415. *Pteridine Studies. Part XVIII.*[1] *The Reduction of Hydroxypteridines.*

By Adrien Albert and Sadao Matsuura.

Reduction, with various agents, of all the mono- and poly-hydroxypteridines is described. Double bonds were reduced, but never a bond adjacent to an oxygen atom. Thus, the 3,4-bond was hydrogenated in 2-hydroxy-, the 5,6-bond in 7-hydroxy-, and 4,7-dihydroxy-pteridine, the 5,6- and 7,8-bonds (together) in 4-hydroxy- and 2,4-dihydroxy-, and the 7,8-bond in 6-hydroxy-, 2,6- and 4,6-dihydroxy-, and 2,4,6-trihydroxypteridine. The reduction, by sodium amalgam, of three 6,7-dihydroxypteridines to 7,8-dihydro-6-hydroxypteridines is a reaction confined to 6,7-dihydroxy-derivatives.

Reductive condensation of glyoxal diethyl monoacetal, and of ethyl glyoxylate ethyl hemiacetal, with 4,5-diaminopyrimidines provided the first direct synthetic methods for 5,6-dihydropteridines.

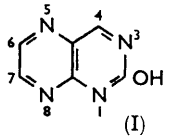
The discovery that 2-hydroxypteridine (I) is reducible in the 3,4-position [2] led us to investigate the reduction of all the mono- and poly-hydroxypteridines. The reagents used were: potassium borohydride, sodium dithionite, potassium (and sodium) amalgam, and hydrogen (over palladium, Raney nickel, and Adams platinum).

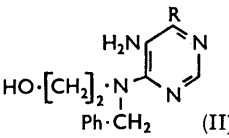
4-Hydroxypteridine was reduced most easily by hydrogen over Raney nickel, and gave a tetrahydro-4-hydroxypteridine. Potassium borohydride produced a mixture of this substance and a dihydro-4-hydroxypteridine, but mainly the former when a large excess of reagent was used. The high solubility of the tetrahydro-compound in water led to low yields with the borohydride, but tetrahydro-4-hydroxy-6-methylpteridine was easily prepared in this way. Sodium amalgam and hydrogen–palladium also gave the above tetrahydro-4-hydroxypteridine along with some unstable substances. That this substance was the 5,6,7,8-tetrahydro-derivative seemed likely from its ultraviolet spectra (all three ionic species) which resembled those of 4,5-diamino-6-hydroxypyrimidine (shifted 10 mμ, or less, to longer wavelengths), as would be expected from the linking of the two amino-groups by a dimethylene residue [2,3] (see Table). That two of the added hydrogen atoms are on nitrogen was confirmed by finding three hydrogen atoms replaceable by deuterium under conditions [4] such that only hydrogen attached to nitrogen or oxygen could be exchanged (see Experimental section).

We then tried to synthesize 5,6,7,8-tetrahydro-4-hydroxypteridine by the general reaction of Brook and Ramage.[5] It was realized that if a free hydroxyl group was present

[1] Part XVII, Albert and Howell, *J.*, 1962, 1591.
[2] Albert and Matsuura, *J.*, 1961, 5131.
[3] Taylor and Sherman, *J. Amer. Chem. Soc.*, 1959, **81**, 2464.
[4] Linderstrøm-Lang, Symposium on Peptide Chemistry, *Chem. Soc. Special Publ.* No. 2, 1955, p. 1.
[5] Brook and Ramage, *J.*, 1955, 896.

at the pyrimidine stage, ring-closure (of the tautomer) would take place in another direction to give an imidazopyrimidine. Hence 4,6-dichloro-5-nitropyrimidine was condensed with 2-benzylaminoethanol to give (after reduction) 5-amino-4-(*N*-benzyl-2-hydroxyethylamino)-6-chloropyrimidine (II; R = Cl). This readily cyclized in phosphorus trichloride, to give 8-benzyl-4-chloro-5,6,7,8-tetrahydropteridine, but the chlorine could not be replaced by a hydroxyl group, and removal of the benzyl group by reduction also replaced the chlorine by hydrogen, giving the known 5,6,7,8-tetrahydropteridine.[6] Accordingly, the chloropyrimidine (II; R = Cl) was converted into the benzyloxy-analogue (II; R = O·CH_2Ph), but this yielded only the hydroxypyrimidine (II; R = OH) when cyclization was attempted with phosphorus trichloride. The synthesis was


(I)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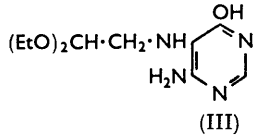

(III)

successful when 4-chloro-6-ethoxy-5-nitropyrimidine was condensed with 2-benzylaminoethanol, to give (after reduction) 5-amino-4-(*N*-benzyl-2-hydroxyethylamino)-6-ethoxypyrimidine (II; R = OEt) which, after treatment with phosphorus trichloride, gave 8-benzyl-5,6,7,8-tetrahydro-4-hydroxypteridine. Sodium, in ammonia, converted this into the desired 5,6,7,8-tetrahydro-4-hydroxypteridine, which was identical with the main reduction product of 4-hydroxypteridine in R_F values, ionization constants, and infrared and ultraviolet spectra.

The dihydro-derivative, produced, in a small amount, in the reduction of 4-hydroxypteridine, was reduced to 5,6,7,8-tetrahydro-4-hydroxypteridine by potassium borohydride or hydrogen and Raney nickel. Thus it must be the 5,6-, 5,8-, 6,7-, or 7,8-dihydro-derivative. Deuterium exchange (see above) disclosed only two mobile hydrogen atoms. One of these is in the hydroxyl group, and thus the choice is narrowed to the 5,6- or 7,8-derivative. The former was synthesized by a new reaction in which 4,5-diamino-6-hydroxypyrimidine was reductively condensed with glyoxal diethyl monoacetal, to give 4-amino-5-(2,2-diethoxyethylamino)-6-hydroxypyrimidine (III) which was cyclized by acid to 5,6-dihydro-4-hydroxypteridine. The latter had ultraviolet spectra very similar to those of 5,6,7,8-tetrahydro-4-hydroxypteridine and unlike those of the reduction product of 4-hydroxypteridine which is hence considered to be 7,8-dihydro-4-hydroxypteridine. In an attempt to prepare this 7,8-isomer, 4,6-dichloro-5-nitropyrimidine was condensed with aminoacetal and the product hydrolysed to 4-(2,2-diethoxyethylamino)-6-hydroxy-5-nitropyrimidine, but neither hydrolysis nor reduction, successful in preparing 7,8-dihydro-2-hydroxypteridine,[2] gave a useful product.

