9. *The Biosynthesis of Pteridines. Part III.*[1] *The Synthesis of* 1-*Deoxy*-1-*pyrimidinylamino*-2-*ketoses.*

By THOMAS NEILSON and H. C. S. WOOD.

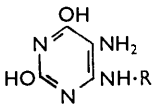
It is suggested that the ketose {III; R = $[CH(OH)]_2 \cdot CH_2 \cdot OH$} is a possible intermediate in the biosynthesis of riboflavin. The synthesis of various ketoses of this type, and of related ketones, is described. The conversion of these compounds into flavins, including riboflavin, and other pteridine derivatives has been carried out.

IT is now generally accepted that a purine, or the derived nucleoside or nucleotide, is the biogenetic precursor of riboflavin in moulds such as *Eremothecium ashbyii* [2] and *Ashbya gossypii*.[3] 5-Amino-4-D-ribitylaminouracil (I; R = D-ribityl) is an intermediate in this transformation, and the formation of riboflavin from this pyrimidine has been described in Part I.[4] Little is known, however, of the intermediate compounds which lie between the purine precursor and the pyrimidine (I; R = D-ribityl).

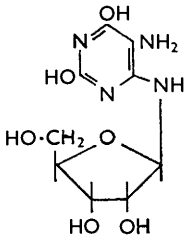
We suggest that a reasonable biosynthetic pathway could involve the following steps: (*a*) ring cleavage of the imidazole ring of a purine nucleoside to give a 5-amino-4-ribosylaminopyrimidine (II), (*b*) Amadori rearrangement of this glycosylamine to the corresponding 1-(substituted amino)-1-deoxypentulose {III; R = $[CH(OH)]_2 \cdot CH_2 \cdot OH$}, and (*c*)

[1] Part II, Cresswell, Neilson, and Wood, *J.*, 1960, 4776.
[2] Maclaren, *J. Bact.*, 1952, **63**, 233; McNutt, *J. Biol. Chem.*, 1954, **210**, 511; 1956, **219**, 365.
[3] Plaut, *J. Biol. Chem.*, 1954, **208**, 513.
[4] Cresswell and Wood, *J.*, 1960, 4768.

stereospecific reduction of the carbonyl group in this ketose to give 5-amino-4-D-ribityl-aminouracil (I; R = D-ribityl). The present paper deals with the synthesis of ketones and ketoses of type (III) and their conversion into flavins (including riboflavin) and other pteridine derivatives.



(I)



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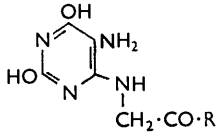


(III)

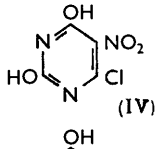
The proposed synthesis for the ketose {III; R = $[CH(OH)]_2 \cdot CH_2 \cdot OH$} involved condensation of 4-chloro-5-nitrouracil (IV) with 1-amino-1-deoxy-D-*erythro*pentulose (V; R = H) to give the 5-nitropyrimidine {VI; R = $[CH(OH)]_2 \cdot CH_2 \cdot OH$}. Reduction of the nitro-group would then give the required ketose. So far as we are aware only one crystalline 1-amino-1-deoxy-D-ketose is known and this is the D-fructose derivative (VII) (isoglucosamine).[5] Experiments were therefore first carried out with this compound which was prepared by catalytic hydrogenation of D-glucosazone, modified from the method described in the literature.[6] Condensing isoglucosamine with 4-chloro-5-nitrouracil[4] (IV) gave the hexulose {VI; R = $[CH(OH)]_3 \cdot CH_2 \cdot OH$} which was characterised as its crystalline oxime. Reduction of the nitro-group with a Raney nickel catalyst gave the corresponding 5-amine {III; R = $[CH(OH)]_3 \cdot CH_2 \cdot OH$} which immediately underwent intramolecular ring-closure to give a pteridine derivative: the course of this reaction is discussed below. It was thus impossible to investigate the reduction of the carbonyl group in the sugar side-chain of this 5-amine. Stereospecific reduction of the carbonyl group in the 5-nitrohexulose {VI; R = $[CH(OH)]_3 \cdot CH_2 \cdot OH$} was achieved, however, by using sodium borohydride in dilute alkali and gave 5-nitro-4-D-sorbitylaminouracil (VIII; R = D-sorbityl) in good yield. The purity of this material was checked by chromatography on paper and on an anion-exchange resin. The product was identical with a sample prepared[1] by an unambiguous method, namely, condensation of D-sorbitylamine with 4-chloro-5-nitrouracil (IV), and differed from the epimeric D-mannitylaminopyrimidine (VIII; R = D-mannityl).[1] Reduction of D-fructose by sodium borohydride has been shown to give an approximately equimolar mixture of D-sorbitol and D-mannitol.[7] It seems likely, therefore, that the stereospecific nature of the reduction of the ketose {VI; R = $[CH(OH)]_3 \cdot CH_2 \cdot OH$} is due to some intereaction between the carbonyl group and the pyrimidine ring, possibly by hydrogen bonding. Reduction of the nitro-group in the pyrimidine (VIII; R = D-sorbityl) by sodium dithionite gave 5-amino-4-D-sorbitylaminouracil (I; R = D-sorbityl) which was converted into 6,7-dimethyl-9-D-sorbityliso-alloxazine (IX; R = D-sorbityl) and into the pyrimido[5,4-*g*]pteridine (X; R = D-sorbityl) by methods previously described by us.[1,8] In each case the products were identical with authentic materials and differed from the epimeric D-mannityl compounds.

