mospheric hydrogenation apparatus and subjected to hydrogen overnight. The catalyst was removed by filtration through solka floc and the filtrate concentrated to afford a solid which was crystallized from EtOAc/Hept to afford 747 mg (80% yield) of the lactone **25**. ^{13}C NMR ($CDCl_3$): 177.99, 156.04, 79.91, 79.56, 50.201, 46.46, 42.47, 28.36, 27.50, 25.94, 24.86, 23.05, 21.93, 20.58, 17.94 ppm. ^1H NMR ($CDCl_3$): 4.50 (d, J = 9.9 Hz, NH), 4.46 (dd, J = 6.2, 9.3 Hz, 1 H), 3.85 (td, J = 10.2, 4.5 Hz, 1 H), 2.6 (m, 1 H), 2.15 (m, 2 H), 1.85 (m, 1 H), 1.60 (m, 3 H), 1.43 (s, 9 H), 1.01 (d, J = 6.9 Hz, 3 H), 0.93 (d, J = 6.3 Hz, 6 H), 0.89 (d, J = 6.7 Hz, 3 H) ppm.

(2*S*,4*S*,5*S*)-2-(2-Propyl)-4-hydroxy-5-[(*tert*-butyloxycarbonyl)amino]-7-methyloctanoic Acid, γ-Lactone (22). A mixture of 457 mg (1.91 mmol) of the azide **20**, 525 mg of $(BOC)_2O$, and a catalytic amount of Pd–C in 10 mL of EtOAc was hydrogenated at 40 psi for 2 h. The catalyst was removed by filtration through solka floc and the product crystallized upon concentration to afford 607 mg (quantitative) of amide **22**. Recrystallization from EtOAc/Hept gave 407 mg of amide **22** along with 200 mg of mother liquor which crystallized upon concentration. $[\alpha]_D$ = −41° (c = 1, ethanol). Mp: 144.5–146 °C. ^1H NMR ($CDCl_3$): 4.55 (d, J = 9.5 Hz, NH), 4.45 (t, J = 6.1 Hz), 3.86 (bm), 2.58 (bm), 2.0–2.35 (m), 1.48 (s, 9 H), 0.96 (d, J = 5.6 Hz, 3 H), 0.94 (m, 9 H) ppm. ^{13}C NMR ($CDCl_3$): 179.0, 156.08, 80.74, 79.54, 51.68, 45.66, 41.77, 29.07, 28.21, 26.25, 24.67, 22.99, 21.77, 20.27, 18.32 ppm. IR (film): 3427, 3320, 1754, 1667, 1677, 1524, 1275, 1200, 1164, 1060, 1035, 675 cm^{-1}. Anal. Calcd for $C_{17}H_{31}NO_4$: C, 65.14, H, 9.97, N, 4.47. Found: C, 65.26, H, 9.92, N, 4.34.

Na/NH_3 Reduction of Amide. The amide **8a** (5.0 g, 16.6 mmole) was dissolved in 20 mL of butylamine and 50 mL of liquid ammonia at −33 °C. Sodium was added slowly so as to maintain the blue color. After 5.5 h the GC showed the reaction to be essentially complete, and NH_4Cl was added and the ammonia allowed to evaporate. The product was isolated with MTBE after the reaction mixture was poured into water. The organic extracts were dried over Na_2SO_4 and concentrated. The GC/MS indicates that some overreduction (4.7%) has taken place because of the presence of an impurity with a m/e = 303. The yields varied from 78 to 89%. The crude product was crystallized from EtOAc/Hept to afford amide **15** melting at 116–119 °C. $[\alpha]_D$ = −13° (c = 0.8, $CHCl_3$). ^1H NMR ($CDCl_3$): 5.42 (m, 2 H), 2.24 (t, J = 6.6 Hz, 2 H), 1.85 (m, 4 H), 1.57 (septet, J = 6.7 Hz, 1 H), 0.98 (d, J = 6.5 Hz, 3 H), 0.96 (d, J = 6.3 Hz, 3 H), 0.86 (d, J = 6.7 Hz, 6 H) ppm. ^{13}C NMR ($CDCl_3$): 177.88, 131.41, 128.42, 54.52, 41.97, 33.20, 30.23, 28.37, 22.28, 20.64, 20.33 ppm. IR: 3510, 3990, 2940, 1670, 1580, 1460, 1375, 1240, 990 cm^{-1}. Anal. Calcd for $C_{12}H_{23}NO$: C, 73.045, H, 11.75, N, 7.099. Found: C, 72.93, H, 11.57, N, 7.22.

