Cercophorins A−C: Novel Antifungal and Cytotoxic Metabolites from the Coprophilous Fungus *Cercophora areolata*

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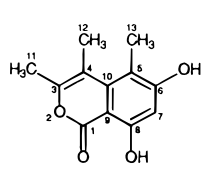
Cercophorins A−C (**3**−**5**), three novel isocoumarin derivatives with antifungal and cytotoxic activities, have been isolated from the coprophilous fungus *Cercophora areolata* (JS 166 = UAMH 7495), a colonist of porcupine dung. Two additional new isocoumarins, decarboxycitrinone (**1**) and 4-acetyl-8-hydroxy-6-methoxy-5-methylisocoumarin (**2**), and a known trichothecene mycotoxin, roridin E (**6**), were also obtained from this species. Compounds **1**−**6** were isolated from ethyl acetate extracts of mycelia and liquid cultures of *C. areolata* through bioassay-guided fractionation and are the first metabolites to be reported from a member of the genus *Cercophora*. Their structures were assigned on the basis of MS, selective INEPT, and 2D-NMR experiments.

The mechanism of fungal antagonism (interference competition) often involves the production of a chemical agent by one species that inhibits the growth of another. The chemistry associated with these interactions remains largely unexplored. Reports of interspecies competition among coprophilous (dung-colonizing) fungi[1,2] have led us to investigate such species as potential sources of natural antifungal agents.[3−5] An isolate of the coprophilous fungus *Cercophora areolata* (JS 166 = UAMH 7495) was shown to display antibacterial and antifungal activity in our assays. Studies of the chemistry of *C. areolata* have resulted in the isolation of two new isocoumarins, decarboxycitrinone (**1**) and 4-acetyl-8-hydroxy-6-methoxy-5-methylisocoumarin (**2**), three novel antifungal 8-hydroxyisocoumarin derivatives, cercophorins A−C (**3**−**5**), and a known trichothecene mycotoxin, roridin E (**6**).[6,7] Roridin E was found to be responsible for most of the anti-*Candida* activity and some of the activity displayed by the extract against competitor fungi. However, a significant portion of the antifungal and antibacterial activity of the extract was due to compounds **1**−**5**. We now report details of the isolation, structure elucidation, and biological activities of these isocoumarin metabolites.



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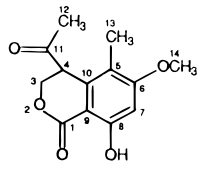

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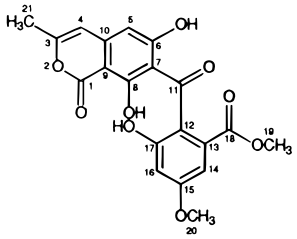

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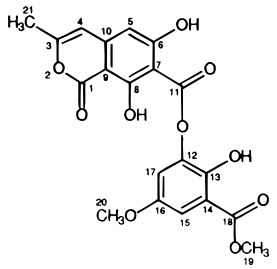

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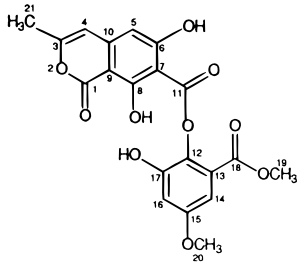

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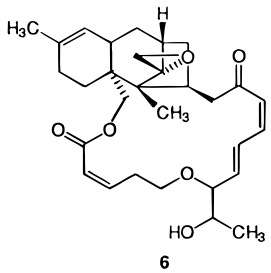

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Results and Discussion

Compounds **1**−**6** were obtained from ethyl acetate extracts of mycelia and liquid cultures of *C. areolata* by silica gel chromatography and reversed-phase HPLC. Analysis of decarboxycitrinone (**1**) by HRFABMS and ^{13}C NMR indicated the molecular formula $C_{12}H_{12}O_4$ (7 unsaturations). The ^1H NMR (Table 1), ^{13}C NMR (Table 2), and DEPT spectra revealed the presence of three aromatic methyl groups, an ester group, an aromatic proton, and two phenolic protons, one of which was intramolecularly hydrogen-bonded. The presence of two phenolic OH groups was confirmed by formation of a mixture of mono- and diacetates upon treatment of **1** with acetic anhydride.

