by a modified method of Lipschitz.[13] They were found to be more active than the parent compound 6-chloro-3,4-dihydro - 7 - sulfamoyl - 1,2,4 - benzothiadiazine 1,1-dioxide. The most active compounds (200–300 times the parent compound) were similar in structure to the most active compounds listed in the preceding paper.

(13) W. L. Lipschitz, Z. Hadidian, and A. Kurpcsar, *J. Pharm. Exper. Therap.*, **79**, 97 (1943).

Acknowledgment. The authors thank W. L. Brown, H. L. Hunter, G. Maciak, and R. M. Hughes for the microanalyses and H. Boaz and D. Woolf for the titrations and infrared data.

The diuretic activities were determined by R. H. Tust of these laboratories.

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[CONTRIBUTION FROM THE DEPARTMENT OF NUTRITION AND METABOLIC DISEASES, THE UPJOHN CO.]

8-Chloroalloxazine, A New Diuretic. Synthesis and Structure

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Received December 27, 1960

8-Chloroalloxazine, a new diuretic, can be obtained in good yield and purity by the condensation of alloxan with 4-chloro-2-aminoaniline (1,2-diamino-4-chlorobenzene) in strongly acidic aqueous solutions or in glacial acetic acid in the presence of boric acid. When the same condensation is carried out in weakly acidic aqueous solution or neutral solvents, 2-hydroxy-6-chloroquinoxaline-3-carboxyureide is the main or exclusive product. Evidence for the structure of these compounds is derived from degradation studies, physical properties, and a comparison of these with the products formed when alloxan and 1,2-diaminobenzene are condensed, the latter reaction giving well characterized compounds.

Diuretic activity of clinical significance is a pharmacodynamic property shown by a limited number of structures,[1] and the recent discovery of such activity in several new types of compounds, including sulfonamyl derivatives and carbonic anhydrase inhibitors,[2] has led to a renewed interest in this aspect of medicinal chemistry. Therefore, the finding by Graham[3] of diuretic activity in rats and dogs when a chloroalloxazine of unknown structure was administered orally seemed to be of sufficient importance to warrant expending considerable effort to determine the chemical nature of the compound responsible for the biological activity. In this paper are described the studies which established the structure of the diuretic and led to unequivocal methods for its preparation.

A compound designated as 7(8)-chloroalloxazine was one of a series of alloxazines and isoalloxazines prepared for the investigation of the possible antitumor activity of riboflavin inhibitors. This compound was prepared according to the method described by Wolf *et al.*,[4] which involves the condensation of alloxan and the appropriate ortho-phenylenediamine. The purity of the 7(8)-chloroalloxazine mentioned by Wolf *et al.* was never established but work presented here indicates the reaction of 4-chloro-2-aminoaniline (I) and alloxan (II) (*cf.* Fig. 1) leads to the formation either of 8-chloroalloxazine (III) or of 2-hydroxy-6-chloroquinoxaline-3-carboxyureide (IV) and that there is no evidence of the formation of 7-chloroalloxazine under any conditions studied by either Wolf *et al.* or us. 2-Hydroxy - 6 - chloroquinoxaline - 3 - carboxyureide (IV) has no diuretic activity, but its presence in some preparations for a time obscured the biological results.

The conditions leading to the formation of 8-chloroalloxazine alone involve the condensation of 4-chloro-2-aminoaniline (I) and alloxan (II) in glacial acetic acid in the presence of boric acid at room temperature or the condensation of these reactants in 1–5*N* hydrochloric acid with heat. If aqueous acetic acid is used even in the presence of boric acid, 2-hydroxy-6-chloroquinoxaline-3-carboxyureide (IV) is formed. If boric acid is eliminated from the glacial acetic acid reaction medium or is present below 0.03 molar equivalents, IV is formed along with III. Similarly, if the hydrochloric acid medium is at an acidity lower than 1*N*, IV is formed as an impurity along with III. It is therefore apparent that 8-chloroalloxazine (III) is formed only under limited conditions of reaction, these being the subject matter of much of this presentation.

