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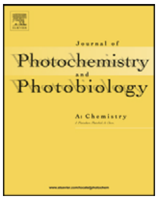

Photodegradation kinetics of androgenic steroids boldenone and trenbolone in aqueous solutions

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ABSTRACT

The subject of this study was photodegradation of boldenone and trenbolone belonging to the class of compounds which demonstrate endocrine disrupting activity. Their decomposition was carried out on the pathway of 254 nm photolysis alone and in the presence of hydrogen peroxide. The influence of pH, oxygen content in the reaction mixture, initial substrate concentration, photon fluence rate and initial concentration of H_2O_2 on the reaction rate was investigated. The effect of radical scavenger on the reaction course was also tested. The obtained results were used to determine kinetic parameters. Quantum yields of boldenone and trenbolone decay were equal to 0.61 ± 0.02 and 0.0029 ± 0.0002, respectively. The estimated rate constant of the reaction of hydroxyl radicals with trenbolone was $(4.3 \pm 0.8) \times 10^9\ M^{-1}\ s^{-1}$.

1. Introduction

Currently, numerous scientific researches indicate that some substances in the environment may interfere with a normal function of the endocrine system of humans and wildlife [1,2]. Minute amounts of these chemicals are able to disrupt the endocrine system and cause cancer to male and female reproductive systems and produce other adverse effects. Therefore, these substances are called endocrine disruptors (EDs).

A particular group of EDs consists of pharmaceuticals based on steroid moiety both of natural and synthetic origin. They are mainly used as contraceptives, drugs applied in menopause, breast cancer and for other medical purposes. A further important group of pharmaceutical hormones are anabolic steroids used in agriculture in animal farming. In EU countries application of growth-promoting hormones is banned in meat production [3] but in other countries they are regularly applied (e.g. USA or Canada) [4]. There is a growing concern that these synthetic hormones are making their way into surface waters and even ground water via human and animal wastes, mainly by their incomplete removal during passage through wastewater treatment plants [4,5]. Natural and synthetic hormones such as 17β-estradiol (E2) and 17α-ethinylestradiol (EE2) are frequently detected in the nanogram per liter ranges [6,7].

Compared with the natural hormones, synthetic hormonal steroids show relatively greater stability in aqueous media and greater resistance to microbial degradation [4]. These properties pose a potential for accumulation and persistence in the environment and endanger consumers with a permanent exposure. It can be presumed that other structurally related xenobiotic hormones used in veterinary treatment show a similar behavior.

Boldenone and trenbolone belong to a group of synthetic steroids with great anabolic potency. Their chemical structures are shown in Fig. 1.

Boldenone (BD) is a steroid which differs from testosterone only by one double bond at the 1-position. It is used mainly as undecylenate ester by bodybuilders and is illicitly administered to racing horses. But first of all it is applied as a growth promoter on meat production farms improving the growth and feed conversion of cattle and therefore might be abused to achieve more efficient meat production. Like other androgenic steroids, BD is classified as a probable human carcinogen [8].

Trenbolone (TB) is characterized by a very high anabolic effect nearly 10 times stronger than that of testosterone propionate [4] and is licensed as a growth promoter in the US and Canada. It is suspected of teratogenic activity as it can induce developmental abnormalities in rat's fetus [9]. The demasculinizing effect in Japanese quail was observed [10] and reproductive alterations in fish living downstream from animal feedlot operations were reported [9]. The problem with TB is more evident taking into account that in the US only several tons is applied every year [4] and that TB can remain in manure piles for more than 270 days [5].

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Nomenclature

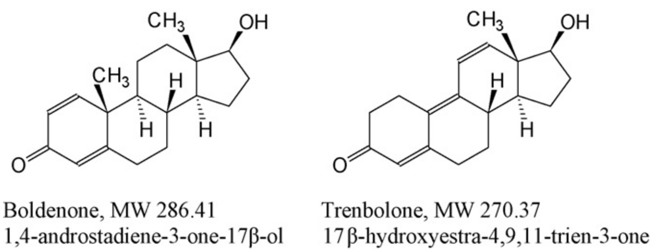
B	optical path length
BD	boldenone
C_i	molar concentration
C_i^0	initial molar concentration
F	fraction of absorbed photon flux
H	hydrogen peroxide
HO_2	hydroperoxide ion
E_0	volumic photon fluence rate
K	dissociation constant
k	rate constant
k_z	I-order apparent rate constant
OH	hydroxyl radical
R	reaction rate
t	time
TB	trenbolone
ε	molar absorption coefficient
λ	wavelength
φ	quantum yield
0	initial conditions

The studies on degradation of hormone steroids in aqueous solutions were performed mainly for estrogens such as estrone (E1), 17β-estradiol (E2), 17α-ethinylestradiol (EE2) and estriol (E3). There were various methods applied for their degradation. Ozonation and ozone-based advanced oxidation processes [6] resulted in efficient removal of the estrogen steroid from the aqueous environment. Worth noting is a very high rate constant of the direct reaction of ozone with dissociated forms of steroids attaining diffusion controlled limit which is 10^4 to 10^5 times greater than with neutral forms of steroids [11]. The application of OH radicals using the H_2O_2/UV system to eliminate the estrogen steroids from aqueous solutions is characterized by rate constants of the order $10^{10}\ M^{-1}\ s^{-1}$ [12,13]. However, photolysis as well as the use of high energy radiation of a low pressure lamp (254 nm) as solar or solar-simulated light is reported to be inefficient [14]. The determined quantum yields are of the order 10^{-2} reaching 0.1 for E2 at radiation from a medium pressure lamp [13]. The direct photolysis using 254 nm radiation can be enhanced in natural waters due to the presence of various photo-oxidants [15].