Selective INEPT experiments were initially conducted in an attempt to solve the structure of **1**. However,

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Table 1. ^{1}H NMR Data for Decarboxycitrinone (**1**), 4-Acetyl-8-hydroxy-6-methoxy-5-methylisocoumarin (**2**), and Cercophorins A−C (**3**−**5**)

position	**1**[a]	**2**[b]	**3**[b]	**4**[a]	**5**[a]
3		4.52 (dd, 3.8, 11.5) 4.89 (dd, 1.1, 11.5)			
4		3.79 (br d, 3.8)	6.15 (s)	6.26 (s)	6.40 (s)
5			6.34 (s)	6.17 (s)	6.17 (s)
6	9.64 (br s, OH)		12.52 (s, OH)	11.73 (br s, OH)	
7	6.48 (s)	6.46 (s)			
8	11.92 (s, OH)	11.25 (s, OH)	12.17 (s, OH)	11.60 (s, OH)	11.70 (br s, OH)
10					
11	2.24 (s)				
12	2.31 (s)	2.12 (s)			
13	2.38 (s)	2.01 (s)		10.38 (s, OH)	
14		3.87 (s)	7.00 (d, 2.3)		6.98 (d, 3.0)
15				7.30 (d, 2.9)	
16			6.60 (d, 2.3)		6.82 (d, 3.0)
17			7.42 (br s, OH)	7.11 (d, 2.9)	
19			3.57 (s)	3.98 (s)	3.71 (s)
20			3.85 (s)	3.81 (s)	3.84 (s)
21			2.23 (s)	2.21 (s)	2.21 (s)

[a] Spectra recorded at 300 MHz in acetone-d_6. [b] Spectra recorded at 300 MHz in $CDCl_3$.

Table 2. ^{13}C NMR Data for Decarboxycitrinone (**1**), 4-Acetyl-8-hydroxy-6-methoxy-5-methylisocoumarin (**2**), and Cercophorins A−C (**3**−**5**)

position	**1**[a]	**2**[b]	**3**[b]	**4**[a]	**5**[a]
1	167.4	169.2	166.6	166.6[c]	166.5[d]
3	150.3	68.6	157.0	156.7	157.0
4	111.0	48.8	104.4	104.9	105.0
5	112.0	116.3	103.4	101.7	101.3
6	164.9	164.6	168.5	162.3	163.4
7	101.9	98.8	110.5	112.1	111.1
8	163.1	163.5	165.1	161.1	161.5
9	100.8	100.5	98.1	101.6	101.5
10	140.2	135.6	143.3	141.3	141.7
11	17.6	204.5	199.1	165.1[c]	165.7[d]
12	17.9	28.7	120.8	143.0	135.0
13	13.8	11.0	132.6	148.8	126.4
14		55.9	108.7	114.8	107.7
15			162.1	111.1	158.7
16			105.6	152.8	108.1
17			156.7	117.1	152.4
18			166.4	170.4	165.9
19			52.3	53.3	52.7
20			55.7	56.3	56.1
21			19.6	19.3	19.3

[a] Spectra recorded in acetone-d_6. [b] Spectra recorded in $CDCl_3$. [c,d] Identical superscripts represent interchangeable assignments.

irradiation of the methyl proton signals afforded ambiguous results due to their proximity, which led to collateral irradiation of neighboring signals. The structure elucidation and NMR assignments were therefore based primarily on results of HMQC and HMBC experiments. HMBC correlations of the signal at 2.24 ppm (H_3-11) with C-4 and with the oxygenated vinylic carbon C-3, of the H_3-12 signal (2.31 ppm) with C-3, C-4, and C-10, and of the H_3-13 resonance at 2.38 ppm with C-5, C-6, and C-10 permitted unambiguous location of the three methyl groups. Incorporation of the remaining atoms to form isocoumarin **1** was based on HMBC correlations of H-7 with C-5, C-6, C-8, and C-9 and correlations of the hydrogen-bonded 8-OH with C-7, C-8, and C-9.

