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THE USE OF POLY-N-VINYLPYRROLIDONE AS THE ADSORBENT FOR THE CHROMATOGRAPHIC SEPARATION OF CHLOROGENIC ACIDS AND OTHER PHENOLIC COMPOUNDS

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SUMMARY

A method is described whereby poly-N-vinylpyrrolidone may be used as an adsorbent for thin-layer chromatography of phenolic compounds. The adsorbent forms hydrogen bonds with phenolic hydroxyl and carboxyl groups, the strength of the adsorbent–phenol binding depending primarily upon the number of these groups present in the molecule, and, for example, permits the separation of chlorogenic acids into the dicaffeoylquinic acids, the caffeoylquinic acids and the feruloylquinic acids. The possible use of this chromatographic system as a structure-diagnostic aid is discussed.

INTRODUCTION

Poly-N-vinylpyrrolidone (PVP) has been used for column and thin-layer chromatography (TLC) of flavonoids, phenolic acids and nucleotides[1–4]. It has been reported to form strong hydrogen bonds with the hydroxyl groups of phenolic compounds[5] and this property has been utilised to remove polyphenols that would otherwise interfere with carbohydrate quantitation[6].

While investigating the chlorogenic acids of green coffee beans, it was found necessary to separate this group of compounds into the feruloylquinic acids (monohydroxyphenols), the caffeoylquinic acids (dihydroxyphenols) and the dicaffeoylquinic acids (tetrahydroxyphenols), but a literature survey did not reveal a suitable chromatographic system. However, it was deduced from the report of Andersen and Somers[5] that at an acidic pH value the strength of a PVP–phenol bond ought to be determined by the number of phenolic hydroxyl groups in the molecule, and that with a suitable solvent system TLC on PVP layers could achieve the required separation of coffee bean chlorogenic acids. This paper describes the establishment of such a TLC system.

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EXPERIMENTAL

It should be noted that the detailed work by Quarmby[1] was not available when these preliminary investigations were carried out.

Materials

The following materials were used. Water-insoluble PVP supplied by GAF Ltd. (Manchester, Great Britain) under the trade name Polyclar AT; calcium sulphate ($CaSO_4 \cdot \frac{1}{2}H_2O$); solutions of the phenolic compounds listed in Table I (5 mg/ml) in 70% 2-propanol.

Preparation of PVP layers

The commercial PVP was passed through a BS 100-mesh sieve, and the coarse particles were discarded. The sieved material was used alone as the adsorbent, and after admixture with anhydrous calcium sulphate binder in the proportions (PVP: $CaSO_4$) 1:1, 2:1, 5:1 and 10:1. The PVP–calcium sulphate mixture was homogenised with distilled water until a smooth paste was obtained, the amount of water and time of homogenisation initially being determined by inspection. The slurry was spread in the conventional manner and the plates were allowed to dry for 15 min at room temperature (19–21°), followed by 30 min at 105°, and allowed to cool to room temperature.

The slurry of PVP alone was prone to settle out, was difficult to spread evenly and disintegrated completely on drying. The PVP–calcium sulphate (10:1) slurry was also unsatisfactory but the other three slurries spread easily and dried with a network of fine cracks on the surface. Since the PVP–calcium sulphate (5:1) layer gave a good solvent front and the most compact spots, it was prepared in the following manner for all subsequent investigations. Calcium sulphate (2.4 g) and PVP (12.0 g) were thoroughly mixed in the dry, 96 ml of distilled water added and the paste homogenised for 1–1½ min. This produced sufficient material to cover five 20 × 20 cm plates to a depth of 500 μm. The plates were developed at room temperature (19–21°) in glass tanks using the solvent system 2-butanone–methyl phenyl ketone–50% acetic acid (5:5:4, by vol.) (BMPKA), which has been reported[7] to give a good separation of several chlorogenic acids on silica gel. The solvent system was allowed to equilibrate with the atmosphere for at least 1 h prior to inserting the plate. A development to 15 cm required 75–80 min, and produced two solvent fronts separated by about 2 cm at this stage. The slower moving solvent front was used for the calculation of R_F values.

Locating reagents

The following reagents were used. Metaperiodate reagent 0.5% (ref. 8); molybdate reagent[9]; diazotised sulphanilic acid[10]; and acidic phloroglucinol reagent, 1% phloroglucinol in 12% hydrochloric acid[10,11].

RESULTS

In Table I are presented the R_F values (measured to the slower-moving solvent front) of 47 phenolic compounds. The ranges quoted are the extreme values taken from at least three separate chromatograms.

TABLE I

THE SEPARATION OF PHENOLIC COMPOUNDS BY TLC ON PVP LAYERS

Experimental conditions: layer, PVP-calcium sulphate (5:1), 500 μm thick; solvent, BMPKA; development, 15 cm at room temperature (19–21°). The R_F values are the extreme values taken from at least three chromatograms.

