

The isolation and synthesis of 3-chloro-4-hydroxyphenylacetamide produced by a plant-associated microfungus of the genus *Xylaria*

Rohan A. Davis,^{a,*} Dianne Watters^b and Peter C. Healy^a

^aChemical Biology Program, Eskitis Institute, Griffith University, Brisbane QLD 4111, Australia

^bCell Biology Program, Eskitis Institute, Griffith University, Brisbane QLD 4111, Australia

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Abstract—Chemical investigations of the fermentation broth from the microfungus *Xylaria* sp. have afforded the new natural product 3-chloro-4-hydroxyphenylacetamide **1** and the previously reported fungal metabolite 3-chloro-4-hydroxyphenylacetic acid **2**. This letter reports the isolation and full spectroscopic characterisation of compounds **1** and **2** by NMR, UV, IR and MS data. The crystal structure and one-pot synthesis of 3-chloro-4-hydroxyphenylacetamide are also reported.
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Several thousand organohalogen compounds have been identified as biosynthetic products from living organisms.¹ Although the oceans are the single largest source of naturally occurring organohalogens, some terrestrial organisms such as plants, fungi, bacteria, insects and lichens have also been identified as producers of halogenated natural products.² Less than 45 organohalogens containing the 3-chloro-4-hydroxyphenyl subunit have been isolated from natural sources to date.^{3–8}

We have recently embarked on a research program looking for new structural and bioactive metabolites from microfungi isolated from Australian endemic plants. Examination of a local rainforest tree, *Glochidion ferdinandi* (family Euphorbiaceae) afforded several microfungal strains, one of which was identified as *Xylaria* sp. (FRR 5657).⁹ This strain, when cultured on damp rice produced two new xanthenes⁹ but when grown in malt extract broth the EtOAc extract yielded, via C18 flash chromatography then phenyl HPLC, the new natural product 3-chloro-4-hydroxyphenylacetamide **1** and the previously reported fungal metabolite, 3-chloro-4-hydroxyphenylacetic acid **2** (Fig. 1).¹⁰ The

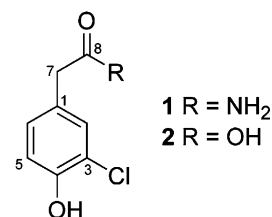


Figure 1. Structures of compounds **1** and **2**.

previously isolated xanthenes⁹ were not produced in the malt extract broth cultures.

Compound **1** was assigned the molecular formula C₈H₈NO₂Cl on the basis of HREIMS,¹¹ ¹H and ¹³C NMR spectral data (see Table 1). The (–)-LRESIMS isotopic pattern¹¹ of **1** confirmed the presence of one chlorine atom. The ¹H NMR spectrum of **1** contained three broad exchangeable singlets [δ 9.88 (1H), 7.36 (1H) and 6.80 (1H)], three aromatic signals [δ 7.20 (d, *J* = 1.5 Hz, 1H), 6.99 (dd, *J* = 8.0, 1.5 Hz, 1H) and 6.87 (d, *J* = 8.0 Hz, 1H)], which were indicative of a 1,3,4-trisubstituted aromatic ring¹² and one methylene singlet [δ 3.24 (2H)]. The ¹³C NMR spectrum of **1** displayed 8 signals of which 7 resonated between 119 and 173 ppm. Analysis of the 2D NMR data (gCOSY, HSQC, gHMBC and ROESY) allowed the structure of **1** to be assigned to 3-chloro-4-hydroxyphenylacetamide. Key HMBC correlations used for the structure

Keywords: Microfungus; *Xylaria* sp.; Natural products; 3-Chloro-4-hydroxyphenylacetamide; 3-Chloro-4-hydroxyphenylacetic acid; X-ray crystal structure; Synthesis.

* Corresponding author. Tel.: +61 7 3875 7587; fax: +61 7 3875 7656; e-mail: r.davis@griffith.edu.au

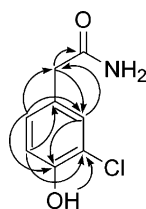
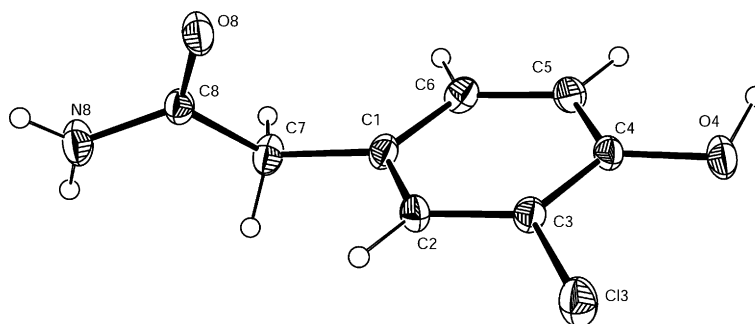
Table 1. NMR data for compounds **1** and **2**^a

