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Short Communication

Thin-layer chromatographic separations of lantadenes, the pentacyclic triterpenoids from lantana (*Lantana camara*) plant

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ABSTRACT

Seven solvent systems of varying suitability are reported for the thin-layer chromatographic separation of lantadenes isolated from the heptatotoxic plant *Lantana camara* var. *aculeata*. Hexane–methanol–ethyl acetate (85:10:5) was found to be the most suitable for the separation of lantadene A, B, C, D, reduced lantadene A and reduced lantadene B. Lantadenes could be detected on thin layers of silica gel G using Liebermann–Burchard reagent, vanillin–sulphuric acid and primulin sprays.

INTRODUCTION

Lantana (*Lantana camara*) triterpenoids constitute a group of pentacyclic compounds called lantadenes, some of which have been shown to elicit hepatotoxicity in animals [1]. In the course of investigations on the purification and characterization of these compounds [2,3], a need arose to develop thin-layer chromatographic (TLC) procedures for the selection of liquid chromatographic systems and monitoring of column effluents, crystallization and purity. Lantadene A (LA), lantadene B (LB), lantadene C (LC), lantadene D (LD), reduced lantadene A (RLA) and reduced lantadene B (RLB) are the lantana triterpenoids which are of biological significance [1]. Previously, we have reported some solvent systems for the resolution of lantadene A from a number of other pentacyclic compounds [4]. Lantadenes differ from the latter compounds in having a side-chain at C-22 (Fig. 1). None of the systems individually was sufficient for the resolution of all the lantadenes, which have very similar polarities (Fig. 1). Here, we report the evaluation of various solvent systems, using silica gel G thin layers, for the resolution of putative hepatotoxins and related compounds from the lantana plant.

EXPERIMENTAL

Silica gel G was obtained from Sisco Research Labs. (Bombay, India). The solvents were of analytical-reagent grade and were freshly distilled before use. Lantadene A, B, C and D, reduced lantadene A and reduced lantadene B were prepared from *Lantana camara* var. *aculeata* (Red Flower variety) as described previously [2,3,5]. The identity of the compounds was confirmed by comparison with authentic standards and spectroscopic analysis. Cho-

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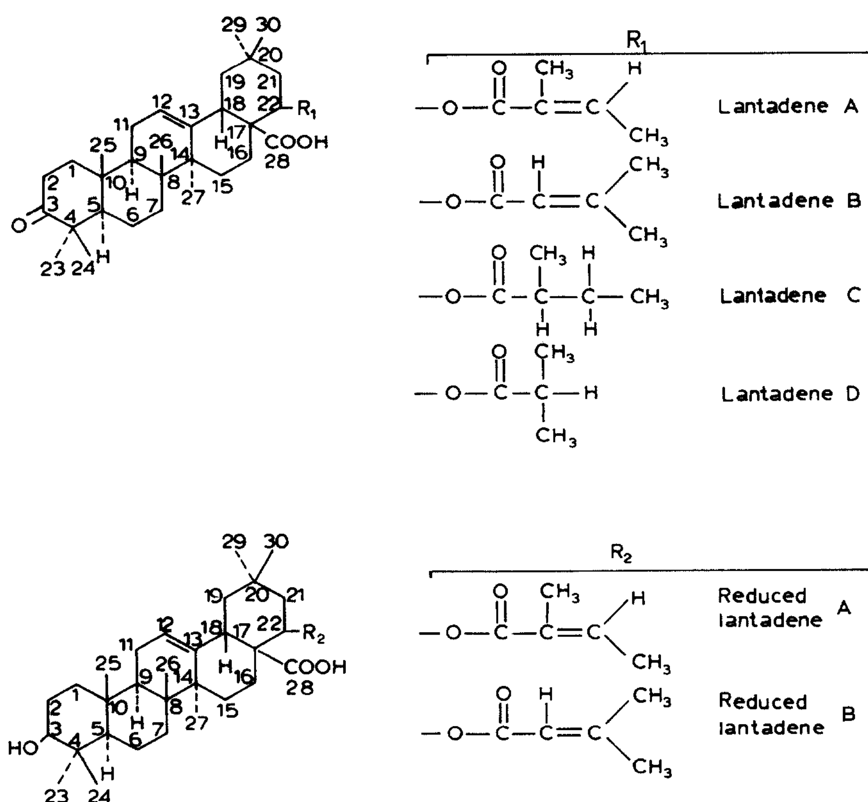

Fig. 1. Structures of lantadenes.

lesterol was purchased from E. Merck (Darmstadt, Germany) and primulin from Sigma (St. Louis, MO, USA).

Thin-layer plates (200 × 200 mm) were coated with silica gel G to a thickness of 0.2 mm, air dried and activated at 110°C for 1 h [6]. Solutions of lantadenes and cholesterol (4 mg/ml) were prepared in chloroform–methanol (2:1) and 10-μl aliquots of each solution were applied to the plate. The following solvent systems were used: (I) light petroleum (b.p. 60–80°C)–ethyl acetate–acetic acid (88:10:2); (II) benzene–methanol–ethyl acetate (85:10:5); (III) chloroform–methanol (97:3); (IV) diisopropyl ether–acetone (70:30); (V) light petroleum (b.p. 60–80°C)–benzene–ethyl acetate–acetic acid (40:80: 28:2); (VI) benzene–diethyl ether (70:30); and (VII) hexane–methanol–ethyl acetate (85:10:5).

The chromatograms were developed at room temperature (ca. 25°C) in 250 × 250 × 120 mm glass tanks, saturated using Whatman filter-paper [6], and air dried. The spots were detected as follows.

(a) Exposure of the developed plates to iodine vapour in an iodine-saturated chamber for *ca.* 10 min yielded yellow spots.

