see Table I. Additional material may be obtained during purification by addition of petroleum ether to the cold benzene solution.

**Dimethylaniline–Boron Trifluoride.**—This substance was prepared and purified in the same manner as the trimethylamine compound: m. p. 90–92° (sealed tube); solubility *ca.* 0.266 g./100 g. of benzene; mol. wt. see Table II.

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## A Color Reaction for Natural Pigments and Phenols

BY HENRY TAUBER AND STEPHEN LAUFER

Recently Tauber, Laufer and Goll[1] described a color test for the anti-bacterial mold pigment citrinin. We are now able to report on certain chemical properties concerning the oxidation product of citrinin. We have also applied this reaction, oxidation with hydrogen peroxide, etc., to a series of natural pigments, and to phenols. We found that some of these substances also give typically colored oxidation products under similar conditions. This indicates that certain phenolic groups are indispensable for this reaction.

TABLE I

SHOWING CHEMICAL DIFFERENCES BETWEEN CITRININ AND ITS OXIDATION PRODUCT

| Reagent | Citrinin | Oxidation product |
|---|---|---|
| Color (in dry state) | Yellow | Reddish-brown |
| Color (in solution at *p*H 7.0) | Yellow | Wine red |
| $FeCl_3$ | Brown | Dark blue |
| NaOH | Yellow | Wine red |
| $H_2SO_4$ | Yellow | Almost colorless (slightly yellow) |
| $AgNO_3$, on boiling | No reaction | Silver mirror |
| $(Ag)_2O\text{-}NH_3$, in cold | No reaction | Black precipitate in cold |
| Basic lead acetate | No precipitate | Heavy reddish-brown |
| Neutral lead acetate | No precipitate | Precipitate |

### Experimental

**Oxidation of Citrinin by Hydrogen Peroxide and Preparation of the Oxidation Product.**—100 mg. of citrinin[1] was dissolved in 1 cc. of dioxane. The solution was brought to 34° and 0.1 cc. of 30% hydrogen peroxide was added. The mixture was stirred for one minute and then cooled in ice water. Thereafter 10 cc. of distilled water was added and the orange red solution was extracted once with 16 cc. of ether and three times with 10-cc. portions of ether. The ether extracts were combined and washed with 10 cc. of distilled water. After evaporation of the ether a yield of 60 mg. of a reddish-brown hygroscopic material was obtained. It was soluble in ether, chloroform, ethyl alcohol, dioxane and slightly soluble in water. It could not be obtained, however, in crystalline form from these solvents. An alcoholic solution of this substance gave a wine red color when adjusted to *p*H 7.0 with dilute sodium hydroxide and on slight acidification the solution turned light yellow, almost colorless. Citrinin solutions are yellow under these conditions. It should be noted that continuous exposure of citrinin to dioxane or boiling citrinin in dioxane effects a gradual oxidation of the substance. It may be seen from Table I that the oxidation product differs in many respects from citrinin; the former appears to be a polyphenolic compound while the latter apparently is a quinoid compound containing one phenolic group only. The structural formula of citrinin is not definitely known yet.[2]

TABLE II

SHOWING COLOR REACTIONS OBTAINED WITH NATURAL PIGMENTS

| Pigment tested | Color with $H_2O_2$ + NaOH | Color with water and NaOH | Color on acidification Experiment | Color on acidification Control |
|---|---|---|---|---|
| | | Anthocyanins | | |
| Red beet | Violet | Yellow | Red | Red |
| Red rose | Intense yellow | Dark brown | Red | Red |
| Deep red dahlia | Light lemon yellow | Deep lemon yellow | Red | Red |
| Red gladiola | Yellow | Dark brown | Pink | Brick red |
| Orange-red dahlia | Deep yellow | Reddish-brown | Brick red | Brick red |
| Blue grape | Yellow | Green | Red | Red |
| | | Flavones | | |
| White dahlia | Yellow | Orange red | Almost colorless | Almost colorless |
| White carnation | Yellow | Yellow | Colorless | Colorless |
| | | Other Natural Pigments | | |
| Citrinin (originally yellow) | Wine red | Orange yellow | Orange yellow | Yellow |
| Penicillin (originally yellow) | Yellow | Yellow | Yellow | Yellow |
| Pigment of *P. Chrysogenium* (originally yellow) | Yellow | Yellow | Yellow | Yellow |
| Actinomycin (originally yellow) | Slightly yellow, almost colorless | Yellow | Yellow | Yellow |

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[1] H. Tauber, S. Laufer and M. Goll, THIS JOURNAL, **64**, 2228 (1942).

[2] F. P. Coyne, H. Raistrick and R. Robinson, *Trans. Roy. Soc.* (London), **220**, 297 (1931).

