ditional support for the conclusion that changes in the 11β-hydroxy acidity cannot completely explain relative glucocorticoid activities. Moreover, Devine and Lack[20] have shown that the hydrogen bonding ability of the hydroxyl proton of 9α-substituted 11β-hydroxyprogesterone derivatives decreases in the order F ≃ Cl > Br > H. If the cortisols behave similarly, there must be additional factors explaining the greater glucocorticoid activity of cortisol relative to 9α-bromocortisol.

Changes in the 11β-OH proton acidity will, however, affect processes other than hydrogen bonding. Thus Bush[21] has pointed out that by contrast to cortisol, which is extensively metabolized to the 11-ketone (cortisone), no C-11 ketone metabolites are found following administration of 9α-fluorocortisol. All of the metabolites are in the reduced form at C-11. This alteration in stability of the C-11 hydroxyl group must also be influenced by electron density changes in the C-11 OH group. Again, Wolff and Hansch[9] have found that the π parameter for 9α substituents is important in predicting biological activity. This is also evidence for an additional mechanism in activity enhancement, since the changes in π could have no discernible effect on the acidity of the 11β-hydroxy group.

Duax, *et al.*,[15] have shown that a major difference between the crystal structure of 9α-fluorocortisol and cortisol lies in the bowing of the A ring toward the α face of the substituted compound. As can be seen from the present electron density results, the largest density changes are in the A ring, but except for the changes at C-4, the shifts in the A ring of 9α-fluoro- and 6α-fluorocortisol are not necessarily in the same direction. These changes at C-4 may well represent the means by which C-9 substituents influence the stereochemistry and decrease of metabolic reduction at the C-4–C-5[21] double bond and could be responsible in part for the observed activity changes.

Although the geometrical distortion induced by 9α-substitution may also be responsible for an enhanced binding of the 3-keto group to the receptor, the changes seen in 6α-fluoro- and 9α-fluorocortisol relative to cortisol do not allow the conclusion that this effect alone explains the observed relative activities.

Conclusions

These calculations have enabled us to conclude that "long-range" effects in neutral steroids (either affecting reactivity or the proclivity for donor–acceptor complex formation) may be due mainly to conformation transmission (both *steric* and *electronic*). This situation is of importance in the interpretation of much of the kinetic and biological data on substituted steroids previously reported. In the case of the biological activity of corticoids, long-range effects between C-9 and C-4, and between C-6 and C-11, appear to be involved in the production of activity enhancing effects.

Interesting areas for future research will be X-ray and CNDO/2 examination of such electronegative substituents as 9α-bromo, 9α-hydroxy, and 9α-methoxy and such electron-donating substituents as 9α-methyl, all of which lead to only weakly active compounds. Another question raised by these results relates to the distortion of substrate bond angles and enzyme–substrate interactions[22] which has been advanced as a mechanism for enzymatic catalysis through stabilization of the transition state. It is clear that a CET mechanism could also be operative in this case and that the catalytic effect could be due not only to geometrical distortion but to changes in charge density at the reaction center which make the reaction proceed more rapidly.

(20) A. B. Devine and R. E. Lack, *J. Chem. Soc.* C, 1902 (1966).
(21) I. E. Bush and V. B. Mahesh, *Biochem. J.*, **69**, 9 (1958).
(22) For a discussion, see W. F. Jencks, "Catalysis in Chemistry and Enzymology," McGraw-Hill, New York, N. Y., 1969, pp 294–319.

A Proton Magnetic Resonance Study of the Conformations of 3′,5′-Cyclic Nucleotides

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Abstract: Complete analyses of the ^1H nmr spectra of the hormonal messenger 3′,5′-cyclic adenosine monophosphate, its dibutyryl derivative, and 3′,5′-cyclic thymidine monophosphate are presented. The ^{31}P–^1H and ^1H–^1H vicinal couplings are consistent with rigid phosphate and ribose ring structures. The phosphate rings are locked in the chair conformation, and the ribose rings are best described as 3′-endo-4′-exo (3′,5′-AMP and dibutyryl-3′,5′-AMP) and 4′-exo (3′,5′-TMP). The different biological activities of 3′,5′-AMP and its dibutyryl derivative cannot be ascribed to conformational differences in the ribose or phosphate rings. These conformations are consistent with those found in the solid state by X-ray crystallography. The measured ^1H–^{31}P couplings are used to formulate an expression for the dependence of such couplings upon dihedral angles.

