347. *Experiments on the Synthesis of Purine Nucleosides. Part XXII. The Synthesis of the α- and β-Forms of 9-Triacetyl-D-ribopyranosido-2-methylthioadenine and Further Studies on the Synthesis of 9-Glycofuranosidopurines.*

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In an endeavour to extend the Schiff-base route to 9-glycofuranosidopurines outlined in Part XVII (*J.*, 1948, 957) to the synthesis of adenosine, 5-*benzoyl* 2 : 3 : 4-*triacetyl* D-*ribose* was condensed with 4 : 6-diamino-2-methylthiopyrimidine, and the product converted into 6-*amino*-4-*triacetyl*-D-*ribosidamino*-5-(2′ : 5′-*dichlorobenzeneazo*)-2-*methylthiopyrimidine* by a method similar to that used for the corresponding arabinoside in Part XVII (*loc. cit.*). Although it had been expected that this product would be a furanoside, it yielded on reduction, thioformylation, and sodium alkoxide cyclisation, followed by reacetylation, 9-*triacetyl*-β-D-*ribopyranosido*-2-*methylthioadenine*; the same glycoside was obtained by an analogous series of reactions from 6-*amino*-4-D-*ribopyranosidamino*-2-*methylthiopyrimidine* prepared by direct condensation of D-ribose and 4 : 6-diamino-2-methylthiopyrimidine. The intermediate *azo-glycoside* in this case was quite distinct from that obtained by the Schiff-base route. Both of these azo-glycosides, when reduced, thioformylated, and cyclised with potassium acetate in methyl cyanide yielded 9-*triacetyl*-α-D-*ribopyranosido*-2-*methylthioadenine*. The significance of these results is discussed, and a probable explanation suggested which also brings into line anomalous results obtained in a re-examination of the L-arabinoside series investigated in Part XVII (*loc. cit.*). The reason for the difficulties experienced with the Schiff-base route to furanosides employing a protecting benzoyl group is pointed out, and a solution of these difficulties proposed.

IN Part XVII of this series (Kenner, Lythgoe, and Todd, *J.*, 1948, 957), we described the synthesis of 9-L-arabofuranosido-2-methylthioadenine by a method depending on the condensation of 4 : 6-diamino-2-methylthiopyrimidine with 5-benzoyl 2 : 3 : 4-triacetyl L-arabinose to a product believed to be a Schiff base. Removal of the acetyl groups from this material by partial hydrolysis yielded, by isomerisation, a furanosidamino-pyrimidine which was then used for synthesising the purine glycoside by the general procedure laid down in earlier papers of this series. The natural development of this Schiff-base route was to apply it to the synthesis of adenosine (9-β-D-ribofuranosidoadenine) by using D-ribose in place of L-arabinose and desulphurising the 9-D-ribofuranosido-2-methylthioadenine so obtained by means of Raney nickel, a method already applied in analogous cases (Part XI; Howard, Lythgoe, and Todd, *J.*, 1945, 556).

D-*Ribose diethyl thioacetal* was prepared by a route analogous to that employed for D-lyxose diethyl thioacetal by Wolfrom and Moody (*J. Amer. Chem. Soc.*, 1940, **62**, 3465), converted into its 5-*benzoyl* derivative and thence into 5-*benzoyl* 2 : 3 : 4-*triacetyl* D-*ribose diethyl thioacetal*. From the last compound the free *aldehydo*-sugar was obtained as a syrup which could not be crystallised; it was condensed with 4 : 6-diamino-2-methylthiopyrimidine, and the crude product used directly to prepare, by the method described in Part XVII (*loc. cit.*), a 6-*amino*-4-*triacetyl*-D-*ribosidamino*-5-(2′ : 5′-*dichlorobenzeneazo*)-2-*methylthiopyrimidine*. This azo-glycoside, which we expected to have a furanose structure, had $[\alpha]_D$ +320° (approx.). Zinc dust reduction, followed by treatment with dithioformic acid, yielded a thioformamido-compound which was cyclised with sodium methoxide in the usual manner. No crystalline product could be isolated directly from the cyclisation reaction, but addition of picric acid precipitated a *picrate* of 9-D-ribosido-2-methylthioadenine. After acetylation the picrate was decomposed by passing it in chloroform solution through a column of activated alumina. The crystalline glycoside so obtained had m. p. 230°, and from its analysis, positive Molisch reaction, ultra-violet absorption spectrum, and the fact that, after deacetylation, it consumed 3 moles of periodate, it was clearly 9-*triacetyl*-D-*ribopyranosido*-2-*methylthioadenine* and not the expected ribofuranoside.

For purposes of comparison, 9-triacetyl-D-ribopyranosido-2-methylthioadenine was synthesised by application of our general synthetic method to 6-*amino*-4-D-*ribopyranosidamino*-2-*methylthiopyrimidine*. The latter compound was first prepared in these laboratories some years ago by Dr. H. T. Howard; although crystalline and apparently homogeneous, the possibility that it may be a mixture of α- and β-isomers is not wholly excluded. The 6-amino-4-triacetyl-D-ribosidamino-5-(2′ : 5′-dichlorobenzeneazo)-2-methylthiopyrimidine prepared from it in the usual manner was commonly obtained as an amorphous powder of $[\alpha]_D$ −100° and was used in this form for the next stage in the synthesis; a small fraction obtained crystalline on one occasion had m. p. 182—183°. This azo-glycoside, then, was clearly different from the supposedly

