TABLE I

QUATERNARY SALTS

Compound	M. p.,[a] °C.	Yield, %	Formula	Analyses,[b] % Calcd. C	Calcd. H	Found C	Found H
II[c]	146 dec.	65	$C_{10}H_{20}INO_4$	34.79	5.84	34.71	5.96
IV[d]	127–128 dec.	60	$C_9H_{16}INO_5 \cdot 0.5H_2O$	33.60	5.27	33.81	5.17
VI[e,f]	174–179 dec.	82	$C_7H_{14}INO$	32.95	5.53	32.60	5.41
VII[e]	182 dec.	67	$C_{13}H_{18}BrNO$	54.94	6.38	54.86	6.20
IX[d]	309–311 dec.	82	$C_7H_{16}INO$	32.70	6.27	32.74	6.15

[a] All melting points are corrected. [b] Analyses by Mrs. G. L. Sauvage. [c] Recrystallized from ethanol. [d] Recrystallized from methanol–ether. [e] Not recrystallized. [f] Howton[9] obtained a quaternary salt which analyzed for one molecule of methanol of crystallization, m. p. 187.6–188°.

liquid, b. p. 80–87° (5–6 mm.), $n^{21}D$ 1.4718.[16] The hydrochloride melted at 155–156°.[16]

Preparation of Quaternary Salts.—The tertiary amines prepared in the above experiments were converted into quaternary salts by allowing benzene solutions of the distilled amine and excesses of the alkyl halide to stand at room temperature for twenty-four hours, the quaternary salts precipitating as they formed. The salts prepared are listed in Table I.

(16) Mills, Parkin and Ward, *J. Chem. Soc.*, 2613 (1927), reported a b. p. of 105° (18 mm.) and a m. p. for the hydrochloride of 157–158°. Riegel and Reinhard, THIS JOURNAL, **48**, 1334 (1926), reported a b. p. of 116–118° (36 mm.).

Summary

A number of previously unreported 4-piperidonium and 4-hydroxypiperidinium salts have been prepared. They were found to exhibit mild curariform activity, and one was found to be effective when administered orally.

ROCHESTER 3, NEW YORK RECEIVED JULY 22, 1948

[CONTRIBUTION FROM THE WELLCOME RESEARCH LABORATORIES]

Isomeric Dihydroxanthopterins[1]

BY GEORGE H. HITCHINGS AND GERTRUDE B. ELION

Dihydroxanthopterin (2-amino-4,6-dihydroxydihydropteridine) has been prepared by three routes[2,3,4] involving the reduction of a pteridine. Purrmann[2] reduced xanthopterincarboxylic acid catalytically and found the resultant dihydro-acid to be readily decarboxylated to dihydroxanthopterin. Totter[3] was able to isolate the same compound from the reduction of leucopterin (2-amino-4,6,7-trihydroxypteridine) by sodium amalgam, and O'Dell, *et al.*,[4] obtained it directly by the catalytic reduction of xanthopterin (III). The latter were the first to assign a structure to the compound. They made the logical suggestion that the two hydrogen atoms might add across the 7,8 double bond to give 7,8-dihydroxanthopterin (V). The synthesis of (V) by an apparently definitive method, however, has led to a dihydroxanthopterin distinct from that formed by reductive methods. For convenience the compound produced by reductive methods will be called α-dihydroxanthopterin and the synthetic compound, β-dihydroxanthopterin.

The existence of two dihydroxanthopterins where a single substance would have been expected recalls the suggestion (not clearly established) that stable tautomers of certain dihydropyrazines may exist.[5] The establishment of this instance of isomerism has important implications in the field of pteridine chemistry; the resistance of the β-dihydroxanthopterin structure to oxidation may have some bearing on the role of oxidative reactions in the synthesis of folic acid.

β-Dihydroxanthopterin was prepared in these laboratories several years ago as a possible intermediate in the synthesis of xanthopterin, which at that time was believed to have a number of important biological functions.[6,7,8] This compound is, indeed, convertible to xanthopterin but in poor yield (20%) and only under relatively drastic conditions (concentrated sulfuric acid at 210° for fifteen minutes). The α-isomer, on the other hand, can be converted to xanthopterin by a wide range of oxidative procedures, quantitatively by exposure to oxygen in alkaline solution with[2] or without platinum catalyst or by alkaline permanganate oxidation at room temperature.[9] Other properties of the two are compared in Table I. In addition to the observed differences in oxidizability the difference in stability to acid and alkali is noteworthy. The α-isomer is gradually decomposed by acid, giving unidentified colored

(1) Presented before the Division of Organic Chemistry at the New York meeting of the American Chemical Society, September 16, 1947.

(2) Purrmann, *Ann.*, **548**, 284 (1941).

(3) Totter, *J. Biol. Chem.*, **154**, 105 (1944).

(4) O'Dell, Vandenbelt, Bloom and Pfiffner, THIS JOURNAL, **69**, 250 (1947).

(5) D'Albe, translator, "Richter's Organic Chemistry," Vol. III, P. Blakiston's Son and Co., Philadelphia, 1923, p. 284.

(6) Tschesche and Wolf, *Z. physiol. Chem.*, **248**, 34 (1937).

