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Nucleophilic Substitution and Racemization of 3-Ethoxy-N-desmethyldiazepam Enantiomers in Acidic Ethanol

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ABSTRACT

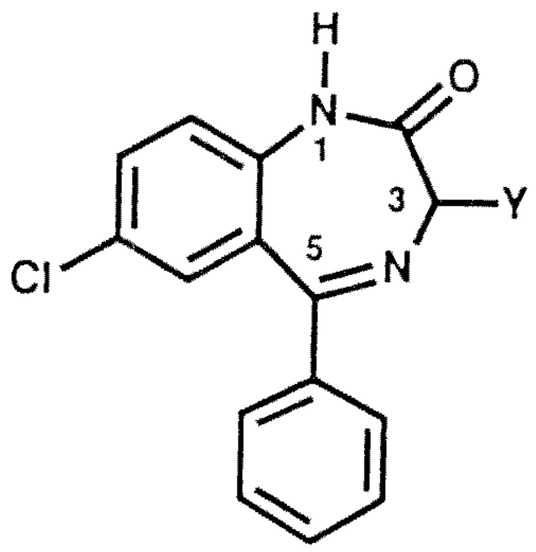
pK_{al} values of 3-ethoxy-N-desmethyldiazepam (3-EtO-NDZ) in ethanol and acetonitrile containing various concentrations of sulfuric acid, determined by spectrophotometry and spectropolarimetry, were found to be 3.4 and 0.63 respectively. Temperature dependent racemization of enantiomeric 3-EtO-NDZ in ethanol containing various acid concentrations was studied by monitoring changes of ellipticity at 365 nm as a function of time on a spectropolarimeter. The racemization reactions were found to follow apparent first order kinetics. Thermodynamic parameters of the racemization reaction were: E_{act} = 16.7 kcal/mol, and at 25℃: $\Delta H^{\ddagger}$ = 16.1 kcal/mol, $\Delta S^{\ddagger}$ = -22.0 cal/K/mol, and $\Delta G^{\ddagger}$ = 22.6 kcal/mol, respectively. The racemization had an isotope effect (k_H/k_D) of 1.9 at 42℃. Based on the results of this report and the results of literature reports on the preferred conformation of enantiomeric 3-substituted-1,4-benzodiazepines, a nucleophilically solvated C3 carbonium ion intermediate resulting from either a P (plus) or a M (minus) conformation is proposed to be an intermediate. This intermediate is responsible for the stereoselective nucleophilic substitution and the subsequent racemization of 3-EtO-NDZ enantiomers in acidic ethanol.

Key words: 3-ethoxy-N-desmethyldiazepam, stereoselective nucleophilic substitution, racemization.

INTRODUCTION

1,4-Benzodiazepines (BDZ's) are the most commonly prescribed psychoactive drugs. 3-Ethoxy-N-desmethyldiazepam (3-EtO-NDZ) is a pharmacologically less active derivative of oxazepam (OX).[1] OX is among the therapeutically used BDZ's that has a hydroxyl group at the C3 position(see structrue and numbering system in Fig. 1). OX is an active metabolite of diazepam(DZ), which is one of the most frequently prescribed drugs[2] for the treatment of anxiety and insomnia, and an adjuvant for anesthesia.[3] Ingestion of OX shortly before, shortly after, or simultaneously with the intake of ethanol may result in the formation of 3-EtO-NDZ in the strongly acidic medium of the stomach. Hence it is of interest to ascertain the properties of 3-EtO-NDZ in acidic medium containing ethanol.

Early studies[4-7] indicated that optically active 3-camphoyloxy-NDZ, 3-hemisuccinyloxy-NDZ and its methyl esters, 3-MeO-NDZ, and 3-O-ethyl-lorazepam undergo racemization in strongly acidic media containing either aqueous or anhydrous methanol or ethanol. A (+)-3-O-ethyl-lorazepam (now known to be the R-enantiomer)[7,8] was reported to undergo racemization at 25℃ with $t_{1/2}$ of 176 min in 10% ethanolic HCl (i.e., ethanol containing 1.2 N HCl) and 71 min in 10% ethanolic HCl/water (4/1, v/v), respectively.[4]



OX, Y = OH

NDZ, Y = H

3-MeO-NDZ, Y = OCH_3

3-EtO-NDZ, Y = OC_2H_5

Figure 1. Structures of N-desmethyldiazepam(NDZ), oxazepam (OX or 3-OH-NDZ), 3-methoxy-N-desmethyldiazepam (3-MeO-NDZ), and 3-ethoxy-N-desmethyldiazepam(3-EtO-NDZ).

These results were discussed in relation to the methanolysis of enantiomeric 3-camphoyloxy-NDZ and 3-hemisuccinyloxy-NDZ and its methyl esters. A rather complex mechanism involving two inversion steps was proposed to be involved in the acid-catalyzed alcoholysis and racemization of (+)-3-hemisuccinyloxy-NDZ, (+)-3-MeO-NDZ, and (+)-3-O-ethyl-lorazepam.[4-6] Recently a considerably more simpler mechanism was proposed in the racemization of enantiomeric 3-MeO-NDZ in acidic methanol.[9]

We describe in this report the results of a kinetic study on the acid-catalyzed racemization of enantiomeric 3-EtO-NDZ in ethanol. Thermodynamic parameters obtained from temperature dependent racemization kinetics also suggested that a nucleophilically solvated C3 carbonium ion of 3-EtO-NDZ in strongly acidic ethanol is an intermediate responsible for the observed racemization. The proposed mechanism appears to be generally applicable to the stereoselective nucleophilic substitutions of enantiomeric 3-alkoxy-BDZ's and their subsequent racemization in strongly acidic alcoholic solvent.

MATERIALS AND METHODS

Materials

Demoxepam (7-chloro-1, 3-dihydro-5-phenyl-2H-1, 4-benzodiazepin-2-one 4-oxide; Ro 5-2092) was generously provided by Dr. Peter F. Sorter of Hoffmann-La Roche, Inc. (Nutley, NJ). 3-O-Acyloxazepam was prepared from demoxepam according to Bell and Childress.[10] 3-EtO-NDZ was prepared by acid-catalyzed ethanolysis of 3-O-acyloxazepam. Deuterated ethanol (C_2H_5OD, 99.5 atom% D) and sulfuric acid (D_2SO_4, 99.5 + atom% D) were purchased from Aldrich Chemical Co. (Milwaukee, WI).

Determination of pK_{al}

Absorbance values at 224, 244, and 292 nm were recorded for solutions of 3-EtO-NDZ (50 μM) in either ethanol or acetonitrile containing various concentrations of H_2SO_4 at room temperature (23 ± 1℃). pK_{al} values were determined by plotting absorbance versus pH and curves were fitted with a curve fitting computer software.

