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Base-catalysed rearrangement of temazepam

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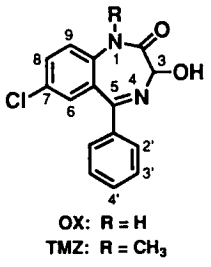
Abstract: Temazepam undergoes a rearrangement reaction in strongly alkaline media to form a cyclic diamide, 7-chloro-1-methyl-5-phenyl-4,5-dihydro-2*H*-benzodiazepin-2,3(1*H*)-dione. Thermodynamic parameters (E_{act}, $\Delta H‡$, $\Delta S‡$, $\Delta G‡$) involved in the rearrangement reaction, studied using either CH_3CN–0.2 N NaOH in H_2O (1:1, v/v) or CH_3CN–0.2 N NaOD in D_2O (1:1, v/v) as the solvent, were similar with an isotope effect (k_H/k_D) of 0.77 ± 0.03. Kinetics of the rearrangement reaction were studied as a function of NaOH concentration, temperature and ionic strength. Results indicated that the rate-determining step did not involve proton exchange with solvent. Mass spectral analysis of the cyclic diamides derived by using either D_2O or $H_2{}^{18}O$ in the solvent mixtures suggested that the formation of the cyclic diamide involved a nucleophilic addition of a hydroxide ion at the C2 carbonyl carbon of temazepam.

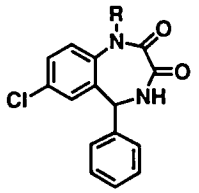
Keywords: *Temazepam; rearrangement; isotope effect; HPLC; kinetics; mass spectral analysis.*

Introduction

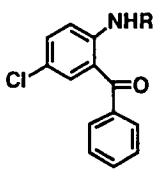
Knowledge of solvent, pH, temperature, or a combination of these parameters under which drugs are unstable helps to avoid degradation in drug formulation, storage, and some analytical conditions. Oxazepam (OX) and temazepam (TMZ) (see structure and numbering system in Fig. 1) are among the most commonly prescribed hypnotic/anxiolytic drugs [1, 2]. Both OX and TMZ are active metabolites of diazepam. There are a large number of pharmacological active 3-substituted 1,4-benzodiazepines and some of these are in clinical use [2, 3].



OX: R = H
TMZ: R = CH_3



OX-RP: R = H
TMZ-RP: R = CH_3



ACBP: R = H
MACBP: R = CH_3

Figure 1
Structures and abbreviations of oxazepam (OX), temazepam (TMZ), rearrangement products OX-RP and TMZ-RP, and decomposition products ACBP and MACBP.

An early study [4] reported that both OX and TMZ undergo rearrangement to form a cyclic diamide (Fig. 1) in ethanol–sodium hydroxide mixtures. The experimental condition required for the rearrangement of OX was considerably more drastic than that for TMZ. The rearrangement of OX in ethanol–0.67 N NaOH (5:6, v/v) requires heating on a steam bath, while rearrangement of TMZ in ethanol–4 N NaOH (20:1, v/v) occurs at room temperature [4]. The structures of the rearrangement products of both OX and TMZ have been characterized [4]. However, the mechanism(s) for the formation of these rearrangement products has not previously been elucidated. The kinetics and mechanism of the base-catalysed rearrangement of TMZ have been studied and are subjects of this report.

Materials and Methods

Materials

Temazepam,7-chloro-1-methyl-5-phenyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one, was generously provided by Sandoz Pharmaceuticals (East Hanover, NJ). 2-Methylamino-5-chlorobenzophenone (MACBP; M^+ at *m/z* 245) was prepared as the major product by methylation of 2-amino-5-chlorobenzophenone (ACBP) with dimethyl sulphate (DMSO) in acetonitrile–10 N NaOH (99:1, v/v) at room

temperature. DMSO, ACBP, deuterium oxide (D_2O, 99.9 atom % D), a sodium deuteroxide (NaOD, 40 wt % in D_2O, 99.9 atom % D) were purchased from Aldrich (Milwaukee, WI). NaOH solutions were diluted from a 10 N NaOH volumetric standard (Fischer Scientific, Pittsburgh, PA). $H_2{}^{18}O$ (99 atom % ^{18}O) was purchased from KOR Isotopes (Cambridge, MA).

Unlabelled TMZ-RP ('RP' stands for rearrangement product), 7-chloro-1-methyl-5-phenyl-4,5-dihydro-2*H*-benzodiazepin-2,3-(1*H*)-dione, was prepared by dissolving 5 mg of TMZ in 2 ml of acetonitrile–0.2 N NaOH (1:1, v/v) and the mixture was left at room temperature for 2 days. The solution was neutralized by adding 0.2 ml of 1 N HCl, followed by 2 ml of 0.2 M Tris–HCl buffer (pH 7.5). TMZ-RP and a minor amount of unreacted TMZ were extracted with 10 ml of ethyl acetate. The ethyl acetate phase was dehydrated with anhydrous magnesium sulphate and evaporated to dryness. TMZ-RP was purified by reversed-phase HPLC as described in the HPLC section below.

Deuterium-labelled TMZ-RP was prepared by dissolving 5 mg of TMZ in 2 ml of CH_3CN–0.2 N NaOD in D_2O (1:1, v/v). ^{18}O-labelled TMZ-RP was prepared by dissolving 5 mg of TMZ in 2 ml of CH_3CN–$H_2{}^{18}O$–10 N NaOH (50:50:1, v/v/v). Each reaction mixture was left at room temperature for 1 week. The reaction mixture was neutralized and processed similarly as described above for the unlabelled TMZ-RP. TMZ-RP was purified by reversed-phase HPLC.

