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The reactivity of vinyl compounds as alkylating agents

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Abstract The reactions of 4-(*p*-nitrobenzyl)pyridine (a trap for alkylating agents with nucleophilicity similar to that of DNA) with the vinyl compounds acrylonitrile, acrylamide, acrylic acid, and acrolein, which can act as alkylating agents of DNA, were investigated. The following conclusions were drawn: (1) vinyl compounds show an alkylating capacity on 4-(*p*-nitrobenzyl)pyridine. The sequence of the alkylating potential was found to be acrylonitrile > acrylamide > acrylic acid > acrolein (alkylation with acrolein was not observed after 3 weeks). The formation of adducts with acrylonitrile was approximately 10- and 100-fold faster than with acrylamide and acrylic acid, respectively, which is consistent with its highly carcinogenic and mutagenic activity. (2) 4-(*p*-Nitrobenzyl)pyridine alkylation reactions by vinyl compounds occur through an enthalpy-controlled Michael addition mechanism. The values for the free energy of activation for these reactions with 4-(*p*-nitrobenzyl)pyridine were: $\Delta^{\ddagger}G°$ (37 °C) acrylonitrile, 98 ± 1 kJ mol^{-1}; acrylamide, 105 ± 2 kJ mol^{-1}; acrylic acid, 109 ± 1 kJ mol^{-1}. (3) Application of Hammett treatment to the kinetic results revealed that these alkylation reactions occur through nucleophilic attack, being moderately accelerated by electron-withdrawing groups.

Keywords Kinetics · Alkylations · Michael addition · 4-(*p*-Nitrobenzyl)pyridine

Introduction

In recent decades, considerable effort has been devoted to studying the carcinogenicity of commonly used chemicals, resulting in lists of substances classified according to their potential carcinogenicity. Among these, alkylating agents are considered archetypical carcinogens because of their ability to react with nucleophilic sites in DNA [1, 2]. Almost all of the heteroatoms in the double helix have the potential to become alkylated. The preferred sites of alkylation in duplex DNA depend strongly on the nature of the alkylating agent [3].

In previous work we studied the in vitro reactivity of several alkylating compounds capable of forming DNA adducts: sorbic acid [4] and sorbates [5], nitrosoureas [6, 7], *p*-nitrostyrene oxide [8], and lactones [9–12]. The results revealed a correlation between the carcinogenicity of the substances and their reactivity with 4-(*p*-nitrobenzyl)pyridine (NBP), a trap for alkylating agents with nucleophilicity similar to that of DNA [13, 14]. There is sufficient evidence from experiments with animals for the carcinogenicity of *β*-propiolactone (BPL) and *β*-butyrolactone (BBL) [15], both readily reacting with NBP. The reaction of BPL, the stronger carcinogen of the two, is six times faster than that of BBL. Alkylation reactions of the nucleoside guanosine by vinyl compounds were also investigated kinetically [16].

Since (1) a correlation between the chemical reactivity (alkylation rate constants) of vinyl compounds (VC) and their capacity to form adducts with biomarkers was found using ultra-fast liquid chromatography (UFLC) to monitor the alkylation reactions [16], (2) to our knowledge no kinetic investigation has been carried out to determine the alkylating capacity of vinyl compounds on the NBP molecule as a model, and (3) the NBP test, besides being very

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simple to use, has a high discriminatory capacity to evaluate the alkylating potential of a reagent, here we were prompted to address these issues.

Results and discussion

No NBP alkylation by acrolein (AC) was observed. The previously investigated blue-colored adducts NBP–acrylonitrile (AN), NBP–acrylic acid (AA), as well as NBP–acrylamide (AM) [18] showed maximum absorption at $\lambda = 560$ nm, $\lambda = 580$ nm, and $\lambda = 570$ nm, respectively. As an example, Fig. 1 shows the increase in absorption caused by the formation of adducts resulting from the reaction between NBP and AA over time until no change in absorbance (A) was observed (because the VCs were in large excess, it may be assumed that all the NBP was consumed). Figure 2a represents typical kinetic runs for the alkylation of NBP by AN, AM, and AA.

