792. *The Biosynthesis of Pteridines. Part IV.*[1] *The Synthesis of Xanthopterin.*

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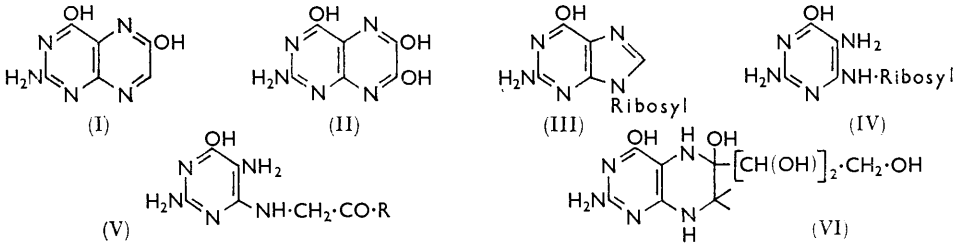
The synthesis of the ketose {V; R = $[CH(OH)]_2 \cdot CH_2 \cdot OH$} and its conversion, *in vitro*, into xanthopterin are described. The relevance of the latter observation to the biosynthesis of xanthopterin is discussed.

THE structures of the insect pigments, xanthopterin (I) and leucopterin (II), are now well established as the result of degradative,[2] synthetic,[3] and spectroscopic [4] studies.

Preliminary studies of the biosynthesis of these pteridines indicated that they can be formed from purines or purine nucleosides through an intermediate 4,5-diaminopyrimidine derivative. Thus, Weygand and Waldschmidt [5] have shown that [2-^{14}C]xanthopterin can be isolated from the wings of *Pierid* butterflies after feeding 2,4,5-triamino-6-hydroxy-[2-^{14}C]pyrimidine to the caterpillars. More recent experiments by Weygand and his collaborators,[6] using a variety of ^{14}C-labelled precursors, have shown that guanosine (III) or its 5′-phosphate can function as a precursor of xanthopterin (I) and leucopterin (II) in the butterfly *Pieris brassicae* L. They suggest that this transformation involves the following steps: (a) ring-cleavage of the imidazole ring of guanosine to give a 5-amino-4-ribosylaminopyrimidine (IV); (b) Amadori rearrangement of this glycosylamine to the corresponding 1-(substituted amino)-1-deoxypentulose {V; R = $[CH(OH)]_2 \cdot CH_2 \cdot OH$}; and (c) cyclisation of this ketose to a polyhydroxyalkylpteridine (VI) which serves as the immediate precursor of xanthopterin.

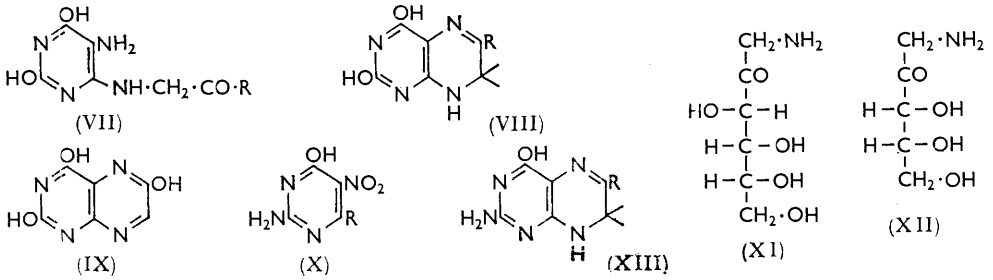
[1] Part III, Neilson and Wood, *J.*, 1962, 44.
[2] Wieland, Metzer, Schöpf, and Bülow, *Annalen*, 1933, **507**, 226; Schöpf and Kottler, *ibid.*, 1939, **539**, 128; Wieland and Tartter, *ibid.*, 1940, **543**, 287; Wieland and Decker, *ibid.*, 1941, **547**, 180.
[3] Purrmann, *Annalen*, 1940, **544**, 182; 1940, **546**, 98.
[4] Pfleiderer and Rukwied, *Chem. Ber.*, 1961, **94**, 118.
[5] Weygand and Waldschmidt, *Angew. Chem.*, 1955, **67**, 328.
[6] Weygand, Simon, Dahms, Waldschmidt, Schliep, and Wacker, *Angew. Chem.*, 1961, **73**, 402.



The present paper deals with the synthesis of the ketose {V; R = $[CH(OH)]_2 \cdot CH_2 \cdot OH$} and its conversion, *in vitro*, into xanthopterin. A preliminary account of this work has already been published.[7]

We have recently reported[1] that the 5-aminopyrimidine {VII; R = $[CH(OH)]_3 \cdot CH_2 \cdot OH$}, which was prepared by catalytic hydrogenation of the corresponding 5-nitropyrimidine, underwent immediate intramolecular cyclisation to give the 7,8-dihydropteridine {VIII; R = $[CH(OH)]_3 \cdot CH_2 \cdot OH$}. On aerial oxidation in alkaline solution this dihydro-compound gave 2,4,6-trihydroxypteridine (IX) in high yield, fission of the polyhydroxy-alkyl side-chain having taken place. The relevance of this observation to the biosynthetic pathway discussed above is readily apparent.

