6. An increase in the alkalinity of the reaction mixture, up to a certain maximum amount, increases the rate of oxidation.

7. A comparison between the results of two experiments recorded by Denis is given.

8. The rate of the addition of the alcoholic solution has no effect on the relative amounts of the oxidation products so far as could be ascertained.

9. An equation has been developed for the relationship between the acetic acid production and the initial concentration of the alkali used when alcohol is oxidized at a given temperature in the presence of potassium hydroxide.

10. By means of this equation it is easy to establish the upper limit of the alkali concentration permitted and still obtain a theoretical yield of acetic acid, *i. e.*, the lower limit of alkali concentration at which oxalic acid forms.

11. By means of this equation it can be shown that at —25° the magnitude of the initial concentration of the potassium hydroxide used would not affect a quantitative yield of acetic acid.

12. An apparatus has been described for the filtering of sludge precipitates in the absence of the carbon dioxide of the air.

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[CONTRIBUTION FROM THE CHEMICAL RESEARCH LABORATORY, THE UPJOHN COMPANY.]

THE YELLOW COLORING SUBSTANCES OF RAGWEED POLLEN.

BY FREDERICK W. HEYL.

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These coloring substances belong to the flavonols and are entirely glucosidic. They are extracted with alcohol; and after preparing an aqueous solution from this alcoholic extract a complete precipitation may be secured with basic lead acetate. The yield amounts to about 7.0 g. from 1150 g. pollen or approximately 0.6%.

The least soluble of these was identified as a quercitin glucoside having the composition $C_{21}H_{20}O_{12}$ and melting at 228–9°. The only sugar obtained upon hydrolysis was glucose. It is therefore isomeric with, and differs from, quercimeritrin and isoquercitrin, which were first isolated by A. G. Perkin[1] from the flowers of *Gossypium herbaceum*. These melt, respectively, at 247–249° and 217–219°. One other isomer is known, having been isolated by Rogerson[2] from the flowers of *Trifolium incarnatum*. Incarnatin melts at 242–245°. The most characteristic behavior of pollen quercitin glucoside on melting is the sharp formation of a cherry-

[1] *J. Chem. Soc.*, **95**, 2181 (1909); Viehoever, Chernoff and Johns, *C. A.*, **12**, 1562 (1918).

[2] *J. Chem. Soc.*, **97**, 1008 (1910).

red oil at the melting point, a peculiarity not noted with any of the above mentioned isomers.

The more soluble fractions of the mixed glucosides contained a glucoside of isorhamnetin. This fraction yielded magnificent crystals of hexagonal prisms, but these were extremely soluble; 2–3 cc. of warm water dissolving a gram or more and yielding a thick unfilterable crystalline mush. Upon hydrolysis it gave isorhamnetin, $C_{16}H_{12}O_7$, a monomethyl derivative of quercitin, and yielded the characteristic beautifully crystalline tetraacetyl-isorhamnetin melting at 197–200°. This substance has been previously isolated[1] from the flowers of *Cheiranthus cheiri* and from red clover flowers.[2] The glucoside of isorhamnetin appears to predominate.

Experimental Part.

Ragweed pollen (approximately 1150 g.) which had been percolated with ether to remove fat, was percolated with alcohol (94%–98%) until the percolate was almost free from color. After distilling off most of the alcohol under diminished pressure this extract was mixed with distilled water and the resin that separated was removed. The aqueous liquor was extracted with ether and the coloring substances were fractionally precipitated by 3 additions of basic lead acetate solution, the yellow precipitates being removed on a Büchner filter, washed with water, and decomposed with hydrogen sulfide, yielding 3 fractions.

On concentrating Fraction I (50 cc.) and cooling, a substance separated in yellow globules. This material was dissolved in a mixture of pyridine and water (1 : 3) but no satisfactory crystallization resulted; on heating and adding an equal volume of glacial acetic acid the substance separated in a crystalline condition having the appearance of wheat. It was very impure, sintering below 170°, becoming soft at 185° and having completely decomposed to a red foam at about 195°. It was insoluble in ether or toluene, but dissolved in boiling nitrobenzene, failing to crystallize satisfactorily. It was purified by boiling with water, to which pyridine was added drop by drop until solution was effected and then collecting the crystals (light yellow needles) which separated from the hot solution. The melting point was raised as follows: 212–220°, 224°, 223–5°, and 224–6°, to a red oil. Yield (0.12 g.).

