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New Designer Anabolic Steroids from Internet

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Introduction

The market of androgenic anabolic steroids is constantly supplied with newly synthesized substances, most of which are just minor alterations to the structure of existing anabolic drugs, nevertheless being presented as 'even better than ever'.

We have studied the metabolic pathways of 5 anabolic 'supplements' Methyl 1-P, Methyl 1-Alpha, Methyl Masterdrol from Legal Gear, ERGO MAX Lean Mass Generator (desoxymethyltestosterone) and Prostanozol from Anabolic Xtreme (**Fig. 1**).

Fig. 1. Steroid supplements emerged in 2005.

As supplement manufacturers are no longer allowed to use the root 'andro' while naming supplement constituents, they have changed it for 'etioallocholane' or something like that and sometimes the names are totally confusing (see below).

Experimental

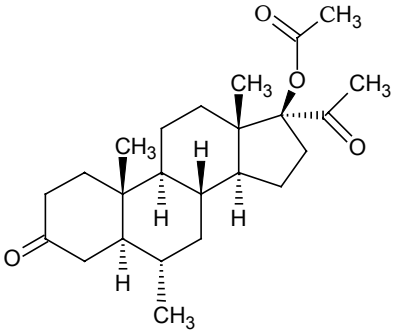
Healthy male volunteers participated in the excretion studies. Urine was subjected to standard screening procedure for total anabolic steroids [1]: to 3 ml of urine 50 µl of internal standard (methyltestosterone) solution, 1 ml of 1M acetate buffer (pH 5.5) and 30 µl of β-glucuronidase from *Helix Pomatia* were added and enzymolysis was performed at 57°C for 3

hrs. After enzymolysis pH was adjusted to 9 by adding carbonate/bicarbonate buffer, and then *ca*. 2 g of anhydrous Na_2SO_4 were added followed by liquid-liquid extraction with 5 ml of diethyl ether. After centrifugation ether layer was evaporated to dryness at 60°C and the residue was derivatized with 50 µl of MSTFA / NH_4I / dithiothreitol (1000:2:4 v/w/w) or, alternatively, MSHFBA / TMSI / TMCS (100:2:5 v/v/v) at 60°C for 20 min. Three µl aliquot was injected in GC-MS Agilent 6890N/5973inert in split mode 1:10 at injection port temperature of 280°C. Separation was performed on a HP-1 Ultra quartz capillary column 12 m × 0.2 mm × 0.33 µm, temperature program was as follows: 190°C (0 min), 2°C/min to 234°C (0 min), 12°C/min to 300°C (4.5 min). Carrier gas (helium) head pressure was 10.1 psi.

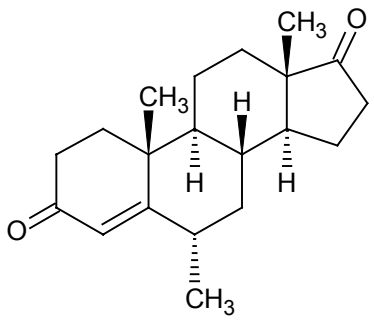
As confirmatory synthesis of the metabolites was not performed, their real structure was not elucidated and was proposed based on molecular weight and fragmentation pattern only.

Results

Methyl 1-P is declared as a mixture of '17-hydroxy-6α-methyl-ethyletiocholan-3,20-dione acetate 50 mg' (**I**) and '6α-methyl-etiocholene-3,17-dione 10 mg' (**II**).



I



II

Compound **I** disappears in course of metabolism most likely due to the loss of 17α-acetyl group thereby having the same biotransformation as **II**. Compound **II** was found to give tetrahydrometabolite with molecular weight 448 amu (as bis-TMS, **Fig. 2**). Except for ions at *m/z* 289 and 304, the spectrum is quite similar to that of TMS-enol derivatives of mesterolone metabolite (1α-methyl-5α-androstane-3α-ol-17-one) and drostanolone metabolite (2α-methyl-5α-androstane-3α-ol-17-one), therefore the metabolite of **II** should be 6α-methyl-5α-androstane-3α-ol-17-one. The metabolite elutes at RRT 0.7383 (relatively to methyltestosterone) and appears in drostanolone metabolite window, shifted left.

