cyclic phosphate rings are in the chair conformation while the furanose rings of 3′,5′-AMP and dibutyryl-3′,5′-AMP are in the 3′-endo-4′-exo conformation and that of 3′,5′-TMP is in the 4′-exo conformation. These conclusions are in general agreement with the crystallographic data for these compounds in single crystals. The similarities in the conformations of 3′,5′-AMP and dibutyryl-3′,5′-AMP suggest that the different biological activities of these compounds do not have a conformational origin.

The relationship between vicinal $^{1}H-^{31}P$ coupling constants and dihedral angle used in the 3′,5′-cyclic nucleotides may be usefully applicable to acyclic nucleotides and oligonucleotides.

A Carbon-13 Nuclear Magnetic Resonance Study of the Conformations of 3′,5′-Cyclic Nucleotides

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Abstract: The carbon-13 nmr spectra of six 3′,5′-cyclic nucleotides have been obtained. The ^{13}C parameters are consistent with the conformations deduced from ^{1}H nmr data. The three-bond carbon–phosphorus couplings give estimates of the trans and gauche couplings for a $^{13}CCOP$ group. The relative utility of $^{1}H-^{31}P$ couplings and $^{13}C-^{31}P$ couplings for conformational analysis is discussed.

In a previous paper,[2] we have investigated the molecular conformation of some 3′,5′-cyclic nucleotides by proton magnetic resonance spectroscopy. Following recent investigations[3-5] of the carbon-13 nmr spectra of nucleosides and nucleotides and the discovery of the usefulness of vicinal $^{13}C-^{31}P$ coupling constants to indicate molecular conformation,[4-6] we have now studied the ^{13}C nmr spectra of some 3′,5′-cyclic nucleotides. The major objectives of this research have been to confirm the molecular conformations indicated by proton nmr, to calibrate the dependence of $^{13}C-^{31}P$ vicinal coupling constants on dihedral angle, and to assess the usefulness of ^{13}C nmr relative to ^{1}H nmr for nucleotide systems. A preliminary report of the data has been communicated.[6]

Experimental Section

The 3′,5′-cyclic nucleotides were obtained from Sigma Chemical Co. and were used as received. The solutions in deuterium oxide were those used previously[2] for the proton nmr study and thus contained 0.1 *M* sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS). The 25.2-MHz ^{13}C nmr spectra were recorded with complete proton decoupling on a Varian XL-100-15 nmr spectrometer in Fourier transform mode. Interferograms were stored in 8K of computer memory, and chemical shifts were measured on 5000-Hz sweep width spectra to an accuracy of ±0.05 ppm. The $^{13}C-^{31}P$ coupling constants were measured from spectra 1000-Hz wide to an accuracy of ±0.25 Hz. A capillary tube concentric with the nmr tube contained the tetramethylsilane (TMS) reference.

Results and Discussion

The ^{13}C chemical shifts and $^{13}C-^{31}P$ coupling constants for the series of 3′,5-cyclic nucleotides[7] studied are given in Table I. The peaks were assigned to specific carbon nuclei by reference to previous research,[3-6] note being taken of the reassignment of the 2′ and 3′ ^{13}C resonances.[4-6] The ^{13}C nmr spectrum of 3′,5′-AMP is shown in Figure 1.

The $^{13}C-^{31}P$ coupling constants are approximately the same in all compounds of the series indicating that the conformations of the ribose and cyclic phosphate rings are similar throughout the series.

It has been demonstrated previously[4-6,8] that vicinal $^{13}C-^{31}P$ coupling constants in nucleotides have a dependence on dihedral angle similar to that of vicinal $^{1}H-^{1}H$[2,9,10] and $^{1}H-^{31}P$[2,10] coupling constants. However, nucleotides studied to date[4,5] have had flexible conformations, and it has only been possible to speculate as to the dihedral angle dependence of such coupling constants.

By analogy with the dihedral angle dependence for $^{1}H-^{1}H$ and $^{1}H-^{31}P$ vicinal couplings and reference to the range of $^{13}C-^{31}P$ vicinal couplings in nucleotides, we would expect a trans $^{3}J_{CP}$ to be large (about 10 Hz) while that of a gauche $^{3}J_{CP}$ should be small (~1–2 Hz). Such vicinal couplings are present in Table I as $J_{H_{2'},P}$

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(2) B. J. Blackburn, R. D. Lapper, and I. C. P. Smith, *J. Amer. Chem. Soc.*, **95**, 2873 (1973).

