Nucleotides. V. Purine Ribonucleoside 2′,3′-Cyclic Carbonates. Preparation and Use for the Synthesis of 5′-Monosubstituted Nucleosides*

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ABSTRACT: Acid-catalyzed ester exchange reactions between diphenyl carbonate and inosine, adenosine, and 5′-*O*-tritylinosine have given good yields of the respective nucleoside 2′,3′-cyclic carbonates. Prior protection of the 5′-hydroxyl group was not advantageous for securing the 2′,3′-carbonates. Inosine 2′,3′-carbonate was treated with β-cyanoethyl phosphate and dicyclohexylcarbodiimide and blocking groups were removed under basic conditions to give inosine 5′-phosphate in good yield. Likewise, treatment of inosine 2′,3′-carbonate with chloromethylphosphonic acid and dicyclohexylcarbodiimide gave inosine 5′-chloromethylphosphonate. Neither of these reactions yielded detectable amounts of 2′(3′)-substituted inosine derivatives.

The ribonucleoside 2′,3′-carbonates are readily deblocked under mildly basic conditions (*e.g.*, within 15 min at pH 8 and 100°) and are thus useful intermediates for the synthesis of 5′-substituted ribonucleosides, in particular, those which are unstable under acidic and/or strongly basic conditions. In this respect the nucleoside carbonates complement the acid-labile 2′,3′-*O*-alkylidene nucleosides which are presently employed as protected intermediates for such syntheses. Attempts to convert uridine to its 2′,3′-carbonate by the present procedure were not successful and resulted, instead, in a direct and convenient synthesis of 2,2′-anhydro-1-β-D-arabinofuranosyluracil.

Selective blocking of the 2′- and 3′-*cis*-hydroxyl groups of ribonucleosides is presently accomplished by formation of 2′,3′-*O*-alkylidene (Hampton, 1961; Chladek and Smrt, 1963; Hampton *et al.*, 1965), arylidene (Chladek and Smrt, 1963; Smith *et al.*, 1964; Cramer *et al.*, 1964; Hampton *et al.*, 1965), or orthoester (Zemlicka, 1964; Jarman and Reese, 1964; Reese and Sulston, 1964) derivatives, all of which are acid labile. Selective introduction of alkali-labile blocking groups at the 2′- and 3′-hydroxyls of ribonucleosides has not yet been described. While 2′,3′-di-*O*-acetyl nucleosides have proved useful alkali-labile intermediates (Brederek *et al.*, 1940; Kenner *et al.*, 1954; Michelson *et al.*, 1956; Chambers and Khorana, 1958), their preparation from nucleosides has required a sequence of three reactions involving initial tritylation of the 5′-hydroxyl (Brederek *et al.*, 1940; Kenner *et al.*, 1954). A potentially more direct route, namely, partial hydrolysis of 2′,3′,5′-tri-*O*-acetyl nucleosides, yields the 5′-*O*-acetyl derivatives unaccompanied by significant amounts of the 2′,3′-di-*O*-acetyl nucleosides (Michelson *et al.*, 1956). Nucleoside 2′,3′-borate complexes can be formed in selective fashion from nucleosides, but their usefulness as protected intermediates is seriously limited by their instability: the borate and phenyl boronate complexes of adenosine, upon phosphorylation, yield significant amounts of adenosine 2′- and 3′-phosphates in addition to the expected 5′ isomer (Ikehara *et al.*, 1964; Yurkevich *et al.*, 1965). Ethyl esters of ribonucleoside 2′,3′-cyclic phosphites are believed to be formed from the reaction of nucleosides with triethyl phosphite (Holy, 1965). Alkaline treatment of these products, however, gives a mixture of nucleoside 2′- and 3′-phosphites (Holy, 1965) and regeneration of the 2′,3′-*cis*-diol system from these would probably require acidic hydrolysis.

This paper reports the direct conversion of inosine and adenosine to alkali-labile 2′,3′-cyclic carbonates and presents evidence that these derivatives can serve as satisfactory intermediates for the synthesis of ribonucleoside 5′-phosphates and related compounds.

