CHROM. 4618

Separation of some synthetic estrogens from natural estrogens by thin-layer chromatography*

Synthetic, non-steroidal estrogens are widely used in the therapy of various endocrine disorders and hormone-dependent cancer. They are also used in agriculture for the fattening of poultry and calves.

Synthetic estrogens such as DES, hexestrol and F6060** have physico-chemical properties which are very similar to those of the natural estrogen estradiol-17β, and there is no published method for the direct chromatographic separation of these compounds. In an investigation it became necessary to determine hexestrol and estradiol-17β separately in uterine tissue[1]. This problem initiated the present work. Since it is known that DES and F6060 (refs. 2 and 3, respectively) severely interfere with the colorimetric determination of natural estrogens, a method which separates synthetic from natural estrogens can be widely applied. The separation of the synthetic estrogens was not within the scope of this investigation.

Chromatographic techniques for the detection of residues of DES in foodstuff have been repeatedly described[4,5]. Thin-layer chromatography of DES and of natural estrogens using eight different solvent systems was described by WALDI[6]. He noted that DES and estradiol-17β had similar mobilities in all systems investigated, except in one (methylene chloride–acetone, 4:1) in which partial separation occurred. More recently SCHULLER[7] described thin-layer chromatography of DES in urine. However, none of these systems were satisfactory for the present purpose.

The substances were run in a number of solvent mixtures on Silica Gel H (Merck) layers. All solvents were of A.R. grade. A 0.25-mm-thick layer of silica gel was prepared with a Desaga spreading device. The plates were air dried and then activated at 100° for 30 min. They were stored in a desiccator until used. Of each test compound 5–10 μg were applied on the plates. The chromatograms were developed to a height of 11–12 cm. Then the plates were air dried, warmed and gently sprayed with a 1% solution of $KMnO_4$ in conc. H_2SO_4. The spots appeared after further warming over a hot plate.

The principal estrogens occurring naturally are estradiol-17β, estrone and estriol. The separation of synthetic estrogens from estradiol-17β has met with difficulties as mentioned above while they are easily separated from estrone and estriol. Table I shows the results obtained with some of the tested solvents. Solvents A, B (ref. 8) and C, which are acid or neutral, are representatives of a large number of solvents which did not separate estradiol-17β from the synthetics. When the solvent systems contained benzene (D and E), separation occurred. Estradiol-17β moved slower than the others, a tendency which increased with increasing benzene content. In alkaline solvents, of which solvent F is one example, separation also occurred. In this case estradiol-17β moved faster than the others. However, in alkaline benzene solvents

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** Trivial and chemical names of the studied compounds: DES is 3,4-bis(4'-hydroxyphenyl)-3-hexene; hexestrol is *meso*-3,4-bis(4'-hydroxyphenyl)-*n*-hexane; F6060 is bis(4'-hydroxyphenyl)-cyclohexylidene methane (the acetyl derivative of this compound is marketed under the name Sexovid®); estradiol-17β is estra-1,3,5(10)triene-3,17β-diol.

TABLE I

R_F VALUES AND SPOT COLORS OF ESTROGENS

Solvents: A, chloroform–acetic acid (85:15); B, chloroform–ethanol (95:5); C, chloroform–ethyl acetate (4:1); D, benzene–ethyl acetate (1:1); E, benzene–ethyl acetate (3:1); F, chloroform–diethylamine (9:1); G, benzene–diethylamine (4:1).

Estrogen	*R_F values in solvent*							*Color (1% $KMnO_4$ in H_2SO_4)*
	A	*B*	*C*	*D*	*E*	*F*	*G*	
Estradiol-17β	0.53	0.37	0.27	0.43	0.25	0.35	0.45	yellow
Hexestrol	0.48	0.32	0.37	0.59	0.44	0.19	0.41	light brown
DES	0.49	0.35	0.37	0.59	0.48	0.19	0.42	lilac
F6060	0.45	0.35	0.30	0.60	0.47	0.11	0.34	brown
Estrone	0.65	0.54	0.62	0.45	0.40	0.52	0.54	light brown
Estriol	0.22	0.08	0.09	0.03	0.07	0.18	0.08	red

(solvent G) no separation was found, the solvent components evidently counteracting each other.

