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Characterization of Autoxidation Products of the Antifungal Compounds Econazole Nitrate and Miconazole Nitrate

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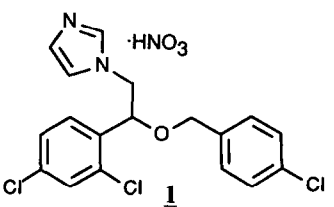
Abstract: *Econazole nitrate* **1** *and miconazole nitrate* **2** *underwent autoxidation in 90% ethanol at 77 °C in the presence of AIBN and oxygen. The benzylic methylene carbons of* **1** *and* **2**, *the benzylic methine carbon of* **2**, *and the imidazole ring of* **2** *were sites of oxidation.*

INTRODUCTION

Econazole nitrate (**1**, 1-[2-[(4-chlorophenyl)methoxy]-2-(2,4-dichlorophenyl)ethyl]-1*H*-imidazole, mononitrate) and miconazole nitrate (**2**, 1-[2-(2,4-dichlorophenyl)-2-[(2,4-dichlorophenyl)methoxy]ethyl]-1*H*-imidazole, mononitrate) are antifungal compounds that are used in pharmaceutical products.[1] A number of reports on various analytical aspects of these compounds have appeared.[2] Some degradation studies have been described,[2h-l] but the isolation and characterization of degradation products of these two compounds have not been reported. The present project was undertaken to identify the products of autoxidation[3] of these two drugs. A solvent system of ethanol/water (90:10) was chosen for these studies to simulate the aqueous lotion and cream formulations that are currently in use.



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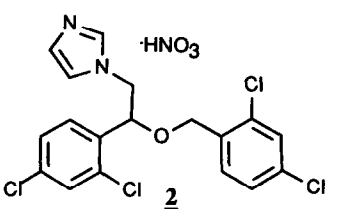

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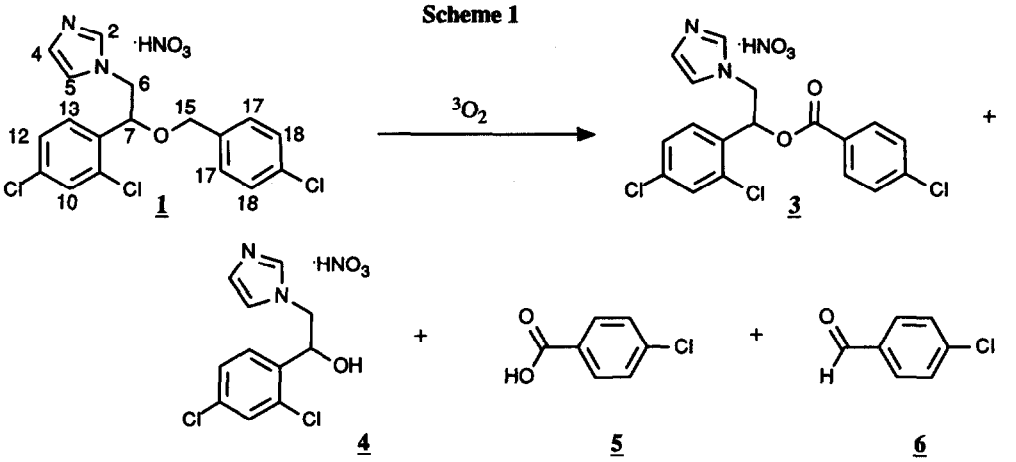
RESULTS AND DISCUSSION

We examined the autoxidation of **1** and **2** in the presence of the free radical initiator, 2,2'-azobis(2-methylpropionitrile) (AIBN).[4] The initiator was used to effect oxidation at lower temperatures and therefore to minimize the amount of secondary degradation of initially formed products. In a typical reaction, **1** or **2** was heated to 77 °C in 90% ethanol in the presence of oxygen and AIBN. The reactions slowed dramatically with roughly 10-15% degradation after ~5 hours when most of the initiator was consumed[4b] (Figure 1). The identified degradation products of econazole nitrate were compounds **3**-**6** (Scheme 1, Table 1) and the identified degradation products of miconazole nitrate were compounds **4**, **7**-**15** (Scheme 2, Table 1).

Scheme 1



Scheme 2

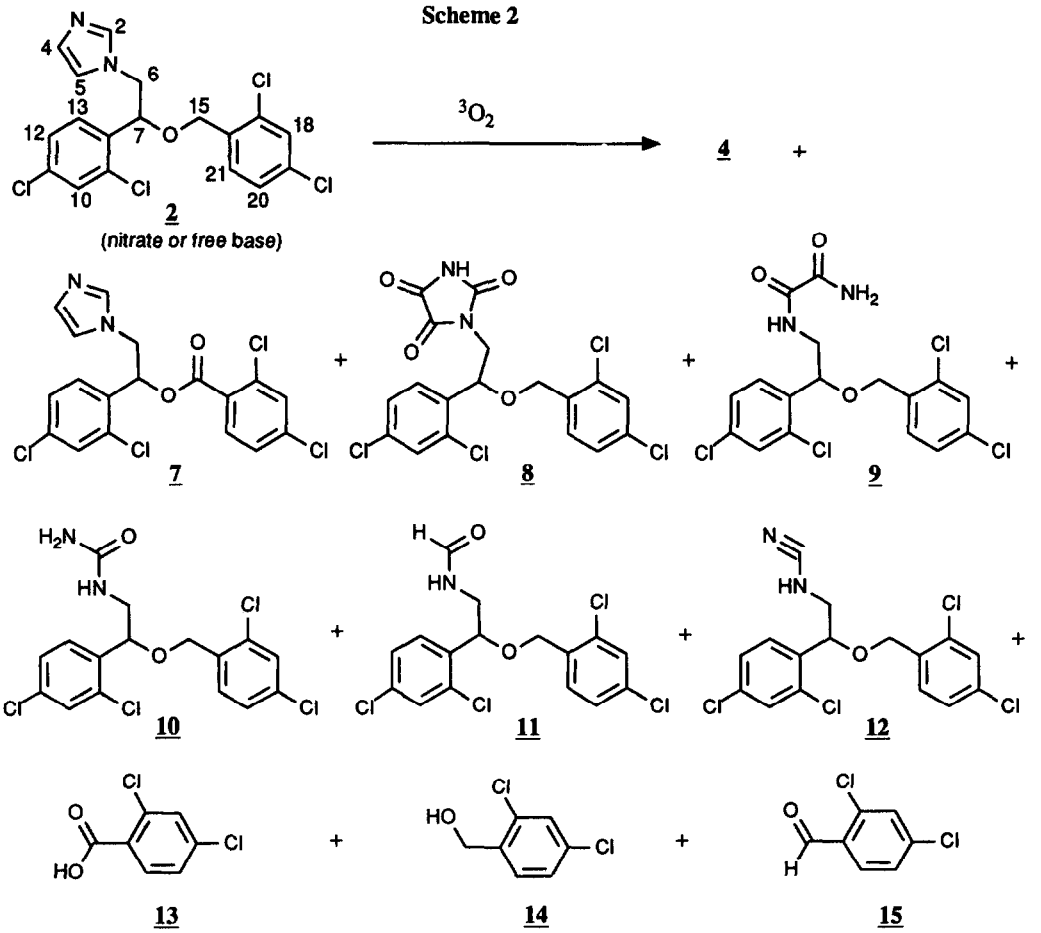


Table 1. Selected Product Distribution Data.

