[Contribution from the Department of Chemistry of the University of Oklahoma]

Amperometric Titrations of Some Flavonoid Compounds with Cupric Sulfate

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Flavone, flavonol, flavane and flavanone compounds were titrated amperometrically in buffered, essentially aqueous solutions with cupric sulfate, using the Sargent–Heyrovsky model XII polarograph. From the recorded data, the ratio of flavonoid to copper for each compound has been obtained. The results indicate the importance of *p*H in the titrations and fit present chelation concepts quite well.

Introduction

Geissman and co-workers[1] have reported studies by classical polarography on representatives of several classes of flavonoid compounds and some of their derivatives. Čapka and Opavský[2] have investigated the polarographic reduction of rutin, quercetin and morin. Clark and Geissman[3] have also investigated polarographically the ability of certain flavonols to form copper complexes and have proposed possible structures for some of these to indicate the formation of intermolecular copper–flavonoid chelation complexes.

The present paper describes studies on flavonoid metal combination through use of amperometric titrations to learn the applicability of this method to the analysis and characterization of these substances.

Experimental

Materials and Methods.—One of the leads from the constant e.m.f. jack of a Sargent–Heyrovsky model XII polarograph was connected to the mercury reservoir of a dropping mercury electrode and the other lead to a saturated calomel electrode. The two half-cells were joined by a bridge containing 3% agar saturated with potassium chloride. The half-cell in which the capillary tip was immersed, consisted of an ordinary 250-ml. electrolytic beaker fitted with a five-hole rubber stopper.

In a typical amperometric titration, 120 ml. of 0.5 *M* sodium acetate buffer solution, whose *p*H had been checked with a Beckman model G *p*H meter, was first degassed with a vigorous stream of purified inert gas such as tank nitrogen. A 5- or 10-ml. aliquot of a 95% ethanolic solution of a flavonoid was then pipetted into the cell and the resulting solution further degassed until a constant current reading was again obtained at the constant voltage setting of the polarograph. Solutions of flavonoids prepared in this way were examined before and after degassing for any appearance of turbidity. Those which remained clear at the *p*H and flavonoid concentration being used were titrated with standard solutions of 0.01 or 0.02 *M* cupric sulfate solution.

The low solubility of 3-hydroxyflavone, and of its sodium salt, in essentially aqueous solutions of even moderately high *p*H, necessitated the use with it of a 50% isopropyl alcohol solution containing 0.2 *M*, *p*H 5, sodium acetate. The solutions of the other flavonoids were prepared as already mentioned in the previous paragraph.

The cupric sulfate solution was added in 0.05- to 0.25-ml. increments from a 5-ml. microburet whose tip inserted through one of the holes of the rubber stopper. After each addition of titrant, the cell solution was mixed by a 1–2 minute degassing period, following which the current reading of the polarograph was recorded. The titrations were carried out at room temperature which varied less than one degree in any one experiment. In some cases it was necessary to perform the titrations at more than one galvanometer shunt ratio or voltage setting of the polarograph. By this expedient, data could be obtained which showed the clearest breaks in the current *vs.* volume curve for a given flavonoid compound.

The flavonoids which were used in this investigation include representatives of the flavone, flavonol and flavanone groups as well as the flavane, 1-epicatechin. The choice of cupric sulfate as titrant was based on the fact that it was found to be very well adapted to amperometric work. Its extremely low half-wave potential, wide range of solubility and relatively high coördinative capacity render it highly suitable for such studies. Aluminum chloride and lead acetate were found to be much less adaptable to amperometric titrations with flavonoid compounds.

The flavonoid compounds studied are listed in Table I. Both the common and descriptive names are given. The source of each compound is listed also.

Table I

List of Flavonoids Studied Amperometrically

Common name	Descriptive name
Flavonols	
Flavonol[a]	3-Hydroxyflavone
Morin[b]	2′,3,4′,5,7-Pentahydroxyflavone
Quercetin[c]	3,3′,4′,5,7-Pentahydroxyflavone
Rutin[d]	Quercetin 3-rhamnosidoglucoside
Flavone	
Glucoluteolin[e]	7-Glucoside of 3′,4′,5,7-tetrahydroxyflavone
Flavanones	
Liquiritigenin[f]	4′,7-Dihydroxyflavanone
Butin[f]	3′,4′,7-Trihydroxyflavanone
Naringenin[g]	4′,5,7-Trihydroxyflavanone
Hesperetin[h]	3′,5,7-Trihydroxy-4′-methoxyflavanone
Dihydroquercetin[i]	3,3′,4′,5,7-Pentahydroxyflavanone
Flavane	
1-Epicatechin[j]	3,3′,4′,5,7-Pentahydroxyflavane

