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Kinetic study of the neutral and base hydrolysis of diketene

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Diketene (4-methylene-2-oxetanone) is inactive as a carcinogen, although it is more reactive toward nucleophiles than the analogs β-propiolactone and β-butyrolactone, both of which are alkylating agents of known carcinogenicity. In the literature the lack of carcinogenic effects has been ascribed to the rapid hydrolysis of diketene, which could preclude its *in vivo* alkylating capacity. In this work, the kinetics of the neutral and alkaline hydrolysis of diketene in aqueous and different water–dioxane media have been studied. The following conclusions can be drawn: (i) The neutral hydrolysis of diketene is slightly faster than that of β-propiolactone and β-butyrolactone. (ii) The hydrolysis reaction of diketene is very slow when compared to its alkylation reaction of a DNA-model nucleophile, suggesting that, contrary to earlier results, competitive hydrolysis is not the cause of its lack of carcinogenicity. (iii) The diketene neutral hydrolysis rate constant increases with the water/dioxane ratio, the opposite occurring in base hydrolysis.
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Keywords: alkylation; diketene; hydrolysis of diketene

INTRODUCTION

In recent decades, considerable effort has been devoted to the study of the carcinogenicity of commonly used chemicals, resulting in lists of substances classified according to their potential carcinogenicity. Among these, alkylating agents are considered archetypical carcinogens because of their ability to react with nucleophilic sites in DNA.[1]

In this line of work, our group has studied the *in vitro* reactivity of several alkylating compounds capable of forming DNA adducts: sorbic acid[2] and sorbates,[3] nitrosoureas,[4,5] and lactones.[6–8] The results obtained reveal a correlation between the carcinogenicity of the substances and their reactivity with 4-*p*-(nitrobenzyl)pyridine (NBP), a trap for alkylating agents with nucleophilicity similar to that of DNA.[9,10] There is sufficient evidence from experiments with animals for the carcinogenicity of β-propiolactone (BPL) and β-butyrolactone (BBL)[11] and both readily react with NBP. The reaction of BPL, the stronger carcinogen of the two, is six times faster than that of BBL. In contrast, there is evidence suggesting the lack of carcinogenicity of γ-butyrolactone (GBL) in experimental animals,[11] and this lactone does not react with NBP, and neither does δ-valerolactone (DVL).

Diketene (DIK; 4-methylene-2-oxetanone; Fig. 1) consists of a four-membered lactone ring adjacent to a methylene function and it can be considered the anhydride of acetoacetic acid. Diketene is common industrial chemical, and its derivatives have versatile applications, including the manufacture of agrochemicals, dyes, pigments, pharmaceuticals (including vitamins), and stabilizers for PVC and polyester.[12] Despite its high chemical reactivity with nucleophilic and electrophilic reagents such as alcohols, aldehydes, amines, and ketones,[12] diketene is inactive as a carcinogen.[13–17]

Van Duuren and Goldschmidt reported that "in the β-lactone series, β-propiolactone and β-butyrolactone are carcinogenic and water-soluble, whereas diketene is inactive [*as a carcinogen*] and water-insoluble. This correlation suggests that solubility plays some role in facilitating the expression of biological activity by these materials."[18] Van Duuren also referred to the rapid hydrolysis of diketene as a possible cause for its lack of carcinogenicity, since it may preclude its *in vivo* reactivity.[14]

Nevertheless, the solubility of diketene in water is around 0.1 M[19] and the values found in the literature for its hydrolysis rate constant in water at 25 °C range from 3.6×10^{-5}[18] to $1.5 \times 10^{-6}\,M^{-1}\,s^{-1}$,[19] the latter being close to that of the carcinogenic β-lactones BPL and BBL (5×10^{-7} and $1.2 \times 10^{-7}\,M^{-1}\,s^{-1}$[20]). These observations suggest that the reason for the lack of carcinogenicity of diketene could be due to some mechanism, other than hydrolysis.

On the other hand, although the hydrolysis of esters has been studied in depth from both the experimental and the theoretical points of view, the hydrolysis of small lactones has not received so much attention. This is also the case with diketene; its neutral hydrolysis has been studied by several authors, with diverging results, while to our knowledge its base hydrolysis has not been studied.

