PROJECT REVIEW

“Characterization of the main metabolites of 17-methylstenbolone and 17 methylmethenolone produced by human hepatocytes and liver fractions”

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New steroids openly appear on the market in products labelled with a rather confusing nomenclature. Once characterized, pharmaceutical grade products not being available, knowledge of the biotransformation pathways essential to an efficient detection of utilization by athletes is difficult to gain since administration to human volunteers should be restricted to the minimum.

The alternative is a reliable in vitro model. Human hepatocytes, fresh or cryopreserved are now available commercially. We have successfully produced and identified phase I metabolites from incubations of human hepatocytes with different steroids, such as 17-methylhydrostanolone and desoxymethyltestosterone (DMT).

The aim of this project is to produce in vitro from human hepatocytes and liver fractions the metabolites of two steroids, the 17-methylated derivatives of stenbolone and its isomer methenolone. The principal metabolites will be synthesized and characterized by NMR and mass spectrometry.

The characterization of metabolites will enable the identification of markers of utilization to be incorporated in routine testing methods. The approach for the chemical synthesis of metabolites will be shared with NMI insuring the distribution to other doping control laboratories. Improving the knowledge of steroid biotransformation is a further benefit from these studies.
Characterization of 17-Methylstenbolone and 17-Methylmethenolone and Identification of Metabolites Produced by Human Hepatocytes and Liver Fractions

WADA Project no. 11A16CA

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Summary

We have synthesized and characterized two designer steroids, 17α-methylmethenolone and 17α-methylstenbolone; the latter is proposed on the internet and two groups have reported different and contradictory results. Incubations with fresh hepatocytes and S9 liver fractions were carried out. Structures were proposed for the main and more relevant metabolites from the GC-MS analysis of their TMS-ether, TMS-enol and TMS-ether (TMS-d$_9$) derivatives in agreement with literature published for the non-methylated analogs methenolone and stenbolone (1-3). A GC-MS/MS SRM method was developed and permitted the detection of these metabolites in an athlete’s sample in which methylstenbolone was detected and also in a reference urine sample provided by WAADS in 2014.

*In vitro* metabolites of 17α-methylmethenolone 1

Three main metabolites 6,17β-dihydroxy-1,17α-dimethyl-5α-androst-1-en-3-one (M2), 16,17β-dihydroxy-1,17α-dimethyl-5α-androst-1-en-3-one (M3) and 2,17β-dihydroxy-1,17α-dimethyl-5α-androst-1-en-3-one (M4) were produced from the incubation of 1 with cryopreserved and fresh hepatocytes or S9 fractions, along with a trace of the 3α-OH metabolite (M1, 3α-SDH).
In vitro metabolites of 17α-methylstenbolone

The incubations of methylstenbolone with hepatocytes and S9 fractions afforded several metabolites. Three, along with the reduced 3α-OH M1 were more important and expected to be present in human urine: 17β, 18-dihydroxy-2,17α-dimethyl-5α-androst-1-en-3-one (M2), 6,17β-dihydroxy-2,17α-dimethyl-5α-androst-1-en-3-one (M3), 16,17β-dihydroxy-2,17α-dimethyl-5α-androst-1-en-3-one (M4).

<table>
<thead>
<tr>
<th>Product</th>
<th>Derivative</th>
<th>Mass Spectrum (EI) Major ions (m/z, abundance)</th>
<th>GC-MS/MS Ion transition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methylstenbolone</td>
<td>TMS-ether, TMS-enol</td>
<td>460 (60%), 221 (11%), 207 (30%), 193 (25%), 143 (45%)</td>
<td>460 =&gt; 220, =&gt; 193, =&gt; 143</td>
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<tr>
<td></td>
<td>TMS-ether</td>
<td>388 (2%), 373 (10%), 331 (7%), 318 (9%), 143 (100%)</td>
<td></td>
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<tr>
<td></td>
<td>Underivatized</td>
<td>316 (25%), 301, 298, 258, 246, 218, 200, 160 (100%), 136, 123</td>
<td></td>
</tr>
<tr>
<td>3α-OH, M1</td>
<td>TMS-ether, TMS-enol</td>
<td>462 (60%), 447 (7%), 419, 332 (6%), 216 (7%), 157 (13%), 143 (100%)</td>
<td>462 =&gt; 419</td>
</tr>
<tr>
<td>18-CH2OH, M2</td>
<td>TMS-ether, TMS-enol</td>
<td>548 (22%), 445 (44%), 354 (15%), 193 (70%), 143 (37%), 103 (10%)</td>
<td>445 =&gt; 193</td>
</tr>
<tr>
<td></td>
<td>TMS-ether</td>
<td>476 (12%), 446 (100%), 143 (95%), 103 (35%)</td>
<td></td>
</tr>
<tr>
<td>16-OH, M4</td>
<td>TMS-ether, TMS-enol</td>
<td>548 (60%), 231 (7%), 218 (7%), 207 (25%), 193 (17%), 147, 117</td>
<td>548 =&gt; 231, =&gt; 218</td>
</tr>
<tr>
<td></td>
<td>TMS-ether</td>
<td>476 (90%), 386 (15%), 330 (22%), 231 (70%), 218 (100%)</td>
<td></td>
</tr>
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References