147. *Experiments on the Synthesis of Purine Nucleosides. Part XI. The Synthesis of* 9-d-*Xylopyranosido-2-methylthioadenine and its Conversion into* 9-d-*Xylopyranosidoadenine.*

By G. A. Howard, B. Lythgoe, and A. R. Todd.

Condensation of *d*-xylose with 4 : 6-diamino-2-methylthiopyrimidine appears to give a mixture of two isomeric *d*-xylosides; only one of these has been obtained crystalline but both give crystalline tetra-acetyl derivatives. The isomeric *d*-xylosides are nitrosated readily in acetic acid solution, yielding isomeric nitroso-xylosides which on reduction and thioformylation yield one and the same 6-*amino*-5-*thioformamido*-4-d-*xylosid-amino*-2-*methylthiopyrimidine*, cyclised by heating in pyridine, borax, or sodium hydroxide solution to 9-d-*xylo-pyranosido*-2-*methylthioadenine*. Acetylation of the thioformamido-compound gives 6-*amino*-5-*thioform-amido*-4-*triacetyl*-d-*xylosidamino*-2-*methylthiopyrimidine*; cyclisation of this substance in pyridine solution gives a product from which, after deacetylation, 9-*d*-xylopyranosido-2-methylthioadenine and the isomeric 6-d-*xylosidamino*-2-*methylthiopurine* can be isolated. Various complications arising in the course of the investigations are described and discussed.

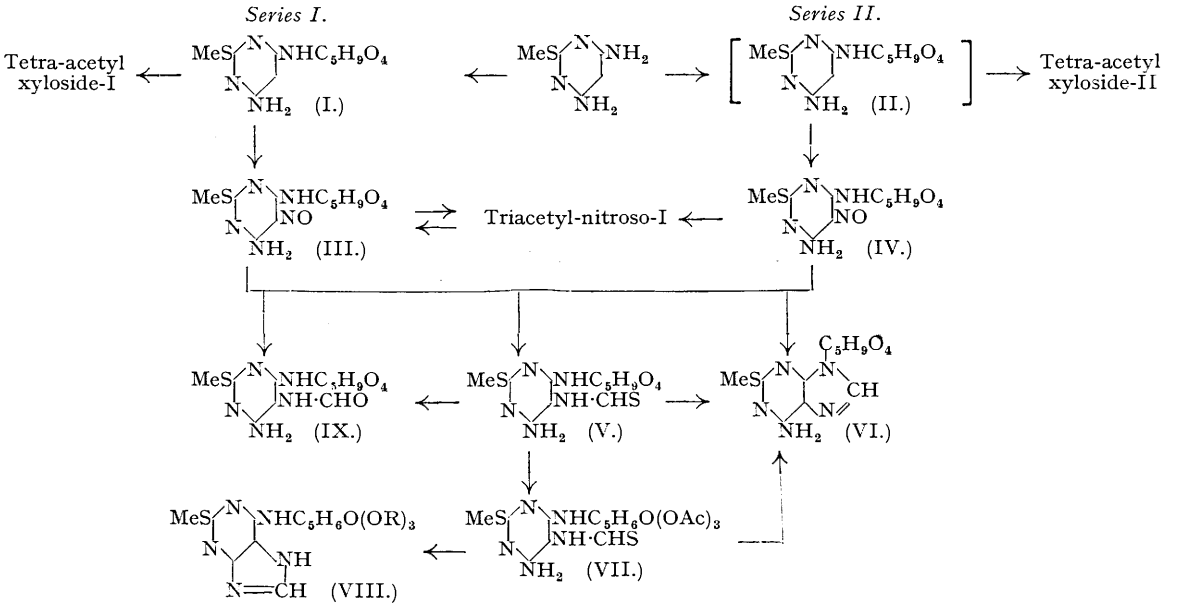
Desulphurisation of 9-*d*-xylopyranosido-2-methylthioadenine with Raney nickel yields 9-*d*-xylopyranosido-adenine identical with the product described in Part IX of this series.

In Part III of this series (J., 1943, 571) the synthesis of 6-amino-4-*d*-xylosidamino-2-methylthiopyrimidine by condensation of *d*-xylose with 4 : 6-diamino-2-methylthiopyrimidine was described. The accessibility of this crystalline xyloside caused it to be selected for our first essay at the synthesis of a 9-glycosidopurine by the method envisaged as a result of earlier model experiments (Part I, J., 1943, 383) and which has since been applied in other cases (Parts VI, IX, X; J., 1944, 318, 652, 657). The attempt was successful and, in fact, the final product, 9-*d*-xylopyranosido-2-methylthioadenine (VI), was the first glycosidopurine synthesised by us. Publication of this synthesis has been delayed until now because of the discovery (cf. Baddiley, Kenner, Lythgoe, McNeil, Todd, and Topham, *Chem. and Ind.*, 1943, **62**, 433) that xylosidisation of 4 : 6-diamino-2-methylthio-pyrimidine gives rise to a mixture of two isomers, and because of other ensuing complications which at the time rendered uncertain the relationship between the final xylosidopurine and the crystalline xylosidaminopyrimidine described in Part III (*loc. cit.*). An extended series of investigations was necessary in order to clarify matters and the results are now reported.

When *d*-xylose is condensed with 4 : 6-diamino-2-methylthiopyrimidine in alcoholic solution in presence of ammonium chloride, and the product worked up by the adsorption procedure described in Part III (*loc. cit.*), the amount of crystalline 6-amino-4-*d*-xylosidamino-2-methylthiopyrimidine which separates directly varies considerably; *e.g.*, starting from 15 g. of *d*-xylose it ranges from 3 g. to 12 g.

