tive observations on a sample of standard hide powder[14] showed it to be isoelectric in the neighborhood of *p*H 5.

Discussion

Wilson has interpreted the two critical points, at *p*H values of 4.7 and 7.7, as the respective isoelectric points of the gel and the sol forms of collagen and gelatin.[15] The fact that the collagen used in the present work received only a mild alkaline treatment with half-saturated calcium hydroxide solution in the course of its preparation, while commercial hide powder and gelatin are given a much more drastic alkaline treatment, suggests that the isoelectric point of native collagen is near *p*H 7.8, and that treatment with alkaline solutions causes some structural change the effect of which is to lower the isoelectric point of the collagen and of the gelatin made from it.[16] The work of Briefer,[17] who found that gelatin made from alkaline pretreated collagen possesses minimum jelly consistency and maximum turbidity at about *p*H 5, while that made from acid pretreated collagen has similar properties at about *p*H 8, is recalled in this connection.

In addition to its unusually small slope, the curve of Fig. 1 displays a further feature of interest when compared to similar curves for other proteins. The curvature in such cases, while slight, is usually convex in the region of positive mobilities and concave in the negative. Just the opposite relations are found in the curve of Fig. 1. No explanation of this difference in shape, which is undoubtedly related to the mode of linkage of the polar groups of the protein, will be attempted at this time. Further work on the cause of the shift in the isoelectric points of collagen and gelatin is in progress in this Laboratory.

The author wishes to acknowledge the valuable assistance of Dr. H.J. Kersten in mounting the vertical microelectrophoresis cell. The cell itself was made by Mr. J. D. Graham of Haddonfield, New Jersey, to whom the author's thanks are also due.

Summary

The isoelectric point of collagen prepared from steer hide with a minimum alkaline treatment has been shown, by measurements of electrophoretic mobility, to be at *p*H 7.8 in buffers of ionic strength 0.005. It is suggested that the shift of the isoelectric point to *p*H 4.7 in the case of commercial gelatin and hide powder is due to some structural change in the protein caused by the alkaline treatment given these materials.

CINCINNATI, OHIO RECEIVED MAY 17, 1939

(14) Kindly furnished by Mr. Louis Cuthbert of The Standard Hide Powder Mfg. Co., Ridgway, Penna.

(15) For further discussion and references see J. A. Wilson, "The Chemistry of Leather Manufacture," Vol. I, The Chemical Catalog Company, New York, N. Y., 1928.

(16) After the completion of this work a paper on the electrophoresis of collagen was presented by Beek and Sookne at the meeting of The American Leather Chemists Association at Shawnee-on-Delaware, Penna., June 7, 1939. Using a horizontal cell and buffers of ionic strength 0.02, they located the isoelectric point of collagen similar to that used in the present work at about *p*H 7, and attributed the shift to a more acid value in the case of commercial gelatins to the hydrolysis of the amide groups during the liming treatment.

(17) Briefer, *Ind. Eng. Chem.*, **21**, 266 (1929).

[CONTRIBUTION FROM CALIFORNIA FRUIT GROWERS EXCHANGE RESEARCH DEPARTMENT]

A Study of the Boric Acid Color Reaction of Flavone Derivatives

BY C. W. WILSON[1]

It was observed in this Laboratory that lemon juice dried in the presence of boric acid produced a brilliant yellow color. Later the color reactive substance was identified as a constituent of Szent-Györgyi's citrin.[2]

This color reaction is sufficiently sensitive to detect 0.004 mg. of citrin or 0.002 mg. of quercitrin in 0.5 ml. of solution. The work here reported is an attempt to determine the specificity of the boric acid color reaction.

Experimental

Several representative flavones[3] and flavanones were purified by recrystallization from dilute alcohol at least three times. Table I shows the structural formulas of some of these substances and their boric acid color reactions.

The hydroxychalcones shown in Table I were prepared by alkaline treatment of naringin and hesperidin. A suspension of 2 g. of naringin or hesperidin with 25 ml. of water was placed in a 50-ml. flask equipped with a stopcock, the flask

(1) Corona Laboratory of California Fruit Growers Exchange, Research Department.

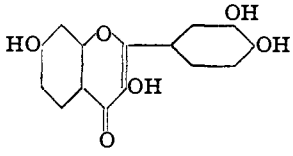
(2) Armentano, Bentsath Beres, St. Rusznyak and Szent-Györgyi, *Deut. med. Wochschr.*, **62**, 1326 (1936).

