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Connecting the Chemical and Biological Reactivity of Epoxides

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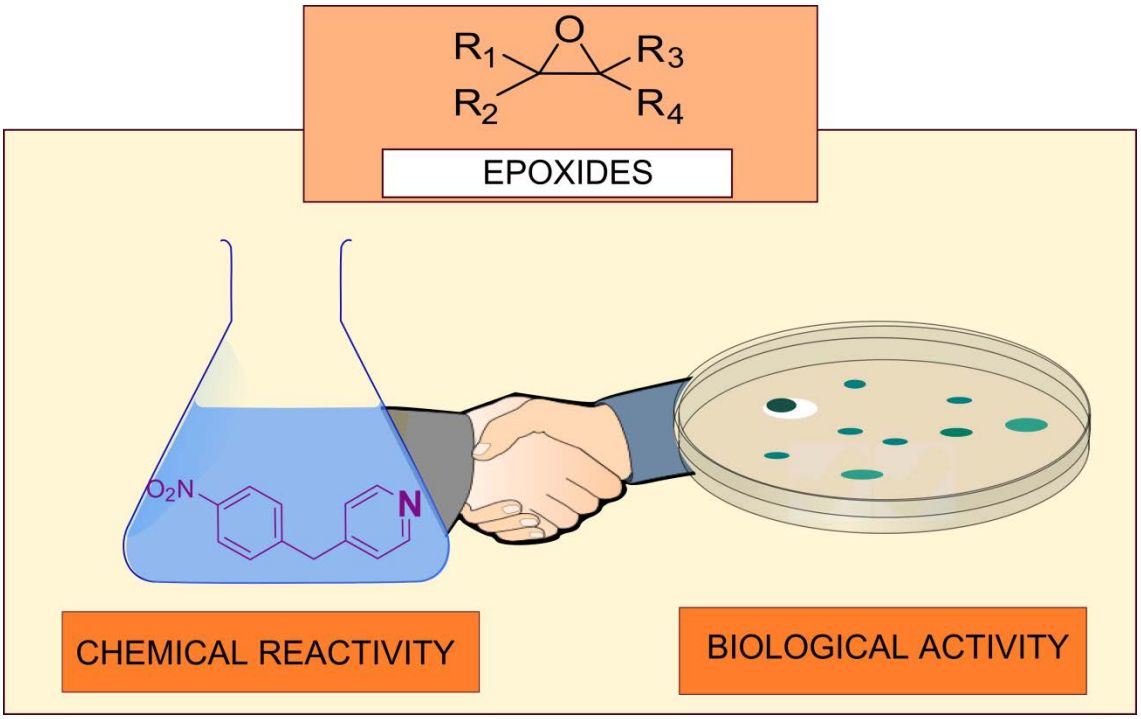
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Abstract

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 adduct and the adduct life (AL) permit the potential of epoxides as bioactive molecules to be rationalized even semi-quantitatively; and iii) alkylation of DNA by epoxides and the 0^6-/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.[1-2,11-14] 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 reactivity. The reactive positions on each epoxide are shown in Scheme 1.

PO as well as 1,2-EB have 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 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 pseudo-first-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. Pseudo-first-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:

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

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 pseudo-first-order rate constant of NBP alkylation is $k'_{alk}, k'_{alk} = k_{alk}\left[NBP\right]$, and k_{hyd}^{AD} and k_{hyd} are the observed pseudo-first-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)[17] 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.

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

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 (Table 1) *(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) 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 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).

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 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 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. Those reactions were found to be faster (k_{hyd}^{AD} , Table 2), for 1,2-EB and PO than for the more asymmetric 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 ºC range and in neutral media. Since the k_{alk}, k_{hyd} and k_{hyd}^{AD} values fitted the Arrhenius equation well, the activation energies were calculated (Table 2).

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] 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]

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

$$\log\left(k_N / k_o\right) = \left(n_N - n_o\right) \cdot s \quad (4)$$

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 a 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 ºC were 3.99, 0.75, 0.85 and 3.21 $\cdot 10^{-6}$ $M^{-1}s^{-1}$ 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 $3.11 \cdot 10^{-6}$ $M^{-1}s^{-1}$ at 37 ºC, 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 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.

$$f = \frac{k_{\text{alk}}\left[\text{NBP}\right]}{\left(k_{\text{alk}}\left[\text{NBP}\right] + k_{\text{hyd}}\right)} \tag{10}$$

$$\text{AL} = \frac{\int_0^\infty \left[\text{AD}\right] dt}{\left[\text{EP}\right]_{\text{o}}} = \frac{k'_{\text{alk}}}{k'_{\text{alk}} + k_{\text{hyd}} - k_{\text{hyd}}^{\text{AD}}} \int_0^\infty \left(e^{-\left(k'_{\text{alk}} + k_{\text{hyd}}\right)t} - e^{-k_{\text{hyd}}^{\text{AD}} t} \right) dt =$$

$$\frac{k'_{\text{alk}}}{\left(k'_{\text{alk}} + k_{\text{hyd}}\right) k_{\text{hyd}}^{\text{AD}}} = \frac{f}{k_{\text{hyd}}^{\text{AD}}} \tag{11}$$

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 $k_{\text{hyd}}^{\text{AD}}$, 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 f and the lower the adduct hydrolysis rate constant, the higher is the AL).

The results (Table 1) show that at 37.5 ºC 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 carcinogeni, as well as the non classified *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,[40] a good tool to investigate quantitative structure-activity relationships with mammalian cell *in vitro*.[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 the discussion of the results.

Chemico-Biological correlations

From the present and previously published results,[9-11,43] some evidence can be gained about qualitative or semi-quantitative 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 formed 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, $\tilde{k}$.

$$\ln MF = n \ln \tilde{k} + \ln m - n \ln D \qquad (15)$$

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 *Schizosaccharomizes pombe* (Figure 3). The good MF/k_{alk} and $MF/E_{a\ alk}$ correlations (See Figure 3a and b)highlight the significant role of the alkylation reaction in the generation of the biological response. 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-/0^6- alkylguanine adducts ratio also proves to be an important factor owing to the strong correlation between 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*.

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 semi-quantitatively.

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 as well as the 0^6-/N7-guanine adduct ratio are directly related to their in vitro mutagenicity.

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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.

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TABLES.

Table 1. Influence of pH on alkylation and hydrolysis rate constants and on the *f* and AL parameters[a]

Compound	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)
PO	**5.12**	1.75	3.07	*n.d.*	0.48	*n.d*
	5.95	1.76	2.28	1.39	0.51	7.27
	6.96	1.68	1.06	5.31	0.76	2.37
1,2-EB	**5.12**	1.32	2.44	*n.d.*	0.50	*n.d.*
	5.95	1.41	1.05	3.66	0.73	3.32
	6.96	1.41	0.8	5.13	0.78	2.53
***trans*-2,3-EB**	**5.06**	0.15	3.11	*n.d*	0.09	*n.d*
	5.20	0.12	1.76	*n.d*	0.12	*n.d*
	6.69	0.15	0.6	1.49	0.33	3.75
***cis*-2,3-EB**	**5.06**	0.14	4.18	*n.d*	0.06	*n.d*
	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 ºC; 7:3 water/dioxane media; n.d.: not detected. [b]Defined as in eq 5. [c]Defined as in eq 6.

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
$E_{a\,hyd}^{AD}$ (**kJ mol^{-1}**)	126 ± 15	110 ± 6	212 ± 32	209 ± 43

Table 3. Substrate constant, *s*, genetic risk equivalent, $k_{n=2,}$ and biological effectiveness of epoxides

Epoxide	*s*	log k_n=2	*Schizosaccharomyces pombe* [f]		Chinese hamster V79 cells [h]
		($M^{-1}s^{-1}$)	MF	Specific activity	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 10^4locus and hour; [g]Ref. 40.

FIGURES

Figure 1.

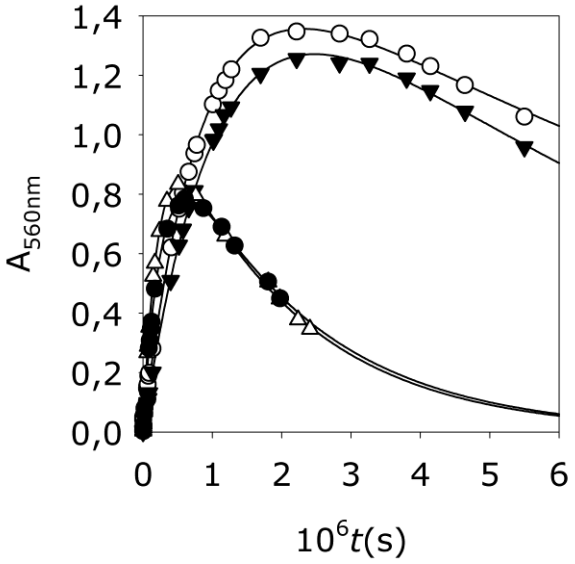


Figure 2.

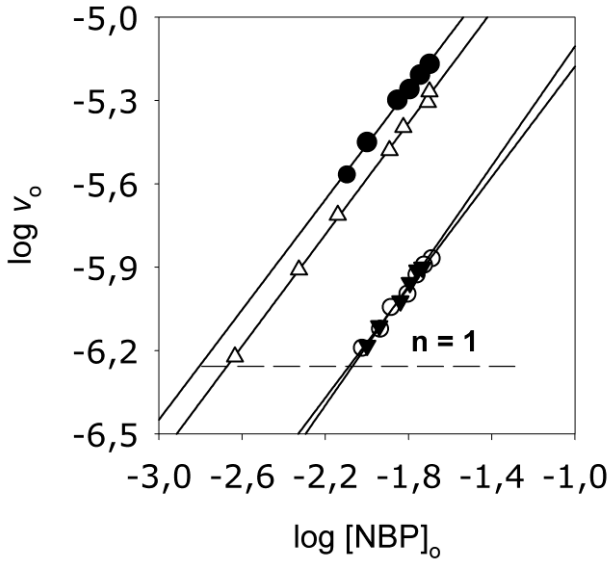


Figure 3.

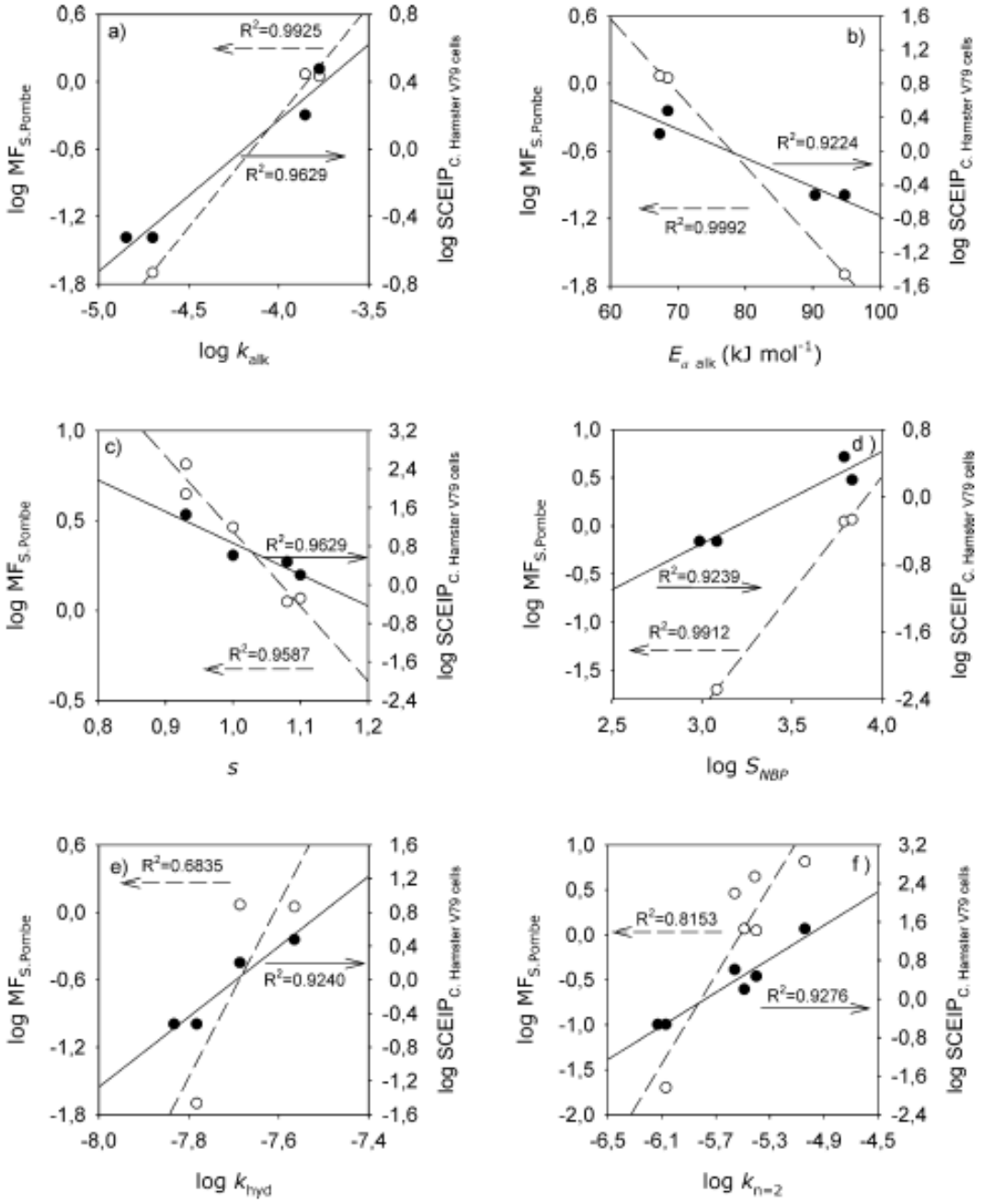

a)
R²=0.9925
R²=0.9629
log MF S.Pombe
log SCEIP C. Hamster V79 cells
log k alk
b)
R²=0.9224
R²=0.9992
E α alk (kJ mol⁻¹)
c)
R²=0.9629
R²=0.9587
s
d)
R²=0.9239
R²=0.9912
log S NBP
e)
R²=0.6835
R²=0.9240
log k hyd
f)
R²=0.8153
R²=0.9276
log k n-2

FIGURE LEGENDS

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

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 (●, □).

SCHEMES LEGENDS

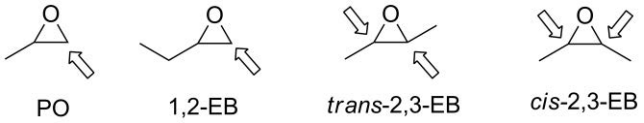
Scheme 1. Epoxides investigated and their reactive positions with NBP

Scheme 2. Method for monitoring the alkylation reactions.

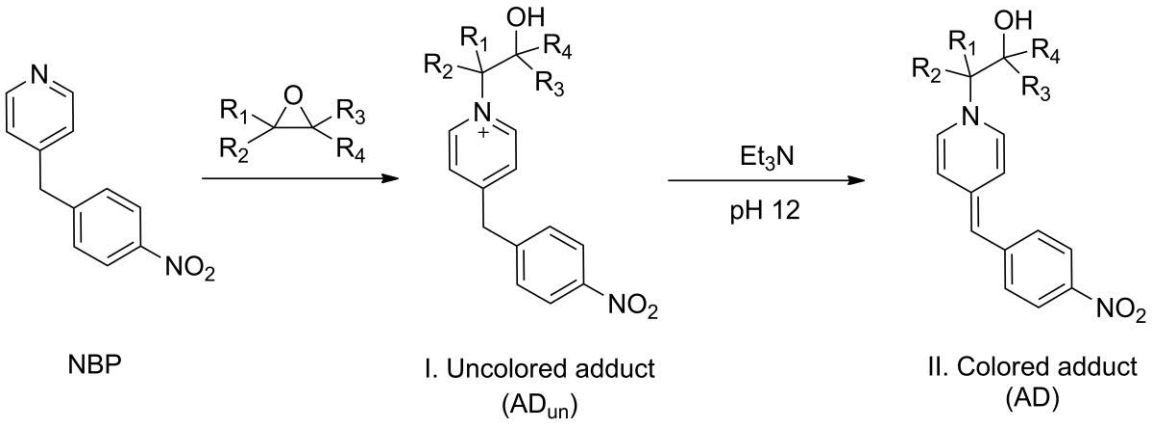
Scheme 3. Mechanism for NBP alkylation by oxiranes.

SCHEMES

Scheme 1.



Scheme 2.



Scheme 3.