Pteridine Studies. Part VII. The Degradation of 4-, 6-, and 7-Hydroxypteridine by Acid and Alkali.*

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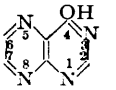
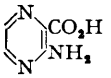
Hot dilute acid and alkali readily degraded 4-hydroxypteridine (I) to 2-aminopyrazine-3-carboxylic acid (II) and its amide; acid also gave rise to 2-aminopyrazine. 7-Hydroxypteridine gave 4 : 5-diaminopyrimidine with alkali, but dilute acid unexpectedly isomerized it to 6-hydroxypteridine.

6-Hydroxypteridine was degraded to 4 : 5-diaminopyrimidine by boiling solutions buffered between pH 6 and 10. There was no action below this range, but above it an extraordinary disproportionation occurred, giving 6 : 7-dihydroxypteridine and 7 : 8-dihydro-6-hydroxypteridine. Under defined conditions the last-named combined with unchanged 6-hydroxypteridine to give two dipteridyls. Ammonia gave a dipteridylamine. Hydroxylamine added across the 7 : 8-double bond of 6-hydroxypteridine to give (finally) 7-amino-6-hydroxypteridine, and it is suggested that this reaction provides a clue to the mechanism of the disproportionation and dimerizations.

IT has been shown that the monohydroxypteridines are, as a class, much more labile than their polyhydroxy-analogues (Albert, Brown, and Cheeseman, *J.*, 1952, 4219), the criteria being the percentages remaining after an hour's refluxing with N-sulphuric acid and N-sodium hydroxide. The nature and quantity of the principal decomposition products have now been determined.

(I)   (II)

4-Hydroxypteridine (I).—Acid decomposition produced 2-aminopyrazine-3-carboxylic acid (II), its amide, and 2-aminopyrazine, in the amounts given in Table 1. Identification was made by mixed melting points and comparison of ultraviolet spectra. The amide was determined by quantitative paper chromatography, developed in water. The amide had the lowest R_F of the four spots; the remaining three spots (the other two pyrazines and

TABLE 1. *Hydrolysis of 4-hydroxypteridine (2 hours at 100°) with* N-H_2SO_4 (3 *equiv.*) *or* N-NaOH (2 *equiv.*)

			Acid hydrolysis (details)				Alkaline hydrolysis (summary)
	$\gamma_{max.}$		$d_{1\,cm.}$ Unknown	Standard	Recovery (%) of	Yield (%) of	Yield (%) of
2-Aminopyrazine	(mμ)	log ε	(4 × 10⁻⁵M)	(2 × 10⁻⁵M)	standard	product	product
(Unsubstituted) as cation	325	3·77	0·113	0·105	90 [a]	53·8	0
-3-carboxylic acid	340	3·76	0·082	0·118	103	34·8	61
-3-amide	350	3·80	0·011	0·126	100	4·4	6
	246	4·06	0·020	0·227	98	4·4	—

[a] Complete recovery could be obtained at pH 1 (instead of 2), but the higher acidity interfered with the electrophoresis.

uncharged starting material) were too close to one another, in this and all other solvents tried, for this technique to be used. However, the other pyrazines were conveniently determined by quantitative paper electrophoresis. From the pK values, it was concluded that pH 5·8 would be suitable for uniquely moving 2-aminopyrazine-3-carboxylic acid to the anode [$pK_a = 3·70$ (basic); < 1 (acidic)]. This was confirmed by a trial run on a mixture of the four substances concerned. The process was then applied to the hydrolysis

* Part VI, S. F. Mason, *J.*, 1955, 2336.

mixture, and the relevant spot was eluted, and analysed as in quantitative paper chromatography (for details of both processes see Experimental section). Similarly, at pH 2·0, 2-aminopyrazine ($pK_a = 3·14$) was uniquely moved to the cathode and analysed.

Alkaline decomposition of 4-hydroxypteridine produced the same acid and amide, but no 2-aminopyrazine (see Table 1). Much 4-hydroxypteridine remained but this could not be determined directly, because it was invisible under the 365-mμ lamp and, although visible, was rapidly photo-decomposed under the 254-mμ lamp. No other spots were found.

7-*Hydroxypteridine.*—Here again, the oxygen-bearing ring was readily opened by acid and alkali. After 4 hours in N-potassium hydroxide at 100°, 42% of 4 : 5-diaminopyrimidine was found by quantitative paper chromatography. This substance was isolated (35%) from the reaction mixture, together with unchanged 7-hydroxypteridine (40%). The latter could not be determined chromatographically, because of its photosensitivity at 254 mμ.

In dilute mineral acids at 37° 7-hydroxypteridine was converted in 5 days into 6-hydroxypteridine in 85% yield. The course of this reaction is apparently: hydrolysis to glyoxylic acid and 4 : 5-diaminopyrimidine (which was detected in the mother-liquor) and re-combination to 6-hydroxypteridine. The latter reaction is known to be favoured by low pH values (Albert, Brown, and Cheeseman, *J.*, 1952, 1620). The identity of the product was confirmed by comparison of all the following properties with material prepared from 2-chloro-4-glycinyl-5-nitropyrimidine, an unambiguous synthesis (Albert *et al.*, *J.*, 1952, 1620): elementary analysis, basic pK_a and hysteresis loop on titration, ultraviolet spectra of cation and of neutral molecule, also the infrared spectrum. The most convenient synthesis of 6-hydroxypteridine has been the condensation of 4 : 5-diaminopyrimidine with ethyl glyoxylate in 2N-sulphuric acid at 20° for 18 hours (Albert *et al.*, *J.*, 1952, 1620), giving 65% of the 6-isomer mixed with 20% of the 7-isomer. The above isomerization indicated that it would be better to conduct the condensation at 37° for 5 days, and an 85% yield of pure 6-hydroxypteridine was obtained in this way.

