

Chemical Reactivity and Biological Activity of Diketene

Rafael Gómez-Bombarelli, Marina González-Pérez, M. Teresa Pérez-Prior, José A. Manso, Emilio Calle, and Julio Casado*

Departamento de Química física, Universidad de Salamanca, E-37008 Salamanca, Spain

Received April 30, 2008

The alkylating potential of diketene (4-methylene-2-oxetanone), the basic unit of many derivatives of pesticides, chemicals, pharmaceuticals, and dyestuffs, was investigated kinetically. The nucleophile 4-(*p*-nitrobenzyl)pyridine (NBP), a trap for alkylating agents with nucleophilic characteristics similar to DNA bases, was used as an alkylation substrate. The alkylation reactions were performed in water/dioxane solvent mixtures. To gain insight into the effect of the hydrolysis of diketene on its alkylating efficiency, alkylation and competing hydrolysis were studied in parallel. Conclusions were drawn as follows: (i) Although diketene, unlike other four-membered ring lactones, is inactive as a carcinogen in experimental animals, it shows an alkylating potential of about 2 orders of magnitude higher than β -propiolactone or β -butyrolactone, which are classified as possibly carcinogenic to humans by the IARC. (ii) The reactivity of diketene as an alkylating agent is enthalpy-controlled. (iii) The fact that the hydrolysis reaction of diketene is slightly faster than those of other four-membered ring lactones shows that diketene is more efficient than β -propiolactone or β -butyrolactone as an alkylating agent, since the hydrolysis of this species poses less competition to the alkylation reaction. (iv) Diketene undergoes acyl fission in the alkylation reaction, which results in an amide bond in the NBP–diketene adduct. The lability of the amide bond as opposed to the amine bonds formed by β -propiolactone and β -butyrolactone could be one of the differential factors responsible for the lack of carcinogenicity of diketene. (v) Ab initio calculations of the energy barriers help to understand the unusual reactivity of diketene.

Introduction

The nitrosation of amino acids leads to the formation of *N*-nitroso products, which decompose to form alkylating agents, able to cause DNA damage (1–3). In previous work (2–4), it was shown that the species resulting from the nitrosation of primary amino acids are the corresponding lactones. Some lactones give rise to alkylating reactions at any of a number of nucleophilic sites in tissues, and because alkylating agents are considered archetypal carcinogens (5), considerable efforts have been devoted to addressing the chemical carcinogenesis caused by these species (6).

We have previously studied the capacity of β -propiolactone (1) (BPL),¹ β -butyrolactone (BBL), γ -butyrolactone (GBL), and δ -valerolactone (DVL) (1) (Figure 1) to form adducts with 4-(*p*-nitrobenzyl)pyridine (NBP), a substrate with nucleophilic characteristics similar to DNA bases (7). A correlation was found between the alkylating potential of these lactones and their carcinogenicity: There is sufficient evidence in experimental animals for the carcinogenicity of BPL and BBL (6), and both of them react readily with NBP. The reaction of BPL, the stronger carcinogen of the two, is 10 times faster than that of BBL (1). In contrast, there is evidence suggesting the lack of carcinogenicity of GBL in experimental animals (6), and this lactone does not react with NBP (1). DVL does not alkylate NBP (1), but there are no data available about its carcinogenicity.

Because α -angelicalactone (AAL) (Figure 1) substantially inhibits the formation of tumors (8, 9), its chemical reactivity

was also investigated and compared with that of carcinogenic lactones. In the same way as for other previously studied lactones, an investigation of the electrophilic potential of AAL was carried out by studying its reactivity with NBP. The formation of the AAL–NBP adduct occurs about 10⁶-fold faster than with BPL, the most effective carcinogenic lactone. It was concluded that the formation of AAL–NBP adducts takes place through an entropy strain-catalyzed mechanism caused by early lactone ring cleavage (10). It has been suggested that the mechanism of inhibition of carcinogen-induced neoplasia by AAL would occur through the inhibition of DNA adduct formation (9). The very fast formation of AAL adducts with nucleophilic substrates could be part of those inhibiting mechanisms.

Diketene (DIK), the anhydride of acetoacetic acid (AcOAcOH), consists of a four-membered lactone ring adjacent to a methylene function (DIK; Figure 1). DIK derivatives have versatile applications, including the manufacture of agrochemicals, dyes, pigments, pharmaceuticals (including vitamins), and stabilizers for PVC and polyester (11). Despite its high chemical reactivity with nucleophilic and electrophilic reagents such as alcohols, aldehydes, amines, and ketones (11), DIK is inactive as a carcinogen. In their series of works on the carcinogenicity of lactones, Van Duuren et al. tested the carcinogenicity of DIK by different methods (12–16).

