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Mechanism of Alkaline Hydrolysis of Diazepam

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Diazepam (**1**) is a frequently prescribed hypnotic/anxiolytic drug in worldwide use. Compound **1** is hydrolyzed in alkaline medium to form 2-methylamino-5-chlorobenzophenone imine (**2**) and 2-methylamino-5-chlorobenzophenone (**3**); the ratio of **2**:**3** increases with increasing NaOH concentration (J. Pharm. Sci. *85*, 745-748, 1996). The mechanism in the conversion of **1** to **2** and **3** via various intermediates is the subject of this report. Results of hydrolysis kinetics and structural identification of some intermediate products indicated an initial hydroxide attack at the C2-carbonyl carbon of **1**, resulting in the formation of a dioxide (**7**, 7-chloro-1,3-dihydro-1-methyl-5-phenyl-2*H*-1,4-benzodiazepin-2,2-dioxide). Compound **7** was characterized by proton NMR spectroscopy and via its monomethyl ether (**8**, 7-chloro-1,3-dihydro-2-hydroxy-2-methoxy-1-methyl-5-phenyl-2*H*-1,4-benzodiazepine). The seven-member diazepine ring of **7** opened at the N1-C2 bond to form a glycinate [**5**, 2-methylamino-5-chloro-α-(phenylbenzylidene)glycinate]. Compound **7** (and/or **5**) underwent an additional hydroxide attack at the C5-N4 imine bond to form a tetrahedral intermediate, which decomposed to form **2** and **3**.

INTRODUCTION

Diazepam (**1**, 7-chloro-1,3-dihydro-1-methyl-5-phenyl-2*H*-1,4-benzodiazepin-2-one, Fig. 1) is a frequently prescribed hypnotic/anxiolytic drug worldwide.[1] The N1-C2 amide bond and C5-N4 imine bond are subjected to hydrolytic cleavages. Some 1,4-benzodiazepines such as demoxepam,[2,3] *N*-desmethyldiazepam,[3] nimetazepam,[4] and 2-oxoquazepam[5] were reported to undergo initial cleavage at the N1-C2 amide bond in alkaline media to form *N*-substituted imine derivatives. However, Han et al.[6] proposed that the initial reaction of **1** in both acidic and alkaline solutions occurs at the C5-N4 imine bond. Broxton and Wright[4] proposed that, relying solely on the dependence of hydrolysis rate on NaOH concentration, **1** was initially hydrolyzed at C5-N4 imine bond in aqueous alkaline solution; the site of cleavage was shifted to the N1-C2 amide bond in the presence of cationic detergent cetyltrimethylammonium bromide (CTAB). A deficiency of the earlier reports[4,6] was the lack of rigorous structural characterization of the initial alkaline hydrolysis product. Recently we described the identification of an *N*-unsubstituted imine **2** as a product formed in alkaline hydrolysis of **1**. We describe in this report the possible pathways involved in alkaline hydrolysis of **1**, leading to the formations of **2** and **3** as final products.

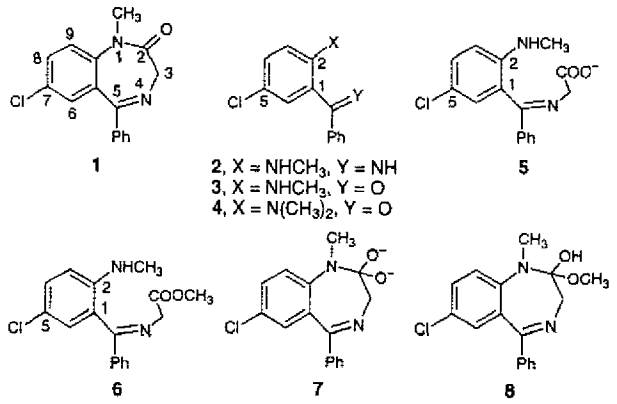

Fig. 1. Structure and abbreviation of diazepam (**1**), 2-methylamino-5-chlorobenzophenone imine (**2**), 2-methylamino-5-chlorobenzophenone (**3**), 2-dimethylamino-5-chlorobenzophenone (**4**), 2-methylamino-5-chloro-α-(phenylbenzylidene)-glycinate (**5**), 2-methylamino-5-chloro-α-(phenylbenzylidene)glycine methyl ester (**6**), 7-chloro-1,3-dihydro-1-methyl-5-phenyl-2*H*-1,4-benzodiazepin-2,2-dioxide (**7**), and 7-chloro-1,3-dihydro-2-hydroxy-2-methoxy-1-methyl-5-phenyl-2*H*-1,4-benzodiazepine (**8**). Compounds **1** to **6** are abbreviated identically as in the earlier report.[9]

EXPERIMENTAL SECTION

Materials

Compound **1** was generously provided by Hoffmann-La Roche Inc. (Nutley, NJ). 2-Methylamino-5-chlorobenzophenone (**3**) was prepared by methylation of 2-amino-5-chlorobenzophenone (Aldrich Chemical Co., Milwaukee, WI).[7] 2-Dimethylamino-5-chlorobenzophenone (**4**) was

prepared as described by Sternbach et al.[8] HPLC grade solvents, C_2H_5OD (EtOD; 99.5+ atom % D), D_2O (99.9 atom % D), and NaOD (40 wt % solution in D_2O, 99.9 atom % D) were obtained from Aldrich Chemical Co. (Milwaukee, WI). Cationic detergent cetyltrimethylammonium bromide (CTAB, 99%) and anionic detergent sodium dodecyl sulfate (SDS, 99%) were obtained from Sigma Chemical Co. (St. Louis, MO). Dimethyl sulfoxide (99.96% D) was obtained from Cambridge Isotope Laboratories, Inc. (Andover, MA).