6-*Hydroxypteridine.*—The reactions of the substance commonly known as 6-hydroxypteridine become clearer if it is borne in mind that it is a pseudo-acid (cf. Albert, Ciba Symposium on the Chemistry and Biology of Pteridines, Churchill, London, 1954, p. 210), and can be converted into the sodium salt of the true acid only after a slow chemical change. This is evident from the hysteresis loop traced out in titration, the addition of alkali giving pH values much higher than those found on back-titration (Albert *et al.*, *J.*, 1952, 1620). No other monoydroxypteridine behaves thus. The anhydrous sodium salt (described below) is normal, but the pseudo-acid, which retains one molecule of water in excess of $C_6H_4ON_4$ up to 180° (where it begins to darken), has a spectrum indicating less conjugation than the other monohydroxylated dihydropteridine. It may well be a dihydroxylated dihydropteridine.

The ionic state of 6-hydroxypteridine in the experiments now to be described has been calculated from the pK_a values for this substance (Albert, Brown, and Cheeseman, *J.*, 1952, 1620) by the equation: Percent ionized $= 100/[1 + \text{antilog}\ (pH - pK_a)]$. Below pH 2, the "pseudo-acid" is the cation ($pK_a = 3·7$) and at pH 5, the neutral molecule of a weak acid ($pK_a = \sim 9·7$). Above pH 5 it enters into slow equilibrium with the anion of the true acid ($pK_a = 6·7$) which can be isolated only as a salt. At pH 7·2 this stronger acid is 75% ionized, but much of the pseudo-acid is also present (non-ionized). At pH 9·2, the ionization of the stronger acid is 99% complete, but only at pH 11 is the pseudo-acid completely converted into the stronger one.

Recognition and separation of the products of decomposition of 6-hydroxypteridine were facilitated by the R_F values and ionization constants assembled in Table 2, and infrared spectra helped to establish identity and purity.

6-Hydroxypteridine was unchanged when refluxed with N-sulphuric acid (1 hour), or with buffer at pH 5 (7 hours). Thus, the cation and the neutral molecule of the pseudo-acid are stable to hydrolysis by H^+. However, refluxing (7 hours) with buffers at pH 7·2 and 9·2 produces, respectively, 34 and 11% of 4 : 5-diaminopyrimidine, with about 1% of 7-hydroxypteridine. No other products were detected, and the unchanged 6-hydroxypteridine was easily recovered at pH 5·5 where it is sparingly soluble. These figures

suggest that the neutral molecule of one of the acids is susceptible to hydrolysis by OH^-.

At pH 10·5, no 4 : 5-diaminopyrimidine was formed. Instead, a new reaction occurred, one not involving degradation of the pteridine skeleton. This reaction was more conveniently studied at higher pH's, and hence discussion of this experiment is deferred to p. 2693.

TABLE 2. *Ionization and chromatographic data used in recognizing and separating products related to 6-hydroxypteridine.*

			Paper chromatography [e] R_F and colour of spot under u.v. lamps					
			Developed in butanol–acetic acid (7 : 3) [f]			Developed in 3% ammonium chloride [p]		
No.	Substance [a]	pK_a	R_F	254 mμ	365 mμ	R_F	254 mμ	365 mμ
	Pyrimidine							
1	4 : 5-Diamino-	6·0 [b]; <0	0·30	D	X	0·65	D	X
2	4-Amino-5-hydroxyacetamido-	(*ca.* 6)	0·20	D	X	0·75	D	X
3	5-Amino-4-carboxymethylamino-	3 [c]; 6·5 [b]	0·15—0·20 [g]	D	X	0·80	D	X
	Pteridine							
4	6-Hydroxy-	3·7 [b]; 6·7 [c,d]	0·25 [h]	B	X	0·45 [m]	B	X
			0·35—0·80	D	X	0·05	D	X
5	7-Hydroxy-	1·2 [b]; 6·4 [c]	0·50	D	X	0·65 [j]	D	X
				B [n]	B		B [n]	B
6	6 : 7-Dihydroxy-	<2 [b]; 6·9 [c]; 10 [c]	0·25 [i]	B	V	0·45 [i]	B	V
7	7 : 8-Dihydro-6-hydroxy-	4·8 [b]; 10·5 [c]	0·30 [j]	D	X	0·45 [i]	D	X
8	7-Amino-6-hydroxy-	See Table 3	0·20 [k]	DV	V	0·30 [k]	DV	V
	Various							
9	8-Hydroxymethylpurine	Table 3	0·45	D	X	0·65 [j]	D	X
10	Substance O	0 [b]; >9 [c]	0·05	Y	Y	O	Y	Y
11	Substance P	<3 [b]; >7 [c]	0·15 [l]	D	X	O	D	X

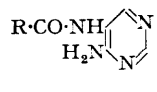
B = Blue fluorescence. D = Dark, against fluorescent background of paper (*i.e.*, absorption without fluorescence). DV = Intermediate between D and V. V = Violet fluorescence. X = Invisible. Y = Yellow fluorescence.

[a] Dissolved in cold 0·1N-sodium hydroxide except No. 2 (water) and 10 (KOH; solution must be used within 5 min. of preparation). [b] Basic. [c] Acidic. [d] See text. [e] Ascending method. R_F's differ from those obtained by the descending method (Albert *et al.*, *J.*, 1952, 1620). [f] pH = 2·7. [g] Streak caused by slow ring-closure in acidic solvent. [h] This spot arises from incipient disproportionation, and is seen only if the compound is kept alkaline. [i] Coincides with No. 4. [j] Coincides with No. 1. [k] Streaks back to origin. [l] Spot is crescent-shaped with the horns pointing downwards. [m] The 0·45 spot may arise from incipient disproportionation and is not seen when working below 20°. When eluted and reapplied, each spot generates the two spots. [n] Change due to photodecomposition after exposure (254 mμ) for a few seconds. [p] pH = 5·5.

