71. *Experiments on the Synthesis of Purine Nucleosides. Part XVI. 9-β-d-Mannopyranosidoadenine. A Proof of the Location of the Sugar Residue in Adenosine.*

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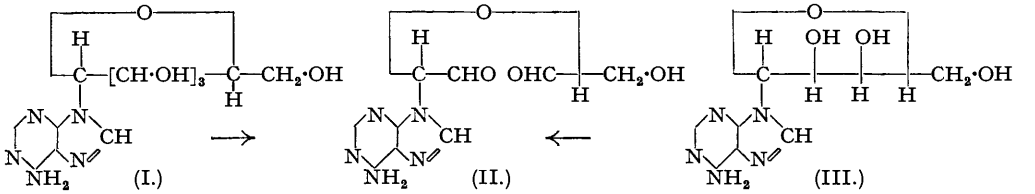
Following the general procedure established in earlier papers of this series, 9-d-*mannopyranosido*-2-*methylthioadenine* has been synthesised and converted by a desulphurisation procedure into 9-d-*mannopyranosidoadenine*. Since the latter compound yields on periodate oxidation a dialdehyde (isolated as its *picrate*) identical with that obtained on similar oxidation of adenosine, it follows that the ribofuranose residue in the natural compound is located at N_9 in the purine skeleton. It also follows that the synthetic mannoside has a β-configuration at the glycosidic carbon atom.

In applying the route for the synthesis of 9-glycosidopurine derivatives described in previous papers (Part IX, *J.*, 1944, 652; Part X, *J.*, 1944, 657; Part XI, *J.*, 1945, 556) attention has so far been directed to pentoside representatives of the series on account of their closer relationship to the naturally occurring nucleosides. The extension of our method to the synthesis of a hexoside analogue forms the subject of the present communication. This work was undertaken not merely with the object of enlarging the range of synthetic adenine glycosides available, but also because it seemed to offer an opportunity of testing by direct chemical methods the N_9 allocation of the sugar residue in adenosine. This allocation, due to Gulland and Holiday (*J.*, 1936, 765), rests at present upon the similarity of the ultra-violet absorption spectra of adenosine and related purine derivatives bearing a substituent on N_9.

Study of the products obtained by oxidation of adenosine and related glycosides with sodium metaperiodate has shown that the glycosidic linkage in adenosine is of the β-type (Part XII, *J.*, 1946, 833). The 9-*d*-glycopyranosidoadenine derivatives so far prepared by our synthetic route have also the β-configuration (Part XII, *loc. cit.*; Part XV, *J.*, 1946, 861) and, since the nature of the method employed permits the formation only of N_9- and not of N_7-glycosidopurines, it seemed likely that by use of a *d*-hexose an authentic 9-β-*d*-hexopyranosidoadenine (I) would

be obtained. Such a compound should give rise on oxidation with sodium metaperiodate to the same dialdehyde (II) as results from the fission of adenosine (III) with the same reagent, provided that (III) is, in fact, a 9-glycoside.



In order to test this we commenced (with Mr. A. Holland) the synthesis of adenine-9-*d*-glucopyranoside, in the hope that the product would prove to be identical with the adenine glucoside first synthesised by Fischer and Helferich (*Ber.*, 1914, **47**, 210); we have already shown (Part XII, *loc. cit.*) that the latter glucoside gives rise on periodate oxidation to the same dialdehyde as does adenosine, but the evidence that the glucose residue is situated at N_9 is, as in the case of adenosine, based on study of the ultra-violet absorption spectrum (Gulland and Story, *J.*, 1938, 259). Work on the synthesis of the authentic adenine-9-*d*-glucopyranoside is not yet complete, as the yields obtained in some of the stages are not altogether satisfactory, so that an account of this work is at present deferred. As a more suitable alternative, the synthesis of 9-*d*-mannopyranosidoadenine was undertaken.

