They said that " recent findings suggest that the receptor sites on blood vessels for the pressor action of the vasopressin are different from the receptors for the vasopressin and oxytocin on uterus and myoepithelial cells." This view agrees with our results.

The authors are indebted to Professor Keijiro Takagi of the University of Tokyo for his advices and encouragement.

Summary

Carbobenzoxylated peptides containing tyrosine inhibited competitively the action of oxytocin on the isolated rat uterus. Among these peptides carbobenzoxy-L-tyrosyl-L-tyrosinate was the most active and 30.4 times as potent as *p*-nitrophenol on the molar basis.

This peptide had non-competitive inhibitions to ACh and barium chloride, but had more active and competitive antagonism to oxytocin when assayed by using their dose-response curves.

On the other hand, it had no inhibition to the avian depressure by oxytocin and to the raising blood pressure by vasopressin.

Di-carbobenzoxy-L-cystinyl-di-L-tyrosine ethyl ester, having a low activity for inhibition of contraction by oxytocin, inhibited about 50 per cent of avian depressure by oxytocin, and almost inhibited the raising blood pressure by vasopressin in dog and rabbit.

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124. **Masaichiro Masui and Hidenobu Ohmori**: Studies on Girard Hydrazones. II.[*1] Polarographic Reduction Mechanisms of Girard Hydrazones.[*2]

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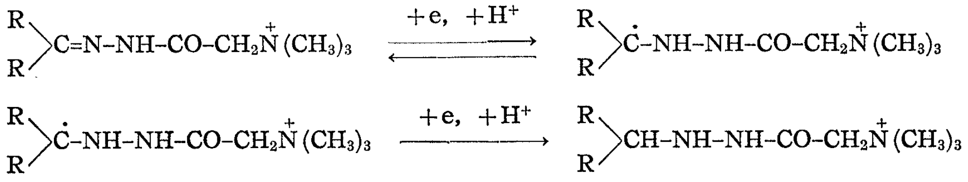
A polarographic reduction process for the Girard-T hydrazones of some aliphatic and alicyclic ketones was proposed by Prelog and Häfliger[1)] to be that shown by equation (1), which was supported by Young.[2)]



$$\begin{gathered}\begin{matrix}\mathrm{R}\\\mathrm{R}\end{matrix}\!\!>\mathrm{C{=}N\text{-}NH\text{-}CO\text{-}CH_2\overset{+}{N}(CH_3)_3}\ \overset{+e,\ +H^+}{\rightleftarrows}\ \begin{matrix}\mathrm{R}\\\mathrm{R}\end{matrix}\!\!>\mathrm{\dot{C}\text{-}NH\text{-}NH\text{-}CO\text{-}CH_2\overset{+}{N}(CH_3)_3} \\ \begin{matrix}\mathrm{R}\\\mathrm{R}\end{matrix}\!\!>\mathrm{\dot{C}\text{-}NH\text{-}NH\text{-}CO\text{-}CH_2\overset{+}{N}(CH_3)_3}\ \xrightarrow{+e,\ +H^+}\ \begin{matrix}\mathrm{R}\\\mathrm{R}\end{matrix}\!\!>\mathrm{CH\text{-}NH\text{-}NH\text{-}CO\text{-}CH_2\overset{+}{N}(CH_3)_3}\end{gathered} \quad (1)$$

[*1] Part I. J. Chem. Soc. in press.

[*2] This paper was read at the 10th Symposium on Polarography in Nagoya, Japan, November 1963.

[*3] Hotarugaike, Toyonaka-shi, Osaka-fu (桝井雅一郎, 大森秀信).

1) V. Prelog, O. Häfliger: Helv. Chim. Acta, **32**, 2088 (1949).

2) J.R. Young: J. Chem. Soc., **1955**, 1516.

This equation was derived from the similar polarographic behaviour of a Girard-T hydrazone to that of an aromatic carbonyl compound.[1] Lund,[3] on the other hand, reported that the polarographic reduction of the compound such as $^{R}_{R'}\rangle C=N-Y-R''$ consumes two electrons per molecule to yield a saturated compound when Y is carbon, but a N-Y bond fission occurs in acid solutions when Y is nitrogen or oxygen, and the fission takes place generally in advance of the saturation. The diffusion current constant of cyclopentanone Girard-T hydrazone in acid solution is quite similar to that of an aldose semicarbazone which is reported of a four-electron reduction.[4] From these two findings, the process (1) proposed for the hydrazone appears to be quite doubtful.

In the present study, we have tried to resolve the polarographic reduction mechanisms for the Girard hydrazones of aliphatic and aromatic ketones. Cyclopentanone and benzophenone were selected as the examples of aliphatic and aromatic ketones respectively, and the study was carried out on Girard-T and Girard-D hydrazones of both ketones.

Experimental and Details

Materials——Girard reagent T, Merck R.G., dried under reduced pressure at room temperature was used without further purification. Girard reagent D was prepared by the known method[5]; m.p. 181°, giving correct analysis. Girard reagent P and semicarbazide hydrochloride were of reagent grade. Cyclopentanone and other ketones were purified by distillation or recrystallization before use. Compounds used for buffer solutions were also of reagent grade and used without further purification.

Buffer Solutions——Britton-Robinson buffer solutions containing $0.1M$ KCl were used for the polarographic and spectrophotometric studies. For the large scale controlled potential electrolysis, those prepared by mixing two solutions (buffer I and II) shown in Table I were used. The polarograms of the compounds studied in these solutions were essentially analogous to those obtained in the Britton-Robinson buffer solutions.

Table I. Buffer Solutions used for the Large Scale Controlled Potential Electrolysis

pH	Buffer I	Buffer II	Ethanol content (%)
1.6	$0.1M$ HCl	$0.2M$ KCl	10
2.0	$0.1N$ H_3PO_4 $0.1M$ KCl		10
6.6	$0.1M$ KH_2PO_4 $0.1M$ KCl	$0.05M$ $Na_2B_4O_7$ $0.1M$ KCl	10
7.0~7.7	$0.2M$ Na_2HPO_4 $0.1M$ KCl	$0.2M$ KH_2PO_4 $0.1M$ KCl	10
11.2	$0.1M$ Na_2HPO_4 $0.05M$ KCl	$0.1M$ NaOH $0.05M$ KCl	10
	$0.2M$ NaOH	$0.2M$ HBO_2 $0.2M$ KCl	50
12.0	$0.1M$ Na_2HPO_4 $0.05M$ KCl	$0.1M$ NaOH $0.05M$ KCl	10

Preparation of Girard-T and Girard-D Hydrazones——About 1 g. of Girard-T or D reagent was refluxed with a carbonyl compound of 2~3 times the mole equivalent in about 10 ml. EtOH until no precipitate appeared when the mixture was cooled. Evaporation of the volatile components under reduced pressure and extraction of the unreacted ketone with Et_2O gave a Girard hydrazone as a colorless crystalline solid, which was recrystallized from EtOH. The Girard hydrazones prepared by this method gave correct analysis. The melting points were; cyclopentanone Girard-T hydrazone, 187°

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3) H. Lund: Acta Chem. Scand., 13, 249 (1959).
4) J.W. Haas, J.D. Storay: Anal. Chem., 34, 145 (1962).
5) M. Viscontini, J. Meier: Helv. Chim. Acta, 33, 1773 (1950).

