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Abstract

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THE USE OF THIN-LAYER CHROMATOGRAPHY FOR THE SEPARATION OF STEROID COMPOUNDS

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Recently a new micromethod of adsorption chromatography on plates, or thin-layer chromatography, has begun to develop. This method has found application chiefly in the investigation of natural products, as well as in organic synthesis.

The essence of the method is as follows: a thin layer of adsorbent (usually silica gel), together with a binder (starch or gypsum) and water, is applied to a small glass plate and activated by heating ([1]). On such plates, with the aid of appropriate solvents, small quantities of substances (5-100γ) can be rapidly (30-90 min.) separated and developed in a manner similar to paper chromatography. The speed of this method, which excludes the influence of the adsorbent on the substance, together with its convenience and simplicity, attracted our attention, and we used it for the identification of steroid compounds.

The possibility of applying thin-layer chromatography to the study of steroid compounds was first mentioned in Stahl's papers ([2]); subsequently it was applied ([3]) to the separation of weakly polar steroids, mainly ethianic acid esters, and also ([4]) to the identification and separation of certain estrogens and, finally, ([5]) to the separation of cholesterol and its esters, cholestendiols, and certain steroid oxiketones.

We investigated a series of steroid compounds that are intermediates in the synthesis of corticosteroid analogs. The experiments were carried out on silica gel-gypsum plates. As solvents, the system cyclohexane–ethyl acetate in various ratios was used. As is known ([6]), in paper chromatography, spraying with concentrated sulfuric acid is used for locating spots of steroid compounds. However, this method had not previously been used for developing chromatograms of steroid compounds on plates. The use of concentrated sulfuric acid for development proved convenient in this series. In almost all cases, spots were obtained that were colored in daylight and fluoresced in ultraviolet light. In addition, in a number of cases development was carried out with solutions of $SbCl_3$, 2,4-dinitrophenylhydrazine (for conjugated ketones), and phosphomolybdic acid (for unconjugated ketones). The value of R_F fluctuated within the limits \$±\$0.05. The results obtained are summarized in Table 1. Below are several examples studied by us of the separation of mixtures of steroid compounds by means of

thin-layer chromatography.

Separation of mixtures of epimers: α- and β-5,6-oxy acetate of dehydroepiandrosterone (Fig. 1a) and α- and β-5,6-oxy acetate of ethinylandrostandiol (Fig. 1b) on a silica gel–gypsum plate.

Thin-layer chromatography was used by us to determine the composition of a mixture of products obtained from the mother liquor during recrystalli-

Table 1

No.	Compound	Cyclohexane–ethyl acetate	R_F	Development with H_2SO_4, daylight	Development with H_2SO_4, u.-v.	Development with $SbCl_5$, daylight	Development with $SbCl_5$, u.-v.
1	Dehydroepiandrosterone	2 : 1	0.28 ± 0.03	+		+	
2	Dehydroepiandrosterone acetate	2 : 1	0.59 ± 0.02	+p		+ϕ	
3	17α-Ethynyl-Δ^5-androstendiol-3β, 17β	2 : 1	0.35 ± 0.02	+p	+	+	
4	3-Acetate-17α-ethynyl-Δ^5-androstendiol-3β, 17β	2 : 1	0.55	+p			
5	3,17-Diacetate-17α-ethynyl-Δ^5-androstendiol-3β, 17β	2 : 1	0.75±0.7	+p	+	+ϕ	+

No.	Compound	Cyclohexane–ethyl acetate	R_F	Development with H_2SO_4, daylight	Development with H_2SO_4, u.-v.	Development with $SbCl_5$, daylight	Development with $SbCl_5$, u.-v.
6	3,17-Diacetate-5,21,21-tribromopregnanetriol-3β, 6, 17β	2 : 1	0.27 ± 0.03	+g weak	+	−	+
7	5α, 6α-Oxide of epiandrosterone acetate	2 : 1	0.34	+z heated	−		
7	5α, 6α-Oxide of epiandrosterone acetate	1 : 1	0.66 ± 0.03	+z heated	−		
8	5β, 6β-Oxide of epiandrosterone acetate	2 : 1	0.38	+z heated	−		
8	5β, 6β-Oxide of epiandrosterone acetate	1 : 1	0.59 ± 0.03	+z heated	−		

