

Aqueous degradation of *N*-(hydroxymethyl)-phthalimide in the presence of specific and general bases. Kinetic assessment of *N*-hydroxymethyl derivatives of nitrogen heterocycles as possible prodrugs

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Abstract: The conversion of *N*-(hydroxymethyl)phthalimide (NHPH) to phthalimide could not be detected within 300 s at pH 9.0, whereas in 0.18 M NaOH complete conversion of NHPH to phthalimide was observed within 50 s. In the presence of 0.2–0.4 M 1,4-diazabicyclo[2.2.2]octane buffer solutions (pH 9.30–9.54), 40–60% conversion of NHPH to phthalimide occurred within 90–120 s. The initial concentration of NHPH affected the extent of conversion of NHPH to phthalimide.

Keywords: *N*-(hydroxymethyl)phthalimide; phthalimide; specific and general bases; formaldehyde; NMR; UV-visible spectrophotometry.

Introduction

Matsui and Aida [1] studied the hydrolysis of *N*-alkylmaleimides including *N*-(hydroxymethyl)maleimide and characterized the hydrolysis products; they did not mention the conversion of *N*-(hydroxymethyl)maleimide to maleimide under the experimental conditions imposed. Recently, the present author studied the kinetics of hydrolysis of *N*-(hydroxymethyl)phthalimide (NHPH) [2], but did not analyse the hydrolysis product(s).

The use of *N*-hydroxymethyl derivatives of nitrogen heterocycles as possible prodrugs has been recently demonstrated by Bansal and co-workers [3] who showed that the *N*-hydroxymethyl derivatives of allopurinol, glutethimide and phenobarbitone were converted rapidly to the parent drug in suspensions of these derivatives in buffer solutions of pH 7; unfortunately, the exact concentrations of buffers used in the reaction mixture were not reported. These studies prompted the author of the present paper to characterize the degradation products of NHPH under different hydrolytic conditions. A few kinetic runs on the aminolysis of NHPH were also carried out and the results of these experiments compared with unpublished results obtained for phthalimide under similar experimental conditions.

Experimental

Materials

Reagent grade chemicals such as NHPH and 1,2-diaminopropane (DAP) were obtained from Aldrich, and phthalimide and 1,4-diazabicyclo[2.2.2]octane (DAZB) were obtained from BDH. All other chemicals used were also of reagent grade.

Analytical instruments

Reaction rates were monitored by a Beckman Model 35 UV-visible spectrophotometer equipped with digital display, a chart recorder Model 24-25 ACC and an electronic temperature control unit. The pH of solutions was measured with a Philips digital pH-meter Model PW 9409 and NMR spectra were recorded by an EM 360 L NMR spectrometer. Data analysis was carried out on a Commodore Professional 3016 microcomputer.

Aqueous degradation of NHPH in the presence of a specific base

NHPH (0.358 g) was dissolved with slight warming in 33.4 ml of H₂O and 5.0 ml of MeCN. To this solution was added 3.1 ml of 0.05 M NaOH; the pH of the resulting solution was 9.00. After 300 s the reaction mixture was made acidic (pH 2.05) by adding 0.5 ml of 1.0 M HCl. After 600 s the reaction mixture was extracted with diethyl ether. Within the extraction period, the aqueous degradation of NHPH in acidic medium would be insignificant, whereas most of the expected hydrolysis product, *N*-(hydroxymethyl) phthalamic acid, would be converted to phthalic acid because the acidic hydrolysis of phthalamic acids is known to be reasonably fast [4, 5]. The ether extract was allowed to evaporate completely at room temperature. The melting point of the white crystalline solid thus obtained was 141–144°C, which is similar to the melting point (142–145°C) of authentic NHPH. The NMR-spectrum (in CDCl₃) of the white solid was similar to that of NHPH.

