BIOORGANIC CHEMISTRY 9, 288–292 (1980)

Proton Inventory Study of the General Base-Catalyzed Hydrolyses of Trifluoroacetanilides by Imidazole

MAKOTO KOMIYAMA AND MYRON L. BENDER

Departments of Chemistry and Biochemistry, Northwestern University, Evanston, Illinois 60201

Received February 16, 1979

The hydrolyses of substituted trifluoroacetanilides were catalyzed by neutral imidazole. The effect of five phenyl substituents (H, *m*-Cl, *p*-Cl, *m*-NO_2, and *p*-NO_2) on the hydrolyses was examined and proton inventory studies were carried out. Catalyses by neutral imidazole exhibited a *positive* $\rho(+0.84)$ and multiproton transfer in the transition state. This reaction involves general base-catalyzed formation of the tetrahedral intermediate by neutral imidazole followed by its breakdown catalyzed by water.

INTRODUCTION

Many studies have been carried out on the nonenzymatic hydrolyses of acetanildes (*1–21*). One important reason for the increasing attention to the hydrolyses of acetanilides is that they can shed new light on the mechanism of hydrolysis of amides catalyzed by serine proteases.

It is widely accepted that the hydrolyses of amides catalyzed by serine proteases involve general base catalysis by the neutral imidazolyl group of histidine and acid catalysis by the imidazolyl cation of histidine (*22, 23*).

In spite of the important role of the imidazolyl group of the histidine residue of serine proteases in the enzymatic hydrolyses of amides, information on the nonenzymatic hydrolyses of anilides catalyzed by imidazole is not sufficient (*1, 19, 20, 24, 25*).

In the present paper, the hydrolyses of five trifluoroacetanilides (H, *m*-Cl, *p*-Cl, *m*-NO_2, and *p*-NO_2) ((**2–6**, respectively) catalyzed by neutral imidazole (**1**), are described. The effect of these phenyl substituents on the rates of hydrolyses catalyzed by **1** is shown. D_2O kinetic solvent isotope effects on the **1**-catalyzed hydrolyses of **2–6** were measured. Furthermore, proton inventory studies, which can ascertain the number of protons transferred in the transition state (*27–29*), were carried out on the **1**-catalyzed hydrolyses of **2** and **6**.

EXPERIMENTAL

Materials. **1** was recrystallized four times from benzene. Trifluoroacetanilides (**2–6**) were synthesized from trifluoroacetic anhydride and the corresponding anilines; **2**, mp 87°C (lit. (*16*) 87–88°C); **3**, mp 70–71°C (lit. (*16*) 66–68°C); **4** mp

0045-2068/80/030288-05$02.00/0

126°C (lit. (*16*) 123–124°C); **5** mp 87°C (lit. (*29*) 88°C); **6** mp 150°C (lit. (*29*) 147°C). D_2O was obtained from Merck (minimum isotopic purity, 99.7 atom%). All water used in the kinetic studies was doubly distilled.

Kinetics. The rate constants of the cleavage of trifluoroacetanilides were determined spectrophotometrically at 70°C by the method of Guggenheim (*30*). The rate constants of the **1**-catalyzed hydrolyses, k_4, were determined from the slope of the straight line between the rate constant of the cleavage and the concentration of **1**, observed in the region of high concentration of **1** (*1, 5, 6, 20*).

The correction of the pH meter reading in D_2O–H_2O mixtures (the difference between the pH reading and the real pH) was made according to the literature (*31*).

RESULTS AND DISCUSSION

Figure 1 shows a plot of the logarithm of k_4 (the catalytic rate constant of *neutral* **1**) versus σ^- as well as a plot of the D_2O solvent isotope effect versus σ^-. From the slope of the straight line between log k_4 and σ^-, the Hammett ρ for k_4 was determined to be $+0.84 \pm 0.04$. This value of ρ is close to the value for the reaction involving the water-catalyzed breakdown of tetrahedral intermediates between trifluoroacetanilides and hydroxide ion (+0.69) (*16*).

The D_2O kinetic solvent isotope effect on k_4 increases as σ^- decreases, and the slope drastically increases (in magnitude) as σ^- decreases. A similar decrease in the magnitude of the D_2O solvent isotope effect with electron-withdrawing phenyl substituents was found in the hydrolyses of trifluoro-*N*-methylacetanilides, where water catalyzes the breakdown of the tetrahedral intermediates between the anilides and hydroxide ion (*9*).

