CHEMICAL & PHARMACEUTICAL BULLETIN

Vol. 29, No. 12 **December 1981**

Regular Articles

Chem. Pharm. Bull.
29(12)3427—3432(1981)

Conformational Analysis of 1-[3-(Adenin-9-yl)propyl]-3-carbamoylpyridinium Cation: a Model Study for the Intramolecular Interaction of Nicotinamide Adenine Dinucleotide

YOKO MIYAMOTO, TOSHIMASA ISHIDA,* and MASATOSHI INOUE

Osaka College of Pharmacy, 2-10-65 Kawai, Matsubara, Osaka 580, Japan

(Received March 26, 1981)

As a model study of the intramolecular interaction between the adenine and pyridinium rings of nicotinamide adenine dinucleotide (NAD$^+$), conformational analysis of 1-[3-(adenin-9-yl)propyl]-3-carbamoylpyridinium cation was carried out by means of semi-empirical energy calculation.

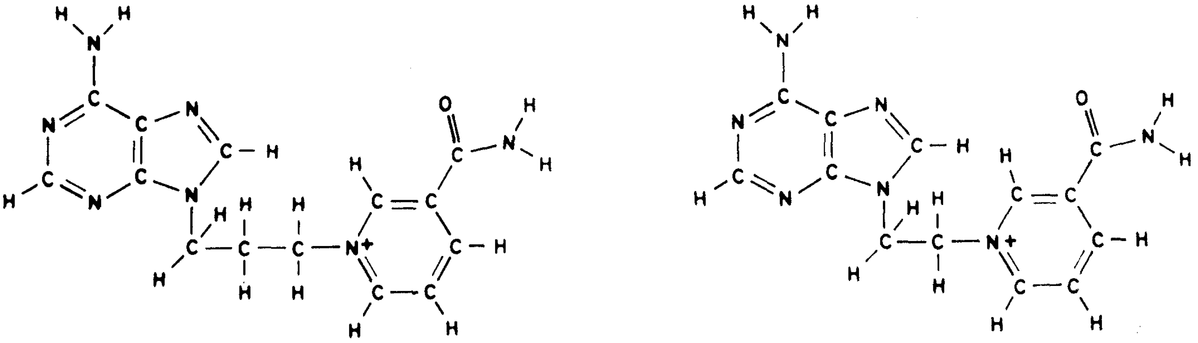
The extended conformation of the molecule, which was observed in the crystal structure, was found to be energetically a metastable one. The most energetically stable conformations seem to be folded forms, especially forms in which the two aromatic rings are stacked; four kinds of stacking modes were observed.

Keywords——pyridinium ring-adenine ring interaction; conformational analysis; semi-empirical energy calculation; PPF method

Introduction

It is well known that nicotinamide adenine dinucleotide (NAD$^+$) acts as a coenzyme in biological oxidation-reduction reactions. In order to clarify the role of the adenine moiety of NAD$^+$ in the catalytic reactions, various spectroscopic studies have been carried out on model compounds.1–3)

We previously reported on the crystal structure of 1-[2-(adenin-9-yl)ethyl]-3-carbamoylpyridinium (AC2N$^+$) chloride,4) in which the adenine ring is not intramolecularly stacked onto



1-[3-(adenin-9-yl)propyl]-3-carbamoylpyridinium (AC3N$^+$)

1-(2-(adenin-9-yl)ethyl)-3-carbamoylpyridinium (AC2N$^+$)

Fig. 1. Chemical Structures of AC2N$^+$ and AC3N$^+$

the pyridinium ring, although it exists in a folded form. From the spectroscopic studies of AC2N$^+$ and related compounds,[5-7] however, it is obvious that an intra- or intermolecular stacking interaction exists between the two aromatic rings. We therefore considered that the conformation observed in the crystal is due to the effect of the crystal lattice packing force and to the shortness of the chain connecting the adenine and pyridinium rings.

We have now carried out the conformational analysis of 1-[3-(adenin-9-yl)propyl]-3-carbamoylpyridinium cation (AC3N$^+$) by means of energy calculation using the PPF (partitioned potential energy function) method; the propylene linkage in the AC3N$^+$ molecule may provide more flexibility between the adenine and pyridinium rings than the ethylene linkage in AC2N$^+$ (Fig. 1).