This work attempts to clarify whether the hydrolysis of diketene poses significant competition to its alkylating potential

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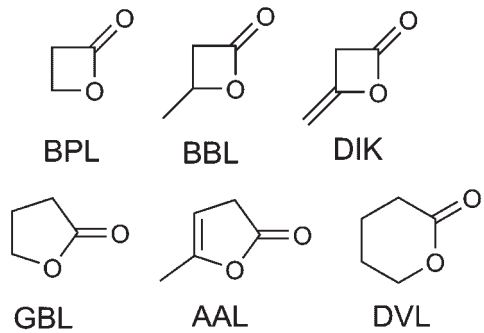


Figure 1. Chemical structure of some lactones

and to compare the hydrolysis reaction of diketene with that of the carcinogenic lactones BPL and BBL.

EXPERIMENTAL

Neutral hydrolysis

The uncatalyzed hydrolysis of diketene was studied at pH = 6.00 using an Metrohm 718 STAT Titrino autotritator which added normalized 0.1 M NaOH to 100 cm^3 of 2.0×10^{-3} M diketene. The total added volume was about 2 cm^3, which means that the effect of the dilution was smaller than the experimental error.

The reaction was studied in aqueous and aquo-organic media, ranging from 9:1 to 4:6 water/dioxane (vol).

Base hydrolysis

The hydroxide-catalyzed hydrolysis of diketene (2.5×10^{-3} M) in excess NaOH (0.01–0.5 M) was studied using a modular 3-syringe Bio-Logic Sequential Mixing Stopped-Flow Spectrometer SFM300 at $\lambda = 400$ nm, where the acetoacetate dianion shows maximum absorbance.

The reaction was studied in aqueous and aquo-organic media, ranging from 9:1 to 4:6 water/dioxane.

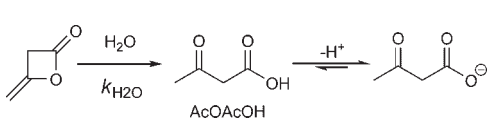
Materials

Diketene (98%) was from Aldrich; dioxane (99%) and NaOH (99%) were from Panreac (Barcelona, Spain).

RESULTS

Neutral hydrolysis

The differential kinetic equation for the neutral hydrolysis of diketene (Eqn (1)) can be deduced from Scheme 1, k_{H_2O} being the second order rate constant for the hydrolysis of diketene. Considering that water is present in large excess the pseudo-first-order approximation allows to integrate Eqn (1), yielding



Scheme 1. Neutral hydrolysis of diketene

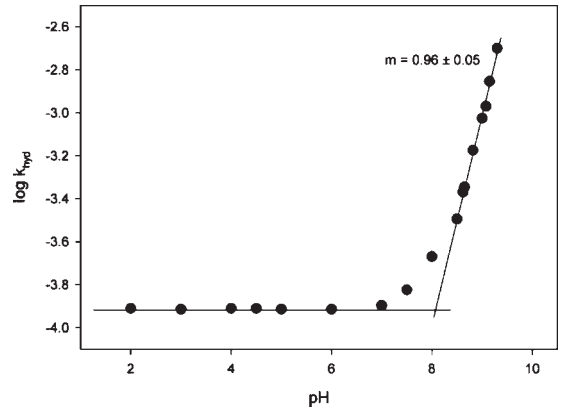


Figure 2. Influence of pH on the experimental rate constant of hydrolysis of diketene. $T = 25\,^{\circ}C$, water/dioxane 8:2 (vol)

Eqn (2), with $k_{1H_2O} = [H_2O]k_{H_2O}$.

$$r = \frac{d[DIK]}{dt} = -\frac{d[AcOAcO^-]}{dt} = -k_{H_2O}[DIK][H_2O] \quad (1)$$

$$[AcOAcO^-] = [DIK]_o\left(1 - e^{-k_{1H_2O}t}\right) \quad (2)$$

The amount NaOH consumed by the titrator is equivalent to the amount of acetoacetic acid formed, so the experimental rate constant $\left(k_{hyd} = k_{1H_2O} + k_{OH}[OH^-]\right)$ – which includes k_{1H_2O} plus catalytic terms; see below – can be calculated by fitting the NaOH consumption to Eqn (3).

$$[AcOAcO^-] = [DIK]_o\left(1 - e^{-k_{hyd}t}\right) \quad (3)$$

Figure 2 plots the experimental hydrolysis rate constant against pH. It can be observed that the hydrolysis of diketene is catalyzed by the hydroxide ion as from pH ~ 8 and is not acid-catalyzed until pH < 2, therefore between 2 < pH < 8, $k_{hyd} = k_{1H_2O}$, while at very high values of pH, the neutral reaction is very slow when compared to the catalyzed one and $k_{hyd} = k_{OH}[OH^-]$.

