929. *The Biosynthesis of Pteridines. Part II.*[1] *The Self-condensation of 5-Amino-4-(substituted amino)uracils.*

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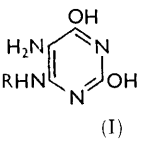
Self-condensation of 5-amino-4-(substituted amino)uracils (I) is shown to give the pyrimido[5,4-*g*]pteridines (II). These compounds were also obtained by condensation of alloxan with the pyrimidines (I). An unambiguous synthesis of one of these pyrimidopteridines eliminates the alternative structure (III).

In Part I [1] the synthesis of 5-amino-4-D-ribitylaminouracil (I; R = D-ribityl) was described. It has been suggested [2] that this compound is an intermediate in the biosynthesis of riboflavin. Biochemical experiments [3] to test this hypothesis gave rather inconclusive results, and only low yields of riboflavin were obtained. We have already noted the ease with which compound (I; R = D-ribityl) undergoes self-condensation, and this reaction may explain the disappointing biological results. We have now identified the self-condensation product as the pyrimido[5,4-*g*]pteridine (II; R = D-ribityl).

The self-condensation product of 5-amino-4-D-ribitylaminouracil (I; R = D-ribityl) and of the analogous uracil derivatives (I; R = Me, $CH_2 \cdot CH_2 \cdot OH$, D-mannityl, and D-sorbityl) were obtained from solutions of the parent pyrimidine which had been exposed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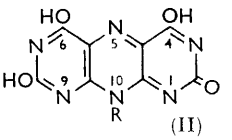
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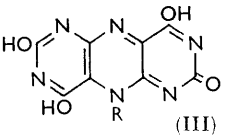
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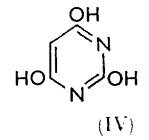
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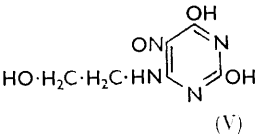
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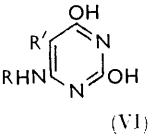
(VI)

to the air. These products were crystalline and yellow, gave an intense blue fluorescence in aqueous solution, and had an ultraviolet absorption spectrum analogous to that of the pyrimido[5,4-*g*]pteridines recently prepared by Taylor and Loux.[4]

Condensation of alloxan with 5-amino-4-D-ribitylaminouracil (I; R = D-ribityl) according to the method of Taylor and Loux [4] gave crystals identical in all respects with those obtained by self-condensation of the pyrimidine. We assign structure (II; R = D-ribityl) to this product. Similar pyrimido[5,4-*g*]pteridines (II; R = Me, $CH_2 \cdot CH_2 \cdot OH$, D-mannityl, and D-sorbityl) were obtained by condensing alloxan with the appropriate pyrimidines (I), and in each case the product was identical with that obtained by self-condensation of the pyrimidine.

The alternative structure (III) for these pyrimidopteridines is eliminated as we have been able to prepare the 2′-hydroxyethyl compound (II; R = $CH_2 \cdot CH_2 \cdot OH$) by an unambiguous method, namely, condensation of barbituric acid (IV) with 2,4-dihydroxy-6-2′-hydroxyethylamino-5-nitrosopyrimidine (V).

In early experiments the D-sorbityl compound was not obtained crystalline and its identity was confirmed by periodate titration to give the crystalline aldehyde (II; R = $CH_2 \cdot CHO$) which was reduced by sodium borohydride to the 2′-hydroxyethylpyrimidopteridine (II; R = $CH_2 \cdot CH_2 \cdot OH$).

[1] Part I, preceding paper.
[2] McNutt, *J. Biol. Chem.*, 1954, **210**, 511; Brown, Goodwin, and Pendlington, *Biochem. J.*, 1955, **61**, 37; Kuwada, Masuda, Kishi, and Asai, *J. Vitaminol (Japan)*, 1958, **4**, 217.
[3] Kishi, Asai, Masuda, and Kuwada, *Chem. Pharm. Bull. (Japan)*, 1959, **7**, 515.
[4] Taylor and Loux, *J. Amer. Chem. Soc.*, 1959, **81**, 2474.

