CONFORMATIONAL STUDY ON RIBONUCLEOSIDE O-PHOSPHONYLMETHYL DERIVATIVES BY ^{1}H NMR SPECTROSCOPY

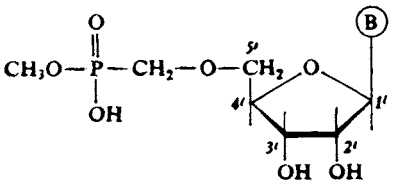
Milena MASOJÍDKOVÁ, Jaroslav ZAJÍČEK, Miloš BUDĚŠÍNSKÝ, Ivan ROSENBERG and Antonín HOLÝ

Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, 166 10 Prague 6

Received October 16th, 1984

Conformational properties of ribonucleoside 5′-O-phosphonylmethyl derivatives have been determined by ^{1}H NMR spectroscopy and compared with those of natural nucleosides and 5′-nucleotides.

O-Phosphonylmethyl derivatives of nucleosides[1] represent novel analogues of natural nucleotides in which the phosphoric acid moiety is bonded to the nucleoside by a methylene bridge-mediated ether bond. Introduction of this grouping does not change the dissociability of the acid residue (important for the interaction with enzymes), and the bond thus obtained is resistant towards chemical hydrolysis and, particularly, towards hydrolysis catalyzed by enzymes of nucleic acid catabolism[1]. Since the sp^3 hybridization of the carbon atom in this group allows in principle a free rotation about the P–C–O bonds, it may be expected that in complexes with enzymes these nucleotide analogues can assume conformations analogous to those of the natural substrates without being able to participate in the reactions at the catalytic site. It has been shown that 5′-isomers of the mentioned type[2] are indeed capable of inter-



I, B = adenin-9-yl
II, B = guanin-9-yl
III, B = inosin-9-yl
IV, B = uracil-1-yl
V, B = 6-azauracil-1-yl
VI, B = cytosin-1-yl

acting with 5'-nucleotidases which they inhibit[1]. Analogues of 5'-triphosphates with a similarly modified α-phosphorus bond inhibit specifically DNA-dependent RNS-polymerase[3] and uridine kinase of leukemic cells[4]. The similarity with natural 5'-triphosphates is indicated also by the fact that the mentioned analogues can transfer the γ-phosphate residue in reaction catalyzed by the latter enzyme[5].

We tried therefore to obtain further information on the conformation of these analogues in solution using ^{1}H NMR spectroscopy. The present work concerns the conformation of the ribose-phosphonate part in the model methyl esters *I* – *VI*. These pyrimidine and purine derivatives, prepared by the previously described procedure[2], were analytically pure and homogeneous according to HPLC.

EXPERIMENTAL

The ^{1}H NMR spectra were measured on a Varian XL-200 (200 MHz) spectrometer at 295 K; concentration about 10 mg of compound in 0·5 ml of solvent. Compounds *I*, *III*, and *V* were dissolved in hexadeuteriodimethyl sulfoxide (DMSO, Aldrich, 99·8% ^{2}H), compounds *II*, *IV*, and *VI* in deuterium oxide (^2H_2O, Aldrich, 99·8% ^{2}H) with tetramethylsilane (TMS) and sodium disilapentanesulfonate (DSS), respectively, as internal standards. The deuterium exchange of hydroxyl and amine protons was effected in hexadeuteriodimethyl sulfoxide by addition of several drops of tetradeuterioacetic acid. Signals were assigned to individual hydrogen atoms on the basis of chemical shifts, multiplicities, and decoupling experiments. The chemical shifts and coupling constants were obtained by first-order analysis from expanded records (2 Hz/cm). Accurate parameters of the sugar part of the molecule were obtained by the simulation procedure using a SPIN program which is a part of the spectrometer program equipment. The fit of the simulated and experimental spectra was ±0·01 ppm and ±0·1 Hz for the δ and J values, respectively.

