ANTHRONE AS A REAGENT FOR THE DETECTION OF GLYCOSIDES AND SAPONINS BY THIN LAYER CHROMATOGRAPHY

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ABSTRACT

Forensic toxicologists often come across poisonous plant species used for purposes of homicide, suicide or criminal abortion. The toxicity of such plant material is attributed to compounds such as alkaloids, terpenoids, lignans, glycosides etc. Anthrone in concentrated sulphuric acid is used as a screening reagent for thin layer chromatography, to locate only glycosides amongst other natural products.

INTRODUCTION

This laboratory has received a few cases with history of deaths due to ingestion of poisonous plant material in various forms. Some of the plants used for criminal purposes such as (vernacular names in parenthesis), Oleander (White kaner), Cerbera Thevetia (Pila kaner), Calatropis Gigentea (Madar), Antiaris toxicarea etc owe their toxicity to steriodal glycosides like Cerberin, Calatropin (oral lethal dose 0.12 mg/kg), Ouabain (oral lethal dose 0.26 mg/kg), α-antiarin (i.v. lethal dose 0.116 mg/kg)[1]. Most of these glycosides have very low fatal doses. Many other natural products like tubers of tapioca Manihot esculenta Crantz (Sakar kanda), seeds of stonefruit jetberry bush and toyon contain cyanogenic glycosides[2,3]. In India, there are over 700 poisonous plant species belonging to over 90 botanical families[4], many of which are misused for purposes like homicide, suicide or abortion. Other plants used for criminal purposes attribute their toxicity to non-glycosidal compounds such as alkaloids, terpenoids, lignans etc. It will be advantageous, therefore, to have a simple thin layer chromatographic (TLC) screening system to detect glycosides amongst the plant poisonous constituents. The reagents reported[5–10] for identification of glycosides are based on reaction product exhibiting fluorescence under UV like steroids, lignans etc. It is rather difficult to differentiate glycosides from vegetable fats (as they also exhibit fluorescence) as well as from free steroids of the plants. Since, in glycosides/saponins, the hydroxyl group on the carbon is combined with one or more sugars via ether linkage[11] (figure 1), it was thought worthwhile to develop a reagent based on reaction with carbohydrate moiety, that will give coloured spots with glycosides. Anthrone (9–10-dihydro-8-oxoanthracene) in concentrated sulphuric acid, when sprayed on developed TLC plates, exhibits green or greenish-blue spots for different glycosides and saponins.

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EXPERIMENTAL

TLC plates were prepared using silica gel G (TLC grade) as adsorbent layer (thickness 250 μm) and activated at 110°C for 30 min.

The glycosides were isolated from plant material by Stass Otto process[12] followed by acid cholroform extraction and evaporated at room temperature (27–32°C).

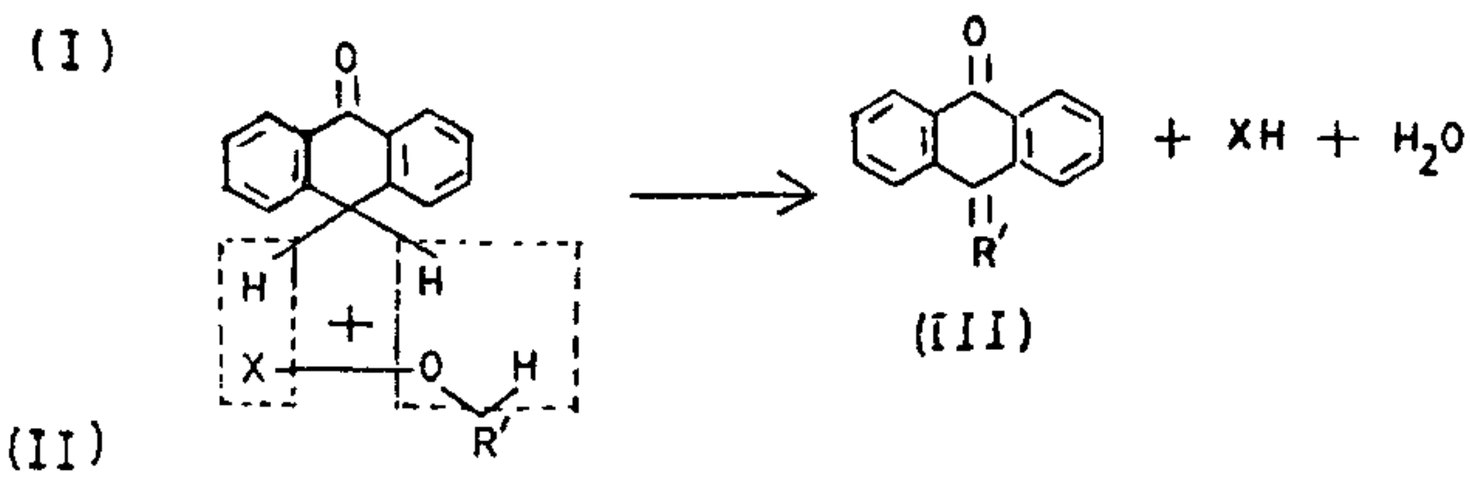
The anthrone spray reagent was prepared by dissolving 2 g anthrone (BDH) in 100 ml concentrated sulphuric acid (AR grade).