The weakness, as a base, of 7,8-dihydro-4-hydroxypteridine (pK_a 0·3) contrasts with the strength of other 7,8-dihydropteridines (Table), notably 7,8-dihydro-4,6-dimethylpteridine (pK_a 6·0; prepared by catalytic hydrogenation of 2-chloro-7,8-dihydro-4,6-dimethylpteridine). As was pointed out for 7,8-dihydro-6-hydroxypteridine,[7] 7,8-dihydropteridines contain the structure of 4-aminopyridine; if they are protonated on $N_{(3)}$, the cations, *e.g.*, (IV), would be capable of the "4-aminopyridine" type of base-strengthening resonance.[8] The "acid amide" type of resonance of a hydroxy-group in the 4-position would be expected to interfere with this resonance.

In an attempted synthesis of 7,8-dihydro-4-hydroxy-6-methylpteridine, 4-acetonylamino-6-chloro-5-nitropyrimidine was hydrolysed to the 6-hydroxy-analogue, but this decomposed profoundly on hydrogenation over Raney nickel.

[6] Brook and Ramage, *J.*, 1957, 1.
[7] Albert and Reich, *J.*, 1961, 127.
[8] Albert, Goldacre, and Phillips, *J.*, 1948, 2240.

Physical properties of pteridines.

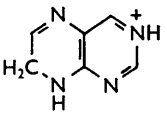
Pteridine	Charge	Ionization [a] (H_2O; 20°) pK_a	Spread (±)	Concn. (M)	Spectroscopy in water $\lambda_{max.}$ (mμ)	log ε	pH
7,8-Dihydro-4,6-dimethyl	0	—	—	—	218, 293	4·27, 3·73	8·2
	+	6·00	0·03	0·005	218, 293	4·13, 3·91	3·
7,8-Dihydro-4-hydroxy	0	—	—	—	248, 367	3·86, 3·68	7
	−	12·13	0·03	S [h]	253, 364	3·97, 3·70	14
	+	0·32	0·05	S [h]	257, 374	3·87, 3·80	−2
5,6-Dihydro-4-hydroxy	0	—	—	—	286	3·78	7
	−	10·29	0·08	0·005	279	3·82	12·3
	+	2·94	0·04	S [i]	258	3·74	0
5,6,7,8-Tetrahydro-4-hydroxy	0	—	—	—	220, 289	4·22, 3·93	7
	−	10·13	0·03	0·005	218, 284	4·33, 3·91	12·3
	+	3·86	0·02	0·005	219, 259	4·33, 3·83	1
5,6,7,8-Tetrahydro-4-hydroxy-6-methyl	0	—	—	—	220, 290	4·26, 3·93	7
	−	9·97	0·06	0·02	217, 284	4·40, 3·91	12
	+	3·84	0·02	0·005	219, 259	4·34, 3·85	1
7,8-Dihydro-2,6-dihydroxy	0	—	—	—	237, 265, 290	4·11, 3·90, 3·46	6
	−	10·22	0·01	S [j]	279, *315*	4·01, *3·70*	12
	+	2·80	0·02	S [k]	257, 317	4·01, 3·86	0
7,8-Dihydro-4,6-dihydroxy	0	—	—	—	274, 318	3·90, 3·69	6·3
	—	9·14	0·02	S [l]	273, *300*	3·83, *3·67*	12
Dihydro-2,7-dihydroxy	0	—	—	—	252, 324	3·50, 3·97	3·5
	−	5·84	0·02	S [m]	258, 340	3·49, 4·01	8
5,6-Dihydro-4,7-dihydroxy	0	—	—	—	217, 275, 328	4·23, 3·78, 3·71	5
	—	8·45	0·02	0·002	264, 310	3·72, 3·59	11
5,6,7,8-Tetrahydro-2,4-dihydroxy	0	—	—	—	298	—	7
	+	~5	—	S [n]	263	—	3
7,8-Dihydro-2,4,6-trihydroxy	0	—	—	—	267, 306	3·90, 4·09	4·8
	—	7·07	0·03	S [o]	223, 272, 306	4·34, 4·05, 3·89	9·1
For comparison							
5,6,7,8-Tetrahydro [b]	0	—			206, 268, 304	4·04, 3·68, 3·81	8·2
	+	6·63			208, 304	4·17, 3·89	—
3,4-Dihydro-2-hydroxy [c]	0	—			248, 317	3·72, 3·89	7
	−	12·6			281, 343	3·98, 3·84	14
	+	0			254, 337	3·78, 3·85	−2
7,8-Dihydro-2-hydroxy [c]	0	—			223, 290	4·35, 3·88	7
	−	~12 [e]			308	3·95	14
	+	3·50			225, 290, *310*	3·86, 3·79, *3·75*	1
7,8-Dihydro-6-hydroxy [d]	0	—			293	3·93	7·4
	−	10·54			305	4·07	13
	+	4·78			292	4·01	2
5,6-Dihydro-7-hydroxy [d]	0	—			271, 319	3·58, 3·70	6
	−	9·94			224, 325	4·34, 3·93	12
	+	3·36			223, 284, 352	4·47, 3·74, 3·71	1
5,6,7,8-Tetrahydro-2-hydroxy [e]	0	—			232, 306	4·09, 3·70	7
	−	12·5			315	3·79	14
	+	4·35			229, 327	4·00, 3·69	1
(4,5-Diamino-6-hydroxypyrimidine)	0	—			280 [g]	3·98	7
	−	9·9			274 [g]	3·95	14
	+	3·6			257	3·79	1

[a] Determined potentiometrically except where marked S (spectrophotometric). [b] From ref. 3. [c] From ref. 2. [d] Brown and Mason, *J.*, 1956, 3443. [e] Unstable in alkali. [f] In 1 and 4 cm. cells; shoulders in italics. [g] Mason (*J.*, 1954, 2071) recorded also a low peak about 371 mμ, but this disappears on further purification. [h] A.w.l. (analytical wavelength), 400 mμ. [i] A.w.l. 290 mμ. A.w.l. 320 mμ. [k] A.w.l. 211 mμ. [l] A.w.l. 335 mμ. [m] A.w.l. 355 mμ. [n] A.w.l. 296 mμ. [o] A.w.l. 225 mμ.

