THE GAIN IN CONFORMATIONAL PURITY AND LOSS IN FLEXIBILITY AS A RESULT OF 3',5' POLYMERIZATION BETWEEN THE COMPONENT MONONUCLEOTIDES – A 300 MHz ^{1}H AND 40.5 MHz ^{31}P NMR COMPARATIVE STUDY OF THE DYNAMIC SOLUTION CONFORMATION OF DINUCLEOSIDE MONOPHOSPHATES AND THEIR COMPONENT MONOMERS

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1. Introduction

The complex 300 MHz ^{1}H and the 40.5 MHz ^{31}P NMR spectra of the dinucleoside monophosphate ApA (I) and the corresponding deoxy-analog dApdA (II) were completely analyzed by extensive computer simulations and the NMR parameters were used to arrive at their dynamic solution conformation. Detailed comparative study of the conformation of the dimers to their component monomeric units were undertaken. Such a study revealed that the purine mononucleotides become conformationally less flexible and achieve a greater degree of conformational purity, as they become integrated into the framework of a dinucleoside monophosphate. Implication of this finding with respect to the conformation of polynucleotides is discussed.

2. Materials and methods

^{1}H spectra of ApA and dApdA, 0.05 M, pD 7.5 in D_2O were obtained at 300 MHz at 27°C in both Fourier transform (FT) and continuous wave (CW) modes, using a Varian HR-300 NMR spectrometer interfaced to a Varian 620f data system. The ^{1}H spectra for the component mononucleotides 3'AMP, 5'AMP, 3'dAMP and 5'dAMP were obtained in the Fourier mode in a 100 MHz NMR system. Fourier transformed ^{31}P spectra for all the above compounds were recorded at a frequency of 40.48 MHz. Details of instrumentation are discussed elsewhere [1,2]. The pD of the mononucleotide solutions was adjusted to be 5.4 so that the phosphate groups would remain as monophosphate monoanions. This situation is most nearly compatible with the ionic state of the phosphate in ApA and dApdA at biological pH values. Spectra were analyzed using the computer program LACOON III. The ^{1}H and ^{31}P spectra of ApA and dApdA along with the corresponding computer simulated spectra are illustrated in fig.1. In view of the complex nature of the spectra, one should notice the remarkable agreement between the observed and computer fitted spectrum. The phosphorus hydrogen couplings were obtained from both ^{1}H and ^{31}p spectra. The coupling constant data for the various protons are compiled in table 1.

3. Results and discussion

In the structures of ApA and dApdA, shown in I and II, the various bonds of the backbone of interest are clearly labelled.

3.1. *Conformation of the phosphodiester backbone*

The minimum energy conformations about the pA O(5')-C(5') and Ap C(3')-O(3') bonds are shown respectively in the Newman projections III, IV, V (g'g', g't', t'g') and VI, VII, VIII (g^-, g^+, t). It has been shown that the conformational distribution about the C(5')-O(5') bond can be computed from the magnitude of the sum Σ' ($J_{5'p} + J_{5''p}$) [3–5]. Population distribution of the conformers about the

