Steroids 76 (2011) 540–547

Contents lists available at ScienceDirect

Steroids

journal homepage: www.elsevier.com/locate/steroids

Seized designer supplement named "1-Androsterone": Identification as 3β-hydroxy-5α-androst-1-en-17-one and its urinary elimination

Maria K. Parr[a,*], Georg Opfermann[a], Hans Geyer[a], Folker Westphal[b], Frank D. Sönnichsen[c], Josef Zapp[d], Dorota Kwiatkowska[e], Wilhelm Schänzer[a]

[a] Center for Preventive Doping Research, Institute of Biochemistry, German Sport University Cologne, Am Sportpark Muengersdorf 6, 50933 Cologne, Germany
[b] State Bureau of Criminal Investigation Schleswig-Holstein, Section Narcotics and Toxicology, 24116 Kiel, Germany
[c] Otto-Diels-Institute of Organic Chemistry, Christian-Albrechts-University, 24098 Kiel, Germany
[d] Institute of Pharmaceutical Biology, Saarland University, 66041 Saarbruecken, Germany
[e] Department of Anti-Doping Research, Institute of Sport, 01-982 Warsaw, Poland

ARTICLE INFO

Article history:
Received 14 September 2010
Received in revised form
23 December 2010
Accepted 2 February 2011
Available online 16 February 2011

Keywords:
1-Androsterone
Metabolism
Steroid profile
Doping control
GC–MS
NMR

ABSTRACT

New analogues of androgens that had never been available as approved drugs are marketed as "dietary supplement" recently. They are mainly advertised to promote muscle mass and are considered by the governmental authorities in various countries, as well as by the World Anti-doping Agency for sport, as being pharmacologically and/or chemically related to anabolic steroids.

In the present study, we report the detection of a steroid in a product seized by the State Bureau of Criminal Investigation Schleswig-Holstein, Germany. The product "1-Androsterone" of the brand name "Advanced Muscle Science" was labeled to contain 100 mg of "1-Androstene-3b-ol,17-one" per capsule. The product was analyzed underivatized and as bis-TMS derivative by GC–MS. The steroid was identified by comparison with chemically synthesized 3β-hydroxy-5α-androst-1-en-17-one, prepared by reduction of 5α-androst-1-ene-3,17-dione with LS-Selectride (Lithium tris-isoamylborohydride), and by nuclear magnetic resonance. Semi-quantitation revealed an amount of 3β-hydroxy-5α-androst-1-en-17-one in the capsules as labeled.

Following oral administration to a male volunteer, the main urinary metabolites were monitored. 1-Testosterone (17β-hydroxy-5α-androst-1-en-3-one), 1-androstenedione (5α-androst-1-ene-3,17-dione), 3α-hydroxy-5α-androst-1-en-17-one, 5α-androst-1-ene-3α,17β-diol, and 5α-androst-1-ene-3β,17β-diol were detected besides the parent compound and two more metabolites (up to now not finally identified but most likely C-18 and C-19 hydroxylated 5α-androst-1-ene-3,17-diones). Additionally, common steroids of the urinary steroid profile were altered after the administration of "1-Androsterone". Especially the ratios of androsterone/etiocholanolone and 5α-/5β-androstane-3α,17β-diol and the concentration of 5α-dihydrotestosterone were influenced. 3α-Hydroxy-5α-androst-1-en-17-one appears to be suitable for the long-term detection of the steroid (ab-)use, as this characteristic metabolite was detectable in screening up to nine days after a single administration of one capsule.

1. Introduction

For a few years, several steroids have been available on the Internet as sport supplements, often sold by bodybuilding sites. Following the classification of the so-called prohormones of testosterone and nandrolone as schedule III controlled substances by the anabolic steroids control act [1] in the USA, more and more products appeared on the market, which contain steroids that have never been marketed as approved drugs [2–6]. Already in 2001, "1-Androstenediol" (5α-androst-1-ene-3ξ,17β-diol) was introduced on the dietary supplement market as prohormone of 1-testosterone (17β-hydroxy-5α-androst-1-en-3-one), quickly followed by 1-androstenedione (5α-androst-1-ene-3,17-dione). Only very limited information is available on the metabolism of these steroids. Information on the in vitro metabolism of 5α-androst-1-ene-3,17-dione and 5α-androst-1-ene-3β,17β-diol is available, albeit with the limited focus on the determination of the 17β-hydroxy and the 3β,17β-dihydroxy analogues, testosterone, dihydrotestosterone and androst-4-ene-3,17-dione [7]. An excretion study with 1-testosterone (**1**) reported the identification of 17β-hydroxy-5α-androst-1-en-3-one (**1**) and 5α-androst-1-ene-3,17-dione (**2**) as metabolites [8]. The structure of the main metabolite in all volunteers remained unconfirmed up to now. Further, while considerations on the influences of

* Corresponding author. Tel.: +49 221 4982 4960; fax: +49 221 497 32 36.
E-mail address: m.parr@biochem.dshs-koeln.de (M.K. Parr).

0039-128X/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.steroids.2011.02.001

the steroid administration on the levels on some endogenous steroids were made in this investigation, they focused only on the testosterone/epitestosterone (T/EpiT) ratio. As the product "1-Androsterone" appeared to contain an active content such as these three above mentioned 1-ene steroids that are covered by the current drug regulations in several countries, e.g. the US and Germany, the product was confiscated. Herein we report the identification of its active content. For elucidation of the urinary elimination post-administration urines are analyzed and the elimination profiles of several metabolites as well as the alterations in the urinary steroid profile are monitored.

2. Experimental

2.1. Supplement

The product "1-Androsterone" of the brand name "Advanced Muscle Science" was seized and provided by the State Bureau of Criminal Investigation Schleswig-Holstein (Kiel, Germany). It was labeled to be a dietary supplement containing 100 mg of "1-Androstene-3b-ol,17-one" per capsule.

