R. Roth and Dr. J. Hedding, University of Bochum, for the generous supply of samples of **1a–d** and the Fonds der Chemischen Industrie for support of this research.

Registry No. **1a**, 14693-11-9; **1b**, 93783-08-5; **1c**, 82093-56-9; **1d**, 60643-98-3; **1e**, 60643-97-2.

Supplementary Material Available: Table III; ^{13}C chemical shift differences $\delta\nu$ (Hz), temperature-dependent line widths (Hz), and calculated rate constants k (s^{-1}) of **1a–d** (2 pages). Ordering information is given on any current masthead page.

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

Mary Bean[1a] and Harold Kohn*[1b]

Department of Chemistry, University of Houston—University Park, Houston, Texas 77004

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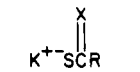
Treatment of mitomycin C (1) with the ambident nucleophile potassium thiobenzoate (**6c**) under reductive conditions (sodium dithionite) at approximately neutral pH led to the formation of predominantly *trans*-aziridine ring-opened mitosene adducts. The ratio of trans to cis derivatives was greater than 9:1. The principal site of reaction in reduced 1 was determined to be carbon-1. The ratio of carbon-1 monosubstituted adducts vs. carbons-1,10 disubstituted adducts was greater than 10:1. The structural identity of each product was confirmed by high-field ^1H NMR studies as well as by additional spectral analyses. In all cases, substitution at carbons-1 and -10 occurred with attack at the sulfur terminus. The implications of these reactions in relation to the mode of action of mitomycin C (1) are discussed. Moreover, various NMR relationships have been established which provide useful information concerning the conformation of these mitosene adducts.

Mitomycin C (1) is a member of a class of antibiotics that exhibit potent, specific antitumor activity.[2] The cytotoxic effects of the mitomycins have been attributed to the bioreductive alkylation of DNA.[3] In the most widely accepted mechanism (Scheme I), attachment of the drug to the genetic material is conjectured to occur at carbons-1 and -10 of 1.[4] Special emphasis has been placed on the potential ability of mitomycin C (1) to cross-link complementary strands of DNA.[2,3]

Studies of the reactions of various nucleophiles[5–13] with 1 under reductive conditions have generally supported this mechanism (Scheme I). Initial covalent linkage to 3 is believed to proceed by an S_N1-type reaction at carbon-1. This pathway suggests the formation of equal amounts of carbon-1 cis- and trans-substituted products. However, a variety of cis/trans product ratios have been observed. Treatment of reduced 1 with either potassium ethyl xanthate[6,7] (**6a**) or potassium ethyl monothiocarbonate[8]



6a, X = S; R = OEt
b, X = O; R = OEt
c, X = O; R = Ph

(**6b**) gave the cis- and trans-1,2-disubstituted mitosenes in the expected 1:1 ratio. On the other hand, reaction of reduced mitomycin C (1) with 5′-guanylic acid led to the isolation of solely the cis adduct,[12] while treatment with d(GpC) gave only the trans derivative after enzymatic degradation.[11]

In this paper, we investigate the role of the nucleophile in determining the eventual stereochemistry at carbon-1 in ring-opened mitosenes. We describe the reaction of mitomycin C (1) with the ambident reagent potassium thiobenzoate[14] (**6c**), a nucleophile similar to that used in previous studies[6–8] but of reduced activity. Under reductive conditions at approximately neutral pH, 1,2-trans-substituted adducts were the predominant products. This surprising result has aided our interpretation of the varying product ratios obtained in related investigations. Moreover, detailed analysis of the high-field ^1H NMR data for these and comparable compounds has suggested that a preferred pyrrolidine ring conformation exists in both cis- and trans-disubstituted mitosene derivatives.

(1) (a) Abstracted from Ph.D. dissertation of this author. Additional structure proof, discussion, and experimental and spectral data may be found in this reference. (b) Alfred P. Sloan Foundation Fellow, 1977–1981. Camille and Henry Dreyfus Teacher-Scholar Grant Recipient, 1977–1982.

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(3) (a) Remers, W. A. "The Chemistry of Antitumor Antibiotics"; Wiley: New York, 1979; Vol. 1, pp 221–276. (b) Crooke, S. T.; Bradner, W. T. *Cancer Treat. Rev.* **1976**, *3*, 121–140. (c) Comis, R. L.; Carter, S. K. *Cancer* (Philadelphia) **1974**, *34*, 1576–1586, and references therein.

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(13) Hashimoto, Y.; Shudo, K.; Okamoto, T. *Tetrahedron Lett.* **1982**, *23*, 677–680. Hashimoto, Y.; Shudo, K.; Okamoto, T. *Chem. Pharm. Bull.* **1983**, *31*, 861–869.

