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1. *Synthesis of 8-Substituted Pteridine Derivatives.*

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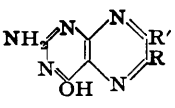
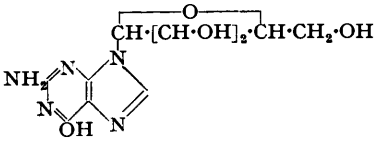
Structural relationships between the natural pteridines, the purine nucleosides, and the flavins suggest that pteridine derivatives bearing a carbohydrate residue attached to $N_{(8)}$ might be expected to occur in Nature. Methods for the synthesis of such compounds have been investigated and the preparation of 5 : 6 : 7 : 8-tetrahydro-6-keto-2 : 4-dimethyl-8-phenylpteridine, 8-ethyl-leucopterin, 4-amino-8-D-glucosyl-5 : 6 : 7 : 8-tetrahydro-6 : 7-diketo-2-methylthiopteridine and some related substances is described.

INTEREST in the derivatives of pteridine has been stimulated in recent years by the discovery that they occur among the insect colouring matters (cf. Gates, *Chem. Reviews*, 1947, **41**, 63) and in the folic acid group of vitamins (cf. Simpson, *Ann. Reports.*, 1946, **43**, 250). A comparison of the naturally occurring pteridine structures (I) with the purine nucleoside guanosine (II) and the *iso*alloxazine riboflavin (III) shows a striking similarity between them in so far as the pyrimidine portion of their respective molecules is concerned. Formally, the guanosine molecule



(I.)



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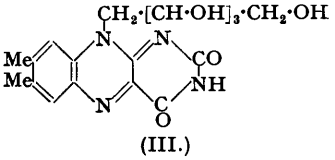
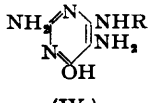


(III.)

might derive from a 2 : 5-diamino-6-hydroxy-4-D-ribosylaminopyrimidine (IV; R = D-ribosyl) by reaction with formic acid, and riboflavin from a closely related substance (IV; R = D-ribityl) by condensation with 3 : 4-dimethyl-*o*-benzoquinone and subsequent deamination. Although there is no evidence that this view has biogenetic significance, the fact that condensation of an intermediate (IV) with an open-chain α-diketone would yield a pteridine derivative bearing a carbohydrate side-chain on $N_{(8)}$, suggested an investigation into the synthesis of compounds of this type. No natural pteridine so far isolated bears a $N_{(8)}$-substituent, but on the above view the natural occurrence of, for example, an 8-*N*-glycoside is at least a possibility (cf. Shive *et al.*, *J. Amer. Chem. Soc.*, 1950, **72**, 2817; Elion *et al.*, *Arch. Biochem.*, 1950, **26**, 337), and in any case it would be of some interest to know whether such a compound would act as a biological antagonist to the purine nucleosides or the flavins.

To this end, investigations were begun on the synthesis of some model compounds in order to explore possible general methods of preparation. We first considered the synthesis of a $N_{(8)}$-phenyl compound starting from the known 5-amino-4-hydroxy-2 : 6-dimethylpyrimidine (V; R = OH, R′ = NH_2) (Andersag and Westphal, *Ber.*, 1937, **70**, 2044) which was prepared



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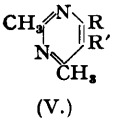

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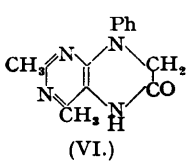

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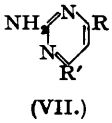
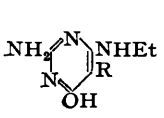


(VII.)

