Nuclear Magnetic Resonance Studies of the Solution Properties of the Antiviral Nucleoside, 1-β-D-Ribofuranosyl-1,2,4-triazole-3-carboxamide, the Corresponding 5′-Phosphate, and Related Triazole Nucleosides†

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ABSTRACT: The solution properties of the antiviral nucleoside, 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide (ribavirin), its 5′-phosphate, and several inactive triazole nucleosides have been determined by proton magnetic resonance at 220 MHz. Ribavirin and the corresponding inactive 5-carboxamide isomer are quite similar in that the furanose conformations slightly favor 3′-endo, the $C_{4'}$–$C_{5'}$ exocyclic conformations are mainly gauche,gauche and the glycosidic conformations are mostly syn. In contrast, the 5′-monophosphate of ribavirin, mechanistically considered to be the active antiviral species, has more 2′-endo furanose pucker, contains more gauche,gauche $C_{4'}$–$C_{5'}$ and $C_{5'}$–$O_{5'}$ rotamers and preferentially adopts the antiglycosidic conformation. Apparently the major criterion for antiviral activity for these compounds resides in a particular spatial distribution of various substituents, governed by location of nitrogens in the heterocycle and site of glycosylation, rather than in a unique solution conformation.

The synthesis and activity of the broad spectrum antiviral nucleoside, 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide (ribavirin, I, Chart I), have been the subject of recent reports (Witkowski *et al.*, 1972; Sidwell *et al.*, 1972). On the basis of nmr[1] studies (Kreishman *et al.*, 1972) and X-ray analysis (Prusiner and Sundaralingam, 1973), the structure of ribavirin was distinguished from an inactive isomer, II. The solid-state furanose and glycosidic conformations were established by Prusiner and Sundaralingam, who also speculated that the antiviral activity of ribavirin may be due to the fact that it can act as a guanosine substitute in hydrogen-bonding interactions with cytidine. In this regard, it has been shown that the mechanism of action for ribavirin (as the 5′-phosphate; Streeter *et al.*, 1973) involves curtailment of GMP biosynthesis by competitive inhibition of IMP-dehydrogenase. In terms of interaction with the active site of this enzyme, ribavirin 5′-phosphate apparently mimics IMP. We have employed ^{1}H nmr (pmr), ^{31}P nmr, and ^{13}C nmr (cmr) techniques in order to obtain information about the relationship of the solution conformation and interaction of ribavirin and ribavirin 5′-phosphate to their antiviral activities. In addition, the solution properties of the inactive 5-carboxamide isomer II were investigated.

Experimental Section

Materials. Syntheses of the triazole derivatives studied here have been reported [I and II, Witkowski *et al.*, 1972; III (ammonium salt), Streeter *et al.*, 1973; IV, Alonso *et al.*, 1970; V and VI, Lehmkuhl *et al.*, 1972]. Deuterated solvents D_2O (99.8% deuterium) and Me_2SO-d_6 (99.5% deuterium) were from Thompson Packard, Inc., Little Falls, N. J. All other chemicals were of highest commercial quality. Samples used in nuclear Overhauser experiments were degassed, purged with nitrogen, then capped, and immediately sealed.

Instrumentation. Pmr spectra were obtained at 60 MHz on a Perkin-Elmer-Hitachi R20A (ambient probe temperature 34°) and at 220 MHz with a Varian HR-220 (probe temperature 15°). Nuclear Overhauser experiments (NOE) were carried out on a Bruker HX-90 spectrometer at 90 MHz. Natural abundance cmr spectra were obtained at 22.6 MHz using either a Bruker HX-90 operating in the Fourier transform (FT) mode with a Nicolet 1074 Digital Equipment Corp. PDP-8/e computer package (4K memory) or a Bruker HX-90e (FT Mode) equipped with a BNC-12 computer. ^{31}P (FT) spectra were obtained on the Bruker HX-90 at 36.4 MHz. Proton chemical shifts were measured from Me_4Si capillaries in the 60- and 90-MHz spectra and from internal Me_4NBr (0.01 M) in the 220-MHz spectra.

Results and Discussion

I. Nucleoside Conformational Properties

Conformation of the Furanose Ring and the Exocyclic $C_{4'}$–$C_{5'}$ Bond. Information about the time-average furanose ring conformation in solution may be obtained from examination of vicinal H–H coupling constants. Table I contains these coupling constants for I and II, obtained by first-order analysis of the 220-MHz spectra. On the basis of these data, the furanose conformations of the biologically active and inactive nucleosides are quite similar. Several interesting features common to both nucleosides arise upon comparison of the data in Table I with the results obtained for several uracil nucleosides and nucleotides (Blackburn *et al.*, 1970; Schleich *et al.*, 1972) where the ribofuranose conformations have been described as an equilibrium mixture of 2′- and 3′-endo puckered forms and the gauche,gauche conformation about $C_{4'}$–$C_{5'}$ is preferred.