2.2. Chemicals and reagents

Testosterone (17β-hydroxyandrost-4-en-3-one), 5α-androstane-3α,17β-diol (Adiol), 5β-androstane-3α,17β-diol (Bdiol), and dehydroepiandrosterone (3β-hydroxyandrost-5-en-17-one) were obtained from NMI (Sydney, Australia). Epitestosterone (EpiT, 17α-hydroxyandrost-4-en-3-one), etiocholanolone (ETIO, 3α-hydroxy-5β-androstan-17-one), androsterone (AND, 3α-hydroxy-5α-androstan-17-one), methyltestosterone (17β-hydroxy-17α-methylandrost-4-en-3-one), LS-selectride (lithium tris-isoamylborohydride, 1 M in tetrahydrofuran), was purchased from Sigma–Aldrich GmbH (Steinheim, Germany), dihydrotestosterone (DHT, 17β-hydroxy-5α-androstan-3-one) from Serva (Heidelberg, Germany), 1-testosterone (**1**, 17β-hydroxy-5α-androst-1-en-3-one) from Thinker Chemicals (Hanghzou, China) and 1-androstenedione (**2**, 5α-androst-1-ene-3,17-dione) from Steraloids (Wilton, USA). d_3-Testosterone, d_3-epitestosterone, d_4-etiocholanolone, d_5-androsterone-glucuronide, and 3α-hydroxy-5β-androst-1-en-17-one were synthesized in our laboratory. β-Glucuronidase from *Escherichia coli* (*E. coli*, >140 U/mL) was obtained from Roche Diagnostics (Mannheim, Germany), N-methyl-N-trimethylsilyl-trifluoroacetamide (MSTFA) from Chem. Fabrik Karl Bucher (Waldstetten, Germany).

All other reagents and solvents were of analytical grade and obtained from Merck (Darmstadt, Germany).

2.3. Synthesis of reference material

Several isomers of 5ξ-androst-1-ene-3ξ,17ξ-diol, including 5α-androst-1-ene-3α,17β- (**5**) and 3β,17β-diol (**6**), were synthesized by reduction from the respective oxo-analogues as described elsewhere [9].

2.3.1. 3ξ-Hydroxy-5α-androst-1-en-17-one (*3* and *4*)

To a stirred solution of 1 mg of 5α-androst-1-ene-3,17-dione (**2**) in 1 mL of absolute diethyl ether 10 μL of LS-selectride solution were added. After 1 h at ambient temperature 1 mL of an aqueous ammonium chloride solution (10% in H_2O) was added, pH∼10 was adjusted by adding solid sodium hydrogencarbonate/potassium carbonate (2:1, w:w) and the mixture was extracted with 5 mL of t-butyl methyl ether (TBME).

2.4. Instrumentation

2.4.1. NMR spectroscopy

The NMR data were obtained using a Bruker DRX 500 instrument, equipped with a 5 mm inverse probehead with actively shielded z-gradient coil. Chemical shifts were given in δ values (ppm) relative to tetramethylsilane. The spectra were recorded at 500 MHz (^{1}H) and 125 MHz (^{13}C) at 298 K using solutions of about 5 mg of each compound in deuterated chloroform. For confirmation of the assumed structures ^{1}H, ^{13}C, and DEPT spectra were measured together with 2D HH-gCOSY, HSQC and HMBC experiments.

2.4.2. GC–MS analyses

The GC–MS analyses of the TMS derivatives were performed on an Agilent 6890N gas chromatograph coupled to an Agilent 5973 inert mass selective detector (MSD) GC–MS system applying the following parameters: column: Agilent Ultra-1 (polysiloxane, 17 m; 0.20 mm i.d.; 0.11 μm film thickness), carrier gas: helium, head pressure: 1 bar, oven temperature program: 0 min 183 °C, +3 °C/min, 0 min 232 °C, +40 °C/min, 2 min 310 °C, injection volume: 3 μL, split 1:16, injection temperature: 300 °C, ionization: 70 eV, EI, full scan mode, 40–800 Da or in case of the urine extract analysis selected ion monitoring. Additionally, the underivatized compounds were analyzed on a GC Agilent 6890 coupled to a MSD Agilent 5973, injection volume: 2 μL, splitless, injection temperature: 300 °C, column: Macherey-Nagel Optima-P-XLB column (diphenyl dimethyl silarylene, 30 m, 0.25 mm i.d., 0.5 μm film thickness), carrier gas: helium, 3 mL/min, oven temperature program: 3 min 60 °C, +40 °C/min, 0 min 260 °C, +5 °C/min, 0 min 330 °C, ionization: 70 eV, EI, data acquisition: full scan mode, 40–400 Da.

2.5. Preparation for GC–MS measurement

The final residues of sample preparation were either derivatized with TMIS reagent (MSTFA/NH_4I/ethanethiol, 1000:2:3, v:w:v) by heating for 20 min at 60 °C as introduced by Donike [10] and Geyer et al. [11] or reconstituted in acetone and injected into the GC–MS. As reference, solutions of the respective steroids in methanol were evaporated to dryness and processed in the same manner.

Relative retention times (RRT) were calculated using testosterone as reference.

2.6. Supplement analysis

For identification of the steroid ingredient 1.0 g of the capsule content was extracted with n−hexane in a Soxhlet apparatus. After crystallization from n−hexane the steroid was characterized by GC–MS and NMR.

2.7. Administration study

An administration study using one capsule of the above-mentioned product was performed in one healthy male volunteer (68 years, 85 kg, p.o.) and urine samples were collected for two weeks. It was approved by Research Ethical Committee at the Sports Institute, Warsaw. The samples were prepared according to the routine steroid screening procedure used in our laboratory [11]. In brief, after addition of 40 μl of the internal standard solution containing methyltestosterone, d_3-testosterone, d_3-epitestosterone, d_4-etiocholanolone and d_5-androsterone-glucuronide 2 mL of urine were incubated at pH 7 with β-glucuronidase from *E. coli* at 50 °C for 1 h. The steroids were extracted with 5 mL of TBME at pH 9.6, the organic layer was evaporated to dryness and analyzed by GC–MS. For quality control blank samples are analyzed in

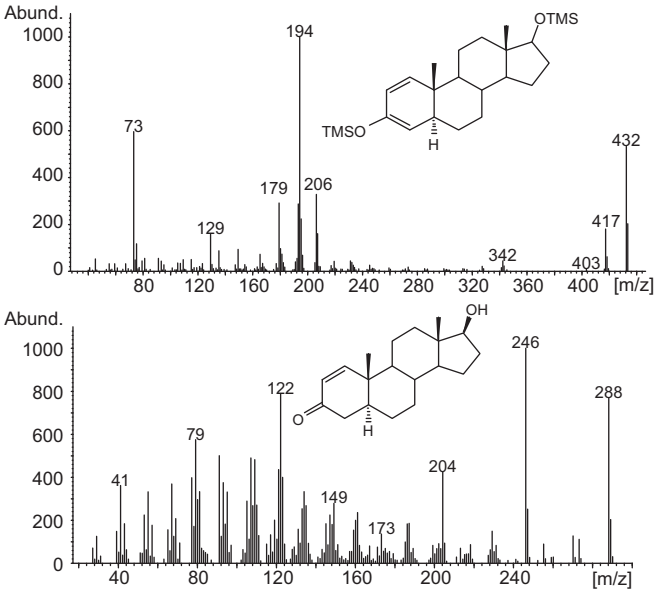


Fig. 1. Mass spectra of 17β-hydroxy-5α-androst-1-en-3-one (1-testosterone, (**1**)), upper bis-TMS, M^+ = 432, lower underivatized, M^+ = 288.

