

Paper chromatography of quercetagenin derivatives

In the course of our investigations of *Vitex agnus castus* seeds, it was found that they contain a flavonoid pigment casticin (5,3'-dihydroxy-3,6,7,4'-tetramethoxy-flavone)¹. It became desirable to follow the progress of the purification of casticin and to compare its R_F values with those of other known flavonoids for the purpose of identification. In most solvent systems that are successfully used with flavonoid pigments², casticin was found to travel near the front and to show considerable tailing. Therefore, for the paper chromatography of casticin, solvent systems such as are used in the steroid field and many other fields³, were applied.

Filter paper (Whatman No. 1) was dipped into a solution of formamide or dimethylformamide in acetone (50 % v/v), the excess of solvent removed by blotting between sheets of filter paper and the samples, ranging in quantities from 1 to 30 γ applied in the form of solutions in acetone. The chromatogram was developed by the descending technique, until the mobile phase had travelled about 30 cm. Mixtures of benzene and decalin, saturated with formamide, or decalin saturated with dimethylformamide, were used as mobile phase. Subsequently the chromatogram was dried in a stream of hot air until the mobile phase had evaporated. Visualisation was achieved by conventional methods as for example by examining the paper in U.V. light or by spraying it with a diazotised solution of sulphanilic acid⁴.

The results, given in Table I, show that satisfactory chromatograms can be obtained with formamide-benzene-decalin, especially if the ratio of benzene-decalin is adjusted to suit the specific requirements. Artemetin and diethylcasticin cannot be separated since their R_F values are too close together. However, the difference between the R_F values obtained with the dimethylformamide-decalin system is large

TABLE I
 R_F VALUES OF QUERCETAGENIN DERIVATIVES

	R_F values in			Colour in U.V. light
	Formamide/ benzene-decalin (1:1)	Formamide/ benzene-decalin (4:1)	Dimethyl- formamide/decalin	
Casticin (5,3'-dihydroxy- 3,6,7,4'-tetramethoxy-flavone)	0.26	0.40	0.0	dark brown
Artemetin (5-hydroxy- 3,6,7,3',4'-pentamethoxy-flavone)	0.81	0.83	0.12	dark brown
Diacetylcasticin (5,3'-diacetoxy- 3,6,7,4'-tetramethoxy-flavone)	0.65	0.72	0.0	blue fluorescence
Hexamethylquercetagenin (3,5,6,7,3',4'-hexamethoxy-flavone)	0.52	0.72	0.0	blue fluorescence
Diethylcasticin (5,3'-diethoxy- 3,6,7,4'-tetramethoxy-flavone)	0.79	0.83	0.22	blue fluorescence

enough to allow distinct separation. Yet this system has the disadvantage that it causes tailing of the above-mentioned substances.

By substituting petroleum ether for decalin in the solvent system formamide-benzene-decalin, the method can be utilised in large scale work. The separation of artemetin and casticin on a column of cellulose powder was carried out to test the method.

Cellulose powder (200 g, Whatman standard grade) was impregnated with formamide (66 g) in acetone (150 ml). Then the acetone was evaporated at 50° under reduced pressure. The cellulose was pressed into a column 4 × 28 cm. The mixture of artemetin (185 mg) and casticin (198 mg), dissolved in a small amount of the mobile solvent, was dropped on the top of the column and the mobile solvent (benzene and 20% v/v petroleum ether 30–40°, saturated with formamide) allowed to pass through the column at the rate of 2 ml/1 min. Ten ml portions were collected and examined by paper chromatography. Fractions 1–7 contained artemetin and fractions 10–20 contained casticin. In fractions 8 and 9 neither flavone could be detected. Fractions 1–7, and 10–20, respectively, were combined, and the solvent evaporated. Of artemetin 190 mg and of casticin 200 mg were recovered. After one crystallisation from benzene-petroleum ether both compounds had the original melting points.

*Institut za kemijo, Medicinska fakulteta,
Ljubljana (Yugoslavia)*

I. BELIČ
J. BERGANT-DOLAR

¹ I. BELIČ, J. BERGANT-DOLAR AND R. A. MORTON, *J. Chem. Soc.*, in the press.

² J. B. HARBORNE, *J. Chromatog.*, 2 (1959) 581.

³ I. M. HAIS AND K. MACEK, *Handbuch der Papierchromatographie*, Band I, VEB Gustav Fischer Verlag, Jena, 1958, pp. 234, 354.

⁴ I. M. HAIS AND K. MACEK, *ibid.*, p. 328.

Received January 23rd, 1961

J. Chromatog., 5 (1961) 455–456

Behaviour of the metals of the 1b group on zirconium phosphate columns and papers

Recent work has revived interest in inorganic ion exchangers and their application¹. Of these, zirconium phosphate was selected for our purpose because of its properties and high capacity. It was prepared according to the method described in the literature².

Columns were made from 6 mm diam. glass tubes constricted at one end and filled to a height of about 40 mm with zirconium phosphate (ZP). Its capacity and distribution coefficients were as previously recorded (capacity = 1.88 mequiv./g).

The zirconium phosphate papers were prepared as follows: Whatman No. 1 paper was impregnated with a solution of 0.2 M $ZrOCl_2 \cdot 8H_2O$ in 4 N HCl, the excess solution drained off and the paper dried at room temperature. These papers were then

J. Chromatog., 5 (1961) 456–457