886. *Pteridine Studies. Part X.* Pteridines with more than One Hydroxy- or Amino-group.*

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Syntheses of new di- and tri-hydroxy- (and amino-)pteridines are described, and a critical discussion of the solubilities of hydroxy- and amino-pteridines is presented. The hysteresis loop traced out by 2 : 6-dihydroxypteridine on titration with alkali (followed by back-titration) is discussed as being due to a further example of covalent hydration.

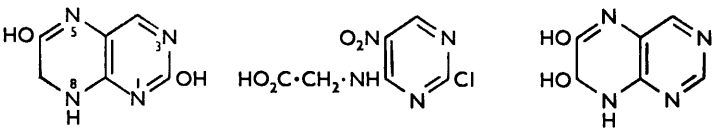
It has been shown that the insertion of a hydroxy- or an amino-group into pteridine greatly decreases the solubility in water, and the explanation has been advanced that this is due to unusually strong O–H–N and N–H–N bonds in the crystal lattice.[1] These are believed to be hydrogen bonds re-inforced by dipoles.[2] The phenomenon has been demonstrated in other heterocyclic systems.[2,3,4] It is known that the insolubilizing group need not be potentially tautomeric,[1] and can even be separated from the nucleus by a methylene group.[5]

The nature of such polyhydroxypteridines as have been examined [1,6] suggests that the addition of each further hydroxy-group continues to decrease the solubility in water, and this is further explored here by the examination of the remaining polyhydroxypteridines.

* Part IX, *J.*, 1956, 3443.

[1] Albert, Brown, and Cheeseman, *J.*, 1952, 4219.
[2] Albert, in "Recent Work on naturally Occurring Nitrogen Heterocyclic Compounds," *Chem. Soc., Special Publ. No.* 3, 1955, p. 126.
[3] Fischer, *Ber.*, 1899, **32**, 498.
[4] (*a*) Albert, *Chem. and Ind.*, 1956, 252; (*b*) Albert and Brown, *J.*, 1954, 2060.
[5] Albert, *Chem. and Ind.*, 1955, 202.
[6] Albert and Brown, *J.*, 1953, 74.

The solubilities of polyamino-heterocycles have not been well explored. In the purine series, the insolubilizing effect of one amino-group is reversed when further such groups are inserted,[4] but this does not appear to be a general rule (cf. 2-amino-, 3-amino-, 2 : 6-diamino-, and 2 : 3-diamino-pyridine, whose solubilities are 1 in 1, <1, 10, and 515 respectively in water at 20°). Hence some polyaminopteridines have been synthesized and examined.



(I) (II) (III)

Hydroxypteridines.—In N-hydrochloric acid, 4 : 5-diamino-2-hydroxypyrimidine combined with ethyl glyoxylate to give 2 : 6-dihydroxypteridine, the 2 : 6-orientation of which was proved when the same substance was obtained by oxidizing 7 : 8-dihydro-2 : 6-dihydroxypteridine [7] (I), prepared from 4-carboxymethylamino-2-chloro-5-nitropyrimidine (II). When the glyoxylate condensation was done at pH 4, a mixture of 2 : 6- and 2 : 7-dihydroxypteridine was produced. This agrees with previous observations that highly acid conditions favour 6-hydroxy-orientation at the expense of the 7-position. Both isomers crystallize with one molecule of water; the 2 : 7-isomer loses this at 180° without further change, but the 2 : 6-isomer decomposes before water is lost. However, there can be no doubt that the pyrazine ring has closed in this " 2 : 6-isomer " because of its formation from (I) which is anhydrous, and because the anion absorbs at so long a wavelength.

The very large difference of 113 mμ between the long-wave ultraviolet absorption peaks of anion and neutral molecule of 2 : 6-dihydroxypteridine points to the formation of a new conjugated double bond in alkali (see Table 1). This reaction is not instantaneous. 2 : 6-Dihydroxypteridine gave two spots in paper chromatography, and each when eluted, equilibrated in acid or alkali, and re-applied to paper developed *both* original spots. 4 : 6-Dihydroxypteridine [6] and xanthopterin [8] also behave thus and, like 6-hydroxypteridine itself, give hysteresis loops on titration with alkali followed by back-titration.[9] The existing evidence suggests that the stable form of the neutral molecule (pK 9·8) of 6-hydroxypteridine is covalently hydrated, and is actually 7 : 8-dihydro-6 : 7-dihydroxypteridine [10] (III), but the stable form of the anion is anhydrous, and corresponds to a stronger acid (pK 6·7). 2 : 6-Dihydroxypteridine is too insoluble to be titrated in this way, but spectrophotometry in a series of buffers revealed a similar hysteresis loop. Thus the absorption at 412 mμ (a peak peculiar to the anion) was plotted against pH, giving two curves depending on whether the substance added to the buffers had been dissolved in water or in alkali (see Figure).

