[CONTRIBUTION FROM THE ROSS CHEMICAL LABORATORY, ALABAMA POLYTECHNIC INSTITUTE]

Some Derivatives of 8-Bromo-6-methylquinoline[1]

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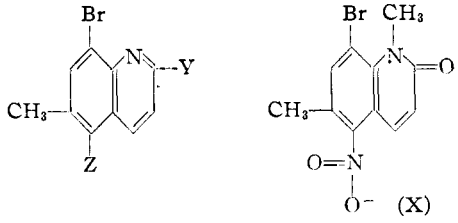
An investigation designed to obtain additional information concerning the synthesis of 8-bromo-6-methylquinoline (I), its properties and methods of conversion into derivatives with established structures was initiated. Methods and procedures previously reported by Capps[2] proved helpful during the preparation and characterization of compounds I through XXI.



I	Y = H, Z = H
II	Y = H, Z = NO_2
III	Y = Cl, Z = H
IV	Y = OH, Z = H
V	Y = H, Z = NH_2
VI	Y = H, Z = CH_3CONH–
VII	Y = H, Z = C_6H_5CONH–
IX	Y = H, Z = AsO_3H_2
XI	Y = Cl, Z = NO_2
XII	Y = Cl, Z = NH_2
XIII	Y = Cl, Z = CH_3CONH–
XIV	Y = Cl, Z = C_6H_5CONH–
XV	Y = Cl, Z = AsO_3H_2
XVI	Y = OH, Z = NO_2
XVII	Y = OH, Z = NH_2
XVIII	Y = OH, Z = CH_3CONH–
XIX	Y = C_6H_5COO–, Z = C_6H_5CONH–
XX	Y = OH, Z = C_6H_5CONH–
XXI	Y = OH, Z = AsO_3H_2

The melting point of 50–52° obtained for I as prepared by a Skraup ring-closure starting with either 4-amino-3-bromotoluene or 4-acetamido-3-bromotoluene corroborated the assumption of T. Ukai[3] regarding the structure of a compound that was prepared by the bromination of a 6-methylquinoline mercurial. A Skraup ring-closure on 4-acetamido-5-bromo-2-nitrotoluene served to show that II resulted from the nitration of I; 4-acetamido-5-bromo-2-nitrotoluene was synthesized by the acetylation of the previously reported 4-amino-5-bromo-2-nitrotoluene.[4] Although earlier investigators added 4-amino-3-bromotoluene nitrate to concentrated sulfuric acid to obtain 4-amino-5-bromo-2-nitrotoluene, we treated 4-amino-3-bromotoluene with a solution of sodium nitrate in concentrated sulfuric acid and also with sulfuric acid (sp. gr. 1.84)–nitric acid (sp. gr. 1.42) solution to obtain 90 and 76% yields, respectively, of the same nitro compound.

Substitution in ring position 2 of both I and II was effected by conversion to N-methylquinolinium salts and subsequent alkaline oxidation to 2-quinolones. The temperature at which dimethyl sulfate reacted with II influenced the amount of N-methylquinolinium salt formed and hence the amount of 2-quinolone produced during the oxidation; increases in temperature from 125 to 140° and from 125 to 175° caused reductions of 50 and 90%, respectively, in quantities of 2-quinolone obtained. X was sufficiently stable to withstand purification by crystallization from methanol. Even though conditions were not established for the purification of 8-bromo-1,6-dimethyl-2-quinolone, both it and purified X reacted with phosphorus pentachloride–phosphorus oxychloride mixtures at 125° to give the corresponding 2-chloroquinolines.

The expected reactivity of the carbon–chlorine bonds in the above mentioned 2-chloroquinolines was demonstrated by establishing conditions for hydrolysis to the corresponding 2-hydroxyquinolines. Hydroxyl and chloro groups in the 2-position of I affected the positions taken by nitro groups during nitration; for the chief nitration product of IV was converted by phosphorus pentachloride–phosphorus oxychloride mixture into the chief nitration product of III, and both of these nitro compounds were shown to be different from XVI and XI.

