Benzaldehyde Derivatives from *Sarcodontia crocea*

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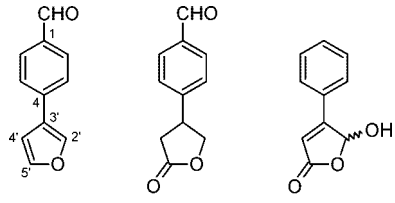
Through bioactivity-guided fractionation, two volatile benzaldehyde derivatives (**1** and **2**) were isolated from a cellulose-matrix culture of the Basidiomycete *Sarcodontia crosea* (syn. *S. setosa*), where the fungus was grown on a growth medium-impregnated thick chromatographic paper. The structures of **1** and **2** were elucidated mainly by 2D NMR techniques as 4-(furan-3-yl)benzaldehyde and 4-(5-oxotetrahydrofuran-3-yl)benzaldehyde, respectively. Compound **1** is a new natural product, while **2** has been synthesized previously. They are considered to contribute to the odor of the fungus, which is an important character for identification of the species. They were weakly antifungal toward several phytopathogenic fungi but less potent against bacteria in microdilution assays.

Sarcodontia Schulzer (Hyphodermataceae, Stereales) is a monotypic genus containing the resupinate white-rot tooth fungus *S. crocea* (Schwein.) Kotl., formerly and better known as *S. setosa* (Pers.) Donk.[1] It grows on old trunks of *Malus* spp. (Rosaceae) parasitically, as tightly attached large, attractive, bright sulfur-yellow hydnoid basidiomes with an intensely sweetish, fruity smell that is said to resemble "anise" or "pineapple".[2] Previous investigations by various workers have identified the species (as *S. setosa*) as an invaluable source of aroma compounds in the biotechnology industry,[3] while it has also been known to produce a series of benzoquinone carboxylic acids named sarcodontic acids.[4,5]

As a part of systematic screening for antimicrobial and/or novel compounds from a collection of wood-inhabiting fungi maintained at Kew,[6,7] a strain of *S. crocea* was found to produce antifungal compounds in large quantity in cultures grown on Sabouraud medium. Subsequent bulk culturing of the strain (KC741) and bioactivity-guided fractionations led to the isolation of two new benzaldehyde derivatives (**1** and **2**). Although the isolation was guided by their activities, it turned out that these compounds may be responsible for the odor of the fungus and that **1** contributes to the color of the fruiting body as well.



1 **2** **3**

Following an initial screening of the collection, a method was sought to achieve more efficient use of the space, in order to maximize the surface area on which fungi would grow in a limited space. It was found that thick chromatographic paper (Whatman 3MM), impregnated with a liquid growth medium, was particularly suited for this purpose, and, being virtually pure cellulose, it offered complete control of nutrient composition for growth. A previous trial with lightweight expanded clay aggregate (LECA) reported as an alternative support to conventional solid-state fermentation for microfungi[8] was not practical with slow-growing species such as *S. crocea*, due to the difficulties in determining the biomass gain during the growth period. The continuous contact of the extracellular content, if any, with the inorganic material in the moist environment and the solvent holdup during the extraction were additional concerns. On the other hand, the paper-matrix culture presented very few such problems and offered ready disposal.

Thus, the long-term culture (75 days) of *S. crocea* resulted in the production of a large quantity of weakly antifungal metabolites. The yields of **1** and **2** (35 and 69 mg/L medium, respectively) far exceeded those recorded for the volatile phenolics produced by this species in the culture fluid, e.g., 2,3-dimethylphenol, in the range 100–1000 μg/L.[3] Interestingly, for compounds with only C, H, and O as the constitutive elements (see below), they were produced only in trace quantity, or none at all, in carbon-rich media such as malt extract, potato-dextrose, and yeast extract–sucrose media for up to a period of three months in the initial screening using agarified media (data not shown). It was noted, however, that the chromatographic paper used as the support had crumbled during the culture period, indicating that this cellulolytic fungus had digested it as a substrate.

