Apiosporamide, a New Antifungal Agent from the Coprophilous Fungus Apiospora montagnei

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J. Nat. Prod., **1994**, 57 (12), 1696-1702• DOI:
10.1021/np50114a012 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 57, No. 12, pp. 1696-1702, December 1994

APIOSPORAMIDE, A NEW ANTIFUNGAL AGENT FROM THE COPROPHILOUS FUNGUS *APIOSPORA MONTAGNEI*

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ABSTRACT.—Bioassay-guided fractionation of an EtOAc extract from the mycelium of the coprophilous fungus *Apiospora montagnei* has furnished a new antifungal metabolite, named apiosporamide [**1**], and the known dihydroisocoumarin *cis*-(3*R*,4*R*)-4-hydroxymellein [**4**]. The structure of **1** was assigned on the basis of HMBC, HMQC, NOESY, and hrms data. Elucidation of the structure was complicated by the fact that several nmr signals appeared only under certain conditions.

Antagonistic interactions among coprophilous fungi (1,2) have led us to investigate such species as unique sources of antifungal agents (2–6). Chemical studies of coprophilous fungal isolates have afforded a variety of new antifungal metabolites, some of which possess novel or rare ring systems (5,6). Investigation of the chemistry of the coprophilous fungus *Apiospora montagnei* Saccardo (JS 140 =UAMH 7489) (Amphisphaeriaceae) has resulted in the discovery of a new antifungal metabolite of mixed biogenetic origin, which we have called apiosporamide [**1**]. The isolation of this compound was guided by bioassays for antifungal activity against early-successional coprophilous fungi. This report provides details of the isolation and structure elucidation of **1**.

RESULTS AND DISCUSSION

An isolate of *A. montagnei* was grown in liquid shake culture at room temperature for one month. The mycelium was separated from the culture broth, extracted with EtOAc, and concentrated to afford an oily residue which exhibited antifungal activity. The extract was subjected to chromatography on Si gel, and active fractions were processed by reversed-phase hplc using a cyanopropyl bonded-phase column to obtain the active principle, apiosporamide [**1**].

Compound **1** is soluble in MeOH and only slightly soluble in $CHCl_3$ or Me_2CO, so initial nmr experiments were conducted in CD_3OD. Hrfabms showed an $(M+H)^+$ ion at *m/z* 430.2217, suggesting several possible molecular formulas for **1**. However, none of these formulas were consistent with the results obtained from ^1H-, ^{13}C-, and DEPT nmr data (see Table 1). The ^{13}C-nmr spectrum of compound **1** in CD_3OD contained only 18 resonances, even when experiments were conducted with increased relaxation delays. These signals corresponded to three oxygenated methine carbons, five aliphatic methines, five methylenes, two methyl groups, two protonated sp^2-carbons, and one oxygenated quaternary carbon.

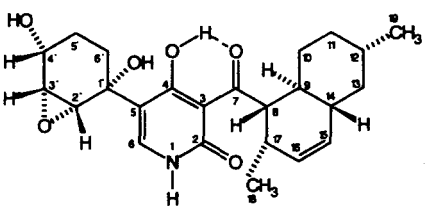

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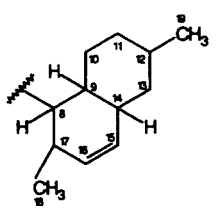
TABLE 1. Nmr Data for Apiosporamide [**1**].

