with 7 ml. of water and 3 ml. of pyruvic acid and allowed to stand at room temperature overnight and at 60° for 2 hr. After dilution with water, the product was extracted with chloroform and the chloroform solution was washed with water, 5% potassium bicarbonate, water, and dried over magnesium sulfate. The solvent was removed *in vacuo* and the residue was chromatographed on 50 g. of neutral alumina and eluted with benzene-chloroform mixtures. From the benzene eluates there was obtained 200 mg. of IV, m.p. 126–131°. Recrystallization from acetone–petroleum ether gave material of m.p. 131–132°, $\lambda_{max}^{CH_3OH}$ 237 mμ, E = 9,900.

*Anal.* Calcd. for $C_{25}H_{32}O_6$: C, 70.08; H, 7.53. Found: C, 69.75; H, 7.78.

From the fractions corresponding to 1–20% chloroform-benzene there was recovered 400 mg. of I (R = O), m.p. 226–229°.

*21-Acetoxy-$\Delta^{4,16}$-pregnadiene-3,11,20-trione* (VI).[9] A solution of 5.0 g. of cortisone acetate-3,20-bissemicarbazone[8] (V) in 100 ml. of acetic acid containing 5 ml. of acetic anhydride was refluxed in a nitrogen atmosphere for 1 hr. The pale yellow to red reaction mixture was concentrated *in vacuo* to a volume of 60 ml. and treated with 30 ml. of water and 15 ml. of pyruvic acid, and allowed to stand at room temperature for 40 hr. and at 60° for 2 hr. After dilution with water, the product was extracted with chloroform and the chloroform solution was washed with water, 5% potassium bicarbonate, water, and dried over magnesium sulfate. The solvent was removed *in vacuo* and the residue was chromatographed on 200 g. of neutral alumina and elution with benzene afforded after crystallization from acetone-ether, 1.37 g. (38%) of VI (R = 0), m.p. 186–187°, $\lambda_{max}^{CH_3OH}$ 237–238 mμ, E = 25,200, identical in the infrared spectrum with an authentic sample.[10]

*Anal.* Calcd. for $C_{23}H_{28}O_5$: C, 71.85; H, 7.34. Found: C, 71.96; H, 7.26.

Further elution of the chromatograph column with 50% benzene–ethyl acetate gave 2.47 g. (61%) cortisone acetate (V), thus affording the $\Delta^{16}$ compound VI in *ca.* 91% yield based on recovered cortisone. In several typical preparations a direct yield of 40 to 46% of VI has been attained.

*11β,21-Dihydroxy-$\Delta^{4,16}$-pregnadiene-3,20-dione* (VII).[11] 21-Acetoxy-$\Delta^{4,16}$-pregnadiene-3,11,20-trione was converted to the corresponding 3,20-bissemicarbazone derivative[8] and reduced with lithium borohydride by the method of Wendler, Huang-Minlon, and Tishler.[14] The reversal of the semicarbazone was carried out as described above with pyruvic acid to give VII, m.p. 153–156°. A sample for analysis was chromatographed on neutral alumina and eluted with chloroform to afford VII crystallized from acetone–petroleum ether, m.p. 159–161°, $\lambda_{max}^{CH_3OH}$ 241 mμ, E = 20,900.

*Anal.* Calcd. for $C_{21}H_{28}O_4$: C, 73.22; H, 8.19. Found: C, 72.83; H, 8.25.

RAHWAY, N. J.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, DUKE UNIVERSITY]

# Synthesis and Antimicrobial Activity of Some Alkyl 3-Phenanthridinols

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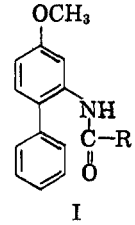
Three 6-alkyl-3-phenanthridinols have been synthesized for antimicrobial testing. A convenient method for the synthesis of the requisite 2-amino-4-methoxybiphenyl was found through the reaction of sodium amide on 3-bromo-4-methoxybiphenyl.