Epoxides 16a and 16b. A solution of 100 mg (0.5 mmol) of the amide **15** in 5 mL of CH_2Cl_2 was cooled to 0 °C and treated with solid KOAc and 0.33 g (1.5 mmol) of *m*-CPBA. The mixture was stirred at 0 °C for 6 h and then allowed to warm to rt and stir overnight. The reaction mixture was quenched with aqueous $NaHSO_3$ and the product isolated with MTBE. The organic layers were dried over Na_2SO_4 and concentrated to afford the epoxide which was shown by GC to be a 12:1 mixture of diastereomers. The epoxide **16b** can be upgraded by crystallization from MTBE. $[\alpha]_D$ = −62° (c = 0.9, $CHCl_3$). Mp: 145–148 °C. ^1H NMR ($CDCl_3$): 6.21, (bs, NH), 6.10, (bs, NH), 2.75 (td, J = 5.9, 2.2 Hz, 1 H), 2.70 (dt, J = 7.3, 2.8 Hz, 1 H), 2.10 (m, 2 H), 1.40 (m, 4 H), 0.97 (d, J = 4.3 Hz, 3 H), 0.95 (d, J = 3.5 Hz, 3 H), 0.94 (d, J = 8.9 Hz, 6 H) ppm. ^{13}C NMR ($CDCl_3$): 177.22, 58.75, 57.08, 50.64, 41.00, 32.47, 30.60, 26.25, 22.80, 22.36, 20.57, 20.03 ppm. IR: 3510, 2890, 2940, 1710, 1680, 1510, 1360 cm^{-1}. Anal. Calcd for $C_{12}H_{23}NO_2$: C, 67.57, H, 10.87, N, 6.57. Found: C, 67.44, H, 10.59, N, 6.75.

Hydrolysis of Epoxy Amide 16b. A solution of 0.935 g of crystallized epoxide in 10 mL of dioxane and 5 mL of 1 M H_2SO_4 was heated to reflux for 2 h. After being cooled to room temperature the reaction mixture was poured into water and the lactone isolated with MTBE. Drying (Na_2SO_4) and concentration of the organic layers afforded 1.0 g of lactone **12a** which crystallized upon standing. GC shows it to be a 95.8/3.3 mixture of the (2*S*,4*R*,5*S*)/(2*S*,4*S*,5*R*) isomers.

Acknowledgment. We would like to thank Fran Eckenrode for developing the GC assay used to separate the diastereomeric hydroxylactones.

Stachybotrins A and B: Novel Bioactive Metabolites from a Brackish Water Isolate of the Fungus *Stachybotrys* sp.

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Stachybotrins A and B (**1** and **2**), two new aromatic alkaloids with antibacterial and antifungal activity, have been isolated from an aquatic isolate of a new species of the genus *Stachybotrys* (CS-710-1). Compounds **1** and **2** were obtained from ethyl acetate extracts of liquid cultures by preparative TLC. The structures were determined primarily by analysis of HMBC, HMQC, COSY, and NOESY experiments.

In the course of our investigations of marine and aquatic fungi as sources of novel biologically active secondary metabolites,[3-6] we examined an isolate of *Stachybotrys* sp. (CS-710-1) collected from brackish water in Florida. This isolate differs significantly from previously known members of the genus *Stachybotrys* and has been established as a representative of a new species.[7] Compounds previously reported from other *Stachybotrys* spp. include trichothecene mycotoxins (satratoxins),[8,9] sterols,[9] and a novel spirobenzofuran that exhibits effects on the com-

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Table I. NMR Data for Stachybotrin A (1) in CD_3OD[a]