Benzoyl chloride reacted with XVII in the presence of sodium hydroxide–water solution to give XIX rather than the expected XX, but alcoholic sodium hydroxide selectively attacked the 2-benzoxy group at room temperature liberating the desired 2-hydroxybenzamidoquinoline.

Experimental

4-Acetamido-3-bromotoluene and 4-Amino-3-bromotoluene.—*p*-Toluidine was acetylated and brominated according to recommendations of Johnson and Sandborn[5] to give 4-acetamido-3-bromotoluene, which was then hydrolyzed. The resulting crude 4-amino-3-bromotoluene was fractionated under reduced pressure through a 36-inch column packed with glass helices.[6]

8-Bromo-6-methylquinoline (I).—4-Acetamido-3-bromotoluene and 4-amino-3-bromotoluene were converted independently into I by means of a Skraup ring-

(1) Presented in part before Southeastern Regional meeting of the American Chemical Society, Oak Ridge, Tennessee, June 10, 1949. Portions of theses presented by Humberto Diaz de Arce and Joseph L. Greene, Jr., to the Graduate School of the Alabama Polytechnic Institute in partial fulfillment of the requirements for the degree of Master of Science are included in this manuscript.

(2) J. D. Capps, THIS JOURNAL, **69**, 176–181 (1947).

(3) T. Ukai, *J. Pharm. Soc. Japan*, **51**, 542–576 (1931); see *C. A.*, **25**, 5427 (1931).

(4) See Claus and Herbabny, *Ann.*, **265**, 367 (1891); G. T. Morgan and A. Clayton, *J. Chem. Soc.*, **87**, 948 (1905); Cohen and Duft, *ibid.*, **105**, 515 (1914).

(5) H. Gilman and A. H. Blatt, "Organic Syntheses," 2nd ed., John Wiley and Sons, Inc., New York, N. Y., Coll. Vol. I, 1941, p. 111.

(6) Todd Scientific Co., Springfield, Pa.

closure under conditions previously reported by Richter and Smith[7] for synthesizing certain substituted quinolines; average yields 36%, based upon quantity of 4-amino-3-bromotoluene used; b. p. 166–179° at 11–16 mm.; m. p. 50–52°, crystallized from petroleum ether.

4-Amino-5-bromo-2-nitrotoluene.[4] A.—4-Amino-3-bromotoluene (28.5 g.) was dissolved in sulfuric acid (200 ml., sp. gr. 1.84) and the resulting solution cooled to 0°. The temperature was maintained below 0° while adding dropwise anhydrous sodium nitrate (10.6 g.) contained in sulfuric acid (60 ml., sp. gr. 1.84) and for an additional thirty minutes; mechanical stirring was employed. Cooling was discontinued for a period of three hours while the temperature of the system spontaneously increased to that of the laboratory. The nitration solution was then poured with stirring into ice-water mixture (2.5 l.) before filtering, washing the residue with water and finally recrystallizing from 95% ethanol; yield approximately 90%; m. p. 120–121°.

B.—4-Amino-3-bromotoluene (24.0 g.) was dissolved in sulfuric acid (75 ml., sp. gr. 1.84) and the resulting solution cooled to −10°. The temperature was maintained below 0° while adding dropwise nitric acid (7.5 ml., sp. gr. 1.42) contained in sulfuric acid (25 ml., sp. gr. 1.84). After allowing temperature of system to spontaneously increase to that of the laboratory, the nitration mixture was processed as in A above; 22.6 g. (76%) yield; m. p. 119–121°.

4-Acetamido-5-bromo-2-nitrotoluene.—Acetic anhydride (10 ml.) was added to a solution of 4-amino-5-bromo-2-nitrotoluene (15.0 g.) in glacial acetic acid (50 ml.) and resulting solution was refluxed for thirty minutes. A solid separated upon pouring with stirring into ice-water mixture (800 ml.). Long, colorless (white) needles were obtained by recrystallizing from 95% ethanol; yield almost theoretical; m. p. 168–169° dried at 118° and 10 mm.

Anal. Calcd. for $C_9H_9BrN_2O_3$: N, 10.26. Found: N, 10.27.

