

Micellar Catalysis of Organic Reactions. XIV* Hydrolysis of Some 1,4-Benzodiazepin-2-one Drugs in Acidic Solution

Trevor J. Broxton,^{A,B} Timothy Ryan^A and Steven R. Morrison^A

^A Department of Organic Chemistry, La Trobe University, Bundoora, Vic. 3083.

^B To whom correspondence should be addressed.

Abstract

Kinetic studies of the acidic hydrolysis of diazepam and nitrazepam were carried out in the presence of micelles of sodium dodecyl sulfate (sds). The hydrolysis of diazepam was shown to occur with biphasic kinetics. This is consistent with initial hydrolysis of the azomethine bond followed by very slow hydrolysis of the amide bond as found for hydrolysis in aqueous solution. Nitrazepam, however, was found to decompose with monophasic kinetics consistent with initial amide hydrolysis. Reactions involving the hydrolysis of the azomethine bond were shown to be independent of acid concentration and subject to inhibition by micelles of sds. Reactions involving amide hydrolysis were shown to be first order in acid concentration and subject to micellar catalysis. The mechanistic change for the hydrolysis of nitrazepam on transfer from water (initial azomethine cleavage) to micelles of sds (initial amide cleavage), was presumably the result of the inhibition of azomethine hydrolysis and the catalysis of amide hydrolysis by the micelles.

Introduction

The study of the effects of micelles on organic reactions is currently of great interest. In particular, there have been many reports of the catalysis of organic reactions.¹ Less widespread are reports of micelle-induced changes of mechanism.

In some cases, different products have been reported for reactions carried out in the presence of micelles compared with reaction in water. This can occur when the product of the reaction in water is unstable in the presence of micelles. For example, the basic hydrolysis of alkyl and aryl *N*-(4-nitrophenyl)carbamates in water at 25°C yields *N*-(4-nitrophenyl)carbamate ion as the stable product. In the presence of micelles of cetyltrimethylammonium bromide (ctab), however, the product was 4-nitroaniline. Decarboxylation of *N*-(4-nitrophenyl)carbamate ion was shown to be strongly catalysed ($\times 45$) by micelles of ctab. Thus, a micelle-induced change of product, but not of mechanism, was observed.²

* Part XIII, *Aust. J. Chem.*, 1984, 37, 977.

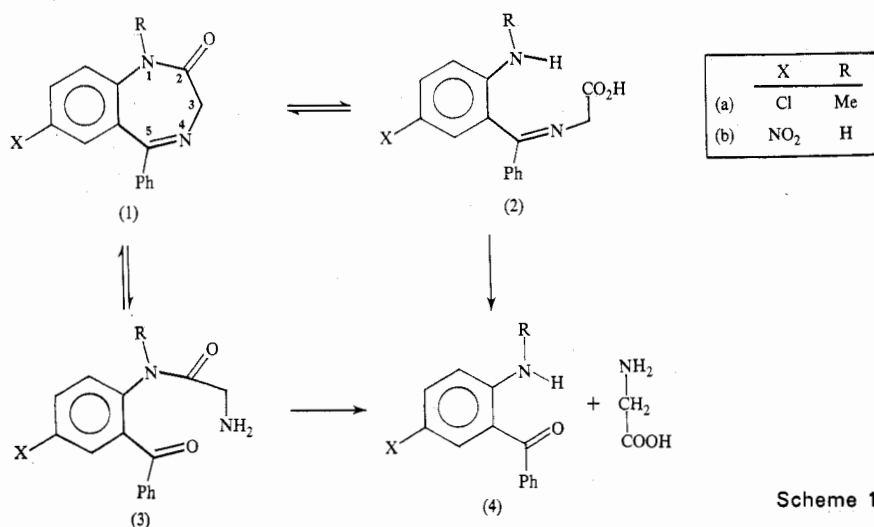
¹ Fendler, J. H., and Fendler, E. J., 'Catalysis in Micellar and Macromolecular Systems' (Academic Press: New York 1975).

² Broxton, T. J., *Aust. J. Chem.*, 1984, 37, 47.

