α-Thiolactones as Novel Intermediates in the Cysteine Conjugate β-Lyase-Catalyzed Bioactivation of Bromine-Containing Cysteine S-Conjugates

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The toxicity of many chemicals is dependent on their enzymatic conversion to reactive intermediates whose formation is associated with cell damage and death.[1] The kidney-selective toxicity of of haloalkenes is associated with hepatic glutathione transferase-catalyzed glutathione *S*-conjugate formation, γ-glutamyltransferase- and dipeptidase-catalyzed hydrolysis of the glutathione *S*-conjugates to cysteine *S*-conjugates, active uptake of the cysteine *S*-conjugates by the kidney, and bioactivation by pyridoxal phosphate-dependent cysteine conjugate β-lyase.[2,3] The β-lyase-dependent bioactivation of *S*-(1-chloroalkenyl)-L-cysteine conjugates affords 1-chloroalkenyl thiolates, pyruvate, and ammonia as products.[2,3] The unstable 1-chloroalkenyl thiolates lose chloride to give thioketenes as thioacylating intermediates.[4] A β-lyase-catalyzed β-elimination reaction from *S*-(1,1-difluoroalkyl)-L-cysteine conjugates gives 1,1-difluoroalkyl thiolates, pyruvate, and ammonia as products.[5] The 1,1-difluoroalkyl thiolates studied thus far lose fluoride to give thioacyl fluorides that react with tissue nucleophiles.[6,7] All 1,1-dichloroalkene-derived cysteine *S*-conjugates that have been studied are nephrotoxic and cytotoxic and mutagenic in the Ames test; in contrast, 1,1-difluoroalkene-derived *S*-conjugates that have been studied are also nephrotoxic and cytotoxic, but are not mutagenic.[8−10]

Recent studies showed, however, that bromine-containing, 1,1-difluoroalkene-derived cysteine *S*-conjugates (Scheme 1, **1a−c**) are mutagenic,[11,12] which challenges the generalization that 1,1-difluoroalkene-derived conjugates are not mutagenic. The observation that conjugates **1a−c** are mutagenic led to a search for alternative bioactivation mechanisms for bromine-containing, 1,1-difluoroalkene-derived *S*-conjugates. We present herein the first evidence for the formation of α-thiolactones as novel intermediates in the β-lyase-catalyzed bioactivation of bromine-containing, 1,1-difluoroalkene-derived cysteine *S*-conjugates.

Incubation of cysteine *S*-conjugates **1a−c**[12] (Scheme 1) with rat kidney homogenates or with a pyridoxal model system (*N*-dodecylpyridoxal in cetyltrimethylammonium micelles[13]), as described previously,[5] and analysis of the reaction mixtures by ^{19}F NMR spectroscopy showed the complete loss of resonances assigned to the conjugates and the formation of inorganic fluoride, but no resonances assignable to organofluorine compounds were observed. Although dihaloacetates are terminal products of bromine-lacking, 1,1-difluoroalkene-derived *S*-conjugates,[5] bromohaloacetates were not detected as products of conjugates **1a−c** by pentafluorobenzyl ester formation[14] and GC−MS analysis.

A search for alternative carbon-containing products formed from bromine-containing cysteine *S*-conjugates led to the detection of glyoxylate **11** as a terminal product. Incubation of conjugates **1a−c** (1 mM) with rat kidney homogenates or with the pyridoxal model system as described above resulted in the formation of 130−160 μM glyoxylate **11**, which was quantified by HPLC analysis.[15] Glyoxylate formation was not detected with the bromine-lacking cysteine *S*-conjugates *S*-(2-chloro-1,1,2-trifluoroethyl)-L-cysteine,[16] *S*-(1,1,2,2-tetrafluoroethyl)-L-cysteine,[17] and *S*-(2,2-dichloro-1,1-difluoroethyl)-L-cysteine;[12] the limit of detection of glyoxylate **11** is 10 μM.

