unsere Technik die Möglichkeit, mehrere Proben nebeneinander auf derselben Platte zu untersuchen. Der Trockentransfer ist auch zum Nachweis dünnschichtchromatographischer Artefakte geeignet, wie z.B. teilweise Zersetzung der Komponente beim Chromatographieren.

Zentrale Forschung, J. R. Geigy A.G., Basel (Schweiz) GUSTAV SZÉKELY

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Quantitative *in situ* fluorometry of plant phenolic compounds on thin-layer plates

Most phenolic compounds, which are widely distributed in plants, exhibit a strong native fluorescence in U.V. light. Of the various analytical methods for plant phenolics[1], fluorometry[2] is considered 100–1000 times more sensitive than spectrophotometry. Many phenolic compounds, such as scopoletin[3], caffeic acid and umbelliferone, can be estimated at concentrations of 10^{-10} to 10^{-11} g/ml which makes fluorometry desirable for determining trace amounts of phenolics in small tissue samples. This report describes the technique, already in use in this laboratory, for quantitative direct fluorometry of some phenolic compounds, especially those present in *Hydrangea* tissues[4,5].

Experimental

The method for separation of phenolic compounds from crude plant extracts has been previously described[6]. Aliquots, 10–20 µl, were spotted quantitatively on TLC plates (20 × 20 cm) which were coated with 0.25 mm thick layers of a mixture of 1:1 (w/w) cellulose powder (Avicel, FMC Corp., Penn.) and Silica Gel G. The TLC plates were developed two-dimensionally using the organic phase of a mixture of benzene–glacial acetic acid–water (2:2:1), for 3 h, in the first direction and 2% aqueous acetic acid in the second direction. The developed plates were thoroughly dried and the fluorescent compounds were located under U.V. light (3600 Å) and lightly marked with pencil. Their location on a two-dimensional TLC plate is shown in Fig. 1.

Quantitative measurement of individual phenolic compounds was carried out with a Turner Filter Fluorometer, Model-111 (Palo Alto, Calif.). The theoretical details for the use of the Turner Fluorometer in scanning fluorescent spots on one-dimensional TLC plates have been described before[2]. For two-dimensional plates each fluorescent compound, already marked with pencil, was designated by the horizontal and vertical