Compound	*Number of hydroxyl groups*	$R_F \times 100$
Class 1, phenols		
Guaiacol	1	88
Syringol	1	88–92
Catechol	2	54–57
Quinol	2	50–54
Resorcinol	2	38–42
Phloroglucinol	3	10–11
Pyrogallol	3	29–32
Class 2, benzaldehydes and cinnamaldehydes		
3-Methoxybenzaldehyde	0	98–100
4-Methoxybenzaldehyde	0	98–100
3,4-Dimethoxybenzaldehyde	0	98–100
3,4,5-Trimethoxybenzaldehyde	0	98–100
Cinnamaldehyde	0	95–98
2-Methoxycinnamaldehyde	0	95–98
2-Hydroxybenzaldehyde	1	67–73
3-Hydroxybenzaldehyde	1	65–68
4-Hydroxybenzaldehyde	1	65–68
3-Methoxy-4-hydroxybenzaldehyde	1	70–74
4-Methoxy-3-hydroxybenzaldehyde	1	68–72
3,5-Dimethoxy-4-hydroxybenzaldehyde	1	78–80
2,4-Dihydroxybenzaldehyde	2	55
3,4-Dihydroxybenzaldehyde	2	48–54
Class 3, amino acids		
Phenylalanine	0	42–46
Tyrosine	1	26–30
DOPA	2	15–19
Class 4, benzoic acids		
2-Hydroxybenzoic acid	1	52–56
3-Hydroxybenzoic acid	1	52–56
4-Hydroxybenzoic acid	1	52–56
3-Methoxy-4-hydroxybenzoic acid	1	58–60
3,5-Dimethoxy-4-hydroxybenzoic acid	1	57–62
2,3-Dihydroxybenzoic acid	2	31–35
2,4-Dihydroxybenzoic acid	2	28–32
2,5-Dihydroxybenzoic acid	2	37–40
2,6-Dihydroxybenzoic acid	2	31–35
3,4-Dihydroxybenzoic acid	2	33–35
3,5-Dihydroxybenzoic acid	2	28–32
3,4,5-Trihydroxybenzoic acid	3	10–12
Class 5, cinnamic acids and chlorogenic acids		
2-Hydroxycinnamic acid	1	46
3-Hydroxycinnamic acid	1	46
4-Hydroxycinnamic acid	1	46

(Continued on p. 264)

TABLE I *(continued)*

Compound	*Number of hydroxyl groups*	$R_F \times 100$
3-Methoxy-4-hydroxycinnamic acid	1	59
4-Methoxy-3-hydroxycinnamic acid	1	59
3,5-Dimethoxy-4-hydroxycinnamic acid	1	70
3-Feruloylquinic acid	1	44–46
3,4-Dihydroxycinnamic acid	2	25–27
3-Caffeoylquinic acid	2	20–22
5-Caffeoylquinic acid	2	16–18
3-Caffeoylquinic acid–potassium–caffeine complex	2	20–23
1,4-Dicaffeoylquinic acid	4	10

DISCUSSION

The PVP–calcium sulphate (5:1) mixture gave compact spots despite the crazed appearance which had been expected to cause considerable diffusion of the spots and disruption of the solvent fronts. Quarmby[1], who used a PVP–calcium sulphate (7:1) mixture, also reported a crazed surface and suggested that the cracks were superficial being partially filled with very fine PVP particles. However, using this layer Quarmby could not obtain a satisfactory separation of a synthetic mixture of flavonoid pigments because of spot disruption and diffusion. It is possible that this unsatisfactory separation was due to the choice of unsuitable solvent systems (ethanol–water–concentrated hydrochloric acid (6:3:1, by vol.); acetic acid–water–hydrochloric acid (30:10:3, by vol.); and 90% formic acid) rather than to an unsatisfactory layer, and there is evidence to show that the solvent system does have a marked effect on the separation obtained.

The R_F values presented in Table I show that the solvent system BMPKA separated the components within a particular class of phenol by the number of phenolic hydroxyl groups present, the greater the number, the lower the R_F value. However, none of the solvent systems used by Quarmby on a PVP–2½% polyvinyl alcohol (PVA) layer gave this type of separation —moreover, the order of separation depended upon the solvent system used. Using the PVP–PVA layer, Quarmby observed solvent demixing and reported that the solvent system acetic acid–water–concentrated hydrochloric acid carried certain compounds, *e.g.* chlorogenic acid, between the two solvent fronts. Although solvent demixing was observed with BMPKA, none of the 47 compounds examined ran between the two solvent fronts, and the various chlorogenic acids had $R_F \times 100$ values between 10 and 46 according to the number of phenolic hydroxyl groups present.