by catalytic, in preference to dithionite, reduction of 4-hydroxy-2 : 6-dimethyl-5-phenylpyrimidine. The above authors converted the aminopyrimidine into the corresponding chloro-

compound by heating it with phosphorus pentachloride and phosphoryl chloride, but the yield was not stated and in our hands the yields obtained were so low (*ca.* 14%) that various modifications were investigated. Chloroacetylation of the aminopyrimidine to give 5-chloroacetamido-4-hydroxy-2 : 6-dimethylpyrimidine (V; R = OH; R′ = NH·CO·CH_2Cl) followed by treatment of this with phosphoryl chloride likewise proved unsatisfactory. Reaction of 4-chloro-5-formamido-2 : 6-dimethylpyrimidine (V; R = Cl; R′ = NH·CHO; Hull, Lovell, Openshaw, and Todd, *J.*, 1947, 41) with aniline, however, followed by hydrolysis, yielded 5-amino-4-anilino-2 : 6-dimethylpyrimidine (V; R = NHPh; R′ = NH_2) which with chloroacetyl chloride was converted into 4-anilino-5-chloroacetamido-2 : 6-dimethylpyrimidine (V; R = NHPh; R′ = NH·CO·CH_2Cl). Cyclisation of this to 5 : 6 : 7 : 8-tetrahydro-6-keto-2 : 4-dimethyl-8-phenylpteridine (VI) was then effected in reasonable yield by using silver carbonate and silver acetate as in Purrmann's classical synthesis (*Annalen*, 1940, **546**, 98).

An 8-alkyl compound would clearly be a better model for an 8-glycoside than an 8-phenyl compound, however, and consequently a satisfactory synthesis of a 4-alkylamino-2-amino-6-hydroxypyrimidine was sought. Reaction of ethylamine with 2-amino-4-chloro-6-methoxypyrimidine (Gabriel and Colman, *Ber.*, 1903, **36**, 3381) caused disubstitution to give 2-amino-4 : 6-bisethylaminopyrimidine (VII; R = R″ = NHEt) and, with 2-amino-4 : 6-dichloropyrimidine under pressure it gave 2-amino-4-chloro-6-ethylaminopyrimidine (VII; R = NHEt; R′ = Cl). The last-mentioned compound was then converted by sodium methoxide into the 6-methoxypyrimidine (VII; R = NHEt; R′ = OMe) but hydrolysis of this to the hydroxy-compound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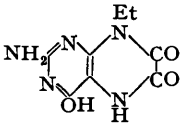
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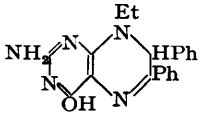
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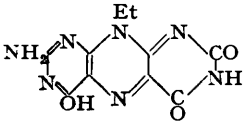
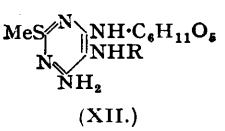

(XI.)

under conditions analogous to those used by Hilbert and Johnson (*J. Amer. Chem. Soc.*, 1930, **52**, 4489) for the hydrolysis of the methoxy-group in 2-keto-6-methoxy-3-tetra-acetylglucosylaminopyrimidine proved troublesome and gave low yields. Finally, 2-amino-4 : 6-dichloropyrimidine was partially hydrolysed with sodium hydroxide to 2-amino-4-chloro-6-hydroxypyrimidine (VII; R = Cl; R′ = OH) and this gave a reasonable yield of 2-amino-4-ethylamino-6-hydroxypyrimidine (VII; R = NHEt; R′ = OH). Nitrosation of this proceeded readily in mineral acid, and reduction of the product (VIII; R = NO) by chemical (dithionite) or catalytic (Pd–$BaSO_4$ in sodium carbonate solution) means gave the amine (VIII; R = NH_2) which was not generally isolated since it was extremely sensitive to atmospheric oxidation. Treatment of the crude amine with chloroacetyl chloride gave 2-amino-5-chloroacetamido-4-ethylamino-6-hydroxypyrimidine (VIII; R = NH·CO·CH_2Cl), but all attempts to cyclise this to a ketopteridine were unsuccessful. However, treatment of (VIII; R = NH_2) with oxalic acid gave 8-ethyl-leucopterin (IX); with benzoin it gave 2-amino-7 : 8-dihydro-8-ethyl-4-hydroxy-6 : 7-diphenylpteridine (X) and, with alloxan, 7-amino-9-ethyl-2 : 3 : 4 : 9-tetrahydro-5-hydroxy-2 : 4-diketo-1 : 3 : 6 : 8 : 9 : 10-hexa-aza-anthracene (a diaza*iso*alloxazine) (XI).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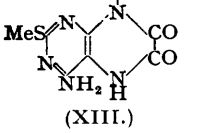
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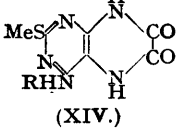
(XIV.)

