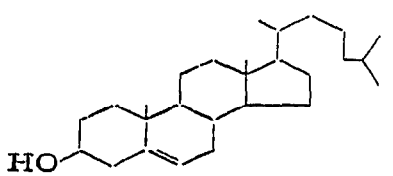
The separation of sterols and corresponding stanols by thin-layer chromatography

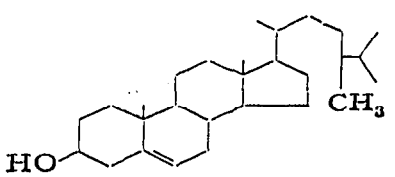
The sitosterols, such as β-sitosterol, occur very often in plants as complex mixtures with the corresponding stanols, mixtures which are difficult to resolve. Studies carried out on the constituents of Israeli peat revealed the presence of an inseparable mixture of β-sitosterol and β-sitostanol. Similar observations have been made by IVES AND O'NEILL[1] with Canadian peat moss (Sphagnum) and by MCLEAN, RETTIE AND SPRING[2] with Scottish peat. In order to prove the presence of sitostanol, the mixture is usually oxidized with chromium trioxide in acetic acid, and the β-sitostanone thus formed is identified. The disadvantages of this method are that the rupture of the double bond in sitosterol present produces polar oxidation products, and that the procedure is time consuming. As the mobilities of sterols and the corresponding stanols are so similar that chromatography makes them practically inseparable (Table II), the applicability of a method first suggested by CARGILL[3] for the separation of cholesterol from related stanols and stanones was investigated.

By this method the sterol mixture is brominated, the stanol remaining unchanged so that it can be separated chromatographically from the brominated sterol. Whilst FABRO[4] used reversed phase paper chromatography for this separation we followed CARGILL[3] in applying thin-layer chromatography. Separations were carried out on plates covered with silica gel G or alumina G (Merck), in 4-solvent systems (see below). The results are summarized in Table I. This method can also be used for the detection of traces of unreduced sterols in products which had been subjected to catalytic reduction.

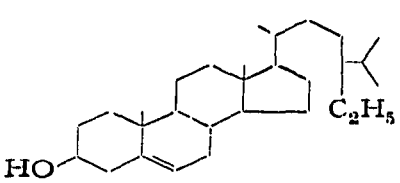
For compasorin, the free sterols were run on alumina G plates, solvent system No. 4 serving as the developer. The R_F values are summarized in Table II.



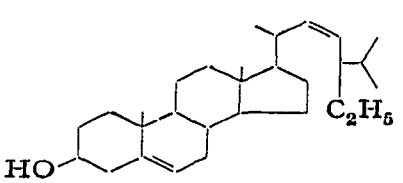
cholesterol



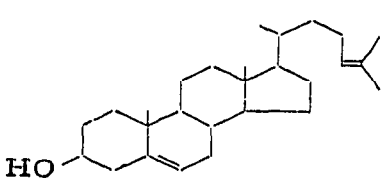
campesterol



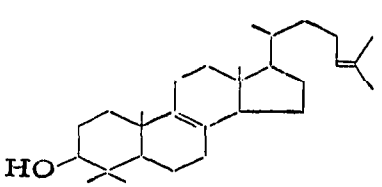
β-sitosterol



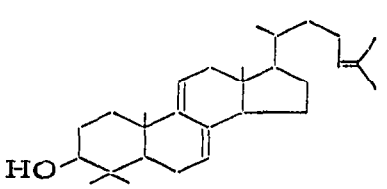
stigmasterol



desmosterol



lanosterol



agnosterol

TABLE I

R_F VALUES OF STANOLS AND BROMINATED STEROLS

The following mixtures were separated: campesterol–campestanol, cholesterol–cholestanol, cholesterol–coprostanol, cholestanol–coprostanol, cholesterol–cholestanol–coprostanol, lanosterol–dihydrolanosterol, stigmasterol–stigmastanol, β-sitosterol–β-sitostanol.

