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Stability of Sulpyrine. V.[1] Oxidation with Molecular Oxygen in the Solid State

SUMIE YOSHIOKA, HIROYASU OGATA, TOSHIO SHIBAZAKI,
and AKIRA EJIMA

National Institute of Hygienic Sciences[2]

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The solid-state decomposition of sulpyrine was investigated at 80° under oxygen gas. The initial rate of decomposition depended on the water content. When the amount of water present was insufficient to dissolve all the sulpyrine, the rate was of zero-order and increased in proportion to the amount of water. On the other hand, in the presence of a larger amount of water, sulpyrine decomposed in an apparent first order reaction, but the rate was dependent on the amount of water.

It is proposed as a mechanism that sulpyrine is hydrolyzed to 4-methylaminoantipyrine (MAA), and then MAA is further oxidized in the aqueous layer. The first order rate constant of decomposition of MAA estimated in saturated solution of sulpyrine in the presence of solid phase was in good agreement with that estimated in sulpyrine solution of lower concentration.

As reaction products, aminopyrine (Am), 4-aminoantipyrine (AA), 4-formylaminoantipyrine (FAA) and 4-formylmethylaminoantipyrine (FMAA) were identified. It is proposed that Am and AA may be formed from MAA by methyl rearrangement, while FAA and FMAA may be formed from MAA and Am, respectively, by oxidation of the methyl to a formyl group *via* intermediates.

Keywords——sulpyrine; decomposition in solid state; water content; kinetics and mechanism; oxidation; decomposition product

In previous studies,[3-5] sulpyrine has been found to undergo hydrolysis and oxidation readily in aqueous solution. 4-Methylaminoantipyrine (MAA), 4,4'-[methylenebis(methylimino)]-diantipyrine (Bis) and 4-(N-hydroxymethyl-N-methyl)aminoantipyrine (HMA) have been detected as hydrolysis products of sulpyrine. On the other hand, it has been found that sulpyrine is readily oxidized to 4-aminoantipyrine (AA), antipyrinyl-4-peroxide (AP) and 4-formylaminoantipyrine (FAA) by oxygen gas in the presence of copper(II) ions. Its high reactivity with water and oxygen gas suggests the possibility of its decomposition in the solid state, but there have been few reports on the stability of sulpyrine in solid dosage forms.

A number of studies have been reported on the decomposition of drugs in solid pharmaceutical systems, but the decomposition mechanisms are poorly understood, and appear to be complex. In the case of water-soluble drugs, the effects of water content or moisture on the decomposition rate have been discussed and a reaction in the adsorbed moisture layer[6-8] or a gas-solid reaction[9] has been proposed as a reaction mechanism, but the processes involved are not well understood.

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[1] Part IV: S. Yoshioka, H. Ogata, T. Shibazaki, and A. Ejima, *Chem. Pharm. Bull.* (Tokyo), **27**, 907 (1979).

[2] Location: *1-18-1 Kamiyoga, Setagaya-ku, Tokyo.*

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

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[5] S. Yoshioka, H. Ogata, T. Shibazaki, and A. Ejima, *Chem. Pharm. Bull.* (Tokyo), **26**, 2723 (1978).

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In this study, the kinetics of decomposition of sulpyrine in the solid state were studied, especially in relation to the effect of water on the rate. Furthermore the reaction products were identified and a reaction mechanism proposed.

Experimental

Materials——Sulpyrine crystals ($C_{13}H_{16}N_3NaO_4S \cdot H_2O$) of J.P. IX grade were ground in a mortar and sieved (80/100 mesh).

Kinetic Studies in a Closed System——Sulpyrine powder (50 mg) was weighed into 10 ml glass ampules, which were sealed under O_2 gas after adding various amounts of water (5 to 200 mg) with a microsyringe. In addition, 5 ml of 2.85×10^{-4} M, 500 μl of 2.85×10^{-3} M and 50 μl of 2.85×10^{-2} M of sulpyrine solution were each placed in 10 ml glass ampules. Similarly, 5 ml of 4.52×10^{-4} M, 500 μl of 4.52×10^{-3} M and 50 μl of 4.52×10^{-2} M of MAA solution were each placed in ampules, then sealed under O_2 gas.

These ampules were stored at 80° in a thermostated chamber and sampled at appropriate intervals. The total amounts of sulpyrine and MAA remaining were determined colorimetrically.[10] The reaction products were 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).

