The Mechanism of the Reaction of Diphenylketene with Bases in Aqueous Solution: Nucleophilic Attack versus General Base Catalysis of Ketene Hydration

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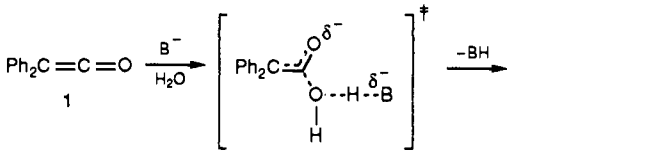
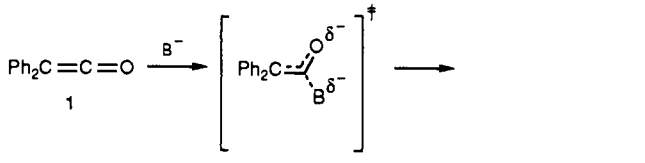
Abstract: Diphenylketene was generated in aqueous solution by flash photolysis, and rates of its decay accelerated by 30 bases of various structure were determined. The rate constants so obtained did not show the regular dependence on basic strength expected if the bases were serving as general base catalysts assisting the attack of water on the ketene, but they did vary with polarizability and steric bulk of the base in the way expected for direct nucleophilic attack of the base on the carbonyl carbon atom of the ketene. Assignment of a direct nucleophilic role to the bases is supported by the formation of amide products in addition to diphenylacetic acid in the reaction of diphenylketene accelerated by ammonia and morpholine, and quantitative analysis of the product ratios shows that these two bases serve only as nucleophiles and that the diphenylacetic acid is formed by uncatalyzed reaction of diphenylketene with solvent water.

Ketenes are interesting and useful substances whose chemistry is receiving renewed attention,[1] stimulated in part by the development of rapid, flash-photolytic techniques for studying these reactive molecules.[2] This has led to a wealth of new information, among which is the discovery that the reaction of ketenes with wholly or partly aqueous solvents is accelerated by bases.[2b,d,3]

This acceleration could be due to general base catalysis of ketene hydration, through a transition state in which the base assists nucleophilic attack of a water molecule on the ketene by removing one of its protons, eq 1, or it could be the result of direct nucleophilic attack by the base itself, eq 2.

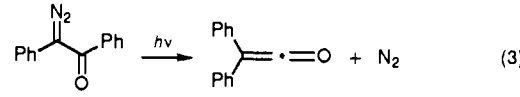


(1)



(2)

In order to distinguish between these two alternatives, we have carried out a detailed study of the reaction of a wide variety of bases with diphenylketene, **1**, generated in wholly aqueous solution by flash photolysis of azibenzil, **2**, eq 3.[2b] Our mechanistic argument is based upon the different reactivity patterns expected



(3)

for bases serving as proton transfer agents, eq 1, and bases serving as nucleophiles, eq 2.[4] We have also made use of the fact that the products of these two kinds of reaction are different (cf. eqs 1 and 2).

Experimental Section

Materials. Azibenzil was prepared by mercuric oxide oxidation of benzil monohydrazone,[5] and 4-(2,2-diphenylacetyl)morpholine was obtained by treating morpholine with diphenylacetyl chloride.[6] All other materials were best available commercial grades.

Ketenes. Flash photolysis was carried out using a system of conventional design that has already been described.[2b] Rates of decay of diphenylketene were determined by monitoring the decrease of ketene absorbance at $\lambda = 267$ nm. The reaction solutions were wholly aqueous and were maintained at 25.0 ± 0.05 °C; initial azibenzil concentrations in these solutions were 2×10^{-5} M. The photochemical conversion of azibenzil to diphenylketene in our apparatus under these conditions was quite efficient, with 80–90% of the azibenzil being consumed in the single flash that was used.

The rate data fit the first-order rate law well, and observed first-order rate constants were obtained by least-squares fitting to an exponential function.

