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A One-step Synthesis of [3-^{2}H]Oxazepam

Shen K. Yang,[a] Ziping Bao,[a] and Magang Shou[b]

*[a]Department of Pharmacology, F. Edward Hébert School of Medicine, Uniformed Services University of the Health Sciences, Bethesda, MD 20814 and [b]Laboratory of Molecular Carcinogenesis, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892

SUMMARY

The proton at 3-position of oxazepam (7-chloro-1,3-dihydro-3-hydroxy-5-phenyl-2*H*-1,4-benzodiazepin-2-one) undergoes a deuterium exchange in deuterated alkaline methanol. A base-catalyzed keto-enol tautomerism is proposed to be responsible for the observed deuterium exchange. This simple method is a considerable improvement of the multi-step synthetic procedure reported in the literature.

Key Words: Oxazepam, keto-enol tautomerism, deuterium exchange, mass spectrometry.

INTRODUCTION

Oxazepam (OX, Figure 1) and diazepam (DZ) are among the frequently prescribed anxiolytic/hypnotic drugs (1). OX is a pharmacologically active metabolite of DZ. In gas chromatography-mass spectrometry (GC-MS) analysis, OX undergoes thermal degradation to lose a water molecule to form 6-chloro-4-phenylquinazoline-2-carboxaldehyde (CPQCA, Figure 1) (2-4). The availability of [3-^{2}H]OX played an important role in the understanding of the mechanism involved in the thermal degradation of OX (2-4). [3-^{2}H]OX was previously synthesized via a 3-step procedure; [3-$^2H_2$]NDZ → [3-$^2H_2$]NDZ 4–oxide → [3-^{2}H]OXA → [3-^{2}H]OX (3, 5). The disadvantages of this 3-step synthetic procedure are: (i) a low overall yield of OX and (ii) the deuterium content of OX is determined by the deuterium content of NDZ (3). To date, a simple procedure to synthesize a NDZ containing ≥90 atom % deuterium at the 3-position is not available. Thus, the preparation of a [3-^{2}H]OX with a high deuterium content at the 3-position cannot yet be achieved by the 3-step synthetic procedure.

Dr. Shen K. Yang (Tel. +301-295-3225, Fax. +301-295-3220)

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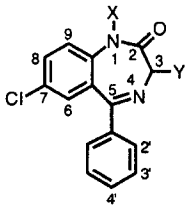
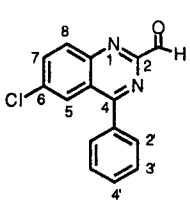
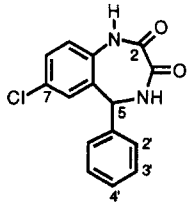


DZ, X = CH_3, Y = H
NDZ, X = Y = H
OX, X = H, Y = OH
OXA, X = H, Y = $OCOCH_3$



CPQCA



OX-RP

Fig. 1. Structure and abbreviation of diazepam (DZ), nordiazepam (NDZ), oxazepam (OX), oxazepam 3-acetate (OXA), 6-chloro-4-phenylquinazoline-2-carboxaldehyde (CPQCA), and 7-chloro-4,5-dihydro-5-phenyl-2H-benzodiazepin-2,3(1H)-dione (OX-RP).

The proton at the 3-position of OX can be considered an α-ketonic proton. There have been extensive studies on the acid- and base-catalyzed deuterium exchange of compounds containing α-ketonic protons (6). Other than a recent report (7), the mechanism of deuterium exchange occurring in a 1,4-benzodiazepine system has not been reported. We recently reported (7) that OXA undergoes, in addition to hydrolysis, a deuterium exchange reaction at 3-position due to a base-catalyzed keto-enol tautomerism. We now report that OX can undergo an efficient deuterium exchange at 3-position in deuterated solvents via a keto-enol tautomerism.

