UNIVERSITY OF MANITOBA

8-HYDROXYPURINE NUCLEOSIDES

by

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To my wife Linda

ACKNOWLEDGEMENTS

It has been my great fortune to be able to work under the direction of Dr. Kelvin Kenneth Ogilvie whose good humor and infinite patience were severely tested during the course of this work.

I would like to thank the Chemistry department of the University of Manitoba for providing financial assistance.

ABSTRACT

This work was designed to determine if the 8-hydroxy derivatives of adenosine and guanosine could be successfully converted to the corresponding 8,2'-O-anhydronucleosides. The method employed utilized the reaction conditions of diphenyl carbonate and sodium bicarbonate in dimethylformamide which had previously been shown to be successful for the synthesis of 2,2'-O-anhydrouridine from uridine.

Both adenosine and guanosine were converted to the corresponding 8-hydroxypurine nucleosides by employing acetyl functions as blocking groups. Following acetylation of adenosine and guanosine, the tri-O-acetylnucleosides obtained were brominated in the 8-position of the purine ring. These 8-halogenated derivatives were treated with excess sodium acetate in refluxing acetic anhydride for 1.5 hours. In the case of adenosine(Scheme IV), 8-hydroxy-N,2',3',5'-tetraacetyl-adenosine was isolated and completely characterized by its' infra red and ultra violet spectra, paper and thin-layer chromatographic data, and the elemental analysis. In the guanosine case(Scheme VII), 8-bromo-N,2',3',5'-tetraacetylguanosine and 8-hydroxy-N,2',3',5'-tetraacetylguanosine were isolated and also fully characterized. Both 8-hydroxy-tetraacetyl derivatives were then deacetylated to produce the corresponding 8-hydroxy-purine nucleosides in good yields.

The 8-hydroxy derivatives of adenosine and guanosine were then subjected to the diphenyl carbonate-sodium bicarbonate treatment resulting in a 74% yield of 8-hydroxyadenosine 2',3'-carbonate(VI) and a 75% yield of 8-hydroxyguanosine 2',3'-carbonate (VII). Attempts to convert these derivatives to the 8,2'-O-

anhydronucleosides using a stronger basic catalyst were unsuccessful. These results showed that an 8-hydroxy function in the purine nucleus is not analogous to the 2-hydroxy function of the pyrimidine nucleosides, at least with respect to the formation of anhydronucleosides.

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ILLUSTRATIONS AND SCHEMES

INTRODUCTION

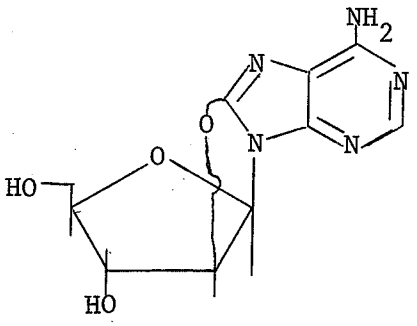
In the past decade a great deal of attention has been focussed upon the chemical and biological nature of the genetic material within the nucleus of the cell. This material, which is responsible for the transmission of genetic information from one living cell to another, is mainly comprised of DNA(deoxyribonucleic acid) and RNA(ribonucleic acid). Mutations in genetic material are believed to be responsible for various mental and physical diseases and it is therefore very important that these nucleic acids are isolated and studied. Furthermore, methods must be developed for the synthesis of natural and modified nucleosides and nucleotides in order to facilitate an understanding of these exceedingly important compounds.

It has been reported recently that modified nucleosides, which do not occur naturally in DNA or RNA, may have significant biological importance. Specifically the uptake of bromine into the nucleosides of RNA from tobacco mosaic virus, has led to a marked decrease in the biological activity of the RNA molecule[1]. Further studies have shown that, although reduced activity is observed in the brominated RNA molecule, halogenation at the 5-position of pyrimidine nucleosides such as uridine and cytidine with bromine or chlorine, results in powerful therapeutic agents[2,3]. In view of these observations many workers[4,5-8] have developed various synthetic methods for the bromination of purine nucleosides from both DNA and RNA. It has also been found that these halogenated derivatives may act as synthetic intermediates in the synthesis of mercapto[4], benzyloxy[9], amino[9] and hydroxy[10,11] substituted nucleosides, the biological importance of which are currently under investigation. This clearly indicates that the chemical modification

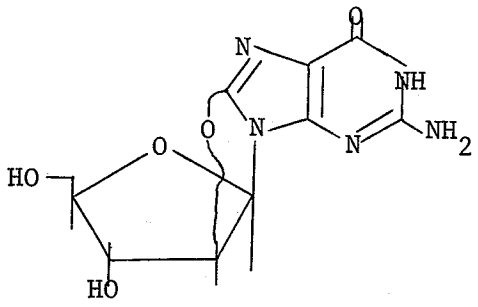
of naturally occurring nucleosides can lead to biologically important compounds. If these new nucleosides can be incorporated into a growing DNA or RNA molecule, they may change its genetic properties[12,14].

A recently recognized area of modified nucleosides which is gaining widespread attention is that of anhydronucleosides, specifically anhydroribonucleosides. These compounds feature, in addition to the N-glycoside bond, a thio ether[15,16], or an ether[11,17-19] linkage between the purine or pyrimidine ring and the sugar ring. The naturally occurring nucleosides rotate freely about the N-glycoside bond[20] but such rotation is prevented in the rigid anhydronucleosides.

A valuable feature of anhydronucleosides is their suitability in nucleotide synthesis. Anhydroribonucleosides such as I and II have the 2'-position of the sugar ring effectively blocked.



I



II

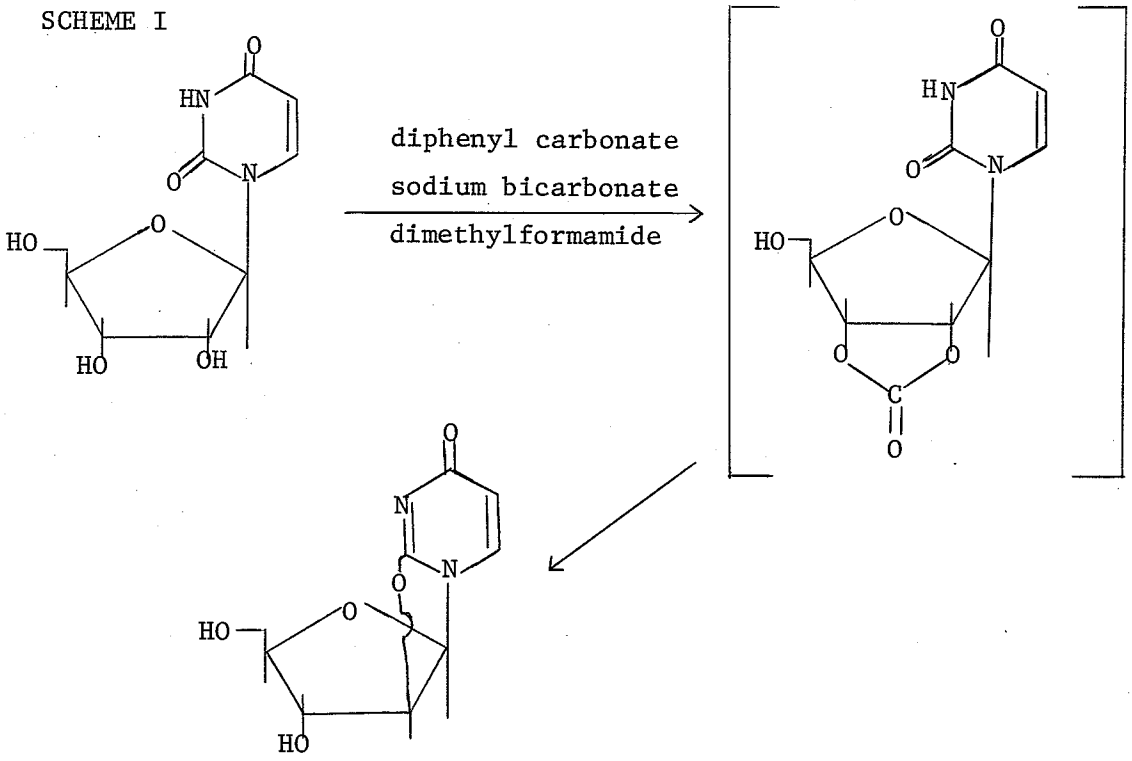
The sugar portion now resembles a deoxyribose sugar for which several versatile methods have been developed to produce oligodeoxyribonucleotides[21,22]. Such methods should be directly applicable to produce anhydroribonucleotides[23]. Since the anhydro bridge can easily be displaced to reproduce the ribose structure[11], the synthesis of anhydroribonucleotides should provide an attractive

route to ribonucleotides. Present methods of ribooligonucleotide synthesis are often laborious and inefficient[24,25].

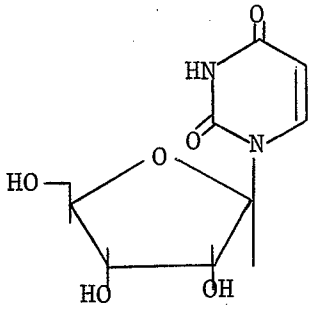
While excellent methods are available for the synthesis of anhydrouridine nucleosides, procedures for the purine anhydronucleosides with 8,2' bridges are quite inefficient[11,17]. The overall yields for conversion of adenosine and guanosine to 8,2'-anhydroadenosine(I) and 8,2'-anhydroguanosine(II) are less than 5%. Throughout the schemes there are numerous problems of separation and purification. In view of the value of these compounds it would be of great advantage if a more efficient synthetic pathway could be established.

In 1966 2,2'-anhydro-1-β-D-arabinofuranosyl uracil was synthesized in 59% yield using uridine and diphenyl carbonate in dimethylformamide with sodium bicarbonate as basic catalyst[26]. It has been suggested that this synthesis probably goes through an intermediate which is uridine-2',3'-carbonate(SCHEME I). Indeed

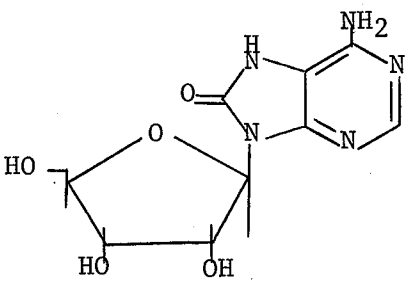
SCHEME I



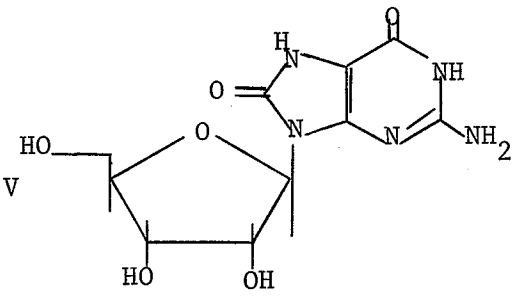
it has been shown[27] that uridine-2',3'-carbonate may be easily converted to the 2,2'-O-anhydronucleoside upon heating in dimethylformamide in the presence of sodium bicarbonate. It would therefore be of considerable interest to investigate the analogy of uridine (III) and the 8-hydroxy derivatives of adenosine(IV) and guanosine(V).



III

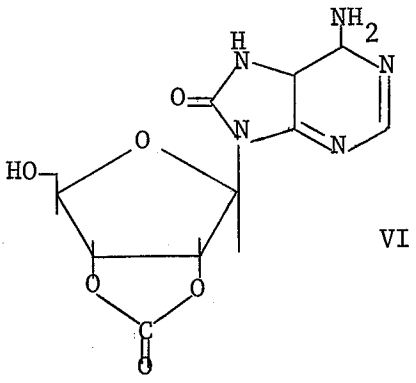


IV

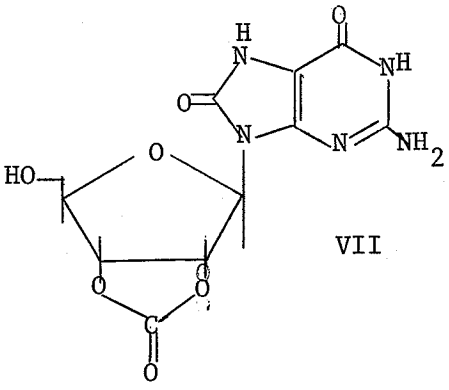


V

If they are analogous, the 8,2'-anhydropurine nucleoside derivatives would be expected when the 8-hydroxy derivatives are treated with the diphenyl carbonate-sodium bicarbonate method used to produce 2,2'-anhydrouridine. However, if the 8-hydroxy-2',3'-carbonate derivatives(VI and VII) result, these might still be converted to



VI



VII

the corresponding anhydronucleosides by further heating in the presence of sodium bicarbonate or a more strongly basic catalyst.

If the 8,2'-anhydropurine nucleosides are not obtainable by the aforementioned methods, it would still be significant to synthesize the 8-hydroxy 2',3'-carbonate of adenosine(VI) and guanosine(VII) in good yields. These derivatives would have the feature of a modified nucleoside which is immediately available for nucleotide synthesis because of the free 5'-hydroxyl. Since the carbonate moiety effectively blocks the 2' and 3'-hydroxyls, this 5'-hydroxy would be the only one which could be phosphorylated or condensed with the 3'-phosphate of another nucleotide. In this manner a guanosine and adenosine nucleoside possessing a hydroxyl at the 8-position may be introduced into growing nucleotide chains. Such nucleotides are of great interest for a number of reasons including their pairing with other nucleotides in the Watson-Crick fashion.