(3) D. E. Dorman and J. D. Roberts, *Proc. Nat. Acad. Sci. U. S.*, **65**, 19 (1970); A. J. Jones, N. W. Winkley, D. M. Grant, and R. K. Robins, *ibid.*, **65**, 27 (1970); A. J. Jones, D. M. Grant, M. W. Winkley, and R. K. Robins, *J. Amer. Chem. Soc.*, **92**, 4079 (1970).

(4) H. H. Mantsch and I. C. P. Smith, *Biochem. Biophys. Res. Commun.*, **46**, 808 (1972).

(5) I. C. P. Smith, H. H. Mantsch, R. D. Lapper, R. Deslauriers, and T. Schleich in "Proceedings of the Fifth Jerusalem Symposium on Quantum Chemistry and Biochemistry," E. Bergmann and B. Pullman, Ed., Academic Press, New York, N. Y., in press.

(6) R. D. Lapper, H. H. Mantsch, and I. C. P. Smith, *J. Amer. Chem. Soc.*, **94**, 6243 (1972).

(7) Abbreviations used: 3′,5′-AMP, adenosine 3′,5′-cyclic phosphate; dibutyryl-3′,5′-AMP, $N^{6},O^{2'}$-dibutyryladenosine 3′,5′-cyclic phosphate; U, uridine; C, cytidine; G, guanosine; T, thymidine.

(8) A. A. Borisenko, N. M. Sergeyev, E. Ye. Nifant'ev, and Yu. A. Ustynyuk, *J. Chem. Soc., Chem. Commun.*, 406 (1972).

(9) T. Schleich, B. J. Blackburn, R. D. Lapper, and I. C. P. Smith, *Biochemistry*, **11**, 137 (1972), and references therein.

(10) R. D. Lapper and I. C. P. Smith, *J. Amer. Chem. Soc.*, **95**, 2880 (1973).

Table I. Carbon-13 Nmr Data for 3′,5′-Cyclic Nucleotides[a]

Carbon	U	C	A	G	T[b]	Dibutyryl-A[c]
	A. Chemical Shifts (ppm downfield from TMS)					
1′	95.67	96.18	92.69	93.20	86.53	90.56
2′	73.03	73.37	73.47	73.27	35.71	74.93
3′	72.90	72.91	72.91	73.03	76.01[e]	73.64
4′	78.16	78.28	78.48	78.30	77.48[e]	76.97
5′	68.32	68.50	68.54	68.60	68.40	68.23
2	152.22	158.18	153.82	154.89	152.51	153.60
4	167.21	167.53	149.05	152.23	167.33	150.04
5	103.42	97.38	119.52	117.65	112.96	123.89
6	143.36	143.08	156.20	159.86	138.91	152.14
8			140.78	139.19		144.27
	B. Coupling Constants, Hz					
Nuclei						
C1′,P	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2
C2′,P	8.0	7.8	7.8	7.8 (8.3)[d]	8.3	8.3
C3′,P	4.5	4.3	4.3	3.8 (3.3)[d]	4.8[e]	4.5
C4′,P	4.5	4.5	4.5	4.8	4.5[e]	3.8
C5′,P	7.0	7.0	7.0	7.3	7.0	7.3

[a] Compounds as sodium salts in D_2O except 3′,5′-UMP (barium salt). [b] Chemical shift of the 5-CH_3 carbon = 12.72 ppm. [c] Chemical shifts of the dibutyryl carbons are as follows: carbonyl, 176.47 and 176.27 ppm; methylene, 40.08, 36.65, 19.76, and 19.13 ppm; methyl, 14.19 ppm. [d] Uncertainty of 0.5 Hz present due to overlap of multiplets. [e] The assignment of these bands and couplings may be reversed.