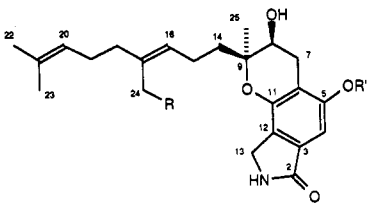
position	^1H	^{13}C	HMBC correlations
2		174.3	
3		132.5	
4	6.74 (s)	101.1	2, 5, 6, 11,[b] 12
5		158.4	
6		113.6	
7	2.65 (dd; 17.6, 7.2)	27.7	5, 6, 8, 9, 11
	2.97 (dd; 17.6, 5.5)		5, 6, 8, 9, 11
8	3.88 (dd; 7.2, 5.5)	68.4	6, 7, 9, 14, 25
9		80.1	
11		150.1	
12		123.8	
13	4.24 (br s, 2 H)	44.2	2, 3, 11, 12
14	1.69 (m)	38.9	8, 9, 15, 16, 25
15	2.27 (m, 2 H)	22.1	9, 14, 16, 17
16	5.30 (dd; 7.4, 7.4)	128.8	14, 15, 17, 18, 24
17		139.7	
18	2.08 (m, 2 H)	36.0	16, 17, 19, 20, 24
19	2.07 (m, 2 H)	27.9	17, 18, 20, 21
20	5.07 (dd; 1.4, 1.4)	125.4	18, 19, 21, 22, 23
21		132.2	
22	1.63 (s)	25.5	20, 21, 23
23	1.55 (s)	17.7	20, 21, 22
24	4.08 (d, 12.2)	59.9	16, 17, 18
	4.05 (d, 12.2)		16, 17, 18
25	1.26 (s)	18.7	8, 9, 14

[a] Proton and ^{13}C NMR data were recorded at 300 and 75 MHz, respectively. HMBC data were recorded at 600 MHz (^1H dimension). [b] A four-bond correlation.

plement cascade.[10] Cultures of this new species exhibited activity in competitive assays against other fungi, and efforts were undertaken to isolate the compound(s) responsible for these effects. Through these studies, we have encountered two new antifungal metabolites which we have named stachybotyrins A and B. Details of the isolation, structure elucidation, and biological activity of these compounds are presented here.

Stachybotrins A and B (1 and 2) were obtained from ethyl acetate extracts of liquid cultures of *Stachybotrys* sp. (CS-710-1)[7] by repeated preparative TLC on silica gel. Stachybotrin A, the major component, was isolated in yields ranging from 10 to 15 mg/L of culture medium. HREIMS (M^+ 401.2189) and carbon-13 NMR data revealed its molecular formula as $C_{23}H_{31}NO_5$ (nine unsaturations). The DEPT data demonstrated that all but four of the protons in the molecule are bound to carbon. A secondary amide group was indicated by a carbon signal at 174.3 ppm, an IR carbonyl absorption at 1684 cm^{-1}, and an exchangeable amide proton singlet observed at 8.30 ppm in DMSO-d_6. Acetylation of stachybotrin A afforded a triacetate, confirming that the other three exchangeable protons were associated with hydroxyl groups. ^1H–^1H COSY data for stachybotrin A permitted the establishment of proton spin systems, and ^{13}C NMR assignments within those systems were afforded by an HMQC[11] experiment. Other important structural information was provided by long-range ^1H–^{13}C correlations detected through an HMBC[12] experiment (Table I).

In the ^1H NMR spectrum of the triacetate, proton signals associated with an oxygenated methine and an oxymethylene unit were shifted downfield (from 3.84 to 5.39 ppm and from 4.06 to 4.52 ppm), thereby indicating that the two carbons (C-8 and C-24 in 1) bear free OH groups



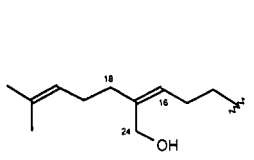
1 R = OH, R' = H

2 R = R' = H

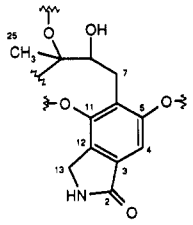
3 R = H, R' = CH_3

in the natural product. The only aromatic proton signal was also downfield-shifted (from 6.74 to 7.05 ppm), suggesting the existence of a phenolic hydroxyl group in stachybotrin A.

