contrasting colour reaction is stable for approximately 48 hr., and may be registered simply by direct photography.

I. Dragúnová
P. Langer

Institute of Endocrinology,
Slovak Academy of Sciences,
Bratislava, Czechoslovakia.
April 19.

[1] Bowden, C. H., Maclagan, N. F., and Wilkinson, J. H., *Biochem. J.*, **59**, 93 (1955).
[2] Fletcher, K., and Stanley, P. G., *Nature*, **175**, 730 (1955).

Detection of Triterpenoid Glycosides on Paper Chromatograms

In the course of our investigations of *Echinocystis lobata* seeds, it became desirable to locate triterpenoid glycosides on paper chromatograms by means of a colour reaction. A modification of the Liebermann–Burchard test described by Neher and Wettstein[1] was unsatisfactory for this purpose. We found that the original Liebermann–Burchard reaction applied to paper chromatograms with comparatively little alterations gave more satisfactory results, permitting a reliable detection of the spots.

The procedure is as follows. The dried paper chromatogram is placed on a glass plate and sprayed with a mixture of equal volumes of chloroform and acetic acid anhydride. A thin layer of concentrated sulphuric acid is spread on a glass plate and the treated filter paper strip laid on it. Additional sulphuric acid is smeared on the top of the strip with a glass rod. After a few minutes, the triterpenoid glycosides develop red-coloured spots. The starting line and the front, as well as the spots, can be marked on the other side of the glass plate in order to obtain the R_F values. This has to be done before the paper is destroyed by the sulphuric acid. Using Whatman No. 1 paper, after 15 min. the destruction is so far completed as to prevent the precise measurement of distances. The sensitivity of the test can be increased by examination of the chromatogram in ultra-violet light. The spots show orange fluorescence. In Table 1 are collected results, observed with pure echinocystic acid glycoside, isolated from *Echinocystis lobata* seeds. Similar results are obtained with methanolic extracts from the seeds.

The presence of the usual plant constituents (for example, oligosaccharides and amino-acids) does not interfere with the reaction. This method has been applied to the detection of triterpenoid compounds of several plants known to contain triterpenes[3]. Ethanolic extracts (70 per cent v/v) were chromatographed with butanol/acetic acid[2] and the chromatograms treated as described above. The results are shown in Table 2.

Table 1. Paper Chromatography of Echinocystic Acid Glycoside

Solvent	R_F	Sensitivity in visible light	Sensitivity in ultra-violet light	Paper
Butanol/acetic acid according to Partridge (ref. 2); descending	0·36	10γ	1γ	Whatman No. 1
Ethyl acetate with 0·08 addition of 1·5 per cent acetic acid and 2 per cent methanol, saturated with water until slight cloudiness appears; ascending	0·08	10γ	1γ	Whatman No. 1

Table 2. Paper Chromatography of Extracts from Triterpene-containing Plants

Plants	Solvent	R_F of glycoside	Paper
Callendula off.	Butanol/acetic acid according to Partridge (ref. 2)	0·8	Whatman No. 1
Arnica montana		no definite spot*	
Tussilago farfara		0·18 and 0·65	

* A coloured trail along the strip from the start to the front.

I. Belič

Chemical Department,
Medical Faculty,
University of Ljubljana,
Yugoslavia.
Feb. 11.

[1] Neher, R., and Wettstein, A., *Helv. Chim. Acta*, **34**, 2283 (1951).
[2] Partridge, S. M., *Biochem. J.*, **42**, 238 (1948).
[3] Brieskorn, C. H., *Planta Medica*, **2**, 35 (1954).

Separation of Pyridine Mono-Carboxylic Acids on Paper Chromatograms

Chemical methods using cyanogen bromide and aromatic amines[1–3] (König reaction) and an acid-indicator[4] for the detection of pyridine carboxylic acids on paper chromatograms have been reported. But the toxic character of cyanogen bromide causes difficulty; and it has been observed that good spots with acid-indicator are often not produced, particularly when the acid concentration is very low. A very convenient method has been found for detecting these acids on the filter paper by spraying with a solution containing cupric ions followed by benzidine; also their efficient separation from the mixture has been achieved.

In our experiments α-, β- and γ-pyridine carboxylic acids were used individually or as a mixture. The paper (Whatman No. 1; 15 cm. × 24 cm.) after development (ascending technique) for 4–6 hr. at 30 ± 0·5° C. with the solvent (as described in Table 1) was completely dried by a current of hot air. It was next sprayed with a 0·2 per cent solution of copper sulphate (anhydrous) in a water–ethanol mixture (5 : 4, by vol.) and dried carefully in an oven kept at about 60° C. The dried paper, on spraying with a 0·1 per cent solution of benzidine in 50 per cent ethanol, revealed well-defined bluish spots against a light-brown background. 10–15 μgm. of each component acid could be separated and detected on the chromatogram.

Copper salts, for example, sulphate, chloride, acetate, etc., have been employed as detection reagents; but copper sulphate has given the most satisfactory result. Butanol with a mobile phase acidified with acetic acid gives the best separation and also raises the respective R_F values when compared to butanol alkalized with ammonia[1]. The other solvent systems studied produced high R_F values but contributed little towards resolution. Better results are given when the chamber is maintained fully saturated with respect to the solvent system.

Table 1. R_F Values of Pyridine Carboxylic Acids

Substance	Solvent			
	n-Butanol/acetic acid/water (4 : 1 : 1)	*n*-Butanol saturated with 1·5 *N* ammonia	*n*-Propanol conc. ammonia water (20 : 1 : 4)	Phenol saturated with water
Picolinic acid	0·63	0·15	0·57	0·56
Nicotinic acid	0·89	0·21	0·61	0·88
*iso*Nicotinic acid	0·76	0·22	0·67	0·90