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RECENT ADVANCES  
IN DOPING ANALYSIS  
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## **Study of Bromantan Metabolites Structure**

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### **Introduction**

On September 1996 bromantan (adamant-2-yl-p-bromophenyl)amine was included by the IOC Medical Commission in the list of the prohibited substances as the stimulant and masking agent. Originally bromantan was synthesized in our country by the army physicians with the aim to keep up soldiers in any extreme conditions and was considered as antihypoxant and immunomodulator. Bromantan belongs to the class of actoprotectors and adaptogens. In terms of its pharmacological action spectrum it shows some antiasthenic effect, increases resistance to overheating, contributes to the restoration of working capacity after physical exercise. The substance is highly lipophilic and is distributed into lipids of brain and fat tissue. It activates lipolysis and facilitates participation of lipids in energy metabolism.

Bromantan has membrane-stabilising action, antioxidant activity and increases immunity even after a single dose (increases the level of B-cells and circulating immune complexes in bloodstream), it is more powerful than Levenizol in terms of its effect on immunity.

Bromantan stimulates synthesis of cytochromes P-450 and thus facilitates detoxifying liver function. In other words, in terms of its pharmacological effect it is a typical actoprotector.

The drug is slowly excreted from the body and is well tolerated. In practice it is often used together with nootropic preparations. After administration in a very high dose (200 mg-1g) appears a certain stimulating and masking effect.

The aim of the present work is the investigation of the bromantan metabolism and the dynamic of the metabolite excretion.

GC-MSD instruments HP 5971 and 5972 were used.

## Results and discussion

Before investigation of the bromantan metabolism and its excretion the mass spectra of the native bromantan and its derivatives were studied. Figure 1 shows the mass spectrum of nonderivatized bromantan and its fragmentation scheme under electron impact. The presence of poliisotopic bromine helps in interpretation of mass spectrum, because all bromine containing ions are easy identified. There are ions at  $m/z$  305, 184, 171. The ions  $m/z$  135, 155, 184 are appeared as the result of the simple bond cleavage, as far as ion 171 is rearrangement ion. In this case the hydrogen migrates from adamantan ring to the charged nitrogen. It should be noted that these main paths of the native bromantan fragmentation shown on the Figure 1, remains the same for the all its derivatives and metabolites.

The substitution of hydrogen at nitrogen for trimethylsilyl (Figure 2) results in the essential increasing of the  $[M-H]^+$  ion intensity ( $m/z$  376). Then this ion can eliminate bromophenyl radical to produce ion at  $m/z$  221. This ion has N-trimethylsilyladamantylimine structure.

As can be seen on figures 3 and 4 the introduction of fluoroacyl radical results in sharp decreasing of molecular ion stability and loosening bond between adamantan ring and nitrogen atom due to high electronegative effect of fluorine atom. High abundance of the peak at  $m/z$  135 is the result of this influence.

For the metabolism investigation bromantan was orally administrated in dose 150 mg. The urine was collected in 2.5 hour and then each day up to two weeks.

Parent bromantan was not found at all. After hydrolysis without derivatization we have seen overload peak with the mass spectrum shown on Figure 5. The molecular weight of this metabolite is more than parent molecule on 16 amu. It means that the hydroxylation takes place. We supposed that hydroxyl group is connected with adamantan group but not phenyl. This assumption is based on the presence of ions at  $m/z$  184 and 171 and the absence of ions at  $m/z$  135 in the spectrum. Instead of the ion at  $m/z$  135 appears prominent ion at  $m/z$  130. The ion appears as the result of water and bromoaniline molecules elimination. This process is shown on the scheme 1.

Some more information was obtained after derivatization. The trimethylsilylation gives huge peak with the mass spectrum shown on Figure 6. The difference on 72 units confirms the presence of hydroxyl groups in metabolite and the ion at  $m/z$  223 - the position of hydroxyl

group in adamantan cycle. The loss of trimethylsilanol molecule from this ion gives rise to ion  $m/z$  133. Additionally six metabolites with the same molecular weight and similar mass spectra but with the essentially decreased concentration were found. The only difference is the increased intensity of the ion at  $m/z$  223. It reflects that hydroxylation took place at others positions of adamantan ring that results in increasing of sterical tension of a molecule.

So it could be supposed that the main metabolite has the hydroxyl group at most distant from phenyl sixth position of adamantan cycle.