	Econazole Nitrate		Miconazole Nitrate	
Temp., °C:	77 ± 1		77.5 ± 1	
Oxygen, psi:	100		100	
AIBN (molar equivalents):	10.0		10.9	
Time, hr:	3.7		3.4	
Oxidation Site	Compound	Mole%	Compound	Mole%
	1	87	**2**	91
Methylene carbon	**3**	3.4	**7**	2.3
Methylene carbon	**4**	1.0	**4**	0.9
Methylene carbon	**5**	1.4	**13**	0.7
Methylene carbon	**6**	1.3	**15**	1.3
Methine carbon			**14**	0.2
Imidazole			**8**	0.3
Imidazole			**9**	0.1
Imidazole			**10**	0.1
Imidazole			**11**	0.6
Imidazole			**12**	0.6

Reversed-phase HPLC methods were used to analyze these reaction product mixtures (Figures 2-3) and to quantitate the amounts of econazole or miconazole and individual degradation products (Table 1 and Figure 1). These HPLC methods used ammonium formate or ammonium acetate mobile phases which were compatible with thermospray HPLC-MS.[5]

Preparative versions of the analytical HPLC methods were used to isolate the degradation products **3**-**5** (Scheme 1) from the econazole nitrate autoxidation product mixture. 4-Chlorobenzaldehyde (**6**) was identified in the econazole product mixture by HPLC-UV and HPLC-MS. The miconazole degradation products **7**-**12** were isolated in a similar manner from a preparative-scale reaction with the free base at 111 °C. This free base product mixture was used for the isolation effort because it contained substantially larger amounts of the same products which were formed in the nitrate reaction (Figure 1). The alcohol **4** was identified in the miconazole product mixture by HPLC-UV and HPLC-MS. 2,4-Dichlorobenzoic acid (**13**), 2,4-dichlorobenzyl alcohol (**14**), and 2,4-dichlorobenzaldehyde (**15**) were identified in the miconazole product mixture by HPLC-UV and GC-MS. The isolated econazole and miconazole products were subsequently characterized by various spectral techniques (see experimental section). The availability of authentic samples of **4**,[1] **5**, **6**, **13**, **14**, and **15** aided these identification efforts. The esters **3** and **7**, which were identified as degradation products of econazole and miconazole, respectively, were previously prepared (as nitrate salts), but spectral data were not reported.[6]

The identified degradation products (Schemes 1-2) resulted from oxidation of the benzylic methylene carbons (Scheme 3) of econazole (**3**-**6**) and miconazole (**4**, **7**, **13**, and **15**), from oxidation of the benzylic methine carbon (Scheme 4) of miconazole (**14**), and from oxidation of the imidazole ring (Schemes 5-6) of miconazole (**8**-**12**).

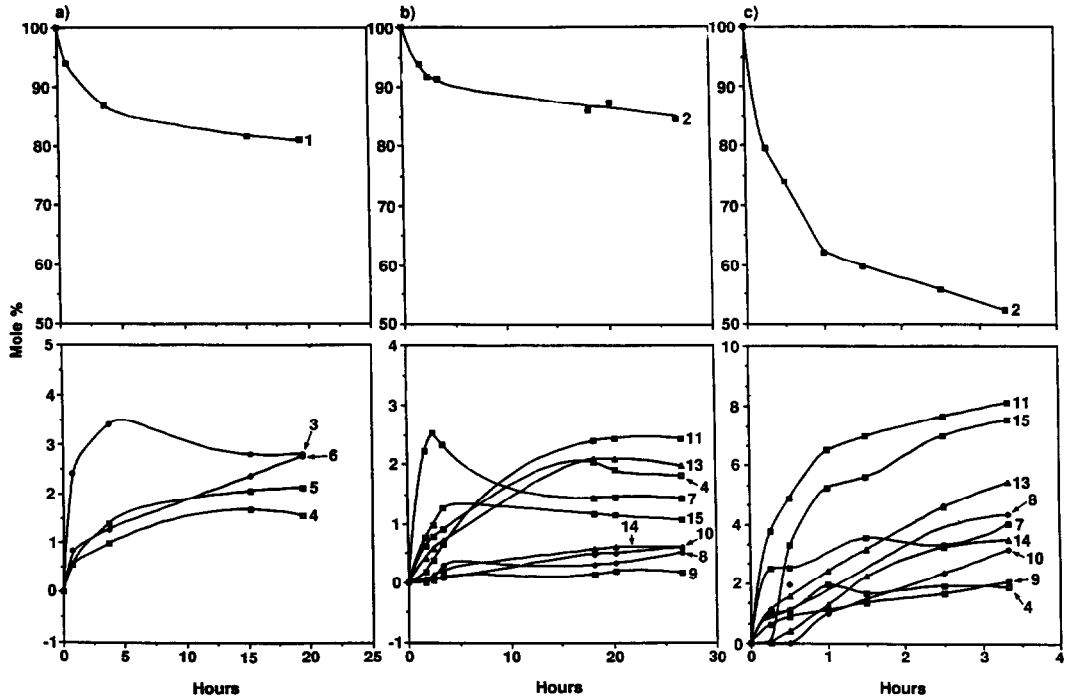


Figure 1. Variation of reactants and products with time: a) econazole nitrate, 77 °C, 100 psi oxygen, 10 molar equivalents of AIBN; b) miconazole nitrate, 77.5 °C, 100 psi oxygen, 10.9 molar equivalents of AIBN; c) miconazole free base, 111 °C, 200 psi oxygen, 5 molar equivalents of AIBN.