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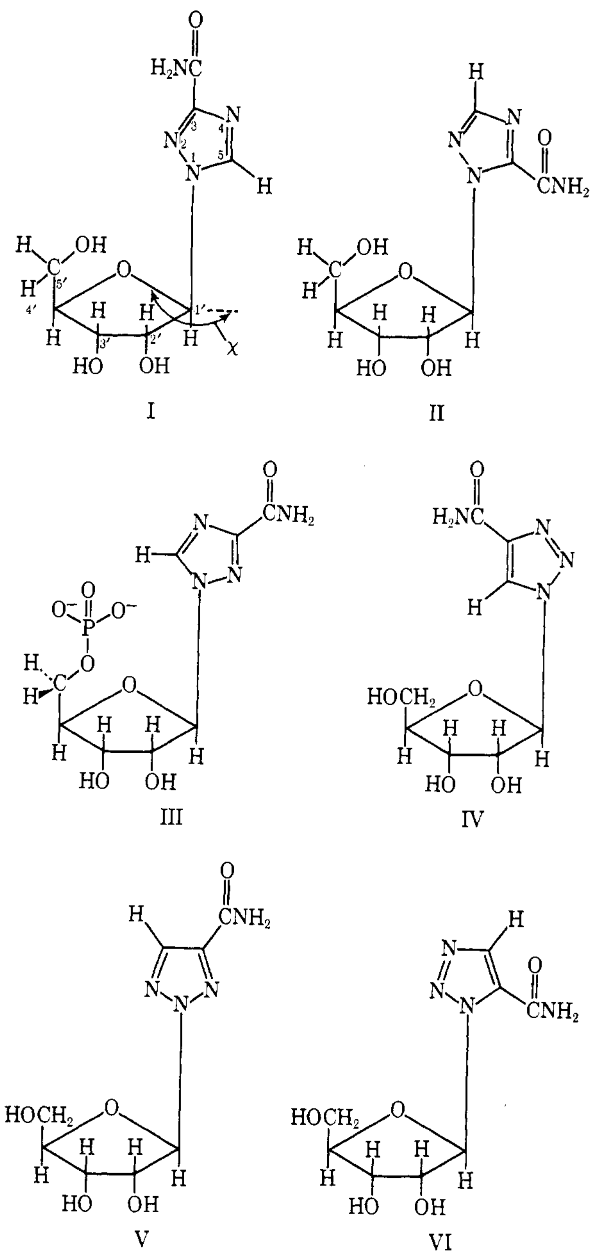
[1] Abbreviations used are: nmr, nuclear magnetic resonance; XMP, nucleoside 5′-phosphates; pmr, proton magnetic resonance; cmr, carbon-13 magnetic resonance. Ribavirin is the U. S. adopted name for 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide, previously referred to as virazole.

Chart I



The major differences in vicinal couplings between the triazole nucleosides I and II and uridine, for example, are 1′–2′ and 4′–5″, being 1.0–1.8 Hz greater and 0.8–1.4 Hz smaller, respectively, for uridine (Schleich *et al.*, 1972; Schweizer *et al.*, 1973). At first glance the smaller ${}^3J_{1'-2'}$ in the triazole compounds would seem to indicate a lower proportion of species with 2′-endo puckering. If such were the case, one would expect to see a corresponding increase in ${}^3J_{3'-4'}$ (Schweizer *et al.*, 1973; Hruska *et al.*, 1971), reflecting the higher population of 3′-endo conformer. However, the values of 5.5 and 6.0 Hz for I and II are quite close to the 5.4 Hz for uridine (Schleich *et al.*, 1972) having roughly equal 2′- and 3′-endo forms. It is of interest to note in Table I that the smaller $J_{1'-2'}$ and larger $J_{3'-4'}$ for II compared with I indicates a slight shift in equilibrium in favor of the 3′-endo conformer in the former. Nucleoside VI, with $J_{1'-2'}$ = 2.5 Hz and $J_{3'-4'}$ = 6.8 Hz, undoubtedly has mainly 3′-endo character.

Although some of the decrease in 1′–2′ coupling constants in the triazole nucleosides may in fact be due to conformational alterations, one must consider a substituent effect due to a change in base from uracil to triazole-3-carboxamide. The latter heterocycle might be considered more electron deficient than uracil, due to the presence of the third nitrogen. Electron-deficient heterocycles attached at C_1 have recently been shown to decrease $J_{1'-2'}$ by as much as 2 Hz in several nucleoside systems (Nesnow *et al.*, 1973; Szekeres *et al.*, 1973).

