SHORT COMMUNICATIONS

PAPER IONOPHORESIS OF CARBOHYDRATES*

II. DETECTION OF NON-REDUCING COMPOUNDS IN SODIUM ARSENITE

By J. L. Frahn†

Paper ionophoresis in $0 \cdot 2$M sodium arsenite at pH $9 \cdot 6$ has proved to be a very useful method for the separation of mixtures of reducing sugars, which are easily detected in the presence of arsenite.[1] The same procedure separates many isomeric rugar alcohols and cyclitols, but the difficulty of detecting non-reducing carbohydrates in the presence of sodium arsenite detracts from its practical value. The method of detection used[1] was difficult and gave uncertain results. The problem has been re-examined, and the three methods of detection described here should meet most requirements.

Detection with ammoniacal silver nitrate[2] is satisfactory for most compounds if the concentration of the reagent is sufficiently high to compensate for the precipitation of silver as silver arsenite. Oxidation of cyclitols to rhodizonic acid with nitric acid, as described for myoinositol on paper chromatograms,[3] permits the detection of most cyclitols on paper in the presence of arsenite. Most compounds containing vicinal glycol groups can be detected by selective oxidation with sodium periodate, followed by the application of benzidine.[4,5] Hartman and Barker[6] used the last method for the detection of tetritols and anhydrotetritols after paper ionophoresis in arsenite, but described it only briefly. A more detailed study of its utility has revealed some interesting features.

Periodates are reduced by trivalent arsenic, especially in slightly acidic solutions, therefore careful control of the conditions is necessary. Acyclic polyols reduce periodate very rapidly and are easily detected, but the results obtained for cyclitols depend on whether the paper strip is acidic or alkaline during the reaction with periodate. If it is alkaline, some cyclitols give *dark blue* instead of white spots when the benzidine is applied, and the others give paler blue spots. This suggests that the cyclitols, or some product of their oxidation by periodate, may retard the reduction of periodate by the arsenite and the paper support. Tests have shown that several cyclitols produce a similar effect on paper strips in the absence of sodium arsenite.

* Manuscript received September 9, 1963.

† Division of Biochemistry and General Nutrition, CSIRO, Adelaide.

1 Frahn, J. L., and Mills, J. A., *Aust. J. Chem.*, 1959, **12**, 65.

2 Hough, L., *Nature*, 1950, **165**, 400.

3 Nagai, Y., and Kimura, Y., *Nature*, 1958, **181**, 1730.

4 Cifonelli, J. A., and Smith, F., *Analyt. Chem.*, 1954, **26**, 1132.

5 Viscontini, M., Hoch, D., and Karrer, P., *Helv. Chim. Acta*, 1955, **38**, 642.

6 Hartman, F. C., and Barker, R., *J. Org. Chem.*, 1963, **28**, 1004.

The tendency of individual cyclitols to give strong blue spots cannot, however, be correlated with the presence of the conformation of hydroxyl groups known[7,8] to promote the formation of stable complexes between periodate ion and cyclic carbohydrates. There is, in addition, no clear relation between the ease of detection of individual cyclitols by the periodate–benzidine method under acidic conditions and the reported rates[9] of the initial stage of their oxidation by periodate in acidic solution.

Corrected values are given for the relative mobilities of some methylated cyclitols in sodium arsenite. The earlier figures[1] for these compounds, which are very difficult to detect by the original method, had been derived from one experiment only.

Experimental

The apparatus and general procedure have been described,[1] but it is preferable to subject the paper strip to a uniform pressure of about 0·4 atm.[10] The compounds were normally applied as 0·1M aqueous solutions and subjected to ionophoresis for 90 min.

