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Studies on 3-Substituted-2-thiohydantoins as Analytical Reagent. II.[1] Polarographic Investigation of Color Reaction of 3-Methyl-2-thiohydantoin

Atsushi Sugii, Masanobu Matsuo, and Yoshihumi Ohara

Faculty of Pharmaceutical Sciences, Kumamoto University[2]

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Color reaction of 3-methyl-2-thiohydantoin (MTH) in alkaline solution was investigated by polarographic method. Hydrogen peroxide and bis(3-methyl-2-thiohydantoinylidene-5) were confirmed in the autoxidation products of MTH and their apparent ratio was determined by this method. Effect of metal ions on the autoxidation of MTH was also studied and a reaction mechanism was proposed.

It has been reported in the preceding paper[1] that the coloration of 3-methyl-2-thiohydantoin (MTH) in alkaline solutions is mainly due to the formation of bis(3-methyl-2-thiohydantoinylidene-5) (Bis-MTH). In addition, we have proposed a possibility of an application of MTH as a highly sensitive reagent for Mn^{2+} which enhanced this coloration even in the presence of trace amount. However, this color reaction is so complicated that sufficient information for its mechanism has not been obtained by the methods reported previously.

In this paper, the polarographic technique has been applied to the further elucidation of the color reaction of MTH and the effects of metal ions. Though there have been few studies on the polarography of thiohydantoin derivatives such as 5,5-diphenyl-2-thiohydantoin,[3] 5,5-diphenyl-2,4-dithiohydantoin[3] and dimethyldithiohydantoin,[4] those of 3-substituted-2-thiohydantoins have not been reported.

Experimental

Apparatus——The DC polarograms were recorded with a Yanagimoto Polarograph Type P8-AP (three electrode). The dropping mercury electrode had the following characteristics in 0.1 M KCl and $h=76$ cm: $m=2.74$ mg/sec with the circuit open and $\tau=2.77$ sec at OV *vs.* SCE. All experiments were carried out at 25±0.1°. A Yanagimoto Controlled Potential Electrolyser Type VE-3 with Pt anode, Pt cathode and SCE for reference electrode were used. Other apparatus were the same as described previously.[1]

Materials and Reagents——MTH, Bis-MTH and bis(3-methyl-2-thiohydantoinyl-5) (Bis(2H)-MTH) were the same as described previously.[1] Dioxane was purified by heating with sodium after heating with hydrochloric acid, then a fraction boiling at 101—101.5° was collected. The composition of the electrolytic solutions for the polarographic measurements was as follows: 0.1 M KCl, 0.025 M $Na_2B_4O_7$, 0.005% gelatine and appropriate concentration of dioxane solution of MTH or its coupling products were contained, and pH was adjusted at 9.8 unless otherwise stated.

Result

Polarography of MTH, Bis-MTH and Bis(2H)-MTH

The typical polarograms of MTH and its coupling products are shown in Fig. 1. The polarogram of MTH after color development in borate buffer solution (pH 9.8) on standing at 25° exhibited a four-step wave with half-wave potential ($E_{1/2}$) +0.05 V, −0.38 V, −0.68 V

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[1] Part I: A. Sugii, Y. Ohara, and K. Kitahara, *Chem. Pharm. Bull.* (Tokyo), 22, 109 (1974).

[2] Location: *Ōe-honmachi, Kumamoto, 862, Japan.*

[3] P. Zuman and M. Kuik, *Naturwissenschaften*, **45**, 541 (1958) [*Chem. Abstr.*, 53, 8886 (1959)].

[4] G. Sartori and A. Liberti, Sbornik Mezinarod. Polarog. Sjezdu v Praze, 1st Congr. 250 (1951) [*Chem. Abstr.*, **46**, 9450 (1952)].

