Synthesis of New Antimicrobial Agents

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SYNTHESIS OF NEW ANTIMICROBIAL AGENTS

A thesis presented to the Committee on Graduate Studies and the Department of Chemistry of Union College, Schenectady, New York, in partial fulfillment of the requirements for the degree of Master of Science.

by Michael J. Burke, M.S., 1975

Approved by

Approved by
Dean
Date
ABSTRACT

Two new compounds have been synthesized in sufficient quantities to be tested for antimicrobial activity. They are:

1. \( \text{N-}[4-(2,2\text{-Dichlorocyclopropyl}]\text{-N-}(3,4\text{-dichlorophenyl})\text{urea} \)

2. \( \text{N,N''-bis}[4-(2,2\text{-Dichlorocyclopropyl})\text{phenyl}]\text{-3,12-diimino-2,4,11,13-tetraazatetradecane-diimidamide} \)

These compounds were prepared in a manner similar to that used to produce known antimicrobial agents of similar structure.

The synthesis of \( 2,2\text{-methylenebis}[4-(2,2\text{-dichlorocyclopropyl})\text{phenol}] \) by condensing \( 4-(2,2\text{-dichlorocyclopropyl}) \) phenol with formaldehyde was also attempted, but without success.
ACKNOWLEDGEMENTS

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I. HISTORICAL

The idea that certain disease causing parasites could be controlled by synthetic chemicals through selective toxicity was first proposed by Ehrlich in the early 1900's. He based his concept on the then recently discovered analgesics and anesthetics which showed selective action on various tissues of the human body. Ehrlich proved his theory of chemotherapy in 1909 when he prepared a series of organic compounds of arsenic which were effective in the treatment of syphilis. These discoveries did much to undermine the theories of Uhlenhuth who held that drugs did not affect the parasite but stimulated the defense mechanism of the host. (Uhlenhuth's theories have recently received some support in the case of certain antiviral drugs.)

Although the mode of action by which these new chemicals worked was not fully understood, an intensive investigation to find new chemotherapeutic agents was undertaken. The search consisted of testing either compounds which were observed in nature to have antimicrobial activity or compounds which were prepared in the laboratory.

A major breakthrough in the understanding of chemotherapeutic agents occurred in 1939 shortly after the discovery of the very effective sulfonamides. D. D. Woods observed that the effectiveness of the sulfonamides could be reduced by introducing 4-aminobenzoic acid (see structure below).
He deduced from these findings that 4-aminobenzoic acid was a necessary coenzyme of the microbial cell and that sulfanilamide with its similar structure (see diagram) could block its function. It was later determined that 4-aminobenzoic acid is not a coenzyme but a precursor of the enzyme folic acid. The selective toxicity arises from the necessity of most bacteria to synthesize folic acid, since they cannot absorb it from dietary sources as mammalian cells do. The conclusions drawn by D. D. Woods seemed to set the stage for a rational approach to the preparation of new chemotherapeutic agents. However, in the years that followed many new compounds were prepared but few were developed into useful chemotherapeutic agents.

Then, in 1939, one of the most outstanding achievements in chemotherapy occurred with the development of penicillin which had been discovered ten years earlier by Fleming. The use of this microbial metabolite opened an entirely new field of investigation which let shortly thereafter to the development of several new antibiotics. Most of the antibiotics in use today are prepared by biological means for
economic reasons. However, almost all have been synthesized in the laboratory and therefore fit well into Ehrlich's concept of chemotherapeutic agents.

The increased knowledge of the biological pathways of microbial life over the past two decades has greatly expanded the field of chemotherapy. The field now includes such chemicals as steroids and antivirals which are produced both biologically and chemically. Although a great deal of knowledge has been accumulated over the years, thousands of chemicals are still screened for each new antimicrobial agent discovered.
II. INTRODUCTION

The object of this research was to synthesize three new compounds similar in structure to the following antimicrobial agents, \( \text{N-}(4\text{-chlorophenyl})\text{-N',}(3,4\text{-dichlorophenyl}) \text{urea, (1); N,N}^{\text{II}}\text{bis}[\text{4-chlorophenyl}]\text{-3,12-diimino-2,4,11,13-tetraazatetradesanediimidamide, (2) and 2,2'}\text{-methylenebis} \ [\text{4-chlorophenol}], (3)\) (see structures below).

