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REACTION OF GUANOSINE DERIVATIVES WITH PHOSPHORUS TRICHLORIDE IN ACETONE

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Summary: 2',3'-Di-O-protected guanosine derivatives (Ia and Ib) were allowed to react with phosphorus trichloride in acetone to give the N^2-(1-methyl-1-phosphono)ethylguanosine derivatives (IIIa and IIIb).

The reaction of an isopropylidene ribonucleoside with phosphorus trichloride in acetone in an open vessel afforded, after hydrolysis and deblocking, the ribonucleoside 5'-phosphate.[1] However, the main product of a similar reaction from 2',3'-O-isopropylideneguanosine (Ia) was not guanosine 5'-phosphate (5'-GMP), but a ribonucleoside 5'-phosphate having a new aglycon. This paper deals with the elucidation of the aglycon structure and the reaction mechanism.

Nucleoside (Ia) (400 mg, 1.24 mmol) was allowed to react with phosphorus trichloride (6 ml) in acetone (35 ml) with stirring at 0° for 2.5 hr. After hydrolysis with ice-water, removal of the protective group in acid solution and desalting with charcoal, the reaction mixture was subjected to the repeated Dowex-1 column chromatography[2] to give a new ribonucleoside 5'-phosphate (IIIa), which showed a single UV absorbing spot[3] having $M_{5'\text{-GMP}}$[4] 1.19 on the paper electrogramm.[5] The electrophoretic behavior reveals that IIIa has an additional dissociable group to 5'-GMP. Enzymatic hydrolysis of IIIa with snake venom 5'-nucleotidase (Trimeresurus flavoviridis Hallowell) yielded a new ribonucleoside (IIIb), which migrated almost the same distance as that of 5'-GMP on the paper electrogramm.[5]

The aglycon (IIIc) was isolated from IIIa as colorless prisms (mp 269-271°) by successive treatments such as the glycosidic fission in acidic solution, the Dowex-1 (chloride) column chromatography and recrystallization from water. The formula, $C_8H_{12}N_5O_4P \cdot H_2O$, was confirmed by the elemental[6] and thermogravimetric[7] analyses, and the field desorption mass spectrometry.[8] The UV spectra[9] of IIIc are more similar to those of N^2-methylguanine[10] than those of 1-methyl-,[10] 3-ethyl-[11] or O^6-ethylguanine,[11] respectively (Fig. 1). The analogous reaction of $N^2,O^{2'},O^{3'},O^{5'}$-tetraacetylguanosine with phosphorus trichloride in acetone, followed by the treatment with hydrochloric acid did not yield IIIc, but guanine. Deamination of IIIa with nitrous acid was unsuccessful. These results afford the evidence that both IIIa and IIIc are the N^2-substituted guanine derivatives.