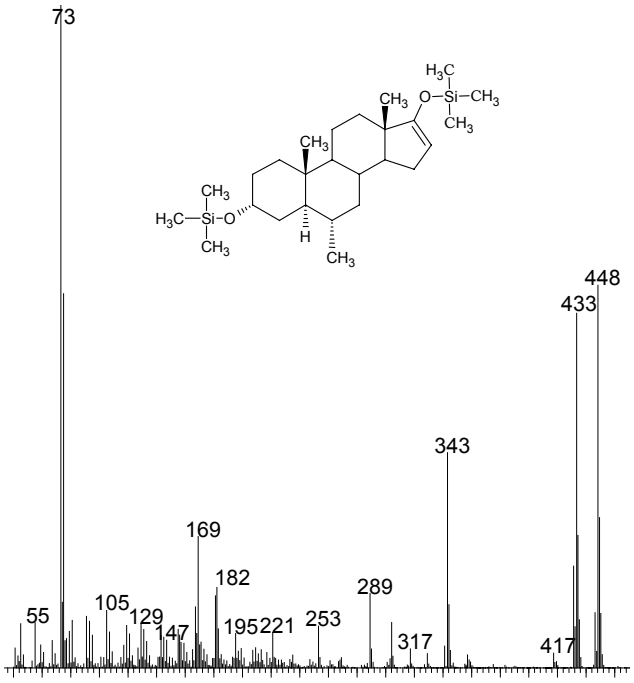
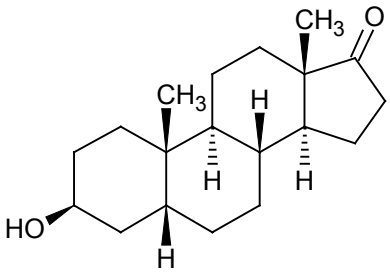


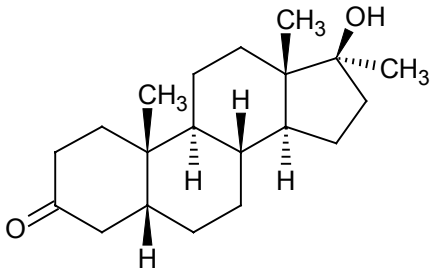
Fig. 2. Mass spectrum of tetrahydrometabolite of **II** as bis-TMS.

Methyl 1-Alpha

Tricky name on the label: 'methyl-1-etiocholenolol epietiocholanolone 20 mg'. Based on mass spectral data, we supposed that it contains the mixture of epietiocholanolone (**III**) and 17α-methyl-17β-hydroxy-5β-androstane-3-one, or 5β-mestanolone (**IV**).



III



IV

We were unable to detect any traces of **III** in urine, probably it converts to etiocholanolone. Compound **IV**, unlike mestanolone, gives dehydrometabolite with molecular weight 446 amu (as bis-TMS, **Fig. 3**), which elutes at RRT 0.7886.

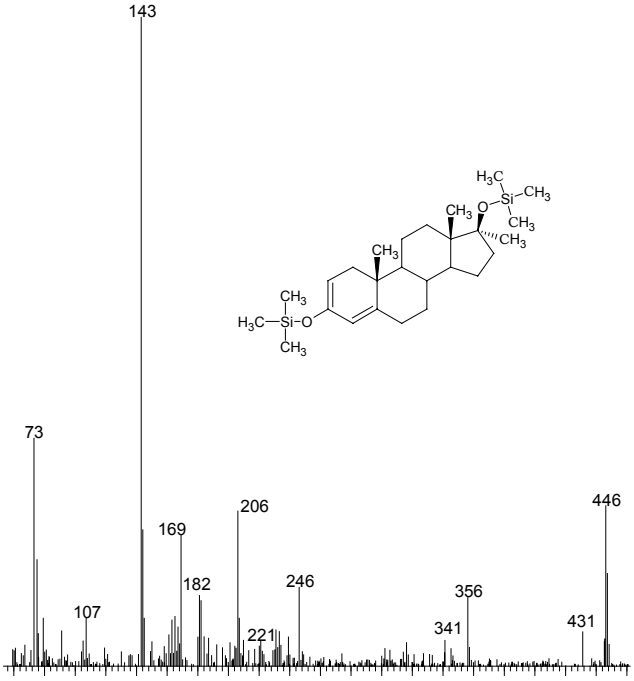
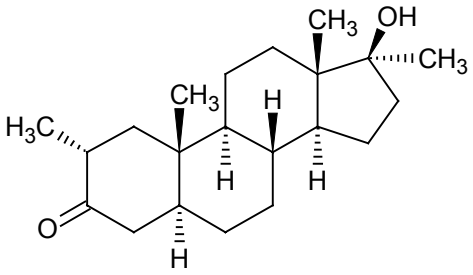


Fig. 3. Mass spectrum of dehydrometabolite of **IV** as bis-TMS.

