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A method for the rapid chromatography of gibberellins

Thin-layer chromatography (TLC) has been used extensively since 1962 for the separation and purification of gibberellins[1–5]. Using the systems which have been described, separations are completed within 1½ to 2 h for a 15-cm solvent pathlength. This relatively rapid rate of separation results in a minimum of diffusion and excellent resolution. Gibberellins can be visualized on the chromatogram by spraying it with ethanolic sulfuric acid, heating for about 10 min and viewing the resulting fluorescent products under longwave UV light.

Although most gibberellins can be separated by adsorption chromatography, partition chromatography is necessary to separate mixtures of GA_1 and GA_3 and mixtures of GA_4 and GA_7. To accomplish these separations, chromatography plates are equilibrated overnight in the aqueous vapor of a two-phase solvent system and then developed in the organic phase.

The purpose of this report is to describe a system for the separation of gibberellins which offers the advantages of speed and convenience over conventional TLC. Excellent resolution and high sensitivity of detection characteristic of TLC are retained in this method. The chromatography medium used is a glass fiber paper impregnated with silica gel (ITLC Chromatography Medium, Gelman Instrument Co.).

The material can be cut and handled like paper. It is stable to the hot, acidic conditions required to induce fluorescence in gibberellins. Development is considerably faster than with conventional TLC, excellent resolution is obtained, and very small quantities of gibberellin can be detected. In addition, gibberellins can be recovered quantitatively for subsequent bioassay or chemical purposes.

Methods

ITLC chromatography medium is available in 20 × 20 cm sheets. These are maintained at 100° for at least 1 h to activate the silica gel. The sheets are cut to desired size, marked and spotted with sample in the usual manner employed for TLC, and developed by ascending adsorption or partition chromatography.

Adsorption chromatography is carried out in the usual manner. Sample is applied to the chromatogram and the sheet is placed in a chromatography tank containing a suitable volume of appropriate solvent.

Partition chromatography requires a 15-min equilibration in aqueous vapor before development in an organic solvent. The chromatography paper is sandwiched between two U-shaped polyethylene spacers about 2 mm in thickness (Fig. 1). These are in turn sandwiched between filter papers saturated with the aqueous phase of a two-phase solvent mixture. The chromatogram, spacers and filter papers are held between two glass plates and the complete assemblage is allowed to stand at least 15 min. The assemblage, still intact, is then placed into the developing solvent and the chromatogram is developed.

Visualization of gibberellins is effected generally in the same manner as with conventional TLC. The chromatogram is sprayed with ethanolic sulfuric acid (95:5) (ref. 4), heated at 100° for 3 min, and viewed under longwave UV light.

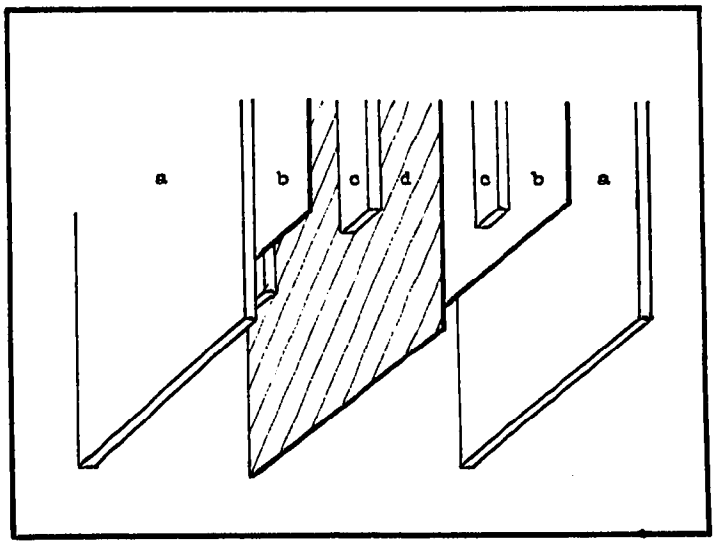

Fig. 1. Assemblage for equilibrating chromatograms prior to partition chromatography. (a) 5 × 20 cm glass plates; (b) 5 × 17 cm Whatman No. 1 filter paper, saturated with aqueous phase; (c) "U" shaped polyethylene spacer, *ca.* 2 mm thick, 5 × 17 cm outside dimensions; (d) glass fiber chromatography paper, 5 × 20 cm. Assemblage is held together with clips or rubber bands.

Results and discussion

The outstanding advantage of this system over TLC is in the speed of separation. Solvent pathlengths of 15 cm generally require 20 min or less for completion. The 15-min equilibration required for partition chromatography compares to overnight equilibration recommended for thin-layer systems[4].

Gibberellins have a greater mobility on this medium than they have on thin-layer chromatograms developed in the same solvent. Table I shows a comparison of R_F values for ten gibberellins developed on Silica Gel G plates in diisopropyl ether–acetic acid (95:5) with the R_F values obtained on silica gel-impregnated glass fiber paper developed in the same solvent mixture. Even the most polar gibberellin used in this study, GA_8, migrated approximately ten times as far on the glass fiber medium as on thin-layer plates and five of the ten gibberellins moved with the solvent front.

