179. *Experiments on the Synthesis of Purine Nucleosides. Part X. A Synthesis of* 9-d-*Ribopyranosidoadenine.*

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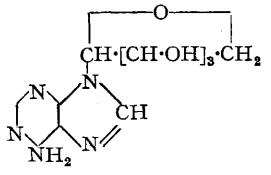
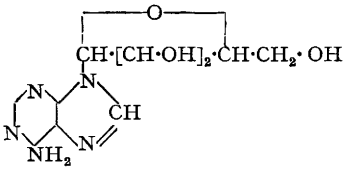
A synthesis of 9-d-*ribopyranosidoadenine* by a method analogous to that used for the corresponding *d*-xyloside (Part IX, preceding paper) is described. The product resembles adenosine (II) but is more resistant to hydrolysis. A point of interest in the synthesis is the absence of evidence of formation of isomeric glycosides from 4 : 6-diaminopyrimidine and *d*-ribose.

In previous papers of this series (Part VI, this vol., p. 318; Part IX, preceding paper) we have described syntheses of two 9-*d*-xylosidopurines, using a general method starting from 6-amino-4-*d*-xylosidaminopyrimidines prepared by direct condensation of *d*-xylose with appropriate 4 : 6-diaminopyrimidines. The successful completion of these syntheses led us naturally to attempt the synthesis of 9-*d*-ribosidoadenine by this method and the present communication records the results obtained. The fact that the purine xylosides so far prepared are pyranosides made it probable that condensation of *d*-ribose with 4 : 6-diaminopyrimidine would yield a product from which ultimately a 9-*d*-ribopyranosidoadenine (I) would be obtained rather than the natural nucleoside adenosine (II) (9-*d*-ribofuranosidoadenine). Nevertheless the possibility that such a direct synthesis might yield adenosine could not be entirely excluded on these grounds. Kuhn and Ströbele (*Ber.*, **1937**, **70**, 773) observed that condensation of *o*-nitroaniline with pentoses yields *N*-pentofuranosides, and although with the diaminopyrimidines xylose yielded pyranosides we had not so far tried other pentoses. Bearing in mind the apparent tendency of ribose to form furanose derivatives as shown by their frequent occurrence in nature, it was not impossible that, unlike xylose, ribose would condense with 4 : 6-diaminopyrimidine to give a furanoside.

(I.) 

 (II.)

Condensation of *d*-ribose with 4 : 6-diaminopyrimidine in alcoholic solution in presence of a small amount of hydrogen chloride in the usual manner gave, in moderate yield, a crystalline 6-*amino*-4-d-*ribosidamino*-*pyrimidine*. In this case no evidence for the formation of an isomeric glycoside was obtained (cf. Part IX, preceding paper); coupling of the pure crystalline riboside or of the crystallisation residues with diazotised 2 : 5-dichloroaniline gave one and the same 6-*amino*-4-d-*ribosidamino*-5-(2′ : 5′-*dichlorobenzeneazo*)*pyrimidine*, readily acetylated to 6-*amino*-4-*triacetyl*-d-*ribosidamino*-5-(2′ : 5′-*dichlorobenzeneazo*)*pyrimidine*. Hydrogenation of the latter in presence of Raney nickel, followed by thioformylation, gave 6-*amino*-4-*triacetyl*-d-*ribosidamino*-5-*thioformamidopyrimidine*. When refluxed in dry pyridine, the thioformamido-compound evolved hydrogen sulphide, but the product did not crystallise readily; the acetyl groups were therefore removed by hydrolysis and when this had been done 9-d-*ribopyranosidoadenine* (I) was readily separated in crystalline form. The structure of the synthetic riboside follows from its hydrolysis to adenine and *d*-ribose, its insolubility in alkali, its absorption of two mols. of reagent and liberation of 1 mol. of formic acid on periodate titration, and finally from its ready deamination to 9-d-*ribopyranosidohypoxanthine*.

