CLXIX. ON THE NON-PROTEIN CYSTEINE IN PLANTS.

PRELIMINARY NOTE ON THE ATTEMPTED ISOLATION OF GLUTATHIONE FROM THE PEA (*PISUM SATIVUM*).

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(Received October 7th, 1926.)

GOLA [1902] was the first to call attention to the colour reaction of some plant tissues with sodium nitroprusside and he suggested that it might be due to the presence of cysteine.

The present communication reports the attempted isolation of the substance responsible for this colour reaction.

Microchemical test. In order to find out which material was the richest in the substance giving the reaction, different plant tissues were compared in the following way. Small slices of tissues were ground with concentrated ammonium sulphate solution; a few drops of a freshly prepared 5 % solution of sodium nitroprusside were added and, after stirring, a drop of dilute ammonia. In many cases a fleeting purple-red colour developed immediately, and this was generally the case with the meristematic tissues of roots, with the ovules, and with young and ripe seeds. The substance responsible for the colour reaction could be extracted with hot water from the above material and was found in the filtrate after precipitation of the proteins.

In comparative tests it was found that the strongest colour reaction was given by the seeds of some *Leguminosae*, such as *Phaseolus multiflorus*, *Pisum sativum*, *Lupinus albus*, *Vicia faba* (not ripe), and also by the young seeds of many other plants, e.g. *Iris*, *Fritillaria*, *Scilla*, *Papaver*, *Acer*, *Melandrium*, *Primula*, *Helianthemum* sp. Much weaker reactions were obtained from the etiolated stems of asparagus, from potato tubers (*Solanum tuberosum*), from the roots of radish, from the latex of *Lactuca sativa* and from the green shells of the unripe fruit of peas and beans.

In some other cases the colour was different; for instance, that given by the scales of onions (*Allium Cepa*) was a violet, but if the juice which was squeezed out from them was mixed with one drop of nitroprusside solution it gave, after addition of ammonia, an intense golden yellow colour. When using the scales of garlic (*Allium sativum*) a bluish sediment appeared after some time. The fresh green leaves gave an olive green colour.

The seeds of the pea were used for the attempted isolation, described

below, of the substance responsible for the colour. An extract of the peas still gave, after precipitation of the proteins, the purple-red colour with nitroprusside and contained organic sulphur. Therefore it seemed probable that a simple compound containing an SH group such as cysteine or glutathione was present, and to decide which of these was actually present extraction and isolation based on Hopkins' method [1921] were undertaken.

ISOLATION OF CYSTEINE FROM THE PEA.

About 50 pounds of ripe green peas were soaked in tap-water for 24 hours and were then extracted three times with boiling water. After the hot extract had been strained through muslin, acetic acid was added and the precipitated proteins were filtered off. To the filtrate (50 l.) a concentrated solution of neutral lead acetate was added with constant stirring until no further precipitate was formed. The subsequent treatment of the lead precipitate was essentially that described by Hopkins for the isolation of glutathione. Believing, however, that part of the substance sought was present in the oxidised form, which (in the case of glutathione) is not thrown down by lead acetate, I precipitated the filtrate from the lead precipitate with mercuric sulphate. Both precipitates were decomposed with H_2S and the filtrate from the sulphide received in each case separate but identical treatment. After removing H_2S a concentrated solution of uranium acetate was added until excess was shown on testing with ferrocyanide solution. After filtering off the precipitate, a concentrated solution of $\text{Ba}(\text{OH})_2$ was added; the precipitate thus formed was filtered off, and the baryta removed from the filtrate by means of H_2SO_4 . The filtrate was precipitated with a 10 % solution of phosphotungstic acid (dissolved in 5 % sulphuric acid), and the filtrate was freed from the excess of phosphotungstic acid by means of a hot concentrated solution of $\text{Ba}(\text{OH})_2$, and the excess of this was removed by H_2SO_4 . The filtrate was now precipitated by acid mercuric sulphate, the precipitate, after being washed with dilute mercuric sulphate, was suspended in water and decomposed with H_2S . The mercuric sulphide was filtered off, and the filtrate freed from excess of H_2S by bubbling hydrogen through it. It was now precipitated with neutral lead acetate, and the precipitate decomposed with H_2S . The last two steps—precipitation by Hg and Pb—were repeated, and, finally, the fluid was neutralised with $\text{Ba}(\text{OH})_2$ and air was blown through until the reaction with nitroprusside quite disappeared. The solution was exactly freed from Ba, and filtered into absolute alcohol. After 24 hours a white precipitate was separated by centrifuging, washed with alcohol and dried in a vacuum desiccator.

The yield was 28 mg. from the first lead precipitate and 200 mg. from the first mercury precipitate.

Properties of the isolated substance. The product which was obtained was a white, non-hygroscopic powder, easily soluble in water and insoluble in alcohol, ether and acetone. When dissolved in a small amount of water it

gave a yellow solution, which, after evaporation in a vacuum desiccator, left a yellow transparent amorphous mass behind and a small amount of a crystalline material which was mainly in the form of small sharp-pointed prisms gathered together in spheroids. After addition of water the crystals remained undissolved and were separated by filtration.