We have been unable to determine with any certainty the factors responsible for this variation. As already reported (Part III, *loc. cit.*), acetylation of the non-crystalline residue gives a product from which a crystalline tetra-acetyl compound can be isolated, identical with the derivative obtained on acetylating the crystalline 6-amino-4-*d*-xylosidamino-2-methylthiopyrimidine; the amount obtained is in general inversely proportional to the amount of crystalline xyloside directly isolated. It was subsequently found that, after acetylation of the resinous portion of the xylosidisation product and separation of the above tetra-acetylxyloside, concentration of the mother-liquors gave a second isomeric tetra-acetylxyloside, evidently corresponding to a second 6-amino-4-*d*-xylosidamino-2-methylthiopyrimidine which had not been isolated and whose presence in varying amount was probably responsible for the variation in the amount of crystalline xyloside which could be isolated directly from the product of xylosidisation of 4 : 6-diamino-2-methylthiopyrimidine. Following the practice adopted in the analogous case of the xylosides of 4 : 6-diaminopyrimidine (Part IX, *loc. cit.*), we designate the two sets of isomers Series I and Series II compounds and distinguish them by adding to their systematic names the suffix I or II. Thus the crystalline xyloside (I) is named 6-amino-4-*d*-xylosidamino-2-methylthiopyrimidine-I and its acetyl derivative 6-acetamido-4-triacetyl-*d*-xylosidamino-2-methylthio-pyrimidine-I, while the new acetyl compound is 6-*acetamido*-4-*triacetyl*-d-*xylosidamino*-2-*methylthiopyrimidine-II*

corresponding to a xyloside-II in the original mixture. Deacetylation of these acetyl derivatives by the Zemplén method gave, respectively, 6-*acetamido*-4-d-*xylosidamino*-2-*methylthiopyrimidine-I* and 6-*acetamido*-4-d-*xylosidamino*-2-*methylthiopyrimidine-II*, both of which yielded on acid hydrolysis one and the same 4-amino-6-acetamido-2-methylthiopyrimidine.



Unlike the glycosides of 4 : 6-diaminopyrimidine and of 4 : 6-diamino-2-methylpyrimidine (Part V, J., 1944, 315), the isomeric 6-amino-4-*d*-xylosidamino-2-methylthiopyrimidines are nitrosated readily in acetic acid solution, so that nitrosation followed by reduction could be used to introduce the 5-amino-group necessary for the further development of the purine synthesis in view. From the crystalline 6-amino-4-*d*-xylosidamino-2-methylthiopyrimidine-I, the purple 5-*nitroso*-6-*amino*-4-d-*xylosidamino*-2-*methylthiopyrimidine-I* (III) was obtained, and the resinous residue left after removing the Series I isomer from the crude xylosidisation mixture yielded on nitrosation a blue 5-*nitroso*-6-*amino*-4-d-*xylosidamino*-2-*methylthiopyrimidine-II* (IV). It is of interest that acetylation of either of these with acetic anhydride in pyridine in the cold gives one and the same 5-*nitroso*-6-*amino*-4-*triacetyl*-d-*xylosidamino*-2-*methylthiopyrimidine-I*; deacetylation of this product by the Zemplén method gave only 5-nitroso-6-amino-4-*d*-xylosidamino-2-methylthiopyrimidine-I. The behaviour of these nitroso-compounds on acetylation recalls the observation by Kuhn and Ströbele (*Ber.*, 1937, **70**, 773) that direct condensation of *l*-arabinose with *o*-nitroxylidine gives two isomeric *N*-arabinosides which on acetylation yield the same triacetyl *l*-arabinoside; no evidence was offered in that case as to the nature of the isomers, although it seems to have been generally assumed that they were α- and β-*l*-arabinosides. It has been observed by us that the amount of 6-acetamido-4-triacetyl-*d*-xylosidamino-2-methylthiopyrimidine-II, isolated after acetylation of the non-crystalline portion of the xylosidisation product from 4 : 6-diamino-2-methylthiopyrimidine, is small as compared with the quantity of nitroso-xyloside-II obtained by nitrosating the same material. This might, of course, be due simply to difficulty of isolation, but in view of the above facts it is also possible that a partial conversion of the Series II into the Series I isomer occurs during acetylation.

Reduction of 5-nitroso-6-amino-4-*d*-xylosidamino-2-methylthiopyrimidine-II (IV) with ammonium sulphide, followed by treatment of the crude product with aqueous sodium dithioformate, gave 6-*amino*-5-*thioformamido*-4-d-*xylosidamino*-2-*methylthiopyrimidine* (V) in fairly good yield. When this compound was first prepared the existence of two isomeric 6-amino-4-*d*-xylosidamino-2-methylthiopyrimidines was not known to us, and the nitroso-compound used had been prepared by nitrosating the total xylosidisation product of 2-methylthiopyrimidine in the belief that it contained only one xyloside whose complete crystallisation was impeded by small amounts of impurity. It was only when the existence of the Series I and Series II isomers was recognised that it was discovered that in fact the nitroso-compound used hitherto was actually the Series II compound and did not correspond to the crystalline 6-amino-4-*d*-xylosidamino-2-methylthiopyrimidine-I. This was an initial cause of confusion in our work and made the subsequent course of the investigation somewhat complex. In the interests of clarity in presentation it is, however, necessary at this stage to indicate that, as was subsequently shown, the thioformamido-compound mentioned above is common to both Series I and Series II, *i.e.*, that the isomerism disappears on reduction of the nitroso-compounds.