The product distributions for the econazole nitrate and miconazole nitrate degradation reactions are summarized in Table 1 (see also Figure 1). The remaining amounts of product material are represented by the small unidentified peaks in the chromatograms of the product mixtures (Figures 2-3). The extent of oxidation of the benzylic methylene carbons of econazole and miconazole was similar (Table 1 and Figure 1). Oxidation of the benzylic methine carbon of miconazole was minimal in comparison to the benzylic methylene carbon (Table 1 and Figure 1). No 4-chlorobenzyl alcohol (<~0.1%) was observed by HPLC in the econazole nitrate product mixture and therefore oxidation of the benzylic methine carbon of econazole may not have occurred to an appreciable extent. No imidazole oxidation products were isolated from the econazole nitrate reaction mixture. However, these latter products may be represented by some of the small unidentified chromatographic peaks in Figure 2.

The process of oxidation of the benzylic methylene carbons of miconazole and econazole probably proceeds by α-autoxidation with a neutral radical chain mechanism[3] as in other arylalkanes such as cumene.[7] The initial products of this autoxidation process with econazole and miconazole are presumed to be the unstable secondary hydroperoxides **16a** and **16b**, respectively (Scheme 3). By analogy to known[8] reactions of α-alkoxybenzyl hydroperoxides, **16a** may decompose (Scheme 3) to ester **3**, alcohol **4**, and aldehyde **6**, and **16b** may decompose to ester **7**, alcohol **4**, and aldehyde **15**. The mechanisms of these decomposition processes may be complex.[8] In the econazole reaction, oxidation of 4-chlorobenzaldehyde (**6**) to 4-chlorobenzoic acid (**5**) and

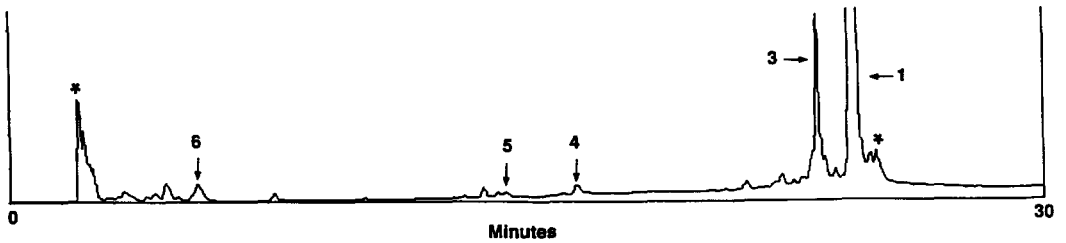


Figure 2. HPLC chromatogram (230 nm) of econazole nitrate autoxidation product mixture (3.7 hr, 77 °C, 100 psi oxygen, 10 molar equivalents of AIBN). The amount of unreacted starting material was 87%. The asterisk indicates peaks in chromatogram of a blank injection.

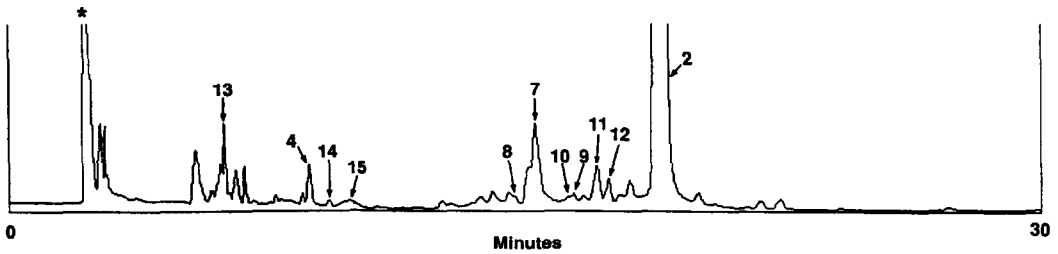


Figure 3. HPLC chromatogram (230 nm) of miconazole nitrate autoxidation product mixture (3.7 hr, 77.5 °C, 100 psi oxygen, 10.9 molar equivalents of AIBN). The amount of unreacted starting material was 91%. The asterisk indicates peaks in chromatogram of a blank injection.

hydrolysis of the ester **3** to **5** and the alcohol **4** probably occurred (Scheme 3). The acid **13** and the alcohol **4** were probably formed in an identical manner in the miconazole reactions (Scheme 3).

The autoxidation of the benzylic methine carbon of miconazole is presumed to proceed in a similar fashion through the hydroperoxide **17** (Scheme 4). This intermediate **17** may be converted to the observed 2,4-dichlorobenzyl alcohol (**14**).

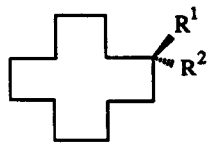
The autoxidation of the imidazole ring of miconazole yielded products **8**-**12**. Little information appears in the literature on the autoxidation (triplet oxygen) of imidazoles.[9] However, imidazoles are known to react with singlet oxygen to form unstable dioxetane and endoperoxide intermediates.[10] The results of our study suggest that these same types of intermediates may be involved in the reaction of imidazoles with triplet (ground state) oxygen (Schemes 5-6).

A lengthy discussion of the mechanisms of autoxidation of the imidazole ring of miconazole is not justified with the limited information that is available. A radical cation chain mechanism may be involved as was suggested for the autoxidation of retinoic acid[11] and other molecules.[3d] Electron-transferred complexes have been proposed[3d,12] as intermediates in autoxidation reactions of some aromatic systems[12a,b] and amines[3d,12c] and they may be involved in the present process as well. The dioxetane **18** (Scheme 5) and the endoperoxide **19** (Scheme 6) are most likely the key intermediates in the formation of the observed products. The dioxetane **18**, by analogy to other dioxetanes,[10c,d,13] may undergo thermolysis to yield a dicarbonyl derivative **20** (Scheme 5) that can be hydrolyzed to compounds such as the isolated formamide **11**. Imidazole endoperoxides were found to be stable at temperatures below -50 °C, but at higher temperatures these compounds either liberated oxygen

energy. The value C^2/C^3, close to 1, indicates the electrophilic nature of the active species.

Cyclododecane **14** is a very convenient substrate for the study of Gif reactions, for the low volatility of the substrate itself and the oxidation products allows excellent mass balances. The main product of the oxidation in GoChAgg system is cyclododecanone **15**, cyclododecanol **16** being observed either in trace amounts or with smaller yields than in GoAgg systems. The mass balance is almost quantitative, and hence no other products are formed in the process. Cyclododecyl pyridines **17** and **18** were not found amongst the reacion products, not even in trace amounts.