The presence of a 4-(hydroxymethyl)-8-methyl-3,7-nonadienyl side chain (subunit **a**) was readily established on the basis of COSY and HMBC data. The key connection of C-17 with C-18 and the location of the hydroxymethyl group were revealed by HMBC correlations of the CH_2-24 proton signal with the carbon signals for C-16, -17, and -18.



a



b

Partial structure **b** was deduced primarily through analysis of the HMBC correlations for H-4, H_2-13, H_2-7, and H_3-25. The H-4 signal correlated with five nonprotonated sp^2 carbons (C-2, C-5, C-6, C-11, and C-12), while H_2-13 showed correlations with C-2, C-3, C-11, and C-12. These results, along with the UV data, suggested that the sp^2 carbons comprised a carbonyl-substituted, pentasubstituted benzene ring. Chemical shift considerations indicated that two of the aromatic ring carbons (C-5 and C-11) are oxygenated (158.4 and 150.5 ppm), and suggested that they have a meta relationship. This meta relationship was confirmed by HMBC correlations of both H_2-7 methylene proton signals with the resonances for C-5, C-6, and C-11. These proton signals also showed correlations to the hydroxylated methine C-8 and to the only aliphatic quaternary carbon C-9 (which must bear CH_3-25) and were vicinally coupled to the methine proton on C-8.

The site of linkage of subunits **a** and **b** was clearly demonstrated by HMBC correlations of the C-25 methyl group protons with C-14, C-8, and C-9 and verified by correlations of H-8 with C-14 and H_2-14 with C-8. These results limited the possible structures to 1 and an alternative structure in which C-9 is linked to the oxygen at position 5. These two possibilities could not be conclusively differentiated by NMR experiments conducted on 1.

The NMR spectral data for the less polar compound stachybotrin B (2; Table II) are nearly identical to those of 1. HREIMS data revealed an elemental composition with one less oxygen atom than 1. The only significant differences in the ^1H NMR spectrum of stachybotrin B compared to the spectrum of 1 are the absence of the vinylic CH_2OH signal and the presence of a third vinyl methyl group singlet (1.54 ppm). An analogous difference

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Table II. NMR data for Stachybotrin B (2) in CD_3OD[a]

position	^1H	^{13}C	position	^1H	^{13}C
2		174.2	14	1.67 (m, 2 H)	38.5
3		132.5	15	2.18 (m, 2 H)	22.6
4	6.74 (s)	100.8	16	5.14 (m, 1 H)	125.5
5		158.0	17		136.2
6		113.5	18	1.95 (m, 2 H)	40.8
7	2.64 (dd; 18.0, 6.0)	27.8	19	2.02 (m, 2 H)	27.8
	2.98 (dd; 18.0, 6.0)		20	5.05 (m, 1 H)	125.3
8	3.87 (dd; 6.0, 6.0)	68.4	21		132.2
9		80.2	22	1.63 (s)	25.8
11		150.2	23	1.55 (s)	17.7
12		124.1	24	1.57 (s)	15.9
13	4.23 (d; 18.0)	44.2	25	1.27 (s)	18.8
	4.20 (d; 18.0)				

[a] Proton and ^{13}C NMR data were recorded at 300 and 75 MHz, respectively.

was observed in the ^{13}C NMR spectrum. Acetylation of 2 afforded a diacetate as indicated by MS and NMR data. The ^1H NMR spectrum of the diacetate again showed significant downfield shifts for the signals corresponding of H-8 and H-4. Thus, the structure of stachybotrin B was proposed as shown in 2. However, as with stachybotrin A, the data failed to distinguish structure 2 from the other possible regioisomer.

The question of regiochemistry for 1 and 2 was addressed by analysis of an HMBC experiment conducted on the methyl ether derivative of 2 (3), which was prepared by treatment of 2 with diazomethane. The position of the resulting methoxy group was established by HMBC correlation of the methoxy methyl proton signal with the signal assigned to C-5. The data also clearly showed a correlation of H_2-13 with a different carbon signal (C-11), demonstrating that C-13 must be ortho to the C-11 oxygen and para to the methoxy group at C-5. In support of the assigned regiochemistry, a NOESY correlation was also observed between the signals for the methoxy group and the aromatic proton. These data permitted assignment of the gross structure of the derivative as 3 and indicated that stachybotrin B has structure 2. Stachybotrin A is proposed to possess the analogous regiochemistry, as shown in 1. The less polar nature of 3 also facilitated an additional confirmatory experiment. The amide protons in 1 and 2 were exchanged with the NMR solvent used (CD_3OD), so no HMBC correlations for either of these protons were observed. However, 3 was sufficiently soluble in $CDCl_3$ to permit selective INEPT[13] irradiation of the nonexchanged amide proton signal at 6.33 ppm, which afforded long-range correlations to all four of the expected carbons (C-2, -3, -12, and -13).