Recently, considerable attention has been directed to the elucidation of the conformations of nucleosides and nucleotides by nuclear magnetic resonance spectroscopy.[2–4] This work has been facilitated by

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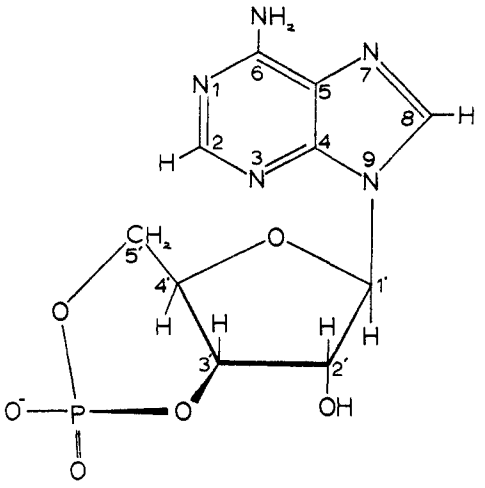

Figure 1. Structure of adenosine 3′,5′-cyclic phosphate.

the advent of spectrometers with larger magnetic fields, improvements in decoupling techniques, and the use of computers to aid in spectral analysis. Previous workers,[5] on the basis of a partial analysis of proton nmr spectra at 60 and 100 MHz with no decoupling experiments, have postulated that the ribose rings of nucleoside 3′,5′-cyclic phosphates have a C3′-endo-C4′-exo conformation. They did not propose a conformation for the cyclic phosphate ring portion of the structure. More recently, a ^{31}P and ^{1}H nmr study[6] has suggested that in the 3′,5′-cyclic nucleotides the cyclic phosphate ring has the twist boat conformation and that the inosine and adenosine derivatives have the base in the anti conformation. The biological importance of one of these compounds,[7] adenosine 3′,5′-cyclic phosphate (3′,5′-AMP)[8] (Figure 1), necessitated that the proposed conformation be verified by a complete spectral analysis and that a more detailed conformational model be proposed. The molecular conformation of 3,′5′-AMP has been elucidated in the solid state by X-ray crystallography,[9] as have the structures of 3′,5′-UMP[10] and 5′-methyleneadenosine 3′,5′-cyclic monophosphonate.[11] For 3′,5′-AMP, two molecules per asymmetric unit were found, one of these having the syn conformation of the base with respect to the ribose ring and the other having the anti conformation. The ribose rings have the same conformation, C4′ being displaced by at least 0.6 Å from the best four-atom plane of the furanose ring in the direction corresponding to the C4′-exo conformation. Both molecules have the cyclic phosphate ring in the chair conformation. 5′-Methyleneadenosine 3′,5′-cyclic monophosphonate, an isosteric analog of 3′,5′-AMP in which O5′ is replaced by a methylene group, was found to be in the syn form and the ribose portion was found to be in the C3′-endo-C4′-exo conformation ($^{3}T_{4}$). 3′,5′-UMP also had two molecules per unit cell, both of which were in the anti range although slightly different. The dihedral angles defining the cyclic phosphate rings of the two molecules are reported as 176 and 174° for ϕ (O5′, O1′), 63 and 60° for ϕ (O5′, C3′), both along the C4′–C5′ bond, and 60 and 62° for ϕ (P, C4′) along the O3′–C3′ bond. In both the communication[10a] and the full paper,[10b] the ribose ring for both molecules was reported as being in the C3′-endo conformation. In a correction[10c] published later, the conformation was stated to be C4′-endo. Sundaralingam,[11] in a table comparing the conformations of cyclic nucleotides, has listed the conformation of 3′,5′-UMP as being C4′-exo-C3′-endo, citing ref 10b. It should be noted that the difference between the conformations commonly referred to as C3′-endo, C4′-exo, C3′-endo-C4′-exo, C4′-exo-C3′-endo are really ones of degree rather than of kind. The conformation C4′-endo would be regarded as being of a different kind. Furthermore, since it has been shown recently that the conformations determined in the solid state do not necessarily correspond to those in solution,[2] a thorough conformational study in solution was required.

