(2) C. W. Leming and G. L. Pollack, *Phys. Rev. B*, 2,3323 (1970).
(3) L. A. Schwalbe, R. K. Crawford, H. H. Chen and R. A. Aziz, *J. Chem. Phys.*, **66**, 4493 (1977).
(4) S. B. Ko, *Jeonbuk National University, Basic Science. Review*, **2**, 87 (1979).
(5) W. K. Kim, *Soongjun Univ.*, Press, 10 (1980).
(6) S. B. Ko and W. K. Kim *Bull. Korean Chem. Soc.*, **1**, 131 (1980).
(7) R. H. Beaumont, H. Chihara and J. A. Morrison, *Proc. Phys. Soc.*, **78**, 1462 (1961).
(8) M. P. Freeman, C. D. Halsey, Jr., *J. Phys. Chem.*, **60**, 1119 (1956).
(9) L. S. Salter, *Trans. Faraday Soc.*, **59**, 657 (1963).
(10) H. H. Chen, R. A. Aziz and C. C. Lim, *Can. J. Phys.* **49**, 1569 (1971).
(11) W. B. Streett and L. A. K. Staveley, *J. Chem. Phys.*, **55**, 2495 (1971).
(12) J. H. Dymond and E. B. Smith, "The Virial Coefficients of Gases", Oxford, London, 1969.
(13) J. A. Barker, R. O. Watts, J. K. Lee, T. P. Schafer and Y. T. Lee, *J. Chem. Phys.*, **61**, 3081 (1974).
(14) M. L. Klein and J. A. Venables, "Rare gas solids", Vol. 2, Academic Press, London, 1977.
(15) G. L. Pollack, *Rev. Mod. Phys.*, **36**, 748 (1964).
(16) G. K. Horton, *Amer. J. Phys.*, **36**, 93 (1968).
(17) H. H. Podgurski and F. N. Davis, *J. Phys. Chem.*, **65**, 1343 (1961).
(18) O. G. Peterson, D. N. Batchelder and R. O. Simmons, *Phil. Mag.*, **12**, 1193 (1965).
(19) A. J. E. Foreman and A. B. Lidiard, *Phir. Mag.*, **8**, 97 (1963).

Theoretical Study of Isotope and Cation Binding Effects on the Hydration of BDNA

Young Kee Kang and Mu Shik Jhon

Department of Chemistry, Korea Advanced Institute of Science, P. O. Box 150, Cheong Ryang Ri, Seoul 131, Korea *(Received January 10, 1981)*

Theoretical studies of the sodium cation binding and the isotope hydration effects on the static model compound B-DNA have been qualitatively elucidated by using empirical potential energy functions. In the first place, the sodium cations bound to phosphate anions and their hydration scheme have been optimized and have given a reasonable agreement with other theoretical results and experimental studies. In the second stage, the isotope effect on the hydration through the substitution of D_2O for H_2O has been carried out by the same procedure. The stabilization of B-DNA has been explained and compared in terms of the sodium cation binding to phosphate anions and its hydration in both cases of H_2O and D_2O.

Introduction

The effect of environmental factors including metal cation binding and the hydration plays important roles in many aspects of biopolymers. Especially metal ions are considered as a key activator widely in biological processes like transcription, translation[1], and replication, and responsible for catalytic activities of enzymes.[2-4]

Interactions of metal ion with nucleic acids and their constituents to a some extent have been investigated.[5-7] Effects of Na^+ and Mg^{2+} ions on the helix-coil transition of DNA[8,9] and dielectric relaxation[10] have been reported. But only simple cases of interactions between metal cation and phosphat e or dimethylmonophosphate anion and its hydration have been studied by *ab initio* self-consistent field molecular orbital methods.[11-13] And a few Monte Carlo (MC) simulations have been performed for the hydration of the phosphate group of DNA[14]. Isotope effect on the hydration tends to stabilize not only native protein conformations but also the helical forms of nucleic acids[15]. Hence D_2O substitution lowers the optical absorbance and raises the melting temperature (T_m) values thereby indicating increased helical stability of DNA[16-19] and *t*-RNA[20].