The aqueous degradation of NHPH was repeated by adding 5.0 ml of 5 M NaOH to the reaction mixture containing 0.358 g of NHPH and 15 ml of MeCN in a total volume of 135 ml of aqueous solvent at 28.5°C. After 50 s 3.0 ml of 10.5 M HCl was added to the reaction mixture; the final pH was 1.10. The reaction mixture was then quickly extracted with diethyl ether. Evaporation of the ether extract gave a white solid with a melting point of 227–229°C which is similar to that (229–230°C) of phthalimide. The NMR spectrum of the white solid product was measured in dimethyl sulphoxide-d₆.

Aqueous degradation of NHPH in the presence of a general base

To a solution containing 39 ml of H₂O and 5 ml of MeCN, 0.358 g of NHPH was added. The solution was warmed slightly to completely dissolve the NHPH. To this solution, 10 ml of 2 M DAZB buffer was added (80% unprotonated DAZB); the pH of the reaction mixture was 9.54. In order to retard or stop the degradation of NHPH, 4 ml of 10.5 M HCl was added to the reaction mixture 120 s after addition of the DAZB buffer; the final pH of the reaction mixture was 0.80. The acidic reaction mixture was then extracted with diethyl ether and the other extract was evaporated to dryness. The solid product obtained did not show a sharp melting point. The NMR spectrum of the product mixture was measured in CDCl₃.

The aqueous degradation of NHPH was also carried out at 28.5°C by adding 18 ml of 2 M DAZB buffer (75% free base) to the clear solution containing 120 ml of H₂O, 15 ml

of MeCN and 0.358 g of NHPH; the pH of the reaction mixture was 9.30. After 90 s, 4 ml of 10.5 M HCl was added to the reaction mixture; the pH was reduced to 2.32. The reaction mixture was then extracted with diethyl ether and the ether extract was evaporated to dryness. The solid product did not show a sharp melting point. The NMR spectrum was measured in dimethyl sulphoxide- d_6 .

Kinetic measurements of aminolysis of NHPH

NHPH strongly absorbs at 300 nm, whereas *N*-(hydroxymethyl)phthalamic acid and *N*-alkylsubstituted phthalamides do not essentially absorb at 300 nm. Therefore, the reaction rates of aminolysis of NHPH were studied spectrophotometrically at 30°C by monitoring at 300 nm, the disappearance of NHPH as a function of time. The desired ionic strength of the reaction medium was kept constant with potassium chloride. The buffer solution of specific pH was prepared just before the start of the kinetic runs. The details of the kinetic measurements and data analysis are described elsewhere [6].

Results

Product characterization of the hydrolytic degradation of NHPH in the presence of a specific base

The hydrolytic degradation of 0.05 M NHPH was studied at pH 9.00 and $>30^\circ\text{C}$. The reaction was carried out for 300 s. The solid reaction product showed a sharp melting point which is similar to that of NHPH. The areas under the peak integrated for protons at δ 5.5 (s, 2 H, N—CH₂—O) and δ 8.1 (m, 4 H, aromatic) revealed about 90% of NHPH in the hydrolysis product mixture. Nearly 10% of the product could be expected to be phthalic acid which was formed due to the hydrolysis of NHPH during the period of 300 s. These results suggest that NHPH could not have been converted to phthalimide in any detectable amount under the experimental conditions imposed.

The aqueous degradation of 0.0145 M NHPH was studied at 0.18 M NaOH and 28.5°C. The reaction was allowed to progress for 50 s. The melting point of the reaction product was similar to that of phthalimide. The NMR spectrum did not show the characteristic peak for the N—CH₂—O group. The value (4.3) of the ratio of the areas under the peaks for protons at δ 8.0 and δ 11.4 was essentially similar to the corresponding value (4.6) obtained for an authentic sample of phthalimide. These observations show the complete (almost 100%) conversion of NHPH to phthalimide within 50 s at 0.18 M NaOH and 28.5°C.