The R_F values of these gibberellins in additional solvent mixtures are shown in Table II. Solvents 1 and 2 are used for adsorption chromatography. Solvent 1 is a

TABLE I

COMPARISON OF GIBBERELLIN MOBILITY (R_F × 100) ON SILICA GEL-IMPREGNATED GLASS FIBER PAPER AND GLASS PLATE-SUPPORTED THIN LAYERS OF SILICA GEL G

Chromatograms were developed in diisopropyl ether–acetic acid (95:5).

	Gibberellin									
	A_1	A_2	A_3	A_4	A_5	A_7	A_8	A_9	A_{13}	A_{14}
Glass fiber	72	32	71	100	100	100	43	100	94	100
Glass plate	11[a]	4[a]	11[a]	37[a]	31[a]	37[a]	4[a]	75[a]	—	50

[a] From MACMILLAN AND SUTER[4].

TABLE II

MOBILITY ($R_F \times 100$) OF SEVERAL GIBBERELLINS ON SILICA GEL-IMPREGNATED GLASS FIBER PAPER DEVELOPED IN DIFFERENT SOLVENTS

Solvents: (1) benzene–acetic acid (95:5); (2) diisopropyl ether–acetic acid (95:5); (3) carbon tetrachloride–acetic acid–water (8:3:5); (4) chloroform–ethyl acetate–water–acetic acid (80:5:80:5); (5) chloroform–acetic acid–water (60:5:40). Solvents 3, 4 and 5 require 15-min equilibration in vapor of aqueous phase prior to development in organic phase.

Solvent	*Gibberellin*									
	A_1	A_2	A_3	A_4	A_5	A_7	A_8	A_9	A_{13}	A_{14}
1	19	12	20	70	61	72	5	97	50	78
2	72	32	71	100	100	100	43	100	94	100
3	0	0	0	70	47	60	0	100	24	82
4	39	0	30	85	100	67	9	100	20	70
5	63	—	77	—	—	—	43	100	—	98

good general purpose mixture for routine separations. Solvent 2 is useful for obtaining separation of relatively polar gibberellins.

Solvents 3, 4 and 5 are partition systems which require equilibration of the chromatogram before development. Solvent 3 is useful for the separation of GA_4 from GA_7; solvent 4 for the simultaneous separation of GA_4 from GA_7 and GA_1 from GA_3; and solvent 5 can be used for separating GA_1 from GA_3.

Figs. 2 and 3 illustrate examples of the practical uses to which this chromatography system can be put in the laboratory. Fig. 2 shows the results of a routine examination of the effluent from column chromatography purification of gibberellins.

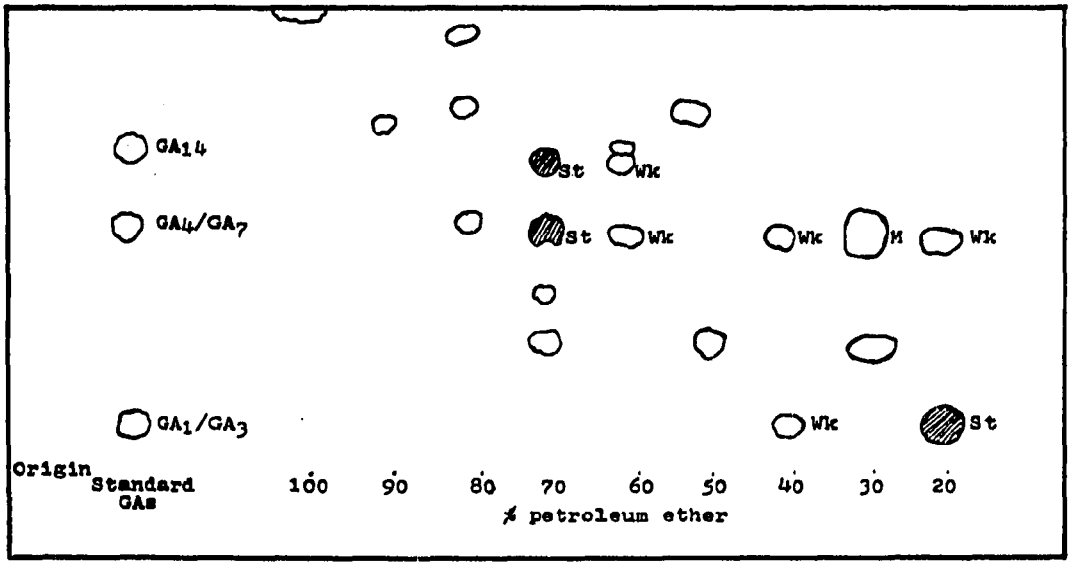

Fig. 2. Sample chromatogram showing fluorescence pattern obtained from effluent fractions of column chromatogram. Ethyl acetate-extractable organic acids from culture filtrates of *Gibberella fujikuroi* were chromatographed on a buffered Celite column[6] by stepwise elution with petroleum ether (60–110° boiling fraction) diluted with diethyl ether in 10% steps. The glass fiber paper chromatogram was developed in benzene–acetic acid (95:5). St = strong fluorescence; M = moderate fluorescence; Wk = weak fluorescence.