The pK_{al} of (3R)-EtO-NDZ was also determined by a spectropolarimetric method. Ellipticity values at 365 nm of ethanol solutions of (3R)-EtO-NDZ containing 0.01 mM to 0.1 M H^+ were determined. pK_{al} was determined by plotting ellipticities (in millidegrees) at 365 nm versus -log [H^+] using a curve fitting computer program.

Chiral Stationary Phase HPLC

HPLC was performed using a Waters Associates (Milford, MA) liquid chromatograph consisting of a Model 6000A solvent delivery system, a Model M45 solvent delivery system, a Model 660 solvent programmer and a Kratos (Kratos Analytical Instruments, Ramsey, NJ) Model Spectraflow 757 uv-vis variable wavelength detector. Samples were injected via a Valco

Model N60 injector (Valco Instruments, Houston, TX).

Enantiomeric pairs of 3-EtO-NDZ were separated by CSP-HPLC using a Pirkle column (either 4.6 mm i.d. x 25 cm or 10 mm i.d. x 25 cm) packed with spherical particles of 5 μm diameter γ-aminopropyl-silanized silica to which R-N-(3, 5-dinitrobenzoyl) phenylglycine was bonded covalently.[8] This was a Hi-Chrom Pirkle covalent phenylglycine HPLC column marketed by Regis Chemical Co. (Morton Grove, IL). Either dioxane/ethanol/acetonitrile/hexane (20/2/1/77, vol ratio; abbreviated as D20EA3) or dioxane/ethanol/acetonitrile/hexane (20/3.33/1.67/75, vol ratio; abbreviated as D20EA5) was used as the mobile phase. Flow rate of mobile phase was 2 ml/min for the 4.6 mm i.d column [t_o = 1.9 min, t_1 = 14.5 min, t_2 = 16.9 min, k_1' (R-enantiomer) = 6.63, k_2' (S-enantiomer) = 7.85, α = 1.18, Rs = 2.12 using D20EA3 as the mobile phase, and t_o = 1.9 min, t_1 = 6.7 min, t_2 = 7.7 min, k_1' (R-enantiomer) = 2.51, k_2' (S-enantiomer) = 3.04, α = 1.21, Rs = 1.62 using D20EA5 as the mobile phase] and 2.8 ml/min for the 10 mm i.d. column [t_o = 6.4 min, t_1 = 24.3 min, t_2 = 27.8 min, k_1' (R-enantiomer) = 2.82, k_2' (S-enantiomer) = 3.37, α = 1.20, Rs = 2.58 using D20EA5 as the mobile phase].

Kinetics of Racemization and Isotope Effect

Absorbance of samples was determined using a 1 cm path length quartz cuvette on a Model DW2000 spectrophotometer (SLM Instruments, Urbana, IL). An enantiomeric 3-EtO-NDZ(57 μmol of dried residue) in a test tube was added with 1 ml of cold (−4℃) ethanol containing acid and followed by mixing for ~10 sec. Isotope effect was studied by using C_2H_5OD and D_2SO_4 instead of C_2H_5OH and H_2SO_4. The resulting solution was transferred into a thermostated micro quartz cuvette with 1 cm path length. Temperature was maintained by passing thermostated water from a circulating water bath. Following the transfer of solution into the thermostated cuvette, 2 min was allowed for the temperature to reach equilibrium. Changes of ellipticity ($\triangle\Phi$, in millidegrees) were subsequently recorded at 365 nm as a function of time on a JASCO Model 500A

Table 1. Temperature and Acid Concentration Dependent Racemization of Enantiomeric 3-EtO-NDZ in Ethanol.

Enantiomer	[H$^+$], N[a]	T(℃)	$t_{1/2}$[b] ± SEM[c](min)	kx 10^3(sec^{-1})
(3S)-EtO-NDZ	1.0	25.0 ± 0.1	72.5 ± 2.1	0.159
(3R)-EtO-NDZ	1.0	25.0 ± 0.1	73.1 ± 0.6	0.158
(3R)-EtO-NDZ	1.0	30.0 ± 0.1	49.4 ± 0.5	0.234
(3R)-EtO-NDZ	0.25	37.0 ± 0.1	26.2 ± 1.2	0.441
(3R)-EtO-NDZ	0.5	37.0 ± 0.1	23.3 ± 1.2	0.496
(3R)-EtO-NDZ	1.0	37.0 ± 0.1	26.3 ± 0.3	0.439
(3R)-EtO-NDZ	1.5	37.0 ± 0.1	25.7 ± 1.3	0.449
(3R)-EtO-NDZ	2.0	37.0 ± 0.1	40.1 ± 1.5	0.288
(3R)-EtO-NDZ	3.0	37.0 ± 0.1	57.4 ± 2.6	0.201
(3R)-EtO-NDZ	4.0	37.0 ± 0.1	86.9 ± 3.3	0.133
(3R)-EtO-NDZ	1.0	42.0 ± 0.1	16.3 ± 0.2	0.709
(3R)-EtO-NDZ[d]	1.0	42.0 ± 0.1	31.3 ± 0.5	0.369
(3R)-EtO-NDZ	1.0	50.0 ± 0.1	8.0 ± 0.1	1.444

[a] The solvent was ethanol containing a particular concentration of H_2SO_4. The acid concentrations are expressed by [H$^+$] in N