3-*O*-Methyl-TMZ was prepared by dissolving TMZ (2 mg) in 2 ml of CH_3CN–0.2 N NaOH (1:1, v/v), followed by the addition of 0.05 ml of $(CH_3)_2SO_4$. The mixture was kept at ambient temperature for 18 h. RP-HPLC analysis indicated that ~72% of TMZ was converted to 3-*O*-methyl-TMZ (*m/z* of M^+ at 314 and base ions at *m/z* 271), the latter had identical UV absorption characteristics as those of TMZ. A 4-*N*-methyl-TMZ-RP (*m/z* of M^+ at 314) was prepared from TMZ-RP under identical conditions as described above. The 4-*N*-methyl-TMZ-RP was earlier prepared by methylation of TMZ-RP in 50% aqueous ethanol containing $(CH_3)_2SO_4$ and 0.32 N NaOH [4]. All solvent mixtures employed in kinetic studies were prepared in screw-capped Teflon-FEP (fluorinated ethylene propylene) flasks and were used within 2 days.

Acidity constant by spectrophotometry

Absorbance of samples was determined using a 1 cm path length quartz cuvette on a DW2000 spectrophotometer (SLM Instruments, Urbana, IL). Absorbance values at 226, 245 and 292 nm were recorded for solutions containing an identical concentration of TMZ (~66 μM) in CH_3CH–H_2O (1:1, v/v) containing various concentrations of HCl at ambient temperature (23 ± 1°C). The wavelengths for monitoring absorbance changes as a function of acid concentration were determined by a difference spectrum [5, 6] between the acidic and the neutral forms of TMZ. Following absorbance vs [HCl] plot, the K_{a1} value was determined by a curve fitting computer software.

Unlike those of OX and lorazepam [7], the absorption properties of TMZ were the same in neutral and alkaline solutions. Gradual changes in the absorption properties of TMZ in alkaline solutions at room temperature were due to the formation of a rearrangement product.

Liquid chromatography

Liquid chromatography was performed using a Waters Associates (Milford, MA) Model M45 solvent pump and a Model 441 absorbance detector (254 nm). The system was fitted with a Vydac C_{18} column (5 μ particles, 4.6 mm i.d. × 25 cm, catalogue no. 201TP54; The Separations Group, Hesperia, CA). For sample analysis in kinetic experiments, methanol–0.02 M phosphate buffer (pH 7.0) (58.5:41.5, v/v) was used as the mobile phase at a flow rate of 1 ml min^{-1}. For purifiation of TMZ-RP for UV–vis absorption and mass spectral analyses, methanol–H_2O (3:2, v/v) was used as the mobile phase, also at 1 ml min^{-1}. HPLC analysis was conducted at ambient temperature (23.5 ± 0.5°C). Samples were injected via a Shimadzu (Shimadzu, Kyoto, Japan) Model SIL-9A automatic sample injector equipped with a water-jacketed sample rack. Temperature of the sample rack was maintained by passing constant-temperature water from a thermostated water circulator. 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 temperature variation was ±0.1°C. The detector signal was recorded with MacIntegrator (a software and hardware pack-

age from Rainin Instruments, Emeryville, CA) on a Macintosh Classic II computer (Apple Computer, Cupertino, CA).

Kinetics of rearrangement

LC Method. In kinetic studies of the rearrangement reaction, each sample containing 130 μg of TMZ, was dissolved in 2 ml of a solvent mixture. The rearrangement reaction was initiated by adding 2 ml of a solvent mixture (pre-equilibrated to the temperature under study) to a test tube containing appropriate amount of dried residues of TMZ. The mixture was then vortexed for ~20 s to dissolve the TMZ. 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 autosampler's thermostated sample rack. Depending on the temperature under study and the rate of the rearrangement reaction, the first sample was injected after the sample vial has been placed in the sample well for 3–10 min to allow the temperature of the solution in the sample vial to reach equilibrium. A sample (10 μl) was subsequently injected for analysis at each sampling interval. Sampling intervals in reversed-phase HPLC analysis ranged from 5.4 to 15.4 min.

Rearrangement $t_{1/2}$ was determined by a curve-fitting computer software following plotting log (AUC of TMZ) vs time. Rearrangement $t_{1/2}$ ($= 0.693/k$) were averages of 2–3 determinations; 2 for $t_{1/2} > 90$ min and 3 for $t_{1/2} < 90$ min.

Spectrophotometric method. Absorbance changes at 316 nm resulting from the rearrangement of TMZ were followed as a function of time on a Model DW2000 spectrophotometer (SLM Instruments, Urbana, IL). The reaction was initiated by adding 2 ml of a solvent mixture into a test tube containing 130 μg of TMZ as dried residues. The mixture was vortexed for ~20 s to dissolve the TMZ. The resulting solution was transferred to a water-jacketed quartz cuvette, which was kept at ambient temperature. Thermostated water was then allowed to circulate through the channel of the metal cuvette holder and the water-jacket of the quartz cuvette. The temperature of the solution reached equilibrium in 1–2 min, depending on the temperature under study. Actual temperature of the solution in the cuvette was determined with a portable digital thermometer fitted with a detachable probe. The temperature variation was ±0.1°C. Within 2 min following placement of sample solution into the cuvette, change in absorbance at 316 nm was continuously recorded as a function of time. The computer software of the spectrophotometer allowed collection of 1000 data points in each kinetic run; the longer the total monitoring time, the longer each sampling interval.

Rearrangement $t_{1/2}$ was determined by a curve fitting computer software, using the equation $A_t = (a - b)\ e^{-kt} + b$, where A_t is the absorbance of TMZ at time t, a is the absorbance at time zero, k is the rate constant ($k = 0.693/t_{1/2}$), and b is the absorbance at the completion of the rearrangmeent reaction, respectively. It should be noted that, experimentally, a is not the absorbance at the start of the reaction, but rather at the start of data collection.

