Cyclization of a Cysteine Conjugate of *N*-(3,5-Dichlorophenyl)succinimide

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N-(3,5-Dichlorophenyl)-2-cysteinylsuccinimide methyl ester hydrochloride (**5**) was prepared from *N*-(3,5-dichlorophenyl)maleimide (**3**) and cysteinyl methyl ester hydrochloride. Attempted neutralization of the cysteine conjugate salt with triethylamine resulted in spontaneous cyclization of **5** to form the more stable 2-(*N*-3,5-dichlorophenylcarbamoylmethyl)-5-carbomethoxy-1,4-thiazine-3-one (**6**). Similar results might be expected *in vivo* should these metabolites of succinimides be formed.

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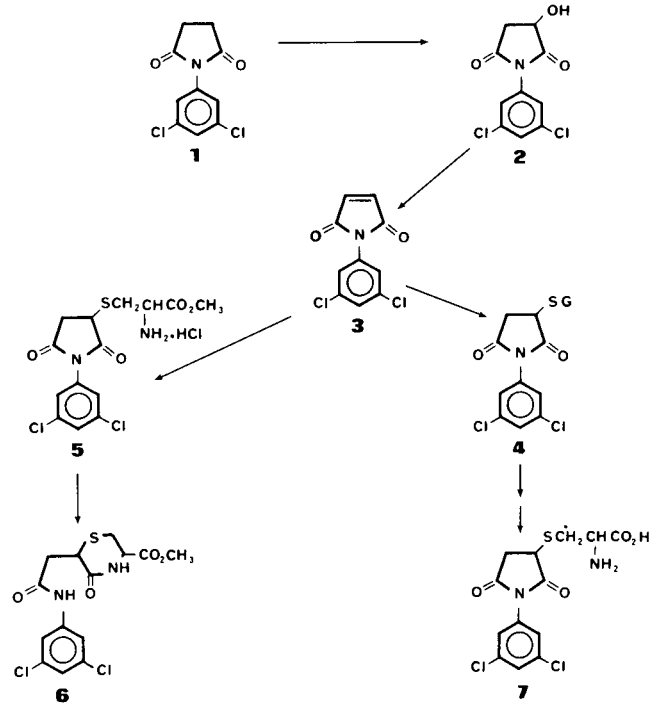
Previous studies from our laboratory have described the nephrotoxicity produced by the experimental agricultural fungicide *N*-(3,5-dichlorophenyl)succinimide (NDPS, **1**) [1-3]. The results of several studies have indicated that NDPS produced nephrotoxicity *via* one or more metabolites [4-6]. Although the identity of the ultimate nephrotoxic species remain unknown, a recent study demonstrated that depletion of renal and hepatic glutathione with diethyl maleate or pretreatment of rats with the cysteine conjugate β-lyase inhibitor aminooxyacetic acid markedly attenuated NDPS-induced nephrotoxicity [7]. These results are consistent with the ultimate nephrotoxic species being a cysteine conjugate of NDPS. Such a finding is important since cysteine conjugates of only a small number of nephrotoxic chemicals have been shown to be the ultimate nephrotoxic metabolite [8].

Ohkawa *et al.* [9] have described the biotransformation pathway of NDPS in rats and dogs. *N*-(3,5-Dichlorophenyl)-2-hydroxysuccinimide (NDHS, **2**) and *N*-(3,5-dichlorophenyl)-2-hydroxysuccinamic acid (NDHSA) are the only identified metabolites of NDPS which have been shown to produce significant nephrotoxicity. These observations have suggested that if a cysteine conjugate of NDPS is the ultimate nephrotoxic species, the conjugate should arise from NDHS and/or NDHSA. In the case of NDHS, a cysteine conjugate could be formed by *in vivo* dehydration of the hydroxyl group of **2** to yield the maleimide **3**. Conjugation of **3** with glutathione followed by enzymatic degradation of **4** would yield the cysteine conjugate **7**.

We had previously prepared **7** *via* addition of cysteine free base to **3**. However, purification of **7** prepared by this method proved quite difficult. Therefore, to improve our yield of **7**, we prepared the methyl ester hydrochloride salt **5** from **3** by addition of cysteinyl methyl ester hydrochloride. Purification of **5** was accomplished using column chromatography techniques with silica gel as the solid support and gradually increasing the polarity of the eluting solvent.

Attempts to neutralize **5** with triethylamine did not yield the expected methyl ester of **7**. Instead, the free base form of **5** underwent an intramolecular transcyclization reaction to yield **6**. Infrared spectra of **6** demonstrated the amide carbonyl stretching band and the lactam carbonyl stretching band as a broad peak centered at 1650 cm^{-1}. The characteristic broad, imide carbonyl stretching bands centered at or near 1700 cm^{-1} were absent in spectra of **6**.

