value for k_{et} and k_{bet} is due to a change in interacting orbitals at r_o.

Electron transfer and back electron transfer involve different pyrylium orbitals. In the forward direction an electron from the carbon–boron bond is donated to the half-filled pyrylium HOMO, while in the back direction an electron from the pyrylium LUMO is transferred to the boranyl radical. We suggest that the aryl groups mediate electron transfer by superexchange similar to the way that bridging ligands appear to operate in bimetallic systems.[19] Thus strong overlap with the pyrylium HOMO enables a fast rate for the forward direction, but for the reverse, a weaker interaction between the LUMO and the occupied orbitals of the aryl groups leads to a diminished maximum rate.

Acknowledgment. Mr. B. Sauerwein helped with the picosecond time scale measurements and Mr. C. Xu with the electrochemistry. Many of the borates used were prepared by Dr. X. Yang. This work was supported by grants from the National Science Foundation (NSF DMR 87-21243, -04130, NSF CHE-88-20271). J.B.M. was supported by PHS GM08276.

Supplementary Material Available: A table containing a list of the borates used and the electrochemical, spectroscopic, and kinetic data (1 page). Ordering information is given on any current masthead page.

(18) Crude estimates based on a classical mechanics model indicate a maximum acceleration of 0.1 Å/ps^2 and damping by viscous resistance of the solvent in a few picoseconds. Also, k_{bet} is unaffected by changing the solvent from benzene (η = 0.65 cP) to *p*-cymene (η = 3.4 cP).

(19) Larson, S. *J. Am. Chem. Soc.* **1981**, *103*, 4034.

Thiophene *S*-Oxides as New Reactive Metabolites: Formation by Cytochrome P450 Dependent Oxidation and Reaction with Nucleophiles

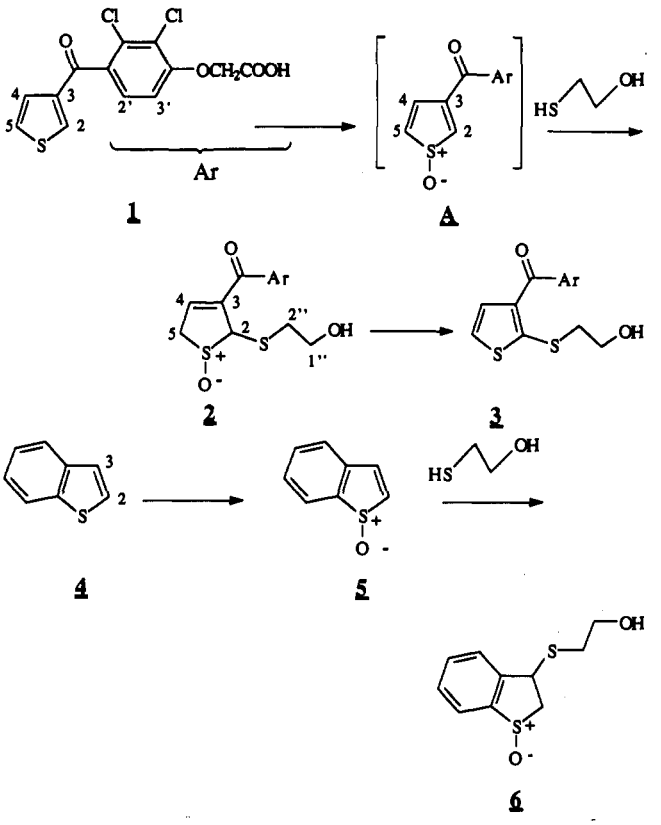
Daniel Mansuy,* Philippe Valadon, Irène Erdelmeier, Pilar Lopez-Garcia, Claudine Amar, Jean-Pierre Girault, and Patrick M. Dansette

Laboratoire de Chimie et Biochimie Pharmacologiques et Toxicologiques, URA 400 CNRS
Université René Descartes, 45 rue des Saints-Pères
75270 Paris Cedex 06, France

Received April 22, 1991

Although there is evidence that several thiophene derivatives cause toxic effects,[1] very little is known not only on the molecular mechanism of these effects but also, in a more general manner, on the oxidative metabolism of the thiophene ring.[2] Recently, it has been shown that the hepatic cytochrome P450 dependent oxidation of a diuretic drug, tienilic acid, and of its isomer with the arylcarbonyl group on position 3 of the thiophene ring, **1**, led to the formation of electrophilic metabolites that covalently bind to hepatic proteins[3] but the nature of which remained unknown.

Scheme I



In an effort to determine the nature of these electrophilic metabolites, we have undertaken the study of the metabolism of thiophene compounds by rat liver microsomes in the presence of a thiol-containing trapping agent.

This communication describes the initial results concerning the study of **1** and of a simpler thiophene derivative **4**. They provide initial evidence for the formation of thiophene *S*-oxides as reactive metabolites of thiophene-containing compounds and for the easy reaction of these reactive metabolites with nucleophiles.

