ml. of dry ether. The rate of addition permitted slow refluxing. Stirring was continued for 15 minutes after the addition was complete. Water was then added with stirring until no more hydrogen was evolved. The solution was cooled to 0° and sulfuric acid (18.6 ml. concd. acid in 100 ml. of water) added. Stirring was continued for an additional hour and the mixture allowed to stand overnight. The ether was discarded and the aqueous layer was added gradually to a solution of 63.2 g. of sodium hydroxide in 200 ml. of water with stirring. The temperature was kept below 10°. The white precipitate was removed and washed with 200 ml. of water. The residue was refluxed with 100 ml. of alcohol and the insoluble material was removed by filtration. While the filtrate was refluxed, 200 ml. of water was slowly added. On cooling the resulting solution to 5°, the alcohol crystallized. The product was recrystallized by this same procedure, yield 90%, m.p. 126–130°. Further recrystallization did not alter the melting point.

Procedure G. 1-Phenyl-2-morpholino-3-methylamino-1-propanol.—α-Morpholino-β-(N-methylbenzylamino)-propiophenone, 13.28 g., in 150 ml. of alcohol was hydrogenated at 75° using a palladium-on-carbon catalyst. The reaction was complete in 1.5 hours. After removing the catalyst, the alcohol was removed by vacuum distillation leaving a glassy residue. The latter was dissolved in 250 ml. of hexane, filtered and the filtrate cooled to 0°. The product separated as white needles, m.p. 96–96.5°. Another recrystallization from hexane gave an 80% yield, m.p. 96.4–96.8°.

Hydrogenolysis of α,β-Dimorpholinoisobutyrophenone to α-Morpholinoisobutyrophenone.—The dimorpholino compound was hydrogenated in glacial acetic acid solution at 80° in the presence of a palladium–carbon catalyst. When hydrogenation was complete, the solution was diluted and filtered. The filtrate was added dropwise to a sodium hydroxide solution kept at 0–5°. The mixture was then extracted with ether. The ether solution was dried and the product precipitated as its hydrobromide. The latter was recrystallized from dry ethanol, yield 80%, m.p. 235–236°.

Anal. Calcd. for $C_{14}H_{19}NO_2 \cdot HBr$: C, 52.01; H, 6.10; N, 4.67. Found: C, 52.30; H, 6.41; N, 4.51.

A sample of the free base was made by adding a cold dilute solution of sodium hydroxide to an aqueous solution of the hydrobromide. The precipitated base after washing with water and drying in vacuum melted at 43.1–43.4°.

Preparation of 1-Phenyl-2-morpholino-2-methyl-1-propanol.—Twelve grams of crude α-morpholinoisobutyrophenone was dissolved in 60 ml. of dry ether and reduced with 1.45 g. of lithium aluminum hydride in 60 ml. of dry ether. Water was then added to decompose the excess reducing agent and the resulting solids dissolved with 8 ml. of sulfuric acid in 50 ml. of water. This solution was added gradually to a solution of 36 g. of sodium hydroxide in 150 ml. of water with stirring and cooling. The ether layer was removed and the aqueous layer again extracted with ether. After removing the ether, the residual oil was dried in a vacuum desiccator over sodium hydroxide. The oil was then dissolved in dry ether and the hydrochloride precipitated with dry hydrogen chloride. Recrystallization from dry alcohol gave a pure product, yield 50%, m.p. 231.3–231.8° dec. The free base was obtained by addition of dilute sodium hydroxide solution to an aqueous solution of the hydrochloride. It was recrystallized from 50% alcohol, m.p. 73.8–74.2°.

Anal. Calcd. for $C_{14}H_{21}NO_2$: C, 71.45; H, 9.00; N, 5.95. Found: C, 71.32; H, 8.95; N, 6.08.

Reaction of α-Bromo-β-(N-methylbenzylamino)-propiophenone Hydrobromide with Acetone.—Fourteen grams of the oil obtained from the bromination of β-(N-methylbenzylamino)-propiophenone hydrobromide was dissolved by shaking with 200 ml. of acetone. Just as the last of the oil dissolved, colorless crystals began to appear. After standing overnight, the mixture was cooled to 0° and filtered. The filtrate had strong lachrymatory properties. The crystals, after washing with acetone and drying, melted at 198–199° and no depression was noted when mixed with β-(N-methylbenzylamino)-propiophenone. Recovery was quantitative.

The possibility that the brominated β-(N-methylbenzylamino)-propiophenone was a perbromide was considered but was thought to be improbable for several reasons. The yields of the crude oil, 60–70%, were not high enough to warrant the assumption that more than one bromine atom was present. When the β-(N-methylbenzylamino)-propiophenone was treated with 1 mole of bromine, the latter was almost completely decolorized, large amounts of hydrogen bromide were evolved and the resulting bromo compound coupled normally with 2 moles of morpholine to give excellent yields of morpholine hydrobromide and α-morpholino-β-(N-methylbenzylamino)-propiophenone.

PHILADELPHIA 4, PENNSYLVANIA

[CONTRIBUTION FROM THE WELLCOME RESEARCH LABORATORIES]

The Identification of "β-Dihydroxanthopterin" as 2,4-Diamino-6-hydroxy-*p*-oxazino(2,3-d)pyrimidine

BY GERTRUDE B. ELION AND GEORGE H. HITCHINGS

RECEIVED FEBRUARY 4, 1952

The "β-dihydroxanthopterin," previously obtained by the cyclization of 5-chloroacetamido-2,4-diamino-6-hydroxypyrimidine, and assigned the 7,8-dihydroxanthopterin structure, has now been identified as 2,4-diamino-6-hydroxy-*p*-oxoazino(2,3-d)pyrimidine. Proof of structure rests on the identification of the hydrolytic cleavage product as 6-carboxymethoxy-2,4,5-triaminopyrimidine, rather than 2,5-diamino-6-hydroxypyrimidyl-4-aminoacetic acid as previously supposed. This identification follows from the reaction of the acid with glyoxal to give 2-amino-4-carboxymethoxypteridine, and synthesis of its ethyl ester from 2,4-diamino-6-chloropyrimidine *via* 5-*p*-chlorobenzeneazo-2,4-diamino-6-carbethoxymethoxypyrimidine. Cyclization of the 6-carbethoxymethoxy-2,4,5-triaminopyrimidine occurs spontaneously in alkaline solution to give a product identical with that obtained from the 5-chloroacetamidopyrimidine.

