The chromatographic differentiation of some deoxy-sugars

A spray reagent containing diphenylamine, aniline, and phosphoric acid was developed by BUCHAN AND SAVAGE[1] for locating hexoses on paper chromatograms. Later this reagent was used by SCHWIMMER AND BEVENUE[2] for the differentiation of 1,4- and 1,6-linked glucosaccharides. A detailed study of the reaction of the reagent with a comprehensive collection of sugars was undertaken by BAILEY AND BOURNE[3] and they defined conditions in which mixed acidic diphenylamine–aniline sprays give, on paper chromatograms, specific colour reactions with uronic acids, aldoheptoses, methylpentoses, and pentoses. We have examined the behaviour of the reagent with a much wider range of deoxy-sugars than was used by BAILEY AND BOURNE[3], who used only methylpentoses (6-deoxyhexoses). Our results indicate that the reagent is useful for the detection and identification, on paper and thin-layer chromatograms and on paper ionophoretograms, of modified sugars of this type.

Experimental

Materials. Except for the following, which were prepared by members of our laboratory, the sugars used were obtained from commercial sources; 3-deoxy-D-*ribo*-hexose, 3-, 4-, and 5-deoxy-D-*xylo*-hexose, 6-deoxy-D-glucose, -L-talose, and -L-idose, 4,6-dideoxy-D-*xylo*-hexose and its 3-O-methyl ether, 2,6-dideoxy-D-*arabino*-hexose, 2,6-dideoxy-3-C-methyl-D-*ribo*-hexose and its 3-O-methyl derivative.

Reagent. Diphenylamine (2 g) and aniline (2 ml) were dissolved in acetone (100 ml) and orthophosphoric acid (80 %, 10 ml) was added. The components of the reagent were B.D.H. reagent-grade chemicals which were not purified further.

Chromatography and ionophoresis. Paper chromatograms were prepared using Whatman No. 1 paper with development by downward irrigation with various solvent systems which included the organic phase of *n*-butanol–ethanol–water (4:1:5), ethyl acetate–*n*-propanol–water (7:1:2), and *n*-butanol–acetic acid–water (70:7:23) (all solvent mixtures are by volume). Whatman No. 3MM paper was used for the ionophoretic separations which were carried out in borate buffer, pH 10, at a potential difference of 26 V cm^{-1}. The paper was supported on a cooled plate. The deoxy-sugars were applied in solution (*ca.* 2 %) in commercial methanol. After development the paper chromatograms and pherograms were air dried, dipped in the diphenylamine–aniline reagent, and heated at 80–85° for 5 min. For thin-layer chromatography silica gel (Kieselgel G nach Stahl, supplied by E. Merck A.G., Darmstadt) was spread on microscope slides and ethyl acetate–methanol (2:3, v/v) was used as solvent system. The developed plates were sprayed with the reagent and then heated at 80–85° for 5 min.

In all cases discrete non-diffuse spots were obtained. The paper chromatograms and pherograms and the thin-layer chromatographic plates were stored in the dark and the colour changes of the spots occurring during 3 days were observed.

Results and discussion

The results obtained from the paper chromatographic and ionophoretic experiments are shown in Table I. It will be seen that coloured spots were obtained with all the deoxy-sugars tested. Irrespective of configuration, monodeoxy-hexoses in which the deoxy-group is similarly located, all give spots with the same colour. It

TABLE I

COLOUR REACTIONS ON PAPER CHROMATOGRAMS AND PHEROGRAMS OF DEOXY-SUGARS AND DIPHENYLAMINE–ANILINE REAGENT

Sugar	*Colour*	
	Immediate	*After 3 days*
2-Deoxy-D-*arabino*-hexose, 2-deoxy-D-*lyxo*-hexose, 2-deoxy-D-*erythro*-pentose	reddish-pink	reddish-brown
3-Deoxy-D-*ribo*-hexose, 3-deoxy-D-*xylo*-hexose	yellow	brownish-yellow
4-Deoxy-D-*xylo*-hexose	yellow	bright green
5-Deoxy-D-*xylo*-hexose	brown (blue tinge)	brown
6-Deoxy-D-glucose, 6-deoxy-L-idose, 6-deoxy-L-talose, 6-deoxy-D-galactose (D-fucose), 6-deoxy-L-mannose (L-rhamnose)	yellow	blue
4,6-Dideoxy-D-*xylo*-hexose	brownish-yellow	green
4,6-Dideoxy-3-O-methyl-D-*xylo*-hexose (D-chalcose)	yellow	green
2,6-Dideoxy-D-*arabino*-hexose	reddish-pink	reddish-brown
2,6-Dideoxy-3-C-methyl-D-*ribo*-hexose (D-mycarose), 2,6-dideoxy-3-C-methyl-3-O-methyl-D-*ribo*-hexose (D-cladinose)	purple	dark green with a blue rim

