Tetrahedron, 1958, Vol. 3, pp. 73–77. Pergamon Press Ltd., London

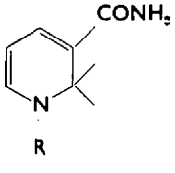
N-METHYL DIHYDRONICOTINAMIDE

R. F. HUTTON* and F. H. WESTHEIMER
Mallinckrodt Laboratories, Harvard University, Cambridge, Mass.

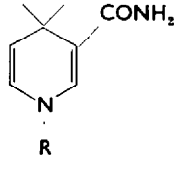
(*Received* 8 *November* 1957)

Abstract—The nuclear magnetic resonance spectra of N-methyldihydronicotinamide and of its 2-deutero and 6-deutero derivatives, amply confirm the 1,4-dihydropyridine structures assigned to these compounds.

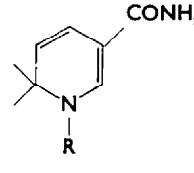
IN 1936 Karrer and Warburg[1] made the important discovery that diphosphopyridine nucleotide (DPN^+) and triphosphopyridine nucleotide are reduced, during enzymatic reactions, in the pyridine ring. They also found that the same reduction can be accomplished with alkaline dithionite. At the time, they suggested that the reduction takes place in the 2-position of the pyridine ring, to produce the structure I, where R represents the adenine—ribose—pyrophosphate—ribose residue of DPN^+. Similar structures were suggested for models of reduced DPN, such as N-methyldihydronicotinamide,[2,3] which can be prepared by alkaline dithionite reduction of the corresponding quaternary salts of nicotinamide. However, following the discovery of direct hydrogen transfer (or deuterium transfer) in the enzymatic reactions[4] of DPN^+, Pullman, San Pietro and Colowick established that the reduction takes place in the 4-position[5] of the pyridine ring; their assignment was independently confirmed by Mauzerall and Westheimer,[6] who used a quite different method of establishing the structure. This latter method depended upon synthesizing 2-deutero-, 4-deutero-, and 6-deutero-nicotinamides, and demonstrating that in enzymatic[7] as well as in non-enzymatic model reactions[6] deuterium was transferred from the 4-position, and only from the 4-position, of the nicotinamide ring. The structure for reduced DPN and for a model compound (N-benzyl dihydronicotinamide) has therefore been firmly established as the 1,4-dihydro compound, II.



I



II



III

* Predoctoral Fellow, National Institutes of Health.

[1] P. Karrer and O. Warburg, *Biochem. Z.* **285**, 297 (1936).
[2] P. Karrer, G. Schwarzenbach, F. Benz and U. Solmssen, *Helv. Chim. Acta.* **19**, 811 (1936); P. Karrer and F. Blumer, *Ibid.* **30**, 1157 (1947).
[3] H. Kuhnis, W. Traber and P. Karrer, *Helv. Chim. Acta* **40**, 751 (1957).
[4] F. H. Westheimer, H. F. Fisher, E. E. Conn and B. Vennesland, *J. Amer. Chem. Soc.* **73**, 2403 (1951); B. Vennesland and F. H. Westheimer, *The Mechanism of Enzyme Action* (Edited by McElroy and Glass). Johns Hopkins Press, Baltimore (1954).
[5] M. Pullman, A. San Pietro and S. P. Colowick, *J. Biol. Chem.* **206**, 129 (1954).
[6] D. Mauzerall and F. H. Westheimer, *J. Amer. Chem. Soc.* **77**, 2261 (1955).
[7] F. A. Loewus, B. Vennesland and D. L. Harris, *J. Amer. Chem. Soc.* **77**, 3391 (1955).

Nevertheless, Karrer and his collaborators have recently[3,8,9,10] repeated their assignment of a 1,2-dihydro structure to reduced DPN^+, on the basis of a set of analogies concerning the similarities and dissimilarities in the acid sensitivity of the model compounds with those of the Hantzsch compounds (i.e. of dihydropyridines which have a hydrogen atom rather than an alkyl group attached to the ring nitrogen atom). We have now determined the proton magnetic resonance spectra of N-methyldihydronicotinamide, N-methyldihydronicotinamide-2-D, N-methyldihydronicotinamide-6-D and of the dihydro compound obtained by reducing nicotinamide methiodide in D_2O. The results of these spectral observations amply confirm the assignment of the 1,4-dihydropyridine structure to these compounds.

