

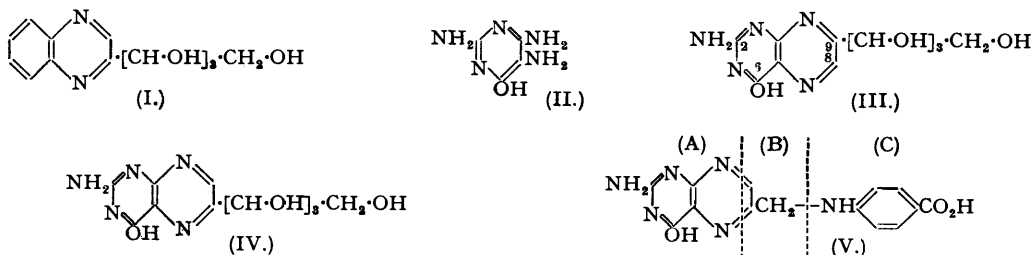
## 16. *The Condensation of 2:4:5-Triamino-6-hydroxypyrimidine with Glucose and Fructose.*

By H. S. FORREST and JAMES WALKER.

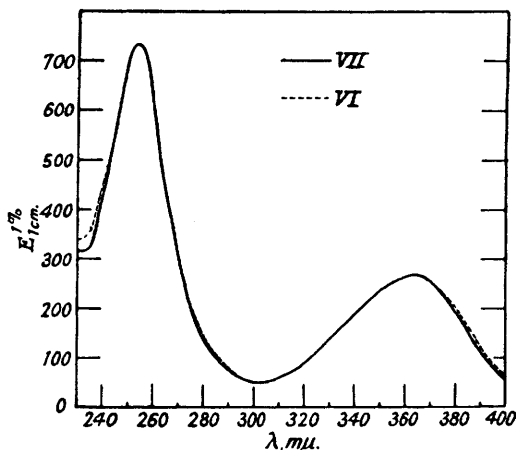
Condensation of 2:4:5-triamino-6-hydroxypyrimidine (II) with glucose and fructose in the presence of hydrazine affords in both cases 2-amino-6-hydroxy-8-D-*arabotetrahydroxybutylpteridine* (IV). Proof of this constitution follows by degradation of the side-chain to a carboxyl group and identification of the products with 2-amino-6-hydroxypteridine-8-carboxylic acid (X). In the absence of hydrazine, glucose and (II) afford the isomeric 2-amino-6-hydroxy-9-D-*arabotetrahydroxybutylpteridine* (III). Glucosone and (II) afford (IV) in the presence of hydrazine and (III) in its absence. Experiments bearing on the possible biogenesis of pteric acid (V) from reductone are recorded. No evidence was obtained of the formation of (V) under the conditions used but evidence indicating the formation of its 9-isomer resulted.

2-TETRAHYDROXY-*n*-BUTYLQUINOXALINE (I) has been obtained by the condensation of *o*-phenylenediamine with glucose (Griess and Harrow, *Ber.*, 1887, 20, 2207), with fructose (Ohle, *Ber.*, 1934, 67, 155), or with glucosone (Fischer, *Ber.*, 1889, 22, 92), and the optimal conditions for the preparation of (I), using either glucose or fructose, have been established by Ohle and Hielscher (*Ber.*, 1941, 74, 13), who effected condensation in the presence of hydrazine, or phenylhydrazine, and boric acid. It appeared to us desirable to extend the scope of this condensation to *o*-diamines of the pyrimidine series, especially 2:4:5-triamino-6-hydroxypyrimidine (II), for two main reasons. In the first place, the tetrahydroxybutyl side-chain in the resulting compounds, (III) or (IV), would be amenable to further chemical manipulation bearing on the synthesis either of substances of the folic acid group or of antagonists to these compounds. In the second place, condensation of (II) with readily available hexoses appeared to us to be worthy of investigation since the formula of pteric acid (V) can be broken up into three parts, one of

which (B) may be derived biogenetically from a triose, (A) being common to (V) and to guanine, and (C) being *p*-aminobenzoic acid.

