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REVERSIBLE AZOMETHINE BOND CLEAVAGE OF NITROFURANTOIN IN ACIDIC SOLUTIONS AT BODY TEMPERATURE

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SUMMARY

A reversible hydrolytic reaction of nitrofurantoin in acidic solutions at body temperature was studied spectrophotometrically. The cleavage reaction of nitrofurantoin took place at the azomethine bond and 5-nitrofurfural produced was in equilibrium with nitrofurantoin. The rate constant of the reverse reaction was greater than that of the forward reaction. The activation energies of the forward and reverse reactions were calculated from Arrhenius type plots to be 18.9 ± 1.2 (S.E.) and 9.94 ± 0.78 (S.E.) kcal/mol, respectively. In addition, the effect of pH on these reactions and the state of ionization of nitrofurantoin were examined.

INTRODUCTION

Reversible ring opening reactions of diazepam at the azomethine bond in acidic media at 37°C have been examined recently (Nakano et al., 1979). No report has been published on the reaction of nitrofurantoin, another drug with an azomethine bond with urinary tract antiseptic activity, in acidic solutions at body temperature. Spectrophotometric studies indicated that a cleavage reaction at the azomethine bond takes place at an appreciable rate even at the body temperature and that 5-nitrofurfural formed is in equilibrium with nitrofurantoin and reverts to nitrofurantoin when the pH value of the media is increased.

Although Burmitz et al. (1976) reported that nitrofurantoin exists in 4 different ionization states over the pH range of 0–14 from their ultraviolet spectrophotometric study, other reports list only one pK_a value (Cadwallader and Bates, 1975; Cadwallader and Jun, 1976). Since it is desirable to identify a reacting species in acidic solutions, spectrophotometric and partition studies were carried out to clarify ionization states of the drug.

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MATERIALS AND METHODS

Materials

Nitrofurantoin was purchased from Sigma Chemicals, St. Louis, whereas nitrofurazone and 5-nitrofurfural were obtained from Tokyo Kasei Kogyo, Tokyo. 1-Aminohydantoin hydrochloride was a generous gift from Norwich-Eaton Pharmaceuticals, Norwich, N.Y. Other chemicals were of reagent grade and were purchased from Wako Pure Chemical Industries, Osaka. Chloroform, dimethylformamide and toluene were distilled before use. 5-Nitrofurfural phenylhydrazone was synthesized and recrystallized from ethanol–water to obtain wine-red needles, mp. 190.0–190.5°C: found C, 57.10; H, 3.87; N, 18.10: calculated values for $C_{11}H_9N_3O_3$; C, 57.10; H, 3.92; N, 18.16. The product had the following characteristics: ν_{max} (Nujol) 3300 (NH) and 1562 (C=N) cm^{-1}; δ (CD_3COCD_3, TMS) 6.88 (1 H, d), 7.15 (5 H, m), 7.51 (1 H, d), 7.86 (1 H, s), and 10.08 (1 H, broad); m/e 231 (M^+).

Kinetic studies

The kinetic studies were carried out spectrophotometrically. Detailed procedures have been described previously (Nakano et al., 1979). All kinetic parameters were estimated by computer using the method of non-linear least-squares (Marquardt, 1964). The reaction mixtures were prepared in the following fashion. Concentrated solutions of nitrofurantoin (9.83×10^{-3}–1.38×10^{-2} M) in dimethylformamide were employed as stock solutions. The stock solutions were diluted 100 times with distilled water prewarmed to the temperature of the study. The resultant solutions were further diluted 2.5 times with 1 N HCl and water to obtain the test solutions which were 0.1 N with respect to HCl. For experiments over the pH range of 3.3–7.8 and at 9.9, McIlvaine buffer and Clark and Lubs NaOH–H_3BO_3–KCl buffer (McKenzie, 1969) were used, respectively.