(decomp.); cyclopentanone Girard-D hydrazone, 202~204°; acetophenone Girard-T hydrazone, 133°; acetophenone Girard-D hydrazone, 185~186°; benzophenone Girard-T hydrazone 185°; benzophenone Girard-D hydrazone, 208~210° (decomp.).

Apparatus——Polarograms were obtained with a Yanagimoto Potential Scanner AP-1 and a Galvarecorder GR-3. The capillary constant of the dropping mercury electrode used was $m^{2/3}t^{1/6}=1.016$ at an open circuit. A Toadempa HM-5 pH-meter with glass-s.c.e. was used for pH measurements. A Hitachi Recording EPS-2 Spectrophotometer was used for UV spectroscopy. IR spectra were obtained by using a Hitachi EPI-2 Spectrophotometer with the salt prisms. A modified Yanagimoto Potentiostat type VE-model 3 with a Yokogawa DC ammeter MPF, class 0.5, was used for large scale electrolysis.

Polarography of Girard Hydrazones——Mixing 0.5 ml. of a stock solution, which was $5\times10^{-3}M$ in a Girard hydrazone in EtOH, with 4.5 ml. of a buffer solution and the solution was deaerated for 3 min. with N_2. When it passed 4 min. after preparing the sample solution, the current-voltage curve was obtained at $25\pm0.1°$. Time zero was taken at the point where half of the pipetting solution was introduced into the buffer. The time lag between the start of the recorder and the time recording the limitting current was corrected. All potentials shown are those against a saturated caromel electrode. 0.004% of Triton X-100 was added to the solution as a maximum suppressor. The pH of the solution was measured at the end of the polarography. The polarographic behaviours of cyclopentanone Girard-T and Girard-D hydrazone are shown in Fig. 1. The wave height in acid solution are of that extrapolated to time zero, because of their noticeable hydrolysis.*[1] Above pH 8 the rate of the hydrolysis is negligibly small for ordinary polarographic measurements. The polarographic behaviours of the above compounds and of other Girard-T hydrazones of aliphatic ketones, except those containing conjugate double bond, are essentially the same as those reported by Prelog and Höfliger.[1)]

The relation between wave height and mercury column height was examined by the log *i vs.* log *h* plot on cyclopentanone Girard-T hydrazone, and the slope of 0.575 and 0.475 were obtained at pH 7 and 11 respectively; poorer lineality was observed at pH 11. The diffusion current constants of cyclopentanone Girard-T and Girard-D hydrazone are compared with that of aldose semicarbazones[4)] (Table II).

Polarographic behaviours of benzophenone Girard-T and Girard-D hydrazone are shown in Fig. 2

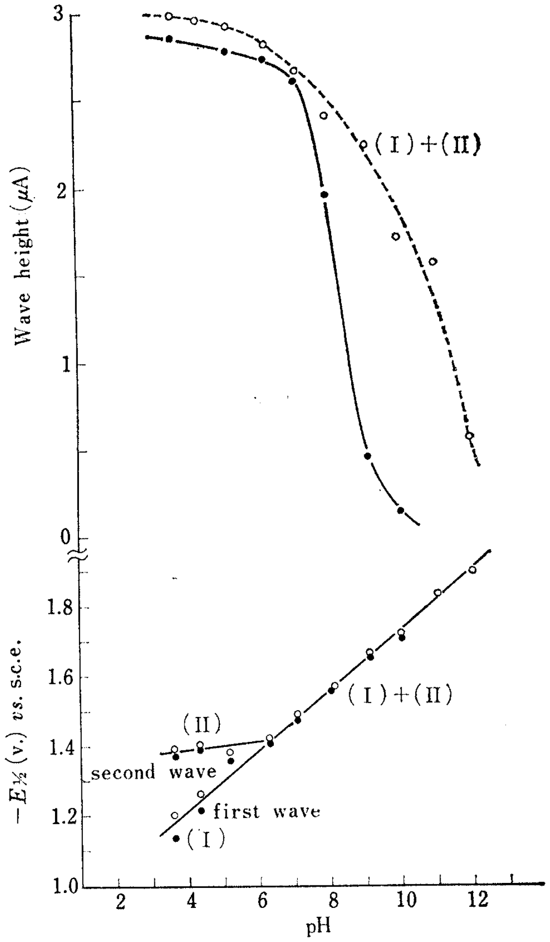

Fig. 1. Variation of Total Wave Height and $E_{1/2}$ with pH for Cyclopentanone Girard-T (open circle) and Girard-D (solid circle) Hydrazone

(I) and (II) indicate the corresponding reactions in the scheme (4).

The $E_{1/2}$ value at pH 5.2 was taken as that of a combined wave, but if the wave is divided into two equal parts, the $E_{1/2}$ values for each wave are on the solid line.

Table II. Diffusion Current Constants of Several Aldose Semicarbazones and Cyclopentanone Girard-T and Girard-D hydrazone

Compd.		$I_d=i_d/Cm^{2/3}t^{1/6}$	pH
Ribose Arabinose Xylose Lyxose	Semicarbazone	6.17~6.30	1.60
Cyclopentanone			
Girard-T hydrazone		5.91	3.25
Girard-D hydrazone		5.66	3.30

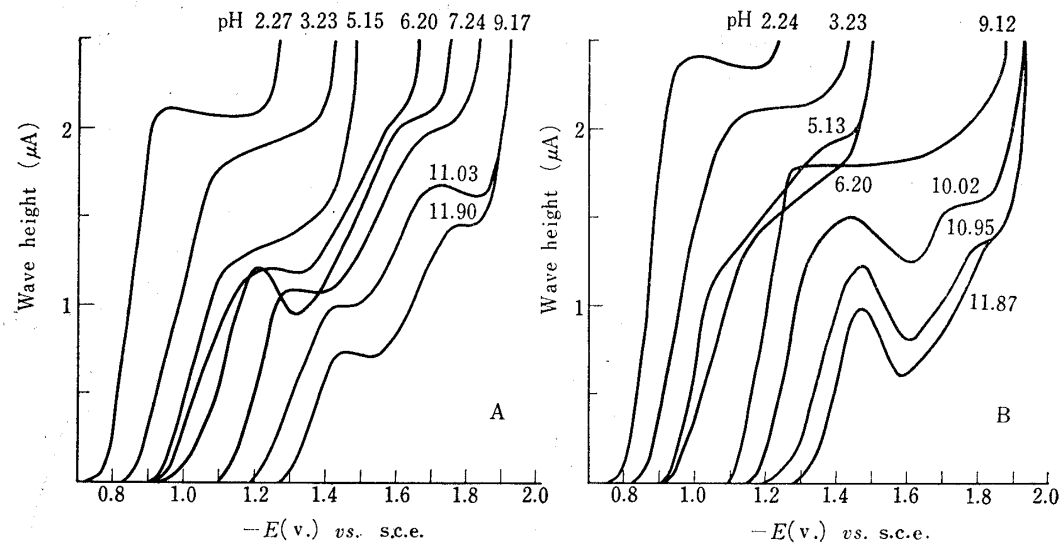

Fig. 2. Polarograms of A : Benzophenone Girard-T Hydrazone, B : Benzophenone Girard-D Hydrazone