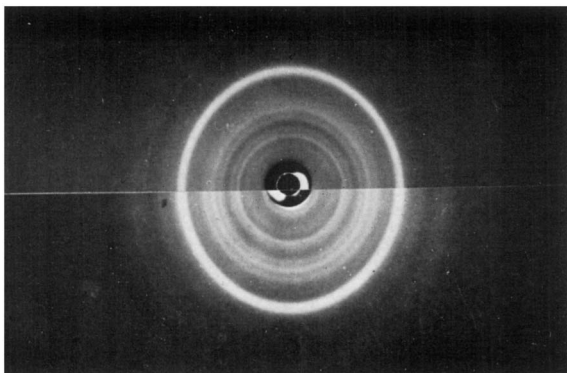


When the conditions developed by Ohle and Hielscher (*loc. cit.*) for the preparation of (I) were applied to (II), glucose afforded a product (VI), having  $[\alpha]_D^{20}$  approximately  $-81.5^\circ$  in *N*-hydrochloric acid or in *N*/10-sodium hydroxide solution, while fructose afforded a substance (VII) with a slightly higher specific rotation,  $[\alpha]_D^{20}$  approximately  $-86.6^\circ$ , under the same conditions. At this point, a paper by Karrer, Schwyzer, Erden, and Siegwart appeared (*Helv.*

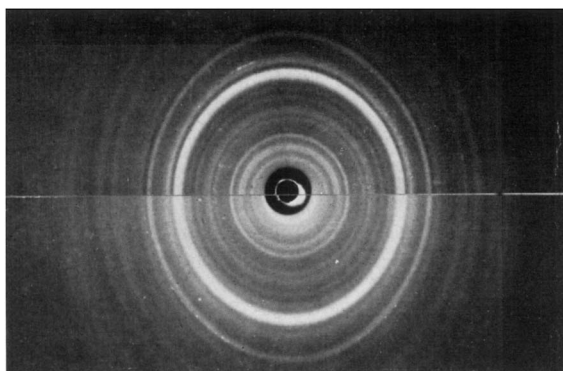


*Chim. Acta*, 1947, **30**, 1031) describing the condensation of (II) with a variety of simple sugars using less elaborate conditions than ours and in the absence of hydrazine. Their product from glucose had  $[\alpha]_D^{20}$   $-68.93^\circ$ , and their product from fructose had  $[\alpha]_D^{20}$   $-46.0^\circ$ , both in *N*/10-sodium hydroxide solution; these figures are significantly different from ours particularly in the case of the product from fructose.\* Although final conclusions were not reached, Karrer and his collaborators considered their products from glucose and fructose to be respectively (III, or IV?) and (IV, or III?), the constitution of their product from glucose being indicated as (III) with some degree of certainty. The lack of concordance between the respective specific rotations recorded by the Swiss authors and our own observed values led us to repeat our own work and we have fully confirmed our own observations. In spite of the slight difference in the optical rotations of (VI) and (VII), these substances gave identical ultra-violet absorption spectra in *N*/10-sodium hydroxide solution (see Fig.), their *X*-ray powder diffractions pattern (Plate, a) showed good agreement, and they were shown to be substantially the same compound, 2-amino-6-hydroxy-8-*D*-arabotetrahydroxybutylpteridine (IV), by oxidative degradation. On treatment with periodic acid, (VI) and (VII) afforded aldehydes, yielding acids (VIII) and (IX) on further oxidation. Alternatively, (VI) and (VII) could be oxidised directly to (VIII) and (IX). The acids (VIII) and (IX) were shown to correspond closely with 2-amino-6-hydroxypteridine-8-carboxylic acid (X), obtained by aerated alkaline hydrolysis of (synthetic) liver *Lactobacillus casei* factor (Stokstad *et al.*, *Ann. N.Y. Acad. Sci.*, 1946, **48**, Art. 5, 269), and to be distinct from

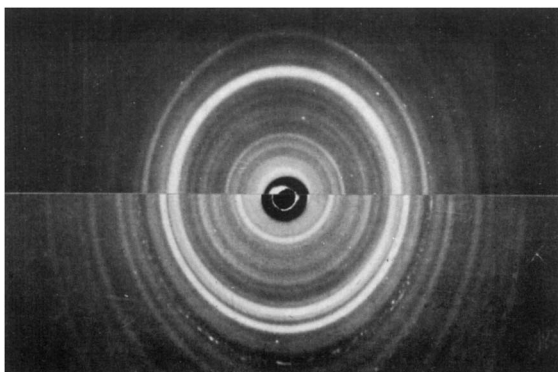
\* Note added in Proof, December 16th, 1948.—Since this paper was submitted, Karrer and Schwyzer (*Helv. Chim. Acta*, 1948, **31**, 782), referring to our preliminary note, have revised the specific rotations for their products from glucose and fructose, substituting  $-72^\circ$  and  $-75.1^\circ$  respectively; there is no indication that the latter figure is maximal since their substance had  $[\alpha]_D$   $-62^\circ$  after the third (penultimate) crystallisation.