Starting material for the synthesis of this mannoside was readily accessible in the crystalline 6-amino-4-*d*-mannosidamino-2-methylthiopyrimidine, prepared as described in Part III (*J.*, 1943, 571). Introduction of a nitrogen into the pyrimidine nucleus at C_5 was achieved by use of nitrous acid (cf. Part XI, *loc. cit.*) giving 5-*nitroso*-6-*amino*-4-d-*mannosidamino*-2-*methylthiopyrimidine*, which was converted by standard methods into 6-*amino*-5-*thioformamido*-4-d-*mannosidamino*-2-*methylthiopyrimidine*. When the latter was subjected to cyclisation with sodium methoxide in ethanol (Part XIII, *J.*, 1946, 852), 9-d-*mannopyranosido*-2-*methylthioadenine* was isolated; no 6-*d*-mannosidaminopurine derivative could be detected in the cyclisation product. The constitution of the product was confirmed by its insolubility in dilute alkali and by deamination to 9-d-*mannopyranosido*-2-*methylthiohypoxanthine*, and its ring-structure was determined by titration with sodium metaperiodate When 9-*tetra-acetyl*-d-*mannopyranosido*-2-*methylthioadenine*, prepared by acetylation of the corresponding mannoside, was refluxed in alcohol with Raney nickel prepared according to Mozingo (*J. Amer. Chem. Soc.*, 1943, **65**, 1013) it underwent desulphurisation (cf. Part XI, *loc. cit.*), and deacetylation of the crude reaction product with methanolic ammonia gave 9-d-*mannopyranosidoadenine*.

This mannoside reacted with sodium metaperiodate with uptake of 2 mols. of oxidant and liberation of 1 mol. of formic acid; the fission product (isolated as the *picrate*) proved identical with samples obtained from similar oxidation of adenosine and of Fischer and Helferich's adenine glucoside. From this identity it follows that the ribofuranose residue in adenosine is located at N_9 in the purine nucleus and that the synthetic mannoside is correctly described as 9-β-*d*-mannopyranosidoadenine.

Experimental.

5-*Nitroso*-6-*amino*-4-d-*mannosidamino*-2-*methylthiopyrimidine*.—6-Amino-4-*d*-mannosidamino-2-methylthiopyrimidine (4 g.; Part III, *loc. cit.*) dissolved in water (200 c.c.) was treated at 0° with sodium nitrite (2·5 g.) and glacial acetic acid (7 c.c.); after 1 hour at 0° the solution was kept at room temperature for a further hour, and the blue precipitate collected and washed with ice-water. Recrystallisation from water gave fine purple needles of the *nitroso*-compound, m. p. 230—231° (decomp.) (2·8 g.) (Found in material dried at 110°: C, 36·4; H, 5·3; N, 18·7. $C_{11}H_{17}O_6N_5S$ requires C, 36·2; H, 5·2; N, 19·2%).

6-*Amino*-5-*thioformamido*-4-d-*mannosidamino*-2-*methylthiopyrimidine*.—The above nitroso-compound (8 g.) was suspended in ice-water (350 c.c.) and after addition of a solution of ammonium sulphide [30 c.c. of ammonia (*d* 0·88) and 350 c.c. of water saturated with hydrogen sulphide at 0°] a current of hydrogen sulphide was passed through the cooled suspension for 6 hours; unchanged nitroso-compound (*ca.* 1 g.) was then removed by filtration. The filtrate was evaporated to dryness under reduced pressure at 25°, the residue extracted with hot water (700 c.c.), and the filtered extract cooled in an atmosphere of nitrogen and treated with sodium dithioformate solution (54 g. of hexahydrate in 180 c.c. of water). After 48 hours the *thioformyl* compound was collected and a further crop obtained by concentration of the mother liquors to 500 c.c. below 25°. Recrystallised from water (charcoal) it formed clusters of colourless needles, m. p. 217—219° (decomp.) (4 g.) (Found in material dried at 110°/0·1 mm. over phosphoric oxide: C, 36·4; H, 5·6; N, 17·5. $C_{12}H_{19}O_5N_5S_2,H_2O$ requires C, 36·6; H, 5·3; N, 17·7%). In one experiment in which sodium dithioformate solution was added to a warm (50°) solution of the crude product of ammonium sulphide reduction of the nitroso-compound, the above thioformyl derivative was accompanied by a small quantity of material, m. p. 231—232° (decomp., without evolution of

hydrogen sulphide). From its behaviour this was presumably 6-*amino*-5-*formamido*-4-d-*mannosidamino*-2-*methylthiopyrimidine* formed by hydrolysis of the thioformyl compound (cf. Part XI, *loc. cit.*) (Found in material dried at 140°/0·1 mm. over phosphoric oxide: C, 38·6; H, 5·0; N, 18·7. $C_{12}H_{19}O_6N_5S,H_2O$ requires C, 38·2; H, 5·5; N, 18·5%).

