Detection of non-reducing carbohydrate with orcinol in phosphoric acid

During work on the fractionation of mucoproteins and their degradation products, it has often been necessary to detect small amounts of combined (non-reducing) carbohydrate. Methods using periodate depend on the availability of pairs of free vicinal hydroxyl groups, and the only really general type of procedure is to use a reagent such as orcinol in a strong acid such as sulphuric acid, which breaks down the polysaccharide[1–3]. This means, of course, that spots or bands have to be eluted from paper before the reaction can be applied. In the modification described here, sulphuric acid is replaced by phosphoric acid and the reaction is done on spot tiles instead of in test tubes. This makes it much more sensitive (down to 0.2 μg), and simple enough to carry out on a large number of samples without undue labour; at the same time, rough quantitation can still be achieved.

When a reagent containing sulphuric acid is used on a spot tile, charring occurs as the acid becomes concentrated by evaporation of water during the heating period. This is avoided by replacing sulphuric with phosphoric acid. The reagent used contained orcinol (100 μg per ml) in 50 % (v/v) phosphoric acid; it was stored at 2° and made up fresh weekly. Spot tiles containing twelve cavities per tile were used. In order to maintain the liquid in the form of compact droplets as the volume decreased by evaporation, the tiles were given a hydrophobic surface by rinsing with dimethyldichlorosilane (5 % v/v) in benzene and allowing to air-dry. To ensure scrupulous cleanness, they were rinsed with water immediately before use, excess water being merely shaken off.

Sample volumes ranged from 10 to 100 μl; since colour development takes place only when excess water has evaporated off, the colour attained is independent of the initial volume. To each sample, one small drop (20 μl) of orcinol reagent was added. Mixing was effected by circular horizontal movement of the tiles, which were then placed for one hour in an oven at 105°. Blanks became pale yellow, whereas a reddish-brown colour developed in the presence of carbohydrate. Within the range 0.2 to 1.6 μg mannose or galactose, the range of tones formed was well differentiated, and casual inspection sufficed for estimation of the amounts present within a factor of two, standards for comparison being run concurrently with the unknowns. With care, estimation within closer limits could be achieved.

Colour development began after about half an hour at 105°; colours did not change markedly if the heating period was prolonged to two hours. At 95°, colour development took more than an hour. At 120°, grey colours were formed, less suitable for visual estimation than the range from pale yellow to deep reddish-brown obtained at 105°.

The colours given by equal amounts of mannose and galactose were indistinguishable; about twice as much glucose or soluble starch, and about eight times as much N-acetylneuraminic acid, were required to produce equivalent colours. Glucosamine hydrochloride in amounts up to 8 μg gave no observable colour, but with 16 μg a faint grey was obtained. Ovomucoid and a bovine plasma mucoprotein fraction gave colours consistent with their hexose contents. Interference by proteins is due, partly at least, to their tryptophan contents. Thus bovine insulin, which is free of tryptophan, gave only a yellow colour in amounts up to 120 μg; 10 μg bovine chymotrypsinogen (5 % tryptophan), on the other hand, gave a pale grey colour.

With tryptophan itself, in amounts above about 1 μg, a pale purple colour was obtained; the same colour was formed with phosphoric acid only, in the absence of orcinol. Such interference by tryptophan seems to be the main difference in specificity between this reaction and the test tube reaction with orcinol in sulphuric acid.

The method has been used in this laboratory by Mr. J. G. BEELEY on fractions obtained by paper electrophoresis after proteolytic degradation of ovomucoid. Cuts of paper (1.6 cm^2) were eluted centrifugally with 100 μl water, and 20 μl samples tested as above. Paper blanks were not appreciable. The method could also be used for rapid testing of fractions obtained by column chromatography.

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Colorimetric identification following tritium recovery from paper*

To assure quantitative recovery of tritium-labeled compounds from paper chromatograms, it is often necessary to elute the material from the paper rather than using scanning techniques. However, various identifying color reagents cannot be used on the developed chromatogram if the eluates are to be counted in a liquid scintillation spectrometer. We have encountered this situation in the isolation of cardiac glycoside metabolites. Glycoside color reagents such as picric acid, *m*-dinitrobenzene, antimony trichloride, and trichloracetic acid markedly quench counts and/or decrease our ability to extract the materials from the developed chromatogram. We have therefore resorted to cutting the developed chromatogram into thin strips throughout its length. Each section is quantitatively extracted, counted and corrected for quench by internal standardization. The picric acid color reagent, which quenches counts about 99%, is then added to each counting vial to verify the location of detectable amounts of carrier compounds used as markers. Investigators may find this color identification after quantitative recovery useful if they are similarly limited by quenching reagents and the counting solution is compatible with color development following radioassay.

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