*Plate a : top half (VII) : bottom half (VI).*

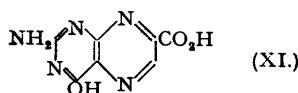
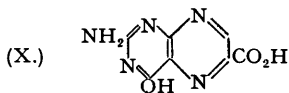


*Plate b : Top half, disodium salt of (IX) ; bottom half, disodium salt of (X) [from (synthetic) liver L. casei factor].*



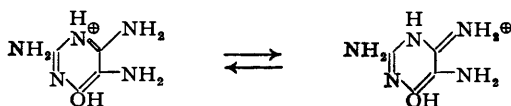
*Plate c : Top half, disodium salt of (X) [from (synthetic) liver L. casei factor] ; bottom half, disodium salt of (XI) (from 2-amino-6-hydroxy-9-methylpteridine). Photographs taken on cylindrical camera, radius 3 cm. using filtered Cu-K $\alpha$  radiation ( $\lambda$  1.54Å.).*

2-amino-6-hydroxypteridine-9-carboxylic acid (XI), obtained by oxidation of 2-amino-6-hydroxy-9-methylpteridine (Mowat *et al.*, *ibid.*, p. 279), by their ultra-violet absorption spectra, those of (VIII), (IX), and (X) showing maxima between 261 and 262  $m\mu$  with  $\epsilon$  of 16,600—16,870, and the corresponding band of (XI) being found at 258  $m\mu$  with  $\epsilon$  of 16,580. Furthermore, the disodium salts of (VIII), (IX), and (X) in aqueous solution all showed the same intense sky-blue fluorescence, while that of (XI) showed a bright green fluorescence in ultra-violet light. X-Ray powder diffraction patterns of the disodium salts of (IX) and (X) showed good agreement (Plate, b), while that of the corresponding salt of (XI) was quite distinctive (Plate, c).

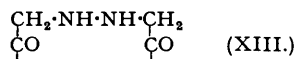
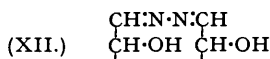


The product formed from glucose under conditions comparable with those used by Karrer *et al.* (*loc. cit.*) has been found to be the 9-isomer (III), by oxidation to (XI), and we have also obtained the same product by the condensation of glucosone with (II) at pH 3.5 and subsequent oxidation; in these observations we are in agreement with Petering and Weisblat (*J. Amer. Chem. Soc.*, 1947, **69**, 2566), who have recently shown also that the orientation of the products produced in these two condensations depends upon the pH of the reaction medium, recalling the formation of *isoxanthopterincarboxylic acid* from (II) and mesoxalic ester in dilute acetic acid and of *xanthopterincarboxylic acid* in 2*N*-sulphuric acid (Purrmann, *Annalen*, 1941, **548**, 284). We have also found that in the presence of hydrazine, but not in the presence of boric acid alone, glucosone reacts with (II) to give the 8-isomer (IV), and we do not ascribe this result to the slight elevation of the pH of the reaction mixture above that obtaining in the absence of hydrazine. In all our experiments with the two hexoses and glucosone, (II) was used as the sulphate in the presence of an equivalent amount, or a slight excess, of sodium acetate, so that condensation was usually effected in the pH region of 3.5—5, *i.e.* in the acetate buffer range.

Among the aminopyrimidines, 4-aminopyrimidines are stronger bases than 5-aminopyrimidines (Gabriel and Colman, *Ber.*, 1899, **32**, 2929; *ibid.*, 1901, **34**, 1237), which can be ascribed to stabilisation of the 4-kation by resonance of the amidinium type, represented in the case of (II) as

