161. *Experiments on the Synthesis of Purine Nucleosides. Part VIII. The Determination of the Lactol Ring Structures of Purine Glycosides by Periodate Oxidation.*

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Periodate oxidation of purine glycosides in which the sugar residue is attached to one of the imidazole nitrogen atoms is shown to follow a course identical with that demonstrated for the simple *O*-glycosides by Jackson and Hudson (*J. Amer. Chem. Soc.*, 1937, **59**, 994). Application of the method permits a convenient determination of the structure of the lactol ring in such *N*-glycosides. The 9-*d*-xylosido-2-methyladenine described in Part VI (this vol., p. 318) is shown to be a pyranoside. Some further possible applications of the method to the determination of structure in the nucleoside and nucleotide fields are considered.

The experiments herein reported had as their object the development of a method for determining whether the 9-glycosidopurine derivatives obtained by application of our general synthetic method (cf. Part VI, *loc. cit.*) were furanosides or pyranosides, a question of considerable importance in view of our intention to apply similar methods of synthesis to the production of adenosine (9-*d*-ribofuranosidoadenine). Ring structures of *N*-glycosides are known with certainty in relatively few cases. Kuhn and Ströbele (*Ber.*, 1937, **70**, 773), relying on tritylation evidence, ascribed a furanoside structure to the *o*-nitroaniline pentosides obtained by their glycosidisation method; as is already known (cf. Watters, Hockett, and Hudson, *J. Amer. Chem. Soc.*, 1939, **61**, 1528), proof obtained in this way is by no means rigorous. Application of the classical methylation method has demonstrated rigidly the pyranoside nature of *d*-glucose-*p*-toluidide (Kuhn and Dansi, *Ber.*, 1936, **69**, 1745) and the furanoside nature of adenosine, guanosine, and uridine (Levene and Tipson, *J. Biol. Chem.*, 1932, **94**, 809; **97**, 491; 1933, **101**, 529). This method, satisfactory in most cases, requires the use of relatively large amounts of material, whereas for our purposes a method conveniently applicable on the decigram scale seemed desirable.

In the *O*-glycoside series such a method is available in the periodate oxidation procedure developed by Jackson and Hudson (*loc. cit.*); titrimetric investigation of the reaction (cf. Hudson *et al.*, *J. Amer. Chem. Soc.*, 1939, **61**, 1530; 1943, **65**, 64), possible once the course of the oxidation is established, gives a method for the semi-micro-determination of ring structure in this series. It seemed possible that this method might be capable of extension to purine glycosides in which the sugar residue is attached to one of the imidazole nitrogen atoms, and experiments were undertaken to decide whether the periodate oxidation of such compounds does, in fact, follow the same course.

Suitable models for our purpose, containing a furanoside structure, were found in adenosine, guanosine, muscle adenylic acid (adenosine-5′-phosphate), and yeast adenylic acid (adenosine-3′-phosphate). As pyranoside models we selected the readily accessible theophylline hexosides and pentosides produced by reaction of theophylline silver with the appropriate pyranose acetohalogen sugar (Fischer and Helferich, *Ber.*, 1914, **47**, 217). These pyranosides, in which the sugar is attached to N_7 in the imidazole ring (Gulland, Holiday, and Macrae, J., 1934, 1639), are of suitable stability to hydrolytic agents (comparable with that of the naturally occurring purine nucleosides); moreover, the ring structure of the sugar residue is known from the mode of formation, and has been verified in the case of theophylline-7-*l*-arabinoside by the unambiguous methylation procedure (Pryde and Williams, J., 1933, 640).

The amount of sodium metaperiodate required for the oxidation of the glycosides investigated, together with the amounts of formic acid liberated in the reaction (both determined titrimetrically), and the terminal

