2-Diazo-3-hydroxy-1-indanone (18a): 69% yield; clear oil; IR (neat) 3440 (br), 2105, 1690 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 3.25 (bd, 1 H, J = 8.1 Hz), 5.98 (d, 1 H, J = 8.1 Hz), 7.46–7.72 (m, 4 H).

2-Diazo-3-hydroxy-3-phenyl-1-indanone (18b) 85% yield, mp 170–171 °C; IR (KBr) 3036, 2100, 1660, 1605, 1350, 1330, 1175, 1040, 780, 765, 700 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 4.70 (s, 1 H), 7.26–7.61 (m, 9 H); ^{13}C NMR (75 MHz, $CDCl_3$) 81.0, 122.3, 124.5, 125.5, 128.2, 128.8, 129.8, 134.4, 134.7, 140.2, 151.5, 186.3. Anal. Calcd for $C_{15}H_{10}O_2N_2$: C, 71.99; H, 4.03; N, 11.19. Found: C, 71.84; H, 4.04; N, 11.10.

2-Diazo-3-hydroxy-3-methyl-1-cyclopentanone (19): IR (neat) 3410, 2100, 1660, 1385, 1330, 1190, 1090 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.65 (s, 3 H), 2.10–2.25 (m, 2 H), 2.34 (dd, 1 H, J = 17.6, 8.7, and 4.2 Hz), 2.67 (ddd, 1 H, J = 17.6, 8.8, and 8.6 Hz), 4.18 (s, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.3, 35.3. 36.9, 68.6, 77.6, 197.3.

Octahydro-2-diazo-3-methoxy-3-phenylpentalen-1-one (20): 80% as a clear oil; IR (neat) 2960, 2890, 1800, 1775, 1455, 1265, 1175, 960, 770, 710 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.12–1.43 (m, 3 H), 1.80–2.02 (m, 3 H), 3.13 (dt, 1 H, J = 7.4 and 7.2 Hz), 3.32 (dt, 1 H, J = 8.4 and 3.3 Hz), 3.50 (s, 3 H), 7.22–7.40 (m, 5 H).

2-Diazo-2,3-dihydro-3-hydroxy-3-methyl-1*H*-pyrrolo[1,2-*a*]indol-1-one (21): 64% yield; mp 130–131 °C; IR (KBr) 3270 (br), 2140, 1645, 1630, 1520, 1390, 1370, 1340, 1320, 1290, 1245 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 2.64 (s, 3 H), 7.10 (d, 1 H, J = 1.6 Hz), 7.17 (dd, 1 H, J = 8.5 and 6.6 Hz), 7.36 (dd, 1 H, J = 8.1 and 6.6 Hz), 7.45 (d, 1 H, J = 8.5 Hz), 7.70 (d, 1 H, J = 8.1 Hz), 9.84 (bs, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 28.8, 107.4, 111.6, 120.8, 122.4, 126.0, 126.7, 132.2, 136.3, 173.3, 189.9. Anal. Calcd for $C_{12}H_9N_3O_2$: C, 64.43; H, 3.99; N, 18.49. Found: C, 63.32; H, 3.94; N, 18.42.

Bicyclo[5.3.0]decane-2,10-dione (24):[16] 51% yield as a colorless oil; IR (neat) 2930, 2860, 1660, 1615, 1450, 1390, 1320, 1230, 1190, 910, 880 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.35–1.50 (m, 4 H), 1.80–2.0 (m, 4 H), 2.05–2.20 (m, 4 H), 2.05–2.20 (m, 1 H), 2.34–2.50 (m, 4 H), 2.75–2.85 (m, 1 H).