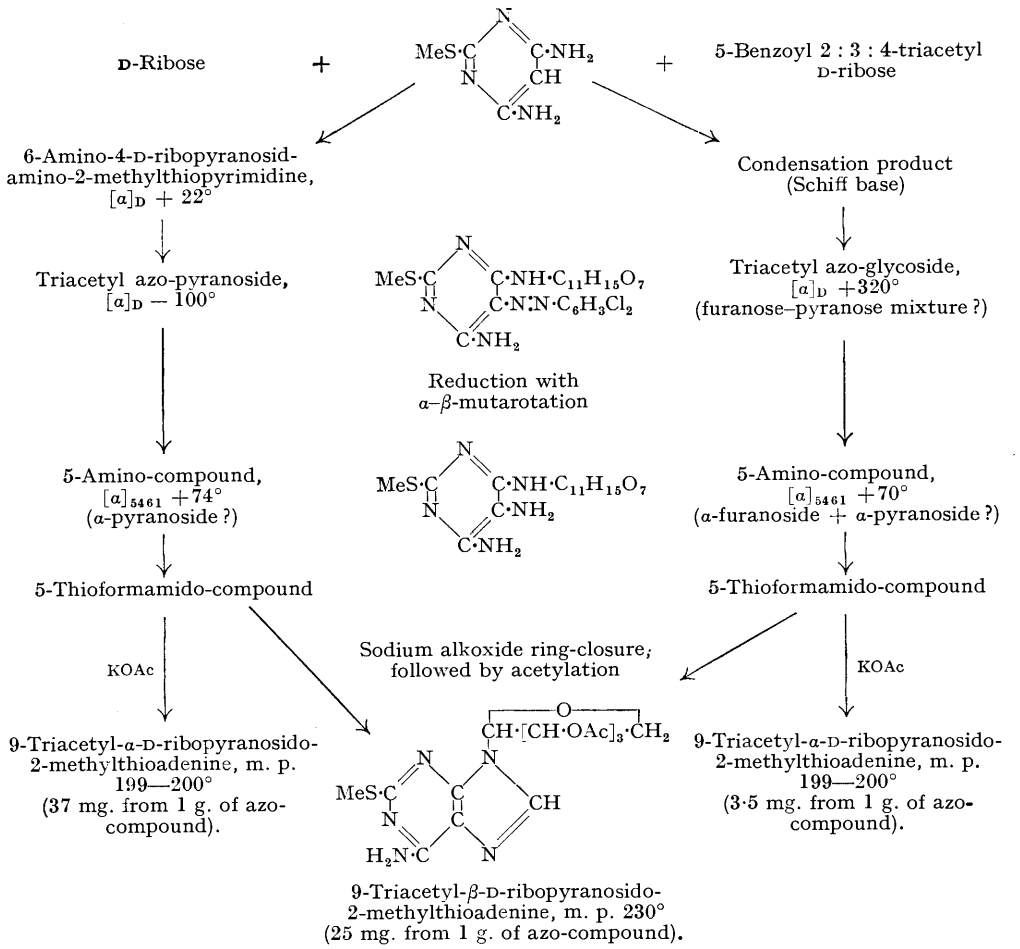
furanose azo-glycoside mentioned above, which had $[\alpha]_D$ +320°, but on reduction, thioformylation, and cyclisation with sodium methoxide, followed by acetylation, it gave a 9-triacetyl-D-ribopyranosido-2-methylthioadenine, m. p. 230°, identical with that described above, and gave it, moreover, in closely similar yield.

These results and other considerations led us to the view that α–β-isomerisation, accompanied by furanose–pyranose interconversion, might be taking place during the alkoxide-catalysed ring-closure. To obviate furanose–pyranose interconversion, it would be necessary to retain the protecting acetyl groups on the sugar residue during cyclisation, but the only method of which we were aware fulfilling these conditions gave large quantities of 6-glycosidaminopurines (cf. Kenner and Todd, Part XIII, *J.*, 1946, 852). Trial experiments, however, showed that potassium acetate in methyl cyanide formed a suitable medium giving, in some cases at least, good yields of 9-glycosidopurines; thus, 6-amino-4-triacetyl-D-xylosidamino-5-thioformamido-2-methylthiopyrimidine cyclised in this medium gave a 45% yield of 9-triacetyl-D-xylopyranosido-2-methylthioadenine, although cyclisation in potassium acetate–acetic acid gave mainly 6-triacetyl-D-xylosidamino-2-methylthiopurine.

The 5-thioformamido-compound, prepared from the, presumably, pyranose 6-amino-4-triacetyl-D-ribosidamino-5-(2′ : 5′-dichlorobenzeneazo)-2-methylthiopyrimidine ($[\alpha]_D$ −100°), was therefore heated with potassium acetate in methyl cyanide. Even after chromatographic purification the product could not be directly crystallised, but it yielded a crystalline picrate, m. p. 130—132°, quite different from the picrate (m. p. 209—210°) of the 9-triacetyl-D-ribopyranosido-2-methylthioadenine (m. p. 230°) obtained previously by alkoxide cyclisation. Decomposition of the picrate yielded a crystalline purine glycoside, m. p. 199—200°. Analysis and periodate titration confirmed that this product was indeed a second 9-triacetyl-D-ribopyranosido-2-methylthioadenine. Its ultra-violet absorption spectrum was indistinguishable from that of the isomeric compound, but it had $[\alpha]_D$ +94°, whereas the isomer of m. p. 230° had $[\alpha]_D$ −34°. Clearly, the purine, m. p. 199—200°, is the α- and that of m. p. 230° is the β-D-*ribopyranoside*. The " A-value " calculated from the molecular rotations is 28,000, which is in reasonable agreement with the value 23,200 found in the D-xylopyranoside series (Howard, Kenner, Lythgoe, and Todd, Part XV, *J.*, 1946, 861). Repetition of these experiments showed that the yields of purine glycoside were reproducible and similar with the two methods of ring-closure, but the potassium acetate–methyl cyanide method always gave the α-glycoside, m. p. 199—200°, without any detectable quantity of the β-isomer, whereas sodium alkoxide gave invariably the β-glycoside. The general theory of the interconversion reactions of *N*-glycosides expounded in Part XIV of this series (Howard, Kenner, Lythgoe, and Todd, *J.*, 1946, 855) provides a reasonable explanation of these phenomena. 5 : 6-Diamino-4-triacetyl-D-ribopyranosidamino-2-methylthiopyrimidine will undergo a rapid mutarotation to an equilibrium mixture consisting largely of the more stable αβ-isomer. Although no definite assessment can yet be made, our observation that this material in solution has an optical rotation of *ca.* + 74° is compatible with its being composed largely of the α-riboside, which would be expected to yield the α-thioformamido-derivative. On cyclisation with potassium acetate in a non-hydroxylic medium, this thioformamido-compound yields the purine α-riboside, m. p. 199—200°. On the other hand, in hot sodium alkoxide solution the acetyl groups are rapidly removed thereby changing the relative stabilities of the α- and β-isomers, and the conditions are extremely favourable for mutarotation, particularly when a proton is removed either from the 5-thioformamido- or from the 4-glycosidamino-group. The production of a purine β-riboside under these conditions is not surprising; all previous syntheses by this general route for purine nucleosides have yielded β-glycosides.