Spectral Analysis

Uv-vis absorption spectra were determined using a 1-cm path length quartz cuvette on a Model DW2000 spectrophotometer (SLM Instruments, Urbana, IL). Direct exposure electron impact and chemical ionization (NH_3) mass spectral analyses were performed on a Finnigan 4500 gas chromatograph-mass spectrometer-data system (Finnigan MAT, San Jose, CA) with a solid probe at 70 eV. The ion source was maintained at 105 °C. Fourier transform ^1H NMR spectral analysis was performed with a Model GEMINI 300 MHz spectrometer (Varian Associates, Palo Alto, CA). The sample was dissolved in $(CD_3)_2SO$ (DMSO-d6). Signals of exchangeable protons were confirmed by the addition of 0.05 mL of D_2O. Chemical shifts are in ppm relative to tetramethylsilane.

High-Performance Liquid Chromatography (HPLC)

HPLC was performed using a Waters Associates (Milford, MA) Model 6000A solvent delivery system and a Spectraflow 757 variable wavelength absorbance detector (Kratos Analytical Instruments, Ramsey, NJ) set at either 232 or 254 nm. HPLC analysis was conducted at ambient temperature. Samples were injected via a Waters Associates Model 717 autosampler. In reversed-phase HPLC, a Zorbax SB-C18 column (3.5 μm particles, 4.6 mm i.d. × 15 cm, Mac-Mod Analytical Inc., Chadds Ford, PA) was used. In normal-phase HPLC, a Zorbax Rx SIL column (5 μm particles, 4.6 mm i.d. × 15 cm, Mac-Mod Analytical Inc., Chadds Ford, PA) was used. Mobile phase compositions are described below in the procedures for analysis and purification. HPLC analysis was conducted at ambient temperature.

Kinetic Analysis

NaOH solutions (0.5-7.5 M) was added to an ethanol (or dioxane) solution of **1** (17-60 μg/mL), with volume ratios ranging from 3:1 to 1:9, in a total volume of 2 mL. After thorough mixing, a 1.2 mL of the mixture was transferred into a water-jacketed cuvette. The temperature of the cuvette was maintained by passing constant-temperature water from a thermostated water circulator. The actual temperature of the solution in the cuvette was measured with a portable digital thermometer fitted with a detachable probe (Thomas Scientific, Swedesboro, NJ). After reaching temperature equilibrium in 1 to 5 min (depending on the temperature of the measurement), absorbance changes of the sample were either continuously monitored at a fixed wavelength or scanned repetitively from 200 to 500 nm at a fixed interval. Wavelength scan rate was 2 nm/s. Pseudo-first order reaction half-times ($t_{1/2}$) of samples in non-deuterated and deuterated solvents were determined from the $A_{\lambda,t}$ (absorbance at wavelength λ and time t) vs. time plot by a curve-fitting computer software (SigmaPlot). Equations used in curve-fitting were either $A_{\lambda,t} = a * \exp(-0.693*t/t_{1/2}) + A_{\lambda,0}$ or $A_{\lambda,t} = a *[1 - \exp(-0.693*t/t_{1/2})] + A_{\lambda,0}$, where $A_{\lambda,t}$ is the absorbance value of the sample at wavelength λ and time t, a is the net absorbance change of the reaction at wavelength λ, and $A_{\lambda,0}$ is the absorbance value at time zero. SigmaPlot is a product of Jandel Scientific (Corte Madera, CA).

Preparation of 7-Chloro-1,3-dihydro-1-methyl-5-phenyl-2*H*-1,4-benzodiazepin-2,2-dioxide (7)

Method A: A crystalline disodium salt of **7** (colorless needles) was prepared from **1** (60 mg in 3 mL of ethanol and 3 mL of 1 M NaOH by heating at 50 °C for 2 h. The solution was stored overnight at 4 °C; **7** and a minor amount of **3** (yellow needles) was formed. Crystalline **7** was harvested by filtration and the contaminating **3** was removed as much as possible by washing with hexane. NMR (DMSO-d6): δ 2.83 (methyl protons of NCH_3 and $NHCH_3$, apparent d, J = 4.8 Hz), 3.68 (CH_2 belonging to **5**, s), 3.72 (H_a of CH_2 belonging to **7**, d, J = 12.7 Hz), 3.98 (H_b of CH_2 belonging to **7**, d, J = 12.7 Hz), 6.52-7.46 (aromatic protons belonging to both **5** and **7**, m), and 10.31 (NH belonging to **5**, m) ppm. Upon the addition of D_2O, the signals for the methyl protons of NCH_3 and $NHCH_3$ became a singlet at 2.82 ppm and the signal for NH proton disappeared. The signal for the doublet at 6.56 ppm (Fig. 2A) disappeared upon the addition of D_2O and it was likely due to an impurity because it was absent in repeated experiments.

