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Steric effect in alkylation reactions by *N*-alkyl-*N*-nitrosoureas: a kinetic approach

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The alkylation reactions of 4-(*p*-nitrobenzyl)pyridine (NBP), a trap for alkylating agents with nucleophilic characteristics similar to DNA bases, by five *N*-alkyl-*N*-nitrosoureas (methyl-, ethyl-, propyl-, butyl-, and allylnitrosourea) were investigated in 7:3 (v/v) water/dioxane medium in the 5.0–6.5 pH range. Decomposition of alkylnitrosoureas (ANU) gives rise to alkyldiazonium ions that yield NBP-R adducts directly or through carbocations in certain instances. The NBP alkylation rate constants by these species were determined. The following sequence of alkylating potential was found: methyl- > ethyl- > allyl- > propyl- > butyl group. Application of Ingold–Taft correlation analysis to the kinetic results revealed that the NBP alkylation reactions occur mainly through steric control. The values of the molar absorption coefficients of the NBP-R adducts also reveal the determinant influence of a steric effect in the formation of alkylation adducts. The kinetic results are consistent with the biological activity of ANU. Copyright © 2008 John Wiley & Sons, Ltd.

Keywords: reactivity of *N*-alkyl-*N*-nitrosoureas; steric effects

INTRODUCTION

Alkylating agents are considered to be archetypical carcinogens.[1,2] Since the decomposition of alkylnitrosoureas (ANU) gives rise to alkyldiazonium ions (RN_2^+) without any need for metabolic activation, they offer an ideal series of compounds in which to examine the effect of variations in chemical structure on alkylating potential.

Previous studies (Chapter 3 of Reference [3]) have shown that some ANUs induce tumors in different organs of rats, but do not have a favorable partition coefficient into organic solvents from water. If physical properties such as solubility and partition coefficient play any role, it must be minor and perhaps limited to effects on potency. Some results[4] have pointed to the structure of the alkyl group adjacent to the nitroso group as the most important determinant of the carcinogenic effect.

Since in spite of its chemical relevance, to our knowledge the chemical reactivity of alkyldiazonium ions as alkylating agents has not been investigated in a comparative quantitative way, here we were prompted to address this issue.

RESULTS AND DISCUSSION

The nucleophile 4-(*p*-nitrobenzyl)pyridine, NBP, a trap for alkylating agents[5] with nucleophilic characteristics similar to DNA bases,[6] was used as the alkylation substrate. This method was previously used by us to investigate the alkylating potential of strong alkylating reagents, such as lactones,[7–10] as well as to determine the reactivity of much weaker alkylating molecules such as sorbates.[11,12]

NBP alkylation reactions by methyl-, ethyl-, propyl-, butyl-, and allylnitrosourea (MNU, ENU, PNU, BNU, and AlNU, respectively) were investigated.

In order to render the NBP soluble, the ANU+NBP alkylation mixtures were prepared in 7:3 (v/v) water/dioxane medium. Reactions were carried out in the 5.0–6.5 pH range. Acetic/acetate buffer was used to maintain pH constant. To monitor the alkylation reactions, 2.4-ml aliquots of the alkylation mixture were removed at different times and added to a cuvette containing 0.6 ml of 99% triethylamine reagent (Et_3N), which stopped the alkylation process and generated a blue color, whose absorbance was measured at the wavelength of maximum absorption (shown below).

The blue NBP adducts showed maximum absorption at $\lambda_{MNU} = 573$ nm, $\lambda_{ENU} = 570$ nm, $\lambda_{PNU} = 570$ nm, $\lambda_{BNU} = 570$ nm, and $\lambda_{AlNU} = 557$ nm. As an example, Fig. 1 shows the increase in absorption caused by the formation of the NBP-adduct with MNU over time until no change in absorbance, *A*, was observed. Because NBP was in large excess, it may be assumed that all the nitrosourea was consumed.

Figure 2 represents typical kinetic runs for the alkylation of NBP by nitrosoureas.

