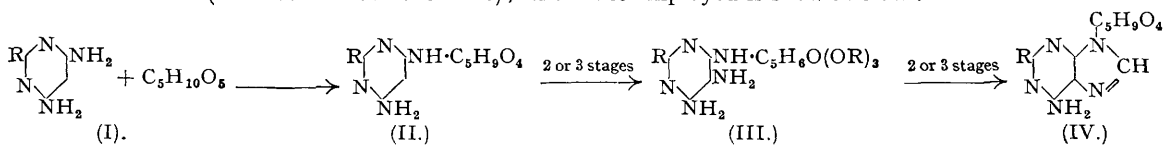
182. *Experiments on the Synthesis of Purine Nucleosides. Part XII. The Configuration at the Glycosidic Centre in Natural and Synthetic Pyrimidine and Purine Nucleosides.*

By J. Davoll, B. Lythgoe, and A. R. Todd.

By a modification of Fischer and Helferich's synthesis of *d*-glucopyranosidoadenine (*Ber.*, 1914, **47**, 210) a 9-*d*-xylopyranosidoadenine has been obtained which is identical in all respects with that described in Part IX (*J.*, 1944, 652). Periodate oxidation of the following pairs of substances gives in each case a dialdehyde common to each pair. Adenosine and adenine-9-β-*d*-glucopyranoside; cytidine and cytosine-3-β-*d*-glucopyranoside; uridine and uracil-3-β-*d*-glucopyranoside. A common (almost certainly β) configuration is therefore present in the nucleosides synthesised from α-acetohalogeno-sugars, in the naturally occurring pyrimidine and purine nucleosides, and in the 9-*d*-pentopyranosidopurines synthesised by the method developed in previous papers of this series.

In previous papers of this series (Parts VI, IX, X, XI; *J.*, 1944, 318, 652, 657; 1945, 556) we have described the preparation by a general synthetic method of a number of 9-glycopyranosidoadenine derivatives closely related to adenosine (9-*d*-ribofuranosidoadenine); the route employed is shown below:



(I). (II.) (III.) (IV.)

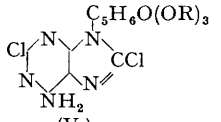
In order to apply this synthetic route to production of nucleosides of the type of adenosine it was clearly necessary to modify the first stage of the synthesis so as to allow production of a 6-amino-4-glycosidaminopyrimidine (II) possessing a furanoside structure, and experiments directed towards this end will be reported shortly. In the meantime it has been shown (Parts IX and XI, *loc. cit.*) that 6-amino-4-glycopyranosidaminopyrimidine derivatives such as (II) may, in some cases at least, be produced in both α- and β-forms. We had hoped that from these isomers the α- and β-forms of the 9-glycopyranosidoadenine derivatives (IV) would be accessible, but experiment showed that, although the αβ-isomerism is retained in a series of derivatives, *e.g.*, in the acetyl derivatives and 5-nitroso- or 5-arylazo-derivatives of (II; R = H or MeS; $C_5H_9O_4$ = *d*-xylosido), only one 5-amino-derivative (III) can be obtained from both series and consequently only one form of the 9-glycosidoadenine derivative (IV; R = H or MeS; $C_5H_9O_4$ = *d*-xylopyranosido). We therefore had to take into account the possibility that the projected synthesis of adenosine by our route might lead either to adenosine or to its αβ-isomer, and it was clearly necessary to investigate methods for determining the configuration at the glycosidic centre both in the natural nucleosides and in the synthetic 9-glycosidoadenine derivatives obtained by our route. Such a determination is also of great importance for other reasons. The stereochemical configuration of the natural nucleosides must be a factor in determining the attachment of, *e.g.*, the adenine nucleotide co-enzymes to their protein apoenzymes in enzymic dehydrogenation and transphosphorylation systems, and in the mode of union of polynucleotide and protein components in the self-duplicating nucleoprotein systems such as the plant viruses.

In the present paper we present the first experimental evidence bearing on this subject so far obtained, former information having been confined to speculation. Levene and Bass ("The Nucleic Acids," 1932, p. 136) state that it is probable that all known purine nucleosides are of the same form in view of the fact that they are all hydrolysed by the same enzyme, and Bredereck ("Fortschritte der Chemie Organischer Naturstoffe," 1938, **1**, p. 131) expresses the opinion that since almost all naturally occurring glycosides belong to the β-series the indications are that a β-glycosidic link is present in the nucleosides. Fischer and Helferich (*Ber.*, 1914, **47**, 210) mentioned their intention of examining the behaviour towards emulsin and similar enzymes of the *N*-glucosides of theophylline and adenine which they prepared by use of acetobromoglucose, but so far as we are aware the results of such experiments have not been published, nor is there in the literature any clear evidence that *N*-glycosidases exhibit the αβ-specificity and insensitivity to the nature of the aglycone which has made carbohydrases of value in configurational studies in the *O*-glycoside series.

