185. *Experiments on the Synthesis of Purine Nucleosides. Part XV. The Configuration of Some Synthetic Purine and Pyrimidine Glycosides.*

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The configuration at the glycosidic carbon atom in a number of synthetic purine and pyrimidine glycosides is deduced from a consideration of optical rotation data. The deductions, where comparable, accord with the results of the experimental determination of configuration described in Part XII (this vol., p. 833). It would appear that in all cases so far encountered where an α-glycoside is obtained by condensation of a sugar with a 4 : 6-diaminopyrimidine derivative, inversion of configuration occurs during the course of any subsequent purine glycoside synthesis so that the final product is always the stable β-glycoside.

An investigation of the oxidation of *N*-glycosides derived from primary amines (including 4-glycosidaminopyrimidines) with sodium metaperiodate and lead tetra-acetate is described. Neither of these reagents can be safely applied to the determination of lactol ring-structures in such compounds.

It has been reported in earlier communications that xylosidisation of 4 : 6-diaminopyrimidine (Part IX; *J.*, 1944, 652) and 4 : 6-diamino-2-methylthiopyrimidine (Part XI; *J.*, 1945, 556) in hot alcoholic solution in presence of acid catalysts yields, in each case, a mixture of isomeric 4-*d*-xylosidaminopyrimidines. In the course of the synthesis of purine xylosides from these products it was observed that the isomerism disappeared on the introduction of an amino-group into position 5 of the pyrimidine nucleus so that each pair of isomers yielded finally only one 9-*d*-xylosidopurine. The 9-*d*-xylosidopurines so obtained have been shown to be pyranosides by the periodate oxidation method (Parts IX and XI, *locc. cit.*). The question naturally arose whether the difference between the isomeric pyrimidine xylosides mentioned above is due to α : β- or to furanose–pyranose isomerism. A definite answer to this question would be very desirable. It would affect the choice of possible methods for synthesising 9-glycofuranosidopurines, and it would be vital to an understanding of the interconversion of the isomers and would probably cast light on a number of interesting features of the behaviour of *N*-glycosides in general. We have sought to answer the question in several ways. An experimental determination of configuration at the glycosidic carbon atom in natural and synthetic purine glycosides has been reported in Part XII (*loc. cit.*) and an interpretation of the interconversion reactions as an aspect of mutarotation in *N*-glycosides has been given (Part XIV; preceding paper) which supports our original view that the pyrimidine xylosides are in fact α : β-isomers and have a pyranoside structure. The present paper records an independent approach to the configurational problem in pyrimidine and purine glycosides by examination of available optical rotation data, together with an account of some attempts to apply oxidation methods to the determination of lactol ring-structure in 4-glycosidaminopyrimidines.

According to modern theory (Kauzmann, Walter, and Eyring, *Chem. Rev.*, 1940, **26**, 339) optical activity results from light absorption in a chromophore being made anisotropic by the asymmetry of neighbouring groups. Chromophores showing absorption bands in the near ultra-violet (*e.g.*, carbonyl) are, on this view, particularly important in determining rotatory power, so that in glycosides containing pyrimidine or purine aglycones optical rotatory power should be closely connected with their ultra-violet absorption. This being so it would be expected that variation in the aglycone in such compounds would exert its main effect on rotatory power through change in ultra-violet absorption, rather than through change in asymmetrical environment of other chromophores, provided that variation of the aglycone did not involve substitution close to the sugar residue. A survey of the rotational data for the substituted aniline-*d*-glucosides given in Table I seems to support this view; of the glucosides showing mutarotation only those in which the direction of mutarotation is towards a more positive value have been included. The molecular optical rotations of these compounds, in which the ultra-violet absorption does not vary widely, are reasonably consistent, exceptions being found, as anticipated, mainly among the *o*-substituted anilineglucosides.