In general, 3′,5′-AMP is a relatively inert compound when applied to intact cells and tissues, but produces significant effects when applied to broken cell preparations. Frequently diacyl derivatives of 3′,5′-AMP have been found to be less active than 3′,5′-AMP in broken cell preparations but more active when applied to intact tissues. This is often explained on the basis that these derivatives penetrate cell walls more rapidly than 3′,5′-AMP, but there is no direct evidence for this.[7] It was considered worthwhile to study the conformation of one of these acyl derivatives, dibutyryl-3′,5′-AMP,[8] to compare it with that of 3′,5′-AMP, since a difference in conformation could be responsible for a difference in reactivity. A cyclic deoxyribonucleotide, thymidine 3′,5′-cyclic phosphate (3′,5′-TMP), was also examined.

Experimental Section

The 3′,5′-cyclic nucleotides were obtained from Sigma Chemical Co. and were used as received. The internal reference, sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS), was obtained from E. Merck, Germany.

The nucleotides were made up in D_2O at a concentration of 0.2 *M* containing 0.1 *M* DSS as internal reference. The pD was adjusted to 7.2 ± 0.1 (pD = meter reading + 0.4) and the solutions lyophilized from D_2O. Spectra were obtained on Varian XL-100 and HR-220 (Canadian 220-MHz NMR Centre, Ontario Research Foundation, Sheridan Park, Ontario) nmr spectrometers. Line positions were measured relative to the internal standard. The spectra were observed at the following temperatures: 30° for the 100-MHz spectra of 3′,5′-AMP and dibutyryl-3′,5′-AMP, 50° for the 100-MHz spectrum of 3′,5′-TMP, and 23° for the 220-MHz spectra.

Results and Discussion

The ^{1}H nmr spectra of 3′,5′-AMP and dibutyryl-3′,5′-AMP were run at 100 and 220 MHz, but in both

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

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(5) (a) C. D. Jardetsky, *J. Amer. Chem. Soc.*, **84**, 62 (1962); (b) M. Smith and C. D. Jardetsky, *J. Mol. Spectrosc.*, **28**, 70 (1968).

(6) M. P. Schweizer and R. K. Robins in ref 4.

(7) G. A. Robison, R. W. Butcher, and E. W. Sutherland, "Cyclic AMP," Academic Press, New York, N. Y., 1971, and references therein.

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

(9) K. Watenpaugh, J. Dow, L. H. Jensen, and S. Furberg, *Science*, **159**, 206 (1968).

(10) (a) C. L. Coulter, *Science*, **159**, 888 (1968); (b) *Acta Crystallogr., Sect. B*, **25**, 2055 (1969); (c) *ibid.*, **26**, 441 (1970).

(11) M. Sundaralingam and J. Abola, *Nature (London), New Biol.*, **235**, 244 (1972); M. Sundaralingam and J. Abola, *J. Amer. Chem. Soc.*, **94**, 5070 (1972).