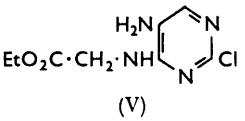
Other Monohydroxypteridines.—6-Hydroxypteridine was reduced with potassium borohydride, sodium dithionite, and hydrogen–palladium, to the same 7,8-dihydro-6-hydroxypteridine, obtained previously by the action of sodium amalgam on 6-hydroxypteridine.[9] The structure of this substance follows from its synthesis [10] from the pyrimidinylglycine derivative (V).

[9] Albert, Brown, and Cheeseman, *J.*, 1952, 1620.
[10] Boon, Jones, and Ramage, *J.*, 1951, 96.

7-Hydroxypteridine has previously been reduced [9] only under alkaline conditions which caused simultaneous ring-opening to an acid, provisionally considered to be 4-amino-5-carboxymethylaminopyrimidine (VI), which gave a methyl ester with methanolic hydrogen chloride. This structure is now confirmed by comparison of the spectrum of the zwitterion of this acid (at pH 6·7) with that of the monocation of 4,5-diaminopyrimidine; they have, respectively, $\lambda_{max.}$ 282 and 284 mμ (log ε 3·93 and 3·94). This acid is cyclized,[9] at a low pH,



(IV)



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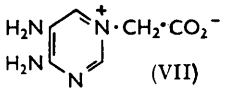

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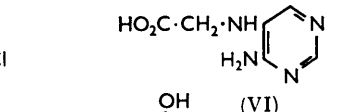

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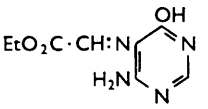


(VIII)

to a dihydro-7-hydroxypteridine which must be the 5,6-dihydro-derivative. It is now found that hydrogenation of 7-hydroxypteridine in alkaline solution over palladium–carbon also gives the pyrimidine acid (VI) (63% yield), but potassium borohydride or potassium amalgam (below 5°) gives 5,6-dihydro-7-hydroxypteridine directly. The following substances were prepared in unsuccessful attempts to synthesize 5,6-dihydro-7-hydroxypteridine: 4,5-diamino-1-carbamoylmethylpyrimidinium iodide (believed to be quaternized on $N_{(1)}$ in analogy with the methylation of 4-aminopyrimidine [11]), 4,5-diamino-1-carboxymethylpyrimidine betaine (VII), and 5-bromo-4-phthaloylglycylaminopyrimidine.

Dihydroxypteridines.—6,7-Dihydroxypteridine was not reduced with potassium borohydride, sodium dithionite, or by hydrogenation over platinum or palladium catalysts. Use of sodium amalgam [9] gave 7,8-dihydro-6-hydroxypteridine.

2,6- and 4,6-Dihydropteridine were reduced by potassium borohydride to 7,8-dihydro-2,6- and -4,6-dihydroxypteridine,[10] which were also prepared from *N*-pyrimidinylglycines similar to (V). Catalytic hydrogenation in alkali over palladium gave the same products, and potassium amalgam acted similarly on 4,6-dihydroxypteridine but destroyed the 2,6-isomer (it also destroyed the likely product, 7,8-dihydro-2,6-dihydroxypteridine).

4,7-Dihydroxypteridine was reduced to the 5,6-dihydro-derivative with potassium borohydride, potassium amalgam, or hydrogenation over palladium. The structure of the dihydro-compound was established by its synthesis from the anil (VIII) formed from 4,5-diamino-6-hydroxypyrimidine and ethyl glyoxylate ethyl hemiacetal.[12] This anil was hydrogenated to 4-amino-5-ethoxycarbonylmethylamino-6-hydroxypyrimidine, a derivative of (VI), which was cyclized by dilute acid to 5,6-dihydro-4,7-dihydroxypteridine.

2,7-Dihydroxypteridine was similarly reduced to a dihydro-derivative, presumably the 3,4-dihydro-derivative because the acid strength was little changed, just as happens when 2-hydroxypteridine is hydrogenated in the 3,4-position.[2] On the other hand, hydrogenation in the 5,6-position is acid-weakening because it causes the pK_a to rise 3·5 and 2·4 units for 7-hydroxy- [13] and 4,7-dihydroxy-pteridine, respectively. An attempt to synthesize 5,6-dihydro-2,7-dihydroxypteridine, through the pyrimidine anil, was unsuccessful. Reaction of 2,7-dihydroxypteridine with sodium dithionite and with sodium metabisulphite gave respectively sodium tetrahydro- and dihydro-2,7-dihydroxypteridinesulphonate. Although the orientations of the sulphonic group are unknown, the overall composition of the products indicates what may be encountered with these reagents in the pteridine series (see ref. 2 for a similar reaction between sodium metabisulphite and 2-hydroxypteridine).

2,4-Dihydroxypteridine absorbed 4 atoms of hydrogen over Adams platinum in acidic

[11] Brown, Hoerger, and Mason, *J.*, 1955, 4035.
[12] Pfleiderer, *Chem. Ber.*, 1959, **92**, 3190.
[13] Albert and Brown, *J.*, 1953, 74.

solution, and over palladium or Raney nickel in alkaline solution. Reduction with sodium amalgam gave the same product, a tetrahydro-derivative, which was highly unstable in air. It had an ultraviolet spectrum very similar to that of 4,5-diamino-2,6-dihydroxypyrimidine ($\lambda_{max.}$ 263 and 260 mμ, respectively, at pH 3). This similarity is also seen in the basic ionization constants (5·0 and 4·5, respectively); hence the reduction product is assigned the constitution 5,6,7,8-tetrahydro-2,4-dihydroxypteridine. Acetic formic anhydride gave the more stable *N*-formyl derivative, considered to be formylated in the 5-position from analogy with tetrahydropteridine[6] and 4,5-diaminopyrimidines.[14] 2,4-Dihydroxypteridine was apparently unaffected by potassium borohydride. Sodium dithionite gave both the above tetrahydro-derivative and a sulphur-containing product, $C_6H_7N_4NaO_5S,H_2O$, believed to be sodium 5,6,7,8-tetrahydro-2,4-dihydroxypteridine-6-sulphonate because it has been shown that the oxidation of 2-amino-5,6,7,8-tetrahydro-4-hydroxypteridine in the presence of such anions as OH^-, SO_3H^-, and CN^- leads to their introduction into the 6-position.[15] This tetrahydro-compound is analogous to a product, obtained by exposing 2-amino-4-hydroxypteridine sulphite to air,[16] which, although described as 2-amino-4-hydroxypteridine-6-sulphonic acid, gave analytical figures much closer to those required by the tetrahydro-derivative. Similarly, a substance described [15] as 2-amino-4-hydroxypteridine-6-carboxamide ($C_7H_6N_6O_2$) appears to be the tetrahydro-derivative (Found: C, 40·0; H, 4·7; N, 40·2. Calc. for $C_7H_6N_6O_2$: C, 40·8; H, 2·9; N, 40·8. Calc. for $C_7H_{10}N_6O_2$: C, 40·0; H, 4·8; N, 40·0%).

