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lew thin-layer chromatographic solvent systems for separating steroid ormones

Three years ago we began research to determine if steroid hormones were ormally present in cow's milk. It led to the development of two one-dimensional 'LC systems for rapidly separating common reference sex and adrenal hormones.)uring this period several different TLC solvent systems were reported for separating teroid hormones[1–5]. But since our solvent systems are different, they may be helpful ɔ other investigators.

Aethods

All solvents were ACS grade and used as obtained from the manufacturer. They ere: anhydrous diethyl ether, petroleum ether, 1-butanol, and formic acid. Silica el G (according to Stahl) was acquired from Brinkmann Instruments, Inc., N.Y. he steroid hormones (Figs. 1 and 2) used in this investigation were purchased from Mann Research Laboratories, Inc., except 4-androsten-17α-ol-3-one, which was cquired from Steraloids, Inc. Plates 0.25 mm thick were prepared using a suspension

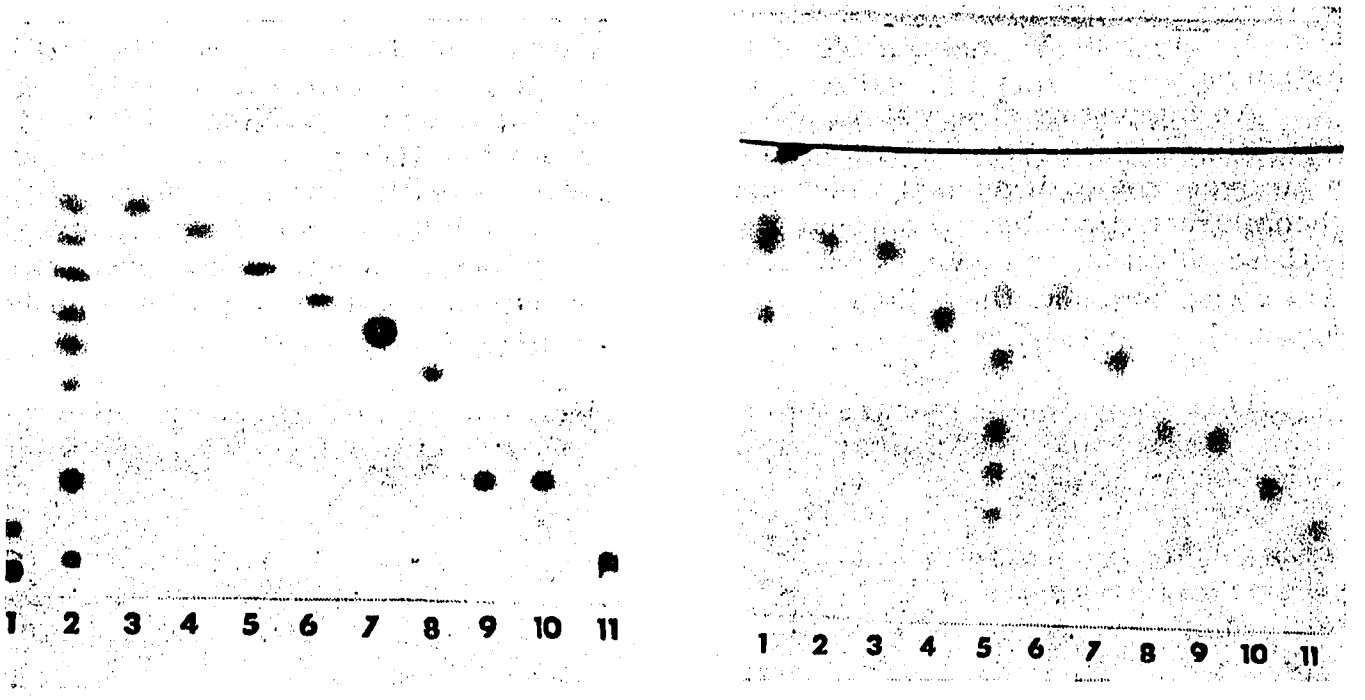

Fig. 1. Separation of sex hormones in system 1. Reference samples: (1) mixture of corticosteroids, (2) mixture of sex hormones, No. 3–11, (3) 1,3,5(10)-estratriene-3,17β-diol 17-acetate, (4) 1,3,5(10)-estratrien-3-ol-17-one 3-acetate, (5) 1,3,5(10)-estratrien-3-ol-17-one, (6) 1,3,5(10)-estratriene-3,17α-diol, (7) 1,3,5(10)-estratriene-3,17β-diol, (8) 4-pregnene-3,20-dione, (9) 4-androsten-17α-ol-3-one, (10) 4-androsten-17β-ol-3-one, and (11) 1,3,5(10)-estratriene-3,16α,17β-triol.

Fig. 2. Separation of corticosteroids in system 2. Reference samples: (1) mixture of sex hormones, (2) 4-androsten-17α-ol-3-one, (3) 4-androsten-17β-ol-3-one, (4) 1,3,5(10)-estratriene-3,16α,17β-triol, (5) mixture of corticosteroids, No. 6–11, (6) 4-pregnene-17α,21-diol-3,11,20-trione 21-acetate, (7) 4-pregnen-21-ol-3,20-dione, (8) 4-pregnene-17α,21-diol-3,11,20-trione, (9) 4-pregnene-11β,17α,21-triol-3,20-dione, (10) 4-pregnene-11β,21-diol-3,20-dione, and (11) 4-pregnen-21-ol-3,11,20-trione.

of 30 g Silica Gel G in 60 ml of 0.01 *M* sodium carbonate[6]. Approximately 1 h before use, a plate was activated at 120° and a developing chamber was saturated with vapors of its solvent.

The reference hormones were dissolved in chloroform–methanol (2:1) and applied to the plates with disposable micropets. All compounds were spotted at a concentration of *ca.* 5 μg, except 4-pregnene-3,20-dione which was about 10 μg.

The two developing solvent systems were: (1) petroleum ether–diethyl ether–formic acid (100:100:2), and (2) petroleum ether–diethyl ether–1-butanol–formic acid (100:50:30:2). Both systems moved 15 cm from the point of spotting. The spots were observed after spraying the plate with H_2SO_4–H_2O (1:1) and heating in an oven at 120° until the organic material colored and charred.

Results and discussion

The hormones were initially grouped as sex and adrenal. System 1 (Fig. 1) was developed to separate sex hormones: system 2 (Fig. 2), adrenal hormones. Excellent separation of the common sex hormones except the α- and β-isomers of 4-androsten-17-ol-3-one (No. 9 and 10) and 1,3,5(10)-estratriene-3,16α,17β-triol (No. 11) is seen in Fig. 1. But in Fig. 2, 1,3,5(10)-estratriene-3,16α,17β-triol (No. 4) separates cleanly with five of the six corticosteroids. Identical R_F values are observed for 4-pregnene-17α,21-diol-3,11,20-trione (No. 8) and 4-pregnene-11β,17α,21-triol-3,20-dione (No. 9). Also, the isomers of 4-androsten-17-ol-3-one (No. 2 and 3) do not separate with system 2.

An interesting observation was recorded in our laboratory. When TLC plates containing reference steroid hormones were sprayed with a H_2SO_4 solution and heated in an oven, colors developed which could be associated with certain hormones. But when extracts from bovine adrenal glands and corpora lutea were chromatographed in these solvents, compounds with identical R_F values to the reference hormones were seen; however, they did not color. This observation cannot be satisfactorily explained.

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