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Acid-catalyzed Reaction of Ethanol and Oxazepam and Its Pharmacological Consequence

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ABSTRACT

Oxazepam (OX), an anxiolytic drug in clinical use, reacts with ethanol in aqueous and anhydrous ethanol solutions and simulated gastric fluid to form 3-*O*-ethyloxazepam (EtOX). In simulated gastric fluid containing 30% (by volume) ethanol, approximately 9.5% of OX is converted to EtOX in 2 hr at 37°C. Studies of this report indicate that the 3-hydroxyl group of OX is substituted by ethanol to form EtOX in acidic media. Pharmacokinetic studies in rats indicate that orally administered OX and EtOX at doses of 35 and 17.5 nmole/kg are absorbed to similar extents. EtOX is eliminated at a slower rate than OX. EtOX is less active than OX in two pharmacological activity tests in mice. It is known that ethanol and OX have additive effects in the central nervous system. The results of this study suggest that ingestion of ethanol shortly before, shortly after, or simultaneously with the intake of OX may result in the formation of EtOX in the strongly acidic medium of the stomach, reducing the pharmacological effects of OX.

Key words : Oxazepam, ethanol, 3-*O*-ethyloxazepam, simulated gastric fluid, acid-catalyzed ethanolysis.

INTRODUCTION

Oxazepam (7-chloro-1,3-dihydro-3-hydroxy-5-phenyl-2H-1,4-benzodiazepin-2-one; OX, Fig. 1) is one of the 1,4-benzodiazepines in clinical use. OX is useful in the management of anxiety, tension, agitation, and irritability in older patients[1]. Alcoholics with acute tremulousness, inebriation, or with anxiety associated with alcohol withdrawal are responsive to OX therapy[1]. OX is an active metabolite of diazepam (DZ), the latter is one of the most frequently prescribed drugs for the treatment of anxiety and insomnia and as an adjuvant for anesthesia[2].

Clinically used 1,4-benzodiazepines such as OX, temazepam (TMZ), lorazepam, and lormetazepam have a hydroxyl group at C3 position and undergo nucleophilic substitution in acidic aqueous and anhydrous alcoholic solutions; the alcohols include straight-chain and branched-chain lower alcohols[3]. A detailed study on the mechanism of acid-catalyzed ethanolysis of TMZ in aqueous and anhydrous ethanol solutions was reported recently[3]. The lability of the C3-hydroxyl group is due to protonation at

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the N4 nitrogen, resulting in a transient C3 carbocation susceptible to nucleophilic attack by alcohols to form alkoxylated products[3].

In this report, we describe (1) the kinetics and mechanism of acid-catalyzed conversion of OX to 3-*O*-ethyloxazepam (EtOX) in anhydrous ethanol and in a mixture of ethanol and simulated gastric fluid (SGF), (2) the pharmacokinetics of OX and EtOX in rats, and (3) the pharmacological activities of the products formed from acid-catalayzed methanolysis and ethanolysis of OX and TMZ in mice. The results suggest that conversion of OX to EtOX in the gastric fluid of humans may reduce the anxiolytic activities of OX.

MATERIALS AND METHODS

I. *Chemicals*

OX (ε_{230}=34.2 $cm^{-1}mM^{-1}$, acetonitrile) and TMZ (ε_{230}=30.2 $cm^{-1}mM^{-1}$, acetonitrile) were generously provided by Wyeth-Ayerst Research (Princeton, NJ) and Sandoz Pharmaceuticals Corp. (East Hanover, NJ), respectively. 3-*O*-methyloxazepam (MeOX), EtOX, 3-*O*-methyltemazepam (MeTMZ), and 3-*O*-ethyltemazepam (EtTMZ) were prepared by acid-catalyzed alcoholysis of OX and TMZ in anhydrous methanol and ethanol, respectively[3]. Sodium pentobarbital was obtained from Butler Co. (Columbus, OH). 2-Amino-5-chlorobenzophenone (ACBP) was obtained from Aldrich Chemical Co. (Milwaukee, WI). Pepsin (Pepsin A, EC 3.4.23.1, 570 units/mg protein) and metrazol were obtained from Sigma Chemical Co. (St. Louis, MO). Acetonitrile, methylene chloride and diethyl ether were HPLC grade solvents and all other reagents were analytical reagent grade.

II. *Kinetics of Ethanolysis*

The kinetics of acid-catalyzed ethanolysis of OX in anhydrous ethanol were studied by reversed-phase HPLC. Typically, OX (260 μg) was dissolved in 1.5 ml of an ethanol solution containing various amounts of concentrated H_2SO_4. The solution was transferred to a sample vial, which was immediately placed in a sample well of a water-jacketed rack in a Shimadzu Model SIL-9A automatic sample injector (Shimadzu Corp., Kyoto, Japan). 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 $\pm$0.1°C.

HPLC 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 Zorbax SB-C18 column (5μ particles, 4.6 mm i.d. x 15 cm; MAC-MOD Analytical Inc., Chadds Ford, PA). The mobile phase was acetonitrile: 0.02 M phosphate buffer (pH 7.0) (1:1, v/v) at a flow rate of 1 ml/min. HPLC analysis was conducted at ambient temperature (23$\pm$1°C). After placing the sample vial in the sample well, each sample was allowed 5 min to reach temperature equilibrium before the injection of the first aliquot. Aliquots (15 μl each) were injected every 5 to 20 min via the autosampler. The detector signal was recorded with MacIntegrator (a software and hardware package from Rainin Instruments Co., Inc., Emeryville, CA) on an Apple Macintosh computer (Apple Computer, Cupertino, CA).

III. *Ethanolysis in Simulated Gastric Fluid*

SGF (pH 1.21) was prepared as described [4]. Briefly, 0.02 g of NaCl, 0.32 g of pepsin, and 0.7 ml of concentrated HCl (12 M) were added to distilled water to make a final volume of 100 ml.