Hydrolysis. To the filtrate hydrochloric acid was added until it was present to the extent of 25 %. After being boiled for eight hours, the solution was cooled in ice and saturated with hydrochloric acid gas. It was placed in the ice-chest, but, as no crystals were deposited after 24 hours, the solution was concentrated under reduced pressure at 40–50° and again saturated with hydrochloric acid gas while being cooled on ice. The glistening white crystals were allowed to settle out for 24 hours in the ice-chest. They were separated by centrifuging, washed with cold concentrated HCl and also with cold alcohol saturated with HCl, and then dried over calcium chloride in a vacuum desiccator. Under the microscope the crystals appeared as long transparent flat parallelograms arranged singly and gathered together in rosettes. There were also present flat prisms unsymmetrically pointed at both ends. The substance melted at 204° (uncorr.) with decomposition, and at 213.5° (uncorr.) after repeated purification. When dissolved in a small amount of water, the crystals soon deposited an insoluble substance in the form of small needles gathered together in spheroids; after evaporation in a vacuum desiccator the residue appeared to be composed of spheroids and hexagonal symmetrical plates. When the substance was heated over a flame on a platinum foil it burned with a greenish flame without melting. It was insoluble in water, alcohol, acetone, ether and acetic acid; soluble in dilute ammonia and mineral acids. When heated in a capillary tube it blackened above 213° without melting. It was found to give the tests for a sulphur-containing compound and also a bluish violet colour with ninhydrin in neutral solution. When dissolved in dilute ammonia and acidified with acetic acid, addition of acetone caused a precipitate of small cubes, and when it was precipitated from its solution by means of mercuric sulphate and the precipitate suspended in water was decomposed with H_2S , a filtrate was obtained which reacted in the same manner with nitroprusside as cysteine.

These properties proved the substance to be cystine.

The mother liquors after the fractional separation of cystine hydrochloride left on evaporation under diminished pressure at 50° a brown amorphous substance which was insoluble in water.

Besides the cystine no other amino-acid could be identified in the products of hydrolysis of the substance isolated from peas, although from some hydrolyses of the original substance a small amount of crystals, resembling the hydrochloride of glutaminic acid, was obtained, melting at 203° without blackening. No further work could be carried out on them on account of the smallness of the yield.

Isolation of non-protein cysteine from grey "maple" peas. The ripe seeds

(25 kg.), without being previously soaked, were ground and extracted with boiling 1 % acetic acid. The extract was purified by the means described above.

The yield of substance was smaller than from the green peas and the only product of hydrolysis that could be identified was cystine.

Isolation of cystine from the leaves of "maple" peas. Although the leaves of the peas gave a negative nitroprusside reaction it was thought that this was not conclusive evidence that cystine was absent and this supposition was justified by the following experiment.

About 10 kg. of fresh green stems together with leaves (fruit and yellow leaves being separated), which were collected before the seeds were ripe, were extracted three times with boiling water. The extract was precipitated with acid mercuric sulphate, and the precipitate decomposed by H_2S . The filtrate from the sulphide was purified by the method above described. A very small amount of substance was finally obtained which gave the nitroprusside reaction and also the reaction for sulphur.

DISCUSSION.

The above described work shows that non-protein cysteine is present in the seeds of peas. On the ground of microchemical examinations it seems likely that this substance is widely distributed in the plant kingdom. On account of the small yield of the substance, it was difficult to establish whether the cysteine and cystine respectively are present in the pea tissues in the free state or bound to some other compound. It is certain, however, that the cysteine which is responsible for the colour reaction with ammoniacal sodium nitroprusside is not in the form of a protein. Although the proteins which were precipitated from the extract by means of phosphotungstic acid, acetic and trichloroacetic acids still gave the colour reaction, the substance responsible for it could be gradually, although not completely, separated by redissolving and reprecipitating the proteins. It is possible that cysteine, or the compound in which it is present, was adsorbed by the protein. On the other hand, in addition to the cysteine, there may also exist in the pea and other plants a protein which, after denaturation, gives the nitroprusside reaction like denaturated egg-albumin [Harris, 1922] or philothion- β [de Rey Pailhade, 1926], but before this can be decided further work must be done.

It is very probable that the cysteine found in the pea is not in the free state but is bound with glutaminic acid, as in yeast and mammalian tissues.

SUMMARY.

1. The pea (*Pisum sativum*) contains non-protein cysteine in the ovules, young and ripe seeds, and in the green shells of the unripe fruit; cystine is present in the leaves.
2. From histochemical studies it is very probable that cysteine is wide-

spread in the higher plants, not only in the meristem as Gola suggested, but also in the "reserve" tissues and in latex.

3. By the microchemical colour reaction of plant tissue with ammoniacal sodium nitroprusside it is not only possible to detect cysteine but also to follow histochemically its oxidation in the living tissues.

4. It is very probable that in the pea the cysteine is bound with glutaminic acid as a dipeptide such as glutathione.

5. The presence of free cysteine and of proteins corresponding to philothion in the pea is not excluded.

In conclusion, the author wishes to express his indebtedness to the Rockefeller Foundation for a fellowship, and also to Sir F. G. Hopkins for his kindness and the interest he has shown in this work.

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