When I and II are condensed in neutral solvents, such as ethanol or cellosolve, or neutral or slightly acidic aqueous media, only IV is formed. This is in harmony with the findings of King and Clark-Lewis[5] and Barlow, Ing, and Lewis,[6] who showed that the reaction of alloxan and *o*-phenylenediamine in neutral solutions yielded 2-hydroxyquinoxaline-3-

(1) For a review of the mercurial, xanthine, and isocytosine diuretics, the reader is referred to R. F. Pitts, *The Physiological Basis of Diuretic Therapy*, Charles C Thomas, Springfield, Ill., 1959.

(2) Carroll A. Handley and John H. Moyer, *The Pharmacology and Clinical Use of Diuretics*, Charles C Thomas, Springfield, Ill., 1959.

(3) B. E. Graham, unpublished communication.

(4) F. J. Wolf, R. H. Beutel, and J. R. Stevens, *J. Am. Chem. Soc.*, **70**, 2572 (1948).

(5) F. E. King and J. W. Clark-Lewis, *J. Chem. Soc.*, 3379 (1951).

(6) R. B. Barlow, H. R. Ing, and I. M. Lewis, *J. Chem. Soc.*, 3242 (1951).

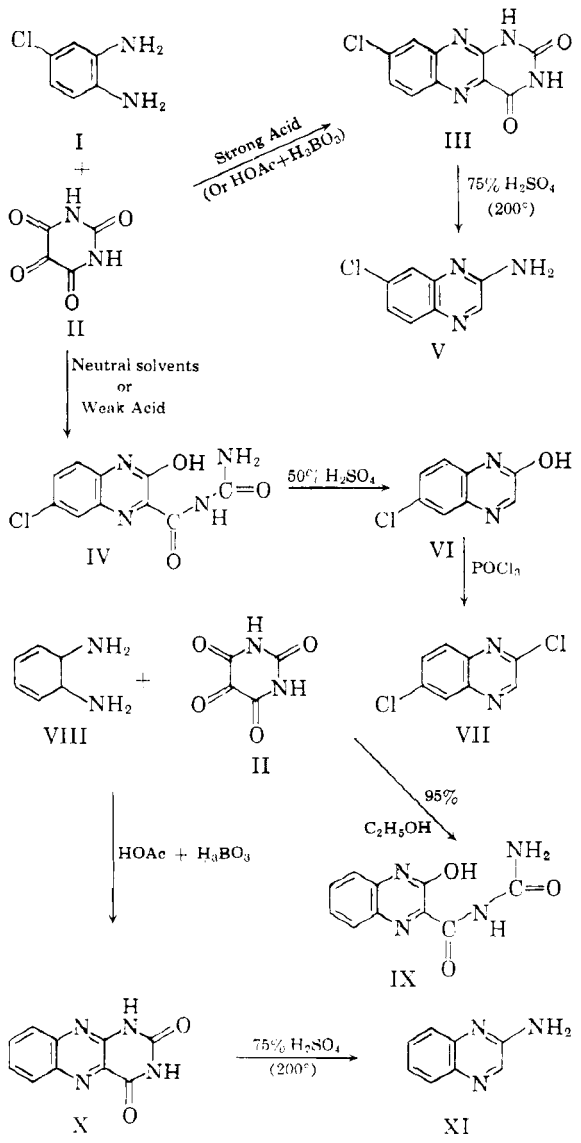

Figure 1

carboxyureide as Hinsberg[7] had suggested rather than an anil as Kuehling,[8] Kuhn *et al.*,[9] and Tishler *et al.*,[10] had maintained.

There is no evidence for the formation of 7-chloroalloxazine under a variety of conditions described here although the 2-hydroxy-6(7)-chloroquinoxaline-3-carboxyureide (IV), which is formed is sterically related to 7-chloroalloxazine. These data indicate that the change of conditions from strongly acidic to neutral or weakly acidic conditions of condensation does alter a sterically directed synthesis in each case.

The purity of the products formed during the reaction was followed by paper chromatography and ultraviolet spectrophotometry, data of which are summarized below. The structures of the respective products of the reaction of I and II, II and VIII were determined by degradation to known quinoxalines which have been synthesized by Crowther, Curd, Davey, and Stacey[11] and Gowenlock, Newbold, and Spring[12] by unambiguous routes.

