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Hydrolytic and Associative Behavior of Aromatic Amides in Aqueous Solution

Kiichiro Kakemi, Hitoshi Sezaki, Masahiro Nakano, Kiichiro Ohsuga, and Takayoshi Mitsunaga

Faculty of Pharmaceutical Sciences, Kyoto University[1]

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Rate constants for alkaline hydrolysis (in 0.1N NaOH) of aromatic amides such as nicotinamide, benzamide, phenylacetamide, cinnamamide, and their corresponding N-alkyl substituted analogs were determined at 25° and 90° in order to investigate the effect of molecular structure on the stability of these amides against hydroxyl ion attack. Stability constants of these aromatic amide complexes with theophylline and 8-methoxycaffeine at 25° were also computed from phase-solubility data. The results have shown that cinnamamides are most stable against hydroxyl ion attack and formed the most stable complexes with the alkylxanthines while phenylacetamides are least stable in alkaline hydrolysis and least associative with alkylxanthines among benzene derivatives. Both hydrolytic behavior and associative tendency of these amides are discussed in relation to the extent of conjugation of the amide group with the rest of the molecule.

Although kinetic studies on the hydrolysis of unsubstituted nicotinamide have been carried out,[2,3] no work seems to have been reported in the literature on the hydrolytic behavior of N-substituted aromatic amides. As a part of investigations to study physico-chemical behavior of aromatic amides in solution,[4,5] the present study is concerned with the measurements of rate constants of hydrolysis of aromatic amides, catalyzed mainly by hydroxyl ion, in order to obtain information on the relationship between the structure and the stability of these amides in aqueous solution, and with examination of associative tendencies of these amides to alkylxanthines in order to elicit certain relationships between chemical structures and interactive tendencies.

Experimental

Materials——All amides and alkylxanthines were the same as reported earlier,[4,5] with the exception of N-ethylnicotinamide, N,N-diethylnicotinamide (nikethamide), and phenylacetamide which were commercial products and purified before use, mp 58°, bp 158° (10 mmHg), and mp 158°, respectively. N,N-Dimethylphenylacetamide was synthesized in this laboratory and vacuum distilled; bp 142° (7 mmHg). Sodium hydroxide, hydrochloric acid, and imidazole were of reagent grade. Chloroform and methanol were of spectroscopic and reagent grade, respectively.

Kinetic Studies——Alkaline Hydrolysis at Elevated Temperatures: One hundred and eighty milliliters of a 0.1N sodium hydroxide solution was preheated in a flask immersed in a constant temperature oil bath maintained at a desired temperature. The amide dissolved in 20 ml of a 0.1N sodium hydroxide solution was then added to the preheated solution and mixed vigorously and the reaction was allowed to proceed. The initial amide concentrations were 2 mM. Since it was shown[2] that there is no primary salt effect in hydrolysis of nicotinamide in strongly alkaline solution, no salt was added to adjust ionic strength. An aliquot of the reaction mixture (5 ml) was sampled at appropriate time intervals, and the reaction was quenched on cooling with ice-water. Unreacted amides were extracted with 5 ml chloroform at 25° and the chloroform layers were appropriately diluted with methanol for ultraviolet measurements with either a Shimadzu QV-50 spectrophotometer or Hitachi Perkin-Elmer spectrophotometer, Model 139. Partition constants of these amides were constant at 25° over the concentration range studied. Determinations of

1) Location: *Yoshida, Sakyo-ku, Kyoto.*
2) P. Finholt and T. Higuchi, *J. Pharm. Sci.*, **51**, 655 (1962).
3) H.H.G. Jellinek and A. Gordon, *J. Phys. Colloid Chem.*, **53**, 996 (1949).
4) M. Nakano and T. Higuchi, *J. Pharm. Sci.*, **57**, 183 (1968).
5) K. Kakemi, H. Sezaki, K. Ohsuga, and M. Nakano, *Chem. Pharm. Bull.* (Tokyo), **16**, 819 (1968).

concentrations of the nicotinamides were made at 263 mμ. Other wavelengths chosen for the determinations of concentration were 270 mμ for the benzamides, 274 mμ for cinnamamide, 280 mμ for N,N-dimethylcinnamamide, and 265 mμ for the phenylacetamides.

Alkaline Hydrolysis at 25°: Weighed samples (2 mM) were dissolved in 200 ml of 0.1N sodium hydroxide solution and stirred for a few minutes to ensure complete dissolution. The solution was then placed in a water-bath maintained at 25°. Extraction and assay procedures were the same as those at elevated temperatures.

