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Alkylating Potential of Styrene Oxide: Reactions and Factors Involved in the Alkylation Process

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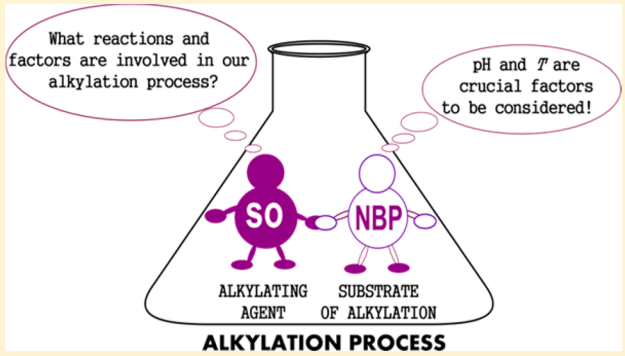
ABSTRACT: The chemical reactivity of styrene-7,8-oxide (SO), an alkylating agent with high affinity for the guanine−N7 position and a probable carcinogen for humans, with 4-(*p*-nitrobenzyl)pyridine (NBP), a trap for alkylating agents with nucleophilic characteristics similar to those of DNA bases, was investigated kinetically in water/dioxane media. UV−vis spectrophotometry and ultrafast liquid chromatography were used to monitor the reactions involved. It was found that in the alkylation process four reactions occur simultaneously: (a) the formation of a β-NBP−SO adduct through an S_N2 mechanism; (b) the acid-catalyzed formation of the stable α-NBP−SO adduct through an S_N2' mechanism; (c) the base-catalyzed hydrolysis of the β-adduct, and (d) the acid-catalyzed hydrolysis of SO. At 37.5 °C and pH = 7.0 (in 7:3 water/dioxane medium), the values of the respective reaction rate constants were as follows: $k_{alk\beta} = (2.1 \pm 0.3) \times 10^{-4}$ M^{-1} s^{-1}, $k_{alk\alpha} = (1.0 \pm 0.1) \times 10^{-4}$ M^{-1} s^{-1}, $k_{hyd}^{AD} = (3.06 \pm 0.09) \times 10^{-6}$ s^{-1}, and $k_{hyd} = (4.2 \pm 0.9) \times 10^{-6}$ s^{-1}. These values show that, in order to determine the alkylating potential of SO, none of the four reactions involved can be neglected. Temperature and pH were found to exert a strong influence on the values of some parameters that may be useful to investigate possible chemicobiological correlations (e.g., in the pH 5.81−7.69 range, the fraction of total adducts formed increased from 24% to 90% of the initial SO, whereas the adduct lifetime of the unstable β-adduct, which gives an idea of the permanence of the adduct over time, decreased from 32358 to 13313 min). A consequence of these results is that the conclusions drawn in studies addressing alkylation reactions at temperatures and/or pH far from those of biological conditions should be considered with some reserve.



INTRODUCTION

The reactivity of styrene-7,8-oxide (SO) has been the subject of intense research from 1900 to the present, mainly due to its industrial applications and biological activity. This oxirane is widely used as a reactive plasticizer or diluent for epoxy resins, in the production of phenethyl alcohol and styrene glycol and its derivatives, and as a precursor for cosmetics, surface coatings, and agricultural and biological chemicals.[1,2] It is also the main *in vivo* mutagenic metabolite of styrene, a compound extensively used in the production of plastic, rubber, fiberglass, pipes, and food containers.[3] Thus, humans and other living organisms are exposed to SO, whose carcinogenic, neurotoxic, and mutagenic effects are well-known.[4]

SO is a potent alkylating agent with the capacity to form adducts *in vivo* and *in vitro* with DNA bases, mainly at the N7-position of guanine.[5] It has been classified as probable carcinogen for humans (2A IARC).[2]

In contrast to other epoxides that only react with nucleophiles through their less hindered carbon, SO can achieve this through the two electrophilic carbons of the oxirane ring, the primary (β) and the secondary (α),[5−10] to form the corresponding adducts.

Regarding the chemical reactivity and biological effectiveness of SO with different nucleophiles, including DNA bases, there are abundant references in the literature.[6,9,11−16] In spite of this, many authors overlook some reactions that may be involved in the alkylation mechanism and, hence, in the alkylation efficacy of SO, such as (a) the potential formation of more than one adduct between SO and the alkylation substrate[5−10,17−20] and (b) the solvolysis reactions of the SO and of the adducts formed.[19] Accordingly, in the present study the chemical reactivity and alkylating potential of SO were investigated in depth. The molecule 4-(*p*-nitrobenzyl)pyridine (NBP), which has several advantages as an alkylation substrate,[21] was used as a nucleophilicity model for DNA.

