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The Transformation of Purines into Pteridines

By A. ALBERT

Department of Medical Chemistry, Australian National University

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It has been demonstrated (Albert, 1954) that 2-hydroxypurine (I), incubated in water with glyoxal at 37°, gives 2-hydroxypteridine (IV), and that other purines can be converted into pteridines. It was suggested that this reaction has biological importance, and recent work has confirmed this (e.g. Ziegler-Günder, Simon & Wacker, 1956). Quantitative aspects of the chemical transformation are explored in the present communication.

MATERIALS

2-Hydroxypurine was obtained by the action of nitrous acid on 2-aminopurine (Albert & Brown, 1954), 2-hydroxy-9-methylpurine by the action of formic acid on 5-amino-2-hydroxy-4-methylaminopyrimidine (Brown & Mason, 1957) and 9-methylpurine by the action of formic acid on 5-amino-4-methylaminopyrimidine (Albert & Brown, 1954).

Hypoxanthine, xanthine and guanine were commercial preparations recrystallized to chromatographic purity and maximal absorption at λ_{max} . (see Mason, 1954).

2-Hydroxy- (Albert, Brown & Cheeseman, 1951), 2,6-dihydroxy- (Albert, Lister & Pedersen, 1956), 4,6-dihydroxy- and 4,6,7-trihydroxy-pteridine (Albert & Brown, 1953) and xanthopterin (Albert & Wood, 1952) were made by standard methods. 6:7:8-Trimethyl-2-pteridone was kindly prepared by Dr D. J. Brown, from diacetyl and 5-amino-2-hydroxy-4-methylaminopyrimidine (Brown & Mason, 1957). Ethyl glyoxylate hemiacetal was prepared from ethyl tartrate (Rigby, 1950). Glyoxylic acid (crystalline) and glyoxal (50% syrup) were purchased. 4:5-Diamino-2-hydroxypyrimidine was prepared as by Johns (1911).

A Hilger Uvispek spectrophotometer with 4 cm. cells was used.

EXPERIMENTAL

2-Hydroxypurine to 2-hydroxypteridine. 2-Hydroxypurine monohydrate (0.46 g.; 0.003 mole), glyoxal (1.38 g. of 50% syrup; 0.012 mole) and water (6.5 ml.) were set aside at 37° for a week, then at 0° for 3 days (final pH was 2). The solid, recrystallized from 50 parts of boiling water (with charcoal), gave a yield of 16% of 2-hydroxypteridine monohydrate. This was chromatographically [on paper, in *n*-butanol-5*N* acetic acid (7:3), also in 3% (w/v) aqueous NH_4Cl] and spectrometrically identical with authentic 2-hydroxypteridine. (Found for material dried at 120°/0.1 mm.: C, 43.6; H, 3.8; N, 33.3. Calc. for $\text{C}_6\text{H}_4\text{ON}_4 \cdot \text{H}_2\text{O}$: C, 43.4; H, 3.6; N, 33.7%.)

From the same quantities of materials plus 5*N*- H_2SO_4 (1.8 ml.) set aside at 37° for 24 hr. (final pH, 0.5), 0.26 g. (52%) of pure 2-hydroxypteridine monohydrate was isolated; but 84% was demonstrated to have been formed, by spectrometric examination (pH 7) of the neutral molecule at 230 and 307 $\text{m}\mu$. When the incubation was prolonged for a week, or when a higher temperature was used, the

yield fell to 39%, and a purple substance, insoluble in boiling water, was also formed.

4:5-Diamino-2-hydroxypyrimidine to 2-hydroxypteridine. Glyoxal (1.38 ml. of 50% syrup) in water (7 ml.) was adjusted to pH 7 with 2N-Na₂CO₃. 4:5-Diamino-2-hydroxypyrimidine (0.38 g.) was added, and the solution set aside at 37° for 24 hr. and refrigerated (final pH, 6.5). 2-Hydroxypteridine monohydrate (0.49 g.; 93%) was deposited as colourless crystals, chromatographically and spectroscopically pure.

2-Hydroxypurine to 2:6-dihydroxypteridine. 2-Hydroxypurine monohydrate (0.154 g.) was dissolved in boiling water (1.5 ml.) and glyoxylic acid monohydrate (0.28 g.) added. The solution was cooled and 2.5N-HCl (1.2 ml.) added. The mixture was set aside at 37° for 72 hr. Without filtration, the precipitate was dissolved in N-KOH (8 ml.) and diluted to 50 ml. with water. Spectrometric examination at 412 m μ . after dilution showed that 84.6% of 2:6-dihydroxypteridine had been formed. (2-Hydroxypurine has $\lambda_{\max}$ 265 in 0.1N-KOH.)