In an investigation of possible stable derivatives of 5-amino-4-D-ribitylaminouracil (I; R = D-ribityl) which might be useful in biological experiments, the formylation of various compounds was investigated. Reduction of the 5-nitropyrimidine (VI; R = Me, R′ = NO_2) with zinc and formic acid readily gave a stable crystalline monoformyl derivative (VI; R = Me, R′ = NH·CHO) of 5-amino-4-methylaminouracil from which the formyl group was removed by aqueous acid at pH 2. Similar treatment of the 2′-hydroxyethyl compound (VI; R = CH_2·CH_2·OH, R′ = NO_2) gave a diformyl derivative (VI; R = CH_2·CH_2·O·CHO, R′ = NH·CHO) of the corresponding amine. We have not, however, been able to prepare a crystalline formyl derivative of the ribitylaminouracil (I; R = D-ribityl).

Experimental

For general detail see Part I.[1] R_F values of the products and ultraviolet absorption spectra are given in the Table below.

5-Nitro-4-(substituted amino)uracils (VI; R′ = NO_2).—4-Methylamino-5-nitrouracil (VI; R = Me, R′ = NO_2), and the 4-2′-hydroxyethylamino- and 4-D-ribitylamino-analogues (VI; R = CH_2·CH_2·OH or D-ribityl, R′ = NO_2) were prepared as described in Part I.[1]

4-D-Mannitylamino-5-nitrouracil (VI; R = D-mannityl, R′ = NO_2).—D-Mannose oxime[5] (2·5 g.) in water (150 c.c.) was shaken with hydrogen and platinum oxide (0·5 g.) until the theoretical amount of hydrogen had been absorbed. The catalyst was filtered off, and the resulting solution of D-mannitylamine was used directly.

Chromatographic behaviour and absorption spectra of the products.

Compound	R_F in solvent * (A)	(B)	$\lambda_{max.}$ (mμ) (ε in parentheses) in H_2O at pH given	
Pyrimidines				
(VI; R = D-mannityl, R′ = NO_2) ...	0·08	0·70	228 (19,200), 324 (11,100)	pH 1
			220 (13,900), 340 (14,500)	pH 13
(VI; R = D-sorbityl, R′ = NO_2)	0·08	0·70	228 (18,500), 322 (11,000)	pH 1
			220 (13,700), 336 (14,300)	pH 13
(VI; R = Me, R′ = NH·CHO)	0·13	0·79	218 (21,500), 266 (17,900)	pH 13
(VI; R = CH_2·CH_2·O·CHO; R′ = NH·CHO)	0·11	0·81	221 (22,500), 268 (17,700)	pH 13
Pyrimido[5,4-g]pteridines				
(II; R = Me)	—	0·23	222 (25,100), 277 (10,900), 418 (24,000)	pH 1
			236 (32,700), 272 (14,400), 431 (35,500)	pH 13
(II; R = CH_2·CH_2·OH)	—	0·31	228 (20,400), 278 (9300), 422 (20,800)	pH 1
			231 (29,100), 272 (13,100), 436 (31,400)	pH 13
(II; R = D-ribityl)	—	0·34	230 (22,600), 280 (10,300), 422 (22,200)	pH 1
			230 (27,100), 272 (11,900), 436 (26,600)	pH 13
(II; R = D-mannityl)	—	0·40	230 (22,100), 280 (10,800), 422 (22,300)	pH 1
			231 (26,500), 272 (11,600), 438 (27,600)	pH 13
(II; R = D-sorbityl)	—	0·42	224 (25,800), 280 (9500), 422 (18,400)	pH 1
			230 (32,100), 272 (16,000), 438 (25,800)	pH 13
(II; R = CH_2·CHO)	0·04	0·33	221 (28,800), 276 (15,200), 404 (20,300)	pH 1
			232 (21,300), 272 (10,000), 436 (23,400)	pH 13

* See Part I.

To the solution of crude D-mannitylamine was added 4-chloro-5-nitrouracil[1] (1·25 g.) in ethanol (100 c.c.). The resulting solution was heated on the steam-bath for 15 min., then cooled, and the mixture of products was separated on an anion-exchange resin as described for the D-ribityl analogue in Part I.[1] Buffer at pH 4 eluted a pyrimidine which crystallised on concentration of the solution to give *4-D-mannitylamino-5-nitrouracil* (1·6 g.). Recrystallisation from water gave needles, m. p. 247° (Found: C, 33·1; H, 5·4; N, 15·3. $C_{10}H_{16}N_4O_9$,1·5H_2O requires C, 33·0; H, 5·3; N, 15·4%), $[\alpha]_D$ −2·5° (*c* 0·22 in 0·05N-NaOH).

