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REVERSIBLE AZOMETHINE BOND CLEAVAGE OF 2′-FLUORO DERIVATIVES OF BENZODIAZEPINES IN ACIDIC SOLUTIONS AT BODY TEMPERATURE

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SUMMARY

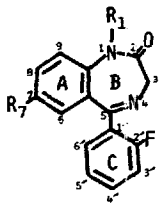
Reversible hydrolytic reactions of flunitrazepam, fludiazepam, and flurazepam in acidic solutions at body temperature were studied spectrophotometrically. The reactions of these drugs apparently took place at the azomethine bonds and the open-ring compounds produced were in equilibrium with the corresponding closed-ring compounds (protonated forms of the parent drugs). Forward and reverse reaction rate constants of these drugs were greater than those of the corresponding non-fluoro benzodiazepines. The greater reactivity in the fluoro derivatives may be attributed to the electronegative effects of fluorine at an ortho position of the 5-phenyl group. The activation energies of the forward and reverse reactions were calculated from Arrhenius-type plots. In addition, the pK_a values of these drugs were determined.

INTRODUCTION

Although pyrazolodiazepinones are easily hydrolyzed at ambient temperatures (Hong et al., 1977), the hydrolytic cleavage of benzodiazepines has been considered to take place only at elevated temperatures (Mayer et al., 1974; Han et al., 1977). However, during permeation studies of benzodiazepines at 30°C, we have noted chemical changes in the receptor solution which was kept acidic. This observation prompted us to investigate the hydrolytic reaction of benzodiazepines in acidic media at 25, 37 and 50°C and the results indicated that diazepam undergoes reversible ring-opening reactions at its azomethine bond (Nakano et al., 1979). We extended our studies to nimetazepam and nitrazepam and found that they are also subject to the same hydrolytic reactions. A short report on the ring-opening reaction of flurazepam under mildly acidic conditions has been published (Hassall et al., 1977).

Since 2′-fluoro derivatives of benzodiazepines; flunitrazepam, fludiazepam, flurazepam

(see formula below) have 2′-fluoro substituents in the 5-phenyl group, the hydrolytic cleavage may proceed at appreciable rates. This communication is concerned with the spectrophotometric investigation of the rate and extent of hydrolyses of these fluoro derivatives in acidic media. The results indicated that the ring-opening reactions took place at faster rates than the corresponding non-fluoro benzodiazepines.



R_1	R_7	
CH_3	NO_2	flunitrazepam
CH_3	Cl	fludiazepam
$CH_2CH_2NEt_2$	Cl	flurazepam

Structures of fluoro benzodiazepines

MATERIALS AND METHODS

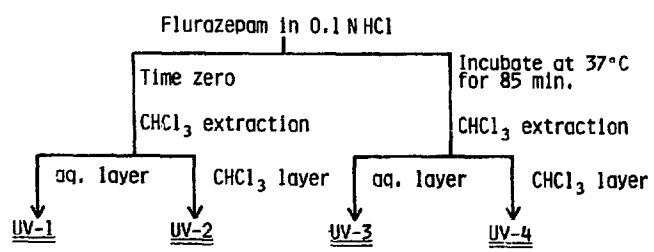
Materials

Flunitrazepam, fludiazepam, and flurazepam were supplied by Nippon Roche K.K., Tokyo, Pharmaceutical Division, Sumitomo Chemicals, Osaka, Japan and Kyowa Hakko Kogyo, Tokyo, respectively. Other chemicals were of reagent grade and were purchased from Wako Pure Chemical Industries, Osaka, Japan. Chloroform and ethanol were distilled before use.

Kinetic studies and pK_a determinations

The kinetic studies and pK_a determinations were carried out spectrophotometrically. Detailed procedures have been described previously (Nakano et al., 1979; Albert and Serjeant, 1971). Reaction rate constants were calculated from changes in absorbance at the fixed wavelengths at their λ_{max} (time scan) because of fast reaction rates relative to a spectral scan speed.

Among the 3 fluoro benzodiazepines, flurazepam was not quantitatively extracted into chloroform upon acidification of its solution. Therefore the molar absorptivity of its chloroform-unextractable species was calculated from the absorbance measurements shown in Scheme 1. After flurazepam solutions in 0.1 N HCl at time zero and at equilibrium were shaken for 3 min with the same volume of chloroform to extract unreacted flurazepam, spectra of the aqueous layer (UV-1 for time zero and UV-3 for the equilibrium state) and the chloroform layer (UV-2 for time zero and UV-4 for the equilibrium state) were obtained. The chloroform/0.1 N HCl partition coefficient and the molar



absorptivity of flurazepam in chloroform saturated with 0.1 N HCl were calculated from absorbance in UV-1 and UV-2. If reaction products are assumed unextractable by chloroform, UV-4 represents flurazepam, the concentration of which could be calculated by the molar absorptivity of flurazepam. Since the absorbance of flurazepam in UV-3 could be calculated by the partition coefficient and the concentration of flurazepam in chloroform, extracted at equilibrium, the concentration and the molar absorptivity of the reaction products could be calculated from the concentration of flurazepam at time zero minus those of flurazepam in chloroform (UV-4) and in the aqueous layer (UV-3) at equilibrium.