Besides TMS-TFA and bis-TFA-derivatives were obtained for the main metabolite. The mass spectra of these derivatives are shown on Figures 7 and 8. In case of TMS-TFA-derivatization we had 90% of trimethylsilyl derivative and only 10 % of TMS-TFA. This phenomenon is explained by sterical difficulties due to the presence of bulked 4-bromophenyl radical.

After bis-TFA derivatization we identified only one chromatographic peak of low intensity, probably due to the same reason.

Besides of monohydroxylated metabolites we additionally found 11 very weak dihydroxylated metabolites. Mass spectrum of most intensive of them is shown on Figure 9. In addition the unusual metabolite was also found. Its concentration in urine is very low. We supposed the formation of this metabolite as the result of the substitution of bromine atom for hydroxyl group. The mass spectrum and structure are shown on Figure 10. This suggestion is confirmed by the fact that the derivative was not found after derivatization of native bromantan..

Figure 11 illustrates dynamic of excretion of bromantan metabolites. It reflects the dependence of density corrected metabolites concentration in urine *versus* time. The rear column corresponds to the main metabolite, middle - sum of the monohydroxylated metabolites except main, and front column - sum of the dihydroxylated metabolites. It could be seen that even after two weeks all the bromantan metabolites are determined.

## **Conclusion**

The bromontan metabolism is mainly characterised by hydroxylation in 6th position of adamantan cycle. The main metabolite is easy determined as a TMS-derivative after enzyme hydrolysis. All determined metabolites could be found even in two weeks after administration of bromantan.

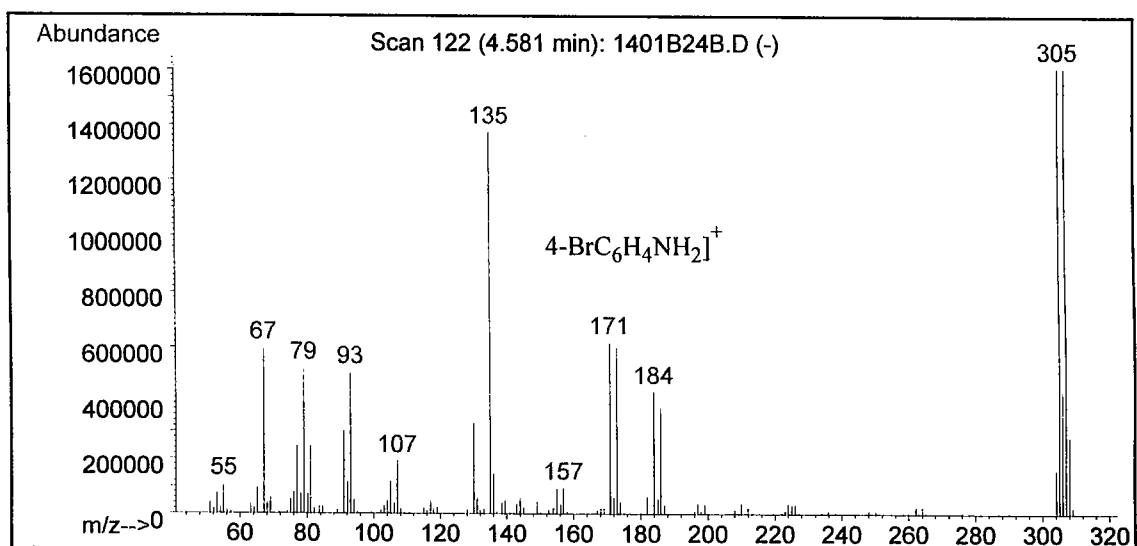
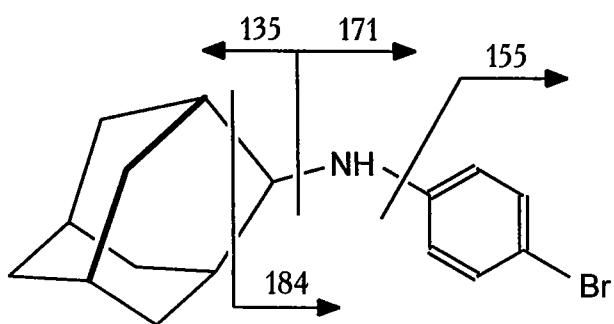


Fig.1. Structure and mass spectrum of bromantan.

