hydroxide and dialyzed until free of inorganic sulfates. Any insoluble material was separated and dried with organic solvents. The soluble portion was concentrated to small volume in dialysis tubing hung in a current of warm air, and then dried from the frozen state. The air-dried samples were analyzed for nitrogen and sulfate sulfur by a modification of the Mease method.[2,18] For substances of low molecular weight, the inorganic sulfates were removed as barium sulfate, and an aliquot of the resultant solution was analyzed for sulfate sulfur.[2,18] Another aliquot was freed from pyridine by repeated evaporation to dryness at room temperature from alkaline solution; it was then analyzed for nitrogen by the Kjeldahl procedure. Control experiments showed that a small fraction (about 0.4%) of the nitrogen of the crude pyridine-chlorosulfonic mixture became non-volatile when the material was heated to 75–78° for two and one-half hours, and progressively more with longer heating. This nitrogenous residue contained some bound sulfate, the S/N ratio being 0.5 to 0.7. Less of this material was formed if the sulfating agent had been isolated and resuspended in pyridine before being heated. Thus an inconstant error, apparently depending upon the nature of the substance being sulfated, detracts from the quantitative significance of the data obtained with small-molecular model substances, as listed in Table II.

In analyzing for sulfate sulfur, the usual conditions of hydrolysis (one hour at 100° in 6 *N* HCl) were found to be not quite sufficient for the quantitative release of the sulfate from certain proteins. A possible explanation is that hydrolysis of sulfated amide groups occurs in such a manner that sulfamic acid is primarily released rather than sulfuric acid.[10] Sulfamic acid is known to be relatively resistant to hydrolysis,[7] and was found to yield only 90% of its sulfate sulfur under our conditions of hydrolysis. When hydrolyzed by refluxing at 120° for sixteen hours, conditions which led to complete breakdown of sulfamic acid, some proteins, notably polyglutamine and β-lactoglobulin, showed sulfate sulfur contents higher by as much as 12%, while others did not. The values in Table III, however, were obtained after one hour of hydrolysis.

All other analytical methods were the same as those used in the preceding study.[2]

Products of Reaction of Pyridine-chlorosulfonic Acid with Alanine.—Five grams of alanine was treated with the pyridine-chlorosulfonic acid reaction product in the usual manner. The mixture was then diluted with water, neutralized, freed from sulfate ions and evaporated to dryness while maintaining an alkaline reaction. The residue was extracted repeatedly with absolute alcohol; 280 mg. of nitrogen dissolved, an equal amount remained in the insoluble fraction. The residue obtained upon evaporation of the alcohol contained 13.8% N, 3.04% sulfate S, 3.50% total S and 0.25% amino N. After acid hydrolysis the amino nitrogen agreed with the total nitrogen. The S/N ratio of 0.12, together with the amino N/N ratio of 0.02 suggests that free as well as terminally sulfated polypeptides must be present. The occurrence of alanine anhydride in this mixture was demonstrated by separating it by high-vacuum sublimation from the crude product; 320 mg. was obtained from 1 g. of product.

Anal. Calcd. for $C_6H_{10}N_2O_2$: N, 19.7. Found: N, 19.8; amino-N, 0.1.

(18) Mease, *J. Res. Nat. Bur. Stand.*, **13**, 617 (1934).

Acknowledgment.—The authors are indebted to S. Ahnger and C. Cleaver for numerous analytical determinations.

Summary

Various proteins were treated with the reaction product of chlorosulfonic acid and pyridine under anhydrous conditions at 70–80° for two and one-half hours. This led to the covalent binding of considerable amounts of sulfate sulfur (up to 10%), corresponding in several proteins to the sulfation of one out of every three amino acid residues. The mode of linkage was ascertained through the use of model substances and proteins or polypeptides rich in certain groups. It was thus shown that, under the condition used, part or all of the aliphatic and phenolic hydroxyl, thiol, primary amide, amino, guanidyl and indole groups were transformed to sulfates or sulfamates. For most proteins, there was a definite correlation between the sum of these groups and the sulfate introduced. The imidazole and carboxyl groups and the peptide linkage did not participate in the reaction.

The stability of the various sulfate linkages in acid and alkali was investigated. Sulfamates of indoles or guanidines appear not to have been described previously.