As reported previously,[3a] oxidation of **1** by rat liver microsomes, in the presence of NADPH, led to metabolites most of which (70%) covalently bound to microsomal proteins, some unknown minor metabolites (30%) being detected by HPLC. Incubations under identical conditions but in the presence of nucleophiles like glutathione or mercaptoethanol led to a dramatic decrease of this covalent binding to proteins[3a] and to a concomitant appearance of new metabolites detected by HPLC. In the presence of 100 μM mercaptoethanol, only one major new metabolite, **2**, appeared. Despite its reactivity [i.e., **2** was rapidly and quantitatively transformed into a new compound, **3**, in acidic conditions (pH ≈ 1), and into other metabolites in the presence of excess mercaptoethanol] metabolite **2** could be isolated by preparative HPLC at neutral pH.[4] The UV, mass ([M + 1]$^+$ = 407), and ^{1}H NMR[5] spectra (Table I) of **3** clearly showed that it was derived from **1** by introduction of the S(CH$_2$)$_2$OH moiety at position 2 (Scheme I). The structure indicated for **2** was based on (i) its very characteristic ^{1}H NMR spectrum, with only one vinylic proton coupled to two geminal protons (H_5), a singlet at 5.44 ppm for H_2, two sets of signals corresponding to the SCH$_2$CH$_2$O moiety, and the signals of the Ar group almost identical with those of **3** (Table I); (ii) its UV spectrum, which was pH-dependent and exhibited bands as expected for a conjugated ketone at low pH and for a highly conjugated anion at high pH (pK ≈ 8.5); (iii)

(1) (a) Mitchell, J. R.; Nelson, W. L.; Potter, W. Z.; Sasame, H. A.; Jollow, J. A. *J. Pharmacol. Exp. Ther.* **1976**, *119*, 41. (b) McMurtry, R. J.; Mitchell, J. R. *Toxicol. Appl. Pharmacol.* **1977**, *42*, 285. (c) Vaughan, D. P.; Tucker, G. F. *Pharm. J.* **1987**, 577. (d) Beaune, P.; Dansette, P. M.; Mansuy, D.; Kiffel, L.; Finck, M.; Amar, C.; Leroux, J. P.; Homberg, J. C. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 551.

(2) (a) Rance, D. J. In *Sulfur-containing drugs and related organic compounds, Chemistry, biochemistry and toxicology, Vol. 1, Part B: Metabolism of sulfur functional groups*; Damani, L. A. Ed.; Ellis Howard Ltd: Chichester, UK, 1989; p 217. (b) Damani, L. A.; Crooks, P. A. In *Metabolic basis of detoxication, metabolism of functional groups*; Jakoby, W. B., Bend, J. R., Cadwell, J., Eds.; Academic Press: New York, 1982; p 69.

(3) (a) Dansette, P. M.; Amar, C.; Smith, C.; Pons, C.; Mansuy, D. *Biochem. Pharmacol.* **1990**, *39*, 911. (b) Dansette, P. M.; Amar, C.; Valadon, P.; Pons, C.; Beaune, P. H.; Mansuy, D. *Biochem. Pharmacol.* **1991**, *40*, 553.

(4) Microsomal incubations of **1**, measurement of covalent binding, and reverse-phase HPLC studies were performed as described previously.[3a]

(5) Cagniant, P.; Cagniant, D.; Paquer, D.; Kirsch, G. In *The Chemistry of heterocyclic compounds, thiophene and its derivatives*; Gronowitz, S., Ed.; John Wiley and Sons: New York, 1985; Vol. 44, Part 2, p 119.

0002-7863/91/1513-7825$02.50/0

Table I. ^{1}H NMR and UV Characteristics of Metabolites 2 and 3

	^{1}H NMR[a]								UV[e] λ, nm (ε, mM^{-1} cm^{-1})
	H_2	H_4	H_5	$H_{2'}$	$H_{3'}$	OCH_2	$H_{2''}$	$H_{1''}$	
2[b]	5.44 (s, 1 H)	7.02 (d, 3.4, 1 H)[d]	3.83[c] 4.57	7.45 (d, 8.8, 1 H)	6.96 (d, 8.8, 1 H)	4.66 (s, 2 H)	3.03 (m, 2 H)	3.89 (t, 6, 2 H)	292 (6.4) pH 7.4 345 (21.2) pH 11
3		7.12 (d, 5.8, 1 H)	7.3 (d, 5.8, 1 H)	7.37 (d, 8.8, 1 H)	6.97 (d, 8.8, 1 H)	4.66 (s, 2 H)	3.33 (t, 6, 2 H)	3.93 (t, 6, 2 H)	240 (20.8), 277 (15.7), 358 (8.2)

