Pyrimidine Nucleoside Conformational Analysis. Nuclear Overhauser Effect and Circular Dichroism Correlations

Phillip A. Hart* and Jeffrey P. Davis

Contribution from the School of Pharmacy, University of Wisconsin, Madison, Wisconsin 53706. *Received June 3, 1970*

Abstract: In a continuing effort to unify the interpretation of various experimental approaches to nucleoside glycosidic conformation in solution we have measured and collated nuclear Overhauser effects, circular dichroism, and ultraviolet absorption spectra on cytidine, 2′,3′-isopropylidenecytidine, uridine, and 2′,3′-isopropylideneuridine in water and organic solvents. 2′,3′-Isopropylideneuridine and 2′,3′-isopropylidenecytidine were found to form nonideal solutions in chlorocarbon solvents. Upon dilution, the typically negative B_{2u} molecular ellipticities of these systems tend toward the positive values characteristic in water and dimethyl sulfoxide. Plausible origins of the concentration dependence of the complex curves resulting in these cases are considered. The NOE results in water and dimethyl sulfoxide indicate that all four nucleosides possess a considerable amount of the syn rotamer in hydrogen bond accepting solvents, with the isopropylidene derivatives favoring this conformation to a higher degree than the underivatized nucleosides. The possible consequences of these nmr observations coupled with new and published CD data are outlined for a previously published monograph of pyrimidine nucleoside B_{2u} molecular ellipticity *vs.* torsion angle.

We have recently reported data[1] derived from nuclear Overhauser effect (NOE) measurements on pyrimidine nucleosides that were viewed as evidence for the existence of substantial amounts of the syn conformation in the conformer equilibrium of some of them. Although the intuitive interpretation of the relative enhancements is that they represent conformer populations, the theory had not been developed that would permit quantitative conformational deductions to be made. Thus the conclusions had to be qualified.

In addition, the syn conformation has been assumed to be a relatively unimportant contributor to pyrimidine nucleoside conformational equilibria, an assumption bolstered by model building, computational, and experimental work.[2-9] The idea that the anti conformation for purine and pyrimidine nucleosides need not be exclusive has, however, been extant since Donohue and Trueblood expressed it,[10] and a recent X-ray analysis of 4-thiouridine, performed by Sanger and Scheit[11a] and quoted in a broader context by Tavale and Sobell,[11b] lends credence to this idea for pyrimidine nucleosides.

Circular dichroism (CD) observations are inherently unable to distinguish single conformers from appropriately weighted mixtures of conformers in the absence of unequivocal conformational models. However, CD studies of a variety of pyrimidine nucleosides led Miles, *et al.*,[9a] to modify somewhat the previous conclusions of Ulbricht,[9b] to establish approximately how molecular ellipticity of the B_{2u} band (the long-wavelength transition of pyrimidine nucleosides) should vary as a function of torsion angle,[12] and to embody the expected trend in a diagram reproduced in Figure 1. The diagram shows that extreme anti conformations of β pyrimidine nucleosides should be characterized by a large positive ellipticity associated with the B_{2u} transition, and that the extreme syn conformation should have a moderately large negative value. The values for the diagram were not all obtained from the same chromophoric system. The high positive values were derived from the experimental values of cytidine, 2′,3′-isopropylidenecytidine, and 1-β-D-arabinofuranosylcytosine on the assumption that these substances adopted the anti conformation in water. The negative values came from ellipticity measurements done on isopropylidenecytidine in dichloroethane, in which case intramolecular 2-keto, 5′-hydroxyl hydrogen bonding was thought to force the adoption of a syn conformation.[13] In addition, measurements on 6-methylcytidine (6-CH_3-C) and 6-methyl-2′-deoxycytidine (6-CH_3-2′-dC) in water, dioxane, and acetonitrile provided the remaining negative values since in those cases the syn conformer is favored because of severe steric interactions between the 6-methyl group and the sugar C-2′, C-3′, and C-5′ hydrogens.

Even though a quantitative relation of relative intramolecular Overhauser enhancements to conformer populations has been developed[14a,b] the experimental technique has not been sufficiently refined to provide data of the accuracy required to employ it rigorously.

* Address correspondence to this author.

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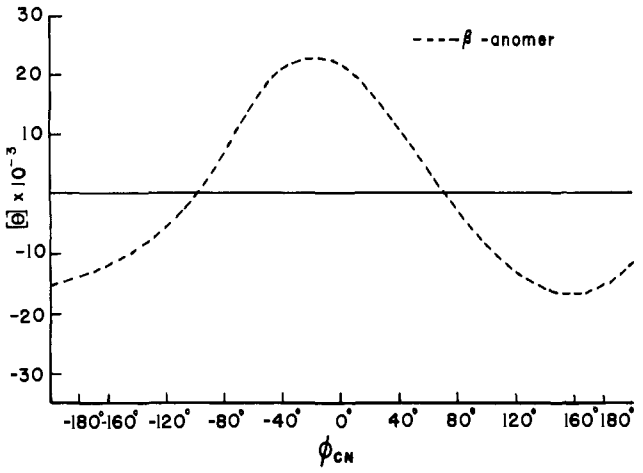

Figure 1. Nomograph of the expected relation of β pyrimidine nucleoside molecular ellipticity to the torsion angle ϕ_{CN}. Taken from ref 9a by permission.

We decided, therefore, to determine NOE enhancements for selected members of the set of nucleosides used by Miles to see if the conclusions reached by the nmr experiment agreed with those reached by the CD measurements. The models chosen were cytidine (C), uridine (U), 2′,3′-isopropylidenecytidine (*i*-C), and 2′,3′-isopropylideneuridine (*i*-U), all in water or polar solvents, and *i*-C in dichloroethane. In view of Miles' work this selection appeared to allow the examination of a series from anti-like to syn-like.

