Investigation of the Configuration and Conformation of *N*-Methyl-*N*-ethylnicotinamide–Adenine Dinucleotide by Nuclear Magnetic Resonance Spectroscopy*,†

Ramaswamy H. Sarma, Maryalice Moore, and Nathan O. Kaplan

ABSTRACT: High and low temperature nuclear magnetic resonance spectra (60 MHz nuclear magnetic resonance system) show that *N*-methyl-*N*-ethylnicotinamide exists as a mixture of *cis* and *trans* isomers, and that there is no preference toward the formation of the *trans* (methyl) isomer. The equal population of the *cis* and *trans* isomers in the product argues against the common notion that steric interactions between the substituent groups on the asymmetrically polarized C–N bond play a significant role in favoring the formation of the *trans* (methyl) isomer. In the present case, steric interactions between the bulky ethyl and pyridyl groups can be precluded, if the amide side chain and the pyridine ring are not coplanar as the X-ray data indicate. High frequency (220 MHz) nuclear magnetic resonance shows that *N*-methyl-*N*-ethylnicotinamide–adenine dinucleotide also exists as a mixture of the *cis* and *trans* isomers, and that in addition, each of the geometric configurations exists as a mixture of two conformers. The two conformers are right- and left-handed folded forms of the dinucleotide. Specifications of the molecular chirality of pyridine dinucleotides show that the right- and left-handed folded conformers are *P* and *M* helices. The present data are in agreement with the view that pyridine dinucleotides in solutions contain significant populations of both *P* and *M* helices. Only if aqueous solutions of *N*-methyl-*N*-ethylnicotinamide–adenine dinucleotide contain significant populations of the *P* and *M* helices (in which the amide side chain is noncoplanar with the pyridine ring) can it give rise to a mixture of two geometric configurations, in which each of the geometric configurations can exist in two different conformers.

Investigation of the conformation of pyridine dinucleotides in aqueous solution (deuterium oxide) by Sarma *et al.* (1968b) and Sarma and Kaplan (1969a) suggested a folded conformation for the dinucleotide as well as dinucleotide analogs involving substitution of the pyridine ring ($CONH_2$, replaced by $COCH_3$, $CO(CH_2)_3CH_3$, CHO, NH_2). Nuclear magnetic resonance data from Sarma *et al.* (1968b), Sarma *et al.* (1968a), and Sarma and Kaplan (1969b) show that in DPN, the adenine C_2H and pyridine C_2H undergoes a larger diamagnetic shielding by the ring current anisotropies of the pyridine and adenine rings, respectively, compared with adenine C_8H and pyridine C_6H. This suggests that in the dinucleotide, the geometry of the folding is such that, on the average, the adenine spends more time with that part of the pyridine ring which contains the amide group, and that the pyrimidine moiety of adenine spends more time with the pyridine ring than does the imidazole part of the purine. In such a conformation, one would expect substituents on the amide nitrogen to interfere sterically with the folding interaction. It has been found, however, that *N*-monomethyl and *N*-monoethyl substitutions do not affect the conformation of DPN, but *N*,*N*-dimethyl substitution causes slight dissociation between base pairs (Sarma *et al.*, 1968b). These observations led us to suggest that only one of the methyl groups in *N*,*N*-dimethylnicotinamide-AD[1] hinders the folding of the rings. Study of the molecular models of the folded conformation shows that the methyl group *trans* to the oxygen is the only methyl group which could hinder the folding. Further, the nuclear magnetic resonance spectra of *N*-monomethyl- and *N*-monoethylnicotinamide-AD point out that only one of the possible rotational isomers is present. From the nuclear magnetic resonance data of LaPlanche and Rogers (1964), it is reasonable to infer that the rotational isomer of the *N*-monoalkylnicotinamide-AD present is the *trans* isomer, *i.e.*, the alkyl group is *cis* to the oxygen of the amide group. The methyl group *trans* to the oxygen apparently causes the steric hindrance observed in the *N*,*N*-dimethylnicotinamide-AD (Sarma *et al.*, 1968b). At this point, it is of interest to recall that the pig brain DPNase does not exchange the nicotinamide of DPN with *N*,*N*-diethylnicotinamide (Sarma *et al.*, 1968b). These observations, which reveal some of the finer stereochemical features of DPN, led us to investigate the configuration and conformation of *N*-methyl-*N*-ethylnicotinamide-AD.

* Contribution No. 690 from the Graduate Department of Biochemistry, Brandeis University, Waltham, Massachusetts 02154, and the Department of Chemistry, University of California, San Diego, La Jolla, California 92037. Requests for reprints should be addressed to University of California. *Received June 9, 1969.* Supported in part by research grants from the American Cancer Society (P-77J) and the National Cancer Institute of the National Institutes of Health (CA-03611).

† This paper contains four four-color figures. To avoid production difficulties, these figures, as well as four-color figures from both accompanying articles, are printed together on pp 551, 552.

[1] Abbreviations used that are not listed in *Biochemistry 5*, 1445 (1966): *N*,*N*-dimethylnicotinamide-AD, *N*,*N*-dimethylnicotinamide–adenine dinucleotide; *N*-methyl-*N*-ethylnicotinamide-AD, *N*-methyl-*N*-ethylnicotinamide–adenine dinucleotide; DSS, sodium 2,2-dimethyl-2-silapentane-5-sulfonate; ADPR, adenosine diphosphoribose.

