volt Densitometer equipped with an incandescent light source (without filter), range switch No. 3, and response No. 5.

This developing system has been used satisfactorily in this laboratory for the semi-quantitative measurement of the related foreign estrogens present in 17α-ethinylestradiol-3-methyl ether.

Preliminary experiments applying this system to estrogen level determinations in urine are promising, and will be reported later.

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1 J. C. TOUCHSTONE, T. MURAWEC AND O. BRUAL, *J. Chromatog.*, 37 (1968) 359.
2 S. HARA AND K. MIBE, *Chem. Pharm. Bull.*, 15 (1967) 1036.
3 B. P. LISBOA AND E. DICZFALUSY, *Acta Endocrinol.*, 40 (1962) 60.
4 L. L. SMITH AND T. FOELL, *J. Chromatog.*, 9 (1962) 339.
5 H. STRUCK, *Mikrochim. Acta*, (1961) 634.
6 D. C. TSILIFONIS AND L. CHAFETZ, *J. Pharm. Sci.*, 56 (1967) 625.
7 R. J. TEMPLETON, W. A. ARNETT AND I. M. JAKOVLJEVIC, *J. Pharm. Sci.*, 57 (1968) 1168.

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Analytical and preparative thin-layer chromatography of flavonol glycosides and their acylated derivatives from pea tissues

It has been shown in recent years that developmental changes are often, in many plants, accompanied or preceded by quantitative or qualitative changes in their flavonoid composition[1–5]. Progress along this front has to some extent been inhibited by the time-consuming nature of the methods available for the separation of the many flavonoid components commonly found in higher plant tissues. Such a situation exists in *Pisum sativum* var. Alaska in which four closely related flavonol compounds show complex and transitory changes associated with developmental events[1]. The methods previously used for this study involved either two-dimensional paper chromatography[6], or selective adsorption on columns of borate-impregnated silica gel[1]. Neither of these methods enabled reliable separation of all the components. This paper describes a one-dimensional thin-layer chromatographic method which can be used for rapid analytical and preparative separation of all four components and which may be of use in other plant systems.

The flavonoids present in green pea leaves are kaempferol-3-triglucoside (KG); kaempferol-3-*p*-coumaroyltriglucoside (KGC); quercetin-3-triglucoside (QG); and quercetin-3-*p*-coumaroyltriglucoside (QGC). The separation is achieved on 20 × 20 cm plates using 0.25–1.0 mm layers of Silica Gel G (Merck) impregnated with a complexing anion, air dried and activated at 110° for 10 min. The anions investigated were borate,

tungstate, molybdate, and acetate[7], and the separation achieved with each is shown in Table I. The most suitable solvent combination was found to be ethyl acetate–methyl ethyl ketone–water–formic acid (40:30:20:10). The preferred impregnating anion was tungstate as it resulted in the best separation and the most compact spots. Furthermore, this anion does not affect the U.V. absorption spectra by means of which the individual components were assayed after elution (Fig. 1). After development the plates were dried at 90° for 6 min and the zones detected under U.V. illumination.

TABLE I

THE SEPARATION OF PEA FLAVONOIDS ON 0.25 mm LAYERS OF SILICA GEL G IMPREGNATED WITH VARIOUS ANIONS

Silica Gel G was slurried with stated concentrations of the sodium salts of the impregnating agents before layering. The acetate impregnated plates were developed twice.

Impregnating agent		R_F *of pea flavonoids*			
		KGC	*QGC*	*KG*	*QG*
Borate	0.01 *M*	0.45	0.41	0.30	0.18
Molybdate	0.01 *M*	0.41	0.34	0.25	0.16
Tungstate	0.01 *M*	0.42	0.36	0.29	0.24
Acetate	0.3 *M*	0.40	0.34	0.26	0.15

Elution was achieved by scraping a zone into absolute methanol, acidifying with 6 *N* HCl, mixing thoroughly and centrifuging. Radioactive substances separated and eluted in this manner can be assayed by evaporating the eluate to dryness in a scintillation vial, taking up in 2 ml of methanol and adding 10 ml of a PPO–toluene scintillator. These methods have been utilised in an investigation of the changes in amount and rate of synthesis of these substances in relation to the effects of various light treatments[8].