Several years ago the preparation was reported of a compound believed to be 7,8-dihydroxanthopterin (V) on the basis of its synthesis from 5-chloroacetamido-2,4-diamino-6-hydroxypyrimidine (VI).[1] This compound was designated as "β-dihydroxanthopterin" since it was found to differ in its physical and chemical properties from the "α-dihydroxanthopterin" obtained by the catalytic reduction of xanthopterin,[2] the sodium–amalgam reduction of leucopterin,[3,4] or the decarboxylation of dihydroxanthopterincarboxylic acid.[5] The outstanding chemical difference between the α- and β-isomers was the ease with which the α-isomer could be

(1) G. H. Hitchings and G. B. Elion, THIS JOURNAL, **71**, 467 (1949).

(2) B. L. O'Dell, J. M. Vandenbelt, E. S. Bloom and J. J. Pfiffner, *ibid.*, **69**, 250 (1947).

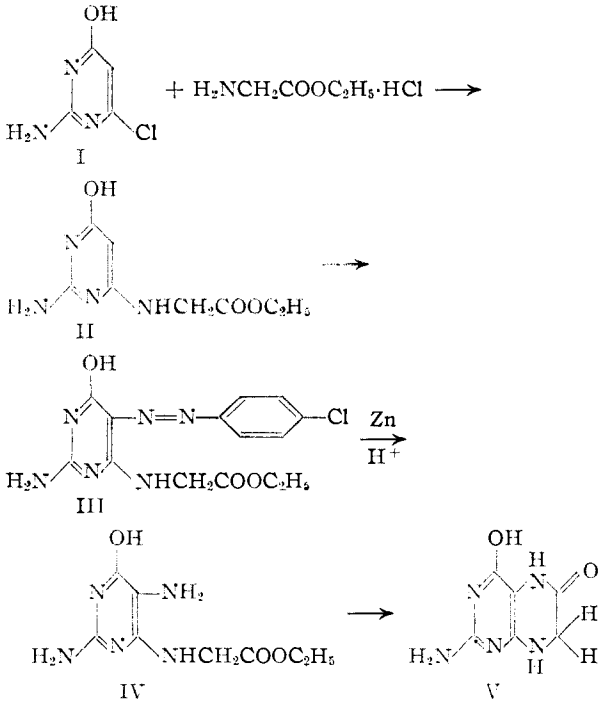
(3) J. R. Totter, *J. Biol. Chem.*, **154**, 105 (1944).

(4) G. B. Elion, A. E. Light and G. H. Hitchings, THIS JOURNAL, **71**, 741 (1949).

(5) R. Purrmann, *Ann.*, **548**, 284 (1941).

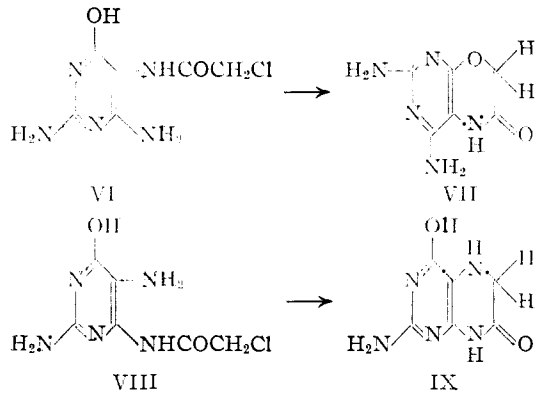
oxidized to xanthopterin as compared with the extreme resistance of the β-isomer to dehydrogenation. The synthesis of other 7,8-dihydropteridines by Boon, *et al.*,[6] and the ease with which these could be oxidized to the corresponding pteridines led to a reëxamination of the problem of the isomeric dihydroxanthopterins.

The first approach was the complete synthesis of 7,8-dihydroxanthopterin by an unequivocal method and the comparison of the product with the α- and β-isomers previously reported. This synthesis was accomplished by the series of reactions (I → V) shown below. While this work was in progress, Boon and Leigh[7] reported a similar synthesis. These

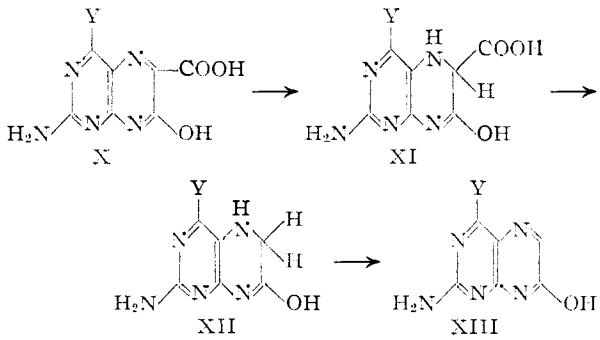


authors coupled I with benzenediazonium chloride before attempting reaction of the chloro compound with glycine ester, and this sequence of reactions is somewhat more productive than that outlined above. In agreement with the findings of Boon the product is identical with α-dihydroxanthopterin.

Elimination of this structure left two leading possibilities for the structure of "β-dihydroxanthopterin," dihydroisoxanthopterin (IX) and the diaminoöxazinopyrimidine (VII). Dihydroisoxanthopterin would be the expected product if the chloroacetamidopyrimidine were VIII rather than VI. This was previously considered to be unlikely[1] on the basis of its physical properties and earlier work on the acylation of 4,5-diaminopyrimidines in general.[8,9,10] However, there remained the more or less unlikely possibility that acyl migration had occurred during the cyclization in aqueous bicarbonate giving IX from VI *via* VIII. For this reason attempts were made to prepare dihydroisoxanthopterin and the reduction of several 7-hydroxypteridines was studied.



