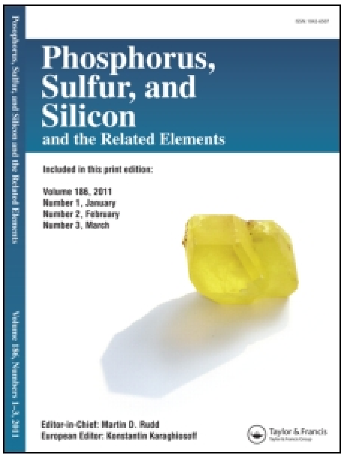

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^{31}P-^{13}C, ^{31}P-^{1}H SPIN-SPIN COUPLING AND STRUCTURAL ASSIGNMENT AND CONFORMATIONAL ANALYSIS OF EPIMERIC THYMIDINE-3'-YL BENZOIN PHOSPHATES

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^{31}P-^{13}C, ^{31}P-^{1}H SPIN-SPIN COUPLING AND STRUCTURAL ASSIGNMENT AND CONFORMATIONAL ANALYSIS OF EPIMERIC THYMIDINE-3′-YL BENZOIN PHOSPHATES

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A pair of epimeric thymidine-3′-yl benzoin phosphates **3** were produced from the ester exchange reaction of oxyphosphorane **1** with thymidine **2**. Their structures were identified by using the ^{1}H, ^{13}C and ^{31}P NMR spectra. The chemical bonds between the ribose 3′-C and α-C atoms to the phosphoryl group were confirmed by ^{31}P-^{13}C and ^{31}P-^{1}H spin-spin coupling. The stereosensitive three-bond coupling constants $^{3}J_{P\text{-}C}$ and $^{3}J_{P\text{-}H}$ in each diastereomer of **3** have been measured, and used for an estimation of trans and gauche conformational analysis. The relative estimated dihedral angle of P-O-C-H was discussed.

Keywords: structural assignment; conformational analysis; thymidine-3′-yl benzoin phosphates

INTRODUCTION

Oxyphosphoranes have been demonstrated as model systems for enzymatic reactions.[1,2] Ester exchange reaction of oxyphosphorane with an unprotected deoxyribonucleoside provided an efficient one-pot phosphorylation procedure for nucleotide synthesis as reported by Zhao *et al.*[3]

Reaction of oxyphosphorane **1** with thymidine **2** generated spiro-oxyphosphorane composed of a five-membered and a six-membered ring, followed by hydrolysis to afford 5′-methyl thymidine mono-phosphate (TMP), dithymidine phosphate (TPT) and unidentified compounds **3** (**Scheme 1**). In this

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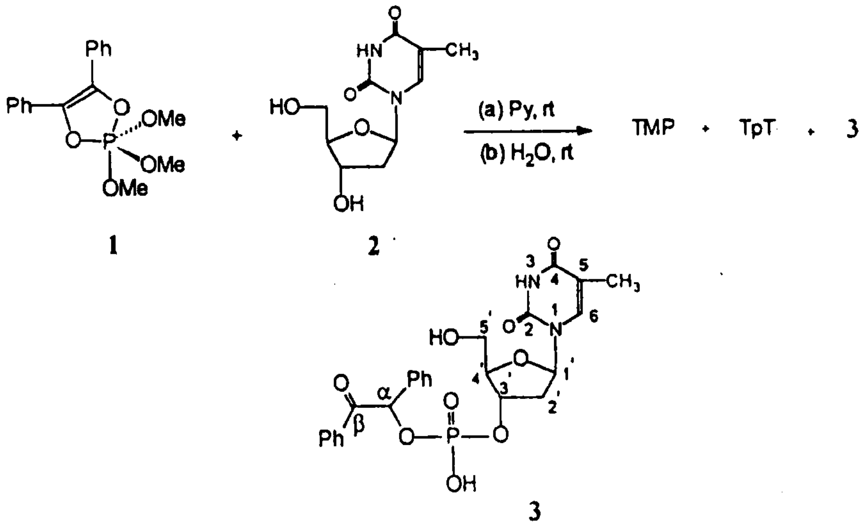


SCHEME 1 Reaction of oxyphosphorane with deoxyribonucleoside.

paper, the structure of **3** was assigned, its conformational analysis was investigated by NMR, and its molecular conformations were studied by measurement of specific ^{31}P-^{13}C, ^{31}P-^{1}H spin-spin coupling constants.

