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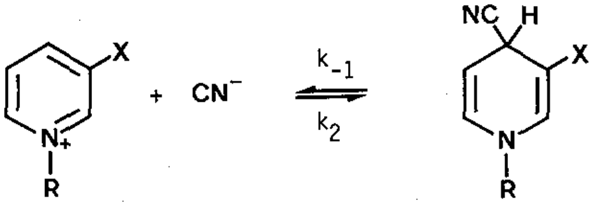
The equilibria and reaction rates of Nicotinamide-adenine-dinucleotide and related compounds with cyanide ion.

Jan Vanbroeckhoven*, 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 strenght 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 (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 usefull 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 CN^- versus H^-) (1).



$$K_A = \frac{k_2}{k_{-1}} \quad \text{the affinity constant}$$

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 (I_a) ; 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl (II_a) and the R group of the natural coenzyme (III_a). The second class consists of 3-acetylpyridine quaternized with the same R groups giving compounds I_b ; II_b and III_b.

The kinetics of the cyanide addition reaction have already been studied with compounds I_a and III_a (4,5). For the other compounds only the affinity constant has been determined, but not in a reproducible way (2,3).

The comparision of the series a and b is usefull 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 strenght 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 (1.10^{-2} M to 5.10^{-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

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

where A_t and A_∞ 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}H-NMR spectrometer and a Jeol JMS-01SG-2 Mass spectrometer.

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

Table 1

^1H and ^{13}C NMR spectral data for the 4-cyano-1,4-dihydropyridines. Solvent DMSO-d_6 ref. TMS.

	Chemical shift δ (ppm).				
	I_a	II_a	III_a[a]	I_b	II_b
H_2	7.38	7.38	7.34	7.79	7.77
		7.35	7.30		7.70
H_4	4.52	4.45	?	4.50	4.40
		4.40	?		4.37
H_5	4.72	4.79	?	4.94	4.97
		4.75	?		4.94
H_6	6.30	6.37	6.46	6.30	6.48
		6.34	6.42		6.46
C_2	138.4	135.5		144.7	142.4
		134.1			141.3
C_3	96.8	99.8		104.0	106.5
		99.3			106.1
C_4	24.9	24.7		24.1	23.8
C_5	96.9	97.5		100.0	100.1
		97.1			99.7
C_6	131.8	130.0		130.8	129.0
		128.5			127.9

a. Solvent D_2O 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 $[M]^{+\cdot}$. 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-

<u>Table 2</u>

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

Product	$\lambda_{max.}$ (nm.)	k_2 ($M^{-1}sec^{-1}$)	k_{-1} (sec^{-1})	K_A (M^{-1})
I_a	340	*1.92 ± 0.05*	*0.69 ± 0.05*	2.8 ± 0.2
II_a	320	14.4 ± 0.3	0.029 ± 0.001	500 ± 20
$III_a{}^c$	320	7.8 ± 0.3	0.029 ± 0.003	270 ± 21
I_b	354	9.8 ± 0.2	0.014 ± 0.001	705 ± 28
$II_b{}^c$	320	74.0 ± 2.2	$5.7\,10^{-4}$ ± $0.4\,10^{-4}$	$13\,10^{4}$ ± $0.8\,10^{4}$
$III_b{}^c$	320	37.2 ± 0.8	$5.5\,10^{-4}$ ± $0.2\,10^{-4}$	$6.7\,10^{4}$ ± $0.3\,10^{4}$

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.

