Sclerotigenin: A New Antiinsectan Benzodiazepine from the Sclerotia of *Penicillium sclerotigenum*

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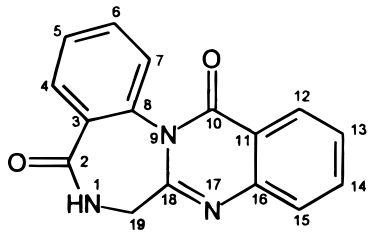
A new benzodiazepine, sclerotigenin (**1**), was isolated from organic extracts of the sclerotia of *Penicillium sclerotigenum* (NRRL 3461) along with two known griseofulvin analogues. The structure of **1** was determined primarily by analysis of ^{1}H NMR, ^{13}C NMR, HMQC, and HMBC data. Compound **1** was the major component of the CH_2Cl_2 extract of *P. sclerotigenum* sclerotia and is responsible for most of the antiinsectan activity of the extract against the crop pest *Helicoverpa zea*.

Many fungi produce reproductive structures called sclerotia to facilitate survival and propagation of the species. We have previously reported a variety of antiinsectan natural products from the sclerotia of *Aspergillus* spp. which may play a role in the longevity of these bodies. Hence, we have undertaken studies of the sclerotia of other fungi as potential sources of antiinsectan metabolites.[1−4] During the course of this work, chemical investigation of the sclerotia of *Penicillium sclerotigenum* Yamamoto (Trichocomaceae) (NRRL 3461) led to the isolation of a new antiinsectan benzodiazepine, which we named sclerotigenin (**1**), and the known compounds griseofulvin and dechlorogriseofulvin.[5,6] Details of these studies are described here.



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Sclerotia of *P. sclerotigenum* were produced by solid-substrate fermentation on corn kernels. The CH_2Cl_2 extract of the sclerotia displayed activity against the corn earworm *Helicoverpa zea*. Fractionation of the extract by Sephadex LH-20 column chromatography, followed by reversed-phase HPLC, afforded sclerotigenin (**1**) as the major antiinsectan component.

Sclerotigenin has the molecular formula $C_{16}H_{11}N_3O_2$ (13 degrees of unsaturation), as deduced from ^{13}C NMR (Table 1), DEPT, and HRFABMS [$(M + H)^+$ at 278.0934, Δ 0.5 mmu] data. The NMR data indicated the presence of one methylene unit, one exchangeable proton, eight protonated sp^2 carbons, and seven nonprotonated sp^2 carbons. Two 1,2-disubstituted benzenoid units and an $NHCH_2$ spin system were identified from the ^{1}H NMR and COSY data, and the NH signal (a broad triplet at δ 7.19) was suggestive of a secondary amide group.

Table 1. NMR Data for Sclerotigenin (**1**) in $CDCl_3$

position	δ_H (multiplicity, J_{HH})[a]	δ_C[b]	HMBC correlations (C-#)
1	7.19 (br t, 6)		3, 19
2		168.2	
3		129.8	
4	7.95 (br d, 7.8)	130.3	2, 6, 8
5	7.53 (m)	129.1	3, 7
6	7.62 (ov. m)	131.4	4, 8
7	7.61 (ov. m)	128.1	3, 5, 8
8		133.7	
10		161.3	
11		121.5	
12	8.29 (dd, 8.0, 1.4)	127.6	10, 14, 16
13	7.52 (m)	127.8	11, 15
14	7.78 (ddd, 8.1, 8.1, 1.4)	135.1	12, 16
15	7.66 (br d, 8.1)	127.3	11, 13
16		146.2	
18		153.6	
19	4.29 (dd, 15, 5.7)	47.0	2, 18
	4.21 (dd, 15, 6.9)		2, 18

[a] Recorded at 600 MHz. [b] Recorded at 75 MHz.

Analysis of HMBC and HMQC data established most of the connectivity of the molecule and led to the assignment of the carbon chemical shifts for **1**. The aromatic proton signal at δ 7.95 (H-4) and the methylene signals for H_2-19 (δ 4.29 and δ 4.21) each correlated to a carbonyl signal at δ 168.2 (C-2), and the triplet at δ 7.19 (NH-1) correlated to another aromatic carbon on the same ring (C-3; δ 129.8). On the basis of these data and chemical shift considerations, C-2 was assigned to an amide carbonyl carbon linking the C-3−C-8 aromatic ring and the $NHCH_2$ group.