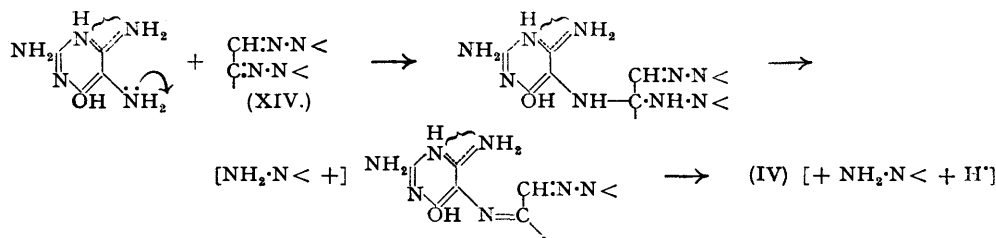


and recalling the greater basicity of 2-amino-pyridine and -quinoline in comparison with the corresponding 3-isomers (Albert and Goldacre, *Nature*, 1944, **153**, 467). The greater electron availability at the 5-amino-group, however, makes this position the point of nucleophilic activity in 4 : 5-diaminopyrimidines with the result that a 5-amino-group is acylated in preference to a 4- (or 2-)amino-group (Isay, *Ber.*, 1906, **39**, 257; Levene and Senior, *J. Biol. Chem.*, 1916, **25**, 617). At low values of pH, when a trivalent kation is formed, the nucleophilic activity of the 5-amino-group will be depressed leading to variable behaviour under such conditions as shown by Petering and Weisblat (*loc. cit.*) and by Purrmann (*loc. cit.*). In the experiments with the two hexoses described in the present paper, (II) is doubtless present in the form of a univalent kation and it remains to interpret, in both cases, how the 2-position in the sugar chain becomes attached to the nitrogen atom of the 5-amino-group in the formation of (IV). At first sight this does not appear to be difficult in the case of fructose and the rôle of hydrazine would appear to be a minor one but for the fact that significant yields of (IV) cannot be obtained in its absence. In the case of glucose one could postulate that glucosealdazine (XII) (Davidis, *Ber.*, 1896, **29**, 2308) undergoes an Amadori type of rearrangement to the hydrazine (XIII), which then reacts with

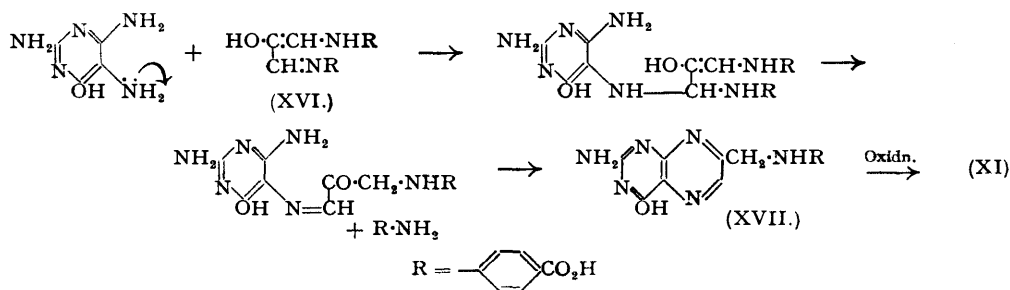


(II) to give (IV), recalling the facile formation of osazones from *N*-arylisoglycosamines (Weygand, *Ber.*, 1940, **73**, 1289). Ohle and Hielscher (*loc. cit.*), rejecting Weygand's suggestion (*loc. cit.*, p. 1268) that (I) is formed from *o*-phenylenediamine and glucose by way of *o*-aminophenylglucoside, Amadori rearrangement, ring-closure, and dehydrogenation, believed glucosephenylosazone to be hydrolysed giving glucosone as the substance combining directly with *o*-phenylenediamine to give (I). This view also is untenable in the light of the experiments

described in the present paper, where glucosone in the absence of hydrazine gives (III) with (II) and glucose and fructose in its presence afford (IV) under comparable conditions of pH. Taking as precedent the relatively facile reduction of glucosephenylosazone to isoglucosamine (1-amino fructose) (Fischer, *Ber.*, 1886, **19**, 1920; Maurer and Schiedt, *ibid.*, 1935, **68**, 2187), when the phenylhydrazine residue in the 1-position undergoes hydrogenolysis between the two nitrogen atoms while that in the 2-position undergoes hydrolysis, indicating the greater susceptibility on the part of the latter residue to ejection by a nucleophilic reagent, we prefer to depict the condensation of (II) with glucose and fructose in the presence of hydrazine as proceeding through a common intermediate (XIV) in the following manner.



