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Note

Separation of some androgens and estrogens by thin-layer chromatography

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During the synthesis of some new androgens (3β,17β-dihydroxy-16-oximino-5-androstene; 3β,17β-dihydroxy-16,17-seco-5-androsteno-16-nitrile; 3β,17β-dihydroxy-17α-picolyl-5-androstene; 3β-acetoxy-17β-hydroxy-17α-picolyl-5-androstene; 3β-hydroxy-17-picolinyliden-5-androstene; 3β-acetoxy-17-picolinyliden-5-androstene; 3β,17β-dihydroxy-16-oximino-17α-picolyl-5-androstene)[1] and estrogens (17β-hydroxy-16-oximinoestrone 3-methyl ether; 16,17-seco-17-oxoestrono-16-nitrile 3-methyl ether)[2], mixtures of particular derivatives were obtained. For the control of the synthesis, it was necessary to identify these derivatives.

Identification was carried out by thin-layer chromatography (TLC) on silica gel layers. Various solvent systems for steroid separations have been described[3–13], but none of them was found to be completely adequate for the separation of the particular mixtures in which we were interested.

This paper describes some modificated developing systems for the TLC of the steroids examined.

EXPERIMENTAL

For the preparation of the thin layers, silica gel G (Merck, Darmstadt, G.F.R.) was used. Silica gel (30 g) was suspended in 75 ml of distilled water and the suspension was coated on to glass plates (20 × 20 cm) with Desaga equipment. The thin layers were dried in air at room temperature and activated for 1 h at 110°.

The mixture of eleven androgens (Table I) and the mixture of five estrogens (Table II) were examined. Solutions of each steroid in chloroform (0.5%) were prepared, and 0.5 μl of each steroid solution and the mixtures were applied to the chromatoplate with a micropipette.

The chromatograms were developed at room temperature in a glass chamber containing 50 ml of solvent mixture without previous saturation, by one- and two-dimensional ascending chromatography. The following solvent systems were used for the separation of androgens:

(A) benzene–acetone (7:1) in the first dimension;
(B) benzene–ethyl acetate (2:1) in the second dimension; and for the estrogens:
(C) benzene–ethyl acetate (7:1);
(D) benzene–acetone (8:1);

TABLE I

R_F VALUES AND COLOURS OF THE ANDROGENS DEVELOPED IN SOLVENT SYSTEMS A AND B

No.	*Androgen*	$R_F \times 100$		*Colour*
		A	*B*	
1	3β,17β-Dihydroxy-17α-picolyl-5-androstene	34	37	Grey
2	3β-Acetoxy-17β-hydroxy-17α-picolyl-5-androstene	78	75	Brown
3	3β-Acetoxy-17-picolinyliden-5-androstene	83	93	Dark red
4	3β-Hydroxy-17-picolinyliden-5-androstene	40	49	Dark red
5	3β,17β-Dihydroxy-16-oximino-17α-picolyl-5-androstene	6	4	Red
6	3β-Hydroxy-16-oximino-5-androsten-17-one	17	27	Pink
7	3β-Acetoxy-5-androsten-17-one	80	94	Violet
8	Lactone of 3β,17β-dihydroxy-16,17-seco-androsten-16-oic acid	24	30	Grey-violet
9	3β,17β-Dihydroxy-16,17-seco-5-androsteno-16-nitrile	13	18	Red
10	3β,17β-Dihydroxy-16-oximino-5-androstene	0	0	Crimson
11	3β-Hydroxy-5-androsten-17-one	42	53	Violet

TABLE II

R_F VALUES AND COLOURS OF ESTROGENS DEVELOPED IN SOLVENT SYSTEMS C–G

No.	*Estrogen*	$R_F \times 100$					*Colour*
		C	*D*	*E*	*F*	*G*	
1	17β-Hydroxy-16-oximinoestrone 3-methyl ether	4	10	13	29	16	Yellow
2	16-Oximinoestrone 3-methyl ether	26	38	57	37	57	Violet
3	16,17-seco-17-Oxoestrono-16-nitrile 3-methyl ether	74	83	94	47	86	Lemon yellow
4	Estrone 3-methyl ether	83	91	98	78	93	Orange
5	Estrone	46	52	80	40	75	Orange-yellow

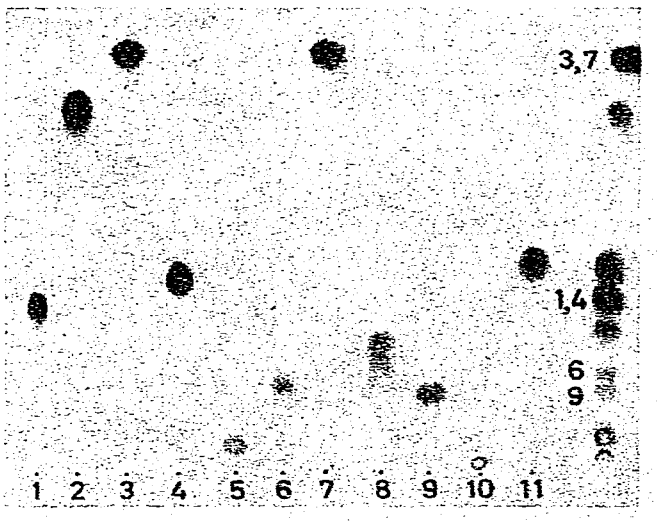

Fig. 1. Chromatogram of androgens 1–11 (Table I) in solvent system A.

