Contributed and Selected

SYNTHESIS OF GLUCOSIDES.*

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Glucosides occur very abundantly in the vegetable kingdom. They have the properties of being hydrolyzed by the action of dilute acids, dilute alkalies, enzymes and at times even by heating with water. They are converted into dextrose and other sugars and into substances of more or less complex constitution.

The glucosides are generally present in the assimilation organs of the plants, i. e., the leaves; very rarely are they found in the reserve organs, the rhizomes or the seeds. Most of the glucosides contain only carbon, hydrogen and oxygen; only a few, such as amygdalin, solanin, etc., contain nitrogen or, like myronic acid and sinalbin, sulphur. Chemically the glucosides must be considered as ester-like compounds of sugars, mostly dextrose. The sugars, however, are not pre-existent in the glucosides but are formed from them by hydrolysis, that is by taking up the elements of water. The amount of water taken up varies considerably. Thus: Salicin takes up one molecule, populin takes up two molecules, hesperidin takes up three molecules, helleborin takes up four molecules, jalapin takes five molecules, sinigrin takes up seven molecules, etc.

For isolating the glucosides no general process can be given on account of the ease with which they are hydrolized. Strong mineral acids and strong basic compounds should be avoided in the manufacturing process as well as prolonged boiling. If the drugs containing the glucosides also contain a hydrolyzing ferment the latter should first be eliminated. This can be done by treating the dried powdered drug with boiling alcohol which contains a small amount of calcium carbonate in order to neutralize any free vegetable acid, and continuing the heating for about one hour. After cooling the liquid is filtered, the alcohol is evaporated at moderate heat or preferably in a vacuum, and from the thick alcoholic residue the glucoside is extracted by boiling with neutral acetic ether; or the alcoholic extract is dissolved in water in the presence of small amounts of calcium carbonate, the solution is filtered and the filtrate evaporated under diminished pressure. The residue is then extracted with alcohol, acetic ether, acetone or other suitable solvents and the resulting solutions allowed to crystallize. At times it is necessary to defecate the alcoholic extract by carefully adding lead acetate solution. The precipitate, thus produced, is then removed by

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filtration, the filtrate is deprived of the lead by hydrogen sulphide gas and the glucoside is allowed to crystallize at moderate heat.

The substances which are produced when hydrolizing glucosides are chiefly hydroxyl compounds (phenols, alcohols, aldehydes, acids) both of the aliphatic and of the aromatic series. About the constitution of these products very little is known at the present time and therefore only few of the glucosides occurring in nature could be synthesized. It was possible, however, to combine alcohols, mercaptols, phenols, etc., directly with dextrose.

Since glucosides contrary to dextrose do not act directly with phenylhydrazine with the formation of osazones, do not reduce Fehling's solution or ammoniacal silver solution, they cannot contain the aldehyde group peculiar to dextrose. They, therefore, can be compared with cane sugar and with the simplest synthetic glucosides—methyl- and ethyl-glucoside.

The glucosides are solid, non-volatile, generally crystallizable compounds which are soluble in water and alcohol with neutral reaction. The enzymes act in a very peculiar way on the glucosides. For instance, emulsin hydrolyzes amygdalin, salicin, esculin, coniferin, etc., but not sinigrin. The enzymes from yeast hydrolyze amygdalin with the formation of amygdonitril glucoside and one molecule of dextrose. Emulsin, however, hydrolyzes amygdalin completely with the formation of two molecules of dextrose and one molecule of benzaldehyde-hydrocyanic acid. The full activity of emulsin obtained from almonds can only be properly explained by assuming the presence of a number of enzymes, none of which, however, so far has been isolated from it. One of these, beta-glucosidase hydrolyzes certain glucosides, another lactase, acts on lactose, a third one, beta-galactosidase, acts on certain galactosides, a fourth one, gentiobiase, acts on gentiobiose, etc.

Notwithstanding our extensive knowledge concerning the hydrolysis of the various glucosides and of the action of the various enzymes up to the present time, as already mentioned, only very few of the glucosides found in nature have been prepared synthetically.

