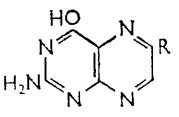
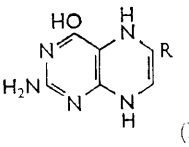
919. *Pteridine Derivatives. Part IX.*[1] 2,6-*Diamino-4-hydroxypteridine and Related Dihydropteridines.*

By Alexander Stuart, D. W. West, and H. C. S. Wood.

An unambiguous synthesis of 2,6-diamino-4-hydroxypteridine is described and the product is shown to be identical with a specimen isolated by van Baalen and Forrest from blue-green algæ. The structure of some related dihydropteridines is examined, and in particular the structure of 7,8-dihydroxanthopterin is confirmed.

van Baalen and Forrest have reported [2] the isolation of 2,6-diamino-4-hydroxypteridine (I; R = NH_2) from *Drosophila melanogaster* and from the blue-green algæ, *Anacystis nidulans* and *Nostoc muscorum* G. They suggest that this pteridine may be an artefact arising from a reactive, naturally occurring dihydropteridine (II or III) by addition of ammonia during the isolation procedure and subsequent oxidation of the resulting tetrahydropteridine. Recently, however, Viscontini and Piraux [3] have suggested that the compound isolated by van Baalen and Forrest does not have structure (I; R = NH_2) but is a 5,8-dihydropteridine derivative (II; R = NH_2). In view of current interest [4] in the chemistry of dihydropteridines we have confirmed structure (I; R = NH_2) for this pteridine derivative by unambiguous synthesis as a preliminary to the study of dihydropteridines and their reactivity.


(I)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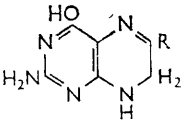
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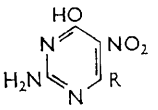
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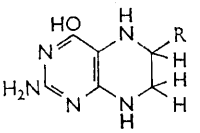
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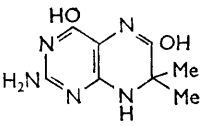

(VI)

Boon and Leigh [5] have described an unambiguous synthesis of xanthopterin (I; R = OH) *via* the 7,8-dihydro-derivative (III; R = OH), and our synthesis of the 6-amino analogue (I; R = NH_2) was based on this approach. Thus 2-amino-4-chloro-6-hydroxy-5-nitropyrimidine (IV; R = Cl) was condensed with aminoacetonitrile to give 2-amino-4-cyanomethylamino-6-hydroxy-5-nitropyrimidine (IV; R = $NH{\cdot}CH_2{\cdot}CN$). Reduction of the nitro-group with sodium dithionite gave the corresponding 5-amine which cyclised spontaneously to give 2,6-diamino-7,8-dihydro-4-hydroxypteridine (III; R = NH_2). Oxidation of this compound with alkaline potassium permanganate gave 2,6-diamino-4-hydroxypteridine (I; R = NH_2) identical with a sample kindly provided by Dr. H. S. Forrest.

An attempt to prepare the 7,8-dihydropteridine (III; R = NH_2) from 2-amino-4-carbamoylmethylamino-6-hydroxy-5-nitropyrimidine (IV; R = $NH{\cdot}CH_2{\cdot}CO{\cdot}NH_2$) by an analogous method gave 7,8-dihydroxanthopterin (III; R = OH) identical with a

[1] Part VIII. Cresswell, Neilson, and Wood, *J.*, 1961, 476.
[2] van Baalen and Forrest, *J. Amer. Chem. Soc.*, 1959, **81**, 1770.
[3] Viscontini and Piraux, *Helv. Chim. Acta*, 1962, **45**, 615.
[4] Viscontini and Weilenmann, *Helv. Chim. Acta*, 1959, **42**, 1854; Wood, Rowan, and Stuart, in "Proceedings of the 3rd International Pteridine Symposium, Stuttgart 1962," Pergamon Press, Oxford, in the press.
[5] Boon and Leigh, *J.*, 1951, 1497.

specimen prepared in similar fashion from 2-amino-4-ethoxycarbonylmethylamino-6-hydroxy-5-nitropyrimidine (IV; R = $NH{\cdot}CH_2{\cdot}CO_2Et$). The nitropyrimidines (IV; R = $NH{\cdot}CH_2{\cdot}CO{\cdot}NH_2$ or $NH{\cdot}CH_2{\cdot}CO_2Et$) were prepared by condensation of aminoacetamide and the ethyl ester of glycine, respectively, with the chloronitropyrimidine (IV; R = Cl).