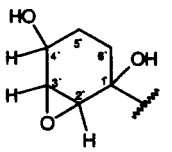
Position	δ_H[a]	δ_H[b]	Multiplicity, J_{HH}[a]	δ_C[c]	δ_C[d]	HMBC[e]	HMBC[f]
1-NH	—	10.46	—	—	—	—	—
2	—	—	—	—	162.2	—	—
3	—	—	—	—	108.4	—	—
4 (OH)	—	11.57	—	—	179.9	—	3, 4, 5, 7
5	—	—	—	—	115.9	—	—
6	7.47	7.66	—	—	140.0	—	2, 4
7	—	—	—	—	211.4	—	—
8	4.33	4.45	dd, 5.7, 11	54.2	53.1	9, 14, 17, 18	3, 7, 9, 10, 14, 18
9	1.48	1.68	dddd, 3, 11, 11, 11	37.7	37.1	8, 10, 13	8, 13, 14
10ax	0.80	0.90	dddd, 3, 11, 11, 11	31.0	30.6	11, 14	—
10eq	1.84	1.93	br dd, 3, 11	—	—	11, 12, 14	11, 12, 14
11ax	0.95	0.99	dddd, 3, 11, 11, 11	36.6	36.2	9, 10, 12, 19	10, 12
11eq	1.67	1.76	m	—	—	9, 13	9, 10
12	1.43	1.56	m	34.4	33.8	14, 19	—
13ax	0.70	0.74	ddd, 12, 12, 12	43.2	42.6	9, 11, 12, 14, 15,	14, 19
13eq	1.67	1.76	m	—	—	19	11, 15
						9, 14	
14	1.73	1.87	m	43.2	42.6	10, 13, 16	9, 10, 13, 15, 16
15	5.31	5.39	m	131.7	131.3	9, 13, 17	9, 13, 17
16	5.51	5.59	m	132.7	132.5	8, 14, 17	8, 14, 17
17	2.74	2.84	m	32.5	31.8	8, 9, 15, 16, 18	9, 16, 18
18	0.74	0.81	d, 7.2	18.4	18.3	8, 16, 17	8, 16, 17
19	0.84	0.90	d, 6.5	22.9	22.8	11, 12, 13	11, 12, 13
1′ (OH)	—	4.35	—	70.5	70.2	—	1′, 2′, 5
2′	3.55	3.54	d, 3.2	60.5	60.0	1′, 6′	1′, 6′
3′	3.32	3.34	dd, 3.2, 3	57.6	57.3	4′, 5′	4′, 5′
4′	4.04	4.08	ddd, 2.9, 5.5, 5.5	67.1	66.4	5′, 6′	—
4′ (OH)	—	3.83	—	—	—	—	—
5′	1.26	1.33	m	25.8	26.0	4′, 6′	1′, 4′, 6′
	1.73	1.87	m	—	—	3′, 4′, 6′	1′, 4′
6′ax	1.61	1.71	ddd, 3, 11, 11	31.6	31.5	1′, 4′, 5′	1′, 4′, 5′, 5
6′eq	2.11	2.17	m	—	—	—	1′, 2′, 4′, 5′, 5

[a]600 MHz, in CD_3OD.
[b]300 MHz, in Me_2CO-d_6.
[c]75 MHz, in CD_3OD.
[d]75 MHz, in Me_2CO-d_6.
[e]600 MHz, in CD_3OD.
[f]600 MHz, in Me_2CO-d_6.

Analysis of COSY, HMQC (7), and HMBC (8) nmr experiments conducted at 600 MHz allowed straightforward elucidation of the partial structures **a** and **b**. The chemical shifts and J values for the mutually coupled oxymethine protons at 3.32 and 3.55 ppm (J=3.2 Hz, C-3′ and C-2′) were characteristic of a 1,2-disubstituted epoxide (5). Treatment of compound **1** with Ac_2O in pyridine yielded a diacetate. The H-4′ proton signal at 4.04 ppm was shifted to 5.14 ppm in the diacetate, indicating that C-4′ bears a free OH group in the parent compound. The lack of any other significant changes in the spectrum suggested that the second acetoxy group is attached to an sp^2-carbon, but



a



b

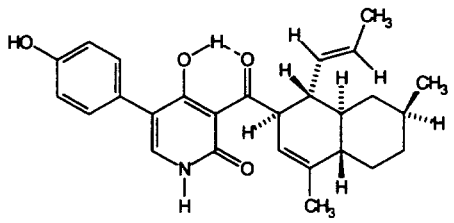
the ^{13}C-nmr spectrum of the natural product (in CD_3OD) did not contain a signal for an oxygenated sp^2-carbon. Moreover, the HMBC experiment showed several correlations for which no corresponding resonances appeared in the ^{13}C-nmr spectrum. These observations, together with the inconsistencies between the hrms and ^{13}C-nmr data, led us to conduct ^{1}H- and ^{13}C-nmr experiments under different conditions.

