

# THE CERIC ACID REACTION WITH PARTICULAR REFERENCE TO SUBERIN.\*

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## INTRODUCTION.

The ceric acid reaction of Doepping (1) as later modified and defined by von Höhnel (4), is recognized by chemists to be a valuable test for the presence of suberin or cutin in plant tissue. This is because of the fact that none of the other cell wall constituents of plants when treated with a strong oxidizing mixture such as nitric acid and potassium chlorate yield similar products of oxidation. We do not know, however, what ceric acid, or the product of this so called ceric acid reaction, really is. It might be thought of as a definite compound, or as a mixture of the fatty acids of which suberin and cutin are composed. Under the first supposition one would naturally infer that the suberized and cuticularized portions of cell walls are very similar in composition. This assumption, however, would be at variance with experimental evidence presented by van Wisselingh (7). The object of this investigation of the product of the ceric acid reaction of suberin is to determine if this product is simply a mixture of the fatty acids of which suberin is known to be composed, and to endeavor to isolate and identify these respective acids.

## HISTORICAL.

The ceric acid reaction was first reported by Doepping (1) in 1843. He obtained a substance which he called cerine, from the bark of the cork oak, *Quercus suber*, by extracting with alcohol or ether. The substance was crystalline, soluble in potassium hydrate and, when treated with concentrated nitric acid, a yellow or golden-yellow, wax-like product was obtained, to which the name ceric acid or cork wax was given. Doepping did not believe that all of the cerine could be extracted with alcohol or ether, because of the fact that bark, after extraction,

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upon being treated with concentrated nitric acid, still yielded a substance similar in appearance and solubility reactions to ceric acid.

Von Höhnel (4), in 1877, modified the reaction by using nitric acid and potassium chlorate. He proposed this as a valuable test for the detection of both suberin and cutin, both substances yielding, when oxidized, oil-like globules which coalesced upon the surface of the liquid. He further characterized ceric acid as melting between 30° and 40°C. and soluble in boiling alcohol, ether, chloroform, benzol, and dilute potassium hydroxide. In von Höhnel's opinion there was no relation between cerine and ceric acid.

In his researches on the bark of the cork oak, Kügler (5) reported the presence of cerine, a higher alcohol, with a melting point of 250°F, stearic acid, phellonic acid, glycerine, and small quantities of coniferin and vanillin. The cork residue, after these substances had been extracted, did not yield ceric acid when oxidized. Van Wisselingh (7), using for the most part microchemical methods, detected both fusible and infusible substances present in the suberized walls of cork, and made special reference to phellonic acid. He considered suberin as a composite product of fatty acids or of analogous substances, such as ethers, glycerides, or compound ethers, and of one or more non-fusible substances, insoluble in chloroform, but which are decomposed by potassium hydroxide.

About the same time, Gilson (3), using the macrochemical methods of organic analysis, isolated three fatty acids, viz., phellonic, a white crystalline substance, melting at 95° to 96°C. suberic semi-liquid at ordinary temperatures and phloionic crystallizing in white needles melting at 120° to 121°C. He also reported the presence of glycerine. In his investigations, the bark of the cork oak, *Quercus suber*, also of *Ulmus campestris*, var. *suberosa*, were used. In the latter, no phloionic acid was found, and only a very small quantity in the former.

Gilson (3) considered suberin to be a mixture of compound ethers slightly fusible and insoluble in such solvents as alcohol, ether, and chloroform, or a product of combination, condensation, or polymerization of the acids or their derivatives. In this respect he was not in agreement with Kügler, who considered suberin to be a fat in the exact sense of the word.

After reviewing and confirming the work of Gilson, Priestly (6) states that we may consider suberin as an aggregate of

variously modified forms, possibly of condensation products or anhydrides of the acids present; also that the suberogenic acids are to a small extent combined with glycerine thus partaking of the nature of true fats.