(7) Totter and Day, *J. Biol. Chem.*, **147**, 257 (1943).

(8) Simmons and Norris, *J. Biol. Chem.*, **140**, 679 (1941).

(9) Elion, Light and Hitchings, forthcoming publication.

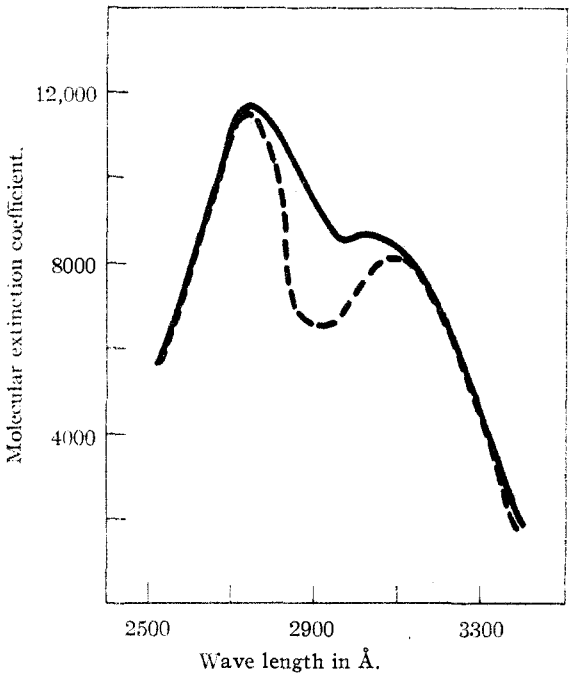


Fig. 1.—Ultraviolet absorption spectra of α-dihydroxanthopterin: —, at *p*H 1.0; ---, at *p*H 3.0.

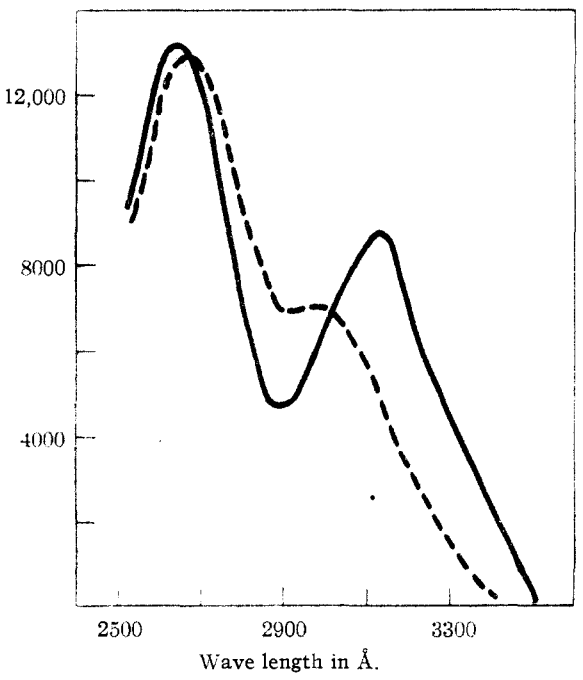


Fig. 2.—Ultraviolet absorption spectra of β-dihydroxanthopterin: —, at *p*H 1.0; ---, at *p*H 3.0.

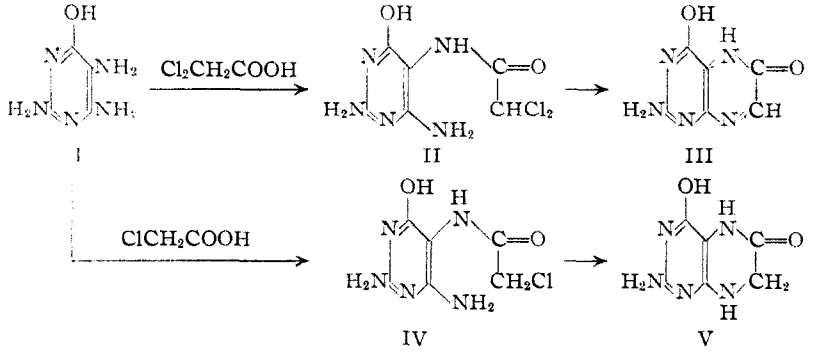
products, but is stable to alkalies. The β-isomer is stable to strong acid, but, as will be shown, the pyrazine ring is cleaved when the compound is warmed with aqueous barium hydroxide. The ultraviolet absorption spectra of the two isomers are similar but distinct (Figs. 1–4). The spectrum of the α-form at *p*H 3 resembles closely that of the β-isomer at *p*H 1, and that of the former at *p*H 1 is similar to that of the latter at *p*H 3 (Figs. 1 and 2). At *p*H 7 the two spectra are dissimilar but at *p*H 11 they are almost indistinguishable (Figs. 3 and 4). The spectra leave little doubt that the two are very closely related.

