114. *Formation of 6-Hydroxypteridines by Condensation of 4,5-Diaminopyrimidines with Chloral.*

By F. Bergmann, M. Tamari, and Hanna Ungar-Waron.

Chloral hydrate reacts with 4,5-diaminopyrimidines, bearing oxygen at position 6, to yield 6-hydroxypteridines. The ready elimination of all three chlorine atoms in this condensation contrasts sharply with the resistance of the corresponding 5-di- and 5-tri-chloroacetamido-derivative to cyclisation. It is assumed that the aldehyde group of chloral condenses first with the 5-amino-group, the latter then inducing formation of an aziridine ring. This ring opens with simultaneous chloride shift, to produce the chloroimido-structure N:CCl·CHCl·OH, in which the hemiacetal group readily condenses with the 4-amino-group of the pyrimidine.

Introduction of a 6- or 7-hydroxyl group into pteridines is usually achieved by condensation of 4,5-diaminopyrimidines with a derivative of glyoxylic acid. Although by proper

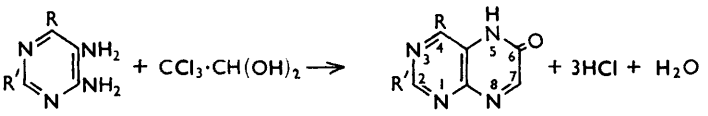
choice of conditions the reaction can be directed so as to yield mainly one of the two possible isomers,[1] the product is often contaminated with small amounts of the other isomer. Purrmann [2] attempted the selective synthesis of 6-hydroxypteridines by use of dichloroacetic instead of glyoxylic acid. This method exploits the greater nucleophilicity of the 5- than of the 4-amino-group,[3] which is responsible for preferential acylation of the former. However, Purrmann's yield of xanthopterin from the intermediate 2,4-diamino-5-dichloroacetamido-6-hydroxypyrimidine was only about 10%.

On the same basis, chloral should undergo first condensation of its aldehyde group with the 5-amino-group, then dehydrohalogenation to close the pyrazine ring, and finally hydrolysis to yield selectively 7-hydroxypteridines. However, by condensation of 2,4,5-triamino-6-hydroxypyrimidine with chloral, Fidler and Wood [4] obtained xanthopterin instead of the expected isoxanthopterin. The present investigation is concerned with the problem of whether this reaction is of general applicability and whether its course is always as in the above example. The following observations were made.

(1) When heated with chloral in a non-aqueous medium, 4,5-diaminopyrimidines do not form pteridines; condensation with chloral hydrate leads exclusively to 6-hydroxypteridines.

(2) The condensation is strongly accelerated, and yields are improved, by the presence of mineral acids. However, the formation of 6-hydroxypteridines does not require acid-catalysis, as demonstrated for the case of 4,5-diaminouracil in Table 1.

(3) The substituent in position 6 of the pyrimidine plays an important role. The reaction represented by following scheme (A) is always successful when R = OH. With

(A) 

4,5-diamino-6-mercaptouracil (R = SH, R' = OH) condensation did take place, but the resulting 2,6-dihydroxy-4-mercaptopteridine was unstable and was not obtained pure. When the 6-position was unsubstituted, as in 4,5-diaminopyrimidine and its 2-hydroxy- or 2-phenyl derivative, the reaction failed. On the other hand, the substituent in position 2 is not critical and the condensation proceeds equally well when R' = OH, SH, Ph, or H (see Table 2). Methyl groups at N-1 and N-3 of the pyrimidine ring are favourable to the reaction (nos. 2, 4, and 5 in Table 2); in particular, the ready formation of compound 4 contrasts with the difficulties encountered by Pfleiderer.[1d]

