487. *Experiments on the Synthesis of Purine Nucleosides. Part XXIV.* 9-D-*Galactosido*-2-*methylthioadenines.*

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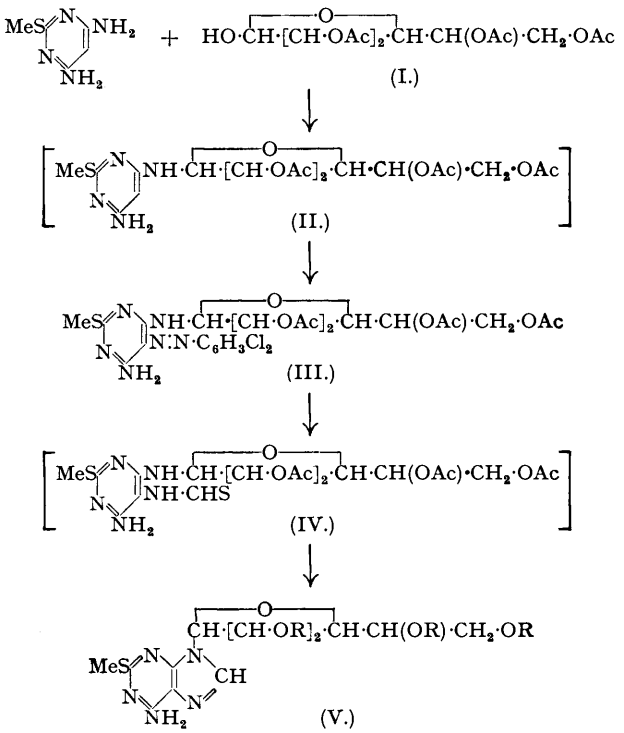
9-D-*Galactopyranosido*-2-*methylthioadenine* has been synthesised by the general method developed in earlier papers of this series. Condensation of 2 : 3 : 5 : 6-tetra-acetyl D-galactofuranose with 4 : 6-diamino-2-methylthiopyrimidine, followed by coupling with diazotised 2 : 5-dichloroaniline, reduction, thioformylation, and cyclisation under conditions which avoided at all stages removal of acetyl groups, yielded a resinous acetylated purine glycoside. Deacetylation of this, followed by treatment with picric acid, yielded 9-D-*galactofuranosido*-2-*methylthioadenine picrate*.

The general procedure for purine nucleoside synthesis developed in early papers of this series gave rise, in general, to pyranosides when the initial step was direct condensation of an unsubstituted aldose with a suitable 4 : 6-diaminopyrimidine. Various modifications of this step were investigated which might yield 6-amino-4-glycofuranosidaminopyrimidines from which, by normal methods, the furanose forms of the purine nucleosides might be synthesised. The most immediately obvious method in the pentose series—condensation of a 5-acylpentofuranose with a 4 : 6-diaminopyrimidine—failed to yield the desired product. As has already been reported (Kenner, Lythgoe and Todd, Part XVII, *J.*, 1948, 957), the possibility that this failure might have been due to too high reactivity of the furanose sugar caused us to investigate the condensation of a fully acetylated furanose sugar, and 2 : 3 : 5 : 6-tetra-acetyl D-galactofuranose was selected as a model compound. This compound condensed with some difficulty with 4 : 6-diamino-2-methylthiopyrimidine and furnished a product which, when deacetylated and coupled with diazotised 2 : 5-dichloroaniline, gave an azo-glycoside. The latter product on acetylation gave a 6-amino-4-(tetra-acetyl D-galactosidamino)-5-(2′ : 5′-dichlorobenzeneazo)-2-methylthiopyrimidine, which differed in its properties from the corresponding pyranose compound and was therefore believed to be a furanoside. Although it clearly indicated a possible route to purine furanosides, this observation was not immediately followed up, largely because another route based on condensation of an *aldehydo*-sugar with a 4 : 6-diaminopyrimidine to yield a Schiff base appeared more promising. The development of the latter method and its final application to the synthesis of adenosine has formed the subject of previous papers (Kenner,

Lythgoe, and Todd, *loc. cit.*; Kenner, Rodda, and Todd, this vol., p. 1613; Kenner, Taylor, and Todd, *ibid.*, p. 1620). It was, however, decided that the route employing acylated furanose sugars, although unlikely to be of preparative value, should be, in due course, explored, and we have therefore studied its application to the synthesis of 9-D-galactofuranosido-2-methylthioadenine.

