Journal of Chromatography, 258 (1983) 258–261
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 15,550

Note

Spray reagents for the detection of steroids and triterpenoids on thin-layer plates

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(First received August 5th, 1982; revised manuscript received November 19th, 1982)

Thin-layer chromatography (TLC) has been applied extensively for the detection and separation of steroids and triterpenoids[1].Various spray reagents for the identification of steroids and triterpenoids on TLC plates have been described, the Liebermann–Burchard reagent[2,3] being the most widely used. Other reagents that have been used for the simultaneous determination of steroids and triterpenoids include chlorosulphonic acid–acetic acid[3], anisaldehyde–sulphuric acid[4,5], carbazole–sulphuric acid[6], molybdophosphoric acid[7,8], tin(IV) chloride[9], antimony(V) chloride[10], antimony(III) chloride–acetic acid[11,12], arsenic chloride–acetic acid[13] and boron trifluoride etherate[14].

In this paper we report two reagents, sulphosalicylic acid and picrylsulphonic acid, which can be used as spray reagents for this purpose. Both reagents give clear colours and permit rapid screening and simultaneous detection of steroids and triterpenoids at very low concentration.

EXPERIMENTAL

Apparatus

TLC plates (0.1 mm) were prepared using silica gel G and a Unoplan apparatus (Shandon, London, Great Britain). The samples were spotted with a 25-μl pipette (Shandon).

Materials

Sulphosalicylic acid reagent. A saturated aqueous solution of the reagent (SD'S, India) was used.

Picrylsulphonic acid reagent. A saturated solution of the reagent (J. T. Baker, Phillipsburgh, NJ, U.S.A.) in glacial acetic acid (analytical-reagent grade,Glaxo) was used, but it was observed that even a 30% solution of the reagent is also capable of producing similar colour reactions, although with a slight diminution of colour intenstity.

Steroids and triterpenoids after development on silica gel G TLC plates were sprayed with the reagents and heated at 120°C for 5 min.

0021-9673/83/0000–0000/$03.00

RESULTS

Both reagents are capable of detecting as little as 0.3 μg of steroids and 0.4 μg of triterpenoids (Table I). The bright colours produced by the phytosterols appears within 3 min and are very distinct. Ring A aromatic steroids and androstadiene derivatives are also capable of developing an intense orange colour that clearly distinguished them from other steroids. Ethynylestradiols show a bright cherry red colour whereas the colours developed by triterpenoids are in general duller and, as with most triterpenoid reagent, the colour reaction with friedlanes is sluggish. In our continuing effort to develop spray reagents[6,14] for steroids and triterpenoids and to investigate the chemistry of colour reactions, we have claimed that the carbazole–sulphuric acid[6] reagent is capable of screening friedlanes from other triterpene skeletons but we should point out that our claim is not always valid because some deviations have been observed.

ABLE I

OLOUR REACTIONS OF STEROIDS AND TRITERPENOIDS WITH SULPHOSALICYLIC ACID AND CRYLSULPHONIC ACID AFTER CHROMATOGRAPHY ON SILICA GEL G THIN-LAYER PLATES