The structure of the products was established by comparison with known compounds and by ultraviolet spectrophotometry, as 6- and 7-hydroxypteridine differ widely in their absorption maxima.[5] No previous information was available for identification of the 2-phenyl derivative, and its structure (no. 7 in Table 2) was derived indirectly in the following way. 4-Hydroxy-2-phenylpteridine was converted by milk xanthine oxidase into the 4,7-dihydroxy-derivative, which differed from the synthetic 4,6-dihydroxy-isomer (Table 3). The divergencies are similar to those for the pair 4,6- and 4,7-dihydroxypteridine.[6] The result of the enzymic oxidation is in agreement with previous observations showing that position 7, but not 6, in the pteridine nucleus is susceptible to enzymic attack.[7]

Special efforts were devoted to the detection of 7-hydroxy-isomers. However, even

[1] (*a*) Albert, *J.*, 1955, 2690; (*b*) Albert, Lister, and Pedersen, *J.*, 1956, 4621; Pfleiderer, *Chem. Ber.*, 1957, **90**, (*c*) 2588, (*d*) 2604.
[2] Purrmann, *Annalen*, 1941, **564**, 98.
[3] Traube, *Ber.*, 1900, **33**, 3035; Wilson, *J.*, 1948, 1157.
[4] Fidler and Wood, *J.*, 1956, 3311.
[5] Albert, Brown, and Cheeseman, *J.*, 1952, 1620.
[6] Albert and Brown, *J.*, 1953, 74.
[7] Bergmann and Kwietny, *Biochim. Biophys. Acta*, 1958, **28**, 613; 1959, **33**, 29.

TABLE 1.

Condensation of 4,5-diaminouracil with chloral hydrate.

No.	Chloral/pyrimidine *	Reagents added	Reaction Temp.	Time (min.)	Yield (%) †
1	3 : 1	—	100°	30	79
2	3 : 1	5% $NaHCO_3$; 0·2 g. KI	100	30	53 ‡
3	2 : 1	5% Na_2CO_3	100	15	0 §
4	1 : 1	2% NaOAc	100	60	57 ¶
5	1 : 1	2% H_2SO_4	90	15	88
6	2 : 1	,,	95	15	86
7	3 : 1	,,	90	15	86

* In all experiments, 0·01 mole of the uracil derivative was used. The volume of the reaction mixture was 100 ml. After completion of the reaction, the mixture was kept for 24 hr. at +4°. The crystals were removed by suction and washed with distilled water. † The yield was determined by comparing the absorption of the crude material at 381 mμ, in 0·1M-phosphate buffer of pH 8·0, with that of an analytically pure sample. ‡ The pH at the end of the reaction was 7. The mixture smelled of chloroform. § The pH at the end of the reaction was 9·6. Chloroform accumulated at the bottom of the vessel. ¶ The pH at the end of the reaction was 5·5.

TABLE 2.

6-Hydroxypteridines, obtained by condensation of 4,5-diaminopyrimidines with chloral hydrate.*

No.	Subst. at 1	2	3	4	Yield (%)	M. p. or decomp.	Purificn.	Form	R_F †	Fluorescence	Ref.
1	—	H	H	OH	85	>340	Repptn.	Spheroids	0·37	Blue	5
2	—	H	Me	OH	89	>340	H_2O	Plates	0·39	,,	19
3	H	OH	H	OH	75	>340	2N-HCl	—	0·38	,,	1*b*
4	Me	OH	H	OH	79	>340	N-HCl	Prisms	0·5	,,	1*d*
5	Me	OH	Me	OH	75	300	H_2O	,,	0·59	,,	1*d*
6 ‡	Me	S	H	OH	77	335	Repptn.	Plates	0·51	,,	—
7	—	Ph	H	OH	85	>330	Aq. AcOH	Rods	0·5	Orange-yellow	—

No.	Found (%) C	H	N	Formula	Required (%) C	H	N
6	39·8	3·1	26·55	$C_7H_5N_4O_2S$	40·0	2·9	26·7
7	59·0	4·0	22·6	$C_{12}H_8N_4O_2,\frac{1}{2}H_2O$	58·9	3·5	22·7

* Condensations were carried out in 2% H_2SO_4 at 100°; no. 2 in N-HCl. Optimum reaction time for nos. 2, 5, 6, and 7, 10 min.; for nos. 1 and 4, 15 min.; for no. 3, 20 min. † In solvent A. ‡ $\lambda_{max.}$ at pH 8·0: 243, 300, and 385 mμ (log ε 4·0, 4·4, and 3·95).

