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Studies on 3-Substituted-2-thiohydantoins as Analytical Reagent. I. Color Reaction of 3-Methyl-2-thiohydantoin

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The color reaction of 3-methyl-2-thiohydantoin in alkaline medium was found to be mainly attributed to the formations of bis(3-methyl-2-thiohydantoinylidene-5) by the autoxidation effected by the dissolved oxygen. The presence of trace of manganese (II) ion showed remarkable effect to this color reaction.

The coloration of 3-substituted-2-thiohydantoins derived from aryl or alkyl isothiocyanate and glycine has been reported[2–5] and a method of the determination of glycine by this reaction has been proposed.[5] However, the informations are insufficient for the reliable presumption of the mechanism of this reaction.

We have found that coloration of 3-methyl-2-thiohydantoin is enhanced by the presence of trace of some metal ions, and some highly sensitive methods of the determinations of the metal ions are expected by the application of these reactions. In an attempt to obtain fundamental informations for the analytical application of this reaction, we investigated the coloration and the influence of the metal ions mainly by spectrophotometric method, on 3-methyl-2-thiohydantoin (MTH) which is most soluble in water among the analogous compounds.

Experimental

Materials[6]——MTH was prepared by the usual method. mp 163—164°, Lit. 161°.[7] *Anal.* Calcd. for $C_4H_6N_2OS$: C, 36.69; H, 4.65; N, 21.52. Found: C, 36.37; H, 4.63; N, 21.20. Mass Spectrum *m/e*: 130 (M^+). Bis(3-methyl-2-thiohydantoinylidene-5) (Bis-MTH) was prepared by Mayer's method[8] and purified by sublimation at 240° (1 mmHg), brown-red powder, mp>350°, IR ν_{max}^{KBr} cm^{-1}: 3180, 2940, 1710, 1610 (C=C), 1460, 1300, 1250, 1100. *Anal.* Calcd. for $C_8H_8O_2N_4S_2$: C, 37.49; H, 3.15; N, 21.86. Found: C, 37.67; H, 3.10; N, 21.69. Mass Spectrum *m/e*: 256 (M^+), 183 (M^+-CH_3NCS). Dioxane solution (10^{-3}M) was prepared for the stock solution. Bis(3-methyl-2-thiohydantoinyl-5) (Bis(2H)-MTH) was prepared from diaminosuccinic acid and methyl isothiocyanate, a method for phenyl derivative[8] being applied. The crude product was washed several times with acetonitrile until the TLC showed one spot. Yellow leaflet, mp>350°, IR ν_{max}^{KBr} cm^{-1}: 3230, 2940, 1730, 1480, 1310, 1230, 1120. *Anal.* Calcd. for $C_8H_{10}O_2N_4S_2$: C, 37.20; H, 3.90; N, 21.69. Found: C, 37.12; H, 3.77; N, 21.48. Mass Spectrum *m/e*: 258 (M^+), 130 [½(M^++2)]. Freshly prepared 10^{-3}M acetonitrile solution was used. N-Methylthiocarbamyl glycine was isolated as the intermediate on the synthesis of MTH. Recrystallization from 50% EtOH gave a white prism, mp 164—166° (decomp.). *Anal.* Calcd. for $C_4H_8N_2O_2S$: C, 32.41; H, 5.45; N, 18.90. Found: C, 32.71; H, 5.67; N, 19.17. Other reagents: All other chemicals used in this study were of reagent grade.

Measurement of Absorption——Ultraviolet (UV) and visible absorption spectra were made with a Hitachi Recording Spectrophotometer EPS-3T and absorbances at a fixed wavelength were measured with a Hitachi Spectrophotometer 139. Quarz or glass cells of 10 mm light path were used.