According to HMBC results, two downfield-shifted signals represented nonprotonated sp^2 carbons that were not members of either benzenoid ring. An aromatic proton belonging to the second disubstituted benzenoid unit (δ 8.29; H-12) revealed an HMBC correlation to one of these signals (δ 161.3; C-10), suggesting the attachment of C-10 to this aromatic ring at C-11. The methylene protons H_2-19 correlated with the second nonbenzenoid sp^2 carbon signal at δ 153.6 (C-18). At this point, two nitrogen atoms, an oxygen atom, and four degrees of unsaturation remained to be assigned. These requirements, in addition to the downfield chemical shifts of carbons C-8, C-16, and C-18, led to the assignment of a fused quinazolino benzodiazepinedione structure for sclerotigenin, as shown in **1**.

The carbon chemical shift assignments for **1** are consistent with spectral data for the fused quinazolino benzodi-

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azepinedione portions of the closely related fungal metabolites asperlicin, a potent nonpeptidal cholecystokinin antagonist isolated from *Aspergillus alliaceus*,[7−10] and benzomalvin A, a substance-P inhibitor isolated from *Penicillium* sp.[11] Compound **1** is most closely related to asperlicin C, which contains a tryptophan unit rather than the glycine moiety present in **1**.[12] Asperlicin appears to be biosynthesized from two equivalents of anthranilate and one each of tryptophan and leucine.[12,13] Sclerotigenin consists of the fused quinazolino benzodiazepinedione portion of the asperlicin structure, suggesting that it is biosynthesized from two anthranilate moieties and a glycine unit.

Although sclerotigenin contains no chiral centers, its ^{1}H NMR spectrum revealed that the C-19 methylene protons are inequivalent (ABX pattern with the amide proton; δ 3.99 and 4.18 in DMSO-d_6 solution). These observations are consistent with NMR behavior reported for some other 1,4-benzodiazepines, including benzomalvins A and D[11,14] and diazepam analogues,[15] but sclerotigenin appears to be the first example containing a secondary amide group. These data and consideration of literature reports suggest that **1** exists as a mixture of enantiomeric conformers for which there is a significant barrier to interconversion, thereby effectively rendering the C-19 protons diastereotopic. In an effort to overcome this barrier, a variable-temperature ^{1}H NMR experiment was conducted in DMSO-d_6 solution. As the probe temperature was increased, the methylene multiplets gradually converged and eventually coalesced to a broad doublet centered at δ 4.13 when the spectrum was measured at 411°K. As expected, the multiplets were again observed when the spectrum was recorded upon return to 298 K. The closest reported analogy to this experiment describes results for three 1,4-benzodiazepines that also lack chiral centers.[15] Although this precedent employed 2-oxo-1,4-benzodiazepines, rather than 5-oxo-1,4-benzodiazepines, the 3-position was similarly unsubstituted. The methylene protons of *N*-desmethyldiazepam (12.3 kcal/mol inversion barrier) resonated as a singlet at room temperature, while those of *N*-methyldiazepam (17.6 kcal/mol barrier) showed an AB pattern that coalesced at elevated temperature. The N-*tert*-butyl diazepam analogue (>24 kcal/mol barrier) also displayed an AB pattern, but these signals did not collapse at temperatures up to 473 K. Although the amide nitrogen in **1** is unsubstituted, the observed behavior of **1** most closely approximates results reported for *N*-methyldiazepam, suggesting that the barrier to interconversion in 5-oxo-1,4-benzodiazepines is somewhat higher. Calculation[16] of the corresponding value for **1** using the variable temperature NMR data described above indicated an interconversion barrier of approximately 20 kcal/mol, which is fully consistent with these conclusions.

Sclerotigenin (**1**) was the major component of the *P. sclerotigenum* extract, and caused a 42% reduction in growth rate relative to controls in assays against first instar larvae of *H. zea* when incorporated into a standard test diet at 200 ppm. Griseofulvin and dechlorogriseofulvin were also isolated from the *P. sclerotigenum* sclerotial extract. These compounds have been previously isolated from a variety of fungi, including *Penicillium* and *Aspergillus* spp.,[4−6] and were identified by comparison of MS and NMR data with literature values.[5,6] In fact, to our knowledge, the only prior report of any chemistry from *P. sclerotigenum* is part of a systematic survey of *Penicillium* chemistry which notes that it produces roquefortine C and griseofulvin.[17] Griseofulvin has been reported to exhibit activity against *H. zea* with potency similar to that of **1**,[18] but since griseofulvin and dechlorogriseofulvin are relatively minor components of the crude extract of *P. sclerotigenum*, the majority of the activity was accounted for by the levels of sclerotigenin present.