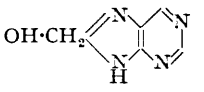
When dissolved in N-sodium hydroxide 6-hydroxypteridine quickly deposited the normal sodium salt which in the solid state was stable at 110° and kept for 6 months at room temperature also in 0·01M-aqueous solution (pH 9) for three days at 20°. When a suspension of the salt in N-sodium hydroxide was left at 20°, the 6-hydroxypteridine all disappeared by the fifth day, its place being taken by 7 : 8-dihydro-6-hydroxy- and 6 : 7-dihydroxypteridine, isolated in 40% and 45% yields respectively (the only other substance present was apparently a dipteridyl, provisionally called substance P, in 8% yield). This reaction is a disproportionation of 6-hydroxypteridine into a product with one more oxygen atom and another with two more hydrogen atoms, and differs from previous disproportionations of hydroxy-heterocyclic compounds where one product loses one oxygen atom and the other loses two hydrogen atoms (*e.g.*, *N*-methyl-5-hydroxyacridan, Albert, "The Acridines," Edward Arnold and Co., London, 1951, p. 187; hydrastinine, McGeogh and Stevens, *J.*, 1934, 1465; cotarnine, Ingold, "Structure and Mechanism in Organic Chemistry," Bell, London, 1953, p. 580).

It was at first thought that this disproportionation may proceed by ring-opening to the pyridine aldehyde (III*a*) followed by a Cannizzaro reaction. If this were so, 4-amino-5-hydroxyacetamidopyrimidine (III*b*) should cyclize in cold N-sodium hydroxide to

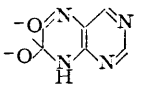
7 : 8-dihydro-6-hydroxypteridine. However, it was found that this pyrimidine, prepared from glycollic acid and 4 : 5-diaminopyrimidine, gave only 8-hydroxymethylpurine (IV) and 4 : 5-diaminopyrimidine, when thus treated. Hence the disproportionation proceeds without ring-opening by attack of a hydroxyl ion on the anion of 6-hydroxypteridine (no neutral molecules are present at this high pH). It is unlikely to be a nucleophilic attack, because that would be expected to occur at position 4 (electron-density diagrams of pteridine show the 4-position to be much poorer than the 7-position in electrons, and the presence of the 6-hydroxy-anion should increase this difference; cf. Albert, *Quart. Rev.*, 1952, **6**, 213). It is more likely that a hydroxyl ion adds across the double bond, to give



(III) (*a*) R = CHO
(*b*) R = CH_2·OH



(IV)



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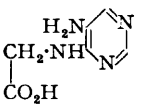


(VI)

the ion (V) [the 7 : 8-bond has the highest degree of double-bond character in the pteridine nucleus, according to recent calculations by C. A. Coulson and T. H. Goodwin (personal communication)]. It is then supposed that the ion (V) transfers a hydride ion from the 7-position to a molecule of starting material which becomes (a dianion of) 7 : 8-dihydro-6-hydroxypteridine, a process which necessarily converts (V) into (an anion of) 6 : 7-dihydroxypteridine.

Another reaction was found which presumably involves an addition across the 7 : 8-double bond : the formation of 7-amino-6-hydroxypteridine from 6-hydroxypteridine and hydroxylamine. The new substance was readily hydrolysed to 6 : 7-dihydroxypteridine by acid or alkali.

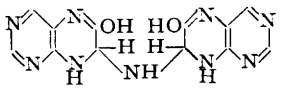
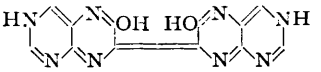
Disproportionation of 6-hydroxypteridine in boiling N-sodium hydroxide was complete in 15 minutes, but gave only 6 : 7-dihydroxypteridine (35% isolated) and substance P (52% isolated). The latter is evidently formed by combination between the 7 : 8-dihydro-6-hydroxypteridine (which in the 20° experiment is largely protected by the poor solubility of its sodium salt at that temperature) and unchanged 6-hydroxypteridine. This is readily shown by the formation of substance P (25%) from these two substances in 0·1N-sodium hydroxide at 20° in 2 days (at this dilution of alkali, the products of disproportionation are barely detectable before 3 days). Substance P is not formed from either 6-hydroxypteridine or the 7 : 8-dihydro-derivative under these mild conditions : both must be present. Likewise it is not formed from the pyrimidine (VI) (see below) and 6-hydroxypteridine under these conditions.

When 7 : 8-dihydro-6-hydroxypteridine was boiled with N-sodium hydroxide for 1½ hours, the ring opened to give 5-amino-4-carboxymethylaminopyrimidine (VI), which rapidly recyclizes in cold 0·1N-hydrochloric acid. These reactions are similar to those given by 5 : 6-dihydro-7-hydroxypteridine (Albert *et al.*, *J.*, 1952, 1620). None of this pyrimidine (VI) was present after the hot disproportionation of 6-hydroxypteridine, 7 : 8-dihydro-6-hydroxypteridine being removed, as substance P, faster than it can be hydrolysed.

The action of boiling 2N-sodium carbonate on 6-hydroxypteridine is similar to that of sodium hydroxide, but much slower. After 4 hours the products were 6 : 7-dihydroxypteridine (15%), substance P (25%), unchanged starting material (32%), and a new reddish-orange dipteridyl, provisionally called substance O (12%). No 4 : 5-diaminopyrimidine or 7 : 8-dihydro-6-hydroxypteridine could be detected.

