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Amodiaquine Analogs. Synthesis of 6-[[3-(*N,N*-Diethylamino)methyl-4-hydroxy]anilino]-5,8-dimethoxy-2,4-dimethylquinoline and Related Compounds†

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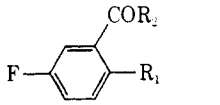
Oxidative amination of 2,4-dimethyl-5,8-quinolinedione (**7**) with 4-amino-α-diethylamino-*o*-cresol gave 6-[[3-(*N,N*-diethylamino)methyl-4-hydroxy]anilino]-2,4-dimethyl-5,8-quinolinedione (**9**), which was reduced to the corresponding 5,8-dihydroxyquinoline **13** with either borane or $Na_2S_2O_4$. Methylation of **13** with Me_2SO_4 gave mainly 6-[[3-(*N,N*-diethylamino)methyl-4-methoxy]anilino]-5,8-dimethoxy-2,4-dimethylquinoline (**15**), purified by column chromatography. Under controlled conditions, methylation of **13** with methyl iodide gave mainly 6-[[3-(*N,N*-diethylamino)methyl-4-hydroxy]anilino]-5,8-dimethoxy-2,4-dimethylquinoline (**16**). The structure of the latter was confirmed by further methylation to give **15** and also by the unambiguous synthesis of **16**. The latter involved the oxidative amination of **7** with 5-amino-2-benzyloxy-*N,N*-diethylbenzamide (**26**) to give 2-benzyloxy-*N,N*-diethyl-5-[(2,4-dimethyl-5,8-dioxoquinolin-6-yl)amino]benzamide (**28**). Reduction of **28** with $Na_2S_2O_4$ gave the corresponding 5,8-dihydroxyquinoline (**29**), which was methylated to give 2-benzyloxy-*N,N*-diethyl-5-[(5,8-dimethoxy-2,4-dimethylquinolin-6-yl)amino]benzamide (**31**) and its 6-*N*-methyl derivative **32**. Reduction of the amide moiety of **31** with a hydride gave **34**, and the removal of the benzyl blocking group of **34** by hydrogenation over a palladium catalyst gave **16**.

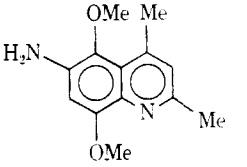
The aminoquinolines represent a group of drugs that possess broad antimalarial activity; the 4-aminoquinolines (chloroquine and amodiaquine) are effective against the plasmodia of erythrocytes but not those of tissue forms, whereas the 8-aminoquinolines (pamaquine and primaquine) act as gametocytocides against plasmodia in both man and mosquito and also act upon primary and secondary tissue schizonts. The 8-aminoquinolines are more toxic than the 4-aminoquinolines, the former showing marked toxicity in people having a deficiency of glucose-6-phosphate dehydrogenase in the erythrocytes.[1] Some 6-[(4-diethylamino-1-methylbutyl)amino]-5,8-dimethoxyquinolines were reported to be as well tolerated by mice and canaries as chloroquine and to be active against *Plasmodium vinckei* and the erythrocytic stages of *Plasmodium cathemerium* as primaquine.[2] In the search for more active and less toxic drugs, we undertook the synthesis of a 6-substituted amino analog of amodiaquine, since amodiaquine is less toxic than chloroquine.

Initially, introduction of the amodiaquine side chain was attempted by the alkylation route. The nitration of *m*-fluorobenzoic acid (**1**) gave the o-nitrobenzoic acid **2**,[3] which was converted to the amide **4** *via* the benzoyl chloride **3**. The alkylation of **5**[4] with **4** in either refluxing xylene in the presence of an acid acceptor or hot DMF to give the corresponding 6-substituted aminoquinoline **6** was unsuccessful as was the fusion of **4** and **5** at 140°.

In another approach oxidative amination of the 5,8-quinolinedione **7**[4] with 4-amino-α-diethylamino-o-cresol (**8**)[5] in ethanol containing acetic acid and cerous chloride to give the quinolinedione **9** occurred readily.‡ The preparation of the blocked quinoline **14** from **9** *via* the quinolinedione **10** and the dihydroxyquinoline **12** was attempted unsuccessfully when treatment of the latter with diazomethane in ether gave only **9** and a monomethylated derivative of **13**. Also, a similar route using the tetrahydropyranyl blocking group was terminated when the conversion of **9** to **11** was effected in poor yield (mass spectrum).



1, $R_1 = R_2 = H$
2, $R_1 = NO_2$; $R_2 = H$
3, $R_1 = NO_2$; $R_2 = Cl$
4, $R_1 = NO_2$; $R_2 = NEt_2$



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Reduction of the 5,8-quinolinedione **9** was effected with both borane and $Na_2S_2O_4$ to give **13**. The latter from the $Na_2S_2O_4$ reduction was confirmed by the mass spectrum. Methylation of this crude sample of **13** was attempted with Me_2SO_4, but the major product from this reaction was identified as the 5,8-quinolinedione **9**. In contrast, the borane reduction product gave a weak mass spectrum, which was attributed to the presence of a boron complex. The surmise was confirmed by elemental analyses. Methylation of this product occurred with Me_2SO_4 but gave a mixture of **15**, **17**, and **19** from which the methyl ether **15** was obtained pure by column chromatography. The successful preparation of **16** involved the reduction of a solution of **9** in $CHCl_3$ with an aqueous solution of $Na_2S_2O_4$ in an oxygen-free drybox to give a solution of **13** in $CHCl_3$, which was evaporated to dryness to give **13** free of inorganic salts. Alkylation of a solution of **13** in DMAc containing sodium hydride with MeI for a shorter period of time than that used in the Me_2SO_4 reaction gave mainly the desired dimethylated product **16**, purified by column chromatography. The isolation of **16** in good yield is remarkable as 31 methylated products are possible in this reaction. The structure of **16** was confirmed by methyl-

