CHROM. 3638

Improved solvent systems for thin layer chromatography of estrogens*

There are many systems for separation of steroids and other compounds by thin layer chromatography (TLC). Many systems[1–3] were developed by following the separation of pure reference compounds. Consequently, some systems are found to be inadequate when applied to extracts of biological materials. The present paper describes solvent systems for TLC developed for the separation of estrogens in extracts of urine. Unknown substances, which were not separated in "conventional" systems, were separated from the estrogens in the systems containing isopropyl ether.

Experimental

Reagents. Solvents were all of reagent grade and redistilled before use. Ethanol (Publicker Industries) was used as obtained from the supplier. Benzene was washed with dilute sulfuric acid, bicarbonate, and water before distillation. Isopropyl ether was stored over ferrous sulfate and distilled prior to use.

Steroids. The steroids were used as obtained from the supplier. Estrone-16 was obtained through the courtesy of Dr. M. N. HUFFMAN, Creighton University, Omaha, Nebr. The 2-methoxy estrogens were kindly supplied by Dr. JACK FISHMAN, Montefiore Hospital, New York, N. Y. The other estrogens were obtained from commercial sources.

Solvent systems. 1. 7 % ethanol in isopropyl ether; 2. 20 % acetone in isopropyl ether; 3. 10 % ethanol in benzene; 4. Ethyl acetate.

These mixtures were changed in the tanks each day.

Methods. Thin layer plates (obtained from Analtech, Wilmington, Delaware) coated with Silica Gel G (250 microns thickness) were used without prior preparation. Steroid solutions made up in *tert.*-butanol (1 mg/ml) were spotted on the plates using Hamilton microsyringes. Plates, 20 × 20 cm size, and spots of 10 μg steroid were used. These were developed at room temperature until the solvent front had reached 0.5 cm from the top of the plate. The time averaged 40 min. After drying at room temperature, estrogen zones were visualized by spraying with the ferric chloride–potassium ferricyanide reagent[4]. Measurements were made from the center of the spots. The R_F values were calculated in the usual manner.

Results and discussion

Each of the estrogens as listed in Table I was run singly and together with other members of the series. The average value for 4 determinations of the R_F for each of these steroids is given in Table I. For illustrative purposes, an extract of normal female urine was also subjected to separation in each of these systems.

In contemporary TLC systems, the estrone zone of extracts as prepared in these laboratories contains a substance which has the retention time of estradiol-17β on gas chromatography. Likewise, the estradiol-17β zone contains unknown substances. It appeared appropriate to investigate other systems. Thus, the described systems

* Supported in part by USPHS Grants HD-01199 & AM-K-14013 and a grant from the Ford Foundation.

TABLE I

R_F (× 100) VALUES FOR ESTROGENS IN DIFFERENT SOLVENTS FOR THIN LAYER CHROMATOGRAPHY
Each figure represents the average of 4 determinations.

Estrogen	*7 % Ethanol in isopropyl ether*	*20 % Acetone in isopropyl ether*	*Ethyl acetate*	*10 % Ethanol in benzene*[2,3]
Substance X	71	67	91	57
Estrone	66	58	93	57
Estrone-16*	64	56	95	56
16-Ketoestrone	65	45	96	51
2-Methoxyestrone	58	52	93	64
Estradiol-17β	51	45	78	40
Estradiol-17α	53	47	70	42
16-Ketoestradiol-17β	42	39	66	55
Estriol	11	7	18	15
Epiestriol	29	23	40	23
2-Methoxyestriol	9	6	16	17

* 3-Hydroxyestra-1,3,5(10)-triene-16-one.

containing isopropyl ether evolved. As seen in Table I, the substance denoted as X migrated with estrone in the systems not containing isopropyl ether. Other materials also separated from the estradiol-17β zone in the systems containing isopropyl ether. For this reason, preliminary purification of the urinary extraction prior to gas chromatography using these systems is carried out in this laboratory.

It will be noted that a greater separation of estriol and epiestriol was obtained in the systems containing isopropyl ether. The system 10 % ethanol in benzene gave the least separation. Different percentages of ethanol in benzene were also investigated, but no separation of X from estrone was obtained. The widest range of separation among substance X, estrone, estradiol-17β and estriol was found in the system 20 % acetone in isopropyl ether. At present, the identity of substance X and other substances separated by the newer systems is unknown.

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Received May 27th, 1968