Product characterization of the aqueous degradation of NHPH in the presence of a general base

The aqueous degradation of 0.038 M NHPH was studied in 0.37 M DAZB buffer (pH 9.54) at $>30^\circ\text{C}$. The reaction was carried out for 120 s. The solid product did not show a sharp melting point, which indicated that the product contained more than one compound. The NMR spectrum of the product did not reveal the characteristic peak of phthalimide at δ 11.4 because of both low solubility of the product in CDCl₃ and the low content ($<30\%$) of phthalimide in the product mixture. The magnitude of the areas under the peaks for protons at δ 7.9 and δ 5.3 revealed the presence of about 60% of NHPH in the product mixture.

This reaction was repeated under slightly different experimental conditions. The reaction mixture containing 0.0133 M NHPH and 0.23 M DAZB buffer (pH 9.30) was

allowed to stand for 90 s at 28.5°C. The reaction was then stopped and the product was characterized spectrally. The NMR spectrum of the product mixture showed peaks at δ 11.4 (characteristic of the presence of phthalimide) and δ 5.13 (characteristic of the presence of NHPH). The hydrolysis of NHPH and phthalimide may be expected to be about 10% within 90 s. The magnitudes of the areas under the peaks for protons at δ 11.4, δ 8.0 and δ 5.1 indicated the presence of NHPH and phthalimide corresponding to about 40 and 60% of the non-hydrolysed substrate, respectively. The product mixture did not show a sharp melting point.

Degradation of NHPH in the presence of buffer solutions of DAZB and DAP

The rates of the reactions of NHPH with DAZB and DAP were studied within the pH ranges of 8.76–9.70 and 8.40–9.03, respectively, at 30°C. The observed pseudo-first-order rate constants, k_{obs} , obtained at varying total buffer concentration, $[B]_{\text{T}}$, and constant pH, obeyed equation (1).

$$k_{\text{obs}} = k_0 + k_n[B]_{\text{T}}, \quad (1)$$

where k_0 and k_n represent the rate constants for buffer-independent and buffer-dependent cleavage of NHPH, respectively. The least squares technique was used to calculate the unknown parameters, k_0 and k_n . These results are summarized in Table 1. The fitting of the observed data to equation (1) is evident from the magnitudes of the standard deviations associated with the calculated parameters, k_0 and k_n (Table 1). The large standard deviations associated with k_0 values for the reactions of DAP with NHPH may be attributed to the insignificant contribution of the k_0 term compared with $k_n[B]_{\text{T}}$ in these reactions.

Discussion

The aqueous degradation of NHPH in the presence of general, as well as specific, bases at $\text{pH} \geq 9.0$ is expected to follow the reaction paths as shown in Scheme 1 where B represents the general base. Hine and Kokesh [7] studied the kinetics of the reactions of

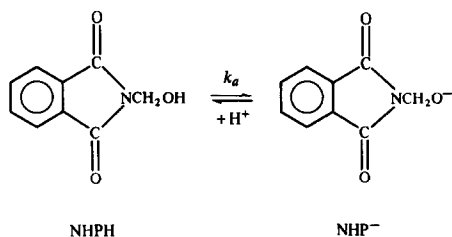
Table 1
Apparent first- and second-order rate constants (\pm SD) for the degradation of NHPH in buffer solutions of DAZB and DAP*

Buffer	pH	$10^4 k_0 \dagger$ $\text{s}^{-1} (\pm \text{SD})$	$10^4 k_n \dagger$ $\text{M}^{-1} \text{s}^{-1} (\pm \text{SD})$	$[B]_{\text{T}}$ range‡	Number of runs
DAZB	8.76	2.36 ± 0.22	6.67 ± 0.32	0.02–1.0	4
	8.99	3.53 ± 0.44	8.34 ± 0.62	0.2–1.0	4
	9.18	5.01 ± 0.42	9.03 ± 0.59	0.2–1.0	4
	9.70	8.76 ± 0.38	5.14 ± 0.53	0.2–1.0	4
DAP	8.40	0.74 ± 0.58	146 ± 2	0.05–0.35	5
	8.55	0.52 ± 1.42	239 ± 6	0.05–0.35	5
	8.76	3.55 ± 1.17	430 ± 5	0.05–0.35	5
	9.03	0.97 ± 3.54	925 ± 14	0.05–0.35	5

