

recrystallized from acetic acid, yielding white needles, m. p. 199–200°.

*Anal.* Calcd. for  $C_9H_{10}ClNO_2$ : Cl, 17.79. Found: Cl, 16.87.

**2-Acetamino-4-chloro-3,6-dimethylphenol.**—A mixture of 87.5 g. of 2-nitro-3,6-dimethylphenol, prepared according to von Auwers,<sup>9</sup> and 150 cc. of redistilled sulfonyl chloride was allowed to stand for one hour. Excess sulfonyl chloride was removed at the water pump to yield 104.5 g. (99.3%) of crude 2-nitro-4-chloro-3,6-dimethylphenol, yielding lemon yellow crystals, m. p. 54–55°. A solution was prepared of 42 g. of this crude nitro compound and 120 g. of sodium carbonate in 1.5 liters of water, and then was added, portionwise, with stirring at 75°, 175 g. of sodium hydrosulfite. The mixture was heated at 90° for twenty minutes, then cooled to room temperature. The amine was collected at the pump, washed with 1 liter of water, dissolved in 500 cc. of ether, dried over calcium chloride, treated with Darco, filtered, and, after concentration to 50 cc., precipitated with 100 cc. of ligroin; yield was 15 g. (42%) of 2-amino-4-chloro-3,6-dimethylphenol, forming buff crystals, m. p. 153–155°. The amine was acetylated with 1:1 acetic anhydride–acetic acid, giving light tan crystals, m. p., 169–171°. Recrystallized from acetic acid, m. p., 169–171°.

**Quinonanil.**—*p*-Hydroxydiphenylamine was oxidized in benzene solution with yellow mercuric oxide, as described by Calm.<sup>10</sup> The crude material was recrystallized once from ligroin, then twice from spectroscopically pure cyclohexane, producing fine orange needles (50% yield), m. p. 94°, with previous softening.

(9) von Auwers, Murbe, Sauerwein, Deines and Schornstein, *Fortschr. Chem., Physik. physik. Chem.*, **18**, No. 2, 37–77 (1924).

(10) Calm, *Ber.*, **16**, 2799 (1883); see, also, Uemura and Abe, *Bull. Chem. Soc. Japan*, **12**, 59 (1937).

*Anal.* Calcd. for  $C_{12}H_9ON$ : N, 7.65. Found: N, 7.68.

**Spectrophotometric Measurements.**—All of the spectrophotometric measurements were made on  $5.0 \times 10^{-5}$  M solutions in 1-cm. cells. The ultraviolet absorption spectra were recorded photographically, using a Hilger rotating sector ultraviolet spectrograph with a photometer attachment, in conjunction with an iron spark. Some check readings were made with Beckman ultraviolet spectrophotometric equipment. Absorption spectra in the visual range were obtained with the General Electric Automatic Recording Spectrophotometer. Specially purified solvents, checked for their transparency down to 2100 Å., were used. We wish to thank Mr. E. E. Richardson and his associates for supplying these solvents, and for making all of the spectrophotometric measurements.

### Summary

The effects of multiple substitution in the oxygen-containing ring of the indoaniline dye, phenol blue, on the absorption characteristics have been determined. In general, the shifts in  $\lambda_{max}$  and  $\epsilon_{max}$  brought about by two or more substituents agree quite closely with values calculated by totalling the shifts for the separate substituent groups.

An exception was noted in the dye from 3,5-dimethylphenol. In this dye, the resonance system is altered because the two methyl groups exert a mechanical interference with the planarity of the dye molecule.

