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Stability of Sulpyrine. IV.[1] Reaction with Glucose in Aqueous Solution

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Glucose accelerated the decomposition of sulpyrine remarkably when it was added to sulpyrine injections. As the reaction products of sulpyrine and glucose, aminopyrine (Am), 4-formylmethylaminoantipyrine (FMAA), and N-[2-(5-hydroxymethyl-2,3,4,5-tetrahydro-2,3,4-trihydroxy)furyl]methyl-N-methylantipyrine (MAAG) were isolated. These products were determined separately by means of high performance liquid chromatography in order to study the kinetics and mechanism of the formation of them.

Methylamino group in 4-methylaminoantipyrine (MAA), a hydrolyzed product of sulpyrine, reacted reversibly with formyl group in glucose and formed an intermediate (MAA-glu). MAA-glu transformed reversibly to MAAG, which decomposed to Am, as follows;

$$\text{MAA} + \text{glucose} \underset{k_2}{\overset{k_1}{\rightleftarrows}} \text{MAA-glu} \underset{k_4}{\overset{k_3}{\rightleftarrows}} \text{MAAG} \xrightarrow{k_5} \text{Am}$$

The dependence of k_3, k_4 and k_5 on pH suggested that these reactions were catalyzed by specific acid.

The reaction pathway of the formation of FMAA were different from that of Am.

Keywords——sulpyrine; aminopyrine; 4-formylmethylaminoantipyrine; glucose; stability; kinetics and mechanism; N-[2-(5-hydroxymethyl-2,3,4,5-tetrahydro-2,3,4-trihydroxy)furyl]methyl-N-methylantipyrine

In the previous study, it has been found that sulpyrine in aqueous solution is decomposed remarkably by oxygen in the presence of trace copper (II) ion.[1,3] Though sulpyrine is considerably stable in aqueous solution of metal free under an atmosphere of nitrogen, the stability may be affected by some additives in preparations. Among salicylate, barbiturate, glucose, *etc.*, which are often added to sulpyrine injections, glucose has been found to accelerate the decomposition of sulpyrine appreciably. This paper presents the kinetics and mechanism of the decomposition of sulpyrine by glucose.

Experimental

Determination of Stability of Sulpyrine Aqueous Solution——Five ml of 5.70×10^{-2} or 2.85×10^{-2} M aqueous solutions of sulpyrine or 4-methylaminoantipyrine (MAA) containing 10 or 20% (w/v) additives, which initial pH values were adjusted at 6.5 with 1 N HCl, were placed into 10 ml glass ampules. The ampules were sealed under N_2 gas. They were heated at 80° in a thermostat chamber and taken out at appropriate intervals. The remained amount of sulpyrine or MAA was determined colorimetrically.[4]

Isolation Procedure of Decomposed Products——A hundred ml of 2.85×10^{-2} M sulpyrine solution containing 20% (w/v) glucose was heated in ampules under N_2 gas at 80° for 8 days. The reaction solution was extracted with chloroform, and the extract was chromatographed on SiO_2. The elution with $CHCl_3$ gave crude powder, which was recrystallized from ethylether to afford colorless needles, mp 110° (compound 1). The successive elution with $CHCl_3$–MeOH (95:5) provided crude powder, which was recrystallized from ethylether to give colorless crystals, mp 105° (compound 2).

The aqueous layer of reaction solution, from which chloroform-soluble compounds were removed, was also concentrated *in vacuo* and then chromatographed on SiO_2. The elution with $CHCl_3$–MeOH (9:1)

1) Part III: S. Yoshioka, H. Ogata, T. Shibazaki, and A. Ejima, *Chem. Pharm. Bull.* (Tokyo), **26**, 2723 (1978).
2) Location: *1-18-1 Kamiyoga, Setagaya-ku, Tokyo.*
3) S. Yoshioka, H. Ogata, T. Shibazaki, and A. Ejima, *Arch. Pharm.*, **312**, 81 (1979).
4) K. Kato, M. Umeda, and S. Tsubota, *Yakuzaigaku*, **24**, 116 (1964).

afforded a viscous oil (compound 4). The successive elution with $CHCl_3$–MeOH (8: 2) afforded an oil, which was crystallized from ethylether as needles, mp 31° (compound 3).

Kinetic Studies of Reaction of MAA and Glucose——The kinetic study was done at 80° for the buffer solutions of MAA, which concentrations were 5×10^{-4} M unless otherwise stated. Buffer systems used were McIlvaine buffer. The ionic strength was maintained at 0.4 with KCl in the pH range 4 to 6.2, and 0.5 at pH 7. The effect of ionic strength on the reaction rate was not observed.

Five ml of the solution were placed into 10 ml glass ampules and heated under N_2 gas in the same manner as in the case of aqueous solutions without buffer components.

