Synthesis and Isomerization of Biindolinones from *Collybia peronata* and *Tricholoma scalpturatum*

Shawn J. Stachel, Mark Nilges,† and David L. Van Vranken*

Department of Chemistry, The University of California, Irvine, California 92697-2025, and Illinois EPR Research Center, 506, South Matthews Avenue, Urbana, Illinois 61801

Received March 3, 1997[⊗]

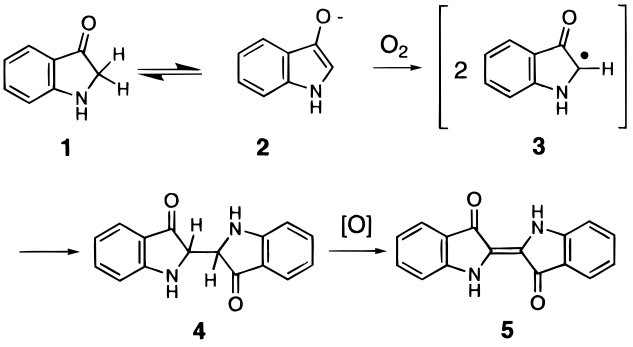
Peronatins A and B and 7,7′-dimethoxyperonatin B, originally isolated from the damaged fruiting bodies of *Collybia peronata* and *Tricholoma scalpturatum*, have been synthesized by oxidative dimerization of 2-alkylindoles. The conversion of peronatin A to peronatin B was shown to be catalyzed by Brønsted acids in chloroform solution and inhibited by triethylamine, implicating a retro-Mannich/Mannich isomerization pathway under these conditions. Attempts to identify or trap out radical or ionic intermediates were unsuccessful.

1. Introduction

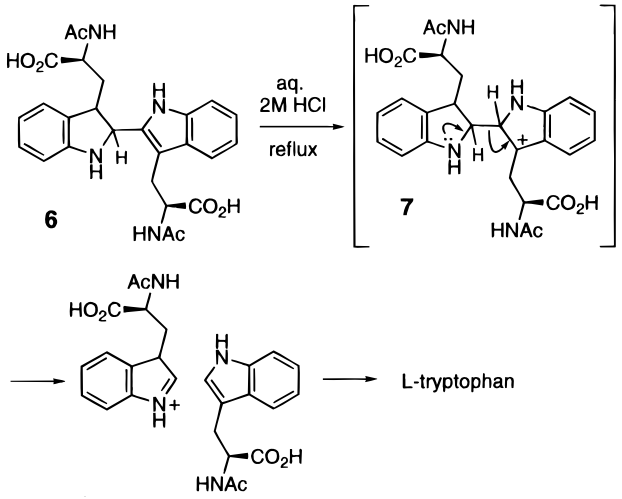
Indole dimers, in the form of indigo, are among the oldest known organic compounds of commercial importance, dating back to 2500 bc.[1,2] The chemistry that underlies indigo technology is surprisingly advanced and exploits enzymatic hydrolysis, bioreduction, and free radical carbon−carbon bond-forming reactions. The mechanism for formation of indigo (**5**) under basic conditions is in accord with Scheme 1.[3] Indoxyl anion **2** can react by single-electron transfer (SET) with molecular oxygen to form a merostabilized indoxyl radical **3**. Merostabilization, known alternatively as captodative (push−pull) stabilization, is anticipated when a radical center is geminally substituted by both electron-donating (e.g., O, N, S) and electron-withdrawing groups (e.g., carbonyls).[4,5] Dimerization of indoxyl radicals produces leucoindigo **4**, a colorless, reduced form of indigo. A second oxidation then leads to formation of the colored dye **5**.

Tryptophan dimers are a second class of important indole dimers. They are formed by exposure of tryptophan derivatives to anhydrous acid.[6−9] In contrast to indigoids, the chemistry of tryptophan dimers is dominated by ionic reactions. We have found that tryptophan dimer **6** may be cleaved under acidic conditions with concomitant amide bond hydrolysis (Scheme 2). This cleavage probably involves a retro-Mannich fragmentation of intermediate **7**.

Scheme 1



Scheme 2



Recently, a series of leucoindigoid natural products were obtained from the damaged fruiting bodies of two fungi (*Collybia peronata* and *Tricholoma scalpturatum*).[10] Four biindolinones were isolated: peronatin A (**8a**), peronatin B (**8b**), 7,7′-dimethoxyperonatin B (**9b**), and 7-hydroxy-7′-methoxyperonatin B (**10**). The isolation of meso isomer 7,7′-dimethoxyperonatin A (**9a**) was not reported. Like indigo, these compounds are not found in the intact plant but are believed to result from enzymatic hydrolysis of an indole precursor and oxidative coupling of the derived indoxyl radicals.[10] Unlike indigo,

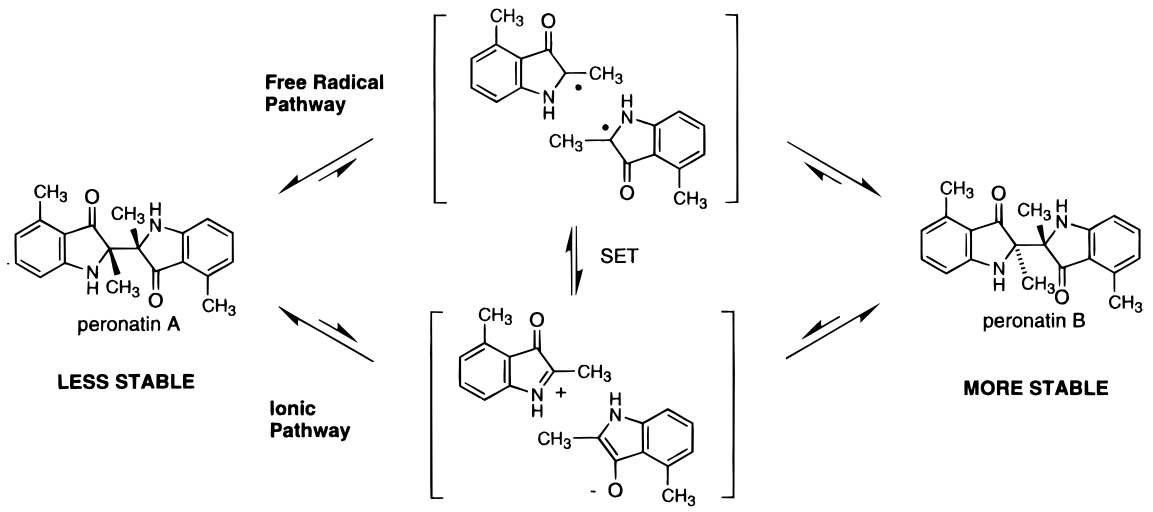
† Illinois EPR Research Center.

⊗ Abstract published in *Advance ACS Abstracts,* June 15, 1997.

