(crystal) structure. In this case, it is clear from Figure 3 that there is no base stacking,[19] similar to the situation observed in dihydrouridine[13] (see ref 19). The structure seems to be stabilized by a combination of hydrogen interactions, van der Waal's, and dipolar forces.

Acid Hydrolysis of the N^1 Substituent. The resistance to acid hydrolysis of the N^1 substituents is surprising since it is known that the hydrogenation of carbons 5 and 6 of uracil facilitates the removal of the sugar from uridine.[20] We were looking for any structural features that might explain this resistance of compound I to acid hydrolysis. Two features in this structure are interesting to note in this connection, namely the long N(1)–C(7) bond and the internal hydrogen bond from N(8) to O(2). The effect of both inter- and intramolecular hydrogen bonding is known not only in altering reaction kinetics, but also in influencing reaction paths.[21,22] Hence, this internal hydrogen bond might be involved in making the N^1 substituent resistant to hydrolysis.

(19) The term "base stacking" seems to be used by different people with different meanings. For example, the structure analysis of dihydrouridine was carried out by two independent groups,[12,13] one of whom[13] consider there is no base stacking in dihydrouridine, but the other[12] suggests that "the base stacking configuration observed here is in many respects similar to that observed in the known planar pyrimidine systems with the carbonyl oxygen atoms O(2) of the two molecules lying either over or close to the rings of adjacent bases." This ambiguity can be removed somewhat by giving a more precise definition for the base stacking in terms of the degree of overlap of the area of the conjugated system on adjacent bases when projected normal to them. The distances between the various atoms on adjacent bases are important, but there are too many such distances to be specified. Hence a stacking distance which is the *shortest* distance between the overlapping π systems might be an appropriate quantity to quote in discussing base stacking. It is realized that the most appropriate quantity for discussive base stacking is the corresponding decrease in energy on forming aggregates, but this is not readily calculable; the percentage degree of overlap and the stacking distance might serve a useful purpose as a crude index of this energy.

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Acknowledgments. R. P. and J. O. thank Dr. D. Harker for valuable and stimulating discussions. S. P. D. and G. B. C. thank Drs. A. Mittelman, E. D. Holyoke, and G. P. Murphy for their keen interest and encouragement in this work. This work was partially supported from Grant CA 14185 from the National Cancer Institute of the U. S. Public Health Service. Our thanks go to Mrs. Winiewicz for excellent technical assistance.

Supplementary Material Available. A listing of observed and calculated structure factors will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 20× reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Journals Department, American Chemical Society, 1155 16th St., N.W., Washington, D. C. 20036. Remit check or money order for $3.00 for photocopy or $2.00 for microfiche, referring to code number JACS-73-8141.

(21) G. C. Pimental and A. L. McClellan, "The Hydrogen Bond," W. H. Freeman, San Francisco, Calif., 1959, pp 184–188.

(22) S. N. Vinogradov and R. H. Linnel, "Hydrogen Bonding," Van Nostrand-Reinhold, New York, N. Y., 1971, p 267.

Conformational Analysis of Cytidine, 1-β-D-(Arabinofuranosyl)cytosine and Their *O'*-Methyl Derivatives by Proton Magnetic Resonance Spectroscopy

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Abstract: The pmr spectra of cytidine, 1-β-D-(arabinofuranosyl)cytosine, and a series of their *O'*-methyl derivatives have been subjected to detailed computer analyses. Conformational analyses profited from the "neighbor anisotropy effect" of the *O'*-CH_3 bonds, since CNDO/2 calculated changes in electron density due to O'-methylation were negligible. It was shown for the vicinal protons that, with a given pentose ring puckering, there is a marked disparity between the values of the dihedral angles calculated from standard stereochemical models and those derived from crystallographic data, and this was taken into account in the present analysis. The correlation between the changes in chemical shifts, due to etherification of one of the pentose hydroxyls, with the values of coupling constants, pointed to the existence of preferred puckered forms, *viz.* $C_{3'}$*endo*–$C_{2'}$endo for the ribose ring, and $C_{2'}$*exo*–$C_{3'}$exo for the arabinose ring. In cytidine derivatives the conformation of the exocyclic 5'-CH_2OH group exhibits a preference for the form gauche–gauche (60–70%), and approximately equal populations for the other two forms. For arabinosylcytosine the gauche–gauche population is in the range 20–35% and the gauche–trans, 40–50%. The influence of a 5'-OCH_3 on the chemical shift of the cytosine H_6 demonstrated the marked preference for the form anti in both nucleosides. It also proved possible to predict preferred conformers for the various *O'*-CH_3 groups. The breadths of the H_5 and H_6 signals were essentially unaffected over the pH range for cytosine ring protonation.

The pioneering studies of Jardetzky[1] and Lemieux[2] on the conformations of nucleosides and nucleotides in solution by means of nmr spectroscopy have since undergone extensive developments, including the use of simulation techniques for analyses of spectra,[3] interpretation of coupling constants between ^1H, ^{13}C,

(1) C. D. Jardetzky, *J. Amer. Chem. Soc.*, **84**, 62 (1962), and references therein.

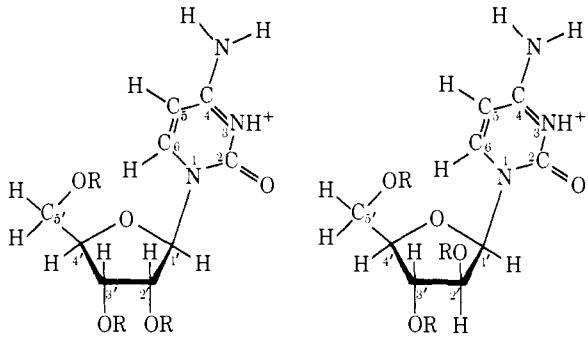
(2) R. U. Lemieux, *Can. J. Chem.*, **39**, 116 (1961).

(3) F. E. Hruska, A. A. Grey, and I. C. P. Smith, *J. Amer. Chem. Soc.*, **92**, 4088 (1970); T. Schleich, B. J. Blackburn, R. D. Lapper, and I. C. P. Smith, *Biochemistry*, **11**, 137 (1972).

^{19}F, and ^{31}P nuclei,[4-6] application of the nuclear Overhauser effect (NOE),[7] as well as improvements in methods of interpretation of chemical shifts in conformational analyses.[8-11]

The present communication describes a further approach to the problem of nucleoside conformation in solution, with the aid of a series of new model compounds and the use of some modifications of normally employed theoretical procedures. The nucleosides selected for this study were cytidine (a normal constituent of nucleic acids) and 1-β-D-(arabinofuranosyl)-cytosine (araC,[12] a potent antimetabolite of considerable therapeutic interest), together with a number of their *O'*-methyl derivatives. The latter analogs proved particularly valuable for this purpose inasmuch as the effects of methylation of successive pentose hydroxyls on the chemical shifts of both the pentose and aglycon ring protons, due to the magnetic anistropy of the

Scheme I



ribonucleosides arabinonucleosides

R = H,CH_3

O'-CH_3 groups, provided supplementary independent data regarding nucleoside conformation, including that of the pentose ring, in both types of nucleosides. It should, furthermore, be emphasized that the conformations of *O'*-methyl nucleosides are also of biological interest[13] because of the widespread natural occurrence of 2'-*O*-methyl nucleosides, particularly in tRNA.[14] The results of a pmr analysis of the conformations of 2'-*O*-methylcytidine and 2'-*O*-methyluridine have already been reported.[15]

The validity of the Karplus relation[16] as a practical tool for the study of conformations in solution was first questioned by Karplus[17] himself, who pointed to the uncertainties involved in the indiscriminate application of this function to the evaluation of angles to an accuracy of several degrees. Even in a semiempirical test of the Karplus relation, the relative values of the constants *A*, *B*, and *C* will be dependent on the ring dimensions, the influence of substituents, the values of the C–C–H angles, etc.[18] A substantial number of measured coupling constants for the vicinal protons of the pentose rings of a variety of nucleosides demonstrates that the constants predicted by Karplus, relative to ethane, should be reasonably applicable to the pentose ring. There has been occasional indiscriminate use of the constants of Abraham, *et al.*,[19] originally proposed for use with six-membered rings. In any event, the validity of the calculated value of a coupling constant derived from the given value for a dihedral angle is usually subject to an error of at least 1 Hz.[20]

Apart from the foregoing, the use of standard stereochemical models for the determination of dihedral angles from the puckering of the pentose ring is subject to errors as large as 15° which, over certain angular ranges, amounts to 2 Hz in the value of the coupling constant (see below). This is undoubtedly related to the fact that certain values of the planar angles of the pentose ring deviate appreciably from those provided *a priori* by most stereochemical models.

In general, the pentose ring conformation may be represented by an equilibrium between various puckered forms, but two types of such equilibria may be distinguished: (a) with comparable populations of different puckered forms; (b) with pronounced preference of a unique puckered form or group of similar conformers. Analyses of pentose conformations are based on assignments of dihedral angles with the aid of standard stereochemical models, and the coupling constants then calculated from the Karplus relation. When no set of such calculated constants corresponded to the measured values, it was concluded that an equilibrium of type (a) exists.[3] Bearing in mind that such an analysis may be subject to at least two errors of unknown magnitude, the results are subject to some reservations.