Gas mixtures of oxygen and nitrogen, which were used to study the effect of the partial pressure of oxygen, were prepared by the method reported by Inoue.[11] Each run was carried out in the dark.

Determination of Molar Ratios of Sulpyrine and MAA in Aqueous Solutions——Five ml of 2.85×10^{-4} to 2.85 M sulpyrine solution heated at 80° under N_2 gas for 1 hr was shaken vigorously with 5 ml of $CHCl_3$ in order to remove MAA, then sulpyrine remaining in the aqueous phase was determined colorimetrically.[10] The absence of Bis and HMA in aqueous solution was confirmed by measuring the NMR spectra of the chloroform extract as reported previously.[3]

Kinetic Studies in an Open System——Sulpyrine powder (50 mg) was weighed into 40 mm.i.d. × 40 mm glass vessels, and stored at 80° at 61, 70 and 81% RH in a constant temperature and humidity chamber (Tabai PR-2A). They were sampled at appropriate intervals and the amounts of sulpyrine and MAA remaining were determined colorimetrically.[10]

Isolation of Reaction Products——MAA (50 mg) was weighed into 10 ml glass ampules and they were sealed under O_2 gas after adding 50 mg of water. Twenty ampules were prepared in the same manner. They were heated at 80° for 3 days.

The combined reaction solution was extracted with $CHCl_3$ after addition of 100 ml of 0.1 N HCl. The extract was chromatographed on SiO_2. Elution with $CHCl_3$–MeOH (99: 1) gave a viscous oil (compound 1). Further elution with $CHCl_3$–MeOH (95: 5) yielded a crude powder, which was recrystallized from ethylether to give colorless crystals, mp 105° (compound 2). Further elution with $CHCl_3$–MeOH (8: 2) gave a powder, which was recrystallized from methanol to give prisms, mp 187° (compound 3).

NaOH (0.2 N, 100 ml) was added to the acidic aqueous layer described above and extracted with $CHCl_3$. The extract was chromatographed on SiO_2. Elution with $CHCl_3$–MeOH (99: 1) and $CHCl_3$–MeOH (95: 5) provided powders, which were recrystallized from ethylether to give needles, mp 110° (compond 4) and crystals, mp 109° (compound 5), respectively.

Compounds 1, 2, 4 and 5 were isolated in the same manner from the reaction solution of sulpyrine (50 mg/50 mg of water) heated under O_2 gas at 80° for 5 days.

Detection of Reaction Products by TLC——The reaction products were detected by TLC. The operating conditions were as follows; plate, precoated PLC plate (silica gel F-254); solvent system, chloroform–methanol (9: 1).

Studies on Reaction Pathways——The following mixtures were heated under O_2 gas at 80° in 10 ml glass ampules: 50 mg of aminopyrine (Am) and 50 mg of water; 50 mg of Am, 30 mg of hydroxymethanesulfonate (OMS) and 50 mg of water; 50 mg of MAA, 30 mg of 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 50 mg of water.

The reaction products were detected by HPLC.

Results and Discussion

Kinetics of Decomposition

The solid-state decomposition of sulpyrine was studied at 80° in ampules sealed under oxygen gas. Figure 1 shows the time course of decomposition of sulpyrine in the presence

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[10] K. Kato, M. Umeda, and S. Tsubota, *Yakuzaigaku*, 24, 116 (1964).

[11] S. Inoue, *Yakugaku Zasshi*, 91, 81 (1971).

of various amounts of water per 50 mg of sulpyrine. The ordinate represents the total amount of sulpyrine and MAA remaining. When less than 30 mg of water was added, the reaction was found to be of zero order. On the other hand, when more than 30 mg of water was added, sulpyrine decomposed in an apparent first-order process. The initial rates of decomposition are plotted against the amount of water added in Fig. 2. The rate was linearly related to the water content in the range below 30 mg, but deviated from this line in the range above 30 mg. These results (Fig. 1 and 2) suggest that in the range below 30 mg sulpyrine decomposes in saturated solution in the presence of the solid phase and zero-order kinetics prevail. First-order kinetics prevail in the range above 30 mg, where all of the sulpyrine is in solution. This consideration may be supported by that the solubility of sulpyrine was estimated to be 50 mg per 24 mg of water spectrophotometrically.