TABLE I

COMPARISON OF PROPERTIES OF DIHYDROXANTHOPTERINS

Reagent	α	β
Hot acetic acid	Decomposition	Recrystallization
O_2 (Pt catalyst)	To xanthopterin	Not oxidized
O_2 (air-alkali)	To xanthopterin	Not oxidized
Permanganate	2/5 mole → xanthopterin 4/5 mole → glycol (?)	2/5 mole → glycol(?)
Barium hydroxide	Barium salt	Ring fission
Ferric chloride	Red color	No color

The first synthesis of xanthopterin by Purrmann[10] was carried out by treatment of 2,4,5-triamino-6-hydroxypyrimidine (I) with dichloroacetic acid, followed by heating the silver salt of the dichloroacetamide (II) in the presence of silver carbonate to effect ring closure to xanthopterin (III). The synthesis of 7,8-dihydroxanthopterin, which is reported here, follows a similar route. The pyrimidine (I) is heated with monochloroacetic acid to give the chloroacetamide (IV) and ring closure follows a relatively mild treatment (aqueous bicarbonate at 95°) to give the dihydroxanthopterin (V). This synthesis is straightforward and the validity of the structures assigned to the intermediate (IV) and product (V) must be regarded as highly probable on *a priori* grounds. However, it is worth while to consider other possible reaction mechanisms and products. Such considerations have led to additional evidence confirming the structure of β-dihydroxanthopterin and incidentally to the discovery of a new heterocyclic, the oxazinopyrimidine, system.



(10) Purrmann, *Ann.*, **546**, 98 (1941).

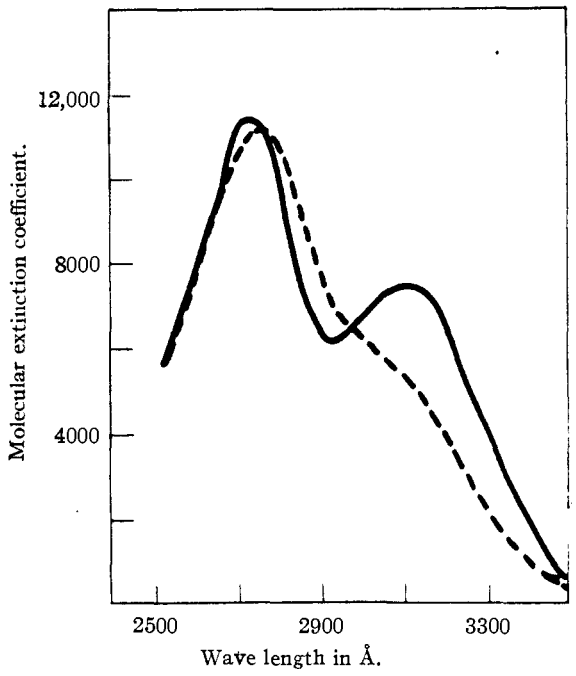


Fig. 3.—Ultraviolet absorption spectra of α-dihydroxanthopterin: —, at *p*H 7.0; ---, at *p*H 11.0.

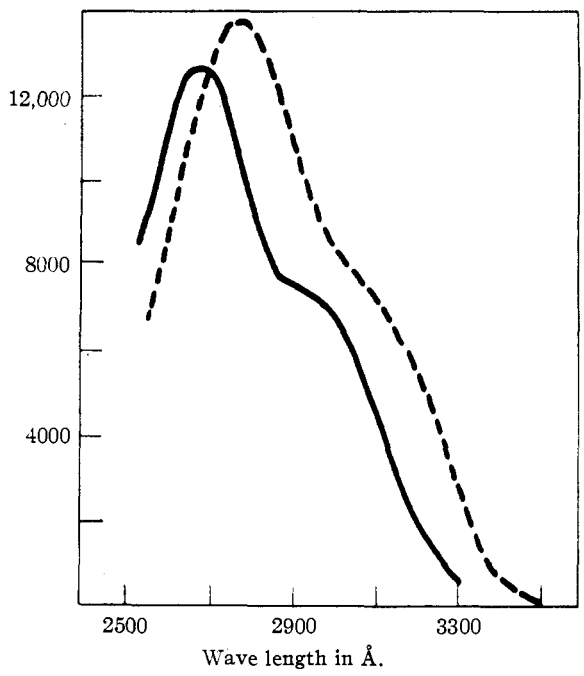


Fig. 4.—Ultraviolet absorption spectra of β-dihydroxanthopterin: —, at *p*H 7.0; ---, at *p*H 11.0.

The allocation of the chloroacetyl group of the intermediate (IV) to the 5′ position rests ultimately on the purine syntheses of Traube[11,12,13] who found 4,5-diaminopyrimidines to be acylated to monoacyl derivatives which could be converted to purines. By methylation of 1,3-dimethyl-2,6-dihydroxy-4 (or 5)-amino-5 (or 4)-formamidopyrimidine, Traube[12] obtained a trimethyl derivative from which caffeine was obtained on ring closure. On the basis of this evidence the formyl group was assigned to the 5′ position, since only the formamido group could provide the acidic hydrogen necessary for methylation. By analogy the other acylamino groups were believed to occupy the 5-position and the greater reactivity of the 5-amino group, in acylations, as compared with the 4 is a generally accepted principle of pyrimidine chemistry,[11,14] to which no exceptions have been published. To this evidence may now be added that afforded by spectrography. Using 2,4-diamino-5-formamido-6-hydroxypyrimidine and the parent triaminohydroxypyrimidine (I) as reference compounds, it is seen that acylation of the 5-amino group results in little change in the absorption spectrum at *p*H 1, but prevents the shift of absorption bands which characterizes the alkaline (*p*H 11) spectrum (Figs. 5 and 6). The chloroacetyl derivative (IV) resembles the formyl derivative so closely as to leave little doubt as to their similarity in structure (Fig. 7). Moreover, the relation of these spectra to those of the parent compounds shows a marked resemblance to that between 5-chloroacetamido-2-amino-4,6-dihydroxypyrimidine (Fig. 8) and 2,5-diamino-4,6-dihydroxypyrimidine (Fig. 9).

