ON THE ATOMIC OR "LOCAL" CONTRIBUTIONS TO PROTON CHEMICAL SHIFTS DUE TO THE ANISOTROPY OF THE DIAMAGNETIC SUSCEPTIBILITY OF THE NUCLEIC ACID BASES.

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Summary. The contribution of the atomic diamagnetic anisotropies to chemical shifts is calculated and compared to the effect of the ring current for protons located in a plane 3.4 Å above the surface of the nucleic acid bases. The contribution is found to be quite significant. The relative role of the two factors and the implications of the additional term for the interpretation of experimental data are considered.

The upfield shift observed in proton magnetic resonance spectra of aromatic molecules associated through vertical stacking has been widely used for the determination of the structure of molecular aggregates. This characteristic variation which is due to the strong anisotropy of the molecular diamagnetic susceptibility of this type of compound has been of particular interest in the study of the secondary structure in solution of polynucleotides [1][2][3] and related systems [4] or of the geometry of complexes between polynucleotides and intercalating agents such as actinomycin [5] or ethidium bromide [6]. For the interpretation of the measured quantities the experimentalists utilize the theoretical values of the contribution of the diamagnetic susceptibility anisotropy to the chemical shift of the protons studied. The comparison of the experimental shift variation with the calculated values for different intermolecular arrangements makes it possible to deduce the most probable of them.

Up to now, the theoretical variations of the chemical shifts ($\Delta\delta$), reported either as isoshielding curves [7][8] or as tables [9], have taken into account for intermolecular distances only the ring current effect. In some cases [10][11][12] experimental studies have suggested, however, that the values of $\Delta\delta$ calculated in this way could be underestimated. Thus e.g. in order to obtain a satisfactory agreement between theory and experiment in the NMR study of the secondary structure of yeast phenylalanine t-RNA in solution Lightfoot et al. [11] had to increase the calculated magnitude of the purine induced shift

by 20%. Similarly, in their study of self assembled 5'-guanosine monophosphate, Pinnavaia et al. [12] measured an association shift for H_8 of 0.95 p.p.m. while the theoretical results [7] predict a shift of only 0.6 p.p.m. for the most probable intermolecular arrangement. This discrepancy between theory and experiment can be attributed either to an underestimation of the ring current intensities in the purines or to the neglect of additional contributions to the chemical shift variation. This second reason seems more plausible as it has been shown that the contribution of the ring current alone does not always reproduce satisfactorily the experimental molecular diamagnetic susceptibility anisotropies and that in the case of aromatic hydrocarbons [13] and in that of biological purines and pyrimidines [14] the calculated values of this quantity are in better agreement with the experimental ones if both the ring current effect and the contribution of the atomic diamagnetic susceptibility anisotropies are included in the computation.

This situation induced us to include the atomic contributions in the calculation of intermolecular $\Delta\delta$ due to the anisotropy of the nucleic acid bases. It has been shown [15][16][17] that these terms may be important in intramolecular approaches but no informations seem available about their influence for intermolecular problems.

Fig. 1 represents isoshielding curves for protons located in a plane 3.4 Å above the molecular surface of cytosine, uracil (representing also thymine), adenine and guanine computed as the sum of the ring current effect evaluated previously [7] and of the contribution of the atomic diamagnetic susceptibility anisotropy. This second term is calculated through the dipolar approximation from theoretical diamagnetic susceptibility tensors, the computational procedure being identical to the one utilized in our study of the conformational dependence of the proton shifts of the ribose in nucleosides and nucleotides [17].

The comparison of the curves of fig. 1 with the corresponding results for the sole effect of the ring current (figs. 1-4 of ref. 7) shows a significant role of the contribution of the atomic anisotropy effect. At the maximum of the isoshielding curves this last contribution amounts uniformly to about 0.35 p.p.m.. Its relative contribution is, however, particularly pronounced for the pyrimidines in which it multiplies e.g. the maximum value due to the ring current effect alone by a factor of 2 for cytosine and of 4 for uracil, to the point that these new results induce us to think that the variations of the chemical shift due to the diamagnetic susceptibility anisotropy of these compounds could be large enough to be measured in favorable experimental conditions, (which was not the case when the ring current effect was the only term taken into account). The present results may imply some modifications in the interpretation of the small variation of the proton chemical shift as a function of concentra-

