

The equilibria and reaction rates of Nicotinamide-adenine-dinucleotide and related compounds with cyanide ion.

Jan Vanbroeckhoven<sup>\*</sup>, Jozef Lepoivre, Frank Alderweireldt

University of Antwerp (RUCA)

Laboratory for Organic Chemistry

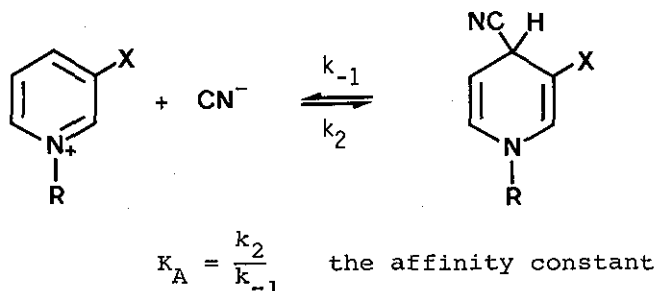
Groenenborgerlaan 171

B 2020 Antwerp Belgium

Abstract : Rate and equilibrium constants for addition of cyanide ion to a series of pyridinium salts have been measured photometrically in aqueous solution at different ionic strength within the pH interval 10 - 13. All the adducts were isolated and characterized by N.M.R. spectroscopy and Mass spectrometry. From the thermodynamic quantities it was shown that the effects of pyridine 1- and 3- substituents on the cyanide affinity are independent of each other. This means that by 1-benzyl-pyridiniumsalts one can predict the reactivity of these pyridine derivative as coenzyme analogue.

As is well known nicotinamide-adenine-dinucleotide ( $\text{NAD}^+$ ) with its reduced form (NADH) is a coenzyme in enzymatic oxidations and reductions. The study of the reactivity and the physical properties of NAD analogues and smaller model

compounds are useful in the understanding of enzymatic reactions. To investigate the affinity of model compounds toward nucleophiles the cyanide addition reaction was examined because of its analogy with the enzymatic reactions. (Addition of  $\text{CN}^-$  versus  $\text{H}^-$ ) (1).



This reaction has already been studied in detail by several investigators (2-5) but there were large discrepancies between the published  $K_A$  values.

The pyridinium salts investigated can be classified into two classes. A first class consists of 3-carbamoylpyridine with different substituents (R) on the pyridine-N-atom, R being benzyl ( $\text{I}_a$ ); 2,3,4,6-tetra-O-acetyl- $\beta$ -D-glucopyranosyl ( $\text{II}_a$ ) and the R group of the natural coenzyme ( $\text{III}_a$ ). The second class consists of 3-acetylpyridine quaternized with the same R groups giving compounds  $\text{I}_b$ ;  $\text{II}_b$  and  $\text{III}_b$ .

The kinetics of the cyanide addition reaction have already been studied with compounds  $\text{I}_a$  and  $\text{III}_a$  (4,5). For the other compounds only the affinity constant has been determined, but not in a reproducible way (2,3).

The comparison of the series a and b is useful to check a possible cross influence of the X and R groups on the investigated reaction.

The kinetic measurements were carried out spectrophotometrically at 20°C and at pH = 12 unless otherwise noted. The ionic strength was maintained at 0.5 M with KCl in order to have results comparable with those of Lindquist (4). The reactions were started by the addition of 0.1 ml substrate solution ( $1 \cdot 10^{-2}$  M to  $5 \cdot 10^{-3}$  M) to 2.90 ml cyanide solution (0.05 M to 0.5 M). In all cases a sufficient excess of cyanide was used so that a pseudo-first-order rate behaviour could be observed. Pseudo-first-order rate constants were evaluated from the slopes of plots of  $\log(A_{\infty} - A_t)$  against time. Second order rate constants were obtained from measured first order rate constants with the expression

$$k_2 = \frac{K_{\text{obs.}}}{(\text{CN}^-) + K_A} - 1$$

where  $A_t$  and  $A_{\infty}$  are the absorbances at time  $t$  and at equilibrium. Equilibrium constants were evaluated at 20°C, using the method of Behme and Cordes (6).

The 4-cyano adducts were prepared according to the method of Wallenfels and Schuly (7). Products were examined using a Jeol JNM-PS-100- $^1\text{H}$ -NMR spectrometer and a Jeol JMS-O1SG-2 Mass spectrometer.