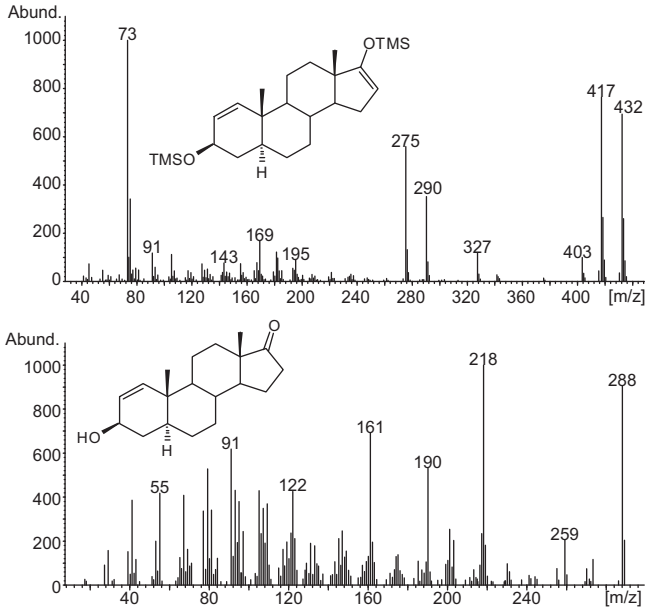


Fig. 3. Mass spectra of 3β-hydroxy-5α-androst-1-en-17-one (**3**), upper bis-TMS, M^+ = 432, lower underivatized, M^+ = 288.

the same manner with every batch to check for the absence of the analytes.

3. Results and discussion

3.1. Synthesis and characterization of reference material

Some of the androst-1-enes, either commercially available or synthesized by reduction from the respective oxo-analogues were analyzed by GC-EI-MS to identify the compounds detected (mass spectra in Figs. 1–5). The generation of the 5ξ-androst-1-ene-3ξ,17ξ-diols was already reported previously. All these epimers were found to reveal very similar mass spectra [3,9]. The retention times of the steroids (as per-TMS derivatives and as underivatized compounds) are listed in Table 1 and the NMR data of selected compounds are shown in Table 2.

The stereochemistry at C-3 and C-17 of 5α-androst-1-ene-3α,17β-diol (**5**) and -3β,17β-diol (**6**) could be deduced from their NMR data: The ^{1}H and ^{13}C NMR shifts of their ring C and D protons and carbons were similar, indicating the same orientation of the 17-hydroxy group. Comparison of this data with those of 17-hydroxy epimeric sterols from the literature [12] clearly identified both compounds as 17β-hydroxy derivatives. The ^{1}H NMR of (**6**) showed for H-3 the same diaxial coupling with H-4$_{ax}$ as found for (**3**). Therefore, (**6**) is 5α-androst-1-ene-3β,17β-diol. This implicated (**5**) to be its 3α-hydroxy epimer, which was furthermore proven by the coupling constants of H-3$_{eq}$ with H-4$_{eq}$ and H-4$_{ax}$, both 4.5 Hz.

The mass spectra of the TMS derivatives of the androstanes with 3-hydroxy-1-ene structure (Figs. 3–5) show fragments with *m*/*z* 142 and 143 (corresponding to C1–C4 of the A-ring) and M^+-142 (corresponding to a neutral loss of C1–C4) and M^+-15-

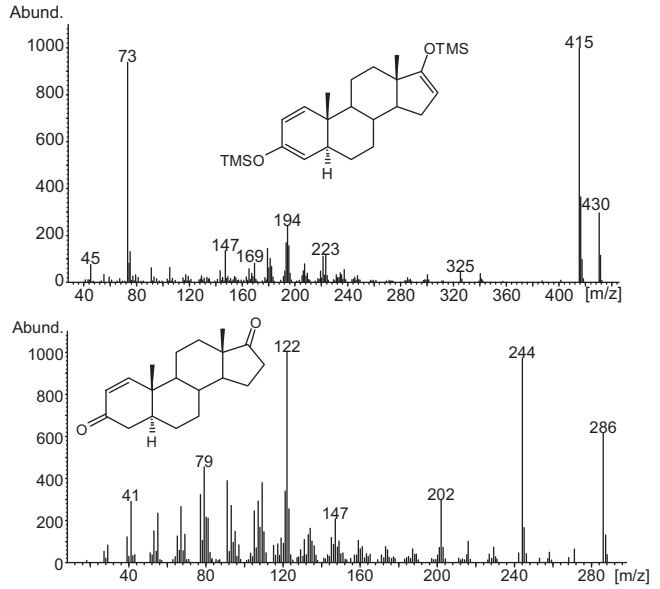


Fig. 2. Mass spectra of 5α-androst-1-ene-3,17-dione (**2**), upper bis-TMS, M^+ = 430, lower underivatized, M^+ = 286.