Attention was then turned to the synthesis of 1-amino-1-deoxy-D-*erythro*pentulose (V; R = H). An attempt to prepare this ketose by catalytic hydrogenation of the osazone of D-arabinose was not successful although a syrup was obtained which gave colour tests[9] characteristic of an Amadori-type product. Synthesis was achieved as follows. D-Arabinose was treated with benzylamine, giving *N*-benzyl-D-arabinosylamine. This

[5] Fischer, *Ber.*, 1886, **19**, 1920.
[6] Maurer and Schiedt, *Ber.*, 1935, **68**, 2187.
[7] Abdel-Akher, Hamilton, and Smith, *J. Amer. Chem. Soc.*, 1951, **73**, 4691.
[8] Cresswell, Neilson, and Wood, *J.*, 1961, 476.
[9] Hodge, *Adv. Carbohydrate Chem.*, 1955, **10**, 188.

compound, on treatment with anhydrous oxalic acid in dry dioxan, underwent an Amadori rearrangement to give the oxalate of the *N*-benzylamino-ketose (V; R = CH_2Ph). Hydrogenation over palladised charcoal removed the benzyl group, affording the required amine (V; R = H) as its crystalline oxalate.

The amine (V; R = H) with 4-chloro-5-nitrouracil (IV) gave the 5-nitropyrimidine {VI; R = $[CH(OH)]_2 \cdot CH_2 \cdot OH$} which was purified by chromatography on an anion-exchange resin. The product was, however, not obtained crystalline and was reduced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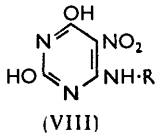
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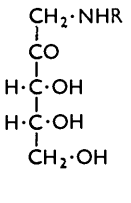
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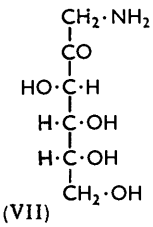
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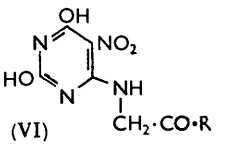
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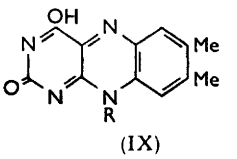
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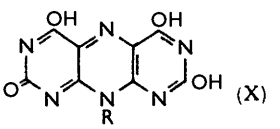

(X)

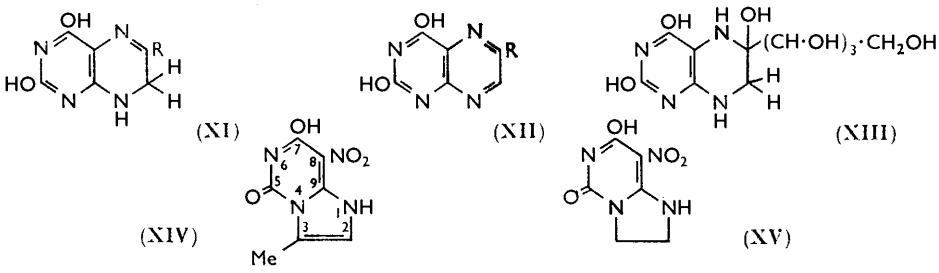
directly with sodium borohydride in alkali to 5-nitro-4-D-ribitylaminouracil (VIII; R = D-ribityl). The purity of this material was confirmed by the methods described above for the D-sorbityl analogue, and its identity was confirmed by comparison with a specimen [4] prepared by condensing D-ribitylamine with 4-chloro-5-nitrouracil. The epimeric 5-nitro-4-D-arabitylaminouracil (VIII; R = D-arabityl) was prepared by condensing D-arabitylamine (from D-arabinose oxime) with 4-chloro-5-nitrouracil (IV), and this was distinct from the product of the sodium borohydride reduction. Reduction of the nitro-group in pyrimidine (VIII; R = D-ribityl) gave 5-amino-4-D-ribitylaminouracil (I; R = D-ribityl) and this was converted into riboflavin (IX; R = D-ribityl) by the methods previously described.[4,8] Similarly, condensation [1] with alloxan gave a pyrimido[5,4-*g*]pteridine (X; R = D-ribityl) identical with an authentic specimen. Preparations of the epimeric D-arabityl flavin (IX; R = D-arabityl) and pyrimidopteridine (X; R = D-arabityl) are also described below.