If the structure (IV) for the chloroacetamido derivative be granted, there remain to be considered the purine (VI) and the oxazine (VII) as possible products of the ring closure. The more remote possibility that one molecule of (IV) might alkylate another with subsequent hydrolysis and the formation of an hydantoin derivative[15] can be eliminated since such reactions could yield at most 50% of the theoretical amount of product whereas in practice a yield of 70% was obtained. The closure of the imidazole ring to give 8-hydroxymethylguanine (VI) would be expected to require much more drastic conditions than those employed.[13] Actually, attempts to form this compound by heating the sodium salt of either (IV) or the corresponding hydroxyacetyl derivative failed to give isolable amounts of this compound. However, (VI) would be expected to have an ultraviolet absorption spectrum nearly identical with that of 8-methylguanine. The dissimilarity of the spectra of the latter (Fig. 10) and β-dihydroxanthopterin (Figs. 2 and 4) is at once apparent.

The oxazine derivative (VII) can be eliminated from consideration in this connection by comparison of β-dihydroxanthopterin with derivatives of the oxazinopyrimidine structure. Compounds related to (VII) but lacking the 4-amino group (thereby excluding the pteridine structure) have been prepared from 4-methyl-5-chloroacetamido-

(11) Traube, *Ber.*, **33**, 1371 (1900).
(12) Traube, *ibid.*, **33**, 3035 (1900).
(13) Traube, *Ann.*, **432**, 266 (1923).
(14) Purrmann, *ibid.*, **548**, 284 (1941).
(15) Russell, Elion and Hitchings, forthcoming publication, p. 474.

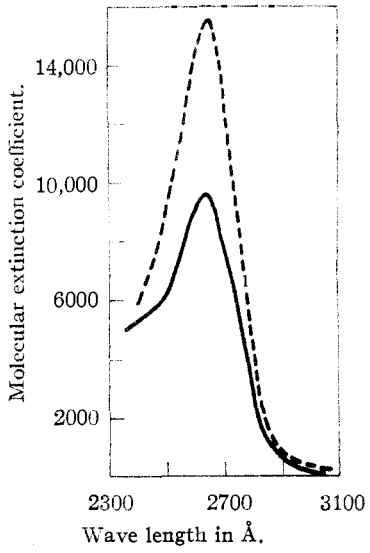


Fig. 5.—Ultraviolet absorption spectra of 2,4-diamino-5-formamido-6-hydroxypyrimidine: —, at *p*H 1.0; ---, at *p*H 11.0.

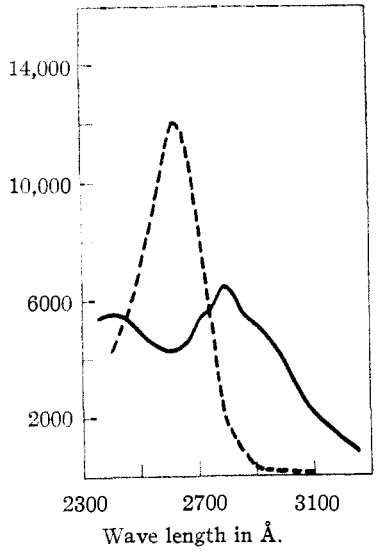


Fig. 6.—Ultraviolet absorption spectra of 2,4,5-triamino-6-hydroxypyrimidine: —, at *p*H 1.0; ---, at *p*H 11.0.

6-hydroxypyrimidines by heating with aqueous barium hydroxide.[15] There is, indeed, spectrographic evidence that the diamino derivative (VII) is formed under these conditions, though it has not yet been obtained in a pure state. Moreover, β-dihydroxanthopterin is unstable under the conditions of formation of the oxazine derivatives. The α-isomer gives only a barium salt, but the β-isomer adds the elements of water to give an acid (VIII) which can be esterified (IX). Both acid and ester revert to β-dihydroxanthopterin, the ester spontaneously in aqueous solution, the acid on warming with mineral acid. The ultraviolet absorption spectrum of the acid (VIII) is given in Fig. 11. It is consistent with that of a pyrimidine with a free amino group in the 5-position (*cf.* Compound 1 in Fig. 5). Moreover, the grouping $-NHCH_2CO_2H$ is indicated by the positive ninhydrin reaction obtained with the product of permanganate oxidation. The spectrum of (IX) is similar to that of the acid (VIII) at *p*H 1 but at *p*H 11 it is that of the β-dihydroxanthopterin indicating rapid and nearly quantitative ring closure.

