[Chem. Pharm. Bull. 22(2) 254—261 (1974)]

UDC 547.567.03 : 543.5

Polarography of Anthraquinones and Dianthrones of Rhubarb[1]

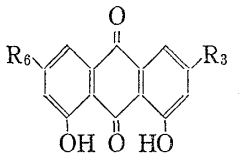
Yutaka Asahi, Kazuo Shinozaki, Masayoshi Mitani, and Hiroshi Ohtsuka

Chemical Research Laboratories, Central Research Division, Takeda Chemical Industries, Ltd.[2]

(Received March 2, 1973)

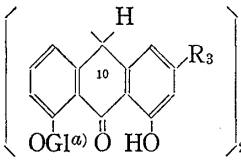
The 1,8-dihydroxyanthraquinones such as emodin (I), physcion (II), crysophanol (III), aloe-emodin (IV) and rhein (V) show reversible 2-electron reduction wave forming the hydroquinones in aqueous isopropanol solutions. Their semiquinones are stable in dimethylformamide. Sennosides (VI) show irreversible 2-electron reduction wave accompanied with 2 adsorption waves, which are ascribed to reductive cleavage of the C–C bridge to the anthrone (VII). The carbonyl in VII is successively reduced to the corresponding alcohol (XI), which seems to dehydrate to the anthracene (XII). Then, the I—V and VI in rhubarb can be determined by polarography.

It has been previously reported[3] that rhubarb contains anthraquinones such as emodin (I), physcion (II), crysophanol (III), aloe-emodin (IV) and rhein (V), and dianthrones such as sennoside A (VIa), sennoside B (VIb), sennoside C (VIc), and sennoside E (VIe), whose diarrhoeal activity is in sequence of sennosides (VI)>V, IV, I>II, III. The present authors intended to determine polarographically the biologically active components (I—VI). Although the polarographic method was more rapid and precise than the biological assay, the correlation was not so good as to replace the latter with the former. Therefore, this paper deals mainly with polarographic behaviors and the electrode reactions of these compounds. Polarographic reductions of the quinones to the hydroquinones have been studied by many investigators in various conditions,[4,5] but no paper has appeared on polarography of dianthrone. Reductive cleavage of the C–C bridge in VI was elucidated by electrolysis in milligram scale in this paper.



I—V

	R_3	R_6
I	CH_3	OH
II	CH_3	OCH_3
III	CH_3	H
IV	CH_2OH	H
V	CO_2H	H



VI

	R_3	10
VIa	CO_2H	*trans*
VIb	CO_2H	*meso*
VIc	CH_2OH	*trans*
VIe	(under investigation)	

a) Gl: glucose, $C_6H_{11}O_5$

Chart 1

Experimental

Chemicals——The pure compounds (I—VI) and samples of rhubarb were offered by Dr. Miyamoto, *et al.*[3] in our laboratories. Stock solutions in iso-PrOH were added to the following buffers to make up test solutions: HCl+0.1N KNO_3 of pH 2, 0.1 M acetate of pH 4—5, 0.1 M phosphate of pH 6.9 and 11, 0.1M

1) Presented in the 14th Symposium on Polarography, Hiroshima, Oct. 1968.
2) Location: *Juso, Higashiyodogawa-ku, Osaka.*
3) M. Miyamoto, S. Imai, M. Shinohara, S. Fujioka, M. Goto, T. Matsuoka, and H. Fujimura, *Yakugaku Zasshi*, **87**, 1040 (1967); T. Matsuoka, *Shoyakugaku Zasshi*, **15**, 113 (1961).
4) *a*) R. Gillard and H.I. Stonehill, *J. Chem. Soc.*, **1952**, 1845, 1857; *b*) L.A. Wiles, *ibid.*, **1952**, 1358; *c*) K.G. Stone and N.H. Furman, *J. Am. Chem. Soc.*, **70**, 3062 (1948); *d*) N.H. Furman and K.G. Stone, *ibid.*, **70**, 3055 (1948); *e*) J.M. Fritsh, T.P. Layloff, and R.N. Adams, *ibid.*, **87**, 1724 (1965); *f*) W. Poethke and H. Behrendt, *Pharm. Zentralhalle*, **104**, 4 (1965).
5) T. Fujinaga, K. Izutsu, K. Umemoto, T. Arai, and K. Takaoka, *Nippon Kagaku Zasshi*, **89**, 105 (1968); K. Yasukouchi, H. Yamaguchi, Y. Ono, and M. Urabe, *ibid.*, **88**, 428 (1967).

borate or 0.1 M NH_4Cl+NH_3 of pH 8—11, 0.1 N NaOH at pH 13. Most chemicals used were the best commercial grade obtainable.

