

446. *The Effect of Hydrazine on the Condensation of Certain α -Ketols and Related Substances with 2 : 4 : 5-Triamino-6-hydroxypyrimidine.*

By H. S. FORREST and JAMES WALKER.

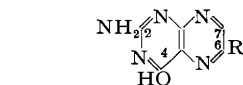
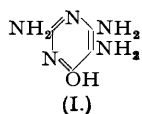
An earlier study of the effect of hydrazine on the condensation of 2 : 4 : 5-triamino-6-hydroxypyrimidine (I) with glucose and fructose has been extended to the condensation of (I) with acetol, methylglyoxal, dihydroxyacetone, and *p*-tolyl-D-isoglucosamine.

THE appearance of a communication by Karrer and Schwyzer (*Helv. Chim. Acta*, 1949, **32**, 423) makes it desirable for us to submit immediately an account of experiments * carried out as an extension of work previously described (Forrest and Walker, *Nature*, 1948, **161**, 308; this vol., p. 79). In our previous communications it was shown that 2 : 4 : 5-triamino-6-hydroxypyrimidine (I) reacts with glucose and fructose in the presence of hydrazine to give in both cases 2-amino-4-hydroxy-6-D-*arab*otetrahydroxybutylpteridine (II). In the absence of hydrazine, glucose and (I) afford the isomeric 2-amino-4-hydroxy-7-D-*arab*otetrahydroxybutylpteridine (III) (cf. also Karrer, Schwyzer, Erden, and Siegwart, *Helv. Chim. Acta*, 1947, **30**, 1031), which, in the light of subsequent events (see below), may have contained some 2-amino-4-hydroxy-7-D-*erythro*-2' : 3' : 4'-trihydroxybutylpteridine (IV), whilst glucosone and (I) afford (II) in the presence of hydrazine and (III) in its absence (this vol., p. 79). The results obtained in the presence of hydrazine were interpreted in terms of an open-chain osazone-type of derivative formed from the sugar component and hydrazine before interaction with (I), otherwise, had the reaction been initiated by condensation between the sugars and (I) before the participation of hydrazine, the hydrazine could not have exerted a controlling influence over the orientation, and the production of the same product (II) from glucose and fructose would have been impossible. Weygand and Bergmann (*Chem. Ber.*, 1947, **80**, 255; cf. also Weygand, *Ber.*, 1940, **73**, 1268) have proposed a mechanism for Ohle and Hielscher's synthesis of 2-D-*arab*otetrahydroxybutylquinoxaline (V) (*Ber.*, 1941, **74**, 13) from *o*-phenylenediamine and glucose in the presence of hydrazine in which *o*-phenylenediaminemonoglucoside is produced initially, thus determining the orientation, and then undergoes Amadori rearrangement to *o*-aminophenylisoglucosamine before the participation of hydrazine and ring-closure to (V).

* Apart from the experiments with *p*-tolylisoglucosamine the work described in the present communication was completed by September 1948, when this collaboration ended with the departure of one of the authors (H. S. F.) to another laboratory.—J. W.

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The equivalence of the two amino-groups in *o*-phenylenediamine obscures the issue in the case considered by Weygand and Bergmann, but their mechanism is untenable in view of the above observations and also in the light of the results presented in this communication.



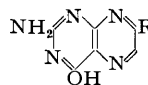
(II; R = [CH·OH]₃·CH₂·OH.)

(VII; R = CH₃.)

(IX; R = CO₂H.)

(X; R = CH₂·OH.)

(XI; R = CH₂·[CH·OH]₂·CH₂·OH.)



(III; R = [CH·OH]₃·CH₂·OH.)

(IV; R = CH₂·[CH·OH]₂·CH₂·OH.)

(VI; R = CH₃.)

(VIII; R = CO₂H.)