Position	Compound 1				Compound 2			
	¹³ C	¹ H (mult., <i>J</i> , int.)	gHMBC	ROESY	¹³ C	¹ H (mult., <i>J</i> , int.)	gHMBC	ROESY
1	128.1				127.5			
2	130.1	7.20 (d, 1.5, 1H)	3, 4, 6, 7	7	130.5	7.19 (d, 1.5, 1H)	3, 4, 6, 7	7
3	119.1				119.1			
4	151.5				151.5			
4-OH		9.88 (br s, 1H)	3	5		9.97 (br s, 1H)	3, 4, 5	5
5	116.3	6.87 (d, 8.0, 1H)	1, 3, 4	6, 4-OH	116.2	6.86 (d, 8.5, 1H)	1, 3, 4	6, 4-OH
6	128.5	6.99 (dd, 8.0, 1.5, 1H)	2, 4, 7	5, 7	128.9	6.98 (dd, 8.5, 1.5, 1H)	2, 4, 7	5, 7
7	40.8	3.24 (s, 2H)	2, 6, 8	2, 6, 8-NH _{trans}	39.2	3.39 (s, 2H)	1, 2, 6, 8	2, 6
8	172.2				173.0			
8-NH _{cis} ^b		6.80 (br s, 1H)						
8-NH _{trans}		7.36 (br s, 1H)		7				
8-OH						12.25 (br s, 1H)		

^a Spectra were recorded in DMSO-*d*₆ at 30 °C.^b *cis* and *trans* Stereochemical assignments for the amide protons are relative to the carbonyl oxygen.

determination of **1** are shown in Figure 2. The structure of **1** was confirmed by X-ray crystallography analysis.¹³ An ORTEP-3¹⁴ representation of 3-chloro-4-hydroxyphenylacetamide is shown in Figure 3. The structural data shows the acetamide group to be twisted with respect to the phenyl ring with the torsion angle C2–C1–C7–C8 = 63.8(4)°. This conformation is likely to be a consequence of inter-molecular hydrogen bonds O4–H4···O8 and N8–H8···O8, and π ··· π interactions between adjacent phenyl rings. Compound **1** was first reported in a patent as a product from a synthetic preparation,¹⁵ however this is the first report of **1** as a natural product.

The minor metabolite 3-chloro-4-hydroxyphenylacetic acid **2** was assigned the molecular formula C₈H₇O₃Cl on the basis of HREIMS,¹⁶ ¹H and ¹³C NMR spectral data (see Table 1). The ¹³C NMR spectrum of **2** was

**Figure 2.** Key HMBC correlations for compound **1**.**Figure 3.** ORTEP plot for compound **1**.

essentially identical to **1**, with only small discrepancies observed (<1.7 ppm). The ¹H NMR spectrum of **2** also showed similarities with **1**, however the exchangeable primary amide signals of **1** were replaced with only one broad exchangeable proton signal at δ 12.25. These data suggested that the primary amide in **1** had been substituted with a carboxylic acid in **2**. Hence compound **2** was determined to be 3-chloro-4-hydroxyphenylacetic acid. This compound has been previously reported as a metabolite from the fungus *Marasmius palmivorus*,¹⁷ however its isolation and full spectroscopic characterisation was not published. Compound **2** is also commercially available and we confirmed by NMR, IR, UV and MS analyses that the natural product was identical to the synthetic material.

The first reported synthesis of **1** involved several steps with the final reaction involving the hydrolysis and dealkylation of an alkoxy protected chlorinated phenyl acetone nitrile.¹⁵ We synthesised **1** from **2** in a one-pot reaction using peptide coupling chemistry.¹⁸ Synthetic **1** was spectroscopically identical in all respects to the natural product.

Compounds **1** and **2** were both tested for cytotoxicity against the cell lines SHSY5Y (human neuroblastoma), HEK293T (SV40 T antigen transformed human embryonal kidney cells), and A549 (human non-small cell lung carcinoma) using the colourimetric sulphorhodamine B

assay.¹⁹ Compounds **1** and **2** showed no cytotoxicity when tested at 2 and 20 µg/mL.

In conclusion, this letter reports the isolation, structure elucidation, crystal structure and one-pot synthesis of the new natural product 3-chloro-4-hydroxyphenylacetamide **1**. The previously reported fungal metabolite 3-chloro-4-hydroxyphenylacetic acid **2** was also isolated and spectroscopically characterised using NMR, UV, IR and MS data.

