acid, was slowly bubbled for 4 h. Subsequent TLC analysis in system B showed small amounts (approximately 10% each) of PE2R and PE1B as well as unreacted **3**.

Nonreductive Reaction of Mitomycin C with Potassium Ethyl Xanthate. To a solution of mitomycin C (100 mg, 0.299 mmol) in Tris·HCl buffer (0.05 M, pH 7.4, 100 mL) was added successively Na_2SO_3 (2.0 g, 1.585 mmol) and an aqueous potassium ethyl xanthate solution (0.05 M, 70 ml, 3.5 mmol). The reaction mixture was kept in a refrigerator at 5 °C for 100 h and then extracted with ethyl acetate (100 mL) eight times. The extract was washed with H_2O. The solvent was removed in vacuo at room temperature to give a dark green-brown residue (149 mg), which was subjected to HPLPLC on a column (30 × 2.5 cm) packed with Whatman LPS-1 silica gel. Compound **5** was isolated by elution with ethyl acetate/$CHCl_3$ (2:1, 15 psi, 7 mL/min UV monitor 310 nm) as a dark violet amorphous substance (28 mg). Unreacted mitomycin C (40 mg) was recovered by elution with acetone. Compound **5** was also formed in fair yield when the molar ratio of 1, Na_2SO_3, and KSC(=S) OC_2H_5 was 1:10:10, however the amount of the minor products was reduced. Only very little **5** was obtained when the reactants were used in 1:1:1 molar ratio.

The homogeneity of **5** was shown by TLC in system C and by the ^1H and ^{13}C NMR spectra. No satisfactory combustion analysis data were obtained because of the decomposition of **5** during drying, however the molecular formula, $C_{18}H_{22}N_4O_6S$, of **5** as determined by the FD-MS spectrum [*m/e* 422 (M^+, relative intensity 92%), 399 (M – MeOH, 100%), and 309 (M – HOCO-NH_2, 6%)] was substantiated by the 360-MHz ^1H NMR spectrum showing signals due to 22 protons and by the ^{13}C NMR spectrum showing signals due to 18 carbons.

5: mp >300 °C, darkening at 208–210 °C; $[\alpha]^{20}_D$ 297° (*c* 0.049, CH_3OH); UV $\lambda_{max}^{CH_3OH}$ nm (ϵ) 253 (25 100), 259 sh (13 000), 356 (27 500).

Treatment of 5 with Hydrogen over Palladium on Carbon To Form 6. To a solution of **5** (13 mg) in ethyl acetate (10 mL) was added 10% Pd on carbon (10 mg). Hydrogen was vigorously bubbled through the mixture at room temperature for 2 h, and then oxygen was passed through the mixture for 5 min. The reaction mixture was filtered and the filtrate was concentrated to dryness (in vacuo), giving a reddish brown residue (9 mg). This was subjected to HPLPLC under conditions similar to those used in the isolation of **5**, and the reaction product (**6**) was obtained as violet needles (1.5 mg). A satisfactory combustion analysis was not obtained, but the molecular formula ($C_{17}H_{18}N_4O_5S$) for **6** could be deduced from the FD-MS spectrum [*m/z* 390 (M^+, relative intensity 100%)] and the NMR spectrum.

6: mp 203–205 °C, $[\alpha]^{20}_D$ +280° (*c* 0.015, CH_3OH); UV $\lambda_{max}^{CH_3OH}$ nm (ϵ), 255 (14 100), 309 (8 300), 345 (3 900).

Acknowledgment. We gratefully acknowledge Dr. W. T. Bradner, Bristol Laboratories, Syracuse, NY, for a gift of mitomycin C and for antitumor testing; J. L. Occolowitz, Eli Lilly Co., Indianapolis, IN for FD mass spectra; Drs. M. Cushman, M. Loudon, and S. Byrn of this department for helpful discussions; use of the NTC-200 and NTC-360 NMR instruments of the Purdue University Biochemical Magnetic Resonance Laboratory (PUBMRL) located in the Department of Chemistry, and supported by NIH, Research Grant RR01077 from the Division of Research Resources; as well as financial support through Research Grant CA25685 from the National Cancer Institute.

Registry No. **1**, 50-07-7; **2a**, 72565-69-6; **2b**, 72565-68-5; **3**, 82264-86-6; **4**, 87495-12-3; **5**, 87495-13-4; **6**, 82246-90-0; potassium ethyl xanthate, 140-89-6.

Studies on the Reaction of Mitomycin C with Potassium Ethyl Monothiocarbonate under Reductive Conditions

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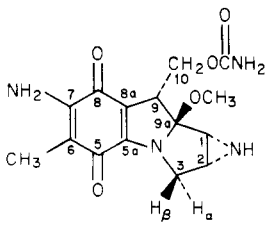
Treatment of mitomycin C (**1**) with the ambident nucleophile potassium ethyl monothiocarbonate (**2**) under reductive conditions (sodium dithionite) at approximately neutral pH at room temperature led to the formation of equivalent amounts of *trans*- (**17**) and *cis*- (**18**) aziridine ring-opened disubstituted mitosene adducts. In both cases substitution at carbons 1 and 10 proceeded with sulfur attack. The structural identity of each product was confirmed by high-field ^1H and ^{13}C NMR spectral analysis as well as by chemical studies. Milder conditions (0–5 °C) led to the isolation of both *trans*- (**22**) and *cis*- (**23**) aziridine ring-opened monosubstituted adducts. Compounds **22** and **23** were converted to the corresponding disubstituted products by treatment with additional **2** and sodium dithionite. The implications of these reactions in relation to the mode of action of mitomycin C (**1**) are discussed.

Mitomycin C (**1**) is a clinically useful antineoplastic antibiotic compound.[2] Although extensive studies indicate that the alkylation of DNA by mitomycin C (**1**) is the primary biological event, the mechanism of action of this drug is poorly understood.[3] A series of mechanisms has been advanced that invokes the participation of both the aziridine and the carbamate moieties in **1**.[4] These sites



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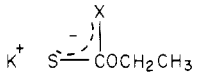
have been considered likely centers for nucleophilic attack.

The reaction of mitomycins with nucleophiles has been reported.[5-13] Special emphasis was placed on the stereochemistry of the aziridine ring-opened products. A surprising result in many of these studies was the predominance of the cis-substituted ring-opened aziridine adducts.[5-8] This finding is diametrically opposed to the observation that simple aziridines with two secondary carbons undergo exclusive ring opening with inversion at the reaction site.[14]

In this paper, we describe the reaction of mitomycin C (1) with the ambident nucleophile, potassium ethyl monothiocarbonate[15] (2), under conditions which may mimic



2, X = O
3, X = S

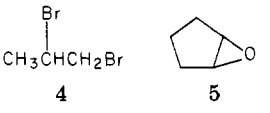
the in vivo process.[3] Multifunctional reagents of this type are suggested as useful aids in mechanistic studies involving mitomycin C (1). Accordingly, treatment of 1 with 2 under reductive conditions at approximately neutral pH led to the isolation of equivalent amounts of cis- and trans-disubstituted products. Milder conditions produced monosubstituted adducts in which the aziridine ring in 1 was selectively cleaved. In all cases, only sulfur-substituted products were obtained. These studies have provided information concerning both the initial reaction site in mitomycin C (1) as well as the mechanism of these transformations.

Results and Discussion

The use of bifunctional reagents in mechanistic studies of mitomycin C (1) has several advantages. First, since DNA contains many ambident nucleophiles, reaction of 1 with a multifunctional reagent may better mimic the drug–receptor site interaction. Second, selective replacement of one of the heteroatoms comprising a resonance stabilized anion allows the fine tuning of the nucleophilicity of the reagent. This alteration may aid in the differentiation of sites (i.e., C-1, C-10, C-9a[9]) in mitomycin C (1) which are susceptible to nucleophilic attack. Third, ambident nucleophiles may function as sensitive diagnostic probes to distinguish initially formed products from those that arise after subsequent rearrangement of the primary adducts.