TABLE 3.

Physical properties of 2-phenylpteridines.

Derivative	$\lambda_{max.}$ (mμ) at pH 8·0	log ε	R_F *	Fluorescence
4-Hydroxy	226, 266, 338	4·09, 4·40, 3·95	0·78	Black-violet
4,6-Dihydroxy	252,† 294, 386	3·86, 3·60, 3·97	0·51	Orange-yellow
	432 ‡	4·30		
4,7-Dihydroxy §	246, 335		0·61	Sky-blue
4,6,7-Trihydroxy	246, 322	4·27, 4·18	0·32	Blue

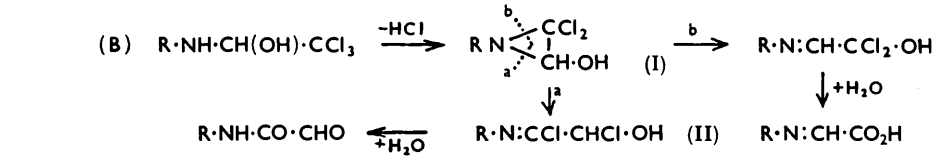
* In solvent A. † Because of the very low solubility of the compound in water, absorption was measured in 0·1M-phosphate buffer of pH 8·0, containing 2·5% of dimethylformamide. ‡ This maximum was measured in pure dimethylformamide. § This product was obtained by enzymic oxidation of 2-phenyl-4-hydroxypteridine. The quantities available did not permit an exact determination of $\varepsilon_{max.}$.

when the condensations with chloral were carried out in the presence of sodium hydrogen carbonate, no trace of a 7-hydroxypteridine was found. Since the latter derivatives are stable in weakly alkaline conditions, the exclusive formation of 6-hydroxypteridines cannot be ascribed to rearrangement of 7-hydroxy-isomers produced initially.[1a]

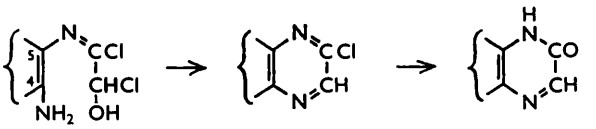
The most surprising aspect of the reaction is the easy elimination of all chlorine atoms of the CCl_3 group. Conversion of chloral into derivatives of dichloroacetic acid is well

known,[8] but the halogen atoms in the remaining CCl_2 group are relatively unreactive. This is reflected in, *e.g.*, the difficult cyclisation of 4-amino-5-dichloroacetamidopyrimidines and excludes these compounds as intermediates in the condensation with chloral. The corresponding trichloroacetamido-derivatives are even more resistant to ring closure (see p. 571).

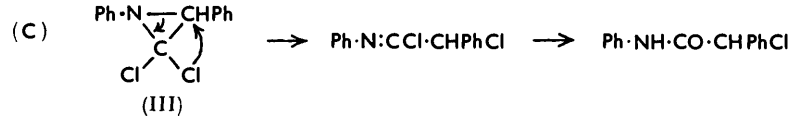
On the other hand, during the condensation of chloral with carbonyl reagents such as semicarbazide [9] and phenylhydrazines,[10] all three chlorine atoms are easily removed to give the corresponding derivatives of glyoxylic acid. Similarly, Sandmeyer,[11] on treating aniline with chloral and hydroxylamine, isolated the oxime Ph·NH·CO·CH=N·OH, whereas aniline and chloral alone form only a Schiff's base or a dianilino-derivative.[12] These observations suggest that the azomethine-nitrogen atom is responsible for the labilisation of all three chlorine atoms of the CCl_3 group. Presumably the primary condensation product is converted into an unstable intermediate, such as an aziridine, which rearranges during ring opening, as indicated in scheme (B).