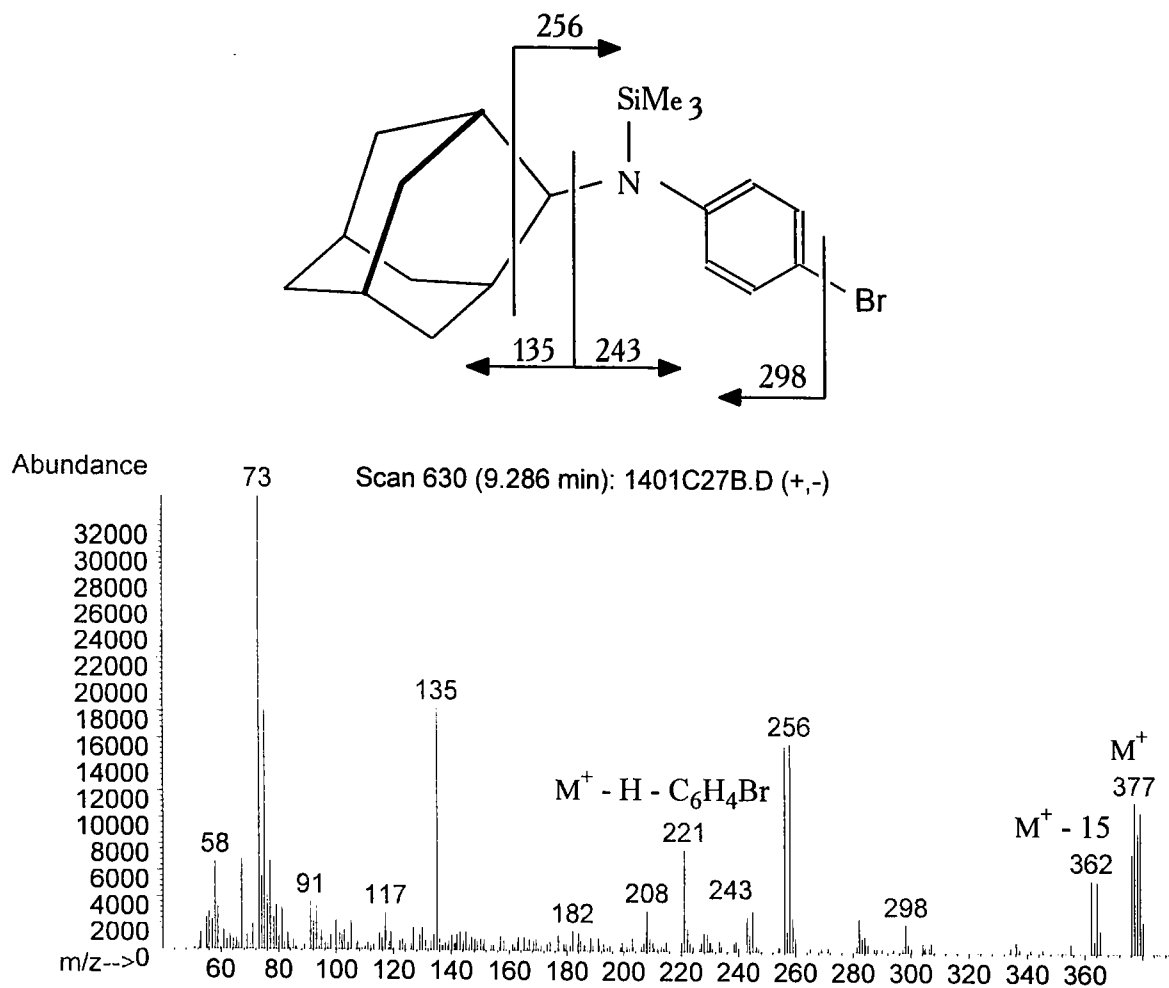


Fig.2. Structure and mass spectrum of N-trimethylsilylbromantan.

