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Connecting the Chemical and Biological Reactivity of Epoxides

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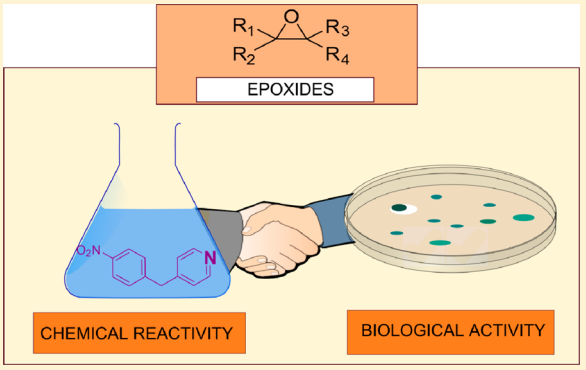
Supporting Information

ABSTRACT: The chemical reactivity of the mutagenic epoxides (EP) propylene oxide (PO), 1,2-epoxybutane (1,2-EB), and *cis*- and *trans*-2,3-epoxybutane (*cis*- and *trans*-2,3-EB) with 4-(*p*-nitrobenzyl)-pyridine (NBP), a bionucleophile model for S_N2 alkylating agents with high affinity for the guanine-N7 position, was investigated kinetically. It was found that three reactions are involved simultaneously: the alkylation reaction of NBP by EP, which yields the corresponding NBP-EP adducts through an S_N2 mechanism, and EP and NBP-EP hydrolysis reactions. PO and 1,2-EB were seen to exhibit a higher alkylating potential than *cis*- and *trans*-2,3-EB. From a study of the correlations between the chemical reactivity (kinetic parameters) and the biological effectiveness of oxiranes, the following conclusions can be drawn: (i) the hydrolysis reactions of epoxides must be taken into account to understand their bioactivity. (ii) The fraction (f) of the alkylating oxirane that forms the adduct and the adduct life (AL) permit the potential of epoxides as bioactive molecules to be rationalized even semiquantitatively; and (iii) alkylation of DNA by epoxides and the O^6-/N7-guanine adduct ratio are directly related to their mutagenicity *in vitro*.



INTRODUCTION

The chemical reactivity of epoxides (also known as oxiranes or alkylene oxides) is an important determinant of their biological effects.[1,2] Epoxides formed *in vivo* endogenously from their parent unsaturated compounds can react with cellular macromolecules such as hemoglobin and nucleic acids or can be biotransformed, either via glutathione-*S*-transferase to give glutathione conjugates or via epoxide hydrolase to generate the corresponding glycols.[3,4] Thus, epoxides have been associated with some genotoxic, mutagenic,[1,5] and carcinogenic effects,[6] mainly due to direct exposure, as has been shown by *in vitro* and *in vivo* assays.[2]

Oxiranes are direct-acting alkylating agents that mainly react with the guanine-N7 position, the most nucleophilic center in nucleic acids.[2,7,8] In keeping with this, the major adducts formed in the reaction with nucleic acids are *N*7-guanine adducts.[7] As a consequence, the alkylating potential of epoxides has attracted much attention, mostly with regard to alkylation mechanisms and structure−activity relationships.

Since 4-(*p*-nitrobenzyl)pyridine, NBP, is a good DNA model for S_N2-reacting alkylating agents with high affinity for the guanine-N7 position,[9,10] it has frequently been chosen to study the alkylating capacity of oxacyclopropanes.[1,2,9,11−14] Nevertheless, since the alkylation of NBP by different alkylating agents may involve the decomposition of these agents or of the adducts formed and/or their solvolysis, the possibility of concurrent parallel reactions should be considered.[9]

Since (i) the mutagenicity of epoxides has been studied previously, (ii) NBP is a trap for alkylating agents with nucleophilic characteristics similar to those of DNA bases, and there are several advantages to using this molecule as a substrate of alkylation,[9] and (iii) to our knowledge the mechanisms of NBP alkylation by oxiranes have not been investigated, taking into account the concurrent parallel reactions able to modulate the main alkylating mechanism, here we were prompted to investigate these issues with the aim of finding possible correlations between chemical reactivity and biological effectiveness.

A kinetic, mechanistic investigation of NBP alkylation reactions by four structurally related 1,2-epoxides was performed. Propylene oxide (PO), 1,2-epoxybutane (1,2-EB), *cis*-2,3-epoxybutane (*cis*-2,3-EB), and *trans*-2,3-epoxybutane (*trans*-2,3-EB) (Scheme 1) were selected to investigate the influence of the position and length of the alkyl substituent on

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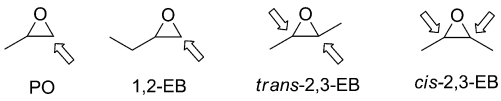
reactivity. The reactive positions on each epoxide are shown in Scheme 1.

Scheme 1. Epoxides Investigated and Their Reactive Positions with NBP



PO as well as 1,2-EB has been classified by the IARC as "probably carcinogenic to humans" (2B) and are used as chemical intermediates for the production of the corresponding glycols and their derivatives (polyglycols, glycol ethers, glycol esters...).[15,16] PO is also used as a food additive and as a fumigant for certain dried fruits and nuts.[16] 1,2-EB is applied in the production of surfactants and as a stabilizer for chlorinated hydrocarbon solvents and gasoline additives.[15] The *cis* and *trans* isomers of 2,3-epoxy butane are not listed by the IARC but share many of the features that make oxiranes genotoxic.

EXPERIMENTAL PROCEDURES

cis-2,3-Epoxybutane (98%), *trans*-2,3-epoxybutane (99%), and 1,2-propylene oxide (99%) were purchased from Alfa Aesar (Kasrlruhe, Germany). 1,2-Epoxybutane (99%), 4-(*p*-nitrobenzyl)pyridine (98%), and triethylamine (99%) were from Sigma-Aldrich (Steinheim, Germany). 1,4-Dioxane was obtained from Panreac (Barcelona, Spain). Water was deionized with a Wasserlab Ultramatic-ecomatic system. The reaction temperature was kept constant with a Lauda Ecoline RE120 thermostat. Numerical treatment of the data was performed using the Sigmaplot 10.0 Systat software.