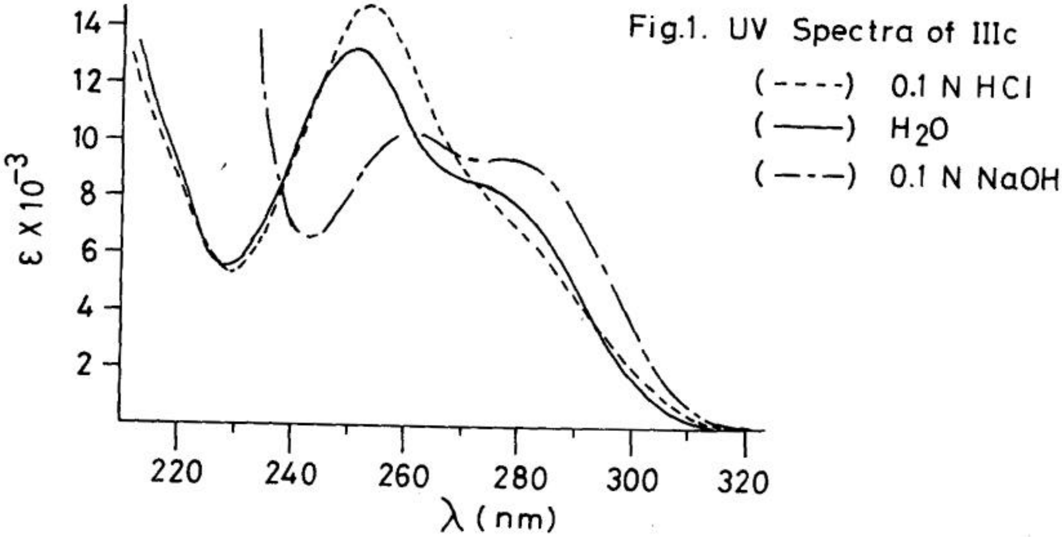

Fig.1. UV Spectra of IIIc

The partial structure of $(HO)_2P(O){-}C(CH_3)_2{-}$ was established by the ^{1}H-NMR[12] (NaOD) [δ 1.60, 1.72 ppm (each 3H, s), J_{HCCP}=11.6 Hz,[13] $2CH_3$], ^{13}C-NMR[12] (NaOD) [δ 25.61 ppm (q, $2CH_3$), 51.62, 57.98 ppm (each s, J_{CP}=144 Hz,[14] $P\text{-}\underline{C}{<}^{C}_{C}$)] and ^{31}P-NMR[15] (NaOD) [δ -20.27 ppm,[16] sept, J_{PCCH}=11.6 Hz,[13] $(HO)_2\underline{P}(O){-}C(CH_3)_2{-}$] signals. The ^{13}C-NMR spectrum showed that the purine skeletal carbons [C-6 (161.18 ppm), C-2 (159.80 ppm), C-4 (153.98 ppm), C-8 (148.55 ppm), C-5 (116.95 ppm)] were existent, and the ^{1}H-NMR spectrum reveals that the C-8 proton (7.78 ppm) is still present. The structure of IIIc was thus determined to be N^2-(1-methyl-1-phosphono)ethylguanine and that of IIIa was identified as 9-(5'-phospho)ribofuranosyl derivative of IIIc. This reaction would present a new route to prepare the aminoalkyl phosphonate from the amino compound.

The analogous reaction of 2',3',5'-tri-O-acetylguanosine (Ib) (1.0 g) with phosphorus trichloride (10 ml) in acetone (100 ml), followed by the treatment with hydrochloric acid and by the Dowex-1 (chloride) column chromatography, afforded IIIc in about 52 % yield. Compound IIIc was also prepared from Ib by the reaction in an atmosphere of nitrogen instead of oxygen. It was thus shown that oxygen in the air is not necessary to the formation of IIIc, that is, oxygen of the phosphonate, $\underline{O}{=}P(OH)_2C{<}$, is not derived from air oxygen in contrast with oxygen of the 5'-phosphate, $\underline{O}{=}P(OH)_2O{-}$,[1] in IIIa.

The addition product of phosphorus trichloride to acetone has been presumed to be II.[17] A plausible mechanism of reaction (Ia→IIIa) would be explained by an initial nucleophilic attack of the 2-amino group to carbon-2 in II, which is synchronized with fission of the C-O bond and elimination of the chloride ion (Chart 1). The reaction of guanosine with tetrakis(hydroxymethyl)-phosphonium chloride has been reported to give the phosphine product.[18] Such a reaction mechanism through the Schiff base could be excluded from the present reaction.