MAA and the reaction products were respectively determined by high performance liquid chromatography (HPLC). The operating conditions were as follows; column: Hitachi Gel 3011, 5 mm i.d. × 50 cm, column temperature: 50°, mobile phase: 73% (v/v) MeOH, flow rate: 0.75 ml/min, detector: UV 254 nm.

Results and Discussion

Effect of Glucose on Stability of Sulpyrine Aqueous Solution

Figure 1 shows the time course of decomposition of 2% sulpyrine solution with some additives at 80°. The remained percent on the ordinate represents the sum amount of sulpyrine and the hydrolyzed product, MAA. Glucose, an aldohexose, caused the decomposition of sulpyrine, while xylitol, a polyhydric alcohol, showed no effect. This suggests that the decomposition of sulpyrine may be attributed to formyl group in glucose.

Furthermore the addition of hydroxymethylsulfonate (OMS) to sulpyrine solution containing glucose inhibited the decomposition of sulpyrine caused by glucose as shown in Fig. 1. This suggests that glucose reacts with MAA, and not with sulpyrine, because sulpyrine is in equilibrium with MAA and the addition of OMS shifts the equilibrium to sulpyrine from MAA as reported previously.[5] This is also supported by the observation that MAA was decomposed by glucose faster than sulpyrine as shown in Fig. 2, which shows the time course of the decomposition of MAA compared with that of sulpyrine. From these results described above it may be reasonable to consider that formyl group in glucose reacts with 4-methylamino group in MAA.

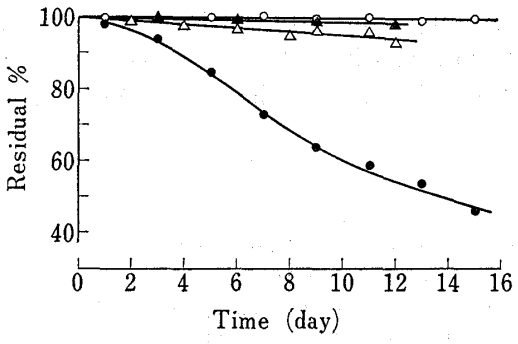


Fig. 1. Decomposition of 2% Sulpyrine Injection

80°, initial pH: 6.5.
○: no additive, ●: 10% glucose,
△: 10% glucose +0.3M OMS,
▲: 10% xylitol.

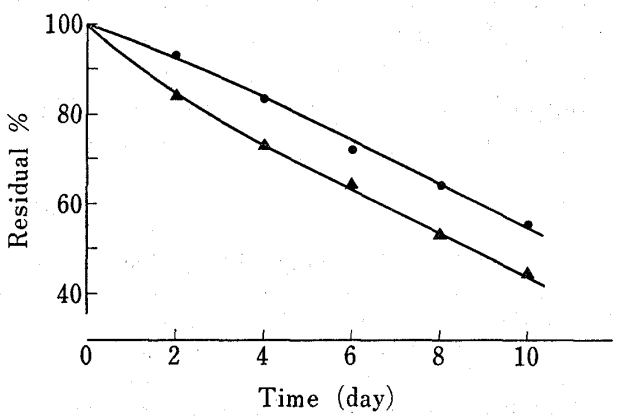


Fig. 2. Decomposition of Sulpyrine and MAA in the Presence of Glucose

80°, initial concentration: 2.85×10^{-2} M, [glucose]: 20%.
●: sulpyrine, ▲: MAA.

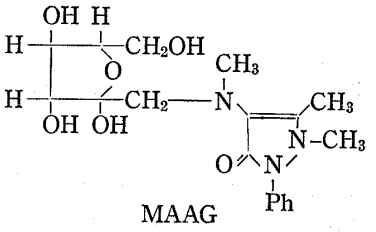
Reaction Products

The reaction products of sulpyrine and glucose in aqueous solution were separated and purified by column chromatography on silica gel. Among four products isolated, compound

5) S. Yoshioka, H. Ogata, T. Shibazaki, and T. Inoue, *Chem. Pharm. Bull.* (Tokyo), **25**, 475 (1977); *idem*, *ibid.*, **25**, 484 (1977).

1, 2 and 3 were identified as aminopyrine (Am), 4-formylmethylaminoantipyrine (FMAA) and 5-hydroxymethylfurfural (5-HMF) respectively by comparison of mp, the nuclear magnetic resonance (NMR) spectra and the mass spectrum (MS) with those of the authentic samples.[6]

Compound 4 was estimated to be N-[2-(5-hydroxymethyl-2,3,4,5-tetrahydro-2,3,4-trihydroxy)furyl]methyl-N-methylantipyrine (MAAG) on the basis of the MS and NMR spectral data, and its structure is represented in Chart 1. The MS of compound 4 showed the molecular peak at *m/e* 379 and the intense peaks at *m/e* 217 and 231. The NMR spectrum showed four singlets at 2.32 (3H), 2.83 (3H), 3.12 (3H) and 3.27 (2H) ppm corresponding to C_3-methyl, 4N-methyl, N_2-methyl and N-methylene respectively, multiplet at 7.2 to 7.7 ppm (5H) corresponding to aromatic protons, and multiplet at 3.5 to 4.2 ppm (5H) corresponding to methine and O-methylene. The structure of compound 4 represented in Chart 1 may be supported by the fact that it is an intermediate in the formation of Am from MAA and decomposes to Am spontaneously as described below.