RESULTS AND DISCUSSION

Scalar ^{31}P-^{13}C and ^{31}P-^{1}H spin-spin coupling constants are very sensitive to interactions between the phosphorus atom and vicinal carbon or hydrogen atoms, and can be used to confirm the binding sites of an oxygen atom in a phosphate. The three-bond coupling constants are dependent on their molecular conformation, since the dihedral angles (Φ) HCOP and CCOP depend on the vicinal $^{3}J_{POCH}$ and $^{3}J_{POCC}$ coupling constants, as shown by the general Karplus equation $J(\Phi) = A(\cos^2\Phi) + B(\cos\Phi) + C$. Thus many structural features can be studied by measuring with $^{3}J_{P\text{-}H}$ and $^{3}J_{P\text{-}C}$.[4,5]

Structural Assignment

Compound **3** has a molecular weight at *m/z* 516 as shown by the negative FAB-MS. Both diastereomers of **3** were identified by ^{1}H, ^{13}C, ^{31}P and ^{31}P-^{1}H COSY NMR spectra.

The ^{13}C resonance signals for $-CH_3$, $-CH_2-$, $-\overset{|}{C}H-$, $-\underset{|}{\overset{|}{C}}-$ can be distinguished and identified by a ^{13}C DEPT (135°) NMR experiment (**Fig. 1**). The complete assignment and analysis of ^1H and ^{13}C resonance signals in the base and ribose ring were made based on ^{13}C, ^1H NMR data of thymidine. The chemical shifts and coupling constant values are listed in **Table I and Table II**. A broad singlet signal of compound **3** in the ^{31}P NMR spectrum at 1.10 ppm was observed. This is consistent with the properties of tetracoordinated phosphorus compounds. Due to the phosphoryl bound at 3′-C and α-C, the ^{13}C, ^1H NMR chemical shifts for 3′-C were shifted downfield about 4.0 and 0.36 ppm relative to thymidine, respectively. The resonance peaks for the α-C at 78.78 ppm ($^2J_{P\text{-}C}$ = 4.70 Hz) and 78.61 ppm ($^2J_{P\text{-}C}$ = 4.79 Hz) were split. Similarly, the peaks for the α-H were also split by phosphorus ($^3J_{P\text{-}H}$ = 8.60, 8.66 Hz).

Compound **3** is actually a pair of epimeric thymidine-3′-yl benzoin phosphates (1 : 1 ratio). This is supported by the integration of the ^1H resonance peaks (1 : 1 ratio). Also two peaks in the ^1H NMR at 4.08 and 3.90 ppm were observed for the 4′-C group, and two peaks in the ^{13}C NMR at 85.23 and 85.15 ppm were observed for the 4′-C atom (Fig. 1).

Also, the ^{31}P-^1H heteronuclear two-dimensional correlation spectrum shown that both the 3′-H and the α-H were conjected to the P atom. This provided additional evidence that the phosphate was linked to the 3′-C of thymidine **2** and

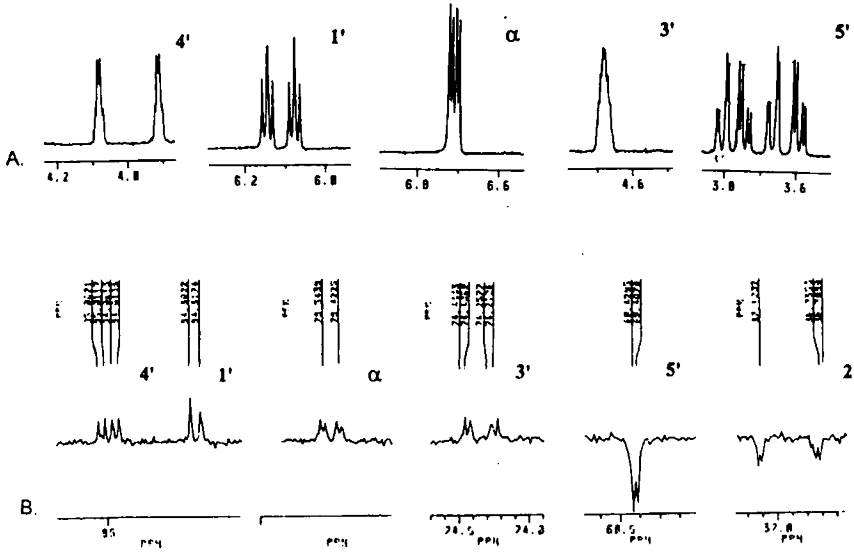


FIGURE 1 NMR spectra of compound **3** a). ^1H NMR spectrum; b). ^{13}C DEPT (135°) spectrum.

