748. *Biosynthesis of Polynucleotides. Part II.* The Synthesis and Properties of Phosphoryl Derivatives of Adenine Glucoside.*

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Syntheses of adenine glucoside-2′, -3′, -4′, and -6′ phosphate are described, and evidence is presented in support of the structures assigned. Adenine glucoside-4′ : 6′ hydrogen phosphate has been prepared and resembles other cyclic phosphates containing a six-membered ring. The -2′, -3′, and -4′ phosphate are not isomerised under conditions which bring about the interconversion of ribonucleoside-2′ and -3′ phosphates.

It is now established that cyclic phosphates of nucleosides are concerned in the chemical [1] and enzymic [2] degradation of ribopolynucleotides and it is also possible that they take part in the biosynthesis of polynucleotides by reversal of enzyme-catalysed degradations.[3,4] It seems reasonable to assume, therefore, that unnatural nucleotides which cannot yield cyclic phosphates or yield relatively stable cyclic phosphates, would be unable to take part in normal polynucleotide metabolism and might serve as a basis for the synthesis of metabolic blocking agents. The natural cyclic phosphates contain two fused five-membered rings and it is of interest to determine whether this ring system is essential to the normal functioning of polynucleotide synthesis and breakdown *in vivo*. With this object in view, we have synthesised the four isomeric phosphoric esters of 9-β-D-glucopyranosyladenine (adenine glucoside).

N^6 : $O^{6'}$-Dityrityl-9-β-D-glucopyranosyladenine was acetylated and, after removal of trityl groups, yielded crystalline 9-(2 : 3 : 4-tri-*O*-acetyl-β-D-glucopyranosyl)adenine. This compound was converted into the 6′-toluene-*p*-sulphonate which, with sodium iodide, yielded a syrup containing covalently bound iodine and considered to be essentially the 6′-iodo-derivative. The fact that covalently bound iodine is present confirms the assumption that the toluenesulphonyloxy-residue replaces the primary hydroxyl group and confirms the structure of the adenine triacetylglucoside. It is interesting that the formation of a *cyclo*nucleoside salt was not observed and we attribute this to the equatorial disposition of the 6′-substituent in the pyranosyl ring.

The adenine triacetylglucoside was phosphorylated by dibenzyl phosphorochloridate, and, after removal of protecting groups, yielded 9-β-D-glucopyranosyladenine-6′ dihydrogen phosphate, identified by the fact that it consumed 2 mols. of periodate with the liberation of 1 mol. of titratable acid. The same material was obtained as the sole product of the phosphorylation of adenine glucoside with phosphorus oxychloride in dry pyridine (cf. Part III [5]). Phosphorylation with phosphorus oxychloride in moist pyridine gave a mixture consisting mainly of the 6′-phosphate together with small quantities of other compounds which were not completely resolved by ion-exchange chromatography. Substantial amounts of the 2′-, 3′-, and 4′-phosphate were not formed, although phosphorylation of adenosine under similar conditions (see Part III [5]) gave appreciable quantities of the 2′- and 3′-phosphate. Newth [6] observed that toluenesulphonylation of an equatorial hydroxyl group takes place readily, as does formation of a carboxylic ester,[7] but this appears not to be the case with phosphorylation. This adds further weight to the

* Part I, preceding paper.

[1] Brown and Todd, *J.*, 1952, 52.
[2] Markham and Smith, *Biochem. J.*, 1952, **52**, 552.
[3] Heppel and Whitfield, *Biochem. J.*, 1955, **60**, 1; Heppel, Whitfield, and Markham, *ibid.*, p. 8.
[4] Barker and Parsons, *Chem. and Ind.*, 1955, 1009.
[5] Barker and Foll, following paper.
[6] Newth, *J.*, 1956, 441.
[7] de la Mare in Klyne's "Progress in Stereochemistry," Butterworths Ltd., London, 1954, Vol. I, p. 61.

belief that the behaviour of adenosine is due to the favourable disposition of the 2′- and 3′-hydroxyl groups for cyclic phosphate formation. We were unable to identify a 4′ : 6′-cyclic phosphate amongst the products of phosphorylating adenine glucoside in moist pyridine but, owing to the small amount of material, resolution was not good enough for the presence of such a compound to be excluded.