Experimental

Energy Calculation——The total energy (E) of a molecule was calculated by using the following equation:

$$E = E_{\mathrm{nb}} + E_{\mathrm{el}} + E_{\mathrm{t}} \qquad (1)$$

where E_{nb}, E_{el} and E_{t} are the nonbonded, electrostatic and torsional energies, respectively. These quantities can be obtained by computing the following equations; all energy units are in kilocalories per mol.

$$E_{\mathrm{nb}} = \sum_{i<j}\sum (-A_{ij}R_{ij}^{-6} + B_{ij}R_{ij}^{-12}) \qquad (2)$$

$$E_{\mathrm{el}} = \sum_{i<j}\sum 332.0 \times Q_i \times Q_j \times R_{ij}^{-1} \times \varepsilon^{-1} \qquad (3)$$

$$E_{\mathrm{t}} = \sum_{k=1}^{5} 0.5 \times V_{o,k} \times (1.0 + \cos X\theta_k) \qquad (4)$$

In Eqs. (2)—(4), R_{ij} is the observed distance (in Å) between atoms i and j, and A_{ij} and B_{ij} are the coefficients in the Lennard-Jones "6—12" potential function. The values used in this work are given in Table I.[8-11] Q_i is the Coulombic charge on atom i, calculated by the CNDO/2 method (see Fig. 2), and ε is the dielectric constant (=4.0). $V_{o,k}$ is the barrier potential for internal rotation about the k-th torsion angle (θ_k). The C–C bonds were assigned a barrier height of 2.5 kcal/mol, and the barrier height of the C–N bond was considered to be negligible in accordance with Lakshminarayanan and Sasisekharan.[8] X is the periodicity of the barrier (=3 for C–C bond, =6 for C–N bond).

TABLE I. Constants for the Nonbonded Potential Energy Equation[a,b]

	A_{ij}(kcal. Å^6.mol^{-1})						B_{ij}(kcal. Å^{12}.mol^{-1})$\times 10^{-3}$					
	C_{al}	C_{ar}	H	$O_{C=O}$	N_{NH_2}	N_{ar}	C_{al}	C_{ar}	H	$O_{C=O}$	N_{NH_2}	N_{ar}
C_{al}	370						286					
C_{ar}	546	874					477	740				
H	128	202	47				38	71	45			
$O_{C=O}$	372	552	124	369			209	470	25	145		
N_{NH_2}	365	553	135	356	125		203	285	32	145	27	
N_{ar}	525	640	143	414	412	456	352	311	28	173	166	181

[a] C_{al}=aliphatic carbon; C_{ar}=aromatic carbon; $O_{C=O}$=carbonyl oxygen; N_{NH_2}=primary amine nitrogen; N_{ar}=aromatic nitrogen

[b] The values given are for the interactions of the pairs of atoms indicated in the appropriate columns and rows.

Minimization of the energy, with the torsion angles as variable parameters, was performed using the Powell algorithm.[12] The minimization was carried out by parabola approximation with 4° intervals and no angle was permitted to vary by more than 12° at each step. All numerical calculations were carried out on the ACOS-900 computer at the Computation Center of Osaka University.

Procedure——The atomic numbering and torsion angles of AC3N$^+$ are presented in Fig. 2. Torsion angle χ, C11–C10–N9–C8, was measured clockwise from C11 to C8 when viewed along the C10–N9 bond, and the other torsion angles, $\omega1$, $\omega2$, $\omega3$ and θ, were also measured in the same manner.

The structural parameters were taken from the X-ray analysis of AC3N$^+$ bromide.[13] Based on various reports, the most reasonable values for the starting torsion angles were determined to be as follows: χ=60°, 120°, 180°, 240°, 300°, $\omega1$=60°, 180°, 300°, $\omega2$=60°, 180°, 300°, $\omega3$=60°, 120°, 180°, 240°, 300°, θ=0°. Thus, 225 different conformations were calculated for the AC3N$^+$ molecule.