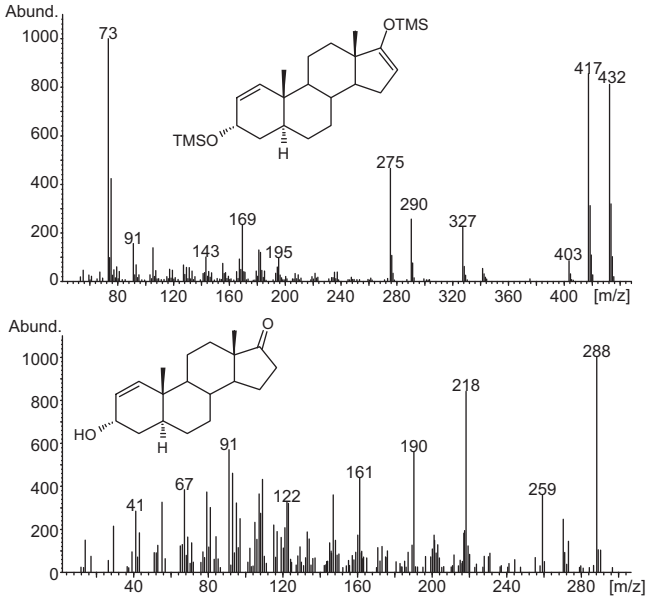


Fig. 4. Mass spectra of main metabolite, identified as 3α-hydroxy-5α-androst-1-en-17-one (**4**), upper bis-TMS, M^+ = 432, lower underivatized, M^+ = 288.

Table 1
Molecular ions (M^+) and relative retention times (RRT) of steroids used as reference (calculated using testosterone, RT(bis-TMS) = 13.01 min, RT(underiv.) = 17.76 min).

Analyte	Bis-TMS		Underivatized	
	RRT	M^+	RRT	M^+
17β-Hydroxy-5α-androst-1-en-3-one (**1**)	0.91	432	0.95	288
5α-Androst-1-ene-3,17-dione (**2**)	0.88	430	0.95	286
3β-Hydroxy-5α-androst-1-en-17-one (**3**)	0.90	432	0.88	288
3α-Hydroxy-5α-androst-1-en-17-one (**4**)	0.89	432	0.87	288
5α-Androst-1-ene-3α,17β-diol (**5**)	0.91	434	0.92	290
5α-Androst-1-ene-3β,17β-diol (**6**)	0.92	434	0.93	290

Table 2
^1H and ^{13}C NMR spectral data of 3β-hydroxy-5α-androst-1-en-17-one (**3**), 5α-androst-1-ene-3α,17β-diol (**5**) and 5α-androst-1-ene-3β,17β-diol (**6**).

	3β-Hydroxy-5α-androst-1-en-17-one (**3**)		5α-Androst-1-ene-3α,17β-diol (**5**)		5α-Androst-1-ene-3β,17β-diol (**6**)	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	137.62	5.906 *dd* (10/2)	140.39	6.078 *d* (10)	137.94	5.917 *dd* (10/2)
2	129.19	5.510 *ddd* (10/2/2)	126.11	5.652 *ddd* (10/4.5/1.5)	128.74	5.492 *ddd* (10/2/2)
3	68.89	4.312 *dddd* (9.5/7/2/2)	64.81	4.097 *dd* (4.5/4.5)	68.85	4.303 *dddd* (9.5/7/2/2)
4	35.91	1.78 *dddd* (12/7/1/1)	34.84	1.79 *m*	35.88	1.76 *m*
		1.52 *m*		1.51 *m*		1.50 *m*
5	43.64	1.43 *m*	39.04	1.56 *m*	43.56	1.45 *m*
6	30.86	1.81 *m*	31.49	1.70 *m*	31.43	1.69 *m*
		1.02 *m*		0.94 *m*		0.90*m*
7	27.95	1.41 *m*	27.86	1.37 *m*	27.98	1.35 *m*
		1.41 *m*		1.33 *m*		1.35 *m*
8	35.32	1.60 *ddd* (11/11/4)	35.83	1.44 *m*	35.66	1.47 *m*
9	51.59	0.89 *m*	51.13	0.87 *m*	51.59	0.84 *m*
10	38.34	–	38.13	–	38.17	–
11	20.68	1.81 *m*	20.72	1.75 *m*	20.86	1.74 *m*
		1.40 *m*		1.37 *m*		1.38 *m*
12	31.66	1.83 *m*	36.71	1.83 *m*	36.68	1.82 *m*
		1.26 *m*		1.07 *m*		1.07 *m*
13	48.04	–	43.11	–	43.13	–
14	51.72	1.30 *ddd* (13/11/6)	51.13	0.99 *m*	50.08	0.99 *m*
15	21.87	1.93 *ddd* (12/9/6)	23.41	1.59 *m*	23.34	1.59 *m*
		1.50 *m*		1.26 *m*		1.26 *m*
16	35.93	2.44 *dd* (19/9)	30.54	2.16 *m*	30.53	2.06 *m*
		2.07 *ddd* (19/19/9)		1.47 *m*		1.43 *m*
17	221.18	–	81.87	3.646 *dd* (8.5/8.5)	81.89	3.634 *dd* (8.5/8.5)
18	14.07	0.876 *s*	11.28	0.753 *s*	11.27	0.747 *s*
19	15.80	0.938 *s*	13.87	0.813 *s*	15.70	0.920 *s*

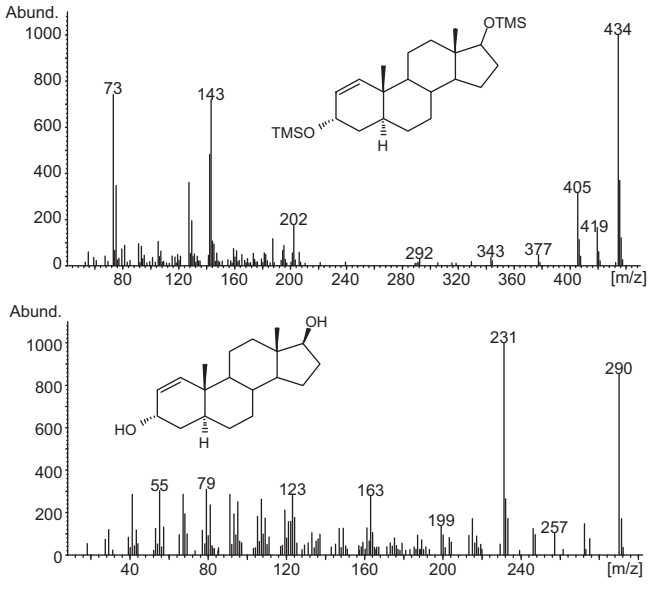


Fig. 5. Mass spectra of metabolite, identified as 5α-androst-1-ene-3α,17β-diol (**5**), upper bis-TMS, M^+ = 434, lower underivatized, M^+ = 290.

