Reaction of *n*-Propanethiol with 3*H*-1,2-Benzodithiol-3-one 1-Oxide and 5,5-Dimethyl-1,2-dithiolan-3-one 1-Oxide: Studies Related to the Reaction of Antitumor Antibiotic Leinamycin with DNA

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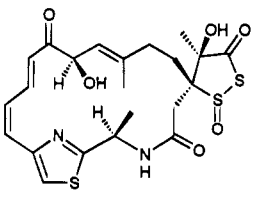
We have studied the reaction of *n*-propanethiol with 3*H*-1,2-benzodithiol-3-one 1-oxide and 5,5-dimethyl-1,2-dithiolan-3-one 1-oxide. The major products isolated from these reactions are the corresponding dithio carboxylic acids. In the case of 3*H*-1,2-benzodithiol-3-one 1-oxide, an unstable hydrodisulfide that decomposes to polysulfides under the reaction conditions is formed. A mechanism involving an unstable oxathiolanone intermediate is proposed for these reactions. We believe that these reactions may serve as useful models for some aspects of the thiol-activated DNA-cleavage chemistry of the antitumor antibiotic leinamycin, a natural product that contains a 1,2-dithiolan-3-one 1-oxide heterocycle.

Introduction

The antitumor antibiotic leinamycin (**1**) is reported to cleave DNA[1] yet does not appear to fall into any of the known classes of DNA-cleaving antibiotics. Leinamycin



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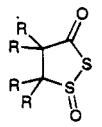

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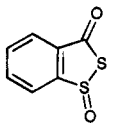

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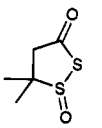

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cleaves DNA *only in the presence of added thiols*, and early experiments by the discoverers of this compound suggest that nucleophilic attack of thiols on the 1,2-dithiolan-3-one 1-oxide heterocycle (**2**) triggers DNA cleavage by this natural product.[1] The mechanism of DNA cleavage by leinamycin is not known.

Little is known about the reactivity of the 1,2-dithiolan-3-one 1-oxide heterocycle in general,[2–6] and to our knowledge no studies examining the reaction of this ring system with sulfur nucleophiles have been reported. Chemical model reactions often have provided information regarding the mechanism of processes involving complex biomolecules.[7] We feel that reactions of simple 1,2-dithiolan-3-one 1-oxides such as 3*H*-1,2-benzodithiol-3-one 1-oxide (**3**)[3] and 5,5-dimethyl-1,2-dithiolan-3-one 1-oxide (**4**) with thiols might serve as useful models for some aspects of the thiol-activated DNA-cleavage chemistry of leinamycin.

Results and Discussion

We find that treatment of **3** with excess *n*-propanethiol and a catalytic amount of triethylamine in dry dichloromethane at 25 °C results in a rapid reaction that affords dithio benzoic acid derivative **5**, 1,2-benzodithiol-3-one (**6**), disulfide **7**, and polysulfides **8** and **9** as products (Scheme 1).

1,2-Benzodithiol-3-one (**6**) probably arises from a reaction analogous to the known reduction of sulfoxides to sulfides upon treatment with thiol and base.[8] Reduction of the sulfoxide functionality occurs with a concomitant oxidation of the thiol, thereby explaining the observed formation of disulfide **7**. The reaction resulting in formation of **5** involves net transfer of the sulfoxide oxygen of **3** to the carboxyl carbon of **5**. Exclusion of molecular oxygen and employment of rigorously dry conditions do not alter the course of the reaction, indicating that the carboxyl oxygen of **5** does not originate from either water or molecular oxygen. A reasonable mechanism that explains the formation of **5**, **8**, and **9** in this reaction involves intramolecular transfer of the oxygen atom via an electrophilic oxathiolanone intermediate (**10**), as shown in Scheme 2.[9,10] Control experiments demonstrate that **5** and **6** are stable under the reaction conditions; thus, **6** does not convert to **5** or vice versa. Oxathiolanones have been proposed as intermediates in a number of reactions,[2,4,11,12] and although they are generally unstable species, one stable oxathiolanone that displays reactivity with thiols identical with that which