The kinetics of acid-catalyzed ethanolysis of OX in SGF containing various volume percentages of ethanol were studied. Typically, OX (260 μg) was dissolved in 0.15 to 1.05 ml of ethanol and SGF (0.45 to 1.35 ml) was subsequ-

ently added. The solution was thoroughly mixed and allowed to reach 37°C in the sample well of the autosampler. Aliquots of samples were analyzed by reversed-phase HPLC as described above.

IV. *Pharmacokinetics in Rats*

Male Sprague-Dawley rats (280-330 gm body weight) were used in pharmacokinetic studies. Rats were allowed free access to food and water prior to the experiments. All rats were fasted overnight prior to use in the experiments.

Each rat was anesthetized by ether. The jugular vein of each rat was then cannulated for blood sampling. Each rat was orally administered a mixture containing equal molar amounts of OX and EtOX (8.75, 17.5, or 35 nmol/kg body weight). At various times following drug administration, 0.5 ml of blood was drawn via the jugular vein, followed by injection of 0.5 ml of normal saline into the tail vein. To each blood sample, 0.522 μg of TMZ (added in 50 μl of methanol) was added to serve as an internal standard for chromatography, followed by 1.5 ml of 0.02 M phosphate buffer (pH 7.0). Following thorough mixing, the mixture was extracted with 5 ml of diethyl ether:methylene chloride (7:3, v/v). The resulting mixture was centrifuged and 4 ml of the organic phase was transferred to a test tube. The aqueous phase was further extracted twice with 5 ml of diethyl ether:methylene chloride (7:3, v/v). The organic phase extracts were combined and evaporated to dryness by blowing with a gentle stream of nitrogen. The residue was dissolved with 0.2 ml of acetonitrile: water (1:1, v/v) for reversed-phase HPLC analysis. Each smple was analyzed twice.

A Hewlett-Packard 1090 HPLC system fitted with a Rheodyne 7010 injection valve, a HP diode-array detector, and a HP 79996 Workstation was used. The separation was performed on a Vydac C18 column (5 μ particles, 4.6 mm i.d. x 25 cm, catalog no. 201TP54; The Separations Group, Hesperia, CA) at room temperature. The detector wavelength was set at 230 nm. The column was eluted with acetonitrile: 0.02 M phosphate (pH 7.0) (3:7, v/v) for the first 5 min, followed with a 5-min linear gradient to acetonitrile: 0.02 M phosphate (pH 7.0) (47.5:52.5, v/v), and the latter mobile phase maintained for an additional 10 min. The flow rate of mobile phase was 1 ml/min.

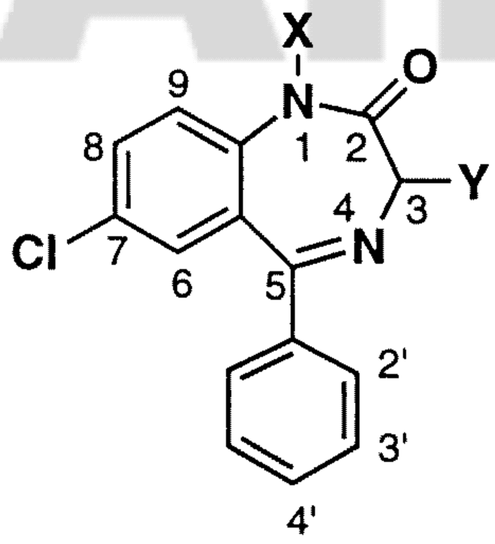
The amounts of EtOX and OX detected in blood samples were quantified by comparing their areas under the curves (AUC) to that of the internal standard. The recovery of both OX and EtOX from blood samples by the extraction procedure described above was 87±4%. The AUC's at 230 nm in HPLC analysis were linearly related to the amount of OX, EtOX and TMZ in the range of 1 to 2000 ng with correlation coefficients of 0.999, 0.998 and 0.999, respectively.

V. *Pentobarbital-induced Sleep Time in Mice*

Adult Swiss Webster mice (male, body weight 20±2 g), obtained from National Cancer Institute (Bethesda, MD), were allowed free access to food and water except during the experimental test period. Pentobarbital-induced sleep time in mice was determined as the time between loss of righting reflex and the moment mice regained this reflex[5]. Each chemical was suspended in 1.5% sodium carboxymethylcellulose and administered by gastric intubation 30 min before intraperitoneal injection of pentobarbital (sodium salt in saline, 50 mg/kg). Groups of 12 mice were used for each chemical and each dose.

VI. *Antimetrazol activity in mice*

Adult Swiss Webster mice (male, body weight 20±2 g) were used. Antimetrazol activity was determined according to Goodman et a1.[6]. Each chemical was suspended in 1.5% sodium carboxymethylcellulose and administered by gastric intubation 30 min before the administration of metrazol. Seizures were elicited by rapid intraperitoneal injection of an aqueous solution of metrazol (110 mg/kg body weight). The volume



DZ, X = Me, Y = H
OX, X = H, Y = OH
MeOX, X = H, Y = OMe
EtOX, X = H, Y = OEt
TMZ, X = Me, Y= OH
MeTMZ, X = Me, Y = OMe
EtTMZ, X = Me, Y = OEt

Fig. 1. Structures and abbreviations of diazepam (DZ), oxazepam (OX), temazepam (TMZ), 3-*O*-methyloxazepam (MeOX), 3-*O*-ethyloxazepam (EtOX), 3-*O*-methyltemzepam (MeTMZ), and 3-*O*-ethyltemazepam (EtTMZ), respectively.

