

## CHAPTER I

### INTRODUCTION

This chapter should be re-read after the remaining chapters have been studied.

ALL plants are made up of a complex organized mixture of chemical substances, both organic and inorganic. As a preliminary to the study of plant chemistry, the student should realize that the chemical compounds which make up the living plant may be approximately grouped into the six following classes. Thus, in later chapters, when reference is made to any plant product, it will be understood, broadly speaking, to which class it belongs, and what relationship it bears to other chemical compounds.

The main classes may be enumerated as follows :

(1) *Carbohydrates*. The simplest members of this class are the sugars, which are aldehydes and ketones of polyhydric alcohols of the methane series of hydrocarbons. The more complex carbohydrates, such as starch, cellulose, dextrans, gums and mucilages, are condensation products of the simpler sugars. The sugars are found in solution in the cell-sap of living cells throughout the plant. Cellulose, in the form of cell-walls, constitutes an important part of the structure of the plant, and starch is one of the most widely distributed solid "reserve materials."

(2) *Vegetable acids*. This term is usually applied to acids and hydroxy-acids derived from the *lower* members of the methane, olefine and acetylene series of hydrocarbons. Such acids as formic, acetic, valeric and caproic are not readily detected in the plant. Nevertheless, it is more than likely that they play an important part in metabolism, for their amino derivatives, glycine, valine, etc. (see section 5) form constituents of practically all proteins. The dibasic and hydroxy-acids, e.g. oxalic, succinic, glutaric, malic, etc., are probably products of oxidation of the sugars in respiration. Aspartic (amino-succinic) and glutaminic (amino-glutaric) acids are also constituents of proteins.

(3) *Fats*. Chemically these are glycerides, that is glycerol esters, of acids derived from the *higher* members of the methane and olefine series of hydrocarbons, and they usually contain a large number of carbon atoms. The fats occur as very fine globules deposited in the cells, especially in the tissues of seeds where they form reserve materials, though they also occur in other parts of plants.

The lecithins, which are compounds of fats with phosphoric acid, are probably present in all living cells and have an important metabolic significance.

The above substances belong to the aliphatic series of organic compounds, that is to the series in which the carbon atoms are united in chains.

(4) *Aromatic compounds.* These are characterized by having the carbon atoms united in a ring as in benzene. They may contain more than one carbon ring, and, moreover, aliphatic groupings may be attached to the carbon ring as side-chains. The number of aromatic substances is very great, and every plant contains representatives of the class. Some members are widely distributed; others, as far as we know, are restricted in their distribution, and may be peculiar to an order, a genus or even a species. This class contains: (a) Phenols, i.e. hydroxy-derivatives of benzene, such as phloroglucinol. (b) Aromatic alcohols, aldehydes and acids derived from benzene; various hydroxy-benzoic acids, such as gallic and protocatechuic acids, are important, since, by condensation, they give rise to tannins. Just as in the case of the carbohydrates, where simpler compounds may become more complex by condensation, the soluble crystalline acids condense to form the complex colloidal tannins. Of other aromatic acids, the amino derivatives, such as phenylalanine and tyrosine, form constituents of proteins. (c) Complex hydrocarbons, the terpenes, accompanied by derivative alcohols, aldehydes, ketones and esters. These form constituents of the "essential oils" obtained from plants by steam distillation, and are responsible for most of the plant scents. (d) Other members which contain more than one ring are the water-soluble yellow, red, purple and blue pigments of plants, the yellow being hydroxy-flavones and flavonols, the remainder, anthocyan pigments.

(5) *Proteins.* This large class contains substances which are in many cases built up of groupings from both the aliphatic and aromatic series. It includes not only the proteins but also their simpler derivatives, the albumoses, peptones and polypeptides. In this case, as before, the simplest derivatives, known as the amino-acids, are synthesized by condensation to form the polypeptides, peptones, albumoses and proteins, in a series of increasing complexity. The amino-acids are compounds, either of the aliphatic, aromatic or heterocyclic (see 6) series, in which one or more hydrogen atoms are replaced by the radicle  $\text{NH}_2$ . They are soluble and crystalline, but after condensing together, the final product, the protein, only exists in either the solid or the colloidal state. Proteins, in the latter condition, constitute the bulk of the complex material, protoplasm;

in the solid state, in the form of grains and granules, they occur as reserve material in the cell.