Kinetic runs were performed in pseudofirst-order conditions, with a large excess of NBP. Acetate and phosphate buffers were used to maintain constant pH. Owing to the insolubility of NBP in water, the alkylation mixtures were prepared by adding 1 mL of epoxide stock solution (0.01−0.03 M in dioxane) to 100 mL of NBP solution (0.01−0.02 M) in 7:3 v/v water/dioxane medium.

To monitor the alkylation reactions, 2.4-mL aliquots of the alkylation mixture were collected at different times and added to a cuvette containing 0.6 mL of 99% triethylamine (Et_3N) to generate the corresponding colored compounds (Scheme 2), after which the absorbance of the AD was measured immediately at λ = 560 nm, where only the adduct absorbed. The deprotonation of AD_{un} by Et_3N is complete and its reaction rate negligible compared with those of alkylation reactions. Pseudofirst-order rate constants were calculated by nonlinear regression analysis of the absorbance/time, A/*t*, data. Detailed reaction conditions are given in the figure and table legends.

RESULTS AND DISCUSSION

As described in the literature,[2,17] steric hindrance governs the reactivity of epoxides, and only when it is almost equal at both carbon atoms are inductive effects observable. Hence, the reaction of monosubstituted oxiranes (e.g., PO and 1,2-EB) with nucleophiles mainly occurs through the less hindered carbon on the oxirane ring and takes place via an S_N2 mechanism. Owing to the increased hindrance, S_N2 reactivity is lower for disubstituted epoxides (e.g., *cis*-2,3-EB and *trans*-2,3-EB). The S_N1 mechanism is only significant for epoxides with vinyl groups conjugated with the oxirane ring.[2,17] As a result, the alkylation of NBP by the compounds investigated here would be expected to be S_N2 reactions.

On the basis of the above considerations, the results obtained here, and those described previously for *p*-nitrostyrene oxide (*p*NSO),[14] the reaction pathway depicted in Scheme 3 can be proposed. Since the reactions through both carbons are indistinguishable, the reaction pathway is also applicable for *cis*- and *trans*-2,3-EB.

Three different reactions are involved in that reaction mechanism: (i) the alkylation of NBP by the epoxide (EP) to give an EP-NBP adduct (AD_{un}); (ii) parallel epoxide hydrolysis to yield the corresponding glycol; and (iii) the consecutive hydrolysis reaction of the AD_{un}.

From the reaction pathway depicted in Scheme 3, and taking into account that the reaction proceeds in an excess of NBP, the integrated rate eq 1 for adduct formation, analogous to that corresponding to the NBP-*p*NSO adduct formation,[14] is readily achieved:

$$A_{AD} = \frac{k'_{alk}[EP]_o \varepsilon l}{(k'_{alk} + k_{hyd}) - k_{hyd}^{AD}}\left[e^{-k_{hyd}^{AD}t} - e^{-(k'_{alk}+k_{hyd})t}\right] \quad (1)$$

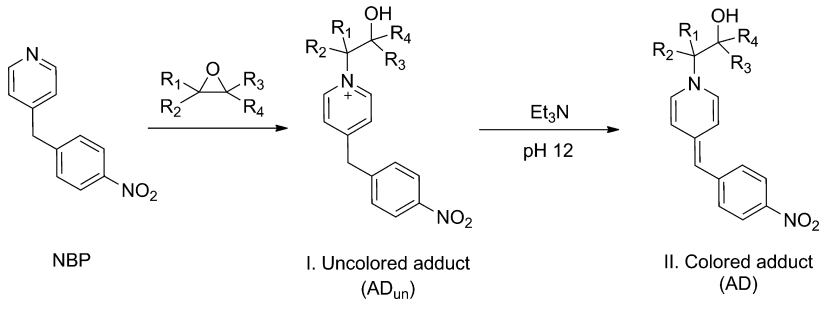
A_{AD} is the absorbance of the colored adduct at time *t*, $[EP]_o$ the initial concentration of epoxide, ε the molar absorption coefficient (λ = 560 nm) of the adduct, and *l* the cuvette path length. The pseudofirst-order rate constant of NBP alkylation is k'_{alk}, $k'_{alk} = k_{alk}[NBP]$, and k_{hyd}^{AD} and k_{hyd} are the observed pseudofirst-order rate constants for the hydrolysis reactions of the adduct and epoxide, respectively.

By nonlinear fitting of the experimental results to eq 1 (see Supporting Information) excellent fits were obtained for all epoxides (Figure 1), which supports the proposed mechanism, as does other experimental evidence described below.

The reaction orders with respect to $[EP]_o$ and $[NBP]_o$ obtained with the initial rate method (IRM)[18] were found to be one, according to an S_N2 mechanism. Figure 2 depicts the plots used for the determination of the kinetic order, *n*, with respect to $[NBP]_o$.

The expression of the initial rate, v_o, in terms of absorbance is given by eq 2.

Scheme 2. Method for Monitoring the Alkylation Reactions



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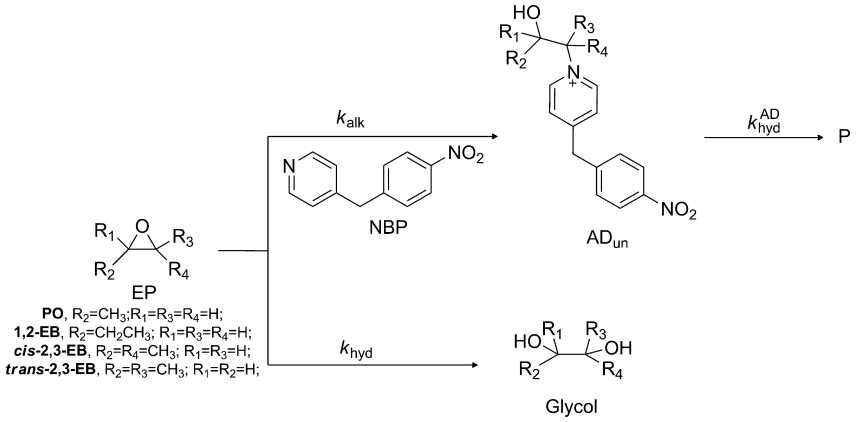
Scheme 3. Pathway for NBP Alkylation by Oxiranes