The first attempts to synthesize glucosides were made by Schuetzenberger (Ann. d. Chem. u. Pharm. CLX, 95) who tried to obtain salicin by the action of triacetyl-dextrose and saligenin sodium. He obtained, however, a substance which was not identical with salicin but which on hydrolyzing with diluted sulphuric acid yielded glucose and saliretin. Later on Michael (Ber. Deutsch. Chem. Ges., 1881, 2097) succeeded in obtaining compounds of dextrose with phenols which behaved exactly like the natural glucosides. The formation of these substances depended on the interaction of acetochlorohydrose or acetrobromohydrose and the alkali salts of phenols. For instance, starting from these substances and salicylaldehyde potassium he obtained helicin and acetic ether. Helicin, which can be obtained from salicin by careful oxidation, therefore was the first natural glucoside produced synthetically. From this comparatively simple glucoside Fisher and Kees (Ber. Deutsch. Chem. Ges., 1885, 1953 and 3481) succeeded in obtaining synthetically the more complex orthocumaraldehyde glucoside. Schiff (Ann. CCXXIV, 19) then tried to produce glucosides by allowing aldehydes and ketones to act on sugar in acetic acid solution. The substances thus obtained were very hygroscopic and were hydrolized already by

water. Emil Fisher (Ber. Deutsch. Chem. Ges., 1893, 2928) finally succeeded in synthesizing glucosides by conducting hydrochloric acid gas into a solution of dextrose in methyl alcohol. The mixture soon lost its property to reduce Fehling's solution and a crystalline product of the composition $C_6H_{11}O_6.CH_3$ which behaved like a natural glucoside and which has been named methyl glucoside was obtained. This process was later on improved by Fisher (Ber. Deutsch. Chem. Ges., 1895, 1145) by using instead of strong hydrochloric acid only very diluted acid and accelerating the reaction with the aid of heat. The dextrose was dissolved in five times its quantity of methyl alcohol which contained only .25 percent of hydrochloric acid and was heated for 50 hours at 100° or until the liquid no longer reduced Fehling's solution. By this process he was able to methylize fructose and sorbose and also to condense these sugars with ketones which could not be done when strong hydrochloric acid was employed. In a similar manner he succeeded in condensing sugars with mercaptanes, etc.

In 1898 A. C. Hill (Proc. Chem. Soc., 1898, 156), noticed that when bottom fermentation yeast prepared and purified by a special process was allowed to act on maltose, the hydrolysis could be carried out only to a certain point, and further that under certain conditions a reconstruction of the maltose apparently took place. This observation was also noticed by several other investigators but it was not until about three or four years ago that Bourquelot and his associates and pupils could prove that enzymes which hydrolyze glucosides can reconstruct them from the products of hydrolysis under certain conditions and further that by the action of enzymes glucosides can be formed, in other words, that enzymes exhibit both a hydrolyzing and synthesizing action. Further that the same enzyme can both hydrolyze and synthesize and that the action is not due to two ferments existing side by side in the same enzymes.

Bourquelot found that while preparing tinctures from certain vegetable drugs with strong alcohol changes take place during the manufacturing process, which could be due only to the action of enzymes, which apparently are not killed by strong alcohols as is generally accepted. Together with Bridel (Journal. Pharm. Chim., 1911, II, 385) he found that emulsin acts hydrolyzing on gentiopicrine in the presence of strong alcohol although the enzyme is not soluble in alcohols of a strength higher than 60 percent. The same holds good for invertase, which is also very resistent against alcohol.

While some enzymes resist the destructive action of ethyl alcohol quite well, they are comparatively easily destroyed by other alcohols. Thus for instance, by dilute methyl alcohol containing 34 to 36 percent by weight of the alcohol, normal propyl alcohol containing 20 to 22 gms. of the alcohol, butyl alcohol containing 6 to 8 percent by weight of alcohol. Bourquelot and Bridel (Journ. Pharm. Chim., 1912) later on found that emulsin not only hydrolyzes but synthesizes and that other enzymes have similar properties. Bourquelot and Bridel (Journ. Pharm. Chim., 1912, 569) then attempted to synthesize salicin from solutions of saligenin and dextrose in 85 percent alcohol in the presence of emulsin, but only betamethyl glucoside was obtained. (Previously they had found that in aqueous solution the hydrolysis of salicin with emulsin ceases when 54.7 percent of salicin had been hydrolyzed.)

Bourquelot soon succeeded in synthesizing quite a number of glucosides, starting from methyl alcohol, ethyl alcohol, butyl alcohol, isobutyl alcohol, geranyl alcohol, etc., and also from some alcohols of the aromatic series, benzyl alcohol, phenylethyl alcohol, cinnamyl alcohol, naphthyl alcohol, etc. (Bourquelot and Bridel, Compt. rend. CLVI, 827). Almost all the glucosides, thus prepared, crystallized quite readily. The beta-geranyl glucoside which Bourquelot and Bridel obtained (Jour. Pharm. Chimie., 1913, 209) was found to be identical in every respect with the glucoside present in pelargonium odoratissimum.