Hydrolysis in the Presence of Imidazole: Imidazole was dissolved in 0.1N NaOH so that its concentration became 4 mM or 40 mM. Weighed amides (2 mM) were then dissolved in 200 ml of the above imidazole solution. The mixture was maintained at 25° in a water-bath. The same procedure as descirbed for alkaline hydrolysis at elevated temperatures was followed. As the blank, the corresponding imidazole solution without containing amides was treated identically.

Acid Hydrolysis at 90°: One hundred and eighty milliliters of 1N HCl was preheated to 90°. Amides dissolved in 20 ml of 1N HCl was then added to the preheated solution and stirred vigorously. While hydrolysis was in progress, an aliquot of the reaction mixture was withdrawn at appropriate time intervals. The same procedures were followed as described for alkaline hydrolysis at elevated temperatures except that the samples, in this case, were made alkaline by adding 10 ml of 2N NaOH before the extraction of unreacted amides.

Solubility Studies——Experimental procedures were the same as those reported previously.[4,5]

Results

Plots of logarithm of (residual amide concentration)/(initial concentration) against time are shown in Fig. 1 for the hydrolysis of N-unsubstituted aromatic amides at 90°. Similar

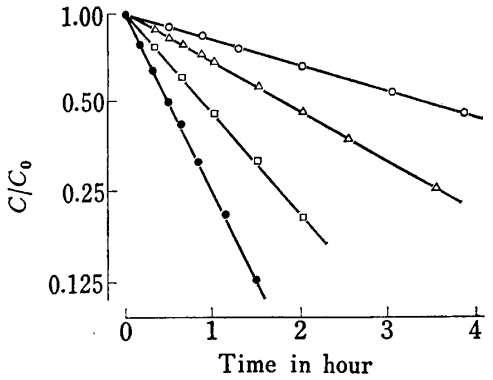

Fig. 1. Hydrolysis of Some Aromatic Amides in 0.1 N NaOH Solutions at 90°

○ : cinnamamide △ : benzamide
□ : phenylacetamide ● : nicotinamide

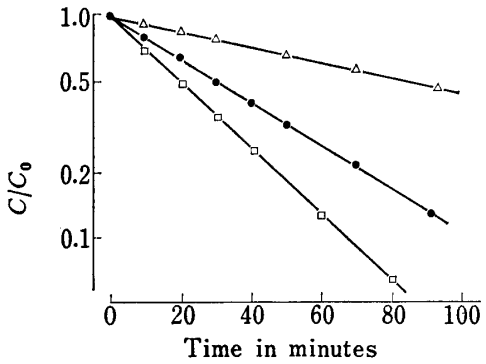

Fig. 2. Hydrolysis of Nicotinamides in 0.1 N NaOH Solutions at 90°

△ : N-methylnicotinamide ● : nicotinamide
□ : N,N-dimethylnicotinamide

Table I. Pseudo-First-Order Rate Constants (hour^{-1}) of Amide Hydrolysis in 0.1N NaOH Solutions

Amide	Rate Constant at 90°	Rate Constant at 25°
Nicotinamide	1.36	0.0301
N-Methylnicotinamide	0.462	—
N,N-Dimethylnicotinamide	2.31	0.0277
N-Ethylnicotinamide	0.292	—
N,N-Diethylnicotinamide	0.104	0.00068[a]
Benzamide	0.385	0.00408
N,N-Dimethylbenzamide	0.347	0.00240
Cinnamamide	0.198	0.00147
N,N-Dimethylcinnamamide	0.219	0.00169
Phenylacetamide	0.770	0.0136
N,N-Dimethylphenylacetamide	0.277	0.00343

a) extrapolated value from measurements at 90° and 50°

plots (Fig. 2) show the effect of N-substitution in the hydrolysis of nicotinamides. The rates of disappearance of these amides from solutions were found to be first order with respect to the amide concentration under the experimental conditions. The rate constants recorded in Table I were computed by the standard procedure on the assumption that the breakdown of the amides is attributed entirely to the hydrolytic cleavage of the amide linkage.

Since N,N-dimethylnicotinamide was found to be exceptionally reactive at 90°, both nicotinamide and N,N-dimethylnicotinamide were examined in greater detail and the results are summarized in Table II. The effects of temperature on rate constants for these amides were illustrated in Fig. 3.

