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.

YONKERS, N. Y. RECEIVED JULY 9, 1949

[CONTRIBUTION FROM THE RESEARCH LABORATORIES, THE UPJOHN CO.]

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-

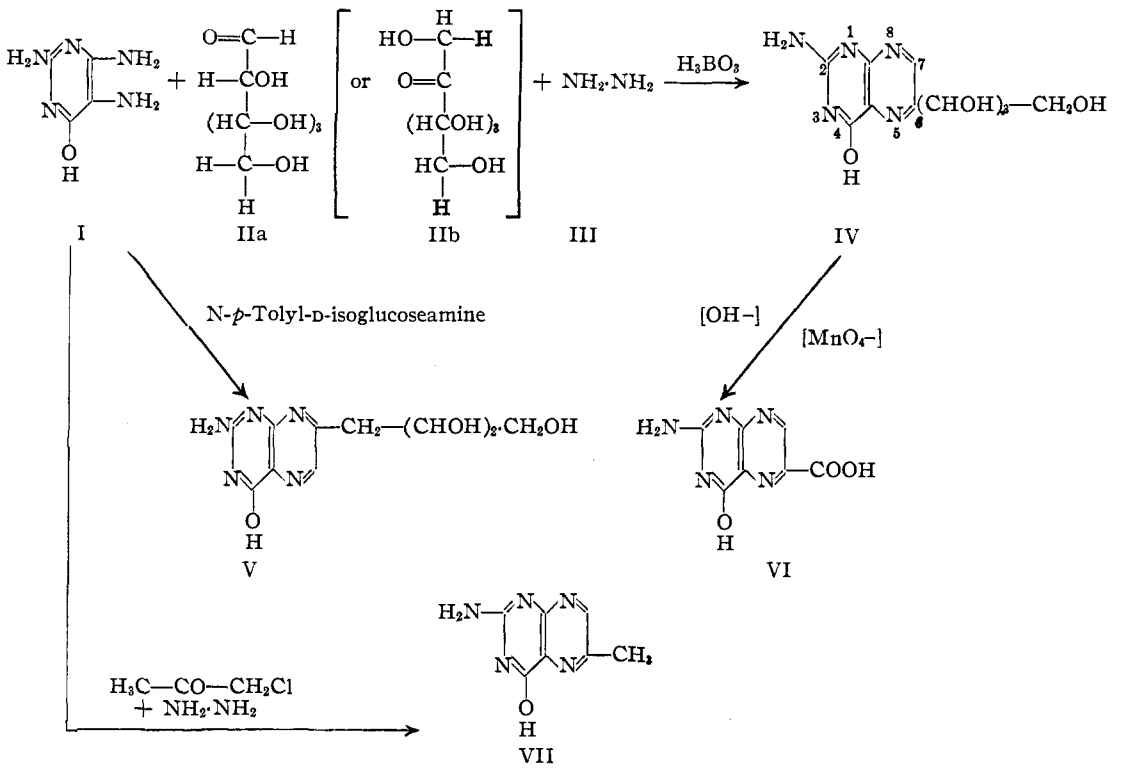
(1) Karrer, Schwyzer, Erden and Siegwart, *Helv. Chim. Acta*, **30**, 1031 (1947).

(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).



pterines differ both qualitatively and quantitatively to such a marked extent that crude isomer mixtures can be directly analyzed, and the conditions governing the course of the synthesis can be readily determined. Some of the conditions necessary for the preparation of the 6-isomer in high purity have been defined.

