Hydrolysis of 2-Oxoquazepam in Alkaline Solution

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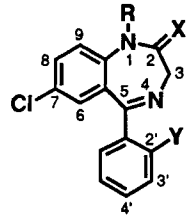
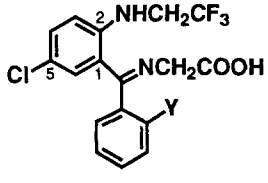
Abstract □ 2-Oxoquazepam [7-chloro-1-(*N*-2,2,2-trifluoroethyl)-5-(2′-fluorophenyl)-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one, OQZ], a major pharmacologically active metabolite of quazepam, was hydrolyzed in NaOH solution to form a sodium salt of 2-(*N*-2,2,2-trifluoroethyl)amino-5-chloro-α-(2′-fluorophenylbenzylidene)glycine. The hydrolysis product was formed via a rapidly established acid–base equilibrium, followed by a rate-determining, ring-opening reaction involving two negatively charged ions. Following neutralization, the hydrolysis product was isolated by reversed-phase HPLC and subsequently identified by its UV–vis absorption and MS analyses. Kinetics of the hydrolysis reaction in acetonitrile/water mixture was studied by reversed-phase HPLC analysis as a function of water content, NaOH concentration, temperature, and ionic strength. In acetonitrile:0.05 N NaOH (1:1, v/v), the hydrolysis of OQZ had an energy of activation of 14.4 kcal/mol and at 25 °C ($\Delta H^{\ddagger}$ = 13.8 kcal/mol, $\Delta S^{\ddagger}$ = −31.2 cal/K/mol, and $\Delta G^{\ddagger}$ = 23.1 kcal/mol).

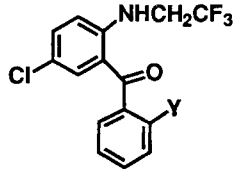
Knowledge of solvent, pH, temperature, or a combination of these parameters under which drugs are unstable helps to avoid degradation in drug formulation, storage, and some analytical conditions. Quazepam (QZ), 2-oxoquazepam (OQZ), and halazepam (HZ) are the 1,4-benzodiazepines shown to be capable of differentiating between central nervous system (CNS) subtypes of benzodiazepine receptors (see structures).[1-10]



NDZ: R = Y = H, X = O
QZ: R = CH_2CF_3, X = S, Y = F
OQZ: R = CH_2CF_3, X = O, Y = F
HZ: R = CH_2CF_3, X = O, Y = H



OQZ-HP: Y = F
HZ-HP: Y = H



TFCFBP: Y = F
TFCBP: Y = H

OQZ is the major pharmacologically active metabolite of QZ.[11-13] OQZ exhibits a higher potency than QZ at the CNS type I receptor sites.[6,7] The 1-*N*-trifluoroethyl substituent appears to be responsible for the receptor subtype selectivity because benzodiazepines lacking the 1-*N*-trifluoroethyl group do not discriminate among the subtypes of benzodiazepine receptors.[3]

The formation of OQZ from QZ in alkaline media was first realized in a study by a polarographic method.[14] It was suggested that OQZ was formed via addition of a hydroxide ion to the C2 thione carbon of QZ.[14] In a strongly alkaline medium, a dimer of QZ was formed between two QZ enthiolate anions and a Hg^{++}.[14] However, hydrolysis product was not detected in the ealier study.

A ring-opening hydrolysis product was formed when *N*-desmethyldiazepam (NDZ) was refluxed for 10 min in ethanol:5 N NaOH (1:2, v/v).[15] OQZ differs from NDZ by a 1-*N*-trifluoroethyl substituent and a fluoro group at 2′ position (see structures). Prior to the study leading to this report, we postulated that the hydrolysis of NDZ was initiated by a nucleophilic attack of a hydroxide ion at the C2 carbonyl carbon. We reasoned that, in the presence of an electron-withdrawing group at N1 position, the hydrolysis reaction of OQZ should proceed much more readily than that of NDZ. We have found that NDZ was stable for days at ambient temperature in acetonitrile/water mixtures containing up to 0.1 N NaOH. Under the same condition, however, OQZ readily underwent hydrolysis, apparently due to the presence of the electron-withdrawing 1-*N*-trifluoroethyl substituent. A detailed study of OQZ hydrolysis in alkaline solution is described in this report.

Experimental Section

Materials—2-Oxoquazepam [7-chloro-1-(*N*-2,2,2-trifluoroethyl)-5-(2′-fluorophenyl)-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one, OQZ; ϵ_{226} 33 100 cm^{-1} M^{-1} at room temperature, acetonitrile] was generously provided by Dr. Allen Barnett of Schering-Plough Corporation, Bloomfield, NJ [EI-MS: M^+ at *m/z* 370 with characteristic fragments at *m/z* 351 (loss of F), 342 (base peak; loss of CO), 335 (loss of Cl), 307 (loss of Cl from fragment at *m/z* 342), and 259 (loss of CH_2CF_3 from fragment at *m/z* 342)]. *N*-Desmethyldiazepam (NDZ) was generously provided by Dr. Peter F. Sorter of Hoffmann-La Roche Inc., Nutley, NJ.