Method B: Compound **1** (60 mg in 3 mL of ethanol) was added to 1 M NaOH (3 mL) and the resulting solution was heated at 50 °C for 2 h. Ethanol was evaporated by blowing the solution with a gentle stream of nitrogen. The remaining aqueous solution was extracted with hexane (3 × 10 mL) to remove **3**, followed by extraction with ethyl acetate (15 mL). The ethyl acetate extract was dehydrated with anhydrous $MgSO_4$ and evaporated to dryness. This procedure resulted in **7** as a white powder. NMR (DMSO-d6): δ 2.83 (methyl protons of NCH_3 and $NHCH_3$, apparent d, J = 4.8 Hz), 3.68 (CH_2 belonging to **5**, s), 3.72 (H_a of CH_2 be-

longing to 7, d, J = 12.6 Hz), 3.98 (H_b of CH_2 belonging to 7, d, J = 12.6 Hz), 6.52-7.49 (aromatic protons belonging to both 5 and 7, m), and 10.32 (NH belonging to 5, m) ppm. Upon the addition of D_2O, the signals for the methyl protons of NCH_3 and $NHCH_3$ became a singlet at 2.82 ppm and the signal for the NH proton disappeared. The origin for the multiplet at 6.79 ppm (Fig. 2B) was not known and it disappeared upon the addition of D_2O.

Methylation Products of 7

Crystalline 7 (2 mg) was added with 1 mL of 10 M NaOH, 1 pellet of NaOH (~10 mg), 5 mL of chloroform, and 0.02 mL of dimethyl sulfate. The mixture was stirred vigorously for 2 h at ambient temperature. Chloroform phase was washed with water (3 × 30 mL), dehydrated with anhydrous $MgSO_4$, filtered, and evaporated to dryness *in vacuo*. The methylation reaction was repeated 20 times in order to obtain sufficient amount of **8** for spectral characterization. Compounds **6** and **8** were initially isolated from the product mixture by repetitive reversed-phase HPLC. The pooled fractions collected in reversed-phase HPLC were each, following removal of solvent, isolated by normal-phase HPLC. In reversed-phase HPLC, the mobile phase was acetonitrile-0.02 M phosphate buffer pH 7 (7:3, v/v) at a flow rate of 1 mL/min. Retention times (in min) were: 3.3 (**1**), 4.8 (**8**), 6.0 (**4**), 6.7 (**3**), and 7.7 (**6**). In normal-phase HPLC, the mobile phase was tetrahydrofuran-hexane (1:9, v/v) at 1 mL/min. Retention times (in min) were: 3.2 (**3**), 3.4 (**4**), 4.7 (**6**), 9.6 (**8**), and 24.1 (**1**). An alternative method to isolate compound **8** was to remove **1** from the product mixture by normal-phase HPLC using tetrahydrofuran-hexane (1:3, v/v) as the mobile phase at 1 mL/min; **1** was eluted at 7.2 min. Chromatographic peaks eluted prior to 7.2 min were collected as one fraction. Methylation products were then separated by normal-phase HPLC using tetrahydrofuran-hexane (1:9, v/v) as the mobile phase at 1 mL/min.

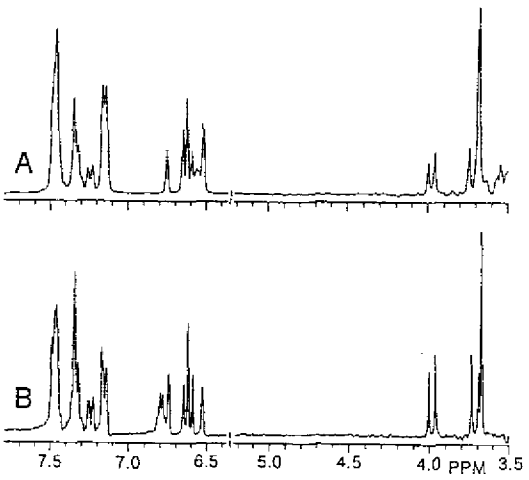

Fig. 2. Proton NMR spectra (300 MHz) of crystalline 7 (A) and powdery 7 (B) in anhydrous DMSO-d6. Signals for methyl protons (2.83 ppm, d, J = 4.8 Hz) and NH protons (10.3 ppm, m) in both samples are not shown. Detailed spectral data and proton assignments are indicated in the Experimental Section.

EI-MS analysis of **8** gave mass ions at *m/z* 316 (M^+, 52.1%), 315 (34.1%), 301 (3.1%), 286 (5.2%), 285 (4.2%), 257 (16.6%), 256 (7.9%), 255 (8.7%), 243 (100%), 228 (74.7%), 193 (36.3%), 179 (7.8%), 165 (20.0%), and 105 (18.8%). CI-MS (NH_3) analysis exhibited an MH^+ ion at *m/z* 317. The ^1H NMR spectral data (DMSO-d6) of **8** (Fig. 5) were: δ 2.61 (3H, NCH_3, d, J = 4.7 Hz), 3.62 (3H, C2-OCH_3, s), 4.02 (H_a of C3-H_2, d, J = 17.6 Hz), 4.21 (H_b of C3-H_2, d, J = 17.6 Hz), 4.98 (1H, C2-OH, m), aromatic protons at 6.70 (1H, d, J = 8.9 Hz), 6.81 (1H, d, J = 2.8 Hz), 7.31 (1H, dd), 7.41-7.46 (3H, m), and 7.59-7.62 (2H, d) ppm. Upon the addition of D_2O, the doublet for NCH_3 became a singlet at 2.58 ppm and the signal for the hydroxyl proton at C2 disappeared.

