426. *Reductone and the Synthesis of Pteridines.*

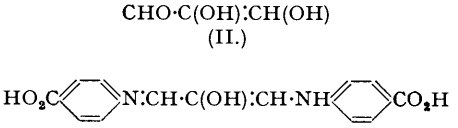
By H. S. Forrest and James Walker.

The possible rôle of reductone in the biosynthesis of pteroic acid and its use in the laboratory synthesis of pteridines are discussed. Possible antagonists of pteroylglutamic acid have been synthesised by condensing a number of sulphur-containing compounds severally with reductone in equimolecular proportions and then allowing the resulting 1 : 1 condensation products to react with 2 : 4 : 5-triamino-6-hydroxypyrimidine. The products from sulphanilylglycine and sulphanilyl-L-glutamic acid produced some inhibition of the growth of a strain of *Streptococcus lactis*.

It has been pointed out in previous communications that the three-carbon atom unit (indicated in bold letters) comprising the ring atoms 7 and 6 and the exocyclic carbon atom of pteroic acid (I) might be provided biogenetically by a triose, and, in the first place, 3-phosphoglyceraldehyde and phosphodihydroxyacetone were indicated as possible precursors (Forrest and Walker, *Nature*, 1948, **161**, 308; this vol., p. 79). The suggestion, however, by O'Meara, McNally, and Nelson (*Nature*, 1944, **154**, 796; *Lancet*, 1947, II, 747) that reductone (II) might be the strongly reducing, non-thiol substance formed in a variety of bacterial cultures during the

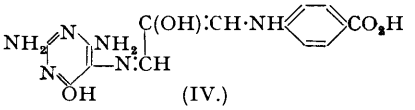
logarithmic phase of growth—the phase during which, incidentally, the antibacterial activity of the sulphonamides is maximal—suggested to us that this substance might well be our postulated triose precursor (Forrest and Walker, *Nature*, 1948, **161**, 721; this vol., p. 79). O'Meara and his colleagues (*Lancet*, 1947, II, 747) showed that streptococci could utilise the condensation product of (II) with *p*-aminobenzoic acid, but not free *p*-aminobenzoic acid, as a source of energy for growth, and it is noteworthy that Nimmo-Smith, Lascelles, and Woods (*Brit. J. Exp. Path.*, 1948, **29**, 264) have shown that only glucose, from which reductone is derivable (von Euler and Martius, *Annalen*, 1933, **505**, 73), and *p*-aminobenzoic acid are required for the synthesis of folic acid by *Streptobacterium plantarum*, glutamic acid being merely stimulatory. O'Meara and his collaborators suggested that *p*-aminobenzoic acid acts as a stabilising agent for reductone in bacterial metabolism, enabling the cell to store reductone and to utilise it as required, presumably after hydrolysis of the reductone–*p*-aminobenzoic acid condensation product to free reductone, and they also suggested that sulphonamides interfere with bacterial growth by combining with reductone and preventing it from becoming available for the use of the cell. The possibility, however, presents itself that *p*-aminobenzoic acid and reductone are first linked together in normal bacterial metabolism and then incorporated into pteroic acid and other factors of the folic acid group, such as pteroylglutamic acid, whereas in sulphonamide bacteriostasis the drug combines with reductone and the condensation product is incorporated into a biologically inert—and perhaps even toxic—analogue of pteroic acid (Forrest and Walker, *Nature*, 1948, **161**, 721). In other words, *p*-aminobenzoic acid and sulphonamides may compete, not for the service of an enzyme as required by the Woods–Fildes theory of sulphonamide bacteriostasis, but, chemically, for a strictly limited supply of pteridine precursors, and the development of sulphonamide resistance in bacteria may be brought about by enhanced production of these precursors in excess of normal requirements. In the light of these considerations the present work was undertaken to investigate the potentialities of reductone in the laboratory synthesis of pteroic acid and analogous substances.

