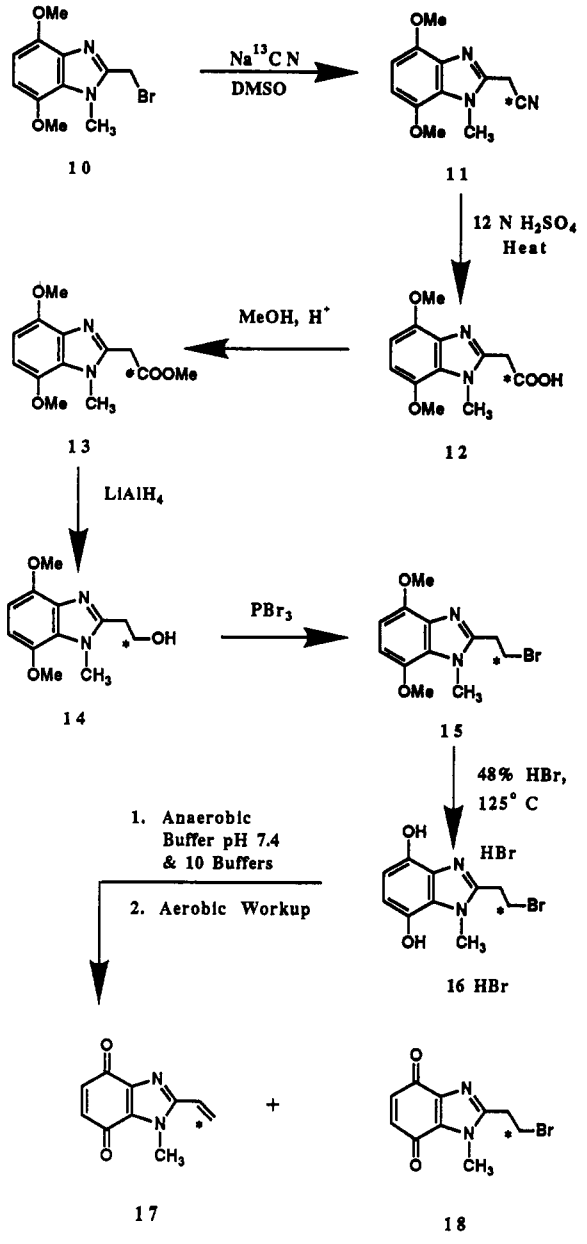
Chart IV



therefore be of the "E2" type wherein both proton and leaving group transfers occur simultaneously to afford the alkene.

The second-order rate constants for buffer catalysis were obtained by replotting the data in Figure1 as $((k_{obsd} - k_{lyate})(K_a + a_H))/[B_T]$ vs a_H where k_{obsd} is the observed rate constant, k_{lyate} is the rate constant at zero buffer, K_a is the dissociation constant of the buffer acid, a_H is the proton activity measured with a pH meter, and $[B_T]$ is the total buffer concentration.[12] The slope and intercept of this plot provide the values of k_{ga} and $k_{gb}K_a$ respectively, where k_{ga} is general acid-catalyzed rate constant and k_{gb} is the general base-catalyzed rate constant. For the acetate buffer system (plot A, Figure 1) the replot provided $k_{ga} = 1.28 \times 10^{-4}$ $M^{-1}s^{-1}$ and $k_{gb} = 2.81 \times 10^{-5}$ M^{-1} s^{-1}. Likewise,

(11) Examples of the realtive elimination capabilities of chloride and bromide are found in ref 1 and in the following refs: (a) Lemus, R. H.; Skibo, E. B. *J. Org. Chem.* **1988**, *56*, 6099. (b) Bingham, R. C.; Schleyer, P. v. R. *J. Am. Chem. Soc.* **1971**, *93*, 3189. (c) Howells, R. D.; McCown, J. D. *Chem. Rev.* **1977**, *77*, 69.

(12) See eq 17 in ref 17 and the accompanying discussion for the graphical method of obtaining general acid and general base second-order rate constants.

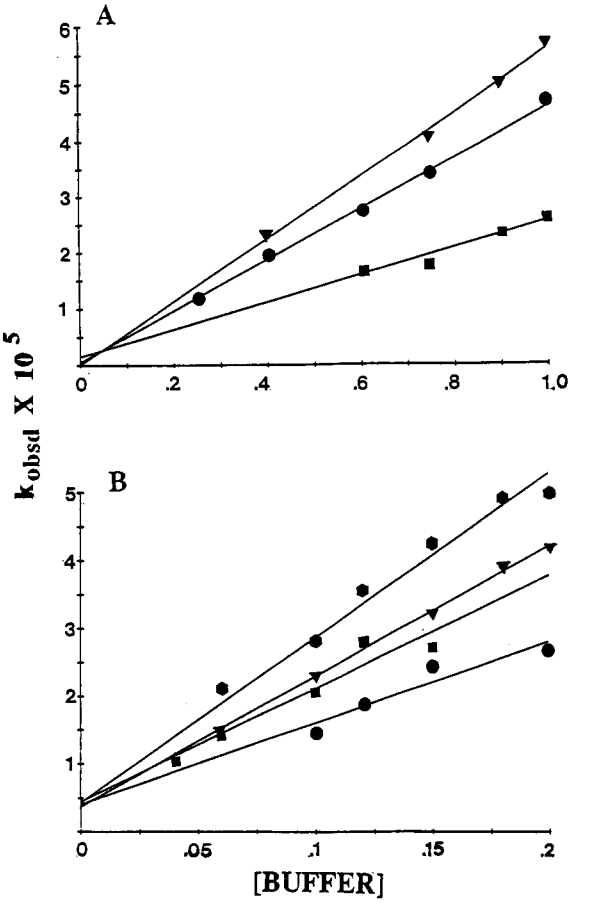


Figure 1. Buffer dilution plots obtained in μ = 1.0 (KCl) anaerobic buffer at 30.0 ± 0.2 °C. Plot A: ▼, pH 5.00; ●, pH 5.5; ■, pH 6.10 acetate buffers. Plot B: ▼, pH 6.4; ●, pH 6.6; ■, pH 6.8; ●, pH = 7.1 phosphate buffer.

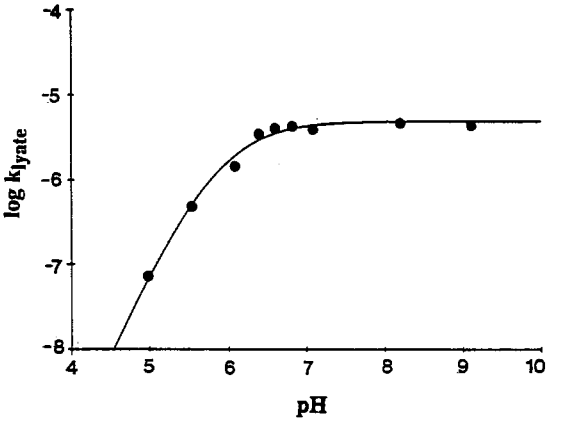


Figure 2. Log k_{lyate} vs pH plot for the conversion of **1** to **2** in anaerobic μ = 1.0 (KCl) aqueous buffers at 30.0 ± 0.2 °C.

the replot of phosphate buffer data in plot B, Figure 1, provided $k_{ga} = 2.947 \times 10^{-4}$ M^{-1} s^{-1} and $k_{gb} = 1.12 \times 10^{-4}$ M^{-1} s^{-1}.

