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Acid-base Equilibria of 1,4-Benzodiazepine 4-Oxides by Spectrophotometry

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Abstract

Chlordiazepoxide (a 1,4-benzodiazepine 4-oxide) is an anxiolytic/hypnotic drug in clinical use. It was reported to be predominantly protonated at the *N*-oxide oxygen in acidic aqueous solutions at pH $\ll$ 4·6 (pKa). We have studied the acid-base equilibria of three 1,4-benzodiazepine 4-oxides (chlordiazepoxide, diazepam 4-oxide, and nordiazepam 4-oxide) by ultraviolet-visible spectrophotometry. The results indicate that chlordiazepoxide is not protonated at the *N*-oxide oxygen, but rather at the nitrogen of an imine bond between C2 carbon and its nitrogen substituent in acidic media.

The fractions of un-ionized and ionized drugs at physiologically relevant pH are important determinants in the extent of drugs absorbed across biological barriers to exert their pharmacological effects. This knowledge is also of value in the development of extraction and analytical procedures in the determination of drugs or metabolites present in various body fluids.

The acid-base equilibria of six 1,4-benzodiazepines including diazepam (Fig. 1), chlordiazepoxide, medazepam, oxazepam, nitrazepam, and lorazepam in methanol : aqueous solution (1:19, v/v) over the pH range 1–13 have been studied by ultraviolet spectrophotometry (Barrett et al 1973). The spectra were interpreted with considerable success by considering them to be superimposed spectra of the two benzene rings, one monosubstituted and one tri-substituted, within the molecules. Diazepam, medazepam, oxazepam, nitrazepam, and lorazepam (pK_a values 1·3–4·4) are protonated in acidic solutions at N4 in the diazepine ring. Chlordiazepoxide, an *N*-oxide, has a pK_a of 4·6 in acidic solutions and the protonation was proposed to occur at the *N*-oxide oxygen (Barrett et al 1973).

Our interest in the acid-base equilibria of 1,4-benzodiazepines originated from studies for understanding the effects of protonation and deprotonation on the nucleophilic substitution and racemization of enantiomeric 3-hydroxy- and 3-alkoxy-1,4-benzodiazepines (Yang & Lu 1992, 1993; Yang 1994a, b). Consistent with the results reported earlier (Barrett et al 1973), we found that 3-hydroxy- and 3-alkoxy-derivatives of diazepam, nordiazepam, 2′-chlorodiazepam, and 2′-chloronordiazepam in strongly acidic aqueous solutions were all protonated at N4 of the diazepine ring. In this report, we describe the acid-base equilibria of chlordiazepoxide, diazepam 4-oxide, and nordiazepam 4-oxide studied by ultraviolet-visible spectrophotometry. The results indicate that the earlier report (Barrett et al 1973) regarding the site of protonation of chlordiazepoxide in an acidic solution was in error.

Materials and Methods

Chemicals and reagents

Demoxepam (nordiazepam 4-oxide) and chlordiazepoxide were generously provided by Hoffmann-La Roche Inc. (Nutley, NJ). Nordiazepam 4-oxide and diazepam 4-oxide are synthetic precursors of oxazepam and temazepam and their 3-*O*-acyl derivatives, respectively (Bell & Childress 1962). Diazepam 4-oxide was prepared by dissolving nordiazepam 4-oxide (100 mg) in 5 mL acetonitrile : 10 M NaOH (200 : 3, v/v), followed by the addition of dimethyl sulphate (0·05 mL). The mixture was stirred under heating at 60°C for 2 h. Following neutralization and extraction, the resulting diazepam 4-oxide (~85% yield) was purified by reversed-phase HPLC (Yang 1994a). Chemical ionization (NH_3) mass spectral analysis (model 4500; Finnigan MAT, San Jose, CA; the ion source was maintained at 105°C) of the purified diazepam 4-oxide indicated mass ions at *m/z* 285 (base peak, loss of oxygen from the M + 1 ion), 301 (M + 1), and 318 (MH-NH_3) and the associated chlorine isotope ions. Britton-Robinson buffer solutions (pH 2–13) were prepared as described by Barrett et al (1973).