Employing the molar absorptivity of nitrofurantoin and 5-nitrofurfural at 365 nm, the concentrations of both species were computed from the observed absorbance in the UV spectra using the following relationship:

$$\log \frac{P_t - P_\infty}{P_0 - P_\infty} = \log \frac{A_t - A_\infty}{A_0 - A_\infty} = -\frac{k_f + k_r}{2.303} t \qquad (1)$$

$$K_{eq} = \frac{k_f}{k_r} = \frac{P_0 - P_\infty}{P_\infty} \qquad (2)$$

where P and A represent fraction or concentration and absorbance, respectively, subscripts 0, t, and ∞ indicate time zero, t and infinity, respectively. The presence of 1-aminohydantoin is not expected to interfere with absorbance measurements since the absorbance of 1-aminohydantoin was negligible above 250 nm. Experiments were carried out in duplicate and the mean values of the rate constants were obtained.

In the synthetic reaction, the stock solutions and test solutions of 5-nitrofurfural and 1-aminohydantoin hydrochloride were similarly prepared. After some portions of the two test solutions were mixed, the reaction mixture was immediately placed in the thermoregulated cell-holder and a series of spectra of the reaction mixture were recorded at predetermined time intervals.

Spectral change in the presence of phenylhydrazine

A 0.2 ml portion of the stock solution of nitrofurantoin (1.38×10^{-2} M) was diluted with distilled water at 37°C to make 20 ml. One ml each of 8% phenylhydrazine hydrochloride solution and 1 N HCl were added to 4 ml of the diluted solution, and then distilled water at 37°C was added to make 10 ml of the reaction mixture. A portion of the reaction mixture was immediately placed in the cell and spectra of the reaction mixture at 37°C were obtained at predetermined time intervals.

In a separate series of experiments, nitrofurantoin solution in 0.1 N HCl was incubated at 37°C and sampled at predetermined time intervals. To each sample, phenylhydrazine hydrochloride solution was added and the mixture was extracted with toluene. The spectra of the organic layer were then recorded.

Identification of phenylhydrazone

A compound formed by addition of phenylhydrazine hydrochloride in nitrofurantoin solution in 0.1 N HCl was extracted into toluene. The compound was separated by preparative thin-layer chromatography on Al_2O_3 using chloroform : cyclohexane (1 : 0.3) as a developing solvent. A spot corresponding to $R_f = 0.43$ was eluted with chloroform to obtain a pure compound for infrared, NMR and mass spectrometric identification.

Spectrophotometry

Spectra obtained by each experiment were compared with those of authentic samples of nitrofurantoin, 5-nitrofurfural and 5-nitrofurfural phenylhydrazone in various solvents. Furthermore, spectra of nitrofurantoin in various buffers (pH 0.9–9.2) were obtained to examine the effect of pH on ionization-states of the drug.

Partition studies

In the examination of the effect of pH on extractability of nitrofurantoin from buffer solutions to chloroform, the aqueous solutions of various pH values were agitated for 3 min with the same volume of chloroform which had been saturated with corresponding buffer solutions. Absorbance of the aqueous layer at partition equilibrium, which is expected to be reached within 3 min, was compared with that of the aqueous solution without extraction to calculate percentage of nitrofurantoin remained in the aqueous layer at partition equilibrium.

RESULTS AND DISCUSSION

The nature of the reaction and properties of the products

The spectral change of nitrofurantoin in 0.1 N HCl at 37°C is shown in Fig. 1. The solution reached its equilibrium in 8 h. The ultraviolet spectrum of the aqueous layer, which was obtained after extraction of the equilibrated solution with chloroform (not shown), indicated a 49.7% decrease in absorbance at 300 nm in contrast to a only 21.4% decrease at a λ_{max} of 365 nm. This observation indicates the presence of at least two species, which markedly differ in their partition coefficients, in the equilibrated solution. The spectrum of the species which remained after chloroform extraction (not shown) was quite similar to that of nitrofurantoin, indicating the presence of unreacted nitrofuran-