The present, two-stage, conformational analysis, involving initially determination of the type of equilibrium between puckered forms, and subsequently the preferred forms prevailing for the compounds under study, profits equally from the changes in chemical shifts due to O'-methylation and the values of the coupling constants. The effect of O'-methylation on chemical shifts also turned out to be useful in analyses of the syn–anti equilibrium.

Experimental Section

Materials. Cytidine was a product of Waldhof Zellstoffabrik (Mannheim, GFR), while 1-β-D-(arabinofuranosyl)cytosine was prepared by J. Giziewicz according to the procedure of Kanai, *et al.*[21] The *O'*-methyl derivatives of cytidine were obtained as

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(5) R. J. Cushley, J. F. Codington, and J. J. Fox, *Can. J. Chem.*, 46, 1131 (1968).
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(9) S. S. Danyluk and F. E. Hruska, *Biochemistry*, 7, 1038 (1968).
(10) M. P. Schweizer, J. T. Witkowski, and R. K. Robins, *J. Amer. Chem. Soc.*, 93, 277 (1971).
(11) M. Remin and D. Shugar, *Biochem. Biophys. Res. Commun.*, 48, 636 (1972).
(12) Typical abbreviations used are: 3'-mC, 3'-*O*-methylcytidine; 2',3'-m_2C, 2',3'-di-*O*-methylcytidine; 2',3',5'-$m_3$araC, 2',3',5'-tri-*O*-methylarabinosylcytosine.
(13) H. Singh and B. G. Lane, *Can. J. Biochem.*, 42, 87, 1011 (1964).
(14) F. T. Tamoaki and B. G. Lane, *Biochemistry*, 7, 3431 (1968).
(15) F. E. Hruska, A. Mak, H. Singh, and D. Shugar, *Can. J. Chem.*, 51, 1099 (1973).
(16) M. Karplus, *J. Chem. Phys.*, 30, 11 (1959).
(17) M. Karplus, *J. Amer. Chem. Soc.*, 85, 2870 (1963).
(18) M. Barfield and D. M. Grant, *Advan. Magn. Resonance*, 1, 187 (1965).
(19) R. J. Abraham, L. D. Hall, L. Hough, and K. A. McLauchlan, *J. Chem. Soc.*, 3699 (1962).
(20) R. D. Lapper, H. H. Mantsch, and I. C. P. Smith, *J. Amer. Chem. Soc.*, 94, 6423 (1972).
(21) T. Kanai, T. Kojima, O. Maruyama, and M. Ichino, *Chem. Pharm. Bull.*, 18, 2569 (1970).

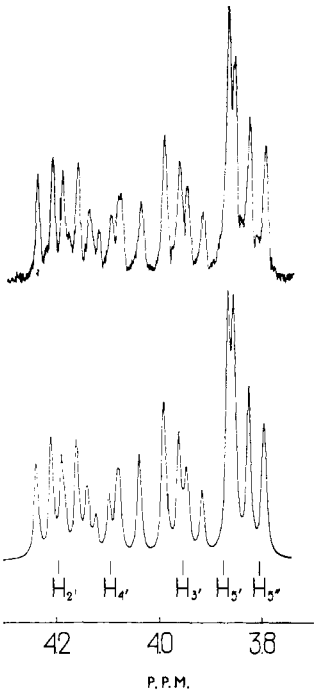

Figure 1. The 100-MHz pmr spectrum of 2',3'-$m_2$araC, 0.15 *M* in 0.15 *N* DCl in D_2O, for the region due to the 2' to 5' protons, at 22°. Chemical shifts are relative to internal DSS. Upper spectrum is that experimentally recorded. Lower spectrum is that obtained by computer simulation.

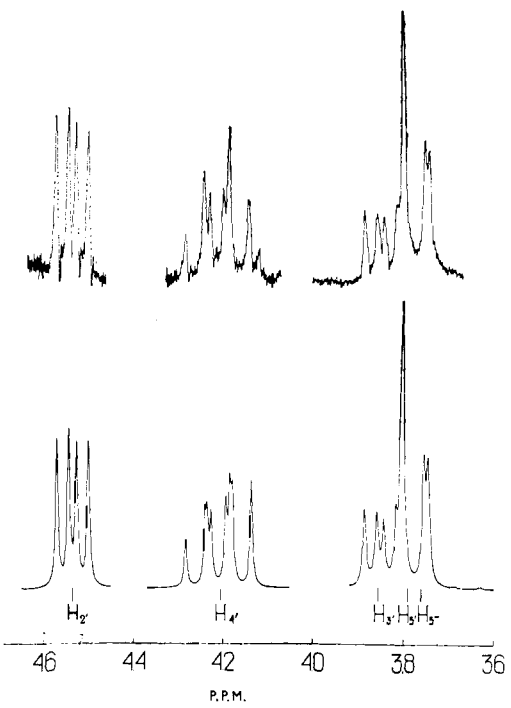

Figure 2. The 100-MHz pmr spectrum of 3',5'-$m_2$araC, 0.15 *M* in 0.15 *N* DCl in D_2O, for the region including the 2' to 5' protons, at 22°. Chemical shifts are relative to internal DSS. Upper spectrum, experimentally recorded; lower spectrum, computer simulated. For both experimental and simulated spectra a twofold higher gain was employed for the $H_{2'}$ and $H_{5''}$.

elsewhere described,[22] and the corresponding *O'*-methyl derivatives of araC according to Darzynkiewicz, *et al.*[23] All the foregoing were converted to the crystalline HCl salts, with defined melting points, according to standard procedures.[22,23]

Methods. The pmr spectra were recorded at room temperature (22 ± 2°) with the aid of a Varian XL-100 and a JNM-4H-100. Samples were dissolved directly in D_2O at concentrations of about 0.15 *M*, the resulting pD being about 1, so that all compounds were in the cationic form. The solutions contained DSS as an internal reference.

Initial chemical shifts and coupling constants were determined analytically[24] for subsequent use in simulation of spectra, carried out with the aid of nmr LAOCN-2. Comparisons of the simulated spectra with the experimental were used to correct the initial parameters. A repeated simulation of the spectra with the aid of corrected parameters led to further correction of the latter. Typical experimental and simulated spectra are presented in Figures 1 and 2. The estimated error in assignments of chemical shifts of H_5, H_6, $H_{1'}$, and CH_3 protons is 0.002 ppm, and for the remaining protons less than 0.005 ppm. The coupling constants J_{5-6} and $J_{1'-2'}$ are accurate to about 0.1 Hz; the accuracy for the other vicinal protons is 0.2–0.4 Hz, and for the geminal protons 0.5 Hz.

Results

Chemical Shifts. The chemical shifts for all protons of the various derivatives are listed in Table I. Table II, in turn, presents the changes in chemical shifts of the various protons resulting from the introduction of a methyl group on a given sugar hydroxyl. The influence of a given methyl substituent on chemical shifts was then examined for all possible pairs of derivatives such that the two members of each pair differed only in that one of them contained an additional methyl group. The second column of Table II lists these specific pairs of derivatives for which the differences in chemical shifts ($\Delta\delta$) are shown.

It will be noted (see Table II) that, both for the ribo and arabinosyl derivatives, the substitution of a methyl on either the 2'-OH or the 3'-OH leads to pronounced shielding (0.3 ppm) of the corresponding $H_{2'}$ or $H_{3'}$, respectively, with simultaneous deshielding of $H_{1'}$ and $H_{3'}$ *or* $H_{2'}$ and $H_{4'}$, respectively. The chemical shifts of the other protons may also be influenced by methylation, *e.g.*, a 3'-*O*-methyl barely affects the chemical shift of $H_{1'}$ in the ribonucleosides, but markedly shields (0.09 ppm) this proton in the arabinonucleosides. Somewhat characteristic is the minimal influence of a 5'-*O*-methyl on the chemical shifts of $H_{1'}$, $H_{2'}$, and $H_{3'}$ as compared to the pronounced deshielding effect on $H_{4'}$ (0.07 for the ribonucleosides and 0.10 ppm for the arabinonucleosides) and the shielding effect on H_6 (0.11 ppm for ribonucleosides and 0.06–0.09 ppm for arabinonucleosides). We shall revert, below, to the significance of the shielding effect on H_6.

Coupling Constants. The coupling constants between the cytosine H_5 and H_6 protons, as well as those for all vicinal and geminal protons of the carbohydrate rings, are presented in Table I. Methylation of a ribonucleoside sugar hydroxyl does not markedly modify the coupling constants (Table I) which reflect ribose ring puckering. Among the arabinonucleoside derivatives, similar values of $J_{1'-2'}$, $J_{2'-3'}$, and $J_{3'-4'}$ are exhibited by those (a) with a 3'-m, group $G^{3'}$, (b) with a 2'-m, but with a free 3'-OH, group $G^{2'}$, (c) with the 5'-OH free or methylated, group G^0.

(22) J. T. Kuśmierek and D. Shugar, *Acta Biochim. Pol.*, **18**, 413 (1971); J. T. Kuśmierek, J. Giziewicz, and D. Shugar, *Biochemistry*, **12**, 194 (1973).