\[
\text{(1)}
\]

\[
\text{(2)}
\]

\[
\text{(3)}
\]

These compounds are commonly referred to as triclocarban or TCC (1), chlorhexidine (2), dichlorophene (3).
The new compounds would have $(2,2\text{-dichlorocyclopropyl})$phenyl groups in place of the chlorophenyl groups in compounds (1) and (2) and $(2,2\text{-dichlorocyclopropyl})$phenol groups in place of the 4-chlorophenol groups in compound (3). These new compounds would have the following structures:

![Chemical Structures](image-url)
The first compound (10) fits into the general category of substituted ureas which were first mentioned as antimicrobial agents by Weinstein and McDonald in 1944\textsuperscript{10}. An intensive study of the substituted ureas by Beaver, Roman and Stoffel in 1957\textsuperscript{11} showed that superior antimicrobial activity is displayed by the phenylureas and that maximum effectiveness is reached with N-(4-chlorophenyl)-N'-(3,4-dichlorophenyl)urea (1). Their work also showed that a significant decrease in antimicrobial activity occurred when the position of the chlorine in the 4-chlorophenyl moiety was changed or when chlorine was replaced by a non-halogen group. The sharp reduction in antimicrobial activity with slight alteration in structure for this class of compounds led them to postulate a "lock and key" mode of action.

The second compound (13) is a bisbiguanide. This class of compounds was first studied for antimalarial activity by Curd and Rose in 1946\textsuperscript{12} and for antimicrobial activity by Rose and Swain in 1956\textsuperscript{13}. The work by Rose and Swain showed that maximum antimicrobial activity for this type of compound was reached when the biguanides were separated by a 5,6 or 7 member carbon chain and the terminal groups were 4-chlorophenyl. Their work led to the extensive use of (2) as a preservative and eye wash.

The third compound (15) is a member of the class of compounds known as bis-phenols which were first recognized
as antimicrobial agents by Beckhold and Ehrlich in 1906. Ehrlich also determined that effectiveness could be increased by chlorinating the phenols. In 1927 the Bayer Company of Germany produced several 2,2-methylenebis halogenated phenols for use as mothproofing agents. Kunz and Gump at the Givaudan Corporation Laboratories began an extensive study of the halogenated bis-phenols in 1937. They developed and improved methods of production for (3), a mildewproofing agent and 2,2'-methylenebis[3,4,6-trichlorophenol] which is currently used in the formulation of antiseptic soaps. Norman (1960) and simultaneously Joswick and Gerhardt (1960) have postulated that the mode of action of 2,2'-methylenebis[3,4,6-trichlorophenol] involves absorption at the cell surface with disruption of permeability which leads to loss of cell contents and eventually death.

The antimicrobial activity of compounds containing a dichlorocyclopropyl group was mentioned by Bruson, Woodbridge and Plant in 1971. Their work described the preparation of nitro-substituted phenyl dihalocyclopropanes to be used as fungicides, insecticides and herbicides.
III. SCHEMES

Scheme 1 - Preparation of Intermediates.
Scheme 2 - Preparation of $N-[4-(2,2\text{-dichlorocyclopropyl})$ phenyl]$-N'-(3,4\text{-dichlorophenyl})$urea. (10)
\[ \left[ N\equiv C-N-C\equiv N \right]^+ - Na^+ \]  + \[ \text{NH}_2 \]  

HCl, water  

\[ \text{Cl}-\text{Cl} \]  \[ \text{NH} \]
\[ \text{H} \]  \[ \text{H} \]  \[ \text{N} \]  \[ \text{C}-\text{N}-\text{C}\equiv\text{N} \]  

nitrobenzene  

\[ \text{Cl}-\text{Cl} \]  \[ \text{NH} \]  \[ \text{NH} \]  \[ \text{H} \]  \[ \text{H} \]  \[ \text{H} \]  \[ \text{H} \]  \[ \text{N} \]  \[ \text{C}-\text{N}-\text{C}\equiv\text{N} \]  

\[ 2 \text{N} \equiv \text{C} \equiv \text{N} \equiv \text{N}^{-} \text{Na}^{+} + \text{H}_{3} \text{N} \left( \text{CH}_{2} \right)_{6} \text{NH}_{3} + 2\text{Cl}^{-} \quad (11) \]