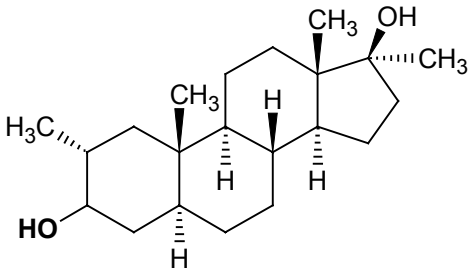
Methyl Masterdrol

Contains 10 mg of '2α,17α-dimethyl-etiocholan-3-one-17-ol' (methasterone, superdrol) (**V**).



V

Methasterone excretes intact (RRT 1.0180) and also gives dihydrometabolite (**VI**) with molecular weight 464 amu (as bis-TMS), RRT 0.8563, which appears in the window of mibolerone tetrahydrometabolite.



VI

Interestingly, according to our excretion studies of 2 volunteers who administered methasterone from Legal Gear (Methyl Masterdrol), parent compound has been excreted mostly intact and only minor amount of dehydrometabolite **VI** was detected. At the same time, in WAADS QA2006_1 urine (Superdrol excretion) the amount of **VI** was 5 times greater than that of parent compound.

The mass spectra of **V** and **VI** are given in **Fig. 4a** and **Fig. 4b**.

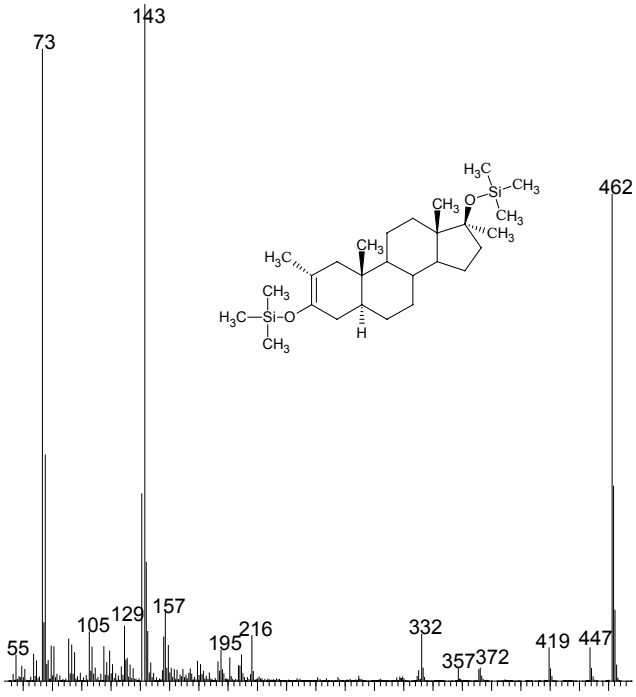


Fig. 4a. Mass spectrum of **V** as bis-TMS.

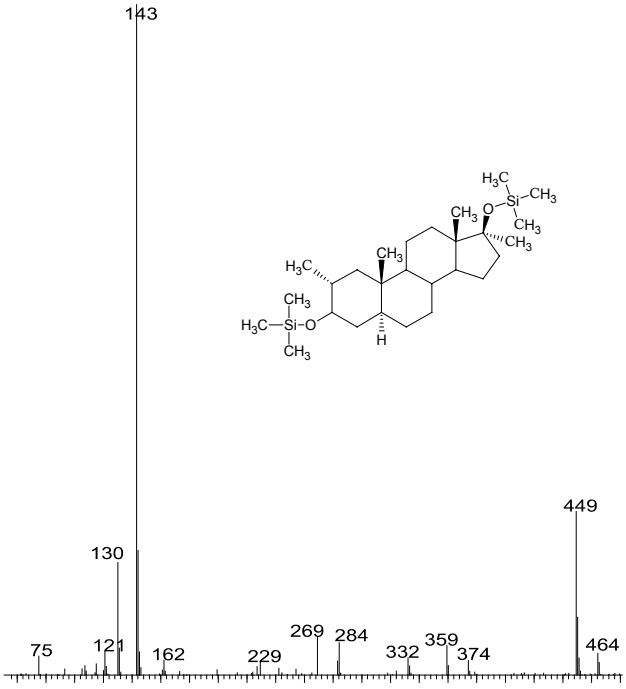
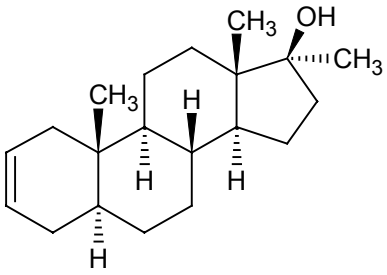