1-(1′-Oxocyclohex-2′-yl)-3,5-nonadiene (25): 9% yield as a colorless oil; IR (neat) 3500, 2960, 2880, 1720, 1700, 1620, 1460, 1410, 1360, 1270, 1060, 980, 810 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.88 (t, 3 H, J = 7.5 Hz), 1.20–1.80 (m, 14 H), 1.80–2.06 (m, 1 H), 2.20–2.60 (m, 4 H), 3.53 (s, 2 H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 13.8, 20.5, 22.1, 24.8, 25.6, 27.4, 28.8, 37.4, 41.7, 43.5, 46.1, 69.4, 202.8, 207.3, 212.6; HRMS calcd for $C_{15}H_{22}O_2$ ($M^+ - H_2O$) 234.1619, found 234.1617.

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Supplementary Material Available: ^1H NMR (300 MHz) and ^{13}C NMR spectra (75 MHz) for all new compounds (9 pages). Ordering information is given on any current masthead page.

Aflavazole: A New Antiinsectan Carbazole Metabolite from the Sclerotia of *Aspergillus flavus*

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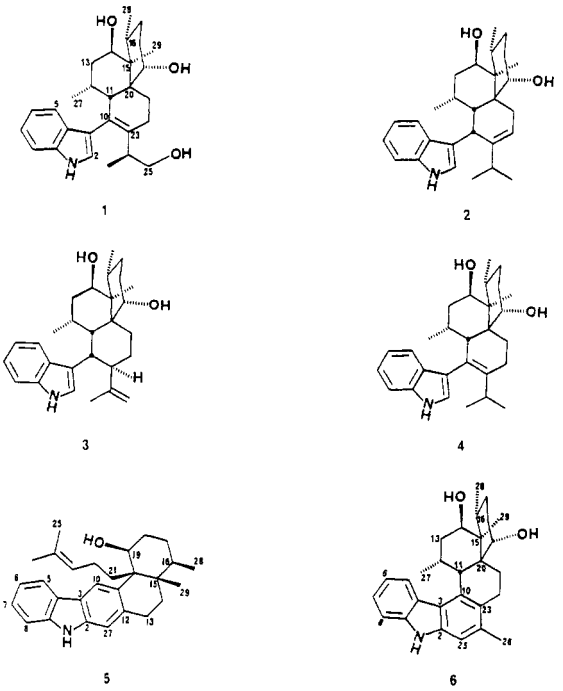
Extracts of the sclerotia of the common mold *Aspergillus flavus* deter feeding by the fungivorous insect *Carpophilus hemipterus*.[1,2] Our initial studies of this phenomenon led to the isolation and structure determination of four aflavinine derivatives (1–4) (Chart I) responsible for most of the antifeedant activity. One additional major antiinsectan indole metabolite was present in the chloroform extract of *A. flavus* sclerotia,[3] but its spectral data were significantly different from those of 1–4. Recently, studies of another *Aspergillus* sp. (*A. tubingensis*) have led us to isolate representatives of a new class of biologically active indole diterpene-derived metabolites containing a carbazole moiety (e.g., 5).[4] Examination of the spectral data for the remaining *A. flavus* metabolite and comparison with the data for 5 have permitted us to assign the structure of this new compound as 6. This metabolite, which we have named aflavazole, appears to be ubiquitous in sclerotial extracts of various strains of *A. flavus* and *A. parasiticus* and incorporates another previously unreported carbazole-containing ring system. Details of the isolation, structure elucidation, and biological activity of 6 are reported here.

Chart I



Sclerotia of *A. flavus* were produced by solid substrate fermentation on corn kernels.[2] The chloroform extract of the sclerotia exhibited insect antifeedant activity and was fractionated by reversed phase flash chromatography, followed by HPLC (C_{18}) to afford aflavazole (6), as well as the aflavinine derivatives 1–4. The molecular formula of 6 was established as $C_{28}H_{35}NO_2$ (12 unsaturations) on the basis of HREIMS and ^{13}C NMR data. Although 6 possessed many spectral similarities with 1–4, it was clear that 6 was not a simple aflavinine derivative. Key differences included the relatively low intensity of the quinolinium ion at m/z 130 in the mass spectrum, and the UV spectrum, which was characteristic of a carbazole unit.[5] The ^{13}C NMR spectrum of 6 indicated the presence of the

