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## Chromatography and detection of some β-D-glucosides

The successful detection of phenolic glucosides on paper chromatograms has depended on the presence of an aglucone which either shows chemical reactivity, especially towards coupling reagents, or interacts with UV light[1–9]. The use of $^{14}C$-labelled glucose or aglucone, supplemented by techniques to confirm the presence of both moieties, has also assisted in the detection of glucosides[10–15]. On thin-layer chromatoplates *p*-coumaryl alcohol 4-O-glucoside, syringin and coniferin have been located using antimony pentachloride in carbon tetrachloride[16]. Whilst investigating the metabolism of simple phenols and their β-D-glucosides in plants we experienced some difficulty in finding a sensitive reagent for locating glucosides on thin-layer plates. Whilst arbutin and the β-D-glucosides of catechol and resorcinol were readily detected with 0.25% ferric chloride/potassium ferricyanide in 50% aqueous alcohol and other reagents, an alternative reagent had to be sought for the remaining β-D-glucosides used in the study. By increasing the proportion of phosphoric acid to aniline in a reagent used to detect mono- and disaccharides on paper chromatograms[17] a sensitive locating reagent for β-D-glucosides on thin-layer plates of silica gel was obtained.

*Materials and methods*

*Thin-layer chromatography.* TLC plates, 20 cm × 20 cm of Silica Gel G (Merck), 0.25 mm thick, were prepared using a BTL applicator. The plates were air-dried for 4 h, stored for 12 h and then activated at 120° for 1 h. The plates were allowed to cool over granular silica gel before use. Solutions of the glucosides in rectified spirit (100 or 1000 μg/ml) were applied to the plates and the plates developed using one of the five solvent systems described below.

The plates were dried at 120° for 10 min and then sprayed with the detecting reagent. After 45 min at 125–130°, the plates were viewed with filtered UV light (Hanovia Fluorescent Lamp, Model 11). The glucosides were visible as light fluorescent spots against a violet background.

*Reagents.* The aryl β-D-glucosides were available from another study and will be reported on elsewhere. Solvents and other chemicals used in the chromatography and detection of the β-D-glucosides were of normal reagent grade and used without further purification. Diethyl ether was saturated with water before use.

*Developing solvents.* The following solvent systems were used: (I) dimethylformamide–chloroform (2:4.5); (II) *n*-butanol–methanol–chloroform (3:1:6); (III) *n*-butanol–ether (1:4.5); (IV) *n*-butanol–dichloromethane (1:2); (V) *n*-butanol–chloroform (1:1.5).

*Detecting reagent.* A solution of 15% (v/v) aniline in *n*-butanol (20 ml) was added to a solution (50 ml) of 30% (v/v) of concentrated phosphoric acid (88–90%, s.g. 1.75) in *n*-butanol. The mixture was shaken well in order to disperse the precipitate formed initially and the clear solution was used as the detecting reagent. If difficulty was experienced with obtaining a clear solution the reagent was filtered before use.

TABLE I

$R_F$ VALUES OF ARYL β-D-GLUCOSIDES

| *Glucoside* | *Solvent system* | | | | |
|---|---|---|---|---|---|
| | *I* | *II* | *III* | *IV* | *V* |
| Phenyl | 0.41 | 0.36 | 0.30 | 0.28 | 0.24 |
| 2-Chlorophenyl | 0.46 | 0.42 | 0.37 | 0.35 | 0.29 |
| 3-Chlorophenyl | 0.46 | 0.40 | 0.36 | 0.29 | 0.26 |
| 4-Chlorophenyl | 0.43 | 0.38 | 0.31 | 0.28 | 0.24 |
| 2,4-Dichlorophenyl | 0.47 | 0.44 | 0.38 | 0.34 | 0.30 |
| 2,6-Dichlorophenyl | 0.57 | 0.52 | 0.47 | 0.40 | 0.39 |
| 2,4,5-Trichlorophenyl | 0.52 | 0.49 | 0.51 | 0.39 | 0.35 |
| Pentachlorophenyl | 0.62 | 0.57 | 0.55 | 0.46 | 0.44 |
| 4-Tolyl | 0.45 | 0.39 | 0.29 | 0.30 | 0.27 |
| 4-Methoxyphenyl | 0.40 | 0.36 | 0.21 | 0.27 | 0.23 |
| 4-Cyanophenyl | 0.39 | 0.34 | 0.21 | 0.24 | 0.21 |
| 4-Nitrophenyl | 0.39 | 0.35 | 0.26 | 0.27 | 0.23 |
| 2-Hydroxyphenyl | 0.32 | 0.33 | 0.29 | 0.25 | 0.20 |
| 3-Hydroxyphenyl | 0.26 | 0.25 | 0.28 | 0.23 | 0.17 |
| 4-Hydroxyphenyl | 0.26 | 0.23 | 0.23 | 0.19 | 0.15 |
| Tetrachloro-4-hydroxyphenyl | 0.12 | 0.29 | 0.38 | 0.25 | 0.21 |

*Discussion*

The main part of our study was concerned with the formation of glucosides in plants treated with synthetic phenols. It was only necessary, therefore, to concern ourselves with chromatographic separation of the parent phenol from its glucoside and other metabolites and sugars which might occur in the treated plant. For this purpose developing solvents such as isopropanol–0.88 ammonia (9:1) and *n*-butanol–acetic acid–water (12:3:5) were adequate, however, the chromatographic separation of individual glucosides from each other with these systems was poor. This is not surprising in view of the dominant hydrophilic character of the glucose moiety. Some results of an investigation of some other solvent systems for improved separation are presented in Tables I and II.