Although **1** was considerably less soluble in $CDCl_3$ and Me_2CO-d_6, ^{1}H- and ^{13}C-nmr spectra were recorded in both solvents, with Me_2CO-d_6 giving the best results. The ^{13}C-nmr spectrum of compound **1** in Me_2CO-d_6 (Table 1) contained 24 resonances; six more than were observed in CD_3OD. These results and the hrfabms data suggested the molecular formula $C_{24}H_{31}NO_6$ (10 unsaturations) for **1**. Analysis of the ^{13}C-nmr and DEPT data obtained in Me_2CO-d_6 showed that the six carbons that had not been detected previously were all sp^2-carbons, one of which was protonated.

Comparison of the ^{13}C-nmr multiplicities and the molecular formula indicated that there must be four exchangeable protons. This conclusion was supported by the ^{1}H-nmr spectrum of **1** recorded in Me_2CO-d_6, which contained two additional sharp singlets at 4.35 and 11.57 ppm, and two broad singlets resonating at 3.83 and 10.46 ppm, all of which are exchangeable. The formation of a diacetate suggested the presence of at least two OH groups. One of the remaining two downfield exchangeable proton signals most likely corresponds to an amide proton, as suggested by ir absorption bands at 1655 and 1645 cm^{-1}, and a ^{13}C-nmr signal at 162.2 ppm.

HMQC and HMBC nmr experiments conducted in Me_2CO-d_6 (Table 1) provided additional long-range C-H correlations not observed in the earlier experiments. The OH proton resonating at 4.35 ppm showed HMBC correlations with signals for C-1′, C-2′, and an sp^2 carbon at 115.9 ppm (C-5), locating the OH at C-1′ and revealing the linkage of C-1′ with both C-2′ and C-5. The HMBC correlation of H-8 (4.45 ppm) with the ketone carbonyl signal at 211.4 ppm (C-7) revealed the connectivity between C-8 and C-7. The hydrogen-bonded OH signal at 11.57 ppm showed HMBC correlations with C-5, C-7, the non-protonated olefinic carbon signal at 108.4 ppm (C-3), and the oxygenated sp^2-carbon resonance at 179.9 ppm (C-4). These correlations suggested the presence of a β-hydroxy-α,β-unsaturated ketone. Correlation of the vinylic proton (H-6; 7.66 ppm) with C-2 and C-4, together with chemical shift considerations and the remaining unsaturation, suggested the linkage of C-6 with C-5 and the amide nitrogen to form a 2-pyridone ring. The amide proton signal did not afford any C-H correlations in the HMBC spectrum or in selective INEPT (9) nmr experiments. In addition, coupling between the N-H and H-6 was not detected by either decoupling or COSY experiments. Thus, these nmr experiments did not provide confirmation of the regiochemistry of the pyridone ring. Ultimately, the regiochemistry of the pyridone moiety of **1** was confirmed by spectral comparison (see Figure 1) with a known compound [**2**, ilicicolin H], which was originally reported as a product of the fungus *Cylindrocladium ilicicola*, with structure and relative stereochemistry verified by total synthesis (10). Compound **2** was also obtained through our investigations of the coprophilous fungus *Gliocladium* sp. (11). Fischerin [**3**], a cytotoxic metabolite recently reported from the ascomycete *Neosartorya fischeri* (Wehmer) Malloch & Cain (12), is a close biogenetic relative of apiosporamide [**1**]. However, its spectral data were not helpful in determining the regiochemistry of **1** due to the presence of an *N*-hydroxy group that significantly affected the ^{13}C-nmr chemical shifts of the pyridone unit.