The experimental rate equation was

$$\text{rate} = \frac{d[AD]}{dt} = \frac{k_{alk}k_{dec}[OH^-][NBP]}{k_{alk}[NBP] + k_{H_2O}[H_2O]}[ANU] = k_1[ANU] \quad (1)$$

where [AD] is the concentration of NBP-R adducts, and k_1 the pseudo-first-order rate constant:

$$k_1 = \frac{k_{alk}k_{dec}[OH^-][NBP]}{k_{alk}[NBP] + k_{H_2O}[H_2O]} \quad (2)$$

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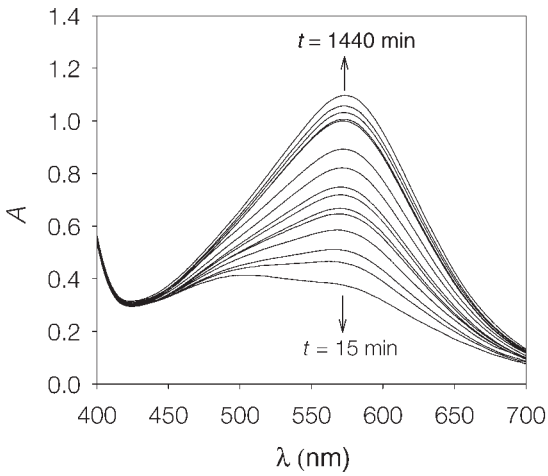


Figure 1. Spectrograms showing the formation of the NBP-adduct with MNU over time in 7:3 water/dioxane medium. Variation in absorbance in the 15–1440 min interval. $[MNU]_o = 1.6 \times 10^{-3}$ M; $[NBP]_o = 0.02$ M; $T = 32.5\,^{\circ}C$; pH = 6.5

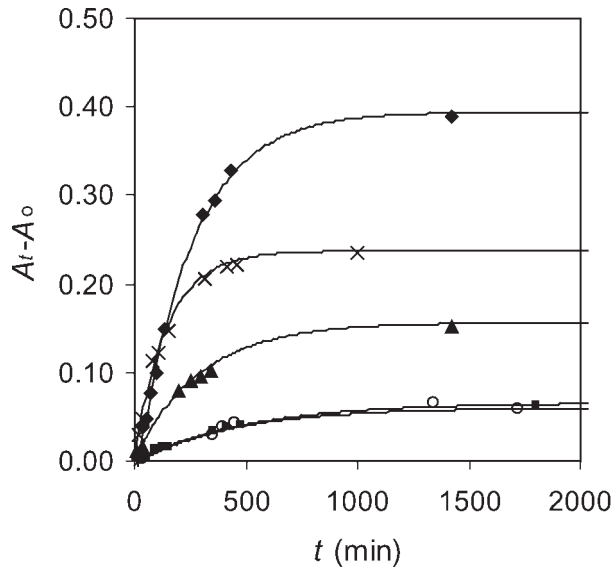


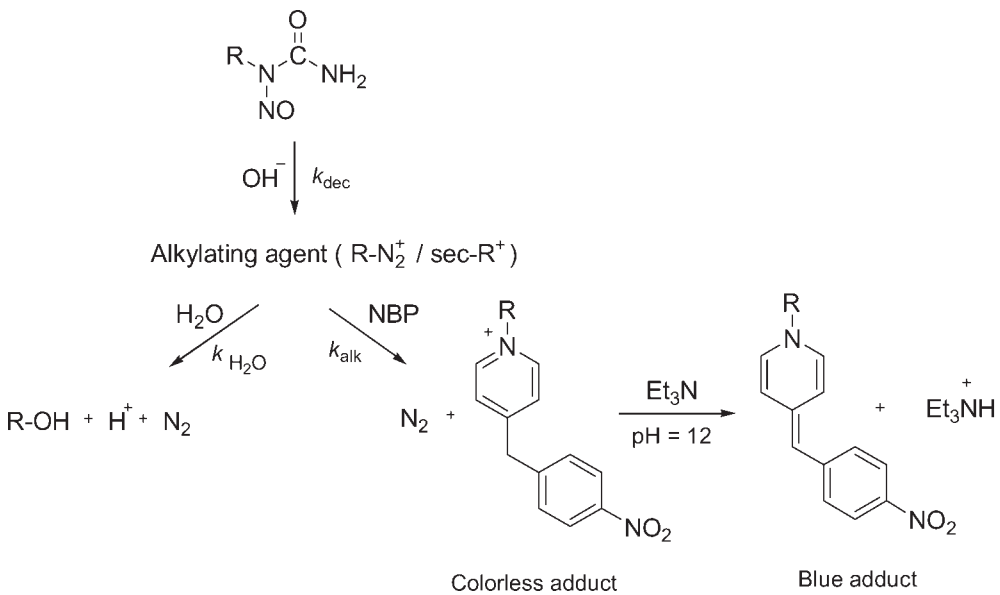
Figure 2. Formation of the NBP-R adducts in 7:3 water/dioxane medium: methyl- (◆), ethyl- (▲), propyl- (■), butyl- (○), and allyldiazonium ion (x); $[ANU]_o = 8 \times 10^{-4}$ M; $[NBP]_o = 0.02$ M; pH = 6.5; $T = 35\,^{\circ}C$. A_o and A_t, designate the absorbance values of the adducts at times, respectively, of zero and t