[b] Average of 3 determinations

[c] SEM = standard error of the mean

[d] Solvent = 0.5 M D_2SO_4 in C_2H_5OD

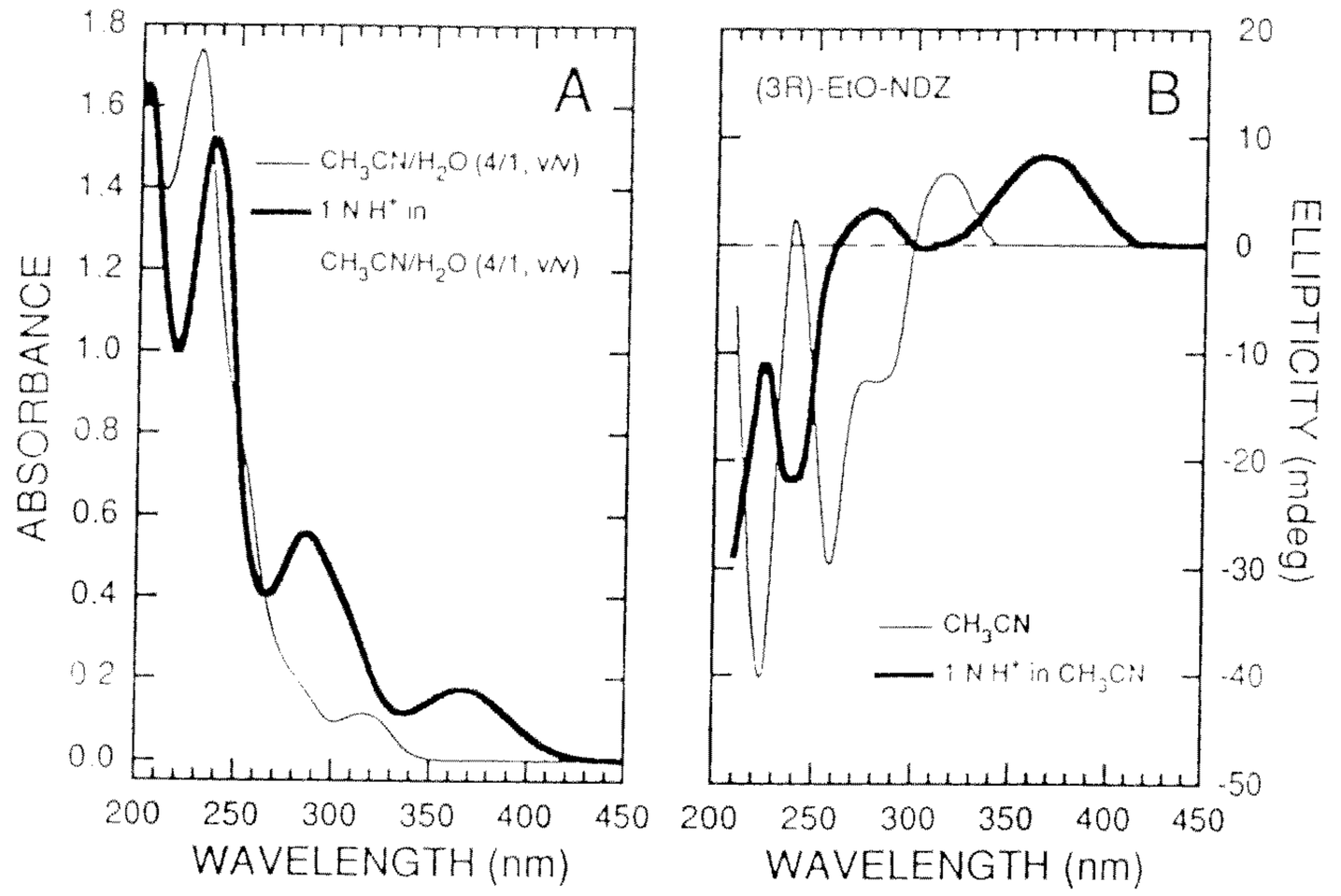


Figure 2. (A). UV-visible absorption spectra of 3-EtO-NDZ (50 μM) in CH_3CN/H_2O (4/1, v/v; thin line curve)and in CH_3CN/H_2O (4/1, v/v) containing 0.5 M H_2SO_4(thick line curve)
(B). CD spectra of enantiomerically pure (3R)-EtO-NDZ in CH_3CN (thin line curve)and in CH_3CN containing 0.5 M H_2SO_4 at 25℃(thick line curve). The ellipticity is in millidegrees (mdeg) for a solution containing 1.0 A_{230} unit of 3-EtO-NDZ per ml of solvent.

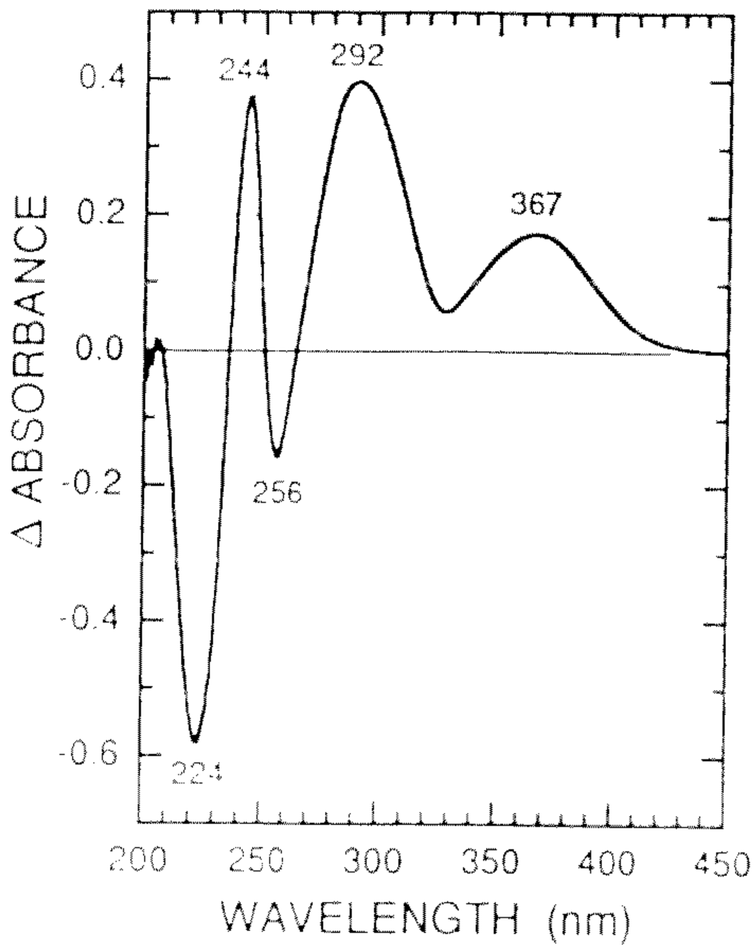


Figure 3. Difference spectrum between the acidic (protonated) forms of 3-EtO-NDZ in CH_3CN/H_2O (4/1, v/v) containing 0.5 M H_2SO_4 and the neutral (unprotonated)forms of 3-EtO-NDZ in CH_3CN/H_2O (4/1, v/v). The spectra of 3-EtO-NDZ (50 μM) were measured at room temperature.

spectropolarimeter (Japan Scientific Co., Easton, MD). The $t_{1/2}$ was determined by plotting log($\triangle\Phi$) versus time. Racemization of 3-EtO-NDZ enantiomers in acidic ethanol followed apparent first order kinetics. 3-EtO-NDZ enantiomers did not undergo detectable racemization in acetonitrile containing 0.5 M H_2SO_4. The slope of the Arrhenius plot ($\log t_{1/2}$ vs. 1/T) was determined by a curve fitting computer software. Absolute values of slopes were identical by plotting either $\log t_{1/2}$ vs. 1/T or logk vs. 1/T. Racemization $t_{1/2}$ of 3-EtO-NDZ enantiomers in ethanol containing 0.125 M to 2 M of H_2SO_4 were studied at 37℃.

Computer and Softwares

Several computer softwares were employed to prepare text, graphics and curve fittings on an Apple Macintosh SE/30 computer. The softwares include Word (Microsoft Corp., Redmond, WA), ChemDraw and Chem3D Plus (Cambridge Scientific, Cambridge, MA), SigmaPlot (Jandel Scientific, Corte Madera,

CA), and Canvas (Deneda Software, Miami, FL).