When 6-amino-5-thioformamido-4-*d*-xylosidaminopyrimidine, prepared as above described, was refluxed in dry pyridine, hydrogen sulphide was evolved, and from the resulting solution 9-d-*xylosido*-2-*methylthioadenine* (VI) was isolated. The constitution of this product follows from its hydrolysis to *d*-xylose and

2-methylthioadenine, its insolubility in aqueous sodium hydroxide, and its deamination to 9-d-*xylosido*-2-*methylthiohypoxanthine*. The yield of purine from the thioformamido-compound was rather low, and other cyclisation media were examined in place of pyridine. Boiling quinoline caused almost complete decomposition, and lutidine was little better than pyridine; better results were obtained when the thioformamido-compound was simply heated for a short time with borax solution or with aqueous sodium hydroxide, although as was to be expected, a considerable amount of 6-*amino*-5-*formamido*-4-d-*xylosidamino*-2-*methylthiopyrimidine* (IX) was formed simultaneously. It seemed possible that the yield of purine derivative might be considerably increased if the thioformamido-compound were acetylated before ring closure, a method used in analogous syntheses described in earlier papers of this series (*loc. cit.*). 6-*Amino*-5-*thioformamido*-4-*triacetyl*-d-*xylosidamino*-2-*methylthiopyrimidine* (VII) was therefore prepared and refluxed in dry pyridine in the normal manner. From the reaction solution 6-*triacetyl*-d-*xylosidamino*-2-*methylthiopurine* (VIII; R = Ac) was readily isolated. It yielded on hydrolysis with ammonia 6-d-*xylosidamino*-2-*methylthiopurine* (VIII; R = H), which was soluble in alkali and could not be deaminated; final proof of its structure was obtained by methylation to 6-d-*xylosidamino*-2-*methylthio*-9-*methylpurine*, hydrolysed by acid to the known 2-methylthio-9-methyladenine. The cyclisation mother-liquors remaining after separation of 6-triacetyl-*d*-xylosidamino-2-methylthiopurine gave on evaporation a resin which was deacetylated by the Zemplén method. From the product, 9-*d*-xylosido-2-methylthioadenine (VI) was isolated, together with a small amount of 6-amino-5-formamido-4-*d*-xylosidamino-2-methylthiopyrimidine.

When 5-nitroso-6-amino-4-*d*-xylosidamino-2-methylthiopyrimidine-I (III) was reduced with ammonium sulphide, and the crude product treated in warm aqueous solution with sodium dithioformate, a crystalline product was obtained which rather surprisingly proved to be 6-amino-5-formamido-4-*d*-xylosidamino-2-methylthiopyrimidine (IX); on acetylation with cold acetic anhydride in pyridine it gave a crystalline *triacetyl* derivative and was hydrolysed by acid to *d*-xylose and 4 : 6-*diamino*-5-*formamido*-2-*methylthiopyrimidine*, identical with a specimen prepared by formylating 4 : 5 : 6-triamino-2-methylthiopyrimidine. Examination of the sample of sodium dithioformate used in the experiment revealed that it had deteriorated somewhat through prolonged keeping, but even so, there had been excess of dithioformate present during the reaction; evidently the thioformamido-compound was so unstable that it was converted into the formamido-compound in the warm alkaline solution even in presence of sodium dithioformate. The reduction of the nitroso-xyloside-I was therefore re-examined more carefully, thioformylation of the crude product being carried out in the cold with sodium dithioformate. The gelatinous product which separated yielded on recrystallisation from water 6-amino-5-thioformamido-4-*d*-xylosidamino-2-methylthiopyrimidine (V), 9-*d*-xylosido-2-methylthioadenine (VI), and 6-amino-5-formamido-4-*d*-xylosidamino-2-methylthiopyrimidine (IX), the last being the major product. Presumably the production of formamido-compound and purine was due partly to the strongly alkaline nature of the reaction medium and partly to the boiling with water involved in recrystallisation.

This result led in turn to a re-examination of the reduction and thioformylation of the nitroso-xyloside-II under similar conditions; in this experiment both the 5-thioformamido- and the 5-formamido-compound were obtained (the former in larger amount) but no purine was isolated. Failure to isolate any purine in a single experiment of this nature is not very surprising, since the amount expected would in any event be small and would probably vary greatly according to the conditions of recrystallisation of the crude product; indeed, it might fairly be stated that according to the conditions, reduction and thioformylation of either of the nitroso-compounds may give rise to any or all of the three products, thioformamido-compound, formamido-compound, and 9-xylosidopurine. In accordance with this view, reductions of nitroso-I and nitroso-II with aluminium amalgam, followed by thioformylation, gave in each case a mixture of products.

From the above experiments it is clear that the Series I and Series II isomers merge into one series when reduction of the 5-nitroso-compounds is carried out. This is strictly analogous to the behaviour of the corresponding isomeric series already reported in the case of the xylosides of 4 : 6-diaminopyrimidine (Part IX; *loc. cit.*) in which the isomerism disappeared on reductive fission of a 5-azo-substituent. The nature of the isomerism of the Series I and Series II compounds remains to be rigidly established and definite evidence is being sought. Meanwhile, we incline to the view that the two series represent α- and β-glycosides.