MAAG

Chart 1

Among these isolated products, 5-HMF has been known as a decomposed product of glucose.[7] In order to study the kinetics and mechanism of the formation of reaction products other than 5-HMF, each product was determined separately by means of HPLC. Fig. 3 shows the chromatogram of the reaction products of 1% sulpyrine solution containing 20% glucose heated at 80° for 8 days. Eight peaks were detected. Peak 2 and 7 were assigned to sulpyrine and MAA respectively. Furthermore peaks 3, 5, 6 and 8 were assigned to 5-HMF, MAAG, FMAA and Am respectively.

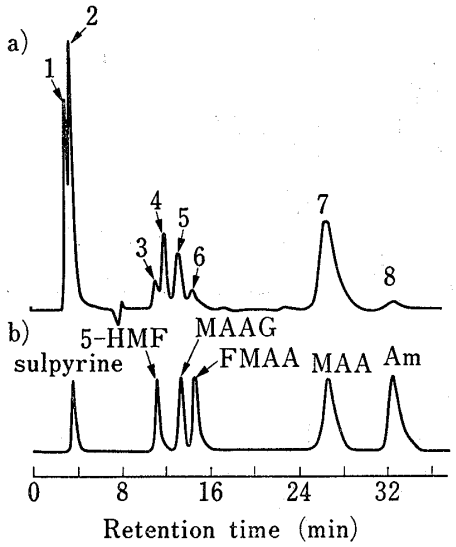


Fig. 3. Chromatogram of Reaction Products of Sulpyrine in the Presence of Glucose

a) 1% sulpyrine solution containing 20% glucose heated at 80° for 8 days.
b) The mixture of standard compounds.

The compound assigned to peak 4 could not be isolated because it transformed to MAA during the isolation. Since peak 4 appeared immediately when MAA was added to glucose solution and shifted to peak 5 gradually as described below, peak 4 may be attributed to an intermediate in the formation of MAAG from MAA and glucose (MAA-glu). Peak 1 may be due to a water-soluble colored substance formed in the decomposition of glucose.

Kinetics of Formation of Aminopyrine

The kinetics of the formation of Am from MAA and glucose was studied in citric acid–phosphate buffer system because the reaction in aqueous solution accompanied pH change. Figure 4 shows the chromatogram of the reaction products of MAA aqueous solution containing 10% glucose heated at 80° for 0, 5 and 10 hr respectively at pH 6.2. The peaks at the retention time 3 to 8 min were assigned to buffer components. MAA-glu was observed even

6) The authentic sample of FMAA was synthesized according to the method reported by Ono, *et al.* S. Ono, R. Onishi, and K. Kawamura, *Yakugaku Zasshi*, **86**, 11 (1966).
7) R.B. Taylor, B.M. Jappy, and J.M. Neil, *J. Pharm. Pharmacol.*, **24**, 121 (1972).