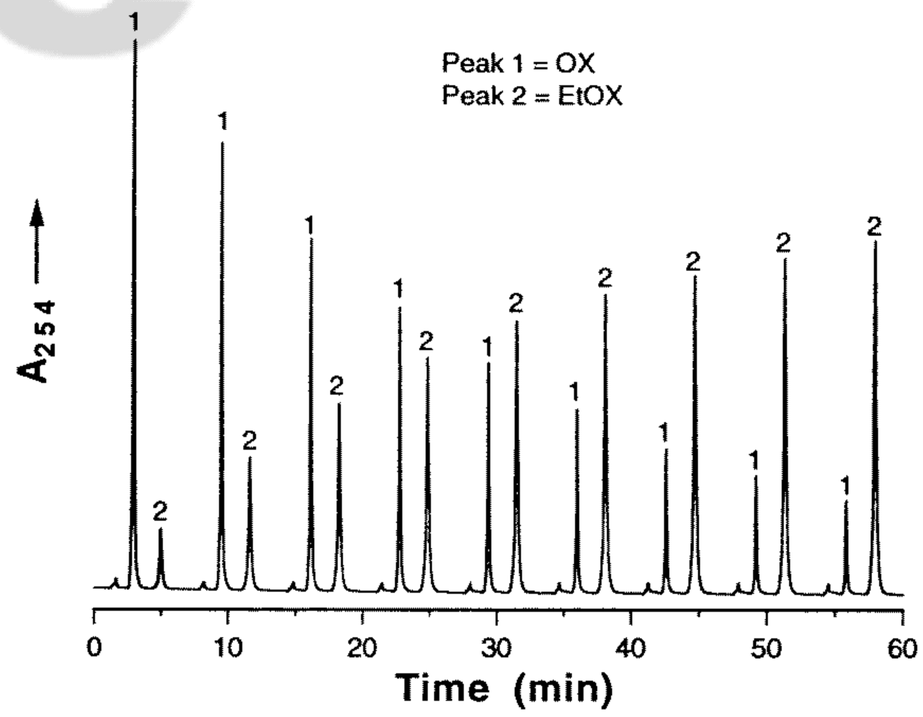


Fig. 2. Reversed-phase HPLC analysis in the continuous monitoring of acid-catalyzed ethanolysis of OX. Peaks 1 and 2 are OX and EtOX, respectively. OX (260 μg) in 1.5 ml of ethanol containing 0.1 M H_2SO_4 was maintained at 37°C. Aliquots (15 μl) were injected for analysis every 6.62 min. In the chromatogram shown, the $t_{1/2}$ in the disappearance of OX was 21.5 min. HPLC conditions are described in Materials and Methods.

of metrazol solution injected into each mouse did not exceed 0.25 ml. The effects of metrazol in the presence and absence of the second drug were: (1) tail straight up, (2) hindleg tonic extensor seizure, (3) survival time, and (4) mortality. Groups of 12 mice were used for each chemical and each dose.

Results of pentobarbital-induced sleep time and antimetrazol activity were analyzed by an independent student's test. $P<0.05$ was considered to be the minimum for a statistically significant difference.

VII. *Spectral Analysis*

The pK_a value of OX in acidic anhydrous EtOH was determined on a Model DW2000 spectrophotometer (SLM Instruments, Urbana, IL) by the method previously described[7,9]. Mass spectral analysis was performed on a Finnigan mass spectrometer system (model 4500 or model 4600; Finnigan MAT, San Jose, CA) with a solid probe by either electron impact at 70 eV or chemical ionization (NH_3); the ion source was maintained at 105°C.

RESULTS AND DISCUSSION

Kinetics of Ethanolysis

The acid-catalyzed ethanolysis of OX in anhydrous ethanol as a function of time was continuously monitored by reversed-phase HPLC analysis (Fig. 2). ACBP, a potential hydrolysis product of OX, was not detectable under the experimental conditions described. Both OX and EtOX are protonated at the N4-nitrogen in a strongly acidic medium[8]. Because the mobile phase used in the reversed-phase HPLC analysis contained a neutral buffer, both OX and EtOX were detected as the unprotonated form. OX underwent a pseudo-first order reaction in acidic anhydrous ethanol. In ethanol containing 0.1 M H_2SO_4 at 37°C, a semi-log plot of the time-dependent disappearance of OX (Fig. 2) yielded an

ethanolysis $t_{1/2}$ of 21.5 min.

Acid-dependent Ethanolysis

The dependence of ethanolysis rate on the concentration of H_2SO_4 in anhydrous ethanol at 37°C is shown in Fig. 3. At acid concentrations less than 1 mM, the rate of ethanolysis was very slow. An apparent plateau of ethanolysis rate was attained at approximately 0.1 M H_2SO_4 (Fig. 3). The K_a value of OX in anhydrous ethanol was estimated to be 7 mM H^+ from the date in Fig. 3. This is consistent with the pK_a value (2.15±0.05) of OX determined at ambient temperature (data not shown) by a spectrophotometric method[7,9]. The spectrophotometric method determind absorbance changes at 291 nm of anhydrous ethanol solutions of OX (80 μ M) within 1 min following the addition of H_2SO_4 to final concentrations ranging from 0.1 mM to 2 M. The results in Fig. 3 are consistent with the conclusion that only protonated form of OX undergoes ethanolysis reaction.