We found, however, that the chlorocarbon solutions of *i*-C were nonideal and that the NOE experiments could not be done on them. In addition, although *i*-U was not sufficiently soluble in the chlorocarbon solvents to even try the nmr experiments, it was sufficiently soluble in chloroform to do the CD experiments for completeness and it too was found to form nonideal solutions. With this information and further CD measurements the role of chiroptical results from "nonassociating" solvents in a correlation diagram can be put into perspective.

Experimental Section

The nucleosides used in this study were Sigma grade Sigma Chemical Co. products. Isopropylidenecytidine was purchased either as the free base or was obtained following neutralization of the hydrochloride by passage through a Dowex-2 OH^- column. The reagent grade solvents were treated as follows: methanol, distilled from Linde 3A molecular sieves; ethanol, distilled from Linde 4A molecular sieves; water, distilled and further deionized; acetonitrile, distilled from P_2O_5 and redistilled; chloroform, washed four times with water, dried over sodium sulfate, and distilled from molecular sieves; 1,2-dichloroethane, dried over sodium sulfate and distilled from molecular sieves; methylene chloride, distilled from calcium hydride; dimethyl sulfoxide, distilled from calcium hydride under vacuum and redistilled under vacuum.

CD measurements were performed on a Cary 60 recording spectropolarimeter fitted with a Model 6002 CD attachment with the slit programmed for a half-band width of 15 Å. Measurements were made at path lengths ranging from 0.1 to 5 cm and for concentrations ranging from 0.912×10^{-5} to 4.55×10^{-3} *M* except where indicated. The CD is recorded as molecular ellipticity, $[\theta]$, in units of deg cm^2 dmol^{-1}, and absorbances never exceeded 2.0. The solutions were not buffered so that ionic strength factors would not enter the NOE–CD comparisons. The resultant pH uncertainties are probably not severe. The instrument was calibrated using (+)-camphorsulfonic acid (Aldrich).

Ultraviolet absorption spectra were taken on a Beckman DK-2 recording spectrophotometer with cells of 1.0-cm path length and the concentration studies in chloroform and water were done on a Cary 14 recording spectrophotometer in cells of 0.1–5.0-cm pathlength. All uv and CD spectra in DMSO were run in a 0.1-cm cell.

Mean degrees of association in solution were measured on a Hewlett-Packard Mechrolab Division Model 301A vapor pressure osmometer using a 37° probe. Recrystallized biphenyl was used as an ideal solute standard in all solvents but in water, in which sodium chloride was used. Standard runs were made before and after each nucleoside run in every solvent and reproducibility of ΔR readings was consistently within 3–5%. Since precise molal osmotic coefficients for biphenyl in the various solvents employed are not available we have presented the osmometry data so that they simply indicate the deviation of *i*-C from ideality in chlorocarbon solvents taking the osmotic coefficient for biphenyl as very nearly one in all cases. The VPO data were identical within experimental error whether the reagent grade solvents were treated or untreated.

The conventional nmr spectra were taken on a Varian A60-A spectrometer. Perdeuteriomethylene chloride, ethanol, methanol, and acetonitrile were Stohler Isotope Corporation products. Pyridine-d_5, benzene-d_6, dimethyl-d_6 sulfoxide, and chloroform-d_1 were from Merck Sharpe and Dohme, and D_2O was purchased from Diaprep, Inc. The NOE experiments were done in coaxial tubes on a Varian HA-100 nmr spectrometer as previously described.[1,15] It was shown that peak height changes correspond well with peak area changes. The symbol f_n^m used to report the NOE data denotes here the fractional enhancement of the resonance m upon irradiation of resonance n. Nmr solvent mixtures are reported as per cent by volume before mixing.

Results

The average degree of association was computed according to Davies and Thomas[16] from thermoelectric vapor pressure osmometry (VPO) measurements. It is apparent from Figure 2 that *i*-C is monomeric in water, methanol, ethanol, and acetonitrile over a wide concentration range and that extensive aggregation occurs with increasing concentration in chloroform and methylene chloride. The highest concentration in each case corresponds closely to a 0.2 *M* solution and the lowest to a 0.005 *M* solution. The average degree of association is plotted against mole fraction of nucleoside since this is the thermodynamically most acceptable concentration definition for such studies. The solubility of *i*-C in dichloroethane was too low to allow a sufficiently wide range of VPO measurements and no experiments were done in DMSO because of its extremely low vapor pressure. The lowest concentration in the VPO studies represents the highest concentration at which the optical measurements were made, and the methods are therefore linked. It is a truism that the ideal high-concentration solutions remain ideal upon dilution. From the CD measurements it is evident that the aggregation of *i*-C at high concentrations is not discontinuous at the spectrophotometric concentrations. The nmr spectral line widths of 2′,3′-isopropylidenecytidine (0.25 *M*) in ethanol-d_6, methanol-d_4, acetonitrile-d_3, D_2O, and DMSO-d_6 varied from 1.0 to 1.6 Hz while in methylene-d_2 chloride and chloroform-d_1 they were about 6.0 Hz. Line widths of the other nucleosides in their respective solvents were close to 1.0 Hz. Table I shows the values of $J_{1'-2'}$; some of the implications of these coupling constants are discussed below. Chemical-shift variations were observed as a function of solvent. We made no detailed study of the concentration dependence of those changes and draw no conclusions from them at this time.