(3) The flavones used in this work were obtained by Mr. A. J. Lorenz from Dr. C. E. Sando of the U. S. Bureau of Chemistry and Soils.

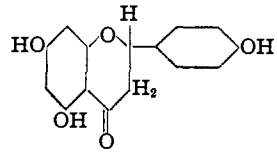
TABLE I

CONFIGURATION OF VARIOUS ORGANIC SUBSTANCES, AND CORRELATION WITH BORIC ACID COLOR REACTION

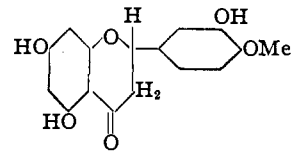
NEGATIVE REACTIONS



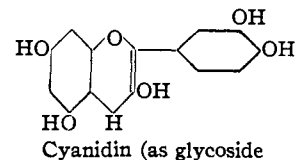
Fisetin



Naringenin

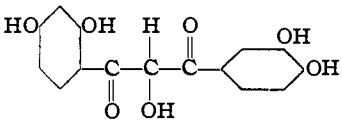


Hesperetin

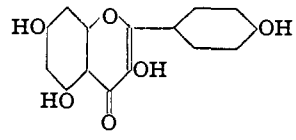


Cyanidin (as glycoside from apple)

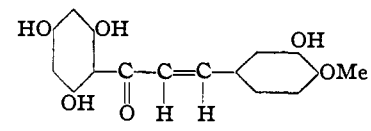
POSITIVE REACTIONS



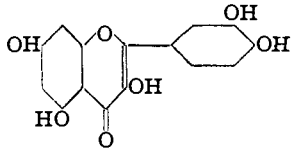
Alkali treated fisetin



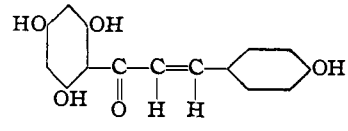
Kaempferol



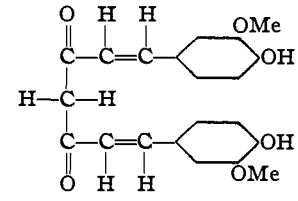
2',4',5,6'-Tetrahydroxy-4-methoxychalcone (from hesperetin)



Quercetin



2',4,4',6'-Tetrahydroxychalcone (from naringenin)



Curcumin. The positive color is pink and not confusable with the flavone color

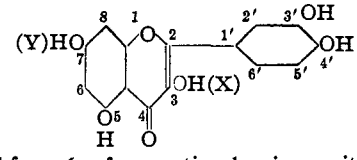
evacuated until the liquid boiled and the stopcock then closed. Into the flask was drawn 25 ml. of 2.5 *N* sodium hydroxide solution. The dark reddish solution was mixed thoroughly and allowed to stand for twenty-four hours at room temperature. It was then poured into 12.5 ml. of 5 *N* hydrochloric acid. The very slightly acid solution was evaporated to dryness in vacuum, and the hydroxychalcone extracted from the residue with absolute methyl alcohol.

The compounds so obtained were water soluble, orange powders.

The reagent used for determining the boric acid reactivity of a compound was made by mixing equal parts of two separate solutions, one composed of 100 ml. of absolute acetone saturated with boric acid, and the other 100 ml. of absolute acetone containing 10 g. of anhydrous citric acid.[4] Sufficient of the mixed solutions was prepared for each day's requirements, since it was found that while each solution was stable indefinitely, a very slow color formation took place in the acidified boric acid solution. This color was sometimes observable after as short an interval as two days.

For carrying out the determination of reactivity, approximately 0.5 mg. of the flavone derivative or other substance was dissolved in about 1 ml. of dry acetone, and the solution divided into two equal portions. To one portion was added about 2 ml. of the boric acid–citric acid–acetone reagent, and the other portion was diluted to an equal volume with a mixture of equal parts of the citric acid–acetone solution and acetone. The colors of the two tubes were compared at the end of a few minutes, and any definitely stronger color in the boric acid containing tube was reported in Table I as a positive reaction.