The structure of β-dihydroxanthopterin appears to be established. No definitive evidence to decide the structure of the α-isomer is available. It is obviously possible to formulate the structure in a great many ways, but most of the formulations, such as (X), would be expected to be tautomeric with (V). Structures (XI) and (XII) represent types which are not necessarily obvious equivalents of (V) and for which some precedents exist. Each would be derived by hydrogenation of a C—N double bond of a xanthopterin of type (XIII) or (XIV). Biltz[16] has shown the existence among substituted uric acids of bond arrangements similar to those of (XI), (XII) and (XIII) while van Veen and Baars[17] have proposed a structure for toxoflavin with a bond arrangement simi-

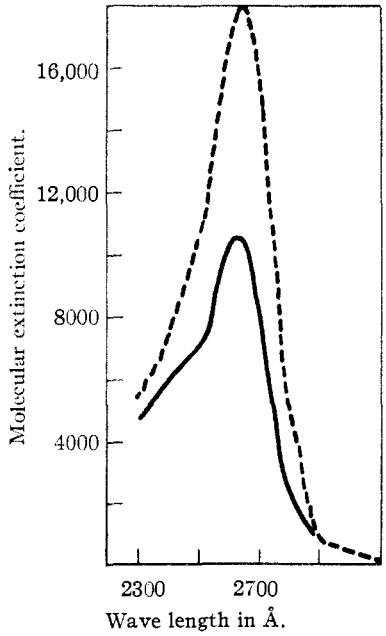


Fig. 7.—Ultraviolet absorption spectra of 2,4-diamino-5-chloroacetamido-6-hydroxypyrimidine: —, at *p*H 1.0; ---, at *p*H 11.0.

lar to that of (XIV). Obviously a considerable body of facts may be required to establish the fine structure of α-dihydroxanthopterin.

(16) Biltz, *J. prakt. Chem.*, **145**, 65 (1936).

(17) van Veen and Baars, *Rec. trav. chim.* **57**, 248 (1938).

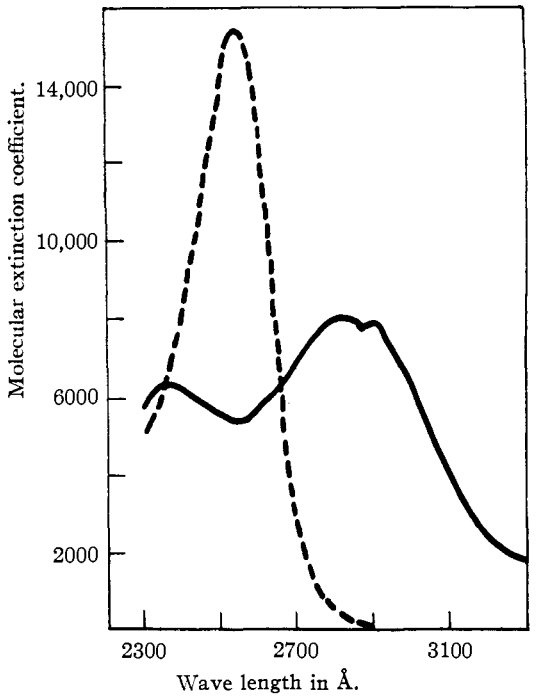


Fig. 8.—Ultraviolet absorption spectra of 2,5-diamino-4,6-dihydroxypyrimidine: —, at *p*H 1.0; ---, at *p*H 11.0.

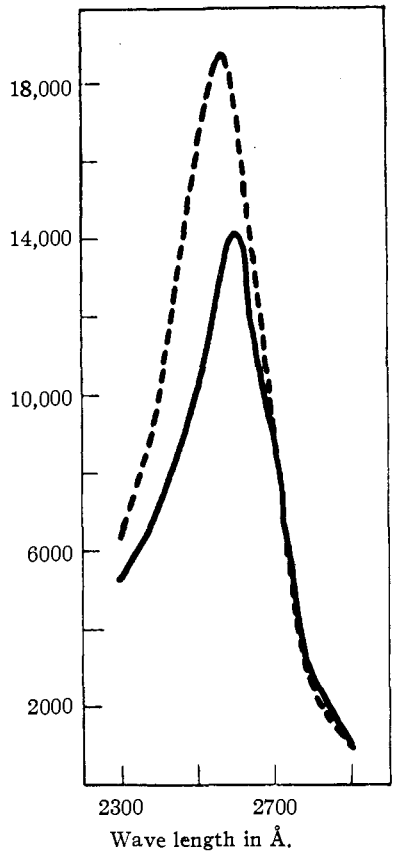
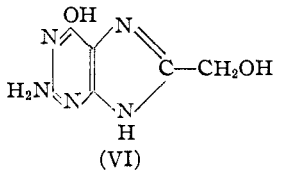
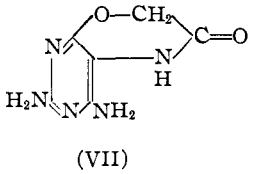
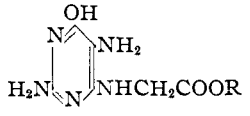
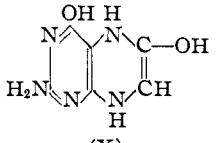


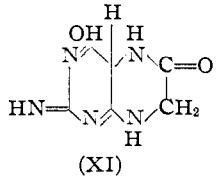
Fig. 9.—Ultraviolet absorption spectra of 5-chloroacetamido-2-amino-4,6-dihydroxypyrimidine: —, at *p*H 1.0; ---, at *p*H 11.0.

