Arugosin F: A New Antifungal Metabolite from the Coprophilous Fungus *Ascodesmis sphaerospora*

Sara M. Hein,[†] James B. Gloer,[*,†] Brenda Koster,[‡] and David Malloch[‡]

Department of Chemistry, University of Iowa, Iowa City, Iowa 52242, and Department of Botany, University of Toronto, Toronto, Ontario, Canada M5S 1A1

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Chemical studies of the coprophilous fungus *Ascodesmis sphaerospora* (JS 247) have led to the isolation of arugosin F (**1**), a new antifungal and antibacterial metabolite. The structure was determined based on NMR and MS data and on comparison with data for known members of the arugosin class. A known xanthone (**2**) was also isolated.

Antagonistic effects among naturally competing coprophilous (dung-colonizing) fungi can serve as a useful guide to the selection of species that produce antifungal agents.[1−3] Fungi of the genus *Ascodesmis* are found almost exclusively on dung of herbivores and carnivores.[4] During our ongoing effort directed toward the discovery of novel, bioactive metabolites from coprophilous fungi, a new compound, arugosin F (**1**), and a known xanthone (**2**) were isolated from *Ascodesmis sphaerospora* Obrist (Ascodesmidaceae). Details of these studies are presented here.

The EtOAc extract of the culture broth of *A. sphaerospora* exhibited antifungal activity and was fractionated by Si gel column chromatography using a stepwise gradient of CH_2Cl_2 in 2-propanol to afford compounds **1** and **2**. The less polar component (**1**) was assigned the molecular formula $C_{15}H_{12}O_5$ (10 unsaturations) based on HREIMS [M^+ at *m/z* 272.0693] and ^{13}C NMR data. The ^{13}C NMR spectrum contained 12 aromatic carbon resonances and signals consistent with the presence of a methyl group (δ 21.9), an aryl ketone carbonyl (δ 197.2), and an acetal or hemiacetal carbon (δ 96.0). These data, along with the number of unsaturations, required the presence of three rings, with at least two being aromatic. The ^1H NMR spectrum revealed the presence of two intramolecularly hydrogen-bonded phenolic OH groups and, together with the ^{13}C NMR data, permitted assignment of 1,2,3-trisubstituted and 1,2,3,5-tetrasubstituted benzene rings. Because of the downfield shifts of the phenolic OH protons (δ 11.5 and 13.2), the ketone was presumed to be located ortho to both phenolic OH groups. DEPT data and the molecular formula indicated the presence of a third exchangeable proton. This required the carbon signal resonating at δ 96.6 to correspond to a hemiacetal, rather than an acetal, group. All of these features were verified by analysis of selective INEPT data (Table 1).

Selective INEPT experiments were used to assign connectivities between the structural subunits described above. Selective INEPT irradiation of each of the hydrogen-bonded phenolic OH protons afforded all of the possible two- and three-bond correlations, leading to assignment of the carbon signals for C-1, C-2, C-11a, C-9, C-10, and C-10a. Irradiation of the aryl methyl signal resulted in polarization transfer to two protonated aromatic carbons (C-7 and C-9), as well as the carbon to which the methyl is attached

Table 1. NMR Data for Arugosin F (**1**, 300 MHz; $CDCl_3$)

position	δ_H (mult., *J* in Hz)	δ_C[a]	selective INEPT correlations (C#)
1		165.4	1, 2, 11a
2	6.72 (dd, 8.2, 1.2)	113.1	
3	7.40 (t, 8.4)	137.8	
4	6.56 (dd, 8.1, 1.2)	110.5	2, 4a, 11a
4a		156.5	
6	6.15 (s)	96.6	4a, 7, 10a
6a		139.1	
7	7.03 (m)	116.3	
8		147.6	
9	6.88 (m)	119.7	7, 10a, 10, 12
10		162.7	9, 10, 10a
10a		116.5	
11		197.2	
11a		113.9	
12	2.40 (s)	21.9	7, 8, 9
1-OH	13.2 (s)		
6-OH	3.68 (br s)		
10-OH	11.5 (s)		

[a] Assignments are consistent with multiplicities established based on DEPT data.