The values of the rate constant k_{H_2O} were obtained in different water/dioxane mixtures at different temperatures, and are shown in Table 1. The activation parameters for the neutral hydrolysis reaction were calculated with the Eyring–Wynne-Jones equation (Table 2).

Table 1. Rate coefficients k_{H_2O} for the neutral hydrolysis of diketene

	$10^6\ k_{H_2O}$ ($M^{-1}\,s^{-1}$)[a]				
Water/dioxane (vol)	15 °C	20 °C	25 °C	30 °C	35 °C
4:6	0.292	0.495	0.810	1.35	2.21
5:5	0.396	0.749	1.21	2.03	3.24
6:4	0.575	0.904	1.43	2.08	3.30
7:3	0.856	1.30	2.03	3.06	4.65
8:2	1.17	1.84	2.77	4.18	6.19
9:1	1.52	2.25	3.54	5.16	7.49
10:0	1.86	2.95	4.50	6.15	9.37

[a] Values are reproducible within 5%.

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Table 2. Activation parameters for the neutral hydrolysis of diketene

Water/dioxane (vol)	$\Delta H^{\ddagger}$ (kJ mol^{-1})[a]	$-\Delta S^{\ddagger}$ (J K^{-1} mol^{-1})[a]
4:6	72.1 ± 1.9	119 ± 4
5:5	74.4 ± 2.6	108 ± 6
6:4	61.4 ± 1.1	151 ± 3
7:3	60.0 ± 0.6	152 ± 2
8:2	58.7 ± 0.4	154 ± 1
9:1	56.8 ± 0.9	158 ± 3
10:0	56.0 ± 1.7	159 ± 6

[a] Values are given with their standard deviations.

Figure 3 shows the existence of an isokinetic $\Delta H^{\ddagger}/\Delta S^{\ddagger}$ relationship for k_{H_2O} in the different media. The existence of the isokinetic relationship has been reported for many reaction series, in particular those that serve to support the common theoretical conceptions. It can serve as an argument – not a proof – that the reactions in question show some similarity, or a common feature; in this case a common mechanism in all the media water/dioxane investigated here.

Base hydrolysis

Diketene reacts with hydroxide ions, affording acetoacetate, which in the conditions used here (excess of NaOH), dissociates to the dianion (Scheme 2).

The differential kinetic equation for the base hydrolysis of diketene is shown in Eqn (4). Its integrated counterpart, assuming that the concentration of hydroxide remains constant due to it being in large excess, is shown in Eqn (5).

$$r = \frac{d[\mathrm{DIK}]}{dt} = -\frac{d[\mathrm{AcOAcO}^{2-}]}{dt} = -k_{\mathrm{OH}}[\mathrm{OH}^-][\mathrm{DIK}] = \\ = -k_{\mathrm{OH}}[\mathrm{OH}^-]\left([\mathrm{DIK}]_o - [\mathrm{AcOAcO}^{2-}]\right) \quad (4)$$

$$[\mathrm{AcOAcO}^{2-}] = [\mathrm{DIK}]_o\left(1 - e^{-k_{\mathrm{OH}}[\mathrm{OH}^-]t}\right) \quad (5)$$

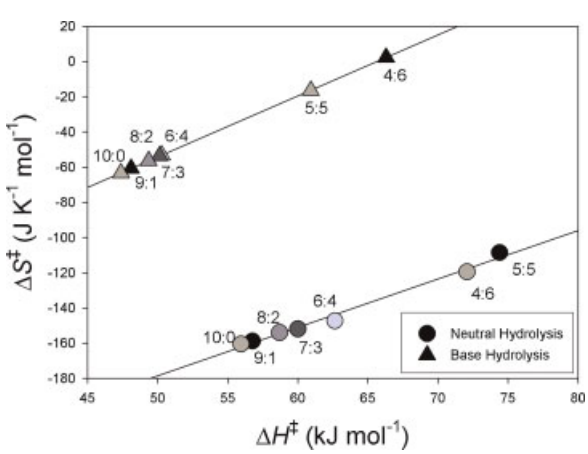
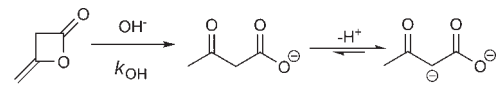