Initially we chose potassium ethyl monothiocarbonate (2) as the nucleophile. This reagent is similar to potassium ethyl xanthate (3) which has been used by Hornemann and co-workers in their mechanistic studies of mitomycin C (1).[10,16] The replacement of one of the sulfur atoms by oxygen in 3 is expected to lead to a reduction in the nucleophilicity of this reagent.

Model Studies. Addition of electrophilic reagents to 2 can in principle lead to both oxygen- and sulfur-substituted products. We have assessed this difference in reactivity by treatment of 2 with 1,2-dibromopropane (4), cyclopentene oxide (5), 1,2-epoxybutane (6), and triethyloxonium tetrafluoroborate (7), respectively.



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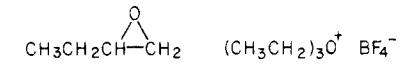

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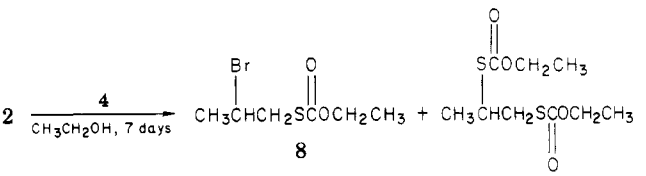
Introduction of 4 into an ethanolic solution containing 2.6 equiv of 2 (55 °C, 7 days) led to the isolation of the mono- (8) and disubstituted (9) products.[17] The observed



8 9

spectral properties for both compounds were consistent with the proposed structures. Of note, the infrared spectra exhibited a characteristic carbonyl absorption at 1720 cm^{-1}. Examination of the ^1H NMR spectra for both compounds revealed that the methylene hydrogens for the propane chain absorbed in the δ 2.95–3.60 region. Moreover, in the ^{13}C NMR spectra the chemical shift values for the corresponding carbon atoms appeared between 37.5–40.8 ppm. These values are consistent with sulfur substitution. Significantly lower field chemical shift values for these absorptions would be expected for the corresponding oxygen-substituted isomer.[18,19] Analysis of the chemical shift values for both the methine hydrogen and carbon atom in the disubstituted adduct 9 led to a similar conclusion. Furthermore, in both 8 and 9 diagnostic downfield signals between 169.8–170.3 ppm for the carbonyl carbons were observed.

Treatment of 2 with an equimolar amount of cyclopentene oxide (5) in a citric acid-buffered ethanolic solution led to the isolation of the ring-opened adduct 10 and a diastereomeric mixture of the disulfide 11. Both sets of products arise from initial sulfur substitution. The trans geometry indicated for 10 is tentative.

Evidence in support of the formation of the diastereomeric disulfides 11 was secured by an independent synthesis of this mixture. Cyclopentene oxide (5) was con-

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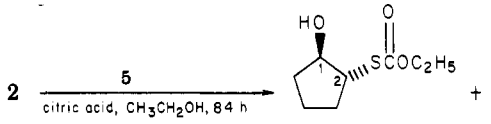

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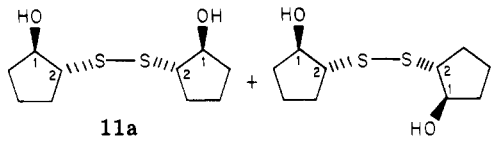
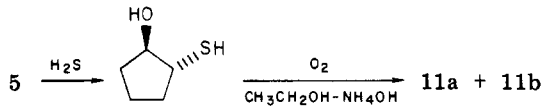


11a 11b

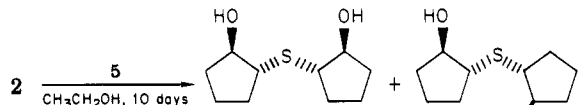
verted to *trans*-2-mercaptocyclopentanol (12) using the



12

procedure of Goodman, Benitez, and Baker.[20] Oxidation[21] of the thiol gave a diastereomeric mixture of bis(2-hydroxycyclopentyl) disulfide (11) which was identical by ^1H and ^{13}C NMR analysis to that obtained from the potassium ethyl monothiocarbonate (2) reaction. Significantly, the proton decoupled ^{13}C NMR spectrum for 11 contained two sets of five lines of nearly equal intensities.

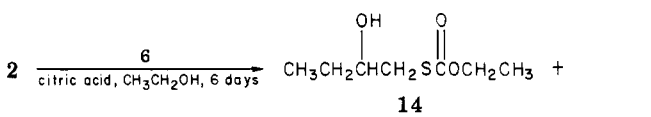
Omission of the citric acid buffer in the reaction of 2 with 5 led to a diastereomeric mixture of bis(2-hydroxycyclopentyl) sulfides[20] (13) as the only isolable product.



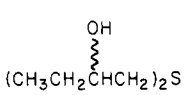
13a 13b

Apparently, under these conditions, solvolysis of the monothiocarbonate moiety readily occurs leading to the eventual formation of the sulfide 13.

The products obtained from the treatment of 1,2-epoxybutane (6) and 2 mirrored those isolated from the cyclopentene oxide (5) reaction. After workup, a complex mixture (>4 compounds by TLC analysis) was obtained. Preparative chromatography led to the isolation of two sets of compounds. The major adduct was identified as 14. An additional product produced in this reaction was the 1:1 diastereomeric mixture of sulfides 15.

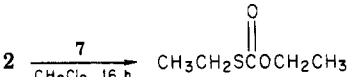


14



15

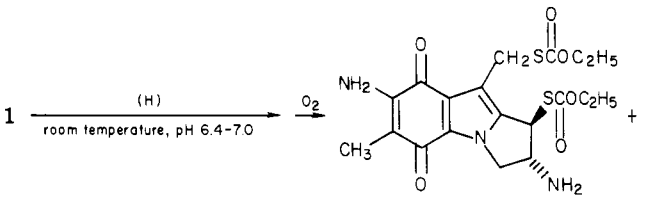
The final alkylating agent examined was triethyloxonium tetrafluoroborate (7). Addition of an equimolar amount of this Meerwein salt to a dichloromethane slurry of 2 led to the high yield formation of ethyl monothiocarbonate[22] (16). The spectral properties for 16 were in agreement with those previously observed in this study.



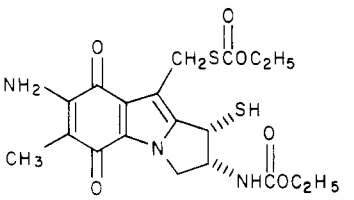
16

In each of the model reactions, no evidence was obtained for the formation of oxygen-substituted compounds. The reaction proceeded at sulfur regardless of the reactivity of the alkylating agent. These results suggest that treatment of mitomycin C (1) with 2 should lead to the initial formation of sulfur-substituted compounds.

Reaction of Mitomycin C (1) with Potassium Ethyl Monothiocarbonate (2). Room Temperature Reactions. To a deoxygenated aqueous solution of mitomycin C (1) and potassium ethyl monothiocarbonate (2) was added a freshly prepared aqueous sodium dithionite solution at room temperature. A violet precipitate formed shortly after the dithionite addition. After 10 min under these reductive conditions, the reaction was quenched with oxygen. The pH of the solution during the reaction remained between 6.4–7.0. TLC analysis of the organic layer after extraction with ethyl acetate indicated the presence of two major compounds (17 and 18). This mixture was purified by thick-layer chromatography. Equal quantities of 17 and 18 were recovered. The structures of 17 and 18 were elucidated with the aid of mass (field-desorption and thermospray-ionization[23]), ultraviolet, infrared, and high-field ^1H (Table I) and ^{13}C (Table II) NMR spectroscopy. The field dispersion and resolution in the ^1H NMR spectra allowed the determination of most chemical shifts and proton–proton coupling constants for 17 and 18. Homo-



17



18

nuclear decoupling experiments aided in the assignment of the remaining chemical shifts and coupling constants. In the case of 18, the values for the coupling constants comprising the doublet of doublets at δ 4.79 were refined by computer simulation studies.

Several key spectral properties for 17 and 18 coupled with chemical studies permitted the complete structural elucidation of these adducts. Both compounds gave protonated molecular ion peaks at m/e 454 by field-desorption and thermospray-ionization mass spectrometry. This value is in agreement with the molecular formula, $C_{19}H_{23}N_3O_6S_2$, for a disubstituted adduct formed by loss of the carbamate group and cleavage of the aziridine ring. In agreement with this finding, the infrared spectra for both adducts contained a strong absorption at 1715–1725 cm^{-1}, characteristic for a thiocarbonate residue.