EXPERIMENTAL

Materials

OX was generously provided by Wyeth-Ayerst Research (Princeton, NJ). Dioxane, acetonitrile (MeCN), dimethylformamide (DMF), tetrahydrofuran (THF), acetone, C_2H_5OD (EtOD; 99.5+ atom % D), CH_3OD (MeOD; 99.5+ atom % D), D_2O (99.9 atom % D), and NaOD (40 wt % solution in D_2O, 99.9 atom % D) were obtained from Aldrich Chemical Co. (Milwaukee, WI).

Methods

Acidity Constant in Alkaline Methanol. The wavelength (352 nm) for monitoring absorbance change as a function of NaOH concentration was determined by a difference spectrum between the alkaline and the neutral forms of OX. Absorbance values at 352 nm of OX (0.32 mM) in methanol containing various concentrations of NaOH (each in triplicate) was determined at ambient temperature (23±1°C), within 2 min following the addition of NaOH, using a 1 cm path length quartz cuvette on a DW2000 spectrophotometer (SLM Instruments, Urbana, IL). Following a plot of absorbance vs. [NaOH], K_a (acidity constant) was determined by a curve fitting program of SigmaPlot (Jandel Scientific, Corte Madera, CA) on an Apple Macintosh computer.

Deuterium Exchange. OX (0.2 mg) in 1 mL of either 1:1 (v/v) mixtures of MeCN:D_2O, acetone:D_2O, DMF:D_2O, THF:D_2O, dioxane:D_2O, MeOD:D_2O, EtOD:D_2O, or an anhydrous MeOD containing various concentrations of NaOD was heated at either ambient temperature (23±1°C), 25.0±0.1°C, or 50.0±0.1°C for various lengths of time. Triplicate samples were performed for each temperature and base concentration. Each reaction mixture was terminated by the addition of 1 mL of 1 M KH_2PO_4 (pH 4.4). OX does not undergo deuterium exchange in solutions with pH < 8. OX was extracted into 3 mL of ethyl acetate. The organic phase was dried with anhydrous $MgSO_4$ and evaporated to dryness. The purity of OX in the residue was determined by reversed-phase HPLC. Whenever necessary, OX was purified by reversed-phase HPLC for mass spectral analysis.

Reversed-Phase HPLC. Reaction products were analyzed by reversed-phase HPLC using a Waters Associates (Milford, MA) Model M45 solvent pump and a Model 441 absorbance detector (254 nm). A Zorbax SB-C18 column (5 μ particles, 4.6 mm i.d. x 15 cm; Mac-Mod Analytical Inc., Chadds Ford, PA) was used. The mobile phase was MeOH:MeCN:0.02 M phosphate buffer pH 7 (10:40:50, v/v/v) at a flow rate of 1 mL/min. HPLC analysis was performed at ambient temperature. Samples were injected via a Shimadzu (Shimadzu Corp., Kyoto, Japan) Model SIL-9A automatic sample injector.

MS Analysis. GC-MS analysis was performed on a Hewlett-Packard 5890 instrument with a 5971 mass selective detector and a HP Vectra QS/20 PC computer using an HP-G1030A MS ChemStation (DOS series) software. A Heliflex (Deerfield, IL) AT-1 silica capillary column (30 cm x 0.25 mm x 1 μm film) was used. Samples were dissolved in dichloromethane (~0.5 μg/mL) and 1 to 5 μL was injected via an autosampler for analysis. Ionization was by electron impact (70 eV). The carrier gas was helium and a temperature program of 13.3 min (30°C/min from 50 to 150°C and then 15°C/min from 150 to 300°C) was used. Direct exposure electron impact MS analysis was performed on a Finnigan 4500 gas chromatograph-mass spectrometer-data system (Finnigan MAT, San Jose, CA) with a direct exposure probe by electron impact at 70 eV. The ion source was maintained at 105°C.

RESULTS AND DISCUSSION

A difference spectrum (Fig. 2A) indicated that changes in absorbance as a function of [NaOH] could be monitored at 352 nm. The pK_a of OX in alkaline methanol was determined to be 11.7 (K_a = 5.4 mM OH^-, Fig. 2B). In 1:10 (v/v) MeCN:aqueous buffer solution, the pK_a of OX was found to be 11.5 (7). In a recent study (7), deprotonation of OXA at N1 was found not to be responsible for the base-catalyzed deuterium exchange and racemization. These results suggest that OX is also

deprotonated at N1 and forms an N1-C2 imine bond in alkaline media. In contrast, Barrett et al. (8) suggested that the C3-hydroxyl group of OX is deprotonated in alkaline aqueous media.

