trans), and 1085 cm.$^{-1}$ (C—O—C). The ultraviolet spectrum is practically identical with that of Ib. *Anal.* Calcd. for $C_{14}H_{14}O_2$: C, 78.48; H, 6.59. Found: C, 78.79; H, 6.70. The compound is more stable than its acetate; when exposed to light it turns brown.

Hydrogenation of Ichthyothereol (Ia). Ichthyothereol (50 mg.) was hydrogenated with 25 mg. of 12% palladium-on-charcoal catalyst, in tetrahydrofurane. Purification was accomplished on a 5-g. silica gel column, eluting by means of a gradient between 50 ml. of benzene and 50 ml. of ethyl ether. Fractions of 5 ml. each were collected. Pure perhydroichthyothereol was present in fractions 6 and 7, as a colorless oil, showing no infrared maxima indicative of unsaturation.

Hydrogenation of Ichthyothereol Acetate (Ib). Hydrogenation was performed in the same way as in the case of Ia. The crude hydrogenated product (100 mg.) was purified on a 10-g. silica gel column, eluting with a gradient between 150 ml. of petroleum ether (b.p. 30–60°) and 150 ml. of benzene. The pure product was present in fractions 16 and 17 (colorless oil, showing no infrared maxima indicative of unsaturation).

Preparation of Ketone III. Perhydroichthyothereol (15 mg.) in acetone (10 ml.) was oxidated with Jones reagent.[12] Isolation via ether gave the crude ketone which was purified by a short-path distillation (bath temp. 100° at 0.07 mm.). This afforded the ketone III as a viscous oil, $[\alpha]_D$ −28°, O.R.D. in methanol, $[\phi]_{320}$ −777°, $[\phi]_{277}$ +643°; amplitude, a = 14.

Interaction and Association of Bases and Nucleosides in Aqueous Solutions. IV. Proton Magnetic Resonance Studies of the Association of Pyrimidine Nucleosides and Their Interactions with Purine[1b]

M. P. Schweizer,[2a] Sunney I. Chan,[2b] and P. O. P. Ts'o[2a]

Contribution from the Department of Radiological Sciences, The Johns Hopkins University, Baltimore, Maryland, and the Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, California.[1a] *Received May 26, 1965*

The mode of self-association of purines has been elucidated by p.m.r. as reported previously. Association of purines is manifested by ring-current magnetic anisotropy effects which result in shifts of the resonance lines to higher field with increasing concentration. Such shifts were not found in the association of nonaromatic uridine, cytidine, and thymidine in solutions or mixtures. However, protons of these pyrimidine nucleosides are shifted upfield with increasing purine concentration; for example, over the purine concentration range 0.0–1.0 m (D_2O, 35°), H-6 and CH_3 of thymidine are shifted by 0.40 and 0.34 p.p.m., respectively. This effect falls off progressively as the proton distance from the ring increases, i.e., H-1′ by 0.31 and H-5′ by 0.08–0.10 p.p.m. The effect of purine on deoxyribose is negligible. Purine-induced changes in the thymidine sugar patterns are also observed. All these data strongly suggest that the purine–nucleoside interaction takes place at the pyrimidine base through vertical ring stacking. The strong self-association of purine in stacks can be reduced by addition of these nonaromatic pyrimidine nucleosides, presumably by insertion or destacking.

Introduction

In the continuing quest for knowledge concerning the molecular basis of nucleic acid structure and interaction, we have recently initiated a program to study systematically the association of bases and nucleosides in aqueous solution. The results from these initial efforts have been reported in the preceding papers of this series.[3–5] The information obtained from the investigation of these monomer systems has provided new insights into possible interactions of the monomeric units in the polymeric nucleic acid state.

Insight into the interactions of the monomers has come from two sources. From osmotic studies[3,4] it was demonstrated that these bases and nucleosides exhibit a high degree of association in neutral aqueous media. It was further established that interactions between purine bases and nucleosides were more favorable than cross-interactions between purine and pyrimidine bases and nucleosides, and that these latter interactions were in turn more favorable than interactions among the pyrimidine bases and nucleosides themselves. From proton magnetic resonance studies[5,6] the mechanism of the self-association of purine and 6-

(1) (a) Contribution No. 3253. Paper presented in part at the Ninth Annual Meeting of the Biophysical Society, San Francisco, Calif., Feb. 1965. (b) This work was supported in part by Grants No. GM 10802-03 and GM-K3-16-704 from the National Institutes of Health, U. S. Public Health Service, and by Grant No. GB-767 from the National Science Foundation.

(2) (a) Department of Radiological Sciences, The Johns Hopkins University; (b) Gates and Crellin Laboratories of Chemistry, California Institute of Technology, Pasadena, Calif.

(3) P. O. P. Ts'o, I. S. Melvin, and A. C. Olson, *J. Am. Chem. Soc.*, **85**, 1289 (1963).

(4) P. O. P. Ts'o and S. I. Chan, *ibid.*, **86**, 4176 (1964).