In pK_a measurements, absorbance was read immeadiately following preparation of solutions to minimize the effect of reactions on absorbance.

RESULTS AND DISCUSSION

The nature of the reactions

The spectral changes of flunitrazepam and flurazepam in 0.1 N HCl at 37°C and shown in Figs. 1 and 2, respectively. The reactions reached equilibria in 60 and 45 min for flunitrazepam and flurazepam, respectively. Similar spectral changes were obtained in fludiazepam, in which the equilibrium was reached in 140 min (not shown). The equilibrated solutions of these drugs were extracted with chloroform. The spectra of the aqueous layers (for example, see Fig. 3, time zero) were quite different from those of the equilibrated solutions (for example, Figs. 1 and 2, time infinity). These observations indicate the presence of at least two species, which markedly differ in the partition coefficients, in the equilibrated acidic solutions of these drugs.

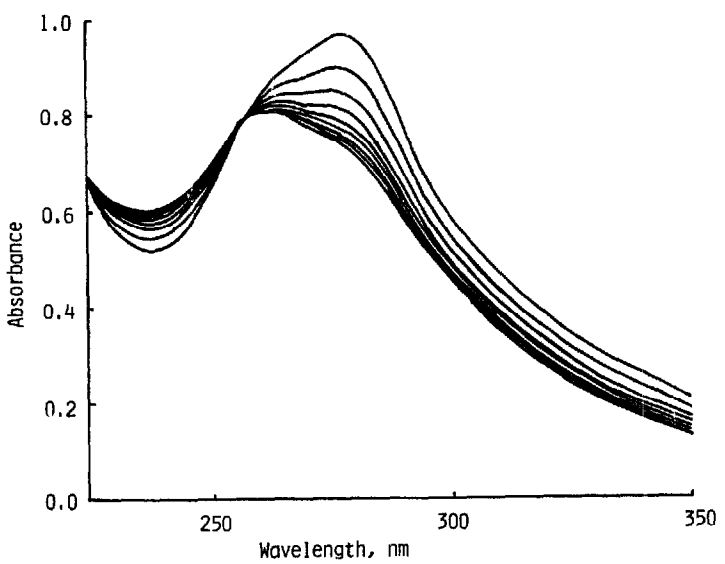


Fig. 1. Typical spectral changes for the hydrolysis of 4.46×10^{-5} M flunitrazepam in 0.1 N HCl at 37°C. Absorbance at 277 nm decreased with time (0, 3, 6, 9, 12, 15, 20, 35 and 60 min (∞) from the top).

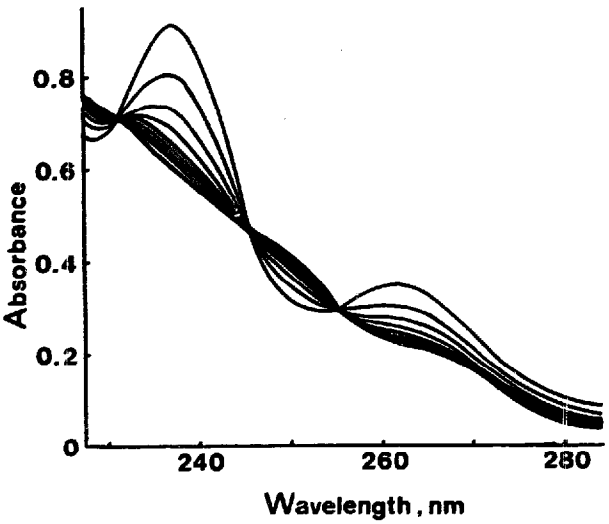


Fig. 2. Typical spectral changes for the hydrolysis of 3.97×10^{-5} M flurazepam in 0.1 N HCl at 37°C). Absorbance at 234.5 nm decreased with time (0, 3, 6, 9, 12, 15, 20, 25 and 45 min (∞) from the top).

A spectrum of the chloroform-unextractable species from flunitrazepam, which was taken immediately following the extraction (Fig. 3, time zero), changed with time to give a spectrum (Fig. 3, time infinity) which was quite similar to that of the equilibrated mixture from the parent drug (Fig. 1, time infinity). A spectrum of the chloroform-unextractable species from fludiazepam similarly changed with time (not shown). Spectra of the aqueous layer of the equilibrated solution of flurazepam immediately following the extraction with chloroform changed to a lesser extent with time (not shown) since flurazepam was not quantitatively extracted into chloroform but gave at equilibrium a spectrum which was quite similar to that of the acidic solution of flurazepam at equilibrium.

