184. *Experiments on the Synthesis of Purine Nucleosides. Part XVIII. A Synthesis of* 9-D-*Glucopyranosidoadenine.*

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9-D-Glucopyranosidoadenine has been synthesised by two methods each using the unambiguous general route to 9-glycosidopurines described in earlier papers of this series. In the first of these, starting from 4 : 6-diamino-2-methylthiopyrimidine and D-glucose, 9-D-*glucopyranosido*-2-*methylthioadenine* was prepared and the methylthio-group removed by treatment with Raney nickel. In the second a more direct synthesis was employed starting from 4 : 6-diaminopyrimidine. The yields in both syntheses were low, but the 9-D-glucopyranosidoadenine, isolated as its picrate, was identical with the adenine glucoside of Fischer and Helferich (*Ber.*, 1914, **47**, 210). This provides additional chemical proof that the sugar residue in adenosine is located at N_9 in the purine skeleton.

In Part XVI (*J.*, 1947, 355) we described the synthesis of 9-β-D-mannopyranosidoadenine, using a method developed in earlier papers which established beyond doubt the position of the sugar residue in the product. One of our objects in preparing this mannoside was to demonstrate directly that in adenosine the ribofuranose residue is attached to N_9 of the purine skeleton, and this aim was accomplished by showing that identical dialdehydes arose from periodate oxidation of the synthetic mannopyranoside on the one hand and of the natural ribofuranoside on the other. The same result could have been achieved with equal validity in an alternative way, which it was our original intention to follow. We had already shown (Davoll, Lythgoe, and Todd, Part XII, *J.*, 1946, 833) that the two sugar residues are present in identical positions in adenosine and in Fischer and Helferich's adenine glucoside, a synthetic product which is obtained by interaction of acetobromoglucose with 2 : 8-dichloroadenine silver, followed by deacetylation and dehalogenation of the product (*Ber.*, 1914, **47**, 210; Davoll, Lythgoe, and Todd, *loc. cit.*). A chemical proof that Fischer and Helferich's compound is in fact a 9-glycoside would therefore establish the same structural feature for adenosine. Accordingly, we commenced the synthesis of 9-D-glucosidoadenine by an unambiguous route, but difficulties in securing satisfactory yields during the later stages caused delay, with the result that the synthesis of the corresponding mannosides, and the proof using the alternative method, were completed first. It seemed desirable in spite of the poor yields obtained to place on record the experiments relating to the synthesis of the glucoside, and a description of this work forms the subject of the present paper.

Condensation of 4 : 6-diamino-2-methylthiopyrimidine and D-glucose by the method described in Part III (*J.*, 1943, 571) gave a crystalline 6-*amino*-4-D-*glucosidamino*-2-*methylthiopyrimidine*, which was used in our first experiments as starting material for a synthesis of 9-D-glucosidoadenine by a route similar to that used for synthesis of 9-D-xylopyranosidoadenine (Part XI, *J.*, 1945, 556). Treatment with nitrous acid converted the 4-D-glucosidamino-compound into a 5-nitroso-derivative which was reduced and the resulting 5-amino-derivative converted without purification into 6-*amino*-5-*thioformamido*-4-D-*glucosidamino*-2-*methylthiopyrimidine*. The last was successfully cyclised by heating it with sodium methoxide in ethanol (Part XIII, *J.*, 1946, 852), giving 9-D-glucosido-2-methylthioadenine. This compound was difficult to isolate in satisfactory yield in pure condition, owing to its high solubility in water and apparently low crystallising power. The methylthio-group was replaced by hydrogen by refluxing an alcoholic solution of the resinous triacetyl derivative with nickel containing adsorbed hydrogen, the acetyl groups then being removed by treatment with methanolic ammonia. The resulting 9-D-glucosidoadenine could not be obtained crystalline, owing to unfavourable solubility and the small amounts available, but treatment with picric acid gave a picrate identical in properties with the picrate of adenine glucoside prepared by Fischer and Helferich's method.