By these studies it has been shown that I and VIII react with II to give similar products, namely alloxazine (X) or 8-chloroalloxazine (III) under acidic conditions and 2-hydroxyquinoxaline-3-carboxyureide or 2-hydroxy-6-chloroquinoxaline-3-carboxyureide under neutral or weakly acidic conditions.

The diuretic activity of 8-chloroalloxazine (III) and related compounds will be published elsewhere.

EXPERIMENTAL

Synthesis of alloxazines. 1. *Alloxazine* (X). A 1.08-g. sample of *o*-phenylenediamine (0.01 mole) was dissolved in 10 ml. of glacial acetic acid. This solution was then added to 20 ml. of glacial acetic acid in which was dissolved 1.60 g. of alloxan monohydrate (II) (0.01 mole) and 0.12 g. of powdered boric acid. The solution was then stirred at room temperature for 4 hr., after which the precipitate was removed and washed with hot water, hot ethanol, and finally ether. Yield: 1.44 g. 67%, m.p. >400°.

Anal. Calcd. for $C_{10}H_6N_4O_2$: C, 56.1; H, 2.8; N, 26.2. Found: C, 55.6; H, 3.1; N, 25.2.

2. *8-Chloroalloxazine* (III). a. *4-Chloro-2-aminoaniline* (I) was prepared by catalytic reduction of 4-chloro-2-nitroaniline using platinum oxide in diethyl ether or ethyl acetate. The diamine was isolated from the reaction mixture after the catalyst had been removed by vacuum distillation of the solvent. It was used without purification.

b. *Condensation of I with alloxan monohydrate* (II) *in glacial acetic acid.* 8-Chloroalloxazine (III) is conveniently prepared by condensing I and II in glacial acetic acid in the presence of boric acid at room temperature as indicated in Fig. 1. I and II are condensed in equimolar quantities (or a slight excess of diamine) in an amount of glacial acetic acid sufficient to dissolve the reactants and with powdered boric acid present in solution in an amount equal to 0.03 molar equivalent or greater. Thus 11.0 g. (0.077 mole) of I and 10.0 g. (0.07 mole) of II were condensed in 150 ml. of glacial acetic acid with 0.64 g. of boric acid (0.01 mole) at room temperature. The mixture was stirred at 40° for 4 hr., after which the solid was removed. The product was washed well with hot water and boiling alcohol to remove acetic acid and boric acid. The yield was 83%—12.9 g., m.p. 330–335°.

Anal. Calcd. for $C_{10}H_5N_4O_2Cl$: C, 48.3; H, 2.01; N, 22.55; Cl, 14.39. Found: C, 47.61; H, 2.33; N, 22.41; Cl, 13.01. This reaction was studied to determine the amount of boric acid necessary to prevent the formation of IV as an impurity. Table I indicates the data obtained using the general method outlined above and involving in each case 0.01 mole of I and II in 0.5 mole of glacial acetic acid. These data indicate that more than 0.03 molar equivalent of boric acid is needed in relation to I and II to obtain III free of IV.

c. *Condensation of* I and II *in hydrochloric acid.* As Wolf *et al.*[4] had indicated the preparation of 7(8)-chloroalloxazine by the condensation of I and II in 0.36*N* hydrochloric acid and Hinsberg[7] had prepared alloxazine by condensing

(7) O. Hinsberg, *Ber.*, **18**, 1228 (1885); *Ann.*, **292**, 245 (1896).

(8) O. Kuehling, *Ber.*, **24**, 2362 (1891); *Ber.*, **26**, 540 (1893).

(9) R. Kuhn and K. Reinemund, *Ber.*, **67**, 1932 (1934).

(10) M. Tishler, J. W. Wellman, and K. Ladenburg, *J. Am. Chem. Soc.*, **67**, 2165 (1945).

(11) A. F. Crowther, F. H. S. Curd, D. G. Davey, and G. J. Stacey, *J. Chem. Soc.*, 1260 (1949).

(12) A. H. Gowenlock, G. T. Newbold, and F. S. Spring, *J. Chem. Soc.*, 622 (1945).