In the various pyrimidine and purine derivatives which have been synthesised, the absorption spectra of the aglycones are sufficiently similar to one another to warrant the view that the molecular rotations of the corresponding glycosides should be comparable in magnitude. That this is indeed so can be seen from Table II, wherein are listed data for the *d*-glucosides of theophylline, adenine, and hypoxanthine, in which the β-configuration may be assumed since they are all prepared from the silver salts of purines and α-acetobromoglucose. Comparison of adenine and theophylline compounds in this way is reasonable, since the aglycones differ in the pyrimidine and not in the iminazole portion of the molecule. Also in Table II the molecular rotation of theophylline-7-(β)-*d*-xylopyranoside, prepared from α-acetochloroxylose, is compared with the rotations of some 9-*d*-xylopyranosidoadenine derivatives, all of which evidently have the β-configuration. Further evidence of the same sort is provided by the molecular rotation values for the dialdehydes formed on periodate oxidation of the purine pentopyranosides in which asymmetry at all centres save the glycosidic carbon atom has been destroyed (Table II). These confirm the β-configuration assigned above to 9-*d*-xylopyranosidoadenine, and in addition indicate that the synthetic 9-*d*-ribopyranosidoadenine is also a

β-glycoside. The above deduction of an identical configuration in the 9-*d*-xylosidoadenine derivatives synthesised by our general synthetic method and in the products obtained using α-acetohalogenosugars is in agreement with the experimental findings recorded in Part XII (*loc. cit.*).

TABLE I.

*Molecular Rotations of Substituted Aniline-*d*-glucosides*

Aglycone.	$[M]_D$.	Solvent.	Ref.	Aglycone.	$[M]_D$.	Solvent.	Ref.
o-Toluidine	−26,800°	Methanol	1	*o*-Phenetidine	− 2,100°	Water	4
m-Toluidine	−27,700	,,	1	*p*-Anisidine	−23,300	Methanol	5
p-Toluidine	−26,200	,,	1	*o*-Anisidine	− 4,300	Water	6
vic.-o-Xylidine	−29,500	,,	1	*p*-Aminobenzoic acid	−33,500	Methanol	2
p-Xylidine	−29,000	,,	1	*p*-Chloroaniline	−11,600	,,	2
as.-o-Xylidine	−26,200	,,	1	*o*-Chloroaniline	−14,600	,,	2
as.-m-Xylidine	−28,600	,,	1	Ethyl *o*-aminobenzoate	+ 1,600	,,	2
s.-m-Xylidine	−29,000	,,	1	*o*-Nitroaniline	+ 7,800	Pyridine	7
α-Naphthylamine ...	−26,400	,,	2	5-Nitro-*o*-4-xylidine	+ 3,800	,,	7
β-Naphthylamine ...	−33,800	,,	3	*o*-Aminophenol	−23,800	Water	8
p-Phenetidine	−28,700	,,	3	*p*-Aminobenzenesulphonamide	−41,200	,,	8

[1] Hanaoka, *J. Biochem. Japan*, 1938, **28**, 109. [2] *Idem, ibid.*, 1940, **31**, 95. [3] Irvine and Gilmour, *J.*, 1910, **97**, 1552. [4] Amadori, *Atti R. Accad. Lincei*, 1931, **13**, 195. [5] *Idem., ibid.*, 1929, **9**, 226. [6] *Idem, ibid.*, 1931, **13**, 195. [7] Kuhn and Ströbele, *Ber.*, 1937, **70**, 777. [8] Kuhn and Birkofer, *ibid.*, 1938, **71**, 625

TABLE II.

		$[M]_D$.	Ref.
Purine Glucosides	Theophylline-7-(β)-*d*-glucopyranoside	− 800°	**1**
	Adenine-9-(β)-*d*-glucopyranoside	− 3,100	1
	Hypoxanthine-9-(β)-*d*-glucopyranoside	0	**1**
Purine Xylosides	Theophylline-7-(β)-*d*-xylopyranoside	−11,100	2
	Adenine-9-*d*-xylopyranoside	− 6,900	3
	2-Methyladenine-9-*d*-xylopyranoside	− 7,300	4
	2-Methylthioadenine-9-*d*-xylopyranoside	− 9,100	5
Dialdehydes from :	Theophylline-7-(α)-*d*-arabopyranoside	−14,900	2
	Theophylline-7-(β)-*d*-xylopyranoside	+14,700	2
	Adenine-9-*d*-xylopyranoside	+10,400 +11,400	3
	Adenine-9-*d*-ribopyranoside	+ 8,800	**6**

[1] Fischer and Helferich, *Ber.*, 1914, **47**, 210. [2] Lythgoe and Todd, *J.*, 1944, 592. [3] Kenner, Lythgoe, and Todd, *ibid.*, p. 652. [4] Baddiley, Lythgoe, and Todd, *ibid.*, p. 318. [5] Howard, Lythgoe, and Todd, *J.*, 1945, 556. [6] Baddiley, Kenner, Lythgoe, and Todd, *J.*, 1944, 657.