oup	*Compound*	*Spray reagent*			
		Sulphosalicylic acid		*Picrylsulphonic acid*	
		Colour observed	*Detection limit (μg)*	*Colour observed*	*Detection limit (μg)*
roids	β-Sitosterol	Pinkish violet	0.3	Violet	0.3
	β-Sitosterol acetate	Pinkish violet	0.3	Violet	0.3
	Stigmasterol	Pinkish violet	0.3	Violet	0.3
	Stigmasterol acetate	Pinkish violet	0.3	Violet	0.3
	α-Spinasterol	Brown	0.4	Grey	0.4
	Cholesterol	Pinkish violet	0.3	Violet	0.3
	Cholesterol acetate	Pinkish violet	0.3	Violet	0.3
	Cholesterol palmitate	Pinkish violet	0.3	Violet	0.3
	Ergosterol	Violet	0.8	Grey	0.6
	Progesterone	Light brown	10.0	Light brown	10.0
	11α-Hydroxyprogesterone	Pink	8.0	Light brown	10.0
	17α-Hydroxyprogesterone	Yellow	10.0	Grey	5.0
	Pregnenolone	Pinkish violet	0.3	Violet	0.3
	Testosterone	Green	5.0	Blue	4.0
	Cortisone	Brown	10.0	Brown	5.0
	Hydrocortisone	Brown	10.0	Brown	5.0
	Estrone	Orange	0.8	Orange	0.6
	Estrone 3-methyl ether	Orange	0.3	Orange	0.4
	Estradiol	Orange	0.4	Orange	0.8
	17α-Ethynylestradiol	Cherry red	0.3	Cherry red	1.0
	17α-Ethynylestradiol 3-methyl ether	Cherry red	0.3	Cherry red	1.0
	cis- androsterone	Blue	3.0	Grey	2.0
	Dehydroepiandrosterone	Pinkish violet	0.6	Pinkish violet	0.8

(Continued on p. 260)

TABLE I *(Continued)*

Group	*Compound*	*Spray reagent*			
		Sulphosalicylic acid		*Picrylsulphonic acid*	
		Colour observed	*Detection limit (μg)*	*Colour observed*	*Detection limit (μg)*
	Androsta-4-en-17β-ol-3-one	Green	1.0	Green	1.0
	5α-Androsta-17β-ol-3-one	Brown	3.0	Grey	2.0
	5α-Androsta-3β-ol-17-one	Violet	1.0	Grey	3.0
	Androsta-1,4-diene-3,17-dione	Orange	3.0	Orange	1.0
	17-Ketal of androsta-1,4-diene-3,17-dione	Orange	3.0	Orange	1.0
	Norethisterone	Violet	1.0	Violet	0.8
	Androsta-4-en-3,17-dione	Green	3.0	Green	1.0
Triterpenoids	Lupeol	Brownish violet	0.4	Greyish violet	0.4
	Methyl betulate	Brownish violet	0.4	Greyish violet	0.4
	β-Amyrin	Brownish violet	0.4	Greyish violet	0.4
	β-Amyrin acetate	Brownish violet	0.4	Greyish violet	0.4
	Taraxerol	Brownish violet	0.4	Greyish violet	0.4
	Taraxerone	Brownish violet	0.4	Greyish violet	0.4
	Taraxerol acetate	Brownish violet	0.4	Greyish violet	0.4
	Multiflorinyl acetate	Brownish violet	0.4	Greyish violet	0.4
	Methyl oleanolate	Brownish violet	0.4	Greyish violet	0.4
	Taraxasterol	Brownish violet	0.4	Greyish violet	0.4
	Taraxasteryl acetate	Brownish violet	0.4	Greyish violet	0.4
	Glutinol	Brownish violet	0.4	Greyish violet	0.4
	Bauernyl acetate	Brownish violet	0.6	Greyish violet	0.5
	Methyl crategolate	Pink	0.6	Pink	0.5
	Methyl ursolate	Brown	5.0	Greyish violet	3.0
	Fridelin	Brown	5.0	Greyish violet	2.0
	Putranjivadione	Brown	4.0	Greyish violet	3.0

With the exception of sulphuric acid-methanol (1:1) reagent[15], which can detect down to 0.005 μg of certain steroids, most of the commonly used reagents have sensitivity limits similar to those of the reagents investigated here. It is interesting that the colours exhibited by sulphosalicylic acid and picrylsulphonic acid are in many instances identical and even the sensitivities are also identical with certain steroids (eleven samples) and triterpenoids (twelve samples). It appears from the present investigation that picrylsulphonic acid, despite of its higher cost, is slightly superior to sulphosalicylic acid with respect to sensitivity.

ACKNOWLEDGEMENT

Thanks are due to CSIR, New Delhi, for Senior Research Fellowship to one of us (P.G.).

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