dissolved in ethyl acetate (25 ml) and chilled to 0° by an ice bath. N,N'-Dicyclohexylcarbodiimide (2.68 g, 0.013 mole) in ethyl acetate (25 ml) was added, and the mixture was stirred for 1 hr at 0°, and then at room temperature overnight. The N,N'-dicyclohexylurea (2.69 g, 92%) was removed and the filtrate was evaporated to give an oil, which solidified on seeding and was crystallized from diisopropyl ether–ethyl acetate–hexane to afford long, almost colorless needles of N-benzyloxycarbonyl-β-*t*-butyl-L-aspartic acid *p*-nitrophenyl ester (4.90 g, 85%): mp 86.5–87.5°; $[\alpha]^{27.0}D$ +1.5° (*c* 1.00, chloroform); $[\alpha]^{27.0}D$ −32.8° (*c* 1.98, methanol); R_f 0.84; ν_{max} 2970 (CH), 1770 and 1700 broad (C=O), 1515 broad (CONH), 1387 and 1367 (*t*-butyl), 1160 (CO), and 742 and 697 (Ph) cm^{-1}; λ_{max} 268 mμ (ε 9720) [lit.[12,13] mp 86°; $[\alpha]^{20}D$ +3° (*c* 3, chloroform); infrared spectrum identical with that of an authentic specimen[14]].

N-Benzyloxycarbonyl-β-*t*-butyl-L-aspartyl-L-phenylalanine Methyl Ester (XII).—To a stirred solution of N-benzyloxycarbonyl-β-*t*-butyl-L-aspartic acid (1.18 g, 0.0036 mole) in acetonitrile (25 ml) was added L-phenylalanine methyl ester hydrochloride (0.935 g, 0.0043 mole), followed by triethylamine (0.493 g, 0.0043 mole). The solution was cooled to −5° by an ice–salt bath, then N,N'-dicyclohexylcarbodiimide (0.763 g, 0.0037 mole) in acetonitrile (5.0 ml) was added, and the reaction was allowed to stand at 4° for 18 hr, followed by 28 hr at room temperature. The N,N'-dicyclohexylurea (0.624 g, 75%) was removed, the solution was evaporated, and the residue was partitioned between ethyl acetate and water. The organic phase was washed with water, aqueous citric acid, water, sodium bicarbonate solution, water, and brine, and dried (Na_2SO_4). On removal of the solvent, the oily residue was redissolved in ether, filtered, and reevaporated to give an oil, which was allowed to stand under hexane in the cold room for 2 months. At this time, the solidified material was crystallized from ethyl acetate–petroleum ether (bp 30–60°) to afford colorless needles of N-benzyloxycarbonyl-β-*t*-butyl-L-aspartyl-L-phenylalanine methyl ester (1.15 g, 66%): mp 77–78°; $[\alpha]^{25.5}D$ −26.0° (*c* 1.00, methanol); R_f 0.85; ν_{max} 3395 (NH), 2975 (CH), 1720 broad (C=O), 1670 and 1525 broad (CONH), 1395 and 1368 (*t*-butyl), 1160 (CO), and 745 and 698 (Ph) cm^{-1}; λ_{max} 248 mμ (ε 273), 252 (363), 257 (418), 264 (341), and 267 (227).

Anal. Calcd for $C_{25}H_{30}N_2O_7$: C, 63.81; H, 6.43; N, 5.95. Found: C, 64.01; H, 6.79; N, 6.02.

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Nucleotides. VI.[1] Conversion of a Ribonucleoside to a Bis(ribonucleoside 5'-)carbonate[2]

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Ribonucleosides in which the ribose moiety bears a single blocking group on the 5' hydroxyl are frequently required as intermediates for syntheses.[3] Blocking groups which have been introduced directly and selectively at the 5' position are restricted to the acid-labile trityl (or methoxy-substituted trityl)[4] and 1''-methoxyisopropyl[5] groups; in addition, the alkali-labile acetyl group has been introduced at the 5' position by acidic treatment of a 5'-O-acetyl-2',3'-O-isopropylidene or benzylidene nucleoside[6,7] and also by hydrolysis of a 2',3',5'-tri-O-acetyl nucleoside.[7,8] The present communication describes a facile, two-step conversion of a ribonucleoside to a bis(ribonucleoside 5'-)carbonate. This novel type of 5'-monosubstituted ribonucleoside should be a useful intermediate for syntheses since it is stable under acidic conditions but readily regenerates the free nucleoside under mildly basic conditions.