142. In case of 17-hydroxy structures (Figs. 1 and 5), the A-ring fragments are quite abundant, while the B/C/D-ring fragments are abundant in the 17-oxo steroids (Figs. 2–4). Additionally, the loss of 15 Da is very dominant in the 17-oxo androstanes. The spectra of the TMS-derivatized 3-oxo-1-enes show characteristic fragments at *m*/*z* 179, 194 and 206 corresponding to the A/B-ring (Figs. 1 and 2).

The mass spectra of the underivatized steroids show a loss of 42 Da for the 3-oxo-1-enes (Figs. 1 and 2), 59 Da for the 1-ene-3,17-diols (Fig. 5) and 70 Da in case of 3β-hydroxy-5α-androst-1-en-17-one (Fig. 3) generating the base peak in the spectra. Additionally, all underivatized 1-enes with an oxo group either at C-3 or C-17 show *m*/*z* 122 in their spectra.

*3.1.1. 3ξ-Hydroxy-5α-androst-1-en-17-one (**3** and **4**)*

The reduction of 5α-androst-1-ene-3,17-dione (**2**) with LS-selectride yielded the two isomeric 3β- (**3**) and 3α-hydroxy (**4**) analogues in a ratio of 85:15. In accordance to the literature [13,14] the reduction of 3-oxo-5α-androst-1-enes with complex borohydrides mainly resulted in 3β-hydroxy isomers which show longer retention times than the 3α-hydroxy isomers. The GC–MS retention times and spectra of the 3β-isomer (**3**) in the mixture and the steroid extracted from the supplement matched and thus confirmed the assignment. Because of very low amounts prepared, separation of the isomers was only performed by GC–MS. Thus, 3α-

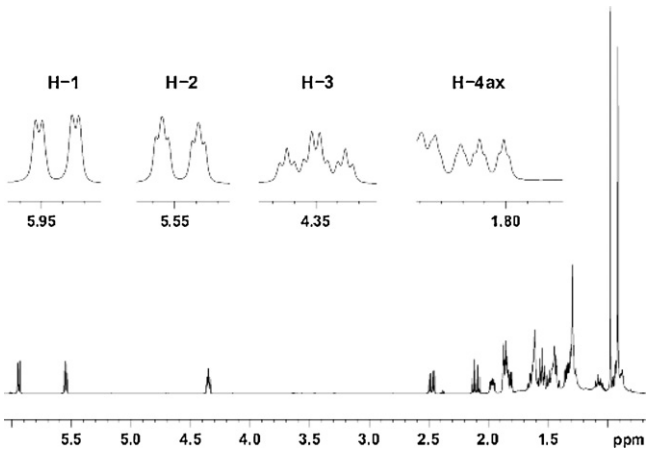


Fig. 6. ^{1}H NMR spectrum (500 MHz, $CDCl_3$, 298 K) of 3β-hydroxy-5α-androst-1-en-17-one (**3**), extracted from the product "1-Androsterone".

hydroxy-5α-androst-1-en-17-one (**4**) was not amenable to NMR analysis.

3.2. Supplement analysis

The total capsule content was determined with 555 mg of powder per capsule. The depletive extraction of 1 g resulted in 205.9 mg of steroid, corresponding to 115 mg/capsule. It was identified as 3β-hydroxy-5α-androst-1-en-17-one (**3**) by NMR and GC–EI–MS (NMR spectrum and data in Fig. 6 and Table 2, GC retention times in Table 1 and mass spectra in Fig. 3). All data of the extracted material complied to that of the synthesized reference (**3**).

Its ^{1}H and ^{13}C NMR data (Table 2) revealed the structure of a hydroxy androstenone. The position of the functional groups could be deduced from the 2D NMR HHCOSY, HSQC and HMBC spectra and led to a 3-hydroxy-androst-1-en-17-one. The H-3 coupling pattern exhibits a pseudo diaxial coupling with H-4_{ax}, $J_{H\text{-}3ax/H\text{-}4ax}$ = 9.5 Hz, indicating a pseudo equatorial position for the hydroxyl group, which therefore is 3β-orientated. The stereochemistry at C-5 followed directly from the comparison of the NMR data with those of 3β-hydroxy-5ξ-androst-1-enes that were synthesized some years ago in our lab [14]. The values for C-3/H-3 and C-5/H-5 matched perfectly with the 5α series. Therefore, the structure of the steroid was identified as 3β-hydroxy-5α-androst-1-en-17-one (**3**).

3.3. Administration study

Following the oral administration of one capsule of "1-Androsterone", corresponding to 115 mg of 3β-hydroxy-5α-androst-1-en-17-one (**3**), to one healthy male volunteer the combined unconjugated and glucuronidated metabolite fractions were analyzed as per-TMS derivatives as used in routine sports doping control. The parent compound (**3**) was detectable in the urines for about two days with the highest concentration detected in the 0–3 h urine. Its 3α-isomer (**4**) was identified by comparison with the unpurified reference obtained by reduction of 1-androstenedione with LS-Selectride. It was found to be the metabolite detectable for the longest time. The highest concentration was found in the 3–7.5 h urine, while its presence could still be confirmed up to seven days (ions used: *m*/*z* 432, 275, and 169) according to the regulations applicable in human sports doping control [15]. Suspicious results in screening analysis were possible for two more days. Applying these criteria, 5α-androst-1-ene-3,17-dione (*m*/*z* 415, 430, and 194) and 1-testosterone (*m*/*z* 194, 432, and 417) were detectable for six and almost four days,

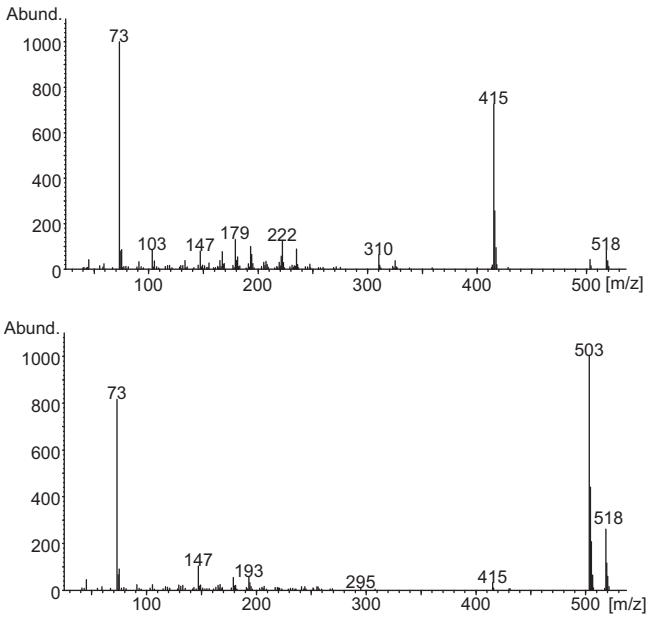


Fig. 7. Mass spectra of hydroxylated metabolites as tris-TMS derivatives upper: HO-Dione1 (M^+ = 518, RRT = 1.14), lower HO-Dione2 (M^+ = 518, RRT = 1.26).