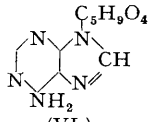
In so far as the αβ-configurations of *N*-glycosides are known at all they have been determined by one of two methods. Some *N*-glycosides show mutarotation, the direction of which can be taken as indicative of the form present. Thus Kuhn and Dansi (*Ber.*, 1936, **69**, 1745) found that the *p*-toluidineglucoside obtained by interaction of glucose and *p*-toluidine in alcohol had $[\alpha]_D^{16}$ − 92·5° ⟶ − 35·5° (*c* = 1 in alcohol), from which they concluded that it belongs to the β-series. However, even with secondary glycosides of the type NHR-sugar, mutarotation does not always occur, *e.g.*, it is absent in the *o*-nitroanilineglycosides (Kuhn and Ströbele, *Ber.*, 1937, **70**, 773) and for glycosides of the type NR_1R_2-sugar it is confined to members containing a strongly basic glycosidic nitrogen atom, *e.g.*, piperidine-*d*-glucoside (Kuhn and Birkofer, *Ber.*, 1938, **71**, 1535). The natural nucleosides and those synthetic nucleosides in which the sugar is attached to one of the nitrogen atoms of a pyrimidine or iminazole nucleus do not exhibit mutarotation, so that no information concerning their αβ-configurations can be gained in this way. The second way in which information has in the past been gained for certain *N*-glycosides seemed more suitable for our purpose. Many *N*-glycosides can be obtained by interaction of a nitrogenous base or one of its metal derivatives with α-acetobromoglucose; by making the reasonable assumption that Walden inversion takes place in such reactions, a β-configuration is deducible for the reaction product. For example, Kuhn and Dansi (*loc. cit.*) found that interaction of α-acetobromoglucose and *p*-toluidine gave a tetra-acetylglucosido-*p*-toluidine identical with that obtained by the acetylation of the *p*-toluidineglucoside mentioned above; both methods of formation lead to formulation of the product as a β-glucoside. On these grounds the theophylline- and adenine-9-*d*-glucosides prepared by Fischer and Helferich, and shown by spectroscopic evidence to possess the structures here assigned (Gulland and co-workers, *J.*, 1934, 1639; 1938, 692), are generally considered to belong to the β-series.

Although a wide variety of theophylline-7-glycopyranosides have been obtained by extension of Fischer and Helferich's procedure, the glucoside mentioned above is the only representative of the adenine-9-glycosides which has been prepared by their method. This is doubtless due in large measure to the fact that the preparation by the method of Fischer (*Ber.*, 1897, **30**, 2220, 2226) of the 2 : 8-dichloroadenine required for these experiments is tedious and impracticable if more than very small amounts are required. The modified procedure described in this paper makes the compound readily accessible; the essential features of the modified

process are employment of purified potassium urate and the use of phosphoryl chloride–dimethylaniline in the preparation of trichloropurine. By using an α-acetohalogenoxylopyranose in place of acetobromoglucose clearly a 9-*d*-xylopyranosidoadenine should be obtained which would be identical or αβ-isomeric with the compound obtained by our own method of synthesis (Part IX, *loc. cit.*). To decide which of these two alternatives is true 2 : 8-dichloroadenine was brought into reaction with α-acetochloroxylose giving in good yield a 2 : 8-*dichloro*-9-*triacetyl*-d-*xylopyranosidoadenine* (V; R = Ac), deacetylated readily to (V; R = H).



(V.)

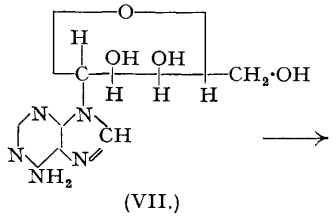


(VI.)