8-Bromo-6-methyl-5-nitroquinoline (II). A.—I (100 g.) dissolved in sulfuric acid (100 ml., sp. gr. 1.84) was added dropwise with stirring at 0° or less to a solution of nitric acid (70 ml., sp. gr. 1.50) in sulfuric acid (100 ml., sp. gr. 1.84). After removing the reaction system from the cooling bath and allowing it to remain suspended in the atmosphere of the laboratory for three or more hours, the temperature of nitration mixture was slowly increased to 70° and maintained for five minutes before discontinuing application of heat. When the temperature of nitration mixture had spontaneously decreased to 40°, it was poured with stirring into a cracked ice–water mixture (3 l.). The solid product obtained was collected on a filter, washed with water, and then dissolved in boiling 95% ethanol. Decolorizing carbon was added, followed by Hyflo Super-Cel[8] and the system was filtered. Pale yellow needles separated upon cooling; yield 87 g. (77%); m. p. 133–34° (dried 100° and 10 mm.).

Anal. Calcd. for $C_{10}H_7BrN_2O_2$: Br, 29.92; N, 10.49. Found: Br, 29.93; N, 10.38.

B.—4-Acetamido-5-bromo-2-nitrotoluene (5.9 g.), arsenic pentoxide (4.0 g.), anhydrous glycerol (6.5 ml.) and sulfuric acid (6.5 ml., sp. gr. 1.84) were mixed well and heated for four hours under a reflux condenser by means of an oil-bath maintained at 173–174°. The cooled mass was poured with stirring into a mixture of cracked ice and water (200 ml.), filtered, and washed with water. Some of the desired II was extracted from the solid residue with 50% by volume sulfuric acid–water solution. After treating the sulfuric acid–water extract with decolorizing carbon and filtering, the new filtrate was combined with the original filtrate from above and the combined filtrates were made basic with sodium hydroxide. A crude solid resulted which was purified by a combination of crystallizations from 95% ethanol and decolorizing carbon treatments; yield 0.4 g., m. p. 133–134°.

(7) F. Richter and G. F. Smith, THIS JOURNAL, **66**, 396 (1944).

(8) A product of Johns-Manville, 22 East 40th St., New York 16, N. Y.

8-Bromo-1,6-dimethyl-2-quinolone.—I (63.5 g.) and dimethyl sulfate (100 ml.) were heated together under a reflux condenser for one and one-quarter hours in an oil-bath maintained at 125°. Water (275 ml.) was added to dissolve quinolinium salt before extracting with diethyl ether three times (100-50-50 ml.) to remove excess dimethyl sulfate. The water solution of the quinolinium salt was then mixed with potassium ferricyanide (325 g.) contained in water (3000 ml.). After increasing temperature of the resulting solution to 40–50°, a solution of potassium hydroxide (46 g.) in water (150 ml.) was introduced dropwise at such a rate as to retain temperature of 40–50°. The needles of quinolone that formed were separated by filtration and washed with water. Attempts to purify the quinolone by recrystallization from organic solvents were unsuccessful; yield 57 g. (79%); m. p. of crude 57–61°.

8-Bromo-2-chloro-6-methylquinoline (III).—8-Bromo-1,6-dimethyl-2-quinolone (31.2 g.) was added in small quantities with shaking to phosphorus oxychloride (65 ml.) at −14°. Phosphorus pentachloride (32 g.) was introduced and the system refluxed in an oil-bath kept at 125° for 1.25 hours. After pouring the mixture with stirring into cracked ice–water (800 ml.), the resultant solid was removed by filtration and washed with water. The washed solid was dissolved in boiling acetone, decolorizing carbon was added followed by Hyflo Super-Cel, and the system was filtered again. Upon cooling the filtrate deposited yellow needles; yield 13.5 g. (43%); m. p. 110–111 (dried 78° and 16 mm.).

Anal. Calcd. for $C_{10}H_7ClBrN$: (Cl + Br), 44.99; N, 5.46. Found: (Cl + Br), 44.77; N, 5.55.