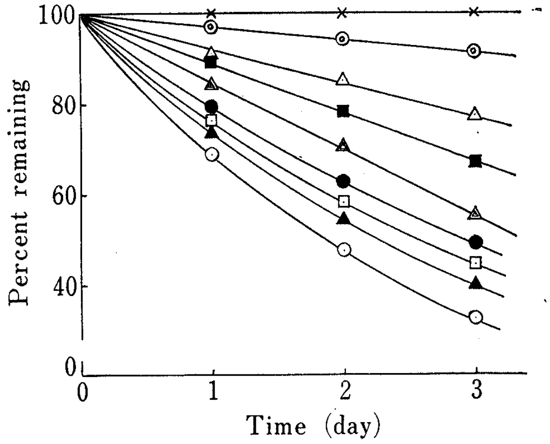

Fig. 1. Decomposition of Sulpyrine under Oxygen Gas

The amount of water added to 50 mg of sulpyrine; ×: 0, ◎: 5, △: 10, ■: 15, ◬: 20, ●: 30, □: 40, ▲: 50, ○: 70 mg.

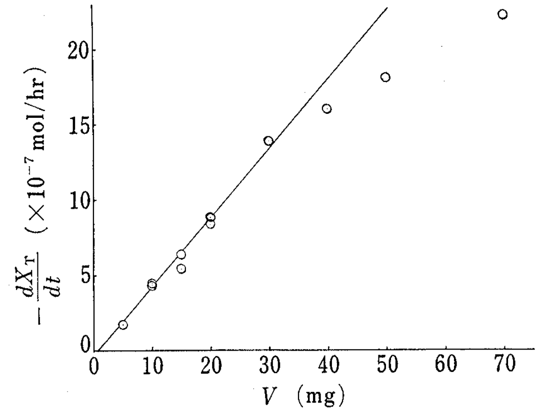

Fig. 2. Effect of Water Content on the Initial Rate of Decomposition of Sulpyrine

Initial amount of sulpyrine: 50 mg.

Sulpyrine has been found to be in equilibrium with the hydrolysis products (MAA, Bis and HMA) in alkaline solution, as reported previously.[3] Under the present conditions, however, only MAA was detected as a hydrolysis product by nuclear magnetic resonance (NMR) spectrometry. The finding that Bis and HMA are not formed, in contrast to the case in alkaline solution, may be due to the differences of temperature and pH.

Consequently, the solid-state decomposition of sulpyrine in the presence of water may be represented as shown in Chart 1, where k_S, k_M and k' represent the decomposition rate constant of sulpyrine in solution, that of MAA in solution and that of solid sulpyrine, respectively. The overall decomposition rate may be written as

$$-\frac{dX}{dt} = VS(k_S F_S + k_M F_M) + k'(X - VS) \tag{1}$$

where X, V and S are the total amount of sulpyrine and MAA, mg of water added, and solubility of sulpyrine in moles per mg of water, respectively, and F_S and F_M are the molar ratio of sulpyrine to the total in solution, and that of MAA, respectively. Since sulpyrine did not decompose in the absence of water, as shown in Fig. 1, k' can be regarded as zero.

In the range of water content below 30 mg, equation (1) can be transformed to equation (2) by substituting k_S', k_M', F_S' and F_M' for k_S, k_M, F_S and F_M respectively.

$$-\frac{dX}{dt} = VS(k_S' F_S' + k_M' F_M') \tag{2}$$

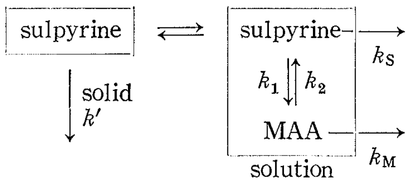

Chart 1

where k_S', F_S', k_M' and F_M' are the rate constant and the molar ratio of sulpyrine and those of MAA in saturated solution, respectively. The value of F_S' was calculated to be 0.952 as described below. Furthermore k_M' can be considered to be equal to the rate constant estimated in MAA solution at a concentration similar to that of MAA in a saturated solution of sulpyrine, 0.160 hr^{-1}, as described below. Consequently k_S' was estimated to be $2 \times 10^{-4}\,\text{hr}^{-1}$ from the slope of the straight line in Fig. 2, and can be regarded as negligible in comparison with k_M'.