Tri- and Tetra-hydroxypteridines.—2,4,6-Trihydroxypteridine [17] was reduced in 77% yield by potassium borohydride (also by potassium amalgam and by hydrogenation over palladium) to 7,8-dihydro-2,4,6-trihydroxypteridine,[18] synthesized from a pyrimidinylglycine similar to (V). The other three isomeric trihydroxypteridines [17] were unaffected by potassium borohydride or hydrogenation over palladium in 0·1N-potassium hydroxide. Potassium amalgam reduced the 4,6,7-isomer to 7,8-dihydro-4,6-dihydroxypteridine,[13] destroyed the 2,6,7-isomer, and left the 2,4,7-isomer unchanged. 2,4,6,7-Tetrahydroxypteridine was unaffected by potassium borohydride or by catalytic hydrogenation, but gave 7,8-dihydro-2,4,6-trihydroxypteridine with sodium amalgam.[17]

2-Amino-4,6-dihydroxypteridine (xanthopterin) was reduced to the known 2-amino-7,8-dihydro-4,6-dihydroxypteridine [19] by potassium borohydride or sodium amalgam in 90% or 75% yield respectively.

Discussion.—Although the reducing reagents were chosen so as to include examples believed to act through hydride ions, protons, and free radicals severally, they generally gave the same product from any given hydroxypteridine. Apart from sodium amalgam, no reagent attacked a double bond to which a hydroxy-group was attached. 2-, 4-, 6-, and 7-Hydroxy-groups caused hydrogenation of, respectively, the following double bonds: 3,4-, 7,8- (and 5,6,7,8-), 7,8-, and 5,6-. When more than one oxygen atom was present, the 6-hydroxy-group was the more influential, followed in turn by the 2-, 7-, and 4-group.

The reduction of 6,7-dihydroxypteridines by sodium amalgam to 7,8-dihydro-6-hydroxypteridines is known also to operate on 8-alkyl derivatives of 6,7-dihydroxypteridine[20] and hence is equivalent to the reduction of an amide group [cf. the similar reductions of acridone (9-hydroxyacridine) and *N*-methylacridone to 9,10-dihydroacridines,[21] whereas 2- and 4-hydroxyquinoline cannot be reduced in this way]. It is notable that oxygen cannot be removed by sodium amalgam from 7-hydroxypteridine itself, or from any other dihydroxypteridine. Evidently this type of reaction depends on a high degree of depletion

[14] Wilson, *J.*, 1948, 1157.
[15] Forrest, Baalen, Viscontini, and Piraux, *Helv. Chim. Acta*, 1960, **43**, 1005.
[16] Viscontini and Weilenmann, *Helv. Chim. Acta*, 1959, **42**, 1854.
[17] Albert, Lister, and Pedersen, *J.*, 1956, 4621.
[18] Boon and Leigh, B.P. 677,342/1952.
[19] Totter, *J. Biol. Chem.*, 1944, **154**, 105.
[20] Elion, Ciba Symposium " Chemistry and Biology of Pteridines," London, Churchill, 1954, p. 49.
[21] Lehmstedt and Hundertmark, *Ber.*, 1931, **64**, 2386.

(owing to the propinquity of the amide group) of electrons from the carbonyl group undergoing reduction.

With the exception of 5,6,7,8-tetrahydro-2,4-dihydroxypteridine, all the reduced substances obtained in the present work were stable on storage, especially as solids. It appears that various other 7,8-dihydro- and 5,6,7,8-tetrahydro-pteridines bearing an amino- or hydroxy-group in the 2-position are also stable in the dry state, although the 7,8-dihydro-derivative is oxidized slowly in acid (and a little faster in alkaline) solution.[22] Dihydroxanthopterin (2-amino-7,8-dihydro-4,6-dihydroxypteridine) is also reasonably stable. The exceptionally rapid oxidation of 5,6,7,8-tetrahydro-2,4-dihydroxypteridine is, however, matched by that of the 5,6,7,8-tetrahydro-derivatives of 2,4-diamino-[3] and 2-amino-4-hydroxy-pteridine,[23] and hence the structural requirements for instability are indicated.

Experimental

Elementary analyses were carried out by the Analytical Section of this Department under Dr. J. E. Fildes. Assessment of yields and purity, and the measurement of ionization constants and ultraviolet spectra, were made as described earlier.[2]

Reductions of 4-Hydroxypteridine.—(*a*) 4-Hydroxypteridine [24] (2·25 g., 0·015 mole) in ethanol (300 ml.) was hydrogenated over Raney nickel (20 g.) at room temperature and pressure. The filtrate was taken to dryness and the residue, crystallized from ethanol, gave 60% of *5,6,7,8-tetrahydro-4-hydroxypteridine*, m. p. 230° (decomp.) (Found, for material dried at 130°/0·1 mm.: C, 47·5; H, 5·45; N, 36·95. $C_6H_8N_4O$ requires C, 47·35; H, 5·3; N, 36·8%).

(*b*) Potassium borohydride (2 g.) was added to a suspension of 4-hydroxypteridine (5 g.) in water (100 ml.) at 20°. Next day, the clear solution was adjusted to pH 5 with hydrochloric acid and filtered. The filtrate, adjusted to pH 10 with potassium hydroxide, was mixed with kieselguhr (15 g.) and dried in a vacuum. The residue was continuously extracted with boiling ethyl acetate, which on cooling deposited 16% of *7,8-dihydro-4-hydroxypteridine* as yellow needles, m. p. 263—265° (decomp.) (Found: C, 48·2; H, 4·05; N, 36·95. $C_6H_6N_4O$ requires C, 48·0; H, 4·0; N, 37·3%).