Through the kindness of Professor Viscontini we have established that our synthetic material (I; R = NH_2) has an infrared spectrum identical with that of the compound formulated as (II; R = NH_2) by Viscontini and Piraux.[3] We have therefore re-examined the evidence on which this formulation is based. They report that on recrystallisation from water, or from aqueous acetic acid,[6] hydrolysis occurs to give 5,8-dihydroxanthopterin (II; R = OH) and that this change is reversible. They also claim that the two compounds (II; R = NH_2 or OH) possess identical ultraviolet absorption spectra and cannot be distinguished on paper chromatography in several different solvent systems. We cannot confirm these observations, as they would involve interconversion of xanthopterin (I; R = OH) and 2,6-diamino-4-hydroxypteridine (I; R = NH_2), which, however, can be readily distinguished by paper chromatography and by thin-layer chromatography, as van Baalen and Forrest [2] have confirmed. The ultraviolet absorption spectra of xanthopterin (I; R = OH) and 2,6-diamino-4-hydroxypteridine (I; R = NH_2) are similar, as would be expected, when measured at pH 13, but they differ considerably at pH 1 (see Table).

Viscontini and Piraux [3] also report that hydrogenation of the compound they formulate as (II; R = NH_2) was terminated after the absorption of 1 mol. of hydrogen. The ultra-

Spectrometry in water.*

Pteridine	$\lambda_{max.}$ (mμ)	ε †	pH
2,6-Diamino-7,8-dihydro-4-hydroxy	274, 319	13,600, 6100	1
	222, 280, *310*	15,050, 10,800, 6800	13
7,8-Dihydroxanthopterin	278, 315	13,850, 10,400	1
	223, 278, *310*	17,500, 12,300, 9000	13
2-Amino-7,8-dihydro-4,6-dihydroxy-7,7-dimethyl	280, 305	12,500, 10,250	1
	222, 280	18,000, 13,300	13
2,6-Diamino-4-hydroxy	271, 377	21,700, 7000	1
	260, 392	16,000, 5200	13
Xanthopterin	230, 260, 356	10,500, 10,000, 4500	1
	255, 392 ‡	18,200, 6920	13
2,6-Diamino-5,6,7,8-tetrahydro-4-hydroxy	270		1
	280		13

* Shoulders in italics. † Where no value for the extinction coefficient is given the compound was too unstable to permit accurate measurement. ‡ Values from Albert and Wood, *J. Appl. Chem.*, 1952, **2**, 591.

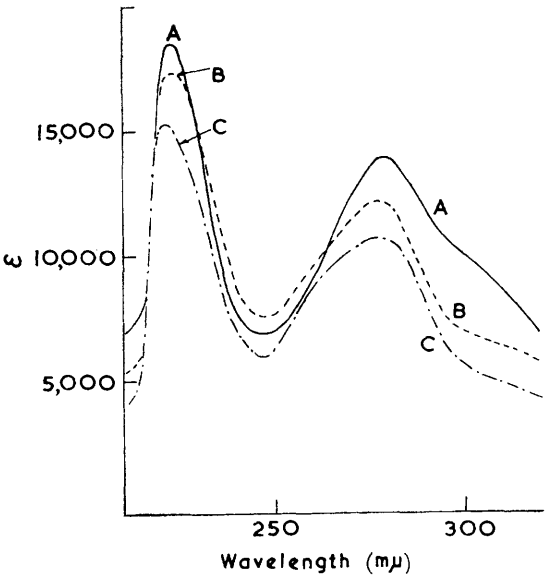
violet absorption spectrum of the reduction product, $\lambda_{max.}$ 275 (ε 13,500) and 316 mμ (ε 5900) at pH 3, is, however, virtually identical with that of the 7,8-dihydropteridine (III; R = NH_2) described above, $\lambda_{max.}$ 274 (ε 13,600) and 319 mμ (ε 6100) at pH 1 (see also Table). In our hands, catalytic hydrogenation of 2,6-diamino-4-hydroxypteridine (I; R = NH_2) with a platinum catalyst resulted in the absorption of 2 mols. of hydrogen to give a solution containing the tetrahydropteridine (V; R = NH_2). This compound was also obtained by a similar reduction (uptake 1 mol.) of the 7,8-dihydropteridine (III; R = NH_2). Re-oxidation of the tetrahydropteridine (V; R = NH_2) took place rapidly on exposure to air to give, finally, 2,6-diamino-4-hydroxypteridine (I; R = NH_2); it proceeds *via* an intermediate which has been identified spectroscopically as the 7,8-dihydro-derivative (III; R = NH_2). This does not exclude the possibility that other, more unstable dihydropteridines may also be involved in this re-oxidation, as is suggested by the work of Kaufman [7] on the re-oxidation of 2-amino-5,6,7,8-tetrahydro-4-hydroxy-6 7-dimethylpteridine.