Carbonates, usually as the five- or six-membered cyclic esters, have been used extensively as protected intermediates in synthesis of carbohydrates (Hough *et al.*, 1960). A 2,3-cyclic carbonate of ribofuranose has been prepared from a 1,5-disubstituted derivative and used for the synthesis of an α nucleoside (Wright *et al.*, 1958). Treatment of methyl ribofuranoside with methyl chloroformate in the presence of base gives what is probably methyl 5-*O*-methoxycarbonyl ribofuranoside 2,3-carbonate (Barker *et al.*, 1960). The only study which appears to have been made of the carbonylation of a nucleoside appears to be that of Baker and co-workers (Baker *et al.*, 1965) who found that reaction of 6-thioinosine with phenyl chloroformate in pyridine re-

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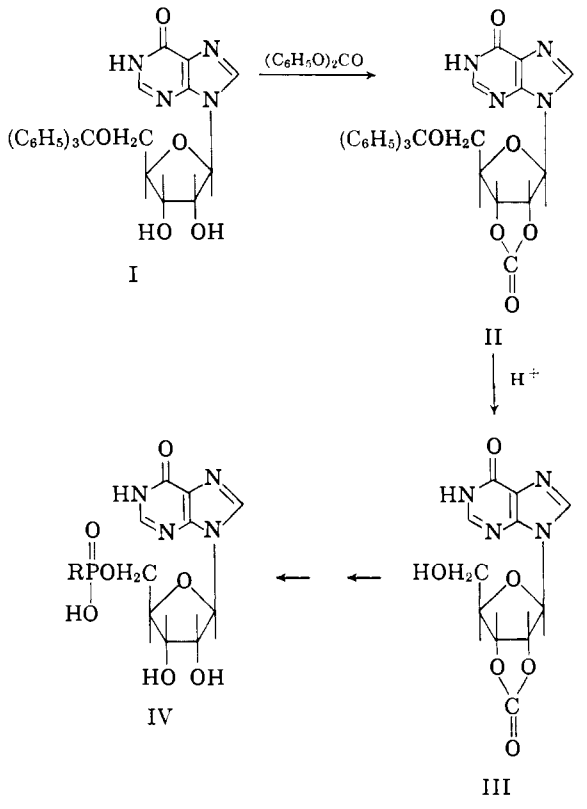
sulted in nonselective introduction of phenoxycarbonate at the 5′ position and cyclic carbonate at the 2′,3′ positions. Fox and co-workers (Fox and Wempen, 1965; Fox *et al.*, 1966) reported that attempted conversion of uridine to its 2′,3′-thiocarbonate with thiocarbonyldiimidazole gave 2,2′-anhydro-1-β-arabinofuranosyluracil; by the use of milder reaction conditions, 5′-*O*-trityluridine has been converted to its 2′,3′-thiocarbonate with thiocarbonyldiimidazole (Ruyle *et al.*, 1965).

Carbonates of carbohydrates have most commonly been prepared by use of phosgene or of chloroformic esters (Hough *et al.*, 1960). Diphenyl carbonate was reported recently by Hough and co-workers (1962) to be useful for the preparation of hexitol carbonates by transesterification. The potential of this relatively stable and nontoxic reagent for the preparation of nucleoside carbonates has been studied in the present work.

The 5′-*O*-trityl derivative of inosine (I), required for attempted unambiguous synthesis of inosine 2′,3′-carbonate (III), was prepared by modifications of the published procedure (Bredereck, 1934)[1] and was further characterized as its 2′,3′-diacetate. With *N*, *N*-dimethylformamide as solvent and sodium bicarbonate as catalyst, as described (Hough *et al.*, 1962), treatment of 5′-*O*-tritylinosine (I) (Scheme I) with slightly more than 1 molar equiv of diphenyl carbonate at 130° afforded a

SCHEME I



[1] Little product was obtained following this method; use of higher temperatures and more solvent, however, gave moderate yields of 5′-*O*-tritylinosine.

good yield of 5′-*O*-tritylinosine 2′,3′-carbonate (II), isolated as a crystalline monophenolate. Removal of the trityl group with aqueous acetic acid gave inosine 2′,3′-cyclic carbonate (III) quantitatively.

Reaction of unprotected inosine with diphenyl carbonate under the above conditions gave, predominantly, material which was indistinguishable from inosine 2′,3′-carbonate (III) prepared from 5′-*O*-tritylinosine. When optimal conditions of time and temperature were employed (Table I), the yield of crystalline, homogeneous inosine 2′,3′-carbonate (III) obtained directly from inosine was 80%.

TABLE I: Reaction of Inosine with Diphenyl Carbonate.

Solvent	Temp (°C)	Catalyst	R_F (Solvent A) and Ratios of Products[a]
Pyridine	112	...	0.35
Pyridine	112	KOH	0.35
Dimethyl sulfoxide	140	$NaHCO_3$	0.35, 0.45 (3:2)
Dimethylformamide	140	. .	0.35
Dimethylformamide	100	$NaHCO_3$	0.35, 0.45 (1:1)
Dimethylformamide	140	$NaHCO_3$	0.45
Dimethylformamide	140	Phenol	0.35, 0.45 (1:2)
Dimethylformamide	140	Bis(*p*-nitrophenyl) hydrogen phosphate	0.35, 0.45 (1:1)

[a] Only the spot of R_F 0.35 gave a positive reaction when the chromatograms were sprayed for *cis*-α-glycol systems (Metzenburg and Mitchell, 1954).