The significance of the different rate constants appearing in Eqn (1) can be seen in Scheme 1. In the case of MNU and ENU the effective alkylating agents should be methyl- and ethyldiazonium ions to give rise NBP-R adducts ($R = CH_3$ and C_2H_5, respectively).[13,14] For the other nitrosoureas, looking at PNU as an example, the propyldiazonium ion resulting from its decomposition undergoes, in competition, elimination to alkenes, concurrent S_N2 substitution and hydride shift yielding a *sec*-carbocation that can undergo partitioning between the associated NBP nuclophile and solvent.[15,16] The method here used to monitoring the alkylation reactions allows one to determine k_{alk} rate constant that represents globally any alkylation mechanisms.

The influence of $[OH^-]$ and [NBP] fits Eqn (2) (as shown in Figs 3 and 4). Eqn (3) (derived from Eqn (2)) shows that the hydrolysis of alkylating ions (rate constant k_{H_2O} in Eqn (1)) is the main reaction responsible for their consumption when the concentration of the alkylation substrate [NBP] tends to zero:

$$\lim_{[NBP]\to 0} \frac{dk_1}{d[NBP]} = \frac{k_{alk} k_{dec} [OH^-]}{k_{H_2O} [H_2O]} \tag{3}$$

By designating the absorbance values of the NBP-R adducts as A_o, A_t, andA_∞ at times, respectively, of zero, t, and infinity (i.e., when the plateau is reached; as shown in Fig. 2), ε_{ANU}, ε_{NBP}, and ε_{AD} being the molar absorption coefficients of the ANU, NBP, and adducts, respectively, and x the adduct concentration,



Scheme 1.

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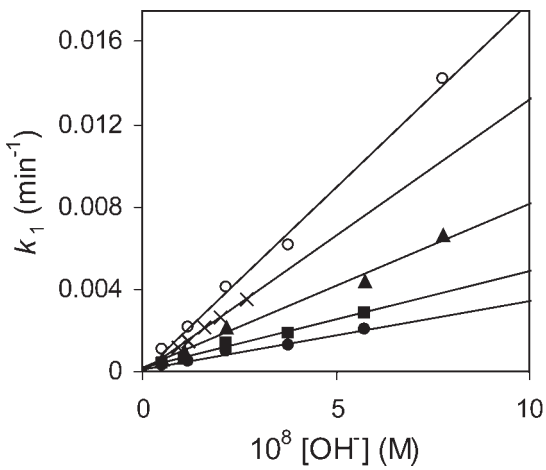

Figure 3. Influence of [OH$^-$] in the alkylation reaction of NBP by MNU at 25.0 °C (●), 27.5 °C (■), 30.0 °C (▲), 32.5 °C (×), and 35.0 °C (○); $[NBP]_o = 0.02$ M; $[MNU]_o = 8 \times 10^{-4}$ M