EXPERIMENTAL PROCEDURES

Styrene oxide (99%), 4-(*p*-nitrobenzyl)pyridine (98%), and triethylamine (Et_3N 99%) were purchased from Sigma-Aldrich (Steinheim, Germany). 1,4-Dioxane (henceforth dioxane) was from Panreac (Barcelona, Spain).

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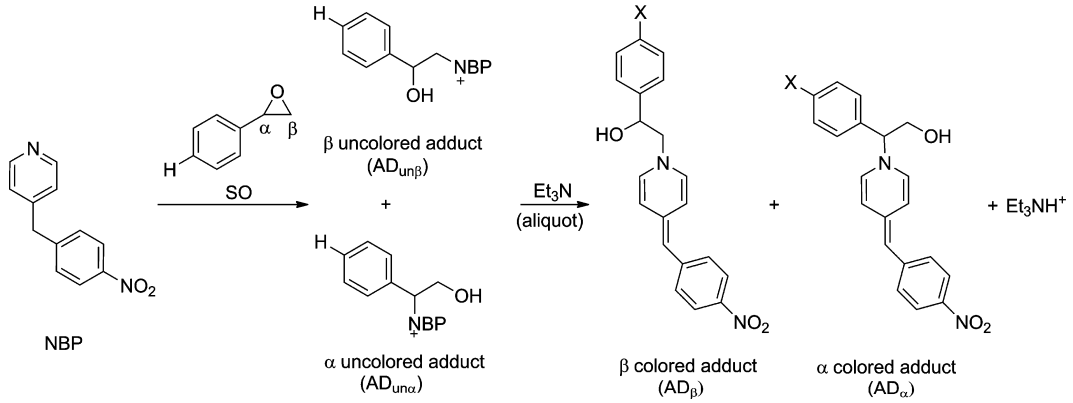
Scheme 1. Alkylation Mechanism of NBP by SO



Caution: Styrene oxide is hazardous and should be handled carefully.

To obtain deionize water, a Milli Q-Gradient (Millipore) system was used. Absorbance measurements were made with a Shimadzu UV-2401-PC spectrophotometer with a thermoelectric six-cell holder temperature control system (±0.1 °C). For pH measurements, a Metrohm 827 pH-meter with a glass electrode was used. The reaction temperature was kept constant (±0.05 °C) with a Lauda Ecoline RE120 thermostat. All kinetic runs were performed in triplicate.

The alkylation reaction of NBP by SO was carried out (i) in an excess of NBP to inhibit the formation of bis-alkylation adducts (1:2 NBP:SO) and to work in pseudo-first-order conditions and (ii) in water/dioxane medium, to render the NBP soluble. Alkylation mixtures were prepared by adding 1.0 mL of 0.005−0.03 M epoxide stock solution (SO in dioxane) to 100 mL of NBP solution (0.01−0.02 M) in water/dioxane medium. Acetate, phosphate, and borate buffers were used when needed to adjust the pH of the alkylation mixture.

Two different complementary techniques were used to investigate the formation and characterization of the SO-NBP adducts kinetically:

Vis Spectrophotometry. At different times, aliquots (2.4 mL) of the alkylation mixture (SO + NBP in a water/dioxane medium) were collected and placed in a cuvette containing 0.6 mL of Et_3N to induce the deprotonation of the uncolored adducts (AD$_{unα}$ and AD$_{unβ}$), yielding the SO-NBP colored adducts (AD$_α$ and AD$_β$; Scheme 1), whose absorbance was measured at λ= 560 nm, where only both adducts absorb. Detailed experimental conditions are given in the figure and table legends. Rate constants were obtained by nonlinear regression analysis of the absorbance/time data.

HPLC. A gradient-controlled Shimadzu ultrafast liquid chromatography system equipped with a diode array detector (UFLC-DAD) was used to monitor the formation of each adduct. Chromatographic separation was achieved with a Mediterranea Sea C18 column (25 cm × 1 cm, 5 μm). Mobile phase A was acetate buffer (pH = 4.75 and 0.1 M), and mobile phase B was acetonitrile. 10% B was held for 5 min, after which a gradient from 10% B up to 16% B was run over 8 min. Then, a gradient of up to 80% B was run over 1 min. 80% B was held for 14 min. The flow rate was set at 1.0 mL min^{-1}, and 200 μL of the alkylation mixture was injected.