The assignment of configuration to the purine and pyrimidine glycosides can be approached in a slightly different way if Hudson's isorotation rules can be applied to such compounds. These rules are usually considered to depend on the validity of van't Hoff's principle of optical independence of asymmetric centres. Deviations from this principle are frequently encountered, and to explain them Kuhn and Freudenberg (*Ber.*, 1931, **64**, 703) introduced the concept of vicinal effects exerted by groups at some distance from an asymmetric centre. On the other hand, according to Gorin, Kauzmann, and Walter (*J. Chem. Physics*, 1939, **7**, 327), Hudson's rules rest on the symmetry of the sugar lactol ring and additivity in vicinal action of the groups attached to it. On either view introduction of a group showing strong ultra-violet absorption (*e.g.*, a pyrimidine or purine nucleus), and therefore probably exerting a strong vicinal effect, will disturb the situation. The magnitude of the vicinal effect of particular groupings is not easy to calculate, but for the purpose of this paper it is sufficient to examine available data in an empirical way. The aniline glycosides would offer a reasonable analogy for purine and pyrimidine glycosides, and Weygand (*Ber.*, 1940, **73**, 1278) has indeed already pointed out the strong vicinal effect of the anilino-residue in polyhydroxyalkylanilines. Unfortunately, the requisite reference compounds and data on α : β-isomers are not available in the aniline series, but substitutes can be found in the phenylglycosides where a very similar vicinal effect should be present. Table III presents the rotational data for several α : β-pairs of phenylglycosides, together with the A and B values calculated from them [A = $(M_\alpha - M_\beta)/2$ characteristic of the aglycone; B = $(M_\alpha + M_\beta)/2$ characteristic of sugar]; in the same table the corresponding values for the methylglycosides are given for comparison (all rotations are taken from Micheel, " Chemie der Zucker und Polysaccharide," Leipzig, 1938). The calculated A and B values for the phenylglycosides show the expected disturbance due to a vicinal effect, which had already been noted by Kuhn and Freudenberg (*loc. cit.*, p. 725). Nevertheless the relationship between the α : β-isomers is still evident, the α-glycosides having a more strongly positive rotation than the β-glycosides in the *d*-series. The A values (*ca.* 25,000—40,000°), which depend on the separation between their molecular rotations, remain fairly characteristic, and are distinctly higher than those calculated for the corresponding methyl compounds; the B values are also increased, but to a lesser degree. Constancy in A and B values cannot, of course, be expected on varying either sugar or aglycone. Mannosides and rhamnosides have been included in Table III because of scarcity of data and because the anomalies in applying isorotation rules to such compounds are not serious for the present purpose. The general conclusion to be drawn is that it is per-

missible to draw conclusions as to α : β-configuration from A and B values in compounds like the phenylglycosides, although strict conformity with isorotation rules cannot be expected.

Such information as is available regarding anilineglycosides appears to confirm these conclusions. Irvine and Gilmour (*J.*, 1909, **95**, 1545) have described two *p*-toluidineglucosides which mutarotate to the same equilibrium value and are almost certainly α : β-isomers. The molecular rotations of the isomers are in accord with the views above recorded (M_α, + 49,500°; M_β, − 26,200°; A, 37,850°, B, 11,650°). Another probable pair of α : β-isomers is to be found in the aniline-*l*-rhamnosides prepared respectively by Hermann (*J. Russ. Phys. Chem. Soc.*, 1905, **37**, 119; *Zentr.*, 1905, I, 1314) and Irvine and McNicoll (*J.*, 1910, 1455), although mutarotation evidence is lacking (M_α, − 12,000°; M_β, 32,800°; A, − 22,400°; B, 10,400°). The A values for these two pairs are in reasonably good agreement with those calculated for the corresponding phenylglycosides (Table III). The two aniline-*d*-glucosides of Irvine and Gilmour (*J.*, 1908, **93**, 1429) do not fit into the same scheme; it is noteworthy, however, that on mutarotation the " α " form is completely converted to the " β." In view of this and of the low A value for the pair (8000°) it seems likely that the one compound is a furanoside and the other a pyranoside (cf. Part XIV, preceding paper).