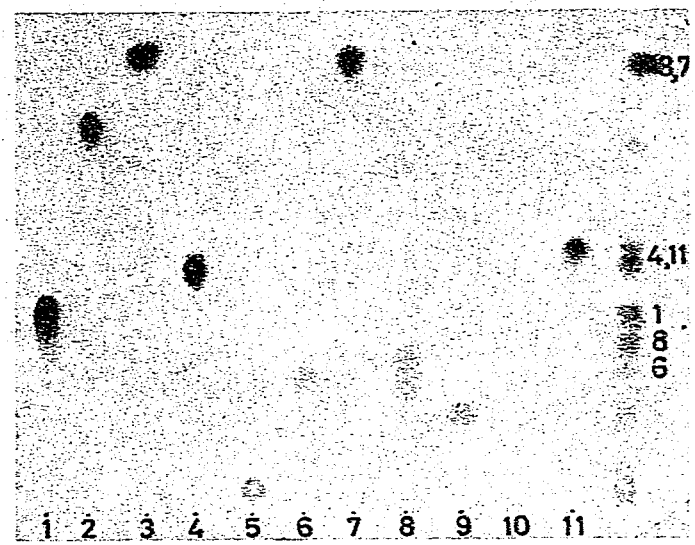

Fig. 2. Chromatogram of androgens 1–11 (Table I) in solvent system B.

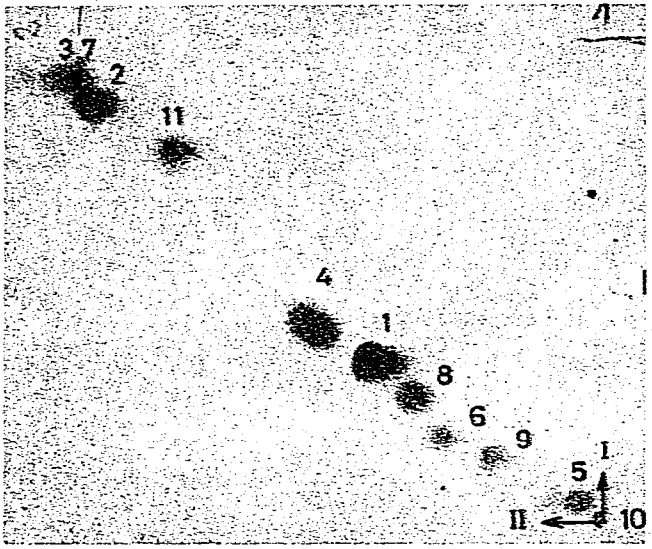

Fig. 3. Two-dimensional chromatogram of androgens 1–11 (Table I). Run I, solvent system A; run II, solvent system B.

(E) chloroform–ethyl acetate (4:1);
(F) *n*-hexane–acetone (2:1);
(G) carbon tetrachloride–ethyl acetate (2:1).

The developed and dried chromatograms were developed by spraying with 50% sulphuric acid in methanol and heating in an oven for 10–15 min at 100–110°.

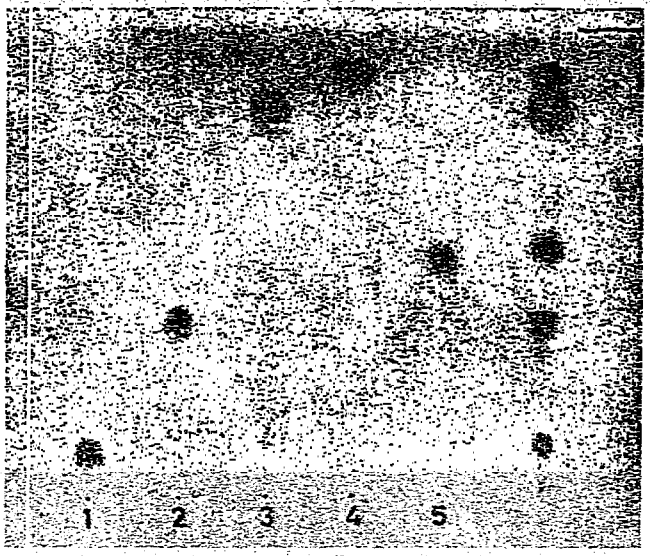

Fig. 4. Chromatogram of estrogens 1–5 (Table II) in solvent system C.

RESULTS

Both solvent systems A and B clearly resolved nine androgens by one-dimensional chromatography (Figs. 1 and 2). The $R_F \times 100$ values for each steroid are recorded in Table I. The same solvents separated ten androgens by two-dimensional chromatography (Fig. 3), but no separation of 3β-acetoxy-17-picolinyliden-5-androstene and 3β-acetoxy-5-androsten-17-one was obtained.

All estrogens were clearly resolved by one-dimensional chromatography with the solvents used (Table II). The best separation was obtained with solvent system C (Fig. 4).

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