Phosphorylation of N^6 : $O^{6'}$-dityrityl-9-β-D-glucopyranosyladenine in pyridine with dibenzyl phosphorochloridate gave a mixture which was separated by ion-exchange chromatography. Three large fractions were obtained which were eluted in the same region as the minor products of the phosphorylation of adenine glucoside in moist pyridine, but no peak corresponding to the 6′-phosphate was obtained. Of the three fractions, one was resistant to periodate and is designated 9-β-D-glucopyranosyladenine-3′ dihydrogen phosphate; the other two each consumed 1 mol. of periodate. In order to distinguish between the last two fractions, the following experiments were carried out.

Adenine glucoside was converted into what we assumed to be crystalline 9-(4 : 6-*O*-benzylidene-β-D-glucopyranosyl)adenine, the assumption being justified by experiments described below. Phosphorylation of this material yielded two nucleotides which were separated by ion-exchange chromatography. One proved to be the 3′-phosphate described above. The other was identical with one of the compounds obtained by phosphorylation of ditritylglucosyladenine. This nucleotide is designated 9-β-D-glucopyranosyladenine-2′ phosphate on the assumption of the structure of the benzylidene compound. No 6′-phosphate was obtained in this experiment. It follows that the benzylidene compound was substituted at position 6′ and one other, not position 3′, since the 3′-phosphate was obtained as described above. Thus the benzylidene derivative is either 9-(2 : 6-*O*-benzylidene- or 9-(4 : 6-*O*-benzylidene-β-D-glucopyranosyl)adenine, and we consider the former to be excluded on steric grounds. The benzylidene derivative was resistant to periodate, as are analogous compounds containing the same bicylic system.[8] We consider that the foregoing results justify the assumption of the structure of the benzylidene compound and therefore also the structures assigned to the nucleotides. The above 2′-phosphate had an R_F value which was lower than those of the other isomers in *iso*pentyl alcohol–sodium phosphate but higher than those of the others in *iso*butyric acid–ammonia. Although it is impossible to give a detailed explanation of this behaviour, it is believed to be connected with the proximity of the phosphate and nitrogenous residue in the 2′-ester. Whereas in adenosine-2′ phosphate these two residues are in true *trans*-positions relative to each other, in the glucoside derivative they almost certainly occupy equatorial positions which would allow of interactions between the groups.

Treatment of adenine glucoside with phenyl phosphorodichloridate gave 9-β-D-glucopyranosyladenine-4′ : 6′ phenyl phosphate. Like methyl α-D-glucopyranoside-4 : 6 phenyl phosphate,[8] the compound was resistant to periodate but, unlike the methyl glucoside cyclic phosphate, the adenine derivative could not be hydrogenated. It was hydrolysed by 80% acetic acid to syrupy 9-β-D-glucopyranosyladenine-4′ : 6′ hydrogen phosphate which was also obtained from the 6′-(dihydrogen phosphate) by treatment with trifluoroacetic anhydride. The properties of adenine glucoside-4′ : 6′ hydrogen phosphate resemble those of other cyclic phosphates containing a six-membered ring,[8,9,10] and, in agreement with this, interconversion of the 4′- and 6′-(dihydrogen phosphate) in acid solution was not observed. We have been unable to obtain any evidence of the formation of cyclic phosphates of adenine glucoside in which adjacent hydroxyl groups of the pyranosyl ring are esterified. Nor have we been able to effect interconversion of the 2′-, 3′- and 4′-phosphate of adenine glucoside with boiling 80% acetic acid or trifluoroacetic anhydride, under which conditions, ribonucleoside-2′ and -3′ phosphate are isomerised. It therefore appears unlikely that 2′-, 3′-, or 4′-phosphate of gluconucleosides could take part in

[8] Baddiley, Buchanan, and Szabo, *J.*, 1954, 3826.
[9] Baddiley and Thain, *J.*, 1952, 3783.
[10] Mosher, Reinhart, and Prosser, *J. Amer. Chem. Soc.*, 1953, **75**, 4899.

reactions *in vivo* in which cyclic phosphates act as intermediates. We are attempting to verify this and to explore the possibility of using such compounds as antimetabolites.