(23) E. Darzynkiewicz, J. T. Kuśmierek, and D. Shugar, *Biochem. Biophys. Res. Commun.*, **46**, 1734 (1972); E. Darzynkiewicz and D. Shugar, manuscript in preparation.

(24) In all instances the H_5, H_6, and $H_{1'}$ signals were "first-order" doublets. It was further assumed that protons $H_{5'}$, $H_{5''}$, and $H_{4'}$ are coupled as in an ABX system, and $H_{2'}$, $H_{3'}$, and $H_{4'}$ as an ABX or XAB system, depending on the derivative.

Table I. Chemical Shifts (in ppm) *vs.* Internal DSS, of the Different Protons and CH_3 Protons in Cytidine (C), Arabinosylcytosine (araC) and Their Various *O'*-Methyl Derivatives, and Proton–Proton Coupling Constants, *J* (in Hz), for Cytidine, Arabinosylcytosine, and Their Various *O'*-Methyl Derivatives

Derivative	H_6	H_5	$H_{1'}$	$H_{2'}$	$H_{3'}$	$H_{4'}$	$H_{5'}$	$H_{5''}$	2'-m	3'-m	5'-m	J_{5-6}	$J_{1'-2'}$	$J_{2'-3'}$	$J_{3'-4'}$	$J_{4'-5'}$	$J_{4'-5''}$	$J_{5'-5''}$
C	8.172	6.270	5.874	4.355	4.225	4.17	3.95	3.83				8.2	3.6	4.8	5.8	2.8	4.3	−12.7
2'-mC	8.200	6.270	5.971	4.06	4.315	4.14	3.965	3.835	3.550			8.2	3.2	5.0	6.2	2.7	4.2	−12.9
3'-mC	8.170	6.265	5.880	4.53	3.915	4.22	3.96	3.815		3.460		8.1	3.5	5.3	6.5	2.8	3.9	−13.1
5'-mC	8.060	6.280	5.885	4.355	4.21	4.24	3.815	3.705			3.442	8.0	3.5	5.0	6.6	2.7	4.6	−11.7
2',3'-m_2C	8.200	6.270	5.990	4.235	4.015	4.195	3.97	3.815	3.561	3.449		8.1	2.7	4.9	6.7	2.8	3.7	−13.4
2',3',5'-m_3C	8.090	6.284	5.984	4.245	4.025	4.27	3.82	3.695	3.551	3.463	3.453	8.0	2.9	5.0	7.2	2.7	4.5	−11.7
AraC	8.083	6.249	6.199	4.445	4.14	4.05	3.93	3.845				8.2	5.2	4.1	5.5	3.2	5.9	−12.3
2'-maraC	8.014	6.228	6.297	4.135	4.23	3.99	3.885	3.79	3.371			8.1	5.3	5.1	5.3	3.5	5.9	−12.3
3'-maraC	8.057	6.244	6.113	4.54	3.87	4.105	3.90	3.845		3.498		8.1	4.5	2.7	4.7	3.3	6.5	−13.0
5'-maraC	7.995	6.260	6.193	4.43	4.125	4.145	3.80	3.75			3.455	8.1	5.3	4.2	4.9	3.8	5.9	−11.7
2',3'-$m_2$araC	8.014	6.235	6.192	4.195	3.955	4.099	3.87	3.80	3.371	3.513		8.0	4.9	3.0	4.6	4.0	6.3	−12.8
3',5'-$m_2$araC	7.990	6.268	6.114	4.54	3.853	4.205	3.793	3.763		3.502	3.466	8.0	4.4	2.7	4.25	4.7	5.9	−11.7
2',5'-$m_2$araC	7.943	6.248	6.300	4.14	4.225	4.105	3.77	3.71	3.382		3.447	8.0	5.0	4.9	5.5	3.3	6.4	−11.7
2',3',5'-$m_3$araC	7.955	6.245	6.198	4.21	3.95	4.21	3.75	3.71	3.366	3.505	3.449	8.2	5.0	2.7	4.4	4.8	5.8	−11.7

Table II. Changes in Chemical Shifts ($\Delta\delta \times 10^2$ ppm), Calculated from the Data in Table I, of the Various Protons and CH_3 Protons in C (Cytidine) and araC (Arabinosylcytosine) Resulting from Introduction of an *O'*-Methyl or an Additional *O'*-Methyl

O'-Methyl group added	Resulting transformation		Resultant change in chemical shift of H_6	H_5	$H_{1'}$	$H_{2'}$	$H_{3'}$	$H_{4'}$	$H_{5'}$	$H_{5''}$	2'-CH_3	3'-CH_3	5'-CH_3
2'-m	C →	2'-mC	−2.8	0.0	−9.7	29.5	−9.0	3.0	−1.5	−0.5			
	3'-mC →	2',3'-m_2C	−3.0	−0.5	−11.0	29.5	−10.0	2.5	−1.0	0.0		1.1	
3'-m	C →	3'-mC	0.2	0.5	−0.6	−17.5	31.0	−5.0	−1.0	1.5			
	2'-mC →	2',3'-m_2C	0.0	0.0	−1.9	−17.5	30.0	−5.5	−0.5	2.0	−1.1		
5'-m	C →	5'-mC	11.2	−1.0	−1.1	0.0	1.5	−7.0	13.5	12.5			
	2',3'-m_2C →	2',3',5'-m_3C	11.0	−1.4	0.6	−1.0	−1.0	−7.5	15.0	12.0	1.0	−1.4	
2'-m	araC →	2'-maraC	6.9	2.1	−9.8	31.0	−9.0	6.0	4.5	5.5			
	5'-maraC →	2',5'-$m_2$araC	5.2	1.2	−10.7	29.0	−10.0	4.0	3.0	4.0			0.8
	3'-maraC →	2',3'-$m_2$araC	4.3	0.9	−7.9	34.5	−8.5	0.6	3.0	4.5		−1.5	
	3',5'-$m_2$araC →	2',3',5'-$m_3$araC	3.5	2.3	−8.4	33	−9.7	−0.5	4.3	5.3		−0.3	1.7
3'-m	araC →	3'-maraC	2.6	0.5	86.	−9.5	27.0	−5.5	3.0	0.0			
	5'-maraC →	3',5'-$m_2$araC	0.5	−0.8	7.9	−11	27.2	−6.0	0.7	−1.3			−1.1
	2'-maraC →	2',3'-$m_2$araC	0.0	−0.7	10.5	−6.0	27.5	−10.9	1.5	−1.0	0.0		
	2',5'-$m_2$araC →	2',3',5'-$m_3$araC	−1.2	0.3	10.2	−7.0	27.5	−10.5	2.0	0.0	1.6		−0.2
5'-m	araC →	5'-maraC	8.8	−1.1	0.6	1.5	1.5	−9.5	13.0	9.5			
	2'-maraC →	2',5'-$m_2$araC	7.1	−2.0	−0.3	−0.5	0.5	−11.5	11.5	8.0	−1.1		
	3'-maraC →	3',5'-$m_2$araC	6.7	−2.6	−0.1	0.0	1.7	−10.0	10.7	8.2		−0.4	
	2',3'-$m_2$araC →	2',3',5'-$m_3$araC	5.9	−1.0	−0.6	−1.5	0.5	−11.1	12.0	9.0	0.5	0.8	

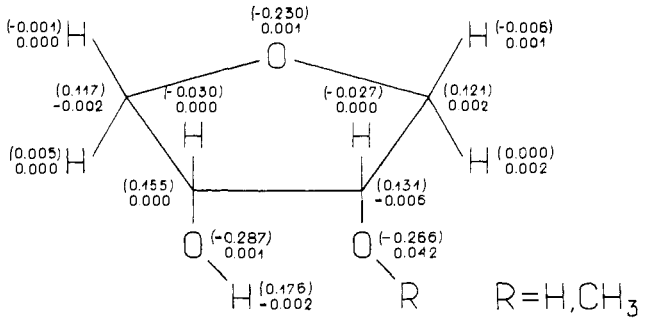

Figure 3. CNDO/2 calculations of the changes in electronic density (in electrons) on the individual atoms of ribose resulting from replacement of 2′-OH by 2′-OCH_3. Values in parentheses represent electron densities prior to introduction of the methyl group.

Observed Correlations. In addition, the results in Table II demonstrate that, in the case of the ribonucleosides, the introduction of a methyl at each of the positions 2′, 3′, and 5′ modifies the chemical shifts of the individual protons in the appropriate groups by values which, to an accuracy of 0.01 ppm, are independent of prior methylation of one or both of the remaining two hydroxyls. For the arabinonucleosides, on the other hand, an analogous effect is observed for those derivatives which, prior to methylation, belong to the same group G and, following methylation, also fall within one of the three groups G defined above.[25] The significance of these regularities will become clearer, below, in relation to the sugar ring conformations. In connection with this, it should likewise be noted that the chemical shifts of the protons of a methyl substituent are modified to only a minor extent on introduction of a second methyl group.