(1) Pettit, F. H. *America's Indigo Blues: Resist-printed and Dyed Textiles of the Eighteenth Century*; Hastings House: New York, 1974.
(2) Leix, A. *Ciba-Rev.* **1938**, *1*, 422.
(3) Russell, G. A.; Kaupp, G. *J. Am. Chem. Soc.* **1969**, *91*, 3851.
(4) Baldock, R. W.; Hudson, P.; Katritzky, A. R.; Soti, F. *J. Chem. Soc., Perkin Trans. 1* **1974**, 1422.
(5) Viehe, H. G.; Janousek, Z.; Merenyi, R.; Stella, L. *Acc. Chem. Res.* **1985**, *18*, 148.
(6) Hashizume, K.; Shimonishi, Y. *Bull. Chem. Soc. Jpn.* **1981**, *54*, 3806−3810.
(7) Omori, Y.; Matsuda, Y.; Aimoto, S.; Shimonishi, Y.; Yamamoto, M. *Chem. Lett.* **1976**, 805.
(8) Stachel, S. J.; Habeeb, R. L.; Van Vranken, D. L. *J. Am. Chem. Soc.* **1996**, *118*, 1225.
(9) Carter, D. S.; Van Vranken, D. L. *Tetrahedron Lett.* **1996**, *37*, 5629.
(10) Pang, Z.; Sterner, O. *J. Nat. Prod.* **1994**, *57*, 852.

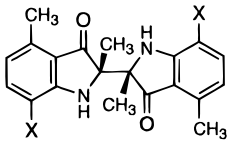
S0022-3263(97)00388-5 CCC: $14.00

Scheme 3

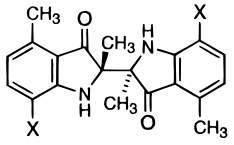


the leuco dimers **8**, **9**, and **10** are resistant to further oxidation because of the 2,2′-methyl substituents.

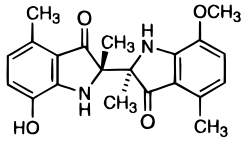
PERONATINS



8a X = H
9a X = OCH_3



8b X = H
9b X = OCH_3

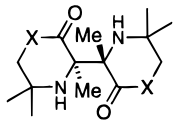


10

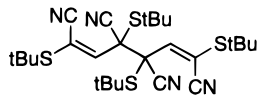
Sterner had previously noted that peronatin A converts slowly to peronatin B in deuteriochloroform.[10] A homolytic dissociation/recombination mechanism was proferred for this reaction since the intermediate radicals should be stabilized by captodative substitution (Scheme 3). However, an acid-catalyzed retro-Mannich/Mannich process (Scheme 3, ionic pathway) would also provide a plausible mechanism since the isomerization was noted in deuteriochloroform where DCl is a common contaminant. Since radical intermediates and ion pairs can interconvert by disproportionation, the nature of the monomeric intermediates does not necessarily reflect the mechanism for dimer cleavage. Despite this complication, and because of our interest in Mannich/retro-Mannich processes in 2,2′-indole dimers, we set out to synthesize the peronatins via a biomimetic pathway and investigate the isomerization of peronatin A to peronatin B.

Specifically, we wished to synthesize the peronatins and methoxyperonatins and answer three questions: (i) Does 7,7′-dimethoxyperonatin A isomerize to 7,7′-dimethoxyperonatin B? (ii) Can acids catalyze the isomerization of peronatin A to peronatin B? (iii) Are persistent radicals present during the isomerization of peronatin A to peronatin B?

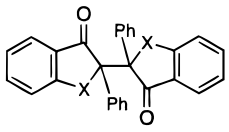
Steric congestion and captodative substitution are known to facilitate homolytic carbon−carbon bond cleavage leading to persistent radicals. For example, at temperatures above 80 °C, the symmetrical dimers **11**−**14** give well-resolved EPR spectra attributable to the monomeric radical species.[11−13] Biindolinone **15** was also proposed to cleave homolytically, but well-resolved EPR signals were not observed.[4] In contrast to the symmetrical dimers, unsymmetrical dimers **16**, **17**, and **18** dissociate to give either radicals or ionic intermediates, depending on the polarity of the solvent.[14−16] Information regarding the mechanism of dimer cleavage was gleaned from kinetic behavior in the case of dimers **11**, **12**, and **16**. In no case has acid been shown to accelerate homolytic cleavage of a symmetrical dimer. In fact, dissociation of **11** has been shown to be **inhibited** at lower pH, presumably due to protonation of the nitrogen.[17]