In the range of water content above that corresponding to the solubility, equation (1) changes to equation (3)

$$-\frac{dX}{dt} = (k_S F_S + k_M F_M) C_T V \tag{3}$$

where C_T represents the total concentration of sulpyrine and MAA. As shown in Fig. 2, the decomposition rate was not constant but increased with the water content. This seems to be ascribable to the change of F_S and F_M with concentration. In order to confirm this, the initial rates of decomposition were determined in sulpyrine solution at concentrations from 2.85×10^{-10} to 2.85×10^{-8} mol per mg of water, where F_S and F_M change substantially. The rates of decomposition were also determined in MAA solutions at concentrations from 4.52×10^{-10} to 4.52×10^{-8} mol per mg of water. Figure 3 shows the relationship between the logarithm of the rate and that of the concentration. The rates determined in sulpyrine solutions of high concentrations are also plotted in Fig. 3. Because a pH change was observed during the reaction, the initial rates were plotted. The linearity and slope of 1 observed in the decomposition of MAA indicate that the decomposition is first order, and the first order rate constant was estimated to be 0.160 hr^{-1}. On the other hand in the case of the decomposition of sulpyrine, the rate deviated from the straight line. This may be due to the variation of F_S and F_M with concentration, which may also account for the deviation shown in Fig. 2. As reported previously,[3] F_S and F_M depend on the concentration according to equation (4)

$$\frac{F_M^2 C_T}{F_S} = \frac{k_1}{k_2} \tag{4}$$

where k_1 and k_2 are the rate constant of hydrolysis of sulpyrine and that of formation of sulpyrine from MAA and OMS, respectively, as shown in Chart 1. F_S in sulpyrine solution at concentrations from 2.85×10^{-10} to 2.85×10^{-6} mol per mg of water were determined by the extraction method[3] and are plotted against the concentration in Fig. 4. The values of F_S agreed with those calculated according to equation (4) from the values of k_1 and k_2 reported previously,[3] as shown by a solid line in Fig. 4. Equation (3) yields equation (5) on substituting $(1\text{-}F_S)$ for F_M.

$$\left(-\frac{dC_T}{dt}\right)\Big/ C_T = (k_S - k_M)F_S + k_M \tag{5}$$

The plot of the initial rate divided by the concentration $(-dC_T/dt)/C_T$ against F_S according to equation (5) was linear, as shown in Fig. 5. This indicates that the deviation shown in Fig. 2 and Fig. 3 can be interpreted in terms of the change of F_S and F_M with concentration. The intercept of the straight line in Fig. 5 gave a value of k_M, 0.157 hr^{-1}, which was in agreement with that estimated for the decomposition of MAA solution, 0.160 hr^{-1}. From the slope k_S was estimated to be almost zero, and thus negligible in comparison with k_M. This is consistent with k_S' obtained in a saturated solution of sulpyrine in the presence of the solid phase.

In conclusion, it was found that sulpyrine decomposed as illustrated in Chart 1, where k' and k_S are negligible in comparison with k_M. Thus sulpyrine is hydrolyzed to MAA in the aqueous layer, and then MAA decomposes further. When all of the sulpyrine is in solution, the decomposition rate increases in proportion to the molar ratio of MAA. When a solid phase of sulpyrine coexists with the aqueous layer, the rate is proportional to the water content present.

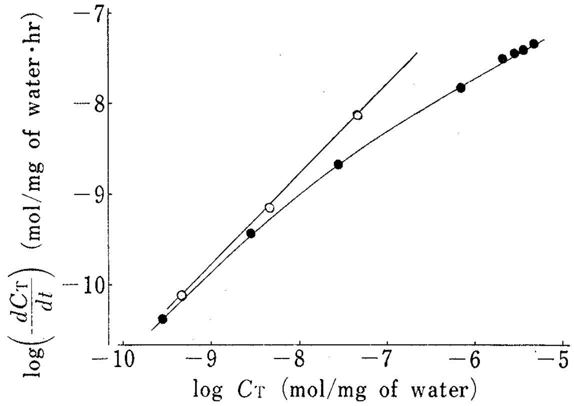

Fig. 3. Relationship of the Logarithm of the Initial Rate *vs.* the Logarithm of the Concentration

○: MAA, ●: sulpyrine.