The HREIMS of **1** suggested a molecular formula of $C_{11}H_8O_2$. The ^{1}H NMR spectrum revealed the presence of seven protons in the aromatic region consisting of a pair of doublets integrating to four protons, three protons that were coupled to each other, and an aldehyde proton. The pattern of splitting of the four aromatic protons at δ 7.91 and 7.67 ppm (d, J = 8.0 Hz) suggested a *para*-disubstituted benzene ring as the partial structure. This was supported by the presence of nine signals in the ^{13}C NMR spectrum consisting of eight in the aromatic region (δ 109.0–144.7 ppm) and an aldehyde carbon (δ 192.0 ppm). On the basis of the HMBC and DEPT spectra two ^{13}C signals at δ 130.8 and 126.5 were assigned to four carbons of the *para*-substituted benzaldehyde ring, C-2(6) and C-3(5), respectively. The remaining four carbons including two oxygenated aromatic carbons (δ_C 109.0, δ_H 6.78; δ_C 126.0; δ_C 140.3, δ_H 7.88; δ_C 144.7, δ_H 7.55) formed a furan ring, accounting for the eight degrees of unsaturation. The attachment of the furan ring at C-3 rather than at the C-2 position followed from an analysis of the ^{1}H–^{1}H COSY spectrum, where a correlation was found between δ 6.78 (H-4′) and 7.55 (H-5′) but not between δ 6.78 and 7.88 (H-2′), implying the presence of two vicinal protons and one isolated methine group within the furanyl moiety. Therefore the structure of **1** was elucidated as 4-(furan-3-yl)benzaldehyde, a new natural product.

The ^{1}H NMR spectrum of **2**, $C_{11}H_{10}O_3$ as found by HREIMS, revealed the presence of two methylene groups, one methine proton,

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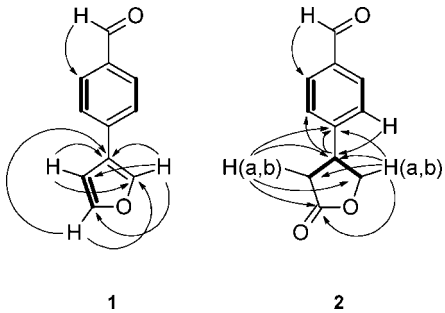
Table 1. Antimicrobial Activities of **1** and **2** against Fungi and Bacteria

	MIC in μg/mL (mM)[a]					
compound	*Botrytis cinerea* IMI160282	*Cladosporium herbarum* IMI300461	*Eurotium amstelodami* FC1021	*Leptosphaeria maculans* IMI266355	*Bacillus subtilis* IMI347329	*Pseudomonas syringae* IMI347448
1	200 (1.17)	200 (1.17)	100 (0.58)	200 (1.17)	800 (4.65)	200 (1.17)
2	200 (1.05)	400 (2.11)	200 (1.05)	400 (2.11)	>800 (4.21)	400 (2.11)
nystatin[b]	50 (0.051)	25 (0.026)	50 (0.051)	100 (0.102)		
chloramphenicol[b]					6.25 (0.019)	3.13 (0.010)

[a] Minimum inhibitory concentrations (MIC) were determined by a microdilution method with four replicates. [b] Positive controls.

four aromatic protons, and one aldehyde proton. The *para*-formylphenyl partial structure was elucidated in the same manner as **1**. Analysis of the ^{1}H–^{1}H COSY spectrum showed the presence of a –CH_2–CH–CH_2– unit. The ^{13}C NMR spectrum of **2** contained an ester carbonyl at δ 176.0, which correlated with all the protons of the –CH_2–CH–CH_2– unit in its HMBC spectrum. A consideration of the seven degrees of unsaturation led to the γ-lactone substructure, which was attached to the 4-position of benzaldehyde. Thus, the methine proton H-3′ at δ 3.88 showed correlations to C-4 and C-3 as well as C-2′, C-4′, and C-5′, while the methylene protons at H-2′ and H-4′ correlated with carbons C-4, C-3′, and C-5′. The correlations observed in the HMBC spectrum were fully in accordance with the proposed structure (Figure 1). Therefore, the structure of **2** was established as 4-(5-oxotetrahydrofuran-3-yl)benzaldehyde. This compound is here for the first time reported from a natural source, although it has been synthesized previously and a comparable ^{1}H NMR data set published.[9] The absolute configuration of the sole chiral center (C-3′) has not been determined.