The proton and carbon NMR data for the pyridinium atoms of the

Table 1

$^1\text{H}$  and  $^{13}\text{C}$  NMR spectral data for the 4-cyano-1,4-dihydropyridines. Solvent  $\text{DMSO-d}_6$  ref. TMS.

|                | Chemical shift $\delta$ (ppm). |                 |                               |                |                 |
|----------------|--------------------------------|-----------------|-------------------------------|----------------|-----------------|
|                | I <sub>a</sub>                 | II <sub>a</sub> | III <sub>a</sub> <sup>a</sup> | I <sub>b</sub> | II <sub>b</sub> |
| H <sub>2</sub> | 7.38                           | 7.38<br>7.35    | 7.34<br>7.30                  | 7.79           | 7.77<br>7.70    |
| H <sub>4</sub> | 4.52                           | 4.45<br>4.40    | ?<br>?                        | 4.50           | 4.40<br>4.37    |
| H <sub>5</sub> | 4.72                           | 4.79<br>4.75    | ?<br>?                        | 4.94           | 4.97<br>4.94    |
| H <sub>6</sub> | 6.30                           | 6.37<br>6.34    | 6.46<br>6.42                  | 6.30           | 6.48<br>6.46    |
| C <sub>2</sub> | 138.4                          | 135.5<br>134.1  |                               | 144.7          | 142.4<br>141.3  |
| C <sub>3</sub> | 96.8                           | 99.8<br>99.3    |                               | 104.0          | 106.5<br>106.1  |
| C <sub>4</sub> | 24.9                           | 24.7            |                               | 24.1           | 23.8            |
| C <sub>5</sub> | 96.9                           | 97.5<br>97.1    |                               | 100.0          | 100.1<br>99.7   |
| C <sub>6</sub> | 131.8                          | 130.0<br>128.5  |                               | 130.8          | 129.0<br>127.9  |

a. Solvent  $\text{D}_2\text{O}$  Ref. Sodium -3-(trimethylsilyl)propanesulfonate (TSP).

adducts are given in Table 1. All carbon and proton chemical shifts of the pyridine moiety are doubled in the II and III derivatives but not in the benzyl derivatives  $I_a$  and  $I_b$ .

This effect is caused by the creation of a new chiral centre at C-4 in the pyridine ring by the addition of the cyanide ion, so that the atoms of the cyanide adduct become diastereotopic. Other examples of such shift differences can be found in the literature (8-10).

The mass spectra of the 4-cyano adducts were characterized by low abundant molecular ions  $[M]^+$ . In the mass spectrum of 3-acetyl-1-benzyl-4-cyano-1,4-dihydropyridine abundant peaks are observed at  $m/z$  213 (R.I. 17) and  $m/z$  122 (R.I. 33). From high resolution measurements, the peak at  $m/z$  213 is due to the molecular ion of 3-acetyl-1-benzyl-1,4-dihydropyridine, which, by loss of a benzyl radical, gives rise to  $m/z$  122. An investigation done in this laboratory revealed a thermal dismutation reaction of the 4-cyano adducts giving the corresponding dihydropyridines (11).

The behaviour of all the compounds under the given circumstances are summarized in tabel 2.

Both the rate and equilibrium of these reactions were found to be independent of the concentration of KOH in the range  $10^{-4}$  to  $10^{-2}$  M. At  $10^{-1}$  M, however, a decrease for  $k_2$  and  $K_A$  was observed together with an increasing absorption band at ca. 420 nm. Most probably at this hydroxide concentration there is a compe-

Table 2

Rate and equilibrium constants for the cyanide addition  
reaction. *b*

| Product                       | $\lambda_{\text{max.}}$ (nm.) | $k_2$ ( $\text{M}^{-1}\text{sec}^{-1}$ ) | $k_{-1}$ ( $\text{sec}^{-1}$ ) | $K_A$ ( $\text{M}^{-1}$ ) |
|-------------------------------|-------------------------------|------------------------------------------|--------------------------------|---------------------------|
| I <sub>a</sub>                | 340                           | $1.92 \pm 0.05$                          | $0.69 \pm 0.05$                | $2.8 \pm 0.2$             |
| II <sub>a</sub>               | 320                           | $14.4 \pm 0.3$                           | $0.029 \pm 0.001$              | $500 \pm 20$              |
| III <sub>a</sub> <sup>c</sup> | 320                           | $7.8 \pm 0.3$                            | $0.029 \pm 0.003$              | $270 \pm 21$              |
| I <sub>b</sub>                | 354                           | $9.8 \pm 0.2$                            | $0.014 \pm 0.001$              | $705 \pm 28$              |
| II <sub>b</sub> <sup>c</sup>  | 320                           | $74.0 \pm 2.2$                           | $5.710^{-4} \pm 0.410^{-4}$    | $1310^4 \pm 0.810^4$      |
| III <sub>b</sub> <sup>c</sup> | 320                           | $37.2 \pm 0.8$                           | $5.510^{-4} \pm 0.210^{-4}$    | $6.710^4 \pm 0.310^4$     |

tition between the addition of hydroxide and cyanide anions (12). Like in the results reported by Lindquist (4) there was a decrease of the absorbance at 340 nm for the adducts of the benzyl salts. The isomerisation products could not be isolated, but the spectral shifts suggest a possible rearrangement of the 4-adduct to a mixture of the 1,2- and 1,6-adducts.