HRFABMS and ^{13}C NMR data for 4-acetyl-8-hydroxy-6-methoxy-5-methylisocoumarin (**2**) indicated the molecular formula $C_{13}H_{14}O_5$ (seven unsaturations). ^{1}H NMR (Table 1) and ^{13}C NMR (Table 2) data revealed the presence of an isolated $CHCH_2O$ unit, two methyl groups, one methoxy group, one intramolecularly hydrogen-bonded phenolic OH proton, an aromatic proton, a ketone, and an ester group. Selective INEPT correlations of H-7 with C-5, C-6, C-8, and C-9, of OH-8 with C-7, C-8, and C-9, of H_3-13 with C-5, C-6, and C-10, and of H_3-14 with C-6 permitted construction of the pentasubstituted benzene ring. Correlations observed between protons of the $CHCH_2O$ unit (H_2-3 and H-4) and C-10, and an additional correlation of H-4 with C-9, required connection of this unit to the benzene ring at C-10. An acetyl group was located at C-4 on the basis of correlations of H_2-3, H-4, and H_3-12 with the ketone carbon C-11. The only remaining carbon (C-1) must link C-9 and O-2 to account for the ester group, thereby completing the assignment of the structure of **2** as shown. The stereochemistry at C-4 was not assigned. Despite their simplicity, neither decarboxycitrinone (**1**) nor 4-acetyl-8-hydroxy-6-methoxy-5-methylisocoumarin (**2**) have been previously reported. Moreover, knowledge of their structures was helpful in determining the structures of cercophorins A−C (**3**−**5**).

Cercophorin A (**3**) has the molecular formula $C_{20}H_{16}O_9$ (13 unsaturations), as determined by ^{13}C NMR (Table 2) and HRFABMS data. ^{1}H NMR (Table 1) and DEPT spectra contained signals for four olefinic or aromatic protons (two of which are meta-coupled), one vinyl methyl group, two methoxy groups, and three phenolic protons (two of which are intramolecularly hydrogen-bonded). The presence of a ketone and two ester functionalities was evident from the ^{13}C NMR spectrum. Long-range correlations obtained from selective INEPT and HMBC experiments (Table 3) permitted construction of partial structures **a** (an isocoumarin subunit) and **b** (a 1,2,3,5-tetrasubstituted benzene ring) (Figure 1).

Partial structure **a** was assigned on the basis of correlations analogous to those observed for OH-8 and for the methyl group at C-3 in decarboxycitrinone (**1**) and an additional HMBC and/or selective INEPT correlations of the hydrogen-bonded 6-OH group with C-5, C-6, and C-7, of H-4 with C-3, C-5, C-9, C-10, and C-21, and of H-5 with C-4, C-6, C-7 and C-9. Partial structure **b** was identified through analysis of HMBC and/or selective INEPT correlations observed between H-16 and C-12, C-14, C-15, and C-17 and between H-14 and C-12, C-13, C-15, and C-16. Correlation of H-14 with a fifth carbon (carboxyl carbonyl C-18), together with a correlation between H_3-19 and C-18, required attachment of a carbomethoxy group at C-13. There were no long-range correlations observed between the two par-

Table 3. HMBC Correlations and Selective INEPT Data for Cercophorins A (**3**), B (**4**), and C (**5**)

^{1}H position	**3**[b]	**4**[a]	**5**[a]
4	C-3, 5, 9, 10[c], 21	C-3, 5, 9, 10, 21	C-3, 5, 9, 10, 21
5	C-4, 6, 7, 9	C-4, 6, 7, 9	C-4, 6, 7, 9
6 (OH)	C-5, 6, 7		
8 (OH)	C-7, 8, 9	C-7,[d] 8,[d] 9[d]	
13		(OH) C-12,[d] 13,[d] 14[d]	
14	C-12, 13,[c] 15, 16, 18		C-12, 13,[c] 15, 16, 18
15		C-13, 14, 16, 17, 18	
16	C-12, 14, 15, 17		C-12, 14, 15, 17
17		C-12, 13, 15, 16	
19	C-18	C-18	C-18
20	C-15	C-16	C-15
21	C-3, 4	C-3, 4	C-3, 4

[a] Spectra recorded in acetone-d_6. [b] Spectra recorded in $CDCl_3$. [c] These correlations were observed only in selective INEPT experiments. [d] Selective INEPT data obtained before separation of **3** and **4** by HPLC.

a **b**

Figure 1. Partial structures **a** and **b**.

tial structures, and only the ketone carbon remained to be located. Thus, subunits **a** and **b** must be connected via the ketone group, leading to assignment of the structure of cercophorin A as **3**. The FAB and EI mass spectral fragmentation patterns for **3** support this structure assignment. Major ions at *m/z* 209 (*m/z* 209.0458 by HRFABMS, $C_{10}H_9O_5$, Δ −0.8 mmu) and 219 (*m/z* 219.0295 by HRFABMS, $C_{11}H_7O_5$, Δ −0.2 mmu) observed in both spectra result from the two possible α-cleavage fragmentations at the ketone carbon C-11 (Figure 2).