Experimental

9-(2 : 3 : 4-*Tri*-O-*acetyl*-β-D-*glucopyranosyl*)*adenine*.—Triphenylmethyl chloride (1·9 g.) was added to adenine glucoside (prepared from N^6-acetylchloromercuripurine and tetra-*O*-acetyl-α-D-glucopyranosyl bromide by Davoll and Lowy's method [11]) (1 g.) in dry pyridine (15 c.c.) at room temperature, and the solution set aside for 7 days, or, in some experiments, heated at 100° for 3 hr. The cooled solution was poured into ice-water (200 c.c.), and saturated aqueous barium chloride was added dropwise to coagulate the precipitated N^6 : $O^{6'}$-ditrityl-9-β-D-glucopyranosyladenine which was collected, washed with water, and dried over phosphoric oxide. This material was heated at 100° with pyridine (10 c.c.) and acetic anhydride (5 c.c.) for 30 min., set aside overnight, and poured into cold water. The precipitate (3 g.) was collected, dried, and boiled for 30 min. with 80% acetic acid (50 c.c.). Triphenylmethyl acetate crystallised on cooling and was removed. The filtrate was poured into water and, after removal of the precipitate, the solvent was removed under reduced pressure. The residual 9-(2 : 3 : 4-*tri*-O-*acetyl*-β-D-*glucopyranosyl*)*adenine*, crystallised from ethanol, had m. p. 132° (Found : C, 47·8; H, 5·2; N, 16·5. $C_{17}H_{21}O_8N_5$ requires C, 48·2; H, 5·0; N, 16·55%).

Toluenesulphonylation of 9-(2 : 3 : 4-*Tri*-O-*acetyl*-β-D-*glucopyranosyl*)*adenine*.—A solution of toluene-*p*-sulphonyl chloride (0·2 g.) and 9-(2 : 3 : 4-tri-*O*-acetyl-β-D-glucopyranosyl)adenine (0·4 g.) in dry pyridine (15 c.c.) was set aside at room temperature for 2 days. Water (8 c.c.) was added followed by saturated aqueous sodium hydrogen carbonate (25 c.c.) at 0° and the solution was extracted with chloroform. The extract was washed successively with cold saturated sodium hydrogen sulphate (20 c.c.) and water (10 c.c.) and dried (Na_2SO_4). Removal of the solvent gave an amorphous solid which was heated at 100° in a sealed tube with acetone (10 c.c.) and sodium iodide (0·2 g.). Crystallisation of sodium toluene-*p*-sulphonate began after 10 min. and was complete in 1 hr. The solid was filtered off and the solvent removed under reduced pressure. The residue was dissolved in water (10 c.c.) and extracted with chloroform (2 × 10 c.c.). The combined extracts were washed with water (10 c.c.) and dried (Na_2SO_4). Removal of the solvent gave a syrup which contained iodine but gave no precipitate with aqueous silver nitrate.

9-β-D-*Glucopyranosyladenine*-6′ *Dihydrogen Phosphate*.—(a) *From* 9-(2 : 3 : 4-*tri*-O-*acetyl*-β-D-*glucopyranosyl*)*adenine*. To the starting material (1·3 g.) in dry pyridine (20 c.c.) at −40°, dibenzyl phosphorochloridate (from 2·5 g. dibenzyl phosphite) in pyridine (15 c.c.) was added with stirring during 3 hr. The solution was set aside at room temperature overnight. Water (10 c.c.) and sodium carbonate (1 g.) were added and the mixture was evaporated under reduced pressure to dryness. The residue was dissolved in chloroform (60 c.c.), the solution filtered, and the filtrate was washed with aqueous sodium carbonate and water and dried (Na_2SO_4). Chloroform was removed under reduced pressure and the residual gum was hydrogenated at room temperature in aqueous ethanol in presence of a mixture of palladium–charcoal and platinum black. The filtrate from the catalyst was evaporated to dryness under reduced pressure. The residue was dissolved in methanol (50 c.c.), methanolic ammonia (50 c.c., saturated at −10°) was added, and the solution set aside at 0° overnight. Removal of the solvent gave the ammonium salt of the nucleotide, chromatographically homogeneous in the *iso*pentyl alcohol–5% sodium phosphate system (See Table). An aqueous solution of the material was percolated through a column of Dowex-1 formate and eluted with formic acid. Removal of the solvent by evaporation and freeze-drying yielded 9-β-D-*glucopyranosyladenine*-6′ *dihydrogen phosphate* (Found : P, 8·3. $C_{11}H_{16}O_8N_5P$ requires P, 8·22%), which gave a *brucine salt* (Found : C, 53·2; H, 6·2; N, 9·55; P, 2·25. $C_{57}H_{68}O_{16}N_9P,7H_2O$ requires C, 53·0; H, 6·35; N, 9·76; P, 2·40%). On being oxidised with sodium metaperiodate, the nucleotide consumed 2·04 mols. of oxidant with the release of 1·1 mol. of titratable acid.