As a preliminary, the synthesis of 9-D-*galactopyranosido*-2-*methylthioadenine* was carried out by our general procedure, partly to have available the intermediate and final products for comparison with the corresponding furanose compounds and partly so that the intermediates might be used as model substances in studying any variation of reaction conditions which the lesser stability of the furanose might necessitate at any step in the projected synthesis; the latter point was of some importance in view of the comparative difficulty of obtaining 2 : 3 : 5 : 6-tetra-acetyl D-galactofuranose in large quantity. 6-Amino-4-(tetra-acetyl D-galactopyranosidamino)-5-(2′ : 5′-dichlorobenzeneazo)-2-methylthiopyrimidine (Kenner, Lythgoe, and Todd, *loc. cit.*) was reduced with zinc dust and acetic acid, and the product thioformylated. The 6-*amino*-4-(*tetra-acetyl* D-*galactopyranosidamino*)-5-*thioformamido*-2-*methylthiopyrimidine* so obtained was cyclised with sodium methoxide giving 9-D-galactopyranosido-2-methylthioadenine. The pyranose nature of the product was confirmed by periodate titration, the consumption of oxidant being 3·08 mols. per mol., of which 1 mol. was used up in oxidising the methylthio-group to a methylsulphinyl group (cf. Howard, Lythgoe, and Todd, *J.*, 1945, 556).

In Part XVII (*loc. cit.*) the product (II) obtained by condensing 2 : 3 : 5 : 6-tetra-acetyl D-galactofuranose (I) with 4 : 6-diamino-2-methylthiopyrimidine was deacetylated before coupling



with diazotised 2 : 5-dichloroaniline to form an azo-glycoside. This procedure, although dangerous because of possible furanose–pyranose interconversion in the acetyl-free glycoside, was adopted at the time because the separation of sugar-free azo-compound from the acetylated azo-glycoside had been virtually impossible by the standard chromatographic method hitherto used in such cases. Further work on the Schiff-base route to purine furanosides (Part XXII, *loc. cit.*), however, indicated that furanose–pyranose interconversion under these conditions was almost certain to occur and that the homogeneity of the " azo-galactofuranoside " described in Part XVII (*loc. cit.*) was accordingly doubtful. It was therefore decided that, to ensure retention of a furanose structure, deacetylation must be avoided at all costs until the glyoxaline ring of the

purine system was closed. On the basis of a series of model experiments on the more accessible pyranose azo-galactoside, a suitable chromatographic method, using neutral alumina as adsorbent and toluene as eluting agent to remove sugar-free material, was devised. By its use 6-*amino*-4-(*tetra-acetyl* D-*galactofuranosidamino*)-5-(2′ : 5′-*dichlorobenzeneazo*)-2-*methylthiopyrimidine* (III) was prepared from the initial crude condensation product without removal of acetyl groups at any stage; this furanose azo-glycoside was entirely different in its rotation and solubility from its pyranose isomer. As expected, it differed also from the product obtained when deacetylation preceded formation of the azo-linkage; the product so obtained (Part XVII, *loc. cit.*) must clearly have been a mixture containing much pyranose material.

Reduction of the acetylated azofuranoside with zinc dust in acetic acid, followed by thioformylation of the unstable amino-compound with precipitated dithioformic acid, gave only low yields of acetylated thioformamido-glycoside accompanied by much sugar-free material. As it was thought that the prolonged boiling with dithioformic acid might be a major factor in producing sugar-free material, model experiments were initiated to find some other thioformylation procedure. Sodium dithioformate gave unsatisfactory results and, rather surprisingly, methyl dithioformate (Levi, *Atti R. Accad. Lincei*, 1929, **9**, 170) did not act as a thioformylating agent; this may have been due to the fact that the ester, a high-melting solid, is polymeric. On the other hand, addition of the calculated amount of acetic acid to methanolic sodium dithioformate at 0° liberated dithioformic acid, apparently as monomer, since it remained almost wholly in solution and was precipitated only when the solution was boiled; this solution of dithioformic acid effectively thioformylates amines at room temperature. By reducing the azo-furanoside with zinc dust and a minimum of acetic acid and thioformylating the product by this method, reasonable yields of crude resinous 6-amino-4-(tetra-acetyl D-galactofuranosidamino)-5-thioformamido-2-methylthiopyrimidine (IV) were obtained. Cyclisation of this product, which was not isolated in a pure state, was effected by heating with fused potassium acetate in acetonitrile solution so that no deacetylation, and hence no furanose–pyranose interconversion, would occur during the reaction. Chromatography then gave a resinous fraction which could not be crystallised but yielded a sparingly soluble *picrate*. From its analysis this material appeared to be the picrate of a 9-(tetra-acetyl D-galactofuranosido)-2-methylthioadenine (V; R = Ac).