Measurement of Oxygen Uptake——Manometric measurements were carried out by usual method using Tabai Warburg apparatus at reaction temperature ±0.01°. After the gas space was replaced with 100%

1) Location: *Ōe-honmachi, Kumamoto.*
2) L.K. Ramanchandran and W.B. Mcconnell, *J. Am. Chem. Soc.*, **78**, 1255 (1956).
3) B. Salvessen, *Medd Norsk. Farm. Selskap*, **18**, 107 (1956) [*C.A.*, **50**, 17316 (1956)].
4) G. Schram, J.W. Schneider, and A. Anderer, *Z. Naturforsch.*, **11b**, 12 (1956) [*C.A.*, **50**, 8789 (1956)].
5) K. Nagendra Nath Reddy and L.K. Ramachandran, *Anal. Chim. Acta*, **32**, 435 (1965).
6) All melthing points were uncorrected.
7) W. Marckwald, M. Neumark, and R. Stelzner, *Ber.*, **24**, 3278 (1891).
8) R. Mayer and H. Thieme, *J. prakt. Chem.*, **27**, 213 (1965).

O_2gas, an alkaline solution placed in a side chamber was transfered to the major chamber where the substrate is placed. The total volume of reaction mixture was 3 ml.

Thin-Layer Chromatography (TLC)——Reaction mixtures were analyzed by TLC under the following conditions. Plate: Wakogel B-5 (250 μ). Solvent system: benzene: ethyl acetate: acetic acid=7: 3: 0.3. Detection: Bis-MTH and other colored substance were marked directly and then I_2 vapor was used. Only gylcine was identified using ninhydrin reagent.

Result

Absorption Spectra

Since we have assumed the color development is probably attributed to the oxidative coupling at 5-position of MTH, the spectral property of MTH was compared with that of the coupling products, namely bis(3-methyl-2-thiohydantoinyl-5) (Bis(2H)-MTH) and bis(3-methyl-2-thiohydantoinylidene-5) (Bis-MTH). The spectral properties of these compounds

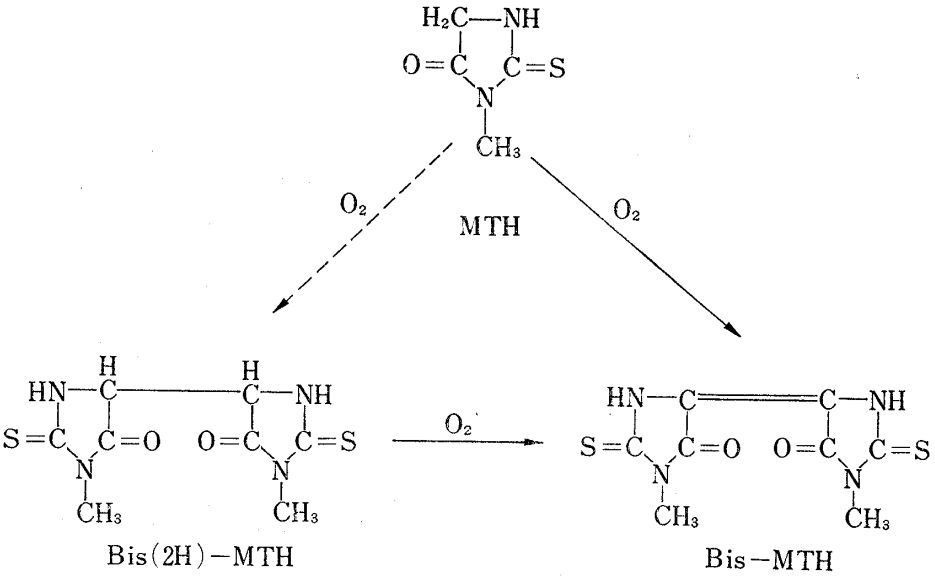

Chart 1

are shown in Fig. 1. The spectrum of MTH after color development in alkaline solution is similar to that of Bis-MTH at same pH and two absorption maxima (330 nm and 500 nm at pH 9.2) were observed. The lower maximum on the spectrum of Bis-MTH is independent of pH but higher maximum shifts to shorter wavelength with decrease of pH and it is observed at 453 nm at pH 5.9. However, in the case of acidic or neutral solution of MTH no absorption is observed at the region of over 300 nm. The spectral property of Bis(2H)-MTH is very similar to that of Bis-MTH in alkaline solution. The intensity and the rate of the color development of MTH are enhanced by the basicity of the solution. The color developments are very rapid even in weak alkaline solution both in Bis(2H)-MTH and Bis-MTH.