The possibility of tautomeric shifts (*e.g.*, II $\rightleftharpoons$ III) occurring in dihydropteridines

[6] Viscontini, personal communication.
[7] Kaufman, *J. Biol. Chem.*, 1961, **236**, 804.

cannot be excluded, and the structures assigned to 7,8-dihydroxanthopterin (III; R = OH) [5,8] and to the 6-amino-analogue (III; R = NH_2) described above must be regarded as ambiguous despite the method of synthesis employed in each case. We have now confirmed the above structures in the following ways:

(*a*) We have prepared an analogue (VI) of 7,8-dihydroxanthopterin (III; R = OH) in which the two hydrogen atoms at position 7 are replaced by methyl groups. Tautomeric shifts of the type (II ⇌ III) are excluded in this molecule. The synthesis was carried out by reaction of the nitrochloropyrimidine (IV; R = Cl) with ethyl α-aminoisobutyrate to give the 5-nitropyrimidine (IV; R = NH·CMe_2·CO_2Et). Reduction of the nitro-group with palladium on charcoal as catalyst gave the 5-amine, which cyclised spontaneously to the dihydropteridine analogue (VI). The ultraviolet absorption spectrum at pH 13 of



Ultraviolet spectra of 7,8-dihydropteridines in water (pH 13).

A, 2-Amino-7,8-dihydro-4,6-dihydroxy-7,7-dimethylpteridine; B, 7,8-Dihydroxanthopterin; C, 2,6-Diamino-7,8-dihydro-4-hydroxypteridine.

this compound is essentially identical with those of 7,8-dihydroxanthopterin (III; R = OH) and the 6-amino analogue (III; R = NH_2) (Fig. 1), and we conclude that these molecules all have similar chromophoric systems.

(*b*) Nuclear magnetic resonance also provides an unambiguous method of determining the structure of dihydropteridines.[9,10] Thus the spectrum of 7,8-dihydroxanthopterin (III; R = OH) in trifluoroacetic acid shows a sharp singlet at τ 5·46 together with a broad, diffuse absorption around τ 1. The latter we attribute to protons bound to nitrogen, and the singlet at τ 5·46 to two equivalent protons attached at C-7. The 6-amino-7,8-dihydropteridine (III; R = NH_2) shows similar absorption at τ 5·10, which is replaced in the 7,7-dimethylpteridine derivative (VI) by a single sharp peak at τ 8·3 corresponding to the two methyl groups.

Xanthopterin (I; R = OH) and 2,6-diamino-4-hydroxypteridine (I; R = NH_2) show no absorption in this region, and each has a simple spectrum with a single peak, at τ 1·25 and 0·92, respectively, which is assigned to the single aromatic proton at C-7.