Recent work[1] has shown that inosine and adenosine can be converted in high yield to the corresponding 2',3'-cyclic carbonates by acid-catalyzed alcohol interchange reactions with 1.2 to 1.5 molar equiv of diphenyl carbonate in dimethylformamide solution. These conversions are analogous to the formation of 2',3'-O-isopropylidene nucleosides by acid-catalyzed alcohol interchange reactions between ribonucleosides and 1 to 2 molar equiv of 2,2-diethoxypropane in dimethylformamide.[9] In these latter reactions, when a large (80-fold) excess of ketal and little or no acid are employed, ribonucleosides can react selectively at the 5' position to form 5'-O-1''-alkoxyalkyl derivatives.[5] Attempts to obtain 5'-O-phenoxycarbonylinosine in analogous fashion, *i.e.*, by use of a large excess of diphenyl carbonate, have, however, given as the primary product only inosine 2',3'-carbonate (**4**) (Table I). Attempted preparation of bis(inosine 5'-)carbonate (**3**) directly from inosine (I) and 0.7 molar equiv of diphenyl carbonate likewise furnished only inosine 2',3'-carbonate (**4**). However, reaction of inosine under more vigorous conditions with 2.0 molar equiv of diphenyl carbonate afforded bis(inosine 2',3'-carbonate 5'-)carbonate (**2**) in 70% yield. The ultraviolet absorption spectra of this compound at pH 2 and 12 were closely similar to those of inosine,[10] indicating absence of substitution on the purine ring. It is known[11] that organic five-membered cyclic carbonates show carbonyl absorption near 1800 cm^{-1}, whereas six-membered and acyclic carbonates absorb near 1760 cm^{-1}. In accord with its proposed structure, compound **2** exhibited maxima at both 1810 cm^{-1} and 1740 cm^{-1}; the spectrum of inosine 2',3'-carbonate,[1] on the other hand, lacks the maximum at 1740 cm^{-1}. The absence in compound **2** of a periodate–oxidizable (*i.e.*, unsubstituted) *cis*-diol system and the characterization of its hydrolysis product **3** (Scheme I) allow unequivocal assignment of the structure of **2**.

Compund **2** was heated at 100° in aqueous buffer, pH 8.0, when paper chromatography indicated that initially a single product was formed and that subsequently this underwent hydrolysis to inosine. The half-lives of **2** and the initial hydrolysis product were approximately 6 min and 35 min, respectively (Table II). The product of partial hydrolysis was isolated

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(2) This work was supported by funds from the National Cancer Institute of Canada and the Medical Research Council (Grant MA-1591).

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(10) G. H. Beaven, E. R. Holiday, and E. A. Johnson, "The Nucleic Acids," Vol. 1, E. Chargaff and J. N. Davidson Ed., Academic Press Inc. New York, N. Y., 1955, p 510.

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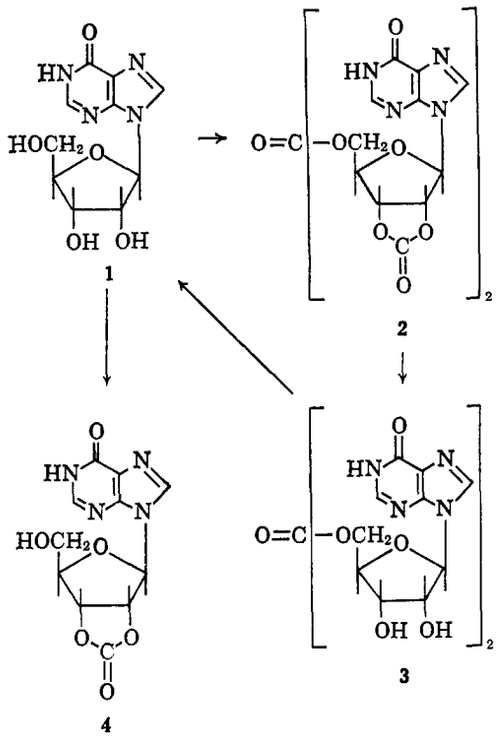
SCHEME I



