THE CONFORMATION OF PYRIMIDINE NUCLEOSIDES. AN APPLICATION OF THE NUCLEAR OVERHAUSER EFFECT.

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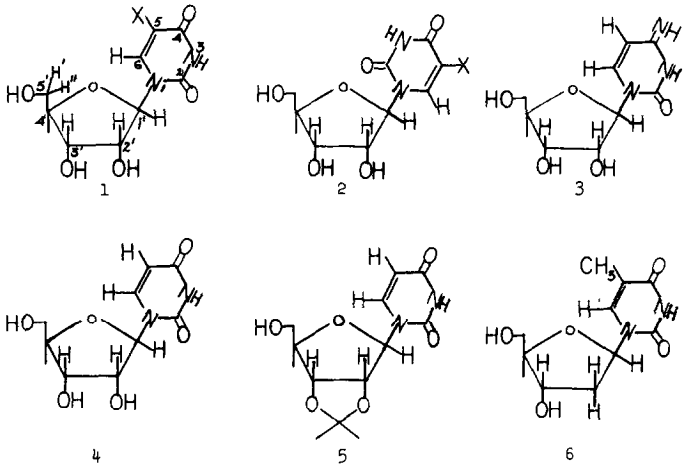
Summary The conformation about the glycosidic bond of cytidine, uridine, 2',3'-isopropylidine uridine and thymidine were studied by observing nuclear Overhauser effects. It is concluded that the syn conformation as well as intermediate-range conformations must be considered as well as the usually accepted anti form.

As well as being intrinsically interesting, knowledge of the conformation of nucleosides and nucleotides about the glycosidic bond may be important in the consideration of the reactions of these species as well as in the consideration of the conformation of di-, tri-, and polynucleotides. We have recently found (Hart and Davis, 1969) that the nuclear Overhauser effect is a useful tool with which to examine the conformation about the glycosidic bond of purine nucleosides and report here our findings in the pyrimidine nucleoside area.

The experimental determination of pyrimidine nucleoside conformation has received considerable attention. On the basis of a large number of optical rotatory dispersion measurements, it has been concluded (Emerson, Swan and Ulbricht, 1967) that the pyrimidine nucleoside conformational equilibrium contains predominantly the anti form (depicted in 1; ϕ_{CN}-30, Donohue and Trueblood, 1960). Further experimental work (Cushley, Watanabe and Fox, 1967; Cushley, Wempen and Fox, 1968) applying proton and fluorine magnetic resonance has been interpreted in terms of a preferred anti conformation for the pyrimidine nucleosides.

Recently, a diagram has been established on the basis of circular dichroism measurements (Miles, et al, 1969) from which one may read the ellipticity of the B_{2u} band of the pyrimidine nucleosides as a function of the torsion angle (ϕ_{CN}). The diagram is based on the assumption that the natural pyrimidine nucleosides adopt the anti conformation. These authors have noted interesting changes in rotation with solvent and structure which they correlate with conformational changes.



As well as the above experimental work, several computational procedures have been performed (Haschemeyer and Rich, 1967; Jordan and Pullman, 1968; Tinoco, Davis and Jaskunas, 1968) and in all cases the anti range of conformations is found to be favored for the pyrimidine nucleosides. All of the above conclusions are contrary to an observation derived from inspection of models (Donohue and Trueblood, 1960) that both the syn and the anti conformers could be allowed.

We undertook nuclear Overhauser effect experiments to determine whether the assumption of preferred anti conformation in the pyrimidine nucleoside series was warranted and have found that under the conditions of our experiments the conformer equilibrium in this series of nucleosides is quite complex and in some cases it is not possible to assign a preferred conformation at all. The nuclear Overhauser effect (NOE) is observed during nuclear magnetic double resonance experiments and has been described elsewhere (Hart and Davis, 1969 and references therein). It is, basically, a method that allows one to discov-