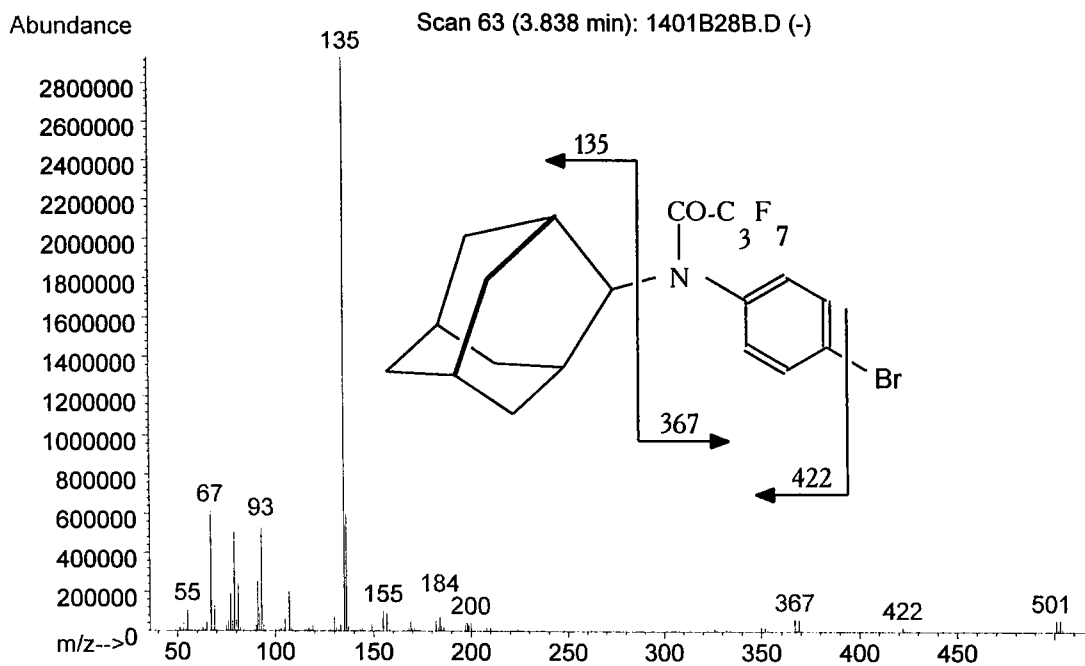


Fig.3. Structure and mass spectrum of N-heptafluorobutyrylbromantan.

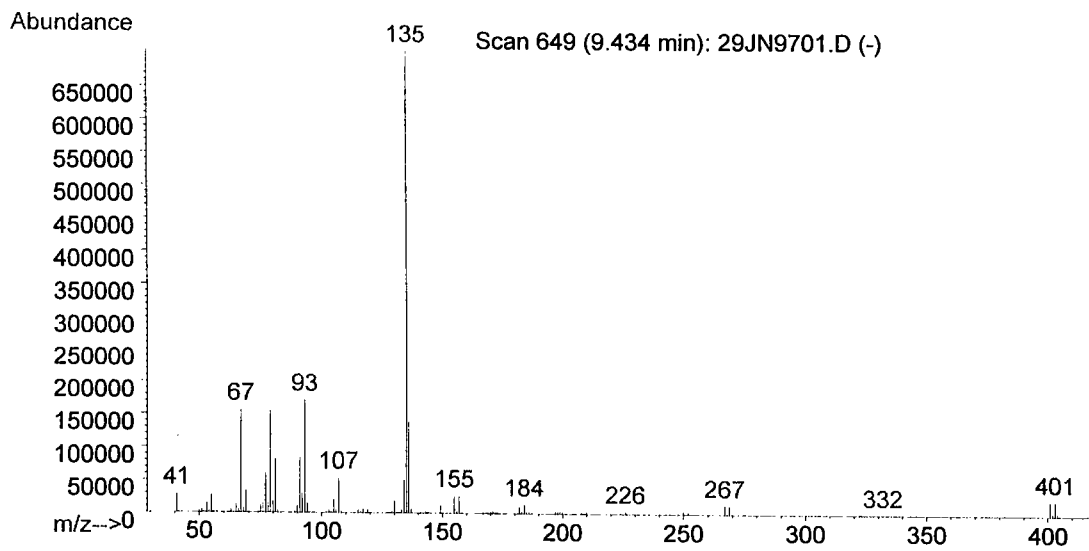
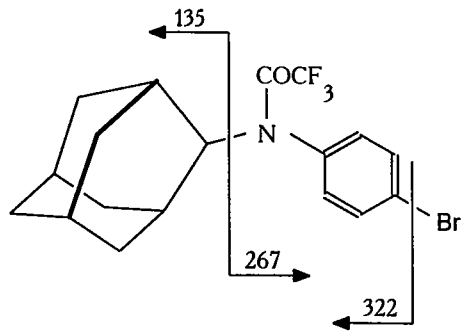


Fig.4. Structure and mass spectrum of N-trifluoroacetylbromantan.