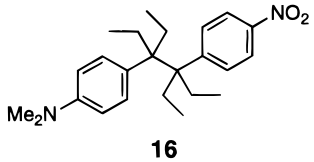
11 X = O
12 X = NH



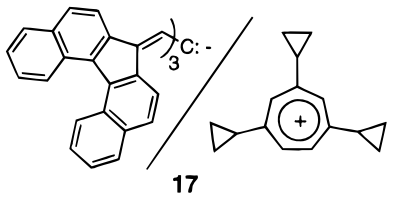
13



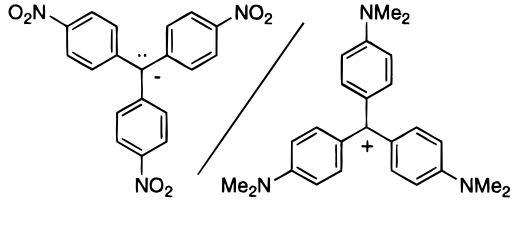
14 X = S
15 X = NH



16

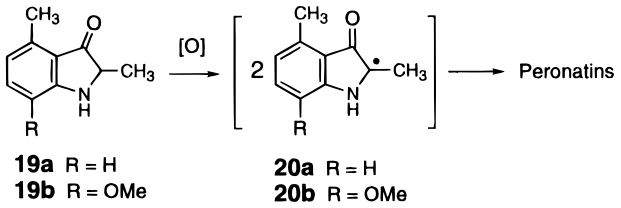


17

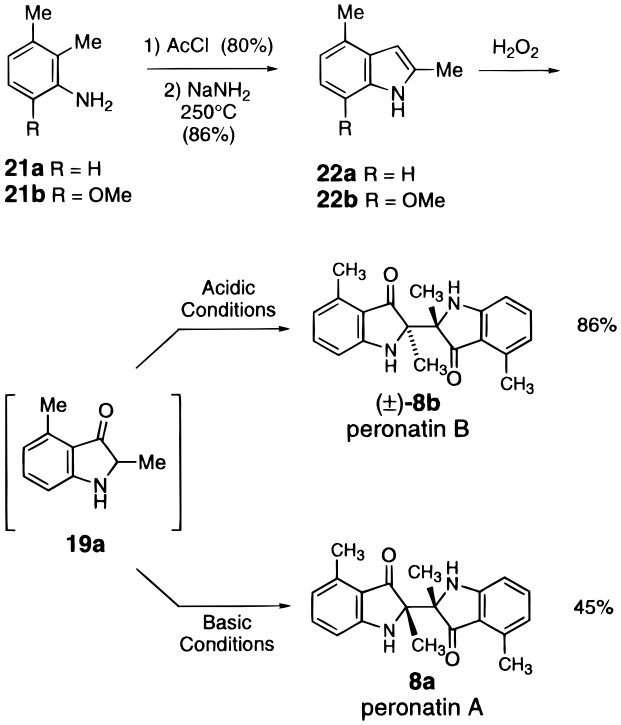


18

Scheme 4



Scheme 5

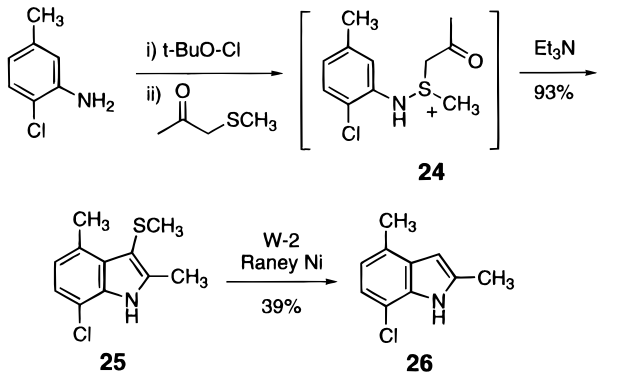


2. Synthesis of the Peronatins and 7,7′-Dimethoxyperonatins

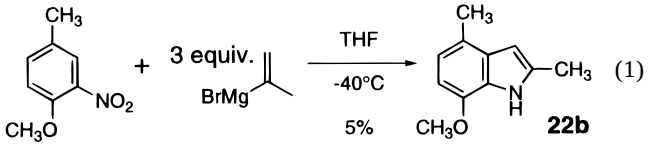
We sought to synthesize the peronatins using a biomimetic coupling reaction involving indolin-2-one intermediates **19a** and **19b** (Scheme 4). Oxidation of 2-substituted indoles with hydrogen peroxide is known to afford biindolinones, presumably via indolin-2-ones and their tautomeric indoxyls.[18] At the outset, it was not clear whether we would have access to both the peronatins by this route. While peronatin A was known to isomerize to peronatin B, there was no evidence for the converse isomerization.

2,4-Dimethylindole was prepared from 2,3-dimethylacetanilide by a modification of the procedure of Baude.[19] Madelung ring closure to give 2,4-dimethylindole was accomplished with sodium amide at 250 °C and proceeded in a gratifying 86% yield (Scheme 5). Oxidative dimerization gave different product mixtures depending on the reaction conditions. When hydrogen peroxide in acetic acid was used, the only dimeric product that could be isolated was (±)-peronatin B. However, when the oxidation is performed with hydrogen peroxide and triethylamine, both peronatin A (45%) and (±)-peronatin B (4%) are formed.

Scheme 6

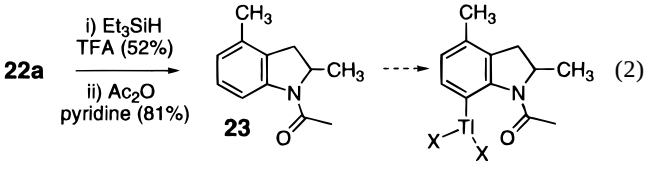


Our next goal was to synthesize the 7,7′-dimethoxyperonatins **9a** and **9b** and assess their relative reactivities. The methoxyperonatins offer a unique means of studying electronic effects on the isomerization of peronatin A to peronatin B since the methoxy groups are distal from the bond-forming/bond-cleaving centers. To exploit the oxidation/dimerization in Scheme 4 for the methoxyperonatins required access to 7-methoxy-2,4-dimethylindole **22b**. Unfortunately, o-alkoxytoluidines such as **21b** are notoriously poor substrates for the Madelung reaction, so several alternative routes were investigated for the synthesis of methoxyindoles. The most direct route to **22b** (addition of 2-propenylmagnsium bromide to 4-methyl-2-nitroanisole) gave the desired indole, albeit in 5% yield (eq 1). Poor yields are to be



(1)

expected from this reaction when the *O*-alkyl group is not bulky, so we turned our efforts to alternative longer, but more efficient, routes to the methoxyindole **22b**. We next sought to introduce oxygen into 2,4-dimethylindole by way of the indoline (eq 2). Indole **22a** was reduced

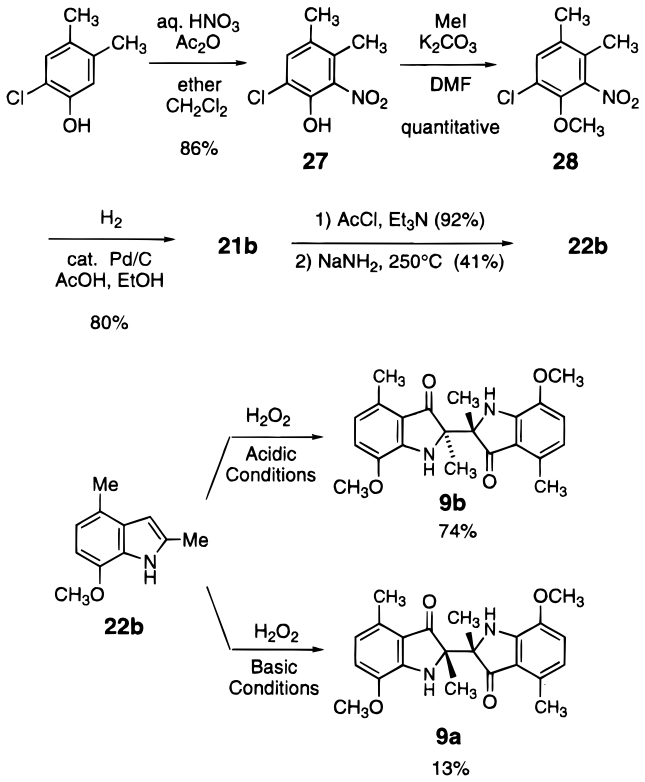


(2)

with triethylsilane in trifluoroacetic acid, and the indoline nitrogen was then acetylated to provide a handle for a directed thallation. Subsequent thallation of **23** with thallium tris(trifluoroacetate) gave intractable mixtures, so we next turned to syntheses of 7-chloro-2,4-dimethylindole by the Gassman route.[20] Sommelet−Hauser rearrangement of **24** afforded 7-chloroindole derivative **25** (Scheme 6). This method can not be used to prepare 7-alkoxyindoles directly because *N*-chloroanisidines are unstable, even at −78 °C. Unfortunately, low yields in the desulfurization to give indole **26** required recycling of unreacted sulfide. This difficulty, combined with

(11) Bennett, R. W.; Wharry, D. L.; Koch, T. H. *J. Am. Chem. Soc.* **1980**, *102*, 2345.
(12) Himmelsbach, R. J.; Barone, A. D.; Kleyer, D. L.; Koch, T. H. *J. Org. Chem.* **1983**, *48*, 2989.
(13) Van Hoecke, M.; Borghese, A.; Penelle, J.; Merenyi, R.; Viehe, H. G. *Tetrahedron Lett.* **1986**, *27*.
(14) Maslak, P.; Narvaez, J. N. *Ang. Chem., Int. Ed. Engl.* **1990**, *29*, 283.
(15) Komatsu, K.; Aonuma, S.; Takeuchi, K.; Okamoto, K. *J. Org. Chem.* **1989**, *54*, 2038.
(16) Arnett, E. M.; Molter, K. E.; Marchot, E. C.; Donovan, W. H.; Smith, P. *J. Am. Chem. Soc.* **1987**, *109*, 3788.
(17) Koch, T. Personal communication.
(18) Piozzi, F.; Langella, M. R. *Gazz. Chim. Ital.* **1963**, 1373.
(19) Noland, W. E.; Baude, F. J. *J. Org. Chem.* **1966**, *1966*, 3321.
(20) Gassman, P. G.; van Bergen, T. J.; Gilbert, D. P.; Cue, B. W., Jr. *J. Am. Chem. Soc.* **1974**, *96*, 5495.