The intramolecular cyclisation of the 5-aminohexulose {III; R = $[CH(OH)]_3 \cdot CH_2 \cdot OH$} was described above. The product from this reaction is the 7,8-dihydropteridine {XI; R = $[CH(OH)]_3 \cdot CH_2 \cdot OH$} which, although unstable in air, was isolated as its crystalline sodium salt. Attempted oxidation of this compound to the fully aromatic 6-polyhydroxyalkylpteridine {XII; R = $[CH(OH)]_3 \cdot CH_2 \cdot OH$} gave as the only isolable product (58%) 2,4,6-trihydroxypteridine (XII; R = OH) that was identified by comparison with an authentic specimen.[10] This oxidation was carried out either by cold alkaline potassium permanganate or by aerial oxidation in alkaline solution. During one experiment with the latter technique 7,8-dihydro-2,4,6-trihydroxypteridine (XI; R = OH) [10,11] separated as an intermediate product. The simplest explanation of this unusual reaction is that covalent hydration of the 5,6-double bond in the dihydropteridine {XI; R = $[CH(OH)]_3 \cdot CH_2 \cdot OH$} occurs, giving a tetrahydropteridine (XIII). Oxidative fission of the polyhydroxyalkyl side chain then gives 7,8-dihydro-2,4,6-trihydroxypteridine (XI; R = OH) which is further dehydrogenated to give 2,4,6-trihydroxypteridine (XII; R = OH). The covalent hydration of 7,8-dihydropteridines, and its possible biological implications, will be discussed in a subsequent paper.

We have been unable to prepare the fully aromatic 2,4-dihydroxy-6-polyhydroxyalkylpteridine {XII; R = $[CH(OH)_3] \cdot CH_2 \cdot OH$} starting from either the 5-aminohexulose {III; R = $[CH(OH)]_3 \cdot CH_2 \cdot OH$} or the corresponding 5-nitrohexulose (VI). Experiments with model compounds, however, did yield a simple 6-alkylpteridine (XII; R = Me) by a

[10] Albert, Lister, and Pedersen, *J.*, 1956, 4621.
[11] Boon and Leigh, B.P. 677,342/1950.

method which did not involve a reactive 7,8-dihydropteridine as an intermediate. This method, first used by Weygand and Bergmann [12] for the synthesis of 2-polyhydroxyalkylquinoxalines, involves preparation of the hydrazone of the ketose. On heating, dehydrogenation of the side-chain occurs before cyclisation, the hydrazine functioning as a hydrogen acceptor. Thus, when the hydrazone of 4-acetonylamino-5-nitrouracil (VI;



R = Me), after reduction of the nitro-group with Raney nickel and hydrogen, was refluxed gently in acetic acid solution cyclisation took place, giving 2,4-dihydroxy-6-methylpteridine (XII; R = Me). This material was identical with a sample prepared by oxidation of the corresponding 7,8-dihydropteridine (XI; R = Me) which, unlike the 6-polyhydroxyalkyl analogue (XI) shows no appreciable tendency to undergo covalent hydration. An attempt to apply this technique to the hydrazone of the 5-nitrohexulose (VI) was not successful.

4-Acetonylamino-5-nitrouracil (VI; R = Me) was prepared by reaction of aminoacetone semicarbazone with 4-chloro-5-nitrouracil (IV), followed by hydrolysis with mineral acid at 37°. Hydrolysis by refluxing dilute mineral acid gave a different product which was also obtained from 4-acetonylamino-5-nitrouracil (VI; R = Me) on treatment with acid. The ultraviolet spectrum of this compound is distinct from that of a 4-alkylamino-5-nitrouracil and we believe that cyclisation has taken place to give the imidazo-[1,2-*c*]pyrimidine derivative (XIV). This reaction is therefore analogous to the cyclisations reported by Ramage and his co-workers [13] and by Lister,[14] and to the cyclisation of 4-2′-hydroxyethylamino-5-nitrouracil (VIII; R = CH_2·CH_2·OH) to the imidazo[1,2-*c*]-pyrimidine derivative (XV) which we now describe.