TABLE I ^{1}H and ^{13}C NMR data for compound **3** (in D_2O)

Position	δ_H *ppm*		δ_C *ppm*	
1′	6.15 (dd)	6.08(dd)	84.64	84.57
2′	2.15 (m)	2.11(m)	37.28	36.92
3′	4.69 (m)	—*	74.54	74.33
4′	4.08 (m)	3.91(m)	85.23	85.15
5′	3.75 (ddd)	3.63(ddd)	60.62	60.60
2			151.08	151.05
4			165.93	—
5			111.00	110.96
6	7.56 (s)	7.50(s)	136.96	—
α	6.71 (d)	6.71(d)	78.78	78.61
β			198.30	—
Me	1.91 (s)	1.90(s)	11.00	—
Ar	7.33–8.03(m)	—	127.25	128.59
			129.03	133.66
			135.14	

*Not distinguishable from its isomer.

TABLE II ^{31}P-^{13}C and ^{31}P-^{1}H coupling constants (Hz)

J/position	*2′*	*3′*	*4′*	*α*	*β*	*Ph*
$^{2}J_{P\text{-}C}$		5.21, 5.32		4.70, 4.79		
$^{3}J_{P\text{-}C}$	2.99, 2.99		6.40, 6.45		1.00 —	1.50 —
$^{3}J_{P\text{-}H}$				8.60, 8.66		

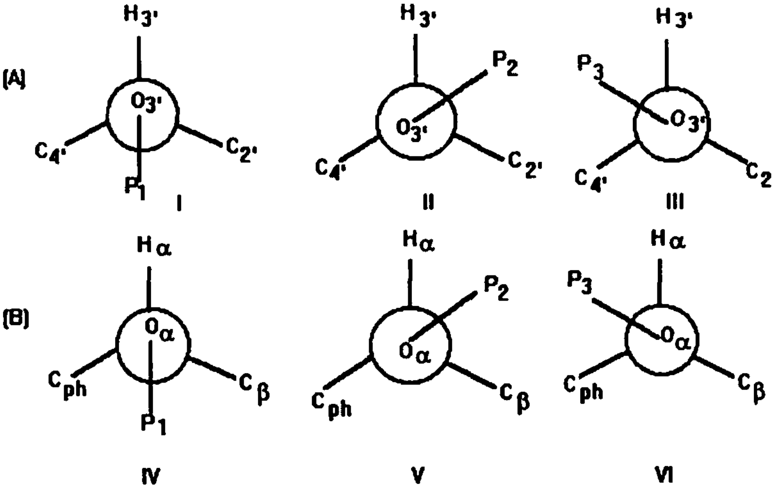
the α-C atom. Moreover the peaks of 2′-C, 4′-C, β-C and Ph-C were split by phosphorus with ^{3}J coupling constants (2.99, 6.40, 1.00, 1.50 Hz) respectively.

Conformational Analysis

There is conformational flexibility about the two C-O bonds ($C_{3'}$-$O_{3'}$, C_α-O_α) with the common phosphorus atom in compound **3**. Generally, only a few of conformers were assumed to be important. The conformational populations were determined by using the observed values of the coupling constants $^{3}J_{P\text{-}C}$. The values of the coupling constants $^{3}J_{P\text{-}C}$ and $^{3}J_{P\text{-}H}$ which related to the associated dihedral angles have been measured and are given in Table II, respectively.

1. Conformation of $C_{3'}$-$O_{3'}$ bond:

The conformations of minimum energy about the $C_{3'}$-$O_{3'}$ bond, which are a weighted average of three constrained and staggered rotamers, are illustrated in Scheme 2 (A). However, it should be noted that there is a possibility of small



SCHEME 2 Classical 60°-staggered rotamer conformations of compound **3**. A. Possible rotational isomers around the $O_{3'}$-$C_{3'}$ bond B. Possible rotational isomers around the Oα-Cα bond.

range of torsion angles centered around some non-classical torsion angles (other than 60°, 180° or 270°). Assuming rapid interconversion between three possible rotamers P_1, P_2 and P_3, which represented the mole fraction of each rotamer, one can calculate their populations from a set of following expressions,[6] where J_g = 2.5 Hz and J_t = 10.0 Hz, which are the three-bond P-C coupling constants when conformer has a gauche ($\Phi = 60°$) or trans ($\Phi = 180°$) orientation, respectively. The $^3J_{P\text{-}C2'}$ values obtained in each epimer of compound **3** were almost equal. Similarly, this is true for $^3J_{P\text{-}C4'}$. Their relative population can be calculated according to the above equations. The ratio of population P_1, P_2 and P_3 is 0.41 : 0.52 : 0.07. This indicated that only two different conformations for P_1 and P_2 exist and their population is almost equal. One favorable arrangement is gauche-gauche conformation for P-$O_{3'}$-$C_{3'}$-$C_{2'}$ group, *i.e.* the P-$O_{3'}$ is gauche to $C_{3'}$-$C_{4'}$ and $C_{3'}$-$C_{2'}$. Another is gauche-trans conformation for P-$O_{3'}$-$C_{3'}$-$C_{4'}$ group, i.e. the P-$O_{3'}$ is trans to $C_{3'}$-$C_{4'}$ and gauche to $C_{3'}$-$C_{2'}$. It is interesting that the calculation values are consistent with the integration ones in ^{1}H NMR spectra.