TABLE I

EFFECT OF H_3BO_3 ON PREPARATION OF 8-CHLOROALLOXAZINE

Glacial Acetic Acid	Moles I	Moles II	Moles H_3BO_3	M.P.	Ratio III/IV By Chromatography
0.5 Mole	0.01	0.01	0	249–250°	1:1
0.5 Mole	0.01	0.01	0.0001	Darkens, 255°; chars, 295°	5:1
0.5 Mole	0.01	0.01	0.0003	Darkens, 290°; chars, 310°	1:0.005
0.5 Mole	0.01	0.01	0.001	333–335°	Only III
0.5 Mole	0.01	0.01	0.03	333–335°	Only III
0.5 Mole	0.01	0.01	0.01	333–335°	Only III

o-phenylenediamine dihydrochloride with II in water, it was decided to investigate the effect of acidity on this reaction. This became more important when it was found that III prepared as above had an m.p. much below the "greater than 360°" mentioned by Wolf. The following procedure was used in this part of our work.

A 1-g. sample of I and 1 g. of II were added to 20 ml. of hydrochloric acid of various normalities and the temperature of the solution brought to 90° and held there for 1 hr. The solution was allowed to cool to room temperature after which it was refrigerated for 14 hr. The solid was removed and washed with hot alcohol and air dried. Table II indicates that it is necessary to have a normality of 2.5 or greater to prepare III free from IV, and that 1*N* hydrochloric acid or greater is required for III with only traces of IV present.

TABLE II

EFFECT OF NORMALITY OF HCl ON PREPARATION OF 8-CHLOROALLOXAZINE

Normality of HCl	Yield	M.P.	Ratio III/IV by Chrom.
0.36[a]	1.38	250–290°	1:2
0.50	1.29	Starts 290° 320–330°	1:0.1
1.0	1.22	Starts 310° 320–330°	Only trace of IV
1.25	1.28	320–330°	Only trace of IV
2.5	1.26	325–330°	Only III
5.0	1.14	325–330°	Only III

[a] Normality used by Wolf *et al.*[4]

The product obtained when 1.0 to 5.0*N* hydrochloric acid was employed as solvent was much more wettable and soluble than the product obtained as indicated under (*b*) above.

Anal. Calcd. for $C_{10}H_5N_4O_2Cl$: C, 48.3; H, 2.01; N, 22.6; Cl, 14.4. Found: C, 47.2; H, 2.3; N, 22.0; Cl, 14.1.

3. *Quinoxalines.* a. *2-Hydroxyquinoxaline-3-carboxyureide* (IX). A 1.08-g. sample of *o*-phenylenediamine (0.01 mole) was combined with 1.3 g. of alloxan monohydrate in solid form and ground together, 40 ml. of 95% ethanol added, and mixture stirred at room temperature for 1 hr. 45 min. A pale green precipitate formed. The solid was removed by centrifugation, washed with ethanol, and dried with ether. Yield: 1.72 g. (74%), m.p. 249–251°.

Anal. Calcd. for $C_{10}H_8N_4O_3$: C, 51.7; H, 3.45; N, 24.2; O, 20.7. Found: C, 52.7; H, 4.3; N, 22.7; O, 21.2.

b. *2-Hydroxy-6-chloroquinoxaline-3-carboxyureide* (IV). A 2.84-g. sample (0.02 mole) of I and 3.2 g. (0.02 mole) of II were dissolved in 150 ml. of 10% acetic acid and the solution stirred at room temperature. A golden precipitate was evident within a few minutes. The reaction was allowed to proceed to completion and after 4 hr. of stirring the solid was removed by centrifugation. It was washed with alcohol and dried with ether. Yield: 5.0 g. (96%), m.p. 249–250°.

Anal. Calcd. for $C_{10}H_7N_4O_3Cl$: C, 45.0; H, 2.63; N, 21.05; Cl, 13.35; O, 17.98. Found: C, 45.3; H, 2.71; N, 20.93; Cl, 13.06; O, 18.69.