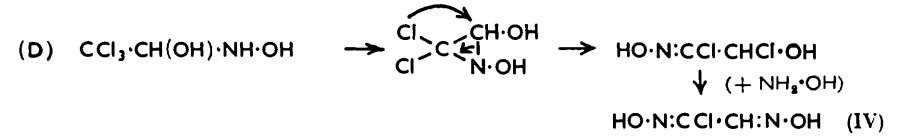
In the case of the 4,5-diaminopyrimidines the reaction follows path (a) and the hemiacetal structure in the intermediate (II) reacts with the 4-amino-group to form the pyrazine ring:



The proposed scheme is supported by Fields and Sandri's observation [13] that aziridines containing a CCl_2 group undergo in water a chloride shift analogous to the transformation (I) ⟶ (II). This is exemplified in scheme (C) for the adduct (III) of a Schiff's base with dichlorocarbenium.



Similarly, chloral oxime with an excess of hydroxylamine forms chloroglyoxime (IV),[14] as shown in scheme (D):

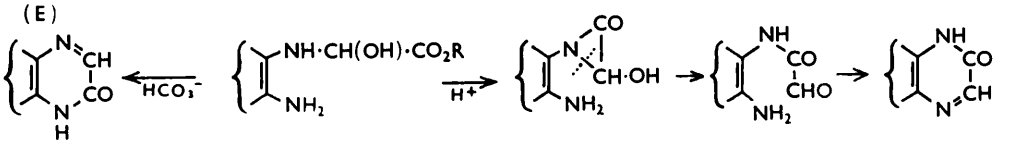


[8] Pinner and Fuchs, *Ber.*, 1877, **10**, 1058; Wallach, *ibid.*, p. 1527.
[9] Darapski and Prabhaker, *Ber.*, 1912, **45**, 2617; Chang and Ulbricht, *J. Amer. Chem. Soc.*, 1958, **80**, 976.
[10] Chattaway and Bennett, *J.*, 1927, 2850; Torres and Martinez-Oller, *Anales Fis. Quim.*, 1944, **40**, 825.
[11] Sandmeyer, *Helv. Chim. Acta*, 1919, **2**, 234.
[12] Rügheimer, *Ber.*, 1906, **39**, 1653.
[13] Fields and Sandri, *Chem. and Ind.*, 1959, 1216.
[14] Hantzsch, *Ber.*, 1892, **25**, 705.

Fidler and Wood,[4] following earlier attempts by Purrmann,[15] condensed 2,4,5-triamino-6-hydroxypyrimidine with chloral in acetic acid containing sodium acetate and isolated an intermediate Schiff's base of the probable structure R·N=CH·CO·NHR (where R represents the properly substituted 5-pyrimidyl radical). In hot 2N-hydrochloric acid, this intermediate yielded xanthopterin. Apparently the 2-amino-group diminishes the reactivity of the 4-amino-substituent to such a degree that the hemiacetal structure CHCl·OH of compound (II) in scheme (B) reacts in weakly acid media preferentially with the 5-amino-group of a second pyrimidine molecule. In the present study we have not succeeded in tracing intermediates in any of the condensations described in Table 2. Whenever the reaction was interrupted before completion, only starting material accompanied the expected end-product. For 4,5-diamino-6-hydroxy-2-phenylpyrimidine the reaction could be carried out in ethanol at +4°, but even under such mild conditions only the pteridine (no. 7 in Table 2) was detected.