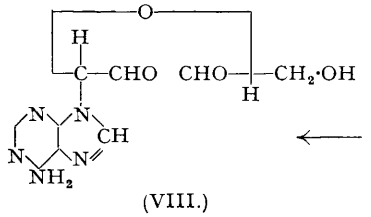
The latter could not be reduced by hydrogen iodide and phosphonium iodide, presumably because the xyloside is more labile to acid hydrolysis than the corresponding glucoside, but when (V; R = OAc) was refluxed in alcohol with Raney nickel containing adsorbed hydrogen (Mozingo, *J. Amer. Chem. Soc.*, 1943, **65**, 1013) it underwent reduction to give a mixture of a monochloro-compound, in all probability 2-*chloro*-9-*triacetyl*-d-*xylopyranosidoadenine*, and 9-*triacetyl*-d-*xylopyranosidoadenine*. Although in these first experiments the yields in this reaction were not very good, owing largely to difficulties in desorbing the reaction product from the catalyst, the method may prove valuable for the dehalogenation of more sensitive glycosides obtained by the Fischer–Helferich procedure. The possibility of isolating the 2-monochloroadenineglycosides by this method may also be of value in offering an approach to the analogues of guanosine; these different aspects are under investigation. The 9-triacetyl-*d*-xylopyranosidoadenine obtained in this way proved to be identical with a sample prepared by reductive removal of the methylthio group from 9-triacetyl-*d*-xylopyranosido-2-methylthioadenine described in Part XI (*loc. cit.*), and yielded on deacetylation with methanolic ammonia a 9-*d*-xylopyranosidoadenine identical with that described in Part IX (*loc. cit.*). The latter is therefore in all probability a β-xyloside. Preliminary evidence has already been described in Parts IX and XI (*locc. cit.*) showing that the adenine-9-*d*-ribopyranoside and 2-methylthioadenine-9-*d*-xylopyranoside belong to the same stereochemical series as (VI), so that our synthetic route apparently gives rise to β-isomerides in all cases so far investigated.

In order to apply the above method directly to determination of the configuration of adenosine, a synthesis of this nucleoside or of its αβ-isomer by the method of Fischer and Helferich would be necessary. No method of preparing the triacylhalogenoribofuranose of known configuration which would be required for this purpose has so far been described, so that an indirect method of establishing the configuration of adenosine was sought.

In Part VIII (*J.*, 1944, 592) we reported that purine glycosides in which the sugar residue is attached to one of the nitrogen atoms of the iminazole ring are oxidised quantitatively with sodium metaperiodate to give dialdehydes which are heterocyclic *N*-analogues of the diglycollic dialdehydes obtained by Jackson and Hudson (*J. Amer. Chem. Soc.*, 1937, **59**, 994) in their experiments with the simpler *O*-methylglycosides; we have already applied this method to determining the lactol ring structures of our synthetic purine-9-glycosides (Parts VI, IX, X, XI, *loc. cit.*) and in establishing the structure of A.T.P. as an adenosine-5′-triphosphate (Lythgoe and Todd, *Nature*, 1945, **155**, 695). As envisaged in Part VIII (*loc. cit.*) this method has proved applicable to the task of relating the configuration at the glycosidic centre in adenosine to that present in the adenine-9-*d*-glucoside of Fischer and Helferich. Oxidation of adenosine (VII) with sodium metaperiodate requires 1 mol. of oxidant, and from the reaction solution a dialdehyde can be isolated in good yield having the composition and reactions expected from the α-(*adenine*-9)-*α′-hydroxymethyldiglycollic dialdehyde* (VIII) and yielding adenine on hydrolysis with mineral acid.



(VII.)



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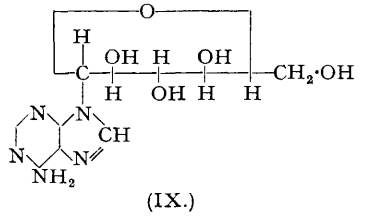
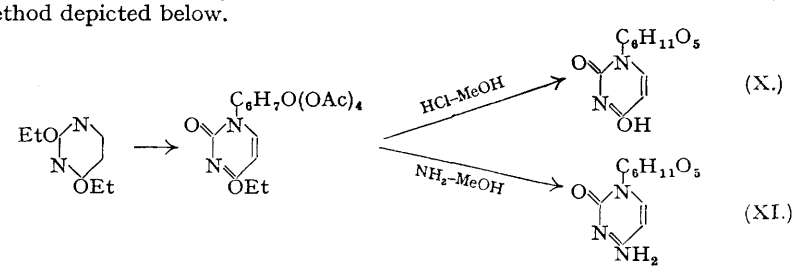


(IX.)

Similar oxidation of adenine-9-β-*d*-glucopyranoside (IX) consumes two mols. of oxidant and liberates 1 mol. of formic acid, giving a dialdehyde identical in composition and properties with that obtained from adenosine. Neither the dialdehyde (VIII) nor its picrate (obtained from oxidation of both adenosine picrate or adenine-9-*d*-glucoside picrate) shows characteristic melting point behaviour, but a comparison of their optical rotations under different pH conditions leaves little doubt of their identity. From this it follows that the configurations at C_1 are identical in adenine-9-*d*-glucoside and in adenosine; the latter is therefore properly described as adenine-9-β-*d*-ribofuranoside.