ALBANY, CALIF. RECEIVED APRIL 8, 1946

[CONTRIBUTION FROM THE MERCK RESEARCH LABORATORIES]

Substituted Sulfaquinoxalines. I. The Isolation and Synthesis of 3-Hydroxy-2-sulfanilamidoquinoxaline and of Related Quinoxalines

BY J. R. STEVENS,[1] K. PFISTER, 3RD, AND F. J. WOLF

The drug 2-sulfanilamidoquinoxaline[2] (I) is of particular interest as a prophylactic agent in avian malaria and exhibits certain unique characteristics not generally found in the sulfa drugs.[3] In experiments on its chronic toxicity in rats, the formation of calculi in the tubule region of the kidneys was observed,[3] and the importance of the structure of this product in considering the metabolic fate of the drug became important.[4]

Examination of the crude calculi indicated that the compound present was not unchanged sulfaquinoxaline although a positive Marshall test established the presence of a free aromatic amino group. Analyses of the purified material proved that it was a hydroxylated derivative of sulfa-

(1) Present address: J. T. Baker Chemical Company, Phillipsburg, New Jersey.

(2) J. Weijlard, M. Tishler and A. E. Erickson, THIS JOURNAL, **66**, 1957 (1944).

(3) A. O. Seeler, C. W. Mushett, C. Graessle and R. Silber, *J. Pharmacol.*, **82**, 357 (1944).

(4) Scudi and Silber, *J. Biol. Chem.*, **156**, 343 (1944), isolated 3-hydroxy-2-sulfanilamidoquinoxaline from the urine of rabbits receiving 2-sulfanilamidoquinoxaline. The metabolic product was identified by degradation with hydrochloric acid to 2,3-dihydroxyquinoxaline.

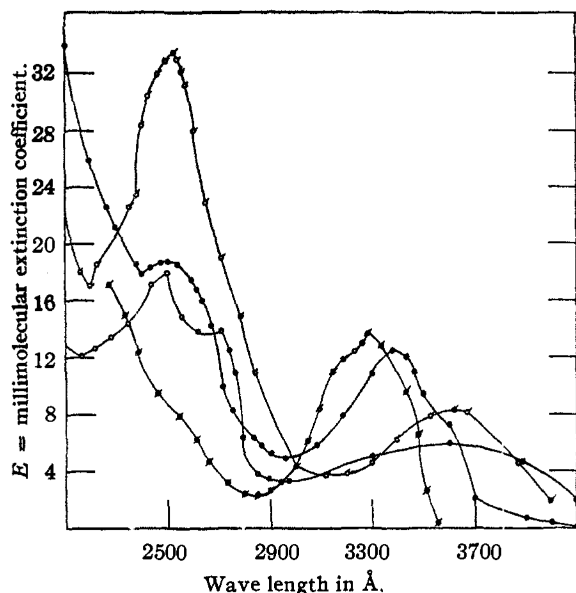


Fig. 1.—●, crude rat kidney calculi in *p*H 6.6 buffer; ♂, 2-sulfanilamidoquinoxaline in *p*H 6.6 buffer; O, 2-sulfanilamidoquinoxaline in 0.1 *N* HCl; ⏀, 2-amino-3-hydroxyquinoxaline in *p*H 6.48 buffer.

The low absorption at 2500 Å. peak can be explained by the effect of the neighboring hydroxyl group on the acidity of N^1 hydrogen since the corresponding peak in the absorption spectrum of I is lowered by increasing the hydrogen ion concentration. The increased absorption of the second peak and its shift to shorter wave lengths was to be expected from comparisons with the absorption spectra of 2,3-dihydroxyquinoxaline [G. Glotz, *Bull. soc. chim.*, [5] 3, 511 (1936)] and of 2-amino-3-hydroxyquinoxaline which show peaks at 3100 and 3300 Å., respectively.