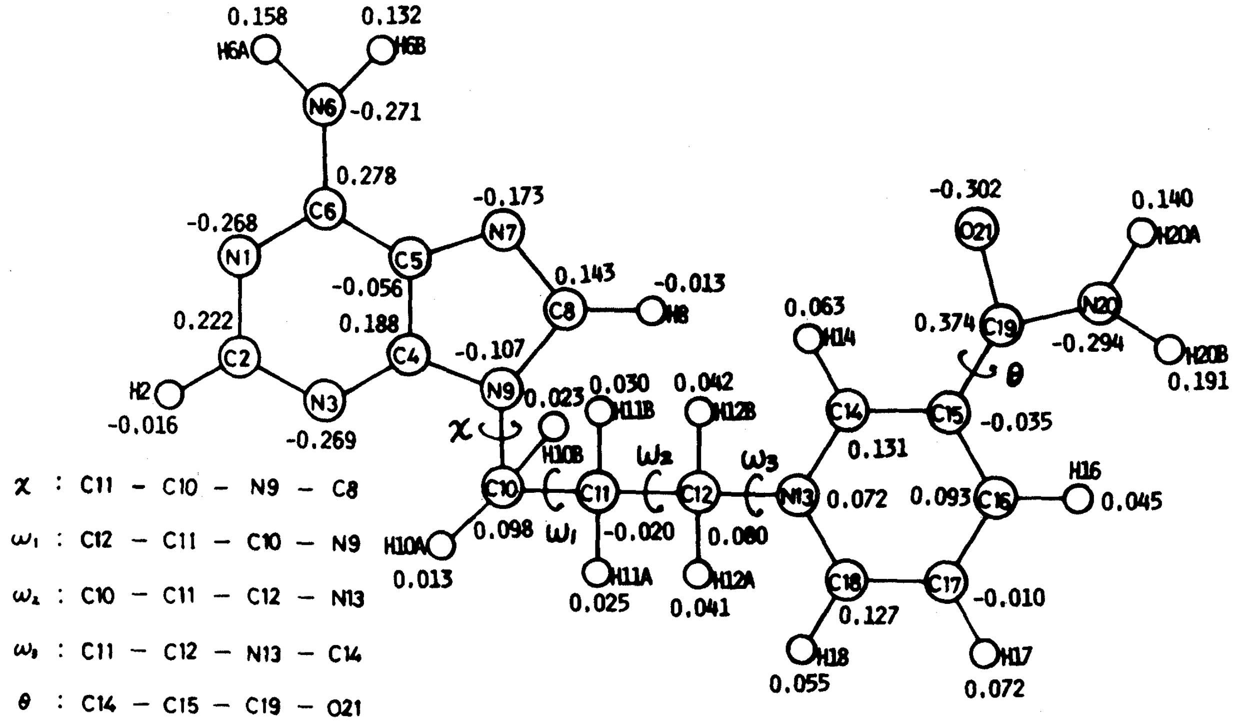


Fig. 2. The Atomic Numbering, Torsion Angles and CNDO/2 Net Electronic Charges (in Electronic Units) for AC3N$^+$

Results and Discussion

As listed in Table II, the conformations having 180° as the starting angle of either ω1 or ω2 unequivocally converged to the extended form, and their energy values were in the range of -9—-12 kcal/mol. Irrespective of the starting values of the remaining torsion angles, the conformations taking the extended form had almost the same energy values, suggesting that these conformations are meta-stable ones.

Figure 3 shows the most energetically stable extended conformation (Fig. 3(a)) and the observed one in the crystal structure (Fig. 3 (b)).[13] The latter conformation has an energy value of -9.39 kcal/mol, and also belongs to the metastable category.

Table II. The Starting and Final Torsion Angles and Their Energy Values of the Energetically Stable Extended Conformations, along with the Angles observed in the Crystal Structure

Starting angle (°)					Final angle (°)					Energy (kcal/mol)	Order
χ	ω1	ω2	ω3	θ	χ	ω1	ω2	ω3	θ		
120	300	180	120	0	136.6	306.0	167.6	92.3	301.2	−12.38	1
240	300	180	120	0	264.6	304.2	184.2	87.9	298.5	−11.85	2
240	300	180	240	0	264.4	305.3	185.1	274.9	297.0	−11.83	3
240	180	60	120	0	242.5	181.1	56.8	78.3	298.0	−11.69	4
120	180	300	120	0	102.9	168.6	296.2	121.6	302.5	−11.56	5
120	180	60	240	0	93.8	180.3	62.2	257.9	299.3	−11.23	6
240	180	300	120	0	242.8	182.0	298.0	100.2	299.8	−11.21	7
60	180	60	60	0	99.8	182.6	59.1	75.6	298.3	−11.13	8
300	180	300	300	0	262.5	180.2	301.2	287.5	296.6	−11.09	9
120	180	300	240	0	108.9	190.4	288.2	271.3	302.1	−11.01	10
					95.5	188.6	178.1	97.4	321.3	−9.39	Crystal

