4(5)-Amino-5(4)-imidazolecarboxamidine Hydrochloride (II).—Adenine sulfate (2 g.) was heated in a sealed tube containing 30 cc. of 6 *N* hydrochloric acid at 150 ± 2° for two hours. The solution was evaporated to dryness and extracted with two 10-cc. portions of warm concentrated hydrochloric acid. To this filtrate was added 30 cc. of ethanol and the solution cooled to induce crystallization. The crystalline deposit was recrystallized three times by dissolving it in warm concentrated hydrochloric acid (*ca.* 2 cc.) and adding alcohol (15 cc.). The yield of pure material was 200 mg. (10%); distribution constant, 0.12 (*n*-butanol–phosphate buffer, *p*H 6.5); ultraviolet absorption spectrum: λ_{max} 287 mμ, log ε 4.03.

Anal. Calcd. for $C_5H_7N_5 \cdot 2HCl$: N, 35.3. Found: N, 35.3.

Conversion of II to Isoguanine. (a) Phosgene Method.—Fifty milligrams of II was dissolved in 5 cc. of *N* sodium hydroxide and phosgene passed through the solution for one hour, during which time three 4-cc. portions of 40% sodium hydroxide were added. The mixture was then aerated for one hour. One cubic centimeter of 2 *N* sulfuric acid was added and the solution heated, decolorized and filtered. On cooling 14 mg. (26%) of material was obtained which was recrystallized from 2 *N* sulfuric acid. The ultraviolet absorption spectrum[24] at various *p*H values was identical with that of isoguanine prepared by another synthesis.[15]

Anal. Calcd. for $(C_5H_5ON_5)_2 \cdot H_2SO_4 \cdot H_2O$: S, 7.6. Found: S, 7.7.

(b) Urea Fusion Method.—Seventy-five milligrams of II (containing 0.97 atom % excess N^{15}) was fused with 410 mg. of urea at 180° for one hour. The cooled melt was extracted with 5 cc. of boiling water and the extract discarded. The residue was recrystallized twice from 2 *N* sulfuric acid; yield 37 mg. (47%). The N^{15} content of this isoguanine sulfate was 0.85 or 88% of that present in II.

Anal. Calcd. for $(C_5H_5ON_5)_2 \cdot H_2SO_4 \cdot H_2O$: S, 7.6. Found: 7.8.

Hydrolytic Degradation.—The hydrolyses of adenine, guanine, xanthine and uric acid were carried out by heating about 1 g. of the purine in 15 cc. of concentrated hydrochloric acid at 180° in a sealed tube for eighteen hours (Table I).

Isolation of Glycine.—The mixture from the above hydrolysis was evaporated to dryness and to the residue was added 10 cc. of 5% sodium hydroxide. The ammonia was removed by aeration, while warming the mixture. One gram of *p*-toluenesulfonyl chloride was added to the alkaline solution and the mixture stirred for six hours at room temperature. The solution was filtered and acidified. The precipitation of *p*-tosylglycine was complete in two hours and the product was collected by filtration. Two recrystallizations from ethyl acetate–petroleum ether gave 30–50 mg. of pure *p*-tosylglycine (Table I).

Decarboxylation of *p*-Tosylglycine.—*p*-Tosylglycine (20 mg.) was mixed intimately with copper powder and heated to 200 ± 5° for one-half hour in a stream of nitrogen. The carbon dioxide was collected in saturated barium hydroxide; yield $BaCO_3$, 14–16 mg. (81–92%).

Oxidation of Adenine (Table II).—Adenine sulfate (400 mg.) was dissolved in 80 cc. of water containing 4 cc. of concentrated sulfuric acid. To the boiling solution was added dropwise over a period of five minutes 2.6 g. of potassium permanganate in 35 cc. of water. Carbon dioxide was collected in saturated barium hydroxide during this time. After the outlet tube had been disconnected the excess permanganate was destroyed with oxalic acid. The solution was filtered, and 160 cc. of glacial acetic acid was added followed by 2 g. of xanthydrol (in 20 cc. methanol). Precipitation of dixanthydrol urea began after one-half hour and was complete after standing overnight. The product was recrystallized three times from glacial acetic acid; yield 120 mg. A sample of ammonia was obtained from the original oxidation mixture (after treatment with oxalic acid) by adding excess alkali and collecting the ammonia in boric acid.