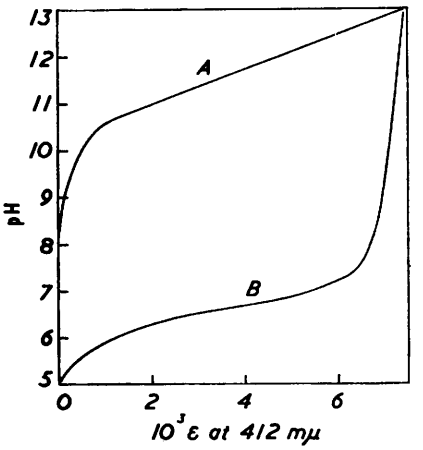
The lower curve (obtained by the rapid adjustment of an alkaline solution to lower pH values) corresponds to an acid of pK ~ 6·7 because 50% of the density (at 412 mμ) of the anionic peak persisted down to pH 6·7. The upper curve (obtained by rapid adjustment of a neutral solution to higher pH values) corresponds roughly to an acid to pH ~ 11·6. However, the upper curve does not have such a theoretically correct shape and it would appear that (as with 6-hydroxypteridine [10]) there are two monoanions as well as two neutral molecules, the anion absorbing at 412 mμ being far the more stable. Further proof that the pK of the stable neutral molecule lies above 9 came from the aqueous solubility which was identical at pH 3·7, 6·3, and 7·9 (20°). The pK of 6·7 represents an equilibrium between the stable anion and the transient neutral molecule, and is assigned to the 6- rather than the 2-hydroxy-group, because the ionization of a 2-hydroxy-group does not induce hysteresis in 2-hydroxy- or 2 : 4- or 2 : 7-di-hydroxy-pteridine. In analogy with 6-hydroxypteridine,[10] the stable neutral molecule would be 7 : 8-dihydro-2 : 6 : 7-trihydroxypteridine in water.

[7] Boon, Jones, and Ramage, *J.*, 1951, 96.
[8] Tschesche and Korte, *Chem. Ber.*, 1952, **85**, 139.
[9] Albert, Ciba Symposium on Chem. and Biol. of Pteridines, Churchill, London, 1954, p. 210.
[10] Brown and Mason, *J.*, 1956, 3443.

But the true structure seems to be yet more complex because even the stable anion (pK 6·7) gives no sign of forming a dianion even at pH 13, whereas the 2 : 7-isomer forms a dianion with pK 10·1 (as the pK of the monoanion is 5·8, the failure of the 2 : 6-isomer to produce a dianion cannot be attributed to a coulombic effect). Thus water may be covalently bound by the pyrimidine ring as well, and the stable neutral molecule would then be 3 : 4 : 7 : 8-tetrahydro-2 : 4 : 6 : 7-tetrahydroxypteridine (IV) (see below).

Condensation of 4 : 5-diaminopyrimidine with ethyl glyoxylate hemiacetal at pH 6 gives a mixture of 6- and 7-hydroxypteridine.[11] However, a method for obtaining the 6-hydroxy-isomer exclusively was described recently,[12] and it is now found that the 7-isomer is formed exclusively and in excellent yield by carrying out the condensation in boiling 2N-sodium carbonate.

The synthesis of trihydroxypteridines will now be discussed. Wieland and Liebig [13] attempted the synthesis of 2 : 4 : 6-trihydroxypteridine from 5 : 6-diamino-2 : 4-dihydroxypyrimidine and glyoxylic acid, but did not examine the product for homogeneity or orientation. By purification through the potassium salt, a product free from the 2 : 4 : 7-isomer



2 : 6-*Dihydroxypteridine. Hysteresis loop caused by slowly equilibrating forms. Readings taken* 60 *sec. after mixing* (4 *cm. cells*).