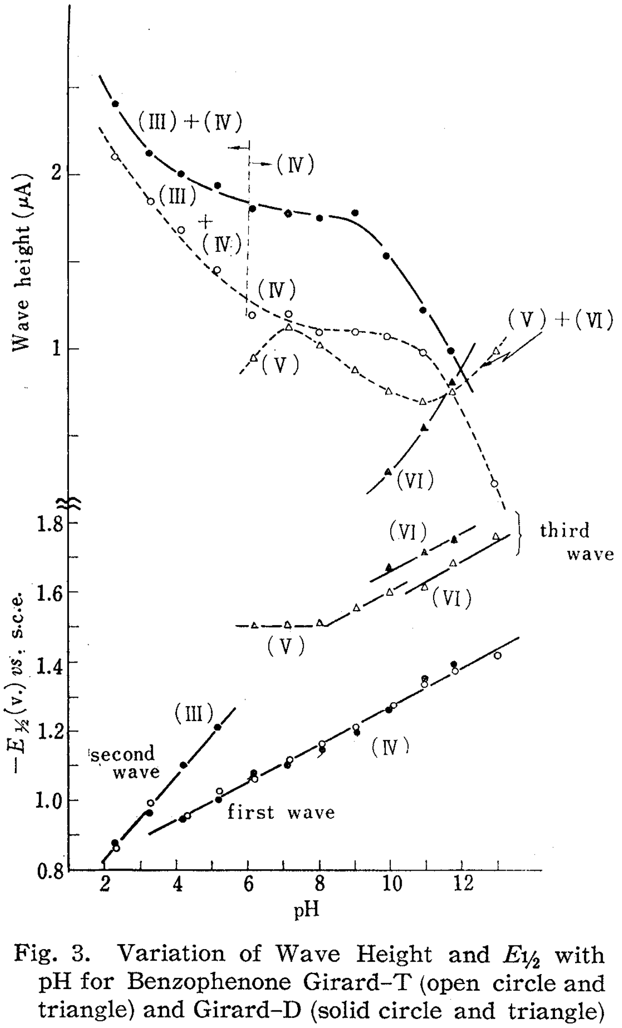

Fig. 3. Variation of Wave Height and $E_{1/2}$ with pH for Benzophenone Girard-T (open circle and triangle) and Girard-D (solid circle and triangle) Hydrazone

(III), (IV), (V), and (VI) indicate the corresponding reactions in the scheme (6).

and 3. In this case, the rate of hydrolysis is negligibly small for ordinary polarographic procedures. Somewhat different polarographic behaviours are noticed between the Girard hydrazones of aliphatic and aromatic ketones.

In the case of the aliphatic series (Fig. 1), two waves are observed in acid solutions, instead only one in aromatic series, and as the pH-dependency of $E_{1/2}$ for the second wave is smaller than that for the first, the latter merges with the former as pH increases. The pH dependency of the first and the combined waves in acid solutions is $-80\sim-100$ mv./pH.

In the case of benzophenone Girard hydrazones (Figs. 2 and 3), only one wave is observed in very acid solutions, but it can appear as two separately discernible waves as pH increases. Contrary to the aliphatic series, the pH-dependency of the $E_{1/2}$ for the second wave, -100 mv./pH, is larger than that for the first wave, $-57\sim-60$ mv./pH, and the second wave becomes smaller with increasing pH value, being disappeared at higher pH region. In neutral and alkaline solutions, a third wave which is not observed in the aliphatic series is developed. As the half-wave potential of the third wave is very negative, only the Girard hydrazones of benzophenone and of benzaldehyde gave a defined wave, but others studied gave only hardly visible or no third wave.

Controlled Potential Electrolysis——Base solutions were deaerated by N_2 and electrolyzed prior to the addition of the weighed sample.

Cyclopentanone Girard-T Hydrazone——202.5 mg. of the hydrazone was dissolved in a base solution (180 ml. of buffer solution of pH 7.6+20 ml. of EtOH), and electrolyzed at -1.6 v. for 80 min. at room temperature. The resulting solution lost the polarographic wave of the hydrazone and consumed 332 coulombs, which corresponds to n=3.97, where n is the number of electrons required per molecule. After being made alkaline with NaOH, the solution was extracted with Et_2O, then dry HCl gas was introduced into the extracts. The colorless precipitate formed was recrystallized from EtOH to give colorless needles (21 mg.), which were identical in IR spectrum with cyclopentylamine hydrochloride prepared by a known method;[6] m.p. 210°; IR λ_{max}^{Nujol} μ: 3.5~5, 6.22, 6.65 (NH_3^+). The low yield of the amine was mainly due to the loss accompanied with the treatment of the small amount of the sample in a dilute solution.

Benzophenone Girard-T Hydrazone——Essentially the same procedure as described above was applied to 101.1 mg. of the hydrazone at pH 1.6, and colorless crystalls (30 mg.) were obtained. From their melting point, 269°, and the results of the elemental analysis, they were found to be benzhydrylamine hydrochloride. IR λ_{max}^{Nujol} μ: 3.5~5 (NH_3^+), 6.2~6.7 (NH_3^+ and aromatic), 13~14.5 (aromatic).

Benzophenone Girard-D Hydrazone——In this case, from 315.6 mg. of the sample electrolyzed at pH 6.6, 176.2 mg. of a white crude product was obtained by the similar procedure as above. Colorless needles were yielded by recrystallization from EtOH, from which benzhydrylamine hydrochloride was sublimed at 140° (4 mm. Hg). The residue is mainly composed of 1-diphenylmethyl-2-dimethylaminoacetylhydrazine hydrochloride. At pH 11.2, 465.6 mg. of the hydrazone consumed electricity corresponding to n=2.5. The resulted solution was made to pH 2 by adding HCl, and evaporated to dryness. From the ethanoholic extract of the residue, colorless crystals (370 mg.) was obtained by evaporation at reduced pressure; m.p. 213° (decomp.) (from EtOH and Et_2O). This was confirmed as 1-diphenylmethyl-2-dimethylaminoacetylhydrazine hydrochloride by elemental analysis and its IR spectrum. *Anal.* Calcd. for $C_{17}H_{22}ON_3Cl$: C, 63.84; H, 6.93; N, 13.14. Found: C, 64.07; H, 6.98; N, 12.90. IR λ_{max}^{KBr} μ: 3.05, 3.19, 3.33 (-NH-), 3.8~4.4 ($-NH^+-$), 5.98, 6.53 (-NH-CO-), 7.23 ($-CO-CH_2-$ or $-CH_2-N^+-$), 13~14.5 (aromatic).

This compound is not stable in EtOH, and seems to decompose to benzhydrylhydrazine and N,N-dimethylglycine ethylester hydrochloride, m.p. 88~89°. Hence it is suitable to use dioxane instead of EtOH in the large scale electrolysis. We got a better yield of this compound from the electrolysis carried out in the mixture of dioxane and phosphate buffer at pH 11.7. This compound gave no polarographic reduction wave under all experimental conditions. All results of the macro scale electrolysis carried out at various conditions are summarized in Table III.

TABLE III. Results of Controlled Potential Electrolysis

Compds.	pH	Applied voltage (v. *vs.* s.c.e.)	n Value Found	Amount of sample (mg./200 ml.)
Cyclopentanone Girard-T hydrazone	7.7	−1.60	3.97[a]	202.5[c]
	11.2	−1.80	4.2	55.3
Cyclopentanone Girard-D hydrazone	7.0	−1.53	4.3	97.2
Benzophenone Girard-T hydrazone	1.6	−1.10	3.7	101.1[c]
	2.0	−1.10	3.3[b]	126.4
	7.3	−1.20[d]	2.2	108.0
	7.3	−1.55[e]	3.0	123.7
	12.0	−1.40[d]	2.4	84.5
	12.0	−1.85[e]	2.5	81.5
Benzophenone Girard-D hydrazone	6.6	−1.25	3.5	315.6[c]
	11.2	−1.80	2.5	465.6[c]

a), b) In order to examine the reproducibility of the coulometry several runs were carried out under the same conditions but the concentration of the sample, $2\sim5\times10^{-3}M$, and 4±0.3 and 3.3±0.1 were obtained for (a) and (b) respectively.

c) Products were isolated from the electrolyzed solution to confirm the process.

d) This potential is that on the plateau of the first wave. When the solution was electrolyzed, the second wave was also disappeared.

e) This potential is that on the plateau of the second wave.

Ultraviolet Absorption Spectra of the Girard Hydrazones in Buffer Solutions——Buffer solutions used were the same as those used for the polarographic study. Owing to the large extinction coefficient of the hydrazones (10,000~20,000), the spectra were measured on $5\times10^{-5}M$ solution. The spectra are shown in Figs. 4, 5, 6, and 7.

Determination of pK_a'——About $1\times10^{-2}M$ solutions of cyclopentanone Girard-T and Girard-D hydrazone were titrated with $0.1M$ NaOH standard solution potentiometrically, and the values of 9.48 and 7.47 were found from the titration curves for the above compounds respectively.

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6) J. Wislicenus, W. Hentschel: Ann., **275**, 325 (1893).

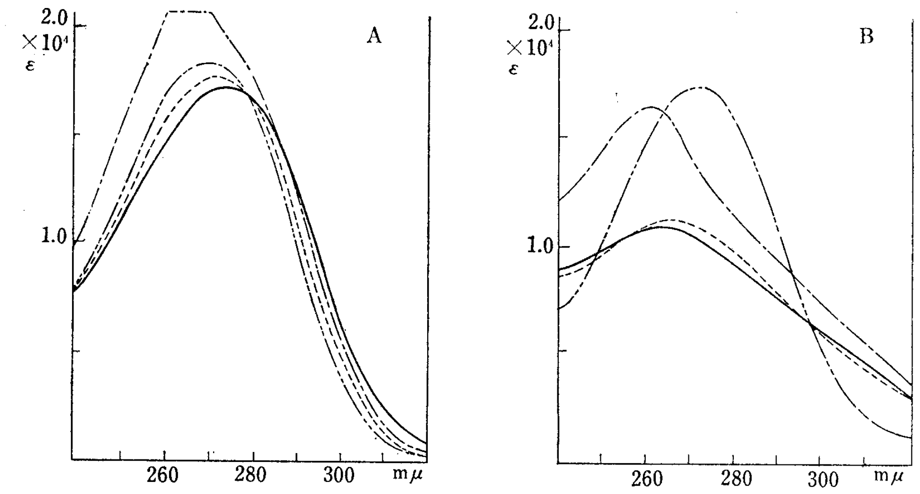
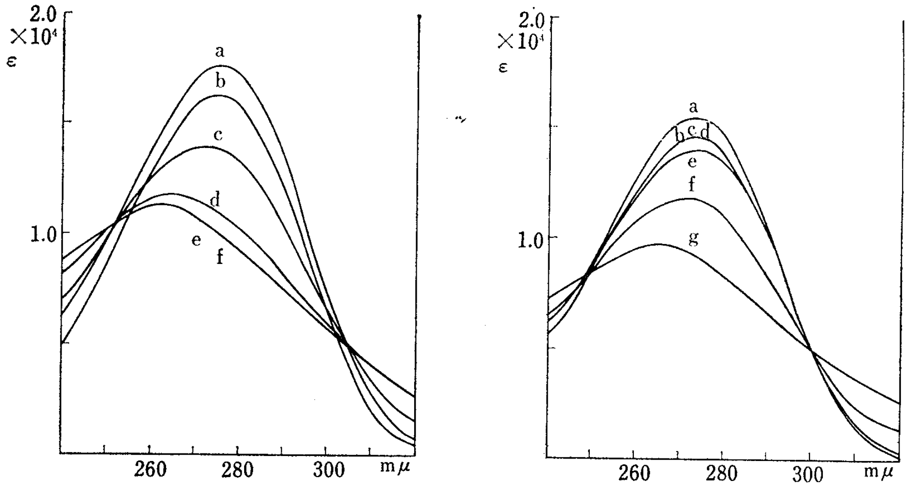


Fig. 4. Ultraviolet Absorption Spectra of Acetophenone Girard Hydrazones and Semicarbazone in A: Water containing 10% Ethanol and B: 0.1N Sodium Hydroxide containing 10% Ethanol

———— Acetophenone Girard-T hydrazone
·············· Acetophenone Girard-D hydrazone
– – – – – Acetophenone Girard-P hydrazone
—··—··— Acetophenone semicarbazone



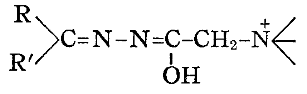
Acetophenone Girard-T hydrazone

Acetophenone Girard-D hydrazone

Fig. 5. Ultraviolet Absorption Spectra of Acetophenone Girard Hydrazones in Buffer Solutions of (a) pH 7.18, (b) 8.11, (c) 9.15, (d) 10.10, (e) 10.95, (f) 12,00, (g) 0.1 N Sodium Hydroxide containing 10% Ethanol

