ANALYSIS OF SUGARS AND URONIC ACIDS USING PAPER CHROMATOGRAPHY (HORIZONTAL MIGRATION)

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The qualitative analysis of the common natural sugars by the application of the horizontal migration method of paper chromatography has recently been reported by Rao and Beri.[1] Each sugar, it was shown, has a characteristic circular R_F value by the determination of which the sugar can be identified. This method has now been applied for the separation and identification of glucuronic and galacturonic acids.

These uronic acids, occurring as they do as integral parts of several polysaccharic substances like pectins, gums, mucilages and hemicelluloses, are widely distributed in nature; yet their identification is a matter of some difficulty. The usual method adopted is to convert them into the corresponding dicarboxylic acid, mucic or saccharic acid as the case may be, and identify the latter. During recent years the barium salts of the acids have become handy for identification, for they are shown to have a definite specific rotation. Rarely the uronic acids themselves have been isolated for purposes of identification, since the isolation is somewhat troublesome and takes a long time; sometimes some of their derivatives like phenyl hydrazone and *p*-bromophenyl hydrazone or salts with organic bases like cinchonine and cinchonidine are prepared. All the above methods are either difficult or time-consuming. It has now been found that the identification of the uronic acids can be conveniently effected by employing the horizontal migration method of paper chromatography.

With two-component solvents like moist butanol, moist phenol, moist *s*-collidine, moist *p*-cresol and moist methyl ethyl ketone, the two uronic acids have given almost the same circular R_F values (Table I). These values, which are in general much lower than those of the sugars, may indicate the presence of the uronic acids but are not, on account of overlapping, capable of establishing their individual identity. Hence three-component solvent systems, *viz.*, ethyl acetate-pyridine-water and ethyl acetate-acetic acid-water, which are reported to give good separations in the case of sugars by the solvent-descending method,[2] have been tried, and the results are

presented in Table II. For purposes of comparison the circular R_F values of the sugars and glucurone with the two three-component solvents have also been determined and included in the table. Of the two solvents only one, namely, ethyl acetate-acetic acid-water, has given a good separation of the acids, the circular R_F values for glucuronic and galacturonic acids being 0·17 and 0·30 respectively. However, the value for *d*-galacturonic acid is very close to that of *d*-galactose (0·32), and is, therefore, not capable of distinguishing the uronic acid from the sugar. The differentiation can, however, be easily made by running the chromatogram, also with the second three-component or any of the two-component solvents.

Since the uronic acids are usually isolated as the barium salts, the behaviour of the latter has also been examined. With two-component solvents they have given almost the same circular R_F values (Table I). However, with the three-component solvents their behaviour is different. Each salt has given rise to two rings instead of one, and at present we are not in a position to offer any satisfactory explanation for the appearance of the two rings. The two rings obtained with either of the two three-component solvents are quite characteristic of the salt and enable its identification, when present alone. But, if both the salts are present, the use of ethyl acetate-acetic acid-water as the solvent is not convenient, since both the rings obtained with one salt overlap the corresponding ring of the other salt. With ethyl acetate-pyridine-water, however, it is possible to identify the two uronates even when they are present together. With this solvent, barium glucuronate gives 0·16 and 0·32 for the circular R_F values, while the galacturonate has 0·12 and 0·24 as its values. With a mixture of the two salts, it is possible to get only three rings, since those with the values of 0·12 and 0·16 overlap each other to some extent. However, the rings with the higher values (0·24 and 0·32) are quite distinct and characteristic of the two individual salts.