Glucosides of solid water-insoluble alcohols may be prepared by using acetone as a solvent and varying quantities of water. Bourquelot further found that primary alcohols are converted into glucosides more readily than secondary, and the latter more readily than tertiary alcohols. All substances containing alcoholic hydroxyl groups form glucosides with dextrose in the presence of emulsin. The compounds obtained are beta-glucosides, and like natural glucosides are levorotatory and hydrolyzable by emulsin. It would be beyond the scope of this paper to relate in detail the excellent work carried out by Bourquelot and therefore only a few more synthesized glucosides may be mentioned. α alcohol-glucosides are prepared by allowing the enzyme α glucosidase present in bottom-fermentation yeast to act on dextrose in alcoholic solutions. The alcoholic strength of the liquid should be weaker than that used for synthesizing β glucosides with emulsin. These α glucosides are dextrorotatory and are not hydrolyzed with emulsin.

When emulsin is allowed to act on galactose in alcoholic solution β ethyl galactoside is formed by the β glucosidase present in emulsin. (Bourquelot and Mougne, Journ. Pharm. Chim., 1914, 157.) Analogous glucosides were obtained when using methyl, benzyl, etc., alcohols.

That some enzymes act synthetically in aqueous solution was shown by allowing emulsin of almonds to act upon a concentrated solution of dextrose at ordinary temperature in the presence of thymol, phenol, etc. (Bourquelot, Herissey and Coirre, Compt. Rend. CLVI, 752.) After allowing to stand for some time the solution was heated to destroy the enzymes, diluted and the undecomposed dextrose was destroyed by fermentation with top fermentation yeast. From the solution thus obtained gentiabiose in pure state could be isolated.

The synthesizing of glucosides is a rather tedious and time-consuming process. For instance, in order to prepare β methyl glucoside, 12 gms. of emulsin are added to a solution of 600 gms. of dextrose in 1020 gms. of methyl alcohol and 440 gms. of water; after allowing the mixture to stand for one month at ordinary temperature about 350 gms. of crude glucoside was obtained which when purified yielded 250 gms. of pure product.

A few words may be said in regard to the reversibility of enzyme action. If a methyl alcoholic solution containing dextrose and β methyl glucoside is treated with emulsin, a state of equilibrium of the synthesizing and hydrolyzing action of the enzyme is obtained. (Bourquelot and Bridel, Compt. rend. CLVI, 957.) When this equilibrium has been attained, further synthesis can be induced by the addition of sugar and on the other hand further hydrolysis may be promoted by the removal of sugar (Bourquelot and Bridel, Compt. rend. CLVIII, 206).

On the strength of numerous experiments Bourquelot arrives at the following conclusions:

1. The rapidity of synthesis increases with the quantity of enzyme present.

2. The action of the enzyme is accelerated by raising the temperature provided, however, the temperature at which the enzyme is destroyed is not exceeded.

3. For equal concentrations of dextrose the proportion converted into glucoside increases with the alcoholic strength and when the latter is kept constant is increased by raising the concentrations of the dextrose up to a limit of 15 to 20 percent.

While the above experiments so far are of little practical value, inasmuch as only a few of the natural glucosides have been prepared up to the present time, they throw considerable light on the chemical changes going on in plant life. The better known enzymes will therefore become a valuable guide in the study of chemistry in the living organism. As long as we only knew that enzymes have a hydrolyzing action on glucosides, they could only be used for detecting glucosides and polysaccharites in plants. Now, since we know, that they have a synthesizing action also, the results obtained by hydrolysis can be verified by those obtained by synthesis. Dextrose is present in all plants. When therefore a plant contains a ferment it points to the presence of the corresponding glucoside. This has been verified in the ericaceæ, the gentians and quite recently in various orchidaceæ. Also in many species of the scrophulariaceæ, especially of the genus linaria, in leguminosæ, proteaceæ, etc. On account of their specific action the enzymes will gradually become the most delicate and certain reagents for the study of the constitution of certain organic compounds. In plant physiology biochemical synthesis is an important factor in the production of food materials. Certain organic compounds which are insoluble in water, form with dextrose soluble compounds. Since dextrose is present in all living organisms it seems to be the best liquifying agent under certain conditions and seems to prevent the formation of certain concretions and to dispose of compounds dangerous to the organism of the plant.

On the other hand the reversibility insures the maintenance of the equilibria necessary for life. In organisms where life is active the liquid media are particularly favorable to hydrolysis, i. e., to the utilization of food materials. In reserve organs, for instance the seeds, the medium becoming gradually less aqueous, is more favorable to synthetic processes. (Bourquelot, Journ. Pharm. Chim., 1914, 361 and 393.)

Whenever the chemical constitution of the products of hydrolysis of natural glucosides shall have been established we shall, without doubt, be in a position to produce such glucosides synthetically.