Polarograph——Yanaco's PA 2 polarograph was used. Characteristics of a dropping mercury electrode were $m=1.103$ mg sec^{-1}, $t=4.28$ sec, and $k_C=1.360$. A normal calomel electrode was used as an external reference electrode. Polarograms were measured at $25+0.1°$ after bubbling the test solution with nitrogen.

Measurements of Dissociation Constants (p*K*)——A solution, 8 ml of 2 mM I (III or IV) in 50% (v/v) iso-PrOH containing 0.4 ml of 0.1 N NaOH was titrated with 0.1 N HCl by Radiometer's titrigraph. The first pK_o values of the hydroxyl groups were estimated to be 7.9 for I and *ca.* 10 for III and IV. No inflection due to second or third pK_o was observed until pH 12 by this method. In presence of 1.25 mM $CuSO_4$ in 2 mM I, pH of the inflection (pK') lowered until 5.7 and 9.9, which suggested formation of copper chelate of I.

Absorbances of the solution of 0.05 mM II—V in 50% iso-PrOH-buffer at 435 and 510 nm were measured by Perkin-Elmer 450 spectrophotometer. The pK_os of II, III, IV, and V were estimated to be 10.1, 9.9, 9.3, and 10.1, respectively, from pH dependence of the absorbance. The pK_o of VIa was also determined to be 11.5 by absorbance at 425 nm in 25% iso-PrOH.

Dissociation constants (pK_R) of dihydro-aloe-emodin (IV') were estimated to be 5, 6.5, and 12 from the pH dependence of absorbance at 430, 402, and 394 nm immediately after 0.05 mM IV in 50% iso-PrOH-buffer was reduced by $Na_2S_2O_4$ under N_2 atmosphere, where borate buffers were omitted because the absorption maximum at pH 8—10 shifted from 402 nm to 394 nm by forming a borate complex with the polyol (IV').

Electrolysis of Sennoside A——1) Setting the Cathodic Potential at -1 V: Two ml of 0.24 mM sennoside A (VIa) in phosphate buffer at pH 6.8 was electrolyzed on a mercury cathode of 1 cm^2 at -1.05 V against a saturated calomel electrode of 50 cm^2 under bubbling N_2. The initial current, 77 μA, decreased down to 13 μA in the course of electrolysis for 75 min. The consumed electricity, 0.107 coulomb, measured by the current-time curve was found to be close to theoretical value, 0.094 coulomb, assuming 2-electron reduction of the C–C bridge in VIa. The electrolyzed solution gave a reduction wave of diffusion current constant (i_D): 5.8 μA mM^{-1} at -1.47 V probably due to 2 moles of rhein-anthrone-8-glycoside (VII). *Anal.* Calcd. for $C_{21}H_{20}O_{10}$ (VII): C, 58.33; H, 4.66; mol. wt., 432.4. Found: C, 58.27; H, 5.06; mol. wt., 450 (vapor pressure osmometer). The ultraviolet spectrum (UV) resembles to that of VI. λ_{max}^{pH8} nm (log ε): 268 (4.28), 310 (4.14), 340 (4.14). Fluorescence activated at 400 nm: λ_{max} 470 nm (very weak).

2) Setting the Cathodic Potential at -1.4 V: Electrolyzing 1.5 ml of 1.26 mM VIa at -1.4 V, 6*F*/mole was consumed indicating the reduction of the C–C and 2 C=O groups. The product gave hardly any polarographic wave. UV λ_{max}^{pH7} nm (log ε): 260 (4.76), 270 (4.68), 352 (3.90), 370 (4.02), 384 (4.00), 405 (3.90). The product is assigned from the UV spectrum to 1,8-dihydroxyanthracene-8-glycoside-3-carboxylic acid (XII), which may be formed by dehydration of 1,8-dihydroxy-9,10-dihydro-9-anthrol-8-glycoside-3-carboxylic acd (XI) showing λ_{max} 260 nm. Fluorescence spectrum activated at 400 nm: λ_{max} 460, 550 nm at pH 7.

Thin-Layer Chromatography (TLC)——Samples were developed on Merck's Silica gel HF_{254} ($20\times20\times0.025$ cm, activated at 100° for 30 min) and detected by UV absorption or heating with H_2SO_4. *Rf* (BuOH: AcOH: $H_2O=5:1:4$): I—V, 0.98; VIa, 0.60. *Rf* (AcOEt): I—IV, 0.9; V—VI, 0. *Rf* ($CHCl_3$): I, IV, 0.1; II, III, 0.9; V, VI, 0. *Rf* (EtOH): I, IV, 0.9; II, III, V, VI, 0.

