on alumina using benzene-hexane (1:1) as the developing solvent. The decarboxylated product, grayanol dimethyl ether (VIII) was obtained in oil, which was not able to purify further due to its poor yield. NMR (in $CHCl_3$) $\tau=9.12$ (terminal Me of C_7H_{15}), 8.73 (intermediate methylenes of C_7H_{15}), 7.75 (aromatic Me), 6.26 (2 OCH_3), 6.17 (1 OCH_3).

Methyl Grayanolate Dimethyl Ether (IX)——Grayanolic acid dimethyl ether (VII) was methylated with CH_2N_2 in Et_2O. The brownish residue obtained on evaporation of the solvent was chromatographed on the silica gel column filled with a small amount of alumina on the top, using benzene as the developing solvent. The methyl ester was obtained as an oil. IR : $\nu_{max}^{CHCl_3}$ 1725 cm^{-1}(ester). NMR (in $CHCl_3$) $\tau=$ 9.15 (terminal Me of C_7H_{15}), 8.75 (intermediate methylenes of C_7H_{15}), 7.65 (aromatic Me), 6.35, 6.28, 6.20 (3 OMe), 6.11 ($COOCH_3$).

The authors wish to thank Professor Emeritus Y. Asahina for his kind advice and encouragement given us throughout this work and for the supply of lichen material.

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Summary

The structure of grayanic acid, $C_{23}H_{26}O_7$, m.p. 186~189° which was isolated from *Cladonia Grayi* MERRILL has been established to be an orcinol-type depsidone as formulated (II).

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162. **Yutaka Asahi** : Polarography of Thioacetazone and its Related Compounds.[*1,*2]

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It has been reported by Dusinsky[1] that thioacetazone (I), an antituberculous chemical, shows a polarographic reduction wave in alkali and two reduction waves in acid, and it is assumed that the wave in alkali ascribes to 2-electron reduction of the azomethine group. The electrode process in acid, however, has not been revealed yet. Polarographic methods for the determination of thioacetazone in tablets have been reported by Mnoucek[2] and Icha.[3]

In this study, the polarographic determination of thioacetazone and its degradation products was an object at the early stage. The polarographic behaviors of thioacetazone and its related compounds such as benzaldehyde thiosemicarbazone (II), S-methyl-

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[*1] Polarographic Studies of Pharmaceuticals. XXVI. Part XXV. Y. Asahi : Bull. Chem. Soc. Japan, 34, 1185 (1961).

[*2] A part of this paper was read at the discussion meeting for polarography (Nov. 1956, in Tokyo).

[*3] Higashiyodogawa-ku, Osaka (朝日 豊).

1) G. Dusinsky : Pharmazie, 8, 897 (1953).

2) K. Mnoucek, E. Knobloch : Czechoslov. farmacie, 2, 306 (1953).

3) F. Icha : *Ibid.*, 2, 308 (1953).

thioacetazone (III), *p*-acetamidobenzaldehyde semicarbazone (IV), *p*-acetamidobenzaldehyde (V), 1-(*p*-acetamidobenzyl)thiosemicarbazide (VI), S-methylisothiourea hydroiodide (VII), and S-ethylisothiosemicarbazide hydrobromide (VIII) have been studied. It was previously reported[4] that thiosemicarbazide gives an anodic wave, which is ascribed to the reaction with mercury ion. It has been confirmed in this study that thioacetazone can be determined by its reduction wave in the presence of the aldehyde (IV) and thiosemicarbazide, and the hydrolysis of the azomethine may be followed by polarography.

Another important subject of this study is the electrode reaction of thiosemicarbazones and its related compounds mentioned above. The reaction processes have been studied in detail by polarography and controlled potential electrolysis. Consequently the reduction waves of thiosemicarbazones in acid media have been ascribed to 4-electron reductions of their cations. A reductive cleavage of the N-N bond is assumed to precede a saturation of the -CH=N- group. The electrode reactions for the second reduction waves of thiosemicarbazones in alkaline media are ascribed to 2-electron reduction of the -CH=N- group in the free base. The second reduction wave of S-methylthioacetazone is considered to be due to 2-electron reduction of the $CH_3S\overset{|}{C}=NH$ group. Anodic waves of thiosemicarbazones are ascribed to formation of mercurous complexes.

Experimental

Materials——S-Methylthioacetazone was prepared by a reaction of thioacetazone with methyliodide in an alkaline medium, m.p. 173°. *Anal.* Calcd. for $C_{11}H_{14}ON_4S \cdot H_2O$: C, 49.23; H, 6.01; H_2O, 6.72; mol. wt., 268.3. Found : C, 49.36; H, 5.82; H_2O, 6.30; mol. wt., 268 (by titration). S-Methylisothiourea hydroiodide and S-ethylisothiosemicarbazide hydrobromide were tendered by Usui and Hashimoto of our laboratories. Thioacetazone, *p*-acetamidobenzaldehyde semicarbazone, and benzaldehyde thiosemicarbazone were offered by Ban of our laboratories. Buffer solutions were prepared with acetate, phosphate, borate, H_2SO_4, HCl and NaOH.