The substitution of ethyl glyoxylate hemiacetal (0.3 g.) for glyoxylic acid lowered the yield to 78.8%. In this instance the remainder of the alkaline solution was adjusted to pH 4.9 at 80° with 0.1N acetic acid (25 ml.) and 0.1N-HCl. The mixture, refrigerated for 4 days, gave 2:6-dihydroxypteridine (0.1267 g.; 71%). Identity was established by infrared and ultraviolet spectroscopy, and by chromatography. 2:7-Dihydroxypteridine could not be detected, but this isomer predominates at pH values above 5 (Albert *et al.* 1956).

Hypoxanthine to 4:6-dihydroxypteridine. Hypoxanthine (0.034 g.), ethyl glyoxylate hemiacetal (0.074 g.) and N-HCl (0.75 ml.) were heated in a sealed tube at 120° for 6 hr. N Potassium hydroxide (1.5 ml.) was added and the solution was diluted to 5 ml. A control solution (0.001 M) of 4:6-dihydroxypteridine monohydrate was made in 0.01N-KOH.

Two bands, each about 8 cm. wide, of the reaction solution (0.1 ml.) were applied to each of two sheets of Whatman no. 1 paper. The control solution (0.1 ml.) was similarly applied to a third paper. The three papers were run as ascending chromatograms in 2N-NH₃ soln. The papers were dried, examined in ultraviolet light at 365 m μ . and then as rapidly as possible at 254 m μ .

The following strips (12 cm. $\times$ 3 cm.) were cut: (a) four containing the 4:6-dihydroxypteridine formed in the reaction; (b) one containing the 4:6-dihydroxypteridine control and (c) one of blank paper from the same R_F location. These strips were eluted overnight with 0.1N-KOH. The eluates from (a) were combined and all eluates were diluted to 10 ml. with 0.1N-KOH: the optical densities of eluates (a) and (b) were measured at 367 m μ . with the eluate (c) as blank. The recovery from the control was 100%. The yield of 4:6-dihydroxypteridine formed from hypoxanthine was 0.49%.

Hydrolysis of guanine to xanthine. Guanine (0.076 g.) and N-HCl (1.5 ml.) were heated for 6 hr. in a sealed tube, chromatographed in 3% (w/v) NH₄Cl and eluted with 0.1N-KOH as above. At 120° 82% of guanine (measured at 274 m μ .) was unchanged and 12% was converted into xanthine (measured at 283 m μ .). The corresponding percentages at 150° were 20 and 47.

Guanine to xanthopterin. Guanine (0.0378 g.), ethyl glyoxylate hemiacetal (0.074 g.) and N-HCl (0.75 ml.) were

heated in a sealed tube for 6 hr. at 110°. The yellow solution was cooled, N-KOH added (1.5 ml.) and made up to 5 ml. with water (solution is 5×10^{-2} M with respect to guanine). Control solutions were made from xanthopterin monohydrate (1.97 mg.) and 2:4:6-trihydroxypteridine (1.8 mg.) in 0.1N-KOH (10 ml.).

Paper chromatograms were run in a similar way to those for hypoxanthine. The recovery of control xanthopterin and 2:4:6-trihydroxypteridine was 99.6 and 102.4% respectively. The yield of these compounds formed from guanine was 0.08 and 0.26%. Measurements were made at 390 m μ . for both substances.

2-Hydroxy-9-methylpurine to 6:7:8-trimethyl-2-pteridone. 2-Hydroxy-9-methylpurine (0.0375 g.), diacetyl (0.086 g.) and 0.01N-HCl (37.5 ml.) were set aside at 37° for 72 hr. To the yellow solution (black if 0.1N acid was substituted) 0.1M-KH₂PO₄ soln. (7.5 ml.) was added, the pH was adjusted to 7.0 with N-KOH, and the solution was diluted to 50 ml. A control solution was made from 6:7:8-trimethyl-2-pteridone (9.5 mg.) in water (25 ml.).

The reaction solution and the control (0.1 ml. of each) were applied as above to paper, which was developed in 3% NH₄Cl. The excised bands were eluted with water, and each eluate was diluted to 10 ml. with water. Spectrophotometry at 327 m μ . showed that 65% of the control had been destroyed in the processing, and a new peak developed at 298 m μ . (apparently 5-amino-2-hydroxy-4-methylaminopyrimidine). If a similar rate of hydrolysis is assumed the yield from 2-hydroxy-9-methylpurine was approximately 7%.