Thus the establishment of an efficient synthetic pathway to either the 8,2'-anhydropurine nucleosides or the 8-hydroxy 2',3'-carbonate purine nucleosides would provide molecules of potential biological importance.

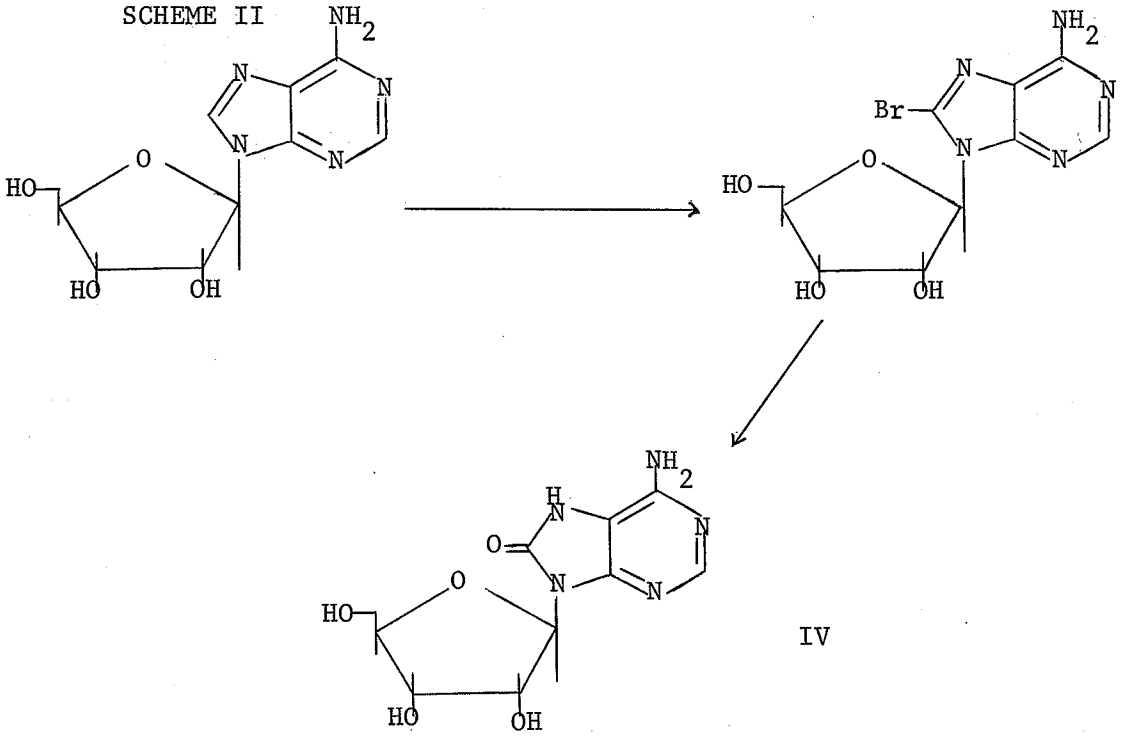
DISCUSSION AND RESULTS

The initial aim of this investigation was to establish whether or not 8-hydroxypurine nucleosides are analogous to the 2-hydroxypyrimidine nucleosides with particular respect to their conversion to the corresponding 8,2'-anhydronucleosides. The proposed route would require the developement of improved techniques for synthesis of the 8-hydroxypurine nucleosides and then subjecting these derivatives to the diphenyl carbonate-sodium bicarbonate method developed by Hampton and Nichol[26].

8-Hydroxyadenosine Nucleoside

The general approach used to synthesize 8-hydroxyadenosine is outlined in SCHEME II. The first stage is the introduction of

SCHEME II



a bromo group into the 8-position of the purine ring followed by a suitable conversion to the 8-hydroxy derivative.

The successful bromination of adenosine in high yields had been reported utilizing bromine-water and aqueous sodium hydroxide[7]. However in attempting to repeat this work the only material in the reaction mixture, detectable on thin-layer chromatography in tetrahydrofuran(R_f=0.36) and on paper chromatography in solvent A(R_f=0.54), was unreacted adenosine. It was noticed, however, that the adenosine did not appear to dissolve in the reaction medium. To effect solution, another attempt was made to brominate the nucleoside, this time employing a small amount of dioxane as well as the bromine-water and aqueous sodium hydroxide. Although the adenosine was completely dissolved, again there was no detectable change in the starting material. A subsequent modification of this procedure by replacing the aqueous sodium hydroxide with ammonium acetate in dioxane to act as a buffer, was also unsuccessful. Although these results were negative, they added further weight to the present criticism[28] of work published by these workers[7].

Recently a method was reported[5] for the bromination of 2',3',5'-tri-O-acetylguanosine. The nucleoside was first suspended in water to form a slurry and then saturated bromine-water was added in small aliquots. An analogous procedure was attempted replacing the 2',3',5'-tri-O-acetylguanosine with adenosine. Although two distinct spots on thin-layer chromatography in tetrahydrofuran(R_f=0.36 and 0.65) were detected, the faster moving compound, identified as 8-bromo-adenosine, was present in a yield of only 14%. After several purifications on thick-layer plates in tetrahydrofuran, the product still appeared to contain traces of unreacted starting

material. Thus it appeared that this method would be unsatisfactory in terms of purity and yield for the synthesis of 8-bromoadenosine.

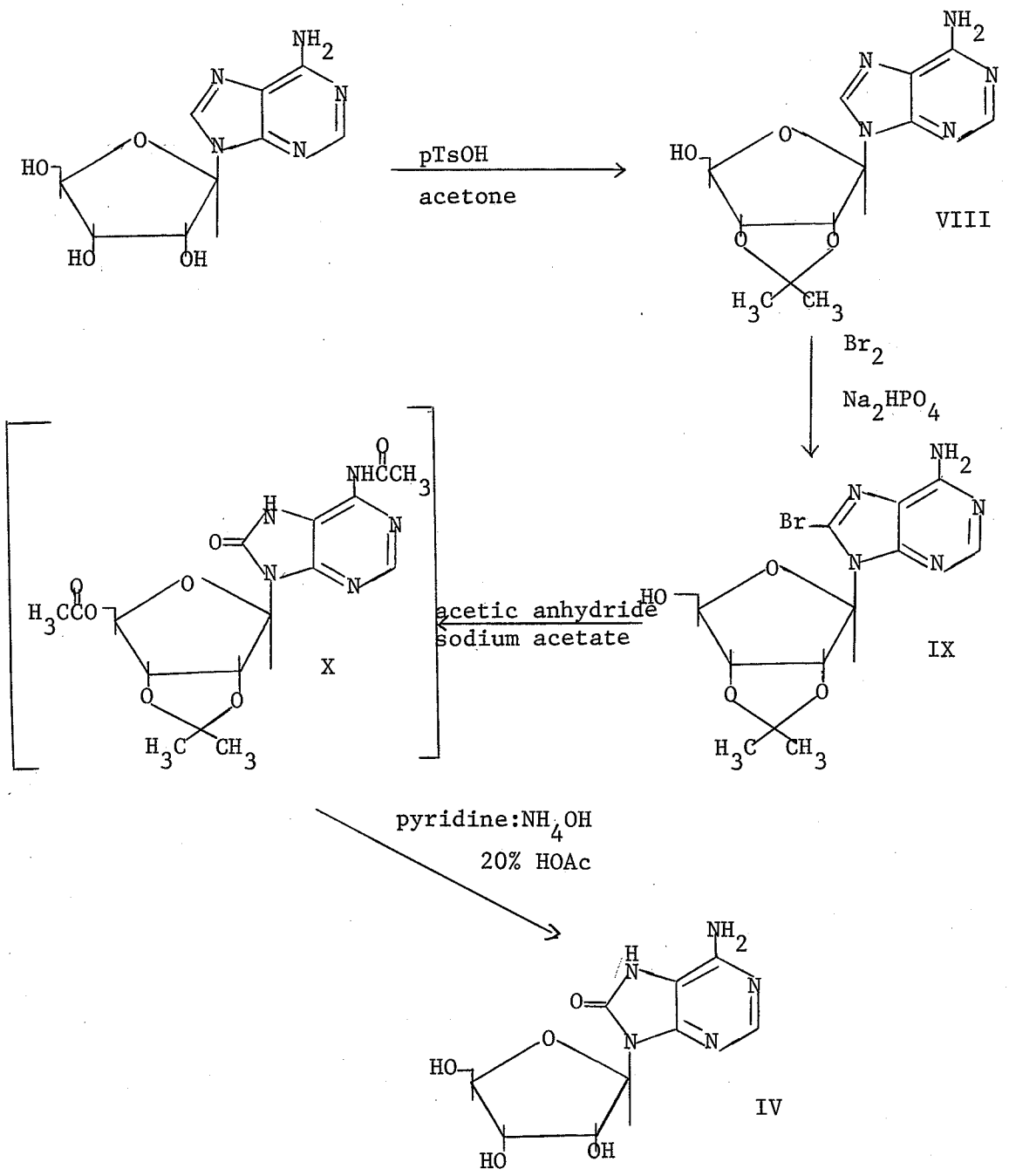
Another procedure was then found which had resulted in the bromination of 2',3'-isopropylideneadenosine in very high yields[7]. By substituting adenosine for 2',3'-isopropylideneadenosine and brominating with liquid bromine in the presence of disodium hydrogen phosphate and dioxane, a 50% yield of 8-bromoadenosine was obtained. This material was treated with sodium acetate in acetic anhydride[11] in an attempt to convert it to the 8-hydroxypurine nucleoside. The reaction products (presumably acetylated 8-hydroxyadenosine and 8-bromoadenosine) were treated with a mixture of pyridine and ammonium hydroxide to remove the acetyl groups. Thin-layer chromatography in tetrahydrofuran indicated that the product mixture contained 8-bromoadenosine(R_f=0.65) and a new product(R_f=0.29). Although this new product appeared to be 8-hydroxyadenosine, it could not be effectively separated from the salts and the unreacted 8-bromoadenosine present in the reaction mixture.

Since it appeared that either adenosine could not be brominated directly or once brominated it could not be successfully purified, it was then decided that perhaps the use of blocking groups on the hydroxyls of the ribose ring would enhance the product's solubility in organic solvents. This would then obviate some of the difficulties of separation, as well as possibly improve the effectiveness of the bromination.

The first method attempted employed the

isopropylidene blocking group on the 2' and 3'-hydroxyls of the ribose ring(SCHEME III). Adenosine was first converted to the