The preferred conformation about the $C_{4'}$–$C_{5'}$ bond (Figure 1) has been studied for numerous nucleosides and nucleotides (Schleich *et al.*, 1972; Remin and Shugar, 1972; Schweizer *et al.*, 1973). From the measured $J_{4'-5'}$, $J_{4'-5''}$ coupling constants and use of appropriate J_0 values for gauche and trans arrangements in the Karplus equation, it is generally found that the gg rotamer predominates. The assignment of Remin and Shugar (1972) for $H_{5'}$ and $H_{5''}$ seems reasonable, that is, the 5′-proton at highest field and with the largest coupling to $H_{4'}$ is the one oriented toward the 3′-OH (in the gg conformation, Figure 1, this is the proton labeled $H_{5''}$). In the case of uridine, for example (depending upon a particular reference), $J_{4'-5'}$ = 2.5–2.9 Hz and $J_{4'-5''}$ = 3.9–4.4 Hz and the gg rotamer population is calculated to be 50–60%. A slight shift toward the gt rotamer is noticed for nucleosides I and II (Table I) in light of the $J_{4'-5''}$ = 5.2–5.3. Such a shift may be due to electrostatic repulsion between the 5′-OH and polar substituents in the aglycone, as previously reported for pyrimidine nucleosides such as 6-methyluridine, 6-oxycytidine, and 6-azauridine (Schweizer *et al.*, 1973). Similar conclusions were reached independently for the latter nucleoside and its 5′-phosphate (Hruska *et al.*, 1973). The shift toward the gt rotamer for I and II could arise from repulsion between

TABLE I: Summary of H–H and H–P Coupling Constants, Hz, for Various Triazole Nucleosides[a] and Ribavirin 5′-Phosphate.[b]

Compound	1′–2′	2′–3′	3′–4′	4′–5′	4′–5″	5′–5″	4′–P	5′–P	5″–P
I	3.5	5.0	5.5	3.3	5.2	−12.5			
II	3.0	5.0	6.0	3.2	5.3	−12.3			
III	4.0	5.0	5.0	4.0[c]	4.0[c]	−11.0[c]	1.5[c]	6.0[c]	5.2[c]
IV[d]	3.8	4.8	4.8	3.5	5.0	−12.5			
V	3.4	5.5	5.5	3.5	5.5	−12.5			
VI	2.5	5.0	6.8	3.3	5.5	−13.0			

[a] Obtained from 220-MHz spectra; 0.16 M in D_2O, 15°. [b] Taken from 220-MHz spectra; 0.43 M in D_2O, pD 7.4, 30°. [c] Taken from best-fit simulated spectra (${}^{31}P$ coupled) using the LAOCOON III program (Castellano and Bothner-By, 1964). Experimentally, it was determined that the sum of $H_{5'}$–P + $H_{5''}$–P = 10.5 Hz (taken from 36.4-MHz ${}^{31}P$ (FT) spectra; sweep width 125 Hz, 400 accumulations). [d] Spectrum obtained at 35° because of solubility limitations.

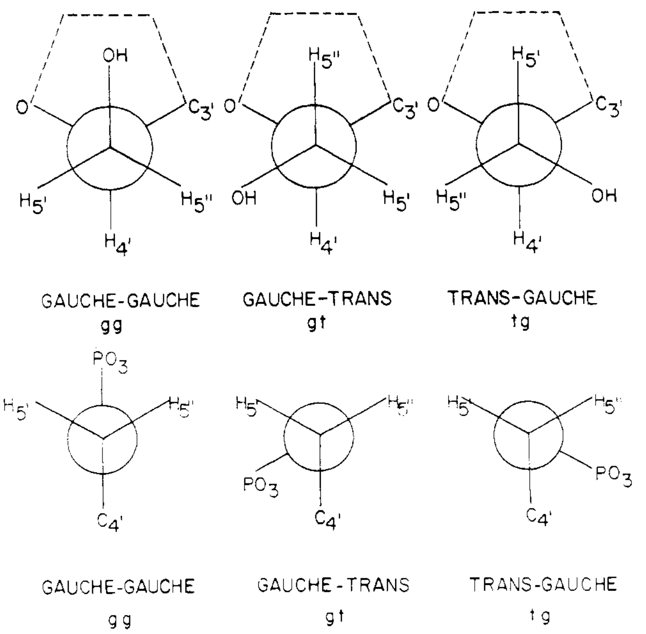


FIGURE 1: (a, top) Staggered conformation about the $C_{4'}$–$C_{5'}$ bond; (b, bottom) classical rotamers about the $C_{5'}$–$O_{5'}$ bond.

5′-OH and N_2 in the triazole base if the glycosidic preferences are as shown in the structural formulae. (Data presented below are substantative of these glycosidic conformations.)

Glycosidic Conformation. Information concerning the syn, anti-glycosyl[2] preferences of the triazole nucleosides was derived from comparison of ribose proton chemical shifts and also from measurement of vicinal C–H coupling constants between base carbons and H_1, as a gauge of rotational position about the glycosidic bond. Finally, the preferred conformations were determined from nuclear Overhauser experiments.

