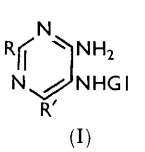
846. *The Synthesis of 7-Glycosylpurines. Part II.* Attempted Syntheses from Pyrimidines.*

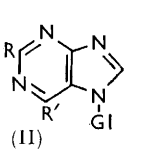
By G. M. Blackburn and A. W. Johnson.

Sugars can be condensed with the 5-amino-group of 4,5-diaminopyrimidines to form the corresponding 5-glycosylamino-derivatives. Acylation of the 5-glycosylaminopyrimidines is difficult because of their low solubility and the lability of the glycoside linkage, properties which have so far prevented cyclisation of the free glycosides. Use of certain *aldehydo*-forms of the sugars in condensations with 4,5-diaminopyrimidines tends to yield pteridines.

Syntheses directed towards the 7-glycosylpurines were described in the preceding paper in which acylhalogeno-sugars were condensed with mercury-derivatives of preformed purines. An alternative approach is now described in which the preparation of 4-amino-5-glycosylaminopyrimidines (I; Gl = glycosyl) is investigated, and from these compounds the 7-glycosylpurines (II) should be obtainable by cyclisation involving incorporation of an extra carbon atom.



(I)



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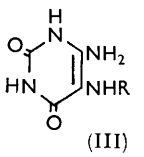


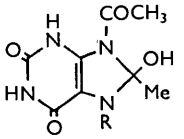
(III)

Many preparations have been described of the 5-amino-4-glycosylaminopyrimidines,[1] which are intermediates for the 9-glycosylpurines, and in consequence it is well established

* Part I, preceding paper.

[1] Kenner, *Fortschr. Chem. org. Naturstoffe*, 1951, **8**, 96; Baddiley, " Nucleic Acids," ed. Chargaff and Davidson, Academic Press, New York, 1955, Vol. I, 137.

that condensation of a free sugar or a suitably protected sugar derivative with a 4,5-diaminopyrimidine will lead to the formation of the 5-glycosylaminopyrimidine (I). This is attributed to the greater electron density at position 5 than at position 4 of the pyrimidine ring. The first approach employed was an extension of the work of Thannhauser and Dorfmüller [2] who condensed glucose with 4,5-diaminouracil in aqueous solution to obtain the 5-glucosyl derivative (III; R = glucosyl) in a yield of about 50%. We have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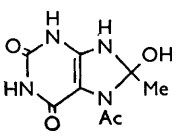
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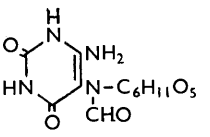
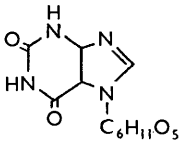

(VI)

found that this figure can be appreciably increased if condensation is effected in an inert atmosphere. When D-ribose was condensed with this pyrimidine, a crystalline ribosyl derivative (III; R = ribosyl) was obtained although in much lower yield. Both glycosyl derivatives with acetic anhydride in the presence of perchloric acid gave products which appeared to possess a fully acetylated pyranose sugar residue and two further acetyl groups on the chromophore. These have been assigned the structure (IV; R = glycosyl) by analogy with " diaminouracil diacetate " [3] (V). Milder acetylation did not lead to crystalline products, and this failure suggested that a pyrimidine derivative with acetylated sugar hydroxyl groups, but with free nuclear amino-groups to allow cyclisation to the purine, could not be obtained by this route. Any method of purine cyclisation applied to the acetyl compounds (IV) would clearly have to involve a preliminary deacetylation. The action of thiourea in hot dimethylformamide on (IV; R = tetra-acetylglucosyl) resulted in complete charring of the glucoside and was abandoned. Heat also failed to cause cyclisation. Better results were obtained with formic acid in dry formamide,[4] and the hexa-acetylglucosyl derivative (IV; R = tetra-acetylglucosyl) gave a deacetylated monoformyl derivative (VI). That this was not a hydrate of glucosylxanthine (VII) was shown by a comparison of its absorption spectrum ($\lambda_{max.}$ 264 mμ, log ε 4·08) with those of related compounds (glucosyl-4,5-diaminouracil, $\lambda_{max.}$ 272 mμ, log ε 4·08; 4-amino-5-formamidouracil,[5] $\lambda_{max.}$ 264 mμ, log ε 4·19) and those of 7-substituted xanthines (7-methylxanthine,[6] $\lambda_{max.}$ 272 mμ, log ε 3·92; xanthosine,[6] $\lambda_{max.}$ 277 mμ, log ε 3·92). Although the detection of the formyl group in compound (VI) by hydrolysis, reduction to formaldehyde, and reaction with chromotropic acid [7] was found to be subject to interference from glucose, the glucose was detected in the hydrolysate by chromatography on paper. Moreover, the absorption maximum at 264 mμ shown by the hydrolysate agreed with diaminouracil rather than xanthine. In view of the lack of success with the glucoside derivative (IV; R = tetra-acetylglucosyl), similar experiments on the less accessible ribose were not performed.