By similar means we have established that uridine and cytidine are respectively uracil- and cytosine-3-β-*d*-ribofuranosides. As reference compounds we employed the uracil- and cytosine-3-β-*d*-glucopyranosides

prepared by Hilbert and co-workers (*J. Amer. Chem. Soc.*, 1930, **52**, 4489; 1936, **58**, 60) from α-acetobromoglucose by the method depicted below.



(X.)

(XI.)

Oxidation of the natural nucleosides involves uptake of 1 mol. of oxidant and no formic acid is liberated; the synthetic glucosides each require 2 mols. of oxidant and liberate 1 mol. of formic acid. From oxidation of cytidine (as the picrate) we isolated in good yield a crystalline *picrate* of α-(*cytosine*-3)-α′-*hydroxymethyldiglycollic dialdehyde*, which had a characteristic decomposition point; a compound of identical composition, decomposition point, optical rotation, and general properties was isolated from oxidative fission of the picrate of cytosine-3-β-*d*-glucopyranoside. From uridine and uracil-3-β-*d*-glucopyranoside we were unable to isolate the crystalline dialdehyde, but we regard the identity of the optical rotations of the two samples as sufficient proof of identity. These identities show that the natural ribofuranosides have the same configuration at the glycosidic centre as that present in the glucopyranosides obtained from α-acetobromoglucose, *i.e.*, they belong to the β-series. The above experiments also constitute proof that the glucose residue in the synthetic compounds is located at N_3, a location assigned hitherto only on the analogy of their mode of formation with that of 3-methyluracil, and on the resistance of the glucosides to hydrolysis by acids.

We are aware that the β-configurations assigned above are not completely rigid as determinations of *absolute* configuration owing to a small degree of uncertainty attaching to the assumption made at the outset, namely, that Walden inversion occurs on reaction of the acetohalogeno-sugar with the base. However, in such closely related compounds as are here considered it is most unlikely that inversion takes place in one case and not in another and we consider that a high degree of certainty attaches to the assignments here made when regarded as determinations of configuration *relative* to that present in the nucleosides synthesised from α-acetohalogeno sugars.

Experimental.

Preparation of Glycosides.

2 : 6-*Dichloro*-8-*hydroxypurine*.—Since the preparation of this substance by the method described by Fischer and Ach (*Ber.*, 1897, **30**, 2208) was found unsatisfactory the following details may prove useful. A filtered solution of potassium urate (51 g. of uric acid in 4 l. of water containing 55 g. of potassium hydroxide) was allowed to cool from 60° to 20° whilst a vigorous current of carbon dioxide was passed, and the precipitated product was freed from alkali by resuspension in cold water saturated with carbon dioxide and washing on the filter with the same medium. After being washed with acetone and dried to constant weight in an air-oven at 110° the product (52 g.) was readily reduced to a fine powder (suitable for use in the next operation). The dry acid potassium urate (16 g.) contained in a Carius tube was mixed thoroughly with redistilled phosphoryl chloride (32 c.c.), and the tube, after being sealed, was heated at 185° for 19 hours. The combined contents of two such tubes were decomposed with cold water and the product was collected and purified by treatment with hot concentrated nitric acid and conversion into the ammonium salt as described by Fischer and Ach. (Yield of pure crystalline ammonium 2 : 6-dichloro-8-hydroxypurine, 9 g.)

2 : 6 : 8-*Trichloropurine*.—The following method is more convenient than the original procedure of Fischer (*Ber.*, 1897, **30**, 2220) and gives better yields. To the above dry ammonium salt (16 g.) covered with redistilled dimethylaniline (32 c.c.) redistilled phosphoryl chloride (380 c.c.) was added and the mixture heated under reflux with exclusion of moisture for 4½ hours, after which excess reagents were removed under reduced pressure, finally at 100°. The residual pale brown gum was decomposed with ice-water (350 c.c. added in one portion) and the mixture kept cold and shaken for some time; some crystalline material separated, together with a dense oil. After addition of more ice the solution was first made strongly alkaline by shaking with sodium hydroxide and then extracted with ether (2 × 350 c.c.). The separated aqueous layer, made strongly acid with hydrochloric acid, was kept for a few hours at 0° and the crude product collected and purified by conversion into the ammonium salt as described by Fischer. (Yield of pure anhydrous ammonium 2 : 6 : 8-trichloropurine, 14·5 g.) This material is suitable for conversion into 2 : 8-dichloroadenine as described by Fischer (*Ber.*, 1897, **30**, 2239).