Attention was next turned to the synthesis of a $N_{(8)}$-glycoside. The above model experiments had been carried out in anticipation of starting from a 2-amino-6-glycosylamino-4-hydroxypyrimidine, but so far the preparation of such a compound has not been found possible (Andrews, Anand, Todd, and Topham, *J.*, 1949, 2490), thus invalidating some of the cyclisation experience gained with the model compounds. However, 5 : 6-diamino-2-methylthio-4-D-tetra-acetylglucosylaminopyrimidine, prepared by a procedure slightly modified from that of Holland, Lythgoe, and Todd (*J.*, 1948, 965), reacted smoothly with chloroacetyl chloride to give 6-amino-5-chloroacetamido-2-methylthio-4-tetra-acetyl-D-glucosylaminopyrimidine (XII; R = CO·CH_2Cl) as its hydrochloride, but this again could not be cyclised to a pteridine. Again (XII; R = H) did not react with benzoin, and with alloxan appeared to give a complex mixture

of products, but in a modified Purrmann synthesis, with ethyl oxalate and sodium methoxide, it gave 4-amino-8-D-glucosylamino-5 : 6 : 7 : 8-tetrahydro-6 : 7-diketo-2-methylthiopteridine (XIII). It is obvious that there is an alternative route for ring formation in this compound, involving the free amino-group in position 6 of the pyrimidine ring to give (XIV), although, by analogy with the cyclisation of 4-glycosylamino-5-thioformamidopyrimidines to 9-glycosylpurines, it was expected that the 8-glycosylpteridine would be at least the major product.

Attempts to determine the structure of the reaction product by nitrous acid treatment were not successful for, in contrast to behaviour of the purines, it has been shown that a free amino-group in position 4 of the pteridine ring is resistant to such treatment (Taylor and Cain, *J. Amer. Chem. Soc.*, 1949, **71**, 2538) and more specifically by us that 4-aminopteridine is unaffected by nitrous acid. Similarly, the ready hydrolysis of the 4-amino-group (*idem, ibid.*) could not be used as a means of identification in this case because of the presence of the acid-sensitive glycosidic link. That the formulation given above is indeed correct was finally shown by periodate titrations. Thus, (XIV; R = glucosyl) would be expected to show an uptake of 5—6 moles of periodate (Howard, Kenner, Lythgoe, and Todd, *J.*, 1946, 861), including 1 mole for the oxidation of the methylthio-group (Howard, Lythgoe, and Todd, *J.*, 1945, 556), whereas (XIII) should take up not more than 3 moles. In fact, when the oxidation was carried out in 50% dimethylformamide at 0°, the compound showed an uptake of 2 moles in 48 hours, followed by a further slow uptake of 1 mole in 10 days, and at room temperature in aqueous suspension with constant shaking there was a fairly rapid (24 hours) uptake of 3 moles, after which oxidation ceased. Model experiments on 4-amino-5 : 6 : 7 : 8-tetrahydro-6 : 7-diketo-2-methylthiopteridine (XIV; R = H), prepared analogously from 4 : 5 : 6-triamino-2-methylthiopyrimidine, were complicated by extreme insolubility, but (XIV; R = H) was unaffected by periodate at 0° and showed a very slow uptake (0·33 mole in 7 days) at room temperature, thus indicating that the ring structure was not a complicating factor in the oxidation.