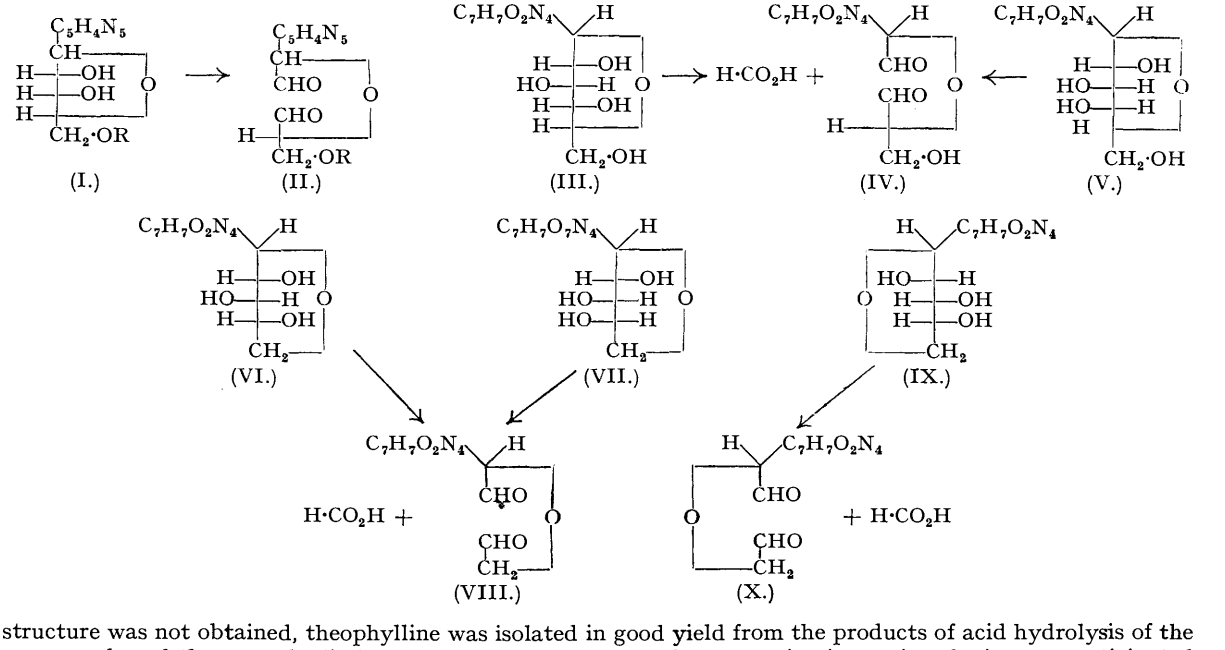
rotations of the reaction solutions are set out in the table. It was previously verified that the parent bases, theophylline, adenine and guanine, do not react with sodium metaperiodate under the conditions employed for oxidation of the glycosides, and that the latter (and hence presumably their scission products) are not sufficiently dissociated to interfere seriously with the estimation of any formic acid liberated in the reaction.

Glycoside.	Reference.	Amount of glycoside (millimols.).	$NaIO_4$ uptake (mols. per mol. of glycoside).	$H{\cdot}CO_2H$ formed (mols. per mol. of glycoside).	Rotation of product * ($[M]_D^{19°} \times 10^{-4}$).
Theophylline-7-(β)-*d*-gluco-pyranoside (III)	Fischer and Helferich, *loc. cit.*	0·4505	2·01	0·985	−1·29°
Theophylline-7-(β)-*d*-galacto-pyranoside (V)	Helferich and Kühlewein, *Ber.*, 1920, **53**, 17.	0·347	2·07	0·955	−1·28
Theophylline-7-(β)-*d*-xylo-pyranoside (VI)	Levene and Sobotka, *J. Biol. Chem.*, 1925, **65**, 463.	0·445	2·02	0·965	+1·47
Theophylline-7-(α)-*d*-arabo-pyranoside (VII)	Present paper.	0·400	1·98	0·98	−1·49
Theophylline-7-(α)-*l*-arabo-pyranoside (IX)	Helferich and Kühlewein, *loc. cit.*	0·445	2·01	0·98	+1·48
Adenosine (I, R = H)	Levene and Tipson, *J. Biol. Chem.*, 1932, **94**, 809.	0·595	1·04	0	—
Guanosine	*Idem, ibid.*, 1932, **97**, 491.	0·6325	1·00	0	—
Muscle adenylic acid (I, R = PO_3H_2)	Levene and Stiller, *ibid.*, 1934, **104**, 299.	0·157	1·01	—	—
Yeast adenylic acid	Levene and Harris, *ibid.*, 1932, **98**, 9; 1933, **101**, 419.	0·169	0	—	—
9-*d*-Xylosido-2-methyl-adenine	Baddiley, Lythgoe, and Todd, this vol., p. 318.	0·318	2·00	0·865	—

* Rotations determined separately on larger samples.

These results are parallel to those obtained with the simpler *O*-glycosides, pyranosides requiring 2 mols. of metaperiodate and liberating 1 mol. of formic acid, whilst furanosides require only 1 mol. of metaperiodate, oxidation taking place without production of formic acid. In the furanosides, the point of attack ($C_{2'}$–$C_{3'}$) of the oxidising agent is shown clearly by the above results, muscle adenylic acid (I, R = PO_3H_2) like adenosine (I, R = H) consuming 1 mol. of the oxidant, whereas yeast adenylic acid, where the hydroxyl at $C_{3'}$ is protected by a phosphoryl residue, fails to react.

