carbonyl oxide account for a rotational barrier which is only correctly predicted at the highest level of sophistication, suggesting that a function calculated in relatively small basis set provides a qualitatively correct electronic description, even if its energy lacks reliability. Finally, the agreement between our results and

reactivity trends emphasizes the usefulness of the still up-to-date valence-bond theory as a tool providing some unique and easily interpretable information.

Ab Initio Quantum Mechanical Calculations of the Magnetic Shielding Constants of the Different Nuclei of Cytosine

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Abstract: The magnetic shielding constants of the different nuclei of cytosine are calculated by an ab initio self-consistent perturbation method utilizing gauge invariant atomic orbitals and a "split valence shell" basis set of Gaussian functions. The comparison of the results obtained with experimental data shows that for each of the four different types of nuclei present in the molecule (H, C, N, O) the theory reproduces correctly the qualitative trends observed and, in many cases, the numerical values of the measured chemical shifts.

In order to be able to carry out reliable calculations on the variation of the chemical shifts of the different nuclei of a nucleoside, a nucleotide, or a nucleic acid, due to conformational changes in that entity and/or to molecular interactions, it is necessary, in the first place, to be able to calculate with a fair degree of accuracy the magnetic shielding constants of these nuclei in the isolated molecules which are the building blocks of the biological entity of interest. For the nucleic acids there are three different units to be considered, namely the phosphate group, the ribose, and the purine and pyrimidine bases.

The semiempirical calculations of magnetic shielding constants, which have been carried out for nucleic acid bases,[1,2] nucleosides,[3] nucleotides,[4] and nucleic acids[5,7] have been concerned only with proton resonances. Moreover, if there is some evidence that such calculations take into account satisfactorily the "through space" or "geometric" effects[8] due to the ring currents[2a,b,9] and the anisotropy of the atomic susceptibility tensors,[9,10] the calculation of the "chemical" contributions,[8] such as the polarization effect, which has to be taken into account in conformational as well as

in intermolecular problems,[2c,d,3,4] appears to be more delicate to carry out, even for protons.

Since it is now currently possible to study the ^{31}P, ^{17}O, ^{15}N, ^{14}N, and ^{13}C as well as ^{1}H nuclear magnetic resonance spectra of nucleosides or nucleotides,[11-14] oligo or polynucleotides,[11e,15,16]

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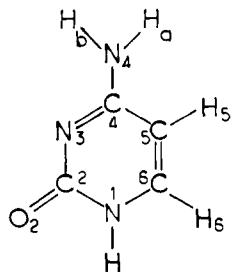(16) Schleich, T.; Cross, B. P.; Smith, I. C. P. *Nucleic Acids Res.* **1976**, *3*, 355–370.

Figure 1. Numbering of atoms in cytosine.

and nucleic acids[17,18] it appears necessary to utilize methods of calculation which are able not only to take into account the above types of interaction but also to give results of the same degree of accuracy for the different species of nuclei present in the molecule.

With these different scopes in mind we have undertaken the calculation of the magnetic shielding constants of the different nuclei present in the nucleic acid constituents in order to contribute to the detailed understanding of the chemical shift variations observed in these molecules. Our first computations have been concerned with the dimethylphosphate anion taken as a model of the phosphate group.[19,20] Since the results obtained for the variation of the magnetic shielding constant of ^{31}P with the molecular conformation and hydration are in satisfactory agreement with experimental data,[19] we can reasonably suppose that the non-empirical self-consistent perturbation method[21] used for these computations will give reliable results also for the other constituting units of nucleic acids and in particular for the nucleic acid bases which are a good test because they are rigid molecules with several distinct carbon, nitrogen, and hydrogen nuclei. They offer therefore the possibility to test the ability of the theory to reproduce correctly the observed variation of the magnetic shielding constant of a given type of nucleus as a function of its location in the molecule and of its state of hydridization (in the case of the nitrogen atoms), in other words to take into account correctly the "chemical" contribution to magnetic shielding constants.[8] To initiate this study we have chosen cytosine, the smallest base.