[a] Distillation Products Industries of Eastman Kodak Co., Rochester, N. Y. [b] L. Light and Co., Ltd., Colnbrook, Bucks, England. [c] Prepared by hydrolysis of rutin. [d] S. B. Penick and Co., New York, N. Y. [e] Courtesy of Dr. Shizuo Hattori, Botanical Institute, University of Tokyo, Japan. [f] Courtesy of Mr. Joseph Pew, Forest Products Laboratory, Madison, Wis. [g] Prepared by hydrolysis of naringin. [h] Courtesy of Sun Kist Growers, Ontario, Calif. [i] Courtesy of Oregon Forest Products Laboratory, Corvallis. [j] Courtesy of Dr. Charles H. Horton, Oak Ridge, Tenn.

Results.—The amperometric titration curves of these flavonoid compounds with the cupric sulfate solution are given in Figs. 1–5. Liquiritigenin gave no indication of interaction with cupric sulfate at *p*H 7.0 or 10.5. In these curves the current, in arbitrary units, is plotted as ordinate. The abscissa is the number of milliequivalents of added reagent per milliequivalent of flavonoid originally present in the solution being titrated. The breaks in the curves show at a glance the ratio of cupric ion

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(1) D. W. Engelkemeir, T. A. Geissman, W. R. Crowell and S. L. Friess, This Journal, **69**, 155 (1947); and T. A. Geissman and S. L. Friess, *ibid.*, **71**, 3893 (1949).

(2) O. Čapka and J. Opavský. *Collection Czechoslov. Chem. Communs.*, **15** [7], 433 (1950).

(3) W. G. Clark and T. A. Geissman, "Biological Antioxidants," ed. by C. G. MacKenzie, Josiah Macy, Jr., Foundation, New York, N. Y., 1948, pp. 92–112; W. G. Clark and T. A. Geissman, *J. Pharm. and Exptl. Therap.*, **95**, 363 (1949).

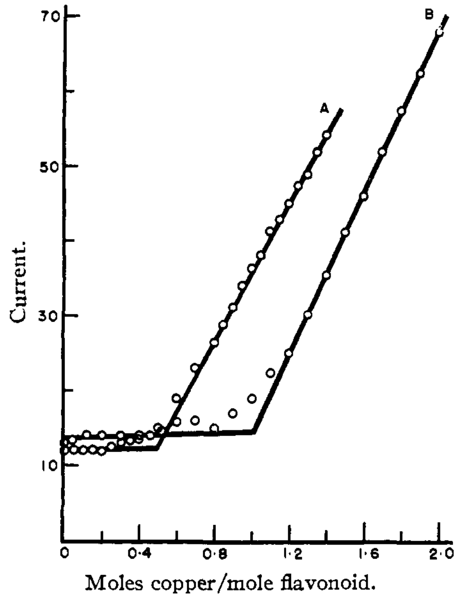

Fig. 1.—A, morin at *p*H 9.3; B, quercetin at *p*H 7.5.

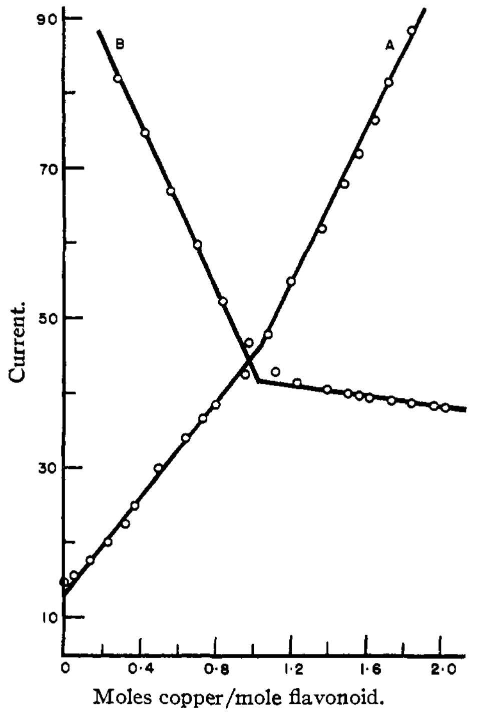

Fig. 2.—A, rutin titrated with CuSO$_4$; B, CuSO$_4$ titrated with Na–rutin.