Equipments——The Yanagimoto pen-recording polarograph model PA 2, the Yanagimoto controlled potential electrolyser, the Beckman pH meter model G, the Beckman Spectrophotometer model DK 2, the Varian A60 NMR spectrophotometer were used. Characteristics of dropping Hg electrodes are described in Table I.

Procedure——Polarograms were taken at 25±0.1° after bubbling N_2 gas, using *N* calomel electrode as an external reference electrode. For A.C. polarography, the *N* calomel electrode was connected with a Hg pool anode by a condenser of 100 μF.

pK Values were measured by variations of absorbancies with pH in the buffer solutions mentioned above or in aq. H_2SO_4 at certain wave length, using Hammett's acidity constants.

D.C. Polarography. Thioacetazone (I)——Thioacetazone afforded two reduction waves with equal height in an aqueous solution of pH 1～4. These waves overlapped together at pH about 5 and the height decreased at higher pH. At pH above 7, another reduction wave appeared at more negative potential (Fig. 1). The total wave heights at pH 4.0, 7.5 and 9.2 were proportional to the concentration of thioacetazone and capillary constant ($m^{2/3}t^{1/6}$). The diffusion current constant k_D was 5.9 (μA. mM^{-1} $mg.^{-2/3}$ $sec.^{1/2}$) at pH below 4 and 2.8 at pH above 8. The 1st wave at pH above 7 had a round maximum and the height at pH 9.2 was independent of the Hg head (30～70 cm.). A linear relationship was observed between half-wave potential ($E_{1/2}$) and pH as shown in Fig. 2. Their slopes, $\Delta E_{1/2}/\Delta$pH, for the 1st wave at pH 1～5, the 2nd wave at pH 1～4, the 1st wave at pH 5～13, and the 3rd wave at pH 7～13 were −0.076, −0.076, −0.06 and −0.03 v., respectively. Their intercepts, E_0, were −0.66, −0.73, −0.74 and −1.42 v., respectively.

In a solution containing 40% EtOH and 0.01% gelatin, the double wave in acid overlaped together. The wave height was proportional to the concentration of thioacetazone (Fig. 5) and as large as 65% of that in aqueous buffers. The slopes of $E_{1/2}$-pH curves for the 1st wave at pH 2～8 and the 2nd wave at pH 7～11 were −0.06 and −0.03 v., respectively. The E_0 values were −0.86 and −1.46 v., respectively.

Thioacetazone gave a small anodic wave, whose height tended to a limiting value of 0.6 μA. at the concentration higher than 0.5 m*M*. The $E_{1/2}$-pH curve in 40% EtOH were given by (+0.20−0.056 pH).

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4) Y. Asahi : Yakugaku Zasshi, **80**, 688 (1960).

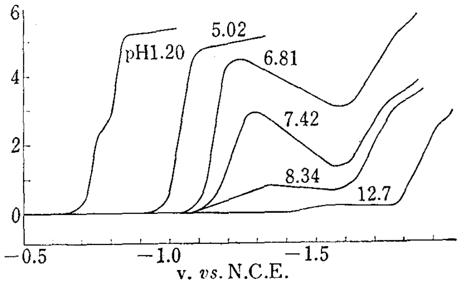


Fig. 1.
Typical Polarograms
of Thioacetazone (I)

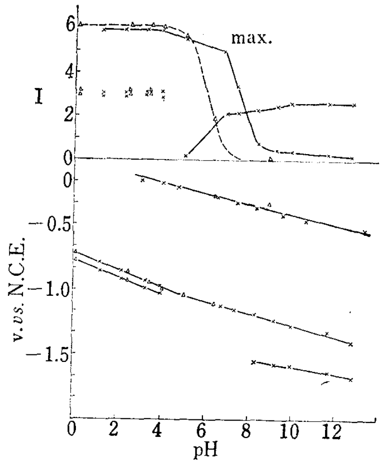


Fig. 2.
$E_{1/2}$-pH Curves and Wave-height (I : μA. mM^{-1} mg$^{-2/3}$ sec$^{1/2}$)-pH Curves of Thioacetazone (×, +) and Benzaldehyde Thiosemicarbazone (△)

×: 0.2 mM, EtOH 1%, k_c=4.28
+: 1.35 mM, EtOH 40%, gelatin 0.01%, k_c=1.70
△: 0.2 mM, gelatin 0.01%, k_c=0.926

Benzaldehyde Thiosemicarbazone (II)——This showed polarogarphic waves similar to those of thioacetazone (I) in aqueous solution, although the 3rd wave of II was not well-defined. The total wave height at pH below 4, k_D 6.1, was larger than that of I because of the difference in their molecular sizes. The $E_{1/2}$ values of II were almost equal to those of I as shown in Fig. 2.