Scheme 7



problems in the halide displacement, led us to return to the Madelung cyclization.

We returned to the original Madelung route to the methoxyindole **22b**. The key aniline derivative **21b** was synthesized from 2-chloro-4,5-dimethylphenol (Scheme 7). Nitration under biphasic conditions followed by methylation with methyl iodide gave the 2-nitroanisole derivative **28**. The chlorine group that was used to block the 6 position of the phenol during the nitration was then removed during reduction of the nitro group to afford aniline **21b** in excellent yield. As expected, Madelung cyclization of **21b** was inefficient, and yields for indole **22b** were typically around 40%. Again, the oxidation/dimerization reaction gave different results under acidic and basic conditions. Acidic conditions (H_2O_2/acetic acid) favor formation of (±)-methoxyperonatin B, **9b**, whereas basic conditions favor formation of methoxyperonatin A. The methoxyperonatins are much less stable than the peronatins. Meso isomer **9a** was so unstable that solutions could not be stored without decomposition. It is quite possible that this compound is formed upon damage to the fruiting bodies of *T. scalpturatum*, but the instability precluded isolation.

3. Relative Stabilities of Peronatin A and Peronatin B

Previously reported ^{1}H NMR data for the peronatins suggested the importance of hydrogen-bonding in the solution conformation of peronatin B, which is not apparent in the less stable peronatin A. The inaccessibility of a similar hydrogen bonding geometry for peronatin A was attributed to an unfavorable interaction between the 2- and 2′-methyl substituents.[10] To put this observation on a more quantitative footing, Monte Carlo searches were performed on the structures of peronatin A and B using Macromodel with MM3* force fields ($CHCl_3$).

Peronatin B possesses a global minimum energy conformation that allows hydrogen bonding between amino hydrogens and carbonyl oxygen atoms, whereas a similar arrangement in peronatin A leads to unfavorable eclipsing interactions between methyl groups (Figure 1). At the AM1 level, the lowest energy conformation of peronatin B is 0.96 kcal/mol lower in energy than the lowest energy conformation of the meso isomer peronatin A. If the free energy of mixing for enantiomers is taken into account (−0.41 kcal/mol at room temperature),[21] an equilibrium ratio of 10:1 in favor of peronatin B is predicted, in qualitative agreement with all observations. These results suggest that the product distribution (mainly peronatin A) obtained from the **oxidative dimerization of 2,4-dimethylindole under basic conditions is under kinetic control, while acidic conditions proceed under thermodynamic control to afford the more stable product, peronatin B**. The relative stabilities of the 7,7′-dimethoxyperonatins **9a** and **9b** mirror the stabilities of **8a** and **8b**, respectively.

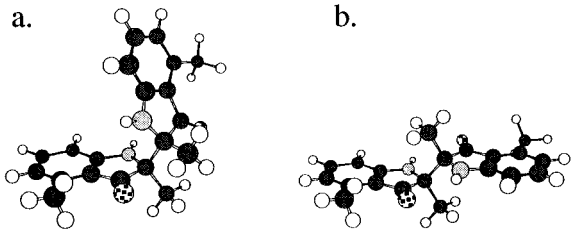


Figure 1. Minimum energy conformation of (a) peronatin A (**8a**) and (b) peronatin B (**8b**).

4. Isomerization of Peronatin A to Peronatin B

In anhydrous chloroform, peronatin A isomerizes slowly to peronatin B (*vide supra*). This isomerization is not clean, and small amounts of unidentified products are formed. A number of methods were explored to address the nature of the intermediates that are present during the isomerization process. While the rate of isomerization increases with temperature, no EPR signals were observed for degassed chloroform solutions of peronatin A or B at temperatures up to 100 °C. Unfortunately, numerous attempts to trap out or react with intermediates (radical or ionic) in the isomerization using TEMPO, isatin,[11] 2,2-diphenylpicrylhydrazyl,[12] and $NaCNBH_3$[22] led either to complex mixtures of products or no reaction. Thus, the identity of the intermediates in this reaction remains elusive.

We have found the isomerization of peronatin A to peronatin B in chloroform to be catalyzed by Brønsted acids and inhibited by amine bases. As shown in Figure 2a, when a solution of peronatin A in chloroform (filtered through anhydrous, activated alumina) is allowed to stand in the dark under nitrogen, the concentration of peronatin A decreases as the concentration of peronatin B increases. In contrast, when 10 mol % camphorsulfonic acid is added to a solution of peronatin A in chloroform, peronatin A rapidly and cleanly isomerizes to form peronatin B (Figure 2b). However, peronatin B is not stable under these conditions and over many hours decomposes to give an array of products. As the peronatin B decomposes, the ratio of peronatin B to peronatin A stays within the range of 10−20:1. If 1 equiv of triethylamine is added to the solution, then neither isomerization or decomposition is observed. Similarly,

(21) Eliel, E. L.; Wilen, S. H. *Stereochemistry of Organic Compounds*; John Wiley & Sons: New York, 1994.
(22) Hassner, A.; Haddadin, M. J. *J. Org. Chem.* **1963**, *28*, 224.

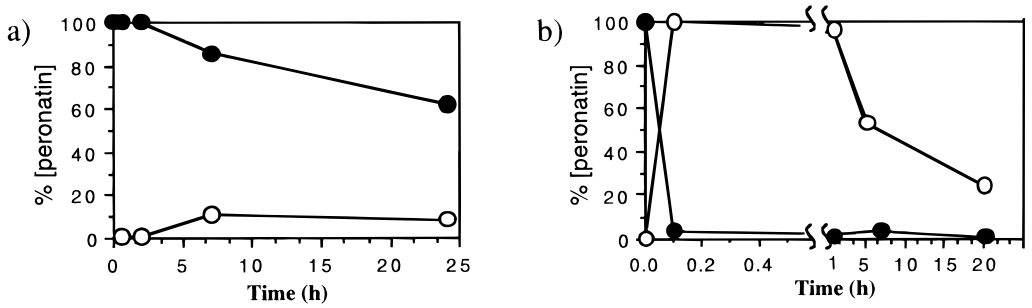


Figure 2. Isomerization of peronatin A to peronatin B expressed as a percentage of the original concentration of peronatin A (−●−, peronatin A; −○−, peronatin B); (a) in chloroform, (b) in chloroform + 10 mol % camphorsulfonic acid (note the broken time scale). Reaction progress was monitored by HPLC using *o*-nitrotoluene as an internal standard.

in THF, peronatin A is stable over 24 h. It is clear from these results that acid accelerates both isomerization and decomposition of the peronatins. At low concentrations of acid, the rate of formation of peronatin B is comparable to the rate of decomposition, whereas at high concentrations of acid the rate for formation of peronatin B is much faster than the rate of decomposition. In nearly stoichiometric quantities, BF_3 catalyzes the isomerization of peronatin A to peronatin B, but it also catalyzes decomposition to a range of products. Similarly, TMSOTf accelerates both isomerization and decomposition; however, triflic acid, produced in the reaction mixture, might be responsible for some of the observed effects.