The relative stereochemistry of apiosporamide [**1**] was assigned on the basis of NOESY data and ^{1}H-^{1}H coupling constants. A NOESY correlation between H-8 and H-14 indicated that these protons are related in a cis-1,3-diaxial fashion. The magnitude of the coupling constant between H-8 and H-9 (11 Hz), and the lack of a NOESY



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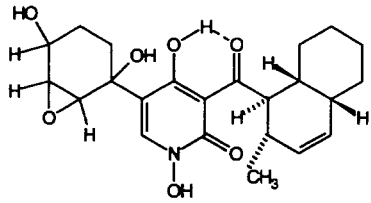

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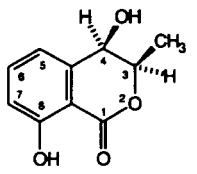

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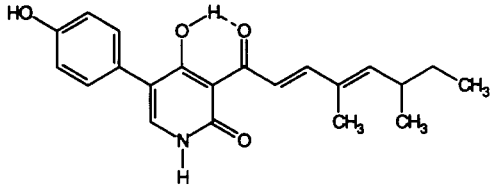

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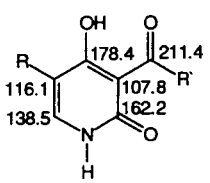
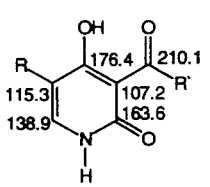
correlation between these protons indicated that they must be trans-diaxial. Similarly, correlations among H-9, H_3-18, and H_{ax}-11 (0.95 ppm) indicated that all of these substituents are axially oriented on the same face of the decalin ring system. A NOESY correlation between H_{ax}-11 and H_3-19 suggested placement of this Me group at an equatorial position and, therefore, cis to H-9. Additional support for this placement was provided by a significant NOESY correlation between H-12 and the axially oriented H-14. Based on these data, and on differences in nmr J values (e.g., $J_{H9\text{-}H14}$ = 11 Hz in **1**, ≤3.9 Hz in **3**) and chemical shifts (e.g., C-14 at 41.9 ppm in **1**, 37.0 ppm in **3**), the relative stereochemistry of the decalin ring juncture of **1** is clearly different from that of **3** as shown.

The relative stereochemistry for the epoxycyclohexanol moiety could not be unambiguously established, although a tentative assignment was made based on the data available. The presence of an 11 Hz coupling between one of the methylene protons at C-5′ and one of those at C-6′ suggested that the ring adopts a pseudo-chair conformation. A strong NOESY correlation between H-2′ and the vinylic proton H-6 indicated that these protons are spatially close. Dreiding molecular models suggest that this correlation is most likely if H-2′ and the pyridone moiety are on the same face of the C-1′–C-6′ ring. A NOESY interaction between H-2′ and H-3′ was consistent with their expected cis relationship, and the absence of a trans-diaxial J value for H-4′ requires that it adopt a pseudoequatorial orientation. The relative stereochemistry for the epoxycyclohexanol portion of fischerin [**3**] was not specified, so direct comparison with



Apiosporamide



Ilicicolin H

FIGURE 1. Comparison of the ^{13}C-nmr chemical shifts of the pyridone units of apiosporamide [**1**] and ilicicolin H [**2**] (in $CDCl_3$).

the identical unit in **1** was not possible. The relative stereochemistry of apiosporamide [**1**] as a whole was not assigned due to a lack of NOESY correlations between the protons of the decalin unit and those of the epoxycyclohexanol moiety.

The absence of six resonances in the ^{13}C-nmr spectrum of apiosporamide [**1**] in CD_3OD was presumed to be due either to metal chelation or to a dynamic phenomenon (e.g., tautomerization) occurring on the nmr timescale. Although the missing resonances were observed in $CDCl_3$ or Me_2CO-d_6 solution, the signals were again absent from the spectrum of the same sample when measured once more in CD_3OD. The missing resonances were also observed when the spectrum was recorded in CD_3OD at 240° K (−33°). These results suggested that a dynamic phenomenon is responsible for the ^{13}C-nmr behavior of **1** in CD_3OD. These nmr observations could be explained by the acceleration in protic solvents of an equilibrium process that is much slower in aprotic solvents. Equilibration among tautomeric forms occurring on the nmr timescale would cause broadening of nmr signals. Support for this conclusion was provided by recording ^{13}C-nmr spectra for **1** in CD_3OD at 10-degree intervals from 298° to 258° K, and at 240° K. As the temperature was decreased, the ^{13}C-nmr signals for the pyridone moiety began to appear at 278° K as broad peaks, in contrast to the sharp signals for the same carbons observed at 240° K. The signals sharpened significantly with decreasing temperature, which is consistent with a decrease in the rate of interconversion among tautomers with decreasing temperature. Interestingly, the ^{13}C-nmr spectra of ilicicolin H [**2**] and fischerin [**3**] in CD_3OD showed all of the expected resonances, including the signals for the pyridone unit. The difference in nmr behavior of **1** and **2** is presumably due to the difference between the substituents at C-5. The extended conjugation present in **2** could have a significant impact on tautomer stability, and would likely affect the rate of interconversion of the tautomers. On the other hand, the difference in the ^{13}C-nmr behavior of **1** and **3** (CD_3OD) is probably due to the *N*-hydroxy group in **3** which prohibits the formation of an hydroxypyridine tautomer.