Fig. 4b. Mass spectrum of **VI** as bis-TMS.

ERGO MAX LMG

According to the label, this supplement contains 10 mg of '17-methyl-delta-2-etioallocholane', or desoxymethyltestosterone [2] (**VII**). Actually, it presents the mixture of 5α/5β-DMT in ratio *ca.* 6:1.



VII

Desoxymethyltestosterone is extensively metabolized via hydroxylation and reduction of double bond to produce at least 8 metabolites with molecular weights (as TMS derivatives)

448 (mono hydroxy, **IX-XI**), 536 (3-oxo dihydro hydroxy, **XV**), 538 (dihydro dihydroxy, **VIII** and **XII**), and 626 amu (dihydro trihydroxy, **XIII** and **XIV**). The parent compound was not detected. The scheme on **Fig. 5** represents proposed metabolic pathways. Silylation with MSTFA/NH_4I/dithiothreitol and MSHFBA/TMSI/TMCS, and methoximation-silylation revealed that there is no oxo group in all metabolites, except metabolite **XV**, as they eluted at the same time and had the same mass spectra regardless of derivatization type.

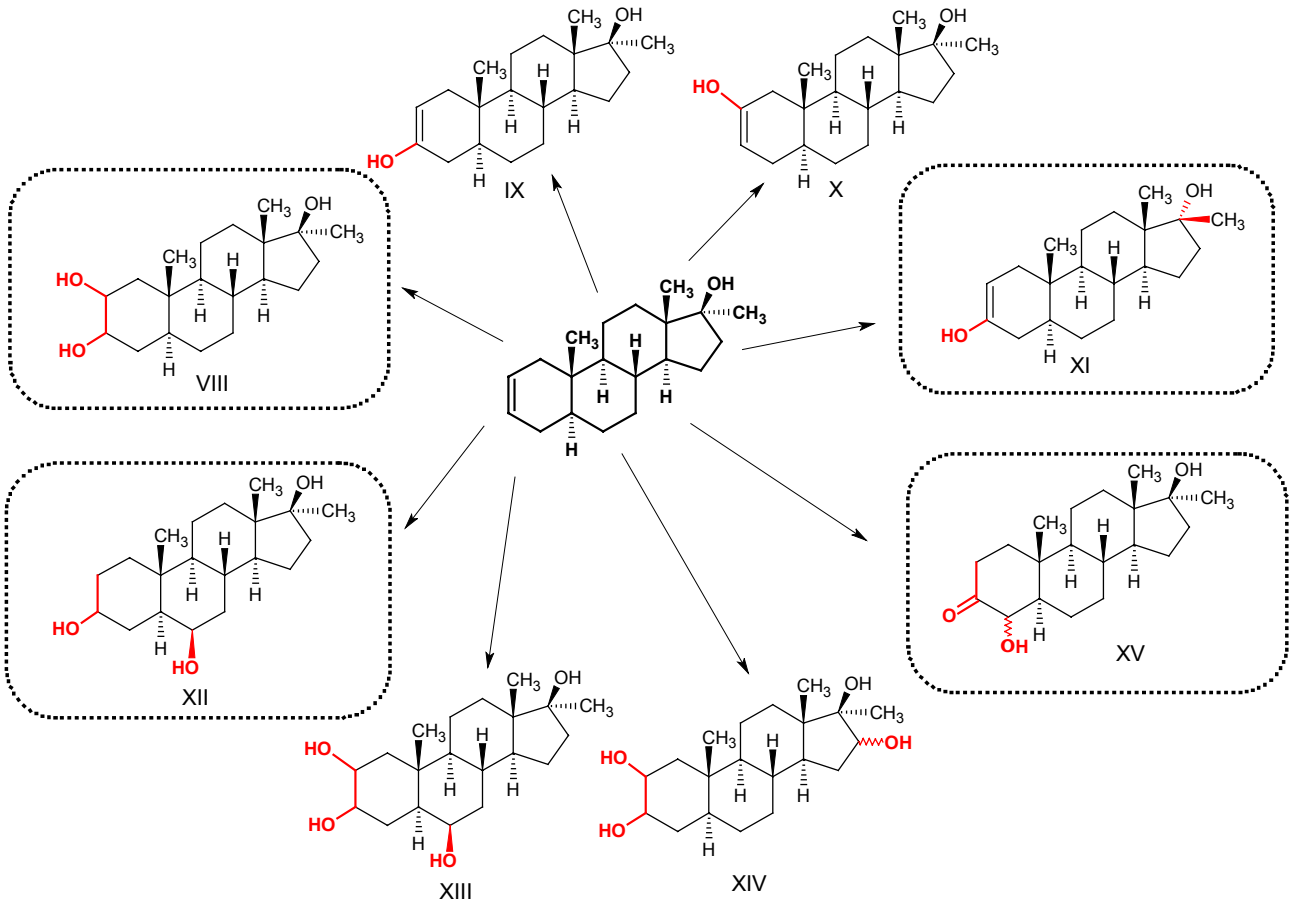