RESULTS

pK_{al} and Solvent Effect

Due to protonation at N4 position,[11] the uv-vis absorption spectrum of 3-EtO-NDZ in CH_3CN/H_2O(4/1, v/v) containing 1 N H^+ was different from that of the unprotonated neutral form (Fig. 2A). The absorption difference spectrum between protonated and unprotonated forms of 3-EtO-NDZ is shown in Fig. 3. The magnitudes of absorption differences at various wavelengths were dependent on the acid concentration. Based on the data shown in Fig. 3, the acid concentration dependent absorptions at 224, 244, and 292 nm of 3-EtO-NDZ (50 μM) in either ethanol or acetonitrile were measured (Fig. 4). Values of pK_{al}(a pH value when concentrations of protonated and unprotonated forms of 3-EtO-NDZ are equal) were determined by fitting the data with a curve fitting program in SigmaPlot. The pK_{al} values in ethanol ($pK_{al} = 0.63 \pm 0.02$) and acetonitrile ($pK_{al} = 3.5 \pm 0.1$) were substantially different (Fig. 4).

The pK_{al} of (3R)-EtO-NDZ was also determined by a spectropolarimetric method (Fig. 5). The pK_{al} value (3.3) is in close agreement with that determined by the spectrophotometric method (Fig. 4B).

Kinetics of Racemization

The acid concentrations chosen for temperature dependent racemization studies were based on the pK_{al} value ($pK_{al} = 0.63$ at $[H^+] = 0.23$ N) in ethanol (Fig. 4A). At 1 N H^+, 81% of 3-EtO-NDZ is present in protonated form. At low concentrations of 3-EtO-NDZ (μM to mM range) and at pH near pK_{al}, the degree of protonation is determined by the pH of the solvent.

Corresponding to absorption differences (Fig. 2A)

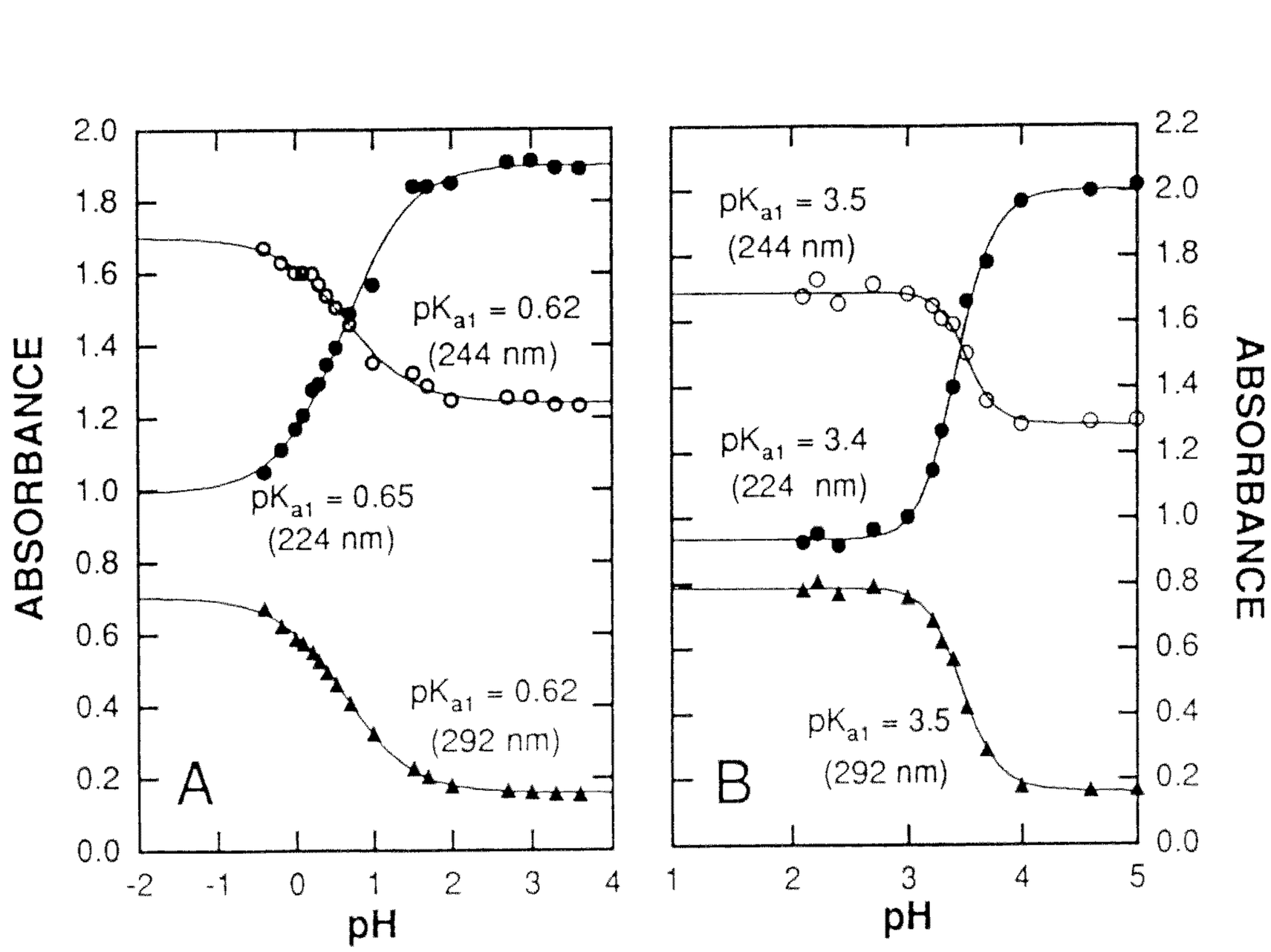


Figure 4. pH Dependence of absorption of 3-EtO-NDZ in ethanol (50 μM; panel A) and CH_3CN (43 μM; panel B) at 224, 244, and 292 nm and at room temperature. Concentrated sulfuric acid was used to prepare ethanol solutions with various pH values.

