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Synthesis of the fungal natural product (−)-xylariamide A

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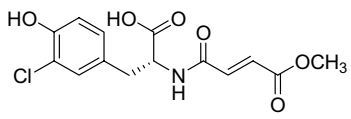
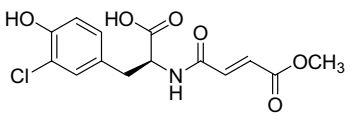
Abstract—The first synthesis of the fungal natural product (−)-xylariamide A **1** is reported. *N*,*O*-Bis(trimethylsilyl)acetamide induced coupling of D-tyrosine with (*E*)-but-2-enedioic acid 2,5-dioxo-pyrrolidin-1-yl ester methyl ester **5** produced the dechloro natural product **6**, which was subsequently monochlorinated using oxone and KCl to yield synthetic **1**. (−)-Xylariamide A **1**, (+)-xylariamide A **2** and (−)-dechloroxylariamide A **6** displayed no cytotoxic or antimicrobial activity.

We have recently reported the isolation and structure elucidation of (−)-xylariamide A **1** from the plant-associated microfungus, *Xylaria* sp. (FRR 5657).[1] Confirmation of the structure and absolute stereochemistry of **1** resulted from the synthesis of (+)-xylariamide A **2**.[1] Both (−)-xylariamide A **1** and (+)-xylariamide A **2** were screened for toxicity in a brine shrimp (*Artemia salina*) lethality assay and only the natural product displayed any activity.[1] Only minute quantities (0.9 mg) of the bioactive natural product **1** were initially isolated from the large-scale fungal fermentation and this prevented more detailed biological evaluations of (−)-xylariamide A. Total synthesis of the chiral chlorinated fungal metabolite **1** appeared to be the best means of obtaining quantities of this compound that would allow a more thorough bioactivity profiling. Herein, we report a short and efficient synthesis of (−)-xylariamide A **1** along with its cytotoxic and antimicrobial screening results (Fig. 1).

Our synthetic approach to **1** was based on similar chemistry to that reported for (+)-xylariamide A **2** where the silylating agent *N*,*O*-bis(trimethylsilyl)acetamide (BSA), was used to form an amide bond between 3-chloro-L-tyrosine and the *N*-succinimide activated ester, (*E*)-but-2-enedioic acid 2,5-dioxo-pyrrolidin-1-yl ester methyl ester **5**.[1,2] A different synthetic route for the natural product **1** had to be designed since we could not find a commercial supplier for 3-chloro-D-tyrosine. Rather than synthesise 3-chloro-D-tyrosine, we decided to react **5** with D-tyrosine in the presence of BSA and follow this coupling reaction with the selective monochlorination of the BSA amide product to yield the natural product **1**. The synthesis of (−)-xylariamide A **1** began with the commercially available (*E*)-but-2-enedioic acid dimethyl ester **3** (Scheme 1), selective monohydrolysis of which using aqueous LiOH in acetone, afforded the previously reported (*E*)-but-2-enedioic acid monomethyl ester (**4**, 87%).[3] Coupling **4** with *N*-hydroxysuccinimide using EDCI in CH_3CN yielded the known (*E*)-but-2-enedioic acid 2,5-dioxo-pyrrolidin-1-yl ester methyl ester (**5**, 40%).[4] (−)-Dechloroxylariamide A **6** was produced by reacting D-tyrosine,



(-)-Xylariamide A **1**



(+)-Xylariamide A **2**

Figure 1. Structures for **1** and **2**.

