EXTENSIVE HYDRATE FORMATION OF 5-NITROFURFURAL IN NEUTRAL AQUEOUS SOLUTIONS: IMPLICATIONS FOR ITS REACTIVITY

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Abstract- In neutral aqueous solutions up to 56% of nitrofurfural was found to be in the geminal diol form. This was concluded from NMR experiments with nitrofurfural in DMSO-d_6 with increasing amounts of D_2O. In DMSO nitrofurfural reacts faster with hydrazine than p-nitrobenzaldehyde does; in water the reverse holds. The decreased reactivity of nitrofurfural in water is caused by the large extent of hydration.

Aliphatic aldehydes easily undergo reversible hydration in neutral aqueous solutions [1]. Reports on hydration of aromatic aldehydes are scarce; due to resonance stabilization of aromatic aldehydes, these compounds do not form gem-diols to a large extent [2]. According to Scott et al. [3] substituted furfurals are hydrated for less than 10% only. Considerable formation of gem-diol has been reported for p-nitrobenzaldehyde (PNBA):20% [4]. In this study we report extensive hydrate formation of 5-nitrofurfural (NFA). This finding may implicate that the observed reactivity of NFA in aqueous solutions needs some correction, especially when comparing reaction rates of NFA with those of other aldehydes.

Within the commercially important group of nitrofurans, of which many are used as antibacterials [5], NFA takes in a key position because of the following: Most of these nitrofurans are synthesized from NFA; secondly they decompose photochemically into NFA[6]. Further NFA is a biologically active compound[7], and an important metabolite of the nitrofurans by their acid hydrolysis in the stomach. E.g. as a result of this acid hydrolysis, 25% of oral doses of nitrofurantoin in the rabbit reaches the blood as NFA[8,9]. Further NFA can irreversibly (photo)bind to human serum albumin (Busker et al., unpublished results), which may be the cause of allergic effects reported [10].

So NFA is a compound of biological relevance, and its chemical behaviour in aqueous solutions needs to be investigated. Hydration seems to be important in this respect, because it may influence the reaction rate of NFA.

In order to determine the extent of hydration of NFA, we recorded ^{1}H-NMR spectra of NFA solutions in DMSO-d_6 with increasing amounts of D_2O. As can be seen in Table 1 the hydrate is formed in the presence of D_2O upto a maximum of 50%. If the 50% mixture was heated from room temperature to 80°C, only the NFA NMR signals remained, whereas after cooling, the gem-diol re-appeared in the original amount. This indicates the reversible character of the hydration.

Table 1. NMR data of 5-nitrofurfural (NFA) in DMSO-d_6 plus varying amounts of D_2O. NFA concentration 2mM. Delta is relative to DSS, for some spectra the relative intensity is given in brackets.

DMSO	D_2O	ALDEHYDIC PROTON	FURYL PROTONS IN ALDEHYDE	FURYL PROTONS IN HYDRATE	ALFA PROTON IN HYDRATE
1	0	9.79	7.84 (1) 7.70 (1)	- -	- -
1	0.5	9.65	7.60 (1) 7.56 (1)	7.48 (0.15) 6.56 (0.15)	5.83 (0.15)
1	1.5	9.59	7.52 7.50	7.40 6.62	5.86
1	3	9.57	7.48	7.37 6.62	5.88
1	20	9.56 (1)	7.48 (2)	7.36 (1) 6.62 (1)	5.91 (1)

The amount of hydrate formation, even 56% at 1°C, is extensive, also compared with the 20% hydration reported for p-nitrobenzaldehyde[8] (PNBA). This can be explained by taking into view the manifest electron-attracting properties of the nitrofuryl- as compared to the p-nitrophenylgroup: pKa 5-nitrofuroic acid = 2.06; pKa p-nitrobenzoic acid = 3.44. The carbonyl-C is consequently electron deficient and can easily react with water.