Experimental

For general detail see Part I.[4]

1-*Amino*-1-*deoxy*-D-*fructose* (*Isoglucosamine*).—D-Glucosazone (20 g.) was made into a paste with acetic acid (100 c.c.), ethanol (50 c.c.), and water (20 c.c.). A palladium oxide–barium sulphate catalyst [15] (5 g.) was added and the mixture was hydrogenated overnight under 3—5 atm. Isoglucosamine acetate (8 g., 60%) was isolated by the recorded method as needles, m. p. 137° (lit.,[6] 137°).

1-*Deoxy*-1-(2,6-*dihydroxy*-5-*nitro*-4-*pyrimidinylamino*)-D-*fructose* {VI; R = [CH(OH)]$_3$·CH_2·OH}.—To a suspension of isoglucosamine acetate (5 g.) in ethanol (30 c.c.) was added a solution from sodium (0·47 g.) in ethanol (50 c.c.), and the mixture was left at room temperature for 1 hr. A solution of 4-chloro-5-nitrouracil (2 g.) in ethanol (50 c.c.) was then added, a yellow solid separating almost immediately. After 30 min. the solid (2 g., 55%) was collected, washed quickly with ethanol (50 c.c.) and ether (2 × 30 c.c.), and dried to give the required ketose as an extremely hygroscopic solid.

To the ketose (335 mg.) in water (10 c.c.) was added hydroxylamine in ethanol (20 c.c.)

[12] Weygand and Bergmann, *Chem. Ber.*, 1947, **80**, 255.
[13] Ramage and Trappe, *J.*, 1952, 4410; Clark and Ramage, *J.*, 1958, 2821.
[14] Lister, *J.*, 1960, 899.
[15] Kuhn and Haas, *Angew. Chem.*, 1955, **67**, 785.

[from the hydrochloride (70 mg.) and sodium (23 mg.)]. The mixture was refluxed for 1 hr. and cooled, the *oxime* of the ketose separating. Recrystallisation from water gave needles (250 mg., 70%), m. p. 291°, $[\alpha]_D$ −49·8° (*c* 0·20 in 0·05N-NaOH) (Found: C, 34·7; H, 4·6; N, 20·1. $C_{10}H_{15}N_5O_9$ requires C, 34·4; H, 4·3; N, 20·1%).

2,6-*Dihydroxy*-5-*nitro*-4-D-*sorbitylaminopyrimidine* (VIII; R = D-sorbityl).—1-Deoxy-1-(2,6-dihydroxy-5-nitro-4-pyrimidinylamino)-D-fructose (1·2 g.) was dissolved in 0·2N-sodium hydroxide (10 c.c.), and sodium borohydride (0·15 g.) in water (10 c.c.) was added with stirring. After 2 hr. at room temperature formic acid was added to destroy the excess of borohydride and ammonia solution was added to pH 9. Paper chromatography showed that this solution contained only one pyrimidine component. The solution was run on a column of an anion-exchange resin (Amberlite CG400; formate form), prepared by washing the resin with ammonium formate buffer (0·1M with respect to formic acid) at pH 9. The column was washed thoroughly with ammonium formate buffers of pH 9 and 7 (0·1M with respect to formic acid). Buffer of pH 4·5 (0·1M with respect to ammonia) eluted the nitropyrimidine in a single sharp band. Concentration of this eluate gave 2,6-dihydroxy-5-nitro-4-D-sorbitylaminopyrimidine (0·8 g., 66%) as needles, m. p. 225°, $[\alpha]_D$ +15° (*c* 0·18 in 0·05N-NaOH). This material was identical (infrared spectra, paper chromatography, mixed m. p., and specific rotation) with a sample prepared [1] from 4-chloro-5-nitrouracil and D-sorbitylamine. The epimeric D-mannityl pyrimidine [1] showed distinct physical constants.

6,7-*Dimethyl*-9-D-*sorbitylisoalloxazine* (IX; R = D-sorbityl).—This was prepared by condensation [8] of 5-amino-4-D-sorbitylaminouracil (from the above 5-nitro-compound) and the dimer of 3,4-dimethyl-*o*-benzoquinone. The product was identical with an authentic sample, m. p. 275° (lit.,[8,16] 275°, 272°), $[\alpha]_D$ −45° (*c* 0·18 in 0·05N-NaOH) (lit.,[8,16] −45·0°, −47·7°).

