New Spraying Reagents for the Detection of Reducing Sugars on the Paper Chromatograms

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(Received September 19, 1952)

Since paper chromatography was first applied to carbohydrates by Partridge,[1] various spraying reagents have been reported for the detection and identification of sugars separated on a paper chromatogram. Acidic solutions of aromatic amines such as aniline[2] and benzidine,[3] or of phenols such as resorcinol and naphthoresorcinol,[4] have been most frequently employed as spraying reagents. Their use depends upon the formation of furfural or its derivative by the action of an acid upon the sugar, and its subsequent condensation with an amine or phenol to give a colored compound. Among them, amine reagent is generally sensitive to aldoses, but is not so sensitive to ketoses and sometimes gives only faint spots. Whereas the sensitivity of phenol reagent to ketoses is excellent, its sensitivity to aldoses is moderately poor. Therefore, for complete detection of components in a mixture of unknown sugars by means of paper chromatography, such disadvantage has been experienced that both amine and phenol reagents must be applied.[5] If, however, aminophenols, carrying amino and phenolic hydroxyl groups, are used instead of an amine or phenol alone, they may be expected to be universal reagents in order to detect both aldoses and ketoses in one spraying operation. In this expectation, several aminophenols have been examined as the color reagents for reducing sugars, and an alcoholic solution of *o*-aminophenol acidified with phosphoric acid has been found to be the most useful one in respect of its universal sensitivity for various types of sugars and its specific coloration.

Experimental

Spraying Reagents: Of aminophenols were examined *o*-, *m*- and *p*-aminophenol, 1-amino-2-naphthol, 2,4-diaminophenol and 2-amino-resorcinol, the last two being used in the form of their hydrochlorides because of the unstability of their free bases. The reagent was prepared before use by dissolving 0.15 g. of each aminophenol in 20 cc. of ethanol and acidifying it with 10 cc. of 50% (w/w) phosphoric acid.

General Procedure: By means of micropipettes, the solutions of various concentrations of a sugar were spotted side by side at intervals of 2 cm. on a starting line of a filter paper sheet (Tōyō filter paper No. 2) so that each spot carried 1, 2, 3, 5, 10, 15 and 20 μg. of the sugar, respectively. The chromatogram was irrigated in the ascending technique with the upper layer of a mixture of butanol, ethanol and water (4:1:5 V/V), the solvent being allowed to advance about 30 cm. from the sugar starting line. The paper was then dried, sprayed with the reagent, and heated in an oven at 105° for a few minuites until a series of sugar spots with successively decreasing intensities was revealed in decreasing order of sugar quantity. The background of the paper remained almost unaffected. Thus, the minimum quantity of the sugar which was detected by each reagent was measured, and the color of the spots was also observed. Experiments were repeated several times for each of L-arabinose, D-xylose, D-ribose, L-rhamnose, D-glucose, D-mannose, D-galactose, D-fructose, L-sorbose, D-tagatose, lactose, sucrose,

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(1) S. M. Partridge, *Nature*, **158**, 270 (1946).
(2) S. M. Partridge, ibid., **164**, 444 (1949).
(3) R. H. Horrocks, ibid., **164**, 443 (1949).
(4) W. G. C. Forsyth, ibid, **161**, 239 (1948).
(5) S. Hirase and C. Araki, *Memoirs of Faculty of Industrial Arts, Kyoto Technical University*, **1**, 19 (1952)

Table 1

The Observed Color and the Sensitivity (in μg. unit) of Each Sugar Class to Various Aminophenol Reagents

Sugar Classes	Aminophenols											
	o-Amino-phenol		*m*-Amino-phenol		*p*-Amino-phenol		1-Amino-2-naphthol		2, 4-Diamino-phenol*		2-Amino-resorcinol*	
Pentose	Blue	1	Brown	2	Reddish brown	5	Blue	5	Dark blue	1	Blue	5
Methyl-pentose	Cherry red	5	Brown	5	Yellowish brown	5	Purple	15	Orange	5	Orange red	15
Aldohexose	Brown	5	Brown	5	Yellowish brown	5	Brown	15	Brown	5	Brown	15
Ketohexose	Lemon yellow	1	Lemon yellow	1	Lemon yellow	1	Lemon yellow	1	Lemon yellow	1	Lemon yellow	1
Lactose	Brown	5	Brown	5	Brown	5	Brown	15	Brown	5	Brown	15
Sucrose	Yellow	1	Yellow	1	Yellow	1	Yellow	1	Yellow	1	Yellow	1
Uronic acid	Blue	1	Reddish brown	1	Reddish brown	1	Blue	1	Blue	1	Blue	1

* u ed as its hydrochloride since its free base was unobtainable.

D-glucuronolactone, D-mannuronolactone and D-galacturonic acid. The results are summarized in the accompanying table.

Results

It will be seen from the table that all of the reagents are more or less effective to detect sugars, though the preliminary expectation is not completely satisfied. Generally speaking, they are particularly sensitive to ketoses and ketose-containing sucrose, giving a yellow color. Uronic acid or its lactone is also detectable with superior sensitivity. With regard to aldoses, pentose can be detected more sensitively than methylpentose and aldohexose, and gives the same coloration as that of uronic acid or its lactone.

Among these reagents, *o*-aminophenol reagent is found to be the most valuable one, for it detects as little as $1\sim5\,\mu$g. of each sugar and yields a specific color with each particular class of sugars. Although 2, 4-diaminophenol reagent also seems to be possessed of these advantages, it is inferior to the former due to its unstability. With *o*-aminophenol reagent, it is easily achieved to decide the sugar class to which an unknown sample belongs, and, moreover, methyl glycosides give the same colorations as those of their parent sugars. For example, methyl xyloside and methyl glucoside give blue and brown color, respectively. Sugar alcohols are lacking in any coloration.

The authors wish to express their hearty thanks to Mr. K. Yamanouchi for his kind assistance in the course of this study.

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