2-(*N*-2,2,2-Trifluoroethylamino)-5-chloro-2′-fluorobenzophenone (TFCFBP; see structure) was prepared by two methods. Method 1 was by treatment with a strong acid, similar to the procedures described earlier.[15-17] OQZ (2 mg) was dissolved in 5 mL of CH_3CN:5 M H_2SO_4 (1:1, v/v), and the resulting solution was heated at 100 °C for 2 h. The yellow product (TFCFBP) was isolated by reversed-phase HPLC for UV–vis absorption and MS analyses. The yield of TFCFBP ranged from 20 to 50% among repeated experiments. Method 2 was to dissolve OQZ (2 mg) in 5 mL of CH_3CN:0.1 N NaOH (1:1, v/v), and the resulting solution was kept at 50 °C for 2 h. The solution containing the hydrolysis product OQZ-HP ("HP" stands for hydrolysis product) was acidified with conc. H_2SO_4 and heated at 50 °C for 30 min. The resulting yellow solution was neutralized with NaOH and subsequently extracted with ethyl acetate. Following evaporation of ethyl acetate, the product (TFCFBP) was isolated by reversed-phase HPLC for UV–vis absorption and MS analyses. The yield of TFCFBP was >98% among repeated experiments. Two methods yielded an identical product [EI-MS: M^+ at *m/z* 331 (base peak) with characteristic fragments at *m/z* 312 (loss of F) and 262 (loss of CF_3)]. An earlier study[15] reported that 2-amino-5-chlorobenzophenone (ACBP) and glycine were formed upon acidification of the hydrolysis product derived by refluxing NDZ in ethanol:5 N NaOH (1:2, v/v).

HPLC—HPLC was performed with a Waters Associates (Milford, MA) model M45 solvent pump and a model 441 absorbance detector (254 nm). A Vydac C18 column (5-μm particles, 4.6 mm i.d. × 25 cm, catalog no. 201TP54; The Separations Group, Hesperia, CA) was used. To analyze samples in kinetic experiments, CH_3CN:0.02 M phosphate buffer (pH 7.0; 65:35, v/v) was used as the mobile phase at a flow rate of 1 mL/min. For purification of OQZ-HP for UV–vis absorption and MS analyses, CH_3CN:H_2O (7:3, v/v) was used as the mobile phase, also at 1 mL/min. HPLC analysis was conducted at ambient temperature

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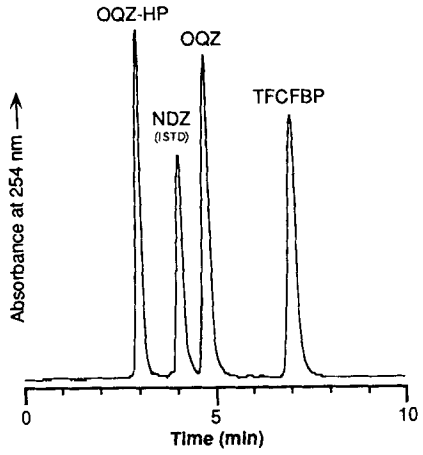


Figure 1—Reversed-phase HPLC separation of OQZ-HP, NDZ, OQZ, and TFCFBP. Chromatographic conditions are described in the *Experimental Section*.

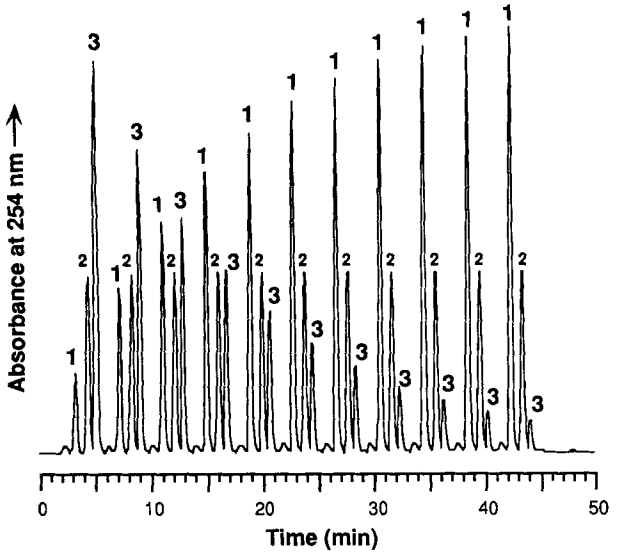


Figure 2—An example of the time course in the monitoring of OQZ hydrolysis by reversed-phase HPLC. Hydrolysis of OQZ was conducted in CH_3CN:0.2 N NaOH (1:1, v/v) at 25 °C. Samples (10 μL each injection) were repetitively injected every 3.92 min. The hydrolysis $t_{1/2}$ of this reaction was 10.4 min.