S-Methylthioacetazone (III)——Acid solutions containing 0.1 mM S-methylthioacetazone and 0.003% gelatin gave a single reduction wave (Fig. 3). The height was proportional to the concentration of III (Fig. 5) and remained constant at pH 1∼9 to give k_D 6.0. The height, however, decreased at pH above

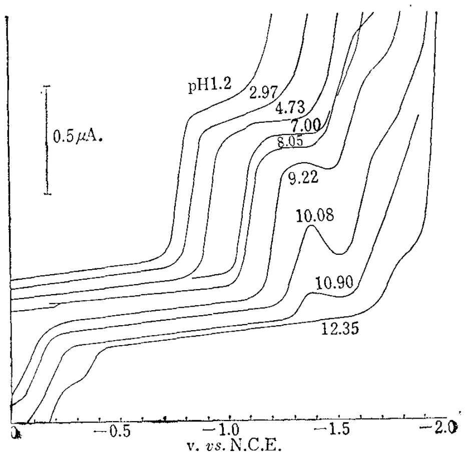


Fig. 3. Polarograms of S-Methylthioacetazone

gelatin 0.01%
0.1 mM
k_c=1.097

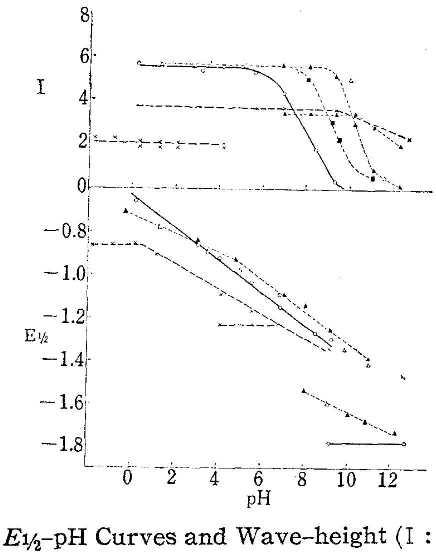


Fig. 4. $E_{1/2}$-pH Curves and Wave-height (I : μA. mM^{-1} mg$^{-2/3}$ sec$^{1/2}$)-pH Curves of S-Methylthioacetazone (------), p-Acetamidobenzaldehyde (- - -), and its Semicarbazone (——)

△: 0.187 mM, k_c 4.4 ▲: 0.1 mM, k_c 1.097
■: 1 mM, gelatin 0.02%, k_c 1.097
×: 0.2 mM, k_c 1.091 ○: 0.2 mM, k_c 1.115

9 as shown in Fig. 4. This compound (Ⅲ) afforded a 2nd reduction wave at −1.5～−1.7 v. in an alkaline solution (Fig. 3). The wave height remained constant at pH 7～10 and decreased at the higher pH. This wave merged into a hydrogen wave in an acid solution and remained even at pH about 12. In alkali, a small anodic wave appeared with a constant height, 0.2 μA., at the range of 0.1～1 m*M* (Fig. 5). The $E_{1/2}$ values for the 1st wave at pH 0～5 and pH 5～11, the 2nd wave at pH 7～13, and the anodic wave at pH 9～13 were given by (−0.72−0.04 pH), (−0.60−0.07 pH), (−1.17−0.046 pH), and (0.65−0.077 pH), respectively.

When the concentration of gelatin was 0.02% and that of Ⅲ was 1 m*M*, the 1st reduction wave was suppressed at pH 8～11. The $E_{1/2}$ values at pH 1～11 were shown by (−0.74−0.065 pH) (Fig. 4). A change of the capillary constant (4.4 and 1.1) caused no essential difference in the polarogram.

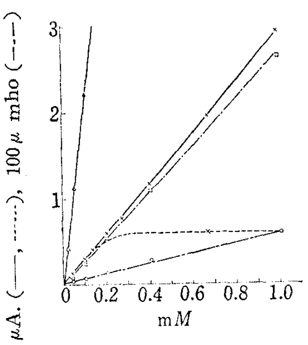


Fig. 5. Calibration Curves of Thioacetazone (Ⅰ), Benzaldehyde Thiosemicarbazone (Ⅱ) and S-Methylthioacetazone (Ⅲ)

——: DC reduction wave

- - -: DC anodic wave —·—: AC reduction wave

+: (Ⅰ) pH 4.08, EtOH 40%, gelatin 0.01%, $k_c=1.70$, $E_{1/2}$ −1.12 (—), −0.07 (····)

△: (Ⅲ) pH 1.3, k_c 4.4, $E_{1/2}$ −0.78;

×: (Ⅱ) pH 2.45, gelatin 0.01%, 0.5*M* KNO_3, k_c 0.926, $E_{1/2}$ −0.83, −0.92

○: (Ⅱ) E_p −0.85 □: (Ⅱ) E_p −0.97

***p*-Acetamidobenzaldehyde Semicarbazone (IV)**——This semicarbazone (Ⅳ) showed a single reduction wave with k_D 5.6 at pH 0～6. The wave height decreased at pH 6～10 as shown in Fig. 4. The 2nd reduction wave appeared at $E_{1/2}$ −1.78 v. at pH above 9. The $E_{1/2}$-pH curve for the 1st wave was represented by $E_{1/2}=(-0.62-0.076\ \text{pH})$.