Table II contains proton chemical shift data for the various compounds under consideration. We have examined the 1,2,3-triazole nucleosides in order to assess the effect of a nitrogen in the base positioned over the sugar ring upon ribose proton chemical shifts. Previously, such a nitrogen proximity effect was elucidated in the case of 6-azauridine *vs.* uridine (Schweizer *et al.*, 1973). The presence of the 6-nitrogen caused shifts of $H_{2'}$ and $H_{3'}$ to lower field by about 0.22 and 0.13 ppm, respectively. Nucleoside V must have a nitrogen atom positioned over the ribose ring. It is likely that nucleoside VI is in the syn conformation, placing a nitrogen over the sugar in this molecule as well, since the carboxamide function cannot be accommodated in the anti conformation due to steric interference with ribose substituents. If VI exists in the syn conformation, the anisotropic carboxamide carbonyl group would be expected to markedly affect the chemical shift of $H_{1'}$. This signal indeed occurs ∼0.5 ppm to lower field in VI than it does in IV or V, providing evidence for the syn conformational assignments.

It is of interest to compare the ribose proton chemical shifts of V and VI with IV, the latter having no apparent conformational restraints. As may be seen in Table II, $H_{2'}$ and $H_{3'}$ of V and VI have similar values for $\delta H_{2'}$ and $\delta H_{3'}$ (albeit the residual HDO resonance overlaps the $H_{2'}$ peak in VI, this quartet must be centered around 1.66 ppm), in both cases these resonances appear at significantly lower field than in IV. An appropriate interpretation of these data, in light of the results with uridine and 6-azauridine, is that a base nitrogen is positioned over the ribose in V and VI, but not in IV, as depicted in the list of structural formulas.

With regard to nucleoside II, the same line of reasoning used for VI may apply for the steric requirements of the carboxamide group. Thus II cannot readily assume the anti conformation in solution, but must exist mainly as the syn conformer. This contention is validated by comparison of $\delta_{H\text{-}1'}$ for I and II. With II in the syn conformation, as was seen in VI, the anisotropic carbonyl of the amide function causes $H_{1'}$ to shift significantly downfield, in this case by 0.7 ppm.

A base nitrogen is positioned over the ribose of II in the syn conformation. Since the ribose proton shifts (other than $H_{1'}$) in both I and II are similar, it is likely that I is also in the syn conformation, otherwise $H_{2'}$ and $H_{3'}$ would appear at higher field than is found. The occurrence of $H_{3'}$ at 0.07 and $H_{2'}$ at 0.04 ppm to higher field in I may be attributed to a slight shift in furanose conformation from 3′-endo in II to 2′-endo in I (see Table I, larger $J_{1'-2'}$ and smaller $J_{3'-4'}$ for I) which results in a greater distance between H_3' and the deshielding influence of N_2. In addition, it is seen below that II has a glycosidic torsional angle near 90°, whereas in I, χ is probably larger. This change in χ, resulting in a change in distance between $H_{2'}$ and $H_{3'}$ and the base nitrogens in I and II, also contributes to the shift changes.

It is of interest to note that the spatial arrangement between H_3 and $H_{1'}$ of II is similar to the situation between H_5 and $H_{1'}$

TABLE II: Summary of Proton Chemical Shifts for Various Triazole Nucleosides[a] and Ribavirin 5′-Phosphate.[b]

	Chemical Shifts (ppm) from Me_4NBr						
Compound	Base Proton	H-1′	H-2′	H-3′	H-4′	H-5′	H-5″
I	5.550	2.873	1.445	1.296	1.010	0.675	0.550
II	4.918	3.568	1.481	1.362	0.990	0.668	0.539
III	5.620	2.873	1.505	1.340	1.186	0.950[c]	0.875[c]
IV[d]	5.440	3.004	1.520	1.256	1.077	0.691	0.560
V	5.006	2.984	1.607	1.402	1.048	0.661	0.527
VI	4.995	3.452	*e*	1.418	1.033	0.643	0.500

[a] Spectra obtained at 220 MHz, 0.16 M in D_2O, 15°. [b] 220-MHz spectra, 0.43 M in D_2O, pD 7.14, 30°. [c] Taken from best-fit simulated spectra (^{31}P coupled) using the LAOCOON III program (Castellano and Bothner-By, 1964). [d] Owing to solubility restrictions, spectra obtained at 35°. [e] H-2′ obscured by HDO at δ 1.655 ppm.

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[2] We adopt a convention for describing rotation about the glycosidic bond as monitored by the torsion angle, χ, as follows. $\chi \equiv 0$ when the C_5–N_1 and $C_{1'}$–$O_{4'}$ bonds are eclipsed. Positive values of χ from 0 to 360° are obtained by counterclockwise rotation about $C_{1'}$–N_1. In analogy to pyrimidine nucleosides (Sundaralingam, 1969), the syn range is centered around χ = 220–230°, whereas the mid-anti range is ≈50–60°.

