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695. *The Detection of Deoxy-sugars, Glycals, and Methyl Pentoses in Paper Partition Chromatography.*

By J. T. Edward and Deirdre M. Waldron.

Colour tests have been found by which deoxy-sugars, glycals, and methyl pentoses may be distinguished from other sugars or sugar derivatives on paper chromatograms. By application of these tests the presence of fucose in serum and urinary mucoprotein has been demonstrated (Waldron, *Nature*, in the press). Some limitations and advantages in conducting spot tests on paper are discussed.

Methyl pentoses and deoxy-sugars may be located on paper chromatograms by spraying them with various reagents (Partridge, *Biochem. J.*, 1948, **42**, 238; *Biochem. Soc. Symposia*, 1950, No. 3, p. 52; Forsyth, *Nature*, 1948, **161**, 239; Hough, Jones, and Wadman, *J.*, 1950, 1702) but researches in progress in our laboratories indicated the need for more specific reagents for distinguishing them from the other reducing sugars. Buchanan, Dekker, and Long (*J.*, 1950, 3162) applied the Feulgen and the Dische reaction to the detection of deoxyribonucleosides, but the methods were inconvenient and relatively insensitive. More recently Buchanan (*Nature*, 1951, **168**, 1091) has employed a spray of cysteine in 3N-sulphuric acid to reveal the presence of deoxy-sugars and deoxyglycosides.

Although the usual aldoses and ketoses are oxidised by periodate to the one-carbon fragments formaldehyde and formic acid (Jackson, "Organic Reactions," 1944, vol. II, p. 345), compounds having a carbon chain lacking some vicinal hydroxyl groups will also yield larger fragments possessing active methyl or methylenic groups. Thus, methyl pentoses give acetaldehyde (Nicolet and Shinn, *J. Amer. Chem. Soc.*, 1941, **63**, 1456), deoxy-sugars malondialdehyde (cf. Manson and Lampen, *J. Biol. Chem.*, 1951, **191**, 95), and deoxypyranosides and deoxy-alcohols such as 2-deoxy-D-dulcitol or 1 : 2-dideoxy-galactose should also yield products with the $\cdot CH_2 \cdot CHO$ grouping. This grouping gives a blue colour with nitroprusside in the presence of secondary amines (Rimini, *Chem. Zentr.*, 1898, II, 277; Lewin, *Ber.*, 1899, **32**, 3308; Neuberg, *Biochem. Z.*, 1915, **71**, 150; Neuberg and Kerb, *ibid.*, 1918, **92**, 96; Fearon and Boggust, *Analyst*, 1951, **76**, 667), and we have found that the compounds mentioned above may be revealed as blue zones when chromatograms are sprayed with aqueous periodate (cf. Buchanan, Dekker, and Long, *loc. cit.*) followed by a solution of sodium nitroprusside and piperazine in aqueous alcohol. Threonine, which is oxidised by periodate to acetaldehyde (Martin and Synge, *Biochem J.*, 1941, **35**, 94), can be distinguished by this test from the other amino-acids. Glycals also react; they are probably oxidised to dialdehydes having the $\cdot O \cdot CH{=}CH \cdot CHO$ grouping; and it is likely that the positive reaction, like those of acraldehyde and cinnamaldehyde (Lewin, *loc. cit.*), depend on the initial addition of the secondary amine to the double bond so that the $\cdot CH_2 \cdot CHO$ grouping is formed.

Unoxidised 2-deoxy-sugars react feebly with nitroprusside–piperazine, presumably because of the small proportion present in the straight-chain form.

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Deoxy-sugars and glycals can be distinguished from methyl pentoses by the deep yellow zones which they give when the chromatogram is sprayed with an acidic solution of *p*-nitroaniline after periodate oxidation. This colour is believed to be due to a condensation of malondialdehyde (which can be furnished by hydrolysis of the ·O·CH=CH·CHO grouping of the oxidised glycal) with the aromatic amine, analogous to the condensations of glutacondialdehyde [Maier-Bode and Altpeter, "Das Pyridin und seine Derivate," W. Knapp, Halle (Saale), 1934, p. 18], which is a vinylogue of malondialdehyde. This test is positive also for deoxyglycosides, including guanine deoxyribofuranoside, which though not initially oxidised by periodate (Brown and Lythgoe, *J.*, 1950, 1990) probably first undergoes hydrolysis to deoxyribose on the paper.