(23.5 ± 0.5 °C). Samples were injected via a Shimadzu (Shimadzu Corporation, Kyoto, Japan) model SIL-9A automatic sample injector equipped with a water-jacketed sample rack. The temperature of the sample rack was maintained by passing constant-temperature water from a thermostated water circulator. The actual temperature of the solution in the sample vial was measured with a portable digital thermometer fitted with a detachable probe (Thomas Scientific, Swedesboro, NJ). The absorbance detector signal was recorded with MacIntegrator (a software and hardware package from Rainin Instruments Company, Inc., Emeryville, CA) on a Macintosh Classic II computer (Apple Computer, Cupertino, CA).

In kinetic studies of the hydrolysis reaction, each sample (2 mL total volume) contained OQZ (ranging from 42.8 to 214 μM) and NDZ (66 μM). NDZ was used as an internal standard for quantification purposes. The detection limit at 254 nm was ~2 ng. The hydrolysis reaction was initiated by adding 2 mL of a solvent mixture (pre-equilibrated to the temperature under study) to a test tube containing appropriate amounts of dried residues of OQZ and NDZ. The mixture was then quickly vortexed for ~15 s to dissolve the OQZ and NDZ. The resulting solution was immediately transferred to a sample vial (pre-equilibrated to the temperature under study) and placed in a sample well of the thermostated sample rack of the autosampler. Depending on the temperature under study and the rate of the hydrolysis reaction, the first sample was injected after the sample vial has been placed in the sample well for 3 to 10 min

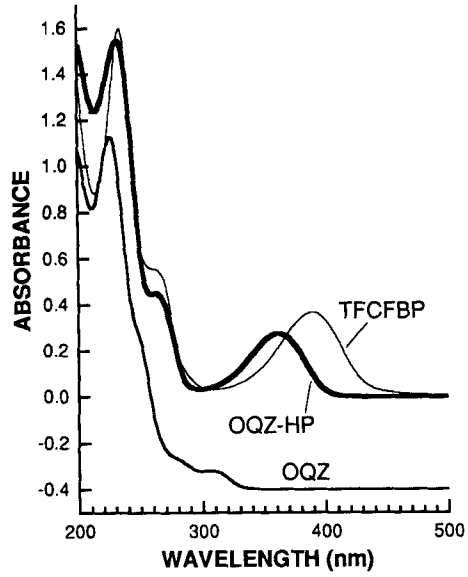


Figure 3—UV–vis absorption spectra of OQZ [43 μM; characteristic λ_{max} at 226, 249 (sh), and 309 nm], OQZ-HP (characteristic λ_{max} at 233, 263, and 362 nm), and TFCFBP (characteristic λ_{max} at 234, 262, and 389 nm) in CH_3CN. The absorbance of OQZ is offset by 0.4 absorbance unit to avoid excessive overlapping of curves.

to allow the temperature to reach equilibrium. A sample (10 μL) was subsequently injected for analysis at each sampling interval. The sampling interval in reversed-phase HPLC analysis ranged from 3.42 to 15.42 min. Detector signals of repetitively injected samples were continuously recorded.

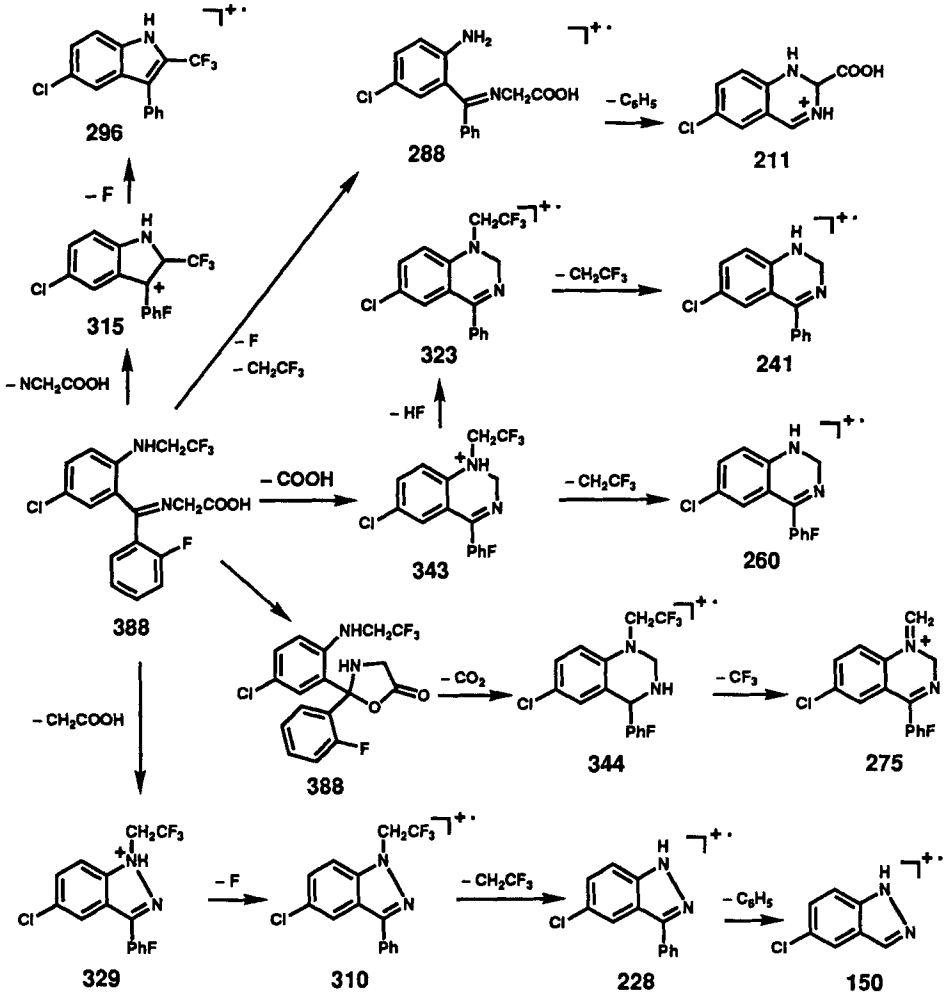
Hydrolysis half-life ($t_{1/2}$) was determined by curve-fitting computer software (either SigmaPlot or Cricket Graph) following plotting of either log (AUC ratio of OQZ/NDZ) or log (AUC or OQZ) versus time. Hydrolysis $t_{1/2}$ (= 0.693/k; k is the rate constant) were averages of two to four determinations: two for hydrolysis $t_{1/2} > 90$ min and three to four for hydrolysis $t_{1/2} < 90$ min. The rate constants are reported as mean ± SD. Error bars used in some figures contained in this report for values of SD were smaller than the size of symbols.