In addition to testing the aglycones (non-sugar parts) shown in Table I, the effect of the presence of sugar groups was checked by comparing the reactivity of two of the glycosides of quercetin; isoquercitrin (sugar group in the position marked X)[5] and quercimeritrin (sugar group in the position marked Y). With quercetin all three gave positive reactions. In these cases, the presence or position of the sugar group was without influence.



Structural formula of quercetin, showing position of sugar group in isoquercitrin (X) and quercimeritrin (Y)

(4) Made by allowing crystalline acid to effloresce completely in air at 30–40°, then heating in a thin layer to 100° for two hours.

(5) Attree and Perkin, *J. Chem. Soc.*, 234–240 (1927).

Since treatment of naringenin and hesperetin with dilute sodium hydroxide caused formation of a substance that gave a positive reaction with the borocitric acid reagent, it was decided to try the effect of the same treatment on fisetin. In this case, also, a positive reacting compound was formed.

Hesperetin heated with 1 volume of concentrated hydrochloric acid in 2 volumes of alcohol formed an orange solution which was carefully neutralized with sodium hydroxide solution and the liquid was evaporated over a steam-bath. The residue was extracted with acetone, and the extract gave a positive reaction with borocitric acid reagent.

U. S. P. tannic acid gave no reaction when treated directly with the borocitric acid reagent, nor after alkaline or acid treatment as described above.

Other substances not shown in Table I but tested in view of their possible occurrence in nature with flavones gave negative reactions. These included coumarin, coumarinic acid, dextrose, alkali treated dextrose, phloroglucinol, quinhydrone, salicylic acid, sucrose, and alkali treated sucrose.

Discussion of Results

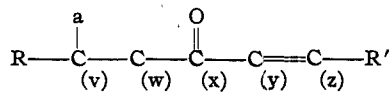
Of the flavone derivatives tested, the flavanones give no color reaction with boric acid, whereas all of the flavones except fisetin gave a reaction. With this one exception, if a substance is established as being a flavone or a flavanone by the cyanidin test, but does not give a color with borocitric acid reagent, the supposition would be that it was a flavanone.

Comparing the lack of reaction of fisetin with the reactivity of quercetin, the importance of the hydroxyl (or some other auxochromic) group in the 5-position is evident.

Comparing the reactivity of kaempferol with the lack of reactivity of naringenin leads to the speculation of whether the OH in the 3-position or the double bond between the 2 and 3 carbon atoms is responsible for the difference.

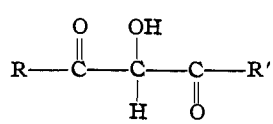
Opening the pyran ring to give the equivalent chalcone definitely establishes the importance of the double bond, and indicates that the pyran ring is unnecessary for color formation.

The configuration of a chalcone suggests that of curcumin, and causes one to feel that the color formation of curcumin with boric acid under similar conditions may be further indicative of the configuration required. If this reactivity is comparable, the benzene ring near the ketone (or quinone) group is unnecessary provided an auxochromic group is attached to the second carbon atom from the C=O group. The indicated configuration for reactivity is

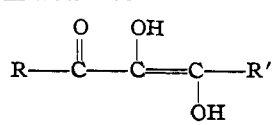


in which "a" is an auxochromic group which may probably be =O, OH, OCH_3, etc., and in which R, C_v and C_w may form a benzene ring, and C_x, C_y and C_z may form a portion of a pyran ring. In all of this work, R′ has been a benzene ring containing either methoxyl or hydroxyl groups.

When the pyran ring of fisetin is opened by treatment with an alkali, the formation of the hydroxyl group in the ortho position on one of the benzene rings fulfils the boric acid color forming requirement of having an OH (or other auxochromic) group on the second carbon atom from a C=O group. The double bond between carbon atoms on the other side of the C=O group is missing in the formula shown in Table I. Compounds of this type, however, are known to exist at least to some extent in enolic forms,[6] and instead of



the enolic form would be



which, when R and R′ have the proper configurations, as is the case in the present instance, would be expected to yield the observed coloration.

Another possible explanation is that in the alkali treatment of the fisetin there are formed other secondary compounds responsible for the color reaction.

Acknowledgment.—The author wishes to express his appreciation to the California Fruit Growers Exchange for the opportunity to carry on and publish this work and to Mr. W. E. Baier, Manager of the Research Department, for his suggestions and interest.