8-Bromo-2-hydroxy-6-methylquinoline (IV).—III (5.0 g.), mixed with a solution of sulfuric acid (10 ml., sp. gr. 1.84) in water (40 ml.), was sealed in an autoclave prior to slowly increasing the temperature to 170–175° and maintaining it for four hours. Water (50 ml.) was added after the temperature had decreased to that of the laboratory, causing a solid to form. Filtration followed by washing with water and recrystallization from acetone gave long needles; yield 3.0 g. (67%); m. p. 177–178° (dried 78° and 1 mm.).

Anal. Calcd. for $C_{10}H_8BrNO$: N, 5.88. Found: N, 5.81.

8-Bromo-1,6-dimethyl-5-nitro-2-quinolone (X).—II (25 g.) was converted into the corresponding dimethyl sulfate addition product and oxidized with 30% hydrogen peroxide under conditions similar to those previously reported by Capps[2] for changing 6-methyl-8-nitroquinoline into 1,6-dimethyl-8-nitro-2-quinolone. The period of heating with dimethyl sulfate was increased to two hours and the oxidation was carried out at 55–65°. X crystallized from methanol as yellow needles; yield 16.5 g. (60%); m. p. 142–143° (dried 25° and 1 mm.).

Anal. Calcd. for $C_{11}H_9BrN_2O_3$: N, 9.43. Found: N, 9.43.

8-Bromo-2-chloro-6-methyl-5-nitroquinoline (XI).—X (16.5 g.) was dissolved in phosphorus oxychloride (30 ml.) at 0°. Phosphorus pentachloride (13.2 g.) was added and the mixture was refluxed for two hours in an oil-bath maintained at 120–130°. After pouring the resultant mixture into cracked ice–water (500 ml.) with stirring, the solid that precipitated was removed by filtration and washed with water. The dried crude XI was dissolved in boiling acetone, treated with decolorizing carbon followed by Hyflo Super-Cel, and then filtered. Yellow needles separated from the filtrate upon cooling; yield 14.8 g. (88%); m. p. 196–197° (dried 78° and 1 mm.).

Anal. Calcd. for $C_{10}H_6ClBrN_2O_2$: N, 9.29. Found: N, 9.43.

8-Bromo-2-hydroxy-6-methylquinoline (XVI).—XI (11 g.) was refluxed for twenty minutes with a solution of sulfuric acid (80 ml., sp. gr. 1.84) in water (80 ml.). A solid formed when the resulting solution was poured into cracked ice–water (700 ml.); this solid was separated by filtration, washed with water and dissolved in boiling 95% ethanol; decolorizing carbon followed by Hyflo Super-

Cel was then added and the system filtered. Fine, yellow needles separated from the filtrate upon cooling; yield almost theoretical; m. p. 210–211° (dried 78° and 1 mm.).

5-Amino-8-bromo-6-methylquinoline (V), 5-Amino-8-bromo-2-chloro-6-methylquinoline (XII) and 5-Amino-8-bromo-2-hydroxy-6-methylquinoline (XVII).—Solutions of II (20.0 g. in reagent grade acetone), XI (10.0 g. in reagent grade acetone), and XVI (5.0 g. in absolute ethanol) were reduced at approximately 50° by shaking with hydrogen at 40 pounds per square inch in presence of Raney nickel catalyst for periods of three, two, and three hours, respectively. After removing the catalyst by filtration in each case, dry hydrogen chloride was passed into the filtrate to precipitate amine hydrochlorides. Separation of the solid amine hydrochlorides by filtration followed by re-solution of them in a minimum quantity of water and neutralization with ammonia gave crude V, XII and XVII. The solvent filtrates that were saturated with hydrogen chloride contained some additional amine which was always recovered by concentration under reduced pressure and treatment with ammonia. Recrystallizations from ethanol–water solutions gave yellow needles V (m. p. 143–144°; dried 100° and 10 mm.), orange needles XII (m. p. 202–203° dec.; dried 78° and 1 mm.), and yellow needles of XVII (m. p. 215–216°; dried 78° and 1 mm.) in yields of almost theoretical, 90 and 89%.