The known compound *cis*-(3*R*,4*R*)-4,8-dihydroxy-3,4-dihydroisocoumarin (**4**; 4-hydroxymellein) was also isolated as a minor constituent of the mycelium of *A. montagnei* (JS 140). Compound **4** was identified through spectral comparisons with literature values (13). A previously known trans isomer of this compound has also been reported from the related species *Apiospora camptospora* (FM 123) (14).

Apiosporamide [**1**] displayed activity against the early successional coprophilous fungus *Ascobolus furfuraceus* (NRRL 6460), causing 58% reduction in radial growth rate relative to controls at 200 μg/disk. In standard disk assays, apiosporamide [**1**] also exhibited zones of inhibition against *Bacillus subtilis* (ATCC 6051) and *Staphylococcus aureus* (ATCC 29213) of 32 and 21 mm, respectively, at 200 μg/disk, but showed no activity against a strain of *Candida albicans* (ATCC 14053) at the same concentration.

To our knowledge, this is the first report of a novel secondary metabolite from a member of the genus *Apiospora*. Apiosporamide [**1**] appears to possess biogenetic similarities with the fungal metabolite tenellin [**5**], a metabolite of the insect pathogenic fungus *Beauveria tenella* (15). Biosynthetic studies showed that compound **5** was derived from polyketide and phenylalanine precursors (16). Accordingly, apiosporamide [**1**] seems likely to be derived from similar precursors with oxidation of the aromatic ring to generate the cyclohexanol epoxide unit in **1**. The decalin portion of the molecule, along with C-2, C-3, and C-7, would likely be derived from seven acetate units, with the C-19 methyl group derived from methionine. The relative stereochemistry of the decalin system and the location of the double bond are consistent with the involvement of a biological Diels-Alder reaction in the biosynthetic pathway. Such a reaction has been proposed as an important step in the formation of several polyketide fungal metabolites

of note (17–20), including the commercial hypocholesterolemic agent mevinolin (17). Interestingly, by analogy to the proposed pathways for solanapyrones A and B (18,19), the relative stereochemistry of compound **1** suggests an *endo* cyclization of an all-trans triene intermediate, while that of **3** would require *exo* cyclization of a similar intermediate. A Diels-Alder step could also be involved in formation of compound **2**, but would require different double-bond locations within the intermediate, with the trisubstituted double bond adopting a *Z*-geometry.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps were determined using a Fisher-Johns micro-melting point apparatus, and are uncorrected. Uv data were recorded in MeOH with a Gilford Response uv-vis spectrometer. Ir data were recorded with a Mattson Cygnus 25 instrument. Hplc separations were conducted using a Beckman Ultrasphere 5 μm cyanopropyl or C_{18} column (10 mm×25 cm) at a flow rate of 2 ml/min with uv monitoring at 215 nm. Cc employed EM Science grade 62 Si gel (60–200 mesh) or Si gel GF_{254} (Fluka). Tlc was performed using glass plates precoated with Si gel F-254 (0.1 mm thickness, E. Merck). Hrfabms data were recorded on a VG ZAB-HF mass spectrometer (thioglycerol matrix), while eims data were obtained at 70 eV using a VG Trio 1 quadrupole mass spectrometer. ^{1}H-Nmr, ^{13}C-nmr, and selective INEPT nmr spectra (optimized for J=4 or 7 Hz) were recorded on a Bruker AC-300 spectrometer. HMBC (optimized for J=8 Hz), HMQC (optimized for J=152 Hz), COSY, and NOESY nmr data were obtained using a Bruker AMX-600 spectrometer. Details of the antifungal bioassays have been described elsewhere (2).