In a preliminary note (*Nature*, 1948, **161**, 308), the present authors have tentatively suggested that either 3-phosphoglyceraldehyde or phosphodihydroxyacetone might function as a triose intermediate in the biogenesis of pteric acid (V). There is evidence to show that many bacteria can synthesise (V) (Sarett, *J. Biol. Chem.*, 1947, **171**, 265) and in some cases this has been related to the *p*-aminobenzoic acid content of the medium (Mayer, *Science*, 1943, **98**, 203; Mills *et al.*, *Proc. Soc. Exp. Biol. Med.*, 1944, **56**, 240). Recent findings also indicate that sulphonamides inhibit the synthesis of (V) from *p*-aminobenzoic acid in the case of enterococci (Lampen and Jones, *J. Biol. Chem.*, 1946, **166**, 435). In this connection, the suggestion by O'Meara, McNally, and Nelson (*Nature*, 1944, **154**, 796; *Lancet*, 1947, ii, 747) that reductone (XV), CHO·C(OH)·CH(OH), may be the strongly reducing, non-sulphydryl, substance formed in bacterial cultures during the logarithmic phase of growth and that the condensation product (XVI) of (XV) with *p*-aminobenzoic acid, but not free *p*-aminobenzoic acid, can act as a source of energy for growth of streptococci, suggested to us that (XV) might indeed be a triose intermediate involved in the biosynthesis of (V). The mechanism of sulphonamide bacteriostasis could then be interpreted in terms of the diversion of (XV) from the synthesis of (V) to the synthesis of biologically inactive derivatives with the sulphonamide drugs. Furthermore, (XV) is already in the correct state of oxidation for direct combination with (II) and *p*-aminobenzoic acid to give (V). We have carried out a considerable number of experiments to test this hypothesis covering a wide range of pH. In each case the product was oxidised directly and, while (XI) has been regularly identified by its ultra-violet absorption spectrum in alkaline solution and by the characteristic fluorescence of its sodium salt in aqueous solution, we have failed to demonstrate so far the production of (X), and hence of pteric acid (V).\* In the formation of the isomeride (XVII) of (V) from the condensation product (XVI) of reductone with *p*-aminobenzoic acid and (II), the course of the reaction may be depicted as follows :



\* Note added in Proof, December 16th, 1948.—Conditions have now been found for the synthesis of pteric acid and related compounds from reductone (Forrest and Walker, *Nature*, 1948, **161**, 721; *J.*, forthcoming communication; Angier *et al.*, *J. Amer. Chem. Soc.*, 1948, **70**, 25).

## EXPERIMENTAL.

**2-Amino-6-hydroxy-8-D-arabotetrahydroxybutylpteridine (IV).**—(A) *From glucose.* To a solution of glucose monohydrate (20 g.) in water (180 c.c.), hydrated sodium acetate (27.2 g.), boric acid (12 g.), and 2 : 4 : 5-triamino-6-hydroxypyrimidine sulphate (25.7 g.) were added in that order. Air was removed from the resulting suspension by displacement with nitrogen, hydrazine hydrate (10 c.c. of 50%) was added, and the mixture, through which nitrogen was bubbled continuously, was heated on the water-bath for 5 hours. It was then cooled and the precipitate (18 g.) was collected and dried. Recrystallisation from 20% acetic acid (norite) afforded a yellow microcrystalline solid (Found, on material dried at 110° in a vacuum : C, 42.5; H, 5.2; N, 24.9. Calc. for  $C_{16}H_{13}O_5N_5$  : C, 42.4; H, 4.6; N, 24.7%).

The substance (VI) could also be recrystallised from *N*/10-hydrochloric acid or from 10% aqueous glycerol. The average optical rotatory power for a number of different preparations, each recrystallised several times, was  $[\alpha]_D^{20} - 81.5^\circ$  in *N*-hydrochloric acid.

(B) *From fructose.* When fructose (18 g.) was substituted for glucose in the above process, a similar product (17 g.) was obtained. The substance (VII) could likewise be recrystallised from the same solvents and behaved in the same manner, separating as a yellow microcrystalline solid (Found, on material dried at 110° in a vacuum : C, 42.5; H, 4.9; N, 24.4%).

The average optical rotatory power for a number of different preparations, each recrystallised several times, was  $[\alpha]_D^{20} - 86.6^\circ$  in *N*-hydrochloric acid.

It is likely that the substance obtained by Karrer *et al.* (*loc. cit.*) from fructose, having  $[\alpha]_D^{20} - 46.0^\circ$  in *N*/10-sodium hydroxide solution, was an impure specimen of (IV). Like urothion (Koschura, *Z. physiol. Chem.*, 1943, 279, 44), to which (IV) probably bears some resemblance, the two substances (VI) and (VII) gave products soluble in organic solvents on acetylation in pyridine with acetic anhydride, but no satisfactory recrystallisation was achieved.

**Oxidation of (IV) with Periodic Acid.**—(A) Recrystallised material (VI) (0.7 g.) was added slowly to a solution of potassium periodate (1.73 g.) in *N*-sulphuric acid (87 c.c.), solution taking place instantaneously. The solution was allowed to stand at room temperature for 30 minutes, and, after adjustment to pH 5—6, the precipitated yellow solid (0.44 g.) was collected. The product was optically inactive and could not be satisfactorily recrystallised. When added to an excess of 2 : 4-dinitrophenylhydrazine in dilute hydrochloric acid it gave an immediate brownish-yellow precipitate of 2-amino-6-hydroxypteridine-8-aldehyde 2 : 4-dinitrophenylhydrazone, which was collected, washed with water, and dried (Found : N, 32.9.  $C_{12}H_8O_5N_9$  requires N, 34.0%).