Keywords: Synthesis; Natural product; (−)-Xylariamide A; (+)-Xylariamide A; (−)-Dechloroxylariamide A; *N*,*O*-Bis(trimethylsilyl)acetamide.
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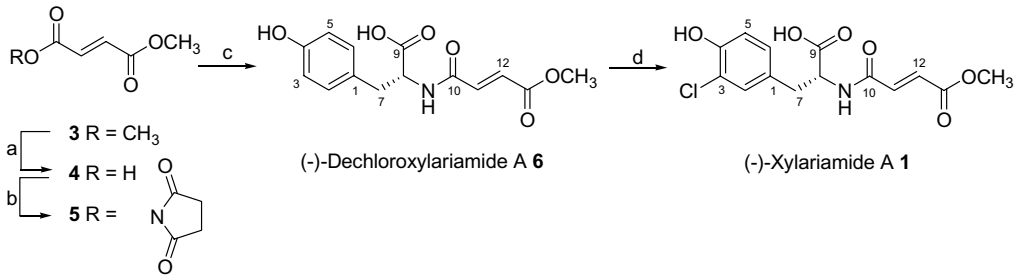

Scheme 1. Total synthesis of (−)-xylariamide A **1**. Reagents and conditions: (a) LiOH, acetone, rt, 1 h (87%); (b) *N*-hydroxysuccinimide, EDCI, CH_3CN, rt, 24 h (40%); (c) D-tyrosine, BSA, DMF, 60 °C, 16 h (54%); (d) oxone, KCl, CH_3CN/H_2O, rt, 72 h (78%).

(*E*)-but-2-enedioic acid 2,5-dioxo-pyrrolidin-1-yl ester methyl ester **5** and BSA in DMF at 60 °C for 16 h.[5] Purification was performed using gel permeation chromatography to afford (−)-dechloroxylariamide A (**6**, 54%). The NMR data for **6** were assigned on the basis of 1D and 2D NMR data analysis. Monochlorination of **6** using oxone and KCl in aqueous CH_3CN,[6] followed by C18 HPLC chromatography yielded pure (−)-xylariamide A (**1**, 78%).[7] The NMR, MS, UV, IR and $[\alpha]_D$ data for synthetic **1** were identical to those reported for the natural product (−)-xylariamide A **1**.[1]

(−)-Xylariamide A **1**, the previously synthesised (+)-xylariamide A **2**[1] and (−)-dechloroxylariamide A **6** were all tested for cytotoxicity against the human cancer cell lines MCF-7 (breast), H460 (nonsmall cell lung) and SF268 (CNS) using the colorimetric sulforhodamine B assay.[8] Compounds **1**, **2** and **6** showed no cytotoxicity after 72 h when tested at 5 and 50 μg/mL. Compounds **1**, **2** and **6** were also tested against a panel of microbial strains known to be associated with nosocomial infection, which included multi-drug resistant *Staphylococcus aureus* (wild type MRSA), *Staphylococcus aureus* (NCCLS 29523), *Escherichia coli* (ATCC 25922), *Enterococcus faecalis* (NCCLS 29212), *Pseudomonas aeruginosa* (ATCC 27853), *Streptococcus pyogenes* (ATCC 19615), *Acinetobacter anitratus* (wild type) and *Candida albicans* (ATCC 60193). Antimicrobial activities were evaluated using a broth microdilution assay[9,10] with each compound screened in a double dilution series from 500 to 1.0 μg/mL. No microbial growth inhibition was observed at any of these concentrations after 20 h of dosing.

In conclusion, this letter reports a simple synthesis of the fungal natural product (−)-xylariamide A **1** from readily available starting materials. (−)-Xylariamide A **1**, (+)-xylariamide A **2** and (−)-dechloroxylariamide A **6** displayed no cytotoxic or antimicrobial activity.

Acknowledgements

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Supplementary data

^1H and ^{13}C NMR spectra and LRESIMS data for compounds **1** and **6**. Supplementary data associated with this article can be found, in the online version at doi:10.1016/j.tetlet.2005.05.114.