Reduction of 4-Hydroxy-6-methylpteridine.—Potassium borohydride (0·66 g., 16H) was added during 5 min. to a solution at 20° of 4-hydroxy-6-methylpteridine [25] (0·96 g.) and potassium carbonate (0·84 g.) in water (6 ml.). After 18 hr., the mixture was warmed at 40° until clear, then taken to pH 9 with 16N-acetic acid. The 5,6,7,8-tetrahydro-4-hydroxy-6-methylpteridine (60% yield), filtered off and recrystallized from water (2 ml.), had m. p. 217—218° (sealed tube) and was very soluble in boiling methanol [the m. p. in the literature [26] (112—116°) is incorrect] (Found, for material dried at 110°/0·01 mm.: C, 50·4; H, 5·9; N, 33·6. Calc. for $C_7H_{10}N_4O$: C, 50·6; H, 6·1; N, 33·7%). It is stable at pH 1 or 12 for several hours, only slowly oxidized in air at 110°, and unaffected by potassium ferricyanide in N-potassium hydroxide at 20°.

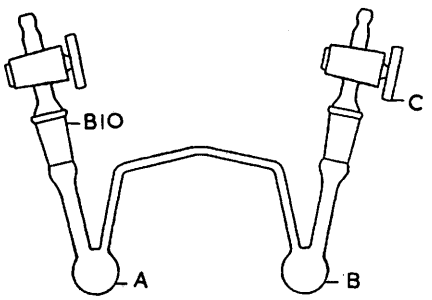
Deuterium Oxide Studies.—The apparatus shown in the Figure was used. These exchange reactions are different in nature from the deutero-reductions previously described.[2] The pteridine (0·25 mmole) and 99·97% deuterium oxide (1·5 g.) were placed in flask A (20 mm. in diameter; the drawing is to scale) and heated until dissolved. Flask A was placed in a carbon dioxide–ethanol bath and evacuated to 0·1 mm. through tap C. The bath was then placed around flask B, and the deuterium oxide distilled into this flask. The contents of flask B were removed, and flask A was heated in an oil-bath at 110°/0·1 mm. for 1 hr. Light water (1·5 g.) was then added to the residue in flask A, which was heated gently until the contents dissolved. Distillation of the water from A to B was then carried out as before, and the D_2O content of the distillate was determined by infrared spectrometry [2] [Found, for 4-hydroxypteridine, used as a control: 0·99 atom of D per molecule. Found, for the products of the reduction of 4-hydroxypteridine: (*a*) the tetrahydro-derivative 2·94; (*b*) the dihydro-derivative, 1·80 atoms of D per molecule].

2-Benzylaminoethanol [with Dr. D. J. Brown].—Benzyl chloride (250 g.) and ethanolamine

[22] Lister, Ramage, and Coates, *J.*, 1954, 4109.
[23] Viscontini and Weilenmann, *Helv. Chim. Acta*, 1958, **41**, 2170.
[24] Albert, Brown, and Wood, *J.*, 1956, 2066.
[25] Albert, Brown, and Cheeseman, *J.*, 1952, 4219.
[26] Blakely, *Biochem. J.*, 1959, **72**, 707.

(500 g.) were stirred on a steam-bath for 22 hr. The mixture was cooled, mixed with 2N-sodium hydroxide (1 l.), and extracted with ether. The extract was washed with water and dried (K_2CO_3) and the ether recovered. The residue was fractionated; 2-benzylaminoethanol (121 g.) distilled at 94—96°/0·02 mm.

5-*Amino*-4-(N-*benzyl*-2-*hydroxyethylamino*)-6-*chloropyrimidine* (II; R = Cl).—2-Benzylaminoethanol (15 g., 0·1 mole) in chloroform (40 ml.) was slowly added, with shaking, to a mixture of 4,6-dichloro-5-nitropyrimidine [10] (19·5 g., 0·1 mole) in chloroform (150 ml.) with sodium hydrogen carbonate (8·4 g., 0·1 mole) in water (30 ml.). When the evolution of carbon dioxide ceased, the chloroform layer was washed with water (50 ml.), dried (Na_2SO_4), and evaporated under reduced pressure, to give an intractable oil (37 g.) which in alcohol at 20°



Apparatus used for deuterium exchange.

over Raney nickel absorbed 3 mol. of hydrogen. The filtered solution, when concentrated to 100 ml. and acidified with ethanolic hydrogen chloride, deposited 34% of 5-*amino*-4-(N-*benzyl*-2-*hydroxyethylamino*-6-*chloropyrimidine hydrochloride*, having m. p. 130° after rapid recrystallization from ethanol below 70° (Found, for material dried at 60°/0·1 mm.: C, 49·6; H, 5·2; Cl, 22·5; N, 17·8. $C_{13}H_{16}Cl_2N_4O$ requires C, 49·5; H, 5·1; Cl, 22·5; N, 17·8%). When this was boiled in ethanol for 5 min., an isomer with different R_F values was formed (Found: C, 49·65; H, 5·2; Cl, 22·6; N, 18·0%).

The first of these isomers (1·5 g.) was added to phosphorus trichloride at 10° and set aside at 20° overnight. The excess of reagent was distilled off at 20° and the residue dissolved in water (30 ml.). This solution was extracted with chloroform (discarded) and then adjusted to pH 7 with sodium hydrogen carbonate and re-extracted with chloroform. Removal of the solvent gave 80% of 8-*benzyl*-4-*chloro*-5,6,7,8-*tetrahydropteridine* which recrystallized from ethanol (5 parts) as colourless needles, m. p. 127° (Found, for material dried at 75°/0·1 mm.: C, 59·9; H, 5·0; Cl, 13·95; N, 21·3. $C_{13}H_{13}ClN_4$ requires C, 59·9; H, 5·0; Cl, 13·6; N, 21·5%). The second isomeric pyrimidine did not undergo this reaction. The chloro-substituent in the pteridine was unaltered when the substance was refluxed with 12N-hydrochloric acid or 2% sodium methoxide.

Metallic sodium was added to a solution of this tetrahydropteridine (0·6 g.) in ammonia (130 ml.) until a blue colour persisted. After an hour, the blue colour was discharged with ammonium chloride, and the ammonia was evaporated. An aqueous solution (3 ml.) of the residue was extracted with chloroform (5 × 10 ml.). The solvent was recovered and the residue, when sublimed, gave 14% of 5,6,7,8-tetrahydropteridine, m. p. 147° (lit.,[6] 146—147°).