As a result of the observation by Steinberg[2] that 3- and 2-phenanthrols possess marked fungistatic activity toward *Aspergillus niger*, research was initiated to determine whether greater activity might be achieved by introduction of substituents into the phenanthrol nucleus[3] or by alteration of the aromatic system.[4] As part of the latter program, we decided to undertaken the synthesis of some 3-phenanthridinols (II). Copp and Walls[5] synthesized the first 6-substituted 3-phenanthridinol derivative (III, R = *p*-$NO_2C_6H_4$) because of their interest in possible trypanocides. The only 6-alkyl-3-phenanthridinol known (II, R = $CH_3$)

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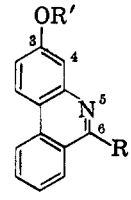



II, R′ = H
III, R′ = $CH_3$

was prepared by Mitsuhashi.[6] It appears that Mitsuhashi followed Copp and Walls[5] both with regard to the cyclization procedure and to the method used in preparing the 2-amino-4-methoxybiphenyl (V). The latter synthesis involved the preparation of 2-nitro-4-methoxybiphenyl by the Gomberg method (in unspecified yield) followed by reduction.

We have found that the desired amine (V) may be obtained in 60% yield from the easily prepared 3-bromo-4-methoxybiphenyl (IV), in only one step, by reaction with sodium amide in liquid

(1) Allied Chemical and Dye Corp. Fellow, 1953–1954. Taken in part from a thesis submitted by P. H. Leake in partial fulfillment of the requirements for the Ph.D. degree at Duke University, 1954. This work was supported in part by the Chemical Corps, Fort Detrick, Md., under contract with Duke University.

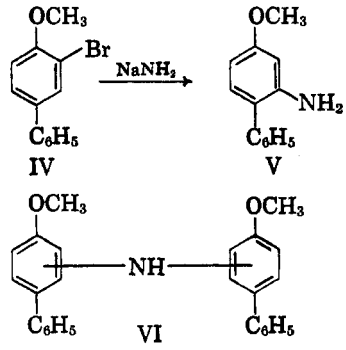
(2) Steinberg, *J. Agr. Research*, **60**, 765 (1940).

(3) Bradsher, Brown, and Leake, *J. Am. Chem. Soc.*, **78**, 4400 (1956).

(4) Bradsher, Brown, and Porter, *J. Am. Chem. Soc.*, **76**, 2357 (1954).

(5) Copp and Walls, *J. Chem. Soc.*, 311 (1950).

(6) Mitsuhashi, *J. Pharm. Soc. Japan*, **II**, 1232 (1951); *Chem. Abstr.*, **46**, 5593 (1952).



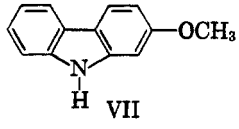

ammonia.[7] As by-products we obtained two less basic compounds having the composition of bis-methoxybiphenylylamines (VI). Acylation of the 2-amino-4-methoxybiphenyl (V) was carried out with acetyl, propionyl, and butyryl chlorides, and the resulting amides (I) cyclized with phosphorus oxychloride.

For the ether cleavage of 3-methoxy-6-methylphenanthridine (III, R = $CH_3$), Mitsuhashi[6] had used hydrochloric acid in sealed tubes at 170°. We have found that the same operation may be carried out more conveniently by refluxing the methoxy compounds (III) in a mixture of hydrobromic and acetic acids.

In order to compare the fungistatic activity of a free base with a related quaternary salt, 5,6-dimethyl-3-hydroxyphenanthridinium chloride was prepared from the 6-methyl-3-phenanthridinol (II, R = $CH_3$).

From the detailed report of the antimicrobial activity of phenanthridinols (experimental part), it will be seen that they fail to inhibit completely the growth of *Aspergillus niger* even at 250 p.p.m., and, with the exception of the phenanthridinium salt, are ineffective toward *B. subtilis* and *E. coli*.

One further experiment of interest was carried out with 2-amino-4-methoxybiphenyl (V). When it was diazotized and the resulting salt treated with sodium azide, a crude azide was formed which upon heating, afforded 2-methoxycarbazole (VIII)[8] in a 63% over-all yield.




