183. *Experiments on the Synthesis of Purine Nucleosides. Part XVII. The Preparation of 4-Glycofuranosidaminopyrimidines, and a Synthesis of 9-L-Arabofuranosido-2-methylthioadenine.**

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A description is given of model experiments on methods for preparing 4-glycofuranosidaminopyrimidines, necessary intermediates in the synthesis of natural purine nucleosides, by our general procedure. Successful methods devised were condensation of acetylated glycofuranoses with 4 : 6-diamino-2-methylthiopyrimidine, and condensation of acylated *aldehydo*-sugars with the same pyrimidine derivative to give Schiff's bases converted into glycosides on deacetylation. Thus 5-*benzoyl* 2 : 3 : 4-*triacetyl* aldehydo-L-*arabinose* was condensed with 4 : 6-diamino-2-methylthiopyrimidine and the acetyl groups removed from the product with methanolic ammonia. Subsequent debenzoylation, azo-coupling, and acetylation gave 6-*amino*-4-*triacetyl*-L-*arabofuranosidamino*-5-(2′ : 5′-*dichlorobenzeneazo*)-2-*methylthiopyrimidine* (VI; R = Ac). From (VI; R = Ac) 9-L-*arabofuranosido*-2-*methylthioadenine* has been synthesised.

In the course of model experiments it was observed that D-xylosyl-1-amine condenses readily with aniline to form aniline-D-xyloside.

THE synthesis of 9-D-ribopyranosidoadenine (Baddiley, Kenner, Lythgoe, and Todd, Part X, *J.*, 1944, 657), a substance differing from natural adenosine only in the size of the lactol ring (Howard, Kenner, Lythgoe, and Todd, Part XV, *J.*, 1946, 861), was effected by the general method developed in this series for purine nucleoside synthesis. The initial step in this method is condensation of a suitable 4 : 6-diaminopyrimidine with an aldose in hot alcoholic solution in presence of an acidic catalyst, and it appears to yield invariably the corresponding 4-glycopyranosidamino-6-aminopyrimidine. In order to utilise the method for the natural nucleosides it was clear that some modification of the initial step to yield a furanoside rather than a pyranoside would be essential. The present paper records some of our model experiments directed to the solution of this problem; they include some novel methods of glycosidisation.

Although the products of condensation of 4 : 6-diaminopyrimidines with sugars in boiling alcoholic solution were pyranosides it seemed possible that condensation in the cold might yield furanosides by analogy with the synthesis of methylglucofuranoside by Fischer (*Ber.*, 1914, **47**, 1980) and the condensation of aniline with D-ribose by Lee, Solmssen, and Berger (U.S.P., 1945, 2,384,102). However, no reaction between diaminopyrimidines and sugars could be observed in the cold. This result was not entirely unexpected, for condensation even in boiling alcohol is sluggish, and some consideration was given to the use of a more reactive sugar derivative. Glycosyl-1-amines, as evidenced by their very ready hydrolysis, tend to eliminate their 1-amino-group, and this tendency should cause them to react with other amines forming *N*-glycosides. A few experiments showed that D-xylosyl-1-amine (de Bruyn and van Leent, *Rec. Trav. chim.*, 1895, **14**, 144) and aniline react in the cold in aqueous alcoholic solution or in alcoholic solution containing hydrogen chloride, ammonia being liberated and a crystalline aniline-D-xyloside produced. This product was similar in both initial optical rotation and velocity of mutarotation to the aniline-D-xyloside prepared by condensation of aniline with aqueous D-xylose at 100° (Weygand, *Ber.*, 1939, **72**, 1663) and despite some discrepancy in m. p. the identity of the two materials was confirmed by their conversion into identical triacetyl derivatives. The formation of *N*-glycosides by this means does not appear to have been previously recorded and the method might be of value in certain cases. It was not further pursued by us, however, since we were unable to condense xylosimine with 4 : 6-diaminopyrimidine in the cold, and other methods described below were already giving promise of success.

An alternative approach to the problem lay in modifying the existing general synthetic route by condensing a 4 : 6-diaminopyrimidine with a 5-acyl pentofuranose instead of with

* *Note added June 22nd*, 1948.—Since this paper was written further investigation has revealed that the Schiff-base route to 4-pentofuranosidaminopyrimidines, depending upon preferential removal of 2-, 3-, and 4-acetyl groups with retention of a 5-benzoyl group, is unreliable in operation. In subsequent experiments using the method described it was found that the 9-arabinosido-2-methylthioadenine isolated has a periodate titre varying between 2 and 3 mols./mol. This unreliability is even more marked in the ribose series. Full details of these extended studies and of modifications in method will be published in future papers. Meanwhile, it is clear that the route here described normally yields mixtures of furanoside and pyranoside, so that the homogeneity of such intermediates as (VI) must remain open to question.