In spite of the successful condensation of sugars with 4,5-diaminouracil in aqueous solution, ethanol was generally preferred as a solvent. It was found that the pyrimidine bases were insufficiently soluble in neutral aqueous solutions; in acid solutions, Amadori rearrangements were favoured and in basic solutions the sugars tended to be charred. Reaction of ethanolic solutions of 4,5,6-triaminopyrimidine [8] or 4,5-diamino-6-hydroxypyrimidine with sugars failed to yield crystalline glycosides but the introduction of

[2] Thannhauser and Dorfmüller, *Ber.*, 1914, **47**, 1304.
[3] Bredereck, Hennig, and Pfleiderer, *Chem. Ber.*, 1953, **86**, 321.
[4] Bendich, Tinker, and Brown, *J. Amer. Chem. Soc.*, 1948, **70**, 3109.
[5] Cavalieri and Bendich, *J. Amer. Chem. Soc.*, 1950, **72**, 2587.
[6] Cavalieri, Fox, Stone, and Chang, *J. Amer. Chem. Soc.*, 1954, **76**, 1119.
[7] Eegriwe, *Z. analyt. Chem.*, 1937, **110**, 22.
[8] Baddiley, Lythgoe, McNeil, and Todd, *J.*, 1943, 383.

2-methylthio-groups into the pyrimidine ring facilitated the reaction. 4,5,6-Triamino-2-methylthiopyrimidine [8] condensed with both D-ribose and D-xylose in hot ethanol in the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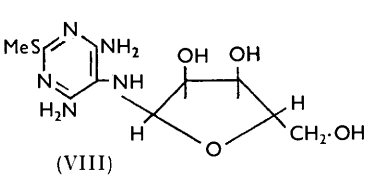
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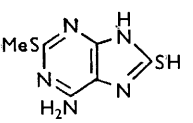

(IX)

presence of ammonium chloride. The reactions were 4—5 times faster in the ribose than in the xylose series, which suggests that the sugar reacts in the aldehyde form, ribose solutions being known [9] to contain a higher proportion of the open-chain sugar. A ribosyl derivative was also obtained in poor yield from 4,5-diamino-6-hydroxy-2-methylthiopyrimidine.[10]

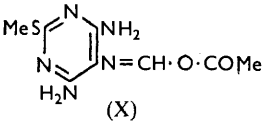
The lability of the sugar residue was shown by the hydrolysis of the ribosyl derivative (VIII) in warm aqueous picric acid, the pyrimidine picrate being isolated and characterised. This ease of hydrolysis of the 5-glycosylaminopyrimidines probably explains their non-formation in aqueous solution unless pteridine formation displaces the equilibrium by removal of the sugar residue. The specific rotation values of the ribosyl derivative in water (−6°) and in pyridine (0°) favoured an α-furanose structure (VIII) as 2-methylthio-7-α-D-ribofuranosyladenine also has zero optical rotation.[11] Berger and Lee [12] found that ribosylamines prepared in hot ethanol have the α-furanose structure. The ribosylamine (VIII) with benzoyl chloride in dry pyridine gave an amorphous tetrabenzoate, which probably contained a ring *N*-benzoyl group because the light absorption was modified from that of the parent. On hydrolysis, the benzoate gave 2,3,4-tri-*O*-benzoyl-D-ribopyranose, which is not incompatible with the furanose structure for the ribosylamine, as 2,3,4-tri-*O*-acetyl-D-ribopyranose has been obtained [13] on hydrolysis of the pyridine acetylation product of *N*-D-ribofuranosylaniline. Attempted acetylation of the ribosylamine (VIII) or the xylose analogue with acetic anhydride under various conditions did not lead to crystalline or even homogeneous products. In one case, an attempted cyclisation with sodium dithioformate led to the 5-thioformamidopyrimidine.

In view of the failure to acetylate the 5-glycosylaminopyrimidines without blocking the nuclear amino-groups, as had been achieved [14] for the 4-glycosylaminopyrimidines, it was subsequently proposed to protect the 4- and 6-amino-groups in compound (VIII) by benzylation until the acetylation of the sugar residue had been achieved. 4,6-Dichloro-2-methylpyrimidine [8] with benzylamine gave 4,6-di-(*N*-benzylamino)-2-methylpyrimidine which proved too insoluble in cold solvents to permit the introduction of the 5-amino-group through the 5-nitroso-compound. Attempted nitration of the di-(*N*-benzylamino)-pyrimidine with acetic acid and nitric acid gave a nitrate rather than a nitro-compound, as catalytic hydrogenation merely gave the parent di(benzylamino)methylpyrimidine.