EXPERIMENTAL

Materials: N-methyldihydronicotinamide was prepared according to the directions of Karrer and his co-workers[2,3,10] except that the compound was extracted from the aqueous reaction mixture with methylene chloride rather than with ether. The compound, crystallized from ethyl acetate, melted at 83·5–85·5° (evacuated capillary); λ_{max} 355 mμ ($\varepsilon = 6680$). Nicotinamide methochloride was reduced with dithionite in D_2O; the resulting dihydro compound (here identified as the 4-deutero derivative showed a m.p. of 85·3–86·8° (evacuated capillary); λ_{max} 355 ($\varepsilon = 6615$).

6-Deuteronicotinamide was prepared by the method reported previously;[6] its n.m.r. spectrum showed an almost complete lack of the peak assigned to the proton in the 6-position. The methiodide, on reduction in H_2O, gave the deuterated dihydro compound, m.p. 82–4° (evacuated capillary); λ_{max} 355 mμ ($\varepsilon = 6520$). 2-Deuteronicotinamide was prepared by the decarboxylation of quinolinic acid-D_2 and formation of the amide in a procedure paralleling that for 6-deuteronicotinamide.[6] Its n.m.r. spectrum completely lacked the peak assigned to the 2-proton in nicotinamide. The methiodide, on reduction, gave a deuterated dihydro compound, m.p. 81–3° (evacuated capillary); λ_{max} 355 mμ ($\varepsilon = 6615$). All of the dihydro compounds showed the characteristic[11] loss of ultraviolet absorption at 355 mμ and the development of a strong absorption at 298 mμ when their solutions were acidified. The deuterated compounds showed a low intensity band around 4·3 μ in the infrared region; this band was absent in the undeuterated compound.

Solutions (~50 per cent) of the dihydro compounds were prepared in D_2O (Stuart Oxygen Co.) or in acetone-D_6 (New England Nuclear Corp.; kindly donated by Dr. A. A. Bothner-By) and sealed in 5 mm tubes containing a sealed, *cyclo*hexane-filled reference capillary. The solutions in D_2O were specially prepared; to remove the proton absorption (resulting from an exchange of the amide protons with solvent) the dihydro compound was twice taken up in D_2O and lyophilized to dryness before final dissolution in D_2O for spectral examination. N.M.R. spectra, taken before each lyophilization, and an ultraviolet spectrum of the final solution demonstrated that the dihydro compound suffered only slight chemical change during this procedure. After 10 days in acetone-D_6, the N-methyl dihydronicotinamide-4-D, which had crystallized on cooling, showed a melting point of 83·0–85·5° and λ_{max} 355 mμ, $\varepsilon = 6560$. Another

[8] L. Kuss and P. Karrer, *Helv. Chim. Acta* **40**, 740 (1957).
[9] P. R. Brook and P. Karrer, *Liebigs Ann.* **605**, 1 (1957).
[10] P. Karrer, *A. Stoll Festschrift.* Basel (1957).
[11] P. Karrer, B. H. Ringier, J. Buchi, H. Fritzsche and U. Solmssen, *Helv. Chim. Acta* **20**, 55 (1937).

sample, all of which remained in solution, showed a λ_{max} 355, $\varepsilon = 5810$, indicating only slight decomposition after twenty days in this solvent.

Spectra. The n.m.r. spectra were observed at 40·01 Mc upon spinning samples, using a Varian Associates model V 4300 B Nuclear Magnetic Resonance spectrometer,

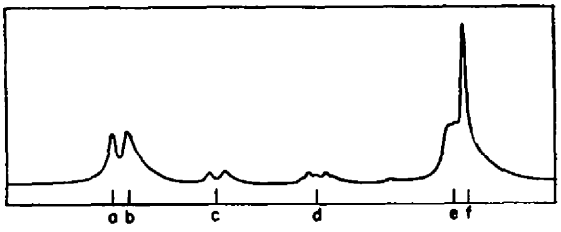


FIG. 1. N.M.R. spectrum of N-methyl-dihydronicotinamide in acetone-D_6. Displacements from the *cyclo*hexane line: *a*, 214 ± 1 c/s; *b*, 207 ± 1 c/s; *c*, 167 ± 1 c/s; *d*, 120 ± 1 c/s; *e*, 59 ± 2 c/s; *f*, 50 ± 2 c/s