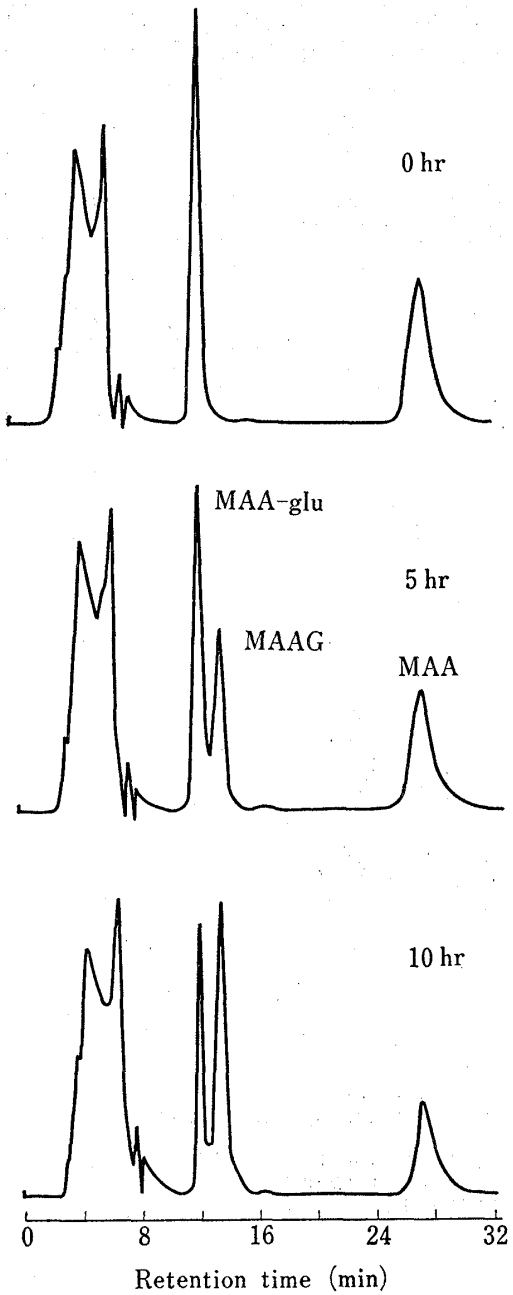


Fig. 4. Chromatogram of Reaction Products of MAA and Glucose

Initial concentration of MAA: 5×10^{-4} M.
pH 6.2, [glucose]: 10%.
Heated at 80° for 0, 5 and 10 hr respectively.

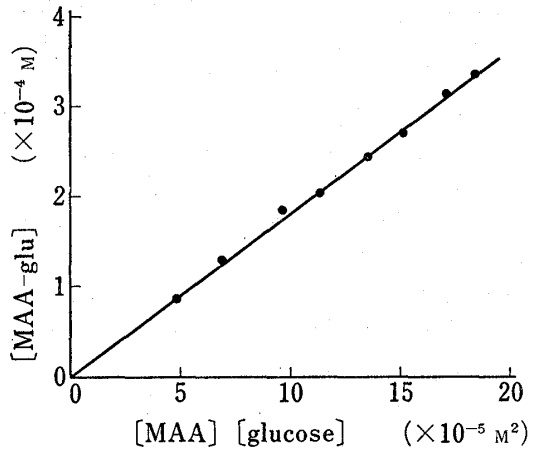


Fig. 5. Equilibrium between MAA and MAA-glu

pH 6.2, room temperature.
Initial concentration of MAA: 5×10^{-4} M.

at the beginning of the reaction and MAAG was gradually formed. The formation of MAA-glu was studied at room temperature since its reaction was very fast at 80°. MAA-glu was formed immediately by adding glucose to MAA solution even at room temp. and the amount of formed MAA-glu depended on the amount of added glucose. Determining MAA-glu formed at various concentrations of glucose at room temp., a linear relationship was observed between the concentration of MAA-glu formed, [MAA-glu], and the product of the concentration of MAA and that of glucose, [MAA][glucose], as shown in Fig. 5. The concentration of MAA-glu was determined by subtracting the concentration of remained MAA from that of added MAA and making a calibration curve for peak-height of MAA-glu. This result suggests that a molar of MAA reacts with a molar glucose reversibly and forms a molar MAA-glu immediately in aqueous solution containing glucose.

The time course of the formation of MAAG from MAA at 80° is represented in Fig. 6. The part in the initial stage is enlarged in Fig. 6-a). The remained percentage on the ordinate represents the sum amount of MAA and MAA-glu because the equilibrated amounts of MAA and MAA-glu may change during determination process. The sum amount of MAA and MAA-glu decreased in apparent first order in the initial stage. The deviation from the first order in the latter stage suggests the reverse reaction of MAAG to MAA or MAA-glu, which is confirmed below. Assuming that MAA-glu is in equilibrium with MAA and glucose, and MAAG is formed from MAA-glu as follows;

$$\text{MAA} + \text{glucose} \underset{k_2}{\overset{k_1}{\rightleftarrows}} \text{MAA-glu} \underset{k_4}{\overset{k_3}{\rightleftarrows}} \text{MAAG}$$

the apparent first order elimination rate constant, k_3', determined from the slope in the initial stage in Fig. 6-a) can be related with the first order rate constant, k_3, and the concentration of glucose, [glucose], according to equation 1.

$$\frac{1}{k_3'} = \frac{1}{k_3}\left(\frac{1+k_1[\text{glucose}]/k_2}{k_1[\text{glucose}]/k_2}\right) = \frac{1}{k_3} + \frac{1}{k_3}\frac{1}{k_1[\text{glucose}]/k_2} \qquad (1)$$

Determining the values of k_3' in the various concentrations of glucose 4 to 20%, the linear correlation was observed between the reciprocal of k_3' and that of the concentration of glucose,