Experimental Section

β-DPN was a commercial preparation from P-L Biochemicals. Nicotinic acid and ethylmethylamine (in the form of the hydrochloride) were purchased from Eastman Organic Chemicals. *N*-Methyl-*N*-ethylnicotinamide and the corresponding DPN analog were prepared in this laboratory.

Synthesis of N-Methyl-N-ethylnicotinamide. A toluene solution of ethylmethylamine was added to an ice cold solution of nicotinoyl chloride prepared according to the procedure of Wingfield *et al.* (1953). After removal of the toluene by distillation, the product was obtained as a colorless, oily liquid by distillation under reduced pressure; bp 93–95° (0.02–0.03 mm). Based on potassium nicotinate as starting material, an overall yield of 61% of the unsymmetrically *N,N*-disubstituted nicotinamide was obtained. The ultraviolet spectrum had peaks with maximum at 262 and 255 mμ (shoulder at 272 mμ). The infrared spectrum was similar to the spectra of *N,N*-dimethylnicotinamide (Sadtler). Analysis of the nuclear magnetic resonance spectra is presented later in this paper.

Synthesis of N-Methyl-N-ethylnicotinamide-AD. A modified procedure of Kaplan and Ciotti (1956) was used. Enzyme units (1240) (one unit of enzyme cleaves 1 μmole of β-DPN in 1 hr) of pig brain DPNase (Zatman *et al.*, 1953) in 30 ml of 0.1 M potassium phosphate buffer (pH 7.2) was added to 120 ml of an aqueous solution of 1 g of β-DPN (1.3 mmoles) and 4 g of *N*-methyl-*N*-ethylnicotinamide (24.5 mmoles), brought to pH 7.2 with 0.1 M NaOH. The reaction mixture was incubated at 38°. Progress of the reaction was followed by making use of the fact that β-DPN reacts with cyanide (Colowick *et al.*, 1951) and alcohol dehydrogenase, whereas the analog reacts only with the cyanide. After 5-hr incubation, the reaction mixture retained 11% of its ability to form cyanide adduct, but can no longer be reduced enzymatically. At this point, the reaction was stopped by the addition of 50% trichloroacetic acid solution until a pH of 3.5 was attained. The precipitated protein was removed by centrifugation, and the analog in the supernatant was isolated according to the procedure of Kaplan and Ciotti (1956). The sample was purified on a DEAE-cellulose column (Whatman DE-11) equilibrated with 0.005 M ammonium carbonate buffer. A linear gradient, formed with 1 l. each of 0.005 M ammonium carbonate and 4% sodium chloride in 0.005 M ammonium carbonate, was used to elute the analog from the column. Under these conditions, the free bases (nicotinamide and *N*-methyl-*N*-ethylnicotinamide) are eluted first, followed by the analog and finally ADPR. The fractions with $OD_{260}:OD_{315}$ ratio (the cyanide adduct of the analog has an ultraviolet maximum of 315 mμ) from 3.3 to 3.9 were combined and then lyophilized. The lyophylized product was dissolved in 10 ml of distilled water and eluted from a Sephadex G-15 column. Chloride-free fractions with an average $OD_{260}:OD_{315}$ ratio of 3.7 were combined and lyophilized; 70 mg of analog was obtained from 1 g of DPN.

The nuclear magnetic resonance spectra were obtained on a high resolution Varian A-60A or HR 220 MHz spectrometer. Shifts were measured with the use of DDS (sodium 2,2-dimethyl-2-silapentane-5-sulfonate, E. Merck AG, Darmstadt, Germany) as an internal standard. The precision of the measurements were within ±0.5 Hz. The samples were lyophilized from D_2O and then dissolved in D_2O (0.1 M, pH 7.0).

Results and Discussion

Because of contributions from the resonance structures I and II (LaPlanche and Rogers, 1964), unsymmetrical substitution of the amide nitrogen of nicotinamide as in *N*-monomethyl- or *N*-monoethylnicotinamide, or as in *N*-methyl-*N*-ethylnicotinamide, provides the possibility for the formation of two geometrical configurations. Direct proof by nuclear magnetic resonance was obtained by Phillips (1955) for restricted rotation about the C–N bond in amides. LaPlanche and Rogers (1964) reported the presence of both *trans* and *cis* monomethylformamides using nuclear magnetic resonance spectroscopy. They could detect, however, only the *trans* isomers in all those cases where the carbonyl group carried a substituent larger than hydrogen. The same authors (1963) also noted that in the case of unsymmetrically *N,N*-disubstituted amides, the bulkier substituent on the nitrogen is *cis* to the formyl hydrogen in formamides, and *trans* to the

PAPER I, FIGURE 3 (opposite page, top left): The molecular model of TPNH in the *P* helical form. The models have been constructed from Courtland atoms. Comparison of Figures 3 and 4 show that the two helical forms are not equivalent. (1) The hydrogen of the $CONH_2$ of the dihydronicotinamide could not hydrogen-bond to the 2′-phosphate oxygens or to any part of ribose. (2) The "A" proton is mainly exposed to the adenine and free from the environment of 2′-phosphate. The base pairs appear to be closer than they are in the *M* helix.