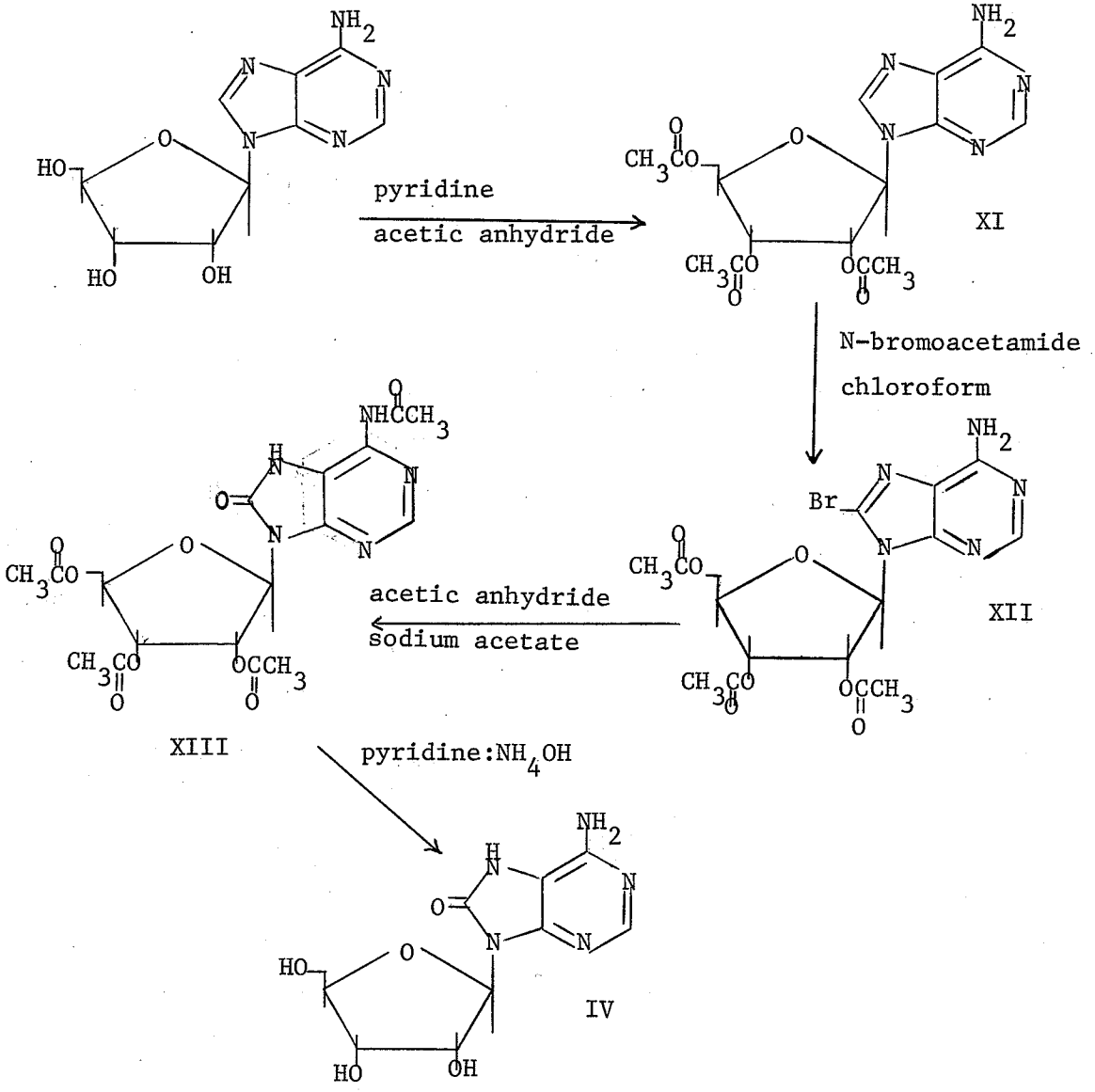
SCHEME III



2',3'-isopropylidene derivative(VIII) in 80% yield using acetone and p-toluenesulphonic acid[29]. This derivative was then brominated in accordance with the reported procedure[7] using liquid bromine in the presence of disodium hydrogen phosphate in dioxane to yield 79% of 8-bromo-2',3'-isopropylidene adenosine(IX). This product was then treated with acetic anhydride containing a ten-fold excess of sodium acetate. Following the normal work-up[11], the residue from this reaction, probably 8-hydroxy-N,5'-diacetyl-2',3'-isopropylideneadenosine(X) although not isolated, was treated with a mixture of pyridine and ammonium hydroxide to remove the 5' and N-acetyl groups and finally with 20% acetic acid to remove the isopropylidene group[30]. The product was a yellow-orange solid which had an R_f value of 0.44 on paper chromatography in solvent A as well as an R_f value of 0.29 on thin-layer chromatography in tetrahydrofuran. This data, coupled with a melting point of 252-256^{o} as well as the appearance of a weak absorption in the infra red spectrum at 5.85μ indicative of a carbonyl absorption, indicated that this compound was identical to the 8-hydroxyadenosine previously reported[11]. However the yield was only 11%. It was therefore clear that this particular route would not be practical for the preparation of the 8-hydroxy-purine nucleoside in the desired yield or purity.

Finally a successful reaction sequence was accomplished as shown in SCHEME IV. Adenosine was acetylated with anhydrous acetic anhydride in pyridine at room temperature[31] to produce a 77% yield of 2',3',5'-tri-O-acetyladenosine(XI). An attempt

SCHEME IV



to brominate the 2',3',5'-tri-O-acetyladenosine in the same manner as reported for the bromination of 2',3',5'-tri-O-acetylguanosine[5] failed to produce any of the desired 8-bromo-2',3',5'-tri-O-

acetyladenosine(XII). However a brominating procedure utilizing N-bromoacetamide in chloroform[4] was successfully employed with a slightly modified work-up for a 73% yield of 8-bromo-2',3',5'-tri-O-acetyladenosine(XII). This product was then refluxed in acetic anhydride in the presence of a ten-fold excess of sodium acetate[11] to yield a new compound which had a melting point almost seventy degrees lower than the starting material. This new compound was purified on thick-layer plates in ether(R_f=0.42) and was obtained in 80% yield. An infra red spectrum showed a general broadening of all peaks in the region 2.75-3.40μ characteristic of N-acetylpurine nucleoside derivatives and a sharp peak at 5.85μ indicative of an 8-CO absorption[32]. On this evidence, chromatographic data and elemental analysis, the structure of this material was assigned that of 8-hydroxy-N,2',3',5'-tetraacetyladenosine(XIII). This was then treated with a mixture of pyridine and ammonium hydroxide(1:3) in a closed flask for seven days to remove the acetyl blocking groups. Following the work-up with chloroform, 8-hydroxyadenosine(IV) was obtained in 94% yield. This represented a yield of 41% of 8-hydroxyadenosine based on adenosine. Previous reports[9] had a maximum yield of 27%.

The 8-hydroxyadenosine synthesized had an ultra violet absorption maximum in neutral solution at 270mμ and a shoulder at 260mμ. This was identical with the 8-hydroxyadenosine previously reported[11] and coupling this information with the chromatographic data, infra red spectrum and elemental analysis it is certain that this is the desired product.

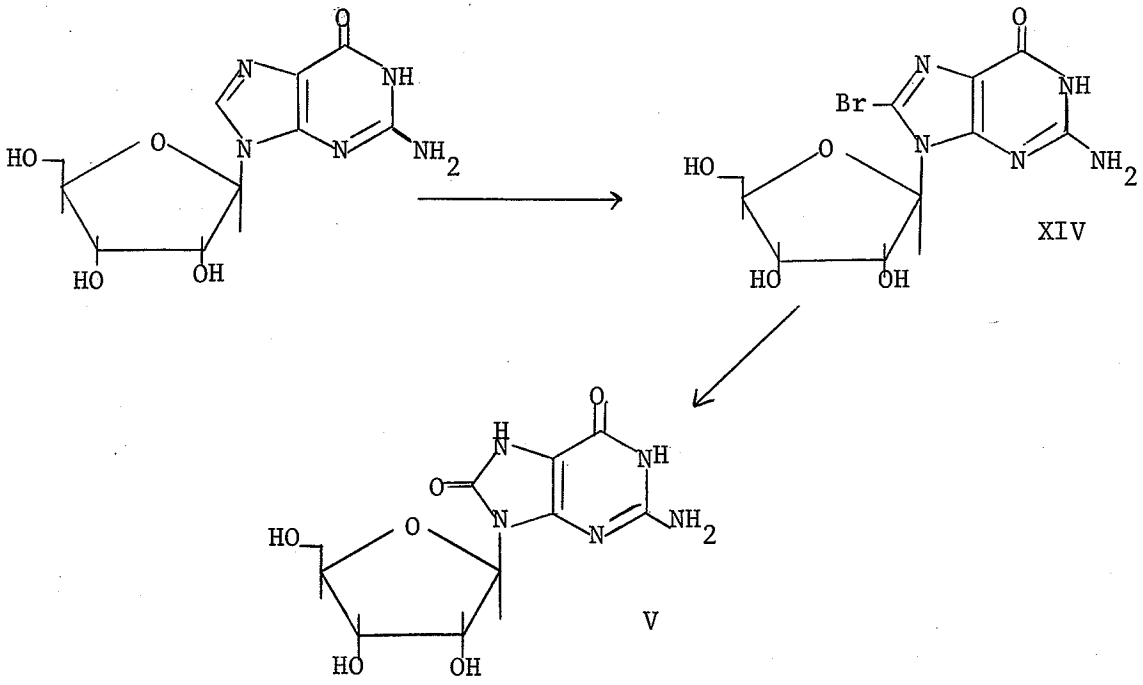
Therefore it appears that the use of acetyl blocking groups provides the most efficient pathway to the synthesis of

8-hydroxyadenosine. Since the various products are well characterized and the yields quite high, this scheme(SCHEME IV) undoubtedly supplies a sufficient amount of the 8-hydroxypurine nucleoside for its practical use in further nucleoside study.

8-Hydroxyguanosine Nucleoside

As in the case of 8-hydroxyadenosine(SCHEME II), the proposed synthesis of 8-hydroxyguanosine consists of first introducing a bromo group into the 8-position of the purine ring, followed by conversion to the 8-hydroxy derivative(SCHEME V).

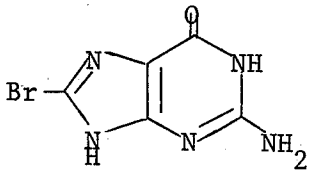
SCHEME V



Initially guanosine was brominated with saturated bromine-water[8] resulting in a 75% yield of 8-bromoguanosine(XIV) contaminated by a small amount of guanosine. In attempting to convert XIV directly to the 8-hydroxy derivative employing 1M sodium hydroxide

at 40°, two compounds of different mobilities on paper chromatography in solventsA(R_f=0.27 and 0.41), C(R_f=0.69 and 0.61) and D(R_f=0.58 and 0.61) were obtained. The faster moving substance was identical to unreacted 8-bromoguanosine(XIV) whereas the slow moving compound was guanosine(R_f(A)=0.28, R_f(C)= 0.61 and R_f(D)=0.58) itself. Attempts to resolve these two compounds on thick-layer plates using chloroform:ethanol(7:3) were unsuccessful as no definite separation was obtained.

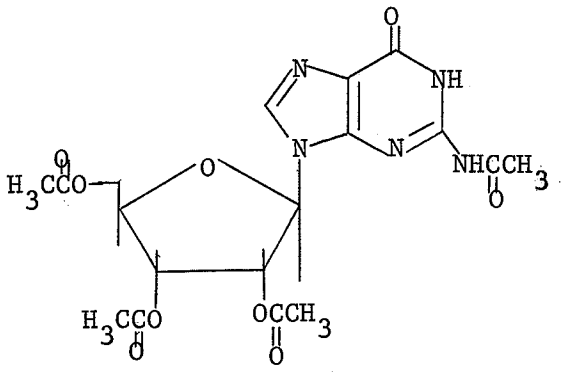
8-Bromoguanosine(XIV) was then treated with sodium acetate in acetic acid according to a previously reported procedure[10]. Following the normal work-up a pale pink powder was obtained which was spectroscopically and chromatographically identical to an authentic sample of 8-bromoguanine(XV). Evidently under



XV

the reaction conditions the N-glycoside bond was cleaved resulting in depurination of the nucleoside. However when 8-bromoguanosine was treated with sodium acetate and acetic anhydride[11] two new substances were detected on thin-layer chromatography in ethyl acetate(R_f=0.14 and 0.35). The faster moving compound, later identified as 8-bromo-N,2',3',5'-tetraacetylguanosine(XVI), was present in 36% yield whereas the slower moving compound, later identified as 8-hydroxy-N,2',3',5'-tetraacetylguanosine (IXX) was present in only 27% yield. In view of these low yields an alternate route was sought.

Since the use of acetyl blocking groups worked so well in the total synthesis of 8-hydroxyadenosine, it was therefore hoped that this approach would also be applicable to the synthesis of 8-hydroxyguanosine. At first guanosine was refluxed with acetic anhydride and pyridine under the identical reaction conditions as those reported for the synthesis of 2',3',5'-tri-O-acetyl-guanosine[33]. However a compound which would not crystallize from any organic solvent, which had a melting point approximately 120° lower than that reported[33] for the 2',3',5'-tri-O-acetyl derivative, and which generally had a far greater mobility in chromatographic systems than the tri-O-acetyl derivative, was produced in a yield of 85.5%. From this information, the elemental analysis and a definite broadening of peaks in the infra red spectrum in the region 2.7-3.4μ, it was concluded that this substance was the N,2',3',5'-tetraacetylguanosine(XVII)

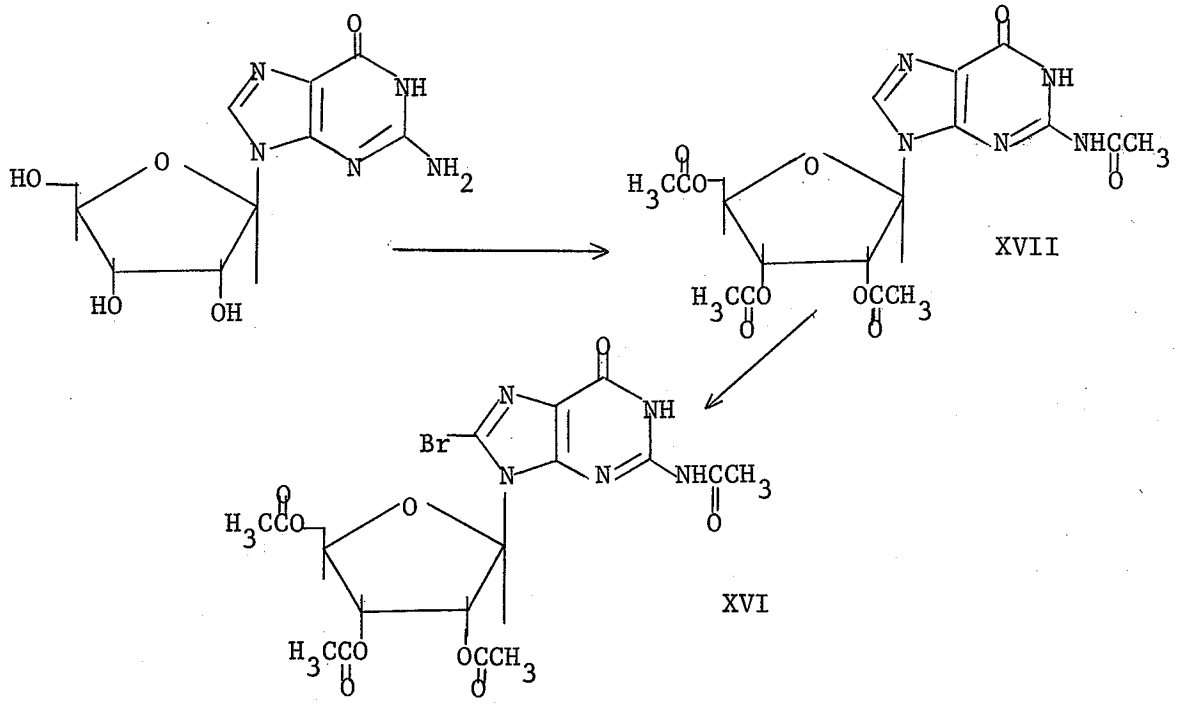


XVII

and not the 2',3',5'-tri-O-acetylguanosine(XVIII) as expected.