The uv spectra of *i*-C show a small bathocromic shift on going from nonassociating solvents (water, methanol,

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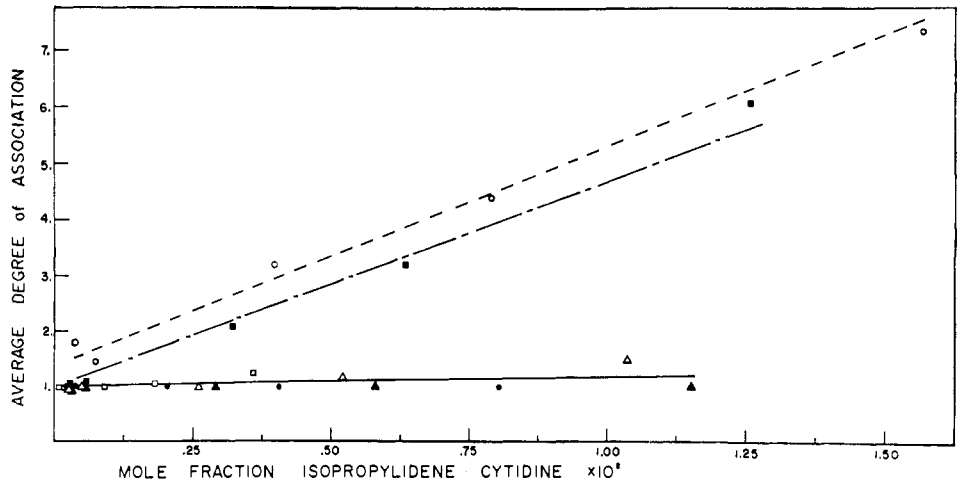

Figure 2. Average degree of self-association of 2′,3′-isopropylidenecytidine as a function of concentration in: $CHCl_3$, ○; MeOH, ●; CH_3CN, △; EtOH, ▲; H_2O, □; CH_2Cl_2, ■. Calculated from vapor pressure osmometry data.

ethanol, and acetonitrile) to associating solvents (chloroform, methylene chloride, and dichloroethane; Table II). The absorbance maxima decrease by approximately 30% on going from hydroxylic solvents to the nonhydrogen-bonding solvents. The ultraviolet spectra of the polar solutions show no concentration dependence, whereas the nonpolar solutions show small absorbance and/or wavelength changes with concentration (Table III), paralleling the concentration dependence shown in the CD experiments (see below).

Table I. Coupling Constants

Nucleoside	Solvent for 0.25 *M* soln	$J_{1',2'}$, Hz
C	D_2O	3.5
	62% benzene-d_6 in DMSO-d_6	2.4
i-C	D_2O	2.8
U	25% pyr-d_5 in D_2O	3.2
	DMSO-d_6	4.6
i-U	75% DMSO-d_6 in D_2O	2.4
	DMSO-d_6	2.4

Table II. Uv Data for 2′,3′-Isopropylidenecytidine

Solvent	Nucleoside molar concn × 10^4	λ_{max}, nm	ϵ_{max}, × 10^{-4}
H_2O	0.87	269	0.765
MeOH	0.42	270	0.780
EtOH	0.40	270	0.777
CH_3CN	1.02	272	0.555
$CHCl_3$	0.82	273	0.550
CH_2Cl_2	0.82	274	0.513
CH_2ClCH_2Cl	0.88	274	0.502

Table III. Uv Data for 2′,3′-Isopropylidenecytidine

Nucleoside molar concn × 10^4	H_2O λ_{max}, nm	H_2O ϵ_{max}, × 10^{-4}	$CHCl_3$ λ_{max}, nm	$CHCl_3$ ϵ_{max}, × 10^{-4}
0.0912	269	0.850	273	0.493
0.455	269	0.850	273	0.503
1.82	269	0.846	273	0.500
9.10	269	0.845	274	0.512
45.5			274	0.525

The circular dichroism spectra of *i*-C in water, methanol, and ethanol are independent of concentration over the range 0.912 × 10^{-5} to 4.55 × 10^{-3} *M* and are identical within 5% (Figure 3). Dimethyl sulfoxide and acetonitrile solutions yield similar concentration-independent spectra, but the position of the positive maximum shifts to longer wavelengths relative to the uv maximum (Figure 3 and Table IV). Solvent effects on CD *vs.* uv maxima have been discussed.[8] A marked change in the CD spectrum of *i*-C occurs when it is run in chloroform, methylene chloride, and

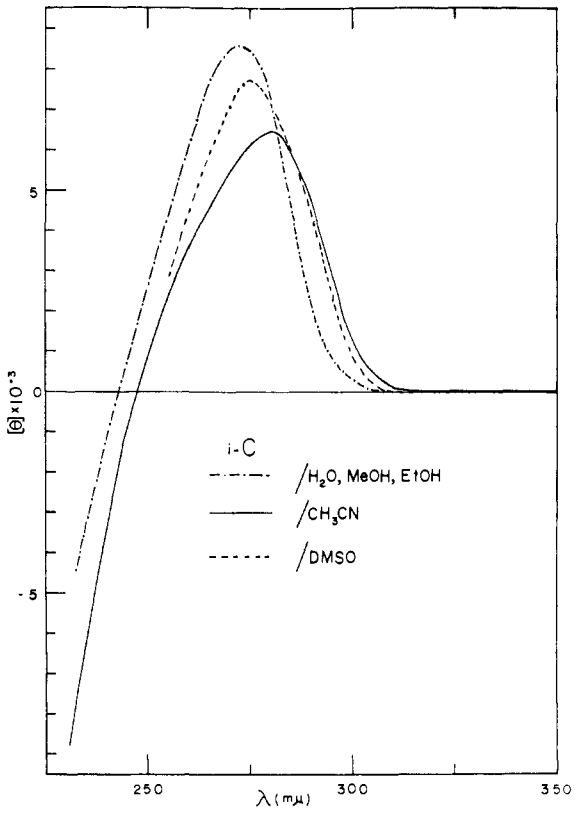

Figure 3. Circular dichroism spectra of 2′,3′-isopropylidenecytidine (*i*-C) in solvents which do not produce concentration-dependent ellipticities. The respective curves are identical within 5% from 0.912 × 10^{-5} *M* to 4.55 × 10^{-3} *M*.