Preliminary experiments, already reported (this vol., p. 79), were of two types. In the first type, the condensation product (III) formed in the reaction between one molecular proportion of reductone and two molecular proportions of *p*-aminobenzoic acid was first isolated and allowed to react with 2 : 4 : 5-triamino-6-hydroxypyrimidine under a variety of conditions. The products were directly oxidised and 2-amino-4-hydroxypteridine-7-carboxylic acid * was obtained in every case. That (III) was actually derived from *p*-aminobenzoic acid and (II) in the ratio 2 : 1 is borne out by further analytical data recorded below, and the constitution (III), involving terminal condensation on the part of the reductone, is borne out by the results of the experiments of the second type, in which reductone was added to a mixture of 2 : 4 : 5-triamino-6-hydroxypyrimidine and *p*-aminobenzoic acid, whereupon a rapid reaction took place. The products on oxidation again afforded 2-amino-4-hydroxypteridine-7-carboxylic acid. The course of this second type of reaction can best be interpreted by assuming reaction of reductone in the 1 and 3 positions with the amino-group of *p*-aminobenzoic acid and the reactive 5-amino-group of 2 : 4 : 5-triamino-6-hydroxypyrimidine respectively, giving (IV),

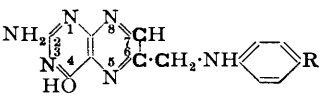


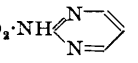
(II.)

(III.)



(IV.)



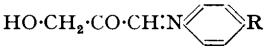
(I.) R = CO_2H
(XIV.) R = $SO_2{\cdot}NH_2$
(XV.) R = $SO_2{\cdot}CH_3$
(XVI.) R = $SO_2{\cdot}NH{\cdot}CH_2{\cdot}CO_2H$
(XVII.) R = $SO_2{\cdot}NH{\cdot}CH(CO_2H){\cdot}CH_2{\cdot}CH_2{\cdot}CO_2H$
(XVIII.) R = $SO_2{\cdot}NH$– 

which then cyclises to the 7-isomeride of pteroic acid. That reductone condenses with aromatic amines in the 1 and 3 positions when presented *simultaneously* with two molecular proportions of amine therefore appears to be clear. The valuable clue that reaction proceeds differently if reductone is allowed to react with only one molecular proportion of aromatic amine *before*

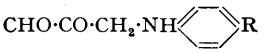
* The Ring Index numbering is now adopted, cf. (I). EDITOR.

the addition of the 2 : 4 : 5-triamino-6-hydroxypyrimidine was obtained by allowing reductone and methyl *p*-aminobenzoate to react with each other in equimolecular proportions for a short time before the addition of 2 : 4 : 5-triamino-6-hydroxypyrimidine, and the product in this case was pteroic acid (I); pteroic acid was also obtained when the 1 : 1 condensation product (V) of reductone with methyl *p*-aminobenzoate was first isolated and then allowed to react with 2 : 4 : 5-triamino-6-hydroxypyrimidine. In the previous communication (this vol., p. 79) it was pointed out that condensation reactions of 2 : 4 : 5-triamino-6-hydroxypyrimidine at the pH of our experiments must be initiated by nucleophilic attack by the amino-group in the 5-position on an electrophilic centre. We would therefore suggest that while the condensation product of reductone with methyl *p*-aminobenzoate is doubtless *methyl* p-(2 : 3-*dihydroxyprop*-2-*enylideneamino*)*benzoate* (V) initially, tautomeric equilibration to an anil of hydroxymethylglyoxal (VI; R = CO_2Me) takes place before the subsequent reaction with 2 : 4 : 5-triamino-6-hydroxypyrimidine, initiated at the 5-amino-group, and cyclisation to (I). That reductone itself is in tautomeric equilibrium with hydroxymethylglyoxal is obvious from the observations of Norrish and Griffiths (*J.*, 1928, 2829). At this point, as indicated in our preliminary note (*Nature*, 1948, **161**, 721), a communication from Angier and his collaborators appeared (*J. Amer. Chem. Soc.*, 1948, **70**, 25; also U.S.P. 2,442,836), describing the preparation of 1 : 1 condensation products of reductone with *p*-aminobenzoic acid and a number of its derivatives, those from *p*-aminobenzoic acid and its ethyl ester and from *p*-aminobenzoyl-L-glutamic acid and its diethyl ester being subsequently condensed with 2 : 4 : 5-triamino-6-hydroxypyrimidine to give pteroic acid and pteroylglutamic acid respectively. The American authors, however, do not appear to have appreciated the possible biogenetic significance of reductone. It also appears probable that they regarded the keto-aldehyde tautomer (VII) as the form undergoing reaction; this, it should be pointed out, would, in the case of (V), certainly react with 2 : 4 : 5-triamino-6-hydroxypyrimidine to give the 7-isomeride of pteroic acid and not pteroic acid (I), in the same way that methylglyoxal and 2 : 4 : 5-triamino-6-hydroxypyrimidine afford 2-amino-4-hydroxy-7-methylpteridine. Backer and Houtman (*Rec. Trav. chim.*, 1948, **67**, 260) have obtained 2-amino-4-hydroxy-7(?)-hydroxymethylpteridine by condensing 2 : 4 : 5-triamino-6-hydroxypyrimidine with hydroxymethylglyoxal in aqueous solution at the boiling-point but their attempt, carried out in the cold, to obtain the same substance, or the 6-hydroxymethyl isomer, from reductone and 2 : 4 : 5-triamino-6-hydroxypyrimidine failed to proceed beyond the unstable initial intermediate, although in our hands the same reaction gave on the water-bath a product affording 2-amino-4-hydroxypteridine-7-carboxylic acid on oxidation (this vol., p. 79).