In the study of natural acid polysaccharides (polyuronides) the paper-chromatographic method of identification of the uronic acids may prove to be quite handy and convenient. The acid hydrolyzate may be neutralized with just the amount of barium hydroxide required for the complete precipitation of sulphuric acid used for the hydrolysis, filtered and the filtrate, which contains sugars and free uronic acid, may be straightaway subjected to paper-chromatographic analysis. As an addition or an alternative, the hydrolyzate may be completely neutralized with barium carbonate, filtered and the filtrate, which contains sugars and barium uronate, may be similarly analyzed using the appropriate solvents,

Experimental

The procedure adopted was just the same as the one already described in our earlier publication (Rao and Beri, *loc. cit.*). With the three-component solvents, however, greater care was found necessary, because slight changes in the relative proportion of the components used to affect the R_F values, as is usual with such systems. In order to keep the composition of the solvent constant right through, firstly the components were taken in exactly measured amounts by means of burette and secondly the evaporation of any of the volatile components was reduced to the minimum by further covering the experimental set-up with another bigger inverted Petri dish. Further, the solvent mixture was prepared only in small amounts so that it was just enough for two or three experiments. It was also found necessary to thoroughly shake the solvent mixture in a separating funnel and allow it sufficient time to attain equilibrium (about half an hour) before it was taken for use. Both with ethyl acetate-pyridine-water and ethyl acetate-acetic acid-water, the lower layer was the discardable aqueous layer and the upper was the solvent mixture for the irrigation of the chromatograms.

Taking Whatman No. 1 circular filter papers (18·5 cm. in diameter) and cutting the tail as described by Rao and Beri (*loc. cit.*), the sugar or the uronic acid solution (1 per cent.) was introduced with the help of a capillary tube as a microdrop at the centre of the filter paper and air-dried. The solvent was taken straight into the Petri dish (15 cm. in diameter) or another small dish placed at its centre in the case of the three-componet solvents, and the filter paper was placed over the Petri dish in the usual way so that the tail hung down into the solvent below. A glass plate was placed over the filter paper and the whole was covered by another inverted bigger Petri dish. The experiments were conducted in a thermostat maintained at 35° C.

When the irrigation was over (30 to 60 minutes depending on the nature of the solvent), the filter paper was removed, the position of the solvent front marked, and dried in an air-oven at 105° for 5 minutes. It was then rapidly and evenly sprayed with a solution of aniline hydrogen phthalate in butyl alcohol and again dried at 105° for 5 minutes. The position taken up by the sugar or the uronic acid was indicated by brown or purplish brown rings. It is worthwhile to note that as a group the pentoses give purplish brown, while the hexoses or the uronic acids derived from them produce brown or dark brown rings, but the shades will be reversed on standing for a few days. By noting the distances through which moved the sugar on the one hand and the solvent on the other, the circular R_F values were calculated as described by Rao and Beri. The values obtained are recorded in the following tables;—

TABLE I

Circular R_F *values at* 35° *with two-component solvents*

	Moist phenol	Moist *n*-butanol	Moist *s*-collidine	Moist *p*-cresol	Moist methyl ethyl ketone
d-Glucuronic acid (obtained by the hydrolysis of gum arabica)	0·24	0·07	0·19	0·06	0·11
d-Galacturonic acid ..	0·27	0·07	0·16	0·08	0·13
Barium glucuronate ..	0·24	0·07	0·18; 0·28	0·08	0·12
Barium galacturonate ..	0·23	0·07	0·24	0·06	0·10

TABLE II

Circular R_F *values at* 35° *with three-component solvents*

	Ethyl acetate-pyridine-water (2 : 1 : 2 by volume)	Ethyl acetate-acetic acid-water (3 : 1 : 3 by volume)
d-Glucose ..	0·59	0·36
d-Galactose ..	0·58	0·32
d-Mannose ..	0·70	0·52
d-Fructose ..	0·67	0·57
l-Rhamnose ..	0·81	0·59
d-Arabinose ..	0·66	0·40
d-Xylose ..	0·77	0·57
Lactose ..	0·56	0·24
Maltose ..	0·67	0·26
d-Glucurone ..	0·92	0·63
d-Glucuronic acid ..	0·26	0·17
d-Galacturonic acid ..	0·25	0·30
Barium glucuronate ..	0·16; 0·32	0·17; 0·34
Barium galacturonate ..	0·12; 0·24	0·12; 0·30

SUMMARY

Using the horizontal migration method of filter paper chromatography, the separation and identification of glucuronic and galacturonic acids and their barium salts have been effected.

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