Experimental Section

General Experimental Procedures. The culture of *P. sclerotigenum* Yamamoto (NRRL 3461) was obtained from the Agricultural Research Service culture collection at the National Center for Agricultural Utilization Research in Peoria, Illinois. *P. sclerotigenum* was received by the ARS culture collection in 1969 from the Commonwealth Mycological Institute as IMI 68616. The culture was isolated from a rotting tuber of a Chinese yam (*Dioscorea batatas* f. *tukune* Makino) from Sasayama, Hyogo Prefecture, Japan. Sclerotia were produced by solid-substrate fermentation of *P. sclerotigenum* on autoclaved corn kernels and harvested using procedures previously described for the production of sclerotia from *Aspergillus* spp.[19] Protocols used for the insect bioassay have been reported elsewhere.[20]

^{13}C NMR and DEPT spectra were obtained using a Bruker AC-300 instrument. ^{1}H NMR, COSY, HMQC, and HMBC experiments were performed on a Bruker AMX-600 spectrometer. HMQC and HMBC data were optimized for $^1J_{CH}$ = 152 Hz and $^nJ_{CH}$ = 8 Hz, respectively. All NMR spectra were recorded using $CDCl_3$ solutions, and chemical shift values were referenced to the corresponding solvent signals (δ_H 7.24/δ_C 77.0). FABMS data were recorded on a VG ZAB-HF mass spectrometer. HPLC separations were accomplished using a Rainin Dynamax-100 Å C_{18} column (5-μm particle size, 1.0 × 25 cm) at a flow rate of 3 mL/min with UV detection at 215 nm.

Isolation of Sclerotigenin (1), Griseofulvin, and Dechlorogriseofulvin. The ground sclerotia (14.5 g) were extracted sequentially with hexane, CH_2Cl_2, EtOAc, and MeOH using 3 × 500 mL of each solvent. The antiinsectan CH_2Cl_2 extract (650 mg) was fractionated on a Sephadex LH-20 column, eluting successively with 4:1 hexane−CH_2Cl_2, 3:2 CH_2Cl_2−acetone, and 4:1 acetone−CH_2Cl_2. Five fractions were collected, and the final fraction (202 mg) was further separated on a second Sephadex LH-20 column, eluting with 3:2:1 hexane−toluene−MeOH to give three subfractions. The third subfraction (29 mg) was purified by reversed-phase HPLC using a linear gradient of 20→100% CH_3CN in H_2O over 25 min to afford sclerotigenin (**1**; 20 mg).

The fourth fraction from the initial Sephadex LH-20 column (70 mg) was separately fractionated on a Sephadex LH-20 column and eluted with 3:2:1 hexane−toluene−MeOH to give four subfractions. The first subfraction (53 mg) was purified by preparative reversed-phase HPLC using a linear gradient of 40→100% CH_3CN in H_2O over 25 min to afford griseofulvin (5 mg) and dechlorogriseofulvin (2 mg). Properties of the latter two compounds have been previously reported.[5,6]

Sclerotigenin (1): white solid; mp 235−238 °C; $[\alpha]_D$ 0° (*c* 0.07 mg/mL, $CHCl_3$); HPLC t_R 16.5 min under the conditions above; UV λ_{max} (MeOH) 235 (ϵ 7800), 271 (3700), 311 (1600); IR (CH_2Cl_2) ν_{max} 3406, 2928, 2854, 1695, 1458, 1263 cm^{-1}; EIMS (70 eV) *m*/*z* 277 (M^+; 100), 276 (64), 248 (39), 220 (32), 192 (20), 102 (25), 76 (25); FABMS (DTT-DTE matrix) *m*/*z* 278 [$(M + H)^+$, 100]; ^{1}H NMR, ^{13}C NMR, and HMBC data, see Table 1; HRFABMS $(M + H)^+$ obsd 278.0934, calcd for $C_{16}H_{11}N_3O_2$ + H, 278.0929.

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