The results of periodate titration of the synthetic 9-*d*-ribopyranosidoadenine serve to indicate its stereochemical relationship to 9-*d*-xylopyranosidoadenine (Part IX, preceding paper). Clearly, if both glycosides have the same configuration at the glycosidic linkage, the periodate scission products should be identical, and, if not, enantiomorphous. Owing to difficulties in isolation with the small amounts employed, the scission product was not obtained in crystalline form, but the optical rotation of the periodate oxidation solution from the riboside was identical in sign and, within the rather wide limit of experimental error, in magnitude with that of the corresponding solution from the xyloside (Part IX, *loc. cit.*) at the same molar concentration. Both glycosides belong, therefore, to the same stereochemical series.

No 6-*d*-ribosidaminopurine was isolated in these synthetic experiments, although its formation was expected by analogy with the simultaneous formation of the 6- and the 9-substituted isomers in the analogous syntheses of the xylosides of adenine and 2-methyladenine (Parts VI and IX, *locc. cit.*). In view of the difficulty encountered in other cases in isolating small amounts of 6-glycosidaminopurines, however, our failure to isolate 6-*d*-ribosidaminopurine does not preclude the possibility of its formation as a by-product.

9-*d*-Ribopyranosidoadenine is very similar to adenosine in its appearance and shows virtually the same absorption spectrum. It has, however, a higher m. p., is less soluble in water, and is, as might be expected, considerably more resistant to hydrolysis by mineral acid. As the synthetic substance may differ from adenosine only in the size of the lactol ring, it may show interesting biological properties. From our results it would seem that the synthesis of adenosine and of other ribofuranosidopurines may be achieved by carrying out the initial condensation of sugar and 4 : 6-diaminopyrimidine with a 5-substituted ribofuranose rather than with *d*-ribose itself. Experiments to this end are in progress.

Experimental.

Activated alumina used throughout was prepared by heating alumina hydrate (British Aluminium Co. Ltd.) to 360° during 5 hrs.

4-*Amino*-6-d-*ribosidaminopyrimidine*.—4 : 6-Diaminopyrimidine (30 g.) and *d*-ribose (15 g.) were refluxed in absolute alcohol (1 l.) containing saturated alcoholic hydrogen chloride (10 c.c.) during 48 hrs. Dry benzene was added at intervals, and water removed azeotropically through a Fenske column with a total reflux variable take-off head. The reaction solution was filtered through activated alumina (1800 g.), and unchanged pyrimidine removed by washing the alumina with absolute alcohol (7 l.). The glycoside was eluted with water (10 l.), and the eluate concentrated in a vacuum to small bulk (100 c.c.) and kept overnight. The *riboside* separated in tiny, colourless, hydrated needles (5·6 g.), m. p. 158° (decomp.), $[\alpha]_D^{23°}$ −32° (*c*, 0·12 in water) (Found : C, 41·1; H, 5·9; N, 20·9. $C_9H_{14}O_4N_4,H_2O$ requires C, 41·5; H, 6·1; N, 21·5%).

A sample of the riboside (60 mg.) was refluxed with N/10-sulphuric acid (5 c.c.) for 30 mins., and the solution neutralised exactly with sodium hydroxide and evaporated to dryness in a vacuum. The residue was dissolved in alcohol (20 c.c.) and poured through a column of activated alumina (10 g.), and the column washed with alcohol (50 c.c.). Evaporation of the alcohol runnings gave 4 : 6-diaminopyrimidine (15 mg.), m. p. 267—268°. The column was now washed with water (50 c.c.), and the washings concentrated to small bulk (5 c.c.). Treated with phenylhydrazine in the usual way, this solution gave *d*-ribosazone (5 mg.), m. p. 163—165°, undepressed by an authentic specimen.