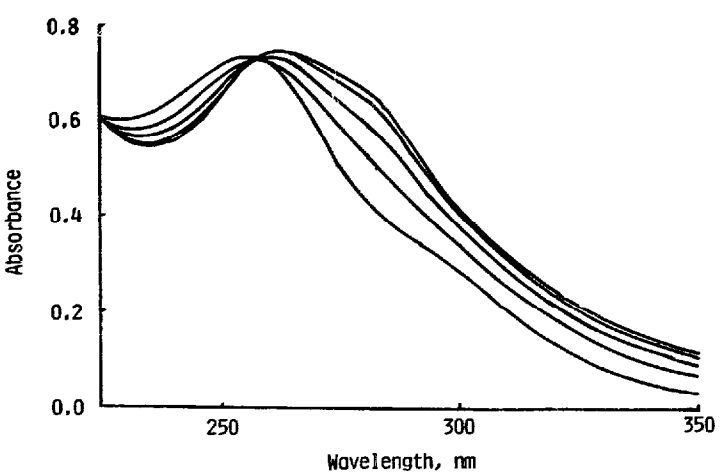


Fig. 3. Typical spectral changes due to the ring-closure reaction of 6.38×10^{-5} M of the chloroform-unextractable species from flunitrazepam in 0.1 N HCl saturated with chloroform at 37°C. Absorbance at 280 nm increased with time (0, 5, 10, 20, and 30 (∞) min from the bottom).

Structural assignment of the chloroform-unextractable species

Although the definite identification of the chloroform-unextractable species produced from the fluoro derivatives is yet to be achieved, the observations ruled out benzophenone derivatives, which are hydrolysis products at both amide and azomethine bonds and which are not expected to be cyclized, from the possible structures of the chloroform-unextractable species. Since assignment of the open-ring structures at the azomethine bond for the structures of the chloroform-unextractable species did not contradict any spectrophotometric and partition characteristics of the species, they are tentatively assigned to the structures of the chloroform-unextractable species.

The chloroform-unextractable species could not be isolated from aqueous solutions as such because of the reversible nature of the reactions in 0.1 N HCl and because of rapid cyclization to form the corresponding parent drugs over the pH ranges above their pK_a values where the extraction of unionized forms into organic solvents is expected. Thus, any attempt to extract the chloroform-unextractable species into organic solvents failed.

Quantitative aspects

The linear relations were obtained in plots according to the equation for reversible first-order reactions (Espenson, 1974). Such a relation obtained in flunitrazepam at 37°C is shown in Fig. 4 as an example. Since reaction rate constants obtained in two determinations under identical conditions did not differ very much from each other, only two experiments were made under each condition, and average values of a set of experiments are shown in Table 1. To indicate a magnitude of standard errors of the mean in the estimation of the rate constants, those of flunitrazepam at 50°C are shown as examples below; $k_f = 9.62 \pm 0.31$ (n = 3), and $k_r = 8.00 \pm 0.07$ (n = 3).

Forward and reverse reaction rate constants (k_f and k_r) of flunitrazepam and fludiazepam, which are structurally 2′-fluoronimetazepam and 2′-fluorodiazepam, respectively,

TABLE 1

RATE CONSTANTS* OF FORWARD AND REVERSE REACTIONS IN 0.1 N HCl AT 3 TEMPERATURES

Drug	Temperature (°C)	Forward (h^{-1})	Reverse (h^{-1})
Flunitrazepam	27	1.55	1.70
	37	3.66	3.18
	50	9.62**	8.00**
Fludiazepam	25	0.326	0.412
	37	0.903	0.954
	50	2.70	2.45
Flurazepam	17	1.08	0.248
	27	3.54	0.775
	37	7.79	1.93

* Average of two determinations, except **, average of 3 determinations.