(C-8). The hemiacetal proton signal showed selective INEPT correlations to C-7 and C-10a, as well as to oxygenated aromatic carbon C-4a. These results required the C-10 OH, the aryl methyl group, and the hemiacetal unit to have a 1,3,5 relative orientation on the tetrasubstituted benzene unit. The hemiacetal carbon must be linked via an oxygen atom to C-4a, which, in turn, must be located on the 1,2,3-trisubstituted aromatic ring in a position meta to the C-1 OH group. The only remaining unit (the ketone carbonyl) must then serve as a bridge linking C-11a and C-10a, thereby completing the assignment of the structure as shown in **1**. This assignment was consistent with the downfield shifts of the two phenolic OH groups. The signals for C-3 and C-4 were assigned based on their chemical shifts, while C-6a was assigned by default to the only remaining nonprotonated carbon signal. Although a small, negative optical rotation was observed, it was not determined whether arugosin F is a single enantiomer. The stereochemistry of the related compounds arugosins A−E has not been reported.[5−7]

On the basis of ^1H NMR, ^{13}C NMR, selective INEPT, and MS data, the second compound ($C_{15}H_{12}O_4$) was identified as 1-hydroxy-6-methyl-8-hydroxymethylxanthone (**2**), a metabolite previously reported from the fungus *Cyathus intermedius*.[8] Results from selective INEPT experiments on the methyl and methylene signals enabled confirmation of their relative positions on the xanthone nucleus. Com-

* To whom correspondence should be addressed. Tel.: (319) 335-1361. Fax: (319) 335-1270. E-mail: james-gloer@uiowa.edu.
† University of Iowa.
‡ University of Toronto.

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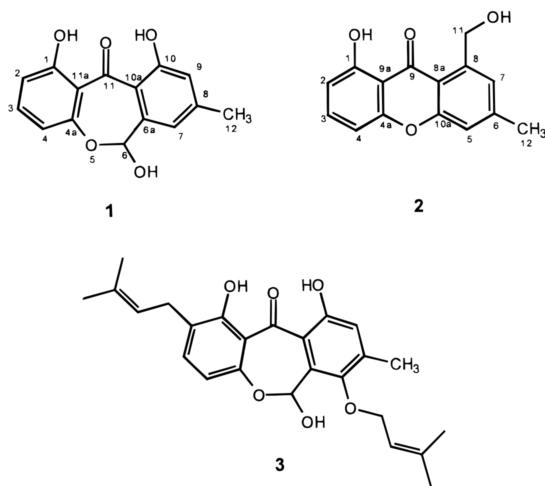
parisons of the data for **2** with literature data[8] verified the structure. ^{13}C NMR data for this compound are presented in the Experimental Section because they have not been previously reported.

Compound **1** exhibited zones of inhibition of 15 and 7 mm in standard disk assays against *Bacillus subtilis* (ATCC 6051) and *Staphylococcus aureus* (ATCC 29213), respectively, at 200 μg/disk. An inhibitory zone of 7 mm was observed for compound **2** against *B. subtilis* when tested at the same level. Antifungal activity was observed in competition assays against the coprophilous fungi *Ascobolus furfuraceus* (NRRL 6460) and *Sordaria fimicola* (NRRL 6459) at 200 μg/disk.[9] Arugosin F (**1**) caused a 50% reduction in radial growth rate of *S. fimicola*, while xanthone **2** caused a 33% reduction. Under similar conditions, compounds **1** and **2** caused 100% and 20% inhibition, respectively, in assays against *A. furfuraceus*.

The name arugosin F was proposed for compound **1** because of its close structural resemblance to arugosins A−E (e.g., **3**), which have been described previously as metabolites of *Aspergillus variecolor,*[5] *A. rugulosus,*[6] and *A. silvaticus.*[7] The compound that most closely resembles arugosin F is arugosin A (**3**). Arugosin F differs from **3** and the other arugosins in the lack of prenyl and oxyprenyl substituents at positions 2 and 7, respectively. This represents the first report of a member of this family of compounds from a genus other than *Aspergillus* and the first report of any secondary metabolite from a member of the coprophilous genus *Ascodesmis*.