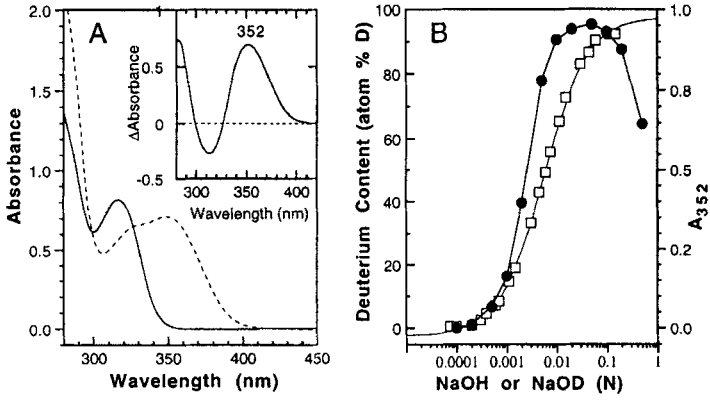


Fig. 2. (A). Ultraviolet-visible absorption spectra of OX in MeOH (——) and MeOH containing 0.01 N NaOH (– – – –) at ambient temperature. The difference spectrum ($A_{alkaline\ MeOH} - A_{MeOH}$) is shown in the inset.
(B). [NaOH]-dependent absorption of OX in MeOH at ambient temperature (□) and [NaOD]-dependent deuterium incorporation into OX in MeOD at 50°C for 16 hr (●).

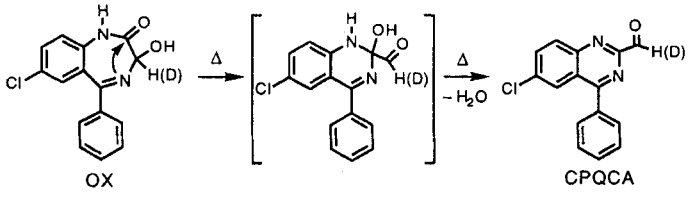


Fig. 3. Mechanism in the formation of CPQCA by thermolysis of undeuterated and 3-deuterated OX observed in GC-MS analysis (2-4).

It was reported (2-4) that OX undergoes thermolysis to form CPQCA by losing a water molecule in GC-MS analysis (Figure 3) and the oxygen of the water is derived from the C2-carbonyl group. The hydrogen at 3-position of OX is retained in the thermolytic product (Figure 3). Because of the capability for unattended operation, GC-MS is ideally suited to study the deuterium incorporation into OX. Figure 4 shows the mass spectra of the thermolytic products of undeuterated and 3-deuterated OX. These mass spectra are consistent with those reported earlier (2-4), with the exception that the highly deuterated OX was not previously available. The percentages of deuterium incorporation into OX can be calculated by the relative ion intensities at either m/z 233 and 234 or m/z 268 and 269; both methods of calculation gave essentially the same results.

The effects of various solvents containing 0.02 N NaOD on the deuterium incorporation into OX at 50°C for 16 hr are shown in Table 1. Deuterated methanol (MeOD), with or without D_2O, gave the