The open-chain formulation of sugar osazones used by Fischer has been supported by Engel (*J. Amer. Chem. Soc.*, 1935, **57**, 2419) and by Chargaff and Magasanik (*ibid.*, 1947, **69**, 1459), and although Haworth ("The Constitution of Sugars," 1929, p. 7) has suggested the possibility of cyclic structures, no final conclusions have been reached (cf. Percival, *Advances in Carbohydrate Chemistry*, 1948, **3**, 23). As the interpretation of our previous results was based on an acyclic osazone-type of structure, it was deemed desirable to extend our study of the effect of hydrazine to condensations involving α -ketols which could form only acyclic osazones. The first case studied was the condensation of (I) with acetol, wherein the product of direct condensation unexpectedly proved to be 2-amino-4-hydroxy-7-methylpteridine (VI); apparently, Karrer and Schwyzer (*loc. cit.*) observed practically no direct condensation between acetol and (I). As condensation reactions of (I) with hydroxycarbonyl compounds are undoubtedly initiated between the nucleophilic 5-amino-group and the electrophilic carbonyl carbon atom (cf. this vol., pp. 79, 2002), the formation of (VI) from acetol must be ascribed to the equilibrium between acetol and lactaldehyde, only the latter undergoing reaction readily with (I). Ordinarily this equilibrium lies far on the side of acetol, as indicated by its production in reactions expected to yield lactaldehyde (Nef, *Annalen*, 1904, **335**, 265; Wohl, *Ber.*, 1908, **41**, 3609; Wohl and Lange, *ibid.*, p. 3612; Hildesheimer, *ibid.*, 1910, **43**, 2804; cf. also Goto, *Bull. Chem. Soc. Japan*, 1940, **15**, 103). When the reaction was repeated with the addition of hydrazine, 2-amino-4-hydroxy-7-methylpteridine (VI) was again produced, apparently exclusively; under comparable conditions Karrer and Schwyzer (*loc. cit.*) claim the formation of the isomeric 2-amino-4-hydroxy-6-methylpteridine (VII). If, however, acetol and hydrazine were set aside together in dilute acetic acid for varying periods of time before the addition of (I), a mixture of 2-amino-4-hydroxy-7- (VI) and 2-amino-4-hydroxy-6-methylpteridine (VII) was obtained, the former predominating; these two substances are not readily separated, but the sodium salts of the mixture of carboxylic acids obtained on alkaline permanganate oxidation were readily separated into the less soluble sodium salt of 2-amino-4-hydroxypteridine-7-carboxylic acid (VIII) and the sodium salt of 2-amino-4-hydroxypteridine-6-carboxylic acid (IX), which are easily characterised by means of their ultra-violet absorption spectra and fluorescence characteristics. As this only partial reversal of the orientation in the presence of hydrazine could be ascribed to the incompleteness of osazone formation between acetol and hydrazine, this difficulty was readily overcome by allowing methylglyoxal to react with hydrazine before admixture with (I); under these conditions the product was 2-amino-4-hydroxy-6-methylpteridine (VII), the orientation being established by oxidation to 2-amino-4-hydroxypteridine-6-carboxylic acid (IX). It will be recalled that the direct reaction between methylglyoxal and (I) affords 2-amino-4-hydroxy-7-methylpteridine (VI) (Mowat *et al.*, *Ann. N.Y. Acad. Sci.*, 1946, **48**, Art. 5, 279; Forrest and Walker, this vol., p. 79), although Karrer and Schwyzer (*loc. cit.*) are of the opinion that the product also contains some 2-amino-4-hydroxy-6-methylpteridine. It is therefore clear, as indicated previously (this vol., p. 79), that condensation in the presence of hydrazine does not proceed by way of the keto-aldehyde (osone) as an intermediate as Ohle and Hielscher (*loc. cit.*) and Karrer and Schwyzer (*Helv. Chim. Acta*, 1948, **31**, 783) have claimed. Karrer and Schwyzer (*ibid.*, 1949, **32**, 429) have now also recognised that the keto-aldehyde cannot be an intermediate.