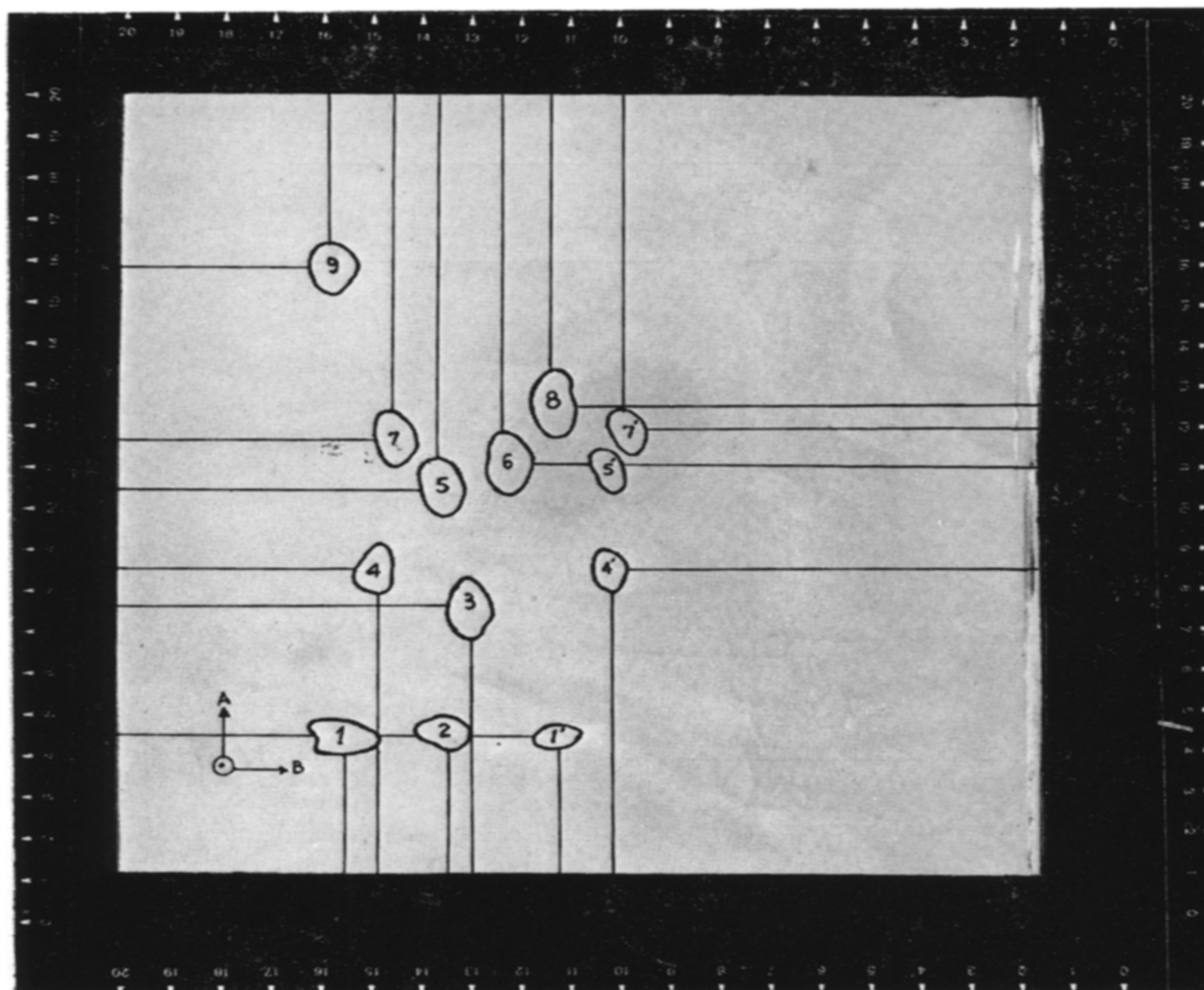

Fig. 1. Two-dimensional TLC of some phenolic compounds (TLC plate accommodated in plate holder). (A) First direction: benzene–acetic acid–water (2:2:1), (organic phase), (B) Second direction: 2% acetic acid. 1 = caffeic acid; 2 = esculetin; 3 = umbelliferone; 4 = *p*-coumaric acid; 5 = sinapic acid; 6 = scopoletin; 7 = ferulic acid; 8 = hydrangetin; 9 = hydrangenol.

coordinates of the plate holder (Fig. 1). The plate holder, which has a fixed scanning speed (20 mm/min), is adjustable in both vertical and horizontal directions; therefore each compound can be positioned opposite the scanning window of the fluorometer for determination of its relative fluorescence intensity. The scanning window has a fixed height (15 mm) and adjustable width (0–3.5 mm). The scanning direction was perpendicular to that of chromatography in the second dimension. The U.V. light source, the primary and secondary filters used with individual compounds and their quantities measurable at different range selector settings (1–30 ×) are given in Table I. In all determinations the instrument was zeroed on a blank area of the plate before scanning the compound of interest, and a uniform base-line was always observed.

Calibration curves of pure standard phenolic compounds were prepared by one-dimensional chromatography of 0.1–10 μg quantities of known compounds and determining their relative fluorescence intensities. These values were read directly

TABLE I

MEASURABLE QUANTITIES OF SOME PHENOLIC COMPOUNDS AT DIFFERENT SELECTOR SETTINGS

U.V. light source (Turner No.), primary and secondary filters (Corning No.): Compounds 1 and 2, lamp 110–850, filters 7 — 54 + 34A, filter 2A; compounds 3–9, lamp 110–855, filters 7 — 54 + 34A + 7 — 60, filter 2A.

No.	*Compound*	*Range selector setting*	*Quantity* (μg)	*Standard deviation* (%)
1	*p*-Coumaric acid	3 ×	0.1 –1	6.2
		10 ×	0.05–5	3.7
2	Hydrangenol[a]	10 ×	0.1 –1	not determined
		30 ×	0.2 –4	
3	Caffeic acid	3 ×	0.1 –1	3.8
		10 ×	0.05–0.6	2.6
4	Chlorogenic acid[b]	3 ×	0.5 –5	4.5
		10 ×	0.1 –1	2.9
5	Ferulic acid	3 ×	1 –10	not determined
		10 ×	0.1 –1	
6	Umbelliferone	1 ×	0.1 –1	3.7
		3 ×	0.05–0.4	2.5
7	Esculetin	1 ×	1 –10	not determined
		3 ×	0.1 –1	
8	Scopoletin	3 ×	1 –10	not determined
		10 ×	0.1 –2	
9	Hydrangetin[c]	3 ×	0.1 –1	5.6
		10 ×	0.05–0.2	4.8

[a] 3,4′-Dihydroxyphenyl-isocoumarin[4].
[b] Chromatographed in *n*-butanol–acetic acid–water (4:1:2).
[c] 7-Hydroxy-8-methoxycoumarin[5].

on the fluorescence dial of the instrument or from the recorded peaks on the chart of the recorder. In few cases the accuracy of the calibration curves was tested by assaying chromatographed aliquots of known concentrations, previously determined spectrophotometrically. The standard deviation ranged from 2.5–6.2% (Table I). TLC on mixed layers of cellulose and Silica Gel G combines the advantages of both

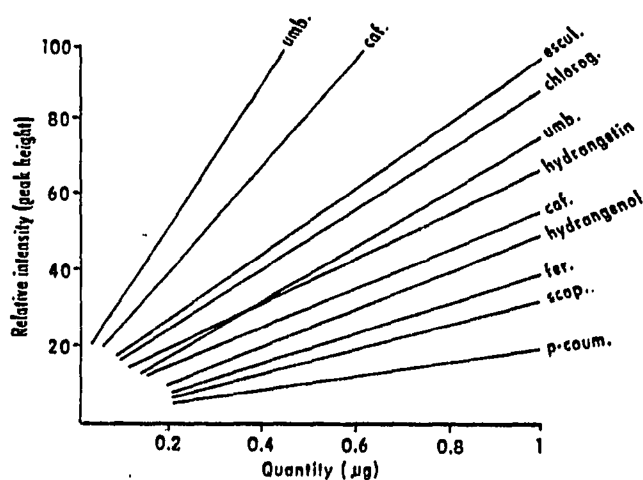

Fig. 2. Calibration curves of fluorescence intensities of some phenolic compounds (for range selector settings, see Table I).

substances and results in good resolution of compact spots. No calculation of the areas under the recorded peaks was necessary since the latter were quite sharp and proportional to sample quantity as shown by the linear correlations in Fig. 2.

Apart from the extreme sensitivity and the relative rapid method of direct fluorometry, there are other advantages: (a) it excludes elution of compounds, which usually results in some quantitative loss, (b) it can be used with other fluorescent phenolic compounds, especially flavones and flavonols, and (c) it is most useful in subsequent specific activity determinations of radioactive metabolites by eliminating solvent quenching problems, which are otherwise unavoidable, during liquid scintillation counting.

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Department of Biology,
Sir George Williams University, Montreal 107, Que. (Canada)

R. K. IBRAHIM

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