ACTA CHEMICA SCANDINAVICA 8 (1954) 859—875

Short Communications

A Method of Detection of N-Dimethylamino Acids on Paper

HANS KIESSLING
and JERKER PORATH

Institute of Biochemistry, University of Uppsala, Uppsala, Sweden

In 1950 Bowman suggested reductive methylation as a method of determining N-terminal amino acids in peptides [1]. Shortly afterwards Ingram [2,3] was able to show its applicability to smaller peptides. The detection of the N-dimethylamino acids formed was based upon the buffering action of these compounds — a method suffering from the disadvantage of being insufficiently specific. We therefore consider it worth while to publish a direct and more reliable procedure which has been used by us for some time.

The detection of the spots is based upon conversion of the dimethylamino acids to betaines by means of methyl iodide:

$$(CH_3)_2N—CHR—COOH + CH_3J \rightarrow (CH_3)_3\overset{+}{N}—CHR—COO^- + H^+ + J^-$$

The iodide remaining in the spot after evaporation of excess methyl iodide may be detected in two ways. Oxidation with chlorine in vapor phase yields iodine which can be detected by spraying with a starch solution (*cf.* Rydon and Smith [4]). An alternative method which we find preferable involves precipitation of silver iodide followed by reduction with a photographic developer. The sensitivity varies considerably, depending on the dimethylamino acids and on the chromatographic system used, but is high enough for the detection of concentrations larger than 5—10 $\mu g/cm^2$. The reaction is particularly sensitive to basic (and sulphur containing) dimethylamino acids where the sensitivity may be higher than that of the ninhydrin reaction of the corresponding unsubstituted amino acids. The darkness of the background varies within wide limits depending on the paper used but all common brands tried have given satisfactory results.

The dried paper is placed in a desiccator, containing a beaker with methyl iodide and left for two hours at 35—40°. The beaker should be placed above the paper to ensure a satisfactory exposure of the paper to the methyl iodide vapor. When the reaction is complete the paper is dried in an oven at 100° C for a few minutes. It is then immersed in an ammoniacal 0.1 *N* silver nitrate solution. The excess of silver nitrate is removed by careful washing with distilled water. The spot may now be developed by a rapid photographic developer (*e. g.* prepared according to the formula of Kodak D 72).

The method may be illustrated by the separation and identification of a mixture of dimethyl*iso*leucine, dimethylleucine, and dimethylphenylalanine (Fig. 1). The paper (Whatman No. 1) had been treated with 0.02 *M* sodium tetraborate solution and dried before application of the dimethylamino acids. The paper chromatogram was developed by a solvent system consisting of 87 % tertiary butanol and 13 % 0.02 *M* sodium tetraborate buffer for a minimum time of 40 hours.

A great number of other amino acids have been methylated, chromatographed and identified in mixtures with untreated amino acids. Amino acids and dimethylamino acids can be detected on the same chromatogram by the multiple dipping technique [5]. The paper is first dipped in a 0.2 % ninhydrin-acetone solution and the color developed in the usual way. The spots are marked with a pencil. The paper is now treated with methyl iodide vapor as described above, dipped in silver nitrate solution and then washed with a strong ammonia solution. The colored spots gradually fade and disappear. The

Fig. 1. Paper chromatogram of dimethyl leucine (1), dimethyl isoleucine (2), dimethyl phenylalanine (3), and a mixture of these acids (4). For details see text.

subsequent treatment with photographic developer reveals the dimethylamino acids, the basic and sulphur containing amino acids as black spots. The identification is simplified by the fact that a dimethylamino acid in butanol-acetic acid-water-systems travels in front of and not widely separated from the corresponding unsubstituted amino acid.

Finally we wish to point out the possibility of using the proposed method for detection of all kinds of substances capable of reacting with methyl iodide with formation of iodide ions (amines, organic sulphides *etc.*). The method has also been used by us to detect ninhydrin-negative impurities in commercial amino acid samples and contaminants obtained in eluates from ion exchange resins.

1. Bowman, R. E. and Stroud, H. H. *J. Chem. Soc.* **1950** 1342.
2. Ingram, V. *Nature* **166** (1950) 1038.
3. Ingram, V. *J. Biol. Chem.* **202** (1953) 193.
4. Rydon, H. N. and Smith, P. W. G. *Nature* **169** (1952) 922.
5. Jepson, J. B. and Smith, I. *Nature* **171** (1953) 43; **172** (1953) 1100.

Received May 12, 1954.

The Use of ^{110}Ag in Quantitative Paper Chromatography of Sugars

MAIRE JAARMA

Institutet för organisk kemi och biokemi, Stockholms Högskola, Stockholm, Sweden

In connection with other investigations it was necessary to determine quantitatively very small amounts of paper-chromatographically separated reducing sugars. The method of Trevelyan *et al.* [1] was used, involving the precipitation in the spots of metallic Ag from $AgNO_3$-acetone solution. The present communication deals with some preliminary studies on the conditions under which reducing sugars can be determined quantitatively with the aid of ^{110}Ag incorporated in $AgNO_3$.

Methods: The sugars were separated in descending chromatograms on sheets of Whatman No. 4 or 54 filter paper, size 30 × 50 cm. The temperature was 20° C and the duration of the chromatography was 24 hours. Two solvents were used: (a) *n*-butanol, pyridine, and water in the ratio 3:2:1.5, and (b) ethyl acetate, glacial acetic acid, and water in the ratio 60:17:17.5[2].

Ag of high purity grade was activated in the Norwegian uranium reactor to a specific activity of about 1.5 mC ^{110}Ag/g Ag. The activated Ag was dissolved in pure nitric acid and the nitrate was prepared. It was then found suitable to dilute this nitrate ten times with inactive $AgNO_3$. The experiments included two aldoses: glucose and galactose, two ketoses: fructose and sorbose, and two disaccharides: maltose and lactose. These sugars were determined singly or in different combinations. It should be mentioned that glucose and fructose were only separated with solvent (b) and that glucose and galactose were not separated from the same solution with these solvents. Small volumes (3—10 μg in one ml) of the sugars to be tested were applied to the papers with an Agla micrometer syringe. In some cases larger quantities, up to 40 μg were used, the spots being allowed to dry between successive 1 μl loadings of the paper. The position of the separated spots were identified with the aid of standard solutions placed on narrow edge strips of the chromatographic paper. R_F values could not be used as the solvent front leaves the paper in about 9 hours and the R_F values in such a case are not sufficiently reproducible. Around the spots,