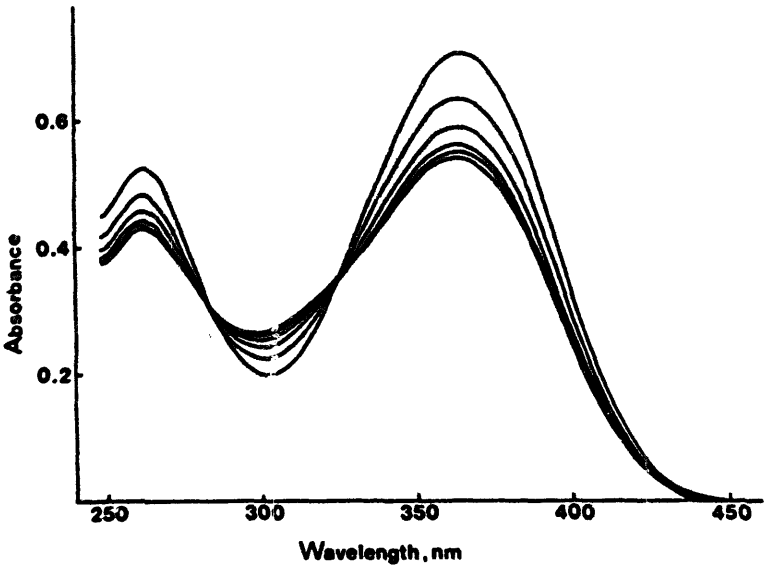


Fig. 1. Typical spectral changes for the hydrolysis of 4.00×10^{-5} M nitrofurantoin in 0.1 N HCl at 37°C. Absorbance at 365 nm decreased with time (0, 1, 2, 3, 5 and 8 h (∞) from the top).

toin in the equilibrated mixture. In addition, the spectrum of equilibrated solution in 0.1 N HCl rapidly changed to that of nitrofurantoin by the addition of 0.4 and 0.04% NaOH and a phosphate buffer, pH 7.4 to adjust pH to 7.4. This result indicates a reversibility of the reaction of nitrofurantoin in acidic media.

The following synthetic reaction gives additional evidence for the reversibility of the reaction. A spectrum of the reaction mixture of 5-nitrofurfural and 1-aminohydantoin hydrochloride with λ_{max} of 307 nm (Fig. 2, 0 h) changed with time to give an equilibrium-state spectrum (Fig. 2, 6.5 h) which was quite similar to that of the equilibrium mix-

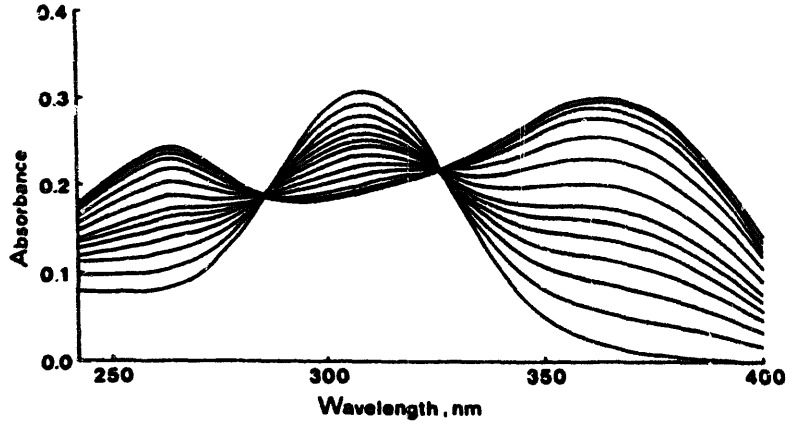


Fig. 2. Typical spectral changes due to the formation of nitrofurantoin with time starting with 6.95×10^{-5} M 5-nitrofurfural and 6.90×10^{-5} M 1-aminohydantoin hydrochloride in 0.1 N HCl at 37°C. Absorbance at 307 nm decreased while that at 263 and 363 nm increased with time (0, 10, 20, 30, 40, 50, 60, 80, 110, 150, 210, 270, 330 and 390 min).

ture of the hydrolytic reaction starting from nitrofurantoin (Fig. 1, 8 h). On the basis of the above observations, the termination of further spectral change after 8 h in Fig. 1 indicates the attainment of an apparent equilibrium state for a reversible reaction rather than the completion of a reaction.