We then examined the condensation of dihydroxyacetone with (I). This condensation was first mentioned by Karrer, Schwyzer, Erden, and Siegwart (*loc. cit.*), who gave no experimental details and stated in a footnote that the product of this reaction was first isolated in the laboratories of F. Hoffmann-La Roche and Co. in Basle; the product, whatever the experimental conditions used in its preparation, was clearly taken to be 2-amino-4-hydroxy-6-hydroxy-methylpteridine (X), and it was subsequently used in a reaction with *p*-aminobenzoylglutamic

acid yielding a product claimed to contain 15% of folic acid, as shown by biological assay (Karrer and Schwyzer, *ibid.*, 1948, **31**, 777). Shortly afterwards, Angier *et al.* (*J. Amer. Chem. Soc.*, 1948, **70**, 3029) pointed out, on the basis of the wealth of experience accumulated by their group in this field, that the reaction of (I) with any three-carbon compound which might be expected to produce initially a hydroxymethyl- or halogenomethyl-dihydropteridine, actually gave primarily the fully aromatised methylpteridine with no substituent on the methyl group; thus (I) afforded 2-amino-4-hydroxy-7-methylpteridine (VI) on condensation with each of the following substances: 1: 3-dichloroacetone, $\beta\gamma$ -dichloropropaldehyde, α -bromotetronic acid, and DL-glyceraldehyde. They therefore inferred that the product obtained by Karrer, Schwyzer, Erden, and Siegwart (*loc. cit.*) from (I) and DL-glyceraldehyde, for which poor analytical results were obtained, was likewise 2-amino-4-hydroxy-7-methylpteridine (VI) and not 2-amino-4-hydroxy-7(or -6?)-hydroxymethylpteridine. Still later, Weygand, Wacker, and Schmied-Kowarzik (*Experientia*, 1948, **4**, 427) reported that diacetoxyacetone and (I) afforded either 2-amino-4-hydroxy-6- (VII) or 2-amino-4-hydroxy-7-methylpteridine (VI), and not 2-amino-4-hydroxy-6-hydroxymethylpteridine (X); furthermore, Weygand *et al.* repeated Karrer and Schwyzer's condensation of diaminoacetone dihydrochloride with (I) and obtained 2-amino-4-hydroxy-7-methylpteridine (VI), whereas Karrer and Schwyzer (*Helv. Chim. Acta*, 1948, **31**, 777) stated that their product was not the expected 2-amino-4-hydroxy-6(?) -aminomethylpteridine but, on the basis of the analytical evidence, 2-amino-4-hydroxy-7(?) -hydroxymethylpteridine. Weygand *et al.* (*loc. cit.*) also obtained either 2-amino-4-hydroxy-6- (VII) or 2-amino-4-hydroxy-7-methylpteridine (VI) from the condensation of 1: 3-bis-(*p*-carboxy-*N*-formanilido)acetone with (I). In our hands the direct condensation of dihydroxyacetone with (I) has afforded 2-amino-4-hydroxy-7-methylpteridine (VI), although a small proportion of the product consisted of a 2-amino-4-hydroxypteridine substituted in the 6-position, as a trace of 2-amino-4-hydroxypteridine-6-carboxylic acid (IX) appeared in the carboxylic acid obtained on oxidation of the crude reaction product with alkaline potassium permanganate; this result is in general accord with the observations of Angier *et al.* (*loc. cit.*) and of Weygand *et al.* (*loc. cit.*), but it is at variance with Karrer and Schwyzer's claim (*Helv. Chim. Acta*, 1949, **32**, 423) that 2-amino-4-hydroxy-6-methylpteridine (VII) is the product of the direct condensation between dihydroxyacetone and (I). When dihydroxyacetone was treated in the cold with hydrazine and then added to (I), the product was 2-amino-4-hydroxy-6-hydroxymethylpteridine (X), affording 2-amino-4-hydroxypteridine-6-carboxylic acid (IX) on oxidation. Karrer and Schwyzer (*ibid.*) have now purified their earlier product from dihydroxyacetone and (I), and state it to be 2-amino-4-hydroxy-6-hydroxymethylpteridine (X); this product is now, for the first time, said to have been prepared in the presence of hydrazine.