The low yields in the above synthesis made it desirable to explore the alternative and more direct method of synthesis starting from 6-*amino*-4-D-*glucosidaminopyrimidine*. This was obtained in satisfactory yield by condensation of the components in ethanol containing hydrogen chloride. It coupled readily with diazotised 2 : 5-dichloroaniline, giving 6-*amino*-5-(2′ : 5′-*dichlorobenzeneazo*)-4-D-*glucosidaminopyrimidine*, and the corresponding *tetra-acetyl* derivative was catalytically reduced to 5 : 6-*diamino*-4-*tetra-acetyl*-D-*glucosidaminopyrimidine*. Treatment with dithioformic acid in ethanol converted this into its 5-thioformyl derivative, but although purified chromatographically the product could not be obtained crystalline, and again the yield was rather poor. Cyclisation with sodium methoxide in ethanol gave a product from which, by

treatment with picric acid, 9-D-glucosidoadenine picrate was obtained, identical with material obtained by the first route.

These experiments demonstrate the nature of Fischer and Helferich's adenine glucoside as a 9-D-glucosidoadenine, and confirm the results of Part XVI (*loc. cit.*), demonstrating that adenosine is a 9-glycosido-adenine.

Experimental.

6-*Amino*-4-D-*glucosidamino*-2-*methylthiopyrimidine*.—4 : 6-Diamino-2-methylthiopyrimidine (22·5 g.), D-glucose (11·3 g.), and ammonium chloride (0·75 g.) were heated under reflux in dry ethanol (300 c.c.) for 12 hours, water generated in the reaction being removed periodically by addition of dry benzene and distillation of the azeotropic mixture through an efficient column. The cooled solution was filtered through activated aluminium oxide (1000 g.) which was washed first with ethanol (3·5 l.) to remove unchanged diamine, and then with water (5 l.). The aqueous eluate was evaporated under reduced pressure to small volume (100 c.c.) and kept overnight; the *glucoside* which had then separated was recrystallised from water. It formed colourless needles, softening at 145° but without definite m. p.; $[\alpha]_D^{20°}$ — 112° (*c*, 4·56 in water) (Found : C, 40·5; H, 6·0; N, 17·1. $C_{11}H_{18}O_5N_4S,\frac{1}{2}H_2O$ requires C, 40·4; H, 5·8; N, 17·1%) (yield, 10 g.).

Hydrolysis. A portion (0·3 g.) of the above glucoside was heated under reflux for 2 hours with 0·1N-sulphuric acid (21 c.c.), the solution neutralised with sodium hydroxide and evaporated to dryness, and the residue extracted with hot ethanol (30 c.c.). The extract was filtered through activated aluminium oxide (15 g.) which was washed with ethanol (45 c.c.), and filtrate and washings united and evaporated, giving 4 : 6-diamino-2-methylthiopyrimidine, characterised as the picrate, m. p. 212° (decomp.) alone or mixed with authentic material. A second portion of glucoside (0·12 g.) was hydrolysed for 2 hours with boiling N-hydrochloric acid (5 c.c.), and then worked up in a similar fashion. The aluminium oxide after being washed with ethanol was eluted with water, the aqueous eluate on treatment with phenylhydrazine hydrochloride and sodium acetate giving glucosazone.

Acetylation. 6-Amino-4-D-glucosidamino-2-methylthiopyrimidine (1 g.), acetic anhydride (4 c.c.), acetyl chloride (0·2 c.c.), and pyridine (15 c.c.) were heated on the steam-bath for 1 hour, the cooled solution treated with ethanol (20 c.c.), and, after 2 hours, solvents were removed under reduced pressure. Crystallisation of the residue from ethanol gave 6-*acetamido*-4-*tetra-acetyl*-D-*glucosidamino*-2-*methylthiopyrimidine* (1 g.) as needles, m. p. 194°; $[\alpha]_D^{17°}$ — 33° (*c*, 1·13 in pyridine) (Found : C, 47·5; H, 5·5; N, 11·0. $C_{21}H_{28}O_{10}N_4S$ requires C, 47·7; H, 5·3; N, 10·6%).

6-*Amino*-5-*thioformamido*-4-D-*glucosidamino*-2-*methylthiopyrimidine*.—To a solution of 6-amino-4-D-glucosidamino-2-methylthiopyrimidine (5 g.) and sodium nitrite (1 g.) in water (150 c.c.), which was cooled to 5°, glacial acetic acid (2 c.c.) was added. After the mixture had been kept at 0° overnight the blue nitroso-compound was collected, washed with cold water, and suspended in ice-cold water (40 c.c.). To the well-cooled suspension ammonium sulphide solution (2·7 c.c. of aqueous ammonia, *d* 0·88, and 40 c.c. of water saturated with hydrogen sulphide at 0°) was added, and a vigorous current of hydrogen sulphide passed through the mixture for 1 hour, after which it was evaporated to dryness under reduced pressure and the residue extracted with hot water (170 c.c. at 90°). The extract, cooled to 40°, was treated with sodium dithioformate solution (4·6 g. hexahydrate in 20 c.c. water) and the solution kept overnight. The *thioformamido*-compound collected and recrystallised from water had m. p. 204—205° (Found : C, 36·3; H, 5·3; N, 17·8. $C_{12}H_{19}O_5N_5S_2,H_2O$ requires C, 36·4; H, 5·3; N, 17·7%) (yield, 2 g.).