[a] 2 and 3 as NH_4^+ salts in D_2O (3 mM, pH around 6.5); δ in ppm/Si$(CH_3)_4$, J in Hz. [b] Data of the major diastereoisomer; the minor one (7%) was not completely characterized but exhibited data similar to that of the major one (5.67 H_2; 6.74 H_4; 7.42 $H_{2'}$; 6.95 $H_{3'}$; 4.66 OCH_2; 2.89 $H_{2''}$; 3.78 $H_{1''}$). [c] Slow exchange of 3.83 (dd, J = 20 and 3.4, 1 H) in D_2O pH 6.5; rapid exchange of 4.57 (dd, J = 20 and 2, 1 H) in D_2O pH 6.5 but slow exchange at pH 3 (easily observed). [d] Doublet (J = 3.4) in D_2O because of partial exchange of the 4.57 H even at pH 3, but quadruplet (J = 3.7 and 1.5) in a CD_3CN–D_2O (DCl) mixture where no exchange of H_5 occurred. [e] In phosphate buffer pH 7.4.

its transformation into 3 at pH 1, with loss of H_2O, which is well-known for dihydrothiophene sulfoxides;[6] and (iv) its mass spectrum with a peak at $[MH - H_2O]^+$ with the expected isotopic cluster (2Cl) and fragments almost identical with those of the mass spectrum of 3.[7]

Oxidation of 4 by rat liver microsomes in the presence of NADPH led to sulfoxide 5 as a major metabolite. After identical incubations but in the presence of mercaptoethanol and at pH 8.5, 5 was rapidly formed but was slowly transformed into a new metabolite, 6. Identification of 5 and 6 were done by comparison with authentic samples prepared by oxidation of 4 by H_2O_2 in CF_3COOH and treatment of 5 by $HOCH_2CH_2S^-$ in CH_3OH.[8]

These results underline the great similarity between the pathways observed for microsomal oxidation of two thiophene derivatives, 1 and 4. In the case of 4, the corresponding *S*-oxide was stable enough (only in diluted solutions) to be detected. Nucleophilic addition of mercaptoethanol to its double bond conjugated with the sulfoxide, which gave 6, was slow enough to be followed by HPLC. In the case of 1, the structures of metabolites 2 and 3 and the similarity observed between the metabolisms of 1 and 4 strongly suggest the intermediate formation of the thiophene *S*-oxide A. Contrary to benzothiophene *S*-oxides, thiophene *S*-oxides are very reactive species and very few of them have been described so far.[9] Thiophene *S*-oxide A should be particularly electrophilic because of the presence of the keto substituent. Therefore, a Michael addition of mercaptoethanol on C-2 of the double bond conjugated with a COAr and sulfoxide functions should be very fast and lead to sulfoxide 2 after protonation of the intermediate anion at position 5. In the absence of a thiol-containing nucleophile added to the incubation mixture, A should react with nucleophilic residues of microsomal protein amino acids, which would explain the observed covalent binding of metabolites of 1 to proteins.[3] Microsomal S-oxidation of 1 is mainly catalyzed by cytochromes P450 since the formation of 2 and the covalent binding of 1 metabolites to proteins are almost completely suppressed in the presence of classical cytochrome P450 inhibitors like CO. The consequences of the formation of this new class of reactive metabolites (thiophene *S*-oxides) for the metabolism and possible toxic effects of thiophene derivatives are under study.

Registry No. 1, 55901-69-4; 2, 136006-18-3; 3, 136006-19-4; 4, 95-15-8; 5, 51500-42-6; 6, 136006-20-7.

(6) (a) Cava, M. P.; Pollack, M. *J. Am. Chem. Soc.* **1966**, *88*, 4112. (b) Takaya, T.; Kosaka, S.; Otsuji, Y.; Imoto, E. *Bull. Chem. Soc. Jpn.* **1968**, *41*, 2086. (c) Buter, J.; Wassenaar, S.; Kellogg, R. M. *J. Org. Chem.* **1972**, *37*, 4045. (d) Jonsson, E. *Ark. Kemi* **1967**, *26*, 357. (e) Klemm, L. H.; Merrill, R. E.; Lee, F. H. W. *J. Heterocycl. Chem.* **1974**, *11*, 535. (f) Geneste, P.; Olivé, J. L.; Ung, S. U. *J. Heterocycl. Chem.* **1977**, *14*, 953.

(7) The largest *m/e* peak of 2 corresponded to $[MH - H_2O]^+$ due to its fast dehydration under mass spectrometric conditions (CI, NH_3).

(8) The chemical preparation and complete characterization of 5 and 6 will be described elsewhere.

(9) Raasch, M. S. In *The chemistry of heterocyclic compounds, thiophene and its derivatives*; Gronowitz, S., Ed.; John Wiley and Sons: New York, 1985; Vol. 44 part 1, p 571. (b) Skaugset, A. E.; Rauchfuss, T. B.; Stern, C. L. *J. Am. Chem. Soc.* **1990**, *112*, 2432–2433.