Weygand, Wacker, and Schmied-Kowarzik (*loc. cit.*) have reported that the condensation of *p*-tolyl-D-isoglucoamine with (I) affords 2-amino-4-hydroxy-7-D-erythro-2': 3': 4'-trihydroxybutylpteridine (IV) and not the expected tetrahydroxybutylpteridine, but no details have yet become available.* We have also investigated this reaction and have ascertained the effect of hydrazine upon the course of the condensation. The direct condensation of *p*-tolyl-D-isoglucoamine with (I) afforded a product having initially $[\alpha]_{\text{D}}^{25} - 69^\circ$ in *n*-hydrochloric acid. On repeated recrystallisation the optical rotation progressively fell and appeared to become constant at $[\alpha]_{\text{D}}^{18} - 22^\circ$, by which time the substance gave acceptable analytical figures for 2-amino-4-hydroxy-7- (IV) or 2-amino-4-hydroxy-6-D-erythro-2': 3': 4'-trihydroxybutylpteridine (XI). Oxidation of the material from the last recrystallisation afforded a mixture of acids, which was separated, *via* the sodium salts, into 2-amino-4-hydroxypteridine-7-carboxylic acid (VIII), giving acceptable analytical figures and showing the characteristic fluorescence and absorption spectrum of this acid, and 2-amino-4-hydroxypteridine-6-carboxylic acid (IX); the analytical results for the latter acid were less satisfactory, but the fluorescence characteristics were unmistakable and the absorption spectrum agreed in every detail with that of the authentic substance. Both 2-amino-4-hydroxy-7- (IV) and 2-amino-4-hydroxy-6-D-erythro-2': 3': 4'-trihydroxybutylpteridine (XI) are therefore formed in the direct condensation of *p*-tolyl-D-isoglucoamine with (I). In sharp contrast, condensation of *p*-tolyl-D-isoglucoamine with (I) in the presence of hydrazine afforded 2-amino-4-hydroxy-6-D-

* *Note added*, 23.7.49.—Since this paper was submitted, a further communication by Weygand, Wacker, and Schmied-Kowarzik has come to hand (*Chem. Ber.*, 1949, **82**, 25). These authors purified their product from the direct condensation of *p*-tolyl-D-isoglucoamine and (I) by precipitation from dilute ammonia solution (charcoal) and crystallisation from water, and record $[\alpha]_{\text{D}}^{20} - 46^\circ$ in 0.1*N*-sodium hydroxide. Moreover, they also carried out this condensation in the presence of hydrazine and record $[\alpha]_{\text{D}}^{20} - 88.5^\circ$ and -83.6° in 0.1*N*-sodium hydroxide for (II), in good agreement with our values of -86.6° , -81.5° , and -83° , observed in *n*-hydrochloric acid.

arabotetrahydroxybutylpteridine (II), having $[\alpha]_D^{22} -83^\circ$ in *n*-hydrochloric acid, in good agreement with our previously recorded values (*loc. cit.*). One crystallisation sufficed to give a pure product affording 2-amino-4-hydroxypteridine-6-carboxylic acid (IX) on oxidation.

The direct condensation of (I) with sugars was first reported by Karrer, Schwyzer, Erden, and Siegwart (*Helv. Chim. Acta*, 1947, **30**, 1031) while we were engaged in a similar study. The use of hydrazine in this condensation, following Ohle and Hielscher's work (*loc. cit.*) with *o*-phenylenediamine, and the recognition of its influence were first reported from this laboratory [Forrest and Walker, *Nature*, 1948, **161**, 308; cf. also this vol., p. 79 (MS. received by the Society on 5.1.48)]. The only other application, to our knowledge, of hydrazine in the pteridine field is contained in the latest paper of Karrer and Schwyzer [*Helv. Chim. Acta*, 1949, **32**, 423 (MS. dated 28.1.49)], which has just come to hand and where they also state: "Leider hatten wir es in unseren früheren Mitteilungen auch unterlassen, darauf hinzuweisen, dass die von uns dargestellten Pteridin-Präparate aus 2,4,5-Triamino-6-oxypyrimidin und Glycerinaldehyd bzw. Dioxyacetone nicht alle in gleicher Weise gewonnen worden sind: einige unter Zusatz von Hydrazin, andere ohne diesen Zusatz. Es hat sich nunmehr gezeigt, dass dadurch auch die Natur der Reaktionsprodukte in einem gewissen Ausmass beeinflusst wird." The necessity for this rectification and the conclusion drawn from it had obviously not been appreciated at the time of their comment [*ibid.*, 1948, **31**, 782 (MS. dated 22.3.48)] on our preliminary note, when they interpreted our results with glucose and fructose in terms of glucosone as a common intermediate.