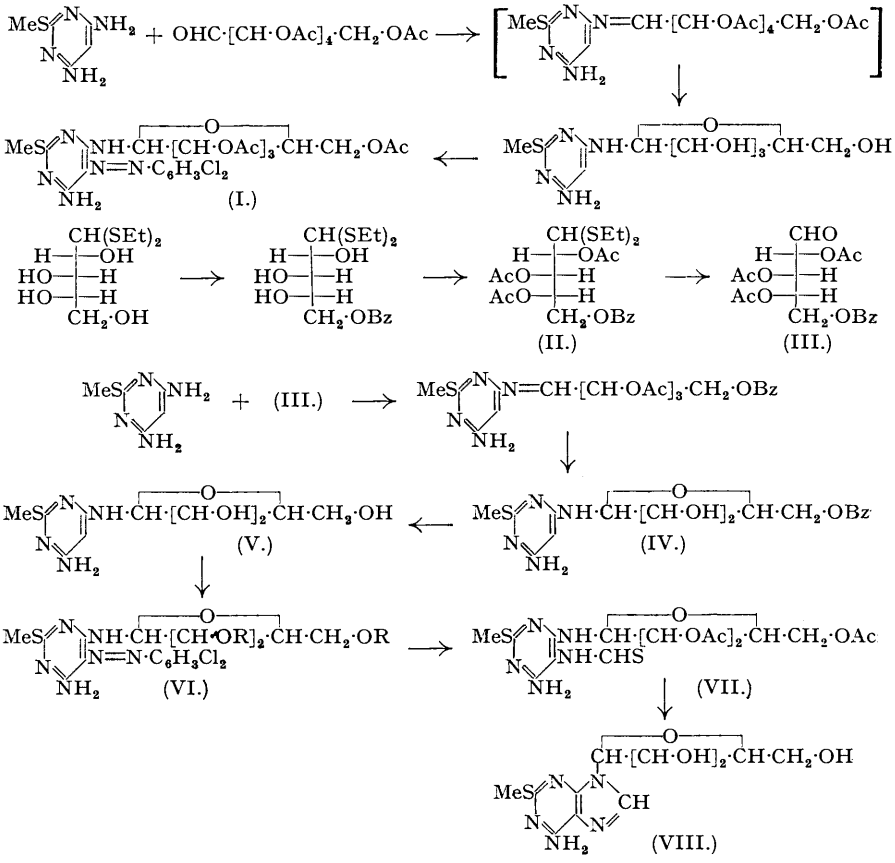
a free sugar. 5-Benzoyl D-ribofuranose was obtained as a syrup by benzoylating 2 : 3-*iso*propylidene methylribofuranoside (Levene and Stiller, *J. Biol. Chem.*, 1934, **104**, 301) and treating the product with dilute acid. Efforts to condense it with 4 : 6-diaminopyrimidine, 4 : 6-diamino-2-methylthiopyrimidine, or 4 : 6-diamino-5-thioformamidopyrimidine by heating in alcoholic solution in presence of acid catalysts by our standard procedure failed. Some reaction appeared to take place, however, since the boiling point of the benzene–alcohol distillates obtained during the experiments indicated that they contained water. The most likely explanation of these results seemed to be a preferential reaction of the furanose sugar component with the alcohol used as solvent. In an attempt to overcome this difficulty methyl cyanide was tried as a reaction medium, but no condensation occurred.

In view of the possibility that the above failures with 5-benzoyl ribose were due to the high reactivity of furanose sugars as compared with their pyranose isomers, the next step was clearly to reduce this reactivity by some means. It was shown that 2 : 3 : 4-triacetyl D-xylopyranose would not condense with 4 : 6-diamino-2-methylthiopyrimidine under the conditions employed successfully for D-xylose (Baddiley, Lythgoe, and Todd, Part III, *J.*, 1943, 571) and this suggested that acetylation of the free hydroxyl groups in a furanose might be a suitable method of deactivation for our purpose. As a model substance 2 : 3 : 5 : 6-tetra-acetyl D-galactofuranose (Compton and Wolfrom, *J. Amer. Chem. Soc.*, 1934, **56**, 1161) was selected. This substance was condensed with 4 : 6-diamino-2-methylthiopyrimidine in boiling alcoholic solution containing a trace of hydrogen chloride, water being removed azeotropically in the usual manner (Part III, *loc. cit.*). The crude product was directly coupled with diazotised 2 : 5-dichloroaniline in pyridine solution and the resulting mixture chromatographed on active alumina. The yellow amorphous product gave analytical results suggesting that it was a mixture of the expected 6-amino-4-tetra-acetyl-D-galactosidamino-5-(2′ : 5′-dichlorobenzeneazo)-2-methylthiopyrimidine with the corresponding sugar-free azo-compound in the proportion 3 : 1. An exactly similar product was obtained when the glycosidisation was repeated using boiling 2-ethoxyethanol as reaction medium; this condensation had to be stopped after only 90 minutes to avoid gross decomposition. Further purification of the products obtained in these reactions by chromatography on alumina was impossible, since it was shown in trial experiments that sugar-free azo-pyrimidines containing a 2-methylthio-substituent, and the corresponding acetylated azo-glycosides, are well-nigh inseparable on columns of this adsorbent. Unacetylated azo-glycosides on the other hand are very firmly adsorbed on alumina and cannot be eluted even with pyridine. Accordingly the crude mixture of azo-compounds obtained in the above condensations was deacetylated and then put on an alumina column which was washed with pyridine: the azo-glycoside then remained at the top of the column as a yellow band which could be extruded, and the glycoside acetylated on the adsorbent by treatment with acetic anhydride in pyridine solution. The product was eluted with ethyl acetate and purified by chromatography on alumina from the same solvent. In this way 6-*amino*-4-*tetra-acetyl*-D-*galactofuranosidamino*-5-(2′ : 5′-*dichlorobenzeneazo*)-2-*methylthiopyrimidine* was obtained as a deep yellow powder. Its formulation as a furanoside is supported by the fact that it is quite different from the corresponding *pyranoside* prepared by coupling 6-*amino*-4-D-*galactopyranosidamino*-2-*methylthiopyrimidine* (prepared by Mr. H. Smith in this laboratory) with diazotised 2 : 5-dichloroaniline and acetylating the product in the normal manner. Application of this method for the production of furanosides to the synthesis of adenosine would, of course, involve the use of the hitherto unknown 2 : 3 : 5-triacetyl ribofuranose. The present inaccessibility of this compound, coupled with the parallel discovery of the following more attractive route to furanosidaminopyrimidines, led us temporarily to suspend exploitation of the method. It may be observed in passing that 2 : 3 : 5 : 6-tetra-acetyl D-galactofuranose did not condense under normal conditions with 4 : 6-diaminopyrimidine or with 4 : 6-diamino-5-thioformamidopyrimidine. It may be that other conditions might be found to effect condensation with the former compound, but this is known to be much less reactive than the 2-methylthio-compound in glycosidisations using free sugars.

Ring-chain tautomerism in *N*-glycosides has been the subject of much speculation and investigation since Schiff (*Annalen*, 1870, **154**, 30) first condensed aniline with D-glucose. On the evidence available up to the present it would appear that, although compounds possessing the open-chain Schiff's base structure may exist as transient intermediates in the mutarotation of *N*-glycosides, they have never been isolated, and all attempts to prepare them lead to the cyclic isomers. These facts suggested a novel method of glycoside synthesis which might prove applicable to the formation of furanosides or pyranosides at will, for it seemed reasonable to suppose that acylated *aldehydo*-sugars might condense with amines to form Schiff's

bases which, on removal of acyl groups, would be at once converted into the isomeric glycosides.