We first studied the effect of different counterions (ligands) in Cu^{II} salts and the concentration of the catalyst on the reaction yield. The yield appeared not to be very sensitive to the nature of the anion and to the amount of cupric salt used (Tables 3 and 4). Cupric chloride and sulphate are poorly soluble in dry pyridine, but the solution becomes homogeneous upon addition of hydrogen peroxide. The reaction rate increases when the concentration of the catalyst is higher, but the final yield was independent of the amount of catalyst present. The rates for ketone formation and hydrogen peroxide decomposition vary in a wide range when



14 : R^1, R^2 = H
15 : R^1, R^2 = O
16 : R^1 = H; R^2 = OH
17 : R^1 = H; R^2 = *o*-pyridyl
18 : R^1 = H; R^2 = *p*-pyridyl

Table 3. Oxidation of Cyclododecane **14** (3.6 mmol) with H_2O_2 (9.5 mmol) in Pyridine Catalyzed by Copper Salts (0.3 mmol). Effect of the Counterion.

Catalyst	14[a]	15 mmol	16	Σ mmol	Σ %	Mass Balance %
$Cu(ClO_4)_2$	3.17	0.40	*traces*	0.40	11.0	100
$Cu(OAc)_2$	3.05	0.32	*traces*	0.32	9.0	94
$CuSO_4$[b]	3.22	0.35	*traces*	0.35	9.7	100
$CuCl_2$[b]	3.15	0.42	*traces*	0.42	11.7	100

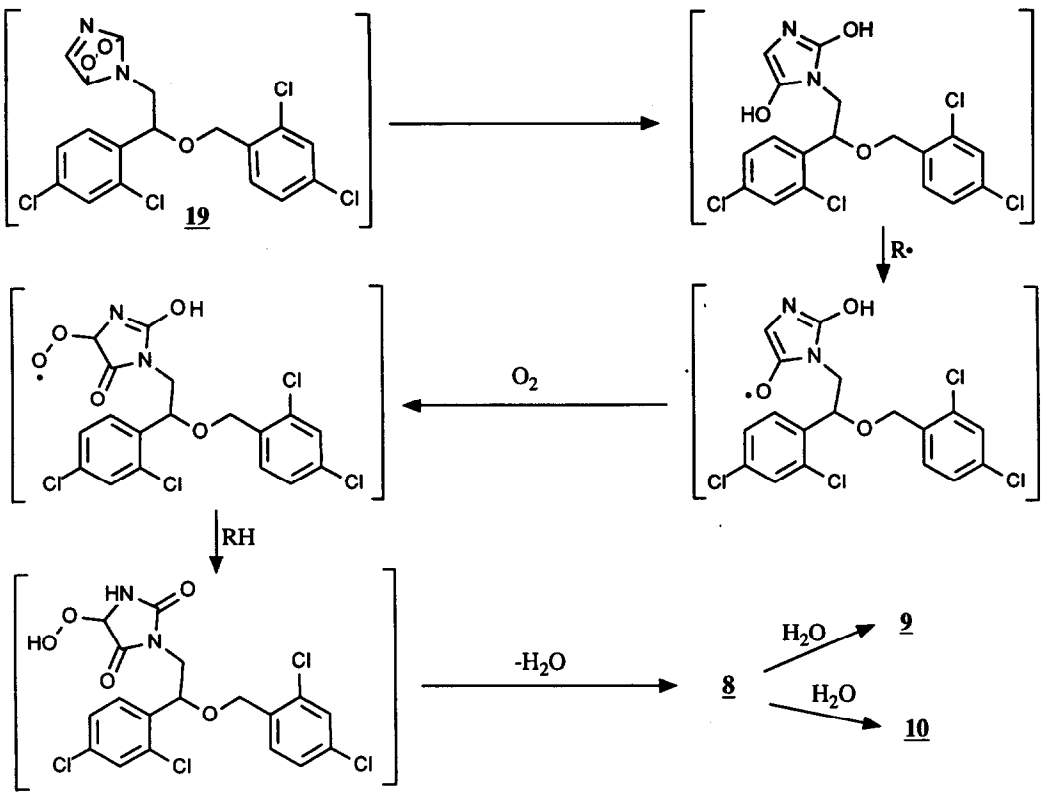
a. Recovered starting material.
b. Salt is not completely soluble in dry pyridine.

Table 4. Oxidation of Cyclododecane **14** (2.0 mmol) with H_2O_2 (19 mmol) in Pyridine Catalyzed by $Cu(ClO_4)_2.6H_2O$. Effect of the catalyst concentration.

$Cu(ClO_4)_2.6H_2O$	14[a]	15 mmol	16	Σ mmol	Σ %	Mass Balance %
0.055	1.75	0.24	0.03	0.27	13.5[a]	101
0.150	1.54	0.29	0.02	0.31	15.5	93.0
0.300	1.65	0.35	0.02	0.37	18.5	101
0.600	1.64	0.35	0.03	0.38	19.0	101
1.400	1.62	0.32	0.05	0.37	18.5	99.5

a. Recovered starting material.

Scheme 6



EXPERIMENTAL DETAILS

Econazole nitrate was obtained from Cilag Chemie (Switzerland). Miconazole nitrate and compound **4** were obtained from Janssen Pharmaceutica (Belgium). The chlorinated benzene derivatives **5**-**6** and **13**-**15** were obtained from Aldrich. Water was obtained from a Millipore water purification system and ethanol was from Pharmco. The solvents used for HPLC were from Burdick and Jackson. Oxygen (Extra Dry Grade) was obtained from Linde Specialty Gases.

The autoxidation reactions were conducted in 600 mL stainless steel reactors (Parr Instruments) which were equipped with magnetic stirrers.

^{1}H NMR spectra (300 MHz) and ^{13}C NMR spectra (75 MHz) were obtained on a General Electric QE-300 (300 MHz) spectrometer in CD_3OD or CD_3SOCD_3 with tetramethylsilane as internal reference. Carbon multiplicities were determined with the DEPT pulse sequence.[14]

Mass spectra were obtained on a Finnigan MAT 8230 mass spectrometer by desorption electron ionization (70 eV, DEI), by desorption chemical ionization (DCI) with isobutane as reagent gas, and by fast atom bombardment (FAB) with glycerol/thioglycerol (1:1) as the matrix. High resolution mass spectra (HRMS) were also obtained on the same instrument. IR spectra were obtained on a Nicolet Model 5DXB FT-IR spectrometer. Microanalyses (C, H, N) were obtained with a Perkin-Elmer 2400 CHN elemental analyzer.

