CLXXXIX. NOTE ON THE ISOLATION OF MESACONIC ACID FROM CABBAGE LEAVES.

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In a paper published by the author some years ago [1923] in conjunction with Prof. Schryver, mention was made of the fact that mesaconic acid had been obtained from among the products extracted from green leaves (cabbage). As it appears that the presence of this substance among naturally occurring products had not previously been reported, it is thought that a rather more detailed account of its discovery might be of interest.

As stated in the original paper, the object of the research was, mainly, to examine the simpler nitrogenous constituents of green leaves, this being the natural extension of the earlier work of Chibnall and Schryver on the leaf-proteins.

The method adopted may be summarised briefly as follows.

90 kg. of fresh leaves were minced, the ether-water extract obtained, and the protein therein flocculated [cf. Chibnall and Schryver, 1921]. The clear solution was treated with alcohol (2 volumes of 95 % alcohol), and the white flocculent precipitate produced was removed; this is termed the "organic phosphate precipitate." After evaporation of the alcohol, the basic substances were precipitated by the usual phosphotungstic acid method. From the filtrate, sulphuric acid and excess of phosphotungstic acid were removed; the filtrate was then saturated with baryta and the barium salts of organic acids present were precipitated by the addition of excess of 95 % alcohol [cf. Dakin, 1921]. This precipitate is designated the "dicarboxylate fraction," consisting as it does mainly of the barium salts of dicarboxylic acids, nitrogenous and non-nitrogenous. It was from the "organic phosphate" and "dicarboxylate" fractions that mesaconic acid was isolated, the greater part being found in the latter.

The barium dicarboxylates were decomposed quantitatively by means of sulphuric acid, the amount necessary having been determined gravimetrically. The filtrate and washings were evaporated to a thick syrup; this was extracted repeatedly with alcohol, whereby the simple organic acids present were separated roughly from the nitrogenous dicarboxylic acids. The alcoholic liquid was evaporated to a thin syrup, and extracted with ether; on evapo-

rating, the ethereal extract partly crystallised. After removing the crystals, the residue was taken up in water, neutralised by the addition of calcium hydroxide, and the calcium salts were precipitated with alcohol. From the calcium salts thus formed, a second crop of crystals of the free acid was obtained, similar to those first separated. The substance was recrystallised from water, giving small colourless prisms, moderately soluble in cold water, readily soluble in hot water and alcohol, but sparingly soluble in ether. The total yield of the recrystallised product was 5.0 g.; M.P. 201°.

An elementary analysis revealed only the presence of carbon, hydrogen and oxygen. The substance was strongly acid, its equivalent, determined by titration with standard alkali, being 65.0. An estimation of the molecular weight by the cryoscopic method in aqueous solution gave a result of 124. The substance was therefore a dibasic acid. Permanganate was rapidly decolorised by the substance, as was also bromine water on warming slightly.

The above properties indicated that the substance was an unsaturated dicarboxylic acid, with a molecular weight of 130. Mesaconic acid, $C_3H_4(COOH)_2$, fulfils these requirements, and has M.P. 202°. The bromine addition product was prepared, and recrystallised from water, being then found to have M.P. 204°, with decomposition, agreeing with the M.P. of the *mesadibromomethylsuccinic* acid obtained on bromination of mesaconic acid. A mixed melting point of the original acid with a sample of pure mesaconic acid had M.P. 201–2°. The acid isolated was therefore certainly mesaconic acid.

As stated above, a further small amount (1 g.) of the acid was obtained from the "organic phosphate" fraction. This fraction consists mainly of the calcium and potassium salts of various inorganic and organic acids, and the mesaconic acid is apparently present as a calcium salt. It is liberated by treatment with dilute sulphuric acid, and is then extracted by alcohol, crystallising out when the alcoholic liquid is mixed with ether and allowed to stand. The total yield of mesaconic acid obtained in the two portions (6 g. from 90 kg. of leaves), is not to be regarded as a measure of the total amount present, as the methods of separation were by no means quantitative.

As far as can be ascertained, mesaconic acid has not previously been met with as a naturally occurring product. It is possibly connected with citric acid, which is a frequent constituent of plants, and was in fact isolated from the "dicarboxylate" fraction in the course of this work. Loss of water from citric acid gives aconitic acid, which is present as the calcium salt in the sugar-cane, sugar-beet, and specially in the leaves of *Adonis vernalis*. This acid is known to pass, by loss of carbon dioxide, into citraconic acid, the optical isomer of mesaconic acid. The change from citraconic acid to mesaconic acid is not easy of explanation; it seems certain, however, that mesaconic acid is present as such in the leaf, and is not an artefact. This view is supported by its isolation from the "organic phosphate" fraction, when only mild reagents, not usually regarded as capable of effecting any intramolecular change, were brought into contact with the acid.

The work on the constituents of green leaves, of which the investigation here described formed a small section, was carried out under the direction of Prof. S. B. Schryver, F.R.S.

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