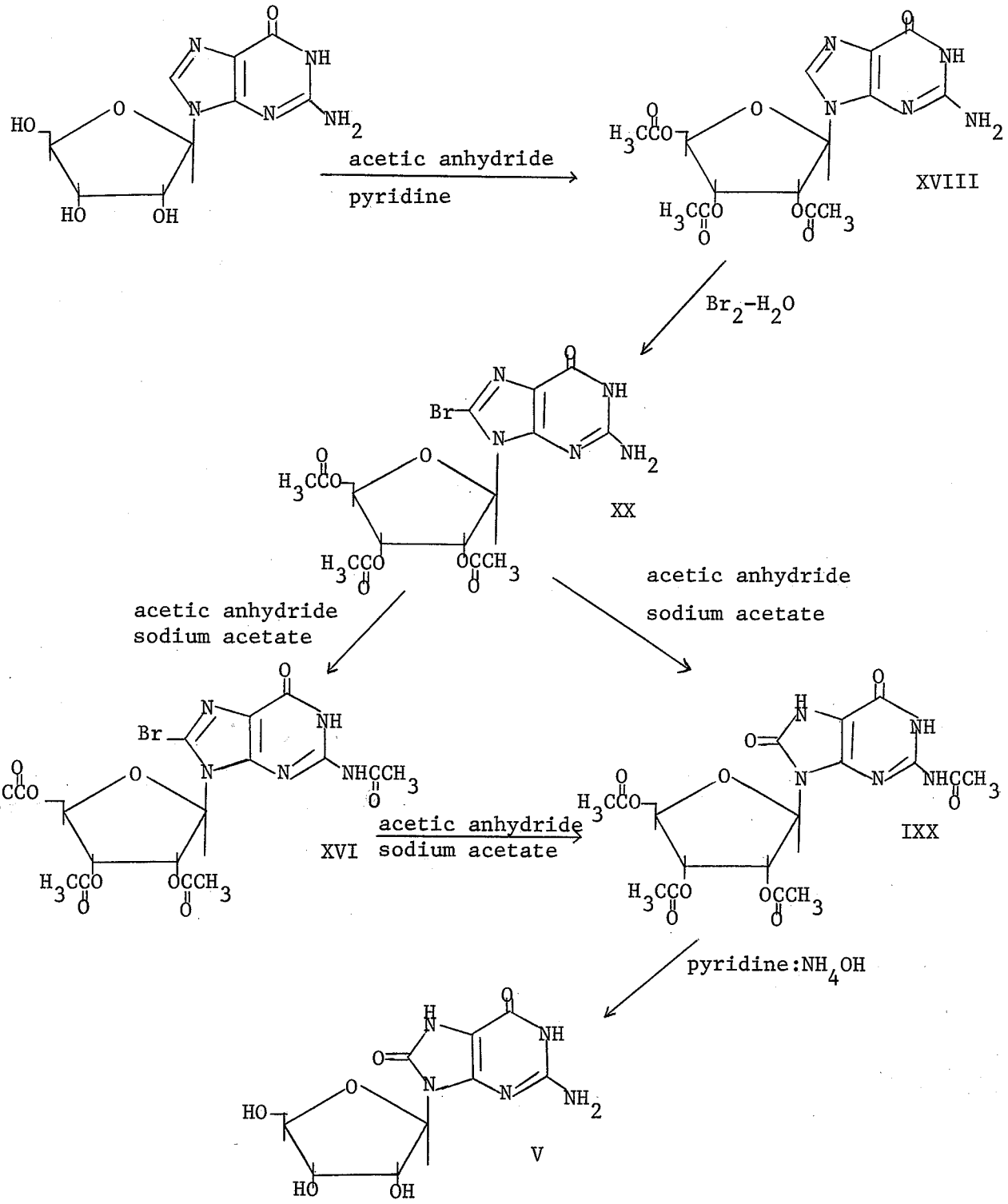
Several attempts were then made to brominate the tetra-acetyl derivative. However cleavage of the N-glycoside bond resulted when either N-bromoacetamide in chloroform[4] or liquid bromine and sodium acetate in acetic acid[4] were employed as methods of bromination. Eventually, using bromine-water[5], 8-bromo-N,2',3',5'-tetraacetylguanosine(XVI) was synthesized from N,2',3',5'-tetraacetylguanosine in 45% yield. Although the tetraacetyl and 8-bromo-tetraacetylguanosine derivatives can be well characterized, the fact that the yield is quite low in the bromination step makes this route(SCHEME VI) impractical.

SCHEME VI



Finally a successful synthetic pathway was established (SCHEME VII) for the synthesis of 8-hydroxyguanosine. Guanosine

SCHEME VII


acetic anhydride
pyridine
XVIII
Br_2-H_2O
XX
acetic anhydride
sodium acetate
acetic anhydride
sodium acetate
XVI
acetic anhydride
sodium acetate
IXX
pyridine:NH_4OH
V

was treated with acetic anhydride and pyridine at room temperature for 54 hours resulting in a 73% yield of 2',3',5'-tri-O-acetylguanosine(XVIII). This was then easily brominated[5] with bromine-water to the 8-bromo-2',3',5'-tri-O-acetylguanosine(XX) in 84% yield. The 8-bromo-2',3',5'-tri-O-acetylguanosine thus produced was identical to an authentic sample and both the triacetyl and the 8-bromo-triacetyl nucleosides crystallized well and were easily characterized.

8-Bromo-2',3',5'-tri-O-acetylguanosine was then treated with acetic anhydride and a ten-fold excess of sodium acetate[11] under the identical reaction conditions as those for the conversion of 8-bromo-2',3',5'-tri-O-acetyladenosine to 8-hydroxy-N,2',3',5'-tetraacetyladenosine. Following the work-up two substances were detected on thin-layer chromatography in ethyl acetate(R_f=0.14 and 0.35). The slower substance, 8-hydroxy-N,2',3',5'-tetraacetylguanosine(IXX) was present in 53% yield whereas the faster substance, 8-bromo-N,2',3',5'-tetraacetylguanosine(XVI) was present in 28% yield. Although the yield of the 8-hydroxy derivative was not high, when the recovered 8-bromo-N,2',3',5'-tetraacetylguanosine(XVI) was subjected to the acetic anhydride-sodium acetate treatment[11], a 55% yield of the 8-hydroxy-N,2',3',5'-tetraacetylguanosine(IXX) resulted. Also 25% of the unreacted starting material was recovered. Therefore the fact that the recovered 8-bromo-tetraacetylguanosine may be reused in the synthesis of the 8-hydroxy derivative, makes this particular route practical for use in the synthesis of 8-hydroxyguanosine.

The 8-hydroxy-N,2',3',5'-tetraacetylguanosine(IXX) thus obtained was deacetylated by reaction with a mixture of pyridine and ammonium hydroxide(1:3) for 53 hours at room temperature in a closed flask. The resulting 8-hydroxyguanosine(V)

was obtained in 83% yield and possessed the characteristic 8-CO absorption in the infra red spectrum[32] at 5.75μ.

Thus the scheme presented(SCHEME VII) avoids the difficulties of the previously reported[10] synthesis of 8-hydroxyguanosine and provides several new compounds(XVI,IXX) which have been characterized for further study.

2',3'-Carbonates of 8-Hydroxypurine Nucleosides

Once prepared, 8-hydroxyadenosine was submitted to the diphenyl carbonate-sodium bicarbonate method of Hampton and Nichol[26]. The reaction resulted in a new substance in 74% yield as well as a recovery of 13% of the starting material. The new compound showed a characteristic absorption in the infra red at 5.52μ indicative of a cyclic, 5-membered carbonate group and a sharp absorption at 5.85μ, revealing that the 8-oxy function was unchanged. From this evidence it appeared that the product was 8-hydroxyadenosine 2',3'-carbonate(VI) and not the 8,2'-anhydropurine nucleoside(I). Further support for this conclusion came from the ultra violet absorption spectrum of this new nucleoside derivative. This product had absorption maxima at 266mμ and 257mμ, which more closely resembled those of 8-hydroxyadenosine than the reported[11] absorption maximum of 260mμ for 8,2'-O-anhydroadenosine.

When 8-hydroxyguanosine was subjected to Hampton and Nichol's procedure, a product having a sharp absorptions in the infra red at 5.55 and 5.85μ was obtained in 62% yield. This evidence, coupled with chromatographic data and the elemental analysis, showed that this compound was 8-hydroxyguanosine-2',3'-carbonate(VIII). Approximately 1% of unreacted starting material was recovered from the reaction mixture as well as a

small amount of a dark brown solid substance. This substance was shown to be a mixture of two products on paper chromatography in solvent A(R_f=0.23 and 0.35). The slower moving compound, present in greatest abundance(5:1), was identified as unreacted 8-hydroxyguanosine and the amount determined spectrophotometrically as 1%. The faster moving compound, although unidentifiable, was shown to have ultra violet absorption maxima at 271 and 261mμ. However, since the reported[17] ultra-violet absorption maxima for 8,2'-O-anhydroguanosine derivatives occur at 247, 251 and 286mμ, it was concluded that this unknown material was not the anhydro derivative.

Since uridine 2',3'-carbonate can be converted to the 2,2'-O-anhydronucleoside[27] by heating in the presence of a basic catalyst, it was conceivable that the 2',3'-carbonates of 8-hydroxypurine nucleosides could be converted to the corresponding 8,2'-anhydro derivatives in an analogous manner. Therefore the 2',3'-carbonates of 8-hydroxyadenosine and guanosine were heated in four different test reactions employing sodium bicarbonate,sodium benzoate and potassium t-butoxide as basic catalysts. The products were then monitored on paper chromatography in solvents C,F and G. In all cases where a product other than the starting material was noted, it was identified as the 8-hydroxy derivative of the particular nucleoside studied. This identification was accomplished by running a spot of the reaction mixture and a spot of the known 8-hydroxypurine nucleoside against one another on each of the chromatograms.

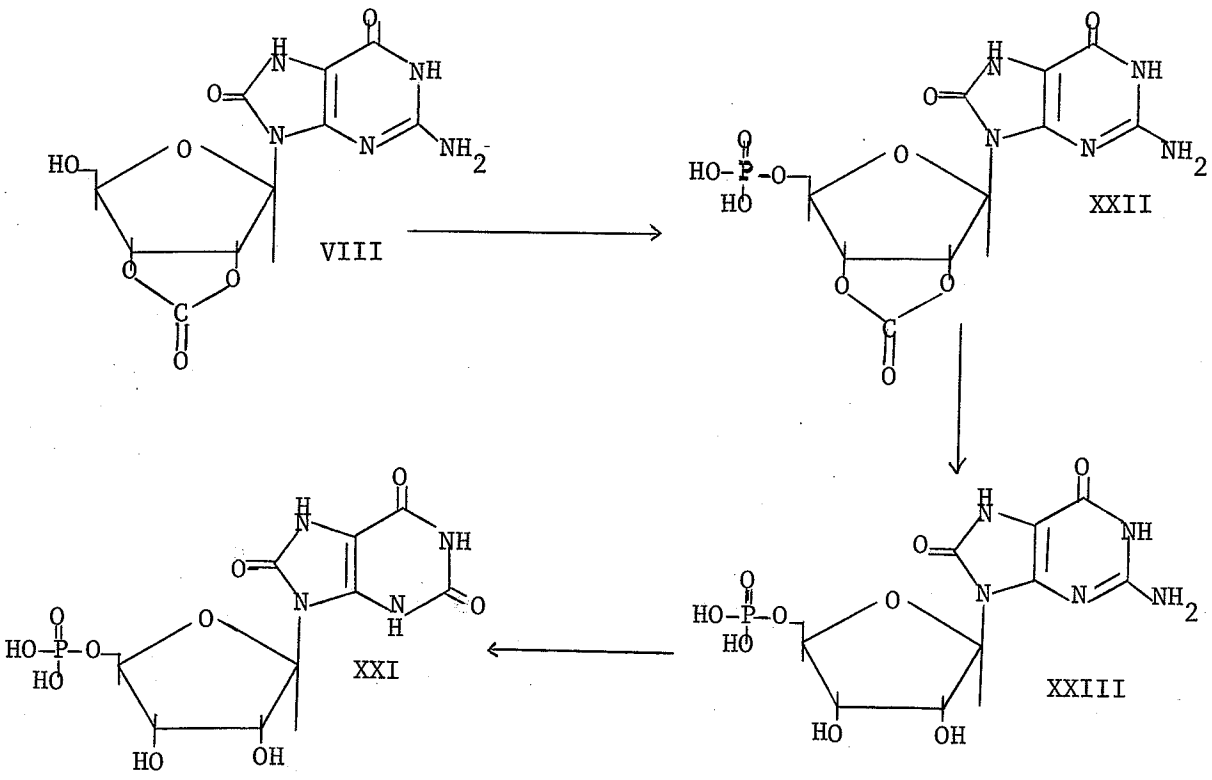
In view of the results of the test reactions as well as the products received from the diphenyl carbonate-sodium bicarbonate reaction with the 8-hydroxypurine nucleosides, it is

evident that the 8-hydroxy function in these purine nucleosides is not analogous to the 2-hydroxy function of the pyrimidine nucleosides, at least with respect to the formation of anhydro-nucleosides. Apparently the 8-hydroxy function of the purine ring is unable to interact with the 2'-position of the ribose ring with the same ease as the 2-hydroxy function of uridine.

