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Note

Separation of some estrogens by thin-layer chromatography

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In a previous paper[1], a mixture of five estrogens was separated by thin-layer chromatography (TLC) on silica gel layers, in addition to a mixture of some androgens. This paper describes the separation of a group of estrogens obtained by further chemical transformations of estrone derivatives[2,3].

EXPERIMENTAL

Thirty grams of silica gel G (Merck, Darmstadt, G.F.R.) were suspended in 60 cm^3 of distilled water and the suspension was coated on to glass plates (20 × 20 cm) with Desaga equipment. The layers were dried in air at room temperature (relative humidity 30–40%) and used for chromatography. Solutions of thirteen estrogens (Table I) in chloroform (0.5%) were prepared, and $5 \cdot 10^{-4}$ cm^3 of each estrogen solution alone and in mixtures were applied to the chromatoplate with a micropipette.

After spotting the samples, the layers were conditioned at room temperature for 1 h in the chromatographic chamber, containing the solvent, and developed by one- and two-dimensional ascending chromatography. The following solvent systems were used for the separations: (A) benzene–acetone (8:1); (B) benzene–ethyl acetate (7:1); (C) chloroform–ethyl acetate (4:1); (D) *n*-hexane–acetone (2:1); (E) cyclohexane–acetone (8:1). For two-dimensional TLC, solvent A was used in the first dimension and solvent E in the second dimension.

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

RESULTS

The silica gel layers were not activated before chromatography, because no substantial differences in the separation efficiency between activated and unactivated layers were observed. The conditioned layers gave sharper spots than the unconditioned layers.

Under the conditions used, solvent system A clearly resolved eleven of the thirteen estrogens by one-dimensional chromatography (Fig. 1). The other solvent

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TABLE I

R_F VALUES AND COLOURS OF ESTROGENS DEVELOPED IN SOLVENT SYSTEMS A–E

No.	*Estrogen*	$R_F \times 100$					*Colour*
		A	*B*	*C*	*D*	*E*	
1	Estrone	56	46	89	56	18	Orange-yellow
2	3-Methoxyestra-1,3,5(10)-trien-17-one	80	72	95	90	54	Orange
3	3-Methoxy-16-oximino-estra-1,3,5(10)-trien-17-one	45	30	73	48	11	Violet
4	3-Methoxy-16-oximino-estra-1,3,5(10)-trien-17β-ol	10	5	18	33	4	Yellow
5	3-Methoxy-17-oxo-16,17-*seco*-estra-1,3,5(10)-triene-16-nitrile	74	65	95	65	25	Lemon yellow
6	3-Methoxy-17-hydroxy-16,17-*seco*-estra-1,3,5(10)-triene-16-nitrile	33	18	55	45	9	Lemon yellow
7	3-Methoxy-17-hydroxy-16,17-*seco*-estra-1,3,5(10)-trien-16-amine hydrochloride	0	0	0	6	0	Grey
8	3-Methoxy-17-hydroxy-16,17-*seco*-estra-1,3,5(10)-trien-16-amine	0	0	2	11	0	Grey
9	3-Methoxy-17-oxa-D-*homo*-estra-1,3,5(10)-trien-16-one	62	42	95	63	22	Lemon yellow
10	3-Methoxy-17-oxa-D-*homo*-estra-1,3,5(10)-trien-16-ol	36	24	63	61	20	Dark red
11	3-Methoxy-16,17-*seco*-estra-1,3,5(10)-trien-16,17-diol	16	7	32	62	9	Orange
12	3-Methoxy-17-oxa-D-*homo*-estra-1,3,5(10)-triene	81	73	95	90	69	Red
13	3-Methoxy-17-aza-D-*homo*-estra-1,3,5(10)-triene-16,17a-dione	40	25	78	41	11	Yellow