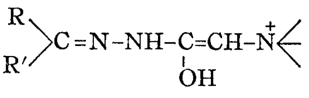
Discussion

Considering the results of the ultraviolet spectroscopic study, the existence of the plausible enol form such as I or II in the solution used for the polarographic study can



$$\begin{matrix}R\\R'\end{matrix}\!>\!C{=}N{-}N{=}\underset{\substack{|\\OH}}{C}{-}CH_2{-}\overset{+}{N}\!<$$

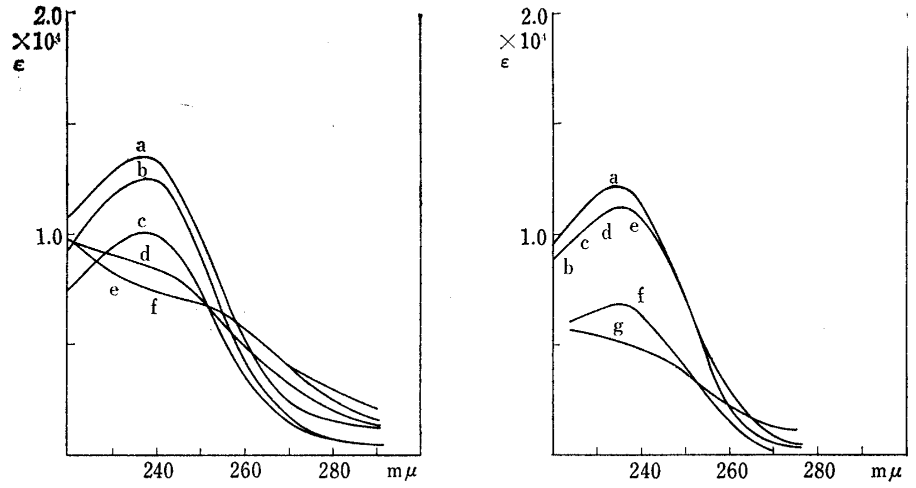
I



$$\begin{matrix}R\\R'\end{matrix}\!>\!C{=}N{-}NH{-}\underset{\substack{|\\OH}}{C}{=}CH{-}\overset{+}{N}\!<$$

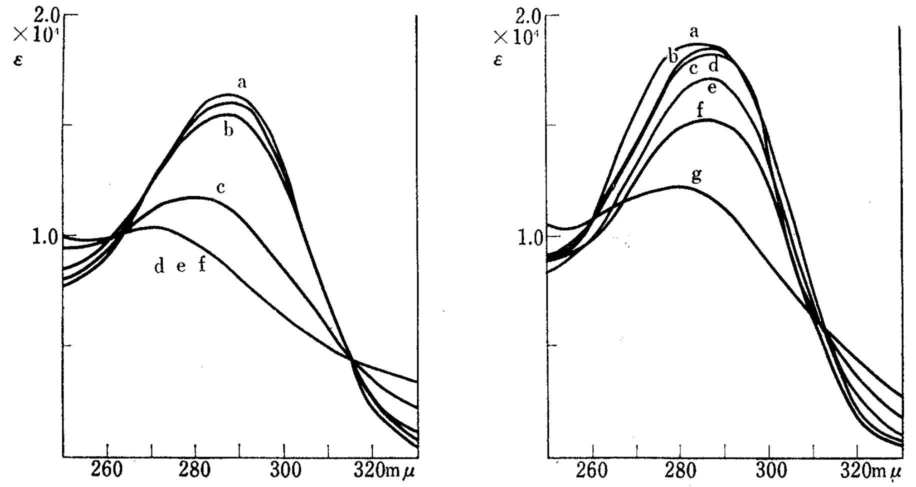
II

be negligible, because of the following three reasons: First, that as the dissociated form of the enol, say of I, is such as III, the absorption spectrum of the compound should



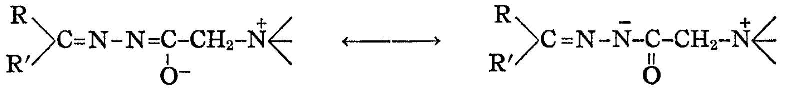
Cyclopentanone Girard-T hydrazone   Cyclopentanone Girard-D hydrazone

Fig. 6. Ultraviolet Absorption Spectra of Cyclopentanone Girard Hydrazones in Buffer Solutions of (a) pH 7.25, (b) 8.25, (c) 9.30, (d) 10.30, (e) 11.20, (f) 12.00, (g) 0.1N Sodium Hydroxide, containing 10% Ethanol



Benzophenone Girard-T hydrazone   Benzophenone Girard-D hydrazone

Fig. 7. Ultraviolet Absorption Spectra of Benzophenone Girard Hydrazones in Buffer Solutions of (a) pH 6.29 and 7.30, (b) 8.18, (c) 9.25, (d) 10.18, (e) 11.08, (f) 12.00, (g) 0.1N Sodium Hydroxide containing 10% Ethanol



$$\mathrm{RR'C{=}N{-}N{=}C(O^-){-}CH_2{-}\overset{+}{N}\!<} \longleftrightarrow \mathrm{RR'C{=}N{-}\overset{-}{N}{-}C({=}O){-}CH_2{-}\overset{+}{N}\!<}$$

Ⅲ

have a bathochromic shift when I changes to Ⅲ, like the case stated by Lees,*et al.*,7) but this shift was not observed for the Girard hydrazones; second, that the difference of the pK_a' values for cyclopentanone Girard-T and Girard-D hydrazone, that is about 2, is not explained by the equilibrium, $\mathrm{I} \rightleftarrows \mathrm{III} + \mathrm{H}^+$; and, third, that if the hydrazones would exist in the form like Ⅱ, Girard-P hydrazone should have a fairly different absorption maximum from those of the corresponding semicarbazone, Girard-T and Girard-D hydrazone, but they are essentially same as seen in Fig 4.

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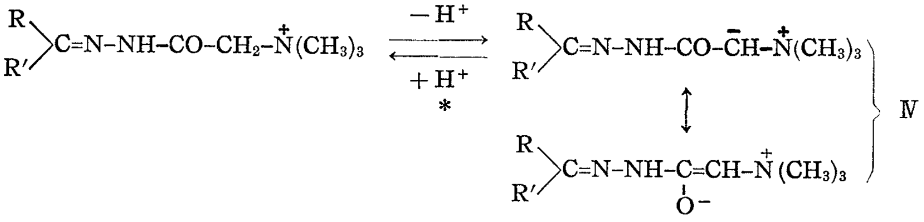
7) W. A. Lees, A. Burawoy: Tetrahedron, 19, 419 (1963).

The variation of absorption waves with pH for cyclopentanone Girard–T hydrazone is the largest at the pH value very close to its pK_a', but in the case of cyclopentanone Girard–D hydrazone, the largest variation occurs at a pH quite apart from its pK_a' value.