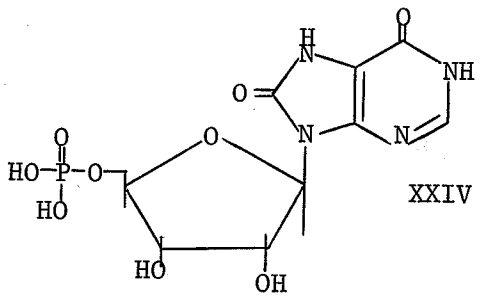
Although the 8,2'-O-anhydronucleosides of adenosine and guanosine proved to be unattainable by the diphenyl carbonate-sodium bicarbonate treatment of the 8-hydroxypurine derivatives, the 2',3'-carbonates of 8-hydroxypurine nucleosides which resulted offer several interesting features which make them valuable in the synthesis of biologically important molecules. Since the 2',3'-carbonate moiety effectively blocks the 2' and 3'-hydroxyls of the ribose ring, the 5'-hydroxyl may then be phosphorylated for the synthesis of 5'-phosphate nucleotides having a hydroxyl group in the 8-position of the purine nucleus. These derivatives would then provide an efficient method for the insertion of a modified nucleoside into a growing nucleotide chain.

A special feature of 8-hydroxyguanosine 2',3'-carbonate is its' close resemblance to 9-ribosyluric acid 5'-phosphate (XXI), a compound found in beef erythrocytes[34]. If the 8-hydroxy nucleoside derivative were phosphorylated in the 5'-position(XXII), the carbonate blocking group removed in dilute base(XXIII) and the amino group in the 2-position oxidized in nitrous acid[35], it may be possible to synthesize 9-ribosyluric acid 5'-phosphate (SCHEME VIII). Instead of oxidation of the amino function, it

SCHEME VIII



could be deaminated[36] to produce 8-hydroxyinosine 5'-phosphate (XXIV)



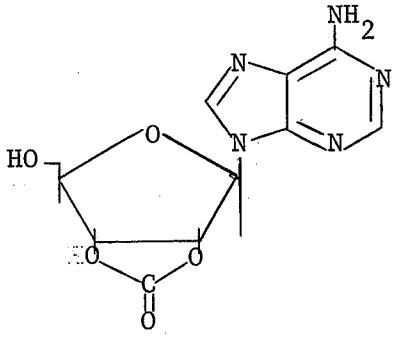
which could have considerable biological importance.

Thus the synthesis of the 2',3'-carbonates of 8-hydroxy-adenosine and guanosine has not only provided molecules of biological interest, but has also led to the synthesis of molecules having potential importance as synthetic intermediates.

2',3'-Carbonates of Purine Nucleosides

A considerable amount of work has been done in the synthesis of 2',3'-carbonates as blocking groups for purine and pyrimidine nucleosides[37-39]. Specifically, in the work of Hampton and Nichol[26] the 2',3'-carbonates of adenosine,inosine and 5'-O-tritylinosine were prepared in good yields. In their work it was noted that the synthesis of adenosine 2',3'-carbonate could not be accomplished under the same experimental conditions as those for the synthesis of inosine 2',3'-carbonate(i.e. dimethylformamide,diphenyl carbonate and sodium bicarbonate at 150° for two minutes). Apparently adenosine was sufficiently basic to inhibit acidic catalysis of the reaction and thus phenol had to be added to force the reaction to proceed rapidly and in high yield.

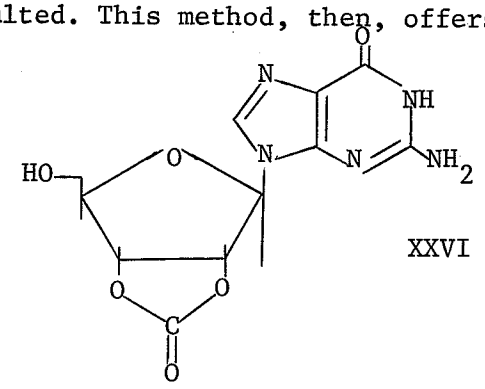
In view of the evidence that 8-hydroxyadenosine was converted in good yield to the 2',3'-carbonate derivative using diphenyl carbonate and sodium bicarbonate and heating the reaction at 150° in dimethylformamide for 30 minutes, it seemed reasonable that adenosine should behave in a similar manner under the same conditions. In fact when adenosine was exposed to these reaction conditions, a 75% yield of adenosine 2',3'-carbonate(XXV)



XXV

resulted. The product was identified by the appearance of a sharp cyclic carbonate absorption in the infra-red spectrum at 5.53μ and a comparison of its' properties with those reported by Hampton and Nichol.

Guanosine was treated under the identical conditions as those for the above synthesis of adenosine 2',3'-carbonate from adenosine and a 77% yield of the 2',3'-carbonate derivative(XXVI) resulted. This method, then, offers a very



XXVI

efficient route to the 2',3'-blocked nucleosides which are thereupon available for further study in nucleotide synthesis.

EXPERIMENTAL

General Methods

Descending paper chromatography was carried out using Whatman 3MM paper. The solvent systems employed were: Solvent A, isopropyl alcohol-concentrated ammonium hydroxide-water(7:1:2); Solvent B, n-butanol-acetic acid-water(5:2:3), Solvent C, 0.5M ammonium acetate-ethanol(3:7, adjusted to pH 3.5 with acetic acid); Solvent D, water(adjusted to pH 10 with ammonia); Solvent E, butanol-water(86:14); Solvent F, 5% ammonium bicarbonate in water; Solvent G, ethanol:water(7:3). The solvents were prepared on a volume basis. Thin-layer chromatography was carried out employing the ascending technique in closed jars which were not coated with absorbent paper. All thin-layer chromatography was run on Eastman Chromagram Sheets 6060, silica gel with fluorescent indicator, on strips 10cm. x 2cm. Thick-layer chromatography was carried out on glass plates(20cm. x 20cm.) coated with a 2mm. thick layer of silica gel DSF-5(Mondray Chemicals Ltd.). Nucleosides and their derivatives were detected on paper chromatograms, thin and thick layer sheets using an ultra-violet light source(Mineralite, output ∿254mμ).

Infra-red spectra were obtained on a Perkin-Elmer 337 recording instrument using KBr disks for sample preparation. Ultra-violet spectra were obtained on a Perkin-Elmer 450 instrument using a concentration of 2.5-3.0mg of compound dissolved in 100ml of solvent. Water or 95% ethanol was used for neutral solutions whereas for pH 1 a buffer of 27ml of 0.2M KCl and 73ml of 0.2M HCl was employed.

Melting points were determined on a Fisher-Johns melting point apparatus and are reported uncorrected. Elemental analyses

were performed by Micro-Tech Laboratories, Skokie, Illinois. Samples submitted to them were prepared by either recrystallization, lyophilization or precipitation from tetrahydrofuran with hexane. The compounds were then heated in a drying apparatus over P_2O_5 at the refluxing temperature of the solvent from which each was obtained.

Reagents and Chemicals

Reagent grade pyridine was distilled from p-toluenesulphonyl-chloride, redistilled from calcium hydride, and stored over Linde Molecular Sieves. Reagent grade acetic anhydride was distilled from phthalic anhydride and stored in the dark. Saturated bromine-water was prepared by the addition of 4.8g of reagent grade bromine to 100ml of water and stored in the dark. N-Bromoacetamide, melting point 101-103°, was prepared according to the method of Oliveto and Gerold[40] in a yield of 48%.

Adenosine nucleoside was purchased from Calbiochem whereas guanosine was purchased from Nutritional Biochemical Corporation. Authentic samples of 8-bromo-2',3',5'-tri-O-acetylguanosine and 8-bromoguanine were purchased from Aldrich. Diphenyl carbonate was also obtained from Aldrich.

Synthetic Methods

8-Bromoadenosine

(i)Adenosine(0.27g, 1mmole) was suspended in 0.1N sodium hydroxide(10ml) and treated with saturated bromine-water(3.3ml). The reaction was stoppered and allowed to stir at room temperature for 96 hours. Thin-layer and paper chromatographic data of the reaction mixture revealed the presence of only one compound which was identical to the starting material.

(ii)Adenosine(0.27g, 1mmole) was dissolved in a mixture

TABLE I. Paper Chromatographic Data of Adenosine Derivatives

Compound	Solvent A	Solvent B	Solvent C	Solvent D	Solvent E	Solvent F	Solvent G
Adenosine	0.54	----	0.76	0.50	0.23	0.52	0.63
2',3',5'-tri-O-acetyladenosine(XI)	----	----	----	----	0.64	----	----
8-bromo-2',3',5'-tri-O-acetyl-adenosine(XII)	0.86	----	----	----	0.78	----	----
8-hydroxy-N',2',3',5'-tetra-acetyl adenosine(XIII)	----	----	0.91	----	----	0.89	0.89
8-hydroxy-adenosine(IV)	0.44	0.29	0.65	0.49	0.17	0.56	0.65
8-hydroxyadenosine 2',3'-carbonate(VI)	----	----	0.85	----	----	0.83	0.79
Adenosine 2',3'-carbonate(XXV)	----	----	0.87	----	----	----	0.76

TABLE II. Thin-layer[+] Chromatographic Data of Adenosine Derivatives

Compound	Solvent				
	Tetrahydrofuran	Ethyl Acetate	Ethanol	Ether	Chloroform:Ethanol(7:3)
Adenosine	0.36	----	0.55	----	0.50
2',3',5'-tri-O-acetyladenosine (XI)	0.68	0.15	----	0.15	----
8-bromo-2',3',5'-tri-O-acetyl-adenosine(XII)	0.84	0.44	----	0.48	----
8-hydroxy-N',2',3',5'-tetra-acetyl-adenosine(XIII)	----	0.60	----	0.42	----
8-hydroxy-adenosine(IV)	0.29	----	0.62	----	0.25
8-hydroxy-adenosine 2',3'-carbonate(VI)	0.87	0.10	0.65	----	0.85
Adenosine 2',3'-carbonate(XXV)	0.60	0.10	0.63	----	0.89

[+] Eastman Chromagram Sheets 6060,silica gel with fluorescent indicator,strips 10cm. x 2cm.

of 0.1N sodium hydroxide(10ml) and dioxane(1ml), and treated with saturated bromine-water(3.3ml). The reaction was stoppered and allowed to stir at room temperature for 96 hours. Again the only detectable substance was identical to adenosine on thin-layer and paper chromatography.

(iii)Adenosine(0.27g, 1mmole) was dissolved in a 1:1 mixture(30ml) of dioxane and 10% ammonium acetate solution, followed by the addition of saturated bromine-water(10ml). The mixture was then stirred at room temperature for 15 hours. Following this time the only substance detectable on thin-layer and paper chromatography was unreacted adenosine.

(iv)Adenosine(0.27g, 1mmole) was suspended in water(10ml) and stirred vigorously at room temperature while saturated bromine-water was added in small aliquots(1ml) at such a rate that the yellow color of the reaction mixture diminished between each addition. After 5 aliquots the yellow color did not diminish and the reaction mixture was then allowed to stir for 12 hours at room temperature during which time the suspended material was dissolved. The reaction mixture was then applied to thick-layer plates and developed once in tetrahydrofuran. The only two bands obtained(R_f=0.36 and 0.65) were scraped off and separately eluted from the silica gel with tetrahydrofuran(100ml). The tetrahydrofuran solution containing the slower moving compound was concentrated to a small volume(3ml) <u>in vacuo</u> and unreacted adenosine(0.19g) precipitated upon addition of hexane. Paper and thin-layer chromatographic data of this substance were identical with those shown(Tables I and II) for the starting material.

The tetrahydrofuran solution containing the faster moving substance was concentrated to a small volume(2ml) <u>in vacuo</u> and a yellow solid(melting point 183-209°) precipitated upon addition

of hexane. The yield was 0.05g(14%). Although the product appeared to be 8-bromoadenosine it still contained unreacted starting material and rechromatography on thick-layer plates in tetrahydrofuran failed to remove this impurity.

(v)Adenosine(0.27g, 1mmole) was dissolved in a 1:1(vol/vol) mixture(30ml) of dioxane and 10% disodium hydrogen phosphate followed by the addition of liquid bromine(0.1ml). The reaction mixture was stirred for 20 hours at room temperature and then extracted with chloroform(100ml). The chloroform extractions were then combined and concentrated to a small volume(2ml) in vacuo. 8-Bromoadenosine(0.17g) precipitated upon addition of hexane. The yield was 50% and the product decomposed upon heating above 200°. The product had a mobility of 0.65 on thin-layer chromatography in tetrahydrofuran and 0.61 on paper chromatography in solvent A.