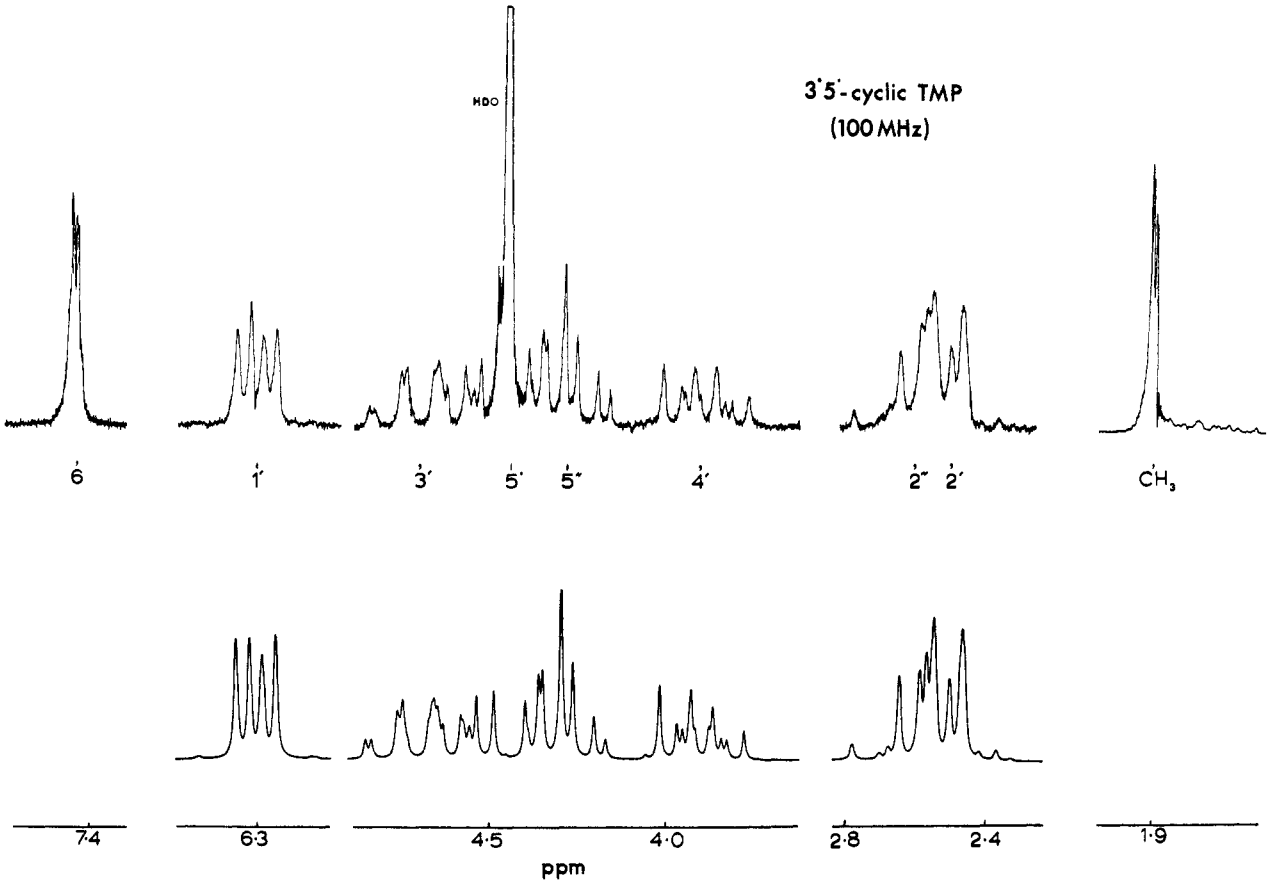

Figure 2. The 100-MHz ^1H nmr spectrum of thymidine 3',5'-cyclic phosphate (0.2 *M* in D_2O, pD 7.2, 50°): upper, observed; lower, simulated.

cases the spectra were too complex to achieve an analysis without reliable estimates of the ^1H–^1H and ^1H–^{31}P coupling constants. The 100-MHz spectrum of 3',5'-TMP was considerably more amenable to analysis; the spin system was less tightly coupled owing to the high-field chemical shift of the protons at position 2'.