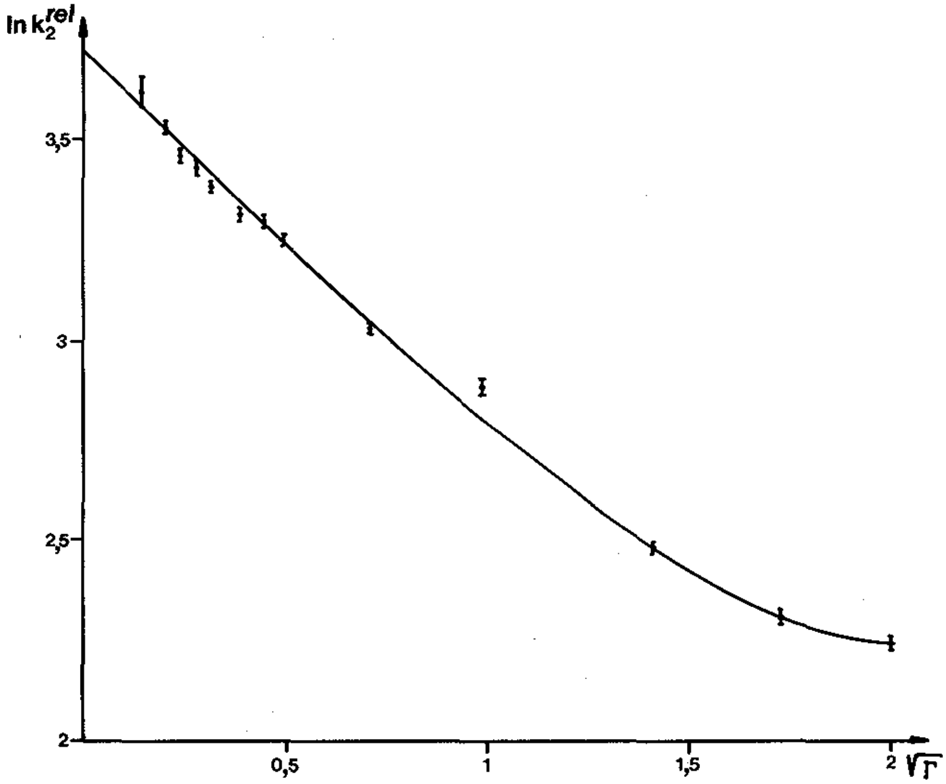
The variation in the ion concentration from 0.02 to 4 M gave a decrease as well in the reaction rate as in the equilibrium constant K_A at 25°C. A similar behaviour has already been observed by Lindquist within the range 0.5 to 3 M (4).
In the range of 0.02 to 0.5 M ionic strenght a linear relationship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

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

where k_r is the reaction rate, Z_A and Z_B the ionic charge of the reacting ions, Γ the ionic strength and α 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 ΔG 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 ± 0.7 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 rougly 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_a	II_a	III_a	I_b	II_b	III_b
ΔH	-6.3 ± 0.4	-5.9 ± 0.4	-6.1 ± 0.6	-11.3 ± 1.0	-11.2 ± 0.9	-11.4 ± 1.9
ΔS	-19.6 ± 1.4	-8.0 ± 1.6	-9.7 ± 2.3	-25.8 ± 3.5	-15.5 ± 3.2	-16.8 ± 6.6
ΔG	-0.59 ± 0.04	-3.62 ± 0.02	-3.24 ± 0.05	-3.82 ± 0.02	-6.86 ± 0.03	-6.47 ± 0.02
E_a	17.0 ± 0.5	14.2 ± 0.6	14.5 ± 0.3	14.1 ± 0.8	12.3 ± 1.4	11.7 ± 0.3
$\Delta H^{\ddagger}$	16.4 ± 0.5	13.6 ± 0.6	13.9 ± 0.3	13.5 ± 0.8	11.7 ± 1.4	11.1 ± 0.3
$\Delta S^{\ddagger}$	-9.4 ± 1.8	-15.0 ± 2.1	-15.0 ± 1.0	-16.0 ± 2.8	-18.2 ± 3.8	-22.1 ± 0.7
$\Delta G^{\ddagger}$	19.1 ± 1.1	18.0 ± 1.2	18.3 ± 0.6	18.2 ± 1.8	17.0 ± 2.8	17.6 ± 0.4
$\Delta G^{\ddagger}_{-}$	19.7 ± 1.1	21.6 ± 1.3	21.6 ± 0.6	22.0 ± 1.8	23.9 ± 2.8	24.1 ± 4.2

In an analogues 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 examinating the cyanide addition reactions for different 3-substituted-benzylpyridinium salts, and after comparing the obtained results with those of I_a and III_a, 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 $°C^{-1}$).

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 ΔH and ΔS 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 ΔS 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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