Accepting the argument advanced earlier as to the irrelevance of the variations in the aglycones and also the strictly limited applicability of the isorotation rules based on the data for the phenylglycosides in Table III, it is possible to draw up a list of the pyrimidine- and purine-xylosides and divide them into two groups, α and β. The molecular rotations are fairly consistent and reasonable A and B values can be calculated from the means (Table IV).

TABLE III.

	M_α.	M_β.	A.	B.
Phenylglycosides (in water or chloroform)				
d-Glucosides	46,200°	−18,200°	32,200°	14,000°
l-Rhamnosides	−25,400	21,000	−23,200	− 2,200
d-Mannosides	29,000	−18,300	23,650	5,300
Tetra-acetyl *d*-glucosides	70,000	−12,300	41,200	28,900
Tetra-acetyl *d*-mannosides	31,300	−26,600	29,000	2,400
Methylglycosides (in water or chloroform)				
d-Glucosides	30,800	− 6,600	18,700	12,100
l-Rhamnosides	−11,100	17,000	−14,100	3,000
d-Mannosides	15,200	−13,400	14,300	900
Tetra-acetyl *d*-glucosides	47,400	− 6,800	27,100	20,300
Tetra-acetyl *d*-mannosides	17,700	−18,100	17,900	− 200

TABLE IV.

		$[M]_D$.	Ref.
β-d-*Xylosides*	Adenine-9-*d*-xyloside	− 6,900	1
	2-Methyladenine-9-*d*-xyloside	− 7,300	2
	2-Methylthioadenine-9-*d*-xyloside	− 9,100	3
	6-*d*-Xylosidamino-2-methylpurine	− 9,000	2
	6-*d*-Xylosidamino-2-methylthiopurine	− 6,300	3
	6-Amino-4-*d*-xylosidamino-2-methylthiopyrimidine-I	− 5,800	3
	Mean	− 7,400	
α-d-*Xylosides*	6-Amino-4-*d*-xylosidaminopyrimidine-I	+37,300	1
	6-Amino-4-*d*-xylosidamino-2-methylpyrimidine	+40,400	2
	Mean	+38,850	

Hence, A = 23,200°; B = 15,800°. (For α- and β-methyl-*d*-xylopyranosides, A = 15,300°; B = 7000°.)

[1] Kenner, Lythgoe, and Todd, *J.*, 1944, 652. [2] Baddiley, Lythgoe, and Todd, *ibid.*, p. 318. [3] Howard, Lythgoe, and Todd, *J.*, 1945, 556.

If these arguments are valid it follows that in synthesising the xylosides of 2-methyladenine (Part VI, *J.*, 1944, 318) an inversion of configuration must have occurred, probably during hydrogenation of the intermediate azo-glycoside, although it escaped detection at the time. The conclusion that in the synthesis of the 2-methylthioadeninexylosides (Part XI; *loc. cit.*) the Series I intermediates have the same stable β-configuration as the final purines is in keeping with the fact that deacetylation of 5-nitroso-6-amino-4-triacetyl-*d*-xylosidamino-2-methylthiopyrimidine (which is formed from either of the isomeric nitroso-xylosides) yields only 5-nitroso-6-amino-4-*d*-xylosidamino-2-methylthiopyrimidine-I. It is also reasonable to conclude that in the condensation of 4 : 6-diaminopyrimidine with *d*-ribose, where only one glycoside was obtained (Part X, *J.*, 1944, 651), that glycoside ($[M]_D$ = − 8300°) is a β-*d*-riboside and it undergoes no inversion in the subsequent stages of the synthesis of 9-β-*d*-ribopyranosidoadenine ($[M]_D$ = − 10,100°).

