Tetrahedron Letters No. 7, pp 683 - 686, 1978. Pergamon Press. Printed in Great Britain.

STRUCTURE OF DIMERS OF NAD$^+$ AND OF N-BENZYL NICOTINAMIDE CHLORIDE.

Jean-Francois BIELLMANN and Claude LAPINTE

Laboratoire associé au CNRS, Institut de Chimie,
1 rue Blaise Pascal, 67008 STRASBOURG (France).

(Received in UK 20 December 1977; accepted for publication 29 December 1977)

Dimers of NAD$^+$ (nicotinamide adenine dinucleotide) and of model compounds have been obtained either by reduction with metals (1) or by electrochemical methods (2, 3, 4). The structures of these dimers which most likely have a 2-2', 4-4' or 6-6' bond between the dihydropyridine rings (5, 6, 7) have never been quite well established. Part of the difficulties may arise from the fact that the absorption spectrum of the dimeric species may be different from the spectrum of the monomeric species due to different symmetry (8). Due to the fact that one of the very strong inhibitors of lactate dehydrogenase arising on storage of NADH, is a dimer (9), it was necessary to establish definitely the structure of the dimers obtained by reduction of NAD$^+$ and of N-benzyl nicotinamide chloride. The dimer of this latter was prepared by reduction with zinc-copper whereas two methods were used to prepare the dimer of NAD$^+$: zinc-copper reduction and electrochemical reduction. The ^{1}H-NMR data measured at 250 MHz and ^{13}C-NMR data at 62.8 MHz are summarised in Table 1 and 2. Only the chemical shifts (ppm, internal standard sodium 3-trimethylsilylpropionate-d_4) of the dihydropyridine system are reported.

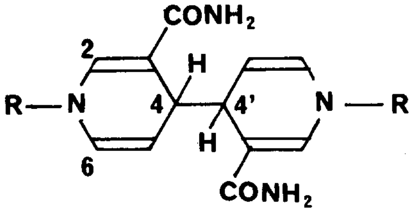
The chemical shift of the proton at high field (underlined in Table 1) in the spectrum of dimers, located between those of dihydro-1,4 and 1,6 pyridine, supports that both dimers are 4-4'. The introduction of a carbon atom as a substituent at C_4 in the dimer induces a shift to lower field compared to the proton at C-4 in the monomer.

In order to confirm unequivocally that the dimers possess a 4-4' link (I), we prepared the dimers from N-benzyl nicotinamide chloride 50 % enriched with deuterium at C-4 (10) as well as from (^{2}H-C4)NAD$^+$ (11). The signal at high field assigned to the proton at C-4 in the dimers was affected by this substitution : the signal at 3,27 ppm for the model dimer had its area reduced to half its normal value and the complex pattern 3,24-2,96 in the NAD$^+$ dimer disappeared completely.

Table 1 : ^{1}H-NMR data

Chemical shifts expressed in ppm from sodium 3-trimethylsilylpropionate-d_4 used as an internal standard

	H-C2	H-C4	H-C5	H-C6	Solvents
N-benzyl 1,4 dihydronicotinamide	7,02	3,00	4,32	5,90	DMSO-d_6
NADH	6,94	2,73	4,52	5,99	D_2O
N-benzyl 1,6 dihydronicotinamide	7,02	6,17	4,70	3,93	DMSO-d_6
model dimer (Zn-Cu)	7,24	3,27	4,33	6,10	DMSO-d_6
dimer of NAD$^+$ (Zn-Cu and electrochemical)	7,02	3,24-2,96	4,38	6,10	D_2O



(I)

Table 2 : ^{13}C-NMR

Chemical shifts expressed in ppm from sodium 3-trimethylsilylpropionate-d_4 used as an internal standard

	C_2	C_3	C_4	C_5	C_6	-CONH$_2$	Solvents
N-benzyl 1,4 dihydronicotinamide	141	99	23	104	131	173	DMSO-d_6
NADH	141	109	26	108	127	173	D_2O
N-benzyl 1,6 dihydronicotinamide	148	99	122	111	52	171	DMSO-d_6
model dimer (Zn-Cu)	139	102	39	103	131	170	DMSO-d_6
dimer of NAD$^+$ (Zn-Cu and electrochemical)	138 and 132	103	40	108	127 and 131	174	D_2O

The ^{13}C NMR spectra support strongly the conclusions derived from ^{1}H NMR spectra. The high field signal (underlined in Table 2) attributed to the sp^{3} carbon is shifted to lower field by the introduction of a carbon as substituent. If the position C-6 were involved in the linkage between the 2 pyridine rings, the signal corresponding to this carbon atom would be found in the region 65 to 70 ppm.