The supposedly furanose acetyl azo-riboside ($[\alpha]_D$ +320°) was now reduced and thioformylated and the product cyclised with potassium acetate in methyl cyanide. Here too the only product isolated was 9-triacetyl-α-D-ribopyranosido-2-methylthioadenine, but the yield was much lower, in our opinion significantly lower, than that from the authentic pyranose acetyl azo-riboside. These results could be explained on the assumption that the 5 : 6-diamino-4-triacetyl-D-ribosidamino-2-methylthiopyrimidine obtained by reducing the azo-compound ($[\alpha]_D$ +320°) is a mixture of α-furanoside and α-pyranoside, an assumption which would not conflict with the fact that its rotation in solution (+70°) does not differ widely from that of the authentic pyranose compound. Thioformylation of this material and cyclisation with potassium acetate in methyl cyanide would give a corresponding mixture of purine ribosides from which only the α-pyranoside can be isolated in crystalline form. Sodium methoxide, on the other hand, by removing the protecting acetyl groups would allow furanose–pyranose interconversion as well as α–β-mutarotation to proceed unchecked during cyclisation, so that

from either starting material identical yields of β-pyranoside would be expected. The results obtained in the ribose series, with our suggested explanation of them, are summarised in the accompanying scheme.



Although there is no proof of the validity of these hypotheses, their plausibility is enhanced by a re-examination of the L-arabinoside series studied in Part XVII (*loc. cit.*). There we had originally applied the sodium alkoxide cyclisation and obtained from the supposedly furanose acetyl azo-arabinoside a 9-L-arabinosido-2-methylthioadenine, which we believed to be a pure furanoside. The complex situation now uncovered in the D-ribose series made a re-examination of the arabinose derivatives imperative. Repetitions of the synthesis described in Part XVII (*loc. cit.*) gave a crystalline nucleoside, but as a rule this product consumed about 2·6 moles of periodate on titration and thus appeared to be a mixture of furanose and pyranose compounds. We were unable to obtain again a product with a periodate titration value as low as that described in Part XVII (*loc. cit.*). Mr. C. W. Taylor in this laboratory has found that when sodium ethoxide is used for cyclisation a similar material (periodate uptake 2·6 mols./mol.) is produced starting from 6-*amino*-4-*triacetyl*-L-*arabinosidamino*-5-(2′ : 5′-*dichlorobenzeneneazo*)-2-*methylthiopyrimidine*, which, prepared as it was in normal fashion from the condensation product of L-arabinose and 4 : 6-diamino-2-methylthiopyrimidine, would be expected to be a pyranose compound. Recrystallisation of this apparently furanose–pyranose mixture of purine glycosides does not separate it into its components, and the periodate uptake remains unchanged. Chromatography of the triacetate, however, yields, with heavy loss, a product from which by deacetylation pure 9-L-*arabopyranosido*-2-*methylthioadenine* can be obtained. The same pyranoside is afforded by potassium acetate–methyl cyanide cyclisation of the pyranose

thioformamido-compound, but the supposedly furanose thioformamido-compound, prepared as described in Part XVII (*loc. cit.*), gives a purine arabinoside with a significantly lower periodate consumption (2·3—2·4 mols./mol.). These phenomena are fundamentally similar to those observed in the ribose series, save that the exceptional complication of α-glycoside production is not encountered and the furanose purine crystallises out with the pyranose instead of remaining in the mother-liquors. Presumably, pure 9-L-arabofuranosido-2-methylthioadenine could be separated, given a sufficient quantity of the material with periodate uptake 2·3—2·4 mols./mol.; this we have not attempted, preferring to seek an unambiguous furanoside synthesis.

The origin of our difficulties in applying the Schiff-base route successfully was clear and their resolution required the retention of the essential protecting group in position-5 of the *aldehydo*-sugar throughout the synthesis, until the glyoxaline ring of the purine system had been formed and furanose–pyranose interchange thereby rendered impossible. Gordon, Miller, and Day (*J. Amer. Chem. Soc.*, 1948, **70**, 1946) have recorded data on the ammonolysis of esters which support our view (Part XVII, *loc. cit.*) of preferential deacetylation as against debenzoylation, but in attempting to retain the protecting 5-benzoyl group on the sugar residue we preferred to use the more easily controlled sodium methoxide technique. By a mild deacylation by this means on the condensation product of *aldehydo*-pentoses, for example, 5-benzoyl 2 : 3 : 4-triacetyl D-ribose, with 4 : 6-diamino-2-methylthiopyrimidine, followed by coupling with diazotised 2 : 5-dichloroaniline and careful chromatography, it was possible to isolate small amounts of azo-glycosides, which undoubtedly retained the benzoyl group intact. They gave satisfactory analytical values and yielded benzoic acid on hydrolysis. Moreover, they did not respond to the Molisch test after reduction of the azo-group unless a preliminary debenzoylation with sodium methoxide was carried out; this behaviour is typical of 5-benzoylpentoses. However, deacetylation and debenzoylation were competitive processes, so that a mixture of azo-compounds was obtained and the situation was further complicated by the presence of unidentified materials which may have contained more than one sugar residue. Indeed, the technical difficulty of separating adequate quantities of pure azo-glycosides containing a 5-benzoyl group on the pentose residue from such mixtures were so formidable that we decided to abandon this route in favour of the strictly analogous one involving an *aldehydo*-sugar bearing a terminal benzyl group instead of a benzoyl group. The results obtained in applying this new modification will be separately reported.

Experimental.

D-*Ribose Diethyl Thioacetal.*—Ethanethiol (36 c.c.) was run into a well-stirred mixture of D-ribose (30 g.) and concentrated hydrochloric acid (36 c.c.; *d* 1·18; previously saturated with hydrogen chloride) at 0° during 15 minutes and stirring was continued for a further ½ hour. The resulting solution was diluted with water (150 c.c.), treated with lead carbonate until no longer acid to Congo-red, filtered, and saturated with hydrogen sulphide. The mixture was again filtered, thoroughly aërated, and treated with excess of silver carbonate. The colourless neutral solution obtained by again passing hydrogen sulphide and filtering through "Hyflo supercel" was concentrated to smaller bulk (150 c.c.), whereupon D-*ribose diethyl thioacetal* (25 g.) separated; a further quantity (6·5 g.) was obtained by concentrating the mother-liquors. Recrystallised from water or aqueous ethanol, it formed colourless needles, m. p. 82—83°, and had $[\alpha]_D^{13}$ −41·5° (*c*, 0·5 in water) (Found: C, 41·8; H, 7·7. $C_9H_{20}O_4S_2$ requires C, 42·2; H, 7·8%).