Structural assignment of products

The initial assignment of 5-nitrofurfural for the structure of the chloroform-extracted species in the equilibrium mixture was based on the observation that the UV spectrum of the chloroform-extracted species in chloroform (λ_{max} at 298 nm) was similar to that of the authentic sample of 5-nitrofurfural. The chloroform-extractable nature of the species suggests the absence of an ionic group in the molecule. Although definite identification of 5-nitrofurfural could not be made because of possible oxidation of the compound during thin-layer chromatographic separation and isolation, the presence of 5-nitrofurfural in the equilibrium mixture may be indicated by the formation of 5-nitrofurfural phenylhydrazone. The spectrum of nitrofurantoin in 0.1 N HCl containing phenylhydrazine showed initial absorption peaks at 365 and 269 nm which are attributed to nitrofurantoin and phenylhydrazine, respectively. Absorbance at 365 and 269 nm decreased with time and a new absorption peak at 459 nm appeared (Fig. 3). A shift of λ_{max} to longer wavelength indicates that the conjugated system of the product is more extended than those of the reactants. Since the spectrum of the authentic sample of 5-nitrofurfural phenylhydrazone shows λ_{max} at 461 nm, it is possible that 5-nitrofurfural was produced by incubation of nitrofurantoin in acidic media before it reacted with phenylhydrazine to produce the phenylhydrazone.

In a separate experiment, phenylhydrazine was added to each of the samples obtained from the acidic reaction mixture at predetermined time intervals and the mixture was

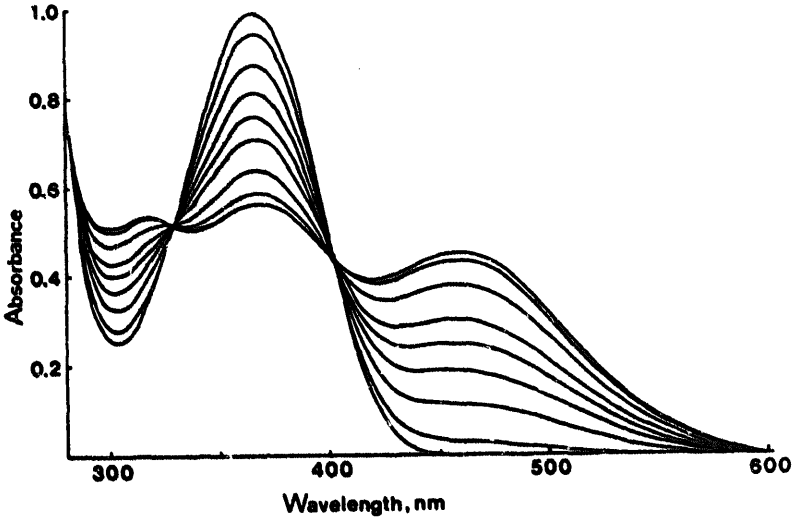
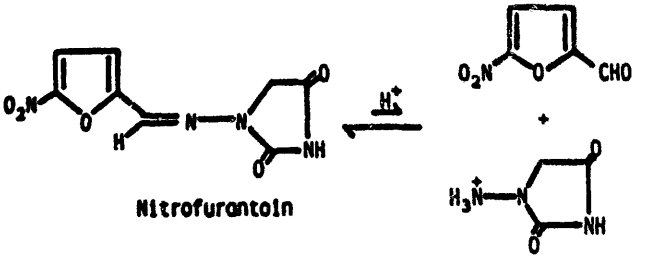


Fig. 3. Typical spectral changes due to the formation of phenylhydrazone with time starting with 5.52×10^{-5} M nitrofurantoin and 5.53×10^{-5} M phenylhydrazine in 0.1 N HCl at 37°C. Absorbance at 365 nm decreased while that at 459 nm increased with time (0, 0.5, 1.5, 2.5, 3.5, 4.5, 6.5, 8.5 and 9.5 h).

extracted with toluene. The absorbance of the toluene extracts at 428 nm increased with time (not shown). It indicates that the amount of 5-nitrofurfural produced increased with time since maximum absorbance of authentic 5-nitrofurfural phenylhydrazone in toluene is at 427 nm. Furthermore, a compound isolated by thin-layer chromatography from reaction products showed identical analytical data in infrared, NMR and mass spectra with those of authentic 5-nitrofurfural phenylhydrazone.

