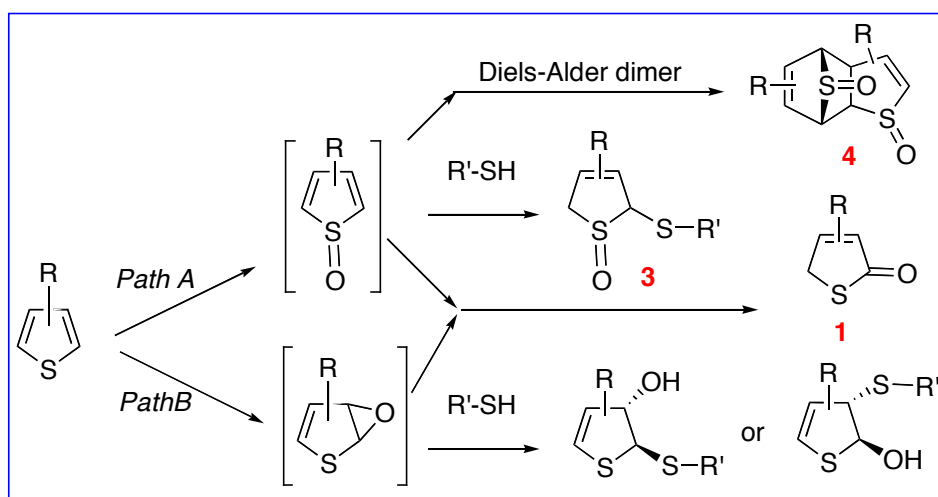


A NEW THIOPHENE METABOLITE: NADPH, O₂ AND THIOL DEPENDENT FORMATION OF A THIOPHENE HYDRATE FROM (S)-MTPPA BY CYP2C9.

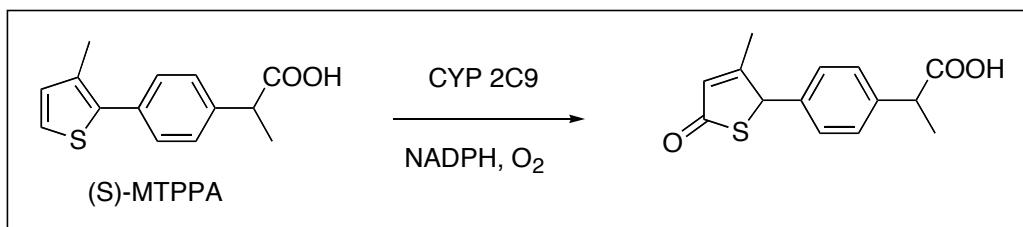
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Thiophenes are metabolized in rat or human liver microsomes to hydroxy-thiophenes¹ and thiophenes sulfoxides which may dimerize or be trapped as nucleophile-adducts². The major metabolites of thiophene are in vivo the N-2-acetylcysteine substituted 2,5-dihydrothiophene-1-oxide³ but in vitro the S-oxide dimer⁴. A number of 2-aryl-thiophenes are however metabolized to the 5-hydroxy derivatives¹. We have also shown that 2-phenyl and 3-phenyl thiophenes are metabolized by rat liver microsomes to thioenones (5-hydroxy-thiophene isomers) and thiophene-sulfoxide dimers, and in presence of glutathione to two type of adducts corresponding to addition of glutathione to **both the intermediate thiophene epoxide(s) and thiophene S-oxide**.

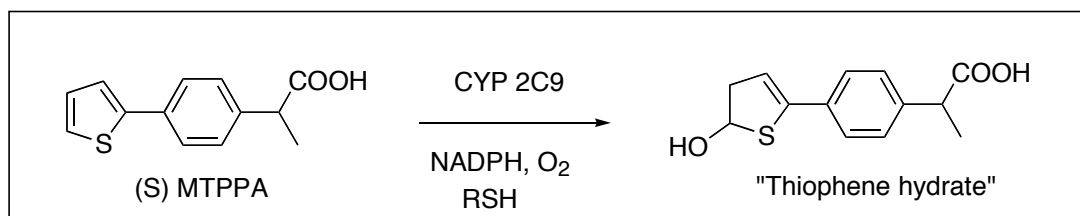


Thus this metabolite was isolated. (S)-MTPPA ((S)-2-[4-(3-methyl-2-thienyl)phenyl]propionic acid) is a NSAID recently introduced. It is metabolized by CYP2C9 into a very major 5-hydroxythiophene metabolite⁶.



The aim of this study was to investigate if a potential epoxide intermediate could be trapped as a glutathione adduct as 2-phenylthiophene. Adding progressive amount (0.1 – 10 mM) GSH to the incubation decreased the formation of the 5-hydroxythiophene metabolite and a new more polar metabolite appeared (see Figure 1 and 2).