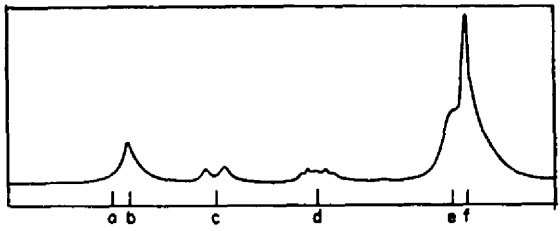


FIG. 2. N.M.R. spectrum of N-methyl-2-deuterodihydronicotinamide in acetone-D_6

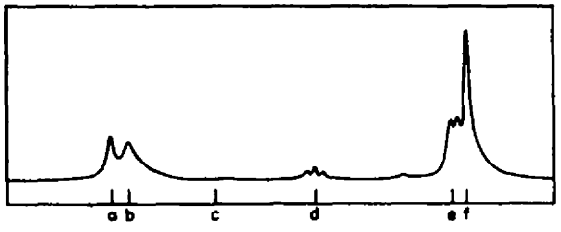


FIG. 3. N.M.R. spectrum of N-methyl-6-deuterodihydronicotinamide in acetone-D_6

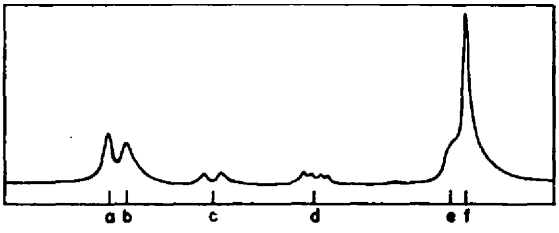


FIG. 4. N.M.R. spectrum in acetone-D_6 of the product obtained by reducing nicotinamide methiodide with dithionite in D_2O; product treated with water before solution in acetone-D_6

equipped with a "Superstabilizer". Line separations, measured by the side-band method,[12] and line positions, referred to the *cyclo*hexane absorption line, are accurate to ±1 c/s.

RESULTS AND DISCUSSION

The high-resolution spectrum for N-methyl dihydronicotinamide in acetone-D_6 is presented in Fig. 1. The spectra for the 2 and 6 deuterated compounds are presented in Figs. 2 and 3; the spectrum of the compound prepared by reduction of nicotinamide methiodide in D_2O is shown in Fig. 4. Since the spectrum (Fig. 4) of nicotinamide methiodide reduced in D_2O is not identical with either the spectrum of the 2-deutero

[12] J. T. Arnold and M. E. Packard, *J. Chem. Phys.* **19**, 1608 (1951).

or of the 6-deutero compound, the reduction product must be that of the 1,4-dihydro structure, II. The same conclusion can be reached independently by the more detailed consideration of the spectra given below.

The spectrum of N-methyldihydronicotinamide in D_2O is not shown, but is identical with that for this compound in acetone-D_6 except that the peak at *b* is totally missing; this experiment identifies this peak as that of the amide hydrogen atoms. The large peak at *f* is that of the N-methyl group, and the shoulder at *e* that of the methylene group. The very small peak, which appears between *d* and *e*, is almost absent in some preparations, and is presumably caused by a trace of impurity, probably water. The peak is greatly accentuated by the addition of water to the saturated solutions of the dihydro compound in acetone-D_6 and no peak was observed at this position (near 85 c/s) in freshly prepared samples in D_2O; in this solvent the H_2O peak is displaced to 120 c/s. At high resolution, we have been unable to obtain a quantitative relationship between peak area and the number of hydrogen atoms at a given position; the differentiation between one, two and three hydrogen atoms is, however, qualitatively clear.