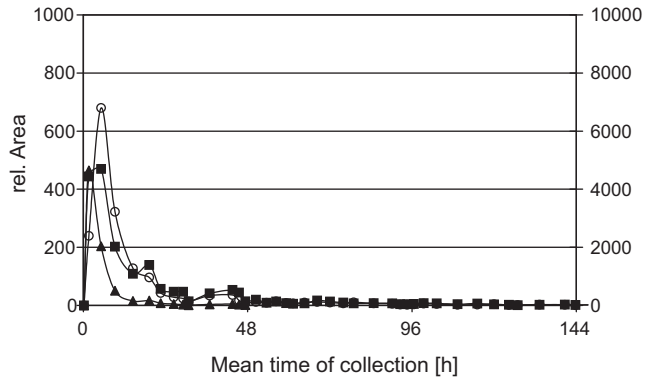


Fig. 8. Elimination kinetics of 3β-hydroxy-5α-androst-1-en-17-one (**3,** ▲, left *y*-axis), 17β-hydroxy-5α-androst-1-en-3-one (**1**, ○, right *y*-axis), and 5α-androst-1-ene-3,17-dione (**2**, ■, left *y*-axis), after one capsule of "1-Androsterone", data shown using midpoints of collection periods and concentrations in 1000-fold peak area of analyte divided by area of internal standard.

respectively. The 5α-androst-1-ene-3ξ,17β-diols (**5**) and (**6**) were unambiguously identified for about one (3β-) and three days (3α-) due to their elution in a region of relatively high background in the chromatograms. An optimized sample pretreatment may eliminate this in case of confirmation of a positive screening result. Positive screening results were obtained for about six (**1**), eight (**2**), four (**5**), and three days (**6**). Additionally, two more metabolites were detected in the early post-administration urines (HO-Dione1 and HO-Dione2). Based on their mass spectra (Fig. 7), structures of androst-1-ene-3,17-diones hydroxylated most likely at position C-18 (OH-Dion1) and C-19 (OH-Dion2) are proposed due to the loss of 103 u (TMS-O-$CH_2^{\bullet}$) from the molecule resulting in *m*/*z* 415.

The elimination kinetics of the above mentioned androst-1-enes are displayed in Figs. 8–11.

In addition, the influence of the administration on the endogenous urinary steroids was monitored. The steroid profile ratios of the corresponding 5α/5β-steroids, namely androsterone/etiocholanolone (AND/ETIO) and 5α-/5β-androstane-3α,17β-diol (Adiol/Bdiol), were significantly increased (Fig. 12). Concomitantly increased concentrations of epiandros-

Table 3
Selected steroid profile data of the post-administration urines and criteria indicative for DHT administration in males.

	Collection period [h]	c (DHT) [ng/mL]	Adiol/Bdiol	AND/ETIO	DHT/ETIO	DHT/EpiT
Decision limit		>21[a]	>1.5	>2.9	>8.2	>0.73
Sample 01	0:00–2:50	20.7	0.4	4.6	18.2	0.3
Sample 02	2:50–7:30	142.6	1.1	18.7	279.5	0.9
Sample 03	7:30–11:00	152.5	3.3	6.7	116.1	1.8
Sample 04	11:00–18:00	101.8	2.9	5.1	98.1	2.0
Sample 05	18:00–20:30	143.2	2.9	4.8	92.7	2.6
Sample 06	20:30–24:40	94.4	2.5	4.6	89.4	2.3
Sample 07	24:40–28:10	96.1	2.0	4.8	102.8	2.4
Sample 08	28:10–30:10	57.7	1.6	4.5	90.4	2.1
Sample 09	30:10–31:20	18.2	1.5	4.3	88.9	1.4
Sample 10	31:20–42:30	66.2	1.1	2.6	60.0	1.5
Sample 11	42:30–44:40	98.9	1.0	2.4	51.5	1.7
Sample 12	44:40–46:20	63.1	0.9	2.3	46.0	1.5
Sample 13	46:20–48:20	27.1	0.8	2.2	36.5	1.0
Sample 14	48:20–52:30	28.4	0.7	2.0	29.9	0.8
Sample 15	52:30–54:30	10.7	0.6	1.7	27.2	0.6
Sample 16	54:30–58:10	22.2	0.5	1.4	29.4	0.7
Sample 17	58:10–60:20	14.8	0.4	1.2	30.4	0.8
Sample 18	60:20–62:10	11.8	0.4	1.2	36.0	0.8
Sample 19	62:10–67:00	13.6	0.4	1.1	30.9	0.7
Sample 20	67:00–69:40	26.0	0.4	1.0	24.2	0.8
Sample 21	69:40–74:30	24.4	0.4	1.2	19.8	0.6

[a] Adjusted for specific gravity of 1.020 g/mL.

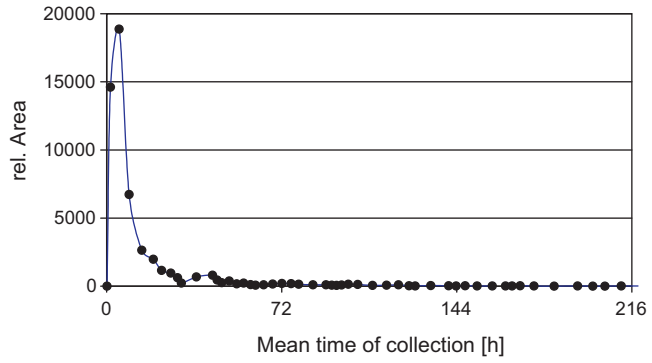


Fig. 9. Elimination kinetics of 3α-hydroxy-5α-androst-1-en-17-one (**4**, ●, main metabolite) after one capsule of "1-Androsterone", data shown using midpoints of collection periods and concentrations in 1000-fold peak area of analyte divided by area of internal standard.