Spectral analysis

UV–vis absorption spectra of samples were determined using a 1 cm path length quartz cuvette on a Model DW2000 spectrophotometer (SLM Instruments, Urbana, IL). Mass spectral analysis was performed on a Finnigan 4500 gas chromatograph–mass spectrometer-data system (Finnigan MAT, San Jose, CA) with a solid probe by electron impact at 70 eV and the ion source was maintained at 120°C.

Results and Discussion

Acidity constant

Due to protonation at N4 position in acidic solutions [7], the UV–vis absorption spectrum of the acidic form of TMZ was different from that of the unprotonated neutral form. Similar to those of 3-*O*-methyl-OX and 3-*O*-ethyl-OX [5, 6], the absorption difference spectrum between protonated and unprotonated forms of TMZ revealed several wavelengths (226, 245 and 292 nm) suitable for monitoring absorbance changes as a function of acid concentration. The acid concentration dependent absorptions of TMZ in CH_3CN–H_2O (1:1, v/v) at 226 nm are shown in Fig. 2. The acidity constant K_{a1} (0.20) was determined by fitting the data with a curve-fitting computer software. K_{a1} values determined at 245 and 292 nm (not shown) were essentially the same as that determined at 226 nm. The result in Fig. 2 indicated that N4-protonated form of

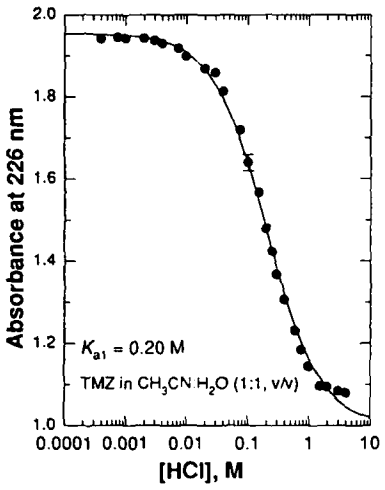


Figure 2
Acid concentration-dependent absorbance changes of TMZ in CH_3CN–H_2O (1:1, v/v) at 226 nm. Absorbance measurements were conducted at 23 ± 1°C. The K_{a1} value (0.20) was obtained by curve fitting-computer software.

TMZ was negligible in neutral and alkaline solutions.

Changes in absorption properties of TMZ in strongly alkaline solutions were due to the formation of a base-catalysed rearrangement product (see below). When TMZ was dissolved in CH_3CN–0.2 N NaOH (1:1, v/v), there was no immediate change in absorption properties. In comparison, both OX and lorazepam exhibited shifts in absorption characteristics when they were dissolved in alkaline solutions [7]. The rapid changes in absorption properties were consistent with the generally observed diffusion-controlled processes in acid–base equilibria. Both OX and lorazepam have a hydrogen at N1 position (Fig. 1). TMZ has a methyl group at N1 position. It appeared tht the N1 hydrogen allowed both OX and lorazepam to form a N1=C2 bond in strongly alkaline solutions. The formation of N1=C2 bond is not possible for TMZ due to the presence of a methyl group at N1 position. The K_{a2} values of OX ($10^{-11.6}$) and lorazepam ($10^{-11.5}$) [7] were likely due to the deprotonation of N1 hydrogen. Hence the changes of absorption properties of OX and lorazepam in alkaline solutions were not due to deprotonation of the C3-hydroxyl group, as suggested by Barrett *et al.* [7]. The results indicate that TMZ does not possess a proton which causes changes in absorption properties upon dissociation.

Rearrangement kinetics by HPLC

TMZ-RP and TMZ were separated by reversed-phase HPLC (Fig. 3). Under the chromatographic conditions, MACBP had a retention time of 32.2 min. Under all experimental conditions employed in this study, the formation of MACBP was below detectable level. Thus the sampling interval in a kinetic study can be as short as 5 min. An example of time-dependent changes in the AUCs of TMZ and TMZ-RP at 254 nm is shown in Fig. 3. This rearrangement reaction was conducted in CH_3CH–0.2 N NaOH in H_2O (1:1, v/v) at 50°C and had a $t_{1/2}$ of 16.2 min (16.2 ± 0.4 min, n = 3). This condition produced minor amounts of unknown products, which did not

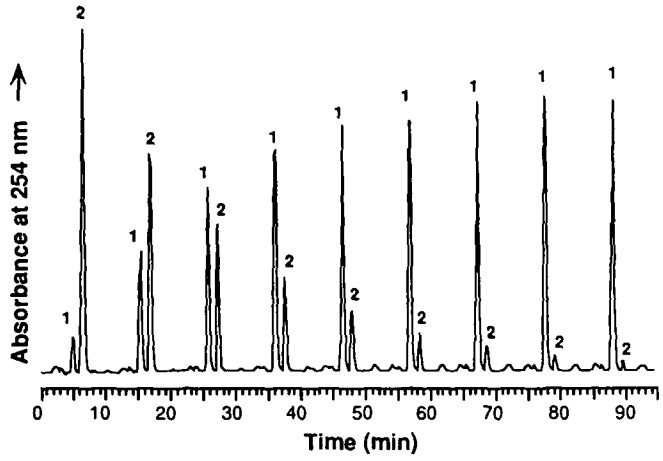


Figure 3
An example of the time course in the continuous monitoring of TMZ rearrangement at 50°C by reversed-phase HPLC. The solvent was CH_3CN–0.2 N NaOH (1:1, v/v). Samples (10 µl each injection) were analysed every 10.42 min. The rearrangement $t_{1/2}$ of this reaction was found to be 16.2 min (16.2 ± 0.4 min, n = 3). Under the chromatographic conditions, MACBP (see structure in Fig. 1) was eluted at 32.2 min.

significantly interfere with the kinetic analysis. At lower concentrations of NaOH and temperature, these minor unknown products were below detectable levels. The exponential increase in the formation of TMZ-RP and the exponential decrease of TMZ can be clearly seen in Fig. 3. Rearrangement $t_{1/2}$ was determined by plotting log (AUC of TMZ) vs time and the resulting data were fitted with curve-fitting computer software. The error involved in repetitively injecting a constant volume (10 μl) of samples was ≤2%. Hence the rearrangement $t_{1/2}$ determined by the reversed-phase HPLC were consistent among repeated experiments.