Attempted synthesis of 8-hydroxyadenosine from 8-bromoadenosine

8-Bromoadenosine(0.35g, 1mmole), from (v), was dissolved in anhydrous acetic anhydride(40ml) containing anhydrous sodium acetate(0.82g, 10mmole) and the mixture refluxed for 1.5 hours. The mixture was then cooled, treated with ethanol(50ml) and concentrated to dryness in vacuo below 35°, using ethanol to aid in the removal of the solvents until there was no detectable odour of acetic acid. Chloroform(50ml) was then added to the residue, insoluble material removed by filtration, and the chloroform filtrate dried over sodium sulphate for 2 hours. The chloroform solution was then filtered, concentrated to dryness in vacuo, and the residue dissolved in a mixture of pyridine(5ml) and ammonium hydroxide(15ml). The mixture was allowed to stir at room temperature for two days in a closed flask. Thin-layer chromatography in tetrahydrofuran indicated two substances (R_f=0.65 and 0.29) were present in the reaction mixture. The

faster substance appeared to be unreacted 8-bromoadenosine whereas the slower material seemed identical to 8-hydroxyadenosine Table II). However attempted separation of these compounds on thick-layer plates in tetrahydrofuran proved worthless as no resolution resulted.

2',3'-Isopropylideneadenosine VIII

p-Toluenesulphonic acid(7.7g) was added to a suspension of adenosine(1.06g, 4mmole) in acetone(150ml, dried and distilled) and stirred at room temperature under anhydrous conditions for 30 minutes. The solution was added to 0.5N aqueous sodium bicarbonate(210ml) and the mixture concentrated to dryness in vacuo below 30°. The resulting residue was treated with chloroform(200ml), insoluble material removed by filtration and the chloroform filtrate concentrated to dryness in vacuo. The residue was then recrystallized from hot water to yield 2',3'-isopropylideneadenosine(0.96g, 3.2mmoles) as a white powder(melting point 219-220°) in a yield of 80%. The product had a mobility of 0.71 on thin-layer chromatography in tetrahydrofuran and 0.74 on paper chromatography in solvent E. The infra-red spectrum showed principal bands at 6.0, 6.25, 7.70, 8.53, and 14.15(broad)μ.

8-Bromo-2',3'-isopropylideneadenosine IX

2',3'-isopropylideneadenosine(0.92g, 3mmole) was dissolved in a 1:1(vol/vol) mixture(90ml) of dioxane and 10% disodium hydrogen phosphate solution, followed by the addition of liquid bromine(0.3ml). The mixture was then allowed to stir for 15 hours at room temperature. The reaction mixture was then extracted with chloroform(250ml) and the chloroform extracts dried over sodium sulphate for 2 hours. The chloroform solution was filtered and concentrated to a small volume(3ml) in vacuo. 8-Bromo-2',3'-isopropylideneadenosine(0.76g, 2.5mmole) precipitated as a white powder(melting point 224-225°) in a yield of 79% upon addition of

hexane. The product had a mobility of 0.85 and 0.47 on thin-layer chromatography in tetrahydrofuran and ethyl acetate respectively, and a mobility of 0.77 on paper chromatography in solvent E. The infra-red spectrum showed principal bands at 6.08, 6.32, 7.00, 7.41, 8.65, 11.77 and 12.51μ.

Attempted synthesis of 8-hydroxyadenosine from 8-bromo-2',3'-isopropylideneadenosine

8-Bromo-2',3'-isopropylideneadenosine(1.15g, 3mmole) was dissolved in anhydrous acetic anhydride(24ml) containing anhydrous sodium acetate(1.53g, 30mmole) and the mixture refluxed for 1.5 hours. Following cooling to room temperature, ethanol(50ml) was added and the mixture concentrated to dryness *in vacuo* below 30° using ethanol to aid in the removal of the solvents, specifically all detectable traces of acetic acid. Chloroform(50ml) was then added to the residue, the insoluble material removed by filtration, and the chloroform filtrate dried over sodium sulphate for 2 hours. After filtering, the chloroform solution was concentrated to dryness *in vacuo*, dissolved in a mixture(60ml) of pyridine and ammonium hydroxide(1:3), and allowed to stir at room temperature for two days in a closed flask. The solution was then concentrated *in vacuo* below 30°, the residue dissolved in 20% acetic acid(40ml) and refluxed for 1.3 hours. The reaction mixture was then concentrated *in vacuo* below 30° and the residue dissolved in tetrahydrofuran(5ml). A yellow-orange solid,melting point 252-256°, precipitated(0.09g) upon addition of hexane, in a yield of 11%. Although the product had identical chromatographic properties as those for 8-hydroxyadenosine shown in Tables I and II, the broadening of all bands in the infra-red spectrum indicated that the product was impure. Attempted purification on thick-layer plates in tetrahydrofuran failed to yield chromatographically pure material.

2',3',5'-Tri-O-acetyladenosine XI

Adenosine(5g, 18.85mmole) was suspended in dry pyridine (100ml) stirred vigorously at room temperature in a closed flask. Anhydrous acetic anhydride(20ml) was added and the solution stirred for 1.5 hours at room temperature in the dark. The solution was then filtered and the filtrate concentrated *in vacuo* below 30° using ethanol to aid in the removal of the solvents. The resulting residue was dissolved in chloroform(50ml) and the solution dried over sodium sulphate for 2 hours. The chloroform solution was then filtered and the filtrate was concentrated to dryness *in vacuo*. The residue was dissolved in hot ethanol and 2',3',5'-tri-O-acetyladenosine(6.02g, 15.4mmole) crystallized as a white powder, melting point 171-172°, in a yield of 81.6%. Paper chromatographic properties are shown in Table I and thin-layer chromatographic properties in Table II.

The ultra violet spectrum in 95% ethanol showed maxima at 258.5mμ(ε 12,650) and 208.0mμ(ε 18,400). The infra red spectrum showed principal bands at 5.60, 5.70, 5.75, 5.95, 6.75, 8.87, 10.50, 13.10 and 13.75μ.

Attempted bromination of 2',3',5'-tri-O-acetyladenosine to 8-bromo-2',3',5'-tri-O-acetyladenosine

2',3',5'-tri-O-acetyladenosine(0.39g, 1mmole) was suspended in water(10ml) and saturated bromine-water slowly added in 1ml aliquots. The yellow color of the solution diminished slightly between each addition. Ten aliquots were employed after which the reaction mixture was allowed to stir at room temperature for 20 minutes. The mixture was then filtered and the solid gathered by filtration was dissolved in ethanol(5ml). The paper and thin-layer chromatographic data for this solid were identical with those listed in Tables I and II for the starting material. The filtrate from the reaction mixture did not contain any

material resembling a nucleoside.

8-Bromo-2',3',5'-tri-O-acetyladenosine XII

2',3',5'-tri-O-acetyladenosine(1g, 2.56mmole) and N-bromoacetamide(1g, 7.25mmole) were added to anhydrous chloroform (25ml) and the mixture refluxed for 5 hours. The resulting red solution was then cooled, concentrated in vacuo below 30° and the residue dissolved in ethyl acetate(100ml). The ethyl acetate solution was extracted first with 20mls of 1M sodium hydrosulphite solution and then with 20mls of a saturated aqueous sodium bicarbonate solution. The ethyl acetate solution was dried over sodium sulphate for 2 hours, filtered and the filtrate concentrated in vacuo to leave a red residue which was dissolved in tetrahydrofuran(3ml) and applied to thick-layer plates. The plates were first developed once in ether and then once in ethyl acetate. The only nucleoside containing band(R_f=0.50) was scrapped off and the material eluted from the silica gel with tetrahydrofuran(100ml). The tetrahydrofuran eluants were concentrated to dryness in vacuo yielding a pale yellow residue which recrystallized from ethanol as a white powder(0.88g, 1.87mmole), melting point 187-188°. The yield of 8-bromo-2',3',5'-tri-O-acetyladenosine was 73% and the product showed no depression upon a mixed melting point determination with an authentic sample. The paper and thin-layer chromatographic properties are shown in Tables I and II respectively.

An ultra-violet spectrum in 95% ethanol showed maxima at 263.0mμ(ε 15,300) and 215.0mμ(ε 23,400). The infra-red spectrum showed principal bands at 3.00, 3.15, 5.65, 5.84, 6.25, 11.60 and 12.40μ.

8-Hydroxy-N,2',3',5'-tetraacetyladenosine XIII

8-Bromo-2',3',5'-tri-O-acetyladenosine(0.39g, 0.83mmole) was dissolved in anhydrous acetic anhydride(8.5ml) containing anhydrous sodium acetate(0.69g, 8.3mmole). The mixture was refluxed for 1.5 hours and then allowed to cool to room temperature. 95% Ethanol(25ml) was added and the mixture allowed to stand overnight at room temperature. The reaction mixture was then concentrated in vacuo below 35° using ethanol to aid in the removal of all detectable traces of acetic acid. The brown residue which resulted was treated with chloroform(30ml), insoluble material removed by filtration, and the filtrate dried over sodium sulphate for 2 hours. The chloroform solution was filtered and the filtrate concentrated to dryness in vacuo. The residue was dissolved in tetrahydrofuran(3ml) and applied to thick-layer plates. After one development in ether, the only detectable band(R_f=0.42) was scraped off and the substance eluted from the silica gel with tetrahydrofuran(100ml). The tetrahydrofuran eluants were combined and concentrated in vacuo to a small volume (2ml) and 8-hydroxy-N,2',3',5'-tetraacetyladenosine(0.30g, 0.67 mmole) precipitated as a white solid upon addition of hexane. The yield was 80% and melting point 110-112°. Paper and thin-layer chromatographic data are listed in Tables I and II.

The ultra violet spectrum in 95% ethanol showed maxima at 288mμ(ε 12.350) and 219.5mμ(ε 24,700). The infra red spectrum showed general broadening in the region 2.75-3.40μ as well as principal bands at 5.71, 5.85, 7.30 and 12.75μ.

Anal. Calcd for $C_{18}H_{21}N_5O_9$: C, 47.89; H, 4.69; N, 15.52
Found: C, 47.43; H, 4.62; N, 15.12

8-Hydroxyadenosine IV

8-Hydroxy-N,2',3',5'-tetraacetyladenosine(0.88g, 1.98 mmole) was dissolved in a mixture(40ml) of pyridine and ammonium hydroxide(1:3) and allowed to stir in a closed flask at room temperature for 7 days. The reaction mixture was then concentrated in vacuo below 30°. The resulting residue was treated with chloroform(50ml) and 8-hydroxyadenosine(0.54g, 1.90mmole) was removed by filtration in a yield of 94%. An analytically pure sample was obtained by recrystallization from water as a finely divided white powder which gradually decomposes upon heating above 220°. Paper and thin-layer chromatographic data are shown in Tables I and II respectively.

The ultra violet spectrum in water showed a maximum at 270mμ(ε 15,800) and a shoulder at 260mμ(ε 13,700). The infra red spectrum showed principal bands at 2.95, 5.75, 5.83, 6.00, 7.25, 9.55, 11.25, 11.82 and 12.91μ.

Anal. Calcd for $C_{10}H_{13}N_5O_5$: C, 42.40; H,4.63; N, 24.73
Found: C, 42.31; H, 4.68; N, 24.64

8-Hydroxyadenosine 2',3'-carbonate VI

8-Hydroxyadenosine(0.10g, 0.35mmole) was dissolved in dimethylformamide(2ml) and treated with diphenyl carbonate(0.10g, 0.46mmole) and sodium bicarbonate(0.001g). The yellow brown solution, which resulted after heating the mixture at 150° for 30 minutes, was cooled and applied to a thick-layer plate. The plate was developed once in ether and then once in tetrahydrofuran resulting in only two bands(R_f=0.25 and 0.50). The band at R_f=0.25 was scraped off and eluted from the silica gel with ethanol(150ml). The ethanol eluants were then concentrated in vacuo to a small volume(5ml) and upon subsequent standing overnight at room temperature, a white powder(0.01g) precipitated. This material was identical in all respects to the starting material, 8-hydroxy-adenosine.

The band at R_f=0.50 was then scraped off and eluted from the silica gel with tetrahydrofuran(100ml). The tetrahydrofuran eluants were combined and concentrated to a small volume(2ml). 8-Hydroxyadenosine 2',3'-carbonate(0.08g, 0.26mmole), precipitated as a white powder upon addition of ether. The product slowly decomposes upon heating above 151° and the yield was 74%. The paper and thin-layer chromatographic properties of 8-hydroxyadenosine 2',3'-carbonate are shown in Tables I and II.

The ultra-violet spectrum in 95% ethanol showed maxima at 266mμ(ε 10,300) and 257mμ(ε 10,200). The infra-red spectrum showed principal bands at 5.58, 5.82, 6.08, 7.37, 8.65, 9.30(broad) and 12.91μ.

Anal. Calcd for $C_{11}H_{11}N_5O_6$: C, 42.72; H, 3.59; N, 22.65
Found: C, 42.40; H, 3.84; N, 20.89

Adenosine 2',3'-carbonate XXV

Adenosine(0.27g, 1mmole) was dissolved in dimethylformamide(1ml) and treated with diphenyl carbonate(0.28g, 1.30mmole) and sodium bicarbonate(0.005g). The mixture was heated at 150° (bath temperature) for 30 minutes, cooled and poured into ether (75ml). The resulting buff-colored precipitate was gathered by filtration, dissolved in ethanol(3ml) and applied to thick-layer plates. The plates were developed once in tetrahydrofuran and the only nucleoside containing band(R_f=0.60) was scraped off and eluted from the silica gel with tetrahydrofuran(100ml). The tetrahydrofuran eluants were combined and concentrated to a small volume(3ml) in vacuo and adenosine 2',3'-carbonate(0.22g, 0.75 mmole) precipitated as a white powder upon addition of hexane. The product occurred in a yield of 75% and slowly decomposed upon heating above 215°. Paper and thin-layer chromatographic data are shown in Tables I and II respectively.