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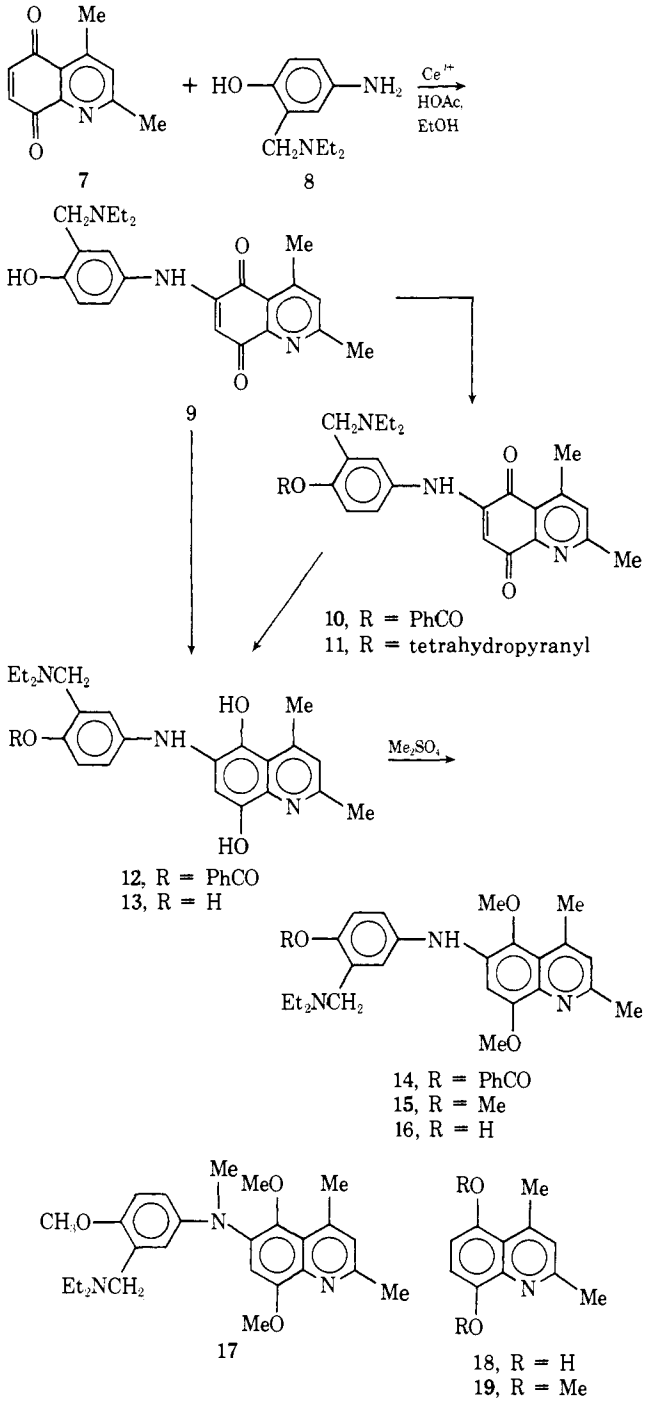
‡The oxidative amination of 5,8-quinolinedione in the 6 position with both aliphatic and aryl amines has been reported; see ref 4 and 6.

ation to give the trimethylated product **15** described above and also by the synthesis of **16** by another route. These results indicate that the methylation of the functional groups of **13** occurs in the following order: (a) 5,8-dihydroxy, (b) cresol OH, and (c) anilino NH (Scheme I).

Scheme I



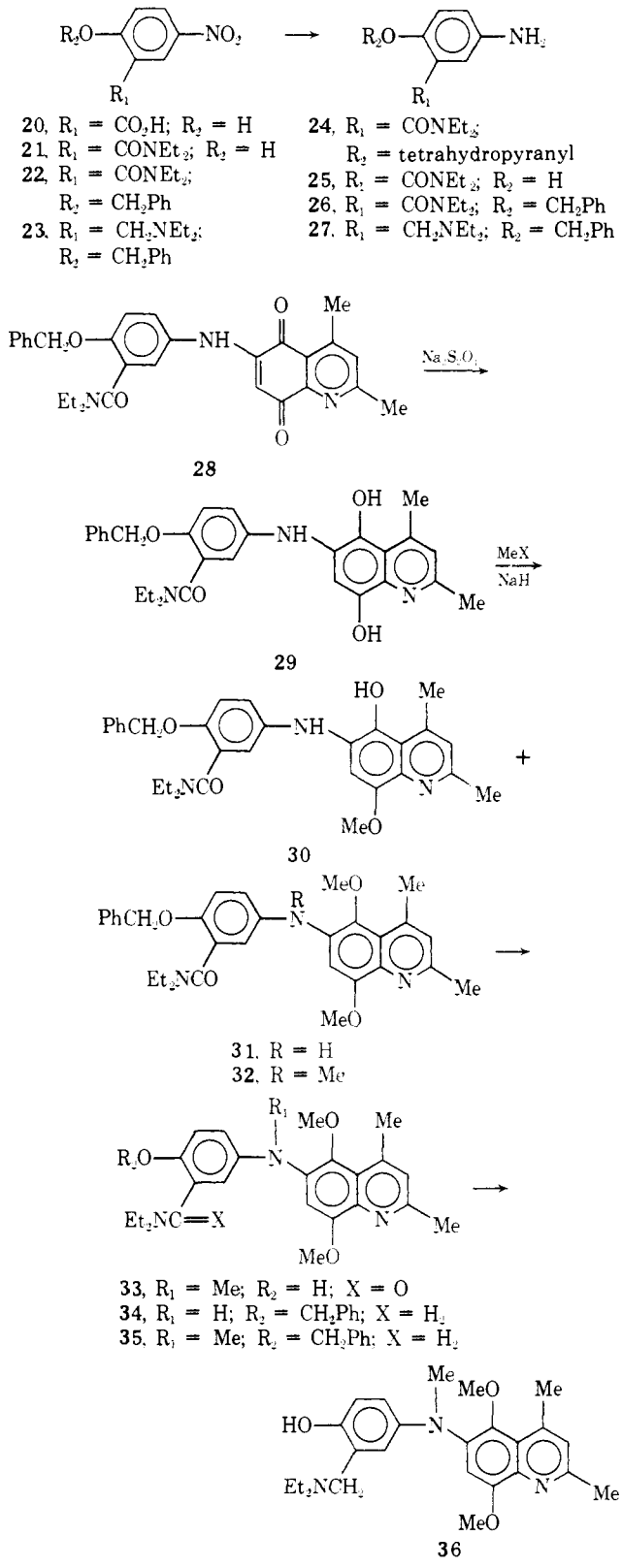
To show that the hydroxy groups of the quinoline ring were preferentially alkylated, it was necessary to prepare an intermediate in which the cresol OH group was blocked with a removable group. A compound that also eliminated from consideration the quaternarization of the tertiary amine function of the side chain involved the preparation of 5-amino-2-benzyloxy-*N,N*-diethylbenzamide (**26**). Treatment of 5-nitrosalicyclic acid (**20**), first with thionyl chloride and then with diethylamine, gave a good yield of the benzamide **21**. The alkylation of **21** with benzyl bromide to give **22** was effected in DMSO containing sodium hydride. The hydrogenation of **22** in the presence of Raney nickel under pressure at room temperature not only reduced the nitro group but also removed the *O*-benzyl blocking group to give the 5-amino-2-hydroxybenzamide **25**, which was also prepared by hydrogenation of **21** in the presence of Raney nickel. The reblocking of the OH group of **25** with 2,3-dihydropyran was unsatisfactory, this reaction giving a mixture of **24** and its (5-hydroxypentylidene)amino derivative.[7] Further investigation showed that the amide function of **22** could be reduced with borane to give **23** and that the nitro group of **23** could be reduced with Raney nickel at atmospheric pressure to give **27**. Based on these results, hydrogenation of a solution of **22** in ethanol with Raney nickel at atmospheric pressure and room temperature gave a good yield of **26** (Scheme II).