The presence of two triplets in the upfield region (δ 1.26–1.37) in the ^1H NMR spectrum of 17 confirmed the

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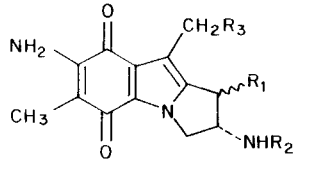
Table I. ^{1}H NMR Assignments of Mitomycin C Derivatives[a]



compd	R^1	R^2	R^3	C_1H	C_2H	C_3H_α	C_3H_β	$C_{10}H_2$	C_6CH_3	OCH_2CH_3	OCH_2-CH_3	C_2NH
17[b,c]	$SCOOCH_2CH_3$	H	$SCOOCH_2CH_3$	4.67; d; 1.9	4.17–4.20; m	4.08; d, d; 2.4, 13.3	4.48; d, d; 6.2, 13.3	4.15; d, ABq; 13.9; 1 H 4.27; d, ABq; 13.9; 1 H	1.83; s	4.25; q; 7.4; 2 H 4.335; q; 7.3; 1 H 4.340; q; 7.3; 1 H	1.28; t; 7.2 1.35; t; 7.3	
20[b]	$SCOOCH_2CH_3$	$COOCH_2CH_3$	$SCOOCH_2CH_3$	4.91; d; 3.0	4.76–4.79; m	4.25; d, d;[d] 2.0,[e] 13.4	4.57; d, d; 5.8, 13.4	4.08; d, ABq; 13.9; 1 H 4.26; d, ABq; 13.9; 1 H	1.82; s	4.13; br q; 7.0; 2 H 4.24; q; 7.3; 2 H 4.34; q; 7.2; 2 H	1.23; t; 7.1 1.27; t; 7.3 1.33; t; 7.2	5.33; br m; 6.3[e]
22[b,f,g]	$SCOOCH_2CH_3$	H	$OCONH_2$	4.60; d; 2.0	4.17–4.20; m	4.10; d, d; 2.5, 13.3	4.47; d, d; 6.1, 13.3	5.26; s	1.84; s	4.34; q; 7.2; 2 H	1.34; t; 7.2	
18[h,i]	SH	$COOCH_2CH_3$	$SCOOCH_2CH_3$	4.79; d, d; 6.6, 7.2[e]	4.99–5.03; m	3.77; d, d; 9.2, 12.6	4.66; d, d; 7.6, 12.6	4.15; d, ABq; 14.1; 1 H 4.27; d, ABq; 14.1; 1 H	1.80; s	4.23; q; 7.1; 1 H 4.24; q; 7.1; 1 H 4.25; q; 7.1; 1 H 4.26; q; 7.1; 1 H	1.26; t; 7.1 1.27; t; 7.1	5.54; br d; 8.6
19[i]	$SCOOCH_2CH_3$	$COOCH_2CH_3$	SH	4.93; d; 6.6	5.17–5.21; m	3.94; d, d;[d] 6.0,[e] 12.9	4.70; d, d; 8.1, 12.9	3.91; d, ABq;[d] 14.1; 1 H 3.96; d, ABq; 14.1; 1 H	1.82; s	4.22; q; 7.2; 1 H 4.23; q; 7.2; 1 H 4.28; q; 7.1; 1 H 4.29; q; 7.3; 1 H	1.28; t; 7.3 1.29; t; 7.2	5.89; br d; 8.3[e]
21[h,i]	$SCOOCH_2CH_3$	$COOCH_2CH_3$	$SCOOCH_2CH_3$	5.18; d; 6.9	5.21–5.25; m	3.83; d, d; 8.3, 12.7	4.68; d, d; 7.4, 12.7	4.15; d, ABq; 13.9; 1 H 4.25; d, ABq; 13.9; 1 H	1.80; s	4.12–4.19;[d] 2 H 4.240; q; 7.2; 1 H 4.245; q; 7.1; 1 H 4.28; q; 7.2; 1 H 4.31; q; 7.2; 1 H	1.25; t[d] 1.26; t; 7.2 1.30; t; 7.2	5.30; br d; 8.3
23[f,i]	SH	$COOCH_2CH_3$	$OCONH_2$	5.11; d; 6.4	5.12–5.19; m	4.06; d, d;[d] 9.2,[e] 12.5	4.62; d, d; 7.9, 12.5	5.28; d, ABq; 12.8; 1 H 5.33; d, ABq; 12.8; 1 H	1.82; s	4.03–4.09;[d] 2 H	1.12; t; 7.0	6.95; br d; 6.70

[a] The number in each entry is the chemical shift value (δ) observed in ppm relative to Me_4Si, followed by the multiplicity of the signal and the coupling constant(s) in Hz. In select cases, the number of hydrogens per signal is indicated. Except where noted all spectra were recorded at 360.1 MHz, and the solvent used was $CDCl_3$. [b] The values reported are for the trans isomer. [c] The signal for the C_2NH_2 protons was located at δ 4.90 (br s). [d] The signal was partially hidden by another signal of similar chemical shift value. [e] The coupling constant was derived or refined by computer simulation. [f] The solvent used was acetone-d_6. [g] The signal for the C_2NH_2 protons was located at δ 4.92 (br s). [h] The spectrum was recorded at 400.1 MHz. [i] The values reported are for the cis isomer.

Table II. 100.6-MHz ^{13}C NMR Assignments of Mitomycin C Derivatives[a]

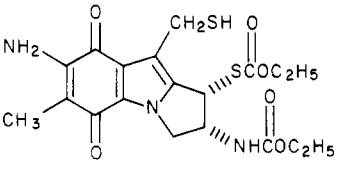
assignment	17	18	19
C_1	53.82[b]	38.01	49.66
C_2	63.41[b]	55.60	56.28
C_3	49.26	49.19	49.33
C_{5a}	145.74[c]	145.76[c]	145.78[c]
C_5	178.18[d]	178.35[d]	178.24[d]
C_6	107.59[c]	107.79[c]	108.07[c]
C_{6a}	7.93	8.01	8.06
C_7	135.45[c]	139.33[c]	135.61[c]
C_8	177.28[d]	177.28[d]	177.10[d]
C_{8a}	115.15[c]	114.41[c]	116.85[c]
C_9	122.39[c]	121.80[c]	121.72[c]
C_{9a}	129.19[c]	129[c,e]	129.41[c]
C_{10}	25.11	25.12	25.21
C_{10}-SC=O	170.89[f]	171.17	
C_1SC=O	169.61[f]		171.30
C_2NHC=O		156[e]	156.07
OCH_2CH_3	64.12	63.66	63.79
OCH_2CH_3	64.81	61.64	61.65
OCH_2CH_3	14.48	14.52	14.61
OCH_2CH_3	14.19	14.30	14.26

[a] The number in each entry is the chemical shift value (δ) observed in ppm relative to Me_4Si. The solvent used was $CDCl_3$. [b,c,d,f] The assignments are tentative and may be interchanged within each class. [e] The signal observed for this resonance was of low intensity. The approximate chemical shift value is reported.

presence of two ethyl monothiocarbonate residues. The corresponding methylene hydrogens for these ethyl residues were located between δ 4.23 and 4.37. Since three distinct quartets were observed, the protons in one of these ethyl groups are diastereotopic. This phenomenon was a frequent occurrence in many of the mitosenes obtained in this study. The methylene protons at carbon-10 were centered at δ 4.21 and appeared as an AB quartet. The relatively high-field chemical shift position for these protons provided evidence for sulfur substitution at this site. Similar analysis of the chemical shift values for the carbon-1 and 2 methine hydrogens indicated that a sulfur substituted ethyl monothiocarbonate and a free amine group were present at these two positions, respectively.[18] The stereochemistry at carbon-1 in **17** was assigned by application of a Karplussian analysis similar to that described in the preceding paper.[16] The proton–proton coupling constants of the pyrrolidine ring indicated that the ethyl monothiocarbonate and the amino groups are trans to one another. In support of this, we note a small coupling between C_2H–C_3H_α, a large coupling between C_2H–C_3H_β, and a small coupling constant between C_2H–C_1H. The proton decoupled ^{13}C NMR spectrum for **17** contained the requisite 19 lines. Key signals included the peaks at 169.61 and 170.89 ppm for the carbonyl carbons of the two ethyl monothiocarbonate residues and the signals at 25.11 and 49.26 ppm for carbons 10 and 1, respectively. The relatively high field chemical shift values for these last two peaks provided additional evidence that sulfur substitution had occurred at both sites.[19]