\[ \text{n-butanol} \rightarrow \begin{array}{c}
\text{H} \quad \text{H} \\
\text{H} \quad \text{H} \\
\text{N} \equiv \text{C} \equiv \text{N} \equiv \text{N} \equiv \text{N} \\
\text{NH} \\
\text{NH} \\
\text{N} \equiv \text{C} \equiv \text{N} \equiv \text{N} \equiv \text{N} \\
\text{NH} \\
\text{NH}
\end{array} \quad (14) \]

\[ \text{N} \equiv \text{C} \equiv \text{N} \equiv \text{N} \equiv \text{N} \equiv \text{N} \\
\text{NH} \\
\text{NH} \quad + \\
\text{H} \quad \text{H} \\
\text{H} \quad \text{H} \\
\text{Cl} \\
\text{Cl}
\]

\[ \text{N} \equiv \text{C} \equiv \text{N} \equiv \text{N} \equiv \text{N} \equiv \text{N} \\
\text{NH} \\
\text{NH} \quad + \\
\text{N} \equiv \text{C} \equiv \text{N} \equiv \text{N} \equiv \text{N} \\
\text{NH} \\
\text{NH} \\
\text{Cl}^{+} \\
\text{Cl}^{-}
\]

\[ \text{nitrobenzene} \rightarrow \begin{array}{c}
\text{Cl} \\
\text{Cl}
\end{array} \quad (13) \]

Scheme 4 - Method II for preparing: N,N'-bis[4-(2,2-dichlorocyclopropyl)phenyl]-3,12-diimino-2,4,11,13-tetraazatetradecanediimidamide.
Scheme 5 - Proposed method of preparing: 2,2'-methylenebis [4-(2,2-dichlorocyclopropyl)phenol].
IV. EXPERIMENTAL

General - Melting points were determined in capillaries using a Thomas Hoover Capillary Melting Point Apparatus, Model No. 6406H and are uncorrected. Catalytic reduction was carried out using a Parr Series 3910 Hydrogenation Apparatus. Spectral characteristics were determined on the following instrumentation: infrared spectra, Perkin-Elmer Model 337 spectrophotometer; nmr spectra, Varian T-60 and HA-100 spectrometers, using TMS as an internal standard; mass spectra JEOL JMS-OLSC mass spectrometer; UV spectra, Cary 15 spectrometer. Gas-liquid chromatography was run on a Hewlett-Packard 5750 instrument. Microanalyses were performed by Instranal Laboratories, Inc., Rensselaer, New York.

Thin Layer Chromatography. I. Materials and Apparatus - Silica Gel F-254 (FM Labs Inc.) precoated 0.25mm plates were used. Experiments were carried out using 20 x 20cm plates in a developing chamber (30 x 27 x 9cm) lined with filter paper. The samples were applied with a microsyringe "Pressure-Lock" (Pierce) and were observed under long and short wave UV light and after exposure to I₂.

II. General Procedure - TLC was used mostly for qualitative evaluation. When estimates of impurities were made the following procedure was used: a known concentration of starting material (usually 5 or 10% by weight of the product) was run simultaneously with the product. Estimates of the
percent of starting material and other unknowns were made by visually comparing spot intensities.

(2,2-Dichlorocyclopropyl)benzene (4), Method I -
Ethenyl benzene (104g, 1 mole), chloroform (120g, 1 mole) benzyltriethylammonium chloride (4g, 0.018 mole) and a 50-50 sodium hydroxide-water solution (200g) were stirred together at 40° for four hours. The resulting mixture was diluted with water (75g) and the layers were separated. The water layer was discarded and the organic layer was fractionally distilled under vacuum. The clear, colorless product (4) (145g, 78%) was collected at 120° under 12mm vacuum. nD 1.5512 (Ref. nD 1.5505)