Mechanism of Elimination. The mechanism of HBr elimination occurs by the process shown in Chart V wherein hydroxide abstracts a proton form neutral **1** while the bromide is eliminating. Other elimination processes include the general base- and specific acid–general base-catalyzed reactions illustrated in Chart VI. These "E2" processes avoid the formation of a carbon anion on an already electron-rich system (E1cB elimination) as well as the formation of a primary carbocation species (E1

Chart V

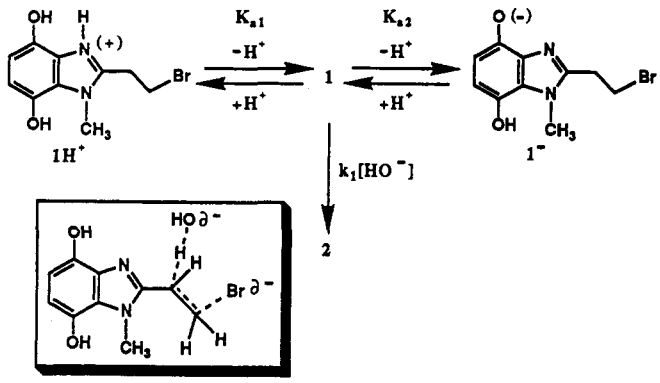
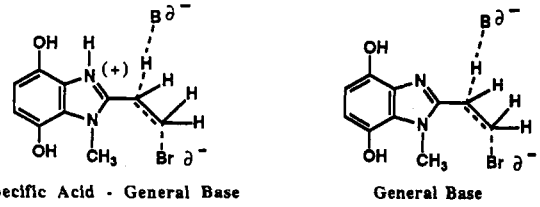


Chart VI



elimination).[9] The evidence which supports E2 processes is discussed in the following paragraphs.

The mechanism in Chart V is consistent with the +2 to 0 slope change observed in the pH-rate profile in Figure 2. Consideration of material balance in all the forms of 1 (= $1H^+ + 1 + 1^-$) and hydroxide-catalyzed elimination of HBr from 1 provides the following rate law:

$$k_{lyate} = \frac{k_1 K_{a1} K_w}{a_H^2 + K_{a1} a_H + K_{a1} K_{a2}} \quad (1)$$

where K_w is the autoprotolysis constant of water,[13] a_H is the proton activity determined with a pH meter, and k_1, K_{a1}, and K_{a2} are constants in Chart V. Equation 1 was computer fit to the data in Figure 2 resulting in the following solution: $k_1 = 245\ M^{-1}\ s^{-1}$, $pK_{a1} = 5.57$, and $pK_{a2} = 6.16$. This solution was used to generate the solid line shown in Figure 2. The kinetically obtained value for the dissociation constants of the protonated benzimidazole (pK_{a1}) is comparable to previously determined values, $pK_{a1} = 4$–6.[2] Acid dissociation from the hydroxyl group of a benzimidazole hydroquinone typically has pK_a values of 7.8 to 8.4.[2a] The value of pK_{a2} obtained from the pH-rate profile (6.16) seems a bit low, but feasible for the dissociation $1 \rightleftarrows 1^- + H^+$.

The pH rate data in Figure 2 could also be explained by loss of bromide from 1^-. Such a mechanism would have to involve the cyclopropyl intermediate shown in Chart I. The ^{13}C scrambling studies described in Experimental Section confirmed that such an intermediate cannot occur. Furthermore, the elimination of HBr also occurs from the *O*-methylated hydroquinone 8 in Chart III and therefore hydroxyl anion formation is not a requirement.

The strongest evidence for the hydroxide-mediated elimination of HBr from neutral 1, inset of Chart V, is the presence of general base catalysis. If acetate and phosphate (pK_a of respective conjugate acids are 4.61 and 6.56) act as general bases in the elimination process, so could the stronger base hydroxide (pK_a of conjugate acid is 15.60).[14] Evidence that hydroxide is acting as a general base was obtained from a Brønsted plot (not shown) wherein log k_{gb} values for the acetate and phosphate buffers, as well as the log of k_1 (Chart V) obtained from the pH-rate profile, are plotted against the respective conjugate acid pK_a values. The plot is linear with a slope of 0.65 and a correlation coefficient of 0.996. Since all three bases lie on the same Brønsted plot, the mechanism involved in elimination must be identical for these bases.

Other observations which support an "E2" process are the absence of deuterium exchange during elimination of HBr from 1 (see Experimental Section) and the slower rates of elimination when the bromide leaving group is substituted with chloride. Thus, the mechanism must involve irreversible proton abstraction in concert with elimination of the leaving group. The presence of general acid-catalyzed elimination of HBr from 1 seemed difficult to reconcile with an "E2" mechanism. The kinetic equivalent of general acid catalysis, specific acid/general acid catalysis illustrated in Chart VI, could be interpreted as an E2 process, however. Equilibrium protonation of 1 to afford $1H^+$ (specific acid catalysis) will electrostatically favor proton abstraction from the 1′ position by the general base. The rapid elimination rates below pH 6, where the predominate species in solution is $1H^+$, are likely due to the specific acid–general base process. For example, elimination of HBr from $1H^+$ in 1 M acetate pH 5.0 buffer proceeds at $5.7 \times 10^{-5}\ s^{-1}$ while the lyate-only rate is $7.3 \times 10^{-8}\ s^{-1}$. A specific acid–general base elimination process involving hydroxide, essentially "water catalyzed" elimination of HBr from 1, was never observed in this study. The hydroxide activity is no doubt too low at the pH range where $1H^+$ is present for this elimination process to occur.

The general acid-catalyzed rate constants calculated from the data in Figure 1 are converted to specific acid–general base-catalyzed rate constants by division by the K_a of the buffer ($\nu = k_{ga}[BH][1]$ for general acid catalysis and $\nu = k'a_H[B^-][1]$ for specific acid–general base catalysis, $\therefore k' = k_{ga}/K_a$). The calculated specific acid–general base-catalyzed rate constant for acetate is $k' = 5.2\ M^{-2}\ s^{-1}$ and that for phosphate is $k' = 1069\ M^{-2}\ s^{-1}$.

Conclusions

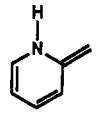
The overall conclusion of the kinetic study is that alkene formation from 1 involves both general base and specific acid–general base-catalyzed elimination of HBr. Formation of a spiro-fused cyclopropyl intermediate is not involved in the elimination process. The specific acid–general base mechanism permits elimination reactions even in acidic (pH < 6) buffer. The facility of benzimidazole alkene formation by this mechanism suggests that it could be useful in the synthesis of nitrogen-containing, electron-rich alkenes.

