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THE REACTION OF GUANOSINE WITH TETRAKIS(HYDROXYMETHYL)PHOSPHONIUM CHLORIDE

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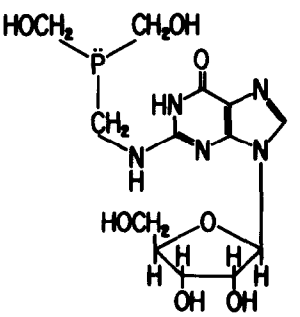
Guanosine is known to react with epoxides (1), lactones (2), and other alkylating agents (3,4) at various positions on the purine ring. The nature and position of the reactions on guanosine have been related to the mutagenicity and carcinogenicity of alkylating agents (5). However, it must be understood that no clearcut relationship exists. Few reactions have been reported to occur at the 2-amino group of guanosine. Formaldehyde (6,7) and glyoxal (8) have been shown to react reversibly at the 2-amino position of guanosine. Glycidaldehyde undergoes an irreversible reaction at the 2-amino position with the formation of a Δ^4-imidazoline ring fused to the pyridine ring of guanosine at the 2-amino and N-1 positions (4).

This report describes the reaction of guanosine with tetrakis(hydroxymethyl)phosphonium chloride (THPC) which gives a 2-amino substituted product, 1, where the phosphonium salt has been reduced to a phosphine. THPC is the chloride salt of a class of phosphonium compounds which are used as chemical flame retardants in certain fabrics. Commercially, the phosphonium salts are copolymerized with ammonia and cross-linked to another phosphonium molecule



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and/or cellulose (9,10). Other studies in this laboratory have been concerned with the carcinogenicity and tumor-promoting properties of THPC and the possible formation of a known human carcinogen, bis(chloromethyl) ether from THPC (11,12).

Guanosine was reacted with THPC in phosphate buffer (0.1M) adjusted to pH 6.5 at 100°C. A precipitate formed after 30 minutes and the pH of the supernatant decreased to 6.0. The yield of 1 was 40% after filtration and thorough washing with water.

The I.R. spectrum had an intense new peak at 1580 cm^{-1} (conjugated hydrogen bonded carbonyl) and changes in the fingerprint region. The U.V. absorption maxima in acid, H_2O, and base exhibited a bathochromic shift of approximately 10nm in comparison with the spectra of guanosine.

U.V. Properties of 1

pH	λmax	ε x 10^{-4}
1	267	1.445
7	266 (280)	1.089
13	266	1.536
	226	1.498

On a G-10 Sephadex column (1.6 x 75 cm), 1, had an elution volume of 104 ml as compared with 260 ml for guanosine. Elemental analysis (C,H,N,P,Cl) of the recrystallized product is in agreement with the assigned structure. The position of attachment of the phosphorus group to the purine ring and the oxidation state of the phosphorus were determined by ^{1}H, ^{31}P, and ^{13}C nmr spectroscopy.

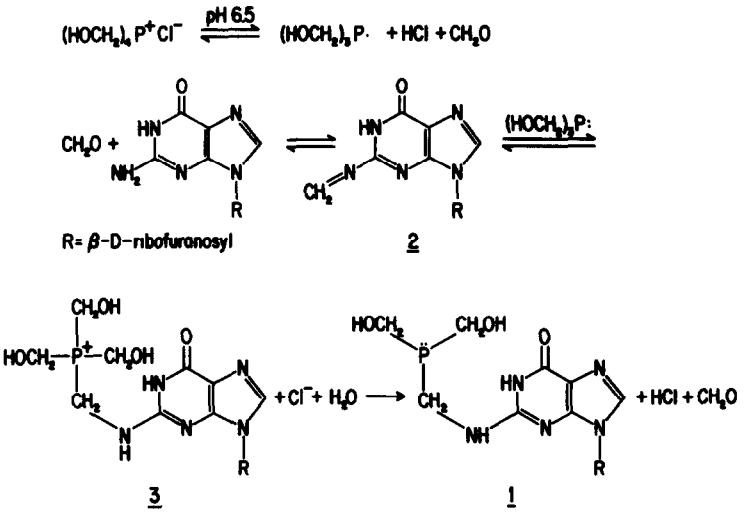
The ^{1}H nmr spectrum in DMSO-D_6 was complex in the ribosyl region, but the N-1 and C-8 protons were still present. The addition of a drop of D_2O exposed a broad peak at 4.75 δ that is probably the 2-amino proton of 1. Guanosines unsubstituted at the 2-amino position normally show these protons at ~ 7 δ (13); the large upfield shift is attributed to the phosphine substituent.

The ^{31}P nmr spectrum of 1 consisted of a single unresolved multiplet at +8.29 ppm (versus H_3PO_4 as external reference). This shift is indicative of a phosphine and compares more closely with tris(hydroxymethyl)phosphine (+25ppm) than with THPC (-27ppm) or tris(hydroxymethyl)phosphine oxide (-48ppm) (14,15).

The ^{13}C nmr (natural abundance in DMSO on a Varian XL100A instrument) was

very informative. Compared with the known spectrum of guanosine (16), two new peaks which correspond to P-$\underline{CH_2}$-NH- and P-$\underline{CH_2}$-OH carbons were observed at δ = 59.9 ppm, J (^{31}P-^{13}C) = 12.8 Hz and δ = 50.8 ppm, J (^{31}P-^{13}C) = 13.4 Hz and integrate at a 1:2 ratio respectively (chemical shifts are relative to TMS). The purine and ribosyl skeletal carbon peaks were not greatly changed and the δ values of them are as follows: C-6 (158.5 ppm), C-2 and C-4 (151.5 ppm), C-8 (137.2 ppm), C-5 (116.9 ppm), C-1' (88.3 ppm), C-4' (85.7 ppm), C-2' (75.0 ppm), C-3' (71.0 ppm), C-5' (62.2 ppm).

The reaction scheme below is consistent with the known behavior of THPC and accounts for the formation of 1:



In this scheme, tris(hydroxymethyl)phosphine, formaldehyde, and hydrochloric acid are formed through a preliminary equilibrium reaction. CH_2O is known to undergo a reversible reaction at the 2-amino function of guanosine forming the Schiff base 2 (6,7). The phosphine reacts with the Schiff base to form a new phosphonium compound 3 which undergoes a conversion to the phosphine product 1 in a manner analogous to the first step. If this reaction occurs with the guanine moiety of DNA in vivo, the known human carcinogen bis(chloromethyl) ether could potentially be formed from HCl and CH_2O liberated during formation of 1 (17). The chloride ion does not play a direct role in the proposed mechanism, and reaction of guanosine with the mixed phosphate/acetate

salt and the oxalate salt of the tetrakis(hydroxymethyl)phosphonium ion also resulted in the formation of 1.

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