(2,2-Dichlorocyclopropyl)benzene (4), Method II -
Ethenyl benzene (104g, 1 mole), chloroform (120g, 1 mole) sodium hydroxide (120g, 3 mole), water (10g) and ethyl dimethyl carbinol (100g) were stirred together at 101° to 105° for 35 minutes. Cooled and added chloroform (60g, 1 mole) and sodium hydroxide (40g, 1 mole). Heated and stirred at 102° to 104° for 45 minutes then quenched in water (500g). Filtered, let stand for one week and separated. The water layer was discarded and the organic layer was fractionally distilled under vacuum. The clear, colorless product (4) (90g, 49%) was collected at 120° under 12mm vacuum. nD 1.5505 (Ref. nD 1.5505)
1-(2,2-Dichlorocyclopropyl)-4-nitrobenzene (5) and 2-nitrobenzene (5a) - A solution of water (13g) in acetic acid (40g) was added slowly to concentrated sulfuric acid (177g, -5^0) keeping the temperature below 0^0. To this solution was added (4) (45g, 0.24 mole) previously cooled to 5^0. To the resulting mixture nitric acid (25g, 0.4 mole) was added over three hours while maintaining a temperature of +3^0 to +5^0. The reaction mixture was then quenched in a mixture of ice (400g) and chloroform (200g). The layers were separated and the aqueous layer was washed with chloroform (100g). The chloroform layers were combined and washed with a water solution of sodium carbonate (300ml, 5%). The sodium carbonate layer was backwashed with chloroform (75g). The combined chloroform extracts were stirred with Drierite (10g), Darco X (2g) and filter-cel (2g) for one hour, filtered and concentrated to an amber oil (5) and (5a), (57g, 102%). glc, (4) (3%); (5a) (29%); (5) (63%); unknown (4%). (Ref. 23 glc (4) (6%); (5a) (27%); (5) (67%).

4-(2,2-Dichlorocyclopropyl)benzenamine (6) - Ethanol (100g, 100% 2B), palladium on carbon (2g, 50% wet) and (5), (5a) (200g, 0.86 mole) were reduced at 40^0-50^0 under 50psi hydrogen pressure. Cooling was required and the theoretical amount of hydrogen was taken up in one and one-half hours. The mixture was then cooled and the alcohol was removed by vacuum distillation. To the residue was added toluene (600g) and a solution of concentrated hydrochloric acid
(24g) in water (440g). The layers were separated and the toluene layer was extracted with a solution of concentrated hydrochloric acid (6g) in water (440g). The combined water extracts were basified with sodium hydroxide (35%) and extracted with chloroform (2 x 300g). The combined chloroform extracts were stirred with Drierite (20g) and Darco X (5g) for one hour, filtered and concentrated under vacuum to a dark oil. The dark oil was triturated with hexane (3 x 500g, hot) and the hexane layers were cooled rapidly in an ice bath. The precipitate was filtered and dried at room temperature under vacuum to give an almost white product (6) (67g, 53%). m.p. 58°-60°; TLC, FTI 2%;

Anal. Calcd. for C₉H₇Cl₂N; Cl, 35.09; N, 6.93.
Found: Cl, 35.25; N, 7.02.

The hexane filtrates were concentrated to dryness and reworked as a second crop.

Second Crop of 4-(2,2-Dichlorocyclopropyl)benzenamine (6) - The residues (50g) from hexane concentrations and untriturated material were again triturated with hexane (3 x 75g, hot). The hexane layers were cooled, filtered and dried to give a brown product (27g), m.p. 52°-55°. This material was stirred with water (150g) and sufficient 1:1 hydrochloric acid-water was added to make a clear dark solution. The solution was then extracted with benzene (3 x 50g) which removed a large amount of the color. The aqueous layer was made basic with sodium hydroxide (35%),
cooled at 5° for one hour, filtered and washed with water (20g). The filter cake was dried under vacuum at room temperature to give a slightly colored product, m.p. 55°-58°. This material was triturated with hexane (3 x 150g, hot) and the hexane layers were cooled (5°) rapidly in an ice bath. The precipitate was filtered, washed with hexane (25g, cold) and dried in a vacuum at room temperature to give an almost white product (6) (12g), m.p. 60°-62°.

4-(2,2-Dichlorocyclopropyl)benzenamine hydrochloride (6b) - Benzene (100g) and (6) (18g, 0.09 mole) were stirred to complete solution and concentrated hydrochloric acid (10g, 0.1 mole) was added slowly with cooling. The resulting mixture was stirred at 15° for one hour, filtered and washed with benzene (2 x 20g). The filter cake was dried under vacuum at 50° to give a white product (6b) (18.8g, 85%), m.p. 220°-230° with decomposition.