Figure 3. $\Delta H^{\ddagger}/\Delta S^{\ddagger}$ correlation for the neutral and base hydrolysis of diketene



Scheme 2. Alkaline hydrolysis of diketene

Figure 4 shows the kinetic profile of a typical experiment and the fitting to Eqn (5). The values of the rate constant k_{OH} were obtained in different water/dioxane mixtures at different temperatures and are shown in Table 3. The activation parameters for the base hydrolysis reaction were calculated with the Eyring–Wynne-Jones equation (Table 4). Figure 3 shows the existence of an isokinetic $\Delta H^{\ddagger}/\Delta S^{\ddagger}$ relationship for k_{OH} in the different media.

DISCUSSION

Neutral hydrolysis

Diketene, like other β-lactones, hydrolyzes at neutral pH and shows no acid-catalyzed hydrolysis until very acidic pH values.[21,22] From Table 1 it can be seen that the rate increases with the water ratio in the medium, suggesting that water stabilizes the transition state more than the reactants, leading to a smaller barrier.

On comparing the activation parameters to those of the carcinogenic β-lactones BPL and BBL (Table 5), it may be seen that the hydrolysis of diketene is only slightly faster than that of BPL and about one order of magnitude faster than that of BBL (k_{H_2O} being 1.1×10^{-6} and 0.2×10^{-6} M^{-1} s^{-1}, respectively as compared to 4.5×10^{-6} M^{-1} s^{-1} for diketene in pure water at 25 °C).[6,20]

These results show that although diketene is in general much more reactive than BPL, this is not the case when referred to hydrolysis. Less reactive, non-carcinogenic GBL, DVL, and AAL do not show hydrolysis in neutral medium.[20]

We have previously determined the rate constants for the reaction of NBP with diketene.[8] The alkylation reaction of NBP by diketene is much faster that its hydrolysis: the alkylation rate constant at 25 °C in 7:3 water/dioxane is 3×10^{-2} M^{-1} s^{-1} (with [NBP] = 0.02 M) as compared to an experimental hydrolysis rate

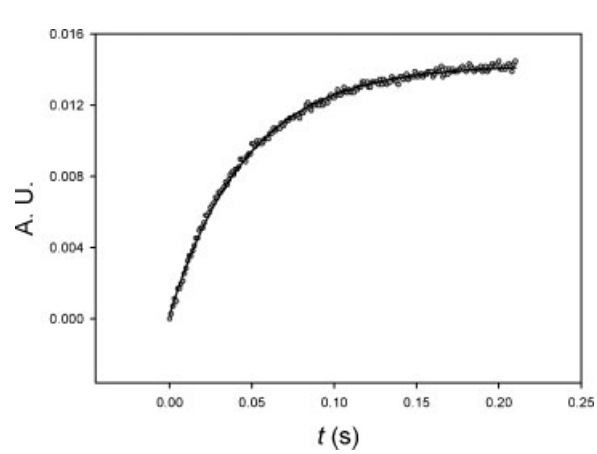


Figure 4. Kinetic profile of a typical kinetic run. [DIK] = 0.005 M, [NaOH] = 0.5 M, water/dioxane 9:1, T = 35 °C

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Table 3. Rate coefficients k_{OH} for the alkaline hydrolysis of diketene

	k_{OH} ($M^{-1}\,s^{-1}$)[a]				
Water/dioxane (vol)	15 °C	20 °C	25 °C	30 °C	35 °C
4:6	7.5	12.0	21.0	32.1	50.5
5:5	7.4	11.6	18.7	29.8	43.4
6:4	7.8	10.9	15.8	22.9	32.3
7:3	7.7	11.1	15.5	23.2	31.1
8:2	7.9	10.9	15.0	23.0	31.6
9:1	7.7	10.5	15.1	22.1	29.6
10:0	7.5	10.4	15.0	21.6	28.7

[a] Values are reproducible within 5%.

constant of $7.8 \times 10^{-5}\,M^{-1}\,s^{-1}$, which renders the amount of competing hydrolysis negligible.

Also, diketene reacts with NBP about a hundred-fold faster than carcinogenic BPL,[6] although the two hydrolyze at very similar rates. These observations suggest that the competing hydrolysis to its alkylating reaction is not likely to be the cause for the lack of carcinogenicity of diketene.

