The Combined Use of ^{1}H and ^{31}P Fast Fourier Transform Nuclear Magnetic Resonance Spectroscopy and Line Shape Computer Simulations to Determine the Torsional Diastereomers Constrained to the C(5′)–O(5′) Bonds of Pyridine Dinucleotides

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Summary A method to determine the torsional diastereomers† constrained to the C(5′)–O(5′) bonds of dinucleotides in general, with special reference to pyridine dinucleotides, is described.

The three staggered conformations, as applied to the C(5′)–O(5′) bond of a mononucleotide are shown in (I), *gauche-gauche* (*gg*), (II), *gauche-trans* (*gt*), and (III), *trans-gauche* (*tg*). A dinucleotide such as β-TPN (IV) or β-DPN (V) can exist in nine different conformations with respect to torsion around each of its C(5′)–O(5′) bonds at the *a*denine (A) and *n*icotinamide (N) ends. They are (1) AC(5′)–O(5′) *gg*-NC(5′)–O(5′) *gg*, (2) AC(5′)–O(5′) *gg*-NC(5′)–O(5′) *gt*, (3) AC(5′)–O(5′) *gg*-NC(5′)–O(5′) *tg*, (4) AC(5′)–O(5′) *gt*-NC(5′)–O(5′) *gg*, (5) AC(5′)–O(5′) *tg*-NC(5′)–O(5′) *gg*, (6) AC(5′)–O5′) *gt*-NC(5′)–O(5′) *gt*, (7) AC(5′)–O(5′) *tg*-NC(5′)–O(5′) *gt*, (8) AC(5′)–O(5′) *gt*-NC(5′)–O(5′) *tg*, and (9) AC(5′)–O(5′) *tg*-NC(5′)–O(5′) *tg*. One can distinguish the above possibilities by using the following coupling constants: $J_{P(A)5'(A)}$, $J_{P(A)5''(A)}$, $J_{P(N)5'(N)}$, and $J_{P(N)5''(N)}$; P(A) and P(N) stand for the ^{31}P attached to the adenine and nicotinamide ends, 5′ and 5″ the geminal hydrogens at C(5′) (IV). These coupling constants cannot be obtained directly from ^{1}H or ^{31}P spectra because of considerable overlap of transitions. We now report a solution to this problem.

(1) ^{31}P spectra of β-TPN and β-DPN were recorded in the Fourier mode using a sufficiently large number of pulses (15030) for a clean noise-free envelope (Figure 1A). This spectrum had no fine features and appeared as a strongly coupled AB quartet with a broad central peak flanked by broad satellites. (2) From the ^{31}P–{^{1}H} spectra of β-TPN and β-DPN, $^2J_{POP}$ and the chemical shifts of P(A) and P(N)

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† The term diastereomer is used to describe all stereoisomers which are not enantiomers (K. Mislow, Introduction to Stereochemistry, W. A. Benjamin, Inc., New York, 1966).