and $J_{H_{4'},P}$. The former couplings are large (8.0 Hz), suggestive of trans couplings. Molecular models of 3′,5′-cyclic nucleotides indicate that a trans POC3′C2′ conformational arrangement can be obtained with the cyclic phosphate ring in a chair or twist boat conformation. X-Ray crystallographic studies of 3′,5′-AMP,[11] 3′,5′-UMP,[12] and 5′-methyleneadenosine 3′,5′-cyclic monophosphonate[13] have indicated that the chair form is present in the solid state. Proton nmr studies of the ^1H–^1H and ^1H–^{31}P coupling constants in these compounds have demonstrated that the cyclic phosphate rings of these compounds have the chair conformation in aqueous solution.[2]

In both conformations $^3J_{C_{4'},P}$ is a gauche coupling. The average $^3J_{C_{4'},P}$ over all data obtained (Table I) is 4.5 Hz and is larger than we would expect for such a gauche coupling. Although the paths are not identical, it is reasonable to suppose that the magnitude of $^3J_{C_{4'},P}$ is approximately double that which would be observed for a single path coupling. Hence $^3J_{CP}$ gauche is approximately 2.3 Hz.

In the chair conformation the phosphorus atom is trans to C2′ with respect to rotation about the OC3′ bond. The large ^{13}C–^{31}P couplings observed in the six compounds studied (8.0 ± 0.3 Hz) thus provide the first reasonable estimate for a trans coupling. This might be expected to vary somewhat with the charge on the phosphate group, but preliminary studies of 5′-UMP over the pH range 3–9 yielded a variation in the three bond ^{13}C–^{31}P couplings of less than 0.2 Hz.[14] However, in dinucleoside monophosphates ^{13}C–^{31}P couplings as large as 10 Hz have been observed.[5] Thus, in view of the small range over which the couplings can vary (0–8 Hz), and in the absence of data on other rigid phosphates, the ^{13}C–^{31}P couplings should be applied with caution to conformational analysis. We suggest a simple relationship of the form $^3J_{CP} = J_0 \cos^2 \gamma$, where J_0 is 8.0 Hz and γ is the $^{13}CCOP$ dihedral angle, for this purpose.

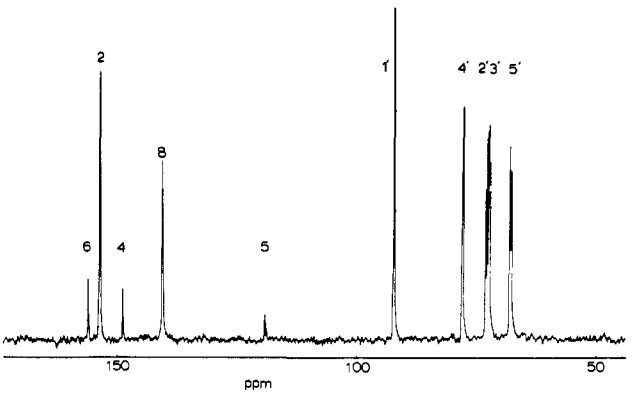

Figure 1. The 25.2-MHz ^{13}C nmr spectrum (with complete proton decoupling) of a 0.15 *M* solution of adenosine 3′,5′-cyclic phosphate (sodium salt) in D_2O, pD 7.2, at 37°. Chemical shifts are in parts per million from external tetramethylsilane.

It is of interest to note that the two geminal ^{13}C–^{31}P couplings in these systems are different in magnitude, $^2J_{C_{4'},P}$ being about 7.0 Hz and $^2J_{C_{3'},P}$ being about 4.3 Hz. This reflects substituent effects and different electron densities along the paths of the couplings.