(b) *From adenine glucoside*. To adenine glucoside (dried at 100°/0·01 mm. for 24 hr.) (0·1 g.) in pyridine (dried with potassium hydroxide and by distillation twice from phosphoric oxide) (5 c.c.) at 0°, phosphorus oxychloride (redistilled immediately before use) (0·075 c.c.) in dry pyridine (0·5 c.c.) was added dropwise with stirring in the absence of atmospheric moisture.

[11] Davoll and Lowy, *J. Amer. Chem. Soc.*, 1951, **73**, 1650.

The solution was set aside at room temperature overnight, then cooled to −10°, and 50% aqueous pyridine was added. N-Sodium hydroxide equivalent to the chloride ions present was added and the solvent removed under reduced pressure. The residue was diluted with water to give an approximately 0·05M-solution with respect to phosphorus, and brought to pH 8 by adding aqueous sodium hydroxide. The solution was chromatographed on a column (5 × 15 cm.) of Dowex-1 formate and eluted as described by Cohn.[12] A single peak was obtained which, after evaporation and freeze-drying, yielded a material having the same R_F value as 9-β-D-glucopyranosyladenine-6′ dihydrogen phosphate in the *iso*pentyl alcohol–5% sodium phosphate system.

Phosphorylation of Ditritylglucosyladenine.—To the crude ditrityl glucosyl adenine described above (prepared from 0·15 g. of adenine glucoside) in pyridine (10 c.c.), phosphorus oxychloride (0·45 c.c.) in pyridine (4·5 c.c.) was added, followed by water (0·045 c.c.) in pyridine (0·45 c.c.). The solution was set aside overnight and 50% aqueous pyridine (5 c.c.) was added. The solution was poured into ice-water (50 c.c.) and saturated aqueous barium chloride was added to coagulate the precipitate which was collected by centrifuging and washed with water. The precipitate was boiled with 80% acetic acid (10 c.c.), triphenylmethyl acetate was removed by filtration of the cooled solution, and the filtrate was poured into water (50 c.c.) and filtered (charcoal). The filtrate was brought to pH 7·4 by addition of hot saturated aqueous barium hydroxide and, after removal of a slight precipitate, the solution was diluted to 1 l. and chromatographed on a column (1 × 10 cm.) of Dowex-1 formate. Three large well-resolved peaks were eluted with 0·1M-formic acid. Fractions were grouped, evaporated, and freeze-dried, giving respectively 9-β-D-glucopyranosyladenine-2′ dihydrogen phosphate which gave a *brucine salt*, m. p. 172—174° (Found: C, 53·3; H, 6·35; N, 9·45; P, 2·42. $C_{57}H_{68}O_{16}N_9P,7H_2O$ requires C, 52·99; H, 6·35; N, 9·76; P, 2·40%); 9-β-D-glucopyranosyladenine-3′ dihydrogen phosphate, which gave a *brucine salt*, m. p. 198—200° (Found: C, 53·1; H, 6·3; N, 9·6; P, 2·2%); 9-β-D-glucopyranosyladenine-4′ dihydrogen phosphate, which gave a *brucine salt*, m. p. 185—190° (Found: C, 53·0; H, 6·15; N, 9·5; P, 2·3%). R_F values are shown in the Table. The 2′- and 4′-dihydrogen phosphate each consumed 1 mol. of sodium metaperiodate; the 3′-dihydrogen phosphate was not oxidised.

R_F Values of derivatives of adenine glucoside.

Adenine glucoside derivative	*iso*Pentyl alcohol–5% sodium phosphate [13]	*iso*Butyric acid–ammonia [14]
Unsubstd.	0·62	0·77
-2′ Dihydrogen phosphate	0·53	0·84
-3′ Dihydrogen phosphate	0·72	0·63
-4′ Dihydrogen phosphate	0·68	0·58
-6′ Dihydrogen phosphate	0·60	0·76
-4′ : 6′ Hydrogen phosphate	0·60	0·91
-4′ : 6′ Phenyl phosphate	—	0·54