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2. Patel, V. F.; Andis, S. L.; Kennedy, J. H.; Ray, J. E.; Schultz, R. M. *J. Med. Chem.* **1999**, *42*, 2588–2603.
3. The commercial reagent (*E*)-but-2-enedioic acid dimethyl ester (**3**, 1.44 g, 10 mmol) was dissolved in acetone (70 mL) at rt and 1 N aqueous LiOH (10 mL, 10 mmol) was slowly added over 15 min to the stirred solution. The reaction was stirred for 1 h, diluted with 2 N HCl (200 mL), saturated with NaCl and then extracted with EtOAc (3 × 200 mL). The EtOAc layer was slowly evaporated and the resulting precipitate was filtered and dried to yield pure (*E*)-but-2-enedioic acid monomethyl ester (**4**, 1.13 g, 87%) as a white amorphous solid; mp 142–143 °C (lit. mp 141–141.5 °C).[1,11,12]
4. (*E*)-But-2-enedioic acid monomethyl ester (**4**, 260 mg, 2 mmol), EDCI (768 mg, 4 mmol) and *N*-hydroxysuccinimide (690 mg, 6 mmol) were dissolved in dry CH_3CN (5 mL) and the reaction mixture was stirred at rt for 24 h. The reaction mixture was pre-absorbed onto silica gel (Alltech 30–40 μm, 60 Å) then loaded into a glass column and flushed with 100% EtOAc (50 mL). The EtOAc wash was evaporated to dryness, the residue re-dissolved in 100% DCM and injected onto a MPLC silica (Alltech 30–40 μm, 60 Å) packed column (20 × 90 mm) using isocratic conditions of 40% EtOAc/60% hexanes at a flow rate of 6 mL/min for 30 min. This yielded pure (*E*)-but-2-enedioic acid 2,5-dioxo-pyrrolidin-1-yl ester methyl ester (**5**, 180 mg, 40%, t_R = 12.0 min) as a white amorphous solid; mp 93–95 °C (lit. mp 93.5–94.5 °C).[1,13]
5. *N*,*O*-Bis(trimethylsilyl)acetamide (878 μL, 3.6 mmol) was added to (*E*)-but-2-enedioic acid 2,5-dioxo-pyrrolidin-1-yl ester methyl ester (**5**, 204 mg, 0.9 mmol) and D-tyrosine (164 mg, 0.9 mmol) in dry DMF (2 mL) and the reaction was heated at 60 °C for 16 h. Upon cooling the reaction solution was poured into 2 N HCl (50 mL), saturated with NaCl then extracted with EtOAc (2 × 50 mL). The EtOAc layer was evaporated to dryness under reduced pressure to yield a yellow gum (250 mg), which was subsequently dissolved in 100% CH_3OH (2 mL) then loaded onto a Sephadex LH-20 open column (45 × 450 mm) and run using 100% CH_3OH as eluent at a flow rate of 4.5 mL/min. All resulting fractions were analysed by TLC and identical

fractions combined to yield pure (−)-dechloroxylariamide A (**6**, 142.6 mg, 54%) as a stable clear gum; $[\alpha]_D^{23}$ −15 (*c* 0.270, CH_3OH); UV (CH_3OH) λ_{max} (log ε) 214 (4.01), 265 (3.58) nm; IR ν_{max} (NaCl) 3600–3100, 1720, 1664, 1546, 1516, 1445, 1349, 1308, 1272, 1232, 1198, 1173, 1113, 1024, 979, 832, 766 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 2.76 (1H, dd, *J* = 14.0, 9.5 Hz, H-7a), 2.98 (1H, dd, *J* = 14.0, 5.0 Hz, H-7b), 3.71 (3H, s, 13-OCH_3), 4.44 (1H, ddd, *J* = 9.5, 7.5, 5.0 Hz, H-8), 6.53 (1H, d, *J* = 15.5 Hz, H-12), 6.64 (2H, d, *J* = 8.5 Hz, H-3, H-5), 7.00 (2H, d, *J* = 8.5 Hz, H-2, H-6), 7.06 (1H, d, *J* = 15.5 Hz, H-11), 8.79 (1H, d, *J* = 7.5 Hz, 8-NH), 9.18 (1H, br s, 4-OH), 12.76 (1H, br s, 9-OH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 35.9 (C-7), 51.9 (13-OCH_3), 54.1 (C-8), 115.0 (2C, C-3, C-5), 127.3 (C-1), 128.5 (C-12), 129.9 (2C, C-2, C-6), 137.1 (C-11), 155.9 (C-4), 162.6 (C-10), 165.4 (C-13), 172.5 (C-9); (−)-LRESIMS *m/z* (rel int.) 134 (10), 216 (5), 248 (5), 278 (5), 292 (100); (−)-HRESIMS *m/z* 292.08401 ($C_{14}H_{14}NO_6$ $[M-H]^-$ requires 292.08268).

