348. *Experiments on the Synthesis of Purine Nucleosides. Part XXIII. A New Synthesis of Adenosine.*

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The extension of the general synthetic route to purine glycosides, developed in earlier papers of this series, to include furanose forms is exemplified by a new synthesis of the natural nucleoside adenosine. 2 : 3 : 4-*Triacetyl* 5-*benzyl* D-*ribose* condenses with 4 : 6-diamino-2-methylthiopyrimidine to give a Schiff base which is deacetylated with complete retention of the 5-benzyl group and then isomerises to a 5-benzylribofuranoside. By following the normal procedure of nucleoside synthesis from this point and removing the protecting benzyl residue and the 2-methylthio-group from the resulting purine glycoside with Raney nickel, 9-β-D-ribofuranosidoadenine is obtained, identical with adenosine prepared from yeast ribonucleic acid.

In earlier papers of this series a general and unambiguous method for the synthesis of purine 9-glycosides was developed which rested on the condensation of an aldose with a suitable 4 : 6-diaminopyrimidine derivative, to give the corresponding 6-amino-4-glycosidamino-pyrimidine, and proceeded by introduction of an amino-group into position 5 of the pyrimidine ring, thioformylation, and cyclisation of the 5-thioformamido-compound so obtained, to yield the required purine glycoside. When it was found that this procedure yielded, in general, pyranose glycosides, whereas the naturally occurring nucleosides have a furanose structure, experiments were initiated with the object of so modifying the initial linkage of sugar to pyrimidine that 6-amino-4-glycofuranosidamino-pyrimidines would be produced; some of these

experiments have already been reported (Part XVII, Kenner, Lythgoe, and Todd, *J.*, 1948, 957). The work carried out during the establishment of the general route to the purine glycopyranosides had, however, not only finally established the structure of the purine and pyrimidine ribonucleosides (Davoll, Lythgoe, and Todd, *J.*, 1946, 833; Howard, Kenner, Lythgoe, and Todd, *ibid.*, p. 861; Lythgoe, Smith, and Todd, *J.*, 1947, 355), but had also made it possible, after the preparation of acetohalogenoribofuranoses (Howard, Lythgoe, and Todd, *J.*, 1947, 1052), to synthesise the naturally occurring nucleosides (Howard, Lythgoe, and Todd, *loc. cit.*; Davoll, Lythgoe, and Todd, *J.*, 1948, 967, 1685) by extension of the methods used earlier for pyrimidine glucosides by Hilbert and Johnson (*J. Amer. Chem. Soc.*, 1930, **52**, 4489) and for adenine glucoside by Fischer and Helferich (*Ber.*, 1914, **47**, 210). Xanthosine was also synthesised by application of the Hofmann degradation to 3-triacetyl-D-ribofuranosidoglyoxaline-4 : 5-dicarboxyamide (Howard, McLean, Newbold, Spring, and Todd, this vol., p. 232).