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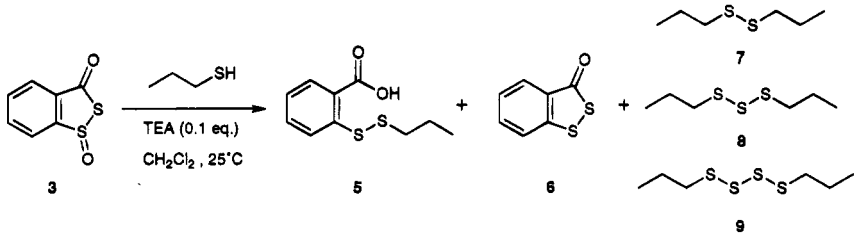
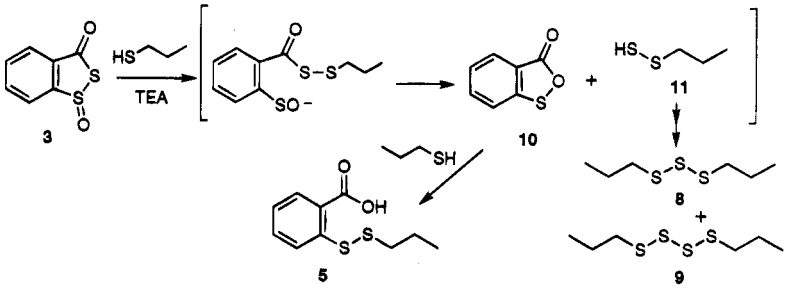
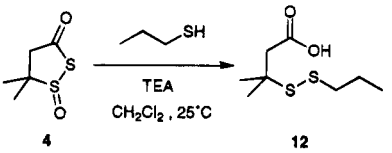
Scheme 1



Scheme 2



Scheme 3



we suggest for **10** has been described.[13] The proposed mechanism predicts the unstable hydrodisulfide **11** as a product of this reaction.[14,15] Using tandem gas chromatography–mass spectrometry, we have identified as products of the reaction between **3** and propanethiol the trisulfide **8** and tetrasulfide **9**, the expected decomposition products of the unstable hydrodisulfide **11**, under our reaction conditions.[15]

We find that the alicyclic 1,2-dithiolan-3-one 1-oxide analog **4**, which closely resembles the sulfur-containing heterocycle of leinamycin, reacts with thiols in a manner analogous to that for **3** (Scheme 3), producing the dithio carboxylic acid product **12**. This result suggests that the formation of dithio carboxylic acid products (e.g. **5**, **12**) in the reaction of thiols with 1,2-dithiolan-3-one 1-oxides is a general phenomenon and that such compounds might therefore be expected as products of the reaction between thiols and the natural product leinamycin.

Identification of the products of thiol-mediated decomposition of 1,2-dithiolan-3-one 1-oxides is a important step toward understanding the chemical mechanism of thiol-dependent DNA cleavage by agents that possess this unusual sulfur-containing heterocycle. Our studies suggest that, in the reaction of thiols with 1,2-dithiolan-3-one 1-oxides, reactive species that may be capable of DNA damage, such as electrophilic oxathiolanones and unstable hydrodisulfides, are generated. Electrophilic species are known to react with nucleophilic sites on DNA, often leading to DNA strand scission.[16] Various "α-effect" nucleophiles, such as hydrazine, hydroxylamine, and methoxylamine, damage DNA through attack at electrophilic sites on the bases[17] and generation of oxygen radicals.[17] Hydrodisulfides formed in the reaction of thiols with 1,2-dithiolan-3-one 1-oxides might damage DNA by a similar mechanism.

It is clear that, similar to certain enediyne antibiotics,[18] thiol-mediated activation of the DNA-cleaving antibiotic leinamycin involves complex and interesting chemistry. We are conducting further mechanistic studies on these and related model reactions and exploring the reaction of **3**, **4**, and other simple 1,2-dithiolan-3-one 1-oxides with DNA.