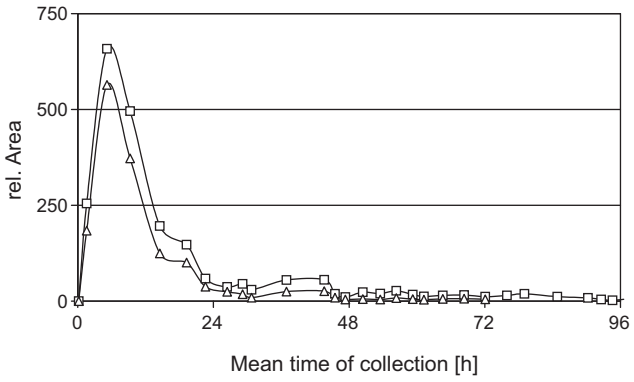
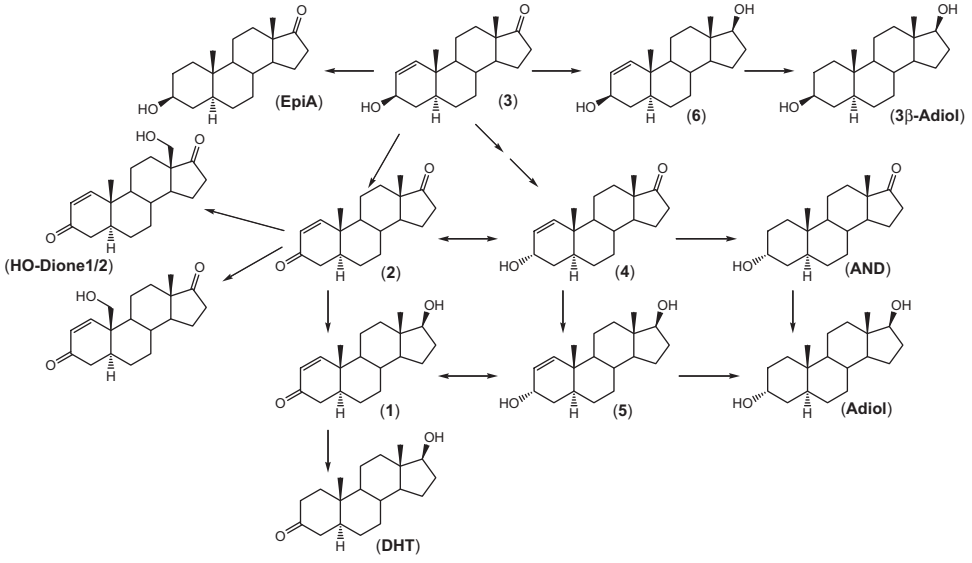


Fig. 10. Elimination kinetics of 5α-androst-1-ene-3α,17β-diol (**5**, □) and 5α-androst-1-ene-3β,17β-diol (**6**, △) after one capsule of "1-Androsterone", data shown using midpoints of collection periods and concentrations in 1000-fold peak area of analyte divided by area of internal standard.



Scheme 1. Proposed metabolism of 3β-hydroxy-5α-androst-1-en-17-one (**3**).

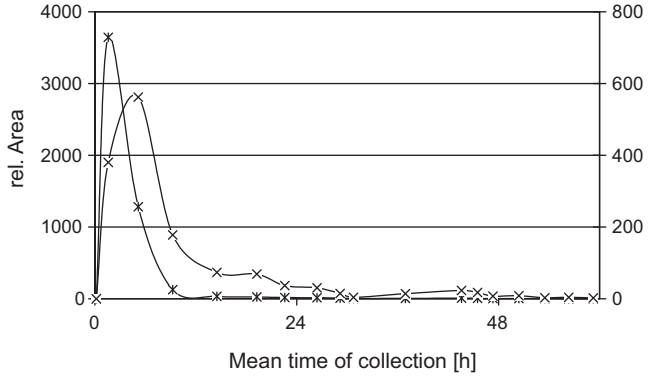


Fig. 11. Elimination kinetics of HO-Dione1 (*, left *y*-axis) and HO-Dione2 (×, right *y*-axis) after one capsule of "1-Androsterone", data shown using midpoints of collection periods and concentrations in 1000-fold peak area of analyte divided by area of internal standard.

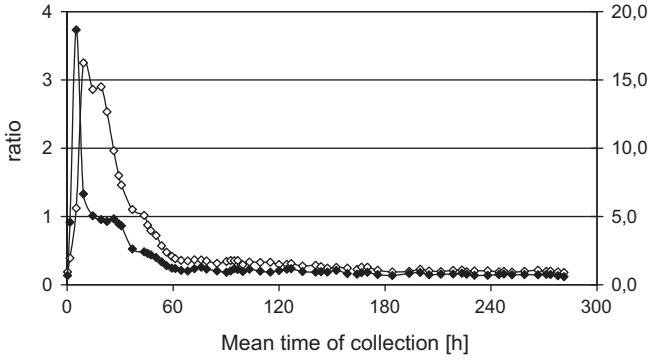


Fig. 12. Time course of AND/ETIO (♦, right *y*-axis) and Adiol/Bdiol ratio (◊, left *y*-axis) after oral administration of one capsule of "1-Androsterone", data shown using midpoints of collection periods.

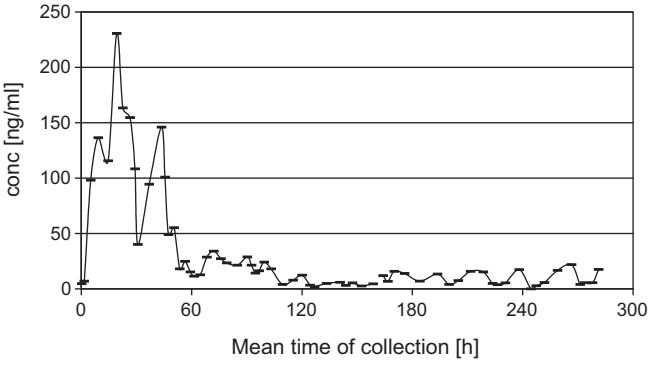


Fig. 13. Time course of EpiAND (—, concentrations not adjusted for density) after oral administration of one capsule of "1-Androsterone", data shown using midpoints of collection periods.

terone (EpiAND, kinetics in Fig. 13) and dihydrotestosterone (DHT) were determined. In human sports doping control the criteria considered as indicative for the administration of 5α-DHT in males as proposed by Donike et al. [16,17] are: a concentration of 5α-DHT > 21 ng/mL (adjusted for specific gravity) together with Adiol/Bdiol > 1.5, AND/ETIO > 2.9, DHT × 1000/ETIO > 8.2 and DHT/EpiT > 0.73. Thus, a potential misinterpretation of the results obtained after 3β-hydroxy-5α-androst-1-en-17-one administration has to be taken into account. All above mentioned criteria are fulfilled in the urines collected up to 30 h post-administration (Table 3). However, an excretion of 1-ene-steroids after 5α-DHT administration has never been reported up to now, allowing for tracing back the administered substance to these steroids. The proposed metabolism is illustrated in Scheme 1. As these data are based on only one administration study future research is needed to reinforce these findings. Additionally the analysis of the sulfoconjugated metabolites has to be addressed to future research.