5-*Benzoyl* D-*Ribose Diethyl Thioacetal.*—Benzoyl chloride (10·4 c.c.) in pyridine (40 c.c.) was added dropwise during 1 hour to a well-stirred solution of D-ribose diethyl thioacetal (25·6 g.) in pyridine (60 c.c.), cooled in an ice–salt mixture. Stirring was continued for 4 hours, and the mixture kept at room temperature overnight, then poured into ice-water (300 c.c.), and stirred for 1 hour. The precipitated 5-*benzoyl* derivative was collected and recrystallised from benzene–light petroleum (b. p. 60—80°) (3 : 1). It formed colourless needles (19 g.), m. p. 100°, $[\alpha]_D^{14°}$ 0° (*c*, 0·16 in chloroform) (Found, in material dried at 65°: C, 53·2; H, 6·7. $C_{16}H_{24}O_5S_2$ requires C, 53·3; H, 6·7%). No formaldehyde could be detected after oxidising a specimen with periodic acid.

5-*Benzoyl* D-*Ribose Phenylhydrazone.*—5-Benzoyl D-ribose diethyl thioacetyl (1·8 g.), cadmium carbonate (4·1 g.), mercuric chloride (4·1 g.), and water (60 c.c.) were stirred vigorously for 30 minutes at room temperature and then for 1 hour at 80°, filtered, cooled, and saturated with hydrogen sulphide. The filtered solution was aërated, treated with excess of silver carbonate, and again filtered. On renewed saturation with hydrogen sulphide at 0°, filtration, aëration, and evaporation to dryness under reduced pressure, 5-benzoyl D-ribose was obtained as a syrup (0·98 g.). Treated with phenylhydrazine hydrochloride in presence of sodium acetate, this product gave 5-*benzoyl* D-*ribose phenylhydrazone* which crystallised from aqueous ethanol as yellow plates, m. p. 159° (Found: N, 8·5. $C_{18}H_{20}O_5N_2$ requires N, 8·1%).

5-*Benzoyl* 2 : 3 : 4-*Triacetyl* D-*Ribose Diethyl Thioacetal.*—Acetic anhydride (50 c.c.) was added dropwise during 1 hour to a stirred ice-cold solution of 5-benzoyl D-ribose diethyl thioacetal (18 g.) in pyridine (80 c.c.). The mixture was stirred for a further 3 hours, kept overnight at room temperature,

then poured into ice-water (800 c.c.), and extracted twice with chloroform. The combined chloroform extracts were washed with, successively, acetic acid (10%), water, sodium hydrogen carbonate, and water, dried, and evaporated under reduced pressure. The *triacetyl* compound crystallised when set aside and was washed with a little hexane and recrystallised from the same solvent, to give colourless needles, m. p. 47—48°, $[\alpha]_D^{15°}$ +14·5° (*c*, 0·15 in chloroform) (Found: C, 54·0; H, 6·2. $C_{22}H_{30}O_8S_2$ requires C, 54·3; H, 6·2%).

5-*Benzoyl* 2 : 3 : 4-*Triacetyl* D-*Ribose*.—The above acetylated thioacetal (15·5 g.) and cadmium carbonate (30 g.) were stirred in acetone (58 c.c.) and then diluted with water (20 c.c.). A solution of mercuric chloride (31·2 g.) in acetone (58 c.c.) was added gradually during 1 hour. The mixture was stirred at room temperature for 20 hours and then for ½ hour at 50—70° before being cooled and filtered through "Hyflo supercel." The filtrate was concentrated, under reduced pressure in presence of a little cadmium carbonate, to a thick oil which was then dissolved in chloroform (200 c.c.). The chloroform solution dried and evaporated gave the *product* as a colourless resin which distilled at 170° (bath temp.)/10^{-4} mm. (Found: C, 57·3; H, 5·3. $C_{18}H_{20}O_9$ requires C, 56·8; H, 5·3%).

6-*Amino*-4-*triacetyl*-D-*ribosidamino*-5-(2′ : 5′-*dichlorobenzeneazo*)-2-*methylthiopyrimidine* ($[\alpha]_D$ +320°), *prepared by the Schiff-base Route*.—A boiling solution of 4 : 6-diamino-2-methylthiopyrimidine (20·2 g.) and ammonium chloride (0·52 g.) in dry ethanol (480 c.c.) was added to the syrupy 5-benzoyl 2 : 3 : 4-triacetyl D-ribose prepared from the corresponding diethyl thioacetal (15·5 g.), and the resulting solution set aside for 2 days. Solvent was removed by evaporation, and the residue extracted four times with chloroform (200 c.c. in all); unchanged diamine (12 g.) remained undissolved. The combined chloroform extracts were evaporated and the residue was set aside for 2 days with methanolic ammonia (320 c.c. of 5N.); the solution was concentrated to 100 c.c. and boiled for 30 minutes with sodium methoxide (1·47 g. of sodium in 30 c.c. of methanol) to remove the benzoyl group completely. Acetic acid (3·5 c.c.) was then added and the whole poured into a neutral solution of diazotised 2 : 5-dichloroaniline (8·1 g.). After 15 minutes the yellow precipitate was collected, washed with water, and dried at 50° *in vacuo*.

The crude product (20 g.) so obtained was dissolved in pyridine (100 c.c.) and adsorbed on active alumina (500 g.; column, 8·5-cm. diam.), and the column washed with pyridine (500 c.c.). The adsorbed azo-compound was next acetylated by stirring the adsorbate with pyridine and acetic anhydride (75 c.c.) in the cold, setting aside overnight, and then stirring for a further hour with addition of fresh acetic anhydride (25 c.c.). Ethanol (75 c.c.) was now added and the cooled mixture stirred for 1 hour. The alumina adsorbate was then extracted five times with equal portions (200 c.c.) of ethyl acetate containing 5% of pyridine. The combined extracts were evaporated, and the residue was redissolved in ethyl acetate (50 c.c.) and chromatographed on activated alumina (200 g.; column, 5-cm. diam.). Washing the column with ethyl acetate eluted the main broad yellow band, and evaporation of the eluate gave a dark reddish resin (6·2 g.). The resin could not be crystallised but, when this was dissolved in hot ethanol and allowed to cool, the *acetyl azo-glycoside* separated as an amorphous orange powder, $[\alpha]_D^{18°}$ +320° ±30° (*c*, 0·3 in chloroform) (Found, in material dried at 80°: C, 45·1; H, 4·6; N, 14·2. $C_{22}H_{24}O_7N_6SCl_2$ requires C, 45·0; H, 4·1; N, 14·3%).