The synthesis of the related ketoses {V; R = $[CH(OH)]_2 \cdot CH_2 \cdot OH$ and R = $[CH(OH)]_3 \cdot CH_2 \cdot OH$} was carried out as follows. Nitration of 2-amino-4-chloro-6-hydroxy-pyrimidine under carefully controlled conditions gave the reactive 5-nitro-derivative (X; R = Cl). Reaction with 1-amino-1-deoxy-D-fructose (isoglucosamine, XI) gave the hexulose {X; R = $NH \cdot CH_2 \cdot CO \cdot [CH(OH)]_3 \cdot CH_2 \cdot OH$} which was characterised as its oxime. Similarly, 1-amino-1-deoxy-D-*erythro*pentulose (XII) gave the corresponding pentulose {X; R = $NH \cdot CH_2 \cdot CO \cdot [CH(OH)]_2 \cdot CH_2 \cdot OH$}, also characterised as its oxime. Reduction of the nitro-group in either of the above pyrimidinylamino-ketoses (X), using a Raney nickel catalyst, gave the corresponding 5-amine (V) which could not be isolated. The reaction mixture in each case was made alkaline with sodium hydroxide solution and allowed to stand at room temperature for three days. Neutralisation of these solutions gave 7,8-dihydroxanthopterin (XIII; R = OH), identified by comparison with an authentic specimen.[8] Prolonged aerial oxidation of the 7,8-dihydroxanthopterin, or treatment with cold alkaline potassium permanganate,[8] gave xanthopterin (I).



We suggest that the mechanism of these reactions involves initial cyclisation of the 5-aminopyrimidine (V) to give a 7,8-dihydropteridine {XIII; R = $[CH(OH)]_3 \cdot CH_2 \cdot OH$ or R = $[CH(OH)]_2 \cdot CH_2 \cdot OH$}. The reaction mixture at this stage showed absorption maxima in the ultraviolet region at 258 and 360 mμ at pH 1, and 282 and 328 mμ at pH 13. These figures compare favourably with those given by Boothe *et al.*[9] for 2-amino-7,8-dihydro-4-hydroxy-6-methylpteridine (XIII; R = Me) which are 252 and 365 mμ at pH 1, and

[7] Stuart and Wood, *Proc. Chem. Soc.*, 1962, 151.
[8] Albert and Wood, *J. Appl. Chem.*, 1952, **2**, 591.
[9] Boothe *et al.*, *J. Amer. Chem. Soc.*, 1948, **70**, 27.

282 and 325 mμ at pH 13. Covalent hydration of the 5,6-double-bond in the dihydropteridines (XIII) then occurs to give tetrahydropteridines (*e.g.*, VI). Albert and his collaborators [10] have established that many pteridines undergo covalent hydration and similar addition reactions. Oxidative fission of the polyhydroxyalkyl side-chain in the tetrahydropteridine (VI) then leads directly to 7,8-dihydroxanthopterin (XIII; R = OH).

The above results appear to support the biosynthetic pathway suggested by Weygand *et al.*[6] It must be borne in mind, however, that xanthopterin can also be formed by the spontaneous oxidation of dihydro- or tetrahydro-folic acid,[11] and could well be an end-product of pteridine metabolism in insects. The radiochemical results of Weygand *et al.*, do not exclude this possibility, and Reynolds and Brown [12] have recently demonstrated that guanosine or its nucleotides can function as precursors of folic acid derivatives in *E. coli*. Whether xanthopterin is formed directly, as suggested by Weygand, or by degradation of a folic acid derivative, the initial pteridine intermediate appears to be a 7,8-dihydropteridine (XIII), since 2-amino-7,8-dihydro-4-hydroxy-6-hydroxymethylpteridine (XIII; R = CH_2·OH) is an efficient precursor of dihydrofolic acid.[13] It is thus possible that our observation, that, *in vitro*, the dihydropteridine {XIII; R = $[CH(OH)]_{2\text{ or }3}$·CH_2·OH} undergoes covalent hydration followed by cleavage of the side-chain to give 7,8-dihydroxanthopterin, is merely one example of the addition reactions undergone by 7,8-dihydropteridines and the instability of the resulting tetrahydropteridines. Further studies of the addition reactions of 7,8-dihydropteridines will be discussed in Part V of this series.[14]

Experimental

For general detail see Part I.[15]

2-Amino-4-chloro-6-hydroxy-5-nitropyrimidine (X; R = Cl).—2-Amino-4-chloro-6-hydroxypyrimidine [16] (5·0 g.) was dissolved in 36N-sulphuric acid (6 ml.) below 45°. Nitric acid (*d* 1·5; 5·3 ml.) was added cautiously with stirring below 45° After ½ hr. the mixture was poured on to ice (20 g.), and the precipitated solid was collected and washed successively with water (2 × 20 ml.), ethanol (20 ml.), and ether (20 ml.) to give the *product* (5·0 g., 91%) as a yellow powder, m. p. >360°, which could not be recrystallised (Found: C, 23·2; H, 2·2; N, 26·4. $C_4H_3ClN_4O_3,H_2O$ requires C, 23·0; H, 2·4; N, 26·8%).