Experimental

Materials.—2,4,5-Triamino-6-hydroxypyrimidine (I) was prepared according to the method of Traube[6] with the modification that the reduction of 2,4-diamino-5-nitroso-6-hydroxypyrimidine was carried out with zinc in hydrochloric acid solution, with the result that I-dihydrochloride was obtained. I-hydrosulfate was prepared from a solution of I or I-dihydrochloride by the addition of dilute sulfuric acid. Hydrazine hydrate (85%) was obtained from Eastman Kodak Co. and used without further purification. D-Glucose was used as the commercial hydrate Cerelose. D-Fructose was obtained from Eastman Kodak Co. L-Sorbose was prepared by the oxidation of sorbitol with *Acetobacter suboxydans* according to the method of Wells, *et al.*[7]

Ultraviolet Absorption Spectra.—Ultraviolet spectra were obtained with a Beckman spectrophotometer. Solvents were 0.1 *N* sodium hydroxide, and 0.08 *N* hydrochloric acid. The latter solvent was chosen because it was found easier to dissolve the materials in acidic media if the 0.1 *N* sodium hydroxide solution was diluted 1:10 with 0.1 *N* hydrochloric acid than to make a solution directly in 0.1 *N* hydrochloric acid. The ratio of the maxima was used as an intrinsic property of the molecule and in the case of crude products it was valuable as an analytical tool regardless of the content of inorganic or other non-absorbing impurities. It was found to be characteristic of the 7- or 6-isomer. *E* 252 mμ/*E* 360 mμ for 7-polyhydroxyalkylpterines in 0.1 *N* sodium hydroxide has been found to be 2.2, and *E* 255 mμ/*E* 365 mμ for 6-polyhydroxyalkylpterines in the same solvent has been found to be 3.2.

In studying the condensation of sugars with I in the presence of an oxidizing agent such as hydrazine three general types of conditions were suggested, based primarily on the observations of Ohle and Hielscher[5]: namely, (a) the condensation in the presence of boric acid, (b) the condensation in the absence of boric acid, and (c) the condensation in the presence of oxidizing agents other than hydrazine. The results with other oxidizing agents—*i. e.*, (c)—can be summarized very briefly in the statement that no good substitute was found, although hydrogen peroxide, benzoyl peroxide, hydroxylamine, methylhydrazine, phenylhydrazine and semicarbazide were tried. Pterines were only obtained with hydroxylamine and the hydrazine derivatives, but the yields were no better than if no oxidizing agent had been used. Hydroxylamine seemed to be useful in directing the synthesis to the 6-form, but it did not affect the

(6) Traube, *Ber.*, **33**, 1371 (1900).

(7) Wells, Stubbs, Lockwood and Roe, *Ind. Eng. Chem.*, **29**, 1385 (1937); **31**, 1518 (1939).

yield over the condensation without any oxidizing agent.

Condensation of 2,4,5-Triamino-6-hydroxypyrimidine with Hexoses in the Presence of Boric Acid and Hydrazine.—Since hydrazine is a reducing agent in alkaline solution, there seemed no reason to study its action above *p*H 7. Therefore the *p*H range was kept at 4.5–7.0, since it was found that strongly acidic solution caused the formation of unreactive salts of I. Three moles of boric acid were always used for one mole of hexose and two moles for one mole of pentose. Its role was not studied in any detail when it was found that it could be eliminated. The volume and type of solvent, the concentration of reactants, and the molar ratio of sugar and hydrazine to pyrimidine were studied. After the identity of the products was proved by careful analysis and alkaline permanganate degradation studies the absorption spectra of the polyhydroxyalkylpterines themselves were relied upon to determine the purity of the products and the isomer composition of the mixture.

It was found that maximum yield was only obtained at *p*H less than 7.0. The reaction at *p*H 5.0 was complete in less than two hours, and was adversely affected by dilution. Using a molar ratio of pyrimidine:sugar:hydrazine of 1:1:1 the yield of product with D-glucose was 38% and with D-fructose and D-sorbose 55–60%. The aqueous ethanol condensation method improved the purity of the 6-isomer obtained from D-glucose, but did not affect the yield of product in the case of any of the hexoses used.

