Thin-layer chromatography of pentacyclic triterpenes

Lately, thin-layer chromatography has been increasingly employed for the separation and identification of tetra- and pentacyclic triterpenes which occur widely in the plant kingdom. In the course of our work on the isolation and characterization of the chemical constituents of different plants, we frequently encountered mixtures of certain typical members of the different groups of pentacyclic triterpene alcohols such as α-amyrin, β-amyrin, lupeol, taraxerol etc., and their corresponding acetates. Triterpene acids such as oleanolic acid, ursolic acid etc., are also of common occurrence. It was often found necessary to identify extremely small amounts of such compounds by TLC.

HUNECK[1] and BERGMANN *et al.*[2] carried out the separation of a large number of tetra- and pentacyclic triterpenes by TLC over aluminium oxide and Alumina G, respectively. Similar studies were carried out by TSCHESCHE *et al*[3]. using Silica Gel G as adsorbent, but in many instances a good resolution was not possible. We also failed to distinguish between a number of triterpenes (see (a–c) below) by TLC over both Silica Gel G as well as Aluminium Oxide G using a number of different solvent systems, including those employed by the previous workers (*loc.cit.*).

(a) α-Amyrin, β-amyrin, lupeol, taraxerol, baurenol, and multiflorenol.

(b) α-Amyrin acetate, β-amyrin acetate, lupeol acetate, taraxerol acetate and baurenol acetate.

(c) Oleanolic acid and ursolic acid.

SUKH DEV AND GUPTA[4] have demonstrated the resolution of certain olefinic sesquiterpenoids by TLC over Silica Gel G impregnated with silver nitrate. In a recent communication[5], TLC over Silica Gel G impregnated with ammoniacal silver nitrate solution has been shown to effect a better resolution than that over Silica Gel G impregnated with silver nitrate, and following this method we were able to separate *cis* and *trans* isomers of certain α,β-unsaturated acids[6]. These methods were, therefore, employed to distinguish between the individual members of each group of compounds mentioned above and these attempts were largely successful. The Tables I–III give the R_F values of the compounds in each group together with the solvent systems employed in each case.

TABLE I

R_F VALUES OF COMPOUNDS IN GROUP (a)
Solvent A: Benzene–chloroform (1:1, v/v).
Solvent B: Benzene–chloroform (2:1, v/v).

Name of compound	R_F *values*			
	Silver nitrate impregnated plates		*Ammoniacal silver nitrate impregnated plates*	
	Solvent A	*Solvent B*	*Solvent A*	*Solvent B*
α-Amyrin	0.67	0.46	0.54	0.38
β-Amyrin	0.62	0.44	0.50	0.36
Lupeol	0.43	0.31	0.39	0.28
Taraxerol	0.57	0.39	0.45	0.33
Baurenol	0.60	0.42	0.48	0.35
Multiflorenol	0.63	0.44	0.51	0.37

TABLE II

R_F VALUES OF COMPOUNDS IN GROUP (b)
Solvent A_1: Benzene–light petroleum (b.p. 60–80°) (2:3, v/v).
Solvent B_1: Benzene–light petroleum (b.p. 60–80°)–chloroform (25:25:1, v/v).

Name of compound	*R_F values*			
	Silver nitrate impregnated plates		*Ammoniacal silver nitrate impregnated plates*	
	Solvent A_1	*Solvent B_1*	*Solvent A_1*	*Solvent B_1*
α-Amyrin acetate	0.72	0.96	0.49	0.85
β-Amyrin acetate	0.68	0.91	0.47	0.82
Lupeol acetate	0.29	0.58	0.26	0.56
Taraxerol acetate	0.59	0.84	0.41	0.77
Baurenol acetate	0.62	0.89	0.43	0.80

TABLE III

R_F VALUES OF COMPOUNDS IN GROUP (c)
Solvent: Benzene–chloroform–methanol (7.5:4:1, v/v).

Name of compound	*R_F values*	
	Silver nitrate impregnated plates	*Ammoniacal silver nitrate impregnated plates*
Oleanolic acid	0.33	0.22
Ursolic acid	0.33	0.28

It will be seen from Tables I and II that these triterpenes and their acetates exhibit the same pattern of resolution. In the case of neutral compounds, TLC over Silica Gel G impregnated with silver nitrate appears to effect a somewhat better resolution than that over Silica Gel G impregnated with ammoniacal silver nitrate, whereas in the case of acidic compounds like oleanolic acid and ursolic acid TLC over silver nitrate impregnated Silica Gel G does not effect any resolution, but some distinction could be made between the two by TLC over Silica Gel G impregnated with ammoniacal silver nitrate. No separation was however achieved in the case of methyl oleanolate and methyl ursolate.

Experimental

Silver nitrate impregnated plates were prepared in the following manner: A slurry of Silica Gel G (E. Merck, 3.0 g) in aq. silver nitrate solution (12.5 %, 3.60 ml) was applied to a glass plate (10 × 20 cm) with an improvised spreader (layer thickness 0.35 mm). The plates were air-dried for 15 min and then activated by heating in an air oven at 100–110° for 45 min–1 h.

Ammoniacal silver nitrate impregnated plates were prepared as follows: A slurry of Silica Gel G (E. Merck) in ammoniacal silver nitrate solution (made by adding 28–29 % ammonia solution to a 5 % aq. silver nitrate solution, drop by drop, till the precipitate first formed completely dissolved), prepared as above, was similarly applied to glass plates and the plates were then air-dried for 15 min and activated by heating

in an air oven at 100–110° for 30–40 min. The compounds in chloroform solution (100–200 μg) were spotted on the glass plates in the usual way.

The solvent was allowed to run up to a height of 14–15 cm from the base line. The plates were then air-dried, sprayed with a solution of acetic anhydride (5 ml) and conc. sulphuric acid (5 ml) in absolute ethanol (90 ml) and heated in an air oven at 130–140° for 15 min. Pink to reddish spots were observed for all these compounds. All these operations were carried out in an air-conditioned room at a temperature of 22–24°.

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Direct fluorimetry of phenolic compounds on thin-layer chromatograms

During work on the phenolic content of parasitised apple leaf tissue, the need arose for quantitative estimations directly after thin-layer chromatography of plant extracts. The method had to be rapid and sensitive to cope with the instability of the compounds and the need to avoid processing large amounts of plant material.

Apparatus

Fluorescence measurements were done on a Chromoscan (Joyce-Loebl Ltd., Middlesex). The fluorescence of compounds (under 365 mμ light) separated on thin-layer chromatograms was enhanced by enclosing them with ammonia vapour in glass cuvettes. Cuvettes were constructed to the dimensions given in Fig. 1, the back plate being 3 mm glass separated by 5 mm glass rod from the window, which consisted of 1.5 mm photographic plate glass. Joints were rendered firm by frosting opposing faces with carborundum powder prior to applying Araldite adhesive (Ciba Ltd., Cambridge). Small lugs were attached to each end of the upper edges of the cuvettes to engage in