Since isoxanthopterin is not reduced readily,[2,5] indirect methods for the preparation of dihydroisoxanthopterin were tried. Isoxanthopterin-6-carboxylic acid (X, Y = OH) was known to lose carbon dioxide on heating to 260°[5] and it seemed possible that reduction of the acid followed by decarboxylation might produce dihydroisoxanthopterin. Reduction of isoxanthopterin carboxylic acid with sodium amalgam or with zinc and alkali resulted in a product which was already partially decarboxylated. On heating to 150°, *in vacuo*, isoxanthopterin was formed. This indicates that dihydroisoxanthopterin is very easily dehydrogenated to isoxanthopterin, and this reactivity is strikingly different from the resistance to oxidation of "β-dihydroxanthopterin." A further investigation was made of the decarboxylation of dihydro-7-hydroxypteridine-6-carboxylic acids using the 2,4-diamino analog (X, Y = NH_2). This pteridine resembles isoxanthopterin-6-carboxylic acid in its method of synthesis and its ultraviolet absorption spectrum[11] but does not lose carbon dioxide when heated at 260°. When X (Y = NH_2) is reduced with zinc and alkali or sodium amalgam, a dihydro compound (XI) (Y = NH_2) is formed which readily decarboxylates at 140°. The product of this decarboxylation appears to be a mixture of XII and XIII (Y =



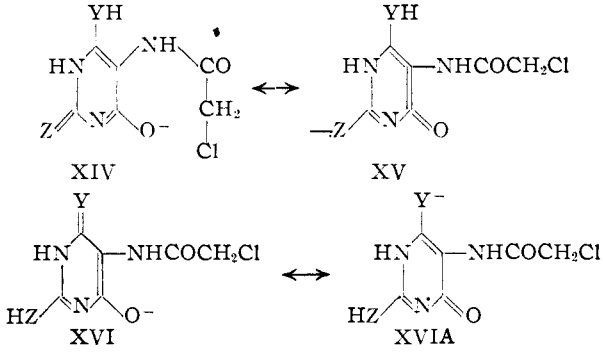
NH_2), which can be converted by alkaline permanganate to XIII (Y = NH_2). It is thus possible by using the 2,4-diamino analog of X, to isolate some of the intermediates on the way to XIII, whereas when Y = OH, the product XI is so unstable that

(6) W. R. Boon, W. G. M. Jones and G. R. Ramage, *J. Chem. Soc.*, 96 (1951).
(7) W. R. Boon and T. Leigh, *ibid.*, 1497 (1951).
(8) W. Traube, *Ber.*, **33**, 1371 (1900).
(9) W. Traube, *ibid.*, **33**, 3035 (1900).
(10) W. Traube, *Ann.*, **432**, 266 (1923).
(11) G. B. Elion, G. H. Hitchings and P. B. Russell, THIS JOURNAL, **72**, 78 (1950).

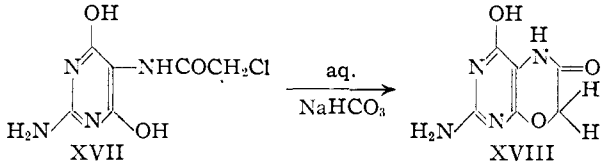
XII and XIII are formed spontaneously. It is interesting to note the effect which reduction and decarboxylation have on the ultraviolet absorption spectrum of X (Y = NH_2). The dihydro derivative XI (Y = NH_2) has a spectrum quite different from X (Y = NH_2), but decarboxylation of XI produces no further change in the spectrum. Thus XI, XII and XIII have essentially identical spectra and the progress of the reactions after the initial reduction to XI therefore cannot be followed spectrophotometrically. This is likewise true when isoxanthopterin-6-carboxylic acid (X, Y = OH) is reduced; the product has the same spectrum as isoxanthopterin (XIII, Y = OH) even though the decarboxylation is not complete. Since the presence or absence of the carboxylic acid grouping has no effect on the ultraviolet absorption spectrum in XI and XII, it may be presumed that it is no longer part of a conjugated system and that XI is a 5,6-dihydro derivative. It is difficult to explain, however, the lack of effect on the spectrum of the dehydrogenation of XII to XIII.

In view of the improbability of IX as the structure of "β-dihydroxanthopterin," the *p*-oxazino-(2,3-d)pyrimidine structure (VII) was reconsidered. The latter structure had been weighed but was discarded[1] when it was found that a number of 4-methyl-5-haloacetamido-6-hydroxypyrimidines did not cyclize in aqueous sodium bicarbonate but formed *p*-oxazino(2,3-d)pyrimidines when heated with aqueous barium hydroxide.[12] By contrast, VI had been closed to "β-dihydroxanthopterin" in bicarbonate solution, whereas heating with barium hydroxide resulted in a ring opening. The product of this ring opening had been postulated to be the carboxylic acid corresponding to the pyrimidine IV, since it could be esterified, appeared to have a 5-amino group, and both acid and ester cyclized spontaneously to reform "β-dihydroxanthopterin." However IV, prepared from III, closes to α-dihydroxanthopterin, and the possibility that the acid might be, in fact, an isomeric substance was once more considered.