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Summary

The acid hydrolysis of purines has been studied by means of tracer elements, and it has been confirmed that glycine arises from the 4,5- and 7-atoms. The oxidation of adenine with potassium permanganate produces urea which is postulated to arise from the 1,7- and 3,9-nitrogen atoms.

The preparation and characterization of 4(5)-amino-5(4)-imidazolecarboximidine are described.

A new synthesis of isoguanine is described.

SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH
NEW YORK, N. Y. RECEIVED JUNE 6, 1949

(24) Cavalieri, Bendich, Tinker and Brown, THIS JOURNAL, **70**, 3875 (1948).

[CONTRIBUTION FROM THE RESEARCH LABORATORY OF GALAT CHEMICAL DEVELOPMENT, INC.]

A New Method for the Isolation of Histamine

BY ALEXANDER GALAT AND HARRIS L. FRIEDMAN

Histamine is usually prepared by the bacterial decarboxylation of histidine in solution, and it is isolated in the form of its dipicrate. The decarboxylation of histidine proceeds readily and in good yield but the isolation of pure histamine by the picrate method offers a number of disadvantages. The purification of the dipicrate by recrystallization from water or dilute alcohol requires large volumes of solvent since the compound is sparingly soluble even at the boiling point. The conversion of the dipicrate into histamine salts, such as the dihydrochloride, involves the treatment of the dipicrate with an excess of hydrochloric acid, removal of the bulk of picric acid by filtration and extraction with an organic solvent to free the product from the last traces of this reagent. Because of the low solubility of the dipicrate these operations must be conducted in hot, dilute solutions which finally must be evaporated to dryness. This method is quite inconvenient for the preparation of relatively large amounts of histamine.

Recently, Vickery described a method for the isolation of histidine involving the precipitation of

this amino acid with *o*-dichlorobenzenesulfonic acid.[1] It seemed of interest to investigate the use of the same reagent for the isolation of histamine. We have found that when an excess of *o*-dichlorobenzenesulfonic acid is added to solutions containing histamine, the disulfonate of this base precipitates in the form of white, well-formed crystals. A nearly quantitative yield was obtained when *o*-dichlorobenzenesulfonic acid was added to solutions of histidine fermented by the method of Ackermann.[2] The new compound is readily soluble in hot water and very sparingly in cold, so that purification was effected conveniently and in good yield. The conversion of the disulfonate into histamine base and its salts was effected with equal ease, as described in the experimental part.

Experimental

Histamine Bis-*o*-dichlorobenzenesulfonate.—Twenty grams of histidine monohydrochloride monohydrate was fermented with *B. coli* organisms according to the method of Ackermann.[2] To the mixture there was added 120 g. of *o*-dichlorobenzenesulfonic acid, prepared by the method of Vickery,[1] and the precipitation completed by allowing the mixture to stand overnight. The crystals of histamine bis-*o*-dichlorobenzenesulfonate were filtered off, washed with water and recrystallized from 400 ml. of boiling water with the addition of activated charcoal. The product, dried at 100°, weighed 47 g. (87%) and melted at 225–227° (cor.).

Anal. Calcd. for $C_{17}H_{17}O_6Cl_4N_3S_2$: C, 36.10; H, 3.00; N, 7.43. Found: C, 36.35; H, 3.05; N, 7.35.

(1) Vickery, *J. Biol. Chem.*, **143**, 77 (1942).

(2) Ackermann, *Z. physiol. Chem.*, **65**, 505 (1910).

Histamine Di-hydrochloride.—A solution of 11.3 g. (0.02 mole) of histamine bis-*o*-dichlorobenzenesulfonate in 100 ml. of boiling *n*-butanol was saturated with hydrogen chloride and cooled to room temperature. The precipitate of histamine dihydrochloride was filtered off, washed with *n*-butanol followed by benzene and finally dried *in vacuo;* yield 3.4 g. (92%), m. p. 242–246° (cor.).