The remainder of this section is devoted to a discussion of the relationship between the mechanisms presented herein and previous work. Specific acid–general base-catalyzed tautomerism of methylated pyridines (Chart VII) is analogous to the catalyzed elimination of HBr from 1 in that base abstraction of a proton occurs from the protonated pyridine nitrogen.[15] If the specific acid–general base-catalyzed reaction of 1 proceeds without loss of the

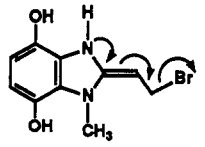
(13) pK_w at 30 °C is 13.83.

(14) pK_w at 30 °C is 15.60 when the concentration of water is taken as 55.5 M rather than the standard state activity of 1, see Jencks, W. *Catalysis in Chemistry and Enzymology*; McGraw-Hill; New York; pp 171–172.

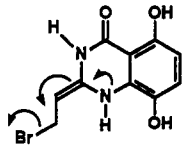
Chart VII



Methyl Pyridine Tautomer



HBr Elimination from Benzimidazole tautomer



HBr Elimination from Quinazolinone tautomer

bromide group, then the product will be the benzimidazole tautomer shown in Chart VII. Elimination of HBr from this tautomer could then afford the alkene product, **2**. Kinetic studies described herein indicate that a tautomeric benzimidazole does not build up in solution and that bromide elimination occurs during proton abstraction. However, a stepwise elimination process actually does occur in the bromoethyl quinazolinone system.[8,16] Thus, the general acid (or specific acid–general base)-catalyzed prototropic shift affords the quinazolinone tautomer shown in Chart VII which undergoes the non-rate-determining loss of bromide. The quinazoline- and benzimidazole-based haloethyl derivatives are essentially identical with respect to elimination chemistry with the only difference being the timing of leaving group loss.

Experimental Section

Elemental analyses were performed by Atlantic Microlab Inc., Norcross, GA. All analytically pure compounds were dried under high vacuum in a heating pistol with refluxing methanol. Melting points are uncorrected and decomposition points were characterized by color darkening without complete melting. All TLC was run with Merck silica gel 60 (F_{254}) plates, employing a variety of solvents. IR spectra were taken as KBr and NaCl thin film pellets; the strongest IR absorbances are reported. Both ^1H and ^{13}C NMR spectra were taken on a 300-MHz spectrometer; chemical shifts of proton spectra are reported relative to TMS. Mass spectral measurements were made with a low-resolution instrument in the electron impact mode.

Kinetic Studies. The kinetic studies were carried out in buffers prepared with doubly distilled water and adjusted to μ = 1.0 with KCl. The following buffer systems were employed to hold pH: acetic acid/acetate (pK_a = 4.61), phosphate monobasic/phosphate dibasic (pK_a = 6.56), and boric acid/borate (pK_a = 9.2). These pK_a values were obtained at 30.0 ± 0.2 °C in μ = 1.0 (KCl) aqueous solutions. Measurements of pH were made with a combination electrode. The hydrolytic studies of the hydroquinones were carried out in anaerobic aqueous buffers employing Thunberg cuvettes as previously described.[17] Both aerobic and anaerobic studies were carried out as follows: A dimethyl sulfoxide stock of the compound to be studied was prepared fresh and 50 μL of this stock was added to 2.95 mL of buffer. The absorbance vs time data were collected on a UV-vis spectrophotometer in thermostated cells held at 30.0 ≙ 0.2 °C. These data were computer-fit to single first-order rate law.

Preparative Anaerobic Reactions. The preparative hydrolysis of 1 and other air-sensitive compounds were carried out in a Thunberg-like reaction vessel equipped with a port to introduce Teflon gas inlet tubes. The buffer (~200 mL) was placed in the bottom port and a dimethyl sulfoxide solution of the hydroquinone HBr salt was placed in the top port. Teflon tubes were passed into both ports and purging carried out with argon gas for 45 min. The argon was saturated with either water or dimethyl sulfoxide before entering the solvents in the bottom and top ports, respectively. The Teflon tubes were removed and the reaction vessel sealed. After equilibrating the buffer to 30 °C the ports were mixed and incubation at 30 °C carried out for the indicated time.

The preparation of new compounds is outlined below in the order found in the text.

2-(*N*-Methyl-3′-chloropropionamido)-3-(3′-chloropropionamido)-1,4-dimethoxybenzene (4). To 1.68 g (7.70 mmol) of 2-(methylamino)-3,6-dimethoxyaniline hydrochloride (**3**) in 40 mL of dry dimethylformamide was slowly added 3.0 mL (32 mmol) of 3-chloropropionyl chloride and 3.2 mL (40 mmol) of pyridine at 5–10 °C. The reaction mixture was stirred at room temperature for 2 h, and the solvent was then evaporated under reduced pressure to ~5 mL. The oily residue was combined with 100 mL water and the resulting solution was buffered to pH 7.00 with bicarbonate. Extraction of this solution with 2 × 100 mL portions of ethyl acetate followed by drying (Na_2SO_4) and concentration *in vacuo* gave a thick colorless liquid. Trituration of this liquid with hexane gave the product as a white crystalline solid: 1.5 g (52%) yield. Recrystallization for analysis and characterization was carried out from ethyl acetate/hexane: mp 134–136 °C; TLC (chloroform/methanol [95:5]) R_f = 0.90; IR (KBr pellet) 3246, 2966, 2837, 1662, 1604, 1494, 1438, 1263, 1101 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.90 and 6.84 (2 H, AB system, J = 9.2 Hz), 3.80 and 3.76 (6 H, 2s), 3.78 (4 H, m), 3.07 (3 H, s), 2.78 (2 H, t, J = 6.5 Hz), 2.6–2.4 (2 H, m); mass spectrum (EI) m/z 362 (M^+, ^{35}Cl, ^{35}Cl), 364 (M^+, ^{35}Cl, ^{37}Cl), 366 (M^+, ^{37}Cl, ^{37}Cl), 299 (M^+ – chloromethyl), 271(M^+ – chloropropionyl). Anal. Calcd for $C_{15}H_{20}Cl_2N_2O_4$: C, 49.59; H, 5.55; N, 7.71. Found: C, 49.42; H, 5.55; N, 7.64.

1-Methyl-2-(2′-chloroethyl)-4,7-dimethoxybenzimidazole (5). To a mixture of 30 mL of acetic acid and 1.5 mL concentrated sulfuric acid was added 1.40 g (3.87 mmol) of **4**, and the reaction mixture was heated at 115–120 °C for 10 h. The reaction solvents were evaporated to ~5 mL of a thick liquid to which 100 mL of water was added followed by buffering to pH 7.00 with bicarbonate. The buffered solution was extracted with 2 × 75 mL portions of ethyl acetate, and the combined extracts were washed with water and dried (Na_2SO_4). The extracts were then concentrated to a small volume, placed on a silica gel column, and purified by flash chromatography using chloroform as eluant. The product fraction was collected and evaporated to a solid residue. Recrystallization from chloroform/hexane (60:40) afforded the desired product **5**: 350 mg (36%) yield; mp 127–128 °C; TLC (chloroform/methanol [95:5]) R_f = 0.80; IR (KBr pellet) 2953, 2839, 1520, 1464, 1259, 1097 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.56 and 6.50 (2 H, AB system, J = 8.4 Hz), 4.06 (2 H, t, J = 7.3 Hz), 4.03, 4.02, and 3.89 (9H, 3s), 3.31 (2 H, t, J = 7.3 Hz); mass spectrum (EI) m/z 254 (M^+, ^{35}Cl), 256 (M^+, ^{37}Cl), 239 ($M^+ - CH_3$), 225 ($M^+ - NCH_3$), 203, 189, 175. Anal. Calcd for $C_{12}H_{15}ClN_2O_2$: C, 56.58; H, 5.93; N, 11.00. Found: C, 56.40; H, 5.96; N, 10.95.