Experimental.

5-Amino-4-hydroxy-2 : 6-dimethylpyrimidine (V; R = OH; R′ = NH_2).—4-Hydroxy-2 : 6-dimethyl-5-phenylazopyrimidine (20 g.) and palladised barium sulphate (2 g.) were shaken in ethanol (250 c.c.) in the presence of hydrogen at atmospheric pressure. The theoretical quantity of hydrogen was absorbed during 3 hours. Filtration, evaporation of the filtrate under reduced pressure in a nitrogen atmosphere, and removal of aniline from the residue by washing it with acetone gave the product (10·5 g.), m. p. 196—197° (Andersag and Westphal, *loc. cit.*, give m. p. 194°).

5-Chloroacetamido-4-hydroxy-2 : 6-dimethylpyrimidine (V; R = OH; R′ = $NH{\cdot}CO{\cdot}CH_2Cl$).—Chloroacetyl chloride (2·14 c.c.) was added to a stirred solution of 5-amino-4-hydroxy-2 : 6-dimethylpyrimidine (3·57 g.) in acetone containing suspended potassium carbonate (1·57 g.). After 2 hours' stirring, the mixture was set aside overnight, whereafter the solid was collected and dried, and then extracted with methanol. The crude product obtained by evaporation of the extract was purified by vacuum-sublimation followed by recrystallisation from ethanol, giving the *amide* as small colourless needles, m. p. 216—217° (Found : C, 44·3; H, 4·9; N, 19·3. $C_8H_{10}O_2N_3Cl$ requires C, 44·5; H, 4·6; N, 19·4%).

Treatment of this product with phosphoryl chloride gave small amounts of 5-amino-4-chloro-2 : 6-dimethylpyrimidine and 5-amino-4-hydroxy-2 : 6-dimethylpyrimidine.

5-Amino-4-anilino-2 : 6-dimethylpyrimidine (V; R = NHPh; R′ = NH_2).—4-Chloro-5-formamido-2 : 6-dimethylpyrimidine (1·21 g.) and aniline (15 c.c.) were heated under reflux for 4 hours. Excess of aniline was removed by distillation, and the solid residue was dissolved in dilute hydrochloric acid, refluxed for 15 minutes, and then cooled and the solution made alkaline. The precipitated solid was collected, washed with cold water, and recrystallised from benzene from which the *product* separated in needles (1·45 g.), m. p. 192—193° (Found : C, 67·7; H, 6·4; N, 25·8. $C_{12}H_{14}N_4$ requires C, 67·3; H, 6·5; N, 26·2%). The same material was similarly prepared from 5-amino-4-chloro-2 : 6-dimethylpyrimidine.

4-Anilino-5-chloroacetamido-2 : 6-dimethylpyrimidine (V; R = NHPh; R′ = $NH{\cdot}CO{\cdot}CH_2Cl$).—Chloroacetyl chloride (0·285 c.c.) was added to a well stirred solution of 5-amino-4-anilino-2 : 6-dimethylpyrimidine (0·77 g.) in acetone containing suspended potassium carbonate (0·26 g.). Next morning, the solid (0·53 g.) was collected, washed with acetone and then water, and finally recrystallised from carbon tetrachloride, from which the *amide* separated in needles, m. p. 139—140° (Found : C, 57·3; H, 5·2; N, 19·6. $C_{14}H_{15}ON_4Cl$ requires C, 57·8; H, 5·2; N, 19·3%). Further small quantities (0·27 g.) were obtained by treating the acetone mother-liquors with more chloroacetyl chloride.