Analytical reversed-phase HPLC analyses of econazole nitrate product mixtures were conducted on a Perkin-Elmer Series 4 instrument with a Hewlett-Packard 1040A diode array UV detector which was set to

monitor 230, 240, and 272 nm. The detector contained a multi-channel DPU board for peak integration. Complete UV spectra were collected at the peak apices. Injections (10 µL) were made with a Perkin-Elmer ISS-100 autosampler. A Rainin *DYNAMAX*® 5 µm C-18 column (4.6 x 250 mm) was used and the temperature was maintained at 30 °C with a Waters Assoc. column oven. The method was a multi-step binary gradient: 60% A/40% B to 5% A/95% B over 20 min (linear), a 6 min hold at 5% A/95% B, 5% A/95% B to 100% B over 1 min (linear), and a 3 min hold at 100% B. The flow rate was 1.2 mL/min. Mobile phase A was 0.025 M ammonium formate in water and mobile phase B was 0.025 M ammonium formate in methanol. A calibration curve was prepared for econazole nitrate and response factors were determined for compounds **4**-**6**.

Analytical reversed-phase HPLC analyses of miconazole product mixtures were conducted with identical hardware except a Waters Assoc. Model 994 multichannel UV detector or a Hewlett-Packard 1040M diode array UV detector/chemstation were used. Peak integration was performed with a Hewlett-Packard HP-3350A laboratory automation system. The method was a multi-step binary gradient: 75% A/25% B to 20% A/80% B over 1 min (linear), 20% A/80% B to 100% B over 25 min (linear), and a 4 min hold at 100% B. The flow rate was 1.2 mL/min. Mobile phase A was 0.1 M ammonium formate in water and mobile phase B was 0.05 M ammonium formate in methanol. Calibration curves were prepared for miconazole nitrate and compounds **13**-**15**. Response factors were determined for compounds **4** and **7**-**12**.

HPLC-MS data for the econazole nitrate autoxidation product mixture (see below) were obtained with a Finnigan TSQ 70 triple stage quadrupole mass spectrometer that was equipped with a Finnigan thermospray ionization interface. The HPLC consisted of two Rainin HPX pumps with an Apple *MACINTOSH*® Plus controller, a Rheodyne 7125 injector (typical injection volume = 20 µL), and a C-18 column (see above). The method was a multi-step binary gradient: 30% A/70% B to 5% A/95% B over 18.75 min (linear), and a 10 min hold at 5%A/95% B. The flow rate was 1.2 mL/min. Mobile phase A was a 0.1 M ammonium acetate in water and mobile phase B was 0.1 M ammonium acetate in methanol. The instrument was scanned from 135 to 500 mass units in 1 s with exclusion of the major mobile phase reagent ions. The auxiliary ionization (corona discharge, 2kV) was employed and the ion source block was set to 270 °C. The thermospray vaporizer temperature was set at 95, 100, or 105 °C. The presence of **3**, **4**, and **6** were determined from positive ion data. The presence of **5**, as well as **3** and **4**, were determined from negative ion data.

HPLC-MS data for the miconazole free base autoxidation product mixture (see below) were obtained with identical hardware except that a Perkin-Elmer Series 410 solvent delivery system was used. The method was a multi-step binary gradient: 75% A/25% B to 20% A/80% B over 1 min (linear), 20% A/80% B to 100% B over 25 min (linear), and a 4 min hold at 100% B. The flow rate was 1.2 mL/min. Mobile phase A was 0.1 M ammonium formate in water and mobile phase B was 0.05 M ammonium formate in methanol. The instrument was scanned from 170 to 870 mass units in 1 s with exclusion of the major mobile phase reagent ions. The auxiliary ionization (corona discharge, 2kV) was employed and the ion source block was set to 280 °C. The thermospray vaporizer temperature was set at 80 °C. The presence of **4** was confirmed by comparison of retention times and mass spectra with the corresponding data for authentic material.

GC-MS data for the miconazole free base autoxidation product mixture (see below) were obtained with a Finnigan TSQ 70 mass spectrometer and a Varian 3400 GC that was equipped with a J & W on-column injector. Helium was used as the carrier gas with a head pressure of 10 psi. The temperature program was 70 to 270 °C at 10 °/min with a final hold at 270 °C for 10 min. The injector was maintained at ambient temperature and the transfer line was maintained at 270 °C. The temperature of the ion source block was 150 °C. Chemical ionization was employed with methane as the reagent gas (0.1-0.5 Torr). The mass spectrometer was scanned from 100 to 500 mass units in 0.5 s. The presence of **13**, **14**, and **15** were confirmed by comparison of retention times and mass spectra with the corresponding data for authentic material.

Preparative HPLC was conducted with a system that consisted of two Rainin HPX pumps with 25 mL/min heads for solvent delivery, a Rainin HPX dispensing pump which was used to inject samples into the head of the column, an Apple *MACINTOSH*® controller, a Rainin *DYNAMAX*® 8 µm C-18 column (41.4 mm x 25 cm), a Gilson 116 dual channel UV detector (typically set to monitor 230 nm and 245 nm, and a Gilson Model 201 automated fraction collector.

Autoxidation of Econazole Nitrate. A typical reaction is described. Econazole nitrate (3.053 g, 6.9 mmol) and AIBN (11.3 g, 68.8 mmol) were dissolved in 400 mL of 90% ethanol. This solution was placed into the Parr reactor and the reactor was pressurized to 100 psi with oxygen and heated to 77 ± 1 °C for 20 hr with stirring. Samples of the reaction mixture were periodically removed and analyzed by HPLC. The amount of

unreacted econazole nitrate was 81%.

Isolation of Autoxidation Products of Econazole Nitrate. The autoxidation products of econazole nitrate were isolated with an isocratic preparative reversed-phase HPLC procedure. The mobile phase was a 0.025 M solution of ammonium formate in water/methanol (1:4). The flow rate was 40 mL/min. The product material from three autoxidation reactions was fractionated in 13 separate injection/separation/collection sequences which averaged 600-700 mg of material per sequence. Specifically, the product mixture from an individual reaction (approximately 400 mL) was concentrated to approximately 30 mL and mixed with 20 mL of the mobile phase. Approximately 10 mL of this solution was then used in an individual injection/separation/collection sequence. Appropriate fractions were combined and rechromatographed as required to obtain pure material. Fractions which contained **3** or **4** were adjusted to pH 8-9 with sodium carbonate. Fractions which contained **5** were adjusted to pH 1-2 with concentrated HCl. The adjusted fractions were then extracted with ether. The ether extracts were washed with water, dried over sodium sulfate, and evaporated to dryness. The following products were obtained:

Compound 3:[6] The residue from the fractions which contained the ester **3** was dissolved in 10 mL of ether and the ether solution was bubbled for a few minutes with hydrogen chloride gas. The ether was evaporated and the crude hydrochloride salt was dissolved in absolute ethanol. Ether was added to begin precipitation and the mixture was then cooled in an ice bath. The resulting ester hydrochloride that precipitated was recrystallized two more times from ethanol/ether. The final material (35 mg) was dried *in vacuo* over P_2O_5 at 40 °C for several days. ^{1}H NMR δ (CD_3SOCD_3) 9.147 (1 H, dd, J = 1.4, 1.4 Hz, H_2), 8.074 (2 H, AA′XX′, J = 8.6 Hz, H_{17}, $H_{17'}$), 7.74 (1 H, d, J = 2 Hz, H_{10}†), 7.71 (1 H, m, H_5†), 7.653 (1 H, m, H_4†), 7.650 (2 H, AA′XX′, J = 8.6 Hz, H_{18}, $H_{18'}$), 7.492 (1 H, dd, J = 8.5, 2.0 Hz, H_{12}), 7.439 (1 H, d, J = 8.5 Hz, H_{13}), 6.489 (1 H, dd, J = 6.7, 4.6 Hz, H_7), 4.846 (2 H, ABX, J = 6.7, 4.6 Hz, H_6), (†assignments are tentative); ^{13}C NMR δ (CD_3OD) 165.16 (s, C_{15}), 141.52 (s, C_{19}), 137.55 (d, C_2), 136.95 (s, C_8†), 134.41 (s, C_{11}†), 133.68 (s, C_9†), 132.50 (d, C_{17}, $C_{17'}$), 130.93 (d, C_{10}†), 130.28 (d, C_{18}, $C_{18'}$), 129.83 (d, C_{13}), 129.32 (d, C_{12}†), 128.67 (s, C_{16}), 124.38 (d, C_4), 121.49 (d, C_5), 72.44 (d, C_7), 52.66 (t, C_6), (†assignments are tentative); MS (DEI) m/z (relative intensity) 394 (0.8, M^+), 396 (0.7, M^+ + 2), 359 (5), 139 (100); MS (DCI) m/z (relative intensity) 395 (100, MH^+), 397 (96, MH^+ + 2), 399 (32, MH^+ + 4). Anal. Calcd for $C_{18}H_{14}N_2O_2Cl_4$: C, 50.03; H, 3.26; N, 6.48. Found: C, 49.48; H, 3.63; N, 6.27.

Compound 4: The hydrochloride salt of the alcohol **4** was prepared as described above. ^{1}H NMR δ (CD_3OD) 8.889 (1 H, br s, H_2), 7.538 (1 H, m, H_5), 7.538 (1 H, m, H_4), 7.516 (1 H, d, J = 2 Hz, H_{10}), 7.466 (1 H, d, J = 8.5 Hz, H_{13}), 7.358 (1 H, dd, J = 8.5, 2.0 Hz, H_{12}), 5.357 (1 H, dd, J = 6.9, 3.1 Hz, H_7), 4.557 (1 H, dd, J = 14.0, 3.1, H_6), 4.390 (1 H, dd, J = 14.0, 6.9 Hz, $H_{6'}$); ^{13}C NMR δ (CD_3OD) 138.18 (s, C_8†), 137.25 (d, C_2), 135.77 (s, C_{11}†), 133.55 (s, C_9†), 130.28 (d, C_{10}†), 129.83 (d, C_{13}†), 128.81 (s, C_{12}†), 124.33 (d, C_4), 120.64 (d, C_5), 69.16 (d, C_7), 55.25 (t, C_6), (†assignments are tentative); MS (DCI) m/z (relative intensity) 257 (100, MH^+), 259 (63, MH^+ + 2), 261 (11, MH^+ + 4). The HPLC-UV data were identical to the corresponding data for authentic **4**.

Compound 5: The isolated material gave HPLC-UV, EI-MS, ^{1}H NMR, and ^{13}C NMR data that were identical to the corresponding data for authentic 4-chlorobenzoic acid.

Autoxidation of Miconazole Nitrate. Miconazole nitrate (3.10 g, 6.5 mmol) and AIBN (11.60 g, 70.6 mmol) were dissolved in 400 mL of 90% ethanol. This solution was placed into the Parr reactor and the reactor was pressurized to 100 psi with oxygen and heated to 77.5 ± 1 °C for 27 hr with stirring. Samples were periodically removed from the reactor and analyzed by HPLC. The amount of unreacted miconazole nitrate at the end of the reaction was 85%. An identical reaction that was conducted without AIBN showed no degradation after 11 days.

Autoxidation of Miconazole Free Base. Miconazole nitrate (9.24 g, 19.4 mmol) and 0.99 N NaOH (19.6 mL, 19.4 mmol) were dissolved in 450 mL of 90% ethanol. The AIBN (15.9 g, 97 mmol) was then added. This solution was divided among three Parr reactors and the reactors were pressurized to 200 psi with oxygen and heated to 111 ± 1 °C for 3.3 hr. Samples were periodically removed and analyzed by HPLC. The amount of unreacted miconazole at the end of the reaction was 52%.

Isolation of Autoxidation Products of Miconazole. The autoxidation products of miconazole were isolated with an isocratic preparative reversed-phase HPLC procedure. The mobile phase was composed of 0.1 M ammonium formate in water (15%) and 0.05 M ammonium formate in methanol (85%). The flow rate was 25 mL/min. The product material from one miconazole free base autoxidation reaction was fractionated in 11

separate injection/separation/collection sequences which averaged 700-800 mg of material per sequence. Specifically, the product mixture from an individual reaction (approximately 450 mL) was concentrated to approximately 120 mL. Approximately 9.1 mL of this solution was then used in an individual injection/separation/collection sequence. Appropriate fractions were combined and rechromatographed as required to obtain pure material. The pH of the final fractions was adjusted to approximately pH 9 with sodium carbonate. The fractions were then extracted with ether. The ether extracts were washed with water and evaporated to dryness. The purity of the final isolated products was established by HPLC. Co-injection of each isolated product with the autoxidation product mixture and comparison of HPLC-UV spectra of isolated samples with corresponding peaks in product mixture were used to insure that the isolated compounds were originally present in the reaction mixture. The following products were obtained:

Compound 7:[6] ^{1}H NMR δ (CD_3OD) 7.885 (1 H, d, J = 8.4 Hz, $H_{21}^{\dagger}$), 7.606 (1 H, d, J = 2.1 Hz, $H_{18}^{\dagger}$), 7.6 (1 H, br s, $H_2^{\dagger}$), 7.552 (1 H, m, $H_{10}^{\dagger}$), 7.469 (1 H, dd, J = 8.4, 2.1 Hz, $H_{20}^{\dagger}$), 7.33 (1 H, m, $H_{12}^{\dagger}$), 7.33 (1 H, m, $H_{13}^{\dagger}$), 7.11 (1 H, br s, $H_5^{\dagger}$), 6.97 (1 H, br s, $H_4^{\dagger}$), 6.564 (1 H, t, J = 5.5 Hz, H_7), 4.598 (2 H, d, J = 5.5 Hz, H_6), (†assignments are tentative); ^{13}C NMR δ (CD_3OD) 164.38 (s, C_{15}), 140.17 (s, $C_{18}^{\dagger}$), 139.2 ($C_2^{\dagger}$), 136.44 (s, $C_{17}^{\dagger}$), 135.93 (s, $C_8^{\dagger}$),), 134.40 (s, $C_{11}^{\dagger}$), 134.29 (s, $C_9^{\dagger}$), 134.02 (d, $C_{21}^{\dagger}$), 132.13 (d, $C_{18}^{\dagger}$), 130.58 (d, $C_{10}^{\dagger}$), 129.97 (d, $C_{13}^{\dagger}$), 129.02 (d, $C_{12}^{\dagger}$), 129.2 ($C_4^{\dagger}$), 128.81 (s, $C_{16}^{\dagger}$), 128.63 (d, $C_{20}^{\dagger}$), 121.5 ($C_5^{\dagger}$), 73.97 (d, $C_7^{\dagger}$), 50.63 (t, $C_6^{\dagger}$), (†assignments are tentative); MS (DEI) m/z (relative intensity) 428 (0.5, $M^{+\cdot}$), 430 (0.6, $M^{+\cdot}$ + 2), 393 (4) 395 (4.6), 397 (1.5), 214 (70), 173 (100), 175 (65), 177 (12); MS (DCI) m/z (relative intensity) 429 (11, MH^+), 431 (14, MH^+ + 2), 433 (7, MH^+ + 4), 197 (100); MS (FAB) m/z (relative intensity) 429 (13, MH^+), 431 (17, MH^+ + 2), 433 (9, MH^+ + 4), 173 (100), 175 (64), 177 (11); HRMS (FAB) m/z calcd for $C_{18}H_{12}N_2O_2Cl_4$ + H^+ 428.9731, found 428.9760.

Compound 8: ^{1}H NMR δ (CD_3SOCD_3) 7.697 (1 H, d, J = 1.9 Hz, $H_{10}^{\dagger}$), 7.609 (1 H, d, J = 1.9 Hz, $H_{18}^{\dagger}$), 7.585 (1 H, d, J=8.3 Hz, $H_{13}^{\dagger}$), 7.530 (1 H, dd, J = 8.3, 1.9 Hz, $H_{12}^{\dagger}$), 7.514 (1 H, d, J = 8.3 Hz, $H_{21}^{\dagger}$), 7.458 (1 H, dd, J = 8.3, 1.9 Hz, $H_{20}^{\dagger}$), 5.091 (1 H, dd, J = 7.7, 4.4 Hz, H_7), 4.446 (2 H, s, H_{15}), 3.756 (1 H, dd, J = 14.1, 7.7 Hz, H_6), 3.690 (1 H, dd, J = 14.1, 4.4 Hz, $H_{6'}$), (†assignments are tentative); ^{13}C NMR δ (CD_3SOCD_3) 159.04 (s, $C_4^{\dagger}$), 157.93 (s, $C_5^{\dagger}$), 154.39 (s, $C_2^{\dagger}$), 134.68 (s, C_8), 134.04 (s, C_{16}), 133.58 (s, C_{11}), 133.12 (s, $C_9^{\dagger}$), 133.12 (s, $C_{17}^{\dagger}$), 133.05 (s, $C_{19}^{\dagger}$), 131.07 (s, C_{21}), 129.32 (d, C_{13}), 128.90 (d, C_{10}), 128.53 (d, C_{18}), 128.01 (d, C_{12}), 127.26 (d, C_{20}), 74.15 (d, C_7), 67.23 (t, C_{15}), 41.90 (t, C_6), (†assignments are tentative); MS (thermospray, no column, 0.075 M ammonium acetate in 3:1 water/methanol) m/z (relative intensity) 478 (58, M + NH_4^+), 480 (100, M + NH_4^+ + 2); MS (DCI) m/z (relative intensity) 461 (1.7, MH^+), 463 (2.2, MH^+ + 2), 285 (100), 287 (64); HRMS (DCI) m/z calcd for $C_{18}H_{12}N_2O_4Cl_4$ + H^+ 460.9630, found 460.9651.

Compound 9: ^{1}H NMR δ (CD_3SOCD_3) 8.723* (1 H, t, J = 6.2 Hz, H_1), 8.046* (1 H, br s, H_3), 7.756* (1 H, br s, $H_{3'}$), 7.620 (1 H, d, J = 1.8 Hz, H_{10}), 7.593 (1 H, d, J = 2.0 Hz, H_{18}), 7.563 (1 H, d, J = 8.3 Hz, H_{21}), 7.510 (1 H, d, J = 8.4 Hz, H_{13}), 7.491 (1 H, dd, J = 8.4 1.8 Hz, H_{12}), 7.424 (1 H, dd, J = 8.3, 2.0 Hz, H_{20}), 5.030 (1 H, dd, J = 6.8, 5.1 Hz, H_7), 4.443 (1 H, AB, H_{15}), 3.444 (2 H, m, H_6) (†assignments are tentative, *signal disappeared when CD_3OD was added); ^{13}C NMR δ (CD_3SOCD_3) 161.70 (s, $C_4^{\dagger}$), 160.32 (s, $C_5^{\dagger}$), 135.67 (s, C_8), 134.32 (s, C_{16}), 133.18 (s, $C_{11}^{\dagger}$), 133.13 (s, $C_9^{\dagger}$), 133.00 (s, $C_{17}^{\dagger}$), 132.92 (s, $C_{19}^{\dagger}$), 130.79 (d, C_{21}), 129.38 (d, C_{13}),128.72 (d, C_{10}), 128.48 (d, C_{18}), 127.7 (d, C_{12}), 127.21 (d, C_{20}), 75.55 (d, C_7), 67.12 (t, C_{15}), 43.25 (t, C_6), (†assignments are tentative); MS (DCI) m/z (relative intensity) 435 (18, MH^+), 437 (25, MH^+ + 2), 439 (12, MH^+ + 4), 259 (100), 261 (64), 263 (11); HRMS (DCI) m/z calcd for $C_{17}H_{14}N_2O_3Cl_4$ + H^+ 434.9837, found 434.9876.