Further evidence of the identity of the course of oxidation with that in the *O*-glycoside series is given by the nature of the scission products formed in the reaction. From oxidation of theophylline-7-(β)-*d*-glucoside (III) a crystalline substance was isolated in good yield, giving the reactions of an aldehyde and having the composition required by a monohydrate of the *dialdehyde* (IV). Although complete degradative proof of



structure was not obtained, theophylline was isolated in good yield from the products of acid hydrolysis of the compound, and there can be little doubt of the correctness of the constitution assigned, since, as anticipated from the identity of configuration at $C_{1'}$ and $C_{5'}$ in (III) and (V), the same compound was obtained by periodate

oxidation of theophylline-7-(β)-*d*-galactoside (V). Oxidation of the pentopyranosides must also follow the expected course, since the same scission product (VIII) is produced from both theophylline-7-(β)-*d*-xyloside (VI) and theophylline-7-(α)-*l*-arabinoside (VII), while the enantiomorphous dialdehyde (X) arises from scission of theophylline-7-(α)-*d*-arabinoside (IX). The dialdehydes (VIII) and (X) could not be isolated crystalline owing to their inconvenient solubilities, and were identified by measurement of optical rotation. The designation of the *d*-glucoside, *d*-galactoside, and *d*-xyloside of theophylline as β-glycosides and of the *d*- and *l*-arabinosides as α-glycosides involves the assumption that, as is usual in such reactions, Walden inversion takes place when the halogen of the acetohalogen sugar is exchanged for the theophylline residue. Although a high degree of probability attaches to this assumption, we are aware that it cannot be regarded as rigorously proved, and it may be pointed out that the validity of those conclusions drawn above which depend on assumption of the configurations at $C_{1'}$ in the theophylline glycosides, is unaffected whether the formation of the latter involves Walden inversion or not, since the mechanism is almost certainly the same in each case.

Having established the course of the oxidation, we could now proceed with the investigation of the ring structure of the synthetic 9-*d*-xylosido-2-methyladenine described in Part VI (*loc. cit.*). As shown in the table, this glycoside was found to consume 2 mols. of sodium metaperiodate and to liberate an amount of formic acid approaching 1 mol.; it is therefore a pyranoside and should be designated as a 9-*d*-xylopyranosido-2-methyladenine; presumably all the other purine and pyrimidine xylosides described in Part VI are also pyranosides.

The procedure here described is not directly applicable in its present form to determination of ring structure of 4-glycosidaminopyrimidine derivatives or of 6-glycosidaminopurine derivatives. The amounts of metaperiodate consumed by, and of formic acid liberated from, these compounds indicate a more extensive breakdown of the sugar residue. A possible explanation is that oxidative scission of the $C_{1'}$–$C_{2'}$ linkage takes place in these cases. Periodate fission of *N*-monosubstituted 1 : 2-amino-alcohol systems is known to occur (Nicolet and Shinn, *J. Amer. Chem. Soc.*, 1939, **61**, 1614; see also Neuberger, J., 1941, 47), although Carter, Glick, Norris, and Philips (*J. Biol Chem.*, 1942, **142**, 449) claim that *N*-benzoylation of sphingosine protects it against attack by periodic acid. Closer examination of the behaviour of our glycosidamino-compounds with sodium metaperiodate is in progress and will be described in a subsequent communication.

Some further important aspects of the periodate oxidation method may be pointed out. It appears to afford an attractive method of settling the ring structure of the sugar residue in other purine ribosides (*e.g.*, uric acid riboside, crotonoside) and in the pyrimidine and purine deoxyribosides from thymus nucleic acid. It should also be capable of affording evidence concerning the location of the phosphoryl residues in the adenosine polyphosphoric derivatives, and of giving information concerning the structures of the more complex breakdown products of ribonucleic acids. Finally, by application of this method approach to the problem of the configuration of the glycosidic linkage in the natural nucleosides should be possible, since periodate degradation of Hilbert and Johnson's 3-*d*-glucosidouracil (*J. Amer. Chem. Soc.*, 1930, **52**, 4489) and of Fischer and Helferich's 9-*d*-glucosidoadenine (*loc. cit.*; Gulland and Story, J., 1938, 259) should give compounds identical or stereoisomeric with those from natural uridine and adenosine respectively, and the β-configuration in the synthetic compounds is known with a high degree of probability from their mode of formation. Investigations along these various lines are in active progress and will be reported in due course.