Product analysis and isotope effect

The UV–vis absorption spectrum of TMZ-RP in CH_3CN was characteristically different from that of TMZ (Fig. 4A). In addition to the loss of an absorption band with λ_{max} at 316 nm [4], a much larger reduction in an absorption band with λ_{max} at 231 nm was observed and this became more apparent in the difference spectrum between the absorption spectra of TMZ and TMZ-RP (Fig. 4B). The changes in absorption properties were apparently due to the saturation of the C5=N4 bond [4] and the addition of a C3=O bond.

Electron impact mass spectra of TMZ-RP derived from rearrangement of TMZ in solvent mixtures CH_3CN–NaOH–H_2O, CH_3CN–NaOD–D_2O, and CH_3CN–NaOH–$H_2{}^{18}O$, respectively, are shown in Fig. 5. The unlabelled TMZ-RP exhibited an intense M^+ at *m/z* 300 and an associated chlorine isotope ion at *m/z* 302. Although TMZ-RP and TMZ have an identical molecular weight, the mass spectrum of TMZ-RP (Fig. 5A) is uniquely different from that of TMZ [2]. Some of the fragment ions that can be readily recognized are: *m/z* 271 (loss of CHO), *m/z* 257 (loss of NHCO), *m/z* 243 (loss of CO from fragment ion at *m/z* 271), *m/z* 228 (loss of CH_3 from fragment ion at *m/z* 234), *m/z* 222 (loss of Cl from fragment ion at *m/z* 257), and *m/z* 193 (loss of Cl from fragment ion at *m/z* 228).

Mass spectral analysis of TMZ-RP derived from the rearrangement reaction of TMZ in CH_3CN–NaOD–D_2O (Fig. 5B) indicated the presence of molecular and fragment ions, all had an additional mass unit than those derived from unlabelled TMZ-RP. A distinctive fragment ion was observed at *m/z* 273 (Fig. 5B). This fragment ion was more intense (relative to that at *m/z* 271) than that derived from unlabelled TMZ-RP (Fig. 5A) and was probably derived from the loss of carbonyl group at C2 position (see Fig. 6). Among repeated experiments, the extent of deuterium incorporation into TMZ-RP varied from 50 to 90%. Unreacted TMZ, recovered from the rearrangement reaction in CH_3CN–NaOD–D_2O, did not contain deuterium by mass spectral analysis.

Mass spectral analysis of TMZ-RP derived

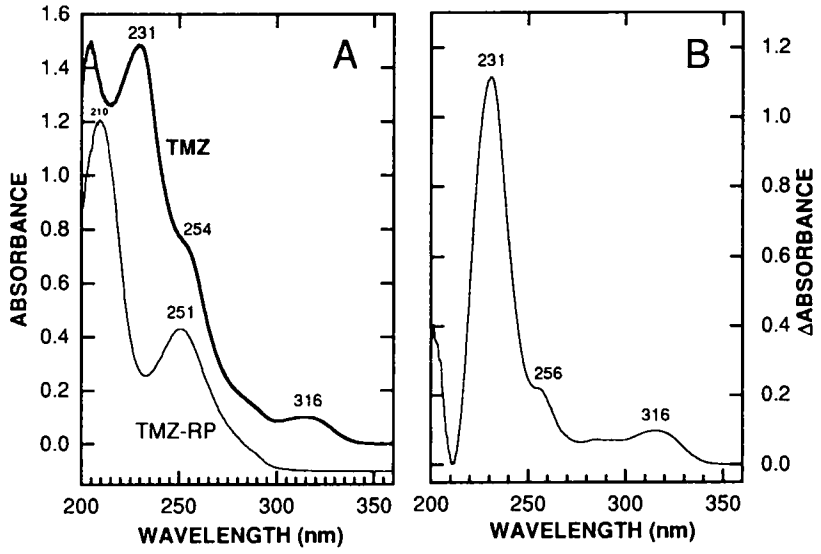


Figure 4
(A) UV–vis absorption spectra of TMZ [43 μM; characteristic λ_{max} at 231, 254 (sh), and 316 nm] and (B) TMZ-RP (characteristic λ_{max} at 210 and 251 nm) in CH_3CN. The absorbance values of TMZ-RP are offset by 0.1 absorbance unit to avoid overlapping of curves.