The second compound isolated from the room temperature reaction was the cis isomer **18**. Techniques used for the structural elucidation of **18** were similar to those described for **17**. In accord with the proposed structure, an absorption at 2405 cm^{-1} in the infrared spectra for the thiol moiety was noted. Moreover, the chemical shift and multiplicity of the apparent doublet of doublets for the carbon-1 methine proton at δ 4.79 in the ^1H NMR spectrum indicated the presence of a free thiol group at this position. The signal for the carbon-2 hydrogen was considerably downfield (δ 4.99–5.03) from that observed in **17**. This shift was an indication of acylation of the amino group during either the reaction or the workup.[18] Finally, the resonances for the carbon-10 methylene hydrogens in **17** and **18** appeared at the same position. Analysis of the coupling constants for the pyrrolidine ring did not permit the determination of the stereochemistry at carbon-1[16] because large proton–proton coupling constants were observed for C_3H_α–C_2H, C_3H_β–C_2H, and C_2H–C_1H. Confirmation of the proposed cis stereochemistry was secured by chemical studies discussed later. Significant features in the ^{13}C NMR spectrum included the appearance of signals at 25.12, 38.01, 156, and 171.17 ppm. These peaks have been assigned to carbon-10, 1, and the carbonyl carbons on the carbamate and thiocarbonate residues, respectively.

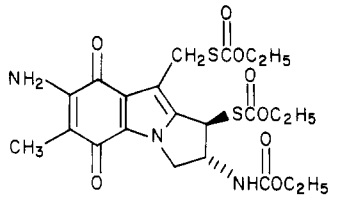
Compound **18** undergoes appreciable rearrangement within days both in the solid state and in solution. The new adduct formed has been assigned structure **19**. At



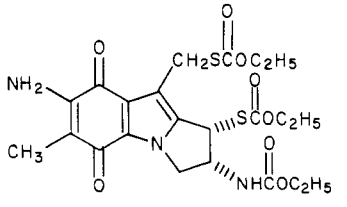
19

this time, we cannot rule out the corresponding carbon-10 sulfide or disulfide adduct. Both field desorption and thermospray ionization mass spectrometry suggested that this compound is isomeric with **18**. An absorption in the infrared spectrum at 2405 cm^{-1} was again noted for the thiol group. The ^1H NMR spectrum for **19** was similar to that observed for **18**. Noticeable points of difference, however, included the appearance of only a doublet pattern at δ 4.93 for the carbon-1 methine hydrogen and the alteration of the AB quartet for the carbon-10 methylene hydrogens. The ^{13}C NMR spectrum was consistent with the proposed structure.[19,24] Whether the conversion of **18** to **19** proceeded by an intra- or intermolecular process is undetermined at this time. This transformation has not been previously observed in the mitosenes.

An important series of reactions confirming the stereochemistry of the aziridine ring-opened products **17** and **18** was the conversion of each of these compounds to the trisubstituted adducts **20** and **21**, respectively. As expected, **17** readily underwent acylation at room temperature with ethyl chloroformate,[25] while **18** required elevated



20



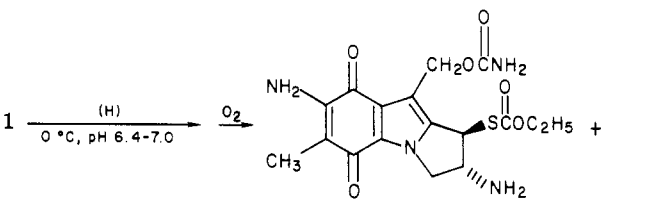
21

(24) The signal cited for the carbamate carbonyl carbon (156.07 ppm) is tentative.

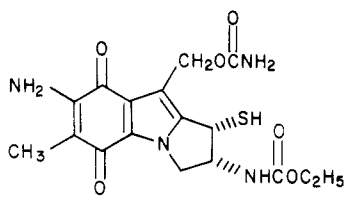
(25) We observed significant formation of **20** along with other products from samples of **17** that were stored in the solid state (few weeks).

temperatures. We were unable to convert **19** to **21**. Use of higher temperatures (60 °C) led to extensive decomposition of the starting material. The mass, infrared, and ^{1}H NMR spectral properties for **20** and **21** are in accord with the proposed structures. Of particular note was the nearly identical proton–proton coupling pattern that was observed for the protons in the pyrrolidine ring in each of the two series of compounds **17**, **20** and **18**, **21**. The formation of two isomeric trisubstituted adducts in these acylation reactions assurred that initial ring opening of the aziridine ring in **1** with potassium ethyl monothiocarbonate (**2**) proceeded with the formation of both the cis- and the trans-substituted products.

0–5 °C Reactions. The reactions of mitomycin C (**1**) with **2** performed at room temperature led to disubstituted adducts in which the aziridine ring was cleaved at carbon-1 and the carbamate group was displaced. To discern which of these two sites was more susceptible to substitution we conducted this reaction at lower temperatures (0–5 °C). The overall yield for the conversion of starting material to products was considerably lower under these conditions. The ethyl acetate layer after extractive workup showed the presence (TLC analysis) of two major products (**22** and **23**), three adducts present in trace amounts as well as unreacted starting material (**1**). Identification of **22** and **23**



22



23

was made on the basis of the observed infrared and ^{1}H NMR spectral data. The ^{1}H NMR spectra obtained for **22** and **23** corresponded well with those obtained for **17** and **18**, respectively. The major difference was the change in chemical shift value for the carbon-10 methylene group. The resonance for these protons appeared in **22** as a singlet at δ 5.26 and in **23** as an AB quartet centered at δ 5.31. Further structural proof for **22** and **23** was provided by the conversion of these compounds to **17** and **19**, respectively. These reactions were run under nearly identical conditions to those described in the room temperature procedure (sodium dithionite, EtOH–H_2O, excess **2**). The ^{1}H NMR spectra obtained for **17** and **19** in these reactions were identical with those previously observed.

Conclusions

Detailed analysis of the ^{1}H NMR spectra along with the chemical studies have permitted the determination of the stereochemistry of a series of ring-opened mitosenes. This information has greatly aided the mechanistic interpretation of these reactions. Several important features of these transformations include the following. First, both the carbon-1 and 10 positions in mitomycins readily undergo substitution at room temperature under reductive conditions. Discrimination between these two sites was made possible only by reducing the temperature of the reaction. Under these milder conditions, preferential substitution at carbon-1 occurred. Second, the carbamate group in mitosenes (i.e., **22** and **23**) was readily displaced under reductive conditions. Third, the isomeric oxygen-substituted ethyl monothiocarbonate adducts were not observed. The products obtained in this study were compatible with direct sulfur substitution at carbon-1 and 10. Fourth, both the cis and the trans ring-opened aziridine products (**17** and **18**) were formed in nearly equal amounts at room temperature.