Hull [15] has cyclised 4,5-diaminopyrimidines with sugar lactones without protecting the sugar hydroxyl groups and accordingly attempts were made to cyclise the ribosylamine (VIII) without prior acylation. Sodium dithioformate, either in water or in phosphate buffer at pH 7·5, gave a high yield of 4,6-diamino-2-methylthio-5-thioformamidopyrimidine, a reaction which involves hydrolysis of the ribose residue. Carbon disulphide in anhydrous pyridine also removed the sugar and gave 8-mercapto-2-methylthioadenine (IX).

[9] Cantor and Peniston, *J. Amer. Chem. Soc.*, 1940, **62**, 2113.
[10] Johns and Baumann, *J. Biol. Chem.*, 1913, **14**, 385.
[11] Friedrich and Bernhauer, *Chem. Ber.*, 1956, **89**, 2507.
[12] Berger and Lee, *J. Org. Chem.*, 1946, **11**, 75.
[13] Howard, Kenner, Lythgoe, and Todd, *J.*, 1946, 855.
[14] Howard, Lythgoe, and Todd, *J.*, 1945, 556.
[15] Hull, *J.*, 1958, 4069.

Diethoxymethyl acetate is known [16] to convert 4,5-diaminopyrimidines into purines under mild conditions. In the present case, the ribosylamine (VIII) gave a small yield of a colourless crystalline compound which was formulated as 5-acetoxymethylideneamino-4,6-diamino-2-methylthiopyrimidine (X) on the basis of analysis and its cyclisation by heat to 2-methylthioadenine with liberation of acetic acid.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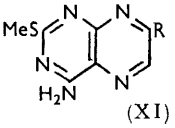
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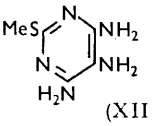
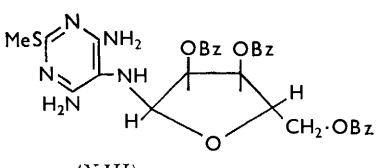

(XII)

Reaction of ethyl orthoformate with the ribosylamine (VIII) in glacial acetic acid gave an orange solid, the analysis and properties of which suggested that it was the hemihydrate of 4-amino-2-methylthio-7-trihydroxypropylpteridine {XI; R = ·[CH(OH)]$_2$·CH$_2$·OH}. The light absorption resembled that of the parent 4-amino-2-methylthiopteridine (XI; R = H), prepared from the triamine (XII) and glyoxal. Other conditions for the cyclisation of the ribosylamine (VIII), *e.g.*, use of alkylformimidoyl ester hydrochlorides, formic acid, and thiourea, were either considered unsuitable in view of the lability of the sugar or gave rise to extensive decomposition.

Attention was then turned to the condensation of *aldehydo*-sugars with 5-aminopyrimidines and in particular with the triamine (XII), as cyclisation of the corresponding 5-ribosylamino-derivative would give 2-methylthio-7-D-ribofuranosyladenine [17] and then 7-D-ribofuranosyladenine [11] itself by desulphurisation. 5-*O*-Trityl-D-ribose has been used in a related condensation [18] in a synthesis of α-ribazole, but attempts to condense it with 4,5,6-triaminopyrimidine in hot ethanol led to the products which, although not fully purified, had the characteristic spectra of pteridines. The use of 5-*O*-benzyl-D-ribose instead of the trityl compound gave similar results. Milder experimental conditions were precluded because of the insolubility of the pyrimidine. Although it was hoped that acylation of the 2-hydroxyl group of the sugar might prevent pteridine formation, condensation of the triamine (XII) with 2,3,4-tri-*O*-acetyl-5-*O*-trityl-D-ribose in hot ethanol again gave a yellow amorphous solid with the properties of a pteridine. Thus the condensation of *aldehydo*-sugars with 4,5-diaminopyrimidines gave pteridines and later attempts at the condensation were therefore carried out with derivatives of the less reactive cyclic forms of the sugar. 2,3,5-Tri-*O*-benzoyl-D-ribofuranose was prepared from 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribose [19] and condensed with the pyrimidine (XII) at room temperature. The product, although amorphous, was shown to be 4,6-diamino-2-methylthio-5-(2,3,5-tri-*O*-benzoyl-D-ribofuranosylamino)pyrimidine (XIII) on the basis of analysis, light absorption, and hydrolysis to the pyrimidine (XII) and 2,3,5-tri-*O*-benzoyl-D-ribose.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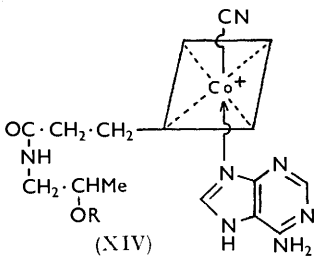
(XIV)