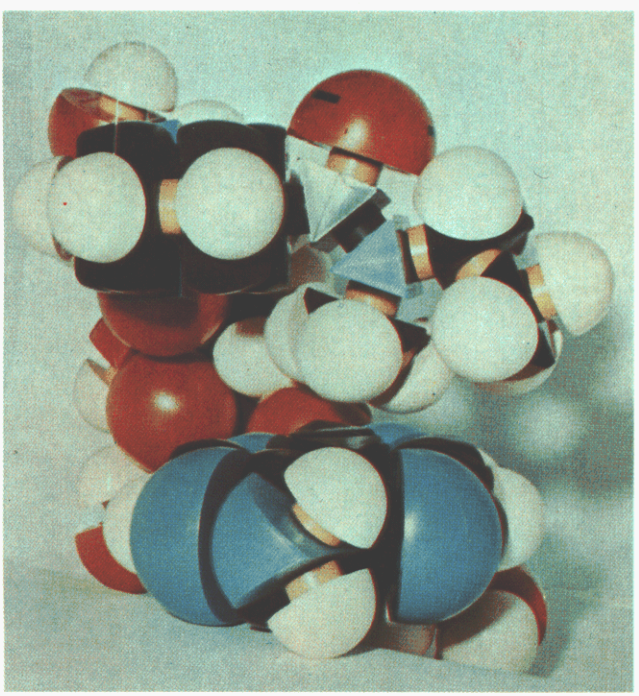
PAPER I, FIGURE 4 (opposite page, top right): The molecular model of TPNH in the *M* helical form. The following are a few important features of the *M* helix of TPNH. (1) The hydrogens of the $CONH_2$ of the dihydronicotinamide could hydrogen bond to any of the three oxygens of the 2′-phosphate. (2) The "B" proton is exposed to the adenine as well as to the environment of the 2′-phosphate. (3) The 2′-phosphate interferes with folding interactions between the dihydropyridine and the adenine.

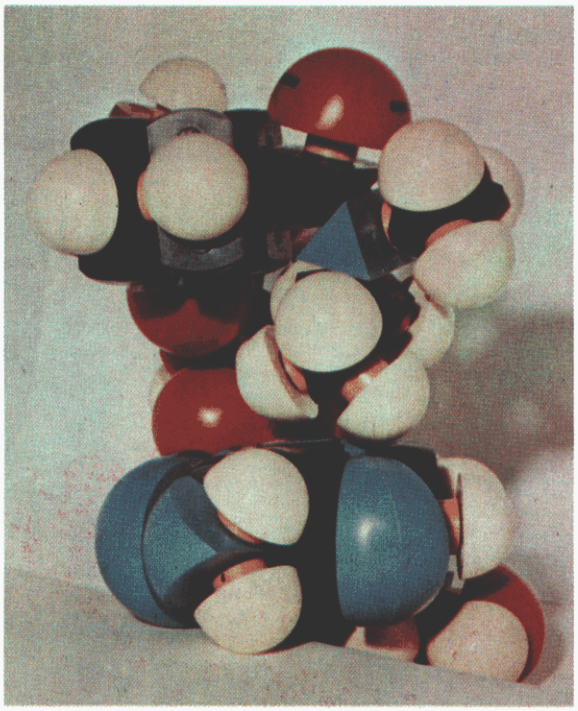
PAPER II, FIGURE 3 (opposite page, bottom left): Relative disposition between adenine and pyridine rings in *trans*-*N*-methyl-*N*-ethylnicotinamide-AD in the right-handedly folded conformation. This is the *P* helix of the dinucleotide analog. Note that the *trans* methyl group is projected toward the adenine ring. The double bond character of the C–N bond prevents free rotation around the C–N bond of the amide side chain. The steric hindrance between the alkyl substituents on amide nitrogens and the pyridine ring would preclude the free rotation of the amide side chain. The folding of the adenine over the pyridine ring would also prevent the free rotation between the C_3 of pyridine and the carbon atom of the amide side chain bearing the oxygen atom. Such preclusion of free rotation would "freeze" the amide side chain and would make possible the existence of a conformer of *trans*-*N*-methyl-*N*-ethylnicotinamide-DPN depicted in Figure 4.

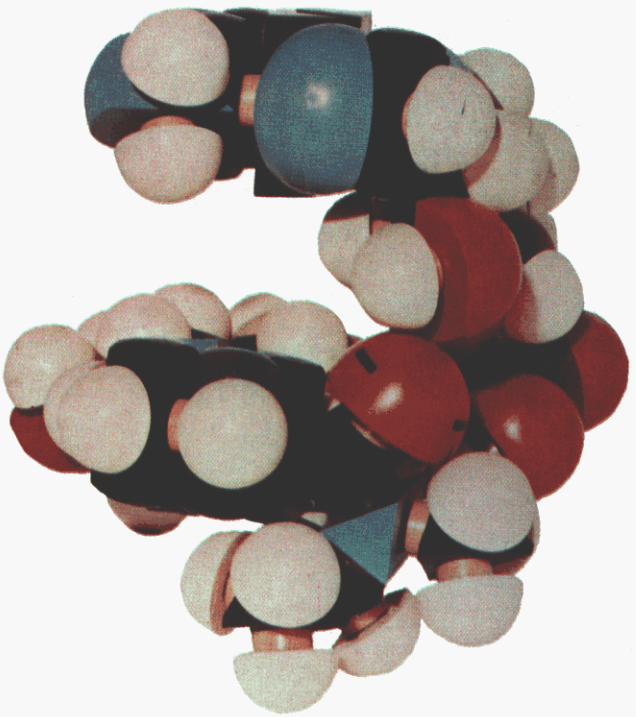
PAPER II, FIGURE 4 (opposite page, bottom right): Relative disposition between adenine and pyridine rings in *trans*-*N*-methyl-*N*-ethylnicotinamide-AD in the left-handedly folded conformation. This is the *M* helix of the dinucleotide analog. Note that the *trans* methyl group is projected away from the adenine ring.