Method

There exist in the literature a number of semiempirical methods which formally take into account the inter- and intramolecular polarization effects as well as the state of hybridization of the different atoms of the chemical entity studied and for many of them the characteristics of its different excited states, an essential feature for the value of the paramagnetic part of the shielding constant. The common characteristic of these methods, which have been derived at different levels of approximation for the neglect of the integrals appearing in the shielding constant's computation, is to have been developed within the framework of all-valence semi-empirical procedures.[22] With this type of

methods it is possible to have a single formalism able to calculate shielding constants for any type of nuclei, but the use of all-valence electron methods introduces differences between the treatment of the shielding constants of protons on one hand and carbons, nitrogens, and oxygens on the other and between these two groups of nuclei and phosphorous 31. While for the hydrogens no electrons are neglected, the 1s electrons are omitted for C, N, and O and for phosphorous 1s, 2s, and 2p electrons are not introduced in the computational treatment. A non-empirical study using pseudopotentials (the non-empirical all-valence electron method) carried out by Ridard et al.[23] on the ^{31}P shielding constant has clearly shown that for the diamagnetic part of the shielding constant the neglect of the inner shells is easily corrected with a good approximation by adding their contribution as obtained from atomic calculations. But for the paramagnetic term of the shielding constant these authors have obtained a ratio of about 10 between all-electron and pseudo-potential results (-347 and -40 ppm, respectively) for $\sigma^{31}P$ in PH_3 and have shown that it is necessary, in order to obtain good results, to orthogonalize, to the inner shells, the molecular orbitals obtained from the va-lence-shell (pseudo-potential) calculations. Since the paramagnetic term is the part of the shielding constant which in most cases determines the variation of this quantity with the exact molecular structure and the location of the nucleus in the molecule (vide infra), it appears necessary to turn to nonempirical methods of calculation if we want to obtain results of the same degree of accuracy for the different types of nuclei present in the molecules. This feature is most probably stressed in the case of molecules like the nucleic acid constituents which contain distinct regions which are either conjugated, saturated, or even formally charged.

Since the utilization of gauge invariant atomic orbitals[21,24] for the computation of magnetic shielding tensors avoids the use of large basis sets[25] it becomes possible to carry out nonempirical calculations on molecules of the size of the nucleic acid constit-uents. Our previous calculations on the magnetic shielding con-stants of the different nuclei of conjugated molecules like form-amide and N-methylformamide[26] or imidazole[27] have shown that the self-consistent perturbation method developed by Ditchfield[21] is able to give satisfactory results for molecules similar to nucleic acids bases.

The self-consistent perturbation method using GIAO has the advantage of giving values of magnetic shielding constants which do not depend on the system of coordinates used for the input of the molecular geometry, not only for the total shielding constant, but also for the diamagnetic and paramagnetic contributions provided that a proper partitioning of the different terms appearing in the expression of shielding tensor elements is made. The $\alpha\beta$ element of the shielding tensor of nucleus N is given by:

$$\sigma_{\alpha\beta}{}^N = \sum_{pq} D_{pq}{}^0 \langle \chi_p | \mathcal{H}^{H_{0\alpha},\mu_{N\beta}} | \chi_q \rangle + \sum_{pq} D_{pq}{}^{H_{0\alpha}} \langle \chi_p | \mathcal{H}^{\mu_{N\beta}} | \chi_q \rangle$$

where $D_{pq}{}^0$ is the unperturbed ground state density matrix obtained from the SCF calculation and $D_{pq}{}^{H_{0\alpha}}$ is the perturbation of the density matrix due to the external magnetic field H_0 directed parallel to the α axis.