It can be seen that the $\Delta H^{\ddagger}/\Delta S^{\ddagger}$ correlation (Fig. 3, ●) shows a large difference for the activation parameters for 6:4 and 5:5 water/dioxane media. This correlates with abrupt changes in the properties of the water–dioxane mixtures, taking place around a ratio of 6:4 (~0.1 dioxane molar fraction (X_{Dio})); for instance, the melting points of the mixtures and the heats of mixing show minima at approximately 6:4.[23]

These sudden changes correlate with the structure of the mixtures; the number of hydrogen bonds, *per* water and dioxane oxygen atom, estimated from X-Ray data, show an inflection point at about $X_{Dio} = 0.1$. At $X_{Dio} < 0.1$, the structure of the solution is that of water, while at $0.1 < X_{Dio} < 0.2$ the formation of small binary clusters consisting of one or two dioxane molecules and several water molecules is observed.[24]

The parameters for the 4:6 medium show an important deviation from the straight line, suggesting that the $\Delta H^{\ddagger}/\Delta S^{\ddagger}$ relationships become complex for media with a higher dioxane ratio. A similar behavior has been observed in the case of BPL and BBL, both of which have N-shaped $\Delta H^{\ddagger}/\Delta S^{\ddagger}$ plots for neutral hydrolysis in water–dioxane mixtures.[25] This curve appears at a water/dioxane ratio of approximately 5:5, and has been classified in terms of the shape of the $\Delta H^{\ddagger}/\Delta S^{\ddagger}$ plot as ${}_{20}A_{35}C_{40}A_{100}$.[26]

Table 4. Activation parameters for k_{OH}

Water/dioxane (vol)	$\Delta H^{\ddagger}$ (kJ mol^{-1})[a]	$-\Delta S^{\ddagger}$ (J K^{-1} mol^{-1})[a]
4:6	66.6 ± 1.0	−3.5 ± 1.3
5:5	60.9 ± 0.2	16.4 ± 0.7
6:4	50.3 ± 0.3	53.0 ± 1.1
7:3	50.2 ± 0.8	53.6 ± 2.8
8:2	49.4 ± 1.4	56.3 ± 4.8
9:1	48.1 ± 0.8	60.8 ± 2.6
10:0	47.4 ± 0.6	63.3 ± 2.2

[a] Values are given with their standard deviations.

Table 5. Comparison of the activation parameters for diketene and BPL and BBL

Water/dioxane (vol)	$\Delta H^{\ddagger}$[a] (kJ mol^{-1})	$-\Delta S^{\ddagger}$[a] (J K^{-1} mol^{-1})	$\Delta G^{\ddagger}$[a] (35 °C) (kJ mol^{-1})
BPL[b]	79 ± 1	95 ± 3	108 ± 1
BBL[b]	83 ± 1	98 ± 3	113 ± 1
Diketene[c]	59 ± 1	156 ± 5	107 ± 1

[a] Values are given within the 95% confidence interval.
[b] Values taken from.[6]
[c] This work.

Base hydrolysis

The values in Table 3 show the variation in k_{OH} with the medium composition. At low temperatures, the variation lies within the experimental error. At higher temperatures, the values of the base hydrolysis rate constant increase as the polarity of the reaction medium decreases. A possible interpretation is that the increase in polarity would stabilize the transition state less than the reactants, since the charge is more delocalized in the transition state.

It can be seen that the $\Delta H^{\ddagger}/\Delta S^{\ddagger}$ correlation for the base hydrolysis (Fig. 3, ▲) also shows a large difference for the activation parameters for 6:4 and 5:5 water/dioxane media. This can be understood in the same terms as those described above.

CONCLUSIONS

(i) The neutral hydrolysis of diketene is slightly faster than that of the carcinogenic analogs BPL and BBL.
(ii) The hydrolysis reaction of diketene is very slow when compared to its alkylation reaction of the DNA-model nucleophile, suggesting that, contrary to earlier results, competitive hydrolysis is not the cause of its lack of carcinogenicity.
(iii) The neutral and base hydrolysis of diketene show a $\Delta H^{\ddagger}/\Delta S^{\ddagger}$ correlation up to a water/dioxane ratio of 5:5 (vol), after which the behavior becomes complex.
(iv) The diketene neutral hydrolysis rate constant increases with the water/dioxane ratio, the opposite occurring in the base hydrolysis.

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