From the theoretical point of view, we have carried out the hydration scheme of the static model compound B-DNA[21] and *t*-RNA[22] without considering counter ion effect. Although in this computation the temperature parameter has not considered, we have obtained the reasonable hydration sites and the stabilization energies due to the hydration, which are in agreement with experimental studies and *ab initio* self-consistent field molecular orbital calculations.

In this paper, we shall elucidate the sodium cation binding scheme of phosphate anions and also incorporated the isotope effect on the hydration of B-DNA by using empirical potential energy functions. The computations

have been limited for the model B-DNA including cations to the presence of water in their first hydration shell and also the temperature parameter is not condidered.

Procedure

The Method. The methods for the optimization of the sodium cation binding, its hydration scheme, and the stabilization energy due to binding, its hydration scheme, and the stabilization energy due to the hydration follow the general formula presented by Perahia *et al.*[21] The interaction energies have been obtained by using the potential energy functions by Caillet and Claverie[23,24] that are composed of three long-range contributions (electrostatic, polarization and dispersion) and a short-range repulsive term. The nature of energy functions and their parameters are discussed elsewhere.[21-24]

(1) Electrostatic Energy: The net atomic charges of the model compound are calculated from Renugopalakrishnan *et al.*[25] and refs 26 and 27. It is assumed that the dielectric constant of this system is unity and the net charge of sodium ion is to be +1. The net charges for H_2O and D_2O obtained from the geometrical data.[28]

(2) Polarization Energy: The polarization energy of a molecule is evaluated as the sum of its surrounding atomic polarization contribution, where the mean polarizability of each atom is calculated from bond polarizabilities[29] according to the weight of the number of electrons[23]. The mean polarizability for sodium cation is obtained from the experimental value[30].

(3) Dispersion and Repulsive Energies: The expression for the dispersion and the repulsive energies is described as the sum of the atom-atom dispersion and repulsive terms[31]. The van der Waals radii of atoms are taken from Bondi[32], while the value of sodium cation is calculated as 1.29 Å from Davis method[33] by using its polarizability. The modification of the repulsive term in ref 21 due to the atomic electron population is neglected for sodium cation. The multiplicative factor in ref 21 for sodium cation is obtained as 2.86 from the experimental result[34] and 1.07 is used for deuterium from the heat of vaporization of D_2[35].

(4) The Case of the Hydrogen Bond: As the interaction due to the hydrogen bond is significant in equilibrium distance and nature in itself, the simple analytical behaviour in the dispersion and repulsive energy function has been refined according to the distance. In this work, we have used $A' = A/4.72$, $C' = C/2.8$ and $\alpha' = 13.83$ for D_2O which agree with other works,[15,28], where A, C and α are equal to 0.24 kcal/mol, 4.7×10^4 kcal/mol and 12.35, as in refs 21 and 22.

The Model Compound of B-DNA. To investigate the sodium cation binding and the isotope effects on the hydration of the B-conformation of DNA, a model compound representing a portion of the B-form of DNA is chosen as the same as that of Perahia *et al.*[21] and presented in Figure 1. It is constituted of an antiparallel double minihelix with three monomer units in each strand. The 5'-3' strand consists of $C_5 \cdot p_3 \cdot G_5 \cdot p_3 \cdot C$ and the second strand carrying the complementary bases. The sugar units are designated successively by S_1, S_2, S_3, the phosphate groups by P and P', and the bases by C, G and C', Each atom is designated by its notation in the monomer unit followed by the designation, with parenthesis, of the unit to which it belongs (*e.g.* $N_7(G)$ denotes the N_7 atom of the base G.).

The cartesian coordinates of this model compound have been obtained from the cylindrical coordinates provided by Arnott *et al.*[36] with refined X-ray diffraction data from the crystalline fiber of B-DNA.

Determination of the Hydration Scheme of B-DNA and Evaluation of the Stabilization Energy Due to the Hydration. The optimum positions of sodium cation bound to phosphate anions have been determined from the known coordinates of three successive atoms, a distance between sodium cation and one anion of phosphate group and their dihed ral angle. The geometrical location of water molecule with respect to the model compound is described as the same as in ref 21. The cartesian coordinates of sodium cation and the water molecule can be obtained by the computational method developed by Thompson[37] with these geometrical parameters.