The composite findings of the studies are in agreement with the concepts of bioreductive alkylation.[4] The formation of **22** and **23** as well as **17** and **18** (Scheme I) is consistent with the mechanism proposed by Moore and Czerniak.[4d] Of particular note, the cis/trans product distribution in these reactions argues that opening of the aziridine ring in mitomycin-based compounds does not occur principally by a S_N2 process under these conditions. Rather our results, as well as those of others,[10,16,26] suggest that ring cleavage proceeds by a S_N1 type pathway even at near neutral pHs. This process may be facilitated by ionization of the hydroxyl group at carbon-8 in **24**.[4d]

Experimental Section

General. Melting points were determined with a Thomas-Hoover melting point apparatus and are uncorrected. Infrared spectra (IR) were run on a Perkin-Elmer Model 237B spectrometer and calibrated against the 1601-cm^{-1} band of polystyrene. Ultraviolet spectra (UV) were obtained on a Hitachi Model 100-80 instrument. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on Varian Associates Models T60 and FT80A instruments as well as by Dr. Bruce Hawkins at the Department of Chemistry, Colorado State University, on a Nicolet NT-360 spectrometer, and by Helga Cohen at the Department of Chemistry, University of South Carolina, on a Bruker WH-400 instrument. Carbon-13 nuclear magnetic resonance (^{13}C NMR) spectra were determined on a Varian Associates FT80A and Bruker WH-400 spectrometers. Chemical shifts are in parts per million relative to Me_4Si, and coupling constants (J values) are in hertz. Low resolution mass spectral data were obtained at an ionizing voltage of 70 eV on a Hewlett-Packard 5930 gas chromatography–mass spectrometer. Thermospray-ionization mass spectrometry[23] was performed by Dr. Marvin Vestal at the Department of Chemistry, University of Houston. The samples were run in aqueous MeOH. Field-desorption mass spectrometry was run by Dr. Robert Cotter at the Department of Pharmacology, The Johns Hopkins University. High-resolution mass spectra were performed by Dr. James Hudson at the Department of Chemistry, University of Texas, on a CEC21-110B double-focusing magnetic-sector spectrometer at 70 eV. Exact masses were determined by peak matching. pH measurements were determined with a Radiometer pHM 26 meter. Elemental analyses were obtained at Spang Microanalytical Laboratories, Eagle Harbor, MI.

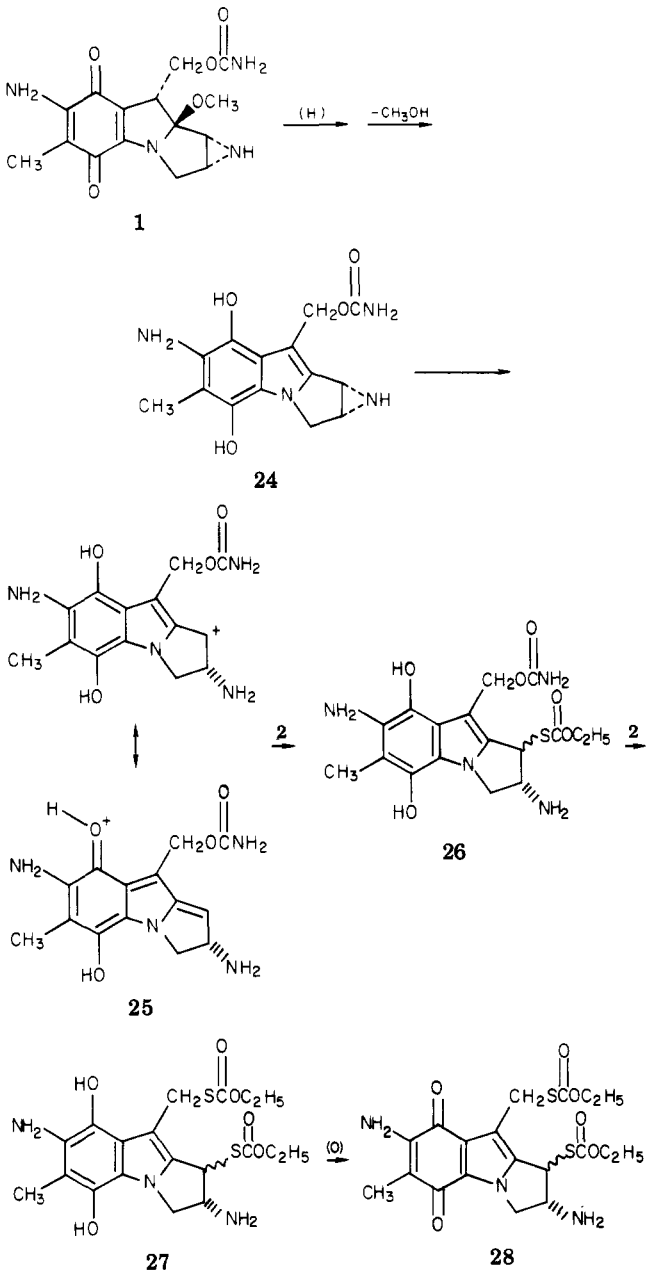
The solvents and reactants were of the best commercial grade available and used without further purification unless noted. When dry CH_2Cl_2 was required, the solvent was distilled from P_2O_5. All H_2O used for the mitomycin C reactions was doubly distilled and deionized.

All reaction were run under nitrogen. Thick-layer preparative chromatographies were run on plates prepared with Merck silica gel 60 PF-254. Thin layer chromatographic analyses were run on Merck precoated silica gel 60F-254 plates (5 × 10 cm).

Potassium Ethyl Monothiocarbonate (2). The procedure of Murphy and Winter[15a] was used to prepare **2**. Carbonyl sulfide was bubbled through a solution of aqueous 50% KOH (4.25 mL, 0.081 mol) and EtOH (7.50 mL, 0.135 mol) until a white, crystalline solid formed (~45 min). After filtering the reaction mixture to remove this solid (mp > 250 °C), addition of the filtrate to Et_2O (250 mL) resulted in the precipitation of the desired compound. The salt was collected, washed with Et_2O (2 × 50 mL), and dried

(26) Tomasz, M.; Lipman, R. *Biochemistry* **1981**, *20*, 5056–5061.

Scheme I



in vacuo: yield 4.39 g (38%); mp 185–187 °C dec (lit.[15b] mp 184.5 °C); IR (Nujol, NaCl) 1585, 1100, 1050, 685 cm^{-1} [15c]; ^1H NMR (Me_2SO-d_6) δ 1.04 (t, 3 H, J = 7 Hz), 3.78 (q, 2 H, J = 7 Hz); ^{13}C NMR (Me_2SO-d_6) 14.86, 59.55, 183.87 ppm.

Reaction of 1,2-Dibromopropane (4) with 2. To a stirred EtOH solution (250 mL) containing **2** (2.31 g, 0.016 mol) was added 1,2-dibromopropane (**4**) (1.26 g, 0.0062 mol). The solution was heated to 55 °C (84 h) and then filtered to remove a white solid. The filtrate was evaporated in vacuo to a pale yellow residue which was triturated with CH_2Cl_2 (2 × 25 mL). Concentration of the combined organic layers in vacuo gave a yellow oil. (The CH_2Cl_2 insoluble portion of the residue was identified as unreacted **2** by ^1H NMR). Bulb-to-bulb distillation of the oil afforded 0.40 g (28%) of colorless **8**: bp 28–30 °C (external temperature, 0.03 torr); IR (neat, NaCl) 2995, 1720, 1165 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.30 (t, 3 H, J = 6 Hz), 1.73 (d, 3 H, J = 6 Hz), 2.95–3.62 (m, 2 H), 3.48–4.45 (m, 3 H); ^{13}C NMR ($CDCl_3$) 14.22 (q, J = 128 Hz), 24.65 (q, J = 128 Hz), 40.76 (t, J = 149 Hz), 47.36 (d, J = 126 Hz), 64.00 (t, J = 149 Hz), 170.03 (s) ppm; mass spectrum, m/e (relative intensity) 149 (5), 148 (9), 147 (100), 146 (76), 119 (32), 75 (69), 74 (85), 73 (44), 61 (56); high-resolution mass spectrum, calcd for $C_6H_{11}BrO_2S$, m/e 225.9664; found, 225.9671.