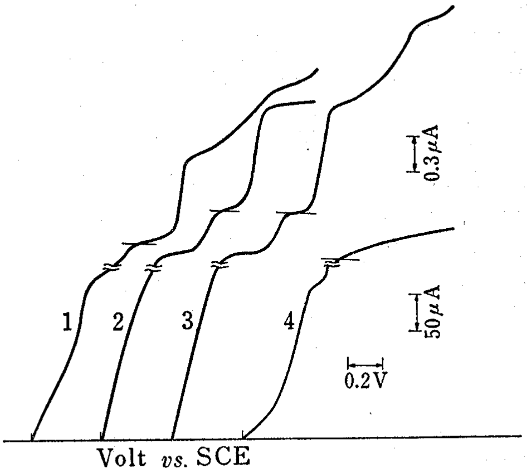

Fig. 1. Typical Polarograms of MTH, Bis-MTH and Bis(2H)-MTH in Borate Buffer Solution Containing 20% (v/v) Dioxane

polarogram recorded: started at +0.2V
1: 5×10^{-3}M MTH (red color),
2: 1×10^{-4}M Bis-MTH
3: 1×10^{-4}M Bis(2H)-MTH,
4: 5×10^{-3}M MTH (colorless)

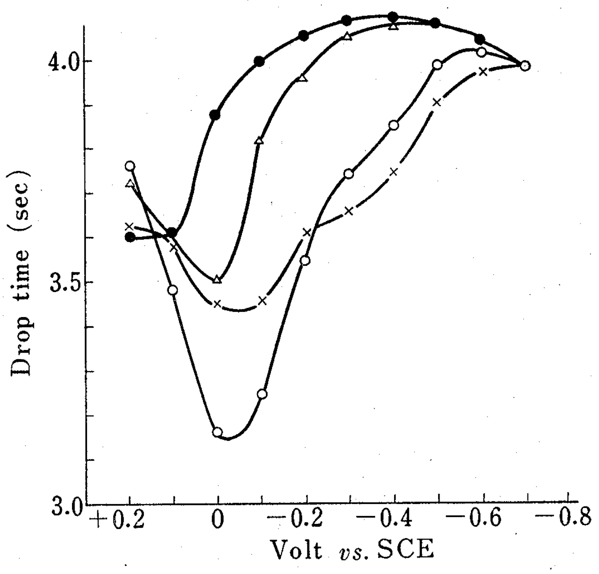

Fig. 2. Electrocapillary Curves of MTH and Bis-MTH

—●—: 0.1M KCl
—△—: 2×10^{-4}M MTH (colorless)
—○—: 2×10^{-4}M MTH (red color)
—×—: 2×10^{-4}M Bis-MTH

and −1.12 V *vs.* SCE, while practically colorless MTH solution prepared under deoxygenated condition at the same pH only gave two anodic waves with $E_{1/2}$+0.05 V and −0.23 V *vs.* SCE.

The heights of the second, third and fourth waves of colored MTH solution increased with standing until some definite time in the electrolytic solution containing dissolved oxygen, and this behavior related to the increase of the red color. The heights of the first and second waves of colorless MTH solution, on the contrary, decreased with standing under oxygenated conditions, and the second wave entirely disappeared when the coloration proceeded. From these facts, it has been concluded that the first wave of colored MTH solution is due to the unoxidized MTH. The anodic waves had an adsorption characteristics as shown on the electro-capillary curve (Fig. 2), which showed suppression at +0.1 V to −0.3 V by the presence of MTH. However, further investigation on the anodic waves was not performed.

Bis-MTH gave a two-step wave with $E_1/_2$ −0.38 V (anodic) and −0.68 V (cathodic) *vs.* SCE, and these waves agreed with the second and third waves of colored MTH solution under the same conditions. The height of anodic wave of Bis-MTH tended to a limit in the concentration higher than 2×10^{-4}M and was proportional to the mercury head. And the plots of $E_{1/2}$ *vs.* pH were given by a straight line $E_{1/2}$=0.32—0.082 pH at pH 4.0—8.6 and −0.38 V at pH 8.6—12.0. Moreover, electrocapillary curve showed adsorption on the dropping mercury electrode at +0.1— −0.6 V. Accordingly, it was assumed that this wave was due to the adsorption of insoluble mercurous salt[5] of Bis-MTH.

While, the height of cathodic wave of Bis-MTH was proportional to the concentration and square root of mercury head, and was assigned to be diffusion control. Plots of log *id-i/i vs.* potential gave straight line with a slope n=2.25, and a slope m=1.64 was obtained from plots of $E_{1/2}$ *vs.* pH. These data indicate that this reduction involves 2-electrons and 2-protons. Moreover, electrode reaction of this cathodic wave was confirmed by the controlled potential electrolysis of Bis-MTH, which was carried out at −0.80 V *vs.* SCE using an acetate buffer solution (pH 4.0) containing 2×10^{-4}M Bis-MTH dioxane solution under a nitrogen gas stream.