The probable hydration sites have been established successively for each water molecule until no more water molecule can be located by a direct binding to the model compound. And we have optimized the locations of sodium cation and bound water molecules by minimizing the interaction energies with the variation of parameters simultaneously by using a quasi-Newton technique suggested by Fletcher[38].

The stabilization energy due to the hydration has been

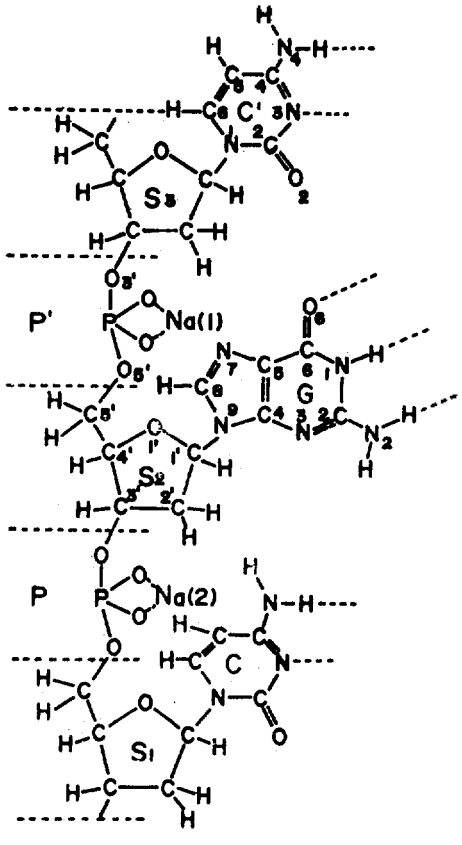

Figure 1. Notations for the model compound.

evaluated by comparing the internal interaction energies between the different groups of the essential subunits of the model compound in Figure 1 in its non-hydrated and hydrated states. In cases in which a water molecule is involved in two hydrogen bonds, it is considered as a part of the subunit to which the binding is the stronger of the two. The hydrated model compound is considered as a 'static supermolecule', a unique entity formed by the substrate and water molecules of the first hydration shell. The optimization processes for D_2O are the same as those for H_2O.

Results and Discussion

We have optimized the locations of total seventeen water molecules, which are projected along the helical axis in Figure 2. The optimized coordination distances between sodium cation and oxygen atoms and the interaction energies of sodium cation and the substrate have been shown in Table 1 and Table 2.

Na^+ Binding to Phosphate Group and Hydration Scheme. It has been turned out that the minor groove sodium cation is bound to four water molecules in octahedral coordination by X-ray crystallographic results on sodium adenylyl-3′,5′-uridine hexahydrate (ApU)[39] and sodium guanylyl-3′,5′-cytidine nonhydrate (GpC)[40]. Thus we have considered sodium cation to be octahedrally coordinated, and optimized the locations of sodium cations and the hydration scheme around them according to the aforementioned technique. This theoretical hydration scheme is in general agreement with infrared studies of Falk *et al.*[41,42] which indicate the fixation of 4-6 water molecules at the phosphate group of DNA.

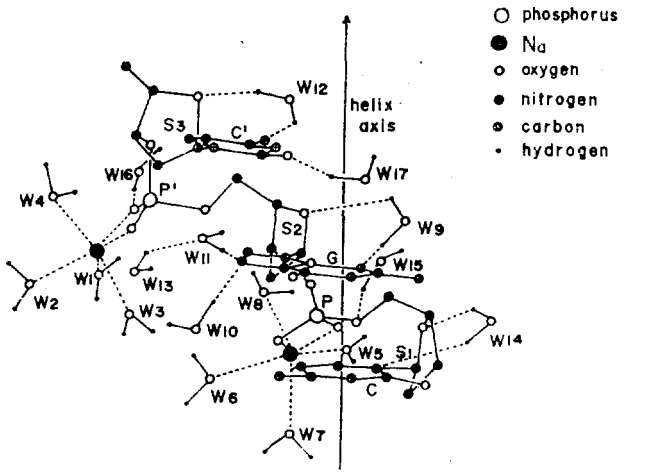

Figure 2. Projection along the helical axis of the model compound indicating the locations of the water molecules of the first hydration shell.