The formation of glyoxylate **11** from conjugates **1a−c** may be rationalized by the formation of thiolates **3a−c**, which may lose fluoride to give thioacyl fluorides **4a−c** (Scheme 1); hydrolysis of **4a−c** would give thionoacetates **6a−c**, which may also be represented as the resonance contributor thiolacetates **7a−c**. The latter species may carry out an intramolecular displacement of bromide to give α-thiolactones **8a−c**. Attack of hydroxide on the carbonyl carbons of α-thiolactones **8a−c**, loss of halide from mercaptoacetates **9a−c**, and hydrolysis of thioaldehyde **10** gives glyoxylate **11**.

To seek confirmatory evidence for the pathway shown in Scheme 1, precursors of proposed intermediates **3a** and **6a/7a** were studied. Haloalkene-derived 2-nitrophenyl disulfides have been studied as precursors of 1-chloroalkenyl thiolates and 1,1-difluoroalkyl thiolates.[4] The reduction of disulfide **2a**,[18] as a precursor of thiolate **3a**, in THF with DABCO, as described earlier,[4] and analysis by ^{19}F NMR spectroscopy resulted in the complete loss of disulfide **2a** and the formation of inorganic fluoride, but no organofluorine compounds were observed; glyoxylate **11** was, however, detected as a product. The hydrolysis of ethyl bromofluorothionoacetate **5a** would be expected to afford intermediates **6a/7a**. Accordingly, reaction of thionoester **5a**[18] (1 mM, 1 h, 37 °C) with 2 N NaOH and HPLC analysis showed stoichiometric formation of glyoxylate **11**. Incubation of bromofluorothioacetate **7a**[18] (1 mM, 1 h, 37 °C) in phosphate buffer (pH 7.4) followed by HPLC analysis also showed stoichiometric formation of glyoxylate **11**.

As noted above, incubation of conjugates **1a−c** with the pyridoxal model system resulted in the complete loss of ^{19}F NMR resonances assigned to conjugates **1a−c**, but less than stoichiometric yields of glyoxylate **11** were formed; in contrast, ethyl bromofluorothionoacetate **5a** and bromofluorothioacetate

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(1) *Bioactivation of Foreign Compounds*; Anders, M. W., Ed.; Academic Press; Orlando, 1985.
(2) Dekant, W.; Anders, M. W.; Monks, T. J. In *Renal Disposition and Nephrotoxicity of Xenobiotics*; Anders, M. W., Dekant, W., Henschler, D., Oberleithner, H., Silbernagl, S., Eds.; Academic Press: San Diego, 1993; p 187.
(3) Dekant, W.; Vamvakas, S.; Anders, M. W. *Adv. Pharmacol.* **1994**, *27*, 115.
(4) Dekant, W.; Urban, G.; Görsmann, C.; Anders, M. W. *J. Am. Chem. Soc.* **1991**, *113*, 5120.
(5) Dekant, W.; Lash, L. H.; Anders, M. W. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 7443.
(6) Hayden, P. J.; Yang, Y.; Ward, A. J. I.; Dulik, D. M.; McCann, D. J.; Stevens, J. L. *Biochemistry* **1991**, *30*, 5935.
(7) Harris, J. W.; Dekant, W.; Anders, M. W. *Chem. Res. Toxicol.* **1992**, *5*, 34.
(8) Green, T.; Odum, J. *Chem.-Biol. Interact.* **1985**, *54*, 15.
(9) Dekant, W.; Vamvakas, S.; Berthold, K.; Schmidt, S.; Wild, D.; Henschler, D. *Chem.-Biol. Interact.* **1986**, *60*, 31.
(10) Vamvakas, S.; Elfarra, A. A.; Dekant, W.; henschler, D.; Anders, M. W. *Mutat. Res.* **1988**, *206*, 83.
(11) Finkelstein, M. B.; Baggs, R. B.; Anders, M. W. *J. Pharmacol. Exp. Ther.* **1992**, *261*, 1248.
(12) Finkelstein, M. B.; Vamvakas, S.; Bittner, D.; Anders, M. W. *Chem. Res. Toxicol.* **1994**, *7*, 157.
(13) Kondo, H.; Kikuchi, J.-I.; Uchida, S.; Kitamikado, T.; Koyanagi, E.; Sunamoto, J. *Bull. Chem. Soc. Jpn.* **1985**, *58*, 675.
(14) Ehrsson, H. *Acta Pharm. Suec.* **1971**, *8*, 113.
(15) Stijntjes, G. J.; te Koppele, J. M.; Vermeulen, N. P. E. *Anal. Biochem.* **1992**, *206*, 334.
(16) Dohn, D. R.; Quebbemann, A. J.; Borch, R. F.; Anders, M. W. *Biochemistry* **1985**, *24*, 5137.
(17) Odum, J.; Green, T. *Toxicol. Appl. Pharmacol.* **1984**, *76*, 306.
(18) The synthesis and characterization of new compounds are described in supporting information.