***p*-Acetamidobenzaldehyde (V)**——This aldehyde (Ⅴ) afforded 2 reduction waves with almost equal height (k_D 1.8) at about pH 4. The slope ($E_{1/4}-E_{3/4}$) was equal to 70 mv. These waves were ascribed to 2 steps of 1-electron reduction wave of the aldehyde. In strong acid solutions, only the 1st wave was observed because the 2nd wave merged into a hydrogen wave. At pH 5～9, the double waves overlapped together to give a 2-electron reduction wave with $k_D=3.7$ and $(E_{1/4}-E_{3/4})=30$ mv. At pH above 9, the wave height decreased (Fig. 4). The $E_{1/2}$ values for the 1st wave at pH 0.5～11 were shown by (−0.83−0.057 pH).

1-(*p*-Acetamidobenzyl)thiosemicarbazide (VI)——This compound (Ⅵ) obtained by a controlled potential electrolysis of thioacetazone, gave an anodic wave. The wave height was proportional to the concentration of Ⅵ. The k_D values were estimated at −5.3 in solutions of pH 4～12 and at −1.0 in *N* H_2SO_4. $E_{1/2}$ values at pH 0.3, 4.0, 6.8, 9.9 and 11.9 were +0.03, +0.03, −0.12, −0.32 and −0.47 v., respectively. $E_{1/2}$-pH curve at pH 5～12 was represented by the equation: $E_{1/2}=0.33-0.066\ \text{pH}$.

Acid solutions of Ⅵ showed an increase of cathodic current at more positive potential than the discharge potential of the buffer, although no distinct wave was observed. This current was assumed to be due to a catalytic hydrogen wave.

S-Methylisothiourea Hydroiodide (VII)——This salt showed a reduction wave with $E_{1/2}$ −1.66～−1.83 v. at pH 6～11. The wave height at pH 6.8 was proportional to the concentration in a range of 0.2～5 m*M*. Its k_D value was estimated at 5.3. At pH above 11, the reduction wave decreased rapidly and anodic waves appeared at $E_{1/2}$ −0.58 and −0.7 v. on the other hand. The total height of these anodic waves increased upto $k_D=3.1$. This change of polarogram was assumed to be due to a decomposition of S-methylisothiourea to methylmercaptane and urea. This salt gives another anodic wave of iodide ion with $E_{1/2}$ −0.06 v. and $k_D=4.4$ at pH 1～11.

S-Ethylisothiosemicarbazide Hydrobromide (VIII)——A solution of 1 m*M* S-ethylisothiosemicarbazide and 0.01% gelatin in 0.1*M* borax gave a reduction wave with $E_{1/2}$ −1.7 v. and k_D 8.3, and an anodic wave with $E_{1/2}$ −0.15 v. and k_D 8.3. The $E_{1/2}$ of the anodic wave at pH 6.8 and 12.9 were 0 v. and −0.27 v., respectively. Their heights remained constant at pH 6.8～12.9. The reduction wave in acid overlapped with the hydrogen wave. No reduction wave was observed at pH 12.9. Neutralizing the alkaline solution, the reduction wave appeared again. Then this compound was assumed to be more stable than S-methylisothiourea.

A. C. Polarography of Benzaldehyde Thiosemicarbazone (II)——Buffer solutions of pH 2.45 containing 0.04～1 m*M* (Ⅱ), 0.5*M* KNO_3 and 0.01% gelatin showed 2 peaks at −0.85 and −0.97 v. in A. C. polarography. The height was proportional to the concentration of Ⅱ as shown in Fig. 5. The slopes of the response curves were 60 and 260 μ mho/m*M* for the 1st and 2nd peaks, respectively.

Controlled Potential Electrolysis. Thioacetazone at pH 6.5——A solution containing 2.4 g. of thioacetazone, 75 ml. of *M* AcOH, 25 ml. of *M* NaOAc and 400 ml. of EtOH was electrolyzed by Hg cathode and Pt anode at −1.2 v. of the controlled cathode potential. The current became negligible after 3860 coulomb, 4*F* (Faraday) per mol., was charged.

Evaporating the resulting solution in vacuum, a colorless crystal (A) was obtained in an yield of 30%. After recrystallization from EtOH, it showed m.p. 212°. *Anal.* Calcd. for $C_{10}H_{14}N_4SO$(1-(*p*-acetamidobenzyl)thiosemicarbazide): C, 50.40; H, 5.92; N, 23.51; S, 13.45; CH_3CO, 18.06. Found: C, 50.79; H, 5.82; N, 23.50; S, 13.47; CH_3CO, 18.44. UV: $\lambda_{max}^{H_2O}$ 240 mμ (log ε 4.38). NMR: τ′ in Me_2SO: 7.97 (Ac), 6.19 (CH_2), 4.8 ($CH_2\underline{NH}$), 2.7 ($CSNH_2$), 2.72 d, 2.50 d (J=9 c.p.s. Ph), 1.42 (NNH), 0.20 (AcNH). Paper partition chromatography: ascending method, filter paper Tōyō No. 7, AcOH-BuOH-H_2O=1:5:4, 20°: Rf 0.53.