Analysis of the Spectra. The 100-MHz proton spectrum of 3',5'-TMP at 50° is shown in Figure 2. The assignment of bands in the spectrum could be made readily in the case of the 2'-CH_2 protons (band at *ca.* 2.5 ppm downfield from DSS), the 5-CH_3 group (1.90 ppm), the 1'-proton (6.3 ppm), and the 6-proton of the base (7.4 ppm), the bands between 3.8 and 4.8 ppm being assigned to the 3', 4', 5', and 5'' protons. Assignments were made by reference to the analyses for deoxyuridine,[2] deoxyadenosine,[12] and thymidine,[12] and by ^1H–^1H and ^{31}P–^1H decoupling experiments. Spectral analysis was carried out by published methods[13–16] using the computer program LAOCNPLT.[17] Determination of the ^{31}P–^1H couplings was aided by examination of molecular models and the knowledge that the trans and gauche $^3J_{^{31}P-^1H}$ are 19–24 and 3 Hz,[18–20] respectively. Other possible ABRX analyses for the spin systems H2'H2''H3'H1' and H5'H5''H4'-H3', and a positive sign for the long-range coupling $J_{1',3'}$, did not yield simulated eight spin spectra consistent with the experimental spectrum. The final iteration of LAOCNPLT on the eight spin system gave an rms error of 0.14 Hz with assignment of 80% of the spectral lines of normalized intensity greater than 0.02. The error on each parameter is *ca.* ±0.05 Hz; chemical shifts and coupling constants are given in Table I.

The 220-MHz ^1H nmr spectrum of 3',5'-AMP is shown in Figure 3. A first-order analysis of the 100- and 220-MHz ^1H spectra of these compounds at 30° was impossible because of tight coupling of their ribose proton spin systems. In both cases it could be seen that the H1'–H2' coupling was small; this allowed assignment of the H2' band. For 3',5'-AMP it should be noted that the H2' band is to high field of the H3' band, in contrast to the normal low-field position in nucleosides but as found for the 3'-nucleotides.[2] Estimates of the proton chemical shifts were made, and the coupling constant values from 3',5'-TMP were used as starting parameters. The computer program LAOCNPLT was used to simulate and iterate upon the spectra at 100 MHz (^{31}P decoupled and coupled) and at 220 MHz (^{31}P coupled) to yield self-consistent parameters and spectra similar to those observed. The final best fit parameters are given in Table I. The values are not as accurate as in the case

(12) R. D. Lapper and I. C. P. Smith, unpublished data.
(13) R. D. Lapper, Ph.D. Thesis, University of Birmingham, Birmingham, England, 1970.
(14) R. J. Abraham and H. J. Bernstein, *Can. J. Chem.*, **39**, 216 (1961).
(15) N. V. Riggs, *Aust. J. Chem.*, **16**, 521 (1963).
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(17) A modified version of LAOCOON III: S. Castellano and A. A. Bothner-By, *J. Chem. Phys.*, **41**, 3868 (1964).
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(19) M. Kainosho, A. Nakamura, and M. Tsuboi, *Bull. Chem. Soc. Jap.*, **42**, 1713 (1969).
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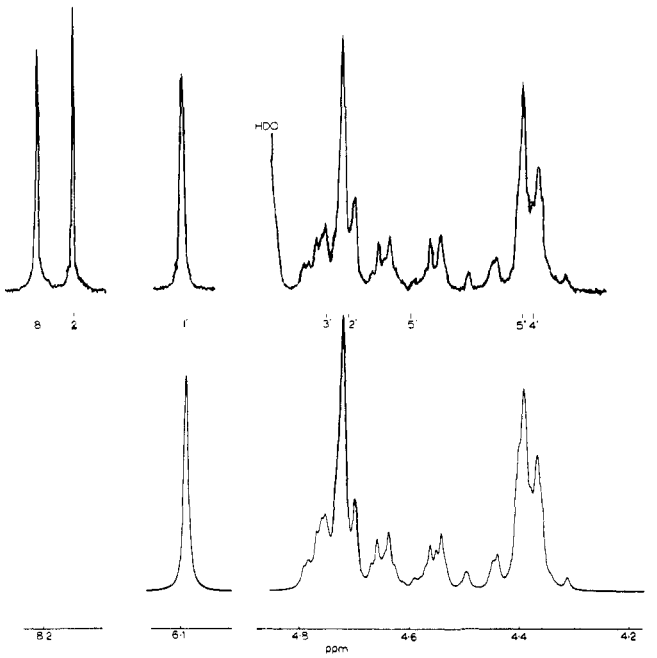

Figure 3. The 220-MHz ^1H nmr spectrum of adenosine 3′,5′-cyclic phosphate (0.2 *M* in D_2O, pD 7.2, 23°): upper, observed; lower, simulated.