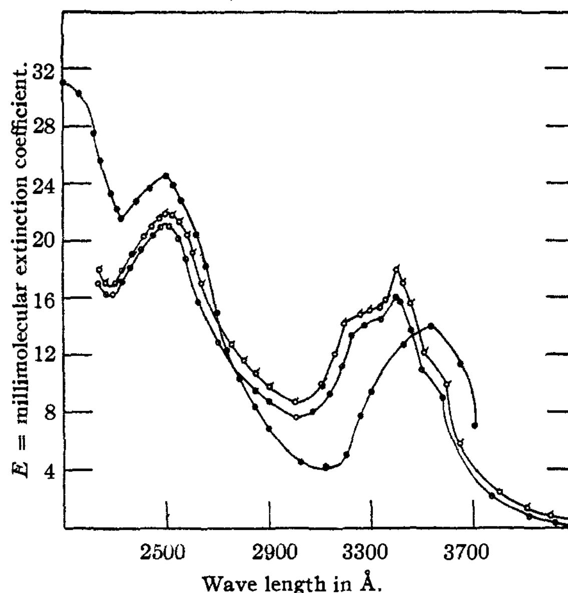


Fig. 2.—♂, synthetic 3-hydroxy-2-sulfanilamidoquinoxaline in *p*H 6.34 buffer; O, natural 3-hydroxy-2-sulfanilamidoquinoxaline in *p*H 6.34 buffer; ●, 3-amino-2-sulfanilamidoquinoxaline in *p*H 6.6 buffer.

quinoxaline, II, and the similarity of the ultraviolet spectra of the metabolite and 2-amino-3-hydroxyquinoxaline (Fig. 1)[5] suggested that the product was 3-hydroxy-2-sulfanilamidoquinoxaline. This structure was unequivocally established by synthesis and comparison of the properties of the synthetic material with those of the natural product (see Fig. 2 for absorption).

The synthesis of 3-hydroxy-2-sulfanilamidoquinoxaline offered considerable difficulty but was finally achieved by the condensation of 2-amino-3-hydroxyquinoxaline, III, with *p*-nitrobenzenesulfonyl chloride followed by reduction of the resulting *p*-nitrosulfonamide, IV. It is interesting to note that the amino group in III was very unreactive toward acylating agents leading to the sulfanilamide derivative. Thus it failed to react with *p*-acetylaminobenzenesulfonyl chloride and with *p*-nitrobenzenesulfonyl chloride vigorous conditions were required for successful condensation.

Two other methods for the synthesis of II were studied. In one scheme 3-methoxy-2-sulfanilamidoquinoxaline was obtained by hydrolysis of the N^4-acetyl compound, VIII, formed in the condensation of 2-chloro-3-methoxyquinoxaline, VI, with N^4-acetylsulfanilamide. However, acid cleavage of the methoxyl group gave only 2,3-dihydroxyquinoxaline and alkaline hydrolysis was ineffective. The required 2-chloro-3-methoxyquinoxaline was obtained along with a trace of 2,3-dimethoxyquinoxaline, VII, by treatment of 2,3-dichloroquinoxaline with sodium methoxide. Attempted preparation of 2-amino-3-methoxyquinoxaline, IX, by amination of VI yielded mainly 2,3-diaminoquinoxaline, X, and only small amounts of the desired product. The reaction of X and excess *p*-acetylaminobenzenesulfonyl chloride gave only 3-amino-2-(N^4-acetylsulfanilamido)-quinoxaline, XI. Attempts to replace the 3-amino group by hydroxyl were unsuccessful. Acid hydrolysis could not be controlled and gave only 2-amino-3-hydroxyquinoxaline, while nitrous acid treatment gave untractable decomposition products. The results of these experiments are indicated in the flow sheet.

Experimental

Purification of Rat Kidney Calculi.—A 50-mg. sample of the calculi[6] containing appreciable quantities of tissue was mixed with 6 ml. of warm 4 *N* ammonium hydroxide, centrifuged and the mother liquor neutralized with 1.5 ml. of 36% acetic acid. The precipitate was separated and washed two times with 1 ml. of water, yielding 34.3 mg., m. p. 268–271°.[7] A sample similarly purified was analyzed.

(5) We are indebted to L. T. Anderson and W. A. Bastedo of this laboratory for determining the extinction coefficients.

(6) Kindly furnished by Dr. C. W. Mushett of the Merck Institute.

(7) All m. p. are uncorrected.