From this limited examination and from data with other tested systems, some structural features responsible for the observed chromatographic data are suggested. Benzene with its aromatic character is less eluotrophic against estradiol-17β with one aromatic nucleus than against the synthetic estrogens which contain two; on the other hand, in alkaline solvents, the acid character of estradiol-17β with one phenolic hydroxyl is less pronounced than of the others which contain two. Thus estradiol-17β is more strongly eluted than the others.

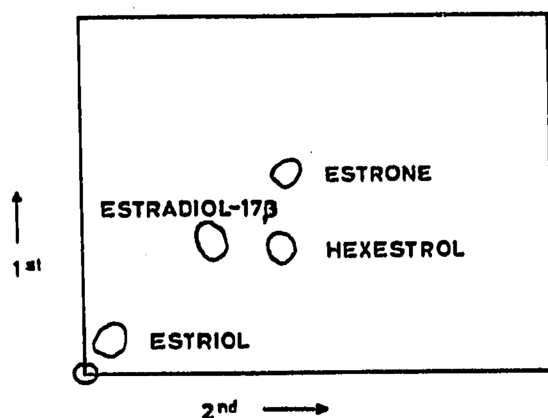

Fig. 1. Two-dimensional thin-layer chromatogram on Silica Gel H. Run 1 was performed with Solvent B (chloroform–ethanol, 95:5) and run 2 with solvent E (benzene–ethyl acetate, 3:1). Of each compound 5 µg were spotted.

Estrone and estriol are not well separated from the synthetic estrogens in the two systems (E and F) which separate estradiol-17β from the synthetics. Only on two-dimensional chromatograms was it possible to separate all three natural estrogens from the synthetic estrogens. In Fig. 1, which is a tracing of a typical chromatogram, hexestrol is well separated from the three natural estrogens which are also well separated from each other. By comparison with the R_F values of Table I, it is easily

seen that DES or F6060 can be separated equally well from the natural estrogens with the same solvent combination.

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1 L. TERENIUS, *Acta Pharmacol. Toxicol.*, 27 (1969) 120.
2 J. B. BROWN, R. D. BULBROOK AND F. C. GREENWOOD, *J. Endocrinol.*, 16 (1957) 41.
3 H. ADLERCREUTZ, C.-J. JOHANSSON AND T. LUUKKAINEN, *Ann. Med. Exptl. Fenniae*, 45 (1967) 269.
4 E. J. UMBERGER, D. BANES, F. M. KUNZE AND S. H. COLSON, *J. Assoc. Offic. Agr. Chemists*, 46 (1963) 471.
5 C. PONDER, *J. Assoc. Offic. Anal. Chemists*, 49 (1966) 489.
6 D. WALDI, in E. STAHL (Editor), *Dünnschicht-Chromatographie*, Springer, Berlin, Göttingen, Heidelberg, 1962, p. 270.
8 P. L. SCHULLER, *J. Chromatog.*, 31 (1967) 237.
7 B. P. LISBOA, *Clin. Chim. Acta*, 13 (1966) 179.

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A simple thin-layer chromatography of cannabinoids by means of silica gel sheets treated with amines

Thin-layer chromatography of cannabis extracts has been applied for detecting, isolating and determining various constituents of the hemp resin[1–4], for examining the composition of cannabis of various origins[1,5], as well as for forensic and toxicological purposes[6–11]. However, most techniques yielding a good separation are relatively complicated and time-consuming for simple and accurate routine testing, some of them requiring previous column purification of the extracts[3,4,10], others involving a multicomponent solvent system[6,7], the preparation of special plates[4,6,9], or their troublesome impregnation[1,2]. Some recent studies indicate that the presence of certain alkaline components in the solvent system, such as ammonia and diethylamine, may improve the separation of cannabinoids[4,7]. The possibility of using as solvent system simply one of the aromatic hydrocarbons has also recently been reported[3,5,12].

This note describes a highly sensitive technique for simple and rapid separation and detection of major cannabinoids, by means of silica gel pre-coated sheets previously dipped in diethylamine or dipropylamine and using toluene (or xylene) as a developing solvent. In this way, the advantages of both the presence of alkalies and of using one single solvent have been joined.

Method

Eastman Chromagram 6060 sheet (ethylene polyterephthalate film coated with