The oxidative amination of **7** with the blocked aniline **26** gave crude **28**, which was purified by column chromatography. A solution of **28** in ethyl acetate was shaken with a solution of $Na_2S_2O_4$ in pH 7 phosphate buffer to give the 5,8-dihydroxyquinoline **29**, the hydrochloride salt of which was isolated as a brittle glass. Although alkylation of **29** with Me_2SO_4 in DMAc gave a complex mixture containing either **30** or an isomer, treatment of **29** with MeI gave a mixture of **31** and **32**, which was separated by column chromatography to give a 51% yield of **32** (M^+ 527) and a 29.8% yield of **31** (M^+ 513) contaminated with a trace amount of **32**. Further work showed that with MeI a shorter reaction time increased the yield of **31** to about 45%. Removal of the benzyl group of **32** to give **33** by hydrogenation in the presence of 5% palladium on charcoal was successful under a hydrogen pressure of 3.6 kg/cm^{-2}. The mass spectrum of this sample indicated that the major component **33** (M^+ 437) was contaminated with minor amounts of **32** (M^+ 527) and corresponding tetrahydro derivatives of both **32** and **33**. The reduction of the amide function of **33** to give **36** was attempted with borane in THF, but the major product of this reaction was the tetrahydro derivative of **36** (M^+ 427). This sample also contained minor amounts of the desired product **36** (M^+ 423) and the tetrahydro derivative of **33** (M^+ 441). Apparently these tetrahydro derivatives resulted from reduction of the pyridine ring by borane, which has also been observed in a related heterocyclic system.[8]

Circumvention of the ring reduction described above was attempted by reversing the order of reductions. However, treatment of the benzyloxy compound **31** with borane gave only a minor amount of **34** (M^+ 499), the major product being its tetrahydro derivative (M^+ 503). The successful preparation of **34** involved the treatment of **31** with excess $NaH_2Al(OCH_2CH_2OCH_3)_2$ in refluxing THF for 2 hr. Removal of the benzyl blocking group of **34** to give **16** by hydrogenation in the presence of a palladium catalyst was successful under a hydrogen pressure of 3.6 kg/cm^{-2}. Also, the preparation of **16** was attempted by converting the mixture of **31** and **32** to a mixture of **34** and **35** and the latter to a mixture of **16** and **36**. However, the mixture of **16** and **36** moved on a silica gel column as a single band and could not be separated. The 5,8-dimethoxyquinolines containing the amodiaquine moiety in the 6 position of the ring are unstable and on silica gel columns readily undergo demethylation followed by oxidation to the corresponding quinolinedione.

Comparison of the pmr spectra of the compounds prepared in this report with those of related but less complex structures[4] allowed the assignment of the positions of the various methyl groups. These spectra were determined on either the free base or the β-resorcylate salt in either DMSO-d_6 or $CDCl_3$ solutions and giving the following

Scheme II



ranges for compounds **15–17**, **31**, **32**, and **36**: δ 1.00–1.40 (CH_3CN), 2.49–2.70 (4-CH_3), 2.77–2.85 (2-CH_3), 3.25–3.33 (6-CH_3N), 3.56–3.78 (5- or 8-CH_3O), and 3.77–3.92 (8- or 5-CH_3O, 4′-CH_3O).

Some of these compounds were tested against lethal, blood-induced *Plasmodium berghei* infections in mice.[9] Previously, activity was observed for some 6-alkylamino-5,8-quinolinediones.[10] In contrast, the 5,8-quinolinedione **9** showed no activity and was toxic at 160 mg/kg, whereas the quinolinedione **28** showed no activity and no toxicity. Results for the blocked amide compound **32** were similar to those obtained for **28**. The cresol methyl ether derivative **15** gave an increase in life span (ILS) of 4.7 days at 320 mg/kg and 5.1 days at 640 mg/kg and showed no toxicity. Borderline activity was observed for the sesquiresorcylate salt of **16**, which showed no toxicity and gave an ILS of 5.9 days at a dose of 640 mg/kg. A 2:1 mixture of **16** and **36** gave an ILS of 3.8 days at a dose of 640 mg/kg, suggesting that the 6-*N*-methyl compound **36** was completely inactive. In contrast, the diresorcylate salt of **16** was inactive both in the *P. berghei* test and the *P. cynomolgi* test in rhesus monkeys.§

Experimental Section&

N,N-**Diethyl-5-fluoro-2-nitrobenzamide (4).** A mixture of 2 (7.47 g, 40.4 mmol) and thionyl chloride (25 ml) was refluxed for 30 min on an oil bath with protection from atmospheric moisture. A homogeneous solution was obtained after 5 min. The cooled solution was diluted with dry benzene (25 ml) and evaporated to give **3** as a viscous oil. A solution of this oil in dry benzene (100 ml) was cooled in ice and treated rapidly dropwise with a solution of diethylamine (7.30 g, 100 mmol) in benzene (10 ml). After 15 min, the slurry was transferred to a separatory funnel and washed successively with H_2O (2 × 100 ml), 2 *N* HCl (2 × 50 ml), H_2O (2 × 50 ml), saturated $NaHCO_3$ solution (2 × 50 ml), and H_2O (4 × 50 ml). The benzene layer was dried over Na_2SO_4, treated with charcoal, filtered through Celite, and evaporated *in vacuo*. The residual clear, pale yellow oil was dried by prolonged oil pump evacuation: yield 9.2 g (94.7%). *Anal.* ($C_{11}H_{13}FN_2O_3$) C, H, N.