In all cases, suitable positive controls, mostly BPL, were used. No tumor was produced by DIK in any of the trials.

Among the factors potentially involved in the carcinogenic character of many alkylating agents are their solubility in water (17). Van Duuren and Goldschmidt reported that “in the β -lactone series, BPL and BBL are carcinogenic and water-soluble, whereas DIK is inactive [as a carcinogen] and water-insoluble. This correlation suggests that solubility plays some

* To whom correspondence should be addressed. Tel: +34 923 294486. Fax: +34 923 294574. E-mail: jucali@usal.es.

¹ Abbreviations: AAL, α -angelicalactone; AcOAcOH, acetoacetic acid; AD, adduct; BBL, β -butyrolactone; BPL, β -propiolactone; DIK, diketene; DVL, δ -valerolactone; GBL, γ -butyrolactone; NBP, 4-(*p*-nitrobenzyl)pyridine.

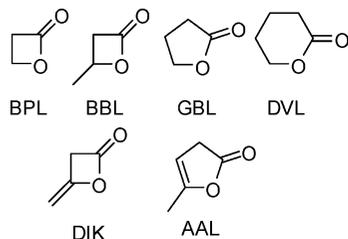
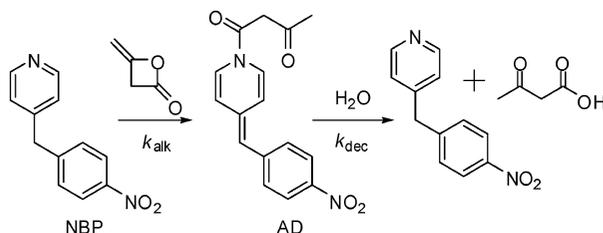


Figure 1. Chemical structures of some lactones.

Scheme 1. Alkylation of NBP by DIK and Subsequent Hydrolysis of the Adduct



role in facilitating the expression of biological activity by these materials" (18). Van Duuren also referred to the rapid hydrolysis of DIK as a possible cause for its lack of carcinogenicity, since it may preclude its *in vivo* reactivity (13).

Nevertheless, the solubility of DIK in water is appreciable; Okeya et al. worked with aqueous solutions of DIK in the 0.15–0.20 M range (19). The values found in the bibliography for the hydrolysis rate constant of DIK in water at 25 °C range from 3.6×10^{-5} (18) to $1.5 \times 10^{-6} \text{ M}^{-1} \text{ s}^{-1}$ (19), the latter being close to that of the carcinogenic β -lactones BPL and BBL (5×10^{-7} and $1.2 \times 10^{-7} \text{ M}^{-1} \text{ s}^{-1}$ (20)). These observations suggest that the reason for the lack of carcinogenicity of DIK could be other than hydrolysis. This, added to the fact that preliminary tests suggested that the NBP–DIK adduct is very short-lived when compared to the lifetimes of the NBP–BPL and NBP–BBL adducts, made the study of the alkylating potential of DIK of biological interest, since the lability of the adducts could be the cause for the said lack of carcinogenicity. Thus, the present study attempts to correlate the reported lack of carcinogenic activity of DIK with its reactivity with NBP—a trap for alkylating agents (7)—and compare this reactivity to other β -lactones of known carcinogenicity.

Experimental Procedures

Given the instability of the NBP–DIK adduct (AD), especially in basic media (see below), the standard NBP test (21, 22) could not be used to study the NBP alkylation by DIK (Scheme 1). Instead, the reaction was monitored by measuring the absorbance of AD in the reaction medium. Thus, absorbance was measured continuously at the wavelength of maximum absorption of AD ($\lambda = 450 \text{ nm}$). Because NBP is insoluble in pure water, the alkylation mixtures were prepared in different water/dioxane media, ranging from 3:7 to 7:3 (vol). Detailed reaction conditions are given in the figure and table legends.

A Shimadzu UV-2401-PC spectrophotometer with a thermoelectric six-cell holder temperature control system ($\pm 0.1 \text{ }^\circ\text{C}$) was used. The hydrolysis of DIK was monitored by titration of the resulting AcOAcOH. The reaction was followed at constant pH using a pH-Stat Metrohm 718 STAT Titrino, which releases NaOH, previously normalized with potassium hydrogen phthalate.