The mechanism of this transcyclization reaction has not been studied in detail. However, it is likely that when the cysteinyl amino group is no longer protonated, the nitrogen atom of the free amino group reacts with the imide carbonyl carbon to form the more thermodynamically stable thiazane-3-one ring. The susceptibility of the succinimide ring to nucleophilic attack is evidenced by the rapidity with which succinimide rings are hydrolyzed as the *p*H of the dissolving media becomes more basic (*i.e.*



*p*H >7.0) [10]. In addition, amino groups of thiol amines are known to undergo intramolecular reactions. For example, Koechel *et al.* [11] have demonstrated thiol amine conjugates of the diuretic agent ethacrynic acid produced diuresis in dogs similar to that produced by the parent compound. However, thiol conjugates of ethacrynic acid without free amino groups were nondiuretic. It was concluded from these studies that once the hydrochloride salt of the amino group became unionized at physiological *p*H (~7.4), the free amino group catalyzed a reverse Michael reaction to liberate ethacrynic acid from the conjugate. Therefore, it is possible that should a cysteine conjugate of a succinimide be formed *in vivo*, it might also undergo an intramolecular transcyclization reaction to yield a thiazane-3-one.

We are currently investigating the nephrotoxic potential of **5** and **6** to detemine if **2** produces nephrotoxicity *via* a cysteine conjugate or transcyclization product.

EXPERIMENTAL

Melting points were determined with a Mel-Temp melting point apparatus and are uncorrected. Infrared spectra were obtained using a Perkin-Elmer model 297 spectrophotometer, while ^{1}H-nuclear magnetic resonance spectra were obtained on a Varian FT-200 (chemical shifts are quoted in ppm downfield from the internal standard tetramethylsilane). Elemental analyses were performed by Atlantic Microlab, Inc., Georgia, USA.

N-(3,5-Dichlorophenyl)-2-cysteinylsuccinimide Methyl Ester Hydrochloride (**5**).

A solution of 2.82 g (0.017 mole) of cysteinyl methyl ester hydrochloride in methanol (20 ml) was added to a solution of 4.0 g (0.017 mole) of *N*-(3,5-dichlorophenyl)maleimide in methylene chloride (20 ml). The resulting solution was stirred at room temperature for 2 hours. Solvent was removed under reduced pressure, and the resulting residue applied to a column containing silica gel (60-270 mesh) and packed in chloroform. The column was eluted subsequently with chloroform (100%) to ethyl acetate (100%) to acetone (100%) and finally ethanol (100%) to yield 6.55 g (96%) of pure **5**, mp 130-131°; ir (potassium bromide): cm^{-1} 3380, 1700, 1570; ^{1}H nmr (deuteriochloroform): δ 2.64 (1H, s, NCOCH-), 2.9 to 3.9 (7H, m, -NCOCH$_2$-, -SCH$_2$, -NH$_3$), 3.58 (3H, s, OCH$_3$), 4.3-4.8 (2H, m, S-CH, CH-CO$_2$), 7.24 (2H, s, C$_2$H and C$_6$H), 7.38 (1H, s, C$_4$H).

Anal. Calcd. for $C_{14}H_{15}Cl_3N_2O_4S$: C, 40.65; H, 3.66; N, 6.77; Cl, 25.71. Found: C, 40.71; H, 3.70; N, 6.75; Cl, 25.65.

2-(*N*-3,5-Dichlorophenylcarbamoylmethyl)-5-carbomethoxy-1,4-thiazane-3-one (**6**).

To a solution of 2.5 g (0.006 mole) of **5** in methanol (30 ml) was added an equimolar amount of triethylamine (0.87 ml). The mixture was stirred overnight at room temperature and allowed to stand for two days. The resulting white precipitate was filtered by suction and washed with methanol to yield 1.53 g (68%) of pure **6**, mp 154-155°; ir (potassium bromide): cm^{-1} 3310, 3200, 3080, 1745, 1650, 1580, 1510, ^{1}H nmr (dimethylsulfoxide-d$_6$): δ 2.99 (Hz, m, -NCOCH$_2$), 3.10 (2H, d, -SCH$_2$), 3.68 (3H, S, -OCH$_3$), 3.97 (1H, t, -CHCO$_2$, J = 6 Hz), 4.42 (1H, m, -COCHS-), 7.25 (1H, s, C$_4$H), 7.61 (2H, s, C$_2$H and C$_6$H), 7.99 (1H, s, -CONH-), 10.31 (1H, s, -CONH-).

Anal. Calcd. for $C_{14}H_{14}Cl_2N_2O_4S$: C, 44.58; H, 3.74; N, 7.43; Cl, 18.79. Found: C, 44.60; H, 3.74; N, 7.41; Cl, 18.85.

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