RESULTS AND DISCUSSION

^{1}H NMR spectra of compounds *I* – *VI* were measured first in hexadeuteriodimethyl sulfoxide. In compounds *II*, *IV*, and *VI* some protons formed strongly interacting spin systems and it was difficult to obtain the NMR parameters. Therefore, these compounds were measured in ^2H_2O, where the dispersion of chemical shifts was more favourable. In order to obtain the most accurate values of chemical shifts and particularly the coupling constants of the sugar part, we performed the simulation analysis of the spectra of *I* – *VI*. To facilitate the analysis, the spectra of the four-spin fragments H-1', H-2', H-3', H-4' and H-3', H-4', H-5', H-5'' were simulated first and the thus-obtained optimized parameters were used as input data for simulation of the complete six-spin system of the ribose protons. The obtained chemical shifts and coupling constants, together with parameters of the other protons in compounds *I* – *VI* are given in Table I.

Signals of protons of the purine and pyrimidine bases occur at the lowest field (δ 8·1 – 8·6 and 5·9 – 8·4, respectively). The ester residue gives two signals: a doublet

Collection Czechoslovak Chem. Commun. [Vol. 50] [1985]

TABLE I

^{1}H NMR parameters of O-phosphonylmethyl derivatives of nucleosides *I*—*VI*

Compound	Solvent	Chemical shifts, ppm											
		H-1′	H-2′	H-3′	H-4′	H-5′	H-5″	H-2	H-5	H-6	H-8	PCH_2	OCH_3
I	DMSO	5·90	4·61	4·23	4·11	3·78	3·71	8·27	—	—	8·56	3·71	3·59
II	^2H_2O	5·90	4·74	4·46	4·30	3·87	3·82	—	—	—	8·36	3·86	3·68
III	DMSO	5·97	4·53	4·20	4·12	3·80	3·74	8·13	—	—	8·57	3·82	3·67
IV	^2H_2O	5·96	4·36	4·31	4·23	3·89	3·78	—	5·92	8·02	—	3·76	3·61
V	DMSO	5·92	4·22	4·00	3·91	3·62	3·50	—	7·48	—	—	3·50	3·46
VI	^2H_2O	5·94	4·35	4·33	4·26	3·95	3·79	—	6·27	8·35	—	3·77	3·60

		Coupling constants, Hz								
		$J_{1',2'}$	$J_{2',3'}$	$J_{3',4'}$	$J_{4',5'}$	$J_{4',5''}$	$J_{5',5''}$	$J_{5,6}$	J_{P,CH_2}	J_{P,OCH_3}
I	DMSO	5·8	4·9	3·3	2·8	4·0	−10·4	—	8·6	10·5
II	^2H_2O	5·5	5·0	4·0	3·2	4·2	−11·2	—	8·6	10·4
III	DMSO	5·0	4·8	4·0	2·5	4·0	−11·3	—	8·6	10·3
IV	^2H_2O	5·1	4·7	4·9	2·6	3·5	−11·3	8·4	8·7	10·4
V	DMSO	3·6	4·8	5·6	3·5	6·2	−10·6	—	8·5	10·3
VI	^2H_2O	3·6	4·9	5·5	2·3	2·6	−11·4	7·9	8·9	10·3

due to the P-CH_2 protons at δ 3·5–3·9 and a doublet of the OCH_3 group at δ 3·4 to 3·7. In both cases the splitting results from the hydrogen–phosphorus interaction.

Of the six protons of the ribose residue, the H-1′ proton forms a markedly separated doublet at the lowest field (δ 5·9–6·0). Signals of the remaining atoms appear at δ 4·75–3·50 in the upfield direction as numbered. Signals of the geminal atoms H-5′ and H-5″ were assigned according to the published convention[6,7], stating that $\delta_{5'} > \delta_{5''}$ and $J_{4'5'} < J_{4'5''}$, H-5″ being gauche with respect to H-4′ and C-3′ atoms in the staggered conformation g_+ (Scheme 1). The non-equivalence of the atoms H-5′ and H-5″ ($\Delta\delta = \delta_{5'} - \delta_{5''}$) is somewhat more marked in the pyrimidine (*IV*–*VI*; $\Delta\delta \approx 0{\cdot}13$) than in the purine (*I*–*III*; $\Delta\delta \approx 0{\cdot}06$) derivatives.