Table I. Chemical Shifts[a] and Coupling Constants[b] for 3′,5′-Cyclic Nucleotides

δ	3′,5′-TMP	3′,5′-AMP	Dibutyryl-3′,5′-AMP
1′	6.301	6.090	6.393
2′	2.497	4.712	5.780
2″	2.589		
3′	4.697	4.752	5.281
4′	3.907	4.373	4.380
5′	4.446	4.600	4.626
5″	4.291	4.392	4.469
2		8.141	8.499
6	7.436		
8		8.206	8.700
CH_3	1.903		
J_{HH}			
1′2′	2.4	0.8	0.6
1′2″	8.9		
1′3′	−0.3	*c*	*c*
2′2″	−13.3		
2′3′	8.0	5.2	5.5
2″3′	10.8		
3′4′	9.2	8.9	9.7
4′5′	4.7	4.8	4.6
4′5″	10.6	10.7	10.3
5′5″	−9.5	−9.7	−9.8
6 CH_3	1.2		
J_{HP}			
1′P	0.5	*c*	*c*
2′P	0.5	*c*	*c*
2″P	0.5	*c*	*c*
3′P	1.7	2.0	2.4
4′P	<0.1	*c*	*c*
5′P	20.4	21.4	20.8
5″P	2.2	1.7	1.0

[a] Chemical shifts are expressed in ppm relative to internal DSS. [b] Coupling constants are in hertz. [c] Not measurable.

of 3′,5′-TMP (±0.1 Hz) and, owing to the complex nature and larger line widths in the observed spectra (even at 50°) over those of 3′,5′-TMP, any small ^{31}P couplings to H1′, H2′, or H4′ were unresolvable. Decoupling of ^{31}P did, however, decrease the width of the H1′ and H2′ bands enough to indicate the presence of small ^1H–^{31}P couplings.

The spectral analyses described above were complex. Consequently, a more detailed description of the procedure has been prepared and is available on request.[1c]

Furanose and Cyclic Phosphate Ring Conformations. Let us consider the vicinal ^1H–^{31}P coupling constants, $J_{H5',P}$, $J_{H5'',P}$, and $J_{H3',P}$, given in Table I. The values of $J_{H5',P}$ are those expected for a trans vicinal coupling[18–20] while the values of $J_{H5'',P}$ and $J_{H3',P}$ are those expected for gauche vicinal couplings.[18–20] Examination of molecular models of 3′,5′-cyclic nucleotides indicates that such couplings can be obtained with the cyclic phosphate ring locked in a chair or a twist boat conformation. The cyclic phosphate ring is clearly locked in one conformation or the other since rapid interconversion of the conformers would average $J_{H5',P}$ and $J_{H5'',P}$; $J_{H3',P}$ would not be significantly affected by such interconversion. However, if the ring were in a twist boat conformation, it would possess sufficient flexibility to exist also in the boat conformation; *i.e.*, the POC5′H5′ and POC5′H5″ dihedral angles could vary from 120 to 180° and from 120 to 60°, respectively. From the dihedral angle dependence of such couplings[18–20] one would thus expect time-averaged values for $J_{H5',P}$ and $J_{H5'',P}$ smaller and larger than those associated with $^3J_{trans}$ and $^3J_{gauche}$, respectively. The observed vicinal POCH couplings are, however, consistent with $^3J_{trans}$ and $^3J_{gauche}$ values, and so we must conclude that the cyclic phosphate ring is locked in the chair conformation and not in the more flexible boat conformation.

