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Note

Thin-layer chromatographic systems for the separation of some estrogen acetates

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Despite the advent of more sophisticated analytical procedures for the separation and characterisation of steroids, thin-layer chromatography (TLC) remains an invaluable aid for rapid, inexpensive, qualitative analysis, and many systems have been developed for the examination of a wide range of these compounds. Although the separation of the "classical" estrogens (estrone*, estradiol-17β and estriol) and related hydroxysteroids has been reported in considerable detail[1–3], much less information is available concerning the behaviour of estrogen acetates in thin-layer systems. While Varon *et al.*[4] compared methods for the detection of free estrogens and their acetates, the investigation was limited to estrone, estradiol-17β and estriol in free and acetylated forms.

This paper reports our experience with three systems used to check the homogeneity of estrogen acetates employed as substrates in studies of enzymic transformations in human and animal tissues, and for the tentative identification of steroid products in such reactions.

MATERIALS AND METHODS

Materials

Water was glass-distilled. All organic solvents were redistilled before use except for cyclohexane which was Spectroscopic Grade. Silica gel G was from Riedel-de Haën (Sellze, Hannover, G.F.R.) Sulphuric acid (sp.gr. 1.84) was Analytical Grade. Estrogen acetates were gifts from Miss P. Carter, Harvard Medical School, Boston, MA, U.S.A., and Professor D. N. Kirk, Medical Research Council Steroid Reference Collection, Great Britain, or they were bought from Sigma (St. Louis, MO, U.S.A.).

* The following trivial names are used in this text: Estrone enol diacetate = 1,3,5(10),16-Estratetraene-3,17β-diol diacetate; 16α-Hydroxyestrone diacetate = 1,3,5(10)-Estratriene-3,16α-diol-17-one diacetate; 16β-Hydroxyestrone diacetate = 1,3,5(10)-Estratriene-3,16β-diol-17-one diacetate; Estradiol-17β 3-monoacetate = 1,3,5(10)-Estratriene-3,17β-diol 3-acetate; Estradiol-17β 17-monoacetate = 1,3,5(10)-Estratriene-3,17β-diol 17-acetate; Estradiol-17β diacetate = 1,3,5(10)-Estratriene-3,17β-diol diacetate; 6-Dehydroestradiol diacetate = 1,3,5(10),6-Estratretaene-3,17β-diol diacetate; Estriol triacetate = 1,3,5(10)-Estratriene-3,16α,17β-triol triacetate; Estrone acetate = 1,3,5(10)-Estratrien-3-ol-17-one acetate; Estrone = 1,3,5(10)-Estratrien-3-ol-17-one; Estradiol-17β = 1,3,5(10)-Estratriene-3,17β-diol; Estradiol-17α = 1,3,5(10)-Estratriene-3,17α-diol; Estriol = 1,3,5(10)-Estratriene-3,16α,17β-triol.

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Thin-layer chromatography

Glass plates (10 × 20 cm) were coated to a thickness of 0.25 mm with a suspension of silica gel (30 g in 60 ml water) using a commercial spreader (Quickfit Instruments, Great Britain). The plates were dried in air at room temperature (≈20°C) before being heated for 1 h at 100°C and stored in a desiccator over silica gel.

Solutions of steroids were prepared in methanol and 30 nmol of each was applied in duplicate or triplicate to the origin of a thin-layer plate. The solvent systems were 2,2,4-trimethylpentane–ethyl acetate (4:1), cyclohexane–acetone (6:1) and cyclohexane–ethyl acetate (6:1) and were poured into each glass tank (internal dimensions *ca.* 6 × 23.5 cm).

The systems were allowed to equilibrate for 2–4 h at room temperature before chromatograms were developed. When the tanks were lined with filter-paper "curtains" the running time of chromatograms was approximately halved, however separation was superior in their absence and they were not routinely used. On completion, the plates were dried in air before being sprayed with a freshly prepared mixture of ethanol–sulphuric acid (1:1) and heated at 110°C for 10–20 min.