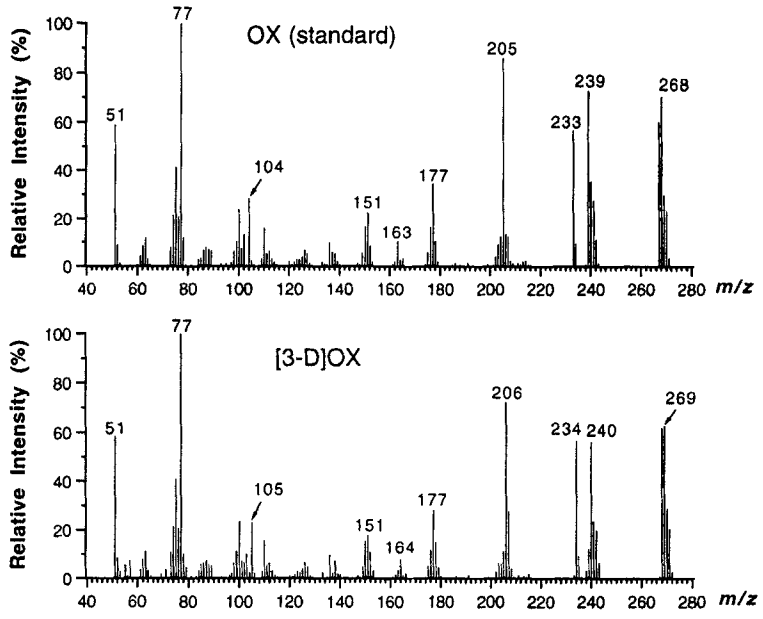


Fig. 4. Mass spectra of the thermolytic products of (A) unlabeled OX and (B) [3-D]OX (97.6 atom % D) by GC-MS. In direct electron impact MS analysis, these compounds exhibited molecular ions at m/z 286 and 287, respectively.

highest deuterium incorporation. EtOD was also effective. THF, DMF, dioxane, MeCN, and acetone were considerably less effective as a co-solvent.

The time-dependent deuterium incorporation into OX in MeOD containing 0.02 N NaOD at 25 and 50°C and 0.1 N NaOD at 50°C is shown in Figure 5. At 50°C, the kinetics of deuterium incorporation was not significantly different for a 5-fold increase in [NaOD]. Curve fitting of the data up to 10 hr indicated an apparent first-order reaction with $t_{1/2}$ of 1.32 hr (0.02 N NaOD) and 1.38 hr (0.1 N NaOD), respectively. At 25°C in 0.02 N NaOD, deuterium incorporation was considerably slower and the reaction was also an apparent first-order reaction with a $t_{1/2}$ of 18.8 hr.

Reversed-phase HPLC analysis of the products formed from OX in 0.02 N NaOD at 50°C for various times (Figure 6) indicated a time-dependent formation of both OX-RP (see structure in Figure 1) and an unknown product (broad peak U in Figure 6). In 0.02 N NaOD at 25°C, the formations of OX-RP and unknown product were insignificant. At 50°C, an increasing amount of OX was converted to OX-RP at [NaOD] > 0.02 N. At [NaOD] = 0.5 N, ~90% of OX was converted to OX-RP. At [NaOD] < 0.005 N, the formation of OX-RP was insignificant, but a significant amount of an unknown product (peak U in Figure 6) was formed. Preliminary results indicated that the unknown product formed from unlabeled OX was not CPQCA, but had a mass spectrum identical to that of CPQCA by GC-MS analysis.

Table 1. Effects of solvents on base-catalyzed deuterium incorporation into OX.

Solvent[1]	Deuterium Content (atom % D)[2]
MeOD	93.1±0.4
MeOD/D_2O (1:1, v/v)	92.8±0.3
EtOD/D_2O (1:1, v/v)	83.7±0.1
THF/D_2O (1:1, v/v)	33.5±0.7
DMF/D_2O (1:1, v/v)	29.4±1.0
dioxane/D_2O (1:1, v/v)	18.0±0.6
MeCN/D_2O (1:1, v/v)	8.3±1.3
acetone/D_2O (1:1, v/v)	4.9±0.7

[1] Each solvent mixture contained 0.02 N NaOD.

[2] Each reaction mixture (1 mL) contained 0.2 mg of OX and was heated at 50.0±0.1°C for 16 hr. Deuterium content of the resulting deuterated OX was determined as CPQCA by GC-MS. Data are mean±SD (n=3).