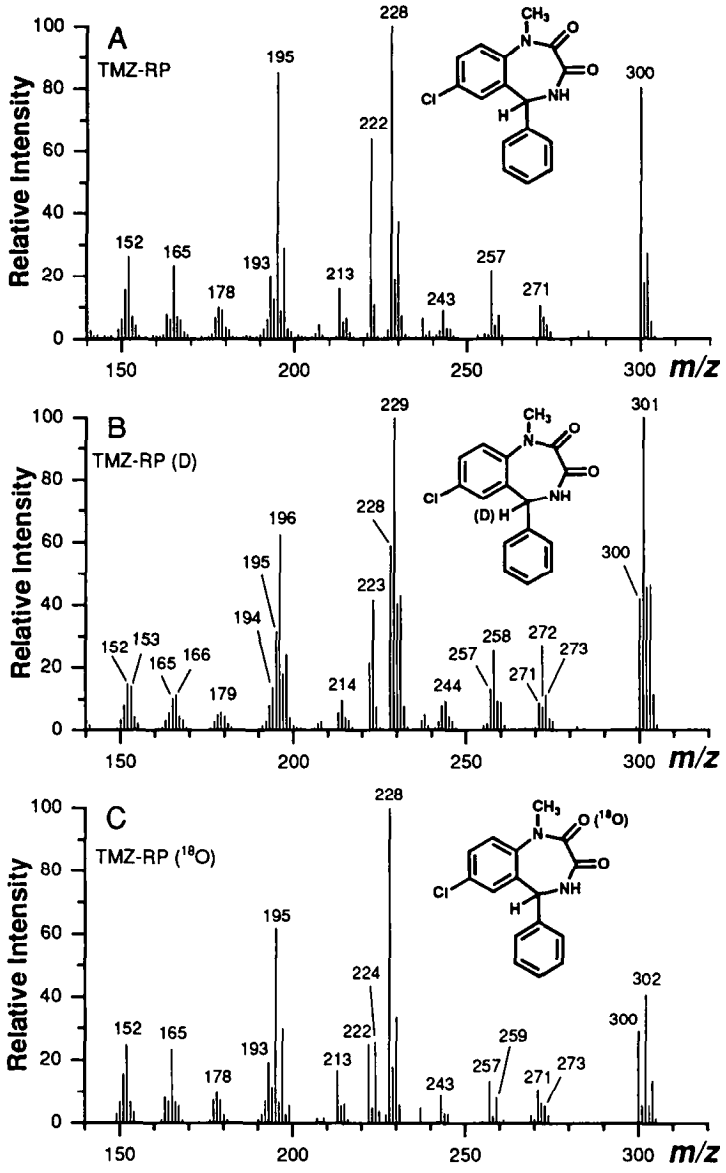


Figure 5
Electron impact mass spectra of unlabelled TMZ-RP (A), deuterium-labelled TMZ-RP (B), and ^{18}O-labelled TMZ-RP (C). See Materials and Methods for the preparation and purification of TMZ-RPs.

from the rearrangement reaction of TMZ in CH_3CN–NaOH–$H_2{}^{18}O$ (Fig. 5C) indicated that, in addition to molecular and fragment ions observed in the mass spectrum of unlabelled TMZ-RP, molecular and fragment ions with two additional mass units were observed at *m/z* 302, 273 (low intensity), 259, and 224. Relative intensities of M^+ at *m/z* 300 and 302 indicated that unlabelled and ^{18}O-labelled TMZ-RPs were present in approximately equal quantity. Since an amide linkage is involved, the ^{18}O-incorporation observed in base-catalysed rearrangement of TMZ is analogous to that observed in base-catalysed hydrolysis of benzamide [8].

Fragmentation pathways leading to the distinctive molecular and fragment ions described above, as well as some of the other major fragment ions are proposed in Fig. 6. Fragment ions derived from both deuterium- and ^{18}O-labelled TMZ-RPs have been taken into consideration and are depicted in the proposed fragmentation pathways. Consistent with an earlier report [4], we also found that TMZ-RP can be methylated to form a 4-*N*-methyl-TMZ-RP (M^+ at *m/z* 314) in an alkaline acetonitrile solution containing $(CH_3)_2SO_4$.

Taken together, the results established TMZ-RP as 7-chloro-1-methyl-5-phenyl-4,5-dihydro-2*H*-benzodiazepin-2,3(1*H*)-dione, in

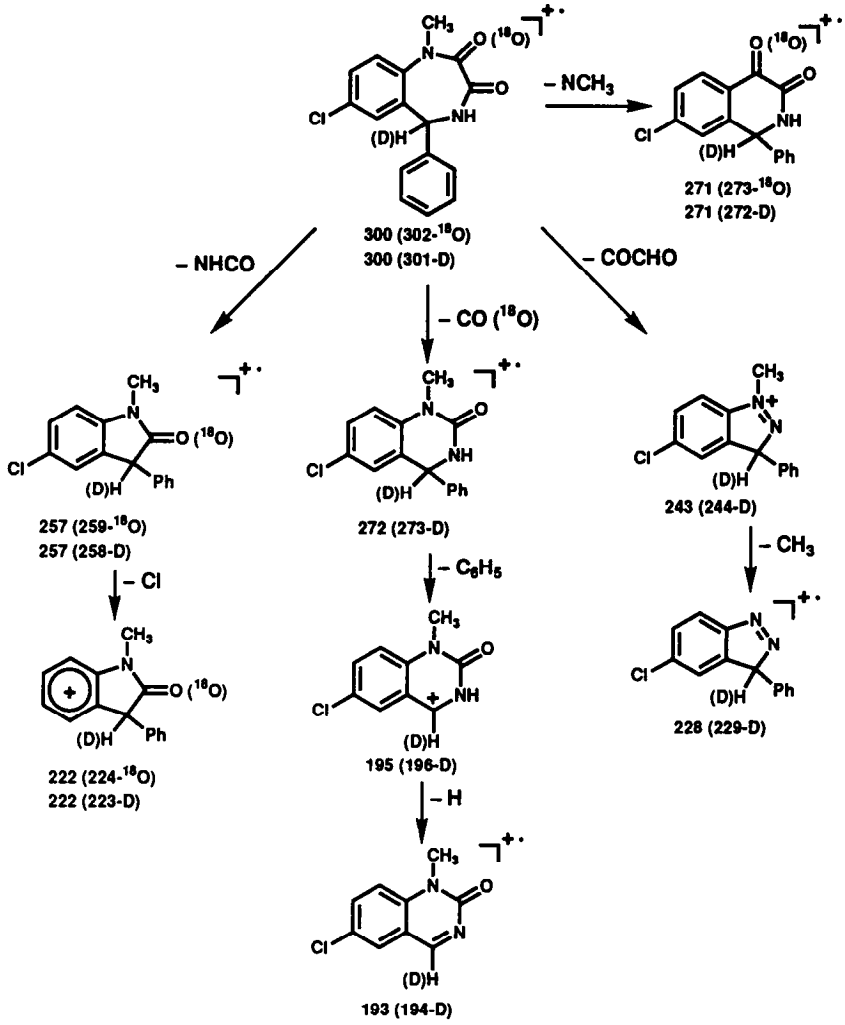


Figure 6
Some fragmentation pathways proposed to account for some of the major mass fragments observed in the electron impact mass spectra of TMZ-RPs shown in Fig. 4. An atom (either D or ^{18}O) in parenthesis indicates an alternative atom in either the molecular or the fragment ion.

agreement with the conclusion reached earlier [4].