The present paper describes aqueous BD and TR degradation studies during 254 nm irradiation alone and in the presence of hydrogen peroxide. The obtained results were used to estimate the decay quantum yield of both studied compounds and rate constant of the reaction of hydroxyl radicals with TB.

2. Materials and methods

Boldenone (BD) (98.8%) was purchased from Sigma–Aldrich, trenbolone (TB) (98%) and *tert*-butyl alcohol (≥99.7%) were from Fluka. Hydrogen peroxide (30% solution), methanol (p.a.) and reagents used to prepare buffers (Na_2HPO_4, KH_2PO_4, NaOH, p.a.) were bought from POCH (Poland).



Boldenone, MW 286.41
1,4-androstadiene-3-one-17β-ol

Trenbolone, MW 270.37
17β-hydroxyestra-4,9,11-trien-3-one

Fig. 1. Chemical structures of studied steroids.

Experiments were carried out in quartz test tubes of the capacity of 10 cm³ (optical path length 0.85 cm), placed in a merry-go-round device which was located between two exposure panels with UVC lamps emitting mainly wavelength at 254 nm. The initial concentrations of the substrates ranged from 4.8×10^{-6} to 1.8×10^{-5} M and from 5×10^{-6} to 1×10^{-4} M for BD and TB, respectively. The details of equipment, the run of the process and the reaction mixture preparation were analogous to those outlined elsewhere [16]. Photon fluence rate entering the reaction space was calculated by uranyl oxalate actinometer [17] and ranged from 4.3×10^{-6} to 10.6×10^{-6} einstein $dm^{-3}\ s^{-1}$ for 2 and 6 lamps, respectively. These fluence values corresponded to irradiance from 11.8 to 29.1 $W\ m^{-2}$.

Spectrophotometric measurements were done on a Unicam apparatus.

The reaction progress was traced by chromatographic analysis of BD and TB decay. The HPLC (Waters) apparatus equipped with a UV diode array detector and Symmetry C18 reverse-phase column, operated in isocratic mode was used. The mobile phase consisted of 30% acetonitrile and 70% of 0.01% (v/v) water solution of phosphoric (V) acid and the flow rate 1 $cm^3\ min^{-1}$ was applied.

Data analysis was performed using Origin software (Microcal Software Inc., USA). The initial reaction rates were calculated by differentiating exponential curve that fitted experimental points (concentration, time) at the correlation factor higher than 0.97.

3. Results and discussion

The spectrophotometric measurements of BD and TB as the basis of photochemical studies were performed and molar absorption coefficients were determined. The absorption spectra of BD and TB in water solutions and emission spectrum of the UVC lamp are shown in Fig. 2.

The maximum of the BD absorption appears at 252 nm which fits very well with the emission of the UVC lamp. TB absorption extends in the whole near-UV range up to nearly 500 nm in the visible range, with two maxima: clear one at 351 nm and broad band centered at 237 nm. The determined molar absorption coefficient ε at 254 nm for BD at pH 5, 7 and 10 is equal to 13,800, 14,570 and 15,240 $M^{-1}\ cm^{-1}$, respectively, and for TB it is 5460, 6300 and 5690 $M^{-1}\ cm^{-1}$, respectively.

The absorption of both compounds slightly depends on pH of the solution, probably due to the interaction of BD and TB chromophores with the solution components. BD and TB can dissociate,

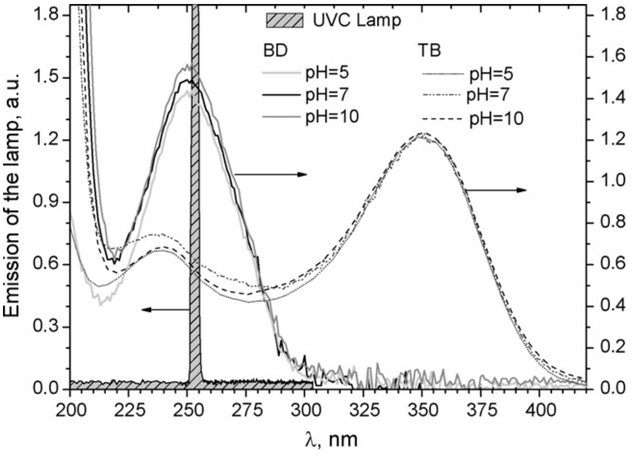


Fig. 2. The absorption spectra of BD and TB in buffered reaction solutions and the emission spectrum of the UVC lamp.