Reaction temperatures were kept constant ($\pm 0.05 \text{ }^\circ\text{C}$) with a Lauda Ecoline RE120 thermostat. All kinetic runs were performed in triplicate. DIK was obtained from Aldrich (98%);

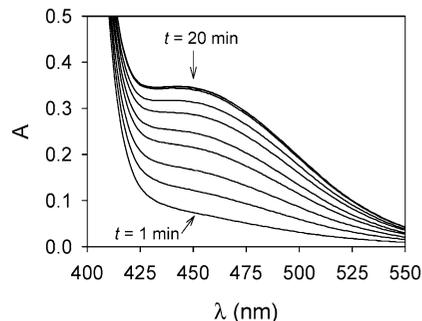


Figure 2. Spectrograms showing the formation of the NBP–DIK adduct along time in 5:5 (vol) water/dioxane medium. $[\text{DIK}]_0 = 5 \times 10^{-3} \text{ M}$, $[\text{NBP}]_0 = 5 \times 10^{-2} \text{ M}$, and $T = 20 \text{ }^\circ\text{C}$.

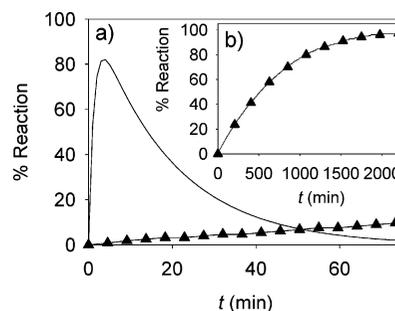


Figure 3. Formation and decomposition of the NBP–DIK adduct and hydrolysis of DIK in 5:5 water/dioxane medium. (a) NBP alkylation and NBP–DIK adduct decomposition (continuous line) as compared to DIK hydrolysis reaction in the same time range (\blacktriangle). % Reaction = $100 \times [\text{AD}]/[\text{DIK}]_0$. (b) Complete DIK hydrolysis. % Reaction = $100 \times [\text{AcOAcOH}]/[\text{DIK}]_0$. NBP alkylation reaction: $[\text{DIK}]_0 = 2 \times 10^{-3} \text{ M}$, $[\text{NBP}]_0 = 5 \times 10^{-2} \text{ M}$, and $T = 20 \text{ }^\circ\text{C}$. DIK hydrolysis reaction: $[\text{DIK}]_0 = 2.5 \times 10^{-3} \text{ M}$ and $T = 20 \text{ }^\circ\text{C}$.

NBP was from Aldrich (98%), and dioxane (99%) was purchased from Panreac.

Numerical treatment of the data was performed using SigmaPlot software, Version 9.0. Quantum calculations (23) were performed at the level of second order Møller–Plesset (MP2) theory with the 6-31++G(d,p) basis set, using the geometries optimized at the B3LYP/6-31++G(d,p) level of theory in the gas phase. The results obtained are almost identical to the corresponding MP2/6-31G++(d,p)/MP2/6-31G++(d,p) values. The integral equation formalism for the polarizable continuum model (IEFPCM) self-consistent reaction field (SCRf) procedure was employed in the solvation calculations with regard to the reactants and transition states. This computational approach has been shown to be valid in the treatment of the alkaline hydrolysis of carboxylic acid esters (24). The program used was Gaussian03 (25). Negative-mode electrospray ionization mass spectra were recorded on a Waters ZQ4000 spectrometer, by direct injection with methanol as solvent.

Results

The NBP–DIK adduct shows maximum absorption at $\lambda = 450 \text{ nm}$. Figure 2 depicts the variation in absorbance along time. Figure 3a shows a typical kinetic run of the alkylation reaction as compared to that of the hydrolysis of DIK in the same conditions (Figure 3a,b). Because the alkylation reaction is much faster (hydrolysis being less than 2% when the concentration of the adduct has reached its maximum), the DIK hydrolysis reaction was neglected.

Figure 3a shows that AD adduct is unstable; through a complete decomposition, it gives NBP and AcOAcOH (Scheme 1). From the mechanism in Scheme 1, eqs 1–3 can easily be deduced (26).

$$\text{rate} = \frac{d[\text{DIK}]}{dt} = -k_{\text{alk}}[\text{NBP}][\text{DIK}] \quad (1)$$

$$\text{rate} = \frac{d[\text{NBP}]}{dt} = -k_{\text{alk}}[\text{NBP}][\text{DIK}] + k_{\text{dec}}[\text{AD}] \quad (2)$$

$$\text{rate} = \frac{d[\text{AD}]}{dt} = -\frac{d[\text{NBP}]}{dt} \quad (3)$$

Because NBP was present in large excess, its concentration was assumed to be constant; hence, eqs 1–3 can be converted to eqs 4 and 5 (where $k_{\text{1alk}} = k_{\text{alk}}[\text{NBP}]$):

$$\frac{d[\text{DIK}]}{dt} = -k_{\text{1alk}}[\text{DIK}] \quad (4)$$

$$\frac{d[\text{AD}]}{dt} = k_{\text{1alk}}[\text{DIK}] - k_{\text{dec}}[\text{AD}] \quad (5)$$

Integration of eqs 4 and 5 and substitution of [AD] by the corresponding absorbance $A = [\text{AD}]l\epsilon$, ϵ being the molar absorption coefficient of the adduct, and l the light path, yield eq 6:

$$A = \epsilon l \frac{k_{\text{1alk}}}{k_{\text{dec}} - k_{\text{1alk}}} [\text{DIK}]_0 (e^{-k_{\text{1alk}}t} - e^{-k_{\text{dec}}t}) \quad (6)$$

Absorbance data were plotted against time. By fitting to eq 6, the k_{1alk} , k_{dec} , and ϵ values were obtained.