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[5] P. Zuman, "Organic Polarographic Analysis," Pergamon Press, London, 1964, p. 20.

Bis(2H)-MTH was identified from the reduction products of Bis-MTH by the both spectrophotometric and thin-layer chromatographic analysis of cathodic solution.

On the other hand, Bis(2H)-MTH showed a three-step wave with $E_{1/2}$—0.38 V (anodic), —0.68 V (cathodic) and —1.12 V (cathodic) *vs.* SCE, and these corresponded to the second, third and fourth waves of colored MTH solution, respectively. The wave of $E_{1/2}$—1.12 V, which was observed on the polarograms of colored MTH and Bis(2H)-MTH solution, corresponded to that of the hydrogen peroxide; the polarogram of colored Bis(2H)-MTH solution agreed with that of equimolar mixture of Bis-MTH and hydrogen peroxide in the same conditions. In addition, the presence of hydrogen peroxide in colored MTH solution was confirmed by the color reaction with potassium iodide and chloroform after deionization with IRA-410 OH-form resin.

Effect of Metal Ions

Wave-heights according to Bis-MTH and hydrogen peroxide on the polarogram of the colored MTH solution were apparently enhanced by the addition of the some metal ions, such as Mn^{2+}, Cu^{2+} and Co^{2+}. As shown in Table I, wave-heights of Bis-MTH increased with the increasing amounts of Mn^{2+}. However, in the presence of higher concentration of Cu^{2+} and Co^{2+} those were decreased. The action on the yield of Bis-MTH increased in the order $Co^{2+} < Cu^{2+} < Mn^{2+}$, as well as that on the color intensity and the rate of the absorption of oxygen.[1] In the case of Bis(2H)-MTH, this action of metal ions was not remarkably observed because it was oxidized immediately to Bis-MTH in alkaline solution. The effect of the reaction time on the yields of Bis-MTH and hydrogen peroxide in the presence of Mn^{2+} was shown in Fig. 3. Reaction time for yielding maximum amounts of Bis-MTH varied with change of MTH concentration, and generally decomposition rate of hydrogen peroxide was faster than that of Bis-MTH in borate buffer solution. Since it is suspected that some

TABLE I. Yield of Bis-MTH in the Presence of Metal Ions

Concentration of MTH (M)	Concentration of metal ions (M)		Yield of Bis-MTH[a] ($\times 10^{-5}$ M)
5×10^{-4}	no addition		0.40
	Mn^{2+}	1×10^{-7}	1.15
		5×10^{-7}	1.85
		1×10^{-6}	2.75
		5×10^{-6}	4.98
		1×10^{-5}	5.55
		5×10^{-5}	5.85
		1×10^{-4}	6.50
	Cu^{2+}	1×10^{-7}	0.43
		5×10^{-7}	1.25
		1×10^{-6}	1.65
		5×10^{-6}	2.94
		1×10^{-5}	3.08
		5×10^{-5}	4.64
		1×10^{-4}	1.28
2×10^{-3}	no addition		1.88
	Co^{2+}	1×10^{-7}	5.40
		5×10^{-7}	5.90
		1×10^{-6}	9.00
		5×10^{-6}	7.60
		1×10^{-5}	5.40
		5×10^{-5}	3.60
		1×10^{-4}	2.80

[a] Determined from the cathodic wave of Bis-MTH produced from MTH solutions after standing 3 hr at 25°.

side reactions between oxidation products of MTH and added metal ions would occur, the polarographic behavior of Bis-MTH in the presence of metal ions has been examined.

As shown in Fig. 4, remarkable changes were observed on the polarograms of Bis-MTH in the presence of Cu^{2+} and Co^{2+}; both wave-heights of Bis-MTH were decreased and additional cathodic waves were observed at $E_{1/2}$—0.82 V for Cu^{2+} and $E_{1/2}$—0.87 V and —1.12 V for Co^{2+}, respectively. The waves produced by the addition of above metal ions increased with increasing amounts of them, and indicated the properties of diffusion controlled limiting current except for the wave of $E_{1/2}$—1.12 V. The wave ($E_{1/2}$—1.12 V) due to the addition of Co^{2+} showed catalytic properties as well as 5,5-diphenyl-2-thiohydantoin.[3]