1-Methyl-2-(2′-bromoethyl)-4,7-dihydroxybenzimidazole Hydrobromide, 1·HBr). A suspension of 350 mg (1.38 mmol) for **5** in 9 mL of 48% hydrobromic acid was heated at 125–130 °C for 5 h. The reaction mixture was then chilled in a refrigerator. The solid product which crystallized from solution was filtered off, washed with ethyl acetate, and recrystallized from methanol/ethyl acetate: 448 mg (93%) yield; mp 231–232 °C; TLC (*n*-butanol/acetic acid/water [5:2:3]) R_f = 0.75; IR (KBr pellet) 3167, 3065, 1548, 1506, 1433, 1273, 1184 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 6.73 (2 H, s), 4.14 (3 H, s), 3.95 (2 H, t, J = 6.3 Hz), 3.72 (2 H, t, J = 6.3 Hz); mass spectrum (EI) m/z 270 (M^+, ^{79}Br), 272 (M^+, ^{81}Br), 190 (M^+ – HBr). Anal. Calcd for $C_{10}H_{11}BrN_2O_2$·HBr: C, 34.11; H, 3.44; N, 7.96; Br, 45.50. Found: C, 34.03; H, 3.42; N, 7.88; Br, 45.33.

1-Methyl-2-(2′-chloroethyl)-4,7-dihydroxybenzimidazole Hydrochloride (1(Cl)·HCl). To a solution of 50 mg (0.14 mmol) of 1·HBr in 50 mL of methanol was added dry HCl gas for 45 min. The reaction mixture was then refluxed for 18 h.

(15) Zoltewicz, J. A.; Kandetzki, P. E. *J. Am. Chem. Soc.* **1971**, *93*, 6562.
(16) Dempcy, R. O.; Skibo, E. B. *Bioorg. Med. Chem.* **1993**, *1*, 39.
(17) Skibo, E. B.; Bruice, T. C. *J. Am. Chem. Soc.* **1983**, *105*, 3304.

Removal of the solvent *in vacuo* afforded **1(Cl)**·HCl solid, which was recrystallized from methanol/ethyl acetate (80:20): 35 mg (94%) yield; mp > 260 °C dec; TLC (*n*-butanol/acetic acid/water [5:2:3]) R_f = 0.75; IR (KBr pellet) 3130 (broad), 2939, 1508, 1307, 1284, 1190 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 6.73 (2 H, s), 4.14 (3 H, s), 4.12 (2 H, t, J = 6.51 Hz), 3.61 (2 H, t, J = 6.51 Hz); mass spectrum (EI mode) m/z 226 (M$^+$, ^{35}Cl), 228 (M$^+$, ^{37}Cl), 190 (M$^+$ - HCl), 175.

Dehydrobromination of 1 in Anaerobic Aqueous Buffer. A solution of 20 mg (0.057 mmol) for 1·HBr in 2 mL of dimethyl sulfoxide was added to 200 mL of buffer (either 0.2 M pH 7.4 phosphate, 0.2 M pH 6.68 phosphate, or 0.2 M pH 9.4 borate all at μ = 1.0 KCl) under strict anaerobic conditions. The reaction mixture was kept at 30 °C for 24 h while maintaining strict anaerobic conditions and then opened to the air and stirred for 30 min. The yellow solution was extracted with 2 × 75 mL portions of chloroform. The extracts were washed with water and dried (Na_2SO_4). Evaporation of the extraction solvent gave a red solid, which was subjected to silica gel flash chromatography employing chloroform as the eluant. The two products, with R_f values of 0.70 and 0.50 in chloroform/methanol (90:10), are as follows. **2-Ethenyl-1-methylbenzimidazole-4,7(1*H*)-dione (6)** was obtained as red crystals: 6.2 mg (58%) yield; mp 168–170 °C (dec); TLC (chloroform/methanol [90:10]) R_f = 0.70; IR (KBr pellet) 3038, 3014, 1678, 1647, 1585, 1473, 1209, 1060 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.68 (4 H, m), 5.81 (1 H, dd, J = 5.5 Hz, J = 7.0 Hz), 4.01 (3 H, s); ^{13}C NMR ($CDCl_3$) 181.03, 179.01, 151.87, 142.22, 137.05, 136.72, 131.03, 126.38, 121.04, 31.81 cps; mass spectrum (EI) m/z 188 (M$^+$), 161 (M$^+$ - CH=CH$_2$), 131, 107, 81. Anal. Calcd for $C_{10}H_8N_2O_2$: C, 63.82; H, 4.29; N, 14.89. Found: C, 63.71; H, 4.25; N, 14.80. **2-(2′-Bromoethyl)-1-methylbenzimidazole-4,7(1*H*)-dione (7)** was obtained as yellow crystals: 1.2 mg (8%) yield; mp 122–124 °C; TLC (chloroform/methanol [90:10]) R_f = 0.5; IR (KBr pellet) 3075, 1656, 1587, 1531, 1473, 1346, 1213, 1080 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 6.66 and 6.59 (2H, AB system, J = 10.4 Hz), 3.66 (3 H, s), 3.83 (2 H, t, J = 6.8 Hz), 3.34 (2 H, t, J = 6.8 Hz); mass spectrum (EI) m/z 268 (M$^+$, ^{79}Br), 270 (M$^+$, ^{81}Br), 189 (M$^+$ - Br), 161, 147. Anal. Calcd for $C_{10}H_9BrN_2O_2$: C, 44.63; H, 3.37; N, 10.41. Found: C, 44.71; H, 3.37; N, 10.37.

2-(2′-Bromoethyl)-4,7-dimethoxy-1-methylbenzimidazole Hydrobromide (8·HBr). The compound 8 (unlabeled **15**) was prepared by the steps illustrated in Chart IV. The HBr salt of 8 was prepared by dissolving 30 mg (0.10 mmol) of 8 in 1 mL of 48% HBr and then heating at 60–65 °C for 15 min. The reaction mixture was cooled to room temperature and chilled for 12 h. The solid product obtained was filtered, washed with ethyl acetate, and dried. Recrystallization from methanol/ethyl acetate afforded the salt of white crystals: 23 mg (61%) yield; mp 180–182 °C; TLC (*n*-butanol/acetic acid/water [5:2:3]) R_f = 0.70; IR (KBr pellet) 2974, 2939, 2841, 1649, 1543, 1502, 1462, 1275, 1107 cm^{-1}; ^{1}H NMR (Me$_2$SO-d_6) δ 7.06 (2 H, s), 4.15, 3.98, and 3.95 (9 H, 3s), 3.93 (2 H, t, J = 6.8 Hz), 3.75 (2 H, t, J = 6.8 Hz).