VII

## EXPERIMENTAL[9]

*2-Amino-4-methoxybiphenyl* (V). The bromination of 4-hydroxybiphenyl was carried out in 84% yield and the 3-bromo-4-hydroxybiphenyl[10] (m.p. 95–96°) obtained was methylated with dimethyl sulfate, affording a 99% yield of 3-bromo-4-methoxybiphenyl (IV),[11] m.p. 76–77.5° (lit.[11] 79°).

Sodium amide was prepared from 29.2 g. of sodium in 3 l. of liquid ammonia, using 1.6 g. of anhydrous ferric nitrate as a catalyst. The sodium amide-ammonia mixture was mechanically stirred in a flask provided with a dry ice condenser while 158 g. of *finely powdered* 3-bromo-4-methoxybiphenyl was added gradually. The reaction mixture became yellow-brown and, in a short while, all of the solid had dissolved. After an additional 5 hr. 100 g. of dry ammonium chloride was added, followed by 500 ml. of ether and 500 ml. of benzene. The excess ammonia was evaporated on the steam bath, the organic solvents being retained by use of a reflux condenser. The resulting solution (A) was extracted with a total of 20 l. of 5% hydrochloric acid, in portions. The acid solution was next neutralized with ammonia and the product extracted with methylene chloride. The methylene chloride extract was dried, filtered, and concentrated. By distillation of the brown residue, 71.8 g. (60%) of 2-amino-4-methoxybiphenyl (V) was obtained, b.p. 163° (3–4 mm.), m.p. 41–43° (lit.[5] b.p. 128–130° at 0.08 mm.).

A sample of the *hydrochloride* was prepared by addition of hydrogen chloride to a methanol solution of V; needles, m.p. 212–213°.

*Anal.* Calcd. for $C_{13}H_{14}ClNO$: C, 66.24; H, 5.99; Cl, 15.04. Found: C, 66.46; H, 5.86; Cl, 15.26.

From the benzene-ether solution (A) above, upon addition of hydrogen chloride, 9.5 g. of a salt was precipitated. The free base was liberated, and crystallized from propyl alcohol as irregular needles, m.p. 168–169°. The analytical values compare well with those calculated for a bis(4-methoxybiphenylyl)-amine.

*Anal.* Calcd. for $C_{26}H_{23}NO_2$: C, 81.86; H, 6.08; N, 3.67. Found: C, 81.65; H, 6.09; N, 3.75.

After precipitation of the above amine from the benzene-ether solution (A), the solvents were evaporated yielding 44.2 g. of a crystalline residue. After several recrystallizations from propyl alcohol, it afforded long colorless needles, m.p. 150–151°. The analytical values compare well with those calculated for bis(4-methoxybiphenylyl)-amine.[12]

*Anal.* Calcd. for $C_{26}H_{23}NO_2$: C, 81.86; H, 6.08; N, 3.67. Found: C, 81.98; H, 6.01; N, 3.61.

*Amides* (I) *from 2-amino-4-methoxybiphenyl.* Acylation of the amine (V) was carried out in ether solution using the acid chlorides in the presence of dry pyridine. The products were recrystallized from petroleum ether.

The *acetamido* derivative (I, R = $CH_3$) was obtained as colorless needles, m.p. 100.5–101° (lit.[6] 95–96°) in 96% yield.

*Anal.* Calcd. for $C_{15}H_{15}NO_2$: C, 74.66; H, 6.27; N, 5.81. Found: C, 74.67; H, 6.41; N, 5.76.

The *propionamido* derivative (I, R = $C_2H_5$) was obtained as colorless needles, m.p. 114–115° (91% yield).

*Anal.* Calcd. for $C_{16}H_{17}NO_2$: C, 75.26; H, 6.71; N, 5.49. Found: C, 75.40; H, 6.90; N, 5.50.

The *butyramido* derivative (I, R = $C_3H_7$) formed colorless plates, m.p. 94–95° (94% yield).