EXPERIMENTAL.

Direct Condensation of Acetol with 2:4:5-Triamino-6-hydroxypyrimidine. Formation of 2-Amino-4-hydroxy-7-methylpteridine (VI).—Acetol (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 sodium acetate (11 g., anhydrous), which was heated on the water-bath and stirred in a slow stream of nitrogen. After 5 hours on the boiling water-bath the suspension was cooled and the yellowish-brown solid (3 g.) was collected, washed, and dried. Crystallisation from 5*N*-sodium hydroxide afforded the sodium salt as pale yellow needles. The product, recovered from the sodium salt by acidification with acetic acid, was obtained as a pale yellow amorphous powder (Found, on material dried at 160° in a vacuum: C, 47.1; H, 4.0. Calc. for $C_7H_7ON_5$: C, 47.5; H, 4.0%).

The substance was shown to be 2-amino-4-hydroxy-7-methylpteridine (VI) and it was identical with the substance previously obtained from (I) and methylglyoxal under similar conditions (this vol., p. 79). Its absorption spectrum in 0.1*N*-sodium hydroxide showed maxima at 250 and 357 $m\mu$., and oxidation in alkaline solution with potassium permanganate afforded 2-amino-4-hydroxypteridine-7-carboxylic acid, which was identified by its absorption spectrum in alkaline solution and by its fluorescence characteristics.

Effect of Hydrazine on the Condensation of Acetol with 2:4:5-Triamino-6-hydroxypyrimidine. Formation of 2-Amino-4-hydroxy-7-(VI) and 2-Amino-4-hydroxy-6-methylpteridine (VII).—A number of experiments were carried out allowing varying times of contact between acetol and hydrazine before reaction with 2:4:5-triamino-6-hydroxypyrimidine. The following is a typical experiment.

Freshly prepared and freshly distilled acetol (3 c.c.) was dissolved in 10% acetic acid (30 c.c.) and treated with 90% hydrazine hydrate (2.5 c.c.). After warming spontaneously to about 35°, the solution was kept at room temperature for 3 hours before being added to a mixture of 2:4:5-triamino-6-hydroxypyrimidine sulphate (10 g.), sodium acetate trihydrate (8 g.), and boric acid (4 g.) in water (40 c.c.), which was stirred on the water-bath in a slow stream of nitrogen. The mixture was heated for 5½ hours, and a bright yellowish-brown product (3.3 g.) separated. It was shown to be a mixture of the 7- and the 6-methyl derivative by oxidation to a mixture of the 7- and the 6-carboxylic acid. The crude product (2 g.) was dissolved in *N*-sodium hydroxide (50 c.c.) and oxidised on the water-bath with excess of potassium permanganate for ½ hour. Excess of permanganate was destroyed with alcohol and, after removal of manganese dioxide, the product (1.9 g.), precipitated with acetic acid, was obtained as a canary-yellow powder after collection at the centrifuge, washing, and drying. The crude mixture of acids was boiled with 2*N*-sodium hydroxide (*ca.* 100 c.c.), and the solution was filtered from a small residue (90 mg.). On cooling, the sodium salt (1.46 g.) of 2-amino-4-hydroxypteridine-7-carboxylic acid (VIII) separated and was readily identified by its absorption spectrum and fluorescence characteristics. On concentration of the mother-liquors to about one-fourth of their bulk and cooling, the sodium salt (0.35 g.) of 2-amino-4-hydroxypteridine-6-carboxylic acid (IX) separated and was similarly identified. Acidification of the final mother-liquors with glacial acetic acid gave a small amount (70 mg.) of crude 2-amino-4-hydroxypteridine-6-carboxylic acid (IX).