The second fraction contained 0.27 g (17%) of colorless **9**: bp 44–45 °C (external temperature, 0.03 torr); IR (neat, NaCl) 2990, 2940, 1720, 1150 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.30 (t, 6 H, J = 8 Hz), 1.36 (d, 3 H, J = 4 Hz), 3.04–3.31 (m, 2 H), 3.38–3.82 (m, 1 H), 4.28 (q, 4 H, J = 7 Hz); ^{13}C NMR ($CDCl_3$) 14.26 (q, J = 128 Hz), 19.43 (q, J = 128 Hz), 37.52 (t, J = 144 Hz), 41.16 (d, J = 159 Hz), 63.48 (t, J = 153 Hz), 63.80 (t, J = 153 Hz), 169.76 (s), 170.33 (s) ppm. The intensity of the signal at 14.26 ppm was approximately double the intensity of the signal located at 19.43 ppm. Mass spectrum, m/e (relative intensity) 253 (0.2), 252 (1), 147 (100), 146 (85), 119 (14), 75 (30), 74 (31), 73 (15), 61 (20); high-resolution mass spectrum, calcd for $C_9H_{16}O_4S_2$, m/e 252.0490; found, 252.0494.

Reaction of Cyclopentene Oxide (5) with 2 and Citric Acid. Cyclopentene oxide (**5**) (1.35 g, 0.0159 mol) was added to an EtOH solution (150 mL) containing **2** (2.31 g, 0.0159 mol) and anhydrous citric acid (1.02 g, 0.0053 mol). The solution was stirred at room temperature (84 h), filtered, and the filtrate was evaporated in vacuo to a yellow solid. Trituration of the residue with CH_2Cl_2 (2 × 25 mL) followed by concentration of the combined organic layers in vacuo afforded a yellow oil. Bulb-to-bulb distillation of the oil gave 0.48 g (16%) of **10**: bp 32–35 °C (external temperature, 0.15 torr); IR (neat,NaCl) 3420, 2960, 2870, 1720, 1155 cm^{-1}; ^1H NMR (400.1 MHz) ($CDCl_3$) δ 1.30 (t, 3 H, J = 7.1 Hz), 1.46–1.55 (m, 1 H), 1.61–1.75 (m, 2 H), 1.76–1.86 (m, 1 H), 1.96–2.05 (m, 1 H), 2.17–2.25 (m, 1 H), 2.98 (d, 1 H, J = 2.3 Hz, exchanges with D_2O), 3.42–3.47 (ddd, 1 H, J = 4.5, 7.8, 8.2 Hz), 4.16–4.23 (m, 1 H), 4.27 (q, 2 H, J = 7.1 Hz); ^{13}C NMR ($CDCl_3$) 14.23 (q, J = 127 Hz), 22.88 (t, J = 133 Hz), 30.16 (t, J = 133 Hz), 33.71 (t, J = 138 Hz), 51.63 (d, J = 151 Hz), 63.68 (t, J = 146 Hz), 80.93 (d, J = 150 Hz), 172.26 (s) ppm; mass spectrum, m/e (relative intensity) 190 (1), 117 (32), 85 (27), 84 (100), 83 (34), 67 (33), 57 (21); high-resolution mass spectrum, calcd for $C_8H_{14}O_3S$, m/e 190.0664; found, 190.0668.

A higher boiling fraction contained a 1:1 mixture of stereoisomers of bis(2-hydroxycyclopentyl) disulfide (**11**): yield 0.05 g (3%); bp 65 °C (external temperature, 0.02 torr); ^{13}C NMR ($CDCl_3$) 21.65, 21.85, 30.19, 30.51, 33.04, 33.22, 56.57, 57.22, 78.31, 78.67 ppm.

***trans*-2-Mercaptocyclopentanol (12).** The title compound was prepared from cyclopentene oxide (**5**) (2.00 g, 0.0238 mol) and H_2S gas by using the procedure of Goodman, Benitez, and Baker[20]; yield 0.83 g (30%); bp 44–46 °C (external temperature, 0.6 torr) (lit.[20] bp 97–98 °C, 15 torr); IR (neat, NaCl) 3350, 2960, 2880, 2550, 1455, 1105 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.15–2.54 (m, 7 H), 3.02 (m, 1 H), 3.48 (s, 1 H, exchanges with D_2O), 4.04 (m, 1 H); ^{13}C NMR ($CDCl_3$) 20.68 (t, J = 134 Hz), 32.07 (t, J = 128 Hz), 33.21 (t, J = 135 Hz), 45.56 (d, J = 141 Hz), 81.45 (d, J = 145 Hz) ppm; mass spectrum, m/e (relative intensity) 118 (23), 100 (100), 85 (50), 83 (45), 67 (57), 57 (34), 56 (31), 55 (34).

Several higher boiling fractions, bp 82–95 °C (external temperature, 0.3 torr), were found to contain mixtures of bis(2-hydroxycyclopentyl) sulfides (**13**) and disulfides (**11**): ^{13}C NMR ($CDCl_3$) 21.31, 21.66, 21.83, 22.51, 30.25, 30.54, 31.71, 32.05, 33.13, 33.28, 33.67, 34.05, 50.47, 53.02, 56.56, 57.28, 78.52, 78.89, 80.02, 81.13 ppm.

Oxidation of 12 to Bis(2-hydroxycyclopentyl) Disulfide (11). Oxygen was bubbled (2 h) through a 95% aqueous EtOH (10 mL) solution containing **12** (0.14 g, 0.0027 mol) and concentrated NH_4OH (2.5 mL). After exposure to air (5 days) the solution was evaporated in vacuo, and the oily residue was then dissolved in CH_2Cl_2 (5 mL), dried (Na_2SO_4), and concentrated in vacuo. Distillation of the residue gave 0.12 g (87%) of a 1:1 mixture of stereoisomers of bis(2-hydroxycyclopentyl) disulfide (**11**): bp 83–85 °C (external temperature, 0.2 torr); IR (neat, NaCl) 3345, 2960, 2870, 1445, 1340, 1070, 1045, 980, 910 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.36–2.40 (m, 12 H), 2.87–3.34 (m, 2 H), 4.08–4.42 (m, 2 H) (the OH proton was not detected); ^{13}C NMR ($CDCl_3$) 21.64 (t, J = 135 Hz), 21.80 (t, J = 135 Hz), 30.24 (t, J = 129 Hz), 30.51 (t, J = 129 Hz), 33.15 (t, J = 135 Hz), 33.27 (t, J = 135 Hz), 56.52 (d, J = 128 Hz), 57.33 (d, J = 128 Hz), 78.64 (d, J = 132 Hz), 78.98 (d, J = 132 Hz) ppm; mass spectrum, m/e (relative intensity) 238 (0.2), 237 (1), 236 (7), 235 (8), 234 (68), 116 (14), 85 (26), 67 (100), 57 (17); high-resolution mass spectrum, calcd for $C_{10}H_{18}O_2S_2$, m/e 234.0748; found, 234.0753.

Bis(2-hydroxycyclopentyl) Sulfide (13). A solution of **2** (1.93 g, 0.0135 mol), cyclopentene oxide (**5**) (1.13 g, 0.0135 mol), and EtOH (250 mL) was stirred at room temperature (10 days),

acidified to pH ~ 6 with ethanolic HCl, and then filtered. The filtrate was evaporated in vacuo and the remaining yellow solid was triturated with CH_2Cl_2 (2 × 50 mL). The combined organic layers were concentrated in vacuo and then distilled to give 0.84 g (60%) of a viscous, colorless oil. ^1H NMR analysis indicated the presence of a 1:1 mixture of stereoisomers of **13**: bp 90–91 °C (external temperature, 0.05 torr); IR (neat, NaCl) 3330, 2965, 2870, 1450, 1070, 985 cm^{-1}; ^1H NMR (400.1 MHz) ($CDCl_3$) δ 1.39–1.52 (m, 4 H), 1.55–1.82 (m, 12 H), 2.00–2.08 (m, 4 H), 2.13–2.22 (m, 4 H), 2.93 (ddd, 2 H, J = 6.6, 8.1, 9.2 Hz), 3.12 (ddd, 2 H, J = 4.8, 8.1, 8.2 Hz), 3.37 (br s, 2 H), 3.81 (br s, 2 H), 4.08 (ddd, 2 H, J = 6.6, 6.6, 6.6 Hz), 4.19 (ddd, 2 H, J = 4.8, 5.6, 5.7 Hz); ^{13}C NMR ($CDCl_3$) 21.31 (t, J = 123 Hz), 22.27 (t, J = 123 Hz), 31.66 (t, J = 121 Hz), 32.04 (t, J = 122 Hz), 33.60 (t, J = 121 Hz), 33.87 (t, J = 122 Hz), 50.57 (d, J = 141 Hz), 52.89 (d, J = 142 Hz), 79.86 (d, J = 150 Hz), 80.84 (d, J = 147 Hz) ppm; mass spectrum, m/e (relative intensity) 204 (1), 203 (4), 202 (25), 184 (78), 117 (48), 101 (32), 100 (40), 89 (38), 84 (100), 83 (69), 67 (45); high-resolution mass spectrum, calcd for $C_{10}H_{18}O_2S$, m/e 202.1027; found, 202.1023.