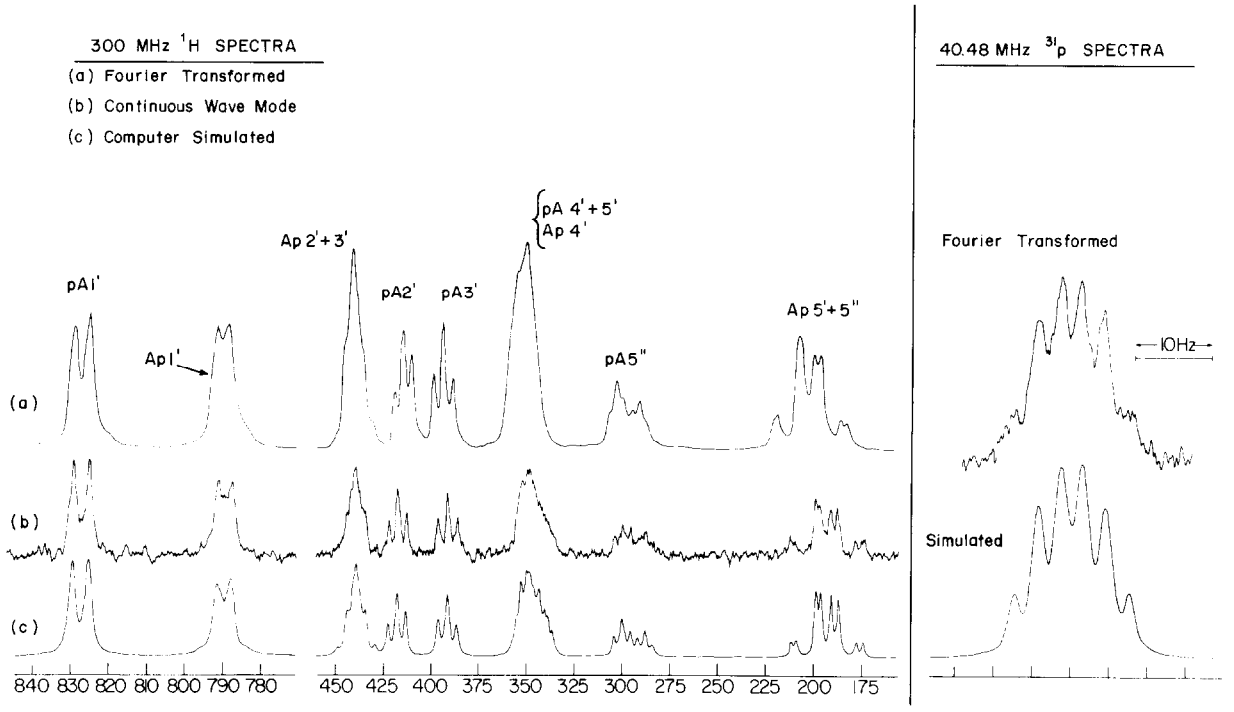


Fig.1. (Left) The diagrams a and b respectively represent the 300 MHz ^{1}HNMR spectra of ApA (0.05 M, pD 7.5, temp. 27°C) obtained by Fourier transform (FT) methods (a), and in continuous wave (CW) modes (b). The diagram c is the computer simulated one. Only the ribose region is shown. The FT spectra were superior in S/N ratio but suffered from a poor resolution of 0.7 Hz because only 8K of the total 16K memory was available to transform a band width of 2500 Hz. The CW spectra was superior in resolution (0.2 Hz) but had only fair S/N ratio. By carefully studying both FT and CW spectra peaks were identified and finally the spectra were simulated to derive the data in table 1. The chemical shifts are expressed in Hz, 300 MHz system, upfield from internal tetramethyl ammonium chloride. (Right) The Fourier transformed (top) and computer simulated (bottom) ^{31}P NMR spectra (40.48 MHz) of dApdA 0.05 M, pD 7.5 and temp. 27°C.

C(3')-O(3') bond can be obtained from the magnitude of $J_{3'p}$ as has been described elsewhere [6–8]. In table 1 we have compiled the results of such calculations. The data show that the C(5')-O(5') bond of the pA and pdA parts of ApA and dApdA shows a clear preference for the g'g' conformer (III), like the component monomers 5'AMP and 5'dAMP. Comparison of the Σ' data (table 1) for the monomers and that for the dimers reveals some important features. Thus the magnitude of Σ' decreases from 10.2 Hz to 7.0 Hz as one goes from 5'dAMP to the pdA part of dApdA (table 1). Also, the value of Σ' decreases from 10.0 Hz to 8.6 Hz as 5'AMP becomes the pA portion of ApA. It has been shown elsewhere [3–5] that a reduction in the magnitude of Σ' is an indication of the increase in the population of g'g' conformer (III). Insofar as the error in the computer fitted value for Σ' is only ± 0.3 Hz, the observed change in the magnitude of Σ' clearly shows that as the mononucleotide fragments become integrated into the framework of dinucleoside monophosphates, the C(5')-O(5') bond loses some of its conformational flexibility, becomes increasingly g'g', thereby achieves a greater degree of conformational purity.

