

## **1-DHEA identification in seized dietary supplement**

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### *Abstract*

For several years, anabolic androgenic steroids have been offered on the “dietary supplement” market. Since 1996, mainly prohormones of testosterone and nandrolone have been available. Following their classification as schedule III controlled substances by the 2004 Anabolic Steroid Control Act, products appeared on the market containing steroids that were never marketed as approved drugs. In the present study, we report the detection of steroids in a product seized by the governmental body. The product “1-Androsterone” of the brand name “Advanced Muscle Science” was labelled to contain 100 mg of “1-Androstene-3 $\beta$ -ol,17-one” per capsule. The product was analyzed underivatized and as bis-TMS derivative by GC-MS. For comparison, 3 $\beta$ -hydroxy-5 $\alpha$ -androst-1-en-17-one (1-DHEA) was prepared by reduction of 5 $\alpha$ -androst-1-ene-3,17-dione with LS-Selectride. After soxhlet extraction of the capsule content with n-hexane, the steroid identity was additionally confirmed by nuclear magnetic resonance spectroscopy. The amount of 3 $\beta$ -hydroxy-5 $\alpha$ -androst-1-en-17-one was determined with 115 mg/capsule. From the knowledge of general steroid metabolism and forced by the indication on the product label that “1-Androsterone “ converts to 1-Testosterone the expected urinary metabolites 3 $\alpha$ -hydroxy-5 $\alpha$ -androst-1-en-17-one, 5 $\alpha$ -androst-1-ene-3 $\alpha$ ,17 $\beta$ -diol, and -3 $\beta$ ,17 $\beta$ -diol were synthesized by reduction of 1-Testosterone or 5 $\alpha$ -androst-1-ene-3,17-dione.

### *Introduction*

For several years anabolic androgenic steroids have been offered on the “dietary supplement” market. In the present study we report the detection of steroids in a product seized by the governmental body.

## *Materials and Methods*

### *Supplement*

The product "1-Androsterone" of the brand name "Advanced Muscle Science" was seized by the governmental body. It was labeled to be a dietary supplement containing 100 mg of "1-Androstene-3 $\beta$ -ol,17-one" per capsule.

### *Supplement analysis*

For identification of the steroid ingredient 1.0 g of the capsule content (555 mg/capsule) was extracted with n-hexane in a Soxhlet apparatus. After crystallization the steroid was characterized by GC-MS and NMR.

### *Preparation for GC-MS measurement*

The final residues were either derivatized with TMIS reagent (MSTFA/ NH<sub>4</sub>I/ ethanethiol, 1000:2:3, v:w:v) by heating for 20 min at 60°C or reconstituted in acetone and injected into the GC-MS.

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

### *Chemicals and reagents*

Testosterone (17 $\beta$ -hydroxyandrost-4-en-3-one) was purchased from NMI (Sydney, Australia), LS-selectride (Lithium trisiamylborohydride, 1 M in tetrahydrofuran), from Sigma-Aldrich GmbH (Steinheim, Germany), and 5 $\alpha$ -androst-1-ene-3,17-dione (1-androstenedione) from Steraloids (Wilton, USA). 17 $\beta$ -Hydroxy-5 $\alpha$ -androst-1-en-3-one (1-testosterone) was obtained from Thinker Chemicals (Hangzhou, China), 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).

### *3-Reduction of 5 $\alpha$ -androst-1-ene-3,17-dione and 1-testosterone*

1-Testosterone or 5 $\alpha$ -androst-1-ene-3,17-dione were reduced with 2-fold molar excess of LS-Selectride. After 1 h at ambient temperature aqueous ammonium chloride solution (10 % in H<sub>2</sub>O) was added, pH~10 was adjusted and the mixture was extracted with 5 mL of t-butyl methyl ether (TBME).

## Instrumentation

### NMR spectroscopy

The NMR data were obtained using a Bruker DRX 500 instrument. Chemical shifts were given in  $\delta$  values (ppm) relative to tetramethylsilane. The spectra were recorded at 500 MHz (1H) and 125 MHz (13C) at 298 K using solutions of about 5 mg in deuterated chloroform.

### 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 (17 m; 0.20 mm i.d.; 0.11  $\mu$ m 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  $\mu$ L, split 1:16, injection temperature: 300°C, ionization: 70 eV, EI, full scan mode, 40-800 Da. Additionally the underivatized compounds were analyzed on a GC Agilent 6890 coupled to a MSD Agilent 5973, injection volume: 2  $\mu$ L, splitless, injection temperature: 300°C, column: Macherey-Nagel Optima-P-XLB column (30 m, 0.25 mm i.d., 0.5  $\mu$ m 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.

## Results and Discussion

The product was found to contain 3 $\beta$ -hydroxy-5 $\alpha$ -androst-1-en-17-one (GC-MS data in tab. 1). Its identity was confirmed by NMR and synthesis (fig. 1). The depletive extraction of 1.0 g of the capsule content revealed an amount of 115 mg/capsule.

The expected urinary metabolites 3 $\alpha$ -hydroxy-5 $\alpha$ -androst-1-en-17-one, 5 $\alpha$ -androst-1-ene-3 $\alpha$ ,17 $\beta$ -diol, and -3 $\beta$ ,17 $\beta$ -diol were synthesized by reduction of 1-Testosterone or 5 $\alpha$ -androst-1-ene-3,17-dione (fig. 1).

The GC-MS data of these steroids were determined as underivatized and TMS derivatized analytes (tab. 1). As common the 3 $\alpha$  and 3 $\beta$  isomers revealed almost identical mass spectra [1].

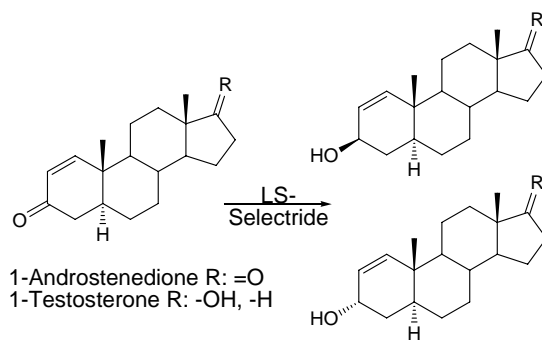


Figure 1: Reduction of 3-oxo-1-enes with LS-Selectride

Table 1: GC-MS data of steroids used as reference (calculated using Testosterone, RT(bis-TMS)=13.01 min, RT(underivatized)=17.76 min)

	RRT (bis-TMS)	Major fragment ions (intensity relative to base peak in brackets)	RRT (under.)	Major fragment ions (intensity relative to base peak in brackets)
3 $\beta$ -hydroxy-5 $\alpha$ -androst-1-en-17-one	0.90	73(100), 432(70), 417 (76), 275(56), 290(36), 169(18)	0.88	218(100), 288(92), 161(68), 91(62), 190(54), 122(42)
3 $\alpha$ -hydroxy-5 $\alpha$ -androst-1-en-17-one	0.89	73(100), 432(82), 417 (85), 275(48), 290(27), 169(24)	0.87	218(86), 288(100), 161(44), 91(58), 190(58), 122(32)
17 $\beta$ -hydroxy-5 $\alpha$ -androst-1-en-3-one	0.91	194(100), 73(60), 432(52), 206(30), 179(28), 417(17)	0.95	288(79), 246(100), 204(43), 122(80), 79(59)
5 $\alpha$ -androst-1-ene-3,17-dione	0.88	415(100), 73 (96), 430(30), 194(26)	0.95	286(62), 244(96), 202(30), 122(100), 79(46)
5 $\alpha$ -androst-1-ene-3 $\beta$ ,17 $\beta$ -diol	0.92	434(100), 143 (74), 129(22), 405(38), 127(24), 73 (74)	0.93	290(88), 231(100), 163(28), 123(28)
5 $\alpha$ -androst-1-ene-3 $\alpha$ ,17 $\beta$ -diol	0.91	434 (100), 143 (80), 129(20), 405(32), 127(35), 73(80)	0.92	

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 to display even improved properties compared to “the old 1-Andro or 1-Test products”. While 1-Testosterone, 5 $\alpha$ -androst-1-ene-3,17-dione, and 5 $\alpha$ -androst-1-ene-3 $\xi$ ,17 $\beta$ -diol are covered by general legislation in several countries, the classification of 3 $\alpha$ -hydroxy-5 $\alpha$ -androst-1-en-17-one is not clarified. Open lists for categorisation as in sports by the WADA list of prohibited substances are desired for the future.

#### Acknowledgements

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#### Remark

More details on identification and metabolism of 3 $\alpha$ -hydroxy-5 $\alpha$ -androst-1-en-17-one are available in:

Parr, M. K., Opfermann, G., Geyer, H., Westphal, F., Sönnichsen, F.D., Zapp, J., Kwiatkowska, D., Schänzer, W. (2010) Seized Designer Supplement named “1-Androsterone”: Identification as 3 $\beta$ -Hydroxy-5 $\alpha$ -androst-1-en-17-one and its Urinary Elimination. *Steroids*, submitted

#### References

- Parr, M. K., Zapp, J., Becker, M., Opfermann, G., Bartz, U., Schänzer, W. (2007) Steroidal isomers with uniform mass spectra of their per-TMS derivatives: synthesis of 17-hydroxyandrost-3-ones, androst-1-, and -4-ene-3,17-diols. *Steroids* **72**, 545-551.