Spectral analysis and determination of pK_a values

The wavelengths for monitoring absorbance changes due to acidification and alkalization were determined by a difference spectrum between the acidic (or alkaline) and the neutral forms as described earlier (Yang & Lu 1993). Absorbance of samples was determined using a 1-cm pathlength quartz cuvette on a model DW2000 spectrophotometer (SLM Instruments, Urbana, IL). Absorbance values at 270 nm (for chlordiazepoxide) and 266 nm (for nordiazepam 4-oxide) of samples in triplicate were recorded for solutions containing an identical amount of each drug in ethanol : Britton-Robinson buffer (1:10, v/v) at ambient temperature (23 ± 1°C). Following an absorbance vs pH plot, the pK_a was determined by a curve-fitting program of SigmaPlot (Jandel Scientific, Corte Medera, CA) on an Apple Macintosh computer.

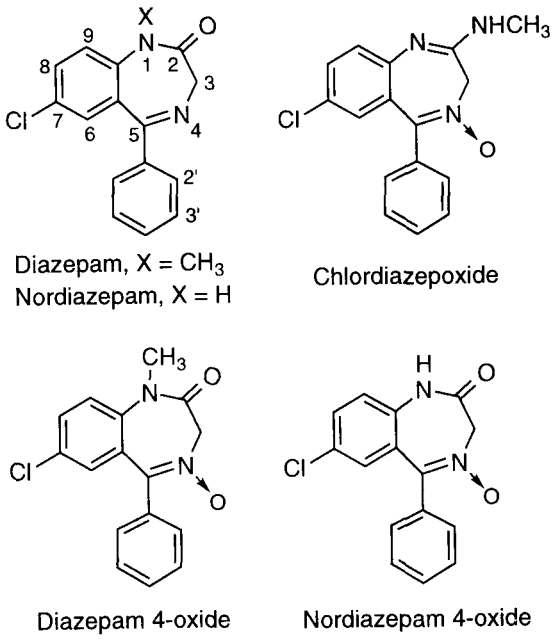

FIG. 1. Structure of diazepam, nordiazepam (*N*-desmethyldiazepam), chlordiazepoxide, diazepam 4-oxide, and nordiazepam 4-oxide (demoxepam).

Results and Discussion

The absorption spectra of chlordiazepoxide in acidic, neutral and alkaline solutions are shown in Fig. 2. The absorption spectra in neutral and alkaline media are essentially identical. The results are consistent with the fact that chlordiazepoxide does not have a dissociable proton. The observed absorption maxima (244, 264, and 310 nm) in neutral and alkaline solutions (Fig. 2) were slightly different from those (250, 260, and 310 nm) reported earlier (Barrett et al 1973).

The neutral-acid difference spectrum of chlordiazepoxide (absorbance of a neutral solution–absorbance of an acidic solution; inset of Fig. 2) indicated that 270 nm was a suitable wavelength for monitoring absorption changes as a function of pH. The pK_a of chlordiazepoxide in acidic solutions was determined to be 4·8 by plotting the absorbance changes as a function of pH (Fig. 3). This pK_a value is similar to a value of 4·6 (Barrett et al 1973) and identical to a value of 4·76 ± 0·05 (MacDonald et al 1972) determined by spectrophotometry, and similar to a value of 4·9 determined by titration using NaOH (MacDonald et al 1972).

The changes in absorption properties of chlordiazepoxide upon acidification were proposed to be due to protonation at the *N*-oxide oxygen (Barrett et al 1973). If the proposed site of protonation applies in general to other 1,4-benzodiazepine 4-oxides, the absorption properties of nordiazepam 4-oxide and diazepam 4-oxide (Fig. 1) in acidic solutions should be different from those in neutral solutions. The absorption spectra of nordiazepam 4-oxide and diazepam 4-oxide in acidic, neutral, and alkaline aqueous solutions are shown in Fig. 4. The absorption spectra of either nordiazepam 4-oxide (Fig. 4A) or diazepam 4-oxide (Fig. 4B) in neutral and acidic media are essentially identical. The results