When the residual solution after the separation of A was acidified with HCl and evaporated to dryness and then extracted with EtOH, a hygroscopic crystal (B), m.p. 242°, was obtained in an yield of 40%. UV: $\lambda_{max}^{H_2O}$ 242.5 mμ ($E_{1\,cm}^{1\%}$ 6750) at pH 4. IR λ_{max}^{Nujol} μ: 3∼5 (NH_3^+), 5.90 (C=O), 6.02 (NH_2), 6.25 (aromatic), 6.58, 6.87……12.0 (*p*-disubstituted benzene). NMR: τ′ in Me_2SO: 7.93 (Ac), 6.06 (CH_2), 2.58 d, 2.39 d (J=9 c.p.s. Ph), 1.2 (NH_3^+), −0.56 (AcNH). Rf 0.46 (a distinct brown spot with the Ninhydrin reaction). pK 9.3 (aliphatic amine). Polarography: No reduction wave was obtained at pH 4∼12. An anodic wave with $E_{1/2}$ −0.26 v. and k_D 0.27 was observed at pH 11.9. It was assumed from these facts that B consisted of *p*-acetamidobenzylamine hydrochloride.

Evaporating the residual solution after the separation of B, a hygroscopic yellow crystal (C), m.p. (decomp.) 170∼180°, was obtained in an yield of 30%. NMR: τ′ in Me_2SO: 6.03 (CH_2), 5.1 (NH_3^+), 1.58 (NH_3^+), 2.75 d, 2.40 d (J=9 c.p.s. Ph). pK 9.3 (aliphatic amine), 4.0 (aromatic amine). Rf 0.14 (a distinct orange spot with the Ninhydrin reaction). It was assumed from these facts that C contained *p*-aminobenzylamine dihydrochloride.

A sulfur containing compound in the fraction (C) was detected as a colorless spot of Rf 0.47 by the $NaN_3 \cdot I_2$ reaction. The same compound was also detected in the residual crystal containing NaCl extracted with EtOH. These compounds were identified with thiourea by IR spectra: λ_{max}^{KBr} μ: 3∼, 6.2, 6.75, 7.0, 9.15, 13.64.

Thioacetazone at pH 8.3——A solution containing 2.4 g. of thioacetazone, 200 ml. of EtOH, and 100 ml. of 0.1*N* NH_4Cl and NH_3 was electrolyzed at −1.8 v. to consume 3.3*F*/mol. From the resulting solution, *p*-acetamidobenzylamine (60%), thiourea (30%), and 1-(*p*-acetamidobenzyl)thiosemicarbazide (40%) were obtained by the manner mentioned above.

Thioacetazone at pH 11.5——When an alkaline solution of thioacetazone (2.4 g. in 200 ml. of EtOH and 100 ml. of 12% NH_3) was electrolyzed at −2.0 v. to consume 2.9*F*/mol., 1-(*p*-acetamidobenzyl)thiosemicarbazide (75%), *p*-acetamidobenzylamine (20%) and thiourea (20%) were obtained.

S-Methylthioacetazone at pH 6.5——A solution containing 1.65 g. of S-methylthioacetazone, 50 ml. of *M* AcOH, 30 ml. of *M* NaOAc, and 150 ml. of EtOH was electrolyzed at −1.2 v. to consume 8*F*/mol.

Table I.

		I	II	III	IV	V	VI	VII	VIII	IX
mol. wt.		236	179	250	220	163	238	90	120	91
D	10^{-6} cm² sec^{-1}	5.8	6.7	5.6	6.0	7.0	5.8	9.3	8.1	9.2
k_D	μA. mM^{-1} mg$^{-2/3}$ sec$^{1/2}$	5.9	6.1	5.6	5.6	3.7	(−5.3)	(5.3)	(8.3)	(−8.2)
n	in acid (or in alkali)	4.0	3.9	3.9	3.8	2.3	(3.6)	(2.8)	(5.1)	(4.5)
pK′	(polarography)	7.5	6.1	10.4	7.8					
pK	(spectroscopy)	−1.1 11.52	−1.0 11.25	5.77	−1.2 13.0			9.6	7.8	1.7
log *k*	M^{-1} sec^{-1}	13.3	12.1	14.3	15.8					
i_a	-μA.	0.5	0.2	1.2						
m	mg. sec^{-1}	1.65	0.607	7.097	0.826	0.794	0.823	0.834	0.834	0.607
t	sec	3.30	4.66	2.83	4.11	4.25	5.03	5.87	5.87	4.66
Area	Å²	26	29	28						
$E_{1/2}$	at pH 4 v *vs.* N.C.E.	−0.96 −1.03	−0.96 −1.03	−0.91	−0.92	−1.06	+0.03			+0.01
	at pH 10	(−.34) −1.74	(−1.7)	−1.32	(−1.78)	−1.40	−0.33	−1.76	−1.7	−0.44
	at pH 0	−0.66 −0.73	−0.66 −0.73	−0.72	−0.62	−0.86	+0.03			0.0

Polarographic Behavior of Thioacetazone (I), Benzaldehyde Thiosemicarbazone (II), S-Methylthioacetazone (III), *p*-Acetamidobenzaldehyde Semicarbazone (IV), *p*-Acetamidobenzaldehyde (V), 1-(*p*-Acetamidobenzyl)thiosemicarbazide (VI), S-Methylisothiourea (VII), S-Ethylisothiosemicarbazide (VIII) and Thiosemicarbazide (IX).