Table IV. Correlation of Ultraviolet Absorption, Circular Dichroism, and Nuclear Overhauser Effect Data for Four Typical Pyrimidine Nucleosides

Nucleoside	Solvent	Molar concn, $\times 10^4$	Uv λ_{max}, mμ	ϵ_{max}, $\times 10^{-4}$	CD λ_{max}, mμ	$[\theta]_{max}$, $\times 10^{-3}$	NOE Solvent	NOE Enhancement, %			
Cytidine	H_2O		268[a]	1.0	271[b]	12.7	D_2O	$f_{1',6}$	$f_{2',6}$	$f_{3',6}$	$f_{5',6}$
								11	13	8	0
	DMSO	9.1	275[c]	0.703	277[c]	14.8	62% benzene-d_6 in DMSO-d_6	$f_{1',6}$	$f_{2',6}$	$f_{3',6}$	$f_{5',6}$
								7	11		3
Isopropylidene-cytidine	H_2O	9.1	269[c]	0.850	271[c]	8.4	D_2O	$f_{1',6}$	$f_{2',6}$	$f_{3',6}$	$f_{5',6}$
								24	4	5	0
	DMSO	9.1	273[c]	0.648	275[c]	7.25	DMSO-d_6	[d]			
Uridine	H_2O		268[a]	1.4	267[e]	8.50	25% pyr-d_5 in D_2O	$f_{1',6}$	$f_{2',3',4',6}$		$f_{5',6}$
								6	18		14
	DMSO	9.1	264[c]	0.945	277[c]	10.1	DMSO-d_6	$f_{1',6}$	$f_{2',3',6}$		$f_{5',6}$
								9	19		4
Isopropylidene-uridine	H_2O		260[f]	0.72	270[e]	4.64	75% DMSO-d_6 in D_2O	$f_{1',6}$	$f_{2',6}$	$f_{3',6}$	$f_{5',6}$
								19	10	4	0
	DMSO	9.1	261[c]	0.967	275[c]	5.0	DMSO-d_6	$f_{1',6}$	$f_{2',6}$	$f_{3',6}$	$f_{5',6}$
								18	10	3	0
	$CHCl_3$	0.365	258[c]	1.05	274[c]	2.9	$CDCl_3$	[g]			
		9.1	259[c]	1.03							

[a] D. W. Miles, R. K. Robins, and H. Eyring, *Proc. Nat. Acad. Sci., U. S.*, **57**, 1138 (1967). Unbuffered neutral solution. [b] D. W. Miles, M. J. Robins, M. W. Winkley, and H. Eyring, *J. Amer. Chem. Soc.*, **91**, 831 (1969). Unbuffered neutral solution. [c] This work. Unbuffered neutral solution. [d] Chemical shifts in DMSO-d_6 do not permit NOE experiments. [e] D. W. Miles, M. J. Robins, R. K. Robins, M. W. Winkley, and H. Eyring, *J. Amer. Chem. Soc.*, **91**, 824 (1969). Unbuffered neutral solution. [f] J. J. Fox, L. F. Cavalieri, and N. Chang, *ibid.*, **75**, 4315 (1953). 0.01 *M* NaOH solution. [g] Low solubility and aggregation in this solvent prohibit NOE experiments.

dichloroethane (Figures 4 and 5), which solvents all produce curves suggestive of the mixing of more than one dichroic transition. Measurements made on *i*-C in each of the three associating solvents between the two extreme concentrations for each solution show that the molecular ellipticity of the B_{2u} band grows progressively more positive in proportion to the dilution of each of the chlorocarbon solutions.

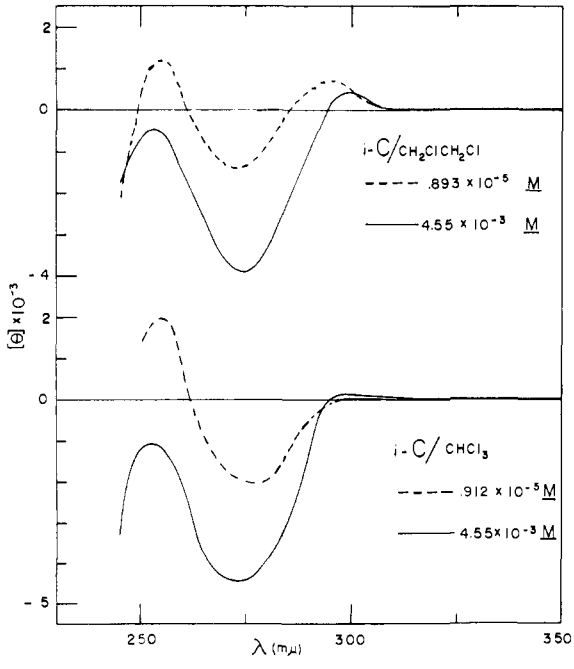

Figure 4. Concentration-dependent circular dichroism spectra of 2',3'-isopropylidenecytidine (*i*-C).