The results of the proton inventory study on k_4 of **2** (the largest D_2O solvent isotope effect) and of **6** (the smallest D_2O solvent isotope effect) are shown in Fig.

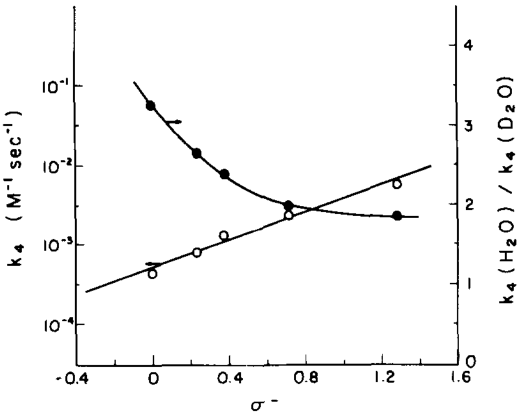

FIG. 1. Plots of the rate constant (k_4) and the D_2O solvent isotope effect versus σ^- in the hydrolyses of trifluoroacetanilides catalyzed by neutral **1** at 70°C.

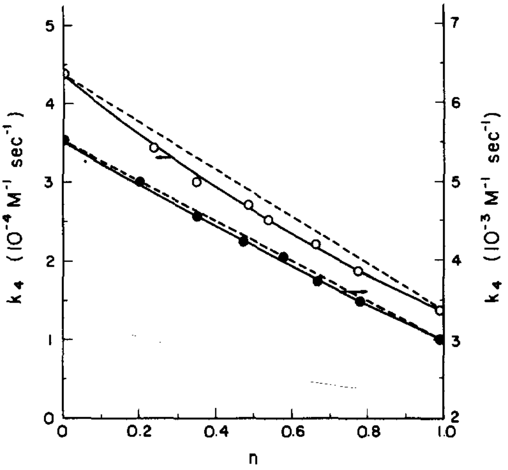

FIG. 2. Plots of the rate constants of the neutral 1-catalyzed hydrolyses of **2** (○) and **6** (●) (k_4) versus the mole fraction of D_2O in D_2O–H_2O mixtures (*n*) at 70°C; dashed lines are theoretical lines corresponding to one-proton transfer; the solid lines are theoretical lines calculated using the assumption that two protons with the same ϕ^T's (0.56 for **2** and 0.74 for **6**) are transferred in the transition state.

2. The plot of k_4 of **2** versus n (the mole fraction of D_2O in D_2O–H_2O mixtures) deviates considerably from a straight line (corresponding to one proton transfer in the transition state, which is depicted by the dashed line in Fig. 2). This fact shows that several protons are transferred in the transition state of the hydrolysis of **2** catalyzed by **1** (*26–28*). The open circles, experimental points, fit the theoretical line (the solid line) calculated using the assumption that two protons are transferred in the transition state and both of them have the same fractionation factor in the transition state (ϕ^T = 0.56). There are many other combinations of ϕ^T's which show curves that almost superimpose the solid line in Fig. 2. Three proton transfers in the transition state cannot be ruled out by the result of this proton inventory, although they are unlikely on entropy grounds. However, it can be definitely stated that two (or more) protons are transferred in the transition state of the hydrolysis of **2** catalyzed by neutral **1**.

The plot of k_4 of **6** versus n also shows a similar deviation from a straight line (corresponding to one proton transfer) as observed in the hydrolysis of **2**. Thus, multiproton transfer in the transition state is likely in the hydrolysis of **6** also. However, the result is not conclusive here as in the hydrolysis of **2**, considering the experimental error.

The proton inventory study shows that catalysis by **1** involves general base rather than nucleophilic catalysis since nucleophilic catalysis should not show multiproton transfers in the transition state. The result agrees with previous indications from spectroscopy (*25*). One of the two protons showing a different ϕ^T from unity is the proton which **1** abstracts from water. This proton abstraction by **1** enhances the nucleophilicity of the water toward the carbonyl carbon atom of anilides, facilitating the formation of the tetrahedral intermediate. The second proton is the one donated from the second water molecule to the nitrogen atom to facilitate breakdown of the tetrahedral intermediate. This proton donation from water is required to avoid the production of the very unstable anilinium anion. Thus, the hydrolysis of trifluoroacetanilides catalyzed by **1** proceeds through (rate determining) general base catalysis by neutral **1**, followed by (general) acid catalysis by water.

In conclusion, the present proton inventory study show that **1** functions as a general base catalyst in the hydrolyses of trifluoroacetanilides. This is the same as the general base catalysis by the imidazolyl group of histidine in serine proteases.