According to Ramsey's original definition[28] the diamagnetic part of the shielding constant is the one which depends only on the unperturbed ground state wave function (first term in the second member of the above equation) while the paramagnetic part contains the perturbed molecular coefficients which depend on the excited states of the molecule (second term of the second member of the above equation).

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Table I. Variation of the Four Terms of the Magnetic Shielding Constants of the Three Nuclei of Water as a Function of the Atomic Coordinates[a]

		x	y	z	σ^1	σ^2	σ^d	σ^3	σ^4	σ^p	σ
A	H1	0.0	0.0	0.0	31.32	−0.23	30.09	0.34	1.99	2.33	32.42
	O	0.0	0.0	0.957	397.2	−5.4	391.8	0.4	−8.5	−8.1	383.7
	H2	0.926	0.0	1.197	21.61	8.48	30.09	0.0	2.33	2.33	32.42
B	H1	5.1	5.0	5.0	31.32	−0.23	30.09	1.67	0.66	2.33	32.42
	O	5.0	5.0	5.957	397.2	−5.4	391.8	−0.8	−7.3	−8.1	383.7
	H2	5.962	5.0	6.197	21.61	8.48	30.09	−1.86	4.19	2.33	32.42

[a] Coordinates in Å and shielding constants in ppm.

When GIAO are utilized for the calculation of the shielding tensor[21] the expression giving the $\alpha\beta$ element of the shielding tensor of nucleus N takes the form:

$$\sigma_{\alpha\beta}^N = (\sigma_{\alpha\beta}^N)^1 + (\sigma_{\alpha\beta}^N)^2 + (\sigma_{\alpha\beta}^N)^3 + (\sigma_{\alpha\beta}^N)^4$$

with

$$(\sigma_{\alpha\beta}^N)^1 = \frac{1}{2c^2} \sum_{pq} D_{pq}^0 \langle \chi_p | (\vec{r}_q \cdot \vec{r}_N \delta_{\alpha\beta} - (\vec{r}_N)_\alpha (\vec{r}_N)_\beta) r_N^{-3} | \chi_q \rangle$$

$$(\sigma_{\alpha\beta}^N)^2 = -\frac{1}{2c^2} \sum_{pq} D_{pq}^0 \langle ((\vec{R}_p - \vec{R}_q) \times \vec{r}_p)_\alpha \chi_p | (\vec{r}_N \times \vec{\nabla})_\beta r_N^{-3} | \chi_q \rangle$$

$$(\sigma_{\alpha\beta}^N)^3 = -\frac{1}{2c^2} \sum_{pq} D_{pq}^0 (\vec{R}_p \times \vec{R}_q)_\alpha \langle \chi_p | (\vec{r}_N \times \vec{\nabla})_\beta r_N^{-3} | \chi_q \rangle$$

$$(\sigma_{\alpha\beta}^N)^4 = -\frac{1}{c} \sum_{pq} D_{pq}^{H_{0\alpha}} \langle \chi_p | (\vec{r}_N \times \vec{\nabla})_\beta r_N^{-3} | \chi_q \rangle$$