Discussion

Effect of O′-Methylation on Electron Density. The changes in chemical shifts due to replacement of an OH by an OCH_3 may be generally interpreted as originating from a "neighbor anisotropy effect" and local electron density variations. The significance of the latter was evaluated by theoretical calculations of the total electron density on the individual atoms of ribose and 2′-*O*-methyl ribose, by means of the CNDO/2 method. The results demonstrated that introduction of the methyl group led to a marked change in electron density only on $O_{2'}$ (+0.042 electron) and $C_{2'}$ (−0.006 electron). The changes in charge on the remaining atoms were of the order of 0.000–0.002 electron, to an accuracy of 0.001 electron (Figure 3). The calculations were based on ribose ring puckering for both models of $C_{3'}$*endo*–$C_{2'}$endo, $C_{3'}$-trans conformations for the $O_{2'}$–H and $O_{2'}$–CH_3 substituents, and the $C_{4'}$-trans conformation for $O_{3'}$–H (see Figure 7).

In the case of purine and pyrimidine bases, a correlation between the total electron density calculated by means of CNDO/2[26] and ^{13}C chemical shifts[27] corresponded to the relation 1 electron ~ 90 ppm. If we relate this result to the correlation existing between the chemical shifts of ring ^{13}C atoms of heterocycles and ^1H, where the latter is directly bonded to the former (40 ppm ~ 1.8 ppm),[28] we may establish the influence of the electron density of a carbon on the chemical shift of a proton bonded to this carbon (0.001 electron ~ 0.004 ppm). Hence a change in electron density on $C_{2'}$ of 0.006 electron should result in a change in chemical shift of $H_{2'}$ of about 0.025 ppm, *i.e.*, an order of magnitude lower than that actually observed. It consequently follows that one may virtually ignore the change in the electron density of the carbon atoms, due to methylation, on the observed chemical shifts of the protons.

Anisotropy of OCH_3. The anisotropic shielding of $H_{1'}$ by COH or $COCH_3$ is reflected in the differing shielding of $H_{1'}$ in the ribonucleosides relative to the corresponding arabinonucleosides. Shielding of $H_{1'}$ in the cis position is about 0.30 ppm with respect to the trans. Such differences cannot be accounted for by any marked changes in the torsion angle about the glycosidic bond, ϕ_{CN} (see below). Similar differences in the chemical shifts of $H_{1'}$ are observed between the α and β anomers of nucleosides.[29] Furthermore, the OCH_3 group differs from the OH by the presence of an additional C–O bond, as well as the methyl C–H bonds which are equally sources of the neighbor anisotropy effect. The anisotropy of the C–O bond has been implicated in shielding effects in cyclohexane derivatives, vinyl ethers[30] and carbohydrates,[31] and calculations of the screening effects of the C–H bond have been reported by ApSimon, *et al.*[32] The subject of the magnetic anisotropy of single bonds has been extensively reviewed by Bothner-By and Pople.[33]

The anisotropic effects of the OCH_3 groups herein considered will clearly be determined by the conformation[34] of this group about the single bond to one of the ring, or exocyclic 5′, carbons. The conformation of a given OCH_3 group will also, in turn, depend on the conformation of the carbohydrate ring, *e.g.*, one would anticipate a different conformation of the OCH_3 group in the case of a $C_{2'}$*endo* form of the sugar ring as compared to $C_{2'}$*exo*. It follows that the data embraced in Table II are a useful source of information regarding the conformations of the ribose and arabinose rings.

Nucleoside Pentose Rings in Solid State. Although due caution must be exercised in extrapolations from the solid state to solutions, there is no valid reason for ignoring crystallographic data in analyses of nucleoside sugar ring conformations in solution by pmr data with the aid of standard stereochemical models. The latter explicitly adopts, for the angles between bonds emanating from a carbon atom, a value of 109.5°, whereas the corresponding angles in crystals of nucleosides vary over the range 100–120°. The import of this is obvious from the fact that the values of these

(25) The single exception to this rule is that for methylation of the 5′-OH, where both the nonmethylated and methylated derivatives belong to the same group.

(26) We are indebted to A. Pohorille and M. Geller for carrying out these calculations; C. Giessner-Prettre and A. Pullman, *Theor. Chim. Acta*, **9**, 279 (1968).

(27) A. J. Jones, D. M. Grant, M. W. Winkley, and R. K. Robins, *J. Amer. Chem. Soc.*, **92**, 4079 (1970).

(28) T. F. Page, T. D. Alger, and D. M. Grant, *ibid.*, **87**, 5333 (1965).

(29) R. J. Cushley, I. Wempen, and J. J. Fox, *ibid.*, **90**, 709 (1968).

(30) J. W. Emsley, J. Feeney, and L. H. Sutcliffe, "High Resolution Nuclear Magnetic Resonance Spectroscopy," Vol. 2, Pergamon Press, Oxford, 1968, pp 700–722.

(31) P. L. Durette and D. Horton, *Carbohyd. Res.*, **18**, 57, 289 (1971).

(32) J. W. ApSimon, W. G. Craig, P. V. Demarco, D. W. Mathieson, L. Saunders, and W. B. Wholley, *Tetrahedron*, **23**, 2339 (1967).

(33) A. A. Bothner-By and J. A. Pople, *Annu. Rev. Phys. Chem.*, **16**, 43 (1965).

(34) Conformation here refers to the population distribution of permissible conformers of this group.

Table III. Dihedral Angles Involving Pentose Ring Hydrogen Atoms in Crystals of 5′-dCMP, Thymidine, and Deoxyadenosine Monohydrate, as Compared with Those Derived from Stick Models

	5′-dCMP[a]				
	LS	VR	Thymidine	Deoxyadenosine monohydrate	From stick models[c]
$\phi_{1'-2'}$	18	11	22	28	20
$\phi_{2'-2''}$	138	134	154	143	140
$\phi_{1'-2''} - \phi_{1'-2'}$	120	123	132	115	120
$\phi_{2'-3'}$	91	89	76	82	93
$\phi_{2''-3'}$	−45	−41	−48	−38	−28
$\phi_{2'-3'} - \phi_{2''-3'}$	136	130	124	120	121
$\phi_{3'-4'}$	−89	−87	−88	−91	−95
Conformation[b]	$C_{3'}$*exo*-$C_{2'}$exo		$C_{3'}$*exo*-$C_{2'}$endo	$C_{3'}$*exo*-$C_{2'}$endo	$C_{3'}$*exo*

[a] Values obtained by two methods, denoted in ref 35 as LS and VR. [b] See ref 39 for description of terms *endo*, endo and *exo*, exo. [c] Reference 3.

angles, for a defined conformation of the pentose ring, are parameters which affect the values of the dihedral angles of the ring. In general, dihedral angles other than those estimated from Dreiding models correspond to defined puckered forms of the pentose ring. Typical examples include 5′-dCMP, thymidine, and deoxyadenosine monohydrate,[35] shown in Table III.

It will be noted from the table that, whereas stereochemical models predict a difference between the dihedral angles for the proton pairs $H_{1'}$–$H_{2'}$ and $H_{1'}$–$H_{2''}$, or $H_{2'}$–$H_{3'}$ and $H_{2''}$–$H_{3'}$, of 120°, for the three aforementioned compounds in the solid state these differences fluctuate over the range 115–135°. The significance of this is best seen from the fact that, for a defined range of ϕ, the differences in coupling constants, J, corresponding to a 15° difference in ϕ, may attain a value of 2.3 Hz. Due care must consequently be exercised in evaluations of dihederal angles for vicinal proton pairs from the pentose conformation (or the converse) as compared to that hitherto exercised in analyses of coupling constants with the aim of establishing pentose conformations. We have therefore profited from certain characteristic regularities in the crystallographic data (see below), which are reflected in the nature of the conformational analysis presented below.

For crystals of typical nucleosides and nucleotides, the pentose ring conformations are such that $C_{2'}$ or $C_{3'}$ lie outside the best plane through the remaining four ring atoms. The ring structure consequently approximates to either the envelope, or the half-chair, form.[36] In general, two types of regularities may be noted from an examination of the X-ray data for most nucleosides and nucleotides.[37]

(a) The pronounced displacement (0.5–0.6 Å) of either $C_{2'}$ or $C_{3'}$ from the best four-atom plane drawn through the remaining ring atoms.

(b) The small scatter of the values for the angles $C_{1'}$–$C_{2'}$–$C_{3'}$, $C_{2'}$–$C_{3'}$–$C_{4'}$, and $C_{4'}$–$O_{1'}$–$C_{1'}$. Particularly pertinent are the values for the angles $C_{1'}$–$C_{2'}$–$C_{3'}$ and $C_{2'}$–$C_{3'}$–$C_{1'}$ (100–103°), which clearly deviate from the standard values of 109.5°, a fact undoubtedly related to (a), above.

It should be emphasized that these regularities prevail not only for syn and anti conformations, but equally for *different* conformations of the pentose ring and for *different* conformations of the exocyclic 5′-CH_2OH group. These different conformations undoubtedly correspond to differences in intermolecular interactions in the solid state, which do not essentially affect the parameters considered in points (a) and (b), above.

The observed effects permit drawing of the following inference: *preferred conformers of the pentose ring in solution exhibit the same regularities, i.e., the pronounced puckering of either $C_{2'}$ or $C_{3'}$ out of the best four-atom plane of the remaining ring atoms, and small deviations of the values for the three ring angles.*

X-Ray diffraction analyses of cytidine and 1-β-D-arabinofuranosyl-4-thiouracil, which are most representative in relation to the compounds embraced in this study, show that both are in the form anti, $C_{3'}$-*endo*, and gauche–gauche.[38]

Ring Conformer Equilibrium Distributions. Starting from the structural regularities in the conformation of pentose rings revealed by X-ray diffraction, we consider two types of conformer equilibria in solution.