Aqueous extract of rhubarb powder (25 mg/50 μl) was separated with TLC or silica gel column. VI was eluted with phosphate buffer (pH 7) and determined by absorbance at 330 nm.

Polarographic Assay of Rhubarb——1) Simple Determination of Anthraquinones and Sennosides: Fine powder of rhubarb (50 mg) was extracted with 10 ml of 0.1 M borate buffer at pH 10 saturated with BuOH on a shaker for 2 hr. Further prolonged extraction for 20 hr caused no progress in the rate of detection. The filtrate was polarographed to measure the wave heights of anthraquinones (I—V and their glycosides) at $E_{1/2}$ -0.7 V, sennosides (VI) at $E_{1/2}$ -0.9 V, and anthrones or flavones at $E_{1/2}$ -1.2 V. General methods using standard addition or calibration curve were applied to the determination.

2) Determination of VI: After extracting fine powder of rhubarb (0.5 g) with 10 ml of 0.1 M phosphate buffer at pH 7 saturated with BuOH, VI and only a part of quinones (I, V and the glycosides) were extracted and were determined by the reduction waves at $E_{1/2}$ -0.8 V and -0.6 V, respectively.

3) Separation of VI by Extraction: The centrifugal sediment of the extract mentioned above was washed with 5 ml of the same solvent. The supernatant (15 ml) was washed with 20 ml of BuOH to eliminate the quinones. The aqueous layer acidified down to pH 2 with oxalic acid (0.2 g) was extracted twice with 10 ml of BuOH, which was again extracted with 10 ml of the buffer at pH 7. The buffer solution was polarographed to measure the wave height of VI at $E_{1/2}$ -0.8 V.

Partition coefficients of VIa in BuOH/buffers were 0.01 at pH 7 and 20 at pH 1, whereas that of III was 1800 at pH 7. Recovery of VIa after the extraction from the buffer (pH 7) was 96%.

4) Paper-Partition Chromatography (PPC): An aqueous extract of rhubarb (50 mg/0.5 ml) spotted on Whatman's filter paper No, 3 MM ($200\times200\times0.33$ mm) was eluted with EtOH–BuOH -0.2 M citrate buffer of pH 6.3 (2: 4: 4).[6] The fractionated pieces of paper were extracted with 10 ml aliquots of the

6) W. Schmid and E. Angliker, *Helv. Chim. Acta*, **48**, 1911 (1965).

buffer of pH 7 saturated with BuOH, which were examined by polarography. The quinones (I—V) were detected mainly as the yellow spots of $Rf>0.5$, whereas VI was detected mostly as the pale yellow or brown spots with UV absorption at $Rf<0.5$. Recovery of VIa from the paper was 98%.

Result and Discussion

Polarography of Anthraquinones

In Aqueous Isopropanol——The anthraquinones (I—V) gave the typical reduction waves in buffer solutions at pH 2—13 containing 20—50% isopropanol and 0.01% gelatin. The wave heights of I at half-wave potential ($E_{1/2}$)—0.78 V in 20% isopropanol at pH 10.3 were proportional to the concentrations in a range of 0.02—0.4 mM. A mixture of I and V gave separate waves whose heights corresponding to the concentrations. Diffusion current constants (k_D) were about 1.8 ($\mu A\ mM^{-1}\ mg^{-2/3}\ sec^{1/2}$) for I—V in aqueous isopropanol and 2.78 for I in aqueous solution. When diffusion coefficient (D) of I in water was estimated to be $5.4\times10^{-6}\ cm^2\ sec^{-1}$, number of electron per a reducible molecule (n) was calculated to be 2. The slopes of current (i) *vs.* potential (E) curve (Eq. 1), were close to the theoretical value (0.03 V) for reversible 2-electron reduction.

$$E = E_{1/2} - \frac{0.059}{n} \log \frac{i}{i_d - i} \qquad \text{Eq. 1}$$

Relationships between $E_{1/2}$ and pH were shown by Fig. 1 and Eq. 2,

$$E_{1/2} = E_0 - \frac{0.059m}{n}\text{pH} \qquad \text{Eq. 2}$$

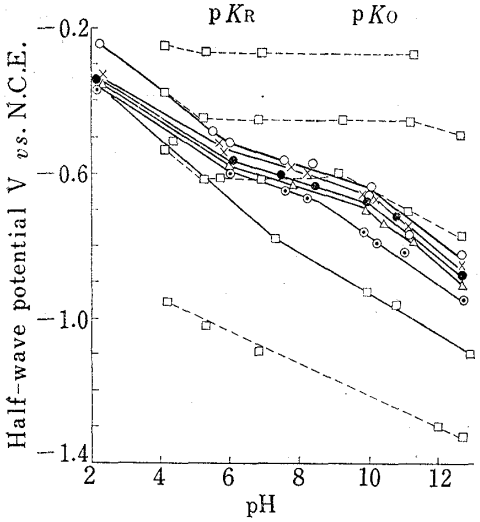


Fig. 1. pH Profiles of Half-wave Potentials ($E_{1/2}$) in Aqueous Buffers (------) or Buffers containing iso-PrOH (——)
⊙: I, △: II, ●: III, ×: IV, ○: V, □: VIa