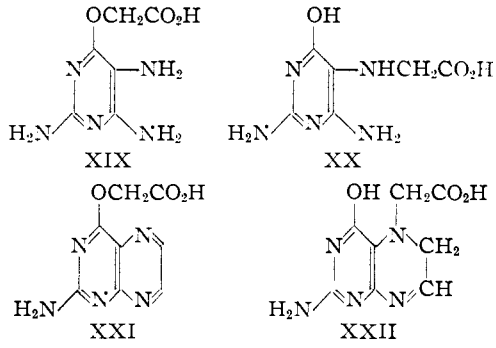
The structural requirements for ring closure and the effect of the quantity of barium hydroxide on the yield of the *p*-oxazino(2,3-d)pyrimidines had been studied for a number of 5-haloacetamido-6-hydroxypyrimidines and α-bromopropionamido-6-hydroxypyrimidines in which there was a methyl group at position 4.[12]



In such cases, ring closure was found possible only when there is a group on position-2 which permits resonance of the XIV ↔ XV type (*e.g.*, Z = NH, O, CH_2N; Y = CH_2). Such resonance increases the acid strength of the 6-hydroxyl group, thereby favoring ring closure. It follows that the presence of a group such as amino or hydroxyl at position 4 (Y = NH, O) would make possible the additional resonance forms XVI and XVIA and further increase the acid strength of the 6-hydroxyl group. As a result, when both Y and Z are amino or hydroxyl groups, the ring closure might require the presence of only a weak base like sodium bicarbonate. To corroborate this theory, the cyclization of 2-amino-5-chloroacetamido-4,6-dihydroxypyrimidine (XVII) with sodium bicarbonate was attempted, and was found to proceed quickly and smoothly to give XVIII, strikingly similar in its ultraviolet absorption spectrum and other properties to "β-dihydroxanthopterin." Attempts to cyclize 5-chloroacetamido-2,4,6-triaminopyrimidine in a similar way gave no isolable products.



Three structural formulas can be written for the pyrimidine which is formed on treatment of "β-dihydroxanthopterin" with barium hydroxide, IV, XIX and XX. Structure IV had been eliminated by synthesis and XX was considered improbable



because of the resistance to oxidation of the parent dihydropteridine. Although 5,6-dihydro-7-hydroxypteridines appear to undergo hydrolytic cleavage at the 7,8-position rather readily[13] they are easily oxidized. The formula XIX was rendered more probable by the observed reaction of the substance with glyoxal giving a pteridine, the analysis and properties of which correspond to those of a compound of formula XXI. The alternative formulation as the 5-carboxymethyl-5,6-dihydropteridine XXII[14] would differ in its analysis (H, 4.03

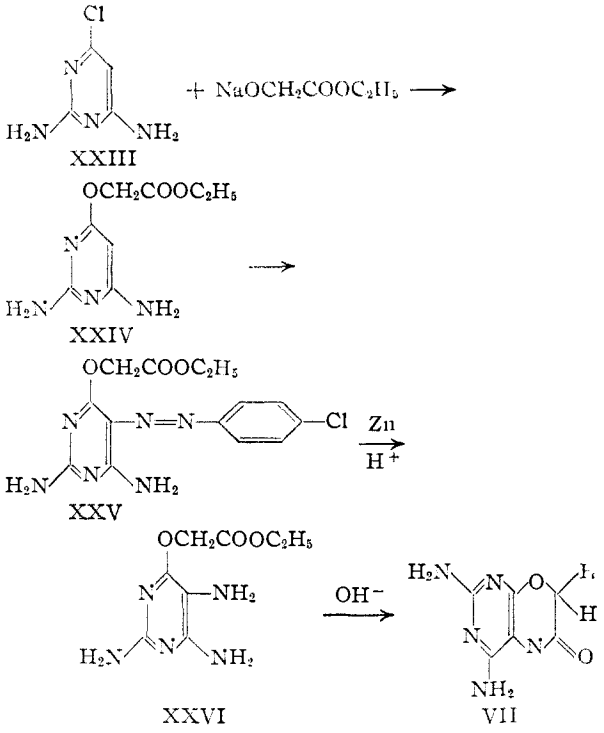
(12) P. B. Russell, G. B. Elion and G. H. Hitchings, *ibid.*, **71**, 474 (1949).

(13) This was suggested by observations during the sodium amalgam reduction of 2,4-diamino-7-hydroxypteridine-6-carboxylic acid (*cf.* Experimental). A more clear-cut demonstration of the ease of this hydrolysis with 5,6-dihydro-7-hydroxypteridine has been formulated by A. Albert, D. J. Brown and G. Cheeseman, *J. Chem. Soc.*, 1620 (1952).

(14) In view of the facility with which electronic transfer occurs during pteridine syntheses (*cf.* R. B. Angier, C. W. Waller, J. H. Boothe, J. H. Mowat, J. Semb, B. L. Hutchings, E. L. R. Stokstad and Y. SubbaRow, THIS JOURNAL, **70**, 3029 (1948)) the dihydro structure could not be eliminated *a priori*.

vs. 3.17). Moreover, the ultraviolet absorption spectrum of the product is nearly identical to that of 2-amino-4-hydroxypteridine. The formation of XXI, like that of 2-amino-4-hydroxypteridine, takes place in two stages. The intermediate has been isolated in the case of 2-amino-4-hydroxypteridine and found to contain two glyoxal residues per molecule of pyrimidine. In both cases cyclization to the pteridine occurred spontaneously in alkaline solution.

Final verification of the structure of VII was obtained by a complete synthesis from 6-chloro-2,4-diaminopyrimidine (XXIII) as follows



The reaction of XXIII with sodium ethyl glycolate does not proceed in good yield due to a side reaction involving self-condensation of XXIII. However, XXIV can be differentiated both from XXIII and its self-condensation product by its ultraviolet absorption spectrum and its extreme water solubility. It was not isolated but was coupled in its crude state; the azo compound XXV was then isolated and characterized. The reduction product XXVI behaves in all respects like the compound formed by the esterification of XIX, closing spontaneously in alkaline solution to VII.

Experimental

Synthesis of 7,8-Dihydroxanthopterin (X).—This procedure is given in outline only, since Boon's procedure[7] appears to be more productive.