The hydrolysis reaction of SO was monitored by UV spectrophotometry. Aliquots of 50−200 μL of the stock solution of the epoxide in dioxane (0.085 M) were added to a thermostated cuvette containing 3.0 mL of the reaction mixture ($HClO_4$ + water + dioxane) at a constant pH. To convert the pH-meter output obtained in water/dioxane mixtures into hydrogen ion concentrations, it is necessary to use a correction factor.[22−24] However, with the solutions studied in this work (7:3 water:dioxane and [$HClO_4$] = 0.1−0.02 M), this correction can be neglected.[24]

Absorbance at λ= 226 nm, where the difference between the signals of SO and its hydrolysis product (styrene glycol, SG)[16] is maximum, was measured until no change was observed.

RESULTS AND DISCUSSION

Reaction Mechanism. In order to determine the reactions, besides SO hydrolysis, involved in the alkylation mechanism, UFLC-DAD analysis was performed. The results obtained revealed the formation of two SO−NBP adducts with different retention times (see Scheme 1). This result is in agreement with their distinctive fragmentation patterns, obtained by analysis of the alkylation mixtures by mass spectrometry in an excess of NBP (m/z = 335, 317, 304 for α-adduct, and m/z = 335, 121 for β-adduct), as well as with previous results obtained with other nucleophiles.[6,13,19,20,25]

Assignment of the UFLC-DAD peaks was performed by comparison of their retention times, UV−vis spectra, and kinetic profiles with those of the β-adduct formed between NBP and *p*-nitrostyrene oxide, a substituted styrene oxide whose alkylating potential has been studied by us previously.[26] The adducts identified were α-NBP−SO (AD$_{unα}$), t_R = 11.0 min, and β-NBP−SO (AD$_{unβ}$), t_R = 8.5 min (see Scheme 1). These two types of adduct also result in alkylation at the N-7 position in the reaction between SO and guanosine, deoxyguanosine, and the single- or double-stranded DNA *in vitro* under physiological conditions.[5,7,8,18]

Figure 1 shows the profile of a typical kinetic run with UFLC-DAD, carried out by monitoring the area of the AD$_{unα}$ and AD$_{unβ}$ peaks over time. As can be observed, while the AD$_{unα}$ signal remains fairly stable along the reaction time, AD$_{unβ}$ undergoes hydrolysis.

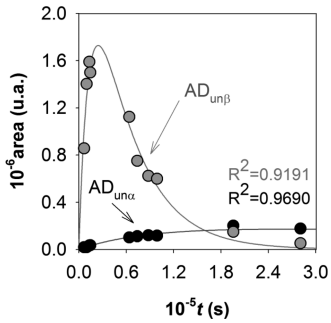


Figure 1. Kinetic profiles obtained by monitoring the variation in the UFLC-DAD peak area, AD$_{unβ}$ (gray circles) and AD$_{unα}$ (black circles), over time in 7:3 (v:v) water/dioxane media. T = 37.5 °C; pH = 7.50; [SO]$_o$ = 1.7 × 10^{-4} M; [NBP]$_o$ = 2.0 × 10^{-2} M; λ = 254 nm.

It should be pointed out that depending on the reaction rates involved in the NBP alkylation process, two different

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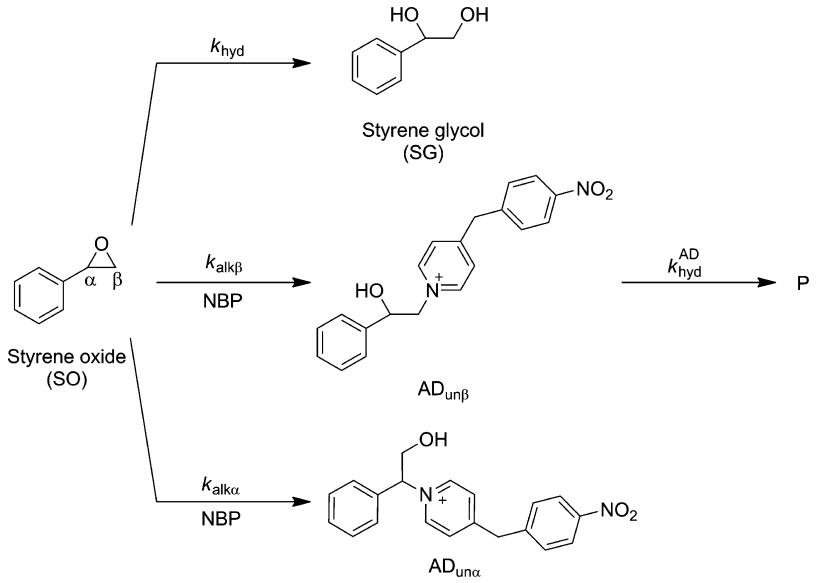
Scheme 2. Reactions Involved in NBP Alkylation by SO in Aquo−Organic Media



approaches can be used: (i) considering only the alkylation reaction; (ii) taking into account all the reactions occurring simultaneously. Since abundant instances of the use of a poor mechanistic framework to determine the alkylating potential of SO can be found in the literature,[5−10,17−20] in our case the second approach was applied (Scheme 2) and included the following: (*a*) the formation of the *α*-NBP−SO and *β*-NBP−SO adducts; (*b*) hydrolysis of the *β*-adduct (the *α*-adduct remained stable along the reaction); and (*c*) SO hydrolysis.