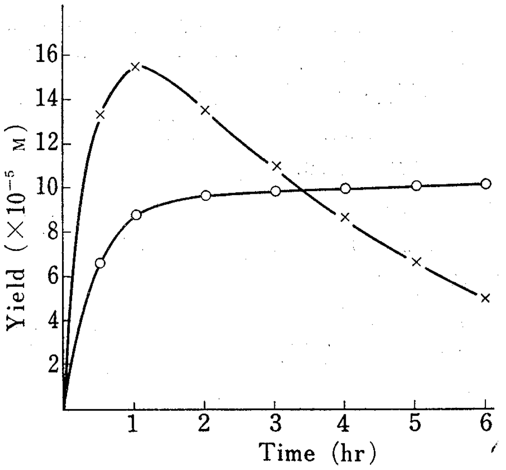

Fig. 3. Time Course of Bis-MTH (-○-) and Hydrogen Peroxide (-×-) Produced from MTH in the Presence of 1×10^{-5}M Mn^{2+}

concentration of MTH: 1×10^{-3}M

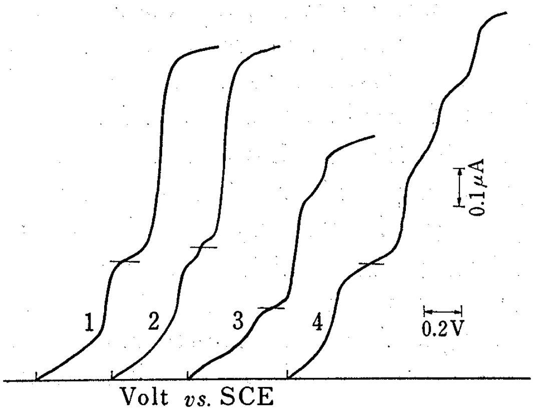

Fig. 4. Polarograms of Bis-MTH in the Presence of Mn^{2+}, Cu^{2+} and Co^{2+}

polarogram recorded: started at +0.2V
concentration of Bis-MTH: 1×10^{-4}M
1: no addition, 2: 5×10^{-5}M Mn^{2+},
3: 5×10^{-5}M Cu^{2+}, 4: 5×10^{-5}M Co^{2+}

On the other hand, the effect of Mn^{2+} on the polarogram of Bis-MTH was less than that of Cu^{2+} and Co^{2+}, and it was negligibly small at the concentration of Mn^{2+} below 10^{-6}M. In the presence of higher concentrations of Mn^{2+} (over 5×10^{-5}M), decrease of the wave-heights of Bis-MTH and appearance of a small wave ($E_{1/2}$—0.50 V) were observed. However, further investigation of latter wave had not been undertaken. The effects of Mn^{2+} on the wave-heights of Bis-MTH were summarized in Table II. In addition, these effects of metal ions were entirely disappeared by the addition of EDTA for masking agent.

TABLE II. Effect of M_n^{2+} on the Polarogram of Bis-MTH

Concentration of Mn^{2+} (M)	Current (μA) Bis-MTH			Mn^{2+}
	$E_{1/2}=-0.38$V	$E_{1/2}=-0.50$V	$E_{1/2}=-0.68$V	$E_{1/2}=-1.50$V
(1) 0	0.254	——	0.520	——
(2) 1×10^{-6}	0.252	——	0.514	——
(3) 1×10^{-5}	0.250	——	0.494	0.032
(4) 5×10^{-5}	0.185	0.065	0.491	0.259
(5) 7.5×10^{-5}	0.185	0.080	0.470	0.368
(6) (3)+EDTA(10^{-4}M)	0.256	——	0.514	——

Discussion

MTH and its oxidation products have shown well defined polarographic waves, and hence the polarographic technique has been applied to the investigation of color reaction of MTH. Hydrogen peroxide and Bis-MTH were confirmed in the autoxidation products of MTH in alkaline solution, and when reaction time reached to 30 minutes their apparent ratio determined by polarography was about 2: 1. The color development of MTH has been accelerated by the addition of small amounts of some metal ions, in which Mn^{2+} showed the most remarkable activity. It is suspected that the metal ions would act as a catalyst on the autoxidation of MTH.