TABLE III: Cmr Parameters for I–III.

Parameter	I[a]	II[b]	III[c]
δ (C=O)	−164.0, −161.8	−160.0	−162.5
C_3	−157.6, −158.4	−151.5	−156.0
C_5	−147.4, −146.3	−148.8	−145.6
$C_{1'}$	−92.8, −92.1	−90.3	−91.8
$C_{2'}$	−76.3, −75.1	−74.5	−74.5
$C_{3'}$	−71.6, −70.7	−71.3	−70.0
$C_{4'}$	−86.3, −85.7	−85.5	−83.9
$C_{5'}$	−62.8, −61.6	−62.5	−64.1
$J_{C_3-H_5}$	11.3		
C_3–H_3		210	
C_5–H_5	216 215		216
C_5–H_3		6.9	
C_5–$H_{1'}$	<3.6, <4.6	<1.5	<4.4
$C_{1'}$–$H_{1'}$	169	170	170
$C_{2'}$–$H_{2'}$	150	152	154
$C_{3'}$–$H_{3'}$	150	152	152
$C_{4'}$–$H_{4'}$	151	147	140
$C_{5'}$–$H_{5'}$[d]	141	140	145
$C_{4'}$–P			10.0[e]
$C_{5'}$–P			6.2[e]

[a] Chemical shifts measured from external C_6F_6 capillary, converted to benzene using the experimentally determined relationship: $\delta_{C_6H_6} = \delta_{C_6F_6(\mathrm{ext})} - 9.9$ ppm, and eventually to Me_4Si using $\delta_{Me_4Si} = \delta_{C_6H_6} - 128.7$ ppm (Stothers, 1972). First value, from 50% w/v D_2O solution; second value 32% w/v Me_2SO-d_6 solution. *J*'s taken from spectra on D_2O (1st value) and Me_2SO-d_6 samples. [b] 28% w/v Me_2SO-d_6 solution. [c] 10% w/v D_2O solution. [d] $J_{C_{5'}-H_{5'},H_{5''}} = \Sigma J_{C_{5'}-H_{5'}} + J_{C_{5'}-H_{5''}}/2$. [e] Obtained from 90-MHz noise-decoupled spectra.

TABLE IV: Nuclear Overhauser Effects Observed for the Base Proton Resonance in Ribavirin and Ribavirin 5′-Phosphate upon Strong Irradiation of the Ribose Protons.

	NOE Enhancement Obsd for H_5 of the Base	
Ribose Proton Irradiated	Ribavirin	Ribavirin-5′-P
$H_{1'}$	22	4
$H_{2'}$	6	0
$H_{3'}$	0	0
$H_{4'}$	0	0
$H_{5'}$, $H_{5''}$	0	0

of uridine. The existence of a measurable $^5J_{5-1'}$ coupling in the latter, as well as in other pyrimidine nucleosides (0.4–0.6 Hz) was used as evidence for the anti conformer in these systems (Hruska, 1971). We have observed a $^5J_{3-1'}$ of 0.6 Hz for II, data which are supportive of a mainly syn conformation for this nucleoside.

Further implications on the glycosidic conformations of I, II, and III may be derived from consideration of vicinal couplings between $H_{1'}$ of the ribose and C-5 of the triazole base as a gauge of the torsional angle about the glycosidic bond. Recently, by relating the dihedral angle $\phi_{C_2-H_{1'}}$ to the measured $^3J_{C_2-H_{1'}}$ values, uridine has been shown to adopt the anti conformation in water (Lemieux *et al.*, 1972), while cytidine is anti and 6-methylcytidine is syn in Me_2SO (Schweizer and Kreishman, 1973). Various ^{13}C chemical shifts and C–H coupling constants were obtained from the natural abundance spectra of I–III (including C–P couplings for III) and are listed in Table III. The assignments used have been previously reported for the carbons of the triazole base (Kreishman *et al.*, 1972) and for ribose carbons in nucleosides (Mantsch and Smith, 1972; Jones *et al.*, 1970a,b).