No.	Sterol (after bromination)	R_F in solvent system					
		1	2		3	4	
		Silica Gel G	Silica Gel G	Alumina G	Silica Gel G	Silica Gel G	Alumina G
1	Campesterol						0.69
2	Campestanol						0.51
3	Cholesterol	0.81	0.78	0.63	0.31	0.44	0.68
4	*allo*-Cholesterol	0.95	0.92	0.77	0.43	0.58	0.82
5	Desmosterol	0.82	0.79			0.47	0.69
6	Cholestanol	0.62	0.60	0.44	0.19	0.27	0.51
7	Coprostanol	0.76	0.66				0.69
8	β-Sitosterol	0.79	0.80	0.63	0.32	0.43	0.68
9	Stigmasterol	0.80	0.76	0.62		0.44	0.68
10	β-Sitostanol (= Stigmastanol)	0.60	0.59	0.44	0.18	0.27	0.50
11	Lanosterol	0.57	0.77			0.59	0.81
12	Dihydrolanosterol	0.50	0.55				0.65
13	Agnosterol	1.00	1.00				0.94

Experimental

Preparation of plates. The suspension required for five plates (20 × 20 cm) was prepared by shaking 50 g of alumina G and 100 ml of water or 30 g of silica gel G and 60 ml of water for 30 sec; it was then spread on the plates with a Desaga thin-layer applicator to give a layer of 0.25 mm thickness. The plates were allowed to dry for 60 min at room temperature and for 30 min at 125°. After cooling, they were placed in a vacuum desiccator.

TABLE II

R_F VALUES OF FREE STEROLS ON ALUMINA G, SOLVENT SYSTEM NO. 4

No.	Sterol	R_F	Colour with	
			$SbCl_3$	$SbCl_5$
1	Campesterol	0.50	Pink	Brown
2	Campestanol	0.51	—	Brown
3	Cholesterol	0.50	Pink	Brown
4	Cholestanol	0.51	—	Brown
5	Coprostanol	0.69	—	Brown
6	*allo*-Cholesterol	0.53	Pink	Brown
7	21-*nor*-Cholesterol[5]	0.52	Pale violet	Brown
8	Desmosterol	0.50	Brown	Brown
9	Lanosterol	0.78	Yellow	Brown
10	Dihydrolanosterol	0.77	Yellow	Brown
11	Ergosterol	0.52	Violet	Brown
12	Agnosterol	0.95	Yellow	Brown
13	β-Sitosterol	0.50	Pink	Brown
14	β-Sitostanol	0.49	—	Brown
15	Stigmasterol	0.52	Pink	Brown

Development. The samples were dissolved in chloroform (1 mg/1 ml of chloroform) and applied with micropipettes along a line 2 cm above the rim of the plate. Development was accomplished in a saturated chamber in four solvent systems (ratios in v/v), (1) benzene–ethyl acetate (2:1), (2) benzene–ethyl acetate (4:1), (3) benzene–ethanol (19:0.2), and (4) benzene–ethanol (19:0.4).

The experiments were performed at room temperature (23–25°). Usually 60 min were required for the solvent to reach a distance of about 12 cm. The plates were removed and the solvent was allowed to evaporate.

Detection. When the plates are heated at 120° for 15 min, the brominated products appear as green spots. Spraying with a saturated chloroform solution of antimony trichloride gave blue spots which turned green and finally grey, except for lanosterol and dihydrolanosterol which gave a yellow colour. The sterols were revealed as brown spots only after spraying with a solution of antimony pentachloride (30 % in chloroform).

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Separation of corticosteroids by thin-layer chromatography on silica gel plates containing tetrazolium blue

Tetrazolium salts, *e.g.* triphenyltetrazolium chloride, tetrazolium blue, etc., are useful reagents for the detection of corticosteroids. Tetrazolium salts in alkaline media are transformed to coloured formazans by corticosteroids, and this is the reaction that serves as the basis of their detection.

When thin-layer chromatography had become generally known and practised, several authors, *e.g.* METZ[1] and NISHIKAZE AND STAUDINGER[2], made use of this reaction after separation by thin-layer chromatography. Unfortunately, the sensitivity of the tetrazolium reaction shown in paper chromatography, *i.e.* 0.2 to 0.5 μg in the case of tetrazolium blue, could not be attained by spraying the surface of the