2 : 8-*Dichloro*-9-*triacetyl*-d-*xylopyranosidoadenine*.—2 : 8-Dichloroadenine silver (10·4 g.) was ground into a thin paste with a little sulphur-free xylene, more xylene (280 c.c.) added, and one-half of the xylene distilled off so as to remove all traces of water. To the suspension, acetochloroxylose (9·8 g.) was added, and the mixture heated under reflux with exclusion of moisture for 4½ hours, filtered hot, and the residual silver chloride washed well with hot xylene (20 c.c.). The combined filtrates, cooled to 40°, were treated with light petroleum (280 c.c.; b. p. 40—60°) and the powdery precipitate collected, washed with more light petroleum, and dried in air. Crystallisation from glacial acetic acid gave the *triacetylxyloside* as colourless needles, united to form pellets, m. p. 228° after sintering at 223° [yield, 7—8 g.; $[\alpha]_D^{16°} = -46·4°$ ($c = 1·71$ in chloroform)] (Found: C, 41·9; H, 3·6; N, 15·2. $C_{16}H_{17}O_7N_5Cl_2$ requires C, 41·6; H, 3·7; N, 15·2%).

2 : 8-*Dichloro*-9-d-*xylopyranosidoadenine*.—The above triacetyl derivative (0·5 g.) dissolved in warm methanol (30 c.c.) was cooled to 0°, treated with methanolic ammonia (70 c.c., saturated at 0°), and set aside at 0° for 3 days. Removal of solvent under reduced pressure and crystallisation of the residue from water gave the *xyloside* as small needles (0·3 g.), m. p. 212° (decomp.) (Found in material dried at 140° in a vacuum: C, 35·5; H, 3·7; N, 20·4. $C_{10}H_{11}O_4N_5Cl_2$ requires C, 35·7; H, 3·3; N, 20·8%).

Dehalogenation of 2 : 8-*Dichloro*-9-*triacetyl*-d-*xylopyranosidoadenine*.—The triacetyl compound (500 mg.), calcium carbonate (250 mg.), and Raney nickel containing adsorbed hydrogen (10 g., prepared according to Mozingo, *loc. cit.*)

were heated together under reflux in alcohol (70 c.c.) for 8 hours. The suspension was cooled, treated with hydrogen sulphide, filtered, and the residue extracted thoroughly (Soxhlet) with hot 95% alcohol containing a small quantity of pyridine. The combined filtrate and extracts were evaporated under reduced pressure, and the residue was dried by evaporation with alcohol and crystallised from the same solvent. The first crop gave on recrystallisation from alcohol 2-*chloro*-9-*triacetyl*-d-*xylopyranosidoadenine* (64 mg.), m. p. 217°, $[\alpha]_D^{14°} = -8{\cdot}0°$ ($c = 0{\cdot}67$ in chloroform) (Found: C, 45·5; H, 4·2; N, 16·1. $C_{16}H_{18}O_7N_5Cl$ requires C, 45·0; H, 4·2; N, 16·3%). The second crop recrystallised from alcohol gave 9-*triacetyl*-d-*xylopyranosidoadenine* (35 mg.) as needles, m. p. 227°, $[\alpha]_D^{14°} = -34{\cdot}8 \pm 2°$ ($c = 0{\cdot}61$ in chloroform) (Found: C, 49·2; H, 4·8; N, 17·9. $C_{16}H_{19}O_7N_5$ requires C, 48·9; H, 4·8; N, 17·8%). By desulphurisation of 9-triacetyl-*d*-xylopyranosido-2-methylthioadenine (150 mg.) as described in Part XI (*loc. cit.*) a triacetyl compound was obtained (yield, 37 mg.) having $[\alpha]_D^{13°} = -31{\cdot}6 \pm 2°$ ($c = 0{\cdot}45$ in chloroform) and m. p. 227° alone or in admixture with the triacetyl compound described above (Found: N, 18·0. Calc. for $C_{16}H_{19}O_7N_5$: N, 17·8%).

9-d-*Xylopyranosidoadenine*.—9-Triacetyl-*d*-xylopyranosidoadenine (70 mg.; obtained by dehalogenation of the 2 : 8-dichloro derivative as described above), dissolved in methanolic ammonia (25 c.c., saturated at 0°), was set aside at 0° for 3 days, evaporated to dryness under reduced pressure, and the residue crystallised from hot water. 9-*d*-Xylopyranosidoadenine separated as colourless plates (20 mg.), m. p. 290° (decomp.) undepressed in admixture with authentic specimens obtained as described in Parts IX and XI (*locc. cit.*). It had $[\alpha]_D^{14°} = -24 \pm 4°$ ($c = 0{\cdot}17$ in water) (Found: N, 26·5. Calc. for $C_{10}H_{13}O_4N_5$: N, 26·2%). Treatment with picric acid gave a picrate, m. p. 223—225° (decomp.); m. p. of the picrate similarly obtained from authentic 9-*d*-xylopyranosidoadenine, 222—224° (decomp.); m. p. of mixture, 222—224° (decomp.).