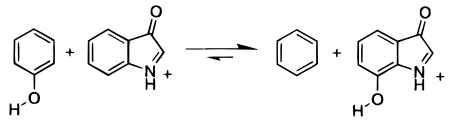
The electron-rich methoxyperonatin A displayed patterns of isomerization and decomposition that were similar to peronatin A, but the reactivity was much higher. 7,7′-Dimethoxyperonatin A could not be stored without the addition of triethylamine as a stabilizer. In the context of an acid-catalyzed retro-Mannich C−C bond cleavage, the decreased stability of 7,7′-dimethoxyperonatin A relative to peronatin A is consistent with the Hammond postulate. The electron-donating *o*-methoxy group should stabilize the α-ketoiminium intermediate[23] (Scheme 3) and the energetically similar endothermic transition state.

We clearly wanted to demonstrate the existence of either radical or ionic species in the reaction mixture. However, evidence for the existence of radical species, ionic species, or both would not have distinguished between a homolytic or heterolytic bond-cleavage mechanism. Kinetic evidence is the single best source of information regarding the initial C−C bond cleavage, and we have shown that the isomerization is acid catalyzed. In theory, acid catalysis does not rule out a homolytic cleavage mechanism. However, since acid-catalyzed homolysis is unprecedented in captodative systems, it seems reasonable to assume that a traditional retro-Mannich/Mannich mechanism is operative.

5. Conclusion

In summary, we have synthesized peronatins A and B and 7,7′-dimethoxyperonatins A and B using a biomimetic indoxyl dimerization. The isomerization of peronatin A to peronatin B is catalyzed by acid and inhibited by base, but firm evidence regarding the intermediates in the isomerization of peronatin A to peronatin B could not be obtained by trapping or EPR spectroscopy. On the basis of these results, we are drawn toward the conclusion that the acid-catalyzed dissociation of peronatin A proceeds by the ionic pathway shown in Scheme 3 and that the uncatalyzed isomerization is not important under ambient conditions. The methoxyperonatins display a similar pattern of reactivity but are much less stable than the peronatins.

(23) Gas phase HF/6-31G*//HF/6-31G* calculations show the following isodesmic reaction to be exothermic by 1.94 kcal/mol.



6. Experimental Section

All reactions were run under an atmosphere of dry nitrogen unless otherwise indicated. Unless otherwise noted, organic solvents were anhydrous and oxygen free. Melting points are uncorrected.

(±)-Peronatin B, 8b. To a stirred solution of 2,4-dimethylindole (0.85 g, 5.86 mmol) in acetic acid (5.9 mL) was added 7 mL of aqueous hydrogen peroxide solution (30% w/w). The reaction was stirred at room temperature for 2.5 h and then adjusted to pH 7 with 10% NaOH followed by extraction with ethyl acetate (3 × 50 mL). The combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo* to afford the crude product. The crude material was adsorbed onto silica gel (3.0 g) and chromatographed on silica gel (5% ethyl acetate/hexanes) to provide peronatin B as a yellow solid (0.76 g, 81%). Spectroscopic data for synthetic peronatin B (IR, ^{13}C NMR, ^1H NMR, LRMS, and HRMS) matched the data reported by Sterner.[10]

8b: mp 183−186 °C (EtOAc); R_f = 0.48 20% ethyl acetate/hexane. Anal. Calcd for $C_{20}H_{20}N_2O_2$: C, 74.96; H, 6.30; N, 8.75. Found: C, 74.87; H, 6.34; N, 8.66.

Peronatin A, 8a. To a stirred solution of 2,4-dimethylindole (0.667 g, 4.70 mmol) in THF (8.0 mL) was added triethylamine (1.42 g, 14.1 mmol) followed by 5.5 mL of aqueous hydrogen peroxide solution (30% w/w). The reaction was stirred at room temperature for 10 days, after which time it was diluted with H_2O (10 mL) and extracted with ethyl acetate (3 × 30 mL). The combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo* to afford the crude product. Chromatography on silica gel (5% ethyl acetate/hexanes) provided peronatin A (0.34 g, 45%) in addition to peronatin B (0.027 g, 4%) and recovered starting material (0.128 g, 19%). Spectroscopic data for synthetic peronatin A (IR, ^{13}C NMR, ^1H NMR, LRMS, and HRMS) matched the data reported by Sterner.[10]

8a: mp 117−120 °C (CH_2Cl_2); R_f = 0.31 in 20% ethyl acetate/hexane. Anal. Calcd for $C_{20}H_{20}N_2O_2$: C, 74.46; H, 6.30; N, 8.75. Found: C, 75.03; H, 6.31; N, 8.74.

7-Methoxy-2,4-dimethylindole, 22b. To a solution of magnesium turnings (0.402 g, 16.53 mmol) in diethyl ether (17 mL) was added dropwise a solution of 2-bromopropene in diethyl ether (5 mL). The solution was stirred for 1 h until all the magnesium was consumed. The freshly prepared organomagnesium reagent was then added, via cannula, to a flask containing 4-methyl-2-nitroanisole (0.92 g, 5.51 mmol) in THF (55mL) at −40 °C. The solution was then stirred at −40 °C for 1 h. The reaction was quenched by pouring over a saturated aqueous solution of ammonium chloride (50 mL).

The solution was then extracted with diethyl ether (3 × 50 mL). The organic layer was dried over $MgSO_4$ and concentrated *in vacuo* to afford the crude product. Chromatography on silica gel (2% ethyl acetate/hexanes) provided 7-methoxy-2,4-dimethylindole (0.0492 g, 5%) as a yellow oil.

22b: $R_f = 0.52$ in 20% ethyl acetate/hexanes; IR (KBr) 3391, 2936, 1524, 1259, 1091, 792 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 8.31 (s, 1H), 6.89 (d, $J = 7.6$ Hz, 1H), 6.61 (d, $J = 7.8$ Hz, 1H), 6.31 (d, $J = 1.2$ Hz, 1H), 4.03 (s, 3H), 2.57 (s, 3H), 2.49 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 143.8, 134.0, 130.0, 125.7, 121.6, 119.3, 101.1, 99.3, 55.3, 18.0, 13.5; LRMS (+ CI) 175 (100), 165 (38), 160 (29), 150 (7), 144 (11), 132 (44); HRMS (+CI) calcd for $C_{11}H_{13}NO$, 175.0997, found 175.0991. Anal. Calcd for $C_{11}H_{13}NO$: C, 75.39; H, 7.48; N, 8.0. Found: C, 75.50; H, 7.56; N, 8.01.

