Journal of Chromatography, 114 (1975) 244–246

CHROM. 8458

Note

Determination of trenbolone and trenbolone acetate® by thin-layer chromatography in combination with a fluorescence colour reaction

KARL-L. OEHRLE, KARL VOGT and BERND HOFFMANN
Institut für Physiologie, Technische Universität, München-Weihenstephan, D 805 Freising (G.F.R.)
(First received February 24th, 1975; revised manuscript received May 24th, 1975)

It has been shown that the synthetic steroid trenbolone acetate® (TBA) (17β-acetoxyoestra-4,9,11-trien-3-one) has anabolic properties[1], and its use as a growth-promoting agent in animal husbandry has been suggested. The major urinary metabolite of TBA has been identified as a conjugated form of the free alcohol trenbolone (TBOH)[2]. In spite of the fact that highly sensitive radioimmunoassay techniques for the determination of TBA and TBOH have been described[3,4], there is still a need for methods that permit the estimation of both compounds in less specially equipped laboratories. The reaction described here is highly sensitive and specific (allowing discrimination against other anabolic agents) and should also allow quantification of TBOH in biological samples (particularly urine) in connection with the use of TBA in animal production. The method involves thin-layer chromatography (TLC) and fluorimetry. It has so far given promising results, which will be reported elsewhere.

MATERIALS AND METHODS

All solvents and reagents used were of analytical grade and were not further purified. Unlabelled TBA and TBOH and [6,7-$^3H_2$]TBOH (58.2 Ci per mmole) were supplied by Roussel-Uclaf (Romainville, France).

One-dimensional TLC was performed on silica gel plates (SIL-G-25 HR; Macherey, Nagel & Co., Düren, G.F.R.) in the system chloroform–ethyl acetate (2:1). The R_F values for TBA and TBOH were 0.68 and 0.34, respectively.

For the location and semi-quantitative estimation of TBA and TBOH, the plates were exposed to the vapour of concentrated hydrochloric acid in a tank and then viewed under UV radiation (≈366 nm). For quantification, the spots were marked and scraped off, and the adsorbent was extracted by elution with ethyl ether (2 × 5 ml). After evaporation of each eluate in a 10-ml tube, 4.0 ml of the acid reagent (see *Quantitative fluorimetry*) were added, and, after the mixture had remained at room temperature for a few minutes, measurements were made with a Turner fluorimeter; the excitation wavelength was 365 nm, which gave max. emission at 498 nm. Standard solutions could also be pipetted directly into the tube, and were treated identically after evaporation of the solvent.

RESULTS AND DISCUSSION

TLC and semi-quantitative determination

TBA and TBOH were spotted on the plates in amounts ranging from 0.5–800 ng. After development and exposure to hydrochloric acid vapour, amounts larger than 100 ng became immediately visible as yellow spots. When viewed under UV radiation, the spots exhibited an intense bright-green fluorescence, making identification and semi-quantitative estimation possible in amounts down to 1 ng. A similar positive reaction was given by trendione (oestra-4,9,11-trien-3,17-dione); other hormonal compounds tested {17α- or 17β-oestradiol, oestrone, equilin, androstenedione, testosterone, androst-16-en-3-one, progesterone, cortisol, corticosterone, ethinyl-oestradiol, stilboestrol, hexoestrol, dienoestrol and zeranol [6-(6,10-dihydroxyundecyl)-β-resorcylic acid μ-lactone]} yielded negative results in amounts of 200 ng. By comparing the radiochemical purity and chromatographic mobilities of ^{3}H-labelled and unlabelled TBOH before and after exposure to hydrochloric acid vapour, it was demonstrated that the molecule was not destroyed by this treatment. Thus, complex formation, with hydrochloric acid is reversible and may involve some enolisation and redistribution of double bonds in the molecule.