2-Ethenyl-4,7-dimethoxy-1-methylbenzimidazole (9). A 10 mg (0.026 mmol) solution of 8·HBr in 7 mL of dimethyl sulfoxide was combined with 100 mL of pH 6.00 acetate buffer under strict anaerobic conditions and then the resulting mixture was incubated at 30 °C for 66 h. The reaction was opened to the air and then extracted with 2 × 50 mL portions of chloroform. The combined chloroform extracts were washed with water and dried (Na_2SO_4). Removal of the solvent gave an oily residue, which eventually formed a semisolid 4.3 mg (75%) yield; TLC (chloroform/ethyl acetate [90:10]) R_f = 0.20; IR (NaCl, thin film) 2995, 2933, 2835, 1523, 1454, 1415, 1261, 1101 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.77 (1 H, dd, J_{cis} = 10 Hz, J_{trans} = 17 Hz), 6.62 (1 H, dd, J_{trans} = 17 Hz, J_{gem} = 1.9 Hz), 6.55 and 6.49 (2 H, AB system, J = 8.5 Hz), 5.66 (1 H, dd, J_{cis} = 10 Hz, J_{gem} = 1.9 Hz), 4.06, 3.97 and 3.89 (9 H, 3s); mass spectrum (EI) m/z 218 (M$^+$), 203 (M$^+$ - CH$_3$), 189, 173, 161.

Dehydrobromination of 1 in Deuterated Phosphate Buffer (pD = 7.05). A solution of 20 mg (0.057 mmol) of 1·HBr in 2 mL of dimethyl sulfoxide and 40 mL of deuterated phosphate buffer (pD = 7.05) were combined under anaerobic conditions and incubated at 30 °C for 24 h. The reaction was opened to the air and stirred for 30 min. The yellow solution was extracted with 2 × 50 mL portions of chloroform, and the extracts were then washed with water and dried (Na_2SO_4). Removal of the solvent gave a red solid, which was purified by preparative TLC on silica gel using chloroform/ethyl acetate (80:20) as eluant: 7.1 mg (66%) yield of **6** and 1.6 mg (10%) yield of **7** were obtained from the purification.

2-Ethenyl-4,7-dihydroxy-1-methylbenzimidazole (2). A 24 mg (0.60 mmol) portion of 60% sodium hydride was washed with 3 × 2 mL portions of pentane and then suspended in 6 mL of tetrahydrofuran. This mixture was combined with 45 mg (0.13 mmol) of 1·HBr suspended in 1 mL of tetrahydrofuran under strict anaerobic conditions. The reaction was stirred at room temperature for 1 h and then opened to the air. Filtration and evaporation of the filtrate afforded a light gray solid, which was washed with chloroform and dried under vacuum: 14 mg (57%) yield; ^{1}H NMR (Me$_2$SO-d_6) δ 9.20 and 9.0 (2 H, 2 bs), 6.96 (1 H, dd, J_{cis} = 11.1 Hz, J_{trans} = 18.4 Hz), 6.40–6.30 (3 H, m), 5.68(1 H, dd, J_{cis} = 11.1 Hz, J_{gem} = 1.62 Hz), 4.03 (3 H, s).

1-Methyl-2-(cyanomethyl)-4,7-dimethoxybenzimidazole-2′-^{13}C (11). To 500 mg (1.76 mmol) of 1-methyl-2-(bromomethyl)-4,7-dimethoxybenzimidazole (**10**) in 30 mL dimethyl sulfoxide was added 105 mg (2.10 mmol) of Na^{13}CN. After stirring at room temperature for 4 h, the reaction mixture was poured over 200 mL of water and extracted with 2 × 100 mL portions of ethyl acetate. The extracts were washed with water and dried (Na_2SO_4). Evaporation of the extraction solvent afforded a light yellow solid product which was recrystallized from chloroform/hexane: 300 mg (74%) yield; mp 201–202 °C; TLC (chloroform/methanol [95:5]) R_f = 0.80; IR (KBr pellet) 2960, 2935, 2837, 2191, 1612, 1525, 1464, 1402, 1263, 1101, 1072 cm^{-1}; ^{1}H NMR($CDCl_3$) δ 6.61 and 6.54 (2 H, AB system, J = 8.5 Hz), 4.12 (3 H, s), 4.07 (2 H, d, $J_{H,^{13}C}$ = 10.8 Hz), 3.96 and 3.91 (6 H, 2s); ^{13}C NMR ($CDCl_3$) 113.8 (t, $J_{H,^{13}C}$ = 10.9 Hz) cps; mass spectrum (EI) m/z 232 (M$^+$), 217 (M$^+$ - CH$_3$), 203 (M$^+$ - NCH$_3$), 189, 175, 161, 147. Anal. Calcd for $C_{12}H_{13}N_2O_2$: C, 62.49; H, 5.64; N, 18.04. Found: C, 62.41; H, 5.66; N, 18.22.

1-Methyl-4,7-dimethoxybenzimidazole-2-acetic acid-2′-^{13}C (12). A suspension of 300 mg (1.29 mmol) of 11 in 4.5 mL of 12 N H_2SO_4 was heated at 125–130 °C with stirring for 1 h. The reaction mixture becomes clear during the heating and afforded a brown crystalline solid product upon cooling to room temperature. The H_2SO_4 salt of **12** was filtered, washed with ethyl acetate, and dried over refluxing methanol in a heating pistol under vacuum: 347 mg (77%) yield; mp 250–253 °C; TLC (*n*-butanol/acetic acid/water [5:2:3]) R_f = 0.60; IR (KBr pellet) 3122–2850 (broad), 1757, 1645, 1548, 1514, 1400, 1273, 1168, 1050 cm^{-1}; ^{1}H NMR (Me$_2$SO-d_6) δ 7.05 (2 H, s), 4.36 (2 H, d, $J_{H,^{13}C}$ = 8.2 Hz), 4.41, 3.97, and 3.95 (9 H, 3s); mass spectrum (EI) m/z 206 (M$^+$ - ^{13}CO$_2$), 191 (M$^+$ - [^{13}CO$_2$ + CH$_3$]), 177 [M$^+$ - (^{13}CO$_2$ + NCH$_3$)], 162, 107. Anal. Calcd for $C_{12}H_{14}N_2O_4 \cdot H_2SO_4 \cdot H_2O$: C, 39.50; H, 4.94; N, 7.63. Found: C, 39.41; H, 4.50; N, 7.89.