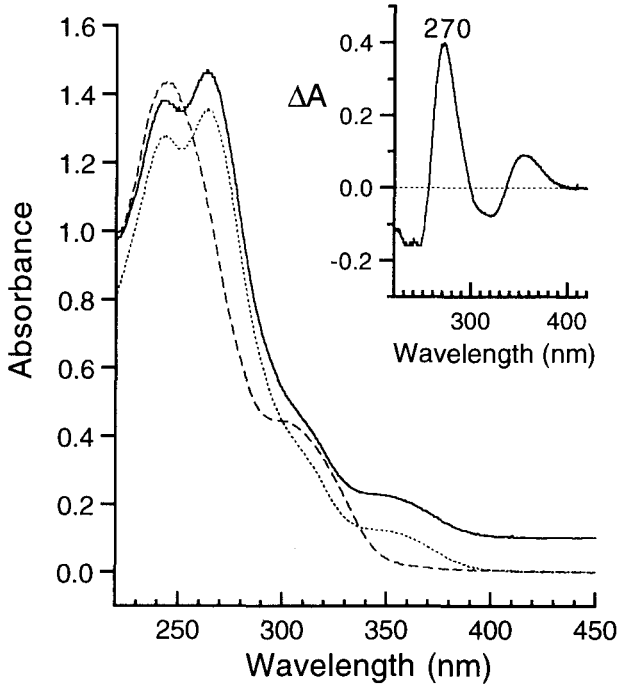

FIG. 2. UV-vis absorption spectra of chlordiazepoxide (15·9 μg mL^{-1}) in acetonitrile : 1 M HCl (1 : 3, v/v) (---; absorption peaks at 244 and 310 nm), acetonitrile : pH 7 buffer (1 : 3, v/v) (—; for offsetting purpose, all absorbance values are increased by 0·1), and acetonitrile : 0·1 M NaOH (1 : 3, v/v) (· · ·). The absorption peaks/shoulders of chlordiazepoxide in neutral and alkaline solutions are at 243, 264, 310, and 356 nm. The inset is a difference spectrum (absorption of a neutral solution–absorption of an alkaline solution).

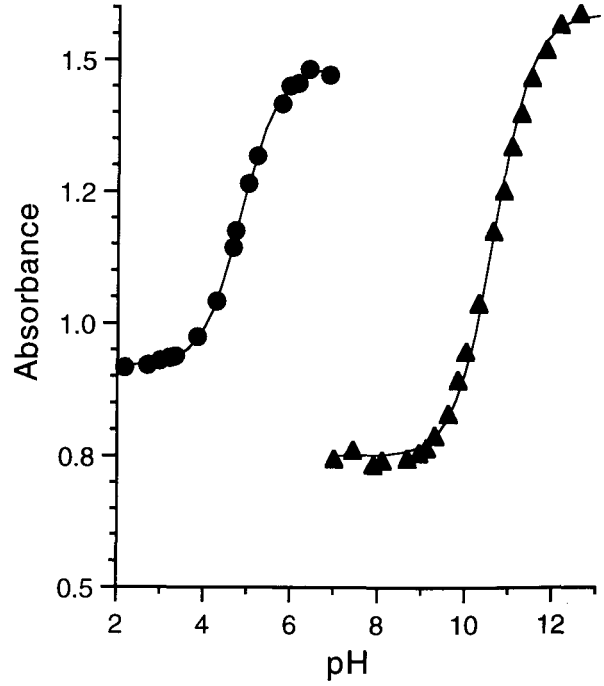

FIG. 3. Absorption changes of chlordiazepoxide (●, at 270 nm; pK_a = 4·8) and nordiazepam 4-oxide (▲, at 266 nm; pK_a = 10·6) as a function of pH in ethanol : Britton-Robinson buffer (1 : 10, v/v) at room temperature (23 ± 1°C). Absorbance values were averages of triplicate samples; the magnitudes of standard deviations were smaller than the sizes of symbols used in the figure. The S-shaped curves were fitted with a curve-fitting computer program.

indicate that the proposed site of protonation for chlordiazepoxide (Barrett et al 1973) is not applicable to the 4-oxides of nordiazepam and diazepam.