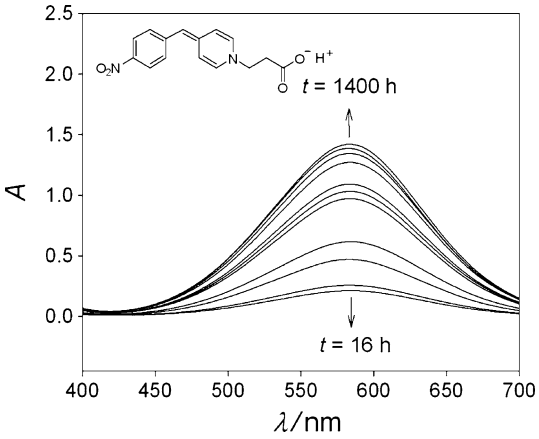


Fig. 1 Formation of the NBP–AA adduct over time. Variation in absorbance in the 16–1,400 h range in 7:3 water/dioxane medium. $[AA]_o = 0.1$ M; $[NBP]_o = 2.5 \times 10^{-4}$ M; $\theta = 35$ °C; pH 3.5

Equation (1) was found to be the rate equation for NBP alkylation by VC.

$$\frac{d[AD]}{dt} = k_{alk}([NBP]_o - [AD])[VC]_o = k'_{alk}([NBP]_o - [AD]) \quad (1)$$

where [AD] represents the concentration of the NBP–VC adduct and $k'_{alk} = k_{alk}\,[VC]_o$ is the pseudo-first order rate constant.

Integrating Eq. (1) and expressing the concentration in terms of absorbance, Eq. (2) is obtained:

$$A_t = A_\infty(1 - \exp(-k'_{alk}t)) \quad (2)$$

where A_t and A_∞ are the values of absorbance of the NBP–VC adducts at times t and infinity, respectively. Figure 2 shows the good fit of the experimental data to Eq. (2) and to its logarithmic form, Eq. (3).

$$\ln\left(\frac{A_\infty}{A_\infty - A_t}\right) = k'_{alk}t \quad (3)$$

Since the time taken for the plateau to be reached was very long, the initial rate method (IRM) was used [19]. The initial rate (v_o) can be written as follows:

$$v_o = \left(\frac{dA}{dt}\right)_{t\to 0} = \varepsilon\, l\, k_{alk}[NBP]_o[VC]_o \quad (4)$$

where ε and l represent the molar absorption coefficient of the adduct and the light path, respectively. The first order rate coefficients obtained from non-linear least squares (Eq. 2) were compared with those derived from the initial rate method (Eq. 4) (Table 1). The good concordance of the results shows that the alkylation reactions are not

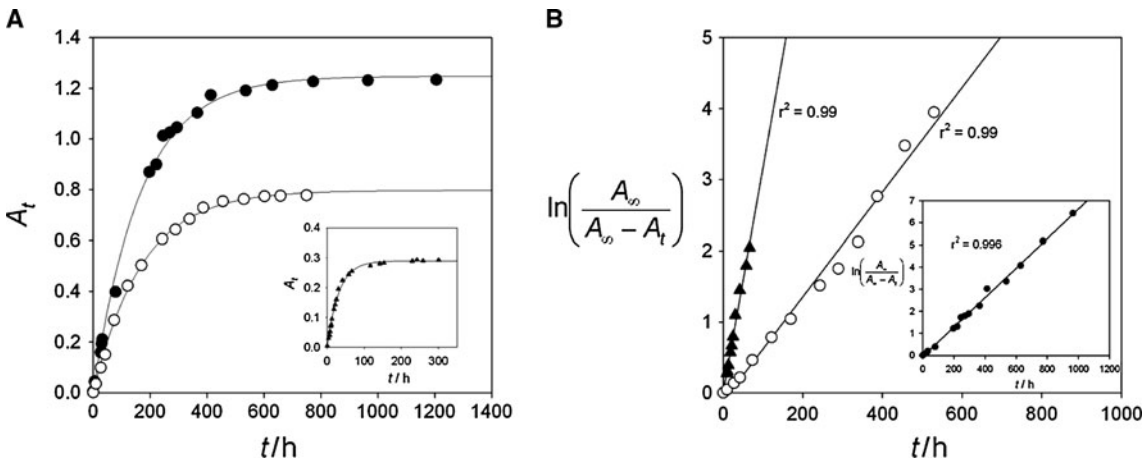


Fig. 2 Kinetic runs for NBP alkylation. **a** Non-linear fits of the experimental results (Eq. 2). **b** Linear fits of the experimental results (Eq. 3). AN (*filled triangle*), AM (*open circle*), and AA (*filled circle*). $[NBP]_o = 2 \times 10^{-4}$ M; $[AA]_o = 1$ M; $[AM]_o = 0.1$ M; $[AN]_o = 0.05$ M; $\theta = 35.0$ °C