(±)-2,4-Dimethylindoline. To a stirred solution of 2,4-dimethylindole (1.0 g, 6.90 mmol) in trifluoroacetic acid (25 mL) was added triethylsilane (11.02 g, 69.0 mmol). The reaction was refluxed for 22 h, after which time the solvent was evaporated *in vacuo*. A 1 N HCl solution (100 mL) was added to the syrup and the resulting mixture extracted with ethyl acetate (2 × 50 mL). The aqueous layer was basified with 2N NaOH and extracted with ethyl acetate (3 × 50mL). The combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The crude material was adsorbed on silica gel (5.0 g) followed by silica gel chromatography (5% ethyl acetate/hexanes) to provide 2,4-dimethylindoline as a clear oil (0.62 g, 61%).

2,4-Dimethylindoline: $R_f = 0.52$ (20% ethyl acetate/hexanes); IR (neat) 3366, 2961, 1601, 1466, 1256, 767 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 6.91 (t, $J = 7.7$ Hz, 1H), 6.51 (d, $J = 7.6$ Hz, 1H), 6.43 (d, $J = 7.6$ Hz, 1H), 3.97 (dd, $J = 7.2$, 15.3 Hz, 1H), 3.76 (s, br, 1H), 3.07 (dd, $J = 8.6$, 15.3 Hz, 1H), 2.53 (dd, $J = 7.9$, 15.5 Hz, 1H), 2.18 (s, 3H), 1.28 (d, $J = 6.4$ Hz, 3H); ^{13}C (125 MHz, $CDCl_3$) δ 150.6, 134.2, 127.5, 127.2, 119.6, 106.6, 54.8, 36.5, 22.5, 18.7; LRMS (CI+) 147 (100), 132 (88), 117 (46); HRMS (CI+) calcd for $C_{10}H_{13}N$ 147.1048, found 147.1051. Anal. Calcd for $C_{10}H_{13}N$: C, 81.57; H, 8.91; N, 9.52. Found: C, 81.63; H, 8.94; N, 9.40.

(±)-*N*-Acetyl-2,4-dimethylindoline, 23. To a solution of 2,4-dimethylindoline (0.90 g, 6.08 mmol) in pyridine (20 mL) was added acetic anhydride (0.96 g, 12.2 mmol). The reaction was stirred at room temperature for 30 min, after which time the solvent was evaporated *in vacuo*. The residue was then dissolved in ethyl acetate (50 mL) and rinsed with 1 N HCl (30 mL). The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. Chromatography on silica gel (1% MeOH/$CHCl_3$) provided *N*-acetyl-2,4-dimethylindoline (0.931 g, 81%) as a white solid.

23: mp 54−56 °C ($CHCl_3$); $R_f = 0.59$ (5% dichloromethane/chloroform); IR (neat) 2974, 1652, 1465, 1395, 1125, 990 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.86 (d, $J = 7.6$ Hz, 1H), 7.05 (t, $J = 7.4$ Hz, 1H), 6.83 (d, $J = 7.6$ Hz, 1H), 4.57 (m, 1H), 3.37 (m, 1H), 2.58 (d, $J = 15.9$ Hz, 1H), 2.21 (s, 3H), 2.19 (s, 3H), 1.20 (d, $J = 6.0$ Hz 3H); ^{13}C (125 MHz, $CDCl_3$) δ 167.8, 141.2, 133.9, 129.3, 126.9, 124.2, 114.3, 55.4, 34.6, 23.0, 21.7, 18.2; LRMS (CI+) 189 (88), 147 (67), 132 (100); HRMS (CI+) calcd for $C_{12}H_{15}CNO$ 189.1154, found 189.1154. Anal. Calcd for $C_{12}H_{15}NO$: C, 76.14; H, 7.99; N, 7.40. Found: C, 76.00; H, 8.05; N, 7.31.

7-Chloro-2,4-dimethyl-3-(methylthio)indole, 25. A solution of 2-chloro-5-methylaniline (5.0 g, 35.3 mmol) in dichloromethane (120 mL) was cooled to −78 °C in a dry ice/acetone bath. *tert*-Butyl hypochlorite (3.76 g, 35.31 mmol) in dichloromethane (15 mL) was then added, and the mixture was stirred for 10 min. The bath temperature was raised to −65 °C, and (methylthio)acetone (3.68 g, 35.31 mmol) was added in dichloromethane (15 mL). The solution was stirred for 1 h followed by the addition of triethylamine (3.57 g, 35.3 mmol). The reaction was quenched with H_2O (50 mL). The aqueous layer was removed, and the organic layer was dried over $MgSO_4$ followed by concentration *in vacuo*. Chromatography on silica gel (5% ethyl acetate/hexanes) provided 7-chloro-2,4-dimethyl-3-(methylthio)indole (7.4 g, 93%) as a red oil.

25: $R_f = 0.40$ (5% dichloromethane/hexanes); IR (neat) 3424, 2916, 1725, 1234, 760 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.33 (s, 1H), 6.97 (d, $J = 7.7$ Hz, 1H), 6.74 (d, $J = 7.8$ Hz, 1H), 2.83 (s, 3H), 2.46 (s, 3H), 2.18 (s, 3H); ^{13}C (125 MHz, $CDCl_3$) δ 140.5, 132.0, 129.4, 128.8, 122.6, 120.5, 113.4, 105.7, 21.9, 18.2, 11.8; LRMS (CI+) 225(100), 210 (61), 166 (11); HRMS (CI+) calcd for $C_{11}H_{12}ClNS$ 225.0379, found 225.0374. Anal. Calcd for $C_{11}H_{12}ClNS$: C, 58.66; H, 5.37; N, 6.22. Found: C, 58.59; H, 5.39; N, 6.21.

7-Chloro-2,4-dimethylindole, 26. To a solution of 7-chloro-2,4-dimethyl-3-(methylthio)indole (1.02 g, 4.52 mmol) in absolute ethanol (20 mL) was added freshly prepared Raney nickel (7.0 g). The mixture was stirred at room temperature for 24 h. The solution was then filtered through a pad of Celite and concentrated *in vacuo*. Chromatography on silica gel (5% dichloromethane/hexanes) provide 7-chloro-2,4-dimethylindole (0.31 g, 38%) as a clear oil.

26: $R_f = 0.42$ (33% dichloromethane/hexanes); IR (neat) 3424, 2907, 1231, 932, 790 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.98 (s, 1H), 6.98 (d, $J = 7.6$ Hz, 1H), 6.76 (d, $J = 7.6$ Hz, 1H), 6.21 (s, 1H), 2.44 (s, 3H); ^{13}C (125 MHz, $CDCl_3$) δ 135.2, 132.5, 130.0, 127.8, 120.5, 120.1, 113.0, 100.0, 18.3, 13.5; LRMS (CI+) 179 (100), 144 (66), 115 (15), 90 (11), 72 (19); HRMS (CI+) calcd for $C_{10}H_{10}ClN$ 179.0502, found 179.0502. Anal. Calcd for $C_{10}H_{10}ClN$: C, 67.02; H, 5.63; N, 7.82. Found: C, 67.11; H, 5.58; N, 7.77.