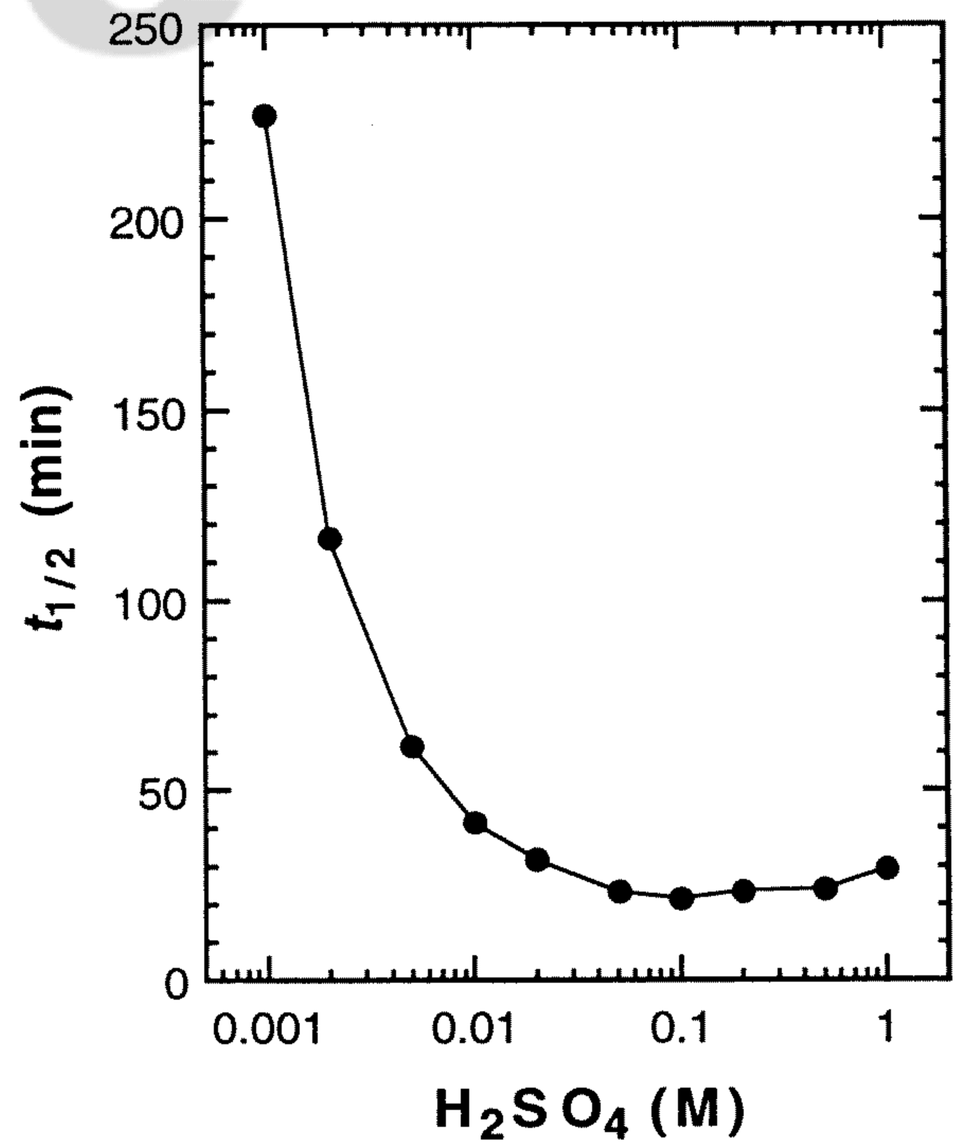


Fig. 3. Dependence of ethanolysis $t_{1/2}$ of OX on acid concentrations at 37°C.

Bond Cleavage in Ethanolysis

The structural changes were studied by mass spectral analysis of an ^{18}O-labeled OX and its ethanolysis product. An ^{18}O-labeled OX was prepared by dissolving EtOX (2 mg) in 2 ml of acetonitrile: $H_2{}^{18}O$ (99 ^{18}O atom %) (3:1, v/v) containing 0.5 M H_2SO_4 at 50°C for 2 hr. The resulting ^{18}O-labeled OX was purified by reversed-phase HPLC and electron impact mass spectral analysis indicated characteristic mass ions at *m/z* 288 (M^+, 14.8%), 286 (M^+, 1.54%), and 257 (loss of $CH^{18}O$, 100%) respectively. Comparing to that of unlabeled OX (M^+ at *m/z* 286 and base fragment ion at *m/z* 257), the mass spectrum of ^{18}O-labeled OX indicated that the ^{18}O-label was associated with the C3-hydroxyl group. The ^{18}O-labeled OX was allowed to react with acidic anhydrous ethanol (or methanol) and mass spectral analysis indicated that the resulting EtOX (or MeOX) did not contain ^{18}O. The results summarized in Fig. 4 established that the

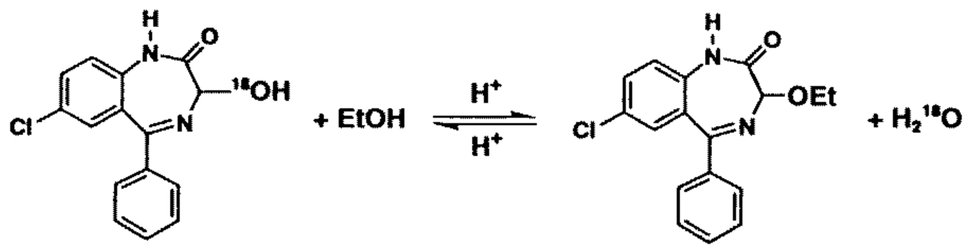


Fig. 4. Bond cleavages involved in acid-catalyzed ethanolysis of OX (forward reaction) and hydrolysis of EtOX (reverse reaction) as revealed by mass spectral analysis of product formations. See text for discussion.

acid-catalyzed ethanolysis of OX occurred via substitution of the C3-hydroxyl group by the ethoxy group of ethanol. An identical conclusion was earlierly reached by the same approach as described above using an ^{18}O-labeled TMZ prepared from EtTMZ and $H_2{}^{18}O$; MeTMZ and EtTMZ derived from the ^{18}O-labeled TMZ did not contain ^{18}O[3].

Table 1. Thermodynamic parameters in acid-catalyzed conversion of OX to EtOX in anhydrous ethanol.