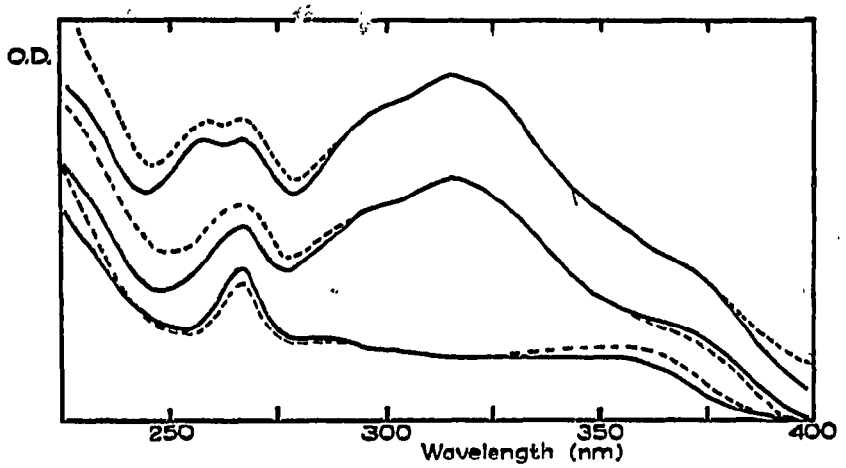

Fig. 1. The U.V. absorption spectra of the flavonoid complexes of pea plants. Upper curves, QGC; middle curves, KGC; lower curves. KG. Solid lines, pure compounds; broken lines, compounds extracted from plant tissue and eluted from silica gel plates. The concentrations were adjusted such that the pure compounds gave O.D. values at 315 nm identical to those of the extracted compounds.

In an attempt to obtain recovery data for this method, crystalline preparations of KG, KGC, and QGC were obtained by scaling-up the method. Plates were prepared as above with 1 mm layers impregnated with 0.01 *M* sodium tungstate. The flavonoids were extracted from 2 kg of green pea shoot tissue using the method of BOTTOMLEY *et al.*[1]. The final extract was strip loaded onto a total of 20 plates and the zones eluted, pooled and rechromatographed. The pooled extracts of the second separation were extracted successively into *n*-butanol, 0.1 *M* NH_4OH, and, after acidification, back into *n*-butanol. A final chromatographic separation yielded eluates of KG, KGC, and QGC which were taken into *n*-butanol and extracted with water to remove any tungstate. The butanolic eluates were then dried down giving micro-crystalline residues of KG, KGC, and QGC. The spectra of these pure compounds are given in Fig. 1 together with spectra of the same compounds separated by the analytical method outlined above.

Using the pure compounds, the recovery rates in the analytical method were as follows: KG, 96–99 %; KGC, 94–97 %; and QGC, 92–96 %. This method is considerably quicker than the previously used methods, and with strip loading it is possible to separate within 45 min, on one plate, sufficient of each component to allow for spectrophotometric determination. Furthermore, pre-purification by solvent extractions is not necessary, thus eliminating the use of alkaline conditions in which the quercetin compounds are unstable. The presence of chlorophylls, carotenoids and aromatic acids do not interfere since these substances all migrate with the solvent front. Sugars, on the other hand, remain on the baseline. The method appears to depend on the degree of hydroxylation of the flavonoid nucleus, and on the presence or absence of acyl and glycosidic residues, and therefore may be of general use in other tissues.

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1 W. BOTTOMLEY, H. SMITH AND A. W. GALSTON, *Phytochemistry*, 5 (1966) 117.
2 R. J. DOWNS AND H. W. SIEGELMAN, *Plant Physiol.*, 38 (1963) 25.
3 J. R. TROYER, *Plant Physiol.*, 39 (1964) 907.
4 H. A. STAFFORD, *Plant Physiol.*, 40 (1965) 130.
5 H. MOHR, *Planta*, 49 (1957) 389.
6 M. FURUYA AND R. G. THOMAS, *Plant Physiol.*, 39 (1964) 634.
7 J. H. HALMEKOSKI, *Suomen Kemistilehti*, 35 (1962) 39.
8 D. B. HARPER AND H. SMITH, *Biochim. Biophys. Acta*, submitted for publication.

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