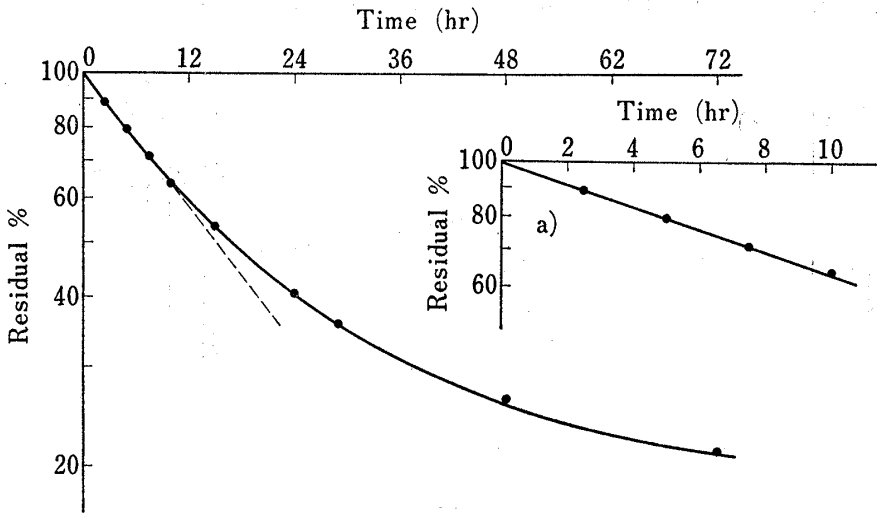


Fig. 6. Reaction of MAA to MAAG

80°, pH 6.2, [glucose]: 10%.
Initial concentration: 5×10^{-4} M.
a) The time course in the initial stage is enlarged.

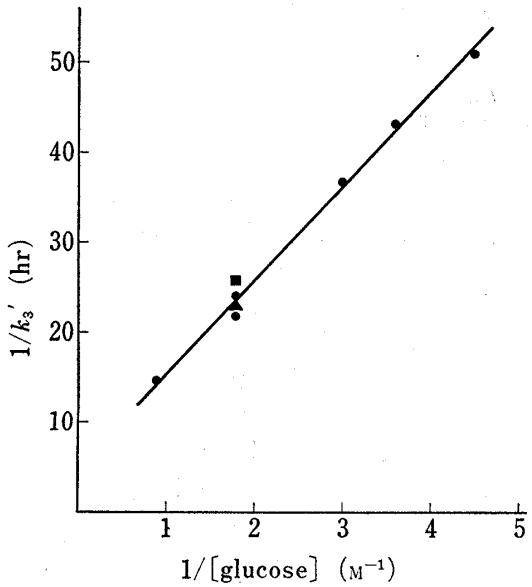


Fig. 7. Relationship between k_3' and Concentration of Glucose

80°, pH 6.2.
Initial concentration of MAA: ●: 5×10^{-4} M
■: 5×10^{-3} M ▲: 2.5×10^{-2} M.

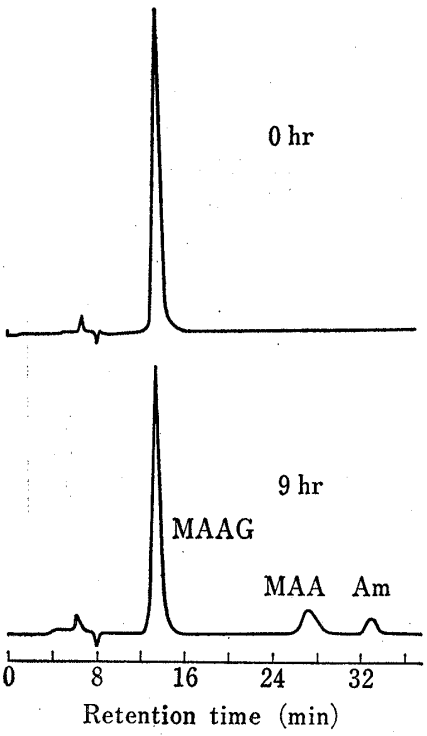


Fig. 8. Chromatogram of Decomposed Products of MAAG

Initial concentration of MAAG: 5×10^{-4} M.
pH 6.2.
Heated at 80° for 0 and 9 hr respectively.

as shown in Fig. 7. The concentration of glucose could be regarded constant during the reaction because the initial concentration of glucose was much larger than that of MAA. The results shown in Fig. 7 may support the mechanism assumed above. From the intercept and slope of the line k_3 and k_1/k_2 at 80° can be calculated respectively.

In order to study the kinetics of the reaction of MAAG, MAAG isolated from the reaction solution of MAA and glucose was heated in citric acid–phosphate buffer. Figure 8 shows the chromatogram of the reaction products of MAAG heated at 80° for 0 and 9 hr respectively. The time courses of the decomposition of MAAG and the formation of Am and MAA are represented in Fig. 9. The decrease of MAAG is plotted on a semilogarithmic scale in Fig.

