90° under N_2 stream for 1 hr. The amount of CO_2 was determined by weighing the Ascarite-tube. The yields of amine **14** and carbamate **52** were determined by gas chromatography using an internal standard method.

Reactions of Amines 13, 14, 21, 34, 37, 44, and 68 with $ClCO_2Et$ in Hexane, Benzene, Chloroform and Nitrobenzene——a) In a three-necked flask equipped with a reflux condenser, cold-trap and a weighed Ascarite-tube serially, an N_2 inlet tube and a dropping funnel, was placed a solution of 1.00 g of amine in 30 ml of hexane, benzene, chloroform or nitrobenzene and the solution was cooled at 5°. To this solution, a solution of 1.00 equiv. of $ClCO_2Et$ in 10 ml of the solvent was dropped during 3 min, with stirring and under a stream of N_2. After 2 hr, the amount of CO_2 evolved was determined. Then, the mixture was heated at the temperature around 80°, and the yields of carbamate, CO_2 and amine were determined.

b) In the similar apparatus, was placed a solution of 1.00 g of amine in 30 ml of hexane, benzene, chloroform or nitrobenzene and the solution was heated at 85—90°. To this solution, a solution of 1.00 equiv. of $ClCO_2Et$ in 10 ml of the solvent was added dropwise during 3 min, with stirring and under a stream of N_2. After 1 hr, the amounts of CO_2, carbamate and amine were determined as described above.

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Methanesulfonic Acid Derivative of N-Alkyl Aniline: Basicity of Aniline and Reactivity[1,2]

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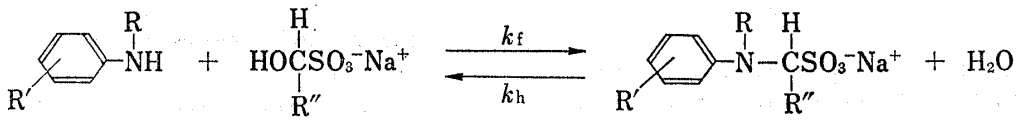
Effects of N-alkyl substituents on the formation and hydrolysis rates of N-substituted aniline methanesulfonic acid derivatives were investigated in connection with substituted aniline derivatives. Their rates were satisfactorily correlated to the basicity of aniline derivatives which was corrected by hydration effect, that is Folkers, *et al.*'s H value.

In a series of studies on the water soluble methanesulfonic acid derivatives (MSD) of aniline homologous drugs, the basicity of the parental aniline has been found to be the dominant factor for the reversible MSD formation, which can be expressed by the following general formula:[4-6]



In the previous studies[4,6] the effect of R′ substituent of aniline on the formation rate constant (k_f, second-order) and the hydrolysis rate (k_h, first order) could be satisfactorily represented by the linear free energy relationship (LFER) between pK_a of substituted anilinium ion and $\log k_f$ or $\log k_h$. The plots for N-methyl aniline, however, deviated positively from the linear relationship on which further explanation was not made.

1) Presented at the 95th Annual Meeting of Pharmaceutical Society of Japan, Nishinomiya, Japan, April 1975.
2) This report constitutes Part VIII of the studies entitled, "Methanesulfonic Acid Derivatives of Drug" where Part VII is in: Y. Kurono, K. Ikeda, and K. Uekama, *Chem. Pharm. Bull.* (Tokyo), **23**, 409 (1975).
3) Location; *Tanabe-dori, Mizuho-ku, Nagoya, 467, Japan.*
4) K. Ikeda, K. Miyata, T. Iwata, F. Kawata, and K. Kurome, *Chem. Pharm. Bull.* (Tokyo), **18**, 440 (1970).
5) K. Ikeda, Y. Kurono, and T. Tukamoto, *Chem. Pharm. Bull.* (Tokyo), **20**, 863 (1972).
6) Y. Kurono, K. Ikeda, and K. Uekama, *Chem. Pharm. Bull.* (Tokyo), **23**, 340 (1975).