The ultra violet spectrum in 95% ethanol showed a maximum at 258.5mμ(ε 11,650). The infra red spectrum showed principal bands at 5.53, 5.75, 6.80, 7.25, 7.51, 8.20(broad) and 12.52 (broad)μ.

8-Bromoguanosine XIV

Guanosine(10g, 35.5mmole) was added to saturated bromine-water(150ml) and the suspension stirred at room temperature for 15 minutes. The suspension was then filtered and the solid material obtained recrystallized from hot water to yield 9.7g of a white powder which had a melting point of 191-194°. This would represent a yield of 75% of 8-bromoguanosine but thin-layer chromatographic data showed a trace of unreacted starting material(R_f=0.50) as well as the main product(R_f=0.60). Attempted purification on thick-layer plates in ethanol failed to completely remove the impurity.

The ultra violet spectrum of the product at pH 1 showed a maximum at 261mμ(ε 16,800) which was identical with that reported for 8-bromoguanosine[4]. The infra red spectrum showed principal bands at 5.90, 6.14, 6.60, 6.83, 7.78, 10.20 and 12.95μ.

Attempted synthesis of 8-hydroxyguanosine from 8-bromo-guanosine

8-Bromoguanosine(0.36g, 1mmole) was dissolved in 1N sodium hydroxide and allowed to stir at 40° for 24 hours. The reaction mixture was then neutralized with Dowex-50W-X8(pyridinium form) filtered and concentrated to a small volume(3ml) *in vacuo* below 30°. The reaction mixture was shown to contain two compounds of different mobilities on paper chromatography in solvents A(R_f=o.27 and 0.41), C(R_f=0.61 and 0.69) and D(R_f=0.58 and 0.61). The faster moving compound was identical to 8-bromoguanosine whereas the slower moving compound was identical to guanosine(Table III).

TABLE III. Paper Chromatographic Data of Guanosine Derivatives

Compound	Solvent A	Solvent B	Solvent C	Solvent D	Solvent E	Solvent F	Solvent G
Guanosine	0.28	----	0.61	0.58	0.04	0.64	0.53
N',2',3',5'-tetra-acetylguanosine (XVII)	0.63	----	0.89	----	0.69	----	----
2',3',5'-tri-O-acetylguanosine (XVIII)	----	----	----	----	0.68	----	----
8-bromo-2',3',5'-tri-O-acetyl-guanosine(XX)	----	----	----	----	0.85	----	----
8-bromo-N',2',3',5'-tetra-acetyl-guanosine(XVI)	0.78	----	0.90	----	0.80	----	----
8-hydroxy-N',2',3',5'-tetra-acetyl-guanosine(IXX)	0.73	----	----	----	0.67	----	----
8-hydroxyguanosine (V)	0.23	----	0.64	0.73	0.10	0.63	0.51
8-hydroxyguanosine 2',3'-carbonate(VII)	----	----	0.75	----	----	0.63	0.59
Guanosine 2',3'-carbonate(XXVI)	----	----	0.75	0.61	----	----	----

TABLE IV. Thin-layer Chromatographic Data of Guanosine Derivatives

Compound	Solvent				
	Tetrahydrofuran	Ethyl Acetate	Ethanol	Ether	Chloroform:Ethanol(7:3)
Guanosine	----	----	0.51	----	0.20
N',2',3',5'-tetra-acetylguanosine (XVII)	0.56	0.13	0.68	----	----
2',3',5'-tri-O-acetylguanosine (XVIII)	0.16	0.02	----	----	----
8-bromo-2',3',5'-tri-O-acetyl-guanosine(XX)	0.48	0.10	----	----	----
8-bromo-N',2',3', 5'-tetra-acetyl-guanosine(XVI)	0.69	0.35	----	----	----
8-hydroxy-N',2',3', 5'-tetra-acetyl-guanosine(IXX)	0.70	0.14	----	----	----
8-hydroxyguanosine (V)	----	----	0.48	----	0.15
8-hydroxyguanosine 2',3'-carbonate(VII)	0.45	----	0.59	----	0.47
Guanosine 2',3'-carbonate(XXVI)	0.16	----	0.47	----	0.51

+ Eastman Chromagram Sheets 6060,silica gel with fluorescent indicator,strips 10cm. x 2cm.

However when the reaction mixture was applied to thick-layer plates and using chloroform:ethanol(7:3) for development, no separation of the two compounds was obtained.

Attempted synthesis of 8-hydroxy-N,2',3',5'-tetra-acetyl-guanosine from 8-bromoguanosine

8-Bromoguanosine(0.18g, 0.5mmole) was dissolved in acetic acid(4ml) containing anhydrous sodium acetate(0.21g, 2.5mmole) and refluxed for 3 hours. The reaction mixture was then cooled and ethanol(25ml) added. The solution was then concentrated to dryness *in vacuo* below 30° using ethanol to remove all detectable traces of acetic acid. The residue was then treated with chloroform(50ml), filtered and the filtrate dried over sodium sulphate for 2 hours. The chloroform solution was filtered and concentrated to a small volume(3ml) *in vacuo* and a slightly pink powder(0.10g) precipitated upon addition of hexane. This product was spectroscopically and chromatographically identical to an authentic sample of 8-bromoguanine.

N,2',3',5'-tetra-acetylguanosine XVII

Guanosine(4.0g, 14.1mmole) was heated at 100° for 17 hours in a mixture of anhydrous pyridine(30ml) and anhydrous acetic anhydride(75ml). The reaction mixture was cooled and then concentrated to dryness *in vacuo* using ethanol to aid in removal of the solvents. The resulting dark brown oily residue was dissolved in methanol(200ml), treated with deactivating charcoal, and heated on a steam bath for a few minutes. The solution was then filtered and the filtrate concentrated to dryness *in vacuo*. The residue was dissolved in tetrahydrofuran(5ml) and N,2',3',5'-tetra-acetylguanosine(4.95g, 10.97mmole) precipitated as a white powder, melting point 104-107°, upon addition of hexane. The yield was 85.5%. An analytically pure sample was obtained by dissolving 100mg of the product in tetrahydrofuran(2ml) and

applying this solution to thick-layer plates. The plates were developed twice in ethyl acetate and the only detectable band (R_f=0.24) scraped off and eluted from the silica gel with tetrahydrofuran(50ml). The tetrahydrofuran eluants were combined and concentrated in vacuo to a small volume(2ml) and the product precipitated upon addition of hexane. The melting point was 107-109°. Paper and thin-layer chromatographic data of N,2',3',5'-tetra-acetylguanosine are shown in Tables III and IV.

The ultra violet spectrum in 95% ethanol showed maxima at 281mμ(ε 11,350), 258.5mμ(ε 15,740) and 253.5mμ(ε 15,800). The infra red spectrum showed a general broadening in the region 2.7-3.4μ as well as principal bands at 5.75, 5.91, 6.22, 6.40, 8.0(broad), 12.30(broad) and 12.71(broad)μ.

Anal. Calcd for $C_{18}H_{21}N_5O_9$: C, 47.90; H, 4.69; N, 15.52
Found: C, 47.90; H, 4.73; N, 15.27

2',3',5'-tri-O-acetylguanosine XVIII

Guanosine(3g, 10.6mmole) was dissolved in a mixture of anhydrous pyridine(160ml) and anhydrous acetic anhydride(75ml). The solution was allowed to stir at room temperature for 54 hours. The reaction mixture was then concentrated to dryness in vacuo, using ethanol to aid in the removal of the solvents. The resulting residue was recrystallized from chloroform to yield 2',3',5'-tri-O-acetylguanosine(3.17g, 7.74mmole) as colorless needles, melting point 231-233°. The yield was 73%. Paper and thin-layer chromatographic properties are listed in Tables III and IV.

The ultra violet spectrum showed a maximum at 255mμ(ε 14,850), a shoulder at 252mμ(ε 14,700) and a shoulder at 271.5mμ(ε 10,300). The infra-red spectrum showed principal bands at 5.70, 5.88, 7.30, 8.10(broad), 8.5 and 12.7μ.

8-Bromo-2',3',5'-tri-O-acetylguanosine XX

2',3',5'-Tri-O-acetylguanosine(1.00g, 2.45mmole) was slowly added to water(35ml) to form a slurry. This slurry was stirred vigorously at room temperature while saturated bromine-water was slowly added in small aliquots(3.5ml) at such a rate that the yellow color disappeared between each addition. Approximately 4 aliquots were required until the yellow color persisted. The reaction mixture was then stoppered and allowed to stir at room temperature for 20 minutes. The mixture was then filtered and the pink solid obtained was immediately recrystallized from acetone:water(1:20) to yield 8-bromo-2',3',5'-tri-O-acetylguanosine(0.95g, 1.96mmole) as small colorless needles. The yield was 84% and melting point 216-217°. This product appeared to be identical in all respects to an authentic sample and its' paper and thin-layer chromatographic data are shown in Tables III and IV.

An ultra violet spectrum at pH 1 showed a maximum at 262mμ(ε 15,950) and a shoulder at 271mμ(ε 13,940). The infra-red spectrum showed principal bands at 2.86, 3.02, 5.70, 5.89, 6.15, 6.29, 10.27, 10.60 and 13.78μ.

Attempted synthesis of 8-bromo-N,2',3',5'-tetra-acetyl-guanosine from N,2',3',5'-tetra-acetylguanosine

(i)N,2',3',5'-tetra-acetylguanosine(0.45g, 1mmole) and N-bromoacetamide(0.45g, 3.2mmole) were added to anhydrous chloroform(8ml) and the solution refluxed for 5 hours. The solution was then concentrated to dryness *in vacuo* and the residue dissolved in ethyl acetate(15ml). The ethyl acetate solution was extracted first with 10ml of 1M sodium hydrosulphite solution and then 10ml of a saturated aqueous sodium bicarbonate solution. The ethyl acetate solution was then dried over sodium sulphate for 2 hours,

filtered, and concentrated to a small volume(2ml) in vacuo. A pink powder(0.29g), which was spectroscopically and chromatographically identical to an authentic sample of 8-bromoguanine, precipitated upon addition of hexane.

(ii)N,2',3',5'-tetra-acetylguanosine(0.45g, 1mmole) was added to a solution of sodium acetate(0.45g, 5.5mmole) and glacial acetic acid(4ml). The mixture was then treated with liquid bromine(0.25ml), stoppered, and heated at 50-60° for 36 hours. The resulting black solution was cooled, ethanol added (10ml), and then concentrated to dryness in vacuo. The black residue was treated with boiling isopropyl alcohol, filtered, and the filtrate concentrated to a small volume(3ml) in vacuo. Upon standing at room temperature a finely divided pink powder (0.19g) precipitated from the alcohol solution. This material was spectroscopically and chromatographically identical to an authentic sample of 8-bromoguanine.

8-Bromo-N,2',3',5'-tetra-acetylguanosine XVI

Method A N,2',3',5'-tetra-acetylguanosine(0.45g, 1mmole) was slowly added with stirring to water(10ml) to form a thick slurry. The slurry was then stirred vigorously at room temperature while saturated bromine-water was slowly added. The addition of bromine-water was made in 1ml aliquots and at such a rate that the yellow color of the reaction mixture diminished between each addition. The total time for complete addition(12 aliquots) was 15 minutes after which the reaction mixture was a deep yellow. The reaction mixture was then allowed to stir for an additional 20 minutes at room temperature. The mixture was filtered and the filtrate concentrated in vacuo below 35° to dryness. The residue which resulted was dissolved in tetrahydrofuran(2ml) and applied to thick-layer plates. The plates were developed once in ethyl acetate and two bands were detected(R_f=0.14 and 0.35). The band

at R_f=0.14 was scraped off and eluted from the silica gel with tetrahydrofuran(150ml). The tetrahydrofuran eluants were combined and concentrated to a small volume(4ml) in vacuo. A white solid(0.15g) which was chromatographically identical to N,2',3',5'-tetra-acetylguanosine(Tables III and IV) was obtained upon addition of hexane, and represented a 33% recovery of the starting material.

The band at R_f=0.35 was scraped off and eluted from the silica gel with tetrahydrofuran(100ml). The tetrahydrofuran eluants were concentrated in vacuo to a small volume(3ml) and 8-bromo-N,2',3',5'-tetra-acetylguanosine(0.24g, 0.45mmole), melting point 102-104°, precipitated as a white powder upon addition of hexane. The yield was 45%. Paper and thin-layer chromatographic data are found in Tables III and IV respectively.

The ultra-violet spectrum in 95% ethanol showed maxima at 286mμ(ε 13,340), 263mμ(ε 17,450) and 259mμ(ε 17,380). The infra-red spectrum showed a general broadening in the region 2.78-3.10μ as well as principal bands at 5.71, 5.87, 6.20, 6.38 and 8.0(broad)μ.