er spatially proximate magnetic nuclei by observing an enhancement in the integrated intensity of the resonance line of one nucleus when the other member of the pair is irradiated with an H_2 field at its resonance position.* The method is ideally suited to the determination of the conformation of both purine (Hart and Davis, 1969) and pyrimidine nucleosides about the glycosidic bond. In the pyrimidine nucleoside case, H-6 is an ideal probe. It is not J coupled with any of the sugar protons, yet as the pyrimidine ring is allowed to rotate through all the possible values of ϕ_{CN}, it is seen that H-6 can be close enough to all the sugar protons with the exception of H-4' and the protons attached to oxygen to allow an NOE. Thus, a sole interaction with H-5' and/or H-5'', would indicate a predominant anti conformation (1) and an exclusive interaction with H-1' would indicate a predominant syn conformation (2). Interactions with H-2' and H-3' would be evidence for intermediate-range conformations.

Materials and Methods The natural pyrimidine nucleosides uridine (4), cytidine (3) and thymidine (6) were chosen for study because the anti conformation is ordinarily assumed for those substances. Isopropylidine uridine (5) was studied because the sugar moiety is conformationally rigid. The models were all commercial samples and were used without further purification. The samples were lyophilized once from D_2O.

The experiments were done in dimethyl sulfoxide-d_6 as well as deuterium oxide to see the effect of self association that has been observed in water (Ts'o, Melvin and Olson, 1963; Ts'o and Chan, 1965; Schweizer, Chan and Ts'o, 1965) compared with the situation in dimethyl sulfoxide in which solvent little or no self association occurs (Katz and Penman, 1966). Both solvents would minimize intramolecular hydrogen bonding and that effect would be eliminated as a factor in lowering the energy of certain conformations involving O-2, OH-2' and O-2, OH-5' hydrogen bonds. Thus, one is able to study conformation as a function of non-bonded interactions only.

* The effect varies as the sixth power of the internuclear distance in rigid intramolecular cases and as the third power of a size parameter in the case of variable distances (Powles, 1963).

TABLE I
Results of The Nuclear Overhauser Effect Experiments

Compound	Solvent[a]	Proton(s) Irradiated (mv at 20 db)	Proton Observed	Percent Enhancement
Cytidine	DMSO-d_6	H-2',3',4'(200)	H-6	18
	DMSO-d_6 (1/1) benzene-d_6[b]	H-1' (200)	H-6	8
		H-2' (200)	H-6	13
		H-3',4',5'(200)	H-6	4
	D_2O	H-1' (100)	H-6	13
		H-2' (100)	H-6	14
		H-3' (100)	H-6	10
		H-4',5'(100)	H-6	0
Uridine	DMSO-d_6	H-1' (150)	H-6	10
		H-2',3'(100)	H-6	13
		H-5' (200)	H-6	10
	D_2O	H-5' (150)	H-6	5
	D_2O (75/25) pyridine-d_5[b]	H-1' (50)	H-6	6
		H-2',3',4'(200)	H-6	18
		H-5' (200)	H-6	12
2',3'-Isopropylidine Uridine	DMSO-d_6	H-1' (100)	H-6	19
		H-2' (200)[c]	H-6	14
		H-3' (200)[c]	H-6	4
		H-5' (200)	H-6	5
	DMSO-d_6[d] (75/25) D_2O	H-1' (150)	H-6	19
		H-2' (150)	H-6	10
		H-3' (150)	H-6	4
Thymidine	DMSO-d_6	H-1' (150)	H-6	7
		H-2' (150)	H-6	18
		H-3' (150)	H-6	8
		H-5' (200)	H-6	4
	D_2O (8/2) DMSO-d_6[d]	H-1' (150)	H-6	5
		H-2' (200)	H-6	16
		H-3' (200)	H-6	8
		H-5' (200)	H-6	4

a sample concentrations approximately 0.25 M
b required to change relative chemical shifts of H-1' and H-5 so that H-1' could be irradiated independantly of H-5.
c positions determined by decoupling H-1'
d insufficient solubility in water.