(a) The equilibrium between conformers of type A ($C_{2'}$*exo*; $C_{3'}$*endo*; $C_{2'}$*exo*-$C_{3'}$*endo*; $C_{3'}$*endo*-$C_{2'}$endo; $C_{3'}$*endo*-$C_{2'}$exo; $C_{2'}$*exo*-$C_{3'}$exo; $C_{2'}$*exo*-$C_{3'}$endo) and those of type B ($C_{2'}$*endo*; $C_{3'}$*exo*; $C_{2'}$*endo*-$C_{3'}$*exo*; $C_{2'}$*endo*-$C_{3'}$endo; $C_{2'}$*endo*-$C_{3'}$exo; $C_{3'}$*exo*-$C_{2'}$exo; $C_{3'}$*exo*-$C_{2'}$endo).[39] All of the foregoing conformers should exhibit pronounced puckering of $C_{2'}$ or $C_{3'}$ out of the best four-atom plane. For the A conformers $C_{2'}$ is below the plane, or $C_{3'}$ above the plane together with the exocyclic 5′-CH_2OH. The reverse situation prevails for the B conformers, *i.e.*, $C_{2'}$ above, or $C_{3'}$ below, the plane.

(b) The equilibrium between conformers within one of the groups A or B.[40]

Conformations of Pentose Rings. In a qualitative approach to the problem of pentose conformation, attention was directed to possible correlations between the behavior of the values of the coupling constants for vicinal protons and the chemical shifts of protons resulting from O′-methylation. In the case of the

(35) M. A. Viswamitra, B. S. Reddy, G. H.-Y. Lin, and M. Sundaralingam, *J. Amer. Chem. Soc.*, **93**, 4565 (1971).

(36) There are instances where another ring carbon, or the ring oxygen, deviates from the best mean-squares plane including the remaining ring atoms, *e.g.*, the α anomers of some nucleosides, but these are outside the scope of those embraced in the present investigation; see M. Sundaralingam, *J. Amer. Chem. Soc.*, **93**, 6644 (1971).

(37) References to X-ray data are listed in C. Altona and M. Sundaralingam, *ibid.*, **94**, 8205 (1972).

(38) S. Furberg, C. S. Petersen, and C. Pomming, *Acta Crystallogr.*, **18**, 313 (1965); W. Saenger, *J. Amer. Chem. Soc.*, **94**, 621 (1972).

(39) The italicized terms *endo* and *exo* refer to pronounced deviations from the plane including $C_{1'}$, $O_{1'}$, and $C_{4'}$ of the order of 0.5–0.6 Å. When these terms are not italicized, the corresponding deviations are of the order 0.1–0.2 Å.

(40) A description of the conformation of the furanose ring in terms of two parameters, based on the concept of pseudorotation, has been advanced by C. Altona and M. Sundaralingam (see ref 37).

Table IV. Calculated Dihedral Angles and Deduced Puckered Forms for Cytosine Ribonucleosides and Arabinonucleosides[a]

Derivatives	$\phi_{1'-2'}$		$\phi_{2'-3'}$		$\phi_{3'-4'}$		Puckering
Ribonucleosides	124	129	39	35	144	153	$C_{3'}$*endo*-$C_{2'}$endo
Arabinonucleosides	41	35	124	139	134	141	$C_{2'}$*exo*-$C_{3'}$exo

[a] The two values correspond to the lowest and highest values of the coupling constant. Because of the approximations involved in these calculations, only one common preferred puckering form is presented for all the ribose derivatives, and similarly for the arabinonucleoside derivatives.

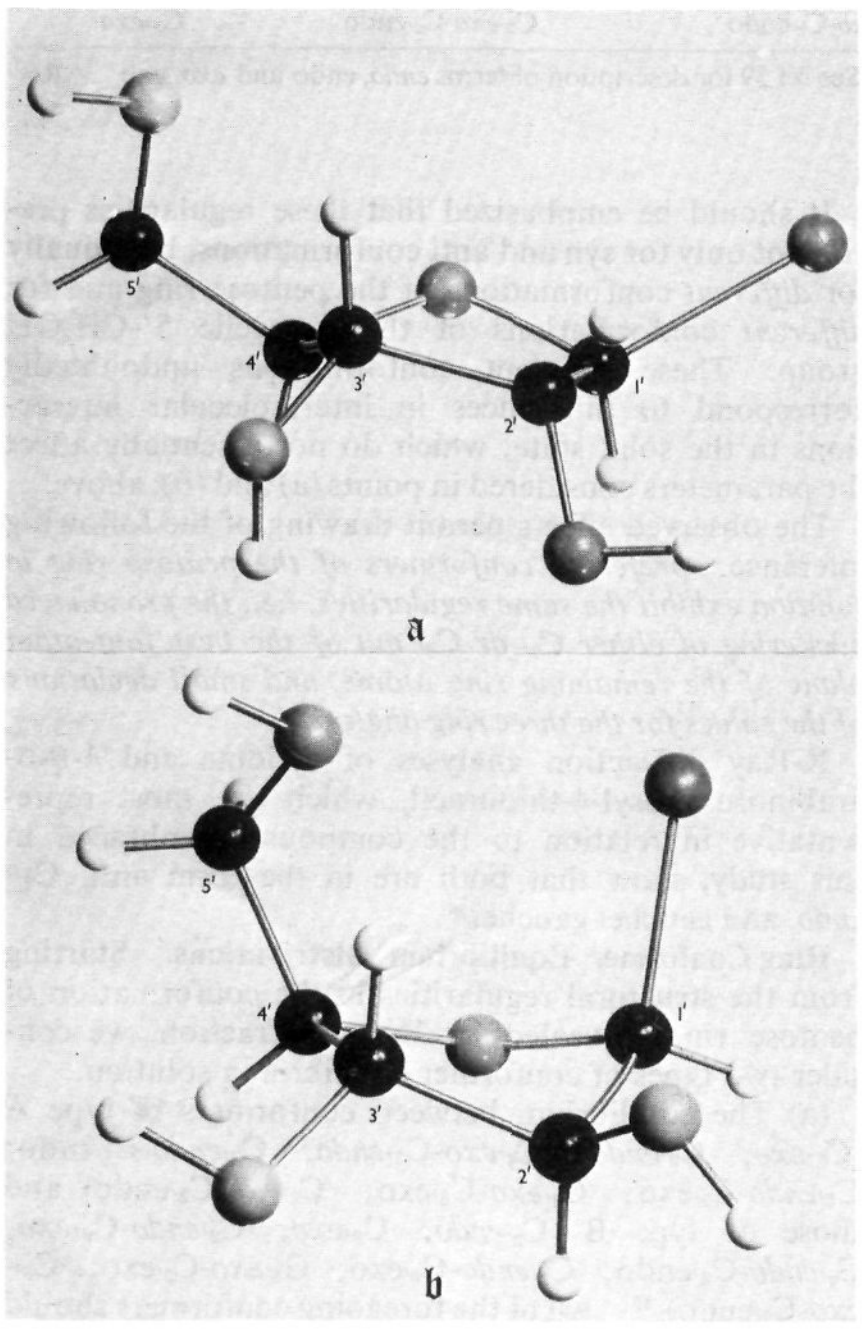

Figure 4. Preferred puckered forms of pentose rings of cytosine nucleosides: (a) ribonucleosides, $C_{3'}$*endo*-$C_{2'}$endo; (b) arabinonucleosides, $C_{2'}$*exo*-$C_{3'}$exo.

ribonucleosides, each methyl only slightly modified the values of $J_{1'-2'}$, $J_{2'-3'}$, and $J_{3'-4'}$, with $J_{1'-2'}$ undergoing a small decrease and $J_{3'-4'}$ a slight increase. Three classes could be differentiated for the arabinonucleosides (Table I and 2nd paragraph of Results): class $G^{3'}$ exhibited decreases in $J_{2'-3'}$ and $J_{3'-4'}$ of about 1 Hz and 0.5–1 Hz, respectively, relative to class G^0; whereas for class $G^{2'}$ the value of $J_{2'-3'}$ increased about 1 Hz with respect to G^0. In view of the negligible changes in electron density accompanying O′-methylation, replacement of an O′–H by O′–CH_3 should not lead to direct modifications in coupling constants, so that any such modifications observed must be due to conformational effects. The similar values of $J_{1'-2'}$, $J_{2'-3'}$, and $J_{3'-4'}$ for the series of ribonucleoside derivatives therefore indicate that O′-methylation hardly affects the ribose conformation; whereas, for the arabinonucleosides, the three classes of J values point to three distinguishable ring conformations. Note, in particular, the similar values of the coupling constants for such derivatives as 2′-mC, 3′-mC, and 2′,3′-m_2C among the ribonucleosides, and 3′-maraC and 2′,3′-$m_2$araC among the arabinonucleosides.