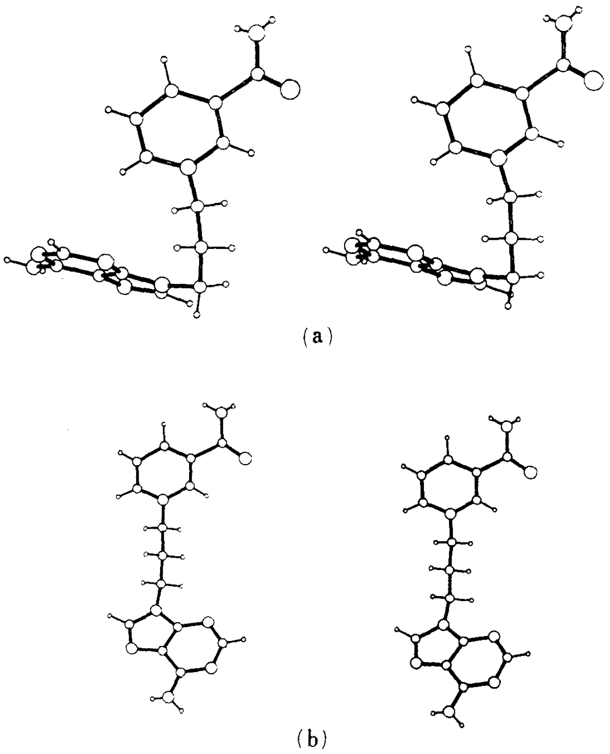


Fig. 3. Stereoscopic View of the Most energetically Stable Extended Conformation (a) and the Conformation observed in the Crystal Structure (b)

The most stable conformations, however, were the folded forms, especially those in which the two aromatic rings are stacked. Ten energetically stable conformations out of 225 different starting sets are listed in Table III, together with their starting angles and final energies. In this table, the letters, A, A′, B and B′, represent the stacking modes between the adenine and pyridinium rings, and the most stable conformations are illustrated in Figs. 4 and 5, in which the molecules are projected onto the adenine ring.

As is obvious from Tables II and III, torsion angles ω1 and ω2 are important for the stacked conformations as well as the extended ones.

In Figs. 4 and 5, the pyridinium rings are very well stacked onto the adenine rings, and the aromatic rings are almost parallel (their dihedral angles=5.5°—9.6°); there are some short contacts less than the normal van der Waals separation (3.4 Å). There are four kinds of adenine-pyridinium ring stacking modes (A, A′, B and B′): these conformations have practically the same energy values (—20——21 kcal/mol). A significant difference between conformers A and A′ or between conformers B and B′ is the orientation of the pyridinium ring relative to the adenine ring: conformer A or B could be related to conformer A′ or B′, respectively, by 180° rotation around the C12-N13 bond. On the other hand, the stacking mode in conformer A or A′ is quite similar to that in conformer B or B′: the stacking orientation of adenine-pyridinium rings in the former is almost identical with that in the latter.

In the conformers A and A′, the pyridinium rings are stacked from below the adenine rings, while in the conformers B and B′, the rings are stacked from above. These results mean that the pyridinium ring could stack well with the upper site as well as the lower site

Table III. The Starting and Final Torsion Angles and Their Energy Values of the energetically Stable Stacked Conformations

Starting angle (°)					Final angle (°)					Energy (kcal/mol)	Order	
χ	ω1	ω2	ω3	θ	χ	ω1	ω2	ω3	θ			
60	60	300	300	0	56.3	80.3	297.9	301.0	312.0	−21.29	1	A
120	300	60	240	0	115.7	297.7	78.0	235.6	306.5	−20.79	2	A
120	300	60	120	0	122.5	297.4	77.2	63.4	293.3	−20.37	3	A′
240	60	300	120	0	239.4	52.9	278.7	124.0	297.3	−20.25	4	B
300	300	60	60	0	297.3	283.0	58.7	55.6	300.8	−20.08	5	B
300	300	60	240	0	302.9	285.1	67.3	239.6	332.1	−20.07	6	B′
60	60	300	240	0	45.1	83.0	303.2	286.2	301.4	−19.92	7	A
300	300	60	120	0	306.6	283.2	55.3	65.9	300.0	−19.89	8	B
60	60	300	120	0	56.0	76.5	297.5	125.4	305.0	−19.65	9	A′
120	300	60	60	0	115.5	287.4	65.7	57.3	300.7	−19.63	10	A′