1-Deoxy-1-(2-amino-6-hydroxy-5-nitro-4-pyrimidinylamino)-D-fructose {X; R = NH·CH_2·CO·$[CH(OH)]_3$·CH_2·OH}.—To a solution of isoglucosamine acetate [17] (2·0 g.) in the minimum of water was added a solution from sodium (0·2 g.) in ethanol (20 ml.) and the mixture was left at room temperature for ½ hr. A suspension of 2-amino-4-chloro-6-hydroxy-5-nitropyrimidine (0·8 g.) in ethanol (200 ml.) was added, and the mixture was refluxed gently for 20 min. On cooling, a pale yellow solid (0·8 g., 57%) separated, and this was collected, washed quickly with ethanol and ether, and dried to give the required ketose as a hygroscopic solid.

To the ketose (0·2 g.) in water (7 ml.) was added hydroxylamine in ethanol (5 ml.) [from the hydrochloride (85 mg.) and sodium (28 mg.)]. The mixture was refluxed for 1 hr. and cooled, the *oxime* of the ketose separating. Recrystallisation from water gave needles (0·18 g., 86%), m. p. >300° (Found: C, 33·5; H, 5·1; N, 22·2. $C_{10}H_{16}N_6O_8,H_2O$ requires C, 33·7; H, 4·9; N, 23·0%).

1-Deoxy-1-(2-amino-6-hydroxy-5-nitro-4-pyrimidinylamino)-D-erythropentulose {X; R = NH·CH_2·CO·$[CH(OH)]_2$·CH_2·OH}.—1-Benzylamino-1-deoxy-D-*erythro*pentulose oxalate (3 g.) in methanol (20 ml.) was added to a suspension of reduced 10% palladium–charcoal (1 g.) in methanol (20 ml.). The mixture was hydrogenated until 1 mol. of hydrogen had been absorbed

[10] Albert, "Current Trends in Heterocyclic Chemistry," Butterworths Scientific Publications, London, 1958, p. 20.
[11] Blakley, *Biochem. J.*, 1957, **65**, 331.
[12] Reynolds and Brown, *J. Biol. Chem.*, 1962, **237**, PC2713.
[13] Brown, Weisman, and Molnar, *J. Biol. Chem.*, 1961, **236**, 2534.
[14] Stuart and Wood, unpublished work.
[15] Cresswell and Wood, *J.*, 1960, 4768.
[16] Forrest, Hull, Rodda, and Todd, *J.*, 1951, 3.
[17] Mauer and Schiedt, *Ber.*, 1935, **68**, 2187.

(12 hr.), the catalyst was removed, and the resulting solution of 1-amino-1-deoxy-D-*erythro*-pentulose oxalate was used directly. A solution of sodium (0·4 g.) in ethanol (40 ml.) was added, and the resulting precipitate of sodium oxalate was filtered off. To the filtrate was added a solution of 2-amino-4-chloro-6-hydroxy-5-nitropyrimidine (0·83 g.) in ethanol (100 ml.). The solution was heated for 20 min. on the steam-bath, filtered, and allowed to cool; the ketose separated as a cream-coloured hygroscopic solid (0·75 g., 58%) which was characterised as its crystalline *oxime* prepared as above (Found: C, 33·0; H, 4·3; N, 25·3. $C_9H_{14}N_6O_7$, $0·5H_2O$ requires C, 33·0; H, 4·6; N, 25·7%).

7,8-*Dihydroxanthopterin* (XIII; R = OH).—(*a*) 1-Deoxy-1-(2-amino-6-hydroxy-5-nitro-4-pyrimidinylamino)-D-fructose (0·7 g.) in water (40 ml.) was hydrogenated over a Raney nickel catalyst (*ca.* 1 g.) until 3 mol. of hydrogen had been absorbed. The catalyst was filtered off, sodium hydroxide solution (2N; 1 ml.) was added, and the solution was left at room temperature for 3 days. Addition of hydrochloric acid (0·5N) to pH 6, followed by concentration *in vacuo*, gave a brown non-crystalline solid (0·2 g.). Recrystallisation from dilute hydrochloric acid gave 7,8-dihydroxanthopterin (0·14 g., 37%), identical (infrared and ultraviolet spectra) with an authentic specimen.[8]

(*b*) 7,8-Dihydroxanthopterin was also obtained, in somewhat lower yield, by similar treatment of 1-deoxy-1-(2-amino-6-hydroxy-5-nitro-4-pyrimidinylamino)-D-*erythro*pentulose.

Xanthopterin (I).—The 7,8-dihydroxanthopterin obtained from the above reactions was oxidised almost quantitatively to xanthopterin on treatment [8] with cold alkaline potassium permanganate solution. Prolonged aerial oxidation in alkaline solution also gave xanthopterin. The product was identical (infrared and ultraviolet spectra, and on paper chromatograms) with an authentic specimen.[8]

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