It has been proposed that the known arugosins result from the biological oxidation of anthrone precursors.[10] A biosynthetic study of *Aspergillus variecolor* metabolites suggested that arugosins A−C and the tajixanthone family of compounds are biogenetically derived from chryosphanol anthrone (1,8-dihydroxy-3-methyl-9-anthrone).[11] Oxidative cleavage of this anthrone, followed by either condensation or cyclodehydration/reduction of an intermediate ketoaldehyde could lead to the formation of arugosin F (**1**) or xanthone **2**, respectively. If this is the case, then it is not surprising that xanthone **2** was also isolated from *A. sphaerospora*, although evidence for an anthrone precursor was not detected.

Experimental Section

General Experimental Procedures. ^{1}H and ^{13}C NMR spectra were recorded using $CDCl_3$ solutions on a Bruker AC-300 spectrometer operating at 300 and 75 MHz, respectively. Residual solvent signals (7.24/77.0 ppm) were used as internal references. Selective INEPT experiments were optimized for $^{n}J_{CH}$ = 7 Hz. EIMS were obtained using a VG Trio 1 instrument operating at 70 eV, and HREIMS data were recorded using a VG ZAB−HF spectrometer.

Fungal Material. The isolate of *A. sphaerospora* was obtained from bison dung collected by B. Koster at the Metro Toronto Zoo, Toronto, Canada, in November 1992. The culture was assigned the accession number JS 247 in the D. Malloch culture collection at the University of Toronto.

Extraction and Purification. Seven 2-L Erlenmeyer flasks, each containing 400 mL of potato dextrose broth (Difco), were individually inoculated with one 1-cm^2 agar plug taken from a stock culture of *A. sphaerospora* maintained at 4 °C on potato dextrose agar. Flask cultures were incubated at 25−28 °C and aerated by agitation on an orbital shaker at 150 rpm for 37 days. The culture filtrate was extracted with EtOAc, and the organic phase was evaporated to give a dark brown solid (312 mg). The extract was subjected to Si gel chromatography (2.5 × 25 cm) with a hexane−CH_2Cl_2 gradient, followed by a CH_2Cl_2−2-propanol gradient, and was separated into 11 fractions. Fractions 2 and 3 (eluted with 100% CH_2Cl_2) contained compounds **1** (3.6 mg) and **2** (2.0 mg), respectively. Other active fractions containing unrelated compounds are still under investigation.

Arugosin F (1): bright yellow solid: mp 163−164 °C; $[\alpha]_D$ − 2.2° (*c* 0.0021 g/mL, $CHCl_3$); UV λ_{max} (MeOH) (log ϵ) 221 (4.0), 274 (3.8), 352 (3.6) nm; IR λ_{max} ($CHCl_3$) 3633, 2945, 1621, 1583, 1448, and 1016 cm^{-1}; ^{1}H,^{13}C, and selective INEPT NMR data, see Table 1; EIMS ions at *m/z* 272 (40, M$^+$), 255 (100), 243 (24), 227 (47), 163 (49), 136 (55), 108 (37), 77 (52); HREIMS obsd $[M]^+$ at *m/z* 272.0693, calcd for $C_{15}H_{12}O_5$, 272.0685.

1-Hydroxy-6-methyl-8-hydroxymethylxanthone (2): ^{13}C NMR ($CDCl_3$, 75 MHz; multiplicities assigned based on DEPT data) δ 22.0 (q, C-12), 65.2 (t, C-11), 106.6* (d, C-2), 110.6* (d, C-4), 112.6 (s, C-9a), 116.5 (s, C-8a), 118.0 (d, C-5), 127.2 (d, C-7), 136.9 (d, C-3), 142.6 (s, C-8), 147.5 (s, C-6), 155.7 (s, C-4a), 158.1 (s, C-10a), 161.9 (s, C-1), 184.3 (s, C-9); other characteristics of **2**, including mp, IR, UV, MS, and ^{1}H NMR data, have been reported previously.[8] (*These assignments may be interchanged.)

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