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- Two 1 L conical flasks each containing 500 mL of malt extract broth (Difco) were each inoculated with one 10 mL aliquot of fungal stock culture grown in malt extract broth for 5 days at 30 °C. The flask cultures were incubated at room temperature on an orbital shaker at 150 rpm for 28 days. The filtered culture broth (1.0 L) was extracted with EtOAc (3 × 500 mL) and the organic phase was dried under vacuum to yield 489 mg of crude extract. The extract was subjected to C18 flash chromatography using a 20% stepwise gradient from 100% H₂O to 100% CH₃OH. The 20% CH₃OH/80% H₂O fraction (22 mg) was further purified by reversed-phase HPLC (Rainin Phenyl 5 µm 10 × 50 mm) using a gradient from 100% aq TFA (0.05%) to 50% CH₃OH/50% aq TFA (0.05%) in 10 min at a flow rate of 4 mL/min. This yielded pure 3-chloro-4-hydroxyphenylacetamide **1** (6.0 mg, *t_R* 5.0 min) and 3-chloro-4-hydroxyphenylacetic acid **2** (1.3 mg, *t_R* 7.5 min).
- 3-Chloro-4-hydroxyphenylacetamide **1** was isolated as stable yellow crystals; mp 160–162 °C; UV (CH₃OH) λ_{\max} (log ϵ) 205 (4.08), 231 sh (3.59), 282 (3.18) nm; UV (CH₃OH + NaOH) λ_{\max} (log ϵ) 206 (4.27), 248 (3.98), 299 (3.62) nm; IR ν_{\max} (NaCl) 3500–3000, 1661, 1606, 1500, 1425, 1393, 1287, 1227, 1179, 937, 901, 824, 802, 668, 650, 571 cm⁻¹; (–)-LRESIMS *m/z* (rel. int.) 184 (100), 186 (33); HREIMS *m/z* 185.02430 (C₈H₈NO₂³⁵Cl M⁺ requires 185.02436).
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- Crystal data for **1** were obtained with a Rigaku AFC7R diffractometer, Mo K α radiation (λ = 0.71073 Å), graphite monochromator, C₈H₈ClNO₂, triclinic, space group P-1, cell dimensions *a* = 5.985(2), *b* = 7.590(3), *c* = 9.395(3) Å, α = 106.0(3), β = 93.18(3), γ = 93.47(3)°, *V* = 408.3(7) Å³, *D*_{calc} = 1.51 g cm⁻³, *Z* = 2, *F*(000) = 192, μ = 0.421 mm⁻¹. Data was collected at 295(2) K using ω –2 θ scans in the range θ = 2.80–27.49°. A total of 2194 reflections were collected, 1871 were unique (*R*_{int} = 0.0227). The structure was refined by full-matrix least squares on *F*². The non-hydrogen atoms were refined anisotropically. Hydrogen atoms were constrained as riding atoms with C–H 0.95 Å, N–H 0.85 Å. The hydroxyl proton was located in a difference Fourier map and constrained as a riding atom with O–H 0.90 Å. *U*_{iso}(H) values were set to 1.2 *U*_{eq} of the parent atom. Atom coordinates, bond lengths, angles and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre: (CCDC No. 253553). Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (Fax: +44(0)-1223-336033; email: deposit@ccdc.cam.ac.uk).
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- 3-Chloro-4-hydroxyphenylacetic acid **2** was isolated as a stable amorphous yellow solid; mp 108–110 °C; UV (CH₃OH) λ_{\max} (log ϵ) 219 (3.66), 282 (3.31) nm; UV (CH₃OH + NaOH) λ_{\max} (log ϵ) 219 (3.68), 249 (3.72), 301 (3.47) nm; IR ν_{\max} (NaCl) 3450, 3300–2800, 1694, 1614, 1579, 1504, 1404, 1334, 1295, 1258, 1205, 1188, 1054, 932, 914, 861, 829, 799, 725, 677, 585 cm⁻¹; (–)-LRESIMS *m/z* (rel. int.) 141 (30), 143 (10), 155 (60), 157 (20), 185 (100), 187 (33); HREIMS *m/z* 186.00806 (C₈H₇O₃³⁵Cl M⁺ requires 186.00837).
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- EDCI (576 mg, 3.0 mmol) and DMAP (24 mg, 0.2 mmol) were added to 3-chloro-4-hydroxyphenylacetic acid **2** (372 mg, 2.0 mmol) in dry CH₃CN (5 mL) and the mixture was stirred at room temperature for 16 h. 25% Aqueous NH₃ (2 mL) was added and the mixture stirred at room temperature for 24 h. The reaction solution was poured into EtOAc (50 mL) then extracted with 2 M HCl (3 × 40 mL). The organic layer was evaporated to dryness then purified by crystallisation using CH₃OH/H₂O (9:1) which yielded pale yellow needles of synthetic **1** (186 mg, 50%).
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