Equations 1−3 can be readily deduced from the mechanism depicted in Scheme 2. In these equations, k_{alk} is the global alkylation rate constant, defined as the sum of the second-order rate constants for alkylation through the *α* and *β* carbons, $k_{alkα}$ and $k_{alkβ}$, respectively. Since NBP is present in a large excess, its concentration can be assumed to be constant, i.e. [NBP] = $[NBP]_o$.

$$r = \frac{d[AD_{un\alpha}]}{dt} = k_{alk\alpha}[NBP][SO] \quad (1)$$

$$r = \frac{d[AD_{un\beta}]}{dt} = k_{alk\beta}[NBP][SO] - k_{hyd}^{AD}[AD_{un\beta}] \quad (2)$$

$$r = \frac{-d[SO]}{dt} = [SO](k_{alk}[NBP] + k_{hyd}) \quad (3)$$

Rate constants were determined using the NBP test[21] (see Scheme 1) by monitoring the variation in absorbance at λ = 560 nm (see above).

Absorbance at time *t* (A_{AD}) can be expressed as the sum of the absorbances of the colored *β*- and *α*-adducts:

$$A_{AD} = A_{AD\beta} + A_{AD\alpha} \quad (4)$$

The absorbance of each adduct at time *t* was obtained from eqs 1 and 2 by applying the Lambert−Beer law to the respective integrated rate equations in terms of concentration:

$$A_{AD\alpha} = \frac{\varepsilon_\alpha l k_{alk\alpha}[NBP][SO]_o}{k_{alk}[NBP] + k_{hyd}}(1 - e^{-(k_{alk}[NBP]+k_{hyd})t}) \quad (5)$$

$$A_{AD\beta} = \frac{\varepsilon_\beta l k_{alk\beta}[NBP][SO]_o}{(k_{alk}[NBP] + k_{hyd}) - k_{hyd}^{AD}}(e^{-(k_{hyd}^{AD})t} - e^{-(k_{alk}[NBP]+k_{hyd})t}) \quad (6)$$

Equation 4 can thus be written as follows:

$$A_{AD} = \frac{a}{b-c}(e^{-ct} - e^{-bt}) + \frac{d}{b}(1 - e^{-bt}) \quad (7)$$

with *a*, *b*, *c*, and *d* being parameters obtained by the nonlinear fitting of the A_{AD}/t data and defined as

$$a = k_{alk\beta}[NBP][SO]_o\varepsilon_\beta l; \quad b = k_{alk}[NBP] + k_{hyd}; \quad c = k_{hyd}^{AD}; \quad d = k_{alk\alpha}[NBP][SO]_o\varepsilon_\alpha l \quad (8)$$

The goodness of the data fit to eq 7, where the parameters *a* and *d* include a first-order reaction dependence on [SO] and [NBP], while the parameter *b* includes a first-order dependence on [NBP], reveals that the formation of the *α*- and *β*-adducts occurs through S_N2' and S_N2 mechanisms, as expected.[6] This, together with the preference of SO for the N7-position of guanine over other nucleophile positions of the DNA bases,[7,8,13,18,27] suggests that NBP is a suitable model to investigate the reactivity of SO with DNA.[21]

The good fitting of the experimental A_{AD}/t data obtained under neutral conditions (Figure 2) to eq 7 supports the proposed mechanism.

Determination of the Kinetic Parameters Involved in the NBP Alkylation Process. As regards the global alkylation rate constant, k_{alk} (eq 3), the value of this was obtained from the slope of the *b*/[NBP] plot ($b = k_{alk}[NBP] + k_{hyd}$), k_{alk} = (3.1 ± 0.4) × 10^{-4} M^{-1} s^{-1} (37.5 °C, pH 7.0). Equation 9 was used to determine $k_{alkβ}$ = (2.1 ± 0.3) × 10^{-4} M^{-1} s^{-1}, and hence $k_{alkα}$ = (1.0 ± 0.1)·10^{-4} M^{-1} s^{-1} (37.5 °C, pH 7.0), assuming the ratio $k_{alkα}/k_{alkβ}$ = 33/67 (obtained previously for the reaction of SO with deoxyguanosine in water/ethanol, 50:50 (v:v), at 37.0 °C)[19] for the alkylation of NBP by SO at 37.5 °C. This value was accepted, since it is known that in protic solvents the $k_{alkα}/k_{alkβ}$ ratio is solvent-independent[11] and is similar for the alkylation of substrates analogous to