The relative stereochemistry and olefin geometry shown for 1 and 2 are proposed on the basis of NOESY data, ^1H NMR coupling constants, and ^{13}C NMR chemical shifts for 3. For example, a strong NOESY correlation between H_{ax}-7 (2.64 ppm) and H_3-25 (1.26 ppm) indicated that these groups are spatially close. This places the H_3-25 methyl group in an axial (or pseudoaxial) position with respect to the dihydropyran ring. A strong correlation between the H-8 and H_3-25 signals provided evidence that H-8 has an equatorial disposition, placing the C-8 hydroxyl group trans to the H_3-25 methyl group. This is consistent with the lack of a large vicinal coupling between H-8 and either of the C-7 protons. The C-16/C-17 double bond was assigned the geometry shown on the basis of the upfield chemical shift of C-24 (15.9 ppm), as well as a strong ^1H–^1H NOESY correlation of the signal for H_2-24 with that of H_2-15 in the absence of a correlation between H_2-15 and H_2-18.

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Compounds 1 and 2 appear to have a mixed biogenetic origin, with the C7-C9/C14-C25 portion arising from farnesyl pyrophosphate and the aromatic ring being derived from the polyketide pathway. Although several triprenylated phenols of this general biogenetic class have been reported as metabolites from other fungi,[9,10] nitrogen-containing examples are rare, and the stachybotrins possess a previously unreported pyrano[2,3-*e*]isoindoline ring system.

Stachybotrins A and B each showed activity in disk assays against *Bacillus subtilis* (ATCC 6051) at 10 μg/disk (8- and 10-mm zones, respectively). They also inhibited the radial growth of the filamentous fungi *Ascobolus furfuraceus* (NRRL 6460) and *Sordaria fimicola* (NRRL 6459) by 50% in centerpoint inoculation assays[15] at 20 and 10 μg/disk, respectively. Disk assays for activity against a strain of *Candida albicans* (ATCC 14053) were negative at 100 μg/disk. Compound 1 was also evaluated for cytotoxicity[14] against three human solid tumor cell lines (nonsmall carcinoma A-549, breast adenocarcinoma MCF-7, and colon adenocarcinoma HT-29), but afforded only mild activity (ED_{50} values 20–30 μg/mL). Further biological evaluation of these compounds is underway.

Experimental Section

General Procedures. NMR spectra were recorded in CD_3OD, and chemical shifts were referenced relative to the corresponding solvent signals (3.30/49.0). Carbon multiplicities were established by DEPT experiments and are consistent with the assignments. The selective INEPT experiment was carried out at 75 MHz optimizing for $^nJ_{CH}$ = 7 Hz. COSY experiments were performed at 300 MHz. All other 2D-NMR data were acquired at 600 MHz (^1H dimension). HMQC and HMBC experiments were optimized for $^nJ_{CH}$ = 135 and 8 Hz, respectively. Procedures used in the antifungal assays have been described previously.[15]

Cultivation of *Stachybotrys* sp. *Stachybotrys* sp. (CS-710-1)[7] was isolated from a twig submerged in Whitewater Bay, Florida. Six 2-L Erlenmeyer flasks, each containing 400 mL of corn meal broth (medium composition: corn meal 1.0%; glucose 1.0%; NaCl, 0.25%; $CaCO_3$, 0.10%) which had been sterilized at 120 °C for 15 min and then cooled to room temperature, were individually inoculated with 1-cm^2 agar plugs taken from stock cultures of *Stachybotrys* sp. (CS-710-1). Flask cultures were inoculated at 25–28 °C and aerated by agitation on an orbital shaker at 150 rpm. The antifungal and antibacterial activity of the culture filtrate reached a maximum after 30 days. The mycelium extract did not display significant activity.

Isolation of Stachybotrins A and B. The culture filtrate (2400 mL) was extracted with EtOAc (5 X 1 L), and the organic phase was dried ($MgSO_4$) and concentrated to afford 400 mg of red oil. The extract was dissolved in 4 mL of acetone and divided into four equal portions. Each portion was subjected to preparative TLC on silica gel GF plates (20 × 20 × 0.1 cm) developing twice with 9:1 $CHCl_3$–EtOH. Major bands observed under UV illumination (254 nm) were collected and extracted with MeOH. Bands 2 (R_f 0.18) and 4 (R_f 0.35) were further purified with the same TLC conditions to afford 31.2 mg of stachybotrin A (1) as a yellow oil and 15.4 mg of stachybotrin B (2) as a white solid.