Table 1 Activation parameters for NBP alkylation by VC

Alkylating agent	$10^5\ k^a_{alk}/M^{-1}\ s^{-1}$ (Eq. 2)	$10^5\ k^a_{alk}/M^{-1}\ s^{-1}$ (IRM, Eq. 4)
Acrylonitrile	17.6 ± 0.5	16.0 ± 0.9
Acrylamide[b]	1.6 ± 0.1	1.10 ± 0.07
Acrylic acid	0.16 ± 0.01	0.21 ± 0.01

[a] Values given within the 95% confidence interval

[b] Values from Ref. [18]. $\theta = 35$ °C

corrupted significantly over the very long times of reactions and supports the use of the initial rate method.

To monitor the formation of the NBP–VC adducts, their molar absorption coefficients must be known. Experiments designed to measure these coefficients were performed with $[VC]_o = 0.1$ M and NBP concentrations in the 4×10^{-5}–4×10^{-4} M range. When absorbance reached a plateau (Fig. 2), it was assumed that the reaction had reached 100%.

At 37.5 °C, the values obtained were $\varepsilon_{580\ nm}$(NBP–AA) = (6,690 ± 120) $M^{-1}\ cm^{-1}$, $\varepsilon_{570\ nm}$(NBP–AM) = (5,400 ± 200) $M^{-1}\ cm^{-1}$, and $\varepsilon_{560\ nm}$(NBP–AN) = (4,500 ± 170) $M^{-1}\ cm^{-1}$. Knowledge of these values should permit easy determination of the concentration of the adducts by simply measuring the absorbance.

Table 2 shows the NBP alkylation rate constants as a function of the temperature. The results reveal the following sequence of alkylating potential: AN > AM > AA. The formation of adducts with AN is approximately 10- and 100-fold faster than with AM and AA, respectively, which is consistent with its high carcinogenic and mutagenic activity [20]. Contrary to some biological biomarkers such as the nucleoside guanosine, no NBP alkylation by AC was observed. In comparison with the results observed with guanosine [14], this result is significant because it is consistent with the fact that acrolein reacts with guanosine through two binding sites: N1 and N2 (this being the reason for the anomalous behavior of acrolein as an alkylating agent) [21, 22]. The absence of these features in NBP could be the reason for the lack of formation of an adduct.

Table 2 Rate constants as a function of temperature for NBP alkylation by VC

θ/°C	$10^5\ k_{alk}/M^{-1}\ s^{-1}$ (AN)[a]	$10^5\ k_{alk}/M^{-1}\ s^{-1}$ (AM)[b]	$10^5\ k_{alk}/M^{-1}\ s^{-1}$ (AA)[a]
15.0	5.3 ± 0.3	–[c]	0.05 ± 0.02
20.0	7.0 ± 0.3	–[c]	0.07 ± 0.03
22.5	8.1 ± 0.7	–[c]	0.08 ± 0.03
25.0	9.6 ± 0.9	0.61 ± 0.08	0.10 ± 0.01
27.5	11.0 ± 0.9	0.72 ± 0.07	0.12 ± 0.01
30.0	12.0 ± 0.9	0.90 ± 0.07	0.15 ± 0.01
32.5	14.0 ± 0.9	1.01 ± 0.07	0.18 ± 0.01
35.0	16.0 ± 0.9	1.10 ± 0.07	0.21 ± 0.01
37.0	17.5 ± 0.3	1.29 ± 0.07	0.23 ± 0.01
37.5	18.0 ± 0.9	1.37 ± 0.07	0.24 ± 0.01
40.0	20.4 ± 0.3	–[c]	0.28 ± 0.02

[a] Values given within the 95% confidence interval

[b] Values from Ref. [18]