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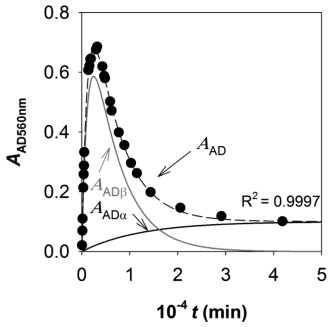

Figure 2. Experimental A_{AD}/t kinetic profiles in the formation of the SO−NBP adducts in 7:3 water/dioxane media (black circles), nonlinear fitting to eq 7 (dashed line), and the corresponding profiles calculated from eqs 5 and 6 for the α-NBP−SO (black line) and β-NBP−SO (gray line) adducts, respectively. λ = 560 nm; T = 37.5 °C; pH = 7.0; $[NBP]_o = 1.99 \times 10^{-2}$ M; $[SO]_o = 7.69 \times 10^{-5}$ M.

guanosine[18−20] by *p*-substituted styrene oxides at the same temperature.

$$k_{alk\beta} = \frac{k_{alk}}{(k_{alk\alpha}/k_{alk\beta}) + 1} \quad (9)$$

With the values of the alkylation rate constants and those of the parameters a and d, the values of ε_β and ε_α (λ = 560 nm) were obtained (eqs 10 and 11): $\varepsilon_\beta = (8 \pm 1) \times 10^3$ M^{-1} cm^{-1} and $\varepsilon_\alpha = (40 \pm 8) \times 10^3$ M^{-1} cm^{-1}, respectively.

$$\varepsilon_\beta = \frac{a}{k_{alk\beta} l [SO]_o [NBP]} \quad (10)$$

$$\varepsilon_\alpha = \frac{d}{k_{alk\alpha} l [SO]_o [NBP]} \quad (11)$$

Regarding the SO hydrolysis reaction, its rate constant was obtained: (i) indirectly, by using the values of parameters b and k_{alk} (eq 12), and (ii) directly, by spectrophotometric measurements (see Experimental Procedures).

$$k_{hyd} = b - k_{alk}[NBP] \quad (12)$$

Since the absorbance, A, of the hydrolysis reaction mixture at time t is the sum of the resulting styrene glycol (SG)[16] and epoxide contributions, eq 13 can be written.

$$A = l[SO]_o(\varepsilon_{SG} + (\varepsilon_{SO} - \varepsilon_{SG})e^{-k_{hyd}t}) \quad (13)$$

Excellent fitting of the experimental data to eq 13 was obtained (Figure 3a). In addition, the SO hydrolysis rate constants determined from eq 13 revealed a high degree of convergence with respect to those calculated from the b parameter (Figure 3b). This supports the methodology used to determine the reaction rate constants involved in the alkylation process.

The values of the β-NBP−SO adduct hydrolysis rate constant, k_{hyd}^{AD} (see Table 1), are given directly by parameter c (eq 8).

The values of the fitting parameters a, b, c, and d, as well as those of $k_{alk\beta}$, $k_{alk\alpha}$, k_{hyd}, and k_{hyd}^{AD}, were used to determine some associated kinetic parameters that may be useful to investigate possible correlations between chemical reactivity and biological activity. Three such useful parameters are the fraction of the alkylating agent that eventually forms the α- and β-adducts, f_α and f_β, respectively (eqs 14 and 15), and the adduct lifetime, AL. The sum $f = f_\alpha + f_\beta$ ranges from 0 to 1, with 0 meaning that

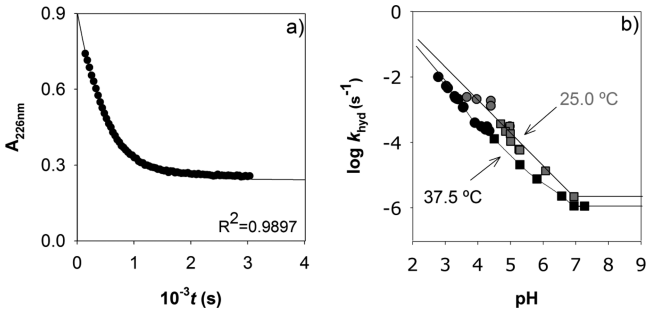

Figure 3. (a) Kinetic profile of a typical kinetic run for SO hydrolysis (experimental data (circles) and fitting to eq 13 (continuous line)). (b) Variation in the SO hydrolysis rate constant with the acidity of the medium in 7:3 (v:v) water/dioxane media obtained directly (circles) and indirectly (squares) at 25.0 °C (gray circles and squares) and 37.5 °C (black circles and squares), respectively.