5 : 6 : 7 : 8-Tetrahydro-6-keto-2 : 4-dimethyl-8-phenylpteridine (VI).—The above chloroacetamido-compound (0·4 g.) was added to a solution of silver acetate (0·14 g.) in boiling water (30 c.c.), followed by silver carbonate (0·3 g.). After 2 hours' refluxing, the black residue was collected by centrifugation and extracted thrice with hydrochloric acid. Addition of excess sodium acetate to the extract precipitated the *pteridine* derivative, which, recrystallised from aqueous ethanol, had m. p. 262—264° (Found : C, 66·0; H, 5·7; N, 22·2. $C_{14}H_{14}ON_4$ requires C, 66·2; H, 5·5; N, 22·1%). Yield, 0·15 g. It was soluble in ethanol, aqueous acetic acid (50%), and aqueous sodium hydroxide, but insoluble in benzene and water.

2-*Amino*-4 : 6-*bisethylaminopyrimidine* (VII; R = R′ = NHEt).—2-Amino-4-chloro-6-methoxypyrimidine (Gabriel and Colman, *loc. cit.*) (2·8 g.) and ethylamine (30 c.c.; 30% w/v) were heated in an autoclave at 150° for 4 hours. Evaporation of the resulting yellow solution under reduced pressure gave an oily residue which was dissolved in dilute hydrochloric acid and precipitated as a solid (2·35 g.), m. p. 125—128°, by addition of excess of sodium hydroxide. The *product* was purified by vacuum-sublimation and recrystallised from benzene, forming feathery needles, m. p. 135·5—136° (Found : C, 53·1; H, 8·4; N, 38·9. $C_8H_{15}N_5$ requires C, 53·0; H, 8·3; N, 38·7%).

2-*Amino*-4-*chloro*-6-*ethylaminopyrimidine* (VII; R = NHEt; R′ = Cl).—2-Amino-4 : 6-dichloropyrimidine (5·0 g.) and ethylamine (8·3 c.c.; 33% w/v) diluted with water (10 c.c.) were heated in an autoclave at 100° for 3 hours. The product was collected from the cooled solution, dissolved in dilute hydrochloric acid, filtered to remove unchanged starting material, and then reprecipitated by addition of sodium hydroxide. Recrystallisation from aqueous ethanol gave prismatic needles of 2-*amino*-4-*chloro*-6-*ethylaminopyrimidine*, m. p. 152·5° (Found : C, 41·7; H, 5·1; N, 32·3. $C_6H_9N_4Cl$ requires C, 41·7; H, 5·2; N, 32·4%).

2-*Amino*-4-*ethylamino*-6-*methoxypyrimidine* (VII; R = NHEt; R′ = OMe).—The above chloro-compound (6·3 g.) in methanol (60 c.c.) was heated with methanolic sodium methoxide (from 1·01 g. of sodium in 15 c.c. of methanol) in an autoclave at 130° for 3 hours. Removal of solvent and crystallisation first from water (charcoal) and then benzene gave the *ether* (5·2 g.) as colourless needles, m. p. 137—138° (Found : C, 49·6; H, 6·9; N, 33·7. $C_7H_{12}ON_4$ requires C, 50·0; H, 7·1; N, 33·3%).

2-*Amino*-4-*ethylamino*-6-*hydroxypyrimidine* (VII; R = NHEt; R′ = OH).—(a) *From* 2-*amino*-4-*ethylamino*-6-*methoxypyrimidine*. Dry hydrogen chloride was passed into a solution of 2-amino-4-ethylamino-6-methoxypyrimidine (0·8 g.) in ethanol (20 c.c.), and the mixture heated under reflux during 3 hours. The syrup produced on evaporation was heated with ethanol (2 c.c.) and the whole set aside overnight. The colourless solid was stirred with excess of cold bicarbonate solution, and the residual solid was recrystallised from ethanol, forming colourless prisms, m. p. 229—230° (Found : C, 46·6; H, 6·1; N, 36·1. $C_6H_{10}ON_4$ requires C, 46·7; H, 6·5; N, 36·4%).