Spectral Analysis—UV-vis absorption spectra of samples in acetonitrile were determined with a 1-cm path length quartz cuvette on a model DW2000 spectrophotometer (SLM Instruments, Urbana, IL). MS analysis was performed on a Finnigan 4500 gas chromatograph-mass spectrometer-data system with a solid probe by electron impact at 70 eV, and the ion source was maintained at 120 °C.

Computer and Software—Several computer software programs were employed to prepare text, graphics, and curve fittings on an Apple Macintosh SE/30 computer. The software includes Word (Microsoft Corporation, Redmond, WA), ChemDraw (Cambridge Scientific, Cambridge, MA), SigmaPlot (Jandel Scientific, Corte Madera, CA), CA-Cricket Graph III (Computer Associates International, Inc., Islandia, NY), and Canvas (Deneba Software, Miami, FL).

Results and Discussion

A reversed-phase HPLC method was developed to separate the hydrolysis products of OQZ (OQZ-HP), NDZ, OQZ, and TFCFBP (Figure 1). NDZ was chosen to serve as an internal standard for simultaneous quantification of both the disappearance of OQZ and the formation of OQZ-HP in a hydrolysis reaction. The choice of NDZ as an internal standard for chromatography was based on the following observations: (*1*) NDZ was stable in CH_3CN:0.2 N NaOH (1:1, v/v) at 50 °C for at least 5 h; and (2) no product of OQZ cochromatographed with NDZ. Inclusion of an internal standard eliminated the need for injecting samples of a constant volume at different times during the hydrolysis reaction. However, due to good repeatability (≤2%) in injecting a constant volume of samples by the autosampler employed in this study, consistent hydrolysis $t_{1/2}$



Scheme 1—Some fragmentation pathways proposed to account for some of the major mass fragments observed in the electron impact mass spectrum of OQZ-HP (PhF and Ph are abbreviations for 2′-fluorophenyl and phenyl groups, respectively).

values were obtained by plotting log (AUC of OQZ) versus time. When NDZ was used as an internal standard, hydrolysis $t_{1/2}$ was obtained by plotting log (AUC ratio of OQZ:NDZ) versus time. Hence, kinetic studies may be carried out by inclusion of an internal standard in the event that an autosampler is not available.

Under the conditions employed to study the hydrolysis reaction of OQZ, the formation of TFCFBP was insignificant and did not interfere with the quantification of either the disappearance of OQZ or the formation of OQZ-HP. A small amount of TFCFBP was formed when OQZ was kept in CH_3CN:0.2 N NaOH (1:1, v/v) at ambient temperature for at least 48 h; the AUC ratio of TFCFBP:OQZ at 254 nm was <0.01 at the end of 48 h. Thus, the sampling interval in a kinetic study of OQZ hydrolysis can be as short as 3.5 min (see Figure 1). An example of a kinetic monitoring of the changes in the AUCs or OQZ and OQZ-HP relative to that of NDZ is shown in Figure 2. This particular reaction had a hydrolysis $t_{1/2}$ of 10.4 min. The exponential increase in the formation of OQZ-HP and the exponential decrease of OQZ can be clearly seen in Figure 2.