(B) Recrystallised material (VII) (1.4 g.) was submitted to the foregoing process and showed identical behaviour (yield, 0.9 g.). The 2 : 4-dinitrophenylhydrazone was prepared in a similar manner (Found : N, 33.3%).

**2-Amino-6-hydroxypteridine-8-carboxylic Acid (X).**—(A) *From the aldehyde obtained on oxidation of (VI).* The crude aldehyde (0.7 g.) was suspended in water (20 c.c.) and treated with 30% hydrogen peroxide (2 c.c.) and 2*N*-sodium hydroxide solution (8 c.c.). The solution was kept at room temperature for 2 hours and then heated on the water-bath for 30 minutes. The product, precipitated on acidification to pH 3 with dilute hydrochloric acid, was collected, washed with water, alcohol, and ether, and dried. The crude material (0.6 g.) was soluble in sodium hydrogen carbonate solution. The disodium salt was obtained by crystallisation (norite) from 2*N*-sodium hydroxide (Found, on material dried at 100° in a vacuum : C, 30.6; H, 1.9. Calc. for  $C_8H_5O_3N_5Na_2 \cdot H_2O$  : C, 31.2; H, 1.9%).

In aqueous solution the disodium salt showed an intense sky-blue fluorescence in ultra-violet light, greatly diminished on addition of a large excess of alkali and quenched on acidification. The ultra-violet absorption spectrum in *N*/10-sodium hydroxide showed two maxima, the sharper of the two being between 261 and 262  $m\mu$  with  $\epsilon$  of 16,600. As routine analytical criteria of identity, absorption in the range 250—270  $m\mu$  together with fluorescence characteristics serve to distinguish this acid from its 9-isomer.

(B) *From the aldehyde obtained on oxidation of (VII).* Crude aldehyde (0.7 g.) was oxidised in the manner described above and the crude acid (0.6 g.) was converted into the disodium salt in the same way (Found, on material dried at 100° in a vacuum : C, 31.7; H, 2.2%).

Fluorescence characteristics were identical with those described above and the ultra-violet absorption spectrum in alkaline solution showed a maximum between 261 and 262  $m\mu$  with  $\epsilon$  of 16,870.

(C) *From (synthetic) liver L. casei factor.* Liver *L. casei* factor ("Lederle", synthetic) (100 mg.) was dissolved in *N*-sodium hydroxide solution (15 c.c.) and refluxed for 4 hours with oxygen bubbling gently through the solution throughout (Stokstad *et al.*, *loc. cit.*). The acid (X) (50 mg.), precipitated on acidification to pH 3, was collected, washed with alcohol and ether, and dried. Fluorescence characteristics were identical with those described above and the ultra-violet absorption spectrum in alkaline solution showed a maximum between 261 and 262  $m\mu$  with  $\epsilon$  of 16,870.

(D) *By direct oxidation of (VI) or (VII).* The substance (3.05 g.) was dissolved in 2*N*-sodium hydroxide solution (40 c.c.) and treated with excess saturated aqueous potassium permanganate. After 30 minutes on the water-bath, excess permanganate was destroyed with alcohol, and the sludge was removed by filtration. The crude acid (2.45 g.), precipitated on bringing the clear yellow filtrate to pH 3, was converted into the disodium salt in the manner described above. Fluorescence and absorption characteristics were identical with those described above.

**2-Amino-6-hydroxy-9-methylpteridine.**—Methylglyoxal (5 c.c.) was added to a suspension of 2 : 4 : 5-triamino-6-hydroxypyrimidine sulphate (18.3 g.) in water (100 c.c.) containing hydrated sodium acetate (19.7 g.). The mixture was heated on the water-bath for an hour, and the product separated as a sodium salt (10 g.) from 5*N*-sodium hydroxide. The sodium salt exhibited a marked blue fluorescence in aqueous solution in ultra-violet light.

**2-Amino-6-hydroxypteridine-9-carboxylic Acid (XI).**—Sodium 2-amino-6-hydroxy-9-methylpteridine (5.6 g.) was dissolved in 2*N*-sodium hydroxide and oxidised on the water-bath with excess saturated aqueous permanganate. After destruction of the excess, the product (3.6 g.) separated on acidification of the filtrate to pH 3. The disodium salt separated from 2*N*-sodium hydroxide as a mass of pale yellow

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needles (Found, on material dried at 100° in a vacuum : C, 33.1; H, 1.5. Calc. for  $C_7H_5O_3N_5Na_2$  : C, 33.5; H, 1.2%).