An attempted cyclisation of the tribenzoylribosylamine (XIII) with formic acid in formamide gave only a small quantity of 2-methylthioadenine, but the 7-ribosyl derivative

[16] Montgomery and Holum, *J. Amer. Chem. Soc.*, 1958, **80**, 404.
[17] Friedrich and Bernhauer, *Chem. Ber.*, 1957, **90**, 1966.
[18] Brink, Holly, Shunk, Peel, Cahill, and Folkers, *J. Amer. Chem. Soc.*, 1950, **72**, 1866.
[19] Kissman, Pidacks, and Baker, *J. Amer. Chem. Soc.*, 1955, **77**, 18.

could not be detected. On the other hand, reaction with ethyl orthoformate and acetic anhydride gave 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribose but the chromophore, obtained as a pink non-crystalline powder, could not be purified.

The greater difficulty of synthesising 7-glycosylpurines than of the 9-analogues is relevant when considering the biogenesis of the nucleotide portion of pseudovitamin B_{12}. The structures of Factor B phosphate [20] (Factor C[21]?) (XIV; R = PO_3H^-) and Factor B phosphoribose [22] [XIV; R = $-\overset{\overset{O^-}{|}}{\underset{\underset{O}{\|}}{P}}-$(3-ribosyl)], which have been isolated from micro-biological sources, lead progressively from Factor B (XIV; R = H) to a hydrated form of pseudovitamin B_{12} lacking the glycosidic linkage if adenine is co-ordinated to the metal.[23,24] Thus formation of the 7-α-ribofuranosyl linkage, which probably involves the substitution at $C_{(1)}$ of phosphate or pyrophosphate by the base, is probably a late stage in the biogenesis and is a consequence of the fixed positions of the sugar and base.

Experimental

4-Amino-5-D-glucosylamino-2,6-dihydroxypyrimidine (III; R = *glucosyl*).—This was prepared by the method of Thannhauser and Dorfmüller,[2] but in improved yield (78% based on the pyrimidine) when the reaction mixture was evaporated in a stream of coal gas to prevent oxidation. The crystalline monohydrate (from 30% aqueous glucose solution) had m. p. 209° (decomp.) (Found: C, 37·1; H, 5·9; loss at 110°, 5·8. Calc. for $C_{10}H_{16}O_7N_4,H_2O$: C, 37·3; H, 5·6; loss, 5·6%). The anhydrous glucoside, obtained at 110° *in vacuo* (Found: N, 18·6. Calc. for $C_{10}H_{16}O_7N_4$: N, 18·4%), had $\lambda_{max.}$ 274 mμ (log ε 4·09) in H_2O.

4-Amino-2,6-dihydroxy-5-D-ribosylaminopyrimidine (III; R = *ribosyl*).—4,5-Diaminouracil sulphate [25] (2 g.) in water (150 c.c.) at 100° was neutralised with dilute aqueous sodium hydroxide to pH 8 and D-ribose (5 g.) was added. The solution was filtered, evaporated under reduced pressure to 15 c.c., and after ½ hr. at 80° was set aside at 0°. The *ribosylamine* (2 g.) crystallised in yellow prisms and was collected, washed with ice-water and then methanol, and dried; it had m. p. 195° (decomp.) (Found: C, 39·1; H, 5·25; N, 20·2. $C_9H_{14}O_6N_4$ requires C, 39·4; H, 5·15; N, 20·4%), $\lambda_{max.}$ 279 mμ (log ε 3·99) in H_2O.

9-Acetyl-8,9-dihydro-2,6,8-trihydroxy-8-methyl-7-D-tetra-acetylglucosylpurine (IV; R = *tetra-acetylglucosyl*).—4-Amino-5-D-glucosidamino-2,6-dihydroxypyrimidine (4 g.) was warmed with acetic anhydride (70 c.c.) containing perchloric acid (2 drops) until completely dissolved and set aside for 3 days at 20°. The pale yellow solution was evaporated at 80° under reduced pressure to 10 c.c. and ethanol (20 c.c.) added. After ½ hr. the solution was evaporated to dryness *in vacuo* and the residue crystallised from ethanol (100 c.c.). The *acetate* was obtained as prisms (4·7 g.), m. p. 249°, $[\alpha]_D^{21}$ +10° (*c* 1·1 in EtOH) (Found: C, 47·8; H, 5·1; N, 10·0; *O*-Ac, 47·3. $C_{22}H_{28}O_{13}N_4$ requires C, 47·5; H, 5·05; N, 10·1; 6 *O*-Ac, 46·4%).

