

# Nucleophilic addition of (bi)sulfite to nitron spin traps: source of possible artifacts in $\text{SO}_3^{\cdot-}$ spin trapping

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Knowledge of the formation of radicals from (bi)sulfites, *in vivo*, are of interest in understanding the allergic and inflammatory responses to environmental sulfoxide exposure. Sulfite trioxide anion radical ( $\text{SO}_3^{\cdot-}$ ) formation was measured in mice, preloaded with the spin trap DEPMPO. Based on spin trapping NMR [1,2], a surprising quantity of reduced products was observed that did not depend on coadministration of oxidizing agents. In the following *in vitro* experiments we observed the formation of single diamagnetic product (P) for DMPO and two diamagnetic products (P1 and P2) for DEPMPO during the reaction with (bi)sulfite even in the absence of an oxidant. Based on the <sup>13</sup>C-, <sup>1</sup>H- and <sup>31</sup>P-NMR (for DEPMPO) data the product P was assigned to 1-Hydroxy-5,5-dimethyl-pyrrolidine-2-sulfonic acid while P1 and P2 to trans- (P1) and cis- (P2) stereoisomers of 5-(Diethoxy-phosphoryl)-1-hydroxy-5-methyl-pyrrolidine-2-sulfonic acid, formed by reversible addition of (bi)sulfite to the double bond of the spin traps. Oxidation of these hydroxylamines results in the formation of the nitroxides identical with earlier described paramagnetic adducts of genuinely trapped  $\text{SO}_3^{\cdot-}$  radical. Based on all the obtained data, a mechanism for a nonradical reaction of (bi)sulfite with DMPO and DEPMPO was suggested. Corresponding equilibrium and rate constants were determined.

The results demonstrate that  $\text{SO}_3^{\cdot-}$  detection using DEPMPO and DMPO spin traps likely reflect nonradical addition reaction except in cases when the required controls unambiguously prove a radical mechanism.

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[1] Khramtsov, V.V. L.J.Berliner, and T.L.Clanton, 1999, *Magn. Reson. Med.* 42, 228-234.

[2] Khramtsov et al. 2001, *Free Rad.Biol.Med.* 30 (10): 1099-1107.