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REACTION OF 3-(β-D-RIBOFURANOSYL)ADENINE WITH PHOSPHORUS OXYCHLORIDE. ISOLATION AND CHARACTERIZATION OF 3(β-D-RIBOFURANOSYL)ADENINE 9,5'(P)-CYCLIC PHOSPHONATE.

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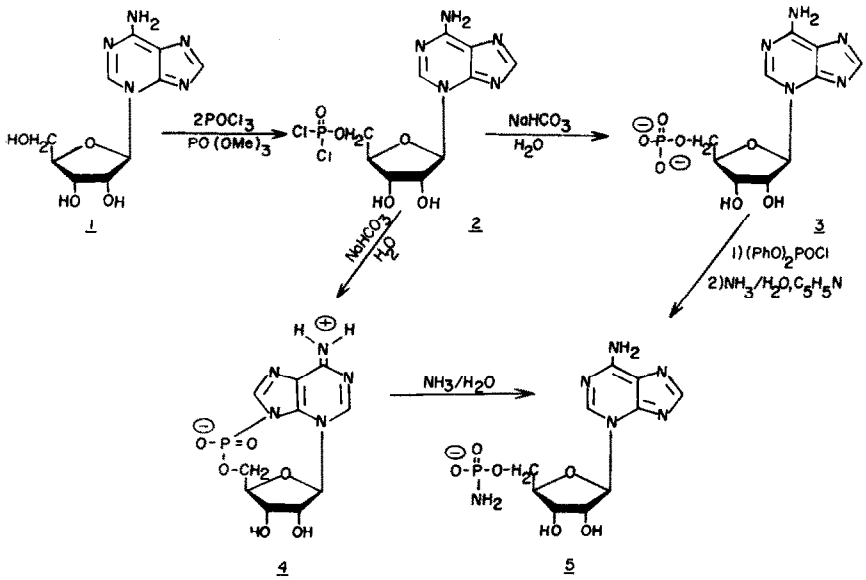
The reaction of phosphorus oxychloride with unprotected nucleosides in trialkyl phosphates as solvent and subsequent hydrolysis provides a convenient route for the preparation of nucleoside 5'-phosphates in high yield.[1] However, due to the polyfunctional nature of $POCl_3$, side reactions can occur, and in at least two cases reported in the literature, cyclic phosphates have been found to be the major product of the reaction. Thus, 9-(β-D-xylofuranosyl)adenine[2] afforded a 38% yield of the corresponding 3',5'-cyclic nucleotide while 9-(2'-O-methyl-β-D-ribofuranosyl)adenine[3] gave a 45% yield of the 3',5'-cyclic phosphate.

In this report evidence is presented to show that the reaction of phosphorus oxychloride with 3-(β-D-ribofuranosyl)adenine[4,5] (1) followed by hydrolysis in aqueous sodium bicarbonate solution yields not only the expected 3-(β-D-ribofuranosyl)adenine 5'-phosphate[4] (3) but also a novel product which has spectral and chemical properties consistent with the structure of 3-(β-D-ribofuranosyl)adenine 9,5'(P)-cyclic phosphonate (4). Compound 4 appears to be the first example of a nucleoside cyclic phosphonate in which a covalent bond exists between a nitrogen atom of the heterocyclic base moiety and the phosphorus atom at the 5'-position of the carbohydrate moiety. The purpose of this communication is to report the data that has been accumulated to date to confirm the structure of this compound as well as to demonstrate its potential synthetic utility.



Finely pulverized 3-(β-D-ribofuranosyl)adenine (20 m moles, 5.4 g) reacted rapidly with a mixture of phosphorus oxychloride (40 m moles, 3.7 ml) in trimethyl phosphate (100 ml) at 0° to form the presumed 5'-phosphorodichloridate (2). Within ten minutes a homogeneous solution was obtained and after 30 minutes the reaction mixture was hydrolyzed in cold (0°) aqueous sodium bicarbonate solution (1M, 125 ml). With continued stirring of the hydrolysis mixture, a solid precipitated (4) which was filtered,[6] washed with cold water, and dried in vacuo (P_2O_5, 25°) [0.91 g (13%), mp 195-197°; Anal.[7] Calcd for $C_{10}H_{12}N_5O_6P \cdot H_2O$: C, 34.59; H, 4.06; N, 20.17; P, 8.92. Found: C, 34.62; H, 4.10; N, 20.11; P, 9.03; Cl, 0.12].