The CD spectra of U and *i*-U manifest a distinct effect of DMSO on the maximum of the positive ellipticity, there being a large shift to higher wavelengths relative to the uv maximum (Table IV; Figure 6). The chloroform solutions of *i*-U show a strongly concentration-dependent CD that changes from a biphasic curve at the higher concentrations to an apparently simple curve

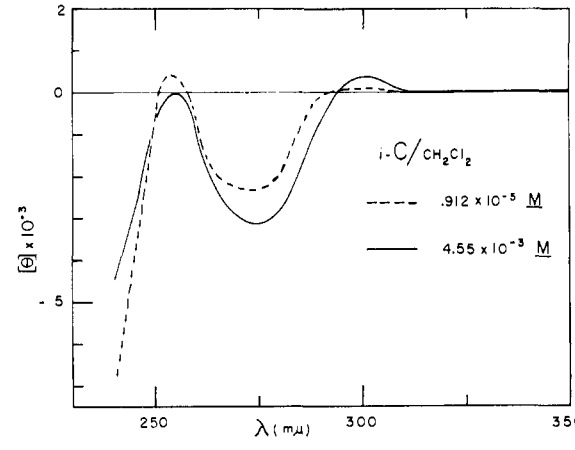

Figure 5. Concentration-dependent circular dichroism spectra of 2',3'-isopropylidenecytidine (*i*-C).

at the lowest concentration employed (Figure 7). The concentration range of *i*-U studied by CD was narrower than in the *i*-C case because of its smaller ellipticity and generally greater ultraviolet absorbance. Neither the position nor the magnitude of the maximum absorbance of *i*-U is noticeably sensitive to solvent or concentration (Table IV).

The CD spectra were studied only in the range of the B_{2u} transition (*i*-C) and the B_{1u}–B_{2u} transitions (*i*-U) because those bands are the most sensitive to glycosidic conformation.[9] Furthermore, comparisons between DMSO and water are impossible below 250 mμ, because DMSO begins to absorb appreciable energy below that point in a 0.1-cm cell. In connection with the DMSO–water comparisons, it is interesting to note that

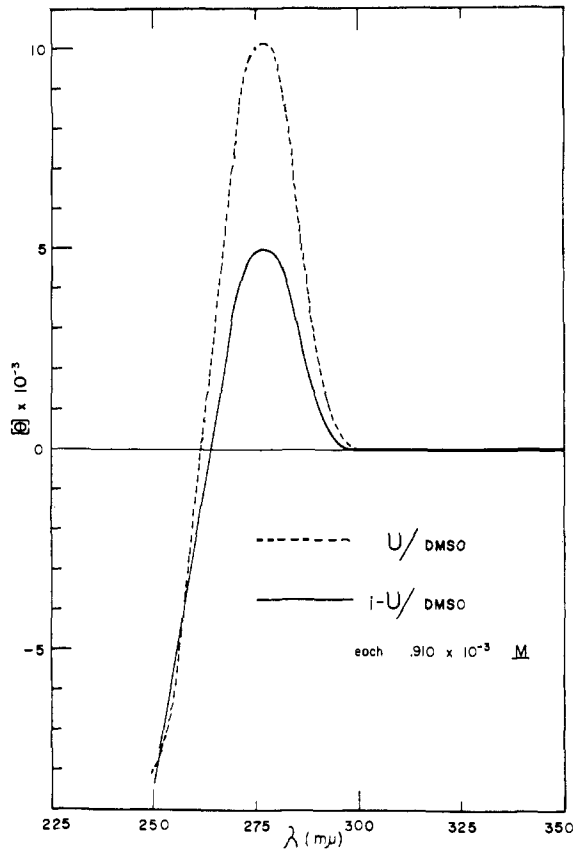

Figure 6. Circular dichroism spectra of uridine (U) and 2′,3′-isopropylideneuridine (*i*-U).

in this series there is not a significant difference between the two solvents as far as molecular ellipticity and curve shape are concerned.

Some of the NOE data herein have been recorded in a preliminary communication.[1] Strict comparisons between NOE and CD data can be made in only four cases, C in water, *i*-C in water, *U* in DMSO, and *i*-U in DMSO. The remaining experiments were run in mixed solvents to allow the separation of certain nuclear magnetic resonances, and to permit the maximum number of enhancements to be measured. Fortunately, the C, *i*-C comparison and the U, *i*-U comparison can be made in the same solvents even though the solvents are different for the two sets. It is unfortunate that the NOE and CD data for all four nucleosides cannot be compared in any one of the nonassociating solvents. However, we regard the similar band shapes and maximum B_{2u} molecular ellipticities for each of the nucleosides in water and DMSO as evidence for little differential effect on confomation by the two soluents.

Discussion

Since *i*-C has been presumed to adopt the syn conformation to a high degree in dichloroethane,[9] we chose this system to obtain NOE data typical of the syn conformation. *i*-C turned out to be not sufficiently soluble in dichloroethane to allow NOE measurements. In addition, though the nucleoside was sufficiently soluble in chloroform and methylene chloride, it was not possible to do the nmr experiment in these solvents because of strong intermolecular association and consequent line broadening.

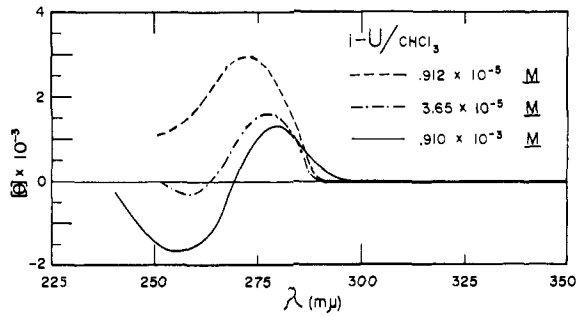

Figure 7. Concentration-dependent circular dichroism spectra of 2′,3′-isopropylideneuridine (*i*-U).