Condensation of 2,4,5-Triaminopyrimidine with Hexoses in the Absence of Boric Acid and Presence of Hydrazine.—The same conditions of study as described above apply to this phase of the work. Figure 1 shows graphically the relationship of the molar ratio of sugar × hydrazine/(pyrimidine)^2 *vs.* yield for D-glucose and L-sorbose. In most cases the reactants were mixed in water at a definite *p*H and then heated at 95°. However in the case where the solvent was aqueous alcohol, the procedure used was to dissolve the reactant in the boiling alcoholic solution and then to carry out the reaction by distillation of the solvent at atmospheric pressure until the volume had been brought to a very small amount, at which time a voluminous precipitate appeared. Since this procedure did not increase the yield, but in the case of the hexose aldoses it did increase the purity of the 6-isomer obtained, it is thought that the condensation reaction was slowed up to the point where the aldose could be converted into a ketose or some common intermediate rapidly enough to prevent the formation of appreciable 7-isomer. This seems to be a logical basis for explaining why ketoses were not appreciably affected by this latter method. Something like an Amadori rearrangement appeared to change the aldose to a ketose.

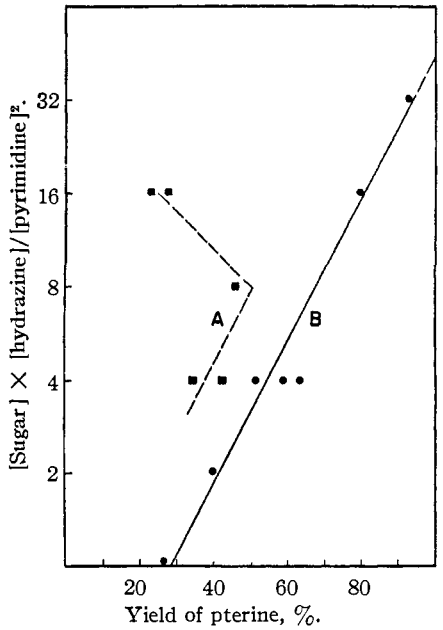


Fig. 1.—Relationship of reactants to yield in reaction without boric acid, time forty-five minutes, temp. 95°: (A), D(+)glucose; (B), L(−)sorbose.

D-Fructose was found to behave in the same manner as L-sorbose, and D-galactose similar to D-glucose in respect to the effect of the conditions described on yield and purity of 6-isomer. Pentose aldoses yielded mixtures of 6- and 7-isomer by the methods described.

The data shown in Fig. 1 were obtained at *p*H 4.5–5.5 and at a reaction time of forty-five minutes. It was found that at 95° the reaction without boric acid required thirty to forty-five minutes for completion. Analytical data together with specific illustration of the methods of preparation and purification of the compounds are given below.

2-Amino-4-hydroxy-6-tetrahydroxybutyl-(D-arabo)-pterine.—2.13 grams of I-dihydrochloride, 1.7 g. of sodium bicarbonate and 4.0 g. of D-glucose monohydrate were mixed with 17 ml. of water; 2.5 ml. of glacial acetic acid and 1.3 ml. of hydrazine hydrate (85%) in 8 ml. of water were added to this solution. The solution in a small erlenmeyer flask was allowed to react on a steam-bath at 90–95° for forty-five minutes. (After twelve minutes of heating a precipitate began to form.) The mixture was cooled and centrifuged, and the precipitate was washed with cold water, hot ethanol and ether (*p*H of mother liquor, 4.9); yield was 1.17 g. Ultraviolet ratio of 0.1 *N* sodium hydroxide solution was 2.99 indicating a product of high 6-isomer content.

One gram of this product was added to 100 ml. of water at 90° and enough ammonia was added dropwise to effect solution; 0.20 g. of Darco G60 was added and the mixture heated fifteen minutes, after which the charcoal was removed by filtration through filter-aid. The filtrate was brought to *p*H 5.0 with acetic acid and cooled to 15°. The precipitate was collected and washed with hot ethanol and ether. The product was dried *in vacuo;* yield 880 mg. *Anal.* Calcd. for $C_{10}H_{13}N_5O_5$: C, 42.4; H, 4.59; N, 24.75. Found: C, 41.2; H, 4.86; N, 24.39. $[\alpha]_D$ −97.26 (0.323% in 0.1 *N* NaOH; *E* 255 mμ/*E* 365 mμ (0.1 *N* NaOH), 2.9.

