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

thin-layer chromatographic plates with an alkaline tetrazolium solution. This sensitivity, however, is attainable when tetrazolium blue is added to the silica gel before the preparation of the thin-layer plates. The details of the method used in these laboratories can be given briefly as follows:

Materials

(a) Tetrazolium blue (REANAL, Budapest), (b) Kieselgel HF_{254} (MERCK, Darmstadt), (c) Solution of sodium alcoholate (10 g reagent grade NaOH dissolved in 100 ml 60 % methanol), (d) Solution of formic acid (2.0 ml of conc. formic acid added to 100 ml of methanol), (e) Solution of "Neatan" (MERCK, Darmstadt).

Method

Tetrazolium blue (100 to 200 mg) and 30 g of Kieselgel HF_{254} are thoroughly mixed, then about 100 ml distilled water is added and the mass is homogenized. A layer of about 0.2 mm thickness is spread with a DESAGA-type equipment on six glass plates of 20 × 20 cm, which are then left to dry at room temperature for at least 24 h.

The corticosteroids are dissolved in ethanol, or chloroform, or dichloromethane, and spotted on the thin layer. The solvent systems chloroform—ethanol (90:10), or dichloromethane–benzene—acetone—ethanol (75:10:10:5), are used to develop the chromatogram (40 to 80 min). These solvent mixtures do not extract tetrazolium blue from the support.

After development of the chromatogram the surface of the plate is sprayed with sodium alcoholate solution. When the coloured spots of the formazan compounds have become visible, further transformation of tetrazolium blue to formazan within the alkaline medium is arrested by careful spraying with a methanolic solution of formic acid. The plates are then allowed to dry, at room temperature, for about 20 to 30 minutes, and sprayed with Neatan solution to fix them. Transfer can be made by the usual method. In the acid solution and without fixation, the silica gel flakes off the plates when completely dry, *i.e.* within one or two hours.

Discussion

In addition to giving greater sensitivity, this method is advantageous because it also allows a more uniform distribution of the tetrazolium reagent. The increased sensitivity might be explained by a more intensive contact being possible between the tetrazolium reagent and the steroid molecules situated at greater depths, as well as those present at the surface.

It is suggested here that, in other cases also, addition of the reagent to the silica gel substrate will bring about an increase in the sensitivity of thin-layer chromatographic methods.

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