*Anal.* Calcd. for $C_{17}H_{19}NO_2$: C, 75.81; H, 7.11; N, 5.20. Found: C, 75.80; H, 7.05; N, 5.14.

*Hydrochlorides of 3-methoxy-6-alkylphenanthridines* (III). Approximately 5 g. of the 2-acylamido-4-methoxybiphenyl (I) was dissolved in 10 ml. of phosphorus oxychloride and refluxed for 30 min. unless the product had crystallized

(7) Gilman and Avakian, *J. Am. Chem. Soc.*, **67**, 349 (1945); Gilman and Nobis, *J. Am. Chem. Soc.*, **67**, 1479 (1945); Gilman, Crounse, Massie, Benkeser, and Spatz, *J. Am. Chem. Soc.*, **67**, 2106 (1945); Gilman, Kyle, and Benkeser, *J. Am. Chem. Soc.*, **68**, 143 (1946). This and other types of cine-substitution reactions are discussed by Bunnett and Zahler, *Chem. Revs.*, **49**, 273 (1951).

(8) This compound has been synthesized by Dr. J. M. Clegg in the laboratory of Dr. P. A. S. Smith, starting from 2-amino-4′-methoxybiphenyl (private communication).

(9) All analyses are by Micro-Tech Laboratories, Skokie, Ill. All melting points are corrected.

(10) Bell and Robinson, *J. Chem. Soc.*, 1127 (1927).

(11) Bell, *J. Chem. Soc.*, 1071 (1930).

(12) On the basis of yields and of the relative basicity of the two secondary amines (VI), it seems likely that the lower-melting one is bis(4-methoxy-2-biphenylyl)-amine and the higher melting one (4-methoxy-2-biphenylyl)-(4-methoxy-3-biphenylyl)-amine.

from solution earlier. The reaction mixture was cooled and the product collected and recrystallized from dilute hydrochloric acid.

*The hydrochloride of the 6-methyl derivative* (III, R = $CH_3$) formed yellow woollike needles, decomposing at 271° (78% yield).

*Anal.* Calcd. for $C_{15}H_{14}ClNO$: C, 69.36; H, 5.43; Cl, 13.65. Found: C, 69.36; H, 5.68; Cl, 13.70.

*The free base of the 6-methyl derivative* (III, R = $CH_3$) crystallized from petroleum ether as short white needles, m.p. 76–77°, (lit.[6] 72–73.5°).

*Anal.* Calcd. for $C_{15}H_{13}NO$: C, 80.69; H, 5.87; N, 6.17. Found: C, 80.55; H, 6.02; N, 6.14.

*The hydrochloride of the 6-ethyl derivative* (III, R = $C_2H_5$) crystallized from methanol-ether as yellow needles which decomposed at 211–211.5°.

*Anal.* Calcd. for $C_{16}H_{16}ClNO$: C, 70.19; H, 5.89; Cl, 12.95. Found: C, 70.01; H, 5.98; Cl, 12.88.

*The hydrochloride of the 6-propyl derivative* (III, R = $C_3H_7$) crystallized as pale yellow needles which melted at 138–139° when heated rapidly, but if placed in the bath at 30° and heated at the rate of four degrees per minute, it melted at 198–199°. The resolidified material melted at 134–136°.

*Anal.* Calcd. for $C_{17}H_{18}ClNO$: C, 70.94; H, 6.30; Cl, 12.32. Found: C, 70.88; H, 6.30; Cl, 12.51.

*6-Alkyl-3-phenanthridinols* (II). Approximately 1 g. of the 3-methoxy-6-alkylphenanthridine was dissolved in a mixture containing 10 ml. of acetic and 10 ml. of 48% hydrobromic acid and the mixture refluxed for about 10 hr. The mixture was cooled and the salt collected. The free base, liberated by the action of ammonia, was obtained in essentially quantitative yield as colorless needles from propyl alcohol.

*The methyl derivative* (II, R = $CH_3$) melted at 305–306° (lit.[6] 325–327°).[13]

*Anal.* Calcd. for $C_{14}H_{11}NO$: C, 80.36; H, 5.30; N, 6.69. Found: C, 80.18; H, 5.36; N, 6.48.