No.	Compound	Cyclohexane–ethyl acetate	R_F	Development with H_2SO_4, daylight	Development with H_2SO_4, u.-v.	Development with $SbCl_5$, daylight	Development with $SbCl_5$, u.-v.
9	3-Acetate-5α, 6α-oxy-17α-ethynyl-androstendiol-3β, 17β	1 : 1	0.80 ± 0.01	+p			
10	3-Acetate-5β, 6β-oxy-17α-ethynyl-androstendiol-3β, 17β	1 : 1	0.69 ± 0.01	+p			
11	Cortisone	2 : 1	0.78	+g	+		
12	17α-Acetate of pregnine	2 : 1	0.51	+g	+		
12	17α-Acetate of pregnine	2 : 3	0.86	+g	+		
13	17-Acetoxy-21,21-dibromo-Δ^4-pregnenol-17β-dione 3,20	2 : 1	0.39	–	–		

No.	Compound	Cyclohexane–ethyl acetate	R_F	Development with H_2SO_4, day-light	Development with H_2SO_4, u.-v.	Development with $SbCl_5$, day-light	Development with $SbCl_5$, u.-v.
13	17-Acetoxy-21,21-dibromo-Δ^4-pregnenol-17β-dione 3,20	2 : 3	0.75 ± 0.05				
14	17-Acetoxy-Δ^4-pregnendione-3,20	2 : 1	0.35	–	–		
14	17-Acetoxy-Δ^4-pregnendione-3,20	2 : 3	0.68 ± 0.03				
15	Δ^4-Androstene-3,17-dione	2 : 1	0.34				
15	Δ^4-Androstene-3,17-dione	2 : 3	0.65 ± 0.03	–	–		

Note. dn. –daylight; u.-v. –ultraviolet light. Coloration: p –pink; ϕ –violet; g –blue; the sign + means development; the sign – means no development. Compounds Nos. 7, 8, and 12 were also developed with phosphomolybdic acid, and compounds Nos. 12–15 with 2,4-dinitrophenylhydrazine.

crystallization of the final product (III) of the reaction (Fig. 2a)

$$\text{(I)} \xrightarrow[\text{Ac}_2\text{O}]{} \text{(II)} \xrightarrow{\text{HOBr}} \text{(III)}$$

as well as for separating an artificial mixture of substances formed successively in the three stages of the synthesis (Fig. 2b).

Fig. 1 and Fig. 2: thin-layer chromatograms

Figure 1: Fig. 1 and Fig. 2: thin-layer chromatograms

It is evident from Fig. 2a that the mixture of products obtained from the mother liquor consists of tribromide III (blue spot) and also some unknown product, possibly a dibromide (blue spot), and does not contain the starting products of the two preceding stages I and II (pink spots).

We were unable to develop with conc. H_2SO_4 17-acetoxy-21,21-dibromo-Δ^4-pregnenol-17β-dione-3,20, 17-acetoxy-Δ^4-pregnendione-3,20, and Δ^4-androstendione-3,17 (Table 1, Nos. 13-15), or to separate a mixture of these compounds in the cyclohexane–ethyl acetate system (2 : 1) and (2 : 3). They were developed with a solution of 2,4-dinitrophenylhydrazine.

Experimental Part

To obtain chromatographic plates, glass plates measuring $11 \times 17.5 \times 0.2$ cm and ground silica gel (195-200 mesh) of grade KSK were used, and medical gypsum was used as the binder. 6.9 g of silica gel and 0.35 g of gypsum were shaken with 18 ml of distilled water. The resulting thick homogeneous mass was poured onto the glass and leveled over its surface with a spatula and by shaking. The plate was left