4-(2,2-Dichlorocyclopropyl)phenol (7) - Water (400g), concentrated sulfuric acid (60ml) and (6) (43.3g, 0.21 mole) were heated and stirred to complete solution 85°-90°, then cooled to 5°. A solution of sodium nitrite (15.6g, 0.23 mole) in water (50ml) was added over one-half hour, then cooled at 5° for one hour. Toluene (425g) and cupric sulfate pentahydrate were added and the mixture was stirred and heated at 70° for two hours during which time N₂ was evolved. The reaction mixture was allowed to cool and separate overnight. The aqueous layer was washed with toluene (175g) and discarded. The toluene layers were
combined and extracted with sodium hydroxide solution (2 x 200g, 10%). The aqueous alkaline layer was acidified with concentrated hydrochloric acid and extracted with chloroform (2 x 350g). The combined chloroform extracts were stirred with Drierite (20g) and Darco X (5g) for one hour and filtered. The chloroform extracts were concentrated to a dark oil which was triturated with heptane (4 x 80ml, hot). The heptane layers were combined and concentrated to a reddish liquid which solidified on standing (7) (19g, 43%), m.p. 51°-54°; TLC, less than 5% (8), two slight unknown impurities.

1:6Hexanediame dihydrochloride (8a) - Benzene (200g) and (8) (58g, 0.5 mole) were stirred to complete solution and cooled to 5°. Ethanolic hydrochloric acid (100g, 36%, 1 mole) were added slowly keeping the temperature below 25°. The mixture was cooled at 5° for 30 minutes and the precipitate was filtered and washed with benzene (3 x 25g). The filter cake was dried to give a white product (8a) (86g, 90%), m.p. 252°-255°.

N-[4-(2,2-Dichlorocyclopropyl)phenyl]-N-(3,4-dichlorophenyl)urea (10) - Ethyl ether (15g) and (9) (5.61g, 0.03 mole) were stirred to complete solution. To this solution was added a solution of (6) (6.06g, 0.03 mole) in ethyl ether (15g). Upon completion of addition, ethyl ether (20g) was added and the mixture was stirred for one and one-half hours. The precipitate was filtered and washed with ethyl
ether (2 x 20g). The filter cake was dried to give an almost white product (10) (9.5g, 80%), m.p. 202°-203° with decomposition. Ethanol (200g) and crude (10) were heated and stirred to complete solution. Darco X (2g) were added and the mixture was stirred for one hour and filtered through filtercel. The filtrates were cooled at 5° overnight, filtered and dried to give a white product (10), (5.6g, 64%), m.p. 207°-208°; nmr (DMSO d$_6$ - TMS) 2.01(d, 2, J=9), 3.07(t, 1, J=10), 7.0-8.1(m, 7), 8.8(S, 2); uv max(EtOH) 268 μm (49,040); ir (KBr) 3310 (N-H), 1650 (C=O, amide), 1590cm$^{-1}$ (N-H); mass spectrum m/e (rel. intensity) M$^+$ 388(2), P+2(3) 253(7), 227(11), 192(59), 187(47), 166(41), 156(52), 130(52).

N-Cyano-N'-[4-(2,2-dichlorocyclopropyl)phenyl] guanidine (12) - Sodium dicyanamide (11) (12g, 0.13 mole), water (125g) and (6) (25.1g, 0.12 mole) were heated and stirred at 90°-95° until a complete solution was formed. Hydrochloric acid concentrated (13.3ml, 0.46 mole) in water (15g) was dripped in over a period of 45 minutes. The mixture was then cooled at 5° for one hour and the precipitate was filtered and washed with hydrochloric acid (7ml, 10%), water (3 x 5ml) and benzene (5ml). The dried filter cake was slurried twice in ethanol (50g, 100% 2b) and once in methanol (50g, hot). Filtered and dried to obtain a white product (12) (9.8g, 37%), m.p. 199°-202°; nmr (DMSO d$_6$-TMS) 2.09 (d, 2, J=9), 3.04 (t, 1, J=10), 6.98(S, 2), 7.0-7.6(m, 4), 9.03 (S, 1); uv max(EtOH) 260 μm (24,570), 223 μm (12,330);
ir(KBr) 3315 (N-H), 2180 (N-C=N), 1645, 1580, 1550, 1511 cm⁻¹; 
mass spectrum, m/e (rel. intensity) M⁺ 268(12) P+2(7), P+4 (1), 232(48), 227(16), 216(12), 198(44), 191(62), 155(56), 
149(44), 130(100).