(b) *From* 2-*amino*-4 : 6-*dichloropyrimidine*. 2-Amino-4 : 6-dichloropyrimidine (10 g.) was refluxed with sodium hydroxide (122 c.c. of N.). After 4 hours, 2-*amino*-4-*chloro*-6-*hydroxypyrimidine* (7·9 g.) (VII; R = Cl; R′ = OH) was precipitated with acetic acid; it had m. p. 261° (Found : C, 33·3; H, 2·9; N, 28·6. $C_4H_4ON_3Cl$ requires C, 33·0; H, 2·8; N, 28·8%). This product and ethylamine (22 c.c.; 31% w/w from decomposition of its hydrochloride) were heated in a sealed tube at 120° for 4 hours. The solid obtained on cooling was dissolved in dilute hydrochloric acid (charcoal), reprecipitated with bicarbonate, and finally crystallised from ethanol, to give prisms, m. p. 227—228°, undepressed on admixture with material prepared by method (a) above.

2-*Amino*-4-*ethylamino*-6-*hydroxy*-5-*nitrosopyrimidine* (VIII; R = NO).—A solution of 2-amino-4-ethylamino-6-hydroxypyrimidine (5 g.) in dilute hydrochloric acid (35 c.c. of 3N. + 50 c.c. water) was cooled to 0° and treated with sodium nitrite (3·75 g.) in water (50 c.c.). The solution was set aside overnight at 0°, then neutralised with sodium carbonate, and the precipitated solid (4 g.) collected; a further quantity (1 g.) was obtained by evaporation of the mother-liquors. Recrystallisation from a large volume of water (4 g./l.) gave the *nitroso*-compound in yellow plates, changing to red-violet on storage or more rapidly on heating, m. p. 265—266° (decomp.) (Found, in material dried at 110° : C, 39·5; H, 5·0; N, 38·5. $C_6H_9O_2N_5$ requires C, 39·2; H, 4·9; N, 38·2%).

2 : 5-*Diamino*-4-*ethylamino*-6-*hydroxypyrimidine* (VIII; R = NH_2).—The above nitroso-compound (3·12 g.) was dissolved in water (50 c.c.) containing sodium hydroxide (4·25 g.) and sodium dithionite (11·2 g.) added during 10 minutes at 60—70°, a vigorous stream of nitrogen being passed through the mixture during the operation. The colour of the solution changed to yellow and a yellow solid was deposited. The mixture was cooled and the pH adjusted to 9·0—9·5; the yellow crystalline 6-*hydroxy*-compound (2·12 g.) was collected, washed with water, and dried, and then had m. p. 236—237° (Found : N, 40·5. $C_6H_{11}ON_5$ requires N, 41·4%). Further purification could not be effected, as the compound decomposed rapidly by oxidation during all attempts at recrystallisation.

2-*Amino*-5-*chloroacetamido*-4-*ethylamino*-6-*hydroxypyrimidine* (VIII; R = NH·CO·CH_2Cl).—2 : 5-Diamino-4-ethylamino-6-hydroxypyrimidine (3·4 g.) in water (250 c.c.) containing sodium hydroxide (1·6 g.) was shaken vigorously with a solution of chloroacetyl chloride (3 c.c.) in light petroleum (100 c.c.; b. p. 40—60°) for 15 minutes with cooling. A cream-coloured solid was deposited and this was collected and washed with water. Recrystallisation from water or methanol gave fine white needles (3·8 g.), m. p. 213—214°, of the *chloroacetamide* (Found, in material dried at 100° : C, 38·8; H, 5·0. $C_8H_{12}O_2N_5Cl$ requires C, 39·1; H, 4·9%).

