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Note

Dimethyl sulphoxide as a spray reagent for the detection of triterpenoids and some steroids on thin-layer plates

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Triterpenoids and steroids have been detected on thin-layer plates using the Lieberman–Burchard reagent[1,2], chlorosulphonic acid–acetic acid[2,3], arsenic trichloride–acetic acid[4] and molybdophosphoric acid[5,6]. Recently, Ghosh and Thakur have reported the use of sulphosalicylic acid, picrylsulphonic acid[7], boron trifluoride etherate and carbazole–sulphuric acid[9] as spray reagents for the screening and detection of steroids and triterpenoids. These methods generally required heating of the plate after spraying with the reagent.

Here, we report the use of dimethyl sulphoxide (DMSO) as a novel spray reagent for the detection of triterpenoids and some steroids on thin-layer plates. Dimethylformamide (DMF) was also found to be useful, although to a lesser extent. Several π-acceptors like *p*-chloranil, *p*-fluoranil, 2,5-dichloro-*p*-benzoquinone, 2,3-dichloro-5,6-dicyanobenzoquinone and chloranilic acid were also examined as detection agents for triterpenoids and steroids and the colour reactions obtained are reported.

EXPERIMENTAL

Materials

Authentic samples of triterpenoids and steroids were obtained from various sources. Some steroidal dosage forms were obtained from the University Medical Centre. Dimethyl sulphoxide (Riedel-de Häen) and dimethylformamide (Merck) were of analytical grade. *p*-Chloranil, *p*-fluoranil, 2,5-dichlorobenzoquinone, 2,3-dichloro-5,6-dicyanobenzoquinone and chloranilic acid were from Pflatz and Baüer and used as received. Other solvents and reagents were of reagent grade.

Spray reagents

The following reagents were employed: I, dimethyl sulphoxide; II, dimethylformamide; III, 0.5% *p*-chloranil in dioxane, followed by DMF or DMSO. IV, 0.5% *p*-fluoranil in dioxane; V, 0.5% 2,3-dichloro-5,6-dicyanobenzoquinone in dioxane. Solutions of 2,5-dichloro-*p*-benzoquinone and chloranilic acid were prepared and used as previously described[10].

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Thin-layer chromatography (TLC)

Triterpenoids and steroids were dissolved in chloroform. The sample was applied to silica gel G (0.2 mm) TLC plates and after development in cyclohexane–ethyl acetate (75:25), the plate was air dried and sprayed.

RESULTS

Agarwal and Elsayed[10] recently reported the use of *p*-chloranil, *p*-fluoranil and 2,5-dichloro-*p*-benzoquinone as detection agents for some alkaloids on thin-layer plates. In the present study, it was found that, with these and other π-acceptors, in the few cases where reaction occurred it was slow. Thus, attempts were made to accelerate and intensify the colour development by spraying the plate with DMF or DMSO[10,11]. When the chromatoplates were sprayed first with DMSO or DMF, all the triterpenoids and some steroids could be visualized immediately. We are not aware of any previous report of DMSO or DMF alone being used as visualizing agent in TLC.

The results of the reactions of triterpenoids with dimethyl sulphoxide are reported in Table I. All the triterpenoids reacted at room temperature to give a silvery white spot. The intensity of the response as well as the detection limit for individual triterpenoids are also given in Table I. Dimethylformamide also reacted with the triterpenoids, although the response was somewhat less intense and the spots tended to disappear with time (½ h). The spots with dimethyl sulphoxide are highly stable (up to several weeks). Other spray reagents like tetramethylguanidine or tetramethylurea were found to be ineffective. The use of π-acceptors, *e.g.*, *p*-chloranil, *p*-fluoranil or 2,5-dichloro-*p*-benzoquinone, was also ineffective for triterpenoids. However, if plates which had been previously sprayed with DMSO were dried and resprayed with

TABLE I

REACTIONS OF TRITERPENOIDS WITH DIMETHYL SULPHOXIDE

Order of increasing response: +, + +, + + +.

Compound	*Reaction with DMSO*	
	Response	*Detection limit (μg)*
α-Amyrin	+ +	1
α-Amyrin acetate	+ +	1
β-Amyrin	+ +	1
β-Amyrin acetate	+ +	1
Lanostenyl acetate	+*	2
Lupeol	+ + +	0.5
Lupeol Acetate	+	2
Oleanolic acid	+ +	1
3-Oxodimethylquinovate	+*	2
Dimethylquinovate	+	2
Quinovaic acid	+	2
Sulphurenic acid	+ + +	0.5

* Develops slowly (*ca.* 5 min).

these reagents, the visibility of the spots was greatly improved because of a contrasting background (purple), and the sensitivity of response was also improved. As the reaction of DMSO with triterpenoids occurs at room temperature, no heating of the plate is necessary. Also the reaction is instantaneous in all cases, except with lanostenyl acetate and oleanolic acid where the spots developed slowly over 5 min.