Product Studies. The products of reaction of diphenylketene in ammonia and morpholine buffers were determined by HPLC analysis using a Waters Novapack C18 column in a Varian Vista 5500 instrument interfaced to a Varian Polychrom 9060 diode array detector. Solvents, either 40/60 (v/v) or 60/40 (v/v) water/methanol, were passed through Millipore glass filters before use, and the instrument was operated at a flow rate of 1 mL min^{-1} and a pressure of 150–180 atm; injection volumes were 200 μL.

In a typical experiment, 3 mL of aqueous buffer was placed in a quartz cuvette and a sufficient quantity (3–10 μL) of stock solution of azibenzil in acetonitrile (Aldrich, HPLC grade) was added to give a final azibenzil concentration comparable to that used for kinetics. In order to minimize photochemical degradation of the azibenzil in this solution by room light, the cuvette was wrapped in aluminum foil. A chromatogram and UV spectrum of a sample of this solution were recorded, and the foil was then removed and the cuvette and its contents were subjected to one flash of ca. 50 μs duration in our conventional flash-photolysis apparatus. The solution in the cuvette was then acidified by adding a few drops of

(1) See, for example: Tidwell, T. T. *Acc. Chem. Res.* **1990**, *23*, 273–279.

(2) (a) Bothe, E.; Meier, H.; Schulte-Frohlinde, D.; von Sonntag, C. *Angew. Chem., Int. Ed. Engl.* **1976**, *15*, 380–381. Bothe, E.; Dessouki, A. M.; Schulte-Frohlinde, D. *J. Phys. Chem.* **1980**, *84*, 3270–3272. (b) Allen, A. D.; Kresge, A. J.; Schepp, N. P.; Tidwell, T. T. *Can. J. Chem.* **1987**, *65*, 1719–1723. (c) Andraos, J.; Kresge, A. J. *J. Photochem. Photobiol. A: Chem.* **1991**, *57*, 165–173. (d) Allen, A. D.; Andraos, J.; Kresge, A. J.; McAllister, M. A.; Tidwell, T. T. *J. Am. Chem. Soc.* **1992**, *114*, 1878–1879. Jones, J., Jr.; Kresge, A. J. *J. Org. Chem.* **1992**, *57*, in press.

(3) (a) Allen, A. D.; Tidwell, T. T. *J. Am. Chem. Soc.* **1987**, *109*, 2774–2780. (b) Allen, A. D.; Stevenson, A.; Tidwell, T. T. *J. Org. Chem.* **1989**, *54*, 2843–2848. Allen, A. D.; Tidwell, T. T. *Tetrahedron Lett.* **1991**, *32*, 847–850.

(4) Jones, R. A. Y. *Physical and Mechanistic Organic Chemistry*, 2nd ed.; Cambridge University Press: New York, 1984; pp 279–284. Jencks, W. P. *Catalysis in Chemistry and Enzymology*; McGraw-Hill: New York, 1969; pp 78–111, 170–182.

(5) Nenitzescu, C. D.; Solomonica, E. *Organic Synthesis*; Wiley: New York, 1943; Collect. Vol. II, pp 496–497.

(6) Rossi, R. A.; Alonso, R. A. *J. Org. Chem.* **1980**, *45*, 1239–1241.

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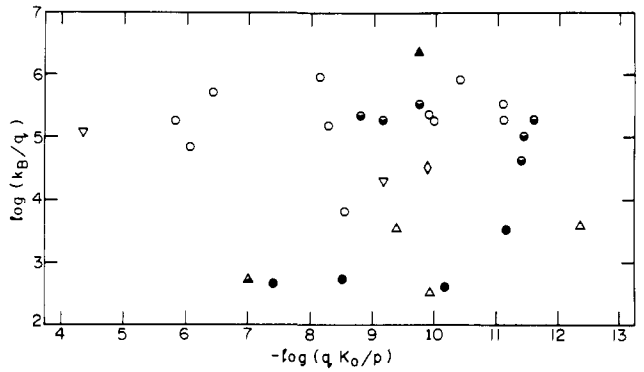


