.14 (m, 2H); 7.22 (dd, *J* = 8, 8 Hz, 1H); 7.41 (d, *J* = 8 Hz, 1H); 8.10 (m, 1H); 11.27 (br s, D_2O exch., 1H). δ_C(125 MHz; $CDCl_3$) 29.5 (q), 108.5 (s), 109.2 (d), 115.0 (d), 122.3 (d), 124.0 (d), 127.4 (s), 140.4 (s), 147.0 (d), 178.8 (s). HRMS (EI) calc. for $C_{10}H_{10}N_2S$ (M^+) 190.0565, found 190.0566. MS-EI, *m/z* 190 (M^+, 100%), 174 (10), 157 (16), 130 (11), 95 (6).