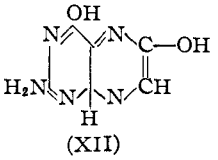

(VI)

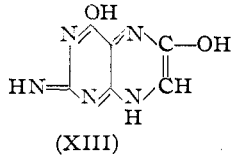

(VII)

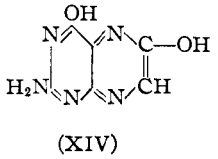

(VIII), R = H
(IX), R = CH_3


(X)


(XI)


(XII)


(XIII)


(XIV)

Experimental

2,4-Diamino-5-chloroacetamido-6-hydroxypyrimidine (IV).—A well-ground mixture of 8 g. of 2,4,5-triamino-6-hydroxypyrimidine[11] and 24 g. of monochloroacetic acid was heated in a boiling water-bath for one hour, with intermittent evacuation of the reaction flask. After cooling, the reaction mixture was leached several times with ether to remove the excess chloroacetic acid and then recrystallized from 300 ml. of 0.2 *M* sodium acetate–acetic acid buffer of *p*H 5. The precipitate after standing overnight at 5° consisted partly of pale yellow platelets and partly of amorphous material. It was washed with water and acetone and air dried. Yield was 12.7 g. (95%). A sample was recrystallized twice from dilute acetate buffer for analysis. The crystalline monohydrate loses its water of crystallization at 130° *in vacuo*, but regains it completely on exposure to air for twenty minutes. This rapid absorption of water from the air probably accounts for the low analytical value obtained for water of crystallization.

Anal. Calcd. for $C_6H_8O_2N_5Cl \cdot H_2O$: C, 30.42; H, 4.23; N, 29.6; H_2O, 7.6. Found: C, 30.64; H, 4.10; N, 29.5; H_2O, 5.7.

β-Dihydroxanthopterin (V).—A solution of 11.9 g. (0.05 mole) of 2,4-diamino-5-chloroacetamido-6-hydroxypyrimidine and 8.4 g. (0.1 mole) of sodium bicarbonate in 350 ml. of water was kept at 95° for two hours. A yellow precipitate formed gradually. After cooling, the product was filtered, washed with water, and dried at 100°. The yield was 6.2 g. (68.5%). It loses no water on drying at 150°. For analysis, a sample was recrystallized from glacial acetic acid.

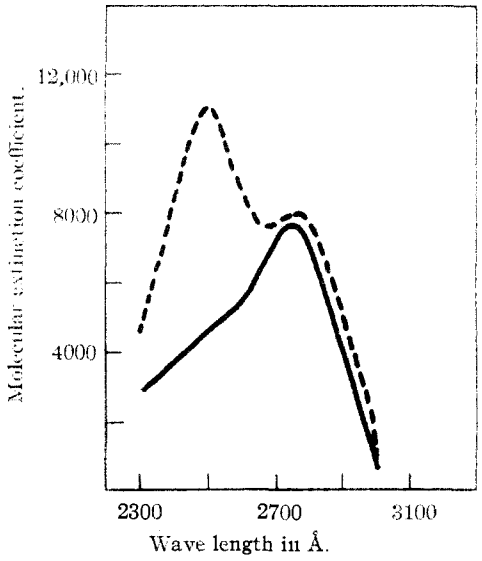


Fig. 10.—Ultraviolet absorption spectra of 8-methylguanine: —, at *p*H 1.0; --- at *p*H 11.0.

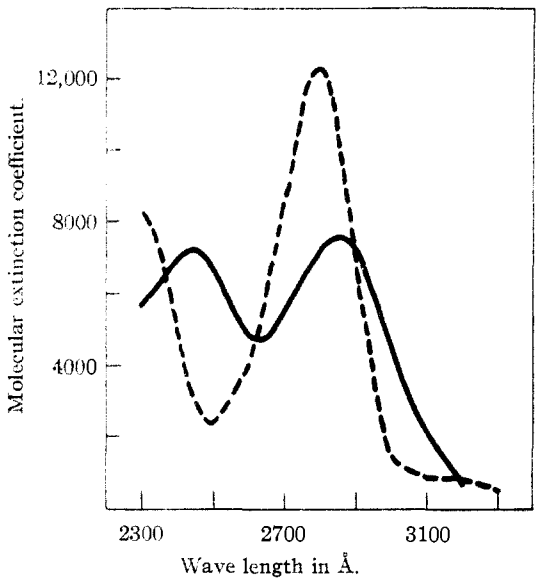


Fig. 11.—Ultraviolet absorption spectra of acid VIII (2,5 - diamino - 6 - hydroxy - 4 - carboxymethylaminopyrimidine): —, at *p*H 1.0; ---, at *p*H 11.0.

Anal. Calcd. for $C_6H_7O_2N_5$: C, 39.79; H, 3.87; N, 38.7. Found: C, 39.77; H, 4.17; N, 38.5.