The hypothesis advanced for the condensation with chloral may also explain why the course of the reaction of 4,5-diaminopyrimidines with glyoxylic acid derivatives depends on the pH.[1] Pfleiderer put forward the view that in neutral media the 5-amino-group of 4,5-diaminopyrimidines is the more reactive, because the nucleophilicity of the 4-amino-group is reduced by mesomerism of the type $NH_2-\overset{4}{C}=\overset{5}{C}-\overset{6}{C}=O \longleftrightarrow \overset{+}{N}H_2=C-C=C-O^-$. Conversely, in acid conditions the 5-amino-group is protonated preferentially and the 4-amino-substituent is now relatively more reactive.[1c,d] On this basis it is, however, difficult to explain why condensations with ethyl glyoxylate hemiacetal should be much faster in acid media. These difficulties can be met by assuming again an aziridine as intermediate [scheme (E)]. Similar considerations may be useful in explaining the conversion of 7- into 6-hydroxypteridines in the presence of strong acid.[1a]

(E)



Finally, it may be noted that condensation of 4,5-diaminouracil with bromal hydrate led to 2,4,6,7-tetrahydroxypteridine. Partial oxidation of hydrogen bromide to free bromine may be responsible for the oxidation of 2,4,6-trihydroxypteridine formed initially. We have indeed found the latter to be oxidised at position 7 by elemental bromine as smoothly as by hydrogen peroxide.[5] However, it also appears possible that oxidation takes place at the intermediate stage (II). In the same manner, condensation of 4,5-diamino-6-hydroxypyrimidine with bromal gave 4,6,7-trihydroxypteridine.

Experimental

Ultraviolet spectra were measured for solutions in 0·1M-phosphate buffer of pH 8·0 on a Beckman D.U. spectrophotometer. Paper chromatograms were developed by the descending method, with the solvents: (A) 95% EtOH–NMe_2·CHO–H_2O (3 : 1 : 1 v/v); (B) propan-2-ol–NMe_2·CHO–25% aq. NH_3 (13 : 5 : 2 v/v); (C) 95% EtOH–H_2O–AcOH (17 : 2 : 1 v/v). Spots were located by their fluorescence under a Mineralight ultraviolet lamp ($\lambda \sim 255$ mμ).

4,5-Diaminopyrimidines were prepared by known procedures: 4,5-diaminouracil,[16] its 3-methyl [17] and 1,3-dimethyl derivative;[18] 4,5-diamino-6-hydroxypyrimidine [19] and its

[15] Purrmann, *Annalen*, 1941, **548**, 284.
[16] Bogert and Davidson, *J. Amer. Chem. Soc.*, 1933, **55**, 1667.
[17] Traube, *Ber.*, 1900, **33**, 3035.
[18] Blicke and Godt, *J. Amer. Chem. Soc.*, 1954, **76**, 2798.
[19] Boon, Jones, and Ramage, *J.*, 1951, 96.

1-methyl [20] and 2-phenyl derivative; [21] and 4,5-diamino-2-thiouracil [22,23] and its 3-methyl derivative.[22]

General Procedure for Condensation with Chloral Hydrate.—A solution or suspension of a 4,5-diaminopyrimidine in 2% sulphuric acid was slowly warmed to 100° and an aqueous solution of chloral hydrate (2 equiv.) was added. The mixture was stirred at 100° for the time specified in Table 1. Precipitation usually started from the hot solution and was completed at +4°. The crude, yellow-to-brown product was filtered off and recrystallised several times, until the chromatogram proved its homogeneity and until the value of $\varepsilon_{max.}$ remained constant. Known pteridines were identified by comparison with authentic samples; new *pteridines* were dried for analysis at 100°.

The product obtained from 4,5-diamino-2-thiouracil showed the following properties: $\lambda_{max.}$ (pH 8·0) 242, 299, and 384 mμ (log ε 4·0, 4·3, and 3·9). These values are so close to those of 4,6-dihydroxy-1-methyl-2-thiopteridine (no. 6 in Table 2) that there can be no doubt as to the identity of the compound as 4,6-dihydroxy-2-thiopteridine. Although the product, purified by five reprecipitations, migrated as a single spot with R_F 0·36 in solvent A, it did not give satisfactory analyses.