2,10-*Dihydro*-4,6,8-*trihydroxy*-2-*oxo*-10-D-*sorbitylpyrimido*[5,4-*g*]*pteridine* (X; R = D-sorbityl).—Condensation of 5-amino-4-D-sorbitylaminouracil with alloxan in acid solution gave the pyrimidopteridine as yellow needles, identical with an authentic specimen.[1]

N-*Benzyl*-D-*arabinosylamine* (with A. G. Wylie).—D-Arabinose (5 g.) and benzylamine (4 g.) in ethanol (50 c.c.) were refluxed for 15 min. The solid which separated after refrigeration overnight was collected and recrystallised from ethanol, to give the *glycosylamine* (5 g., 60%) as needles, m. p. 117—118° (decomp.), $[\alpha]_D$ −4·0° (*c* 1·0 in MeOH) (Found: C, 60·3; H, 6·9; N, 5·7. $C_{12}H_{17}NO_4$ requires C, 60·2; H, 7·2; N, 5·9%).

1-*Benzylamino*-1-*deoxy*-D-erythro*pentulose Oxalate* (V; R = CH_2Ph) (with A. G. Wylie).—To a solution of the above glycosylamine (5 g.) in dry dioxan (70 c.c.) was added a cold solution of anhydrous oxalic acid (1·8 g.) in dioxan (50 c.c.). A white gelatinous precipitate was formed almost immediately and this was dissolved by addition of water (10 c.c.) and gentle warming for a few minutes. On refrigeration overnight crystals separated. These were collected and identified as benzylamine hemioxalate,[17] m. p. and mixed m. p. 180°. The filtrate was evaporated to dryness *in vacuo* to a pale yellow solid which recrystallised from ethanol to give 1-*benzylamino*-1-*deoxy*-D-erythro*pentulose oxalate* (3 g., 40%) as needles, m. p. 145—146° (decomp.), $[\alpha]_D$ +5·2° (*c* 0·33 in 0·05N-NaOH) (Found: C, 50·8; H, 5·8; N, 4·2. $C_{14}H_{19}NO_8$ requires C, 51·1; H, 5·8; N, 4·3%). The compound gave a deep purple colour with *o*-dinitrobenzene in alkaline solution.[18]

1-*Amino*-1-*deoxy*-D-erythro*pentulose Oxalate* (V; R = H).—1-Benzylamino-1-deoxy-D-*erythro*pentulose oxalate (2·8 g.) in ethanol (50 c.c.) was added to a suspension of reduced 10% palladium–charcoal (1 g.) in ethanol (20 c.c.). The mixture was hydrogenated until 1 mol. of hydrogen had been absorbed, the catalyst removed, and the filtrate evaporated *in vacuo*. Crystallisation of the resulting gum from methanol–ethanol gave the *pentulose oxalate* (1 g., 50%) as needles, m. p. 70°, $[\alpha]_D$ −1·0° (*c* 0·20 in 0·05N-NaOH) (Found: C, 34·8; H, 5·9; N, 5·9. $C_7H_{13}NO_8$ requires C, 35·1; H, 5·5; N, 5·9%), that gave a deep purple colour with *o*-dinitrobenzene in alkaline solution.[18]

2,6-*Dihydroxy*-5-*nitro*-4-D-*ribitylaminopyrimidine* (VIII; R = D-ribityl).—To a solution of 1-amino-1-deoxy-D-*erythro*pentulose oxalate (2·1 g.) in ethanol (40 c.c.) and water (10 c.c.) was added a solution from sodium (0·4 g.) in ethanol (40 c.c.). Sodium oxalate was removed and to the filtrate was added 4-chloro-5-nitrouracil (0·83 g.) in ethanol (30 c.c.). The mixture was heated on the steam bath for 15 min., the ethanol removed *in vacuo*, and the pH of the remain-

[16] Euler, Karrer, Malmberg, Schopp, Benz, Becker, and Frei, *Helv. Chim. Acta*, 1935, **18**, 522.
[17] Holleman, *Rec. Trav. chim.*, 1894, **13**, 411.
[18] Fearon and Kawerau, *Biochem. J.*, 1943, **37**, 326.

ing aqueous solution adjusted to pH 10 with aqueous ammonia. This solution was chromatographed on an anion-exchange resin as described above. Buffer of pH 4 eluted the pyrimidine in a narrow band. Concentration of the eluate *in vacuo* gave the 5-nitro-ketose {VI; R = $[CH(OH)]_2 \cdot CH_2 \cdot OH$} as a jelly that gave a deep red colour with *o*-dinitrobenzene in alkaline solution [18] and appeared homogeneous on paper chromatograms.