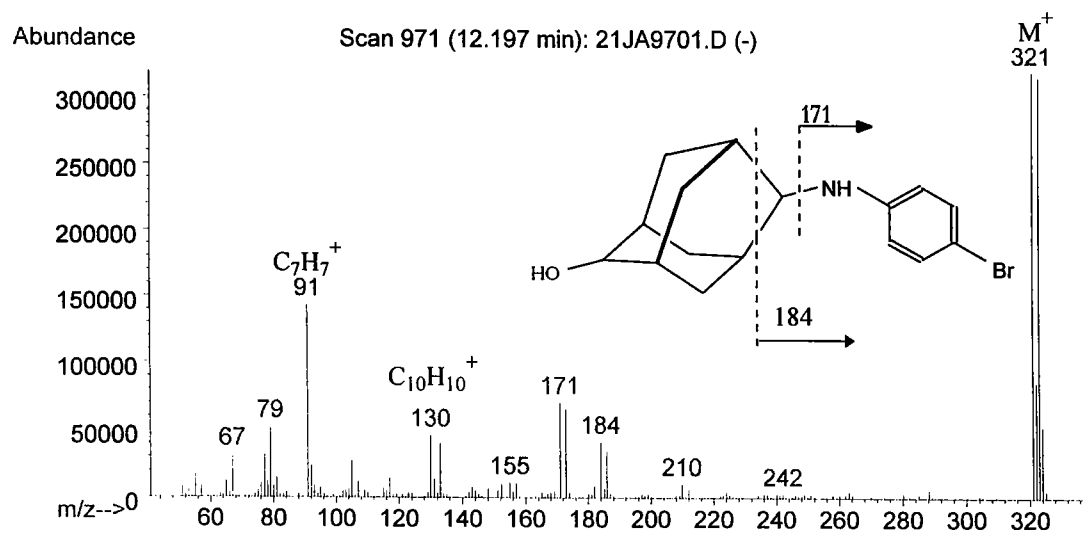
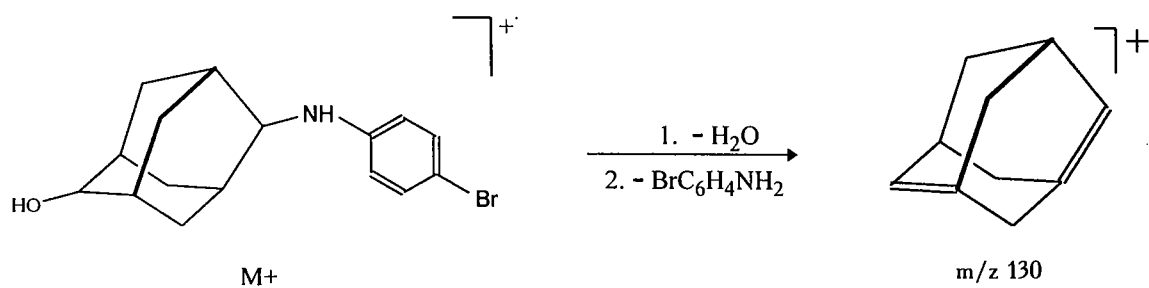


Fig. 5. Structure and mass spectrum of bromantan non derivatized metabolite.



Scheme 1.