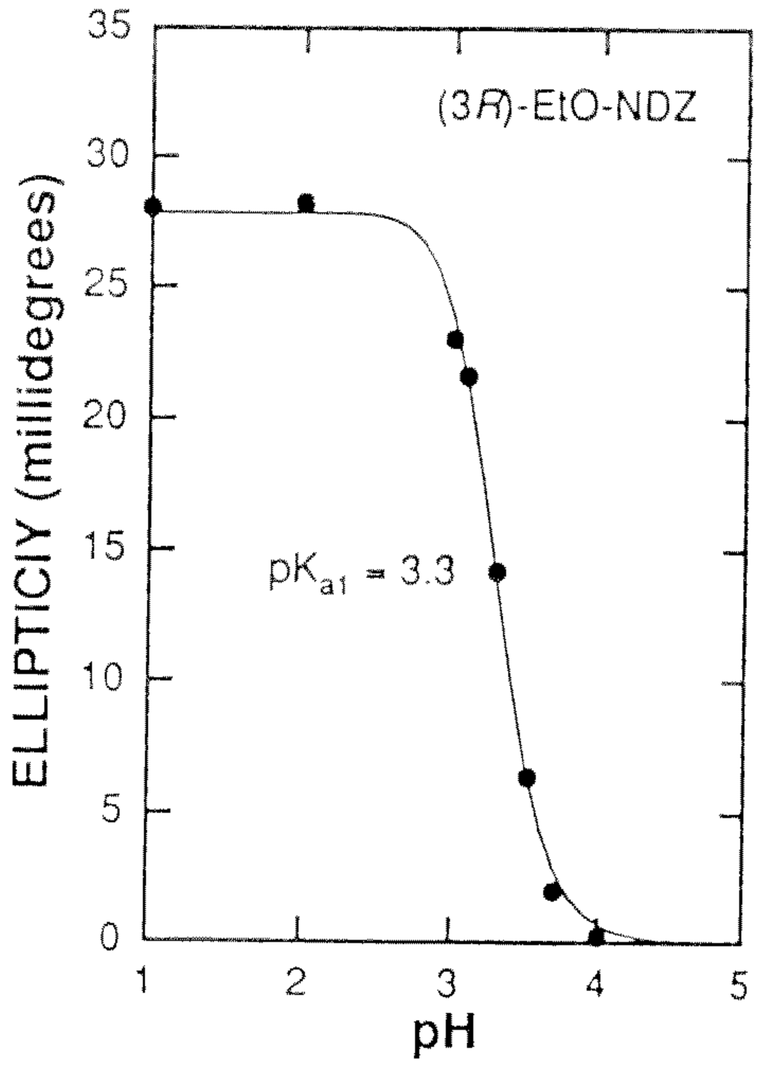


Figure 5. pH Dependence of ellipticity of (3R)-EtO-NDZ in acetonitrile(86 μM)at 365 nm and at 25℃. Concentrated sulfuric acid was used to prepare ethanol solutions with various pH values.

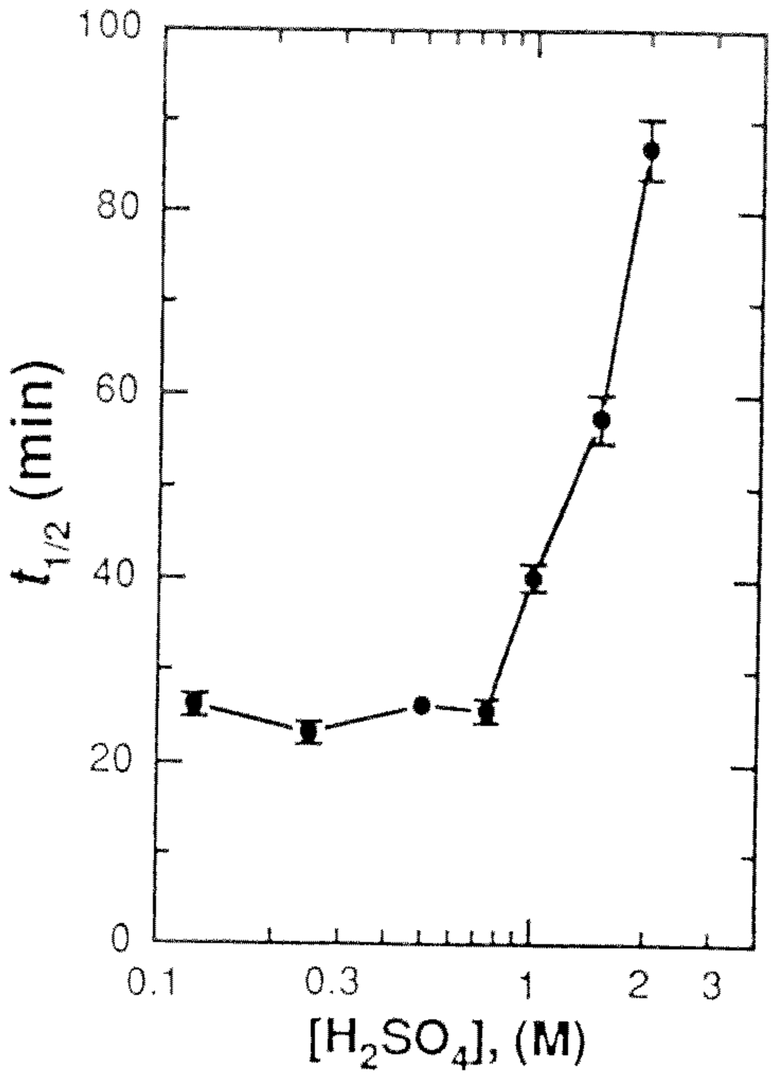


Figure 6. Dependence of racemization $t_{1/2}$ of (3R)-EtO-NDZ on the acid concentration in ethanol at 37℃.

, CD spectra of protonated and unprotonated forms of (3R)-EtO-NDZ were also characteristically different (Fig. 2B). Based on the data shown in Fig. 2B, at least two wavelengths (245 and 365 nm) were suitable for monitoring changes in ellipticity during racemization of an enantiomeric 3-EtO-NDZ. Because of substantially lower noise, 365 nm was chosen as the monitoring wavelength in studies described in this report.

Half-lives of racemization of 3-EtO-NDZ enantiomers in ethanol containing 0.5 M H_2SO_4 were determined at various temperatures (Table 1). Because acetonitrile is a poor nucleophile, neither protonated nor unprotonated forms of 3-EtO-NDZ enantiomers undergo racemization in acetonitrile. Unprotonated forms of 3-EtO-NDZ enantiomers did not undergo racemization in ethanol.

The R and S enantiomers of 3-EtO-NDZ had the same racemization $t_{1/2}$ in ethanol containing 0.5 M H_2SO_4 at 25℃ (Table 1). Hence only one 3-EtO-NDZ enantiomer (the R-enantiomer) was used to determine racemization $t_{1/2}$ in ethanol containing 0.5 M H_2SO_4 at 25℃ (Table 1). Arrhenius plot of the temperature dependence of $t_{1/2}$ in the racemization of (3R)-EtO-NDZ yielded an energy of activation (E_{act}) of 16.7 kcal/mol. Other thermodynamic parameters of the racemization were (25℃): $\Delta H^{\ddagger}$ = 16.1 kcal/mol, $\Delta S^{\ddagger}$ = -22.0 cal/K/mol), and $\Delta G^{\ddagger}$ = 22.6 kcal/mol, respectively.