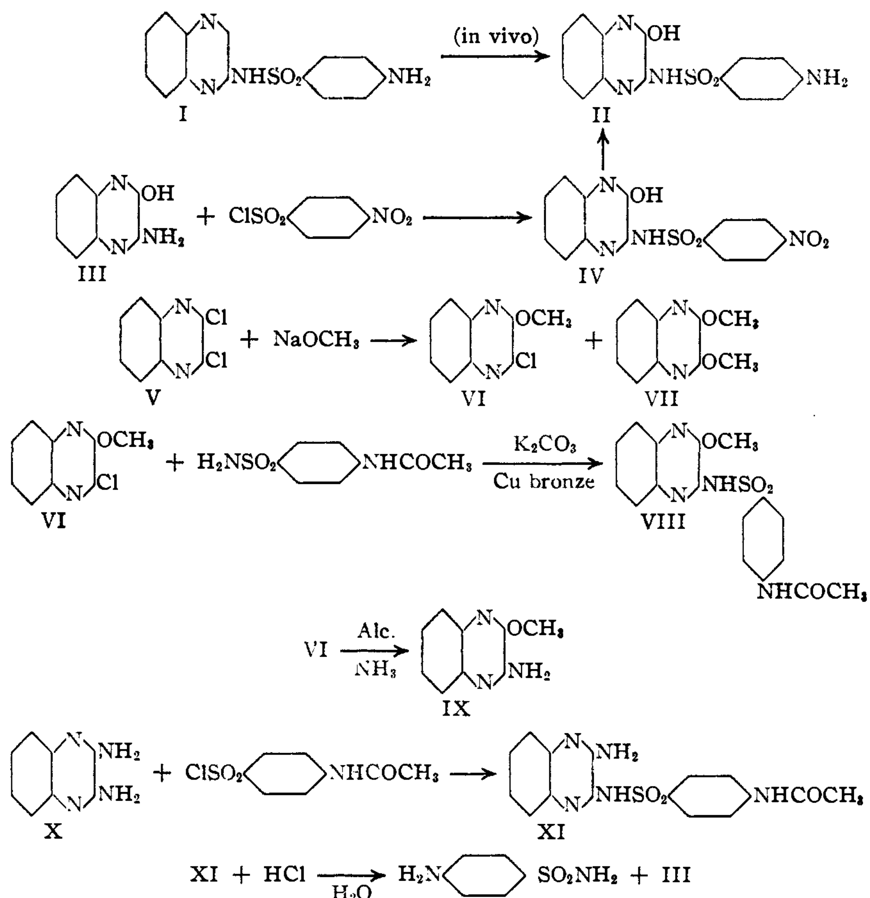
Anal.[8] Calcd. for $C_{14}H_{12}N_4SO_3$: C, 53.16; H, 3.83; N, 17.71. Found: C, 52.83; H, 4.03; N, 17.06; ash, 1.

The ultraviolet absorption of this sample gave a curve (Fig. 1) which approximated the curve finally obtained from the pure material. A ferric chloride test in acetone was negative. A 0.5-mg. portion of the material dissolved in 1.5 ml. of 2.5 *N* hydrochloric acid, cooled and treated with 0.2 ml. of 10% sodium nitrite solution followed by 0.2 ml. of 10% ammonium sulfamate gave a red color when poured into 2 ml. of 2.5 *N* sodium hydroxide containing 100 mg. of β-naphthol.

The product, 31.6 mg., was dissolved in 2.5 ml. of warm pyridine and separated from the insoluble substance (6 mg.). Addition of ether precipitated 22.9 mg. of material.

Anal. Calcd.: N, 17.71. Found: N, 17.35.

For further purification, 11.5 mg. was dissolved in 0.6 ml. of hot 6 *N* ammonium hydroxide; on cooling the solution the ammonium salt separated in fine white needles. The mother liquor was separated and the ammonium salt dissolved in 0.5 ml. of water and precipitated by the addition of 0.2 ml. of 36% acetic acid, yielding 5.5 mg. of white lustrous material. The ultraviolet absorption curve is shown in Fig. 2.



Anal. Calcd.: C, 53.16; H, 3.83. Found: C, 53.44; H, 3.96.

Synthesis of 3-Hydroxy-2-sulfanilamidoquinoxaline

2,3-Diaminoquinoxaline, X, from Cyanogen and *o*-Phenylenediamine.—The diamine was prepared by the method of Hinsberg[9] in 44% yield. It was recrystallized from pyridine to form beautiful yellow platelets which do not melt at 340°.

Anal. Calcd. for $C_8H_8N_4$: C, 59.97; H, 5.03. Found: C, 59.85; H, 5.01.

2,3-Diaminoquinoxaline, X, from 2,3-Dichloroquinoxaline.—A mixture of 5.0 g. (0.025 mole) of 2,3-dichloroquinoxaline (see below), 200 ml. of methanol and 40 ml. of liquid ammonia was heated in a glass lined steel bomb at 150° for twelve hours. The reaction mixture was concentrated to dryness and the residue recrystallized from pyridine to give 2.8 g. of material similar to that obtained above.

To confirm the identity of the two compounds, equimolecular quantities of the diamines and phenanthroquinone were refluxed in glacial acetic acid.