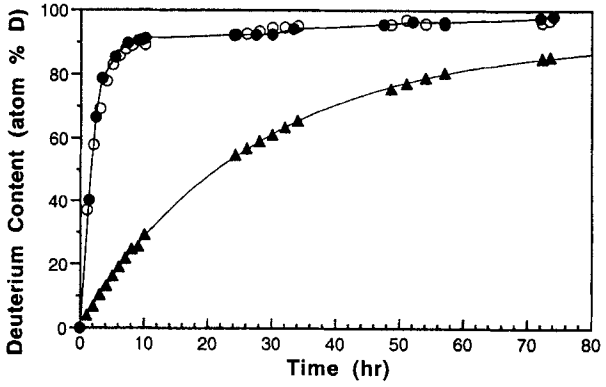


Fig. 5. Kinetics of deuterium exchange of OX in MeOD containing 0.1 N NaOD (●) and 0.02 N NaOD (○) at 50°C, and 0.02 N NaOD at 25°C (▲).

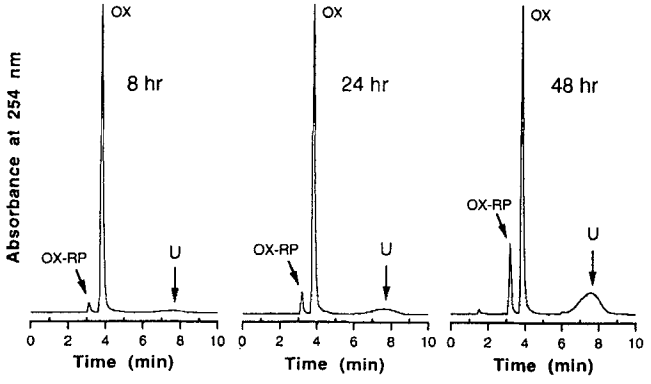


Fig. 6. Reversed-phase HPLC analysis of products formed from OX in MeOD containing 0.02 N NaOD at 50°C for 8, 24, and 48 hr. OX-RP is a rearrangement product of OX and an unknown decomposition product was eluted as a broad peak (labeled as U).

The [NaOD]-dependent deuterium exchange of OX in MeOD at 50°C for 16 hr is shown in Fig. 2B. A 95% deuterium incorporation was obtained with 0.05 N NaOD. The extent of deuterium incorporation decreased at [NaOD] > 0.05 N. Deuterium incorporation at half maximum occurred at 2.5 mM NaOD, a base concentration lower than the K_a value (5.4 mM OH^-) of OX determined by spectrophotometry (Figure 2B). The increase in absorbance at 352 nm upon the addition of NaOH (Figure 2B) occurs instantaneously. In contrast, the deuterium incorporation into OX is a relatively slow process (Fig. 2B). The results indicate that the observed deuterium exchange is not caused by a simple acid-base equilibrium.

Undeuterated and deuterated OX-RP were prepared from OX by heating undeuterated and deuterated methanol solutions containing 0.5 N NaOH and NaOD, respectively, at 50°C for 24 hr. The OX-RP had an ultraviolet absorption spectrum (not shown) similar to that of the base-catalyzed rearrangement product of temazepam (9, 10). Temazepam is N-methyloxazepam. Both GC-MS and direct electron impact MS analyses indicated that the undeuterated and deuterated OX-RP had the expected molecular ions at m/z 286 and 287, respectively. The deuterium atom of the deuterated OX-RP is expected to be at C5 position, similar to that found in the deuterated rearrangement product of temazepam (10).

The results of time-dependent deuterium incorporation (Figure 5) and reversed-phase analysis (Figure 6) suggested that a high [NaOD] and a temperature greater than 50°C should be avoided to prepare [3-D]OX. An experiment was conducted to test the effects of reaction cycles on the extent of deuterium incorporation (Table 2). In MeOD containing 0.02 N NaOD, 3 cycles of an 8 hr reaction at 50°C increased the deuterium incorporation from 84.6 to 95.2 atom % D. However, significant amounts of OX-RP and the unknown product were formed (see Figure 6). At 50°C, it was not possible to achieve a complete (100%) deuterium incorporation without significant decomposition. Seven cycles of a 24 hr reaction at ambient temperature (23±1°C) resulted in ~83% deuterium incorporation (Table 2). Since there was no significant decomposition at ambient temperature, additional cycles of 24-hr reactions may be carried out to achieve a higher degree (>90%) of deuterium incorporation.