Racemization $t_{1/2}$ of (3R)-EtO-NDZ at 37℃ in ethanol containing 0.125 M to 0.75 M of H_2SO_4 was found to be relatively constant (Fig. 6). However, the rate of racemization is substantially decreased (with increasing $t_{1/2}$) by increasing the [H_2SO_4] in ethanol (Table 1 & Fig. 6).

Isotope Effect

The effect of a heavy isotope (deuterium) on the racemization rate of (3R)-EtO-NDZ was studied by using C_2H_5OD containing 0.5 M D_2SO_4 at 42℃. The temperature was chosen on the basis of the data obtained by using C_2H_5OH and H_2SO_4 (Table 1). Racemization in C_2H_5OD/D_2SO_4 was considerably slower than that in C_2H_5OH/H_2SO_4 (Table 1). Racemization $t_{1/2}$ in C_2H_5OD/D_2SO_4 was found to be 31.3 ± 0.5 min. The

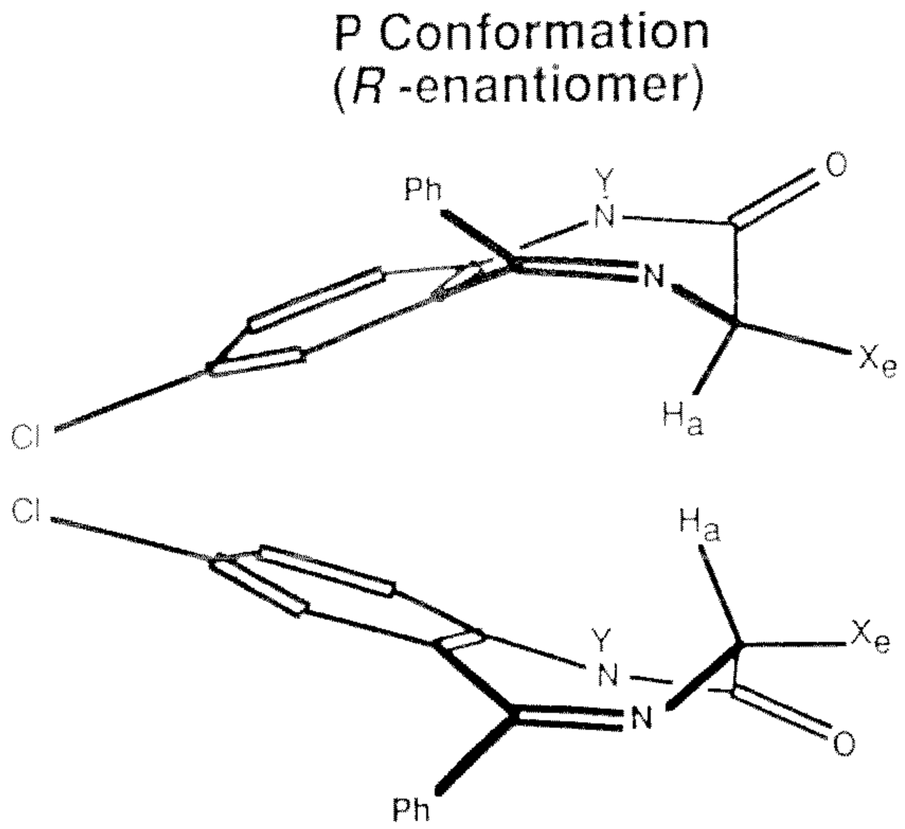


Figure 7. P and M conformations of unsubstituted (X = H) and 3-substituted 1, 4-benzodiazepines. For 3-EtO-NDZ, X = OC_2H_5 and Y = H, respectively. Subscripts a and e indicate quasiaxial and quasiequatorial positions, respectively.

results indicated that the nucleophilic substitution reaction had an isotope effect (k_H/k_D) of 1.9.

DISCUSSION

Based on the results of temperature dependent kineties and of the isotope effect in the racemization of enantiomeric 3-MeO-NDZ, we have recently proposed[9] a considerably simpler mechanism for the nucleophilic substitution and racemization of enantiomeric 3-MeO-NDZ than that proposed by other investigators.[4-6] We had proposed the following sequence of events occurring in the racemization of enantiomeric 3-MeO-NDZ in strongly acidic methanol: (1) the N4-protonated 3-MeO-NDZ enantiomer is extensively solvated by methanol, (2) a nucleophilically solvated C3 carbonium ion is formed as an intermediate, (3) a solvent-assisted nucleophilic substitution by methanol then takes place. The nucleophilic substitution by methanol is stereoselective because the intermediate is preferentially oriented in favor of a nucleophilic attack from one stereo heterotopic face of the C3 carbon. The highly favored ori-entation for stereoselective nucleophilic attack results from the preferred conformation (M conformation for S-enantiomer and P conformation for R-enantiomer; Fig. 7) of a 3-MeO-NDZ enantiomer. Racemization is then observed as a consequence of the stereoselective nucleophilic substitution.

We had previously suggested that the racemization reaction could be classified as a nucleophilically assisted and a mixed S_N1 and S_N2 reaction. Results of this report indicated that the acid-catalyzed racemization kinetics of enantiomeric 3-EtO-NDZ in ethanol followed a similar mechanism to that described for an enantiomeric 3-MeO-NDZ in acidic methanol.[9]

Our interest in studying the properties of 3-EtO-NDZ originated from a preliminary observation that a chemical reaction between OX (or other 3-OH-BDZ's) and ethanol can occur when ethanol is mixed with OX in a strongly acidic medium such as the stomach juice. Hence the pharmacological properties of a 3-OH-BDZ may be significantly altered by coingestion of ethanol. Among 3-OH-BDZ's, oxazepam, lorazepam, and lormetazepam are clinically used drugs. A 3-EtO-BDZ may also be converted to a 3-OH-BDZ by an acid-catalyzed nucleophilic substitution reaction in an aqueous medium. The possibility of this *ethanol-drug coingestion interaction* and its pharmacological/therapeutic consequences have heretofore not been investigated.

The pK_{al} of 3-EtO-NDZ in acetonitrile is ~5.4-fold higher than that in ethanol (Figs. 4 and 5). 3-EtO-NDZ (Figs. 4 and 5) and 3-MeO-NDZ[9] have essentially the same pK_{al} in acidic acetonitrile. The pK_{al} of 3-EtO-NDZ in ethanol and 3-MeO-NDZ in methanol were 0.63 (Fig. 4A) and 1.3,[9] respectively. Thus hydrogen ions in alcohols appear to be more readily available for protonation at N4 position of 3-MeO-NDZ than protonation at N4 position of 3-EtO-NDZ.

Alcohols are known to form hydrogen bonds among themselves and with hydrogen ions.[12] Because of hydrogen bonding, 3-MeO-NDZ and 3-EtO-NDZ are more extensively solvated in either methanol or ethanol than in acetonitrile. However, ethanol appears to be more effective than methanol in shielding an alkoxy-NDZ from protonation by H^+.