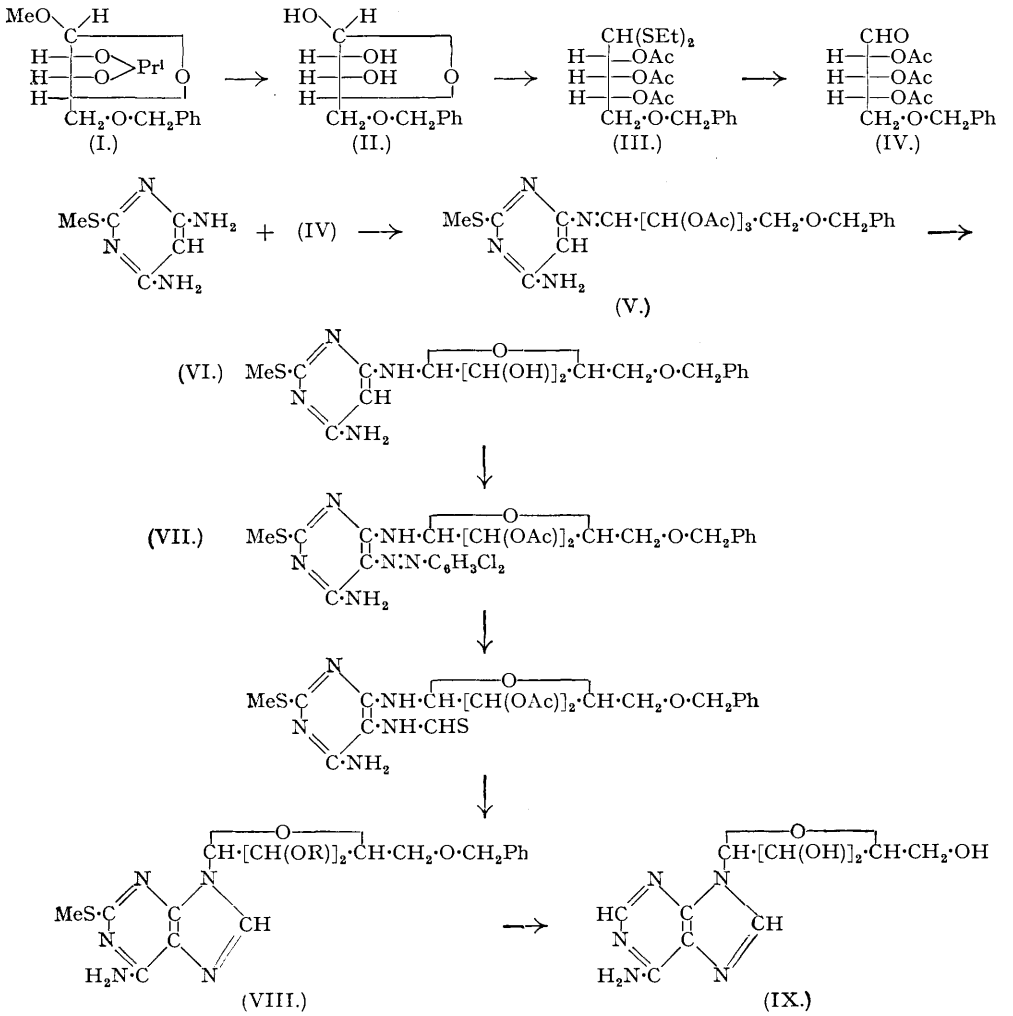
These syntheses, although valuable in individual cases, are of limited application and their accomplishment did not in any way reduce the importance of extending our general synthetic procedure to cover the 9-glycofuranosidopurines, since such a method is an essential part of the scheme of investigations on nucleosides and nucleotides upon which we are engaged. The successful extension of the general procedure to the synthesis of adenosine forms the subject of the present memoir.

Initial attempts to prepare 6-amino-4-pentofuranosidaminopyrimidines by condensation of 5-benzoyl 2 : 3 : 4-triacetyl pentoses with 4 : 6-diaminopyrimidines to give Schiff bases, followed by hydrolytic removal of the acetyl groups and consequent isomerisation to 5′-benzoylpentofuranosides, were only partly successful. The unsatisfactory results were due, in part, to the fact that some debenzoylation always occurred as well as deacetylation, even under the mildest conditions, and, in part, to other complications arising from furanose–pyranose interconversion and α–β-isomerisation at subsequent stages in the syntheses. These investigations have been described elsewhere in detail (Kenner, Lythgoe, and Todd, *J.*, 1948, 957; also, preceding paper) and led to the conclusion that the difficulty of preparing adequate quantities of pure furanosidamino-pyrimidines by this means was so great that the use of a benzoyl residue as a protecting group in position 5 of the pentose residue should be abandoned. It was evident, however, from the work which had been done, that the Schiff-base route to purine glycofuranosides was sound in principle, and that it could be successfully applied if the 5-position in the *aldehydo*-sugar derivative were protected by a group which could be retained intact until the glyoxaline ring of the purine had been closed and furanose–pyranose interconversion thereby prevented. For this purpose we elected to use a benzyl residue, since it would undoubtedly be retained under conditions bringing about complete removal of acetyl groups and could be removed finally by hydrogenation.