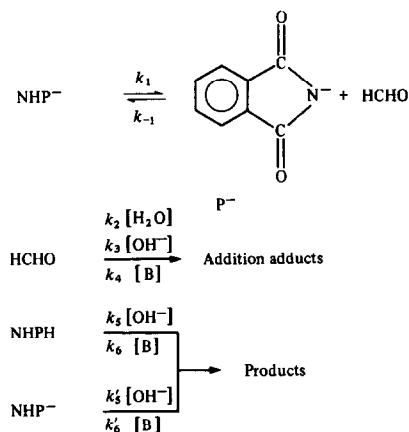
* Conditions: 30°C; ionic strength 1.0 M; 1% MeCN in the aqueous reaction mixture; $[\text{C}_6\text{H}_4(\text{CO})_2\text{NCH}_2\text{OH}]_0 = 2 \times 10^{-4}$ M.

† Calculated from equation (1) as described in the text.

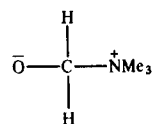
‡ Total buffer concentration range.



Scheme 1



formaldehyde (CH₂O) with H₂O and Me₃N and found the values of $k_2 [H_2O]$, k_1 and k_{-1} to be 14 s^{-1} , $3.4 \times 10^3 \text{ s}^{-1}$ and $1.3 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$, respectively. The rate constants, k_1 and k_{-1} represent the rate constants for the expulsion of Me₃N from 1 and the reaction of Me₃N with CH₂O, respectively. The pK_a of phthalimide (9.5 at 30°C) is about 0.5 pK units smaller than that of Me₃NH⁺ ($pK_a = 9.95$) [8]; hence the values of k_1 and k_{-1} may be considered to be of the order of 10^3 s^{-1} and $10^7 \text{ M}^{-1} \text{ s}^{-1}$, respectively. The value of k_3 of $3.6 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$ is obtained from the work of Guthrie [9]. The values of $k_3 [OH^-]$ would be $3.6 \times 10^1 \text{ s}^{-1}$ and $6.5 \times 10^5 \text{ s}^{-1}$ at pH 9.0 and 0.18 M NaOH, respectively. The value of $k_{-1} [P^-]$ will depend upon $[P^-]$. At an initial concentration of NHPH, $[NHPH]_0$, of 0.05 M, the formation of about 1% of product (P⁻) will produce about $5 \times 10^{-4} \text{ M P}^-$. The value of $k_{-1} [P^-]$ would be about 10^3 s^{-1} at $[P^-] = 10^{-4} \text{ M}$. On the other hand, at $[NHPH]_0 = 0.0145 \text{ M}$, the 100% completion of k_1 -step (Scheme 1) would make $k_{-1} [P^-]$ of the order of 10^5 s^{-1} . This may be considered as the maximum value of $k_{-1} [P^-]$ within the limits of the experimental conditions. These analyses indicate that, in the absence of a general base and at pH 9.0, $k_{-1} [P^-]/k_3 [OH^-] > 10^2$, whereas at $[OH^-] = 0.18 \text{ M}$, $k_{-1} [P^-]/k_3 [OH^-] < 1$. It is therefore evident that at pH 9.0 and $[NPH]_0 = 0.03\text{--}0.05 \text{ M}$, the conversion of NHPH to phthalimide should not occur, whereas at $[OH^-] = 0.18 \text{ M}$, the complete conversion of NHPH to phthalimide should occur within 1 s. The observed results are in agreement with these predictions.