4-*Amino*-6-d-*ribosidamino*-5-(2′ : 5′-*dichlorobenzeneazo*)*pyrimidine*.—A solution of 2 : 5-dichloroaniline (3·3 g.) in a mixture of water (150 c.c.) and concentrated hydrochloric acid (10 c.c.) was cooled and diazotised in the usual way with sodium nitrite (1·5 g.). Finely powdered 4-amino-6-*d*-ribosidaminopyrimidine (5·5 g.) was made into a paste with a little water and added to the diazo-solution; all solid then dissolved. The solution was promptly neutralised with sodium hydrogen carbonate and after 1 hour the precipitated *azo-riboside* was collected, washed with water, and dried. Recrystallised from alcohol–pyridine, it formed yellow needles (5 g.), m. p. 225—226° (decomp.) (Found in material dried at 140° : C, 43·8; H, 4·5; N, 20·3. $C_{15}H_{16}O_4N_6Cl_2$ requires C, 43·4; H, 3·9; N, 20·2%).

4-*Amino*-6-*triacetyl*-d-*ribosidamino*-5-(2′ : 5′-*dichlorobenzeneazo*)*pyrimidine*.—(1) 4-Amino-6-*d*-ribosidamino-5-(2′ : 5′-dichlorobenzeneazo)pyrimidine (5 g.) was dissolved in dry pyridine (100 c.c.), acetic anhydride (15 c.c.) added, and the solution set aside overnight. Alcohol (20 c.c.) was added and after 1 hour the solution was evaporated in a vacuum, and the residue recrystallised from benzene. The *acetyl* derivative formed yellow needles (4·5 g.), m. p. 138° after sintering at 128° (Found in material dried at 100° : C, 46·3; H, 4·2; N, 15·8. $C_{21}H_{22}O_7N_6Cl_2$ requires C, 46·6; H, 4·1; N, 15·5%). $[\alpha]^{21°}$ −213° (*c*, 1·106 in chloroform; Wratten No. 29 filter).

(2) After removal of crystalline riboside from the reaction product of 4 : 6-diaminopyrimidine and *d*-ribose (15 g.) as above described, the aqueous mother-liquor was treated with diazotised 2 : 5-dichloroaniline in presence of sodium hydrogen carbonate. After 1 hour the crude azo-compound was collected, dried, and acetylated as described under (1) above. After destruction of the excess of acetic anhydride with alcohol and evaporation in a vacuum the residue was dissolved in ethyl acetate and chromatographed on activated alumina; development and elution were carried out with ethyl acetate. From the eluate an acetylazo-glycoside, m. p. 138°, was obtained (2·1 g.) identical with that already described; no evidence of any isomer was obtained.

4-*Amino*-6-*triacetyl*-d-*ribosidamino*-5-*thioformamidopyrimidine*.—The above acetylazo-compound (4 g.) was suspended in ethyl acetate (100 c.c.) and hydrogenated at 100°/100 atm. during 4 hrs., a Raney nickel catalyst being used. The hydrogenation solution was filtered from catalyst and evaporated in a vacuum, the residue taken up in ethyl acetate (25 c.c.) and filtered, and light petroleum (200 c.c.; b. p. 60—80°) added. The precipitated 5-amino-compound was collected, dissolved in alcohol (150 c.c.), refluxed with dithioformic acid (from 30 g. of the sodium salt) for 1 hour, a second portion of acid (from 10 g. of the sodium salt) added, heating continued for a further hour, and the mixture left overnight. Unchanged acid was filtered off and washed with hot alcohol. Filtrate and washings were evaporated in a vacuum. The residual resin crystallised on addition of alcohol and was recrystallised from alcohol. It formed colourless hydrated needles (1·6 g.) which sintered at 148° and decomposed with evolution of hydrogen sulphide at 158° (Found : C, 42·3; H, 5·0; N, 15·8. $C_{16}H_{21}O_7N_5S$, $1·5H_2O$ requires C, 42·3; H, 5·2; N, 15·4%).