Cercophorins B (**4**) and C (**5**) coeluted from a Sephadex LH-20 column. Separation of **4** and **5** was accomplished by reversed-phase semipreparative HPLC, using a solvent system of 0.01 M NH_4OAc in MeOH. However, sharp hydrogen-bonded OH signals that were observed in the ^{1}H NMR spectrum of the mixture of **4** and **5** appeared broadened in the spectra of the pure compounds, even when the spectra were obtained in $CDCl_3$ or DMSO-d_6. Thus, long-range correlations to these OH signals in the spectra of the purified metabolites were not observed. Selective INEPT experiments conducted on the mixture prior to HPLC separation did afford some important correlations from the 8-OH and 13-OH proton signals of **4** (Table 3). However, the 6-OH signal for **4** and **5** and the 8-OH signal for **5** were broad, even in the mixture, and did not show any selective INEPT correlations.

A molecular formula of $C_{20}H_{16}O_{10}$ (13 unsaturations) for cercophorin B (**4**) was established by HRFABMS, HREIMS, and ^{13}C NMR (Table 2) data, differing from that of cercophorin A (**3**) by the addition of one oxygen atom. The presence of the same isocoumarin subunit shown in partial structure **a** (Figure 1) and found in compound **3** was confirmed by HMBC and/or selective INEPT correlations analogous to those observed for **3** (Table 3). NMR signals for the 1,2,3,5-tetrasubstituted benzene ring in **4** were assigned on the basis of HMBC

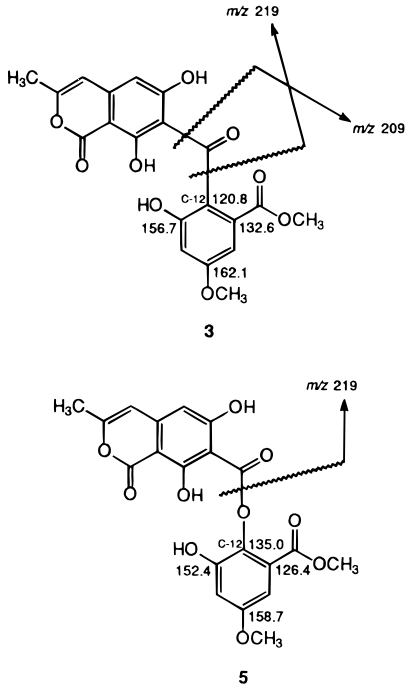

Figure 2. Selected MS fragmentations and ^{13}C NMR chemical shift differences for cercophorins A (**3**) and C (**5**).

and/or selective INEPT correlations of H-15 with C-13, C-14, C-16, C-17, and the carboxyl carbonyl C-18, of H-17 with C-12, C-13, C-15, and C-16, and of the intramolecularly hydrogen-bonded 13-OH group with C-12, C-13, and C-14. The 1,2,3,5-tetrasubstituted benzene ring and the isocoumarin subunit account for all but two oxygen atoms and a carboxyl carbon to which no long-range correlations were observed. Chemical shift considerations indicate that C-7 is not oxygenated, while C-12 must be bound to oxygen. Therefore, the two partial structures must be connected by a carboxyl group, leading to the structure of cercophorin B as shown in **4**.

On the basis of HRFABMS and ^{13}C NMR data (Table 2), cercophorin C (**5**) was determined to be a structural isomer of cercophorin B (**4**). The ^{1}H NMR (Table 1) and DEPT spectra of **5** were nearly identical to those of cercophorin A (**3**). However, the ketone carbonyl signal in the ^{13}C NMR spectrum of **3** was again replaced by a signal resonating near 165 ppm, suggesting an additional ester or acid functionality in **5**. The observation of a major ion at *m/z* 219 in the FABMS of **5** (Figure 2), as well as HMBC and/or selective INEPT correlations analogous to those observed for the isocoumarin moiety in **3** and **4** (Table 3), suggested that this subunit was also present in cercophorin C (**5**).