Kinetic analysis by spectrophotometry

The difference spectrum (Fig. 4B) between the absorption spectra of TMZ and TMZ-RP indicated that the base-catalysed rearrangement reaction of TMZ could also be studied by monitoring absorbance changes at a wavelength at or near the absorption bands centering at 231, 256 and 316 nm, respectively. The result of an experiment, carried out by monitoring absorbance changes at 316 nm with TMZ (217 μM) in CH_3CN–0.2 N NaOH (1:1, v/v) at 50°C, is shown in Fig. 7. This particular reaction had a rearrangement $t_{1/2}$ of 16.0 min (16.1 ± 0.3 min, n = 3).

The concentration of TMZ is zero at the completion of a base-catalysed rearrangement reaction. Because the rearrangement reaction is relatively slow, it is less time-consuming and experimentally simpler to determine the rearrangement $t_{1/2}$ by the HPLC method (Fig. 3) than by the spectrophotometric method (Fig. 7). Under the same experimental condition, there was no significant difference in the rearrangement $t_{1/2}$ determined by either the HPLC method (Fig. 3) or the spectrophotometric method (Fig. 7). In general, the HPLC method requires less than half the time to determine a rearrangement $t_{1/2}$ than the spectrophotometric method. The detailed kinetic studies described below were all carried out by the HPLC method.

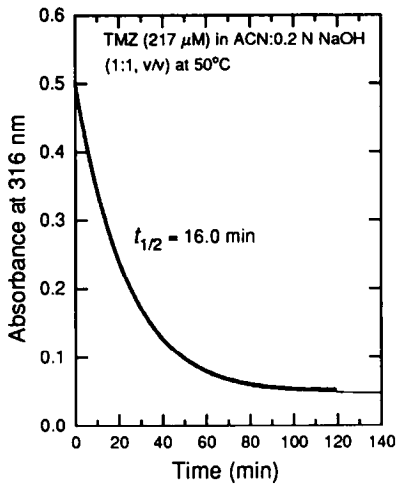


Figure 7
An example of the time course in monitoring the disappearance of TMZ (thick line curve consisting of 1000 absorbance values) in CH_3CN–0.2 N NaOH (1:1, v/v) at 50°C. The rearrangement $t_{1/2}$ of this reaction was found to be 16.0 min (16.1 ± 0.3 min, n = 3). The thin line curve, which essentially overlaps with the data points, was obtained by a computer curve-fitting program.

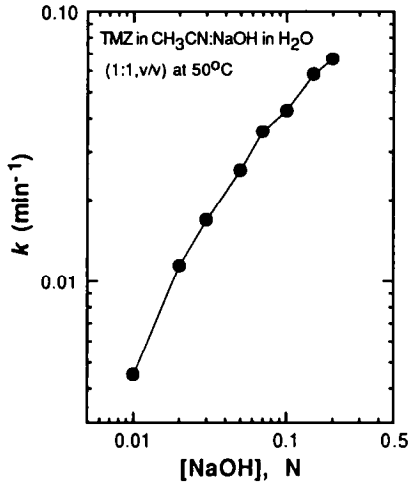


Figure 8
Dependence of rearrangement rate of TMZ (217 μM) on the net [NaOH] in CH_3CN–H_2O (1:1, v/v) at 50°C.

Factors influencing rearrangement rates

The rate of TMZ rearrangement in alkaline solutions depended on the concentrations of both TMZ and NaOH. By keeping TMZ constant at 217 μM in CH_3CN–H_2O (1:1, v/v) containing a large molar excess of NaOH (ranging from 0.01 to 0.2 N), the rearrangement reactions at 50°C all followed apparent first-order kinetics. In a solvent mixture containing CH_3CN–H_2O (1:1, v/v), the highest concentration of NaOH that could be used was 0.2 N; above which the solution became immiscible. The rate constant increased nonlinearly as the [NaOH] of the solvent increased (see log$k$ vs [NaOH] plot in Fig. 8). A break in the rate of increase of rearrangement rate occurred at [NaOH] ≈ 0.03 N.

The mechanism of the rearrangement reaction was further probed by studying the dependence of rearrangement rate on the ionic strength. In CH_3CN–0.2 N NaOH (1:1, v/v) solutions containing 0, 0.25 and 0.5 M of NaCl, the $t_{1/2}$ of TMZ rearrangement at 50°C were 25.2 ± 1.2, 28.2 ± 0.7 and 26.8 ± 1.3 (n = 3), respectively. Thus the reaction rate was essentially independent of the ionic strength.

The thermodynamic parameters, determined by an Arrhenius plot of data obtained from temperature dependence of rate constant k in the rearrangement reaction of TMZ in CH_3CN–0.2 N NaOH (1:1, v/v) (see log$k$ vs 1000/T plot in Fig. 9), were found to be: E_{act} = 22.5 kcal mol^{-1} and at 25°C: $\Delta H‡$ = 21.9 kcal mol^{-1}, $\Delta S‡$ = −5.2 cal K^{-1} mol^{-1} and $\Delta G‡$ = 23.5 kcal mol^{-1}, respectively. The enthalpy of activation was relatively small, indicating a relatively small energy required to form the transition state. The relatively small negative entropy indicates a slight gain of orderliness in the transition state. The negative $\Delta S‡$ also suggested that the transition state was solvated.