The UV–vis absorption spectrum of OQZ-HP in CH_3CN was characteristically different from that of OQZ (Figure 3). OQZ-HP had a broad absorption band in the visible region with a λ_{max} at 362 nm and, hence, a light yellow color in CH_3CN. We have observed by reversed-phase HPLC analysis (Figure 1) that OQZ-HP slowly underwent a spontaneous decompositon to form TFCFBP when OQZ-HP was stored in CH_3CN at 4 °C. TFCFBP exhibited a more intense yellow color than OQZ-HP due to its absorption band centering at 389 nm (Figure 3). Absorption

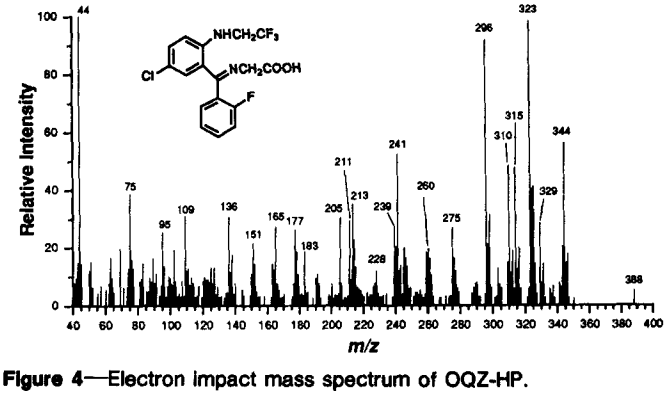


Figure 4—Electron impact mass spectrum of OQZ-HP.

bands between ~350 and 410 nm are observed in various substituted benzophenones.[18]

EI-MS analysis of OQZ-HP indicated a weak M^+ at m/z 388 and some characteristic fragment ions (Figure 4). Some of the fragment ions that can be readily recognized are as follows: m/z 343 (loss of COOH), m/z 329 (loss of CH_2COOH), m/z 315 (loss of NCH_2COOH), m/z 296 (loss of NCH_2COOH and F). Fragmentation pathways leading to these and other major fragment ions are proposed in Scheme 1. A tautomeric 5,5-disubstituted oxazolidone intermediate is proposed to account for the formation of fragment ions at m/z 344 and m/z 275. The 5,5-disubstituted oxazolidone intermediate may be the precursor in the subsequent formation of TFCFBP. Taken together, the results unequiv-

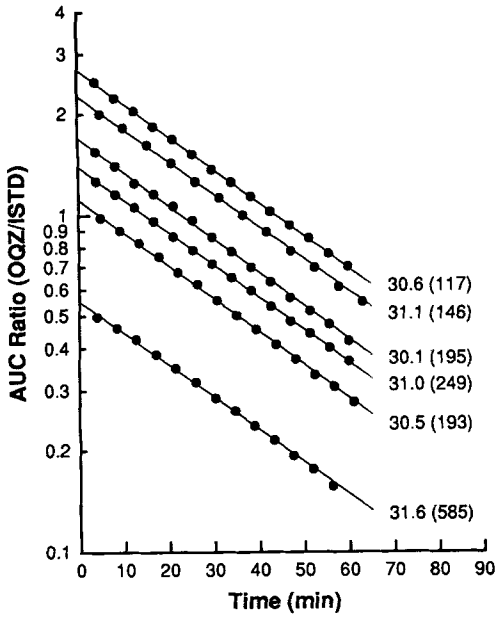


Figure 5—Dependence of hydrolysis rate on the concentration of OQZ in CH_3CN:0.1 N NaOH (3:1; v/v) at 23.5 ± 0.5 °C. The concentration of OQZ ranged from 42.8 to 214 μM. Disappearance of OQZ was expressed by the normalized AUC ratio of [OQZ]:[ISTD]. The hydrolysis $t_{1/2}$ (in min) determined from each set of experimental data and the [NaOH]:[OQZ] molar ratio (in parenthesis) in each experiment are indicated.

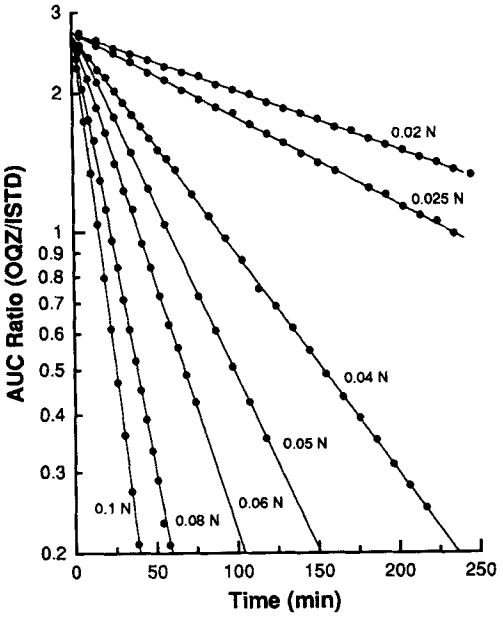


Figure 6—Dependence of hydrolysis rate of OQZ (214 μM) on [NaOH] in CH_3CN:H_2O (1:1; v/v) at 25 °C. Disappearance of OQZ was expressed by the normalized AUC ratio of [OQZ]:[ISTD]. The final concentration of NaOH for each determination is indicated.

ocally established the structure of OQZ-HP as 2-(*N*-2,2,2-trifluoroethyl)amino-5-chloro-α-(2′-fluorophenylbenzylidene)-glycine.

In the initial phase of the kinetic studies, we established that the rate of OQZ hydrolysis in alkaline solution depended on the concentrations of both OQZ and NaOH (see results below). Thus, OH^- was probably both a catalyst and a reactant. For the purpose of elucidating the mechanism of hydrolysis, we carried out a series of experiments, and the results are described below.