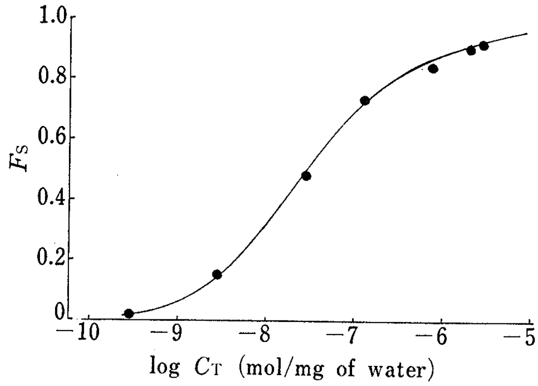

Fig. 4. Relationship of the Molar Ratio of Sulpyrine *vs.* the Logarithm of the Total Concentration

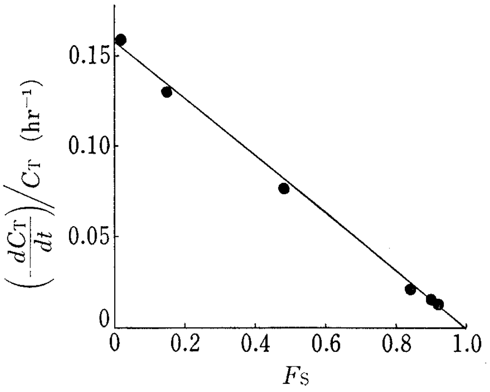

Fig. 5. Relationship of the Ratio of the Initial Rate to the Total Concentration *vs.* the Molar Ratio of Sulpyrine

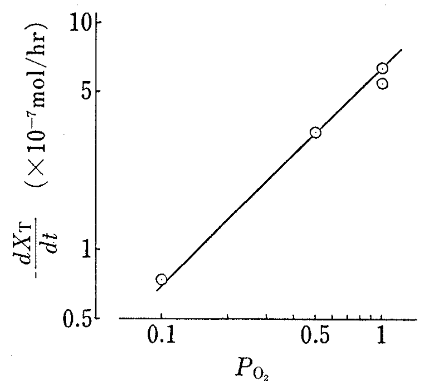

Fig. 6. Effect of the Partial Pressure of Oxygen on the Initial Rate of Solid-state Decomposition of Sulpyrine

The amount of water added to 50 mg of sulpyrine: 15 mg.

The further decomposition of MAA, which follows the hydrolysis of sulpyrine, seems to involve oxidation because MAA has been found to be reactive with oxygen gas.[4,5] Thus, the effect of oxygen on the solid-state decomposition of sulpyrine was studied. The initial rates were determined under various partial pressures of oxygen (Fig. 6). The rate seems to be of first order in the partial pressure of oxygen. This suggests that the oxidation mechanism differs from that in the oxidation of MAA by oxygen gas in the presence of copper(II) ions, where the rate was of 0.5 order in the partial pressure of oxygen, as reported previously.[4,5]

The solid-state decomposition of sulpyrine was further studied under air in the presence of moisture in an open system. The time courses showed a pattern characteristic of an autocatalytic reaction, unlike that seen in the oxidation in ampules (Fig. 7). Though the rates depended on the relative humidity, the value of the reaction order in humidity, n, calculated according to a Freundlich isotherm, was not constant and increased with increase in the relative humidity. The change of n was larger as the relative humidity approached the critical relative humidity, which was estimated to be approximately 81% by means of the method reported by Yamamoto *et al.*[12] These discrepancies between the reaction pattern and the effect of water in open and closed systems seem to be due to a difference in water adsorption mechanism and other factors; the changes are complex, and require further study.

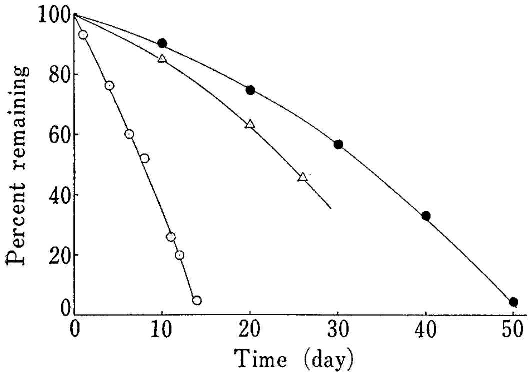

Fig. 7. Solid-state Decomposition of Sulpyrine at 80° in the Presence of Moisture

RH; ●: 61%, △: 70%, ○: 81%.

Oxidation Products and Their Formation in Concentrated Solutions

Five compounds were isolated as reaction products from MAA solution (50 mg/50 mg of water) after storage at 80° for 3 days. Among them, compounds 2, 3, 4 and 5 (numbered in the experimental section) were identified as 4-formylmethylaminoantipyrine (FMAA), FAA, Am and AA respectively by comparison of mp, NMR spectra and MS spectra with those of authentic samples.