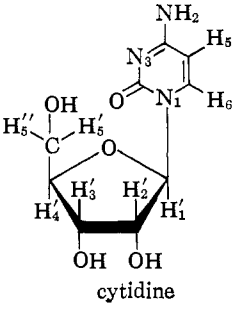
(5) S. I. Chan, M. P. Schweizer, P. O. P. Ts'o, and G. K. Helmkamp, *ibid.*, **86**, 4182 (1964).

(6) Similar experimental findings for purine and nucleotides have been independently observed by Jardetzky. See O. Jardetzky, *Biopolymers Symp.*, **No. 1**, 501 (1964).

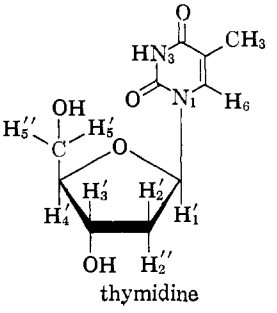
Table I. Concentration Dependence of Chemical Shifts for Cytidine, Thymidine, and Uridine Protons at 35°[a]

Concn., *m*	Chemical shifts from SDSS, c.p.s. H-5	H-6	H-1′	(H-2′, H-3′, H-4′)			H-5′	H-5″
				A. Cytidine				
0	363.5	469.8	354.3		252.8		230.0	233.8
0.78[b]	359.5	467.4	352.5		251.3		230.0	233.5
	Δδ +4.0	+2.4	+1.8		+1.5		0.0	+0.3
	CH_3	H-6	H-1′	H-2′, H-2″	H-3′	H-4′	H-5′	H-5″
				B. Thymidine				
0	111.8	457.5	376.4	141.8	268.3	241.2	226.4	228.7
0.35[b]	111.8	456.9	373.9	139.8	267.3	239.2	224.2	228.7
	Δδ 0.0	+0.6	+2.5	+2.0	+1.0	+2.0	+2.2	0.0
	H-5	H-6	H-1′	H-2′	H-3′	H-4′	H-5′	H-5″
				C. Uridine				
0	353.0	469.7	353.8	259.7	253.2	246.9	228.0	233.4
0.77[b]	350.1	468.2	350.9	257.6	251.9	245.1	226.7	230.9
	Δδ +2.9	+1.5	+2.9	+2.1	+1.3	+1.8	+1.3	+2.5

[a] Solvent D_2O. Numbering of the nucleoside atoms shown, *e.g.*, with cytidine and thymidine.



cytidine



thymidine

[b] Concentrations approaching limits of solubility.

methylpurine was elucidated to be that of vertical ring stacking. The positions of the proton resonances in these molecules were noted to shift to higher fields with increase in the concentration of the bases. In view of the aromaticity of these two bases, these shifts were attributed to ring-current magnetic anisotropy of neighboring molecules in stacks of various sizes. A good correlation was established between the experimental shifts and calculated shifts based upon population distributions of the associated species obtained from analysis of the osmotic data.

We wish to report here proton magnetic resonance results obtained on the self-association of cytidine, thymidine, and uridine, their cross-interactions with one another, and their cross-interactions with purine. Purine was intended as a substitute for the naturally occurring purine nucleosides owing to solubility restrictions.

Experimental Section

Materials. Purine, uridine, cytidine, thymidine, and 2-deoxy-D-ribose of the highest available purity were purchased from Sigma Chemical Co., St. Louis, Mo. Deuterium oxide, 99.5 mole %, was obtained from Matheson Coleman and Bell. Eastman Organic supplied the sodium 2,2-dimethyl-2-silapentane-5-sulfonate (SDSS).

Instrumentation. N.m.r. spectra were recorded on both Varian Associates A-60 and DP-60 spectrometers. Normal probe temperature for the A-60 spectrometer was 35 ± 1°. An A-60 spectrometer equipped with a Varian V-6057 variable temperature accessory was used in conjunction with the variable temperature studies.[7] Chemical shifts were measured in c.p.s. either from SDSS in D_2O as an external standard or from liquid tetramethylsilane (TMS) sealed off in capillary tubing. The data presented in Figure 4 were obtained using the DP spectrometer at 56.4 Mc., and the chemical shifts here were referred to external chloroform. In all cases, the shifts can be measured with a precision of ±1 c.p.s. The reported shifts have not been corrected for bulk susceptibility but it is estimated that this correction would not exceed 2–3 c.p.s.

The apparent pH values of the various nucleoside solutions (D_2O solvent) were in the range 6–7.5.

Assignment of the nucleoside proton resonances were taken from Varian Associates Catalog.[8] The spectral assignment of the proton resonances in purine has recently been firmly established.[9,10]

Results and Discussion

Pyrimidine–Pyrimidine Interactions. The concentration dependence of the chemical shifts for cytidine, thymidine, and uridine protons are presented in Table I. Each nucleoside was studied up to its solubility limit in D_2O. Shifts at infinite dilution were obtained by graphical extrapolation of the measured shifts to

(7) One of us (S. I. C.) is indebted to the Chemistry Department of California State College at Los Angeles for the use of this instrument.

(8) "High Resolution N.M.R. Spectra," Vol. II, Varian Associates, Palo Alto, Calif., 1960.

(9) M. P. Schweizer, S. I. Chan, G. K. Helmkamp, and P. O. P. Ts'o, *J. Am. Chem. Soc.*, **86**, 696 (1964); S. Matsuura and T. Goto, *Tetrahedron Letters*, **No. 22**, 1499 (1963).