(b) Liebermann–Burchard spray reagent (LBR) was prepared by mixing 90 ml of acetic anhydride with 10 ml of sulpuric acid with cooling [7,8]. The sprayed plates were heated at 110°C and observed after 5 and 10 min for the appearance of spots. The plates were examined at 366 nm using a UV cabinet. Spots for 3-hydroxylantadenes (RLA and RLB) and cholesterol appeared within 5 min; they were purple and reddish purple and gave golden and pinkish yellow fluorescence, respectively, at 366 nm. The sports for 3-keto compounds, *viz.*, LA, LB, LC and LD, appeared after heating for 10 min and were brownish purple.

(c) Vanillin–sulphuric acid spray reagent was prepared by dissolving 0.5 g of vanillin in 100 ml of sulphuric acid–ethanol (40:10) [6]. The sprayed plates were heated at 120°C, observed after 5 and 10 min and examined at 366 nm using a UV cabinet. The rate of appearance of spots for the different compounds and their colours were the same as with LBR.

(d) A stock solution of primulin was prepared by dissolving 0.1 g of primulin in 100 ml of water. To prepare the spray reagent, 1 ml of stock solution was diluted with 100 ml of acetone–water (4:1). Immediately after spraying, the wet chromatograms were observed at 366 nm in a UV cabinet [9]. All the lantadenes and cholesterol gave pale blue fluorescent spots.

RESULTS AND DISCUSSION

The R_F values of different compounds are known to vary considerably with various factors such as layer thickness, chamber saturation, air humidity and source of adsorbents [6]. Hence it is advisable to use hR_{st} values relative to some suitable reference compound of comparable structure [6]. The hR_{st} values for the lantadenes in relation to cholesterol as a reference compound are given in Table I.

All the solvent systems except V were suitable for the resolution of LA and LB. LA, LB and LC had the same hR_{st} value in solvent system V. Solvent systems I, V and VII gave very compact spots and thus the resolution was very sharp. Solvent system II also gave fairly compact spots but less so than those obtained with I, V and VII. The separations of LA and LC and of LB and LD were difficult. Only solvent systems VI and VII resolved LA and LC, VII being the most suitable. LB and LD could be separated in solvent systems IV, V, VI and VII. Like LA and LB, RLA and RLB could be separated in all the solvent systems except V. Two other solvents systems, light petroleum (b.p. 60–80°C)–ethyl acetate (60:40) and hexane–ethyl acetate (60:40), were fairly useful for the separation of LA and LB and of RLA and RLB, but they had limited utility for the resolution of other combinations of lantadenes. Amongst all the solvent systems, only VII was suitable for the separation of all the lantadenes investigated. The only limitation of this system was that the differences in the hR_{st} values of LA and LC and of RLA and RLB were very small (Table I). This problem could be overcome by applying "long-run" TLC [10] using 400 × 200 mm plates. This was more advantageous than "multiple-development" TLC recommended by some workers for difficult separations [6].

All the solvent systems used were suitable for the

TABLE I

hR_{st} VALUES FOR LANTADENES USING CHOLESTEROL AS A REFERENCE COMPOUND

The hR_{st} value for cholesterol was taken as 100. The R_F values for cholesterol in the different solvent systems were: I, 0.48; II, 0.82; III, 0.88; IV, 0.89; V, 0.55; VI, 0.70; and VII, 0.79.

Compound	hR_{st}						
	I	II	III	IV	V	VI	VII
Lantadene A (22β-angeloyloxy-3-oxoolean-12-en-28-oic acid)	99	84	60	78	109	51	30
Lantadene B (22β-dimethylacryloyloxy-3-oxoolean-12-en-28-oic acid)	85	74	48	70	109	46	21
Lantadene C (22β-(*S*)-2′-methylbutanoyloxy-3-oxoolean-12-en-28-oic acid)	99	84	60	78	109	50	33
Lantadene D (22β-isobutyroyloxy-3-oxoolean-12-en-28-oic acid)	85	74	48	58	102	43	25
Reduced lantadene A (22β-angeloyloxy-3β-hydroxyolean-12-en-28-oic acid)	69	68	43	78	95	43	19
Reduced lantadene B (22β-dimethylacryloyloxy-3β-hydroxy-12-en-28-oic acid)	63	58	32	70	95	36	16

separation of RLA and RLB from cholesterol. The difference in the hR_{st} values of LA, LC and cholesterol was only marginal in system I. Similarly, the difference in hR_{st} values of LD and cholesterol was very small in system V (Table I). The order of appearance of coloured spots using both LBR and vanillin–sulphuric acid sprays was: cholesterol > 3-hydroxylantadenes > 3-ketolantadenes. These spray reagents gave spots of different colours with 3-hydroxy- and 3-ketolantadenes. In addition, the spots for 3-hydroxylantadenes gave a golden fluorescence at 366 nm, which further facilitated their differential identification. The primulin spray gave the same type of spots (pale blue) with all the lantadenes and cholesterol. However, this detection system had the advantage of being non-destructive [8,9] and hence was suitable for preparative TLC.

All the lantadenes and cholesterol could be detected at levels down 2 μg using primulin and vanillin–sulphuric acid spray. The detection limit for all the compounds using LBR was 5 μg. Detection using iodine vapour was comparatively less sensitive. 3-Ketolantadenes could be detected at levels down to 5 μg, but the detection of 3-hydroxylantadenes and cholesterol required nearly 10-μg applications on the plates.

The reported TLC procedures would be useful in tackling different separation problems in the preparation of lantadenes and related compounds from the lantana plant. They would also find use in investigations on the metabolism and disposition of lantadenes in animal systems.

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