Figure 1. Bronsted-type plot for the reaction of diphenylketene with bases in wholly aqueous solution at 25 °C, ionic strength = 0.10 M: ◊, NH_3; ○, RNH_2; ⊖, R_2NH; ●, R_3N; △, RO^-; ▲, RS^-; ▽, N_3^-, CN^-; ▼, HPO_4^{2-}.

concentrated perchloric acid, and a chromatogram of the acidified solution and UV spectra of all detectable products were recorded; wavelengths in the range λ = 234–263 nm were used for detection. Flashed reaction mixtures were acidified in order to convert the diphenylacetic acid product into its un-ionized form; if this were not done, the acid in its carboxylate form was eluted at the same time as buffer components and quantitative analysis could not be performed. Products were identified by spiking with authentic samples, and product absorbance ratios were converted into concentration ratios using extinction coefficients determined here.

Further details of experimental techniques are available in the thesis upon which this report is based.[7]

Results

Rates of reaction of diphenylketene were measured in wholly aqueous buffer solutions of 30 bases. Series of buffer solutions of constant stoichiometric buffer ratio and constant ionic strength but varying total buffer concentration were used. Buffer concentrations were varied by factors ranging from 3 to 100, and replicate measurements (2–17) were made at each buffer concentration. These data are summarized in ref 7.

In all cases, observed first-order rate constants proved to be linear functions of buffer concentration,[8] and measurements made at different buffer ratios showed that the buffer base was the reactive buffer component. The data were therefore fitted to the rate law given in eq 4. Least-squares analysis gave the bimolecular

$$k_{obs} = k_o + k_B[B] \quad (4)$$

rate constants, k_B, listed in Table I and provided values of k_o in good agreement with expectation on the basis of the known[2b] specific rates of reaction of diphenylketene with water, k_{H_2O}, and hydroxide ion, k_{HO^-}, according to eq 5; hydroxide ion concen-

$$k_o = k_{H_2O} + k_{HO^-}[HO^-] \quad (5)$$

trations required for this purpose were calculated from literature values of the relevant pK_a's (Table I) and activity coefficients recommended by Bates.[10]

Discussion

Kinetics. The rate constants determined here for the reaction of diphenylketene with the base component of aqueous buffer solutions are displayed in the form of a Bronsted plot in Figure 1.[11] It may be seen that there is no correlation between rate

(7) Andraos, J. Ph.D. Thesis, 1991, University of Toronto.

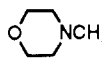
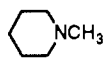
(8) Adjustments for buffer failure[9] were made where required.

(9) Keeffe, J. R.; Kresge, A. J. *Investigations of Rates and Mechanisms of Reactions*, Bernasconi, C. F., Editor; Wiley-Interscience: New York; Techniques of Chemistry, Vol. VI, Part 1, Chapter XI.

(10) Bates, R. G. *Determination of pH. Theory and Practise*; Wiley-Interscience: New York, 1973; p 49.

(11) The following statistical factors were used in constructing this plot: NH_3, $p = 4$, $q = 1$; RNH_2, $p = 3$, $q = 1$; R_2NH, $p = 2$, $q = 1$; R_3N, $p = 1$, $q = 1$; NH_2NH_2, $p = 3$, $q = 2$; $NH(CH_2CH_2)_2NH$, $p = 2$, $q = 2$; $N(CH_2CH_2)_3N$, $p = 1$, $q = 2$; RO^-, $p = 1$, $q = 1$; N_3^-, $p = 1$, $q = 2$; HPO_4^{2-}, $p = 2$, $q = 3$; CN^-, $p = 1$, $q = 1$.