It was readily shown in preliminary experiments that benzaldehyde condenses with 4 : 6-diamino-2-methylthiopyrimidine in alcoholic solution although the Schiff's base was not isolated in crystalline form. When 2 : 3 : 4 : 5 : 6-penta-acetyl glucose was substituted for benzaldehyde condensation occurred at room temperature in alcoholic solution, more readily in presence of a little ammonium chloride. The Schiff's base was not isolated, the condensation product being at once deacetylated by means of sodium methoxide or methanolic ammonia. Attempts to crystallise the resulting glucoside failed and it was coupled in the usual manner with diazotised 2 : 5-dichloroaniline and the product acetylated giving 6-*amino*-4-*tetra-acetyl*-D-*glucopyranosidamino*-5-(2′ : 5′-*dichlorobenzeneazo*)-2-*methylthiopyrimidine* (I), identical with a specimen prepared in similar fashion from authentic crystalline 6-amino-4-D-glucopyranosidamino-2-methylthiopyrimidine (prepared by Mr. A. Holland in the course of other work in this laboratory). It is probable that the crude resinous glucoside obtained by deacetylating the Schiff's base is a mixture of α- and β-isomers, and in accordance with this view the acetylated azo-glucoside, isolated in a pure state, represented only part of the crude acetylated product. The production of a glucopyranoside by this method was expected but it contrasts with the production of *N*-acetyl-D-glucofuranosylamine by the action of ammonia on penta-acetyl *aldehydo*-D-glucose (Hockett and Chandler, *J. Amer. Chem. Soc.*, 1944, **66**, 957; Niemann and Hays, *ibid.*, 1945, **67**, 1302). It is interesting to note that the increased reactivity of 4 : 6-diamino-2-methylthiopyrimidine as compared with 4 : 6-diaminopyrimidine is again shown by the failure of the latter compound to condense with penta-acetyl *aldehydo*-glucose; 4 : 6-diamino-5-thioformamido-2-methylthiopyrimidine also fails to condense.



Adaptation of this new method of glycosidisation to the synthesis of furanosides by protecting the hydroxyl at C_5 in the sugar chain during the tautomeric change leading to the glycoside was

envisaged. The necessary differential acylation at C_5 in the *aldehydo*-sugar is simplest in the pentoses where the hydroxyl concerned is primary, and the benzoyl group seemed to fill the requirements of a protecting group, since it is readily removed by sodium methoxide but is stable to methanolic ammonia under conditions which cause complete removal of acetyl groups. For reasons of economy L-arabinose was chosen for our model experiments. *5-Benzoyl 2 : 3 : 4-triacetyl L-arabinose diethylthioacetal* (II), readily obtained from 5-benzoyl L-arabinose diethylthioacetal (Lieser and Schweizer, *Annalen*, 1935, **519**, 271), was converted by standard methods into *5-benzoyl 2 : 3 : 4-triacetyl* aldehydo-L-*arabinose* (III), a syrup which could not be crystallised. Condensation of (III) with 4 : 6-diamino-2-methylthiopyrimidine in alcoholic solution in presence of ammonium chloride gave a product which, largely freed from unreacted diamine by dissolution in chloroform, was deacetylated by treatment in the cold with methanolic ammonia. To avoid any complications arising from possible incomplete deacetylation or partial debenzoylation the product, presumably for the most part the glycoside (IV), was treated with hot sodium methoxide solution. As it was expected that the resulting product (V) would be very labile no attempt was made to remove unchanged diamine by alumina treatment, and the crude product was coupled directly with diazotised 2 : 5-dichloroaniline. The *azo-glycoside* (VI; R = H) was readily separated from sugar-free azo-diamine by chromatography on activated alumina, dry pyridine being used as solvent; the sugar-free compound passed rapidly through the column while the glycoside was firmly held and could subsequently be eluted by washing with pyridine to which sufficient water had been added to deactivate the alumina. The resinous product obtained was freed from other impurities by acetylation and subsequent chromatography; *6-amino-4-triacetyl-L-arabofuranosidamino-5-(2′ : 5′-dichlorobenzeneazo)-2-methylthiopyrimidine* (VI; R = Ac) was thus obtained as a yellow powder; the pure *azo-glycoside* (VI; R = H) was obtained from the acetyl derivative by treatment with methanolic ammonia. As an alternative the crude azo-glycoside adsorbed on alumina could be acetylated *in situ* and eluted as the triacetyl derivative (VI; R = Ac); both methods gave comparable yields.

For purposes of comparison the pyranose compounds corresponding to (VI) were prepared. Condensation of 4 : 6-diamino-2-methylthiopyrimidine with L-arabinose in the ordinary way gave a syrup which was coupled with diazotised 2 : 5-dichloroaniline and the product acetylated. Chromatographic purification gave *6-amino-4-triacetyl-L-arabopyranosidamino-5-(2′ : 5′-dichlorobenzeneneazo)-2-methylthiopyrimidine* as yellow prisms quite distinct from (VI; R = Ac). Deacetylation gave the yellow *6-amino-4-L-arabopyranosidamino-5-(2′ : 5′-dichlorobenzeneazo)-2-methylthiopyrimidine* which was not identical with (VI; R = H). There was thus good reason to believe that the azo-compounds (VI; R = H or Ac) were in fact furanosides. The only alternative, *viz.*, that they were α- or β-isomers of the pyranosides obtained by the direct glycosidisation process, seemed unlikely. Clearly, however, the simplest way to settle this point conclusively was by completion of the projected purine synthesis and application of the periodate oxidation method for determining lactol ring-structure (cf. Part XV, *loc. cit.*) to the synthetic purine glycoside.