\[ \text{N,N}^{11}-\text{bis}[4-(2,2-\text{Dichlorocyclopropyl})\text{phenyl}] \text{-3,12-diimino-2,4,11,13-tetraazatetradecanediimidamide (13)} \]

Nitrobenzene (30g), (12) (8.3g, 0.31 mole) and (8a) (2.8g, 0.15 mole) were stirred and heated at 140°-145° for four hours. Cooled to 85°, filtered and dried the filter cake under vacuum at 50°. The crude product (13) was slurried in a solution of acetone (75g) and acetic acid (7g). The product (13) was still brown in color so it was slurried in ethanol (300 g, hot, 100% 2B), filtered and dried at 70° to give a light tan product (13) (2.3g, 20%) m.p. 220°-225° with decomposition; nmr (DMSO d₆-D₂O, 4:1, TMS) aromatic to aliphatic should be 1:2.5. Found 1:5.47; ir 1640 cm⁻¹ (C=NH); uv max(EtOH) 263.5 μm (24,370), 233.5 (18,140); TLC, large amount of (13), large amount of impurity at the origin.

\[ \text{N,N}^{11}-\text{1,6-hexanediylbis}[N-\text{cyano-guanidine}] \text{(14)} \] - Sodium dicyanamide (11) (17.8g, 0.2 mole), (8a) (18.8g, 0.1 mole) and n-butanol were stirred and heated at reflux (117°) for eight hours, then cooled to room temperature and filtered. The filter cake was washed with isopropyl alcohol and dried at 65°. The dried cake was slurried in water (700ml, 75°), filtered (75°) and dried at 65°. The crude product (14)
was slurried in methanol (400ml, 50°), filtered at room temperature and dried at 65° to give a tan product (14) (14g, 58%), m.p. 205°-208° (Ref. 24 m.p. 208°-212°); TLC showed only one slight impurity.

**N,N-tetrazatetradecanediimidamide (13),**

*Scheme 4* - Nitrobenzene (100g), (6b) (14.2g, 0.06 mole) and (14) (7.5g, 0.03 mole) were heated and stirred at 140° for four hours, then cooled to room temperature, filtered and dried under vacuum at 60°. The crude product was slurried twice in benzene (100g) and dried at 60° to obtain a brown product (13) (15.5g). The crude product (13) (11g) was slurried in a hot 50-50 water-methanol mixture (200g). Filtered (hot), washed with acetone (10g), ethyl ether (10g) and dried at 75° to obtain a light tan product (13) (4g, 22%). nmr 1.38(S,8) 2.08(d,4,J=9), 2.80-3.50(m,6), 4.04(S,12), 7.0-7.75(m,8); ir(KBr) 3310 (N-H), 1640, 1580, 1540, 1510 cm⁻¹; mass spectrum, m/e (re. intensity) 226(4), 191(35), 155(19), 130(25), 36(100). TLC only slight unknown impurity at origin.

**Anal. Calcd for C₂₈H₃₈Cl₆N₁₀:** C, 46.23; H, 5.27; Cl, 29.24. Found: C, 46.55; H, 5.33; Cl, 29.37.

**2,2'-methylenebis[4-(1,1,3,3-tetramethylbutyl)phenol]** - A solution (-10°) of 4-(1,1,3,3-tetramethylbutyl)phenol (24g, 0.115 mole) in methanol (35g) and water (3.5g) was added to sulfuric acid (60ml, 0°). The mixture was stirred
and cooled to -5\(^\circ\) and formaldehyde (9g, 38\%, 0.055 mole) was added dropwise. After the addition of a few drops a sticky material began to form and at the end of the addition the entire mixture was thick and unworkable. The experiment was terminated.
V. DISCUSSION AND RESULTS