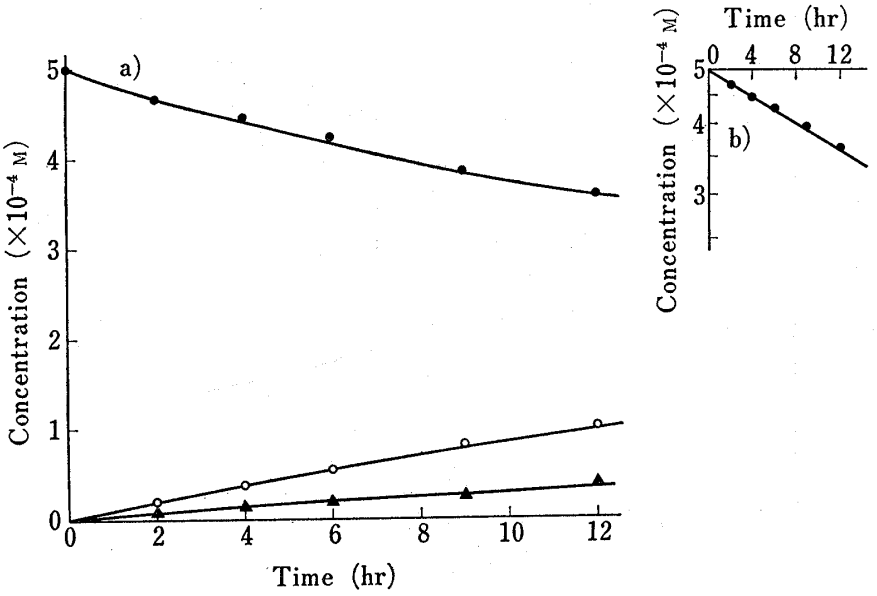


Fig. 9. Decomposition of MAAG to Am and MAA

80°, pH 6.2.
●: MAAG ○: MAA ▲: Am
b) Semilogarithmic scale.

9-b). MAAG decomposed spontaneously in first order in the initial stage, and Am and MAA were formed as follows;

$$\text{MAA} \xleftarrow{k_4} \text{MAAG} \xrightarrow{k_5} \text{Am}$$

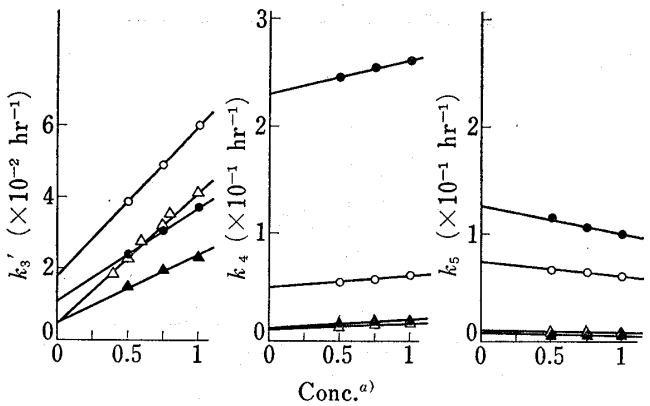


Fig. 10. Effect of Concentration of Buffer Components on $k_3'^{b)}$, k_4 and k_5

●: pH 4, ○: pH 5, △: pH 6.2, ▲: pH 7.
a) The concentration of undiluted buffer solution is represented as 1.
b) [glucose]: 10%.

The fact that MAA-glu was not detected can be explained on the basis that the concentration of glucose was so small that the equilibrium between MAA and MAA-glu lied so far to MAA. The values of k_4 and k_5 can be determined respectively because the apparent first order elimination rate constant represents the sum of k_4 and k_5, and the ratio of k_4 to k_5 is represented in terms of the ratio of the amount of formed MAA to that of formed Am.

$$k_4/k_5 = [\text{MAA}]/[\text{Am}]$$

From the results described above it may be concluded that Am is formed from MAA and glucose as follows;

$$\text{MAA} + \text{glucose} \underset{k_2}{\overset{k_1}{\rightleftarrows}} \text{MAA-glu} \underset{k_4}{\overset{k_3}{\rightleftarrows}} \text{MAAG} \xrightarrow{k_5} \text{Am}$$

The values of k_3', k_4 and k_5 were affected by the concentrations of buffer components as shown in Fig. 10. The values extrapolated to zero in Fig. 10 were plotted against pH values in Fig. 11 and 12. It is troublesome to determine k_3 and k_1/k_2 at 80° from the intercept and slope of the line in Fig. 7 because of the dependence on the concentrations of buffer components. Therefore k_3 represented in Fig. 11 was estimated by substituting the value of k_1/k_2 determined at room temp. into equation 1 assuming that the value at 80° is the same as that at room temp. The pH profile of k_3 may be similar to the estimated one. As shown in Fig. 11 and 12 the reversible reaction of MAA-glu to MAAG and the reaction of MAAG to Am seem to be catalyzed by specific acid.