were obtained.[1] (3) The ^{1}H spectrum of β-NMN (β-nicotinamide mononucleotide)[2] gave values for $J_{\mathrm{P(N)5'(N)}}$, $J_{\mathrm{P(N)5''(N)}}$, $J_{\mathrm{4'(N)5'(N)}}$, $J_{\mathrm{4'(N)5''(N)}}$, $J_{\mathrm{5'(N)5''(N)}}$, and the chemical shifts of 4′, 5′, and 5″ protons for the mononucleotide. (4) ^{1}H and ^{1}H–{^{31}P} spectra of 5′AMP (adenosine 5′-monophosphate) and 5′ADP (adenosine 5′-diphosphate) were recorded using a 16K transform until a noise-free envelope was obtained. Complete analysis of the ribose region of 5′AMP and 5′ADP was carried out using line shape simulation program LAME. The analysis yielded the following values: (i) 5′AMP, 0·1M, pH 8·2; $J_{1'2'}$ 5·7, $J_{2'3'}$ 5·1, $J_{3'4'}$ 3·7, $J_{4'5'}$ 3·2, $J_{4'5''}$ 3·6, $J_{4'0}$ ±1·4, $J_{5'\mathrm{P}}$ ±4·5, $J_{5''\mathrm{P}}$ ±4·5 and $J_{5'5''}$ 10 Hz. (ii) 5′ADP, 0·1M, pH 5·2. $J_{1'2'}$ 5·6, $J_{2'3'}$ 5·1, $J_{3'4'}$ 3·8, $J_{4'5'}$ 3·0, $J_{4'\mathrm{P}\alpha}$ ±1·2, $J_{4'\mathrm{P}\beta}$ ±1·0, $J_{5'\mathrm{P}\alpha}$ ±5·2, $J_{5''\mathrm{P}\alpha}$ ±5·2, $J_{5'\mathrm{P}\beta}$ *ca.* 1, $J_{5''\mathrm{P}\beta}$ *ca.* 1 and $J_{5'5''}$ −10 Hz. The chemical shifts of 4′, 5′ and 5″ protons of 5′ADP were 122·1, 107·1 and 106·6 Hz downfield from Me$_4$NCl. The coupling constant data indicate that in both 5′AMP and 5′ADP the torsional diastereomers constrained to the C(5′)–O(5′)[2,3] and C(4′)–C(5′)[4] bonds are predominantly *gauche–gauche*‡ and that ribose exists as a mixture of C(3′)*endo* and C(2′)*endo* conformations[4] undergoing interconversion *via* pseudorotation. (5) Now the relevant data obtained above for the component mononucleotides and the coupling constants $^3J_{\mathrm{POCH(trans)}} = 25 \pm 3$ Hz, $^3J_{\mathrm{POCH(gauche)}}$ 3 ± 2 Hz,[3] could be used as initial estimates as the values for the dinucleotide and the ^{31}P spectra for the nine different conformations listed earlier can be simulated. Unfortunately an absolute spectral assignment of the 5′,5″ hydrogens cannot be made at present and thus a distinction between *gt* and *tg* rotamers should not be made. Hence the nine conformations can be reduced to the following four representative cases: (a) AC(5′)–O(5′)*gg*-NC(5′)–O(5′)*gg*, (b) AC(5′)–O(5′)*gg*-NC(5′)–O(5′)*gt*, (c) AC(5′)–O(5′)*gt*-NC(5′)–O(5′)*gg*, and (d) AC(5′)–O(5′)*gt*–NC(5′)–O(5′)*gt*. The spectra for the above four cases were simulated using parameters (see caption to Figure 2) which are approximated from the mononucleotides. These preliminary calculations shows that only case (a) could possibly give the observed ^{31}P n.m.r. spectrum (compare Figure 1A with 2a). A series of calculations were undertaken to refine the spectrum in Figure 2a and to reproduce the observed one (Figure 1A). The final result is shown in Figure 1B along with the parameters. The fact that the sum $J_{5'\mathrm{P}} + J_{5''\mathrm{P}}$ is small in itself suggests that the individual couplings must be small and therefore the predominant conformation of the pyridine dinucleotide is all *gauche* with respect to the C(5′)-O(5′) bond. A more precise determination of the extent to which this conformation is preferred cannot be achieved unless $\nu\delta_{5'5''}$ in the proton spectrum can be increased.