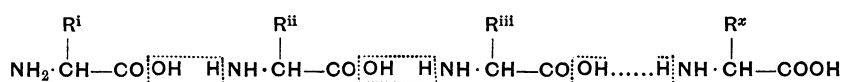
(6) *Plant bases*. This class contains (a) the amines or substitution products of ammonia. Sometimes the hydrogen of ammonia is substituted by a group of some complexity which leads to the production of a compound of the heterocyclic type, i.e. with a ring containing both carbon and nitrogen atoms. The pyrrole ring is an example which occurs in the amino-acid, proline, in certain alkaloids (see below), and in the pigment chlorophyll. (b) Purines. In connection with these substances we need to consider two more heterocyclic rings, i.e. the pyrimidine and the iminazole. The former may be regarded as the condensation product of urea, which is possibly present in small quantities in plants, and an unsaturated acid, e.g. acrylic acid. The pyrimidine ring is present in some purines, the iminazole in the amino-acid, histidine. The remaining purines contain a condensed pyrimidine and iminazole ring. Certain of the purines become condensed together, in combination with phosphoric acid and a pentose sugar, to form the nucleic acids. The latter, in combination with proteins, as nucleoproteins, form a constituent, as their name implies, of the nucleus. (c) The alkaloids are substances of considerable complexity, containing various heterocyclic rings. Unlike the simpler bases, they are restricted to a certain extent in their distribution.

It is not possible to include all classes of plant substances in the above list and many others, such as the sulphur compounds, sterols, phytin, etc., are referred to in the later chapters. It should be borne in mind that the importance of a compound in plant metabolism is not estimated by the amount of it occurring in the plant. Frequently, most important substances occur in such small quantities that they are difficult to detect.

In order to appreciate the subject of plant chemistry, the plant, which is familiar as a botanical entity, must be interpreted in chemical terms. The principal classes of the more essential and widely distributed compounds found in plants have already been indicated on the broadest basis, so that they may now be referred to without additional comment.

From the botanical point of view, the plant may be regarded as a structure composed of many living protoplasmic units enclosed in cell-walls and combined together to form tissues. There are also certain tissues, known as dead tissues, which assist in giving rigidity to the plant. All these structural elements may, in time, be translated into terms of chemical compounds.

One of the chemical processes most frequently met with in the plant is that of synthesis by condensation, with elimination of water, of large complex molecules from smaller and simpler molecules. The formation of cellulose, for instance, is a case in point. Cellulose has the composition  $(C_6H_{10}O_5)_n$  and, on hydrolysis with dilute acids, it yields glucose as a final product. Hence it is concluded that the complex molecule of cellulose is built up from the simpler carbohydrate by condensation. The synthesis of proteins from amino-acids affords another example. These acids contain either an aliphatic or aromatic nucleus (let it be  $R$ ), and one or more carboxyl and amino groups. Condensation takes place in the plant, with elimination of water, according to the following scheme:



The products of such condensation, the proteins, vary among themselves according to the number and kind of amino-acids which take part in the synthesis.

Two important results arise from this process. First, the substances formed by condensation have molecules of a very large size; secondly, whereas the simple compounds, sugars and amino-acids, are soluble, crystalline and diffusible, the condensation products are either insoluble, e.g. cellulose, or exist in the colloidal state, as is the case of many proteins and other plant constituents. As these very large molecules do not dialyze, they remain where they are synthesized, and build up the solid structure of the plant, as for instance, the cell-walls.

Matter in the colloidal state is of very great importance in the plant and is probably responsible for many of the properties of "living" material. Thus it will not be out of place, though it will be referred to again in a later chapter, to make at this point a few remarks on the colloidal state. It has been known for some time that certain metals, e.g. gold and silver, and also certain metallic hydroxides and sulphides, e.g. ferric hydroxide and arsenious sulphide, though insoluble in water under ordinary conditions, can, by special methods, be obtained as solutions which are clear to the unaided vision. Such solutions are termed colloidal. Investigation has shown that the matter is not present in true solution, but in a very finely divided state, i.e. as particles many times larger than simple molecules, but smaller than the particles obtainable by mechanical means of division. Such solutions are known as artificial colloidal solutions, but there are a number of organic substances, with very large molecules, such as proteins, starch, gums, agar, etc., which at

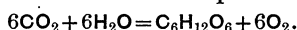
once dissolve in water giving colloidal solutions. The main feature of the colloidal state is that the system consists of two phases, or conditions of matter. In the case of the artificial colloidal solutions first mentioned, one state is solid, the gold particles; the other state is liquid, the water. The solid is known as the dispersed phase, and the water as the continuous phase, and such colloidal solutions are termed suspensoids. In the case of proteins, starch, etc., both phases are liquid: the dispersed phase, a concentrated solution of protein, etc.; the continuous phase, a dilute solution of protein, etc. Such colloidal solutions are known as emulsoids.