In both reactions, the orange precipitates appearing in twenty to thirty minutes were recrystallized from glacial acetic acid as hair-fine orange needles melting above 330°. Both preparations gave a brilliant purple color with concentrated sulfuric acid.

Anal. Calcd. for phenanthro[9,10-b.]quinoxalo[2,3-e.]-pyrazine,[9] $C_{22}H_{12}N_4$: N, 16.86. Found: N, 16.60.

2-Amino-3-hydroxyquinoxaline,[9,10] III.—A solution of 20 g. (0.125 mole) of 2,3-diaminoquinoxaline in 300 ml. of 2.5 *N* hydrochloric acid was heated on a steam-bath for one hour; decolorizing charcoal was added and the heating continued for ten minutes. The solution was filtered and the product precipitated by the addition of 140 ml. of 6 *N* ammonium hydroxide. The crude product was purified by dissolving in 300 ml. of 1.25 *N* sodium hydroxide, treating with decolorizing charcoal and precipitating with glacial acetic acid. The white powder, yield 19.6 g. (97.2%) did not melt at 350°.

The ultraviolet absorption curve is given in Fig. 1.

Anal. Calcd. for $C_8H_7N_3O$: C, 59.60; H, 4.38; N, 26.07. Found: C, 59.66; H, 4.49; N, 26.20.

3-Hydroxy-2-*p*-nitrobenzenesulfonamidoquinoxaline, IV.—A solution of 11.0 g. (0.0685 mole) of 3-hydroxy-2-aminoquinoxaline in 2 l. of pyridine, dried over barium oxide, was refluxed and 44.0 g. (0.199 mole) of *p*-nitrobenzenesulfonyl chloride added in portions over one hour. After refluxing for one half hour, the dark solution was concentrated under vacuum and water was added. The yellow crystalline material (contaminated with a tarry substance in some runs) was filtered, dissolved in 500 ml. of hot 5% sodium hydroxide, treated with Norite, filtered and precipitated with acetic acid. The yellow-orange powder was dissolved in 400 ml. of cold 3 *N* ammonium hydroxide and again treated with Norite and acidified. The yellow crystalline material, 5.8 g. (24.5%) melted with decomposition at 310°.

It was readily soluble in hot dioxane, pyridine and glacial acetic acid and difficultly soluble in ethano land

(8) Microanalyses were kindly performed by R. N. Boos, W. K. Humphrey, E. H. Meiss, E. Thornton, L. Rosalsky and J. McGregor of this laboratory.

(9) Hinsberg and Schwantes, *Ber.*, **36**, 4039 (1903).

(10) Bladin, *ibid.*, **18**, 666 (1885).

36% acetic acid. A sample precipitated from pyridine by ether retained one molecule of pyridine when dried at 100° and 3 mm.

Anal. Calcd. for $C_{14}H_{10}N_4SO_5 \cdot C_5H_5N$: C, 53.64; H, 3.55. Found: C, 53.25; H, 3.90.

A sample, recrystallized from ethanol, was easily freed of the solvent.

Anal. Calcd. for $C_{14}H_{10}N_4SO_5$: C, 48.55; H, 2.91. Found: C, 48.39; H, 3.08.

3-Hydroxy-2-sulfanilamidoquinoxaline, II.—A mixture of 5.7 g. (0.0165 mole) of 3-hydroxy-2-(*p*-nitrobenzenesulfonamido)-quinoxaline, 18 g. of iron powder (0.34 gr. atom), 1.2 ml. of dilute (1:1) hydrochloric acid and 1.5 liters of ethanol was refluxed with vigorous stirring for six hours. After adding 1.0 g. of Norite the hot solution was filtered through Supercel. On cooling the orange solution gave lustrous clusters of nearly colorless rods, weighing 4.3 g. (82.6% of theory), melting with decomposition at 275–278°.

For purification the product was dissolved in 180 ml. of hot 3 *N* ammonium hydroxide, filtered from a little insoluble material and cooled. The white needles of the ammonium salt were filtered off, taken up in hot water and precipitated with acetic acid. The nearly white product weighed 1.3 g. (30% recovery). From the filtrate 1.55 g. (36%) of less pure material was recovered by acidification with acetic acid.

The ultraviolet absorption curve is given in Fig. 2.

Anal. Calcd. for $C_{14}H_{12}N_4SO_2$: C, 53.16; H, 3.82; N, 17.71. Found: C, 53.39; H, 3.85; N, 17.68.