Ammonia behaved differently : a solution of 6-hydroxypteridine in 2N-ammonia rapidly deposited large yellow crystals of the ammonium salt, but within 5 minutes these began to change into a white, amorphous precipitate (substance N). Analyses indicated a formula $(C_6H_4ON_4)_2NH_3$, and when gently warmed with N-hydrochloric acid or 0·1N-sodium hydroxide the substance gave 6-hydroxypteridine (90% yield) and ammonia. However, substance N is not a salt or loosely bound adduct of ammonia and 6-hydroxypteridine, because none of the latter could be detected by paper chromatography. Also, substance N did not dissolve in boiling acetic acid, in which 6-hydroxypteridine is very soluble.

Dr. S. F. Mason reports that substance N shows 8 bands (in the "fingerprint region" of the infrared) that are present in neither 6-hydroxypteridine nor its sodium salt; also there is a strong carbonyl band near 1600 cm.$^{-1}$. The most likely constitution seems to be that of the dipteridylamine (VII), formed by the addition of one molecule of ammonia across the 7 : 8-double bond of 6-hydroxypteridine to give 7-amino-7 : 8-dihydro-6-hydroxypteridine and further reaction of this with another molecule of 6-hydroxypteridine. This mechanism receives support from the formation of 7-amino-6-hydroxypteridine instead of substance N when an oxidizing agent is present. Less attractive would be the formulation of substance N as an analogue of (*a*) diacetonamine or (*b*) dibarbiturylmethylamine (Möhlau and Lither, *J. prakt. Chem.*, **1906**, **73**, 475); these would require respectively one and two of the oxygen atoms to be present as water of crystallisation, but water is not evolved even at 240°.

(VII)   (VIII)

The orange substance, $C_{12}H_8O_2N_8$ (substance O), is formed when 6-hydroxypteridine is refluxed with (*a*) 2N-sodium carbonate (0% in 30 minutes, 5% in one hour, 12% in 4 hours), (*b*) aqueous hydroxylamine (5% in 30 minutes), or (*c*) M-ammonium hydrogen sulphide (50% in 10 minutes). The helpful role of reducing conditions is explained by the increased yield obtained when 6-hydroxypteridine and 7 : 8-dihydro-6-hydroxypteridine were refluxed together in 2N-sodium carbonate (18% in 30 minutes); the dihydro-compound alone was ineffective. It may be supposed that the 7-methylene group in the dihydropteridine adds across the 7 : 8-double bond of 6-hydroxypteridine to give 7 : 8 : 7′ : 8′-tetrahydro-6 : 6′-dihydroxy-7 : 7′-dipteridyl, which loses two hydrogen atoms to give substance O (VIII).* This 7-methylene group proved active enough to couple with diazotized sulphanilic acid.

Substance O could not be reduced by hydrogen over platinum at 20°, sodium amalgam, sodium borohydride, sodium dithionite, or tin and acid. In cold 0·1N-potassium hydroxide it rapidly absorbed oxygen from the air, giving two equivalents of 6 : 7-dihydroxypteridine. When air was excluded, a ruby-red dipotassium salt was formed. Substance O is unaffected by boiling N-hydrochloric acid. No water was lost at 250°. Formula (VIII) with its long conjugated pathway, two acidic groups, and lack of benzenoid character (regained on oxidation) fits these facts well. Unfortunately, as with substances N and P, the poor solubility prevented determination of molecular weight.

Substance P ($C_{12}H_{10}O_2N_8$) also is formed by the union of 6-hydroxy- and 7 : 8-dihydro-6-hydroxy-pteridine (see above). This reaction is reversible, for when the substance is boiled with 0·1N-sodium hydroxide, traces of 6-hydroxy- and 7 : 8-dihydro-6-hydroxypteridine were obtained (boiling with water or N-hydrochloric acid was ineffective). It is a diacidic base, and the fact that it is a stronger acid than substance O was used in their separation. Substance P does not auto-oxidize in alkaline solution. Chemical oxidation did not produce substance O, but gave two equivalents of 6-hydroxypteridine. It is suggested, in analogy with the action of hydroxylamine (see p. 2697), that the NH-group of 7 : 8-dihydro-6-hydroxypteridine has added across the 7 : 8-double bond of 6-hydroxypteridine to give 7 : 8 : 7′ : 8′-tetrahydro-6 : 6′-dihydroxy-7 : 8′-dipteridyl (IX). The corresponding 8 : 8′-dipteridyl which is a symmetrical hydrazine is less likely in view of low $\lambda_{max.}$ (Table 3). An analogy exists between the formation of substance P and alloxantin, but the latter is a glycol (Beilstein's Handbuch, 4th Ed., 2nd. Supp. *XXVI*, 335) whereas P is an acid. Nor is P likely to be formed by an aldol-like condensation, because neither 6-hydroxypteridine nor the 7 : 8-dihydro-derivative condenses with benzaldehyde in 0·1N-sodium hydroxide (2 days at 20°).

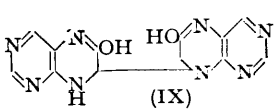

(IX)

Table 3 lists some physical constants of substances described in this paper. The similarity of the spectra of 8-hydroxymethylpurine to those of 8-methylpurine (Mason,

* The hydrogen atoms have been placed at positions 3 : 3′ to give the longest conjugated pathway, but could conceivably be 1 : 1′ or 8 : 8′.

J., 1954, 2072) assisted identification and sharply differentiated this substance from the isomers, 5 : 6-dihydro-7- and 7 : 8-dihydro-6-hydroxypteridine (Albert *et al.*, *J.*, 1952, 1620).

TABLE 3.

