

directly with methanol-chloroform mixtures too high values for gangliosides are obtained.

Rittenberg, Howe and Chargaff<sup>5</sup> have suggested the existence of both gangliosides and mucolipids (strandin) in brain tissue, as they found that strandin contained amino acids, which was not the case of gangliosides. When strandin, prepared by the method used by them, was chromatographed on a cellulose column<sup>6</sup> with chloroform-methanol mixtures, gangliosides were isolated in the effluents, while on the top of the column a substance remained which gave strong positive reactions for sialic acid (Bial's reagent) and amino-acids (ninhydrin). After hydrolysis of the substance all the common amino acids were indicated by paper chromatography. A further evidence for the heterogeneity of strandin is that Folch *et al.*<sup>7</sup> showed only amino acids in strandin prepared with the partition dialysis method (procedure C) but not in strandin prepared by two other procedures.

Until further data are given for strandin we have to consider it is a ganglioside to which other substances are associated. Gangliosides consist of a hydrophobic and a hydrophilic portion and may be able to act as protecting colloids for, *e.g.*, polypeptides in organic solvents and cerebroside in water.

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1. Folch, J., Arsove, S. and Meath, J. A. *J. Biol. Chem.* **191** (1951) 819.
2. Klenk, E. and Lauenstein, K. *Hoppe-Seyler's Z. physiol. Chem.* **291** (1953) 249.
3. Rosenberg, A., Howe, C. and Chargaff, E. *Nature* **175** (1956) 234.
4. Svennerholm, L. *Colloque sur les Lipidoses du Systeme Nerveux Central*, Anvers 1955.
5. Svennerholm, L. *J. Neurochem.* **1** (1956) 42.
6. Svennerholm, L. *Unpublished*.
7. Gardell, S. *Acta Chem. Scand.* **7** (1953) 207.
8. Svennerholm, L. *Ibid.* **8** (1954) 1108.
9. Svennerholm, L. *Acta Soc. Med. Uppsaliensis* **61** (1956) 00.
10. Zilliken, F., Braun, G. A. and György, P. *Arch. Biochem. and Biophys.* **54** (1955) 564.
11. Klevstrand, R. and Nordahl, A. *Acta Chem. Scand.* **4** (1950) 1320.
12. Blix, G., Lindberg, E., Odin, L. and Werner, I. *Nature* **175** (1955) 340.

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## Structure of the 1:1 Compound Pyridine — Iodo Monochloride

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It has been suggested that the 1:1 complexes probably present in the liquids containing pyridine and halogens are non-planar: The halogen atom directly linked to nitrogen is assumed to lie in the ring plane whereas the second halogen atom (bearing a negative charge) was expected to form an electrostatic link with the (positive) nitrogen atom and to lie outside the ring plane<sup>1,2</sup>. Unfortunately the yellow precipitate obtained by adding water to a solution of iodine in pyridine has been shown to contain water and the very unstable compound obtained in the absence of water does not appear to have the 1:1 composition<sup>3</sup>. Our X-ray work has therefore started with the iodo monochloride compound<sup>4</sup>, the yellow, needle-shaped crystals of which (m. p. 132° C) turned out to be monoclinic — space group  $P2_1/c$  — with the parameters  $a = 4.25$ ,  $b = 12.29$ ,  $c = 14.07$ ,  $\beta = 94.4^\circ$ . Using trial and error methods approximate  $y$  and  $z$  parameters of the iodine atoms (occupying a fourfold position) could easily be determined and signs of the structure factors evaluated which made possible a preliminary Fourier synthesis referring to the  $(0kl)$ -zone.

In Fig. 1a the refined electron density map is reproduced. The pyridine ring drawn in the figure is derived not from the direct synthesis but is slightly modified according to the results of a difference synthesis with subtraction of the contribution to structure factors from the halogen atoms. The  $(h0l)$ -synthesis in its final form is given in Fig. 1b. Here the overlapping of two pyridine rings forms an obstacle to a more precise determination especially of the carbon coordinates. A difference synthesis with subtraction of the contribution of the iodine atoms led to a  $z$  coordinate of the nitrogen atom which did not deviate more than 0.004 from the value obtained in the first projection. Neglecting not observed reflexions, the  $R$  factors for the two zones are 0.12 and 0.14, respectively.