$$^3J_{P\text{-}C2'} = P_1J_g + P_2J_g + P_3J_t \qquad (1)$$

$$^3J_{P\text{-}C4'} = P_1J_g + P_2J_t + P_3J_g \qquad (2)$$

$$1 = P_1 + P_2 + P_3 \qquad (3)$$

2. Conformation of C_α-O_α bond:

The three minimum energy models for conformation about C_α-O_α system are shown by Newman projections in Scheme 2(B). Table II displayed the values of stereosensitive coupling constants $^3J_{P\text{-}C\beta}$, $^3J_{P\text{-}CPh}$ and $^3J_{P\text{-}H\alpha}$. The relative ratio of possible conformations P_1, P_2 and P_3 was calculated from the J values, equation (1), (2) and (3) and the reference to the range of ^{13}C-^{31}P coupling constants in nucleotides. The estimated ratio of $P_1:P_2:P_3$ equals to 1:0:0. This means that the gauche conformation IV is the dominant conformer. For this orientation, the O_α-P is gauche to C_α-C_β and C_α-C_{Ph}. This suggested that the alone conformation probably arised from the increasing of steric hindrance. In molecular terminal of compound **3**, the carbonyl group with benzene ring (at the β position) are located in a common plane, and have conjugated interaction between them. Moreover, due to oxygenphilicity of phosphate, it is possible that the position of P(O)OH group is near by the carbonyl group and formation of association between two groups would increase the steric hindrance in the molecules.

In addition, according to the Karplus curve, the coupling constant $^3J_{P\text{-}H}$ between the hydrogen and phosphorus of H-C-O-(PO_2) system in the nucleotide in an aqueous solution is 1.5 to 4.5 Hz, when the C-H is gauche to O-P ($\Phi = 60°$), and the value is about 23.5 Hz when the C-H is trans to O-P ($\Phi = 180°$).[4]

By analysis of the above data and the observed vicinal coupling value of $^3J_{P\text{-}H}$ (8.6 Hz), it is possible to estimate the corresponding P-O_α-C_α-H_α dihedral angle to be about 50° or 130°. The former possibility (50°) may be eliminated, because only the gauche conformation existed in this case.

CONCLUSION

Compound **3** was assigned to a pair of epimeric thymidine-3′-yl benzoin phosphates (ratio 1:1) based on its ^{31}P, ^1H, ^{13}C NMR spectra. The conformations were estimated by ^{31}P-^{13}C and ^{31}P-^1H spin-spin coupling. One of the epimers is in a gauche conformation at both P-$O_{3'}$-C_3 and P-O_α-C_α system. The other one has the trans rotamer at P-$O_{3'}$-$C_{3'}$ and a gauche rotamer in the P-O_α-C_α system. The dihedral angle H_α-C_α-O_α-P is nearly 130° in both epimers. Stereosensitive three-bond coupling constants $^3J_{P\text{-}C}$ and $^3J_{P\text{-}H}$ could offer a valuable tool for the conformational analysis.

EXPERIMENTAL SECTION

Reaction and Purification for Products:

One molar equivalent (1.0 mmol) of oxyphosphorane **1** and thymidine **2** dissolved in dry pyridine (8 mL) was stirred under N_2 at 23 °C for 12 h. The solvent was removed at reduced pressure and below 30 °C. Some precipitate was removed by centrifugation, and the aqueous solution was applied to DEAE-cellulose column chromatography, which was eluted with a linear gradient of NH_4HCO_3 buffer. The appropriate peaks were collected and lyophilized respectively to afford TMP in a yield of 63%, TPT in 10% and compound **3** in 26%.

NMR and MS Spectra:

^1H, ^{13}C NMR spectra were obtained on a Bruker AM 500 FT spectrometer. ^{13}C NMR spectra were recorded at 125 MHz under complete proton decoupling and DEPT (135 °) conditions. Proton-decoupling ^{31}P NMR spectra and ^{31}P-^1H heteronuclear two-dimensional spectrum were taken on a Bruker AC-200P spectrometer operating at 81 MHz. The ^{31}P-^1H correlation provided by an XHCORR experiment was recorded with 2048 (t_2) × 512 (t_1) data points. All the measurements were performed at room temperature.

Mass spectra were run on a Finnigan MAT 90 MS double focusing mass spectrometer operated in the negative FAB mode.

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