These results indicate unequivocally that in the dihydropteridines (III; R = OH or NH_2) the two protons are attached to a saturated carbon atom. The alternative 5,8- or

[8] O'Dell, Vandenbelt, Bloom, and Pfiffner, *J. Amer. Chem. Soc.*, 1947, **69**, 250.
[9] Viscontini, Merlini, and Philipsborn, *Helv. Chim. Acta*, 1963, **46**, 1181.
[10] Pastore, Friedkin, and Jardetzky, *J. Amer. Chem. Soc.*, 1963, **85**, 3058.

5,6-dihydro-structures would give a spectrum with absorption from the single proton at C-7 in the region τ 1·65—2·25.[9,11]

Experimental

Yields of substances that have no definite m. p. refer to the stage when they appear homogeneous in paper chromatography. Chromatograms were developed by the ascending technique, the solvents being (A) butan-1-ol–5N-acetic acid (7 : 3); (B) 3% aqueous ammonium chloride; (C) propan-1-ol–water–concentrated ammonia (40 : 20 : 1), and (D) methanol–butan-1-ol–water–benzene (2 : 1 : 1 : 1), and were viewed in filtered ultraviolet light. Whatman No. 1 paper was used for solvent systems A, C, and D, and Whatman No. 20 paper for system B.

Ultraviolet Spectra.—These were determined with an Optika recording grating spectrophotometer (model CF4) and on a Unicam manual instrument (model S.P. 600) on aqueous solutions of standard pH.

Nuclear Magnetic Resonance Spectra.—These were determined with a Perkin-Elmer spectrometer operating at 40 Mc./sec. Solutions (10% approx.) in trifluoroacetic acid were used with tetramethylsilane as internal reference.

2-Amino-4-chloro-6-hydroxy-5-nitropyrimidine (IV; R = Cl).—The published procedure [12] was modified as follows. 2-Amino-4-chloro-6-hydroxypyrimidine [13] (5·0 g.) was dissolved in 36N-sulphuric acid (18 ml.) below 15°. Nitric acid (*d* 1·5; 5·6 ml.) was added slowly, with stirring below 0°. The mixture was worked up as before to give the product (5·0 g., 76%), $\lambda_{max.}$ 298 mμ in ethanol.

A sample was routinely checked by conversion to the 4-benzylaminopyrimidine as described below.

2-Amino-4-benzylamino-6-hydroxy-5-nitropyrimidine (IV; R = NH·CH_2Ph).—2-Amino-4-chloro-6-hydroxy-5-nitropyrimidine (0·5 g.) in ethanol (100 ml.) was added to a solution of benzylamine (0·6 g.) in ethanol (10 ml.) and the mixture heated on the steam-bath for 20 min. The solid, which separated on cooling, was collected, washed with water, ethanol, and ether and dried. Recrystallisation from boiling dimethylformamide and ethanol gave *2-amino-4-benzylamino-6-hydroxy-5-nitropyrimidine* as needles (0·6 g., 88%), m. p. 334—335° (decomp.) (Found: C, 50·6; H, 4·3; N, 26·6. $C_{11}H_{11}N_5O_3$ requires C, 50·6; H, 4·2; N, 26·8%).

Aminoacetonitrile.[14]—Glycollonitrile (7·0 g. of 70% aqueous solution) was added slowly to aqueous ammonia (*d* 0·88; 120 ml.) at 0°. The flask was shaken gently, stoppered, and left at room temperature overnight. Ethanol (150 ml.) was added and this was evaporated, together with excess of ammonia, *in vacuo*. The residual, pale yellow liquid (approx. 5 g.) was used directly.

2-Amino-4-cyanomethylamino-6-hydroxy-5-nitropyrimidine (IV; R = NH·CH_2·CN).—Aminoacetonitrile (5 g.) in ethanol (25 ml.) was added slowly, with stirring, to a solution of 2-amino-4-chloro-6-hydroxy-5-nitropyrimidine (10 g.) in ethanol (400 ml.) and the mixture heated on the steam-bath for 20 min. On cooling, a cream solid separated which crystallised (charcoal) from 2N-ammonia solution to give the *nitrile* (8·4 g., 76%), m. p. >300° (Found: C, 34·4; H, 3·0; N, 39·7. $C_6H_6N_6O_3$ requires C, 34·3; H, 2·9; N, 40·0%).