RESULTS AND DISCUSSION

Proton NMR Characterization of 7

In an earlier study,[9] the intermediate product formed from **1** in ethanol-1 M NaOH (1:3, v/v) at 50 °C for 2 h was converted to **5** in a pH 8 buffer. The structure of **5** was elucidated via its methyl ester **6**.[9] The immediate precursor of **5** has been characterized as **7** in this study (see below). In this study, **7** was prepared either as a crystalline or a powdery product. EI-MS analysis of **7** gave a fragmentation pattern similar to that of **5**[9] and the molecular weight could not be directly determined by either EI-MS or CI-MS. Compound **7** was hygroscopic and consequently **5** was found a contaminant in all preparations of **7**. The amount of **5** increased with time when **7** was stored under desiccation. Prolonged storage of **7** resulted in the formation of **3**.

The uv-vis absorption characteristics of **7** differ from those of **5**.[9] ^1H NMR spectra of freshly prepared crystalline and powdery **7** in DMSO-d6 exhibited similar characteristics (Fig. 2). Absorption peaks at 3.65-4.05 ppm indicated that both samples were a mixture of two compounds; one compound contained equivalent methylene protons (singlet at 3.68 ppm) and another compound contained non-equivalent protons (doublets at 3.72 and 3.98 ppm). The numerical values for ^1H NMR spectral data of crystalline and powdery **7** in DMSO-d6 are described in the Experimental

Section.

The detections of equivalent methylene protons (singlet at 3.68 ppm), non-equivalent methylene protons (doublets at 3.72 and 3.98 ppm), and a secondary amino proton (multiplet at 10.3 ppm) in both crystalline **7** (Fig. 2A) and powdery **7** (Fig. 2B) suggested the presence of both ring form (**7**) and open-chained form (**5**) in anhydrous DMSO-d6. Although freshly prepared crystalline and powdery **7** were used in repeated spectral measurements, substantial amount of **5** was detected (Fig. 2) upon dissolution of **7** in anhydrous DMSO-d6. Compound **5** may be formed due to the trace amount of water present in the commercially purchased "anhydrous" DMSO-d6. In a pH 8 solution, **7** was irreversibly converted to **5**.[9]

The non-equivalent methylene protons exhibited in Figs. 2A and 2B were similar to those of **1**, in which non-equivalent methylene protons at C3 exhibited an AB pattern (doublets at 3.78 and 4.84 ppm, J = 10.8 Hz) in DMSO-d6.[10] The spectral data of the non-equivalent protons at C3 of **1**[10] were consistent with the results described in other reports.[11,12]

Compound **5** was previously elucidated via its methyl ester **6**.[9] The results described above indicated that **7** was a disodium salt of a dioxide containing an intact diazepine ring. The structure of **7** was further characterized via a monomethyl ether **8** (see below).

Methylation Products of 7

Crystalline **7** was used to prepare methylation products by reaction with dimethyl sulfate in an alkaline solution. Unreacted **7** was removed as **5** in the aqueous phase following extraction with ethyl acetate and a subsequent wash with water.[9] The reaction yielded products labelled as peaks a, b, c, d, and e in reversed-phase HPLC analysis (Fig. 3). Peak a of Fig. 3 contained exclusively **1**, indicating that **7** could be protonated and lose a water molecule to form **1** under the experimental condition. Peak b contained predominantly **8**. The major component contained in peak c was **4**. Peak d contained exclusively **3**. The major component in peak e was identical to **6** reported earlier.[9] Compound **8** was collected by repetitive reversed-phase HPLC on a C18 column and further purified by normal-phase HPLC on a silica gel column. The uv-vis absorption spectrum of **8** (Fig. 4) has similar characteristics as that of **7** reported earlier.[9]

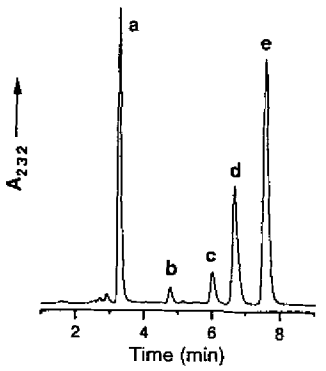

Fig. 3. Reversed-phase HPLC chromatogram of the methylation products formed from crystalline **7**. The identities of chromatographic peaks are described in the text.

In order to test if **5** could be recyclized to form **7** in a strongly alkaline solution, compound **7** was converted to **5** in a pH 8 buffer as previously described.[9] The resulting **5** was used in the methylation reaction in a strongly alkaline solution. Compound **8** was found a minor product by reversed-phase HPLC analysis. In normal-phase HPLC analysis reported earlier,[9] **8** was not detected because it was a very minor product. Compound **8** was not formed when **6** was used as a reactant in the methylation reaction. The results indicated that **8** was not a recyclization product of **6**. Taken together, the results suggested that **5** could be partially recyclized to form **7** in strongly aqueous alkaline solutions.