The vicinal ^1H–^{31}P coupling constants observed in these molecules allow us to estimate, with more certainty than was previously possible,[2] the dihedral angle dependence of H–C–O–P proton–phosphorus vicinal coupling constants in nucleotides. White and Verkade[20] have obtained plots of POCH vicinal coupling constants against dihedral angle for a number of cyclic phosphates, phosphites, and thiophosphates. Their plots for cyclic phosphates consist of points corresponding to dihedral angles of approximately 60, 120, and 180°. It has been shown[21] that vicinal ^1H–^1H coupling constants depend on dihedral angle (ϕ) according to a relationship such as

$$J_{ij} = J_A \cos^2 \phi + J_B \cos \phi + B \qquad (1)$$

where J_A, J_B, and B are constants. In the case of such ^1H–^1H couplings in nucleosides and nucleotides, a reduced expression of the form

$$J_{ij} = J_0 \cos^2 \phi + B \qquad (2)$$

has been found more useful,[2] where ϕ is the H–C–C–H dihedral angle. Because ^{31}P–^1H couplings can be considerably larger than the corresponding ^1H–^1H couplings, we feel that the more general equation (1) is preferable in this case. Hence, taking the average values for J_{gauche} (1.8 Hz) and J_{trans} (20.9 Hz) obtained from the three 3′,5′-cyclic nucleotides studied, and

(21) (a) M. Karplus, *J. Chem. Phys.*, **30**, 11 (1959); (b) *J. Amer. Chem. Soc.*, **85**, 2870 (1963). (c) We should point out that in ref 21a and 21b the functional form suggested was $J = A + B \cos \phi + C \cos 2\phi$. This is equivalent to eq 1 which we find convenient to use.

neglecting B, we obtain

$$^3J_{H,P} = 16.3 \cos^2 \theta - 4.6 \cos \theta \quad (3)$$

Such a relationship must be used with caution and only in a semiquantitative manner since many assumptions are included. For example, (a) J_A and J_B are constant over all ranges of θ (this is not so in the case of J_0, eq 2, for the similar relationship applicable to vicinal $^1H-^1H$ coupling constants); (b) the bond angles in the cyclic phosphate ring are such that the observed trans and gauche $^1H-^{31}P$ couplings reflect angles of 180 and 60°, respectively; and (c) such a relationship can be more generally applied to acyclic systems possessing vicinal $^1H-^{31}P$ coupling constants.

A full discussion of the dihedral angle dependence of vicinal proton–proton coupling constants in furanose rings has been given previously.[2] An examination of molecular models for 3′,5′-cyclic nucleotides indicates that the furanose ring is held rigid by the cyclic phosphate ring. With the cyclic phosphate ring in the chair conformation the number of possible furanose ring conformations is severely limited. Those possible are given in Table II, where the quoted dihedral angles were measured from stick models. The expected $^1H-^1H$ coupling constants associated with a particular conformation were calculated according to the equation

$$J_{ij} = J_0 \cos^2 \phi - B \quad (4)$$

where $J_0 = 9.27$ ($0° < \phi < 90°$), $J_0 = 10.36$ ($90° < \theta < 180°$), and $B = 0.28$ Hz.[2]

Comparison of the proton–proton coupling constants in Table I with the calculated coupling constants in Table II leads us to the conclusion that the furanose rings of 3′,5′-AMP and dibutyryl-3′,5′-AMP are locked in conformations best described as 3′-endo-4′-exo, while that of 3′,5′-TMP is locked in a conformation best described as 4′-exo. It should be noted that in the latter case the observed $J_{2'3'}$ and $J_{2''3'}$ couplings are larger than expected for a 3′-endo-4′-exo or 4′-exo conformation. This may well be explained by the fact that eq 4, having been derived for a ribofuranose ring with C2′ attached to an oxygen atom, cannot be applied to the 2′ part of a deoxyribose ring, where O2′ is replaced by a proton, without slight modification of the J_0 and B constants. The change in electronegativity of substituent at C2′ and the probable change in the geminal H–C–X (X = O or H) angle may well account for the anomalously large coupling constants to H2′ and H2″.