Acetylation. Acetylated with pyridine and acetic anhydride in the usual way the above thioformamido-compound (6·5 g.) gave 6-*amino*-5-*thioformamido*-4-*tetra-acetyl*-D-*glucosidamino*-2-*methylthiopyrimidine*, forming crystals from ethanol–chloroform, m. p. 218—219° (Found : C, 43·8; H, 4·9; N, 12·6. $C_{20}H_{27}O_9N_5S_2$ requires C, 44·0; H, 4·9; N, 12·8%) (Yield, 7 g.).

9-D-*Glucopyranosido*-2-*methylthioadenine*.—The acetyl-free thioformamido-compound (200 mg.) and sodium methoxide (30 mg.) were dissolved in dry ethanol (16 c.c.) and the solution heated under reflux in a slow stream of nitrogen for 5 hours, then kept at 0° for 2 hours and evaporated to dryness under reduced pressure. The residue was dissolved in cold water, N-sulphuric acid (0·7 c.c.) added, and the solution warmed with barium carbonate for a few minutes, filtered, and evaporated to dryness. Recrystallisation from ethanol gave the *glucoside* as a light brown powder, m. p. 173—176° (Found : C, 39·9; H, 5·7; N, 19·5. $C_{12}H_{17}O_5N_5S,H_2O$ requires C, 39·9; H, 5·3; N, 19·4%) (yield, 100 mg.).

6-*Amino*-4-D-*glucosidaminopyrimidine*.—4 : 6-Diaminopyrimidine (18 g.) and D-glucose (9 g.) were heated under reflux in dry ethanol (500 c.c.) containing hydrogen chloride (6 c.c. of ethanol saturated with dry hydrogen chloride at 0°) for 40 hours, with azeotropic removal of water. Filtration through activated aluminium oxide as usual, followed by washing with ethanol, elution with water, and evaporation of the aqueous eluate, gave 6-*amino*-4-D-*glucosidaminopyrimidine* as colourless needle-clusters which on rapid heating melted at 191°, resolidified, and finally melted at 208—209° (Found : C, 41·8; H, 6·4; N, 19·3. $C_{10}H_{16}O_5N_4,H_2O$ requires C, 41·4; H, 6·2; N, 19·3%) (yield, 8 g.).

6-*Amino*-4-*tetra-acetyl*-D-*glucosidamino*-5-(2′ : 5′-*dichlorobenzeneazo*)*pyrimidine*.—A suspension of the above glucoside (5·5 g.) in water (50 c.c.) was treated with a solution of 2 : 5-dichlorobenzenediazonium chloride, prepared in the usual way from 2 : 5-dichloroaniline (4 g.) in water (120 c.c.) containing concentrated hydrochloric acid (25 c.c.) and sodium nitrite (1·7 g.). The solution was then immediately neutralised with sodium hydrogen carbonate solution, and after standing overnight the red azo-compound was collected and dried (yield, 7·4 g.). This crude material was dissolved in pyridine (150 c.c.), treated with acetic anhydride (37·5 c.c.), and, after standing overnight, excess of acetic anhydride was decomposed with ethanol (60 c.c.) and solvents removed under reduced pressure. The residue was dissolved in ethyl acetate and filtered through a column of activated aluminium oxide from which washing with the same solvent removed the desired substance, which was then obtained by evaporation of the eluate and crystallisation of the residue from benzene. 6-*Amino*-4-*tetra-acetyl*-D-*glucosidamino*-5-

(2′ : 5′-*dichlorobenzeneazo*)*pyrimidine* separated as pellet-like needle aggregates, m. p. 236—237°; $[\alpha]_D^{18°}$ — 360° (*c*, 3·76 in chloroform) (Found: C, 45·8; H, 4·3; N, 13·7. $C_{23}H_{24}O_8N_6Cl_2,H_2O$ requires C, 45·9; H, 4·3; N, 14·0%) (yield, 4·5 g.).