where number of proton per a reducible molecule (m) was estimated from the slope ($0.059\ m/n$) to be 2, 1 and 2 in acid, neutral and alkaline regions, respectively. The pH values of turning points in an alkaline region were close to pK_0 values of the hydroxyls in I—V and those in an acid region were close to dissociation constants (pK_R) of the hydroquinones. At $pH<pK_R<pK_0$, the electrode reactions of I—V are assumed to be 2-electron 2-proton reduction of the undissociated polyhydroxyanthraquinones (Q) to the corresponding hydroquinones (H_2Q).

$$Q + 2e + 2H^+ \rightleftarrows H_2Q$$

At $pK_R<pH<pK_0$, the undissociated quinones (Q) are reduced into the hydroquinones dissociated to the monoanions (HQ^-) by taking 2 electrons and 1 proton per molecule.

$$Q + 2e + H^+ \rightleftarrows HQ^-$$

At $pH>pK_0>pK_R$, the anionic quinones (Q^-) are reduced into the anionic hydroquinones (H_2Q^-) by taking 2 electrons and 2 protons.

$$Q^- + 2e + 2H^+ \rightleftarrows H_2Q^-$$

The reduction wave of II—V changed to a redox wave having a constant height and a persistent half-wave potential by partial reduction with sodium dithionite in 0.2M triethanol-

amine buffer at pH 8.4 and recovered by successive aeration. Whereas dihydro-emodin (I′) gave an oxidation wave at more positive potential than that of reduction wave of I by *ca.* 0.35 V in the buffer solutions containing acetate, phosphate, ammonium and sodium hydroxide at pH 4—13. Therefore, the redox systems of II—V are considered to be strictly reversible, whereas that of I seems to be quasi-reversible. The half-wave potentials of the 9,10-anthrahydroquinones (I′—V′) shifted to more positive potentials by *ca.* 0.12 V in presence of borate probably due to formation of the borate complexes with I′—V′. The tautomerism[4] of 9,10-anthrahydroquinones to 9-hydroxy-10-anthrones was not evidently observed in case of I′—V′.

Linear relationships between the half-wave potentials of many substituted anthraquinones and sum of the Hammett's *para* substituent constants (σ_p) including both the polar inductive effect and the resonance effect have been reviewed,[7] where the total polar reaction constants (ρ_π) were +0.09 V at pH 1.25 in 70% ethanol and +0.14 V in sulfuric acid–acetic acid (1:9). Similarly the half-wave potentials of I—V at pH 5.7 in 37% isopropanol (Table I) correlate nicely with the sum of the σ_p values for the 3- and 6-substituents, the ρ_π value being +0.08 V (Fig. 2), but poorly with the *meta* substituent constants (σ_m) excluding the resonance effects. Therefore, the 3- and 6-substituents in the 1,8-dihydroxyanthraquinones (I—V) seem to have not only the polar inductive effect but also the resonance effect on the reduction potentials of the quinones, whereas the steric effect may be negligible.

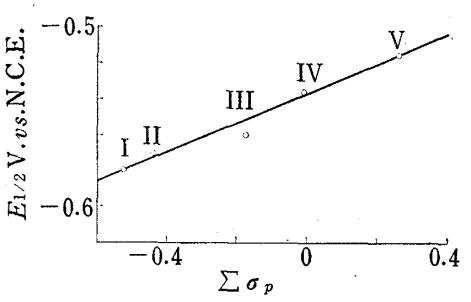


Fig. 2. Correlation of Half-wave Potential ($E_{1/2}$) of 1,8-Dihydroxyanthraquinones (I—V) at pH 5.7 in 37% Isopropanol with Sum of the Substituent Constants ($\sum\sigma_p$) at the 3- and 6-Positions

σ_p[7]: CH_3, −0.170; OH, −0.357; OCH_3, −0.268; CH_2OH, −0.01; CO_2H, 0.265. $\Sigma\sigma_p$: I, −0.170—0.357; II, −0.170—0.268; III, −0.170; IV, −0.01; V, 0.265

In aqueous solutions, α-hydroxyanthraquinones give a reversible 2-electron reduction wave with the theoretical slope, whereas β-hydroxyanthraquinones show larger slope suggesting more stable semiquinones.[4] The behaviors of 1,8-dihydroxyanthraquinones (I—V) resemble those of α-hydroxyanthraquinones.[4]

In Dimethylformamide——Emodin (I) in dimethylformamide (DMF) with 0.1M tetramethylammonium bromide at pH 9.6 gave 2 steps of 1-electron reduction at −0.63 and −1.2 V with equal height. Decreasing contents of DMF in DMF-water, the both steps shift together to overlap finally in 30% (v/v) DMF. The total wave height varied with viscosity of the solution: k_D 3.0 in DMF and 1.8 in 50% DMF.

