random noise generator, were described earlier.[3] The chemical shifts reported here have been corrected to carbon disulfide as external reference by the relations $\delta_C = \delta_C^{\text{dioxane}} + 125.5$ ppm, or $\delta_C = \delta_C^{CH_2Cl_2} + 138.9$ ppm, and were reproducible to ±0.1 ppm. In the 3-methylcyclopentanols, the assignments of the carbons 3 and 4 are somewhat uncertain. The same is true of C-3, C-4, and C-5 in the 3-methylcyclopentyl acetates because the resonances are very close together.

A Nuclear Magnetic Resonance Study of the Conformation of β-Cyanuric Acid Riboside. Further Evidence for the Anti Rotamer in Pyrimidine Nucleosides[1a]

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Abstract: A complete analysis of the 100- and 220-MHz nmr spectra of β-cyanuric acid riboside, a compound in which an α-keto group on the base must lie over the ribose ring, is presented. A model for the molecular conformation is deduced. The changes induced in the ribose hydrogen resonances (relative to corresponding resonances in uridine) by the presence of the α-keto group demonstrate the correctness of previous conclusions that uridine and β-pseudouridine exist in aqueous solution in the anti conformation (with respect to rotation about the glycosyl bond).

In an effort to elucidate the structures of nucleic acids in solution considerable attention has been focused on conformational studies of nucleotides and nucleosides by nmr spectroscopy.[2–12] Although X-ray diffraction studies indicate that most of these compounds prefer the anti rotamer about the point of attachment of the base to the ribose ring,[13] it is important to demonstrate that this also holds in aqueous solution. Nmr studies of corresponding nucleosides and nucleotides, *e.g.*, uridine and uridine 5′-phosphate, as functions of temperature[9] and pH[2] have suggested quite strongly that this is so. We report here a detailed nmr study of α-*N*-(β-cyanuric acid)-D-ribofuranoside (β-cyanuric acid riboside, β-CAR),[14] Figure 1, in which a keto group must lie over the ribose ring. Previous studies have suggested that if nucleosides or nucleotides existed in the syn conformation, appreciable changes in the chemical shifts of the ribose hydrogens, particularly those at positions 2′ and 3′, should occur.[11] On comparison of the nmr data for β-CAR with those of uridine[12] such large changes are manifest, providing confirmation of the previous assertions.

Recently detailed nmr studies of the coupling between the ribose hydrogens of β-pseudouridine, β-ψ,[10,11] uridine, U,[12] and α-pseudouridine, α-ψ,[15] indicated that the ribose ring adopts none of the conventional ring puckered conformations, but interconverts rapidly between them with approximately equal residence times in each. Also, a slight preference for the gauche–gauche rotamer of the exocyclic CH_2OH group was indicated.[10–12,15] Similar treatment of the nmr data for β-CAR indicates that the ribose ring has a slight preference for the $C_{2'}$-exo and/or $C_{3'}$-endo conformations of the ribose ring, and for one of the rotamers in which the CH_2OH group points away from the base (gauche–trans or trans–gauche). As was found for β-ψ, α-ψ, and U, the ribose conformation of β-CAR is essentially temperature independent.

Experimental Section

β-CAR was synthesized according to Robins and Winkley.[14] Nmr spectra were obtained on Varian HA-100 and HR-220 instruments using standard techniques. After adjustment of pH the sample was triply lyophilized from D_2O to minimize the interfer-