PROCEDURE

After spotting the extracted plant material, the TLC plate was developed in the solvent system hexane:acetone (7:3) according to the standard TLC procedure. After drying the solvent, the plate was sprayed with 2% w/v anthrone reagent.

RESULTS AND DISCUSSION

Results are shown in tables 1 and 2 (using different solvent systems). Green or greenish-blue spots appered on the TLC plate after spraying the reagent. The brown spots may be due to resins and other plant ingredients. Sensitivity of the reagent was 50 ng. The probable reaction can be presented as



(I) Anthrone (II) X—O(R′)H is glycoside . (III) Reaction product. X-Aglycone moiety. R′– Carbohydrate residue.

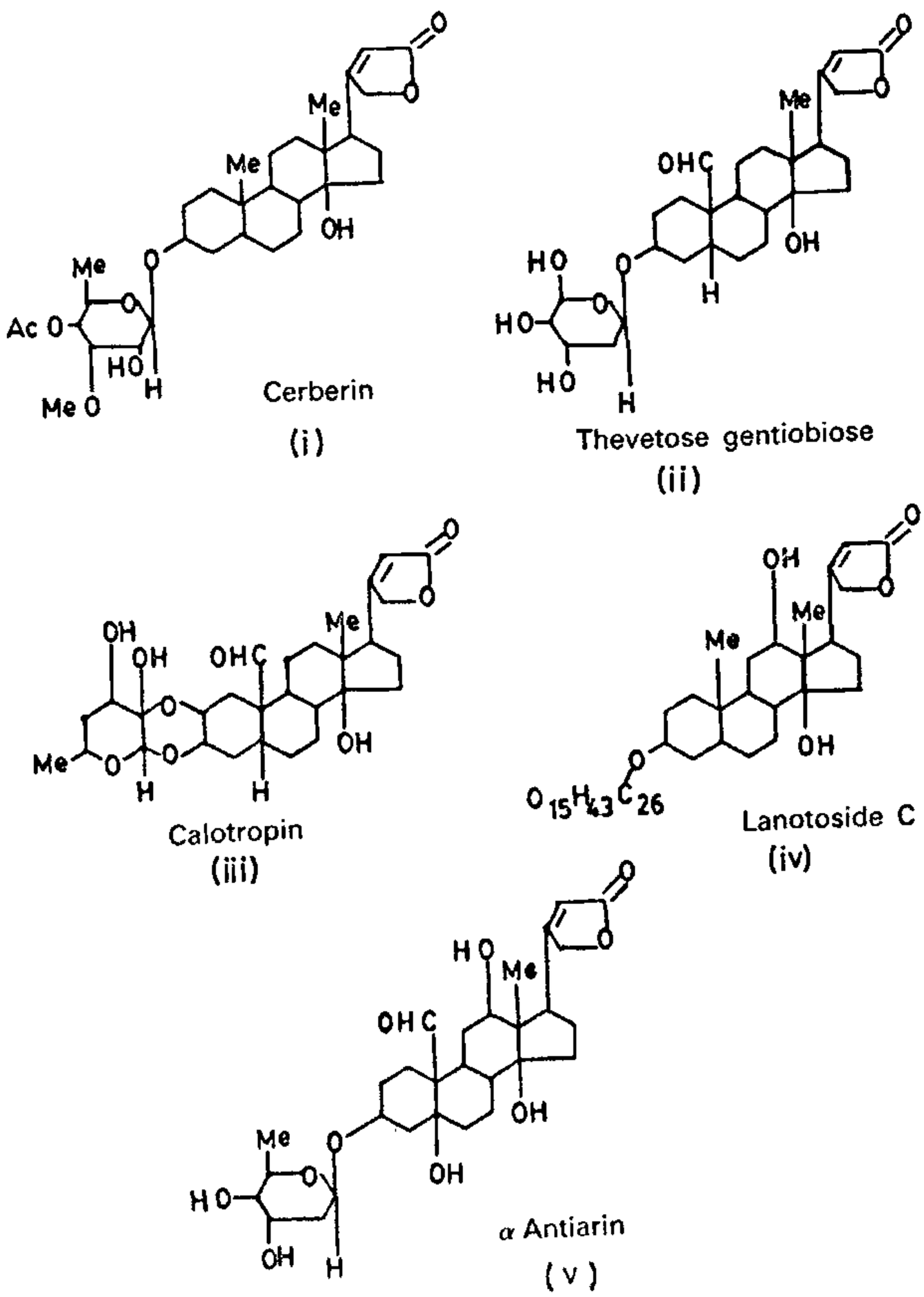


Figure 1.

Table 1 *TLC results using hexane: acetone solving system*

Sample Botanical name (Vernacular name)	Colour of the spot	R_f	Relative intensity	Compounds so far reported
Cerberin Thevetia (Pila Kaner)	a) Green	0.10	(s)	
	b) Green	0.25	(s)	
	c) Blue-Green	0.43	(vs)	Cerberin[1,14]
	d) Dark-brown	0.53	(s)	Thevetin[1,15]
	e) Dark-brown	0.58	(s)	Thevetoxin[15]
	f) Blue-green	0.66	(vs)	(All glycosides)
	g) Dark-brown	0.73	(s)	
	h) Violet	0.88	(ms)	
Calatropis Gigentea (Madar)	a) Blue-green	0.14	(vs)	