(10) F. J. Bullock and O. Jardetzky, *J. Org. Chem.*, **29**, 1988 (1964).

zero nucleoside concentration. The concentration shifts (shifts with increase in solute concentration) are all upfield; however, in all cases they are small and roughly of the same magnitude to be expected for over-all increases in the diamagnetic susceptibility of the bulk solutions.

The concentration dependence of the shifts in nucleoside mixtures was also investigated. Cytidine was added to a 0.1 *m* thymidine solution in D_2O and the proton resonances of thymidine were monitored as a function of the cytidine concentration. These results are tabulated in Table II. It can be seen that increasing the cytidine concentration has only a slight shielding effect, again probably due to increase in the bulk susceptibility of the solution.

Table II. Thymidine Proton Chemical Shift Dependence upon Cytidine Concentration at 35°[a]

Cytidine, *m*		Chemical shifts from SDSS,[b] c.p.s. CH_3	H-6	H-1′	H-2′, H-2″	H-3′	H-5″
0		111.8	457.1	375.3	141.0	267.9	228.6
0.71		109.9	455.1	373.0	139.7	266.5	228.8
	Δδ	+1.9	+2.0	+2.3	+1.3	+1.4	−0.2

[a] Solvent: D_2O; 0.1 *m* thymidine. [b] Thymidine H-4′ and H-5′ resonances obscured by cytidine sugar proton bands.

Thus, in contrast to the large concentration shifts previously reported for the proton resonances of the purine bases, the concentration shifts for the pyrimidine nucleosides under study are negligible. However, cytidine, uridine,[3] and thymidine[11] have all been shown to associate in aqueous solutions by osmotic pressure lowering. Therefore, we should interpret this result on the basis that these pyrimidine nucleosides do not support ring currents as do the aromatic purine bases, so that their interactions cannot be monitored by proton magnetic resonance *via* the effect of the ring-current magnetic anisotropy. To support this contention, we note that the self-association of the aromatic pyrimidine molecule has recently been studied by p.m.r. in the same manner as the purine bases.[12]

Purine–Pyrimidine Interactions. As an indication of purine–pyrimidine interactions we have studied the effect of purine on the proton resonances of thymidine, uridine, and cytidine.

Table IIIa summarizes the gross purine effect upon thymidine protons. A more detailed presentation of the data is given in Figure 1. Marked upfield shifts are noticed, particularly for the thymine base protons and the anomeric proton H-1′. As one proceeds around the deoxyribose ring from C-1′ to C-5′, however, there is a progressive drop off or decrease in the magnitude of these upfield shifts, indicating that the purine–pyrimidine nucleoside interaction is preferentially localized at the pyrimidine base of the nucleoside so that the purine ring-current magnetic anisotropy is principally felt by the base protons. The direction of the purine-induced shifts plus their variation with distance for the respective protons from the apparent

(11) J. A. Schellman and T. Solie, University of Oregon, private communication.
(12) S. I. Chan and J. Nelson, unpublished results.

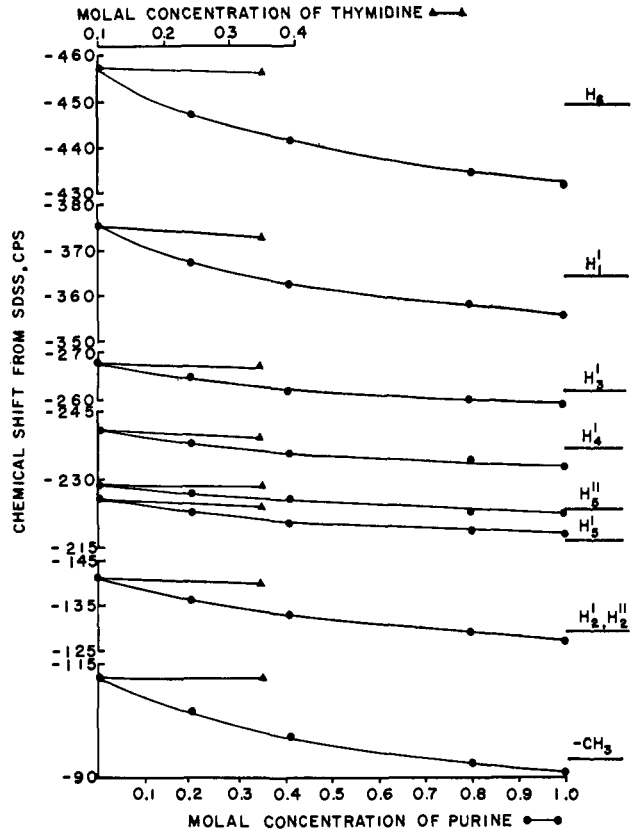


Figure 1. Chemical shift dependence of thymidine protons upon thymidine (-▲-▲-) and upon purine concentration (-●-●-) at 35° in D_2O. Shifts measured from external SDSS. Magnetic field increases from top to bottom along ordinate. Spectra obtained at 60 Mc.

site of interaction suggests that the interaction is that of vertical ring stacking of the pyrimidine and purine bases.[5]

The magnitudes of the purine-induced shifts for the base protons in thymidine at the highest purine concentration studied here are 0.40 and 0.34 p.p.m. for H-6 and CH_3, respectively. These shifts are roughly 50–60% of those found for the protons of purine and 6-methylpurine under similar conditions. Purine–pyrimidine associations have been shown to be less favorable than purine–purine interactions by our earlier osmotic studies.[3] Our present n.m.r. results appear to be consistent with these findings.