In view of the evidence described below, that the ester interchange reactions with diphenyl carbonate are acid catalyzed, the product obtained from acidic detritylation of 5′-*O*-tritylinosine 2′,3′-carbonate (II) could conceivably be the 3′,5′-carbonate, even though six-membered cyclic carbonate esters are predicted to be less stable than the corresponding five-membered rings (Hough *et al.*, 1960). To investigate this possibility, and to determine the utility of ribonucleoside 2′,3′-cyclic carbonates for the synthesis of nucleoside 5′-phosphates, the inosine carbonate was phosphorylated in pyridine solution with β-cyanoethyl phosphate by the procedure of Tener (1961). The cyanoethyl and carbonyl groups were removed from the product by alkaline treatment. The homogeneous nucleotide

thereby obtained (yield, 55%) was identified as inosine 5′-phosphate (IV, R = OH) by its behavior as a *cis*-α-glycol when treated with sodium periodate, by its ultraviolet absorption spectra, paper chromatography, electrophoresis in borate buffer, and its facile cleavage to inosine by the specific 5′-nucleotidase of *Trimeresurus flavoviridis* venom[2] (Mizuno *et al.*, 1961). Since the above inosine carbonate was completely stable in pyridine solution under the conditions of the phosphorylation,[3] it is concluded to be substituted by carbonyl at the C-2′ and C-3′ positions.

Inosine 2′,3′-carbonate (III) was readily hydrolyzed to inosine under mildly alkaline conditions (Table II): its half-life in aqueous buffer, pH 8, at 100° was 5 min, and in 1 N ammonia at 100° hydrolysis was complete within 2 min. In contrast, at pH 1 and 100°, hydrolysis of the glycosidic linkage occurred more rapidly than did hydrolysis of the carbonate ester. The 2′,3′-cyclic carbonate function significantly stabilized the glycosidic bond towards acidic hydrolysis.

TABLE II: Hydrolysis of Inosine 2′,3′-Carbonate.

Buffer	Time (min)	R_F and Ratio of Products[a]
pH 8.0, 0.1 M	2	0.35, 0.45 (1:3)
pH 8.0, 0.1 M	5	0.35, 0.45 (1:1)
pH 8.0, 0.1 M	15	0.35
0.1 M HCl in aqueous 50% acetic acid (pH 1.0)	30	0.45
0.1 M HCl in aqueous 50% acetic acid (pH 1.0)	60	0.42, 0.45 (1:5)

[a] All spots were negative when sprayed for *cis*-α-glycol systems except the spot of R_F 0.35.

It has been concluded (Hough *et al.*, 1962) that sodium bicarbonate acts as a basic catalyst for ester exchange between diphenyl carbonate and carbohydrates in dimethylformamide solution. However, good yields in such reactions have been reported with resorcinol as solvent and no added catalyst (Hochstetter, 1912), and in the present work, inosine did not react with diphenyl carbonate with pyridine as solvent, even after addition of a trace of potassium hydroxide. In dimethylformamide, reaction of the inosine did not occur if sodium bicarbonate was omitted. This suggested that the actual catalyst might be phenol formed by hydrolysis of diphenyl carbonate. When phenol was substituted for sodium bicarbonate, conversion of inosine to its 2′,3′-carbonate in fact occurred, and at a comparable rate. The conversion was promoted equally well by di-*p*-nitrophenyl hydrogen phosphate.

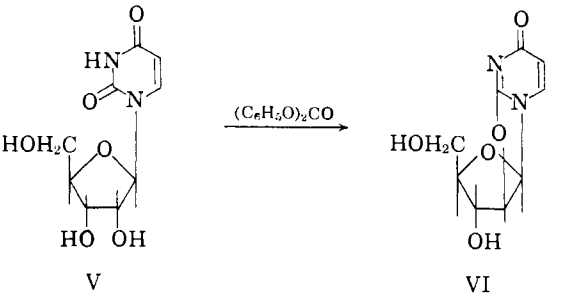
Adenosine underwent no reaction under the conditions which effected selective formation of the 2′,3′-carbonate from inosine. Presumably adenosine (pK_a = 3.5) is sufficiently basic to inhibit acidic catalysis of the reaction. In line with this view, when 1 molar equiv of phenol was added, adenosine was rapidly converted to a cyclic carbonate in 90% yield. This ester readily reformed adenosine when warmed in methanol. Deamination of the adenosine carbonate with nitrous acid gave a single product which possessed the ultraviolet absorption and paper chromatographic properties of inosine 2′,3′-carbonate (III) and which showed a similar rate of alkaline cleavage to inosine as did authentic inosine 2′,3′-carbonate (Table III). The present product, therefore, appears to be adenosine 2′,3′-carbonate rather than the 3′,5′ isomer.