Solutions of VI, XIII and XVIII in 95% ethanol were treated with volumes of hydrochloric acid (sp. gr. 1.19) equal to quantities of solid VI, XIII and XVIII taken before refluxing for periods of six hours, three hours and three hours. Relatively pure samples of V, XII and XVII were obtained by diluting the refluxed systems with water, neutralizing with ammonia, filtering, washing with water, and finally recrystallizing from ethanol–water solutions.

Anal. Calcd. for $C_{10}H_9BrN_2$: N, 11.82. Found: N, 11.72. Calcd. for $C_{10}H_8ClBrN_2$: N, 10.32. Found: N, 10.20. Calcd. for $C_{10}H_9BrN_2O$: N, 11.07. Found: N, 10.91.

5-Acetamido-8-bromo-6-methylquinoline (VI), 5-Acetamido-8-bromo-2-chloro-6-methylquinoline (XIII) and 5-Acetamido-8-bromo-2-hydroxy-6-methylquinoline (XVIII).—V, XII and XVII were acetylated by heating with solutions of acetic anhydride in acetic acid for thirty minutes; 0.8 ml. of acetic anhydride in 10 ml. of acetic acid, 1.7 ml. of acetic anhydride in 3.3 ml. of acetic acid, and 1.7 ml. of acetic anhydride in 1.7 ml. of acetic acid were used, respectively, per gram of V, XII and XVII. The reaction mixtures were decomposed by pouring with stirring into cracked ice–water (100 ml., 17 ml. and 17 ml. per gram of starting amine) before filtering and washing with water. A combination of decolorizing carbon treatments in ethanol–water solutions and crystallizations was employed to purify the crude acetamido derivatives. Yields of 60, 79 and 83% with melting points of 237–238° (VI) (dried 140° and 10 mm.), 277–278° (XII) (dried 78° and 1 mm.), and 292–293° (XVIII) (dried 78° and 1 mm.) resulted.

Anal. Calcd. for $C_{12}H_{11}BrN_2O$: N, 10.04. Found: N, 9.94. Calcd. for $C_{12}H_{10}ClBrN_2O$: N, 8.93. Found: N, 8.75. Calcd. for $C_{12}H_{11}BrN_2O_2$: N, 9.49. Found: N, 9.56.

5-Benzamido-8-bromo-6-methylquinoline (VII), 5-Benzamido-8-bromo-2-chloro-6-methylquinoline (XIV) and 5-Benzamido-2-benzoxy-8-bromo-6-methylquinoline (XIX).—V, XII and XVII were shaken independently with benzoyl chloride and 10% sodium hydroxide–water solution until the oils that first formed solidified; 1 ml. of benzoyl chloride with 10 ml. of sodium hydroxide solution, 3 ml. of benzoyl chloride with 10 ml. of sodium hydroxide solution and 3 ml. of benzoyl chloride with 10 ml. of sodium hydroxide solution were used, respectively, per gram of V, XII and XVII. The crude benzamido derivatives were removed by filtration, washed with water, and purified by the application of a combination of decolorizing carbon treatments in ethanol–water solutions with crystallizations from either ethanol–water solutions or absolute ethanol. Yields of 90% or better were obtained with melting points of 229–230° (VII) (dried 118° and 8 mm.), 223–224° (XIV) (dried 78° and 1 mm.), and 215–216° (XIX) (dried 78° and 1 mm.).

Anal. Calcd. for $C_{17}H_{13}BrN_2O$: N, 8.21. Found: N, 8.00. Calcd. for $C_{17}H_{12}ClBrN_2O$: N, 7.46. Found: N, 7.64. Calcd. for $C_{24}H_{17}BrN_2O_3$: N, 6.07. Found: N, 6.11.