ROCHESTER 4, N. Y.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF CALIFORNIA, LOS ANGELES]

## Flavanones and Related Compounds. IV. The Reduction of Some Naturally-Occurring Flavones at the Dropping Mercury Electrode

BY D. W. ENGELKEMEIR,<sup>1</sup> T. A. GEISSMAN, W. R. CROWELL AND S. L. FRIESS

Methods suitable for the analysis of small amounts of fresh plant tissue for their content of flavonoid substances are of particular interest in connection with studies on the genetical factors which control the synthesis of these substances in the organism. While spectroscopic and colorimetric methods are applicable to the anthocyanin pigments, their application to the analysis of acyanic tissues for flavone derivatives leaves much to be desired. An examination is being made of the behavior of compounds of this class (flavones, flavanones, chalcones and benzalcoumaranones) when they are reduced at the dropping mercury electrode. The only work of a related nature so far reported is the study of Adkins and Cox<sup>2</sup> on the polarographic reduction of a number of chromones, including flavone. Since the compounds in which we are interested are polyhydroxy compounds (and ethers and glycosides of these), further information was needed concerning the effect of the type and position of such substituents upon

the reducibility of compounds of the classes mentioned above.

The results reported in the present paper deal with several naturally-occurring flavonols (3-hydroxyflavones) and some of their derivatives. These are quercitrin (I), quercetin (II), quercetin-3',4',5,7-tetramethyl ether (III), quercetin pentamethyl ether (IV), quercetin pentaacetate (V), apigenin (VI) and flavonol (VII). The aims of this phase of the study were as follows: (1) To determine the half-wave potentials, or  $\pi_{1/2}$  values, at various pH's. (2) To determine the relationship between flavone concentrations ( $C$ ) and wave heights ( $h$ ) in the concentration range  $10^{-3}$  to  $10^{-4}$  molar. (3) To study the influence of the nature of the group in the 5-position (H, OH, OCH<sub>3</sub>, OCOCH<sub>3</sub>) on the half-wave potential. (4) To determine the values of the diffusion coefficients,  $D$ , in the Ilkovic equation  $i_D = 605nD^{1/2}m^{2/3}t^{1/6}C$ , where  $n$  is the number of electrons involved in the electrode reaction,  $D$  the diffusion coefficient and  $C$  the concentration of the diffusing substance,  $m$  the mass of mercury dropping per second, in

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(2) Adkins and Cox, *THIS JOURNAL*, **60**, 1151 (1938).

TABLE I

	$\pi^{1/2}$ vs. S. C. E.			$i_D$		
	pH 5.6	pH 7.7	pH 10.4	$Cm^{3/2}t^{1/6}$	pH 5.6	pH 7.7
Quercitrin (I)	-1.46	-1.58	-1.81	3.1	2.9	3.4
Quercetin (II)	-1.53	-1.62	-1.81	3.0	2.73 <sup>a</sup>	2.1
Quercetin tetramethyl ether (III)	-1.41 <sup>b</sup>	-1.47	-1.59	1.9	1.6	1.82
Quercetin pentamethyl ether (IV)	-1.27 <sup>c</sup>	-1.43	-1.54 <sup>d</sup>	1.8	1.8 <sup>e</sup>	2.0 <sup>f</sup>
Quercetin pentaacetate (V)	-1.10	-1.16	-1.72	1.8 <sup>g</sup>	1.9 <sup>g</sup>	3.2 <sup>h</sup>
Apigenin (VI)	-1.57	-1.63	-1.85	2.9	2.8	2.5
Flavonol (VII)	-1.25	-1.33	-1.45	2.08	2.06	2.02

<sup>a</sup> In calculating values of  $i_D/Cm^{3/2}t^{1/6}$  more significant figures were retained when a smooth curve with a well-defined diffusion current was obtained. <sup>b</sup> The curve in this case appeared to be made up of two inflections of about equal height. The "break" between the two is barely noticeable. Approximate individual half wave potentials were -1.33 and -1.47 v. <sup>c</sup> 0.01% gelatin added to suppress maximum. <sup>d</sup> Addition of 0.01% gelatin had little effect on the maximum, which was of the rounded hump variety. <sup>e</sup> Wave height measured on curve before the addition of gelatin, which reduced corrected wave height from 39.5 mm. to 20 mm. <sup>f</sup>  $i_D$  measured after the maximum had been passed. <sup>g</sup> A small wave about  $1/6$  of the height of the main wave was observed at about -1.21 v. in pH 5.6 buffer, and at -1.33 v. in pH 7.7 buffer. This wave height was not included in calculating  $i_D/Cm^{3/2}t^{1/6}$ . <sup>h</sup> A small wave of about the same height as noted in <sup>g</sup> was observed before the maximum at about -1.50 v.

milligrams, and  $t$  the drop time in seconds. (5) To determine the reversibility of the reduction step and the number of electrons involved in this step. Of these (4) remains to be dealt with more thoroughly in future work, but the experimental

data so far obtained make it possible to draw useful conclusions regarding the other points.