4. Conclusions

The present results demonstrate that another "prohormone" with 1-ene structure is now available on the market. It is advertised to be "pro anabolic" and displays "even improved properties compared to the old 1-Andro or 1-Test products". While 1-testosterone, 5α-androst-1-ene-3,17-dione, and 5α-androst-1-ene-3ξ,17β-diol are covered by general legislation in several countries, the classification of 3β-hydroxy-5α-androst-1-en-17-one is not explicitly specified. In sports it is covered by the WADA list of prohibited substances [18] by the wording "and other substances with a similar chemical structure or similar biological effect(s)", and therefore, its use by athletes is prohibited.

Acknowledgements

The Manfred Donike Institut für Doping Analytik e.V., Cologne, and the Federal Ministry for the Interior, Berlin, are acknowledged for financial support of the study.

References

[1] US Drug Enforcement Administration. Anabolic steroids control act. US Drug Enforcement Administration; 2004.
[2] Catlin DH, Sekera MH, Ahrens BD, Starcevic B, Chang YC, Hatton CK. Tetrahydrogestrinone: discovery, synthesis, and detection in urine. Rapid Commun Mass Spectrom 2004;18:1245–9.
[3] Parr M, Geyer H, Opfermann G, Schänzer W. Prescription drugs and new anabolic steroids in nutritional supplements. In: Schänzer W, Geyer H, Gotzmann A, Mareck U, editors. Recent advances in doping analysis (12). Köln: Sport und Buch Strauß; 2004. p. 71–80.
[4] Sekera MH, Ahrens BD, Chang YC, Starcevic B, Georgakopoulos C, Catlin DH. Another designer steroid: discovery, synthesis, and detection of 'madol' in urine. Rapid Commun Mass Spectrom 2005;19:781–4.
[5] Parr MK, Opfermann G, Schänzer W. Detection of new 17-alkylated anabolic steroids on WADA 2006 list. In: Schänzer W, Geyer H, Gotzmann A, Mareck U, editors. Recent advances in doping analysis (14). Köln: Sport und Buch Strauß; 2006. p. 249–58.
[6] Rodchenkov G, Sobolevsky T, Sizoi V. New designer anabolic steroids from internet. In: Schänzer W, Geyer H, Gotzmann A, Mareck U, editors. Recent advances in doping analysis (14). Köln: Sport und Buch Strauß; 2006. p. 141–50.
[7] Reilly CA, Crouch DJ. Analysis of the nutritional supplement 1AD, its metabolites, and related endogenous hormones in biological matrices using liquid chromatography–tandem mass spectrometry. J Anal Toxicol 2004;28:1–10.
[8] Zhang L, Zhang Y, Xie M, Liu X, Wang J, Yan Y, et al. Excretion study with different Preparations of 1-Testosterone. In: Schänzer W, Geyer H, Gotzmann A, Mareck U, editors. Recent advances in doping analysis (14). Köln: Sport und Buch Strauß; 2006. p. 179–88.
[9] Parr MK, Becker M, Bartz U, Opfermann G, Schänzer W. Synthesis of isomers of 5-androstan-17-ol-3-one, androst-1- and -4-ene-3,17-diol and their characterisation with GC/MS DPhG-Jahrestagung 2005. Mainz: Deutsche Pharmazeutische Gesellschaft; 2005. p. 120.
[10] Donike M. N-Methyll-N-trimethylsilyl-trifluoroacetamide a new silylating agent from series of silylated amides. J Chromatogr 1969;42:103.
[11] Geyer H, Schänzer W, Mareck-Engelke U, Nolteernsting E, Opfermann G. Screening procedure for anabolic steroids-control of hydrolysis with deuterated androsterone glucuronide and studies with direct hydrolysis. In: Schänzer W, Geyer H, Gotzmann A, Mareck-Engelke U, editors. Recent advances in doping analysis (5). Sport und Buch Strauss: Köln; 1998. p. 99–102.
[12] Ciuffreda P, Casati S, Manzocchi A. Complete H-1 and C-13 NMR spectral assignment of 17-hydroxy epimeric sterols with planar A or A and B rings. Magn Reson Chem 2004;42:360–3.
[13] Schänzer W, Donike M. Metabolism of anabolic-steroids in man – synthesis and use of reference substances for identification of anabolic-steroid metabolites. Anal Chim Acta 1993;275:23–48.
[14] Parr MK, Zapp J, Becker M, Opfermann G, Bartz U, Schänzer W. Steroidal isomers with uniform mass spectra of their per-TMS derivatives: synthesis of 17-hydroxyandrostan-3-ones, androst-1-, and -4-ene-3,17-diols. Steroids 2007;72:545–51.

[15] World Anti-Doping Agency. WADA Technical document TD2003IDCR, 1.2 ed. World Anti-Doping Agency; 2004.
[16] Donike M, Ueki M, Kuroda Y, Geyer H, Nolteernsting E, Rauth S, et al. Detection of dihydrotestosterone (DHT) doping: alterations in the steroid profile and reference ranges for DHT and its 5alpha-metabolites. J Sport Med Phys Fit 1995;35:235–50.
[17] Geyer H, Schänzer W, Mareck-Engelke U, Donike M. Changes of the urinary steroid profile after sublingual application of dihydrotestosterone. In: Donike M, Geyer H, Gotzmann A, Mareck-Engelke U, editors. Recent advances in doping analysis (3). Köln: Sport und Buch Strauss; 1996. p. 215–30.
[18] World Anti-Doping Agency. The 2010 prohibited list. World Anti-Doping Agency; 2009.