6-[[**3-(***N,N*-**Diethylamino)methyl-4-hydroxy]anilino]-2,4-dimethyl-5,8-quinolinedione (9).** A solution of **7** (7.70 g, 41.2 mmol), **8**·2HCl (11.0 g, 41.2 mmol), $CeCl_3 \cdot 7H_2O$ (1.5 g, 4.1 mmol), and anhydrous NaOAc (3.4 g, 41.2 mmol) in EtOH (400 ml) was stirred for 24 hr with free access to the air. The volatiles were removed *in vacuo*, leaving a dark purple gum. A solution of the gum in $CHCl_3$ (400 ml) was shaken with saturated $NaHCO_3$ solution (200 ml), filtered through a glass wool plug to remove a grey inorganic solid, washed with H_2O (200 ml), and evaporated to give a porous purple glass. A solution of the glass in $CHCl_3$–MeOH (98.2) was chromatographed on a coarse silica gel column (300 g, 70–320 mesh). The desired **9** was collected in four chromatographically homogeneous fractions: yield 13.6 g (87.3%); melting point indefinite, glass softens gradually above 65°. The structure was confirmed by its mass, pmr, and infrared spectra. A solution of the glass (1.5 g) in EtOH (10 ml) was diluted with ether (500 ml) and treated with a slight excess of 3 *N* ethanolic HCl. The precipitated purple solid was collected by filtration under N_2 and dried *in vacuo* over P_2O_5: yield 1.59 g; melting point indefinite, gradual softening and decomposition above 140°. *Anal.* ($C_{22}H_{25}N_3O_3 \cdot 2HCl \cdot H_2O$) C, H, Cl, N. In another experiment this product was obtained as a partial hydrochloride hemihydrate. *Anal.* ($C_{22}H_{25}N_3O_3 \cdot 0.75HCl \cdot 0.5H_2O$) C, H, Cl, N.

6-[[**3-(***N,N*-**Diethylamino)methyl-4-hydroxy]anilino]-5,8-dihydroxy-2,4-dimethylquinoline (13) Boron Complex.** A solution of **9**·0.75HCl·0.5H_2O (2.0 g, 4.8 mmol) in H_2O (75 ml) was neutralized to pH 7–8 by the addition of 1 *N* NaOH. The mixture was extracted with $CHCl_3$ (4 × 100 ml) and the combined, dried (Na_2SO_4) extract was evaporated to dryness. The resulting purple glassy residue was dried *in vacuo* over P_2O_5. This solid (1.8 g) was dissolved in dry THF (15 ml), and the solution was treated under N_2 with a solution of 1 *M* borane in THF (5 ml). After standing for 30 min, a second 5-ml portion of borane was added. After an additional 10 min, MeOH (20 ml) was added, and when the vigorous gas evolution subsided, the solution was chilled in Dry Ice and evaporated to dryness (oil pump). A solution of the residue in THF (30 ml) was treated with concentrated HCl (1 ml), but the gummy orange precipitate remained intractable

§L. H. Schmidt, private communication.

&Melting points were determined on a Mel-Temp apparatus. The mass and pmr spectra, respectively, were determined with a Hitachi Perkin-Elmer RMU-6D-3 spectrometer and a Varian A-60A spectrometer using tetramethylsilane as an internal reference. Silica gel was obtained from Brinkmann Instruments, Inc., and thin-layer chromatograms were usually developed with mixtures of $CHCl_3$ and MeOH. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.

after trituration and cooling. The residue was dissolved by the addition of MeOH (20 ml), and the solution was evaporated *in vacuo*. After treatment of this material with MeOH (30 ml) again, the residual orange-red glass was pulverized and dried *in vacuo* over P_2O_5: yield 2.17 g (86%). Although the mass spectrum of this product showed the presence of **13** (M^+ 381, weak) and the absence of significant amounts of **9**, the low volatility indicated that this sample was a boron complex. *Anal.* ($C_{22}H_{27}N_3O_3 \cdot 1.8CH_3OH \cdot 0.5B \cdot 2.1HCl$) C, H, B, Cl, N.

6-[[3-(*N,N*-Diethylamino)methyl-4-methoxy]anilino]-5,8-dimethoxy-2,4-dimethylquinoline (15). (**A**) A solution of **16** (2.80 g, 6.84 mmol) in dry DMAc (25 ml) was treated with a suspension of NaH (0.18 g, 7.5 mmol) in DMAc (25 ml). After stirring under N_2 for 5 min, a solution of methyl iodide (0.98 g, 6.9 mmol) in DMAc (25 ml) was added dropwise, and the mixture was allowed to stand for 9 hr. The reaction was diluted with H_2O (10 ml), adjusted to pH 7–8 (paper) with concentrated HCl, and evaporated to dryness *in vacuo*. A solution of the residue in $CHCl_3$ was extracted with $NaHCO_3$ solution and H_2O and after drying (Na_2SO_4) was evaporated to dryness: yield 3.1 g. A solution of this material in $CHCl_3$–MeOH (9:1) was chromatographed on a coarse silica gel column (300 g). In addition to recovered **16** (0.74 g, 26%), the desired trimethylated product **15** was obtained in several fractions totaling 1.20 g (41.6%). A solution of this sample in MeOH (12 ml) and Et_2O (20 ml) was treated dropwise with a solution of β-resorcylic acid (0.88 g) in Et_2O (200 ml). The initially gummy precipitate hardened into a tractable solid after stirring for several minutes. A solution of the solid in MeOH (8 ml) was added dropwise to vigorously stirred Et_2O (400 ml), and the precipitated glassy solid was dried *in vacuo* over P_2O_5 at room temperature for 48 hr and at 65° for 30 min: yield 1.5 g (59% recovery); melting point foams above 90°. The structure of this product was confirmed by its mass and pmr spectra. Also, both spectra showed strong $CHCl_3$ peaks. *Anal.* ($C_{25}H_{33}N_3O_3 \cdot 1.5C_7H_6O_4 \cdot 2CHCl_3$) C, H, N.