FERMENTATION OF THE FUNGAL CULTURE.—The isolate of *A. montagnei* employed in this work was originally obtained from mouse dung collected in the Reserve National de Faunes de la Pointe de l'est Magdalen Ils, Quebec, Canada on 13 June, 1990 by D. Malloch. A culture of this fungus has been deposited in the University of Alberta Mycological Herbarium (UAMH) and designated as UAMH 7489. Twenty 2-liter Erlenmeyer flasks, each containing 400 ml of Difco potato dextrose broth, were inoculated with several 1-cm^2 agar plugs obtained from stock cultures of *A. montagnei*. The cultures were incubated at room temperature (25–28°) while being aerated on orbital shakers at 150 rpm.

ISOLATION AND CHARACTERIZATION OF APIOSPORAMIDE [**1**].—Filtration of the culture medium afforded the mycelium, which was dried at room temperature and extracted with EtOAc (4×500 ml). The combined mycelial extracts were evaporated under reduced pressure to afford a brown oil with antifungal activity (3.9 g). Part of the extract (1.6 g) was subjected to Si gel cc (100 g) eluting with increasing concentrations of MeOH in $CHCl_3$ to afford 65 fractions. Similar fractions (as shown by tlc) were combined. Fractions 51–62 (233 mg, eluted with 20% MeOH/$CHCl_3$) were further purified by reversed-phase hplc (MeOH-H_2O, 57:43) to give apiosporamide (**1**, 14.2 mg) as a white solid.

Apiosporamide [**1**].—Mp 240–250° (dec); hplc R_t 15.3 min under the above conditions; $[\alpha]_D$ −97.4° (c=0.0004 g/ml, MeOH); uv λ max (MeOH) 210 (ε 15700), 231 (11400), 275 (4200), 330 (7100) nm; ir ν max ($CHCl_3$) 3400, 1655, 1645, 1590 cm^{-1}; eims *m/z* 411 [$(M-H_2O)^+$, 13], 375 (3), 301 (13), 266 (27), 248 (100), 230 (54), 212 (54), 202 (38), 188 (16), 163 (36), 164 (20), 134 (30), 106 (44), 91 (60), 55 (67); ^{1}H and ^{13}C nmr (Me_2CO-d_6, CD_3OD), see Table 1; ^{13}C nmr ($CDCl_3$) δ 211.4 (s, C-7), 178.4 (s, C-4), 162.2 (s, C-2), 138.5 (d, C-6), 131.2 (d, C-16), 131.0 (d, C-15), 116.1 (s, C-5), 107.8 (s, C-3), 70.8 (s, C-1′), 63.6 (d, C-4′), 58.0 (d, C-2′), 55.4 (d, C-3′), 52.9 (d, C-8), 41.9 (d, C-14), 41.7 (t, C-13), 36.2 (d, C-9), 35.3 (t, C-11), 33.1 (d, C-12), 31.2 (d, C-17), 29.9 (t, C-6′), 28.6 (t, C-10), 26.0 (t, C-5′), 22.5 (q, C-19), 18.0 (q, C-18); hrfabms, observed 430.2217 $(M+H)^+$; calcd for $C_{24}H_{31}NO_6$+H, 430.2230 (Δ=1.3 mmu).

ACETYLATION OF APIOSPORAMIDE [**1**].—A sample of **1** (1.0 mg) was dissolved in Ac_2O-pyridine (1:1). The reaction vessel was capped and allowed to stand overnight at room temperature. The solvent was evaporated to afford the diacetate as an oil, which exhibited the following ^{1}H-nmr data (300 MHz; CD_3OD) δ 7.50 (1H, s, H-6), 5.49 (1H, m, H-16), 5.31 (1H, m, H-15), 5.14 (1H, m, H-4′), 4.32 (1H, dd, J=5.7 and 11.3 Hz, H-8), 3.95 (1H, d, J=3.8 Hz, H-2′), 3.47 (1H, m, H-3′), 2.74 (1H, m, H-17), 1.98 (3H, s, CH_3COO), 1.94 (3H, s, CH_3COO), 1.10–1.90 (13H, overlapping m, H_2-5′, H_2-6′, H-9, H_2-10, H_2-11, H-12, H_2-13, H-14), 0.82 (3H, d, J=6.5 Hz, H_3-19), 0.73 (3H, d, J=7.2 Hz, H_3-18).

