Title: SEVERAL UNSTABLE COMPOUNDS IN PLANTS

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[illegible] CONDITIONS IN PLANTS

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Lipmann and his collaborators /2/ have established important role of acylphosphates in the metabolism of bacteria; in fact, they have separated from bacteria and identified acetylphosphate in the form of its silver salt.

Labile phosphorus compounds of the acylphosphate type are very unstable and they are decomposed under conditions standard for the determination of inorganic phosphorus. The inorganic phosphorus, determined by the ordinary Fiske and Subbarow method, is actually a mixture of inorganic phosphorus and phosphorus of labile compounds /3/.

Lipmann and Tuttle have suggested two micromethods for the determination of acylphosphate phosphorus.

The first is based on the solubility of the calcium salt of acylphosphate in an alcohol-water medium (25% alcohol). The inorganic phosphorus is completely precipitated by calcium chloride at pH = 8.

The difference between the "inorganic phosphorus" determined by the Fiske-Subbarow method and the real inorganic phosphorus represents acylphosphate phosphorus /4/.

1 g of green leaves was quickly triturated along with 5 ml of ice water; 5 ml of 12% trichloracetic acid were added; and the mixture was centrifuged. 1 ml of the centrifuged liquid was neutralized with ammonium acetate solution to give a weak violet color with thymol blue. (The ammonium acetate solution was prepared by mixing 10 ml of 25% ammonia, add 4 ml of glacial acetic acid, 10 ml of a 0.4 M solution of sodium bicarbonate, and 75 ml of water). Then 2 ml of a 0.5 M solution of calcium chloride in 40% alcohol were added. This made the reaction mixture yellow.

Addition of the ammonium acetate solution changed the color of the reaction mixture once more to become a weak violet. Subsequently, 5 drops of a 0.04 M solution of bicarbonate were added, and the resulting solution was allowed to stand for 3 minutes to permit complete precipitation. Then centrifuging was carried out and the centrifugal liquid was decanted; the residue was washed with a 2 ml solution of calcium chlorides of the same concentration, and the centrifuging was carried out again.

The entire operation was conducted in the course of 15-20 minutes from the beginning of the trituration of the leaf at a temperature of 5-0°.

The washed residue was dissolved in 1 drop of strong hydrochloric acid, and the phosphorus was determined by the usual method. The amount of phosphorus found was taken to be actual inorganic phosphorus.

In parallel with this operation, an equal part of the original solution was used to determine phosphorus by the Fiske-Subbarow method. The difference between the inorganic phosphorus determined according to Fiske and Subbarow and the real inorganic phosphorus determined by the author's procedure should represent the amount of acylphosphate phosphorus.

A great number of experiments using this method were conducted by the author, but she did not find acylphosphates in any one of them. This included determinations carried out on Tradescantia (spiderwort), Pelargonium (geranium), cyclamen, fuchsia, etc.

In several experiments, before disintegration, the leaves of Tradescantia were frozen with liquid air and treated with chilled trichloroacetic acid in order to protect the acylphosphates from the destructive action of enzymes. Again, no acylphosphates were detected.

To study methods of determination and behavior of acylphosphates this scientists synthesized acetylphosphate /4/, which was then determined with sufficient accuracy with the above method.

Now, an aqueous solution of the sodium salt of acetylphosphate is stable enough and can be kept for a long time in a neutral medium in the

refrigerator. Such a solution, containing 325 γ of labile phosphorus per ml, was prepared. After standing in the refrigerator for 17 days, it was found to retain 200 γ/ml of labile phosphorus corresponding to 60% of unchanged acetylphosphate.

The dry silver salt of acetylphosphate, when stored in a desiccator in darkness for many months, did not decompose.

The author then assumed that in plants containing enzymes, decomposition during disintegration occurred in plants containing acylphosphates faster than it was possible to analyse the latter. To check this assumption, leaves of Transcantia were triturated in the presence of a 0.0001 M solution of sodium salt of acetylphosphate and allowed to stand 30 minutes at 25°, after which an analysis was carried out. Partial decomposition of the acetylphosphate occurred, but 63% of it remained unaffected.