By using a carefully controlled two-dimensional technique, Quarmby reported satisfactory separation of the flavonoid aglycones of *Rhododendron* sp. and *Vaccinium myrtilus*, but Wrolstad[3], who replaced the PVA binder by soluble PVP, could not obtain a satisfactory separation of related glycosides. Even when one of the same solvents was employed (glacial acetic acid–water–concentrated hydrochloric acid (30:10:3, by vol.), Wrolstad reported irregular solvent fronts which spread the pigments in an irregular manner without any separation. However, Wrolstad was able to improve established chromatographic techniques for flavonoid pigments, obtaining

more compact spots, by incorporating insoluble PVP (10%) into cellulose layers. Compact spots were a noteworthy feature of the PVP–calcium sulphate/BMPKA system. To obtain a satisfactory separation using insoluble PVP, it is necessary to match carefully the properties of the solvent system and the adsorbent to the solubility characteristics of the compounds to be separated. The choice of such a compatible solvent system as BMPKA was almost entirely fortuitous.

Fig. 1 and the R_F values in Table I show that this chromatographic system gave the desired separation of chlorogenic acids and a similar separation of several other

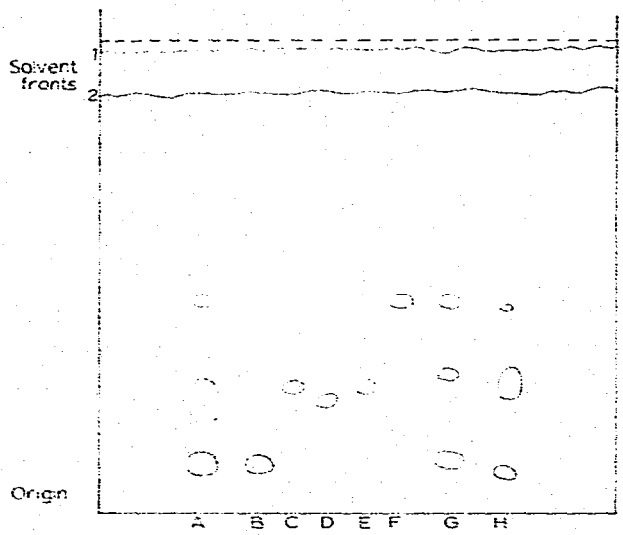

Fig. 1. Tracing of a thin-layer chromatogram of concentrated extracts of chlorogenic acids from green coffee beans and standard chlorogenic acids. Layer, PVP–calcium sulphate (5:1); solvent system, BMPKA; development, 15 cm at room temperature. A and H = Concentrated extracts of chlorogenic acids from green Robusta and green Arabica coffee beans, respectively; B = 1,4-dicaffeoylquinic acid; C = 3-caffeoylquinic acid; D = 5-caffeoylquinic acid; E = 3-caffeoylquinic acid–potassium–caffeine complex; F = 3-feruloylquinic acid; G = mixture of B, C and F.

types of phenolic compound. While it is clear that within a class the separation is dependent upon the number of phenolic hydroxyl groups present, it is also clear that individual classes do not behave identically. Comparison of the results for Classes 1 and 2 with the results for Class 4 shows that a carboxyl group retards the molecule by about the same amount as an extra hydroxyl group. The binding of carboxyl groups to PVP was also noted by Andersen and Somers[5]. The lower R_F values of the amino acids (Class 3) are doubtless due to lower solubility in the solvent system.

Because of the overlap between classes, *e.g.* tyrosine, $R_F \times 100 = 26–30$, and 3,4-dihydroxycinnamic acid, $R_F \times 100 = 25–27$, the use of this chromatographic system as a structure-diagnosing aid is limited by a requirement for prior knowledge of the class of phenol to which the unknown belongs. Although the use of ninhydrin, acidic phloroglucinol reagents[10,11] and dinitrophenylhydrazine allow easy characterisation of amino acids, aldehydes and ketones, it is not so easy to distinguish simple phenols from benzoic or cinnamic acids. Acid–base indicators are not entirely reliable

due to the difficulty of removing completely the acetic acid from the developed chromatogram. Even if the class of phenolic compound is known, this chromatographic system cannot immediately distinguish between, for example, *o*-, *m*-, and *p*-dihydroxyphenols, or between monohydroxyphenols and hydroxymethoxyphenols. The use of structure-specific locating reagents such as metaperiodate, molybdate and diazotised sulphanilic acid[8–10] are necessary to make such distinctions.

CONCLUSIONS

The chromatographic system PVP–calcium sulphate (5:1) and BMPKA separates the chlorogenic acids into dicaffeoylquinic, caffeoylquinic and feruloylquinic acid fractions.

This system, used in conjunction with structure-specific locating reagents may be used as a structure-diagnostic aid.

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