Compared with those in acidic and neutral media, the absorption bands of nordiazepam 4-oxide in an alkaline solution were shifted to longer wavelengths (Fig. 4A). Reversed-phase HPLC analysis using a neutral aqueous mobile phase (Yang 1994a) indicated that nordiazepam 4-oxide was stable in an alkaline solution (pH 13; data not shown). The alkali-neutral difference spectrum of nordiazepam 4-oxide (inset of Fig. 4A) indicated that 266 nm was a suitable wavelength for monitoring absorption changes as a function of pH in the alkaline region. The pK_a of nordiazepam 4-oxide was determined to be 10·6 by plotting the absorbance changes as a function of pH (Fig. 3).

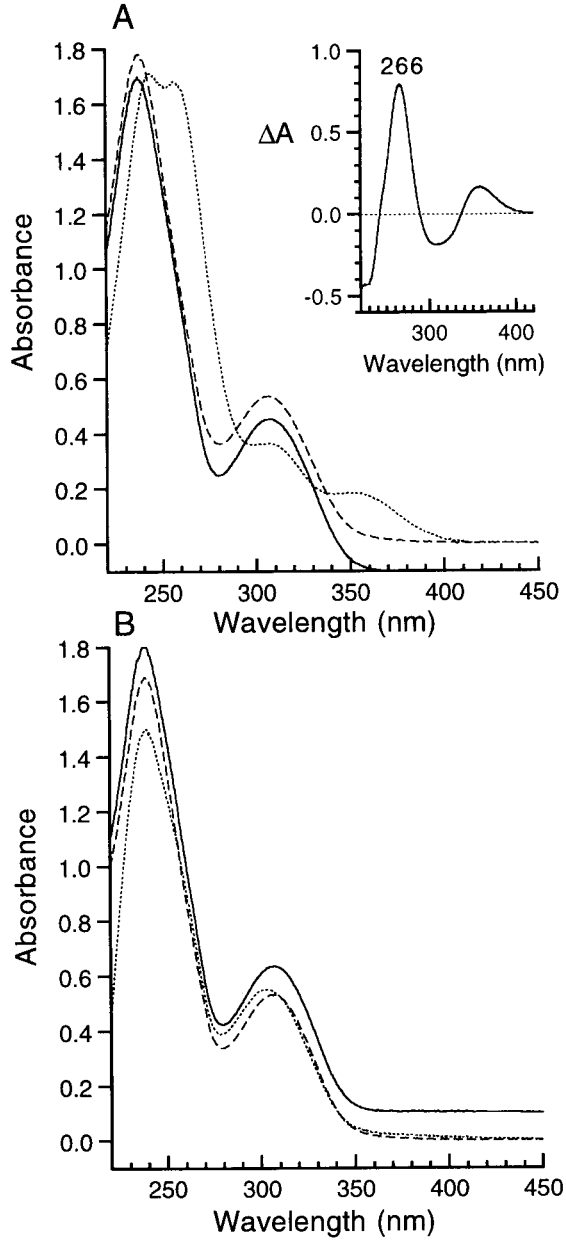

FIG. 4. A. UV-vis absorption spectra of nordiazepam 4-oxide (17·3 $\mu g\ mL^{-1}$) in acetonitrile : 1 M HCl (1 : 3, v/v) (---; absorption peaks at 237 and 308 nm), acetonitrile : pH 7 buffer (1 : 3, v/v) (—; absorption peaks at 237 and 308 nm; for offsetting purpose, all absorbance values are decreased by 0·1), and acetonitrile : 0·1 M NaOH (1 : 3, v/v) (···; absorption peaks/shoulders at 244, 258, 309 and 356 nm). The inset is a difference spectrum (absorption of an alkaline solution–absorption of a neutral solution). B. UV-vis absorption spectra of diazepam 4-oxide (16·3 $\mu g\ mL^{-1}$) in acetonitrile : 1 M HCl (1 3, v/v) (---; absorption peaks at 239 and 306 nm), acetonitrile : pH 7 buffer (1 : 3, v/v) (— absorption peaks at 239 and 306 nm; for offsetting purpose, all absorbance values are increased by 0·1), and acetonitrile : 0·1 M NaOH (1 : 3, v/v) (···; absorption peak/shoulder at 239 and 302 nm).