A point of considerable interest in the present work is that cyclisation of 6-amino-5-thioformamido-4-*d*-xylosidamino-2-methylthiopyrimidine, carried out several times under varied conditions, gave exclusively 9-*d*-xylosido-2-methylthioadenine (VI); no trace of 6-*d*-xylosidamino-2-methylthiopurine (VIII) was ever obtained. On the other hand, cyclisation of 6-amino-5-thioformamido-4-triacetyl-*d*-xylosidamino-2-methylthiopyrimidine (VII) gave a mixture of the 9-xylosido- and the 6-xylosidamino-purines. The production of two isomeric purine xylosides from acetylated thioformamido-compounds had been observed before (*e.g.*, Parts VI and IX, *loc. cit.*) although it was unexpected on the basis of the model experiments on the synthesis of 9-alkylpurines (Part I, J., 1943, 383). In Part IX (*loc. cit.*) it was suggested that the explanation might lie in the effect of chelation involving the glycosidic NH group and the carbonyl of the acetyl residue at C_2 in the sugar chain. On this view it would follow that cyclisation of an unacetylated thioformamido-glycoside would lead exclusively to the 9-glycosidopurine, since chelation would no longer be possible. In this sense present results lend considerable support to the above suggestion.

Application of the periodate oxidation method (Part VIII; *loc. cit.*) for determination of lactol ring structure to the synthetic 9-*d*-xylosido-2-methylthioadenine led to a consumption of 3 mols. of periodate and the liberation of 1 mol. of formic acid. Of the periodate used, only 2 mols. were used for oxidation of the sugar chain, the third

presumably oxidising the methylthio-group to a methylsulphoxido-group. It was established in a control experiment that 2-methylthioadenine itself absorbed 1 mol. of periodate. It follows therefore that the synthetic glycoside (VI) is correctly described as 9-d-*xylopyranosido*-2-*methylthioadenine*.

In view of the production of isomeric xylosides on condensing *d*-xylose with 4 : 6-diaminopyrimidine and with 4 : 6-diamino-2-methylthiopyrimidine and of the disappearance of this isomerism in the course of purine xyloside synthesis from them, it was of some importance to know whether or not the 9-*d*-xylopyranosidoadenine described in Part IX (*loc. cit.*) and the 9-*d*-xylopyranosido-2-methylthioadenine now described belonged to the same stereochemical series. A possible method of settling this point appeared to be the desulphurisation procedure using Raney nickel saturated with hydrogen recently developed by Mozingo (*J. Amer. Chem Soc.*, 1943, **65**, 1013) provided that it could be applied to 2-methylthiopurines.

As a preliminary model, 2-methylthio-9-methyladenine was refluxed in alcoholic solution with Raney nickel; 9-methyladenine was produced in good yield. 9-*Triacetyl*-d-*xylopyranosido*-2-*methylthioadenine* treated in exactly the same way yielded a product, deacetylated to 9-*d*-xylopyranosidoadenine, identical with a specimen synthesised from 4 : 6-diaminopyrimidine. It follows that these two xylosides belong to the same stereochemical series. To that series also belong, in all probability, the synthetic 9-*d*-xylopyranosido-2-methyladenine and 9-*d*-ribopyranosidoadenine as described in previous papers (Part IX, *loc. cit.*; Part X, J., 1944, 657). Whether the configuration of these compounds is that of α- or β-glycosides and whether it corresponds to that of the natural purine nucleosides should be capable of determination by application of the method of periodate oxidation to appropriate natural and synthetic glycosides; the results of experiments on these lines will be reported in a subsequent paper.

Experimental.

Activated alumina used throughout was prepared by heating alumina hydrate (British Aluminium Co., Ltd.) to 360° during 5 hrs.

Condensation of d-*Xylose with* 4 : 6-*Diamino*-2-*methylthiopyrimidine*.—The following modification of the procedure described in Part III (*loc. cit.*) was used: *d*-Xylose (15 g.), 4 : 6-diamino-2-methylthiopyrimidine (30 g.), and ammonium chloride (1 g.) were refluxed in absolute alcohol (250 c.c.) for 30 mins. in a flask fitted with an 18″ Fenske column and a reflux ratio head. A mixture of benzene and alcohol (1 : 1, total 500 c.c.) was added in portions, and water removed by distillation as the ternary mixture. Slow distillation was continued during 4 hours, and the orange-coloured mixture cooled and poured through a column of activated alumina (2 kg.). The column was washed with alcohol (3·5 l.) to remove unchanged 4 : 6-diamino-2-methylthiopyrimidine and then eluted with cold water (5 l.). The aqueous eluate was worked up as in Part III (*loc. cit.*). The crystalline 6-amino-4-*d*-xylosidamino-2-methylthiopyrimidine-I (3—12 g.; m. p. 190—192°) had $[\alpha]_D^{17.5°}$ −20° (*c* = 0·15 in water).

An attempt to condense 4 : 6-diamino-2-methylthiopyrimidine with *d*-xylose by heating in aqueous solution was made; although some evidence of condensation was obtained, no pure xyloside could be isolated and the method appeared of little practical value.

6-*Acetamido*-4-*triacetyl*-d-*xylosidamino*-2-*methylthiopyrimidine-II*.—The crude xyloside mixture (15 g.) from *d*-xylose and 4 : 6-diamino-2-methylthiopyrimidine (see above) was dissolved in pyridine (150 c.c.), and a mixture of acetic anhydride (40 c.c.) and acetyl chloride (1 c.c.) added. There was a noticeable rise in temperature and the mixture was set aside overnight, heated on the steam-bath for 40 mins., cooled, and excess of acetic anhydride–acetyl chloride destroyed by adding alcohol and allowing it to stand for 1 hr. The resulting solution was evaporated, the residue dissolved in a minimum of hot alcohol, and the solution cooled; 6-acetamido-4-triacetyl-*d*-xylosidamino-2-methylthiopyrimidine-I (10 g.; m. p. 225—226°) separated. Evaporation of the alcoholic mother-liquors gave a resin which was dissolved in methanol (*ca.* 25 c.c.) at 40°, and water added slowly until a faint turbidity appeared. 6-*Acetamido*-4-*triacetyl*-d-*xylosidamino*-2-*methylthiopyrimidine*-II (1 g.) separated in pellet-like aggregates of colourless needles; recrystallised several times from a mixture of alcohol and ethyl acetate, it had m. p. 217—218°, $[\alpha]_D^{18°}$ +48·4° (*c* = 2·354 in chloroform) (Found: C, 47·6; H, 5·1; N, 12·1. $C_{18}H_{24}O_8N_4S$ requires C, 47·3; H, 5·3; N, 12·3%).