Table 1. Influence of Temperature on the Rate Constants of the Reactions Involved in the Alkylation of NBP by SO and Values of Some of the Associated Kinetic Parameters[a]

T (°C)	25.0	27.5	30.0	32.5	35.0	37.5
$10^4 k_{alk\beta}$ (M^{-1} s^{-1})	0.7	0.9	1.1	1.3	1.5	2.1
$10^4 k_{alk\alpha}$ (M^{-1} s^{-1})	0.2	0.3	0.5	0.4	0.7	1.0
$10^4 k_{alk}$ (M^{-1} s^{-1})	0.9	1.2	1.6	1.7	2.2	3.1
$10^6 k_{hyd}$ (s^{-1})	1.0	0.8	0.8	0.8	2.8	4.2
$10^6 k_{hyd}^{AD}$ (s^{-1})	0.5	0.6	1.0	1.5	1.9	3.1
f_β	0.50	0.57	0.54	0.62	0.42	0.40
f_α	0.13	0.18	0.24	0.19	0.19	0.20
f	0.63	0.75	0.79	0.81	0.61	0.60
AL_β (min)	17377	15209	8419	6810	3622	2185

[a] 7:3 (v:v) water/dioxane media; pH = 7.0.

all the initial epoxide is hydrolyzed and with 1 meaning that the epoxide reacts exclusively with the NBP, yielding alkylation adducts.[21] These parameters have been correlated previously with the mutagenic potency of different compounds.[21,26,28−31]

The adduct lifetime gives an idea of the permanence of the adduct over time. Thus, it is only meaningful for the unstable β-adduct. AL_β is defined as the area under the curve $[AD_\beta]/t$ per unit of alkylating agent concentration (eq 16).[21,26,28−31] Consequently, it can be calculated from the area under the curve $A_{AD\beta}/t$ (i.e., Figure 2), as shown in eq 16. Calculation of this parameter is based on the rate constants of the three reactions involved, k_{hyd}, k_{alk}, and k_{hyd}^{AD}. As may be seen from eq 16, AL_β is also related to the alkylating efficacy, f_β (i.e., the higher the f_β value and the lower the adduct hydrolysis rate constant, the higher the AL_β).

$$f_\alpha = \frac{k_{alk\alpha}[NBP]}{(k_{alk}[NBP] + k_{hyd})} = \frac{k_{alk\alpha}[NBP]}{b} \quad (14)$$

$$f_\beta = \frac{k_{alk\beta}[NBP]}{(k_{alk}[NBP] + k_{hyd})} = \frac{k_{alk\beta}[NBP]}{b} \quad (15)$$

$$AL_\beta = \frac{\int_{t=0}^{t=\infty} [AD]_\beta \, dt}{[SO]_o} = \frac{\int_{t=0}^{t=\infty} A_{AD\beta} \, dt}{\varepsilon_\beta l [SO]_o} = \frac{k_{alk\beta}[NBP]}{(k_{alk}[NBP] + k_{hyd})k_{hyd}^{AD}} = \frac{f_\beta}{k_{hyd}^{AD}} = \frac{f_\beta}{c} \quad (16)$$

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Influence of Temperature on the Kinetic Parameters Involved in the Alkylation Process. Table 1 shows the values of the above parameters determined in the 25.0−37.5 °C range.

As can be observed, whereas the values of f_α and f_β do not show any clear trend with temperature, AL_β is strongly influenced by it, being higher at lower temperatures. This means that the stability of the β-adduct is higher at lower temperatures; that is, a greater accumulation of the β-adduct occurs with time.

It can also be seen that the $k_{alk\beta}/k_{alk\alpha}$ ratio is inversely related to temperature, showing an increase from 67/33 to 78/22 with a decrease in temperature from 37.5 to 25.0 °C. This trend, which seems to be common for the reaction of SO with nucleophiles similar to NBP in protic solvents,[6] highlights the relevant role played by temperature on the alkylating capacity of SO. This result also suggests that any conclusions drawn about the chemicobiological correlations established from alkylation reactions with styrene oxide at temperatures far from those of biological conditions should be taken with some reserve.

The activation parameters calculated with the Arrhenius and Eyring−Wynne−Jones equations, $E_{a\beta}$ = (64 ± 4) kJ mol^{-1}; $E_{a\alpha}$ = (99 ± 5) kJ mol^{-1}; $\Delta G^{\#}_{\beta(25.0\ °C)}$ = (96 ± 8) kJ mol^{-1}; and $\Delta G^{\#}_{\alpha(25.0\ °C)}$ = (99 ± 19) kJ mol^{-1}, revealed that the formation of the activated intermediate state of the α-adduct is enthalpy-controlled whereas that of the β-adduct is not.