9-(4 : 6-O-*Benzylidene*-β-D-*glucopyranosyl*)*adenine.*—Adenine glucoside (1·25 g.), anhydrous zinc chloride (3 g.), and benzaldehyde (15 c.c.) were shaken together at room temperature for 24 hr. The resulting solution was added dropwise with vigorous stirring to ether (250 c.c.), and the solid was collected, washed with ether and dried. The dry solid was dissolved in Cellosolve (20 c.c.), sodium hydroxide (1·25 g.) in water (10 c.c.) was added, the mixture was set aside for 10 min., and carbon dioxide was passed through it until neutral to litmus and thereafter for 5 min. The precipitate was removed and washed with hot Cellosolve, and the combined filtrates were concentrated to 5 c.c. Water (25 c.c.) was added and the 9-(4 : 6-O-*benzylidene*-β-D-*glucopyranosyl*)*adenine* was collected, washed with a little water, and crystallised from aqueous ethanol (yield, 1 g.). It had m. p. 300—301° (Found: C, 56·05; H, 5·05; N, 18·2. $C_{18}H_{19}O_5N_5$ requires C, 56·1; H, 4·94; N, 18·18%). The material was not oxidised by sodium metaperiodate over a period of 3 days.

Phosphorylation of 9-(4 : 6-O-*Benzylidene*-β-D-*glucopyranosyl*)*adenine.*—The material (0·355 g.) was phosphorylated as described for ditritylglucosyladenine. After decomposition of excess of phosphorus oxychloride, the solution was neutralised with aqueous sodium carbonate and the solvents were removed under reduced pressure. The residue was dissolved in a little

[12] Cohn, *J. Amer. Chem. Soc.*, 1950, **72**, 1471.
[13] Carter, *J. Amer. Chem. Soc.*, 1950, **72**, 1466.
[14] Magasanik, Vischer, Doniger, Elson, and Chargaff, *J. Biol. Chem.*, 1950, **186**, 37.

aqueous dioxan, and 30% aqueous acetic acid (40 c.c.) was added and the solution heated at 90° for 4 hr. The cooled solution was evaporated to dryness under reduced pressure, and the residue was dissolved in water, brought to pH 8 by adding saturated aqueous barium hydroxide, diluted to 2 l., and chromatographed as previously described on a column (3 × 20 cm.) of Dowex-1 formate. Materials from the two fractions obtained were isolated as described above and had R_F values (see Table) identical with the 2′- and 3′-phosphate respectively.

9-β-D-*Glucopyranosyladenine-4′ : 6′ Phenyl Phosphate.*—To adenine glucoside (0·67 g.) in dry pyridine (25 c.c.) phenyl phosphorodichloridate (0·48 g.) in dry pyridine (10 c.c.) was added dropwise with stirring at room temperature and the solution was set aside overnight. Water (25 c.c.) was added, and, after 2 hr. the solution was concentrated under reduced pressure and pyridine was largely removed by distillation with ethanol. The residue was dissolved in aqueous ethanol and percolated through a column of Amberlite IR-4B (OH form). Removal of the solvents from the eluate gave 9-β-D-*glucopyranosyladenine-4′ : 6′ phenyl phosphate* (0·35 g.) which crystallised from aqueous ethanol in needles, m. p. 272—275° (decomp.) (Found : C, 46·8; H, 4·25; P, 7·0. $C_{17}H_{18}O_7N_5P$ requires C, 46·9; H, 4·14; P, 7·1%).

9-β-D-*Glucopyranosyladenine-4′ : 6′ Hydrogen Phosphate.*—(*a*) The 4′ : 6′-(phenyl phosphate) described above (0·4 g.) was boiled with 80% acetic acid (25 c.c.) for 30 min. Acetic acid was removed by distillation with ethanol. The residue was dissolved in water, brought to pH 8, and chromatographed on Dowex-1 formate. The product from the main fraction was homogeneous on paper chromatograms (see Table) and travelled at approximately the same speed as adenosine-2′ : 3′ hydrogen phosphate on electrophoresis at pH 7·4. It was converted into the *barium salt* (Found : C, 31·6; H, 2·95; N, 16·3; P, 7·15. $C_{22}H_{26}O_{14}N_{10}P_2Ba$ requires C, 30·97; H, 3·04; N, 16·4; P, 7·27%).

(*b*) 9-β-D-Glucopyranosyladenine-6′ dihydrogen phosphate (0·05 g.) was set aside overnight at room temperature with trifluoroacetic anhydride (0·5 c.c.). Evaporation of trifluoroacetic anhydride gave a gum which was triturated with dry ether to yield a yellowish-white solid. This was set aside for 30 min. in saturated ethanolic ammonia (5 c.c.). The solution was evaporated to dryness, and the residue was washed with acetone and dissolved in water. It behaved on electrophoresis and paper chromatography in the same way as the material prepared from the 4′ : 6′-(phenyl phosphate).

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