2,6-Diamino-7,8-dihydro-4-hydroxypteridine (III; R = NH_2).—The above nitrile (6·0 g.) suspended in water (150 ml.) was heated on the steam-bath, while solid sodium dithionite was added in portions until the pyrimidine had completely dissolved. Excess of sodium dithionite was destroyed by the addition of 2N-hydrochloric acid (10 ml.) when a pink solid separated. After cooling, this was collected and washed successively with water, ethanol, and ether to give the *7,8-dihydropteridine* (4·7 g., 91%), which crystallised readily from 0·1N-hyrochloric acid, m. p. >300° (Found: C, 36·3; H, 5·0; N, 42·3. $C_6H_6N_6O,H_2O$ requires C, 36·4; H, 5·1; N, 42·4%).

2,6-Diamino-4-hydroxypteridine (I; R = NH_2).—The above 7,8-dihydropteridine (0·4 g.) was dissolved in N-sodium hydroxide solution (8·0 ml.) and 0·2M potassium permanganate solution (10 ml.) was added dropwise over 10 min. After 20 min. at room temperature, solid

[11] Philipsborn, Stierlin, and Traber, " Proceedings of the 3rd International Pteridine Symposium, Stuttgart, 1962," Pergamon Press, Oxford, in the press.
[12] Stuart and Wood, *J.*, 1963, 4186.
[13] Forrest, Hull, Rodda, and Todd, *J.*, 1951, 3.
[14] Menge, *J. Amer. Chem. Soc.*, 1934, **56**, 2197.

sodium dithionite was added to destroy excess of permanganate, and the coagulated precipitate of manganese dioxide was filtered off ("Filtercel" pad). The precipitate was washed with hot water (10 ml.) and the combined filtrate and washings were brought to pH 7 with 2N-hydrochloric acid and allowed to cool. The orange, non-crystalline pteridine (0·29 g., 73%) was purified *via* the sodium salt by dissolving in 2N-sodium hydroxide solution (15 ml.), adding charcoal and heating on the steam-bath for 15 min. Filtration, followed by refrigeration of the filtrate, gave the *sodium salt* of 2,6-diamino-4-hydroxypteridine as yellow-orange needles (0·2 g., 45% overall) (Found: C, 26·7; H, 4·6; N, 30·8. $C_6H_5N_6NaO,4H_2O$ requires C, 26·5; H, 4·8; N, 30·9%).

The 7,8-dihydropteridine may also be oxidised by stirring it at room temperature for 4 hr. in alkaline solution with manganese dioxide.

The pteridine (from the sodium salt) could be recovered unchanged after heating with water, or with aqueous acetic acid. On paper chromatography the following R_F values were obtained (values for xanthopterin in parentheses): Solvent A, 0·08 (0·14); B, 0·33 (0·37); C, 0·23 (0·17); D, 0·23 (0·33).

Catalytic Hydrogenations.—Hydrogenation of 2,6-diamino-4-hydroxypteridine and of its 7,8-dihydro-derivative were carried out at normal temperature and pressure with solutions in 0·5N-sodium hydroxide and Adams catalyst. The resulting solution in each case showed an ultraviolet spectrum ($\lambda_{max.}$ 268 mμ at pH 1) typical of a tetrahydropteridine.

2-Amino-4-carbamoylmethylamino-6-hydroxy-5-nitropyrimidine (IV; R = $NH{\cdot}CH_2CONH_2$). —Aminoacetamide hydrochloride [15] (2·2 g.) was dissolved in water (25 ml.), sodium bicarbonate (1·68 g.) was added, and the solution was stirred until effervescence ceased. The resulting solution was added slowly, with stirring, to a solution of 2-amino-4-chloro-6-hydroxy-5-nitropyrimidine (1·9 g.) in ethanol (100 ml.) and the mixture heated on the steam-bath for 20 min. The product was purified by solution in dilute aqueous ammonia and reprecipitation with 2N-hydrochloric acid to give the *pyrimidine* as needles (1·9 g., 83%), m. p. >300° (Found: C, 31·7; H, 3·8; N, 36·6. $C_6H_8N_6O_4$ requires C, 31·6; H, 3·5; N, 36·8%).