where $\vec{r}_N$ and $\vec{r}_q$ refer to the distance of the electron to nucleus N and to the nucleus carrying the orbital χ_q, respectively, and $\vec{R}_q$ refers to the position of the nucleus of the atom carrying the orbital χ_q. The scrutinization of the expression of the different integrals which appear in the four formulae listed above shows that $(\sigma_{\alpha\beta}^N)^1$ and $(\sigma_{\alpha\beta}^N)^2$ have in every case values which are independent of the system of coordinates. The variation of $(\sigma_{\alpha\beta}^N)^3$ with the origin of the gauge is due to the presence of the product $(\vec{R}_p \times \vec{R}_q)$ in the equation. In the case of $(\sigma_{\alpha\beta}^N)^4$ it is $D_{pq}^{H_0}$, the perturbed density matrix, which depends upon the system of coordinates through the expression of the perturbation operator $\mathcal{H}^{H_0}$. Therefore we have proposed[29] to take $(\sigma_{\alpha\beta}^N)^d = (\sigma_{\alpha\beta}^N)^1 + (\sigma_{\alpha\beta}^N)^2$ and $(\sigma_{\alpha\beta}^N)^p = (\sigma_{\alpha\beta}^N)^3 + (\sigma_{\alpha\beta}^N)^4$ in order to have a partition into diamagnetic and paramagnetic contributions which are both invariant with the gauge as is $\sigma_{\alpha\beta}^N$. This partitioning has the additional advantage of giving equal values of $(\sigma_{\alpha\beta}^N)^d$ and $(\sigma_{\alpha\beta}^N)^p$ as of $\sigma_{\alpha\beta}^N$ for chemically equivalent nuclei while each of the four contribution to $\sigma_{\alpha\beta}^N$ differ for each nucleus. In order to illustrate the variation of the four terms with the atomic coordinates we report in Table I the results obtained for water with two locations A and B of the origin of the system of coordinates. The tabulated values clearly show for each of the hydrogens $(\sigma_{\alpha\beta}^N)^1$ and $(\sigma_{\alpha\beta}^N)^2$ are equal in the two calculations but that only the sum of the two terms is identical for the two equivalent protons of the molecule. The values reported show also that $(\sigma_{\alpha\beta}^N)^3$ and $(\sigma_{\alpha\beta}^N)^4$ have different values for the two locations of the origin and for the two protons of the molecule.

The partitioning into diamagnetic and paramagnetic contributions as defined above might appear rather artificial since σ^p contains a term which depends upon the unperturbed density matrix as σ^d. But for a given type of nucleus like carbon-13 or hydrogen the measured chemical shifts have been plotted against the calculated atomic populations (ref 30 and references therein)

Table II. Nuclear Magnetic Shielding Constants (σ in ppm) and Atomic Populations (Q in Electrons) in Cytosine

	σ^d	σ^p	σ	Q
N1	335.33	−152.15	183.18	8.078
C2	241.25	−179.52	61.73	4.753
N3	327.89	−262.89	65.01	7.927
C4	244.94	−191.15	53.80	5.165
C5	257.63	−122.02	135.61	6.506
C6	252.80	−177.35	75.45	5.748
N4	330.98	−87.31	243.67	7.964
O2	406.65	−393.78	12.87	8.691
H5	37.383	−3.684	33.699	0.676
H5	29.987	−2.919	27.068	0.642
H(NH$_2$)$_a$	38.944	−2.507	36.437	0.629
H(NH$_2$)$_b$	38.129	−2.370	35.759	0.620
H(N1)	38.644	−5.639	33.005	0.601

with variable success; therefore, it is interesting to compare the calculated value of the shielding constants as well as the term of this quantity which explicitly depends only upon the ground-state molecular wave function with the atomic population computed from the same molecular wave function since it might give meaningful indications on the limitations of such charge-shift correlations.

The basis set used for the computation of the molecular wave function and for the integrals which appear in the equations giving the shielding tensor is a "split valence shell" one which has given us satisfactory results in previous studies on smaller molecules.[26,27] The orbital exponents and the contraction coefficients are taken from studies of Roos and Siegbahn[31] for carbon, nitrogen, and oxygen and from Huzinaga[32] for the hydrogens. The numerical values actually utilized are reported elsewhere.[33]

The geometry used as input is the one measured by Barker and Marsh[34] in the crystal.