### Experimental

All measurements were made on a modified Heyrovsky polarograph constructed under the supervision of one of us (W.R.C.). The temperature of the solutions in the polarograph cells was maintained at  $25.0 \pm 0.1^\circ$  in all runs.

**Buffers.**—The following buffer solutions were made up:

	Components	pH
A	0.4 M (CH <sub>3</sub> ) <sub>4</sub> NOH-HOAc	5.0
B	0.4 M (CH <sub>3</sub> ) <sub>4</sub> NOH-H <sub>3</sub> PO <sub>4</sub>	7.0
C	0.4 M (CH <sub>3</sub> ) <sub>4</sub> NOH-H <sub>3</sub> BO <sub>3</sub>	9.0

These were diluted to 50% with purified *i*-propyl alcohol before use in the polarographic runs. The buffers so prepared had the following characteristics:

Buffer solution (diluted 50% by vol. with <i>i</i> -C <sub>3</sub> H <sub>7</sub> OH)	pH by	
	Quinhydrone electrode	Glass electrode
A	5.56	...
B	7.64	7.73
C	..	10.4

In the following discussion, the word "buffer" will refer to the solutions 50% in *i*-propyl alcohol, with pH values of 5.6, 7.7 and 10.4.

**Procedure.**—Solutions of the flavones to be reduced were made up in the appropriate buffer and freed of oxygen by the use of nitrogen purified by passage through a chromous chloride<sup>3</sup> tower. Each deaeration was conducted for twenty to thirty minutes prior to starting a run. All potentials were measured against the saturated calomel electrode (S.C.E.).

### Results

In Table I is presented a summary of the experimental data.