The coupling constants $^3J_{5,6}$ in the pyrimidine bases, as well as the phosphorus–hydrogen coupling constants $^2J_{P,CH_2}$ and $^3J_{P,OCH_3}$ of the ester moiety, are constant (about 8·0, 8·5, and 10·5 Hz, respectively) throughout the whole series of compounds (Table I). From the conformational point of view, only the coupling constants of the ribose protons are interesting. Whereas the $J_{2'3'}$ values are for all compounds in the narrow range 4·7–5·0 Hz, more marked differences occur with $J_{1'2'}$ and $J_{3'4'}$. For the purine derivatives *I*–*III* $J_{1'2'}$ is greater than $J_{3'4'}$, whereas the reverse is true ($J_{1'2} < J_{3'4'}$) for the pyrimidine derivatives *IV*–*VI* (except derivative *IV*, where $J_{1'2'} \approx J_{3'4'}$). Long-range coupling constants $^4J_{H,H}$ are generally very small and make the signals only a little broader.

Conformation

Conformational properties of the furanose rings and population of rotamers about the $C_{(5')}$—$C_{(4')}$ bond were determined from the vicinal interactions of ribose hydrogen atoms (Table I). Conformational parameters of the ribonucleotides *I*–*VI* were calculated using the program of Guschlbauer[8]. Using the relationship (*1*) between the five endocyclic torsion angles in the five-membered ring

$$\tau_i = \tau_m \,.\, \cos\left(P + (i - 2) \,.\, 144\right), \quad i = 1, \ldots 5 \qquad (1)$$

the torsion angles $\phi_{H_1'H_2'}$, $\phi_{H_2'H_3'}$, and $\phi_{H_3'H_4'}$ were calculated for the pucker amplitude τ_m in the range 30° to 50° at the 1° steps and for the whole possible range of the phase angle *P* at the 3° steps. The coupling constants $J_{H_1'H_2'}$, $J_{H_2'H_3'}$, and $J_{H_3'H_4'}$ for the corresponding angles $\phi_{H'H'}$ were then calculated by the Karplus equation (*2*)

$$J_{H_iH_j} = A \,.\, \cos^2 \phi_{H_iH_j} + B \,.\, \cos \phi_{H_iH_j} + C \qquad (2)$$

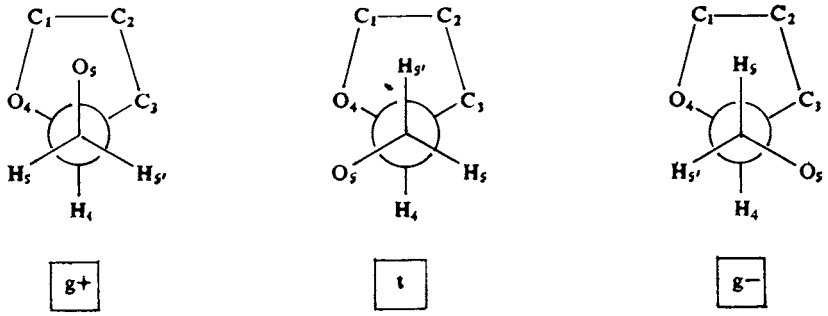
with parameters $A = 10{\cdot}2$, $B = -0{\cdot}8$ and $C = 0$ which for ribonucleosides are known to give the best agreement of pucker amplitude values τ_m and mostly also of the phase angle *P* with those derived from the X-ray data[8]. From the experimental

values of $(J_{1'2'} + J_{3'4'})$ and $J_{2'3'}$ one single value fitting the pucker amplitude τ_m was then determined, along with two possible phase angles ${}^{N}P$ and ${}^{S}P$ (${}^{S}P = 180 - {}^{N}P$), describing two possible conformations in the S or N region of the pseudorotation cycle. For these conformations (characterized by ${}^{S}P$ and ${}^{N}P$) the respective coupling constants ${}^{S}J_{1'2'}$, ${}^{S}J_{3'4'}$ and ${}^{N}J_{1'2'}$, ${}^{N}J_{3'4'}$ were then calculated. Since the observed $J^{exp}_{H_iH_j}$ are time-averaged, the populations ${}^{N}X$ and ${}^{S}X$ of the conformers ${}^{N}P$ and ${}^{S}P$ can be determined from $J^{exp}_{1'2'}$ and $J^{exp}_{3'4'}$ by the relationship (*3*) and (*4*)

$$J^{exp}_{1'2'} = {}^{N}X \,.\, {}^{N}J_{1'2'} + {}^{S}X \,.\, {}^{S}J_{1'2'} \qquad (3)$$

$$J^{exp}_{3'4'} = {}^{N}X \,.\, {}^{N}J_{3'4'} + {}^{S}X \,.\, {}^{S}J_{3'4'} \,. \qquad (4)$$