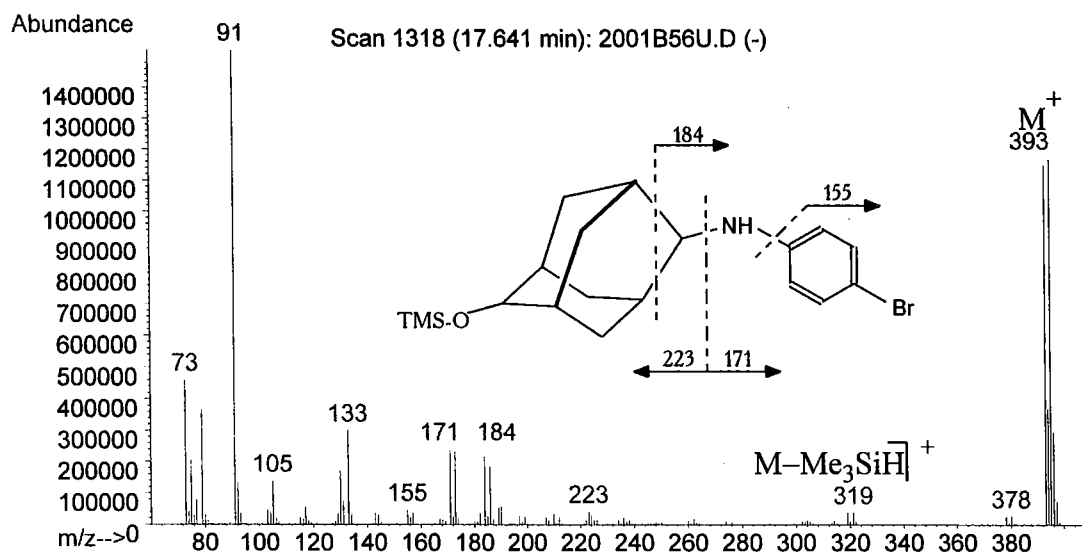


Fig.6. Structure and mass spectrum of bromantan TMS derivatized metabolite.

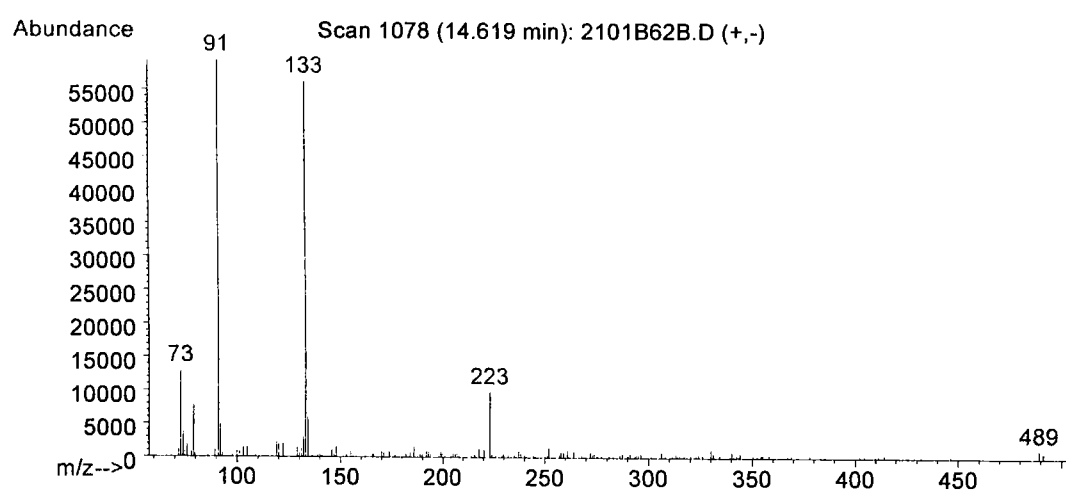
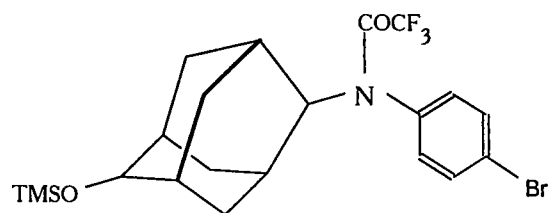


Fig. 7. Structure and mass spectrum of bromantan TMS-TFA derivatized metabolite.

