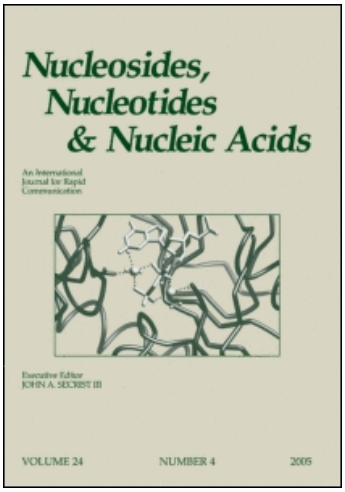

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Conversion of Nucleosides to Cyclic Dinucleoside Dipyrophosphates

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CONVERSION OF NUCLEOSIDES TO CYCLIC DINUCLEOSIDE DIPYROPHOSPHATES

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ABSTRACT : Treatment of 2'-deoxynucleosides with $POCl_3$ and DMF leads to the formation of the title compounds as major products.

INTRODUCTION

Most methods for the preparation of nucleoside di- and triphosphates involve the preparation of activated nucleoside monophosphates followed by substitution of the leaving group by a monophosphate or diphosphate[1]. These methods often require two or more steps and carefully controlled reaction conditions. On the other hand, relatively simple one-flask syntheses also can be achieved by using $POCl_3$ and alkylammonium phosphate salts[2,3]. A typical procedure for the synthesis of a nucleoside di- or triphosphate is by the *in situ* preparation of nucleoside phosphorodichloridate, known as a Yoshikawa intermediate[4], using $POCl_3$, followed by addition of a DMF solution of an alkylammonium phosphate or pyrophosphate salt. This method has also been widely applied for the preparation of many nucleotide analogues[3]. The reported yields for nucleotides vary widely between 20 % and 80 %.

In this paper, we report an unusual product encountered while following a standard procedure for nucleotide synthesis from the nucleoside 2'-deoxycytidine and its 2'-(fluoromethylene)-analogue. This product is a cyclic dinucleoside dipyrophosphate (FIG.1).

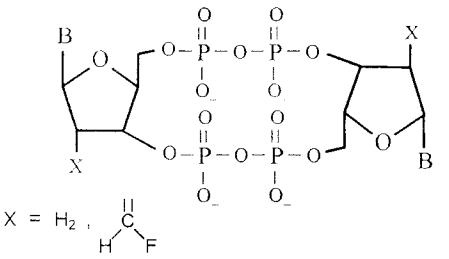

Figure. 1

In our system, this cyclic compound was the major product, while the expected product, the nucleoside 5'-diphosphate, was formed in minor quantities. Knorre and his colleagues[5] had already described the formation of these cyclic compounds under other conditions, but had not succeeded in isolating them.

RESULTS AND DISCUSSION

The presence of this material was first observed by its electrophoretic behavior ; it migrated more rapidly than picrate at both pH 4.5 (R_p = 1.5) and at pH 7.9 (R_p = 2.0). Since the cytosine residues will be half-protonated at pH 4.5, these data suggest no change in ionization of the phosphate groups over this pH range. The R_p values suggest a net charge of 4- at pH 8 since a nearly identical mobility is shown by ATP at this pH (TABLE 1). The other evidence suggesting the structure shown in FIG.1 is as follows : a) hydrolysis with barium hydroxide gave 2'-deoxycytidine-3',5'-diphosphate as the only product ; b) the negative ion FAB-MS spectrum using glycerol as matrix showed the molecular ion for the tetrasodium salt of the product from 2'-deoxycytidine at 825 (M-1) ; c) the ^{31}P nmr spectrum showed a pair of doublets in the range -11 ~ -12 ppm. This chemical shift range is characteristic of the cyclic dinucleoside dipyrophosphate[5] and contrasts with the chemical shift for related compounds as shown in TABLE 1. The isomeric cyclic molecule linked 3',3' and 5',5' (rather than 3',5') has, by contrast, a pair of singlets in the same chemical shift range[5] ; d) elemental analysis gave satisfactory values for carbon and hydrogen ; e) the uv spectrum shows the expected λ_{max} at 270 nm with an extinction coefficient of 15,300 $M^{-1}cm^{-1}$. Attempts to carry out enzymatically catalyzed hydrolysis using snake venom phosphodiesterase 1, DNase 1, or nucleoside 3',5'-cyclicphosphodiesterase were unsuccessful.