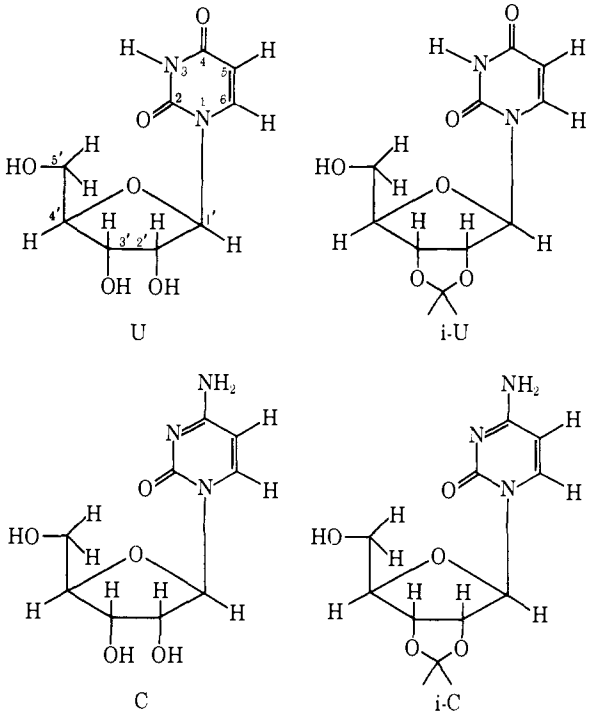

Figure 8. Structural formulas of uridine (U), 2′,3′-isopropylideneuridine (*i*-U), cytidine (C), and 2′,3′-isopropylidenecytidine (*i*-C).

The fact of self-association of *i*-C in the low polarity solvents at the moderately high concentrations of the nmr measurements casts a doubt on the soundness of the use of *i*-C in dichloroethane as a spectroscopic model for the syn conformation and it became necessary to determine whether aggregation phenomena were responsible for this compound's observed ellipticities in nonpolar media.

When degree of association derived from the VPO measurements is plotted against mole fraction of *i*-C (Figure 2) it becomes apparent that significant aggregation occurs in chloroform and in methylene chloride (perhaps to a lesser extent in the latter) and that virtually no self-association occurs in water, methanol, ethanol, and acetonitrile. The CD and NOE data obtained from solutions in the latter solvents (or their deuterated analogs) are therefore interpreted as monomer data.

The uv and CD studies of *i*-C as a function of concentration carried the examination of self-association into the low-concentration region. There is a small bathochromic shift and a distinct reduction in the ab-

sorbance correlated with decreasing solvent polarity (Table II). Further, the absorbance and wavelength changes serve to differentiate the associating and nonassociating solvents. Acetonitrile is anomalous in this study as it falls into the group of associating solvents on the basis of uv data, but into the group of nonassociating solvents on the basis of the VPO measurements. The anomaly is not serious, however, for acetonitrile has been shown to cause hypochromicity relative to water in 9-pyranosyladenine without leading to aggregation.[17] Hypochromicity can, of course, be associated with aggregation[18a,b] and in that case the remaining solvents seem to be appropriately grouped.

The complex concentration-dependent CD spectra of *i*-C and *i*-U (Figures 4, 5, and 7) in chlorocarbon solvents are difficult to interpret rigorously. In general, the observed curve shapes suggest the appearance of a positive curve at the expense of a negative curve on going to lower concentrations. The complexity of the *i*-C curves is not completely removed even at the lower concentrations, while the lower concentrations of *i*-U in chloroform are characterized by distinctly simplified spectra and the lowest concentration curve shows very little of the negative contributor. The *i*-C curves in chloroform differ markedly from their water solution counterpart[9] in the 300–240-mμ range. On the other hand, the *i*-U curves at higher concentrations in chloroform are not fundamentally different from their polar solvent counterparts, there being a positive and negative portion in both solvent types between 300 and 240 mμ. The lower wavelength Cotton effect in this range has been associated with a B_{1u} transition[8] in the uridine series and possible reasons for not seeing a corresponding Cotton effect in some cytidine derivatives[9] are either that the transition does not exist in those derivatives or that the transition gives a positive Cotton effect. In the latter case, the single positive curve that is seen in the cytidine derivatives might actually be an unresolved summation of the B_{1u} and B_{2u} transitions. Magnetic circular dichroism studies verify this conclusion.[19] The similarity between the *i*-U CD spectra in polar and nonpolar solvents may be coincidental, however, since the biphasic nature of the chloroform curve disappears as the concentration is reduced. The pronounced, DMSO-induced shift of the B_{2u} ellipticity maximum to higher wavelength relative to the uv absorbance maximum—a shift also caused to a lesser extent by water, dioxane, and acetonitrile—is probably due to a selective solvent effect on the B_{2u} and B_{1u} transitions. Such effects have been discussed.[8]