This study allows us to assess the potential of ^{13}C nmr spectroscopy in relation to ^1H nmr for nucleotides. Because of the smaller range of ^{13}C–^{31}P couplings (0–10 Hz)[4,5,10] compared with that of ^1H–^{31}P couplings (0–22 Hz) and the correspondingly less sensitive dependence of ^{13}C–^{31}P vicinal couplings on dihedral angle, the information to be gained from the ^{13}C–^{31}P couplings is less reliable and is best used to confirm conclusions drawn from accurate ^1H nmr data. The advantage that ^{13}C nmr has over ^1H nmr lies in the larger range of chemical shifts. As can be seen from 3′,5′-UMP, 3′,5′-CMP, and 3′,5′-GMP, where no accurate proton data could be obtained owing to the tight coupling of their proton spin systems,[2] it is possible to use ^{13}C nmr to extend conclusions drawn from ^1H nmr of 3′,5′-AMP, dibutyryl-3′,5′-AMP, and 3′,5′-TMP[2] to other 3′,5′-cyclic nucleotides. To date, therefore, ^1H nmr is more useful in determining conformation of nucleotides than is ^{13}C nmr. ^{13}C nmr is, however, of considerably more use in the case of polymers, such as polynucleotides,[4,5,15] where the proton nmr spectra may well be totally unanalyzable to yield ^1H–^1H and ^1H–^{31}P coupling constants. Owing to the range of ^{13}C chemical shifts, the utility of proton noise decoupling, and the first-order nature of ^{13}C–^{31}P couplings, ^{13}C nmr gives considerable insight into the backbone conformations of polynucleotides, as has already been demonstrated for polyuridylic acid.[4,5,15] With further studies on ^{13}C–H and ^{13}C–^{13}C couplings, ^{13}C nmr may reach the level of reliability enjoyed by ^1H nmr for determining conformations.

Conclusion

The three bond ^{13}C–^{31}P couplings for a series of 3′,5′-cyclic nucleotides indicate that all members of the

(11) K. Watenpaugh, J. Dow, L. H. Jensen, and S. Furberg, *Science*, **159**, 206 (1968).
(12) C. L. Coulter, *ibid.*, **159**, 888 (1968); *Acta Crystallogr., Sect. B*, **25**, 2055 (1969); *ibid.*, **26**, 441 (1970).
(13) M. Sundaralingam and J. Abola, *J. Amer. Chem. Soc.*, **94**, 5070 (1972).
(14) B. J. Blackburn and I. C. P. Smith, unpublished data.
(15) G. Govil and I. C. P. Smith, *Biopolymers*, in press.

series have similar conformations of the cyclic phosphate and ribose rings. This conclusion is consistent with the ^1H nmr data,[2] which showed that the cyclic phosphate ring is locked in the chair conformation. The conformational information obtainable from ^{13}C chemical shifts and $^{13}C-^{31}P$ coupling constants is shown to be less reliable than that available from proton nmr data in the case of mononucleotides. In larger molecules such as dinucleoside monophosphates and polynucleotides[4,5] the ^{13}C nmr data are more useful.

A ^{13}C and ^1H Nuclear Magnetic Resonance Study of the Conformations of 2′,3′-Cyclic Nucleotides[1a]

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Abstract: The ^1H nmr spectra of the 2′,3′-cyclic nucleotides of uridine, cytidine, adenosine, and guanosine were analyzed to second order to yield $^1H-^1H$ and $^1H-^{31}P$ coupling constants. Fourier-transformed ^{13}C nmr spectra yielded $^{13}C-^{31}P$ coupling constants. The coupling constants indicate that the ribofuranose and cyclic phosphate rings are in rapid equilibrium between puckered forms and that the average conformations do not have planar ribofuranose rings. In the conformational equilibria the pyrimidine nucleotides tend toward the 3′-endo (2′-exo) conformers, that of adenosine tends (less) toward the 2′-endo (3′-exo) conformers, whereas that of guanosine manifests no significant preference for any of the principal conformers. Increasing the temperature from 30 to 75° decreases the observed conformational preferences. The exocyclic hydroxymethyl groups of the pyrimidine nucleotides demonstrate a preference for the unsymmetrical trans–gauche (or gauche–trans) rotamer, whereas those of the purine nucleotides prefer the gauche–gauche rotamer. The conformations of these compounds in aqueous solution are more flexible than in the solid state where rigid planar ribofuranose rings have been observed.