In the undecoupled spectrum of nucleoside II, the C_5 resonance is a well-resolved doublet with a spacing of 6.9 Hz, due to vicinal coupling with H_3. There is no evidence of further splitting due to interaction with $H_{1'}$. The $\nu_{1/2}$ of either leg of the C_5 signal is 1.5 Hz, thus $^3J_{C_5-H_{1'}}$ must be less than 1.5 Hz, which means, from consideration of ϕ_{C-H} *vs.* $^3J_{C-H}$ relationships (Lemieux *et al.*, 1972) that $\pm 70° < \phi_{C_5-H_{1'}} \leq \pm 110°$. Inspection of space-filling molecular models indicates that steric contact between the 5-carboxamide and furanose substituents in II makes the ±110° limit unlikely, so that the range of ϕ values is probably more likely between ±70 and 80°, the corresponding torsion angles, χ, thus being in the syn range (centered either about 165 or 325°) in agreement with the conclusions reached above from ribose proton shifts. If the value of $\phi_{C_5-H_{1'}} = +(70 \text{ to } 80°)$ (clockwise rotation looking down the glycosyl bond) is considered with the aid of models, it is seen that the N_2 of the triazole is right above the $H_{2'}$. With reference to Table II, it is seen that the chemical shift of $H_{2'}$ in II is not found at low field as is the case for V and VI; therefore, it appears that $\phi = -(70 \text{ to } 80°)$ is a more likely solution, with $\chi = 310$–$320°$.

With regard to I and III, the C_5 peak is a widely spaced doublet of *ca.* 215 Hz due to coupling with the directly bonded H_5. No further splitting is observable. Here the $\nu_{1/2}$ of a given doublet member is *ca.* 4.5 Hz for both I and III. Since there is a contribution to the line widths of I and III from C–H dipolar relaxation, it is not possible to obtain precise information about the glycosidic conformation of these molecules from these studies. It can be noted however, that it is unlikely that the dihedral angle, $\phi_{C_5-H_{1'}}$, is close to cis or trans extremes ($^3J_{C-H} = 6$–8 Hz; Lemieux *et al.*, 1972) because of the small $^3J_{C_5-H_{1'}}$ values. (A coupling of 3.0–3.5 Hz would be at least partially resolved under the present experimental conditions; thus it is likely that $J_{C_5-H_{1'}}$ for I and III is less than 3 Hz so that $\phi_{C_5-H_{1'}}$ probably is within the range ±30 to ±130°.)

The glycosidic conformation for I can be unambiguously assigned based on results from proton–proton Overhauser enhancement experiments (Son *et al.*, 1972; we thank a referee for suggesting these studies). In these experiments, one saturates a proton resonance and observes the resulting change in another proton's magnetization. Examination of space-filling models shows that in I the base proton H_5 is very close to the ribose $H_{1'}$ proton in the syn conformation and close to $H_{5'}$ and $H_{5''}$ protons in the anti conformation. As shown in Table IV, results of our experiments indicate that a nuclear Overhauser enhancement of 22% was observed for the base proton resonance when the $H_{1'}$ proton was irradiated in I and a slight increase of 6% was observed when $H_{2'}$ was irradiated. No measurable peak area increase in H_5 was noted upon irradiating $H_{5'}$, $H_{5''}$.

It is noteworthy that the time average dynamic conformation of I, with essentially equal contributions from 2′- and 3′-endo forms to the furanose, with a shift toward the gt rotamer about $C_{4'}$–$C_{5'}$ and with a preference for the syn glycosidic form, differs from the static situation elucidated by Prusiner and Sundaralingam (1973). These workers described two crystalline forms of ribavirin, one being 3′-endo, gg, and normal anti, the other 2′-exo, gt, and intermediate between anti and syn.

II. Conformation and Interaction of Ribavirin 5′-Phosphate (III)

Since it appears to be the 5′-phosphate rather than the nucleoside itself which is involved with blocking GMP biosynthesis by inhibiting IMP-dehydrogenase (Streeter *et al.*, 1973), it was of interest to study the conformational features of the nucleotide.

In terms of the furanose conformation, III is very similar to the nucleoside I, with a slightly higher proportion of 2′-endo conformer, judging from the larger $J_{1'-2'}$ (Table I). However, with respect to other conformational features, several significant differences between I and III are evident. Associated with the slight 2′-endo preference is a concomitant switch to more gg exocyclic rotamer about $C_{4'}$–$C_{5'}$, as may be seen from $J_{4'-5'} = J_{4'-5''} = 4.0$ Hz. This alteration apparently accompanies a change in glycosidic conformation from syn in the nucleoside I to anti in the 5′-monophosphate (see the structural formulas above), reducing the possible repulsion between 5′-OH and N_2 in the former. Additional evidence for the anti conformation in III comes from consideration of the electric field effect of the ionized phosphate function. Previously it was shown (Schweizer *et al.*, 1968) that the preferred glycosidic conformation for the 5′-phosphates of pyrimidine and purine nucleosides could be deduced by monitoring the differential effect of the phosphate ionization upon the chemical shifts of the base protons. Accordingly, the chemical shift dependence of the H_5 proton of III upon pD was assessed over the range 5.9–7.8 which encompasses the secondary phosphate ionization. The H_5 resonance moved 0.05 ppm to lower field over this range, which is similar to the behavior exhibited by certain base protons of 5′-mononucleotides oriented in the anti conformation (Schweizer *et al.*, 1968). From these data it is possible to state that the preferred glycosidic conformation for III is anti.