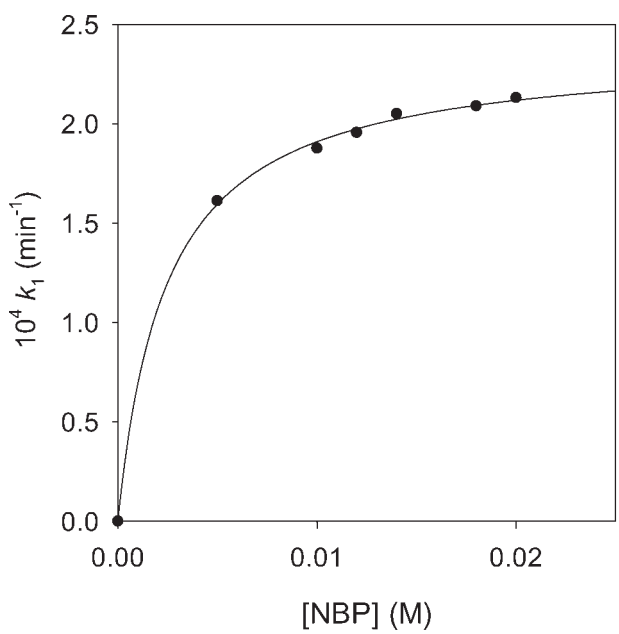

Figure 4. Influence of [NBP] in the alkylation reaction of NBP by MNU at 25 °C in 7:3 water/dioxane medium. $[MNU]_o = 8 \times 10^{-4}$ M; pH = 5.72

Eqns (4)–(6) can be written as

$$A_o = \varepsilon_{ANU}[ANU]_o + \varepsilon_{NBP}[NBP] \quad (4)$$

$$A_t = \varepsilon_{ANU}([ANU]_o - x) + \varepsilon_{NBP}[NBP] + x\varepsilon_{AD} \quad (5)$$

$$A_\infty = \varepsilon_{AD}[ANU]_o + \varepsilon_{NBP}[NBP] \quad (6)$$

Integration of Eqn (1) gives

$$A_t - A_o = (A_\infty - A_o)(1 - e^{-k_1 t}) \quad (7)$$

Figure 2 shows the fitting of the experimental data to Eqn (7).

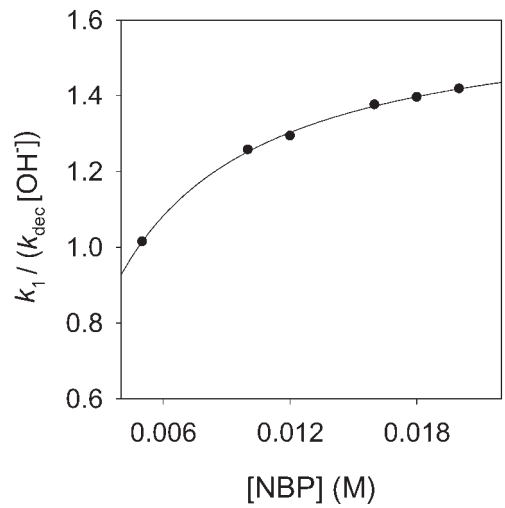

Figure 5. Fitting of the results to Eqn (8). $[MNU]_o = 8 \times 10^{-4}$ M; pH = 5.72

Eqn (2) can be written in the form:

$$\frac{k_1}{[OH^-]k_{dec}} = \frac{k_{alk}/k_{H_2O}[NBP]}{k_{alk}/k_{H_2O}[NBP] + [H_2O]} \quad (8)$$

Figure 5 plots the $k_1/([OH^-]\,k_{dec})$ values against those of [NBP]. The k_{alk}/k_{H_2O} ratios for the different alkyldiazonium ions were calculated by a nonlinear optimization method. Since the k_{dec} values were needed to be known, ANU decomposition reactions were investigated directly. These reactions were monitored spectrophotometrically at the wavelengths of maximum absorption for each of the ANUs studied here ($\lambda_{MNU} = 251$ nm, $\lambda_{ENU} = 248$ nm, $\lambda_{PNU} = 244$ nm, $\lambda_{BNU} = 244$ nm, and $\lambda_{AINU} = 243$ nm).

In the 5.0–7.0 pH range, the following rate equation was observed for ANU decomposition:

$$-\frac{d[ANU]}{dt} = k_{dec}[OH^-][ANU] = k'[ANU] \quad (9)$$

where k' is the pseudo-first-order rate constant (in this pH range, the decomposition reaction of ANU is not catalyzed by the buffer[17]).

Figure 6 shows a typical kinetic run for the decomposition of MNU. Experiments at different pHs were carried out (Fig. 7), the slope $\alpha = 1$ revealing first order with respect to the concentration of OH$^-$.