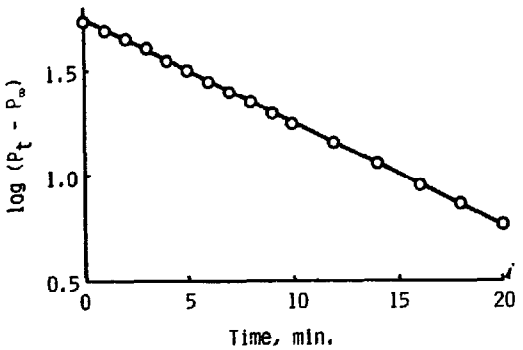


Fig. 4. Change of concentration of unreacted flunitrazepam (initial concentration, 4.53×10^{-5} M) with time in 0.1 N HCl at 37°C, where P_t and P_∞ represent per cent of flunitrazepam remaining unreacted at time t and time infinity, respectively.

were greater than those of nimetazepam ($k_f = 0.61$, $k_r = 0.82\ h^{-1}$ at 37°C) and diazepam ($k_f = 0.15$, $k_r = 0.30\ h^{-1}$ at 37°C), respectively, but the k_f/k_r ratios of the fluoro derivatives were smaller than those of corresponding non-fluoro benzodiazepines. These results may be explained by the increased reactivity in the fluoro derivatives because of their electron deficient state of the 5-carbon caused by the presence of strongly electronegative fluorine. On the other hand, the open forms of the drugs once produced are cyclized comparatively slowly possibly because of the steric hindrance of a fluoro substituent in spite of expected increased reactivity caused by fluorine for the reverse reaction.

The rate constants of flunitrazepam, which has a nitro group at 7, were 3–4 times greater than those of fludiazepam, which has a chlorine at 7, because of a stronger electron-withdrawing tendency of the nitro group than chlorine. Similar substituent effects were observed between diazepam and nimetazepam. The rate constant in the acid-hydrolysis of *m*-nitrobenzoate has been reported to be greater by the same degree than that of *m*-chlorobenzoate (Timm et al., 1958). The fact that the forward reaction rate constant of flurazepam was greater than that of fludiazepam may be explained by the proposition that the methyl group on amide nitrogen of fludiazepam is expected to affect the A-ring (see formula) to increase its electron density more than the cationic diethylaminoethyl group, though a definite explanation can not be presented because the reactivity is dependent on both electronic and steric factors of a reactant and the relative magnitude of the

TABLE 2

ACTIVATION ENERGIES OF FORWARD AND REVERSE REACTIONS IN 0.1 N HCl

Drug	Forward $E_a \pm$ S.E. (kcal/mol)	Reverse $E_a \pm$ S.E. (kcal/mol)
Flunitrazepam	15.0 ± 0.9	13.6 ± 0.4
Fludiazepam	16.6 ± 0.9	14.1 ± 0.6
Flurazepam	15.8 ± 0.9	17.4 ± 0.6

steric effect can not be deduced from only a few data obtained. Nevertheless, the fact that the k_f/k_r ratio of flurazepam was greater than that of fludiazepam may be attributed to the steric hindrance of the diethylaminoethyl group of flurazepam.

From the data obtained so far, the major factor influencing the reactivity of the azomethine bond cleavage in acidic solutions appears to be the electron density of 5-carbon. When 5-carbon is in an electronically deficient state, the reactivity of this reaction may be increased even if other factors such as strains in the ring system may also influence the rate.

Effect of temperature

The rate constant vs temperature plots for data obtained at 3 temperatures for the 3 drugs were linear (not shown) and the activation energies calculated are tabulated in Table 2.

pK_a values of fluoro derivatives of the benzodiazepines

The pK_a values were calculated to be 1.71 ± 0.003, 2.29 ± 0.009 and 1.53 ± 0.01 (S.E.) for flunitrazepam, fludiazepam and flurazepam, respectively. The smaller pK_a values for flunitrazepam than that of fludiazepam may be explained by less electron density in 4-nitrogen in flunitrazepam. These values were somewhat smaller than reported pK_a values of 1.82 for flunitrazepam (Boxenbaum et al., 1978) and 1.90 for flurazepam (Rudy and Senkowski, 1974). These values may not be very accurate unless great care has been taken to eliminate errors caused by these ring-opening reactions which take place at the pH region of pK_a measurements.

General discussion

At body temperature, the reversible reactions involving hydrolyses of 4,5-azomethine bonds of the fluoro derivatives of the benzodiazepines are expected to take place in a manner similar to that of diazepam, nimetazepam and nitrazepam, Since the magnitude of the reaction rates differ greatly among these drugs, this reaction is greatly dependent on the nature of substituents at 1, 5 and 7 positions of benzodiazepines.

The experimental data obtained in the present in vitro study suggest that after administration of these drugs, some parts of the drug dissolved in the stomach are quickly hydrolyzed to form the open-ring compounds because of the acidic pH values of stomach contents. Because of large k_f values, i.e. short half-life values, hydrolytic cleavage is expected to reach equilibrium in a short time. When the open-ring compounds are emptied from the stomach into the intestine, they revert back to parent drugs upon increase in the pH value of the media. Therefore, there will be little loss in drug bioavailability.

However, measurements of dissolution rates of these drugs from solid dosage forms in simulated gastric fluids have to be made with a prior knowledge that a part of dissolved drugs is hydrolyzed to form the open-ring compounds which exhibit different absorption spectra.

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