An important point in connexion with the colloidal state is that the molecules, or aggregates of molecules, forming the dispersed phase are so large that they exhibit some of the phenomena of surface energy, electrical charge, etc., associated with matter in mass. These properties come to be of considerable importance, when we consider how large a surface is presented by matter in this state in comparison with its mass.

A material in the plant upon which much interest naturally centres is the protoplasm and the nucleus. It has been shown that the protoplasm consists, chemically, largely of proteins in the colloidal state. It is itself a liquid, and embedded in it are substances of various chemical constitution, in the form of granules of solid matter and also liquid globules. Numerous chemical reactions are continually taking place in the protoplasm throughout the cell, and since many of these reactions can take place both simultaneously and independently, the protoplasm must have some form of organized structure. Though many phenomena of "life" may be accounted for by the physical and chemical properties of such substances as proteins, it is impossible to say, with our present knowledge, how far all "living" phenomena may yet be explained in this way.

Some of the main lines of metabolic syntheses which take place in the plant will next be considered. A fundamental fact which should be borne in mind is that the green plant synthesizes all the complex materials of which it is composed from the simple compounds, carbon dioxide, water and certain inorganic salts. The most important factor, perhaps, which figures in plant metabolism, is chlorophyll. The green pigments of chlorophyll are esters of complex organic acids containing the elements carbon, hydrogen, oxygen, nitrogen and magnesium. They have the remarkable power of absorbing the radiant energy of the sun's rays and of transforming it into chemical energy, by means of which carbon dioxide and water are combined to form some organic compound, possibly formaldehyde, from which a simple carbohydrate is readily synthesized.

If now the initial and final products of carbon assimilation be considered in detail, it will be seen that the process is one of reduction :



This is confirmed by the fact that oxygen is evolved in the process. Moreover, the plant accumulates a store of energy, since the final product, the carbohydrate, has a higher potential energy than the system, water and carbon dioxide. Hence carbon assimilation, in addition to providing a basis of organic material as a starting-point for all the main metabolic functions, also provides a source of chemical energy by means of which reactions in other directions are brought about.

The setting free of this accumulated energy constitutes the process of respiration, which is, in reality, an oxidation of carbohydrate taking place in tissues throughout the plant. It is the converse of carbon assimilation, in that oxygen is absorbed and carbon dioxide and water are formed. Thus these two processes, both so fundamental and essential to the metabolism of the green plant, are constantly taking place side by side in the same cell.

The first-formed carbohydrate, which is probably a hexose, is condensed in the plant, on the general lines we have previously indicated, to form more complex disaccharides and polysaccharides, such as maltose, cane-sugar, starch, cellulose, etc. Some of these products, such as the disaccharides, form true solutions and may be present in the cell-sap; others, such as cellulose and starch, are present in the solid state, though they contain considerable quantities of water. Others, again, such as dextrin and gum, are present in the colloidal state. Thus, given an initial carbohydrate and a source of energy, we may proceed to indicate the other main lines of syntheses in the plant.

The next most important line of syntheses is probably that which gives rise to the nitrogen-containing constituents of the plant. Nitrogen is absorbed by the green plant in the form of nitrates and ammonium salts, but the processes which lead to the synthesis of some of the simplest nitrogen-containing compounds, such as the amino-acids, are still very obscure. Aliphatic and aromatic acids of various kinds are abundantly present in the tissues, but the reactions by which the  $\text{NH}_2$  groups are introduced are by no means clear. There is little doubt, however, that once the amino-acids are formed, condensation takes place as already indicated, and more complex molecules, termed polypeptides, arise. Such polypeptides have now been synthesized artificially by the condensation of amino-acids. From the polypeptides, by further stages of condensation, the albumoses, peptones, and finally proteins are produced.

