Conformation of nucleosides: II. Dipole moments and conformation of nucleosides

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Summary: The solubilities of uracil and thymine nucleosides in dioxane, about 0.02 M at room temperature, have been found adequate to make possible dipole moment measurements. Such data have been obtained for four nucleosides with the predominant conformation anti (uridine, ribose thymine, deoxyuridine, thymidine), and one with the conformation syn (6-methyluridine). The experimental values have been compared with those calculated theoretically in relation to nucleoside conformation.

The stereochemistry of mononucleosides and mononucleotides, particularly in aqueous medium, is a matter of some importance in relation to the structure of oligo and polynucleotides. Nuclear magnetic resonance and circular dichroism are at present the principal experimental methods which have provided data indicating that nucleosides (1,2, 3,4) and nucleotides (5) in aqueous medium exist largely in the anti conformation. However, some recent measurements by means of the Overhauser nuclear effect (6), and relaxation methods based on absorption of ultrasound in solution (7), point to the presence in some nucleosides of an appreciable fraction with the conformation syn.

A recent extensive theoretical study by Kang (8), who calculated the dependence of the dipole moments of several nucleosides on the value of ϕ_{CN} for the glycosidic bond, suggested to us the utility of obtaining some experimental data on the dipole moments of nucleosides. According to Kang (8), the difference in dipole moments between the anti and syn conformations of a given nucleoside, depending on the conformation at $C_{1'}$, may attain a value as high as several Debye units, although theoretically calculated values subsequently reported by Berthod and Pullman (9) are not in agreement with this conclusion in the case of 2'-deoxyuridine.

It is rather surprising that no dipole moment measurements for nucleosides have hitherto been reported. Indeed, in a number of instances (e.g. ref. 10) it was found necessary to assume that the dipole moment of a nucleoside may be approximated by that for the

Table 1: Dipole moments of some nucleosides in dioxane at 25.0°C ± 0.05°C.

Nucleoside	μ (D)
2'-deoxyuridine	5.25 ± 0.01
Thymidine	5.25 ± 0.01
Uridine	5.01 ± 0.02
Thymine ribose	4.86 ± 0.02
6-methyluridine	4.48 ± 0.03

corresponding methylated heterocyclic base. This unsatisfactory situation stems in large part from the fact that the poor solubility of unsubstituted nucleosides in solvents of low polarity poses serious technical problems of measurement.

We have now found that uracil and thymine nucleosides are sufficiently soluble in dioxane (saturating concentration at room temperature about 0.02 M) to permit of reasonably accurate measurements of dipole moments. Deoxyuridine was a Loba Chemie (Vienna) product, thymidine was obtained from Merck (Darmstadt, G.F.R.), uridine from Koch-Light (England), ribose thymine from Calbiochem (Lausanne). All four were A grade products, and were further recrystallized twice from ethanol and checked for chromatographic homogeneity. We are indebted to Dr. A.Holy for Institute of Organic Chemistry and Biochemistry for a gift of 6-methyluridine. The dioxane employed was purified according to standard procedures (11). Dielectric constant measurements were carried out with a DM-01 Dipolemeter (WTW, German Federal Republic). A Zeiss (Jena, German Democratic Republic) interferometer was employed for determinations of differential refractive index. Dipole moments were calculated according to the procedure of Guggenheim (12). Further details are described elsewhere (13).

The dipole moment values for several nucleosides are exhibited in Table 1. These include uridine, ribose thymine (5-methyl uridine) 2'-deoxyuridine and thymidine, all four of which are generally accepted as being predominantly in the anti configuration; and 6-methyluridine, shown by NMR and CD to be predominantly syn (1,4).

The identical dipole moment values for 2'-deoxyuridine and thymidine appear to point to the identity of the contributions of the

carbohydrate moieties. This is, however, true only to a first approximation, for the dipole moments of various 5-methyluracil derivatives have been found to be slightly lower than those of the corresponding uracils (13). Consequently the conformations of the two nucleosides probably differ to a small extent, in accord with NMR data, which show the absence in thymidine of any difference in chemical shift between $H_{2'}$ and $H_{2''}$, whereas in deoxyuridine $\Delta\delta$ is about 0.1 ppm (14).

By contrast, the difference between uridine and ribose thymine is appreciably greater than the difference between uracil and thymine (13). Since NMR spectroscopy has shown similar conformations for the ribose rings in uridine and ribose thymine (15), the difference in dipole moments may reflect either a difference in conformation of the exocyclic 5'-CH_2OH, or a difference in the values of ϕ_{CN}.

The problem now is to evaluate the conformations of the nucleosides on the basis of the measured dipole moments. The value for thymidine corresponds, from the theoretical curve of Kang (8) to a ϕ_{CN}, using the nomenclature of Sundaralingam (16), of 0°, in accordance with the conformation anti. A similar conclusion may be drawn with respect to 2'-deoxyuridine. It must, however, be noted that Kang (8) assumed a $C_{3'}$-endo conformation for the deoxyribose moiety of thymidine, whereas more recent 220 MHz NMR measurements (14) suggested that the deoxyribose conformation in 2'-deoxyuridine and thymidine (in D_2O) is predominantly 3'-endo,2'-endo.

The foregoing is, of course, based on the assumption that the conformation of nucleosides in aqueous medium is identical with that in dioxane. It will consequently be necessary to examine the NMR spectra of thymidine and 2'-deoxyuridine in dioxane, a project which is now under way. Nonetheless, it should be noted that for several nucleosides, including thymidine, adenosine and deoxyadenosine, the coupling constants J(1' - 2',2") have been reported to be virtually the same in such widely differing solvents as D_2O, DMSO-d_6 and pyridine-d_5 (e.g. ref.17). On the other hand, the values of J(1' - 2') for cytidine and uridine have been reported to exhibit solvent-dependent effects (16). The theoretical calculations take no account of solute-solvent interactions.

More pertinent is the difference between uridine and 6-methyluridine. Direct comparison between these two is not valid, because of the 6-methyl substituent in the latter. Fortunately the dipole moment of 6-methyluracil itself has now been measured experimentally, the value being 4.7 D (13). Hence the dipole moment for 6-methyluridine (a syn

conformer) is lower than that for the parent pyrimidine, as compared to the increased value for uridine (an anti conformer) relative to uracil, = 4.1 D (13), thus qualitatively in agreement with the calculations of Kang (8).

The foregoing results, and their comparison with the theoretical calculations, point to the utility of further dipole moment measurements on those nucleosides with sufficient solubility in dioxane. This, in turn, will require more extensive theoretical calculations, with due regard to the different possible conformations of the pentose moieties. Furthermore, the availability of dipole moment data for nucleosides should make possible a more precise evaluation of their potential contribution to the forces responsible for the conformation and stability of oligo and polynucleotides. Solubility of various nucleosides in dioxane and other solvents of low polarity are therefore being examined with a view to extension of the foregoing data.

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