The decomposition of ribonucleic acids catalyzed by pancreatic ribonuclease involves a two-step mechanism.[2] The first step is cleavage of phosphorus–oxygen bonds in the backbone chain of the ribonucleic acid by transesterification to yield 2′,3′-cyclic nucleotides; the second is hydrolysis of the cyclic nucleotides to yield specifically 3′-ribonucleotides. The degradation of ribonucleic acids by alkali, although believed to occur by a similar two-step mechanism, is less specific and yields mixtures of 2′- and 3′-ribonucleotides.[3]

Recent studies have investigated the mechanism of this enzymatic decomposition[4,5] with a special interest in the stereochemistry of the second step of the reaction[4] and the enthalpy of hydrolysis of various 2′,3′-cyclic nucleotides.[6] The conformations of the 2′,3′-cyclic nucleotide intermediates and the influence of stereochemistry on the ribonuclease degradation of ribonucleic acids is not fully understood.

Only limited X-ray crystallographic data related to the 2′,3′-cyclic nucleotides are available.[7,8] Since such data are necessary for static conformations, they may not always reflect the situation in solution. We have examined the conformations of several 2′,3′-cyclic nucleotides (2′,3′-UMP, 2′,3′-CMP, 2′,3′-AMP, 2′,3′-GMP[9]) in aqueous solution with the aid of proton and carbon-13 nmr spectroscopy. Of particular interest are the conformations of the furanose and cyclic phosphate rings; the $^1H-^{31}P$ and $^{13}C-^{31}P$ coupling constants are particularly helpful in this case. A preliminary report of this work has appeared.[10]

Experimental Section

The 2′,3′-cyclic nucleotides were purchased from Schwarz Bioresearch and Sigma Chemical Co. and were used without further purification. Whenever possible the nucleotides were studied as their sodium salts, although the barium salt of 2′,3′-UMP and the pyridinium salt of 2′,3′-GMP were studied, the latter being converted to the pyridinium salt because of the insolubility of the barium salt. The internal reference, sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS), was a product of E. Merck, Germany. The nucleotides were 0.15 *M* in D_2O containing 0.08 *M* DSS. The pD of each solution was adjusted to 7.2 (pD = pH + 0.4[11]) by the addition of small amounts of dilute NaOD and DCl. The samples were lyophilized from D_2O to reduce the concentration of HDO. Proton and ^{13}C nmr spectra were obtained on a Varian XL-100-15 spectrometer with fast Fourier transform accessory. Proton noise decoupling was utilized in the case of the ^{13}C spectra. The spectra were run at 30° (^1H) and 37° (^{13}C).

Results and Discussion

Complete ^1H nmr spectra were taken at 100 MHz. Assignments to specific hydrogens were made by refer-

(1) (a) Issued as NRCC No. 13165; (b) NRCC Postdoctoral Fellow, 1970–1972, Department of Chemistry, Liverpool, England.
(2) E. A. Barnard, *Annu. Rev. Biochem.*, **38**, 677 (1969).
(3) H. R. Mahler and E. H. Cordes, "Biological Chemistry," Harper and Row, New York, N. Y., 1966.
(4) D. A. Usher, D. I. Richardson, Jr., and F. Eckstein, *Nature (London)*, **228**, 663 (1970).
(5) G. C. K. Roberts, E. A. Dennis, D. H. Meadows, J. S. Cohen, and O. Jardetzky, *Proc. Nat. Acad. Sci. U. S.*, **62**, 1151 (1969), and references therein.
(6) S. A. Rudolph, E. M. Johnson, and P. Greengard, *J. Biol. Chem.*, **246**, 1271 (1971).
(7) C. L. Coulter and M. L. Greaves, *Science*, **169**, 1097 (1970).
(8) W. Saenger and F. Eckstein, *J. Amer. Chem. Soc.*, **92**, 4712 (1970).
(9) Abbreviations used: 2′,3′-UMP, uridine 2′,3′-cyclic monophosphate; C, cytidine; A, adenosine; G, guanosine.
(10) (a) I. C. P. Smith, H. H. Mantsch, R. D. Lapper, R. Deslauriers, and T. Schleich in "Proceedings of the Fifth Jerusalem Symposium on Quantum Chemistry and Biochemistry," E. Bergmann and B. Pullman, Ed., Academic Press, New York, N. Y., in press; (b) R. D. Lapper, H. H. Mantsch, and I. C. P. Smith, *J. Amer. Chem. Soc.*, **94**, 6243 (1972).
(11) P. K. Glascoe and F. A. Long, *J. Phys. Chem.*, **64**, 188 (1960).