The whole synthesis was now repeated with fresh material and the crude cyclisation product split into three fractions by chromatography. Each fraction was separately deacetylated, but the products did not crystallise. The deacetylated middle fraction, however, yielded a sparingly soluble picrate which, from its analysis, appeared to be the expected *picrate* of 9-D-galactofuranosido-2-methylthioadenine (V; R = H) and on periodate titration it consumed 6·38 mols. of oxidant per mol. This periodate consumption is in agreement with the formulation of the product as a hexofuranoside, which one would expect to consume *ca.* 6 mols. of periodate per mol. Under precisely similar conditions 9-D-galactopyranosido-2-methylthioadenine formed a sparingly soluble, but different, picrate (periodate consumption 3·06 mols. per mol.).

The deacetylated first fraction also gave a sparingly soluble picrate which was evidently a mixture of the above glycoside picrate and sugar-free material. The third fraction did not yield a sparingly soluble picrate under the same conditions; it was finally obtained as a hygroscopic brownish resin. This resinous material was obviously impure, but it gave analytical values which suggested that it may have contained a considerable proportion of uncyclised 5-formamido-pyrimidine glycoside formed by a side-reaction from the thioformamido-compound.

The formulation of the above-mentioned picrate as that of 9-D-galactofuranosido-2-methylthioadenine rests upon its analysis, periodate titration, the similarity in ease of formation and solubility between it and the picrate of the 9-D-galactopyranoside, and the production of 9-glycosidopurines in analogous cases by the cyclisation procedure employed (cf. Kenner, Rodda, and Todd, *loc. cit.*). Owing to lack of material and the arduous nature of the synthetic route, a more extended examination of the final product has not been undertaken. The virtual certainty that it is correctly formulated as a 9-glycoside, however, justifies publication of our findings in their present form. Their main interest lies in the demonstration that synthesis of purine glycofuranosides by a route starting with acetylated furanose sugars is possible. The method is hardly likely to be of any practical value, partly because of low yields and partly because the sugar derivatives employed would normally be available only through the corresponding aceto-halogenofuranoses. Under such conditions it would always be at a disadvantage as compared with either the modified Fischer method (Davoll, Lythgoe, and Todd, *J.*, 1948, 967) or the Schiff-base route (Kenner, Taylor, and Todd, *loc. cit.*) to purine glycofuranosides.

Experimental.

6-Amino-4-(tetra-acetyl D-*galactopyranosidamino)-5-thioformamido-2-methylthiopyrimidine.*—A solution of 6-amino-4-(tetra-acetyl D-galactopyranosidamino)-5-(2′ : 5′-dichlorobenzeneazo)-2-methylthiopyrimidine (2 g., Kenner, Lythgoe, and Todd, *loc. cit.*) in ethyl acetate (100 c.c.) was boiled under reflux in a nitrogen atmosphere with vigorous stirring. Zinc dust (12 g.) was added and then, dropwise during 15 minutes, a mixture of acetic acid (1·2 c.c.) and ethyl acetate (12 c.c.). The mixture was stirred and heated under reflux for 1 hour, and the resulting colourless solution filtered from zinc. The filter residue was washed with warm ethyl acetate, and the combined filtrate and washings were evaporated under reduced pressure in a stream of nitrogen. The residual resin was redissolved in a little ethyl acetate, and the 5-amino-glycoside precipitated as an amorphous powder (1·15 g.) by adding light petroleum (b. p. 40—60°).

The crude 5-amino-compound (2 g.) was dissolved in methanol (150 c.c.) at room temperature in a nitrogen atmosphere. A solution of sodium dithioformate (1·75 g.) in methanol (50 c.c.) was added, followed at once by acetic acid (0·53 g., 1 equiv.) in methanol (15 c.c.). Most of the liberated dithioformic acid remained in solution, and only a small amount of polymeric material separated. The mixture was left overnight, then boiled under reflux for 1 hour to precipitate the residual dithioformic acid, and filtered. The filtrate was evaporated under reduced pressure, and the residue dissolved in ethyl acetate and put on a column of neutral alumina. The column was washed with ethyl acetate and chloroform and then eluted with pyridine. The eluate was evaporated and the product recrystallised from ethanol. The *5-thioformamido-glycoside* formed small colourless needles, m. p. 145—148°, and had $[\alpha]_D^{15}$ +38° (±10°) (*c*, 1·10 in chloroform) (Found, in material dried at 100°/4 mm.: C, 43·8; H, 5·2; N, 12·6. $C_{20}H_{27}O_9N_5S_2$ requires C, 44·0; H, 5·0; N, 12·8%).