*The ethyl derivative* (II, R = $C_2H_5$) melted at 280–281°.

*Anal.* Calcd. for $C_{15}H_{13}NO$: C, 80.69; H, 5.87; N, 6.27. Found: C, 80.51; H, 6.11; N, 6.52.

The propyl derivative (II, R = $C_3H_7$) melted at 231–232°.

*Anal.* Calcd. for $C_{16}H_{15}NO$: C, 80.98; H, 6.37; N, 5.90. Found: C, 80.63; H, 6.47; N, 6.14.

*2-Hydroxy-5,6-dimethylphenanthridinium chloride.* Five hundred milligrams of 6-methyl-3-phenanthridinol (II, R = $CH_3$) was dissolved in 10 ml. of nitrobenzene and heated with 1 ml. of dimethyl sulfate at 150° for 15 min. The nitrobenzene was steam distilled, and the resulting mixture filtered. To one half of the filtrate, concentrated hydrochloric acid was added, precipitating 220 mg. (79%) of a brownish solid, m.p. 244–245°. The analytical sample was obtained from dilute hydrochloric acid as needles, m.p. 245–246° (dec.), having the composition of a hydrate.

*Anal.* Calcd. for $C_{15}H_{14}ClNO.H_2O$: N, 5.04; Cl, 12.76. Found: N, 5.31; Cl, 12.87.

*2-Methoxycarbazole* (VII). This procedure was modeled after that of Smith and Brown.[14] A solution of 3.3 g. of 2-amino-4-methoxybiphenyl in a warm (40–50°) solution of dilute sulfuric acid (3 ml. of concentrated sulfuric acid in 25 ml. of water) was cooled in an ice water bath, precipitating the amine sulfate. The mixture was diazotized by addition of a solution containing 1.38 g. of sodium nitrite in 15 ml. of water. The excess nitrous acid was removed by addition of urea, and 0.25 g. of Norit was added to the mechanically stirred solution. After 0.5 hr., the Norit was filtered off and a solution containing 1.8 g. of sodium azide in 10 ml. of water was added dropwise, with stirring, to the cold solution. Nitrogen was evolved immediately and a red oil precipitated. After the reaction mixture had stood in the hood overnight, the red oil was extracted with ether. The ethereal extract was dried and concentrated and the residue dissolved in 25 ml. of purified kerosine. The kerosine solution was poured into 100 ml. of kerosine maintained at 180–190°. The reaction mixture was maintained at this temperature for 5 min. and then allowed to cool slowly to room temperature. Tan plates, 2.1 g. (63%) crystallized from the kerosine solution, m.p. 233–235°. After a single recrystallization from alcohol (Norit), the material was obtained as colorless plates, m.p. 235–236° (lit.[8] 234–234.5°).

*Anal.* Calcd. for $C_{13}H_{11}NO$: C, 79.16; H, 5.62; N, 7.10. Found: C, 79.48; H, 5.68; N, 7.00.

*Antimicrobial testing.* The testing procedures have been outlined in an earlier publication.[15] All of the simple phenanthridinols (II, R = $CH_3$, $C_2H_5$, $C_3H_7$) failed to inhibit completely the growth of *A. niger*, *B. subtilis*, and *E. coli* at 250 p.p.m. The 3-hydroxy-5,6-dimethylphenanthridinium chloride was no more effective toward *A. niger*, but inhibited *B. subtilis* completely at 100 p.p.m. and *E. coli* at 200 p.p.m.

*Acknowledgment.* We are indebted to Mrs. Barbara Bayless for carrying out these tests.

DURHAM, N. C.

(13) Although our product was of analytical purity, it did not show the higher melting point which has been reported.

(14) Smith and Brown, *J. Am. Chem. Soc.*, **73**, 2438 (1951).

(15) Brown, Bradsher, Morgan, Tetenbaum, and Wilder, *J. Am. Chem. Soc.*, **78**, 384 (1956).