To our surprise it did not contain GSH, Use of 5-Tritium labeled (S)-MTPPA showed that contrary to the thiolenone, the new metabolite was radioactive and retained the tritium. Establishment of its structure by multidimensional NMR (see table 1 and spectrum 1 and 2) and MS shows that it is a **5-hydroxy-4,5-dihydro-thiophene derivative, formally a “thiophene hydrate”**.



A neutral pH this new metabolite is relatively **stable** (T_{1/2} # 3 days). It is acid labile (20 mM TFA, T_{1/2} < 15 min) and dehydrates to the parent thiophene. GSH can be replaced by other thiols (N-Acetylcysteine or mercaptoethanol).

Potential mechanism of formation

Two mechanistic pathways are shown on Scheme 1 and Scheme 2:

Scheme 1 shows a thiophene 4,5-epoxide intermediate which would be common for formation of the thiolenone and the thiophene hydrate. We favor this pathway.

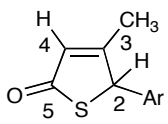
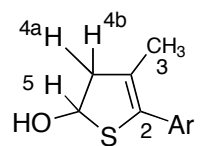
Scheme 2 on the other hand uses a thiophene 1-oxide as intermediate. Although on paper the mechanism is plausible, since thiols are better nucleophile than water it is astonishing that no thiophene 1-oxide-thiol adduct could be trapped, contrary to the case of 2-phenylthiophene, a simpler analog of MTPPA. Thus we believe that this pathway is unlikely.

Finally this mechanism is not specific of (S)-MTPPA, since recent work shows that 2-phenylthiophene and 2-thienyl-4-benzoic acid also can be metabolized in presence of GSH or mercaptoethanol to the corresponding “thiophene hydrate”

1) Neau E. et al. *Biochemical Pharmacol.* 1990; 39: 1101-07; 2) Mansuy D. et al. *J. Am.Chem. Soc.* 1991; 113: 7825-26; 3) Dansette P.M. et al. *Biochem. Biophys. Res. Commun.* 1992; 186: 1624-30; 4) Treiber A. et al. *J. Am. Chem. Soc.* 1997; 119: 1565-71. 5) Dalvie, D.K et al. *Chem. Res. Toxicol.*; 2002; 15: 269-299. 6) Taguchi et al. *Xenobiotica*, 1999; 29: 899-907.

Methods: (S)-MTPPA was labeled in CH₂Cl₂ by exchange with tritiated TFA obtained by mixing TFA anhydride with tritiated water and similarly exchange with deuterated TFA yielding 5-deutero (S)-MTPPA thiophene. Metabolic oxidation of (S)-MTPPA (0.2 mM) was performed by recombinant CYP2C9 expressed in yeast in presence of an NADPH generating system.. Incubations in presence glutathione (0.1 - 6 mM), mercaptoethanol (10 mM) or N-acetylcystein (6 mM) were compared. Scale up to 40 ml incubation allowed isolation of enough metabolite for characterisation by NMR and mass spectroscopy. NMR: 250 MHz and 500 MHz (Brucker), HPLC-ESI MS: Surveyor and LCQ Advantage (ThermoFinnigan). HPLC were on Hypersil MOS (5 μ, 250 x 4.6 mm) or BDSX C8 columns (5 μ, 150 x 4.6 mm). Sample preparation used SepPak C18 or Oasis columns (Waters).

Table 1: selected NMR values for the 2 metabolites of (S)-MTPPA, the thioenone, and the new “thiophene hydrate”

			
H (C)	H	H	C
2	5;52, s	-----	129.3
4a	6.20, s	2.91, d $J_{4a-4b}=17.7$ Hz	55
4b	-----	3.34, dd $J_{4a-4b}=17.7$ Hz, $J_{4-5}=5.9$ Hz	
5a	-----	$J_{4-5}=5.9$ Hz	81
5b	-----	-----	
1'	-----	-----	145.4
2'	7.27, d $J_{2'-3'}=7.9$ Hz	7.38, d $J_{2'-3'}=7.9$ Hz	130.1
3'	7.35, d $J_{2'-3'}=7.9$ Hz	7.42, d $J_{2'-3'}=7.9$ Hz	131.4
4'	-----	-----	130.7
1''	-----	-----	185.7
2''	3.75	3.69, q $J_{2''-3''}=7.1$ Hz	50.7
3''	1.48, d $J_{2''-3''}=7.1$ Hz	1.43, d $J_{2''-3''}=7.1$ Hz	21
3-Me	1.96, s	1.90, s	17.6