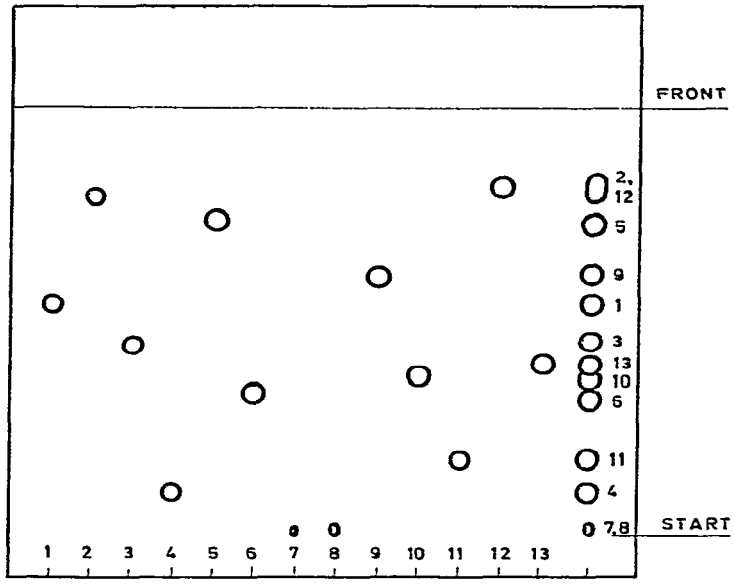

Fig. 1. Chromatogram of estrogens 1–13 (Table I) in solvent system A.

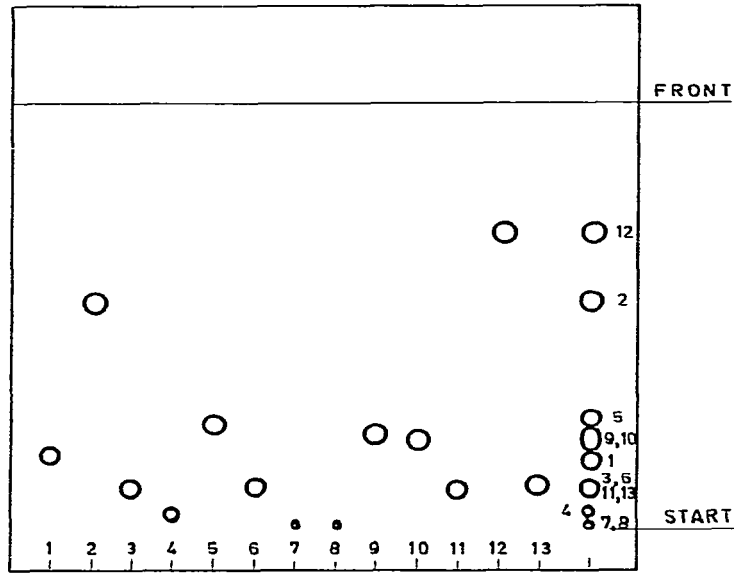

Fig. 2. Chromatogram of estrogens 1–13 (Table I) in solvent system E.

systems tested resolved 6–10 estrogens (the $R_F \times 100$ values for each estrogen are given in Table I). Only solvent system E separated 3-methoxyestra-1,3,5(10)-trien-17-one (2) from 3-methoxy-17-oxa-D-*homo*-estra-1,3,5(10)-triene (12) (Fig. 2). Therefore, solvent systems A and E were used for two-dimensional chromatography, and twelve estrogens were separated (Fig. 3).

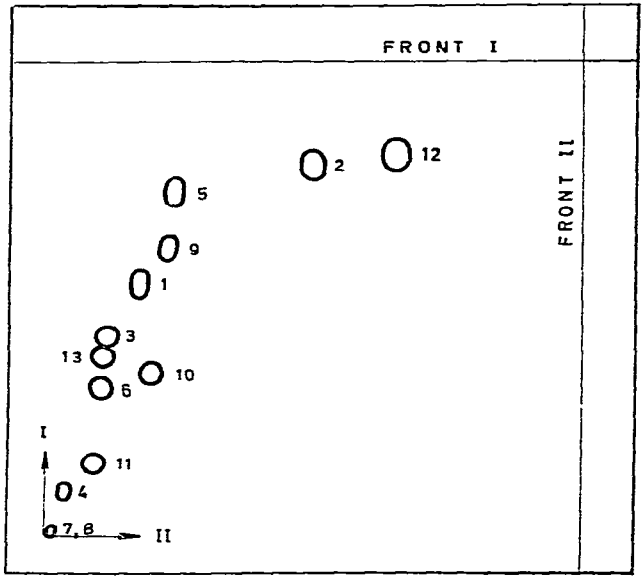

Fig. 3. Two-dimensional chromatogram of estrogens 1–13 (Table I). Run I, solvent system A; run II, solvent system E.

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