Table II. Pseudo-First-Order Rate Constants ($hour^{-1}$) of Nicotinamides

Amide	In 0.1N NaOH solution at					
	50°	40°	25°	25° with 4 mM imidazole	25° with 40 mM imidazole	In 1N HCl at 90°
Nicotinamide	0.154	0.0825	0.0301	0.0308	0.0252	0.693
N,N-Dimethylnicotinamide	0.198	0.0866	0.0277	0.0277	0.0256	0.115

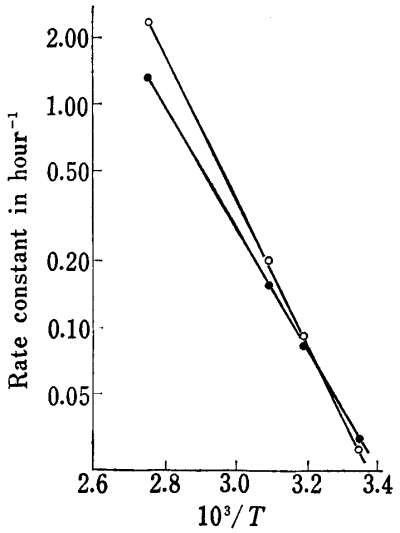

Fig. 3. Arrhenius Plot for the Hydrolysis of Nicotinamides in 0.1N NaOH Solutions

○ : N,N-dimethylnicotinamide
● : nicotinamide

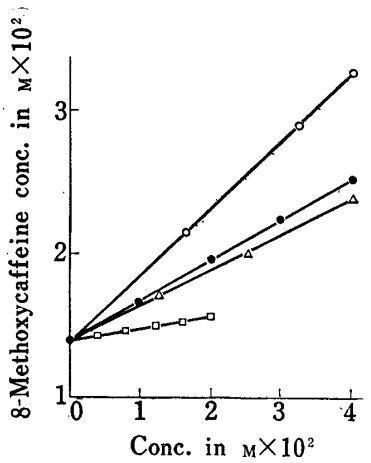

Fig. 4. Solubility Diagrams for 8-Methoxycaffeine in the Presence of Aromatic Amides at 25° in Water

○ : cinnamamide
● : nicotinamide
△ : benzamide
□ : phenylacetamide

Solubility diagrams of 8-methoxycaffeine in the presence of N-unsubstituted aromatic amides are shown in Fig. 4. Based upon such data apparent 1:1 stability constants, computed[6] as a measure of interactive capacity of aromatic amides with theophylline and 8-methoxycaffeine, are presented in Table III together with some of the results reported earlier in order to elicit the structural dependence of the associative tendency of aromatic amides.

Discussion

It is apparent in Fig. 1 that the stability of aromatic amides against hydrolysis catalyzed by hydroxyl ion appears to be related to the structure of amides. Among the three amides

6) T. Higuchi and K.A. Connors, *Advan. Anal. Chem. Instr.*, 4, 117 (1965).

Table III. Apparent 1:1 Stability Constants (M^{-1}) of Theophylline and 8-Methoxycaffeine Complexes at 25° in Water

Amide	Theophylline	8-Methoxycaffeine
Nicotinamide	13	28[b]
N-Methylnicotinamide	13	25[b]
N,N-Dimethylnicotinamide	3.4	5[b]
Benzamide	12[a]	21
N-Methylbenzamide	13[a]	—
N,N-Dimethylbenzamide	2[a]	4
Cinnamamide	32[a]	63
N,N-Dimethylcinnamamide	30[a]	54
Phenylacetamide	4.2	6.3
N,N-Dimethylphenylacetamide	5.8	6.6

a) from reference 4 *b*) from reference 5

having a benzene ring, the amide group joined directly to a benzene ring through an olefinic group (cinnamamide) is most stable, that joined directly to a benzene ring (benzamide) being of intermediate stability, and that joined to a benzene ring through an aliphatic group (phenylacetamide) being least stable against hydroxyl ion attack. Generally, the more highly conjugated is the group joined to the amide group, the more stable are the aromatic amides against hydroxyl ion attack. Reduced reactivity of amide groups in the presence of a neighboring double bond or an aromatic ring may be attributed to resonance stabilization which makes the formation of a tetrahedral intermediate prior to hydrolytic cleavage difficult. Nicotinamide which is a hetero aromatic amide was found to be more reactive than its benzene counterpart.