The detection of non-equivalent methylene protons at C3 (doublets at 4.01 and 4.21 ppm, Fig. 5) indicated that **8** retained the seven-member diazepine ring of **1**. The signals for protons of a hydroxyl group and a methoxy group at C2 indicated that **8** was derived from **7**. Following storage of a dried residue of **8** in the refrigerator (−4 °C) for 6 months,

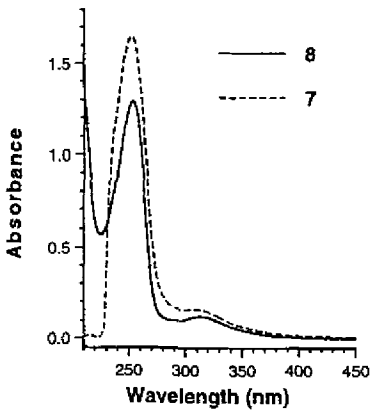

Fig. 4. Uv-vis absorption spectra of **8** (—, in acetonitrile at ambient temperature) and **7** [---, in ethanol-1 M NaOH (1:3, v/v) at 50 °C]. The absorption spectrum of **7** is taken from an earlier report[9] for ready comparison.

reversed-phase HPLC analysis indicated the formations of **1** (~2%, resulting by the loss of CH_3OH from **8**) and **6** (~17%, resulting by ring opening of **8**). The results provided additional support to the assigned structure of **8**. The results described above indicated that **8** was 7-chloro-1,3-dihydro-2-hydroxy-2-methoxy-1-methyl-5-phenyl-2*H*-1,4-benzodiazepine.

Kinetics and Thermodynamic Parameters in the Conversion of 1 to 7

The kinetics in the conversion of **1** to **7** was monitored by absorption changes at either 255 or 342 nm.[9] At a final NaOH concentration of 0.75 M, pseudo-first order reaction $t_{1/2}$'s at 50 °C were found to be 5.3 ± 0.3 min (n = 4) [solvent = ethanol-1 M NaOH (1:3, v/v)], 3.6 ± 0.1 min (n = 4) [solvent = ethanol-1.5 M NaOH (1:1, v/v)], 1.2 ± 0.1 min (n = 3) [solvent = ethanol-3 M NaOH (3:1, v/v)], 0.9 ± 0.1 min (n = 4) [solvent = ethanol-3.75 M NaOH (4:1, v/v)], and 0.8 ± 0.1 min (n = 5) [solvent = ethanol-7.5 M NaOH (9:1, v/v)]. The results indicated that the observed reaction rate increased with increasing percentage of ethanol in the reaction mixture. At the end of 10 reaction $t_{1/2}$s, reversed-phase HPLC analysis of the products formed indicated that **1** was essentially completely (>99.9%) converted to **7**. Thus the **1** → **7** conversion in strongly aqueous alkaline solvents was essentially irreversible.

NaOH-dependent reaction rates were determined with reaction mixtures containing 1:1 or 1:3 volume ratios of ethanol-*x* M NaOH at 25 °C or 50 °C (Table 1). At 25 °C in 1:3 volume ratios of ethanol and NaOH, the reaction rate was proportional to NaOH concentration with a slope of 2.42 in the plot of $\log(0.693/t_{1/2})$ vs. [NaOH] (Table 1). At 50 °C in 1:3 and 1:1 volume ratios of ethanol and NaOH, the reaction rates were also proportional to NaOH concentration with slopes of 2.25 and 2.47, respectively, in the plots of $\log(0.693/t_{1/2})$ vs. [NaOH] (Table 1). Extrapolation to 0% ethanol gave a slope of 2.03. Thus the reaction order with respect to hydroxide ion was ~2.0.

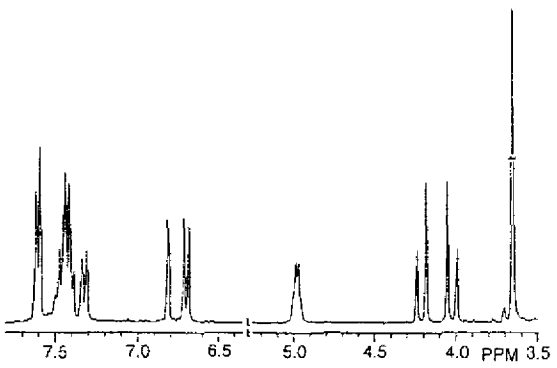

Fig. 5. Proton NMR spectrum (300 MHz) of **8** in DMSO-d6. Signal for methyl protons of NCH_3 (2.61 ppm, d, J = 4.7 Hz) is not shown. Detailed spectral data and proton assignments are indicated in the Experimental Section.

Table 1. [NaOH]-Dependent Pseudo-first Order Hydrolysis $t_{1/2}$ of **1** in Ethanol-NaOH Mixtures

[NaOH] (M)[a]	$t_{1/2}$ (min)[a]		
	E1W3 at 25 °C	E1W3 at 50 °C	E1W1 at 50 °C
0.35	-	-	23.3 ± 0.1 (3)
0.375	129.3 ± 3.4 (2)	25.2 ± 2.3 (3)	-
0.5	-	-	9.5 ± 0.4 (3)
0.5625	-	10.5 ± 0.6 (3)	-
0.75	25.2 ± 0.4 (7)	5.3 ± 0.3 (7)	3.6 ± 0.1 (5)
0.938	14.6 ± 0.2 (4)	-	-
1.0	-	-	1.7 ± 0.1 (5)
1.125	-	2.1 ± 0.1 (5)	-
1.5	4.5 ± 0.2 (5)	1.1 ± 0.1 (5)	-
1.875	2.5 ± 0.2 (5)	-	-
3.0	0.84 ± 0.02 (5)	-	-
3.75	0.52 ± 0.04 (5)	-	-
Slope[b]	2.419	2.250	2.472
r^2 [c]	0.9997	0.9998	0.9997

[a] E1W3 and E1W1 abbreviate for 1:3 and 1:1 (v/v) ethanol-NaOH mixtures. Net NaOH concentration in the reaction mixture is listed. Reaction $t_{1/2}$'s are mean ± SD and the number of determinations are indicated in parenthesis.