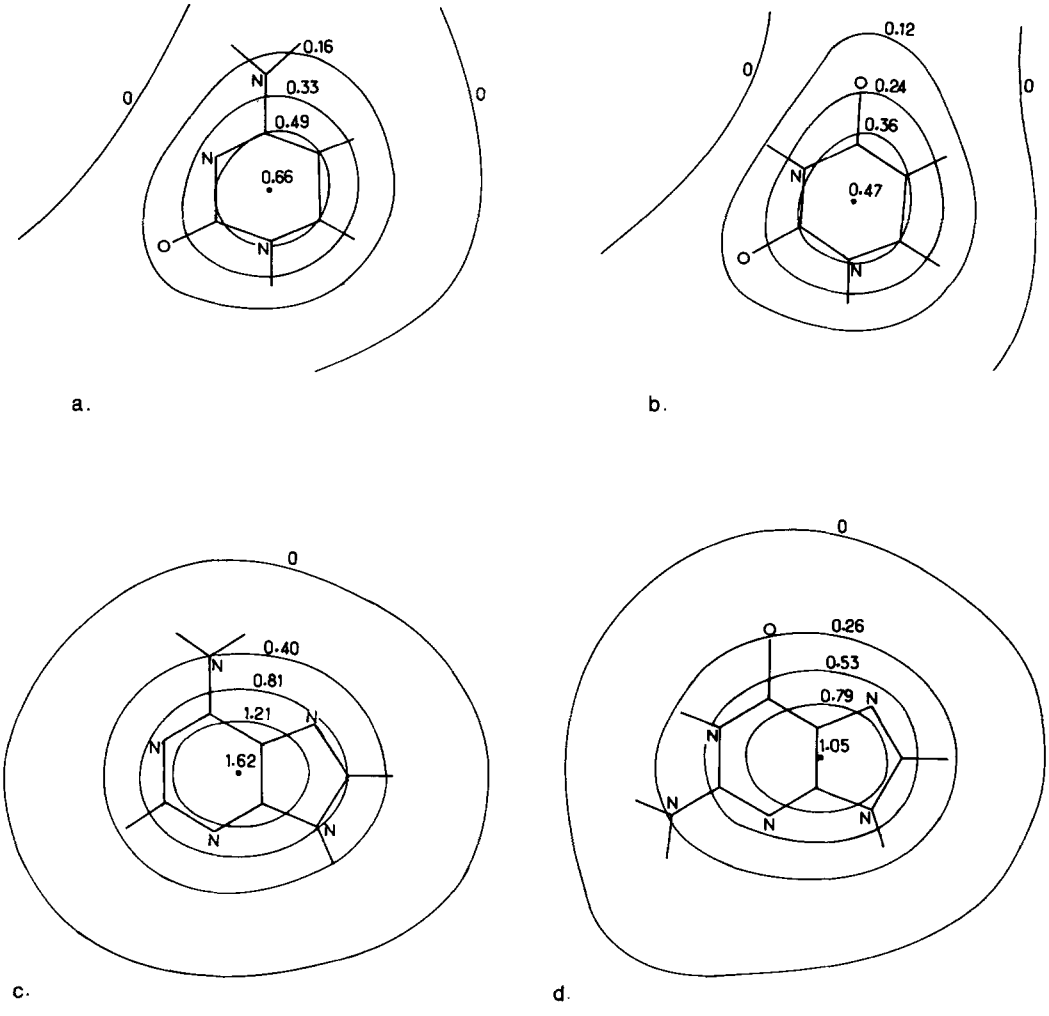

Figure 1. - Intermolecular shielding values ($\Delta\delta$ in p.p.m.) due to the sum of the contributions of the ring current and of the atomic diamagnetic susceptibility anisotropies, in a) cytosine, b) uracil, c) adenine, d) guanine (in a plane 3.4 A distant from the molecular surface).

tion for pyrimidines in water [18][19]. In addition it may be useful to note that the isoshielding curves for cytosine and uracil are no longer regular circles, in particular for $\Delta\delta = 0$.

For the purines the variation in $\Delta\delta$ brought about by the introduction of the atomic contributions represents, at the maximum, an increase of about 25% in adenine and 40% in guanine with respect to the ring current effect alone. We may note also that for guanine the curves corresponding to relatively large

values are somewhat more extended than previously toward the six membered ring; in particular the location of the maximum is shifted toward this ring with respect to its location in the computations limited to the ring current effect. The present results substantiate thus the conclusions of ref. [10-12,20] that the variations of the chemical shifts due to the ring current effect underestimate $\Delta\delta$ due to stacking. They indicate moreover that for the two purines and at the intermolecular distances under consideration, the effect of the contribution of the atomic diamagnetic susceptibility anisotropy is of the appropriate order of magnitude to bring about a reasonable agreement between theory and experiment. The here included curves may thus replace advantageously for future studies those of ref. 7.

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