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NOTES

A Novel Spray Reagent for the Detection of Carcinogens on Thin Layer Chromatography

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The paper describes the use of a sensitive spray reagent tribromoacetic acid to detect steroids on thin layer plates.

A number of spray reagents for the detection of steroids on Silica Gel G thin layer plates are known[1]. Acetic anhydride-sulphuric acid detects Δ^5-3-sterols and a number of steroids and triterpene glycosides. Antimony (III) chloride acetic acid detects steroids in addition to diterpenes. Other spray reagents such as ceric sulphate-sulphuric acid, chlorosulphonic acid-acetic acid, cinnamaldehyde-acetic anhydride-sulphuric acid, phosphoric acid, trifluoro-acetic acid, CIBA, vanillin-phosphoric acid and zinc chloride are used for detecting steroids. *Meta*-dinitrobenzene is used only for detecting 17-keto steroids, 4-hydroxy benzaldehyde-sulphuric acid for 3-keto steroids unsubstituted in the 2-position, isonicotinic acid hydrazide for Δ^4-3-ketosteroids, *p*-phenylene diamine phthalic acid for 3-keto steroids conjugates, picric acid-perchloric acid for Δ^5-3-β hydroxy steroids, resorcyl aldehyde-sulphuric acid for 16-dehydro steroids and sodium hydroxide for Δ^4-3-keto steroids. Molybdophosphoric acid is used only for reducing steroids while methylene blue detects only sulphate esters. Sulphosalicylic acid, picryl sulphonic acid, dimethyl sulphoxide, carbazole-sulphuric acid, anisaldehyde-sulphuric acid are used for detecting steroids and triterpenes[2–5]. Chloramine-T-sulphuric acid, mercury (II) iodide and rubeanic acid are used to detect steroids[6–8]. This paper describes the use of another sensitive spray reagent, tribromoacetic acid to detect steroids on thin layer plates.

A number of 20 × 20 cm glass plates were coated with a silica gel *G* slurry which was prepared by mixing thoroughly one part by mass of silica gel *G* with two parts by volume of water, spreading being carried out by the method of Davidek and Prochazka[9]. After drying in air, the plates were stored and baked in an oven for 45 minutes at 110°C when required for use. The mixture of benzene-ethyl acetate (10 : 1 v/v) was used as solvent system I in a chamber in which the atmosphere was saturated. Similarly a mixture of benzene-ethyl acetate (5 : 1 v/v) was used as the solvent system II in the same chamber. A 14 cm run on the thin layer chromatographic plates took 25 minutes. The spray reagent was the 25% solution of tribromoacetic acid in chloroform.

When tribromoacetic acid/chloroform was used, the thin layer chromatographic plates were heated for five minutes at 110°C in order to make the spots visible. The R_f values, colour reactions with this spray reagent and the colour in the ultraviolet light are given in Table 1.

TABLE 1

THIN LAYER CHROMATOGRAPHIC RESULTS

Compound	R_f value in		Colour with naked eyes	Fluorescence in UV light
	Solvent I	Solvent II		
4-Androsten-3, 17-dione	0.08	0.16	Yellowish brown	Yellowish green
Cholesterol	0.19	0.34	Brownwish green	Green
5-β-cholestan-3α-ol	0.18	0.40	Pinkish brown	Yellowish green
Dehydro epiandrosterone	0.05	0.11	Yellowish brown	Yellowish green
Dehydro epiandrosterone acetate	0.28	0.49	Light yellow	Yellowish green
Estrone	0.18	0.32	Brown	Greenish yellow
Estradiol	0.05	0.08	Brown	Yellowish green
Methyl testosterone	0.06	0.11	Yellow	Yellowish green
Pregnenolone acetate	0.36	0.59	Light yellow	Green
Stigmasterol	0.30	0.24	Light brown	Light green
Testosterone	0.03	0.10	Light brown	Green
Testosterone acetate	0.20	0.78	Light brown	Green

Evidence put forward in the earlier communications[10-11] showed that terpenoids and steroids can be detected on thin layer plates upto the concentration of .5 μg by spraying the activated plates with a novel reagent $AsCl_3$/AcOH. It was also described that the only simple way in which this reagent can show this reaction for detecting terpenoids is due to the complex forming ability of arsenic which can be further enhanced due to the activation by the nicely located neighbouring group[12] in the molecule. The study has also been extended by using another simpler reagent tribromoacetic acid/chloroform which is even more sensitive than the first spray reagent. This according to author's view represents a rare example of spray reagent which has been used to detect steroids. Saturated solution of this reagent has been found to be sensitive reagent for the detection of steroids on thin layer plates. When saturated solution of this was used as a spray reagent, the thin layer chromatographic plates were heated for five minutes at 110°C in order to make the spots visible.

The colour reactions with this spray reagent and the colours in the ultraviolet light are given in Table 1. The colours produced are reported at 5 μg concentration, although the reagent appears to be sensitive at concentration down to 1.5 μg for many steroids. Tribromoacetic acid reacts probably with functional groups like hydroxyl or carbonyl in ring *A* and *D* of the steroid molecule. The rate of the reaction is quite fast (i.e. it can be observed within five minutes) when at least one of the functional groups is hydroxyl or carbonyl in ring *A* or *D*. This is evident from the fact that estradiol, methyl testosterone, cholesterol, dehydroepiandrosterone acetate, testosterone can be detected within five minutes and the slight change in structure is also reflected by the change in the colour of the spot observed. When the hydroxyl function is esterified or replaced by an acetyl function, the reaction becomes slow and the colour spots are observed only after ten minutes. This is clear from the detection of testosterone acetate and pregnenolone acetate.

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