The optimum average distances $Na^+\cdots O$ (water) and $Na^+\cdots O$ (phosphate anion) are 2.44 Å (2.43 Å for D_2O) and 2.18 Å, respectively, and in good agreement with the average cation-oxygen bond lengths observed in crystals containing polycoordinated sodium cation[39,40,43,44] and *ab initio* self-consistent field molecular orbital calculations[11-13]. The interaction energies between sodium cation and its coordinated waters are shown in Table 3, and its average value for $Na^+\cdots H_2O$ is −20.8 kcal/mol which is a reasonable value com pared with the experimental result[34]. For $Na^+\cdots D_2O$ system we obtained −21.2 kcal/mol.

The remaining two water molecules, W15 and W16, are bound between oxygen atoms in phosphate and sugar groups, and their binding energies are −11.3 kcal/mol and −13.3 kcal/mol, respectively, which are similar to the values in ref 21, and in the deuterated cases we have obtained −11.4 kcal/mol and −13.8 kcal/mol, respectively.

The hydration scheme of nucleosides of this model compound is nearly similar to that of Perahia *et al.*[21] but one additional water molecule W17 attached to $O_2(C')$ whose interaction energy is −13.1 kcal/mol and −13.6 kcal/mol for H_2O and D_2O, respectively. But the binding energies of W10 and W12, −12.0 kcal/mol and −8.3 kcal/mol, respectively, are less than those in ref 21. This may be explained by the influence of the bound water molecules around so-

TABLE 1: The Optimized Coordination Distances between Sodium and Oxygen Atoms (The Values in Parentheses are for D_2O)

	Ligand atom	Distance(Å)
Na(1)	O (P′)	2.18
Na(1)	O (W1)	2.42 (2.44)
Na(1)	O (W2)	2.42 (2.44)
Na(1)	O (W3)	2.41 (2.41)
Na(1)	O (W4)	2.47 (2.44)
Na(2)	O (P)	2.18
Na(2)	O (W5)	2.41 (2.38)
Na(2)	O (W6)	2.42 (2.42)
Na(2)	O (W7)	2.46 (2.64)
Na(2)	O (W8)	2.46 (2.43)

Average Na-O (W) distance; 2.44 (2.43)

TABLE 2: The Interaction Energies of Sodium and the Substrate (Units in kcal/mol)

	E_{el}	E_{pol}	E_{dsp}	E_{rep}	E_{tot}
Na (1)	−151.7	−34.2	−11.9	35.2	−162.6
Na (2)	−146.5	−33.7	−11.8	34.6	−157.4

TABLE 3: Interaction Energies between Na^+ and Its Coordinated Waters (Units in kcal/mol)

	E_{el}	E_{pol}	E_{dsp}	E_{rep}	E_{tot}		$E_{average}$
Na(1)-$(H_2O)_4$	−66.2	−23.9	−9.3	16.9	−82.5		
Na(2)-$(H_2O)_4$	−67.5	−24.0	−9.3	17.2	−83.6	−166.1	−20.8
Na(1)-$(D_2O)_4$	−67.0	−25.7	−9.8	18.3	−84.2		
Na(2)-$(D_2O)_4$	−67.7	−25.3	−9.4	17.2	−85.2	−169.4	−21.2

TABLE 4: Interaction Energies between the Different Groups of the Model Compound in Their Hydrated (Left-Hand Triangle) and Non-hydrated (Right-hand one) States (Units in kcal/mol)