In this study the effect of N-alkyl substituent, R, on k_f and k_h was investigated on several secondary anilines and the results were satisfactorily correlated to the basicity of aniline derivative by introducing Folkers, *et al.* 's H value.[7]

Results and Discussion

Formation Rate

The relationship between pK_a of N-alkyl aniline homologue and log k_f is represented in Fig. 1 (No. 22—26). At the same time the results for R'-substituted aniline derivatives were plotted in connection with N-alkyl substituent anilines.[6] In parallel to the solid line on which the plots of *meta* and *para* substituted aniline (notation: ○) are located, a dotted line is drawn with a distance of 0.301 (=log 2) on which the plots of intramoleculary H-bonded *ortho* substituted aniline (notation: ⊙) are plotted. On the deviations from the linear relationship explanations were presented for each case in the previous article.[6]

The plots for N-alkyl aniline obtained in this study can not be explained from the simple pK_a value. In spite of the decrease of reactive hydrogen due to R-substitution the reaction rate is higher than the expectation from pK_a value. According to Folkers, *et al.*,[7] a correction for the hydration to amine is necessary to correlate the basicity of primary and secondary amine to Taft's substitution constant, σ^*. They postulated $n\cdot H$ term to pK_a, where n is the number of hydration and H is the empirical constant, −1.12. The n is then 3 and 2 for

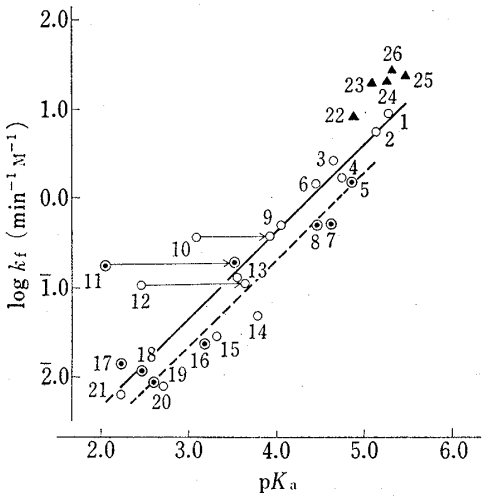


Fig. 1. Relationship between log k_f at pH 7.4 (37°) and pK_a of Corresponding Anilinium Ion

Numbers refer to aniline derivatives containing the follo-w ing substituent.

1: *p*-OC_2H_5	10: *m*-COO^-	19: *o*-Cl
2: *p*-CH_3	11: *o*-COO^-	20: *p*-$COCH_3$
3: H(aniline)	12: *p*-COO^-	21: *p*-SO_2NH_2
4: *m*-CH_3	13: *m*-Cl	22: N-CH_3
5: *o*-OH	14: *m*-SO_3^-	23: N-C_2H_5
6: *o*-CH_3	15: *p*-SO_3^-	24: N-C_3H_7
7: *o*-OCH_3	16: *o*-F	25: N-*n*-C_4H_9
8: *o*-OC_2H_5	17: *o*-$COOCH_3$	26: N-*n*-C_5H_{11}
9: *p*-Cl	18: *o*-SO_3^-	

Results from No. 1 to No. 21 are the same as those reported in: Y. Kurono, K. Ikeda, K. Uekama, *Chem. Pharm. Bull.* (Tokyo), **23**, 340 (1975).

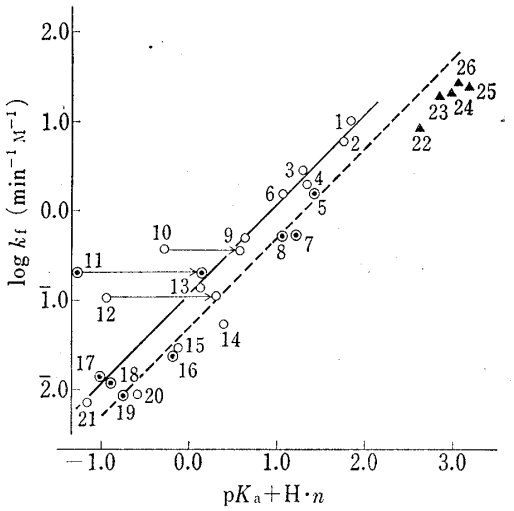


Fig. 2. Relationship between log k_f and $pK_a+H\cdot n$

Numbers correspond to those in Fig. 1.