Evaporating the resulting solution, any substance insoluble in H_2O was not obtained. In the extract of the residual solid with EtOH, *p*-acetamidobenzylamine and *p*-aminobenzylamine were detected by the paper chromatography and the alkali titration.

1-(*p*-Acetamidobenzyl)thiosemicarbazide at pH 6.5——A solution of this compound (0.3 g. in 100 ml. of EtOH and 20 ml. of acetate buffer of pH 4) was electrolyzed at −1.8 v. to consume 270 coulomb. The original compound was recovered entirely from the resulting solution.

S-Methylisothiourea Hydroiodide at pH 8——A solution of this salt in borate buffer (1.3 g./100 ml.) was electrolyzed at −1.8 v. to consume 6*F*/mol. From a extract of the resulting solution with $CHCl_3$, 0.5 g. of tris(methylthiomethyl)amine was obtained. *Anal.* Calcd. for $C_6H_{15}NS_3$: C, 36.51; H, 7.66; N, 7.10; S, 48.73. Found : C, 36.15; H, 7.44; N, 6.78; S, 47.90. IR $\lambda_{\max}^{\text{liq.}}$ μ : 3.36, 3.44, 3.54 (ν_{CH}), 7.00 (δ_{CH_2}), 7.50 (δ_{CH_3}), 7.88, 9.10, 9.17 (ν_{CN}), 10.20, 11.20, 13.20 (ν_{CS}). NMR : τ in $CDCl_3$: 7.87 (CH_3S), 5.95 (SCH_2N). The Ninhydrin and the $NaN_3 \cdot I_2$ reactions were positive for this compound.

The aqueous layer extracted with $CHCl_3$ was distilled with KOH. The distillate was collected in *N* HCl. Evaporating the acid solution, 0.3 g. of NH_4Cl was obtained.

Discussion

Kinetic Current

The relationships between pH and the reduction wave height of thiosemicarbazones resemble the dissociation curves of acids and bases as shown in Figs. 2 and 4. However the pK′ values estimated by the pH value, in which the wave height is a half of maximum height, are smaller than pK values of the thiol groups and larger than those of the bases, whose pK values are estimated by spectrophotometry. A similar phenomenon is observed in the case of S-methylthioacetazone and *p*-acetamidobenzaldehyde semicarbazone which have no thiol group. The limiting current has characters of kinetic currents in the pH range in which the current is sensitive to pH. It was revealed from these facts that a protonation of the base on the electrode precedes an electron transfer in acid media. The rate constants (*k*) of protonation were evaluated from the difference between pK′ and pK according to Koutecky's equation:[5] $\log k = 2(\text{pK}' - 0.2) - \text{pK} - \log t$ where *t* is drop time (Table I). In acid media, the rate of protonation will be far larger than that of electron transfer, so that the limiting current is controlled by a diffusion. In neutral and alkali media, the limiting current has natures of kinetic currents because both rates will become comparable.

The round maximum at pH above 7 can not be suppressed by gelatin and the height does not exceed the diffusion currents. Similar features were often observed in kinetic currents.[*4] These phenomena will be ascribed not to a catalytic current or an ordinary maximum wave but to a decrease of kinetic current. Further discussion in detail is not presented here.

Cathodic Waves of Thiosemicarbazones

Thiosemicarbazones and semicarbazones described in this paper show similar reduction waves, but thiosemicarbazides give no reduction wave. It is easily assumed that the reduction takes place in the -CH=NNH- group.

Ordinarily diffusion constant (*D*) is calculated by the equation : $D = 8.85 \cdot 10^{-5} M^{-1/2}$ ($cm^2 \cdot sec^{-1}$) where *M* is molecular weight, so that *D* value of thioacetazone is estimated at $5.8 \cdot 10^{-6}$. The number (*n*) of electron required for the reduction of a molecule of thioacetazone was estimated at 2 for each wave of 3 steps according to Ilkovič equation : $n = k_D/(607\, D^{1/2})$ where k_D is diffusion current constant ($\mu A.\ mM^{-1}\ mg^{-2/3}\ sec^{1/2}$). The same *n* value was also revealed from the fact that the slope of each wave, $\Delta E/\Delta \log [i/(i_d - i)]$: −0.03 v., was nearly equal to the theoretical value ($RT/2F$). Since benzaldehyde thiosemicarbazone shows A.C. polarograms, the electrode process is assumed to be reversible.

[*6] For example : J. Korta : "Advances in Polarography" I. 359 (1960). Pergamon Press, London.

[5] J. Koutecký, R. Brdička : Collection Czechoslov. Chem. Communs., **12**, 337 (1947).