In cutin, Fremy and Urbain (2) reported the presence of two fatty acids, stearocutic and oleocutic. All investigators agree that phellonic acid is lacking in cutin. On the other hand, van Wisselingh, in his summary of his researches on cutin and suberin, definitely states that there are no acids in suberin identical with the steareocutic and oleocutic of cutin. When both substances were heated in glycerin at 300°C. the residue from suberin is readily soluble in chromic acid while that from cutin is not. According to this author, both kinds of lamella yield ceric acid when oxidized with nitric acid and potassium chlorate. Cerine has never been mentioned as a constituent of cutin.

Since there appears to be a general agreement especially among the latter investigators that suberin and cutin are distinctly different substances, the question arises as to whether the ceric acid obtained in each case is the same compound, or varies according to its source.

#### EXPERIMENTAL.

When the cerine, which, according to Doepping (1) could be extracted from cork by hot alcohol or ether, was oxidized with nitric acid and potassium chlorate, the product obtained softened, but did not melt at the boiling temperature of water, whereas the product of oxidation of the cork residue, after the cerine was removed, or of the entire cork lamella, including the cerine, melted between 30° and 40°C. Evidently, similar observations led von Höhnel (4) to disregard cerine as the source of ceric acid. Since suberin and cutin are recognized to be aggregates of fatty acids, it seemed reasonable to assume that their products of oxidation may likewise be mixtures of the same fatty acids of which they are composed. The ceric acid obtained from the suberin of cork was investigated from this point of view.

The work of Gilson (3) was repeated, using a granulated cork, in order to gain familiarity with the details of analysis and also for the purpose of securing samples of the various acids for later comparison. The same method of analysis was

adapted as far as possible in the analysis of ceric acid produced from the ceric acid reaction.

The ceric acid was prepared by macerating granulated cork in a mixture of equal parts of a concentrated, aqueous solution of potassium chlorate and nitric acid specific gravity 1.42. The mixture was heated to boiling and the reaction allowed to continue until the cork was completely disintegrated and oxidation was complete. At this stage a layer of golden yellow wax-like substance fused together and spread over the surface of the liquid. After cooling upon ice it solidified and became brittle. The wax was then removed and washed with distilled water until no trace of nitrates remained; about 20 grams being prepared in this way. It was then dissolved in 250 cc. of boiling alcohol and filtered. Only the portion which was completely soluble in boiling alcohol was used in the analysis.

After filtering sufficient solid potassium hydroxide was added to make a 3 percent solution and the acid was saponified by refluxing for one hour, care being taken to keep the solution distinctly alkaline to litmus paper. With the addition of the alkali, the color quickly changed, even with slight heating, from a straw yellow to dark brown. After refluxing and cooling upon ice a flocculent brownish white precipitate settled to the bottom of the flask. The precipitate was removed by filtering, washed with alcohol and recrystallized three times out of hot alcohol, by cooling. The alcohol was then removed by heating on a water bath. When some of the residue was then treated with a solution of iodine and potassium iodide and afterwards a few drops of 12 percent sulphuric acid or with chloro-zinc-iodide the typical rose or rose violet color indicative of potassium phellonate appeared.

The precipitate was further purified by boiling in a 25 percent solution of sodium chloride made alkaline with potassium hydroxide until the greater portion of the coloring matter was removed. It was then taken up in hot, distilled water and sulphuric acid added in excess to set free the acid from the potassium salt. After filtering, and washing to remove sulphates the residue was dried on a water bath; then recrystallized several times from alcohol, finally from chloroform, and allowed to stand in a desiccator over sulphuric acid for several days. The melting point was found to vary from 93° to 95°C. This is below the melting point of phellonic acid which is given as 95° to 96°C. When the residue was treated with chloro-zinc-

iodide particles were noticed which were yellow to yellowish brown in color, whereas the greater part of the preparation showed the rose or rose violet characteristic of phellonic acid and its salts, with the exception of the copper salt which colors brown. It was thought probable that this impurity was due to the fatty acid derived from the oxidation of the cerine. This acid appeared to be similar in solubility reactions to the phellonic and its potassium salt. It was very difficult to remove this impurity. After crystallizing again three times from hot alcohol, and the same number of times from boiling chloroform, a product was obtained which melted at 95°C. When a portion of this acid was thoroughly mixed with an equal quantity of known phellonic acid which had previously been extracted from cork, the melting point remained unchanged. The evidence appeared to be sufficient to conclude that phellonic acid had been isolated from the product of the ceric acid reaction.