Table II gives the colour reactions and detection limits of steroids with various spray reagents. DMSO gave grey spots with some steroids while DMF was generally ineffective as it gave white spots with only cholestan-3β,5α,6β-triol and diosgenin. The use of π-acceptors was more successful with steroids, spraying with *p*-chloranil giving purple colourations with 17β-estradiol, dienesterol, estrone and reddish brown spots with stilbesterol and ethisterone. Overspraying the plate with DMSO or DMF resulted in intense orange spots for 17β-estradiol, dienestrol, estrone, stilbestrol and 2α-xanthatocholestan-3-one, while dehydroepiandrosterone and 3-acetamidocholest-3,5-diene appeared as yellow and grey spots respectively. *p*-Fluoranil (IV) gave yellow spots with 17β-estradiol, dienestrol and estrone. 2,3-Dichloro-5,6-dicyanobenzoquinone (V) gave green spots for 17β-estradiol, estrone and stilbesterol and a faint yellow spot for dienesterol. However, the use of this reagent is not recommended as (i) it is toxic, (ii) the spots fade with time and (iii) spots are difficult to detect as the reagent produces a very dark background with silica gel. In the cases of both *p*-fluoranil and 2,3-dichloro-5,6-dicyanobenzoquinone, detection was not significantly improved by overspraying the plate with DMF or DMSO. Other π-acceptors such as 2,5-dichloro-*p*-benzoquinone and chloranilic acid were found to be totally ineffective, either alone or in combination with DMF or DMSO.

The following compounds gave no reaction with any of the spray reagents studied testosterone propionate, progesterone, 5β-androsterone-3,17-diene, pregnenolonemethyl ether, androstenalione, 17-α-hydroxyprogesterone, 16-dehydroprogesterone d-norgestrel, chloramidinon acetate, cortisone acetate, cholest-l-ene and digoxin.

The described use of DMSO or DMF as spray reagent may not be exclusive to triterpenoids and steroids and other classes of compound may also react with them. In our laboratory, however, when these spray reagents were used for the detection of alkaloids no response could be seen.

In conclusion, DMSO is useful as a spray reagent for detecting triterpenoids on thin-layer plates. The use of *p*-chloranil followed by overspraying with DMSO is recommended for the detection of certain steroids. In either case, no heating of the thin-layer plate is necessary.

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TABLE II

REACTIONS OF STEROIDS WITH SPRAY REAGENTS

Order of increasing response: +, + +, + + +.

Compound	*DMSO*		*Chloranil* + DMSO*		*p-Fluoranil*		*2,3-Dichloro-5,6-dicyanobenzoquinone*	
	Color response	*Detection limit (μg)*	*Colour response*	*Detection limit (μg)*	*Colour response*	*Detection limit (μg)*	*Colour response*	*Detection limit (μg)*
17β-Estradiol			Orange (+ +)	2.0	Yellow (+)	4.0	Green (+ +)	2.0
Cholesterol	Grey (+ + +)	0.5						
Stigmasterol	Grey (+ + +)	0.5						
β-sitosterol	Grey (+ + +)	0.5						
Dienesterol**	Grey (+ +)	1.0	Orange (+ + +)	2.0	Yellow (+ + +)	2.0	Yellow (+)	4.0
Dehydroepiandrosterone			Yellow (+)	4.0				
Estrone			Orange (+ + +)	1.0	Yellow (+ +)	2.0	Green (+ +)	2.0
Dexamethasone***	Grey	2.0						

	(+ +)						
Stilbesterol[§]	Grey (+ +)	2.0	Orange (+ +)	2.0		Green (+)	2.0
Ethisterone[§§]	Grey (+ +)	2.0	Grey (+ +)	2.0			
2α-Xanthatocholestan-3-one	Grey (+ + +)	1.0	Orange (+ +)	2.0			
Cholestan-3β,5α,6β-triol	Grey (+ +)	1.0					
2α-Thiocyanatocholestan-3-one	Grey (+ +)	2.0					
Cholest-1-en-3β-ol	Grey (+ +)	2.0					
3-Acetamidocholest-3,5-diene			Grey (+)	2.0			
Diosgenin	Grey (+ +)	1.0					

* 17β-Estradiol, dienesterol and estrone gave purple, while stilbesterol and ethisterone gave reddish brown, spots when sprayed with *p*-chloranil only.
** Tablets, B.P. 1 mg per tablet (Evans Medical).
*** Tablets, 0.5 mg per tablet (Merck Sharp & Dohme).
§ Tablets, 1 mg per tablet (Imarsel).
§§ Tablets, 25 mg per tablet (Evans Medical).

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