TABLE III: Hydrolysis of Deamination Product from Adenosine 2′,3′-Carbonate.

Time (min)	R_F Values of Components	Ratios of Components
0	0.45	...
5	0.45, 0.35	5:4
15	0.45, 0.35	1:10
30	0.35	...

When conversion of uridine (V) (Scheme II) to its

SCHEME II



V VI

2′,3′-carbonate was attempted by the present procedure, 2,2′-anhydro-1-β-D-arabinofuranosyluracil (VI) was obtained in 60% yield. This cyclonucleoside was obtained recently when preparation of uridine 2′,3′-thiocarbonate was attempted by reaction of uridine with thiocarbonyldiimidazole (Fox and Wempen, 1965). Either uridine 2′,3′-carbonate or 2′-phenoxycarbonyl uridine is evidently an intermediate in the present conversion.

The usefulness of nucleoside 2′,3′-carbonates as synthetic intermediates is further illustrated by the preparation of inosine 5′-chloromethylphosphonate (IV, R = CH_2Cl). Treatment of inosine 2′,3′-carbonate

[2] The enzyme preparation which was used has been shown to have no action on nucleoside 2′- or 3′-phosphates (Mizuno *et al.*, 1961).

[3] The carbonate was recovered unchanged from a solution in pyridine after 24 hr at 25°.

(III) in anhydrous pyridine solution with chloromethylphosphonic acid and excess of dicyclohexylcarbodiimide was followed by hydrolysis of the carbonate by addition of water and warming. The desired product was separated from chloromethylphosphonic acid by anion exchange chromatography and isolated in good yield as a homogeneous lithium salt.

Experimental Section

Methods

Melting points (capillary method) are uncorrected. Paper chromatography was carried out on Whatman No. 1 paper by the ascending method. Solvent systems were (A) 1-butanol–acetic acid–water (5:2:3) and (B) isoamyl alcohol–5% aqueous disodium hydrogen phosphate (1:1). Ratios of chromatographic components of mixtures were visually estimated from the intensity of their absorption of ultraviolet light (254 mμ). Some important R_F values are given in Table IV. Papers were sprayed with molybdate to detect phosphates (Burrows *et al.*, 1952) and with periodate–starch for *cis*-α-glycols (Metzenburg and Mitchell, 1954).

TABLE IV: Paper Chromatography.

Compound	R_F Values in Solvent Systems A	B
Hypoxanthine	0.42	0.51
Inosine	0.35	0.64
Inosine 2′,3′-cyclic carbonate	0.45	
Adenosine	0.47	
Adenosine 2′,3′-cyclic carbonate	0.58	
Ribose	0.40	0.85
Inosine 5′-phosphate	0.08	0.74

Paper electrophoresis was carried out on Whatman No. 3MM paper at agradient of 20 v/cm. Ultraviolet absorption spectra were recorded on a Cary Model 15 spectrophotometer. Microanalyses were by Dr. A. Bernhardt, Mülheim, Germany. All analytical samples were dried at 100° for 8 hr over P_2O_5 *in vacuo*. Dimethylformamide was distilled after drying over anhydrous calcium sulphate. Pyridine was dried over sodium hydroxide, then over calcium hydride, and distilled.

Inosine 2′,3′-Carbonate from Inosine. A. PRELIMINARY EXPERIMENTS. Inosine (0.1 g, 0.37 mmole) was dissolved in the solvents shown in Table I and heated with diphenyl carbonate (0.1 g, 0.46 mmole) for 30 min in the presence or absence of a catalyst (0.005 g). The results are shown in Table I.

B. PREPARATION. Inosine (1.0 g, 3.7 mmoles) was dissolved in dimethylformamide (5 ml) under reflux and treated with sodium hydrogen carbonate (0.05 g) and diphenyl carbonate (1.04 g, 4.9 mmoles). Refluxing was continued for 2 min and the solution was poured while still hot into ether (200 ml). The finely dispersed white precipitate was filtered off, washed with ether, and crystallized from a mixture of aqueous 5% acetic acid (10 ml) and methanol (5 ml). Inosine 2′,3′-carbonate (0.88 g, 80%) separated as colorless needles mp 261–265° (dec). This product was chromatographically pure and was used for the preparation of inosine 5′-phosphate. The melting point was raised to 273–274° (dec) after several recrystallizations from methanol–acetic acid.

Anal. Calcd for $C_{11}H_{10}N_4O_6$: C 44.91; H 3.40; N 19.02. Found: C 44.56; H 3.84; N 18.82.

