



cysteic acid and the other compound. If thiomalic acid is present instead of cysteine as —SH group carrier, new materials are also formed. They give but a weak brownish colour with ninhydrin, as might have been expected, and glutamic acid is liberated on hydrolysis.

Hydrogen peroxide, replacing bromine as oxidizing agent, produces identical but weaker spots; autoxidation of the solution gives only trails due to incomplete reaction.

Nothing is known about the chemical link between the reacting groups; one of these must be the sulphur atom, since unexpected spots do not appear after bromine treatment in the absence of sulphhydryl groups in the amino-acid solution, but α_1 and α_2 are always present when cysteine alone is oxidized. The other linking group might be the amino group, but no proof can be given as yet; the facts supporting this hypothesis are the slowness of the appearance of the ninhydrin spots and their weakness when compared with those of the amino-acids obtained after hydrolysis of an equivalent amount of product; this suggests the unmasking of amino groups. On the other hand, deamination of the compound giving the β -spot (see diagram) according to Consden, Gordon and Martin⁵ leads to the complete disappearance of both amino-acids after hydrolysis; this might be due to the liberation of the presumably protected amino groups in the strong acid solution (6N hydrochloric acid) in which the reaction takes place.

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Detection of Deoxyribonucleosides on Paper Chromatograms

THE paper chromatography of deoxyribonucleosides has been described by several workers¹⁻⁵. The compounds can be located on the chromatogram by examination under ultra-violet light^{1,6,7}, by a microbiological method^{2,3,5} or by means of the Feulgen or Dische reactions⁴. A colour reaction between deoxyribonucleic acid and cysteine in sulphuric acid has been reported by Dische^{8,9}. This has now been applied to the detection of deoxyribonucleosides on paper chromatograms. While all the deoxyribosides, as well as deoxyribose itself, give the reaction, neither ribose nor the ribonucleosides can be detected by this method.

The reagent consists of a solution of cysteine hydrochloride (0.5 gm.) in 3N sulphuric acid (100 c.c.). The air-dried chromatogram is sprayed with the solution. After 5-10 min. at 85°, pink spots appear. Quantities of 10-20 μ gm. can be easily detected when the compounds are introduced to the chromatogram in 1 per cent solution. Spots containing more than about 50 μ gm. of the compound are pink with a brown centre. The purine deoxyribosides react more rapidly than the pyrimidine derivatives, especially at 75°, due to their labile glycosidic linkage. There is, however, no difficulty in detecting the pyrimidine compounds. This is a distinct advantage over the earlier chemical tests, in which colour development with pyrimidine deoxyribosides is slow (in the Feulgen and Dische reactions), or which lead to gross decomposition of the filter paper (Dische reaction)⁴. A certain amount of care is necessary in handling chromatograms after treatment at 85°. The colours fade rapidly on exposure to light, and if a permanent record is required it is advisable to photograph the chromatogram immediately after development of the colour.

The R_F values of the naturally occurring deoxyribonucleosides in two solvent systems are given below. The butanol solvent gives fairly good resolutions of the pyrimidine deoxyribosides, while the alkaline phosphate system resolves the purine derivatives.

Compound	n-Butanol saturated with water	5 per cent Na ₂ HPO ₄ in 180-amy alcohol (ref. 10)
Cytosine deoxyriboside	0.23	0.77
5-Methylcytosine deoxyriboside (ref. 11)	0.25	0.76
Thymine deoxyriboside	0.51	0.78
Uracil deoxyriboside	0.38	0.79
Adenine deoxyriboside	0.35	0.55
Guanine deoxyriboside	0.21	0.62
Hypoxanthine deoxyriboside	0.23	0.70
2-Deoxyribose	0.36	0.87
2-Deoxygalactose	0.23	0.86

It is hoped that this technique, together with the phosphate spray of Hanes and Isherwood¹² and the other procedures mentioned above, will facilitate the rapid analysis of compounds obtained by the degradation of nucleic acids and in synthetic work in this field.

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