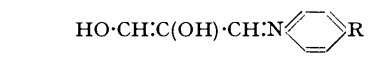
In accordance with the theory of sulphonamide bacteriostasis outlined above, sulphonamide drugs are pictured as being incorporated into biologically inert analogues of pteroic acid. Such analogues, however, would be likely to inhibit the growth of organisms requiring preformed factors of the folic acid group, such as pteroylglutamic acid; in other words, they would be antagonists of pteroylglutamic acid, although they would not be expected to inhibit markedly the growth of sulphonamide-sensitive organisms. Reductone was condensed in equimolecular proportions with sulphanilamide, *p*-methylsulphonylaniline, sulphanilylglycine, sulphanilyl-L-glutamic acid, diethyl sulphanilylglutamate, and sulphadiazine with the production of N^4-(2 : 3-*dihydroxyprop*-2-*enylidene*)*sulphanilamide* (VIII), p-(2 : 3-*dihydroxyprop*-2-*enylideneamino*)*phenyl methyl sulphone* (IX), N-p-(2 : 3-*dihydroxyprop*-2-*enylideneamino*)*benzenesulphonylglycine* (X), N-p-(2 : 3-*dihydroxyprop*-2-*enylideneamino*)*benzenesulphonylglutamic acid*



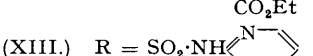
(VI.)



(VII.)



(V.) R = CO_2Me
(VIII.) R = $SO_2{\cdot}NH_2$
(IX.) R = $SO_2{\cdot}CH_3$
(X.) R = $SO_2{\cdot}NH{\cdot}CH_2{\cdot}CO_2H$
(XI.) R = $SO_2{\cdot}NH{\cdot}CH(CO_2H){\cdot}CH_2{\cdot}CH_2{\cdot}CO_2H$
(XII.) R = $SO_2{\cdot}NH{\cdot}CH(CO_2Et){\cdot}CH_2{\cdot}CH_2{\cdot}CO_2H$



(XIII.) R = $SO_2{\cdot}NH$

(XI), the *monoethyl* ester (XII) of (XI), and N^1-2-*pyrimidyl*-N^4-(2 : 3-*dihydroxyprop*-2-*enylidene*)-*sulphanilamide* (XIII). These condensation products were then in turn allowed to react with