The crude product from 4,5-diamino-6-thiouracil [24] and chloral exhibited absorption peaks (pH 8·0) at 239, 299, and 414 mμ. On a paper chromatogram, it showed a main spot with R_F (solvent A) 0·61 in addition to other components. However, the material decomposed during recrystallisation or reprecipitation. Therefore an analytically pure sample could not be obtained, although the value of the long-wave absorption maximum suggests the presence of the desired 4-mercaptopteridine derivative.

4-*Hydroxy*-2-*phenylpteridine.*—A solution of 4,5-diamino-6-hydroxy-2-phenylpyrimidine [21] (2 g.) and glyoxal bisulphite (3 g.) in 2N-hydrochloric acid (160 ml.) was refluxed for 1 hr. A dark by-product which had been precipitated was filtered off from the hot solution and the pH of the filtrate was adjusted to 6 by dropwise addition of concentrated aqueous ammonia. The *product* (1·8 g., 85%), precipitated on cooling of the ammoniacal solution, was recrystallised from 50% acetic acid, forming plates, decomp. >310°, easily soluble in acetic acid or dioxan, very slightly soluble in water (Found: C, 64·4; H, 3·7; N, 25·4. $C_{12}H_8N_4O$ requires C, 64·3; H, 3·6; N, 25·0%).

4,6,7-*Trihydroxy*-2-*phenylpteridine.*—4,5-Diamino-6-hydroxy-2-phenylpyrimidine (4 g.) and ethyl oxalate (8 ml.) in ethylene glycol (80 ml.) were refluxed for 2 hr. The *product*, precipitated on cooling, recrystallised from acetic acid as prisms, m. p. (sublimation) 320—325° (3·9 g., 76%) (Found: C, 52·65; H, 3·6. $C_{12}H_8N_4O_3,H_2O$ requires C, 52·6; H, 3·65%).

Di- and Tri-chloroacetyl Derivatives of 4,5-*Diaminopyrimidines.*—4-*Amino*-5-*dichloroacetamido*-6-*hydroxypyrimidine.* 4,5-Diamino-6-hydroxypyrimidine [20b] (2·6 g.) and dichloroacetic acid (20 ml.) were heated to 120°/200 mm. for 1 hr., then poured into water (100 ml.); the precipitate recrystallised from water as needles (3·6 g., 78%), decomp. 295—300°, $\lambda_{max.}$ (pH 8·0) 259 mμ (log ε 3·9), R_F 0·18 in solvent A and 0·45 in solvent C (Found: N, 22·85. $C_6H_6Cl_2N_4O_2,\frac{1}{2}H_2O$ requires N, 22·8%).

This product did not cyclise when heated in molten potassium acetate to 320° for 20 min. Longer heating produced extensive decomposition.

4-*Amino*-5-*dichloroacetamido*-6-*hydroxy*-2-*phenylpyrimidine.* When the appropriate diamino-derivative [21] (1 g.) was heated in dichloroacetic acid (4 ml.) at 85—95°, it slowly dissolved. Soon afterwards the acylation product started to crystallise. After 1 hour's heating at ~90° the mixture was diluted with water (40 ml.). The *product* (1·3 g., 84%) crystallised from a large volume of acetic acid in rods, m. p. 311° (sublimation), R_F 0·9 in solvent B, $\lambda_{max.}$ (pH 8·0) 269 mμ (Found: C, 46·6; H, 3·3; N, 18·1. $C_{12}H_{10}Cl_2N_4O_2$ requires C, 46·0; H, 3·2; N, 17·9%).

The amide (0·3 g.) was heated with molten sodium acetate (3 g.) at 240—250° for 20 min. The cake was dissolved in N-sodium hydroxide, filtered, and acidified with acetic acid. The product was a mixture of starting material and free diamine, but did not contain even traces of 4,6-dihydroxy-2-phenylpteridine.

