

DETECTION OF CHOLEST-5-ENE-3 β ,7 β -DIOL IN WOOL WAX*

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The hydrolysis products of freshly secreted wool wax have recently been investigated by amplified distillation¹ and chromatography.^{2,3} Hydrocarbons, aliphatic acids, monohydric aliphatic alcohols, α,β -diols, and cholesterol and lanosterol have been identified.

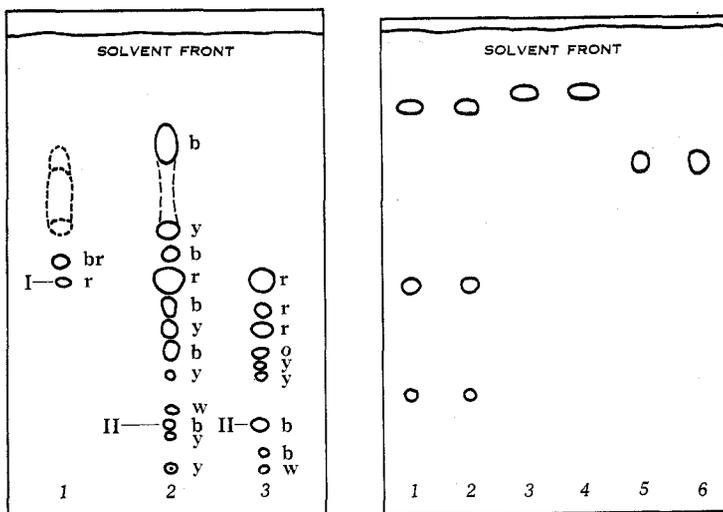


Fig. 1

Fig. 2

Fig. 1.—Thin-layer chromatogram of B.P. wax (1), tip wax (2), and lipid extracted from scoured Merino wool (3). Spots (250 μ g) were developed in benzene/ethyl acetate (2 : 1 v/v). Plates were sprayed with a saturated solution of antimony trichloride in chloroform and viewed under u.v. light (366 m μ). Colours: *b*, blue; *br*, brown; *o*, orange; *r*, red; *w*, white; *y*, yellow.

Fig. 2.—Spots 1, 3, 5 (cholest-5-ene-3 β ,7 β -diol) and 2, 4, 6 (component II recovered from thin-layer chromatogram of tip wax) applied to plate and treated with acetic anhydride (spots 1, 2), acetyl chloride (3, 4), and benzoyl chloride (5, 6) at 110° for 10 min. Developing solvent: benzene/ethyl acetate (2 : 1, v/v).

In commercial wax, obtained by centrifuging a wool scouring liquor, oxidation products of the sterols, viz., cholest-5-en-3 β -ol-7-one, cholestane-3 β ,5 α ,6 β -triol, cholest-7-en-3 β -ol, cholesta-3,5-dien-7-one, lanost-8-en-3 β -ol-7,11-dione, and lanost-8-en-3 β -ol-7-one, have also been identified.⁴

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¹ Murray, K. E., and Schoenfeld, R., *Aust. J. Chem.*, 1955, **8**, 424.

² Horn, D. H. S., *J. Sci. Food Agric.*, 1958, **9**, 632.

³ Downing, D., Kranz, Z., and Murray, K. E., *Aust. J. Chem.*, 1960, **13**, 80.

⁴ Milburn, A., Truter, E., and Woodford, P., *J. Chem. Soc.*, 1956, 1740.

Generally, however, the auto-oxidized wool waxes, e.g., wax from the tip portion of wool staples (tip wax) and wax unseparated in the centrifugal process have received little attention. As the oxidation products play an important role in wax technology^{5,6,7} and may contain components valuable as starting materials in steroid synthesis, we are studying these products.

This communication reports the detection and characterization of cholest-5-ene-3 β ,7 β -diol by R_F values and *in situ* derivatives on thin layer chromatoplates.

TABLE I
 R_C VALUES OF CHOLEST-5-ENE-3 β ,7 β -DIOL AND COMPONENT II ON THIN-LAYER CHROMATOGRAPHY PLATES

Spots were detected by spraying with antimony trichloride (saturated solution in chloroform)

Developing System	R_C Value	
	Cholest-5-ene-3 β ,7 β -diol	Component II
Benzene/ethyl acetate/acetic acid (7:3:1)	0.64	0.64
Benzene/dioxan/acetic acid (75:20:2)	0.52	0.52
Benzene/ethyl acetate (2:1)	0.23	0.23

Figure 1 shows a thin-layer chromatogram of various waxes. The plates were prepared with a commercial applicator using Silica Gel G (supplied by E. Merck) and were activated by drying at 105° for 30 min. In B.P. wax, (Fig. 1, spot 1) component I formed characteristic coloured complexes with antimony trichloride, phosphomolybdic acid, and sulphuric acid and was identified as cholesterol. In B.P. wax there were no components more polar than cholesterol.

In tip wax, (Fig. 1, 2), several strongly polar compounds were detectable with a variety of reagents. Many of these compounds had R_F values similar to those of known wax compounds and were not investigated further.

However, R_F values and colour reactions indicated that component II (Fig. 1, 2, 3) was not one of the previously reported oxidation products of wool wax.

Component II was shown to be cholest-5-ene-3 β ,7 β -diol from the following:

- (i) R_C^* values of synthetic cholest-5-ene-3 β ,7 β -diol (prepared by Wintersteiner's⁸ method) and component II were identical in three developing systems (Table 1).

* R_C = (distance moved by test component)/(distance moved by cholesterol).

⁵ Anderson, C. A., and Wood, G. F., *Nature*, 1962, **193**, 742.

⁶ Anderson, C. A., *J. Amer. Oil Chem. Soc.*, 1963, **40**, 333.

⁷ Anderson, C. A., *J. Text. Inst.*, 1962, **53**, T401.

⁸ Wintersteiner, O., and Ruigh, W. L., *J. Amer. Chem. Soc.*, 1942, **64**, 2453.

- (ii) Derivatives of synthetic diol and component II formed *in situ* on TLC plates with different reagents under different conditions had identical R_F values after being developed (Fig. 2).
- (iii) Colour reactions of the two compounds with phosphomolybdic acid, antimony trichloride, and dichlorofluorescein were identical. The immediate reaction and the intense blue colour formed after spraying the spots with antimony trichloride solutions are particularly characteristic of Δ^5 -7-hydroxy-sterols.⁹

Semi-quantitative estimation of the amount of cholest-5-ene-3 β ,7 β -diol in various waxes was obtained by visual comparison of the size and colour of the sprayed spots with those of known quantities of the synthetic compound. The amounts in waxes from various sources were: solvent degreasing process 0.4%; tip wax 0.6%; and lipid extracted from detergent scoured wool 3%.

⁹ Barr, T., Heilbron, I. M., Parry, E. G., and Spring, F. S., *J. Chem. Soc.*, 1936, 1437.