The antimicrobial activities of **1** and **2** were tested against four fungal and two bacterial strains by a ×2 microdilution method.[6] They showed weak antifungal and very little antibacterial activities (Table 1). Compound **1** exhibited generally more potent activity than **2** against the test strains. These compounds were fungitoxic by autobiography on TLC plates during isolation when 10 μg of chromatographic fractions was applied; however, this observation may be attributable to the higher sensitivity of the method rather than the degradation of the test compounds in the aqueous medium, as opposed to the case of, for example, the isocoumarin (–)-epoxyartemidin.[10] Thus, solutions of **1** and **2** in water in the dark at 25 °C for 3 days gave no noticeable change, and a TLC analysis of the re-extracted ethyl acetate-soluble part gave the original **1** and **2** alone. Compounds **1** and **2** bear a close structural resemblance to 5-hydroxy-4-phenyl-2(5*H*)-furanone (**3**), and their antifungal activities are also comparable.[11] Several fungitoxic benzaldehyde derivatives of fungal origin are known, including 4-chloro-3,5-dimethoxybenzaldehyde[12] and 4-prenyloxybenzaldehyde and its 3-hydroxy derivative.[13] Furthermore, a small number of antifungal volatile metabolites, isolated from fungi, have been reported. These include 6-pentyl-2*H*-1-pyrone,[14,15] 2-phenylethanol,[16] and tiglic and senecioic acids.[17] They all are considered to play a role in the viability and survival fitness of the producing organisms in their respective growing environments. They all possess relatively simple structures and, therefore, serve as ideal lead compounds.



1 2

Figure 1. Selected ^{1}H–^{1}H COSY (bold) and HMBC (arrows) correlations of **1** and **2**.

Compound **1** exhibited a characteristic odor, although not exactly identical to that of fresh fruiting bodies of *S. crocea*, which is said to be "anise/pineapple-like".[2] On the other hand the odor of **2** could be described as faint almond- or cinnamon-like. It is likely that these compounds, in addition to a number of lower alcohols, aldehydes, esters, and phenols as a mixture, are responsible for the specific odor that is an important taxonomic criterion of this species.[1,3] In this respect it is interesting to note that low levels of 4-butenolide and benzaldehyde have been detected previously in the culture fluid of this species, grown on a glucose–asparagine–yeast extract liquid medium for 20 days, although they may not necessarily be the biosynthetic precursors or the degradation products of **1** and **2**.

Experimental Section

General Experimental Procedures. Melting points were recorded using an Electrothermal apparatus and are uncorrected. UV absorbance spectra were obtained with a Shimadzu UV-1601 spectrophotometer. NMR spectra were acquired using a Bruker DPX 400 spectrometer in $CDCl_3$ at 20 °C, with TMS as the internal standard (400.1 MHz for proton and 100.6 MHz for carbon). Chemical shifts are expressed in ppm. Low-resolution mass spectra were recorded using a Fisons MD800 (single quadrupole) and a Micromass Quattro II (triple quadrupole) mass spectrometer, in positive EI mode. HRMS were obtained on a Finnigan MAT 95XP mass spectrometer also in positive EI mode. Analytical TLC was run on silica gel F_{254} precoated plates (Merck 1.05554) with hexane–EtOAc–MeOH (60:40:1), and spots were detected by UV light and bioautography;[18] precoated preparative TLC (0.5 mm thickness, Merck 1.05744) was used with the same solvent system and UV detection. Final purification of **1** and **2** was carried out using a Waters HPLC system consisting of a 600 pump, a 717_{PLUS} autosampler, and a 996 photodiode array detector. A Genesis C_{18} column (Jones Chromatography, 10 mm i.d. × 250 mm, particle size 4 μm) was used at 30 °C, eluting with linear gradient mixtures of MeOH in H_2O at a flow rate of 4 mL/min.

Fungal Strain. The fruiting body of *Sarcodontia crocea* was found growing on an old tree of a *Malus* sp. (Rosaceae) in West Molesey, Surrey, UK, in July 1995 and identified by Dr. Brian Spooner of the Mycology Section, Jodrell Laboratory, Royal Botanic Gardens, Kew. The dried specimen was deposited in the mycological herbarium of the Gardens (accession number K(M)30835). The mycelial culture (strain KC741) raised from uncontaminated tissue of the same specimen was maintained on malt extract-agar medium under oil at 16 °C.

Fermentation, Extraction, and Isolation. The seed culture was prepared on a medium containing 200 mL of Sabouraud dextrose broth (Difco) in a 500 mL conical flask inoculated with homogenized, well-grown malt extract-agar culture and shaken at 140 rpm at 25 °C for 17 days. A 100 mL portion of this preculture was then added to each of two 3 L flasks containing 300 mL of the same Sabouraud dextrose broth and loosely packed, shredded Whatman chromatography paper (3MM, ca. 110 g), previously sterilized at 121 °C for 15 min, and mixed. The admixture was stationarily incubated at 25 °C for 75 days with light–dark periods of 12:12 h under artificial illumination (fluorescent white light, 4 × 40 W).