A. **2-Amino-4-carboxymethylamino-6-hydroxypyrimidine** (II).—A mixture of 3 g. of 2-amino-4-chloro-6-hydroxypyrimidine[15] and 6 g. of ethyl glycinate hydrochloride was heated at 135° for one hour, then at 145° for 1.5 hours. The reaction mixture was extracted with alcohol, ether was added and the resultant precipitate was leached with cold acetone. The ultraviolet absorption spectrum of the insoluble material (1.2 g.) indicated the presence of two substances. When it was treated with 20 ml. of water at room temperature the extract appeared to contain a single substance identifiable as the carbethoxymethylaminopyrimidine by the similarity of its spectrum to that of 2,4-diamino-6-hydroxypyrimidine. The estimated yield was 600–700 mg.

Ultraviolet absorption spectrum of 2,4-diamino-6-hydroxypyrimidine: At *p*H 1, λ_{max} 265 mμ, E_m 20,000. At *p*H 11, λ_{max} 265 mμ, E_m 12,500.

Ultraviolet absorption spectrum of aqueous solution of II (dilution 1:2500): At *p*H 1, λ_{max} 268 mμ, O.D. 0.55. At *p*H 11, λ_{max} 268 mμ, O.D. 0.47.

Ultraviolet absorption spectrum of 2-amino-4-chloro-6-hydroxypyrimidine (I): At *p*H 1, λ_{max} 286 mμ. At *p*H 11, λ_{max} 275 mμ.

B. **7,8-Dihydroxanthopterin** (V).—The aqueous solution of the above crude 2-amino-4-carbethoxymethylamino-6-hydroxypyrimidine was treated with diazotized *p*-chloroaniline (0.35 g.) at *p*H 5 at 0° for one hour. The bright yellow azo compound was filtered off, washed with water and dried *in vacuo* (0.35 g.).

The above azo compound was dissolved in 50% boiling aqueous methanol, 750 mg. of zinc dust and 1 ml. of 6 *N* sulfuric acid were added and the solution was boiled for five minutes and filtered. On chilling 7,8-dihydroxanthopterin precipitated (100 mg.). The compound was purified by solution in 2 *N* sodium hydroxide and precipitation with acetic acid, and was crystallized as the sulfate[1] for analysis.

Anal. Calcd. for $C_6H_7N_5O_2 \cdot {}^1/_2H_2SO_4 \cdot {}^1/_2H_2O$: C, 30.1; H, 3.8. Found: C, 30.2; H, 3.7.

Ultraviolet absorption spectrum: At *p*H 1, λ_{max} 276, 305 mμ, E_m 11,700, 8700; λ_{min} 297 mμ. At *p*H 11, λ_{max} 276, 310 (inflection) mμ, E_m 11,300, 5300.

On standing, the aqueous filtrates from the reaction mixture and purification deposited xanthopterin, identified by its ultraviolet absorption spectrum.

Reduction of 2,4-Diamino-7-hydroxypteridine-6-carboxylic Acid. A. With Zinc and Alkali.—To a solution of 5 g. of 2,4-diamino-7-hydroxypteridine-6-carboxylic acid in 100 ml. of 2 *N* sodium hydroxide was added 10 g. of zinc dust. The mixture was shaken for twenty minutes at room temperature, then heated to 50° and filtered. The warm filtrate was acidified with 12 ml. of concentrated hydrochloric acid, cooled, and the pale yellow precipitate collected. It was purified by solution in 50 ml. of 1 *N* sodium hydroxide, filtration and acidification to *p*H 3 with hydrochloric acid. The precipitate was centrifuged off, washed with water, alcohol and ether and dried in a vacuum desiccator (3.8 g.).

Anal. Calcd. for $C_7H_8N_6O_3 \cdot H_2O$: C, 34.7; H, 4.1; N, 34.7. Found: C, 35.4; H, 4.1; N, 34.5.

Ultraviolet absorption spectrum: At *p*H 1, λ_{max} 298, 336 mμ, E_m 11,200, 12,700; λ_{min} 265, 310 mμ. At *p*H 11, λ_{max} 255, 342 mμ; E_m 11,800, 13,400; λ_{min} 250 295 mμ.

B. **With Sodium Amalgam.**—To a solution of 2.2 g. of 2,4-diamino-7-hydroxypteridine-6-carboxylic acid in 15 ml. of 0.67 *N* sodium hydroxide was added, in small portions, sodium amalgam prepared from 1.4 g. of sodium and 50 g. of mercury. The temperature was kept below 45° by occasional immersion in cold water. One hour after all the amalgam had been added, the mercury was filtered off, washed with 50 ml. of water and the alkaline filtrate acidified to *p*H 5 with acetic acid. The mixture was chilled for a short time and the pale yellow precipitate filtered off, washed with water and dried in a vacuum desiccator (1.75 g.). After purification by solution in 0.5 *N* sodium hydroxide and acidification with acetic acid, the product (1.5 g.) had an ultraviolet absorption identical with the product obtained above by reduction with zinc.

Anal. Calcd. for $C_7H_8N_6O_3 \cdot H_2O$: C, 34.7; H, 4.1; N, 34.7. Found: C, 35.2; H, 4.4; N, 34.4.

The mother liquors from the first precipitation deposited a gray crystalline precipitate after standing several hours. This product (250 mg.) had an ultraviolet absorption spectrum different from that of the dihydropteridine and closely resembling that of a pyrimidine. Recrystallization from 2000 parts of hot water resulted in a partial change in the spectrum in the direction of that of the primary reduction product. The analysis is the same as that of the hydrate of the dihydropteridine and indicates that this compound may have been formed by the ring opening of 2,4-diamino-7-hydroxydihydropteridine-6-carboxylic acid.

Anal. Calcd. for $C_7H_{10}N_6O_4$: C, 34.7; H, 4.1; N, 34.7. Found: C, 35.4; H, 4.1; N, 35.0.