The relationship between the furanose ring and cyclic phosphate ring conformations should be further confirmed by the H4′–H5′ and H4′–H5″ coupling constants, the former being a gauche and the latter a trans vicinal coupling in the inferred ring conformations. The $J_{4'5''}$ couplings are clearly those expected for a trans arrangement. The $J_{4'5'}$ couplings (4.6–4.8 Hz) are, however, larger than might be expected from eq 4 for a gauche conformation (2.0 Hz). The angle indicated by the observed values when using eq 4 is about 43° which further indicates that while the conformational conclusions reached are qualitatively correct, uncertainties occur both in the bond angles in these molecular systems and in the equations used to relate dihedral angle to coupling constant.

The conclusions drawn from the proton nmr results are compatible with the solid-state conformations found for 3′,5′-cyclic nucleotides from X-ray crystallography.[9-11] They are also compatible with the values of three-bond $^{13}C-^{31}P$ couplings in these compounds.[3,4,22] They contrast sharply with those reached for the 2′,3′-cyclic phosphates, which have mobile ribose and cyclic phosphate rings.[3,4,23] It is not possible from the present nmr parameters to speculate as to the conformation of the base about the C1′–N glycosidic bond, and so no comparison between solution and solid state data can be made in this regard. With an accurate analysis of the proton spectrum at hand, this conformational aspect is presently under study[24] using the nuclear Overhauser effect.[25]

We have also examined the proton nmr spectra of 3′,5′-CMP, 3′,5′-UMP, and 3′,5′-GMP but in each case have found it impossible, because of the very tight coupling of their spin systems, to achieve reliable analyses.

Table II. Dihedral Angles of Various Possible Ribose Conformations[a] and the Expected Spin–Spin Coupling Constants for 3′,5′-Cyclic Nucleotides

Atoms	ϕ, deg	J, Hz
O-Endo conformation		
1′,2′	150	7.5
1′,2″	30	6.7
2′,3′	0	9.0
2″,3′	120	2.3
3′,4′	170	9.8
3′-Endo conformation		
1′,2′	100	0.0
1′,2″	20	7.9
2′,3′	40	5.2
2″,3′	160	8.9
3′,4′	170	9.8
4′-Exo conformation		
1′,2′	120	2.3
1′,2″	0	9.0
2′,3′	30	6.7
2″,3′	150	7.5
3′,4′	170	9.8
3′-Endo-4′-exo conformation		
1′,2′	110	0.9
1′,2″	10	8.7
2′,3′	35	5.9
2″,3′	155	8.2
3′,4′	170	9.8
4′-Exo-O-endo conformation		
1′,2′	135	4.9
1′,2″	15	8.4
2′,3′	15	8.4
2″,3′	135	4.9
3′,4′	170	9.8

[a] Measured from stick models of 3′,5′-cyclic nucleotides.

Conclusion

Analysis of the ^1H nmr data for 3′,5′-TMP, 3′,5′-AMP, and dibutyryl-3′,5′-AMP demonstrates that the furanose and cyclic phosphate rings are rigid, in contrast to those of the 2′,3′-cyclic nucleotides. The

(22) R. D. Lapper, H. H. Mantsch, and I. C. P. Smith, *J. Amer. Chem. Soc.*, **95**, 2878 (1973).
(23) R. D. Lapper and I. C. P. Smith, *ibid.*, **95**, 2880 (1973).
(24) G. Govil and I. C. P. Smith, unpublished results.
(25) (a) R. E. Schirmer, J. H. Noggle, J. P. Davis, and P. A. Hart, *J. Amer. Chem. Soc.*, **92**, 3266 (1970); (b) P. A. Hart and J. P. Davis, *ibid.*, **94**, 2572 (1972); (c) R. E. Schirmer, J. P. Davis, J. H. Noggle, and P. A. Hart, *ibid.*, **94**, 2561 (1972).

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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(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.

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