6-*Amino*-4-D-*ribopyranosidamino*-2-*methylthiopyrimidine* (Experiment by Dr. H. T. Howard).—A solution of D-ribose (10 g.), 4 : 6-diamino-2-methylthiopyrimidine (20 g.), and ammonium chloride (0·6 g.) in absolute ethanol (120 c.c.) was heated under reflux for 30 minutes in a flask fitted with an 18″ Fenske column and a reflux-ratio head. A mixture of benzene and ethanol (1 : 1) was added gradually and water removed as the ternary mixture by slow distillation during 4 hours. The solution was now poured on activated alumina (1 kg.), and excess of 4 : 6-diamino-2-methylthiopyrimidine removed by washing with alcohol (2 l.). The glycoside was eluted with cold water (5 l.), and the eluate concentrated to small bulk and set aside at 0° for several days. 6-*Amino*-4-D-*ribopyranosidamino*-2-*methylthiopyrimidine* separated and was recrystallised from water; it formed rosettes of colourless needles (6·2 g.), m. p. 138—140°, $[\alpha]_D^{18°}$ +22° (*c*, 0·25 in water) (Found: C, 39·2; H, 6·0; N, 17·5; loss on drying at 110°, 5·5. $C_{10}H_{16}O_4N_4S,H_2O$ requires C, 39·2; H, 5·9; N, 17·6; loss on drying, 5·8%).

6-*Amino*-4-*triacetyl*-D-*ribosidamino*-5-(2′ : 5′-*dichlorobenzeneazo*)-2-*methylthiopyrimidine* ($[\alpha]_D$ −100°).—A neutral solution of diazotised 2 : 5-dichloroaniline (3·2 g.) was added to 6-amino-4-D-ribopyranosidamino-2-methylthiopyrimidine (4·1 g.) in water (10 c.c.), and after 30 minutes the yellow precipitate was collected, washed with water, and dried *in vacuo*. The product was dissolved in pyridine (36 c.c.) containing acetic anhydride (9 c.c.), and the solution set aside overnight. Ethanol (9 c.c.) was then added and after 1 hour the solution was evaporated under reduced pressure, and the residual resin dissolved in ethyl acetate (75 c.c.) and adsorbed on a column of activated alumina (180 g.; 5-cm. diam.). Washing the column with ethyl acetate eluted most of the coloured material which formed a broad yellow band, and evaporation of the eluate yielded a yellow resin. When this resin was dissolved in hot ethanol and the solution allowed to cool, the *acetyl azo-glycoside* separated as a yellow powder (4·5 g.), $[\alpha]_D^{17°}$ −100° ±8° (*c*, 0·13 in chloroform) (Found, in material dried at 80°: C, 45·1; H, 4·2; N, 13·8. $C_{22}H_{24}O_7N_6SCl_2$ requires C, 45·0; H, 4·1; N, 14·3%). When an ethyl acetate solution of this material evaporated slowly, the compound separated on one occasion as long needles, m. p. 182—183°, $[\alpha]_D^{15°}$ −320° ±20° (*c*, 0·13 in chloroform) (Found, in material dried at 100°: C, 45·6; H, 4·3; N, 13·6%).

Rotation of Crude 5 : 6-*Diamino*-4-*triacetyl*-D-*ribosidamino*-2-*methylthiopyrimidines prepared from the Above Azo-glycosides*.—Parallel reductions with zinc dust and acetic acid were carried out on each of the above azo-glycosides (0·5 g.). The 5-amino-glycosides obtained were resins; their optical rotations were determined in chloroform (80 c.c.). The product obtained from the azo-glycoside ($[\alpha]_D$ +320°) had $[\alpha]_{5461}^{20°}$ +70°, whilst that from the azo-glycoside ($[\alpha]_D$ −100°) had $[\alpha]_{5461}^{20°}$ +74°.

9-*Triacetyl*-β-D-*ribopyranosido*-2-*methylthioadenine*.—(a) *From triacetyl azo-riboside* ($[\alpha]_D$ +320°). Zinc dust (16 g.) was stirred vigorously with a solution of the azo-compound (2 g.) in boiling ethyl acetate (60 c.c.), and a mixture of acetic acid (8 c.c.) and ethyl acetate (80 c.c.) was gradually added during 1 hour. The solution was decanted and the residue extracted thrice with hot ethyl acetate. The combined solution and extracts were evaporated under reduced pressure (nitrogen), and the resinous amino-compound obtained was dissolved in methanol (100 c.c.) and cooled to 0°. A freshly prepared solution of dithioformic acid (1·4 g. of sodium dithioformate in 10 c.c. of methanol, treated at 0° with

0·4 g. of acetic acid in 10 c.c. of methanol) was added and the mixture kept at 0° for 1 hour and then set aside at room temperature overnight. A fresh portion of dithioformic acid (from 0·7 g. of sodium salt) was added, and the mixture heated under reflux for 1 hour and evaporated to dryness under reduced pressure. The residue was extracted four times with ethyl acetate (100 c.c. in all), and the solution concentrated to 20 c.c., poured on a column of neutral alumina (70 g.; 3·5-cm. diam.), washed with ethyl acetate (260 c.c.), and finally eluted with pyridine (130 c.c.). Evaporation of the eluate gave 6-amino-5-thioformamido-4-triacetyl-D-ribosidamino-2-methylthiopyrimidine as a pale yellow resin (676 mg.) which did not crystallise and was used directly for further work.