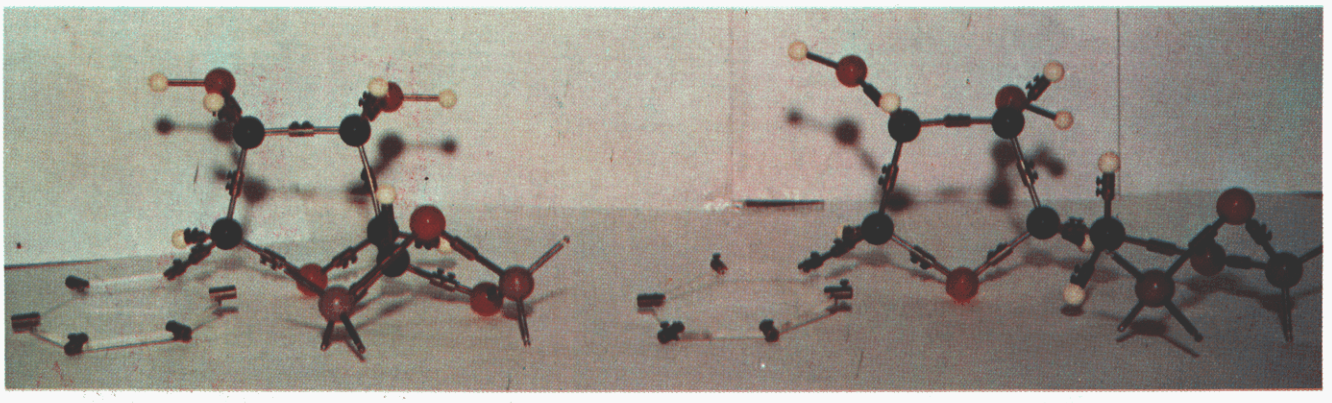

B
A

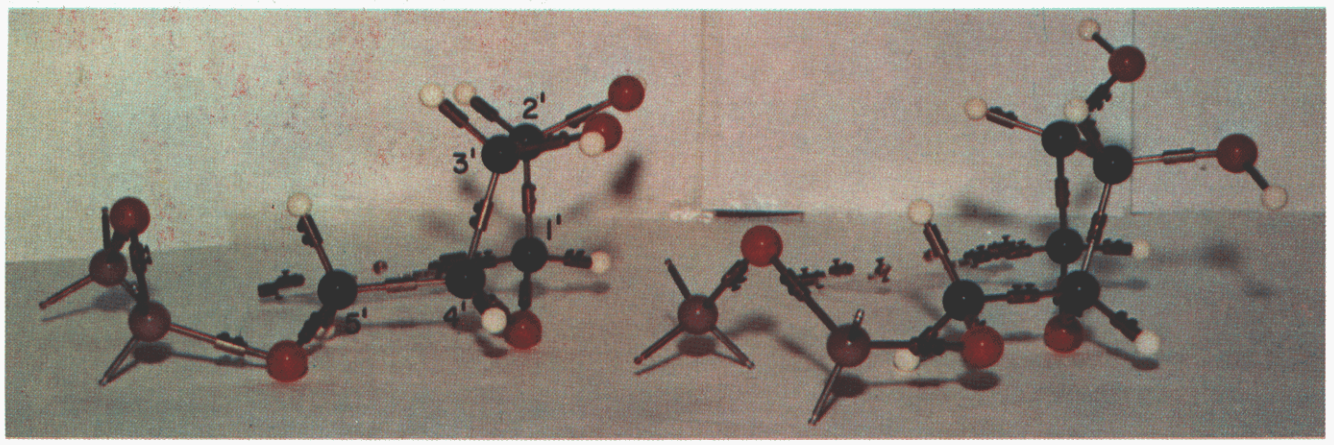

B
A

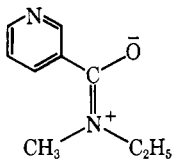




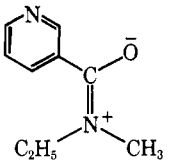





2'
3'
1'
5'
4'



I
trans (methyl)



II
cis (methyl)

acetyl methyl group of acetamides, in the preferred isomer. These results are accounted for on the basis of steric interactions between substituent groups.

Configurations of N-Methyl-N-ethylnicotinamide. According to the observations of LaPlanche and Rogers (1963, 1964) the preparation of *N*-methyl-*N*-ethylnicotinamide was expected to result in either completely the *trans* (methyl) isomer or predominantly the *trans* isomer. Figure 1 is the 60 MHz nuclear magnetic resonance spectrum of the product at 35°. The signals, designated A in Figure 1, are an overlapping pair of triplets from the CH_3 groups of the *cis* and *trans* C_2H_5. The signals B and C are the resonance from the *N*-methyl groups of the two geometric configurations. The group of signals D are a pair of quartets from the CH_2 of the *cis* and *trans* C_2H_5. These observations indicate that the *N*-methyl-*N*-ethylnicotinamide is a mixture of the *cis* and *trans* isomers. Figure 2 shows the spectrum of the product at 90°. At 90°, the pair of overlapping triplets (A in Figure 1) have coalesced to a triplet, the signals B and C have coalesced to a singlet, and the pair of quartets (D in Figure 1) have given place to a distinct quartet, indicating rapid rotation about the C–N bond at 90° on the nuclear magnetic resonance time scale. The *perfect symmetry* of the overlapping pair of triplets (A in Figure 1) indicates that *there is no preference toward the formation of the trans isomer and that both the cis and trans isomers are present in an equal amount in the product.* Note that the nonsymmetrical appearance of the resonance from *cis* and *trans* N–CH_3 groups (B and C in Figure 1) is due to the fact that one of the peaks contains the area from part of the overlapping pair of quartets.