Parameter	0.1M H_2SO_4 in EtOH	0.5M H_2SO_4 in EtOH	0.5M D_2SO_4 in EtOD
$t_{1/2}$ at 25°C (min)[a]	55.5±0.2	71.6±0.6	111.5±7.5
$t_{1/2}$ at 30°C (min)[a]	34.8±0.4	43.7±0.5	73.6±5.5
$t_{1/2}$ at 37°C (min)[a]	21.5±0.3	24.4±0.3	36.5±4.2
$t_{1/2}$ at 43°C (min)[a]	11.6±0.1	14.1±0.2	24.8±2.3
$t_{1/2}$ at 50°C (min)[a]	7.7±0.1	8.5±0.4	13.7±0.5
Slope[b]	3.341	3.572	3.505
r^{2c}	0.9929	0.9992	0.9974
E_{act} (kcal/mol)[d]	15.3	14.9	16.0
$\triangle H^{\ddagger}$ (kcal/mol)[e]	14.7	14.3	15.4
$\triangle S^{\ddagger}$ (cal/mol)[e]	-26.6	-28.0	-25.0
$\triangle G^{\ddagger}$ (kcal/mol)[e]	22.6	22.6	22.9

[a]Half-life ($t_{1/2}$, mean±SD of 3 determinations) of substitution reaction was determined by reversed-phase HPLC method.

[b]Slope in Arrhenius plot (log $t_{1/2}$ vs. 1000/T).

[c]Correlation coefficient in Arrhenius plot.

[d]Determined from the slope of Arrhenius plot.

[e]Values calculated for temperature at 25°C.

Temperature and Isotope Effects in Ethanolysis

Ethanolysis half-life ($t_{1/2}$) of OX in anhydrous ethanol containing 0.1 and 0.5 M H_2SO_4 were determined at several temperatures (Table 1). The slopes determined from Arrhenius plots yielded the thermodynamic parameters (E_{act}, $\triangle H^{\ddagger}$, $\triangle S^{\ddagger}$, and $\triangle G^{\ddagger}$) as shown in Table 1. The negative values of $\triangle S^{\ddagger}$ are similar to those in acid-catalyzed ethanolysis of TMZ[3] and indicated a gain of orderliness in the transition state. The transition state probably, as earlierly proposed[3], consisted hydrogen bonds between N4-protonated OX and a number of attacking nucleophiles (EtOH), resulting in increased orderliness relative to the separated molecules. Substitution of a heavy isotope (EtOD containing 0.5 M D_2SO_4) slowed down the ethanolysis reaction ($k_H/k_D \approx 1.6$), but did not significantly altered the numerical values of the thermodynamic parameters (Table 1). Thus, the rate-determining step of the ethanolysis reaction required a proton transfer, similar to that found in the acid-catalyzed ethanolysis of TMZ[3].

Kinetics of Ethanolysis in Simulated Gastric Fluid

The acid-catalyzed ethanolysis of 3-hydroxy-1,4-benzodiazepines is a reversible reaction; the 3-alkoxy group of 3-alkoxy-1,4-benzodiazepines can be substituted by water in strongly acidic media[3,10]. In a strongly acidic aqueous ethanol solution, an equilibrium is reached and the net concentration of OX and EtOX do not undergo further changes. In SGF containing up to 30% (v/v) ethanol at 37°C, the percentage of EtOX formed increased with increasing percentages of ethanol (Fig. 5). In SGF containing 30% (v/v) ethanol, ~9.5% of OX was converted to EtOX in 2 hr. The residence time of drugs in the stomach of humans varies between 20 min to several hours, depending on a large number of factors[11]. The results in Fig. 5 indicated that a portion of OX may be converted to EtOX under the acidic environment of the stomach when OX and EtOH are concurrently ingested.