1-Methyl-4,7-dimethoxybenzimidazole-2-acetic Acid-2′-^{13}C Methyl Ester (13). To 345 mg (0.94 mmol) of **12** in 20 mL of dry methanol was added 0.5 mL of concentrated sulfuric acid, and the reaction mixture was refluxed for 4 h. The solvent was removed under reduced pressure and the residue was dissolved in 50 mL of water. The solution was buffered to pH 7.00 and extracted with 2 × 50 mL portions of ethyl acetate. The extracts were washed with water and dried (Na_2SO_4). Evaporation of the extraction solvent gave an oily residue which was dissolved in 2 mL of diethyl ether. Addition of 10 mL of hexane to the ether solution gave **13** as a white crystalline product: 169 mg (68%) yield; mp 82–83 °C; TLC(chloroform/ethyl acetate [80:20]) R_f = 0.40; IR (KBr pellet) 3007, 2955, 2837, 1701, 1529, 1467, 1269, 1138, 1099 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.57 and 6.51 (2 H, AB system, J = 8.5 Hz), 4.01 (2 H, d, $J_{H,^{13}C}$ = 9.8 Hz), 4.00 (3 H, s) 3.95 and 3.89 (6 H, 2s), 3.71 (3 H, d, $J_{H,^{13}C}$ = 3.9 Hz); ^{13}C NMR($CDCl_3$) 169.6 (doublet of triplet, $J_{H,^{13}C}$ = 9.0 Hz, $J_{^{13}C,CH_3}$ = 3.9 Hz) cps; mass spectrum (EI) m/z 265 (M$^+$), 250 (M$^+$ - CH$_3$), 236 (M$^+$ - NCH$_3$), 205 (M$^+$ - ^{13}COOCH$_3$). Anal. Calcd for $C_{13}H_{16}N_2O_4$: C, 59.23; H, 6.08; N, 10.56. Found: C, 58.50; H, 6.12; N, 10.32.

1-Methyl-2-(2′-hydroxyethyl)-4,7-dimethoxybenzimidazole-2′-^{13}C (14). To a suspension of 56 mg (1.47 mmol) of lithium aluminum hydride in 20 mL of refluxing dry ether was added a solution of 165 mg (0.62 mmol) of **13** in 40 mL dry ether over 20-min period. After the addition was complete, the reaction mixture was refluxed for 1 h. The reaction was cooled to room temperature and 10 mL of ethyl acetate was added to decompose

excess of lithium aluminum hydride. The reaction mixture was acidified with 10 mL of 4 N HCl and shook well. The acidic aqueous layer was separated and neutralized to pH = 7.0 with 4 N NaOH and then extracted with 2 × 75 mL portions of ethyl acetate. The extracts were washed with water and dried (Na_2SO_4). Removal of solvent and recrystallization from ethyl acetate/hexane afforded 14 as white crystals: 90.5 mg (61%) yield; mp 132–133 °C; TLC (chloroform/methanol [95:5]) R_f = 0.75; IR (KBr pellet) 3149, 2939, 2843, 1525, 1473, 1400, 1265, 1105, 1047 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.55 and 6.52 (2H, AB system, J = 8.5 Hz), 4.18 (2 H, doublet of triplets, $J_{H,H}$ = 5.6 Hz, $J_{H,^{13}C}$ = 150 Hz), 3.98 (3 H, s), 3.97 and 3.89 (6 H, 2s), 3.00 (2 H, q, $J_{H,H}$ = 5.6 Hz); ^{13}C NMR ($CDCl_3$) 58.80 (triplet of triplet, $J_{H,^{13}C}$ = 145.2 Hz, $J_{H,^{13}C}$ = 4.5 Hz) cps; mass spectrum (EI) m/z 237 (M^+), 222 ($M^+ - CH_3$), 206 ($M^+ - OCH_3$), 190 ($M^+ - CH_3{}^{13}CH_2OH$). Anal. Calcd for $C_{12}H_{16}N_2O_3$: C, 61.16; H, 6.80; N, 11.81. Found: C, 60.09; H, 6.84; N, 11.61.

1-Methyl-4,7-dimethoxy-2-(2′-bromoethyl)benzimidazole-2′-^{13}C (15). A mixture of 120 mg (0.50 mmol) of 14 and 0.55 mL (5.8 mmol) of phosphorus tribromide in 10 mL of chloroform was heated at reflux for 3 h. The reaction mixture was then cooled to room temperature and diluted with 100 mL of diethyl ether. The white solid which precipitated out was filtered off, dissolved in 50 mL of water, and then filtered. The filtrate was buffered to pH 7.00 with sodium bicarbonate and extracted with 2 × 50 mL portions of ethyl acetate. The extracts were washed with water and dried (Na_2SO_4). Removal of the solvent afforded a solid product, which was recrystallized from chloroform/hexane: 98 mg (65%) yield; mp 115–116 °C; TLC (chloroform/ethyl acetate [80:20]) R_f = 0.50; IR (KBr pellet) 2949, 2928, 2833, 1520, 1462, 1257, 1180, 1095 cm^{-1}; ^1H NMR ($CDCl_3$) 3.88 (2 H, doublet of triplets, $J_{H,H}$ = 7.4 Hz, $J_{H,^{13}C}$ = 150 Hz), 4.0 (3H, s), 3.95 and 3.89 (6 H, 2s), 3.41 (2 H, q, $J_{H,H}$ = 7.4 Hz, $J_{H,^{13}C}$ = 7.4 Hz); ^{13}C NMR ($CDCl_3$) δ 32.40 (triplet of triplet, $J_{H,^{13}C}$ = 155.5 Hz, $J_{H,^{13}C}$ = 5.3 Hz); mass spectrum (EI) m/z 299 (M^+, ^{79}Br), 301 M^+, ^{81}Br), 284 ($M^+ - CH_3$), 270 ($M^+ - NCH_3$), 204. Anal. Calcd for $C_{12}H_{15}BrN_2O_2$: C, 48.35; H, 5.04; N, 9.33. Found: C, 48.21; H, 5.05; N, 9.24.

1-Methyl-2-(2′-bromoethyl)-4,7-dihydroxybenzimidazole Hydrobromide-2′-^{13}C (16·HBr). A mixture of 80 mg (0.27 mmol) of 15 and 2 mL of 48% hydrobromic acid was heated at 120–30 °C for 5 h and then chilled in a refrigerator for 2 h. The solid hydrobromide salt which crystallized from solution was filtered, washed with ethyl acetate, and dried. Recrystallization of the product from methanol/ethyl acetate afforded light yellow crystals: 76 mg (80%) yield; mp 230–231 °C, IR (KBr pellet) 3173, 3063, 2930, 1558, 1506, 1300, 1267, 1188, 1060 cm^{-1}; ^1H NMR (Me_2SO-d_6) 6.72 (2 H, s), 3.96 (2 H, doublet of triplets, $J_{H,^{13}C}$ = 153.4 Hz, $J_{H,^{13}C}$ = 6.7 Hz), 4.14 (3 H, s), 3.71 (2 H, t, J = 4.9 Hz); ^{13}C NMR ($CDCl_3$) 30.22 (triplet of triplet, $J_{H,^{13}C}$ = 158.2 Hz, $J_{H,^{13}C}$ = 5.0 Hz) cps; mass spectrum (EI) m/z 271(M^+, ^{79}Br), 273 (M^+, ^{81}Br), 191($M^+ - HBr$), 176 [$M^+ - HBr - CH_3$). Anal. Calcd for $C_{10}H_{11}BrN_2O_2$·HBr: C, 34.30; H, 3.43; N, 7.94. Found: C, 34.37; H, 3.45; N, 8.00.