Detection with Ammoniacal Silver Nitrate.—The paper strip is dried at 100°, but prolonged heating is avoided. It is sprayed at once, on both sides, with a solution made by adding conc. ammonia to aqueous silver nitrate solution (about 17% w/w) until the precipitate has just redissolved. The formation of yellow silver arsenite on the paper facilitates uniform application of the reagent. Reducing sugars and some alditols may appear almost immediately, at room temperature, as faint grey spots. The paper is heated at 100° for 3–5 min, then it is examined and any spots that have developed are outlined in pencil. It is returned to the oven, but is kept under observation, and any further spots are marked as they appear. After 10–15 min, when the background begins to darken and lessen the contrast with the spots, the paper is fixed by being dipped into aqueous sodium thiosulphate (10%), washed thoroughly in water, and dried. Some of the faintest spots may disappear during fixing. Excess silver reagent should be discarded at once, to avoid the formation of an explosive precipitate.[11]

Sugar alcohols containing more than four hydroxyl groups react strongly, but glycerol and the tetritols give only faint spots. Cyclitols generally react only weakly, but are detectable with care. Most methyl glycosides give a very weak reaction and are preferably applied as 0·25M solutions.

Detection of Cyclitols as Rhodizonic Acid.—The dried paper is dipped into a mixture of conc. nitric acid and ethanol (1 : 9 v/v), allowed to drain, and heated at 95° for 10 min. It is then sprayed with a solution of calcium chloride (5%) in 50% aqueous ethanol and heated at 95° for several minutes.

Allo-, *cis*, and (−)-inositol react very readily, giving moderately strong, pink spots that are stable for at least one year. Myo- and epi-inositol give a similar, but rather weaker reaction. Scylloinositol is barely detectable in this way, but it, quebrachitol, and neo- and mucoinositol may be detected as weakly fluorescent spots when the paper is exposed to ultraviolet light from a Philips HPW lamp (emission at 350 mμ).

Detection by the Periodate–Benzidine Method.—(i) Under acidic conditions. A solution of sodium metaperiodate (0·5%) in 0·2M acetic acid is sprayed quickly and evenly over the dried paper strip at the rate of about 3 ml/100 cm^2. It is then set aside at room temperature (about 20°) for about 3 min (not longer). The paper is then sprayed with a solution of benzidine (0·5%) in a mixture of ethanol and glacial acetic acid (4 : 1 v/v). Oxidizable compounds appear as white or

7 Barker, G. R., and Shaw, D. F., *J. Chem. Soc.*, 1959, 584.

8 Barker, G. R., *J. Chem. Soc.*, 1960, 624.

9 Angyal, S. J., and McHugh, D. J., *J. Chem. Soc.*, 1957, 1423.

10 Frahn, J. L., and Mills, J. A., *Aust. J. Chem.*, 1964, **17**, 256.

11 Vasbinder, H., *Pharm. Weekblad*, 1952, **87**, 861.

cream spots on a pale blue background. The spots are marked with a copying pencil, as the background colour fades to light buff within 30 min.

All sugar alcohols give clear, strong spots, and ethylene glycol, other vicinal diols, and glycerol are also detectable. Of the cyclitols, only muco- and (−)-inositol, quebrachitol, and *cis*-quercitol give a strong reaction. Myo-, allo-, and *cis*-inositol, and bornesitol are detectable only with difficulty. Epi-, neo-, and scylloinositol usually cannot be detected, although they may sometimes appear as slightly deeper blue spots on the pale blue background.

(ii) Under alkaline conditions. For the detection of some cyclitols not detectable by method (i) the dried paper is sprayed with aqueous sodium metaperiodate (0·5%) at the rate of about 3 ml/100 cm^2. The moist paper is then more alkaline than pH 9. The paper is set aside at room temperature for 15–20 min, as the periodate is reduced fairly slowly under these conditions, then it is sprayed with the benzidine reagent. The normal, pale blue background (which fades within an hour) is produced, but the cyclitols are revealed as deeper blue spots.

Epi-, myo-, and scylloinositol, and bornesitol give very strong spots that persist for days. Allo-, neo-, muco-, *cis*-, and (−)-inositol, and *cis*-quercitol also give blue spots, but they are only slightly deeper in colour than the background.

Corrected Mobilities for Methylated Cyclitols.—The following relative mobilities in sodium arsenite (M_R values) replace those given in Part I[1]: 3-*O*-methyl-(+)-inositol (pinitol), 0·25; 2-*O*-methyl-(−)-inositol (quebrachitol), 0·15; 1-*O*-methylmyoinositol (bornesitol), 0·11; 5-*O*-methylmyoinositol, 0·15.