The thus-obtained values of τ_m, ${}^{N}P$, ${}^{N}X$, ${}^{S}P$, and ${}^{S}X$ for the compounds *I*–*VI* are given in Table II. The populations of the rotamers g_+, *t*, and g_- (Scheme 1) were



SCHEME 1

determined from the experimental values of $J_{4'5'}$ and $J_{4'5''}$ using the published[9] equations (*5*)–(*7*) and are also included in Table II.

$$g_+ = (13 - (J_{4'5'} + J_{4'5''}))/10 \qquad (5)$$

$$t \;\; = (J_{4'5''} - 1{\cdot}5)/10 \qquad (6)$$

$$g_- = (J_{4'5'} - 1{\cdot}5)/10 \qquad (7)$$

As seen from Table II, compounds *I*–*VI* have practically the same pucker amplitude $\tau_m = 40°$. Also the conformational types in the equilibrium ${}^{3}_{2}T \rightleftharpoons {}^{2}_{3}T$ (or ${}^{3}T_2 \rightleftharpoons {}^{2}T_3$) are the same throughout the whole series of compounds. Some differences occur in the population of both types. Whereas the ${}^{2}_{3}T$ type prevails in the purine nucleotides

TABLE II

Calculated conformational parameters of O-phosphonylmethyl derivatives *I*—*VI*

Compound	Conformational equilibrium	Pseudorotational parameters					Rotamer populations		
		τ_m	^NP	^NX	^SP	^SX	g_+	t	g_-
I	${}^3_2T \rightleftharpoons {}^2_3T$	40	3	0·36	177	0·64	0·62	0·25	0·13
II	${}^3T_2 \rightleftharpoons {}^2T_3$	40	9	0·42	171	0·58	0·56	0·27	0·17
III	${}^3_2T \rightleftharpoons {}^2_3T$	41	0	0·44	180	0·56	0·65	0·25	0·10
IV	${}^3T_2 \rightleftharpoons {}^2T_3$	43	13	0·49	167	0·51	0·69	0·20	0·11
V	${}^3_2T \rightleftharpoons {}^2_3T$	41	3	0·61	177	0·39	0·33	0·47	0·20
VI	${}^3_2T \rightleftharpoons {}^2_3T$	40	3	0·60	177	0·40	0·81	0·11	0·08

TABLE III

Calculated conformational parameters of selected nucleosides and 5′-nucleotide monophosphates

Compound[a]	Conformational equilibrium	Pseudorotational parameters					$C_{(5')}$—$C_{(4')}$ Rotamer populations		
		τ_m	^NP	^NX	^SP	^SX	g_+	t	g_-
				Nucleosides					
Ado	${}^3T_2 \rightleftharpoons {}^2T_3$	40	6	0·37	174	0·63	0·66	0·19	0·15
Guo	${}^3T_2 \rightleftharpoons {}^2T_3$	40	9	0·41	171	0·59	0·65	0·18	0·17
Ino	${}^3T_2 \rightleftharpoons {}^2T_3$	38	12	0·40	168	0·60	0·61	0·24	0·15
Urd	${}^3E \rightleftharpoons {}^2E$	40	18	0·58	162	0·42	0·57	0·29	0·14
Z^6Urd	${}^3T_2 \rightleftharpoons {}^2T_3$	38	12	0·62	168	0·38	0·42	0·41	0·17
Cyt	${}^3E \rightleftharpoons {}^2E$	39	19	0·61	161	0·39	0·59	0·28	0·13
				5′-Nucleotides					
AMP	${}^3T_2 \rightleftharpoons {}^2T_3$	39	9	0·39	171	0·61	0·68	0·16	0·16
GMP	${}^3T_2 \rightleftharpoons {}^2T_3$	40	12	0·39	168	0·61	0·57	0·22	0·21
IMP	${}^3T_2 \rightleftharpoons {}^2T_3$	37	9	0·40	171	0·60	0·58	0·21	0·21
UMP	${}^3T_2 \rightleftharpoons {}^2T_3$	40	6	0·42	174	0·58	0·75	0·15	0·10
Z^6UMP	${}^3E \rightleftharpoons {}^2E$	38	15	0·57	165	0·43	0·23	0·46	0·31
CMP	${}_2T \rightleftharpoons {}_3T$	43	3	0·51	183	0·49	0·76	0·14	0·10