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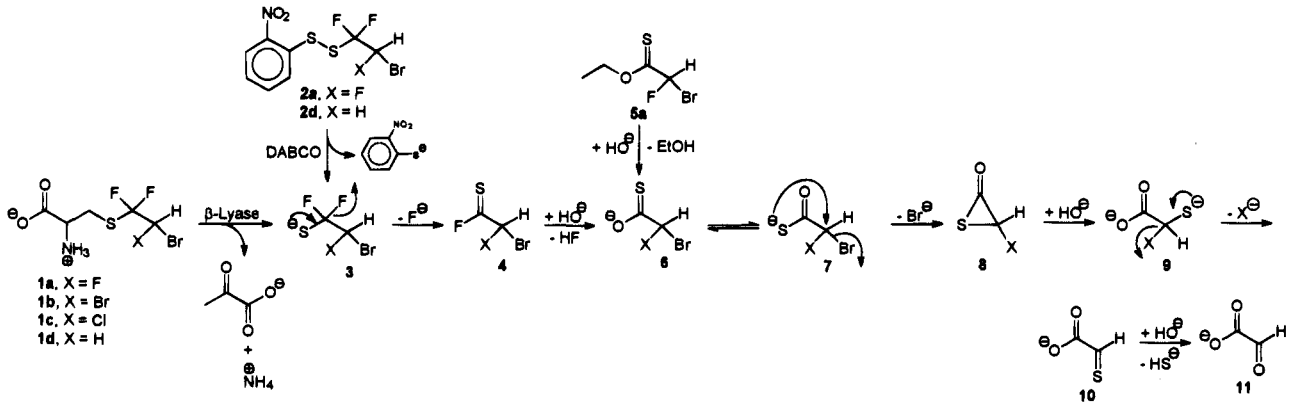
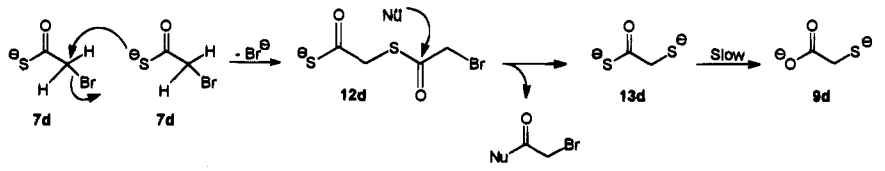
Scheme 1



Scheme 2



7a gave stoichiometric yields of glyoxylate **11**. Therefore, the low stoichiometric yields of glyoxylate **11** from conjugates **1a–c** must be associated with the diversion of 1,1-difluoro-2-bromo-2-haloethanethiolates **3a–c** or bromohalothionoacetyl fluorides **4a–c** to other products. Other terminal products have not been identified, but polymeric material that was insoluble in phosphate buffer and organic solvents (THF, MeOH, EtOH, CH_2Cl_2) was formed from conjugates **1a–c**. Elemental analysis (C, H, S, N, Br, F) of the polymeric material did not provide insight into nature of the products.