When the reaction of *p*-acetylaminobenzenesulfonyl chloride and 3-hydroxy-2-aminoquinoxaline was attempted, only the unchanged amine could be recovered. When these reaction mixtures were heated at temperatures above 70°, rapid darkening accompanied by decomposition of the acid chloride occurred.

2,3-Dichloroquinoxaline, V.—The procedure is essentially that of Hinsberg.[11] A mixture of 60 g. (0.37 mole) of 2,3-dihydroxy-quinoxaline (obtained in 85% yield by refluxing an ethanol solution of *o*-phenylenediamine and ethyl oxalate and purifying the crude product by dissolving in dilute sodium hydroxide, treating with decolorizing charcoal and precipitating with dilute acid), and 154 g. (0.735 mole) of phosphorus pentachloride was heated with good stirring on an oil-bath at 140° allowing the phosphorus oxychloride to distill off. The mixture gradually became homogeneous and the temperature rose to 160°. At the end of one and one-quarter hours it was poured into 600 g. of ice and water and filtered. The light brown precipitate was air dried and recrystallized from 800 ml. benzene with charcoal treatment. The product, 52 g. (75%), separated as white colorless needles of m. p. 147–150°.

2-Chloro-3-methoxyquinoxaline, VI.—In a 1-liter two-necked flask equipped with a sealed stirrer and small Soxhlet extractor was placed 70 g. (0.35 mole) of 2,3-dichloroquinoxaline and 400 ml. of dry methanol. In the Soxhlet thimble was placed 19.5 g. of 95% sodium methoxide (0.34 mole). After refluxing for sixteen hours approximately half of the sodium methoxide had dissolved. The thimble was broken and the remainder was washed into the flask with 350 ml. of methanol and refluxing continued for four hours. The hot mixture was filtered. From the precipitate 15.7 g. of pure 2,3-dichloroquinoxaline was obtained. Upon cooling the filtrate an additional 16.0 g. of essentially pure 2,3-dichloroquinoxaline, m. p. 142–146°, was obtained. The mother liquor was concentrated to half its volume and on cooling 16.1 g. (43.0%) of 2-chloro-3-methoxy-quinoxaline, m. p. 74–75°, was obtained. The filtrate contained 14 g. of material, m. p. 60–90°, from which it was possible by careful fractionating to isolate 2,3-dimethoxyquinoxaline, m. p. 92–93°.

Anal. 2,3-dimethoxyquinoxaline, VII. Calcd. for $C_{10}H_9N_2O_2$: C, 63.25; H, 5.28. Found: C, 63.48; H, 5.59.

2-Amino-3-methoxyquinoxaline, IX, from 2-Chloro-3-methoxyquinoxaline.—A mixture of 19.6 g. (0.1 mole) of 2-chloro-3-methoxy-quinoxaline, 400 ml. of methanol and 20 g. of anhydrous ammonia was heated in a glass lined steel bomb at 165° for eight hours. The reaction mixture was concentrated to dryness and the residue extracted with 150 ml. of hot ethyl acetate. The insoluble fraction, about 10 g., freed from ammonium chloride and recrystallized from pyridine was 2,3-diaminoquinoxaline. The ethyl acetate solution was concentrated to dryness. The residue, 2.5 g., was recrystallized from nitromethane, m. p. over 260°.

Anal. Calcd. for 2-amino-3-methoxyquinoxaline, $C_9H_9N_3O$: C, 61.72; H, 5.18. Found: C, 61.42; H, 5.43.

2-Amino-3-methoxyquinoxaline, IX, from 3-Hydroxy-2-aminoquinoxaline.—The hydroxyamine, 4.5 g. (0.0283 mole) was dissolved in 75 ml. of 5% sodium hydroxide by warming. The warm solution was filtered and cooled in ice. Dimethyl sulfate, 7 ml. (0.0765 mole) was added portionwise with occasional shaking over thirty to forty-five minutes. A white precipitate formed and thickened as the addition progressed. After standing one hour in ice, the reaction mixture was filtered and the precipitate washed with dilute sodium hydroxide and water, yielding 4.2 g. (86%), m. p. 260–265°. It was soluble in cold 2.5 *N* hydrochloric acid, slightly soluble in ethyl acetate and readily soluble in hot pyridine from which it was recrystallized as small white crystals, m. p. 264–270° (with sublimation).

Anal. Calcd. for $C_9H_9N_3O$: C, 61.75; H, 5.18; N, 23.93. Found: C, 61.46; H, 5.17; N, 24.13.