in 55% yield after treatment of 2 with aqueous sodium bicarbonate under carefully controlled conditions. The infrared spectrum of this compound included a maximum at 1760 cm^{-1} corresponding to carbonyl absorption of an acyclic carbonate, but the spectrum showed no absorption due to a five-membered cyclic carbonate. The ultraviolet spectra were identical with those of 2 and thus indicated lack of substitution on the purine ring. Periodate titration[12] revealed that for each mole of inosine moiety 1 mole of *cis*-α-glycol was present. The 5′-hydroxyl is thereby indicated to be the only point of substitution and the product of partial hydrolysis of 2 is concluded to be bis(inosine 5′-)carbonate (**3**).

Experimental Section

Melting points (capillary method) were determined in an Electrothermal apparatus and are corrected. Analyses were by Dr. A. Bernhardt, Mülheim, Germany, on samples dried at 100° *in vacuo* for 8 hr over P_2O_5. Ultraviolet spectra were determind with a Cary Model 15 spectrophotometer. Infrared spectra were determined in KBr disks in a Perkin-Elmer 137B spectrophotometer. N,N-Dimethylformamide was purified by treatment with Drierite and distillation. Paper chromatography was carried out on Whatman No. 1 paper using *n*-butyl alcohol–acetic acid–water (5:2:3) as solvent. Relative amounts of components were estimated visually by inspection of chromatograms in ultraviolet light (Corning filter No. 9863). Inosine, inosine 2′,3′-carbonate, bis(inosine 2′,3′-carbonate)-5′-carbonate, and bis(inosine 5′-)carbonate had R_f values 0.35, 0.45, 0.38, and 0.17, respectively, in the above solvent system.

Attempted Conversion of Inosine to 5′-O-Phenoxycarbonylinosine.—Diphenyl carbonate (5 g) was dissolved in warm dimethylformamide (5 ml) and di-*p*-nitrophenyl hydrogen phosphate (0.05 g) was added. The mixture was cooled to room temperature and inosine (0.1 g) was added. The suspension was stirred for 30 min at room temperature, a sample was withdrawn, and the ether-insoluble portion was subjected to paper chromatography. The process was repeated at 70, 110, and 140°. Results are shown in Table I.

(12) J. S. Dixon and D. Lipkin, *Anal. Chem.*, **26**, 1092 (1954).

TABLE I

Temp of reaction, °C	R_f and ratio of products
20	0.35[a]
70	0.35[a]
110	0.35,[a] 0.45 (1:1)
140	0.45

[a] Gave a positive reaction when sprayed for *cis*-α-glycol systems.[11]

Attempted Direct Conversion of Inosine to Bis(inosine 5′-)carbonate.—A suspension of inosine (0.2 g) and $NaHCO_3$ (0.01 g) in dimethylformamide (0.6 ml) was warmed to 85° and diphenyl carbonate (0.11 g) added. Heating was continued for 5 hr after which the suspension was poured into ether (30 ml) and the precipitate filtered off. Chromatography of this product showed two components of R_f values 0.45 and 0.35 in a ratio of 3:2. Only the spot of R_f 0.35 reacted positively when sprayed for *cis*-α-glycol systems.

Bis(inosine 2′,3′-carbonate 5′-)carbonate.—Diphenyl carbonate (0.8 g, 3.74 mmoles) and sodium hydrogen carbonate (0.02 g) were added to a solution of inosine (0.5 g, 1.86 mmoles) in dimethylformamide (2.5 ml). The mixture was heated at 140° (bath temperature) for 1.5 hr, then poured into a mixture of water (10 ml) and ethanol (40 ml). A small amount of insoluble material was filtered off and the filtrate chilled. Bis(inosine 2′,3′-carbonate 5′-)carbonate (0.39 g, 68%) separated as fine, colorless needles, mp 237–238°. Recrystallization from methanol raised the melting point to 237–239°. The product had an R_f value of 0.38 and reacted negatively when sprayed for *cis*-α-glycol systems.