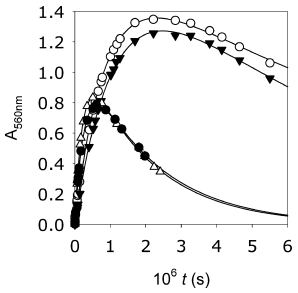


Figure 1. Kinetic profile of the evolution of the EP-NBP adduct along time. The points correspond to the experimental data, and the continuous lines correspond to the data obtained by nonlinear regression fitting to eq 1. $[PO]_o = 5.59 \times 10^{-5}$ M (Δ); $[1,2\text{-EB}]_o = 6.31 \times 10^{-5}$ M (●); $[cis\text{-}2,3\text{-EB}]_o = 2.08 \times 10^{-4}$ M (○); $[trans\text{-}2,3\text{-EB}]_o = 2.21 \times 10^{-4}$ M (▼); $[NBP]_o = 1.99 \times 10^{-2}$ M; 7:3 water/dioxane media; T = 37.5 °C; pH 7.0.

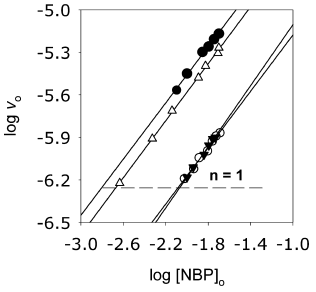


Figure 2. Reaction order, n, with respect to $[NBP]_o$ for the reaction of PO(Δ); 1,2-EB(●); *cis*-2,3-EB (○); and *trans*-2,3-EB(▼).

$$\log v_o = \log\left(\lim_{t\to o}\frac{\Delta A}{\Delta t}\right) = \log(k_{alk}\varepsilon l) + n\log[NBP]_o + m\log[EP]_o \quad (2)$$

With NBP in a large excess, the mass spectra of the reaction mixtures afforded m/z fragments in accordance with the formation of a 1:1 NBP-EP adduct for all the epoxides under study (m/z for the molecular ion peaks were 287.1 for epoxibutane adducts and 273.1 for NBP-PO). No peaks of other possible adducts such as 2:1 were observed.

Molar Absorption Coefficients of the NBP-EP Adducts. The determination of the molar absorption coefficients, ε, for each NBP-EP adduct is important since it permits direct calculation of all the reaction rate constants involved in the alkylation process. To determine ε values, a series of kinetic runs were carried out for each epoxide with different $[NBP]_o$ values (see Supporting Information).

The values of the molar absorption coefficients for *cis*- and *trans*-2,3-EB adducts were $\varepsilon = (2.5 \pm 0.1) \times 10^4$ $M^{-1}cm^{-1}$ and $\varepsilon = (2.27 \pm 0.03) \times 10^4$ $M^{-1}cm^{-1}$, respectively (they were calculated according to the method described by Barbin et al.,[19] previously used by us with *p*-nitrostyrene oxide[14]). The value for 1,2-EB ($\varepsilon = (2.2 \pm 0.1) \times 10^4$ $M^{-1}cm^{-1}$) was lower than that of PO ($\varepsilon = (2.6 \pm 0.1) \times 10^4$ $M^{-1}cm^{-1}$), as has also been found in other solvent mixtures.[20,21] The differences observed among the molar absorption coefficients, up to 20%, highlight the importance of using each adduct's own absorption coefficient and not the same value for all of them, as done by other researchers (see below).

Kinetic Analysis. *Influence of pH.* The values for the rate constants reported in Table 1 show that while the alkylation reactions were not influenced by pH in the range studied, epoxide hydrolysis reactions are acid-catalyzed, and adduct hydrolyses reactions are base-catalyzed (i.e., negligible in acidic media; see above).

Table 1. Influence of pH on Alkylation and Hydrolysis Rate Constants and on the f and AL Parameters[a]

compd	pH	$10^4\ k_{alk}$ ($M^{-1}s^{-1}$)	$10^6\ k_{hyd}$ (s^{-1})	$10^7\ k_{hyd}^{AD}$ (s^{-1})	f[b]	10^{-4} AL[c] (min)
	5.12	1.75	3.07	n.d.	0.48	n.d.
PO	5.95	1.76	2.28	1.39	0.51	7.27
	6.96	1.68	1.06	5.31	0.76	2.37
	5.12	1.32	2.44	n.d.	0.50	n.d.
1,2-EB	5.95	1.41	1.05	3.66	0.73	3.32
	6.96	1.41	0.8	5.13	0.78	2.53
	5.06	0.15	3.11	n.d.	0.09	n.d.
trans-2,3-EB	5.20	0.12	1.76	n.d.	0.12	n.d.
	6.69	0.15	0.6	1.49	0.33	3.75
	5.06	0.14	4.18	n.d.	0.06	n.d.
cis-2,3-EB	5.20	0.16	3.33	n.d.	0.09	n.d.
	6.69	0.15	0.65	1.19	0.28	3.90

[a] T = 37.5 °C; 7:3 water/dioxane media; n.d.: not detected. [b] Defined as in eq 5. [c] Defined as in eq 6.

As can be observed, the less substituted compounds show higher k_{alk} values, the alkylation rate constants for PO and 1,2-EB being 10-fold greater than those for the 2,3-EB isomers. This is in accordance with the reactivity reported in literature

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for these epoxides with ammonia[2] and with DNA bases.[21,22] The PO alkylation of deoxiguanosine is faster than that by 1,2-EB, and the PO alkylation of guanosine is about three times faster than that by *trans*-2,3-EB.[22,23] Thus, the NBP method can be considered a suitable model for studying the reactivity of oxiranes.

Our results (Table 1) differ from those reported by Hemminki et al.[23] for the alkylation of NBP because those authors failed to find major differences in the reactivities (k_{alk}) of epoxides of different chain lengths. Two possible causes of this discrepancy could be that (i) in their investigation, Hemminki's group considered the alkylation reaction to be the only reaction involved and that (ii) those authors did not take into account the fact that the values of the molar absorption coefficients were different for each NBP-EP adduct, which can differ by up to 20%.