The simplicity and accuracy of the periodate oxidation method for the determination of lactol ring-structure and configuration of purine glycosides (Part VIII, *J.*, 1944, 592; Part XII, *loc. cit.*) made a study of the applicability of this method, or of some modification, to the 4-glycosidaminopyrimidines attractive. Various observations recorded in the literature suggested that the method might not apply to glycosides containing an NH group; for example, Karrer and Mayer (*Helv. Chim. Acta*, 1937, **20**, 407) found that periodate causes

complete breakdown of ethyl *N*-carbethoxyglucosaminate, and a similar observation was made by Neuberger (*J.*, 1941, 47) in the case of ethyl *N*-benzoylglucosaminate. On the other hand, it has been reported by Nicolet and Shinn (*J. Amer. Chem. Soc.*, 1939, **61**, 1615) that *N*-acetylserine is only very slowly attacked by periodic acid, and Niemann and Hays (*ibid.*, 1940, **62**, 2960) showed that *N*-acetyl-*d*-glucosylamine behaves normally with the same reagent. The oxidation of a number of NH-glycosides was therefore examine titrimetrically. The results listed in Table V show that, in general, complete oxidation of the glycosides occurred, pentosides consuming *ca.* 4 and hexosides *ca.* 5 mols. of oxidant per mol. of glycoside. The values obtained were moderately consistent but the unexplained excessive uptakes of *o*-nitroaniline-*d*-xyloside and 6-acetamido-4-*d*-xylosidamino-2-methylpyrimidine-I would tend to destroy any confidence in the method applied to an unknown compound. It is, of course, evident that the method in any case, although it might distinguish a hexoside from a pentoside, could give no information as to lactol ring-structure or configuration. Furanosides and pyranosides presumably give the same products and all asymmetric centres are destroyed in the oxidation which in all probability follows the course shown in the scheme below.

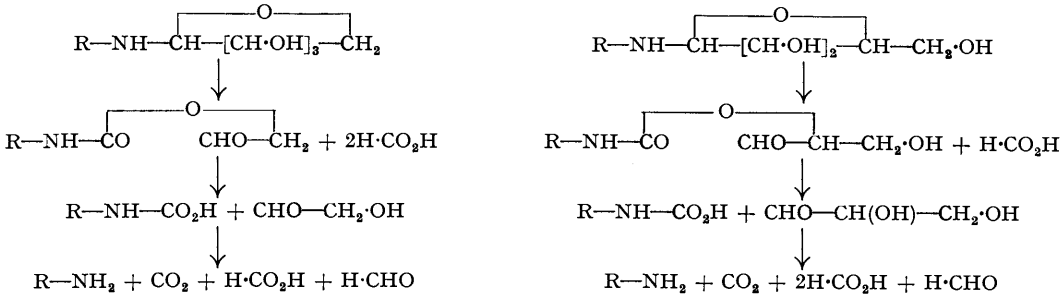

TABLE V.

Compound.	Mols. of oxidant per mol. (approx.). Sodium metaperiodate.	Lead tetra-acetate.
o-Nitroaniline-*d*-xyloside	4—5	3
o-Nitroaniline-*l*-arabinoside	4	4—5
p-Carboxyaniline-α-*d*-ribopyranoside	—	3
o-Nitroaniline-*d*-glucoside	5	4
p-Toluidine-*d*-glucoside	5	5—6
6-Acetamido-4-*d*-xylosidamino-2-methylthiopyrimidine-I	5—6	3·5
4-Amino-6-acetamido-2-methylthiopyrimidine	1	0·5

Oxidation of the same series of glycosides with lead tetra-acetate was next examined. The results, also indicated in Table V, are, like those obtained using periodate, unsatisfactory, although it is of interest to note that in a number of cases the oxidation stops, or at any rate becomes very slow, after the first stage in the scheme set out above. If this were general, lead tetra-acetate might be used to differentiate between furanosides and pyranosides. Unfortunately the extent of oxidation appears to depend on the nature of the aglycone, so that *p*-toluidineglucoside shows an unduly large uptake, while aniline-*d*-ribopyranoside undergoes such rapid and extensive oxidation to deep red products that the reaction cannot be followed quantitatively. The general conclusion to be drawn from these experiments is that neither periodate nor lead tetra-acetate can be employed satisfactorily to determine lactol ring-structure of glycosides of unknown structure containing a free NH group attached to the glycosidic carbon atom. In certain cases some information might be obtained using lead tetra-acetate (*e.g.*, it would seem from Table V that 6-acetamido-4-*d*-xylosidamino-2-methylthiopyrimidine-I is almost certainly a pyranoside), but little confidence could be reposed in the method when applied to unknown compounds.