Direct Condensation of Methylglyoxal with 2:4:5-Triamino-6-hydroxypyrimidine. Formation of 2-Amino-4-hydroxy-7-methylpteridine (VI).—See this vol., p. 79.

Effect of Hydrazine on the Condensation of Methylglyoxal with 2:4:5-Triamino-6-hydroxypyrimidine. Formation of 2-Amino-4-hydroxy-6-methylpteridine (VII).—A solution of methylglyoxal (1.2 g.) in water (20 c.c.) was treated with hydrazine hydrate (2 c.c.; 90%) and, after 25 minutes at room temperature, the mixture was added to a suspension of 2:4:5-triamino-6-hydroxypyrimidine sulphate (4.6 g.) in water (50 c.c.) containing sodium acetate (2.75 g.; anhydrous) and boric acid (2 g.), which was stirred on the water-bath in a gentle stream of nitrogen for 4 hours. After cooling, the product (2.2 g.) was collected. After crystallisation as the sodium salt from 5*N*-sodium hydroxide the substance was recovered as a pale yellow amorphous powder (Found, on material dried at 160° in a vacuum; C, 47.4; H, 4.1. Calc. for $C_7H_7ON_5$: C, 47.5; H, 4.0%).

The substance was shown to be 2-amino-4-hydroxy-6-methylpteridine (VII); its absorption spectrum in 0.1N-sodium hydroxide solution showed maxima at 252 and 363—364 μ ., and oxidation in alkaline solution with potassium permanganate afforded 2-amino-4-hydroxypteridine-6-carboxylic acid (IX), which was identified by its absorption spectrum and fluorescence characteristics.

When methylglyoxal was added to a suspension of 2 : 4 : 5-triamino-6-hydroxypyrimidine sulphate in water containing sodium acetate, boric acid, and hydrazine hydrate, the product consisted essentially of 2-amino-4-hydroxy-7-methylpteridine (VI) with only a small proportion of the 6-methyl isomer (VII), owing to the rapid direct reaction between methylglyoxal and 2 : 4 : 5-triamino-6-hydroxypyrimidine.

Direct Condensation of Dihydroxyacetone with 2 : 4 : 5-Triamino-6-hydroxypyrimidine. Formation of 2-Amino-4-hydroxy-7-methylpteridine (VI).—To a solution of crude dihydroxyacetone (5 g.) (Underkofler and Fulmer, *J. Amer. Chem. Soc.*, 1937, **59**, 301), estimated by titration (Shaffer and Hartmann, *J. Biol. Chem.*, 1920, **45**, 365) to contain 85%, in water (60 c.c.) there were added 2 : 4 : 5-triamino-6-hydroxypyrimidine sulphate (5.1 g.) and sodium acetate (2.5 g.; anhydrous), and the mixture was heated on the water-bath under nitrogen for 2 hours. After cooling, the crude product (2.5 g.) was collected, washed, and dried. Part of the crude product was set aside for oxidative degradation and the rest was purified through the sodium salt. The crude substance (2.25 g.) was dissolved in 2N-sodium hydroxide (33 c.c.), and the filtered solution was treated with an equal volume of filtered 50% sodium hydroxide solution. The crystalline material which separated was collected after chilling in the ice-chest, and the process was repeated. The product (1.05 g.) was recovered from the twice recrystallised sodium salt as a light-yellow powder (Found : C, 47.9; H, 3.8. Calc. for $C_7H_7ON_5$: C, 47.5; H, 4.0%).