*b* All reactions are followed spectrophotometrically at the indicated wave length. Ionic strength : 0.5 M. Temperature : 20°C. pH = 12 except for *c* : pH = 10.  
The equilibrium was reached within 30 min.

The variation in the ion concentration from 0.02 to 4 M gave a decrease as well in the reaction rate as in the equilibrium constant  $K_A$  at 25°C. A similar behaviour has already been observed by Lindquist within the range 0.5 to 3 M (4). In the range of 0.02 to 0.5 M ionic strength a linear relationship between  $\log k_r$  and  $\sqrt{I}$  is found as predicted by the Debye-Hückel law for reactions between ions of opposite charge (Fig. 1).

$$\log k_r = 2\alpha Z_A Z_B \sqrt{I}$$

where  $k_r$  is the reaction rate,  $Z_A$  and  $Z_B$  the ionic charge of the reacting ions,  $I$  the ionic strength and  $\alpha$  a constant which for water at 25°C equals 0.509.

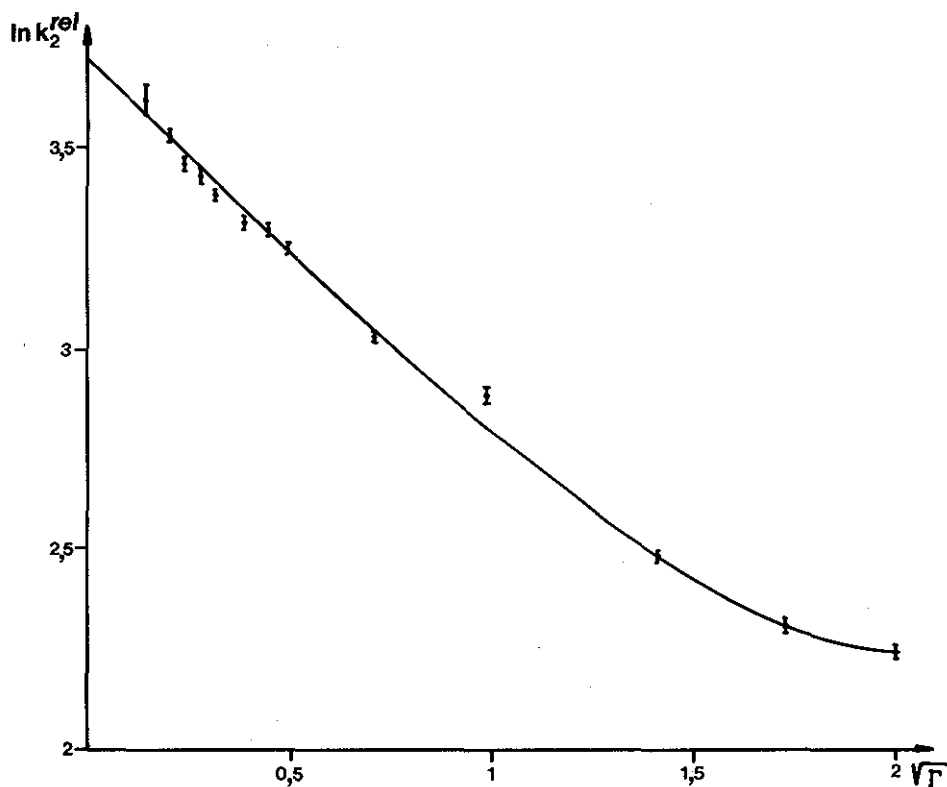
The backward rate constant was not influenced by changing the ionic strength, since the backward reaction is a decomposition of an electrically neutral substance.

The influence of the temperature on the  $k_2$  and  $K_A$  values for the different salts was also determined at 10°, 15°, 25° and 30°C. The  $K_A$  value is decreased at higher temperature. This reflects the fact that the cyanide addition reaction is exothermic.