As for the effect of N–substitution on the reactivity of amides (see Table I), it is generally observed for aliphatic amides that the reactivity of the amides decreases with the increasing number and size of the N–substituent.[7] This trend is evident in the phenylacetamides while it is not so clear in the benzamides. In the cinnamamides, although no significant difference in the reactivity was observed between the N–unsubstituted and the N,N-dimethyl derivative, the reverse phenomenon was observed. In nicotinamide, the N,N-dimethyl analog was exceptionally unstable at higher temperatures being much more so than the N-unsubstituted. This unusual effect, however, diminished at lower temperatures suggesting its heat of activation is larger than that of the N–unsubstituted. This effect is clearly seen in Fig. 3.

Although imidazole has been known to catalyze some of the hydrolytic cleavage of esters,[8] its effect on amide hydrolysis under the present experimental condition appears to be rather opposite to it. Imidazole served as an inhibitor in the present experiments. The results may be rationalized by the formation of a weak complex which is less reactive towards hydroxyl ion attack. Similar observation has been reported on alkaline hydrolysis of a cinnamate ester.[9] Less inhibitory effect of imidazole in the alkaline hydrolysis of N,N-dimethylnicotinamide compared with that of nicotinamide may be attributed to its less tendency to form a complex in comparison with the unsubstituted amides.[4]

The hydrolysis of amides in an acid medium was performed only with nicotinamide and its N,N-dimethyl analog. In 1N HCl these amides were expected to exist in the totally protonated form at the pyridine ring. The unusual reactivity of N,N-dimethylnicotinamide as observed in the hydroxyl ion catalyzed hydrolysis at elevated temperatures was not observed in the acid hydrolysis under the experimental conditions, the N,N-disubstituted being less

7) *a*) U. Mazzucats, A. Foffani, and G. Cauzzo, *Ann. Chim.* (Rome), **50**, 512 (1960) through *C.A.*, **54**, 23643*g* (1960); *b*) H. Morawetz and P.S. Otaki, *J. Am. Chem. Soc.*, **85**, 463 (1963).
8) T.C. Bruice and G.L. Schmir, *Arch. Biochem. Biophys.*, **63**, 484 (1956).
9) J.A. Mollica and K.A. Connors, *J. Am. Chem. Soc.*, **89**, 308 (1967).

reactive than the parent compound. It remains to be seen that what factors are responsible for the marked temperature effect on the alkaline hydrolysis of N,N-dimethylnicotinamide as compared with that of the unsubstituted amide.

The relationship between the complexing tendency and molecular structure of amide is apparent in Fig. 4. Among the four N-unsubstituted amides, cinnamamide exhibited the largest associative tendency toward 8-methoxycaffeine and theophylline followed by nicotinamide and benzamide, and phenylacetamide being least associative. These associative tendencies appear to be related to the extent of conjugation as was the case with a number of systems studied previously.[10] While in cinnamamide the amide group is conjugated with the styrene group, no such conjugation of the amide group with the benzene ring is possible in phenylacetamide.

The reduced associative tendency due to the effect of N,N–disubstitution is pronounced in benzamides and nicotinamides, while the effect is less obvious in both phenylacetamides and cinnamamides. The reduced associative ability of the N,N–dimethyl analogs of benzamide and nicotinamide may be rationalized by the hypothesis that these molecules are not planar because of the difficulty in maintaining a planar structure due to the presence of bulky substituents.[4,5] In cinnamamides, both the N–unsubstituted and N,N–disubstituted derivative can take a planar structure,[4] whereas both phenylacetamide and its N,N–dimethyl analog assume nonplanar structures with respect to the benzene ring. The importance of planarity of molecules for strong binding tendency has already been discussed elsewhere.[4]

From both kinetic and equilibrium studies discussed above, it may be concluded for some simple systems (such as benzamide, phenylacetamide, and cinnamamide) that an amide whose amide group is conjugated with a styrene group or a benzene ring assumes a resonance stabilized planar structure, and consequently it is more stable against hydroxyl ion attack and forms a more stable complex with alkylxanthines than that without such conjugation. The fact that reactivity and associative tendency do not necessarily correlate well in all aromatic amides studied here, however, suggests that the extent of conjugation is only one of the factors which may influence the hydrolytic and associative behavior of organic molecules in solution. For instance, steric and inductive effects will, no doubt, also contribute to such behaviors in solution. Further, the difference in relative importance among such factors for both associative and reactivity capacity is not likely to permit us to observe such generalization as made above for all similar systems.

10) *a*) M. Nakano and T. Higuchi, the 115th Annual Meeting of Academy of Pharmaceutical Sciences of APhA, Miami, Florida, May 1968; *b*) M. Nakano, Ph. D. Thesis, University of Wisconsin, Madison, Wis., 1967.