chromatography xylene saturated with formamide appeared to be the best solvent system. Resolution of *p*-chlorocresols from the parent cresols and of 6-chloro-2-methylphenol from 4,6-dichloro-2-methylphenol could not be achieved.

The application of TLC technique as an analytical tool in following the extent of chlorination is suggested.

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Received October 22nd, 1964

Separation of sterols and corresponding stanols on thin layers of silica impregnated with silver nitrate

In natural materials, such sterols as cholesterol and β-sitosterol very often occur as inseparable mixtures with the corresponding stanols. IKAN AND KASHMAN[1] have found a mixture of β-sitosterol and β-sitostanol in Israeli peat. Similar observations have been made by MCLEAN, RETTIE AND SPRING[2] with Scottish peat and by IVES AND O'NEILL[3] with Canadian peat moss.

In a previous communication[4] we have shown that by bromination of such mixtures, the unchanged stanols were easily separated on thin layers from the brominated sterols. In the present study the method applied by AVIGAN, DE GOODMAN AND STEINBERG[5] and MORRIS[6] for the fractionation of sterols, and by IKAN[7] for the separation of tetracyclic triterpenes on thin layers of silica impregnated with silver nitrate has been extended to include sterol–stanol mixtures. The sterols and the corresponding stanols had been shown to have practically the same R_F values on thin layers of silica gel G. However, on silica gel G impregnated with silver nitrate, the R_F values of the sterols were sufficiently different from the stanols. The R_F values and the colours obtained by spraying with 50 % sulfuric acid are summarized in Table I.

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

Experimental

Preparation of plates. The suspension for five plates (20 × 20 cm) was prepared by shaking 30 g of silica gel and 60 ml of water for 30 sec and applied uniformly to a thickness of 0.25 mm with a Desaga applicator. After 30 min at room temperature, the plates were heated in an oven at 125–130° for 45 min. After cooling they were

TABLE I

SEPARATION AND DETECTION OF STEROLS AND STANOLS BY THIN-LAYER CHROMATOGRAPHY

No.	Sterol	R_F on silica gel G		Colours with 50 % H_2SO_4 (after charring)	
		Treated with $AgNO_3$	Untreated	Silica gel treated with $AgNO_3$	Silica gel untreated
1	Campesterol	0.23	0.25	black	violet
2	Campestanol	0.26	0.25	brown	brown
3	Cholesterol	0.23	0.25	black	violet
4	Cholestanol	0.26	0.25	brown	brown
5	Allocholesterol	0.28	0.29	black	violet
6	Coprostanol	0.42	0.35	brown	brown
7	Demosterol	0.14	0.25	brown	brown
8	β-Sitosterol	0.23	0.25	black	violet
9	β-Sitostanol	0.32	0.25	brown	brown
10	Stigmasterol	0.23	0.25	black	violet
11	Lanosterol	0.41	0.42	brown	brown
12	Dihydrolanosterol	0.45	0.42	brown	violet
13	Agnosterol	0.89	0.92	brown	brown
14	Dihydroagnosterol	0.95	0.92	brown	violet

sprayed with concentrated aqueous-methanolic silver nitrate solution, 5 % relative to silica gel, and then activated at 120° for 30 min.

This method permits impregnation of only part of the plate, which can thus be used for comparative chromatography.

Development. The samples were dissolved in chloroform and applied with micropipettes along a line 2 cm above the rim of the plate. The experiments were performed at room temperature (25–27°). Chloroform was used as mobile phase. It was allowed to rise a distance of 15 cm. The plates were removed and the solvent was evaporated in air.

Detection. The sterols were detected by spraying with 50 % sulfuric acid, followed by heating in an oven at 150° for 10–15 min.

Acknowledgements

The authors wish to thank Prof. A. KUKSIS, Department of Biochemistry, Queen's University, Kingston, Ontario, for a sample of campesterol, and Mr. I. COHEN for technical assistance.

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Received October 20th, 1964