(**B**) A solution of **13** boron complex obtained from **9**·0.75HCl·0.5H_2O (2.00 g, 4.82 mmol) was methylated with dimethyl sulfate (1.22 g, 9.80 mmol) in DMAC (20 ml) in the presence of sodium hydride (0.82 g, 34 mmol) for 10 hr. After acidification and evaporation of the reaction mixture, column chromatography [silica gel, $CHCl_3$–MeOH (9:1)] of the residue gave two fractions. One was identified as **19** (160 mg, 15.3%) by its mass spectrum (M^+ 217). The second band (1.22 g, 60.4%) was shown by its pmr spectrum to be a 5:1 mixture of **15** (M^+ 423) and **17** (M^+ 437). Elution of the column with $CHCl_3$–MeOH (4:1) gave another crude sample (0.32 g) that was tentatively identified as a tetrahydro derivative of **9** (M^+ 383). A solution of the mixture of **15** and **17** (1.22 g) in $CHCl_3$–MeOH (4:1) was eluted from another silica gel column (300 g). The main band containing **15** (0.50 g) was dissolved in absolute EtOH (5 ml) containing ~2 *N* ethanolic HCl (2 ml). The solution was added dropwise from a pipet to Et_2O (600 ml). The precipitated solid was collected by filtration under N_2, dissolved in EtOH (5 ml), and reprecipitated from fresh Et_2O (750 ml). The tan powder was dried *in vacuo* over P_2O_5: yield 0.58 g; melting point indefinite, softens and foams at 135–140°. The structure of this product was confirmed by its mass and pmr spectra. Also, these spectra indicated the presence of Et_2O. *Anal.* ($C_{25}H_{33}N_3O_3 \cdot 1.7C_4H_{10}O \cdot 2HCl$) C, H, Cl, N.

A sample was also dried *in vacuo* over P_2O_5 at 56° for 60 hr. *Anal.* ($C_{25}H_{33}N_3O_3 \cdot 1.5C_4H_{10}O \cdot 2HCl$) C, H, N.

6-[[3-(*N,N*-Diethylamino)methyl-4-hydroxy]anilino]-5,8-dimethoxy-2,4-dimethylquinoline (16). (**A**) Inside a N_2-filled glove box, a solution of **9** (13.4 g, 35.3 mmol) in $CHCl_3$ (400 ml) was shaken vigorously for 10 min with a solution of excess $Na_2S_2O_4$ (15 g) in pH 7 phosphate buffer (150 ml). The $CHCl_3$ layer was drained into a N_2-filled flask, and the solvent was evaporated *in vacuo* (oil pump) without exposure to air: yield 13.5 g. Under a N_2 atmosphere a solution of this crude sample (**13**) in dry DMAc (100 ml) was treated with a slurry of NaH (2.8 g, 116 mmol, prepared from 4.9 g of 57% oil dispersion by washing with petroleum ether) in DMAc (50 ml). After stirring for 5 min, a solution of methyl iodide (15.1 g, 106 mmol) in DMAc (25 ml) was added dropwise, and the solution was stirred under N_2 for 5 hr. Water (10 ml) was added, and when the effervescence subsided, concentrated HCl was added dropwise to adjust the mixture to pH 7–8 (paper). The volatiles were removed *in vacuo;* a solution of the gummy residue in $CHCl_3$ (300 ml) was extracted with aqueous $NaHCO_3$ (200 ml) and with H_2O (200 ml). The dried (Na_2SO_4) $CHCl_3$ layer was evaporated to give a dark porous glass: yield 14.6 g. A solution of the glass in $CHCl_3$–MeOH (95:5) was chromatographed on a column of coarse silica gel (300 g, 70–230 mesh). The first major component, collected in two fractions totaling 7.30 g (50.6%), was identified by tlc and mass spectral analysis as **16**. A portion (2.0 g, 4.9 mmol) of this sample was dissolved in Et_2O (25 ml) containing MeOH (2 ml). A solution of β-resorcylic acid (1.5 g, 9.8 mmol) in Et_2O (250 ml) was added dropwise with vigorous stirring, and the brick-red precipitate was collected by filtration under N_2. The solid was washed well with Et_2O (3 × 100 ml) and dried *in vacuo* over P_2O_5 at room temperature for 18 hr and at 65° for 30 min: yield 2.2 g (70.1% as the sesquiresorcylate); melting point indefinite, sinters gradually and foams above 115°. *Anal.* ($C_{24}H_{31}N_3O_3 \cdot 1.5C_7H_6O_4$) C, H, N.

The second major component obtained from the column in several fractions totaling 2.56 g (17.4%) was identified as **15** (see above).

(**B**) A solution of **34** (2.27 g, 4.55 mmol) in EtOH (100 ml) was hydrogenated over 5% palladium-on-carbon catalyst (500 mg) for 4 hr at an initial pressure of 3.5 kg/cm^{-2}. The dark yellow solution was filtered under N_2 through a Celite pad into a solution of β-resorcylic acid (1.40 g, 9.10 mmol) in EtOH (25 ml). The orange-red solution was evaporated *in vacuo* (oil pump) to give a brittle solid residue: yield 3.04 g. Elemental analysis of this material indicated that it contained excess resorcyclic acid. A solution of the powdered solid in MeOH (15 ml) was added dropwise to vigorously stirred Et_2O (500 ml). The precipitated rust-colored solid was collected by filtration under N_2, washed thoroughly with Et_2O, and dried *in vacuo* over P_2O_5 at room temperature for 48 hr and at 65° for 2 hr: yield 2.39 g (69.7%); melting point foams up ~110°. *Anal.* ($C_{24}H_{31}N_3O_3 \cdot 2C_7H_6O_4 \cdot 2H_2O$) C, H, N.

***N,N*-Diethyl-2-hydroxy-5-nitrobenzamide (21).** A stirred mixture of **20** (16.4 g, 90.0 mmol) and excess thionyl chloride (32 g) in benzene (500 ml) was refluxed gently for 3.5 hr. The cooled solution was added slowly with stirring to an ice-cold solution of excess diethylamine (82 g) in benzene (250 ml). After standing for 16 hr, the volatiles were removed *in vacuo*. A suspension of the residue in H_2O (400 ml) was adjusted to pH ~3 with concentrated HCl, and the crude solid was collected by filtration and washed with cold H_2O. The solid was resuspended in H_2O (600 ml) to which 50% NaOH was added slowly until complete solution was obtained. This solution was treated with charcoal, filtered through Celite, and acidified to pH 3 with concentrated HCl. The precipitated solid was collected by filtration and recrystallized from 2:1 H_2O–EtOH (600 ml) to give long, pale yellow needles: yield 18.4 g (86.0%); mp 170–171°. The analytical sample was obtained by recrystallization from 1:1 H_2O–EtOH: mp 171–172°. *Anal.* ($C_{11}H_{14}N_2O_4$) C, H, N.

