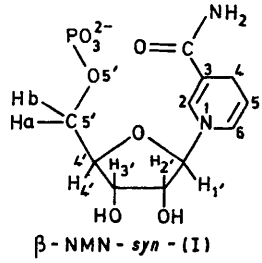
Complete Analysis of the Fast Fourier Transformed 100 MHz ^{1}H Nuclear Magnetic Resonance Spectra of Oxidized and Reduced Pyridine Mononucleotides

By RAMASWAMY H. SARMA* and RICHARD J. MYNOTT

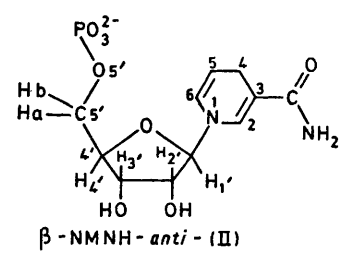
(*Department of Chemistry, State University of New York at Albany, Albany, New York* 12222)

Summary Complete analysis of the fast Fourier transformed ^{1}H n.m.r. spectra of oxidized and reduced pyridine mononucleotides show significant differences in their solution conformation.

THE ^{1}H 8K fast Fourier transformed (FFT) 100 MHz n.m.r. spectrum of oxidized pyridine mononucleotide, β-NMN (I), along with the LAOCN III computer simulated ribose region is shown in the Figure. The chemical shifts and coupling constants at two pH values are shown in the Table. The previous assignment[1] of the C (1′) H resonance of reduced coenzyme (*e.g.* β-NMNH; II) was found to be erroneous. The chemical shifts of the various protons of β-NMN (0·4—0·0025M) and β-NMNH (0·1—0·01M) showed a concentration dependence of less than 1—2 Hz indicating that these nucleotides do not undergo intermolecular stacking. The magnitude of the coupling constants



β-NMN-*syn*-(I)



β-NMNH-*anti*-(II)

J_{5a}–^{31}P and J_{5b}–^{31}P for β-NMN and β-NMNH (Table) show, according to Tsuboi *et al.*,[2] that the torsional isomer

TABLE

Chemical shifts and coupling constants of β-NMN and β-NMNH; 0·1 M-*solution* 100% D_2O. *Data from* 100 MHz *spectra.*

		Chemical shifts (δ/Hz)			Coupling constants (J/Hz)		
Nucleotides	Proton	pH 8·3	pH 4·0	Δδ pH 8·3→4·0	Nuclei Coupled	pH 8·3	pH 4·0
β-NMN	2-H	640·9	628·7	12·2	2–4	1·5	1·5
	4-H	580·9	581·2	0	2–5	N.d.	N.d.
	5-H	512·6	512·6	0	2–6	N.d.	N.d.
	6-H	615·6	610·9	4·7	4–5	8·2	8
	1′-H	300·4	304·4	−4·0	4–6	1·5	1·5
	2′-H	148·1	139·0	9·1	5–6	6·3	6·3
	3′-H	129·2	127·0	2·2	1′–2′	5·7	5·2
	4′-H	142·0	147·9	−5·9	2′–3′	5·0	5·0
	5a′-H	101·3	112·8	−11·4	3′–4′	2·1	2·6
	5b′-H	84·2	97·3	−13·1	4′–5a′	2·6	2·4
					4′–5b′	2·0	2·3
					5a′–^{31}P	4·2	4·4
					5b′–^{31}P	4·4	5·1
					5a′–5b′	12·1	12·1
β-NMNH[b]	2-H	397·5	396·1	1·4	2–6	1·7	1·6
	6-H	304·6	300·2	4·4	5–6	8·2	8·1
	5-H	184·3	183·7	0·6	4–6	1·6	1·8
	2 × 4-H	−11·7	−12·0	0	4–5	3·5	3·4
	1′-H	170·4	171·9	−1·5	2–5	Not resolved	
	2′-H	115·1	109·3	3·8	2–4		
	3′-H	106·4	104·7	1·7	1′–2′	6·8	6·8
	4′-H	90·0	92·2	−2·2	2′–3′	5·4	5·4
	5a′-H	67·5	79·5	−12·0	3′–4′	1·9[c]	1·8
	5b′-H[d]	67·5	79·5	−12·0	4′–5a′[d]	4·0	3·0
					4′–5b′		
					5a′–^{31}P[d]	4·8	5·1
					5b′–^{31}P		

[a] Chemical shifts are accurate to at least 0·2 Hz; coupling constants to 0·1 Hz. For β-NMNH (low pH), chemical shifts are accurate to 0·3 Hz, coupling constants to 0·2 Hz. [b] pH 8·1 and 5·0. [c] From 220 MHz n.m.r. spectrum. [d] No difference in chemical shifts observable between 5a′-H and 5b′-H even at 220 MHz.