Reaction of 1,2-Epoxybutane (6) with 2. A solution containing **2** (2.31 g, 0.0159 mol), **6** (1.14 g, 0.0159 mol), citric acid (1.02 g, 0.0053 mol), and EtOH (150 mL) was stirred at room temperature (6 days), filtered, and evaporated in vacuo. The semisolid residue was triturated with CH_2Cl_2 (2 × 25 mL), and the combined organic layers were concentrated in vacuo. Thin-layer analysis of this material indicated the presence of at least four compounds. Distillation at 51 °C (external temperature, 0.15 torr) did not afford pure compounds. The mixture was chromatographed on thick-layer silica gel plates by using cyclohexane–ethyl acetate (1:2) as the eluent.

A minor zone (R_f 0.33) was identified as a 1:1 mixture of stereoisomers of **15**: yield 0.19 g (7%); IR (neat, NaCl) 3370, 2930, 2970, 2885, 1465, 1120, 1020, 970 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.95 (t, 6 H, J = 6.0 Hz), 1.22–1.90 (m, 4 H), 2.15–2.72 (m, 4 H), 3.09 (br s, 2 H), 3.30–3.80 (m, 2 H); ^{13}C NMR ($CDCl_3$) 9.93 (q, J = 128 Hz), 29.09 (t, J = 127 Hz), 29.17 (t, J = 127 Hz), 39.69 (t, J = 139 Hz), 40.25 (t, J = 139 Hz), 71.16 (d, J = 154 Hz), 71.90 (d, J = 154 Hz) ppm. The signal at 9.93 ppm was slightly larger than nearby signals. Mass spectrum, m/e (relative intensity) 180 (0.1), 179 (0.2), 178 (2), 120 (64), 102 (29), 87 (20), 62 (100), 61 (26), 59 (65), 55 (53), 54 (19); high-resolution mass spectrum, calcd for $C_8H_{18}O_2S$, m/e 178.1027; found, 178.1030.

The major zone (R_f 0.63) isolated was identified as **14**: yield 0.72 g (25%); IR (neat, NaCl) 3410, 2975, 2940, 1715, 1180, 1150 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.98 (t, 3 H, J = 7 Hz), 1.30 (t, 3 H, J = 7 Hz), 1.32–1.82 (m, 2 H), 2.54–3.06 (m, 3 H), 3.37–3.90 (m, 1 H), 4.25 (q, 2 H, J = 7 Hz); ^{13}C NMR ($CDCl_3$) 9.79 (q, J = 128 Hz), 14.18 (q, J = 127 Hz), 29.03 (t, J = 127 Hz), 37.77 (t, J = 140 Hz), 63.80 (t, J = 153 Hz), 72.17 (d, J = 154 Hz), 171.32 (s) ppm; mass spectrum, m/e (relative intensity) 178 (0.6), 121 (83), 120 (100), 77 (71), 61 (58), 59 (96), 48 (87); high-resolution mass spectrum, calcd for $C_7H_{14}O_3S$, m/e 178.0664; found, 178.0664.

Ethyl Monothiocarbonate (16). To a vigorously stirred CH_2Cl_2 (30 mL) suspension of **2** (2.00 g, 0.0139 mol) was added 2.68 g (0.0139 mol) of triethyloxonium tetrafluoroborate (**7**). The resulting slurry was stirred at room temperature (16 h) and then filtered. Evaporation of the filtrate in vacuo followed by distillation of the residual liquid afforded 1.70 g (91%) of colorless ethyl monothiocarbonate (**16**): bp 145–147 °C [lit.[22] bp 156 °C (760 torr)]; IR (neat, NaCl) 2990, 2940, 1720, 1150 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.30 (t, 6 H, J = 7 Hz), 2.83 (q, 2 H, J = 8 Hz), 4.20 (q, 2 H, J = 7 Hz); ^{13}C NMR ($CDCl_3$) 14.06 (q, J = 127 Hz), 14.83 (q, J = 128 Hz), 25.00 (t, J = 145 Hz), 62.99 (t, J = 148 Hz), 170.71 (s) ppm.

Anal. Calcd for $C_5H_{10}O_2S$: C, 44.75; H, 7.51; S, 23.89. Found: C, 44.46; H, 7.52; S, 23.72.

Reactions of Mitomycin C (1) with 2. In a typical reaction, an aqueous solution (50 mL) of **1** (50 mg, 0.15 mmol) was purged with N_2 (10 min) at 0–5 °C or room temperature as indicated. The N_2 bubbling was continued until the reaction's termination. To this solution were added **2** (0.13 g, 0.90 mmol) and then $Na_2S_2O_4$ (50 mg, 0.29 mmol) freshly dissolved in H_2O (1 mL). After stirring the reaction mixture at the desired temperature (10 min), the reaction was terminated by bubbling O_2 through the solution. The reaction mixture was extracted with ethyl acetate (3 × 50 mL), and the combined organic layers were dried (Na_2SO_4) and then evaporated to dryness in vacuo. The remaining residue was chromatographed on thick-layer silica gel plates (40 × 20 cm) using the eluents indicated. Solvent systems available for thin-layer analysis and preparative chromatography were: A, 1-octanol/acetone/ligroin (90–110 °C) (2:5:5); B, ethyl acetate/hexanes (1:4); C, 2-propanol/ethyl acetate/hexanes (2:5:5); D, 2-propanol/ethyl acetate/hexanes (1:5:5); and E, 2-propanol/ethyl acetate/hexanes (1:10:10).

Room Temperature Reaction. The preceding procedure described for a typical reaction was performed at room temperature. Thin-layer analysis of the residue with system A as the eluent indicated the presence of two violet colored compounds, **17** (R_f 0.62) and **18** (R_f 0.73). Preparative thick-layer chromatography in system B afforded the pure compounds.

The purity of **17** was demonstrated in system A (R_f 0.63) and system B (R_f 0.42): yield 12.06 mg (18%); mp 120–123 °C; IR ($CHCl_3$) 3520, 3395, 2990, 2930, 1715, 1610, 1575, 1390, 1350, 1150, 1090, 1070, 1020 cm^{-1}; UV (MeOH) λ_{max} nm (ϵ) 205 (13 060), 255 (11 940), 309 (6 590), 349 (2 300); thermospray-ionization mass spectrum, m/e (relative intensity) 457 (1), 456 (11), 455 (38), 454 (100), 452 (40), 451 (33), 348 (15), 116 (16); field-desorption mass spectrum, m/e 454, 453.

The purity of **18** was demonstrated in system A (R_f 0.72) and system B (R_f 0.75): yield 11.92 mg (17.7%); mp 155–160 °C; IR ($CHCl_3$) 3520, 3395, 3015, 2405, 1725, 1610, 1575, 1505, 1220, 1180, 1155, 740, 670 cm^{-1}; UV (MeOH) λ_{max} nm (ϵ) 206 (10 100), 255 (8 500), 310 (4 800), 351 (1 600); field-desorption mass spectrum, m/e 454, 453, 421, 420.

Thin-layer analysis with system A as the eluent of **17** after standing in the solid state for several weeks indicated the formation of a new compound, **20** (R_f 0.72). Chromatography of the mixture using system B as the eluent afforded the violet colored **20**; mp 60–64 °C; IR ($CHCl_3$) 3520, 3400, 3020, 2990, 1730, 1710, 1675, 1615, 1575, 1510, 1395, 1355, 1270, 1170, 1080, 1025 cm^{-1}; UV (MeOH) λ_{max} nm (ϵ) 204 (6 600), 253 (5 900), 307 (3 700), 345 (1 500); field-desorption mass spectrum, m/e 525.