In aqueous solution the disodium salt showed a bright green fluorescence in ultra-violet light, greatly diminished on addition of a large excess of alkali and quenched on acidification. The ultra-violet absorption spectrum in *N*/10-sodium hydroxide solution showed the usual two bands, the more intense having a maximum at 258  $m\mu$  with  $\epsilon$  of 16,580.

*Direct Condensation of Glucose with 2 : 4 : 5-Triamino-6-hydroxypyrimidine. Formation of 2-Amino-6-hydroxy-9-D-arabotetrahydroxybutylpteridine and Oxidation to (XI).*—Glucose monohydrate (22 g.), 2 : 4 : 5-triamino-6-hydroxypyrimidine sulphate (18.2 g.), and sodium acetate (11.6 g.) were heated in aqueous solution (175 c.c.) under nitrogen on the water-bath for 5 hours. The solid product was collected and crystallised from *N*/10-hydrochloric acid, when a microcrystalline yellow solid separated,  $[\alpha]_D^{17}$   $-52^\circ$  in *N*-hydrochloric acid. From its rotation, this material was probably a crude preparation of (III). Karrer *et al.* (*loc. cit.*) record  $[\alpha]_D^{20}$   $-68.93^\circ$  in *N*/10-sodium hydroxide and Ptering and Weisblat (*loc. cit.*) record  $[\alpha]_D^{20}$   $-70.9^\circ$  in *N*-sodium hydroxide for what is presumably this substance.

Direct oxidation of the above material (1.8 g.) with alkaline permanganate gave a crude product (1.3 g.), which was converted into the disodium salt. Fluorescence characteristics and ultra-violet absorption showed it to be the disodium salt of (XI).

*Condensations with Glucosone.*—(i) *Direct condensation.* To an aqueous solution (70 c.c.) of glucosone (*ca.* 7 g.) were added hydrated sodium acetate (9.7 g.) and 2 : 4 : 5-triamino-6-hydroxypyrimidine sulphate (9 g.), and the mixture was heated for 3 hours on the water-bath in an atmosphere of nitrogen. Direct oxidation of the product with alkaline permanganate yielded (XI), characterised as its disodium salt by fluorescence characteristics and ultra-violet absorption.

(ii) *In presence of boric acid.* The preceding experiment was repeated (*ca.* 2 g. of glucosone and other reagents in proportion) in the presence of boric acid (1.2 g.). The product, on oxidation with alkaline permanganate, again afforded (XI).

(iii) *In presence of hydrazine.* To a mixture of sodium acetate (5.5 g.), 2 : 4 : 5-triamino-6-hydroxypyrimidine sulphate (5.1 g.), boric acid (2.4 g.), and 50% hydrazine hydrate (2 c.c.) under nitrogen in water (50 c.c.), a 10% aqueous solution of glucosone (*ca.* 3.6 g.) was finally added, and the mixture was heated on the water-bath for 3 hours. On oxidation of the product with alkaline permanganate (X) was isolated in this instance and characterised as the disodium salt, which showed its characteristic sky-blue fluorescence and ultra-violet absorption.

*Experiments with Reductone.*—(i) *Condensation product (XVI) with p-aminobenzoic acid* (compare O'Meara *et al.*, *loc. cit.*). Reductone (0.44 g.) (*v.* Euler and Martius, *Annalen*, 1933, 505, 73) was dissolved in a little water and added to a warm saturated solution of *p*-aminobenzoic acid (1.37 g.) in dilute acetic acid (75 c.c.). The solution was allowed to come to room temperature and kept in the refrigerator overnight. The orange-coloured solid was collected and washed well with dilute acetic acid, with water, and dried (yield, 1.05 g.). The substance was almost completely insoluble in organic solvents and could not be purified by recrystallisation. It darkened at about 200° and melted at 240—250° (Found : N, 8.3. Calc. for  $C_{17}H_{14}O_5N_2$  : N, 8.6%).

(ii) *Condensation of (XVI) with (II).* (a) The above compound (XVI) (1.82 g.) was added to a hot solution of 2 : 4 : 5-triamino-6-hydroxypyrimidine sulphate (1.38 g.) in water (100 c.c.) containing sodium acetate (0.9 g.) and the mixture was heated in an atmosphere of nitrogen for 3 hours. The mixture was then cooled and the product collected (2.2 g.). The crude substance (1 g.) was oxidised directly with alkaline permanganate affording a crude acid (110 mg.). Crystallisation from 2*N*-sodium hydroxide afforded an insoluble by-product (50 mg.) together with the disodium salt of (XI) (50 mg.), which exhibited its characteristic bright green fluorescence and ultra-violet absorption spectrum (maximum at 258  $m\mu$ ).