The proposed reaction mechanism (Scheme 1) holds that NBP is regenerated along the reaction. If the decomposition of AD affords NBP and AcOAcOH, then when the reaction is over all of the initial NBP must have been regenerated. To confirm that no NBP was actually being consumed, the reaction was carried out in excess DIK. It was observed that when the reaction had finished and more DIK was added to the mixture, the reaction started again, proving that NBP was still present and that all of the DIK had been depleted (Figure 4). This behavior, from the standpoint of the conversion of DIK to AcOAcOH, could be considered as a catalyzed hydrolysis reaction. This is coherent with the observed fact that pyridine is a powerful catalyst for the hydrolysis of carboxylic anhydrides (27). In addition, Briody and Satchell have reported that pyridine is a nucleophilic catalyst for the hydrolysis of DIK (28). It seems reasonable to expect that NBP, a pyridine derivative, would have a similar effect. The formation of AcOAcOH was confirmed by UV-vis spectra, as well as by the decrease in pH.

Formation of the NBP–DIK Adduct. Table 1 gives the values of the NBP alkylation rate constant (k_{alk}) in different water/dioxane media at several temperatures. Figure 5 shows the good fit of the k_{alk} values to the Eyring equation (29).

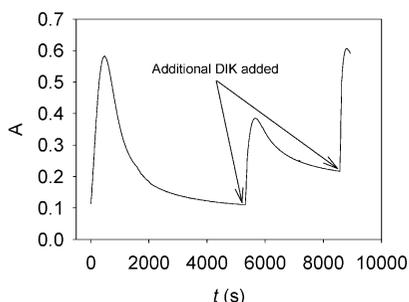


Figure 4. Formation and decomposition of the NBP–DIK adduct in excess DIK in 4:6 water/dioxane. Additional DIK was added at $t = 5500$ s and $t = 8500$ s. $[\text{DIK}]_0 = 0.2$ M, $[\text{NBP}]_0 = 1 \times 10^{-2}$ M, and $T = 35$ °C. Subsequent additions of DIK to $[\text{DIK}] = 0.4$ M and $[\text{DIK}] = 0.6$ M are also shown.

Table 1. Rate Constants of NBP Alkylation by DIK as a Function of Temperature and Medium Composition

water/dioxane (vol)	k_{alk} ($\text{M}^{-1} \text{s}^{-1}$) ^a				
	15.0 °C	20.0 °C	25.0 °C	30.0 °C	35.0 °C
3:7	0.035	0.047	0.061	0.073	0.094
4:6	0.080	0.102	0.141	0.182	0.220
5:5	0.156	0.195	0.260	0.3214	0.410
6:4	0.292	0.336	0.438	0.517	0.602
7:3	1.05	1.32	1.51	1.95	2.30

^a Values of the rate constants are reproducible to 3%.

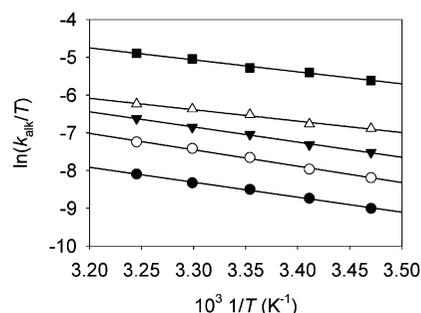


Figure 5. Eyring plots for NBP alkylation reactions by DIK in different water/dioxane media: 7:3 (■), 6:4 (△), 5:5 (▼), 4:6 (○), and 3:7 (●) (vol).

Table 2. Activation Parameters for NBP Alkylation by DIK as a Function of Medium Composition

water/dioxane (vol)	ΔH^\ddagger ^a (kJ mol ⁻¹)	$-\Delta S^\ddagger$ ^a (J K ⁻¹ mol ⁻¹)	ΔG^\ddagger ^a (35 °C) (kJ mol ⁻¹)
3:7	33 ± 1	158 ± 4	82 ± 2
4:6	36 ± 2	140 ± 5	79 ± 3
5:5	33 ± 1	144 ± 3	77 ± 2
6:4	26 ± 2	167 ± 5	77 ± 3
7:3	26 ± 2	152 ± 5	73 ± 3

^a Values are given within the 95% confidence interval.