Histamine Diphosphate.—A hot solution of 11.3 g. (0.02 mole) of histamine bis-*o*-dichlorobenzenesulfonate in 50 ml. of water was treated with a hot solution of 6.3 g. (0.01 mole) of barium hydroxide octahydrate in 25 ml. of water. The mixture was cooled and the precipitate of barium *o*-dichlorobenzenesulfonate filtered off and washed with water. To the filtrate was added 6.0 g. of 85% phosphoric acid diluted with 10 ml. of water. The solution was then concentrated *in vacuo* to a small volume and histamine diphosphate precipitated by the gradual addition of methanol; yield 5.5 g. (90%), m. p. 130°.

Histamine Base.—A hot solution of 11.3 g. (0.02 mole) of histamine bis-*o*-dichlorobenzenesulfonate in 50 ml. of water was mixed with a hot solution of 6.3 g. (0.01 mole) of barium hydroxide octahydrate in 25 ml. of water, the mixture cooled and the precipitate filtered off and washed with cold water. The filtrate was concentrated to a small volume, cooled and a small additional amount of the barium salt removed by filtration. The filtrate was evaporated to dryness *in vacuo*, the sirup dissolved in 50 ml. of absolute isopropyl alcohol, treated with charcoal, filtered and evaporated *in vacuo* to dryness. The residue crystallized on cooling and seeding; yield: 1.90–1.95 g. (85–87%), m. p. 82–85° (sealed tube).

Summary

A new method for the isolation of histamine is described. Histamine is precipitated as bis-*o*-dichlorobenzenesulfonate which is readily converted in good yield into the histamine base or its salts.

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Studies in Pterine Chemistry. I. 2-Amino-4-hydroxy-6-polyhydroxyalkylpterines

BY H. G. PETERING AND J. A. SCHMITT

Karrer, Schwyzer, Erden and Siegwart[1] reported that aldoses (IIa) and ketoses (IIb) may be condensed with 2,4,5-triamino-6-hydroxypyrimidine (I) to form 7- and/or 6-polyhydroxyalkylpterines (IV). These authors did not clearly indicate which of the isomers was obtained in any case, but they did appear to accept the hypothesis that aldoses formed 7-isomers and ketoses formed 6-isomers. The fact that D-glucose forms a 7-isomer with I was confirmed by Petering and Weisblat,[2] but neither the latter authors nor Karrer, *et al.*,[1] offered proof which could gainsay the claim of Weygand, *et al.*,[3] that the product of D-fructose (IIb) and (I) is 2-amino-4-hydroxy-7-(2′, 3′, 4′)-trihydroxybutylpterine (V) and not 2-amino-4-hydroxy-7-tetrahydroxybutyl-(D-arabo)-pterine. Thus the method of Karrer, *et al.*,[1] and that of Petering and Weisblat[2] yields 7-isomers rather than the 6-isomer, and the nature of the condensation product of D-glucose or D-fructose and I is still in doubt.

Forrest and Walker[4] have reported the synthesis of 2-amino-4-hydroxy-6-tetrahydroxybutyl-(D-arabo)-pterine from D-glucose or D-fructose and I in the presence of hydrazine (III) and boric acid by the application of the method of Ohle and Hielscher[5] for the preparation of 2-tetrahydroxybutylquinoxaline. We also have studied this method and wish to present our findings which extend the reports of Forrest and Walker in a number of ways and which are in disagreement with the latter in some respects. The data presented here depend on the fact that the authors have found that the absorption spectra of 6- and 7-alkyl-

(1) Karrer, Schwyzer, Erden and Siegwart, *Helv. Chim. Acta*, **30**, 1031 (1947).

(2) Petering and Weisblat, THIS JOURNAL, **69**, 2566 (1947).

(3) Weygand, Wacker, and Schmied-Kowarzik, *Experientia*, **IV**, 427 (1948).

(4) Forrest and Walker, *Nature*, **161**, 308 (1948); *J. Chem. Soc.*, 79 (1949).

(5) Ohle and Hielscher, *Ber.*, **74**, 13 (1941).