We have previously demonstrated some examples where the fine details of the mechanism of basic hydrolysis of amides are affected by micelles of ctab.^{3,4} For the hydrolysis of *N*-aryl-*N*-phenylbenzamides, a change of mechanism from rate determining solvent-assisted C–N bond breaking in water to rate determining hydroxide ion attack in ctab was observed.³ In the case of *N*,*p*-dimethyl-*N*-phenylbenzamide, basic hydrolysis in water occurred via a slow protonation of the nitrogen atom in the tetrahedral intermediate followed by fast C–N bond cleavage, whereas in ctab the rate-determining step was solvent-assisted C–N bond-breaking.⁴

We are now interested in the investigation of systems in which a more dramatic mechanistic change is possible. We looked for a system where two possible sites of attack were available. Such a system is the benzodiazepinone nucleus (1), where nucleophilic attack may occur at either the 2-position, leading to amide hydrolysis [(1) → (2)] or at the 5-position, leading to azomethine cleavage [(1) → (3)] (Scheme 1).

Previous studies, in water, of the acidic hydrolysis of benzodiazepines of therapeutic interest have been reported. These include studies of the hydrolysis of 7-chloro-1-methyl-5-phenyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one (diazepam) (1a)^{5–7} and 7-nitro-5-phenyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one (nitrazepam) (1b).^{8,9}



Scheme 1

In each case, the hydrolytic mechanism involves cleavage initially of the 4,5 azomethine bond and then of the 1,2 amide bond to give the appropriate aminobenzophenone (4) and glycine (Scheme 1). For other benzodiazepines, e.g., oxazepam⁵ and chlordiazepoxide,¹⁰ cleavage of the 1,2 amide bond has been reported to precede

³ Broxton, T. J., Fernando, D. R., and Rowe, J. E., *J. Org. Chem.*, 1981, **46**, 3522.

⁴ Broxton, T. J., and Duddy, N. W., *Aust. J. Chem.*, 1979, **32**, 1717.

⁵ Han, W. W., Yakatan, G. J., and Maness, D. D., *J. Pharm. Sci.*, 1977, **66**, 573.

⁶ Nakano, M., Inotsume, N., Kohri, N., and Arita, T., *Int. J. Pharm.*, 1979, **3**, 195.

⁷ Mayer, W., Erbe, S., Wolf, G., and Voigt, R., *Pharmazie*, 1974, **29**, 700.

⁸ Han, W. W., Yakatan, G. J., and Maness, D. D., *J. Pharm. Sci.*, 1977, **66**, 795.

⁹ Inotsume, N., and Nakano, M., *J. Pharm. Sci.*, 1980, **69**, 1331.

¹⁰ Han, W. W., Yakatan, G. J., and Maness, D. D., *J. Pharm. Sci.*, 1976, **65**, 1198.

cleavage of the 4,5 azomethine bond. Thus, the mechanism of hydrolysis of benzodiazepines is sensitive to substituents on the aromatic ring and on the amide nitrogen.

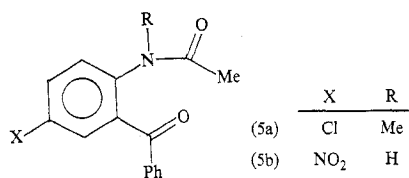
Depending on the order of bond cleavage, intermediates (2) or (3) can be formed during the hydrolysis (Scheme 1). If the 4,5 azomethine bond is cleaved first, intermediate (3) is formed, while if the initial hydrolysis step occurs at the 1,2 amide bond, intermediate (2) is formed. In acidic solution, it has been proposed^{5,6} that intermediate (3) would accumulate in solution because the reversal of the first step would be inhibited since the nucleophilicity of the amino group would be reduced as a result of protonation. Thus, the reaction should display biphasic kinetics if initial azomethine hydrolysis occurs. Intermediate (2), however, is not expected to accumulate in acidic solution. This is consistent with many reports in the literature that lactam formation from amino acids like intermediate (2), is facile.^{11,12} Thus, if initial amide hydrolysis occurs, the rapid equilibration should result in monophasic kinetics.