The whole culture was extracted with $CHCl_3$–MeOH (2:1, v/v), 2.5 L per flask, when the mycelia with a hint of yellow had covered the paper matrixes completely. The combined organic phase was concentrated in vacuo at 40 °C, and the residue (brown oil, 623.8 mg) was chromatographed over silica using mixtures of $CHCl_3$–Me_2CO (99:1,

98:2, 95:5, 90:10, 80:20, and 60:40). Among a total of 32 fractions collected, two clusters of antifungal activities were observed in fractions 8 and 9 (combined yield 43.5 mg), and 17 and 18 (80.6 mg), against the test strain *Cladosporium herbarum* IMI300461. On the analytical TLC run in parallel with the bioautography the major UV-absorbing spots were found corresponding to the activities. The combined fractions 8 and 9 were further purified by reversed-phase semipreparative HPLC, eluting with a linear gradient of 50 to 100% MeOH in H_2O over 20 min. Compound **1** eluted at 14.6 min, yielding 27.7 mg of yellow solid. Fractions 17 and 18 were combined and subjected to preparative TLC, followed by RP semipreparative HPLC with a linear gradient of MeOH in H_2O, 25 to 75% over 20 min. Compound **2** eluted at 14.3 min and was obtained as a colorless solid (55.5 mg).

4-(Furan-3-yl)benzaldehyde (1): yellow solid (MeOH–H_2O); mp 34–36 °C; UV (MeOH) λ_{max} (log ε) 235 (sh) (2.61), 257 (sh) (2.57), 300 (2.93) nm; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 10.02 (1H, s, C*H*O), 7.91 (2H, d, J = 8.0 Hz, H-2, H-6), 7.88 (1H, m, H-2′), 7.67 (2H, d, J = 8.0 Hz, H-3, H-5), 7.55 (1H, m, H-5′), 6.78 (1H, m, H-4′); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 192.0 (C, *C*HO), 144.7 (CH, C-5′), 140.3 (CH, C-2′), 139.0 (C, C-4), 135.4 (C, C-1), 130.8 (CH, C-2, C-6), 126.5 (CH, C-3, C-5), 126.0 (C, C-3′), 109.0 (CH, C-4′); EIMS *m/z* 172 $[M]^+$ (97), 171 (99), 115 (100); HREIMS *m/z* 171.0439 (calcd for $C_{11}H_7O_2$ {R-CHO → $[R\text{-}C{\equiv}O^{\bullet}]^+$}, 171.0441).

4-(5-Oxotetrahydrofuran-3-yl)benzaldehyde (2): colorless flakes (MeOH–H_2O); mp 76–77 °C; UV (MeOH) λ_{max} (log ε) 253 (2.92) nm; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 10.02 (1H, s, C*H*O), 7.90 (2H, d, J = 8.5 Hz, H-2, H-6), 7.42 (2H, d, J = 8.3 Hz, H-3, H-5), 4.71 (1H, dd, J = 7.8, 8.8 Hz, H-2′a), 4.31 (1H, dd, J = 7.5, 9.2 Hz, H-2′b), 3.88 (1H, m, H-3′), 2.99 (1H, dd, J = 8.8, 17.6 Hz, H-4′a), 2.70 (1H, dd, J = 8.5, 17.6 Hz, H-4′b); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 191.8 (C, *C*HO), 176.0 (CO, C-5′), 146.8 (C, C-4), 136.3 (C, C-1), 130.9 (CH, C-2, C-6), 127.8 (CH, C-3, C-5), 73.7 (CH_2, C-2′), 41.5 (CH, C-3′), 35.9 (CH_2, C-4′); EIMS *m/z* 190 $[M]^+$ (27), 132 (87), 131 (100); HREIMS *m/z* 190.0621 (calcd for $C_{11}H_{10}O_3$, 190.0624).

Biological Assays. The fungus *Cladosporium herbarum* IMI300461 was used as the test strain to trace the activity on the TLC bioautography.[18] A microdilution method[6] was used for the determination of minimal inhibitory concentrations of **1** and **2**, in four replicates per concentration, per test strain, in the range 1.56 to 800 μg/mL. The positive controls nystatin and chloramphenicol for fungi and bacteria, respectively, were obtained from Sigma (Poole, Dorset, UK) and tested in the same concentration range as **1** and **2**. The fungal and bacterial species used in the assays are listed in Table 1 together with the strain numbers.

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