To facilitate the interpretation of results, it was necessary to choose a concentration range of NaOH so that the hydrolysis reaction could be studied under pseudo-first-order kinetics. The results (Figure 5) were obtained with OQZ concentrations ranging from 41.8 to 214 μM at ambient temperature (23.5 ± 0.5 °C) in CH_3CN:0.1 N NaOH (3:1; v/v). The average hydrolysis $t_{1/2}$ of these reactions was 30.8 ± 0.5 min. Hence, the reaction was independent of the OQZ concentration up to 214 μM under the experimental conditions used.

In a solvent mixture containing CH_3CN:H_2O (3:1; v/v), the highest concentration of NaOH that could be used was ~0.025 N; above this NaOH concentration, the solution was not homogeneous and became cloudy upon mixing. To extend the range of NaOH concentration that could be examined, the rate of OQZ hydrolysis was further studied at 25 °C in a 1:1 volume ratio of CH_3CN:H_2O containing final concentrations of NaOH ranging from 0.02 to 0.1 N and [NaOH]:[OQZ] molar ratios ranging from 93 to 467 (Figure 6). Under the experimental conditions, the hydrolysis reaction followed apparent first-order kinetics. A fivefold increase in [NaOH] (from 0.02 to 0.1 N) resulted in a 24-fold increase in the rate of hydrolysis (the hydrolysis $t_{1/2}$ decreased from 248 min to 10.4 min).

The rate constant k (or hydrolysis $t_{1/2}$) and [NaOH] were found to be linearly related by plotting log k versus pH (or log $t_{1/2}$ versus pH). The straight lines (slopes ≈ 2), resulting from plotting log k versus pH from data obtained with both 3:1 and 1:1 volume ratios of acetonitrile:water, were essentially parallel to each other (Figure 7). The straight line resulting from data obtained with ≥3:1 volume ratio of CH_3CN:H_2O could be expressed by $k = 4.05 \times 10^{-28} \times 10^{2.08 \times pH}$, and that in 1:1 volume

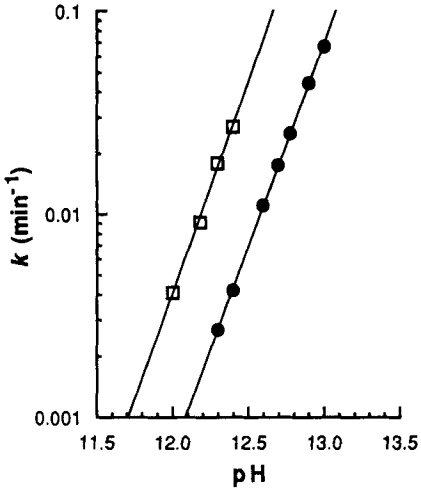


Figure 7—Dependence of log k or OQZ (214 μM) on [NaOH] in CH_3CN:H_2O [(□) 3:1 or (●) 1:1; v/v] at 25 °C. The concentration of NaOH is expressed by pH, which is equal to 14 + log [NaOH]. The slopes of the two lines are (□) 2.08 and (●) 2.01.

ratio of CH_3CN:H_2O by $k = 5.49 \times 10^{-28} \times 10^{2.01 \times pH}$. The hydrolysis reactions of OQZ, the rates of which were determinable by the HPLC method described in this study, occurred in solutions with pH between 12 and 13. At pH < 12, the rate constant k became very small. A slope of 2, revealed by plotting log k versus pH (Figure 7), indicated that the overall reaction was second order in hydroxide ion.[19]

The mechanism of the hydrolysis reaction was further probed by studying the dependence of hydrolysis rate on the ionic strength (μ) in CH_3CN:0.1 N NaOH (1:1, v/v). NaCl was used to vary the ionic strength of the solvent. The rate of OQZ hydrolysis was found to increase with increasing $\sqrt{\mu}$ of the solvent mixture (Figure 8). The solubility of OQZ decreased up to 40% due to increased concentration of NaCl in the solvent mixture. However, the amounts of OQZ and NDZ dissolved were sufficient to allow the determination of hydrolysis $t_{1/2}$. When the solvent mixture contained <0.25 M NaCl, log k increased linearly with a positive slope with increasing $\sqrt{\mu}$ (Figure 8). These results

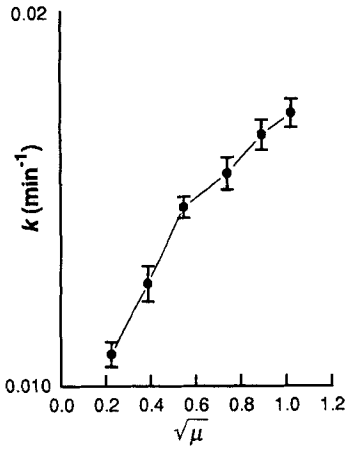


Figure 8—Dependence of log k of OQZ (214 μM) on $\sqrt{\mu}$ in CH_3CN:0.0625 N NaOH (1:4; v/v) containing (data points from left to right) 0, 0.1, 0.25, 0.5, 0.75, and 1 M NaCl, respectively, at 25 °C.