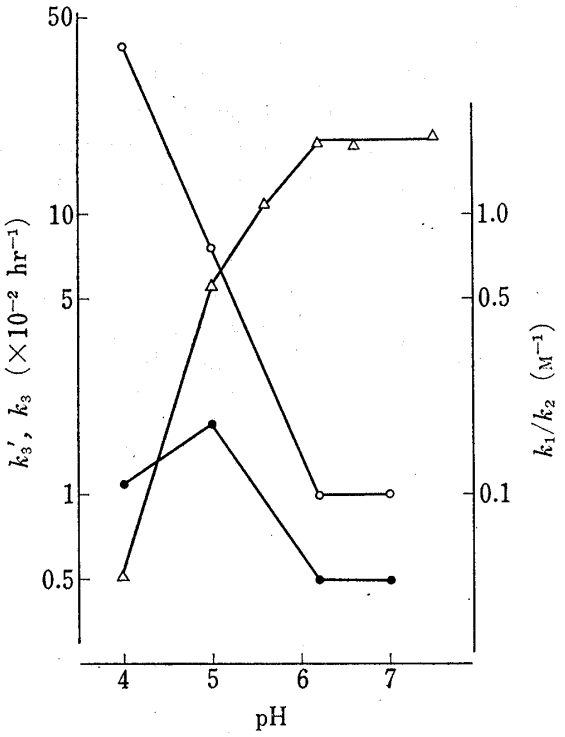


Fig. 11. pH Profile of k_3', k_3 and the Ratio of k_1 to k_2

●: k_3'[a], ○: k_3[b], △: k_1/k_2 at room temp.
a) [glucose]: 10%, 80°.
b) See text.

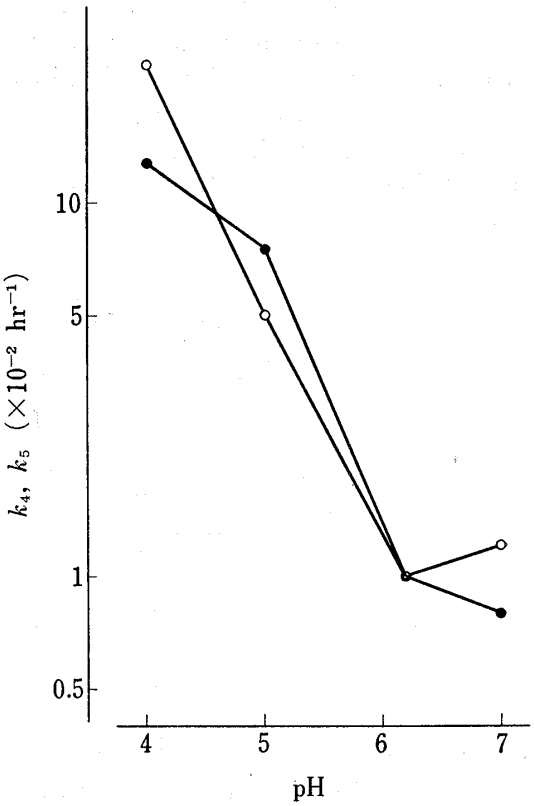


Fig. 12. pH Profile of k_4 and k_5

○: k_4, ●: k_5.
80°.

Table I. Formation of FMAA in Aqueous Solution of MAA and Glucose[a]

$[\text{MAA}]_0$[b] (M)	[FMAA]/$[\text{MAA}]_0$[c] Concentration of glucose (w/v)	
	5%	10%
2.8×10^{-1}	2.8%	4.6%
2.8×10^{-2}	1.9	3.7
1×10^{-2}	1.3	2.6
5×10^{-3}	0	0

a) Heated for 10 days at 80°.
b) The initial concentration of MAA.
c) The ratio of FMAA formed to the initial concentration of MAA.

Formation of 4-Formylmethylaminoantipyrine

FMAA was isolated as a reaction product of sulpyrine injection containing glucose described above, but FMAA was not formed in citric acid-phosphate buffer solution. Therefore MAA was heated with glucose at 80° for 10 days in the absence of buffer components and the ratio of the concentration of FMAA formed to the initial concentration of MAA was determined and represented in Table I. The ratio of FMAA formed seems to increase as the initial concentration of MAA and that of glucose increase.

Since FMAA does not decompose any more under this condition and is not formed from MAAG, the reaction pathway of the formation of FMAA may be different from that of Am.