6-*Acetamido*-4-d-*xylosidamino*-2-*methylthiopyrimidine-II*.—The tetra-acetyl compound (250 mg.) was allowed to stand for 3 days with methanolic ammonia (25 c.c., saturated at 0°), and the solution evaporated to dryness under reduced pressure. The resinous residue was dissolved in boiling absolute alcohol, and the solution set aside to cool. The crystalline 6-*acetamido*-4-d-*xylosidamino*-2-*methylthiopyrimidine-II* which separated was recrystallised from alcohol; colourless needles (130 mg.), m. p. 175—180° (Found, in material dried at 100°/1 mm.: C, 43·4; H, 6·0; N, 16·9. $C_{12}H_{18}O_5N_4S$ requires C, 43·6; H, 5·5; N, 17·0%). The compound gave a marked depression in m. p. when mixed with 6-acetamido-4-*d*-xylosidamino-2-methylthiopyrimidine-I (Part III, *loc. cit.*), and on acid hydrolysis yielded the known 4-amino-6-acetamido-2-methylthiopyrimidine, identified by m. p. and mixed m. p.

5-*Nitroso*-6-*amino*-4-d-*xylosidamino*-2-*methylthiopyrimidine-I*.—6-Amino-4-*d*-xylosidamino-2-methylthiopyrimidine-I (4 g.) was dissolved in hot water (200 c.c.), cooled rapidly to 5°, and sodium nitrite (2·5 g.) and glacial acetic acid (7 c.c.) added. The mixture was kept in an ice-bath for 45 minutes, then for a similar period at room temperature, and finally was filtered ice-cold. The blue filter residue was washed with ice-water (100 c.c.) and recrystallised from water. The *nitroso*-compound formed fine purple needles (3·1 g.), m. p. 237° (Found, in material dried at 140°/1 mm.: C, 37·7; H, 5·3; N, 22·1. $C_{10}H_{15}O_5N_5S$ requires C, 37·9; H, 4·8; N, 22·1%).

Acetylation of this product (0·5 g.) with acetic anhydride (1 c.c.) in pyridine (5 c.c.) in the cold, by shaking it for 15 mins. then leaving it overnight and working it up in the usual manner, gave 5-*nitroso*-6-*amino*-4-*triacetyl*-d-*xylosidamino*-2-*methylthiopyrimidine-I* (0·48 g.), crystallising from alcohol–ethyl acetate in small green needles, m. p. 192—193° (Found, in material dried at 140°/1 mm.: C, 43·7; H, 4·8; N, 15·3. $C_{16}H_{21}O_8N_5S$ requires C, 43·4; H, 4·8; N, 15·8%). Hydrolysis of this product by Zemplén's method gave 5-nitroso-6-amino-4-*d*-xylosidamino-2-methylthiopyrimidine-I, m. p. 237°.

5-*Nitroso*-6-*amino*-4-d-*xylosidamino*-2-*methylthiopyrimidine-II*.—The resinous residue (*ca.* 30 g.) left after separation of the crystalline xyloside-I from the xylosidisation product of 4 : 6-diamino-2-methylthiopyrimidine was dissolved in water (200 c.c.) and nitrosated as in the previous experiment with sodium nitrite (9 g.) and glacial acetic acid (17 c.c.). The *product* separated from water as a blue gel, which on drying in a desiccator gave a blue solid (9·5 g.), m. p. 197° (decomp.) (Found, in material dried at 140°/1 mm.: C, 37·6; H, 5·1; N, 21·4. $C_{10}H_{15}O_5N_5S$ requires C, 37·9; H, 4·8; N, 22·1%).

Acetylation of this material (1 g.) in the manner described above for the Series I isomer gave 5-nitroso-6-amino-

4-triacetyl-*d*-xylosidamino-2-methylthiopyrimidine-I (0·85 g.), m. p. 190—191°; mixed m. p. with an authentic specimen (m. p. 192—193°), 192°.

6-*Amino*-5-*thioformamido*-4-d-*xylosidamino*-2-*methylthiopyrimidine*.—(A) A suspension of 5-nitroso-6-amino-4-*d*-xylosidamino-2-methylthiopyrimidine-II (14 g.) in water (540 c.c.) was cooled to 0°, and an ice-cold saturated solution of hydrogen sulphide in aqueous ammonia [36 c.c. of ammonia (*d* 0·880) + 540 c.c. of water] added with shaking. Hydrogen sulphide was passed through the mixture for 50 mins., and the resulting pale yellow solution evaporated to dryness under reduced pressure. The residue was shaken with water (1800 c.c.) at 90°, precipitated sulphur removed by filtration, and aqueous sodium dithioformate (54 g. in 180 c.c. of water) added to the filtrate. The *thioformamido*-compound began to separate almost immediately; it was collected after 18 hrs. and recrystallised from water (charcoal); colourless hydrated needles (11·2 g.), m. p. 208° (decomp.). Water of crystallisation was very firmly held, and was only partly removed after drying for 7 hrs. at 140°/1 mm. (Found, in material dried for 5 hrs. at 140°/1 mm.: C, 36·5; H, 5·1; N, 19·1. $C_{11}H_{17}O_4N_5S_2,H_2O$ requires C, 36·2; H, 5·2; N, 19·2%).