RESULTS

The principal results of these transformations are summarized in Table 1. In several experiments, paper chromatography followed by elution and spectrophotometry was used because of the small yields. It is noteworthy that 2N-NH₃ soln. had no effect upon 4:6-dihydroxypteridine and xanthopterin, whereas it rapidly forms a secondary amine with 6-hydroxypteridine, even at 20° (Albert, 1955).

DISCUSSION

The conversion of purines into pteridines has no preparative value (because both are made from the same intermediates), but is of interest for the light which it may shed on the biological origin of natural pteridines. Provided that a purine is suitably substituted, the imidazole ring opens readily. For example, 2-hydroxypurine (I) gives 4-amino-5-formamido-2-hydroxypyrimidine (II) at 20° and pH 5 (2-mercaptapurine behaves similarly) (Albert & Brown, 1954). The formyl derivative (II) loses the formyl group more and more rapidly as it becomes autocatalysed by H⁺ ions from the liberated formic acid, giving 4:5-diamino-2-hydroxypyrimidine (III) (Albert & Brown, 1954). It is shown in the Experimental section that 4:5-diamino-2-hydroxypyrimidine combines with glyoxal to give 2-hydroxypteridine (IV) in 93% yield at 37° and pH 7. This is typical of a general reaction,

which proceeds under physiological conditions regardless of substituents in the pyrimidine and requires no catalyst. 1:2-Diketones, aldehyde acids and keto acids react similarly to glyoxal. The hydroxyl group, derived from aldehyde acids, goes to the 6 position of pteridines under acid (and perhaps enzymic) conditions, but sometimes to the 7 position when the pH is raised (Albert, Brown & Cheeseman, 1952).

In Table 1 the results cover these three consecutive reactions, i.e. the purine, two-carbon fragment and solvent were mixed and the yield of the pteridine was measured. Expt. 1 shows a yield of 16% of 2-hydroxypteridine from 2-hydroxypurine and glyoxal in water at 37°. The rate-limiting reaction is (II) → (III), so that (II) accumulated (Albert & Brown, 1954). A much better yield (84%) was obtained in Expt. 2, under more acid conditions. 2-Hydroxypteridine is itself sensitive to acid, and forms an insoluble purple substance, which appears to be a dimer (Albert *et al.* 1951): thus the yield fell on longer incubation (Expt. 3).

Dihydroxypteridines, however, are not affected by acid, and hence a longer incubation was given when glyoxylic acid and 2-hydroxypurine were combined, and a yield of 85% of 2:6-dihydroxypteridine resulted (Expt. 4). Ethyl glyoxylate hemiacetal gave similar results (Expt. 5) and, since it is more accessible than glyoxylic acid, was used in Expts. 6 and 7.

A hydroxyl group in the 6 (or 8) position of purine does not facilitate degradation to the same extent as in the 2 position. Even at 120°, hypoxanthine gave only 0.5% of 4:6-dihydroxypteridine (Expt. 6).

Since 12% of guanine was converted into xanthine at 120° in *N* hydrochloric acid in 6 hr. the reaction between guanine and ethyl glyoxylate was carried out at 110°. However, the reactivity of the degradation products of xanthine proved to be greater than those of guanine because more 2:4:6-trihydroxypteridine than the 2-amino-4:6-dihydroxy compound (xanthopterin) was produced, and the total yield was small (Expt. 7).

When the 9 position in a purine is alkylated, as in naturally occurring purines, considerations of valency lead to a different type of product with glyoxal and diketones. Thus to give a pteridine with diacetyl, 5-amino-2-hydroxy-4-methylaminopyrimidine (V), from the degradation of 2-hydroxy-9-methylpurine, must surrender a hydrogen from the hydroxyl group, and submit to a quinonoid rearrangement of bonds as in riboflavin. The product, 6:7:8-trimethyl-2-pteridone (VI) was obtained in only moderate yield (Expt. 8), because this arrangement of bonds gives a strained molecule in the pteridine series; in the presence of acid the reverse reaction, to (V) and diacetyl, also occurs.

It is interesting to consider the course that the transformation would follow in alkaline media.