Substance	Solubility in H_2O (100°), 1 in	pK_a (in H_2O), spread, and concn. (20°)	Spectrophotometry in H_2O: $\lambda_{max.}$ (mμ)	log $\varepsilon_{max.}$ (mol.)	pH
7-Amino-6-hydroxypteridine	5000	—	233; 310; 323; 339	4·01; 4·24; 4·35; 4·16	4·8; 6·0
anion	—	(about 8)	241; 329; 343	4·20; 4·29; 4·20	10; 13
5-Amino-4-carboxymethylaminopyrimidine	30	—	290	4·05	4·74 [d]
cation	—	6·54 ± 0·07 (0·03M)	—	—	1 [e]
anion	—	2·9 [a]	253; 289	3·88; 3·85	8·8
8-Hydroxymethylpurine	14	—	266	4·01	6·1
cation	—	2·62 ± 0·02 (0·02M)	265	3·94	0
anion	—	8·79 ± 0·01 (0·02M)	275	4·01	11·4
Substance N	20,000	—	—	—	—
Substance O	>100,000	—	—	—	—
cation	—	(about 1) [b]	297; 419; 445	3·81; 4·63; 4·63	0
anion	—	—	460; 490 [c]	—	13
Substance P	>100,000	—	—	—	—
cation	—	—	292	4·33	1
anion	—	—	299	4·30	13

[a] Approximate, because of tendency for ring to close in acid. [b] Determined spectrometrically. [c] Only approximate because of rapid oxidation, even in de-aerated solvent and covered cells. [d] At this pH, 97% is zwitterionic. [e] Ring-closure to 7 : 8-dihydro-6-hydroxypteridine is rapid at this pH.

EXPERIMENTAL

Microanalyses were by Mr. P. R. W. Baker, Beckenham. The yields of substances that lack a m. p. refer to the stage at which they became chromatographically homogeneous (on paper). All substances were dried at 120°, unless otherwise specified.

Hydrolysis of 4-Hydroxypteridine.—4-Hydroxypteridine (0·1482 g.; Albert, Brown, and Cheeseman, *J.*, 1951, 474) was refluxed with N-sulphuric acid (3 ml., 3 equiv.) or N-sodium hydroxide (2 ml.) for 2 hr., then diluted to 60 ml., and added to 0·01M-phosphate buffer (10 ml.; pH 6). This solution was adjusted to pH 6, and then diluted to 100 ml. (thus it became 0·01M with reference to 4-hydroxypteridine). 0·005M-Solutions were prepared from 2-aminopyrazine, its 3-carboxylic acid, and the corresponding amide, adjusted to the same pH and same content of phosphate and sulphate. Care was taken to choose a pH where only one ionic species could be present. For determination of the amide (by paper chromatography), the digest and the standard (0·02 ml. of each) were applied separately with an " Agla " micrometer syringe to a 24" sheet of Whatman No. 1 filter paper, 1" above the lower edge. This was developed with water (ascending method). The dried chromatograms were examined in light of λ 254 mμ (Thermal Syndicate's lamp T/M5/369E, with Chance Brothers' filter OX7/19874). The standard and the unknown spots were marked in pencil and cut out as strips 1" × 4½", with the spot near to one end which tapered to a point. A blank strip in the same R_F area was also cut out. These three strips were separately eluted with 0·1N-hydrochloric acid as described by Brimley and Barrett (" Practical Chromatography," London, Chapman and Hall, 1953). The eluates (2—3 ml.) were diluted to 5 ml. and examined in a Hilger " Uvispek " photoelectric spectrophotometer at $\lambda_{max.}$ (the eluate from the blank strip was used in the reference cell). The results are given in Table 4. The experiment was run (in duplicate) in the dark to avoid contamination with the photolytic products of 4-hydroxypteridine.

For the determination of 2-aminopyrazine and its 3-carboxylic acid (by paper electrophoresis), the digest and a 0·005M-standard (0·02 ml. of each) were similarly applied (1" apart) to the central line of a No. 1 Whatman paper. Buffer solution (phosphate pH 2·00 and 5·80 respectively) was allowed to rise by capillarity from both ends of the paper. When the two fronts had met, the current (2·6 mA, 220 V, direct) was passed for 3½ hr. The paper was dried at 110° for 5 min. and the spots were outlined in pencil under 365-mμ light (an ordinary Woods lamp). The standard, blank, and unknown strips were cut out as above, eluted overnight

with 0·1N-hydrochloric acid (for 2-aminopyrazine) or with 0·01M-phosphate buffer of pH 6·0 (for the carboxylic acid), and examined as above. The results are given in Table 1.

Alkaline Hydrolysis of 7-Hydroxypteridine.—7-Hydroxypteridine (Albert *et al.*, *J.*, 1952, 1620) was refluxed with N-potassium hydroxide, under the conditions used for the 4-isomer. The resulting solution was adjusted to pH 3·0 with N-sulphuric acid and diluted to 100 ml. A 0·005M-solution of 4 : 5-diaminopyrimidine was prepared with the same pH and concentration of potassium sulphate. These were applied to papers (as above), two of which were developed by chromatography in 3% aqueous ammonium chloride, and two in *n*-butanol (2 vols.)–5N-acetic acid (1 vol.). Brief examination under the 254-mμ lamp, which is somewhat destructive for 4 : 5-diaminopyrimidine, enabled the spots to be marked, and they were eluted and submitted to spectrophotometry as above. The results are given in Table 4.

TABLE 4. *Alkaline hydrolysis of 7-hydroxypteridine to 4 : 5-diaminopyrimidine.*

Developer	$\lambda_{max.}$ (mμ)	log ε	$d_{1\ cm.}$ Unknown (4×10^{-5}M)	$d_{1\ cm.}$ Standard (2×10^{-5}M)	Recovery (%) of standard	Yield (%) of product
Ammonium chloride (3% aq.) ...	284	3·937	0·120	0·138	80 [a]	44
Butanol–5N-AcOH (2 : 1)	,,	,,	0·110	0·142	82	39

[a] 4 : 5-Diaminopyrimidine is sensitive to the wavelength (254 mμ) used to render the spot visible.