The stepwise reductions of quinones (Q) to the dianionic hydroquinones ($Q^=$) *via* anionic radicals of the semiquinone (Q^-) have been observed in non-aqueous solution.[5]

$$Q \xrightarrow{e} Q^- \xrightarrow{e} Q^=$$

In the case of I—V the radicals were also demonstrated by electron spin resonance in the course of electrolysis in DMF.[8]

A.C. Polarography of Emodin——Emodin (I) in an aqueous buffer at pH 10.3 containing 0.01% gelatin gave 2 peaks with summit potentials (E_S) −0.75 and −1.0 V. The former was close to $E_{1/2}$ and the height (i_S) was proportional to the concentration (2.7 m mho/mM) in a range of 0.01—0.07 mM and tended to a limit (0.3 m mho) in a range of 0.1—0.2 mM.

7) P. Zuman, "Substituent Effects in Organic Polarography," Plenum Press, New York, N.Y., 1967, pp. 271—307, 46—48.
8) T. Terao, Y. Asahi, and M. Shintani, *Takeda Kenkyusho Ho*, **31**, 170 (1972).

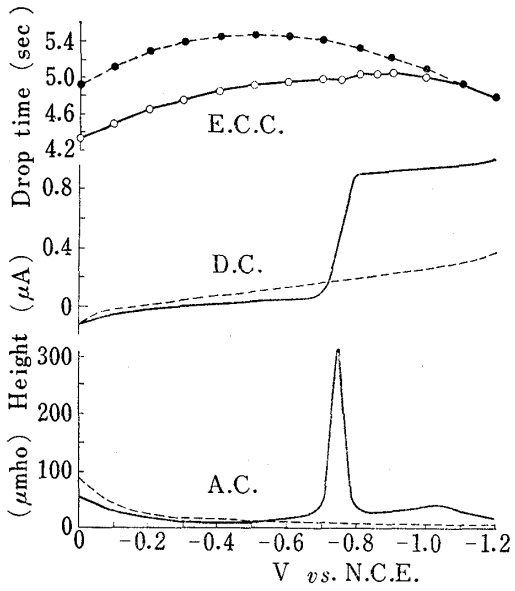


Fig. 3. Electrocapillary Curves (E.C.C.), D.C. Polarograms (D.C.) and A.C. Polarograms (A.C.) of Emodin (I) in Aqueous Buffer of pH 10

——: 0.2 mM ——: 0 mM

These facts reveal the reversible electrode reaction accompanied with adsorption process.

The second small peak (20 μ mho/0.1 mM) is assigned to a tensammetric wave due to desorption of the molecule adsorved on the electrode since residual current of D.C. polarogram and electrocapillary curve are suppressed in the range of 0——1 V by presence of 0.2 mM I in buffer.

Poethke[4] has reported that I gives cathodic and anodic spikes on oscillopolarogram.

Polarography of Sennosides

Sennoside A in Aqueous Buffers——VIa gave 3—4 steps of reduction waves in aqueous buffer solutions with 0.01% gelatin. The first small step appeared at $E_{1/2}$ −0.26 V in pH 4—11 with limiting current (i_l) smaller than 0.1 μA/0.1 mM and the second small step at −0.45 V in pH 5—13 with i_l<0.15 μA/0.1 mM are assigned to adsorption waves. The third main wave appeared at $E_{1/2}$ −0.53——0.71 V in pH 4—11. Total heights of these 3 steps at pH 4—13 were

Table I. Half-wave Potentials ($E_{1/2}$: −V *vs.* N.C.E.) and Diffusion Current Constants (k_D: μA mM^{-1} $mg^{-2/3}$ $sec^{1/2}$) of 1,8-Dihydroxyanthraquinones (I—V) and Sennoside A(VIa) at 25°