8-*Ethyl*-*leucopterin* (IX).—2 : 5-Diamino-4-ethylamino-6-hydroxypyrimidine (1·5 g.; freshly prepared) was ground intimately with oxalic acid (8·9 g.), and the mixture (under slight vacuum) was heated in an oil-bath. At 120—140°, the mass fused and water was evolved; when this evolution ceased (30 minutes), the temperature was raised to 260° for 15 minutes. The cooled mass was then extracted with warm water (150 c.c.), and the residual solid dissolved in sodium hydroxide solution (150 c.c. of 0·1N.). The solution was boiled with charcoal and filtered into a boiling solution of dilute hydrochloric acid (150 c.c. of 0·1N.). On cooling, a brownish-yellow microcrystalline powder was deposited. Repetition of this procedure gave 8-*ethyl*-*leucopterin* as a yellow-brown microcrystalline solid (Found, in material dried at 140° : C, 43·5; H, 4·0; N, 30·9. $C_8H_9O_3N_5$ requires C, 43·1; H, 4·0; N, 31·5%).

2-*Amino*-8-*ethyl*-7 : 8-*dihydro*-4-*hydroxy*-6 : 7-*diphenylpteridine* (X).—The above freshly prepared diamino-compound (0·5 g.) and benzoin (0·8 g.) were refluxed in a mixture of ethanol (5 c.c.) and acetic acid (3 c.c.) for 2 hours. Cooling caused the deposition of yellow crystals (0·7 g.) which were collected; the mother-liquors, poured into water, gave a further quantity of yellow solid (0·4 g.) which was combined with the above crystals. The crude product was purified by dissolution in hot sodium hydroxide

solution and filtration into excess of dilute acetic acid. The *diphenylpteridine* was obtained as yellow needles on recrystallisation from aqueous pyridine (30%) (Found : C, 69·5; H, 5·5; N, 20·3. $C_{20}H_{19}ON_5$ requires C, 69·6; H, 5·5; N, 20·3%). It was insoluble in water but slightly soluble in ethanol or acetic acid.

7-Amino-9-ethyl-2 : 3 : 4 : 9-tetrahydro-5-hydroxy-2 : 4-diketo-1 : 3 : 6 : 8 : 9 : 10-hexa-aza-anthracene (XI).—The nitroso-compound (0·5 g.) was hydrogenated in sodium carbonate solution (10 c.c.; saturated) with palladised barium sulphate and, after filtration and evaporation to dryness *in vacuo*, the residue was dissolved in a mixture of glacial acetic acid (5 c.c.) and ethanol (10 c.c.); alloxan (0·5 g.) was added and the whole was refluxed for 2 hours. The solution was then evaporated to dryness and the residue triturated with water and collected (0·8 g.). It was then dissolved in boiling sodium hydroxide (10 c.c. of 2N.), and the solution filtered and then acidified to pH 5 with acetic acid. The yellow microcrystalline *product* was collected and recrystallised from aqueous pyridine or water, from which it tended to separate in semi-colloidal form (Found, in material dried at 150°/0·1 mm. : C, 44·3; H, 3·3; N, 34·9. $C_{10}H_9O_3N_7$ requires C, 43·6; H, 3·3; N, 35·6%).

6-Amino-2-methylthio-5-nitroso-4-tetra-acetyl-D-glucosidaminopyrimidine.—6-Amino-4-D-glucosylamino-2-methylthio-5-nitrosopyrimidine (2·58 g.) (Holland, Lythgoe, and Todd, *loc. cit.*) was kept at room temperature for 36 hours with acetic anhydride (5·4 c.c.) in pyridine (50 c.c.). Ethanol (25 c.c.) was then added (temperature, <10°) and after 2 hours the solution was evaporated to dryness under reduced pressure. Ice-water was added and the green product (3·45 g.) was collected and washed with water. It was purified by passing its solution in ethyl acetate through a column of neutral alumina. Evaporation of the main eluate gave the *nitroso*-compound as blue needles, m. p. 154° (Found : C, 44·1; H, 4·8; N, 13·9. $C_{19}H_{25}O_{10}N_5S$ requires C, 44·3; H, 4·8; N, 13·6%).