The completion of a purine glycoside synthesis from (VI; R = H or Ac) presented certain new features. Hitherto the necessary 5-amino-group has been introduced into 6-amino-4-glycosidamino-2-methylthiopyrimidines by nitrosation followed by reduction (Part XI, *loc. cit.*; Part XVI, *J.*, 1947, 355); the use of azo-compounds as intermediates has so far been avoided in the case of derivatives containing a 2-methylthio-group. For the present synthesis we considered the nitrosation route but found that 6-amino-4-L-arabofuranosidamino-2-methylthiopyrimidine (V) gave a water-soluble nitroso-derivative, resembling in this respect the corresponding arabopyranoside and ribopyranoside (observation by Mr. H. T. Howard). Neither this product nor its acetyl derivative could be obtained in crystalline form. Attempts to obtain the acetyl deriative in a purer condition by acetylating (V) before nitrosation again yielded only a green syrup in poor yield. The use of nitroso-compounds as synthetic intermediates was therefore abandoned, although it is probable that a synthesis employing them might be developed by further work. Attention was instead devoted wholly to the use of the azo-compound (VI; R = Ac) as an intermediate for nucleoside synthesis. This involved an extended study of the reductive fission of the azo-group by various methods, since it was thought that the 2-methylthio-group would make the usual catalytic hydrogenation procedure troublesome.

A possible solution of the problem seemed to lie in treatment of (VI; R = Ac) in alcoholic solution with massive quantities of Raney nickel containing adsorbed hydrogen so as to cause simultaneous fission of the azo-group and removal of the 2-methylthio-group. When this

method was applied to 6-amino-4-triacetyl-D-xylopyranosido-5-(2′ : 5′-dichlorobenzeneazo)-2-methylthiopyrimidine in a model experiment, the expected 5 : 6-diamino-4-D-xylopyranosidaminopyrimidine was formed, although in rather low yield. An extended series of experiments showed, however, that high yields were unobtainable and the results were very variable; no method of controlling the reaction could be devised. The method proved equally unsatisfactory when applied to (VI; R = Ac). The indifferent results were doubtless due, in part, to formation of nickel complexes and the ready oxidation of the products, but the unusual reduction method, which probably does not cause simple fission of the azo-linkage in the manner of a normal hydrogenation, was almost certainly a contributory factor.

The important question of the mechanism of hydrogenolysis by Raney nickel under the conditions used in the normal desulphurisation method (Mozingo, *J. Amer. Chem. Soc.*, 1943, **65**, 1013) is not here directly relevant, and detailed consideration of it is deferred. Various observations suggest that it involves attack by atomic hydrogen with intermediate formation of free radicals; for example, Mozingo, Spencer, and Folkers (*J. Amer. Chem. Soc.*, 1944, **66**, 1859) record production of 43% of *N*-ethylaniline from hydrazobenzene under these conditions.

The unsatisfactory results obtained in these experiments led us to examine the application of normal catalytic hydrogenation procedures to 5-arylazopyrimidines containing a 2-methylthio-substituent. Using hydrogen at atmospheric pressure, a Raney nickel catalyst, although effective, had to be used in such large quantities that it was impracticable. The sulphur-resistant palladium–polyvinyl acetate catalyst of Kavanagh and Nord (*J. Amer. Chem. Soc.*, 1943, **65**, 2121) also brought about reduction of the azo-compound, but the low maximum concentration of catalyst made its use inconvenient. However, using only moderate quantities of Raney nickel, hydrogenation at 80° under 100 atmospheres' pressure was shown to be effective in model experiments. Simultaneously an independent approach was made using chemical methods of reduction, and zinc dust in weakly acid media was found to be effective.

Application of the zinc dust or catalytic methods of reduction to (VI; R = Ac), followed by treatment of the crude reduction product with dithioformic acid, gave 6-amino-5-thioformamido-4-triacetyl-L-arabofuranosidamino-2-methylthiopyrimidine (VII) as a colourless resin. Cyclisation of (VII) proceeded normally by boiling in alcoholic solution with sodium alkoxide, giving 9-L-*arabofuranosido*-2-*methylthioadenine* (VIII). The location of the sugar residue in (VIII) follows from its mode of preparation and is borne out by its insolubility in alkali, and the similarity between its absorption spectrum and that of 9-D-xylopyranosido-2-methylthioadenine. Its furanoside structure was rigidly established by periodate titration (Part VIII, *J.*, 1944, 592), 2 mols. of oxidant being consumed without production of formic acid; under similar conditions 2-methylthioadenine consumes 1 mol. of periodate (Part XI, *loc. cit.*).

The evidence presented defines the structure of the 9-L-arabofuranosido-2-methylthioadenine in all respects save for the configuration at the glycosidic carbon atom. In the absence of suitable data for comparison, the optical rotations of the glycoside and its periodate fission product do not provide reliable evidence on this point. The β-configuration might, however, be expected on general grounds since mutarotation to the β-form is normally found after reduction of 5-arylazopyrimidine intermediates (Part XIV, *J.*, 1946, 855).

The synthetic method described should be capable of extension to other 9-glycofuranosido-2-methylthioadenines and, since it has already been shown that 9-glycopyranosido-2-methylthioadenines can be converted into 9-glycopyranosidoadenines by desulphurisation with Raney nickel (Parts XI and XVI, *locc. cit.*), the results so far obtained indicate a route to the synthesis of adenosine itself. Experiments designed to effect such a synthesis are in progress.

Experimental.

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

Reaction of D-*Xylosyl*-1-*amine with Aniline.*—(1) Aniline (2·5 g.) and D-xylosyl-1-amine (4·05 g.) (De Bruyn and Van Leent, *loc. cit.*) were dissolved in water (20 c.c.) and alcohol (7 c.c.) and left for 12 days. Extraction with ether (3 portions of 150 c.c.) followed by evaporation of the ether yielded a syrup which solidified and crystallised from alcohol in fine needles (0·37 g.), m. p. 131° (Found in material dried at 56°: C, 58·6; H, 6·9; N, 6·2. Calc. for $C_{11}H_{15}O_4N$: C, 58·7; H, 6·7; N, 6·2%).