The crude reaction product (0.25 g.) was oxidised in dilute sodium hydroxide solution with excess of potassium permanganate on the water-bath for 30 minutes, affording a crude acid (0.16 g.). Crystallisation from 2N-sodium hydroxide afforded the sodium salt (100 mg.) of 2-amino-4-hydroxypteridine-7-carboxylic acid (VIII), identified by its green fluorescence in aqueous solution and by the maximum in its absorption spectrum in 0.1N-sodium hydroxide at 258 μ .; the mother-liquors, on acidification with acetic acid, afforded traces only of a precipitate, which showed a sky-blue fluorescence in 0.1N-sodium hydroxide and therefore contained some 2-amino-4-hydroxypteridine-6-carboxylic acid (IX).

Effect of Hydrazine on the Condensation of Dihydroxyacetone with 2 : 4 : 5-Triamino-6-hydroxypyrimidine. Formation of 2-Amino-4-hydroxy-6-hydroxymethylpteridine (X).—Hydrazine hydrate (2.3 c.c.; 90%) was added to an aqueous solution (20 c.c.) of crude dihydroxyacetone (3.5 g.), and the mixture was kept at room temperature for 30 minutes before being added to a suspension of 2 : 4 : 5-triamino-6-hydroxypyrimidine sulphate (5.1 g.) in water (60 c.c.) containing sodium acetate (3.1 g.; anhydrous) and boric acid (2.4 g.). The resulting mixture was heated under nitrogen on the water-bath for 2 hours, and the product (2 g.) was thereafter collected, washed, and dried. The pure substance, 2-amino-4-hydroxy-6-hydroxymethylpteridine (X), separated from a large volume of water (charcoal) as a pale yellow powder (Found, on material dried at 160° in a vacuum : C, 43.4; H, 3.6; N, 35.7. Calc. for $C_7H_7O_2N_5$: C, 43.5; H, 3.6; N, 36.3%).

Oxidation with potassium permanganate in the usual way afforded 2-amino-4-hydroxypteridine-6-carboxylic acid (IX), identified by the blue fluorescence of its sodium salt in aqueous solution and by the maximum in its absorption spectrum in 0.1N-sodium hydroxide at 261—262 μ ..

Direct Condensation of p-Tolyl-D-isoglucoamine with 2 : 4 : 5-Triamino-6-hydroxypyrimidine. Formation of 2-Amino-4-hydroxy-7- (IV) and 2-Amino-4-hydroxy-6-D-erythro-2' : 3' : 4'-trihydroxybutylpteridine (XI).—A mixture of *p*-tolyl-D-isoglucoamine (19.8 g.) (Weygand, *Ber.*, 1940, **73**, 1269), 2 : 4 : 5-triamino-6-hydroxypyrimidine sulphate (21 g.), sodium acetate trihydrate (22.5 g.), and boric acid (6 g.) in water (270 c.c.) was heated in a stream of nitrogen for 6 hours on the water-bath. A greyish-brown solid separated and some tarry material floated on the surface. The product was collected at the centrifuge, washed with acetone and alcohol until the washings were colourless, and then with ether, and dried, affording a buff-coloured powder (15 g.). The crude material was dissolved in *N*-hydrochloric acid (240 c.c.), and some black insoluble material was separated. The dark brown solution was treated twice with charcoal (3.25 g. and 2 g.), and the resulting yellow solution was diluted (to 1800 c.c.) but no precipitate was obtained until after neutralisation with ammonia, whereupon a pale yellow product (10.72 g.) was obtained, having $[\alpha]_D^{25}$ -69° in *N*-hydrochloric acid.

Part of the product (3 g.) was crystallised thrice (average recovery, 68%) from hot 0.1N-hydrochloric acid, affording a micro-crystalline solid, $[\alpha]_D^{19}$ -47° in *N*-hydrochloric acid. Better purification was achieved by recrystallising the remainder (6.4 g.) from 20% aqueous acetic acid, two recrystallisations affording a product with $[\alpha]_D^{19}$ -43.4° in *N*-hydrochloric acid. After five further recrystallisations (average recovery, 78%), the pale yellow product had $[\alpha]_D^{19}$ -22° , unaltered by the last recrystallisation (Found, on material dried at 140° in a vacuum : C, 44.4; H, 5.1. Calc. for $C_{10}H_{13}O_4N_5$: C, 44.9; H, 4.9%).

