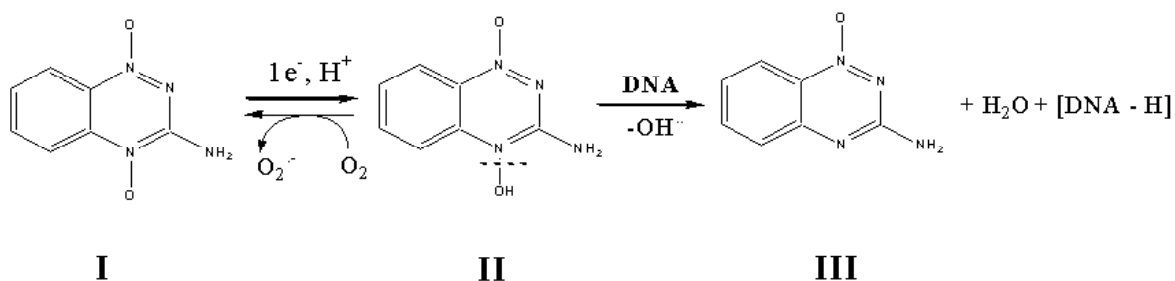


## TANDEM MASS SPECTROMETRY STUDY OF METABOLIC TRANSFORMATIONS OF ANTITUMOR DRUG TIRAPAZAMINE

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Tirapazamine (**I**) is a promising antitumor agent that selectively damages oxygen-poor tumor cells. The suggested mechanism of the drug's metabolism (Scheme 1) involves the formation of intermediate **II**, which has never been detected or directly characterized. In the present study, tandem mass spectrometry methods were used to study the mechanism of transformations of **I** and for analysis of mixtures of tirapazamine with its metabolites. Compound **I**, as well as its metabolites, 3-amino-1,2,4-benzotriazine 1-oxide (**III**), 3-amino-1,2,4-benzotriazine 4-oxide (**IV**) and 3-amino-1,2,4-benzotriazine (**V**) were characterized by EI, CI, APCI and ESI methods. It was shown that protonation of tirapazamine results exclusively in **II**<sup>+</sup> ions regardless of the method of protonation. A loss of OH radical from these ions gives rise to molecular ions of 1-oxide, **III**<sup>+</sup>.



Scheme 1. Proposed mechanism of metabolic transformation of tirapazamine **I**.

Neutralization-reionization mass spectrometry (NR MS) experiments with **II**<sup>+</sup> ions resulted in the formation of neutrals that are stable in the mass spectral time frame (>5 ns). This is the first direct experimental evidence for the existence and intrinsic stability of neutral **II**. The NR mass spectrum of **II**<sup>+</sup> ions (Figure 1) showed intense signal due to **[I+H-OH]**<sup>+</sup> ions. We demonstrated that their formation was a result of the loss of OH from neutral **II**. The activation energy for this process was estimated as ~14 kcal mol<sup>-1</sup>, which is a reasonable value for the second step of the metabolic transformation of **I**.

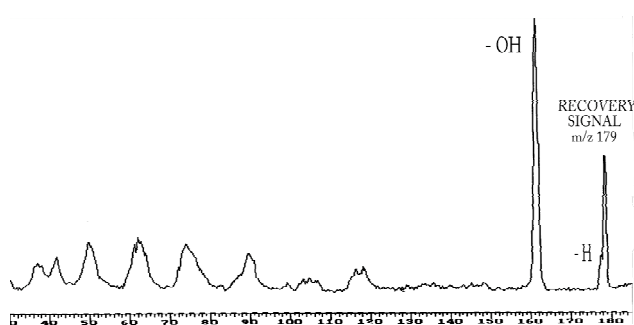


Figure 1. Neutralization-reionization mass spectrum of **II**<sup>+</sup> ions.

LC/MS/MS procedures of separation and identification of **I** and its metabolites were developed. They are based on the fact that collision-induced dissociation mass spectra of protonated isomeric monoxides produced structure-specific fragment ions. Selected reaction monitoring was the most efficient way of recognition of isomeric monoxides.

The dissociation characteristics of **[M+H]**<sup>+</sup> ions of compounds **III**, **IV** and **V** indicated that most likely more than one heteroatom was involved in protonation. The NR MS experiments with **[III+H]**<sup>+</sup> and **[V+H]**<sup>+</sup> ions demonstrated the stability of their neutral analogues. Based on the experimental observations and the results of our quantum chemical calculations, it was concluded that stable **[III+H]**<sup>+</sup> and **[V+H]**<sup>+</sup> radicals are those having an extra hydrogen atom at one of the ring N-atoms.