Oxidation of Glucosamine and Galactosamine with Ninhydrin to Arabinose and Lyxose and their Identification with Paper Chromatography

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The identification of the natural amino-hexoses was formerly a difficult task. While the hydrochloride of glucosamine yielded characteristic crystals that of galactosamine, because of its high solubility, was very difficult to obtain. The first way to identify small quantities of them was given with the use of paper chromatography as worked out by Partridge [1] and Aminoff and Morgan [2]. Collidine cf the highest degree of purity is necessary.

We found this technique reliable, and at the same time we tried to apply paper chromatography to the oxidation products of glucosamine and galactosamine.

The fact that the hexosamines under the same conditions as the amino acids react with ninhydrin to give blue coloured compounds has been known for a considerable time [3]. This reaction has been used for the detection of the sugars in paper chromatography by Aminoff and Morgan [2], Pratt and Auclair [4] and others.

When ninhydrin is allowed to act upon amino acids, an aldehyde with one carbon atom less than the original acid is formed. If the same type of reaction is assumed to take place with the hexosamines the aldehyde formed must be a pentose, for D-glucosamine it must be D-arabinose and for D-galactosamine (chondrosamine) D-lyxose.

These two pentoses can easily be separated by means of paper chromatography, if a mixture of butanol and ethanol in proportions 9 : 1 is used as solvent. Thus it would be very simple to identify the pentoses from a reaction mixture. This also proved to be the case.

200 mg of glucosamine and 180 mg of ninhydrin were dissolved in 150 ml of water and hydrochloric acid was added to pH 4.7. The mixture was placed in a boiling water bath for half an hour. The blue coloured solution was filtered to remove a brownish precipitate and the mother liquor was evaporated *in vacuo* to dryness. The residue was taken up in 1 ml of water and the solution was subjected to chromatography in the following way. Spots containing 0.002 – 0.005 ml were placed on the starting line of a chromatographic paper (Munktell OB). On each side of the testspot there were placed spots containing 100 γ of arabinose. The chromatogram was run in butanol-ethanol 9 : 1 mixture for 18 hours at 22° C. After drying, the paper was sprayed with an aniline trichloroacetate reagent, suggested by Dr Erik Vasseur [5]. In the test a strong spot appeared at the same distance from the starting line as the spots of arabinose. If the experiment was carried out in the same way with galactosamine and if lyxose was used instead of arabinose, a spot corresponding to the lyxose spots was obtained.

The technique of using this principle for the identification of aminosugars in mucopolysaccharides will be dealt with in another communication.

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3. Deetjen, H., and Fränkel, E. *Münch. Med. Wochschr.* **61** 467.
4. Pratt, J. J. jr., and Auclair, J. L. *Science* **108** (1948) 213.
5. Vasseur, E. Personal communication.

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