A second test by which glycals and deoxy-sugars can be distinguished from methyl pentoses consists in spraying the chromatogram with an acid solution of *p*-dimethylaminobenzaldehyde or ninhydrin. On gentle heating, the former compounds give rise to blue or grey zones, the glycals showing an initial pink colour. On more prolonged heating pentoses and ketoses form coloured zones, but aldohexoses and methyl pentoses do not (cf. Partridge, *loc. cit.*). It is probable that the condensation of the *p*-dimethylaminobenzaldehyde takes place with products resulting from the acid-catalysed rearrangement of the deoxy-sugars or glycals (cf. Deriaz, Stacey, Teece, and Wiggins, *J.*, 1949, 1222; Allerton, Overend, and Stacey, *J.*, 1952, 255; Newth, *Adv. Carbohydrate Chem.*, 1952, **2**, 85), since these products (kindly furnished by Professor Stacey and Dr. Shafizadeh) react as do the sugars, whereas aldol and other compounds containing the ·CH_2·CHO group fail to give a perceptible colour.

Buchanan's cysteine–sulphuric acid reaction (*loc. cit.*) probably involves a preliminary rearrangement of the deoxy-sugars and is positive also for glycals.

Specific tests for glycals are furnished by spraying chromatograms with ethanolic hydrochloric acid which on gentle warming gives zones initially pink but changing to brown; or with alcoholic ferric chloride which gives brown zones changing to black. Since the glycals are easily separable from their corresponding deoxy-sugars by paper chromatography, these tests may prove useful in following their reactions.

The tests described above are all much more sensitive when carried out on filter paper rather than in solution (cf. Feigl, "Specific and Special Reactions for Use in Qualitative Analysis," Nordemaa, New York, 1940, p. 162), except in the case of some very volatile compounds. Thus nitromethane reacts like acetaldehyde with nitroprusside–piperazine in solution but unlike acetaldehyde cannot be tested on paper, because it evaporates before the slow reaction takes place. The retention of the more volatile acetaldehye on paper, which recalls the difficulty frequently encountered in removing from paper chromatograms the last traces of polar developing solvents, is probably due to solution in the adsorbed water, or to direct hydrogen bonding to cellulose.

A second advantage in conducting spot tests on paper lies in the generally increased rate of reactions. Thus 2-deoxy-D-glucose, because of the *trans*-α-glycol system of its pyranose ring, is oxidised more slowly than 2-deoxy-D-galactose in dilute solution (cf. Jackson, *loc. cit.*), and requires about two hours before the maximum colour is attained after addition of the nitroprusside–piperazine solution. However, on paper maximum colour is obtained after 10—15 minutes' oxidation. It seems likely that after the periodate solution has been sprayed on the chromatogram, evaporation of the solvent results in an extremely concentrated solution, in which oxidation takes place more rapidly. Such an effect would perhaps explain the observation of Hough (*Nature*, 1950, **165**, 400) that on paper even polyhydric alcohols react with ammoniacal silver nitrate.

Experimental

Unless otherwise stated, the paper chromatograms were developed by the ascending technique, with pyridine–amyl alcohol–water as solvent (Werner and Odin, *Uppsala Läk. Forsch.*, 1949, **1/2**, 69) and Whatman No. 1 filter paper.

Test A. Periodate Oxidation, followed by Treatment with Nitroprusside–Piperazine.—The dried paper chromatograms were sprayed lightly with saturated aqueous sodium metaperiodate (1 vol.) diluted with water (2 vols.). After 10 minutes at room temperature, a solution of

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saturated aqueous sodium nitroprusside (1 vol.), water (3 vols.), and ethanol saturated with piperazine (20 vols.), was sprayed on the paper. Methyl pentoses, deoxy-sugars, and glycals gave rise to blue zones in 5—10 minutes, and thereafter slowly faded. The sensitivity to deoxy-sugars was slightly improved by adding to the second spray an equal volume of 1% ethylene glycol in methanol, in order to destroy excess of periodate. The minimum quantity detectable in spot tests is shown in the Table. On chromatograms, because of diffusion of the zones, sensitivity was diminished five- to ten-fold.

Secondary bases such as piperidine and diethylamine (cf. Rimini, *loc. cit.*; Lewin, *loc. cit.*) could be substituted for piperazine if this test was carried out in solution. However, they were unsatisfactory for tests on paper because of their volatility. Samples of diethylamine frequently gave rise to a blue colour with nitroprusside alone, presumably because they had undergone some oxidation to acetaldehyde; this compound was detected also in old specimens of triethylamine and diethylaminoethanol.