	R_3	R_6	% iso-PrOH	$E_{1/2}$ pH 2.2	5.7	7.5	10	13	k_D av.
I	CH_3	OH	37	0.35	0.58	0.64	0.76	0.95	1.8
II	CH_3	OCH_3	37	0.35	0.57	0.63	0.70	0.90	1.7
III	CH_3	H	37	0.33	0.56	0.60	0.68	0.87	1.8
IV	CH_2OH	H	37	0.32	0.54	0.58	0.65	0.85	1.8
V	CO_2H	H	37	0.23	0.49	0.56	0.64	0.83	1.9
VIa	CO_2H	H	25	0.17	0.30	0.57	0.42		*a)*
				0.36	0.61	0.78	0.92	1.33	1.3
				0.93	1.07	1.19	1.28	1.61	0.4*c)*
VIa			0	*b)*	0.26	0.26	0.26		*a)*
					0.44	0.45	0.46	0.48	*a)*
					0.62	0.61	0.65	0.77	2.0
					1.02	1.11	1.22	1.33	0.7*c)*

a) adsorption wave *b)* insoluble *c)* kinetic current

proportional to the concentration and had reasonable temperature coefficient (1.7% deg^{-1}) to be assigned to diffusion current with k_D 2.0. Assuming $D=3\times10^{-6}$ cm^2 sec^{-1} from the molecular weight of 863, the number of electrons (n) were estimated to be round 2. The fourth step at −1 V in pH 4—7 was assumed to be a kinetic current because of its large temperature coefficient (5% deg^{-1}).

No A.C. polarogram of VIa in an aqueous buffer solution was observed at the potential (−0.2——1 V) where D.C. polarogram was observed (Fig. 4, No. 3). The small A.C. wave at −1.2 V was assigned to a tensammetric wave due to desorption of VIa on the electrode because the suppressed electrocapillary curve recovered at the same potential (Fig. 4, No.

1 and 3). Therefore the electrode reaction is found to be irreversible and to be affected by adsorption of VIa.

Effect of Alcohols and Surfactants——The reduction wave of VIa (0.2 mM) in a phosphate buffer at pH 7 was suppressed in a certain potential range to result in a minimum wave by presence of a certain concentration of alcohols or surfactants such as 40—50% ethanol, 20—50% isopropanol, 5—7.4% (saturated) butanol, 2.3—3.8% isoamyl alcohol, saturated octanol in 30% ethanol, and 0.01—0.1% polyoxyethylene sorbitan monooleate ("Tween 80"). The minimum concentrations of alcohols to cause the suppression decrease with elongating the alkyl chains, which are effective to the adsorption on the electrode. The range of current suppression started at about −0.7 V spread out upto −0.4——0.9 V with increasing the concentration of isopropanol or with lowering the concentration of VIa (0.2—0.05 mM) in 25% isopropanol.

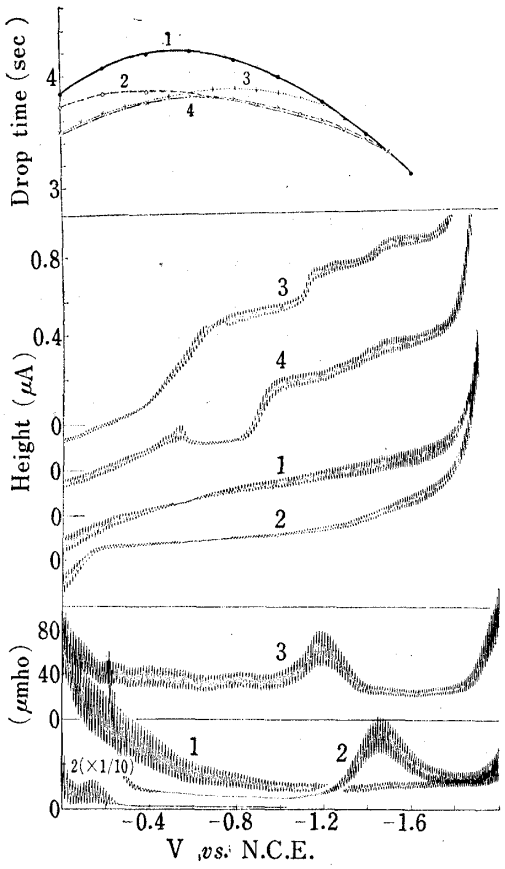


Fig. 4. Electrocapillary Curves (upper), D.C. Polarograms (middle) and A.C. Polarograms (lower) of Sennoside A (VIa)

1 : buffer of pH 6.8
2 : the buffer containing 25% iso-PrOH
3 : 0.1 mM VIa in the buffer (1)
4 : 0.1 mM VIa in the solution (2)
capillary constants: m=2.00, t=3.64, k_e=1.97