Compound 10: ^{1}H NMR δ (CD_3SOCD_3) 7.621 (1 H, d, J=8.4 Hz, $H_{13}^{\dagger}$), 7.609 (1 H, d, J = 2.2 Hz, $H_{18}^{\dagger}$), 7.593 (1 H, d, J = 2.2 Hz, $H_{10}^{\dagger}$), 7.516 (1 H, d, J = 8.4 Hz, $H_{21}^{\dagger}$), 7.474 (1 H, dd, J = 8.4, 2.2 Hz, $H_{20}^{\dagger}$), 7.456 (1 H, dd, J = 8.4, 2.2 Hz, $H_{12}^{\dagger}$), 6.094* (1 H, t, J = 5.8 Hz, H_1), 5.481* (2 H, br s, H_3), 4.857 (1 H, dd, J = 6.5, 4.6 Hz, H_7), 4.443 (2 H, AB, H_{15}), 3.32 (2 H, m, H_6), (†assignments are tentative, *signal disappeared when CD_3OD was added); ^{13}C NMR δ (CD_3SOCD_3) 158.24 (s, C_2), 135.98 (s, $C_8^{\dagger}$), 134.45 (s, $C_{16}^{\dagger}$), 133.19 (s, $C_{11}^{\dagger}$), 132.92 (s, $C_{19}^{\dagger}$), 132.89 (s, $C_9^{\dagger}$), 132.89 (s, $C_{17}^{\dagger}$), 130.79 (d, C_{21}), 129.45 (d, C_{13}), 128.64 (d, C_{10}), 128.48 (d, C_{18}), 127.54 (d, C_{12}), 127.26 (d, C_{20}), 77.02 (d, C_7), 67.03 (t, C_{15}), 43.54 (t, C_6), (†assignments are tentative); MS (DCI) m/z (relative intensity) 407 (88, MH^+), 409 (100, MH^+ + 2), 411 (45, MH^+ + 4), 364 (11), 366 (14), 368 (8), 231 (39), 233 (26); MS (FAB) m/z (relative intensity) 407 (81, MH^+), 409 (100, MH^+ + 2), 411 (50, MH^+ + 4); HRMS (FAB) m/z calcd for $C_{16}H_{14}N_2O_2Cl_4$ + H^+ 406.9888, found 406.9892.

Compound 11: ^{1}H NMR δ (CD_3OD; chemical shifts are given for the major *Z* conformer (see structure **11**), atoms indicated by "**" represent the minor *E* conformer[15]) 8.028 (1 H, s, H_2), 7.918** (1 H, s, H_2), 7.553 (1

H, d, J=8.3 Hz, H_{13}†), 7.540 (1 H, d, J = 8.3 Hz, H_{21}†), 7.472 (1 H, d, J = 1.9 Hz, H_{10}†), 7.411 (1 H, d, J = 1.9 Hz, H_{18}†), 7.375 (1 H, dd, J = 8.3, 1.9 Hz, H_{20}†), 7.330 (1 H, dd, J = 8.3, 1.9 Hz, H_{12}†), 5.019 (1 H, dd, J = 7.2, 4.4 Hz, H_7), 4.496 (2 H, s, H_{15}), 3.574 (1 H, dd, J = 14.0, 4.4 Hz, H_6), 3.501 (1 H, dd, J = 14.0, 7.2 Hz, $H_{6'}$), (†assignments are tentative); ^{13}C NMR δ (CD_3OD; chemical shifts are given for the major *Z* conformer (see structure **11**), atoms indicated by "**" represent the minor *E* conformer[15]) 168.00** (d, H_2), 163.83 (d, H_2), 136.66 (s, C_8†), 135.61 (s, C_{16}†), 135.58 (s, C_{11}†), 135.22 (s, C_{19}†), 135.02 (s, C_9†), 134.89 (s, C_{17}†), 131.89 (d, C_{21}†), 130.36 (d, C_{13}†), 130.32 (d, C_{10}†), 129.93 (d, C_{18}†), 128.76 (d, C_{12}†), 128.31 (d, C_{20}†), 78.67** (d, H_7), 77.80 (d, H_7), 69.07** (t, H_{15}), 68.96 (t, H_{15}), 47.43** (t, H_6), 43.28 (t, H_6), (†assignments are tentative); MS (DCI) m/z (relative intensity) 392 (87, MH^+), 394 (100, MH^+ + 2), 396 (44, MH^+ + 4), 216 (70), 218 (46); MS (FAB) m/z (relative intensity) 392 (80, MH^+), 394 (100, MH^+ + 2), 396 (50, MH^+ + 4); HRMS (FAB) m/z calcd for $C_{16}H_{13}N_1O_2Cl_4$ + H^+ 391.9779, found 391.9799.

Compound 12: ^1H NMR δ (CD_3SOCD_3) 7.704 (1 H, d, J = 8.6 Hz, H_{21}), 7.684 (1 H, d, J = 2.0 Hz, H_{10}), 7.622 (1 H, d, J = 2.2 Hz, H_{18}), 7.567 (1 H, d, J=8.6 Hz, H_{13}), 7.534 (1 H, dd, J = 8.6, 2.0 Hz, H_{12}), 7.487 (1 H, dd, J = 8.6, 2.2 Hz, H_{20}), 7.14* (br, s, H_1), 4.957 (1 H, dd, J = 0.0, 3.5 Hz, H_7), 4.520 (2 H, AB, H_{15}), 3.266 (1 H, dd, J = 14.0, 3.5 Hz, H_6), 3.171 (1 H, dd, J = 7.0, 3.5 Hz, $H_{6'}$), (*signal disappeared when CD_3OD was added); ^{13}C NMR δ (CD_3SOCD_3) 134.58 (H_8), 134.14 (s, H_{16}), 133.41 (s, C_9†), 132.97 (s, C_{17}), 132.87 (s, C_{11}†), 132.87 (s, C_{19}†), 130.87 (d, C_{21}), 129.51 (d, C_{13}), 128.86 (d, C_{10}), 128.43 (d, C_{18}), 127.79 (d, C_{12}), 127.26 (d, C_{20}), 116.92 (s, C_2), 76.95 (d, C_7), 67.26 (t, C_{15}), 48.76 (t, C_6), (†assignments are tentative); IR (KBr) 226.5 cm^{-1} (C≡N stretch); MS (DCI) m/z (relative intensity) 389 (78, MH^+), 391 (100, MH^+ + 2), 393 (45, MH^+ + 4); MS (FAB) m/z (relative intensity) 389 (62, MH^+), 391 (100, MH^+ + 2), 393 (67, MH^+ + 4); HRMS (FAB) m/z calcd for $C_{16}H_{12}N_2O_1Cl_4$ + H^+ 388.9782, found 388.9764.

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