Table 1 gives the values of k_{dec} measured for the decomposition of the five nitrosoureas investigated here. These results are consistent with those measured earlier in water medium.[17,18] Table 2 shows the values of the activation parameters $\Delta H^\#$ and $\Delta S^\#$ for the decomposition reactions.

As is known,[19,20] the existence of an isokinetic relationship can serve as an argument – but not proof – that the reactions studied share a common feature. The meaning of the isokinetic relationship is the existence of a compensation effect between the values of enthalpy, $\Delta H^\#$, and the entropy of activation, $\Delta S^\#$, such that the Gibbs' energy of activation, $\Delta G^\#$, is approximately constant.

The results shown in Fig. 8 support the idea of a common mechanism.

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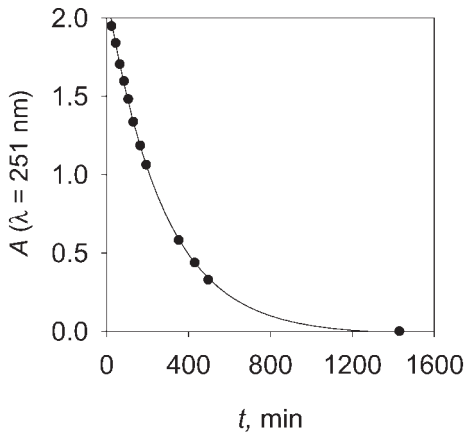

Figure 6. Decomposition of MNU at 32.5 °C in 7:3 water/dioxane medium. $[MNU]_o = 4 \times 10^{-4}$ M; pH = 6.49

Table 2. Activation parameters for decomposition reactions of *N*-alkyl-*N*-nitrosoureas in 7:3 water/dioxane medium

N-Alkyl-*N*-nitrosourea	$\Delta H^{\#}$ (kJ mol^{-1})[a]	$\Delta S^{\#}$ (J mol^{-1} K^{-1})[a]
MNU	104 ± 2	159 ± 8
ENU	116 ± 2	193 ± 6
PNU	116 ± 2	192 ± 7
BNU	113 ± 1	191 ± 5
AlNU	105 ± 2	186 ± 10

[a] Values are given with their standard deviations.

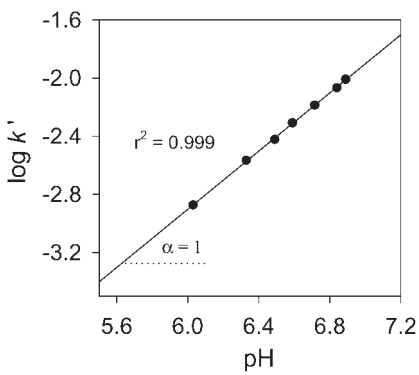

Figure 7. Variation in the MNU decomposition rate constant with the acidity of the medium. $[MNU] = 4 \times 10^{-4}$ M; $T = 32.5$ °C

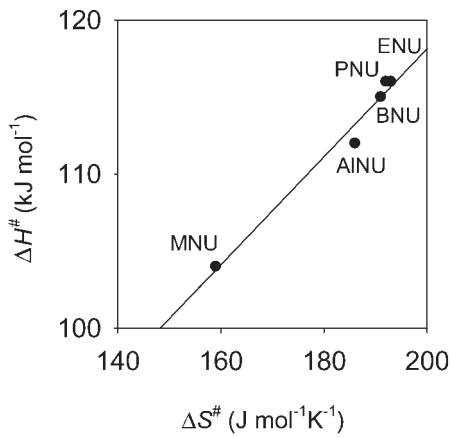

Figure 8. Isokinetic relationship in decomposition reactions of *N*-alkyl-*N*-nitrosoureas

Table 3 gives the k_{alk}/k_{H_2O} values for the alkylation/hydrolysis reactions studied here.