2-Benzyloxy-*N,N*-diethyl-5-nitrobenzamide (22). A suspension of NaH (2.70 g, 112 mmol) in DMSO (100 ml) was added to a solution of **21** (25.5 g, 107 mmol) in DMSO (100 ml). After the disappearance of the gray solid a solution of benzyl bromide (22.9 g, 134 mmol) in DMSO (50 ml) was added dropwise during 20 min. The reaction mixture was stirred at room temperature for 1 hr, at 60–65° for 2 hr, and at ~50° while the DMSO was removed *in vacuo*. A solution of the residue in benzene (500 ml) was extracted (3 × 150 ml) with 3% aqueous Na_2CO_3 (w/v) followed by water (2 × 200 ml). After filtration the benzene layer was evaporated to dryness *in vacuo*. The residual gum was dissolved in Et_2O (250 ml), and the solution was stirred for about 5 min after which spontaneous crystallization occurred. The mixture was diluted slowly with hexane (400 ml), and the dense white crystals were collected by filtration and dried *in vacuo* over P_2O_5: yield 31.6 g (89.8%); mp 100–101°. *Anal.* ($C_{18}H_{20}N_2O_4$) C, H, N.

2-Benzyloxy-*N,N*-diethyl-5-nitrobenzylamine Hydrochloride (23). A solution of **22** (0.50 g, 1.5 mmol) in THF (25 ml) was treated under N_2 with 1 *M* solution of BH_3 in THF (2 ml). The resulting solution was refluxed gently for 1 hr with separation of solid on the walls of the flask after about 15 min. The mixture was cooled in ice and treated with cold 6 *N* HCl (20 ml). Most of the THF was removed *in vacuo*, and the aqueous residue was adjusted to pH 10–11 with NaOH and extracted with Et_2O (2 × 75 ml). The Et_2O extract was washed with H_2O (50 ml), dried, and evaporated to dryness to give flat off-white platelets. This solid was dissolved in ethanolic HCl (10 ml) and the resulting solution was evaporated to dryness *in vacuo*. The residue was triturated with hexane and collected by filtration under N_2: yield 0.34 g (64.2%); mp 133–135° (M^+ 314).

5-Amino-*N,N*-diethyl-2-hydroxybenzamide Monohydrochloride (25). (**A**) A solution of **21** (1.0 g, 5.2 mmol) in EtOH (40 ml) was hydrogenated over Raney nickel catalyst (~1 g) at atmospheric pressure. The theoretical quantity of H_2 was absorbed in

5 hr. The mixture was filtered under N_2, and the clear, colorless filtrate was acidified with a slight excess of 2 *N* ethanolic HCl. The solution was evaporated to dryness, and the white solid residue was triturated thoroughly with Et_2O (200 ml). The solid was dried *in vacuo* over P_2O_5: yield 0.96 g (93.2%); mp 255° with extensive prior decomposition. *Anal.* ($C_{11}H_{16}N_2O_2$·HCl) C, H, Cl, N.

(**B**) A solution of **22** (0.20 g, 0.61 mmol) in EtOH was hydrogenated over Raney nickel catalyst in a Parr shaking apparatus for 36 hr at an initial H_2 pressure of 3.0 kg/cm^{-2}. Subsequent workup as in A described above gave a granular white solid that was shown by tlc to be the debenzylation product: yield 0.14 g (94% as the HCl salt); mp 245–248°.

5-Amino-2-benzyloxy-*N,N*-diethylbenzamide Monohydrochloride (26). A solution of **22** (0.20 g, 0.61 mmol) in EtOH (20 ml) was hydrogenated at atmospheric pressure over Raney nickel catalyst (~1 g). The theoretical volume of H_2 was absorbed in about 5 hr. The catalyst was removed by filtration under N_2, and the colorless filtrate was evaporated to dryness *in vacuo*. A solution of the residue in absolute EtOH (10 ml) was acidified with ~2 *N* ethanolic HCl (0.5 ml) and diluted with Et_2O (200 ml). The precipitated white solid was dried *in vacuo* over P_2O_5: yield 0.13 g (60.5%); mp 194–195° dec. *Anal.* ($C_{18}H_{22}N_2O_2$·HCl·0.83H_2O) C, H, Cl, N.

5-Amino-2-benzyloxy-*N,N*-diethylbenzylamine Dihydrochloride (27). A mixture of **23** monohydrochloride (0.25 g, 0.71 mmol) and Raney nickel (~0.5 g) in EtOH (50 ml) was hydrogenated under atmospheric pressure until the theoretical volume of H_2 for reduction of the nitro group was absorbed. The solution was filtered under N_2, and the filtrate was evaporated *in vacuo*. A solution of the residue in EtOH (5 ml) containing ~5 *N* ethanolic HCl (0.2 ml) was diluted slowly with Et_2O (200 ml). The granular, light tan precipitate was collected under N_2 and dried *in vacuo* over P_2O_5: yield 0.18 g (71% as the dihydrochloride salt); mp 152–155° with foaming (M^+ 284).