In addition to other HMBC and selective INEPT correlations listed in Table 3, the meta-coupled protons H-14 and H-16 of the 1,2,3,5-tetrasubstituted benzene ring showed correlations with C-15 and also with C-12, one of two remaining oxygenated carbons. In contrast to cercophorin B (**4**), the non-hydrogen-bonded 17-OH in **5** did not show any long-range correlations. In order for **5** to be different from **4**, C-17 must bear the OH group, and C-12 must, therefore, be the acylated carbon. Thus, cercophorins B (**4**) and C (**5**) differ only in the site of acylation of the tetrasubstituted aromatic ring. On the basis of these data, the structure of cercophorin C was assigned as shown in **5**. Two small-scale attempts

to convert cercophorin A (**3**) to cercophorin C (**5**) via a Baeyer−Villiger reaction yielded complex, inseparable mixtures of aromatic products.

Differences in the ^{13}C NMR chemical shifts of the 1,2,3,5-tetrasubstituted benzene ring in cercophorin C (**5**) (a downfield shift of C-12 and upfield shifts of C-13, C-15, and C-17; see Figure 2) relative to cercophorin A (**3**) due to oxygenation at C-12 are consistent with the assigned structure for **5**. These shifts were also compared with aromatic ring carbon shifts calculated for a model compound (2-acetoxy-3-hydroxy-5-methoxybenzoic acid methyl ester) and were within 3.5 ppm of the calculated values.[8]

A known tricothecene mycotoxin, roridin E (**6**), was also isolated from *C. areolata* and was identified on the basis of evaluation of ^1H NMR, ^{13}C NMR, HMQC, HMBC, and MS data and on comparison of the 1-D NMR data with literature values.[9]

Cercophorins A−C (**3**−**5**) inhibit the growth of the early successional coprophilous fungi *Sordaria fimicola* (NRRL 6459) and *Ascobolus furfuraceus* (NRRL 6460) at 200 μg/disk in centerpoint inoculation disk assays.[5] Decarboxycitrinone (**1**) caused a 100% reduction in radial growth against both fungi, while 4-acetyl-8-hydroxy-6-methoxy-5-methylisocoumarin (**2**) and cercophorins A (**3**), B (**4**), and C (**5**) caused 46, 34, 10, and 51% growth reduction against *S. fimicola*, respectively. Roridin E (**6**) caused a 100% reduction in growth of *S. fimicola* and *A. furfuraceus* and an inhibition zone of 29 mm against *Candida albicans* (ATCC 14053) at the same concentration. In standard disk assays, cercophorin A (**3**) was the most potent of the new compounds against *Bacillus subtilis* (ATCC 6051) and *Staphylococcus aureus* (ATCC 29213), affording zones of inhibition of 26 and 16 mm, respectively, at 200 μg/disk. Cercophorins A, B, and C showed only limited cytotoxicity in the NCI tumor cell line bioassay panel, displaying average GI_{50} values of 9.4, 26.2, and 20.8 μg/mL, respectively.

Compounds **1** and **2**, like most natural isocoumarins, are proposed to be biosynthetically derived from acetate via the polyketide pathway.[10] Cercophorins A−C (**3**−**5**) are more unusual from a biogenetic standpoint. Formation of these metabolites could be accounted for by condensation of an isocoumarin moiety (or a precursor) with a tetraketide comprised of a modified orsellinic acid unit. If this is the case, their structural relationship implies that the ester **5** arises from the ketone **3** via an oxidation process. Literature precedents for other systems[11] suggest that this process could involve epoxidation of an enol form of **3** at C11-C12, with subsequent ring-opening at the epoxide C−C bond. It seems more likely, however, that both **3** and **5** arise from a single nonaketide anthraquinone-type precursor. Oxidative cleavage of such a precursor to give **3** could occur in a fashion analogous to that shown to be involved in the biosynthesis of the benzophenone sulochrin from the anthraquinone questin.[12] Compound **5** would arise if this step were preceeded by a similar oxidation of the opposite ketone carbonyl of the anthraquinone precursor, followed by recondensation of the resulting acid and phenolic groups, as in the biosynthesis of depsidones.[13] However, neither of these proposed biosynthetic routes would readily rationalize the formation of compound **4**, unless this metabolite arises from **5** through a (presumably intramolecular) transesterification process.