The appearance of new or complex CD bands has been treated in several contexts and attributed to several specific phenomena. The enhanced ellipticity of helical polynucleotides relative to the ellipticity of independent monomer components has been related to intra- and intermolecular base–base interactions. In that case, the interacting chromophores become nearly the sole determinant of ellipticity.[20-22] If these interactions are between identical chromophores, exciton splitting can result and one observes closely spaced CD curves of equal magnitude and opposite sign. Exciton coupling has been given considerable theoretical treatment.[20-25] The appearance of exciton splitting has been noted in the stacking of nucleic acid and polynucleotide bases associated with helix formation,[26-28] in the interaction of dyes bound to dissymmetric macromolecules,[29] and in the self-association of several chlorophyll and protochlorophyll pigments.[30] If the interaction is between different chromophores with similar uv absorption maxima, the resultant interaction has been called by Urry[31,32] a reciprocal relation to describe the mutual effect of one chromophore on the other. Reciprocal relations are seen, for example, in the intramolecular association of the purine and pyridine moieties of NAD or NADH and the purine and isoalloxazine moieties of FAD. Both exciton splitting and reciprocal relations are special cases of electronic dipole–dipole coupling. Complex circular dichroism spectra have been observed in simpler systems as well and have been variously ascribed to vibronic coupling,[33] the presence of more than one conformation type,[34-36] and the existence of several solvate types.[37,38] In these cases it is usual to observe unsymmetrical biphasic CD curves.

The curve shapes seen in the present experiments are too complex to allow one to factor out exciton contributions and since the chromophores are identical one need not consider reciprocal relations. If the trends in curve shape with decreasing concentration can be interpreted in terms of the diluting-out of self-association, then the dissociation of aggregates is more extensive in the case of *i*-U than of *i*-C and the negative ellipticity observed for both is likely intrinsic only to the aggregates. The gradual enhancement of the positive contributor at the expense of the negative is a good indication of the emergence of some new form (probably monomer) that exists only at a low, limiting, concentration.[39] Whatever species this is, it is apparently more prevalent in the case of *i*-U at the low concentration limit. These CD experiments do not reveal the struc-

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tures of the aggregates, but it is clear that the B_{2u} ellipticity of *i*-C and *i*-U in chlorocarbons at the higher concentrations cannot be used as a reference for the syn conformation.

Observations with regard to known *monomeric* model systems can now be made succinctly. The larger f_1^6 of *i*-C in D_2O (Table IV) relative to f_1^6 of C in D_2O indicates a greater magnetic dipole–dipole[40] interaction between H-1′ and H-6 in the former substance and demonstrates that *i*-C possesses a higher proportion of the syn conformer in water. The smaller observed values of f_2^6, and f_3^6, for *i*-C relative to C support the same conclusion. The NOE experiments indicate that of U and *i*-U in DMSO, *i*-U has a greater proportion of the syn conformer. Because of the very similar nucleoside CD curves in water and DMSO this comparison would probably be found to hold in water if NOE experiments in that solvent were possible.

The trends in these NOE experiments are reflected in the values of the H-1′, H-2′ coupling constants (Table I). The smaller $J_{1',2'}$ values for the isopropylidene derivatives possibly indicate a decrease in the C–H_1′, C–H_2′ dihedral angle, a consequent change of the pyrimidine ring from a pseudoaxial to a pseudoequatorial configuration, and slightly less steric interaction between H-2′ and O-2. Such an explanation must be qualified, however, by knowledge that the relationship of dihedral angle to coupling constant[41,42] varies when substituents of varying electronic properties are involved.[43,44]

The molecular ellipticities of *i*-C and *i*-U are smaller than those of C and U in both water and DMSO. These values, characteristic of the isopropylidene derivatives, reflect a change in the torsion angle distribution as required by the NOE data, a small change in the sugar conformation, and a possible vicinal effect of the isopropylidene group.[45] We assume that the torsion angle change is the major determinant of the observed ellipticity change. Vicinal effects of 2′ and 3′ substituents are usually negligible[9b] and the small sugar conformational change is not expected to directly dominate the observed ellipticity differences. Thus, the qualitative trend is toward smaller ellipticities correlated with a greater proportion of the syn conformer.

Conclusion

Nmr observations of the type presented here allow an approximate identification of nucleoside glycosidic conformation in solution which is not based on theoretical predictions about unsolvated species,[46] model building, simple chemical shift and coupling constant analysis, or solid state X-ray determinations. The theory developed by Schirmer[14a] shows that if enough pairs of spins in a given molecule are probed by double resonance experiments, their NOE's provide unequivocal evidence of the proximity of those sets which produce substantial enhancements and the separation of those which do not.

On the basis of multiple intramolecular NOE experiments, then, *i*-U and *i*-C appear to be characterized by a predominance of the syn conformer in water and in DMSO and the conformational populations of U and C appear to be weighted less toward this conformation. Along with published and new CD data these results suggest (subject to the assumptions made above) that the B_{2u} ellipticities associated with the syn and anti conformations of unsubstituted pyrimidine nucleoside chromophores differ only in positive magnitude.

This conclusion is inconsistent with Miles' assignment of the negative ellipticities of 6-CH_3-U[8], 6-CH_3-C, and 6-CH_3-2′-dC[9a] to a sterically required syn conformation, and one is hard pressed to explain these negative values unless the β-6-methyl derivatives aggregate in water, acetonitrile, and dioxane, or the methyl substituent itself perturbs the B_{2u} Cotton effect. With regard to the second alternative, the small change in pyrimidine nucleoside ultraviolet spectra caused by 5- and 6-methyl groups has led to the expectation[9a] of little alkyl substituent effect on the pyrimidine B_{2u} ellipticity. Clear examples of a large alkyl substituent effect on optical activity exist, however, among the aromatic steroids.[47,48] Both 1-methyl- and 4-methylestradiol show distinctly different chiroptical properties from estradiol, yet the corresponding ultraviolet spectra are nearly identical. As there is virtually no opportunity for conformation changes to accompany such methyl substitution in the estradiol nucleus, the ellipticity and rotatory changes can only be ascribed to a direct, electronic effect. Thus, the negative B_{2u} ellipticities of the three aforementioned 6-methylpyrimidine nucleosides may well be due to a direct substituent effect and not solely to a substituent-induced conformation.