The chemistry described above and in Scheme 1 predicts that hydrolysis of the halogen-lacking α-thiolactone **8d** would afford mercaptoacetate **9d**. Incubation of *S*-conjugate **1d**[18] (1 mM) with rat kidney homogenates or with the pyridoxal model system or of disulfide **2d**[18] with THF and DABCO as described above gave inorganic fluoride as the only assignable resonance by ^{19}F NMR spectroscopy; pentafluorobenzyl ester formation[14] and GC–MS analysis showed the appearance of mercaptoacetate **9d** (1 mM **1d** gave 90 μM mercaptoacetate **9d**). Incubation of bromothioacetate **7d**[19] (1 mM, 1 h, 37 °C) in phosphate buffer (pH 7.4) also gave mercaptoacetate **9d**.

An alternative route to mercaptoacetate **9d** from bromine-containing cysteine *S*-conjugates has been considered (Scheme 2): bimolecular attack of thiolate **7d** or **7d** (or its conjugate acid) may displace bromide and afford thiolester **12d**; nucleophilic attack by thiolate **7d** or another nucleophile would give mercaptothiolacetate **13d**.[20] With the unimolecular pathway, mercaptoacetate **9d** is formed as a terminal product, whereas with the bimolecular reaction, mercaptothiolacetate **13d** would be formed. Phase-transfer analysis[14] did not show the formation of pentafluorobenzyl mercaptothiolacetate; the limit of detection of mercaptothiolacetate **13d** is 10 μM. Moreover, mercaptothiolacetate **13d** (1 mM) underwent little hydrolysis (<5%, pH 8.0, 37 °C, 5 h) to mercaptoacetate **9d**, indicating that a bimolecular reaction cannot account for mercaptoacetate **9d** formation.

The data imply that α-thiolactones are intermediates in the conversion of bromine-containing cysteine *S*-conjugates **1a–c** and disulfide **2a** to glyoxylate **11** (Scheme 1). α-Thiolactones **8a–c** may arise by intramolecular displacement of bromide by anionic sulfur in thiolacetates **7a–c**. Also, as described above, the net 1,2 migration of sulfide required for the formation of mercaptoacetate **9d** from conjugate **1d** or disulfide **2d** provides strong analogy for the intermediacy of **3d–8d**. Although displacement of bromide within oxyanions **6a–c** would give α-thionolactones, this was considered unlikely because the soft thiolates **7a–c** would be more nucleophilic in displacing bromide than the hard oxyanions **6a–c** and because the calculated heats of formation of α-thiolactone **8a** and of the corresponding α-thionolactone favor α-thiolactone **8a** by about 13 kcal/mol (heats of formation in the gas phase were calculated by the MOPAC 6.0 program with an AM1 Hamiltonian). Furthermore, no derivatization product of thionoglycolic acid, the expected product of this alternative pathway, was observed by GC–MS.

This is the first example of the formation of xenobiotic-derived α-thiolactones. Only two α-thiolactones are known.[21,22] The biological properties of α-thiolactones have apparently not been reported, and their role in the observed mutagenicity of bromine-containing cysteine *S*-conjugates merits investigation. The novel origin of α-thiolactones described in this paper may, however, be exploited to study the formation and the chemical and biological properties of α-thiolactones.

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Supporting Information Available: Synthesis and characterization of **1d, 2a,d, 5a,** and **7a** (2 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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(19) Randhawa, H. S.; Walter, W. *Bull. Chem. Soc. Jpn.* **1978**, *51*, 1579.
(20) Satsumabayashi, S.; Takahashi, H.; Tanaka, T.; Motoki, S. *J. Org. Chem.* **1973**, *38*, 3953.
(21) Schaumann, E.; Behrens, U. *Angew. Chem., Int. Ed. Engl.* **1977**, *16*, 722.
(22) Schaumann, E.; Lange, B.; Reinholdt, K. *J. Chem. Soc., Chem. Commun.* **1983**, 797.