Table I. Rate Constants for the Reaction of Diphenylketene with Bases in Aqueous Solution at 25 °C[a]

base	pK_a(BH)	k_B (M^{-1} s^{-1})
NH_3	9.25[b]	3.53×10^4
$NCCH_2NH_2$	5.34[c]	1.76×10^5
$CF_3CH_2NH_2$	5.59[d]	6.98×10^4
NH_2OH	5.95[e]	5.03×10^5
$NC(CH_2)_2NH_2$	7.80[f]	1.51×10^5
NH_2NH_2	7.97[g]	1.82×10^6
$(CH_2OH)_3CNH_2$	8.07[h]	6.48×10^3
$CH_3O(CH_2)_2NH_2$	9.40[d]	2.38×10^5
$HO(CH_2)_2NH_2$	9.50[i]	1.83×10^5
$CH_3O(CH_2)_3NH_2$	9.92[d]	3.92×10^5
$CH_3(CH_2)_3NH_2$	10.60[j]	3.52×10^5
CH_3NH_2	10.62[k]	1.94×10^5
	8.49[l]	2.22×10^5[m]
	8.84[n]	1.84×10^5
	9.73[o]	6.89×10^5
	11.08[p]	4.06×10^4[m]
	11.12[q]	1.03×10^5[m]
	11.27[r]	1.91×10^5
	7.41[s]	4.57×10^2[m]
	8.82[t]	1.07×10^3[m]
	10.19[u]	4.01×10^2
	11.15[v]	3.39×10^3
$(CF_3)_2C(OH)O^-$	6.65[w]	x
$(CF_3)_2CHO^-$	9.39[y]	3.37×10^3[m]
$(CF_3)_2C(CH_3)O^-$	9.93[y]	3.35×10^2
$CF_3CH_2O^-$	12.40[y]	3.94×10^3[m]
N_3^-	4.65[z]	1.17×10^5
HPO_4^{2-}	7.20[aa]	1.04×10^3
CN^-	9.22[bb]	1.96×10^4
$HOCH_2CH_2S^-$	9.72[cc]	2.43×10^6[m]

[a] Ionic strength = 0.10 M ($NaClO_4$). [b] Bates, R. G.; Pinching, G. D. *J. Res. Natl. Bur. Stand.* **1949**, *42*, 419–430. [c] Stevenson, G. W.; Williams, D. *J. Am. Chem. Soc.* **1958**, *80*, 5943–5947. [d] Love, P.; Cohen, R. B.; Taft, R. W. *J. Am. Chem. Soc.* **1968**, *90*, 2455–2462. [e] Lumme, P.; Lahermo, P.; Tummavuori, J. *Acta Chem. Scand.* **1965**, *19*, 2175–2188. [f] Soloway, S. S.; Lipschitz, H. *J. Org. Chem.* **1958**, *23*, 613–615. [g] Sallavo, K.; Lumme, P. *Suomen. Chem.* **1967**, *40B*, 155–162. [h] Datta, S. P.; Grzybowski, A. K.; Weston, B. A. *J. Chem. Soc.* **1963**, 792–796. [i] Bates, R. G.; Pinching, G. D. *J. Res. Natl. Bur. Stand.* **1951**, *46*, 349–352. [j] Evans, A. G.; Hamann, S. D. *Trans. Faraday Soc.* **1951**, *47*, 34–40. [k] Everett, D. H.; Wynne-Jones, W. F. K. *Proc. R. Soc. A* **1941**, *177*, 499–516. [l] Hetzer, H. B.; Bates, R. G.; Robinson, R. A. *J. Phys. Chem.* **1966**, *70*, 2869–2872. [m] Weighted average values of determinations at two different buffer ratios. [n] Krishnamurthy, M.; Babu, K. S.; Muralikrishna, U. *Curr. Sci.* **1988**, *57*, 598–600. [o] Hetzer, H. B.; Robinson, R. A.; Bates, R. G. *J. Phys. Chem.* **1968**, *72*, 2081–2086. [p] Horwitz, J. P.; Rila, C. C. *J. Am. Chem. Soc.* **1958**, *80*, 431–437. [q] Bates, R. G.; Bower, V. E. *J. Res. Natl. Bur. Stand.* **1956**, *57*, 153–157. [r] Searles, S.; Tamres, M.; Block, F.; Quarterman, L. A. *J. Am. Chem. Soc.* **1956**, *78*, 4917–4920. [s] Hall, H. K. *J. Phys. Chem.* **1956**, *60*, 63–70. [t] Paoletti, P.; Stern, J. H.; Vacca, A. *J. Phys. Chem.* **1965**, *69*, 3759–3762. [u] Hall, H. K. *J. Am. Chem. Soc.* **1956**, *78*, 2570–2572. [v] Lobo, S. T.; Murty, T. S. S. R.; Robertson, R. E. *Can. J. Chem.* **1976**, *54*, 3607–3613. [w] Wooley, E. M.; Hepler, L. G.; Roche, R. S. *Can. J. Chem.* **1971**, *49*, 3054–3056. [x] No reaction with buffer observed. [y] Arrowsmith, C. H.; Kresge, A. J.; Tang, Y. C. *J. Am. Chem. Soc.* **1991**, *113*, 179–182. [z] Ahrland, S.; Avsar, E. *Acta Chem. Scand.* **1975**, *29A*, 881–889. [aa] Grzybowski, A. K. *J. Phys. Chem.* **1958**, *62*, 550–555. [bb] Izatt, R. D.; Christensen, J. J.; Pack, R. T.; Bench, R. *Inorg. Chem.* **1962**, *7*, 828–831. [cc] Irving, R. J.; Nelanders, L.; Wadso, I. *Acta Chem. Scand.* **1964**, *18*, 769–787.