The results show: (i) the following sequence of reactivity (expressed as k_{alk}/k_{H_2O}, as shown in Scheme 1) for diazonium ions (directly or through alkyl cations): $MN_2^+ > EN_2^+ > AlN_2^+ > PN_2^+ > BN_2^+$; (ii) a higher value of k_{alk}/k_{H_2O} for the allyldiazonium ion as compared with those obtained for propyl- and butyldiazonium ions. This can be rationalized in terms of the electronic structure of that ion. Since unlike the propyldiazonium ion (with the same number of carbon atoms) the allyldiazonium ion has two possible electrophilic sites[21] (C1 and C3 positions), the reactivity of AlN_2^+ should be appreciably greater than that of PN_2^+ (approx. twofold greater), as was indeed observed (this assumption requires that the NBP alkylation reaction by AlN_2^+ be more sensitive to the particular structure of this ion than its competitive solvent reaction); (iii) the hydrolysis of the

Table 1. Decomposition rate constants (k_{dec} in Scheme 1) as a function of temperature for *N*-alkyl-*N*-nitrosoureas in 7:3 water/dioxane medium

T (°C)	10^{-4} k_{MNU}	10^{-4} k_{ENU}	10^{-4} k_{PNU} (M^{-1} min^{-1})[a]	10^{-4} k_{BNU}	10^{-4} k_{AlNU}
25.0	4.2 ± 0.1	2.4 ± 0.2	2.14 ± 0.03	2.56 ± 0.03	4.81 ± 0.01
27.5	5.5 ± 0.1	3.6 ± 0.2	3.1 ± 0.1	4.00 ± 0.02	7.6 ± 0.1
30.0	7.9 ± 0.1	4.5 ± 0.5	4.5 ± 0.3	5.68 ± 0.04	10.9 ± 0.2
32.5	11.7 ± 0.2	7.4 ± 0.6	7.0 ± 0.1	8.38 ± 0.07	15.0 ± 0.2
35.0	16.0 ± 0.3	12.6 ± 0.4	10.2 ± 0.3	12.4 ± 0.1	23.4 ± 0.3

[a] Values are given within the 95% confidence interval.

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Table 3. Alkylating potential of alkyldiazonium ions, expressed as the alkylation rate/hydrolysis rate ratio

Alkyldiazonium ion	k_{alk}/k_{H_2O} (35 °C)[a]
Methyl	12 878 ± 102
Ethyl	10 809 ± 179
Propyl	4691 ± 61
Butyl	3204 ± 117
Allyl	8169 ± 167

[a] Values given with their standard deviations.

alkyldiazonium ions is not sufficiently effective to prevent alkylation; (iv) the kinetic results are consistent with the known fact that the biological activity of ANU decreases when their molecular size increases, hindered ANU being biologically inactive.[3,22]

In order to analyze the influence of steric and polar effects on the relative alkylating potential of alkyldiazonium ions (expressed as k_{alk}/k_{H_2O}), the Ingold–Taft correlation equation[23] was applied:

$$\log \frac{(k_{alk}/k_{H_2O})_{RN_2^+}}{(k_{alk}/k_{H_2O})_{CH_3N_2^+}} = \sigma^* \rho^* + E_s \qquad (10)$$

The values for σ^* (polar constant) and E_s (steric constant) were taken from the literature.[24,25]

Correlations with E_s, on the one hand, and with σ^*, on the other, were used to check whether these reactions were controlled mainly by steric or mainly by polar effects. Figure 9 shows the respective plots, together with the plot representing the influence of steric and polar effects jointly (Eqn (10)).

The results show that: (i) owing to the positive sign of the reaction constant ρ^* the NBP alkylation reactions by RN_2^+ must occur through nucleophilic attack; (ii) these reactions are mainly controlled by the size of the alkyl R groups; (iii) the deviation