A, Solution in water added to buffers, whose pH's *were chosen* (*by trial*) *to give about* 10% *progressive change in extinction. B, Solution in* 0·01N-*potassium hydroxide treated similarly.*

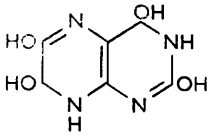
has now been obtained, and the orientation confirmed by obtaining the same substance by the action of sodium amalgam on tetrahydroxypteridine and oxidation of the product, 7 : 8-dihydro-2 : 4 : 6-trihydroxypteridine.[14] This is an established method for selectively removing a 7-hydroxy-group.[11,15]

2 : 4 : 7-Trihydroxypteridine has been obtained from its 6-carboxylic acid.[16] A direct synthesis was attempted from the reactants used to make the 2 : 4 : 6-isomer, but at pH 5 or above. The product, as in the similar synthesis of *iso*xanthopterin,[17] was an anil. This was cyclized with boiling 2N-ammonia, to 2 : 4 : 7-trihydroxypteridine which could not be obtained homogeneous. However, a satisfactory product was obtained by the alkaline hydrolysis of 4-amino-2 : 7-dihydroxypteridine, which was easily prepared from triamino-2-hydroxypyrimidine and ethyl glyoxylate.

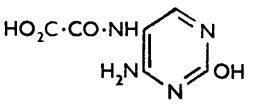
As 4 : 6 : 7-trihydroxypteridine is known,[6] only the 2 : 6 : 7-isomer remained to be made. By heating 4 : 5-diamino-2-hydroxypyrimidine (dry or in water) with oxalic acid or methyl oxalate, also by oxidizing 2 : 6- or 2 : 7-dihydroxypteridine, we obtained a substance which, after recrystallization six times from water (the only suitable solvent), gave elementary analyses consonant with the hemihydrate of the required substance. Nevertheless, it

[11] Albert, Brown, and Cheeseman, *J.*, 1952, 1620.
[12] Albert, *J.*, 1955, 2690.
[13] Wieland and Liebig, *Annalen*, 1944, **555**, 146.
[14] Boon and Leigh, B.P. 677,342/1950.
[15] Totter, *J. Biol. Chem.*, 1944, **154**, 105.
[16] Tschesche and Korte, *Chem. Ber.*, 1951, **84**, 801.
[17] Albert and Wood, *J. Appl. Chem.*, 1953, **3**, 521.

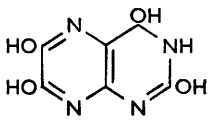
titrated with alkali as a mixture of substances with pK's 6·4 and 3·5. When it was first dissolved in alkali and back-titrated the same pK's were obtained (evidence that it was not hydrolysed by hydrogen ions, the 0·001-M-solution being acidic). No hysteresis was observed during these titrations. In chromatography (in 3% aqueous ammonium chloride) it gave two spots, one fluorescing blue (R_F 0·50) and a dark, absorbing spot (R_F 0·75) (both in light of λ 254 mμ). No known di-, tri-, or tetra-hydroxypteridine gives a dark spot. When the dried paper was kept in the dark for a day, the dark spot changed to a blue fluorescence even when there had been no prior irradiation. However, when the paper was kept moist, the change was exceedingly slow. Separate elution with water of both spots (whether in the dark or the fluorescent condition) and reapplication to paper produced from each eluate a blue fluorescent spot at R_F 0·50, and a dark spot at R_F 0·75 which fluoresced after dry storage. Thus the evidence suggests that the material produced is a mixture of two components which freely enter into equilibrium with one another. The chromatographic behaviour was the same whether the substance was applied to paper in acid, neutral, or alkaline solution.



(IV)



(V)



(VI)

The two most likely explanations of these phenomena are that 2 : 6 : 7-trihydroxypteridine can exist only in equilibrium with (i) its precursor 4-amino-2-hydroxypyrimid-5-yloxamic acid (V), or (ii) a covalently hydrated form, *e.g.*, (VI). The first explanation may seem unlikely, for the three isomeric trihydroxypteridines are stable, unchanged by boiling acid or alkali (as also are tetra- and the six di-hydroxypteridines [1]). Nevertheless it is supported by the formation of 4 : 5-diamino-2-hydroxypyrimidine in boiling acid. Against the second explanation, 6-hydroxypteridines are not hydrated when the 7-position is occupied by a hydroxyl group.[11] No conclusion can yet be reached.

Attempts to make 2 : 6 : 7-trihydroxypteridine by the action of nitrous acid on 2-amino-6 : 7-dihydroxypteridine led to profound decomposition. An improved synthesis of tetrahydroxypteridine is described.