9-Acetyl-8,9-dihydro-2,6,8-trihydroxy-8-methyl-7-D-tri-O-acetylribosylpurine (IV; R = *tri-acetylribosyl*).—4-Amino-2,6-dihydroxy-5-D-ribosylaminopyrimidine (2 g.) in acetic anhydride (30 c.c.) containing perchloric acid (1 drop) was warmed. Only partial solution was effected and considerable charring occurred. After ½ hr. the mixture was filtered and ethanol (100 c.c.) added to the filtrate which was set aside for ½ hr. and evaporated (to 5 c.c.) under reduced pressure. The *acetate* separated and was crystallised from ethanol–chloroform as crystals (0·25 g.), m. p. 270° (decomp.) (Found: C, 46·8; H, 4·65; N, 11·6. $C_{19}H_{24}O_{11}N_4$ requires C, 47·1; H, 5·0; N, 11·6%).

4-Amino-5-(N-formyl-N-glucosylamino)-2,6-dihydroxypyrimidine (VI).—9-Acetyl-8,9-dihydro-2,6,8-trihydroxy-8-methyl-7-D-tetra-acetylglucosylpurine (above; 5 g.), formic acid

[20] Barchielli, Boretti, di Marco, Julita, Migliacci, Minghetti, and Spalla, *Biochem. J.*, 1960, **74**, 382.
[21] Ford, Holdsworth, Kon, and Porter, *Nature*, 1953, **171**, 148; Porter, *Proc. Nutrition Soc.*, 1953, **12**, 106.
[22] Dellweg and Bernhauer, *Arch. Biochem. Biophys.*, 1957, **69**, 74.
[23] Bernhauer, Becher, and Wilharm, *Arch. Biochem. Biophys.*, 1959, **83**, 248.
[24] Johnson and Kay, *J.*, 1960, 2979.
[25] Traube, *Ber.*, 1900, **33**, 1371.

(0·7 g.), and dry formamide (40 c.c.) were heated in a sealed tube at 160° during 3 hr. The cooled solution was filtered and evaporated to dryness *in vacuo* at 80°. The solid residue was extracted with hot methanol (20 c.c.) and the solution decolorised (charcoal) and cooled. A white deposit was filtered off and the filtrate diluted with acetone (20 c.c.) and set aside at 0°. The white semicrystalline precipitate was removed, washed with acetone, and crystallised from a little methanol. The *formamido-compound* (0·6 g.) had m. p. 226° (effervescence), solidifying and remelting at about 250° (Found: C, 40·1; H, 5·1; N, 16·5. $C_{11}H_{16}O_8N_4$ requires C, 39·8; H, 4·85; N, 16·85%), $\lambda_{max.}$ 264 mμ (log ε 4·08) in H_2O.

This derivative (10 mg.) was heated in water (1 c.c.) containing concentrated hydrochloric acid (0·1 c.c.) at 90° during 3 hr. Paper chromatography of the hydrolysate on Whatman No. 1 paper in butanol–acetic acid–water (4 : 1 : 5) and development with aniline phthalate [26] gave a brown spot at R_F 0·07, and in phenol–water one at 0·37, both identical with those of D-glucose controls.

The hydrolysate was diluted to 10 c.c. with water and basified to pH 9 with alkali. The ultraviolet absorption was a maximum at 264 mμ (xanthine, 240 and 277 mμ at pH 9).

4-*Amino*-6-*hydroxy*-5-*xylosylaminopyrimidine*.—A suspension of 4,5-diamino-6-hydroxypyrimidine (0·3 g.) and D-xylose (0·3 g.) in absolute ethanol (20 c.c.) was heated under reflux until complete dissolution was obtained. The solution was filtered, evaporated to 10 c.c., and cooled. The pale yellow amorphous *product* (0·15 g.) separated and was collected. It had m. p. 164—166° (decomp.) (Found: C, 40·8; H, 5·95. $C_9H_{14}O_5N_4,\frac{1}{2}H_2O$ requires C, 40·5; H, 5·65%), $\lambda_{max.}$ 214 and 276 mμ (log ε 4·23 and 3·93) in EtOH.

4,6-*Diamino*-2-*methylthio*-5-D-*ribosylaminopyrimidine* (VIII).—4,5,6-Triamino-2-methylthiopyrimidine [8] (1·3 g.) and D-ribose (1·2 g.) were heated under reflux in absolute ethanol (20 c.c.) containing a trace of ammonium chloride as catalyst. After 10 min. a precipitate began to be formed and after a further 10 min. the mixture was cooled and filtered. The *ribosylamine* (2 g.) crystallised from methanol; it then had m. p. 183° (corr.), $[\alpha]_D^{22}$ −5·8° ± 1° (*c* 2 in H_2O), 0° ± 1° (*c* 1 in pyridine) (Found: C, 39·4; H, 5·4; S, 10·3. $C_{10}H_{17}O_4N_5S$ requires C, 39·6; H, 5·65; S, 10·55%), $\lambda_{max.}$ 217 and 275 mμ (log ε 4·43 and 3·92) in H_2O.

