EVIDENCE FOR THE FORMATION OF S-OXYGENATED SPECIES DURING THE HYDROGEN PEROXIDE OXIDATION OF 2-THIOIMIDAZOLES

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2-Thioimidazoles in the presence of hydrogen peroxide (H_2O_2) inactivate the physiologically important hemoproteins thyroid peroxidase (Engler et al 1983) and lactoperoxidase (Doerge, 1986). Reactive S-oxygenated 2-thioimidazole intermediates are thought to be responsible for this irreversible (suicide) inhibition. However these labile species to date have not been fully characterised.

In an attempt to identify the initial reaction products formed when 2-thioimidazoles are oxidised by H_2O_2 we have used liquid chromatography-mass spectrometry (LC-MS).

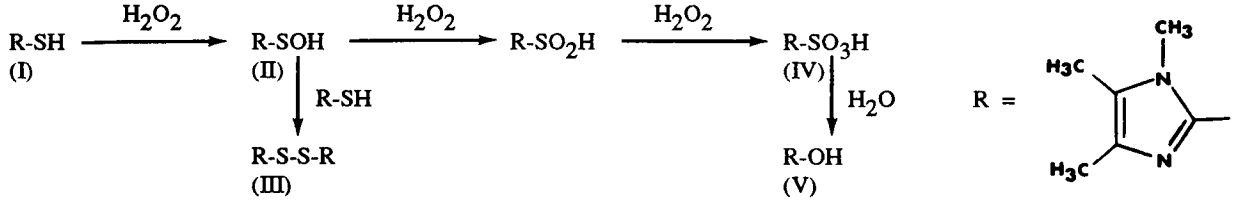
H_2O_2 (5 μL, 100 volumes) was mixed with 1,4,5-trimethyl-2-thioimidazole (I, 10 μL, 10mM) in phosphate buffer (1mL, pH 7.4) and left at room temperature for 10 minutes, after which an aliquot (25 μL) of the mixture was analysed by LC-MS. Chromatography was carried out on a Partisil 5 μ ODS3 column (250 x 4.6 mm) eluted with acetonitrile-ammonium acetate pH 3 (10:90). The column eluent initially passed into a UV-detector (260 nm) and then into a Finnigan Mat quadropole mass spectrometer (scan range 105-305 amu) via a thermospray interface (tip temperature 80 ° C).

Although both the UV and mass-chromatograms indicated the presence of at least four components apart from the initial reactants in the reaction mixture, the relative intensities of the peaks in the respective chromatograms were different. The mass spectral data for the components are summarised in Table 1.

TABLE 1: LC-MS data for the oxidation of I.

Component	Retention Time (min)	Ions (m/z)	Assignment
II	6.2	159(M+H),143,111	mono-oxygenated I
III	8.1	283(M+H)	disulphide of I
IV	13.2	191(M+H)	tri-oxygenated I
I	14.7	143(M+H)	I
V	15.5	127(M+H)	oxygen analogue of I

These data are consistent with the proposed mechanism for the oxidation of 2-thioimidazoles (Karkhanis and Field, 1985) involving the initial formation of an S-monooxygenated species.



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