Such a change in the time average conformation of an important backbone bond such as C(5')-O(5') would, in turn, be expected to affect the geometries of other backbone bonds. From extensive studies of such conformationally aberrent nucleotides as 8-Br-5'AMP, 8-CH_3S-5'AMP, 8-aza-5'AMP and 8-aza-5'GMP, we have shown [9,10] that the backbone of nucleotides is engineered in such a way that pertur-

Table 1
Coupling constants for dApdA and ApA (pD 7.5) and component monomers (pD 5.4)

Values (Hertz)	dApdA		3'dAMP	5'dAMP	ApA		3'AMP	5'AMP
	dAp	pdA			Ap	pA		
H(1')-H(2')	8.7	7.0	8.0	7.3	4.2[a]	4.3[a]	6.2	5.7
H(1')-H(2'')	5.5	6.7	6.2	6.3				
H(2')-H(2'')	−14.0	−13.9	−14.2	−14.1				
H(2')-H(3')	5.6	6.6	5.9	6.3	5.2	5.1	5.2	5.2
H(2')-P(3')			0.8[b]				1.1	
H(2'')-H(3')	2.3	4.1	2.9	3.4				
H(3')-H(4')	2.4	4.0	2.5	3.0	5.0	4.9	2.9	3.7
H(3')-P(3')	5.6		7.4		8.0[b]		7.9	
H(4')-H(5')	3.3	2.5	3.1	3.8	2.3	2.5[b]	2.4	3.1
H(4')-H(5'')	3.3	2.8	3.7	3.8	3.8	3.5	3.3	3.1
Σ[c]	6.6	5.3	6.8	7.6	6.1	6.0	5.7	6.2
H(4')-P(5')		2.7		1.7		2.0		1.7
H(5')-P(5')		3.5		5.2		4.3		5.0
H(5'')-P(5')		3.5		5.0		4.3		5.0
Σ'[d]		7.0		10.2		8.6		10.0
H(5')-H(5'')	−12.0	−12.0	−13.1	−13.0	−12.8	−11.6	−12.7	−12.0
% Populations of:[e]								
g'g' (III)		94		77		86		78
g^- (VI) + g^+ (VII)	86		76		72		73	
gg (IX)	64	77	62	54	69	70	73	68
^{2}E (XII, C(2') endo)[f]	91	83	88	82	50	52	75	69
S[f]	79	74	77	73	42	43	62	57

[a] The values reported for JH(1')-H(2') do not agree with the ones in [22–24]. This is because in earlier investigations [22–24] the magnitude of JH(1')-H(2') was obtained from first order analysis of complex 60 or 100 MHz spectra.

[b] The J values are estimated from the spectra and are subject to ± 0.3 Hz error.

[c] Σ = JH(4')-H(5') + JH(4')-H(5'').

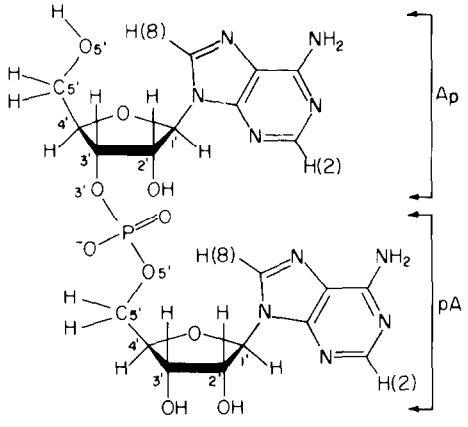
[d] Σ' = JH(5')-P(5') + JH(5'')-P(5').