[20] (*a*) Pfleiderer, *Chem. Ber.*, 1959, **92**, 3190; (*b*) Elion, *J. Org. Chem.*, 1962, **27**, 2478.
[21] Bergmann, Kalmus, Ungar-Waron, and Kwietny-Govrin, *J.*, 1963, 3729.
[22] Traube, *Annalen*, 1904, **331**, 71.
[23] Elion, Bürgi, and Hitchings, *J. Amer. Chem. Soc.*, 1952, **74**, 411.
[24] Levin, Kalmus, and Bergmann, *J. Org. Chem.*, 1960, **25**, 1752.

One-step Condensation of 4,5-Diaminouracil with Dichloroacetic Acid to 2,4,6-Trihydroxypteridine.—4,5-Diaminouracil (1·4 g.), dichloroacetic acid (20 ml.), and potassium iodide (0·1 g.) were kept at 110°. After 3 min., the pyrimidine dissolved and a short time thereafter 4-amino-5-dichloroacetamidouracil began to be precipitated. The temperature was raised slowly to 170—180° and kept at this level for 3 hr. The mixture was then cooled and the product precipitated by addition of water (30 ml.). After 3 crystallisations from 2N-hydrochloric acid, the product (0·7 g., 39%) showed R_F 0·39 in solvent A and the absorption maxima (225, 265, and 381 mμ) characteristic of 2,4,6-trihydroxypteridine.

This procedure failed with other 4,5-diaminopyrimidines.

4-Amino-5-trichloroacetamidouracil.—4,5-Diaminouracil (6 g.) and trichloroacetic acid (24 g.) were heated at 100°/100 mm. for 1 hr. The *product* was precipitated by addition of dioxan and crystallised from water in prisms (10 g., 85%), decomp. >330°, $\lambda_{max.}$ (pH 8·0) 264 mμ (log ε 4·15), R_F 0·76 in solvent A (Found: C, 25·3; H, 1·7; N, 18·9. $C_6H_5Cl_3N_4O_3$ requires C, 25·1; H, 1·75; N, 19·5%).

When this compound was heated in a molten mixture of sodium and potassium acetate at 280° for 20 min., extensive carbonisation set in. The dark mixture was extracted with N-sodium hydroxide and the filtrate was acidified with glacial acetic acid. The gelatinous material that was precipitated on cooling contained, besides starting material, ~1% of 2,4,6,7-tetrahydroxypteridine, which was identified on a paper chromatogram (R_F 0·27 in solvent A) and by $\lambda_{max.}$ 330 and 342 mμ. An authentic sample showed the same R_F value and $\lambda_{max.}$ (pH 8·0) 332 and 346 mμ.[25]

4-Amino-6-hydroxy-2-phenyl-5-trichloroacetamidopyrimidine.—4,5-Diamino-6-hydroxy-2-phenylpyrimidine (4 g.) and trichloroacetic acid (12 g.) were heated *in vacuo* at 100° for 1 hr. The *product* was precipitated by addition of water and recrystallised from butan-1-ol as plates (5·4 g., 90%), $\lambda_{max.}$ 227 and 280 mμ, R_F 0·94 in solvent A (Found: C, 41·9; H, 2·8; N, 16·3. $C_{12}H_9Cl_3N_4O_2$ requires C, 41·4; H, 2·6; N, 16·1%).

This compound was recovered unchanged when heated at 280° in sodium–potassium acetate. On the other hand, when warmed at 90° with 2% sulphuric acid, it was split to the free diaminopyrimidine.

Condensation of 4,5-Diamino-6-hydroxypyrimidine with Bromal.—4,5-Diamino-6-hydroxypyrimidine (2·4 g.), freshly distilled bromal, b. p. 174° (16 ml.), and water (1·5 ml.) were heated under reflux for 30 min., cooled, and diluted with methanol (50 ml.). When left overnight in the refrigerator, the solution deposited a brown-red material (1·2 g.), which was dissolved in alkali and reprecipitated by hydrochloric acid. The R_F of the purified product (0·32 in solvent A) and its absorption spectrum [$\lambda_{max.}$ (pH 8·0) 241, 317, and 330 mμ)] were identical with those of authentic 4,6,7-trihydroxypteridine.[6]