Aminopteridines.—All the monoaminopteridines are known,[19] but only the 2 : 4- and 4 : 7-diaminopteridines.[20,1] The 4 : 6-isomer has now been obtained by the action of ammonia on the chlorination product of 4 : 6-dihydroxypteridine. 2 : 6-, 2 : 7-, and 6 : 7-Dihydroxypteridines gave no chloro-compounds with phosphorus halides, and were destroyed if conditions were severe. 6 : 7-Diaminopteridine could not be obtained by the action of cyanogen or dithio-oxamide on 4 : 5-diaminopyrimidine. The spectra of the neutral molecules of 4 : 6- and 4 : 7-diaminopteridines are almost identical with those of the dianions of the corresponding dihydroxypteridines,[6] a relation established for the mono-substituted analogues.[19]

The only known triaminopteridine is the 2 : 4 : 7-isomer, obtainable in very small yield by heating the 6-carboxylic acid.[20b] It has now been obtained in good yield from 2 : 4 : 7-trihydroxypteridine, *via* the trichloropteridine. The 4 : 6 : 7-isomer was prepared similarly.

Solubility.—Table 2 lists the solubilities of all possible hydroxypteridines (except 2 : 6 : 7-trihydroxypteridine which is not yet known beyond doubt, see above). Only three substances in Table 2 show hysteresis during titration (6-hydroxy- and 2 : 6- and 4 : 6-dihydroxy-pteridine), and two of these are abnormally insoluble. The averages (these two being set aside) show that addition of each hydroxy-group to pteridine further increases insolubility. The figures are compatible with the hypothesis advanced earlier that " 6-hydroxypteridine " may be in reality covalently hydrated to the dihydroxypteridine (III),

[18] Bertho and Bentler, *Annalen*, 1950, **570**, 127; Schöpf, Reichert, and Riefstahl, *ibid.*, 1941, **548**, 82.
[19] Albert, Brown, and Wood, *J.*, 1954, 3832.
[20] (*a*) Mallette, Taylor, and Cain, *J. Amer. Chem. Soc.*, 1947, **69**, 1814; (*b*) Osdene and Timmis, *J.*, 1955, 2036.

Table 1. *Physical properties of pteridines.*

Pteridine	Ionization[c] (H_2O; 20°) pK_a	spread ±	concn. M	Solubility in H_2O 20° 1 in	100°	Spectroscopy in water[j] $\lambda_{max.}$ (mμ)	log ε	pH
2 : 6-Dihydroxy[a]	—	—	—	110,000[d]	4500	235, 299	4·22, 3·79	3·9 or 7·8
monoanion (anhyd.)	6·7[d]	—[h]	0·00002	—	—	225, 246, 412	4·41, 4·23, 3·87	13
monoanion (hydrated[a])	11·6[d]	—[h]	0·00002	—	—	—	—	—
2 : 7-Dihydroxy	—	—	—	1,400	100	258, 328	3·99, 4·11	3·6
monoanion	5·83	0·02	0·005	—	—	282, 343, 359	3·54, 4·42, 4·43	7·9
dianion	10·07	0·06	0·005	—	—	224, 271, 343, 353	4·50, 3·78, 4·25, 4·20	12
2 : 4 : 6-Trihydroxy	—	—	—	7,400	400	223, 248, 300, 364	4·04, 3·99, 3·16, 3·73	3·5
monoanion	5·73	0·03	0·0007	—	—	222, 265, 379	4·15, 4·05, 3·83	7·5
dianion	9·41	0·02	0·0007	—	—	234, 274, 394	4·15, 3·07, 3·80	13[k]
2 : 4 : 7-Trihydroxy	—	—	—	12,000[d]	1400	—	—	—
monoanion	3·61	0·02	0·0005	—	—	225, 248, 275, 335	4·42, 3·92, 3·86, 4·13	13[k]
2 : 4 : 6 : 7-Tetrahydroxy	—	—	—	58,000[d]	7000	233, 292, 346	4·27, 3·88, 4·11	13[k,l]
4 : 6-Diamino	—	—	—	24,000[d]	940	263, 375	4·18, 3·81	6·7
monocation	4·37[d]	0·03	0·0002	—	—	254, 284, 376	4·09, 3·87, 3·90	2·4
4 : 7-Diamino	—	—	—	5,000	300	241, 339	4·38, 4·05	7·0
monocation	4·97[f]	0·03	0·001	—	—	233, 257, 284, 343	4·24, 4·22, 3·69, 4·14	3·0
2 : 4 : 7-Triamino	—	—	—	4,500	200	227, 257, 350	4·54, 4·13, 4·17	8·5
monocation	6·30	0·04	0·001	—	—	255, 275, 342	4·16, 3·84, 4·28	4·3
4 : 6 : 7-Triamino	—	—	—	12,500[d]	450	227, 256, 284, 345	4·33, 4·17, 3·63, 4·11	8·5
monocation	5·57	0·02	0·001	—	—	224, 245, 353	4·20, 4·21, 4·26	3·6
2 : 4 : 6 : 7-Tetramino[b]	6·86[d,g]	0·03	0·0001	13,000[d]	—	—[b]	—	—
2-Amino-6 : 7-dihydroxy	—	—	—	—	—	244, 348	4·26, 4·09	13[k]
4-Amino-2 : 7-dihydroxy	—	—	—	—	—	223, 256, 344	4·42, 4·04, 4·12	13[k]