These experimental data suggest that the reaction of nitrofurantoin in acidic media may be represented as shown in Scheme 1. Although some attempts were made to isolate



Scheme 1. Reversible azomethine bond cleavage of nitrofurantoin in acidic media.

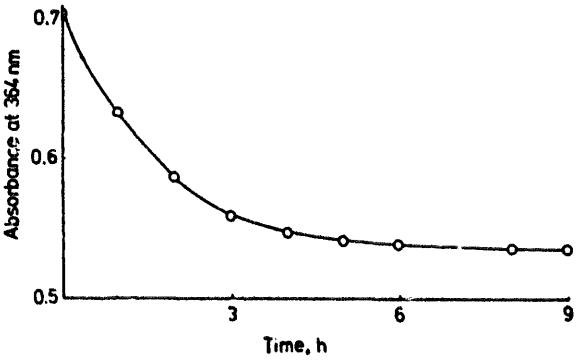
1-aminohydantoin, which is expected to be produced with 5-nitrofurfural, they failed because of its unextractable nature into organic solvents. Nonetheless, 1-aminohydantoin may be expected to be present along with 5-nitrofurfural in the equilibrated mixture since nitrofurantoin was regenerated from the equilibrated mixture by addition of 0.4 and 0.04% NaOH and a buffer at pH 7.4 to adjust pH to 7.4 and nitrofurantoin was synthesized from 5-nitrofurfural and 1-aminohydantoin in the acidic solution.

Quantitative aspects

A plot of absorbance against time is given in Fig. 4. The linear relation was obtained when the equation for reversible first-order reaction (Eqn. 1) is applied (Fig. 5). The forward and reverse reaction rate constants were calculated to be 0.135 and 0.443 h^{-1}, respectively. The fact that the reverse reaction rate constant was greater than the forward reaction rate constant indicates that the concentration of nitrofurantoin is greater than that of 5-nitrofurfural at equilibrium. The percentage of nitrofurantoin at equilibrium was calculated to be 76.9 of an initial concentration of nitrofurantoin.

Effect of temperature

The rate constants at 25°C and 50°C determined from spectral changes similar to those at 37°C are presented in Table 1 along with the calculated percentage of nitrofurantoin present in equilibrated mixtures. Table 1 shows that the reverse reaction rate constants are also greater than the forward reaction rate constants at 25°C and 50°C and that the percentage of nitrofurantoin present at equilibrium decreases with increasing temperature. The energies of activation calculated from the Arrhenius equation were 18.9 ± 1.2 (S.E.) kcal/mol for the forward reaction and 9.94 ± 0.78 (S.E.) kcal/mol for the reverse reaction.



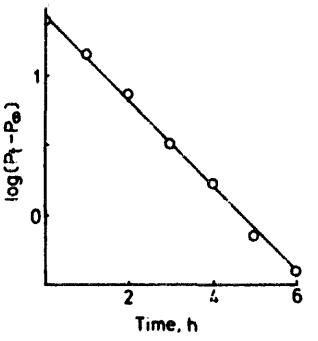


Fig. 4. Change in absorbance of nitrofurantoin (4.00×10^{-5} M) with time in 0.1 N HCl at 37°C.

Fig. 5. Change of concentration of unreacted nitrofurantoin (initial concentration, 4.00×10^{-5} M) with time in 0.1 N HCl at 37°C, where P_t and P_∞ represent per cent of nitrofurantoin remaining unreacted at time t and time infinity, respectively.