5-Benzyl 2 : 3-iso*propylidene methyl*-D-*ribofuranoside* (I) was prepared from 2 : 3-*iso*propylidene methyl-D-ribofuranoside (Levene and Stiller, *J. Biol. Chem.*, 1934, **104**, 301) by benzyl chloride and potassium hydroxide in xylene; it was also obtained, in lower yield, by adding benzyl chloride to a solution of the riboside and sodium in liquid ammonia. Hydrolysis with dilute acid yielded *5-benzyl* D-*ribofuranose* (II) as a syrup, whose structure was confirmed by acetylation followed by removal of the benzyl group by hydrogenolysis; acetylation of the product so obtained gave the known 1 : 2 : 3 : 5-tetra-acetyl D-ribofuranose. From 5-benzyl D-ribofuranose, 2 : 3 : 4-*triacetyl* 5-*benzyl* D-*ribose diethyl thioacetal* (III) was obtained by interaction with ethanethiol in presence of hydrochloric acid, followed by acetylation, and from it 2 : 3 : 4-*triacetyl* 5-*benzyl* D-*ribose* (IV) was prepared as a colourless syrup by the normal procedure.

Condensation of (IV) with 4 : 6-diamino-2-methylthiopyrimidine in ethanol containing a little ammonium chloride gave a glassy product, presumably the Schiff base (V), which was deacetylated with methanolic ammonia, to convert it into the required glycoside (VI), and coupled with diazotised 2 : 5-dichloroaniline in the usual way. The crude azo-compound was acetylated and the 6-*amino*-4-(2′ : 3′-*diacetyl*-5′-*benzyl*-D-*ribofuranosidamino*)-5-(2″ : 5″-*dichlorobenzeneazo*)-2-*methylthiopyrimidine* (VII) purified by chromatography. Complete separation of (VII) from a small amount of adhering sugar-free material (*ca.* 10%) is rather tedious, but for use in the next step complete removal of the latter is not essential since sugar-free impurities are readily eliminated in the later stages. Model experiments having shown that the benzyl group in 5-benzyl pentoses is not affected by zinc dust and acetic acid, (VII) was reduced with this reagent, and the resulting 5-amino-compound directly thioformylated. The resinous thioformamido-glycoside so obtained was cyclised using sodium methoxide, but the purine glycoside (VIII; R = H) showed no tendency to crystallise. It was, however, clear that the most direct proof of the validity of the synthesis would be the production of adenosine (IX) itself, whose

structure is known. It was, therefore, decided to proceed directly to adenosine without isolating (VIII; R = H) in a pure state. The resinous material was acetylated and the acetyl derivative



(VIII; R = Ac) heated under reflux in ethanol with Raney nickel to effect simultaneous removal of the benzyl and the methylthio-group. Deacetylation of the product furnished adenosine (9-β-D-ribofuranosidoadenine) (IX), identical with a sample of the natural nucleoside prepared by hydrolysis of yeast ribonucleic acid.

The synthesis of adenosine in this way establishes the validity of the Schiff-base route, and it is clear that it should be generally applicable to other purine glycofuranosides, including those derived from deoxypentoses, provided that the appropriate 5-benzyl sugar derivatives can be made available.

Experimental.

*5-Benzyl 2 : 3-iso**Propylidene Methyl-D-ribofuranoside.—Method I.* 2 : 3-*iso*Propylidene methyl-D-ribofuranoside (5 g.; Levene and Stiller, *loc. cit.*) was dissolved in dry xylene (50 c.c.), finely powdered potassium hydroxide (20 g.) and benzyl chloride (4 g.) were added, and the mixture was stirred at 80° for 4 hours. Water was now added, the mixture evaporated *in vacuo*, and the residue extracted with ether. The ethereal extract was dried over sodium sulphate, the ether removed, and the residue distilled in a high vacuum. Aftr traces of benzyl chloride and benzyl alcohol had been removed at a bath temp. of 50°, the *riboside* distilled at 95—100° (bath temp.)/10^{-4} mm. as a colourless syrup (6 g.), $[\alpha]_D^{17°}$ −36° ±2° (*c*, 1·3 in chloroform) (Found: C, 65·3; H, 7·8. $C_{16}H_{22}O_5$ requires C, 65·3; H, 7·5%).