Subs. dried in vacuum at 130° 0.1174: CO_2, 0.2322; H_2O, 0.0466.
Calc. for $C_{21}H_{20}O_{12}$: C, 54.3; H, 4.3. Found: C, 53.95; H, 4.45.

The original aqueous solution from which the crude glucoside separated could be concentrated to a syrup without further crystallization. It contained a mixture of extremely soluble uncrystallizable glucosides. This syrup was taken up with 5% sulfuric acid and heated upon the steam

[1] A. G. Perkin, *J. Chem. Soc.*, 69, 1658 (1896).
[2] Power and Salway, *Ibid.*, 97, 245 (1910).

bath until the separation of insoluble yellow substance was complete. This, when filtered off on a gooch crucible, weighed 1.097 g. It was discolored. It was redissolved in ammonium hydroxide solution and reprecipitated with hydrochloric acid. The precipitate was dissolved as far as possible in ether, and the ether solution was extracted with water, ammonium carbonate, and then with potassium carbonate, which took out a considerable part of the material in a deep orange colored solution. The last mentioned extract was acidified and the yellow precipitate filtered off on suction and washed with water. It melted with decomposition at 286°. It was recrystallized with dilute alcohol[1] from which it separated in microscopic crystals melting at 281–286° (C 58.3, H 3.75). The remainder was acetylated and the colorless acetyl derivative obtained proved to be comparatively soluble, most of it remaining in the mother liquor as a syrup. The crystalline part (0.05 g.) softened at 144° and melted at 148–149°. Upon recrystallization it softened at 146° and melted at 149–151°. The yellow substance therefore is not quercitin but a mixture.

The yellow hydrolytic cleavage products having been filtered off, the filtrate after clarifying with lead subacetate showed the presence of 0.534 g. glucose by the Munson-Walker process. The phenylglucosazone melted at 203–4° and pentose sugars were absent, it being impossible to obtain even a trace of phloroglucide by the quantitative procedure.

Upon concentrating the filtrate from the lead sulfide after decomposing the *second lead salt* two crystalline deposits A and B, consisting chiefly of yellow globules, were obtained. A (1.1 g.), was repeatedly recrystallized from water but the melting point remained 170–211°. B (1.0 g.), when fractionally crystallized from water, gave a small crop of needles of the glucoside melting at 221–224°, but from the mother liquors material similar to A separated. By crystallization from water and pyridine a small yield of pure yellow glucoside (0.3 g.) melting at 222–5° was obtained from A, and B yielded the same product, m. p. 228–9°.

The glucoside here obtained was hydrolyzed with unsatisfactory results: 0.2733 g. was heated on the steam bath with dilute alcohol in a covered beaker in the presence of approximately 5% sulfuric acid, and with the occasional addition of alcohol. The solution was then concentrated and alcohol completely removed, several additions of water being made to keep the volume constant. After standing overnight, 0.1579 g. yellow cleavage product was filtered off on a gooch crucible (57.8%). The filtrate yielded pure *d*-phenyl-glucosazone melting at 205–6°.

[1] The mother liquors from this substance contained alteration products which were very soluble in water yielding red solutions that no longer gave yellow solutions with alkali. Potassium hydroxide intensified the red color.

The yellow cleavage product was twice crystallized from 95% alcohol and melted at 311–313°, but was not identical with the product later obtained which melted at 314–315°, as a mixture of the two melted at about 288°. After drying *in vacuo* at 130°, it was analyzed.

Subs., 0.0814: CO_2, 0.1773; H_2O, 0.0259.
Calc. for $C_{15}H_{10}O_7$: C, 59.6; H, 3.3. Found: C, 59.4; H, 3.6.

The substance agrees in all its properties with quercitin. When mixed with quercitin from *Zygadenus* (m. p. 305°), the mixture melted at 305–7°. It yielded a colorless acetyl derivative that crystallized characteristically from alcohol and melted at 191–3°. When mixed with acetyl quercitin the melting point was not depressed.

Although the calculated yield of quercitin from the equation

$$C_{21}H_{20}O_{10} + H_2O = C_{15}H_{10}O_7 + C_6H_{12}O_6,$$

should be 65% (found 57.8), there is no doubt but that the substance is a quercitin glucoside.