9-d-*Mannopyranosido*-2-*methylthioadenine*.—The thioformyl compound (180 mg.) and sodium methoxide (30 mg.) were heated under reflux in dry ethanol (10 c.c.) with exclusion of moisture for 4 hours, and the mixture set aside at room temperature for 1 hour. The precipitate, collected and recrystallised from water (charcoal), gave 9-d-*mannopyranosido*-2-*methylthioadenine* as long fine colourless needles, m. p. 287—288° (decomp.) (86 mg.), whose solubility in water was not increased by adding sodium hydroxide; $[\alpha]_D^{13°}$ + 58° (*c*, 0·464 in 0·1N-hydrochloric acid) (Found in material dried at 110°/0·1 mm. over phosphoric oxide: C, 39·9; H, 5·4; N, 19·2. $C_{12}H_{17}O_5N_5S,H_2O$ requires C, 39·9; H, 5·3; N, 19·4%. Found in material dried at 140°/0·1 mm. over phosphoric oxide: C, 42·0; H, 4·7. $C_{12}H_{17}O_5N_5S$ requires C, 42·0; H, 4·9%). From the mother liquors further small quantities of the same material were obtained, but complete purification of this was difficult owing to its contamination with 6-amino-5-formamido-4-*d*-mannosidamino-2-methylthiopyrimidine. No evidence for the presence of 6-*d*-mannosidamino-2-methylthiopurine in the cyclisation product could be obtained.

Hydrolysis. 9-*d*-Mannopyranosido-2-methylthioadenine (60 mg.) dissolved in sulphuric acid (4 c.c. of N) was heated under reflux for 4 hours, the solution cooled and neutralised with sodium hydroxide, and the precipitated 2-methylthioadenine filtered off and recrystallised from water (20 mg.); m. p. 296—297°, undepressed in admixture with an authentic specimen. The filtrate, treated with phenylhydrazine hydrochloride (30 mg.) and sodium acetate (60 mg.) and heated to 50°, gave, on cooling, *d*-mannose phenylhydrazone, m. p. 189—190°, undepressed in admixture with authentic material.

Periodate titration. Amount of anhydrous mannoside used, 32·5 mg. Periodate consumed after 60 hours, 2·97 mols. per mol. of mannoside, of which 1 mol. is to be ascribed to oxidation of the methylthio-group (Part XI, *loc. cit.*). Formic acid liberated, 0·98 mol. per mol. of mannoside. The compound is therefore a pyranoside.

9-d-*Mannopyranosido*-2-*methylthiohypoxanthine*.—A solution of the adenine mannoside (114 mg.) in water (10 c.c.) and hydrochloric acid (5 c.c. of N) was treated at 65° with sodium nitrite (229 mg.). After 20 minutes at this temperature, gas evolution had ceased; a further quantity (115 mg.) of sodium nitrite was then added and the heating continued for a further 10 minutes. The solution was finally cooled, neutralised with sodium hydroxide, concentrated under reduced pressure to 15 c.c., and set aside for 60 hours. The product, collected and recrystallised from water, gave 9-d-*mannopyranosido*-2-*methylthiohypoxanthine* (25 mg.) as colourless needles, m. p. 219—220° (decomp.) after sintering at 176° (Found in material dried at 110°/0·1 mm. over phosphoric oxide: C, 40·1; H, 5·0; N, 16·0. $C_{12}H_{16}O_6N_4S,H_2O$ requires C, 39·8; H, 5·0; N, 15·5%).