In 0.01 N aqueous hydrochloric acid the product showed λ_{max} 248 mμ (ε 11,200) and λ_{min} 219 mμ (ε 1970). In 0.01 N aqueous sodium hydroxide, it showed λ_{max} 253 mμ (ε 11,810) and λ_{min} 224 mμ (ε 2076).

Stability of Inosine 2′,3′-Carbonate. Inosine 2′,3′-cyclic carbonate (0.001 g) was heated at 100° in buffer solutions (1 ml) of Table II. The buffer of pH 8 was made from Tris and HCl. After the hydrolysis for 1 hr in 0.1 M HCl (Table II) a weak nonultraviolet-absorbing spot corresponding in R_F value to ribose was detected using solvent system B and dipping the papers in an acetone solution of silver nitrate followed by ethanolic potassium hydroxide (Smith, 1960). Under identical hydrolysis conditions inosine gave a much more intense spot.

Inosine 5′-Phosphate. Inosine 2′,3′-carbonate (0.315 g, 1.07 mmoles) was dissolved in pyridine (12.5 ml) at room temperature, treated with a pyridine solution of β-cyanoethyl phosphate (2.5 ml, 1 mmole/ml), and evaporated to dryness *in vacuo*. The oily residue was dissolved in pyridine (20 ml) and again evaporated to dryness after which the residue was dissolved in pyridine (10 ml), treated with dicyclohexylcarbodiimide (1g, 4.9 mmoles), and stored at room temperature for 48 hr. Water (3 ml) was added and after 1 hr the solution was evaporated to dryness <30°. The residue was treated with water (20 ml) and dicyclohexylurea was filtered off. The filtrate was treated with aqueous NaOH (20 ml, 1 N), refluxed for 1 hr, filtered, and rapidly passed through a column (3 × 10 cm) of Dowex 50 (H^+) ion-exchange resin which had been previously cooled to 0°. The column was washed with chilled water (200 ml) and the combined eluate was adjusted to pH 10 with barium hydroxide. The suspension was centrifuged to remove barium phosphate. The supernatant was diluted with ethanol (500 ml) and after 1 hr the flocculent precipitate was collected by centrifugation, washed with ethanol, and redissolved in water (50 ml). A small amount of undissolved material was removed and the barium salt was reprecipitated with ethanol (100 ml). The precipitate was collected by centrifugation, washed with ethanol, and dried *in vacuo* over sodium hydroxide to yield barium inosine 5′-phosphate (0.337 g, 54%). The product was indistinguishable from authentic inosine 5′-phosphate on paper chromatography in solvent B and reacted positively when the paper was sprayed for *cis*-α-glycol systems. Its mobility (10 cm/hr) on paper electrophoresis in 1 M borate buffer, pH 6.5,

was identical with that of inosine 5′-phosphate. No other product could be detected by paper chromatography or electrophoresis. Chromatography in system B showed that the product (0.002 g) was completely hydrolyzed by the 5′-nucleotidase of *T. flavoviridis* venom (0.001 g) on incubation at 37° for 1 hr in pH 8.5 Tris buffer (0.5 ml, 0.1 M) and aqueous magnesium chloride (0.5 ml, 0.03 M). In 0.01 N NaOH the product showed λ_{max} 254 mμ (ε 11,840) and λ_{min} 221 mμ (ε 2760).

5′-O-Tritylinosine. A suspension of inosine (4 g, 14.9 mmoles) in pyridine (50 ml) was heated under reflux and a solution of trityl chloride (4.5 g, 16.8 mmoles) in pyridine (20 ml) was added over a period of 2 hr. After the final addition refluxing was continued for 1 hr and methanol (5 ml) was added. The solution was evaporated *in vacuo* and the residual yellow gum was dissolved in chloroform (20 ml). The solution was washed with water (2 × 10 ml), filtered, and treated with petroleum ether (100 ml, bp 40–50°). The precipitated gum was dissolved in ethanol (30 ml) and treated with water (150 ml). After standing overnight the gum crystallized. Recrystallization from chloroform–benzene gave 5′-*O*-tritylinosine (2.0 g, 27%) as thin colorless needles, mp 200–210°. Repeated recrystallization from chloroform gave a product of mp 231–234° [reported by Bredereck (1934), 231–232°]. In 0.01 N NaOH the product had λ_{max} 254 mμ (ε 12,300) and λ_{min} 240 mμ (ε 10,410).