The foregoing analysed material (148 mg.) was dissolved in 0.1N-sodium hydroxide solution (12 c.c.) and oxidised with excess of potassium permanganate on the water-bath, 5 minutes being allowed after a stable permanganate colour was attained. The product, isolated in the usual way, was dissolved in dilute sodium hydroxide solution (ca. 17 c.c.) and 1.5 volumes of filtered 50% sodium hydroxide solution were added. The solid (109 mg.) which separated on cooling was collected after standing for 36 hours in the ice-chest, and the colourless mother-liquors remained clear on acidification with acetic acid. The solid was recrystallised from 2N-sodium hydroxide, affording a crop (A) (57.6 mg.) and mother-liquors (B). The material (A) showed a turquoise-blue fluorescence in aqueous solution, and further recrystallisation of this product from 2N-sodium hydroxide (8 c.c.) afforded the sodium salt (47.7 mg.) of 2-amino-4-hydroxypteridine-7-carboxylic acid (VIII), exhibiting its characteristic bright green fluorescence in aqueous solution and distinctive absorption spectrum. The free acid was analysed (Found, on material dried at 160° in a vacuum : C, 40.7; H, 3.1. Calc. for $C_7H_5O_3N_5$: C, 40.6; H, 2.4%).

The mother-liquors (B) were acidified with acetic acid and the precipitate (34.2 mg. was collected

(Found, on material dried at 160° in a vacuum: C, 38.8; H, 2.2. Calc. for $C_7H_5O_3N_5$: C, 40.6; H, 2.4%). Despite the poor value for carbon, this substance showed properties identical with those of 2-amino-4-hydroxypteridine-6-carboxylic acid (IX); the sodium salt showed the typical intense sky-blue fluorescence in aqueous solution and the ultra-violet absorption spectrum in alkaline solution agreed in every detail with that of the authentic substance.

Effect of Hydrazine on the Condensation of p-Tolyl-D-isoglucosamine with 2:4:5-Triamino-6-hydroxypyrimidine. Formation of 2-Amino-4-hydroxy-6-D-arabotetrahydroxybutylpteridine (II).—A mixture of *p*-tolyl-D-isoglucosamine (13.15 g.), 2:4:5-triamino-6-hydroxypyrimidine sulphate (14 g.), sodium acetate trihydrate (15 g.), boric acid (6 g.), and hydrazine hydrate (4 c.c.; 90%) in water (140 c.c.) was heated on the water-bath in a slow stream of nitrogen for 6 hours. The clear brown solution deposited a reddish-brown solid (6.94 g.), which was collected, washed, and dried. The entire crude product was dissolved in *N*-hydrochloric acid (100 c.c.), and the solution was treated twice with charcoal (2.5 g. and 3 g.), diluted to 0.1*N.*, and neutralised with ammonia, affording a pale yellow product (4.66 g.), $[\alpha]_D^{25} - 83^\circ$ in *N*-hydrochloric acid; this specific rotation was unaltered when the substance (2 g.) was recrystallised from 20% acetic acid (recovery, 73%) (Found, on material dried at 140° in a vacuum: C, 42.7; H, 5.1. Calc. for $C_{10}H_{13}O_5N_5$: C, 42.4; H, 4.6%).

The observed specific rotation is in good agreement with our previously observed values, -81.5° and -86.6° , for the same substance prepared from glucose and fructose respectively. Oxidation with potassium permanganate in the usual way afforded 2-amino-4-hydroxypteridine-6-carboxylic acid (IX), identified by the sky-blue fluorescence of its sodium salt in aqueous solution and by the maximum in its absorption spectrum in 0.1*N*-sodium hydroxide at 261–262 $m\mu.$; the product of this condensation was therefore 2-amino-4-hydroxy-6-D-arabotetrahydroxybutylpteridine (II).

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