TABLE III

ULTRAVIOLET ABSORPTION DATA ON 8-CHLOROALLOXAZINE AND RELATED COMPOUNDS IN 0.1*N* NaOH

		mμ	a_M
1.	8-Chloroalloxazine	219	31,300
		262.5	38,570
		335	5,550
2.	Alloxazine	215	27,900
		260	36,100
		332	5,700
3.	2-Hydroxy-6-chloroquinoxaline-3-carboxyureide	231	30,600
		316	5,300
		348	3,300
4.	2-Hydroxyquinoxaline-3-carboxyureide	237	22,000
		292	3,000
		352	5,150
5.	2-Amino-7-chloroquinoxaline	244	29,500
		356	7,200
6.	2-Aminoquinoxaline	242	28,700
		358	5,680
7.	2-Hydroxy-6-chloroquinoxaline	240	26,100
		353	7,550

c. *2-Amino-7-chloroquinoxaline* (V). A 2.75-g. sample of 8-chloroalloxazine was dissolved with stirring in 17 ml. of 75% sulfuric acid preheated to 200°. The temperature was held at 195–205° for 10 min., during which strong foaming occurred. The temperature was then reduced to 165–175° for 15 min., 135–145° for 60 min., and finally 120° for 20 min. The sulfuric acid solution was poured over crushed ice and the ice allowed to melt. A greenish brown precipitate formed containing mainly unreacted starting material. The entire mixture was extracted with four 100-ml. portions of ether to remove any hydroxy quinoxaline present. This was never a significant amount. The unchanged material was removed by centrifugation, washed, dried, and weighed (1.10 g.). The supernatant was made slightly alkaline (*p*H 8) with sodium hydroxide and again extracted with ether (6 × 100 ml.). The alkaline ether extracts were combined, washed with 5% sodium carbonate, water, and dried over sodium sulfate. Upon concentration to dryness, the 2-amino-7-chloroquinoxaline was obtained in 60% yield (1.2 g.). This was recrystallized from ethanol:benzene, yielding a light yellow-tan colored solid (m.p. 199–200°).

Anal. Calcd. for $C_8H_6N_3Cl$: C, 53.5; H, 3.3; N, 23.4; Cl, 19.8. Found: C, 53.9; H, 3.2; N, 23.7; Cl, 19.6.

d. *2-Hydroxy-6-chloroquinoxaline* (VI). A 1.0-g. sample of IV was dissolved in 20 ml. of 50% sulfuric acid and heated at 135° for 30 min., when bubbling ceased, after which the mixture was poured onto crushed ice. The tan precipitate was separated and washed with 5% sodium bicarbonate, water, and alcohol. It was dried over calcium chloride in a dessicator. Yield: 0.61 g. The precipitate was recrystallized from ethyl acetate. m.p. 300–305°.

Anal. Calcd. for $C_8H_5N_3OCl$: C, 53.2; H, 2.8; N, 15.5; Cl, 19.7. Found: C, 52.7; H, 2.8; N, 15.3; Cl, 19.5.

e. *2,6-Dichloroquinoxaline* (IX). A 200-mg. sample of recrystallized VIII was treated with 3 ml. of phosphorus oxychloride for 10 min. at 90°. The excess phosphorus oxychloride was removed by distillation. The oily residue was mixed with ice water and the precipitate thus formed was removed and recrystallized from ethanol. The white crystalline product weighed 100 mg., m.p. 153–155°.

Anal. Calcd. for $C_8H_4N_2Cl_2$: C, 48.2; H, 2.0; N, 14.1; Cl, 35.7. Found: C, 47.81; H, 1.87; N, 13.16; Cl, 35.28.

f. *2-Aminoquinoxaline.* A 2.75-g. sample of alloxazine was degraded in 75% sulfuric acid in the same manner as indicated for III above. In this case the amount of unchanged material was 1.18 g., giving a yield of 68% (0.68 g.). The yellow solid was recrystallized from benzene:ether, giving long, needleshaped crystals melting at 155–157°. In contrast to 2-amino-7-chloroquinoxaline, the 2-aminoquinoxaline is more soluble in benzene than ether.

Anal. Calcd. for $C_8H_7N_3$: C, 66.1; H, 4.83; N, 29.0. Found: C, 68.5; H, 5.1; N, 28.5.

Comparison of physical properties. 1. *Ultraviolet spectra.* Table III gives the ultraviolet spectral data of compounds III, X, IV, IX, V, XI, and VI.

These data indicate the similarity of the related compounds and the fact that the substitution of the chlorine does cause some alteration in the absorption spectra.

