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Electrophoresis of the Red Beet Pigments

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The chemical nature of the red beet pigments is still unknown, and they have not yet been isolated in a pure state. They have been classified as "nitrogenous anthocyanins" since the pioneer investigation by Schudel [1], who discovered the nitrogen content and the glycosidic character of the pigments. Other workers have isolated different preparations of "betanin chloride", which all differ in their composition [2-5]. The formulae hitherto proposed are all based on the assumption that "betanin" really is an anthocyanin derivative. So far, there has been no proof of that assumption.

The complexity of the red beet pigments was later demonstrated by the aid of adsorption chromatography [6]. At least eleven coloured zones (yellow, orange, and red) could be observed on the column. The absorption spectra of the different components were determined, but no chemical investigations were made.

A similar separation of the red beet pigments can be effected by means of paper electrophoresis. Within the pH interval 2—7, they form relatively stable, *negatively* charged ions. A typical electrophoresis strip from fresh red beet sap is shown on Fig. 1. In a 0.1 M citrate buffer of pH 5.5, a good separation is obtained in 3—4 hours (Munktell 20/150 paper strips of size 5 × 35 cm; 220 V). At least seven coloured zones can be observed, of which one red and one bright yellow are always the strongest ones. The different zones seem to separate in roughly the same order as on an adsorption column [6]. The main yellow component is very easily destroyed and appears only in fresh beet sap. Different varieties of the red beet seem to contain the same coloured components, though differing in their relative amounts.

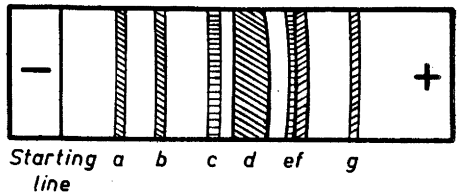

Fig. 1. Typical electrophoresis strip of fresh red beet sap in 0.1 M citrate buffer at pH 5.5. Paper: Munktell 20/150; 220/35 volts/cm; 3 hours at room temperature. Visible zones: a: *red, very weak;* b: *red, weak;* c: *orange, very weak;* d: *red, very strong;* e: *orange, weak;* f: *yellow, strong;* g: *yellow, very weak.*

At pH values below 2, a weak tendency to migrate towards the cathode can be observed, but observation is made difficult by the rapid destruction of the beet pigments in strongly acid solution. Thus, the pigments seem to have an isoelectric point at about pH 2. The exact position of the isoelectric point has not been determined, but it seems to be about the same for all the coloured fractions. In their behaviour during electrophoresis, the beet pigments differ from the common anthocyanins, which form positively charged ions except in alkaline solution. The extremely low value of the isoelectric point indicates that the molecule is dominated by strongly acidic groups, such as carboxyls. From methylation experiments, previous workers have concluded that the "betanin" molecule must contain one or two carboxyl groups [2,4].

The results obtained on electrophoresis have been confirmed by experiments with ion exchange resins. The red colour of beet

sap is firmly adsorbed on an anion exchanger, but rather little on a cation exchanger. Anthocyanins from fruits or berries behave in the opposite way.

In practice, paper electrophoresis can be used to detect the addition of red beet sap to fruit juices, as only the beet pigments migrate towards the anode in weakly acid solution. An addition of 10 % of red beet sap is easily detected.

Electrophoresis on cellulose columns has been used with some success in attempts to isolate the beet pigments. A very simple apparatus built after the same principle as that described by Porath [7] was used. The buffer solutions were volatile, consisting of 0.1 M formic acid and 0.1 M pyridine in varying proportions. The separation was generally carried out at a pH about 3.5, because the pigments then can be separated from most proteins and amino acids, which move in the opposite direction. The "main red zone" was eluted from the electrophoresis column and further purified by adsorption on a talc column [6]. Lyophilisation (evaporation from a frozen solution) was generally used for concentration of the solutions, since the pigments are easily changed even during vacuum distillation at low temperature. The dark red solid fractions obtained by this method are at present being further investigated.

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Added in proof: In a recent communication (*Naturwiss.* **43** (1956) 159). O. T. Schmidt and W. Schönleben have arrived at similar results, and, in addition, shown that the beet pigments do not contain nitrogen.

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The Cuprous Salts of some N,N-Disubstituted Dithiocarbamic Acids and their Degree of Polymerisation

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Among the cuprous salts of the N,N-disubstituted dithiocarbamic acids, only the diethyl compound is mentioned in the literature. It was first prepared by Cambi and Coriselli [1]. They treated the sodium salt of diethyldithiocarbamic acid in inert atmosphere with cuprous oxide, stirring for about twelve hours; the salt was recrystallized from chloroform + petroleum ether. Fredga [2] prepared cuprous diethyldithiocarbamate by shaking thiuramdisulphide with copper bronze in chloroform solution, at room temperature. The compound isolated by him consisted of yellow, glistening needles, with a molecular weight of about 1 000 (determined in boiling chloroform). At about the same time, Tamminen and Hjelt [3] obtained a mixture of the cupri- and cuprous compounds, by boiling tetraethylthiuramdisulphide in benzene solution with metallic copper.

It was the author's intention to synthesize more of such compounds, and examine their polymerity. An X-ray examination of the cuprous diethyldithiocarbamate was begun in The Inorganic Department of this Institute by Hesse [4]; about the same time a molecular weight determination in boiling carbon disulphide was made, and later, an ultracentrifugation according to Archibald's method. It appeared that the true molecule consists of four formula units. The determination of the structure of the complex now completed, evidently shows that the molecule is tetrameric and that the four copper atoms are situated in the corners of a tetrahedron.

When the cuprous dithiocarbamates were prepared, the thiuramdisulphide was shaken with an excess of copper bronze in carbon disulphide until the colour of the solution became yellow or yellow-brown. After the copper bronze had settled, the solution was filtered and the cuprous complexes were isolated by means of their different solubilities. Several