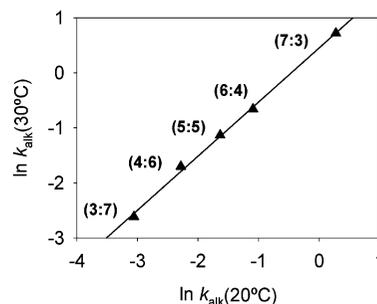


Figure 6. Palm–Vizgert plot for the NBP alkylation reaction by DIK in different water/dioxane media.

$$k = \frac{kT}{h} e^{\Delta S^\ddagger/R} e^{-\Delta H^\ddagger/RT} = \frac{kT}{h} e^{-\Delta G^\ddagger/RT} \quad (7)$$

Table 2 reports the values of the activation parameters. Figure 6 depicts the good fitting of the results to the Palm–Vizgert correlation (30) (see Discussion). The negative-mode electrospray ionization mass spectrum of the adduct shows a mass/charge ratio = 297.1, which is coherent with the suggested structure.

Decomposition of the NBP–DIK Adduct. It was observed that the decomposition of the adduct underwent general base catalysis by NBP, such that k_{dec} was the result of two contributions (eq 8), the adduct hydrolysis reaction ($k_{\text{H}_2\text{O}}$) and the hydrolysis catalyzed by NBP (k_{NBP}):

$$k_{\text{dec}} = (k_{\text{H}_2\text{O}} + k_{\text{NBP}}[\text{NBP}]][\text{H}_2\text{O}] \quad (8)$$

The existence of general base catalysis was tested with AcONa, as shown in Figure 7. The intercept corresponds to

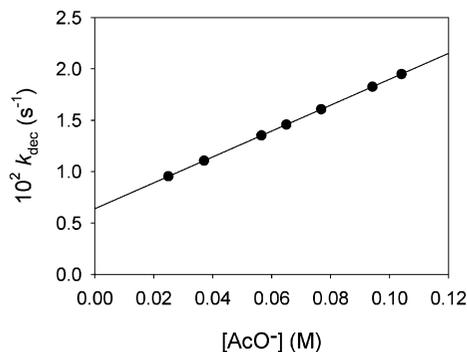


Figure 7. Catalytic effect of AcONa on the hydrolytic decomposition of the NBP–DIK adduct in 5:5 water/dioxane medium. $[\text{DIK}]_0 = 6 \times 10^{-3} \text{ M}$, $[\text{NBP}]_0 = 0.13 \text{ M}$, and $T = 25 \text{ }^\circ\text{C}$.

Table 3. Rate Constants for the Hydrolysis of the NBP–DIK Adduct as a Function of Temperature and Medium Composition

water/dioxane (vol)	$10^5 k_{\text{H}_2\text{O}} (\text{M}^{-1} \text{s}^{-1})^a$				
	15.0 $^\circ\text{C}$	20.0 $^\circ\text{C}$	25.0 $^\circ\text{C}$	30.0 $^\circ\text{C}$	35.0 $^\circ\text{C}$
3:7	0.59	0.83	1.13	1.48	1.94
4:6	1.06	1.40	1.75	2.47	3.11
5:5	1.85	2.41	3.34	4.54	6.23
6:4	3.24	4.68	6.54	8.13	11.1
7:3	10.5	14.8	19.3	26.1	34.6

^a Values are reproducible to 5%.

Table 4. Catalytic Coefficients, k_{NBP} , for the NBP-Catalyzed Decomposition of the NBP–DIK Adduct as a Function of Temperature and Medium Composition

water/dioxane (vol)	$10^4 k_{\text{NBP}} (\text{M}^{-2} \text{s}^{-1})^a$				
	15.0 $^\circ\text{C}$	20.0 $^\circ\text{C}$	25.0 $^\circ\text{C}$	30.0 $^\circ\text{C}$	35.0 $^\circ\text{C}$
3:7	0.49	0.68	0.97	1.32	1.86
4:6	0.96	1.27	1.78	2.45	3.54
5:5	1.29	1.94	2.50	3.23	4.47
6:4	2.00	2.64	3.48	4.74	6.25
7:3	4.66	6.33	7.64	9.29	12.3

^a Values of the rate constants are reproducible to 5%.

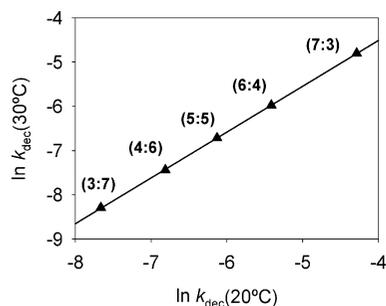


Figure 8. Palm–Vizgert plot for the decomposition of the NBP–DIK adduct in water/dioxane media.

the value of k_{dec} in the absence of AcONa. Table 3 shows the values of $k_{\text{H}_2\text{O}}$ at different temperatures and in different media. Table 4 gives the values of the catalytic coefficient k_{NBP} . Figure 8 depicts the good fitting of the k_{dec} values to the Palm–Vizgert correlation (see Discussion).

Theoretical Results. Table 5 shows the calculated activation energy for acyl and alkyl cleavage in the reaction of BPL, BBL, and DIK with NBP, together with the experimental value.

Discussion

Formation of the NBP–DIK Adduct. The k_{alk} values for DIK (Table 1) are much higher than those observed for BPL

Table 5. Energy Barriers for the Reaction of NBP with BPL, BBL, and DIK

lactone	$E_a (\text{kJ mol}^{-1})$		
	experimental ^a	calcd acyl	calcd alkyl
BPL (alkyl fission) ^b	44	116	33
BBL (alkyl fission) ^b	50	123	55
DIK (acyl fission)	29	31	102

^a In 7:3 water/dioxane medium. ^b From ref 1.

Table 6. Activation Parameters for NBP Alkylation by BPL, BBL, and DIK in 7:3 Water/Dioxane Medium

water/dioxane (vol)	ΔH^\ddagger ^a (kJ mol ⁻¹)	$-\Delta S^\ddagger$ ^a (J K ⁻¹ mol ⁻¹)	ΔG^\ddagger ^a (35 $^\circ\text{C}$) (kJ mol ⁻¹)
BPL ^b	41 ± 2	148 ± 6	87 ± 2
BBL ^b	47 ± 2	148 ± 6	93 ± 2
DIK ^c	26 ± 2	152 ± 5	71 ± 2

^a Values are given within the 95% confidence interval. ^b Values are taken from ref 1. ^c This work.

and BBL (1): $1.5 \text{ M}^{-1} \text{ s}^{-1}$ for DIK, as compared with 8×10^{-3} and $7 \times 10^{-4} \text{ M}^{-1} \text{ s}^{-1}$ for BPL and BBL at 25 $^\circ\text{C}$ in 7:3 water/dioxane medium. Table 6 shows the values of the activation parameters for the NBP alkylation reaction by BPL, BBL, and DIK. As can be observed, they suggest that the reactivity of these lactones is enthalpy-controlled (31) (the same ΔS^\ddagger values for the three lactones are consistent with their analogous geometry).

The values reported in Tables 1 and 6 show that DIK reacts with NBP about 2 orders of magnitude faster than BPL or BBL, both of these being carcinogenic lactones, and that the hydrolysis of DIK is only slightly faster than that of the other two lactones. Thus, DIK shows a much higher alkylating potential (expressed as the alkylation rate constant) and a much higher efficiency of alkylation (expressed as the alkylation rate constant/hydrolysis rate constant ratio).

The existence of an isokinetic relationship could serve as an argument—but not as proof—that the reactions studied share a common feature (32). A mathematical formulation of the isokinetic effect is the linear relationship between two series of $\log k$ values measured at two temperatures: T_1 and T_2 . Thus, $\log k(T_2) = a + b \cdot \log k(T_1)$. Another formulation of the isokinetic relationship is the existence of a compensation effect between the values of enthalpy, ΔH^\ddagger , and the entropy of activation, ΔS^\ddagger , such that the Gibbs' energy of activation, ΔG^\ddagger , is approximately constant (33). The results shown in Figure 6 support the idea of a common mechanism for the NBP alkylation reactions by DIK in different water/dioxane media, ranging from 3:7 to 7:3 (vol).

Decomposition of the NBP–DIK Adduct. Contrary to what occurs with BPL and BBL and other alkylating agents, the adduct formed by DIK readily undergoes hydrolysis in the reaction conditions used here. The cause could lie in the fact that the β -ketoamide bond formed by the acyl fission on DIK is more easily cleaved than the amine bond formed by alkyl fission on BPL and BBL (Figure 9).