Stachybotrin A (1): ^1H NMR, ^{13}C NMR, and HMBC data, see Table I; HPLC t_R 2.95 min (5 μm Beckman C_{18} Ultrasphere column; 250 × 4.6 mm; 90:10 MeOH–H_2O; 1.0 mL/min); $[\alpha]_D$ = +8.8° (*c* 0.61 g/dL; MeOH); IR (neat) 3262, 2918, 1684, 1611, 1462, 1358, 1049, 1025, 1006 cm^{-1}; UV_{max} 220 (ε 17 000), 254 (6400), 302 nm (2900); EIMS (70 eV) *m/z* 401 (M^+; rel int 24), 383 (10), 332 (7), 314 (4), 248 (12), 232 (26), 216 (18), 190 (23), 178 (57), 147 (19), 121 (21), 93 (24), 69 (100); HREIMS obsd for $C_{23}H_{31}NO_5$ 401.2189, calcd 401.2202.

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Stachybotrin B (2): ^1H and ^{13}C NMR data, see Table II; HPLC t_R 4.05 min; mp 178–180 °C dec; $[\alpha]_D$ = +39.1° (c 0.11 g/dL; MeOH); IR (neat) 3293, 2919, 1653, 1608, 1457, 1350, 1080 cm^{-1}; EIMS (70 eV) m/z 385 (M^+; rel int 36), 311 (22), 232 (13), 216 (16), 190 (23), 178 (38), 177 (30), 147 (9), 123 (13), 109 (15), 95 (16), 81 (25), 69 (100); HREIMS obsd for $C_{23}H_{31}NO_4$ 385.2270, calcd 385.2254.

Acetylation of Stachybotrin A. A sample of stachybotrin A (4 mg) was dissolved in 0.2 mL of 1:1 pyridine-Ac_2O. The mixture was stirred and heated at 70 °C for 3 h and then allowed to stand at room temperature for 48 h. H_2O (1 mL) was added, and the solution was then extracted with $CHCl_3$ (2 × 2 mL). The $CHCl_3$ solution was dried and evaporated to afford 5 mg of yellow oil. Further purification was accomplished by preparative TLC on silica gel plates (12 × 4 × 0.1 cm) with $CHCl_3$–acetone (95:5) as the eluent. The MeOH extract of the band at R_f 0.7 was evaporated to afford the triacetate (2.5 mg; 48% yield) as a colorless oil with: ^1H NMR signals at 7.09 (s, 1 H), 5.39 (t, J = 7.4 Hz; 1 H), 5.16 (t, J = 5.0 Hz, 1 H), 5.05 (br m, 1 H), 4.52 (br s, 2 H), 4.37 (s, 2 H), 3.02 (dd, J = 18.0, 5.0 Hz, 1 H) 2.70 (dd, J = 18.0, 5.0 Hz, 1 H), 2.31 (s, 3 H), 2.26 (m, 2 H), 2.04 (s, 3 H), 2.03 (m, 4 H), 1.94 (s, 3 H), 1.67 (m, 2 H), 1.65 (s, 3 H), 1.55 (s, 3 H), 1.35 ppm (s, 3 H); IR (neat) 2918, 1734, 1646, 1602, 1570, 1450, 1380, 1228, 809 cm^{-1}; EIMS (70 eV) 526 $[(M - H)^+$, rel int (0.2), 468 (0.4), 418 (0.8), 414 (0.3), 360 (1.4), 332 (1.2), 316 (2.1), 300 (7.9), 274 (3.8), 258 (18), 230 (4.2), 216 (11), 177 (4.9), 129 (4.1), 95 (6.2), 69 (12), 55 (16), 43 (100).

Acetylation of Stachybotrin B. The procedure described above was repeated using 4 mg of stachybotrin B to afford the diacetate (2.2 mg; 45% yield): EIMS (70 eV) m/z 469 (M^+, rel int 0.3), 424 (0.4), 369 (6.8), 353 (1.7), 318 (1.3), 300 (5.1), 285 (2.1), 258 (7.9), 236 (11), 213 (3.6), 185 (4.6), 152 (8.1), 97 (66), 83 (39), 69 (57), 55 (66), 43 (100).