	S1	*C*	*P*	*S2*	*G*	*P'*	*S3*	*C'*
S1		5.8	−13.7	3.0	−0.7	−3.9	1.9	−1.3
C	1.0 (0.9)		−9.7	1.4	−14.7	−2.2	0.9	−0.1
P	−14.3 (−14.4)	−10.4 (−10.5)		−13.6	−2.2	7.8	−3.2	2.4
S2	3.3 (3.3)	1.4 (1.4)	−14.7 (−14.9)		−0.8	−14.2	2.4	2.4
G	−6.4 (−6.6)	−17.8 (−18.0)	−2.8 (−2.9)	−1.6 (−1.7)		−2.3	−1.6	−9.8
P'	−3.7 (−3.6)	−2.0 (−2.0)	5.5 (5.4)	−14.3 (−14.0)	−7.0 (−7.3)		−11.7	−1.1
S3	1.9 (1.9)	1.0 (1.0)	−3.2 (−3.2)	2.3 (2.3)	−1.5 (−1.7)	−13.0 (−13.1)		5.3
								Total-78.3
C'	−1.4 (−1.5)	0.4 (0.4)	2.0 (2.1)	−3.4 (−3.8)	−20.0 (−20.3)	−4.0 (−3.7)	2.5 (2.1)	
	Total	−110.5	(−122.5)					

TABLE 5: The Interaction Energies of Sodium Cations and the Phosphate Groups (Units in kcal/mol)

	E_{el}	E_{pol}	E_{dsp}	E_{rep}	E_{tot}
Na(1)-*P'*	−134.2	−52.4	−12.2	36.0	−162.8
Na(2)-*P*	−128.4	−50.7	−11.5	34.2	−156.4

dium cation and W17 bound to O_2 (*C'*), respectively. Also, we have obtained −12.2 kcal/mol and −9.0 kcal/mol for the deuterated W10 and W12. In this work, we have optimized the reasonable number of water molecules interacting with the bases (1–2 with cytosine, 3–4 with guanine), which is in agreement with result of ultrasonic study[45].

Stabilization of the Model Compound B-DNA Due to Sodium Cations and Hydration. The interaction energies between the different groups (sugars, phosphates and bases) in both their hydrated and non-hydrated states are presented in Table 4 (the values in parentheses correspond to those for D_2O). We have considered each sodium cation as a part of phosphate groups *P* and *P'*. The left-hand triangle of the table concerns the hydrated state and the right-hand one, the non-hydrated one.

In the case of non-hydrated state of Na^+-B-DNA, there is a decrease of the interaction energy between the different groups in the order of 64 kcal/mol compared with B-DNA in ref 21, which has mainly from the interaction between phosphate group and the other ones, *i.e.*, the decrease of the electrostatic and the polarization energies due to cation binding. But the model compound of Na^+-B-DNA* is considered to have another contribution to the stabilization compared with B-DNA, which is the binding energies of sodium cations to phosphate groups. In Table 5, these interaction energies are represented. $Na^+(1)$ interacts with the phosphate *P'* in the order of 163 kcal/mol and $Na^+(2)$ with *P* in 156 kcal/mol, hence the interaction energy between the sodium cations and the phosphate groups in about 319 kcal/mol. Therefore the non-hydrated state of Na^+-B-DNA is more stabilized than B-DNA in the order of 254 kcal/mol which corresponds to about twice as much as the total interaction energy between the different groups of the non-hydrated B-DNA. From this result, we can see that the counter-ion may be a predominant factor in the stabilization of the DNA structure.

The stabilization through the hydration of Na^+-B-DNA is brought about by two different effects; (1) the interaction energies between groups, and (2) the interaction energy between sodium cation and its coordinated water molecules, both due to the introduction of water into the system. In Table 4, the first type of effect is shown, which is less than that of B-DNA in the order of 88 kcal/mol. The stabilization of the hydrated Na^+-B-DNA produced by this type of effect is thus about 32 kcal/mol compared with 55 kcal/mol in B-DNA. As it can be seen in Table 3 that the second effect amounts to −166.1 kcal/mol, the overall stabilization energy of the hydrated Na^+-B-DNA is about −596 kcal/mol which includes the interaction energy between Na^+ and the phosphate group.

The hydrated Na^+-B-DNA is stabilized more than the non-hydrated Na^+-B-DNA in the order of 198 kcal/mol and about eight times as much as the hydrated B-DNA. The overall comparison between the interaction energies is listed in Table 6. The precise amount of this stabilization energy may be questionable, of course, what is certainly significant, however, is its relatively large value and perhaps still more the information which it may produce on the nature of the interactions involved in the stabilization of the DNA structure.