All this may result in a lower availability of the free aldehyde group and thus NFA in water would react less fast with for instance amines. To investigate this the reaction of NFA and of PNBA with hydrazine was studied in aqueous and non-aqueous solutions. A solution of NFA (1 mM), or PNBA (1 mM) with hydrazine HCl (10 mM) was made in phosphate buffer pH 7 or in DMSO. After certain time intervals samples were applied on a HPLC system (column 3 RP 18, eluent water:methanol:perchloric

acid = 500:500:1). The reaction of NFA was followed by the formation of the hydrazone, the only product (absorption at 390 nm); PNBA was determined directly by its absorption at 270 nm. As can be seen in Fig. 1 the reaction of both NFA and PNBA, both in buffer and in DMSO proceed according to pseudo first order kinetics. This can be explained as follows:

$$H_t \underset{}{\overset{\longleftarrow}{\longrightarrow}} A_t + H_2O \qquad A_t + HZ \xrightarrow{k} P_t + H_2O$$

H_t, A_t, P_t, and HZ represent the concentration at reaction time t of the hydrate H, the aldehyde A, the reaction product P and hydrazine HZ. (HZ, being $10.A_t$ at t=0, and $>10.A_t$ during the reaction, is considered constant).

$$dP_t/dt = k.A_t \qquad (1)$$

With x being the fraction of hydrated A, the following is valid:

$$1-x = A_t/(A_t + H_t) \qquad (2)$$

Substitution of (2) in (1) gives:

$$dP_t/dt = k.(1-x).(A_t + H_t) \qquad (3)$$

With $A_0 = A_t + H_t + P_t$, (3) changes into:

$$dP_t/dt = k.(1-x).(A_0 - P_t) \qquad (4)$$

and after integration one becomes:

$$\ln(A_0 - P_t) = -k.(1-x).t - \ln A_0 \qquad (5)$$

According to Sayer [4] both the hydration and dehydration reactions of PNBA are fast: the rate constant of hydration is 3.0 min^{-1}. From NMR observations we estimate that the rate constant for the hydration of NFA is even larger. So when comparing with the rate of the reaction with hydrazine (Table 2), x can be considered constant during the reaction because the hydration equilibrium is always quickly established. This results in a pseudo first order reaction with hydrazine, also in aqueous solution (Eq. 5). Now if hydration is extensive, x will be large, and the observed rate constant k.(1-x) becomes small.

As can be seen in Table 2 the reaction of NFA with hydrazine in aqueous solution is slower than that of PNBA. However in non-aqueous DMSO NFA reacts faster. So the high extent of hydration of NFA results in a smaller observed reaction rate in aqueous solutions. This may have important consequences for NFA e.g. as a metabolite of nitrofurans used as a drug. In effect the apparently smaller reaction rate of NFA by hydration does not change its intrinsic reactivity but results in a longer lifetime only. This longer lifetime enables NFA to reach sensitive target tissue far from its site of formation where its reaction may lead to adverse biological effects.

Table 2. Pseudo first order rate constants (min^{-1}).

NFA in buffer	0.077 + 0.003
NFA in DMSO	0.181 + 0.005
PNBA in buffer	0.162 + 0.008
PNBA in DMSO	0.112 + 0.004

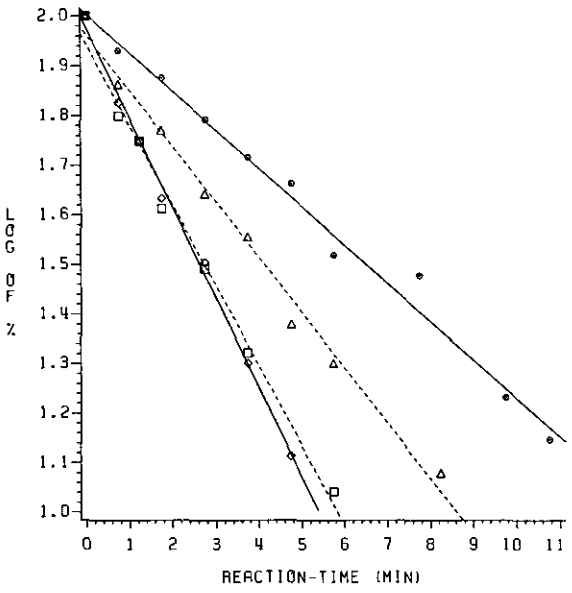

Fig 1. Log of molar percentage of aldehyde present versus reactiontime. NFA (1 mM) or PNBA (1 mM) was incubated with 10 mM hydrazine HCl.

—•—•— NFA in buffer —◇—◇— NFA in DMSO
- □- □- PNBA in buffer -△ -△ - PNBA in DMSO

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