Test B. Periodate Oxidation, followed by Condensation with p-*Nitroaniline.*—After periodate oxidation the chromatograms were sprayed with a solution made up of 1% *p*-nitroaniline in ethanol (4 vols.) and concentrated hydrochloric acid (1 vol.). Deep yellow spots rapidly appearing against the pale yellow background indicated deoxy-sugars and glycals; these could be more easily detected in ultra-violet light as yellow fluorescent zones against a brown background. When the chromatograms were sprayed with 5% methanolic sodium hydroxide, the zone became green; the sensitivity in spot tests is shown in the Table.

Sensitivity (in μg.) of colour reactions for methyl pentoses, deoxy-sugars, and glycals.

Sugar	Test A	Test B	Test C	Test D
Fucose	4	—	—	—
Rhamnose	1	—	—	—
2-Deoxy-D-ribose	2	0·1	0·1	—
2-Deoxy-D-glucose	2	0·1	0·2	—
2-Deoxy-D-galactose	1	0·1	0·2	—
3-Deoxy-L-xylose	1	0·1	7	—
α-Methyl-2-deoxy-D-ribopyranoside	10	0·1	0·1	—
α-Methyl-2-deoxy-D-galactopyranoside	50	0·1	0·1	—
β-Methyl-3-deoxy-L-xyloside	—	3	7	—
β-Guanine-2-deoxy-D-ribofuranoside	—	1	0·5	—
2-Deoxy-D-dulcitol	1	—	—	—
1 : 2-Dideoxy-D-galactose	5	—	—	—
D-Ribal	0·2	0·1	0·7	0·1
D-Glucal	1	0·2	0·2	0·1
D-Galactal	1	0·1	0·1	0·1
3 : 4 : 6-Triacetyl D-glucal	—	—	—	—

Other methods which were employed to reveal the malondialdehyde resulting from the oxidation of deoxy-sugars were: spraying with alcoholic ferric chloride (cf. Claisen, *Ber*, 1903, **36**, 3664); spraying with diazotised *p*-anisidine (Sanger and Tuppy, *Biochem. J.*, 1951, **49**, 463) and making alkaline with ammonia fumes (cf. Claisen, *loc. cit.*); and exposure to sulphur dioxide followed by a spray of Schiff's reagent (cf. Buchanan, Dekker, and Long, *loc. cit.*). However, none of these methods was as sensitive as the one above.

Test C. Treatment with p-*Dimethylaminobenzaldehyde in Acid.*—Chromatograms were sprayed with a mixture of 1% *p*-dimethylaminobenzaldehyde in ethanol (4 vols.) and concentrated hydrochloric acid (1 vol.) and heated in an oven at 90°. In 30 seconds deoxypentoses, deoxypentosides, and D-ribal had given rise to bluish- or purplish-grey spots; deoxyhexoses, deoxyhexosides, glucal, and galactal had given pinkish-grey spots. 3-Deoxy-L-xylose and β-methyl-3-deoxy-L-xyloside responded slightly more slowly (cf. Allerton, Overend, and Stacey, *loc. cit.*), to give grey colours. When heating was prolonged for 2 minutes, xylose and arabinose (1—2 μg.) gave grey zones. The sensitivity of the test for materials spotted on paper is shown in the Table. Substitution of ninhydrin for *p*-dimethylaminobenzaldehyde resulted in a test of the same sensitivity, but with more bluish colours.

Test D. Treatment with Alcoholic Hydrochloric Acid.—Chromatograms were sprayed with concentrated hydrochloric acid (1 vol.) diluted with ethanol (4 vols.). On heating in an oven at 90°, pink zones changing rapidly to brown indicated the presence of glycals. The spray of alcoholic ferric chloride was slightly less sensitive.

Chromatography of Glycals and Deoxy-sugars.—Glycals and deoxy-sugars were submitted to ascending chromatography at 20° with Partridge's solvent system butanol–acetic acid–water (*loc. cit.*), and located with *p*-dimethylaminobenzaldehyde spray. The R_F values were: D-

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galactal, 0·54; D-glucal, 0·61; D-ribal, 0·77; 2-deoxy-D-galactose, 0·32; 2-deoxy-D-glucose, 0·35; 2-deoxy-D-ribose, 0·45.

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