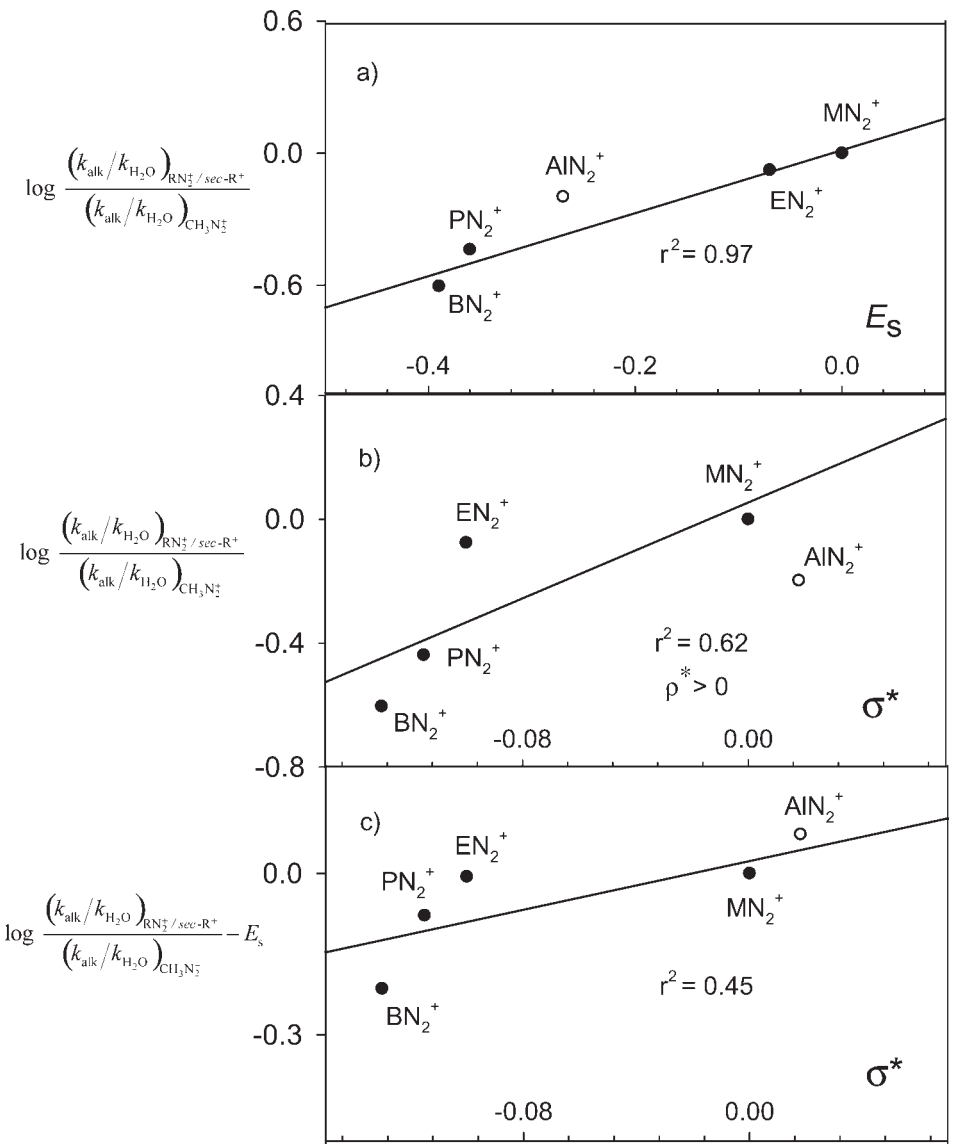

Figure 9. Influence of (a) steric; (b) polar, and (c) steric + polar effects on the reactivity of alkyldiazonium ions as alkylating agents

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Table 4. Molar absorption coefficients of the NBP-R adducts

NBP-R adduct	λ (nm)	ε ($M^{-1}\,cm^{-1}$)
NBP-Me	573	521 ± 30
NBP-Al	557	282 ± 20
NBP-Et	570	204 ± 8
NBP-Bu	570	93 ± 11
NBP-Pr	570	65 ± 8

shown by the allyldiazonium ion is quite understandable due to its above-cited particular electronic structure.

Molar absorption coefficients of the NBP-R adducts

We were also interested in knowing the molar absorption coefficients (ε) of the NBP-R adducts. Knowledge of these values should permit easy determination of the concentration of adducts by simply measuring the absorbance.

Experiments were performed with [NBP] = 0.02 M and ANU concentrations in the 4×10^{-4}–1.5×10^{-3} M range.

The correlations between $A_\infty - A_o$ values and those of $[ANU]_o$ give the values of the molar absorption coefficients shown in Table 4.

The structures of the NBP-R adducts obtained by geometry optimization (as shown in Experimental Section) revealed (Fig. 10) that the values of ε increase when the size of RN_2^+ decreases (the second highest value for NBP-Al must be due to the particular electronic structure of the allyl group; refer to comments given above). The greater the non-planarity in the NBP-R adducts, disrupting the π-electron cloud to interlink the two phenyl rings, the smaller the values of ε (Fig. 11).

A similar argument can be invoked to rationalize the fact that the biological activity of ANUs decreases when their molecular size increases:[3,22] the smaller the alkyl group of the diazonium ions, the stronger the linkage between DNA nucleophile sites and the electrophilic alkyldiazonium ions.