The potassium salt of phellonic acid prepared from the acid gives the distinguishing rose or rose violet color with the iodine reagents, much better than the crude salt. This salt also gives the color reaction much more readily than the acid. When small particles showing the rose violet color were removed from the iodine solution and placed in a drop of water, the color promptly disappeared. It also disappeared from the iodine solution upon warming, and reappeared upon cooling.

The analysis was continued with the first filtrate following Gilson's (3) method and both suberic and phloionic acids were isolated. These acids forming the product of the ceric acid reaction, appeared to be identical with those obtained from cork by the extraction method. Only a very small quantity of phloionic acid was secured, not enough for confirmatory melting point determinations. The fine needles which crystallized out of hot water, upon cooling, could not be distinguished from those isolated from the cork extract. Less attention, however, has been directed to these acids because no tests have been discovered for their detection which would be adapted for microchemical use. It is for this reason that so much attention has been given to phellonic acid, more particularly to its potassium salt. The color which is given with the iodine reagents is so distinctive that it can be detected in very minute quantities; yet there is less than one fourth as much phellonic acid as suberic acid in the suberine of cork, according to Gilson (3).

## SUMMARY AND CONCLUSION.

The results of former investigations (Gilson (3), van Wiselingh (7), Priestley (6), regarding the nature of suberin would indicate that it is an aggregate made up of a number of fatty acids in varying degrees of polymerization. It may also be inferred that these acids are in some form in which they are insoluble in the common organic solvents. It is evident that they do not occur as acids or as salts, because none of them are present in the alcoholic extract of cork. When suberized lamellæ are treated with a strong oxidizing reagent such as nitric acid and potassium chlorate these acids fuse together forming a substance commonly referred to as "ceric acid," or cork wax. In the state into which they fuse they manifest a common property toward the action of such organic solvent as hot chloroform, alcohol, ether and benzol, but are insoluble in water.

It could undoubtedly be possible to detect and isolate other fatty acids in the oxidation product of suberin. No attention has been given to the identity of the acid which would be derived from cerine. It has been my particular interest to determine if the three fatty acids reported by Gilson (3) to be present in suberine still maintained their identities in the oxidation product of this substance. This has been done. The term "ceric acid reaction" is evidently misleading and should more properly be designated as the "oxidation reaction," because only a very small part of the fatty acids incorporated in the oxidation product of suberin, comes from the cerine.

There is considerable reason to believe that the composition of suberine may vary in different plants depending upon the conditions under which it is formed. Gilson was not able to isolate any phloënic acid from elm bark. If this proposition be true, it must follow that the oxidation products must also show wide variation when applied to different types of suberized lamellæ.

Even with our present conception of the constitution of cutin, there is no reason to believe that the oxidation product of this substance has very much in common with that of suberine. Fremy and Urbain (2) have reported two fatty acids present in cutin of Agave, viz. oleocutic and steareocutic. While the first may resemble in some respects the suberic acid of suberin, the steareocutic appears to be entirely different

from any of the suberogenic acids yet known. The oxidation reaction with respect to cutin requires further investigation.

Possibly, this interpretation of the ceric acid reaction may aid in further investigations of suberized lamellæ, particularly in tissue where the amount of suberin may be relatively small. In such cases there is difficulty at times in differentiating the action of the iodine reagents upon cellulose and suberized lamellæ after macerating in concentrated potassium hydrate. Von Höhnel erred in this connection and von Wisselingh (8) pointed out his mistake. By saponifying the fusion products of oxidation with a 3 to 5 percent solution of alcoholic potash, and cooling, the potassium phellonic salt will precipitate out, if suberin is present. Separate the precipitate from the liquid by filtering and wash several times with 95 percent alcohol. To a small portion of the residue on a glass slide or watch glass add a few drops of chloro-zinc-iodide. If the potassium salt of phellonic acid is present it may readily be detected by the characteristic rose or rose violet color of the disc-like crystals, which commonly form in clumps or aggregates.

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