Isotope Effect on the Hydration of Na^+-B-DNA. The interation energies due to the isotope effect on the hydration of Na^+-B-DNA are illustrated in Tables 1, 3, 4 and 6.

TABLE 6: Comparison of Interaction Energies between Na^+ Free and Bound B-DNA (Units in Kcal/mol)

	Non-hydrated		Hydrated	
	Na^+ bound	Na^+ free	Na^+ bound	Na^+ free
E_g[a]	−78.3	−143.3	−110.5 (−122.5)[c]	−198.9
E_i[b]	−319.2		−485.3 (−488.6)[c]	
$E_{overall}$	−397.5	−143.3	−595.8 (−611.1)[c]	−198.9

[a] E_g denote the total interact on energies between the different groups. [b] E_i denote the interaction energy between Na^+ and the phosphate group for the non-hydrated Na^+-B-DNA, and the sum of the interaction energies between sodium cation and its coordinated water and the interaction energy between Na^+ and the phosphate group for the hydrated Na^+-B-DNA. [c] Values in parentheses correspond to those for D_2O.

We, of course, assumed that the overall differences in the intermolecular forces are resulted from the different configurations of H_2O and D_2O in the ground-state vibrational levels. The optimized coordination distances between sodium and the oxygen atoms of the deuterated waters are shown Table 1 and their average value, 2.43 Å, shows no remarkable difference compared with that in the case of water.

The differences in the interaction energies of the hydration of Na^+ and between the different groups due to the isotope effect are 3.3 kcal/mol and 12 kcal/mol, respectively (see Tables 3 and 4). The overall stabilization energy through the substitution of D_2O for H_2O is thus about 15 kcal/mol which is much the same as the stacking energy of the neighbouring bases or the interaction energy between sugar and phosphate groups.

Conclusions

In spite of the questionable amount of the stabilization energy of B-DNA, it seems nevertheless that the present study accounts at least on the qualitative level for the schemes of the sodium cation binding and the hydration in the static B-DNA, which is in good agreement with *ab inito* self-consistent field molecular calculations and X-ray crystallographic results.

By comparing the interaction energies both in the hydrated state and in the non-hydrated one, which include the binding energies of sodium cations to the phosphate groups or the interaction energy between sodium cation and its coordinated water molecules, Na^+-B-DNA is known to be singificantly stabilized more than B-DNA.

For the more realistic hydration structure around DNA and the stabilization energy due to the hydration, the conformational change and the interaction between two complementary strands of double-helical structure of the DNA must be considered, and the temperature parameter is also needed.

Acknowledgement. This work was supported in part by Korea Research Center for Theoretical Physics and Chemistry.