After **18** (7.27 mg) was allowed to stand for several hours either in ethyl acetate solution or in the solid state, thin-layer analysis in system A indicated the formation of a new compound **19** (R_f 0.66). Chromatography of the **18**/**19** mixture on thick-layer silica gel plates with system E as the eluent afforded 3.42 mg (47%) of the red-purple **19**: mp 88–94 °C; IR ($CHCl_3$) 3520, 3400, 3020, 2980, 2940, 2405, 1710, 1615, 1575, 1510, 1395, 1360, 1220, 1180, 1160, 750 cm^{-1}; UV (MeOH) λ_{max} nm (ϵ) 204 (5 600), 270 (4 000), 305 (2 300) 346 (815); thermospray-ionization mass spectrum, m/e (relative intensity) 455 (11), 454 (23), 453 (37), 418 (6), 183 (8), 155 (9), 142 (25), 117 (27), 116 (100); field-desorption mass spectrum, m/e 454, 453, 382, 381.

0–5 °C Reaction. All reagent quantities were increased 4-fold over those previously described for the typical reaction. The reaction was run at 0–5 °C. Thin-layer analysis of the ethyl acetate extract in system A indicated the presence of at least 2 major [**22** (R_f 0.35) and **23** (R_f 0.24)] and four minor (R_f 0.04, 0.10, 0.41, and 0.63) red-purple to violet colored compounds. Preparative thick-layer chromatography of the reaction mixture on silica gel plates in system C allowed separation of the major from the minor components. The two major compounds were then further purified by applying the mixture to a thick-layer silica gel plate and eluting with system D.

Compound **22** was isolated in 5% yield (12.28 mg): mp 100–104 °C; IR (KBr) 3440, 3360, 2990, 2930, 1720, 1670, 1610, 1510, 1395, 1335, 1245, 1175, 1150, 1105, 940, 905 cm^{-1}; UV (MeOH) λ_{max} nm 205, 253, 305, 335; field-desorption mass spectrum, m/e 408.

Compound **23** was isolated in 3.5% yield (8.56 mg): mp 179–182 °C; IR (KBr) 3440, 3340, 2980, 2920, 1710, 1605, 1505, 1390, 1335, 1240, 1100, 945, 900 cm^{-1}; UV (MeOH) λ_{max} nm 205, 266, 304, 338. A parent ion was not observed by field-desorption mass spectrometry.

Reaction of 17 with Ethyl Chloroformate. To a tetrahydrofuran (5 mL) solution of **17** (6.71 mg, 0.0148 mmol) were added with a syringe triethylamine (2.70 μL, 0.193 mmol) and ethyl chloroformate (1.42 μL, 0.0148 mmol). After stirring at room temperature (30 min), the solution was evaporated to dryness in vacuo. Thin-layer analysis of the red-violet residue in system A indicated the presence of two compounds, R_f 0.72 and 0.63.

Preparative chromatography of the mixture in system F afforded 3.65 mg (47%) of pure **20** (R_f 0.72, system A).

Reaction of 18 with Ethyl Chloroformate. Compound **18** (5.98 mg, 0.0132 mmol) dissolved in tetrahydrofuran (5 mL) was treated with triethylamine (6.10 μL, 0.0436 mmol) and ethyl chloroformate (3.80 μL, 0.0396 mmol) at 52 °C (3 h). The reaction mixture was then evaporated to dryness in vacuo. Thin-layer analysis in system A of the violet residue indicated the presence of two compounds, R_f 0.72 and 0.66. Preparative thick-layer chromatography in system E afforded 3.38 mg (49%) of **21** (R_f 0.72, system A): mp 84–87 °C; IR ($CHCl_3$) 3510, 3370, 3020, 1735, 1715, 1615, 1575, 1505, 1390, 1355, 1270, 1210, 1175, 1155, 1090 cm^{-1}; UV (MeOH) λ_{max} nm 205, 255, 308, 345; field-desorption mass spectrum, *m/e* 525.

Reaction of 22 with 2. Compound **22** (9.78 mg, 0.0240 mmol) was dissolved in 5 mL of ethanol–water (1:1). After purging the solution with N_2 (10 min), **2** (25.42 mg, 0.1765 mmol) and then an aqueous $Na_2S_2O_4$ (30.67 mg, 0.1763 mmol) solution (1 mL) were added. The reaction mixture was stirred at room temperature (10 min) with continuous N_2 bubbling. Oxygen was passed through the solution (5 min) to terminate the reaction. Extraction with ethyl acetate (3 × 5 mL) followed by drying (Na_2SO_4) and evaporation of the combined organic layers in vacuo gave a violet colored solid. Preparative thick-layer chromatography of this solid in system E afforded 5.76 mg (53%) of compound **17**, R_f 0.63 (system A).

Reaction of 23 with 2. The preceding procedure was adopted using **23** (6.56 mg, 0.0161 mmol), **2** (17.05 mg, 0.1184 mmol), and an aqueous $Na_2S_2O_4$ (20.57 mg, 0.1182 mmol) solution (1 mL). Preparative thick-layer chromatography of the evaporated ethyl acetate extract in system E afforded 1.93 mg (27%) of compound **19**, R_f 0.67 (system A).

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Registry No. 1, 50-07-7; 2, 35832-93-0; 4, 78-75-1; 5, 285-67-6; 6, 106-88-7; 8, 87483-16-7; 9, 87483-17-8; 10, 87483-18-9; 11a, 87483-19-0; 11b, 87508-68-7; 12, 5449-08-1; 13a, 87508-69-8; 13b, 87508-70-1; 14, 87483-20-3; *meso*-15, 87483-21-4; (*R**,*R**)-15, 87483-22-5; 16, 3554-12-9; 17, 87483-23-6; 18, 87483-24-7; 19, 87483-25-8; 20, 87483-26-9; 21, 87508-71-2; 22, 87483-27-0; 23, 87483-28-1; carbonyl sulfide, 463-58-1; citric acid, 77-92-9.

Approaches to Azepines: A New Azepine by the Photolysis of Dimethyl *p*-Azidosalicylate

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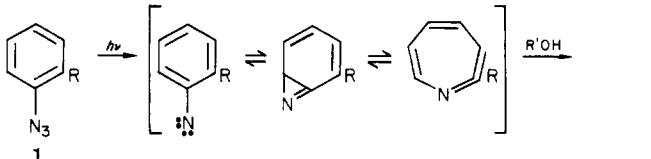
We have generated 3-methoxy-4-carbomethoxyphenylnitrene and 3,4-dimethoxyphenylnitrene under various conditions, in a search for new azepines. Unexpectedly, only the former, by photolysis of dimethyl *p*-azidosalicylate, gave an azepine. Intramolecular coordination of the nitrene to the carbonyl group being impossible, electronic rather than steric effects are implicated. The product, methyl 2,4-dimethoxy-3*H*-azepine-5-carboxylate was hydrolyzed to 2,3-dihydro-4-methoxy-2-oxo-1*H*-azepine-5-carboxylic ester and acid.

The photolysis of phenyl azide in methanol gives 2-methoxy-3*H*-azepine (**1** (R = H) → **2** (R = H, R′ = Me)).[1,2]



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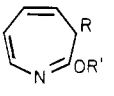

2

2-Alkoxyazepine production is reportedly facilitated by an electron-withdrawing group, e.g., COOMe ortho but not para to the azido group[2,3] because of an electronic effect enhancing the electrophilicity of the intermediate nitrene.[2] It has otherwise been proposed that coordination to the *o*-carbonyl group promotes formation of the azepine.[4] In support of the former explanation and contrary to expectation,[5] we found that methyl 4-azido-2-methoxybenzoate (dimethyl *p*-azidosalicylate, **3**) on photolysis in methanol gives methyl 2,4-dimethoxy-3*H*-azepine-5-carboxylate (**4**) in fair yield, convertible by standard methods[6,7] to the azepinones **5** (R = Me and H). No 4,7-dimethoxy isomer was detected, indicating high specificity and demonstrating the ability of a para ester group that

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