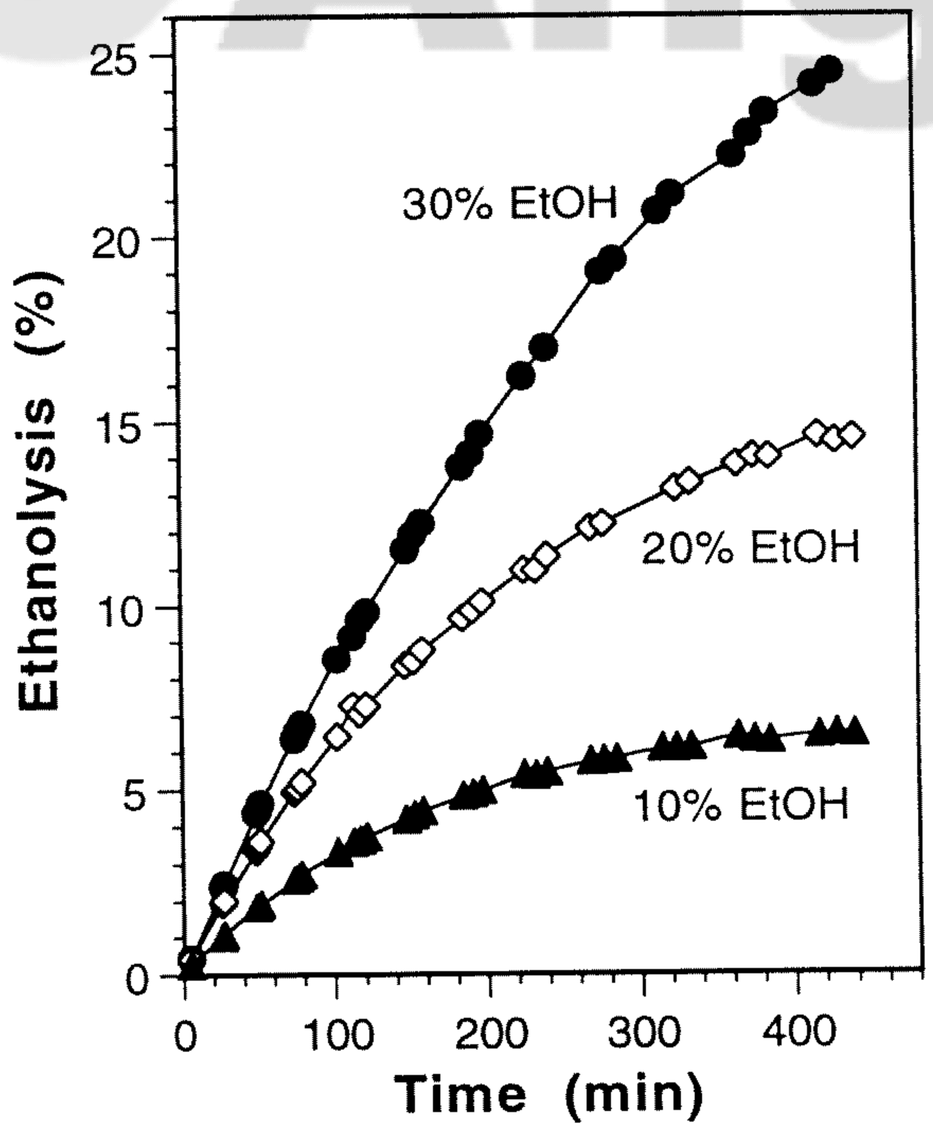


Fig. 5. Time-dependent ethanolysis of OX in SGF containing 10, 20 and 30% (by volume) ethanol at 37°C. Each curve contained data from triplicate samples.

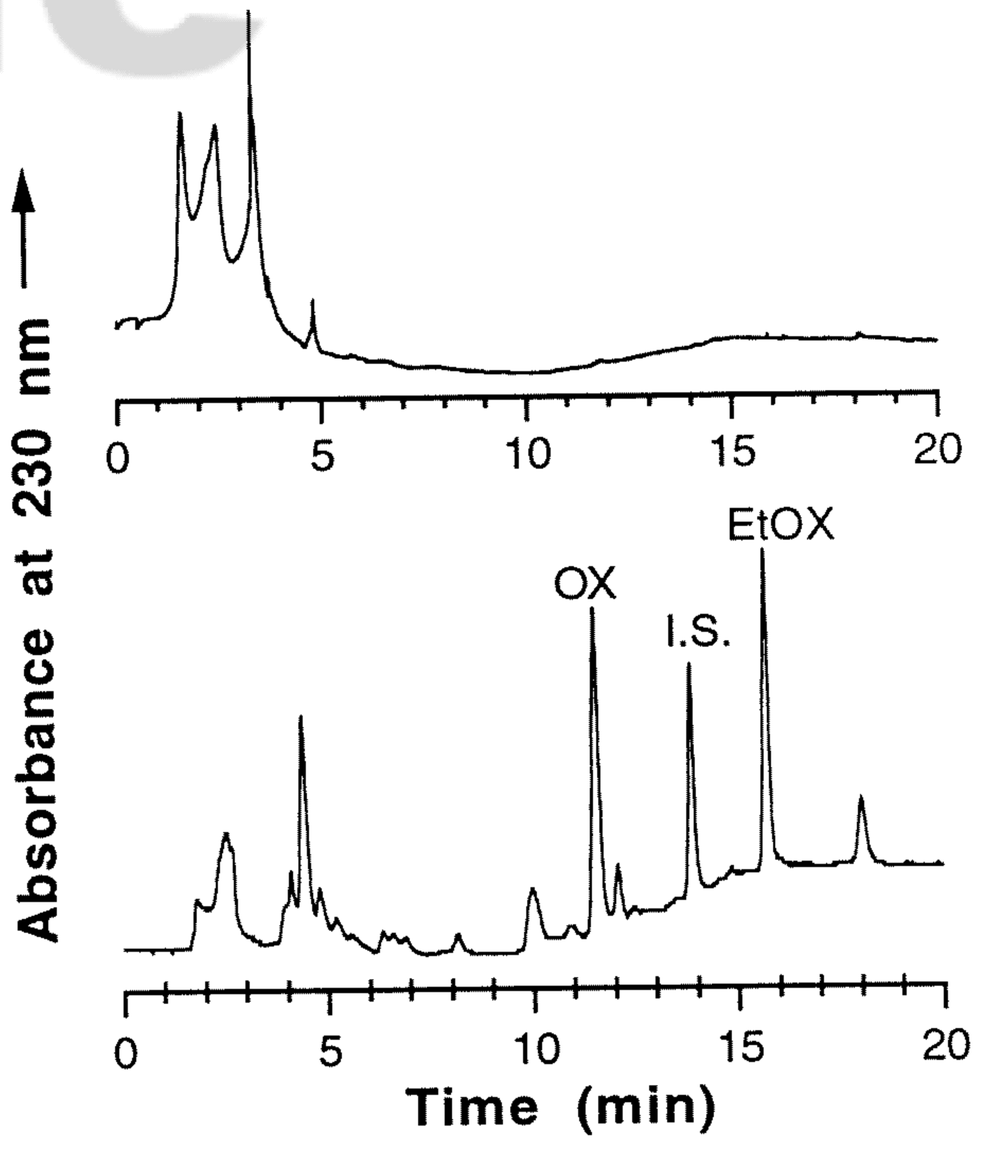


Fig. 6. Reversed-phase HPLC analysis of organic solvent extracts of a blood sample from an untreated rat (upper chromatogram) and of a blood sample taken at 180 min following intraperitoneal treatment of a rat with a mixture of equal molar amounts (17.5 nmol/kg) of OX and EtOX (lower chromatogram). The internal standard (I.S.) was TMZ. HPLC conditions and experimental procedures are described in Materials and Methods.

Reaction Mechanism of Alcoholysis and Hydrolysis

The results of acid-catalyzed ethanolysis of OX described above are consistent with a general reaction mechanism (eq.1)[3].

$$B-OR_1+R_2O-H \underset{H^+}{\overset{H^+}{\rightleftharpoons}} B-OR_2+R_1O-H$$

Where B represents a 1,4-benzodiazepine nucleus lacking a C3-substituent, R_1=H or alkyl and R_2=H or alkyl; R_1 does not necessarily equal to R_2. When $R_1=R_2$, the reaction is a homonucleophilic substitution reaction. When $R_1 \neq R_2$, the reaction is a heteronucleophilic substitution reaction. The preferred conformation of the benzodiazepine ring determines the preferred site of substitution at the stereoheterotopic C3 carbon[3,7,9,12,13].