(2) Aniline (1·85 g.) and D-xylosyl-1-amine (3·0 g.) were shaken for 10 days with dry methanol (50 c.c.) and ethanolic hydrochloric acid (5 c.c. of N). The product, recrystallised from alcohol (0·82 g.), had m. p. 131°, undepressed by material prepared as in (1) above.

The glycoside was hydrolysed by refluxing for 30 minutes with N/10-sulphuric acid yielding aniline, identified as tribromoaniline, and D-xylose, identified as tetra-acetyl β-D-xylose.

Aniline-D-xyloside, prepared according to Weygand (*loc. cit.*), had m. p. 140°; a mixed m. p. with the

xyloside (m. p. 131°) described above was 133—135°. The crystalline form of the two specimens was identical, as was their mutarotation behaviour when examined in aqueous solution (*c*, 2·5 at 18°):

Time (mins.)	3	4	5	7	10	15	20	25	30
$[\alpha]_D$, xyloside, m. p. 131°	−66·4°	−63·6°	−61·5°	−60·6°	−56·5°	−56·2°	−55·1°	−55·1°	−54·9°
$[\alpha]_D$, xyloside, m. p. 140°	−67·2°	−66·2°	−65·2°	−63·3°	−61·0°	−59·6°	−58·4°	−58·4°	−58·0°

On acetylation with acetic anhydride in pyridine solution in the cold, both samples of xyloside gave the same product, m. p. and mixed m. p. 148—149°.

5-*Benzoyl* 2 : 3-iso*Propylidene Methyl*-D-*ribofuranoside*.—2 : 3-*iso*Propylidene methyl-D-ribofuranoside (19·2 g. : 0·01 mol.) (Levene and Stiller, *loc. cit.*), dissolved in pyridine (25 c.c.), was slowly treated with a solution of benzoyl chloride (15·15 g.; 0·011 mol.) in chloroform (25 c.c.) with shaking, the temperature being kept below 30°. After 2 days, solvent was evaporated, the residue treated with ice-water, and the mixture extracted several times with chloroform. The combined chloroform solutions were washed with ice-cold N-sulphuric acid, ice-cold aqueous sodium carbonate (10%), and ice-water (three times with each), and dried (Na_2SO_4). The syrup obtained on evaporation was distilled at 139°/2 × 10^{-3} mm. (yield, 22·2 g.; 75%) (Found in material redistilled at 170°/0·01 mm.: C, 63·1; H, 6·7. $C_{16}H_{20}O_6$ requires C, 62·4; H, 6·5%).

5-*Benzoyl* 1 : 2 : 3-*Triacetyl* D-*Ribose*.—5-Benzoyl 2 : 3-*iso*propylidene methyl-D-ribofuranoside (7·5 g.) was heated in boiling hydrochloric acid (100 c.c. of 0·04N) for 2½ hours. The syrup remaining after evaporation of the filtered solution, which had been shaken with barium carbonate, was extracted with warm *n*-butanol (70 c.c.). Thorough evaporation of the extract yielded a thick resin (5·7 g.; 92%); this was used in subsequent experiments as 5-benzoyl ribose. For identification the product (0·57 g.) was dissolved in pyridine (5 c.c.) and acetic anhydride (2·5 c.c.) and left overnight. Alcohol (3 c.c.) was added, and after 2 hours the liquid was evaporated, the residue dissolved in hot alcohol (5 c.c.), and the 5-*benzoyl* 1 : 2 : 3-*triacetyl* D-*ribose*, which crystallised on cooling, collected and recrystallised from alcohol; colourless plates (0·22 g.), m. p. 117—118° (Found: C, 56·8; H, 5·5. $C_{18}H_{20}O_9$ requires C, 56·8; H, 5·3%).

6-*Amino*-4-*tetra-acetyl*-D-*galactofuranosidamino*-5-(2′ : 5′-*dichlorobenzeneazo*)-2-*methylthiopyrimidine*.—Tetra-acetyl-D-galactofuranose (0·7 g.) (Compton and Wolfrom, *loc. cit.*) and 4 : 6-diamino-2-methylthiopyrimidine (0·94 g.) were dissolved in boiling alcohol (25 c.c.), to which an alcoholic solution of hydrogen chloride (0·2 c.c. of 1·9N) had been added. Water was removed as the benzene–alcohol azeotrope through a 20 cm. Fenske column with reflux ratio head, and heating continued for 25 hours during 4 days. The resulting solution was then subjected to one of the following treatments.

(*a*) Solvent was evaporated and the residue extracted with chloroform. After evaporation of the chloroform extract the residue was dissolved in pyridine (10 c.c.) and treated with a neutral diazotised aqueous solution of 2 : 5-dichloroaniline (0·73 g.). Next day, after dilution with water (100 c.c.), the solid was collected, dried in a vacuum, and extracted with hot benzene (40 c.c.). The extract was adsorbed on alumina (40 g.) and the column (35 × 3 cm.) developed with ethyl acetate. The main broad yellow band was washed through rapidly and yielded a yellow solid (0·29 g.). This was evidently a mixture and could not be separated into its components by fractional crystallisation from benzene–alcohol (Found in material dried at 60°: C, 44·2; H, 3·9; N, 16·0. Calc. for a mixture containing 76% of $C_{25}H_{28}O_9N_6Cl_2S$ and 24% of $C_{11}H_{10}N_6Cl_2S$: C, 44·2; H, 3·9; N, 15·8%).