2 : 4 : 5-triamino-6-hydroxypyrimidine, affording N^4-(2-*amino*-4-*hydroxy*-6-*pteridylmethyl*)*sulphanilamide* (XIV), p-(2-*amino*-4-*hydroxy*-6-*pteridylmethyl*)*aminophenyl methyl sulphone* (XV), N-p-(2-*amino*-4-*hydroxy*-6-*pteridylmethyl*)*aminobenzenesulphonylglycine* (XVI), N-p-(2-*amino*-4-*hydroxy*-6-*pteridylmethyl*)*aminobenzenesulphonylglutamic acid* (XVII), and N^1-2-*pyrimidyl*-N^4-(2-*amino*-4′-*hydroxy*-6′-*pteridylmethyl*)*sulphanilamide* (XVIII), respectively. In the ultimate analysis of these latter compounds the difficulties familiar in this field were again encountered (cf. Wieland and Purrmann, *Annalen*, 1940, **544**, 163), low carbon and high hydrogen values being obtained under ordinary conditions of drying and combustion. Recourse was therefore had to drying specimens at 140—160° before microanalysis, and under these conditions reasonably satisfactory results were obtained. Proof of the constitutions of (XIV)—(XVIII) was obtained by cleavage in aerated alkali, 2-amino-4-hydroxypteridine-6-carboxylic acid being formed in each case, and a further approximate analytical check on the compounds was obtained by colorimetric determination of the primary aromatic amine formed simultaneously.

Tests for *in vitro* activity against *Strep. hæmolyticus*, *Staph. aureus*, and *Bact. coli* were kindly carried out by Dr. A. T. Fuller on compounds (XIV)—(XVIII) but, as expected, none showed significant activity against these organisms, with the possible exception of (XIV) and (XV) which showed some activity in broth cultures of hæmolytic streptococci when the implants

Effect of (XVI) *and* (XVII) *on the growth of* Streptococcus lactis (N.C.T.C. 2700).

Concn. of pteroylglutamic acid. 10^{-9} g./c.c.	Concn. of drug. 10^{-6} g./c.c.	Turbidity * (Spekker). (XVI.)	(XVII.)
0	0	0·49	0·49
0·04	0	0·60	0·60
0·04	0·4	0·58	0·50
0·04	1·6	0·50	0·47
0·04	6·3	0·49	0·40
0·04	25	0·39	0·18
0·04	100	0·09	0·00
0·4	0	0·71	0·71
0·4	0·4	0·69	0·72
0·4	1·6	0·68	0·70
0·4	6·3	0·60	0·63
0·4	25	0·48	0·58
0·4	100	0·09	0·12

The basal medium used was that of Luckey, Briggs, and Elvehjem (*J. Biol. Chem.*, 1944, **152**, 157) except that sodium citrate was substituted for sodium acetate.

* Observed after incubation at 37° for 18 hours.

were small and growth was scanty. Compounds (XVI) and (XVII) were kindly examined in some detail by Miss J. M. Horton for activity against a strain of *Strep. lactis*. Both compounds inhibited the growth of this organism and the inhibition was reversed by pteroylglutamic acid; a representative series of observations is recorded in the Table. From these figures it does not appear that either compound can be regarded as a powerful antagonist of pteroylglutamic acid, but we have no basis of comparison with other known folic acid antagonists. Indeed, markedly different results would be expected with other strains of *Strep. lactis* as these vary widely in their folic acid requirements (cf. Stokes, Keresztesy, and Foster, *Science*, 1944, **100**, 522) and hence in their responses to folic acid antagonists. In view of the reported activity of folic acid antagonists in neoplastic growth, Professor Alexander Haddow kindly examined compounds (XIV), (XVII), and (XVIII) for activity on the Walker carcinoma 256 in the rat, but no inhibitory effect was observed.

Experimental.

p-(3-p′-*Carboxyanilino*-2-*hydroxyprop*-2-*enylideneamino*)*benzoic Acid* (III).—The product resulting from the condensation of one molecular proportion of reductone with two molecular proportions of *p*-aminobenzoic acid in hot dilute acetic acid solution has been further characterised (Found : C, 59·1; H, 4·6; N, 8·3. Calc. for $C_{17}H_{14}O_5N_2$: C, 62·6; H, 4·3; N, 8·6. Calc. for $C_{17}H_{14}O_5N_2,H_2O$: C, 59·3; H, 4·6; N, 8·1%).

Methyl p-(2 : 3-*Dihydroxyprop*-2-*enylideneamino*)*benzoate* (V).—Equimolecular amounts of reductone and methyl *p*-aminobenzoate were allowed to condense under conditions essentially the same as those used by Angier *et al.* (*loc. cit.*) for analogous cases. The *substance* separated from absolute alcohol in yellow rhombs, m. p. 196° after darkening at 180° (Found : C, 60·0; H, 5·3; N, 6·6. $C_{11}H_{11}O_4N$ requires C, 59·7; H, 5·0; N, 6·3%).