A chloroform solution of 4-(*N*-benzyl-2-hydroxyethylamino)-6-chloro-5-nitropyrimidine, obtained as above from 4,6-dichloro-5-nitropyrimidine (10 g.), was washed with water, dried (Na_2SO_4), and evaporated. Benzene (15 ml. portions) was repeatedly distilled (at 30°) from the residual oil to remove all the chloroform. A solution prepared from sodium (2·5 g.) and benzyl alcohol (70 ml.) was added to the residue and the whole was heated at 100° for 10 min. Solid carbon dioxide was added to the cooled mixture, to decompose the alkoxide, and then water (50 ml.). The mixture was adjusted to pH 6 and the benzyl alcohol removed by steam-distillation (4 hr.). The solution was extracted with benzene, which was recovered, and the residue was hydrogenated at 20° in alcohol over Raney nickel. The filtrate was concentrated to 40 ml. from which ethanolic hydrogen chloride precipitated 5-*amino*-4-(N-*benzyl*-2-*hydroxyethylamino*)-6-*benzyloxypyrimidine hydrochloride* as needles (3%), m. p. 203° (Found: C, 62·5; H, 6·0; N, 14·35. $C_{20}H_{22}N_4O_2$,HCl requires C, 62·1; H, 6·0; N, 14·5%). This substance, treated with phosphorus trichloride as was the 6-chloro-analogue (see above), gave 50% of

5-*amino*-4-(N-*benzyl*-2-*hydroxyethylamino*)-6-*hydroxypyrimidine*, m. p. 140—142° (from water at pH 9·7) (Found: C, 59·7; H, 6·3; N, 21·0. $C_{13}H_{16}N_4O_2$ requires C, 60·0; H, 6·2; N, 21·5%).

5,6,7,8-*Tetrahydro*-4-*hydroxypteridine*.—2-Benzylaminoethanol (3·8 g. in 10 ml. of methanol) was added to 4-chloro-6-ethoxy-5-nitropyrimidine [27] (2·5 g. in 15 ml. of methanol) at 0°, then set aside at 20° overnight. The solvent was removed and water (50 ml.) added to the residue. The mixture was extracted with benzene (2 × 25 ml.), and the benzene layer washed with water to remove benzylaminoethanol. The benzene was recovered at 20°. The residual oily 4-(*N*-benzyl-2-hydroxyethylamino)-6-ethoxy-5-nitropyrimidine was hydrogenated in ethanol at 20° over Raney nickel. The filtrate was taken to dryness and the last trace of ethanol removed with benzene. Phosphorus trichloride (15 g. in 30 ml. of benzene) was added to the residual oil (in 30 ml. of benzene) at 4° and the mixture set aside at 20° for 2 days. The benzene layer was discarded and ice (50 g.) added to the residue. The solution was extracted with benzene (discarded) and adjusted to pH 7 with sodium hydrogen carbonate. Crystallization of the precipitate from ethanol gave 26% of 8-*benzyl*-5,6,7,8-*tetrahydro*-4-*hydroxypteridine* as colourless needles, m. p. 170° (Found: C, 64·15; H, 5·75; N, 23·1. $C_{13}H_{14}N_4O$ requires C, 64·45; H, 5·8; N, 23·1%). This substance (1 g.), in fine powder, was added to liquid ammonia (130 ml.), followed by sodium until the solution became deep blue. After 30 min., the colour was discharged with ammonium chloride. After the ammonia had evaporated, water was added to the residue and the solution adjusted to pH 6. The solution was extracted with chloroform (discarded), and the aqueous layer evaporated to dryness under reduced pressure. Crystallization of the residue from water gave 54% of 5,6,7,8-*tetrahydro*-4-*hydroxypteridine*, m. p. 234° (decomp.) (Found, for material dried at 135°/0·1 mm.: C, 47·25; H, 5·6; N, 36·35%).

5,6-*Dihydro*-4-*hydroxypteridine*.—4,5-Diamino-6-hydroxypyrimidine [28] (1·3 g.) and glyoxal diethyl monoacetal [29] (1·7 g.) were hydrogenated at 20° in ethanol (200 ml.) over Raney nickel. The filtrate was taken to dryness and the residue, recrystallized from acetone, gave 35% of colourless 4-*amino*-5-(2,2-*diethoxyethylamino*)-6-*hydroxypyrimidine* (III), decomp. 142° (Found, for material dried at 65°/0·1 mm.: C, 49·1; H, 7·6; N, 23·15. $C_{10}H_{18}N_4O_3$ requires C, 49·55; H, 7·5; N, 23·15%). This substance (0·84 g.) was boiled with 0·5N-hydrochloric acid (10 ml.) for 1 min. and then cooled at once. The solution, adjusted to pH 5 and cooled overnight, gave 88% of 5,6-*dihydro*-4-*hydroxypteridine* as colourless needles (from water), decomp. 230° (Found, for material dried at 20°/0·1 mm.: C, 46·4; H, 4·35; N, 36·5. $C_6H_6N_4O,0·25H_2O$ requires C, 46·6; H, 4·25; N, 36·2%).

4-(2,2-*Diethoxyethylamino*)-6-*hydroxy*-5-*nitropyrimidine*.—Aminoacetal (5·4 g.) in water (50 ml.) was adjusted to pH 8 with acetic acid. Sodium hydrogen carbonate (6 g.) was added, and the solution was dropped into a cold, shaken solution of 4,6-dichloro-5-nitropyrimidine (7·9 g.) in chloroform (50 ml.). After 2 hr., the chloroform layer was added to N-sodium hydroxide (200 ml.), and nitrogen was bubbled through the solution at 100° for 20 min. (during this time the chlorine atom was replaced by a hydroxyl group). The solution was extracted with benzene (discarded) and then adjusted to pH 6. The precipitate, recrystallized from ethanol, gave 27% of 4-(2,2-*diethoxyethylamino*)-6-*hydroxy*-5-*nitropyrimidine*, m. p. 118—120° (Found: C, 42·35; H, 6·1. $C_{10}H_{16}N_4O_5,0·5H_2O$ requires C, 42·75; H, 6·1%).

7,8-*Dihydro*-4,6-*dimethylpteridine*.—2-Chloro-7,8-dihydro-4,6-dimethylpteridine [30] (0·92 g.) was hydrogenated (1 mol.) in 50% ethanol at 20° over 5% palladium–carbon (0·5 g.) and magnesium oxide (0·8 g.). The filtrate was taken to dryness and the residue dissolved in water (15 ml.) and repeatedly extracted with chloroform. The lower layer was dried, and the solvent recovered. The residue (0·68 g.) recrystallized from benzene, then sublimed at 95°/0·1 mm., giving 7,8-*dihydro*-4,6-*dimethylpteridine*, m. p. 135° (decomp.) (Found: C, 59·2; H, 6·15; N, 34·0. $C_8H_{10}N_4$ requires C, 59·25; H, 6·2; N, 34·55%).