In Fig. 1 are shown representative polarograms

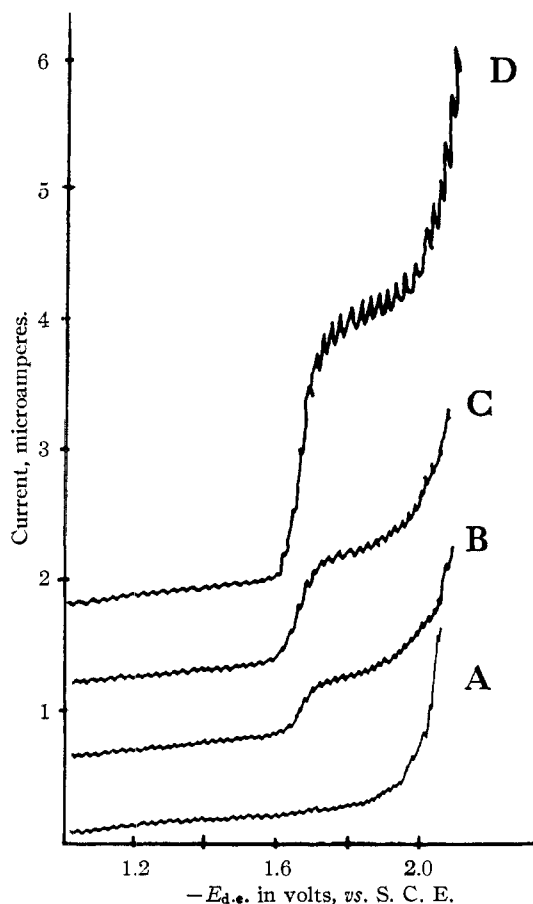


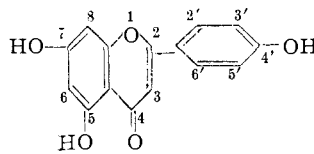
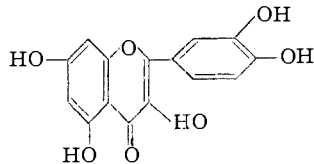
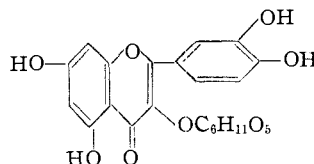
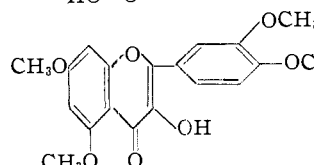
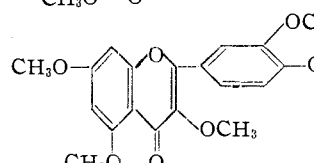
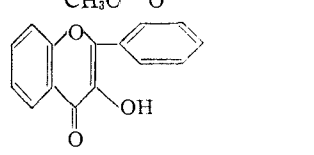
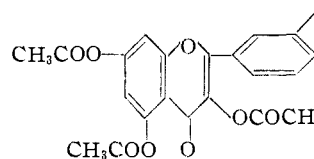
Fig. 1.—Quercetin, pH 7.7; condenser out, sensitivity  $1/10$ ; at -1.80 v.,  $m^{3/2}t^{1/6} = 1.135$ : A, buffer blank; B, concn.  $1.27 \times 10^{-4} M$ ; C, concn.  $2.50 \times 10^{-4} M$ ; D, concn.  $5.03 \times 10^{-4} M$ .

(3) Stone and Skavinski, *Ind. Eng. Chem., Anal. Ed.*, **17**, 495 (1945).

for quercetin at varying concentrations in the buffer of  $pH$  7.7.

All of the values for  $\pi_{1/2}$  are corrected for the  $IR$  drop of the solution and of the sliding contact in the instrument.

**Shift of Half-Wave Potential with Changes in Structure.**—The structures of the flavones studied are shown below with their  $\pi_{1/2}$  values at  $pH$  7.7

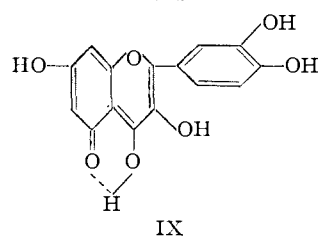
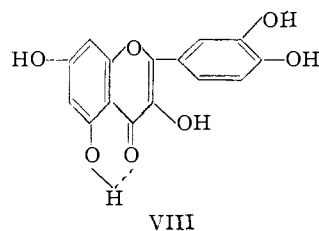
	$\pi_{1/2}$ v. at $pH = 7.7$
	VI    -1.63
	II    -1.62
	I    -1.58
	III   -1.47
	IV   -1.43
	VII   -1.33
	V    -1.16

Since more negative  $\pi_{1/2}$  values correspond to more difficult reduction (probably of the carbonyl group, as shown later), the  $\pi_{1/2}$  data in this restricted group of compounds lead to certain conclusions regarding the effect of the substituents studied on the readiness of flavone reduction.

The relatively slight effect of the 3-hydroxyl group is seen by comparing III and IV, II and VI, and I and II. Here, methylation or glyco-

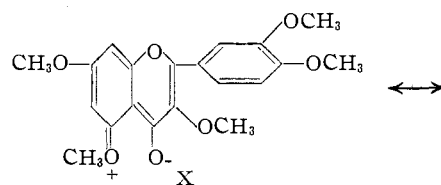
sylation of the 3-hydroxyl group results in a slightly greater ease of reduction of the resulting flavone as compared with the otherwise corresponding flavone having the free 3-hydroxyl group. Quercitrin and quercetin pentamethyl ether both have negative values of  $\pi_{1/2}$  lower by 0.04 v. than quercetin and quercetin 3',4',5,7-tetramethyl ether, respectively. This difference is small, however, and may not be due to the presence or absence of the 3-hydroxyl group *per se*, as will be shown below from the study of the effect of  $pH$  on the  $\pi_{1/2}$  values of 3-hydroxylated flavones.