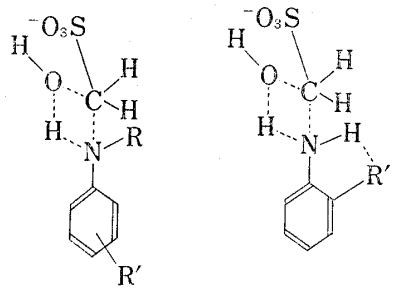


Chart 1. Reaction Intermediate

left; N-substituted aniline MSD
right; intramolecularly H-bonded aniline MSD

7) E. Folkers and O. Runquist, *J. Org. Chem.*, **29**, 830 (1964).

primary and N-alkyl aniline, respectively. Figure 2 shows the plots of log k_f against $pK_a+n\cdot H$. As is seen in Fig. 2 the plots for N-alkyl aniline are near the extrapolation of dotted line of the intramolecularly H-bonded anilines. This may be acceptable considering the structure of the reaction intermediate postulated because the number of reactive H on N becomes half by the substituent R on N. The intramolecularly H-bonded *ortho* substituted anilines have also only one reactive H.[6] Accordingly the downward deviation from the extrapolation may be attributed to the steric hindrance of substituent R in addition to the effect of intramolecularly H-bonded structure.

The linear relationship between $pK_a+n\cdot H$ and log k_f may be satisfied as follow. Folkers, *et al.*'s equation can be represented as equation 1 where ρ_K^* is the parameter for dissociation constant of anilinium ion, K_a. Equation 2 is the LFER expression of the reaction where ρ_R^* is the parameter for the reaction.

$$pK_a^\circ = \rho_K^* \sum\sigma^* + H\cdot n + pK_a \quad (1)$$

$$\log k_f = \rho_R^* \sum\sigma^* + \log k_f^\circ \quad (2)$$

Because all of the σ^* values are not known, the elimination of $\sum\sigma^*$ term from equation 1 and 2 gives the following equation 3 which consequently rationalizes the linear relationship between log k_f and $pK_a+H\cdot n$.

$$\log k_f = -\frac{\rho_R^*}{\rho_K^*}(pK_a+H\cdot n) + \frac{\rho_R^*}{\rho_K^*} pK_a^\circ + \log k_f^\circ \quad (3)$$

Hydrolysis Rate

Figure 3 shows the relationship between log k_h and pK_a, where again the plots for N-alkyl aniline markedly deviate from the linear relationship. Figure 4 represents the relationship between log k_h and $pK_a+H\cdot n$ where the n for MSD of primary aniline is 2 and that of N-alkyl aniline is 1. The statistical calculation gave a slope of 0.993 and $r=0.995$. The linear relationship may also be rationalized by the equation similar to equation. Values pK_a for MSD is not known, so the values for intact anilinium ions were used. However the linear relationship obtained may be accounted by the supposition of the following relation where α is the term related to the unknown σ^* values for $-CH_2SO_3^-$ group of MSD.

$$pK_a \text{ of MSD} = pK_a \text{ of intact anilinium ion} + \alpha \quad (4)$$

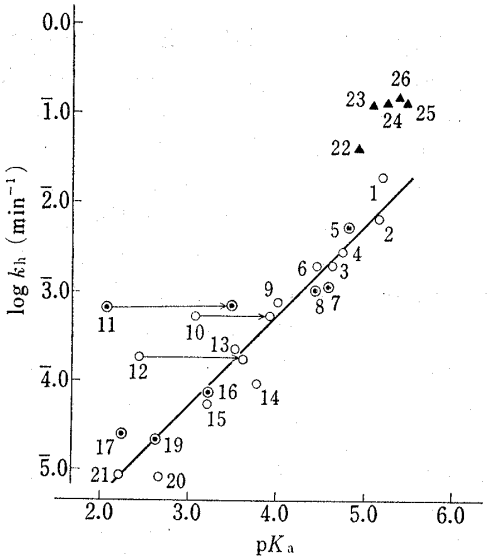


Fig. 3. Relationship between log k_h at pH 7.4 (37°) and pK_a of Corresponding Anilinium Ion

Numbers correspond to those in Fig. 1.