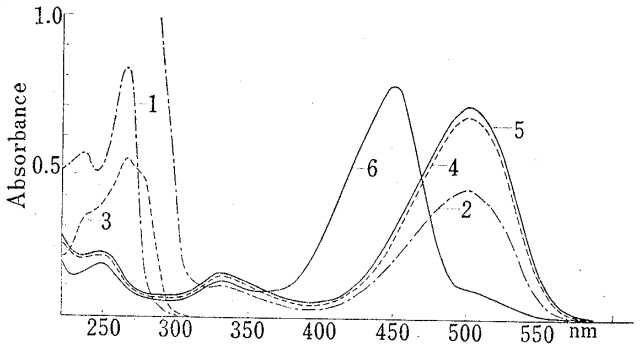

Fig. 1. Absorption Spectra of MTH (——), Bis (2H)-MTH (·····) and Bis-MTH (———)

1: MTH (5×10^{-5}M), pH 6.0[a]
2: MTH (3×10^{-5}M), in 0.02M $Na_2B_4O_7$,[a] pH 9.2, after standing 2 hr at 25°
3: Bis(2H)-MTH (2×10^{-5}M), pH6.90[a]
4: Bis(2H)-MTH (2×10^{-5}M) in 0.02M $Na_2B_4O_7$, pH 9.2[a]
5: Bis-MTH (2×10^{-5}M) in 0.02M $Na_2B_4O_7$, pH 9.2[b]
6: Bis-MTH (2×10^{-5}M), pH 5.9[b]
a) containing 10% acetonitrile *b*) containing 10% dioxane

Effect of Dissolved Oxygen

The effect of dissolved oxygen on the color reaction of MTH was investigated by spectrophotometry. The color intensity of both MTH and Bis(2H)-MTH in alkaline solution containing dissolved oxygen was compared with that of in the deaerated alkaline solution. As shown in Table I, color intensity was remarkably reduced by the deaeration with N_2 in both MTH and Bis(2H)-MTH. However, in the case of Bis-MTH the effect of the deaeration was not observed. Accordingly, the contribution of dissolved oxygen to the color development was confirmed.

TABLE I. Effect of Dissolved Oxygen on the Color Development of MTH, Bis(2H)-MTH and Bis-MTH

Sample	Alkaline solution (0.02 M)	Standing time at 25°	Absorbance		
			Deaeration[a]	Without deaeration	λ, nm
MTH	$Na_2B_4O_7$	2 hr	0.085	0.180	500
(2×10^{-3} M)	NH_4OH	2 hr	0.049	0.525	505
	NaOH	2 hr	0.028	0.260	515
Bis(2H)-MTH[b]	$Na_2B_4O_7$	10 min	0.215	0.675	500
(2×10^{-5} M)					
Bis-MTH[c]	$Na_2B_4O_7$	10 min	0.685	0.683	500
(2×10^{-5} M)					

a) Sample and alkaline solutions were bubbled with nitrogen for 30 min prior to mixing, respectively.
b) containing 10% acetonitrile
c) containing 10% dioxne

Effect of Metal Ions

Effect of several kinds of metal ions on the color reaction of MTH was examined. Since the rate of color development in weak alkaline solution (*e.g.* 0.025 M $Na_2B_4O_7$) was very slow, the acceleration of color development by the addition of some metal ions such as Mn^{2+}, Cu^{2+} and Co^{2+} was observed. This effect was observed by the presence of trace of these ions. The activity of Mn^{2+} was the strongest, namely even in the presence of 10^{-7}—10^{-8} M, the activity was clearly observed. In the case of Cu^{2+}, when its concentration is higher than 10^{-4} M, the activity was not clearly observed due to the formation of black precipitation which is considered to be a complex composed of MTH and Cu^{2+}.