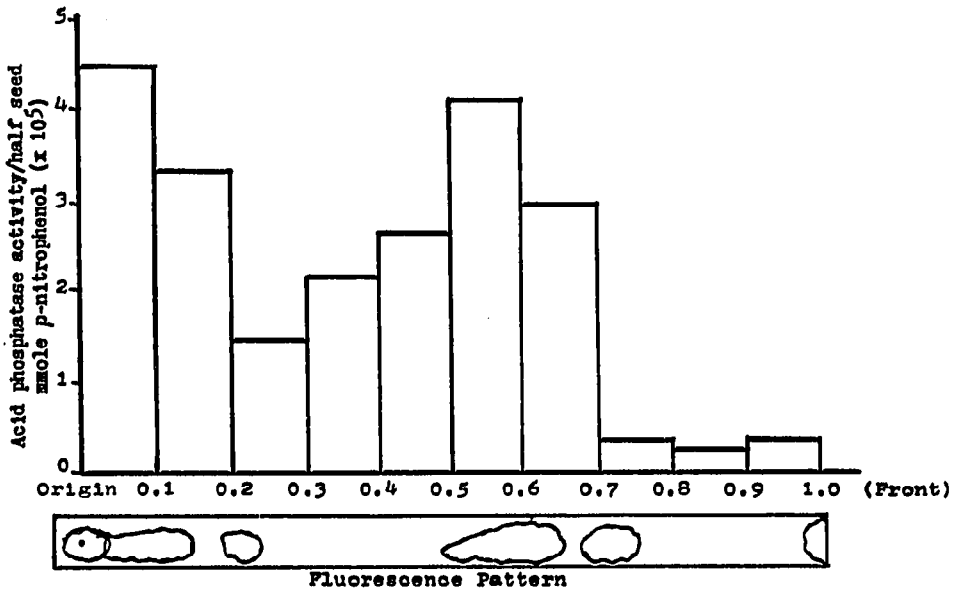

Fig. 3. Results of a bioassay for gibberellin-like substances eluted from a silica gel–glass fiber paper chromatogram. Ethyl acetate-extractable organic acids from culture filtrates of *Gibberella fujikuroi* were used as a source of gibberellin-like substances.

A buffered Celite column was loaded with organic acids produced by *Gibberella fujikuroi* and developed by stepwise elution with petroleum ether diluted with diethyl ether[6]. Portions of the effluent fractions were chromatographed on the glass fiber medium and the fluorescent pattern examined and recorded on this diagram.

Fig. 3 demonstrates how this material can be used for the purification of gibberellin-like materials in association with a bioassay. In this case, *Gibberella fujikuroi* was grown on a potato-dextrose broth medium and ethyl acetate-soluble organic acids were extracted from the culture filtrate[6]. A portion of the extract was chromatographed in solvent 1 and the chromatogram was cut into ten transverse strips, corresponding to ten R_F zones. A vertical strip was left on one side of the chromatogram and used for visualizing and locating gibberellin-like fluorescent materials. Each of the ten strips was washed with 10 ml acetone. The acetone was evaporated to dryness and a portion of the residue was tested in the barley endosperm acid phosphatase assay[7].

Two two major peaks of activity shown in Fig. 3 correspond in R_F values to GA_1/GA_3 and GA_4/GA_7. These are the most prevalent gibberellins in this extract when the fungus is grown under these conditions.

Subsequent examination of the strips of chromatographic material which had been washed with acetone revealed no fluorescent materials. Acetone appears to elute quantitatively gibberellin-like substances from this medium, without leaving an adsorbent residue in the eluate.

Conclusions

Silica gel-impregnated glass fiber paper is suitable for the purification of gibberellins and gibberellin-like materials. It can be used in connection with bioassays and for monitoring column effluents. It offers the advantages of speed and convenience over conventional TLC methods.

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1 G. W. ELSON, D. F. JONES, J. MACMILLAN AND P. J. SUTER, *Phytochemistry*, 3 (1964) 93.
2 D. F. JONES, J. MACMILLAN AND M. RADLEY, *Phytochemistry*, 2 (1963) 307.
3 T. KAGAWA, T. FUKINBARA AND Y. SUMIKI, *Agr. Biol. Chem. (Tokyo)*, 27 (1963) 598.
4 J. MACMILLAN AND P. J. SUTER, *Nature*, 197 (1963) 790.
5 G. SEMBDNER, R. GROSS AND K. SCHREIBER, *Experientia*, 18 (1962) 584.
6 K. C. JONES, C. A. WEST AND B. O. PHINNEY, *Phytochemistry*, 7 (1968) 283.
7 K. C. JONES, *Plant Physiol.*, 44 (1969) 1695.

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