The regularities described above (Table II and 3rd paragraph of Results) may be interpreted as follows. If, prior to methylation of a given hydroxyl, the compounds exhibit similar pentose conformations, then the changes in chemical shifts of the individual protons are independent of whether another of the hydroxyls was previously methylated or not. Since the changes in chemical shifts due to methylation are dependent on the conformation of the O'-CH_3 group, in line with the anisotropic nature of the effect, it follows that the O'-CH_3 conformation is determined by the pentose conformation, but is not directly affected by a neighboring O'-CH_3 group.

Bearing in mind the low probability that, in the case of an equilibrium of pentose conformers of the type A ⇌ B with comparable populations of A and B, the effect of one O'-CH_3 group on the conformation of a second would be so small, it appears unlikely that such an equilibrium exists. Another argument against the existence of such an equilibrium is the fact that, in some instances, O′-methylation is virtually without effect on the pentose conformation. The obvious conclusion is that the conformer equilibrium is weighted in favor of a defined type of conformers, *i.e.*, with preference for a defined type of puckering, or a group of conformers with similar puckering.

It follows that the assignment of an average conformation to the sugar ring would provide a first approximation to the favored conformers. Application of the Karplus relation, with the aid of the Karplus constants, to the measured values of $J_{1'-2'}$, $J_{2'-3'}$, and $J_{3'-4'}$ was employed to calculate the corresponding dihedral angles, which are presented in Table IV. For the ribose ring of the cytidine derivatives the calculated dihedral angles point to a conformation approximating $C_{3'}$*endo*-$C_{2'}$endo (Figure 4a). For the araC derivatives the dihedral angles correspond to $C_{2'}$*exo*-$C_{3'}$exo (Figure 4b).

Frequent attempts to interpret the values of the coupling constants between vicinal protons, on the basis of the Karplus relation for a variety of nucleosides and nucleotides, were made to show the existence of an equilibrium between various puckered forms *via* an eclipsed form, with conformers of type A and B equally populated. This is equivalent to acceptance of conformers of type A and B as energetically equivalent. If we consider only the interaction of substituents on the 2′ and 3′ positions of the ribonucleosides, this conclusion may appear reasonable. However, since the

decisive factor which determines the sugar ring conformation includes the interactions of all the substituents with the ring, as well as the interactions between the substituents themselves, it should not be overlooked that the principal substituent on the pentose ring is the aglycon and that the interaction between them decidedly influences the pentose ring conformation.[41] A comparison of conformers of type A with those of type B, bearing in mind the orientation of the aglycon relative to the pentose moiety, reveals that, in the first case, the base adopts a quasiaxial orientation and, in the second, quasiequatorial.

Conformation of Exocyclic 5′-CH_2OH. The conformation of the exocyclic 5′-CH_2OH group with respect to the $C_{4'}$–$C_{5'}$ bond may be derived from an analysis of the coupling constants $J_{4'-5'}$ and $J_{4'-5''}$. Assuming the existence of three classical conformations, or of three such conformers corrected for O–O repulsion (see Figure 5), Blackburn, *et al.*, applied the Karplus relations to calculate the population of the gauche–gauche conformation.[42] Subsequently, specific assignments of the $H_{5'}$ and $H_{5''}$ signals to the protons $H_{5'B}$ and $H_{5'C}$ made it possible to establish the populations of all three rotamers for a variety of nucleosides and nucleotides.[11] This was based on the experimentally observed difference in sensitivities of the chemical shifts (δ) of $H_{5'}$ and $H_{5''}$ to introduction of a 3′-phosphate group, from which the following assignments were deduced: $H_{5'} \equiv H_{5'B}$ and $H_{5''} \equiv H_{5'C}$. The foregoing appears to be fairly general in the light of the following two regularities noted for many nucleosides:[11] $\delta(H_{5'}) > \delta(H_{5''})$ and $J_{4'-5'} < J_{4'-5''}$. The source of these regularities is discussed below.

On the basis of available crystallographic data on the conformation of the exocyclic 5′-CH_2OH group, it was further assumed that each of the three forms is uniformly distributed over the region between a fixed classical conformer and that resulting from 15° O–O repulsion. The populations, P, of the three forms may then be derived from the solution of the following three equations.

$$J_{4'-5'B} = P_I J_{IB} + P_{II} J_{IIB} + P_{III} J_{IIIB}$$

$$J_{4'-5'C} = P_I J_{IC} + P_{II} J_{IIC} + P_{III} J_{IIIC}$$

$$1 = P_I + P_{II} + P_{III}$$

The magnitudes of $J_{IB}, \ldots J_{IIIC}$ are simply the averages over the appropriate angular region of the values of the coupling constants calculated from the Karplus relation, *i.e.*

$$J(\phi_1,\phi_2) = \frac{1}{\phi_1 - \phi_2}\int_{\phi_1}^{\phi_2} J(\phi)\mathrm{d}\phi$$

where

$$J(\phi) = A + B\cos\phi + C\cos 2\phi$$

Establishment of the constants A, B, and C was based on the empirical form of the Karplus function for ethane[43] which, for three different angles, assumes the following values: $J(0°) = 14.7$ Hz, $J(90°) = 0$, $J(180°) = 18$ Hz. Correction of these by taking account of the electronegativities of the $O_{5'}$ and $O_{1'}$ oxygens and the $C_{3'}$ carbon[44] led to the following values: $J(0°) = 9.1$ Hz, $J(90°) = 0$, $J(180°) = 11.1$ Hz. From these one may directly calculate the constants: $A = 5.05$ Hz, $B = -1.0$ Hz, $C = 5.05$ Hz. The foregoing is based on the obvious fact that the measured values of $J_{4'-5'}$ and $J_{4'-5''}$ are averages.

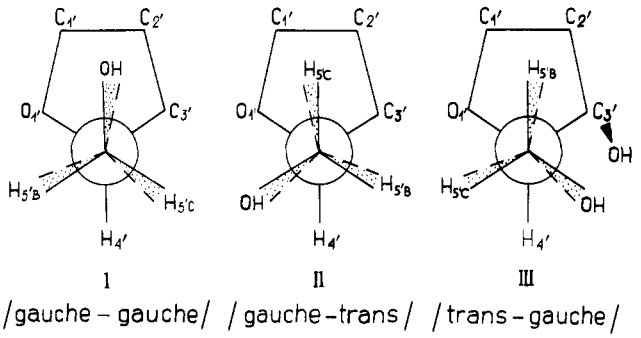

Figure 5. Newman projections along the $C_{4'}$–$C_{5'}$ bond of the three preferred conformers of the exocyclic 5′-CH_2OR group (R = H or CH_3), uniformly distributed between classical rotamers (——) and those estimated from O–O repulsion (– – –).

The resulting calculated populations of the three rotamers for the various *O′*-methyl ribonucleosides and arabinonucleosides, and the parent cytidine and arabinosylcytosine, are listed in Table V.

Table V. Population Distribution (in %) of the Gauche–Gauche (g–g), Gauche–Trans (g–t), and Trans–Gauche (t–g) Conformers of the Exocyclic 5′-CH_2OR (R = H or Me) Groups of Cytidine, Arabinosylcytosine, and Their Various *O′*-Methyl Derivatives

Derivative	P_{g-g}	P_{g-t}	P_{t-g}
C	64	19	17
2′-mC	66	18	16
3′-mC	69	14	17
5′-mC	61	23	16
2′,3′-m_2C	71	12	17
2′,3′,5′-m_3C	62	21	17
araC	37	41	22
2′-maraC	34	42	25
3′-maraC	28	49	23
5′-maraC	30	43	28
2′,3′-$m_2$araC	22	49	30
3′,5′-$m_2$araC	18	45	37
2′,5′-$m_2$araC	30	48	22
2′,3′,5′-$m_3$araC	18	44	38

Note from the table the very marked preference, in the case of the ribonucleosides, for the form gauche–gauche (60–70%) and approximately equivalent populations of gauche–trans and trans–gauche. By contrast, the arabinonucleosides exhibit a considerably lower proportion of the form gauche–gauche (20–35%), with a preponderance of gauche–trans (40–50%) as compared to trans–gauche.

The appreciably lower population of the gauche–

(41) This is also exemplified by the marked differences in ultraviolet absorption spectra between 1-methylcytosine and cytosine nucleosides, and the variations in absorption spectra of the latter with the nature of the carbohydrate component; see J. J. Fox and D. Shugar, *Biochim. Biophys. Acta*, **9**, 369 (1952).

(42) B. J. Blackburn, A. A. Grey, I. C. P. Smith, and F. E. Hruska, *Can. J. Chem.*, **48**, 2866 (1970).

(43) R. M. Lynden-Bell and N. Sheppard, *Proc. Roy. Soc., Ser. A*, **269**, 385 (1962); M. Barfield and D. M. Grand, *Advan. Magn. Resonance*, **1**, 149 (1965).

(44) H. Spiesecke and W. G. Schneider, *J. Chem. Phys.*, **35**, 722 (1961).