[a] Covalently monohydrated. [b] We thank Dr. E. C. Taylor of Princeton University for this substance, and understand that he is reporting on its spectroscopy. [c] Determined potentiometrically except where otherwise shown (for details see Albert and Phillips, *J.*, 1956, 1294). [d] Determined spectrophotometrically. [e] We thank Mr. G. Timmis for this substance. [f] Cf. 2 : 4-diaminopteridine, 5·32. [g] Monocation: analytical wave-length was 265 mμ. [h] Approx., see Fig. [j] Hilger "Uvispek" photoelectric spectrophotometer (1 cm. cells). [k] Polyanion. [l] This replaces earlier values[1] on material now known to be impure.

and " 2 : 6-dihydroxypteridine " to the tetrahydroxypteridine (IV). This argument, which ignores steric factors, must not be taken too far, but suggests that equilibrium does not favour the hydrate of 4 : 6-dihydroxypteridine.

The addition of one primary amino-group to pteridine greatly decreases solubility (all four monoaminopteridines [1,19] fall in the range 1 in 1400—1500); for 2 : 4-, 4 : 6-, and 4 : 7-diaminopteridines solubilities are 1 in 3000, 24,000, and 5000 respectively (average 11,000); for the two triaminopteridines 1 in 4500 and 12,500; and for tetra-aminopteridine 1 in 13,000. Thus, although there are not so many data as for the hydroxypteridines, it is seen that one primary amino-group has a marked insolubilising effect which, unlike the case for hydroxypteridines, is only moderately intensified by further additions of the same group.

TABLE 2. *Solubilities of hydroxypteridines in water at* 20°.

	1 in		1 in		1 in
Unsubstituted	7	2 : 4-Dihydroxy	800	2 : 4 : 6-Trihydroxy ...	7400
		2 : 6-Dihydroxy	110,000	2 : 4 : 7-Trihydroxy ...	12,000
2-Hydroxy	600	2 : 7-Dihydroxy	1400	4 : 6 : 7-Trihydroxy ...	27,000
4-Hydroxy	200	4 : 6-Dihydroxy	5500	Average	15,000
6-Hydroxy	3500	4 : 7-Dihydroxy	4000	Tetrahydroxy	58,000
7-Hydroxy	900	6 : 7-Dihydroxy	3000		
Average 400 (omitting 6-hydroxy)		Average 3000 (omitting 2 : 6-dihydroxy)			

EXPERIMENTAL

Microanalyses were by Mr. P. R. W. Baker, Beckenham. The yields refer to the stage at which substances became chromatographically homogeneous (on paper), 3% aqueous ammonium chloride or butanol–5N-acetic acid (7 : 3) being eluants. Substances were dried at 120° unless otherwise specified.

2 : 6-*Dihydroxypteridine.*—(*a*) 4 : 5-Diamino-2-hydroxypyrimidine [21] (1 g.) in N-hydrochloric acid (30 ml.) was refluxed with ethyl glyoxylate hemiacetal [22] (2 g.) for 30 min., cooled and neutralized, giving 80% of 2 : 6-*dihydroxypteridine monohydrate*. It was boiled with 50 parts of water (rejected) and recrystallized from 100 parts of boiling N-sulphuric acid in which it is far more soluble than in water but from which it is deposited uncombined. From 5000 parts of water it formed colourless crystals, decomp. >250° (Found, for material dried at 150°/0·01 mm.: C, 39·6; H, 3·25; N, 30·4. $C_6H_4O_2N_4,H_2O$ requires C, 39·6; H, 3·3; N, 30·8%). 2 : 6-Dihydroxypteridine was unchanged when shaken in 0·1N-sodium hydroxide or 2N-ammonia in air for 11 hr. The sodium and potassium salts are highly soluble in water to yellow solutions with an intense green fluorescence, which fades in daylight, but are stable in the dark at 20°.