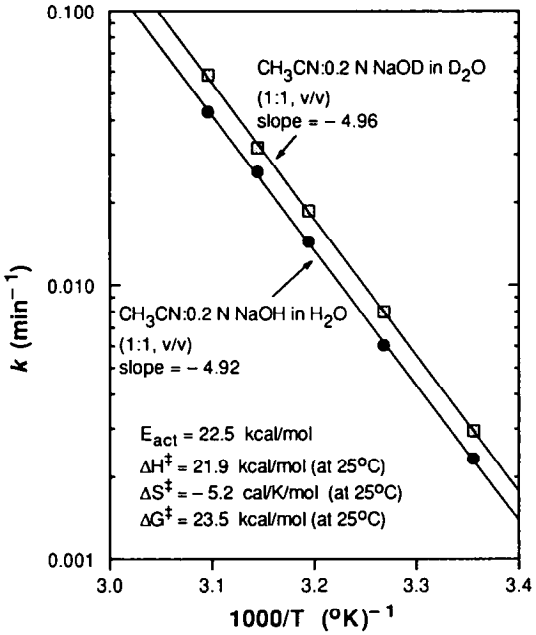


Figure 9
Arrhenius plot for temperature dependence of rearrangement rate constant k of TMZ in alkaline solution. The solvents were either CH_3CN–0.2 N NaOH in H_2O (1:1, v/v) or CH_3CN–0.2 N NaOH in D_2O (1:1, v/v).

The temperature-dependent rearrangement reaction was further studied using CH_3CN–0.2 N NaOD in D_2O (1:1, v/v) as the solvent (Fig. 9). The reaction rates became faster when NaOH in H_2O was replaced by NaOD in D_2O. However, the slopes in the log$k$ vs $1000/T$ plots were essentially the same (Fig. 9). The isotope effect (k_H/k_D) was 0.77 ± 0.03 over the temperature range studied. The thermodynamic parameters were: E_{act} = 22.7 kcal mol^{-1} and at 25°C: $\Delta H\ddagger$ = 22.1 kcal mol^{-1}, $\Delta S\ddagger$ = −4.1 cal K^{-1} mol^{-1} and $\Delta G\ddagger$ = 23.3 kcal mol^{-1}, respectively; these were essentially the same as those found using nondeuterated solvent. These results indicated that the rate-determining step in the rearrangement reaction did not involve a proton exchange with solvent [9]. Whenever proton transfer is involved in the rate-determining step, the rate constant is expected to decrease when H_2O is replaced by D_2O [9].

Reaction mechanism

Based on the results described above, three possible mechanisms in the rearrangement of TMZ in strongly alkaline media are described in Fig. 10. One mechanism (scheme A) is via an initial deprotonation at C3—OH, followed by a nucleophilic attack at C2 carbonyl carbon by a hydroxide ion. The second mechanism (scheme B) involves an irreversible enolization of C2 keto group, followed by a hydroxide addition at the C2 of an enolate ion. The third mechanism (scheme C) is via an initial nucleophilic attack at C2 carbonyl carbon by a hydroxide ion. These alternative mechanisms are each discussed below.

The initial deprotonation of C3—OH (scheme A) is essential in one of the three alternate mechanisms. The C3-hydroxyl groups in OX and lorazepam are suggested to be deprotonated in alkaline media with pK_{a2} of 11.6 and 11.5, respectively [7]. However, the mechanism in the deprotonation of OX and lorazepam in strongly alkaline solutions indicated by Barrett *et al.* [7] might not be correct (see discussion in Acidity Constant section above). Deprotonation of the C3—hydroxyl group of TMZ could not be demonstrated by the spectrophotometric method due to the lack of [NaOH]-dependent absorption changes. The pK_a values of simple alcohols are on the order of 14–16 [10]. We have found that TMZ can be *O*-methylated in CH_3CN–0.2 N NaOH–$(CH_3)_2SO_4$ (40:40:1, v/v/v), indicating that the proton in C3-OH of TMZ is ionizable.