9-*d*-*Glucopyranosidoadenine*.—The preparation of this glycoside has been described by Fischer and Helferich (*loc. cit.*) and by Gulland and Story (*J.*, 1938, 259), but the following observations seem worth recording. Acetobromoglucose (15·5 g.) and dry finely divided 2 : 8-dichloroadenine silver (11·4 g.), reacting together in dry xylene (145 c.c.) for 6 hours, gave 2 : 8-dichloro-9-tetra-acetyl-*d*-glucopyranosidoadenine separating as small needles from glacial acetic acid, m. p. 212—213°; the yield (10·5 g., pure) is considerably better than that obtained by Fischer and Helferich. Deacetylation and dehalogenation of the product were carried out substantially as described by those authors; we were, however, successful in obtaining the 9-*d*-glucopyranosidoadenine virtually ash-free by direct crystallisation of the crude reduction product. The pure material melted at *ca.* 207—210°, resolidified, and remelted at 275—280° (decomp.); $[\alpha]_D^{14°} = -9{\cdot}7° \pm 0{\cdot}6°$ ($c = 0{\cdot}18$ in water) (Found: C, 44·3; H, 5·2; N, 23·4; ash, 0·3. Calc. for $C_{11}H_{15}O_5N_5$: C, 44·5; H, 5·1; N, 23·5%). The mother liquors from which the above material separated gave in the usual manner the picrate, m. p. 246° (decomp.) (Found in material dried at 110° in a vacuum over phosphoric oxide: C, 37·6; H, 3·8; N, 20·9. Calc. for $C_{11}H_{15}O_5N_5,C_6H_3O_7N_3,H_2O$: C, 37·6; H, 3·7; N, 20·6%).

Synthetic Pyrimidine Nucleosides.—3-*d*-Glucopyranosidocytosine picrate was prepared according to Hilbert and Jansen (*J. Amer. Chem. Soc.*, 1936, **58**, 60) and had m. p. 214° (decomp.) (Found in material dried at 100° in a vacuum over phosphoric oxide: C, 38·6; H, 3·8; N, 16·9. Calc. for $C_{10}H_{15}O_6N_3,C_6H_3O_7N_3$: C, 38·3; H, 3·6; N, 16·7%). 3-*d*-Glucopyranosidouracil, prepared by the method of the above authors, was obtained and used as the hemihydrate, m. p. 206° (decomp.; slow heating) (Found: C, 42·5; H, 5·1; N, 10·3. Calc. for $C_{10}H_{14}O_7N_2,\frac{1}{2}H_2O$: C, 42·5; H, 5·3; N, 9·9%). After being dried in a vacuum over phosphoric oxide at 110° it had m. p. 206—208° (decomp.); $[\alpha]_D^{15°} = +22{\cdot}2°$ ($c = 1{\cdot}52$ in water).

Natural Nucleosides.—Adenosine and its picrate were prepared from an aqueous pyridine hydrolysate of yeast nucleic acid (Bredereck, Martini, and Richter, *Ber.*, 1941, **74**, 694) and isolated by the method of these authors. They were dried at 110° in a vacuum over phosphoric oxide before use. Cytidine picrate obtained from a similar hydrolysate by using the isolation procedure of Levene and Bass (" Nucleic Acids," 1931, p. 163) was obtained as the monohydrate after drying at 80° and had m. p. 183° (decomp.) (Found: C, 36·5; H, 3·7; N, 17·5. Calc. for $C_9H_{13}O_5N_3,C_6H_3O_7N_3,H_2O$: C, 36·7; H, 3·7; N, 17·1%). Uridine was isolated from the hydrolysate of yeast nucleic acid as described by Bredereck, Martini, and Richter, m. p. 164·5°, and was dried in a vacuum at 110° over phosphoric oxide.

Oxidative Fission of Glycosides with Sodium Metaperiodate.

The values of $[\alpha]_D$ given refer to the anhydrous fission products.