[c] No data available

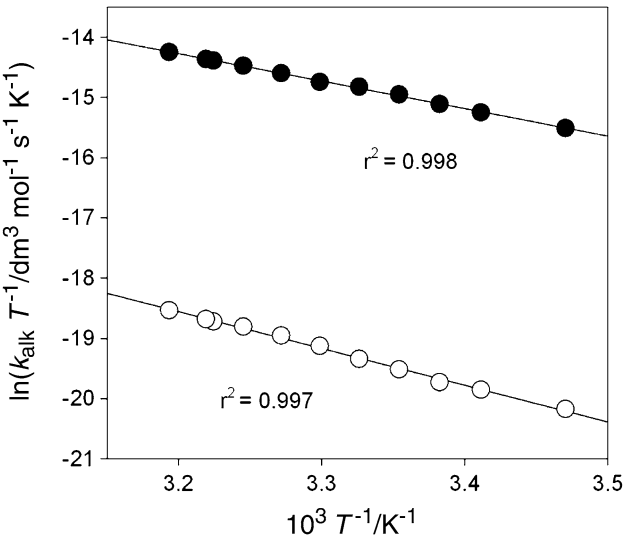


Fig. 3 Eyring plots for NBP alkylation reactions by AN (*filled circle*) and AA (*open circle*) in 7:3 water/dioxane medium

A good fit of the k_{alk} values to the Eyring–Wynne-Jones equation [23] is shown in Fig. 3.

$$\ln(k_{alk}/T) = \ln(k_B/h) + \Delta^{\ddagger}S/R - (\Delta^{\ddagger}H/RT) \quad (5)$$

The values of the activation parameters shown in Table 3 clearly demonstrate that NBP alkylation reactions by VC are essentially enthalpy-controlled.

As is known, the Hammett correlation [24] (Eq. 6) is frequently used to analyze the substituent's effect on the rate constant of a reaction series. Although this equation was derived originally for substituents in the benzene ring, it can be applied even for acyclic derivatives if the rigid shape of the molecule secures separation of the substituent from the reaction center.

$$\log k_{alk} = \rho\sigma_p + \log k_o \quad (6)$$

In Eq. (6), k_{alk}s are the alkylation rate constants for each of the VC, and ρ is the reaction constant.

Since olefinic trans derivatives are correlated simply [25] by the constant σ_p, which includes both inductive and mesomeric effects of the substituent group, σ_p values of −0.05, 0.31, and 0.71 for the carboxylate, amide, and cyano groups, respectively [26], were used.

The excellent correlation observed (Fig. 4) shows that NBP alkylation reactions by VC are mainly controlled by

polar effects. The positive ρ value means that these reactions occur through a nucleophilic mechanism, being moderately accelerated by electron-withdrawing groups. This also indicates that substituents affect this reaction in the same way as they do the ionization of substituted benzoic acids, i.e., the transition state is proceeding from neutral reactants to a negatively charged substituted product. The magnitude of the slope parameter is likely tied to the through-bond distance from the reactive center and the substituent, which is shorter in these vinylic compounds than it is in X-benzoic acids.

Since nucleophilic addition to α,β-conjugated systems is favored through a Michael mechanism [27], the high value of $\rho = 2.48$ obtained here is consistent with that nucleophilic attack by NBP.

Table 3 Activation parameters for NBP alkylation by VC

Alkylating agent	$\Delta^{\ddagger}H^{\circ c}$/ kJ mol^{-1}	$-\Delta^{\ddagger}S^{\circ c}$/ J K^{-1} mol^{-1}	$\Delta^{\ddagger}G^{\circ c}$ (37 °C)/ kJ mol^{-1}
Acrylonitrile[a]	38 ± 1	194 ± 3	98 ± 1
Acrylamide[b]	45 ± 2	193 ± 9	105 ± 2
Acrylic acid[a]	51 ± 1	189 ± 4	109 ± 1
Acrolein[a]	No reaction was observed		

[a] This work

[b] Values from Ref. [18]