Recovery of 7-Hydroxypteridine and 4 : 5-Diaminopyrimidine.—After a similar alkaline hydrolysis, but with N-sodium hydroxide, the solution was cooled to 20° and the pure sodium salt of 7-hydroxypteridine filtered off after 10 min. The filtrate was adjusted to pH 10 with sodium hydrogen carbonate and evaporated to dryness. The residue was exhaustively extracted with alcohol, and the extract decolorized (carbon) and concentrated, giving pale 4 : 5-diaminopyrimidine, m. p. and mixed m. p. 200—201°.

Action of Acid on 7-Hydroxypteridine.—7-Hydroxypteridine (1 g.; fine powder) and 2N-sulphuric acid (15 ml., 4·5 equiv.) were set aside at 37° for 5 days. The solution was decolorized with acid-extracted charcoal. Sodium citrate (2 g.) was added and enough 6N-sodium hydroxide to give pH 5·5. The deposit of 6-hydroxypteridine (0·93 g.) was chromatographically homogeneous (on paper). It was recrystallized from 230 parts of water (Found: for material dried at 120°/0·1 mm.: C, 43·2; H, 3·5; N, 33·7. Calc. for $C_6H_4ON_4,H_2O$: C, 43·4; H, 3·6; N, 33·7%).

6-Hydroxypteridine (Recommended Synthesis).—4 : 5-Diaminopyrimidine (11 g.), ethyl glyoxylate hemiacetal (20 g., 1·3 equiv.; Rigby, *J.*, 1950, 1912) and 2N-sulphuric acid (180 ml.) were kept at 37° for 5 days. The dark solution was shaken with acid-washed carbon (1 g.). The filtrate was taken to pH 5·5 with sodium citrate (10 g.) and 10N-sodium hydroxide (about 11 ml.). The precipitate was refluxed with water (100 ml.) and filtered at the b. p., then recrystallized from 240 parts of boiling water (yield 85% of monohydrate). [6-Hydroxypteridine monohydrate (dried at 110°) is to be understood in all the following preparations where "6-hydroxypteridine" is mentioned.]

Action of Buffer Solutions on 6-Hydroxypteridine.—6-Hydroxypteridine (0·083 g., 0·0005 mole) was refluxed with 0·05M-buffers (20 ml.; acetate pH 5·00, phosphate pH 7·20, borate pH 9·18) for 7 hr. All solutions remained clear. They were adjusted to pH 2·0 with N-hydrochloric acid (because precipitates were formed at intermediate values) and diluted to 50 ml. with 0·01N-hydrochloric acid. They were submitted to paper-electrophoresis (see under 4-hydroxypteridine, above) in pH 4·00 acetate buffer (0·1M), with 4 : 5-diaminopyrimidine (0·005M) as standard. Brief examination under the 254-mμ lamp showed that the 4 : 5-diaminopyrimidine cation (pK_a 6·0) had run much further than the 6-hydroxypteridine. Strips of paper were cut, as before, and eluted, and the eluate measured spectrometrically. The recovery of the standard was 80—84% from these papers, and the calculations were based on this.

Sodium Salt of 6-Hydroxypteridine.—N-Sodium hydroxide (10 ml., 2 equiv.) was added to 6-hydroxypteridine (0·83 g.), suspended in water (5 ml.). The solution deposited yellow crystals of the *sodium salt* which were filtered off after an hour at 0°, washed with alcohol, and dried at 110° (0·8 g.) (Found, for material dried at 110° and 0·01 mm. and burnt over V_2O_5: C, 42·3; H, 2·5; N, 33·25. $C_6H_3ON_4Na$ requires C, 42·4; H, 1·8; N, 33·0. Gain, on exposure, to const. wt.: 17·8%. $C_6H_3ON_4Na,2H_2O$ requires 17·5%).

Action of Cold Sodium Hydroxide Solution.—6-Hydroxypteridine (2·5 g., 0·015 mole), dissolved in cold N-sodium hydroxide (30 ml., 2 equiv.), was stored at 20° for 5 days. The suspension was clarified by warming to 50°. The solution was taken to pH 2 (metanil-yellow) with

5N-sulphuric acid (8 ml.), and filtered after an hour at 20°. The precipitate of 6 : 7-dihydroxypteridine (1·1 g.) recrystallized from 300 parts of water (Found: C, 43·6; H, 2·2; N, 34·7. Calc. for $C_6H_4O_2N_4$: C, 43·9; H, 2·45; N, 34·2%). The filtrate was adjusted to pH 8·5 [$(HO{\cdot}CH_2)_3C{\cdot}NH_2$; 0·5 g.] * and N-sodium hydroxide (12 ml.), and filtered. The precipitate was rapidly recrystallized (as the sodium salt) from boiling N-sodium hydroxide (15 ml.), dissolved in hot water (12 ml.), and adjusted to pH 8·5, giving 7 : 8-dihydro-6-hydroxypteridine (0·88 g.), which recrystallized from 400 parts of water (Found: C, 48·2; H, 4·4; N, 36·6. Calc. for $C_6H_6ON_4$: C, 48·0; H, 4·0; N, 37·3%). The identity of these two substances with unambiguously synthesized material (Albert *et al.*, *J.*, 1952, 1620) was further confirmed by comparison of ionization constants and ultraviolet and infrared spectra. The filtrate from the sodium salt was adjusted to pH 5 with acetic acid and filtered at 100°. The precipitate was recrystallized (as *substance P dihydrochloride*) from N-hydrochloric acid (9 parts) giving large prisms (0·27 g.) (Found, for material dried at 20°/0·1 mm.: C, 35·7; H, 4·0; O, 15·8; N, 27·6; Cl, 17·4. $C_{12}H_{10}O_2N_8,2HCl,2H_2O$ requires C, 35·4; H, 4·0; O, 15·7; N, 27·5; Cl, 17·4%). The crystals were dissolved in 12 parts of boiling 0·1N-hydrochloric acid and adjusted to pH 5 with sodium citrate and hydroxide. The free *base* was filtered off at the b. p. It was insoluble in all solvents tried, and remained unchanged at 350° (Found, for material dried at 120°: C, 48·3; H, 3·4; N, 37·3. $C_{12}H_{10}O_2N_8$ requires C, 48·3; H, 3·4; N, 37·6%). There was no loss on drying further at 150°. More data on substance P are on p. 2698.