Conforming with this pattern, the integrations of the 220 MHz nuclear magnetic resonance spectrum of the DPN analog (*vide infra*, Figure 7), *N*-methyl-*N*-ethylnicotinamide-AD, indicate that the population density of the two geometric configurations of the analog is the same. The equal population of the *cis* and *trans* isomers in the product is evidence against steric interactions between the substituent groups on the asymmetrically polarized C–N bond playing any significant role in controlling the formation of either *cis* or *trans* isomer. Steric interactions between the bulky ethyl and pyridyl groups in the *cis* isomer is avoided if the pyridine ring does not lie in the same plane as the amide group. Indeed the X-ray data of Wright and King (1954) show that in nicotinamide, the $CONH_2$ group is not in the same plane as the pyridine ring. Examination of the space filling model of *N*-methyl-*N*-ethylnicotinamide shows clearly that in both *trans* methyl and *cis* methyl configurations, there is considerable steric hindrance between the pyridine ring and the alkyl substituents on the amide nitrogen if the amide side chain is coplanar to the pyridine ring. When the plane of the amide side chain is perpendicular to the plane of the pyridine ring, there is no steric hindrance.

de Kowalewski (1960) and Hatton and Richards (1960, 1962) as well as LaPlanche and Rogers (1963) have reported that in the case of *N*-alkyl-substituted amides the methyl group *cis* in relation to the carbonyl oxygen appears at field higher than the same groups *trans* to the carbonyl oxygen. The chemical shifts of the *cis* and *trans* isomers in the present studies could be obtained by making use of this criterion. In view of the fact that in *N*-methyl-*N*-ethylnicotinamide we did not observe any preferential formation of either of the geometric configurations, and that the orientation of substituents on the asymmetrically polarized C–N bond could influence the chemical shifts, we would hesitate to assign chemical shifts based on this criterion in the present case without further experimentation.

PAPER II, FIGURE 5 (opposite page, top left): Relative disposition between adenine and pyridine rings in *cis*-*N*-methyl-*N*-ethylnicotinamide-AD in the right-handedly folded conformation. This is the *P* helix of the dinucleotide analog. Note that the ethyl group is projected toward the adenine ring.

PAPER II, FIGURE 6 (opposite page, top right): Relative disposition between adenine and pyridine rings in *cis*-N-methyl-N-ethylnicotinamide-AD in the left-handedly folded conformation. This is the *M* helix of the dinucleotide analog. Note that the ethyl group is projected away from the adenine ring.

PAPER III, FIGURE 5 (opposite page, middle): Relative disposition between the side chain at C_4' and the pyridine base in the oxidized coenzyme (left) and the reduced coenzyme (right). The photograph shows the portion of the coenzyme which contains the pyridine base, the ribose adjacent to it the accompanying diphosphate backbone. We have not indicated whether the orientation of the pyridine base with respect to the ribose is *anti* or *syn*. This has been done by removing the $CONH_2$ side chain from the pyridine ring. Our continuing research shows that the geometric relation between the pyridine ring and ribose is *syn* (Sarma and Kaplan, 1969b). It should also be realized that the diphosphate group could orient in different conformations. However, the photographs clearly show that a change in the conformation of the ribose dramatically affects the distance between the pyridine base and the side chain at C_4'. In the oxidized coenzyme, the conformation of the ribose adjacent to the pyridine ring is most likely C_3'-*exo*-C_4'-*endo* (see Figure 6). In the reduced coenzyme, the conformation of the ribose is most likely C_2'-*endo*-C_3'-*exo* (see Figure 6). Inspection of the photographs shows that the distance between the side chain at C_4' and the pyridine base is considerably less in the oxidized coenzyme (left) compared to that in the reduced coenzyme (right). It could also be seen that the dihedral angle between $C_1'H$ and $C_2'H$ (*trans*) has increased as one goes from the oxidized to the reduced coenzyme. The molecular models were designed by Dr. C. D. Jardetzky from X-ray data.

PAPER III, FIGURE 6 (opposite page, bottom): Photographs of the models in Figure 5 taken in an angle to illustrate the difference between the conformations of the riboses. The conformations of the ribose on the left (DPN^+) is C_3'-*exo*-C_4'-*endo*, *i.e.*, C_1', C_2' and O are in the same plane, C_3' is puckered away from C_5', C_4' is puckered toward C_5'. The conformation of the ribose on the right (DPNH) is C_2'-*endo*-C_3'-*exo*, *i.e.*, C_1', O, and C_4' are in the same plane, C_3' is puckered away from C_5', C_2' is puckered toward the C_5'.