Results and Discussion

In Table II are reported the magnetic shielding constants of the different nuclei of cytosine as well as the diamagnetic and paramagnetic components (as defined above) of this quantity. The examination of the three sets of numbers giving the values of σ^d, σ^p, and σ respectively shows that σ^d undergoes large variations with the type of the atom, carbon or oxygen for example, but that for a given type of nuclei, ^{13}C or ^{15}N and even to a lesser extend ^{1}H, it is the value of σ^p which determines the chemical shift of a particular atom. For example, we see that $\sigma_{N4}^d < \sigma_{N1}^d$ and that the final reverse order of their total shielding constants is due to the larger absolute value of σ_{N1}^p compared to σ_{N4}^p. Similarly the downfield shift of H(N1) compared to H(NH$_2$) is due to the larger paramagnetic term of the shielding constant of that atom. Since σ^p appears to be determining for the relative ordering of the shielding constants of the different nuclei and since this term introduces all the molecular excited states it is not surprising that the shielding constant σ and the atomic population Q do not run parallel even when the two series of quantities are calculated from the same molecular wave function as in the present case. But if we compare the variations of σ^d and Q which both depend upon

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the ground state only we see from Table II that we have H6 < H5 < H(NH$_2$) < H(N1) < C2 < C4 < C6 < C5 < N3 < N4 < N1 < O2 for σ^d and H(N1) < H(NH$_2$) < H6 < H5 < C2 < C4 < N3 < N4 < N1 < O2 for Q. Therefore the present data confirm our previous results concerning ^{7}Li[29] on the absence of relation between the variation of the shielding constant of the nucleus of an atom with its electronic population. The situation is not improved if we consider separately each type of nucleus since it is for the different protons of the molecule that we obtain a different order of increasing σ^d and Q.

For the different protons of the molecule the calculated values of the shielding constants are larger by several parts per million than those calculated in the case of imidazole[27] which is as cytosine a conjugated heterocycle. This systematic difference is due to the geometry used as input, since the CH and NH bond lengths given by Barker and Marsh[34] (0.86–1.01 Å) are shorter than those reported for similar molecules and in particular for imidazole[35] (1.05–0.03 Å); we have shown in the case of formamide[26] that a shortening of 0.03 Å of a CH or NH bond produces an increase of about 0.6–0.7 ppm of the corresponding proton shielding constant. With the situation being identical for the different protons of the molecule, the comparison of the differences between the shielding constants of the five hydrogens of the molecule with the differences between the chemical shifts measured for these nuclei is meaningful.

For the CH protons our computations give $\sigma_{H6} < \sigma_{H5}$ in agreement with experimental data[36,37] but the theoretical value of the difference $\sigma_{H5} - \sigma_{H6}$ is much larger than the measured one (6.6 and 1.75 ppm, respectively). For the NH protons theory and experiment agree on the value of 3 ppm for the difference between $\sigma_{H(N1)}$ and the average of the two $\sigma_{H(NH_2)}$,[38] the latter being upfield, but disagree on the location of the resonances of these protons with respect to those of the CH since we have $\sigma_{H6} < \sigma_{H(N1)} < \sigma_{H5} < \sigma_{H(NH_2)}$ from theory and $\sigma_{H(N1)} < \sigma_{H6} < \sigma_{H(NH_2)} < \sigma_{H5}$ from experiment. The explanation of this discrepancy is that in the conditions of the experiment of Coletta et al.[37] the NH protons are hydrogen bonded to the oxygen atom of dimethyl sulfoxide used as solvent and that their resonance lines are therefore shifted downfield with respect to their location in the isolated molecule. If we suppose that the protons are shifted downfield by 3–4 ppm due to the intermolecular hydrogen bonds,[13d,26,27,39] the agreement between theory and experiment is restored.

For the carbon atoms we calculate $\sigma_{C4} < \sigma_{C2} < \sigma_{C6} < \sigma_{C5}$ in agreement with measurements on cytosine[37,40] as well as on cytidine and cytidine phosphates.[14,40] In addition the calculated values of the carbon's chemical shifts are ranging between 165 and 83 ppm downfield with respect to methane ($\sigma_{C_{calcd}} = 219$ ppm[27]) for measured values of 97 to 174 ppm[40] (if we suppose that the carbons of benzene are shifted 131.5 ppm[41] downfield from the carbon of methane). If C5 is taken as reference the chemical shifts of C6, C2, and C4 are 60, 74, and 80 ppm from theory and 50, 65, and 74 ppm[37] or 64, 73, and 77 ppm[40] from experiment. The comparison of the calculated and measured shifts shows a good agreement between the present results and the experimental data concerning the carbon atoms.