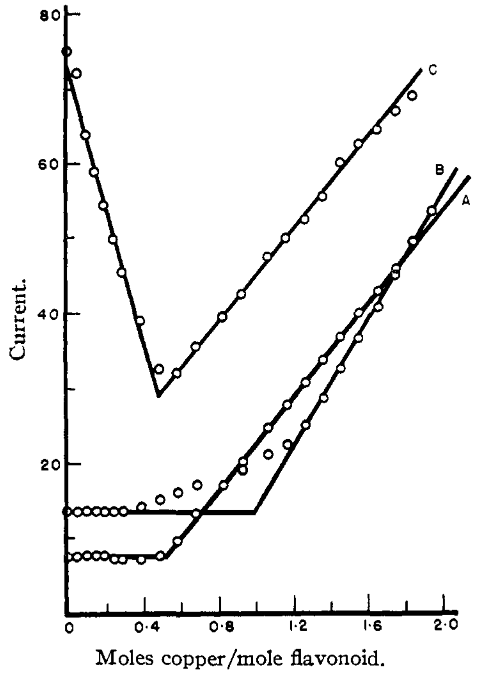

Fig. 3.—Glucoluteolin: A, *p*H 6.5, 0.10 volt; B, *p*H 10, 0.10 volt; C, *p*H 6.5, 1.7 volt.

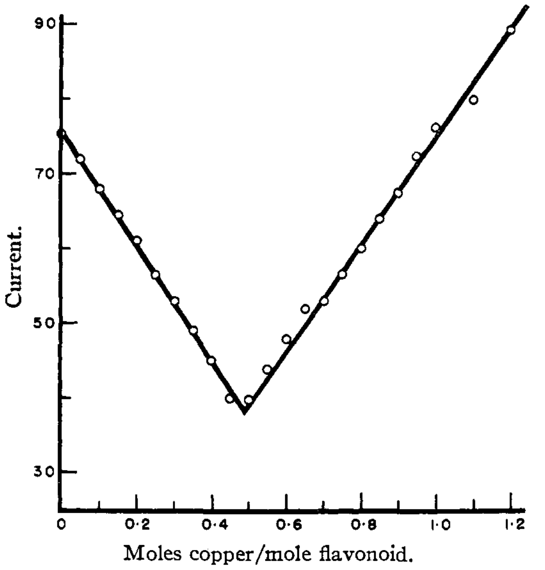

Fig. 4.—Hesperetin at *p*H 9, 1.85 volt.

to flavonoid in the chelate complex formed. Table I summarizes those flavonoids studied whose curves indicated the possibility of their complexing cupric ion, at the *p*H given, in the ratio of two flavonoid units per unit of copper.

TABLE II

FLAVONOID:COPPER = 2:1

Compound	Apparent *p*H
Flavonol	5
Morin	9.3
Glucoluteolin	6.5
Butin	8.9
Hesperetin	9.0
Dihydroquercetin	6.5
1-Epicatechin	8.0, 11

Table III summarizes those flavonoids studied which gave curves indicative of a possible 1:1 chelation with cupric ion at the *p*H given.

TABLE III

FLAVONOID:COPPER = 1:1

Compound	Apparent *p*H
Quercetin	7.5
Rutin	7.0
Glucoluteolin	10.0
Dihydroquercetin	10.0

In Table IV are listed those flavonoids whose

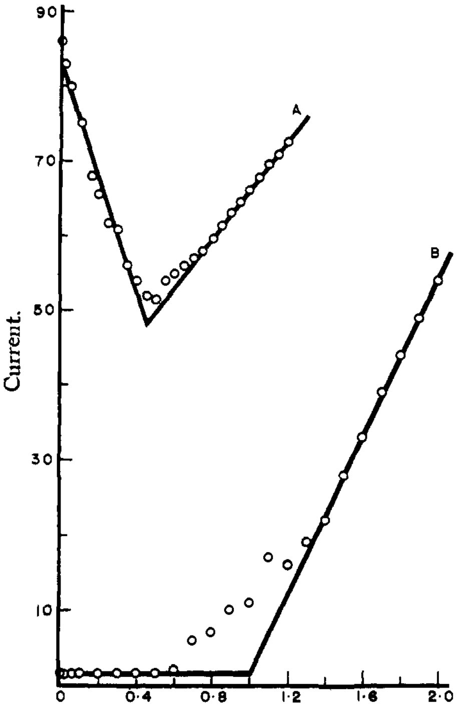

Fig. 5.—Dihydroquercetin: A, *p*H 6.5, 1.75 volt; B, *p*H 10.0, 0.10 volt.

current volume curves showed no breaks at the *p*H values indicated.