The resinous thioformamido-compound (400 mg.) was dissolved in ethanol (20 c.c.), sodium methoxide (95 mg.) added, and the mixture heated under reflux for 4 hours and then evaporated to dryness under reduced pressure. The residue was dissolved in water (7 c.c.) and neutralised with dilute hydrochloric acid, and the flocculent precipitate spun off and washed with water (4 × 3 c.c.). The combined filtrate and washings were mixed with picric acid (7 c.c. of a saturated ethanolic solution) and set aside overnight at room temperature. The yellow precipitate was extracted with boiling water (50 c.c.), and the extract concentrated to half bulk and set aside. The crystalline picrate so obtained (100 mg.) had m. p. 160—165° (decomp.). It was dissolved in pyridine (9 c.c.), and acetic anhydride (1·5 c.c.) added. After 16 hours ethanol (1·5 c.c.) was added, and the mixture set aside for 1 hour and then evaporated to dryness under reduced pressure. The residue was dissolved in chloroform (15 c.c.) and poured on a column of activated alumina (10 g.; 1-cm. diam.) which retained the picric acid, the acetylated purine glycoside being washed through the column with chloroform (100 c.c.). Evaporation of the chloroform washings, followed by recrystallisation of the residue from alcohol, gave 9-*triacetyl-β*-D-*ribopyranosido*-2-*methylthioadenine* as colourless prisms (20 mg.), m. p. 230° (Found, in material dried at 100°: C, 46·7; H, 5·2; N, 15·6. $C_{17}H_{21}O_7N_5S$ requires C, 46·5; H, 4·8; N, 15·9%). The compound was insoluble in alkali, had $[\alpha]_D^{17°}$ −35° ±3° (*c*, 0·36 in pyridine) and in ethanol showed absorption maxima at 2345 A. (ε, 24,300) and 2760 A. (ε, 15,700). A small amount (8·2 mg.) was deacetylated with sodium methoxide (2 mg.) in methanol (10 c.c.). After removal of methanol the residue was dissolved in water, made slightly acid, and oxidised with 0·25M-sodium metaperiodate (1 c.c.); in 40 hours the consumption of periodate was complete at 3·0 mols./mol. The riboside yielded with alcoholic picric acid a picrate, m. p. 209—210° (decomp.).

(b) *From triacetyl azo-riboside* ($[\alpha]_D$ −100°).—The triacetyl azo-compound (1·5 g.) was converted into the corresponding 5-thioformamido-compound and cyclised with sodium methoxide as described above. On being worked up in the same manner, 9-triacetyl-*β*-D-ribopyranosido-2-methylthioadenine (17 mg.), m. p. 230°, was obtained. A mixture of this product with that obtained by method (*a*) showed no depression in m. p.

Cyclisation of 6-*Amino*-5-*thioformamido*-4-*triacetyl*-D-*xylopyranosidamino*-2-*methylthiopyrimidine with Potassium Acetate in Methyl Cyanide.*—A solution of the thioformamido-compound (160 mg.; *cf.* Part XI, *loc. cit.*) in dry methyl cyanide (10 c.c.) was heated under reflux for 14 hours with freshly fused potassium acetate (98 mg.; 3 mols.). The residue remaining after evaporation of the solvent was extracted with chloroform (5 c.c.). Removal of chloroform, followed by replacement with ethanol (2 c.c.), yielded 9-triacetyl-D-xylopyranosido-2-methylthioadenine (47 mg.); recrystallised from alcohol it had m. p. 192°, undepressed by an authentic specimen (m. p. 192—193°). The original alcoholic mother-liquor, treated with sodium ethoxide, yielded a small additional amount of product as 9-D-xylopyranosido-2-methylthioadenine (12 mg.).

9-*Triacetyl-α*-D-*ribopyranosido*-2-*methylthioadenine.*—(a) *From* 6-*amino*-4-*triacetyl*-D-*ribosidamino*-5-(2′ : 5′-*dichlorobenzeneazo*)-2-*methylthiopyrimidine* ($[\alpha]_D$ +320°). The acetyl azo-compound (2·0 g.) was converted into the resinous 5-thioformamido-glycoside as described for the preparation of 9-triacetyl-*β*-D-ribopyranosido-2-methylthioadenine (above). The thioformamido-compound (676 mg.), fused potassium acetate (412 mg.), and dry methyl cyanide (50 c.c.) were heated under reflux for 14 hours with exclusion of moisture, and the solution was evaporated to dryness under reduced pressure. The residue was thrice extracted with chloroform (40 c.c. in all), and the extracts were evaporated, yielding a yellow resin (486 mg.) which did not crystallise. It was accordingly re-dissolved in chloroform (10 c.c.) and adsorbed on neutral alumina (50 g.; column, 3·5-cm. diam.). The main pale yellow band was washed through with chloroform (200 c.c.) and chloroform–ethanol (9 : 1; 150 c.c.), the eluate evaporated, the residue (210 mg.) dissolved in warm ethanol (3 c.c.), and saturated ethanolic picric acid (3 c.c.) added. The picrate which separated was washed with cold alcohol (3 × 1 c.c.) and recrystallised from water; the final product (70 mg.) had m. p. 128—130°. The picrate was dissolved in chloroform (10 c.c.) and decomposed by adsorption on alumina in the usual manner, yielding a pale brownish resin (32 mg.) which was dissolved in a minimum of warm benzene, *n*-hexane being then added to incipient turbidity. When the mixture was kept for several days in the ice-chest, 9-*triacetyl-α*-D-*ribopyranosido*-2-*methylthioadenine* separated as clumps of colourless micro-crystals (7 mg.) and was recrystallised from the same solvent mixture. The purified material was insoluble in alkali and had m. p. 199—200° and $[\alpha]_D^{18°}$ +94° ±5° (*c*, 0·148 in pyridine) (Found, in material dried at 100°: N, 15·7. $C_{17}H_{21}O_7N_5S$ requires N, 15·9%). In ethanol the substance showed absorption maxima at 2345 A. (ε, 23,800) and 2755 A. (ε, 15,800). A portion of the glycoside (6·217 mg.) was deacetylated with sodium methoxide (2 mg.) in the usual way, and the product oxidised with 0·25M-periodate (1 c.c.); after 40 hours the consumption of periodate corresponded to 3·0 mols./mol., confirming the pyranose nature of the synthetic product. The picrate prepared by treatment by alcoholic picric acid had m. p. 130—132°.

(b) *From* 6-*amino*-4-*triacetyl*-D-*ribosidamino*-5-(2′ : 5′-*dichlorobenzeneazo*)-2-*methylthiopyrimidine* ($[\alpha]_D$ −100°). The azo-compound (0·76 g.) was reduced, thioformylated, and cyclised with potassium acetate in methyl cyanide as described in (*a*) above. 9-Triacetyl-*α*-D-ribopyranosido-2-methylthioadenine (28 mg.) was obtained, having m. p. 199—200°, undepressed in admixture with the material obtained by method (*a*).