The compound is soluble in about 5000 parts of boiling water, readily in warm 2.5 *N* hydrochloric acid, from which the monohydrochloride crystallizes on cooling as needles.

Anal. Calcd. for $C_6H_7O_2N_5 \cdot HCl$: Cl, 16.3. Found: Cl, 16.7.

The sulfate crystallized in colorless prisms from 3 *N* sulfuric acid as a tetrahydrate. Three moles of water were lost at 120°, leaving a monohydrate which does not lose the remaining mole of water at 150°.

Anal. $(C_6H_7O_2N_5)_2 \cdot H_2SO_4 \cdot 4H_2O$: Calcd. for 3 H_2O, 10.03. Found: 10.03 (after drying at 150° for two hours). Calcd. for $(C_6H_7O_2N_5)_2 \cdot H_2SO_4 \cdot H_2O$: C, 30.13; H, 3.76. Found: C, 30.44; H, 3.77.

The picrate crystallized in bundles of small, dark yellow needles when aqueous picric acid was added to a hot aqueous solution of β-dihydroxanthopterin. After recrystallization from 30% aqueous acetic acid containing 0.3% picric acid, the picrate sinters at about 185° and melts with decomposition at about 265°.

Oxidation of β-Dihydroxanthopterin.—Attempts to oxidize β-dihydroxanthopterin to xanthopterin catalytically in alkaline solution or in glacial acetic acid, as described by O'Dell, *et al.*, for the oxidation of α-dihydroxanthopterin,[4] led only to recovery of the starting material. With silver oxide considerable decomposition occurred.

Treatment of β-dihydroxanthopterin with neutral permanganate solution resulted in the consumption of two equivalents of permanganate to form a soluble compound with no absorption band in the ultraviolet. This product, which is presumably a glycol, gives a strong ninhydrin reaction after being boiled with alkali.

A solution of 250 mg. of β-dihydroxanthopterin in 5 ml. of concentrated sulfuric acid was heated at 210° for fifteen minutes. The mixture was allowed to cool and then poured over 25 g. of ice. An amorphous brown precipitate was centrifuged off. The supernatant liquid was neutralized to about *p*H 6 with sodium hydroxide solution and the amorphous orange precipitate removed at once by centrifugation. This precipitate, after purification, consisted of 50 mg. of xanthopterin, as shown by its ultraviolet absorption spectrum. The neutralized reaction mixture, on longer standing, deposited 40 mg. of unreacted β-dihydroxanthopterin also.

Alkaline Degradation of β-Dihydroxanthopterin to the Acid (VIII).—A solution of 0.4 g. (0.0022 mole) of β-dihydroxanthopterin in 15 ml. of hot water containing 0.63 g. (0.002 mole) of barium hydroxide octahydrate was heated in a boiling water-bath for six hours and allowed to stand at room temperature overnight. The reaction mixture was diluted to 50 ml. and treated with an excess of sodium sulfate to remove the barium ion. After removal of the barium sulfate the solution was acidified with acetic acid. The yellow crystalline precipitate which formed was filtered off, washed with water and alcohol and air dried (0.2 g.). A sample of this precipitate was purified for analysis by solution in very dilute sodium hydroxide, treatment with carbon and acidification of the hot solution with acetic acid. On cooling, bunches of small colorless needles were formed. The compound does not melt below 350°. It loses its water of crystallization at 120°.

Anal. Calcd. for $C_6H_9N_5O_3 \cdot 1.5H_2O$: C, 31.86; H, 5.32; N, 31.0; H_2O, 11.95. Found: C, 32.33; H, 6.06; N, 31.0; H_2O, 12.20.

When the degradation product (VIII) was boiled with 0.2 *N* hydrochloric acid for a few minutes and the solution neutralized with sodium acetate, the precipitate which formed was identified as β-dihydroxanthopterin by its absorption spectrum.

Esterification of the Acid (VIII).—To 12.5 ml. of absolute methanol containing 1 g. of hydrogen chloride was added 150 mg. of VIII. The solution was allowed to stand at room temperature overnight and then evaporated to dryness *in vacuo* below 40°. The solid yellow residue was dissolved in 3 ml. of absolute methanol and precipitated by the slow addition of 125 ml. of absolute ether. The yield of the ester dihydrochloride was 180 mg. (83%). The compound is converted to β-dihydroxanthopterin by heating to about 200°, by solution in alkali, or simply on standing in aqueous solution. Attempts to purify the ester further led in general to less pure material presumably because of the tendency to spontaneous ring closure.

Anal. Calcd. for $C_7H_{11}N_5O_3 \cdot 2HCl$: C, 29.38; H, 4.55; Cl, 24.8. Found: C, 29.95; H, 4.50; Cl, 24.0.

The ultraviolet absorption spectrum of the ester (IX) is almost identical with that of the acid (VIII) at *p*H 1 (Fig. 11). When an attempt was made to measure the spectrum of IX at *p*H 11, the spectrum was found to be identical with that of β-dihydroxanthopterin at *p*H 11 (Fig. 4). This ring closure was confirmed when the

alkaline solution was reacidified to *p*H 1 and the spectrum found to be identical with that of β-dihydroxanthopterin at *p*H 1 (Fig. 2).