Girard–T hydrazones have a quaternary ammonium group, and this group has a much larger electron-withdrawing effect than $-N(CH_3)_2$ group does. The following schema (2) and (3) are proposed, therefore, for the dissociation of Girard–T and Girard–D hydrazones respectively.

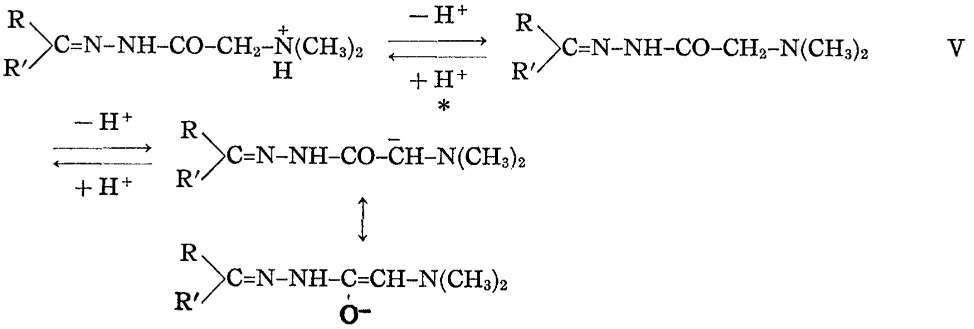
For Girard–T hydrazone;



(2)

* $pK_a'=9.48$ for cyclopentanone Girard–T hydrazone.

For Girard–D hydrazone;

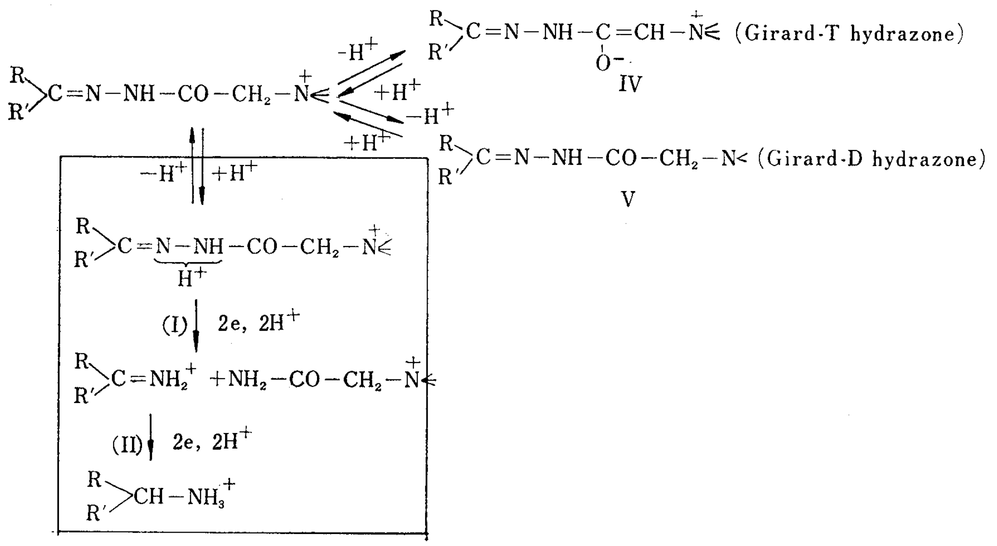


(3)

* $pK_a'=7.47$ for cyclopentanone Girard–D hydrazone.

A similar relation between ultraviolet absorption spectrum and dissociation constant has been observed in INH and the related compounds.[8]

From those results described above, it is clear that the scheme (1) proposed by Prelog and Häfliger must be abandoned, and for the Girard–T and Girard–D hydrazones of aliphatic ketones a much more complicated scheme (4) is proposed instead.



(4)

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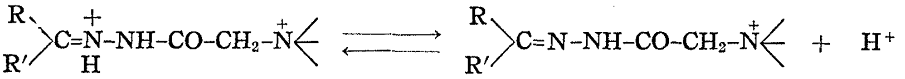
8) K. Nagano, H. Tsukahara, H. Kinoshita, Z. Tamura: This Bulletin, **11**, 797 (1963).

Rectangle represents electrolysis reactions occurring at the electrode surface.

The scheme (4) represents the followings; (a) by the controlled potential electrolysis of cyclopentanone Girard–T and Girard–D hydrazone, four electrons per molecule are consumed, and cyclopentylamine was isolated from the electrolyzed solution of the Girard–T hydrazone; (b) over the whole pH range studied, $E_{1/2}$ of the first wave depends linearly on pH, and the second wave in acid solution is also dependent on pH though the degree is smaller; (c) the decrease of wave height in alkaline solutions can be understood by a plausible assumption that Ⅳ and Ⅴ are more difficultly reducible than their protonized forms, because of their charge effect, and this coincides with the fact that Girard–D hydrazone having a smaller pK_a' value diminishes its wave height at the lower pH than Girard–T hydrazone; (d) inspite of the diminished wave height at pH 11, the controlled potential electrolysis of cyclopentanone Girard–T hydrazone consumed electricity corresponding to a four–electron reduction, which indicates that the more easily reducible form is supplied from the more difficultly reducible form Ⅳ until the total amount of the hydrazone is reduced; (e) except what stated in (c), the polarographic behaviours of cyclopentanone Girard–T and Girard–D hydrazones are closely similar with each other.

Equilibrium as equation (5) looks like possible to explain the diminished wave height in alkaline solutions by assuming the form in the right hand side is hardly reducible.



$$\begin{array}{l}\mathrm{R}\\ \mathrm{R'}\end{array}\!\!>\mathrm{C{=}\underset{H}{\overset{+}{N}}{-}NH{-}CO{-}CH_2{-}\overset{+}{N}}\!< \;\rightleftarrows\; \begin{array}{l}\mathrm{R}\\ \mathrm{R'}\end{array}\!\!>\mathrm{C{=}N{-}NH{-}CO{-}CH_2{-}\overset{+}{N}}\!< \;+\; \mathrm{H^+} \qquad (5)$$

But this view is not correct, because the pK_a' for the equilibrium (5) is not more than 1 as shown by the kinetic study on the hydrolysis of cyclopentanone Girard–T hydrazone.*[1] Even if the pK_a' is assumed to be 1, the proton recombination rate constants for the Girard–T and Girard–D hydrazone calculated by the method described by Koutecký and Bridička[9] become unreasonably high, 10^{21} and $10^{16}\,M^{-1}$ sec.$^{-1}$, respectively. Although the rate constant calculated by such polarographic method is apt to be a higher value than the actual,[10] those values should be said to be extraordinarily large compared with the theoretical upper limit for a second order reaction, $10^{13}\,M^{-1}$ sec.$^{-1}$, predicted by the Onsager's theory.[11,12] Moreover, by equation (5), it is impossible to explain the difference between the pH ranges where the decrease of the wave height occurs for cyclopentanone Girard–T and Girard–D hydrazone.