Fig. 1. a–solvent system: cyclohexane-ethyl acetate (1 : 1); run 1 hour 15 min.; development with conc. sulfuric or phosphomolybdic acid.
1–5α, 6α-oxide of epiandrosterone acetate, $R_F = 0.69$ (Table 1, No. 7); 2–5β, 6β-oxide of epiandrosterone acetate, $R_F = 0.62$ (Table 1, No. 8); 3–mixture of α- and β-5,6-oxides of epiandrosterone acetate; α-oxide $R_F = 0.73$; β-oxide $R_F = 0.64$ (Table 1, Nos. 7, 8).
b–same system, development with conc. sulfuric acid. 1–3-acetate-5α, 6α-oxide-17α-ethynylandrostane-3β, 17β, $R_F = 0.82$ (Table 1, No. 9); 2–3-acetate-5β, 6β-oxide-17α-ethynylandrostenediol-3β, 17β, $R_F = 0.68$ (Table 1, No. 10); 3–mixture of α- and β-oxides (Table 1, Nos. 9 and 10), α-oxide $R_F = 0.83$; β-oxide $R_F = 0.66$.

Fig. 2. a–silica gel-gypsum plate; solvent system cyclohexane-ethyl acetate (2 : 1), run 1 hour 20 min.; development with conc. sulfuric acid and irradiation with UV light.
1–17α-ethynyl-Δ^5-androstenediol-3β, 17β, $R_F = 0.35$ (Table 1, No. 3), pink spot; 2–3,17-diacetate-17α-ethynyl-Δ^5-androstene-3β, 17β, $R_F = 0.74$ (Table 1, No. 5), pink spot; 3–3,17-diacetate-5,21,21-tribromopregnanetriol-3β, 6, 17β, $R_F = 0.30$ (Table 1, No. 6), blue spot; 4–mixture of products obtained from the mother liquor after recrystallization III, $R_F = 0.30$ (Table 1, No. 6) and $R_F = 0.45$, unknown product. Spots blue.
b–same system, run 55 min., development with conc. sulfuric acid. 1–17α-ethynyl-Δ^5-androstenediol-3β, 17β, $R_F = 0.35$ (Table 1, No. 3); 2–3-acetate-

17α-ethynyl-Δ^5-androstenediol-3β,17β, R_F = 0.55 (Table 1, No. 4); 3–3,17-diacetate-17α-ethynyl-Δ^5-androstenediol-3β,17β, R_F = 0.68 (Table 1, No. 5); 4—mixture of products (Table 1, Nos. 3, 4, and 5), for No. 3 R_F = 0.32; for No. 4 R_F = 0.54; for No. 5 R_F = 0.68.

for 20 min. on a horizontal surface, dried (20 min.), and activated in a drying oven at 105–110° for 1 hour. It was then cooled and stored in a desiccator over blue gel (silica gel impregnated with cobalt salts).

At a distance of 1.5 cm from the lower edge of the plate, starting points 1.5-2 cm apart were applied with a sharp pencil. The substances under study were dissolved in 0.2 ml of ether or chloroform, and about 0.07 ml of solution was applied to the plate with a micropipette (approximately 10–20 γ). The plate was placed vertically in a glass accumulator jar (13 × 18 × 23 cm) with solvent so that it was immersed only 0.5 cm in the liquid, and the jar was closed with a ground glass plate. When the liquid had risen 9-10 cm (from 30 min. to 1.5 hours), the plate was removed, the solvent front was marked, and the plate was dried at room temperature or in a drying oven at 40–50°, and then developed.

Spraying with conc. H_2SO_4 was used as the developing agent. In almost all cases colored spots appeared immediately, although in some cases it was necessary to heat the plate to 80°. For determination of a conjugated double bond, spraying was carried out with a solution of 2,4-dinitrophenylhydrazine ([3]), prepared as follows: 150 mg of 2,4-dinitrophenylhydrazine were dissolved in 25 ml of water and 22 ml of conc. HCl; the resulting solution was diluted with water to 100 ml. For determination of carbonyl compounds, spraying with a 10% solution of phosphomolybdic acid in alcohol was used, and after spraying the plate was heated for 1 min at 110° until spots appeared ([3]).

Thus, the possibility has been demonstrated of separating certain steroid compounds by means of thin-layer chromatography. Conc. H_2SO_4 was used as the developing agent. Two examples are given of the separation of epimeric oxides of steroid compounds.

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