LVI. THE ISOLATION FROM CABBAGE LEAVES OF A CARBOHYDRATE, HITHERTO UNDESCRIBED, CONTAINING THREE CARBON ATOMS.

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THE substance described in the following paper was obtained during the investigation of the water-soluble constituents of cabbage leaves. The main object of the research was the isolation of the simpler nitrogenous substances present, but non-nitrogenous substances obtained were also examined, it being thought that these might throw some light on the derivation of the nitrogenous bodies.

During the course of the work, the method for separation of amino-acids and peptides from sugars by the carbamate method [Buston and Schryver, 1921] has been elaborated and rendered more complete; fractions containing 8–12 % nitrogen have been produced, which await further investigation. Amongst non-nitrogenous substances isolated may be mentioned citric and mesaconic acids. The latter does not appear so far to have been isolated as a natural product, and its preparation from cabbages may throw some light on the formation of citric acid from glucose. These points will form the subject of a later communication.

The product described in this paper appears to be of sufficient interest to warrant a separate mention.

The method adopted was briefly as follows:

An ether-water extract of 90 kilos. of the minced leaves was obtained, and the protein present flocculated as described by Chibnall and Schryver [1921]. The clear solution was then precipitated successively with alcohol, phosphotungstic acid, and baryta-alcohol; finally the major part of the nitrogenous substances was removed by repeated treatments with baryta and carbon dioxide in the presence of alcohol, according to the method described by the authors [1921].

The liquid remaining after this treatment was treated with sulphuric acid to remove barium quantitatively, and evaporated to a syrup. The syrup was left standing for two or three days under three times its weight of 90 % alcohol, by which time a fair quantity of crystalline matter had separated. This was sucked off on the pump, and dried on a porous plate. 36 g. of pale brown, somewhat sticky crystals were obtained, containing 0.9 % nitrogen. By treating in a Soxhlet for six hours with absolute alcohol, the colouring

matter and nitrogenous substances were removed, leaving 25 g. of white crystalline material. Of this, 51 % was found to be inorganic matter, mainly potassium chloride. The crystals were boiled with glacial acetic acid and filtered hot; on cooling, a bulky crop of fine white needles separated. These were removed and dried on a plate; weight obtained, 9.5 g. The mother liquor, on evaporation, gave a further crop of 1.2 g. The crystals still contained small amounts of potassium chloride, but were freed from this by repeated recrystallisation from glacial acetic acid. The total yield was about 10 g. from 90 kilos. of leaves.

The substance was finally obtained as a white crystalline powder, readily soluble in water and in hot glacial acetic acid; but practically insoluble in alcohol and other organic solvents. The substance possessed a faint but perceptible sweetish taste. Its melting point was 148°.

The combustions gave the following figures:

C = 33.44, 33.19 %; H = 7.30, 7.49 %; O = 59.26, 59.32 %.

A molecular weight determination in aqueous solution by the freezing point method gave: $M = 111.4$. The above figures indicate the formula to be $C_3H_8O_4$. (This requires C = 33.34 %; H = 7.40 %; O = 59.26 %. Mol. wt. 108.)

The only constitutional formula to satisfy this would be that of a trihydroxy-ether:

To test this, it was decided to prepare the benzoyl derivative. 0.3 g. of the substance, treated in the ordinary way with benzoyl chloride and alkali, and extracted with ether, gave, on evaporation of the ether solution, 1.3 g. of a colourless, almost solid syrup. This was granulated with ligroin and could then be recrystallised from benzene. 0.7 g. white crystals were thus obtained; M.P. 52°–53°. Combustion of this gave: C = 68.20 %, H = 5.11 %, O = 26.69 %. Molecular weight in benzene solution, 434. (The tri-benzoyl derivative requires C. 68.58 %; H = 4.78 %; O = 26.68 %. Mol. wt. 420.)

The benzoyl derivative must therefore contain three benzoyl groups, and have the formula:

So that the original substance must contain three hydroxy groups, and have the formula given above.

Examination of this formula will show that the central carbon atom is asymmetric; the substance obtained, however, was optically inactive. This

may have been due to racemisation produced by the hot glacial acetic acid, or by the long contact with baryta in the earlier stages of separation.

The formula also suggests the derivation of the substance by the condensation of formaldehyde and glycollic aldehyde (which may itself be regarded as the first condensation product of formaldehyde), reacting in the hydrated form:

It might thus be regarded as a simple disaccharide, glycollic aldehyde and formaldehyde being regarded as diose and monose respectively. Its formation from this source would thus make it appear to be formed as an alternative to a triose.

Various experiments were carried out to test for disaccharide-like properties in the substance. It showed no reducing properties, and gave no osazone. After heating with 5–10 % hydrochloric acid for some time, the neutralised solution still did not reduce Fehling's solution, though it reduced permanganate on warming. No formaldehyde could be detected. The substance therefore differs from normal disaccharides in its great stability (resembling more the ethers, in which class its formula also places it). Possibly the smallness of the molecule is the cause of its stability.

It is interesting to note further that by condensation with a molecule of glyoxylic acid a pyrone ring, which forms the basis of many naturally occurring substances such as the anthocyanins, might be produced:

Various similar condensations of the substances or its derivatives could be suggested.

SUMMARY.

An account is given of the isolation from cabbage leaves of a carbohydrate hitherto undescribed, of the formula $\text{C}_3\text{H}_8\text{O}_4$. This crystallises in needles, M.P. 148° which are readily soluble in cold water and hot glacial acetic acid, but insoluble in most other organic solvents. It yields a tri-benzoyl derivative, formula $\text{C}_3\text{H}_5\text{O}_4(\text{C}_6\text{H}_5\text{CO})_3$; M.P. 52° – 53° . The carbohydrate therefore has the constitution $\text{CH}_2(\text{OH}) \cdot \text{CH}(\text{OH}) \cdot \text{O} \cdot \text{CH}_2(\text{OH})$.

A possible mode of formation from formaldehyde in the plant is outlined, and it is suggested that it may form a stage in the making of a pyrone ring, which is an important constituent of the molecules of the anthocyanins.

REFERENCES.

- Buston and Schryver (1921). *Biochem. J.* 15, 636.
 Chibnall and Schryver (1921). *Biochem. J.* 15, 60.