5′-O-Tritylinosine 2′,3′-Diacetate. Crude 5′-*O*-tritylinosine (0.1 g, 0.2 mmole) was dissolved in a mixture of acetic anhydride (2.5 ml) and pyridine (1.5 ml) and stored overnight at room temperature. The solution was poured into water (20 ml) and the white precipitate was filtered off, dried, and crystallized from benzene to yield 5′-*O*-tritylinosine 2′,3′-diacetate (0.097 g, 0.165 mmole, 82%) as colorless silky needles mp 192–193°. In alcohol the product showed λ_{max} 254 mμ (ε 10,970) and λ_{min} 242 mμ (ε 8110).

Anal. Calcd for $C_{33}H_{30}N_4O_7$: C 66.68; H 5.05; N 9.43. Found: C 66.54; H 5.03; N 9.65.

5′-O-Tritylinosine 2′,3′-Carbonate. 5′-*O*-Tritylinosine (0.2 g, 0.40 mmole) was mixed with diphenyl carbonate (0.1 g, 0.47 mmole) and sodium hydrogen carbonate (0.005 g) and dissolved in dimethylformamide (0.5 ml). The resulting solution was heated at 140° (bath temperature) for 1 hr after which it was poured into chloroform (20 ml) which was washed with water (3 × 10 ml). A little of the crystalline product separated at this stage and was filtered off. The filtrate was evaporated to dryness and the pale yellow gum was crystallized from chloroform–benzene. The combined yield of crystalline material was 0.18 g (71% yield).

5′-*O*-Tritylinosine 2′,3′-carbonate phenolate was obtained as colorless needles of the monohydrate, mp 228–229° (dec). In 0.01 N ethanolic HCl the product showed only end absorption with an inflection at 243 mμ (ε 11,850); however, in 0.01 N ethanolic NaOH the product showed a peak of λ_{max} 252 mμ (ε 12,320) and λ_{min} 241 mμ (ε 10,000). Acidification of this solution reproduced the acid spectrum.

Anal. Calcd for $C_{30}H_{24}N_4O_5 \cdot C_6H_5OH \cdot H_2O$: C, 66.69; H, 4.94; N, 8.64. Found: C, 66.73; H, 4.70; N, 8.81.

The phenolate (0.1 g) was dissolved in chloroform–5% methanol (5 ml) and applied to a column of silica gel (1 × 10 cm). Elution with chloroform (50 ml) and evaporation of the eluate gave a colorless crystalline compound, mp 38°, with an odor of phenol. Admixture with phenol did not depress the melting point. Further elution with chloroform–4% methanol (100 ml) and evaporation of the eluate gave a gum which was crystallized from chloroform–petroleum ether containing a little methanol. 5′-*O*-Tritylinosine 2′,3′-carbonate was obtained as colorless needles (0.052 g), mp 169–173°.

Anal. Calcd for $C_{30}H_{24}N_4O_6 \cdot 0.5H_2O$: C, 66.07; H, 4.59; N, 10.27. Found: C, 65.61; H, 4.94; N, 10.79.

In 0.01 N ethanolic HCl the compound showed λ_{max} 249 mμ (ε 13,700) and 243 mμ (ε 14,780) and λ_{min} 248 mμ (ε 13,650) and 241 mμ (ε 14,720). In 0.01 N ethanolic NaOH it showed λ_{max} 251 mμ (ε 15,100) and 244 mμ (ε 15,220) and λ_{min} 247 mμ (ε 14,940) and 241 mμ (ε 15,150).

5′-*O*-Tritylinosine 2′,3′-carbonate (0.05 g) was dissolved in chloroform (1 ml) and treated with phenol (0.02 g) in the cold. 5′-*O*-Tritylinosine 2′,3′-carbonate phenolate began to precipitate immediately as fine colorless needles (0.035 g), mp 229–230°. The melting point was not depressed on admixture with the phenolate isolated from reaction of 5′-*O*-tritylinosine with diphenyl carbonate.

Inosine 2′,3′-Carbonate. 5′-*O*-Tritylinosine 2′,3′-carbonate phenolate (0.1 g, 0.157 mmole) was dissolved in a mixture of acetic acid (1.5 ml) and water (0.25 ml) and warmed at 100° for 2 hr. The solution was treated with water (5 ml) and the precipitated triphenylcarbinol was filtered off. The filtrate was evaporated to dryness and the residue was crystallized from methanolic acetic acid (5%) to yield inosine 2′,3′-carbonate (0.046 g, 99%) as colorless needles mp 277–279° (dec). The melting point was not depressed on admixture with the product of reaction of inosine with diphenyl carbonate. The two products were indistinguishable on paper chromatography in solvent system A. In 0.01 N HCl the product showed λ_{max} 248 mμ (ε 11,540) and λ_{min} 219 mμ (ε 2070). In 0.01 *N* NaOH it showed λ_{max} 253 mμ (ε 11,950) and λ_{min} 224 mμ (ε 2360).