9-d-*Ribopyranosidoadenine*.—The above thioformamido-compound (1 g.) was dissolved in dry pyridine (4 c.c.) and refluxed in a slow stream of nitrogen until hydrogen sulphide evolution ceased (*ca.* 14 hours). Solvent was removed in a vacuum, and the residue dissolved in a little ethyl acetate. As no crystals separated within a few hours, the ethyl acetate was removed, the residual resin dissolved in methanolic ammonia (70 c.c. saturated at 0°), and the solution kept for 60 hours. Methanol was removed on the steam-bath, and hot alcohol (20 c.c.) added to the residue. The solid which separated was recrystallised from water (yield 150 mg.). The *riboside* formed long needles which contained variable amounts of water of crystallisation. On heating it sintered somewhat at 234—235° and melted with decomp. at 254° (Found in material dried at room temp. : C, 42·7; H, 5·8; N, 25·2. $C_{10}H_{13}O_4N_5,H_2O$ requires C, 42·2; H, 5·3; N, 24·6%. Found in material dried at 140° for 4 hours : N, 26·1. $C_{10}H_{13}O_4N_5$ requires N, 26·2%). In a second experiment in which deacetylation was carried out with sodium methoxide in methanol–chloroform, 2·5 g. of the thioformamido-compound gave 350 mg. of 9-*d*-ribopyranosidoadenine; no other crystalline glycoside was isolated.

The synthetic riboside was insoluble in alkali, had $[\alpha]_D^{20°}$ −38° (*c*, 0·28 in water), and its absorption spectrum in N/10-hydrochloric acid showed a maximum at 2610 A. (ε, 19,200) and in N/10-sodium hydroxide a maximum at 2610 A. (ε, 12,600). Although unchanged on refluxing for 1 hour with N/10-sulphuric acid, the substance (50 mg.) was hydrolysed by boiling for 6 hours with N-sulphuric acid (5 c.c.). The hydrolysis solution was neutralised with dilute aqueous sodium hydroxide, and adenine picrate precipitated by adding the calculated amount of hot aqueous picric acid. It formed long yellow needles (50 mg.), m. p. 290° (decomp.). The filtrate was evaporated to dryness, and the residue extracted with boiling alcohol. The extract was evaporated, and the residue dissolved in pyridine (0·5 c.c.) and acetic anhydride (0·05 c.c.) and left at 0° overnight. On addition of a little cold water and neutralisation with sodium bicarbonate β-tetra-acetyl-*d*-ribose (*ca.* 4 mg.) separated. It had m. p. 110°, undepressed in admixture with an authentic specimen (m. p. 110°).

Periodate titration (cf. Part VIII, this vol., p. 592). Amount used, 0·0758 g. Periodate consumed, 2·01 mols./mol.; formic acid liberated, 0·99 mol./mol. Rotation of final solution +0·10° (*c*, 0·152; *l*, 2 dm.).

9-d-*Ribopyranosidohypoxanthine*.—9-*d*-Ribopyranosidoadenine (200 mg.) and sodium nitrite (400 mg.) were dissolved in water (8 c.c.) at 65°, and acetic acid (0·6 c.c.) added, gas being evolved; the temperature was maintained at 65° for 15 mins. The solution was neutralised to phenolphthalein with sodium hydroxide and evaporated to dryness in a vacuum, the residue warmed on the steam-bath for a few minutes with acetic anhydride (4 c.c.) in pyridine (10 c.c.), and the mixture left overnight. Solvent was removed in a vacuum, and the resinous residue extracted with three successive portions of boiling chloroform (30 c.c. in all). The combined extracts were evaporated on the steam-bath, and the oil so obtained deacetylated by boiling for 30 mins. with aqueous barium hydroxide (25 c.c. of a solution containing 20 g. in 300 c.c.). The calculated amount of sulphuric acid to remove barium was added, barium sulphate centrifuged off, and the

solution concentrated to small bulk in a vacuum; 9-d-*ribopyranosidohypoxanthine* then separated in colourless platelets (80 mg.), m. p. 259—260° (decomp.) (Found in material dried at 100°: C, 44·4; H, 5·0; N, 21·4. $C_{10}H_{12}O_5N_4$ requires C, 44·8; H, 4·5; N, 20·9%).

Grants and gifts of materials from Roche Products Ltd. and Imperial Chemical Industries Ltd. are gratefully acknowledged.

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[*Received, September 6th*, 1944.]