From Fig. 2, the linearity between the color intensity and the concentration of the metal ion was observed in both Mn^{2+} and Cu^{2+}. The absorption maximum of alkaline MTH solution (pH 9.2) at 500 nm shifted to 514 nm by the addition of Mn^{2+}, and similar behavior was observed in the case of Bis-MTH. In addition, the effect of Mn^{2+} on the absorption spectra disappered completely by addition of equivalent amount of EDTA to Mn^{2+}.

Oxygen Uptake of MTH

The rate of oxygen uptake of MTH in alkaline solution was measured by the standard manometric method with Warburg apparatus, in the presence of Mn^{2+}, Cu^{2+} and Co^{2+}. The results were shown in Fig. 3. By plotting $\log V_\infty - V_n$ against time, where V_∞ was the maximum amount of oxygen uptake determined from Fig. 3 and V_n was the amount of oxygen uptake at certain time t_n, straight line was obtained with respect to each metal ion after initial lag phase from 80 min to 240 min. Accordingly, the autoxidation of MTH follows first order kinetics, hence the rate of the absorption of oxygen was calculated by the following equation, and its results are shown in Table II.

$$k = \frac{2.303}{t_n} \cdot \frac{\log (V_\infty - V_n)}{V_\infty}$$

Each of the first order rate constant was the average of the straight line portion, the effect on the rate of the absorption of oxygen increases in the order $Co^{2+}<Cu^{2+}<Mn^{2+}$, as well as that on the intensity of the color.

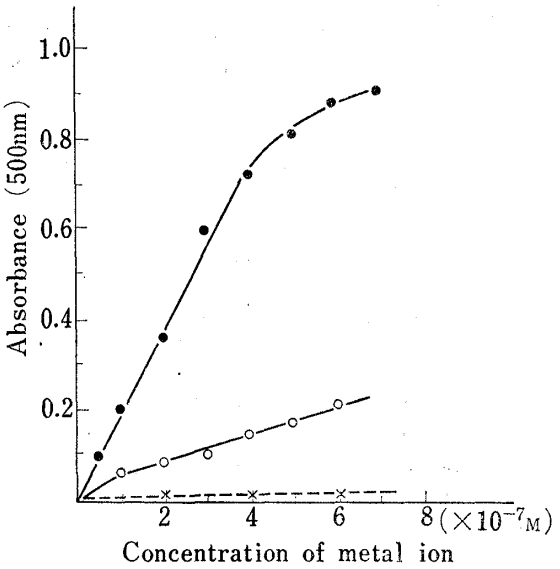

Fig. 2. Effect of Metal Ions on the Color Development of MTH

Each absorbance was measured after standing for 2 hr at 35° against reagent blank.
concentration of MTH: 5×10^{-4}M in 0.025M $Na_2B_4O_7$
—●—: Mn^{2+} —○—: Cu^{2+} ···×···: Co^{2+}

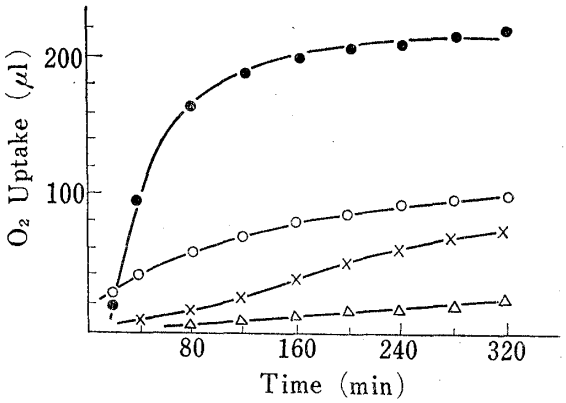

Fig. 3. Oxygen Uptake of MTH in the Presence of Metal Ions

under 100% O_2 gas at 20°
concentration: MTH=3.3×10^{-3}M, metal ion=3.3×10^{-5}M, $Na_2B_4O_7=1.7\times10^{-2}$M
—●—: MTH + Mn^{2+} —×—: MTH + Co^{2+} —○—: MTH+Cu^{2+} —△—: MTH