Similarly, Table IIIb summarizes the effect of purine or uridine. A more detailed presentation of the data is given in Figure 2. As expected, the purine-induced shifts are independent of the nucleoside concentration under conditions in which the solution is flooded with purine. Thus, the uridine protons in 0.11 (Table IIIb) and 0.21 *m* (Figure 2) uridine solutions exhibit similar purine-induced shifts in mixtures containing 1 *m* purine.

The magnitude of the purine effect upon uridine is smaller than that observed for thymidine. It is possible that methylation of the pyrimidine base enhances the interaction of the nucleoside with purine. The methylation of purine at the C-6 position, for example, has been shown[4] to increase the self-association tendencies of 6-methylpurine relative to purine. However, it is probable that the depicted difference in

Table III. Summary of Nucleoside Proton Chemical Shift Dependence upon Purine Concentration at 35°[a]

Purine, *m*	Chemical shifts from SDSS, c.p.s.							
	CH_3	H-6	H-1′	H-2′, H-2″	H-3′	H-4′	H-5′	H-5″
				A. 0.1 *m* Thymidine				
0	111.8	457.1	375.3	141.0	267.9	240.9	225.6	228.6
1.0	91.4	433.0	356.5	127.4	259.5	233.0	218.5	222.9
(0.0–1.0)	Δδ +20.4	+24.1	+18.8	+13.6	+8.4	+7.9	+7.1	+5.7
(0.0–1.0)	Δδ 0.34 (p.p.m.)	0.40	0.31	0.23	0.14	0.13	0.12	0.095
	H-5	H-6	H-1′	H-2′	H-3′	H-4′	H-4′	H-5″
				B. 0.11 *m* Uridine				
0	352.7	469.7	353.5	259.7	253.0	246.7	227.8	233.0
1.0	333.8	455.6	340.6	254.3	248.0	241.9	223.8	227.8
(0.0–0.1)	Δδ +18.9	+14.1	+12.9	+5.4	+5.0	+4.8	+4.0	+5.2
(0.0–1.0)	Δδ 0.32 (p.p.m.)	0.24	0.22	0.090	0.083	0.081	0.066	0.086
	H-5	H-6	H-1′	H-2′, H-3′, H-4′		H-5′	H-5″	
				C. 0.11 *m* Cytidine				
0	363.0	469.5	354.0	252.6		230.0	233.7	
1.0	335.6	449.0	340.0	244.4		224.6	227.6	
(0.0–1.0)	Δδ +27.4	+20.5	+14.0	+8.2		+5.4	+6.1	
(0.0–1.0)	Δδ 0.46 (p.p.m.)	0.34	0.23	0.14		0.09	0.10	

[a] Solvent, D_2O.

the purine effect on uridine and thymidine may at least be partially due to steric reasons. That steric factors can be important may be seen by comparing

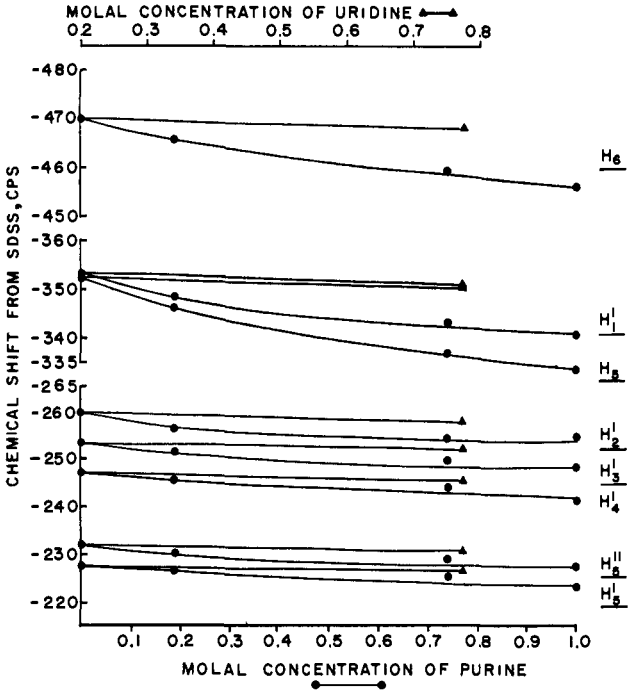


Figure 2. Chemical shift dependence of uridine protons upon uridine (-▲-▲-) and upon purine concentration (-●-●-) at 35° in D_2O. Shifts measured from external SDSS. Field increases from top to bottom along ordinate. Spectra obtained at 60 Mc.

the purine-induced shifts on the base protons in both uridine and cytidine (see Table IIIb,c.) In both cases, the effect on H-5 is greater than on H-6 by ~0.1 p.p.m. It is reasonable to ascribe this difference to steric effect of the sugar moiety attached at N-1 limiting the distance of approach of the purine molecule to the vicinity of H-6. In the case of thymidine it is possible, then, that the larger purine-induced shifts for H-6 may be partially due to the steric effect of the methyl group so that the interacting purine molecule is oriented on the average closer to the deoxyribose ring.