5-Benzamido-8-bromo-2-hydroxy-6-methylquinoline (XX).—XIX (1 g.) was dissolved in a solution of sodium hydroxide (5 g.) in 95% ethanol (50 ml.) by shaking at room temperature. The resulting solution stood overnight at room temperature before it was diluted with water (200 ml.). A saturated aqueous solution of sodium bicarbonate was added in small quantities with shaking until further precipitation of XX ceased. After filtration and washing with water, the solid was dissolved in 95% ethanol (boiling); decolorizing carbon was added followed by Hyflo Super-Cel and the system was filtered. The filtrate was heated to boiling and one-fourth its volume of water added. White needles appeared upon cooling; these were removed by filtration, washed with an ethanol–water solution, and dried at 78° and 1 mm.; yield 0.8 g.; m. p. 265–266°.

Anal. Calcd. for $C_{17}H_{13}BrN_2O_2$: N, 7.48. Found: N, 7.59.

8-Bromo-6-methyl-5-quinolinearsonic Acid (IX), 8-Bromo-2-chloro-6-methyl-5-quinolinearsonic Acid (XV) and 8-Bromo-2-hydroxy-6-methyl-5-quinolinearsonic Acid (XXI).—V, XII and XVII were diazotized and converted into arsonic acids according to procedure reported by Capps and Hamilton[9] for converting certain 2-chloro-aminoquinolines into 2-chloroquinolinearsonic acids. IX, XV and XXI resulted in yields of 6.9, 2.3 and 28%, respectively, with melting points of 276–277° dec. (IX) (dried 118° and 10 mm.), 290–292° dec. (XV) (dried 78° and 16 mm.), and above 300° (XXI) (dried 78° and 1 mm.).

Anal. Calcd. for $C_{10}H_9BrNAsO_3$: As, 21.65. Found: As, 21.64. Calcd. for $C_{10}H_8ClBrNAsO_3$: N, 3.68; As, 19.69. Found: N, 3.65; As, 19.70. Calcd. for $C_{10}H_9BrNAsO_4$: N, 3.87; As, 20.69. Found: N, 3.86; As, 20.44.

Summary

8-Bromo-6-methylquinoline was prepared by a Skraup ring-closure on 4-amino-3-bromotoluene and converted into several derivatives. The structures of seventeen of these derivatives were established by showing that 8-bromo-6-methylquinoline nitrates chiefly in the 5-position to yield 8-bromo-6-methyl-5-nitroquinoline. No 8-bromo-2-hydroxy-6-methyl-5-nitroquinoline nor 8-bromo-2-chloro-6-methyl-5-nitroquinoline was isolated from the nitration products of either 8-bromo-2-hydroxy-6-methylquinoline or 8-bromo-2-chloro-6-methylquinoline. Three other compounds were prepared by the use of well characterized reactions leaving little doubt regarding their structures.

It was shown that temperature changes in certain ranges markedly affect the reaction between 8-bromo-6-methyl-5-nitroquinoline and dimethyl sulfate. The reactivity of carbon–chlorine bonds in 8-bromo-2-chloro-6-methylquinoline and 8-bromo-2-chloro-6-methyl-5-nitroquinoline was demonstrated by hydrolysis to 2-hydroxyquinolines. Alcoholic sodium hydroxide solution selectively attacked the benzoxy group in 5-

(9) J. D. Capps and C. S. Hamilton, THIS JOURNAL, **60**, 2105 (1938).

benzamido - 2 - benzoxy - 8 - bromo - 6 - methylquinoline at room temperature in preference to the benzamido group.

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[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF CHAS. PFIZER AND CO., INC.]

Bis-(α-hydroxystreptomycyl)-amine, a Toxic Derivative of Streptomycin[1]

BY I. A. SOLOMONS AND PETER P. REGNA

During investigation of the composition of residues remaining after streptomycin processing and purification, a streptomycin derivative was isolated that proved to be highly toxic when injected intravenously into mice. The present paper deals with the isolation of this compound, its identification as bis-(α-hydroxystreptomycyl)-amine (II), and its subsequent synthesis. The substance was of interest in connection with its possible bearing on observed minor variations in the toxicity of different preparations of streptomycin and dihydrostreptomycin.