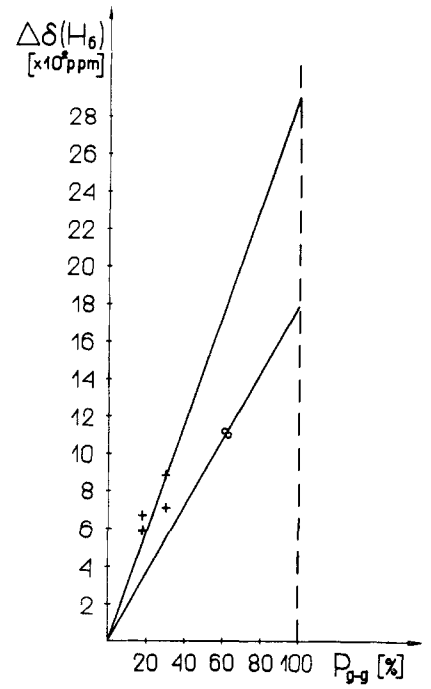

Figure 6. Dependence of change in chemical shift of H_6 (due to replacement of a 5′-OH by 5′-OCH_3) on the gauche–gauche population of the exocyclic 5′-CH_2OCH_3 group for cytosine ribonucleosides (○) and arabinonucleosides (+). The experimental points are taken from the data in Tables II and V. The straight lines represent the semiempirically expected relationship.

gauche form in arabinonucleosides as compared to the ribonucleosides may be correlated with the following two facts.

(a) The estimated 1.5–2-fold shorter distance in arabinonucleosides between the exocyclic $O_{5'}$ in the form gauche–gauche and the cytosine ring C_6, assuming the anti conformation for both arabinonucleosides and ribonucleosides. This distance is dependent on the conformation of the pentose ring.

(b) The O–O repulsion between the exocyclic $O_{5'}$ and the "up" pentose $O_{2'}$ in the arabinonucleosides, negligible in the case of the ribonucleosides.

The source of the observed regularities referred to above, *viz.* $\delta(H_{5'}) > \delta(H_{5''})$ and $J_{4'-5'} < J_{4'-5''}$, poses a separate problem. This phenomenon can be related to the dependence of $(\delta(H_{5'}) - \delta(H_{5''}))$ on the gauche–gauche population, which is approximately linear for both the ribonucleosides and arabinonucleosides. This, in turn, implies that the shielding effect of $C_{3'}$–$O_{3'}$ on $H_{5''}$, which occurs for the form gauche–gauche, is one of the main factors responsible for the observed differences between $\delta(H_{5'})$ and $\delta(H_{5''})$ and the dependence of this difference on the gauche–gauche population. As concerns the inequality $J_{4'-5'} < J_{4'-5''}$, this may be accounted for: (a) in those instances where the gauche–gauche form is favored, by the geometry of this form, for which the mean dihedral angles $\phi_{4',5'}$ and $\phi_{4',5''}$ are such that $\phi_{4',5'} > \phi_{4',5''}$; (b) when the gauche–gauche form is not favored, by the higher population of gauche–trans relative to trans–gauche.

Conformation about the Glycosidic Bond. Establishment of the conformation about the N_1–$C_{1'}$ bond in nucleosides has hitherto encountered serious difficulties. Some progress has recently been made by application of the nuclear Overhauser effect,[7] but even this is hampered by the lack of sufficient information about the conformation of the pentose rings.

We here approach this problem by making use of the anisotropy of the $O_{5'}$–CH_3 group, which will affect the value of chemical shift of H_6 if the nucleoside conformation is anti, but not if it is syn. Furthermore the change in chemical shift of H_6 should be dependent on the population of the form gauche–gauche and the pentose conformation.

The dependence of the change in chemical shift of H_6 ($\Delta\delta(H_6)$) on the gauche–gauche population (P_{g-g}) of derivatives with a 5′-*O*-methyl is shown in Figure 6. Assuming, as appears reasonable, that the effect of a 5′-*O*-methyl on the chemical shift of H_6 manifests itself when the exocyclic group is in the form gauche–gauche, and is negligible with the other two forms, straight lines have been drawn through the experimental points for the C and araC derivatives so that both pass through the intersection of the coordinate axes. Extrapolation of these lines gives the values of $\Delta\delta(H_6)$ corresponding to $P_{g-g} = 100\%$, bearing in mind that the changes in the chemical shift of H_6 resulting from introduction of a 5′-*O*-methyl are not due to a change in the equilibrium syn–anti, since the chemical shift of $H_{1'}$, both in the C and araC derivatives, is unaffected by such methylation, and is very sensitive to a change in the syn–anti equilibrium.

Consequently, the slopes of the lines, or the values of $\Delta\delta(H_6)$ corresponding to $P_{g-g} = 100\%$, will depend on the population of the form anti and the distance between the exocyclic $O_{5'}$ and H_6, dependent in turn on the pentose conformation. The dependence of $\Delta\delta(H_6)$ on this distance will, of course, be rather complex since the shielding of H_6 is due to OCH_3 magnetic anisotropy.

Note that the derivatives 2′,3′,5′-$m_3$araC, 3′,5′-$m_2$araC, 2′,5′-$m_2$araC, and 5′-maraC, all with comparable pentose conformations, fit reasonably well to the straight line drawn through the intersection of the coordinate axes, indicating that the proportion of the population anti is similar for all three. The fact that an additional methyl on the 2′-O does not appreciably perturb the equilibrium syn–anti emphasizes the preference for the form anti. The high values of $\Delta\delta(H_6)$, about 0.29 ppm, for these derivatives corresponding to $P_{g-g} = 100\%$ implies that the form anti is strongly favored. Finally, bearing in mind that the population of the form anti is conserved on introduction of a 5′-*O*-methyl, one may anticipate that 2′-maraC, 3′-maraC, 2′,3′-$m_2$araC, and araC itself will all exhibit a decided preference for the form anti.

An analogous analysis was carried out for the cytidine derivatives, using experimental points for 5′-mC and 2′,3′,5′-m_3C (Figure 6), profiting from the observation that 5′-O-methylation again is without effect on the equilibrium syn $\rightleftharpoons$ anti ($\Delta\delta(H_{1'}) \simeq 0$). The value of $\Delta\delta(H_6)$ for $P_{g-g} = 100\%$ is, in this case, 0.18 ppm, in line with the approximately 1.5–2-fold greater $O_{5'}$–H_6 distance in the ribonucleosides as compared to the arabinonucleosides, in agreement with the analysis of the pentose conformations (see above), hence not due to any change in syn $\rightleftharpoons$ anti equilibrium. The obvious conclusion is that the form anti is favored here as well, and (by means of the same arguments used for the arabino-

nucleosides) applies also to 2′,3′-m_2C and cytidine itself.

Probably similar conclusions apply to 2′-mC and 3′-mC in view of the marked similarity of the ribose conformation of these derivatives with that of the others.[45,46]

Conformations of *O*′-Methyl Groups. The changes in proton chemical shifts resulting from the introduction of methoxy groups will also depend on the conformations of the methyl substituents about the C–O bonds. Prediction of the magnitude of the neighbor anisotropy effect on chemical shifts is associated with considerable difficulties, but the data at our disposal do permit some qualitative conclusions, bearing in mind that the observed modification in chemical shift for a given proton will also depend on its displacement from the anisotropic group. In the following discussion, reference should be made to Table II and Figure 7, as well as to CPK space-filling models.

(1) Ribonucleosides. (a) 3′-*O*-Methyl. The negligible changes in chemical shifts of $H_{5'}$, $H_{5''}$, and $H_{1'}$ point to low populations of the forms trans-$C_{2'}$ and trans-$H_{3'}$, respectively. By contrast, the marked deshielding of $H_{2'}$ by the 3′-*O*-methyl is consistent with a preference for the form trans-$C_{4'}$.

(b) 2′-*O*-Methyl. The imperceptible change in chemical shift of the 2′-*O*-methyl protons on introduction of a 3′-*O*-methyl suggests a preference for the form trans-$C_{3'}$. If the conformation were trans-$C_{1'}$, one would anticipate a marked effect of a 3′-*O*-methyl on the chemical shift of the 2′-*O*-methyl and *vice versa*. No such effect is observed. Furthermore, if the conformation were trans-$H_{2'}$, this would result in a marked effect on $H_{4'}$, which is not the case.

(c) 5′-*O*-Methyl. The minimal change in chemical shift of $H_{3'}$ on introduction of a 5′-*O*-methyl testifies to the low population of trans-$H_{5'B}$ in the form gauche–gauche, and of trans-$H_{5'C}$ for the form trans–gauche of the exocyclic group. The slightly greater effect on $H_{5'}$, as compared to $H_{5''}$, is probably due to the conformation trans-$H_{5'C}$ in the form gauche–trans.

(2) Arabinonucleosides. (a) 2′-*O*-Methyl. The net observed shielding of H_6, $H_{5'}$, and $H_{5''}$ due to introduction of a 2′-*O*-methyl is consistent with a conformation for the latter of trans-$H_{2'}$. The slightly greater effect on $H_{5''}$ relative to $H_{5'}$ is due to the higher population of the form gauche–trans of the exocyclic group.

(b) 3′-*O*-Methyl. The virtually negligible effect of a 3′-*O*-methyl on $H_{5'}$ and $H_{5''}$ argues against the form trans-$C_{2'}$, whereas the marked effect on $H_{1'}$ indicates some participation of the form trans-$H_{3'}$. That the effect on $H_{1'}$ is not due to any change in ϕ_{CN} is testified to by the fact that there is only a negligible change in chemical shift of H_6.