2-Benzyloxy-*N,N*-diethyl-5-[(2,4-dimethyl-5,8-dioxoquinolin-6-yl)amino]benzamide Three-Fourths Hydrate (28). A solution of **22** (28.4 g, 86.6 mmol) in EtOH (1200 ml) was hydrogenated at atmospheric pressure over Raney nickel catalyst (~20 g). When the uptake of H_2 ceased, the colorless solution of the resulting aniline (**26**) was filtered under N_2 through a Celite pad into a well-stirred mixture of **7** (16.2 g, 86.6 mmol) and $CeCl_3$·7H_2O (3.2 g, 8.7 mmol) in EtOH (250 ml). The resulting red-purple solution was stirred for 26 hr with free access to the air followed by evaporation to dryness *in vacuo*. A solution of the residue in $CHCl_3$ (750 ml) was shaken with 10% NaOAc solution (250 ml) and with H_2O (250 ml). The $CHCl_3$ layer was evaporated *in vacuo* to give a porous purple glass. A solution of this material in $CHCl_3$–MeOH (98:2) was chromatographed on a silica gel H column (800 g) that was poured, washed, and eluted with this same solvent. The main product band was collected in four arbitrary fractions totaling 39.1 g (93.9%). Two fractions (4.4 g, 10.5%) were contaminated with an unidentified material and were discarded. The remaining two fractions (34.7 g, 83.4%) were chromatographically homogeneous. The analytical sample was dried at 65° for 4 hr *in vacuo* over P_2O_5: melting point indefinite but softens gradually above 90°. *Anal.* ($C_{29}H_{29}N_3O_4$·0.75H_2O) C, H, N.

2-Benzyloxy-*N,N*-diethyl-5-[(5,8-dimethoxy-2,4-dimethylquinolin-6-yl)amino]benzamide (31) and 2-Benzyloxy-*N,N*-diethyl-5-[[*N*-(5,8-dimethoxy-2,4-dimethylquinolin-6-yl)-*N*-methyl]amino]benzamide (32). Working inside a glove box that had been purged with N_2, a solution of **28**·0.75H_2O (15.7 g, 31.6 mmol) in $CHCl_3$ (250 ml) was shaken with a solution of excess $Na_2S_2O_4$ (15 g) in pH 7 phosphate buffer (400 ml) until the color changed from deep purple to orange. Some difficulty was encountered with a stable emulsion that collected near the interface, and this material was discarded. The clear orange $CHCl_3$ layer was transferred to a flask, and the solvent was evaporated *in vacuo* (oil pump) without exposure to air. The residual porous glass was dried *in vacuo* overnight: yield 11.3 g (74% as **29**). A suspension of NaH [1.12 g, 46.6 mmol, prepared from 57% oil dispersion (1.97 g) by washing with petroleum ether] in dry DMAc (50 ml) was added to a stirred solution of the crude **29** (11.3 g, 23.3 mmol) in DMAc (50 ml). After 10 min, the mixture was treated dropwise during 15 min with a solution of methyl iodide (6.63 g, 46.6 mmol) in DMAc (50 ml). The mixture was stirred under N_2 for 4 hr, then H_2O (20 ml) and concentrated HCl (1 ml) were added, and the volatiles were removed *in vacuo*. A solution of the dark residual gum in $CHCl_3$ (200 ml) was shaken with 10% K_2CO_3 solution (100 ml) and H_2O (100 ml), dried over Na_2SO_4, and evaporated to dryness. A solution of the residue (12.2 g) in $CHCl_3$–MeOH (98:2) was chromatographed on a short column of coarse silica gel (250 g) to remove some very dark, unidentified resinous materials as well as some regenerated **28**. Those fractions containing mainly **31** and **32** were combined (11.4 g) and eluted with $CHCl_3$–MeOH (99:1) from a column of silica gel H (600 g). Based on mass spectral data and tlc, the first two fractions (total 2.87 g) contained mainly **32**. The amber glass was dried *in vacuo* over P_2O_5 at room temperature: melting point indefinite. *Anal.* ($C_{32}H_{37}N_3O_4$·1.5H_2O) C, H, N.

Continued elution gave **31** as a diffuse band that was collected in a number of fractions. Five of these fractions (total wt 4.30 g) were identified by mass spectral analysis and tlc to be mainly **31**. Five other fractions (total wt 2.16 g) appeared to have more than trace amounts of both **28** and **32** as contaminants. These were combined and chromatographed on a third silica gel column (250 g) using $CHCl_3$–MeOH (99:1) as elution solvent. This column gave an additional 1.2 g of **31**. Thus, the total yield of the essentially pure **31** was 5.4 g (45%) (M^+ 513).

6-[[4-Benzyloxy-3-(*N,N*-diethylamino)methyl]anilino]-5,8-dimethoxy-2,4-dimethylquinoline (34). A solution of $NaAlH_2(OCH_2CH_2OCH_3)_2$ (50 ml, ~175 mmol) in dry THF (50 ml) was added dropwise during 15 min to a solution of **31** (5.40 g, 10.5 mmol) in THF (250 ml) with external cooling in an ice bath. The resulting solution was refluxed under N_2 for 3 hr, cooled in an ice bath, and diluted by slow addition of H_2O (100 ml). Most of the THF was removed by evaporation under reduced pressure, and 50% NaOH (50 ml) was added to the aqueous residue to dissolve the gummy white aluminum compounds. The solution was extracted with Et_2O (2 × 300 ml); the extract was washed with H_2O (200 ml), dried over Na_2SO_4, and evaporated *in vacuo* to give a red gum: yield 4.3 g. A solution of the gum in $CHCl_3$–MeOH (95:5) was chromatographed on a short column of coarse silica gel (200 g). The desired compound **34** was collected in several fractions totaling 2.98 g (56.8%). Three of these (2.27 g) were shown by tlc to be essentially pure, and they were used as an intermediate without further purification.