(*b*) A solution of sodium (0·023 g.) in alcohol (5 c.c.) was added and the solution boiled for 30 minutes before being poured into a neutral diazotised solution of 2 : 5-dichloroaniline (0·97 g.). After 15 minutes the precipitated solid was filtered off, washed with water, and dried at 50° (2·55 g.). It was dissolved in pyridine (25 c.c.) and adsorbed on alumina (50 g.) Washing the column with pyridine eluted a yellow band containing 4 : 6-diamino-5-(2′ : 5′-dichlorobenzeneazo)-2-methylthiopyrimidine (1·08 g.). The top portion of the column was removed, diluted with a little pyridine, and left overnight with acetic anhydride (10 c.c.). Alcohol (10 c.c.) was then added and the mixture kept for 1 hour in a bath of cold water. The solution was decanted and the alumina extracted with cold ethyl acetate–pyridine (9 : 1.; 200 c.c. in 6 portions). The solution and extracts were evaporated, taken up in ethyl acetate (15 c.c.) and chromatographed on alumina (50 g.; column 25 × 2 cm.). The broad yellow band which was first eluted contained an amorphous material which separated from alcohol as a yellow powder of indefinite m. p. *ca.* 140°; $[\alpha]_D^{20°}$ − 335° (± 20°) (*c*, 0·17 in chloroform) (Found in material dried at 80°: C, 45·3; H, 4·3; N, 12·8. $C_{25}H_{28}O_9N_6Cl_2S$ requires C, 45·5; H, 4·2; N, 12·7%).

6-*Amino*-4-D-*galactopyranosidamino*-2-*methylthiopyrimidine* (by Mr. H. Smith).—Ammonium chloride (1 g.), D-galactose (8 g.), and 4 : 6-diamino-2-methylthiopyrimidine (24 g.) were refluxed in absolute alcohol (300 c.c.) for 8 hours in a flask fitted with a Fenske column with reflux ratio head, water being removed azeotropically with benzene. Next day the mixture, which contained a small amount of crystalline material, was adsorbed on active alumina (1 kg.) which was washed with alcohol to remove unreacted diamine. Elution with water (2·5 l.) and concentration to small bulk caused crystallisation of the *galactoside* in pellet-like aggregates of colourless hydrated needles (10·15 g.; 72%). On being heated, the recrystallised product appeared to lose water at 95—96° and had m. p. *ca.* 176° (decomp.); $[\alpha]_D^{20°}$ − 58° (± 0·6°) (*c*, 0·3507 in water) (Found in material dried at 100°/0·1 mm.: C, 40·8; H, 5·7; N, 17·6. $C_{11}H_{18}O_5N_4S$ requires C, 41·4; H, 5·7; N, 17·6%).

6-*Amino*-4-*tetra-acetyl*-D-*galactopyranosidamino*-5-(2′ : 5′-*dichlorobenzeneazo*)-2-*methylthiopyrimidine*.—6-Amino-4-D-galactopyranosidamino-2-methylthiopyrimidine (0·5 g.) was suspended in water (5 c.c.) and treated with a diazotised solution of 2 : 5-dichloroaniline (0·4 g.), followed immediately by excess of aqueous sodium bicarbonte. After 1 hour the precipitated solid was filtered off, washed with water, and dried at 60° (0·62 g.). It was kept overnight in pyridine (10 c.c.) and acetic anhydride (3 c.c.); alcohol (5 c.c.) was then added, and after 1 hour the solvent was evaporated. The residue, dissolved in ethyl acetate, was chromatographed on a column of alumina (100 g.; 3 cm. bore). Evaporation of the eluate

of the broad yellow band yielded a resin (0·52 g.) which, on crystallisation from alcohol (10 c.c.) containing a little ethyl acetate, gave fine orange needles, m. p. 242—243°; $[\alpha]_D^{20°}$ — 443° (± 10°) (*c*, 0·26 in chloroform) (Found in material dried at 140°: C, 45·0; H, 4·5; N, 12·4. $C_{25}H_{28}O_9N_6Cl_2S$ requires C, 45·5; H, 4·2; N, 12·7%).

6-*Amino*-4-*tetra-acetyl*-D-*glucopyranosidamino*-5-(2′ : 5′-*dichlorobenzeneazo*)-2-*methylthiopyrimidine*.—(a) *From penta-acetyl* aldehydo-D-*glucose*. Ammonium chloride (0·03 g.; 0·6 mol.) and 4 : 6-diamino-2-methylthiopyrimidine (1·26 g.; 8·0 mols.) were dissolved in boiling absolute alcohol (40 c.c.) and 2 : 3 : 4 : 5 : 6-penta-acetyl D-glucose (0·78 g.; 2·0 mols.) (Wolfrom, *J. Amer. Chem. Soc.*, 1929, **51**, 2188) added, the mixture being shaken until solution was complete. After 24 hours the solvent was evaporated and the residue left for two days with methanolic ammonia (30 c.c. of 5N). The residue obtained on evaporation was dissolved in alcohol (25 c.c.) and adsorbed on a column of alumina (30 g.; 3 cm. bore), which was washed with alcohol (70 c.c.) to remove unchanged diamine (1·06 g.; 6·8 mols.) and eluted with water (70 c.c.). No crystalline glucoside separated in 3 days from the eluate concentrated to 8 c.c. A neutral diazotised solution of 2 : 5-dichloroaniline (0·32 g.; 2 mols.) was added and the precipitated azo-compound collected after ½ hour and dried (0·48 g.; 51%). The crude material was acetylated by being kept overnight with pyridine (4 c.c.) and acetic anhydride (2 c.c.). The resinous product was chromatographed on alumina (40 g.; 2·5 cm. bore) in ethyl acetate; evaporation of the eluate from the broad yellow band yielded an orange resin (0·51 g.; 39%). After three recrystallisations from alcohol, the product formed needles, m. p. 218—219°; $[\alpha]_D^{20°}$ — 456° (± 40°) (*c*, 0·08 in chloroform) (Found in material dried at 100°: C, 45·8; H, 4·0; N, 12·5. $C_{25}H_{28}O_9N_6Cl_2S$ requires: C, 45·5; H, 4·2; N, 12·7%).

(b) *From* 6-*amino*-4-D-*glucopyranosidamino*-2-*methylthiopyrimidine*. The crystalline glucoside (0·64 g.; prepared by Mr. A. Holland) was mixed with a diazotised solution of 2 : 5-dichloroaniline (0·4 g.) and rapidly neutralised by addition of an aqueous solution of sodium bicarbonate (2 g.). After 1 hour the azo-compound was collected. Recrystallised from alcohol–pyridine it had m. p. 268°. A portion of the crude material (0·15 g.) was left overnight with pyridine (3 c.c.) and acetic anhydride (1 c.c.). Alcohol (2 c.c.) was then added, the solvent evaporated after 1 hour, and the residue recrystallised from benzene; fine yellow needles, m. p. 220—221°, undepressed in admixture with material prepared as in (a) above; $[\alpha]_D^{20°}$ — 486° (± 10°) (*c*, 0·25 in chloroform).