TABLE 1. ^{31}P chemical shifts and electrophoretic mobilities of nucleotides

Compound	Chemical shift, ppm (in D_2O, pD 7)	R_p (mobility relative to picrate, at pH 4.5)
5'-CMP	+ 3.99 [a]	0.3
5'-CDP	-11.13, -6.94 [b]	0.7
5'-CTP	-7.25, -11.51, -22.77 [b]	1.2
5'-ATP	-7.33, -11.45, -22.66 [b]	1.45
Deoxycytidine 3',5'-diphosphate	+ 3.5, + 4.1	0.9

[a] D. G. Gorenstein, A. M. Wyrwicz & J. Bode, *J. Am. Chem. Soc.*, 1976, **98**, 2308.
[b] R. J. Labotka, T. Glonek & T. C. Myers, *J. Am. Chem. Soc.*, 1976, **98**, 3699.

The mechanism of formation of this molecule presumably proceeds through a Vilsmeier-Haack reagent acting on the nucleoside 3',5'-diphosphate* to produce, preferentially, the meta phosphate intermediate at the 5'-position[6]. Self-condensation of this species leads to the observed product (FIG.1).

EXPERIMENTAL

(E)2'-(fluoromethylene)-2'-deoxycytidine was supplied by Marion Merrell Dow Inc. Anhydrous DMF was an Aldrich product. Trimethylphosphate and $POCl_3$ were freshly distilled before use. Paper electrophoresis was performed on Schleicher & Schuell 589 white ribbon paper using 0.1 M ammonium formate buffer (pH 4.5) and 0.05 M ammonium bicarbonate buffer (pH 7.9) at 1.2 $kVcm^{-1}$ for 1~1.5 hours. Column chromatographies were performed on Sigma DEAE Sephadex A-25, Dowex 50W (50X8-200) and Sephadex G-10 using 30 x 2.5 cm, 30 x 1 cm and 45 x 1.7 cm columns. TLC was carried out on Silica Gel 60F254 (Aldrich) on aluminum plates with UV detection. ^{31}P NMR spectra were recorded at 121.5 MHz (Bruker MSL-300). Negative ion FAB-MS were obtained using a Finnigan MAT-900. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. Microanalyses were performed by Galbraith Analytical Laboratories, Knoxville, Tenn.

Phosphorylation of 2'-deoxycytidine

2'-Deoxycytidine (0.5 g, 2.2 mmoles) was dissolved in 5 ml of trimethylphosphate under nitrogen. $POCl_3$ (3 ml, 33 mmoles) was added to the solution which was then stirred

* There is sufficient water in the "anhydrous" reagents to allow formation of this molecule.

for 3 hours at 5 ^{0}C. DMF (10 ml) was added dropwise and the solution stirred for 1 hour. Ice water (50 ml) was added and the mixture neutralized with 1 N NaOH. The trimethylphosphate solution was then extracted with ethyl ether (3 x 60 ml) to remove trimethylphosphate. A 5 ml portion (10 % of the total) of the aqueous solution was applied to a DEAE ion exchange column (30 x 2.5 cm) equilibrated with 0.01 M NH_4HCO_3. It was eluted with a linear gradient (0.01 M to 1.2 M). We observed 3 minor components and one major peak eluting last. The major peak was collected and evaporated to give a white solid. The solid was again dissolved in 3ml of water and passed through a Sephadex G-10 column (45 x 1.7 cm) using 0.05 M NH_4HCO_3 solution (20 ml / hour). Two peaks were observed in a 90 : 10 ratio. The first peak (major) was collected and freeze-dried to give a white solid (7 mg). The same procedure was repeated for the rest of the aqueous solution in 10 % aliquots. The white solid was converted to the potassium salt using a Dowex 50W column (30 x 1 cm). Freeze-drying gave a total yield of about 70 mg (7.7 %) of the cyclic dinucleoside dipyrophosphate. The compound is not very stable in aqueous solution ; it decomposes to form the nucleoside 3',5'-diphosphate. However, the compound is quite stable in the solid form at -20 ^{0}C. The low yield is the result of three factors : a) lack of optimization ; b) overlapping peaks during chromatography ; c) instability. The elution profile of the initial reaction suggests a crude yield in the range of 30 %.

^{31}P NMR (D_2O) : -11.1 (d, J_{pop} = 14.5 Hz), -11.9 (d, J_{pop} = 11.8 Hz) ; UV : λ_{max} (water) 272 (ε 15,300) ; $[\alpha]_D$ = + 15.4^0 (c 0.13, water) ; Anal. Calcd. for $C_{18}H_{22}O_{18}N_6P_4K_4{\cdot}24H_2O$, C : 16.34, H : 5.33, Found, C : 16.59, H : 5.86 ; R_p (mobility relative to picrate) : 1.5 at pH 4.5, 2.0 at pH 7.9.

We also carried out the reaction with (E)-2'-(fluoromethylene)-2'-deoxycytidine with similar results; that is, the cyclic dimer was the predominant product while our original objective, the 5'-nucleoside diphosphate, was isolated in only about 8 % yield. It gave ^{1}H nmr data in essential accord with those reported by van der Donk et al.[7] We have also recorded ^{13}C and ^{31}P nmr data for this molecule.[8]

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