Fig. 5. Metabolic pathways of DMT (main metabolites are boxed).

Detection of DMT metabolites is highly affected by urine matrix components which may strongly interfere with most DMT metabolites excreted in low concentrations. For instance, the presence of large amounts of dihydroxy xanthenone (*m/z* 357, 372), occurring in some urines, completely mask metabolite **XI** due to close *m/z* ratios and coelution. Common 11β-hydroxyandrosterone has the same or very close RT as metabolite **XI** that also makes its detection difficult. The mass spectra of TMS derivatives of DMT are non-informative due to low abundance of the molecular ion (**Fig. 6–8**). Since confirmatory synthesis was not performed, the real structure of the metabolites is not known.

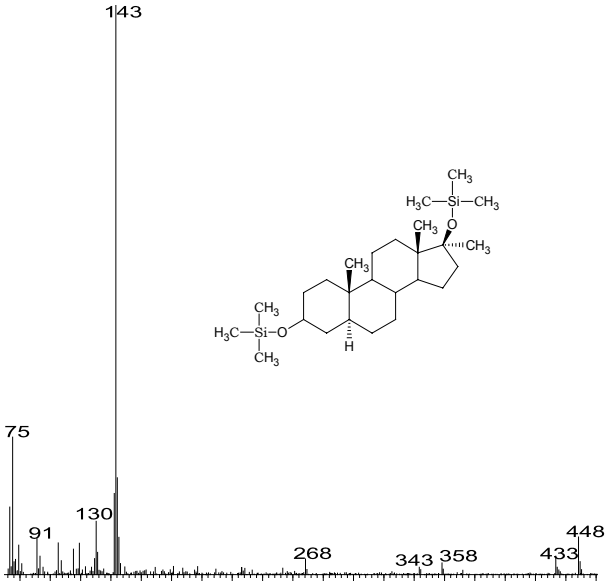


Fig. 6. Mass spectrum of mono hydroxy metabolite of DMT as bis-TMS, RRT 0.8632.

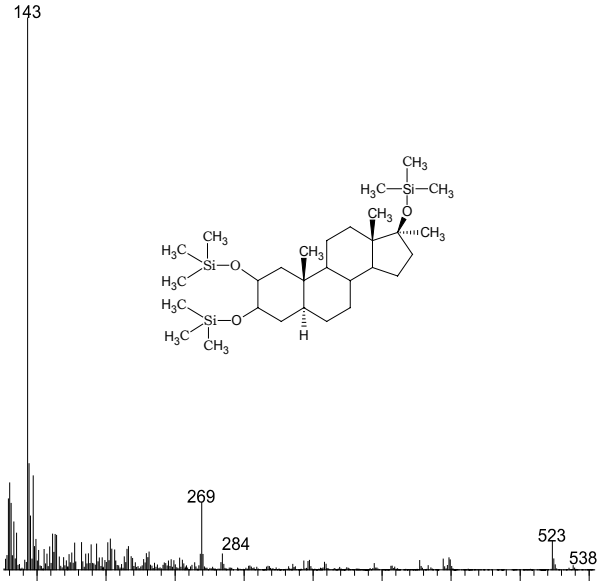


Fig. 7. Mass spectrum of dihydro dihydroxy metabolite of DMT as tris-TMS, RRT 1.0264.