Anal. Calcd for $C_{18}H_{20}BrN_5O_9$: C, 40.77; H, 3.80; Br, 15.07 N, 13.21

Found: C, 40.92; H, 3.82; Br, 14.70; N, 13.17

Method B 8-Bromo-2',3',5'-tri-O-acetylguanosine(1g, 2.05 mmole) was added to anhydrous acetic anhydride(20ml) containing anhydrous sodium acetate(1.68g, 20.5mmole) and the mixture refluxed for 1.5 hours. The reaction mixture was then cooled and and 95% ethanol (20ml) added. The mixture was stoppered and allowed to sit at room temperature overnight. Following this, the mixture was concentrated to dryness in vacuo below 40° using ethanol to aid in the removal of all traces of acetic acid. The resulting

brown residue was treated with chloroform(25ml), insoluble material removed by filtration, and the chloroform solution dried over sodium sulphate for 2 hours. The chloroform solution was then filtered and the filtrate concentrated to dryness in vacuo. The oily red residue was dissolved in tetrahydrofuran (3ml) and applied to thick-layer plates which were subsequently developed once in ethyl acetate. Only two bands were detected (R_f=0.20 and 0.35). The band at R_f=0.35 was scraped off and eluted from the silica gel with tetrahydrofuran(100ml). The tetrahydrofuran eluants were then combined and concentrated in vacuo to a small volume(3ml) and 8-bromo-N,2',3',5'-tetra-acetylguanosine(0.30g, 0.57mmole) precipitated as a white powder, melting point 101-104°, upon addition of hexane. The yield was 28% and a mixed melting point determination with the product of Method A showed no depression. All chromatographic and spectrophotometric data were identical with those recorded for the 8-bromo-N,2',3',5'-tetra-acetylguanosine prepared in Method A.

Method C 8-Bromoguanosine(0.36g, 1mmole) was added to anhydrous acetic anhydride(10ml) containing anhydrous sodium acetate(0.84g, 10mmole) and the mixture refluxed for 1.5 hours. Following the identical work-ups as Methods A and B, the two bands(R_f=0.20 and 0.35) were obtained on thick-layer plates in ethyl acetate. The isolation of the faster moving band gave 8-bromo-N,2',3',5'-tetra-acetylguanosine in 36% yield which was identical to the products synthesized in Methods A and B.

8-Hydroxy-N,2',3',5'-tetra-acetylguanosine IXX

Method A The slower moving band(R_f=0.20) from the thick-layer plates of Method B preparation of 8-bromo-N,2',3',5'-tetra-acetylguanosine, was scraped off and eluted from the silica

gel with tetrahydrofuran(100ml). The tetrahydrofuran eluants were then concentrated to a small volume(3ml) in vacuo and 8-hydroxy-N,2',3',5'-tetra-acetylguanosine(0.15g, 1.09mmole) precipitated as a white solid, melting point 131-134°, upon addition of hexane. The yield was 53%. Paper and thin-layer chromatographic data are shown in Tables III and IV.

The ultra-violet spectrum in 95% ethanol showed maxima at 303mμ(ε 8,290) and 265.5mμ(ε 15,510). The infra-red spectrum showed general broadening in the region 2.8-3.2μ and principal bands at 5.70, 5.90, 6.35, 7.00, 7.28, 8.0(broad), 9.55 and 13.10μ.

Anal. Calcd for $C_{18}H_{21}N_5O_{10}.0.5H_2O$: C, 45.38; H, 4.65; N, 14.70

Found: C, 45.23; H, 4.44; N, 14.62

Method B The 8-bromo-N,2',3',5'-tetra-acetylguanosine prepared above(Method A, B or C) was treated under the identical conditions as those for the preparation of 8-hydroxy-N,2',3',5'-tetra-acetylguanosine from 8-bromo-2',3',5'-tri-O-acetylguanosine (Method B and then Method A(above)). Following the identical work-up a 55% yield of 8-hydroxy-N,2',3',5'-tetra-acetyl-guanosine(melting point 129-132°) was obtained as well as a 25% recovery of 8-bromo-N,2',3',5'-tetra-acetylguanosine. The 8-hydroxy derivative was identical in all chromatographic and spectrophotometric properties as those reported above in Method A and a mixed melting point determination of the two identical products showed no depression.

Method C The slower moving band(R_f=0.20) from the thick-layer plates of Method C preparation of 8-bromo-N,2',3',5'-tetra-acetylguanosine was scraped off and eluted from the silica gel with tetrahydrofuran(100ml). The tetrahydrofuran eluants were combined and concentrated to a small volume(3ml) in vacuo and 8-hydroxy-N,2',3',5'-tetra-acetylguanosine, which was identical in all respects to the products of Methods A and B, precipitated as a white powder in 27% yield on addition of hexane.

8-Hydroxyguanosine V

8-Hydroxy-N,2',3',5'-tetra-acetylguanosine(0.96g, 2.06 mmole) was dissolved in a mixture(100ml) of pyridine and ammonium hydroxide(1:3) and the solution was stirred at room temperature in a closed flask for 53 hours. The reaction mixture was then concentrated to dryness in vacuo below 30°. The resulting residue was dissolved in water(10ml) and this solution applied to thick-layer plates which were subsequently developed twice in chloroform:ethanol(7:3). The only nucleoside containing band (R_f=0.22) was scraped off and eluted from the silica gel with ethanol in a soxhlet apparatus. The ethanol solution was then concentrated to dryness in vacuo, the residue dissolved in water (100ml), frozen in a dry ice-acetone bath and lyophilized to dryness. The 8-hydroxyguanosine(0.51g, 1.71mmole) obtained was a white solid which decomposed gradually upon heating above 180°. The yield was 83%. The paper and thin-layer chromatographic properties of 8-hydroxyguanosine are shown in Tables III and IV.

The ultra-violet spectrum in water showed maxima at 294mμ(ε 7,490) and 247mμ(ε 8,990). The infra-red spectrum showed principal bands at 5.75, 5.88, 6.13, 6.25, 6.60 and 13.21μ.

Anal. Calcd for $C_{10}H_{13}N_5O_6.H_2O$: C, 37.86; H, 4.77; N, 22.08
Found: C, 37.38; H, 4.61; N, 21.75

8-Hydroxyguanosine 2',3'-carbonate VII

8-Hydroxyguanosine(0.30g, 1mmole) was dissolved in dimethyl-formamide(6ml) and treated with diphenyl carbonate(0.28g, 1.30 mmole) and sodium bicarbonate(0.006g). The mixture was then heated at 150° (bath temperature) for 30 minutes. The reaction mixture was cooled, poured into ether(100ml), and the brown precipitate gathered by filtration. This material was then dissolved in hot ethanol(7ml) and applied to thick-layer plates. The plates were developed once in ether and then twice in chloroform:ethanol (7:3). Two bands which moved from the origin (R_f=0.15 and 0.44) were detected. The band at R_f=0.15 was scraped off and eluted

from the silica gel with ethanol(100ml). The ethanol eluants were combined and concentrated to a small volume(2ml) in vacuo and a white powder(0.003g) precipitated upon standing at room temperature. This was identical chromatographically (Tables III and IV) and spectrophotometrically with 8-hydroxyguanosine and represented a 1% recovery of the unreacted starting material.

The band at R_f= 0.44 was scraped off and eluted from the silica gel with ethanol in a soxhlet apparatus. When the ethanol solution was concentrated to a small volume(5ml) in vacuo, 8-hydroxyguanosine 2',3'-carbonate(0.20g, 0.62mmole) precipitated as a white powder which slowly decomposed upon heating above 240°. The yield was 62%. Paper and thin-layer chromatographic data are shown in Tables III and IV respectively.

The ultra-violet spectrum in 95% ethanol showed maxima at 247.5mμ(ε 11,400) and 295.5mμ(ε 8,740). The infra-red spectrum showed principal bands at 5.55, 5.85, 5.98, 6.18, 6.92, 7.35, 8.65(broad), 9.40(broad) and 11.15μ.

Anal. Calcd for $C_{11}H_{11}N_5O_7 \cdot 0.5H_2O$: C, 39.53; H, 3.62; N, 20.95

Found: C, 39.92; H, 3.54; N, 20.08

On the thick-layer plates above, an immovable brown band remained at the origin. This was scraped off and eluted from the silica gel with dimethylformamide(100ml). A small known volume of this dimethylformamide solution was applied to a paper chromatogram and developed in solvent A. Two distinct bands(R_f=0.50 and 0.65) were detected after development of the chromatogram for 3 days. Each band was eluted separately with water. A spectrophotometric determination of each showed, that the slower moving substance, present in largest excess(5:1), had two maxima in its' ultra-violet spectrum(293 and 246.5mμ),

corresponding to 8-hydroxyguanosine in a yield of 1%. However the faster moving compound had ultra-violet absorption maxima at 271 and 261mμ which did not correspond to the starting material(V), the major product(VII) or the values reported for the 8,2'-O-anhydroguanosine derivatives[17].

Guanosine 2',3'-carbonate XXVI

Guanosine(0.28g, 1mmole) was dissolved in dimethylformamide (1ml) and treated with diphenyl carbonate(0.28g, 1.30mmole) and sodium bicarbonate(0.005g). The mixture was heated at 150°(bath temperature) for 30 minutes, cooled to room temperature and poured into ether(75ml). The resulting brown precipitate was then treated with hot tetrahydrofuran(50ml), filtered, and the filtrate concentrated to dryness in vacuo. The residue was dissolved in ethanol-water(1:1) and guanosine 2',3'-carbonate crystallized(0.24g, 0.77mmole) as slightly pink flakes which decomposed slowly upon heating above 246°. The yield was 77%. Paper and thin-layer chromatographic data of guanosine 2',3'-carbonate are shown in Tables III and IV.

The ultra-violet spectrum in 95% ethanol showed maxima at 273mμ(ε 8,680) and 254.5mμ(ε 13,150). The infra-red spectrum showed principal bands at 5.54, 5.93, 6.58, 6.77, 8.55(broad) and 9.22(broad)μ.

Anal. Calcd for $C_{11}H_{11}N_5O_6.0.5H_2O$: C, 41.51; H, 3.80; N, 22.01

Found: C, 42.25; H, 4.31; N, 21.66

Experimental test reactions of the 2',3'-carbonates of 8-hydroxypurine nucleosides

The following reaction mixtures were set up to test the possibility of converting the 2',3'-carbonates of 8-hydroxypurine nucleosides to 8,2'-anhydronucleosides.

Reaction (a) 8-hydroxyadenosine 2',3'-carbonate(10mg) was treated with dimethylformamide(0.5ml) and sodium bicarbonate(0.001g).

Reaction (b) 8-hydroxyadenosine 2',3'-carbonate(10mg) was treated with dimethylformamide(0.5ml) and sodium benzoate(0.001g).

Reaction (c) 8-hydroxyadenosine 2',3'-carbonate(10mg) was treated with dimethylformamide(0.5ml) and potassium t-butoxide (0.001g).

Reaction (d) 8-hydroxyadenosine 2',3'-carbonate(10mg) was treated with t-butyl alcohol(0.5ml) and potassium t-butoxide(0.001g).

Reaction (a') 8-hydroxyguanosine 2',3'-carbonate(10mg) was treated with dimethylformamide(0.5ml) and sodium bicarbonate (0.001g).

Reaction (b') 8-hydroxyguanosine 2',3'-carbonate(10mg) was treated with dimethylformamide(0.5ml) and sodium benzoate(0.001g).

Reaction (c') 8-hydroxyguanosine 2',3'-carbonate(10mg) was treated with dimethylformamide(0.5ml) and potassium t-butoxide (0.001g).

Reaction (d') 8-hydroxyguanosine 2',3'-carbonate(10mg) was treated with t-butyl alcohol(0.5ml) and potassium t-butoxide (0.001g).

The reaction mixtures involving dimethylformamide were heated at 150° for 30 minutes whereas the reaction mixtures involving t-butyl alcohol were heated at 80° for 30 minutes. The resulting solutions were spotted on paper chromatograms which were subsequently developed in solvents C, F and G. A standard reference spot of the corresponding 8-hydroxypurine nucleoside was run against each. The results are summarized in Tables V and VI.

TABLE V. Paper Chromatographic Results of Test Reactions on the 2',3'-Carbonates of 8-Hydroxypurine Nucleosides

Compound	Solvent C	Solvent F	Solvent G
8-Hydroxyadenosine(IV)	0.79	0.56	0.65
8-Hydroxyguanosine(V)	0.64	0.63	0.51
8-Hydroxyadenosine 2',3'-carbonate(VI)	0.85	0.83	0.79
8-Hydroxyguanosine 2',3'-carbonate(VII)	0.75	0.63	0.59

TABLE VI. Paper Chromatographic Results of Test Reactions on the 2',3'-Carbonates of 8-Hydroxypurine Nucleosides

Reaction	Solvent C	Solvent F	Solvent G
(a)	0.78+0.86	0.54+0.85	0.65+0.80
(b)	0.79+0.87	0.55+0.83	0.63+0.79
(c)	0.78+0.85	0.53+0.82	0.66+0.82
(d)	0.80+0.86	0.54+0.81	0.65+0.80
(a')	0.48+0.62	0.47+0.58	0.62
(b')	0.53+0.65	0.51+0.60	0.61
(c')	0.49+0.62	0.47+0.57	0.58
(d')	0.51+0.62	0.44+0.57	0.50

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