Table 1. Transformation of purines into pteridines

Expt. no.	Purine starting material	1:2-Dicarbonyl substance	Temp.	Solvent	Time (hr.)	Pteridine product	Yield (%)
1	2-Hydroxypurine	Glyoxal	37°	Water	168	2-Hydroxypteridine	16
2	2-Hydroxypurine	Glyoxal	37	<i>N</i> -H ₂ SO ₄	24	2-Hydroxypteridine	84
3	2-Hydroxypurine	Glyoxal	37	<i>N</i> -H ₂ SO ₄	168	2-Hydroxypteridine	39*
4	2-Hydroxypurine	Glyoxylic acid	37	<i>N</i> -HCl	72	2:6-Dihydroxypteridine	85
5	2-Hydroxypurine	Ethyl glyoxylate hemiacetal	37	<i>N</i> -HCl	72	2:6-Dihydroxypteridine	79
6	6-Hydroxypurine	Ethyl glyoxylate hemiacetal	120	<i>N</i> -HCl	6	4:6-Dihydroxypteridine	0.49
7	2-Amino-6-hydroxypurine	Ethyl glyoxylate hemiacetal	110	<i>N</i> -HCl	6	2-Amino-4:6-dihydroxypteridine [xanthopterin]	0.08
						2:4:6-Trihydroxypteridine	0.26
8	2-Hydroxy-9-methylpurine	Diacetyl	37	0.01 <i>N</i> -HCl	72	6:7:8-Trimethyl-2-pteridone	~7

* Plus a quantity of the purple substance (? dimer) formed by the action of acid on 2-hydroxypteridine (Albert *et al.* 1951).

9-Methylpurine is easily split by alkali (to 5-amino-4-methylaminopyrimidine) (Albert & Brown, 1954), and so is 9-ribosylpurine (Brown, 1957). In general, those purines so substituted as to give no ionizable hydrogen undergo this decomposition, e.g. 7-methyladenine or 1:7-dimethylhypoxanthine. A very few purines, notably 9-methyladenine and adenosine, fulfil this structural requirement and yet are not split by boiling π sodium hydroxide. The choice of the suitable dicarbonyl substance for these alkaline condensations is limited. However, ethyl glyoxylate hemiacetal survived boiling 2N sodium carbonate for an hour, giving a yield of 77% of 7-hydroxypteridine from 4:5-diaminopyrimidine (Albert *et al.* 1956), and a little 8-methyl-7-pteridone (VII) was obtained from 9-methylpurine. The 1:2-diketones, although stable to alkali, introduce difficulties in satisfying valency.

The products from 7-methylpurines and glyoxylic acid should have quite another character, because Brown & Mason (1957) showed that 4-amino-5-methylaminopyrimidine and glyoxylic acid (except in strong acid) give a dihydropteridine, 5:6-dihydro-6:7-dihydroxy-5-methylpteridine (VIII).

(VII)

(VIII)

Biological implications. It has been suggested (Albert, 1954) that the biological origin of pteridines is from the union of naturally occurring 1:2-dicarbonyl substances (such as glyoxylic, pyruvic and oxaloacetic acids) and 4:5-diaminopyrimidines, and that these pyrimidines arise by the degradation of natural purines or purine ribosides. Some confirmation of these hypotheses has recently been published. [2- 14 C]2:4:5-Triamino-6-hydroxypyrimidine was fed to pierid caterpillars; [2- 14 C]xanthopterin was isolated from the wings (Weygand & Waldschmidt, 1955). More relevant is the transformation of a purine into a pteridine in larvae of the amphibian *Xenopus*. These were intradermally injected with [2- 14 C]guanine, then ten days later the skins were extracted with π ammonia solution at 20° and chromatographed. A blue-fluorescing spot was oxidized to 2-amino-4-hydroxypteridine-6-carboxylic acid, which was radioactive (Ziegler-Günder *et al.* 1956).

The purine $\rightarrow$ pteridine reaction may be significant in the regulation of growth, because some

pteridines have powerful and specific growth-regulating properties. For example, formyltetrahydropteroylglutamic acid is essential, in traces, for driving the mitosis of mammalian cells past metaphase into anaphase (Jacobson, 1952, 1954). Also, traces of xanthopterin enormously increase the mitotic rate in mammalian kidneys, which grow far beyond their normal size (Haddow, 1947, and personal communication). Although cells contain a comparatively large amount of purines, the amount of pteridines is usually minute. Hence it is conceivable that natural growth could be accelerated by facilitating reactions of the type (I) $\rightarrow$ (IV) through a local abundance of either 1:2-dicarbonyl substances or of the transforming enzyme(s).

SUMMARY

1. 2-Hydroxypurine reacts with 1:2-dicarbonyl substances, such as glyoxal or glyoxylic acid, to give the corresponding pteridines in good yield.

2. Guanine and hypoxanthine undergo similar transformations, but less readily.

3. The evidence that naturally occurring pteridines arise from purines (or purine derivatives) is reviewed, and it is suggested that this reaction may regulate growth in certain circumstances.

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