(b) 2 : 4 : 5-Triamino-6-hydroxypyrimidine sulphate (1.25 g.) was dissolved in an aqueous solution (50 c.c.) of sodium hydrogen carbonate (0.84 g.) and treated with a suspension of (XVI) (0.91 g.) in aqueous sodium hydrogen carbonate (0.84 g. in 50 c.c.). The mixture was heated on the water-bath under nitrogen for 3 hours. The product (1.1 g.), precipitated on acidification to pH *ca.* 3, was oxidised directly with alkaline permanganate, affording crude material (0.55 g.), separated by crystallisation from 2*N*-sodium hydroxide into alkali-insoluble by-product (50 mg.) and the disodium salt of (XI) (0.5 g.), which showed its characteristic fluorescence and ultra-violet absorption.

(c) The substance (XVI) (1.3 g.) and 2 : 4 : 5-triamino-6-hydroxypyrimidine hydrogen sulphite (1.3 g.) were heated in 80% acetic acid (50 c.c.) under nitrogen for 2 hours and the solid (1.5 g.) was collected after cooling. The crude substance (1 g.) was oxidised directly, and the product (0.2 g.) was separated into alkali-insoluble material (140 mg.) and the disodium salt of (XI) (50 mg.), identified by its fluorescence and absorption spectrum.

(d) When the preceding experiment was repeated using *N*/10-hydrochloric acid as solvent in place of 80% acetic acid, a similar result was obtained.

(iii) *Condensation of (II) with p-aminobenzoic acid and free reductone.* (a) To a solution of (II) (1.9 g.) in water (50 c.c.) containing sodium acetate (1.23 g.) and *p*-aminobenzoic acid (1.03 g.), heated on the water-bath in an atmosphere of nitrogen, there was added an aqueous solution (10 c.c.) of reductone (0.66 g.). The solution remained clear for a few seconds and then a brown solid was rapidly precipitated. After 2½ hours on the water-bath, the product (2.1 g.) was collected, washed with water, alcohol, and ether, and dried. The crude material (1 g.) was oxidised and the product (0.2 g.), on treatment with 2*N*-sodium hydroxide, yielded insoluble by-product (50 mg.) and the disodium salt of (XI) (70 mg.), exhibiting its characteristic green fluorescence and a maximum in its absorption spectrum at 258  $m\mu$ .

(b) The preceding experiment was repeated in the presence of boric acid (1.05 g.) at approximately 40° under nitrogen. After a few seconds again a brown precipitate was thrown down. After 24 hours at 37° the product was collected. When oxidised, it (1 g.) afforded an alkali-insoluble product (50 mg.) and the disodium salt of (XI) (70 mg.), exhibiting the characteristic green fluorescence and a maximum in its absorption spectrum at 258  $m\mu$ .

As the possibility was not excluded that *p*-aminobenzoic acid played no part in the preceding reactions (iii a and iii b), the following experiments were carried out.

(iv) *Direct condensation of (II) with reductone.* (a) To a solution of 2 : 4 : 5-triamino-6-hydroxypyrimidine sulphate (1.26 g.) in aqueous sodium hydrogen carbonate (0.82 g. in 35 c.c.) was added an aqueous solution of reductone (0.44 g. in 10 c.c.). The mixture was heated under nitrogen on the water-bath for 3 hours and the brown product (0.33 g.), which commenced to separate within about 10 seconds of mixing, was collected and dried. Direct oxidation with alkaline permanganate afforded a crude acid (0.15 g.), which yielded the disodium salt of (XI) on crystallisation from 2*N*-sodium hydroxide, exhibiting the characteristic green fluorescence and a maximum in its absorption spectrum at 258  $m\mu$ .

(b) A solution of 2 : 4 : 5-triamino-6-hydroxypyrimidine hydrogen sulphite (1.3 g.) in 80% acetic acid (35 c.c.) was mixed with a solution of reductone (0.44 g.) in the same solvent (10 c.c.), and heated on the water-bath under nitrogen for 3 hours. The crude dark brown product (0.43 g.) was collected and oxidised directly. The crude acid (0.22 g.) was crystallised from 2*N*-sodium hydroxide and again afforded the disodium salt of (XI), which exhibited its characteristic fluorescence and a maximum at 258  $m\mu$  in its ultra-violet absorption spectrum.

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