2. *Chromatographic behavior.* Table IV gives the R_f values of the compounds described here using circular paper chromatography and two solvent systems. By using two solvent systems, it is possible to determine some aspects of structure. Thus, in the case of IV and IX, Solvent B shows a distinct difference in R_f values whereas Solvent A did not. The change from VI to VII is evident in the R_f in each solvent solvent.

TABLE IV

CHROMATOGRAPHIC CHARACTERISTICS

Compound	No.	R_f[a] A[b]	R_f[a] B[c]	Ultraviolet Fluorescence
8-Chloroalloxazine	III	0.81	0.39	Yellow-gold
Alloxazine	X	0.74	0.26	Gold
2-OH-6-Cl-Quinoxaline-3-carboxyureide	IV	0.65	0.51	Green-yellow
2-OH-Quinoxaline-3-carboxyureide	IX	0.67	0.36	Gold
2-NH_2-7-Cl-quinoxaline	V	0.90	0.88	Light blue
2-NH_2-quinoxaline	XI	0.85	0.81	Light blue
2-OH-6-Cl-quinoxaline	VI	0.90	0.69	Light blue
2-6-dichloroquinoxaline	VII	—	0.97	Dark blue

[a] R_f values measured from spot of origin to center of band. Location of bands determined by fluorescence under ultraviolet lamp. Solvents: [b] A. *n*-Butyl alcohol, 6; pyridine, 4; water, 3 (v./v./v.). [c] B. *n*-Butyl alcohol, 50; piperidine, 1; water, 10 (v./v./v.).

Acknowledgment. The authors wish to express their appreciation to D. G. Thomas, M. A. Rebenstorf, J. Korman, and H. H. Fall for assistance and advice in carrying out certain aspects of this work. Elementary analyses were obtained by the Analytical Chemistry Laboratory under W. A. Struck.

KALAMAZOO, MICH.

[CONTRIBUTION FROM THE DEPARTMENT OF BIOLOGICAL SCIENCES, STANFORD RESEARCH INSTITUTE]

Potential Anticancer Agents.[1] LVII. Synthesis of Alkylating Agents Derived from 6-Amino-6-deoxy-D-glucose and 5-Amino-5-deoxy-D-ribose

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Received December 27, 1960

The synthesis of a series of one-armed mustards of 6-amino-6-deoxy-D-glucose is described in order to investigate the effect of structure on the antitumor activity. The effect of change in the sugar on antitumor activity was also investigated by the synthesis of the two-armed and one-armed mustards of 5-amino-5-deoxy-D-ribose.

An earlier paper in this series[2] presented a rationale for the design of specific enzyme inhibitors. As part of the rationale, it was proposed that a nitrogen mustard, attached to a substrate as carrier, might operate as a specific, irreversible enzyme inhibitor. It was further suggested that a "one-armed" mustard could be as good as, or possibly even better than, the corresponding "two-armed" mustard as an alkylating agent. A later paper[3] described the synthesis for biological evaluation of a "one-armed" mustard, a "two-armed" mustard, and a "mono" mustard of 6-amino-6-deoxy-D-glucose, namely 6-[(2-chloroethyl)ethylamino]-6-deoxy-D-glucose hydrochloride (I), 6-[bis(2-chloroethyl)amino]-6-deoxy-D-glucose hydrochloride (II), and 6-(2-chloroethylamino)-6-deoxy-D-glucose hydrochloride (III), respectively.

The one-armed mustard (I) and two-armed mustard (II) were both inactive against Sarcoma 180, and Adenocarcinoma 755.[4] The one-armed mustard (I) showed considerable activity against Leukemia

(1) This work was carried out under the auspices of the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, Public Health Service, Contract No. SA-43-ph-1892. The opinions expressed in this paper are those of the authors and are not necessarily those of the Cancer Chemotherapy National Service Center. For the preceding paper in this series, see L. O. Ross, W. W. Lee, M. G. M. Schelstraete, L. Goodman, and B. R. Baker, *J. Org. Chem.*, 26, 3021 (1961).

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(4) We wish to thank Dr. Joseph Greenberg, and staff of this Institute for the test data, performed under contract with the Cancer Chemotherapy National Service Center.