The good fitting to the Palm–Vizgert correlation suggests that the decomposition of the adduct occurs through a common mechanism in all media, its rate increasing with the water:dioxane ratio (the adduct is stable in anhydrous media). Because DIK is water-soluble and because its hydrolysis is slow enough not to compete with its alkylating ability (see Figure 3), which in vitro is much higher than that of BPL and BBL, its lack of carcinogenicity could be explained in terms of the instability of the adducts that it may form with DNA. DIK could react rapidly with the nucleophilic sites in DNA (such as nitrogen

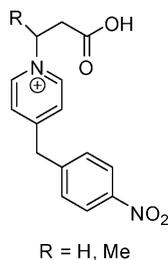


Figure 9. Adducts formed by BPL (R = H) and BBL (R = Me) with NBP.

atoms of purine and pyrimidine nucleotides), but because the adducts would have a very short life, they would decompose into the free nucleotide and AcOAcOH, the latter of which is in turn metabolized in the organism. In view of the observed dependency of the rate constants on the medium composition, and the presence in the cellular environment of high concentrations of phosphate buffer—capable of producing general base catalysis—the decomposition of adducts in the biological medium should be even faster than what was observed here.

Theoretical Results. The results shown in Table 5 suggest that a change occurs in the reaction mechanism of DIK in comparison with other β -lactones. BPL and BBL are known to react with water in neutral medium and with pyridine by alkyl fission (18), whereas DIK reacts by acyl fission with the nitrogen atoms of heterocyclic aromatics (11). The values obtained for the energy barriers further confirm that DIK reacts by forming an amide bond through acyl fission that is less stable than the amine bonds formed by BPL and BBL by alkyl fission. Our results also suggest that the MP2/6-31G++(d,p)//B3LYP/6-31++G(d,p) level of theory and the IEFPCM SCRF methods afford valid results for the semi-quantitative treatment of this type of reaction.

Conclusions

(i) Although DIK, unlike other four-membered ring lactones, is inactive as a carcinogen in experimental animals, it shows an alkylating potential of about 2 orders of magnitude higher than BPL or BBL, which are classified as possibly carcinogenic to humans by the IARC (6), since there is sufficient evidence of their carcinogenicity in experimental animals. (ii) The reactivity of DIK as an alkylating agent is enthalpy-controlled. (iii) The fact that the hydrolysis reaction of DIK is slightly faster than those of other four-membered ring lactones shows that DIK is more efficient as an alkylating agent, since the hydrolysis of this species poses less competition to the alkylation reaction. (iv) DIK undergoes acyl fission in the alkylation reaction, which results in an amide bond in the NBP–DIK adduct. The lability of the amide bond as opposed to the amine bonds formed by BPL and BBL could be one of the differential factors responsible for the lack of carcinogenicity of DIK. (v) Ab initio calculations of the energy barriers help to understand the unusual reactivity of DIK.

Acknowledgment. We thank the Spanish Ministerio de Ciencia e Innovación (CTQ2007-63263), as well as the Spanish Junta de Castilla y León (Grant SA040A08) for supporting the research reported in this article. R.G.B. thanks the Ministerio de Ciencia e Innovación, and M.G.P. also thanks the Junta de Castilla y León for Ph.D. grants. Thanks are also given for the valuable comments made by the referees.