4-*Acetonylamino*-6-*hydroxy*-5-*nitropyrimidine* was prepared by setting aside at 20° a solution of 4-acetonylamino-6-chloro-5-nitropyrimidine [27] in 50% aqueous ethanol. It formed needles (from alcohol), m. p. 207° (decomp.) (Found: C, 39·2; H, 4·3. $C_7H_8N_4O_4$ requires C, 39·6; H, 3·8%).

Reductions of 6-*Hydroxypteridine*.—(*a*) 6-Hydroxypteridine monohydrate [31] (0·33 g., 0·002

[27] Boon and Jones, *J.*, 1951, 591.
[28] Albert, Brown, and Cheeseman, *J.*, 1951, 474.
[29] Fischer and Baer, *Helv. Chim. Acta*, 1935, **18**, 514.
[30] Lister and Ramage, *J.*, 1953, 2234.
[31] Albert, *J.*, 1955, 2690.

mole) was dissolved in cold 0·1N-sodium hydroxide (22 ml.), and potassium borohydride (0·055 g., 4H) added. The clear solution evolved no gas. Next day the mixture was acidified to pH 7 (phosphoric acid) and chilled for 4 hr. The precipitate, dried at 110°, was found, from physical constants, to be pure 7,8-dihydro-6-hydroxypteridine (90%) (Found: C, 48·2; H, 4·0. Calc. for $C_6H_6N_4O$: C, 48·0; H, 4·0%).

(*b*) 6-Hydroxypteridine monohydrate (1 g., 0·006 mole) was dissolved in N-sodium carbonate (30 ml.) at 60°, and sodium dithionite (0·8 g., 4H) added (transient deep orange colour). Agitation with air being avoided, the mixture was brought rapidly to the boil, then cooled at once, and adjusted to pH 7 (phosphoric acid). Next day, the precipitate was filtered off and boiled with water (20 ml.) for 3 min., to remove salts. The suspension was chilled and the solid filtered off and boiled for 2 min. with N-hydrochloric acid (10 ml.) to expel sulphur dioxide. The solution was adjusted to pH 7 and chilled, giving chromatographically pure 7,8-dihydro-6-hydroxypteridine (80%) (Found: C, 47·7; H, 4·0%).

(*c*) Hydrogenation at 20° in 0·1N-sodium hydroxide over palladium–carbon gave 63% of 7,8-dihydro-6-hydroxypteridine.

Reductions of 7-Hydroxypteridine.—The poor solubility of the sodium salt of 7-hydroxypteridine in cold water led to the choice of potassium reagents. (*a*) Potassium borohydride (0·055 g., 4H) was added at 25° to a solution of 7-hydroxypteridine [17] (0·3 g., 0·002 mole) in 0·5N-potassium carbonate (8 ml.). After 6 hr., more of the borohydride (0·055 g.) was stirred into the paste. Next day, the mixture was brought to pH 5 with acetic acid, chilled, and filtered. The solid, recrystallized from water (40 ml.), gave 80% of 5,6-dihydro-7-hydroxypteridine (Found: C, 47·6; H, 4·1%).

(*b*) To a stirred ice-cold suspension of 7-hydroxypteridine (0·15 g.) in water (10 ml.), 4% potassium amalgam (5 g.) was added (maximum temperature, 5°). After 10 min. the top layer was isolated and brought to pH 7 with hydrochloric acid. The precipitate, recrystallized from water, gave 43% of 5,6-dihydro-7-hydroxypteridine (Found: C, 48·25; H, 3·9%).

4,5-Diamino-1-carbamoylmethylpyridinium Iodide.—4,5-Diaminopyrimidine [32] (0·22 g.), iodoacetamide (0·37 g., 1 equiv.), and alcohol (5 ml.) were refluxed for 1 hr. Calcium carbonate (0·1 g.) was added and the suspension refluxed for 1 hr., giving 67% of *4,5-diamino-1-carbamoylmethylpyrimidinium iodide hemihydrate*, which was recrystallized from 7 parts of water and dried at 20° (Found: C, 23·85; H, 3·77; I, 41·6; loss at 110°, 2·8. $C_6H_{10}IN_5O,0·5H_2O$ requires C, 23·7; H, 3·65; I, 41·7; $0·5H_2O$, 2·95%).

4,5-Diamino-1-carboxymethylpyrimidine Betaine (VII).—4,5-Diaminopyrimidine (0·22 g.), iodoacetic acid (0·37 g., 1 equiv.), and 2N-sodium carbonate (1·5 ml.) were heated at 98° for 20 min., chilled, and filtered, giving 70% of *4,5-diamino-1-carboxymethylpyrimidine betaine hemihydrate* which was recrystallized from 23 parts of water and dried at 20°. It chars about 300° and is very soluble in N(but not in 0·1N)-sodium hydroxide (Found: C, 40·5; H, 5·1; N, 31·6; loss at 110°, 5·4. $C_6H_8N_4O_2,0·5H_2O$ requires C, 40·7; H, 5·1; N, 31·6; $0·5H_2O$, 5·1%). It is unchanged when refluxed with an excess of N-sodium hydroxide for an hour.

5-Bromo-4-phthaloylglycylaminopyrimidine.—Phthaloylglycyl chloride [33] (1·3 g.) and 4-amino-5-bromopyrimidine [34] (1 g.) were refluxed for 1 hr. in pyridine (5 ml.). The pyridine was recovered and water (20 ml.) added to the residue. The precipitate, triturated with sodium hydrogen carbonate solution and recrystallized from ethanol, gave *5-bromo-4-phthaloylglycylaminopyrimidine*, m. p. 223—225° (Found: C, 46·2; H, 2·6; N, 15·3. $C_{14}H_9BrN_4O_3$ requires C, 46·55; H, 2·5; N, 15·5%). Attempted fission by hydrazine gave 4-amino-5-bromopyrimidine.