As shown in Table I and Fig. 4, Cu^{2+} and Co^{2+} not only accelerate the oxidation of MTH, but also react with Bis-MTH. Although the reactivity of Mn^{2+} with Bis-MTH is smaller than that of Cu^{2+} or Co^{2+}, when Mn^{2+} concentration is higher than 5×10^{-5} M it is not negligible. Probably this may be correlated to the red-shift of Bis-MTH1) in the presence of Mn^{2+}. As the effects of these metal ions were entirely masked by the addition of EDTA, the reaction products of metal ions with Bis MTH are assumed to be complexes.

On the other hand, it is well known that metal ions act as a catalyst on the decomposition of hydrogen peroxide.6) The behavior of metal ions on the autoxidation of MTH is very complex, so it is difficult to explain their action unequivocally.

Bis(2H)-MTH, which has been presumed to be an intermediate in this autoxidation, was confirmed by means of controlled potential electrolysis of Bis-MTH. However, it could not be identified directly in colored MTH solution, because it was rapidly oxidized to Bis-MTH and hydrogen peroxide in alkaline media.

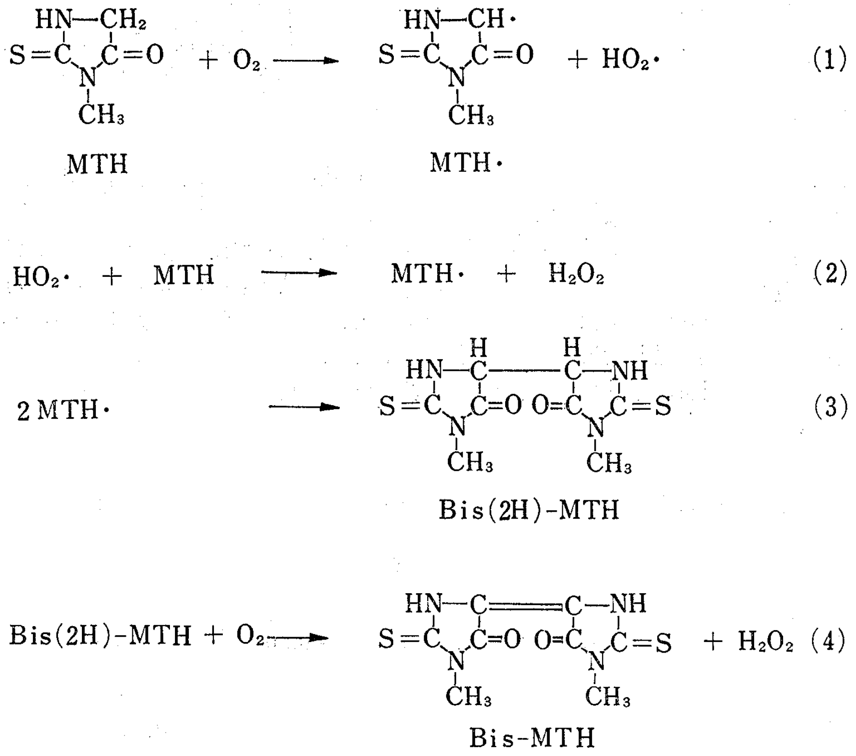

Chart 1

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6) T. Shiokawa and S. Suzuki, *Nippon Kagaku Zasshi*, **72**, 12 (1951).

It has been known that the autoxidation of phenols[7] is caused by radicals, and the production of hydrogen peroxide by the autoxidation of hydroquinones[8] has been well known. Consequently, we assumed that the autoxidation of MTH may proceed by similar reaction mechanism, and proposed the following pathway. Bis(2H)-MTH produced in the first step (Eq. 1—3) will be oxidized to Bis-MTH by the manner as well as the first step. The fact that hydrogen peroxide is also produced in the oxidation of Bis(2H)-MTH may supports this pathway. However, the second step is very fast even in the absence of metal ions, so that metal ions may act as a catalyst on the first step of this autoxidation. In addition to above pathway, it is necessary to take into accounts a possibility of direct oxidation of MTH with the hydrogen peroxide produced.

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7) A. Fairbourn and E.A. Lucken, *Proc. Chem. Soc.*, **1960**, 67 [*Chem. Abstr.*, **54**, 16186 (1960)].
8) T.H. James, J.M. Snell, and A. Weissberger, *J. Am. Chem. Soc.*, **60**, 2084 (1938).