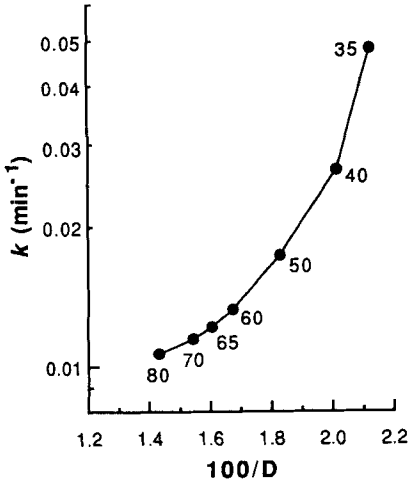


Figure 9—Dependence of log k of OQZ (214 μM) on 100/D in various volume ratios of CH_3CN:H_2O containing 0.05 N final concentration of NaOH at 25 °C (D is the weighted dielectric constant of the solvent; see text for explanation). The volume percentage of water contained in the solvent mixture is indicated next to each data point.

suggested that the rate-determining step was probably due to a reaction between two negatively charged ions.[19] A positively charged ion is not expected to be formed in the hydrolysis reaction of OQZ in alkaline solution. The rate constant of a reaction between a neutral (or dipolar) molecule (e.g., OQZ) and a negatively charged ion (e.g., OH^-) is expected to be independent of the ionic strength of the solvent.[19]

The hydrolysis reaction was further studied in solvents containing 0.05 N NaOH and various volume ratios of acetonitrile and water. Depending on the amount of OQZ used, the total volume of a solvent mixture with acetonitrile had to be at least 5 to 20% to dissolve OQZ. Figure 9 is a plot of log k versus 100/D, where D is the weighted dielectric constant of the CH_3CN/H_2O mixture. Dielectric constants of acetonitrile and water are 37.5 and 80.1, respectively.[20] The weighted dielectric constant of a 3:7 volume ratio of CH_3CN:H_2O, for example, is equal to 37.5 × 0.3 × 0.7822 + 80.1 × 0.7 × 0.9982, where 0.7822 and 0.9882 are densities of acetonitrile and water at 20 °C, respectively.[20] The results in Figure 9 indicate that the hydrolysis rate became progressively slower with increasing percentages of water (or increasing dielectric constant) in the solvent mixture. The rate-determining step in a reaction either between a neutral (or a dipolar) molecule (i.e., OQZ) and a negatively charged ion (i.e., OH^-) or between two negatively charged ions is expected to result in a straight line with a negative slope when log k is plotted

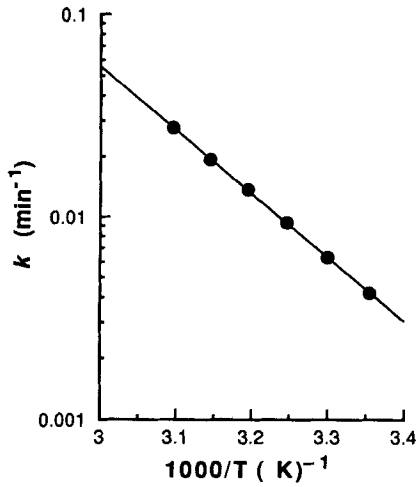
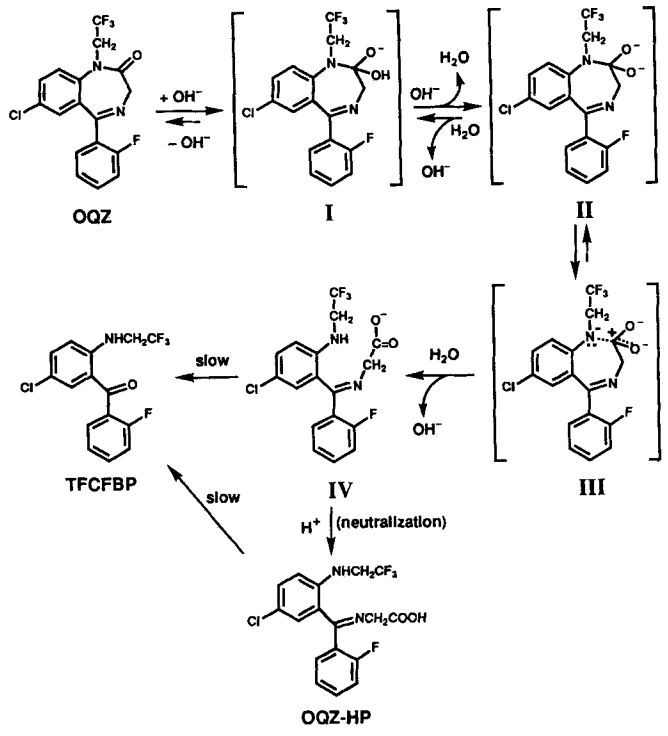