Hydrolysis of the product (196 mg.) by heating under reflux with 0·1N-sulphuric acid for 90 mins., followed by neutralisation and concentration to small bulk in a vacuum, gave 4 : 6-diamino-5-thioformamido-2-methylthiopyrimidine. On heating, it melted at 235—236°, evolved hydrogen sulphide, then resolidified and again melted at 293°; this behaviour was also shown by an authentic specimen prepared as described in Part I (*loc. cit.*) and by a mixture of the two.

(B) In a later experiment carried out after the isolation of 6-amino-5-formamido-4-*d*-xylosidamino-2-methylthiopyrimidine on reduction and thioformylation of the Series I nitroso-compound (see below), the mother-liquors remaining after removal of the thioformamido-compound yielded on concentration 6-amino-5-formamido-4-*d*-xylosidamino-2-methylthiopyrimidine, m. p. and mixed m. p. 232°.

Formation of 9-d-*Xylopyranosido*-2-*methylthioadenine by Cyclisation of* 6-*Amino*-5-*thioformamido*-4-d-*xylosidamino*-2-*methylthiopyrimidine*.—(1) *In pyridine*. A solution of the thioformamido-compound (2 g.) in dry pyridine (165 c.c.) was heated under reflux for 21 hrs., hydrogen sulphide evolution then being very slow. The solution was concentrated to small bulk (5 c.c.) under reduced pressure, absolute alcohol (150 c.c.) added, and the solution set aside overnight. A small amount of unchanged starting material which separated was filtered off, and the filtrate evaporated to dryness. The resinous residue was dissolved in a little hot water (charcoal); the *purine xyloside* separated on cooling as colourless needles (125 mg.), m. p. 278° (decomp.) (Found, in material dried at 140°/1 mm.: C, 42·5; H, 4·8; N, 22·1. $C_{11}H_{15}O_4N_5S$ requires C, 42·2; H, 4·8; N, 22·4%). The crystallisation mother-liquors yielded on concentration more unchanged starting material (200 mg.) and an uncrystallisable resin.

The xyloside was insoluble in dilute sodium hydroxide and had $[\alpha]_D^{19°}$ −29° (*c* = 0·03 in water). Its absorption spectrum in N/20-hydrochloric acid showed a maximum at 2710 A. (ε, 15,700), and in N/20-sodium hydroxide maxima at 2355 A. (ε, 17,600) and 2755 A. (ε, 13,700).

Hydrolysis. The xyloside (121 mg.) was refluxed with N-sulphuric acid (4 c.c.) for 3 hrs., cooled, diluted with an equal volume of water, neutralised with N-sodium hydroxide (4 c.c.), and the solution set aside overnight. 2-Methylthioadenine separated; recrystallised from water, it formed needles (41 mg.), m. p. 295° undepressed by an authentic specimen. The hydrolysis mother-liquors were evaporated to dryness under reduced pressure, and the residue heated for 1 hr. at 100° with acetic anhydride (1 c.c.) and anhydrous sodium acetate (60 mg.). Excess of acetic anhydride was removed by evaporation under reduced pressure, and water (5 c.c.) added; recrystallisation of the water-insoluble portion from alcohol gave β-tetra-acetyl *d*-xylose (37 mg.), m. p. 123—124° undepressed in admixture with an authentic specimen.

Periodate titration. Amount of xyloside used, 0·126 g. Mols. of sodium periodate used per mol. of xyloside 3·01; mol. of formic acid liberated per mol. of xyloside, 0·985.

Periodate titration of 2-methylthioadenine. Amount of purine used, 21 mg. Mols. of sodium periodate used per mol. of purine, 1·05.

(2) *In borax solution*. A solution of the thioformamido-compound (0·5 g.) in water (10 c.c.) and saturated borax solution (20 c.c.) was boiled for 15 mins. and allowed to cool. As no crystalline material separated, the solution was evaporated to dryness and the residue recrystallised from water, giving 9-*d*-xylopyranosido-2-methylthioadenine (55 mg.; m. p. 278°). The mother-liquors on standing deposited 6-amino-5-formamido-4-*d*-xylosidamino-2-methylthiopyrimidine (126 mg.; m. p. 232°).

(3) *In aqueous sodium hydroxide*. Aqueous 3N-sodium hydroxide (1 c.c.) was added to a solution of the thioformamido-compound (0·2 g.) in water (25 c.c.), and the mixture boiled for 5 mins. Concentration of the neutralised solution yielded successively 9-*d*-xylopyranosido-2-methylthioadenine (30 mg.) and 6-amino-5-formamido-4-*d*-xylosidamino-2-methylthiopyrimidine (50 mg.).

9-d-*Xylopyranosido*-2-*methylthiohypoxanthine*.—9-*d*-Xylopyranosido-2-methylthioadenine (88 mg.) was dissolved in N-sulphuric acid (16 c.c.), sodium nitrite (0·23 g.) added, and the solution maintained at 35° until gas evolution had virtually ceased (25 mins.). Sodium hydroxide was added to neutrality, and the solution concentrated to 20 c.c. 9-d-*Xylopyranosido*-2-*methylthiohypoxanthine* separated and was recrystallised from water; colourless needles (20 mg.), m. p. 255° (Found, in material dried at 140°/1 mm.: C, 42·3; H, 4·2; N, 17·6. $C_{11}H_{14}O_5N_4S$ requires C, 42·0; H, 4·3; N, 17·8%).