Reaction Pathways

In conclusion the reaction pathways of sulpyrine and glucose may be summarized in Chart 2. MAA-glu can be regarded as a compound formed by bonding of a formyl group in glucose and a methylamino group in MAA on the basis of the fact that it is formed from MAA and glucose reversibly and transforms to MAAG reversibly. The similar compound has been reported in amino-carbonyl reaction of *p*-toluidine and glucose.[8] Arabonic acid may be expected to be formed when MAAG decomposes to Am. Though its formation was not confirmed in this study, its detection has been reported in the reaction of *p*-toluidine and glucose.[8]

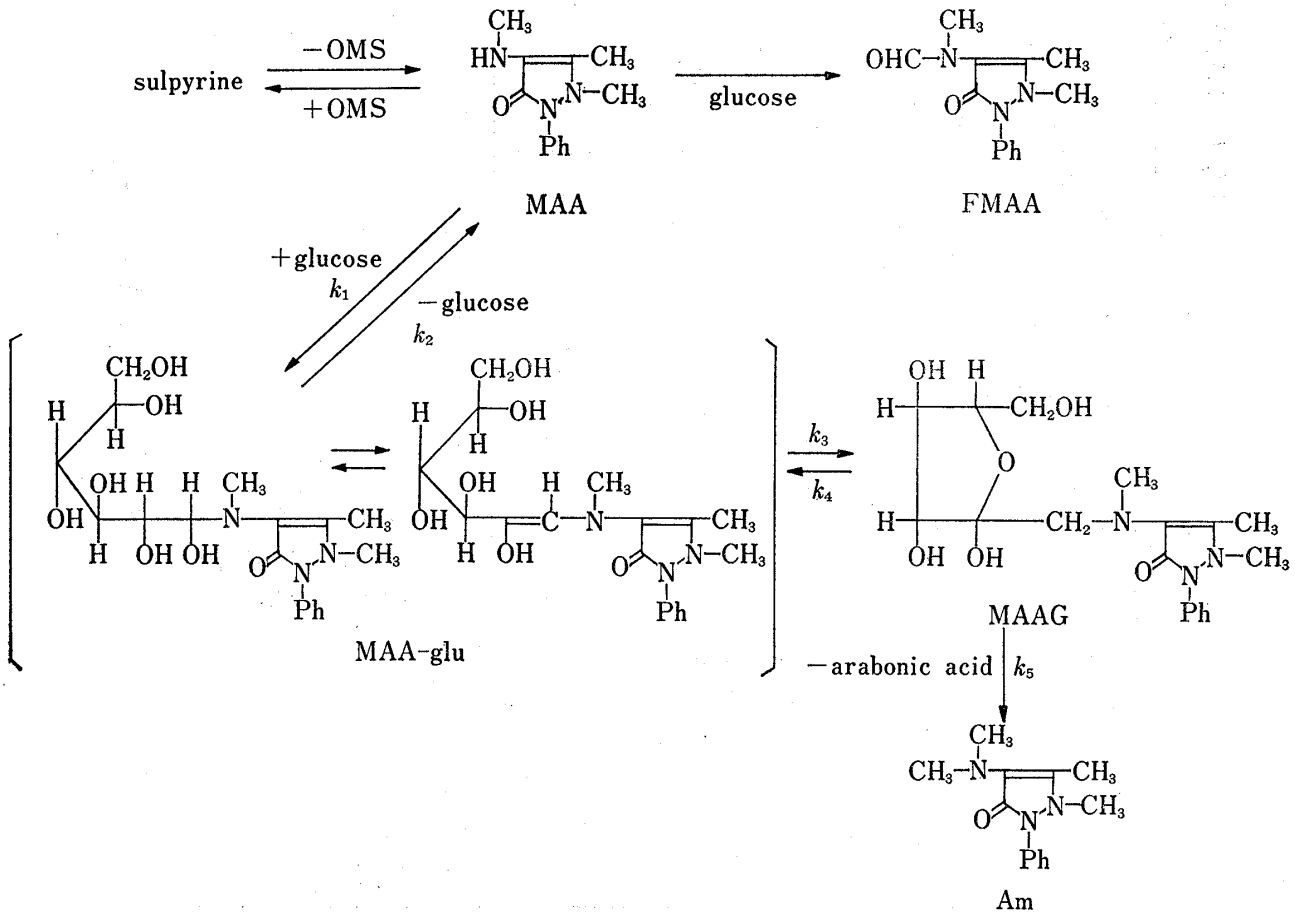


Chart 2

In order to study whether other secondary amines are decomposed by glucose and form the corresponding tertiary amines similarly to MAA, the decomposition of N-methylaniline

8) N. Kinae, Y. Nakamura, M. Abe, and T. Ozawa, *Yakugaku Zasshi*, **98**, 153 (1978).

by glucose was tried under the same conditions. However the decomposition of N-methylaniline was not observed. This may be ascribable to the difference of the reactivities of the secondary amino groups.

Acknowledgement The authors wish to express their thanks to Dr. S. Sueyoshi of this institute for helpful discussions.