The results of the nuclear Overhauser effect experiments are recorded in Table I.* A typical experiment involved first establishing a 'standard' trace of the resonance of an appropriate proton by observing the resonance band while a secondary field (H_2) in a signal-free part of the spectrum was turned on. Then with the first H_2 off, the appropriate proton signal was observed while an H_2 of the same intensity was imposed at the frequency of a second proton. The effect of the H_2 irradiation was expressed as the percentage peak height difference between the 'standard' and the experimental trace. A positive enhancement greater than four percent was taken as evidence for a nuclear Overhauser effect. The measurement of peak heights rather than peak areas is valid as no signal width variations are observed in these experiments.

Discussion of The Results Cytidine: The observed interaction of H-6 with H-1', H-2' and H-3' in both solvents makes it necessary to include the syn conformation as well as intermediate range conformations in any consideration of the cytidine conformer equilibrium. There was no observed interaction of H-6 and H-5' - H-5''. This negative observation may indicate that the pure anti conformer is unimportant or may simply indicate that the 5' hydrogens are rotated away from the pyrimidine ring. The presence of benzene would serve to enhance self-association as well as to allow another type of solute-solvent interaction. One of the two possibilities may account for the apparently restricted conformational situation evidenced by the non-interaction of H-6 with H-3'.

Uridine: It is possible that the uridine conformational equilibrium is composed of an even richer variety of conformers than cytidine because H-6 interacts with H-1', H-2' and/or H-3' and H-5' - H-5''. In this case also, the syn conformation as well as intermediate-range conformations must be considered, though the extent of the intermediate range can not be assessed as the H-2', H-3' chemical shifts are the same and it is not possible to differentiate between them.

* A Varian HA 100 NMR spectrometer run in frequency sweep mode was used. Coaxial sample tubes were used to minimize possible additional relaxation mechanisms by the hexamethyl disilizane lock sample. Samples were de-gassed by multiple freeze-pump-thaw cycles.

Isopropylidine Uridine: Only H-1' and H-2' interact with H-6 in this model indicating that in this case too the syn conformation must be considered. It is possible to state in the present case that there is no interaction of H-6 with H-3' as the latter resonance is distinguishable from H-2'. The non-interaction of H-6 with H-5' - H-5'' may indicate an unfavorable conformation about the C-4' - C-5' bond or may be a function of the change from the more flexible furanose conformation of uridine (Jardetzky, 1960) to the rigid furanose conformation in the isopropylidine derivative. Models show that in the more rigid case, there is a possibility of increased non-bonded interactions between H-2' and H-6 that might restrict rotation of the pyrimidine ring into the anti range.

Thymidine: The apparently strong interaction of H-6 with H-2' and the apparently weaker interaction of H-6 with H-1' and H-3' tempt one to speculate that the pure syn conformation is disfavored and that an intermediate-range conformation is important. It must be stressed, however, that it is not, at present, possible to make quantitative judgements based on the enhancement values. We are treating similar data quantum mechanically to allow those judgements to be made. The appropriate conclusion at this stage of our investigations is that a syn-like conformation must be considered for thymidine. In this case too, the non-interaction of H-6 and H-5' - H-5'' may be a function of the conformation about the C-5', C-4' bond. However, contrary to the pyrimidine ribosides the pyrimidine 2'-deoxyribosides are characterized by an O-endo sugar conformation (Jardetzky, 1961; Lemieux, 1961) in which the pyrimidine ring is more equatorial than it is in the riboside series. The result of that difference is that H-6 is a minimum of 3.5 A^{o} from H-3' and 3.25 A^{o} from H-5 or H-5'', distances that might preclude a nuclear Overhauser effect.*

Although the recorded enhancements can not, at this time be used as a quantitative indication of the conformer population, we feel that the results afford a strong qualitative indication

* The distances were determined by measurement of the internuclear distances of Framework Molecular Models using a planar pyrimidine ring.

that subtle differences in the sugar moiety affect the conformer equilibrium and that several major conformers must be considered for the natural nucleosides cytidine, uridine and thymidine. In particular, under the conditions of these experiments, we feel that the anti conformation can not be assigned an exclusive role, in keeping with Donohue and Trueblood's original contention.

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