Based on the pK_{al} of 3-EtO-NDZ in ethanol (Fig. 4A), the protonated forms of 3-EtO-NDZ can be calculated to constitute 81, 89.5, and 94.5% of the total 3-EtO-NDZ in ethanol containing 1, 2, and 4 N H^+, respectively. We have also found that, as ordinarily found in acid-base equilibrium, the increase in absorbance at 224 nm was essentially instantaneous (within seconds) when an ethanol solution of 3-EtO-NDZ was mixed with a strong acid. Hence, at $pH < pK_{al}$ and in the absence of other factors, the racemization $t_{1/2}$ is expected to be fairly independent of $[H^+]$. The observed decrease in the rate of racemization at $[H^+] > 1.5$ N is probably due to increased $[HSO_4^-]$ and $[SO_4^=]$ which form ionic bonds with protonated N4 nitrogen of 3-EtO-NDZ. Thus a layer of ion pairs is formed around the protonated 3-EtO-NDZ, making the penetration of attacking nucleophiles (ethanol) more difficult. This shielding by ion pairs is probably responsible for the decreased rate of racemization (i.e., decreased rate of nucleophilic attack) of 3-EtO-NDZ enantiomers in ethanol containing more concentrated sulfuric acid (at $[H^+] > 1.5$ N).

Even in the absence of an asymmetric center, BDZ's exist as conformational racemates.[13-16] For example, DZ and NDZ each exists in P (plus) and M (minus) conformations, which are mirror images of each other (Fig. 7).[13 16] The M conformation binds preferentially to the BDZ receptors.[15] Because 3-substituents are preferentially in quasiequatorial position (Fig. 7),[13 16] the R- and S-enantiomers of 3-substituted BDZ's are predominantly in P and M conformation, respectively (Fig. 7). According to Decorte et al.,[15] (3R)-EtO-NDZ is expected to exist predominantly (>97%) in P conformation. In Fig. 8, a highly ethanol-solvated (3R)-EtO-NDZ predominantly in P conformation is depicted as intermediate I.

In intermediate I of Fig. 8, (3R)-EtO-NDZ is shown with two bifurcated hydrogen bonds[12] between the N4 hydrogen and the oxygens of two attacking nucleophiles, and one hydrogen bond between the hy-

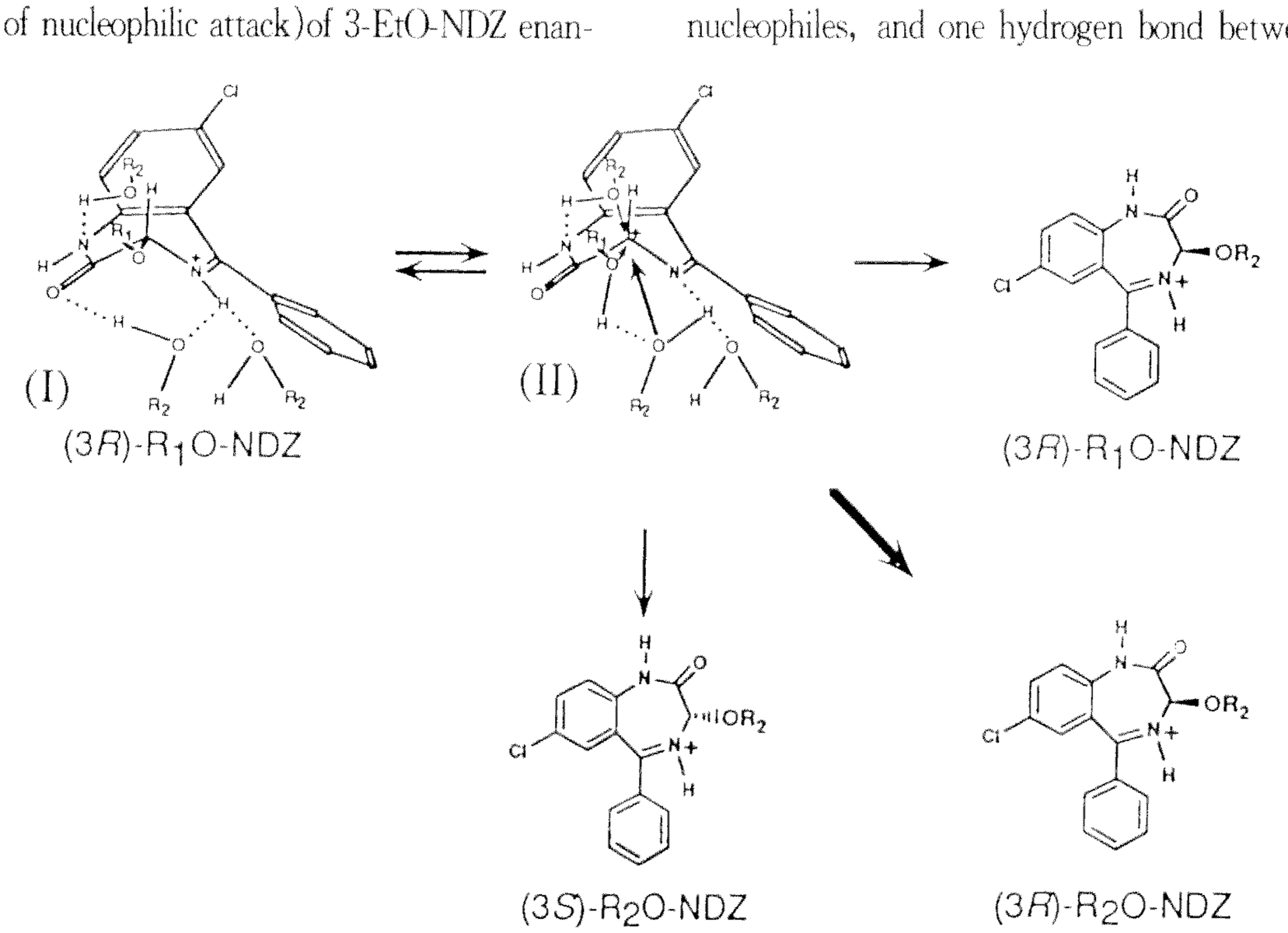


Figure 8. Possible intermediates of solvated and protonated (3R)-EtO-NDZ (in P conformation) in ethanol leading to stereoselective nucleophilic substitution and racemization. R_1O and R_2O ($R_1 = R_2$ = ethyl group) indicate the leaving and attacking nucleophile, respectively. Arrows indicate origin of nucleophilic attack at C3. For simplicity, only 3 of many possible attacking nucleophiles (R_2OH) are shown. Three enantiomeric products, which remain protonated at N_4 position, of a stereoselective nucleophilic substitution are shown. Similar mechanism is proposed for (3S)-EtO-NDZ.

drogen of an attacking nucleophile and the oxygen of a leaving ethoxy group. It can be seen in Fig. 8 that the solvation is expected to be less extensive, due to steric crowding, in the region including C3 hydrogen, N1 nitrogen, and C3 carbon. The C2 carbonyl oxygen is electronegative, therefore it is expected to be also involved in hydrogen bonding with solvent ethanol. However, since C2 carbonyl oxygen does not form a hydrogen bond with the leaving ethoxy group of the N4-protonated 3-EtO-NDZ, such hydrogen bond probably does not play a significant role, if any, in the nucleophilic substitution of 3-EtO-NDZ. In Fig. 8, R_2OH represents one of many possible nucleophiles and is not limited to ethanol. The possibilities of (1) acid-catalyzed alcoholysis of 3-EtO-NDZ in alcohols other than ethanol, and (2) nucleophilic substitution by nucleophiles other than alcohols are currently being investigated in our laboratory.