Table 3 gives the thermodynamic parameters for the cyanide addition to the different salts. Very striking results can be derived from the  $\Delta G$  values :

$$\Delta G(I_a) - \Delta G(I_b) = \Delta G(II_a) - \Delta G(II_b) = \Delta G(III_a) - \Delta G(III_b) = 3.23 \pm 0.7 \text{ kcal/mole, a constant value which is due to the change}$$

Fig. 1



Relationship between  $\log k_r$  and  $\sqrt{\Gamma}$ .

in the 3-substituent and independent of the 1-substituent. Also from the less accurate  $\Delta G^\ddagger$  values a roughly constant change of 1 kcal/mole, induced by the variation of the 3-substituent alone, can be estimated. The  $\Delta\Delta G$  value of 3,23 kcal/mol between compounds a and b is reflected in a 250 fold increase for the affinity constant (see Table 2), when one changes the 3-substituent from carbamoyl to an acetylsubstituent.

Table 3

Thermodynamic parameters for the cyanide addition to pyridinium salts at 20°C. *d*

|                       | I <sub>a</sub>   | II <sub>a</sub>  | III <sub>a</sub> | I <sub>b</sub>   | II <sub>b</sub>  | III <sub>b</sub> |
|-----------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| $\Delta H$            | -6.3 $\pm$ 0.4   | -5.9 $\pm$ 0.4   | -6.1 $\pm$ 0.6   | -11.3 $\pm$ 1.0  | -11.2 $\pm$ 0.9  | -11.4 $\pm$ 1.9  |
| $\Delta S$            | -19.6 $\pm$ 1.4  | -8.0 $\pm$ 1.6   | -9.7 $\pm$ 2.3   | -25.8 $\pm$ 3.5  | -15.5 $\pm$ 3.2  | -16.8 $\pm$ 6.6  |
| $\Delta G$            | -0.59 $\pm$ 0.04 | -3.62 $\pm$ 0.02 | -3.24 $\pm$ 0.05 | -3.82 $\pm$ 0.02 | -6.86 $\pm$ 0.03 | -6.47 $\pm$ 0.02 |
| $E_a$                 | 17.0 $\pm$ 0.5   | 14.2 $\pm$ 0.6   | 14.5 $\pm$ 0.3   | 14.1 $\pm$ 0.8   | 12.3 $\pm$ 1.4   | 11.7 $\pm$ 0.3   |
| $\Delta H^\ddagger$   | 16.4 $\pm$ 0.5   | 13.6 $\pm$ 0.6   | 13.9 $\pm$ 0.3   | 13.5 $\pm$ 0.8   | 11.7 $\pm$ 1.4   | 11.1 $\pm$ 0.3   |
| $\Delta S^\ddagger$   | -9.4 $\pm$ 1.8   | -15.0 $\pm$ 2.1  | -15.0 $\pm$ 1.0  | -16.0 $\pm$ 2.8  | -18.2 $\pm$ 3.8  | -22.1 $\pm$ 0.7  |
| $\Delta G^\ddagger$   | 19.1 $\pm$ 1.1   | 18.0 $\pm$ 1.2   | 18.3 $\pm$ 0.6   | 18.2 $\pm$ 1.8   | 17.0 $\pm$ 2.8   | 17.6 $\pm$ 0.4   |
| $\Delta G_-^\ddagger$ | 19.7 $\pm$ 1.1   | 21.6 $\pm$ 1.3   | 21.6 $\pm$ 0.6   | 22.0 $\pm$ 1.8   | 23.9 $\pm$ 2.8   | 24.1 $\pm$ 4.2   |

In an analogous way the  $\Delta\Delta G^\ddagger$  stays for a 5 fold increase in the reaction rate. The  $\Delta G_-^\ddagger$  values are a measure for the backward rate constants.

Consequently by examining the cyanide addition reactions for different 3-substituted-benzylpyridinium salts, and after comparing the obtained results with those of I<sub>a</sub> and III<sub>a</sub>, one can predict the change in reactivity and equilibrium for the

*d* The values for the free energy and enthalpy and those of activation are given in kcal/mole.

Values for the entropy and entropy of activation are given in e.u. (cal °C<sup>-1</sup>).

corresponding coenzyme analogues. Up to now this correspondence of the results obtained with coenzyme model compounds was only assumed.

As can be seen in table 3 constant increments between the corresponding a and b compounds are also found in the  $\Delta H$  and  $\Delta S$  values. This is a good proof for the independent and large electronic influence of the 3-substituent on the stability of both the pyridinium and the dihydropyridine system.

On the contrary, the influence of the N-substituents only results in the scattering of the  $\Delta S$  values within the a and b series. This is probably due to different solvation of the nitrogen atom in both substrate and reaction product.

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