This crude material (~ 600 mg., as estimated by the intensity of ultraviolet absorption) was dissolved in 2N-sodium hydroxide (2 c.c.), and an aqueous solution (5 c.c.) of sodium borohydride (0·08 g.) was added. The product was isolated and purified as described above for the D-sorbityl analogue; 2,6-dihydroxy-5-nitro-4-D-ribitylaminopyrimidine (300 mg.) was obtained as a white, non-crystalline solid, m. p. 202° (lit.,[4] 203—204°), $[\alpha]_D$ +5·0° (*c* 0·21 in 0·05N-NaOH) (lit.,[4] +4·5°). The infrared spectrum of this material was identical with that of an authentic sample [4] prepared by condensing D-ribitylamine and 4-chloro-5-nitrouracil and differed from that of the D-arabityl epimer described below.

Riboflavin (IX; R = D-ribityl).—5-Amino-4-D-ribitylaminouracil (from the above 5-nitro-compound) was converted into riboflavin by the methods previously described by us; [4,8] the product formed orange needles, m. p. 288° (lit.,[4] 289°), $[\alpha]_D$ −116° (*c* 0·53 in 0·1N-NaOH) (lit.,[19] −115°). Its infrared spectrum was also identical with that of an authentic sample.

2,10-*Dihydro*-4,6,8-*trihydroxy*-2-*oxo*-10-D-*ribitylpyrimido*[5,4-*g*]*pteridine* (X; R = D-ribityl).—Prepared from the above 5-nitro-4-D-ribitylaminopyrimidine by the known method,[1] the pyrimidopteridine was obtained as yellow plates (from water), m. p. >325°, $[\alpha]_D$ −33° (*c* 0·2 in 0·05N-NaOH) (lit.,[1] −26·0°).

D-*Arabitylamine*.—This was prepared by catalytic reduction of D-arabinose oxime [20] in presence of a platinum catalyst. The resulting solution, after removal of the catalyst, was used directly as described below.

4-D-*Arabitylamino*-2,6-*dihydroxy*-5-*nitropyrimidine* (VIII; R = D-arabityl).—To an aqueous solution (50 c.c.) of crude D-arabitylamine (from 2 g. of oxime) was added 4-chloro-5-nitrouracil (1·15 g.) in ethanol (50 c.c.). The resulting solution was left at room temperature for 24 hr. A small amount (100 mg.) of solid separated and this was collected and identified as 4-amino-5-nitrouracil.[21] Concentration of the filtrate *in vacuo*, followed by the addition of ethanol (100 c.c.), gave the nitropyrimidine (1·5 g., 78%) which crystallised from aqueous ethanol as needles, m. p. 185°, $[\alpha]_D$ −17·5° (*c* 0·22 in 0·05N-NaOH) (Found: C, 35·2; H, 4·5; N, 18·0. $C_9H_{14}N_4O_8$ requires C, 35·3; H, 4·6; N, 18·3%), $\lambda_{max.}$ 228 (ε 19,000) and 322 mμ (ε 10,900) at pH 1, 220 (ε 12,100) and 335 mμ (ε 13,400) at pH 13.

9-D-*Arabityl*-6,7-*dimethylisoalloxazine* (IX; R = D-arabityl).—Condensation [8] of 5-amino-4-D-arabitylaminouracil (from the above 5-nitro-compound) with the dimer of 3,4-dimethyl-*o*-benzoquinone gave 9-D-arabityl-6,7-dimethylisoalloxazine (29%) as orange needles, m. p. 307—308° (lit.,[22] 303°), $[\alpha]_D$ +65° (*c* 0·09 in 0·05N-NaOH).

10-D-*Arabityl*-2,10-*dihydro*-4,6,8-*trihydroxy*-2-*oxopyrimido*[5,4-*g*]*pteridine* (X; R = D-arabityl).—Condensation of 5-amino-4-D-arabitylaminouracil (from reduction of 250 mg. of the corresponding 5-nitro-compound) with alloxan 0·13 g.) in acid solution [1] gave the *pyrimidopteridine* (200 mg., 67%) as yellow needles (from N-hydrochloric acid), m. p. >325°, $[\alpha]_D$ −76° (*c* 0·20 in 0·05N-NaOH) (Found: C, 41·6; H, 3·8; N, 21·8. $C_{13}H_{14}N_6O_8$ requires C, 41·8; H, 3·7; N, 22·0%).