Method II. 2 : 3-*iso*Propylidene methyl-D-ribofuranoside (2 g.) in dry ether (1 c.c.) was added to liquid ammonia (60 c.c.). Sodium (0·25 g.) was added to the solution, and the mixture stirred until the

sodium dissolved and the blue colour disappeared. Benzyl chloride (1·7 c.c.) was now added, and the ammonia was allowed to evaporate. Dry air was blown through the flask for 2 hours, the residue dried over phosphoric oxide and extracted with ether, the extract evaporated, and the product distilled as above. The yield was 1·6 g. (Found : C, 66·0; H, 7·6%).

5-Benzyl D-*Ribofuranose*.—5-Benzyl 2 : 3-*iso*propylidene methyl-D-ribofuranoside (2·3 g.) was heated on a boiling water-bath for 3 hours with 50% aqueous ethanolic hydrochloric acid (50 c.c. of 0·05N). The acid was neutralised with barium carbonate, and the filtered solution evaporated under reduced pressure. The residue was extracted with chloroform, the extract washed with a little water, dried (Na_2SO_4), and evaporated. The *product* (1·6 g.) was a pale yellow syrup which could not be distilled or crystallised; it had $[\alpha]_D^{18°}$ −8·5° (*c*, 1·5 in alcohol) (Found : C, 59·6; H, 6·9. $C_{12}H_{16}O_5$ requires C, 60·0; H, 6·7%).

A sample of 5-benzyl D-ribofuranose (2 g.) prepared in this way was acetylated in the usual manner with acetic anhydride (6 c.c.) in pyridine (12 c.c.). The syrupy acetyl compound (2·7 g.) so obtained was dissolved in ethanol and hydrogenated under atmospheric pressure using a palladised charcoal catalyst. Catalyst was removed by filtration, the solution evaporated, and the residue acetylated with acetic anhydride (2 c.c.) in pyridine (6 c.c.). The product was purified by distillation under reduced pressure and finally by recrystallisation from ethanol, whereupon 1 : 2 : 3 : 5-tetra-acetyl D-ribofuranose (1 g.) was obtained, having m. p. 57—58°, undepressed in admixture with an authentic specimen (m. p. 58°; Howard, Lythgoe, and Todd, *J.*, 1947, 1052).

2 : 3 : 4-*Triacetyl* 5-*Benzyl* D-*Ribose Diethyl Thioacetal*.—5-Benzyl D-ribofuranose (3·8 g.) was dissolved in a mixture of dioxan (0·25 c.c.) and concentrated hydrochloric acid (5 c.c.; *d* 1·19), the solution cooled to 0°, and ethanethiol (3 c.c.) added in three portions with shaking. After being shaken for a further 10 minutes the mixture was set aside at room temperature for 15 minutes and then poured into excess of ice-cold saturated aqueous sodium hydrogen carbonate, and the whole extracted with chloroform. The chloroform extracts, dried (Na_2SO_4) and evaporated, gave crude 5-benzyl D-ribose diethyl thioacetal as a yellow syrup (3 g.) which was acetylated in the usual way with acetic anhydride (10 c.c.) in pyridine (30 c.c.). The product distilled at 170—180° (bath temp.)/10^{-4} mm. as a colourless syrup (1·3 g.) which did not crystallise; it had $[\alpha]_D^{18°}$ +9° (*c*, 0·87 in chloroform) (Found : C, 56·4; H, 7·1. $C_{22}H_{32}O_7S_2$ requires C, 55·9; H, 6·8%).