Experimental Section

All chemicals were purchased from Aldrich Chemical Co. unless otherwise noted. TLC was performed on silica gel plates (0.25 mm) with F_{254} fluorophore (Merck). Visualization of compounds was achieved with UV light or phosphomolybdic acid staining. Column chromatography was performed using 230–400 mesh silica gel (Merck) with technical grade solvents that were distilled prior to use. Methylene chloride and triethylamine were distilled from CaH_2 prior to use. Gas chromatography–mass spectrometry (GCMS) experiments employed a Hewlett-Packard Model 5890A gas chromatograph in tandem with a Hewlett-Packard Model 5890B detector. High-resolution mass spectrometry was performed at the Midwest Center for Mass Spectrometry (University of Nebraska–Lincoln).

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Reaction of *n*-Propanethiol with 3*H*-1,2-Benzodithiol-3-one 1-Oxide (3). In a typical procedure, **3**[3] (65 mg, 0.35 mmol) was dissolved in dry methylene chloride (3 mL) with stirring at room temperature under nitrogen gas. To this solution was added *n*-propanethiol (0.32 mL, 3.5 mmol, 10 equiv) followed by dry triethylamine (5 μL, 0.03 mmol, 0.1 equiv). When all starting material was consumed (<2 h), the solvent was evaporated under a stream of nitrogen, the resulting residue resuspended in ethyl acetate, the suspension filtered to remove insoluble material, and the resulting ethyl acetate soluble mixture separated by column chromatography on silica gel (0–100% ethyl acetate–hexane). An inseparable mixture of nonpolar products (16 mg) isolated from the column was characterized by GCMS and identified as the disulfide **7** (M^+ *m/e* 150), trisulfide **8** (M^+ *m/e* 182), and the tetrasulfide **9** (M^+ *m/e* 214). High-resolution mass spectrometry of the polysulfide mixture confirmed the presence of the disulfide **7**, trisulfide **8**, and tetrasulfide **9**. HRMS (EI; *m/e*): calcd for **7** ($C_6H_{14}S_2$, M^+) 150.0538, found 150.053, calcd for **8** ($C_6H_{14}S_3$, M^+) 182.0259, found 182.0255, calcd for **9** ($C_6H_{14}S_4$, M^+) 213.9980, found 213.9981.

Column chromatography of the reaction mixture using $CHCl_3$–methanol (95:5) allows isolation of a relatively polar compound, *o*-(propyldithio)benzoic acid (**5**; 23 mg, 29%): ^1H NMR (500 MHz, $CDCl_3$) δ 10.93 (br s, 1H), 8.23 (dd, J = 8 Hz, 0.8 Hz, 1H), 8.15 (dd, J = 8 Hz, 0.8 Hz, 1H), 7.61 (dt, J = 8, 1.6 Hz, 1H), 7.27 (t, J = 8 Hz, 1H), 2.70 (t, J = 7 Hz, 2H), 1.71 (sextet, J = 7 Hz, 2H), 1.00 (t, J = 7 Hz, 3H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 171.6, 143.2, 133.6, 132.5, 125.8, 125.7, 125.1, 40.4, 22.4, 13.2; IR (CCl_4) 3000 (br), 2973, 1697, 1558, 1461, 1416, 1275 cm^{-1}; HRMS (EI; *m/e*) calcd for $C_{10}H_{12}O_2S_2$ (M^+) 228.0280, found 228.0279. The spectral data match those for material prepared by the method of Field and Giles.[19]

A product of intermediate polarity on TLC relative to **7**, **8**, **9**, and **5** was isolated from the column and identified as 3*H*-1,2-benzodithiol-3-one[3] (**6**, 9 mg, 15%): ^1H NMR (500 MHz, $CDCl_3$) δ 7.97–7.40 (m, 4H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 193.5, 148.2, 133.4, 129.1, 127.3, 125.6, 124.6; HRMS (EI; *m/e*) calcd for $C_7H_4OS_2$ (M^+) 167.9704, found 167.9703.