Decarboxycitrinone (**1**) is closely related to decarboxydihydrocitrinone, previously isolated from *Penicillium citrinum*,[14] but contains a double bond in the lactone ring. 4-Acetyl-8-hydroxy-6-methoxy-5-methylisocoumarin (**2**) is an O-methylated derivative of 4-acetyl-6,8-dihydroxy-5-methylisocoumarin, an antifungal metabolite previously reported from *Aspergillus viridinutans*.[15] By contrast, no close analogs to cercophorins A (**3**), B (**4**), and C (**5**) have been reported in the literature. To our knowledge, compounds **1**−**6** are the first natural products to be reported from a member of the fungal genus *Cercophora*.

Experimental Section

General Procedures. ^1H NMR and ^{13}C NMR data were obtained on a Bruker AC-300 instrument. NMR spectra were recorded in acetone-d_6 or $CDCl_3$, and chemical shifts were referenced relative to the corresponding acetone (2.04 ppm/29.8 ppm) or chloroform (7.24 ppm/77.0 ppm) signals. Carbon multiplicities were established by DEPT experiments and are consistent with the assignments. HMQC and HMBC experiments were conducted on a Bruker AMX-600 spectrometer and were optimized for $^1J_{CH} = 152$ Hz and $^nJ_{CH} = 8$ Hz, respectively. All selective INEPT experiments were optimized for $J = 7$ Hz. FAB and EIMS data were recorded on a VG ZAB-HF mass spectrometer. Semipreparative HPLC purifications employed Beckman Ultrasphere C_{18} columns (5-μm particles, 250 × 10 cm) at a flow rate of 2.0 mL/min with UV detection at 215 nm. Preparative HPLC separations were accomplished using a Rainin Dynamax-60A C_{18} column (8-μm particles, 21.4 mm × 25 cm) at a flow rate of 10 mL/min with UV detection at 215 nm. Details of the antifungal bioassays have been described elsewhere.[4,5]

Cultivation of *C. areolata*. The isolate of *C. areolata* employed in this study (JS 166) was obtained from a sample of porcupine dung collected by D. Malloch near Bird Lake, Muskoka District, Ontario, in May 1990. A subculture has been deposited at the University of Alberta Mycological Herbarium Collection and was assigned the accession number UAMH 7495. Twelve 2-L Erlenmeyer flasks, each containing 400 mL of potato dextrose broth (Difco), were each inoculated with two 1-cm^2 agar plugs taken from stock cultures of *C. areolata*. Flask cultures were incubated at 25−28 °C and aerated by agitation on an orbital shaker at 160 rpm for 30 days.

Isolation of Decarboxycitrinone (1), 4-Acetyl-8-hydroxy-6-methoxy-5-methylisocoumarin (2), Cercophorins A−C (3−5), and Roridin E (6). The filtered broth (4800 mL) was extracted with EtOAc (10 × 250 mL), and the organic phase was dried ($MgSO_4$) and concentrated to yield 3.3 g of a dark red oil. The extract was subjected to silica gel column chromatography with a step gradient from 0 to 40% (v/v) MeOH in $CHCl_3$, and 8-mL fractions were collected. Similar fractions (as determined by TLC) were pooled and bioassayed. Active fractions were further purified by Sephadex LH-20 column chromatography eluting with hexane−toluene−MeOH (3:1:1), followed by reversed-phase semipreparative HPLC using a gradient from 20 to 70% MeOH in 0.01 M NH_4OAc over 20 min and then

70−100% MeOH in 0.01 M NH_4OAc over 2 min. HPLC fractions were concentrated *in vacuo* to afford 4-acetyl-8-hydroxy-6-methoxy-5-methylisocoumarin (**2**; 6.5 mg), cercophorin B (**4**; 4.5 mg), and cercophorin C (**5**; 21.2 mg).

The dried mycelial material was extracted with EtOAc (4 × 250 mL), followed by filtration and concentration of the solvent to yield 2.0 g of a dark red solid. This extract was processed by silica gel and Sephadex LH-20 column chromatography as described above and then by preparative HPLC using a gradient from 70 to 100% MeOH in H_2O over 45 min to afford decarboxycitrinone (**1**; 36.5 mg), cercophorin A (**3**; 15.6 mg), and roridin E (**6**; 9.6 mg).