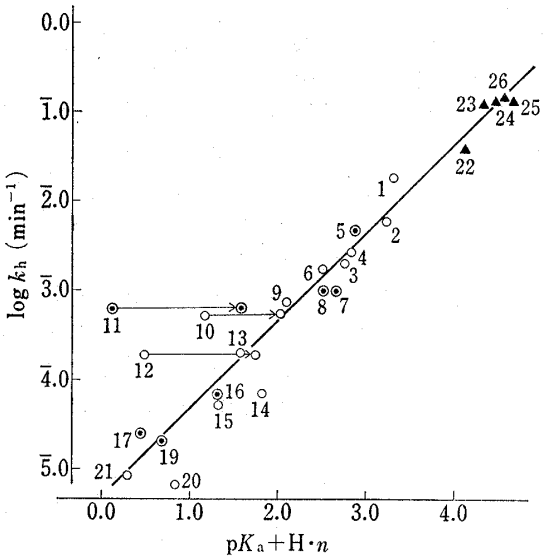


Fig. 4. Relationship between log k_h and $pK_a+H\cdot n$

Numbers correspond to those in Fig. 1.

Experimental

Synthesis of N-Substituted Aniline MSD——Synthesis was followed to the method of Neelakantan, *et al.*[8]

Kinetic Techniques——Kinetic procedures for the determinations of formation and hydrolysis rate constants were the same as employed in the previous study.[6] Reaction temperature was maintained at 37°.

pK_a Measurement——The pK_a of N-*n*-pentylaniline was determined by spectrophotometrical method at 25°.[6] Values pK_a of other aniline derivatives were cited from literature.[7,9]

8) L. Neelakantan and W.H. Hartung, *J. Org. Chem.*, **24**, 1943 (1959).
9) A. Albert and E.P. Serjeant, "Ionization Constants of Acids and Bases," John Willy & Sons Inc., New York, N. Y., 1962, p. 148.

[Chem. Pharm. Bull. 24(2) 352—355 (1976)]

UDC 547.458.09 : 577.159.04

Effect of Zymosan on Hepatic Drug Metabolism in Mice. II.[1] Identification of Active Component of Yeast Cell Walls

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(Received June 4, 1975)

Three glucan and a mannan preparations from baker's yeast (*Saccharomyces cerevisiae*) were examined for their effects on aminopyrine N-demethylase activity and cytochrome P-450 content of liver, and phagocytic activity in mice. Dextran and pustulan were also examined.

Among the polysaccharides tested, insoluble glucan mostly decreased cytochrome P-450 content and aminopyrine N-demethylase activity, and stimulated phagocytic activity. Alkaline soluble glucan, mannan, and pustulan decreased a little of cytochrome P-450 at high dose, whereas water-soluble glucan residue produced by acetolysis affected neither cytochrome P-450 content nor phagocytosis.

It has been reported that some modifiers of the reticuloendotherial (RE) system prolong the barbiturate-sleeping time and depress the metabolism of drugs.[3] Wooles, *et al.* had shown that prolonged intravenous administration of zymosan produced a marked prolongation of the barbiturate-sleeping time in mice,[3c] but decreased only 11% the metabolism of pentobarbital in liver slices.[3e] In previous work[1] we investigated the mechanism of zymosan-induced depression of the drug metabolism in mouse liver and found that the activities of aminopyrine N-demethylase, *p*-nitroanisole O-demethylase, and aniline aromatic hydroxylase were all markedly depressed and concomitantly cytochrome P-450 content was decreased.

Since zymosan is an insoluble cell wall complex of yeast consisting of polysaccharides as the major component, proteins, lipids, and inorganic elements,[4] it became necessary to clarify what component of zymosan would affect the drug-metabolizing enzyme system.

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2) Location: *a*) *Aobayama, Sendai*; *b*) *1–18–1, Kamiyoga, Setagaya-ku, Tokyo.*
3) *a*) S.C. Samaras and N. Diets, Jr., *Federation Proc.*, **12**, 122 (1953); *b*) W.R. Wooles and J.F. Borzelleca, *J. Reticuloendothel. Soc.*, **1**, 354 (1964); *c*) W.R. Wooles and J.F. Borzelleca, *ibid.*, **3**, 41 (1966); *d*) F.J. DiCarlo, L.J. Haynes, C.B. Countinho, and G.F. Phillips, *ibid.*, **2**, 360 (1965); *e*) D.W. Barnes and W.R. Wooles, *ibid.*, **7**, 684 (1969); *f*) W.R. Wooles and A.E. Munson, *ibid.*, **9**, 108 (1971); *g*) D. Gaillard, B. Pipy, and R. Derache, *Biochem. Pharmacol.*, **23**, 1245 (1974).
4) F.J. DiCarlo and J.V. Fiore, *Science*, **127**, 756 (1958).