The dimer of N-benzylnicotinamide seems to be present as one single stereoisomer meso or dl, as indicated by the sharpness of NMR signals. No trace of the other diastereoisomer could be detected. In contrast diastereoisomers of NAD^{+} were detected in the ^{1}H NMR spectrum where a complex pattern appeared at high field. Moreover in the ^{13}C NMR spectrum of this dimer, two signals may be attributed to C_2 and to C_6. The ratio of the different diastereoisomers varied slightly according to the preparation method. However all the species detected were 4-4' dimers.

Due to the possibility that thermal interconversion of the dimers linked at different positions of the dihydropyridine rings (4-6' ⇄ 4-4' ⇄ 6-6' etc.) may occur through homolysis of the bond between the two rings (12, 13, 14) care was taken to avoid any heating during the isolation. Isomerisation of the 4-4' dimers was also attempted by heating them to 60°, but in none of these experiments any dimer involving links at C-2 and C-6 could be detected.

Recently the dimers of N-methyl substituted pyridinium salts were found to possess a 4-4' or a 2-4' link between the dihydropyridine ways (13, 14). The presence of a bulky substituent at the nitrogen for the compounds studied in the present paper, appears to reduce the reactivity of both the C-2 and C-6 centers and in these cases the reaction product is a 4-4' dimer.

We thank Boehringer Mannheim, Germany, for financial support and Dr. G. Weimann and Mr. E. Haid, BM - Biochemica Werk Tutzing, for helpful discussions and continued interest in this work and Mr. F. Hemmert and Mr. R. Graff for the NMR spectra.

REFERENCES

(1) U. EISNER and J. KUTHAN, Chem. Rev., 72, 1 (1972).

(2) W. BURNETT and A.L. UNDERWOOD, Biochem., 7, 3328 (1968).

(3) C.O. SCHMAKEL, K.S.V. SANTHANAM and P.J. ELVING, J. Amer. Chem. Soc., 97, 3083 (1975).

(4) D. THEVENOT and R. BUVET, J. Electroanal. Chem., 39, 429 (1972).

(5) O. MUNN and J. DIEDEREICHSEN, Justus Liebigs A. Chem., 538, 195 (1939).

(6) H. DIEKMANN, G. EINGLETT and K. WALLENFELS, Tetrahedron, 20, 281 (1969).

(7) K. WALLENFELS and M. GELLRICH, Chem. Ber., 92, 1406 (1959).

(8) H.H. JAFFE and M. ORCHIN, Theory and Applications of Ultraviolet Spectroscopy, John Wiley and Sons, New York, 1962, p. 35-146.

(9) J.F. BIELLMANN and C. LAPINTE, to be published.

(10) J.F. BIELLMANN and H.J. CALLOT, Bull. Soc. Chim. France, 1154 (1968).

(11) A. SAN PIETRO, J. Biol. Chem., 217, 579 (1955) and A.P. ZENS, P.T. FOGLE, T.A. BRYSON, R.B. DUNLAP, R.R. FISHER and P.D. ELLIS, J. Amer. Chem. Soc., 98, 3760 (1976).

(12) P. ATLANI, J.F. BIELLMANN, R. BRIERE, J. LEMAIRE and A. RASSAT, Tetrahedron, 28, 2827 (1972).
P. ATLANI, J.F. BIELLMANN, R. BRIERE and A. RASSAT, Tetrahedron, 28, 5805 (1972).
P. ATLANI and J.F. BIELLMANN, C.R. Acad. Sci. Paris, 271, 688 (1970).

(13) I. CARELLI, M.E. CARDINALI, A. CASINI and A. ARNONE, J. Org. Chem., 41, 3967 (1976).

(14) F.T. Mc NAMARA, J.W. NIEFT, J.F. AMBROSE and E.S. HYUSER, J. Org. Chem., 42, 988 (1977).