Fission of Adenosine.—The titrimetric investigation of this reaction has already been described (Part VIII; Lythgoe and Todd, *loc. cit.*). The product of the fission was readily isolated as follows. Adenosine (413 mg.), suspended in 0·246 M-sodium metaperiodate (10 c.c.), was set aside at room temperature for 24 hours and the *α-(adenine-9)-α′-hydroxymethyldiglycollic dialdehyde* collected, washed with water, and dried at room temperature in a vacuum over phosphoric oxide for 48 hours; it then had a composition corresponding to a dihydrate (yield, 275 mg.) (Found: C, 40·5; H, 4·9; N, 23·1. $C_{10}H_{11}O_4N_5,2H_2O$ requires C, 39·9; H, 5·0; N, 23·2%). Attempts to desolvate this material at higher temperatures or to recrystallise it from water resulted in decomposition. In the estimation of aldehyde groups by the method of Ripper (*Monatsh.*, 1900, **21**, 1079) 1 mol. of the substance combined with 0·05 mols. of potassium bisulphite. It showed no definite m. p., but gradually darkened on being heated. When dissolved in 0·1N-hydrochloric acid (25·1 mg. in 3 c.c. total volume) the solution showed a change in rotation for which at present no satisfactory explanation can be offered: $[\alpha]_D^{19°} = +36{\cdot}4°$ (initial value) ⟶ $-15°$ (final value after 24 hours). After hydrolysis of the dialdehyde by heating under reflux for 5 hours with concentrated hydrochloric acid, adenine was isolated from the product as the picrate, m. p. and mixed m. p. 296°. By setting aside adenosine picrate (420·7 mg.) suspended in 0·246M-sodium metaperiodate (5 c.c.) and water (5 c.c.) at room temperature for 24 hours (sodium metaperiodate used: 1·04 mols./mol. adenosine) the *picrate* of α-(adenine-9)-α′-hydroxymethyldiglycollic dialdehyde was obtained as a light yellow powder with no characteristic m. p. After being dried at 110° in a vacuum over phosphoric oxide it had the composition of a monohydrate (Found: C, 37·8; H, 3·2; N, 21·7. $C_{10}H_{11}O_4N_5,C_6H_3O_7N_3,H_2O$ requires C, 37·6; H, 3·1; N, 21·9%). The picrate had $[\alpha]_D^{14°} = -21{\cdot}2°$ ($c = 1{\cdot}4$ in 0·1N-sodium bicarbonate).

Polarimetric Investigation of the Fission.—The rotation of the dialdehyde is strongly affected by pH of the solution; the following values observed for the fission solutions are reproducible only under the conditions described. (*a*) Adenosine (252·4 mg.) dissolved in hot water (20 c.c.) and cooled to 20° was treated with 0·246M-sodium metaperiodate (5 c.c.), made up to 30 c.c. with water, and set aside at room temperature for 48 hours; titration then showed that the reaction was complete; under these conditions no separation of dialdehyde takes place. The solution had $[\alpha]_D^{20°} = -31{\cdot}7°$. (*b*) Adenosine (91·7 mg.) dissolved in hot water (7 c.c.) was cooled to 30°, treated with 0·249M-sodium metaperiodate (2 c.c.), made up to 10 c.c. with water, and set aside at room temperature for 24 hours. A portion of the fission solution (2 c.c.) was treated with 0·1465N-formic acid (0·47 c.c. ≡ 1 mol./mol. adenosine) and aqueous sodium iodate (1 c.c. containing 13·6 mg. ≡ 1 mol./mol. adenosine), these amounts being chosen to give concentrations of dialdehyde and electrolytes equivalent to those resulting in a parallel experiment using adenine glucoside. The solution had $[\alpha]_D^{14°} = -20{\cdot}7°$. (*c*) Adenosine (180 mg.), 0·75N-formic acid (0·9 c.c.), sodium iodate (133 mg.), and 0·249M-sodium metaperiodate (7·3 c.c.) were mixed (total vol., 10 c.c.) and set aside for 36 hours. A portion of the solution (2 c.c.) was then treated with 0·3N-hydrochloric acid (1 c.c.). The solution had $[\alpha]_D^{15°} = -10{\cdot}9°$ (constant during 10 hours).

Fission of Adenine 9-d-*Glucopyranoside. Titrimetric Investigation.*—Amount of glucoside used, 98·6 mg. (in a total volume of 10 c.c. containing 5 c.c. of 0·249M-sodium metaperiodate). Amount of periodate consumed after 42 hours at room temperature, 1·98 mols./mol. glucoside. Formic acid produced, 0·98 mol./mol. glucoside. The pure dialdehyde could not be isolated from fission of the glucoside, since larger concentrations of periodate had to be employed than were necessary with adenosine and the product was considerably contaminated with inorganic material. Thus when the glucoside (160 mg.), suspended in 0·249M-sodium metaperiodate (5 c.c.), was set aside for 24 hours at room temperature and then treated with aqueous sodium hydroxide (≡ 1 mol./mol. glucoside), crude dialdehyde (15 mg.) containing *ca.* 4% of ash was deposited. Dissolved in 0·1N-hydrochloric acid, this material showed a change of rotation of the same type as displayed by the dialdehyde obtained from adenosine, although initial and final values were naturally somewhat smaller owing to the presence of the inorganic contaminant. The fission product picrate was isolated as follows. The glucoside picrate (215 mg.), suspended in 0·249M-sodium metaperiodate, was set aside at room temperature for 72 hours, and the product collected, washed, and dried at 110° in a vacuum over phosphoric oxide (Found : C, 38·0; H, 3·2; N, 21·6. 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%). This material had $[\alpha]_D^{14°} = -20·7°$ ($c = 1·46$ in 0·1N-sodium bicarbonate), and showed the same behaviour on heating as did the dialdehyde picrate obtained from adenosine picrate.