6-*Amino*-4-*triacetyl*-L-*arabinosidamino*-5-(2′ : 5′-*dichlorobenzeneazo*)-2-*methylthiopyrimidine* (" *Pyranose*" *Azo-compound*).—L-Arabinose (5 g.), 4 : 6-diamino-2-methylthiopyrimidine (10 g.), and ammonium chloride (0·3 g.) were heated under reflux in absolute ethanol (80 c.c.) for 1 hour in a flask

fitted with an 18" Fenske column with reflux-ratio head. A mixture of benzene and ethanol (1 : 1; 200 c.c.) was added in portions, and water removed as the ternary mixture by slow distillation. After 8-hours' heating the solution was set aside overnight and worked up in the usual way by adsorption on alumina and elution of the condensation product with water. Concentration of the eluate yielded the glycoside as a resin which could not be crystallised and was used directly for the next step. Dissolved in water (80 c.c.) it was added to a neutral solution of diazotised 2 : 5-dichloroaniline (5·4 g.), and after 1 hour the precipitated azo-compound (9·9 g.) was collected, washed with water, and dried. The crude azo-glycoside was acetylated with acetic anhydride (15 c.c.) in pyridine (45 c.c.) by setting the mixture aside overnight. Ethanol (20 c.c.) was added, and after 2 hours the solution was evaporated under reduced pressure and the residue dissolved in ethyl acetate and chromatographed on neutral alumina in the usual manner. The *triacetyl azo-arabinoside* was eluted from the column with ethyl acetate, and evaporation of the eluate yielded it as an amorphous or micro-crystalline powder (2·7 g.). On dissolution of it in ethanol containing a little ethyl acetate and slow evaporation of the solution, this material yielded small orange prisms, m. p. 213—214°, $[\alpha]_D^{20°}$ −209° (*c*, 0·42 in chloroform) (Found, in material dried at 140°: C, 44·7; H, 4·2; N, 14·3. $C_{22}H_{24}O_7N_6SCl_2$ requires C, 45·0; H, 4·1; N, 14·3%).

6-*Amino*-4-L-*arabinosidamino*-5-(2' : 5'-*dichlorobenzeneazo*)-2-*methylthiopyrimidine*.—Methanolic sodium methoxide (50 mg. of sodium in 2 c.c. of methanol) was added to a solution of the above acetyl azo-arabinoside (0·5 g.) in chloroform (3 c.c.), and the mixture kept overnight at room temperature. The deacetylated *azo-arabinoside* which separated was recrystallised from pyridine–alcohol; it formed flat orange-coloured prisms, m. p. 233—234° (Found, in material dried at 140°: N, 17·9. $C_{16}H_{18}O_4N_6SCl_2$ requires N, 18·2%).

9-L-*Arabopyranosido*-2-*methylthioadenine* (*Sodium Methoxide Cyclisation*).—The above "pyranose" triacetyl azo-arabinoside (13 g.) was reduced with zinc dust in acetic acid, and the product thioformylated by the method described above for the analogous triacetyl azo-ribosides. The resinous thioformamido-glycoside (8·3 g.) was cyclised by heating under reflux for 5 hours in dry ethanol (400 c.c.) containing sodium methoxide (1·6 g.). Evaporation of the solution and crystallisation of the residue from water gave a purine arabinoside (1·4 g.), m. p. 268—270°. Three further recrystallisations gave finally a product *A* (487 mg.), m. p. 273—274°, which, on periodate titration, consumed 2·67 mols./mol., unchanged by further recrystallisation (Found: C, 42·0; H, 5·1; N, 22·5. Calc. for $C_{11}H_{15}O_4N_5S$: C, 42·2; H, 4·8; N, 22·4%). From the original crystallisation mother-liquor, by concentration, a quantity of glycoside (200 mg.) was obtained which had m. p. 284° and showed a periodate uptake of 2·9 mols./mol. Recrystallisation left the m. p. unchanged and the product, which is considered to be pure 9-L-*arabopyranosido*-2-*methylthioadenine*, showed a periodate uptake of 3·03 mols./mol. (Found: C, 42·3; H, 4·9; N, 22·6. $C_{11}H_{15}O_4N_5S$ requires C, 42·2; H, 4·8; N, 22·4%); $[\alpha]_D^{17°}$ +34° ±10° (*c*, 0·058 in water).

Since product *A*, although evidently a mixture of furanose and pyranose forms, could not be separated into its components by recrystallisation, a portion (200 mg.) was acetylated by shaking for 6 hours with acetic anhydride in pyridine and keeping the solution overnight before working up. The product (265 mg.), in a small amount of chloroform, was poured on a column (15 × 1·5 cm.) of neutral alumina. The column was washed with chloroform containing methanol (1%), and the washings were collected in two arbitrary fractions. Each fraction was evaporated, and the residue dissolved in chloroform and again chromatographed in the same way, so that finally four different fractions were obtained from the original material. Each of these fractions was then treated as follows. The solvent was removed under reduced pressure, and the glassy residue dissolved in methanol (5 c.c.) containing sodium methoxide (5 mg.). The crystalline 9-L-arabinosido-2-methylthioadenine which separated was collected and submitted to periodate oxidation; succeeding fractions (in order of elution) consumed the following amounts of periodate: 2·84, 2·95, 2·89, 2·30 mols./mol. These results support the view that the original product *A* is a furanose–pyranose mixture, and suggest that, given larger amounts of material, it might well be possible to isolate the pure components; further experiments in this direction were not carried out.

Cyclisation of "Pyranose" 6-*Amino*-5-*thioformamido*-4-*triacetyl*-L-*arabinosidamino*-2-*methylthiopyrimidine with Potassium Acetate in Methyl Cyanide*.—The thioformamido-compound (607 mg.), prepared as described above starting from the condensation product of L-arabinose and 4 : 6-diamino-2-methylthiopyrimidine, was heated under reflux for 14 hours with fused potassium acetate (370 mg.) in dry methyl cyanide (50 c.c.). The resulting solution was evaporated to dryness and the residue thrice extracted with chloroform (40 c.c. in all); the combined extracts were poured on neutral alumina (35 g.; column, 3-cm. diam.), and the glycoside was washed through with more chloroform (300 c.c.). Evaporation of the pale yellow chloroform solution gave a resin which was set aside overnight in methanol (3 c.c.) with sodium methoxide (3 mg.). The 9-L-arabinosido-2-methylthioadenine which separated (72 mg.) had m. p. 273—274° and consumed 2·85 mols. of periodate per mol. in 90 hours (Found: N, 22·4. Calc. for $C_{11}H_{15}O_4N_5S$: N, 22·4%).