5-*Benzoyl* 2 : 3 : 4-*Triacetyl* L-*Arabinose Diethylthioacetal*.—Acetic anhydride (100 c.c.) was added slowly to an ice-cold stirred solution of 5-benzoyl L-arabinose diethylthioacetal (36 g.; Lieser and Schweizer, *Annalen*, 1935, **519**, 271) in dry pyridine (150 c.c.), and the mixture left overnight and then poured into ice-water (2 l.). Extraction with chloroform and evaporation of the dried extract gave a syrup which was dissolved in warm methanol. Water was added until the solution became slightly opalescent. The *triacetyl* compound slowly separated in colourless prisms (42 g.), m. p. 44—45°, unchanged on further recrystallisation; $[\alpha]_D^{14°}$ — 26·8° (*c*, 2·0 in chloroform) (Found: C, 54·8; H, 6·3. $C_{22}H_{30}O_8S_2$ requires C, 54·3; H, 6·2%).

5-*Benzoyl* 2 : 3 : 4-*Triacetyl* L-*Arabinose*.—Cadmium carbonate (30 g.) and 5-benzoyl 2 : 3 : 4-triacetyl L-arabinose diethylthioacetal (15·5 g.) were stirred with acetone (57·6 c.c.) and water (20 c.c.), and a solution of mercuric chloride (31·2 g.) in acetone (57·6 c.c.) slowly dropped in. After 15 hours' stirring at room temperature with occasional addition of fresh cadmium carbonate, the temperature was raised to 50° for 15 minutes, and to 70° for a further 15 minutes. The cooled mixture was filtered, and the residue well washed with acetone. The combined filtrate and washings were evaporated and the residue was repeatedly evaporated with dry acetone, extracted with chloroform (200 c.c.), and the extract dried (Na_2SO_4). Evaporation of the extract gave 5-*benzoyl* 2 : 3 : 4-*triacetyl* L-*arabinose* as a clear syrup which gave a positive Schiff's test, but could not be crystallised. Distillation at 170° (bath temp.)/3×10^{-4} mm. gave a yellowish syrup (Found: C, 57·7; H, 5·5. $C_{18}H_{20}O_9$ requires C, 56·8; H, 5·3%).

6-*Amino*-4-*triacetyl*-L-*arabofuranosidamino*-5-(2′ : 5′-*dichlorobenzeneazo*)-2-*methylthiopyrimidine*.—(1) Ammonium chloride (0·56 g.) and 4 : 6-diamino-2-methylthiopyrimidine (20·2 g.) were dissolved in boiling absolute alcohol (480 c.c.) and added to syrupy 5-benzoyl 2 : 3 : 4-triacetyl L-arabinose prepared from the mercaptal (15·5 g.), and the resulting solution was left for 20 hours. The solution was evaporated and the residue extracted with chloroform (160 c.c.), leaving unchanged diamine (13·8 g.). The chloroform extract was evaporated, the residue left for 2 days with methanolic ammonia (320 c.c. of 5N) to remove the acetyl groups, and the solution evaporated. A solution of sodium (1·47 g.) in methanol (200 c.c.) was added, and the whole boiled for 30 minutes, cooled, and concentrated to 80 c.c. After addition of glacial acetic acid (3·05 c.c.) the solution was poured into a neutral diazotised solution of 2 : 5-dichloroaniline (6·5 g.) and the precipitated yellow solid (15·5 g.) collected after 15 minutes, washed with water, and dried. The crude azo-compound was dissolved in pyridine (200 c.c.) and adsorbed on a column of alumina (500 g.; 8·5 cm. bore), which was then washed with pyridine to remove sugar-free azo-compound. The upper part of the column containing the adsorbed azo-glycoside was extruded, washed with water (80 c.c.), and eluted with pyridine–water (9 : 1; 1·2 l.). Evaporation of the eluate gave a resin which was left overnight with acetic anhydride (15 c.c.) and pyridine (30 c.c.). Excess of acetic anhydride was decomposed with alcohol, the solution evaporated, and the residue chromatographed on active alumina (180 g.; column 5·5 cm. bore) in ethyl acetate solution. The resinous acetylated *azo-glycoside* (5·85 g.; 31%) passed through as a pure yellow band. On dissolution in hot alcohol and allowing the solution to cool, the product was obtained as a yellow amorphous powder, m. p. *ca.* 135°; it had $[\alpha]_D^{20°}$ — 124° (± 10°) (*c*, 0·25 in chloroform) (Found in material dried at 100°: C, 45·3; H, 4·4; N, 13·9. $C_{22}H_{24}O_7N_6Cl_2S$ requires C, 45·0; H, 4·1; N, 14·3%).

(2) The crude azo-glycoside (1 g.) obtained as in (1) above was stirred with pyridine (25 c.c.) and adsorbed on an alumina column (30 × 3 cm.) which was well washed with pyridine. The upper half of the column was extruded and left overnight with acetic anhydride (10 c.c.) and pyridine (20 c.c.). Unchanged acetic anhydride was decomposed by alcohol (10 c.c.) and the product eluted with hot ethyl acetate. Purification by chromatography gave acetylated azo-glycoside (0·4 g.; 33%), identical with that obtained by (1).

6-*Amino*-4-L-*arabofuranosidamino*-5-(2′ : 5′-*dichlorobenzeneazo*)-2-*methylthiopyrimidine*.—A solution of the above acetylated azo-glycoside (0·3 g.) in chloroform (8 c.c.) and saturated methanolic ammonia (20 c.c.) was left for 3 days then evaporated to dryness. Recrystallisation of the residue from alcohol yielded the *azo-glycoside* as yellow prisms, m. p. 217—218° (Found in material dried at 140° : C, 41·2; H, 4·1; N, 17·7. $C_{16}H_{18}O_4N_6Cl_2S$ requires C, 41·6; H, 3·9; N, 18·2%).