2-Amino-4-ethoxycarbonylmethylamino-6-hydroxy-5-nitropyrimidine (IV; R = $NH{\cdot}CH_2{\cdot}CO_2Et$).—Glycine ethyl ester hydrochloride reacted similarly with the nitro-chloropyrimidine. The *ester* (89%) was recrystallised from water to give needles, m. p. 360—370° (decomp.) (Found: C, 37·5; H, 4·3; N, 27·6. $C_8H_{11}N_5O_5$ requires C, 37·4; H, 4·3; N, 27·2%).

7,8-Dihydroxanthopterin (III; R = OH).—(*a*) 2-Amino-4-carbamoylmethylamino-6-hydroxy-5-nitropyrimidine (0·5 g.) suspended in water (20 ml.) was heated on the steam-bath while solid sodium dithionite was added in portions. The pyrimidine dissolved to give an almost clear solution and on cooling a cream-coloured solid separated. This was collected and recrystallised from 0·1N-hydrochloric acid to give 7,8-dihydroxanthopterin (0·35 g., 89%) as needles, m. p. >320° (Found: C, 36·3; H, 4·5; N, 35·7. Calc. for $C_6H_7N_5O_2H_2O$: C, 36·2; H, 4·6; N, 35·2%).

The crystalline *sodium salt* (96%) was obtained by dissolving 7,8-dihydroxanthopterin in the minimum of warm 2N-sodium hydroxide solution and cooling rapidly (Found: C, 32·9; H, 3·5; N, 32·2. $C_6H_6N_5O_2Na,H_2O$ requires C, 32·6; H, 3·6; N, 31·7%). This material was unstable and rapidly oxidised in air to give xanthopterin.

(*b*) 2-Amino-4-ethoxycarbonylmethylamino-6-hydroxy-5-nitropyrimidine similarly gave 7,8-dihydroxanthopterin (84%).

Ethyl α-Aminoisobutyrate.—The free ester was prepared from the hydrochloride [16] by solution in 20% sodium hydroxide solution, extraction with ether, and fractional distillation of the extract to give a colourless oil, b. p. 146—147°, n_D^{20} 1·4170 (lit.,[16] n_D^{17} 1·4169).

2-Amino-4-(2-ethoxycarbonylisopropylamino)-6-hydroxy-5-nitropyrimidine (IV; R = $NH{\cdot}CMe_2{\cdot}CO_2Et$).—2-Amino-4-chloro-6-hydroxy-5-nitropyrimidine (4·52 g.) was dissolved in dimethylformamide (25 ml.) and ethyl α-aminoisobutyrate (6·18 g.) was added. The mixture was kept at 37° for 4 days and then poured into water. The yellow solid which separated was collected and recrystallised from water to give the *pyrimidine* (4·3 g., 64%) as needles, m. p. 247—249° (Found: C, 42·0; H, 5·35; N, 25·3. $C_{10}H_{15}N_5O_5$ requires C, 42·1; H, 5·3; N, 24·6%).

2-Amino-7,8-dihydro-4,6-dihydroxy-7,7-dimethylpteridine (VI).—2-Amino-4-(2-ethoxycarbonylisopropylamino)-6-hydroxy-5-nitropyrimidine (1·98 g.) was suspended in water (75 ml.) and

[15] Karmas and Spoerri, *J. Amer. Chem. Soc.*, 1952, **74**, 1580.
[16] Zelinsky and Kulikov, *Z. Physiol. Chem.*, 1911, **73**, 459.

hydrogenated at room temperature and pressure over 10% palladium on charcoal. The catalyst was filtered off from the otherwise clear solution and was extracted with boiling water (2 × 40 ml.). The combined filtrate and extracts were evaporated to dryness *in vacuo* and the resulting solid recrystallised from water to give the *dimethylpteridine*, m. p. >320° (Found: C, 45·9; H, 5·4; N, 34·0. $C_8H_{11}N_5O_2$ requires C, 45·9; H, 5·3; N, 33·5%).

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