The acid-base equilibrium (pathway A) and reactions (pathways B and C) of OX in alkaline methanol are proposed in Figure 7. Spectrophotometric measurements indicated that neutral and alkaline forms of OX (Figure 2A) interconvert instantaneously upon the change of pH. Hence, deprotonation of N1 hydrogen and ionization of C2-carbonyl oxygen to form a N1-C2 imine bond do not contribute to the base-catalyzed deuterium exchange reaction. We propose that the deuterium

Table 2. Effects of reaction cycles on base-catalyzed deuterium exchange of OX.

Reaction Condition[1]	Deuterium Content (atom % D)[2]
0.02 N NaOD in MeOD at 50°C for 8 hr	
1 cycle	84.6 ±0.3
2 cycles	90.5 ±0.1
3 cycles	95.2 ±0.7
0.02 N NaOD in MeOD at ambient temperature for 24 hr	
1 cycle	23.2±2.3
2 cycles	48.0±1.8
3 cycles	61.1±0.7
4 cycles	66.4±1.8
5 cycles	69.5±1.7
6 cycles	73.1±2.0
7 cycles	83.3±0.4

[1] Each reaction cycle indicates a reaction with the indicated solvent, temperature, and reaction time, followed by neutralization and extraction of reaction product.

[2] Deuterium content of the resulting deuterated OX was determined as CPQCA by GC-MS. Data are mean±SD (n = 3).

exchange is caused by a base-catalyzed keto-enol tautomerism (pathway B), similar to that proposed for OXA (7). A dianion may be formed in equilibrium with the enol form (pathway B). OX undergoes deuterium exchange at a relatively slow rate. Another relatively slow base-catalyzed reaction is the rearrangement of OX to form OX-RP (pathway C). The mechanism of base-catalyzed conversion of OX to OX-RP is expected to be similar to the base-catalyzed rearrangement of temazepam, the latter is initiated by the addition of a hydroxide ion at the C2-carbonyl carbon (10). The formation of an unknown product (Figure 6) is not indicated in Figure 7.

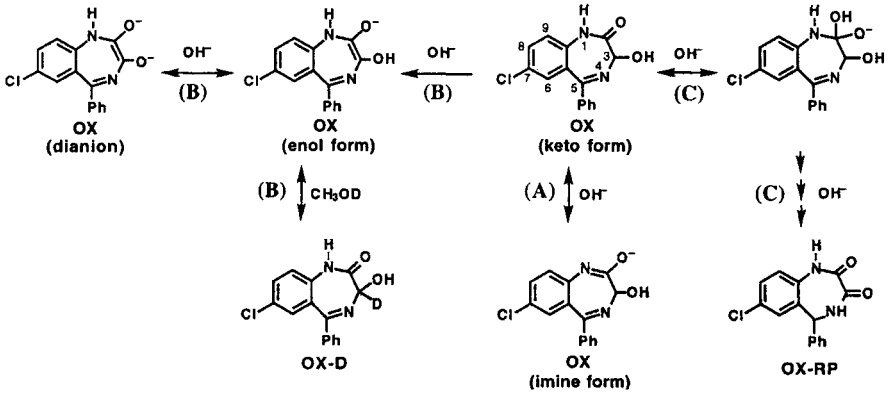


Fig. 7. Proposed acid-base equilibrium of OX (A), the keto-enol tautomerism responsible for the observed deuterium exchange (B), and base-catalyzed formation of OX-RP (C) in alkaline media. See text for discussion.

In conclusion, a simple one-step procedure is described to prepare [3-D]OX containing >90 atom % D in deuterated alkaline methanol at 50°C. However, a small but significant fraction of OX undergoes decomposition at 50°C. By carrying out more than seven cyles of the same reaction at ambient temperature, a [3-D]OX containing >90% atom % D may also be prepared without significant decomposition. The method may be generally applicable to the preparation of other 3-deuterated 1,4-benzodiazepines.

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