constant and basic strength, as might be expected if the bases were functioning as proton transfer agents, catalyzing the nucleophilic attack of water on the ketene according to eq 1. This figure, on the other hand, does show the scatter that would be produced by the base functioning as a nucleophile according to eq 2, in which case reactivity would be governed by factors such as polarizability and steric hindrance in addition to basic strength. The pattern shown by Figure 1 is in fact reminiscent of the scatter diagram given by the classic reaction of nucleophiles with *p*-nitrophenyl acetate.[12]

Most of the bases used in the present study are amines, and the bulk of those are primary amines. But even so structurally homogeneous a group as this shows no correlation between rate constant and basic strength: least-squares analysis of the data for the 11 examples of this structural group listed in Table I gives a Bronsted coefficient statistically indistinguishable from zero, $\beta = 0.038 \pm 0.095$, and a correlation coefficient, $r = 0.131$, which indicates no significant linear dependence of log (k_B/q) upon log (qK_a/p). Leaving out the three most deviant bases, hydroxylamine, hydrazine, and tris(hydroxymethyl)methylamine, offers some improvement, for now $\beta = 0.080 \pm 0.030$ and $r = 0.731$, but the correlation is still a very poor one. Similarily poor correlations are produced by the data for secondary amines, $\beta = -0.154 \pm 0.102$ and $r = 0.600$, and tertiary amines, $\beta = 0.178 \pm 0.134$ and $r = 0.678$. The present data thus give no evidence of the regular dependence of reaction rate upon basic strength to be expected if the bases reacting with diphenylketene were functioning as proton transfer agents according to eq 1.

The present data, on the other hand, do show a general pattern of primary amines being more reactive than secondary, and secondary amines being more reactive than tertiary. This is mechanistically significant because the reactivity order is usually opposite to this when amines serve as proton transfer agents, i.e. in cases of genuine general base catalysis, tertiary amines are usually more reactive than secondary, and secondary more reactive than primary.[13] This reactivity order for general base catalyzed reactions is readily understandable in terms of the Principle of Non-perfect Synchronization.[14] The amine reactivity order found here thus supports a mechanistic assignment in which these bases engage in direct nucleophilic attack on the ketene according to eq 2.

In such a nucleophilic reaction, tertiary amines would of course be expected to be less effective than secondary, and secondary less effective than primary because of steric hindrance. The nucleophile attacks the lowest unoccupied molecular orbital of the ketene functional group, which lies in the plane of the ketene σ-bonded framework.[1] Substituents attached to the ketene group also lie in this plane, and, if they are bulky, they will impede approach of the nucleophile; this is evident, for example, in the striking unreactivity of di-*tert*-butylketene.[3a,15] The substituents in the present case are phenyl groups, which are also large, and steric factors are consequently expected to play an important role. Additional manifestations of this effect may be seen in the lower reactivity of 2-methylpiperidine compared to piperidine, the lower reactivity of $(CF_3)_2C(CH_3)CO^-$ compared to $(CF_3)_2CHCO^-$, and the comparative unreactivity of tris(hydroxymethyl)methylamine.