9-*Tetra-acetyl*-d-*mannopyranosido*-2-*methylthioadenine*.—The adenine mannoside (230 mg.), pyridine (15 c.c.), and acetic anhydride (1·5 c.c.) were shaken together for 3½ hours; dissolution was then complete. Next day the solution was concentrated to 5 c.c., treated with alcohol (10 c.c.), and after 20 minutes evaporated to dryness under reduced pressure. The residue was again evaporated with alcohol and then crystallised from the same solvent, giving the *tetra-acetyl* derivative as plates (275 mg.), m. p. 182—183° (Found in material dried in a vacuum at 110°: C, 46·9; H, 4·9; N, 13·8. $C_{20}H_{25}O_9N_5S$ requires C, 46·9; H, 4·9; N, 13·7%).

9-d-*Mannopyranosidoadenine*.—The tetra-acetyl derivative (200 mg.) in alcohol (30 c.c.) was refluxed with Raney nickel containing adsorbed hydrogen (4 g.; Mozingo, *loc. cit.*) and the solution filtered. The nickel was thoroughly extracted with boiling alcohol (Soxhlet), extracts and filtrate were combined, suspended nickel was removed by centrifugation, and the clear solution was evaporated to dryness in a vacuum. The gummy residue was set aside at 0° for 3 days with methanolic ammonia (30 c.c. saturated at 0°), then filtered and evaporated to dryness under reduced pressure. Recrystallisation from water gave 9-d-*mannopyranosidoadenine* (43 mg.) as long fine needles, m. p. 174·5—176·5° (decomp. at 229°); $[\alpha]_D^{16°}$ + 35° (calc. as anhydrous material) (*c*, 0·325 in water) (Found in material dried at 140°/0·1 mm. over phosphoric oxide: C, 43·1; H, 5·4; N, 23·2. $C_{11}H_{15}O_5N_5,\frac{1}{2}H_2O$ requires C, 43·1; H, 5·2; N, 22·9%). The *picrate*, obtained by mixing alcoholic solutions of the two compounds, formed platelets, m. p. 219° (Found in material dried over phosphoric oxide at 140°/0·1 mm.: C, 39·0; H, 3·9; N, 21·1. $C_{11}H_{15}O_5N_5,C_6H_3O_7N_3$ requires C, 38·8; H, 3·6; N, 21·3%).

Fissions with Sodium Metaperiodate.—9-d-*Mannopyranosidoadenine*. *Titrimetric and polarimetric investigation*. Amount of glycoside used, 17·6 mg. of hemihydrate (in a total volume of 3 c.c. containing 0·645 c.c. of 0·249M-sodium metaperiodate). Amount of periodate consumed, 1·95 mols. per mol. of glycoside. Amount of formic acid liberated, 0·95 mol. per mol. of glycoside. Rotation of solution (final value, after 36 hours at 14°, calculated for anhydrous fission product), $[\alpha]_D^{14°}$ — 20·8°. This value is in good agreement with the values obtained under closely parallel conditions (Part XII, *loc. cit.*) with adenosine (— 20·9°) and with Fischer and Helferich's adenine glucoside (— 21·3°).

9-d-*Mannopyranosidoadenine picrate*. The anhydrous picrate (124 mg.) was kept for 96 hours at room temperature with 0·238M-sodium metaperiodate (7 c.c.); titration then showed fission to be complete. Amount of periodate consumed, 2·0 mols. per mol. of glycoside. The powdery dialdehyde picrate was collected, washed with water, and dried over phosphoric oxide at 110°/0·1 mm. (73 mg.) (Found: C, 37·6; H, 2·9; N, 22·0. Calc. for $C_{10}H_{11}O_4N_5,C_6H_3O_7N_3,H_2O$: C, 37·6; H, 3·1; N, 21·9%); $[\alpha]_D^{17°}$ — 20° (*c*, 1·437 in 0·1N-sodium bicarbonate), in agreement with the value of — 21·2° found for the dialdehyde picrate from adenosine and — 20·7° for that from adenine glucoside (Part XII, *loc. cit.*). The product showed the same behaviour on heating as did the picrates mentioned above.

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