Due to hydrogen bonding, the attacking nucleophiles (R_2OH) are in place, ready to replace the leaving nucleophile (R_1OH). The actual nucleophilic substitution takes place following the transient formation of intermediate II (Fig. 8). As depicted in intermediates I and II, the formation of (3R)-R_2O-NDZ is expected to be favored over the formation of (3S)-R_2O-NDZ, the latter exists predominantly in M conformation. This chain of events results in a stereoselective nucleophilic substitution. The resulting (3S)-R_2O-NDZ also undergoes 3S-stereoselective nucleophilic substitution by a mechanism similar to that depicted for (3R)-EtO-NDZ. The subsequent stereoselective nucleophilic substitutions of both (3R)-R_2O-NDZ and (3R)-R_2O-NDZ eventually lead to racemization of the initial (3R)-EtO-NDZ. The mechanism depicted in Fig.8 can therefore be classified as a nucleophilically assisted and a mixed S_N1 and S_N2 reaction, similar to that proposed for the acid-catalyzed racemization of enantiomeric 3-MeO-NDZ in acidic methanol.[9]

The thermodynamic parameters, determined by an Arrhenius plot of data obtained from temperature dependence of racemization $t_{1/2}$ (Table 1), are consistent with the model proposed in Fig. 8. The enthalpy of activation($\triangle H^{\ddagger}$) of the racemization reaction was found to be 16.1 kcal/mol. This relatively small enthalpy of activation is consistent with the difference in energy between (1) that required to break hydrogen bonds and the partial breakage of a C-O bond in 3-EtO-NDZ and (2) that released by partial formation of a C-O bond between the attacking nucleophile and C3 carbon and the formation of hydrogen bonds in the transition state (intermediate II in Fig. 8). Each hydrogen bond formation releases 2-10 kcal/mol.[12] This analysis of possible bond breaking and formation indicates that the energy required to form the transition state is relatively small.

The entropy of activation ($\Delta S^{\ddagger}$) of the racemization reaction was found to be -22.0 cal/K/mol. This relatively large negative entropy indicated a gain of orderliness in the transition state. The proposed model of transition state (intermediate II in Fig. 8) is consistent with the negative entropy of activation. Intermediate II consists of hydrogen bonds between the N4-protonated 3-EtO-NDZ and a number of attacking nucleophiles (R_2OH), bringing about increased orderliness relative to the separated molecules.

The proposed transition state (intermediate II in Fig. 8) requires a transient deprotonation of the N-H bond at the N4 position of 3-EtO-NDZ and the breakage of an O-H bond in the attacking nucleophile R_2OH. Thus the nucleophilic substitution reaction carried out in C_2H_5OD/D_2SO_4 is expected to be slower than that in C_2H_5OH/H_2SO_4 and this was actually observed (Table 1). An isotope effect (k_H/k_D) of 1.9 was found in the racemization of (3R)-EtO-NDZ at 42℃ (Table 1). Because the O-D bond in C_2H_5OD is stronger than that in CH_3OD, the isotope effect in the acid-catalyzed racemization of an enantiomeric 3-EtO-NDZ in C_2H_5OD is expected to be greater than that of an enantomeric 3-MeO-NDZ in CH_3OD. The observed isotope effects [1.9 for 3-EtO-NDZ (Table 1) and 1.6 for 3-MeO-NDZ[9] at 42℃] is consistent with the prediction. These results are consistent with the mechanism proposed in Fig. 8.

In conclusion, we propose: (1) a highly solvated and hydrogen-bonded intermediate of 3-EtO-NDZ exists in acidic ethanol, (2) the solvating ethanol (a nucle-

ophile) reacts at the C3 position of 3-EtO-NDZ, (3) ethanol undergoes nucleophilic attack in a stereoselective manner due to the conformational preference of either (3R)-EtO-NDZ (in P conformation) or (3S)-EtO-NDZ (in M conformation), and (4)enantiomers of 3-EtO-NDZ undergoes racemization as a consequence of the stereoselective nucleophilic substitution. The relatively simple mechanism proposed in Fig. 8 can be applied to explain the ethanolysis of 3-O-ethyl-lorazepam observed in acidic ethanol.[4] Relative to those proposed earlier,[4-6] our proposed mechanism(Fig. 8) is considerably simpler. The proposed mechanism can also be extended in concept to explain the mechanism involved in the acid-catalyzed exchange of hydroxyl group of temazepam[17, 18] and hydrolysis of alkoxy-BDZ's in strongly acidic aqueous solutions.

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3-乙氧基-N 去甲基安定對映異構體在酸性乙醇中的親核取代和消旋作用

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摘　要

3-乙氧基-N 去甲基安定(3-EtO-NDZ;diazepam＝安定)在含不同硫酸濃度的乙醇和乙腈中的 pK_{al} 值用光譜法和旋光光譜法作了測定,其結果分別為 3.4 和 0.63。3-EtO-NDZ 之對映異構體在含各種不同酸濃度的乙醇中與溫度相關的消旋過程,用旋光光譜儀選擇其圓二色譜在 365 nm 波長處強度的改變為時間函數的方法作了測定。發現其消旋反應屬於顯見一級動力學過程。消旋反應之熱力學參數分別為:E_{act} = 16.7 kcal/mol, 及在 25°C 時 $\Delta H^{\ddagger}$ = 16.1kcal/mol, $\Delta S^{\ddagger}$ = -22.0 cal/K/mol 和 $\Delta G^{\ddagger}$ = 22.6 kcal/mol。該消旋反應有一同位素效應(k_H/k_D),在 42°C 時為 1.9。基於本文所報導的結果及文獻所記述之對映異構性在 C3 位有取代基時的 1,4-苯并二氮䓬類的傾向構象,設想提出所觀察之現象是由正(P)或負(M)構象所致的一個親核性經溶劑化的 C3 陽碳離子為中間體。該中間體導致了 3-EtO-NDZ 對映異構體在酸性乙醇中所發生的立體選擇性親核取代反應及隨後的消旋過程。