Anal. Calcd for $C_{23}H_{18}N_8O_{13}\cdot 2H_2O$: C, 42.45; H, 3.38; N, 17.23. Found: C, 42.63; H, 3.62; N, 17.83.

In 0.01 *N* HCl the product showed λ_{max} 248 mμ (ε 11,400) and λ_{min} 219 mμ (ε 1780). In 0.01 *N* NaOH it showed λ_{max} 253 mμ (ε 11,920) and λ_{min} 224 mμ (ε 2100). Carbonyl absorption was at 1810 and 1740 cm^{-1} (carbonate) and 1700 cm^{-1} (purine ring carbonyl).

Hydrolysis of Bis(inosine 2′,3′-carbonate 5′-)carbonate.—The carbonate (0.05 g) was warmed at 100° in pH 8.0 tris(hydroxymethyl)aminomethane–HCl buffer (0.1 *M*, 5 ml). Samples were withdrawn after the periods shown in Table II and their composition was determined by paper chromatography. All spots except that of R_f 0.38 reacted positively toward the spray for *cis*-α-glycols.

TABLE II

Period of hydrolysis, min	R_f and ratios of components
0	0.38
5	0.38, 0.17 (3:2)
10	0.38, 0.35, 0.17 (1:1:7)
15	0.35, 0.17 (1:5)
30	0.35, 0.17 (4:3)

Bis(inosine 5′-)carbonate.—Bis(inosine 2′,3′-carbonate 5′-)carbonate (0.1 g) was warmed on a boiling water bath in 5% aqueous sodium hydrogen carbonate (10 ml) for 15 min. (Chromatography indicated that the yield of the required compound was maximal at this time.) The solution was acidified with acetic acid (2 ml) and concentrated to *ca.* 1 ml under reduced pressure. Addition of ethanol (20 ml) and cooling gave a white precipitate (0.05 g, 54%). Crystallization of this from methanol yielded colorless, silky needles (0.04 g), mp 213–217°.

Anal. Calcd for $C_{21}H_{22}N_8O_{11}\cdot 2H_2O$: C, 42.16; H, 4.34; N, 18.72. Found: C, 42.30; H, 4.53; N, 18.25.

In 0.01 *N* HCl λ_{max} was 248 mμ (ε 11,300) and λ_{min} was 219 mμ (ε 2050). In 0.01 *N* NaOH λ_{max} was 253 mμ (ε 11,920) and λ_{min} was 224 mμ (ε 2130). Carbonyl absorption was at 1760 cm^{-1} (carbonate) and 1690 cm^{-1} (purin-6-one). Inosine 2′,3′-carbonate had carbonyl maxima at 1820 and 1710 cm^{-1}.

Paper chromatography indicated that the product was unchanged after heating for 1 hr at 100° in 50% aqueous acetic acid; after 2 hr at 100° a trace of a new spot of R_f 0.45 appeared; this component could be hypoxanthine. When heated for 5 min in 1 *N* NH_4OH at 100° the product was completely converted to material of R_f 0.35.

When a 2.5 x 10^{-5} M aqueous solution of the product was titrated at 25° with sodium periodate[13] the uptake of periodate (2 molar equiv) was the same as that by the same volume of a 5 x 10^{-5} M solution of inosine. A difference of 5% would have been detectable under these conditions. The time required (30 min) for complete reaction with periodate was the same for both nucleosides.

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Attempted Synthesis of a 3-Deoxy-3-phthalimido-D-ribopyranose Derivative. Formation of Furanose Derivatives