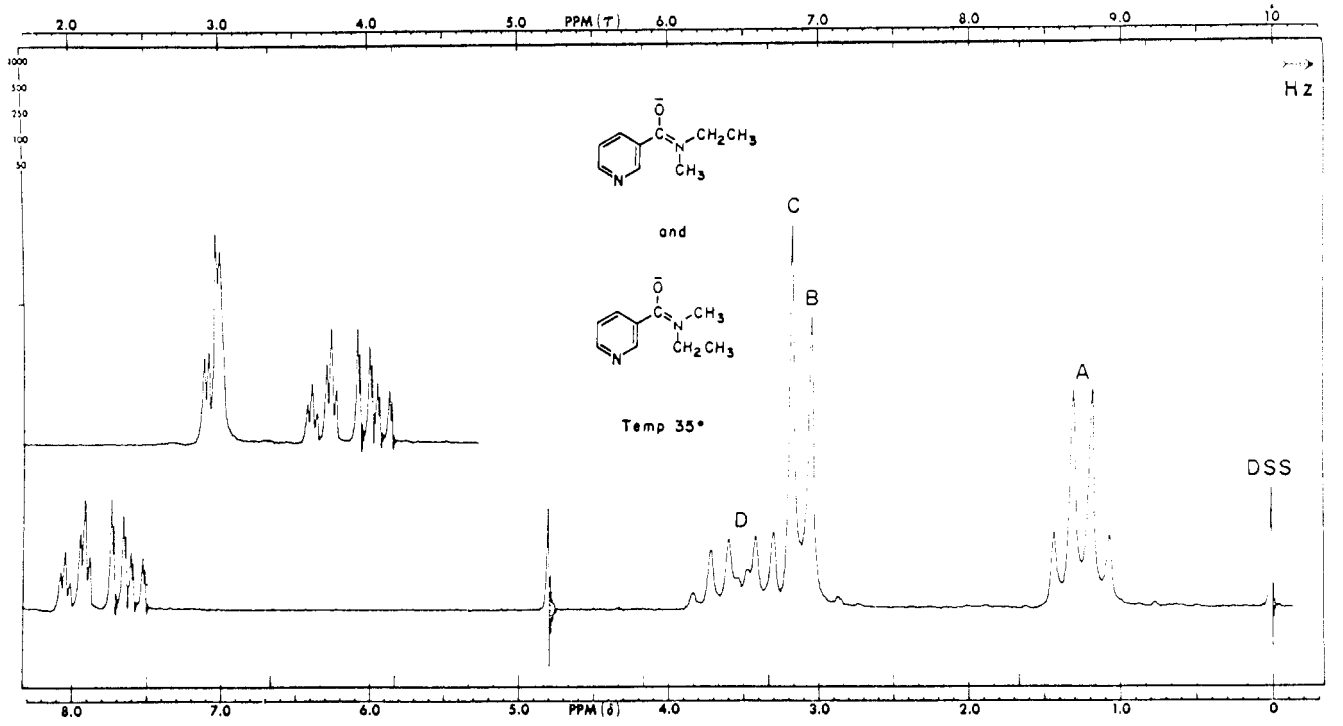


FIGURE 1: Nuclear magnetic resonance spectrum of *N*-methyl-*N*-ethylnicotinamide taken in a 60 MHz nuclear magnetic resonance system at 35° at a sweep width of 500 Hz, the sweep offset being 100 Hz. The internal standard was DSS. The spectrum clearly shows that the compound is a mixture of the *cis* and *trans* isomers, both being present to an equal extent.

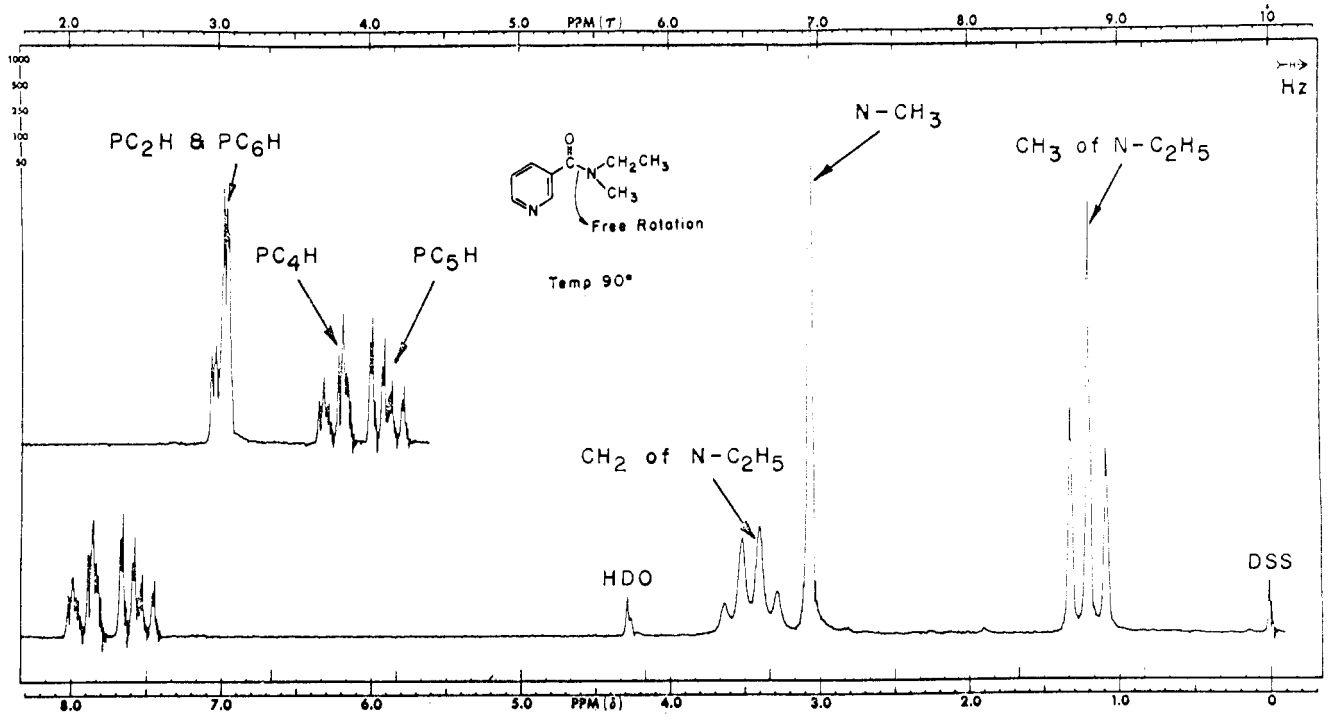


FIGURE 2: Nuclear magnetic resonance spectrum of *N*-methyl-*N*-ethylnicotinamide taken in a 60 MHz nuclear magnetic resonance system at 90° at a sweep width of 500 Hz, the sweep offset being 100 Hz. The internal standard was DSS. Compare this spectrum with that in Figure 1.