[e] What is given in this table is the % populations of the *preferred* conformer. The populations of the alternate conformers i.e. the non-preferred ones, can be obtained by deducting the value reported here from 100; e.g. the combined populations of the g't' and t'g' rotamers about C(5')-0(5') bond of the pdA part of dApdA is 6% (i.e. 100-94); in the same way, the population of the ^{3}E conformer for the Ap part of ApA is 50% (i.e. 100-50).

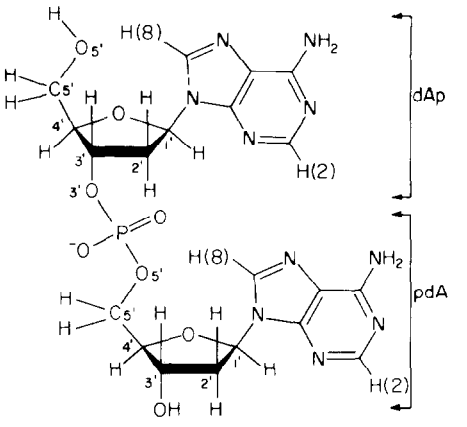
[f] For the deoxy series, the ^{2}E and S populations are calculated from $J_{1'2'} + J_{1'2''}$ as described in [19].

bation of a stereochemical relation along any of the backbone bonds, in turn, in general, perturbs the other geometric relations so that the molecule undergoes a conformational adjustment. The data in table 1 shows that in the present case, the increase in the conformational purity that C(5')-0(5') bond achieves, as the monomers become dimers, also creates differences in the magnitudes of rotational preferences along other bonds. For example, the value of $J_{3'p}$ in 3'dAMP and the dAp part of dApdA are 7.4 and 5.6 Hz respectively. Contrary to the deoxy series, the magnitude of $J_{3'p}$ in 3'AMP and the Ap part of ApA remains constant. The error in the measurement of $J_{3'p}$ values is only ± 0.2 Hz. The observed magnitude of $J_{3'p}$ in all the compounds examined suggest [6–8] that the 3' phosphate group prefer gauche (g^-, g^+, VI, VII) orientations.

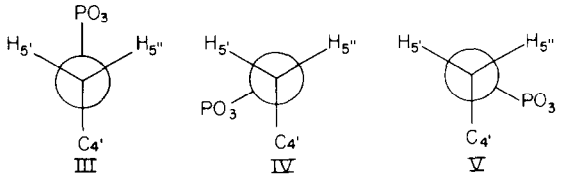
The ^{1}H and ^{31}P NMR methods do not ordinarily enable to determine whether the 3' phosphate group prefers to occupy g^- (VI) or g^+ (VII) position. However, in the favorable case in which ribose is ^{2}E (XII) and the 3' phosphate group is g^+ (VII), an almost in plane 'W' relation exists between the phosphorus atom and the 2' (H). Such a 'W' relation would be



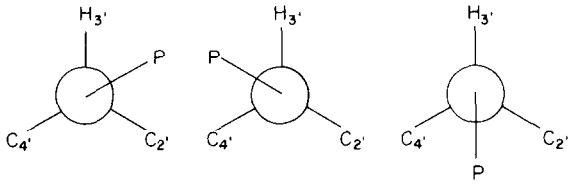
ApA
I



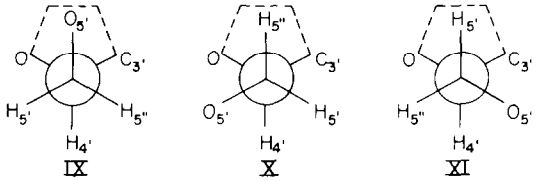
dApdA
II



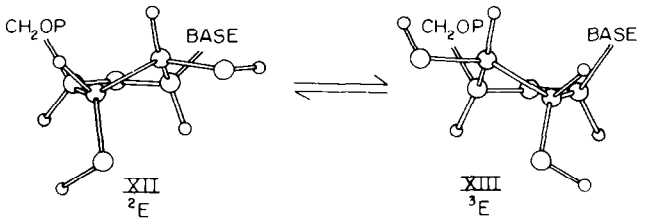
III (GAUCHE)'-(GAUCHE)' g' g'
IV (GAUCHE)'-(TRANS)' g' t'
V (TRANS)'-(GAUCHE)' t' g'