Dehydrobromination Experiment of ^{13}C-Labeled 1 (16·HBr). A solution of 20 mg (0.057 mmol) of labeled 16·HBr in 2 mL of dimethyl sulfoxide was added to 200 mL of pH 9.4 borate buffer under strict anaerobic conditions. The reaction was stirred at 30 °C for 66 h and then opened to the air and neutralized with 4 N HCl to pH 7.0. The neutralized reaction was stirred for 30 min, and then extracted with 2 × 50 mL portions of chloroform. The extracts were washed with water, dried (Na_2SO_4), and then concentrated to a red solid. Preparative TLC of the solid on silica gel using chloroform/ethyl acetate (80:20) as the eluent afforded products 17 and 18. Properties of 17 (red crystals): 6.1 mg (57%) yield, mp 166–168 °C; TLC (chloroform/ethyl acetate [80:20]) R_f = 0.50; IR (KBr pellet) 3036, 3016, 1678, 1647, 1585, 1473, 1209, 1066 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.68 (1 H, octet, $J_{H,Hgem}$ = 1.3 Hz, $J_{H,Htrans}$ = 17.2 Hz, $J_{H,^{13}C}$ = 162.0 Hz), 6.71–6.61 (3 H, m), 5.81 (1 H, octet, $J_{H,Hgem}$ = 1.3 Hz, $J_{H,Hcis}$ = 11.0 Hz, $J_{H,^{13}C}$ = 162.0 Hz), 4.00 (3H, s); ^{13}C NMR ($CDCl_3$) 126.05 (triplet of doublets, $J_{H,^{13}C}$ = 163.15 Hz, $J_{H,^{13}C}$ = 4.6 Hz); mass spectrum (EI) m/z 189 (M^+), 162 [$M^+ - CH={}^{13}CH_2$), 132, 118, 107. Anal. Calcd for $C_{10}H_8N_2O_2$·0.25 H_2O: C, 62.52; H, 4.42; N, 14.46. Found C, 63.01; H, 4.29; N, 14.54. Properties of 18 (yellow crystals): 1.3 mg (8%) yield; mp 122–124 °C, TLC (chloroform/methanol [90:10]) R_f = 0.50; ^1H NMR ($CDCl_3$) δ 6.66 and 6.63 (2 H, dd, J = 10 Hz) 3.87 (2 H, triplet of doublet, $J_{H,H}$ = 7.4 Hz, $J_{H,^{13}C}$ = 155 Hz), 3.99 (3 H, s), 3.36 (2 H, q, $J_{H,H}$ = 7 Hz, $J_{H,^{13}C}$ = 7.0 Hz).

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Villiramulins A and B: New Phenol Derivatives from *Piper villiramulum*

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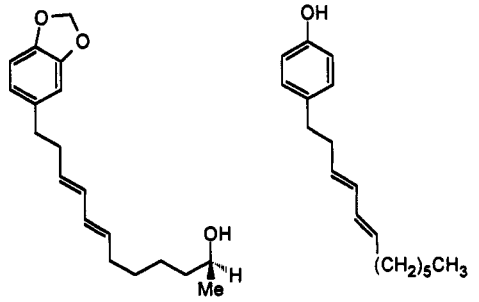
Two new phenol derivatives, villiramulin A (1) and B (2), have been isolated from the Panamanian plant *Piper villiramulum* and assigned structures on the basis of a variety of spectroscopic data. After a model study wherein the (+)- and (−)-*O*-methylmandelate esters of 2-heptanol were shown to present significantly different ^{1}H NMR spectra, villiramulin A was esterified with both of these acids. On the basis of the ^{1}H NMR data of the resulting esters, the S stereochemistry was assigned to the natural product. Finally, villiramulin B was shown to be significantly active in a bioassay that measures repellency to a captive colony of leafcutter ants.

Leafcutter ants are among the most destructive insects in the New World tropics and subtropics. However, even though leafcutters collect material from numerous plant species to serve as a substrate for their mutualistic fungus, still other plants within their range remain essentially unharmed. During studies of the chemical ecology of leafcutter ants we have isolated numerous natural products from avoided leaves.[1] In particular, plants of the genus *Piper* have given many novel compounds,[2] prompting further interest. In this paper, we report an investigation of the Panamanian shrub *Piper villiramulum*, which has resulted in the isolation and identification of two novel alkyl phenols.

The $CHCl_3$ extract of a sample of *P. villiramulum* leaves was subjected to dry column chromatography. Two of the resulting fractions exhibited ant-repellent activity against a captive *Atta cephalotes* colony.[3] The more polar fraction was further purified, first by flash column and then by reverse phase chromatography, and gave a pure compound ultimately assigned the structure of villiramulin A (1). Purification of the less polar fraction by successive flash and radial dispersion chromatography resulted in the isolation of a second, related natural product now characterized as villiramulin B (2).



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The first unknown, villiramulin A, gave a molecular ion of *m/z* 302.1901 in its high resolution mass spectrum, corresponding to a molecular formula of $C_{19}H_{26}O_3$ and seven degrees of unsaturation. The appearance of resonances for ten sp^2 hybridized carbons in the ^{13}C NMR spectrum indicated that six degrees of unsaturation could be attributed to the presence of one aromatic ring and two C–C double bonds. The last degree of unsaturation could then be satisfied by assignment of one additional ring. Only 25 hydrogens could be identified through the ^{1}H and DEPT spectra (Table I), indicating that one substituent must be a hydroxyl group.

Table I. ^{1}H and ^{1C} NMR Data for Villiramulin A

C/H	^{13}C	^{1}H	selective INEPT correlations
1	23.53 (q)	1.18 (d; 6.2)	2
2	68.12 (d)	3.78 (m)	
3	32.53 (t)[a]	1.41 (m)	
4	25.33 (t)	1.41 (m)	
5	29.39 (t)	1.41 (m)	
6	34.70 (t)[a]	2.06 (m)	4, 5, 7, 8
7	130.92 (d)[b]	5.56 (m)[a]	
8	132.57 (d)[b]	6.03 (m)[b]	
9	130.37 (d)	6.03 (m)[b]	
10	131.05 (d)	5.56 (m)[a]	
11	35.64 (t)	2.34 (dt; 7.9, 6.9)	
12	39.22 (t)	2.61 (t; 8.0)	10, 1′, 2′
1′	135.77 (s)		
2′	121.09 (d)	6.67 (s)	1′, 3′, 4′
3′	145.56 (s)		
4′	147.48 (s)		
5′	108.07 (d)[c]	6.72 (d; 7.9)	
6′	108.86 (d)[c]	6.62 (d; 7.9)	2′
7′	100.70 (t)	5.91 (s)	

[a–c] Assignments of resonances with the same superscripts may be interchanged.

The initial assignment of three partial structures was based mainly on coupling information found in the ^{1}H NMR and ^{1}H–^{1}H COSY spectra. For example, the three aromatic resonances gave rise to a distinctive 1,2,4-substitution pattern. The two ortho substituents were added to the aromatic ring after the presence of a methylenedioxy group was noted (δ 5.91 (s, 2H) and 100.70 ppm (t)). Because no unassigned sp^2 resonances have a chemical shift indicating an oxygen substituent, the last aromatic substituent must be an alkyl group, leading to partial structure A.