6-[[3-(*N,N*-Diethylamino)methyl-4-hydroxy]anilino]-5,8-dimethoxy-2,4-dimethylquinoline (16) and 6-[[3-[(*N,N*-Diethylamino)methyl]-4-hydroxy-*N*-methyl]anilino]-5,8-dimethoxy-2,4-dimethylquinoline (36) Triresorcylate. A solution of a mixture of **34** and **35** (3.40 g) in EtOH (75 ml) was hydrogenated over 5% palladium on carbon (100 mg) in a Parr shaking apparatus at an initial pressure of 3.6 kg/cm^{-2}. Tlc showed only partial debenzylation within 24 hr. After additional catalyst (500 mg) was added, the hydrogenation was complete within 6 hr. The catalyst was filtered off under N_2, and the filtrate was evaporated *in vacuo* to give a red glassy solid: yield 2.2 g. A solution of this material in $CHCl_3$–MeOH (95:5) was chromatographed on a silica gel H column (200 g) that was eluted with the same solvent. The fractions that contained **16** and **36** were combined: yield 2.0 g. A 1.75-g portion of this material was dissolved in benzene (100 ml), and a solution of β-resorcylic acid (0.66 g, 4.3 mmol) in Et_2O (50 ml) was added dropwise with stirring. The precipitated β-resorcylate salt was collected in two crops as a red solid: yield 2.0 g. However, pmr and mass spectral analyses indicated that in addition to **16** and **36** a third component was present, which was identified as 6-[[3-[(*N,N*-diethylamino)methyl]-4-hydroxy-*N*-methyl]anilino]-2,4-dimethyl-5,8-quinolinedione (M^+ 395). The presence of this quinolinedione accounted for the deep red color of the samples and their solutions. To remove the impurity, a solution of the β-resorcylate salt in $CHCl_3$ (150 ml) was shaken with saturated $NaHCO_3$ solution (2 × 75 ml). The mixture of regenerated free bases (1.35 g) obtained by evaporating the $CHCl_3$ was dissolved in $CHCl_3$–MeOH (95:5) and chromatographed on a short silica gel H (125 g) column. Seven fractions totaling 1.32 g (98% recovery) were collected, and three of these (total 0.83 g) appeared to be essentially free of the quinolinedione (tlc). A solution of thse combined fractions in Et_2O (75 ml) was treated with a solution of 2 equiv of β-resorcylic acid in Et_2O (50 ml). The yellow solid was collected by filtration under N_2 and dried *in vacuo* over P_2O_5 at 65° for 3 hr: yield 1.04 g; melting point sinters above 110°. Based on elemental analyses and on its pmr and mass spectra, this mixture was calculated as two parts of **16** and one part of **36**, the whole as a triresorcylate salt. The mass spectrum showed only a trace of quinolinedione impurity. *Anal.* [($C_{24}H_{31}N_3O_3$)$_2$·$C_{25}H_{33}N_3O_3$·3$C_7H_6O_4$] C, H, N.

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Synthesis and Antimicrobial Activity of Aliphatic Nitro Compounds

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A series of simple aliphatic nitro compounds has been synthesized and tested for antimicrobial activity *in vitro*. Some of the compounds inhibit the growth of a broad range of fungi and bacteria including species of *Pseudomonas*. The most active compounds are alcohols containing the grouping $-CBrNO_2-$. Replacement of bromine by hydrogen, chlorine, or alkyl groups diminishes the activity. Structure–activity relationships are discussed.

In the course of a synthetic program based on aliphatic nitro compounds we noted antimicrobial activity in a group of aliphatic halogenonitro alcohols. Low concentrations of these compounds inhibit the growth of a broad range of organisms including species of *Pseudomonas*. Pseudomonads are notoriously insensitive to many bacteriostatic agents, including some commonly used preservatives and antiseptics, and are of marked medical and economic importance. We have therefore explored, and now report on, the synthesis and antimicrobial activity of a series of these compounds. A preliminary communication describing 2-bromo-2-nitropropane-1,3-diol, one of the most active members of the series, has already appeared.[1]

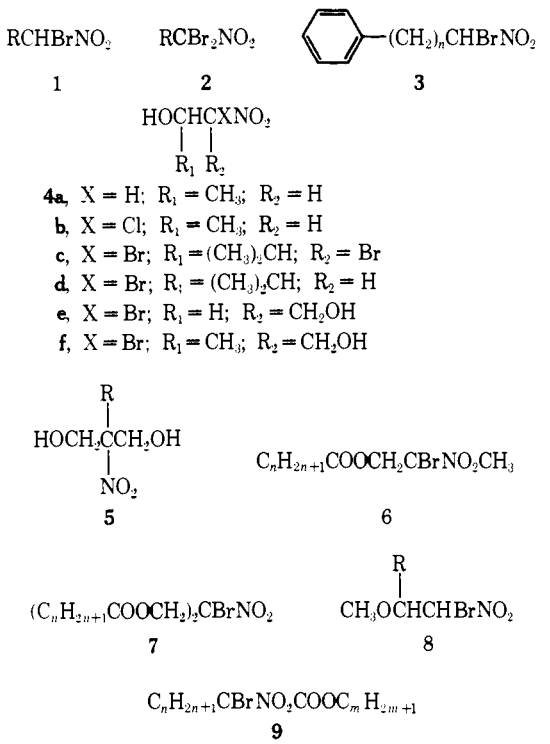
Results

The general formulas of the compounds studied are given in Chart I. The parent straight-chain 1-nitroalkanes ($C_nH_{2n+1}NO_2$, n = 1–6 and 8), at a concentration of 1000 μg/ml, did not inhibit the growth of any of the test organisms listed in Table I. The introduction of a bromine atom adjacent to the nitro group (**1**) enhanced the activity of these compounds somewhat but, with the exception of bromonitromethane (**1**, R = H), none of these derivatives was inhibitory to the bacteria at concentrations lower than 200 μg/ml.

Bromonitromethane, a volatile, highly irritant liquid, inhibited all the organisms at considerably lower concentrations but gave inconsistent results. This was attributed to the volatility of the compound, and it was not tested further. 1-Bromo-1-nitrooctane (**1**, R = C_7H_{15}), the next most active of this group, inhibited *Candida albicans* and the dermatophytes at 16.6 μg/ml but was much less active against the other organisms.

The dibromo analog of this compound, 1,1-dibromo-1-nitrooctane (**2**, R = C_7H_{15}) was no more active although the other dibromonitroalkanes were somewhat more inhibitory than their monobromo analogs. Attachment of a benzene nucleus to the alkyl chain of these compounds did not enhance activity against the pseudomonads, although 1-bromo-1-nitro-2-phenylethane (**3**, n = 1) and 1-bromo-1-nitro-3-phenylpropane (**3**, n = 2) inhibited some of the other organisms at 16.6 μg/ml. Bromonitrophenylmethane (**3**, n = 0) and 1-bromo-1-nitro-2,2-diphenylethane failed to inhibit any of the test organisms at concentrations less than 50 μg/ml.

Chart I



The unhalogenated nitro alcohols (**4**, X = H), in which R_1 ranged from H to C_6H_{13} and R_2 from H to C_4H_9, also possessed relatively low antimicrobial activity, generally requiring a concentration greater than 1000 μg/ml for growth inhibition (*e.g.*, **4a**, Table I), while none of the chloro analogs (**4**, X = Cl) was inhibitory at less than 200 μg/ml (*e.g.*, **4b**, Table I). However, when one or two bromine atoms were introduced adjacent to the nitro group, antimicrobial activity became much more pronounced. This is illustrated (Table II) by reference to the sensitivity