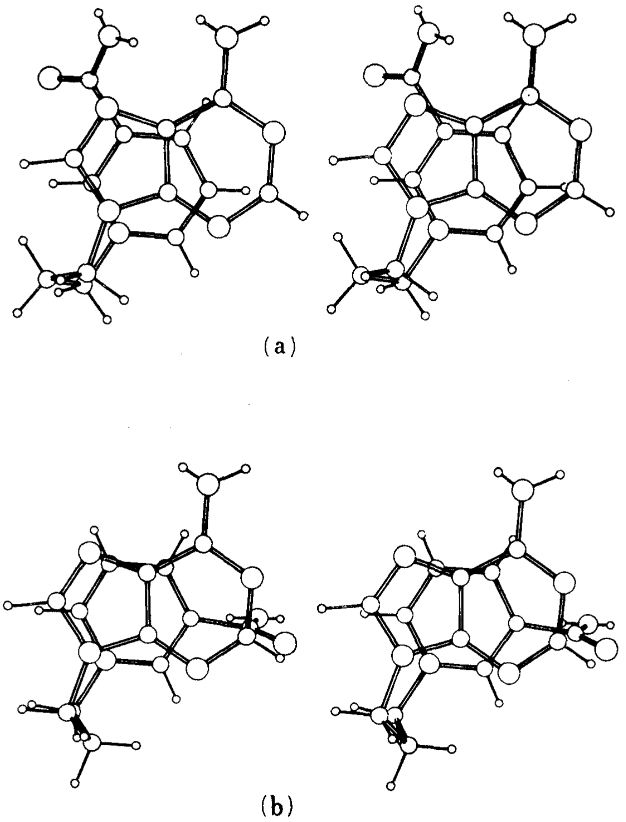


Fig. 4. Stereoscopic View of the Most energetically Stable Stacked Conformations, projected onto the Adenine Ring

(a) conformer A, (b) conformer A′.

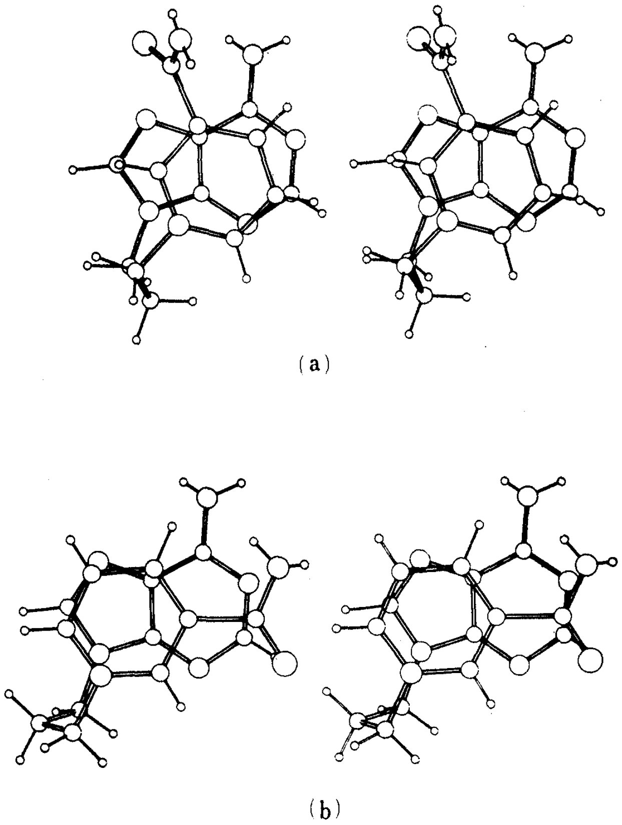


Fig. 5. Stereoscopic Views of the Most energetically Stable Stacked Conformations, projected onto the Adenine Ring

(a) conformer B, (b) conformer B′.

of the adenine ring; in other words, the pyridinium ring could interact with both sites of the adenine ring with the same stacking forces.

On the other hand, the torsion angle θ did not affect these stacked conformations to any significant extent and their energy values were essentially unaffected by different starting values of θ.