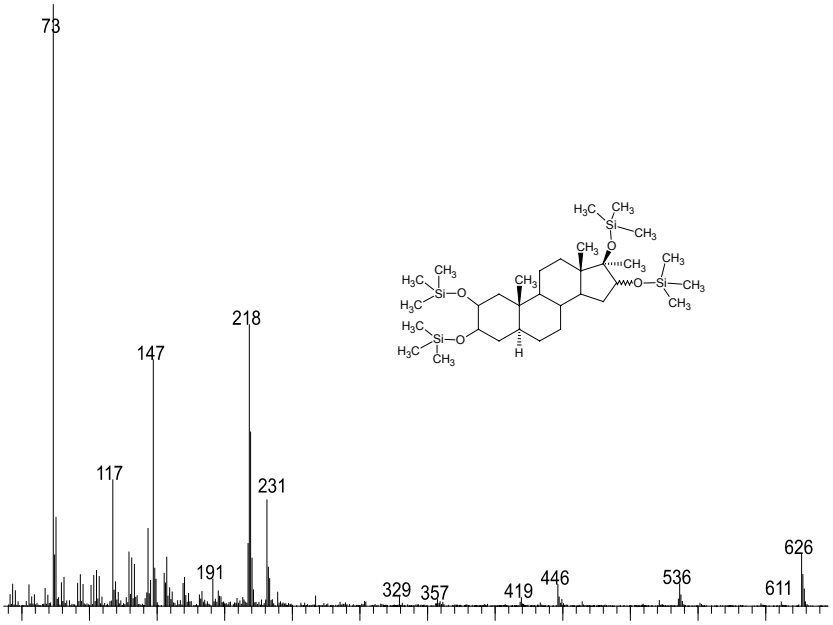
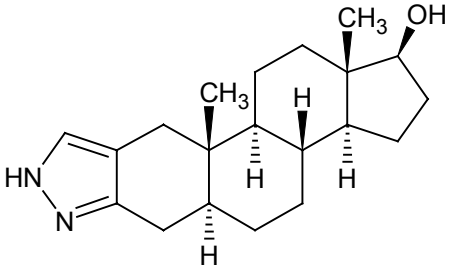


Fig. 8. Mass spectrum of metabolite **XIV** as tetrakis-TMS, RRT 1.1792.

Prostanozol

Contains '[3,2-c]pyrazole-5α-etioallocholane-17β-tetrahydropyranol 25 mg' (**XVI**).



XVI

Prostanozol is actually stanozolol without 17α-methyl group. The metabolism includes oxidation of 17-hydroxy function and hydroxylation at different positions as shown in **Fig. 9**.