Anal. Calcd for $C_{11}H_{10}N_4O_8$: C, 44.91; H, 3.40; N, 19.02. Found: C, 44.68; H, 3.70; N, 19.16.

Adenosine 2′,3′-Cyclic Carbonate. Adenosine (0.5 g, 1.87 mmoles) was dissolved in dimethylformamide (2.5 ml) and treated with phenol (0.17 g, 0.182 mmole) and diphenyl carbonate (0.6 g, 0.28 mmole). The mixture was heated at 150° (bath temperature) for 30 min and poured into ether (100 ml) while still hot. The fine buff-colored precipitate was filtered off and washed with ether. The product (0.52 g, 96%) was chromatographically pure in system A and did not react when sprayed for cis-α-glycol systems. Attempts to purify it by recrystallization from methanol resulted in the formation of adenosine as indicated by paper chromatography. A small sample was recrystallized by rapid solution in dry chloroform and evaporation *in vacuo* until crystal-

lization commenced to yield adenosine 2′,3′-cyclic carbonate as colorless needles, mp 218–220° (dec). In ethanol the product showed λ_{max} 258 mμ (ε 13,500) and λ_{min} 227 mμ (ε 640).

Anal. Calcd for $C_{11}H_{11}N_5O_5$: C, 45.07; H, 3.76; N, 23.88. Found: C, 44.73; H, 3.91; N, 24.08.

A picrate was prepared and crystallized from methanol as yellow needles, mp 198–200°.

Anal. Calcd for $C_{17}H_{14}N_8O_{12}$: C, 39.10; H, 2.68; N, 21.44. Found: C, 38.66; H, 2.63; N, 20.59.

Deamination of Adenosine 2′,3′-Carbonate. Adenosine 2′,3′-carbonate (0.01 g) was dissolved in aqueous acetic acid (50%, 1 ml) and treated with sodium nitrite (0.05 g). After standing overnight at room temperature, the solution showed an absorption maximum at 257 mμ. Additional sodium nitrite (0.1 g) was added. After 6 hr the absorption maximum was at 254 mμ, indicating substantial conversion to a hypoxanthine derivative.

The solution was adjusted to pH 8.0 with ammonia and heated at 100°. At intervals the solution was analyzed in solvent A (Table III).

2,2′-Anhydro-1-β-D-arabinofuranosyluracil. Uridine (0.5 g, 2.05 mmoles) was dissolved in dimethylformamide (1.0 ml) and treated with diphenyl carbonate (0.57 g, 2.65 mmoles) and sodium hydrogen carbonate (0.01 g). The mixture was heated at 150° (bath temperature) for 30 min and poured into ether. The precipitated gum when crystallized from methanol gave the 2,2′-anhydroarabinosyluracil as colorless prisms (0.27 g, 59%) mp 238–244° [reported by Fox and Wempen (1965), 246–248°]. The melting point was not depressed on admixture with authentic 2,2′-anhydroarabinosyluracil.

Anal. Calcd for $C_9H_{10}N_2O_5$: C, 47.81; H, 4.42; N, 12.38. Found: C, 47.61; H, 4.38; N, 12.24.

In 0.01 *N* HCl the compound showed λ_{max} at 249 mμ (ε 8540) and 222 mμ (ε 8690) and λ_{min} at 233 mμ (ε 6630) and 211 mμ (ε 6540). In 0.01 N NaOH it had λ_{max} at 254 mμ (ε 7760) and λ_{min} at 238 mμ (ε 6190). The above absorption characteristics in 0.01 N HCl closely resemble those reported for the authentic nucleoside in water (Yung and Fox, 1961).

Inosine 5′-Chloromethylphosphonate. Inosine 2′,3′-carbonate (0.885 g, 3.01 mmoles) was dissolved in dry pyridine (40 ml) and treated with dicyclohexylcarbodiimide (3.5 g, 17.1 mmoles) followed by chloromethylphosphonic acid (0.80 g, 6.18 mmoles). After 24 hr water (20 ml) was added and after a further 3 hr the mixture was evaporated to dryness. Water (25 ml) was added, dicyclohexylurea was filtered off, and the filtrate was treated with concentrated ammonium hydroxide (1 ml) and heated at 100° for 5 min. The mixture was evaporated to dryness and the residue was dissolved in water (1.0 ml) and applied to a column (3 × 22 cm) of Dowex 1 (Cl^-) ion-exchange resin. The column was eluted with 1 l. of 0.1 M LiCl–0.025 M sodium tetraborate. In the spectrophotometer this eluate showed fluorescence (negative absorption) in the 270-mμ region as did chloromethylphosphonic acid. Elution with 0.1 M LiCl (1 l.) gave a small peak of material of R_F value 0.25 in solvent system A. The spot reacted as a phosphate toward the molybdate spray test (Burrows *et al.*, 1952).