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7. Oxone (105 mg, 0.171 mmol) was added to (−)-dechloroxylariamide A (**6**, 50 mg, 0.171 mmol) in CH_3CN (5 mL) and H_2O (15 mL) then KCl (20 mg, 0.257 mmol) was added in two equal portions over 1 h and the reaction mixture was stirred at rt for 72 h, then poured into 2 N HCl (50 mL), saturated with NaCl then extracted with EtOAc (2 × 50 mL). The EtOAc layer was evaporated to dryness under reduced pressure to yield a yellow gum (76 mg). This material was dissolved in DMSO (750 μL) and CH_3OH (250 μL) and purified by preparative HPLC on a Thermo Hypersil C18 BDS 5 μm 143 Å column (21.2 × 150 mm) using isocratic conditions of 40% CH_3OH/60% aqueous TFA (0.2%) at a flow rate of 6 mL/min. This yielded pure (−)-xylariamide A (**1**, 43 mg, 78%) as a stable clear gum; $[\alpha]_D^{23}$ −16 (*c* 0.250, CH_3OH) (lit. $[\alpha]_D^{24}$ −22 (*c* 0.060, CH_3OH));[1] UV (CH_3OH) λ_{max} (log ε) 208 (4.01), 220 sh (3.93), 276 (3.36) nm; IR ν_{max} (NaCl) 3500–3200, 1712, 1665, 1549, 1512, 1440, 1345, 1294, 1237, 1196, 1173, 1057, 1024, 976, 824, 766, 667 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 2.78 (1H, dd, *J* = 14.0, 9.0 Hz, H-7a), 2.99 (1H, dd, *J* = 14.0, 5.0 Hz, H-7b), 3.71 (3H, s, 13-OCH_3), 4.46 (1H, ddd, *J* = 9.0, 8.5, 5.0 Hz, H-8), 6.54 (1H, d, *J* = 15.5 Hz, H-12), 6.84 (1H, d, *J* = 8.5 Hz, H-5), 6.97 (1H, dd, *J* = 8.5, 1.5 Hz, H-6), 7.05 (1H, d, *J* = 15.5 Hz, H-11), 7.17 (1H, d, *J* = 1.5 Hz, H-2), 8.80 (1H, d, *J* = 8.5 Hz, 8-NH), 9.94 (1H, br s, 4-OH), 12.82 (1H, br s, 9-OH), ^{13}C NMR (125 MHz, DMSO-d_6) δ 35.5 (C-7), 52.0 (13-OCH_3), 53.8 (C-8), 116.4 (C-5), 119.1 (C-3), 128.6 (2C, C-6, C-12), 128.9 (C-1), 130.2 (C-2), 136.9 (C-11), 151.6 (C-4), 162.7 (C-10), 165.4 (C-13), 172.3 (C-9); (−)-LRESIMS *m/z* (rel int.) 168 (20), 170 (7), 250 (10), 252 (3), 282 (10), 284 (3), 312 (10), 314 (3), 326 (100), 328 (33); (−)-HRESIMS *m/z* 326.04252 ($C_{14}H_{13}NO_6{}^{35}Cl$ $[M-H]^-$ requires 326.04369).

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