6-*Amino*-4-*triacetyl*-L-*arabopyranosidamino*-5-(2′: 5′-*dichlorobenzeneazo*)-2-*methylthiopyrimidine*.—Ammonium chloride (0·3 g.), L-arabinose (5 g.), and 4 : 6-diamino-2-methylthiopyrimidine (10 g.) were heated in boiling absolute alcohol (80 c.c.) for 1 hour. The flask was then fitted with a Fenske column with reflux ratio head and the solution refluxed for 8 hours, water being removed azeotropically with benzene. Next day the mixture was adsorbed on active alumina (500 g.) which was washed with alcohol (3·5 l.); evaporation of the washings gave unchanged diamine (5 g.). The glycoside was eluted with water (6 l.) and the eluate concentrated to 80 c.c. As no crystals separated, a portion (three-tenths = 0·01 mole of sugar) of this solution was treated with a neutral diazotised solution of 2 : 5-dichloroaniline (1·62 g.), and the yellow precipitate (3·3 g.) collected and dried. Acetylation of this by leaving it overnight with acetic anhydride (5 c.c.) in pyridine (15 c.c.) and purification of the product by chromatography in ethyl acetate solution gave the acetylated *azo-glycoside* (2·7 g.; 46%) as an orange yellow powder. Slow crystallisation from alcohol containing a little ethyl acetate gave the compound in orange prisms which sintered somewhat at 160° and melted at 213—214°; $[\alpha]_D^{20°}$ — 209° (± 5°) (*c*, 0·42 in chloroform) (Found in material dried at 100° : C, 44·7; H, 4·2; N, 14·3. $C_{22}H_{24}O_7N_6Cl_2S$ requires C, 45·0; H, 4·1; N, 14·3%).

6-*Amino*-4-L-*arabopyranosidamino*-5-(2′ : 5′-*dichlorobenzeneazo*)-2-*methylthiopyrimidine*.—Sodium methoxide (from 0·05 g. of sodium in 3 c.c. of methanol) was added to the above triacetyl compound (0·5 g.) in chloroform (3 c.c.) and the mixture left overnight and worked up in the usual manner. The *azo-glycoside* crystallised from pyridine–alcohol in flat orange prisms, m. p. 233—234° (Found in material dried at 140° : N, 17·9. $C_{16}H_{18}O_4N_6Cl_2S$ requires N, 18·2%).

9-L-*Arabofuranosido*-2-*methylthioadenine*.—(1) Zinc dust (8 g.) and 6-amino-4-triacetyl-L-arabinofuranosidamino-5-(2′ : 5′-dichlorobenzeneazo)-2-methylthiopyrimidine (1 g.) were stirred vigorously in boiling ethyl acetate (80 c.c.), and acetic acid (4 c.c.) in ethyl acetate (40 c.c.) added dropwise during 1 hour. The liquid was then decanted, the zinc extracted with warm ethyl acetate (2 × 50 c.c.), and the combined solution and extracts were evaporated to dryness in a nitrogen atmosphere and the resin triturated with *n*-hexane. The insoluble residue was dissolved in ethyl acetate (60 c.c.), dithioformic acid (from 4 g. of sodium salt) added, and the mixture refluxed for 2 hours before the addition of a second portion of dithioformic acid (from 2 g. of sodium salt). Heating was continued for a further hour and the mixture set aside overnight. The solution was decanted, the residue extracted with boiling alcohol (4 × 50 c.c.), and the combined solution and extracts were evaporated to dryness. The residue was dissolved in ethyl acetate (25 c.c.) and chromatographed on a column of neutral alumina (35 g.; 3 cm. bore), the column being developed with ethyl acetate (80 c.c.) before elution with pyridine (100 c.c.); evaporation of the eluate gave a colourless resin (450 mg.) consisting mainly of the thioformamido-compound (IX) contaminated with some sugar-free material.

The dried crude thioformamido-compound (450 mg.) was heated under reflux with sodium methoxide (53 mg.) and absolute alcohol (20 c.c.) in a nitrogen atmosphere for 4 hours. The solution was evaporated, water (6 c.c.) was added, and the mixture set aside for 2 days at 0°. The 9-L-*arabofuranosido*-2-*methylthioadenine* which separated (138 mg.; 26% of theory calculated on acetylated azo-glycoside) was collected and recrystallised from hot water; it then formed colourless needles, m. p. 274° (decomp.); $[\alpha]_D^{13°}$ + 46° (± 10°) (*c*, 0·13 in water) (Found in material dried at 140° : C, 42·3; H, 4·9; N, 22·8. $C_{11}H_{15}O_4N_5S$ requires C, 42·2; H, 4·8; N, 22·4%). Ultra-violet absorption : In N/20-hydrochloric acid, maxima at 2200 A. (ε, 15,900) and 2680 A. (ε, 16,300). In N/20-sodium hydroxide, maxima at 2340 A. (ε, 21,400) and 2755 A. (ε, 15,500).

Periodate titration. 46·7 Mg. of glycoside in water + 5 c.c. of sodium metaperiodate (0·238M) made up to 25 c.c. total volume. Periodate consumed ≡ 2·1 mols.; liberation of acid, nil. Rotation of solution after oxidation, + 0·03° (± 0·02°) (*l*, 1 dm.), i.e. $[M]_D^{13°}$ of fission product, + 5000° (± 3000°).

Note. As the presence of sugar-free material in the crude thioformamido-compound causes wastage of reagent it was found desirable in later runs to employ twice the amount of sodium methoxide used in the above preparation.

(2) The acetylated azo-furanoside (1 g.) and Raney nickel (0·2 g.) were stirred with ethyl acetate (100 c.c.) and hydrogen under 90 atmospheres at 80° for 8 hours. The liquid was decanted, the nickel washed with cold alcohol, and the solution filtered with supercel; subsequent operations were as in (1) above. Yield of crude thioformamido-compound, 325 mg. Yield of 9-L-arabofuranosido-2-methylthioadenine, 61 mg.

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