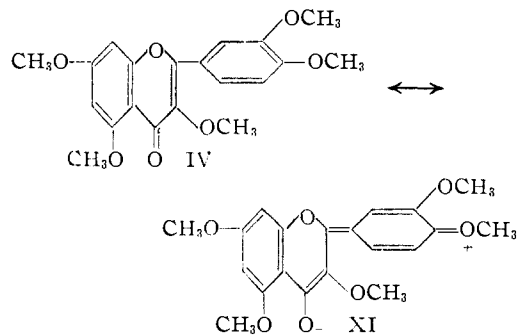
A more profound effect on the carbonyl reducibility is shown by those substituted flavones having the 4',5-dihydroxyl grouping. Although it is not possible to separate the individual effects of the 4' and 5-hydroxyl groups from the data given, it seems probable that the 5-hydroxyl group predominates. Thus, the most likely explanation for the difficult reducibility of I, II and VI is that in these compounds there is strong hydrogen bonding between the 4- and 5-positions, involving such forms as (in the case of II) VIII and IX



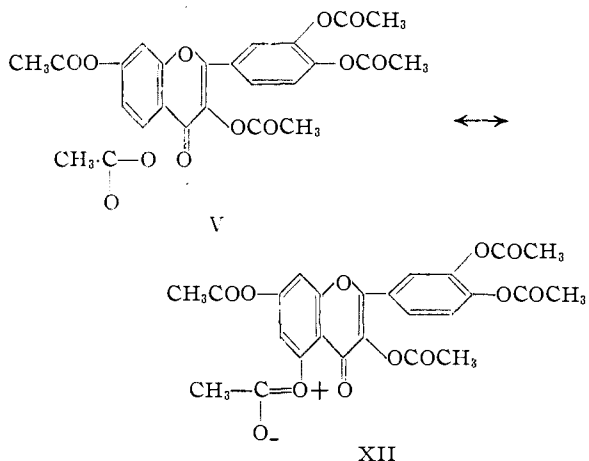
Secondarily, both the 4'- and 5-hydroxyl groups, or for that matter methoxyl or acetate groups too, can enter into resonance structures which lend  $-O^-$  character to the carbonyl group and probably render reducibility more difficult.

In this latter connection, the large drop in  $\pi_{1/2}$  values observed in the case of quercetin pentaacetate as compared with quercetin pentamethyl ether indicates that factors other than hydrogen bonding play an important part in modifying the ease with which these substances are reduced. In the case of the more difficultly reduced pentamethyl ether, the carbonyl group can participate





in resonance of the kind shown in X and XI, while the greatly reduced basicity of the oxygen atoms attached to the aromatic nuclei in the pentaacetate as a result of forms such as XII prevents stabilization of this sort in V.



**Shift of Half-Wave Potential with Changes in pH.**—A consideration of the curves showing the relationship between  $\pi_{1/2}$  and pH, given in Fig. 2, furnishes further information regarding the factors which contribute to the ease of reduction of these substances. The curve of flavonol is

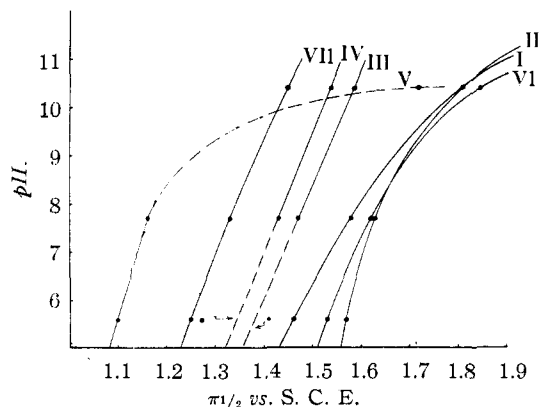
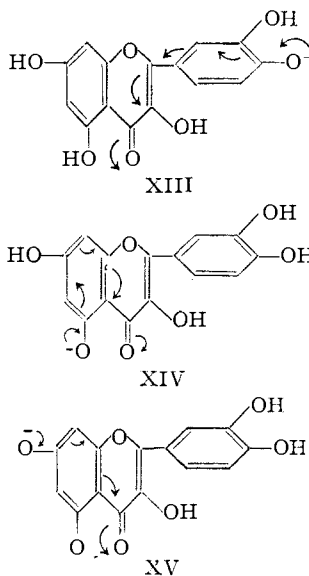