[c] Values are given with their standard deviations

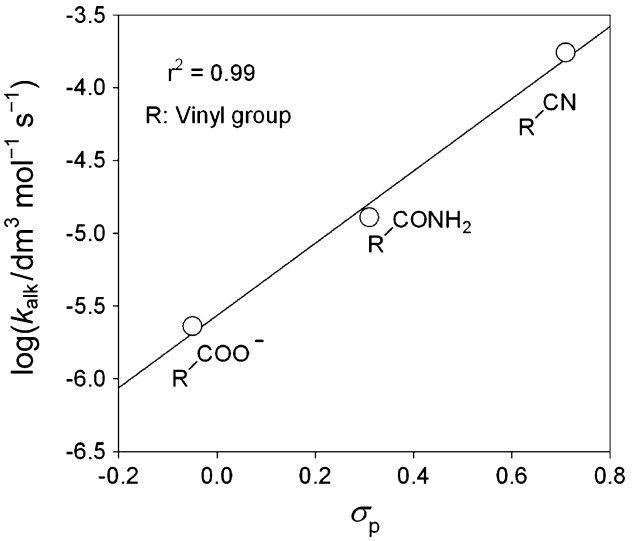


Fig. 4 Influence of the polar effects on the reactivity of vinyl compounds as alkylating agents

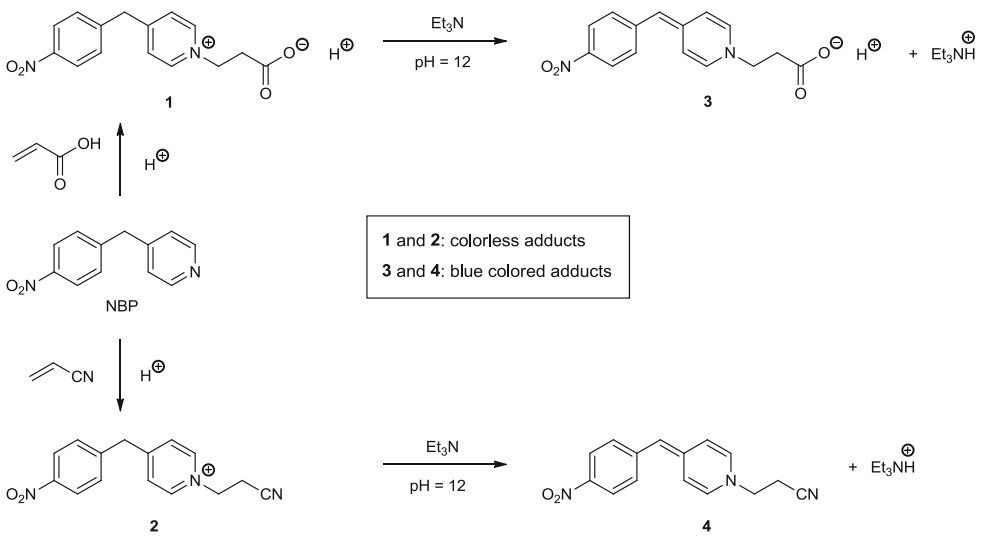
Experimental

Acrylonitrile (99%), triethylamine (Et_3N, 99%), and NBP (98%) were obtained from Aldrich. Acrolein (98%) was purchased from Sigma. 1,4-Dioxane and acrylic acid (99%) were Panreac reagents. Caution: Because AN is probably carcinogenic to humans [17], it should be handled carefully.

The reaction temperature was kept constant (±0.05 °C) with a Lauda Ecoline RE120 thermostat. A Crison Micro pH 2000 pH meter was used to perform pH measurements (±0.01). Water was deionized with a MilliQ-Gradient device (Millipore). Numerical treatment of the data was performed using the Wolfram Mathematica® 7 software.

The NBP alkylation reactions by VC were carried out at the pH alkylation mixture. To monitor these reactions, 2.4 cm^3 aliquots of the alkylation mixture (NBP + VC) were withdrawn at different times and added to a cuvette containing 0.6 cm^3 of 99% Et_3N to stop the alkylation process (Scheme 1), measuring absorbance at the wavelength of maximum absorption of the adducts **3** and **4**.

Scheme 1



A Shimadzu UV-2450-PC spectrophotometer with a thermoelectric six-cell holder temperature control system (±0.1 °C) was used. To render NBP soluble, the alkylation mixtures were prepared in 7:3 (vol.) water/dioxane medium. Detailed reaction conditions are given in the figure and table legends.

To check the structures of the adducts **1** and **2**, positive-mode electrospray ionization mass spectra were obtained with mass/charge ratios of 287.1 and 268.1, respectively, which are coherent with the suggested structures (calc. 287.10 and 268.11, respectively). A Waters ZQ4000 apparatus was used.

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