A solution of the riboside in warm water was treated with one equivalent of picric acid. 4,5,6-*Triamino*-2-*methylthiopyrimidine picrate* separated on cooling and crystallised from water as orange prisms, m. p. 209—210° (corr.) (Found: C, 33·2; H, 2·9; N, 27·6. $C_{11}H_{12}O_7N_8S$ requires C, 33·0; H, 3·0; N, 27·8%).

4,6-*Diamino*-2-*methylthio*-5-D-*xylosylaminopyrimidine*.—By the same procedure, D-xylose (1 g.) and the triamine (1·1 g.) gave a crystalline precipitate after being heated under reflux during 40 min. in ethanol. The *xylosylamine* (1·5 g.) was obtained as prisms, m. p. 204° (decomp.), from absolute methanol (Found: C, 39·8; H, 6·4; S, 10·5%), $\lambda_{max.}$ 216 and 274 mμ (log ε 4·26 and 3·81) in H_2O.

4,6-*Diamino*-2-*methylthio*-5-D-*ribosylaminopyrimidine Tetrabenzoyl Derivative*.—The above ribosylamine (0·6 g.) in dry pyridine (40 c.c.) was treated with benzoyl chloride (1·4 c.c.) at 0° and set aside at 22°. After 3 days the solution was reduced to 5 c.c. and mixed with water (50 c.c.); the product solidified and was dissolved in hot benzene (15 c.c.) which was diluted with light petroleum until turbid and then cooled. The solution was later decanted from a red oil and diluted with light petroleum, to give a pale yellow solid. This *derivative* was purified by repeated chromatography in benzene on acid-washed alumina and then precipitated from benzene with light petroleum as an amorphous cream-coloured solid (1·1 g.) (Found: C, 63·5; H, 4·95. $C_{38}H_{33}O_8N_5S$ requires C, 63·4; H, 4·6%), $\lambda_{max.}$ 232 and 252 mμ (log ε 4·72 and 4·52) in ethyl acetate.

The tetrabenzoylribosylamine (0·3 g.) was hydrolysed by heating it in acetone (20 c.c.), water (10 c.c.), and concentrated hydrochloric acid (2 c.c.) for 5 hr. The acetone was then distilled off and the aqueous solution extracted with chloroform (2 × 15 c.c.). The combined extracts were washed successively with dilute hydrochloric acid, sodium hydrogen carbonate solution, and water, dried ($MgSO_4$), and evaporated *in vacuo*. The residue crystallised from aqueous ethanol as needles (80 mg.), m. p. 135—137°, $[\alpha]_D^{23}$ −39° ± 2° (*c* 1 in $CHCl_3$). 2,3,4-Tri-*O*-benzoyl-D-ribopyranose [27] has m. p. 135—137°, $[\alpha]_D$ −42·2° (*c* 1·4 in $CHCl_3$).

4-*Amino*-6-*hydroxy*-2-*methylthio*-5-D-*ribosylaminopyrimidine*.—A solution of D-ribose (1·0 g.) and 4,5-diamino-6-hydroxy-2-methylthiopyrimidine (1·1 g.) in absolute ethanol (20 c.c.) was

[26] Partridge, *Nature*, 1949, **164**, 443.
[27] Fletcher and Ness, *J. Amer. Chem. Soc.*, 1954, **76**, 760.

heated under reflux during 4 hr., evaporated to half-volume, and cooled. The *ribosylamine* separated as a finely divided solid, was removed, and recrystallised from aqueous acetone as plates (150 mg.), m. p. 175—176° (Found: C, 39·3; H, 5·3; N, 18·4. $C_{10}H_{16}O_5N_4S$ requires C, 39·45; H, 5·3; N, 18·4%), $\lambda_{max.}$ 235 and 281 mμ (log ε 4·04 and 3·91) in 0·01N-HCl, 210, 269, and 349 mμ (log ε 4·16, 4·08, and 3·64) in 0·01N-NaOH.

4,6-*Di*(*benzylamino*)-2-*methylpyrimidine*.—4,6-Dichloro-2-methylpyrimidine [8] (2 g.) and benzylamine (6·6 c.c.) were heated in a sealed tube at 200° during 3 hr. After cooling, the crystalline mixture was extracted twice with hot water and recrystallised from ethanol. The *product* (1·9 g.) had m. p. 191·5° (Found: C, 74·8; H, 6·25; N, 18·8. $C_{19}H_{20}N_4$ requires C, 75·0; H, 6·6; N, 18·4%).