(15) H. S. Forrest, R. Hull, H. J. Rodda and A. R. Todd, *J. Chem. Soc.*, 3 (1951).

Ultraviolet absorption spectrum before recrystallization: At *p*H 1, λ_{max} 280 mμ, E_m 13,000; λ_{min} 250 mμ. At *p*H 11, λ_{max} 278 mμ, E_m 8,000; λ_{min} 250 mμ. After recrystallization: At *p*H 1, λ_{max} 280, 335 mμ, E_m 12,100, 2,500. At *p*H 11, λ_{max} 278, 340 mμ, E_m 8,000, 2,900.

Decarboxylation of 2,4-Diamino-7-hydroxydihydropteridine-6-carboxylic Acid.—A 575-mg. portion of the main sodium amalgam reduction product of 2,4-diamino-7-hydroxypteridine-6-carboxylic acid was heated at 140° for two hours. The loss in weight was 26.0%; the theoretical loss for CO_2 and H_2O is 25.6%. The product has an ultraviolet absorption spectrum which is essentially unchanged from that of the 2,4-diamino-7-hydroxydihydropteridine-6-carboxylic acid; analysis indicates the loss of some hydrogen as well as the CO_2 and H_2O.

Anal. Calcd. for $C_6H_8N_6O$: C, 40.0; H, 4.4. Calcd. for $C_6H_6N_6O$: C, 40.5; H, 3.4. Found: C, 40.1; H, 3.7.

2,4-Diamino-7-hydroxypteridine (XIII, Y = NH_2).—To a solution of 90 mg. of the decarboxylation product of 2,4-diamino-7-hydroxydihydropteridine-6-carboxylic acid, described above, in 1 ml. of 2 *N* sodium hydroxide was added slowly 8.4 ml. of 0.04 *M* potassium permanganate. The slight excess of permanganate was removed with a few particles of sodium sulfite. The manganese dioxide was filtered off and the filtrate acidified to *p*H 5 with acetic acid. After chilling the precipitate was centrifuged off, washed with water and dried in a vacuum desiccator (55 mg.).

Anal. Calcd. for $C_6H_6N_6O \cdot H_2O$: C, 36.7; H, 4.1; N, 42.8. Found: C, 37.0; H, 4.3; N, 42.2.

Ultraviolet absorption spectrum: At *p*H 1, λ_{max} 295, 338 mμ, E_m 12,200, 14,400; λ_{min} 265, 310 mμ. At *p*H 11, λ_{max} 257, 340 mμ, E_m 12,000, 14,100; λ_{min} 250, 290 mμ.

Reduction of Isoxanthopterin-6-carboxylic Acid.—A solution of 1 g. of isoxanthopterin-6-carboxylic acid[5] in 5 ml. of 2 *N* sodium hydroxide was shaken with 2 g. of zinc dust at room temperature for 20 minutes. The mixture was filtered, the zinc washed with 50 ml. of water and the filtrate adjusted to *p*H 2 with hydrochloric acid. The precipitate was centrifuged off, redissolved in 25 ml. of water by the addition of 3 ml. of 2 *N* sodium hydroxide, filtered and reprecipitated with 4 ml. of 2 *N* hydrochloric acid. The product was collected by centrifugation, washed with water, alcohol and ether and dried in a vacuum desiccator. On the basis of the analytical figures the product appears to be a mixture of dihydroisoxanthopterin-6-carboxylic acid (XI, Y = OH) and its decarboxylation products (XII, XIII, Y = OH).

Anal. Calcd. for $C_7H_7N_5O_4$: C, 37.3; H, 3.1; N, 31.1. Calcd. for $C_6H_7N_5O_2$: C, 39.7; H, 3.9; N, 38.7. Calcd. for $C_6H_5N_5O_2$: C, 40.2; H, 2.8; N, 39.1. Found: C, 38.6; H, 3.2; N, 34.1.

Ultraviolet absorption spectrum: At *p*H 1, λ_{max} 288, 340 mμ, O.D. at 10 mg. per l., 0.57, 0.75; λ_{min} 260, 308 mμ. At *p*H 11, λ_{max} 253, 275 (inflection), 340 mμ, O.D. at 10 mg. per l. = 0.61, 0.22, 0.77; λ_{min} 245, 290 mμ.

When the reduction was heated at 150° for eight hours there was a loss in weight of 5.1%. The product then had an analysis and an ultraviolet absorption spectrum like that of isoxanthopterin.[16]

Anal. Calcd. for $C_6H_5N_5O_2$: C, 40.2; H, 2.8; N, 39.1. Found: C, 40.3; H, 3.1; N, 38.2.

Ultraviolet absorption spectrum: At *p*H 1, λ_{max} 288, 340 mμ, E_m 10,000, 13,100; λ_{min} 260, 302 mμ. At *p*H 11, λ_{max} 250, 275 (inflection), 342 mμ, E_m 10,000, 3600, 12,700; λ_{min} 245, 290 mμ.

2-Amino-4,6-dihydroxy-*p*-oxazino(2,3-d)pyrimidine (XVIII).—A solution containing 300 mg. of 2-amino-5-chloroacetamido-4,6-dihydroxypyrimidine hydrate[1] and 230 mg. of sodium bicarbonate in 10 ml. of water was heated in a boiling water-bath for one hour. A precipitate began to form after the first ten minutes. After one hour, the mixture was brought to *p*H 5 by the addition of acetic acid and cooled. The precipitate was filtered, washed with water and acetone and dried at 110° (145 mg.).

Anal. Calcd. for $C_6H_6N_4O_3 \cdot H_2O$: C, 36.0; H, 4.0; N, 28.0. Found: C, 36.1; H, 4.3; N, 28.0.

Ultraviolet absorption spectrum: At *p*H 1, λ_{max} 258, 308 mμ; E_m 12,400, 9800; λ_{min} 280 mμ. At *p*H 11, λ_{max} 267, 300 (inflection) mμ, *E* 11,800, 8000.