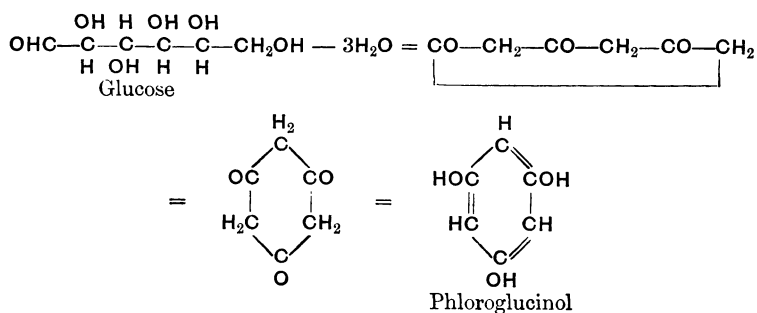
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Another line of syntheses is that which leads to the production of the fats and allied substances. The fats are mainly glycerides of acids of the methane and olefine series, such as butyric, palmitic and oleic acids. Like all other plant products the fats must either directly or indirectly arise from the carbohydrates. There is evidence that the origin is fairly direct, as, for instance, in fatty seeds when the fats take the place of sugars in ripening. The sugars, as we know, are aldehydes of the polyhydric alcohols of the methane series. It has been suggested, though the actual stages have not been ascertained, that by various oxidation and reduction processes, the sugars yield fatty acid residues which then condense to form the fatty acids of high molecular weights present in fats. By a converse process, the fats, especially when they are stored as reserve materials in seeds, are broken up, and sugars are again formed which pass to other parts of the germinating seedling, and are there used in other synthetic processes.

A third main line of syntheses is that which gives rise to the aromatics of the plant. Since no ring compound is absorbed by the green plant, it follows that by some process the aliphatic structure must be transformed into the aromatic. Thus, for instance, the trihydric phenol, phloroglucinol, might at some stage be formed from a hexose by conversion of the aliphatic chain into a closed ring:



There is evidence that aromatic compounds, such as phloroglucinol, tannins, flavones and anthocyanins are synthesized in the leaves, and that sugar-feeding, by floating leaves in sugar solutions, leads to the increase of aromatics in the tissues. When the ring structure has been once synthesized, further changes can take place either by the addition of side-chains to the ring or by the condensation of two or more rings. In this way the great multitude of aromatic products present in the higher plants may arise.

Thus the cell can be pictured as a colloidal solution of proteins endowed with the properties of matter in mass and surrounded by a permeable cell-wall of cellulose. The colloidal solution contains liquid and solid particles of very varied chemical composition. In the protoplasm are spaces, vacuoles, filled with cell-sap also containing many and various substances in solution. Throughout the protoplasm, which probably has an organized structure, many kinds of chemical reactions are continually in progress, some being the converse of others, as for instance those of oxidation and reduction which can take place side by side in the same cell.

Next will be considered the chemical reactions by which the various metabolic changes in the plant are brought about. How are these processes controlled and how do they take place?

There is a large group of organic substances, termed enzymes, many of which are present in every plant. They have a certain characteristic in common, i.e. they bring about chemical reactions in the plant without undergoing any permanent change: in other words they are organic catalysts. Many of these reactions, which take place in the cell at ordinary temperatures with considerable rapidity, need prolonged heating at high temperatures when brought about by artificial means. Enzymes can generally be extracted from the plant by water, especially if the tissues are thoroughly disintegrated. Their chemical constitution is at present unknown, and they are usually destroyed by temperatures greater than 60° C. Moreover, many of the processes which they control in the plant can be brought about by them *in vitro* under suitable conditions, and it is by means of such experiments that information as to their rôle in plant metabolism has been ascertained. The majority of known enzymes control both hydrolysis and its converse, synthesis by condensation with elimination of water, but under artificial conditions hydrolysis most frequently occurs. The enzyme, diastase, for instance, found in all starch-containing plants hydrolyzes *in vitro* starch to dextrin and maltose. Similarly the enzyme, maltase, hydrolyzes maltose into glucose. Other enzymes hydrolyze proteins into amino-acids, and others, again, hydrolyze fats into fatty acids and glycerol.