From the conformational analysis of $AC3N^+$ molecule, it could be considered that the extended conformation observed in the crystal structure is mainly due to intermolecular effects in the crystal packing. On the other hand, Johnson *et al.*[14] also reported that the dicationic form of $AC3N^+$ protonated at N1 (AH^+C3N^+) takes a folded conformation in the AH^+C3N^+ bromide hydrobromide crystal structure, and this conformation is due to the electrostatic interaction between the N1 atom as a center of positive charge and the polarizable oxygen atom (021). In the energy calculations of AH^+C3N^+ having the torsional values listed in Table II and III as its starting angles, the most energetically stable conformation was the folded form ($\chi=121.5°$, $\omega1=293.6°$, $\omega2=51.6°$, $\omega3=69.7°$ and $\theta=299.7°$), which is more energetically stable by 2.1 kcal/mol than the one observed in the crystal structure (see Figure 6). This conformation is energetically unstable (energy value $=-5.45$ kcal/mol), and the overlapping area between the adeninium and pyridinium rings is small, compared with the stacked conformations of $AC3N^+$. This result could be due to the electrostatic repulsion between the positively charged aromatic N1 and N13 atoms.

From the results of hypochromicity studies at the 260 nm band in NAD^+ and model compounds having a positive charge, Leonard *et al.*[6] concluded that the plane-parallel conformation and/or the approach of the positive charge to the adenine ring bring about hypochro-

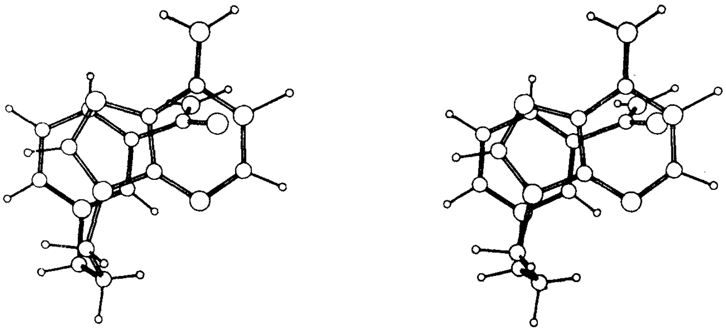


Fig. 6. Stereoscopic View of the Most energetically Stable AH$^+$ C3N$^+$ Conformation, projected onto the Adenine Ring

mism, and AC3N$^+$ exhibits hypochromism similar to that of NAD$^+$. The insights obtained in this work, therefore, provide important information as to the possible interaction between the adenine and pyridinium rings of NAD$^+$.

Conclusion

The major results of this work are as follows. (1) The stacking interaction between adenine and pyridinium rings brings about an energetically stable conformation. (2) Four kinds of stable conformations exist that exhibit adenine-pyridinium ring stacking. (3) The pyridinium ring can stack equally well on either side of the adenine ring.

Acknowledgement We would like to thank Professor K. Tomita and Dr. S. Fujii, of Osaka University, for providing the energy calculation program and for their valuable advice.

References and Notes

1) D. Voet, *J. Am. Chem. Soc.*, **95**, 3763 (1973).
2) J.C. Greenfield, N.J. Leonard, and R.I. Gumport, *Biochemistry*, **14**, 698 (1975).
3) R.J. Suhadolnik, M.B. Lennon, T. Uematsu, J.E. Monahan, and R. Baur, *J. Biol. Chem.*, **252**, 4125 (1977).
4) The 100th Annual Meeting of the Pharmaceutical Society of Japan, Tokyo, Apr. 1980.
5) J.M. Siegel, G.A. Montgomery, and R.M. Bock, *Arch. Biochem. Biophys.*, **82**, 288 (1959).
6) N.J. Leonard, T.G. Scott, and P.C. Huang, *J. Am. Chem. Soc.*, **89**, 7173 (1967).
7) J.A Secrist III and N J. Leonard, *J. Am. Chem. Soc.*, **94**, 1702 (1972).
8) A.V. Lakshminarayanan and V. Sasisekharan, *Biopolymers*, **8**, 475 (1969).
9) V. Renugopalakrishnan, A.V. Lakshminarayanan, and V. Sasisekharan, *Biopolymers*, **10**, 1159 (1971).
10) N. Yathindra and M. Sundaralingam, *Biopolymers*, **12**, 297 (1973).
11) O.E. Miller, Jr. and J.A. Andersen, *Biopolymers*, **14**, 2159 (1975).
12) M.J.D. Powell, *Computer J.* **7**, 155 (1964).
13) P.L. Johnson, C.A. Maier, and I.C. Paul, *J. Am. Chem. Soc.*, **95**, 5370 (1973).
14) P.L. Johnson, J.K. Frank, and I.C. Paul, *J. Am. Chem. Soc.*, **95**, 5377 (1973).