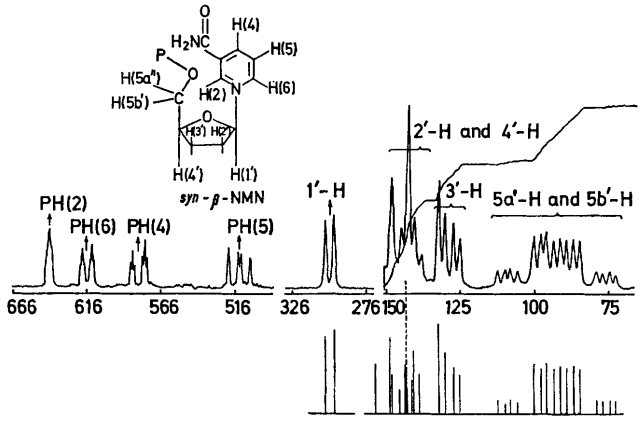


FIGURE. 8K *Fourier-transformed* ^{1}H *n.m.r. spectrum of* 0·1M-*solution of* β-NMN *in* '100%' D_2O, pH 8·3; *number of pulses* 500; *pulse width* 40 μs; *sampling frequency* 2000 Hz s^{-1}. P = *pyridine. Chemical shifts are expressed in* Hz *downfield from* $Me_4N^+Cl^-$; 100 MHz *n.m.r. system. The spectrum was recorded using a Digilab* FTS-NMR-3-system interfaced to a Varian HA-100D NMR *spectrometer, with a* ^{19}*F lock. The total memory of the data system was* 132K *permitting a maximum of* 32K *transforms* (*double precision* 32 *bits per word length*). *Bottom part shows the computer simulated spectrum of the ribose region of* β-NMN. *One of the transitions visible in the simulation was obscured by the* HDO *resonance.*

constrained to the O(5′)–C(5′) bond of β-NMN and β-NMNH is predominently *gauche–gauche*. Calculations[3] of the torsional isomer population around the C(5′)–C(4′) bond from the $J_{4'-5a'}$ and $J_{4'-5b'}$ values show that over 90% of β-NMN molecules exist in the *gauche–gauche* form constrained to the C(5′)–C(4′) bond whereas in the case of β-NMNH at pH 8·0 only 50% of the molecules exhibit *gauche–gauche* conformation. A qualitative application of Karplus equation to the $J_{1'-2'}$, $J_{2'-3'}$, and $J_{3'-4'}$ (Table) values of β-NMN and β-NMNH indicate that the ribose fragment of both compounds exists as an equilibrium mixture of 3′-*endo*- and 2′-*endo*-structures, the 2′-*endo*-population being larger for β-NMNH. The pH profiles for β-NMN (pH range 2—9, increments of 0·5 pH units) reveal that the protonation of the phosphate group (the pK of the phosphate group was determined by ^{31}P n.m.r. measurements by the method in Sarma and Mynott[4]) perturbs both pyridine 2-H and 6-H resonance, the perturbation of 2-H being about three times larger than that of 6-H. Thus the data clearly indicate that β-NMN prefers to exist in the *syn*-form (I), as has been suggested by Sarma and Kaplan[5] from crude pH studies. Qualitative calculations show that about 78% of the molecules exist in the *syn*- and 22% in the *anti*-conformation. No extensive ^{31}P and ^{1}H n.m.r. studies as a function of pH is possible for β-NMNH since this nucleotide decomposes at pH values below 6·0. However, the FFT mode of operation enabled us to obtain high quality spectra very quickly at pH 5·0. The data for β-NMNH (Table) indicate that this nucleotide preferentially exists in the *anti* conformation (II), contrary to the predictions of Sarma and Kaplan[5] from

ring current considerations. The data in the Table make possible a complete analysis by ^{13}C FFT n.m.r. methods of the ^{13}C n.m.r. spectra of β-NMN and β-NMNH by proton off-resonance spin-decoupling.[6]

This research was supported by grants from the National Science Foundation and the National Institutes of Health.

(*Received, 22nd May* 1972; *Com.* 870.)

[1] W. L. Meyer, H. R. Mahler, and R. H. Baker, jun., *Biochem. Biophys. Acta*, 1962, **64**, 353; O. Jardetzky and N. G. Wade-Jardetzky, *J. Biol. Chem.*, 1966, **241**, 85; R. H. Sarma and N. O. Kaplan, *ibid.*, 1969, **244**, 771; W. A. Catteral, D. P. Hollis and C. F. Walter, *Biochemistry*, 1969, **8**, 4032; R. H. Sarma and N. O. Kaplan, *ibid.*, 1970, **9**, 539.
[2] M. Tsuboi, S. Takahashi, Y. Kyogoku, H. Hayatsu, T. Ukita, and M. Kainosho, *Science*, 1969, **166**, 1504.
[3] F. E. Hruska, A. A. Grey, and I. C. P. Smith, *J. Amer. Chem. Soc.*, 1970, **92**, 4088.
[4] R. H. Sarma and R. J. Mynott, *Org. Magnetic Resonance*, in the press.
[5] R. H. Sarma and N. O. Kaplan, *Biochem. Biophys. Res. Comm.*, 1969, **36**, 780.
[6] B. Birdsall, N. J. M. Birdsal, and J. Feeney, *J.C.S. Chem. Comm.*, 1972, 316.