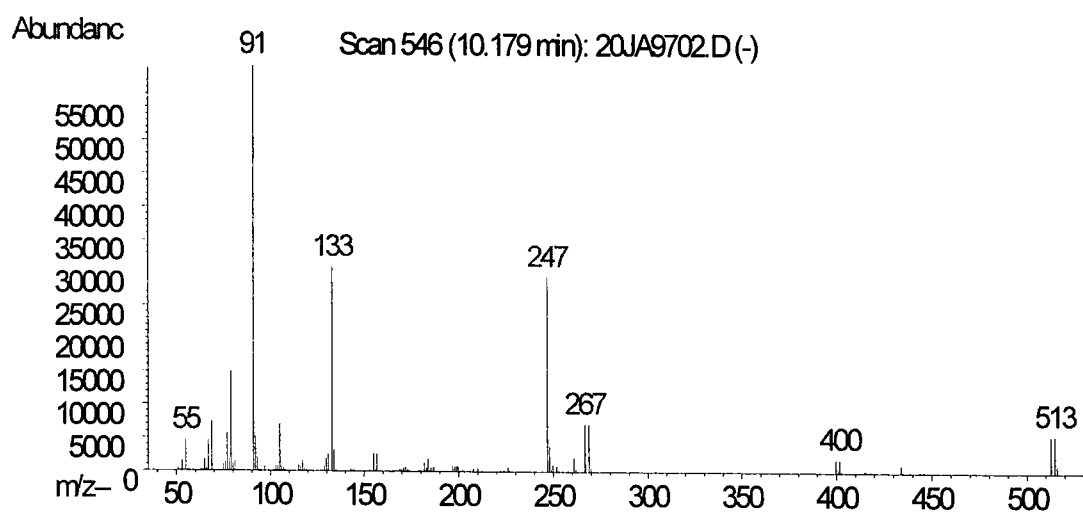
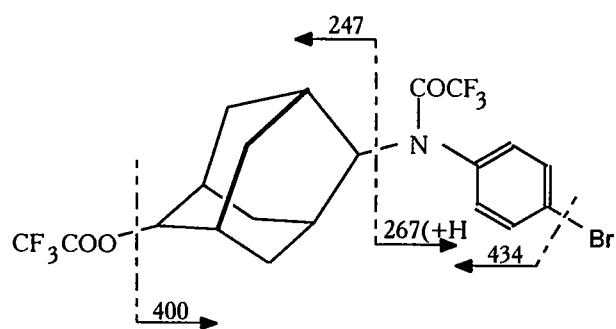


Fig.8. Structure and mass spectrum of bromantan bis-TFA derivatized metabolite.

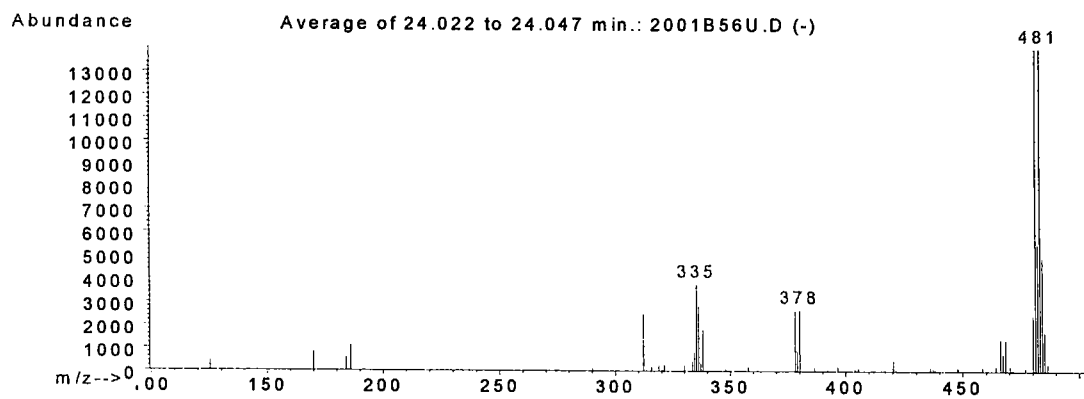
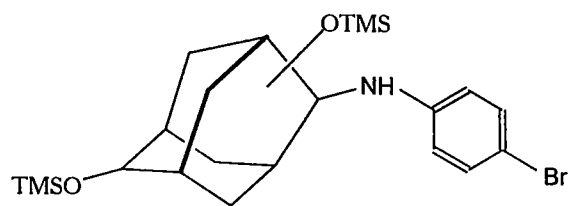


Fig.9. Structure and mass spectrum of dihydroxybromantan, bis-TMS.