References

(1) G. I. Eichhorn, "Inorganic Biochemistry", Vol. 2. Elsevier, Amsterdam, 1973.
(2) C. J. Gray, "Enzyme-Catalysed Recations", Van Nostrand Reinhold Co., London, 1971.
(3) A. S. Mildvan, "The Enzymes", P. D. Boyer, Ed., 3rd Ed., Vol. 2, Academic Press, New York, 1970.
(4) M. Friedman, "Protein-Metal Interactions", Ed., Plenum Press, New York, 1974.
(5) D. Perahia, A. Pullman and B. Pullman, *Theoret. Chim. Acta*, **43**, 207 (1977).
(6) R. M. Izatt, J. J. Christensen and J. H. Rytting, *Chem. Rev.*, **71**, 439 (1971).
(7) S. J. R. Phillips, *Chem. Rev.*, **66**, 501 (1966).
(8) M. T. Record, Jr., *Biopolymers*, **14**, 2137 (1975).
(9) M. T. Record, Jr., C. P. Woodbury and T. M. Lohman, *Biopolymers*, **15**, 893 (1976).
(10) M. Sakamoto, R. Hayakawa and Y. Wada, *Biopolymers*, **17**, 1507 (1978).
(11) B. Pullman, A. Pullman and H. Berthod, *Int J. Quant. Chem., Quant. Bio. Symp.*, **5**, 79 (1978).
(12) D. S. Marynick and H. F. Schaefer III, *Proc. Natl. Acad. Sci. USA*, **72**, 3794 (1975).
(13) B. Pullman, N. Gresh, H. Berthod and A. Pullman, *Theoret. Chim. Acta.*, **44**, 151 (1977).
(14) E. Clementi, G. Corongiu and F. Lelj, *J. Chem. Phys.*, **70**, 3726 (1979).
(15) S. Lewin, "Displacement of Water and Its Control of Biochemical Reactions", Academic Press, London, 1974.
(16) S. Lewin, *Archs Biochem. Biophys.*, **115**, 62 (1966).
(17) S. Lewin, *Studia Biophys*, **4**, 29 (1967).
(18) S. Lewin and B. A. Williams, *Archs Biochem. Biophys.*, **144**, 1 (1971).
(19) S. Lewin, *J. Theoret. Biol.*, **17**, 181 (1967).
(20) S. Lewin and J. R. Stow, *Biochem. J.*, **122**, 48 (1971).
(21) D. Perahia, M. S. Jhon and B. Pullman, *Biochim. Biophys. Acta*, **471**, 349 (1977).
(22) K. Kim and M. S. Jhon, *Biochim. Biophys. Acta*, **565**, 131 (1979).
(23) J. Caillet and P. Claverie, *Acta Cryst.*, **A31**, 448 (1975).
(24) J. Caillet and P. Claverie, *Biopolymers*, **13**, 601 (1974).
(25) V. Renugopalakrishnan, A. V. Lakshminarayanan and V. Sasisekharan, *Biopolymers*, **10**, 1159 (1971).
(26) H. Berthod and A. Pullman, *J. Chem. Phys.*, **62**, 942 (1965).
(27) B. Pullman and A. Pullman, "Quantum Biochemistry", Wiely Interscience, New York, 1963.
(28) G. Nemethy and H. A. Scheraga, *J. Chem. Phys.*, **41**, 680 (1964).
(29) R. J. W. Le Fèver, "Advances in Physical Organic Chemistry", Vol. 3, p. 1-90, Academic Press, New York, 1965.
(30) K. Fajans, "Encyclopedia of Chemistry", Clark and Hawley Eds., p. 764, Reinhold Publ. Co., New York, 1957.

(31) M. J. Huron and P. Claverie, *J. Phys. Chem.*, **76**, 2123 (1972).
(32) A. Bondi, *J. Phys. Chem.*, **68**, 441 (1964).
(33) A. Bondi, *J. Phys. Chem.*, **68**, 441 (1964).
(33) B. W. Davis, *J. Colloid Interface Sci.*, **59**, 420 (1977).
(34) I. Džidić and P. Kebarle, *J. Phys. Chem.*, **74**, 1466 (1970).
(35) J. A. Dean Ed., "Langes Handbook of Chemistry", 11th Ed., McGraw-Hill Book Co., New York, 1973.
(36) S. Arnott and D. W. L. Hukin, *Biochem. Biophys. Res. Commun.*, **47**, 1504 (1972).
(37) H. P. Thompson, *J. Chem. Phys.*, **47**, 3407 (1967).
(38) R. Fletcher, "FORTRAN Subroutines for Minimization by Quasi-Newton Methods", A. I. R. E. Report R7125, 1972.
(39) N. C. Seeman, J. M. Rosenberg, F. L. Suddath, J. J. P. Kim and A. Rich, *J. Mol. Biol.*, **104**, 109 (1976).
(40) J. M. Rosenberg, N. C. Seeman, R. O. Day and A. Rich, *J Mol. Biol.*, **104**, 145 (1976).
(41) M. Falk, K. A. Hartman, Jr. and R. C. Lord, *J. Amer. Chem. Soc.*, **85**, 391 (1963).
(42) M. Falk, A. G. Poole and C. G. Goymour, *Can. J. Chem.* **48**, 1536 (1970).
(43) N. Camerman and J. K. Fawcett, *J. Mol. Biol.*, **107**, 601 (1976).
(44) G. E. Blank, J. Pletcher and M. Sax, *Acta Cryst., Sect. B*, **31**, 2584 (1975).
(45) S. K. Sadykhova and F. I. Braginskaya, *Biophysics, U. R. S. S., Engl. Ed.*, **20**, 15 (1975).