A comparison of the spectra shows that the peak at *a* must be that of the hydrogen atom at position 2, since this peak is absent in the 2-deutero compound (Fig. 2), and the peak at *c* must be that of the hydrogen atom at position 6 since this peak is absent in the 6-deutero compound (Fig. 3). Structures I and III have methylene groups, rather than single hydrogen atoms, at positions 2 and 6 respectively. The n.m.r. spectra identify the peaks at *a* and *c* as those for single hydrogen atoms at positions 2 and 6, and therefore eliminate structures I and III from consideration. The identification of the reduction product as the 1,4-dihydro structure, II, is thus confirmed.

The above argument is strongly reinforced by the fine structure of the spectra. The peak at *a* in Fig. 1 (for the undeuterated N-methyldihydronicotinamide) can be identified, without reference to any other spectrum, as due to the hydrogen in position 2. The peak is not split, and is the only peak for a single hydrogen atom which is not split.* Only for the 2-hydrogen atom is there no adjacent hydrogen, and therefore the peak at *a* must correspond to this atom. This fact alone eliminates structure II from consideration, although (without other evidence) it does not eliminate structure III. This identification confirms that which was also made by an examination of the spectrum (Fig. 2) of the 2-deutero compound. This second identification of the peak corresponding to the hydrogen at position 2 is most important, because it shows conclusively that there has been no migration of deuterium from the 2-position during the preparation of the 2-deutero compound; Karrer[9,10] postulated such migrations to reconcile our data (and his own) with the hypothesis of the 1,2-dihydro structure. Since there has been no rearrangement in the preparation of the 2-deutero compound, a rearrangement in the preparation, by similar methods, of the 6-deutero compound becomes highly unlikely.

The rest of the fine structures are consistent with the assignment of structure. The

* Under still higher resolution than that here presented, peak *a* (and each peak of *c*) develops a partially resolved doublet structure ($J < 2$ c/s); such secondary splitting is of the magnitude observed for cross-ring interactions[13,14,15] and is considerably smaller than the primary splittings ($J = 9$ c/s). Furthermore, the secondary splitting of peak *a* is absent from the spectrum of the 6-deutero compound, and the corresponding splitting of each of the peaks of *c* is absent from the spectrum of the 2-deutero compound.

[13] *Technical Information Bulletin* Vol. 2, No. 1 Varian Associates, Palo Alto, Cal. (1957).
[14] R. Hutton, unpublished results.
[15] H. S. Gutowsky, C. H. Holm, A. Saika and G. A. Williams, *J. Amer. Chem. Soc.* **79**, 4596 (1957).

peak at *b* has already been identified as that of the NH_2 group. For N-methyldihydronicotinamide itself, the peak at *c* (previously identified as that of the hydrogen atom in position 6) is split into a doublet by the adjacent hydrogen atom at 5. The peak at *d* is that for the hydrogen atom at position 5, and has been split by the single hydrogen atom at 6 and the adjacent methylene group at 4 into a pair of partially-superimposed triplets.

The fine structure of the 2-deutero compound (Fig. 2) is essentially unchanged from that of the parent compound, in keeping with the fact that the 2-hydrogen atom is not adjacent to any other of the hydrogen atoms. The fine structure of the 6-deutero compound (Fig. 3) is changed in two respects: the peak at *d* (for the 5-hydrogen atom) which previously consisted of a pair of triplets, has now been simplified (since there is no hydrogen atom at position 6) to a triplet. Secondly, the expected splitting of the methylene group is now clear; it consists of a doublet produced by the interaction of the adjacent hydrogen atom in position 5. In the other spectra this splitting is not clear, perhaps because of a small cross-ring interaction[13,14,15] with the hydrogen atom at position 6.

The spectrum of N-methyldihydronicotinamide, obtained by reducing nicotinamide methiodide in D_2O, is shown in Fig. 4. The hydrogen atoms on the amide have been partially restored by solution in water prior to taking the spectrum. The shoulder at *e* has been diminished in size, since deuterium has been introduced, during reduction, at position 4, and the peak at *d* is a pair of doublets, split by the hydrogen atom at position 6 and the single hydrogen atom at position 4.

Acknowledgements—The authors are indebted to Dr. A. A. Bothner-By for his valuable help in interpreting these spectra, and to the National Institutes of Health for financial support.