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Detection of steroids in thin-layer chromatography

The anisaldehyde spray reagent (0.5 ml anisaldehyde, 1 ml conc. sulfuric acid, 50 ml glacial acetic acid) which we have used for detection of bile acids on thin-layer chromatograms[1] gives a variety of colors, depending on the structure of the particular bile acid. This color variability has been useful in assigning structures to unidentified bile acids[2]. We have extended the use of this reagent to detection of a number of other steroids. The results obtained are the basis of this communication.

The steroids (5–10 μg) were spotted on Silica Gel G plates and the chromatogram developed with isooctane–isopropyl ether–acetic acid (2:1:1). The plates were sprayed with the anisaldehyde reagent and kept at 125° for 10 min. The color development was carried out in this manner in order to test the reagent under experimental conditions. Subsequently, groups of similar steroids were also chromato-

TABLE I

COLORS OBTAINED WITH VARIOUS STEROIDS USING ANISALDEHYDE SPRAY REAGENTS

Compound	*Color*	R_F *value*	
		Isooctane–isopropyl ether–acetic acid (2:1:1)	*Benzene–ethyl acetate (4:3)*
Sterols			
Cholesterol	Purple	0.58	0.69
Coprostanol	Purple	0.63	
Sitosterol	Purple	0.56	
Ergosterol	Brown	0.56	0.60
Lanosterol	Purple	0.60	
Desmosterol	Purple	0.53	
Zymosterol	Brown	0.44	
Other steroids			
Δ^4-Cholesten-3-one	Orange	0.56	0.73
3,5-Cholestadiene	Brown	0.90	
7β-Hydroxy-Δ^4-cholesten-3-one	Orange	0.43	0.49
3β,5α,6β-Trihydroxycholestane	Brown	0.22	0.09
Cholesteryl chloride	Purple	0.83	0.80
Cholesteryl laurate	Purple	0.85	
Cholesteryl methyl ether	Purple	0.75	
Androgens			
Androsterone	Purple	0.30	0.50
Epiandrosterone	Purple	0.30	0.50
Testosterone	Brown	0.20	0.41
Estrogens, progestins			
Progesterone	Orange	0.23	0.60
Δ^5-Pregnen-3β-ol-20-one	Purple	0.33	0.50
5α-Pregnane-3β,20β-diol	Purple	0.31	0.43
Estrone	Brown	0.30	0.63
Estriol	Dark purple	0.10	0.12

graphed in benzene–ethyl acetate (4:3) in an attempt to further differentiate specific compounds.

The results are presented in Table I. Among the sterols only zymosterol and ergosterol give colors other than purple. The brown color observed with 3,5-cholestadiene suggests that a conjugated double bond system is responsible for the shade of color obtained. The color obtained with zymosterol cannot be explained on this basis, however, in view of the purple obtained with lanosterol which has the same double bond system, although the 4,4'-methyl groups in the latter compound may exert an effect. The orange color observed with cholestenone and 7β-hydroxycholestenone is similar to the colors obtained with keto bile acids[1]. The brown color obtained with 3β,5α,6β-trihydroxycholestane is probably due to an unsaturated compound obtained as a result of dehydration caused by the spray reagent.

The brown color obtained with testosterone differentiates it from androsterone. The brown color obtained with testosterone and that obtained with estrone indicate that, in the absence of a side chain, the presence of a keto group is insufficient to give the orange shade observed with cholestenone and keto bile acids. This suggestion is given credence by the orange color seen with progesterone.

Our results indicate the further usefulness of the anisaldehyde spray reagent in detection and possibly differentiation of steroids in thin-layer chromatography.

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