should be stressed, however, that reproducible results can be obtained only if the temperature range of 80–85° for heating the papers is adhered to strictly. Although yellow coloured spots were obtained initially with all the 3-, 4-, and 6-deoxy-hexoses examined, the colour changes on storage of the chromatograms from these three classes of deoxy-hexose were different and served to differentiate between them. BAILEY AND BOURNE[3] reported that the two methylpentoses (6-deoxy-hexoses) (*i.e.* fucose and rhamnose) which they used gave orange coloured chromatographic spots with the diphenylamine–aniline reagent. Under the conditions of our experiments a true orange colour was not observed with any of the five methylpentoses we examined. From the results with 2,6-dideoxy-D-*arabino*-hexose, and 4,6-dideoxy-D-*xylo*-hexose and its 3-O-methyl derivative, it appears that the final colour produced is that given respectively by the corresponding 2- and 4-deoxy-hexose rather than that of the 6-deoxy-hexose. C-Branching in the dideoxy-hexose leads to different colours. As

TABLE II

COLOUR REACTIONS ON THIN LAYER PLATES OF DEOXY-SUGARS* AND DIPHENYLAMINE–ANILINE REAGENT

Sugar	*Colour*		
	Immediate	*After 1 day*	*After 3 days*
2-Deoxy-D-*arabino*-hexose	reddish-brown	unchanged	unchanged
3-Deoxy-D-*ribo*-hexose	brownish-yellow	unchanged	unchanged
4-Deoxy-D-*xylo*-hexose	yellow	turquoise	bright green
5-Deoxy-D-*xylo*-hexose	brown	blue-brown	brown
6-Deoxy-D-glucose	yellow	blue	blue (unchanged)

* The compounds listed in this table are frequently referred to by their less systematic names of 2-, 3-, 4-, 5-, and 6-deoxy-D-glucose.

further sugars of these types become available, it will be of interest to see if these conclusions are general.

In Table II are listed the colour changes obtained on treating thin-layer silica gel chromatograms of the monodeoxy-hexoses commonly known as the monodeoxy-glucoses, with the diphenylamine–aniline reagent. In each case the final colour obtained was the same as was found with the paper chromatogram. However, the time taken to give the final colour was much shorter than with the paper chromatograms, and it was possible to differentiate within a short time between all five monodeoxy-glucoses by their colour reaction on thin-layer plates.

TABLE III

RATES OF MIGRATION ON PAPER DEVELOPED WITH *n*-BUTANOL–ETHANOL–WATER (4:1:5, ORGANIC PHASE) OF THE MONODEOXY-GLUCOSES

Monodeoxy-glucose	$R_{GLUCOSE}$
2-	2.48
3-	2.41
4-	1.86
5-	2.88
6-	2.66

For reference, the relative rates of migration on paper of these deoxy-sugars are indicated in Table III. It will be seen that for those compounds which move at similar rates the colour reaction with the diphenylamine–aniline reagent provides a useful additional means of identification.

Acknowledgements

The following colleagues are thanked for gifts of deoxy-sugars: Mr. P. J. BEYNON AND Drs. P. M. COLLINS, B. FLAHERTY, B. GOUGH AND E. J. HEDGLEY. We are also indebted to Dr. HEDGLEY for helpful discussions. This work was carried out as part of a programme of research supported by the Medical Research Council.

Chemistry Department, Birkbeck College, S. MCNALLY
University of London, London (Great Britain) W. G. OVEREND

1 J. L. BUCHAN AND R. I. SAVAGE, *Analyst*, 77 (1952) 401.
2 S. SCHWIMMER AND A. BEVENUE, *Science*, 123 (1956) 543.
3 R. W. BAILEY AND E. J. BOURNE, *J. Chromatog.*, 4 (1960) 206.

Received June 21st, 1965