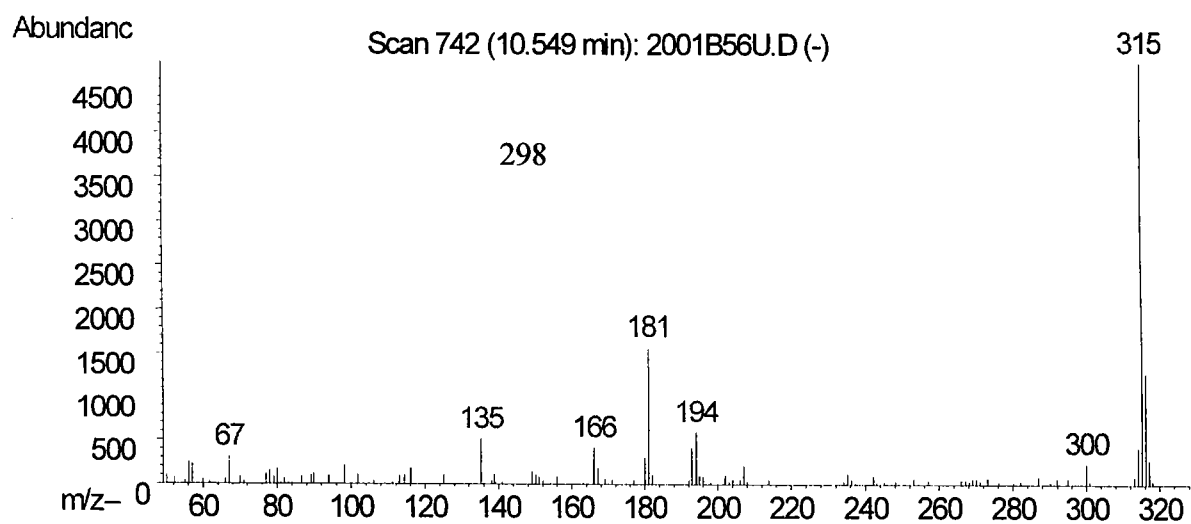
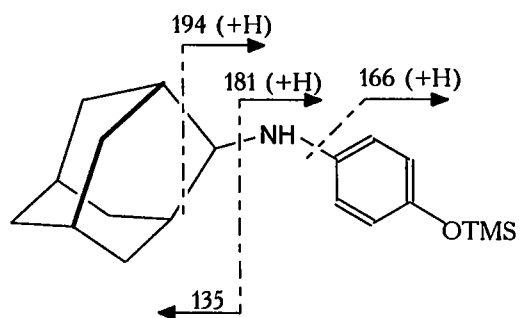


Fig.10. Structure and mass spectrum of hydroxylated debrominebromantan, TMS.

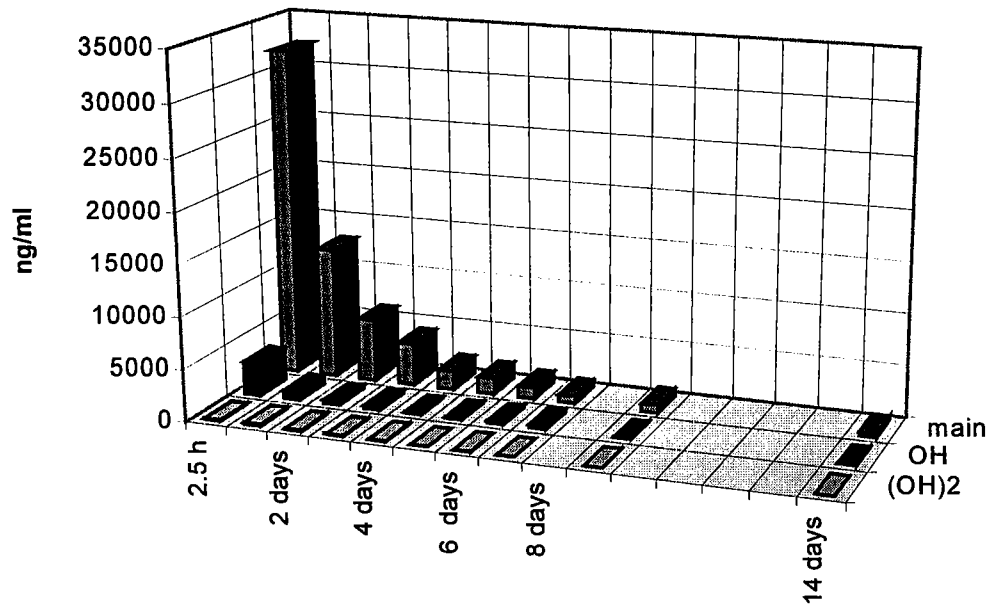


Fig.11. Excretion of bromantan metabolites.