Quantitative fluorimetry

TBOH and TBA developed a characteristic fluorescence at 498 nm (excitation at 365 nm) in the presence of an alcohol (methanol or ethanol) and acid, regardless of whether the compound was used directly or had undergone TLC and exposure to hydrochloric acid vapour. It was demonstrated, as shown in Fig. 1, that the intensity of fluorescence depended on the type of acid used and the ratio of acid to alcohol. There was almost no response with sulphuric acid under the conditions used, and, despite the fact that the highest readings were obtained when 50% phosphoric acid in methanol was used as the acid medium, hydrochloric acid was chosen for further work, as the low blank values obtained with this acid made for more convenient

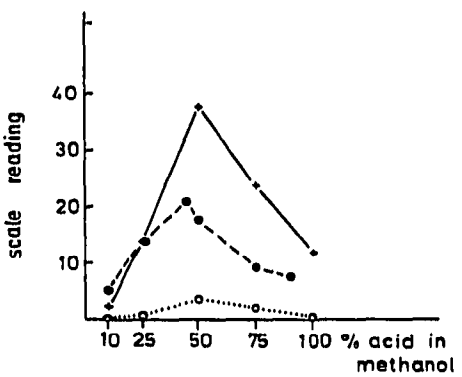
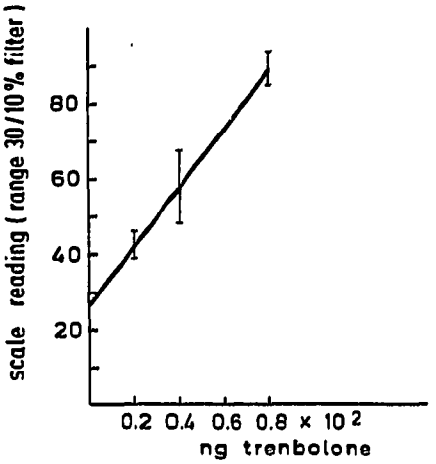


Fig. 1. Determination of trenbolone (400 ng) by fluorimetry. Development of fluorescence in relation to type of acid used and the ratio of acid to alcohol. +—+, H_3PO_4; ●---●, HCl; ○···○, H_2SO_4.



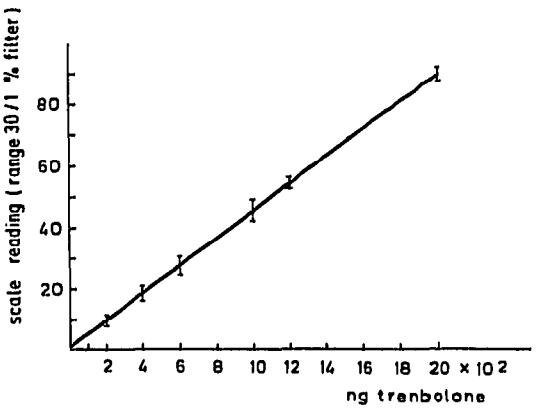


Fig. 2. Calibration graph for trenbolone (20–80 ng), showing mean values (10 determinations) ± standard deviation.

Fig. 3. Calibration graph for trenbolone (200–2000 ng), showing mean values (10 determinations) ± standard deviation.

instrument readings. The final "acid reagent" was 75% 6 *M* hydrochloric acid in methanol (44.2% concentrated acid in methanol); calibration graphs obtained under these conditions were linear within the ranges tested 20 to 80 ng and 200 to 2000 ng (see Figs. 2 and 3).

REFERENCES

1 P. van der Wal and P. L. M. Berende, *J. Anim. Sci.*, 37 (1973) 255.
2 J. Pottier, H. Busigny and I. A. Grandadam, *J. Anim. Sci.*, 37 (1973) 256.
3 H. Karg, H. J. Kyrein and B. Hoffmann, *J. Anim. Sci.*, 37 (1973) 256.
4 B. Hoffmann, K. H. Heinritzi, H. J. Kyrein, K. L. Oehrle, G. Oettel, E. Rattenberger, K. Vogt and H. Karg, *Z. Tierphysiol., Tierernähr., Futtermittelk.*, Suppl. 6 (1975) in press.