Interfacial tension of mercury measured by the drop time was suppressed by presence of 25% isopropanol at the potential down to −1.5 V (Fig. 4, No. 2) and further suppressed in addition of 0.1 mM VIa down to −0.6 V (Fig. 4, No. 4). Residual current in D.C. polarogram of the buffer solution was also suppressed by presence of isopropanol in a range of −0.1——1.5 V (Fig. 4, No. 1 and 2). Tensammetric waves due to adsorption and desorption of isopropanol were observed in the A.C. polarogram at −0.1 and −1.5 V, respectively, between which the residual current was suppressed again (Fig. 4, No. 1 and 2). It is revealed from these facts that the alcohols or surfactants adsorbed on the electrode suppress not only the residual current but also the reduction current of VIa. It is noteworthy that no effect of surfactants is observed on the reversible reduction waves of the anthraquinones (I—V).

Apparent half-wave potential ($E_{1/2}'$) of VIa was hardly affected by the presence of 25% isopropanol at pH 2—5 whereas shifted more negative by 0.2—0.3 V than those in aqueous buffer solutions at pH 7—11. The shifted reduction wave of VIa at pH 10.7 was assumed to be a diffusion current, since the heights were proportional to the concentration of VIa (0.05—1 mM) and also to square root of the mercury head (70—90 cm). The constant (k_D) decreased with the increase of viscosity of aqueous alcohols: k_D 1.6 in 7.4% butanol and k_D 1.3 in 25% isopropanol.

At pH 12—13, the main reduction wave of VIa split into 2 steps, of which the second step at more negative potential increased in cost of the first step at higher pH which is assigned to the 1-hydroxyl anion having pK_0 11.5.

Polarography of Sennoside B—E——The homologues (VIb and VIe) had polarographic behavior similar to that of VIa. Namely, VIb and VIe (0.2 mM) in aqueous buffer at pH 6.7 gave a main reduction wave at $E_{1/2}$ −0.6 V accompanied with an adsorption wave at

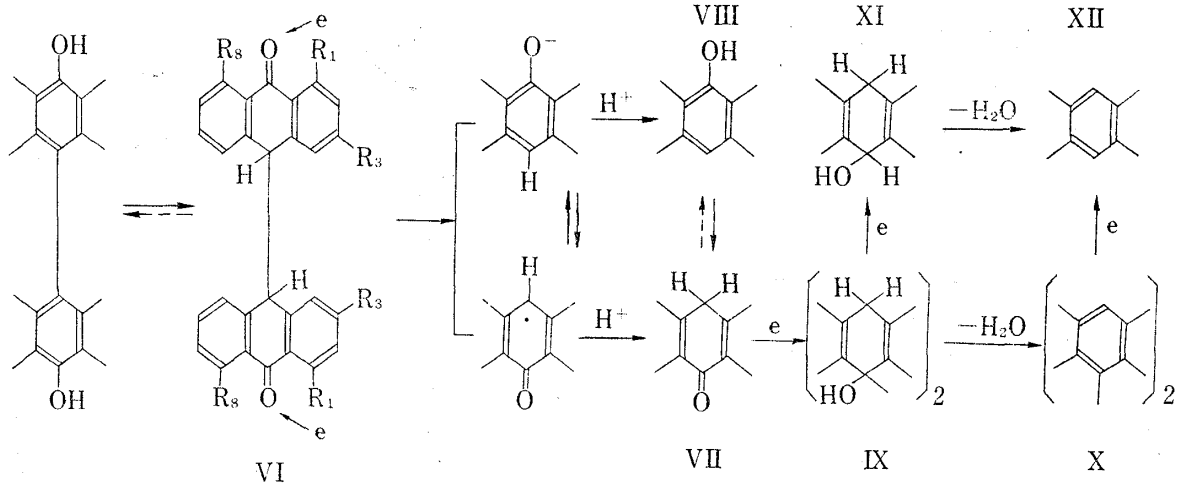
E_a −0.45 V and a second reduction wave at $E_{1/2}$ −1.09 V with a nature of kinetic current. The waves were again deformed by the adsorption of isopropanol or butanol on the electrode to give $E_{1/2}'$ −0.83 V (VIb) or −0.93 V (VIe) in 25% isopropanol at pH 7.

The polarographic behavior of VIc, 3′-hydroxymethyl homologue of VIa, was similar to but somewhat different from those of VIa. Namely, the half-wave potentials were more negative by 0.1 V than those of VIa and the kinetic current showed about 3 times higher than that of VIa. The reduction wave of VIc at pH 7 was hardly affected by 50% ethanol, 25% isopropanol, 7% butanol and 4% isoamyl alcohol, but deformed by octanol saturated in 30% ethanol or by 1% "Tween 80".