Table IV

Compound	Apparent *p*H
Liquiritigenin	7.0; 10.5
Butin	7.0
1-Epicatechin	6.5

Many of the titrations were carried out considerably below the half-wave potentials of the flavonoids tested, but above the applied voltage at which cupric ion normally is reduced. Under these conditions, reversed L-shape curves were obtained, as expected. Figure 1 is typical of curves of this type. In Fig. 2 the effect of reversing the order of addition of the reactants is shown, and in this titration the break in the curve is more readily apparent when the flavonoid is added to the copper solution.

Curves A and B in Fig. 3 show that more chelating groups become available as the *p*H is increased, the ratio of flavonoid:copper being 2:1 at *p*H 6.5 and 1:1 at *p*H 10. At higher applied voltage both flavonoid and copper are reduced, the complex showing the minimum reduction current; and a V-shaped curve, C in Fig. 3, is obtained.

Discussion

Clark and Geissman,[3] by polarographic studies, have shown that chelation of flavonoid compounds with metals occurs. These studies show disappearance or diminution of the normal copper wave and appearance of a new wave (the chelate) at a more negative half-wave potential in mixtures containing cupric ion and a number of flavonoid compounds. Those workers concluded that in the flavonoid compounds the important complex-forming elements were the 3′,4′-dihydroxy grouping and the 3-hydroxy-4-keto grouping. Included among other possibilities, however, are the 5-hydroxy-4-keto grouping. Clark and Geissman report the actual isolation and purification of the copper complex of 3-hydroxyflavone. They found its elementary analysis to be in accord with a chelated structure with a 2:1 flavonol–copper ratio, and propose a chelate structure for the copper complex.

On this basis, the flavonoids 3-hydroxyflavone, butin, naringenin, hesperetin, morin and 1-epicatechin contain only one major potential chelating site, and theoretically would be capable of forming intermolecular chelate complexes in a ratio of two flavonoids to one copper. The breaks obtained, in the graphs drawn from the amperometric titration data of these compounds *vs.* cupric sulfate, indicate that the contribution of cupric ion to the net polarographic current was not significant until an amount was added to the cell equal to one-half the molar concentration of flavonoid material present. Since no such breaks were obtained from similar titrations at the same *p*H range in which chelatogenic flavonoids or other chelating materials were absent, it appears that, under the conditions used, cupric ion interacts in some manner with the chelatogenic flavonoids tested. The data shown in Table II for these compounds thus seem to fit well the concept of possible chelation as suggested by Clark and Geissman.[3] Particularly in the determinations between *p*H 5–9, it accounts quite satisfactorily for the sharpness of the curve breaks which were obtained at the stoichiometric points required for intermolecular chelation.

The inclusion of glucoluteolin, dihydroquercetin and 1-epicatechin in two tables is discussed in a later paragraph.

Flavonoids such as glucoluteolin, dihydroquercetin, quercetin and rutin possess two potential chelating sites, and on the basis of the chelation concept, theoretically should be capable of forming intermolecular chelate rings in a ratio of one flavonoid to one copper. Table III lists the apparent *p*H values at which the amperometric titrations of these flavonoid compounds yielded data showing this 1:1 ratio.

The results reported in this study on rutin and quercetin also check with those of Hörhammer and Hänsel.[4] These workers used a photometric method to show that the colored complexes of these two compounds with zirconium (+4), aluminum (+3), and copper (+2) are mainly 1:1 complexes.

Reference to the amperometric curves for glucoluteolin at *p*H 10 (Fig. 3) and dihydroquercetin at *p*H 10 (Fig. 5), shows the extrapolation involved in computing the curve beaks for these two compounds with equimolar amounts of cupric ion. Comparison with Table II and the amperometric titration curves for the glucoluteolin and dihydroquercetin at *p*H 6.5 indicates that these same di-chelatogenic flavonoids give somewhat sharper breaks for the interaction with copper, at lower

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(4) L. Hörhammer and R. Hänsel, *Arch. Pharm.*, **285**, 438 (1952).

apparent pH values, in a flavonoid:copper ratio of 1:1.