This pyrimidine (2 g.) was added in portions to a stirred mixture of glacial acetic acid (10 c.c.) and 93% nitric acid (5 c.c.) at room temperature. After 1½ hr. the solution was poured on crushed ice (50 g.), and the precipitate triturated till solid. The *nitrate* crystallised from ethanol as prisms (1·5 g.), m. p. 167° (Found: C, 61·9; H, 5·75; N, 19·1. $C_{19}H_{20}N_4,HNO_3$ requires C, 62·1; H, 5·75; N, 19·05%).

The nitrate (0·4 g.) was hydrogenated in ethanol at atmospheric pressure over Raney nickel. The product (0·3 g.) had m. p. 192° undepressed on admixture with the parent pyrimidine.

Attempted Cyclisation of 4,6-*Diamino*-2-*methylthio*-5-D-*ribosylaminopyrimidine*.—(i) *With sodium dithioformate*. A solution of the ribosylamine (0·4 g.) in a buffer at pH 7·5 (50 c.c.) was treated with sodium dithioformate (0·2 g.) in small portions with shaking and then set aside at 0°. Pale yellow crystals separated (0·18 g.; m. p. 231°). After recrystallisation from water they had m. p. 233° alone or mixed with 4,6-diamino-2-methylthio-5-thioformamidopyrimidine [8] of m. p. 234° (Found: S, 29·0. Calc. for $C_6H_9N_5S_2$: S, 29·8%). The corresponding xylosylamine did not react with this reagent in ethanol in 5 hr., and was recovered unchanged.

(ii) *With carbon disulphide*. Carbon disulphide (2·0 c.c.) was added to a solution of the ribosylamine (1·0 g.) in warm pyridine (25 c.c.) and warmed on a steam-bath during 1 hr. The excess of carbon disulphide was then distilled off and the residual solution heated under reflux at 115° during ½ hr. and then evaporated to dryness *in vacuo* at 60°. The solid residue recrystallised from ethanol and was 8-*mercapto*-2-*methylthioadenine* (0·3 g.), m. p. >310° (Found: C, 34·0; H, 3·0; S, 30·6. $C_6H_7N_5S_2$ requires C, 33·8; H, 3·3; S, 30·1%).

(iii) *With diethoxymethyl acetate*. The ribosylamine (3 g.) was stirred in diethoxymethyl acetate [16] (12 c.c.) at room temperature until complete dissolution was effected and then set aside. After 5 hr., ethanol (30 c.c.) was added followed by light petroleum (50 c.c.) to precipitate a red gum. This was dissolved in hot ethanol (100 c.c.) and cooled to 0°. After 2 days, a precipitate was removed and recrystallised from ethanol. *Acetoxymethylideneamino*-4,6-*diamino*-2-*methylthiopyrimidine* (X) (500 mg.) had m. p. 259°, solidifying and remelting at 290° (Found: C, 40·3; H, 4·4. $C_8H_{11}O_2N_5S$ requires C, 39·8; H, 4·6%). On fusion at 270°, a sample (100 mg.) cyclised, with evolution of acetic acid (smell), to 2-methylthioadenine [8] which crystallised (m. p. 290°) from ethanol (Found: C, 39·3; H, 3·7; S, 17·8. Calc. for $C_6H_7N_5S$: C, 39·8; H, 3·9; S, 17·7%).

(iv) *With ethyl orthoformate*. The ribosylamine (0·45 g.) and ethyl orthoformate (1 c.c.) in glacial acetic acid (20 c.c.) were heated on a steam-bath during 7 hr., then evaporated *in vacuo* at 70°. The residue was obtained from ethanol as an orange powder, m. p. *ca.* 290° (decomp.) (Found: C, 41·1; H, 5·0; loss at 110°, 1·45. $C_{10}H_{13}O_3N_5S,\frac{1}{2}H_2O$ requires C, 41·1; H, 4·85; H_2O, 3·1%), $\lambda_{max.}$ 210, 273, and 363 mμ (log ε 4·31, 4·07, and 3·61) in EtOH, and was probably 4-*amino*-2-*methylthio*-7-(1,2,3-*trihydroxypropyl*)*pteridine hemihydrate*.

(v) *Acetylation and thioformylation*. The dry riboside (1·9 g.) was shaken in dry pyridine (30 c.c.) and acetic anhydride (8 c.c.) during 12 hr. at room temperature, then poured into ethanol (30 c.c.). After 1 hr. the solvents were evaporated *in vacuo*, leaving a pale yellow gum, which was extracted with chloroform. The extracts were dissolved in ethanol (30 c.c.), sodium dithioformate (2·0 g.) was added, and after 1 hr. the solution was diluted with water (30 c.c.) and set aside at 0°. The yellow precipitate was collected and recrystallised from water as needles (0·52 g.), m. p. 230°; mixed with a sample of 4,6-diamino-2-methylthio-5-thioformamidopyrimidine (above) it had m. p. 231°.