5 : 6-Diamino-2-methylthio-4-tetra-acetyl-D-glucosylaminopyrimidine.—Zinc dust (10 g.) was added to a solution of the above nitroso-compound (5 g.) in boiling ethanol (150 c.c.) containing glacial acetic acid (5 c.c.) and heating was continued for 10 minutes. Filtered and cooled, the solution deposited a yellowish solid (3·7 g.) which was collected and washed with ice-water to remove inorganic material. Two recrystallisations from ethanol gave the diamine as pale yellow needles, m. p. 214° (Found, in material dried at 110° : C, 45·4; H, 5·5; N, 13·9. Calc. for $C_{19}H_{27}O_9N_5S$: C, 45·5; H, 5·4; N, 14·0%).

6-Amino-5-chloroacetamido-2-methylthio-4-tetra-acetyl-D-glucosylaminopyrimidine Hydrochloride (XII; R = $CO{\cdot}CH_2Cl$).—Chloroacetyl chloride (1·7 g.) was added to a solution of the above compound (5 g.) in dry chloroform (200 c.c.). After 8 hours, most of the chloroform was removed *in vacuo* and ether (5 vols.) was added. The precipitated solid (6 g.) was collected and recrystallised from ethyl acetate from which the chloroacetyl derivative *hydrochloride* separated as a white microcrystalline powder, m. p. 163—164° (decomp.) (Found, in material dried at 100° : C, 41·6; H, 4·7; N, 11·4. $C_{21}H_{28}O_{10}N_5SCl,HCl$ requires C, 41·0; H, 4·8; N, 11·4%). Dissolution in water followed by rapid neutralisation with sodium hydrogen carbonate solution yielded the free base as fine white needles, m. p. 138—139°.

4-Amino-8-D-glucosylamino-5 : 6 : 7 : 8-tetrahydro-6 : 7-diketo-2-methylthiopteridine (XIII).—Freshly prepared 5 : 6-diamino-2-methylthio-4-tetra-acetyl-D-glucosidaminopyrimidine (0·5 g.) was dissolved in boiling ethanol (25 c.c.); ethyl oxalate (1 g.) and sodium methoxide (0·56 g.) were added. There was an immediate reaction and a yellow granular solid was deposited. After 1½ hours on the water-bath, this solid was collected, washed with ethanol, and dried. It was dissolved in cold water, and the pH of the solution adjusted to 6 with acetic acid. On cooling, yellow needles (0·25 g.) were deposited. Recrystallisation from water gave the *product* as pale yellow needles, m. p. 230—240° (decomp.) (Found, in material dried for 2 days at 140°/10^{-3} mm. : C, 39·0; H, 5·1; N, 16·9. $C_{13}H_{17}O_7N_5S,H_2O$ requires C, 38·5; H, 4·7; N, 17·3%).

The product gave a strongly positive Molisch reaction and in alkaline solution it exhibited an intensely blue fluorescence in ultra-violet light.

4-Amino-5 : 6 : 7 : 8-tetrahydro-6 : 7-diketo-2-methylthiopteridine (XV).—A solution of ethyl oxalate (0·73 g.) in ethanol (15 c.c.) containing sodium ethoxide (from 0·46 g. of sodium) was heated to boiling and then added to a saturated solution of 4 : 5 : 6-triamino-2-methylthiopyrimidine (1·9 g.) in alcohol (35 c.c.), causing immediate deposition of a yellow solid. After 16 hours at room temperature this solid (2 g.) was collected and purified by dissolution in aqueous ammonia, treatment with charcoal, and reprecipitation with dilute hydrochloric acid. The *product* finally separated from dimethylformamide in pale yellow needles (Found, in material dried at 120° : C, 37·5; H, 3·3; N, 30·8. $C_7H_7O_2N_5S$ requires C, 37·3; H, 3·1; N, 31·1%). Alkaline solutions exhibited an intense blue fluorescence in ultra-violet light.

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