	b) Orange	0.34	(mf)	Uscharin[16,17]
	c) Orange	0 51	(f)	Calotoxin[16,17]
	d) Dark-brown	0 62	(s)	Calactin[16]
	e) Brown	0.71	(f)	Calotropin[1,17]
	f) Blue-green	0.77	(vs)	Gigantin[18]
	g) Violet	0.97	(m)	
Nerium Odorum Apocynaceae (White Kaner)	a) Blue-green	0 34	(vs)	
	b) Blue-green	0.50	(vs)	Nerin[14]
	c) Pink	0.54	(f)	Neriodorin[20]
	d) Blue	0.57	(s)	Neriodorein[20]
	d) Blue-green	0.61	(vs)	Karabin[20]
	f) Violet	0.70	(ms)	(All glycosides)

vs = very strong > s = strong > ms = medium strong > m = medium mf = medium faint > f = faint.

Table 2 *TLC results using butanol: ethyl acetate: formic acid solvent system*

Sample Botanical name (Vernacular Name)	Colour of the spot	R_f	Compound reported
Strychnos Nux Vomica Linn. (Kuchla)	Green	0.39	Loganin
Croton	Green	0 41	Crotonside
Cycus Circinalish	Yellow-Green	0.47	Cycasus
Digitonin (Pure crystals)	Green	0.15	
Saponin (Crystaline)	Green	0.19	Saponin glycosides[18].

This reagent is reported for colorimetric determination of sugars[13]. Since glycosides are isolated from plant material by acid chloroform extraction, the possibility of other soluble carbohydrates interfering with anthrone reagent is elminated.

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