4-*Amino*-2-*methylthiopteridine*.—A solution of glyoxal sodium bisulphite (2 g.) and 4,5,6-triamino-2-methylthiopyrimidine (2·8 g.) in N-sulphuric acid (40 c.c.) was heated under reflux

during 1 hr., basified with ammonia solution, filtered (charcoal), and cooled. The brown *pteridine* was collected and recrystallised from water as pale yellow needles (1 g.), m. p. 208° (Found: C, 43·1; H, 3·5; N, 36·1. $C_7H_7N_5S$ requires C, 43·5; H, 3·6; N, 36·3%), $\lambda_{max.}$ 206, 245, 275, and 356 mμ (log ε 4·11, 4·05, 4·28, and 3·81) in EtOH.

4,6-*Diamino*-2-*methylthio*-5-(2,3,5-*tri*-O-*benzoyl*-D-*ribofuranosylamino*)*pyrimidine* (XIII).—A solution of 2,3,5-tri-*O*-benzoyl-D-ribofuranose (3 g.; prepared from the 1-*O*-acetyl compound [19] by an adaptation of the method of Ness *et al.*[28]) and 4,5,6-triamino-2-methylthiopyrimidine (1·3 g.) in absolute ethanol (50 c.c.) was shaken at room temperature during 14 hr., heated at 50° during 1 hr., and evaporated to dryness *in vacuo* at room temperature. The residue was extracted with hot chloroform (2 × 100 c.c.), and the extracts were washed successively with dilute hydrochloric acid, sodium hydrogen carbonate solution, and water, and dried (Na_2SO_4). The solid obtained on evaporation of the chloroform did not crystallise but was repeatedly chromatographed on acid-washed alumina in benzene–chloroform (3 : 1). The *ribosylamine* (2·1 g.) was precipitated from benzene with light petroleum as an amorphous white powder, $[\alpha]_D^{23}$ 0° ± 1° (*c* 1·3 in $CHCl_3$) (Found: C, 64·0; H, 5·2. $C_{31}H_{29}O_7N_5S,C_6H_6$ requires C, 64·0; H, 5·1), $\lambda_{max.}$ 227 and 274 mμ (log ε 4·67 and 4·26) in EtOH.

This ribosylamine (0·3 g.) was hydrolysed as described previously for the tetrabenzoylribosylaminopyrimidine. 2,3,5-Tri-*O*-benzoylribofuranose was obtained from pyridine–water as needles, and recrystallised from ethanol (85 mg.; m. p. and mixed m. p. 102—103°). The dilute acid washings of the chloroform extract of the hydrolysate were neutralised, evaporated, and extracted with hot ethanol. Addition of 5% aqueous picric acid (1 c.c.) gave 4,5,6-triamino-2-methylthiopyrimidine picrate (40 mg.), which recrystallised from water as prisms, m. p. and mixed m. p. 209°.

Attempted Cyclisations of 4,6-*Diamino*-2-*methylthio*-5-*tri*-O-*benzoyl*-D-*ribofuranosylaminopyrimidine.*—(i) *With formamide and formic acid.* The tribenzoylribosylamine (600 mg.) in dry formamide (20 c.c.) was heated in a sealed tube with formic acid (0·15 c.c.) at 165° during 2 hr. Methanethiol was formed. The solution was filtered to remove some charred material and evaporated *in vacuo* at 80°. The residue was extracted with cold water (extract A) and then with hot water (extract B). The water-insoluble material did not give any characterisable product after chromatography in chloroform on acid-washed alumina. Extract B, on cooling, gave a crystalline precipitate (40 mg.) of 2-methylthioadenine, m. p. and mixed m. p. 289°, $\lambda_{max.}$ 233 and 275 mμ (log ε 4·32 and 4·04) in water. Extract A gave a chloroform-soluble extract which was chromatographed on alumina. No fraction collected had light absorption maxima in the range 260—300 mμ, nor did one give a positive Molisch test. Extract A, after extraction with chloroform, did not give a precipitate with aqueous picric acid.

(ii) *With ethyl orthoformate and acetic anhydride.* The tribenzoylribosylamine (500 mg.) was refluxed in ethyl orthoformate (7 c.c.) and acetic anhydride (4·5 c.c.) during 1 hr. and the resulting red solution cooled and evaporated to dryness *in vacuo*. The residual gum was dissolved in 4 : 1 benzene–chloroform (20 c.c.), and chromatographed on acid-washed alumina (51 × 2·6 cm.). A crystalline product was obtained by elution with 4 : 1 benzene–chloroform (70 c.c.) and recrystallised from methanol as plates (15 mg.), m. p. 127—128° (corr.) undepressed on admixture with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose. No other fractions from the chromatogram were identified.

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[28] Ness, Diehl, and Fletcher, *J. Amer. Chem. Soc.*, 1954, **76**, 763.