Table IV. Dependence of 2-Deoxy-D-ribose Proton Chemical Shifts upon Purine Concentration at 35°[a]

Purine, *m*	Chemical shifts from SDSS, c.p.s.		
	H-2′, H-2″	H-3′, H-4′, H-5′, H-5″	H-1′, H-1″
0	113.5	227.5	318.0
1.0	109.5	223.0	313.0
	Δδ +4.0	+4.0	+5.0

[a] Solvent, D_2O; 0.19 *m* 2′-deoxy-D-ribose.

The effect of added purine upon the protons of cytidine is summarized in Table IIIc. The induced shifts here are intermediate between those observed for thymidine and uridine.

Purine Effect on 2-Deoxy-D-ribose. To confirm the above interpretation of the purine-induced shifts on the proton resonances of the pyrimidine nucleosides, the effect of purine on 2-deoxy-D-ribose protons has also been studied. These results are summarized in Table IV. Two points are noteworthy. First, the shifts are small and are similar in magnitude to those observed for H-5′ and H-5″ of the pyrimidine nucleosides. Second, in contrast to the gradual attenuation of effect from H-1′ to H-5′ observed for the pyrimidine nucleosides, the effect is essentially uniform for all

the protons in the 2-deoxy-D-ribose. Since no interaction is expected between purine and 2-deoxy-D-ribose, the small high-field shifts observed for the protons of the deoxyribose are probably due to an increase in the bulk magnetic susceptibility of the solution.

Temperature Studies. The effect of temperature on the interaction of purine with thymidine and uridine was also investigated and is summarized in Table V. The data indicate that purine-induced shifts at the higher temperature (53°) are *only* reduced by 1–6 c.p.s. with respect to the 25° data. For exothermic association of purine and pyrimidine nucleosides, increasing the temperature should result in reduced stacking tendencies. This means that at elevated temperatures the competing processes of purine–purine interactions and pyrimidine–pyrimidine nucleoside interactions are reduced concurrently with the cross-interactions of purine and pyrimidine nucleoside. In other words, the activities of purine and pyrimidine nucleosides at a given concentration will be increased at higher temperature, even though the equilibrium constants for the purine–pyrimidine nucleoside associations are probably lowered somewhat. Apparently these two factors offset each other to the extent that the n.m.r. results exhibit only a small temperature effect. There are some preliminary data suggesting that purine–purine interactions are more exothermic than purine–pyrimidine nucleoside interactions.[3]

Table V. Effect of Temperature on the Purine-Induced Shifts of Nucleoside Base Protons and H-1′

	Purine-induced shifts, Δδ, c.p.s.					
	CH_3		H-6		H-1′	
Temp., °C.	0.4 *m* purine	0.8 *m* purine	0.4 *m* purine	0.8 *m* purine	0.4 *m* purine	0.8 *m* purine
	A. 0.2 *m* Thymidine					
53	10.0	16.3	9.8	16.1	6.3	11.3
25[a]	13.8	19.7	16.0	22.0	13.0	16.5
53 − 25	−3.8	−3.4	−6.2	−5.9	−6.7	−5.2
	H-5		H-6		H-1′	
	0.4 *m* purine	0.8 *m* purine	0.4 *m* purine	0.8 *m* purine	0.4 *m* purine	0.8 *m* purine
	B. 0.2 *m* Uridine					
53	10.0	16.4	7.8	12.6	6.0	10.4
25[a]	12.7	18.8	11.0	13.8	10.5	13.2
53 − 25	−2.7	−2.4	−3.2	−1.2	−4.5	−2.9

[a] Probe temperature (±1°) obtained by observing the splitting of ethylene glycol proton resonances.

Other Spectral Changes Induced by Purine. In addition to the purine-induced shifts reported above, the proton n.m.r. spectrum of thymidine was found to be further influenced by purine in the sugar–proton region. The 100 Mc. spectra[13] of the deoxyribose proton region of (a) 0.1 *m* thymidine and (b) 0.1 *m* thymidine in 1.0 *m* purine are reproduced in Figure 3 for comparison. The resonance patterns in both spectra are, from left to right, due respectively to H-3′, H-4′, (H-5′, H-5″), and (H-2′, H-2″).

(13) We are indebted to Mr. Ross Pitcher of Varian Associates' Applications Laboratory, Pittsburgh, Pa., for obtaining the 100 Mc. spectra.

