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Note

Thin-layer chromatographic separation of oestrogen sulphates*

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Very few thin-layer chromatography (TLC) systems separate satisfactorily individual oestrogen monosulphates[1–4]. Among the more important oestrogen monosulphates are oestrone sulphate (E_1S), oestradiol-17β-3-sulphate ($E_2\beta$-3S) and oestradiol-17β-17-sulphate ($E_2\beta$-17S) which are present in high concentrations in the circulation of a number of mammalian and avian species. In the course of our investigation of these oestrogen monosulphates in the domestic hen, we have developed two TLC systems which, when used in combination, give good separation of E_1S, $E_2\beta$-3S and $E_2\beta$-17S.

MATERIALS AND METHODS

All solvents were reagent grade and were used as obtained. Steroids were purchased from Steraloids, Wilton, NH, U.S.A. Aluminium plates, 20 × 20 cm, coated with 0.20 mm silica gel 60 F_{254} were purchased from Brinkman Instruments, Rexdale, Canada. TLC system 1 (see Table I) was developed in this laboratory. TLC system 2 was a modification of a system described by Sarfaty and Lipsett[3]. The chromatography tank (length 27 cm, width 7 cm, height 20 cm) was lined with filter paper which was wetted with the solvent system as the latter was poured into the tank. Only 5-min equilibration was required in either system provided the filter paper was wetted.

Oestrogen standards dissolved in methanol (20–30 μg/0.01 ml) were applied to the plate either singly or in combination, and the plate was developed, in sequence, twice in system 1, once in system 2, and once again in system 1 (Table I); each time the solvent front was allowed to migrate 16.5 cm from the line of sample application. The plate was air-dried between developments. The times taken for one development in system 1 and system 2 were approximately 65 min and 50 min, respectively, at 24°C. The steroid spots on the plate (with fluorescing background) were located visually under UV. After the final development, the locations of the spots were verified by spraying with 10% sulphuric acid in methanol and then heating to 100 C for 5–10 min or until yellowish-red spots appeared.

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TABLE I

TLC SEPARATION OF E_1S, $E_2\beta$-3S AND $E_2\beta$-17S

System 1: ethanol–chloroform–3% ammonia (45:90:1.8); system 2: ethanol–ethyl acetate–30% ammonia (45:90:1.8). Figures in parentheses are R_F values: distance travelled by the compound after successive developments divided by the distance travelled by the solvent front (16.5 cm).

Development	*System*	*E_1S*	*$E_2\beta$-3S*	*$E_2\beta$-17S*
1st	1	4.7* (0.29)	4.1 (0.25)	3.5 (0.21)
2nd	1	7.3 (0.44)	6.4 (0.39)	5.8 (0.35)
3rd	2	10.6 (0.64)	9.7 (0.59)	8.9 (0.54)
4th	1	11.6 (0.70)	10.7 (0.65)	9.7 (0.59)

* Cumulative distance travelled (cm) after successive developments.

RESULTS AND DISCUSSION

System 1 was designed primarily for the separation of E_1S from $E_2\beta$-3S and system 2 was designed for the separation of $E_2\beta$-17S. As indicated in Table I, after the first two developments in system 1, E_1S and $E_2\beta$-3S (0.9 cm apart) were better separated than $E_2\beta$-3S and $E_2\beta$-17S (0.6 cm apart). However, after the third development, in system 2, the three oestrogen sulphates were almost equally separated (approx. 0.9 cm apart). The fourth development in system 1 only marginally improved the separation of $E_2\beta$-3S and $E_2\beta$-17S and therefore is optional in routine work.

Under the same conditions, unconjugated oestrogens ran near the solvent front, the disulphates of oestradiol-17β ran just above the origin, and the oestrogen glucuronides hardly moved. However, for group separation of the oestrogens (free, sulphates and glucuronides) the system described by Sarfaty and Lipsett[3] is recommended.

Our present systems do not separate the isomer oestradiol-17α-3-sulphate from $E_2\beta$-3S or oestradiol-17α-17-sulphate from $E_2\beta$-17S. We know of no TLC system which does so.

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