The product (compound 1) seems to be an adduct of molecular oxygen with MAA (MAAO) on the basis of the MS, NMR and IR spectral data. The MS spectrum of MAAO showed

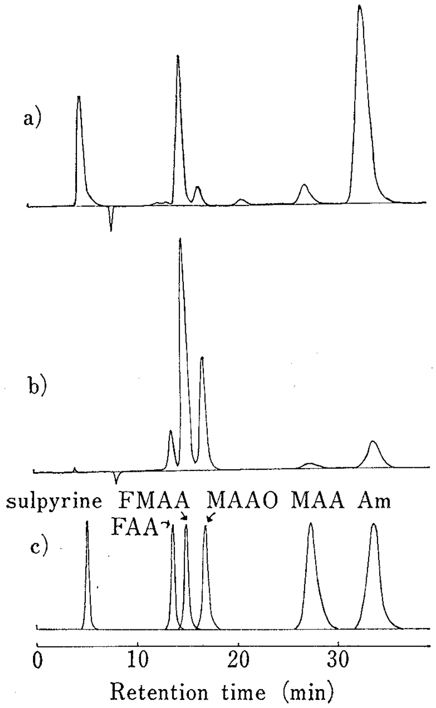

Fig. 8. High Performance Liquid Chromatogram of Reaction Products

a): sulpyrine solution (50 mg/50 mg of H_2O) heated at 80° for 5 days.
b): MAA solution (50 mg/50 mg of H_2O) heated at 80° for 3 days.
c): the mixture of standard compounds.

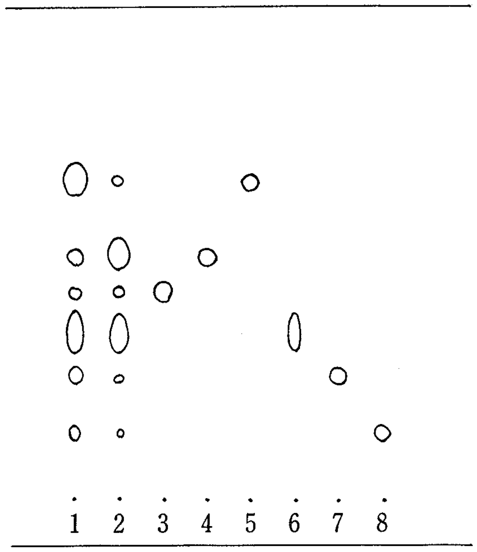

Fig. 9. Thin-Layer Chromatogram of Reaction Products

1): MAA solution (50 mg/50 mg of H_2O) heated at 80° for 3 days.
2): sulpyrine solution (50 mg/50 mg of H_2O) heated at 80° for 5 days.
3): MAA, 4): Am, 5): MAAO, 6): FMAA, 7): AA, 8): FAA.

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[12] R. Yamamoto and T. Takahashi, *Shionogi Kenkyusho Nempo*, **4**, 455 (1954).

a molecular peak at *m/e* 249 ($C_{12}H_{15}N_3O_3$). In the NMR spectrum (in $CDCl_3$), each of three singlet peaks corresponding to N_2-methyl, C_3-methyl and 4N-methyl of MAA changed to two peaks with a constant ratio. This may be interpreted on the basis that free rotation of the C_4–N bond is hindered by the addition of oxygen to the 4N atom. The IR spectrum showed a broad band at 3300 cm^{-1}, which may be assigned to –NH or –OH. Though the structure of MAAO has not been confirmed, it can be regarded as an intermediate in the oxidation of MAA to FAA, because it was found to transform to FAA.

FMAA, Am, AA and MAAO were also isolated from sulpyrine solution (50 mg/50 mg of water) heated at 80° foı 5 days. FAA was not isolated because of its low yield. Figure 8 shows the HPLC patterns of the reaction solutions of sulpyrine and MAA, and Fig. 9 shows the TLC patterns of them. AA was not detected by HPLC under these operating conditions, but five products including AA were detected by TLC in both sulpyrine and MAA reaction solutions. In order to clarify the formation mechanism of the products, the time course of each product in sulpyrine and MAA solutions was determined by HPLC (Fig. 10 and 11, respectively). These figures do not show the time courses of all products, but only those of the products which could be detected by HPLC. Though AA seems to be a major product in both cases, it could not be determined by HPLC. The ratio of the formation of each product in sulpyrine solution was different from that in MAA solution. Am was the major product other than AA in the case of sulpyrine solution, while FMAA and MAAO were major products in the case of MAA solution.