N^4-(2 : 3-*Dihydroxyprop*-2-*enylidene*)*sulphanilamide* (VIII).—Prepared in a similar manner from equimolecular proportions of reductone and sulphanilamide, the *substance* separated from 50% alcohol in tiny yellow needles, m. p. 220° after darkening at 180° (Found, on material dried at 100° in a vacuum : C, 44·2; H, 4·3; N, 11·1. $C_9H_{10}O_4N_2S$ requires C, 44·6; H, 4·1; N, 11·6%).

p-(2 : 3-*Dihydroxyprop*-2-*enylideneamino*)*phenyl Methyl Sulphone* (IX).—Prepared in a similar manner from *p*-methylsulphonylaniline, the *compound* crystallised from 50% alcohol in irregular yellow plates, m. p. 236—238° (Found : C, 50·2; H, 4·6; N, 6·0. $C_{10}H_{11}O_4NS$ requires C, 49·8; H, 4·6; N, 5·8%).

N-p-(2 : 3-*Dihydroxyprop*-2-*enylideneamino*)*benzenesulphonylglycine* (X).—Prepared in a similar manner from sulphanilylglycine (Dewing *et al.*, *J.*, 1942, 242), the *compound* separated from 50% alcohol in yellow needles, m. p. 220° after darkening at 206° (Found : N, 9·2. $C_{11}H_{12}O_6N_2S$ requires N, 9·3%).

N-p-(2 : 3-*Dihydroxyprop*-2-*enylideneamino*)*benzenesulphonylglutamic Acid* (XI).—Reductone (2 g.) was added to a solution of sulphanilyl-L-glutamic acid (6 g.) (Dewing *et al.*, *loc. cit.*) in alcohol (30 c.c.) containing glacial acetic acid (1·2 c.c.). Next day the yellow precipitate (7 g.) was collected. The *substance* separated from alcohol in yellow rectangular plates, m. p. 120—126° (Found : C, 45·7; H, 4·7; N, 7·3. $C_{14}H_{16}O_8N_2S$ requires C, 45·2; H, 4·3; N, 7·5%).

Ethyl Hydrogen N-p-(2 : 3-*Dihydroxyprop*-2-*enylideneamino*)*benzenesulphonylglutamate* (XII).—(i) A solution of acetylsulphanilyl chloride (11·7 g.) in acetone was added to a well-stirred suspension of sodium hydrogen carbonate (4·2 g.) in acetone (30 c.c.) containing diethyl glutamate (10 g.), prepared from the hydrochloride (Chiles and Noyes, *J. Amer. Chem. Soc.*, 1922, **44**, 1802). The mixture was stirred for an hour and kept overnight at room temperature. The acetone solution was then filtered and evaporated, whereupon the product (16 g.) crystallised on treatment with a little alcohol. *Diethyl* N-p-*acetamidobenzenesulphonylglutamate* separated from benzene in felted needles, m. p. 119° (Found : N, 7·6. $C_{17}H_{24}O_7N_2S$ requires N, 7·0%).

(ii) Deacetylation was effected by dissolving the compound (10 g.) in 4N-alcoholic hydrochloric acid (100 c.c.) and allowing the solution to stand at room temperature for 56 hours. Most of the alcohol and the hydrogen chloride were removed in a vacuum at room temperature, and the residue was poured into water and carefully neutralised with ammonia. The oil which separated was recovered by extraction with ethyl acetate but, on isolation, the deacetylated material could not be induced to crystallise. The crude material was therefore dissolved in water (700 c.c.) containing concentrated hydrochloric acid (3·5 c.c.) and treated with reductone (2·5 g.). The mixture was stirred at room temperature for 2 hours and then kept in the refrigerator overnight. The *half-ester* (9·6 g.) separated from absolute alcohol in yellow needles, m. p. 188° [Found : C 48·1, 47·6; H, 4·9 5·4; N, 7·0, 7·1. $C_{16}H_{20}O_8N_2S$ requires C, 48·0; H, 5·0; N, 7·0. $C_{18}H_{24}O_8N_2S$ (diethyl ester) requires C, 50·5; H, 5·6; N, 6·6%].