Future work may show the β portion of Miles' diagram to be correct to the extent that a negative ellipticity is associated with a pyrimidine syn conformation and a positive one with the anti. Rogers and Ulbricht have recently presented ORD data for O^2,5′-cyclothymidine, O^2,3′-cyclouridine, O^2,2′-cyclouridine, and O^6,5′-cyclo-6-hydroxyuridine.[49] These models are in the torsion angle order from required syn to required anti and the observed molecular amplitudes vary from large negative to large positive values. The chromophores are different in this series, however, and it has already been pointed out that arguments about the noncyclic nucleosides based on information from the cyclic series must be regarded as tentative.[9a]

The data presently available, correlated empirically, do not lead necessarily to the Miles diagram. Given the present state of experimental information for pyrimidine nucleosides, at most it is warranted to say that syn rotamers elicit a smaller ellipticity than anti rotamers. Further definition of structural influences on the chiroptical properties of pyrimidine nucleosides is required.

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Reduction of Δ^{24} of Lanosterol in the Biosynthesis of Cholesterol by Rat Liver Enzymes. I. The Addition of a 24-Pro-*S* Proton[1]

J. B. Greig,[2a] K. R. Varma,[2b] and E. Caspi*

Contribution from the Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts 01545. *Received June 17, 1970*

Abstract: The side chain of 17α,20,24*R*-$^{3}H_3$-$^{14}C_5$-cholesterol biosynthesized from 4*R*-(2-^{14}C,4-^{3}H)-mevalonic acid by rat liver enzymes was cleaved with an adrenal enzyme preparation and the 1,5-$^{14}C_2$-3*R*-$^{3}H_1$-isocaproic acid encompassing carbons 22–27 of cholesterol was isolated. The $^{14}C_2$,$^{3}H_1$-isocaproic acid was degraded, without disturbing the stereochemistry of the isotopic hydrogen, to 1*S*-3-^{14}C,1-^{3}H-isobutyl alcohol. The isobutyl alcohol was oxidized with NAD^+ and yeast alcohol dehydrogenase (YADH) to 3-^{14}C,1-^{3}H-isobutyraldehyde with complete retention of tritium. Since we proved that NAD^+–YADH oxidation of isobutyl alcohol proceeds with the abstraction of the 1-pro-*R* proton it follows that the isobutyl alcohol has the 1*S* stereochemistry. Hence the ^{14}C,^{3}H-cholesterol must have the 24*R* stereochemistry. This implies that a 24-pro-*S* proton adds to Δ^{24} of lanosterol in the biosynthesis of cholesterol by rat liver enzymes. It was also demonstrated with the use of samples of 1-$^{3}H_1$-isobutyl alcohols that no significant isotope effects were detected in the NAD^+–YADH oxidation of the alcohol to isobutyraldehyde by the procedures employed.

The biosynthetic transformation of lanosterol (**1**) to cholesterol (**2**) is a multistep process which involves among other transformations the reduction of the C-24 double bond.[3] In this paper we concern ourselves with the mode of reduction of this double bond in a rat liver enzyme system,[4a,b] and particularly with the stereochemistry of addition of the hydrogen at C-24. At the time that this work was undertaken it was already known[5a] that the biosynthesis of squalene, lanosterol, and cholesterol from exogenously supplied mevalonic acid (MVA) proceeds with the stereospecific retention of, respectively,[5a] six, five, and three 4-pro-*R* protons of MVA. Of the three protons retained in cholesterol the presence of the one at C-17 was proven experimentally.[5a] The other two were thought to be located at C-20[5b] and -24,[4b] which we later confirmed.

The terminal methyls of lanosterol (and of cholesterol (**2**)) differ in that one originates from C-2 and the other from C-3′ of MVA. From the work of Birch, *et al.*,[6a] it was inferred that the geometry at Δ^{24} of lanosterol is that shown in **1** (the heavy dots indicate carbons derived from C-2 of MVA, and the encircled protons indicate hydrogens originating from the 4-pro-*R* position of MVA). Subsequently the hypothesis was confirmed experimentally.[6b]

The sequence of biosynthetic reactions in the transformation of lanosterol to cholesterol is unknown. However, to simplify discussion we will for the present assume that demosterol (**4**) is the immediate precursor of cholesterol. It follows therefore that acquisition by demosterol of two hydrogens at C-24 and -25 will give cholesterol. We will now analyze the possible mechanisms of this reduction from the point of view of events at C-24. The addition of a hydrogen at C-24 can occur either from the front or back of the double bond. In a backside attack the encircled C-24 proton thought to be derived from the 4-pro-*R* position of MVA will be pushed forward as in **5** and **6**. If the overall reduction entails a cis addition of two hydrogens, the resulting cholesterol will have the configuration shown in **5**. On the other hand if the reduction is a trans process the biosynthetic cholesterol will have the configuration as in **6**. By a similar reasoning, should the attack at C-24 occur from the front side, a cis or trans reduction will result in cholesterols **7** and **8**, respectively. Hence, for the determination of the overall mechanism of the reduction of Δ^{24} it is necessary to define the prochirality at C-24[4a] and C-25[4b] of the biosynthetic cholesterol.

We focused first on the events taking place at C-24 which are described in this paper.[4a] Our plan of attack was to biosynthesize cholesterol from MVA stereospecifically labeled with isotopic hydrogen at the 4-pro-*R* position and then to determine the configuration at C-24. The most direct approach would have been to biosynthesize cholesterol from 4*R*-4-$^{2}H_1$-MVA and

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