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Chemical investigations of the fermentation broth from 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 paper reports the isolation and full spectroscopic characterisation of compounds 1 and 2. The crystal structure and one-pot synthesis of 3-chloro-4-hydroxyphenylacetamide is also reported.
The isolation and synthesis of 3-chloro-4-hydroxyphenylacetamide produced by a plant-associated microfungus of the genus *Xylaria*

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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 paper 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 is also reported.

Keywords

Microfungus; *Xylaria* sp.; natural products; 3-chloro-4-hydroxyphenylacetamide; 3-chloro-4-hydroxyphenylacetic acid; X-ray crystal structure; synthesis
Several thousand organohalogen compounds have been identified as biosynthetic products from living organisms.\textsuperscript{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.\textsuperscript{2} Less than 45 organohalogens containing the 3-chloro-4-hydroxyphenyl subunit have been isolated from natural sources to date.\textsuperscript{3-8}

We have recently embarked on a research program looking for new chemistry and bioactive metabolites from microfungi isolated from Australian endemic plants. Examination of a local rainforest tree, \textit{Glochidion ferdinandi} (family Euphorbiaceae) afforded several microfungal strains, one of which was identified as \textit{Xylaria} sp. (FRR 5657).\textsuperscript{9} This strain, when cultured on damp rice produced two new xanthones\textsuperscript{9} 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 \textsuperscript{1} and the previously reported fungal metabolite, 3-chloro-4-hydroxyphenylacetic acid \textsuperscript{2}.\textsuperscript{10} The previously isolated xanthones\textsuperscript{9} were not produced in the malt extract broth cultures.

Compound \textsuperscript{1} was assigned the molecular formula C\textsubscript{8}H\textsubscript{8}NO\textsubscript{2}Cl on the basis of HREIMS,\textsuperscript{11} \textsuperscript{1}H and \textsuperscript{13}C NMR spectral data (see Table 1). The (-)-LRESIMS isotopic pattern\textsuperscript{11} of \textsuperscript{1} confirmed the presence of one chlorine atom. The \textsuperscript{1}H NMR spectrum of \textsuperscript{1} contained three broad exchangeable singlets [\(\delta\) 9.88 (1H), 7.36 (1H) and 6.80 (1H)], three aromatic signals [\(\delta\) 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 ring\textsuperscript{12} and one methylene singlet [\(\delta\) 3.24 (2H)]. The \textsuperscript{13}C NMR spectrum of \textsuperscript{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 determination of 1 are shown in Figure 2. The structure of 1 was confirmed by X-ray crystallography analysis. An ORTEP-3 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-O8 = 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, 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, 1H and 13C NMR spectral data (see Table 1). The 13C NMR spectrum of 2 was 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 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 acetonitrile.
synthesised 1 from 2 in a one-pot reaction using peptide coupling chemistry. 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. Compounds 1 and 2 showed no cytotoxicity when tested at 2 and 20 μg/mL.

In conclusion, this paper 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.

Acknowledgements

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References

10. 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. 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, tR 5.0 min) and 3-chloro-4-hydroxyphenylacetic acid 2 (1.3 mg, tR 7.5 min).
11. 3-Chloro-4-hydroxyphenylacetamide 1 was isolated as stable yellow crystals; m.p. 160-162 °C; UV (CH3OH) λmax (log ε) 205 (4.08), 231 sh (3.59), 282 (3.18) nm; UV (CH3OH+NaOH) λmax (log ε) 206 (4.27), 248 (3.98), 299 (3.62) nm; IR νmax (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).
13. Crystal data for 1 were obtained with a Rigaku AFC7R diffractometer, Mo Kα 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, Dcalc = 1.51 g cm⁻³, Z = 2, F(000) = 192, μ = 0.421 mm⁻¹. Data was collected at 295(2) K using ω-2θ scans in the range θ = 2.80 - 27.49°. A total of 2194 reflections were collected, 1871 were unique (Rint = 0.0227). The structure was refined by full-matrix least squares on F². 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. Uiso(H) values were set to 1.2 Ueq 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).
16. 3-Chloro-4-hydroxyphenylacetic acid 2 was isolated as a stable amorphous yellow solid; UV (CH3OH) λmax (log ε) 219 (3.66), 282 (3.31) nm; UV (CH3OH+NaOH) λmax (log ε) 219 (3.68), 249 (3.72), 301 (3.47) nm; IR νmax (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 (\(\text{C}_8\text{H}_7\text{O}_3\text{Cl}\) \(\text{M}^+\) requires 186.00837).


18. EDCI (576 mg, 3.0 mmol) and DMAP (24 mg, 0.2 mmol) were added to 3-chloro-4-hydroxyphenylacetic acid 1 (372 mg, 2.0 mmol) in dry \(\text{CH}_3\text{CN}\) (5 mL) and the mixture was stirred at room temperature for 16 h. 25% aqueous \(\text{NH}_3\) (2 mL) was added and the mixture stirred at room temperature for 24 h. The reaction solution was poured into \(\text{EtOAc}\) (50 mL) then extracted with 2M HCl (3 \(\times\) 40 mL). The organic layer was evaporated to dryness then purified by crystallisation using \(\text{CH}_3\text{OH}/\text{H}_2\text{O}\) (9:1) which yielded pale yellow needles of synthetic 1 (186 mg, 50%).


![Figure 1. Structures for compounds 1 and 2.](image1)

![Figure 2. Key HMBC correlations for compound 1.](image2)
Figure 3. ORTEP plot for compound 1.
Table 1. NMR data for compounds 1 and 2.

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<th>Position</th>
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<th>Compound 2</th>
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<td>8-OH</td>
<td>12.25 (brs, 1H)</td>
<td>12.25 (brs, 1H)</td>
</tr>
</tbody>
</table>

$^a$ 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.