Experimental.

Preparation of Materials.—Acetylated theophylline glycosides were prepared by Gulland and Macrae's modification (J., 1933, 662) of Fischer and Helferich's original procedure (*loc. cit.*); except in the preparation of tetra-acetyl-7-(β)-*d*-glucoside, the acetochloro-sugar was used instead of the acetobromo-sugar in the reaction with theophylline silver. Triacetyl theophylline-7-(β)-*d*-xyloside, described by Levene and Sobotka as an amorphous powder, $[\alpha]_D$ −21·9° (*c*, 4·25 in methyl alcohol), was obtained as plates from ethyl alcohol, m. p. 147—148°, $[\alpha]_D^{18\cdot5°}$ − 23·9° (*c*, 3·3 in chloroform) (Found: C, 49·5; H, 5·0. Calc. for $C_{18}H_{22}O_9N_4$: C, 49·3; H, 5·0%).

Triacetyl theophylline-7-(α)-*d*-arabinoside formed plates from ethyl alcohol; m. p. 215—216°, $[\alpha]_D^{18°}$ −36·7° (*c*, 3·0 in chloroform). Pryde and Williams (*loc. cit.*) give $[\alpha]_{5461}^{21°}$ +42·1° (*c*, 0·605 in chloroform) for the enantiomorph; our own preparation of the enantiomorph had m. p. 215—216°, $[\alpha]_D^{18°}$ +36·6° (*c*, 3·0 in chloroform); triacetyl theophylline-7-(α)-*dl*-arabinoside separated from an equimolecular solution of the enantiomorphs in ethyl alcohol, m. p. 233—234°, $[\alpha]_D^{19°}$ ±0° (*c*, 3·92 in chloroform).

Theophylline glycosides, obtained by deacetylation of the acetates in the usual manner, were dried to constant weight at 140° in a vacuum over phosphoric oxide before use. Theophylline-7-(β)-*d*-xyloside, needles (hydrated) from water, m. p. 249—251° (decomp.); the anhydrous material had $[\alpha]_D^{18°}$ −35·5° (*c*, 1·78 in water); Levene and Sobotka (*loc. cit.*) give m. p. 229°, $[\alpha]_D$ −27·4° (*c*, 3·61 in water).

Theophylline-7-(β)-d-*arabinoside* separated as needles (hydrated) from water, m. p. 264—267°; the anhydrous material had $[\alpha]_D^{18°}$ −36·3° (*c*, 2·15 in water) (Found: C, 46·1; H, 5·1; N, 17·9. $C_{12}H_{16}O_6N_4$ requires C, 46·1; H, 5·1; N, 17·9%). Our preparation of the enantiomorph had m. p. 272—274°, $[\alpha]_D^{19°}$ +36·3° (*c*, 2·04 in water); for the latter, Helferich and Kühlewein (*loc. cit.*) record m. p. 276—277°, $[\alpha]_D^{18°}$ +33·87° (*c*, 2·5 in water), and Pryde and Williams (*loc. cit.*) give m. p. 282—284°, $[\alpha]_{5461}^{17°}$ +39·9° (*c*, 2·5 in water).

Adenosine and guanosine were prepared from B.D.H. yeast nucleic acid by hydrolysis with aqueous pyridine (Bredereck, Martini, and Richter, *Ber.*, 1941, **74**, 694) and dried to constant weight at 110° in a vacuum over phosphoric oxide before use. Muscle adenylic acid, for the gift of which we are deeply indebted to Dr. F. Dickens, was a sample obtained from Messrs. Fraenkel and Landau. It was similarly treated before use. The yeast adenylic acid used was a sample obtained through the courtesy of Messrs. Roche Products Ltd.