• Abstract published in *Advance ACS Abstracts*, November 15, 1993.

(1) For recent papers in this series, cf.: (a) Galinis, D. L.; Wiemer, D. F.; Cazin, J. *Tetrahedron*, **1993**, *49*, 1337. (b) Chen, T.-B.; Galinis, D. L.; Wiemer, D. F. *J. Org. Chem.* **1992**, *57*, 862. (c) Chen T.-B.; Wiemer, D. F. *J. Nat. Prod.* **1991**, *54*, 1612.

(2) (a) Green, T. P.; Galinis, D. L.; Wiemer, D. F. *Phytochemistry* **1991**, *30*, 1649. (b) Green, T. P.; Wiemer, D. F. *Phytochemistry* **1991**, *30*, 3759. (c) Chen, T.-B.; Green, T. P.; Wiemer, D. F. *Tetrahedron Lett.* **1992**, *33*, 5673. (d) Green, T. P. Ph.D. Thesis, 1990, University of Iowa, Iowa City.

(3) Hubbell, S. P.; Howard, J. J.; Wiemer, D. F. *Ecology*, **1984**, *65*, 1067.

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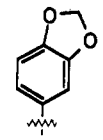
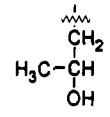


A



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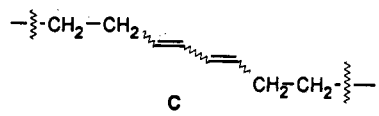


C

The second and third partial structures also were proposed on the basis of ^{1}H NMR coupling data. A methine resonance at δ 3.78, whose chemical shift indicated a geminal OH group, was coupled to a methyl group at δ 1.18 in the ^{1}H–^{1}H COSY spectrum. A COSY experiment also indicated that this methine was further coupled to a methylene group at δ 1.41 and allowed assembly of the second partial structure (**B**). The final partial structure accounts for a pair of overlapping vinylic multiplets at δ 6.03 and 5.56, each integrating for two hydrogens and demonstrating coupling to each other in the COSY spectrum. These data indicated that the two C–C double bonds were disubstituted and conjugated. This diene unit was expanded to include two flanking -CH_2CH_2- groups based on several correlations observed in the COSY experiment. For example, the methylene resonances at δ 2.34 and 2.06 were coupled to the vinylic resonances at δ 5.56 and 6.03, respectively. The additional coupling of these methylene groups to two other methylene groups (δ 2.61 and 1.41) established the third fragment, partial structure **C**.

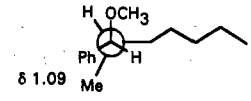
The connectivity of partial structures **A** and **C** was assigned on the basis of ^{1}H–^{13}C coupling information obtained from a series of selective INEPT experiments (Table I). Correlation of the methylene resonance at δ 2.61 with aromatic carbon resonances at 135.77 and 121.09 ppm allowed a joining of partial structure **C** to the aromatic ring. Selective INEPT data also allowed connection of the remaining unassigned fragments of the molecule, one -CH_2CH_2- group and partial structure **B**, to the alkyl chain completing the overall skeletal assignment.

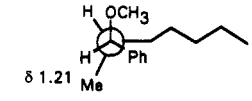
The stereochemical issues of this structure elucidation were resolved using a combination of spectroscopic and synthetic methods. The first issue, double bond geometry, was established through ^{1}H NMR decoupling experiments conducted to lessen the complexity of the vinylic resonances. Irradiation of the aliphatic resonances at δ 2.06 and 2.34, in separate experiments conducted in C_6D_6, resulted in collapse of the multiplets at δ 6.03 and 5.56, giving essentially broadened doublets. The remaining coupling constant in these doublets was determined to be 13.7 Hz, indicating a trans geometry about the two double bonds.

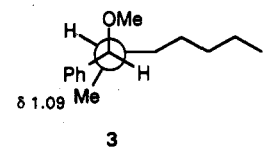
The configurational assignment of C2 was more difficult to establish, but ultimately was determined through a combination of synthetic transformations and spectroscopic studies. The Mosher–Trost[4] method for assigning absolute configuration of secondary alcohols appeared to offer a viable means of assigning this stereochemistry through preparation of diastereomeric ester derivatives with nonracemic acids. However, because this method has been applied primarily to cyclic compounds, a model study with 2-heptanol was conducted before reactions were attempted with the natural product itself.

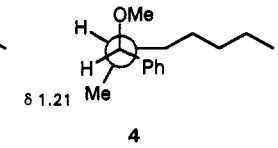
Following the procedure of Hassner and co-workers,[5] the *R* enantiomer of 2-heptanol was esterified in separate experiments with both enantiomers of *O*-methylmandelic acid. After workup of the separate reactions, the respective diastereomers were obtained in pure form and high yields, without the need for chromatographic separation. The ^{1}H NMR spectra of both diastereomers were recorded for comparison.

On the basis of the model proposed by Trost et al.,[4b] drawings of "extended Newman projections" of the *R,S* (**3**) and *R,R* (**4**) diastereomers indicate that the chemical shifts of the methyl groups should be different as a result of the shielding effect of the phenyl ring. As predicted by the model, the methyl resonance of the *R,S* diastereomer (δ 1.09) was shifted upfield relative to that of the *R,R* diastereomer (δ 1.21). This substantial chemical shift difference for the two methyl groups validated the applicability of this method to villiramulin A.

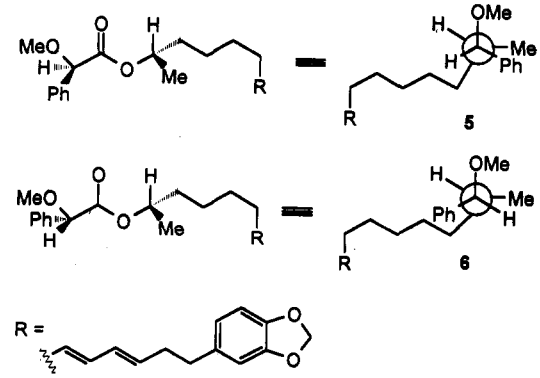








To determine the stereochemistry of villiramulin A, separate reactions were conducted with the natural product and each enantiomer of the acid. Again both diastereomers were obtained in relatively pure form and in high yield. Esterification of the natural product with the *R* enantiomer of the acid gave an ester with an upfield chemical shift (δ 1.09) for the methyl resonance relative to the corresponding resonance (δ 1.20) of the diastereomeric ester obtained from reaction of the natural product with the *S* acid. To account for this chemical shift difference, and the direction in which these methyl resonances are shifted, the "extended Newman projection" of the *R* acid diastereomer must be drawn as shown in structure **5**. On the basis of this drawing, C2 of the natural product would be assigned an *S* configuration.



Once this assignment has been made, the "extended Newman projection" of the *S,S* diastereomer (**6**) would predict that the methylene group of the natural product