A. Preparation of Intermediates

The first intermediate (4) was obtained in good yield by following a procedure described by Makosza and Wawrzyniewicz. This reaction gives better yields than methods previously described by Doering which required anhydrous conditions because of the rapid hydrolysis of dichlorocarbene:

\[ :\text{CCl}_2 + 2\text{OH}^- \rightarrow \text{CO} + 2\text{Cl}^- + \text{H}_2\text{O} \]

The system used by Makosza and Wawrzyniewicz involves a unique two-phase reaction in which only 7% of the dichlorocarbene generated is lost by hydrolysis. The benzyltriethyl ammonium chloride is converted to the hydroxide by sodium hydroxide. The hydroxide which is insoluble in both the aqueous and organic phases migrates to the interface where it reacts with chloroform to form the quaternary ammonium derivative of the trichloromethyl anion. The quaternary ammonium compound diffuses into the organic phase where it is converted into the dichlorocarbene and benzyltributylammonium chloride. The dichlorocarbene reacts with ethenyl-benzene and the benzyltributyl ammonium chloride migrates back to the aqueous layer completing the cycle. The reactions are shown below:

\[
\begin{align*}
\text{Et}_3\text{NCH}_2\text{Ph}^+ \text{Cl}^- + \text{NaOH} & \rightleftharpoons \text{Et}_3\text{NCH}_2\text{Ph}^+ \text{OH}^- + \text{NaCl} \\
\text{Et}_3\text{NCH}_2\text{Ph}^+ \text{OH}^- + \text{CHCl}_3 & \rightleftharpoons \text{Et}_3\text{NCH}_2\text{Ph}^+ \text{CCl}_3^- + \text{H}_2\text{O} \\
\text{Et}_3\text{NCH}_2\text{Ph}^+ \text{CCl}_3^- & \rightleftharpoons \text{Et}_3\text{NCH}_2\text{Ph}^+ \text{Cl}^- + :\text{CCl}_2
\end{align*}
\]
The desired intermediate (4) was also prepared by following a procedure described by Bruson and Plant\textsuperscript{20}. The yield (49%), using this procedure, was lower than that obtained with the Makosza and Wawrzyniewicz procedure and the separation of layers after the water quench was hampered by the formation of an emulsion.

The mixture was filtered in an attempt to break the emulsion but a considerable amount of time (one week) was required for adequate separation.

Intermediate (4) was then nitrated using a procedure described by Sterling Winthrop Research Institute\textsuperscript{23}. A mixture of isomers (5) (63%), (5a) (29%), starting material (4) (3%) and an unknown material (4%) was obtained. This mixture was reduced without purification or separation of the isomers.

It was possible to obtain a good quality (6) (ETI 2%) almost free of its isomer (6a) because of the solubility of (6) and the insolvency of (6a) in an acid solution. A small portion of (6) (m.p. 58\textdegree{}-60\textdegree{}) was further purified by recrystallizing from hexane. This material (m.p. 62\textdegree{}-64\textdegree{}) was pure by TLC and was used as a reference standard in later TLC work.

The phenol intermediate (7) was obtained by diazotizing the amine (6) to the diazonium salt and hydrolysis to (7) according to a procedure supplied by Sterling Winthrop Research Institute\textsuperscript{23}.\textsuperscript{23}
Intermediate (8) was supplied by Sterling Winthrop Research Institute and was converted in good yield (90%) to high quality (8a) by the addition of ethanolic hydrochloric acid to a benzene solution of (8).

B. N-[4-(2,2-Dichlorocyclopropyl)phenyl]-N-(3,4-dichlorophenyl)urea (10)

This material was obtained in good yield and quality by reacting (6) with (9) in ethyl ether.

Extensive biological testing has been done on a series of compounds similar in structure to (10). It is hoped that by comparing the biological results obtained on the newly synthesized compound (10) with that of similar compounds an evaluation of the effect of the (2,2-dichlorocyclopropyl) group can be made.

Preliminary biological tests indicate that compound (10) is not as effective as the known antimicrobial agent (1) at inhibiting the growth of Streptococcus Pyrogenes and Staphylococcus Aureus but that it does inhibit growth at sufficiently low concentrations (1.95 mcg/ml) to warrant further study. It also showed that compound (10) is about as effective as (1) at inhibiting the growth of certain fungi.