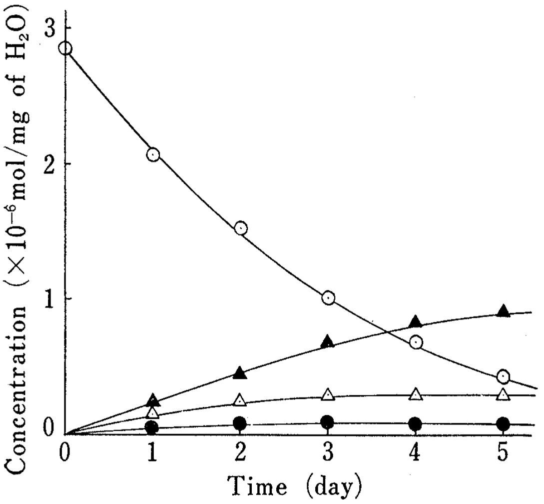

Fig. 10. Decrease of Sulpyrine and Formation of Reaction Products at 80°

○: sulpyrine+MAA, ▲: Am, △: FMAA, ●: MAAO.

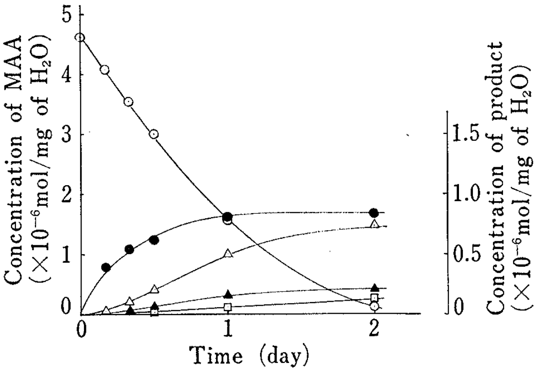

Fig. 11. Decrease of MAA and Formation of Reaction Products at 80°

○: MAA, ▲: Am, △: FMAA, ●: MAAO, □: FAA.

The pathways of formation of these products may be summarized as shown in Chart 2. Am and AA may be formed from MAA by methyl rearrangement. On the other hand FAA and FMAA may be formed from MAA and Am, respectively, by oxidation of the methyl to a formyl group, *via* intermediates. In order to confirm the proposed pathways, the effect of DPPH, a radical, on the reaction was studied. The oxidation of Am was also studied under the same conditions. As shown in Table I, the addition of DPPH to the reaction solution accelerated the reaction, and this supports the possobility of a radical reaction. Figure 12, which shows the HPLC pattern of reaction solution of Am, indicates that FMAA is formed in Am solution *via* an intermediate (AmO). Though the structure of AmO has not been confirmed, it may correspond to MAAO, the intermediate in the oxidation of MAA to FAA. Furthermore, the addition of hydroxymethanesulfonate (OMS) to Am solution was found to inhibit the oxidation of Am to FMAA as shown in Table I. This may account for the difference between the ratios of formation of products in sulpyrine solution and those in MAA solution shown in Fig. 10 and 11. It seems reasonable to consider that in sulpyrine solution OMS which is formed when sulpyrine hydrolyzes to MAA, inhibits the oxidation of Am to FMAA and that of MAA to FAA, so that Am accumulates significantly. These results support the reaction pathways shown in Chart 2.

In the previous studies on the copper(II) ion-catalyzed oxidation of sulpyrine by molecular oxygen,[4,5] antipyrinyl-4-peroxide (AP), AA and FAA were characterized as oxidation products, while Am and FMAA were not detected. In this study, however, Am, AA and FMAA were major products, while AP was not detected. It was suggested in the