6-*Amino*-5-*thioformamido*-4-*triacetyl*-d-*xylosidamino*-2-*methylthiopyrimidine*.—The above thioformamido-compound (6·5 g.) was acetylated by shaking for 30 mins. with pyridine (140 c.c.) and acetic anhydride (17 c.c.) and leaving the solution overnight. Excess of acetic anhydride was decomposed with alcohol (50 c.c.), and the solution concentrated to small bulk under reduced pressure. The *triacetyl* derivative separated on standing, and was recrystallised from alcohol; colourless needles (4·4 g.), m. p. 209° (Found: C, 43·0; H, 5·1; N, 14·2. $C_{17}H_{23}O_7N_5S_2$ requires C, 43·1; H, 4·9; N, 14·8%).

Cyclisation of 6-*Amino*-5-*thioformamido*-4-*triacetyl*-d-*xylosidamino*-2-*methylthiopyrimidine*.—The above triacetyl derivative (3 g.) was refluxed in pyridine (15 c.c.) during 7 hrs. The brown solution was evaporated to dryness under reduced pressure, and the resinous residue dissolved in absolute alcohol (15 c.c.). On standing, a brownish crystalline solid separated; this was collected (A), and the mother-liquor (B) worked up as described below.

(A) Recrystallisation from absolute alcohol (charcoal) gave colourless needles (0·48 g.) of 6-*triacetyl*-d-*xylosidamino*-2-*methylthiopurine*, m. p. 254° (Found, in material dried at 100°/1 mm.: C, 46·0; H, 4·9; N, 15·4. $C_{17}H_{21}O_7N_5S$ requires C, 46·5; H, 4·8; N, 15·9%).

(B) The alcoholic mother-liquor was evaporated to dryness, leaving a brown resin which was dissolved in dry chloroform (10 c.c.), and a solution of sodium (0·25 g.) in methanol (10 c.c.) added. On the following day the mixture was extracted with water (60 c.c.), the aqueous extract treated with charcoal and concentrated to *ca.* 40 c.c. On standing, 9-*d*-xylopyranosido-2-methylthioadenine (120 mg., m. p. and mixed m. p. 278°) separated (Found: C, 42·0; H, 4·6; N, 21·9. Calc. for $C_{11}H_{15}O_4N_5S$: C, 42·2; H, 4·8; N, 22·4%). Further concentration of the mother-liquors gave material, m. p. *ca.* 218°, yielding after several recrystallisations 6-amino-5-formamido-4-*d*-xylosidamino-2-methylthiopyrimidine, m. p. 232°.

6-d-*Xylosidamino*-2-*methylthiopurine*.—The above 6-triacetyl-*d*-xylosidamino-2-methylthiopurine (218 mg.) was dissolved in methanol (25 c.c.) saturated at 0° with ammonia, and the solution set aside for 3 days. The product which had separated was collected, and a further crop was obtained by concentrating the mother-liquors. Recrystallised from

water, the *xyloside* formed colourless, hydrated needles, m. p. 235°, $[\alpha]_D^{17.5°}$ −20° (*c* = 0·15 in water) (Found: C, 40·4; H, 5·1; N, 21·0. $C_{11}H_{15}O_4N_5S,H_2O$ requires C, 39·9; H, 5·2; N, 21·1%).

Readily soluble in sodium hydroxide, the xyloside was unaffected by nitrous acid; its absorption spectrum in N/20-hydrochloric acid showed maxima at 2490 A. (ε, 30,800) and 2950 A. (ε, 19,800), and in N/20-sodium hydroxide at 2355 A. (ε, 16,400) and 2880 A. (ε, 15,000). Hydrolysed by boiling with dilute sulphuric acid, the substance yielded 2-methylthioadenine (m. p. and mixed m. p. 295°) and *d*-xylose, isolated after acetylation as β-tetra-acetyl *d*-xylose (m. p. and mixed m. p. 123—124°).

Final proof of structure was obtained by methylation: The xyloside (213 mg.) was suspended in absolute alcohol (50 c.c.), and a solution of sodium (0·016 g.) in alcohol (2 c.c.) added, followed by methyl iodide (0·05 c.c.). The solution was refluxed for 2 hrs., then evaporated to dryness and the residue recrystallised from water. 6-d-*Xylosidamino*-2-*methylthio*-9-*methylpurine* was obtained as colourless needles (40 mg.), m. p. 214° (Found: C, 40·9; H, 5·5; N, 20·2; loss at 140°/1 mm., 8·8. $C_{12}H_{17}O_4N_5S,2H_2O$ requires C, 40·6; H, 5·6; N, 19·7; loss on drying, 9·0%). The methylated material was hydrolysed by refluxing for 2 hours with dilute sulphuric acid. On neutralising the resulting solution, 2-methylthio-9-methyladenine separated, m. p. 262° undepressed in admixture with an authentic specimen.

Reduction of 5-*Nitroso*-6-*amino*-4-d-*xylosidamino*-2-*methylthiopyrimidine-I with Ammonium Sulphide.*—Reduction of the nitroso-compound-I (3·5 g.) was carried out as described above for the Series II isomer, and to the hot aqueous solution of the product sodium dithioformate (6 g. in 50 c.c. of water) was added. On concentration of the solution to 60 c.c. and allowing it to stand, 6-*amino*-5-*formamido*-4-d-*xylosidamino*-2-*methylthiopyrimidine* separated; recrystallised from water (charcoal), it formed colourless hydrated needles, m. p. 232° (Found: C, 37·8; H, 5·5; N, 20·5; S, 10·0. $C_{11}H_{17}O_5N_5S,H_2O$ requires C, 37·8; H, 5·4; N, 20·1; S, 9·6%). Acetylation with acetic anhydride in pyridine in the cold gave a *triacetyl* derivative, m. p. 190—191° after slight sintering at 188° (Found: C, 45·1; H, 5·4; N, 15·3. $C_{17}H_{23}O_8N_5S$ requires C, 44·6; H, 5·0; N, 15·3%).

