A sensitive reagent for detecting 2-deoxysugars and 3-deoxypolyols

WARAVDEKAR AND SASLAW[1] have described a sensitive colour reaction for 2-deoxyribose in which the sugar is oxidized with periodic acid to give malondialdehyde. Malondialdehyde is condensed with thiobarbituric acid to give a red compound, which is estimated spectrophotometrically (Fig. 1). It has been found that a spot test based on these reactions will detect 2-deoxyribose, 2-deoxyglucose and 3-deoxyxylitol on paper chromatograms. On theoretical grounds other 2-deoxysugars and 3-deoxypolyols should give the same reaction.

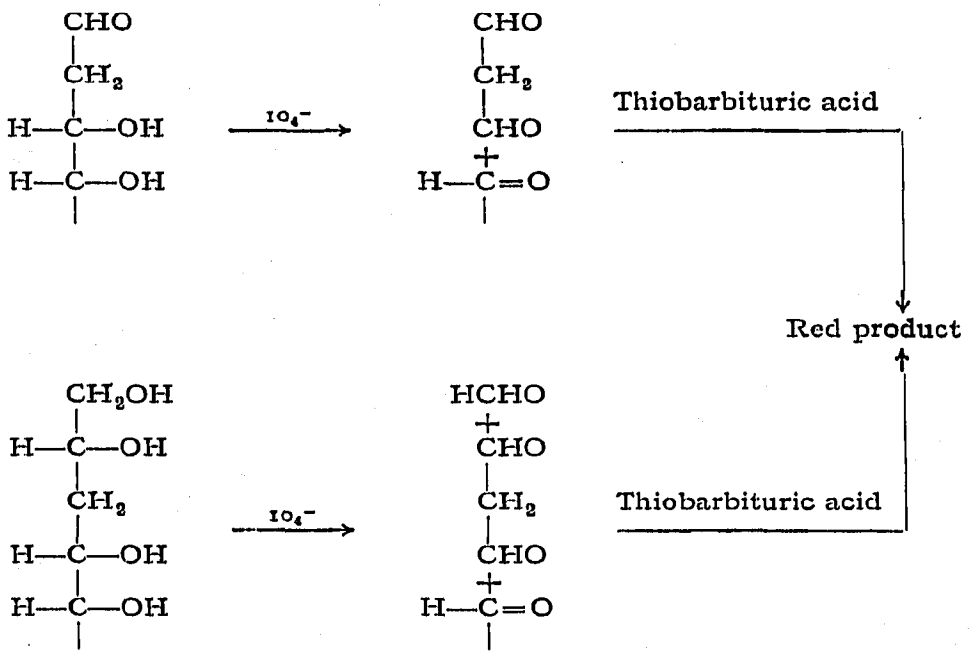

Fig. 1. Periodate–thiobarbituric acid colour reaction with 2-deoxysugars and 3-deoxypolyols.

Method

Reagent A. 1 vol. of 0.1 *M* periodic acid solution mixed with 20 vols. of acetone immediately before use.

Reagent B. 0.01 *M* ethylene glycol in ethanolic solution saturated with thiobarbituric acid.

The paper is dipped in reagent A and the acetone is allowed to evaporate or is removed with the aid of a blower. The paper is then dipped in reagent B, dried in the same way and heated at 60° for 10–20 min. Compounds yielding malondialdehyde on periodate oxidation give brilliant red spots on a pale yellow background. Under U.V. light the spots fluoresce a brilliant orange on a violet background.

Alternative method

If, for any reason, it is desirable to avoid the presence of formaldehyde in the paper, the periodate can be removed with arsenious acid.

Reagent A. As above.

Reagent B. Arsenious acid in acetone solution prepared by shaking 1 g of sodium

arsenite in 100 ml of acetone previously acidified with 0.66 ml of 10 *N* HCl and filtering the solution. The filtrate is diluted to 500 ml.

Reagent C. Ethanol saturated with thiobarbituric acid.

The paper is dipped successively through reagents A, B and C, with drying after each dip. It is then heated as above with identical results.

Solutions of 3-deoxyxylose, 3-deoxyxylitol, 2-deoxyribose and 2-deoxyglucose (0.2 *M*–0.003 *M*) were applied as 4 μl spots (about 1 cm diam.) to strips of Whatman No. 1 chromatography paper. 3-Deoxyxylitol, 2-deoxyribose and 2-deoxyglucose could be readily detected down to 1 μg/sq.cm. At the highest concentration used, 3-deoxyxylitol showed a red "halo" because of incomplete cleavage in the centre of the spot. The spot from 0.1 *M* 3-deoxyxylose was faint and at lower concentrations undetectable.

None of the following sugars gave reactions: glucose, fructose, sorbose, xylose, xylulose and 3-deoxyxylulose.

Biochemistry Department, University of Otago Medical School, Dunedin (New Zealand) P. J. ANDERSON

1 V. S. WARAVDEKAR AND L. D. SASLAW, *J. Biol. Chem.*, 234 (1959) 1945.

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Thin-layer chromatography of vitamin B_{12} and its analogues

Vitamin B_{12}, isolated from microbial fermentation, is often accompanied by its analogues, some of which have no biological activity for either man or animals. The separation of these compounds is difficult because they are chemically closely related to cyanocobalamin.

The known methods for the separation of vitamin B_{12} from its analogues include the use of column chromatography on cellulose[1] and ion exchangers[2–8], electrophoresis[9,10], and paper chromatography[6,11–17].

Recently, there have been some reports on the use of thin-layer chromatography on silica gel for the separation of these compounds[19,20].

This report relates to a new method for separation of some vitamin B_{12} compounds on thin layers of alumina.

Experimental and results

The experiments were performed with the following cobalamins: cyanocobalamin (B_{12}), factor B_{12III}, pseudovitamin B_{12}, factor A, factor B, factor V(nB), and a mixture of them in water with an addition of NaCN (5 % aqueous solution) to obtain the dicyano complexes. During chromatography dicyano complexes decomposed into the monocyano forms. Different mixtures of the following solvents were used: