Traditional Chinese medicine and sports drug testing: identification of natural steroid administration in doping control urine samples resulting from musk (pod) extracts

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ABSTRACT

The administration of musk extract, that is, ingredients obtained by extraction of the liquid secreted from the preputial gland or resulting grains of the male musk deer (eg, Moschus moschiferus), has been recommended in Traditional Chinese Medicine (TCM) applications and was listed in the Japanese pharmacopoeia for various indications requiring cardiovascular stimulation, anti-inflammatory medication or androgenic hormone therapy. Numerous steroidal components including cholesterol, 5α-androst-3,17-dione, 5β-androstane-3,17-dione, androsterone, etiocholanolone, epiandrosterone, 3β-hydroxy-androst-5-en-17-one, androst-4-ene-3,17-dione and the corresponding urea adduct 3α-ureido-androst-4-en-17-one were characterised as natural ingredients of musk over several decades, implicating an issue concerning doping controls if used for the treatment of elite athletes. In the present study, the impact of musk extract administration on sports drug testing results of five females competing in an international sporting event is reported. In the course of routine doping controls, adverse analytical findings concerning the athletes’ steroid profile, corroborated by isotope-ratio mass spectrometry (IRMS) data, were obtained. The athletes’ medical advisors admitted the prescription of TCM-based musk pod preparations and provided musk pod samples for comparison purposes to clarify the antidoping rule violation. Steroid profiles, IRMS results, literature data and a musk sample obtained from a living musk deer of a local zoo conclusively demonstrated the use of musk pod extracts in all cases which, however, represented a doping offence as prohibited anabolic–androgenic steroids were administered.

INTRODUCTION

Traditional Chinese Medicine (TCM) has been employed for the treatment of various disease symptoms including, for instance, asthma bronchiale, cough and skin disorders.1 2 The potential risk of adverse analytical findings in the course of doping controls has also been discussed particularly for TCM preparations used for the attenuation of the common cold, containing, for example, Ephedrae Herba as a major component of Sho-seiryu-to,5 Kakkon-to4 and Ma Huang.5 These herbal products were shown to contain ephedrine and its structural analogues such as norephedrine, pseudoephedrine and cathine that would lead to a positive test result if urinary threshold levels are exceeded. Sources and applications of steroid hormone-containing remedies were rarely reported; however, few articles were published particularly concerning the composition6 7 and use of musk pod and its extracts as TCM in cases of health conditions necessitating cardiovascular and/or β-adrenergic stimulation,3 anti-inflammatory therapeutics9 or androgenic hormone intervention.6 7 10 Musk is the dried secretion from the preputial follicles of the male musk deer, which are located in a small sac (resulting from an infolding of the skin) in close proximity to the preputial orifice (figure 1A).11 This sac (or pod), which contains the brownish musk, comprises a small canal debouching close to the preputial orifice that allows the controlled release of the pungent product by the animal. Upon removal of moisture, the material converts into small, dark, reddish-brown musk grains. In comprehensive studies,6 7 the lipid constituents of musk were elucidated and numerous steroidal components were characterised as summarised in table 1.

Urinary steroidal hormones have been of great importance in clinical diagnosis and so called steroid profiles have been the subject of research and routine application for several decades. Initiated by the seminal studies by Shackleton et al using thin-layer chromatography and direct reflectance densitometry (which required a full week of staff ing to analyse six urine specimens),12 the use of column chromatography,13–17 and eventually gas chromatography/mass spectrometry (GC-MS)18–20 provided the required speed, sensitivity and robustness to establish doping control analytical procedures concerning urine steroid analysis.21 22 In case of atypical (ie, suspicious) analytical results, confirmatory measurements using gas chromatography/combustion/isotope-ratio mass spectrometry (GC/C/IRMS) are conducted to differentiate naturally deviating steroid profiles from those being the result of legitimate circumstances or illicit medications.23 24 In the present study, the detection of unusual urinary steroid profiles of five female athletes is reported, which was shown to be in agreement with the administration of musk (pod) extracts containing a mixture of different
natural androgens. Carbon isotope ratios of steroidal analytes proved their exogenous nature, and the presence of 3α-ureido-androst-4-en-17-one as a typical component of musk lipids was demonstrated by high-resolution/high-accuracy tandem mass spectrometry and comparison to chemically synthesised reference material.

**EXPERIMENTAL**

**Urine sample and musk (pod) extract analysis**

Doping control urine samples were collected as regular sports drug testing specimens in pre/out-of-competition and in competition periods of a tournament (for details, see, *Case Vignette* in the Results section). Following the reporting of adverse analytical findings for the administration of endogenous steroid(s), three samples of TCM formulations presumably consisting of musk extract and the remainders of two musk pods (figure 1B) were provided by the responsible team physician for clarification of the antidoping rule violation.

Urine samples were analysed according to validated doping control procedures for steroid-profile determination (applying specific gravity adjustments to 1.020 according to established regulations) using GC-MS and isotope-ratio mass spectrometry (IRMS)-based confirmation assays as described elsewhere. Quality controls for steroid profile and IRMS analyses were measured and met the relevant acceptance criteria. Aliquots of ethanolic extracts of 20–50 mg of musk (pod) preparations were also subjected to these methods to qualitatively and quantitatively measure the target compounds. In addition, liquid chromatography – high-resolution/high-accuracy (tandem) mass spectrometry (LC-MS/MS) was used to identify the analyte 3α-ureido-androst-4-en-17-one in urine and musk (pod) extracts. Aliquots of urine or musk samples were diluted (1:10) in 0.2% formic acid and injected into the LC-MS/MS system using the same setup as reported for the characterisation of the target analyte after chemical synthesis (*vide infra*).

**Chemical synthesis and characterisation of 3α-ureido-androst-4-en-17-one**

In order to unambiguously confirm the structure of 3α-ureido-androst-4-en-17-one, reference material was synthesised according to published methods. In brief, dehydroepiandrosterone (DHEA) was converted into its 17-ethylene ketal by means of ethylene glycol under acidic conditions, followed by oxidation of the 3-hydroxy function to yield androst-4-en-3-one-17-ethylene ketal. This intermediate was treated with sodium borohydride to reduce the 3-oxo group, and the subsequent incubation with urea under acidic conditions yielded the desired product of 3α-ureido-androst-4-en-17-one, which was finally purified by flash chromatography.

Characterisation of the reference material was performed with one- and two-dimensional nuclear magnetic resonance spectroscopy (NMR) and high-resolution/high-accuracy mass spectrometry. NMR analyses were conducted on a Bruker AV 600 instrument (Bruker, Karlsruhe, Germany) equipped with a 5 mm inverse probe head (z-gradient coil). All spectra were recorded at room temperature from solutions of approximately 10 mg/ml analyte concentration and calibration was conducted using the solvent residual peak as reference signal. Liquid chromatography high-resolution/high-accuracy (tandem) mass spectrometry was done using a Thermo (Bremen, Germany) open Accela ultrahigh-performance liquid chromatograph equipped with a Thermo Hypersil Gold analytical column (2.1 x 50 mm, particle size 1.9 μm), which was connected to a Thermo Q Exactive mass spectrometer (MS). The LC solvents used were 0.2% formic acid (A) and acetonitrile (B) and gradient elution was done starting at 90% A, decreasing to 0% A in 10 min, followed by re-equilibration at 90% A for 2.5 min. The flow rate was set to 200 μl/min. The MS was operated using positive heated-electrospray ionisation and two acquisition modes were employed: (A) full scan MS (m/z 100–1000) and (B) product ion scan MS (referred to as targeted higher energy collision-induced dissociation (HCD) of m/z 331 at a collision energy of 25 eV. The ion source was operated at 250°C using an ionisation voltage of +5 kV, and nitrogen for the curved linear ion trap was obtained from a nitrogen generator (CMC.
Instruments, Eschborn, Germany). The MS was calibrated using the manufacturer's calibration mixture (containing caffeine, the tetrapeptide MRFA and ultrasmark) allowing for mass accuracies <5 ppm for the period of analysis.

**Authentic musk**

Authentic musk was obtained from a live male of *M moschiferus*. The animal was born on 18 June 2007 in the Leipzig zoological garden (Leipzig, Germany). The ARKS number ('Animal Record Keeping System') of the zoo is M089999. Within this system, individuals can be identified unambiguously by the number of a transponder implant. The particular specimen of *M moschiferus* has the number 96800000479985. The animal was still alive and in healthy state during the experimental period. Taxonomically and physiologically, *M moschiferus* represents a ruminant. The presumed natural diet of this species mostly encompasses moss and lichen. As it is virtually impossible to reproduce the animal in captivity, the corresponding animals are fed a diverse variety of domestic vegetables such as cabbage, carrots, potatoes etc. Most important for the data presented hereafter, this diet neither feature C-4 plants in significant amounts nor contains significant sterol or steroid sources other than plants.

A small amount of musk was sampled from the preputial gland of the anaesthetised animal on 26 November 2008. Anaesthesia was induced in order to facilitate adjustment and therapy of a broken leg. Until further analysis, the musk sample was stored in a sealed Eppendorf polyethylene tube (1 ml) at −18°C. Upon arrival in the laboratory, the musk aliquot (27 mg) was dissolved in 2 ml of absolute ethanol. The preparation was vigorously shaken on a Vortex device for several minutes and subsequently stored in a sealed glass tube at −18°C.

**RESULTS AND DISCUSSION**

**Case vignette – details**

Four persons of a total of 21 athletes composing a team participating in the FIFA Women World Cup 2011, Germany were urine tested for doping control purposes out-of-competition (OOC) on day X. All samples returned negative results and the team played its first match on day X+3 when two athletes were selected for doping controls. One of these two specimens exhibited unusual steroid profile data necessitating a comprehensive IRMS analysis, which yielded an adverse analytical finding demonstrating the exogenous nature of etiocholanolone and other steroids. Due to these repetitive findings, the international federation (FIFA Antidoping) decided to conduct doping controls on the entire team after the following match another four days later (X+11). The team consisted of 19 remaining athletes since two individuals were meanwhile excluded due to the aforementioned antidoping rule violations. Out of these 19 doping controls, three specimens were tested positive with abnormal steroid profile data and IRMS-derived evidence for the administration of exogenous steroid preparations. These three athletes were not tested in any of the earlier doping controls.

The persons in charge of the team (representatives, team physicians) were invited to comment on the adverse analytical findings and contribute to the clarification of the scenario. The explanation provided was based on an accident that occurred 13 days prior to the first OOC doping controls (X-13), where a lightning stroke hit the goal post on the training ground. An unknown/undisclosed number of athletes were subsequently subjected to a Traditional Chinese Medicine therapy based on musk pod formulations, which was supposedly administered on the days X−4, X+3, X+7 and X+11. Aliquots of these products and remainders of musk pod preparations were provided for further analysis and investigation. A summary of the Case Vignette timeline is presented in figure 2.

**Analytical results – urine samples**

In the course of routine doping controls, steroid profile analyses of all urine samples are generated to indicate, among others, whether steroidal hormones of natural (endogenous) nature were administered. Commonly, the concentrations of testosterone (T), epitestosterone (EpiT), androsterone (A), etiocholanolone (E), 5α-androstan-3α,17β-diol (5α-Adiol), 5β-androstan-3α,17β-diol (5β-Adiol) and DHEA are determined and corresponding diagnostic ratios as, for example, T/EpiT, A/E, 5α-Adiol/5β-Adiol etc are calculated. Threshold levels are valid for urinary concentrations of the glucuronides of A and E with 10000 ng/ml, DHEA with 100 ng/ml, T and EpiT with 200 ng/ml. In addition, the ratio of the glucuronide conjugates of T/E should not exceed 4. If any of the threshold values is exceeded, additional testing with IRMS analysis is recommended.25

In the following, the steroid profile data of six doping control urine samples of the above-mentioned scenario with
corresponding IRMS results are presented (table 2). Particular emphasis is put on samples 1a and 1b, which belong to the same athlete from two different testing occasions with 1a being the OOC sample from day X and 1b the in competition (IC) specimen from day X+3 (ie, the day of the second musk pod therapy (figure 2)). The four doping control samples collected on day X on the training site of the team in question yielded unsuspicious results concerning their steroid profiles and no follow-up studies were required. On day X+3, two specimens including sample 1b were obtained and 1b was subjected to IRMS analysis due to abnormal etiocholanolone concentrations exceeding 10 000 ng/ml (after specific gravity adjustment to 1.020). The IRMS result regarding etiocholanolone concentration of approximately 16 939 ng/ml, but all 19 urine samples were subjected to IRMS analyses due to the prior two findings, enabling the detection of a total of three additional athletes having received endogenous steroids. Besides the commonly conducted steroid analyses, doping control urine samples were analysed for the presence of the urea conjugate 3α-ureido-androst-4-en-17-one, which is an abundant component of musk pod extracts. Owing to the lack of commercially available reference material, 3α-ureido-androst-4-en-17-one was synthesised and characterised for identification purposes (vide infra). In order to facilitate its detection in biological matrices, product ion scan experiments were conducted providing characteristic fragments at m/z 271, 253 and 81 (figure 5A). The ions at m/z 271.2052 (error: −1.1 ppm) and 253.1947 (error: −1.6 ppm) are suggested to resemble the precursor ion (M+H)+ at m/z 331.2376 (error: −1.1 ppm) as having received endogenous steroids.

Consequently, IRMS confirmatory measurements were conducted reinforcing the exogenous nature of A, E, 5α-Adiol, and Epit as shown in table 3. Based on these repetitive findings of antidoping rule violations within one team, all remaining 19 athletes were eventually called in for doping control urine sample collections on day X+7, which resulted in an additional three adverse analytical findings (samples 3–5, tables 2 and 3). Here, only in one sample (4), an atypical steroid profile result was observed with the etiocholanolone concentration of approximately 16 939 ng/ml, but all 19 urine samples were subjected to IRMS analyses due to the prior two findings, enabling the detection of a total of three additional athletes having received endogenous steroids.

### Table 2  Steroid profile data of six doping control urine specimens collected in the course of a world championship tournament. Atypical results are shown in bold. Due to the broad working range and the extremely elevated concentrations of, for example, etiocholanolone (E), the concentrations listed below are to be considered as semiquantitative estimates

<table>
<thead>
<tr>
<th>Sample</th>
<th>Test type</th>
<th>pH</th>
<th>Specific gravity (g/ml)</th>
<th>A (ng/ml)</th>
<th>E (ng/ml)</th>
<th>5α-Adiol (ng/ml)</th>
<th>5β-Adiol (ng/ml)</th>
<th>T (ng/ml)</th>
<th>Epit (ng/ml)</th>
<th>DHEA (ng/ml)</th>
<th>T/Epit</th>
<th>A/E</th>
<th>5α-Adiol/5β-Adiol</th>
<th>Epit/Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a OOC</td>
<td>5.4</td>
<td>1021</td>
<td>2192</td>
<td>1465</td>
<td>21</td>
<td>33</td>
<td>4</td>
<td>13</td>
<td>32</td>
<td>0.34</td>
<td>1.50</td>
<td>0.63</td>
<td>1.60</td>
<td>X</td>
</tr>
<tr>
<td>1b IC</td>
<td>5.3</td>
<td>1013</td>
<td>6857</td>
<td>10941</td>
<td>57</td>
<td>147</td>
<td>22</td>
<td>26</td>
<td>91</td>
<td>0.83</td>
<td>0.63</td>
<td>0.39</td>
<td>2.17</td>
<td>X+3</td>
</tr>
<tr>
<td>2 IC</td>
<td>5.1</td>
<td>1024</td>
<td>3981</td>
<td>16762</td>
<td>26</td>
<td>93</td>
<td>4</td>
<td>198</td>
<td>19</td>
<td>0.02</td>
<td>0.24</td>
<td>0.28</td>
<td>0.13</td>
<td>X+7</td>
</tr>
<tr>
<td>3 IC</td>
<td>6.0</td>
<td>1027</td>
<td>3017</td>
<td>7972</td>
<td>21</td>
<td>68</td>
<td>2</td>
<td>103</td>
<td>28</td>
<td>0.01</td>
<td>0.38</td>
<td>0.31</td>
<td>0.20</td>
<td>X+11</td>
</tr>
<tr>
<td>4 IC</td>
<td>4.9</td>
<td>1030</td>
<td>6253</td>
<td>18939</td>
<td>66</td>
<td>180</td>
<td>4</td>
<td>175</td>
<td>85</td>
<td>0.02</td>
<td>0.37</td>
<td>0.36</td>
<td>0.38</td>
<td>X+11</td>
</tr>
<tr>
<td>5 IC</td>
<td>4.9</td>
<td>1013</td>
<td>2762</td>
<td>9067</td>
<td>24</td>
<td>75</td>
<td>2</td>
<td>94</td>
<td>29</td>
<td>0.02</td>
<td>0.30</td>
<td>0.32</td>
<td>0.26</td>
<td>X+11</td>
</tr>
</tbody>
</table>

*Concentrations are specific gravity adjusted to 1.020.

5α-Adiol, 5α-Androstane-3α,17β-diol; 5β-Adiol, 5β-Androstane-3α,17β-diol; A, androsterone; DHEA, dehydroepiandrosterone; E, etiocholanolone; Epit, epitestosterone; IC, in competition; OOC, out-of-competition; T, testosterone.

### Table 3  IRMS results of doping control urine samples. Data representing an adverse analytical finding are illustrated in bold

<table>
<thead>
<tr>
<th>Sample</th>
<th>Test type</th>
<th>δ13C values of target analytes</th>
<th>δ13C values of endogenous reference compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A (‰)</td>
<td>E (‰)</td>
</tr>
<tr>
<td>1a OOC</td>
<td>5.4</td>
<td>−19.9</td>
<td>−21.1</td>
</tr>
<tr>
<td>1b IC</td>
<td>5.3</td>
<td>−22.4</td>
<td>−26.6</td>
</tr>
<tr>
<td>2 IC</td>
<td>5.1</td>
<td>−25.3</td>
<td>−27.1</td>
</tr>
<tr>
<td>3 IC</td>
<td>6.0</td>
<td>−22.5</td>
<td>−26.4</td>
</tr>
<tr>
<td>4 IC</td>
<td>4.9</td>
<td>−23.1</td>
<td>−26.0</td>
</tr>
<tr>
<td>5 IC</td>
<td>4.9</td>
<td>−23.8</td>
<td>−26.5</td>
</tr>
</tbody>
</table>

*Signal too low (<1nA).
†Peak interference detected by gas chromatography/mass spectrometry.
physician being responsible for the team members in question provided three different traditional Korean medicine preparations (allegedly containing musk) as well as the remainders of two musk pods for chemical analysis. While the ethanolic extracts of the three therapeutic formulations did not contain any anabolic–androgenic steroid arguably representing adulterated material\(^3\) (data not shown), the GC-MS and LC-MS/MS analyses of the musk pods and respective grains revealed the presence of at least 17 steroidal substances (table 4, samples 1 and 2), nine of which are prohibited according to WADA's 2011 regulations,\(^3\) namely 5α-androstan-3α,17α-diol, androsterone, 5α-androstan-3α,17β-diol, 5α-androstan-3β,17α-diol, DHEA, epitestosterone, EpiT, androstenedione and testosterone. The composition and relative concentration of the determined androgens is well in agreement with literature data and represents the typical signature of ruminant steroid patterns.\(^3\)–\(^4\) Moreover, the relative abundances of the steroids detected in the musk (pod) specimens provided by the athlete's team physician convincingly matched the steroid pattern determined from a reference musk grain sample collected from a live musk deer domiciled in a German zoo (table 4, sample 3), corroborating the assumption that the samples 1 and 2 represent authentic musk deer specimens. By means of GC/C/IRMS, the carbon isotope ratios of the three steroidal analytes androsterone, etiocholanolone and 5β-androstan-3α,17α-diol extracted from samples 1 and 2 were determined at −28.7‰, −28.4‰ and −28.7‰, respectively, further supporting the authenticity of the musk specimens.

Finally, all three samples were tested for the presence of the characteristic urea-conjugated analogue to 3α-hydroxy-androsterone-4-ene-3β,17β-diol (3α-ureido-androsterone-4-ene-17-one). In all specimens, the target analyte was identified by its accurate mass (error <5 ppm) and diagnostic product ions (m/z 271, 255 and 81) generated by means of CID of the protonated molecule (M+H)\(^+\) at m/z 331.

**Chemical synthesis and characterisation of 3α-ureido-androst-4-ene-17-one**

Reference material of 3α-ureido-androsterone-4-ene-17-one was synthesised according to established procedures.\(^3\)\(^1\) The aimed structure was confirmed by \(^1\)H NMR spectroscopy including \(^1\)H–\(^1\)H TOCSY and NOESY correlation spectra and comparison to literature data,\(^3\)\(^1\) with the NOE interactions outlining the desired stereochemistry at C-3: \(^1\)H NMR (pyridine-d\(_5\), 600 MHz): δ (ppm)=0.31 (m, 1H, H-9), 0.61 (m, 1H, H-7), 0.76 (s, 3H, H-18), 0.85 (s, 3H, H-19), 0.91 (m, 1H, H-14), 1.11 (m, 1H, H-12), 1.15 (m, H-11), 1.27 (m, 1H, H-1 ax.), 1.34 (m, 1H, H-11), 1.36 (m, 1H, H-8), 1.39 (m, 1H, H-1 eq.), 1.59 (m, 1H, H-7), 1.69 (m, 1H, H-15), 1.71 (m, 1H, H-15), 1.74 (m, 1H, H-2 ax.), 1.80 (m, 1H, H-12), 1.91 (m, 1H, H-6), 1.95 (m, 1H, H-2 eq.), 2.00 (m, 1H, H-16), 2.08 (m, 1H, H-6), 2.39 (m, 1H, H-16), 4.65 (s, 1H, H-3), 5.47 (d, J=4.9 Hz, 1H, H-4), 6.21 (s, 2H, NH\(_2\)), 6.81 (d, J=8.0 Hz, 1H, NH).

The accurate mass analysis of the protonated molecule of the prepared 3α-ureido-androsterone-4-ene-3β,17β-diol yielde d m/z 331.2580 (C\(_{20}\)H\(_{35}\)O\(_2\)N\(_2\), error: 0.1 ppm), further confirming the suggested structure of the synthesised reference material.

**CONCLUSIONS**

The administration of endogenous steroids to athletes competing in an international sporting event was demonstrated, indicated by atypical steroid profiles in initial testing procedures and confirmed by isotope-ratio mass spectrometry.

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**Analytical results – musk-containing material**

In the course of investigations concerning the unusual steroid profile results and corresponding adverse analytical findings, the
doping control analytical procedures. In cooperation with the responsible team representatives and the International Sport Federation (FIFA), the use of musk pod formulations was identified as the source of the steroidal substances, which contained nine prohibited androgens and further a urea conjugate of androstan-4-ene-3,17-dione. Due to the undisputed antidoping rule violation in five cases, sanctions between 14 and 18 months were imposed on the athletes and the team is further excluded from the forthcoming FIFA Women World Cup.

The herein presented observations underline the importance of an adequate education of athletes and their medical staff concerning all therapeutics being considered for the athletes’ treatment, even if (or particularly when) these medications belong to the so called ‘traditional medicines’. It might be worthwhile issuing a general warning to athletes, doctors, paramedics and international federations concerning ‘traditional medicine’ preparations, which do not indicate their ingredients as those might contain prohibited substances and, thus, might lead to violations of the World Anti-Doping Code followed by necessary sanctions.

**Contributors**

WS, HG and UF conducted the steroid profile analyses of musk pod preparations including data interpretation; DT, JG and CR performed doping control analyses with steroid profiling and IRMS measurements, RH provided authentic preparations including data interpretation; JD coordinated the analyses with steroid profiling and IRMS measurements, RH provided authentic preparations including data interpretation; JD coordinated the synthesis of reference steroids and LC-MS/MS analyses, and JD coordinated the doping control procedures and the overall case management.

**Acknowledgements**

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**Competing interests**

None.

**Provenance and peer review**

Not commissioned; externally peer reviewed.

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### Table 4  Steroids identified by gas chromatography/mass spectrometry and LC-MS/MS in two musk (pod) samples provided by the athletes’ team physician and a reference musk grain specimen collected from a live musk deer in a German zoo

<table>
<thead>
<tr>
<th>Steroid</th>
<th>Sample 1</th>
<th>Sample 2</th>
<th>Sample 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Musk pod tissue and grains</td>
<td>Musk grains</td>
<td>Musk grains Zoo Leipzig</td>
</tr>
<tr>
<td><strong>%</strong></td>
<td>µg/g</td>
<td>%</td>
<td>µg/g</td>
</tr>
<tr>
<td>5α-Androstan-3α,17β-diol</td>
<td>9.6</td>
<td>1358</td>
<td>12.5</td>
</tr>
<tr>
<td>5α-Androstan-3α,17β-diol*</td>
<td>0.9</td>
<td>122</td>
<td>1</td>
</tr>
<tr>
<td>3α-Hydroxy-androstan-4-en-17-one</td>
<td>4.8</td>
<td>679</td>
<td>10</td>
</tr>
<tr>
<td>Androsterone*</td>
<td>3.0</td>
<td>512</td>
<td>5.1</td>
</tr>
<tr>
<td>Etiocholanolone</td>
<td>30.2</td>
<td>4272</td>
<td>29.2</td>
</tr>
<tr>
<td>5α-Androstan-3α,17β-diol*</td>
<td>0.1</td>
<td>17</td>
<td>0.1</td>
</tr>
<tr>
<td>5β-Androstan-3α,17β-diol</td>
<td>2.3</td>
<td>306</td>
<td>5.8</td>
</tr>
<tr>
<td>5α-Androstan-3β,17β-diol*</td>
<td>7</td>
<td>987</td>
<td>4.1</td>
</tr>
<tr>
<td>3β-Hydroxy-androst-4-en-17-one</td>
<td>2.7</td>
<td>383</td>
<td>2.4</td>
</tr>
<tr>
<td>DHEA*</td>
<td>0.3</td>
<td>49</td>
<td>0.1</td>
</tr>
<tr>
<td>Epiandrosterone*</td>
<td>1.9</td>
<td>269</td>
<td>1.9</td>
</tr>
<tr>
<td>Epitestosterone*</td>
<td>0.3</td>
<td>48</td>
<td>0.1</td>
</tr>
<tr>
<td>Androstan-4-ene-3,17-dione*</td>
<td>0.5</td>
<td>67</td>
<td>0.1</td>
</tr>
<tr>
<td>Testosterone*</td>
<td>&lt;0.01</td>
<td>2</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>15.7</td>
<td>2227</td>
<td>10.6</td>
</tr>
<tr>
<td>5α-Cholesterol</td>
<td>4.5</td>
<td>634</td>
<td>2</td>
</tr>
<tr>
<td>Other steroids</td>
<td>16</td>
<td>2263</td>
<td>14.5</td>
</tr>
<tr>
<td>3α-Ureido-androst-4-en-17-one</td>
<td>†</td>
<td>†</td>
<td>†</td>
</tr>
<tr>
<td>Sum</td>
<td>14.194</td>
<td>35.494</td>
<td>19.558</td>
</tr>
</tbody>
</table>

*Steroids prohibited according to the WADA Prohibited List 2011, S1.1b.†

†Identified but not quantified.

DHEA, dehydroepiandrosterone.

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### What this study adds

- Antidoping rule violation with natural steroids of animal origin was uncovered
- Source of the steroidal preparation was determined as musk pod extract
- Characteristic steroid distribution and steroid derivatives were identified, synthesised and their presence proven by mass spectrometry

**REFERENCES**