2 : 3 : 4-*Triacetyl* 5-*Benzyl* D-*Ribose*.—2 : 3 : 4-Triacetyl 5-benzyl D-ribose diethyl thioacetal (3·7 g.) was dissolved in a mixture of acetone (40 c.c.) and water (4 c.c.), yellow mercuric oxide (8 g.) was added, and the mixture stirred vigorously while a solution of mercuric chloride (9 g.) in acetone (20 c.c.) was run in during 1 hour. Stirring was continued for 24 hours at room temperature, and the mixture filtered. The filtrate was evaporated under reduced pressure, and traces of water removed from the residual syrup by distillation with benzene. The residue was extracted with chloroform (3 × 50 c.c.), and the extract washed with 40% aqueous potassium iodide (50 c.c.) and then with water and dried (Na_2SO_4). Chloroform was removed by evaporation, and the aldehydo-*sugar* purified by distillation at 150° (bath temp.)/10^{-3} mm. It formed a colourless syrup (2·6 g.), $[\alpha]_D^{17°}$ −4·2° ±0·8° (*c*, 0·65 in chloroform) (Found : C, 59·4; H, 6·4. $C_{18}H_{22}O_8$ requires C, 59·0; H, 6·0%).

6-*Amino*-4-(2′ : 3′-*diacetyl*-5′-*benzyl*-D-*ribofuranosidamino*)-5-(2″ : 5″-*dichlorobenzeneazo*)-2-*methylthiopyrimidine*.—Ammonium chloride (0·3 g.), 4 : 6-diamino-2-methylthiopyrimidine (11·4 g.), and 2 : 3 : 4-triacetyl 5-benzyl D-ribose (6·6 g.) were dissolved in boiling absolute ethanol (270 c.c.), and the solution was kept at room temperature for 24 hours and then evaporated under reduced pressure. The residue was extracted with chloroform, unchanged diamine remaining undissolved, and the extract on evaporation yielded a yellowish glass (8·8 g.). This crude Schiff base was dissolved in methanol (200 c.c.) previously saturated with ammonia at 0°, and the solution set aside for 2 days and then evaporated, the deacetylated glycoside being thus obtained as a syrup.

A portion of this syrup (4·3 g.) was dissolved in pyridine (100 c.c.) and poured into a neutral diazotised solution of 2 : 5-dichloroaniline (1·85 g.); after 2 hours at 0° the solution was diluted with water (600 c.c.), and the precipitated azo-compound (4·5 g.) collected, washed with water, and dried. The dried material was acetylated in the cold with acetic anhydride (5 c.c.) in pyridine (10 c.c.), and the product chromatographed on neutral alumina (140 g.; column, 4-cm. diam.), the column being washed with benzene, then with benzene containing increasing amounts of chloroform, and finally with chloroform alone. The main bulk (1·1 g.) of the product was eluted by washing with chloroform (900 c.c.) containing 0·5% of methanol. Evaporation of the eluate, followed by dissolution in ethanol and precipitation with hexane, gave an amorphous orange-yellow product which, from its analysis, appeared to be the desired azo-glycoside contaminated with *ca.* 10—12% of sugar-free azo-compound (Found: C, 50·1; H, 4·1; N, 15·0%). In order to obtain a pure specimen, the above material was deacetylated with sodium methoxide in the usual manner and chromatographed on neutral alumina, the column being thoroughly washed with ethyl acetate. The washed column was extruded and the adsorbed material acetylated directly with cold acetic anhydride in pyridine solution. The acetylated product was adsorbed on a column of neutral alumina, and the *azo-glycoside* eluted by washing with ethyl acetate. Purified by precipitation from ethanolic solution with hexane, it was obtained as an orange-yellow powder which softened and melted at *ca.* 90° (Found : C, 51·2; H, 4·0; N, 13·4. $C_{27}H_{28}O_6N_6SCl_2$ requires C, 51·0; H, 4·4; N, 13·2%), $[\alpha]_D^{16°}$ +660° ±60° (*c*, 0·048 in chloroform).