3-Methoxy-2-sulfanilamidoquinoxaline.—A mixture of 25 g. of 2-chloro-3-methoxyquinoxaline (0.126 mole), 35 g. of N^4-acetylsulfanilamide (0.164 mole), 35 g. of ground anhydrous potassium carbonate, 1.0 g. of copper bronze and 200 ml. of nitrobenzene was heated gradually and refluxed for fifteen minutes. The reaction mixture was cooled and filtered and the light tan precipitate was washed well with benzene and then with ether to remove nitrobenzene.

The precipitate was slurried with 600 ml. of water and 100 ml. of 2.5 *N* sodium hydroxide. The dark solution was treated with decolorizing charcoal and made acid with acetic acid. As the solution becomes acid a black tar first precipitates followed by lighter material. The precipitate was taken up in 175 ml. of hot glacial acetic acid and filtered. The insoluble portion, 7.4 g., was mainly 2,3-dihydroxyquinoxaline. The dark acetic acid solution was poured into 1.5 liters of water with vigorous stirring, yielding 10 g. of tan powder. This was taken up in 400 ml. of hot acetone, filtered from 0.65 g. of insoluble material and the acetone solution poured into 1.5 liters of water. The black gum which separated was taken up in 400 ml. of hot 4 *N* ammonium hydroxide, the solution treated with decolorizing charcoal and neutralized with acetic acid. A grayish blue precipitate, wt. 6.8 g., was obtained.

Purification of this material was quite difficult and it was hydrolyzed by heating with 125 ml. of 2.5 *N* sodium hydroxide in the steam cone for three hours, decolorizing charcoal being added during the last hour. The precipitate obtained by neutralizing with acetic acid was taken up in 100 ml. of 6 *N* ammonium hydroxide, treated again with decolorizing charcoal and precipitated with acetic acid. The khaki-green precipitate, wt. 3.5 g. (8.4%) was taken up in 100 ml. of hot 6 *N* ammonium hydroxide and the solution, after decolorizing charcoal treatment, was boiled until a light tan material separated. The solution was cooled and filtered. The mother liquor was again boiled until a precipitate appeared, cooled and filtered. The crops, weight 1.23 g., m. p. 263–264°, were combined and 0.15 g. recrystallized from acetic acid was analyzed.

Anal. Calcd. for 3-methoxy-2-sulfanilamidoquinoxaline, $C_{15}H_{16}N_4SO_3$: C, 54.54; H, 4.27; N, 16.95. Found: C, 54.28; H, 4.48; N, 17.01.

The material was unaffected by heating with sodium methoxide in methanol in a sealed tube at 210° for eight hours. When heated with 2.5 *N* hydrochloric acid on a

(11) Hinsberg and Pollak, *Ber.*, **29**, 784 (1896).

steam cone for one hour, 2,3-dihydroxyquinoxaline was obtained.

3-Amino-2-sulfanilamidoquinoxaline, XI.—A suspension of 6.4 g. (0.04 mole) of 2,3-diaminoquinoxaline in 125 ml. of dry pyridine was warmed to 35° and 9.2 g. (0.039 mole) of *p*-acetylaminobenzenesulfonyl chloride added gradually over forty-five minutes. The turbid mixture was warmed for one hour at 50°, treated with decolorizing charcoal, and concentrated *in vacuo* to a thick gum. By working with water a pale yellow granular precipitate was obtained which was twice dissolved in 3 *N* ammonium hydroxide, treated with decolorizing charcoal and precipitated with acetic acid. The product, 10.8 g. (75.6%), darkened at 250° and sintered and decomposed at 260°. An analytical sample recrystallized from glacial acetic acid behaved similarly.

Anal. Calcd. for 3-amino-2-(N^4-acetylsulfanilamido)-quinoxaline, $C_{16}H_{15}N_5SO_3$: C, 53.77; H, 4.25; N, 19.60. Found: C, 53.75; H, 4.49; N, 19.75.

When two or more equivalents of *p*-acetylaminobenzenesulfonyl chloride were utilized, the same compound was obtained. Attempts to replace the amino group by hydroxyl by treatment with nitrous acid gave unidentifiable tars.

Hydrolysis of the acetyl group was effected by heating 8 g. of compound in 80 ml. of absolute ethanol and 40 ml. of concentrated hydrochloric acid for one hour at reflux. The reaction mixture was cooled and the precipitated white hydrochloride dissolved in 5 *N* ammonium hydroxide and precipitated with acetic acid. After two such purifications, the yield of pale yellow material was 5.0 g. (70.8%); it darkened at 260° and sintered and decomposed at 275–280°.