*9-*D*-Galactopyranosido-2-methylthioadenine.*—Sodium methoxide (65 mg.) was added to a solution of the above thioformamido-glycoside (260 mg.) in ethanol (20 c.c.) and the mixture was boiled under reflux in an atmosphere of nitrogen for 4 hours. The solution was treated with charcoal and allowed to cool, whereupon the *purine galactoside* separated as very small colourless crystals; concentration of the mother-liquors gave a further quantity of the same product (total yield, 50 mg.). Recrystallised from aqueous ethanol (50%), 9-D-galactopyranosido-2-methylthioadenine was obtained as colourless hydrated needles, m. p. 236—237°, which retained water of crystallisation tenaciously (Found, in material dried at 150°/4 mm. for 6 hours: C, 39·8; H, 5·5; N, 19·2. $C_{12}H_{17}O_5N_5S,H_2O$ requires C, 39·9; H, 5·3; N, 19·4%). The substance had $[\alpha]_D^{17}$ +25° (±10°) (*c*, 0·29 in water). Light absorption in ethanol: maximum, 2750 A. (ε = 15,500); minimum, 2500 A. (ε = 5450). Addition of picric acid to an ethanolic solution precipitated the *picrate* which dissolved in boiling water and separated again as a yellow powder which on heating decomposed at 280° (Found: C, 37·5; H, 3·6; N, 19·2. $C_{12}H_{17}O_5N_5S,C_6H_3O_7N_3$ requires C, 37·8; H, 3·5; N, 19·6%). On periodate titration the picrate absorbed 3·06 mols. of oxidant per mol.

6-Amino-4-(tetra-acetyl D-*galactofuranosidamino)-5-(2′ : 5′-dichlorobenzeneazo)-2-methylthiopyrimidine.*—Tetra-acetyl-D-galactofuranose (10 g.; Schlubach and Prochownik, *Ber.*, 1930, **63**, 2298) and 4 : 6-diamino-2-methylthiopyrimidine (15 g.) were heated under reflux in dry ethanol (200 c.c.) to which had been added concentrated sulphuric acid (0·3 g.) during 40 hours, water formed during the reaction being removed in the usual way by periodic addition of a benzene–ethanol mixture and slow distillation through a 20-cm. Fenske column fitted with a reflux-ratio head. Unchanged 4 : 6-diamino-2-methylthiopyrimidine separated on cooling and was collected by filtration. A further quantity of the same material was obtained by evaporating the filtrate under reduced pressure and extracting the residue with chloroform; most of the sugar-free pyrimidine remained undissolved (total recovery, 11 g.). The chloroform extract was evaporated and the residue dissolved in pyridine (70 c.c.). A neutral solution of diazotised 2 : 5-dichloroaniline (6·4 g.) was added and the mixture set aside for 3 hours before adding water (600 c.c.) and collecting the crude precipitated azo-derivative. The dried crude product was extracted with warm toluene, and the toluene solution allowed to cool, whereupon a quantity of sugar-free azo-compound separated. The mixture was filtered, the filtrate evaporated, and the residue dissolved in ethyl acetate and put on a column of neutral alumina (200 g.). Washing with ethyl acetate eluted the main broad red band from the column. The eluate was evaporated and the residue extracted with cold toluene which left a small amount of sugar-free material undissolved. The toluene solution was now chromatographed on neutral alumina (200 g.), the column being washed with toluene (1 l.). The broad red band which remained at the top of the column was pushed out and eluted with a mixture of pyridine and ethanol (1 : 1). Evaporation of the eluate gave an orange-red resin which was dissolved in toluene (25 c.c.), and light petroleum (b. p. 40—60°) was added gradually, giving a reddish-orange powder (2·83 g.) with an indefinite m. p. (115—140°) (Found, in material dried at 90°/0·1 mm.: N, 13·0. $C_{25}H_{28}O_9N_6Cl_2S$ requires N, 12·7%). Further purification was effected by dissolution in ether and careful reprecipitation with light petroleum, the *azo-furanoside* being obtained as an orange powder, m. p. 120—140° (Found, in material dried at 90°/0·1 mm.: C, 45·4; H, 3·9; N, 12·6. $C_{25}H_{28}O_9N_6Cl_2S$ requires C, 45·5; H, 4·3; N, 12·7%). The substance gave a positive Molisch test and had $[\alpha]_D^{16}$ +60° (±20°) (*c*, 0·095 in chloroform).