The mother liquors (pyridine + water) which accumulated during the purification of this quercitin glucoside contained more than 0.5 g. glucosidic material. It was hydrolyzed and the yellow cleavage product converted into the colorless acetyl derivative. The crude product melted at 165–180° and after several recrystallizations at 188–195°. A methoxyl determination here indicated that it consisted of a mixture of acetyl-isorhamnetin and acetylquercitin, the former predominating.

Subs., 0.0511 (182–192°): AgI, 0.0170. Found: OCH_3, 4.4.

The filtrate from B upon concentration yielded 2.0 g. of an insoluble yellow product C as it was concentrated to a syrup, hydrolysis evidently being effected by a small amount of free acid present. The insoluble yellow cleavage product was filtered off and the filtrate made acid with 5% sulfuric acid to complete the hydrolysis and heated on the steam bath and again filtered from a further slight quantity of badly contaminated cleavage product. The acid filtrate on thorough examination contained nothing but glucose.

The product C (2.0 g.) was boiled twice with smaller volumes of water and then the insoluble residue was boiled with one liter of water and filtered hot. From the filtrate a small quantity (0.07 g.) of micro-crystalline material separated. Dried at 120°, it softened at 255° and melted to a black oil at 260°. It is readily soluble in ammonia, yielding a yellowish green color, which turned to a burnt yellow when the ammonia is expelled (C, 61.15; H, 3.9). It is probably the same material as the insoluble part.

The insoluble part was dissolved in boiling 95% alcohol, and water was added to opalescence and then on cooling the material separated in amorphous balls melting at 293–6°. It weighed 0.6 g. For further purifica-

tion the material was acetylated with acetic anhydride in the presence of a drop of pyridine. By 5 crystallizations from alcohol the melting point of this colorless acetyl derivative that crystallized in needles was elevated from 191–2° to 197–200°.

Subs., 0.0667: CO_2, 0.1444; H_2O, 0.0247.

Subs., 0.0921: AgI, 0.0466.

Calc. for $C_{16}H_8O_7(COCH_3)_4$: C, 59.5; H, 4.1; OCH_3, 6.4. Found: C, 59.05; H, 4.15; OCH_3, 6.7.

The pure acetyl derivative was hydrolyzed by boiling with 5% sulfuric acid in dilute alcohol and filtering after removing the alcohol on the steam bath. The recovered yellow substance separated from 95% alcohol in curved prims and melted sharply at 314–315° to a black oil.

Subs., 0.079: CO_2, 0.1768; H_2O, 0.0288.

Calc. for $C_{16}H_{12}O_7$: C, 60.7; H, 3.8. Found: C, 61.05; H, 4.1.

This substance appears to be isorhamnetin, one of the monomethyl quercitins, and the purification through the acetyl derivative did not change the results of the combustion.

The *third lead precipitate* was decomposed with hydrogen sulfide and the filtrate from the lead sulfide was concentrated to a syrup but no crystals resulted. The syrup, when subjected to acid hydrolysis, yielded only calcium sulfate.

The writer is greatly indebted to Mr. Clayre Pomeroy for the analytical work.

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[CONTRIBUTION FROM THE MELLON INSTITUTE AND THE SCHOOL OF CHEMISTRY, UNIVERSITY OF PITTSBURGH.]

PHTHALIC ACID DERIVATIVES; CONSTITUTION AND COLOR. XVI.[1] PHENOLTETRABROMO-PHTHALEIN AND SOME OF ITS DERIVATIVES.

BY DAVID S. PRATT, F. B. DOANE AND A. W. HARVEY.

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The preparation of various halogenated phenolphthaleins has been considered important from two standpoints, *i. e.*, to provide additional indicators of this excellent type for analytical work and to further our knowledge of the effect on absorption spectra brought about by the substitution of important atoms in phthalic acid derivatives. Since the corresponding chlorine and iodine derivatives of phenolphthalein have been prepared it was interesting to study the intermediary bromine analogues.

Phenoltetrabromo-phthalein with the halogen in the anhydride ring was probably obtained in an impure condition by Rupp,[2] but details are lacking

[1] THIS JOURNAL, 40, 1425 (1918).

[2] *Arch. Pharm.*, 249, 56 (1911).