Fig. 2.—Plot of  $\pi_{1/2}$  vs. pH for: I, quercitrin; II, quercetin; III, quercetin tetramethyl ether; IV, quercetin pentamethyl ether; V, quercetin pentaacetate; VI, apigenin; VII, flavonol.

nearly a straight line over the entire pH range studied.<sup>4</sup> While the points at pH 5.6 deviate from a straight line in the cases of quercetin tetra- and pentamethyl ether, the points at the two higher pH's can be connected by lines which are substantially parallel with the curve for flavonol. However, in the latter two cases, the  $\pi_{1/2}$  values at pH 5.6 are uncertain due to the irregularities noted in footnotes *b* and *c* to Table I. The curve for quercetin pentaacetate at low pH's has a slope slightly greater than the three already mentioned; but at higher pH's it changes rapidly until the  $\pi_{1/2}$  value at pH 10.4 is nearly as great as that for quercetin. This is undoubtedly due to the hydrolysis at higher pH of the acetyl groups; whether the  $\pi_{1/2}$  value at pH 10.4 is that of some discrete acetyl derivative of quercetin or of a mixture of partially acetylated molecules is unknown.

The curves for those derivatives which contain free hydroxy groups in positions 4', 5 and 7 are interesting, in that while at low pH's they are substantially parallel with the curve for flavonol, at higher pH's they deviate markedly from this slope, their  $\pi_{1/2}$  values showing a greater increase with increasing pH than those of the compounds which contain no free hydroxyl groups in the 4', 5 and 7 positions. These positions bear a unique relationship to the carbonyl group at 4, since negative charges arising by ionization of the 4', 5 or 7 hydroxyl groups can resonate with the carbonyl group as shown in XIII, XIV and XV



(4) For a reversible electrode reaction, the curve showing the relationship of  $\pi_{1/2}$  to pH should be a straight line of slope  $-0.059$  (for a one-electron change). Moderate departure from a straight line, as in the case of VII, does not necessarily mean that pH changes result in modification of the structure (e. g., ionization of  $-\text{OH}$  groups) in such a way as to influence the reducibility of a group taking part in an electrode reaction, but may be due to (a) some degree of irreversibility of the reaction and (b) the fact that the pH values used are nominal values only, being those for alcoholic solutions in which the function is not clearly defined.

This resonance undoubtedly contributes to the stabilization of the carbonyl group toward reduction at the dropping mercury electrode. It should be noted that there is no way in which the ionization of the 3-hydroxyl group can exert a similar influence upon the carbonyl group, since resonance of the kind shown in XIII to XV is impossible in such a case.

**Variation of Wave Height with Flavone Concentration.**—For this study, quercetin was chosen as a model substance, using the *pH* 7.7 buffer and a condenser to minimize fluctuations in the polarograms. A graph of wave height (plotted as diffusion current, in microamperes) versus concentration (in millimoles/liter) demonstrated that perfect linearity exists over the range 0.1–1.3 millimoles/liter.

**The Reversibility of the Reduction (with Quercetin).**—To examine whether the reduction is reversible, and to determine the number of electrons involved in the electrode reaction, use is made of the equation for a reversible, symmetrical wave:

$$E_{1.e.} = \text{const.} - \frac{0.059}{n} \log \frac{i}{i_D - i} \quad (25^\circ)$$

If  $\log i/(i_D - i)$  is plotted against the corresponding values of  $E_{d.e.}$ , the curve should be a straight line if the reaction is reversible. If the curve is not a straight line, a tangent to it at  $E_{d.e.} = \pi_{1/2}$  should have a slope approximately equal to  $-0.059/n$ , where  $n$  is the number of electrons involved in the reduction.

