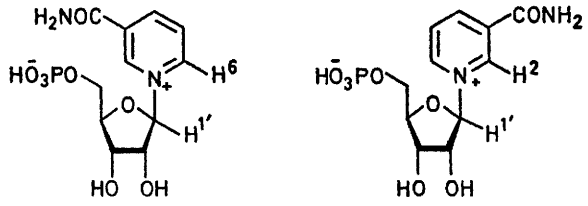
# Solution Conformation of Nicotinamide Mononucleotide: a Nuclear Overhauser Effect Investigation

By WILLIAM EGAN and STURE FORSÉN
(*Division of Physical Chemistry, The Lund Institute of Technology, Chemical Center*, S-220 07 *Lund* 7, *Sweden*)
and JOHN JACOBUS*
(*Department of Chemistry, Clemson University, Clemson, South Carolina* 29631)

*Summary* Evidence, based on the nuclear Overhauser effect, is presented which supports a model for nicotinamide mononucleotide (NMN) in which the *syn* and *anti* conformers about the nicotinamide N-1 and ribose C-1 bond are equally, or nearly equally, populated.

EXTENSIVE studies of the pyridine nucleotides have been carried out[1] to elucidate their conformation in solution. Nicotinamide mononucleotide (NMN) has been used as a




*syn* *anti*

model system. Regarding rotation about the nicotinamide–ribose linkage, it has been concluded[2] that NMN exists in the *syn* conformation,† a conclusion subsequently applied in defining the geometry of nicotinamide adenine dinucleotide,[2] for which the *syn* disposition of the nicotinamide ring is similarly claimed. We present here evidence, based on nuclear Overhauser effect[3] studies, that this previous assignment of a *syn* geometry to NMN is incorrect.

Most simply stated, the exclusive or near exclusive population of the *syn* conformer predicts (a) an enhancement at 1′-H upon irradiation of 6-H that is large relative to that obtained upon irradiation of 2-H and (b) a negligible enhancement of 2-H upon irradiation of 1′-H. The experimental findings, a large enhancement‡ at 2-H upon irradiation of 1′-H (*ca.* 0·24) and equal enhancements (*ca.* 0·15) at 1′-H upon irradiation of either 2-H or 6′-H, render claims to an exclusive, or even highly preferential, population of the *syn* geometry untenable.

The data do, however, appear to be most simply accounted for by a model in which the *syn* and *anti* conformers are equally or nearly equally populated [based on the equality of $f_{1'}$ (6) and $f_{1'}$ (2)] and interconverting rapidly on the nuclear Overhauser effect time scale, $1/T_1$, where $T_1$ is the longitudinal (spin-lattice) relaxation time.§

Studies to determine the ribose-ring conformation and to apply quantitatively our Overhauser effect results on NMN are now in progress.¶

(*Received*, 7*th November* 1972; *Com.* 1870.)

† The *syn* conformation is defined as that one in which the dihedral angle, 6-H–N–C-1–1′-H, is 0°.

‡ In keeping with current terminology,[3] the nuclear Overhauser effects are given as the fractional enhancement of the resonance of spin *d* when the resonance of the spin(s) *s* are saturated, $f_d(s)$ = (area of *d* when *s* saturated − equilibrium area of *d*)/equilibrium area of *d*.

§ If the *syn* and *anti* conformers were not under conditions of rapid exchange on this time scale, then separate resonances for the various conformers would be expected. This would normally be the case for the Region I discussed in ref. 3b.

¶ The complete NOE effect enhancements between all ribose protons and 2-H and 6-H, in both directions, have been performed and will be published in the complete account of this work.

[1] For a recent review of $^1$H n.m.r. conformational studies on the pyridine dinucleotides, see A. F. Casy, 'PMR Spectroscopy in Medicinal and Biological Chemistry,' Academic Press, New York, 1971, p. 361. These studies have recently come under criticism (J. Jacobus, *Biochemistry*, 1971, **10**, 161). Studies based on fluorescence measurements have also been criticized (O. Jardetsky and N. G. Wade-Jardetsky, *J. Biol. Chem.*, 1966, **241**, 85).

[2] R. H. Sarma and N. O. Kaplan, *Biochem. Biophys. Res. Comm.*, 1969, **36**, 1969; R. H. Sarma and R. J. Mynott, *J.C.S. Chem. Comm.*, 1972, 975, 977.

[3] (a) For a full discussion of the origin and application of the nuclear Overhauser effect see J. H. Noggle and R. E. Schirmer, 'The Nuclear Overhauser Effect: Chemical Applications,' Academic Press, New York, 1971; (b) *Cf.* R. E. Schirmer, J. P. Davis, J. H. Noggle, and P. A. Hart, *J. Amer. Chem. Soc.*, 1972, **94**, 2561; (c) P. A. Hart and J. P. Davis, *ibid.*, p. 2572.