The effects of micelles on the acidic hydrolysis of amide and azomethine bonds has been reported. Micelles of sodium dodecyl sulfate (sds) have been reported to inhibit both the acidic hydrolysis of amides¹³ and azomethine groups.¹⁴ The magnitude of inhibition, however, varies and it was decided to investigate the effects of micelles of sds on the mechanism of hydrolysis of diazepam (1a) and nitrazepam (1b). Since the site of initial hydrolysis of benzodiazepines is finely balanced and since amide and azomethine hydrolyses are subject to different degrees of inhibition by micelles of sds, we hoped to find examples where the mechanism of hydrolysis of benzodiazepines changed on transfer from water to the micelle.

Results and Discussion

(a) Diazepam

Han⁵ has proposed that in aqueous solution the initial event is hydrolysis of the azomethine bond forming intermediate (3a) in equilibrium with diazepam (1a). Subsequent hydrolysis of (3a) to (4a) occurs much more slowly.⁵ We measured the rate of loss of diazepam at 280 nm and determined the percentage of diazepam present at equilibrium (by measuring the absorbance at 280 nm initially, i.e. due to diazepam, and at equilibrium). In addition, we measured the absorbance at 280 nm for compound (5a) as a model for intermediate (3a). Compound (5a) and intermediate (3a) differ only in the replacement of the NH₂ group by H; it would thus seem reasonable that the ultraviolet spectra be similar. By this method, the percentage of diazepam at equilibrium was found to be 65%. This value was almost independent of acid



¹¹ Hendrickson, J. B., Cram, D. J., and Hammond, G. S., 'Organic Chemistry' 3rd Edn, p. 513 (McGraw-Hill: New York 1970).

¹² Sternbach, L. H., and Reeder, E., *J. Org. Chem.*, 1961, **26**, 4936.

¹³ Linda, P., Stener, A., Cipiciani, A., and Savelli, G., *J. Chem. Soc., Perkin Trans. 2*, 1983, 821.

¹⁴ Behme, M. T. A., and Cordes, E. H., *J. Am. Chem. Soc.*, 1965, **87**, 260.

concentration within the range 0.013–0.5 M HCl and is within 10% of the value obtained by extrapolation of Nakano's results⁶ to 68°C. Nakano determined the percentage of diazepam at equilibrium by the chemical separation of the equilibrium mixture. This result gave us some confidence in the u.v. method and in our assumption about the similarity of the u.v. spectra of (5a) and (3a).

In an equilibrium such as (1a) \rightleftharpoons (3a) the rate constant obtained by monitoring (1a) loss is in fact the sum of forward and reverse reactions ($k_1 + k_{-1}$).¹⁵ By using the value for the measured percentage of diazepam at equilibrium k_1 , the rate of the forward reaction (1a) \rightarrow (3a) was calculated from the observed rate. These results are in Table 1.

Table 1. Observed rate constants for the first phase of the acidic hydrolysis of diazepam (1a) at 68.5°C

Loss of diazepam was followed at 280 nm. A very slow subsequent reaction was observed but it was too slow to obtain reliable rate constants. Rate constants for (1a) \rightarrow (3a) calculated as described in text

[HCl] (M)	$10^4 k_1 / \text{s}^{-1}$ for [sds]/mM of				
	0 ^A	40 ^B	100 ^B	200 ^B	300 ^B
0.26	3.8			0.88	
0.13	3.5			0.84	
0.066	3.8			0.84	
0.026	3.8			0.84	
0.013	4.2	1.2	1.0	0.90	0.92

^A In absence of sds 65% diazepam at equilibrium.

^B In presence of sds 40% diazepam at equilibrium.

The rate of cleavage of the azomethine bond is independent of acid concentration within the range 0.013–0.26 M HCl. Tight isosbestic points were obtained at 224, 256 and 270 nm, indicating the absence of any accumulation of an intermediate during the establishment of the equilibrium between (1a) and (3a).

The percentage of diazepam present in equilibrium was similarly calculated in the presence of sds. By using these results, rate constants k for the forward reaction (1a) \rightarrow (3a) in the presence of sds were calculated from the observed rates. These results (Table 1) show that the reaction is inhibited by micelles of sds. The rate–sds profile showed a plateau between 200 and 400 mM sds, which indicates complete solubilization of diazepam in sds micelles at 200 mM sds. Tight isosbestic points were obtained at 225, 252 and 272 nm as for reaction in water. Furthermore, the rate of conversion (1a) \rightarrow (3a) in the presence of 200 mM sds was independent of acid concentration within the range 0.013–0.26 M HCl.

