*Journal of Chromatography*, 92 (1974) 179–181


CHROM. 7308

## Note

# Thin-layer chromatography of 6,7-substituted coumarins

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(Received November 6th, 1973)

Following our investigations into the coumarins of the Umbelliferae[1-5], it was necessary to find a method by which 6,7-substituted coumarins could be separated and identified. We chose thin-layer chromatography (TLC), which has been applied with success to the separation of furanocoumarins. The TLC separation of some 6,7-substituted coumarins has been described (*e.g.* refs. 6–8), but no systematic investigation of the chromatographic conditions necessary for the separation of the compounds of interest was known to the authors.

The present paper describes a method by which the seven compounds shown in Table I can be separated and identified.

The reference compounds umbelliferon, coumarin, aesculetin and scopoletin were commercially available. Herniarin was synthesized by methylating umbelliferon with dimethyl sulphate in the presence of potassium iodide. Isoscopoletin was synthesized from aesculin by the procedure of Seka and Kallir[9] (m.p. 177°, corr.).

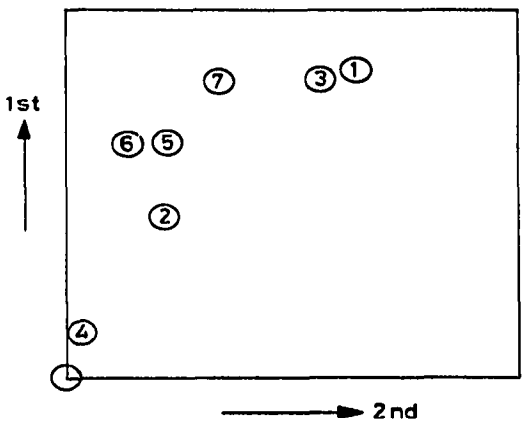
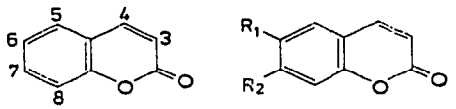



Fig. 1. Two-dimensional chromatogram of the coumarin derivatives. 1 = Coumarin; 2 = umbelliferon; 3 = herniarin; 4 = aesculetin; 5 = isoscopoletin; 6 = scopoletin; 7 = scoparon.

TABLE I

$R_F$ VALUES AND SPOT COLOURS OF 6,7-SUBSTITUTED COUMARINS



| No. | Coumarin derivative | | | $R_F$ values × 100 | | Colour of fluorescence | | |
|---|---|---|---|---|---|---|---|---|
| | Name | $R_1$ | $R_2$ | Solvent 1 (twice) | Solvent 2 | UV light (254 nm) | UV light (254 nm) + NaOH | Pauly's reagent |
| 1 | Coumarin | H | H | 86 | 61 | – | green | yellow |
| 2 | Umbelliferon | H | OH | 39 | 20 | blue | green-blue | orange |
| 3 | Herniarin | H | $OCH_3$ | 81 | 54 | violet | green-blue | orange |
| 4 | Aesculetin | OH | OH | 11 | 2 | blue-green | green | orange |
| 5 | Isoscopoletin | $OCH_3$ | OH | 61 | 20 | blue | pink | pink |
| 6 | Scopoletin | OH | $OCH_3$ | 60 | 12 | blue | green-blue | orange |
| 7 | Scoparon | $OCH_3$ | $OCH_3$ | 81 | 31 | violet | blue | – (pink) |

Scoparon was made according to Gattermann[10]. The identities of the compounds were checked by NMR spectroscopy.

The following chromatographic conditions gave a complete separation of the seven compounds.

A 35-g amount of Kieselgel H (Merck, Darmstadt, G.F.R.) was mixed with 70 ml of distilled water and the slurry was applied to 20×20 cm glass plates with a Desaga spreading device. The thickness of the layers was 0.3 mm. The coated plates were air-dried for 20 min at room temperature and were then activated in a drying oven for 1 h at 110 °. The samples of reference compounds were applied in chloroform and methanol solutions. The chromatograms were first developed twice in toluene–acetone (95:5) (Solvent 1). They were then air-dried and further developed in Solvent 2 (the organic layer of chloroform–acetic acid–water (4:1:1) after thorough mixing). After drying, the spots were observed in UV light (254 nm) before and after spraying with 0.5% potassium hydroxide in methanol. In addition the colours of the spots on the chromatograms after spraying with Pauly's reagent were used in the identification[11,12]. The results obtained are shown in Fig. 1 and Table I.

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