VI GAUCHE (g^-)
VII GAUCHE (g^+)
VIII TRANS (t)



IX GAUCHE–GAUCHE gg
X GAUCHE – TRANS gt
XI TRANS–GAUCHE tg



XII ^2E ⇌ XIII ^3E

expected to produce a long range four bond $^{31}P(3')$-$^{1}H(2')$ coupling of maximum magnitude of $\simeq$ 2.7 Hz [11–13], should all the molecules were populated in $^{2}E.g^{+}$ conformation. It is probably safe to conclude that a $^{31}P(3')$-$^{1}H(2')$ coupling of magnitude of $\simeq$ 1 Hz would be observed, should at least 50% of $^{2}E.g^{+}$ conformers contribute toward the time average conformation of the 3′ phosphate group. In the cases of ApA, and dApdA, the observed $^{31}P(3')$-$^{1}H(2')$ coupling are of the order of $\simeq$ 0.5 Hz and it appears that the computed percentages of the total gauche populations (VI, VII) in table 1 for these compounds mostly reflect the population of the g^{-} conformer. The observed reduction in the value of $J_{3'p}$ as one goes from 3′dAMP to the dAp part of dApdA obviously reflect an increase in gauche populations as a result of dimerization. The observation that in 3′AMP and the Ap part of ApA have identical value for $J_{3'p}$ does not necessarily mean that the 3′ phosphate group has identical orientation in 3′AMP and ApA, because g^{-} and g^{+} orientations have identical dihedral angle relationships between the $^{31}P(3')$ and $^{1}H(3')$ atoms, i.e. in 3′AMP the phosphate group may be g^{+} and in ApA, it may be g^{-}. In fact, unlike in ApA, we have observed a four bond $^{31}P(3')$-$^{1}H(2')$ coupling of 1.1 Hz in 3′AMP, indicating that g^{+} orientation has really become accessible to this mononucleotide. NMR data on UpU, ApG, and ApU at 70°C [14] indicate a $^{31}P(3')$-$^{1}H(2')$ coupling in these compounds of magnitude greater than 1.0 Hz, suggesting that g^{+} conformation becomes accessible even in the dinucleoside monophosphates.

In the fractional population calculations for the conformer distribution of the 3′ phosphate group (table 1 we have assumed that for nucleic acid components, all the three conformers i.e. g^{-} (VI), g^{+} (VII) and t (VIII), are accessible in aqueous solutions. Because in the crystal structures [15] one has not observed the t (VIII) conformation and theoretical calculations [16] do not suggest the presence of such orientations, one may process the $J_{3'p}$ coupling data by completely excluding the t conformation. If this were the case the $J_{3'p}$ values in table 1, suggest that for the class of compounds examined the 3′ phosphate group occupies g^{+}, g^{-} domains in which the time average H(3′)-C(3′)-O(3′)-P(3′) dihedral angle varies from ± 50 to ± 60 degrees. A reduction in the value of $J_{3'p}$, as it happens when one goes from 3′dAMP to dApdA, indicates an increase in the above dihedral angle. The present approach, like the previous one, suggests the same difference the orientation of the 3′ phosphate group between 3′AMP and the Ap part of ApA.