The second method for the determination of acylphosphates, suggested by Lipmann and Tuttle, is based on the ability of hydroxylamine to form hydroxamic acids with anhydrides of acids. In the presence of iron chlorides, the hydroxamic acids, which form according to the formula shown below, give brightly colored products.

$$RCOOPO_3H_2 + H_2NOH \rightarrow RCONHOH + H_3PO_4$$

In conducting this reaction with plant material, A. M. Kuzin and M. Ya. Chkolnik /6/ obtained a color similar to the one that hydroxamic acids form with iron chlorides, and on the basis of this made an assumption concerning the presence of acylphosphate compounds in plants.

The author studied this reaction in much detail. The intensity of the color produced by the green plants with hydroxylamine and iron chloride proved to be excessive in comparison with the color produced with a standard solution of synthetic acetylphosphate.

For example, in Transcantia, 0.5% of labile phosphorus were found by the "hydroxamic" method, whereas the material actually contained only about 0.3%.

This discrepancy arises from the fact that this reaction is not specific

[illegible] the intensive detection of the presence of ether, as [illegible] which give a color with hydroxylamine and iron chloride, this color being analogous to the color produced by hydroxamic acid with iron chloride.

In conducting this reaction with a destroyed leaf structure (as a result of trituration before introducing hydroxylamine) a color was not produced. It was produced only as a result of the simultaneous trituration of the leaf and introduction of hydroxylamine.

This suggests that in the given reaction the influence of some enzyme system is felt. An attempt to inhibit such systems by the introduction of neutral hydrazine of semicarbazide showed that in the presence of these substances and iron chloride a color similar to the color obtained with hydroxamic acid and iron chloride was produced.

The interaction with semicarbazide or hydrazine in the case of the destroyed leaf structure does not produce a color with iron chloride.

Experiments conducted in order to investigate the interaction of synthetic acetylphosphate either with semicarbazide or with hydrazine followed by a subsequent interation with iron chloride gave negative results; that is, no colored products were obtained. Therefore, it follows that plants contain substances giving color reactions similar to the "hydroxamic" reaction, although these substances are not acylphosphates.

The author suggested that the substances responsible for the similar color are of the phenolquinone type. It is known that these substances are contained extensively in plants and that, according to the work of V. I. Palladin /7/, Oparin /8/, and others, they participate in oxidation and reduction systems.

As a result of trituration of the leaf, its structure is broken, intensifying the oxidation reaction in which substances of the quinone type participate to a large degree.

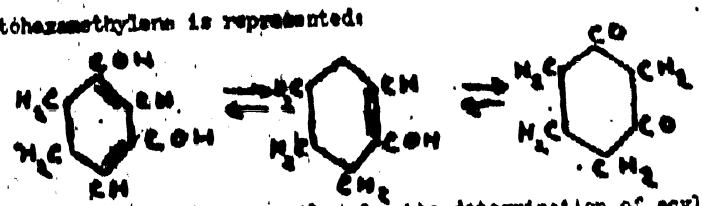
These substances are very active and in the instant during which the leaf is pulverized in the presence of enzymes, they are rapidly changed (oxidized and condensed).

[illegible] in the presence of semicarbazide, hydrazine, or hydroxylamine, [illegible] enzyme systems which is specific in this manner.

Substances of phenol-quinoid structure remain free and probably have in their structure carbonyl groups which react with amino groups of the compounds indicated above. Compounds such as semicarbazone, hydrazones, and oximes, giving colored products, and precipitating with iron chloride a color similar to that obtained with hydroxamic acid and iron chlorides. In confirmation of the above assumption, p-benzoquinone in a concentration of 0.001 M was tested, and it interacted with semicarbazide and iron chloride to produce a red coloration.

An aqueous solution of dihydroresorcin was also colored in the presence of hydroxylamine as a result of the addition of iron chloride to create a gradually deepening cherry red coloration.

For dihydroresorcin, a typically tautomeric transfer into diketohexamethylene is represented:



The "hydroxamic" micromethod for the determination of acylphosphates as suggested by Lipmann and Tuttle is not specific for use with plant tissues, since similar colors can be obtained when quinones and phenols are present in the plants.

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