References

- Manso, J. A., Pérez-Prior, M. T., García-Santos, M. P., Calle, E., and Casado, J. (2005) A kinetic approach to the alkylating potential of carcinogenic lactones. *Chem. Res. Toxicol.* 18, 1161–1166.
- García-Santos, M. P., Calle, E., and Casado, J. (2001) Amino acid nitrosation products as alkylating agents. *J. Am. Chem. Soc.* 123, 7506–7510.
- García-Santos, M. P., González-Mancebo, S., Hernández-Benito, J., Calle, E., and Casado, J. (2002) Reactivity of amino acids in nitrosation reactions and its relation to the alkylating potential of their products. *J. Am. Chem. Soc.* 124, 2177–2182.
- García-Santos, M. P., Calle, E., and Casado, J. (2003) A method for the kinetic study of amino acid nitrosation. *Polyhedron* 22, 1059–1066.
- Lawley, P. D. (1984) Carcinogenesis by alkylating agents. In *Chemical Carcinogens* (Searle, C. E., Ed.) p 428, American Chemical Society, Washington, DC.
- IARC (1999) *Re-Evaluation of Some Organic Chemicals, Hydrazine and Hydrogen Peroxide*, pp 367, 1103, and 1317, IARC Monograph 71, IARC, Lyon, France.
- Shephard, S. E., and Lutz, W. K. (1989) Nitrosation of dietary precursors. *Cancer Surv.* 8, 401–421.
- Ioannou, Y. M., Wilson, A. G., and Anderson, M. W. (1982) Effect of butylated hydroxyanisole, alpha-angelica lactone, and beta-naphthoflavone on benzo(alpha)pyrene-DNA adduct formation in vivo in the forestomach, lung, and liver of mice. *Cancer Res.* 42, 1199–1204.
- Huber, W. W., McDaniel, L. P., Kaderlik, K. R., Teitel, C. H., Lang, N. P., and Kadlubar, F. F. (1997) Chemoprotection against the formation of colon DNA adducts from the food-borne carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) in the rat. *Mutat. Res.* 376, 115–122.
- Fernández-Rodríguez, E., Manso, J. A., Pérez-Prior, M. T., García-Santos, M. D. P., Calle, E., and Casado, J. (2007) The unusual ability of alpha-angelicalactone to form adducts: A kinetic approach. *Int. J. Chem. Kinet.* 39, 591–594.
- Clemens, R. J. (1986) Diketene. *Chem. Rev.* 86, 241–318.
- Van Duuren, B. L., Nelson, N., Orris, L., and Palmes, E. D. (1963) Carcinogenicity of epoxides, lactones, and peroxy compounds. *J. Natl. Cancer Inst.* 31, 41–55.
- Van Duuren, B. L. (1969) Carcinogenic epoxides, lactones, and halo-ethers and their mode of action. *Ann. N. Y. Acad. Sci.* 163, 633–650.
- Van Duuren, B. L., Langseth, L., Goldschmidt, B. M., and Orris, L. (1967) Carcinogenicity of epoxides lactones and peroxy compounds. 6. Structure and carcinogenic activity. *J. Natl. Cancer Inst.* 39, 1217.
- Van Duuren, B. L., Langseth, L., Orris, L., Teebor, G., Nelson, N., and Kuschner, M. (1966) Carcinogenicity of epoxides, lactones, and peroxy compounds. IV. Tumor response in epithelial and connective tissue in mice and rats. *J. Natl. Cancer Inst.* 37, 825–838.
- Van Duuren, B. L., Langseth, L., Orris, L., Baden, M., and Kuschner, M. (1967) Carcinogenicity of epoxides, lactones, and peroxy compounds. V. Subcutaneous injection in rats. *J. Natl. Cancer Inst.* 39, 1213–1216.
- Lijinsky, W. (1992) *Chemistry and Biology of N-Nitroso Compounds*, Cambridge University Press, Cambridge, GB.
- Van Duuren, B. L., and Goldschmidt, B. M. (1966) Carcinogenicity of epoxides, lactones, and peroxy compounds. III. Biological activity and chemical reactivity. *J. Med. Chem.* 9, 77–79.
- Okeya, S., and Kawaguchi, S. (1984) Kinetic-studies of the hydrolysis of diketene catalyzed by the chloropalladium(II) complex. *Bull. Chem. Soc. Jpn.* 57, 1217–1221.
- Pérez-Prior, M. T., Manso, J. A., García-Santos, M. D., Calle, E., and Casado, J. (2005) Reactivity of lactones and GHB formation. *J. Org. Chem.* 70, 420–426.
- 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.
- Epstein, J., Rosenthal, R. W., and Ess, R. J. (1955) Use of p-(4-nitrobenzyl)-pyridine as analytical reagent for ethylenimines and alkylating agents. *Anal. Chem.* 27, 1435–1439.
- Levine, I. (1991) *Quantum Chemistry*, Prentice Hall, New York.
- Zhan, C.-G., Landry, D. W., and Ornstein, R. L. (2000) Energy barriers for alkaline hydrolysis of carboxylic acid esters in aqueous solution by reaction field calculations. *J. Phys. Chem. A* 104, 7672–7678.
- Fritsch, M. J., (2004) *Gaussian 03*, Revision B.05, Gaussian, Inc., Wallingford, CT.
- Laidler, K. J. (1987) *Chemical Kinetics*, 3rd ed., Harper Collins, New York.
- Bender, M. L. (1960) Mechanisms of catalysis of nucleophilic reactions of carboxylic acid derivatives. *Chem. Rev.* 60, 53–113.

- (28) Briody, J. M., and Satchell, D. P. (1965) Acylation. XVI. Spontaneous and catalysed hydrolysis of diketene. *J. Chem. Soc., Chem. Commun.* 3778–3785.
- (29) Connors, K. A. (1990) *Chemical Kinetics, the Study of Reaction Rates in Solution*, VCH, New York.
- (30) Palm, V. A., and Vizgert, R. V. (1962) Correlation of Rate Constants in Alkaline Hydrolysis of Aromatic Sulfoacid Esters, as Dependent on Structure of these Compounds. *Dokl. Akad. Nauk SSSR* 142, 1091–1097.
- (31) Carpenter, B. K. (1984) *Determination of Organic Reaction Mechanisms*, pp 123–158, Wiley, New York.
- (32) Exner, O. (1988) *Correlation Analysis of Chemical Data*, p 106, Plenum, New York.
- (33) House, J. E. (1997) *Principles of Chemical Kinetics*, p 162, WCB Publishers, Dubuque.

TX800153J