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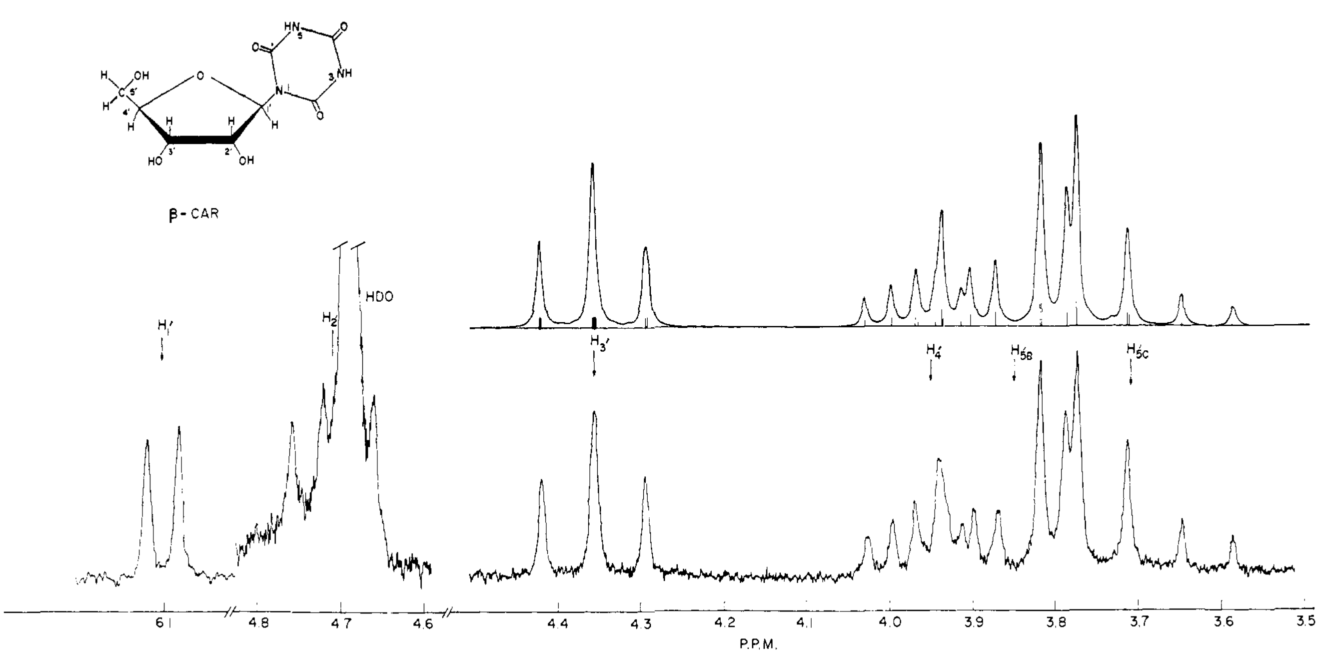

Figure 1. (a) The 100-MHz nmr spectrum of β-CAR in D_2O (pD 8.0, 0.11 *M*, 30°). Chemical shifts are relative to internal sodium 3-(trimethylsilyl)propanesulfonate. The quartet of the $H_{2'}$ resonance is partially obscured by the HDO resonance, but becomes measurable at higher temperatures; (b) simulated 100-MHz spectrum of the region due to the ribose protons 3'–5', using the 30° data of Table I.

ing HDO peak. Spectral analysis and simulation were performed on an IBM 360/50 computer using a modified version[10,11] of the program LAOCOON .II[16]

Results and Discussion

The nmr spectrum of β-CAR at 100 MHz is shown in Figure 1. The resulting chemical shifts and coupling constants at 30 and 75° are given in Table I; the corresponding data for uridine[12] are included for comparison. Peak assignments were confirmed by spin decoupling.

Table I. Chemical Shifts and Coupling Constants of β-Cyanuric Acid Riboside[a] and Uridine[b]

	Chemical shifts, ppm				Coupling constants, Hz		
	β-CAR		U[b]		β-CAR		U[b]
	30°	75°	28°		30°	75°	28°
$H_{1'}$	6.10	6.11	5.90	1',2'	3.9	4.0	4.4
$H_{2'}$	4.71	4.74	4.34	2',3'	6.4	6.1	5.3
$H_{3'}$	4.36	4.36	4.22	3',4'	6.6	6.2	5.5
$H_{4'}$	3.95	3.96	4.13	4',5'B	3.2	3.4	3.0
$H_{5'B}$	3.85	3.84	3.91	4',5'C	6.2	6.0	4.4
$H_{5'C}$	3.72	3.74	3.80	5'B,5'C	−12.8	−12.4	−12.7

[a] The chemical shifts and coupling constants are estimated to be accurate to within 0.01 ppm and 0.1 Hz, respectively. [b] Reference 12.

A. Spin–Spin Coupling Constants and the Ribose Ring Conformation. By means of the Karplus equation[17] relating vicinal coupling constants to dihedral angles it is possible to estimate the conformation of the ribose ring. This can be done despite the known limitations of the relationship,[18] because the various puckered conformations have very different coupling constants.[7,10,11,19] For example, the $C_{2'}$-endo conformation would have, for $J_{1',2'}$, $J_{2',3'}$, and $J_{3',4'}$, values around 9.5, 4.3, and 0.4 Hz, respectively, whereas in the $C_{3'}$-endo conformation these values would be 0.4, 4.3, and 9.5 Hz, respectively (Table II).

Table II. Measured Dihedral Angles (φ) and Calculated Coupling Constants for Various Ribose Ring Conformations of β-Cyanuric Acid Riboside[b]

Atom out of plane[a]	$\phi_{1'2'}$, deg	$J_{1'2'}$, Hz	$\phi_{2'3'}$, deg	$J_{2'3'}$, Hz	$\phi_{3'4'}$, deg	$J_{3'4'}$, Hz
$C_{2'}$-endo	165	9.5	45	4.3	105	0.4
$C_{3'}$-exo	145	6.7	40	5.0	100	0
$H_{2'}$, $H_{3'}$ eclipsed	120	2.3	0	9.0	120	2.3
$C_{3'}$-endo	105	0.4	45	4.3	165	9.5
$C_{2'}$-exo	100	0	40	5.0	145	6.7
Obsd, 30°		3.9		6.4		6.6

[a] Endo means the atom is located on the same side of the plane defined by $C_{1'}O_{1'}$ and $C_{4'}$ as the $C_{4'}$–$C_{5'}$ bond. Exo means that it is found on the opposite side. [b] Angles and expected coupling constants from ref 11.