[a] Solutions in ^2H_2O; guanosine in N^2H_3.

–III, the pyrimidine nucleotides *IV – VI* exist predominantly in the ${}^{3}_{2}T$ conformation. The preferred orientations of the 5′-O-phosphonylmethyl nucleoside methyl ester follow from the population of rotamers about the $C_{(5')}—C_{(4')}$ bond. As seen from Table II, the rotamer g_+, with *gauche* arrangement of the H-4′, H-5′, and H-5″ atoms and the oxygen atom O-5′ pointing above the furanose ring, largely predominates in all the compounds except *V*. This means that the ester moiety, or at least a part of it, is located above the furanose ring and that purine, and probably also pyrimidine, bases prefer the sterically more favourable *anti*-conformation. A different conformational behaviour was observed with the 6-azauracil derivative: since this compound exists predominantly in the rotamer *t*, there are no steric reasons for a preferential *anti*-orientation of the base.

A very similar conformational behaviour has been found[8] for natural nucleosides whose conformational data are listed in Table III, together with the parameters of natural 5′-nucleotides calculated by the above-mentioned method from the published[10–14] vicinal coupling constants. Comparison of these parameters with those in Table II shows that in the studied solutions the conformation of the ribonucleotide analogues *I – VI* is very probably similar to that of natural 5′-nucleotides.

We are indebted to Dr W. Guschlbauer of CEN de Saclay, France, for making available the program allowing the complete conformational analysis of pentofuranose nucleosides.

REFERENCES

1. Holý A. in the book: *Phosphorus Chemistry Directed Towards Biology* (S. J. Stec, Ed.), p. 53. Pergamon Press, Oxford 1980.
2. Holý A., Rosenberg I.: This Journal *47*, 3447 (1982).
3. Horská K., Rosenberg I., Holý A., Šebesta K.: This Journal *48*, 1352 (1983).
4. Veselý J., Rosenberg I., Holý A.: This Journal *47*, 3464 (1982).
5. Veselý J., Rosenberg I., Holý A.: This Journal *48*, 1783 (1983).
6. Remin M., Shugar D.: Biochem. Biophys. Res. Commun. *48*, 636 (1972).
7. Davies D. B., Rabczenko A.: J. Chem. Soc., Perkin Trans. 2, *1975* 1703.
8. Guschlbauer W.: Biochim. Biophys. Acta *610*, 47 (1980).
9. Davies D. B. in the book: *Progress in Nuclear Magnetic Resonance Spectroscopy* (J. W. Emsley, J. Feeney, L. H. Sutcliffe, Eds), Vol. 12, p. 135. Pergamon Press, Oxford 1978.
10. Lee C. H., Evans D. F., Sarma R. H.: FEBS (Fed. Eur. Biochem. Soc.) Lett. *51*, 73 (1975).
11. Tran-Dinh S., Guschlbauer W.: Nucleic Acid Res. *2*, 873 (1975).
12. Chachaty C., Zemb T., Langlet G., Tran-Dinh S., Buc H., Morange M.: Eur. J. Biochem. *62*, 45 (1976).
13. Wood D. J., Hruska F. E., Mynott R. J., Sarma R. H.: Can. J. Chem. *51*, 2571 (1973).
14. Davies D. B., Danyluk S. S.: Biochemistry *13*, 4417 (1973).

Translated by M. Tichý.