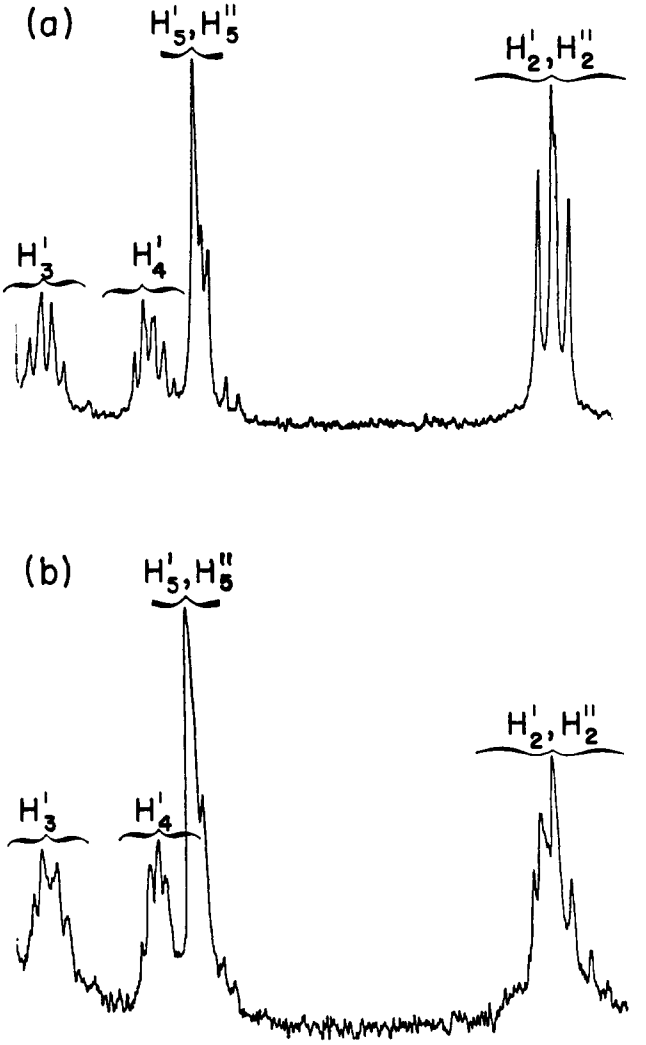


Figure 3. Effect of purine upon spectral patterns of thymidine sugar proton resonances. 100 Mc. spectra of (a) 0.1 *m* thymidine and (b) 0.1 *m* thymidine and 1.0 *m* purine; D_2O solvent.

Line broadening is evident for all the thymidine resonances in the mixture, as expected for formation of molecular aggregates.

The addition of purine apparently also alters the spectral features of the (H-2′, H-2″) proton resonance. Lemieux[14] has indicated that the H-2′ and H-2″ protons in thymidine itself are nearly equivalent. Accordingly, the proton resonance pattern for H-2′ and H-2″ protons of the 2′-deoxyribose ring is adequately predicted on the basis of an A_2MX spin–spin splitting scheme (M ≡ H-3′ and X ≡ H-1′). A quartet of equal intensity would then be expected for the 2′-protons of thymidine, as observed in Figure 3a. From the triplet of the H-1′ resonance (intensity ratio 1:2:1), we obtained $|J$H-1′, (H-2′, H-2″)$|$ = 7.0 c.p.s. as an average coupling of H-1′ with (H-2′, H-2″) in agreement with Lemieux.[14] From the (H-2′, H-2″) resonance pattern, then, $|JH$-3′, (H-2′, H-2″)$|$ = 5.5 c.p.s. As seen in Figure 3b, the addition of purine introduces further splitting of the (H-2′, H-2″) resonance pattern. This more complex spin multiplet for the (H-2′, H-2″) protons in the mixture undoubtedly arises from the changeover of the correct description for the four-spin system from

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

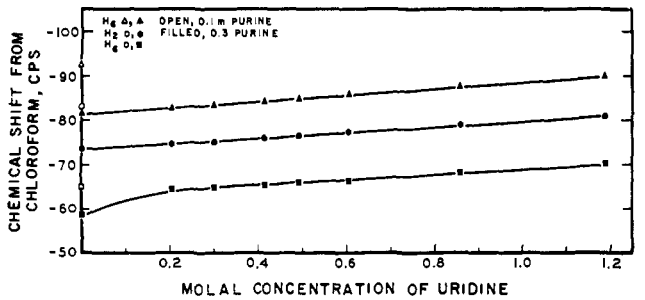


Figure 4. Effect of uridine concentration upon purine proton chemical shifts at 25° in H_2O. Shifts obtained at 56.4 Mc. from external chloroform; filled symbols 0.3 *m* purine, open symbols on ordinate due to 0.1 *m* purine.

the A_2MX to the ABMX scheme. This change in coupling scheme most certainly has its origin in the fact that H-2′ and H-2″ are no longer equivalent in the purine–nucleoside complex. Molecular models show that the 2′ proton *trans* to H-1′, thus closer to the base, should be influenced by the purine ring in the complex to a larger extent than the 2″ proton *cis* to H-1′. Therefore, the aforementioned nonequivalence of (H-2′, H-2″) is to be expected. It is observed that the H-1′ resonance pattern is not markedly altered in the mixture compared to the nucleoside solution alone. However, the coupling pattern of H-1′ with (H-2′, H-2″) should not change appreciably as a result of a small increase in the nonequivalence of the latter protons.

Whether or not the conformation at C-1′ of the nucleoside is affected by the association of purine cannot be concluded from our data; however, conformation changes at C-2′ and C-3′ are unlikely since the notable spectral changes occur only in the (H-2′, H-2″) region.