In both aqueous solution and in the presence of sds, it was found that when the pH of the equilibrium mixture (1a) \rightleftharpoons (3a) was increased to pH 7, diazepam (1a) was reformed in quantitative yield.

This suggests that hydrolysis of diazepam follows the (1a) \rightleftharpoons (3a) \rightarrow (4a) pathway, both in aqueous solution and in the presence of sds. Thus, initial attack occurs at C5, leading to azomethine cleavage in both cases.

The position of the equilibrium between diazepam and intermediate (3a) was displaced slightly in favour of the intermediate in the presence of 200 mM sds relative

¹⁵ Pannetier, G., and Souchay, P., 'Chemical Kinetics' p. 166 (Elsevier: Amsterdam 1967).

to the situation in water. Since the rate of conversion (1a) \rightarrow (3a) is reduced in the presence of sds, an increase in the proportion of (3a) at equilibrium must be the result of a greater reduction in the rate of conversion (3a) \rightarrow (1a). This may be the result of different conformations of (3a) in aqueous solution and in the negatively charged micelle. However, recyclization of (3a) requires the amino group to be unprotonated. In acidic solution, (3a) is in equilibrium with the corresponding protonated form. This protonated form would be favoured in micelles of sds because of the electrostatic stabilization provided by the micelle. Thus, the recyclization of (3a) would be inhibited relative to the situation in water.

(b) *Nitrazepam*

Han⁸ proposed that in aqueous solution initial hydrolysis occurs at the azomethine linkage. We have determined the percentage of nitrazepam present at equilibrium as a function of acid concentration. This was done as for diazepam, compound (5b) being used as a model for intermediate (3b). We found that above 0.1 M HCl, the equilibrium was displaced completely towards intermediates (3b), while at lower acid concentrations, significant amounts of nitrazepam existed in equilibrium with (3b). These range from 13% nitrazepam at 0.05 M HCl, through 25% at 0.025 M HCl to 38% at 0.012 M HCl.

The variation of the percentage of nitrazepam at equilibrium as a function of acid concentration probably reflects the lower basicity of nitrazepam, which contains a nitro group, compared with diazepam which has a chloro substituent. Furthermore, less reactant is present at equilibrium for nitrazepam than for diazepam. This can be explained by the fact that the positive charge in protonated nitrazepam is conjugated with the nitro group whereas in intermediate (3b) the positive charge is not conjugated with the nitro group. Thus, the destabilizing effect of the strong electron-withdrawing nitro group is more severe for nitrazepam than for intermediate (3b). For diazepam, the chloro group is electron-releasing by resonance and hence stabilizes the protonated starting material more than intermediate (3a) for which the positive charge and the chloro group are not conjugated.

Table 2. Observed first-order rate constants for the acidic hydrolysis of nitrazepam (1b) at 68.5°
The loss of nitrazepam was followed at 280 nm. Rate constants for (1) \rightarrow (3) as described in text, percentage of nitrazepam at equilibrium being used

[HCl] ^A (M)	0	0 ^B	10 ⁴ k ₁ /s ⁻¹ for [sds]/mM of					
			20	40	100	200 ^C	300	400
0.527 (0)	6.95	6.36				9.60 (9.56)		
0.264 (0)	7.08	6.44				6.02 (6.26)		
0.132 (0)	7.72	6.61				4.40 (4.43)		
0.066 (13)	7.70	6.0				3.05 (3.33)		
0.026 (25)	6.90	5.30				2.20 (2.02)		
0.013 (38)	5.5	3.9	3.51	2.99	2.32	1.70	1.7	1.8

^A Percentage of nitrazepam at equilibrium in aqueous acidic solution in parenthesis.

^B Constant ionic strength, 0.527 M (KCl).

^C Values in parenthesis followed production of benzophenone (4b) at 364 nm.

By using these results, the rate of conversion (1b) \rightarrow (3b) (Table 2), was calculated from the observed rate of establishment of equilibrium. The rate of hydrolysis of

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts



Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research



With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips



Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS

Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS

Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS

Sync your system to PACER to automate legal marketing.