Adenosine (9-β-D-*Ribofuranosidoadenine*).—The above acetyl azo-riboside (1 g. containing *ca.* 12% of sugar-free material) and zinc dust (8 g.) were stirred vigorously in boiling ethyl acetate (80 c.c.), and a mixture of acetic acid (4 c.c.) in ethyl acetate (40 c.c.) was added dropwise during 30 minutes. The solution was decanted from zinc, the latter twice extracted with warm ethyl acetate, and the combined solution and extracts were evaporated to dryness under reduced pressure (nitrogen). The resin obtained was triturated with light petroleum (b. p. 60—80°), and the insoluble portion dissolved in methanol (100 c.c.) and cooled to 0°. An ice-cold solution of sodium dithioformate (0·8 g.) in methanol (5 c.c.) was added, followed by acetic acid (0·2 g.) in methanol (5 c.c.), and the mixture set aside for 4 hours. A further quantity of dithioformic acid (from 0·4 g. of sodium dithioformate) was added in the same way, and the whole kept overnight and then heated under reflux for 1 hour. Evaporation under reduced pressure gave a residue which was extracted thoroughly with ethyl acetate, and the extracts were

evaporated. The solid residue was dissolved in hot benzene (50 c.c.) and adsorbed on a column of neutral alumina (30 g.; 2-cm. diam.). The column was washed with benzene until the washings were colourless, and the thioformamido-glycoside then eluted with pyridine (100 c.c.); evaporation of the eluate gave a brittle resin (0·3 g.) which could not be crystallised.

The resinous thioformamido-glycoside was heated under reflux in absolute ethanol (20 c.c.) with sodium methoxide (60 mg.) under nitrogen for 4 hours. The resulting solution was neutralised with acetic acid and evaporated to dryness, giving the crude purine glycoside as a brownish resin, which did not crystallise. It was accordingly acetylated by shaking it for 24 hours with acetic anhydride (1 c.c.) in pyridine (5 c.c.), the mixture being worked up in the usual manner. The product was dissolved in ethanol (30 c.c.), Raney nickel (3 g.) added, and the whole boiled for 2 hours and filtered. The nickel was extracted (Soxhlet) with a mixture of ethanol, water, and pyridine (90 : 5 : 5) for 12 hours, and the extract combined with the ethanolic filtrate. Evaporation yielded a glassy residue which was deacetylated with sodium methoxide (15 mg.) in methanol (15 c.c.). The methanol was distilled off and replaced by water (10 c.c.), acetic acid being added to neutrality. When this solution evaporated slowly at room temperature under reduced pressure, a quantity of crystalline material (largely sugar-free purine contaminated with some nickel complex) separated and was filtered off. Hot saturated aqueous picric acid was added to the filtrate. Adenosine picrate separated and, after two recrystallisations from water, formed yellow needles (30 mg.), m. p. 181—185° (decomp.), alone or mixed with an authentic specimen (m. p. 181—185°) (Found: C, 38·5; H, 3·4; N, 22·3. Calc. for $C_{10}H_{13}O_4N_5,C_6H_3O_7N_3$: C, 38·7; H, 3·2; N, 22·6%).

To prepare adenosine itself, the picrate (100 mg.) was acetylated in the usual way with acetic anhydride (0·3 c.c.) in pyridine (1·5 c.c.), and the product dissolved in chloroform and decomposed by adsorption on neutral alumina. The picric acid-free product was eluted with chloroform (100 c.c.) containing 1% of ethanol and deacetylated with sodium methoxide (10 mg.) in methanol (2 c.c.). Adenosine crystallised directly from the hydrolysis solution and was recrystallised from water (yield, 38 mg.). It had m. p. 230—231°, undepressed in admixture with an authentic specimen (m. p. 230—231°) from natural sources, and had $[\alpha]_D^{18°}$ −62·1° (*c*, 0·34 in water) (Found, in material dried at 100° for 12 hours: C, 45·0; H, 4·8; N, 26·3. Calc. for $C_{10}H_{13}O_4N_5$: C, 45·0; H, 4·9; N, 26·2%). On periodate titration the uptake of oxidant in 48 hours was 0·98 mol./mol.

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