(6) A. M. Buswell, W. H. Rodebush and M. F. Roy, THIS JOURNAL, **59**, 1767 (1937).

Summary

Citrus juices have been observed to give a yellow color when dried with boric acid.

This yellow color forming substance is concentrated in the citrin fraction of Szent-Györgyi.

Many flavone derivatives have been found to give the color reaction. Of non-flavone substances commonly found in plant tissues, none have so far been found to give a confusable color reaction.

The color reaction has been correlated with molecular constitution.

ONTARIO, CALIF. RECEIVED MAY 2, 1939

[CONTRIBUTION FROM THE AVERY LABORATORY OF CHEMISTRY OF THE UNIVERSITY OF NEBRASKA]

Organoselenium Compounds. I. Diphenylselenium Dihydroxides and Diphenylselenides

BY C. KENNETH BANKS[1] AND CLIFF S. HAMILTON

In order to investigate some of the therapeutic properties of organoselenium compounds, a series of compounds in which the effect of the valence of selenium could be studied, as well as the effect of changes in substituent groups, was prepared. This paper deals with some of the derivatives of diphenylselenium dihydroxide and diphenylselenide derived from the phenoxyalkanols, as these compounds have led to some therapeutically valuable arsenicals.

The reaction of Alquist and Nelson[2] and Kunckell[3] by which anisole and its homologs were condensed with selenium oxychloride to give *bis*-(*p*-alkyloxyphenyl)-selenium dichlorides was extended to three other ethers, β-phenoxyethanol, γ-phenoxypropanol and α-methyl-β-phenoxyethanol. The first two gave ether insoluble gums, while the last gave a crude crystalline mass. The crude products were purified by dissolving in chloroform, treatment with activated charcoal and "Celite," and reprecipitation with benzene. By means of this procedure all three crystallized in stable forms. The dichlorides hydrolyzed to the dihydroxides in the presence of sodium carbonate, all three of which were white crystalline solids.

The above dihydroxides, as well as *bis*-(4-methoxyphenyl)-selenium dihydroxide, were then nitrated using fuming nitric acid (sp. gr. 1.5) at 0° as the nitrating agent. A large excess of acid was required for complete nitration, probably due to the formation of an alcohol dinitrate of the dinitrodietherdiphenylselenium dinitrate. The gum formed when the nitration mixture was poured over ice was solidified by neutralizing the excess acid with sodium carbonate and boiling. These compounds proved to be of the formula *bis*-(3-nitro-4-R-phenyl)-selenium dihydroxide, R representing the ether group. The position of the nitro group was shown by cleaving with hydriodic acid, giving *o*-nitrophenol.

The nitro compounds were reduced catalytically to the diamines, using Raney catalyst.[4] At 40 pounds (2.67 atm.) pressure and room temperature the selenium was also reduced. The amines were of the general formula *bis*-(3-amino-4-R-phenyl) selenide.

Of theoretical interest was the extension of the reaction to another type of compound, acetanilide. The reaction was first tried in ether but it was found that the product was a double salt of acetanilide and selenium oxychloride. As the salt was soluble in chloroform, the reaction was run in that medium. After five days, a thick gum separated. This gum was hydrolyzed with sodium carbonate, giving a water insoluble white compound which, on purification, proved to be *bis*-(4-acetaminophenyl)-selenium dihydroxide. This compound was reduced to the corresponding diphenyl selenide and then hydrolyzed to the free amine. The last two compounds have been prepared by Theobald[5] from sulfonamide and selenic acid. The acetylated compound was obtained in two crystalline modifications, one melting at 216° as reported by Theobald, the other at 176°. The two forms were chemically identical.

Experimental

1. Diphenylselenium Dichlorides.—One-half mole of the aromatic ether was treated with selenium oxychloride according to the procedure of Alquist and Nelson.[2] The solvent was decanted from the gum or crystals formed and

(1) Parke, Davis and Company Fellow.

(2) F. N. Alquist and R. E. Nelson, THIS JOURNAL, **53**, 4033 (1931).

(3) Kunckell, *Ber.*, **28**, 609 (1895).

(4) M. Raney, U. S. Patent 1,628,190 (1927).

(5) E. Theobald and P. Theobald, French Patent 794,192 (1936); German Patents 631,100, 631,570, 631,571, 631,572, 632,073, 633,344 (1936).