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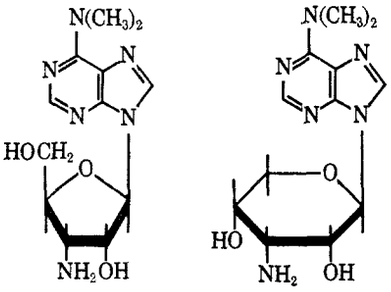
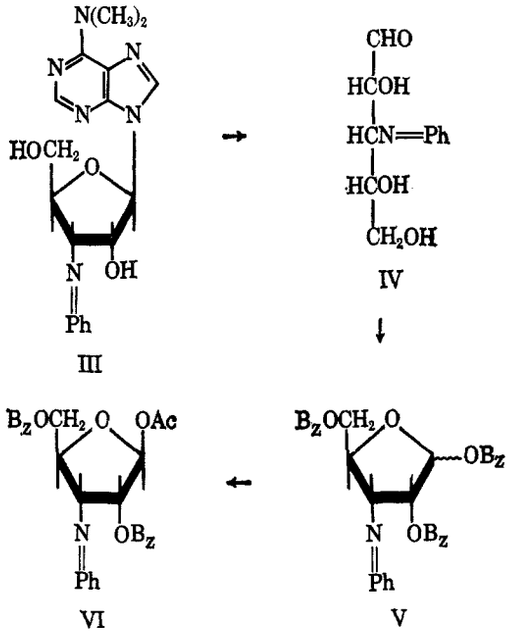
Analogs of the aminonucleoside I[1] derived from the antibiotic puromycin are of interest because of the antitumor[2] and trypanocidal[3] properties exhibited by I in experimental animals. As part of our program for the synthesis of aminonucleoside analogs, we undertook to prepare the 9β-3-amino-3-deoxy-D-ribopyranosyl derivative (II) of 6-dimethylaminopurine. Although our attention was diverted to other areas before the synthesis of II could be successfully achieved, several observations which were made during this study appear worthy of note.



I II

In principle, the synthesis of II requires the preparation of a suitably blocked derivative of 3-aminoribopyranose, its conversion to a 1-halo sugar, and condensation of this halogenose with a chloromercuripurine derivative. At the inception of our study, it seemed likely that the required 3-aminoribopyranose could be prepared by procedures analogous to those reported for the preparation of ribopyranose derivatives. Of particular interest was the procedure of Jeanloz, Fletcher, and Hudson,[4] whereby ribose was benzoylated at low temperature in pyridine solution to give a high yield of ribopyranose tetrabenzoate anomers. Since it had already been indicated[5] that N-phthaloyl amino sugar derivatives were best suited for the transformations required to produce aminonucleosides, our work was directed towards the preparation of a 3-phthalimidoribopyranoside and required as starting material 3-phthalimido-3-deoxy-D-ribose (IV). This compound was obtained by acid-catalyzed cleavage of the N-phthaloylaminonucleoside III.[5] After some study (see the Experimental Section), it was found that hydrolytic cleavage could best be achieved (79% yield) by a 15-min treatment of a suspension of III with Amberlite IR-120[6] [H] resin at reflux temperature.[7]

Benzoylation of IV according to the procedure of Jeanloz, Fletcher, and Hudson[4] gave amorphous tribenzoate (V) in 79% crude yield. Acetolysis of this crude product afforded a mixture from which it was possible to isolate crystalline material in 61% yield. This product, after further purification, was then identified as the known[5,8] 1-*O*-acetyl-2,5-di-*O*-benzoyl-3-deoxy-3-phthalimido-β-D-ribofuranose (VI). Thus, contrary to experience with ribose itself,[4] 3-phthalimidoribose affords on benzoylation, at least as the major product, a structure having the furanoid configuration.



Ph = phthaloyl; Bz = benzoyl

As an alternative approach, we next investigated the product that might be obtained on acetonation of IV. Conceivably, acetonation could lead to the furanoside VII or the pyranoside VIII, or a mixture of both. When 3-phthalimidoribose (IV) was treated with acetone in the presence of copper sulfate and ethanesulfonic acid,[9] there was obtained an 85% yield of a crystalline isopropylidene derivative of unknown ring size. In order to determine the ring configuration, the isopropylidene derivative was dephthaloylated[8] by treatment with butylamine in refluxing methanol. The re-

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(6) Amberlite IR-120 is the trademark of the Rohm and Haas Co.

(7) This procedure was based on that used by J. X. Khym, D. G. Doherty, and W. E. Cohn [*J. Am. Chem. Soc.*, **76**, 5523 (1954)] for the cleavage of ribose 5-phosphate from adenosine 5′-phosphate.

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(9) Method of B. R. Baker and R. E. Schaub, *J. Am. Chem. Soc.*, **77**, 5900 (1955).