5-Nitro-4-D-sorbitylaminouracil (VI; R = D-sorbityl, R′ = NO_2).—Similar reduction of D-glucose oxime[6] (4·0 g.) followed by condensation with 4-chloro-5-nitrouracil (1·6 g.) and ion-exchange chromatography of the crude product gave *5-nitro-4-D-sorbitylaminouracil*

[5] Fischer and Hirschberger, *Ber.*, 1889, **22**, 1155.
[6] Wohl, *Ber.*, 1893, **26**, 730; Wolfrom and Thompson, *J. Amer. Chem. Soc.*, 1931, **53**, 622.

(1·8 g.) as needles (from water), m. p. 224—225° (Found: C, 34·3; H, 4·9; N, 16·0. $C_{10}H_{16}N_4O_9,H_2O$ requires C, 34·0; H, 5·1; N, 15·8%), $[\alpha]_D$ +17·5° (*c* 0·20 in 0·05N-NaOH).

Pyrimido[5,4-g]*pteridines* (II).—Methods (*a*) and (*b*) below are typical of the preparation of these compounds. The other pyrimidopteridines listed were prepared in analogous fashion, and in each case the product from self-condensation [method (*b*)] of the 5-aminopyrimidine (I) was shown by ultraviolet and infrared spectroscopy, and by paper chromatography, to be identical with that produced by the alloxan condensation [method (*a*)].

2,10-*Dihydro*-4,6,8-*trihydroxy*-10-2′-*hydroxyethyl*-2-*oxopyrimido*[5,4-g]*pteridine* (II; R = CH_2·CH_2·OH).—(*a*) 4-2′-Hydroxyethylamino-5-nitrouracil [1] (0·5 g.) in hot water (12 c.c.) was treated with zinc dust (1·6 g.) and 10N-sulphuric acid (1·5 c.c.). The mixture was refluxed gently for 10 min. and filtered while still hot. The zinc residues were washed with water (10 c.c.), the filtrate and washings were combined, and alloxan monohydrate (0·4 g.) was added. A deep purple colour was formed immediately. The mixture was refluxed for 2 hr., during which the purple colour faded; when it was then cooled a yellow solid separated. Recrystallisation from water gave the *pyrimidopteridine* (0·69 g.) as yellow plates, m. p. >325° (Found: C, 38·0; H, 3·7; N, 26·5. $C_{10}H_8N_6O_5,H_2O$ requires C, 38·7; H, 3·3; N, 27·1%).

Similar results were obtained by reducing the nitro-group by sodium dithionite in alkali.[1]

(*b*) A solution of the diformyl derivative of 5-amino-4-2′-hydroxyethylaminouracil (0·37 g.) in 0·1N-hydrochloric acid (50 c.c.) was refluxed for 6 hr. (the formyl groups are hydrolysed during this procedure and the parent amine undergoes self-condensation). The solution was concentrated to *ca.* 10 c.c. and cooled, the pyrimidopteridine (0·25 g.) crystallising as yellow plates. The ultraviolet and infrared spectra of this material were identical with those of a sample prepared by method (*a*).

This self-condensation may also be carried out, less conveniently, by reduction of the 5-nitropyrimidine (VI; R = CH_2·CH_2·OH, R′ = NO_2) by sodium dithionite and alkali,[1] and aerating the resulting solution.

(*c*) Barbituric acid (0·5 g.) and 4-2′-hydroxyethylamino-5-nitrosouracil (0·5 g.) in glacial acetic acid (15 c.c.) containing one drop of 12N-hydrochloric acid were heated for 9 hr. at 160° in a sealed tube. After cooling, the solution was filtered and 2N-sodium hydroxide was added to pH 5. The yellow product (0·35 g.) which separated was recrystallised several times from 2N-hydrochloric acid, to give the pyrimidopteridine as bright yellow needles, identical with that prepared as in (*a*) and (*b*) above.

(*d*) To a solution of the aldehyde (II; R = CH_2·CHO) (60 mg.) in 0·2N-sodium hydroxide (10 c.c.) was added an aqueous solution (5 c.c.) of sodium borohydride (15 mg.). The mixture was left overnight, and then 2N-hydrochloric acid was added to give pH 3. A yellow solid separated and was recrystallised from water, to give the 10-2′-hydroxyethylpyrimidopteridine (36 mg.) as yellow needles, m. p. >325°, identical with a sample prepared by method (*a*).

