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IN DOPING ANALYSIS
(6)

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The Position of the Hydroxy Group in the Main Bromantane Metabolite
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The position of the hydroxy group in the main bromantane metabolite

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Introduction
In 1996 bromantane (I) was added to the IOC list of forbidden substances after several positive cases were observed doping the 1996 Olympic Games in Atlanta. It has been used by athletes for its immune- and psychostimulating properties\(^1\). Bromantane was not added to the list as a stimulant but as a masking agent, because its main metabolite appeared to coelute with epitestosterone in screening procedures for anabolic steroids. Because bromantane is used at a very high dose, the metabolite interferes the gas chromatography/mass spectrometry of epitestosterone, so the T/E ratio can not be determined. Although Ueki et al\(^2\) concluded that neutral n-pentane extraction efficiently eliminates the bromantane metabolite, bromantane is still registered as a masking agent. Ayotte et al (unpublished results) reported that the main metabolite coeluting with epitestosterone is a monohydroxy derivative. The location of the hydroxy group was not known besides the fact that it is located on the adamantane part of the molecule (II).

\[ \text{I} \quad \text{II} \]
In this study an excretion experiment is performed after administration of one tablet of bromantane to a male volunteer. The urine samples were analyzed for the bromantane metabolites. To resolve the hydroxylation position of the main metabolite, the mass spectra of the metabolites of bromantane are compared with mass spectra from two model compounds, 1- and 2-adamantol (III and IV respectively), which are hydroxylated at different positions on the adamantane structure.

![OH](image)

III  IV

** Experimental 
Urine samples were collected from one male volunteer (age 27) after administration of half a tablet of bromantane (kindly provided by the Moscow Antidoping Centre, Russia). The dose was unknown. Urine samples were stored at -20°C until analysis was performed. Sample clean-up was performed applying respectively C\textsubscript{18}-solid phase extraction, selective enzymatic hydrolysis with β-glucuronidase, alkaline liquid-liquid extraction with diethyl ether and derivatization to TMS-derivatives by MSTFA/NH\textsubscript{4}I/ethanethiol (1000:2:3; v/w/v). Analysis of bromantane was done after clean-up from a tablet after liquid-liquid extraction with diethyl ether. GC/MS analysis was performed by splitless injection in a HP5890 gas chromatograph equipped with a HP Ultra-1 column (18 m, i.d. 0.20 mm, film thickness 0.11 μm) and a HP 5972 MSD.
Results

Mass spectrometry of bromantane

The full scan mass spectrum of bromantane is shown in figure 1. Because one bromine atom is present in the structure, fragments which retained the bromine show a so called "bromine pattern". A bromine pattern can be observed for ions at m/z 305/307 (M⁺), 184/186, 171/173, 155/157 respectively.

Excretion study

The normalized excretion curve (based on the molecular ion at m/z 305) of the main bromantane single hydroxy metabolite is shown in figure 2. The amount of excreted bromantane metabolite is normalized because the dose of administered bromantane is unknown. The maximum excretion is achieved around 6-7 hours after administration. Until 30 hours, detection of the metabolite was still possible in a low concentration. The urine sample collected at 6-7 hours was screened for, besides the main metabolite also for the other single hydroxy metabolites by applying SIM for the ion at m/z 393 (M⁺) (figure 3). The main metabolite (M1) was detected at 18 minutes. Three more single hydroxy metabolites were detected (M2-4).

Mass spectrometry of adamantanes

Fragmentation to [M-57]⁺ of 1-adamantole-TMS (figure 4) can be explained as follows³:
The \([M-73]^+\) is explained by the loss of the trimethylsilyl fragment. \([M-89]^+\) appears to be the result of -OTMS loss from the molecule. This fragmentation is not coupled to the loss of a proton from a nearby carbon atom as can be observed in mass spectrometry of TMS-derivatives of steroids. It is suggested that loss of a proton from neighboring secondary carbon atoms is energetically unfavorable. The proton loss should be favorable from tertiary carbon atoms, as it is the case in 2-adamanole (figure 5). The fundamental fragmentation difference between 1- and 2-adamanole can be extrapolated to the mass spectra of the single hydroxylated bromantane metabolites.

**Mass spectrometry of 4 bromantane metabolites**

Analogous to 2-adamanole, a minor \([M-90]^{++}\) fragment is observed in the mass spectrum of \(\text{M1}\) and \(\text{M3}\) (respectively figures 6 and 8). Applying the hypothesis described before, this should be explained by a secondary -OTMS group on the adamantane-part of the molecule. The significant intensity of the \([M-89]^{++}\) fragment is observed in the spectra of \(\text{M2}\) and \(\text{M4}\) (respectively in figure 7 and 9), analogous to 1-adamanole, leading to the conclusion that the -OTMS on \(\text{M2}\) and \(\text{M4}\) is tertiary positioned. Additional evidence of the tertiary position of -OTMS on the adamantane structure in \(\text{M2}\) and \(\text{M4}\) is given by the presence of \([M-57]^+\) in the spectra. Also a \([M-74]^{++}\) can be observed in the spectra of \(\text{M1}\) and \(\text{M3}\), explained by the loss of TMSH which also proves the secondary position of -OTMS.

In summary, the 4 detected single hydroxy metabolites in the urine samples of this bromantane excretion experiment can be divided in two groups according to their hydroxyl-position on the adamantane structure. Metabolites \(\text{M1}\) and \(\text{M3}\) are secundairy hydroxy-metabolites (V).
There are two possible diastereomeric structures (respectively $\alpha$ and $\beta$) for secondary hydroxy metabolites. Metabolites M2 and M4 are tertiary hydroxy metabolites (VI), also showing two diastereomeric possibilities (respectively $\gamma$ and $\delta$). A complete structure identification has not been possible so far. Additional experiments will take place in order to obtain more precise information on bromantane metabolism.

![Chemical Structure](image)

**Literature**


Figure 2: Normalized excretion curve of the main single hydroxy metabolite of bromantane, calculated by area of the ion m/z 305 (M⁺) (SIM)
Figure 3: Extracted ion chromatogram of the ion at m/z 305, showing 4 diastereomeric single hydroxy metabolites numbered in reversed order of intensity.

EIC on mass 393
Figure 4: Full scan mass spectrum of TMS-derivative of 1-adamantole.
Figure 7.
Full scan mass spectrum of TMS-derivative of brominated metabolite M2.

- [M-89]⁺
- [M-57]⁺
- [M-15]⁺
- OTMS
- 223
- 184
- 155
- 171
- 91
Figure 8: Full scan mass spectrum of TMS-derivative of bromantane metabolite M3.
Figure 9: Full scan mass spectrum of TMS-derivative of bromantane metabolite M4.

[M-89]+ 394/396

M+ 393/395

OTMS

223

171

184

Br

N

H

155

171

223