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Table I. Proton and Carbon NMR Data for Aflavazole (6)[a]

C/H	NMR ^{1}H	NMR ^{13}C	selective INEPT correltns	NOESY correltns
1				
2		140.29		
3		119.57		
4		123.88		
5	7.95 (br, d; 8.0)	122.33	3, 4, 7, 9	11, 12
6	7.14 (br dd; 8.0, 7.1)	119.64	4, 8	
7	7.27 (br dd; 7.8, 7.1)	125.09	5, 9	
8	7.39 (br d; 7.8)	111.67	4, 6	
9		141.53		
10		135.80		
11	4.18 (br d; 7.1)	40.05	3, 10*, 12*, 15, 19*, 21, 23, 27	5, 18ax
12	2.86 (m)	32.79	11, 13, 14, 20, 27	5
13ax	2.38 (m)	36.43		16, 28
13eq	1.68 (br dd; 12.9, 2.2)		14	
14	4.22 (dd; 12.9, 3.4)	72.48	12*, 15, 16, 20	21, 29
15		44.94		
16	2.60 (m)	32.60	15	13ax
17ax	1.26 (m)	28.72		28
17eq	1.78 (m)			
18ax	1.37 (br dd; 12.7, 1.9)	25.22		11
18eq	1.84 (m)			
19	3.84 (br d; 2.9)	71.47	15, 17	
20		45.23		
21	2.15 (m)	23.05	19, 20, 22	14, 27, 29
	2.75 (br dd; 11.0, 7.1)		11, 20	
22	2.89 (br dd 17.6, 7.1)	30.77	10, 20, 23, 24	26
	2.39 (m)			27
23		126.40		
24		135.13		
25	7.12 (br s)	110.25	3, 23, 26	
26	2.35 (br s)	20.68	23, 24, 25	
27	0.64 (d; 7.6)	19.34	11, 12, 13	
28	1.19 (d; 6.8)	13.70	15, 16, 17	29
29	1.33 (s)	19.83	14, 15, 20	

[a] Data were recorded in CD_3OD at 360 and 90.7 MHz, respectively. Carbon assignments are based on multiplicities and on selective INEPT results. NOESY Correlations between scalar-coupled protons have been omitted. Additional selective INEPT correlations (noted with asterisks) were detected in a parallel set of experiments performed by using $CDCl_3$ as the solvent.

requisite 12 aromatic carbons. Due to the absence of evidence for any other multiple bonds, compound **6** must contain three additional rings beyond the carbazole moiety.

Proton and carbon NMR data for **6** are provided in Table I. Axial or equatorial orientations for the protons were assigned where possible on the basis of proton–proton coupling constants. Evidence for a 2,3,4-trisubstituted carbazole substructure and a fragment matching the intact C11–C20 portion of the aflavinine ring system (e.g., **1**) was supplied by analysis of ^{1}H NMR decoupling and COSY data, along with selective INEPT[6,7] experiments summarized in Table I. Comparison with the relevant data for **5**[4] and several aflavinine derivatives[3,8] supported these assignments. In addition, the ^{1}H NMR and selective INEPT data clearly showed the presence of an isolated ethylene unit and an aromatic methyl group exhibiting benzylic coupling to a broad aromatic C–H singlet at 7.12 ppm. Since there are no other sp^2 carbons, the aromatic methyl group must be attached to the carbazole system.