For the nitrogen nuclei the calculated values of the magnetic shielding constants give the order $\sigma_{N3} < \sigma_{N1} < \sigma_{N4}$. The ex-

perimental results available in the literature although concerning not cytosine itself but cytidine[13a-c,f] or cytidine 5'-phosphate[13a,c] also give this ordering. The calculated value of the difference $\sigma_{N4} - \sigma_{N1}$ (60.5 ppm) is in good agreement with the measured ones ($\simeq 59$ ppm[13a-c,f]) but for the $\sigma_{N1} - \sigma_{N3}$ and $\sigma_{N4} - \sigma_{N3}$ differences we calculate 118 and 179 ppm respectively while the measured values are of about 55–60 ppm.[13a-c,f] The discrepancy may be attributed partly to the cytosine–cytosine and cytosine–solvent hydrogen bonds since calculations and measurements have shown that the resonances of NH and NH2 nitrogen nuclei are shifted downfield by intermolecular hydrogen bonding while the resonance of the pyridine-like nitrogens, such as N3, are shifted upfield by such interactions.[13b,d,e,f,26,27,42] The calculated ^{15}N chemical shifts with respect to the nitrogen of NH$_3$ ($\sigma_{N_{calcd}} = 274$ ppm[27]) are 91, 209, and 30 ppm for N1, N3, and N4, respectively, for measured values of about 170, 225, and 110 ppm;[13,43] therefore it appears that the calculated value of σ_{N3} is of a good order of magnitude. Since intermolecular interactions cannot be responsible for shifts as large as 60 ppm it seems that the calculations overestimate the upfield shift of pyrrole and aniline-like nitrogens when compared to pyridine-like ones, a feature already noticed in the case of imidazole.[27]

For the oxygen atom of the molecule the calculated value of the shielding constant corresponds to a downfield shift of 370.9 ppm with respect to water ($\sigma_{O_{calcd}} = 384$ ppm (see Table I)) (in the vapor state since the calculations are carried out for isolated molecules and since liquid water is extensively hydrogen bonded). To our knowledge the chemical shift of ^{17}O has not been measured for cytosine but the corresponding data are available for the oxygens of the carbonyls of uridine which should not be very different from O2 of cytosine and are 250–300 ppm[12] with respect to liquid water, and therefore 280–300 ppm with respect to vapor water.[45] These values are of the order of magnitude of the shift calculated for the oxygen of cytosine since they are measured in water and are consequently lowered by several tens of parts per million by the upfield shift due to the water–carbonyl hydrogen bonds.[26,46]

Concluding Remarks

The comparison between the measured spectra and the theoretical results is altogether satisfactory for each of the different types of nuclei of cytosine. It confirms previous results[19,27] on the possibility of carrying out, with a basis set of moderate size, calculations which are able to give all the qualitative trends of the observed chemical shifts, and in many cases values in numerical agreement with the measurements, for molecules which are the building blocks of biological macromolecules. The capability of theory to give good results for the variation of shielding constants with the location in the molecule, of the nucleus considered as in the present work, with intermolecular interactions,[26,27,29] and with molecular conformation[19] is quite encouraging for the future. We can resonnably expect that it will be possible, using the results of computations to contribute to the interpretation of the experimental data, on nucleic acids in particular, in which the three factors can contribute to the measured quantity. It is important to be able to obtain such results with relatively small basis sets since the size of the molecules which have to be considered when one is interested in NMR of nucleic acids prevents the use of the large basis sets which in the case of small molecules give the insurance that the calculated values will not be qualitatively further modified by the inclusion of additional basis functions.

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