Polarographic Reduction——It has been known[9] that sennoside A (VIa) is reduced to rheinanthrone-8-glycoside (VII) by hydrogen on palladium or dithionite, and partially to the anthracene (XII) by zinc with acid. Anthrone (VII′) is in equilibrium with a trace of anthrol (VIII′) and converts to anthrol in photo-irradiation.[10] Anthrone is reduced to dianthranyl (X′) *via* pinacol (IX′) and to anthracene (XII′) *via* 9,10-dihydroanthrol (XI′) by zinc with alkali.[10]

As mentioned formerly, VI gives 2-electron reduction wave with 2 adsorption prewaves and a post-wave having kinetic nature. Polarographic reduction of VIa in the third step forms the anthrone (VII), which is successively reduced to the anthracene (XII) in the post-wave. These products in the electrolysis are the same in the chemical reduction. The 2-electron reduction waves including the prewaves are, therefore, assigned to reductive cleavage of the C–C bridge. Generally single C–C bonds are hardly reduced in polarography. The C–C bridge conjugated in the carbonyls seems to cleave probably through ketyl radical formed by initial attack of electrons on the carbonyls.

The reduction potential of the post-wave is close to that of anthrone (VII′). The step is ascribed to reduction of the carbonyls in 2 moles of VII. The kinetic nature and the small wave height in VI are due to the equilibrium of active anthrone (VII) and inactive anthrol (VIII) on the electrode.



R_1: OH, R_3: CO_2H, R_8: O-glucose, R_3': CO_2H (VIa, VIb) or CH_2OH (VIc)
VII′—XII′: R_1, R_3, R_8: H

Chart 2

9) A Stoll, B. Becker, and A. Helfenstein, *Helv. Chim. Acta*, 33, 316 (1950); L. Hörhammer, H. Wagner, and I. Köhler, *Arch. Pharm.*, **292**, 591 (1959).
10) G.E. Risinger and C.W. Eddy, *Chem. Ind.* (London), **1963**, 570; N. Kanamura and S. Nagakura, *Bull. Chem. Soc. Japan*, 39, 1355 (1966); H. Baba and T. Takemura, *ibid.*, 37, 1241 (1964).

Analysis of Anthraquinones and Dianthrones in Rhubarb

1,8-Dihydroxyanthraquinones and 1,8-dihydroxyanthrones in drugs have been determined by the reduction waves at −0.4 and −1.2 V, respectively in acetate buffer, in which each homologues can not be distinguished.[4f] Rhubarb contains a few percent of anthraquinones (I—V) and sennosides (VI). A principle of the diarrhoeal activity in mice seems to be VI.[3] Determination of VI besides I—V is necessary to evaluate the drug.

Inspecting the pH $-E_{1/2}$ curves (Fig. 1), the best condition for polarographic analyses of I—VI was found to be isopropanol solution at about pH 7—10, where I, V and VIa give separate waves although mixture of I—V will give an overlapped wave. Mixtures of I and VIa in 25% isopropanol at pH 10 gave well defined waves with heights proportional to the individual concentrations (0.02—1 mM) within variation coefficient of 1% (method 1 described in the experimental). The anthraquinones (I—V), sennosides (VI) and anthrones (or flavones) in rhubarb can be extracted with the alkaline buffer containing 25% isopropanol or 7% butanol and determined by their reduction waves. Especially the anthraquinones, abundant components of rhubarb, can be easily determined by this method within 3 hr. Correlation between the contents of anthraquinones and the diarrhoeal activity,[3] reciprocal of half effective dose in mice ($1/ED_{50}$), was poor as correlation factor (r) of 0.3 for 20 lots of rhubarb.

The procedure including the extraction with neutral buffer is suitable for the determination of VI because of less disturbance by the predepolarizing quinones (method 2).

For more accurate determination of VI, it was preferable to clean up VI from I—V by the extraction with butanol (method 3). It took about 5 hr and the precision was within ±2%. The content of VI in rhubarb measured by this method correlated fairly (r=0.8) with the diarrhoeal activity ($1/ED_{50}$). We hesitated, however, to use the physical method in place of biological assay since the correlation was somewhat tenuous among different sources of rhubarb and mice to be tested.

The reduction wave of VI was masked by foreign substances in a degraded rhubarb which can be separated by PPC (method 4). Since it took about 10 hr and the precision was not good (±5%), this procedure had comparable merits to the colorimetry after separation by PPC.[3]

Acknowledgement The authors express their thanks to Dr. S. Tatsuoka for permission of this publication. Thanks are due to Dr. M. Miyamoto and his colleagues for offering materials and their informations and also to Mr. M. Yamazaki for his biological assay.