Though the equilibrium (5) is neglected in the bulk of the solutions used in the present study, an equilibrium like (5) must be considered at the electrode surface as a pre–equilibrium[13] to explain the pH–dependency of the half wave potential. Two mechanisms have been proposed to such case. One is a concerted reaction mechanism as applied to the electrolytic reduction of aromatic carbonyl compounds by Elving and Leone,[14] and the other is a pre–protonation mechanism by Mairanovskii,[13] who ascribed the unexpectedly large pre–protonation of a compound to the higher hydrogen ion and substrate concentrations at the electrode surface at the potential than those in the bulk of the solution. In scheme (4) and (6), we depicted them with Mairanovskii's theory[13] in mind. However, it is difficult to decide experimentally which mechanism should be predominant in the present case.

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

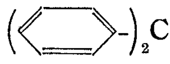
[10] *a*) R. Bridička : Z. Electrochem., **64**, 16 (1960). *b*) I. M. Kolthoff, J. J. Lingane : "Polarography," 274 (1952), Interscience Publishers, New York.

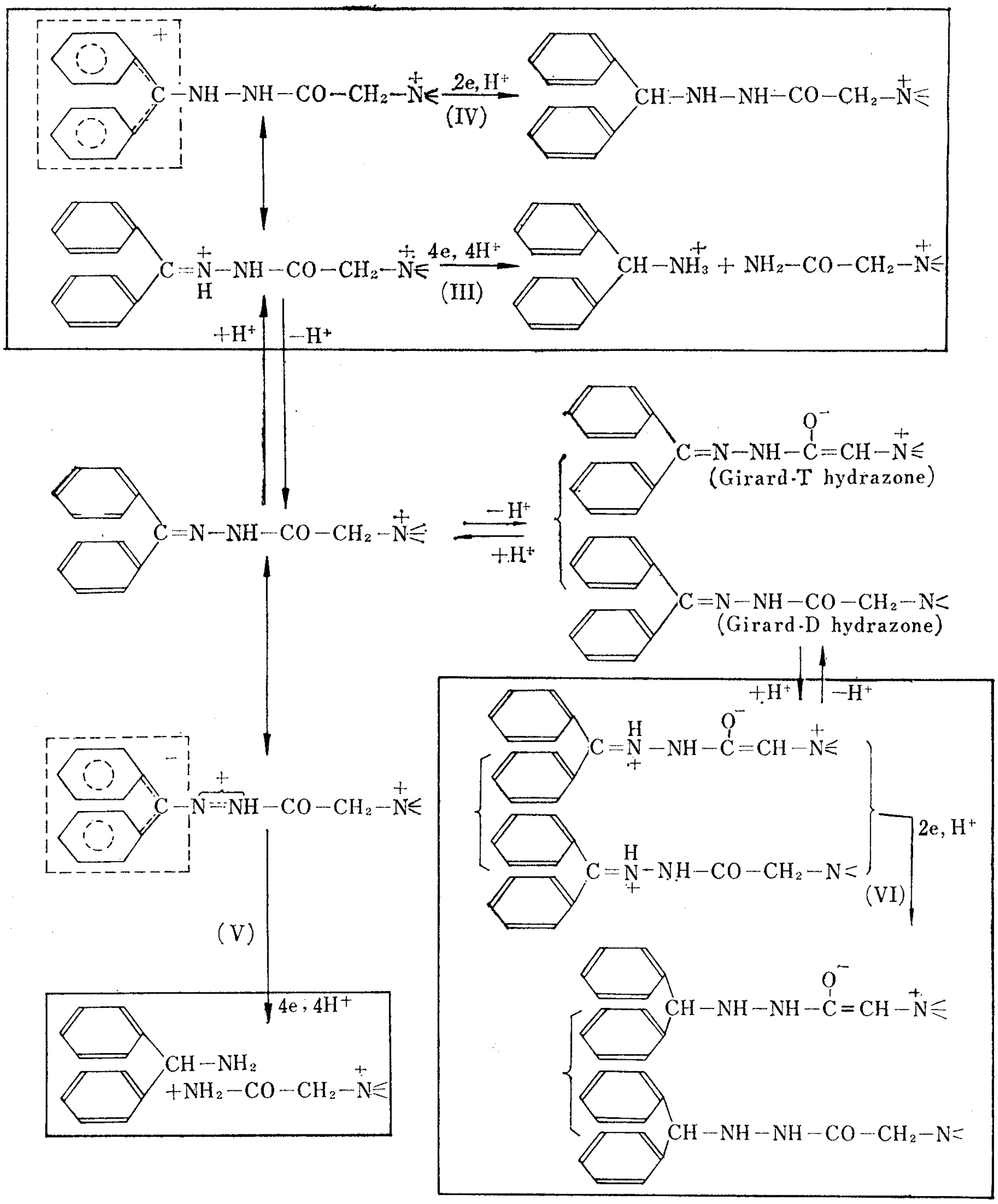
[11] L. Onsager : J. Chem. Phys., **2**, 599 (1934).

[12] R. G. Pearson, R. L. Dillon : J. Am. Chem. Soc., **75**, 2439 (1953).

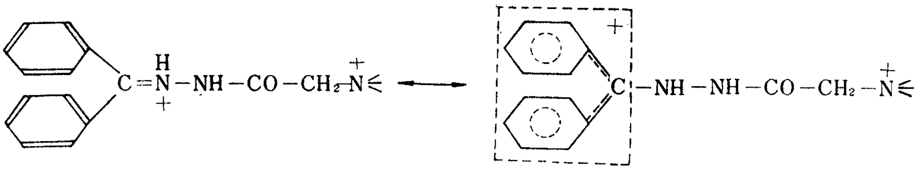
[13] S. G. Mairanovskii : J. Electroanal. Chem., **4**, 166 (1962).

[14] P. J. Elving, J. T. Leone : J. Am. Chem. Soc., **80**, 1021 (1958).

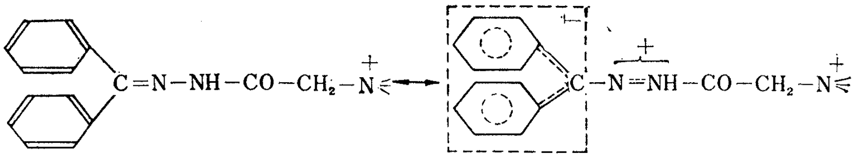
For the polarographic reduction of benzophenone Girard-T and Girard-D hydrazone, the following scheme (6) can be proposed. A large body of consistent evidence favours scheme (6): (a) In very acid solutions, the controlled potential electrolysis gave benzhydrylamine after consuming electricity corresponding to nearly four electrons permolecule. (b) The pH-dependency of $E_{1/2}$ of the first wave in very acid solutions (Fig. 3, curve Ⅲ)) is −100 mv. per pH, being about the same value to that for the first wave of various aliphatic Girard Hydrazones (−80∼−100 mv./pH); from (a) and (b), the line (Ⅲ) in Fig. 3 may be ascribed to the reaction (Ⅲ) in scheme (6), but in this case the supposed primary reduction product, $C{=}\overset{+}{N}H_2$, would be spontaneously reduced at the potential, because the resonance effect of the phenyl group makes its reduction potential be more positive than the case of cyclopentanone derivatives, so that the single wave is only developed. (c) By considering the reduction of the C=N double bond is easier than the case of aliphatic compounds, because of the resonance effect as shown in equation (7), the reaction (Ⅳ) in scheme (6) is thought to occur as well as the reaction