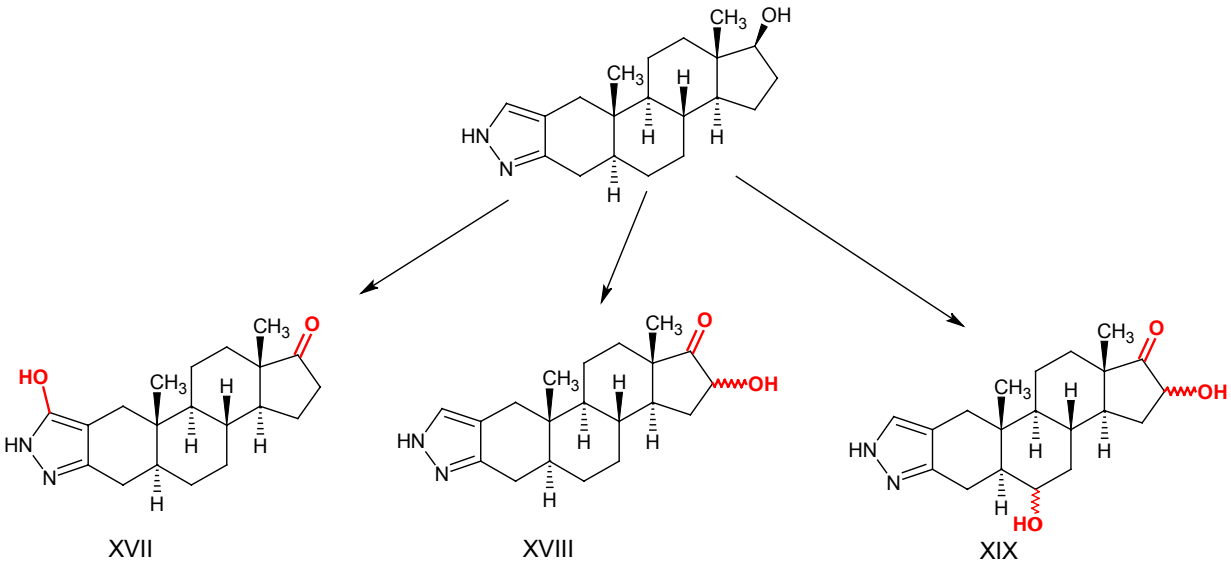


Fig. 9. Metabolism of prostanozol. Metabolite **XVIII** might be hydroxylated either at C-6 or C-16.

The main metabolite is **XVIII** (molecular weight 544 amu as tris-TMS). Metabolites **XVII** and **XIX** (molecular weights 544 and 632 amu as tris-TMS and tetrakis-TMS, respectively) are excreted in comparable amounts and are about twice as less than metabolite **XVIII**. Eluting in the corticosteroid area, metabolites **XVIII** and especially **XVII** are difficult to detect due to chromatographic interferences and also are susceptible to GC column aging process like stanozolol metabolites. Nevertheless, metabolite **XIX** shows better chromatographic properties with no tailing and elutes after corticosteroid area.

The mass spectra of prostanozol metabolites as respective TMS-enol derivatives are presented in **Fig. 10-12**. Interestingly, the ion at *m/z* 254 is characteristic of metabolite **XVII** only. Therefore, as in case of stanozolol, this metabolite should be 3'-hydroxy. At the same time, metabolites **XVIII** and **XIX** do not have ion at *m/z* 254 in their mass spectra that is likely to indicate no hydroxylation at C'-3 or C-4 positions.

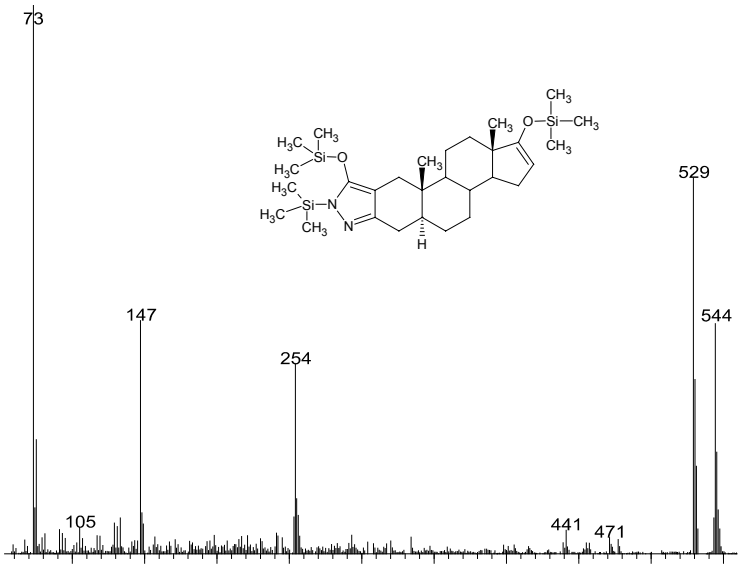


Fig. 10. Mass spectrum of **XVII** as tris-TMS, RRT 1.2174.