C. N,N\textsuperscript{11}-1,6-Hexanediylbis[N-cyano-guanidine] (14)

First attempts to prepare this compound using sodium dicyanamide (11) supplied by American Cyanamide gave a poor quality product (m.p. 186°-197°). This melting point
is lower than that reported by Rose and Swain\textsuperscript{13} (m.p. 202°-203°) and Lorenz\textsuperscript{24} (m.p. 208°-212°). Sodium dicyanamide (assayed 99\%) provided by Sterling Winthrop Research Institute was used in subsequent experiments and gave a product that melted at 205°-208° and showed only slight impurities by TLC. This material was used to synthesize compound (13), Scheme 4.

D. N-Cyano-N-[4-(2,2-dichlorocyclopropyl)phenyl]guanidine (12)

This material was prepared in sufficient quantity and quality by applying a procedure used by Lorenz\textsuperscript{24} to prepare N-cyano-N-(4-chlorophenyl)guanidine. This material was used in the unsuccessful attempt to obtain (13), Scheme 3.

E. N,N\textsuperscript{1}-bis[4-(2,2-Dichlorocyclopropyl)phenyl]-3,12-diimino-2,4,11,13-tetraazatetradecanediimidamide (13)

This product was synthesized according to Scheme 3 but could not be adequately purified. The infrared spectrum of this material showed the absence of (N=C=N) which indicates that all the cyano compound (12) was reacted or removed by purification. The high ratio of aliphatic to aromatic hydrogens shown by the nmr suggests the presence of (8a). TLC indicated the presence of a large amount of the desired product and a large amount of an unknown impurity which showed up at the origin.
The desired product (13) was synthesized according to Scheme 4 in sufficient quantity and quality to be tested for antimicrobial activity. If good antimicrobial activity is observed this compound (13) will be tested for biodegradability. It is hoped that the (2,2-dichlorocyclopropyl) phenyl groups in compound (13) will render it more biodegradable and will produce less toxic degradation products than compound (2). The use of compound (2) may be limited because of its decomposition to toxic 4-chlorobenzenamine.

F. 2,2-Methylenebis[4-(2,2-dichlorocyclopropyl)phenol] (15)

It was originally intended that this compound be prepared by condensing (7) with formaldehyde in the presence of sulfuric acid. This procedure has been used by Faith to condense 4-chlorophenol to (3). It was necessary to run this reaction at -5° to 0° and with an excess of 4-chlorophenol in order to avoid polymerization. Since it was determined, by the direction of nitration, that the 2,2-dichlorocyclopropyl group is activating the possibility of polymerization was greatly increased. In light of this it was decided to attempt the condensation of readily available 4-(1,1,3,3-tetramethylbutyl)-phenol to 2,2-methylenebis 4-(1,1,3,3-tetramethylbutyl)phenol in order to determine the feasibility of this type reaction on activated phenols. This attempt resulted in apparent rapid polymerization and it was decided that sufficient quantities of the desired product could not be prepared by this method.
IR Spectrum of Compound (10)
NMR Spectrum of Compound (10)
UV Spectrum of Compound (10)
Mass spectrum of compound (12) prepared, following Scheme 3.
IR Spectrum of Compound (12)

<table>
<thead>
<tr>
<th>Frequency (cm⁻¹)</th>
<th>Absorbance</th>
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<tr>
<td>3500</td>
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</tr>
<tr>
<td>2900</td>
<td>2</td>
</tr>
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</tr>
<tr>
<td>650</td>
<td>5</td>
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**Note:** The spectrum shows absorption peaks at various wave numbers.
UV Spectrum of Compound (12)
Prepared by: Follow-up Scheme 3
NMR Spectrum of Compound (13)
**Method II (N) 1.0**

Mike Burke
1-47 Bu

<table>
<thead>
<tr>
<th>0.9</th>
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<tr>
<td>0.7</td>
<td>n, ( T )</td>
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1.17 mg \( \rightarrow \) 100 cc
5 cc \( \rightarrow \) 50 cc

UV Spectrum of Compound (13)
Prepared by Following Scheme 3

<table>
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</table>

\( \lambda_{\text{mm}} \)
IR Spectrum of Compound (13)
NMR Spectrum of Compound (13)
Prepared by Following Scheme 4
UV Spectrum of Compound (13)
Prepared by Following Scheme 4


23. Sterling Winthrop Research Institute, Private Communication.