Decarboxycitrinone (1): white solid; mp 208−210 °C; HPLC t_R 31.6 min (88:12 MeOH−H_2O); UV (MeOH) 250 (ϵ 4800), 338 (ϵ 2800); IR ν max 3586, 2950, 2830, 1671, 1241 cm^{-1}; FABMS (3-NBA matrix) *m/z* 221 ([M + H]$^+$, 100), 220 (71), 205 (7); EIMS *m/z* 220 (M$^+$, 100), 205 (46), 191 (12), 177 (50); ^1H NMR and ^{13}C NMR data, see Tables 1 and 2; HRFABMS found 221.0799 (M + H)$^+$, calculated for ($C_{12}H_{12}O_4$ + H)$^+$, 221.0813.

4-Acetyl-8-hydroxy-6-methoxy-5-methylisocoumarin (2): white solid; mp 110−112 °C; $[\alpha]_D$ −36° (*c* 0.006 g/mL, MeOH); HPLC t_R 15.4 min (66:34 MeOH−H_2O); UV (MeOH) 232 (ϵ 4100), 265 (ϵ 4200), 313 (ϵ 2800); IR ν max 2928, 2855, 1718, 1670, 1617, 1158 cm^{-1}; FABMS (DTT/DTE matrix) *m/z* 251 ([M + H]$^+$, 11), 207 (3), 167 (9), 119 (83), 103 (62), 85 (100); EIMS *m/z* 250 (M$^+$, 33), 208 (38), 190 (100), 179 (31), 162 (22); ^1H NMR and ^{13}C NMR data, see Tables 1 and 2; HREIMS found 250.0824 (M)$^+$, calcd for $C_{13}H_{14}O_5$, 250.0841.

Cercophorin A (3): white solid; mp 147−150 °C dec; HPLC t_R 26.5 min (84:16 MeOH−H_2O); UV (MeOH) 253 (ϵ 8400), 318 (ϵ 6900), 348 (ϵ 4900); IR ν max 2950, 2928, 1718, 1684, 1630, 1354, 1241 cm^{-1}; FABMS (thioglycerol) *m/z* 401 ([M + H]$^+$, 20), 369 (15), 341 (30), 219 (100), 209 (86); EIMS *m/z* 400 (M$^+$, 2), 351 (18), 341 (100), 219 (36), 209 (64); ^1H NMR and ^{13}C NMR data, see Tables 1 and 2; HMBC and selective INEPT data, see Table 3; HRFABMS found 401.0867 (M + H)$^+$, calculated for ($C_{20}H_{16}O_9$ + H)$^+$, 401.0873.

Cercophorin B (4): yellow solid; mp 99−101 °C; HPLC t_R 26.7 min (100% MeOH); UV (MeOH) 252 (ϵ 9400), 333 (ϵ 6500); IR ν max 2987, 2855, 1720 (sh), 1688, 1619 cm^{-1}; FABMS (glycerol) *m/z* 439 ([M + Na]$^+$, 75), 417 ([M + H]$^+$, 28), 399 (60), 299 (74), 219 (68), 172 (100); EIMS *m/z* 416 (M$^+$, 2), 372 (29), 340 (29), 267 (13), 236 (10); ^1H NMR and ^{13}C NMR data, see Tables 1 and 2; HMBC and selective INEPT data, see Table 3; HRFABMS found 439.0660 [(M + Na]$^+$), calcd for ($C_{20}H_{16}O_{10}$ + Na)$^+$, 439.0641; HREIMS found 416.0728 (M)$^+$, calcd for $C_{20}H_{16}O_{10}$, 416.0743.

Cercophorin C (5): yellow solid; mp 102−105 °C; HPLC t_R 24.9 min (100% MeOH); UV (MeOH) 247 (ϵ 9100), 327 (ϵ 4200); IR ν max 2950, 1726, 1689, 1620, 1354, 1197 cm^{-1}; FABMS (glycerol) *m/z* 439 ([M + Na]$^+$; 12), 417 ([M + H]$^+$; 3), 399 (21), 379 (12), 355 (36), 219 (39), 116 (100); EIMS *m/z* 372 ([M − CO_2]$^+$; 100), 340 (91), 312 (24), 297 (14), 269 (14); ^1H NMR and ^{13}C NMR data, see Tables 1 and 2; HMBC and selective INEPT data, see Table 3; HRFABMS found 439.0661 (M + Na)$^+$, calcd for ($C_{20}H_{16}O_{10}$ + Na)$^+$, 439.0641.

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