Pharmacokinetics of OX and EtOX in Rats

For the purpose of assessing relative rate of absorption and elimination, a mixture of equal molar amounts of OX and EtOX was administered by gastric intubation to a rat. Following anesthesia by diethyl ether, blood samples were drawn and processed for reversed-phase HPLC analysis (Fig. 6). The amounts of OX and EtOX in blood samples were quantified with the aid of an internal standard (Fig. 6). The time-dependent changes of OX and EtOX in blood samples following intragastric administration of an OX-EtOX mixture (35, 17.5, or 8.75 nmol/kg, each dose contains the same molar amount of OX and EtOX) to a rat are shown in Fig. 7. The experiments with each dose of OX-EtOX were carried out with 3 different rats. The relative blood concentration of OX and EtOX in each of the

three rats receiving the same dose of OX-EtOX were similar at doses of 35 and 17.5 nmole/kg. However, since variations of actual blood concentrations of OX and EtOX in three rats varied up to 110%, the results at 8.75 nmole/kg were not as clear.

The time to reach peak drug concentration by using a dose of 35 nmol/kg was considerably shorter than that using a dose of 17.5 nmol/kg. However, in both cases the blood concentration of OX was higher than that of EtOX at early times and the relative blood concentrations of OX and EtOX were reversed ~2 hours later. The elimination $t_{1/2}$ of EtOX was considerably longer than that of OX (Fig. 7). The results are consistent with the earlier finding that OX is eliminated primarily by glucuronidation of the hydroxyl group[14-16]. Since EtOX does not have a free hydroxyl group, it does not form a glucuronide conjugate unless it is *O*-deethylated.

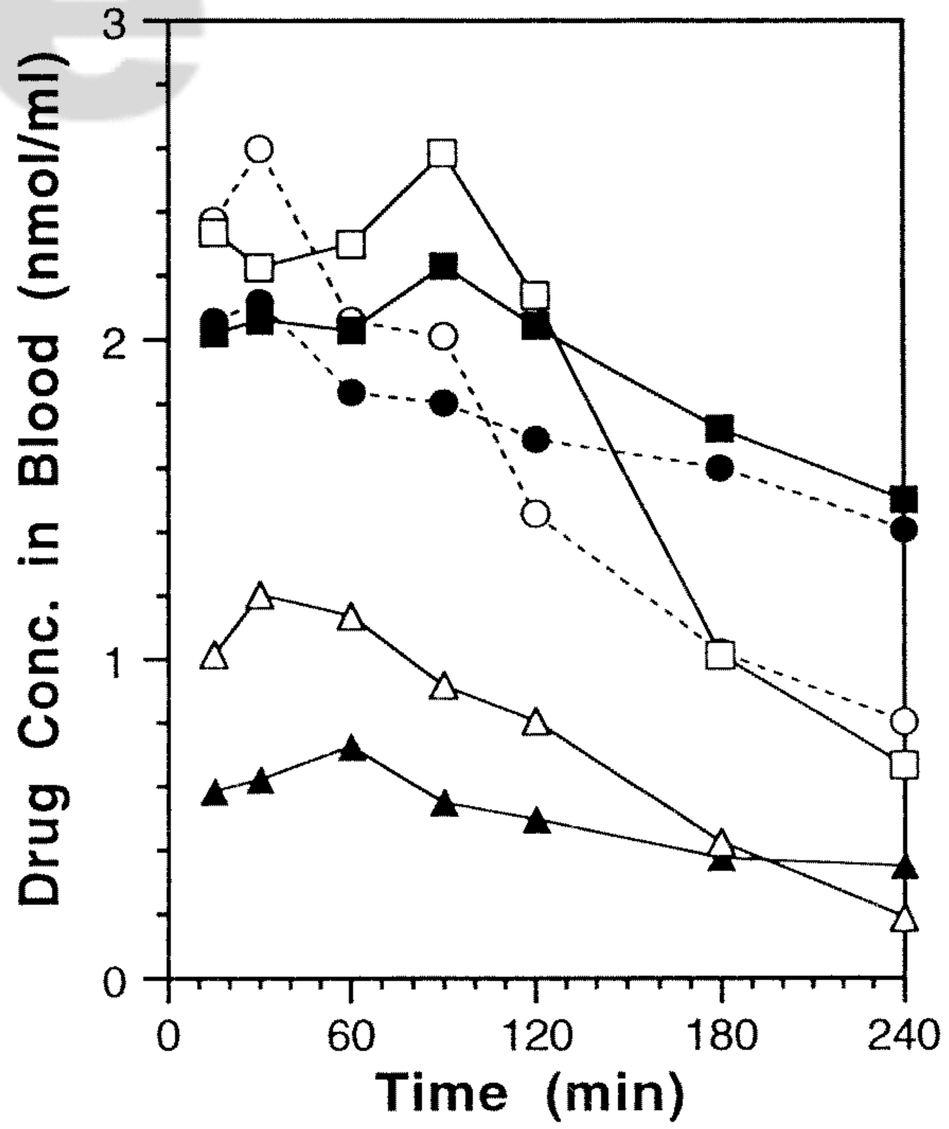


Fig. 7. Pharmacokinetics of OX and EtOX in rats. A mixture containing equal molar amounts of OX (open symbols) and EtOX (closed symbols) (○ and ●, 35 nmol/kg; □ and ■, 17.5 nmol/kg; △ and ▲, 8.75 nmol/kg) was administered by gastric intubation(averages of data from 3 rats in each group). Following anesthesia with diethyl ether, blood samples were taken at various times and prepared for reversed-phase HPLC analysis as described in Materials and Methods.