The connectivity of the molecule was assigned on the basis of additional selective INEPT experiments (Table I), which provided information regarding two- and three-bond CH correlations. Irradiation of the proton at C-11 verified its correlation with appropriate carbon signals assigned to the C11–C20 spin system and also resulted in polarization transfer to three aromatic carbon singlets at 119.57, 135.80, and 126.40 ppm (carbons 3, 10, and 23, respectively). This result revealed that C-11 must be directly attached to the carbazole nucleus (at C-10). The downfield shift of the proton signal for H-11 (4.18 ppm) can be accounted for by aromatic ring current deshielding effects. The aromatic methyl protons (H_3-26) correlated with three aromatic carbon signals at 126.40, 135.13, and 110.25 ppm (carbons 23, 24, and 25, respectively), thereby locating the methyl group meta to C-11 and confirming its position ortho to the aromatic proton at C-25. Irradiation of one of the downfield proton signals corresponding to the isolated ethylene unit (H-22; 2.89 ppm) resulted in polarization transfer to the three aromatic carbons at 135.80, 126.40, and 135.13 ppm (carbons 10, 23, and 24), thus allowing linkage of C-22 to C-23. Irradiation of one of the other proton signals in this ethylene unit (H-21; 2.15 ppm) afforded polarization transfer to C-19 and C-20 (71.47 and 45.23 ppm) indicating linkage of C-20 to C-21. Connection of these two carbons leads to assignment of structure **6** for aflavazole, which possesses a previously undescribed ring system. The numbering system shown for aflavazole was chosen to coincide with that used for the aflavinines. Aflavazole is closely related to the aflavinines and probably arises from biogenetic cyclization of an aflavinine precursor. Exposure of aflavinines **1–4** to the extraction conditions does not yield compound **6**, and direct HPLC analysis of the fresh chloroform extract confirmed that this new compound is not an artifact of the isolation process.

The relative stereochemistry shown for aflavazole was assigned by analogy to that of the aflavinines and was supported by the results of NOESY experiments summarized in Table I. Despite some ambiguities caused by the presence of overlapping signals, the NOESY data are in accord with these assignments. NOESY correlations of H-5 with H-11, H_3-29 with H-14, and one of the protons at C-21 with both H-14 and H_3-27 support the stereochemical assignment for the terminal ring of the ring system. The relative stereochemistry proposed for the adjoining ring is implied by the observed correlations of H-11 with H-18_{ax}, H_3-29 with H-17_{ax}, and H_3-29 with H_3-28. These results, and the absence of a trans-diaxial coupling for H-19, support the suggestion that the relative stereochemistry of **6** corresponds to that of the aflavinines. The NOESY correlations are also consistent with the adoption of a chair-chair conformation by this portion of the molecule.

Aflavazole displays significant antifeedant activity against the fungivorous beetle *Carpophilus hemipterus* when incorporated into a standard test diet at 100 ppm and is second only to dihydroxyaflavinine (**1**) among *A. flavus* indole diterpenoid sclerotial metabolites in activity against *C. hemipterus*.[2] When incorporated into the diet at concentrations found in *A. flavus* sclerotia (200–600 ppm), virtually complete feeding deterrence is observed.

Although the aflavinines **1–4** are abundant in the 11 strains of *A. flavus* and *A. parasiticus* we have analyzed, high concentrations of aflavazole are less common among these strains. Like the aflavinines, aflavazole occurs almost exclusively in sclerotia, and liquid shake cultures of *A.*

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flavus do not contain significant amounts of this metabolite.

Experimental Section

General. Strains of *A. flavus* (e.g., NRRL 13462) were obtained from the Agricultural Research Service (ARS) collection at the USDA Northern Regional Research Center in Peoria, IL. The sclerotia were prepared by solid substrate fermentation on autoclaved corn kernels using general procedures that have been previously described[2] and were stored at 4 °C until extracted. Proton and carbon NMR data were obtained in CD_3OD or $CDCl_3$ on a Bruker WM-360 spectrometer, and chemical shifts were recorded by using the signal for the residual protiated solvent (3.30 ppm for CD_3OD) as a reference. Carbon multiplicities were established by a delayed decoupling experiment. All long-range C–H correlations were obtained through selective INEPT experiments. Proton signals studied with the selective INEPT technique were individually subjected to four separate experiments, optimizing for 4, 7, 10, or 15 Hz. Details of other experimental procedures and insect bioassays have been described elsewhere.[2-4]