Interestingly, when only 1 equiv of *n*-propanethiol (based on moles of **3**) is employed in this reaction, a product inseparable from **5**, but whose exact mass and NMR are consistent with that expected for *o*-(propyltrithio)benzoic acid is observed. This product is suggestive of attack of propyl hydrodisulfide (**11**) on the putative oxathiolanone intermediate (**10**): HRMS (EI; *m/e*) calcd for $C_{10}H_{12}O_2S_3$ (M^+) 260.0001, found 259.9993.

The remainder of the mass balance in these reactions is obtained as a virtually insoluble precipitate that is extremely polar as judged by TLC. Degassing the reaction mixture by bubbling prepurified nitrogen gas through the solution for 15 min prior to adding the thiol has no significant effect on the reaction rate or products.

5,5-Dimethyl-1,2-dithiolan-3-one 1-Oxide (4). To 5,5-dimethyl-1,2-dithiolan-3-one[6c] (220 mg, 1.5 mmol) in acetic acid (1.6 mL) was added 30% hydrogen peroxide (0.2 mL). The resulting solution was stirred for 45 min at 40 °C. The reaction mixture was poured into cold water (10 mL) and extracted twice with chloroform (10 mL). The organic layer was washed three times with water and dried ($MgSO_4$) and the solvent removed under vacuum. The material was purified by silica gel column chromatography (0–50% ethyl acetate–hexane) to yield 5,5-dimethyl-1,2-dithiolan-3-one 1-oxide (**4**; 220 mg, 89%) as a tan oil: ^1H NMR (500 MHz, $CDCl_3$) δ 3.45 (d, J = 17 Hz, 1H), 2.90 (d, J = 17 Hz, 1H), 1.61 (s, 3H), 1.46 (s, 3H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 200.8, 64.3, 49.3, 21.9, 21.7; IR (neat) 1726, 1091 cm^{-1}; HRMS (EI; *m/e*) calcd for $C_5H_8O_2S_2$ (M^+) 164.2373, found 163.9968.

Reaction of *n*-Propanethiol with 5,5-Dimethyl-1,2-dithiolan-3-one 1-Oxide (4). To 5,5-dimethyl-1,2-dithiolan-3-one 1-oxide (**4**; 60 mg, 0.37 mmol) and triethylamine (26 μL, 0.18 mmol) in methylene chloride (1.5 mL) was added *n*-propanethiol (332 μL, 3.7 mmol). The resulting mixture was stirred for 4 h at room temperature under a nitrogen atmosphere. The solvent was evaporated under a stream of nitrogen gas, and the products were isolated by column chromatography on silica gel (0–100% ethyl acetate–hexane followed by 0–5% methanol–methylene chloride). The dithio carboxylic acid product **12** was obtained as a tan oil (38 mg, 50%): ^1H NMR (500 MHz, $CDCl_3$) δ 2.71 (t, J = 7 Hz, 2H), 2.69 (s, 2H), 1.69 (sextet, J = 7 Hz, 2H), 1.46 (s, 6H), 0.99 (t, J = 7 Hz, 3H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 175.1, 48.4, 45.6, 42.8, 27.3, 22.7, 13.1; IR ($CHCl_3$) 3104 br, 2966, 2933, 2867, 1717, 1460, 1415 cm^{-1}; HRMS (EI; *m/e*) calcd for $C_8H_{16}O_2S_2$ (M^+) 208.0593 found 208.0599.

Similar to the reaction of **3** with thiols, a small amount of reduced (*S*-deoxy) heterocycle is observed as a product of this reaction and the remainder of the mass balance is found as polar material. Efforts are currently underway to identify these potentially important polar products.

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Supplementary Material Available: Figures giving ^1H and ^{13}C NMR spectra of compounds **4** and **12** (5 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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