Table IV contains NOE data supportive of the anti conformation for III. Only slight enhancement was observed for the base proton upon irradiation of the $H_{1'}$ and no enhancements were observed upon irradiation of the other ribose protons. It is shown below that the conformations about the $C_{4'}$–$C_{5'}$ and $C_{5'}$–$O_{5'}$ bonds are predominantly gg, positioning the phosphate over the ribose ring and preventing free rotation of the base about the glycosidic bond. In light of the phosphate ionization effects which provide evidence for the anti conformation, the H_5 base proton should be directed toward the $H_{3'}$ or $O_{4'}$. Since no NOE enhancement was observed except when irradiating $H_{1'}$, we suggest that in the anti conformation of III, the base plane is oriented along the $C_{1'}$–$O_{4'}$ bond such that $\chi \cong 0$.

With respect to conformation of the phosphate group, it is seen in Table III that the $^3J_{C_{4'}-P}$ of 10 Hz indicates that the phosphorus and $C_{4'}$ atoms are oriented essentially trans about the $C_{5'}$–$O_{5'}$ bond (Mantsch and Smith, 1972). Additional information about conformation about the $C_{5'}$–$O_{5'}$ bond (Figure 1b) may be obtained from consideration of $^3J_{5'-P}$ and $^3J_{5''-P}$ (Table I). Experimentally, one observes a broad triplet resonance in the Fourier transform ^{31}P spectrum of III with $\Sigma {}^3J_{5'-P} + {}^3J_{5''-P} = 10.5$ Hz. From the best-fit simulated spectrum, ^{31}P coupled, $^3J_{5'-P} = 6.0$, and $^3J_{5''-P} = 5.2$ Hz. Using the relationship derived by Hruska *et al.* (1973), relating the fraction of gg rotamer about the $C_{5'}$–$O_{5'}$ bond to the sum of $J_{5'-P}$ and $J_{5''-P}$ it is estimated that in the case of III, the gg rotamer is present to the extent of 70%. Thus the backbone conformation of III, being predominantly gg about both $C_{4'}$–$C_{5'}$ and $C_{5'}$–$O_{5'}$ bonds, is similar to 5′-AMP and 5′-ADP previously reported (Sarma *et al.*, 1973). The stereochemical features of III are summarized in the structural formulas.

III. Nucleoside Interactions

Self-Association. Association of nucleosides and nucleotides in aqueous solution has been monitored by pmr (Broom *et al.*, 1967; Schweizer *et al.*, 1968). The characteristic concentration and temperature-depending shifts observed were manifestation of the ring-current diamagnetic anisotropy of vertically interacting aromatic rings in these systems. We have studied the effects of concentration upon the 220-MHz pmr spectra of I. Over the range 0.04–1.0 M in D_2O, H_5 and $H_{1'}$ shifted upfield *ca.* 0.02 ppm, which can be accounted for in terms of change in bulk susceptibility.

Similarly, H_5 of I and both H_3 and $H_{1'}$ of II were insensitive to temperature alteration from 30 to 80°. The chemical shift independence of concentration and temperature may be interpreted in terms of no significant self-association for these nucleosides in water. However, these measurements would reflect interaction only if the base can sustain a ring current in the applied field and thereby could alter the magnetic environment of a proton on a nearby molecule. The magnitude of the current induced in the triazole ring is not known. If it is low, the possibility exists that weak association as found for pyrimidine nucleosides occurs (Ts'o *et al.*, 1963), but the interaction cannot be detected by these nmr methods.

Interaction of Ribavirin with Cytidine. The ability of ribavirin (as the 5′-phosphate) to inhibit IMP-dehydrogenase is apparently related to its structural similarity to inosine, thus acting in a feedback loop (Streeter *et al.*, 1973). Prusiner and Sundaralingam (1973) have suggested that ribavirin's similarity to guanosine may be manifested in hydrogen bonding base pairing interactions with cytidine. We have investigated the possibility that ribavirin and cytidine might display evidence of hydrogen-bonding interactions in Me_2SO. In the case of guanosine and cytidine, such an interaction was indicated by the large downfield shifts of N–H protons involved in the hydrogen bonding (Katz and Penman, 1966).

Various mixtures of I and cytidine were studied, where the molar ratios were 1:1, 2:1, and 1:2. In comparing the chemical shifts and line widths of the various protons in these mixtures with the homogeneous solutions of I or cytidine, no changes were observed. We conclude that if hydrogen bonding interactions of a base-pairing nature occur between I and cytidine, these interactions must be much weaker than that found between guanosine and cytidine in Me_2SO.