Effect of pH

The preliminary studies indicated that spectral change similar to that shown in Fig. 1 was observed in the citrate–Na_2HPO_4 buffer solution at pH 3.0. However, the change in absorbance was smaller than that in 0.1 N HCl indicating that the reaction proceeded to a lesser extent. Over a pH range of 5.4–9.9, little change in absorbance with time was observed. As indicated earlier, 5-nitrofurfural, once formed in acidic solutions, reverted to nitrofurantoin when the pH of media was raised.

State of ionization of nitrofurantoin

When the effect of pH on spectra was examined, λ_{max} of nitrofurantoin was at 264 and 365 nm over pH 0.6–5.6 whereas it was at 277 and 385 nm over pH 7.8–9.9. This indicates that the electronic state of nitrofurantoin changes at about pH 7. In order to identify a reacting species in acidic solutions, measurements of absorbance and partition coefficients, which are dependent on chemical species present, were carried out. Partition studies and measurement of spectrum were carried out quickly. Time for equilibrium was 3 min and degree of degradation of the drug during this period is expected to be negli-

TABLE 1

RATE CONSTANTS FOR FORWARD AND REVERSE REACTIONS OF NITROFURANTOIN IN 0.1 N HCl AT 3 TEMPERATURE VALUES

Temperature (°C)	Rate constants (h^{-1})		Nitrofurantoin at equilibrium (%)
	Forward	Reverse	
25	0.035 ± 0.009	0.193 ± 0.009	86.0
37	0.135 ± 0.026	0.443 ± 0.026	76.9
50	0.462 ± 0.073	0.834 ± 0.073	64.3

gible even at pH 0.6. Decrease in absorbance was observed around pH 7 when absorbance at 250 nm was plotted against pH (not shown). In the partition studies, the fractions of nitrofurantoin remaining in the aqueous layer were almost constant at 83–85% over pH 0.6–5.6 whereas they were 97.4 and 100% at pH 7.8 and 9.2, respectively (not shown). These results indicate that a chemical species which is predominant at pH 7.8 and 9.2 is different from that over pH 0.6–5.6 and that a chemical species which is predominant over pH 0.6–5.6 is expected to be an unionized form because of higher partition coefficient over pH 0.6–5.6 than that at pH 7.8 and 9.2. A chemical species extracted into chloroform showed the same spectrum as nitrofurantoin in chloroform. On the other hand, nitrofurazone, which is an analog of nitrofurantoin, showed no change in absorbance with pH over a pH range of 0.6–9.2, and thus the drug is expected to remain unionized over the pH range. These observations indicate that nitrofurantoin exists in an unionized form over the pH range of 0.6–5.6, and that a part of an unionized species is extracted with chloroform. Changes in absorbance and partition coefficient with pH at around pH 7 may be attributed to the deprotonation of the imido hydrogen because such a change was not observed in a pH profile of absorbance of nitrofurazone at about pH 7. According to the present study, protonation at the α-position (nitrogen in the azomethine bond) is expected to become predominant only in strongly acidic solutions (pH < 0.6).

These observations are in accord with pH dependency of nitrofurantoin solubility in water (Chen et al., 1976) but in contrast with the report of Burmitz et al. (1976) who suggested pK_{a_1} (protonation at azomethine nitrogen) at 3.5, pK_{a_2} (protonation at hydantoin nitrogen) at 7.8, and pK_{a_3} (deprotonation of imide proton) at higher pH values.

GENERAL DISCUSSION

At body temperature, the reversible reaction involving the hydrolysis of the azomethine bond of nitrofurantoin is expected to be a major reaction in an acidic solution. The present in vitro study suggests the following reactions in vivo: after administration of a dosage form of nitrofurantoin, some nitrofurantoin dissolved in the acidic stomach contents is hydrolyzed into 5-nitrofurfural and 1-aminohydantoin. When 5-nitrofurfural is emptied from the stomach into the intestine, it reacts with 1-aminohydantoin to regenerate nitrofurantoin upon an increase in pH of the medium. Since the hydrolytic reaction may proceed beyond equilibrium if 5-nitrofurfural, which is produced by equilibrium reaction, is preferentially absorbed in the stomach, an additional experiment on the possible absorption of 5-nitrofurfural in the stomach is required.

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