(*b*) Potassium permanganate (0·7 g.) in water (50 ml.) was added dropwise to a stirred solution of 7 : 8-dihydro-2 : 6-dihydroxypteridine [7] (1 g.) in 0·2N-sodium hydroxide (90 ml.). After 30 minutes' further stirring, the mixture was filtered and adjusted to pH 5, giving 40% of 2 : 6-dihydroxypteridine monohydrate, identical with the above on chromatography in three solvents.

2 : 7-*Dihydroxypteridine.*—Ethyl glyoxylate hemiacetal (1·2 g., 1 equiv.) was added to 4 : 5-diamino-2-hydroxypyrimidine (1·25 g., 0·01 mole) in water (25 ml.) at 50°. The mixture was set aside at 20° for 24 hr. (initial and final pH 4). The precipitate (1·63 g.) was refluxed with 70 parts of water. The filtrate deposited 45% of 2 : 7-dihydroxypteridine, purified as the *sodium salt* by recrystallization from 2 equivs. of boiling N-sodium hydroxide. The pale crystals were washed with alcohol (Found, for material dried at 110°/0·01 mm.: C, 29·75; H, 2·5; N, 22·8; loss at 150°, 15·3. $C_6H_2O_2N_4Na_2,2H_2O$ requires C, 29·5; H, 2·5; N, 22·95; loss, 14·8%). This salt was dissolved in boiling water (no fluorescence), adjusted to pH 4, giving 2 : 7-*dihydroxypteridine* which forms colourless crystals from 100 parts of boiling water (Found, for material dried at 110°/0·01 mm.: C, 39·6; H, 3·2; N, 30·7; loss at 180°, 10·0. $C_6H_4O_2N_4,H_2O$ requires C, 39·6; H, 3·3; N, 30·8; loss, 9·9%). It was unchanged after 1 hr. at 175° (chromatographic tests) but became brown slowly at 275°.

That part of the initial precipitate which was sparingly soluble in water was again boiled with 70 parts of water (discarded), giving 45% of 2 : 6-dihydroxypteridine monohydrate,

[21] Johns, *Amer. Chem. J.*, 1911, **45**, 79; modified by Brown, *J. Appl. Chem.*, 1957, in the press.
[22] Rigby, *J.*, 1950, 1912.

identical with material described above. A condensation in phosphate buffer (pH 7) gave 65% of the 2 : 7- and 25% of the 2 : 6-isomer.

7-*Hydroxypteridine* (*Recommended Synthesis*).—4 : 5-Diaminopyrimidine (1·1 g., 0·01 mole) in 2N-sodium carbonate (10 ml.) was refluxed with ethyl glyoxylate hemiacetal (2·2 g., 50% excess) in a boiling-water bath for 1 hr. (final pH 9·5). The mixture was cooled and set aside at 20° for an hour. The sodium salt was filtered off, dissolved in boiling water (17 ml.) containing N-sodium hydroxide (0·5 ml.), and brought to pH 2·5 with 5N-sulphuric acid, whilst hot. The 7-hydroxypteridine [11] (77%) recrystallized from 76 parts of water.

2 : 4 : 6-*Trihydroxypteridine*.—To 5 : 6-diamino-2 : 4-dihydroxypyrimidine hemisulphate (6 g.), dissolved in 78% w/w sulphuric acid (72 ml.) at 90°, was added ethyl glyoxylate hemiacetal (6·6 g.). After 2 min. at 90°, water (300 ml.) was added and the mixture refrigerated. The crystals and potassium carbonate (8·5 g.) were dissolved in boiling water (100 ml.). The potassium salt was filtered from the chilled solution, dissolved in boiling water, brought to pH 4 with citric acid, and recrystallized from water, giving yellow 2 : 4 : 6-trihydroxypteridine (50%), decomp. 360—380° (Found : C, 39·8; H, 2·4; N, 31·3. Calc. for $C_6H_4O_3N_4$: C, 40·0; H, 2·2; N, 31·1%). A colourless modification was obtained by running a solution in 2N-ammonia through alumina, and acidifying, but this reverted on recrystallization.