The enhanced reactivity of the α-effect amines, hydroxylamine and hydrazine, found here is also characteristic of a direct nucleophilic reaction.[16a] It is significant as well that the thiolate ion, $HOCH_2CH_2S^-$, is the most reactive of the bases used here: sulfur species such as this are commonly found to be especially good nucleophiles, a property generally attributed to the polarizability of the sulfur atom.[16b]

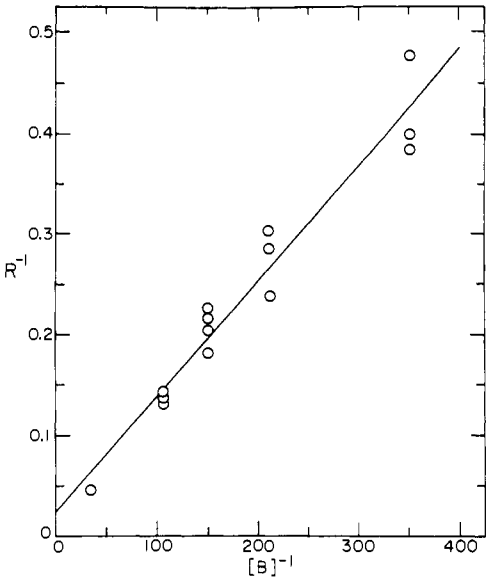


Figure 2. Relationship between amide to acid product ratios, R, and base concentration for the reaction of diphenylketene with aqueous morpholine buffer solutions at 25 °C; data are plotted according to eq 8.

The pattern of reactivity observed in the present study thus indicates that the bases used here are reacting with diphenylketene by direct nucleophilic attack. This contrasts with the conclusion reached in a recent study of the alcoholysis of diphenylketene catalyzed by "concave" pyridines where the pyridines were assigned general base catalytic roles.[17] These concave pyridines, however, are substances in which the basic site is severely hindered and direct nucleophilic attack is consequently very difficult.

Reaction Products. This assignment of a direct nucleophilic role to the bases reacting with diphenylketene in aqueous solution is supported by the results of a product study. As eq 1 shows, diphenylacetic acid will be formed if the base acts as a proton transfer agent assisting the attack of water, whereas the diphenylacetyl derivative of the base will be formed if the base reacts as a nucleophile according to eq 2. We examined the products of reaction of diphenylketene in the presence of two bases, ammonia and morpholine, and in both cases found the corresponding amides to be present, as expected on the basis of the direct nucleophilic reaction.

These spent reaction mixtures also contained diphenylacetic acid, but it can be shown by a quantitative analysis of the amide to acid product ratios, R, that this diphenylacetic acid was formed by the uncatalyzed reaction of diphenylketene with solvent water, eq 6, and not by the general base catalyzed process of eq 1. These

$$Ph_2C{=}C{=}O + H_2O \rightarrow Ph_2CHCO_2H \qquad (6)$$

product studies, just as the kinetic measurements, were done in a series of buffer solutions of constant buffer ratio and constant ionic strength but varying buffer concentration. At the acidities employed ($pH = 9.4$ for the series using ammonia as the base and $pH = 8.6$ for that using morpholine), contributions from catalysis by hydroxide ion were negligible, and R is then related to base concentration according to eq 7. This expression takes into account the possibility that only a fraction, ω, of the base molecules are

$$R = \frac{[\text{amide}]}{[\text{acid}]} = \frac{\omega k_B[B]}{k_{H_2O} + (1 - \omega)k_B[B]} \qquad (7)$$

reacting with diphenylketene by direct nucleophilic attack to give amide according to eq 2 and that the remaining fraction, $1 - \omega$,

(12) Jencks, W. P.; Carriuolo, J. *J. Am. Chem. Soc.* **1960**, *82*, 1778–1786.