4-*Amino*-5-*hydroxyacetamidopyrimidine* (III*b*).—4 : 5-Diaminopyrimidine (0·90 g.; Brown, *J. Appl. Chem.*, 1952, **2**, 239), glycollic acid (cryst.; 1·9 g., 3 equiv.), and water (3 ml.) were heated in an open vessel at 100° for 6 hr. Phosphate buffer (2 ml.; 0·1M; pH 7) was added, then 2N-sodium carbonate until the pH at 100° was 7·5. The product was evaporated and extracted with boiling alcohol (30 ml.). The extract deposited 4 : 5-diaminopyrimidine (0·5 g.) on refrigeration, and the filtrate from this, concentrated *in vacuo* to 3 ml., deposited 4-*amino*-5-*hydroxyacetamidopyrimidine* (0·1 g.), decomp. ~150° to 8-hydroxymethylpurine (Found: C, 42·2; H, 4·7; N, 33·7. $C_6H_8O_2N_4$ requires C, 42·8; H, 4·8; N, 33·3%). It was not quite homogeneous chromatographically, but on attempted recrystallization it partly cyclized to 8-hydroxymethylpurine.

8-*Hydroxymethylpurine* (IV).—Higher yields of this substance were obtained as follows. Ethyl glycollate (13 g., 4 equiv.) and 4 : 5-diaminopyrimidine (3·3 g.) were heated in an open flask at 140° for 2 hr. The excess of ester was recovered *in vacuo*, and the residue recrystallized from water (25 ml.; carbon). The filtrate (pH 3) was adjusted to pH 5·5 before cooling, and yielded 8-*hydroxymethylpurine* (60%), m. p. 262° (effervescence), soluble in 220 parts of cold water. It recrystallized from 200 parts of alcohol (Found: C, 48·2; H, 3·7; N, 37·5. $C_6H_6ON_4$ requires C, 48·0; H, 4·0; N, 37·3%).

7-*Amino*-6-*hydroxypteridine*.—Hydroxylamine hydrochloride (0·84 g., 2 equiv.) was added to a solution of 6-hydroxypteridine (1 g.) and sodium carbonate (1·3 g.) in water (50 ml.). The mixture was refluxed for 30 min. and filtered at 100° from substance O (0·05 g., see below). The filtrate was adjusted to pH 5·5 with sodium citrate and 5N-sulphuric acid, warmed to 100°, and filtered. The colourless precipitate of 7-amino-6-hydroxypteridine (65%) was dissolved in N-sodium hydroxide (2 equiv.) and refrigerated. The *sodium salt* was washed with alcohol and air-dried at 20° (Found: C, 32·5; H, 3·7; N, 31·7. $C_6H_4ON_5Na,2H_2O$ requires C, 32·6; H, 3·6; N, 31·7%). This was dissolved in 100 parts of boiling water and acidified to pH 5·5, and the almost colloidal *base* was collected. It becomes brown about 315° without melting, and is poorly soluble in organic solvents (Found: C, 44·4; H, 3·0; N, 42·3. $C_6H_5ON_5$ requires C, 44·2; H, 3·1; N, 42·9%). It was completely hydrolysed to 6 : 7-dihydroxypteridine when refluxed with 2 equivalents of N-sodium carbonate (1 hr.), N-sodium hydroxide (10 min.), or N-hydrochloric acid (5 min.).

Action of Sodium Hydroxide at 100°.—6-Hydroxypteridine (1·66 g., 0·01 mole) and N-sodium hydroxide (20 ml., 2 equiv.) were gently refluxed for 15 min., adjusted to pH 9·0 with glycine (0·3 g.) and 5N-sulphuric acid, and filtered at 100°. The residue (crude substance P) was purified through the hydrochloride as above (0·86 g.). The filtrate was taken to pH 4 with sodium citrate (0·3 g.) and 5N-sulphuric acid, and filtered after chilling, giving pure 6 : 7-dihydroxypteridine (0·5 g.)

5-*Amino*-4-*carboxymethylaminopyrimidine* (VI).—7 : 8-Dihydro-6-hydroxypteridine (0·9 g.) and N-sodium hydroxide (12 ml., 2 equiv.) were refluxed for 90 min., cooled, and seeded with

* Buffers of suitable p*K* were used here and elsewhere to sharpen the separations and increase the yields. Trial experiments were conducted which established that they did not initiate chemical change.

the sodium salt of the starting material (0·15 g. recovered by filtration). The filtrate was placed in an ice-bath and carefully brought to pH 5 with acetic acid, and the precipitated *5-amino-4-carboxymethylaminopyrimidine* (75%) recrystallized from 30 parts of water (Found: C, 43·0; H, 4·8; N, 33·6. $C_6H_8O_2N_4$ requires C, 42·9; H, 4·8; N, 33·3%). There was no apparent change at 300°, but chromatography showed that the substance had completely cyclized (no decarboxylation). In 0·1N-hydrochloric acid (2 equiv.) at 20°, ring closure was half-complete in about 1 hr., and complete in 6 hr. (determined by neutralizing and chromatographing samples in ammonium chloride solution); the 7 : 8-dihydro-6-hydroxypteridine was isolated at pH 8·5 (95%).