2-Chloro-6-nitro-4,5-dimethylphenol, 27. To a stirred solution of $NaNO_3$ (5.43 g, 63.9 mmol) in 4.6 M HCl (129 mL) was added a solution of 2-chloro-4,5-dimethylphenol (10.0 g, 63.9 mmol) dissolved in 3:2 diethyl ether:dichloromethane (387 mL). Acetic anhydride (0.72 g, 7.02 mmol) was added dropwise, and the biphasic reaction was stirred for 15 min, after which time the reaction was essentially complete. The aqueous layer was removed and extracted with diethyl ether (50 mL). The combined organic layers were then rinsed with H_2O (50 mL). The organic layer was dried over Na_2SO_4 and concentrated *in vacuo* to afford the crude product. Chromatography on silica gel (5% ethyl acetate/hexane) provided 2-chloro-6-nitro-4,5-dimethylphenol as a yellow solid (11.0 g, 86%).

27: mp 124−126 °C (EtOAc); $R_f = 0.41$ 20% ethyl acetate/hexane; IR (KBr) 3432, 1528, 1378, 1181, 1048, 874, 756 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.11 (s, 1H), 7.34 (s, 1H), 2.31 (s, 3H), 2.26 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 145.3, 139.1, 134.2, 130.8, 130.2, 119.3, 19.6, 15.8; LRMS (+CI) 201 (100), 196 (14), 184 (86), 171(7), 154 (36), 140 (6), 128 (9), 120 (15); HRMS (+CI) calcd for $C_8H_8NO_3Cl$, 201.0193, found 201.0188. Anal. Calcd for $C_8H_8NO_3Cl$: C, 47.76; H, 4.01; N, 6.97. Found: C, 47.84; H, 3.97; N, 6.94.

2-Chloro-6-nitro-4,5-dimethylanisole, 28. To a stirred solution of 2-chloro-6-nitro-4,5-dimethylphenol (10.27 g, 50.98 mmol) in DMF (100 mL) was added potassium carbonate (7.04 g, 54.6 mmol). The solution immediately turned red in color. Methyl iodide (7.96 g, 56.1 mmol) was then added dropwise, and the reaction was stirred at room temperature for 4.5 h. The reaction was quenched with H_2O (500 mL) and extracted using diethyl ether (3 × 150 mL). The combined organic layers were washed again with H_2O (200 mL). The organic layer was dried over $MgSO_4$ and concentrated *in vacuo* to afford the crude product. Chromatography on silica gel (5% ethyl acetate/hexanes) provided 2-chloro-6-nitro-4,5-dimethylanisole as a yellow solid (10.98 g, 100%).

28: mp 35−36 °C (EtOAc); $R_f = 0.62$ in 20% ethyl acetate/hexane; IR (KBr) 2945, 1540, 1285, 1064, 960, 910, 757, 735 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.26 (s, 1H), 3.91 (s, 3H), 2.26 (s, 3H), 2.13 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 147.6, 145.4, 134.7, 132.2, 127.5, 125.1, 62.4, 19.3, 13.9; LRMS (+CI) 216 (61) $[MH]^+$, 199 (100), 185 (10), 169 (7), 154 (7), 137 (10); HRMS (+CI) calcd for $C_9H_{10}NO_3Cl$, 215.0349, found 215.0314. Anal. Calcd for $C_9H_{10}NO_3Cl$: C, 50.22; H, 4.69; N, 6.51. Found: C, 50.24; H, 4.68; N, 6.48.

2-Methoxy-5,6-dimethylaniline, 21b. 2-chloro-6-nitro-4,5-dimethylanisole (5.0 g, 23.20 mmol) was dissolved in ethanol (100 mL) in a Parr flask. Acetic acid (5 mL) was added followed by 10% Pd/C (1.0 g). The flask was then charged to 60 psi with hydrogen and shaken in a Parr apparatus at room temperature 8 h. The reaction was then filtered through a pad of Celite and concentrated *in vacuo* to afford the crude

product. Chromatography on silica gel (5% ethyl acetate/hexanes) provided 2-methoxy-5,6-dimethyaniline as a clear oil (2.61 g, 80%).

21b: R_f = 0.48 20% ethyl acetate/hexane; IR (KBr) 3468, 2937, 1609, 1241, 789, 761 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.59 (d, J = 8.0 Hz, 1H), 6.56 (d, J = 8.4 Hz, 1H), 3.82 (s, 3H), 2.21 (s, 3H), 2.07 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 145.2, 133.9, 128.6, 120.8, 118.6, 107.2, 55.3, 19.6, 12.7; LRMS (+CI) 151 (100), 136 (78); HRMS (+CI) calcd for $C_9H_{13}NO$ 151.0997, found 151.0993. Anal. Calcd for $C_9H_{13}NO$: C, 71.48; H, 8.67; N, 9.27. Found: C, 71.53; H, 8.68; N, 9.32.

***N*-(2,3-Dimethyl-6-methoxyphenyl)acetamide.** To a stirred solution of 2-methoxy-5,6-dimethylaniline (1.85 g, 12.33 mmol) in anhydrous THF (100 mL) at room temperature was added triethylamine (1.87 g, 18.5 mmol) followed by acetyl chloride (1.06 g, 13.57 mmol). The mixture was stirred at room temperature for 3 h. The precipitated triethylamine hydrochloride was filtered off, and the filtrate was concentrated *in vacuo*. The residue was then dissolved in ethyl acetate (100 mL) and washed with 1 N HCl (50 mL) and then H_2O (50 mL). The organic layer was dried over $MgSO_4$ and concentrated *in vacuo* to afford the crude product. Chromatography on silica gel (2% MeOH/$CHCl_3$) afforded N-(2,3-dimethyl-6-methoxyphenyl)acetamide as a white solid (2.20 g, 92%).

***N*-(2,3-Dimethyl-6-methoxyphenyl)acetamide:** mp 146−148 °C ($CHCl_3$); R_f = 0.66 in 10% MeOH/$CHCl_3$; IR (KBr) 3248, 2919, 1663, 1478, 1087, 745 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6, 40 °C) δ 8.95 (s, 1H), 6.99 (d, J = 8.3 Hz, 1H), 6.73 (d, J = 8.3 Hz, 1H), 3.69 (s, 3H), 2.16 (s, 3H), 1.99 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6, 40 °C) δ 167.9, 152.8, 134.9, 128.0, 127.6, 125.1, 108.3, 53.3, 22.5, 19.2 14.4; LRMS (+CI) 193 (100), 176 (13), 162 (6), 151 (80), 136 (80), 131 (5), 119 (7); HRMS (+CI) calcd for $C_{11}H_{15}NO_2$ 193.1103, found 193.1102. Anal. Calcd for $C_{11}H_{15}NO_2$: C, 68.35; H, 7.83; N, 7.25. Found: C, 68.25; H, 7.88; N, 7.22.

