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Note

Detection of trenbolone residues in meat and organs of slaughtered animals by thin-layer chromatography

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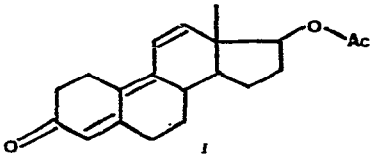
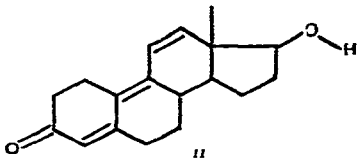
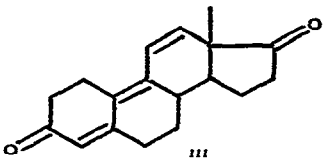
17-Acetoxyestra-4,9,11-trien-3-one, usually known as trenbolone acetate® or TBA (I), is a synthetic steroid with anabolic properties[1–3]. For treatment of cattle, this compound is implanted alone (Finaplix®: 300 mg of TBA) or in association with estradiol (Revalor®: 140 mg of TBA + 20 mg of estradiol). Studies with the ^{3}H-labelled molecule[4] showed that implant resorption is very slow resulting in an extended anabolic action. However, 2 or 3 months after implantation, residues found in carcasses are still of the order of parts per billion (10^{9}) (ref. 5). Trenbolone or TBOH (II) resulting from the hydrolysis of TBA is the main metabolite found in meat and organs of treated cattle. 4,9,11-Estratriene-3,17-dione(III), the oxidation product of TBOH, is also found in small amounts[4] and unchanged TBA can only be detected in fatty tissue[6]. Although a very specific and sensitive radioimmunoassay has been described[7] for the determination of TBOH residues in meat, it was necessary to develop a sensitive detection procedure that does not require expensive equipment.



I



II



III

EXPERIMENTAL

Solvents and reagents

All of the solvents used were of analytical grade and were redistilled in an

all-glass apparatus. Silica gel 60 TLC plates were purchased from E. Merck (Darmstadt, G.F.R.) and were activated at 110° for 1 h and stored in a desiccator until required. Pure trenbolone was kindly provided by Roussel-Uclaf (Romainville, France).

Extraction and clean up

Five grams of tissue were sampled and homogenized in 5 ml of distilled water. The homogenate was extracted three times with 10 ml of diethyl ether. After evaporation of the ether layer under nitrogen at 40°, the residue was dissolved in 3 ml of methanol and washed three times with 3 ml of light petroleum (b.p. 40–60°). The methanol solution was then dried and the residue, dissolved in 3 ml of chloroform, was washed with 1 ml of aqueous sodium hydroxide (0.1 *N*). The organic layer was again dried.

TLC development and detection

The residue from the clean-up procedure was dissolved in 0.1 ml of diethyl ether–light petroleum (b.p. 40–60°) (6:4) and wholly spotted on the chromatographic plate. A 10-μl portion of a solution of TBOH in methanol (1 μg/ml) was used as standard. The chromatogram was developed in the first dimension with chloroform–ethyl acetate (2:1) in an unlined tank, and in the second dimension with chloroform–propionic acid (95:5) ($R_{F1} = 0.22$, $R_{F2} = 0.36$). After drying the plate under a cold stream of air, the plate was sprayed with concentrated phosphoric acid–ethanol (1:1) and immediately examined under UV radiation at 366 nm. Trenbolone appeared as a highly fluorescent yellow spot.

RESULTS AND DISCUSSION

The detection procedure described is highly sensitive. The limit of detection is of the order of 250 pg, four times more sensitive than the detection with hydrochloric acid proposed earlier[8]. Trenbolone is specifically detected by our procedure: progresterone and testosterone, two natural hormones chemically similar to trenbolone, did not interfere. Experiments performed with meat from animals of different age and sex showed that no naturally occurring substances impair the analytical results.

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