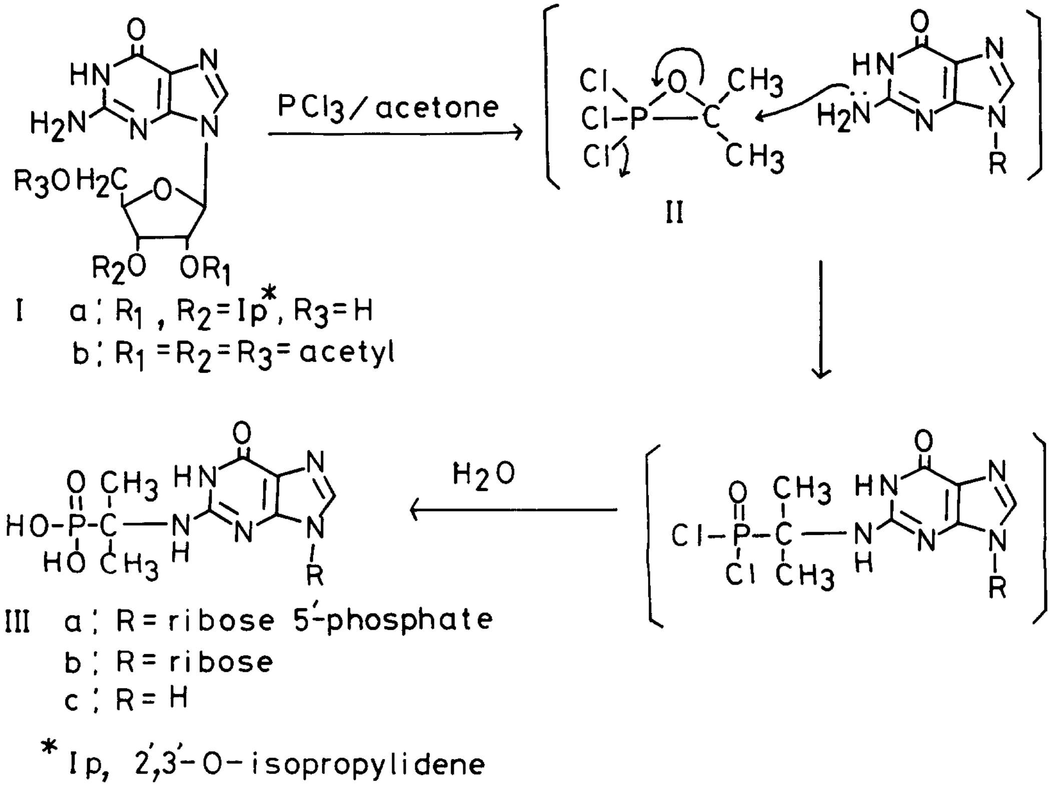

Chart 1

The exceptional reaction of isopropylideneguanosine with phosphorus trichloride in acetone might be attributed to the smaller pKa value (1.6)[19] of the 2-amino group than those (3.5 and 4.2)[19] of the 6-amino group of isopropylidene-adenosine and the 4-amino group of isopropylidenecytidine,respectively. These two amino groups having the larger values would react preferentially with phosphorus trichloride to give $RNHPCl_2$, which would be hydrolyzed to reproduce the parent RNH_2 compound.

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References and Footnotes

1. M. Honjo, R. Marumoto, K. Kobayashi and Y. Yoshioka, Tetrahedron Letters, 3851 (1966).
2. At first, the reaction mixture was applied onto the Dowex-1 (formate) and the peak fractions eluted with 0.25 M $HCOONH_4$ were collected and desalted with charcoal. Second: the Dowex-1 (chloride) and 0.003 N HCl + 0.055 M NaCl. Third: the Dowex-1 (formate) and 4 N HCOOH + 0.2 M $HCOONH_4$.
3. $\lambda_{max}^{pH\ 1}$ 261.9 nm, $\lambda_{max}^{pH\ 7}$ 258.5 nm, $\lambda_{max}^{pH\ 11}$ 261.7 nm.
4. Mobility ratio to 5'-GMP.
5. 0.025 M Phosphate buffer (pH 7.5), 22 V/cm.
6. Anal. Calcd for $C_8H_{12}N_5O_4P \cdot H_2O$: C, 33.00; H, 4.85; N, 24.05; P, 10.67. Found: C, 32.68; H, 4.74; N, 23.79; P, 11.31.
7. The thermogravimetric analysis showed the presence of one mole of water, which was further confirmed to be crystallization water by the X-ray powder diffraction pattern.
8. The field desorption mass spectrum [M^+ (m/e) 273] was measured with a GCMS-6020-ECI spectrometer.
9. $\lambda_{max}^{0.1\ N\ HCl}$ nm (ε): 253.5 (14800), 280 sh (6900), $\lambda_{max}^{H_2O}$ nm (ε): 251.5 (13300), 275 sh (8400), $\lambda_{max}^{0.1\ N\ NaOH}$ nm (ε): 261 (10300), 278 (9400).
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12. The ^1H-NMR (TMS as reference) and ^{13}C-NMR (dioxane as reference) spectra were measured with a Hitachi R-42 FT spectrometer.
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15. The ^{31}P-NMR spectrum (H_3PO_4 as reference) was measured with a R-900 FT NMR spectrometer.
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