For β-ψ and U these coupling constants were all in the region of 5 Hz, indicating a time-averaged conformation. For β-CAR, however, they are tending in the direction expected for either the $C_{3'}$-endo or $C_{2'}$-exo conformations. The fact that they still deviate significantly from the values expected for any one of the puckered conformations indicates that a rapid equilibrium is still taking place, but with the populations of the various conformers weighted more heavily toward $C_{2'}$-exo and $C_{3'}$-endo. The lack of a significant temperature dependence for either chemical shifts or coupling constants demonstrates that, just as with U and β-ψ, the energy differences between the various ring conformations of β-CAR are very small.

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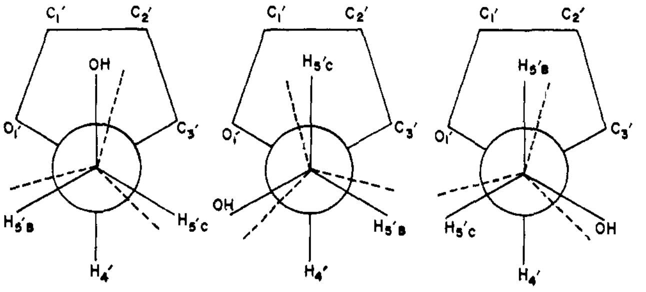
Table III. Limits for the Relative Populations of Rotational Isomers about the $C_{4'}$–$C_{5'}$ Bond of β-CAR

	Classical staggered rotamers				Rotamers with 15° O–O repulsions			
J_0 for $0 \le \phi \le 90°$	9.27	9.27	9.27	9.27	9.27	9.27	9.27	9.27
J_0 for $90 \le \phi \le 180°$	10.36	10.36	12.00	12.00	10.36	10.36	12.00	12.00
$J_{4'5'B}$	3.2	6.2	3.2	6.2	3.2	6.2	3.2	6.2
$J_{4'5'C}$	6.2	3.2	6.2	3.2	6.2	3.2	6.2	3.2
P_I	0.32	0.32	0.45	0.45	0.06	0.06	0.28	0.28
P_{II}	0.52	0.16	0.43	0.12	0.62	0.28	0.45	0.54
P_{III}	0.16	0.52	0.12	0.43	0.32	0.66	0.27	0.18

B. Spin–Spin Coupling Constants and the Conformation of the Exocyclic CH_2OH Group. Previously it has been shown that estimates of the rotamer population about the 4′–5′ exocyclic bond may be made, despite the limitations of the Karplus equation and uncertainties about the geometry of the energy minima and the assignment of the two 5′ resonances.[10–12,15] We have applied the method described in detail by Blackburn, *et al.*[12] Calculations were done for the classical rotamers, and for a rotamer set with dihedral angles expanded by 15° due to O–O repulsions, Figure 2. Both assignments of the 5′-resonances were tried, and the various J_0 values applied by Blackburn, *et al.*,[12] were used. The ranges of populations resulting from this treatment are shown in Table III. Thus, in contrast to β-ψ and U, β-CAR does not show a preference for the gauche–gauche rotamer. Particularly when the nonclassical rotamers are used as a basis, a definite preference for the gauche–trans or trans–gauche rotamer is indicated. This is presumably due to repulsion between the 5′-hydroxyl and one of the α-keto groups of the base.



I (GAUCHE-GAUCHE) II (GAUCHE-TRANS) III (TRANS-GAUCHE)

Figure 2. Possible conformations of the exocyclic CH_2OH groups of β-CAR. The dotted lines indicate nonclassical geometries resulting from oxygen–oxygen repulsion.

C. Ribose Hydrogen Chemical Shifts and the Sugar–Base Torsion Angle. The differences between the chemical shifts of the 2′, 3′, and 4′ hydrogens in β-CAR and U (Table I) show the effect of the magnetic anisotropy of the keto groups present on the base of β-CAR. Whereas $H_{2'}$ and $H_{3'}$ are deshielded, $H_{4'}$, is appreciably shielded, and the two $H_{5'}$ are barely affected. It is tempting to use these data to verify one of the various models for the magnetic anisotropy of a keto group[20–22] but the flexibility of the molecule obviates this. The large effects on $H_{2'}$, $H_{3'}$, and $H_{4'}$ do demonstrate, however, the expected trends to be observed in a pyrimidine nucleoside in the syn conformation, and provide justification for previous conclusions that U and β-ψ have the anti conformation.[10–12]

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