Condensation of 4,5-Diaminouracil with Bromal.—4,5-Diaminouracil (3·1 g.), bromal (18 ml.), and water (3·5 ml.) were heated under reflux for 30 min. Purification as before gave 2 g. (46%) of yellowish crystals, R_F 0·27 in solvent A, $\lambda_{max.}$ (pH 8·0) 288, 332, and 344 mμ, corresponding to the values of authentic 2,4,6,7-tetrahydroxypteridine.[25]

Oxidation of 2,4,6-Trihydroxypteridine with Bromine.—2,4,6-Trihydroxypteridine (1 g.) was dissolved in warm 2% sulphuric acid (700 ml.). The solution was rapidly cooled, and with vigorous stirring, bromine (0·7 ml.) was added from a side-tube at 25°. The solution was next refluxed for 10 min., then concentrated *in vacuo*. When left overnight in the cold, the concentrate deposited yellowish crystals (0·6 g., 50%) of 2,4,6,7-tetrahydroxypteridine, R_F 0·27 in solvent A, $\lambda_{max.}$ (pH 8·0) 332 and 345 mμ.

Enzymic Oxidation of 4-Hydroxy-2-phenylpteridine to 4,7-Dihydroxy-2-phenylpteridine.—Purified milk xanthine oxidase was obtained through the courtesy of Professor F. Bergel and Dr. D. A. Gilbert of the Chester Beatty Institute of Cancer Research, London. At pH 8·0 and 28°, an enzyme dilution of 1 : 4000 produced 1 μg. per ml. per min. of uric acid when 65 μmole of xanthine served as substrate.

Catalase (Worthington) was added in all enzyme experiments at a concentration of 1 unit/ml. This amount is sufficient to decompose 1 mg. of hydrogen peroxide per ml. per min. when the substrate concentration is ~1·5 mM.

For oxidation of 4-hydroxy-2-phenylpteridine the following conditions were used: enzyme, 1 : 1000; substrate, 7·1 μg./ml. (32 μmole); 0·01M-phosphate buffer of pH 8·0; temperature

[25] Kwietny and Bergmann, *J. Chromatog.*, 1959, **2**, 162.

28°. The progress of the reaction was measured at 270 mμ, where the absorption diminishes, and at 335 mμ, where it increases, during oxidation. The relative rate of the reaction was 2·1 (rate of xanthine oxidation at the same enzyme concentration = 100).

After the reaction had stopped, the solution was concentrated *in vacuo* and subjected to paper chromatography. The product of enzymic oxidation (R_F 0·61 in solvent A) differed from the isomeric 4,6-dihydroxy-2-phenylpteridine (R_F 0·51) in its fluorescence and absorption characteristics (see Table 3).

The solubility of 4,6-dihydroxy-2-phenylpteridine (no. 7 in Table 2) in water or phosphate buffer is of the order of 0·1 μg./ml. Therefore it is not possible to measure the enzymic reaction spectrophotometrically, in the conditions described before. When to the pteridine dissolved in dimethylformamide, phosphate buffer was slowly added, a solution was obtained containing 7·5 μg./ml. of substrate in a solvent mixture containing 2·5% of dimethylformamide. This concentration of the organic solvent inhibited xanthine oxidase by about 30%. However, the (presumably very small) enzymic rate could not be determined beyond doubt, because the solution deposited the pteridine when kept for a few hours.

This study was supported by the United States National Institutes of Health. The authors thank Professor A. Albert and Dr. D. J. Brown of the National Australian University, Canberra, for a generous gift of some of the pteridines, required for identification, and Professor F. Bergel and Dr. D. A. Gilbert of the Chester Beatty Institute of Cancer Research, London, for the purified xanthine oxidase.

Department of Pharmacology,
The Hebrew University, Hadassah Medical School,
Jerusalem, Israel.

[*Received, April 22nd,* 1963.]