3.2. *The conformation about the C(4′)-C(5′) bonds*

There are two classes of C(4′)-C(5′) bonds; one class belongs to 5′AMP, 5′dAMP, the pA and pdA parts of ApA and dApdA; the other class belongs to 3′AMP, 3′dAMP, the Ap and dAp parts of ApA and dApdA. These two classes are clearly labelled in I and II. The Newman projections IX (gg), X (gt) and XI (tg) show the minimum energy conformations about the C(4′)-C(5′) bonds. The population distribution of these conformers were calculated from the magnitude of $\Sigma (J_{4'5'} + J_{4'5''})$ as described elsewhere [3–5] and the data are compiled in table 1. It is seen that the C(4′)-C(5′) bond in all the cases examined, in general, displays preference to exist in the gg (IX) conformation. And no significant difference in the gg population is detectable between 3′AMP and the Ap part of ApA on the one hand, and between 3′dAMP and the dAp part of dApdA, on the other. This is entirely expected because here one is dealing with a part of a structure which does not participate in a linkage during the formation of the dimer. On the other hand, the gg population about the C(4′)-C(5′) bond of 5′dAMP undergoes over a 20% increase in being incorporated into dApdA, the magnitude of Σ changing from 7.6 to 5.3 Hz (table 1).

In this respect the pair 5′AMP and ApA display a striking contrast. No change in the population distribution about C(4′)-C(5′) occurs as 5′AMP becomes incorporated into the pA part of ApA (table 1).

3.3. *The sugar ring conformation*

We have shown [17] that whether one uses the traditional Karplus approach of a ^{2}E (XII) $\rightleftarrows$ ^{3}E (XIII) equilibrium [1,18] or the pseudorotational approach of a S $\rightleftarrows$ N equilibrium [19], one could obtain only qualitative information about the sugar conformation from ring coupling constants. In order to avoid confusion we have suggested to use the terms ^{2}E and S as well as ^{3}E and N synonymously [6–8, 10]. In table 1 are presented the computed populations of ^{2}E and S conformers for the present series of compounds. These calculations were performed as described in

[17,19]. The observed differences in the population of the ^{2}E and the S conformers for a given nucleotide is an indication of the qualitative nature of the two approaches [10,17]. In this paper we are interested only in determining the direction of the shift in conformer populations as the mononucleotide fragments become dimers. We have shown elsewhere that *shift in the population distribution of sugar ring conformers manifest precisely in ring coupling constant changes and relative changes in populations, as opposed to actual populations, can be computed accurately* [7,10, 20].

Inspection of the data for the sugar conformers in table 1 reveals some amazing facts about the conformational characteristics of ribo- and deoxyribonucleoside monophosphates. Comparison of the ^{2}E population in 3′dAMP and the dAp part of dApdA and that in 5′dAMP and the pdA part of dApdA shows that dimerization has essentially no effect on the time average distribution of ^{2}E and ^{3}E conformers. In this respect, 3′AMP, 5′AMP and ApA provide a contrast. It is seen that (table 1), the ^{2}E population decrease by 20–25% (i.e. the ^{3}E population increase by 20–25%) as one moves from 5′AMP and 3′AMP to the respective fragments of ApA, so much so in ApA, at 27°, both ribose moieties exist as 50:50 mixtures of ^{2}E:^{3}E conformers. Prima facie it appears that as a result of dimerization, the ribose moiety has lost some of its conformational purity. This happens because in the monoribonucleotides in aqueous solution the preferred conformation for the sugar ring is ^{2}E and we believe that in the polyribonucleotides the conformation may be essentially ^{3}E. Hence, as one moves from the mononucleotides to the dimer the ^{2}E:^{3}E ratio changes from 70:30 to 50:50. It is anticipated that in the triribonucleotides such as pApApAp, the ^{2}E:^{3}E ratio will change significantly in favor of the ^{3}E conformer. Such a conclusion receives support from our finding that in poly A, the ribose moiety shows an outspoken preference for the ^{3}E conformer [21].