Diazepam 4-oxide does not have a dissociable proton and hence it does not possess a pK_a in the alkaline region. Although there were no major shifts in absorption bands, minor changes in the absorption properties of diazepam 4-oxide were observed upon alkalization (Fig. 4B). Preliminary results by reversed-phase HPLC analysis (data not shown) indicated that the minor changes observed in the absorption spectrum of diazepam 4-oxide upon alkalization were due to the formation of some yet unidentified decomposition products.

Since both 4-oxides of nordiazepam and diazepam in neutral and acidic solutions have essentially the same absorption properties (Fig. 4), they are either not protonated or the protonations in acidic solutions do not result in significant absorption changes from those in neutral solutions. The protonation, a diffusion-controlled process, probably occurs at the C2 carbonyl oxygen. Protonation at the carbonyl oxygen in acidic solutions is known to occur in carbonyl compounds (Carey & Sundberg 1990). Protonations at the C2 carbonyl oxygens of nordiazepam 4-oxide and diazepam 4-oxide are not expected to alter significantly the absorption properties because of the lack of conjugation between the aromatic ring and the C2 carbonyl group. Since protonation of nordiazepam 4-oxide or diazepam 4-oxide does not cause changes in absorption properties, the absorption changes in chlordiazepoxide upon acidification cannot be due to protonation at the *N*-oxide oxygen.

Except for red shifts of 4–5 nm, the neutral-acid difference spectrum of chlordiazepoxide (inset in Fig. 2) is closely similar to the alkali-neutral difference spectrum of nordiazepam 4-oxide (inset in Fig. 4A). The similarity in the difference spectra suggests similar structural differences. We propose that nordiazepam 4-oxide in an alkaline solution with $pH > pK_a$ exists predominantly in the anionic form, with an N=C double bond (an imine bond) between N1 and C2 (Fig. 5), similar to the anionic structure suggested for nitrazepam (pK_a=10·8) in an alkaline solution (Barrett et al 1973). Diazepam 4-oxide in an alkaline solution can not form an imine bond between C1 and N2 because of a methyl substituent at N1. In a neutral solution, chlordiazepoxide exists predominantly in the un-ionized state with an N=C double bond between N1 and C2 (Fig. 5). Because chlordiazepoxide's neutral-acid difference spectrum is similar to nordiazepam 4-oxide's alkali-neutral difference spectrum, it is likely that the N1–C2 bond of chlordiazepoxide also exists predominantly as a single bond in an acidic solution with $pH < pK_a$. To account for the observed results, an amidine-imine tautomerism (Fig. 6) is proposed for chlordiazepoxide in neutral media. Structure I (amidine form) predominates at $pH > pK_a$ in neutral and alkaline solutions and structure III (an iminiun ion derived from imine II) predominates at $pH < pK_a$. The predominant structures of nordiazepam 4-oxide, diazepam 4-oxide, and