ACKNOWLEDGMENTS

The authors acknowledge valuable comments from Dr. R. L. Schowen at the University of Kansas and Dr. R. M. Pollack at the Universite des Science et Techniques due Languedoc. This work was supported by grants from the National Science Foundation (CHE76-14283), an American Cancer Society Institutional award, and the Hoffmann–LaRoche.

REFERENCES

1. Preliminary communication; M. KOMIYAMA AND M. L. BENDER, *J. Amer. Chem. Soc.* **100**, 5977 (1978).
2. S. S. BIECHLER AND R. W. TAFT, JR., *J. Amer. Chem. Soc.* **79**, 4927 (1957).
3. M. L. BENDER AND R. J. THOMAS, *J. Amer. Chem. Soc.* **83**, 4183 (1961).
4. P. M. MADER, *J. Amer. Chem. Soc.* **87**, 3191 (1965).
5. S. O. ERIKSSON AND C. HOLST, *Acta Chem. Scand.* **20**, 1892 (1966).
6. S. O. ERIKSSON AND L. BRATT, *Acta Chem. Scand.* **21**, 1812 (1967).
7. R. L. SCHOWEN AND G. W. ZUORICK, *J. Amer. Chem. Soc.* **88**, 1223 (1966).
8. R. L. SCHOWEN, H. JAYARAMAN, L. KERSHNER, AND G. W. ZUORICK, *J. Amer. Chem. Soc.* **88**, 4008 (1966).
9. L. D. KERSHNER AND R. L. SCHOWEN, *J. Amer. Chem. Soc.* **93**, 2014 (1971).
10. R. L. SCHOWEN, C. R. HOPPER, AND C. M. BAZIKIAN, *J. Amer. Chem. Soc.* **94**, 3095 (1972).
11. D. DRAKE, R. L. SCHOWEN, AND H. JAYARAMAN, *J. Amer. Chem. Soc.* **95**, 454 (1973).
12. C. R. HOPPER, R. L. SCHOWEN, K. S. VENKATASUBBAN, AND H. JAYARAMAN, *J. Amer. Chem. Soc.* **95**, 3280 (1973).
13. R. M. POLLACK AND M. L. BENDER, *J. Amer. Chem. Soc.* **92**, 7190 (1970).
14. C. O'CONNOR, *Quart. Rev. Chem. Soc.* **24**, 553 (1970).
15. R. H. DEWOLFE AND R. C. NEWCOMB, *J. Org. Chem.* **36**, 3870 (1971).
16. C. E. STAUFFER, *J. Amer. Chem. Soc.* **94**, 7887 (1972).
17. R. M. POLLACK AND T. C. DUMSHA, *J. Amer. Chem. Soc.* **95**, 4463 (1973).
18. M. KOMIYAMA AND M. L. BENDER, *J. Amer. Chem. Soc.* **99**, 8021 (1977).
19. J. W. HENDERSON AND P. HAAKE, *J. Org. Chem.* **42**, 3989 (1977).
20. M. KOMIYAMA AND M. L. BENDER, *Bioorg. Chem.* **7**, 133 (1978).
21. M. KOMIYAMA AND M. L. BENDER, *Bioorg. Chem.* **8**, 141 (1979).
22. M. L. BENDER AND J. V. KILLHEFFER, *CRC Crit. Rev. Biochem.* **1**, 149 (1973).
23. D. M. BLOW, *Accounts Chem. Res.* **9**, 145 (1976).
24. C. E. STAUFFER, *J. Amer. Chem. Soc.* **96**, 2489 (1974).
25. R. M. POLLACK AND T. C. DUMSHA, *J. Amer. Chem. Soc.* **97**, 377 (1975).
26. A. J. KRESGE, *Pure Appl. Chem.* **8**, 243 (1964).
27. V. GOLD, *Advan. Phys. Org. Chem.* **7**, 259 (1969).

28. R. L. SCHOWEN, "Isotope Effects on Enzyme-Catalyzed Reactions" (W. W. Cleland, M. H. O'Leary, and D. B. Northrop, Eds.), p. 64, University Park Press, Baltimore, Md., 1977.
29. M. J. SAXBY, *Mass. Spectrom.* **2,** 835 (1969).
30. E. A. GUGGENHEIM, *Philos. Mag.* **2,** 538 (1926).
31. K. B. J. SCHOWEN, J. K. LEE, AND R. L. SCHOWEN, paper presented at the 10th Midwest Regional Meeting of the American Chemical Society, University of Iowa, Iowa City, 1974.