The cyclic phosphonate (4) was found to be stable in the solid state but labile when dissolved in either dimethyl sulfoxide or buffered aqueous solution. The lability of the compound initially hindered attempts to obtain NMR spectra by conventional methods but on the other hand the instability in solution lends support to the proposed structure. Indeed, inspection of Corey-Pauling-Koltun space-filling models demonstrates that a considerable amount of strain is present in the molecule due to non-bonded interactions and that small deviations from normal in bond angles and/or bond lengths are necessary in order to accommodate the structure. In addition, the positively-charged heterocyclic base would not only be a good leaving group but also would enhance the susceptibility of the phosphorus atom to nucleophilic attack.

Due to the instability of 4 in solution, the 100 MHz ^1H NMR spectrum in DMSO-d_6 was obtained in the Fourier transform mode. The relevant portion of the spectrum is shown in Figure 1. The low-field absorptions at 9.35 and 10.7 δ downfield from an internal tetramethylsilane reference are assigned to the protons of the exocyclic amino group. This assignment is due in part to the rapid disappearance of these signals upon addition of D_2O to a freshly prepared sample of 4 in DMSO-d_6. In the spectrum of the nucleoside (1) in DMSO-d_6 the amino protons appear as a broad absorption centered at 8.22 δ[5]. The fact that the NH_2 protons are extensively deshielded in the product as compared to the starting nucleoside and occur as two distinct singlets indicates that the positive charge is localized on the amino group. The peak at 8.5 δ is assigned to the two heterocyclic base protons (H_2 and H_8) which occur as superimposable singlets. The addition of D_2O not only caused the disappearance of the NH_2 protons but also led to a resolution of the peak at 8.5 δ and indeed two singlets were observed at 8.35 and 8.4 δ. The anomeric proton (H_1') appears as a doublet ($J \approx 2$ Hz) at 5.76 δ. In comparison, the coupling constant for the anomeric proton of 1 in DMSO-d_6 is 7 Hz.[5] The low, broad absorbance centered around 5.45 δ is assigned to the free 2' and 3'-hydroxyl groups while the complex pattern centered around 4.2 δ can be attributed to the remaining non-exchangeable protons of the carbohydrate moiety. Finally, the peak at 3.5 δ is due to water of hydration in the sample of 4 as was also indicated by the microanalysis.

The undecoupled ^{31}P NMR spectrum of 4 in DMSO-d_6 was obtained in the Fourier transform mode at 40.5 MHz. The spectrum revealed a single absorption peak at 11.0 ppm upfield from an external 85% ortho phosphoric acid (OPA) reference signal. From the line width of the signal, it was evident that the phosphorus atom was coupled to other nuclei but the couplings were small (on the order of 4-5 Hz). Previously reported studies[8,9] on the ^{31}P NMR spectra of simple nucleotides and related compounds were carried out in D_2O solution, and it is of interest that the chemical shift values of the phosphorus atom in the compounds studied were found to occur in certain narrow regions depending upon the type of compound under consideration.[9] Thus, at pH 3,

the chemical shift of the phosphorus atom of 2',3'-cyclic nucleotides occurred around -20 ppm downfield from an external OPA reference while 5'-mononucleotides were found at -0.2 to -0.4 ppm and 3',5'-cyclic nucleotides occurred at 1.5 to 2.1 ppm. While it is likely that the ^{31}P chemical shift value of 4 would be somewhat different in D_2O rather than DMSO-d_6, the observed value of 11.0 ppm is simply too far out of line with the data reported above for D_2O solution for 4 to be a simple mono- or cyclic nucleotide.