By deacetylation of this material with methanolic sodium methoxide 6-*amino*-4-D-*glucosidamino*-5-(2′ : 5′-*dichlorobenzeneazo*)*pyrimidine* was obtained. Recrystallised from ethanol–pyridine it had m. p. 225° (decomp.) (Found: C, 42·9; H, 4·0; N, 18·8. $C_{16}H_{18}O_5N_6Cl_2$ requires C, 43·1; H, 4·0; N, 18·9%). The same compound was also obtained by purification of the crude material from the coupling reaction described above.

5 : 6-*Diamino*-4-*tetra-acetyl*-D-*glucosidaminopyrimidine*.—The acetylated azo-compound (0·5 g.) dissolved in ethyl acetate was hydrogenated in presence of Raney nickel during 7 hours at 100°/100 atmospheres. The catalyst was removed by filtration, washed with ethanol, and filtrate and washings evaporated under reduced pressure. Recrystallisation of the residue from ethyl acetate gave the *product* as clusters of needles, which softened at 80° but had no characteristic m. p. (Found: C, 47·0; H, 5·6; N, 15·2. $C_{18}H_{25}O_9N_5$ requires C, 47·5; H, 5·5; N, 15·4%). For preparation of larger quantities the crude hydrogenation product was freed from 2 : 5-dichloroaniline by dissolving it in ethyl acetate and adding light petroleum. The precipitated material was collected and washed with light petroleum and was then sufficiently pure for use in the preparation of 9-D-glucopyranosidoadenine.

9-D-*Glucopyranosidoadenine*.—(a) *From* 9-D-*glucopyranosido*-2-*methylthioadenine*. The 2-methylthio-compound (170 mg.) was acetylated with pyridine and acetic anhydride in the usual manner, giving the acetyl derivative as a resin, which was dissolved in ethanol (42 c.c.) and heated under reflux for 2 hours with Raney nickel (2·5 g.) prepared according to Mozingo (*J. Amer. Chem. Soc.*, 1943, **65**, 1013). After 24 hours the solution was filtered and the residual nickel extracted with boiling ethanol (Soxhlet). Filtrate and extracts were combined and evaporated, and the residue dissolved in methanolic ammonia (30 c.c. saturated at 0°) and kept for 3 days. Solvents were then removed under reduced pressure, the residue dissolved in water, and the solution filtered from a little insoluble material and treated with saturated aqueous picric acid. On cooling, adenine glucoside picrate separated as needles (103 mg.), m. p. 246° (decomp.) alone or mixed with a specimen prepared by Fischer and Helferich's method (Found: C, 38·8; H, 3·5; N, 21·6. Calc. for $C_{11}H_{15}O_5N_5,C_6H_3O_7N_3$: C, 38·8; H, 3·4; N, 21·3%)

(b) *From* 5 : 6-*diamino*-4-D-*glucosidaminopyrimidine*. Crude diamino-compound [from hydrogenation of 0·5 g. of 6-amino-4-tetra-acetyl-D-glucosidamino-5-(2′ : 5′-dichlorobenzeneazo)pyrimidine] was dissolved in ethyl acetate (40 c.c.) and shaken for 24 hours with dry dithioformic acid (from 5 g. of $NaS{\cdot}CHS,6H_2O$). Unchanged acid was then filtered off and washed with ethyl acetate, and filtrate and washings evaporated to small volume and passed through a column of neutral activated aluminium oxide (20 g.). The column was washed successively with ethyl acetate (30 c.c.), chloroform (30 c.c.), and dry pyridine (70 c.c.), and the pyridine eluate evaporated to dryness under reduced pressure. The dry resin (168 mg.) was heated under reflux for 3 hours in absolute ethanol (12 c.c.) containing sodium methoxide (18·2 mg.), a slow current of nitrogen being passed through the solution. The residue left by evaporation of the resulting solution under reduced pressure was dissolved in water, neutralised with 0·1N-sulphuric acid, and the solution treated with saturated aqueous picric acid. 9-D-Glucopyranosido-adenine picrate (100 mg.) separated as needles, m. p. 245° (decomp.) alone or in admixture with authentic material (Found: C, 39·1; H, 3·6; N, 21·4%).

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