[b] Slope of the linear regression line in the plot of $\log(0.693/t_{1/2})$ vs. net NaOH concentration in reaction mixture.

[c] Correlation coefficient of the linear regression line in the plot of $\log(0.693/t_{1/2})$ vs. net NaOH concentration in reaction mixture.

Temperature-dependent reaction $t_{1/2}$'s were determined with reaction mixtures containing 1:3 volume ratios of ethanol-1 M NaOH at 6 temperatures. The $t_{1/2}$'s were 25.2 ± 0.4 min (n = 5) at 25 °C, 18.6 ± 0.3 min (n = 4) at 30 °C, 12.1 ± 0.4 min (n = 4) at 37 °C, 8.8 ± 0.2 min (n = 3) at 43 °C, 5.3 ± 0.3 min (n = 7) at 50 °C, and 4.5 ± 0.2 min (n = 5) at 55 °C. Arrhenius plot [$\log t_{1/2}$ vs. 1000/T, where T is temperature in Kelvin] yielded a slope of 2.42 (r^2 = 0.9979). The activation parameters were: E_{act} = 11.1 kcal/mol and at 25 °C, $\Delta H^{\ddagger}$ = 10.5 kcal/mol, $\Delta S^{\ddagger}$ = -38.6 cal/K/mol, and $\Delta G^{\ddagger}$ = 22.0 kcal/mol. The large negative value of $\Delta S^{\ddagger}$ suggested the formation of an activated complex resulting from reaction of hydroxide ion and **1**.

Reaction $t_{1/2}$ of **1** in C_2H_5OD-1 M NaOD in D_2O (1:3, v/v) at 50 °C was found to be 3.4 ± 0.1 min (n = 6). In C_2H_5OH-1 M NaOH (1:3, v/v) at 50 °C, the reaction $t_{1/2}$ was 5.3 ± 0.3 min (n = 7). Thus the deuterium kinetic isotope ef-

fect (k_H/k_D) was 0.62. The results on the reaction order (~2.0) with respect to hydroxide ion in 100% aqueous solution (Table 1) and the deuterium kinetic isotope effect (k_H/k_D = 0.62) indicated that the reaction mechanism was similar to those described for alkaline hydrolysis of *p*-nitroacetanilide and *p*-formylacetanilide.[13] The slopes of the log*k* vs. log[OH^-] plots at high NaOH concentrations for *p*-nitroacetanilide and *p*-formylacetanilide were ~2. The deuterium kinetic isotope effect (k_H/k_D) for *p*-nitroacetanilide was 0.61.[13] Thus the kinetic data indicated that, in alkaline hydrolysis of **1**, the initial reaction involved a hydroxide ion attack at the carbonyl carbon of the N1-C2 amide bond and followed by the formation of a dianion (see reaction mechanism discussed below).

Kinetics and Thermodynamic Parameters in the Conversion of 7 to 2 and 3

In alkaline hydrolysis of **1**, two kinetic processes can be monitored by spectrophotometry.[4,9] The fast kinetic process is characterized by increases of absorption bands centered at 255 and 342 nm, with a simultaneous decrease of an absorption band centered at 236 nm.[9] The slow kinetic process is characterized by an increase of an absorption band centered at ~410 nm, owing to the formation of **3** at [NaOH] ≤ 1 M.[4,9] In ethanol-5 M NaOH (1:3, v/v) at higher than 50 °C, an increase of an absorption band centered at 400 nm is observed, owing to the formations of both **2** and **3**.[9] By reversed-phase HPLC analysis, the formations of **2** and **3** from **1** in ethanol-5 M NaOH (1:3, v/v) at 50 °C appeared to follow zero-order kinetics.[9]