From the above results it may safely be

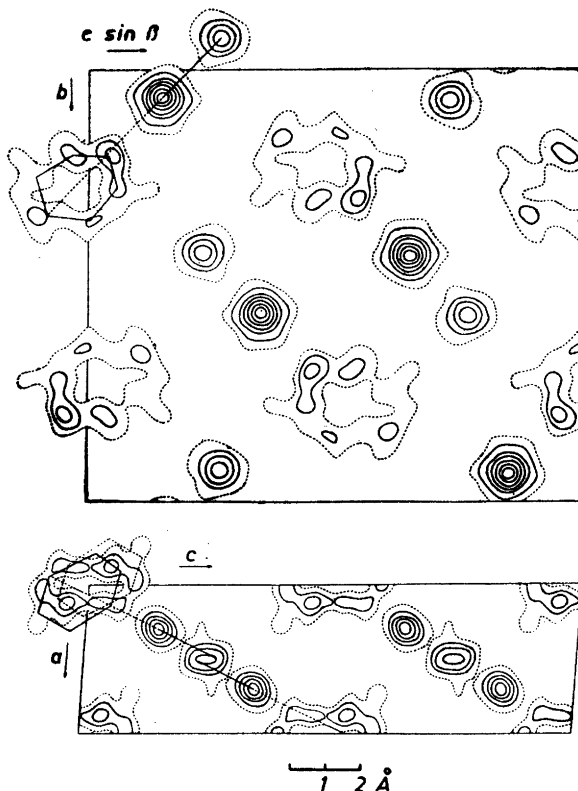


Fig. 1. a. Fourier projection along a-axis. b. Fourier projection along b-axis. Approximate relative position of two overlapping pyridine rings is indicated. In both projections the ratio of contour line distances is:  $N(C) : Cl : I = 1 : 2 : 5$ .

concluded that the I, Cl and N atoms are situated at least very nearly on a straight line. The projection 1a together with considerations of ring dimensions<sup>5</sup> strongly suggest that the carbon atom opposite to the nitrogen atom is also situated on this line. The I, Cl and N parameters are: I(0.298, 0.068, 0.151); Cl(0.509, 0.072, 0.268) and N(0.141, 0.201, 0.047). From these values the following distances result: I—Cl = 2.51 Å, N—Cl = 2.2 Å.

Published structure analyses of addition compounds of ethers and amines with halogens<sup>6-8</sup> show a linear arrangement O (or N)—Hal.—Hal. in contrast to suggestions earlier made. The experimental findings do not appear to strengthen the suggestion that the "outer" halogen atom attains a strong negative charge. In

the case of ethers a small lengthening only of the Hal.—Hal. bond is observed, whereas in the case of amines the lengthening is considerably greater. This lengthening may at least partly be due to a weakening of the double bond character<sup>9</sup> of the Hal.—Hal. bond present in the free molecules. The strong interaction between the oxygen (nitrogen) atom and the nearest halogen atom is evident from the short separation between these atoms, this effect being greatest in the case of nitrogen. It appears probable that electrons associated with the atoms forming an O—Hal. or N—Hal. bond are partially promoted to d orbitals.

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1. Mulliken, R. S. *J. Am. Chem. Soc.* **72** (1950) 600.
2. Mulliken, R. S. *J. Am. Chem. Soc.* **76** (1954) 3869.
3. Chatelet, M. *Ann. Chim.* [11] **2** (1934) 12.
4. Zappi, E. V. and Fernandez, M. *Anales asoc. quim. argentina* **27** (1939) 102.
5. Almenningen, A., Bastiansen, O. and Hansen, L. *Acta Chem. Scand.* **9** (1955) 1306.
6. Hassel, O. and Hvoslef, J. *Acta Chem. Scand.* **8** (1954) 872.
7. Hassel, O. and Hvoslef, J. *Acta Chem. Scand.* **10** (1956) 138.
8. Eia, G. and Hassel, O. *Acta Chem. Scand.* **10** (1956) 139.
9. Mulliken, R. S. *J. Am. Chem. Soc.* **77** (1955) 884.

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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<sup>1</sup>, 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<sup>2-5</sup>. 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<sup>6</sup>. 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<sup>6</sup>. 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.

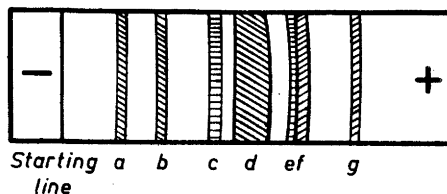


Fig. 1. Typical electrophoresis strip of fresh red beet sap in 0.1 M citrate buffer at pH 5.5. Paper: Munktell 20/150; 220/35 volts/cm; 3 hours at room temperature. Visible zones: a: red, very weak; b: red, weak; c: orange, very weak; d: red, very strong; e: orange, weak; f: yellow, strong; g: yellow, 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<sup>3,4</sup>.

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