Powdered sodium amalgam (4%; 20 g.) was added during 10 min. to finely divided tetrahydroxypteridine (1 g.; see below) suspended in water (15 ml.; under nitrogen). After 20 min., water (25 ml.) was added and warmed until a clear solution could be decanted from the mercury (under nitrogen). The supernatant liquid was acidified to pH 4 and cooled and the precipitate of 7 : 8-dihydro-2 : 4 : 6-trihydroxypteridine (0·9 g.) filtered off and found to be homogeneous. It has been prepared in another way.[14] Oxidation with potassium permanganate [11] gave 0·25 g. of 2 : 4 : 6-trihydroxypteridine.

2 : 4 : 7-*Trihydroxypteridine*.—Triamino-2-hydroxypyrimidine sulphate [23] (4 g.) and sodium acetate (20 g.) in acetic acid (20 ml.) and water (400 ml.) were refluxed with ethyl glyoxylate hemiacetal (4 g.) for 1 hr. The semicolloidal precipitate was dissolved in N-sodium hydroxide (with minimal heating) and poured into excess of boiling 2N-hydrochloric acid. The precipitate, boiled with water, gave 70% of 4-*amino*-2 : 7-*dihydroxypteridine* which is far less soluble in water than 2 : 4 : 7-trihydroxypteridine (Found, for material dried at 110°/0·1 mm. : C, 40·3; H, 2·7; N, 39·25. $C_6H_5O_2N_5$ requires C, 40·2; H, 2·8; N, 39·1%).

This amine (1 g.) was refluxed for 5 hr. with 6N-sodium hydroxide (20 ml.). Next day the sodium salt was filtered off, dissolved in boiling water (40 ml.), and acidified with acetic acid. The precipitate was purified as the potassium salt from 0·7N-potassium carbonate solution. Final recrystallization from water gave colourless crystals (75% calc. on amine) of 2 : 4 : 7-trihydroxypteridine (Found : C, 40·1; H, 2·6; N, 30·9%).

Attempted Preparation of 2 : 6 : 7-*Trihydroxypteridine*.—(*a*) 4 : 5-Diamino-2-hydroxypyrimidine [21] (1 g.), methyl oxalate (2 g.), and water (25 ml.) were heated under reflux in boiling water for 100 min. The solution became clear within 10 min., after which crystals were deposited (95% yield). These were repeatedly recrystallized from 1200 parts of water giving pale yellow crystals (dried over P_2O_5 at 20°) (Found : C, 36·8; H, 3·1; N, 28·95; loss at 200°, 4·3. $C_6H_4O_3N_4,H_2O$ requires C, 36·4; H, 3·0; N, 28·3; H_2O, 9·1%). Specimens dried at 20° and 200° were chromatographically identical. Boiling with N-sulphuric acid gave a little 4 : 5-diamino-2-hydroxypyrimidine.

(*b*) 4 : 5-Diamino-2-hydroxypyrimidine (4 g.) and oxalic acid dihydrate (30 g.) were intimately mixed, heated in a wide-mouthed flask to 165° during 30 min. and maintained there for 2 hr. The product was dissolved in alkali, then acidified to pH 4. The solid was recrystallized four times from water and dried (P_2O_5 at 120°) (Found : C, 37·7; H, 2·9; N, 29·5. $C_6H_4O_3N_4,0{\cdot}5H_2O$ requires C, 38·1; H, 2·7; N, 29·6%). The products from both reactions are identical.

(*c*) A solution of 2 : 6-dihydroxypteridine monohydrate (0·47 g.) in 5N-sulphuric acid (12 ml.) and 30% hydrogen peroxide (8 ml.) was set aside for 3 days. The precipitate (78%) was identical with the above.

(*d*) 2 : 7-Dihydroxypteridine (0·5 g.) in cold nitric acid (*d* 1·5; 1·5 ml.) was set aside for a day. The deposit was boiled with water (12 ml.) and adjusted to pH 4 with sodium citrate and hydroxide. The precipitate (28%) was identical with the above.

2-*Amino*-6 : 7-*dihydroxypteridine*.—The following is an improvement on the fusion process.[24]

[23] Bendich, Tinker, and Brown, *J. Amer. Chem. Soc.*, 1948, **70**, 3112.
[24] Wieland, Tartter, and Purrmann, *Annalen*, 1940, **545**, 209.