**Hydrogen Peroxide Color Reactions with Anthocyanins.**—The anthocyanins were prepared by the following method: Samples of the finely ground plant material were extracted with 5 volumes of 95% ethyl alcohol by boiling for three minutes. The filtered extracts were evaporated in vacuum. 200-mg. samples of the crude materials were dissolved in 5 cc. of ethyl alcohol containing 0.5 cc. of *N* HCl. The solution was centrifuged and a small amount of insoluble matter was discarded. From the alcoholic solution the anthocyanins were precipitated with a large excess of ether. The precipitated anthocyanin chlorides were redissolved in alcohol and again precipitated with ether and dried in vacuum.

In the following is described in detail the hydrogen peroxide–sodium hydroxide color reaction as given by the anthocyanin of red beet: To 2.5 mg. of the pigment in 0.5 cc. of 50% ethyl alcohol was added 0.3 cc. of 3% hydrogen peroxide. The mixture was agitated for one minute. On addition of 0.3 cc. of 0.2 *N* sodium hydroxide the color turned reddish-violet and on addition of 0.5 cc. of 0.2 *N* sulfuric acid the solution became red. The red pigment was fairly stable in acid solution. In the control tube in which water was used instead of hydrogen peroxide a yellow color was formed. In Table II are recorded a series of color reactions as given by other pigments. The carotenoids physalien, capsanthin, and cryptoxanthin (not listed in Table II) gave no color under similar conditions.

**Color Reactions with Other Pigments.**—With other pigments the technique of the reaction was carried out in the same manner. In the controls water was used instead of hydrogen peroxide. The results are summarized in Table II. The color reaction may also be carried out directly on the alcoholic extracts of plants. In the case of molds the ether extracts of the media to which some water is added may be employed.

For the tests on flavanols 0.5 cc. of the alcoholic extracts of the flowers were used. Penicillin and actinomycin[3] were very kindly furnished by Merck and Company, Inc. For the pure carotenoids we are grateful to Dr. L. Zechmeister of the California Institute of Technology.

**Color Reactions with Polyphenols.**—Compounds containing phenolic groups in positions 3 and 5 readily give color reactions at room temperature with 3% peroxide and 0.2 *N* sodium hydroxide. Thus phloroglucinol (1 mg. in 0.5 cc. 95% ethyl alcohol) gives a violet color and orcinol gives a pink color. Phenol, catechol, resorcinol, thymol, salicylic acid and cresol give no color. Hydroquinone and pyrogallol give a yellow color, being directly affected by the alkali. Substances of a quinone structure and having one phenolic group like citrinin apparently undergo certain oxidative changes by hydrogen peroxide treatment, which make the intense and rapid color reaction possible.

In the case of phloroglucinol maximum color formation (intense violet) takes place two to three minutes after the addition of sodium hydroxide. Then the intensity of the color gradually decreases and the solution becomes colorless. On slight heating, however, maximum color intensity is obtained immediately. At the beginning of the reaction the color is reversible. The case is similar with the color forming pigments.

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## The Conductance of Aqueous Solutions of Magnesium Perchlorate

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In the course of some investigations on nonaqueous solutions of magnesium perchlorate, of which the remarkably high solubilities in various organic solvents were measured by Willard and Smith,[1] we noticed that no conductance data were available for the aqueous solutions of this salt. These we therefore determined for the range of concentrations from 0.001 equivalent per liter to saturation (6.588 equivalents per liter[1]). Several series of solutions were prepared, some with the anhydrous salt, some with the hexahydrate as obtained from the G. F. Smith Co., some with carefully recrystallized hexahydrate derived from G. F. Smith anhydrous salt. All solutions were prepared in calibrated glassware, successive dilutions being carried out on a weight basis with the usual buoyancy corrections. A Washburn conductivity cell was used with an oil thermostat kept at 25°. A Wolff potentiometer–Wheatstone bridge combination of high precision[2] was used, the alternating current being provided by a microphone hummer and, in part of the work, by an R. C. A. beat-frequency audio oscillator. Conductivity water of specific conductance of less than $10^{-6}$ mho was used throughout. Table I gives the equivalent conductances interpolated for the series of round concentrations

Table I

Conductances of Aqueous Solutions of Magnesium Perchlorate at 25°

| Concentrations, equivalents per liter | Equivalent conductances in mhos | Concentrations, equivalents per liter | Equivalent conductances in mhos |
|---|---|---|---|
| 0 | (128.5) | 0.5 | 79.4 |
| 0.001 | 120.8 | 0.7 | 75.7 |
| .002 | 118.2 | 1.0 | 71.0 |
| .005 | 112.6 | 2 | 59.1 |
| .01 | 108.0 | 3 | 49.4 |
| .02 | 103.9 | 4 | 40.5 |
| .05 | 98.3 | 5 | 31.4 |
| .07 | 95.7 | 6 | 22.6 |
| .1 | 93.1 | 6.588 | 17.5 |
| .2 | 87.4 | | |

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(3) S. A. Waksman and M. Tishler, *J. Biol. Chem.*, **142**, 519 (1942).

(1) H. H. Willard and G. F. Smith, This Journal, **45**, 286 (1923).

(2) O. Wolff, *Z. Instrumentenkunde*, **21**, 227 (1901); **22**, 224 (1902); etc.