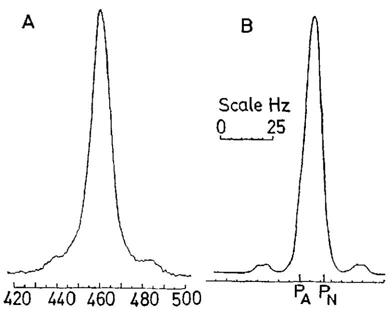


FIGURE 1.(A) *Fourier transformed* ^{31}P n.m.r. (40·48 MHz) *spectrum* (*only the pyrophosphate region is shown*) *of a* 0·25M *aqueous solution of* β-TPN *in* 3 × 10^{-3}M EDTA (15030 pulses). *Scales are given in* Hz *upfield from external* 85% H$_3$PO$_4$. (B) *Simulated spectrum for case* (*A*) *after refinement of parameters. Final parameters:* Chemical shifts P(A) = 454·3, P(N) = 465·8 (*both in* Hz *upfield from* 85% H$_3$PO$_4$). 4′ (A) = 120·4, 5′(A) = 106·8, 5″(A) = 106·4, 4′(N) = 120·4, 5′(N) = 101·3, 5″(N) = 84·2 (*all in* Hz *downfield from internal* Me$_4$NCl. Coupling constants, in Hz, between nuclei indicated: P(A)P(N) = 20·3, P(A)4′(N) = 0·8, P(A)4′(A) = 1·4, P(A)5′(A) = 4·7, P(N)4′(N) = 1·4, P(N)4′(A) = 0·8, P(N)5′(N) = 4·3, P(N)5″(N) = 4·5, 4′(N)5′(N) = 2·6, 4′(N)5″(N) = 2·0, 4′(A)5′(A) = 2·6, 4′(A)5″(A) = 2·0, 5′(A)5″(A) = −12·1, 5′(N)5″(N) = −12·1. *The linewidths in the simulated and observed spectra were* 9 *and* 10·3 Hz *respectively.*

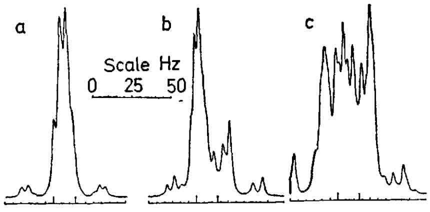
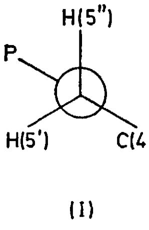
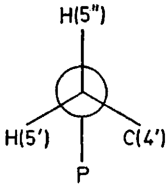


FIGURE 2. *Initially simulated* ^{31}P *spectrum of representative cases* (*a*), (*b*), *and* (*c*). *The parameters used are:* Chemical shifts: P(A) = 453·2, P(N) = 466·8 (*both in* Hz *upfield from external* 85% H$_3$PO$_4$). 5′(A) = 106·6, 5″(a) = 106·6, 5′(N) = 101·3, 5″(N) = 84·2 (*all in* Hz *from internal* Me$_4$NCl). Coupling constants, in Hz, between nuclei indicated: P(A)5′(A) = P(A)5″(A) = 5·0 (*gauche*), P(N)5′(N) = 4·2 (*gauche*), P(N)5″(N) = 4·4 (*gauche*), 5′(A)5″(A) = −10, P(A)P(N) = 20·3, 5′(N)5″(N) = −12·1. P(N)5″(N) = P(A)5″(A) = P(N)5′(N) = P(A)5′(A) = ±25·0 (*trans.*). *All other coupling constants were assumed to be zero.*



(I)



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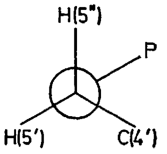
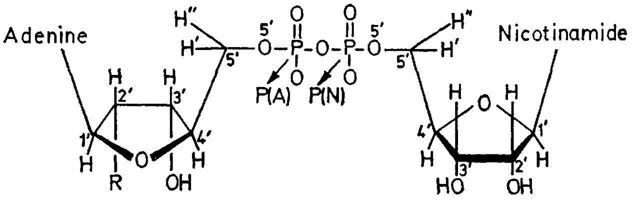


(III)



(IV) = (β-TPN) R = OPO$_3^{-2}$

(V) = (β-DPN) R = OH

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‡ In the case of 5′AMP and 5′ADP we have discovered a four-bond P–H coupling ($^4J_{\mathrm{P-4'}}$) of 1·4 Hz and it is our belief that this coupling is suggestive of the 'zig-zag' spatial relationship between the ^{31}P and the 4′ hydrogen encountered when the molecule is oriented *gauche-gauche* about both C(4′)–C(5′) and C(5′)–O(5′).

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[2] R. H. Sarma and R. J. Mynott, *J.C.S. Chem. Comm.*, 1972, 975.
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