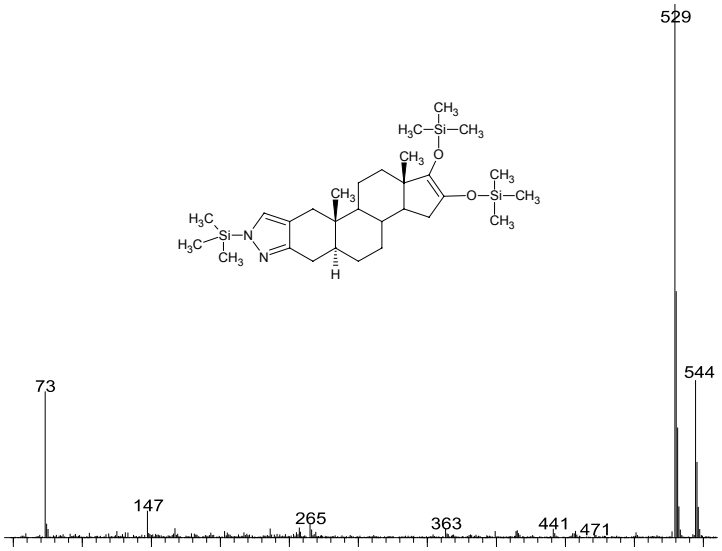


Fig. 11. Mass spectrum of **XVIII** as tris-TMS, RRT 1.2660.

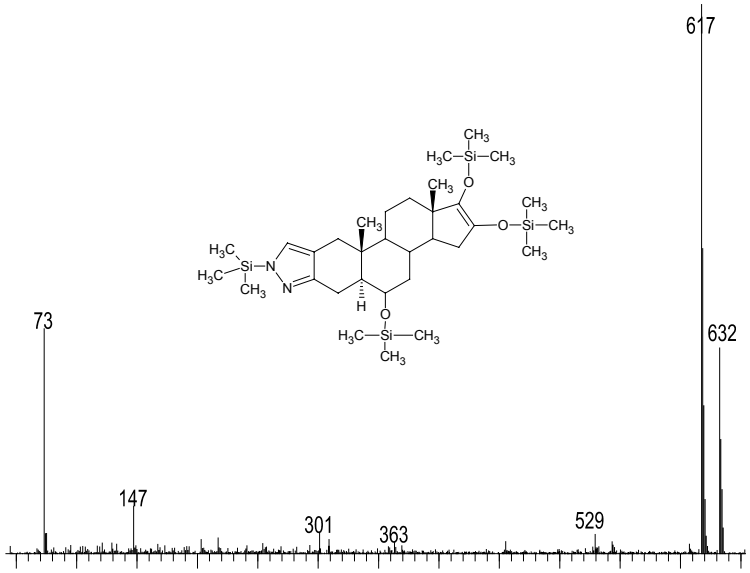


Fig. 12. Mass spectrum of **XIX** as tetrakis-TMS, RRT 1.3171.

Conclusion

The metabolic pathways of five new designer anabolic steroids (Methyl 1-P, Methyl 1-Alpha, Methyl Masterdrol, Desoxymethyltestosterone and Prostanozol) were studied.

The supplements **Methyl 1-P** and **Methyl 1-Alpha** contain two steroid components each. The main metabolite of Methyl 1-P is 6α-methyl-5α-androstane-3α-ol-17-one that appears in drostanolone metabolite window. Active component of Methyl 1-Alpha, supposedly 5β-mestanolone, gives one dehydrometabolite. **Methasterone** is excreted intact and gives one dihydro metabolite. **Desoxymethyltestosterone** extensively metabolizes and is the most 'complicated' steroid to detect as its metabolites have non-informative electron ionization mass spectra and are strongly interfered with urine matrix components. **Prostanozol** gives 3 hydroxylated metabolites, 2 of which (mono hydroxy) are also difficult to detect due to matrix effects, poor mass spectra and noticeable tailing as TMS derivatives. The dihydroxy metabolite of prostanozol is preferable for detection by GC-MS. For detection prostanozol metabolites a method of LC-MS looks very promising.

References

[1] M. Donike, H. Geyer, A. Gotzmann, M. Kraft, F. Mandel, E. Nolteernsting, G. Opfermann, G. Sigmund, W. Schanzer, J. Zimmermann. *Dope Analysis* // Proceedings of I.A.F. World Symposium on Doping in Sport, Florence, May 10-12, 1987.

[2] M.H. Sekera, B.D. Ahrens, Y.C. Chang, B. Starcevic, C. Georgeakopoulos, D.H. Catlin. Another Designer Steroid: Discovery, Synthesis and Detection of 'Madol' in Urine // Rapid Commun. Mass Spectrom. 2005, 19(6), 781-784.