TABLE I: Chemical Shifts of Adenine C_8H, C_2H, and $C_1'H$ of ADPR, DPN, and *N*-Methyl-*N*-ethylnicotinamide-AD.[a]

Compound	Adenine Peaks (Hz)		
	C_8H	C_2H	C'H
ADPR	512.0	495.0	370.8
DPN	504.0	486.0	363.0
N-Methyl-*N*-ethylnicotinamide-AD	508	488	366

[a] Internal standard, DSS; all measurements were made on a 0.1 M solution in D_2O at 35° in a high resolution Varian Model A-60A spectrometer.

Configurations and Conformations of N-Methyl-N-ethylnicotinamide-AD. The *N*-methyl-*N*-ethylnicotinamide-AD provides a unique case in which the dinucleotide analog could, in principle, exist in four distinct spatial isomers. This is due to the fact that the dinucleotide itself exists as an equilibrium mixture of a right-handed and left-handed folded conformation (Cahn *et al.*, 1966; Sarma and Kaplan, 1970), in which the adenine and pyridine rings are stacked in parallel planes (Sarma *et al.*, 1968a,b, 1969), and the amide side chain is not on the same plane as the pyridine ring. The geometry of the folding is such that the adenine spends more time with that part of the pyridine ring which contains the amide side chain. Hence, each of the two possible geometric isomers could exist in two conformers, *i.e.*, the *trans* isomer can have its methyl group projected toward the adenine ring, as in a right-handed folded conformation (*P* helix, Figure 3), or away from the adenine ring, as in a left-handed folded conformation (*M* helix, Figure 4). The *cis* methyl isomer also can have its ethyl group projected toward the adenine ring (*P* helix, Figure 5) or away from the adenine ring (*M* helix, Figure 6). This would result in a total of four conformers from the two geometric isomers. At room temperature, the product could be a mixture of four conformers, and hence would have four environmentally nonequivalent methyl groups, and four nonequivalent ethyl groups.

This could be expected to result in a proton magnetic resonance spectrum, which would contain four individual signals from the four NCH_3 groups, four triplets and four quartets from the four NC_2H_5 groups. It is evident that many of these signals will overlap, because the differences in chemical shifts among some of the isomers are very small. Figure 7 is the nuclear magnetic resonance spectrum of *N*-methyl-*N*-ethylnicotinamide-AD taken in a 220 MHz nuclear magnetic resonance system. The group of signals designated B and C in Figure 1 has become four individual signals in Figure 7, indicating four nonequivalent NCH_3 groups. The four individual signals designated B_1, B_2, C_1, and C_2 in Figure 7 appear as a pair of doublets, but this cannot be due to any long range spin coupling, because coupling constants are independent of the resonance frequency of the oscillator and hence, if the arrangement was due to long range coupling such splitting would have appeared in the 60 MHz nuclear magnetic resonance spectrum, and this was not observed. In view of the above we have surmised that the four individual signals

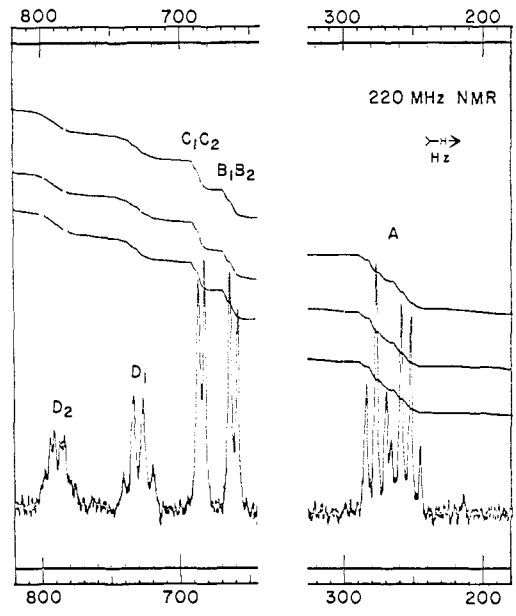


FIGURE 7: Nuclear magnetic resonance spectrum of *N*-methyl-*N*-ethylnicotinamide-AD taken in a Varian HRSC-IX super-conducting solenoid 220 MHz nuclear magnetic resonance system at a sweep width of 1000 Hz using DSS as an internal standard; sweep offset 100 Hz. Note that the integration of the signals was performed in the downfield direction because the large signals from HDO and the ribose protons appear just upfield from the *N*-alkyl protons.