Mixtures of simple alcohols or dimethylformamide with chloroform, dichloro-

TABLE II

$R_F$ VALUES OF β-D-THIOGLUCOSIDES

| *Thioglucoside* | *Solvent system* | | | | |
|---|---|---|---|---|---|
| | *I* | *II* | *III* | *IV* | *V* |
| Phenyl | 0.51 | 0.43 | 0.32 | 0.34 | 0.33 |
| 4-Tolyl | 0.54 | 0.47 | 0.36 | 0.37 | 0.35 |
| 4-Chlorophenyl | 0.52 | 0.44 | 0.38 | 0.36 | 0.33 |
| 4-Nitrophenyl | 0.48 | 0.41 | 0.33 | 0.36 | 0.30 |
| Ethoxythiocarbonyl | 0.53 | 0.46 | 0.41 | 0.39 | 0.34 |
| N-Methylthiocarbamoyl | 0.31 | 0.26 | 0.19 | 0.16 | 0.13 |
| N,N-Dimethylthiocarbamoyl | 0.42 | 0.35 | 0.16 | 0.23 | 0.19 |

methane or diethyl ether proved to be the most effective for partial resolution of the glucosides investigated. The resolving power of a solvent mixture improved with the use of alcohols of increased chain length; at the same time the mobility of the glucosides was depressed. The mobility of the glucosides was greatest in solvent systems based on diethyl ether as a component and least with chloroform. The $R_F$ values of the glucosides were depressed with increasing proportion of diethyl ether, dichloromethane or chloroform in the mixture with a slight improvement in resolution. On the other hand the addition of acetic acid or methanol enhanced the $R_F$ values of the glucosides but caused some deterioration in resolving power.

The reagent used for the detection of the glucosides differs from that of HIMES *et al.*[17] in so far that it contains a considerable excess of phosphoric acid. Reducing the proportion of the solution of concentrated phosphoric acid in *n*-butanol to 40 ml leads to the formation of a bulky precipitate; the precipitate can be removed by filtration and the filtrate used without loss of sensitivity. Larger reductions in the quantity of phosphoric acid in the spray not only lead to precipitate formation but to loss of sensitivity of the reagent towards glucosides, but not towards glucose. All glucosides were visible at the 0.1–0.2 $\mu$g level as fluorescent spots under filtered UV light whilst larger amounts (1.0–2.0 $\mu$g) of glucoside were visible as grey-brown spots on a white background under ordinary daylight conditions.

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1 R. S. ANDREWS AND J. B. PRIDHAM, *Nature*, 205 (1965) 1213.
2 A. HUTCHINSON, C. ROY AND G. H. N. TOWERS, *Nature*, 181 (1958) 841.
3 R. D. PALMER AND C. O. GROGAN, *Weeds*, 13 (1965) 219.
4 V. C. RUNECKLES AND K. WOOLRICH, *Phytochemistry*, 2 (1963) 1.
5 J. B. PRIDHAM, *Nature*, 182 (1958) 795.
6 J. B. PRIDHAM, *Phytochemistry*, 3 (1964) 493.
7 J. B. PRIDHAM AND M. J. SALTMARSH, *Biochem. J.*, 87 (1963) 218.
8 H. THIEME, *Pharmazie*, 19 (1964) 725.
9 A. WAGNER AND P. PFLEGEL, *Pharmazie*, 19 (1964) 638.
10 H. D. KLÄMBT, *Planta*, 57 (1961) 391.
11 H. D. KLÄMBT, *Nature*, 196 (1962) 491.
12 T. KOSUGE AND E. E. CONN, *J. Biol. Chem.*, 234 (1959) 2133.
13 C. R. SWANSON, R. E. KADUNCE, R. H. HODGSON AND D. S. FREAR, *Weeds*, 14 (1966) 319.
14 E. W. THOMAS, *Dr. Phil. Thesis*, Oxford, 1964.
15 G. H. N. TOWERS, A. HUTCHINSON AND W. A. ANDREAE, *Nature*, 181 (1958) 1535.
16 K. FREUDENBERG AND J. M. HARKIN, *Phytochemistry*, 2 (1963) 189.
17 J. B. HIMES, L. D. METCALFE AND H. RALSTON, *Anal. Chem.*, 33 (1961) 364.

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