RESULTS AND DISCUSSION

The chromatographic behaviour of nine estrogen acetates, the three "classical" estrogens and estradiol-17α has been examined on silica gel plates in three solvent systems and the results are summarised in Table I.

TABLE I

TLC OF ESTROGEN ACETATES AND ESTROGENS IN THREE SOLVENT SYSTEMS

The solvent systems were: (1) trimethylpentane–ethyl acetate (4:1); (2) cyclohexane–acetone (6:1); (3) cyclohexane–ethyl acetate (6:1). A 30-nmol amount of steroid in methanol was applied to each origin and ascending chromatograms were developed at room temperature. Compounds were located by spraying with ethanol–H_2SO_4 (1:1) followed by heating at 110 C for 10–20 min.

Compound	R_F* (*n* = 3, *except where indicated*)			*Colour*
	1	*2*	*3*	
Estrone acetate	0.36 (4)	0.44	0.28	Yellow-orange
Estrone enol acetate	0.44	0.61	0.51	Orange
16α-Hydroxyestrone diacetate	0.16	0.25	0.15	Red
16β-Hydroxyestrone diacetate	0.26 (5)			Orange
Estradiol-17β 3-monoacetate	0.12 (6)	0.20	0.11	Orange
Estradiol-17β 17-monoacetate	0.26	0.28	0.24	Orange
Estradiol-17β diacetate	0.56	0.60	0.42 (4)	Yellow-orange
6-Dehydroestradiol diacetate	0.39	0.54	0.44	Lime-yellow
		0.50	0.41	Orange-pink
Estriol triacetate	0.30	0.31	0.16	Mauve
Estrone	0.18	0.19	0.16	Yellow
Estradiol-17α	0.10	0.12	0.10	Orange
Estradiol-17β	0.08	0.10	0.08	Yellow-orange
Estriol	Origin	Origin	Origin	Mauve

* Values represent the mean R_F of at least three determinations—standard deviations are not included but were all less than 10%.

Generally good separations were achieved in trimethylpentane–ethyl acetate, especially of the epimeric 16-hydroxyestrone diacetates. Unfortunately there was insufficient 16β-hydroxyestrone diacetate to permit its further examination in the cyclohexane systems, where the remaining estrogen acetates showed overall patterns of separation similar to those found in trimethylpentane–ethyl acetate, except for 6-dehydroestradiol diacetate. While the last-named compound appeared to be homogeneous in that system, it was resolved into two distinct components in cyclohexane–acetone and cyclohexane–ethyl acetate. Lack of material prevented characterisation of the authentic steroid.

The range of bright colours obtained after heating chromatograms sprayed with ethanol–sulphuric acid was of some use in assigning tentative identifications in conjunction with R_F values and they retained considerable intensity for up to 2 days if the chromatograms were covered with clean, dry glass plates. For example, estrone, 16α-hydroxyestrone diacetate and estriol triacetate shared similar R_F values in cyclohexane–ethyl acetate, but the differences in colour allowed discrimination. When these compounds were analysed in cyclohexane–acetone or trimethylpentane–ethyl acetate, they were completely separated. Small to moderate differences in the shade of colours produced after treatment with reagent were noted between chromatograms, particularly in respect of the mono- and diacetylated estrogens. Examination of the chromatograms under UV light, before and after spraying with reagent, was of little value in detection and was not routinely carried out.

In all three systems, the free estrogens remained close to or at the origin in the case of estriol, and were fairly well demarcated from the acetylated compounds.

The solvent mixtures described have proved useful for checking the purity of substrates before the investigation of enzyme-catalysed transformations in human and animal tissues, and for the provisional identification of steroid products from such reactions. They have also been employed to monitor the acetylation of polyols and may be of value in preparative TLC for "cleaning-up" compounds prior to gas–liquid chromatography, or for applications to column chromatography which frequently enhances resolution.

We have not investigated colour reactions for the *in situ* characterization of estrogens[2] nor have we explored the extensive range of reagents available for detection of these compounds as an aid to their recovery[4].

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