7,8-*Dihydro*-2,4-*dihydroxy*-6-(D-arabo*tetrahydroxybutyl*)*pteridine* {XI; R = $[CH(OH)]_3 \cdot CH_2 \cdot OH$}.—1-Deoxy-1-(2,6-dihydroxy-5-nitro-4-pyrimidinylamino)-D-fructose (1 g.) in water (10 c.c.) was hydrogenated over Raney nickel (0·5 g.) for 12 hr. An off-white material separated during this time and was dissolved by the addition of 2N-sodium hydroxide (2 c.c.). The catalyst was removed and the filtrate was refrigerated overnight. The *sodium salt* of the dihydropteridine (0·4 g., 45%) separated (Found: C, 39·4; H, 4·7. $C_{10}H_{13}N_4NaO_6$ requires C, 39·2; H, 4·3%). This compound was extremely unstable and attempted recrystallisation proved impossible.

2,4,6-*Trihydroxypteridine* (XII; R = OH).—The above 7,8-dihydropteridine (from 1 g. of the 5-nitro-ketose) was dissolved in 0·2N-sodium hydroxide (15 c.c.) at room temperature and oxygen was bubbled through the solution for 3 days. Acidification with hydrochloric acid

[19] Kuhn and Rudy, *Ber.*, 1935, **68**, 169.
[20] Ruff, *Ber.*, 1898, **31**, 1573.
[21] Bitterli and Erlenmeyer, *Helv. Chim. Acta*, 1951, **34**, 835.
[22] Weygand, *Ber.*, 1935, **68**, 1282.

precipitated a bright yellow solid (300 mg., 58%). Recrystallisation from boiling water gave 2,4,6-trihydroxypteridine as yellow needles, m. p. 360—380° (decomp.) (lit.,[10] 360—380°). This material was identical (infrared and ultraviolet spectra) with an authentic specimen.[10]

During one oxidation which was carried out in more concentrated solution, 7,8-dihydro-2,4,6-trihydroxypteridine (XI; R = OH) separated after 1—2 days. This compound has previously been prepared in an unambiguous fashion.[10,11] The oxidation can also be carried out, less satisfactorily, by treatment of the 7,8-dihydropteridine {XI; R = $[CH(OH)]_3 \cdot CH_2 \cdot OH$} with cold alkaline potassium permanganate solution.

4-Acetonylamino-2,6-dihydroxy-5-nitropyrimidine (VI; R = Me).—Aminoacetone semicarbazone hydrochloride [23] (4·62 g.) was added to a solution from sodium (0·64 g.) in ethanol (40 c.c.) and left at room temperature for 30 min. To this solution was added 4-chloro-5-nitrouracil (2·66 g.) in ethanol (60 c.c.), a precipitate being formed almost immediately. The mixture was stirred, water (20 c.c.) was added, and the yellow solid was collected. Recrystallisation from water gave *4-acetonylamino-2,6-dihydroxy-5-nitropyrimidine semicarbazone* (4·0 g., 96%) as pale yellow needles, m. p. >300° (Found: C, 32·5; H, 4·4; N, 33·6. $C_8H_{11}N_7O_5,0{\cdot}5H_2O$ requires C, 32·6; H, 4·1; N, 33·4%).

This semicarbazone (1 g.), suspended in N-hydrochloric acid (50 c.c.), was kept at 37° for 3 days. Then, on refrigeration overnight, a solid (300 mg.) separated and concentration of the mother liquors gave a further 300 mg. (total 76%). Recrystallisation from water gave *4-acetonylamino-2,6-dihydroxy-5-nitropyrimidine* as prisms, m. p. 273° (Found: C, 36·8; H, 3·7; N, 24·2. $C_7H_8N_4O_5$ requires C, 36·9; H, 3·5; N, 24·6%).

4-Acetonylamino-2,6-dihydroxy-5-nitropyrimidine (400 mg.) and hydrazine hydrate (0·15 c.c.) in ethanol (60 c.c.) were heated on the steam bath, the gum which was formed initially slowly dissolving. On cooling, 4-acetonylamino-2,6-dihydroxy-5-nitropyrimidine hydrazone separated as *its hydrazine salt* (400 mg., 83%), that recrystallised from water as needles, m. p. >300° (Found: C, 30·5; H, 4·8; N, 40·2. $C_7H_{10}N_6O_4,N_2H_4$ requires C, 30·6; H, 5·1; N, 40·8%).