Influence of the Acidity of the Medium on the Kinetic Parameters Involved in the Alkylation Process. A series of experiments was performed in media of different acidities in the 5.0 < pH < 8.0 range, because experimental limitations did not allow the reaction rate constants from eqs 7 and 8 to be determined outside this range. (Since at pH < 5.0 the values of the hydrolysis rate constant are equal to or higher than those of the pseudo-first-order alkylation rate constant, calculation of the individual values is not possible. At pH > 7.7 some unidentified byproducts appear.) For the reaction at the β carbon of SO, it was observed that the alkylation rate constant was not subject to any influence of pH. The β-NBP−SO adduct was significantly hydrolyzed in neutral aquo-organic media (Table 2), whereas the hydrolysis of the β-adduct is basic-catalyzed and can be considered negligible in acid aquo-organic media (Table 2), as occurs with other oxiranes.[26,30] In contrast, alkylation through the α carbon is acid-catalyzed (Table 2), with the protonated transition state probably being favored by the positive charge stabilization on that atom,[9,32] such that the α-NBP−SO adduct formed remains stable along the reaction (Figure 2). It should be noted that although consideration of the stabilities of the adducts formed by SO is important, in the literature they are usually overlooked.[7,27,33]

Table 2. Influence of pH on the Rate Constants of the Reactions Involved in the Alkylation of NBP by SO and Associated Kinetic Parameters[a]

pH	5.29	5.81	6.57	6.95	7.27	7.69
$10^5 k_{alk\beta}$ (M^{-1} s^{-1})	7.30	7.15	7.09	6.98	7.29	7.20
$10^5 k_{alk\alpha}$ (M^{-1} s^{-1})		4.27	1.94	1.81	0.71	0.58
$10^5 k_{alk}$ (M^{-1} s^{-1})		11.42	9.03	8.79	8.00	7.78
$10^6 k_{hyd}$ (s^{-1})	20.89	7.08	2.03	1.05	0.89	0.19
$10^7 k^{AD}_{hyd}$ (s^{-1})		0.79	2.59	4.81	8.44	10.43
f_β	0.07	0.15	0.37	0.50	0.59	0.83
f_α		0.09	0.10	0.13	0.06	0.07
f		0.24	0.47	0.63	0.65	0.90
AL (min)		32358	23874	17377	11635	13313

[a] 7:3 water/dioxane media; T = 25.0 °C.

The hydrolysis of styrene oxide is also influenced by the acidity of the medium. In the pH 2−8 range, the rate constant k_{hyd} can be expressed as the sum of the spontaneous and catalytic hydrolysis rate constants (eq 17).

$$k_{hyd} = k^{\circ}_{hyd} + k_{H^+}[H^+] \quad (17)$$

In 7:3 water/dioxane medium the values of k_{hyd} at 37.5 and 25.0 °C are given by the expressions $k_{hyd37.5}$ = (2.5 ± 0.8) × 10^{-6} + (18 ± 1) [H^+] (s^{-1}) and $k_{hyd25.0}$ = (1.0 ± 0.1) × 10^{-6} + (6.5 ± 0.5) [H^+] (s^{-1}) (Figure 3b).

The reaction order with respect to [H^+], determined in pH < 5 acidic media at 25.0 and 37.5 °C, was found to be one.

The importance of the concurrent SO hydrolysis in the study of NBP alkylation by the epoxide is such that at pH < 5 we observed that the value of the above parameter $b = k_{alk}$[NBP] + k_{hyd} gave the value of k_{hyd} directly because it was significantly higher than that of k_{alk}[NBP]. In spite of this, in the literature it is unusual to find studies that take SO hydrolysis into account (see above).

As can be observed (Table 2), all the associated kinetic parameters are clearly influenced by the acidity of the medium, except f_α. This means that a small fraction of the initial SO, around 9−13%, is converted into α-adduct regardless of the pH of the medium. Additionally, the fraction of β-adduct formed is substantially influenced by pH and is higher than that of the α-adduct. Thus, the fraction of total adducts formed increases from 24% to 90% of the initial SO in the pH 5.81−7.69 range. This means that in acid media SO hydrolysis is predominant, whereas in neutral−basic media adduct formation predominates, particularly that of the β-adduct (the $k_{alk\beta}/k_{alk\alpha}$ ratio increases from 63/37 to 93/7 due to an increase in the pH of the medium from 5.8 to 7.7). This is important because it could be related to the alkylating activity of styrene oxide in the neutral biological medium. The results highlight the importance of choosing the appropriate kinetic approach and of considering all the reactions involved in the alkylation process. Neglecting them would lead to mistaken conclusions about the SO alkylating potential. Despite this, scrutiny of the literature shows how frequently this is the case;[5−10,17−20] that is, when using the NBP test or another spectroscopic method, the increase in absorbance at a fixed time is considered to be a measure of the reactivity or alkylating potential of SO[34−37] (this alkylating potential has sometimes even been used to establish correlations between chemical and biological activity).[34,36]

Another interesting result is that although the f_β value declines with the acidity of the medium, β-adduct stability clearly increases with it.