(c) 5′-*O*-Methyl. As in the case of the ribonucleosides, the small effect of a 5′-*O*-methyl on $H_{3'}$ excludes trans-$H_{5'B}$ in the form gauche–gauche and trans-$H_{5'C}$ in the form trans–gauche. On the other hand, the more pronounced effect, as compared to the ribonucleosides (above), on $H_{5'}$ relative to $H_{5''}$ favors the conformation trans-$H_{5'C}$ in the highly populated form gauche–trans. In agreement with this is the pronounced deshielding of $H_{4'}$ by the 5′-*O*-methyl, which is slightly greater in the arabinonucleosides than the ribonucleosides.

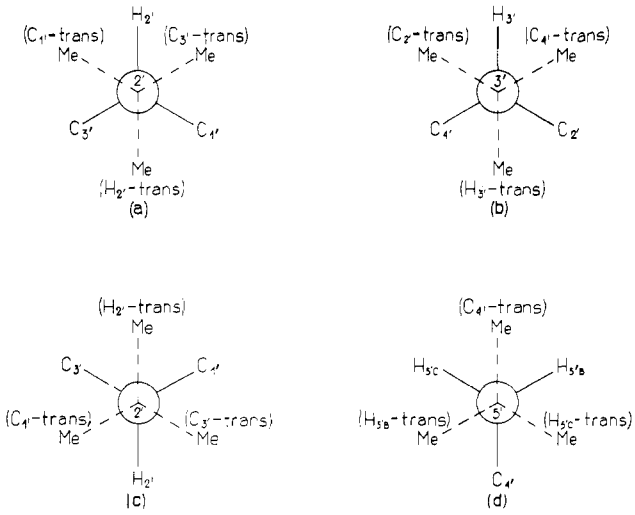

Figure 7. Newman projections about the bonds O_i–C_i (i = 2′, 3′, 5′) of the three classical rotamers of *O*′-methyl groups for cytosine ribonucleosides (a, b, and d corresponding to 2′-, 3′-, and 5′-*O*-methyl) and arabinonucleosides (c, b, and d corresponding to 2′-, 3′-, and 5′-*O*-methyl).

Finally, it should be noted that the use of the coupling constant $J_{H_i-C_i-O_i-CH_3}$ (i = 2′, 3′, 5′), the value of which is <0.2 Hz, for the establishment of the *O*′-CH_3 conformations,[15] involves some degree of uncertainty because of the absence of data on the values of coupling constants *via* a zig-zag pathway.

Influence of pH. In view of the intimate dependence of nucleoside conformation on the interaction between the pentose ring and the aglycone, it appeared of interest to examine the effect of pH on conformation. In the case of cytidine (and also araC), a change in pH from neutral to acid medium leads to protonation of the cytosine ring N_3 ($pK \simeq 4.1$), with accompanying modification in electronic structure. This might also bring into play presumed tautomerism of the cytosine ring.

The spectral parameters for cytidine were measured at three pH values, 1.5, 4, and 6, corresponding to the protonated, half-protonated and neutral forms, respectively. No detectable change in chemical shift of $H_{1'}$ was observed, pointing to the invariancy of the equilibrium syn–anti. The value of $J_{1'-2'}$ increased by only 0.2 Hz in going from pH 1.5 to 6, showing that the accompanying modification of the ribose conformation was small.

The values of $J_{4'-5'}$ and $J_{4'-5''}$ were not detectably altered over the above pH range, testifying to maintenance of the exocyclic 5′-CH_2OH conformation. The observed changes in chemical shifts of $H_{5'}$ and $H_{5''}$, due to the increased shielding of $H_{5'}$ and $H_{5''}$ by about 0.015–0.020 ppm at pH 6 relative to pH 1.5, are too small to be considered as resulting from a shift in the syn–anti equilibrium.

Furthermore, the values of the coupling constants for the pentose protons of araC at pH 8 ($J_{1'-2'}$ = 4.8 Hz; $J_{2'-3'}$ = 4.0 Hz; $J_{3'-4'}$ = 5.1 Hz; $J_{4'-5'}$ =

(45) An analogous analysis has been made of the spectra of the corresponding *O*′-methyluridines, and also leads to the conclusion that these compounds are all predominantly in the anti form.

(46) In a recent paper [W. Hutzenlaub and W. Pfleiderer, *Chem. Ber.*, **106**, 665 (1973)] on the synthesis of the *O*′-benzyl derivatives of cytidine, the published pmr spectra for 2′-, 3′-, and 5′-*O*-benzylcytidines demonstrate chemical shifts for the H_6 of 8.07, 8.04, and 7.79 ppm, respectively. This may be interpreted only as a strong preference for the anti form.

3.3 Hz; $J_{4'-5''}$ = 5.8 Hz[47]) are so close to the corresponding values at pH 1.5 (Table I) as to indicate at most only minor modifications in the conformation of the arabinose ring, as well as of the exocyclic 5'-CH_2OH.

Apart from the foregoing, it is worth drawing attention to the breadths ($\nu_{1/2}$) of the H_5, H_6, and $H_{1'}$ lines. To an accuracy of ±0.2 Hz, these are unaltered over the pH range 1.5–6, contrary to the observations of Lee, *et al.*,[48] and to their conclusion that cytidine exists to the extent of 15% in the imino form. Our own results are in agreement with those of Wong, *et al.*,[49] who showed that the pH dependence of the H_5 line width is probably due to the presence of paramagnetic ion impurities.

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Rates of Nonenzymatic Deamidation of Glutaminyl and Asparaginyl Residues in Pentapeptides

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Abstract: The synthesis of 42 peptides that contain glutaminyl or asparaginyl residues is reported. The deamidation rates of these peptides have been measured in pH 7.4, I = 0.2, 37.0° phosphate buffer. These deamidation half-times vary between 18 days and 9 years and show the effects of intramolecular steric hindrance and charge of the residues beside the glutaminyl or asparaginyl residue. These findings are discussed in reference to the hypothesis that deamidation serves as a molecular timer of protein turnover and of organismic development and ageing.

It has been suggested that sequence-dependent nonenzymatic amide hydrolysis, deamidation, of glutaminyl and asparaginyl residues in peptides and proteins may serve as a general molecular timer of biological processes.[1] The usefulness of deamidation as a molecular timer depends upon the width of the distribution function of deamidation rates that is available in peptides and proteins. We report here the synthesis of 24 pentapeptides of the type Gly*XxxGlnYyyGly and 18 pentapeptides of the type Gly*XxxAsnAlaGly and measurement of the deamidation rates of these peptides in pH 7.4, I = 0.2, 37.0° phosphate buffer. Deamidation of these peptides seems to be first order in peptide concentration at a peptide concentration of 0.0010 *M*. The half-time for deamidation varies from 96 to 3400 days for the glutaminyl peptides and from 18 to 500 days for the asparaginyl peptides.

Experimental Section

Peptide Synthesis. The peptides were synthesized by the usual methods of Merrifield solid-phase peptide synthesis;[2-7] 1% cross-linked polystyrene resin, Boc-L-alanine, Boc-nitro-L-arginine, Boc-*p*-nitrophenyl L-asparaginate, Boc-β-Bz-L-aspartic acid, Boc-*S*-Bz-L-cysteine, Boc-γ-Bz-L-glutamic acid, Boc-*p*-nitrophenyl L-glutaminate, Boc-glycine, Boc-*im*-TOS-L-histidine, Boc-L-isoleucine, Boc-L-leucine, Boc-ε-CBz-L-lysine, Boc-L-methionine, Boc-L-phenylalanine, Boc-L-proline, Boc-*O*-Bz-L-serine, Boc-*O*-Bz-L-threonine, Boc-L-tryptophan, Boc-*O*-Bz-L-tyrosine, and Boc-L-valine were used in the synthesis. Part of these reagents were obtained from Fox Chemical Co. and part were synthesized in our laboratory by standard methods.[5] The Boc-glycine used for the amino terminal residue in each peptide was labeled at the carboxyl carbon with $^{14}C^{8}$ at 0.5 Ci/mol. Boc-nitro-L-arginine was coupled in dimethylformamide, DMF, for 2 hr with dicyclohexylcarbodiimide, DCC; Boc-*p*-nitrophenyl L-asparaginate and Boc-*p*-nitrophenyl L-glutaminate were coupled overnight in DMF + 1% v/v acetic acid; all other residues were coupled for 2 hr in CH_2Cl_2 with DCC. Boc groups were removed with trifluoroacetic acid, TFA, CH_2Cl_2, and anisole at 49:49:2 (v/v); 1% dithioethane was added to this mixture when the peptide contained tryptophan.[9] Resin peptides were neutralized by triethylamine in CH_2Cl_2 at 15:85 (v/v); 1 g of resin with 0.17 m*M* Boc-glycinate on it was used for each synthesis. Occasionally during the syntheses the resin did not show a negative reaction by a ninhydrin test[10] after the coupling reaction. These

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The experiments described herein were performed by J. W. Scotchler and J. H. McKerrow in partial fulfillment of the requirements of the Ph.D. degree as described in ref 15 and 16. All of the experiments on glutaminyl peptides were performed by Scotchler and all of those on asparaginyl peptides were performed by McKerrow. Separate manuscripts with these students as principal authors were originally submitted. These manuscripts were combined at the suggestion of the editors.

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