ISOLATION AND IDENTIFICATION OF **4**.—Fractions 10–12 (128 mg), eluting from the Si gel column with 2% MeOH/$CHCl_3$, were re-chromatographed on a Si gel column packed with $CHCl_3$. The column was eluted with a $CHCl_3$/Me_2CO gradient. Fractions 6–11 (23 mg) were combined and further purified by reversed-phase hplc (60% MeOH/H_2O; C_{18}) to obtain compound **4** (3.3 mg; hplc R_t 8.6 min). This compound was identified by comparison of its properties with literature values (mp, ms, ^{1}H nmr, $[\alpha]_D$) (13).

ACKNOWLEDGMENTS

We thank the National Institutes of Health (AI 27436 and CA 01571) for financial support. Additional support for J.B.G. in the form of an Alfred P. Sloan Foundation Fellowship is also gratefully acknowledged. We also thank Prof. J.C. Vederas, University of Alberta, for a helpful suggestion.

LITERATURE CITED

1. D.T. Wicklow and B.J. Hirschfield, *Can. J. Microbiol.*, **25**, 855 (1979).
2. J.B. Gloer and S.M. Truckenbrod, *Appl. Environ. Microbiol.*, **54**, 861 (1988).
3. H.A. Weber, D.C. Swenson, J.B. Gloer, and D. Malloch, *Tetrahedron Lett.*, **33**, 1157 (1992).
4. Y. Wang and J.B. Gloer, *J. Nat. Prod.*, **56**, 341 (1993).
5. H.A. Weber, N.C. Baenziger, and J.B. Gloer, *J. Org. Chem.*, **56**, 4567 (1988).
6. H.A. Weber and J.B. Gloer, *J. Org. Chem.*, **53**, 4355 (1991).
7. A. Bax and S. Subramanian, *J. Magn. Reson.*, **67**, 565 (1986).
8. M.F. Summers, L.G. Marzilli, and A. Bax, *J. Am. Chem. Soc.*, **108**, 4285 (1986).
9. A. Bax, *J. Magn. Reson.*, **57**, 314 (1984).
10. M. Matsumoto and H. Minato, *Tetrahedron Lett.*, 3827 (1976).
11. A.A. Alfatafta, "New Antifungal and Antiinsectan Natural Products from Coprophilous and Scleroium-producing Fungi," Ph.D. Thesis, University of Iowa, Iowa City, IA, 1994, pp. 53-54.
12. H. Fujimoto, M. Ikeda, K. Yamamoto, and M. Yamazaki, *J. Nat. Prod.*, **56**, 1268 (1993).
13. M. Devus, J. Bousquet, A. Kollmann, and M. Barbier, *Phytochemistry*, **19**, 2221 (1980).
14. L. Camarda, L. Merlini, and G. Nasini, *Phytochemistry*, **15**, 537 (1976).
15. C.K. Wat, A.G. McInnes, D.G. Smith, J.L. Wright, and L.C. Vining, *Can. J. Chem.*, **55**, 4090 (1977).
16. E. Leete, N. Kowanko, and R.A. Newmark, *Tetrahedron Lett.*, 4103 (1975).
17. R.N. Moore, G. Bigam, J.K. Chan, A.M. Hogg, T.T. Nakashima, and J.C. Vederas, *J. Am. Chem. Soc.*, **107**, 3694 (1985).
18. H. Oikawa, T. Yokota, T. Abe, A. Ichihara, S. Sakamura, Y. Yoshizawa, and J. Vederas, *J. Chem. Soc., Chem. Commun.*, **17**, 1282 (1989).
19. H. Oikawa, T. Yokota, A. Ichihara, and S. Sakamura, *J. Chem. Soc., Chem. Commun.*, **17**, 1284 (1989).
20. D.E. Cane, W. Tan, and W.R. Ott, *J. Am. Chem. Soc.*, **115**, 527 (1993).

Received 22 July 1994