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Note

Thin-layer chromatography of lantadene A and some related triterpenoids

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Lantadene A (22β-angeloyloxyoleanolic acid), a triterpenoid compound, is the toxic principle of *Lantana camara*[1,2]. No satisfactory thin-layer chromatographic (TLC) procedure is available to separate this compound from other related constituents of plants, *viz.*, α-amyrin, β-amyrin, oleanolic acid, ursolic acid and their derivatives[3,4]. During our studies on the metabolism of lantadene A in animals a need arose to separate it from cholesterol also. In this article we report TLC procedures suitable for the resolution of α-amyrin, β-amyrin, oleanolic acid, ursolic acid, their methyl and acetate derivatives, lantadene A and cholesterol.

EXPERIMENTAL

Silica gel G was obtained from BDH, Bombay, India. The solvents were of analytical grade and freshly distilled before use. α-Amyrin, β-amyrin, oleanolic acid, ursolic acid, α-amyrin acetate, β-amyrin acetate, oleanolic acid methyl ester, ursolic acid methyl ester, oleanolic acid methyl ester acetate and ursolic acid methyl ester acetate were kindly supplied by Dr. S. K. Nigam, National Botanical Research Institute, Lucknow, India. Lantadene A was prepared according to Barton *et al.*[5]. Cholesterol was purchased from E. Merck, Darmstadt, G.F.R.

The thin-layer plates (200 × 200 mm) were coated with silica gel G to a thickness of 0.2 mm and air-dried. Just before use, the plates were activated at 110°C for 1 h. Solutions of terpenoids and cholesterol (0.2–0.3%) were prepared in boiling ethanol and 50 μl of each solution was applied on the plate. Seven different solvent systems were used:

(I) Benzene–diethyl ether (80:20)
(II) Benzene–methanol–ethyl acetate (119:14:7)
(III) Light petroleum (b.p. 60–80°C)–benzene–ethyl acetate–acetic acid (40:80:28:2)
(IV) Light petroleum–diethyl ether–acetic acid (90:10:1)
(V) Diisopropyl ether–acetone (75:30)
(VI) Chloroform–methanol (93:7)
(VII) Ethyl acetate–*n*-hexane (30:20)

The chromatograms were developed at room temperature (≈ 20°C), air-dried and the spots detected by: (1) exposure of the plates to iodine vapour in an iodine-

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TABLE I

THIN-LAYER CHROMATOGRAPHY OF SOME TRITERPENOIDS

Compound	R_F *value*					*Detection*	
	I	*II*	*III*	*IV*	*V*	*Liebermann–Burchard reagent*	*Chlorosulphonic acid–acetic acid*
α-Amyrin	0.57	0.77	0.69	0.20	0.92	Pink	Pink
β-Amyrin	0.57	0.77	0.69	0.20	0.92	Pink	Pink
α-Amyrin acetate	0.91	0.96	0.93	0.54	0.93	Brown*	Pink
β-Amyrin acetate	0.91	0.93	0.92	0.54	0.92	Brown*	Pink
Oleanolic acid	0.14	0.40	0.43	0.10	0.43	Pink	Violet
Ursolic acid	0.14	0.38	0.43	0.10	0.43	Blue	Violet
Oleanolic acid methyl ester	0.48	0.66	0.62	0.14	0.62	Pink	Pink
Ursolic acid methyl ester	0.52	0.66	0.60	0.17	0.60	Pink	Pink
Oleanolic acid methyl ester acetate	0.91	0.93	0.90	0.32	0.90	Pink	Pink
Ursolic acid methyl ester acetate	0.91	0.95	0.90	0.32	0.90	Pink	Pink
Lantadene A	0.38	0.51	0.42	0.13	0.42	**	Violet
Cholesterol	0.46	0.64	0.52	0.15	0.52	Blue	Violet

* Spot appeared after keeping at 100°C for 10 min.

** A brown spot appeared on keeping at 100°C for 20 min.

saturated chamber for 10 min, yielding yellow spots; (2) spraying with vanillin–sulphuric acid–ethanol [0.5 g vanillin in 100 ml sulphuric acid–ethanol (40:10)], followed by heating the sprayed plates at 120°C for 10 min[6]; (3) spraying with acetic anhydride–sulphuric acid (Liebermann–Burchard reagent; 10 ml acetic anhydride mixed with 90 ml sulphuric acid with cooling), followed by heating the sprayed plates at 100°C for 5 min; (4) spraying with chlorosulphonic acid–acetic acid (1:3) spray, followed by heating the plates at 120°C for 10 min[7], giving pink or violet spots.

RESULTS

The R_F values in the different solvents systems and detection of eleven terpenoids and cholesterol are shown in Table I. None of the solvent systems described is suitable for the separation of α- and β-amyrin. The acetates of these compounds could just be resolved in solvents II, III, V and VII. Oleanolic acid and ursolic acid had the same R_F values in solvents I, III, IV, V and VI. However, they could be discerned in systems II and VII. Methyl esters of oleanolic acid and ursolic acid had different R_F values in solvents I, III, IV and V, and the esters and acetates of these acids could be separated in systems II and VII. All the solvent systems except IV and VI are suitable for the separation of lantadene A from cholesterol and the triterpenoids studied. The most satisfactory solvent system for the separation of α-amyrin, β-amyrin, oleanolic acid and ursolic acid from their methyl and acetate derivatives, lantadene A and cholesterol was II, benzene–methanol–ethyl acetate (119:14:7). All the compounds studied could be detected by iodine vapours, vanillin–sulphuric acid–ethanol or chlorosulphonic acid sprays. Lantadene A did not give any colour with Liebermann–Burchard reagent presumably because of the absence of a 3-β-hydroxyl group[8].

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