Synthesis of Substance P from Components.—6-Hydroxypteridine (0·34 g.) and 7 : 8-dihydro-6-hydroxypteridine (0·30 g.) in 0·1N-sodium hydroxide (80 ml.) were left at 20° for 2 days. The clear solution was boiled, adjusted to pH 5, and filtered at 100°. The precipitate was purified through the hydrochloride as above (yield, 0·15 g.).

Action of Sodium Carbonate.—6-Hydroxypteridine (0·5 g., 0·003 mole) and 2N-sodium carbonate (15 ml., 10 equiv.) were refluxed for 4 hr. (pH was 10·5 at start and finish), filtered from substance O (0·06 g., see below) and cooled, then substance P (0·11 g.) was filtered off. The filtrate was adjusted to pH 2·0 (metanil-yellow) with sulphuric acid and filtered at once from 6 : 7-dihydroxypteridine (0·08 g.). The filtrate was adjusted to pH 5·5 (sodium citrate and hydroxide) and chilled and the 6-hydroxypteridine (0·16 g.) filtered off.

Reaction with Ammonia.—6-Hydroxypteridine (0·82 g.) was dissolved in 2N-ammonia (40 ml., 30 equiv.) at 35°, and set aside at 20°. Next day, the suspension was gently refluxed for 5 min. and filtered at the b. p., giving *substance N* (0·3 g.) as a white powder. The filtrate was refluxed again next day and deposited 0·1 g. more. The filtrate from this, brought to pH 5·5, gave 6-hydroxypteridine (0·4 g.). None of the usual products of alkaline decomposition was formed. Substance N is insoluble in all solvents, and in cold 0·1N-sodium hydroxide or hydrochloric acid. It becomes brown at 270° without melting [Found: C, 46·0; H, 3·4; N, 39·5. $(C_6H_4ON_4)_2,NH_3$ requires C, 46·0; H, 3·5; N, 40·3%].

When substance N (0·25 g.) was refluxed with N-hydrochloric acid (5 ml., 3 equiv.) for 5 min., and the solution adjusted to pH 5·5, 6-hydroxypteridine (0·24 g.) was obtained. Similar treatment with 0·1N-sodium hydroxide, but for 30 sec. only, gave 6-hydroxypteridine (0·23 g.).

Potassium ferricyanide (0·33 g., 1 equiv.) and 6-hydroxypteridine (0·08 g.) were dissolved, in that order, in 2N-ammonia (4 ml.) and set aside at 20° for 2 days. The solution was adjusted to pH 5·5. The precipitate, dissolved in N-sodium hydroxide, gave the sodium salt of 7-amino-6-hydroxypteridine (0·06 g., 55%; dried at 20°; dihydrate).

Substance O.—6-Hydroxypteridine (1 g.) and M-ammonium hydrogen sulphide (60 ml., 10 equiv.) were refluxed for 10 min. and filtered at the b. p. giving substance O (0·5 g.). The filtrate contained 7-amino-6-hydroxypteridine (0·2 g.) and a precursor of substance O, which produces it (0·2 g.) when acidified to pH 5 and exposed to air. It was shown by infrared spectra to be identical with the two specimens prepared by other methods (see above). This substance, recrystallized from cold N-potassium hydroxide (2 equiv.) as the *dipotassium salt* (long ruby needles), which was washed with alcohol, and dried at 20°/0·01 mm. (Found: C, 35·1; H, 2·7; N, 27·5; loss at 120°, 9·4. $C_{12}H_6O_2N_8K_2,2H_2O$ requires C, 35·3; H, 2·5; N, 27·45; loss, 8·8. Found, for material dried at 120°: C, 38·6; H, 2·2. $C_{12}H_6O_2N_8K_2$ requires C, 38·7; H, 1·6%). It was suspended in water, adjusted to pH 7 with phosphoric acid, boiled for 5 min., and filtered at 100°, giving *substance O* as reddish-orange crystals (Found: C, 48·3; H, 2·8; N, 37·5. $C_{12}H_8O_2N_8$ requires C, 48·6; H, 2·7; 37·8%). It is unchanged at 350°. It is insoluble in 2N-ammonia, or organic solvents. The solution in 0·1N-sodium hydroxide is glutinous. The salts with mineral acids are lemon-yellow and highly insoluble (the hydrochloride requires 7000 parts of boiling N-hydrochloric acid to dissolve it: ten-fold dilution with water partly liberates the neutral molecule).

Substance P.—Two syntheses of this substance have already been given. It was not formed when 6-hydroxypteridine and 5-amino-4-carboxymethylaminopyrimidine (0·08 g. of each) were set aside at 20° in 0·1N-sodium hydroxide (20 ml.) for 2 days.

0·1M-Potassium permanganate (4·4 ml., equiv. to 2H) was added dropwise, at 20°, to substance P (0·2 g.), and kieselguhr (0·05 g.) in 0·1N-sodium hydroxide (28 ml.). The insoluble material was extracted with boiling water (3 ml.), and the combined filtrates were adjusted at once to pH 5·5; pure 6-hydroxypteridine (0·2 g.) was precipitated.

Substance P (0·08 g.) in 0·1N-sodium hydroxide (10 ml.) was unchanged at 20° for 27 hr., but when it was refluxed therein for an hour, paper chromatography clearly revealed spots characteristic of 6-hydroxypteridine and 7 : 8-dihydro-6-hydroxypteridine. After 2 hours'

refluxing with N-sodium hydroxide (4 equiv.), a spot characteristic of 6 : 7-dihydroxypteridine (arising from disproportionation of 6-hydroxypteridine) also appeared.

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