Further elution with 0.5 M LiCl (1 l.) gave a large peak of R_F 0.33 in solvent system A and R_F 0.63 in solvent system B. The spot reacted positively when sprayed for *cis*-α-glycol systems and negatively when sprayed for phosphate. The eluate was evaporated to dryness and LiCl was removed by extraction of the residue with acetone in a Soxhlet apparatus. The residue was dissolved in methanol (5 ml) and acetone (25 ml) was added. The white precipitate was centrifuged, redissolved in methanol (5 ml), and reprecipitated with acetone (25 ml). The lithium salt of inosine chloromethylphosphonate (0.59 g, 46%) was obtained as a colorless powder, mp >300°. The compound showed a mobility (10.0 cm/hr) identical with that of inosinic acid on paper electrophoresis in pH 3.5 formate buffer. In pH 6.5 1 M borate buffer, it showed a mobility of 7.5 cm/hr. An aqueous solution of product gave no precipitate with silver nitrate, indicating the absence of chloride ion.

Anal. Calcd for $C_{11}H_{13}ClLiN_4O_7P\cdot 2H_2O$: C, 31.26; Cl, 8.39; H, 4.03; N, 13.28; P, 7.34. Found: C, 31.07; Cl, 8.11; H, 4.09; N, 12.72; P, 7.30.

In 0.01 N aqueous NaOH the compound showed λ_{max} at 253 mμ (ε 11,850) and λ_{min} at 224 mμ (ε 2720). In 0.01 N aqueous HCl it showed λ_{max} at 249 mμ (ε 10,220) and λ_{min} at 221 mμ (ε 3020).

Acknowledgments

The authors thank Dr. J. J. Fox for a sample of authentic 2,2′-anhydro-1-β-D-arabinofuranosyluracil and Dr. A. Nomura for 5′-nucleotidase from *T. flavoviridis* venom.

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Aminoacyl Nucleosides. III. A Novel Rearrangement: Conversion of N^6-(α-Aminoacyl)adenines into N-(6-Purinyl)amino Acids*

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ABSTRACT: A spontaneous conversion of N^6-glycyladenine (IV) to N-(6-purinyl)glycine (VI) is described. N^6-Glycyladenine (IV) in aqueous solution over a period of several hours at room temperature, or in a few minutes at 100°, loses the elements of ammonia and forms a crystalline cyclic intermediate which has been assigned the structure, 3*H*-7,8-dihydro-8-oxoimidazo-[2,1-*i*]purine (V). The elemental analysis and ultraviolet and infrared absorption spectra of the product are consonant with this assignment. In neutral solution, compound V undergoes ring opening and rearrangement to yield N-(6-purinyl)glycine (VI). Treatment of V with 0.5 N hydrochloric acid produces mainly 5-aminoimidazole-4-carboxamide (VIII) and another compound which appears to be a carboxamidine (IX) as well as a small amount of VI. Preliminary studies suggest that these reactions are common to all N^6-(α-aminoacyl)-adenines.

These results led us to investigate the properties of N^6-chloracetyladenine (VII). This compound is also unstable in aqueous solution as N^6-glycyladenine and forms an identical cyclic intermediate V. The significance of these findings is enhanced by recent reports of the isolation of N^6-(N-formyl-α-aminoacyl)-adenosines from an enzymic digest of yeast soluble ribonucleic acid (s-RNA). N^6-(N-Carbobenzoxyglycyl)-adenine (III) is stable to boiling water; thus the possibility arises that a function of the formyl residue on the α amino groups of the natural compounds is to provide chemical stability.

We have reported the isolation of a class of nucleosides from enzymic hydrolysates of yeast soluble ribonucleic acid (s-RNA) which has been identified as N^6-(N-formyl-α-aminoacyl)adenosines (Hall, 1964; Hall and Chheda, 1965). Corroborative evidence for the presence of such amino acid nucleoside derivatives in yeast s-RNA was provided by the isolation of a derivative of N^6-(N-formylthreonyl)adenine from a mild acid hydrolysate of yeast s-RNA (Hall and Chheda, 1966). The N^6-(N-formyl-α-aminoacyl)adenosines are labile compounds which have made it difficult to affect quantitative recovery using the above isolation techniques. Further, the high order of chemical reactivity of the aminoacyladenosine derivatives raises the question

* From the Department of Experimental Therapeutics, Roswell Park Memorial Institute, Buffalo, New York 14203. *Received February 9, 1966.* This investigation has been supported in part by a grant from the National Cancer Institute, U. S. Public Health Service (CA-05697). Papers one and two in this series are Hall (1964) and Hall and Chheda (1965), respectively.