The ratios of these preparations varied from 2.9 to 3.1 and $E^{g/l.}_{1 cm.}$ (0.1 *N* NaOH) for similar preparations varied from 71.1–76.7 at 254 mμ and 24.3–24.7 at 365 mμ.

2-Amino-4-hydroxy-6-tetrahydroxybutyl-(L-xylo)-pterine.—2.13 grams of I-dihydrochloride, 1.7 g. of sodium bicarbonate and 3.6 g. of L-sorbose were dissolved in 25 ml. of water. To this solution 2.5 ml. of acetic acid and 1.3 ml. of hydrazine hydrate (85%) were added. The mixture was heated on the steam-bath at 90° for forty-five minutes, after which it was cooled and the precipitate was collected. It was washed well with water and hot ethanol and dried; yield 1.78 g.; ultraviolet ratio in 0.1 *N* sodium hydroxide was 3.0.

One gram of this product was extracted in a Bailey–Walker extraction unit with 110 ml. of water for three and one-half hours. The extract was made alkaline with ammonia to *p*H 11 and the resulting solution was decolorized with 0.2 g. of Darco G60.

The filtrate was brought to *p*H 5.0 with acetic acid and cooled. The precipitate was collected and washed with hot ethanol and ether. The precipitate was dried *in vacuo;* yield 640 mg.; ultraviolet ratio in 0.1 *N* sodium hydroxide = 3.0. *Anal.* Calcd. for $C_{10}H_{13}N_5O_5$: C, 42.4; H, 4.59; N, 24.75. Found: C, 41.3; H, 5.10; N, 23.08. $[\alpha]_D$, −69.0 (0.393% in 0.1 *N* sodsum hydroxide; *E* 354 mμ/*E* 365 mμ (0.1 *N* sodium hydroxide), 3.0. In general this ratio has been found to vary from 2.9–3.2 and the $E^{g/l.}_{1 cm.}$ in a manner similar to those found for 6-tetrahydroxybutyl-(D-arabo)-pterine preparations.

Oxidation with Alkaline Permanganate

To indicate the basis of the analysis of the ultraviolet absorption spectra for isomer composition the following data obtained by oxidative degradation to the corresponding 6-(or 7)- carboxypterine are presented.

a. 6.1 mg. of 2-amino-4-hydroxy-7-tetrahydroxybutyl-(D-arabo)-pterine was dissolved in 50.0 ml. of 0.1 *N* sodium hydroxide and the solution heated to 70°. A concentrated solution of barium permanganate was added dropwise until a permanent purple color remained after one hour of heating at 70°. The solution was cooled and to it formaldehyde was added dropwise to discharge the purple color. The mixture was filtered through filter-aid and the filter-cake was washed with a small amount of 0.1 *N* sodium hydroxide. The volume was made to 60.0 ml. and the ultraviolet absorption spectrum of a 1:10 dilution was analyzed. The original compound had an ultraviolet ratio of 2.4.

b. In a similar way 6.05 mg. of a preparation with ultraviolet ratio of 3.2 was oxidized and the ultraviolet absorption spectrum of the oxidized product analyzed.

The ultraviolet curves for the carboxypterines described above are given in Fig. 3, and show the variations in the spectra between the 7-isomer and the 6-isomer. These curves check very well with those reported for 7-carboxypterine and 6-carboxypterine by Mowat, *et al.*,[8] and Wittle, *et al.*[9] The oxidation product of a mixture of 6- and 7-tetrahydroxybutylpterines gives ultraviolet curves intermediate between a and b.