Anal. Calcd. for 3-amino-2-sulfanilamidoquinoxaline, $C_{14}H_{13}N_5SO_2$: C, 53.33; H, 4.15; N, 22.21. Found: C, 53.19; H, 4.10; N, 22.45.

Hydrolysis could also be accomplished by heating with 2.5 *N* sodium hydroxide but when 2.5 *N* hydrochloric acid was used only 2-amino-3-hydroxyquinoxaline was obtained.

Acknowledgment.—The authors are indebted to Dr. R. T. Major and Dr. Max Tishler for their kind encouragement, advice and criticism. Mr. R. M. Wilson, Jr., Mr. C. A. Robinson and Miss L. H. Falk assisted with the preparation of the compounds.

Summary

Material present in the kidneys of rats fed 2-sulfanilamidoquinoxaline was identified by synthesis as 3-hydroxy-2-sulfanilamidoquinoxaline. Efforts to synthesize this compound also led to the preparation of 3-methoxy-2-sulfanilamidoquinoxaline, 3-amino-2-sulfanilamidoquinoxaline, 2-chloro-3-methoxyquinoxaline, 2-amino-3-methoxyquinoxaline and 2,3-dimethoxyquinoxaline.

RAHWAY, N. J. RECEIVED FEBRUARY 8, 1946

[CONTRIBUTION FROM THE CHEMOTHERAPY DIVISION, STAMFORD RESEARCH LABORATORIES, AMERICAN CYANAMID COMPANY]

Studies in Chemotherapy. XIV. Antimalarials. The Synthesis of Substituted Metanilamides and Related Compounds[1]

BY J. P. ENGLISH, J. H. CLARK, R. G. SHEPHERD, H. W. MARSON, J. KRAPCHO AND R. O. ROBLIN, JR.

2-Sulfanilamido-5-chloropyrimidine (Fig. 1, X = Cl) and its bromine analog (Fig. 1, X = Br) were prepared[2] in the course of attempts to obtain sulfanilamides more active than sulfadiazine (Fig. 1, X = H) in experimental malaria. These compounds proved to be unusual in that, unlike sulfadiazine and the other sulfanilamides tested, their antimalarial activity was only partially prevented by *p*-aminobenzoic acid.[3] Other sulfanilamidohalogen heterocycles did not show this behavior and we therefore explored other possibilities in attempting to develop this new antimalarial activity.

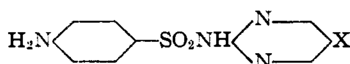


Fig. 1.—2-Sulfanilamidopyrimidines

To investigate the effects of isomerism, 2-metanilamidopyrimidine[4] (Fig. 2, XVII) and 2-metanilamido-5-chloropyrimidine (Fig. 2, XIX) were prepared. These compounds, isomers of

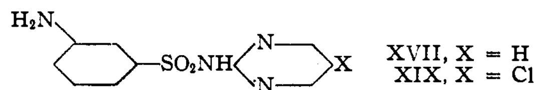


Fig. 2.—2-Metanilamidopyrimidines

sulfadiazine and its 5-chloro substitution product, proved to be highly active in sporozoite-induced *Plasmodium gallinaceum* infections in chickens,[5] and representative N^1-substituted metanilamides and orthanilamides, as well as variously substituted 2-phenylsulfonamidopyrimidines, were synthesized to study the relation of structure to activity in these series. The compounds prepared in this research are listed in Table I together with their antimalarial activities. Several known compounds are also included there for purposes of comparison.

The nitro compounds, from which the metanilamides and *m*-aminobenzamido compounds were prepared by reduction, are listed in Table II (see Experimental). These, with a few exceptions, were synthesized by the reaction of the appropriate acid chloride with the amine. The phenylsulfonamidoheterocycles in Table I were also prepared by this general procedure using benzenesulfonyl chloride. Anhydrous pyridine

(1) Presented in part before the Medicinal and Organic Sections of the American Chemical Society, Atlantic City, April 8–12, 1946.

(2) English, Clark, Clapp, Seeger and Ebel, THIS JOURNAL, **68**, 453 (1946).

(3) Waletzky and Brackett, unpublished results.

(4) The nomenclature is that proposed by Crossley, Northey and Hultquist, THIS JOURNAL, **60**, 2217 (1938).

(5 Brackett and Waletzky, in preparation.