4. Conclusion

As the 3′ and 5′ purine mononucleotides become integrated into the framework of a dinucleoside monophosphate, the backbone becomes less flexible and the system achieves a greater degree of conformational purity*. It is reasonable to expect this trend of increase in conformational purity to continue as the system goes from dimers to oligomers to polymers, at which stage in aqueous solutions, one would expect the backbone to be overwhelming conformationally pure. From the presently observed trends one may conclude that in aqueous solutions the 3′, 5′ polymers of purine ribonucleotides and deoxyribonucleotides will have an almost conformationally pure backbone in which C(4′)-C(5′) is gg (IX), C(5′)-O(5′) is g′g′ (III) and C(3′)-O(3′) is g$^+$/g$^-$ VI, VII). The only significant difference that is expected between the above polyribonucleotides and polydeoxyribonucleotides is in the sugar conformation, the former is expected to display ^{3}E pucker, and the latter ^{2}E pucker.

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References

[1] Sarma, R. H. and Mynott, R. J. (1973) J. Amer. Chem. Soc. 95, 1641.
[2] Sarma, R. H. and Mynott, R. J. (1972) Org. Magnetic Res. 4, 577.

* Note added in proof: Professor M. Sundaralingam has arrived at the same conclusions (personal communication to R. H. Sarma) from extensive theoretical studies, as well as from in depth comparative study of the X-ray data on a large number of nucleic acids and the components.

[3] Hruska, F. E., Wood, D. J., Mynott, R. J. and Sarma, R. H. (1973) FEBS Lett. 31, 153.
[4] Wood, D. J., Hruska, F. E., Mynott, R. J. and Sarma, R. H. (1973) Can. J. Chem. 51, 2571.
[5] Wood, D. J., Mynott, R. J., Hruska, F. E. and Sarma, R. H. (1973) FEBS Lett. 34, 323.
[6] Lee, C. H. and Sarma, R. H. (1974) FEBS Lett. 43, 271.
[7] Lee, C. H. and Sarma, R. H. (1974) J. Amer. Chem. Soc. (in press).
[8] Lee, C. H. and Sarma, R. H. (1974) Proceedings, The Fourth Annual Harry Steenback Symposium (S. T. Rao and M. Sundaralingam, eds.) University Park Press, Baltimore, Md. USA (in press).
[9] Sarma, R. H., Lee, C. H., Evans, F. E., Yathindra, N. and Sundaralingam, M. (1974) J. Amer. Chem. Soc. 96, 7337.
[10] Lee, C. H., Evans, F. E. and Sarma, R. H. (1974) J. Biol. Chem. (in press).
[11] Hall, C. D. and Malcolm, R. B. (1972) Can. J. Chem. 50, 2092.
[12] Donaldson, B. and Hall, L. D. (1972) Can. J. Chem. 50, 2111.
[13] Sarma, R. H., Mynott, R. J., Hruska, F. E. and Wood, D. J. (1973) J. Amer. Chem. Soc. 95, 6457.
[14] Lee, C. H. and Sarma, R. H. (1974) Unpublished results.
[15] Sundaralingam, M. (1968) Biopolymers 6, 189.
[16] Pullman, B., Perahia, D. and Saran, A. (1972) Biochimica Biophysica Acta 269, 1.
[17] Evans, F. E. and Sarma, R. H. (1974) J. Biol. Chem. 249, 4754.
[18] Hruska, F. E. (1973) Proc. Intern. Symp. Conformation Biol. Mol. Polymers – Symp. Quantum Chem. and Biochem. (Bergmann, E. D. and Pullman, B., eds.) Vol. 5, 345, Academic Press.
[19] Altona, C. and Sundaralingam, M. (1973) J. Amer. Chem. Soc. 95, 2333.
[20] Evans, F. E. and Sarma, R. H., submitted to J. Amer. Chem. Soc.
[21] Evans, F. E. and Sarma, R. H. (1974) Biopolymers 13, 2117.
[22] Ts'o, P. O. P., Kondo, N. S., Schweizer, M. P. and Hollis, D. P. (1969) Biochemistry 8, 997.
[23] Chan, S. I. and Nelson, J. H. (1969) J. Amer. Chem. Soc. 91, 168.
[24] Hruska, F. E. and Danyluk, S. S. (1968) J. Amer. Chem. Soc. 90, 12.