2-Amino-4-hydroxy-6-methylpterine.—Two and four-tenths grams of I-hydrosulfate and 1.06 g. of sodium carbonate were mixed in 25 ml. of water. To this solution were added 1.0 ml. of glacial acetic acid and 1.2 ml. of hydrazine hydrate (85%). The mixture was cooled in an ice-bath. To this cool mixture was added 2.0 g. of monochloroacetone in 5 ml. of alcohol. The mixture was kept cool (25°) during the ensuing reaction, and the *p*H was kept at 6.0 with small additions of solid sodium bicarbonate. A rapid reaction continued and the mixture was kept cool until a red-colored precipitate began to form. It was then heated on a steam-bath for forty-five minutes and cooled. An equal volume of ethanol was added and the mixture centrifuged. The precipitate was washed well with methanol and finally dissolved in 45 ml. of 10% ammonia water. The solution was warmed to 75° and the dark residue was removed by centrifugation. The solution was decolorized with 0.2 g. of Darco G60, and the filtrate brought to *p*H 5.0 with acetic acid. The cooled mixture was centrifuged and the precipitate washed with warm methanol. It was dried with acetone and ether and *in vacuo;* yield 700 mg.

Anal. Calcd. for $C_7H_7N_5O$: N, 39.6. Found: N, 39.3. *E* 252 mμ/*E* 360 mμ (0.1 *N* sodium hydroxide), 3.2.

Discussion

In view of the fact that the $[\alpha]_D$ of the pterine obtained by the condensation of I and D-glucose in the presence of hydrazine is identical with that obtained by the condensation of I and D-glucosone,[6] there seems little doubt that the side-chain is 1′,2′,3′,4′-tetrahydroxybutyl. This means that hydrazine must act as an oxidizing agent and there is no chance for the formation of CH_2 at C_1'.

It has been found that the reaction of I and IIa or IIb in the presence of hydrazine does not require boric acid as an assisting agent. The data obtained also suggest that the reaction requires equimolar quantities of the sugar and hydrazine, and that the action of hydrazine is more rapid and complete with ketoses than with aldoses. The fact that the reaction can be made to go to the 6-isomers with aldoses indicates a possible Amadori rearrangement. In fact it has been found that D-xylose if first treated with aniline or toluidine to give D-arylaminoxyloketose the reaction proceeds smoothly to a 6-isomer, whereas D-xylose or any other pentose yields only a mixture of isomers by any of the methods which were satisfactory for D-glucose. Similarly the preparation of 2-amino-4-hydroxy-6-methylpterine indicates that simple 6-alkylpterines may be readily prepared by the condensation of a suitable ketone and pyrimidine in the presence of hydrazine.

If the data for the aldoses and ketoses are analyzed in such a way that the expression log [sugar × hydrazine/(pyrimidine)^2] is plotted against % yield, the striking difference in behavior of the ketoses and aldoses is shown (Fig. 1).

Increasing either sugar or hydrazine increases the percentage yield based on the pyrimidine for L-sorbose or D-fructose, but for D-glucose this is not true. In the latter case an excess of either component beyond the value where sugar × hydrazine/(pyrimidine)^2 is greater than 8 results in a lowering of the yield.

The conditions for directing the synthesis to the 6-isomer for ketose are broad. In general the *p*H should be kept at 4–7, and the temperature above 90° for rapid reaction. Small volume of aqueous medium is desirable, for it facilitates isolation since the 6-isomer is more soluble than the 7-isomer. Boric acid is of no value.

The conditions for the synthesis of 6-isomer from I and aldose hexoses in the presence of hydrazine include low volume of aqueous medium—*i. e.*, high concentration of reactants—and a slight excess of hydrazine and/or sugar; the molar ra-

(8) Mowat, Boothe, Hutchings, Stokstad, Waller, Angier, Semb and SubbaRow, THIS JOURNAL, **70**, 16 (1948).

(9) Wittle, O'Dell, Vandenbelt and Pfiffner, *ibid.*, **69**, 1786 (1947).

