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Separation of free oestrogens and their monosulfate esters by thin layer chromatography

Interest in the sulfate esters of oestrogens in the maternal and fetal circulation and in ovarian tissues led to development of thin layer chromatographic systems for the separation and isolation of these conjugated steroids.

Previous studies of thin layer chromatography (TLC) of conjugated steroids have described the group separation of free steroids, steroid glucuronides and steroid sulfates[1]. Other investigators have also reported systems for the separation of neutral steroid sulfates[2–4], and two TLC systems for phenolic steroid sulfates have been described[5]. In this report we describe four solvent systems for the separation of the 3-monosulfate-esters of oestrone, 17β-oestradiol and oestriol by TLC.

Experimental

Reagents. All reagents were of analytical grade. Organic solvents were redistilled before use. Reagent grade ammonium hydroxide was used in the solvent systems.

Steroids. Oestrone-3-sulfate, 17β-oestradiol-17-sulfate and oestriol-3-sulfate were generous gifts from HANS FEX, Aktiebolaget Leo, Sweden. 17β-Oestradiol-3-sulfate was prepared by reduction of oestrone-3-sulfate with sodium borohydride. Dehydroepiandrosterone sulfate, oestrone, 17β-oestradiol and oestriol were purchased from Mann Research Laboratories, New York. Oestriol-16(17) glucuronoside was purchased from Sigma Chemical Co., St. Louis. The oestrogen sulfates were recrystallized from methanol and ether. The other steroids were used without further purification.

Method. The spreader for coating the plate was manufactured by Desaga, Brinkmann, U.S.A. The plates, 200 × 100 × 3.7 mm, were coated to 0.5 mm thickness with Silica Gel H (E. Merck), activated at 110° for one hour and stored in a dessicator over calcium chloride.

Solvent systems

(A) Benzene–methanol–ammonia (64:32:4)

(B) Benzene–methanol–chloroform–ammonia (24:30:42:4)

(C) Ethyl acetate–methanol–methylcyclohexane–ammonia (44:30:22:4)

(D) Methylene chloride–methanol–ammonia (70:22:8).

Solutions of the steroid samples in methanol (1 mg/ml) were applied at a rate of 50 μg of steroid as spots on TLC plates. To ensure saturation of the atmosphere the enclosed jars were lined with Whatman No. 1 filter paper 30 min before development of the chromatograms. The steroids were detected by spraying the air-dried plates with a 2 % solution of phosphomolybdic acid in ethanol followed by heating at 90° for 10 min. On some occasions the organic sulfate moiety was detected with the methylene blue reagent[2].

Results and discussion

Each steroid (Table I) was run simultaneously on a single plate in ten different experiments. The R_S value for each steroid was calculated as:

$$R_S = \frac{\text{distance from starting point to center of spot}}{\text{distance from starting point to center of } \textit{free} \text{ oestriol spot}}$$

TABLE I

R_F AND R_S (FREE ESTRIOL) FOR ESTROGENS AND THEIR 3-MONO-SULFATE ESTERS*

System		*Oestriol-3-SO_4*	*17β-Oestradiol-3-SO_4*	*Oestrone-SO_4*	*DHA-SO_4*	*Oestriol*	*17β-Oestradiol*	*Oestrone*
A	R_F	0.22 ± 0.03	0.30 ± 0.04	0.37 ± 0.05	0.37 ± 0.05	0.46 ± 0.05	0.52 ± 0.04	0.62 ± 0.03
	R_S	0.49 ± 0.04	0.66 ± 0.04	0.80 ± 0.04	0.80 ± 0.04	—	1.13 ± 0.03	1.34 ± 0.03
B	R_F	0.21 ± 0.04	0.29 ± 0.04	0.36 ± 0.04	0.38 ± 0.04	0.56 ± 0.05	0.71 ± 0.04	0.80 ± 0.04
	R_S	0.34 ± 0.03	0.52 ± 0.03	0.67 ± 0.03	0.68 ± 0.02	—	1.26 ± 0.04	1.42 ± 0.03
C	R_F	0.20 ± 0.02	0.32 ± 0.02	0.37 ± 0.02	0.36 ± 0.02	0.59 ± 0.02	0.74 ± 0.02	0.77 ± 0.02
	R_S	0.35 ± 0.02	0.55 ± 0.02	0.63 ± 0.03	0.61 ± 0.02	—	1.24 ± 0.03	1.32 ± 0.03
D	R_F	0.29 ± 0.04	0.34 ± 0.04	0.42 ± 0.03	0.40 ± 0.03	0.65 ± 0.02	0.85 ± 0.03	0.92 ± 0.03
	R_S	0.43 ± 0.05	0.53 ± 0.05	0.68 ± 0.05	0.64 ± 0.05	—	1.27 ± 0.06	1.36 ± 0.08

* Mean of 10 determinations ± S.D.

The mean R_F and R_S values and their standard deviations for each steroid in the four different solvent systems are shown in Table I. Oestriol-16(17) glucuronide stayed on or very near the starting spot in each solvent system. The R_F and R_S values of 17β-oestradiol-3-sulfate and 17β-oestradiol-17-sulfate were identical in all solvent systems.

In order to test the effect of hydrogen ion concentration on the mobility of the oestrogen sulfates, ammonia was replaced in each system with equal amounts of either distilled water or glacial acetic acid. Although these changes in pH did not appreciably alter any of the R_F or R_S values, considerable tailing of the oestrogen sulfates occurred in the neutral and acidic systems. When the solvent systems contained ammonia all of the free estrogens and their sulfate esters migrated as compact spots without tailing.

None of the solvent systems were capable of adequately separating oestrone sulfate from dehydroepiandrosterone sulfate when developed unidirectionally. These two sulfoconjugated steroids were separated only when bidimensional chromatography was carried out in solvent systems A or B followed by development at a right angle in system D.

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