(13) Bell, R. P. *The Proton in Chemistry*, 2nd ed.; Cornell University Press: Ithaca, NY, 1973; pp 217–219.

(14) Bernasconi, C. F. *Adv. Phys. Org. Chem.* **1992**, *27*, 119–238.

(15) Kabir, S. H.; Seikaly, H. R.; Tidwell, T. T. *J. Am. Chem. Soc.* **1979**, *101*, 1059–1060.

(16) Jencks, W. P. *Catalysis in Chemistry and Enzymology*; McGraw-Hill: New York, 1969; (a) pp 107–111, (b) pp 85–92.

(17) Luning, U.; Baumstark, R.; Schyja, W. *Leibigs Ann. Chem.* **1991**, 999–1002.

are serving as general bases, giving diphenylacetic acid product according to eq 7. Taking reciprocals of both sides of eq 7 leads to eq 8, which predicts a linear relationship between $1/R$ and

$$\frac{1}{R} = \frac{1-\omega}{\omega} + \frac{k_{H_2O}}{\omega k_B}\frac{1}{[B]} \quad (8)$$

$1/[B]$, with slope and intercept parameters from which ω and k_B may be obtained, the latter by making use of the known value of k_{H_2O} (=275 s^{-1}).[2b]

The experimental data for both bases conformed to this relationship well; this is illustrated for the case of morpholine in Figure 2. Least-squares analysis gave parameters which led to $\omega = 0.98 \pm 0.04$ and $k_B = (3.73 \pm 0.41) \times 10^4$ M^{-1} s^{-1} for ammonia and $\omega = 0.98 \pm 0.02$ and $k_B = (2.45 \pm 0.17) \times 10^5$ M^{-1} s^{-1} for morpholine. These rate constants agree well with the more precise values obtained from kinetic measurements, $k_B = (3.53 \pm 0.08) \times 10^4$ M^{-1} s^{-1} for ammonia and $k_B = (2.22 \pm 0.01) \times 10^5$ M^{-1} s^{-1} for morpholine, and both values of ω are, to a rather small statistical uncertainty, indistinguishable from unity. This analysis therefore shows that these two bases are functioning primarily as nucleophiles, with very little if any contribution from reaction as general base catalysts.

Acknowledgment. We are grateful to the Natural Sciences and Engineering Research Council of Canada, the donors of the Petroleum Research Fund, administered by the American Chemical Society, and the U.S. National Institutes of Health for financial support of this work.

Registry No. 1, 525-06-4; **2**, 3469-17-8; NH_3, 7664-41-7; $CNCH_2NH_2$, 540-61-4; $CF_3CH_2NH_2$, 753-90-2; NH_2OH, 7803-49-8; $CN(CH_2)_2NH_2$, 151-18-8; NH_2NH_2, 302-01-2; $(CH_2OH)_3CNH_2$, 77-86-1; $CH_3O(CH_2)_2NH_2$, 109-85-3; $CH_2HOCH_2NH_2$, 141-43-5; $CH_3O(CH_2)_3NH_2$, 5332-73-0; $CH_3(CH_2)_3NH_2$, 109-73-9; CH_3NH_2, 74-89-5; $(CF_3)_2C(OH)O^-$, 62394-21-2; $(CF_3)_2CHO^-$, 44870-01-1; $(CF_3)_2C(CH_3)O^-$, 130935-17-0; $CF_3CH_2O^-$, 24265-37-0; N_3^-, 14343-69-2; HPO_4^{2-}, 14066-19-4; $HO(CH_2)_2S^-$, 57966-62-8; morpholine, 110-91-8; thiomorpholine, 123-90-0; piperazine, 110-85-0; 2-methylpiperidine, 109-05-7; piperidine, 110-89-4; pyrrolidine, 123-75-1; *N*-methylmorpholine, 109-02-4; *N*-methylpiperidine, 626-67-5; 1,4-diazabicyclo[2.2.2]octane, 280-57-9; 1-azabicyclo[2.2.2]octene, 100-76-5; cyanide, 57-12-5.