TABLE II. Effect of Temperature on the Rate Constant for Oxygen Uptake of MTH in the Presence of Metal Ions

Temperature (°C)	k (sec^{-1})		
	Mn^{2+}	Cu^{2+}	Co^{2+}
25	3.13	1.63	0.76
30	3.52	1.85	1.03
35	3.76	1.93	1.51
40	4.14	2.25	1.96

concentration; MTH=3.3×10^{-3}M, metal ion=3.3×10^{-5}M, $Na_2B_4O_7=1.7\times10^{-2}$M

Oxygen Uptake of Bis(2H)-MTH and Bis-MTH

The behavior of Bis(2H)-MTH and that of Bis-MTH in the uptake of oxygen in various alkaline solutions were examined in the state of suspension or solution similarly to the case of MTH. As shown in Fig. 4, oxygen uptake of Bis(2H)-MTH in $Na_2B_4O_7$ was scarecely observed. However, in 0.01N NaOH more rapid oxygen uptake was observed than in the case of MTH, and it was increased in the presence of Mn^{2+}. In the case of Bis-MTH, small amount of oxygen uptake was observed in $Na_2B_4O_7$, whereas in 0.1N NaOH oxygen was absorbed very rapidly, and the rate of the absorption was increased in accord with the increase of the concentration of alkali and also by the addition of Mn^{2+} as in the case of Bis(2H)-MTH and MTH. From this fact, it was confirmed that Bis-MTH which was produced from MTH in strong alkaline solution was decomposed by dissolved oxygen.

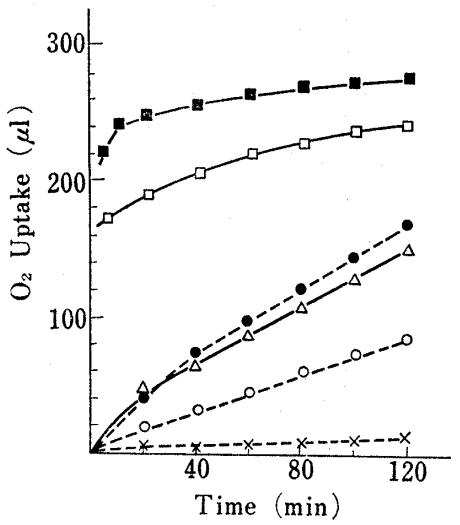

Fig. 4. Oxygen Uptake for Bis(2H)-MTH (——) and Bis-MTH(·····) in Various Alkaline Solutions

under 100% O_2 gas at 35°
Each vessel contained 1.2×10^{-2} mmole Bis(2H)-MTH or 3.9×10^{-2} mmole Bis-MTH.
—△—: 0.017M $Na_2B_4O_7$ (suspension), —□—: 0.01N NaOH,
—■—: 0.01N NaOH+Mn^{2+} (3.3×10^{-5}M),
···×···: 0.017M $Na_2B_4O_7$ (suspension), ···○···: 0.1N NaOH,
···●···: 0.1N NaOH+Mn^{2+} (3.3×10^{-5}M)