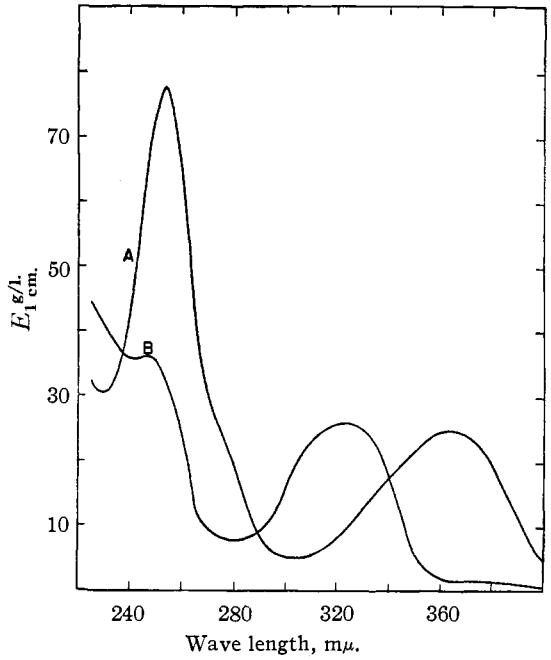


Fig. 2.—Ultraviolet absorption spectra of 2-amino-4-hydroxy-6-tetrahydroxybutyl-(D-arabo)-pterine: (A) in 0.1 *N* sodium hydroxide; (B) in 0.08 *N* hydrochloric acid.

tio of sugar × hydrazine/(pyrimidine)[2] should not exceed 8. The *p*H should be kept at 4–7 as in the case of ketoses. An alternate method of preparing 6-isomers of high purity involves the use of aqueous ethanolic medium, the reaction being carried out during the distillation of the solvent at atmospheric pressure.

Under any condition it seems that hydrazine acts as an oxidizing agent with the probable formation of ammonia in the reaction. We have found that all hexoses studied react normally, while all pentose aldoses do not. If, however, the pentoses are converted to ketoses, then the directed synthesis to 6-isomer proceeds smoothly.

The value of the absorption spectra of the polyhydroxyalkylpterines in elucidating the isomer problem can readily be seen from the data of Figs. 2 and 3. More details of the physical properties of pterines will be published later.

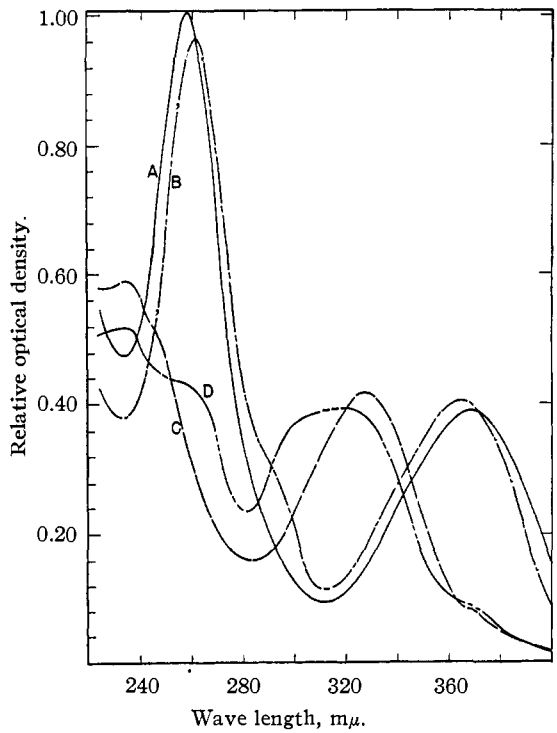


Fig. 3.—Ultraviolet absorption spectra of permanganate oxidation products from polyhydroxyalkylpterine: 2-amino-4-hydroxy-7-tetrahydroxybutylpterine (A) in 0.1 *N* sodium hydroxide, (C) in 0.08 *N* hydrochloric acid: 2-amino-4-hydroxy-6-tetrahydroxybutylpterine, (B) in 0.1 *N* sodium hydroxide and (D) in 0.08 *N* hydrochloric acid.

Summary

Methods for the direct synthesis of 2-amino-4-hydroxy-6-tetrahydroxyalkylpterines from the corresponding aldo- and ketohexoses are described and discussed.

Evidence is presented to show that the ratio *E* 255 mμ/*E* 365 mμ in 0.1 *N* sodium hydroxide of 6-tetrahydroxybutylpterines is 3.2 and is characteristic of these compounds. This fact is used in studying conditions affecting the isomer composition of the products obtained.

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