Hydrolytic Fission of 2,4-Diamino-6-hydroxy-*p*-oxazino-(2,3-d)pyrimidine to the Acid (XIX).—One gram of 2,4-diamino-6-hydroxy-*p*-oxazino(2,3-d)pyrimidine was heated with 25 ml. of concentrated ammonium hydroxide in a sealed tube at 120° for 20 hours. The excess ammonia was then evaporated on the steam-bath and the insoluble orange residue filtered off (320 mg.). This residue had the same ultraviolet absorption spectrum as the starting material. The alkaline filtrate was acidified by the addition of acetic acid and the precipitate collected. This product was identical in ultraviolet absorption spectrum with the acid obtained by the degradation of the oxazinopyrimidine with barium hydroxide.[1]

2-Amino-4-hydroxypteridine.—This compound was prepared by Mowat, *et al.*,[17] by the condensation of 2,4,5-triamino-6-hydroxypyrimidine dihydrochloride with glyoxal at 70°. The crude product was dissolved in 10 *N* sodium hydroxide and precipitated by acid for purification before further examination. In this Laboratory when the crude product was recrystallized from hot water or dilute hydrochloric acid solution it gave an analysis corresponding to the diglyoxalyl derivative of the pyrimidine.

Anal. Calcd. for $C_8H_7N_5O_3$: C, 43.4; H, 3.2; N, 31.7. Found: C, 42.9; H, 3.4; N, 31.3.

The ultraviolet absorption spectrum differs from that of 2-amino-4-hydroxypteridine at *p*H 1, λ_{max} 235, 270, 330 mμ. At *p*H 11 it has a spectrum identical with that of 2-amino-4-hydroxypteridine (see below).

A solution of the primary condensation product was treated with phenylhydrazine giving a yellow phenylhydrazone containing two molecules of phenylhydrazine per molecule of pyrimidine.

Anal. Calcd. for $C_{20}H_{19}N_9O$: C, 59.7; H, 4.7. Found: C, 60.1; H, 5.0.

When the primary product was dissolved in 2 *N* sodium hydroxide and precipitated by acidification, 2-amino-4-hydroxypteridine was obtained.

Anal. Calcd. for $C_6H_5N_5O$: C, 44.2; H, 3.1; N, 43.0. Found: C, 44.1; H, 3.3; N, 43.2.

The ultraviolet absorption spectrum has the following characteristics: At *p*H 1, λ_{max} 312 mμ, E_m 8150; λ_{min} 270 mμ. At *p*H 11, λ_{max} 252, 358 mμ, E_m 22,000, 7600; λ_{min} 295 mμ.

2-Amino-4-carboxymethoxypteridine (XXI).—To a suspension of 100 mg. of 2,4,5-triamino-6-carboxymethoxypyrimidine in 10 ml. of water was added 0.3 ml. of a 30% aqueous solution of glyoxal and 0.6 ml. of 2 *N* sodium hydroxide to bring the *p*H to 6. The mixture was allowed to stand at room temperature for two hours, and then heated on the steam-bath for 45 minutes. At the end of this time, the solid was completely in solution. At *p*H 1, the ultraviolet absorption spectrum of this solution was almost identical with that of diglyoxalylpyrimidine intermediate formed in the preparation of 2-amino-4-hydroxypteridine: λ_{max} 235, 270, 330 mμ. At *p*H 11, the spectrum resembled that of 2-amino-4-hydroxypteridine: λ_{max} 252, 360 mμ. After standing overnight at room temperature, the solution was evaporated to 6 ml. in a stream of air and then chilled at 0° overnight. The crystalline cream-colored precipitate was filtered off, washed with 3 ml. of ice-water and dried at 105° (58 mg.). It was recrystallized from 10 ml. of water. The analysis and spectrum indicate that ring closure to the pteridine had occurred.

Anal. Calcd. for $C_8H_7N_5O_3$: C, 43.5; H, 3.2; N, 31.7. Found: C, 43.2; H, 3.4; N, 31.6.

Ultraviolet absorption spectrum: At *p*H 1, λ_{max} 313 mμ, E_m 7700; λ_{min} 270 mμ. At *p*H 11, λ_{max} 252, 360 mμ, E_m 21,200, 7,100; λ_{min} 290 mμ.

6-Carbethoxymethoxy-2,4-diaminopyrimidine (XXIV).—A mixture of 2.35 g. (0.016 mole) of 6-chloro-2,4-diaminopyrimidine,[18] 2.15 g. (0.017 mole) of sodium ethyl glycolate in 8 ml. of ethyl glycolate was heated at 110° for 14 hours. The mixture was cooled, diluted with 100 ml. of anhydrous ether and filtered. The precipitate was leached with 50

(16) G. B. Elion and G. H. Hitchings, THIS JOURNAL, **69**, 2553 (1947).

(17) J. H. Mowat, J. H. Boothe, B. L. Hutchings, E. L. R. Stokstad, C. W. Waller, R. B. Angier, J. Semb, D. B. Cosulich and Y. Subba-Row, *ibid.*, **70**, 14 (1948).

(18) E. Büttner, *Ber.*, **36**, 2227 (1903).

ml. of absolute ethanol and the insoluble residue (1.9 g.) filtered off. The residue contained 0.57 g. (0.0097 mole) of sodium chloride, as determined by the chloride analysis. The alcoholic extract was taken to dryness and the syrupy residue dissolved in 17 ml. of water. This aqueous solution showed an ultraviolet absorption spectrum different from the starting pyrimidine and different from 2,4-diamino-6-hydroxypyrimidine. This solution was used directly for the next step.

Ultraviolet absorption spectrum of XXIV (at a dilution of 1:1000): At *p*H 1, λ_{max} 276 mμ, O.D. 0.99. At *p*H 11, λ_{max} 275 mμ, O.D. 0.75.