2 : 4 : 5-Triaminopyrimidine[25] (0·5 g.) and oxalic acid dihydrate (1 g.) were refluxed in water (12 ml.) for 90 min. The precipitate was collected next day and boiled with water (40 ml., rejected), taken up in N-sodium hydroxide, brought to pH 4 with acetic acid, and centrifuged. This sequence was repeated twice, and the product (0·4 g.) washed with alcohol, and ether (Found, for material dried at 130°/0·01 mm. : C, 40·0; H, 2·9; N, 38·8. Calc. for $C_6H_5O_2N_5$: C, 40·2; H, 2·8; N, 39·1%).

2 : 4 : 6 : 7-*Tetrahydroxypteridine*.—Existing methods, which use oxalic acid,[18] gave poor yields and the product was hard to purify. The following was satisfactory : 4 : 5-Diamino-2 : 6-dihydroxypyrimidine (8 g.), diethyl oxalate (16 ml.), and dried ethylene glycol (200 ml.) were refluxed for 2 hr., then cooled to 100°, and water (400 ml.) was added. The precipitate was purified as the potassium salt from 0·5N-potassium carbonate. This salt was dissolved in water and acidified, and the precipitated tetrahydroxypteridine (45%) boiled repeatedly with water (Found, for material dried at 140°/0·01 mm. : C, 36·6; H, 2·05; N, 28·4. Calc. for $C_6H_4O_4N_4$: C, 36·7; H, 2·05; N, 28·6%).

4 : 6-*Diaminopteridine*.—Finely divided 4 : 6-dihydroxypteridine[6] (1 g.) and phosphorus pentachloride (8 g., in phosphoryl chloride, 40 ml.) were refluxed at 135° for 4 hr. Excess of solvent was removed *in vacuo* at 100°, and ice added to the residue. The resulting solution was adjusted to, and held at, pH 7 with 10N-sodium hydroxide. The solid was filtered off, and the filtrate extracted with chloroform (4 × 30 ml.). The solid was extracted separately to avoid an emulsion. The combined extracts were dried (Na_2SO_4) and evaporated below 35°. As the residue (0·5 g.) slowly polymerized on attempted purification, it (0·35 g.) was heated with aqueous ammonia (*d* 0·88; 10 ml.) at 140° for 4 hr., then cooled. The precipitate, dissolved in water (120 ml.) and concentrated, gave yellow crystals (0·1 g.) of 4 : 6-*diaminopteridine* (Found, for material dried at 130° : C, 44·4; H, 3·7; N, 51·6. $C_6H_6N_6$ requires C, 44·4; H, 3·7; N, 51·8%).

2 : 4 : 7-*Triaminopteridine*.—2 : 4 : 7-Trihydroxypteridine (2 g.) and phosphorus pentachloride (10 g.) in phosphoryl chloride (100 ml.), treated similarly, gave a residue that was recrystallized from ether (by concentration), giving pale needles (77%) of 2 : 4 : 7-*trichloropteridine*, m. p. 133° (Found : C, 30·85; H, 0·8; N, 23·9; Cl, 44·8. $C_6HN_4Cl_3$ requires C, 30·6; H, 0·4; N, 23·8; Cl, 45·2%).

The trichloropteridine (1 g.) and ammonia (*d* 0·88; 10 ml.) were treated as above. The precipitate was crystallized from 0·3N-ammonia (180 ml.), giving 50% of yellow 2 : 4 : 7-triaminopteridine, not melting at 250° (Found, for material dried at 110° : C, 36·6; H, 4·2; N, 49·2. Calc. for $C_6H_7N_7,H_2O$: C, 36·9; H, 4·65; N, 50·25. Found, for material dried at 150°/0·01 mm. : C, 41·1; H, 3·5; N, 54·7. Calc. for $C_6H_7N_7$: C, 40·7; H, 4·0; N, 55·3%). It was chromatographically identical, in the two solvents, with a specimen synthesized by the older method.[20b]

4 : 6 : 7-*Triaminopteridine*.—4 : 6 : 7-Trihydroxypteridine[6] (1 g.), treated similarly, gave yellow-brown needles of 4 : 6 : 7-*trichloropteridine* (23%), m. p. 179—181°, loss occurring through emulsification (Found : C, 30·8; H, 0·4; N, 24·0; Cl, 45·2%). It was aminated as above. The product, extracted with boiling water (210 ml.; then concentrated) gave pale crystals (0·2 g.) of 4 : 6 : 7-*triaminopteridine* (Found, for material dried at 150° : C, 41·0; H, 3·7; N, 54·5%).

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[25] Albert, Brown, and Cheeseman, *J.*, 1951, 474.