α-Dihydroxanthopterin.—This isomer was prepared by the reduction of leucopterin by sodium amalgam[3] and by the decarboxylation of dihydroxanthopterincarboxylic acid.[2] The ultraviolet absorption spectra of the samples prepared in these two ways are identical and agree with the published spectrum of the product prepared by O'Dell, *et al.*,[4] by the catalytic reduction of xanthopterin (Figs. 1 and 3).

The sample prepared by the reduction of leucopterin was purified *via* the sodium salt as described by Elion, Light and Hitchings[9] and then recrystallized from 250 parts of hot water. The pale yellow elongated prisms which precipitated on cooling contained one mole of water after being dried at 100°. One half of the water of crystallization is lost at 130°, the other half at 150°. When the anhydrous compound is exposed to the air, it quickly regains one-half mole of water to form the stable hemihydrate.

Anal. Calcd. for $C_6H_7O_2N_5 \cdot H_2O$: C, 36.2; H, 4.52; H_2O, 9.05. Found: C, 36.44; H, 4.44; H_2O (dried at 130° for two hours), 4.52; H_2O (dried at 150° for two hours), 9.10.

When α-dihydroxanthopterin was boiled with glacial acetic acid, the solid material as well as the solution turned dark red. This red product no longer has the spectrum of α-dihydroxanthopterin and has not yet been identified. Its analysis is: C, 36.90; H, 3.42; N, 31.4. Calcd. for $C_7H_5N_5O_4$: C, 37.2; H, 3.54; N, 31.0.

An attempt to cleave α-dihydroxanthopterin by hot barium hydroxide solution led only to the precipitation of an insoluble barium salt which remained unchanged.

The sulfate monohydrate was prepared by the addition of 4 ml. of concentrated sulfuric acid to a solution of 0.5 g. of α-dihydroxanthopterin in 25 ml. of 2 *N* sulfuric acid. It was filtered, washed with acetone and air dried.

Anal. Calcd. for $(C_6H_7O_2N_5)_2 \cdot H_2SO_4 \cdot H_2O$: C, 30.13; H, 3.76. Found: C, 29.77; H, 3.73.

The picrate, formed on the addition of one volume of saturated aqueous picric acid solution to a hot aqueous solution of α-dihydroxanthopterin, crystallized as pinkish-orange needles and prisms. It turned brown at about 330° and did not melt at 370°.

Oxidation of α-Dihydroxanthopterin.—The spontaneous oxidation of this isomer to xanthopterin in alkaline solution as well as its catalytic oxidation in glacial acetic acid have been previously described.[2,4] It is also oxidized to xanthopterin by silver oxide[3] and by alkaline potassium permanganate.[9] The oxidation with permanganate proceeds beyond the xanthopterin stage if more than two equivalents are used, forming a soluble substance which may be a glycol since it no longer exhibits the ultraviolet absorption band characteristic of the pyrimidine ring structure. After alkaline solutions of this substance have been boiled, the resulting solutions give a strong ninhydrin reaction.

2,5-Diamino-4,6-dihydroxypyrimidine.—This compound was prepared by the method of Traube[18] except that the reduction of the 2-amino-5-nitroso-4,6-dihydroxypyrimidine was carried out more easily and in better yield by addition of an excess of sodium hydrosulfite to the ammoniacal suspension of the unisolated nitroso compound rather than by the use of hydrogen sulfide.

2-Amino-5-chloroacetamido-4,6-dihydroxypyrimidine.—A mixture of 5 g. of 2,5-diamino-4,6-dihydroxypyrimidine and 13 g. of chloroacetic anhydride was heated in a boiling water bath for one hour, cooled, leached several times with ether and filtered. The insoluble residue was then leached with 150 ml. of sodium acetate–acetic acid buffer of *p*H 5, filtered, washed with water and acetone and dried at 100°. The yield of this crude material was 6.7 g. (80%). A sample was recrystallized from 150 parts of hot water for analysis and dried at 100°.

Anal. Calcd. for $C_6H_7N_4O_3Cl \cdot H_2O$: C, 30.44; H, 3.8. Found: C, 30.85; H, 4.05.

8-Methylguanine.—This was prepared by the method of Traube[18] from 2,4-diamino-5-acetamido-6-hydroxypyrimidine.

Ultraviolet Absorption Spectra.—The spectra were measured with a Beckman spectrophotometer using solutions containing 10 mg. per liter. For solutions of *p*H 1, 0.1 *N* hydrochloric acid was used; for *p*H 3, 0.001 *N* hydrochloric acid; for *p*H 7, a Sörensen phosphate buffer; for *p*H 11, a glycine–sodium hydroxide buffer.

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Summary

The synthesis of 7,8-dihydroxanthopterin from 2,4 - diamino - 5 - chloroacetamido - 6 - hydroxypyrimidine is reported. This isomer, called β-dihydroxanthopterin, differs from the α-dihydroxanthopterin, which is formed by the reduction of pteridine derivatives, in several chemical properties. It is more resistant to oxidation and more stable to acid but more readily cleaved by alkali than the α-isomer. The significance of these observations in relation to the arrangement of the double bonds in pteridine derivatives is discussed.

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(18) Traube, *Ber.*, **26**, 2556 (1893).