In Fig. 3 values of  $\log i/(i_D - i)$  vs.  $E_{d.e.}$  are plotted for quercetin in a typical run. The curve has a slight but definite curvature, indicating that the reaction is not completely reversible. A tangent to the curve at the value of  $E$  corresponding to  $\pi_{1/2}$  has a slope of  $-0.056$ . Thus, to a fairly good approximation the electrode reaction involves a one-electron change.

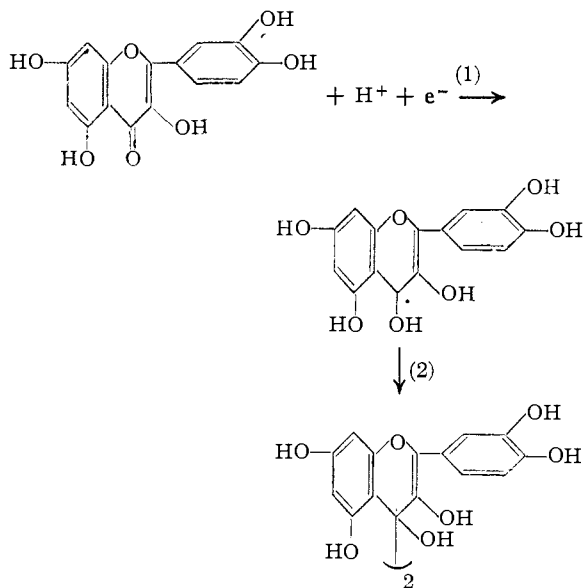
In terms of an actual electrode reaction, a one-electron change might involve the reactions shown, where (1) is the electrode reaction, and (2) is a subsequent reaction taking place in the surrounding medium:

Reactions of this sort, involving the bimolecular reduction of carbonyl compounds, are well known. Examples that might be quoted are the reduction of benzophenone to benzopinacol with magnesium and magnesium iodide,<sup>5</sup> the bimolecular reduction of flavanone with magnesium and hydrochloric acid,<sup>6</sup> and the reduction of such ketones as benzalacetophenone by metal combinations.<sup>7</sup>

(5) Gomberg and Bachmann, *THIS JOURNAL*, **49**, 236 (1927).

(6) Freudenberg and Orthner, *Ber.*, **55B**, 1748 (1932); Geissman and Clinton, *THIS JOURNAL*, **68**, 700 (1946).

(7) Allen and Blatt in "Organic Chemistry," edited by Gilman, John Wiley and Sons, New York, N. Y., 1943, p. 677.



Further work is in progress in this Laboratory on the influence of structure on the behavior of flavones and related compounds at the dropping mercury electrode.

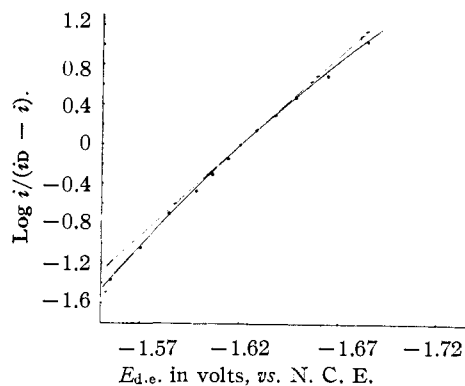


Fig. 3.—Plot of  $\log i/(i_D - i)$  vs.  $E_{d.e.}$  for quercetin, *pH* 7.7: at  $E = \pi_{1/2} = -1.62$  v.,  $(1/\text{slope}) = -0.056$  v.

## Summary

Studies on the polarographic reduction of a number of hydroxylated flavones and some of their derivatives have shown (a) the relationship between structure and half-wave potential at several *pH* values, (b) the strict linearity of the relationship between wave-height and concentration in one typical case, and (c) the probable course of the reaction involving a one-electron change in an electrode reaction which is nearly a reversible one.

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