B_1, B_2, C_1, and C_2 in Figure 7 originate from the four possible conformers discussed above. The signals B_1B_2 and C_1C_2 should belong to the two possible geometric configurations in which the NCH_3 could exist. According to the criterion for the assignment of NCH_3 groups in *N*-alkyl-substituted amides (LaPlanche and Rogers, 1963; de Kowalewski, 1960; Hatton and Richards, 1960, 1962), the upfield peak (B_1B_2) belongs to the NCH_3 which is *cis* to the carbonyl oxygen, and the downfield peak (C_1C_2) belongs to the NCH_3 which is *trans* to the carbonyl oxygen. However, we hesitate to assign chemical shifts based on this criterion for reasons already presented. But what is significant is the fact that the area of B_1B_2 is the same as that occupied by C_1C_2, indicating the equal population of the two geometric configurations of the DPN analog. This substantiates our view that steric interactions between the substituent groups on the asymmetrically polarized C–N bond do not play any role in favoring the formation of the *trans* (methyl) isomer. Furthermore, the various substituents on the asymmetrically polarized C–N bond could comfortably coexist if the amide side chain and the pyridine ring are noncoplanar (Sarma *et al.*, 1968b), as the X-ray data (Wright and King, 1954) and molecular models indicate.

The group of signals designated A in Figure 1 comes as a multiplet in Figure 7. If the product was a mixture of just two geometrical isomers (with no conformers) the CH_3 of NC_2H_5 groups would have appeared as a pair of triplets instead of as a multiplet. This multiplet is the result of overlapping resonance from the CH_3 of the four NC_2H_5 groups of the four different conformers. The group of signals designated D in Figure 1 come as D_1 and D_2 in Figure 7. These are the quartets from the CH_2 of NC_2H_5 of the two geometric configurations—the difference in chemical shifts of the CH_2 between the two

conformers of each geometric configurations is so small that they overlap onto each other's resonance. As expected, the area of the multiplet A is the same as the total area occupied by B_1, B_2, C_1, and C_2. Also, the total area of signals D_1 and D_2 is two-thirds of the multiplet A, or of the area covered by B_1, B_2, C_1, and C_2.

Intramolecular Interactions between the Base Pairs of N-Methyl-N-ethylnicotinamide-AD. Table I contains the chemical shift data on adenine C_8H, C_2H, and $C_1'H$ of ADPR, DPN, and *N*-methyl-*N*-ethylnicotinamide-AD. Comparison of the data shows that in the dinucleotide analog the adenine protons are shifted to lower fields compared with those in DPN. The downfield shift indicates that there is less intramolecular association between base pairs in the analog compared to DPN. They are, however, shifted upfield as compared to the same protons in ADPR. This indicates that the pyridine ring of the analog, despite heavy substitutions of the amide nitrogen, diamagnetically shields the adenine protons through ring current magnetic anisotropy, thereby indicating that in the analog the base pairs are juxtaposed to each other in parallel planes. At high temperature, the signals move downfield. The unfolding of the dinucleotide at elevated temperatures (Sarma *et al.*, 1968b; Jardetzky and Wade-Jardetzky, 1966) is partially responsible for the downfield shift. As a result of this unfolding, there is no longer any diamagnetic shielding by mutually induced ring currents from the adenine and pyridine π systems.

Acknowledgments

We thank Dr. W. D. Phillips of the E. I. du Pont de Nemours Co. for numerous discussions on the contents of this paper. We are also grateful to him for arranging to record the 220 MHz nuclear magnetic resonance spectrum and to Mr. F. V. Ferrari for technical assistance.

References

Cahn, R. S., Ingold, C., and Prelog, V. (1966), *Angew. Chem. Intern. Ed. Engl. 5*, 385.

Colowick, S. P., Kaplan, N. O., and Ciotti, M. M. (1951), *J. Biol. Chem. 191*, 447.

de Kowalewski, D. G. (1960), *Arkiv. Kemi 16*, 373.

Hatton, J. V., and Richards, R. E. (1960), *Mol. Phys. 3*, 253.

Hatton, J. V., and Richards, R. E. (1962), *Mol. Phys. 5*, 139.

Jardetzky, O., and Wade-Jardetzky, N. G. (1966), *J. Biol. Chem. 241*, 85.

Kaplan, N. O., and Ciotti, M. M. (1956), *J. Biol. Chem. 221*, 823.

LaPlanche, L. A., and Rogers, M. T. (1963), *J. Am. Chem. Soc. 85*, 3728.

LaPlanche, L. A., and Rogers, M. T. (1964), *J. Am. Chem. Soc. 86*, 337.

Phillips, W. D. (1955), *J. Chem. Phys. 23*, 1373.

Sadtler, The Sadtler Standard Infrared Spectra, Vol. 9, 8761; Vol. 19, 18773.

Sarma, R. H., Dannies, P., and Kaplan, N. O. (1968a), *Biochemistry 7*, 4359.

Sarma, R. H., and Kaplan, N. O. (1969a), *J. Biol. Chem. 244*, 771.

Sarma, R. H., and Kaplan, N. O. (1969b), *Biochem. Biophys. Res. Commun. 36*, 780.

Sarma, R. H., and Kaplan, N. O. (1970), *Biochemistry 9*, 539.

Sarma, R. H., Phillips, W. D., and Kaplan, N. O. (1969), *Biophys. J. 9*, FAM-F3.

Sarma, R. H., Ross, V., and Kaplan, N. O. (1968b), *Biochemistry 7*, 3052.

Wingfield, H. N., Harlan, W. R., and Hanmer, H. R. (1953), *J. Am. Chem. Soc. 75*, 4364.

Wright, W. B., and King, G. S. D. (1954), *Acta Cryst. 7*, 283.

Zatman, L. J., Kaplan, N. O., and Colowick, S. P. (1953), *J. Biol. Chem. 200*, 197.