Until fairly recently the fact escaped notice that such reactions are reversible, and that these enzymes *in situ* in the plant may, according to the conditions, control not only the hydrolytic but also the corresponding synthetic process. The latter may also be brought about, though not readily, *in vitro*. This, and other evidence, leads us to believe that enzymes in the plant control the reactions in both directions.



Hydrolysis, and synthesis with elimination of water are not however the only processes catalyzed by enzymes. There is another type of these catalysts, the oxidizing enzymes, which bring about oxidation of substances in the plant, notably of aromatics. In addition, there is the enzyme, zymase, which decomposes sugar with the production of alcohol and carbon dioxide.

The question which now arises is—How many reactions in the plant are catalyzed by enzymes? It is conceivable that a greater number of enzymes may exist than are at present known, but that they are unable to be extracted by our present methods of isolation. A certain number of reactions probably take place in the cell-sap between the substances in solution; others are catalyzed by enzymes which are supposed to be intimately connected with the protoplasm, but there are an enormous number to which there is at present no clue as to how they are brought about, such, for instance, as the synthesis of carbohydrates from carbon dioxide and water, and the formation of the benzene ring from the open carbon chain. Such processes are usually said to be controlled by the “living protoplasm,” but what exactly is the significance of this expression is at present beyond our knowledge.

Finally, also, little is known of the question as to how the various lines of metabolic syntheses in different parts of plants are regulated and correlated with each other. Some of the phenomena involved are shortly outlined as follows. There is undoubtedly, under suitable conditions, a constant synthesis of sugars in the leaves. In all probability aromatic substances are also synthesized in the same organs, for there is evidence that there is an increase of these compounds in the leaf if translocation through the petiole is prevented. It is possible that amino-acids also are formed in the leaf. The above products are constantly translocated to the growing organs as material for growth. They may, nevertheless, be temporarily stored in the tissues where they have been synthesized, and of this there is evidence in at least one case, e.g. starch in the leaf. But, apart from the immediate use for growth, there is in practically every plant, some tissue where, owing to some unknown stimulus (causing probably changes in permeability of the cell-membranes), accumulation of compounds occurs. This accumulation is characteristic of organs from which growth will take place when it is impossible for the plant to obtain fresh supplies by carbon assimilation, as, for example, of bulbs, rhizomes, tubers, buds, seeds, fruits and woody tissues. In these cases, in due time, the products stored supply the growing shoots.

During storage, simple sugars, amino-acids, etc. have been condensed to form insoluble, colloidal, or large molecules of starch, fats, aleurone, cane-sugar, etc. These will remain until they are hydrolyzed by enzymes when they can supply the growing shoots. Such stores are termed "reserve materials." The actual stimuli involved in bringing about and regulating this storage are unknown, but they are probably connected with the life cycle of the particular plant under consideration and its adaptation to external conditions.

## REFERENCES

1. **Abderhalden, E.** Handbuch der biochemischen Arbeitsmethoden. Berlin, 1920- .
2. **Abderhalden, E.** Biochemisches Handlexikon. Berlin, 1911.
3. **Allen's Commercial Organic Analysis.** London, 1924-28. 5th ed.
4. **Bertrand, G., and Thomas, P.** Practical Biological Chemistry. Translated by H. A. Colwell. London, 1920.
5. **Cole, S. W.** Practical Physiological Chemistry. Cambridge, 1928. 8th ed.
6. **Czapek, F.** Biochemie der Pflanzen. Jena, Bd. 1, 1913, Bd. 2, 1920, Bd. 3, 1921.
7. **Haas, P., and Hill, T. G.** The Chemistry of Plant Products. London, 1928. 4th ed.
8. **Kostytschew, S.** Lehrbuch der Pflanzenphysiologie. Bd. 1. Chemische Physiologie. Berlin, 1926.
9. **Kostytschew, S.** Pflanzenatmung. Berlin, 1924.
10. **Palladin, V. I.** Plant Physiology. Edited by B. E. Livingston. Philadelphia, 1918.
11. **Plimmer, R. H. A.** Practical Organic and Biochemistry. London, 1926. 4th ed.
12. **Rosenthaler, L.** Grundzüge der chemischen Pflanzenuntersuchung. Berlin, 1923.
13. **Wehmer, C.** Die Pflanzenstoffe. Jena, 1911.
14. **Wester, D. H.** Anleitung zur Darstellung phytochemischer Uebungspräparate. Berlin, 1913.