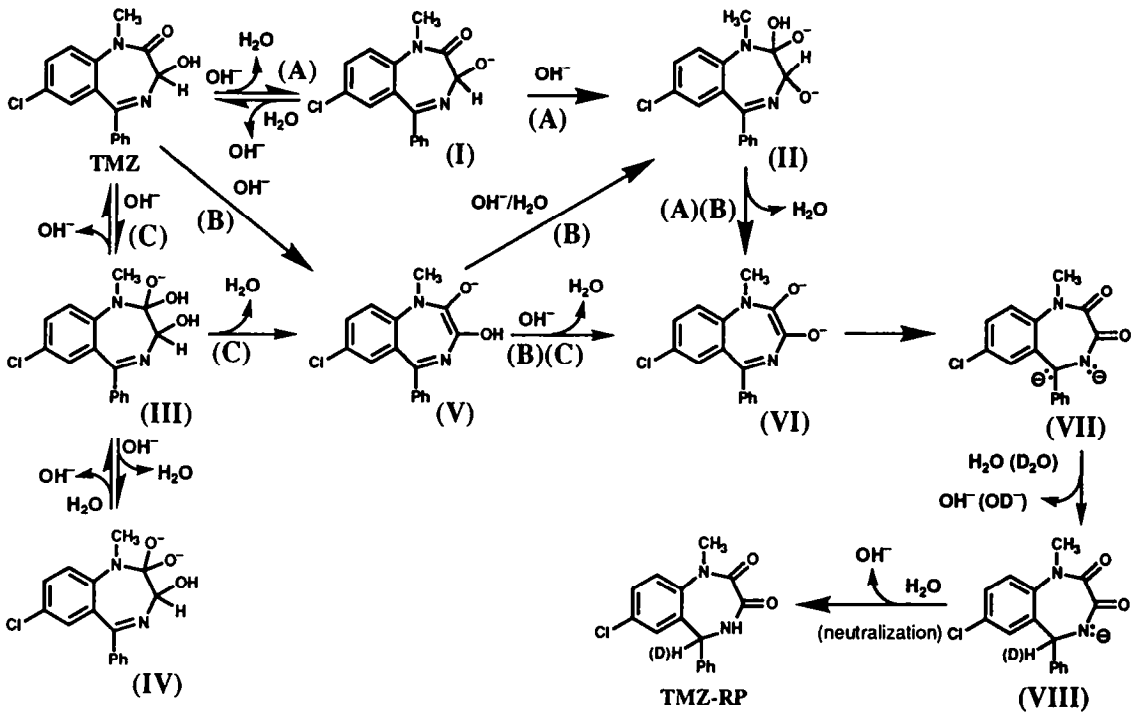


Figure 10
Alternate mechanisms proposed for the formation of TMZ-RP in the rearrangement of TMZ in a strongly alkaline medium. Incorporation of ^{18}O occurs in the conversion of either **I** → **II** → **VI**, **V** → **II** → **VI**, or **TMZ** → **III** (in equilibrium with **IV**) → **V**. Deuterium incorporation occurs in the conversion of **VII** to **VIII**. See text for discussion.

Intermediate **II**, formed by nucleophilic attack at C2 by OH^-, is consistent with the observed ^{18}O-incorporation in the final rearrangement product. Loss of a water molecule leads to the formation of **VI**. Addition of proton by solvent water to **VII** yield **VIII**. In this reaction scheme, the rate-determining step probably occurs in the **I** → **II** → **VI** pathway.

The second mechanism (scheme B) involves an initial enolization of ketone group at C2 to form **V**. Due to the presence of a high concentration of NaOH, the enolization is expected to be essentially irreversible [11]. Reversible TMZ ↔ **V** would have caused a proton transfer from solvent water at C3. Intermediate **V** may lead to the formation of either **VI** (by deprotonation of C3 enol group) directly or via the formation of **II**. The latter pathway (**V** → **II**) is energetically unfavourable. The pathways described above are inconsistent with the experimental findings. Because (1) the TMZ → **V** → **VI** pathway would not have lead to ^{18}O-incorporation in the final rearrangement product and (2) the formation of **II** from **V** would have caused a proton transfer to C3 from the solvent and this is inconsistent with the increased rearrangement rate in deuterated solvent (see data in Fig. 9). Thus the alternate mechanism described in scheme B can be ruled out.

The third mechanism (scheme C) involves an initial nucleophilic attack at C2 carbonyl carbon to form **III**, which is in equilibrium with **IV** with two equivalent oxygens at C2. Loss of a water molecule from **III** yields **V** (an enolate). The proton of C3=OH in **V** (an enol) is readily ionized to form the dianion **VI**. The pK_a values of enols are 9–11 [11, 12]. The TMZ → **III** (↔ **IV**) → **V** → **VI** pathway is consistent with the observed ^{18}O-incorporation in the final rearrangement product. Subsequent rearrangement of **VI** and protonation of **VII** by solvent water are consistent with the deuterium-incorporation in TMZ-RP. The anion **VIII** is the deprotonated form of TMZ-RP in strongly alkaline media. Neutralization of reaction medium results in the neutral molecule TMZ-RP. In this reaction scheme, the rate-determining step probably occurs in the TMZ → **III** → **V** → **VI** pathway.

The mechanisms discussed above were based on the following observations and considerations. (1) Two of the three alternate mechanisms (schemes A and C) were consistent with the incorporation of ^{18}O and deuterium in the rearrangement product. (2) Stable intermediate(s) with UV–vis absorption properties different from those of TMZ and TMZ-RP in alkaline media were not detected. Thus **V** and **VI**, whose UV–vis absorption properties are expected to be different from those of TMZ and TMZ-RP, did not accumulate as intermediates in the rearrangement reaction. (3) TMZ, isolated from an incomplete rearrangement reaction in a D_2O–NaOD–CH_3CN mixture, was devoid of deuterium. Thus there was no proton transfer prior to the rate-determining step. (4) The reaction pathways **I** → **II** → **VI** and TMZ → **III** → **V** were irreversible because reversible reactions would have produced a TMZ-RP containing a higher atom% of ^{18}O than was actually observed. Furthermore, reversible reactions would have produced a kinetic isotope effect (i.e. $k_D > k_H$), contrary to what was observed experimentally. (5) A solvated activated complex with a structure similar to either **V** or **VI** was consistent with the observed energy of activation (22.5 kcal mol^{-1}) involved in the rearrangement reaction. An enol (or enolate ion) is in a higher energy state than a ketone [11]. (6) The reduction in crowdedness from **II** to **VI** (or **III** to **V**) was consistent with the small negative entropy of activation (−5.2 cal K^{-1} mol^{-1}) observed. (7) The possible activated complex and the resulting product carried the same charge. This was consistent with the observed rearrangement rates which were not sensitive to the changes in the ionic strength of the solvent. (8) The results (Fig. 9) were consistent with a general observation that deuteroxide ions in D_2O undergo nucleophilic attacks on carbon about 20–50% faster than do hydroxide ions in H_2O [9].

The percentages of neutral and deprotonated forms of TMZ in the alkaline solutions employed in this study are not known. Thus one or both mechanisms depicted in schemes A and C (Fig. 10) may be operative in the base-catalysed rearrangement of TMZ. Base-catalysed rearrangement of OX observed earlier [4] probably follows the same mechanism(s) as described for the base-catalysed rearrangement of TMZ.

Conclusions

Temazepam undergoes a rearrangement reaction in strongly alkaline media to form a

cyclic diamide, 7-chloro-1-methy-5-phenyl-4,5-dihydro-2*H*-benzodiazepin-2,3(1*H*)-dione. The rearrangement reaction involves a nucleophilic addition of a hydroxide ion at the electrophilic C2 carbonyl carbon of temazepam. The rate-determining step does not involve exchange of proton with solvent.

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