Spectrum of 6-chloro-2,4-diaminopyrimidine: At *p*H 1, λ_{max} 298 mμ. At *p*H 11, λ_{max} 282 mμ.

6-Carbethoxymethoxy-5-*p*-chlorobenzeneazo-2,4-diaminopyrimidine (XXV).—To the above aqueous solution was added a *p*-chlorobenzenediazonium chloride solution (prepared from 220 mg. of *p*-chloroaniline and 125 mg. of sodium nitrite) and 2 g. of sodium bicarbonate. The mixture was kept at 0° overnight and the bright yellow precipitate collected, washed with water and dried in a vacuum desiccator (230 mg.). A portion of this azo compound was purified by solution in 8 ml. of absolute ethanol at room temperature, filtration and dilution with 6 ml. of water.

Anal. Calcd. for $C_{14}H_{15}N_6O_3Cl$: C, 48.2; H, 4.3; N, 24.1. Found: C, 47.8; H, 3.8; N, 24.6.

2,4-Diamino-6-hydroxy-*p*-oxazino(2,3-d)pyrimidine (VII).—A boiling solution of 100 mg. of the crude azo compound (XXV) in 20 ml. of 50% aqueous ethanol was treated with 500 mg. of zinc dust and 2 ml. of 2 *N* hydrochloric acid. After boiling for five minutes, the solution was filtered. A small aliquot of this solution was examined spectrophotometrically. At *p*H 1 the spectrum closely resembled that of XIX[1]; at *p*H 11, that of "β-dihydroxanthopterin." Reacidification of the alkaline solution showed that the spectrum in acid solution now corresponded to that of "β-dihydroxanthopterin," indicating that ring closure had occurred in alkaline solution.

The reaction mixture was made alkaline with 3 ml. of 2 *N* sodium hydroxide, a slight precipitate filtered off and the solution acidified to *p*H 5 with acetic acid. After chilling, the precipitate was centrifuged off, washed with water, alcohol and ether and dried in a vacuum desiccator (23 mg.). After recrystallization from water, from which it crystallized very slowly, the compound was found to be identical in all respects with "β-dihydroxanthopterin."

Anal. Calcd. for $C_6H_7N_5O_2$: C, 39.7; H, 3.9. Found: C, 39.4; H, 4.0.

Ultraviolet absorption spectrum: At *p*H 1, λ_{max} 263, 312 mμ, E_m 13,200, 8800; λ_{min} 290 mμ. At *p*H 11, λ_{max} 275 mμ, E_m 13,700.

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

Acknowledgment.—We are indebted to Samuel W. Blackman and Nicholas Martinez, Jr., for the microanalyses reported here.

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[CONTRIBUTION FROM THE STERLING CHEMISTRY LABORATORY, YALE UNIVERSITY, AND THE DEPARTMENT OF CHEMISTRY, BUCKNELL UNIVERSITY]

The Dehydration of Some Glycols Derived from 1-Cyclohexyl-1-phenylethane[1]

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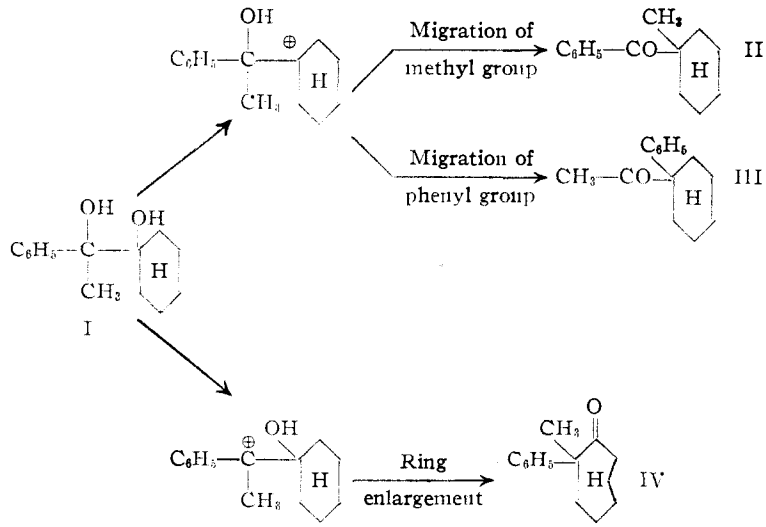
RECEIVED DECEMBER 19, 1951

1-(Cyclohexanol-1)-phenylethanol and 1-cyclohexyl-1-phenyl-1,2-ethanediol have been prepared by the performic acid hydroxylation of the corresponding olefins. It was found that the dehydration of cyclohexylphenylmethylcarbinol gave a mixture of α-cyclohexylidene-ethylbenzene and α-cyclohexylstyrene. The dehydration of the glycols showed no abnormalities due to the steric hindrances of the cyclohexyl and phenyl groups.

In connection with earlier studies[3] on glycol rearrangements, it was felt of interest to investigate the rearrangement of 1-(cyclohexanol-1)-1-phenylethanol (I), an open chain analog of the glycols studied in the tetralin and decalin series.[3,4] Depending on which hydroxyl group was removed first, three products were considered possible.

The glycol (I) was prepared by the hydroxylation of α-cyclohexylidene-ethylbenzene (V). Although it has been reported[5] that the dehydration of cyclohexylmethylphenylcarbinol gave (V), we found upon ozonolysis of the resulting hydrocarbon that the product was a mixture of about 10% α-cyclohexylidene-ethylbenzene (V) and 90% α-cyclohexylstyrene (VI). No ozonolysis products were found which would indicate that the double bond had migrated out of conjugation into the cyclohexane ring.



(1) This paper is based in part on the thesis presented by Charles A. Russell to the Graduate School of Yale University in partial fulfillment of the requirements for the degree of Doctor of Philosophy, and in part on the thesis submitted by Luther T. Stroup to the faculty of Bucknell University in partial fulfillment of the requirements for the degree of Master of Science.

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