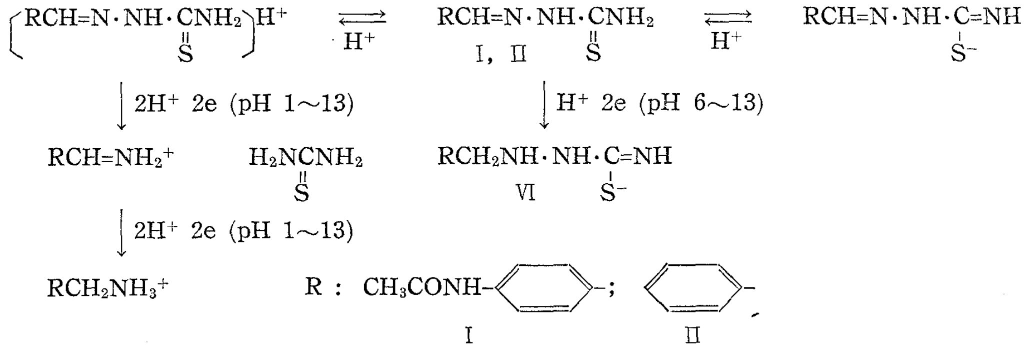
The numbers of proton (m) for the reversible reduction of a molecule can be calculated by the equation : $\Delta E_{1/2}/\Delta \text{pH} = mRT/nF$. The m values of the first, second and third waves of thioacetazone were estimated at 2, 2 and 1, respectively.

It has been reported by Lund[6] that semicarbazones, phenylhydrazones and oximes in acid solutions consume 4 electron per molecule and a reductive splitting of the N–O or N–N bond precedes a saturation of the $\rangle$C=N– group while ketimines and aniles undergo 2-electron to the corresponding secondary amines. Gardner[7] and Φsterud[8] have also described that reduction waves of oximes are ascribed to the 4-electron reduction of the cations to the amines after the oximes were protonated on the electrode.

These reduction processes of semicarbazones and oximes will be also prospected in the case of thiosemicarbazones. As mentioned above, the reduction waves of thioacetazone in acid media are assumed to be due to 4-electron reduction of the -CH=NNH- group. If the saturation of the -CH=N- group would take place at the first step, the reductive splitting of the N-N bond will not occur at the second step because 1-(*p*-acetamidobenzyl)thiosemicarbazide formed by 2-electron reduction of thioacetazone does not undergo further reduction. Therefore it is appropriate to consider that the reductive cleavage of the N-N bond precedes the saturation of the azomethine.

The consideration for kinetic current has suggested that the reduction waves in acid media are ascribed to the reduction of the cation. The first reduction wave of thioacetazone is considered to be due to 2-electron reduction of the cation to *p*-acetamidobenzylidene immonium ion ($RCH{=}NH_2^+$) and thiourea. The second step is ascribed to the 2-electron of the ketimine to the benzylamine ($RCH_2NH_3^+$).

The reduction wave of thioacetazone in alkaline media is assumed to be due to 2-electron reduction of the azomethine group of free base as postulated by Dusinsky.[1]



In the course of controlled potential electrolysis of thioacetazone, 1-(*p*-acetamidobenzyl)thiosemicarbazide (A), *p*-acetamidobenzylamine (B), *p*-aminobenzylamine (C), and thiourea were obtained. A was produced by 2-electron reduction of thioacetazone while B and thiourea were formed by 4-electron reduction, and C would be formed by acid hydrolysis of B. A was a major product in alkaline media while it was a minor one in acid media. Presence of the 2 steps of reduction waves in alkaline media suggests that the reduction products in alkaline media (pH 8,3 and 11.5) at −1.8~−2.0 v. will be mainly B and thiourea with a small amount of A. The results in the electrolysis were qualitatively agreed with this suggestion. The major product on the electrolysis in acid media at −1.2 v. is the same expected by polarography. The formation of a minor product (A) can not be understood with the polarographic behaviors. This

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6) H. Lund : Acta Chem. Scand., **13**, 249 (1959).
7) H. J. Gardner, W. P. Georgans : J. Chem. Soc., **1956**, 4180.
8) Th. ϕsterud, M. Prytz : Acta Chem. Scand., **10**, 451 (1956).

discrepancy may be caused by the differences between polarography and the electrolysis of concentrated solutions on the mercury pool electrode.

Cathodic Waves of S-Methylthioacetazone

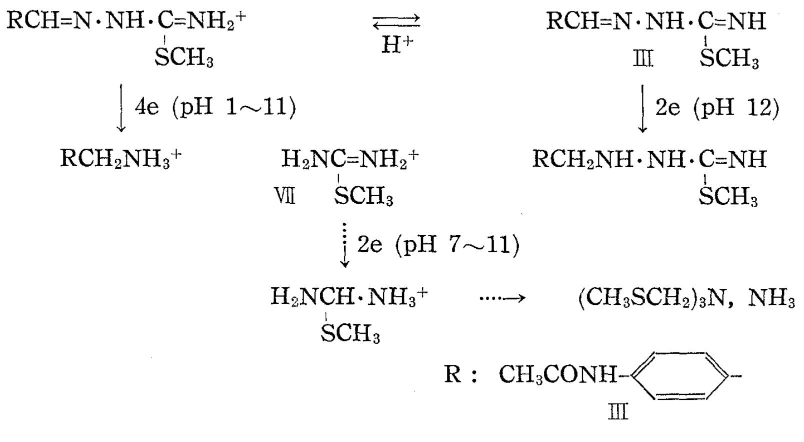
The first reduction wave of S-methylthioacetazone has a diffusion current constant similar to the total wave height of thioacetazone. This reduction is assumed to consume 4-electrons per molecule. In the course of controlled potential electrolysis of S-methylthioacetazone at pH 6.5 *p*-acetamidobenzylamine was obtained. The electrode reaction, therefore, is considered to be a cleavage of the N-N bond and a saturation of the azomethine as similar as that of thioacetazone.