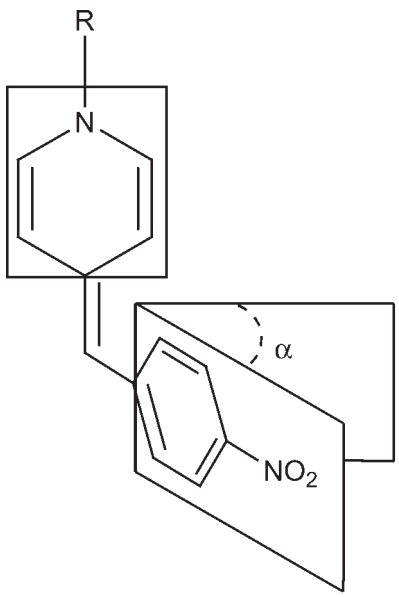

Figure 10. Lack of coplanarity in the NBP-R adducts

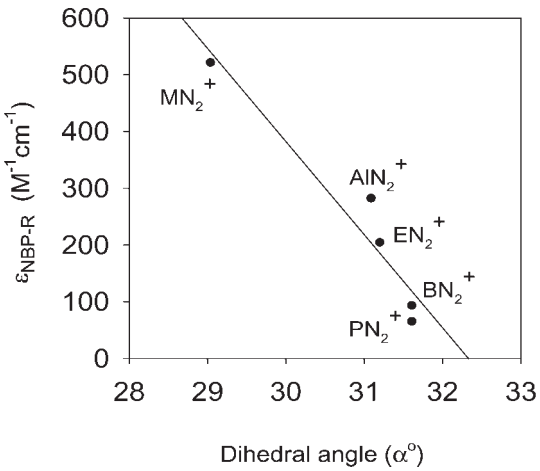

Figure 11. Correlation between the structure of NBP-R adducts and their molar absorption coefficients, ε

CONCLUSIONS

- The alkylation reactions of the nucleophile NBP by *N*-alkyl-*N*-nitrosoureas occur through a mechanism that includes the decomposition of ANU to alkyldiazonium ions (RN_2^+) that yield NBP-R adducts directly or through carbocations in certain instances.
- The sequence of reactivity of RN_2^+ ions as direct/indirect NBP alkylating agents is: methyl- > ethyl- > allyl- > propyl- > butyldiazonium ion. Application of Ingold–Taft treatment to the kinetic results revealed that the NBP alkylation reactions by diazonium ions occur under steric control. This may explain why the biological activity of ANU decreases when their molecular size increases (hindered ANUs are biologically inactive).
- The values of the molar absorption coefficients for the NBP-R adducts also reveal the determinant influence of steric effect in the formation of alkylation adducts.

EXPERIMENTAL

General remarks

A Shimadzu UV-2401-PC spectrophotometer with a thermoelectric six-cell holder temperature control system (±0.1 °C) was used.

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 (Millipore).

All kinetic runs were performed in triplicate.

Numerical treatment of the data was performed using the 7.1.44 Data Fit software. Geometry optimization of the NBP-R adducts was carried out with the Chem3D Ultra Molecular Modeling and Analysis software, version 9.0, and with Gaussian 03W Client Pro 9.0. The PM3 semiempirical method was used.

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Procedures: synthesis of *N*-alkyl-*N*-nitrosoureas

N-Methyl-*N*-nitrosourea and *N*-ethyl-*N*-nitrosourea were obtained from Sigma.

N-Propyl-*N*-nitrosourea, *N*-butyl-*N*-nitrosourea, and *N*-allyl-*N*-nitrosourea were prepared from the respective *N*-alkylureas. *N*-butylurea was obtained from Fluka while *N*-propylurea and *N*-allylurea were Alfa and Merck products, respectively.

PNU, BNU, and AlNU were prepared as by Werner.[26] The respective *N*-alkylureas (approx. 1 g) were dissolved in water and 10 g of sodium nitrite was added. The mixture was cooled to below 0 °C and ice-cold sulfuric acid (10%) was added dropwise during continuous stirring. The nitrosoureas precipitated as yellow crystals. After vacuum filtration, the crystals collected were repeatedly washed with cool water and then desiccated.

Acknowledgements

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