Studies on the Linear Free Energy Relationship in Methyl Cinnamates by ^{1}H-NMR Spectrometry

Sang Chul Shim and Joon Won Park

Department of Chemistry, Korea Advanced Institute of Science and Technology, Seoul 131, Korea
(Received January 30, 1981)

Chemical shift values of α-proton of *trans*- and *cis*-methyl cinnamates are well correlated with σ (σ_I, σ_R^o), and and (F, R) (r=0.999−0.879). It is observed that (1) the degree of variation of δH_α value by varying the substituents in *trans*-cinnamates is similar to that of *cis*-cinnamates (ρ_{trans}=0.296, ρ_{cis}=0.284), (2) resonance contribution is larger in the *trans*-cinnamates than that in the *cis*-cinnamates, but inductive contribution is reversed, (3) for *m*-substituted derivatives, resonance contribution is very small compared to that for *p*-substituted derivatives.

Introduction

Attempts have been made by many workers to relate ^{1}H chemical shifts of various type of compounds to specific substituent properties such as electronegativity[1] or dipole moment[2] as well as to correlate them with more general expressions of substituent ability to affect electron density such as, for example, the well known Hammett σ constants and variations[3] thereof. Explanations involving either the magnetic[4] or electric field[5] arising in a substituent have been advanced.

Wittstruck and Trachtenberg[6] found that perturbations in their chemical shifts are caused mainly by inductive and resonance effects rather than by electric and magnetic field effects in the case of cinnamic acid. Wehrli, *et al.*[7] calculated π-electron density of vinyl proton by LCAO-MO method, and they could observe that there is a linear correlation between chemical shift of vinly proton and electron density on the side chain of *trans*-cinnamic acid (r=0.77). It is found by Katritzky and Swinbourne[8] that there is a good correlationship between the chemical shifts of α-proton and Hammett σ-constants of ring substituents in cinnamic acids (r=0.973 in dimethyl sulfoxide, r=0.912 in trifluoroacetic acid), but less so for the β-protons (r=0.850). They proposed that steric and magnetic anisotropic effects (possibly including ring current) are the cause of the poor relationship for the β-protons.

In order to exclude the possibility of interfering effect of hydroxyl group and to increase the solubility in organic solvents, the acid is esterified. Thus we determined the chemical shifts of vinyl protons of *trans*- and *cis*-methyl cinnamates, and corerlated them with Hammett equation (eq. 1) and its variations (eq. 2, eq.3).[9-11]

$$\delta H_{\alpha,x}=\rho\sigma+\delta H_{\sigma,0} \quad \text{(eq. 1)}$$
$$\delta H_{\alpha,x}=\rho_I\sigma_I+\rho_R\sigma_R+\delta H_{\alpha,0} \quad \text{(eq. 2)}$$
$$\delta H_{\alpha,x}=fF+rR+\delta H_{\alpha,0} \quad \text{(eq. 3)}$$
$$\lambda_p=(\rho_R/\rho_I)_p \quad \text{(eq. 4)}$$
$$\lambda'_p=(r/f)_p \quad \text{(eq. 5)}$$
$$\lambda_m=(\rho_R/\rho_I)_m \quad \text{(eq. 6)}$$
$$\lambda_m'=(r/f)_m \quad \text{(eq. 7)}$$
$$N_p=(\lambda_p)_{trans}/(\lambda_p)_{cis} \quad \text{(eq. 8)}$$
$$N_p'=(\lambda_p')_{trans}/(\lambda_p')_{cis} \quad \text{(eq. 9)}$$
$$N_m=(\lambda_m)_{trans}/(\lambda_m)_{cis} \quad \text{(eq. 10)}$$
$$N_m'=(\lambda_m')_{trans}/(\lambda_m')_{cis} \quad \text{(eq. 11)}$$

where F and R are the substituent constants corresponding