In summary, our studies show that both the antiviral nucleoside I and its inactive isomer II are closely related in solution. With respect to furanose conformation, rotation about the exocyclic $C_{4'}$–$C_{5'}$ bond and glycosidic conformation, comparison of these nucleosides with uridine shows that the exocyclic conformations are shifted toward gauche,trans. Both triazole nucleosides are syn as compared with anti-uridine. On the other hand, ribavirin 5′-phosphate, considered to be mechanistically the active species in terms of inhibiting IMP-dehydrogenase, has different solution properties than I or II, having slightly more 2′-endo furanose pucker, predominantly gg about both $C_{4'}$–$C_{5'}$ and $C_{5'}$–$O_{5'}$ bonds, and in the anti glycosidic conformation.

Since it is highly unlikely that the 5′-phosphate of II, not available for study, could adopt the anti conformation because of intimate contact between the 5-carboxamide in the base and ribose substituents, it is reasonable to conclude that the preferred glycosidic conformation would be syn as in II.

Inspection of II (more correctly, the 5′-phosphate of II) and III in the structural formulas above serves to illustrate that, although both have potentially the same hydrogen-bonding capabilities, the spatial arrangement of substituents in the aglycone with respect to the ribose is quite different because of the point of ribose attachment. Compound III resembles IMP and therein undoubtedly lies the activity in terms of binding to IMP-dehydrogenase.

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References

Alonso, G., Garcia-López, M. T., Garcia-Muñoz, G., Madroñero, R., and Rico, M. (1970), *J. Heterocycl. Chem. 7*, 1269.

Blackburn, B. J., Grey, A. A., Smith, I. C. P., and Hruska, F. E. (1970), *Can. J. Chem. 18*, 2866.

Broom, A. D., Schweizer, M. P., and Ts'o, P. O. P. (1967), *J. Amer. Chem. Soc. 89*, 3612.

Castellano, S., and Bothner-By, A. A. (1964), *J. Chem. Phys. 41*, 3863.

Hruska, F. E. (1971), *Can. J. Chem. 49*, 2111.

Hruska, F. E., Smith, A. A., and Dalton, J. G. (1971), *J. Amer. Chem. Soc. 93*, 4334.

Hruska, F. E., Wood, D. J., Mynott, R. J., and Sarma, R. H. (1973), *FEBS (Fed. Eur. Biochem. Soc.) Lett. 31*, 153.

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

Jones, A. J., Grant, D. M., Winkley, M. W., and Robins, R. K. (1970b), *J. Phys. Chem. 74*, 2684.

Katz, L., and Penman, S. (1966), *J. Mol. Biol. 15*, 220.

Kreishman, G. P., Witkowski, J. T., Robins, R. K., and Schweizer, M. P. (1972), *J. Amer. Chem. Soc. 94*, 5894.

Lehmkuhl, F. A., Witkowski, J. T., and Robins, R. K. (1972), *J. Heterocycl. Chem. 9*, 1195.

Lemieux, R. U., Nagabhushan, T. L., and Paul, B. (1972), *Can. J. Chem. 50*, 773.

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

Nesnow, S., Miyazaki, T., Khwaja, T., Meyer, R. B., Jr., and Heidelberger, C. (1973), *J. Med. Chem. 16*, 524.

Prusiner, P., and Sundaralingam, M. (1973), *Nature (London), New. Biol. 244*, 116.

Remin, M., and Shugar, D. (1972), *Biochem. Biophys. Res. Commun. 48*, 636.

Sarma, R. H., Mynott, R. J., Hruska, F. E., and Wood, D. J. (1973), *Can. J. Chem. 51*, 1843.

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

Schweizer, M. P., Banta, E. B., Witkowski, J. T., and Robins, R. K. (1973), *J. Amer. Chem. Soc. 95*, 3770.

Schweizer, M. P., Broom, A. D., Ts'o, P. O. P., and Hollis, D. P. (1968), *J. Amer. Chem. Soc. 90*, 1042.

Schweizer, M. P., and Kreishman, G. P. (1973), *J. Magnetic Res. 9*, 334.

Sidwell, R. W., Huffman, J. H., Khare, G. P., Allen, L. B., Witkowski, J. T., and Robins, R. K. (1972), *Science 177*, 705.

Son, T., Guschlbauer, W., and Guéron, M. (1972), *J. Amer. Chem. Soc. 94*, 7903.

Stothers, J. B. (1972), Carbon-13 NMR Spectroscopy, New York, N. Y., Academic Press, p 49.

Streeter, D. G., Witkowski, J. T., Khare, G. P., Sidwell, R. W., Bauer, R. J., Robins, R. K., and Simon, L. N. (1973), *Proc. Nat. Acad. Sci. U. S. 70*, 1174.

Sundaralingam, M. (1969), *Biopolymers 7*, 821.

Szekeres, G. L., Robins, R. K., Dea, P., Schweizer, M. P., and Long, R. A. (1973), *J. Org. Chem. 38*, 3277.

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

Witkowski, J. T., Robins, R. K., Sidwell, R. W., and Simon, L. N. (1972), *J. Med. Chem. 15*, 1150.