7,8-Dihydro-2,4-dihydroxy-6-methylpteridine (XI; R = Me).—4-Acetonylamino-2,6-dihydroxy-5-nitropyrimidine (300 mg.), suspended in water (25 c.c.), was hydrogenated overnight over Raney nickel (0·5 g.). Sodium hydroxide solution was added to pH 13 and the solution was warmed to dissolve the precipitate which had been formed. The catalyst was removed and on cooling a crystalline sodium salt separated. Neutralisation with 2N-hydrochloric acid and gentle warming gave the *7,8-dihydropteridine* (160 mg., 67%) as pale yellow needles, m. p. >300° (Found: C, 46·8; H, 4·5; N, 31·0. $C_7H_8N_4O_2$ requires C, 46·7; H, 4·5; N, 31·1%), $\lambda_{max.}$ 228 (ε 11,200), 267 (ε 15,400), and 350 mμ (ε 4500) at pH 1, 226 (ε 23,000), 276 (ε 13,000), and 318 mμ (ε 6500) at pH 13.

2,4-Dihydroxy-6-methylpteridine (XII; R = Me).—(*a*) The above 7,8-dihydropteridine (120 mg.) was dissolved in 0·1N-sodium hydroxide (25 c.c.). 0·2M-Potassium permanganate solution (2·2 c.c.) was added dropwise at room temperature in 2—3 min. The manganese dioxide was removed by filtration and the solution was acidified. The solid which separated was collected and recrystallised from water, to give the pteridine (80 mg., 67%) as colourless needles, m. p. >300° (Found: C, 47·0; H, 3·1; N, 31·7. Calc. for $C_7H_6N_4O_2$ C, 47·2; H, 3·4; N, 31·5%). This pteridine has previously been prepared [24] by deamination of the 2-amino-analogue.

(*b*) 4-Acetonylamino-2,6-dihydroxy-5-nitropyrimidine hydrazone (200 mg.) in water (25 c.c.) was hydrogenated over Raney nickel (0·5 g.) for 24 hr. The catalyst was removed, and acetic acid added to the filtrate to give a 2N-solution (with respect to acetic acid) which was then refluxed for 1 hr. Water and acetic acid were removed *in vacuo*, leaving a gum which crystallised from water to give the pteridine (90 mg., 64%) as colourless needles. The infrared and ultraviolet spectra of this material were identical with those of the pteridine prepared by method (*a*).

4,5-Dihydro-7-hydroxy-3-methyl-8-nitro-5-oxoimidazo[1,2-c]pyrimidine (XIV).—4-Acetonylamino-2,6-dihydroxy-5-nitropyrimidine semicarbazone (2 g.) was refluxed in 2N-hydrochloric acid (50 c.c.) for 15 min. On cooling, a yellow solid separated and this was recrystallised from water, to give the *imidazopyrimidine* (1·2 g., 80%) as needles, m. p. 265° (Found: C, 39·8; H,

[23] Boon and Leigh, *J.*, 1951, 1497.
[24] Angier, Boothe, Mowat, Waller, and Semb, *J. Amer. Chem. Soc.*, 1952, **74**, 408.

3·2; N, 26·8. $C_7H_6N_4O_4$ requires C, 40·0; H, 2·9; N, 26·7%), $\lambda_{max.}$ 224 (ε 10,600), 268 (ε 5500), and 360 mμ (ε 11,600) at pH 1, 238 (ε 12,000) and 394 mμ (ε 9700) at pH 13.

An identical product was obtained in similar fashion from 4-acetonylamino-2,6-dihydroxy-5-nitropyrimidine.

1,2,3,5-*Tetrahydro-7-hydroxy-8-nitro-5-oxoimidazo*[1,2-*c*]*pyrimidine* (XV).—4-2′-Hydroxyethylamino-5-nitrouracil [4] (2 g.) was refluxed in N-hydrochloric acid (200 c.c.) for 15 min. On cooling, a solid separated which recrystallised from water to give the *imidazopyrimidine* (1·4 g., 70%) as needles, m. p. 275° (Found: C, 33·5; H, 3·7; N, 25·5. $C_6H_6N_4O_4,H_2O$ requires C, 33·3; H, 3·7; N, 25·9%), $\lambda_{max.}$ 228 (ε 24,000) and 322 mμ (ε 13,500) at pH 1, 221 (ε 15,800) and 334 mμ (ε 16,400) at pH 13. This ultraviolet spectrum is almost identical with that of the starting material, but the infrared spectra were quite distinct.

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