In contrast to the alkylation reactions, the hydrolysis reaction rates (k_{hyd}; Table 1) for the different epoxides did not reveal large differences. *cis*-2,3-EB showed the strongest change with the acidification of the media, the sequence being as follows: *cis*-2,3-EB > *trans*-2,3-EB >1,2-EB ~ PO, in accordance with the results observed in aqueous acidic media.[2,24−27]

Since hydrolysis reactions of NBP-EP adducts are base-catalyzed, they are negligible in acidic media. These reactions were found to be faster (k^{AD}_{hyd}, Table 1) for 1,2-EB and PO than for the more symmetric 2,3-EB isomers.

Temperature Dependence. The influence of temperature on the alkylation and hydrolysis rate constants was investigated in the 32.5−45.0 °C range and in neutral media. Since the k_{alk}, k_{hyd}, and k^{AD}_{hyd} values fitted the Arrhenius equation well, the activation energies were calculated (Table 2).

Table 2. Activation Energies for NBP Alkylation, EP Hydrolysis, and Adduct Hydrolysis Reactions

activation energies	PO	1,2-EB	*trans*-2,3-EB	*cis*-2,3-EB
$E_{a\ alk}$ (kJ mol^{-1})	68 ± 4	67 ± 3	90 ± 6	95 ± 5
$E_{a\ hyd}$ (kJ mol^{-1})	97 ± 8	76 ± 6	184 ± 99	135 ± 31
E^{AD}_{ahyd} (kJ mol^{-1})	126 ± 15	110 ± 6	212 ± 32	209 ± 43

The values of the rate constants (Table 1; also see Supporting Information) reveal that monosubstituted epoxides are more reactive than disubstituted ones. It can also be observed that the alkylation reactions of NBP by epoxides are more favored than those of epoxide hydrolyses and much more than those of NBP-EP hydrolyses.

Since the PO- and 1,2-EB-NBP adducts are more labile than the 2,3-EB-NBP adducts (*cis*- and *trans*- isomers), it can be suggested that the adduct hydrolysis reaction would be initiated by an attack by a water molecule on the carbon attached directly to the nitrogen atom, less hindered on the PO- and 1,2-EB-NBP adducts than on the 2,3-EB isomers.

Chemical Parameters and Biological Effects. *Chemical Parameters: Rate Constants, Selectivity Factors, Alkylating Capacity, and Effectiveness.* Since (i) NBP is a suitable model for the guanine-N7 position, (ii) the alkylating capacity of many substances seems to be a determinant factor in their biological activity, and (iii) to our knowledge no clear correlations between the chemical reactivity of oxiranes as alkylating agents and their biological activity have been reported, we were prompted to address these issues.

To this end, in addition to the kinetic parameters k_{alk}, $E_{a\ alk}$, and k_{hyd}, two selectivity factors were taken into account: (i) the chemoselectivity, S_{NBP}, of epoxides toward NBP was evaluated as the ratio of the alkylation and hydrolysis rate constants (eq 3);[28] and (ii) the Swain-Scott substrate constant *s*, which provides basic information about the selectivity of alkylation in reactions with different nucleophiles, was obtained from eq 4, where k_N and k_o are the second-order rate constants for reactions of a common electrophile with nucleophiles, *N*, of nucleophilic strengths n_N, and water ($n_o = 0$), respectively.[29] To estimate *s*, we considered hydrolysis competition with NBP: thus $k_N = k_{alk}$ and $n_N = n_{NBP} = 3.5$.[30]

$$S_{NBP} = \frac{k'_{alk}}{k_{hyd}}\frac{[H_2O]}{[NBP]} \tag{3}$$

$$\log(k_N/k_o) = (n_N - n_o)\cdot s \tag{4}$$

The S_{NBP} values ($2.6 < \log S_{NBP} < 4.0$) and *s* constants ($s > 0.7$) obtained here (see Supporting Information) are typical of S_N2 mechanisms.[28,31,32] The *s* values are in good agreement with those previously found for ethylene and propylene oxides.[2] Both high selectivities and *s* values are indicative of an enhanced S_N2 character and the preference of the alkylating agents to react with highly nucleophilic N centers in DNA (e.g., N7-G, N3-A), this being related to a low genotoxicity and a high N7-/O^6-alkylguanine ratio.[8,9,31,33,34]

The alkylation rate constant, $k_{n=2}$, for an $n = 2$ substrate (centers in DNA with low reactivity, such as O^6-guanine) is usually considered to be proportional to the genetic risk. It has been correlated with the mutagenicity of alkylating agents and has been estimated using the Swain-Scott equation (eq 4).[2,33] The values obtained here for $k_{n=2}$ at 37.5 °C were 3.99, 0.75, 0.85, and $3.21\cdot10^{-6}$ M^{-1}s^{-1} for PO, *trans*-2,3-EB, *cis*-2,3-EB, and 1,2-EB, respectively. The $k_{n=2}$ values for PO and 1,2-EB are in accordance with those reported in the literature (3.33 and 3.11 × 10^{-6} M^{-1}s^{-1} at 37 °C, respectively).[2]

Two useful parameters of the reactivity of alkylating agents are the fraction, *f*, of the alkylating agent that finally forms the adduct and the adduct life, AL.[14] These have been correlated with the mutagenic potency of different compounds.[9,14,35−37] The *f* parameter represents the ratio of the initial epoxide that forms the adduct (eq 5) instead of being diverted to different products (in the parallel hydrolysis reaction). Thus, *f* ranges from 0 to 1, 0 meaning that all the initial epoxide is hydrolyzed and 1 that the epoxide reacts exclusively with the NBP, yielding the adduct.

$$f = \frac{k_{alk}[NBP]}{(k_{alk}[NBP] + k_{hyd})} \tag{5}$$

$$AL = \frac{\int_0^\infty [AD]dt}{[EP]_o} = \frac{k'_{alk}}{k'_{alk} + k_{hyd} - k^{AD}_{hyd}}$$

$$\int_0^\infty (e^{-(k'_{alk}+k_{hyd})t} - e^{-k^{AD}_{hyd}t})dt = \frac{k'_{alk}}{(k'_{alk} + k_{hyd})k^{AD}_{hyd}}$$

$$= \frac{f}{k^{AD}_{hyd}} \tag{6}$$

The adduct life, or alkylating effectiveness, is defined as the area under the kinetic profile of the reaction per unit of alkylating agent.[9,14,35−37] This parameter comprises the rate constants of the three reactions involved in the mechanism, k_{hyd}, k_{alk}, and k^{AD}_{hyd}, and gives an idea of the permanence of the

adduct along time. As seen in eq 6, it is also related to the alkylating efficacy, f (i.e., the higher the f value and the lower the adduct hydrolysis rate constant, the higher is the AL).