The results show a glucoluteolin:copper ratio of 2:1 at pH 6.5, but 1:1 at pH 10. The same was found for dihydroquercetin. These indicate the importance of the pH factor in the resulting ratio of flavonoid to copper and possibly may indicate the participation of one potential chelating site at the more acid pH and two sites at the higher pH. Grimaldi and White,[5] in a recent study on the use of quercetin as a colorimetric reagent for determination of zirconium, likewise reported that the mole ratio of quercetin to zirconium in the complex could be either 2:1 or 1:1, depending on experimental conditions.

As shown in Tables II and IV, both butin and 1-epicatechin apparently fail to complex copper at a pH near neutrality, but do so at higher pH values.

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(5) F. S. Grimaldi and C. E. White, Abstracts, 124th A.C.S. Meeting, 3B (Sept., 1953).

NOTES

Acetylenes. I. Mixed Dihalides and Halohydrins from Butynediol[1]

By William J. Bailey[2] and Edward Fujiwara[3]

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In a research program designed to study the effect of structure on the properties of hydrocarbon polymers,[4] polydimethyleneacetylene was desired not only to determine the effect of triple bonds on the properties of a polymer but also to serve as a source for the synthesis of all-*cis*- and all-*trans*-polybutadiene. The most logical starting material for the synthesis of this acetylene polymer appeared to be some derivative of the readily available 2-butyne-1,4-diol (I).

Johnson[5] found that 1,4-dichloro-2-butyne (II) would not react with magnesium to form a Grignard reagent or a polymer. Apparently the chlorine atoms, although reactive to nucleophilic displacements, are not reactive enough to free radical attack by magnesium to form a Grignard reagent under ordinary conditions. Johnson[5] also found that 1,4-dibromo-2-butyne (III) would not form a Grignard reagent. Apparently the first trace of Grignard reagent was extremely reactive and coupled with itself to coat the magnesium with a thin coating of a polymer, preventing any further reaction. It appeared, then, that if the dichloro compound II was not reactive enough to form a Grignard and the dibromo derivative III was too reactive, some mixed dihalide should have an intermediate reactivity so that one halo group would form a Grignard but the other halo group would not immediately couple with it. Three mixed dihalides were synthesized and their reactivities were studied.

Treatment of 2-butyne-1,4-diol (I) with a slight excess of thionyl chloride in pyridine produced a 61% yield of 4-chloro-2-butyne-1-ol (IV) plus a 15% yield of the 1,4-dichloro-2-butyne (II). Since the chlorohydrin IV was comparatively easy to prepare, it was used as the starting material for the other halohydrins. Thus, IV plus sodium bromide in methanol gave a 62% yield of 4-bromo-2-butyne-1-ol (V), while IV plus sodium iodide in acetone gave a 95% yield of the solid 4-iodo-2-butyne-1-ol (VI). IV reacted only slowly with alcoholic silver nitrate, while both V and VI gave an immediate precipitate with the same reagent.

Reaction of the chlorohydrin IV with phosphorus tribromide in chloroform produced a 70% yield of 1-chloro-4-bromo-2-butyne (VII). Similarly, the iodohydrin VI plus phosphorus trichloride produced a 37% yield of the highly reactive 1-chloro-4-iodo-2-butyne (VIII), while VI plus phosphorus tribromide gave a 90% yield of the solid 1-bromo-4-iodo-2-butyne (IX).

When the chlorobromo derivative VII was treated with magnesium in ether, a Grignard reagent resulted. The presence of the Grignard reagent was indicated by the violent reaction upon the addition of water and the formation of color with Michler ketone.[6] Apparently, it is the bromo group of VII that is reactive enough to form the Grignard, confirming Johnson's explanation of the lack of formation of a Grignard reagent with dibromobutyne III. In a similar manner, the chloroiodo compound VIII would form a Grignard, but the 1-bromo-4-iodo-2-butyne (IX) would not. Apparently, IX behaved very much like III in this reaction.

In view of the recent[7] synthesis of butatriene by treatment of III with zinc in diethylene glycol diethyl ether at 80°, the formation of the Grignard reagent from VII is extremely interesting. Ap-

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(1) Presented in part at the 118th Meeting of the American Chemical Society, Chicago, Ill., September, 1950.

(2) Department of Chemistry, University of Maryland, College Park, Md.

(3) Office of Naval Research Fellow, 1949–1951.

(4) W. J. Bailey and H. R. Golden, This Journal, **75**, 5418 (1954).

(5) A. W. Johnson, *J. Chem. Soc.*, 1009 (1946).

(6) H. Gilman and F. Schulze, This Journal, **47**, 2002 (1952).

(7) W. M. Schubert, T. H. Liddicoet and W. A. Lanks, *ibid.*, **76**, 1929 (1954).