N^1-2-*Pyrimidyl*-N^4-(2 : 3-*dihydroxyprop*-2-*enylidene*)*sulphanilamide* (XIII).—A solution of reductone (2·5 g.) in water (30 c.c.) was added with stirring to a suspension of finely powdered sulphadiazine (6·3 g.) in water (700 c.c.) containing concentrated hydrochloric acid (3·5 c.c.). Stirring was continued for 2 hours, and the product (8 g.) was collected after the mixture had been kept overnight. The *compound* separated from ethylene glycol in yellow needles, m. p. 260° after darkening at 240° (Found : C, 49·1; H, 4·4; N, 17·7. $C_{13}H_{12}O_4N_4S$ requires C, 48·8; H, 3·8; N, 17·5%).

p-(2-*Amino*-4-*hydroxy*-6-*pteridylmethyl*)*aminobenzoic Acid* (*Pteroic Acid*) (I).—(*a*) A solution of reductone (0·44 g.) and methyl *p*-aminobenzoate (0·75 g.) in alcohol (40 c.c.) was heated under reflux on the water-bath for 15 minutes, and then treated with a suspension of 2 : 4 : 5-triamino-6-hydroxypyrimidine sulphate (1·3 g.) in water (30 c.c.) containing sodium acetate (0·8 g.; anhydrous). The resulting mixture was heated on the water-bath under nitrogen for 5 hours; a brown solid slowly separated. After cooling, the crude product (1·1 g.) was collected, washed with water, alcohol, and ether, and dried. On hydrolysis by aerated alkali this material afforded an acid having the fluorescence characteristics and absorption spectrum, in alkaline solution, of 2-amino-4-hydroxypteridine-6-carboxylic acid, together with a primary aromatic amine; the same acid was isolated as the product of direct oxidation in alkaline solution with potassium permanganate.

(*b*) The condensation product (V) (2·2 g.), 2 : 4 : 5-triamino-6-hydroxypyrimidine sulphate (2·5 g.), and sodium acetate (2·5 g., anhydrous) were powdered together and then heated on the water-bath in alcohol (100 c.c.) for 2 hours. The suspension so obtained was filtered and the residue was washed with water, alcohol, and ether, and dried. The crude brown product (2 g.) was dissolved in 0·02N-sodium hydroxide solution (1 l.), and the solution was filtered after adjustment to pH 7. The filtrate was adjusted to pH 3, and the precipitated product was collected. The processes of dissolution and reprecipitation were repeated twice with the addition of decolorising charcoal (0·3 g.), affording a yellow amorphous product (0·22 g.) (Found, on material dried at 160° in a vacuum : C, 53·2; H, 4·1; N, 27·8. Calc. for $C_{14}H_{12}O_3N_6$: C, 53·8; H, 3·8; N, 26·9%).

The absorption spectrum of the substance in 0·1N-sodium hydroxide solution showed maxima at 255 mμ. ($E^{1\%}_{1\,cm.}$ 840), 275 mμ. ($E^{1\%}_{1\,cm.}$ 755), and 365 mμ. ($E^{1\%}_{1\,cm.}$ 290), in good agreement with the values recorded by Wolf *et al.* (*J. Amer. Chem. Soc.*, 1947, **69**, 2757) and by Waller *et al.* (*ibid.*, 1948, **70**, 20). Cleavage of the substance (80 mg.) in aerated boiling N-sodium hydroxide solution (8 c.c.) for 5 hours afforded an acid (40 mg.), identified by its absorption spectrum and fluorescence characteristics as 2-amino-4-hydroxypteridine-6-carboxylic acid, together with a primary aromatic amine, determined colorimetrically by the Bratton–Marshall method (*J. Biol. Chem.*, 1939, **128**, 537) on an aliquot of the filtrate from the acidified hydrolysis mixture (Found, in terms of *p*-aminobenzoic acid calibration curve : 40 mg. Calc. : 35 mg.).