Prepared in analogous fashion were:

2,10-*Dihydro*-4,6,8-*trihydroxy*-10-*methyl*-2-*oxopyrimido*[5,4-g]*pteridine* (II; R = Me), yellow needles (from water), m. p. >325° (Found: C, 38·2; H, 2·3; N, 29·3. $C_9H_6N_6O_4,H_2O$ requires C, 38·6; H, 2·9; N, 30·0%).

2,10-*Dihydro*-4,6,8-*trihydroxy*-10-D-*ribityl*-2-*oxopyrimido*[5,4-g]*pteridine* (II; R = D-ribityl), yellow plates (from water), m. p. >325° (Found: C, 38·3; H, 4·7; N, 20·8. $C_{13}H_{14}N_6O_8,1·5H_2O$ requires C, 38·1; H, 4·2; N, 20·5%), $[\alpha]_D$ −26·0° (*c* 0·17 in 0·05N-NaOH).

2,10-*Dihydro*-4,6,8-*trihydroxy*-10-D-*mannityl*-2-*oxopyrimido*[5,4-g]*pteridine* (II; R = D-mannityl), yellow needles (from water), m. p. >325° (Found: C, 36·4; H, 4·6; N, 17·8. $C_{14}H_{16}N_6O_9,3H_2O$ requires C, 36·0; H, 4·8; N, 18·0%), $[\alpha]_D$ +17° (*c* 0·33 in 0·05N-NaOH).

2,10-*Dihydro*-4,6,8-*trihydroxy*-10-D-*sorbityl*-2-*oxopyrimido*[5,4-g]*pteridine* (II; R = D-sorbityl), yellow needles (from water), m. p. >325° (Found: C, 37·6; H, 4·4; N, 18·4. $C_{14}H_{16}N_6O_9,2H_2O$ requires C, 37·5; H, 4·5; N, 18·7%), $[\alpha]_D$ +61° (*c* 0·28 in 0·05N-NaOH).

10-*Formylmethyl*-2,10-*dihydro*-4,6,8-*trihydroxy*-2-*oxopyrimido*[5,4-g]*pteridine* (II; R = CH_2·CHO).—To a solution of crude 10-D-sorbitylpyrimidopteridine (II; R = D-sorbityl) (180 mg.) in water (50 c.c.), sodium metaperiodate (650 mg.) in water (10 c.c.) was added. Yellow crystals separated during 2 days at room temperature. Recrystallisation from water gave the *pyrimidopteridine* (120 mg.) as bright yellow plates, m. p. >325° (Found: C, 38·9; H, 2·3; N, 27·3. $C_{10}H_6N_6O_5,H_2O$ requires C, 38·9; H, 2·6; N, 27·3%).

5-*Formylamino*-4-*methylaminouracil* (VI; R = Me, R′ = NH·CHO).—4-Methylamino-5-nitrouracil (0·33 g.) was dissolved in 98% formic acid (20 c.c.) and treated at 90° with zinc

dust (0·5 g.). When the initial reaction had subsided, the mixture was heated for a further 10 min. at 90° and filtered hot. Immediate addition of ethanol (25 c.c.) and ether (200 c.c.) to the filtrate precipitated the pyrimidine as a white solid (0·25 g.). Recrystallisation from water gave the *formyl compound* (0·19 g.) as needles, m. p. >325° (Found: C, 39·0; H, 4·4; N, 30·1. $C_6H_8N_4O_3$ requires C, 39·1; H, 4·4; N, 30·4%).

5-*Formylamino*-4-2′-*formyloxyethylaminouracil* (VI; R = CH_2·CH_2·O·CHO, R′ = NH·CHO).—Similar reduction of the 4-2′-hydroxyethylamino-5-nitrouracil (0·5 g.) by zinc dust (0·6 g.) in 98% formic acid (20 c.c.) and refluxing of the solution for 8 hr. gave the *diformyl derivative* (0·22 g.) as colourless needles, m. p. >325° (Found: C, 39·7; H, 4·3; N, 23·3. $C_8H_{10}N_4O_5$ requires C, 39·7; H, 4·2; N, 23·1%).

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