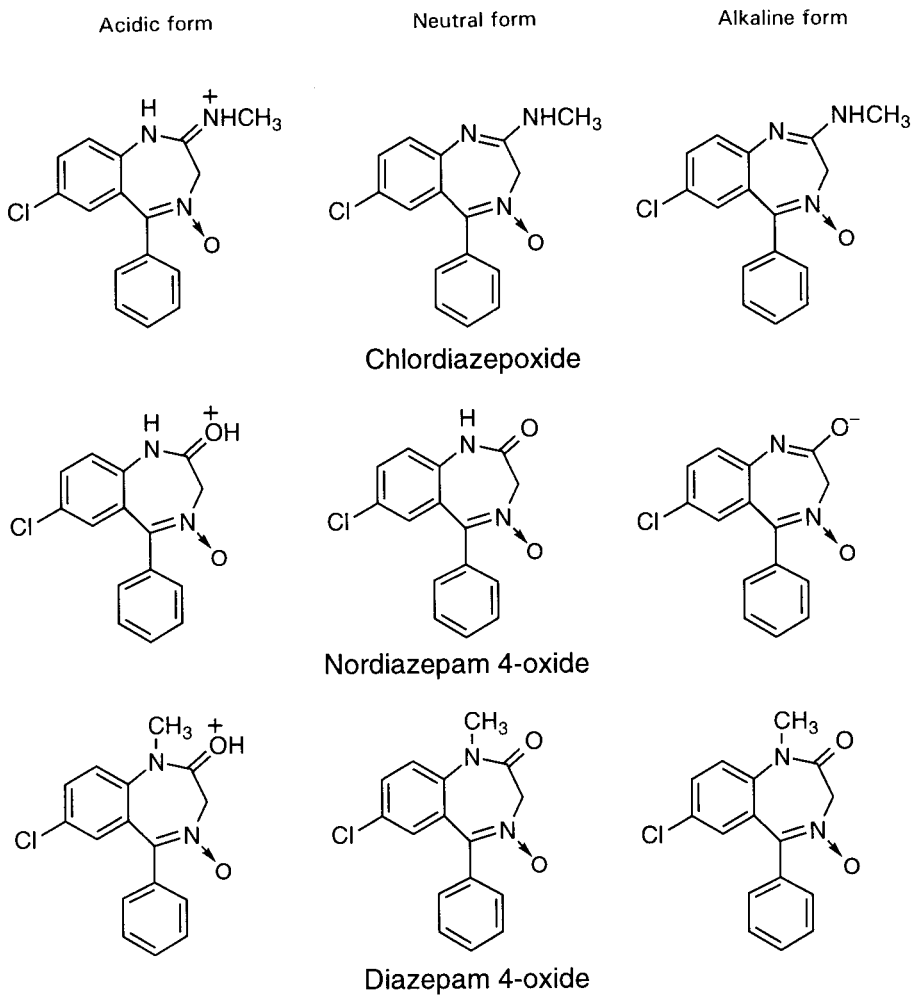

FIG. 5. Proposed structures of chlordiazepoxide, nordiazepam 4-oxide, and diazepam 4-oxide in acidic, neutral and alkaline solutions.

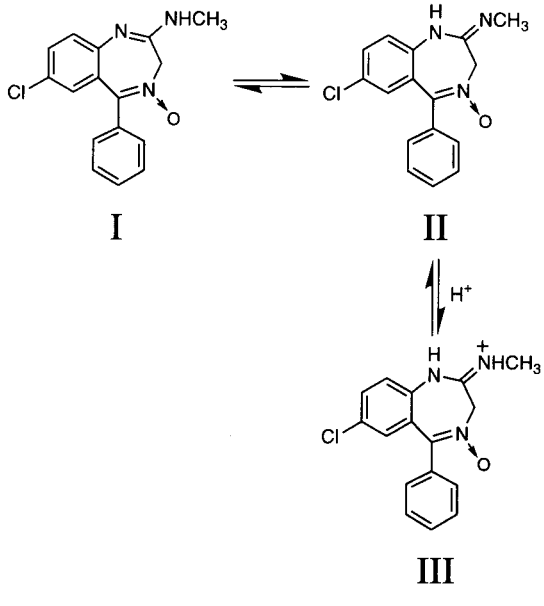

FIG. 6. Proposed amidine-imine tautomerism of chlordiazepoxide. The amidine form (structure I) predominates at pH > pK_a in neutral and alkaline solutions and the iminiun ion III (a protonated imine II) predominates at pH < pK_a in acidic solutions.

chlordiazepoxide in acidic, neutral, and alkaline solutions proposed in Fig. 5 are consistent with the experimental results observed in this study.

In conclusion, our results suggest that 1,4-benzodiazepine 4-oxides such as nordiazepam 4-oxide, diazepam 4-oxide, and chlordiazepoxide are not protonated in acidic solutions at the *N*-oxide oxygen. In acidic aqueous solutions with pH $\ll$ pK_a, chlordiazepoxide is protonated predominantly at the nitrogen of an imine bond between C2 carbon and its nitrogen substituent, whereas nordiazepam 4-oxide and diazepam 4-oxide are probably protonated at the C2 carbonyl oxygen.

Acknowledgements

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