EXPERIMENTAL.

Oxidations with Sodium Metaperiodate.—A weighed quantity (100—200 mg.) of glycoside was suspended in sodium metaperiodate solution (20 c.c. of *ca.* 0·25M) in a graduated flask, and the mixture left at room temperature. After *ca.* 20 hours the contents of the flask were diluted to 50 c.c. and unchanged metaperiodate estimated iodimetrically on an aliquot; further estimations were made at suitable intervals until a constant value was reached.

o-Nitroaniline-l-*arabinoside* (Kuhn and Ströbele, *loc. cit.*). Uptake of oxidant (mols./mol.): 21 hours, 4·14; 96 hours, 4·2; 168 hours, 4·2.

o-Nitroaniline-d-*glucoside*. Uptake of oxidant (mols./mol.): 22 hours, 4·88; 48 hours, 4·95; 96 hours, 4·99.

o-Nitroaniline-d-*xyloside* (Kuhn and Ströbele, *loc. cit.*). Uptake of oxidant (mols./mol.): 24 hours, 3·66; 48 hours, 4·16; 172 hours, 4·52; 168 hours, 4·75.

p-*Toluidine*-d-*glucoside* (Kuhn and Dansi, *Ber.*, 1936, **67**, 1749). Uptake of oxidant (mols./mol.): 18 hours, 5·02; 47 hours, 5·14.

6-*Acetamido*-4-d-*xylosidamino*-2-*methylthiopyrimidine-I* (Baddiley, Lythgoe, and Todd, *J.*, 1944, 318). Uptake of oxidant (mols./mol.): 72 hours, 5·18; 144 hours, 5·56.

In the course of oxidations of *o*-nitroanilineglycosides, methylenebis-*o*-nitroaniline was always precipitated, doubtless as a result of interaction of formaldehyde and *o*-nitroaniline produced by complete oxidation of the sugar residues; in the same way, *p*-toluidineglucoside gave on oxidation with periodate 1 : 3 : 5-tri-*p*-tolyl-1 : 3 : 5-hexahydrotriazine.

Oxidations with Lead Tetra-acetate.—The substance to be oxidised (50—100 mg.) was suspended in a solution of lead

tetra-acetate (50 c.c. of 0·0588M) in pure acetic acid, and the mixture maintained at 23° in a thermostat. Aliquots (2 c.c.) were removed from time to time and unchanged lead tetra-acetate estimated iodimetrically.

o-*Nitroaniline*-l-*arabinoside*. Uptake of oxidant (mols./mol.): 1 hour, 0·99; 3 hours, 1·89; 9·5 hours, 3·06; 34 hours, 4·03; 48 hours, 4·36; 53 hours, 4·45; 71 hours, 4·50.

o-*Nitroaniline*-d-*glucoside*. Uptake of oxidant (mols./mol.): 3 hours, 0·45; 10 hours, 1·79; 23 hours, 2·45; 34 hours, 2·91; 49 hours, 3·36; 97 hours, 4·02; 120 hours, 4·14; 168 hours, 4·4.

o-*Nitroaniline*-d-*xyloside*. Uptake of oxidant (mols./mol.): 3 hours, 1·75; 23 hours, 2·63; 46 hours, 2·95; 54 hours, 3·05; 71 hours, 3·30.

p-*Toluidine*-d-*glucoside*. Uptake of oxidant (mols./mol.): 5 hours, 4·96; 25 hours, 5·83; 101 hours, 6·42. In other experiments the uptake ranged between 5 and 6 mols./mol.

6-*Acetamido*-4-d-*xylosidamino*-2-*methylthiopyrimidine*-*I*. Uptake of oxidant (mols./mol.): 3 hours, 0·22; 22 hours, 2·20; 48 hours, 3·22; 71 hours, 3·74; 120 hours, 4·10; 168 hours, 4·50.

p-*Carboxyaniline*-d-*ribopyranoside* (Lee, Solmssen, and Berger, U.S.P. 2,384,102). Uptake of oxidant (mols./mol.): 3 hours, 2·38; 23 hours, 2·9; 45 hours, 3·14; 49 hours, 3·16; 53 hours, 3·25.

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