N^4-(2-*Amino*-4-*hydroxy*-6-*pteridylmethyl*)*sulphanilamide* (XIV).—The condensation product (VIII) (2·5 g.), 2 : 4 : 5-triamino-6-hydroxypyrimidine sulphate (2·5 g.), and sodium acetate (2·5 g.; anhydrous) were powdered together and heated on the water-bath in alcohol (100 c.c.) for 2 hours. The crude product (3·4 g.) was collected, washed with water, alcohol, and ether, and dried. It was dissolved in hot N-aqueous ammonia at a concentration of 1 mg./c.c., and the hot solution was treated with decolorising charcoal (1 mg. per mg. of crude product) and filtered. The filtrate was acidified at the b. p. with glacial acetic acid, and the hot acidified solution was filtered and cooled, affording a brownish-yellow solid. Dissolution and reprecipitation were repeated twice affording ultimately a yellow microcrystalline *compound* (Found, on material dried at 140° in a vacuum : C, 44·3; H, 3·9; N, 29·1. $C_{13}H_{13}O_3N_7S$ requires C, 45·0; H, 3·7; N, 28·3%).

The absorption spectrum in 0·1N-sodium hydroxide solution showed maxima at 259 mμ. ($E^{1\%}_{1\,cm.}$ 975)

and 365 mμ. ($E^{1\%}_{1\,cm.}$ 275). Hydrolysis of the substance (100 mg.) in aerated N-sodium hydroxide (10 c.c.) afforded 2-amino-4-hydroxypteridine-6-carboxylic acid (60 mg.), identified by its absorption spectrum and fluorescence characteristics, together with a primary aromatic amine, determined colorimetrically as before (Found : 51 mg. Calc. : 49·6 mg.).

p-(2-*Amino*-4-*hydroxy*-6-*pteridylmethyl*)*aminophenyl Methyl Sulphone* (XV).—The condensation product (IX) (4 g.), 2 : 4 : 5-triamino-6-hydroxypyrimidine sulphate (4 g.), and sodium acetate (4 g., anhydrous) were powdered together and treated as in the preceding case of the sulphanilamide analogue. The crude brown product (3·9 g.) was purified in the manner described in the preceding experiment (1 g. yielding *ca.* 300 mg. of purified material), a yellow *substance* being obtained (Found, on material dried at 150° in a vacuum : C, 48·5; H, 4·1. $C_{14}H_{14}O_3N_6S$ requires C, 48·6; H, 4·0%).

The absorption spectrum in 0·1N-sodium hydroxide showed a maximum at 259 mμ. ($E^{1\%}_{1\,cm.}$ 770) with a " shoulder " at 270 mμ. ($E^{1\%}_{1\,cm.}$ 735) and a maximum at 365 mμ. ($E^{1\%}_{1\,cm.}$ 275). Hydrolysis of the substance (100 mg.) in aerated alkali in the usual way gave 2-amino-4-hydroxypteridine-6-carboxylic acid (55 mg.), identified by its absorption spectrum and fluorescence characteristics, together with a primary aromatic amine, determined colorimetrically (Found : 51 mg. Calc. : 49·6 mg.).

As an alternative method of purification, crystallisation (charcoal) of the crude reaction product (1 g.) from N-sodium hydroxide (50 c.c.) gave yellow needles (0·2 g.) of the sodium salt. Dissolution in very dilute sodium hydroxide and treatment with excess of acetic acid then afforded the same product as above in the form of a light yellow powder (0·14 g.).

N-p-(2-*Amino*-4-*hydroxy*-6-*pteridylmethyl*)*aminobenzenesulphonylglycine* (XVI).—The condensation product (X) (3 g.), 2 : 4 : 5-triamino-6-hydroxypyrimidine sulphate (2·5 g.), and sodium acetate (2·5 g.) were powdered together and treated in the usual way in alcohol (120 c.c.). After being heated under reflux for 2 hours the suspension was poured into water, the pH was adjusted to 3, and the product (2·4 g.) was collected, washed, and dried. The crude material was purified in the manner described for the sulphanilamide derivative, three treatments affording a light yellow *substance* (Found, after drying at 160° in a vacuum : C, 44·3; H, 4·0; N, 24·0. $C_{15}H_{15}O_5N_7S$ requires C, 44·4; H, 3·7; N, 24·2%).