The results (Table 1) show that at 37.5 °C and physiological pH, for PO and 1,2-EB 70−80% of the initial concentration of the alkylating agent yields the adduct, whereas the remaining 20−30% is hydrolyzed to form glycol. In contrast, for the 2,3-EB isomers the highest percentage of epoxide is hydrolyzed. In general, f decreases with increasing temperature, in agreement with the increase in the hydrolysis rate constant.

As shown in Table 1 (also see Supporting Information) AL increases when pH and temperature decrease. The effect of pH was attributed to the fact that, being base-catalyzed, adduct hydrolysis reactions are negligible at acid pH. Regarding the influence of temperature, this is due to the decrease in f as well as to the increase in the adduct hydrolysis rate constant with temperature.

Biological Effectiveness of Oxiranes. PO and 1,2-EB, classified by the IARC as "probably carcinogenic," as well as the nonclassified *cis*- and *trans*-2,3-EB have been reported to be weak mutagens in different organisms, including the TA100 and TA1535 strains of *Salmonella typhimurium*.[5,38,39] They also induce sister-chromatid exchanges (SCE) in Chinese hamster V79 cells.[40] Table 3 shows that mutagenicity decreases inversely to the hydrocarbon chain length and is enhanced by electron-withdrawing substituents. The greater mutagenic potential of the *cis*-regioisomer found here compared with that of its *trans*-counterpart is consistent with results reported for other disubstituted epoxides, whose *trans*-isomer frequently lacks mutagenicity.[38,39] The same behavior has also been observed for such activity in the SCE test, a good tool to investigate quantitative structure−activity relationships with mammalian cells *in vitro*.[40]

Table 3. Substrate Constant, s, Genetic Risk Equivalent, $k_{n=2}$, and the Biological Effectiveness of Epoxides

			Schizosaccharomyces pombe[f]		
epoxide	s	log $k_{n=2}$ ($M^{-1}s^{-1}$)	*MF*	specific activity	Chinese hamster V79 cells[g] SCEIP
EO	0.93[a]	−5.41[e]	4.44	0.148	
PO	1.08[b]	−5.40[b]	1.12	0.037	3
1,2-EB	1.10[b]	−5.49[b]	1.17	0.039	1.6
trans-2,3-EB	0.85[b]	−6.13[b]	0.02	0.0004	0.3
cis-2,3-EB	0.88[b]	−6.07[b]			0.3
*p*NSO	1.13[c]	−5.04[c]			
ECH	0.93[d]	−5.04[e]	6.53	0.218	28.8
glycidol	1.00[d]	−5.56[e]	2.9	0.097	4.1

[a]Ref 41. [b]This work. [c]Ref 14. [d]Ref 29. [e]Ref 2. [f]Ref 42. *MF*: mutation frequency; mutants per 10^4 survivors per mM. Specific activity, mutation frequency per 10^4 locus and hour. [g]Ref 40.

The oxirane SCE-inducing potencies (SCEIP) and mutagenicity, as well as their Swain-Scott substrate constant, s, and $k_{n=2}$ values, are summarized in Table 3. Data for other epoxides, such as ethylene oxide (EO), *p*-nitrostyrene oxide (*p*NSO), epichlorohydrine (ECH), and glycidol, have also been included to facilitate discussion of the results.

Chemico-Biological Correlations. From the present and previously published results,[9,11,43] some evidence can be gained about the qualitative or semiquantitative correlations between the chemical reactivity and biological effects of oxiranes.

Since (i) the adducts formed by the four epoxides are quite stable (i.e., the AL values were large; see Table 1); (ii) the AL values are higher than those observed with other alkylating agents;[9,14,35−37] (iii) 2,3-EB regioisomers are mainly hydrolyzed, whereas PO and 1,2-EB form adducts in higher proportions; and (iv) the higher the adduct proportion and its stability, the higher the probability of its biological effects being effective, the possibility of the adducts being accumulated *in vivo* over a long period and thus exerting their biochemical activity could be significant.[9] As a consequence of this, it could be expected that PO and 1,2-EB would show greater effectiveness than *cis*- and *trans*-2,3-EB, as indeed is the case. Thus, for epoxides that form highly stable adducts, as in the present case, it seems that the f parameter is a more influential determinant than AL with regard to biological effectiveness.

To investigate possible quantitative correlations, the model of Hakura et al.[44] was chosen because those authors attributed the observed mutation frequency, *MF*, to a chemical kinetic process, such as modification of the target, which occurs at a given rate, $\tilde{k}$.

$$\ln MF = n \ln \tilde{k} + \ln m - n \ln D \qquad (7)$$

Here, k_{alk} obtained with NBP (a model nucleophile for guanine-N7 position) or $k_{n=2}$, which describes the rate of reaction between alkylating agents and *O*-centers in DNA, were considered to be the rate constants of modification of the target site.

We first focused our attention on the correlations between the chemical parameters and mutagenic response observed in *Schizosaccharomyces pombe* (Figure 3). The good MF/k_{alk} and $MF/E_{a\ alk}$ correlations (see Figure 3a and b) could be attributed to a significant role of N7-guanine alkylation in the generation of the biological response for the oxiranes, for which N7-guanine is the main adduct formed. This is in agreement with the results obtained by Hooberman et al.,[1] who found a good correlation between mutagenicity in strain TA100 and chemical reactivity with NBP. Whereas small adducts such as N7-methylguanine and N7-ethylguanine have been characterized as well-tolerated by cells, larger N7-alkylguanine lesions can have potent biological activities including cytotoxicity and mutagenicity.[45]

Since the formation of N7-guanine adducts cannot be used in isolation mutagenic response or as a surrogate for other biological processes,[7] we also looked for other correlations. A correlation was found with $k_{n=2}$ (see Figure 3f). Although poor, its existence highlights the importance of the formation of O-adducts in the biological effects caused by the epoxides studied here (although O-adducts are minor products for oxirane alkylation, they are more mutagenic than N7-alkylguanine[9]).

The N7-/O^6-alkylguanine adducts ratio also proves to be an important factor owing to the strong correlations MF/s and MF/S_{NBP} (see Figure 3c and d). A low degree of correlation was found with MF/k_{hyd} (Figure 3e). Since the hydrolysis reactions are not directly involved in any modification of the target sites, this result supports the validity of the Hakura model. Finally, good correlations were observed between SCEIP in Chinese hamster V79 cells and all the chemical parameters, including $k_{n=2}$ (Figure 3). This reinforces the conclusions drawn using the mutagenic frequency in *Schizosaccharomyces pombe*.

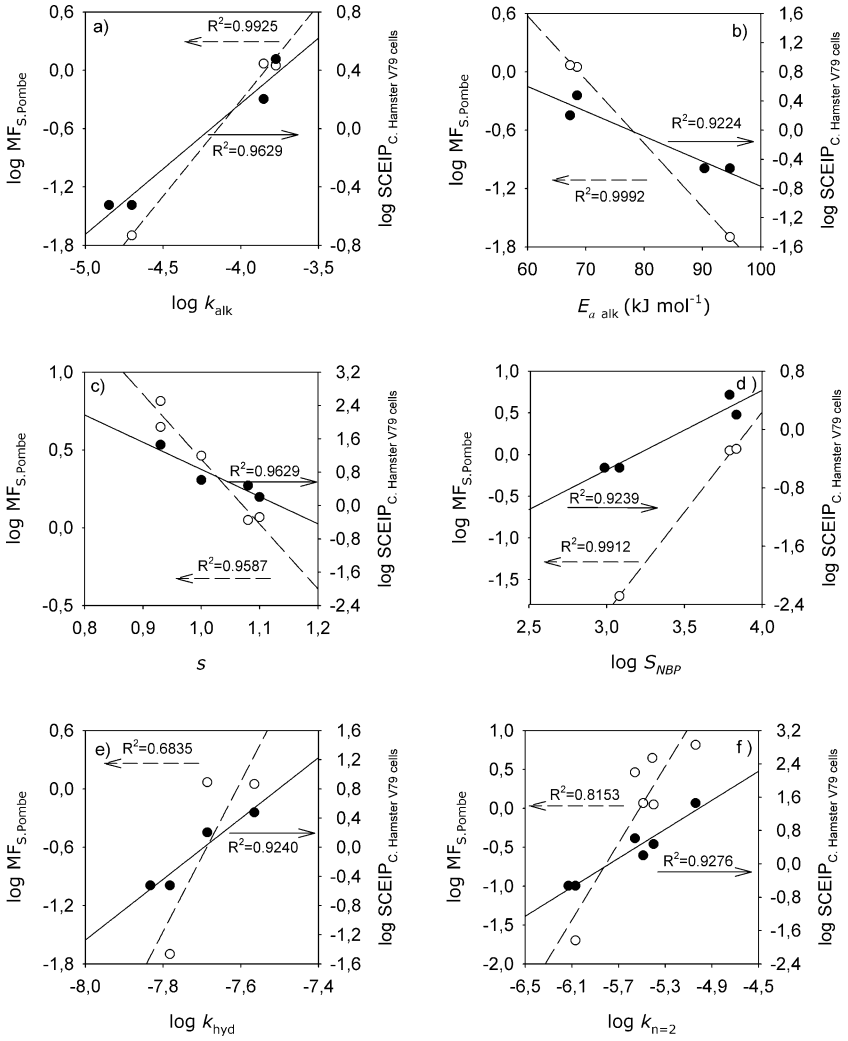

Figure 3. Correlations between chemical parameters, (a) k_{alk}, (b) $E_{a\ alk}$, (c) s, (d) S_{NBP}, (e) k_{hyd}, and (f) $k_{n=2}$, and mutation frequency in *Schizosaccharomyces pombe* (○, - - -) and SCE induction potency in Chinese hamster V79 cells (●, —).

CONCLUSIONS

(i) The alkylation of 4-(*p*-nitrobenzyl)pyridine (NBP) by epoxides (EP) (propylene oxide (PO), 1,2-epoxybutane (1,2-EB), *cis*-2,3-epoxybutane (*cis*-2,3-EB), and *trans*-2,3-epoxybutane (*trans*-2,3-EB)) yields the corresponding NBP-EP adducts through an S_N2 mechanism.

(ii) The alkylating potentials of PO and 1,2-EB are higher than that of 2,3-EB.

(iii) The hydrolysis reactions of epoxides must be taken into account for a better understanding of their bioactivity.

(iv) The fraction, *f*, of the alkylating oxirane that forms the adduct and the adduct life, AL, permits the potential of epoxides as a cause of their bioactivity to be rationalized, even semiquantitatively.

(v) The good qualitative and quantitative correlations between the chemical reactivity (kinetic parameters) of epoxides and their bioactivity suggest that the alkylation of DNA by epoxides and the O^6-/N7-guanine adduct ratio are directly related to their *in vitro* mutagenicity.

ASSOCIATED CONTENT

Supporting Information

Non-linear fitting of the experimental results to eq 1; determination of the molar absorption coefficients of the NBP-EP adducts; and influence of temperature on the reaction rate constants and chemical parameters. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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ABBREVIATIONS

NBP, 4-(*p*-nitrobenzyl)pyridine; PO, propylene oxide; 1,2-EB, 1,2-epoxybutane; *cis*-2,3-EB, *cis*-2,3-epoxybutane; *trans*-2,3-EB, *trans*-2,3-epoxybutane

REFERENCES

(1) Hooberman, B. H., Chakraborty, P. K., and Sinsheimer, J. E. (1993) Quantitative structure-activity relationships for the mutagenicity of propylene oxides with Salmonella. *Mutat. Res., Genet. Toxicol. 299*, 85−93.

(2) Ehrenberg, L., and Hussain, S. (1981) Genetic toxicity of some important epoxides. *Mutat. Res., Rev. Genet. Toxicol. 86*, 1−113.

(3) Faller, T. H., Csanády, G. A., Kreuzer, P. E., Baur, C. M., and Filser, J. G. (2001) Kinetics of propylene oxide metabolism in microsomes and cytosol of different organs from mouse, rat, and humans. *Toxicol. Appl. Pharmacol. 172*, 62−74.

(4) Lee, M. S., Faller, T. H., Kreuzer, P. E., Kessler, W., Csanády, G. A., Pütz, C., Ríos-Blanco, M. N., Pottenger, L. H., Segerbäck, D., Osterman-Golkar, S., Swenberg, J. A., and Filser, J. G. (2005) Propylene oxide in blood and soluble nonprotein thiols in nasal mucosa and other tissues of male Fischer 344/N rats exposed to propylene oxide vapors-relevance of glutathione depletion for propylene oxide-induced rat nasal tumors. *Toxicol. Sci. 83*, 177−189.

(5) Castelain, P., Criado, B., Cornet, M., Laib, R., Rogiers, V., and Kirsch-Volders, M. (1993) Comparative mutagenicity of structurally related aliphatic epoxides in a modified *Salmonella*/microsome assay. *Mutagenesis 8*, 387−393.

(6) Van Duuren, B., Orris, L., and Nelson, N. (1965) Carcinogenicity of epoxides lactones and peroxy compounds II. *J. Natl. Cancer Inst. 35*, 707−717.

(7) Boysen, G., Pachkowski, B. F., Nakamura, J., and Swenberg, J. A. (2009) The formation and biological significance of N7-guanine adducts. *Mutat. Res., Genet. Toxicol. Environ. Mutagen. 678*, 76−94.

(8) Uziel, M., Munro, N. B., Sue Katz, D., Vo-Dinh, T., Zeighami, E. A., Waters, M. D., and Griffith, J. D. (1992) DNA adduct formation by 12 chemicals with populations potentially suitable for molecular epidemiological studies. *Mutat. Res., Rev. Genet. Toxicol. 277*, 35−90.

(9) Gómez-Bombarelli, R., González-Pérez, M., Calle, E., and Casado, J. (2012) Potential of the NBP method for the study of alkylation mechanisms: NBP as a DNA-model. *Chem. Res. Toxicol. 25*, 1176−1191.

(10) Hemminki, K. (1983) Nucleic acid adducts of chemical carcinogens and mutagens. *Arch. Toxicol. 52*, 249−285.

(11) Hemminki, K., and Falck, K. (1979) Correlation of mutagenicity and 4-(*p*-nitrobenzyl)-pyridine alkylation by epoxides. *Toxicol. Lett. 4*, 103−106.

(12) Kim, J. H., and Thomas, J. J. (1992) Use of 4-(nitrobenzyl)-pyridine (4-NBP) to test mutagenic potential of slow-reacting epoxides, their corresponding olefins, and other alkylating agents. *Bull. Environ. Contam. Toxicol. 49*, 879−885.

(13) Turchi, G., Bonatti, S., Citti, L., Gervasi, P. G., Abbondandolo, A., and Presciuttini, S. (1981) Alkylating properties and genetic activity of 4-vinylcyclohexene metabolites and structurally related epoxides. *Mutat. Res. 83*, 419−430.

(14) González-Pérez, M., Gómez-Bombarelli, R., Pérez-Prior, M. T., Manso, J. A., Céspedes-Camacho, I. F., Calle, E., and Casado, J. (2011) Reactivity of *p*-nitrostyrene oxide as an alkylating agent. A kinetic approach to biomimetic conditions. *Org. Biomol. Chem. 9*, 7016−7022.

(15) IARC (1999) *Re-evaluation of Some Organic Chemicals, Hydrazine and Hydrogen Peroxide*, 45−233, IARC monograph 71, IARC, Lyon, France.

(16) IARC (1994) *Some Industrial Chemicals*, pp 73−159, IARC monograph 60, IARC, Lyon, France.

(17) Parker, R. E., and Isaacs, N. S. (1959) Mechanisms of epoxide reactions. *Chem. Rev. 59*, 737−799.

(18) Casado, J., López-Quintela, M. A., and Lorenzo-Barral, F. M. (1986) The initial rate method in chemical kinetics: Evaluation and experimental illustration. *J. Chem. Educ. 63*, 450−452.

(19) Barbin, A., Bérériat, J.-C., Croisy, A., O'Neill, I. K., and Bartsch, H. (1990) Nucleophilic selectivity and reaction kinetics of chloroethylene oxide assessed by the 4-(*p*-nitrobenzyl)pyridine assay and proton nuclear magnetic resonance spectroscopy. *Chem.-Biol. Interact. 73*, 261−277.

(20) Agarwal, S. C., Van Duuren, B. L., and Kneip, T. J. (1979) Detection of epoxides with 4-(*p*-nitrobenzyl) pyridine. *Bull. Environ. Contam. Toxicol. 23*, 825−829.

(21) Hammock, L. G., Hammock, B. D., and Casida, J. E. (1974) Detection and analysis of epoxides with 4-(*p*-nitrobenzyl)-pyridine. *Bull. Environ. Contam. Toxicol. 12*, 759−764.

(22) Roe, R., Paul, J. S., and Montgomery, P. O. B. (1973) Kinetics and mechanism of the N7-hydroxyalkylation of guanosine. *J. Heterocycl. Chem. 10*, 859−865.

(23) Hemminki, A., Väyrynen, T., and Hemminki, K. (1994) Reaction kinetics of alkyl epoxides with DNA and other nucleophiles. *Chem.-Biol. Interact. 93*, 51−58.

(24) Minerath, E. C., and Elrod, M. J. (2009) Assessing the potential for diol and hydroxy sulfate ester formation from the reaction of epoxides in tropospheric aerosols. *Environ. Sci. Technol. 43*, 1386−1392.

(25) Long, F. A., and Pritchard, J. G. (1956) Hydrolysis of substituted ethylene oxides in H_2O^{18} solutions. *J. Am. Chem. Soc. 78*, 2663−2667.