Fission of Glycosides with Sodium Metaperiodate.—(a) *Titrimetric investigation.* The glycoside (0·1—0·7 millimol.) was dissolved in a convenient volume of hot water in a graduated flask, the solution cooled rapidly to 25°, treated with 0·2655M-sodium metaperiodate (5 c.c.), diluted with water to a known volume, and set aside at 19°. After a suitable interval an aliquot was removed, and the unchanged metaperiodate estimated iodometrically by the method of Barneby (*J. Amer. Chem. Soc.*, 1916, **38**, 330), using 0·1N-sodium arsenite; this process was repeated till no further uptake of

metaperiodate took place. Formic acid was then estimated in an aliquot by dilution with water and titration to the methyl-red end-point with 0·01N-sodium hydroxide (cf. Jackson and Hudson, *J. Amer. Chem. Soc.*, 1939, **61**, 1530). Experience of the effect of concentrations of reactants and of the nature of the glycoside used enabled the time required for completion of the reaction to be gauged, and so decreased the number of titrations required; *e.g.*, oxidation of guanosine (179 mg. in 250 c.c. of water) was complete after 26 hours; oxidation of 9-*d*-xylosido-2-methyladenine (89·3 mg. in 250 c.c. of water) took 60—70 hours for completion. Titration of muscle and yeast adenylic acids was carried out in the same manner, 1 equiv. of sodium hydroxide being used to effect dissolution in the first place.

(b) *Polarimetric investigation.* Theophylline pentosides (*ca.* 500 mg.) were dissolved in water (25 c.c.) containing an excess (*ca.* 10%) of sodium metaperiodate, and the rotation of the solution was followed ($l = 2$) till constancy was attained. Investigation of the theophylline hexosides was carried out in the same way, but the initial concentration of glycoside could not conveniently be increased beyond 4 mg./c.c. owing to a tendency of the scission product to separate from solution; on this account the use of a 4-dm. tube for measurement of rotations was found necessary.

Isolation of α-(*Theophylline*-7)-α′-*hydroxymethyldiglycollic Aldehyde.*—(a) *From theophylline*-7-(β)-d-*glucopyranoside.* The glucoside (1 g.) in water (15 c.c.) was allowed to stand with addition of 0·2655M-sodium metaperiodate (13 c.c.) at 15° for 60 hours, and the monohydrate of the *dialdehyde* collected and recrystallised from water. It separated as dense crystalline aggregates from concentrated solutions, or as needles from dilute solutions; yield, 0·8 g. On rapid heating, it melted with evolution of water vapour at 177—179°; on slow heating, sintering took place at 177—179°; m. p. 207—208° (decomp.); $[\alpha]_D^{19°}$ −42° (c = 0·25 in water) (Found: C, 43·4; H, 4·9; N, 17·0. $C_{12}H_{14}O_6N_4,H_2O$ requires C, 43·9; H, 4·9; N, 17·1%). Attempts to dehydrate the substance at 160° in a high vacuum over phosphoric oxide were unsuccessful. Schiff's test was positive. Aqueous solutions gave with phenylhydrazine acetate a white precipitate, recrystallisation of which could not be effected. An attempt to convert the substance into a crystalline strontium salt of the corresponding dicarboxylic acid by oxidation with bromine in presence of strontium carbonate was unsuccessful.

Estimation of aldehyde groups. The above monohydrate (63·6 mg.; 0·194 millimol.), treated with potassium hydrogen sulphite according to Ripper's method (*Monatsh.*, 1900, **21**, 1079), combined with 0·390 millimol. of the hydrogen sulphite (Calc. for 2CHO groups: 0·388 millimol.).

Hydrolysis. The dialdehyde monohydrate (200 mg.) was refluxed for 1½ hours with 10% hydrochloric acid (10 c.c.), the solution evaporated under reduced pressure at 40°, and the residue dissolved in water and again evaporated. Crystallisation from water (3 c.c.) gave theophylline as needles, m. p. 266·5—267·5°, undepressed on admixture with an authentic specimen of theophylline, m. p. 267—268°; yield, 75 mg.

(b) *From theophylline*-7-(β)-d-*galactopyranoside.* The galactoside (650 mg.) in water (15 c.c.) was set aside at 18° with addition of 0·2640M-sodium metaperiodate (16 c.c.). The dialdehyde monohydrate (450 mg.), collected after 60 hours, separated from water in the same crystalline form as the material obtained by route (a) above, and alone and in admixture with this material showed the same behaviour on heating; m. p. 207—208°, $[\alpha]_D^{19°}$ −41° (c, 0·25 in water) (Found: C, 44·1; H, 5·2%).

Attempts were made to isolate the product from sodium metaperiodate fission of theophylline-7-(α)-*l*-arabopyranoside. Periodate and iodate were removed from the reaction solution by addition of barium chloride, and excess of barium removed as barium sulphate by addition of sodium sulphate solution. The residue from evaporation of the solution was extracted with warm alcohol, evaporation of which left the fission product as a gum, very soluble in water and alcohol. Crystallisation of this material could not be effected.

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