

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.

Keywords

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

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.

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 (Scheme 1), selective monohydrolysis of which using aqueous LiOH in acetone, afforded the previously reported (*E*)-but-2-enedioic acid monomethyl ester (**4**, 87% yield).³ Coupling **4** with *N*-hydroxysuccinimide using EDCI in CH₃CN 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, (*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% yield). Monochlorination of **6** using oxone and KCl in aqueous CH₃CN,⁶ followed by C18 HPLC chromatography yielded pure (-)-xylariamide A (**1**, 78%).⁷ The NMR, MS, UV, IR and [α]_D data for synthetic **1** were identical to those reported for the natural product (-)-xylariamide A **1**.¹

(-)-Xylariamide A **1**, the previously synthesized (+)-xylariamide A **2**¹ and (-)-dechloroxylariamide A **6** were all tested for cytotoxicity against the human cancer cell lines MCF-7 (breast), H460 (non small cell lung), and SF268 (CNS) using the colourimetric sulphorhodamine 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 nosocomial microbial strains 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 micro dilution assay^{9,10} with each compound screened in a double dilution series from 500 µg/mL to 1.0 µg/mL. No microbial growth inhibition was observed at any of these concentrations after 20 h of dosing.

In conclusion, this paper 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 ¹³C NMR spectra and LRESIMS data for compounds **1** and **6**. Supplementary data associated with this article can be found in the online version.

References and notes

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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 (1.44 g, 10 mmol) was dissolved in acetone (70 mL) at rt and 1N 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 2N HCl (200 mL), saturated with NaCl 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% yield) 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 CH₃CN (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% yield, *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 2M HCl (50 mL), saturated with NaCl then extracted with EtOAc (2 × 50 mL). The EtOAc layer was evaporated to dryness under

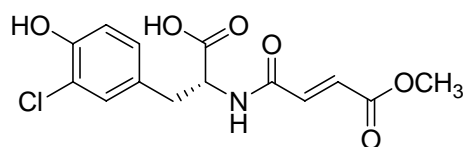
reduced pressure to yield a yellow gum (250 mg) which was subsequently dissolved in 100% CH₃OH (2 mL) then loaded onto a Sephadex LH-20 open column (45 × 450 mm) and run using 100% CH₃OH as eluent at a flow rate of 4.5 mL/min. All resulting fractions were analysed by TLC and identical fractions combined to yield pure (-)-dechloroxylariamide A (**6**, 142.6 mg, 54% yield) as a stable clear gum; $[\alpha]_D^{23}$ -15° (*c* 0.270, CH₃OH); UV (CH₃OH) 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-OCH₃), 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, brs, 4-OH), 12.76 (1H, brs, 9-OH); ¹³C NMR (125 MHz, DMSO-*d*₆) δ 35.9 (C-7), 51.9 (13-OCH₃), 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 (C₁₄H₁₄NO₆ [M-H]⁻ requires 292.08268).

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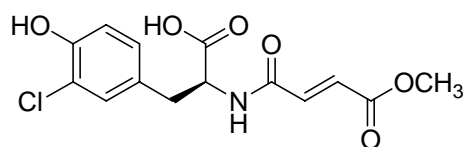
7. Oxone (105 mg, 0.171 mmol) was added to (-)-dechloroxylariamide A (**6**, 50 mg, 0.171 mmol) in CH₃CN (5 mL) and H₂O (15 mL) then KCl (20 mg, 0.257 mmol) was added in two equal portions over 1 h and the reaction mixture was stirred at rt for 72 h, then poured into 2M HCl (50 mL), saturated with NaCl 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 μ L) and CH₃OH (250 μ L) and purified by preparative HPLC on a Thermo Hypersil C18 BDS 5 μ m 143 Å column (21.2 × 150 mm) using isocratic conditions of 40% CH₃OH/60% aqueous TFA (0.2%) at a flow rate of 6 mL/min. This yielded pure (-)-xylariamide A (**1**, 43 mg, 78% yield) as a stable clear gum; $[\alpha]_D^{23}$ -16° (*c* 0.250, CH₃OH) (lit. $[\alpha]_D^{24}$ -22° (*c* 0.060, CH₃OH));¹ UV (CH₃OH) 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-OCH₃), 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, brs, 4-OH), 12.82 (1H, brs, 9-OH), ^{13}C NMR (125 MHz, DMSO- d_6) δ 35.5 (C-7), 52.0 (13-OCH₃), 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 (C₁₄H₁₃NO₆³⁵Cl [M-H]⁻ requires 326.04369).

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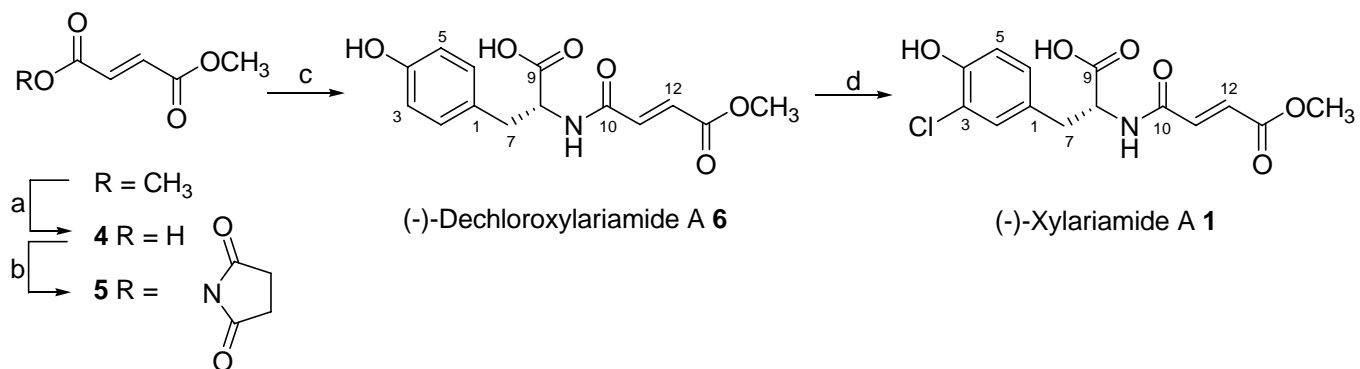


(-)-Xylariamide A 1



(+)-Xylariamide A 2

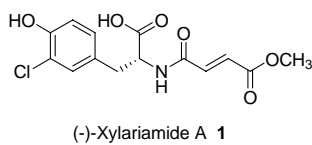
Figure 1. Structures for **1** and **2**.



Scheme 1. Total synthesis of (-)-xylariamide A 1. Reagents and conditions: (a) LiOH, acetone, rt, 1 h (87%); (b) *N*-Hydroxysuccinimide, EDCI, CH₃CN, rt, 24 h (40%); (c) D-tyrosine, BSA, DMF, 60 °C, 16 h (54%); (d) Oxone, KCl, CH₃CN/H₂O, rt, 72 h (78%).

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