(26) Kirkovsky, L. I., Lermontov, S. A., Zavorin, S. I., Sukhozhenko, I. I., Zavelsky, V. I., Thier, R., and Bolt, H. M. (1998) Hydrolysis of genotoxic methyl-substituted oxiranes: Experimental kinetic and semiempirical studies. *Environ. Toxicol. Chem. 17*, 2141−2147.

(27) Pritchard, J. G., and Long, F. A. (1956) Kinetics and mechanism of the acid-catalyzed hydrolysis of substituted ethylene oxides[1]. *J. Am. Chem. Soc. 78*, 2667−2670.

(28) Ninomiya, S., Kohda, K., and Kawazoe, Y. (1984) Studies on chemical carcinogens and mutagens . XXV. Chemoselectivity of alkyl sulfonates toward 4-(*p*-nitrobenzyl)pyridine (NBP) in phosphate buffer. *Chem. Pharm. Bull. (Tokyo) 32*, 1326−1332.

(29) Swain, C. G., and Scott, C. B. (1953) Quantitative correlation of relative rates. Comparison of hydroxide ion with other nucleophilic reagents toward alkyl halides, esters, epoxides and acyl halides[1]. *J. Am. Chem. Soc. 75*, 141−147.

(30) Spears, C. P. (1981) Nucleophilic selectivity ratios of model and clinical alkylating agents by 4-(4′-nitrobenzyl)pyridine competition. *Mol. Pharmacol. 19*, 496−504.

(31) Vogel, E. W., Nivard, M. J. M., Ballering, L. A. B., Bartsch, H., Barbin, A., Nair, J., Comendador, M. A., Sierra, L. M., Aguirrezabalaga, I., Tosal, L., Ehrenberg, L., Fuchs, R. P. P., Janel-Bintz, R., Maenhaut-Michel, G., Montesano, R., Hall, J., Kang, H., Miele, M., Thomale, J., Bender, K., Engelbergs, J., and Rajewsky, M. F. (1996) DNA damage, and repair in mutagenesis and carcinogenesis: implications of structure-activity relationships for cross-species extrapolation. *Mutat. Res., Fundam. Mol. Mech. Mutagen. 353*, 177−218.

(32) Kawazoe, Y., Tamura, N., and Yoshimura, T. (1982) Studies on chemical carcinogens. XXIII. A simple method for characterization of the alkylating ability of compounds by using 4-(*p*-nitrobenzyl)-pyridine. *Chem. Pharm. Bull. (Tokyo) 30*, 2077−2086.

(33) Walles, S. A. S. (1980) Determination of reaction rate constants for alkylation of 4-(*p*-Nitrobenzyl)pyridine by different alkylating agents. *Toxicol. Lett. 5*, 161−167.

(34) Simmon, V. F. (1979) In vitro assays for recombinogenic activity of chemical carcinogens and related compounds with Saccharomyces cerevisiae D3. *J. Natl. Cancer Inst. 62*, 901−909.

(35) Pérez-Prior, M. T., Gómez-Bombarelli, R., González-Pérez, M., Manso, J. A., García-Santos, M. P., Calle, E., and Casado, J. (2010) Reactivity of the mutagen 1,4-dinitro-2-methylpyrrole as an alkylating agent. *J. Org. Chem. 75*, 1444−1449.

(36) Arenas-Valgañón, J., Gómez-Bombarelli, R., González-Pérez, M., González-Jiménez, M., Calle, E., and Casado, J. (2012) Taurine-nitrite interaction as a precursor of alkylation mechanisms. *Food Chem. 134*, 986−991.

(37) Pérez-Prior, M. T., Gómez-Bombarelli, R., González-Pérez, M., Manso, J. A., García-Santos, M. P., Calle, E., and Casado, J. (2009) Sorbate-nitrite interactions: acetonitrile oxide as an alkylating agent. *Chem. Res. Toxicol. 22*, 1320−1324.
(38) Wade, D. R., Airy, S. C., and Sinsheimer, J. E. (1978) Mutagenicity of aliphatic epoxides. *Mutat. Res., Genet. Toxicol. 58*, 217−223.
(39) von der Hude, W., Seelbach, A., and Basler, A. (1990) Epoxides: comparison of the induction of SOS repair in *Escherichia coli* PQ37 and the bacterial mutagenicity in the Ames test. *Mutat. Res., Fundam. Mol. Mech. Mutagen. 231*, 205−218.
(40) von der Hude, W., Carstensen, S., and Obe, G. (1991) Structure-activity relationships of epoxides: induction of sister-chromatid exchanges in Chinese hamster V79 cells. *Mutat. Res., Fundam. Mol. Mech. Mutagen. 249*, 55−70.
(41) Silvari, V., Haglund, J., Jenssen, D., Golding, B. T., Ehrenberg, L., and Törnqvist, M. (2005) Reaction-kinetic parameters of glycidamide as determinants of mutagenic potency. *Mutat. Res., Genet. Toxicol. Environ. Mutagen. 580*, 91−101.
(42) Migliore, L., Rossi, A. M., and Loprieno, N. (1982) Mutagenic action of structurally related alkene oxides on *Schizosaccharomyces pombe*: The influence, 'in vitro', of mouse-liver metabolizing system. *Mutat. Res., Genet. Toxicol. 102*, 425−437.
(43) Turchi, G., Bauer, C., Bronzetti, G., Citti, L., Corsi, C., Fassina, G. F., Gervasi, P. G., Lippi, A., Nieri, R., Abbondandolo, A., Berti, G., and Mastrorilli, E. (1983) Mutagenicity of 3 structurally related epoxides, with defined stereochemical configuration, in *Saccharomyces cerevisiae* and in V79 Chinese hamster cells. *Mutat. Res., Genet. Toxicol. 117*, 213−224.
(44) Hakura, A., and Kawazoe, Y. (1986) Studies on chemical carcinogens and mutagens. XXXVI. Apparent activation energy from mutagenic modification induced in *E. coli* by alkylating agents. Estimation from mutation frequency. *Chem. Pharm. Bull. (Tokyo) 34*, 1728−1734.
(45) Gates, K. S., Nooner, T., and Dutta, S. (2004) Biologically relevant chemical reactions of N7-alkylguanine residues in DNA. *Chem. Res. Toxicol. 17*, 839−856.