Polarimetric Investigation.—(*a*) The fission solution obtained in the titrimetric investigation above had $[\alpha]_D^{14°} = -23·1°$. After addition of sodium hydroxide (≡ 1 mol./mol. formic acid produced in the fission) it had $[\alpha]_D^{14°} = -29·2°$ in fair agreement with the value recorded under (*a*) in the polarimetric investigation of adenosine. (*b*) For more accurate comparison the glucoside (18·0 mg.) suspended in 0·249M-sodium metaperiodate (0·64 c.c.) and water (up to 3 c.c. total volume) was set aside for 24 hours at 15°. The solution had $[\alpha]_D^{14°} = -21·3°$ in good agreement with the value of −20·7° obtained under identical conditions with adenosine (see (*b*) above). (*c*) Adenine glucoside (40 mg.) and 0·249M-sodium metaperiodate (2 c.c.) were mixed and set aside for 29 hours at room temperature, and 0·3N-hydrochloric acid (1 c.c.) was added. The solution had $[\alpha]_D^{14°} = -10·3°$ (constant), in agreement with the value of −10·9° obtained for the solution from adenosine as in (*c*) above.

Fission of Cytidine Picrate.—The picrate (236·5 mg., monohydrate) suspended in water (5 c.c.) and 0·2775M-sodium metaperiodate (5 c.c.) was set aside at room temperature for 48 hours (the reaction was then complete) and made up to 30 c.c. with water. Mols. oxidant used/mol. cytidine, 0·99. The yellow fission product was collected and recrystallised from water, giving the *picrate* of *α-(cytosine-3)-α′-hydroxymethyldiglycollic dialdehyde* as a crystalline powder (82 mg.) showing a fairly sharp decomposition point at 212—214° (rapid heating); $[\alpha]_D^{18°} = +57·5°$ ($c = 1·07$ in pyridine) (Found in material dried in a vacuum at 110° : C, 37·9; H, 3·2; N, 18·3. $C_9H_{11}O_5N_3,C_6H_3O_7N_3$ requires C, 38·3; H, 3·0; N, 17·9%). In the estimation of aldehyde groups by Ripper's method the substance combined with 1·80 mols. of potassium bisulphite/mol.

Fission of 3-d-*Glucopyranosidocytosine Picrate.*—Method as above. Amount of glucoside picrate used, 300·4 mg. Mols. periodate used/mol. glucoside, 2·10. Yield of dialdehyde picrate, 110 mg. (Found in material dried at 110° in a vacuum : C, 37·9; H, 3·2; N, 18·0. Calc. for $C_9H_{11}O_5N_3,C_6H_3O_7N_3$: C, 38·3; H, 3·0; N, 17·9%). In the estimation of aldehyde groups by Ripper's method the picrate combined with 1·86 mols. of potassium bisulphite/mol. It decomposed at 210—215° (rapid heating) and had $[\alpha]_D^{18°} = +56·7°$ ($c = 0·45$ in pyridine), in agreement with the properties of the dialdehyde picrate obtained from fission of cytidine picrate.

Fission of Uridine and of 3-d-*Glucopyranosidouracil.*—Amount of uridine used, 167·7 mg. Mols. of periodate consumed/mol. of uridine, 0·98. Mols. of formic acid produced/mol. of uridine, nil. Rotation of final solution (carried out using 341·4 mg. uridine in 10 c.c. total volume) $[\alpha]_D^{14°} = +16·0°$. Amount of 3-*d*-glucopyranosidouracil used, 140·3 mg. (hemihydrate). Mols. of periodate consumed/mol. of glucoside, 1·98. Mols. of formic acid produced/mol. of glucoside, 1·05. Rotation of final solution (carried out by using 149·9 mg. glucoside hemihydrate in 10 c.c. total volume), $[\alpha]_D^{15°} = +16·0°$.

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