Figure 10—Arrhenius plot for temperature dependence of log k in the hydrolysis of OQZ. The hydrolysis reaction was conducted in CH_3CN:0.05 N NaOH (1:1, v/v). Slope, −3.15; E_{act} = 14.4 kcal/mol; $\Delta H^{\ddagger}$ = 13.8 kcal/mol (at 25 °C); $\Delta S^{\ddagger}$ = −31.2 cal/K/mol (at 25 °C); $\Delta G^{\ddagger}$ = 23.1 cal/mol (at 25 °C).

versus 1/D.[19] Although the results in Figure 8 indicate that the rate-limiting step involved two negatively charged ions, the results in Figure 9 seem to be contradictory. However, the result that rate constant k increases with increasing 1/D (Figure 9) may be due to selective solvation of reactants by water molecules. The solvation became more extensive with increasing percentage of water in the solvent mixture. Selective solvation of reactants by water probably increased the effective distance between the reactants, thereby decreasing the rate of nucleophilic attack by OH^- at the C2 carbonyl carbon of OQZ.[19]

The thermodynamic parameters, determined by an Arrhenius plot of data obtained from temperature dependence of rate constant k in OQZ hydrolysis in CH_3CN:0.05 N NaOH (1:1, v/v) (Figure 10), were energy of activation (E_{act}) = 14.4 kcal/mol and at 25 °C, enthalpy ($\Delta H^{\ddagger}$) = 13.8 kcal/mol, entropy ($\Delta S^{\ddagger}$) = −31.2 cal/K/mol, and Gibbs free energy ($\Delta G^{\ddagger}$) = 23.1 kcal/mol. The E_{act} is relatively small, indicating that a relatively small energy is required to form the transition state. The relatively large negative $\Delta S^{\ddagger}$ indicated a gain of orderliness in the transition state. This is expected for a reaction resulting in an addition product from separated molecules. The large negative $\Delta S^{\ddagger}$ also suggested that the transition state was extensively solvated.

Based on the results described above, the mechanism of hydrolysis of OQZ in alkaline solution is proposed in Scheme 2. Results shown in Figures 8 and 9 indicate that the formation of intermediate I was not the rate-determining step. We propose that intermediate I is formed via a rapidly established acid–base equilibrium at pH > 12. This is consistent with the findings that a higher concentration of NaOH favors the formation of intermediate I, thereby increasing the rate of OQZ hydrolysis. The result that log k increases linearly with a positive slope with increasing $\sqrt{\mu}$ (Figure 8) indicates that the deprotonation reaction of intermediate I by a hydroxide ion is involved in the rate-determining step in the formation of product IV. The dianion (intermediate II) may undergo ring opening via intermediate III to form product IV. Intermediate III is probably the transition state of the hydrolysis reaction. The structure of the transition state (intermediate III) suggests an intermediate structure in which a N-C bond is about to be broken and a C-O bond (leading to the formation of the C=O bond at C2 poistion of OQZ) is about to be formed. The magnitude of $\Delta H^{\ddagger}$ (Figure 10) is consistent with the energy required to form the transition state. The proposed mechanism (Scheme 2) is also consistent with the finding (Figure 7) that the overall hydrolysis reaction is second order in hydroxide ion.



Scheme 2—Proposed mechanism for the formation of OQZ-HP in the hydrolysis of OQZ in alkaline medium. Structures in brackets indicate proposed intermediates that were not directly isolated and characterized. Both IV and OQZ-HP slowly decomposed to form TFCFBP by a mechanism not yet known.

The proposed mono- and di-anions (intermediates I and II, respectively, in Scheme 2) as the intermediates in the hydrolysis of OQZ are similar to the anions proposed in the hydrolysis of benzamide,[21] coumarins,[22] and acyl cyanides[23] in alkaline solutions. The existence of anions as intermediates is consistent with the results shown in Figures 8 and 9. It has been suggested that reactions in which ionic charge is created are facilitated by solvents having high dielectric constant and by high ionic strength.[24] The results depicted in Figure 8 (log k increases with increasing $\sqrt{\mu}$) are consistent with the proposed formations of anions shown in Scheme 2.

Conclusions

We have established that the electron-withdrawing 1-*N*-trifluoroethyl substituent of OQZ facilitates a nucleophilic attack by a hydroxide ion at the C2 carbonyl carbon. Among 1,4-benzodiazepines, QZ, OQZ, and HZ possess unique selectivity for the CNS type I receptors.[1-10] An earlier report[14] and our preliminary results[25] indicate that QZ and HZ (see structure), both of which contain an electron-withdrawing 1-*N*-trifluoroethyl substituent, underwent similar nucleophilic addition reaction in alkaline media. The electrophilic character of the C2 carbonyl carbon induced by the electron-withdrawing properties of the 1-*N*-trifluoroethyl substituent may be related to the unique receptor subtype selectivity of 1-*N*-trifluoroethylated 1,4-benzodiazepines.

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25. Preliminary results indicate that in CH_3CN:0.2 N NaOH (1:1, v/v) at 25 °C, the hydrolysis $t_{1/2}$ of HZ and QZ are ~18 and <7 min, respectively. QZ reacted with hydroxide ion to form OQZ, which was hydrolyzed to form OQZ-HP.

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