Isolation and Properties of Aflavazole (6). Ground *A. flavus* sclerotia (NRRL 13462, 24.7 g) were exhaustively extracted with hexane, followed by chloroform, and the chloroform extract (513 mg) was subjected to flash chromatography on a reversed phase column (1 × 10 cm; C_{18}; 40–63 μm particles) using a step gradient from 50 to 70% MeOH–H_2O in 5% increments. Compound **6** was contained in fractions eluting at 60% MeOH. Separation of the resulting fractions by reversed phase HPLC (5-μm C_{18} column; 250 × 10 mm; 70:30 MeOH–H_2O at 2.0 mL/min) afforded compound **6** (8.6 mg) as a light yellow crystalline solid with the following properties: mp 156–160 °C dec; $[\alpha]_D$ +2.8° (*c* 0.35, MeOH); HPLC retention time under the above conditions, 25.3 min; UV (MeOH) 341 (ε 1600), 327 (1300), 297 (7600), 263 (7500), 243 (17 300), 219 (15 300); ^1H NMR, ^{13}C NMR, selective INEPT, and NOESY data, Table I; EIMS (70 eV) 417 (M^+; rel intensity 100), 399 (37), 381 (13), 316 (40), 284 (8), 272 (13), 258 (32), 254 (16), 244 (36), 231 (42), 217 (62), 204 (29), 194 (47), 182 (20), 168 (21), 130 (60); HREIMS, obsd 417.2694, calcd for $C_{28}H_{35}NO$, 417.2669.

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Silicon-Modified Metal–Ammonia Reduction of Fluorene

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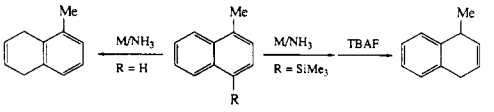
The metal–ammonia reduction of aromatic and polynuclear aromatic compounds, known as the Birch reduction when alcohols are employed as proton sources, represents an important method for the synthesis of hydroaromatic compounds.[1] The regiochemistry of this reduction is dictated primarily by the electron density distribution in the anionic intermediates. We have been exploring methods for regiochemical control in this reaction and have shown that a trimethylsilyl group can be used to alter regiochemistry, and, after its subsequent removal, afford a "misoriented" Birch reduction product.[2] For example, 1-methylnaphthalene (**1**, R = H) reduces



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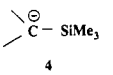
exclusively in the unsubstituted ring providing **2**, whereas reduction of 1-methyl-4-(trimethylsilyl)naphthalene followed by desilylation with tetrabutylammonium fluoride (TBAF) affords only **3**. Presumably, the driving force for this change in regiochemistry comes from the stabilization of negative charge by an α-silicon (**4**).[3] This study shows



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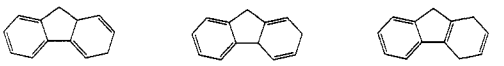
that remote silyl substituents, which are not α to a site bearing a negative charge in the reaction intermediate, can also be used to alter the course of metal–ammonia reduction.

The metal–ammonia reduction of fluorene was reinvestigated[4] after an earlier report[5] suggested the 3,9a-dihydro isomer **5** to be the primary product, in conflict with HMO calculations that predicted **6**. In fact, the reduction of fluorene produces a mixture of dihydro isomers **6** and **7** in 39% and 37% yields, respectively, together with 8%



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of an unidentified tetrahydro product and 11% recovered starting material. The dihydro products were not easily isolable, and analytical samples were obtained by trapping off the gas chromatograph. Reduction of 9,9-dimethylfluorene also produced a mixture of dihydro isomers, analogous to **6** and **7**, in 38% and 24% yields, respectively.

In contrast, reduction of 9,9-bis(trimethylsilyl)fluorene produced the single dihydro isomer **8** together with a minor amount of recovered starting material. Subsequent treatment with TBAF in THF for 30 min provided 1,4-dihydrofluorene (**7**) as a crystalline solid. Similar reduction, but with excess metal and in the presence of *tert*-butyl alcohol, afforded the tetrahydro isomer **9**, which also underwent smooth desilylation with TBAF to produce a crystalline solid (**10**).

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