DNA Binding Properties of *cis*-$[Pt(NH_3)(C_6H_{11}NH_2)Cl_2]$, a Metabolite of an Orally Active Platinum Anticancer Drug

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Abstract: The compound *cis,trans,cis*-$[Pt(NH_3)(C_6H_{11}NH_2)(OC(O)C_3H_7)_2Cl_2]$ (**1**) is the prototypical member of a new class of orally active platinum anticancer drugs. A major metabolite of this compound, formed after ingestion, is *cis*-$[Pt(NH_3)(C_6H_{11}NH_2)Cl_2]$ (**2**). We have used enzymatic digestion/HPLC analysis to investigate the spectrum of adducts formed by the reaction of this Pt(II) reduction product with calf thymus DNA. The major adduct (54%) formed is an intrastrand cross-link involving adjacent guanosine residues, followed in frequency by interstrand or long range intrastrand cross-links also involving guanosine nucleosides (18%). Unlike cisplatin, **2** forms d(ApG) intrastrand cross-links to only a minor extent (8%). The presence of the cyclohexylamine ligand gives rise to two orientational isomers of the platinated d(GpG) moiety, differing with respect to the positioning of the cyclohexyl group toward either the 3′ or the 5′ direction of the phosphodiester linkage. These isomers were observed for platination of calf thymus DNA (2:1 ratio) as well as shorter oligonucleotides. The coordination sites of platinum in the adducts were identified by studies of the reaction products of **2** with d(GpG). ^1H and ^{195}Pt NMR spectroscopy revealed that platinum is bonded to the N7 positions of the two guanine bases. The individual d(GpG)-**2** orientational isomers were synthesized and isolated from reagents in which both the ammine ligand and the N7 position of the 3′-guanine base were labeled with ^{15}N. Comparison with the ^{15}N–^{15}N coupling constants in the $^{15}N\{^1H\}$ NMR spectrum of the two isomers allowed for determination of the stereochemistry at the platinum metal center. From this information the orientational isomer having the cyclohexyl group directed toward the 3′ end of the platinated strand was identified as the more abundant of the two d(GpG)-**2** isomers formed in the reaction of **2** with calf thymus DNA. This isomer is less disruptive to the hydrogen bonding between the NH_3 ligand and the 5′-phosphate group, a structural feature previously identified as being important in the major cisplatin adducts with DNA. Two orientational isomers were also formed upon reaction of **2** with the dodecanucleotide d(TCTAGGCCTTCT), which contains a single d(GpG) platination site. Separation and purification of the two platinated dodecanucleotide orientational isomers allowed for construction of modified M13 genomes containing a single isomer of each of the two d(GpG)-**2** adducts. Each purified isomer was incorporated into a gapped heteroduplex, providing the two corresponding isomers of the site-specifically platinated M13 genomes. Studies of the replication of these platinated genomes with T7 DNA polymerase revealed differences in the position within the genome at which DNA synthesis is inhibited. The *cis*-$[Pt(NH_3)(C_6H_{11}NH_2)\{d(GpG)\text{-}N7(1), \text{-}N7(2)\}]$ orientational isomers inhibited DNA replication less efficiently than the parent cisplatin complex, allowing more (10–15% versus 8% for cisplatin) translesion synthesis.

Introduction

Two rational approaches to the development of new therapies for cancer are to investigate the molecular and cellular biological events that lead to tumorogenesis[1] and to study the mechanism of action of known chemotherapeutic agents. Platinum complexes are used in the treatment of ovarian and bladder cancers and have become indispensable for testicular cancer.[2,3] A long term objective in our laboratory is to elucidate the molecular basis for the anticancer activity of these platinum complexes. This research has focused on DNA as the primary target of the major platinum anticancer drug, *cis*-$[Pt(NH_3)_2Cl_2]$, (*cis*-DDP or cisplatin),[4] and

(1) For a recent dicussion, see: *Science*, **1992**, *254*, No. 5035.

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(3) Loehrer, P. J.; Einhorn, L. H. *Ann. Intern. Med.* **1984**, *100*, 704.

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