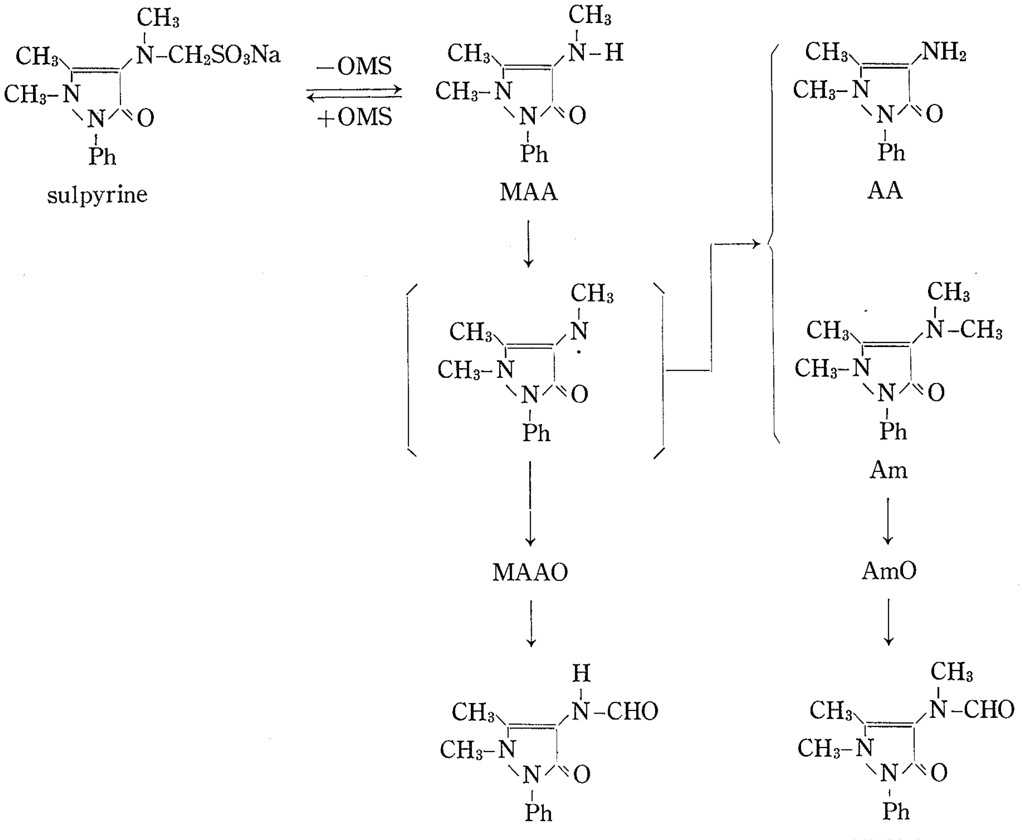

Chart 2

Table I. Effects of DPPH and OMS on the Decomposition Reactions of MAA, Sulpyrine and Am

	Additive	Relative rate[a]
MAA[b]	—	1
	DPPH	2.0
Sulpyrine[c]	—	1
	DPPH	1.9
Am[d]	—	1
	OMS	0.3

a) Relative rate is the ratio of the amount decomposed in the presence of the additive to that in its absence.
b) Fifty mg of MAA was heated with or without 30 mg of DPPH in the presence of 50 μl of water and 50 μl of EtOH for 1 day.
c) Fifty mg of sulpyrine was heated with or without 30 mg of DPPH in the presence of 50 μl of water and 50 μl of EtOH for 2 days.
d) Fifty mg of Am was heated with or without 30 mg of OMS in the presence of 50 μl of water for 6 days.

previous study[5] that in the copper(II) ion-catalyzed oxidation, hydrogen peroxide was formed when MAA was decomposed to AA, and subsequently took part in the oxidation of AA to AP. On the other hand in the oxidation of MAA in the absence of copper(II) ions reported in this paper, it is suggested that AP is not formed, because AA may be formed by methyl rearrangement of MAA, without hydrogen peroxide formation.

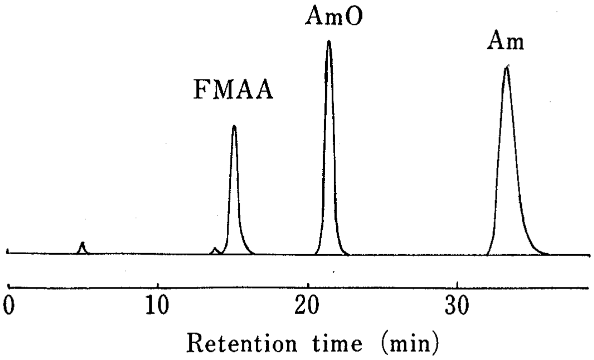

Fig. 12. High Performance Liquid Chromatogram of Reaction Products of Aminopyrine

Am solution (50 mg/50 mg of H_2O) heated at 80° for 6 days.

Though the isolation of reaction products and the study of the reaction pathways described above were carried out with concentrated solutions of sulpyrine or MAA, it is conceivable that the same reactions may occur when the solid phase of sulpyrine coexists with an aqueous layer, since the same reaction products were detected by TLC in both systems.