7-Methoxy-2,4-dimethylindole, 22b. To a dry 250 mL round-bottom flask containing *N*-(2,3-dimethyl-6-methoxyphenyl)acetamide (1.30 g, 6.74 mmol) under N_2 was added 95% sodamide (0.73 g, 17.7 mmol). The mixture was heated slowly to 250 °C. The brown melted material was then cooled to 100 °C followed by the addition of H_2O (20 mL). The mixture was then refluxed gently for 15 min. The mixture was partitioned between water (50 mL) and diethyl ether (100 mL). The aqueous layer was removed and extracted with diethyl ether (3 × 100 mL). The combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo* to afford the crude product. The crude material was adsorbed on silica gel (10 g) and chromatographed on silica gel (5% ethyl acetate/hexanes) to provide 7-methoxy-2,4-dimethylindole as a yellow oil (0.479 g, 41%).

22b: R_f = 0.52 20% ethyl acetate/hexanes; IR (KBr) 3391, 2936, 1524, 1259, 1091, 792 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.31 (s, 1H), 6.89 (d, J = 7.6 Hz, 1H), 6.61 (d, J = 7.8 Hz, 1H), 6.31 (d, J = 1.2 Hz, 1H), 4.03 (s, 3H), 2.57 (s, 3H), 2.49 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 143.8, 134.0, 130.0, 125.7, 121.6, 119.3, 101.1, 99.3, 55.3, 18.0, 13.5; LRMS (+CI) 175 (100), 165 (38), 160 (29), 150 (7), 144 (11), 132 (44); HRMS (+CI) calcd for $C_{11}H_{13}NO$ 175.0997, found 175.0991. Anal. Calcd for $C_{11}H_{13}NO$: C, 75.39; H, 7.48; N, 8.0. Found: C, 75.50; H, 7.56; N, 8.01.

(±)-7,7′-Methoxyperonatin B, 9b. To a stirred solution of 7-methoxy-2,4-dimethylindole (0.184 g, 1.02 mmol) in acetic acid (1.0 mL) was added 1.2 mL of aqueous hydrogen peroxide solution (30% w/w). The reaction was stirred at room temperature for 30 min and then adjusted to pH 7 with 10% NaOH followed by extraction with chloroform (3 × 20 mL). The combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo* to afford the crude product. The crude material was adsorbed onto silica gel (3.0 g) and chromatographed on silica gel (50% chloroform/hexane) to provide 7,7′-methoxyperonatin B as a yellow solid (0.144 g, 74%). Spectroscopic data for synthetic 7,7′-dimethoxyperonatin B (IR, ^{13}C NMR, ^{1}H NMR, LRMS, and HRMS) matched the data reported by Sterner.[1]

9b: mp 186 °C dec ($CHCl_3$); R_f = 0.33 in $CHCl_3$. Anal. Calcd for $C_{22}H_{24}N_2O_4$: C, 69.44; H,6.36; N, 7.37. Found: C, 69.27; H, 6.45; N, 7.26.

7,7′-Methoxyperonatin A, 9a. To a stirred solution of 7-methoxy-2,4-dimethylindole (0.479 g, 2.74 mmol) in THF (11 mL) was added triethylamine (3.0 g, 21.5 mmol) followed by 8.0 mL of aqueous hydrogen peroxide solution (30% w/w). The reaction was stirred at room temperature for 72 h, after which time it was diluted with H_2O (10 mL) and extracted with chloroform (3 × 30 mL). Triethylamine (5 mL) was added to the combined organic extracts to prevent premature isomerization. The combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo* to afford the crude product. Preparative TLC chromatography on silica gel (10% ethyl acetate/hexane/1% triethylamine) provided the desired 7.7′-methoxyperonatin A (0.068 g, 13%) as a yellow solid.

9a: R_f = 0.21 20% ethyl acetate/hexane; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.68 (d, J = 7.9 Hz, 1H), 6.44 (d, J = 7.8 Hz, 1H), 4.54 (s, 1H), 3.73 (s, 3H), 2.49 (s, 3H), 1.67 (s, 3H); ^{13}C (500 MHz, $CDCl_3$) δ 202.0, 150.4, 144.0, 131.1, 120.5, 116.8, 86.2, 55.6, 23.5, 17.3. The instability of this compound prevented complete characterization.

Isomerization Experiments, General. Conversion of peronatin A to peronatin B was monitored by analytical reversed-phase HPLC. Stationary phase: Rainin C_{18} microsorb. Mobile phase: A, 50 mM triethylammonium acetate buffer, pH 7.50; B, CH_3CN. Gradient elution: 40−70% B over 30 min, 254 nm detection.

Isomerization of 8a to 8b in $CHCl_3$. Peronatin A (0.034 g, 0.10 mmol) was dissolved 5 mL of a chloroform solution (filtered through anhydrous, activated, basic alumina) containing 0.02 M *o*-nitrotoluene as an internal standard. The solution was allowed to stand in the dark under nitrogen. Aliquots were removed, the chloroform was evaporated, and the samples were redissolved in an equal volume of THF for HPLC analysis.

Isomerization of 8a to 8b in $CHCl_3$ in the Presence of Base. Peronatin A (0.034 g, 0.10 mmol) was dissolved 5 mL of a chloroform solution (filtered through anhydrous, activated, basic alumina) containing 0.02 M *o*-nitrotoluene as an internal standard. To this solution was added triethylamine (0.01 g, 0.10 mmol). The solution was allowed to stand in the dark under nitrogen. Aliquots were taken, the chloroform evaporated, and the samples redissolved in an equal volume of THF for HPLC analysis. No isomerization was observed over 24 h.

Isomerization of 8a to 8b in $CHCl_3$ in the Presence of Acid. Peronatin A (0.017 g, 0.05 mmol) was dissolved 2.5 mL of a chloroform solution (filtered through anhydrous, activated, basic alumina) containing 0.02 M *o*-nitrotoluene as an internal standard. To this solution was added a 0.01 M camphorsulfonic acid solution (0.4 mL 0.005 mmol) in chloroform. The solution was allowed to stand in the dark under nitrogen. Aliquots were taken, the chloroform was evaporated, and the samples were redissolved in an equal volume of THF for HPLC analysis.

Reaction of 8b in $CHCl_3$ in the Presence of Acid. Peronatin B (0.017 g, 0.05 mmol) was dissolved 2.5 mL of a chloroform solution (filtered through anhydrous, activated, basic alumina) containing 0.02 M *o*-nitrotoluene as an internal standard. To this solution was added a 0.01 M camphlorsulfonic acid solution (0.4 mL 0.005 mmol) in chloroform. The solution was allowed to stand in the dark under nitrogen. Aliquots were taken, the chloroform was evaporated, and the samples were redissolved in an equal volume of THF for HPLC analysis.

Acknowledgment. We are grateful to Professor Tad Koch for helpful discussions. This material is based upon work supported by the Camille and Henry Dreyfus New Faculty Awards Program and the National Science Foundation under Grant No. CHE-9523521.

JO970388P