The absorption spectrum in 0·1N-sodium hydroxide showed maxima at 261 mμ. ($E^{1\%}_{1\,cm.}$ 815) and 365 mμ. ($E^{1\%}_{1\,cm.}$ 235). Cleavage of the substance (75 mg.) in aerated alkali afforded 2-amino-4-hydroxypteridine-6-carboxylic acid (28 mg.), identified by its absorption spectrum and fluorescence characteristics, together with a primary aromatic amine, determined colorimetrically (Found : 27 mg. Calc. : 32 mg.).

N-p-(2-*Amino*-4-*hydroxy*-6-*pteridylmethyl*)*aminobenzenesulphonylglutamic Acid* (XVII).—The condensation product (XII) (8·6 g.), 2 : 4 : 5-triamino-6-hydroxypyrimidine sulphate (7·4 g.), and sodium acetate (7·4 g.; anhydrous) were powdered together and heated in alcohol (400 c.c.) for 2 hours on the water-bath. About half of the alcohol was distilled off and the residue was treated with water (700 c.c.), the crude product (8·4 g.) being then collected, washed, and dried. The entire crude product was dissolved in water (5 l.) containing N-sodium hydroxide (100 c.c.), on the water-bath. The dark brown solution was adjusted carefully to pH 7, cooled, and filtered. The filtrate was then adjusted to pH 3, and the precipitate was collected. It was then dissolved in N-aqueous ammonia (600 c.c.), and the solution was treated with charcoal (2 g.), filtered, heated to the b. p. and then treated with water (3 l.) containing excess of acetic acid. The flocculent precipitate was collected, and the processes of dissolution in ammonia and reprecipitation were repeated once more, affording a pale yellow *substance* (3 g.) (Found, on material dried at 160° in a vacuum : C, 45·3; H, 4·2; N, 20·2. $C_{18}H_{19}O_7N_7S$ requires C, 45·3; H, 4·0; N, 20·5%).

The absorption spectrum in 0·1N-sodium hydroxide showed maxima at 262 mμ. ($E^{1\%}_{1\,cm.}$ 680) and 365 mμ. ($E^{1\%}_{1\,cm.}$ 200). Hydrolysis of the substance (93 mg.) in aerated alkali afforded 2-amino-4-hydroxypteridine-6-carboxylic acid (30 mg.), identified by its absorption spectrum and fluorescence characteristics, together with a primary aromatic amine, determined colorimetrically (Found : 30 mg. Calc. : 33·6 mg.).

N^1-2-*Pyrimidyl*-N^4-(2-*amino*-4-*hydroxy*-6-*pteridylmethyl*)*sulphanilamide* (XVIII).—When the conditions used in the preceding condensations were applied to (XIII), the expected reaction did not take place and the following conditions were therefore employed. The condensation product (XIII) (6·4 g.), 2 : 4 : 5-triamino-6-hydroxypyrimidine sulphate (6·4 g.), and sodium acetate (6·4 g.; anhydrous) were powdered together and heated, with continual stirring under nitrogen, in ethylene glycol (100 c.c.) at 140° for an hour. The dark brown mixture, after cooling, was treated with water, the pH was adjusted to about 3, and the resulting precipitate (8·4 g.) was collected, washed, and dried. The crude product (1·8 g.) was dissolved in hot 2N-aqueous ammonia (900 c.c.) and treated with charcoal (1·8 g.). The filtered solution was heated to the b. p., acidified with acetic acid, filtered hot, and cooled. The solid (0·8 g.), so obtained, was collected and the same process was repeated, affording a light-yellow *substance* (Found, on material dried at 160° in a vacuum : C, 47·2; H, 3·7; N, 29·9. $C_{17}H_{15}O_3N_9S$ requires C, 48·0; H, 3·5; N, 29·7%). The absorption spectrum in 0·1N-sodium hydroxide showed maxima at 259 mμ. ($E^{1\%}_{1\,cm.}$ 870) and 367 mμ. ($E^{1\%}_{1\,cm.}$ 220). Hydrolysis of the substance (92 mg.) in aerated alkali afforded 2-amino-4-hydroxypteridine-6-carboxylic acid (40 mg.), identified in the usual way, together with a primary aromatic amine, determined colorimetrically (Found : 36 mg. Calc. : 37·6 mg.).

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