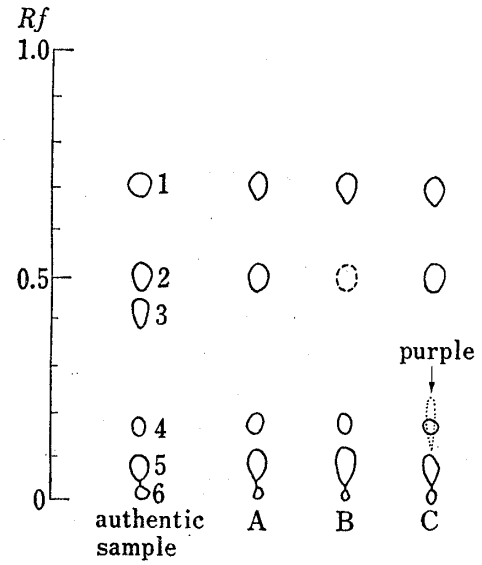

Fig. 5. TLC of MTH in Alkaline Solutions

Samples (A—C) were spotted after standing for 2 hr. concentration of MTH: 2×10^{-3}M
A: 0.02M $Na_2B_4O_7$, B: 0.02M NaOH, C: 0.02M $Na_2B_4O_7$ +Mn^{2+} (5×10^{-5}M)
authentic sample 1: Bis-MTH, 2: MTH, 3: Bis(2H)-MTH, 4: N-methylthiourea, 5: N-methylthiocarbamyl glycine, 6: glycine
plate: Wakogel B-5, solvent system: benzene–EtOAc–HOAc (7: 3: 0.3)

TLC of Degradation Products

Edward, *et al.*[9] have reported that a black solid produced by the oxidation of 2-thiohydantoin is assumed to be a bisform, which is considered to correspond to the coupling product of MTH, and in addition, this compound is decomposed successively by adsorbed oxygen to give oxalic acid and thiourea. The degradation products of MTH and Bis-MTH in alkaline solutions were examined by thin-layer chromatography (TLC). Bis-MTH, N-methylthiourea, N-methylthiocarbamyl glycine were detected from colored MTH solution, however, Bis(2H)-MTH was not detected.

In the presence of Mn^{2+}, the existence of a purple colored substance was confirmed and its color did not change in acidic media. Further investigation of this substance was not carried out because the amount of this substance was too little and its color faded gradually on standing. In the degradation products of Bis-MTH, N-methylthiourea and glycine were detected and oxalic acid was identified by the gas chromatography[10] after the methylation.

Discussion

Based on the data on UV spectra and TLC, the color reaction of MTH in alkaline solution is confirmed to be attributed to the formation of Bis-MTH by the oxidative coupling of MTH. Previously, Nagendra Nath Reddy, *et al.*[5] have reported that the coloration of 3-phenyl-2-thiohydantoin (PTH) is based on the formation of its ammonium salt by the reaction with ammonia. However, their data on infrared (IR) and UV spectrum and elemental analysis on the compound obtained as a principle of the color reaction can be accepted, provided that

9) J.T. Edward and S. Nielsen, *J. Chem. Soc.*, **1959**, 2327.
10) Gas chromatographic conditions were as follows; column: 20% diethylene glycol succinate, 3 m×4 mm, column temp.: 110°.

the substance isolated is a mixture of ammonium salt of PTH and coupling product of PTH which corresponds to Bis-MTH. Dissolved oxygen was found to give essential effect to the development of color and the presence of some metal ions, particulary that of Mn^{2+} accelerated the color reaction. However, the colored substance produced in the presence of the metal ion was not investigated. The effect of Mn^{2+} presented here may offer a possibility of an application of MTH as a highly sensitive analytical reagent for the determination of trace of Mn^{2+}. The red-shift of the absorption maxima of MTH and Bis-MTH in $Na_2B_4O_7$ by the addition of Mn^{2+} may be concerned with the complex formation of Bis-MTH with Mn^{2+}.

Bis(2H)-MTH is considered to be an intermediate in this reaction, however, its existence in the colored MTH solution was not confirmed by TLC probably due to its rapid autoxidation. We can conclude that the formation of Bis-MTH and degradation of both MTH and Bis-MTH occur simultaneously in the color reaction of MTH. The further investigation for the reaction mechanism will be studied later.

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