The product, m. p. 232° (200 mg.), was hydrolysed by boiling with dilute hydrochloric acid (1 c.c. of N + 10 c.c. of water) for 1 hr. Considerable darkening occurred, and the solution was cooled, neutralised, and concentrated to 2 c.c. The brown solid which separated was recrystallised from water (charcoal), giving almost colourless prisms which on heating melted at *ca.* 254° then resolidified and again melted between 280° and 290°. The same behaviour on heating was shown by a sample of 4 : 6-*diamino*-5-*formamido*-2-*methylthiopyrimidine* prepared by heating 4 : 5 : 6-triamino-2-methylthiopyrimidine with 98% formic acid (Found: C, 36·1; H, 4·7; N, 35·4. $C_6H_9ON_5S$ requires C, 36·2; H, 4·5; N, 35·2%) and by a mixture of the hydrolysis product with this substance.

In another experiment reduction of the Series I nitroso-compound (1 g.) was repeated under similar conditions, and the crude product treated with aqueous sodium dithioformate in the cold. The solid obtained yielded on fractional crystallisation from water 6-amino-5-formamido-4-*d*-xylosidamino-2-methylthiopyrimidine (263 mg.), 6-amino-5-thioformamido-4-*d*-xylosidamino-2-methylthiopyrimidine (91 mg.), and 9-*d*-xylopyranosido-2-methylthioadenine (70 mg.).

Reductions with Aluminium Amalgam.—Series I. The nitroso-xyloside-I (0·58 g.) was suspended in water at 50°, aluminium amalgam (2 g.) added, and the mixture left overnight then filtered, the aluminium hydroxide being washed with hot water. Combined filtrate and washings were treated with sodium dithioformate in the usual manner, and the crystalline product which separated yielded on fractionation 6-amino-5-thioformamido-4-*d*-xylosidamino-2-methylthiopyrimidine (200 mg.) and 9-*d*-xylopyranosido-2-methylthioadenine (10 mg.). In a second experiment no purine was isolated, the product being a mixture of the 5-formamido- and the 5-thioformamido-compound.

Series II. The nitroso-xyloside-II (2 g.) was reduced with aluminium amalgam in a similar manner, and the product thioformylated. Fractional crystallisation yielded 9-*d*-xylopyranosido-2-methylthioadenine (0·3 g.) and 6-amino-5-formamido-4-*d*-xylosidamino-2-methylthioadenine (0·25 g.).

Desulphurisation of 2-*Methylthio*-9-*methyladenine* (by Dr. J. Baddiley).—A solution of purine (0·2 g.) in alcohol (15 c.c.) was refluxed for 2 hrs. with Raney nickel (2 g., prepared according to Mozingo, *loc. cit.*) and set aside for 24 hrs. The nickel was filtered off and extracted with boiling alcohol. Combined filtrate and extracts were evaporated to dryness, and the residue recrystallised from water. 9-Methyladenine was obtained as colourless prisms, m. p. 300° undepressed by an authentic specimen prepared by Krüger's method (*Z. physiol. Chem.*, 1894, **18**, 434).

Conversion of 9-d-*Xylopyranosido*-2-*methylthioadenine into* 9-d-*Xylopyranosidoadenine.*—A suspension of 9-*d*-xylopyranosido-2-methylthioadenine (172 mg.) in pyridine (15 c.c.) was shaken with acetic anhydride (1·5 c.c.) until dissolved (2 hrs.), and the solution left overnight; it was then concentrated to 5 c.c., and alcohol (10 c.c.) added. After standing for a short time, the solution was evaporated, and the residue recrystallised from aqueous alcohol. 9-*Triacetyl*-d-*xylopyranosido*-2-*methylthioadenine* (200 mg.) was obtained as colourless needles, m. p. 192—193° (Found, in material dried at 140°/1 mm.: C, 46·5; H, 4·9; N, 15·9. $C_{17}H_{21}O_7N_5S$ requires C, 46·5; H, 4·8; N, 15·9%).

The above triacetyl derivative (183 mg.) was dissolved in absolute alcohol (30 c.c.), and the solution refluxed for 2 hrs. with Raney nickel (1·8 g., prepared according to Mozingo, *loc. cit.*) and left overnight. The supernatant liquid was decanted and the nickel extracted with absolute alcohol (Soxhlet). The combined extracts and supernatant liquid were evaporated in a vacuum, and the residue dissolved in methanolic ammonia (25 c.c. saturated at 0°). After 3 days the crystalline precipitate was collected and recrystallised from water; colourless needles, m. p. 293—294° undepressed in admixture with 9-*d*-xylopyranosidoadenine (m. p. 294—295°) synthesised by the method described in Part IX (*loc. cit.*) (Found: C, 45·4; H, 5·2; N, 26·1. Calc. for $C_{10}H_{13}O_4N_5$: C, 45·0; H, 4·9; N, 26·2%).

The authors thank the Department of Scientific and Industrial Research for a maintenance allowance held by one of them (G. A. H.), and Imperial Chemical Industries Ltd. and Roche Products Ltd. for grants and gifts of materials.

The University, Manchester.
University Chemical Laboratory, Cambridge. [*Received, May 23rd,* 1945.]