In ethanol-5 M NaOH (1:3, v/v) at 50 °C, the conversion of **1** to **7** was essentially complete in less than 1 min (Table 1). The slower kinetic process was due to an additional reaction of **7** in the alkaline medium, resulting in the formations of **2** and **3**.[9] This slower kinetic process of **1** in ethanol-5 M NaOH (1:3, v/v) was studied by continuously monitoring the increase of absorbance at 380 nm (which detects the formations of both **2** and **3**) and at 415 nm (which detects the formation of primarily **3**). An initial pseudo-first order increase, followed by a zero-order increase in absorbance at both 380 nm and 415 nm, was observed and the reaction $t_{1/2}$'s of the initial pseudo-first order process were not dependent on the wavelength of detection (Fig. 6). Curve-fitting of the data using equation $A_t = a * exp(-0.693 * t/t_{1/2}) + b * t + c$ gave similar $t_{1/2}$'s from data obtained at both 380 and 415 nm for the initial pseudo-first order reaction. In the equation above, A_t is absorbance at either 380 nm or 415 nm at time t, b is the slope of the linear portion of the curve, and a and c are constants depending on the concentration of the sample. The slope of the linear portion of the curve increased with increasing temperature. Temperature-dependent reaction $t_{1/2}$'s were determined with reaction mixtures containing 1:3 volume ratios of ethanol-5 M NaOH at five temperatures: 33.0 ± 0.5 min (n = 3) at 37 °C, 21.3 ± 0.6 min (n = 3) at 43 °C, 10.6 ± 0.7 min (n = 4) at 50 °C, 6.7 ± 0.2 min (n = 5) at 55 °C, and 4.7 ± 0.5 min (n = 3) at 60 °C. Arrhenius plot [log$t_{1/2}$ vs. 1000/T, where T is temperature in Kelvin] yielded a slope of 3.919 (r^2 = 0.996) and extrapolation of the curve-fitted straightline gave a $t_{1/2}$ of 111.4 min at 25 °C. The activation parameters were: E_{act} = 17.9 kcal/mol and at 25 °C, $\Delta H^{\ddagger}$ = 17.3 kcal/mol, $\Delta S^{\ddagger}$ = -18.6 cal/K/mol, and $\Delta G^{\ddagger}$ = 22.9 kcal/mol. The relatively large negative value of $\Delta S^{\ddagger}$ indicated an activated complex resulting from a reaction between hydroxide ion and **7** (and/or **5**).

Effect of Detergents

In 1:3 volume ratios of either dioxane-0.5 M NaOH or ethanol-0.5 M NaOH, in which the 0.5 M NaOH solution either contained or did not contain 12 mM CTAB (or 0.2 M SDS), time-dependent uv-vis absorption spectra of **1** at 50 °C (data not shown) were essentially the same as those of **1** in ethanol-1 M NaOH (1:3, v/v) reported earlier.[9] Thus, the results of both our earlier[9] and present reports indicated that **1** was hydrolyzed in aqueous alkaline media by initial attack of hydroxide ion at the N1-C2 amide bond, regardless of the presence or absence of CTAB and SDS. The results are in contrast to an earlier study reporting the shift of initial site of alkaline cleavage from C5-N4 imine bond to N1-C2 amide bond in the presence of CTAB.[4]

Single wavelength monitoring at either 255 nm or 342 nm at 50 °C indicated the following pseudo-first order reac-

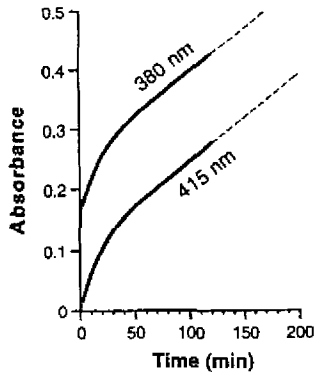

Fig. 6. Hydrolysis kinetics of **1** (86 μg/mL) in ethanol-5 M NaOH (1:3, v/v) at 50 °C. Reactions were continuously monitored at either 380 or 415 nm. Concentration of **1** in the reaction mixture was 186 μg/mL. Each thick line curve consisted of 1000 data points. Broken line curves were obtained by curve-fitting as described in the text.

tion $t_{1/2}$'s of **1** in various solvents: 25.2 ± 0.2 min (n = 5) in ethanol-0.5 M NaOH (1:3, v/v), 12.3 ± 0.2 min (n = 5) in ethanol-0.5 M NaOH/12 mM CTAB (1:3, v/v), and 90.5 ± 3.2 min (n = 3) in ethanol-0.5 M NaOH/0.2 M SDS (1:3, v/v). At 50 °C, the reaction $t_{1/2}$ was 11.3 ± 0.3 min (n = 5) in dioxane-0.5 M NaOH (1:3, v/v), 5.5 ± 0.1 min (n = 5) in dioxane-0.5 M NaOH/12 mM CTAB (1:3, v/v), and 49.6 ± 1.4 min (n = 5) in dioxane-0.5 M NaOH/0.2 M SDS (1:3, v/v). Compare to ethanol as a co-solvent present in 25% by volume, dioxane increased the hydrolysis rate by ~2-fold. In the presence of 0.2 M SDS (an anionic detergent), the hydrolysis rate was more than 3-fold slower. In the presence of 12 mM CTAB (a cationic detergent), the hydrolysis rate increased more than 2-fold. In contrast, an earlier study[4] reported that a 12 mM CTAB caused a 9.3-fold and a 18.4-fold increase, respectively, in the rate of hydrolysis of **1** at 74.5 °C in aqueous solutions containing 0.0738 M and 0.277 M NaOH, respectively. We could not compare our results to those in the earlier report[4] because the exact solvent composition was not unambigously described in the earlier report.

Reaction Mechanism

Based on the results described above, the reaction mechanism involved in the conversion of **1** to **2** and **3** as final products in aqueous alkaline media is proposed in Fig. 7. The initial reaction is the attack of a hydroxide ion at the C2-carbonyl carbon, followed by abstraction of a hydroxyl proton to form a dianion **7**. The dianion **7** may be opened to form **5**, which may be recyclized to form **7** in the strongly alkaline medium. The proposed formations of the initial reaction products were supported by: (1) a reaction order of ~2 with respect to hydroxide ion in 100% aqueous solution, (2) deuterium kinetic isotope effect (k_H/k_D) of 0.62, (3) a relatively large negative value of the entropy of activation, (4) proton NMR spectral data of **7** in DMSO-d6, (5) the formation of **7** from **5** via recyclization, (6) structural characterization of methyl ester **6** formed from **5**,[9] and (7) structural characterization of monomethyl ether **8** formed from **7**.