Pharmacological Activities in Mice

DZ, OX, and TMZ significantly prolonged the pentobarbital-induced sleep time, while MeOX, EtOX, and EtTMZ were considerably less effective (Table 2). MeOX, EtOX, and EtTMZ were also considerably less active in protecting the metrazol-induced seizures and these are consistent with the results reported earlier [17]. The results indicated that acid-catalyzed reactions of both OX and TMZ with ethanol produced pharmacologically less active products. Unlike three other 3-*O*-alkyl derivatives included in this study, MeTMZ was less active than DZ, OX, and TMZ in the prolongation of pentobarbital-induced sleep time and had similar antimetrazol activity as those of DZ, TMZ, and OX in mice (Table 2).

CONCLUSIONS

The results of this study suggest that a significant conversion of OX to EtOX may occur in the acidic medium of the stomach following consumption of ethanol shortly before, shortly after, of simultaneously with the ingestion of OX. The actual extent of EtOX formation in the stomach is expected to vary, depending upon a number of physiological factors such as the exact ethanol and food contents. EtOX is less effective than OX in the prolongation of pentobarbital-induced sleep time in mice. EtOX is also less effective than OX in protecting metrazol-induced seizure in mice. At the same dose in rats, EtOX reaches a slightly lowered peak concentration in blood than OX. However, EtOX exhibits a considerably longer elimination half-life in rats than OX. It is known that ethanol and OX have additive effects in the central nervous system[1]. The results of this study su-

Table 2. Prolongation of pentobarbital (PB)-induced sleep time and antimetrazol activity of OX, TMZ, and their 3-*O*-alkyl derivatives in mice[a]

Drug & Dose[b]	Time to lose righting reflex (min)	Sleep Time (min)	Seizure[c] (%)	Mortality (%)
PB (50 mg/kg)	4.30±0.68	15.6±4.31	—	0
DZ (10 mg/kg)+PB	2.12±0.30	200.8±57.4[d]	—	0
DZ (5 mg/kg)+PB	2.72±0.33	108.5±53.8[d]	—	0
OX (10 mg/kg)+PB	2.25±0.32	106.8±29.4[d]	—	0
OX (5 mg/kg)+PB	3.10±0.50	84.0±34.1[d]	—	0
MeOX (10.5 mg/kg)+PB	3.48±0.93	81.5±30.6[d]	—	0
MeOX (5.3 mg/kg)+PB	3.70±0.97	30.5±12.6[d]	—	0
EtOX (11 mg/kg)+PB	3.55±0.62	47.8±30.7[d]	—	0
EtOX (5.5 mg/kg)+PB	4.85±0.67	33.3±14.7[d]	—	0
TMZ (10.5 mg/kg)+PB	2.78±0.32	130.7±58.1[d]	—	0
TMZ (5.3 mg/kg)+PB	3.13±0.48	129.4±48.4[d]	—	0
MeTMZ (11 mg/kg)+PB	2.83±0.43	76.4±36.6[d]	—	0
MeTMZ (5.5 mg/kg)+PB	2.62±0.98	62.9±24.8[d]	—	0
EtTMZ (11.5 mg/kg)+PB	3.28±0.43	47.6±40.9[d]	—	0
EtTMZ (5.7 mg/kg)+PB	4.83±1.20	30.6±23.5[d]	—	0
Metrazol (110 mg/kg)+PB	—	—	100	100
DZ (9.94 mg/kg)+MTZ	—	—	0	0
DZ (4.97 mg/kg)+MTZ	—	—	0 (1)[f]	0
OX (10.0 mg/kg)+MTZ	—	—	0	0
OX (5.0 mg/kg)+MTZ	—	—	0 (1)[f]	0
MeOX (10.5 mg/kg)+MTZ	—	—	92 (1)[f]	67
MeOX (5.3 mg/kg)+MTZ	—	—	100	73
EtOX (11 mg/kg)+MTZ	—	—	0 (4)[f]	0
EtOX (5.5 mg/kg)+MTZ	—	—	25 (6)[f]	0
TMZ (10.5 mg/kg)+MTZ	—	—	0	0
TMZ (5.3 mg/kg)+MTZ	—	—	0 (1)[f]	0
MeTMZ (11 mg/kg)+MTZ	—	—	0	0
MeTMZ (5.5 mg/kg)+MTZ	—	—	0 (3)[f]	0
EtTMZ (11.5 mg/kg)+MTZ	—	—	100	58
EtTMZ (5.7 mg/kg)+MTZ	—	—	100	83

[a]Experiments were conducted as described in the Experimental Section; 12 mice were used in each group and each dose.

[b]In benzodiazepine-pretreated groups, PB and MTZ were administered at 50 mg/kg and 110 mg/kg, respectively.

[c]Appearance of tonic hindleg extensor component of the seizure.

[d]$P<0.01$.

[e]$P>0.05$.

[f]The number in parenthesis is the number of mice with appearance of tail straight up component of the seizure.

ggest that the pharmacological effects of OX itself in humans may be considerably reduced by co-ingestion of alcohol.

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乙醇與oxazepam在胃酸中之化學反應及其對藥效之影響

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摘　要

Oxazepam是用作治療焦慮之藥物。Oxazepam (OX)在酸性水溶液，無水乙醇，或模擬胃液中與乙醇起化學反應，產生3-*O*-ethyloxazepam (EtOX)。OX在37°C含有30%(體積比)乙醇之模擬胃液中，2小時後約有9.5%轉換成EtOX。本研究結果指出，OX在酸性溶液中其3位碳上之羥基被乙醇之乙氧基取代而形成EtOX。以大白鼠作藥物代謝動力學比較之結果顯示，口服OX與EtOX之吸收程度差別不大，而EtOX之排除速率比OX較慢。以小白鼠所作兩種藥效試驗之結果顯示，EtOX之藥效比OX較弱。文獻中報導同時服用酒精與OX對中樞神經系統會有加成性效應。本研究結果顯示在飲酒前後或同時進服OX可能會在胃液的酸性條件下產生EtOX而導致OX本身之藥效降低。