The pK_2 of DAZB (9.10 at 30°C [10]) is about 0.9 pK units smaller than that of Me_3NH^+ . The second-order rate constant for the reaction of CH_2O with OH^- is about 4 times smaller than that with Me_3N , despite the fact that pK_a of H_2O is about 5.7 pK units larger than that of Me_3NH^+ . Thus, it may not be unreasonable to assume that the second-order rate constant for the reaction of CH_2O with Me_3N is not significantly larger than that with DAZB. The value of $k_4 [B]$, where B represents unprotonated DAZB, is therefore of the order of $10^6 s^{-1}$ at $[B] = 0.2-0.3 M$ and pH 9.30-9.54. However, the value of $k_{-1} [P^-]$ is expected to be $<10^5 s^{-1}$ at a $[NHPH]_0$ range of 0.0133-0.038 M. This shows that 0.01-0.04 M NHPH should be completely converted to phthalimide within 1 s at $[B] = 0.2-0.3 M$ and pH 9.30-9.54. But the observed results indicate that only 40-60% of NHPH has been converted to phthalimide within 90-120 s. This shows that the assumption that $k_4 [B] \gg k_{-1} [P^-]$ under the experimental conditions is probably not correct. The value of $k_{-1} [P^-]$ is expected to increase with increase in $[NHPH]_0$. This is reflected in the observed yields for the conversion of NHPH to phthalimide; these yields are 60% at 0.0133 M NHPH and 40% at 0.038 M NHPH.

It is interesting to note that the values of the rate constants for the expulsion of H_2O from $HOCH_2OH$ and OH from $HOCH_2O^-$ are $1 \times 10^{-4} s^{-1}$ and $3 \times 10^2 s^{-1}$, respectively, [9]. This shows that the conversion of NHPH to phthalimide would be greatly reduced at a low pH where NHPH could not produce a significant amount of NHP^- .

The detailed kinetic analysis of the observed data on kinetics of the reactions of NHPH with DAZB and DAP revealed that DAP reacted with both NHPH and NHP^- while DAZB reacted with only NHPH. The calculated values of the second-order rate constants (k) for the reactions of NHPH with DAZB ($10^3 k = 2.54 \pm 0.03 M^{-1} s^{-1}$) and DAP ($10^3 k = 51.5 \pm 3.0 M^{-1} s^{-1}$) are not significantly different from the corresponding unpublished values of $10^3 k = 2.70 \pm 0.90 M^{-1} s^{-1}$ and $10^3 k = 66.3 \pm 18.8 M^{-1} s^{-1}$, respectively, obtained for phthalimide. These kinetic results suggest either that NHPH was completely converted to phthalimide in a fast step followed by relatively slow aminolysis of phthalimide, or that nucleophilic reactivity of DAZB and DAP towards NHPH and phthalimide are not significantly different. The latter assumption is conceivable from the fact that $\sigma_{CH_2OH}^*$ ($= 0.56$ [11]) is not very much different from σ_H^* ($= 0.49$ [11]).

Conclusions

The *N*-hydroxymethyl derivatives of nitrogen heterocycles might enhance the aqueous solubility, compared with that of the parent nitrogen heterocycle, and thus these derivatives could be used as possible prodrugs. However, extensive studies under physiological conditions would be needed to determine whether these prodrugs would be easily converted *in vivo* to parent drug compounds. The results described in this paper show that the conversion of *N*-hydroxymethyl derivatives of nitrogen heterocycles to parent nitrogen heterocycles in an aqueous medium would be affected by (i) pH of the reaction medium and (ii) basicity of the general base present in the reaction mixture. The magnitudes of the rate constants (obtained from the literature) for the addition of specific and general bases to CH_2O to form the addition adducts and for the expulsion of the leaving groups from addition adducts might be used as a tool to predict the possible percentage conversion of *N*-hydroxymethyl derivatives to parent nitrogen heterocycles under certain experimental conditions.

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