The second reduction wave of S-methylthioacetazone at pH 7~10 resembles the reduction waves of S-methylisothiourea and S-ethylisothiosemicarbazide. These waves are, therefore, assumed to be due to the reduction of S-alkylisothiourea group.

The k_D value for the second wave of S-methylthioacetazone was about a half of that for its first wave. The n value for the second wave was assumed to be 2.

The electrolysis of S-methylthioacetazone failed to isolate S-containing compounds and consumed much amounts of electricity. On the electrolysis of S-methylisothiourea, tris(methylthiomethyl)amine and ammonium chloride were obtained. Methyl diaminomethyl sulfide postulated as the 2-electron reduction product, may be too unstable to isolate. Complicate decompositions and additional reductions might be occur in these electrolyses. Then these polarographic reduction processes could not be confirmed by the electrolyses.

The $E_{1/2}$ value of S-methylthioacetazone at pH about 12 resembles also that of thioacetazone. The reduction wave of S-methylthioacetazone is observed, as shown Fig. 3, even in a strong alkaline solution (pH 12) in which S-methylisothiourea and S-ethylisothiosemicarbazide fail to give the reduction wave. Therefore the wave at pH 12 may be ascribed the reduction of the benzylidene-imino group in the free base as similar as thioacetazone.



Anodic Waves

It has been previously reported[4] that thiosemicarbazide reacts with 1 mole of mercurous or silver ion in acid media and 4 mole in alkaline media. The anodic wave of S-ethylisothiosemicarbazide in alkali will be also ascribed to the reaction with mercurous ion which forms in an anodic dissolution of the mercury electrode. The anodic waves of 1-(*p*-acetamidobenzyl)thiosemicarbazide resemble those of thiosemicarbazide, so that their electrode reactions are assumed to be similar.

Thiosemicarbazones such as thioacetazone, S-methylthioacetazone, and benzaldehyde thiosemicarbazone give a small anodic wave. It has been described by

Haugas[9] that thiosemicarbazones give complexes such as $RCH=N\cdot N=C(SAg)NH_2\cdot AgNO_3$. Then the anodic waves of thiosemicarbazones are considered to be due to the formation of mercurous complexes on the mercury electrode. The complexes will be adsorbed on the mercury surface to disturb further reaction, so that the wave height is limited to a small value in a higher concentration. The surface area of electrode occupied by a molecule of the complex was estimated from the limiting current according to Brdička,[10] and resembles the cross-sectional area of molecule. It is cunsidered from these facts that the mercurous complex forms a mono molecular layer on the electrode.

Analytical Application

A polarographic determination of thioacetazone in a tablet has been reported by Icha[3] who recommends an acetate buffer of pH 4.7 and ethanol as a solvent. The other buffers at pH below 5 may be utilized since the wave height is independent of pH and largest in this condition. As a maximum suppressor, 0.01% gelatin may be used. In this condition, the determination of thioacetazone is not disturbed by thiosemicarbazide. Thioacetazone and *p*-acetamidobenzaldehyde can be separately determined in *N* hydrochloric acid or borate buffer of pH 10. Acid hydrolysis of thioacetazone can be followed by this method and the kinetic study will be reported in the other paper.

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Summary

Thioacetazone gives 2 steps of 2-electron reduction waves in acid media, which are ascribed to a reductive cleavage of the N-N bond in the protonated thioacetazone and a saturation of the -CH=N- group, respectively. Precedence of the protonation of base on the electrode causes in a kinetic current. In alkaline media, thioacetazone gives other reduction wave due to a 2-electron reduction of the -CH=N- group in the free base, and an anodic wave which are ascribed to the formation of a mercurous complex on the electrode.

The reduction waves of benzaldehyde thiosemicarbazone and *p*-acetamidobenzaldehyde semicarbazone and the first reduction wave of S-methylthioacetazone are also ascribed to the reaction as similar as that of thioacetazone.

The second reduction wave of S-methylthioacetazone as well as the reduction waves of S-methylisothiourea and S-ethylisothiosemicarbazide are assumed to be due to the reduction of the $RS\overset{|}{C}=NH$ group.

p-Acetamidobenzaldehyde shows 2 steps of 1-electron reduction waves at pH about 4, which overlap together and diminish at higher pH.

Thiosemicarbazides in alkali give an anodic wave due to a reaction with mercurous ion.

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[9] E. A. Haugas, B. W. Mitchell : J. Pharm. and Pharmacol., **10**, 451 (1956).

[10] R. Brdička : Z. Elektrochem., **48**, 778 (1942).