Breakdown of the Purine Stacks by Pyrimidine Nucleosides. Since purine interacts with the pyrimidine nucleosides *via* ring stacking in the same manner as for the self-association of purine it would be of interest to know whether the purine stacks are broken down by the pyrimidine nucleosides or whether the pyrimidine nucleosides merely insert between or adhere to the ends of existing purine stacks.

In an effort to answer these questions we may exploit the magnetic "inertness" of the pyrimidine nucleosides. The breakdown of and/or insertion of pyrimidine nucleoside into purine stacks should lead to a diminution of the purine concentration shifts. This deshielding of the purine protons in purine–nucleoside mixtures compared with purine solutions of the same concentration has been noticed in the experiments cited in the previous sections. For instance, in mixtures containing 0.97 *m* purine and 0.1 *m* cytidine, thymidine, or uridine, the purine proton resonances are generally shifted 4–6 c.p.s. to lower fields compared to a 0.97 *m* purine solution. This result suggests that there is indeed breakdown of the purine aggregates and/or insertion of pyrimidine nuclosides in between the purine stacks.

In Figure 4, the proton resonance shifts of a 0.3 *m* purine solution are reported as a function of successively higher concentrations of uridine. These data, which were obtained at 25° and 56.4 Mc., indicate that as uridine is added to 0.3 *m* purine the purine resonances are progressively shifted to lower fields. The open symbols on the ordinate at zero uridine concentration represent proton chemical shifts of 0.1 *m* purine relative to chloroform. At 1.2 *m* uridine,[15] the H-2 and H-6 resonances of 0.3 *M* purine are within 2 c.p.s. of the 0.1 *m* values. Interestingly enough, H-8 in this same solution has shifted 5 c.p.s. downfield beyond the 0.1 *m* purine value. A closer examination of the data reveals that H-8 is, for some peculiar reason, being deshielded faster by uridine than either H-2 or H-6 at low uridine concentrations. In fact, it is in the uridine concentration range 0.0 to 0.2 *m* that H-8 is shifted downfield 5 c.p.s. more than H-2 and H-6. Beyond 0.2 *m* uridine, all three of the purine protons are deshielded to the same degree per incremental increase in uridine concentration. It is possible that, in addition to the "destacking" effect of uridine as manifested by the deshielding of the H-2, H-6, and H-8 protons of purine, there is also hydrogen bonding between uridine and purine at N-7 or N-9 of the imidazole ring. The additional deshielding of the H-8 proton may then be rationalized on this basis. At any rate, the above results demonstrated that the strong purine–purine interaction as monitored by n.m.r. can be decreased by addition of "inert" uridine to the solution.

Conclusion

In this paper we have presented additional evidence to support and reaffirm the concept that purine and pyrimidine bases and nucleosides interact *via* vertical ring stacking of the bases in aqueous solution. A comparison of the purine-induced shifts of the pyrimidine base protons with the concentration shifts previously reported for the protons of purine and 6-methylpurine suggests that the magnitude of the purine–pyrimidine interactions is ~50–60% of the purine–purine interactions. An attempt was also made to study pyrimidine–pyrimidine interactions in this work by investigating the association of the pyrimidine nucleosides both in homogeneous solution and in mixtures. The lack of ring-current magnetic anisotropy in the pyrimidine bases of these nucleosides prevented us from monitoring these interactions by proton magnetic resonance.

The above observations for the monomeric bases and nucleosides provide an important basis for interpreting the proton n.m.r. spectra of these bases in the nucleic acid state. The proton magnetic resonance spectra of a purine or pyrimidine base is strongly dependent upon its magnetic environment, and in nucleic acids would therefore be dependent upon whether its neighbor is a purine or pyrimidine base. The high-resolution n.m.r. spectrum of denatured DNA was recently observed by McDonald, Phillips, and Penman.[16] A 7 c.p.s. splitting was noted for the thymine methyl protons. In the light of our present observations it is tempting to attribute this splitting to thymine molecules "locked" in two different types of magnetic environment along the polynucleotide strand, namely, those in the vicinity of purine and pyrimidine bases.

(15) Purine enhances the solubility of uridine beyond its normal solubility in D_2O.

(16) C. C. McDonald, W. D. Phillips, and S. Penman, *Science*, **144**, 1234, (1964).

It would have been difficult to predict beforehand the magnitude of this splitting for the thymine protons in these concentrated DNA solutions (at 95°) on the basis of the purine–pyrimidine nucleoside shifts reported in this work. Shifts of 5–10 c.p.s. were observed in mixtures of 0.1 *m* thymidine and 0.2 *m* purine.

Jardetzky[6] has compared the proton resonance shifts of 0.2 *M* mononucleotide solutions with the corresponding polynucleotide solutions (0.2 *M* in mononucleotides). The differences, termed "polymerization shifts," were found to be 20–40 c.p.s. upfield for base protons and H-1′ of the AMP–poly-A pair. From our earlier work[5] upfield shifts for purine over the concentration range 0.0–0.2 *m* were 12–20 c.p.s., while over the range 0.0–1.0 *m*, 30–40 c.p.s. values were obtained. The magnitude of these latter shifts, originating from association of monomers, is therefore comparable to the "polymerization shifts." It is to be noted that the bases in the polymer are held rigidly at a certain intramolecular distance from each other.