Both the ^{1}H and ^{31}P NMR spectra were observed to change dramatically with time. In the proton spectrum, all the peaks initially observed decreased in intensity and new absorptions occurred. Likewise, in the phosphorus spectrum the initially observed peak decreased in intensity and a new peak appeared at -0.07 ppm downfield from the OPA reference. In addition, changes were observed in the ultraviolet spectra of 4 in both dimethyl sulfoxide and buffered aqueous solution. In dimethyl sulfoxide, 4 initially had a UV maximum at 283 nm but with time the absorbance maximum not only decreased in intensity but also shifted to longer wavelengths. After approximately three hours the maximum had shifted to 290 nm and the absorbance at the maximum was 70% of its original value. The wavelength of maximum absorbance and the broad shape of the curve at the end of three hours were both very similar to that obtained for the nucleoside (1) in DMSO (288 nm). In 0.037 M phosphate buffer (pH 7.5) comparable results were obtained. The UV maximum of 4 in this case initially occurred at 278 nm, but with time it shifted to shorter wavelengths and decreased in intensity. After three hours the maximum absorbance appeared at 275 nm and was 88% of the original value. At pH 7, the monophosphate (3) has a UV maximum at 275 nm.

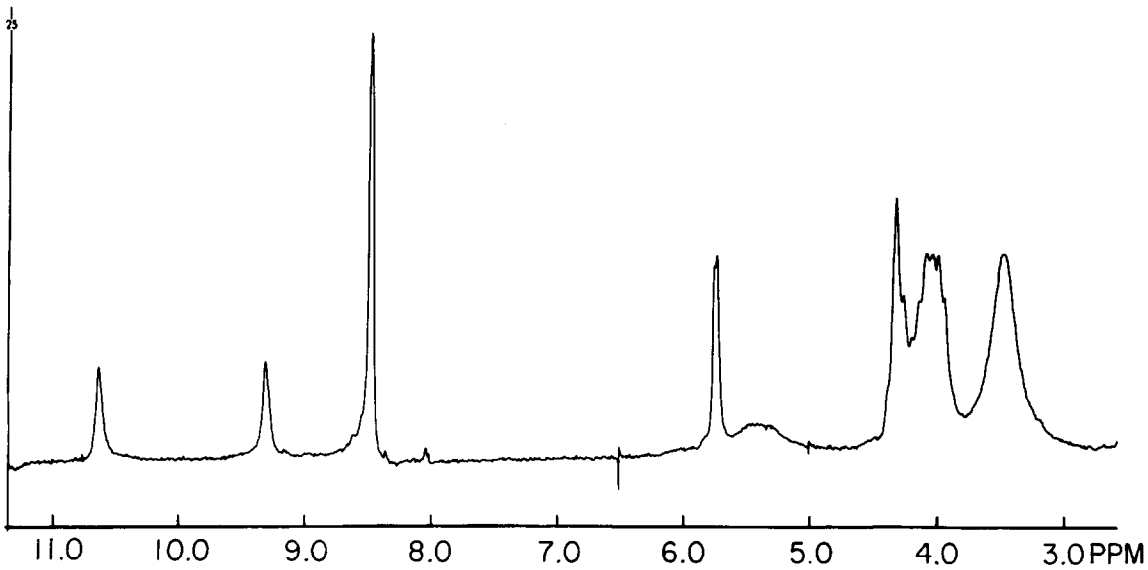


Figure 1. 100 MHz ^{1}H NMR spectrum of 3-(β-D-ribofuranosyl)adenine 9,5'(P)-cyclic phosphonate in DMSO-d_6 obtained in the Fourier transform mode.

Further support for the structure of compound 4 was obtained from the results of its reaction with aqueous ammonia. In concentrated aqueous ammonia at room temperature, 4 rapidly reacted to yield a single product which was conclusively identified as 3-(β-D-ribofuranosyl) adenine 5'-phosphoramidate (5). In addition to having the correct microanalysis,[7] the identity of 5 was confirmed by preparing an authentic sample from 3 according to the general procedure of Michelson.[10] After ion-exchange chromatography, both samples were identical by ^{1}H NMR, IR, UV, chromatography and electrophoresis. In dilute solutions of aqueous ammonia, two products were observed -- the major product again was the 5'-phosphoramidate (5) while the minor product was identified by chromatography as the monophosphate (3).

The cyclic phosphonate, in some respects, resembles the nucleoside 5'-phosphorimidazolidates.[11] The phosphorimidazolidates are synthetically-useful intermediates since the imidazole moiety can be displaced by a variety of nucleophiles. The major difference here, of course, is that the heterocyclic base moiety of 4 would serve as the leaving group. Since ammonia in aqueous solution reacts preferentially at the phosphorus atom of 4 to yield 5, it appears likely that other nucleophiles will react specifically at the same position. This area of research as well as other aspects of this novel compound are currently being explored and will be reported at a later date.

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