9-(Tetra-acetyl D-*galactofuranosido)-2-methylthioadenine Picrate.*—6-Amino-4-(tetra-acetyl D-galactofuranosidamino)-5-(2′ : 5′-dichlorobenzeneazo)-2-methylthiopyrimidine (1·1 g.) was reduced with zinc dust and acetic acid in ethyl acetate solution, and the product thioformylated exactly as described for the corresponding pyranose isomer (see above). The thioformamido-glycoside was obtained as a yellowish resin (0·43 g.) which could not be crystallised and was therefore use directly without further purification for the next stage in the synthesis.

The resinous thioformamido-glycoside (0·41 g.) was dissolved in dry acetonitrile (50 c.c.). Freshly fused potassium acetate (0·37 g.) was added and the solution stirred and heated under reflux for 10 hours, by which time evolution of hydrogen sulphide had practically ceased. The mixture was filtered and evaporated, and the residue taken up in chloroform and put on a column of neutral alumina (18 × 1·5 cm.). The column was washed with chloroform which slowly eluted the lowest narrow yellowish band;

evaporation of the eluate gave a resin *A* (110 mg.). Further elution with chloroform containing 5% ethanol (100 c.c.) gave a second resin *B* (140 mg.), which, although it contained carbohydrate material (positive Molisch reaction), did not form a picrate and was clearly not a purine glycoside. Resin *A* could not be crystallised; it was dissolved in ethanol (4 c.c.), boiled with charcoal, filtered, and cooled, and a saturated solution of picric acid in ethanol (1 c.c.) was added. There was immediate precipitation of a yellow *picrate* (39 mg.) which was purified by dissolution in ethyl acetate and reprecipitation with light petroleum (b. p. 40—60°). The product was apparently amorphous and melted rather indefinitely at *ca.* 120° (Found, in material dried at 75°/4 mm.: C, 43·2; H, 4·1; N, 15·0. $C_{20}H_{25}O_9N_5S,C_6H_3O_7N_3$ requires C, 42·2; H, 3·8; N, 15·1%).

9-D-*Galactofuranosido-2-methylthioadenine Picrate.*—The preparation of the crude acetylated 5-thioformamidofuranoside (0·5 g.) and its cyclisation by heating with potassium acetate in acetonitrile were carried out precisely as in the previous experiment. The crude cyclisation product was put on a column of neutral alumina as before and the column washed with chloroform (*ca.* 100 c.c.) which eluted a brownish resin *C* (160 mg.). Further washing with chloroform (200 c.c.) yielded a second resinous fraction *D* (180 mg.). A third Molisch-positive fraction *E* (240 mg.) was obtained by eluting the column with pyridine..

Resin *C* was dissolved in ethanol (charcoal), filtered, and cooled. Methanolic barium methoxide (0·8 c.c.; 0·155N.) was added to effect deacetylation. The deacetylated product separated out in part from the solution, but the main bulk (70 mg.) was obtained as a resin by neutralising the solution and evaporating to dryness. This residue gave a sparingly soluble picrate but, from analysis (Found: N, 18·1%) and periodate titration (8·6 mg. consumed 0·227 ml. of 0·268M-sodium metaperiodate), it was evidently not homogeneous; the resin consisted presumably of purine glycoside mixed with considerable amounts of other impurities.

Resin *D* was deacetylated in the same way as *A*, by methanolic barium methoxide (0·8 c.c.; 0·155N.). A small amount of amorphous material separated; this was filtered off, the filtrate evaporated, the residual resin which could not be crystallised dissolved in ethanol (4 c.c.), and saturated ethanolic picric acid (1 c.c.) added. The precipitated *picrate* (60 mg.) was collected after 24 hours. It could not be obtained in a crystalline state and was purified by precipitation from ethanolic solution with light petroleum (b. p. 40—60°). The product was an amorphous yellow powder, which softened somewhat at 170° and melted with decomposition at 250° (Found, in material dried at 90°/5 mm.: C, 38·1; H, 3·9; N, 19·2. $C_{12}H_{17}O_5N_5S,C_6H_3O_7N_3$ requires C, 37·8; H, 3·5; N, 19·6%).

Resin *E* was deacetylated in the same way as resins *C* and *D*, but the product did not give a sparingly soluble picrate. Attempted purification by fractional precipitation from ethanolic solution with light petroleum (b. p. 40—60°) gave a buff-coloured hygroscopic powder which rapidly changed to a brownish gum (Found, in material dried at 90°/5 mm.: C, 39·2; H, 5·8; N, 18·5. Calc. for $C_{12}H_{17}O_5N_5S,1\frac{1}{2}H_2O$: C, 38·9; H, 5·4; N, 18·9%). The fact that the analytical values obtained correspond to those calculated for the purine glycoside plus $1\frac{1}{2}$ mols. of H_2O suggests that the material is not homogeneous and contains a considerable amount of uncyclised 5-formamido-compound.

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