The major question, however, is whether or not the properties of adenine and guanine can be approximated by purine as a substitute. Owing to solubility restrictions, it has not been possible until quite recently to obtain data on adenine–adenine or adenine–thymine interactions at the nucleoside level in water. Now, however, we are able to take advantage of the high solubility of 2′-O-methyladenosine to study these interactions. The data indicate that increase in concentration of 2′-O-methyladenosine does result in upfield shifts for adenine protons in homogeneous solution and for thymine and adenine protons in nucleoside mixtures. The values were about the same as the purine-induced shifts at the same concentration.[17]

The results of this communication lead quite logically to studies of base–base interactions in mono-, di-, and oligonucleotides. These investigations are currently in progress.

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(17) A. D. Broom, M. P. Schweizer, and P. O. P. Ts'o, unpublished results.

Communications to the Editor

The Effects of Steric Compression on Chemical Shifts in Half-Cage and Related Molecules[1,2]

Sir:

The effects of steric compression, both intermolecular[3] and intramolecular,[4] on chemical shifts have been reported numerous times. In general, a low-field shift (*ca.* 0.1–0.6 p.p.m. for protons attached to carbon) is observed, irrespective of the nature of the atom which is in juxtaposition to the proton being examined.

We now report a new kind of steric compression effect, *viz.*, a shielding of one of the protons in a CH_2 group when the other proton is strongly compressed by an oxygen function. We also report some unusually large deshielding effects (1–4 p.p.m.).

The compounds we have examined (I–IV) have the half-cage or *endo,endo*-fused skeletons related to the birdcage hydrocarbon.[5] With their rigid geometries and enormous H–H or H–O steric oppositions, they are ideally suited for the study of effects of steric compression on chemical shifts. These compounds are already known to display C–H stretching bands at abnormally high frequencies ascribed to a strong compression of at least one CH group.[5b,d]

As is clear from the data summarized in Table I, the inside protons are strongly deshielded. The α proton, H_a, in the O-outside half-cage alcohol I-OH (τ 5.52) is at much lower field than the corresponding proton in the *endo,exo*-fused isomer IX-OH (τ 6.52) or in the simpler model compound, *exo*-norborneol (VII, τ 6.48). This observed $\Delta\delta$ of *ca.* 1 p.p.m. is a minimum figure for the size of the steric effect on chemical shift because of the effect of magnetic anisotropy of nearby

(1) (a) Research was supported in part by National Science Foundation Grants No. G-7423 and GP 3780. (b) Research was sponsored in part by the U. S. Army Research Office (Durham). (c) Research was supported in part by a grant from the Petroleum Research Fund administered by the American Chemical Society. Grateful acknowledgement is hereby made to the donors of this fund.

(2) Reported in part at the Symposium on Cagelike Molecules at the 148th National Meeting of the American Chemical Society, Chicago, Ill., Sept. 1, 1964.

(3) (a) The effect of pressure on the chemical shifts of compounds in the gaseous state has been studied: S. Gordon and B. P. Dailey, *J. Chem. Phys.*, **34**, 1084 (1961); W. T. Raynes, A. D. Buckingham, and H. J. Bernstein, *ibid.*, **36**, 3481 (1962); (b) solvent effects are also relevant to this problem: A. A. Bothner-By, *J. Mol. Spectry*, **5**, 52 (1960); A. D. Buckingham, T. Schaefer, and W. G. Schneider, *J. Chem. Phys.*, **32**, 1227 (1960); T. Schaefer, W. F. Reynolds, and T. Yonemoto, *Can. J. Chem.*, **41**, 2969 (1963).

(4) C. Reid, *J. Mol. Spectry*, **1**, 18 (1957); W. Nagata, T. Terisawa, and K. Tori, *J. Am. Chem. Soc.*, **86**, 3746 (1964); D. R. Arnold, D. J. Trecker, and E. B. Whipple, *ibid.*, **87**, 2596 (1965). Many other papers, too numerous to enumerate here, refer to small low-field shifts, which have often been ascribed to van der Waals interactions.

(5) (a) S. Winstein, *Experientia Suppl.*, **2**, 137 (1955); (b) L. de Vries and S. Winstein, *J. Am. Chem. Soc.*, **82**, 5363 (1960); (c) P. Bruck, D. Thompson, and S. Winstein, *Chem. Ind.* (London), 405, 590 (1960); (d) D. Kivelson, S. Winstein, P. Bruck, and R. L. Hansen, *J. Am. Chem. Soc.*, **83**, 2938 (1961); (e) R. Howe and S. Winstein, *ibid.*, **87**, 915 (1965); (f) compounds I-OH, m.p. 131–132°, and II-OH, m.p. 108–109°, have been previously reported.[5a–c] The III-OH, m.p. 197–198°, was obtained by lithium aluminum hydride reduction of the half-cage ketone, m.p. 165–167°, and IV-OH, m.p. 90–91°, was similarly prepared from the corresponding ketone, m.p. 70–71°, 90–91° (two melting points). Satisfactory carbon and hydrogen analyses were obtained for the new compounds reported.