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Note

General spray reagent for the detection of steroids on thin-layer plates

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The Lieberman–Burchard reaction with acetic anhydride–sulphuric acid is used for the detection of Δ^5-3-hydroxysterols, and other steroids and triterpene glycosides[1], and forms the basis of a widely used quantitative method for determining cholesterol in biological materials[2]. Other reagents such as *p*-toluenesulphonic acid–sulphuric acid[3], iron(III) chloride–glacial acetic acid–concentrated sulphuric acid[4], and Lewis acids, such as antimony chloride–acetic acid, arsenic chloride–acetic acid[5], cerium(IV) sulphate–sulphuric acid, chlorosulphonic acid–acetic acid, cinnamaldehyde–acetic anhydride–sulphuric acid, orthophosphoric acid, trifluoroacetic acid and vanillin–phosphoric acid, have been proposed for the detection and determination of sterols. Specific reagents, such as *m*-dinitrobenzene for 17-keto steroids, methylene blue for sulphate esters of steroids, *p*-hydroxybenzaldehyde for 3-keto steroids unsubstituted in the 2-position and isonicotinic acid hydrazide for Δ^4-3-keto steroids have been reported[1]. The last reagent has recently been reported to detect *o*-dihydroxyphenolic compounds[6]. Molybdophosphoric acid is used to detect reducing steroids, but being non-specific this reagent detects most of the reducing substances. It has recently been recommended for the determination of cholesterol and cholesterol esters in blood serum[7].

In this paper we report a very sensitive reagent, chloramine-T–sulphuric acid, which is capable of detecting various types of steroids at levels of 0.2–5 μg.

PROCEDURE

A 2% solution of chloramine-T in concentrated sulphuric acid is sprayed on silica gel G plates that have been, spotted with various steroids in different concentrations. The colours produced are observed after 5 min at room temperature and also after heating at 110° for 5 min. The colours observed and the limits of detection are given in Table I.

TABLE I

COLOUR REACTIONS OF STEROIDS WITH CHLORAMINE-T–SULPHURIC ACID REAGENT ON SILICA GEL G THIN-LAYER PLATES

Compound	*Colour observed after 5 min*		*Limit of detection (μg)*
	At room temperature	*At 110°*	
Estriol	Purple	Dark purple	0.5
Estriol 3-sulphate	Purple	Purple	0.5
Estriol 3-methyl ether	Purple	Purple	0.5
β-Estradiol	Yellow	Brown	1
β-Estradiol 3,17–dipropionate	Light yellow	Light brown	2
β-Estradiol 3,17-diacetate	Light yellow	Light brown	2
β-Estradiol 3-methyl ether	Light yellow	Brown	1
Estrone	Yellow	Brown	1
Estrone 3-sulphate	Yellow	Light brown	2
Estrone β-D-glucuronide	Light yellow	Light brown	2
Progesterone	Yellow	Light brown	2
17α-Hydroxyprogesterone	—	Yellow	5
Pregnenolone	Yellow	Brown	1
Testosterone	Yellow	Dark brown	0.5
Methyltestosterone	Deep yellow	Reddish brown	0.5
Testosterone acetate	—	Light yellow	5
Testosterone propionate	—	Yellow	5
Dehydroepiandrosterone	Dark purple	Purple	0.2
Cholesterol	Brown	Dark brown	0.5
Cholesterol acetate	—	Dark brown	0.5
Solasodine	Purple	Purple	0.5
Solanine	—	Purple	0.5

RESULTS AND DISCUSSION

Table I shows that whereas most but not all of the steroids are detected at room temperature, all are detected when the thin-layer plates are heated at 110° for 5 min. The reaction was most sensitive with dehydroepiandrosterone, when a dark purple colour was observed at room temperature. However, on heating, the intensity of the purple colour decreased slightly. Estriol and its derivatives, such as the sulphate ester and 3-methyl ether, and the steroidal alkaloid solasodine also gave a purple colour at room temperature and also on heating. Other steroids gave a yellow colour, which changed to brown on heating.

The negative reactions given by testosterone acetate and testosterone propionate at room temperature, compared with the positive reactions given by testosterone and 17α-methyltestosterone, indicates that a hydroxyl group at C-17 is more important for the reaction than the Δ^4-3-keto group, which is probably deactivated owing to charge delocalization. Positive reactions were given at room temperature by Δ^5-3β-hydroxy steroids, *e.g.*, dehydroepiandrosterone and pregnenolone, which contain a non-conjugated carbonyl group and a hydroxyl group. The positive reactions given by progesterone and estrone and its derivatives at room temperature are attributable to the presence of a non-conjugated carbonyl group in these compounds. The negative reaction given by 17α-hydroxyprogesterone at room temperature may

be ascribed to hindrance of the carbonyl group at C-20 by the neighbouring hydroxyl group at C-17. Steroids containing a substituted hydroxyl group, such as testosterone acetate, testosterone propionate, cholesterol acetate and solanine (glycoalkaloid with a steroid nucleus), gave positive reactions on heating, apparently due to the hydrolytic action of sulphuric acid freeing the substituted hydroxyl group. From these comparisons, it appears that a free hydroxyl group or a non-conjugated carbonyl group is important for the sensitivity of the reaction. It may be assumed that the strong oxidizing action of chloramine-T under acidic conditions[8] causes a properly oriented hydroxyl group to become oxidised to a carbonyl group, which reacts with the chromogenic reagent. Determinations of different steroids have shown that this reaction is at least five times more sensitive than the Lieberman–Burchard reaction.

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