(6)


(7)

(Ⅲ), and, if it may be assumed that the pH-dependence of the reduction potential for the reaction (Ⅳ) is smaller than for the reaction (Ⅲ), the fraction of the reduction (Ⅳ) may increase as the pH of the solution increases. This assumption is strongly supported by the n values, 3.7, 3.3, and 2.2, found with the coulometric studies carried out at pH 1.6, 2, and 7.3 as seen in Table Ⅲ. The line (Ⅳ) in Fig. 3 is, therefore, ascribed to the reaction (Ⅳ). The isolated products from the electrolyzed solution in the macro scale electrolysis described above, and the decrease of the wave height with increasing pH in acid region as seen in Fig. 3 also support this view. (d) As a resonance, like


(8)

equation (8), may contribute to the structure of the hydrazones, the four-electron reduction (Ⅴ) in scheme (6) is thought to occur at a relatively less negative potential, and the pH-independent line (Ⅴ) in Fig. 3 is believed to correspond to the reaction (Ⅴ). The coulometric results obtained at pH 7.3 for benzophenone Girard-T hydrazone (Table Ⅲ) favour this view. The wave for the reaction (Ⅴ) is not observed in acid solution, because it is masked by the hydrogen discharge wave. Since the equilibriums (2) and (3) moves to the right as pH increases, the contribution of the reaction (Ⅴ) to a polarographic wave height must be diminished at a higher pH region. The decrease of the total wave height in neutral and alkaline regions, and the fact that the wave corresponding to the reaction (Ⅴ) is not observed distinctly with benzophenone Girard-D hydrazone which have a smaller pK_a' may be ascribed to it. (e) In relatively strong alkaline solutions, benzophenone Girard-T and Girard-D hydrazone show a decreasing wave (Ⅳ) and an increasing wave (Ⅵ) as the pH increases. The pH-dependency of $E_{1/2}$ for the wave (Ⅳ) is closely similar to that of the wave (Ⅵ). The n values for the two waves found by the coulometry on the Girard-T hydrazone at pH 12.0 are approximately equal (Table Ⅲ), though the potentials used are somewhat different. With the Girard-D hydrazone the n value at pH 11.2 is also found to be about 2.5, and from the electrolyzed solution 1-diphenylmethyl-2-dimethylaminoacetylhydrazine is isolated as a main product. These are best explained by considering the reduction takes place mainly through two two-electron processes (Ⅳ) and (Ⅵ) in scheme (6), which correspond to the waves (Ⅳ) and (Ⅵ), respectively. Where the process (Ⅳ) occurs, another process (Ⅲ) should take place at the same time, and the fraction will apt to increase with increasing applied negative potential and/or with decreasing pH. Really, the n value is always larger than two, and a production of benzhydrylamine is recognized in the crude product as a minor component by an infrared spectrum.

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Summary

Polarographic reduction processes of Girard hydrazones of aliphatic and aromatic ketones were studied. From the results of controlled potential electrolysis, the two-electron reduction process proposed by Prelog and Häfliger for Girard-T hydrazones of aliphatic and alicyclic ketones was found to be not correct, and a mechanism shown in scheme (4) containing a four-electron reduction process was proposed instead. In the case of the Girard hydrazones of aromatic ketones, the electrode reaction processes seem to be more complicated than the aliphatic series: A reduction mechanism shown as scheme (6) containing four and two-electron reduction processes has been proposed. These mechanisms can explain the polarographic behaviours of Girard hydrazones most adequately. In connection with this study, ultraviolet absorption spectra of Girard hydrazones were measured under various conditions. The molecular forms of Girard hydrazones under the experimental conditions have been discussed from the results.

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125. Hiroyuki Inouye, Toshio Arai, und Yukiko Miyoshi :

Über die Bestandteile der Pyrolazeen. XIV.[*1]
Über die Struktur des Monotropeins.[*2]

(*Pharmazeutische Fakultät der Universität Kyoto*[*3])

Im Jahre 1923 wurde von Bridel[1)] aus dem den Pyrolazeen gehörenden Saprophyt, *Monotropa hypopithys* L. ein Stoff isoliert, welches Monotropein genannt wurde. Nach ihm liegt in diesem Stoff ein saures Glucosid vor, welches durch Einwirkung von Emulsin oder Mineralsäure eine eigentümliche blaue Färbung gibt. In der Folgezeit erwähnt die Literatur aber keine wichtige Arbeit über diesen Stoff außer einer über sein papierchromatographisches Verhalten.[2)]

In den letzten Mitteilungen dieser Reihe berichteten wir nun über die Isolierung dieses Glucosids aus den verschiedenen einheimischen Vertretern der Pyrolazeen, einschließlich der Gattungen *Pyrola*, *Chimaphila*, *Monotropa* und *Monotropastrum*.[3)]

In der vorliegenden Arbeit wird die Struktur des Monotropeins untersucht werden. Monotropein kristallisiert aus Wasser als Monohydrat vom Schmp. 170~173° sowie $[\alpha]^{17}_{D}$ $-127.7°$ (H_2O) und aus Methanol als Monomethanolat vom Schmp. 160~163°. Auf Grund zahlreichen Analysen trifft für Monotropein (I) die Summenformel $C_{16}H_{22}O_{11}$ mit einem

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[*1] XIII. Mitteil. : Yakugaku Zasshi, **84**, 444 (1964).

[*2] Diese Arbeit wurde im 7. Symposium über die Chemie der organischen Naturstoffe Japans (Fukuoka, den 17. Oktober 1963) vorgetragen. Ein Teil dieser Arbeit wurde auch in Tetrahedron Letters, No. 16, 1031 (1963) veröffentlicht.

[*3] Yoshida-Konoe-cho, Sakyo-ku, Kyoto (井上博之, 新井敏夫, 三好由紀子).

1) M. Bridel : Compt. rend., **176**, 1742 (1923); *Idem* : Bull. soc. chim. biol., **5**, 722 (1923).

2) A.R. Trim, R. Hill : Biochem. J., **50**, 510 (1952).

3) H. Inouye, T. Arai, Y. Yaoi, M. Ogawa : Dieses Bulletin, **12**, 255 (1964) ; H. Inouye, T. Arai : *Ibid.*, **12**, 533 (1964) ; *Idem* : Yakugaku Zasshi, **84**, 444 (1964) ; H. Inouye, T. Arai, Y. Yaoi : *Ibid.*, **84**, 337 (1964).