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Note

Spray reagent for steroids and triterpenoids on thin-layer plates

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A number of spray reagents for the selective and non-selective detection of triterpenoids and steroids[1] on silica gel G thin-layer plates are known. The Liebermann–Burchard reaction with acetic anhydride–sulphuric acid is widely used and other reagents claimed to be effective in the detection of both classes include chlorosulphonic acid–acetic acid[2], antimony trichloride–acetic acid[3,4] and arsenic trichloride–acetic acid[5]. The use of molybdophosphoric acid[6,7] as a very sensitive reagent, although non-specific, is well established.

In this paper we describe another very sensitive spray reagent, carbazole–sulphuric acid, which is capable of detecting triterpenoids in amounts as low as 0.8 μg and steroids at levels of 0.2–6 μg.

EXPERIMENTAL

The reagent is prepared by adding 2 ml of concentrated sulphuric acid per 10 ml of a 1% solution of highly purified carbazole (BDH, Poole, Great Britain; recrystallised six times from benzene) in ethanol. Steroids and triterpenoids, after development on silica gel G thin-layer plates (0.1 mm), are sprayed with the reagent and heated at 120°C for 5 min.

RESULTS AND DISCUSSION

The observed colours and the limits of detection are presented in Table I, which shows that only two out of thirty steroidal samples fail to respond even at very much higher concentrations. The colour reaction with phytosterols develops within 3 min, whereas almost all other steroids show no coloration, thereby providing a convenient method for the identification of phytosterols in sample mixtures. The range of colours indicates the diagnostic value of this reagent in differentiating different classes of steroids. The chemistry behind the colour reaction is not clear and it is obvious that neither the stereochemistry of the ring junctions nor the nature of the side-chains and functional characteristics in the molecule can be responsible for the observed coloration.

Amongst the triterpenoids no coloration is observed for samples with the friedelane skeleton, but seven other skeletons *viz.*, lupane, oleanane, ursane, taraxerane, glutinane, taraxasterane and bauerane, responded well. Possibly this is the

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TABLE I

COLOUR REACTIONS OF STEROIDS AND TRITERPENOIDS WITH CARBAZOLE–SULPHURIC ACID REAGENT ON SILICA GEL G THIN-LAYER PLATES

Type	*Compound*	*Colour on heating for 5 min at 120°C*	*Limit of detection (μg)*
Steroids	β-Sitosterol	Pinkish violet	0.2
	β-Sitosterol acetate	Pinkish violet	0.2
	Stigmasterol	Pinkish violet	0.2
	Stigmasterol acetate	Pinkish violet	0.2
	α-Spinasterol	Pinkish violet	0.2
	Cholesterol	Pinkish violet	0.2
	Cholesterol acetate	Pinkish violet	0.2
	Cholesterol palmitate	Pinkish violet	0.2
	Ergosterol	Green	0.5
	Progesterone	No coloration	–
	11α-Hydroxyprogesterone	Light yellow	6.0
	17α-Hydroxyprogesterone	Light brown	6.0
	Pregnenolone	Pinkish violet	0.2
	Testosterone	Brown	6.0
	Cortisone	Light blue	1.2
	Hydrocortisone	Light blue	1.2
	Estrone	Light brown	4.0
	Estrone 3-methyl ether	Orange	0.8
	Estradiol	Deep orange	0.4
	17α-Ethinylestradiol	Pinkish violet	1.2
	17α-Ethinylestradiol 3-methyl ether	Pinkish violet	1.6
	cis-Androsterone	Light brown	0.4
	Dehydro-epi-androsterone	Pinkish violet	1.6
	Androsta-4-ene-17β-ol-3-one	Light green	1.6
	5α-Androsta-17β-ol-3-one	Light green	1.6
	5α-Androsta-3β-ol-17-one	Light green	4.0
	Androsta-1,4-diene-3,17-dione	Light brown	4.0
	17-Ketal of androsta-1,4-diene-3,17-dione	Light brown	4.0
	Norethisterone	Light brown	1.6
	Androsta-4-ene-3,17-dione	No coloration	–
Triterpenes	Lupeol	Reddish brown	0.8
	Methyl betulate	Reddish brown	0.8
	β-Amyrin	Reddish brown	0.8
	β-Amyrin acetate	Reddish brown	0.8
	Taraxerol	Reddish brown	0.8
	Taraxerone	Reddish brown	0.8
	Taraxerol acetate	Reddish brown	0.8
	Multiflorinyl acetate	Reddish brown	0.8
	Methyl oleanolate	Reddish brown	0.8
	Taraxasterol	Reddish brown	0.8
	Taraxasteryl acetate	Reddish brown	0.8
	Glutinol	Reddish brown	0.8
	Bauernyl acetate	Reddish brown	0.8
	Methyl crategolate	Reddish brown	0.8
	Methyl ursolate	Reddish brown	0.8
	Friedelin	No coloration	–
	Epi-friedelinol	No coloration	–
	Putranjivadione	No coloration	–

only report of the differentiation of friedelane derivatives from other triterpenoids through a colour reaction. The sterochemistry of A/B ring junction and the staggered arrangement of the 4- and 5-methyl groups compared with the usual 4,4-dimethyl arrangement in other classes of triterpenoids are probably responsible for the non-participation of friedelane derivatives in colour reactions with this reagent. It is expected that samples with the filicane skeleton, the only system bearing a resemblance to friedelane skeleton, will also exhibit a negative reaction but a lack of samples prevented us from testing this suggestion. It appears that triterpenoids having a *gem*-dimethyl arrangement at C-4 with proper ring A/B stereochemistry and a free hydroxyl or carbonyl group or groups capable of producing a hydroxyl function under the hydrolytic action of sulphuric acid are important in determining the sensitivity of the reaction, as all other triterpenoids examined respond equally and produce the same coloration in spite of the varying stereochemistry of the ring junctions.

It is worth mentioning that the same reagent is capable of producing colorations with other terpenoids also. A few representative samples have already been examined and more are awaiting confirmation. It is expected that this reagent will be versatile for detecting terpenoids and steroids, in addition to its accepted use in the carbohydrate field[8].

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