Methylation of Stachybotrin B. An ethereal solution of CH_2N_2 (3.5 mL) was added to a solution of 2 (10 mg) in 0.5 mL of MeOH. After standing at rt for 24 h, the solvents were evaporated and the residue was subjected to preparative TLC on silica gel (100- × 50- × 0.25-mm plates) eluting twice with 95:5 $CHCl_3$–MeOH. The major band at R_f 0.6 was collected and extracted with MeOH. Filtration and concentration of the resulting solution afforded 6.1 mg of the methyl ether derivative 3 (59% yield) as a colorless oil with: NMR assignments (based in part on HMQC and HMBC data); ^1H NMR 6.73 (s, 1 H; H-4), 5.13 (br t, 1 H, 6.0; H-16), 5.05 (br t, 1 H, 6.0; H-20), 4.28 (d, 1 H, 15.4; H-13), 4.24 (d, 1 H, 15.4; H-13), 3.88 (s, 3 H; C5-OMe), 3.87 (m, 1 H; H-8), 2.95 (dd, 1 H, 18.0, 5.1; H-7), 2.65 (dd, 1 H, 18.0, 6.9; H-7), 2.17 (m, 2 H; H-15), 2.03 (m, 2 H; H_2-19), 1.95 (br t, 2 H; H_2-18), 1.65 (m, 2 H; H_2-14), 1.62 (br s, 3 H; H_3-22), 1.56 (s, 3 H; H_3-24), 1.54 (s, 3 H; H_3-23), 1.28 ppm (s, 3 H; H_3-25); ^{13}C NMR, 174.1 (C-2), 160.3 (C-5), 150.0 (C-11), 136.2 (C-17), 132.6 (C-3), 132.2 (C-21), 126.0 (C-12), 125.4 (C-16), 125.3 (C-20), 114.4 (C-6), 96.7 (C-4), 80.3 (C-9), 68.2 (C-8), 56.3 (C-5-OMe), 44.2 (C-13), 40.8 (C-18), 38.4 (C-14), 27.7 (C-19, C-7), 25.8 (C-22), 22.6 (C-15), 18.8 (C-25), 17.7 (C-23), 15.9 (C-24).

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Supplementary Material Available: ^1H NMR spectra of stachybotrins A and B and the ^{13}C NMR spectrum of stachybotrin A (2 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

Reactivity and Synthetic Applications of Bis(iminophosphoranes). One-Pot Preparation of Pyrido[2,3,4-*de*]quinazolines and Benzo[*de*][1,6]naphthyridines

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Aza-Wittig reaction of bis(iminophosphorane) 4 with 2 equiv of isocyanate leads directly to pyrido[2,3,4-*de*]quinazolines 5. Iminophosphoranes 8, available from 4 and 1 equiv of the appropriate isocyanate, react either with 1 equiv of isocyanate or ketene to give pyrido[2,3,4-*de*]quinazolines 9 or benzo[*de*][1,6]naphthyridines 10, respectively. Bis(iminophosphorane) 4 by sequential treatment with aldehydes and isocyanates yielded 2,3-dihydropyrido[2,3,4-*de*]quinazolines 17.

The intramolecular version of the aza-Wittig-type reaction has attracted considerable attention recently because of its high potential for the synthesis of a wide variety of nitrogen heterocycles, which can be attributed in good measure to the rapid progress in the preparation of functionalized iminophosphoranes, and several interesting heterocyclization reactions involving iminophosphoranes have been reviewed.[1] These compounds can be easily converted through aza-Wittig reactions with isocyanates, carbon dioxide, or carbon disulfide into functionalized heterocumulenes which exhibit a rich chemistry of unusual synthetic promise. However, the chemistry of bis(iminophosphoranes) remains almost unexplored.[2] Bis(iminophosphoranes) are expected to have synthetic potential because they provide a reaction system in which the two iminophosphorane groups can react either with a reagent having two functionalities or with two separate reagents bearing the same functionality (mode A). It is expected that the utility of the bis(iminophosphoranes) could be improved if the two iminophosphorane moieties show different reactivity toward the same functionality. In this

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