Intermediate product **7** (and/or **5**) undergoes an additional attack by hydroxide ion at the C5 of the C5-N4 imine bond to form intermediate c (Fig. 7). Both pathway A (**7** → **5** → intermediate c) and pathway B (**7** → intermediate b → intermediate c) are plausible mechanisms leading to the formation of intermediate c. Intermediate c is probably the immediate precursor of **2** and **3** in strongly alkaline solution. In 1:3 volume ratios of ethanol-NaOH reaction mixture at 50 °C, **3** is essentially the only product formed at [NaOH] ≤ 1 M.[9] The formation of **2** increased with increasing concentration of NaOH in the ethanol-NaOH reaction mixture.[9]

The proposed formation of intermediate c and its subsequent decomposition to form **2** and **3** were supported by: (1) the spectrophotometric observation of a pseudo-first order reaction of **7** (and/or **5**) in ethanol-5 M NaOH (1:3, v/v), followed by a zero-order reaction (Fig. 6), (2) zero-order formations of **2** and **3** from **7** (and/or **5**) revealed by reversed-phase HPLC analysis,[9] (3) both the pseudo-first order and zero-order reactions were temperature dependent, and (4) a relatively large negative value of the entropy of activation associated with the pseudo-first order reaction.

Cleavage of C(OH)-NH bond of intermediate c (Fig. 7), leading to the formation of **3**, is an expected consequence in the extensively studied base-catalyzed hydrolysis of imines.[14] Compound **2** is slowly converted to **3** in aqueous alkaline media.[9] However, cleavage of NH-CH_2 bond of intermediate c (Fig. 7) is a highly unusual reaction leading to the formation of **2** and acetate. It is possible that, following an initial hydroxide attack at C2-carbonyl carbon, subsequent cleavage of N4-C3 bond may also occur in strongly alkaline solutions for some other 1,4-benzodiazepines.

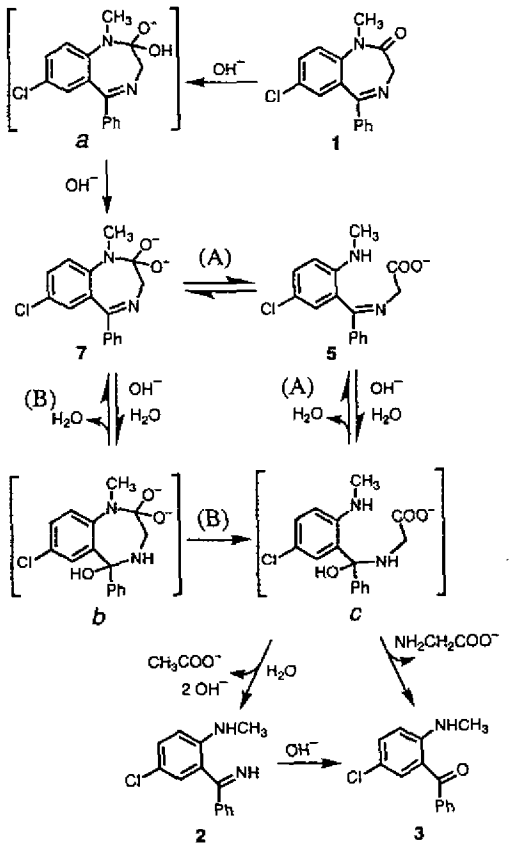

Fig. 7. Proposed mechanism in alkaline hydrolysis of 1. Proposed intermediates shown in brackets were not isolated for characterization. See text for discussion.

CONCLUSION

The results of this report indicated that the proposed hydrolytic attack at C5-N4 imine bond of **1** in aqueous alkaline solutions by two earlier reports[4,6] was in error. The change of initial hydrolysis via the C5-N4 imine bond to initial hydrolysis via the N1-C2 amide bond in the presence of a cationic detergent CTAB proposed in an earlier report[4] was also in error. The errors were made apparently due to the lack of rigorous structural characterization of the initial alkaline hydrolysis product(s).

Results of this report on the hydrolysis kinetics and structural identification of some intermediate products indicated an initial hydroxide attack at the C2-carbonyl carbon of **1**, resulting in the formation of a dioxide **7**. Compound **7** was characterized by proton NMR spectroscopy and via its monomethyl ether **8**. The seven-member diazepine ring of **7** opened at the N1-C2 bond to form a glycinate **5**. Compound **5** might be present in equilibrium with **7** in strongly alkaline solutions. Compound **7** (and/or **5**) underwent an additional hydroxide attack at the C5-N4 imine bond to form a tetrahedral intermediate, which slowly decomposed to form **2** and **3**; the ratio of 2:3 increases with increasing NaOH concentration.[9]

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Key Words

Diazepam; Alkaline hydrolysis; 7-Chloro-1,3-dihydro-1-methyl-5-phenyl-2*H*-1,4-benzodiazepin-2,2-dioxide; 2-Methylamino-5-chlorobenzophenone imine; 2-Methylamino-5-chlorobenzophenone; Mass spectral analysis; Proton NMR.

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