Initial purification of bis-(α-hydroxystreptomycyl)-amine was accomplished by converting the streptomycin residues to the corresponding hydrochlorides and vacuum freeze-drying the product. Trituration with small quantities of methanol removed a major portion of the streptomycin trihydrochloride and other extraneous substances. From the residue, a reineckate was crystallized and this was converted to amorphous bis-(α-hydroxystreptomycyl)-amine sulfate.

When dilute aqueous solutions of bis-(α-hydroxystreptomycyl)-amine sulfate were prepared, the compound was found to hydrolyze predominantly to streptomycin which was ascertained by increases in maltol analysis,[1a] biological potency and the minimum lethal dose in mice. Moreover, it was observed that the hydrolysis is accelerated in acid solutions and retarded in alkaline solutions. Bis-(α-hydroxystreptomycyl)-amine sulfate and its hydrolysis products were compared with streptomycin by paper chromatography, using the method of Winsten and Eigen.[2] The position of the components on the paper after development were detected by pressing the paper chromatograms on agar plates seeded with *B. subtilis*. In addition, the spots were detected in the same position by spraying the paper with sodium nitroprusside–potassium ferricyanide reagent after a method of Horne and Pollard[3] which detects the streptidine moiety of the streptomycin molecule. It was found (Fig. 1) that the intact material does not move from the point of application on the paper under the conditions used, while after mild hydrolysis the only chromatographically detectable component is streptomycin. These results showed that streptomycin was the only biologically active or streptidine-containing component in the toxic compound.

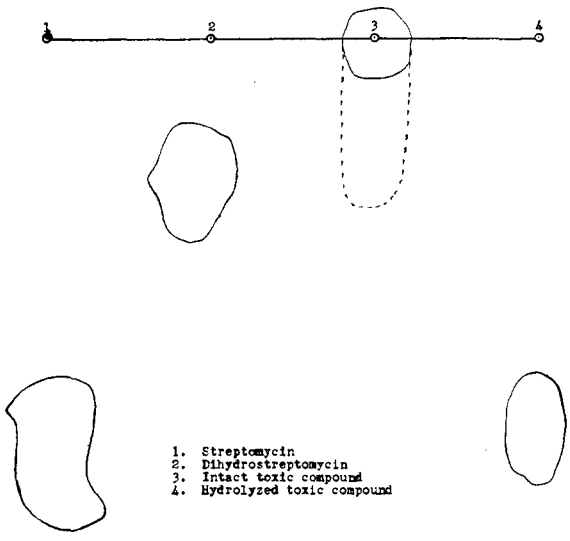

Fig. 1.—Chromatographed on Whatman #4 paper, descending system, using *n*-butanol–*p*-toluene sulfonic acid–piperidine solvent system; developed overnight; spots identified with streptidine spray.

Infrared and ultraviolet absorption spectra of bis-(α-hydroxystreptomycyl)-amine are very similar, in general, to those of streptomycin. On the other hand, polarographic analysis shows it to be significantly different. Whereas the presence of a free aldehyde group in streptomycin causes a definite break in the curve at a half-wave potential ($E_{1/2}$) of −1.45 volts,[4] an analysis of the toxic compound shows no break. Since it had been established by paper chromatography that bis-(α-hydroxystreptomycyl)-amine contains streptomycin, this indicated that the streptomycin was joined to another component through its carbonyl group.

Since acid is consumed during the hydrolysis of bis-(α-hydroxystreptomycyl)-amine, a measure of the consumption was used in calculating an empirical formula weight of 1450 for the compound. The hydrolysis in this experiment was performed

(1) Presented before the Division of Medicinal Chemistry at the Meeting of the American Chemical Society at Atlantic City, September, 1949.

(1a) G. E. Boxer, V. C. Jelinek and P. M. Leghorn, *J. Biol. Chem.*, **169**, 153 (1947).

(2) W. A. Winsten and E. Eigen, THIS JOURNAL, **70**, 3333 (1948).

(3) R. E. Horne, Jr., and A. L. Pollard, *J. Bact.*, **55**, 231 (1948).

(4) G. B. Levy, P. Schwed and J. W. Sackett, THIS JOURNAL, **68**, 528 (1946).