Reduction of Dihydroxypteridines.—2,6-Dihydroxypteridine,[17] reduced with potassium borohydride as above, gave 80% of 7,8-dihydro-2,6-dihydroxypteridine (Found: C, 43·3; H, 3·75; N, 33·7. Calc. for $C_6H_6N_4O_2$: C, 43·4; H, 3·65; N, 33·7%). Hydrogenation over palladium or Adams platinum in 0·1N-sodium hydroxide gave the same substance in 73% and 85% yield, respectively. 4,6-Dihydroxypteridine [13] gave 96% of 7,8-dihydro-4,6-dihydroxypteridine with potassium borohydride (Found: C, 43·15; H, 3·6; N, 33·5%). With potassium amalgam, and with hydrogen over palladium, it gave the same product in 72% and 79% yield, respectively.

[32] Brown, *J. Appl. Chem.*, 1952, **2**, 239.
[33] Gabriel, *Ber.*, 1907, **40**, 2674.
[34] Chesterfield, McOmie, and Sayer, *J.*, 1955, 3478.

2,7-Dihydroxypteridine [17] and potassium borohydride similarly gave 84% of a *dihydro-2,7-dihydroxypteridine* that recrystallized from water (Found: C, 43·45; H, 3·75; N, 33·65%). Potassium amalgam gave 64% of the same product. Sodium dithionite in boiling 0·5N-sodium hydroxide gave, after strong acidification with hydrochloric acid, 60% of yellow needles of *sodium tetrahydro-2,7-dihydroxypteridinesulphonate*, decomp. 245° (Found, for material dried at 65°/0·1 mm.: C, 27·05; H, 2·7; S, 11·1. $C_6H_7N_4NaO_5S$ requires C, 26·7; H, 2·6; S, 11·85%). Sodium metabisulphite (0·6 g.), added to 2,7-dihydroxypteridine (0·32 g.) in N-sodium hydroxide (1·5 ml.) and set aside at 20° for 5 hr., gave *disodium dihydro-2,7-dihydroxypteridinesulphonate* (0·15 g.), m. p. 310° (Found, for material dried at 60°/0·1 mm.: C, 23·3; H, 2·4; N, 18·3; S, 10·9. $C_6H_4N_4Na_2O_5S,H_2O$ requires C, 23·4; H, 2·0; N, 18·1; S, 10·4%).

4,7-Dihydroxypteridine [12] and potassium borohydride (as 7-hydroxypteridine, above) gave 85% of 5,6-*dihydro-4,7-dihydroxypteridine* as pale yellow needles, decomp. 300° after recrystallization from water (Found: C, 43·5; H, 3·9; N, 33·4. $C_6H_6N_4O_2$ requires C, 43·4; H, 3·65; N, 33·7%). Hydrogenation over palladium gave 67% of the same product. 4-Amino-5-ethoxycarbonylmethyleneamino-6-hydroxypyrimidine (1 g.), prepared from ethyl glyoxylate ethyl hemiacetal and 4,5-diamino-6-hydroxypyrimidine as in ref. 12, was hydrogenated (1 mol.) in ethanol (250 ml.) over Raney nickel at 20°. The filtrate was taken to dryness. The residue, recrystallized from ethanol, gave 52% of *4-amino-5-ethoxycarbonylmethylamino-6-hydroxypyrimidine*, decomp. 138° (Found, for material dried at 65°/0·1 mm.: C, 45·2; H, 5·8; N, 26·35. $C_8H_{12}N_4O_3$ requires C, 45·3; H, 5·7; N, 26·4%). This substance (0·3 g.) was heated in N-hydrochloric acid (4 ml.) on a steam-bath for 1 hr. under nitrogen. The precipitate, recrystallized from water, gave 80% of 5,6-dihydro-4,7-dihydroxypteridine (Found: C, 43·2; H, 3·7; N, 33·7%).

2,4-Dihydroxypteridine [28] (0·5 g., 0·003 mole) was reduced with 4% sodium amalgam (9 g.) under nitrogen. Aqueous 2,3-dimercaptopropan-1-ol (0·001 mole, 3 ml.) was added as an antioxidant and the mercury was decanted. The solution was adjusted to pH 5 with acetic acid, chilled, and centrifuged. The slimy precipitate was washed at the centrifuge with 0·0001M-dimercaptopropanol (3 × 50 ml.) and dried at 20°/0·1 mm., giving 63% of 5,6,7,8-*tetrahydro-2,4-dihydroxypteridine* (Found: N, 33·2. $C_6H_8N_4O_2$ requires N, 33·3%). A second preparation of the (unwashed) precipitate was dried over P_2O_5 at 20° and then dissolved in formic acid (50 ml.). Acetic formic anhydride [35] (5 ml.) was added, and the mixture set aside at 20°. Next day the filtrate was taken to dryness under reduced pressure. The residue, recrystallized from water, gave 28% of *5-formyl-5,6,7,8-tetrahydro-2,4-dihydroxypteridine*, m. p. 300° (decomp.) (Found: C, 40·55; H, 4·1; N, 27·2. $C_7H_8N_4O_3,0·5H_2O$ requires C, 41·0; H, 4·4; N, 27·05%). Tetrahydro-2,4-dihydroxypteridine, prepared as above, absorbed 80% of the theoretical requirement of oxygen when shaken in air at 20°.

Sodium dithionite (4·8 g.) was boiled with 2,4-dihydroxypteridine (1·64 g.) in 0·1N-sodium hydroxide (100 ml.) for 2 min. then cooled, and centrifuged. The precipitate, washed with 30%, 45%, then 60% ethanol under nitrogen and dried at 0·1 mm., gave 54% of 5,6,7,8-tetrahydro-2,4-dihydroxypteridine. The supernatant liquid from the precipitate was concentrated under reduced pressure, at 20°, to 50 ml. and diluted with ethanol (50 ml.). A small precipitate was discarded, and the filtrate concentrated to 30 ml. and diluted with ethanol (60 ml.), giving colourless *sodium tetrahydro-2,4-dihydroxypteridine-6-sulphonate* (from 30% ethanol) which slowly decomposed at 80° (Found, for material dried at 20°/0·1 mm.: C, 24·75; H, 3·1; N, 19·15; S, 10·65. $C_6H_7N_4NaO_5S,H_2O$ requires C, 25·0; H, 3·15; N, 19·4; S, 11·1%).

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Department of Medical Chemistry, Institute of Advanced Studies,
Australian National University, Canberra. [*Received, October 16th*, 1961.]

[35] Béhal, *Compt. rend.*, 1889, **128**, 1460.