A consequence of the above is the strong influence of the acidity of the medium on the reactivity of styrene oxide as an alkylating agent, which underscores the importance of this analysis in the search for possible chemicobiological correlations.

Influence of the Composition of the Reaction Medium on the Alkylation Process. Figure 4a shows the strong dependence of $k_{alk\beta}$ and $k_{alk\alpha}$ on the dielectric constant, D, of the reaction medium in different water/dioxane mixtures,

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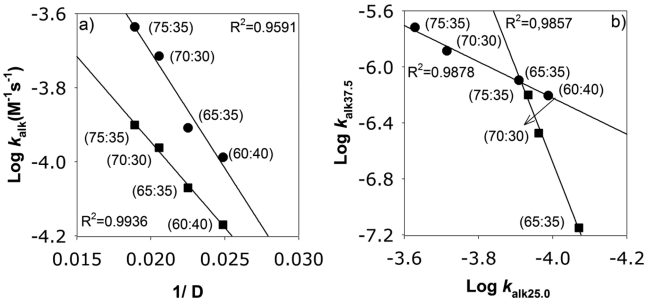

Figure 4. (a) Variation in the alkylation rate constants (37.5 °C) with the dielectric constant of the reaction medium, and (b) isokinetic correlations in the formation of both the α- and β-adducts (black squares and black circles, respectively) in media of different composition.

with the alkylation rate increasing upon increasing D. This behavior, consistent with a reaction between polar molecules in which the activated complex is more polar than the reagents,[38] supports the proposed mechanism.

As is known,[39] the existence of an isokinetic relationship can serve to defend the argument that the reactions of a series share a common mechanism. The members may differ in the identities of a functional group, the length of a side chain, the composition of the solvent, and so on. In this work we considered the solvent composition, that is, the percentage of dioxane in the reaction medium. A mathematical formulation of the isokinetic effect is the linear relationship between two series of log k_{alk} values measured at two temperatures $T1$ and $T2$: log $k_{alk}(T2) = a + b \log k_{alk}(T1)$.

It should be noted that the meaning of the isokinetic relationship is the existence of a *compensation effect* between the values of the enthalpy, $\Delta H^{\#}$, and entropy of activation, $\Delta S^{\#}$, such that the Gibbs free energy of activation, $\Delta G^{\#}$, is approximately constant. The results shown in Figure 4b support the idea of common mechanisms of formation of both the α- and β-SO−NBP adducts, respectively bimolecular nucleophilic S_N2' and S_N2 substitutions, across the whole composition range of the solvent mixture.

CONCLUSIONS

(i) NBP alkylation reactions through the α and β carbons of SO occur via $S_N2'$and S_N2 mechanisms, respectively.

(ii) Neglecting some of the reactions involved in the alkylation process would lead to mistaken conclusions about the alkylating potential of SO.

(iii) Temperature and pH are crucial factors because: (*a*) The lifetime of the unstable β-adduct is strongly influenced by temperature, and hence, the accumulation of this adduct is greater at lower temperatures; (*b*) The hydrolysis of the β-NBP−SO adduct is basic-catalyzed. The formation of the α-NBP−SO adduct is acid-catalyzed, with the α-NBP−SO adduct remaining stable along the reaction. The hydrolysis of SO is acid-catalyzed.

(iv) Any conclusions drawn in studies addressing alkylation reactions at temperature and/or pH far from those found in biological conditions should be considered with some reserve when searching for chemicobiological correlations.

(v) The existence of an isokinetic relationship between alkylation rate constants in different water/dioxane ratios reveals a compensation effect that supports the idea of common mechanisms of formation of both adducts across the whole composition range of the solvent mixture.

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Notes

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ABBREVIATIONS

A_{AD}, sum of the absorbances of the colored β- and α-adducts at time t; AD_α, colored α-NBP−SO adduct; AD_β, colored β-NBP−SO adduct; $AD_{un\alpha}$, uncolored α-NBP−SO adduct; $AD_{un\beta}$, uncolored β-NBP−SO adduct; AL, adduct lifetime; D, dielectric constant; f_α and f_β, fraction of the alkylating agent that eventually forms the α- and β-adducts, respectively; NBP, 4-(*p*-nitrobenzyl)pyridine; SG, styrene glycol; SO, styrene-7,8-oxide; UFLC-DAD, ultrafast liquid chromatography system equipped with a diode array detector

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