Cyclisation of "Furanose" 6-*Amino*-5-*thioformamido*-4-*triacetyl*-L-*arabinosidamino*-2-*methylthiopyrimidine with Potassium Acetate in Methyl Cyanide*.—5-Benzoyl 2 : 3 : 4-triacetyl L-arabinose (from 7·86 g. of the diethyl thioacetal; Part XVII, *loc. cit.*) was added to a hot solution of 4 : 6-diamino-2-methylthiopyrimidine (10·2 g.) and ammonium chloride (0·26 g.) in dry ethanol (250 c.c.). After 3 days at room temperature the solvent was removed *in vacuo*, and the residue extracted with chloroform (120 c.c. in three portions). The resin obtained on evaporating the chloroform extract was dissolved in dry methanol (100 c.c.), and sodium methoxide (0·75 g.) added. After 1 hour acetic acid (0·2 c.c.) was added and the solution poured into a neutral diazotised solution of 2 : 5-dichloroaniline (4·8 g.). The crude precipitated azo-compound was collected at once and dried at 50° *in vacuo* (15·5 g.). It was then dissolved in dry pyridine (150 c.c.) and adsorbed on activated alumina (250 g.; column, 8-cm. diam.), the column being washed with dry pyridine (400 c.c.). The upper, coloured portion of the column was removed and stirred at 0° with more pyridine, whilst acetic anhydride (75 c.c.) was added dropwise. Next day ethanol (75 c.c.) was added, and after 1 hour the alumina was extracted with ethyl acetate (6 × 150 c.c.), a little ethanol (50 c.c.) being added before the final extraction. The crude acetylated

azo-arabinoside obtained on evaporating the extracts was purified by chromatography on neutral alumina (200 g.; column, 3·5-cm. diam.) in ethyl acetate, and finally by precipitation from ethereal solution by *n*-hexane; it formed an orange-yellow powder (1·8 g.) and was directly reduced in the usual manner with zinc dust in ethyl acetate–acetic acid. The crude 5-amino-arabinoside was thioformylated in methanol, yielding the resinous 5-thioformamido-compound (400 mg.). This material was cyclised by boiling under reflux for 14 hours with fused potassium acetate (240 mg.) in dry methyl cyanide (40 c.c.). Worked up in the manner described in the preceding experiment, the cyclised material yielded a 9-L-arabinosido-2-methylthioadenine (28 mg.), m. p. 274° (Found: N, 22·4. Calc. for $C_{11}H_{15}O_4N_5S$: N, 22·4%). During 70 hours it consumed 2·3 mols. of periodate per mol.

6-*Amino*-4-(5′-*benzoyl*-D-*ribofuranosidamino*)-5-(2″: 5″-*dichlorobenzeneazo*)-2-*methylthiopyrimidine*.—5-Benzoyl 2 : 3 : 4-triacetyl D-ribose (from 5 g. of the diethyl thioacetal) was added to a hot solution of 4 : 6-diamino-2-methylthiopyrimidine (6·5 g.) and ammonium chloride (0·165 g.) in dry ethanol (160 c.c.). After 40 hours the mixture was evaporated under reduced pressure, and the residue extracted with chloroform (3 × 20 c.c.), unchanged 4 : 6-diamino-2-methylthiopyrimidine (4·3 g.) being insoluble. The chloroform extract was evaporated, the residue dissolved in dry methanol (60 c.c.), and sodium methoxide (0·2 g.) added. After 1½ hours, the whole was poured into a neutral diazotised solution of 2 : 5-dichloroaniline (3·1 g.). The granular orange-yellow precipitate (10 g.) was washed, dried, and extracted with warm, dry benzene (55 c.c.); the residue (5·1 g.) consisted largely of sugar-free azo-compound. The benzene extract was poured on alumina (200 g. of Spence Grade H + 5% of water; column, 5·5-cm. diam.), and the chromatogram developed with benzene (400 c.c.). Dry ether (350 c.c.) eluted an orange band, and evaporation of the eluate gave an orange resin (1·56 g.). This resin was again dissolved in ether (50 c.c.) and chromatographed on alumina (150 g. of Spence Grade H + 2·5% of water; 5·5-cm. diam.), the column being washed with ether. The first material passing through the column was partly acetylated, but elution of the remaining dark orange band from the top of the column, with ether containing methanol (5%), and evaporation gave the *benzoyl azo-riboside* as an orange amorphous powder (0·6 g.) (Found in material dried at 70°: C, 49·1; H, 4·0; N, 15·1. $C_{23}H_{22}O_5N_6SCl_2$ requires C, 48·8; H, 3·9; N, 14·8%).

6-*Amino*-4-(5′-*benzoyl*-2′: 3′-*diacetyl*-D-*ribofuranosidamino*)-5-(2″: 5″-*dichlorobenzeneazo*)-2-*methylthiopyrimidine*.—Acetic anhydride (0·5 c.c.) was added to a solution of 6-amino-4-(5′-benzoyl-D-ribofuranosidamino)-5-(2″: 5″-dichlorobenzeneazo)-2-methylthiopyrimidine (0·5 g.) in pyridine (3 c.c.), and the mixture set aside overnight. Ethanol (0·5 c.c.) was then added and after 1 hour the whole was evaporated to dryness under reduced pressure. The residue was dissolved in benzene (20 c.c.), chromatographed on neutral alumina (75 g.; column, 3-cm. diam.) and developed with benzene (500 c.c.) and benzene–ether (5 : 1; 250 c.c.). This treatment washed out two yellow bands, and the remaining yellow band was eluted by washing with benzene–ether (3 : 1; 100 c.c.). The benzene–ether eluate gave the *benzoyl diacetyl* compound as an orange resin (0·19 g.) (Found, in material dried at 70°: C, 50·3; H, 4·5; N, 13·4. $C_{27}H_{26}O_7N_6SCl_2$ requires C, 50·0; H, 4·0; N, 12·9%). A narrow deep-orange band still remained on the column and could be eluted with ether; it consisted largely of the unacetylated starting material.

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