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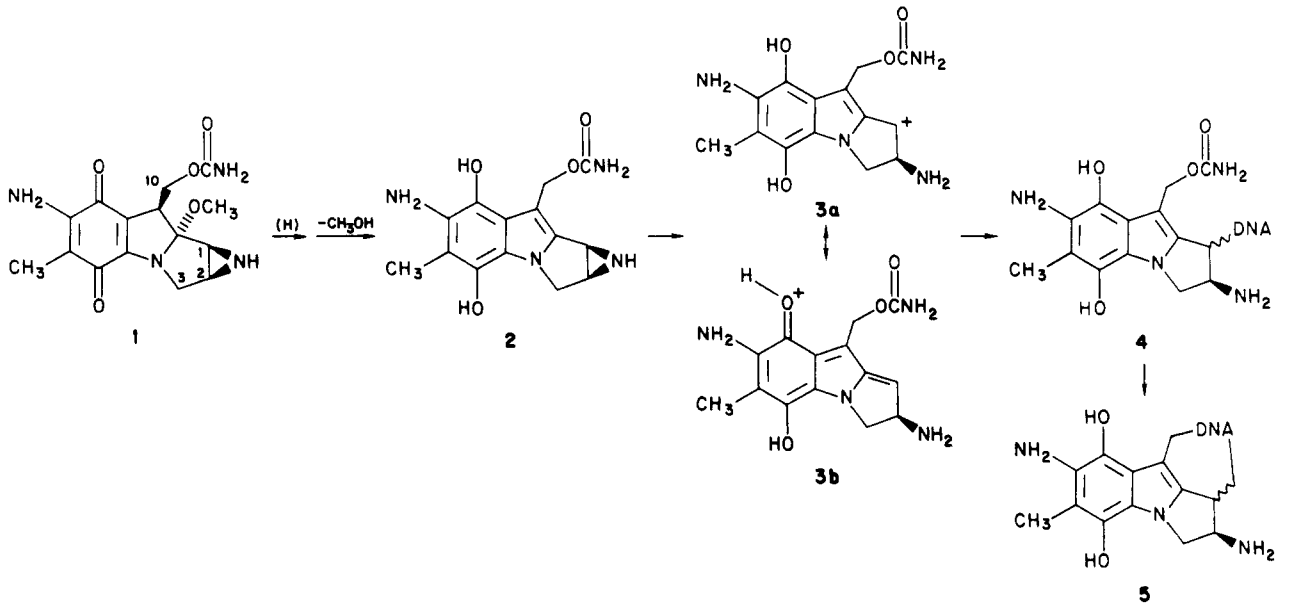
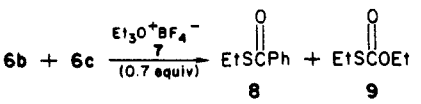
Scheme I

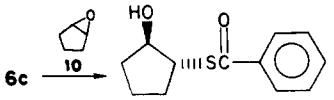


Results

Model Studies. The nucleophilicity of potassium thiobenzoate (**6c**) vs. potassium ethyl monothiocarbonate (**6b**) was evaluated by reacting the two salts with 0.7 equiv of triethyloxonium tetrafluoroborate (**7**) at 0–5 °C. The product mixture was analyzed by ^{1}H NMR and determined to be a 30:70 mixture of ethyl thiobenzoate (**8**) and ethyl monothiocarbonate[8,15] (**9**), respectively. Therefore, the ethyl monothiocarbonate anion **6b** was noticeably more reactive toward the powerful alkylating agent **7** than was the thiobenzoate anion **6c**.



Model studies were also conducted to determine the reactivity of sulfur vs. oxygen in **6c**. Treatment of **6c** with both **7** and **10** gave **8**[8,15] and **11**, respectively. Both products

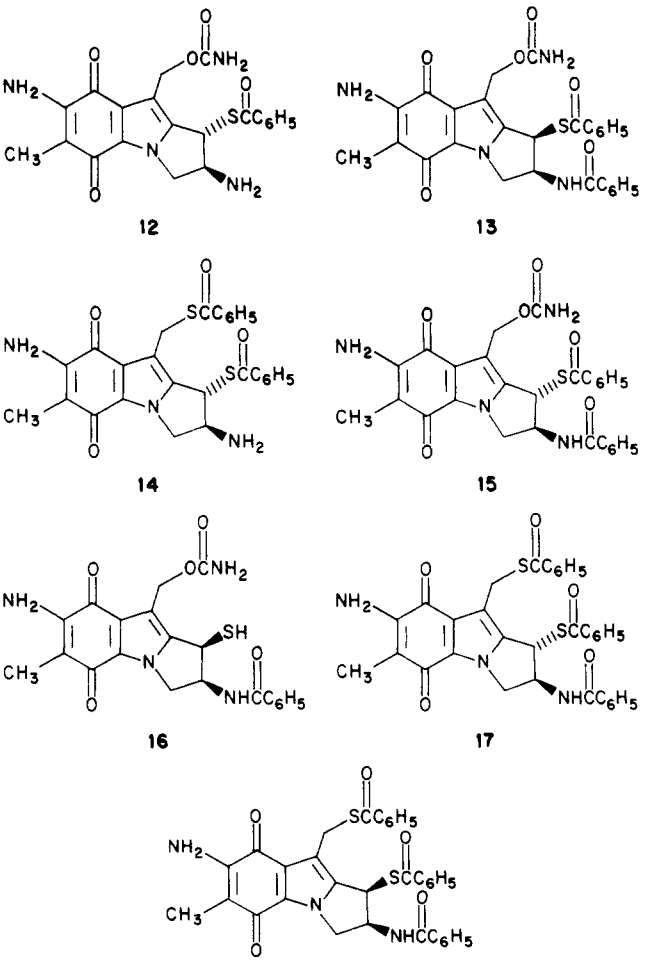


showed a strong absorption at 1655 cm^{-1} in the IR for the carbonyl stretching vibration indicating sulfur substitution.[16] Also consistent with sulfur attack was the chemical shift for the carbonyl carbon atom between 191.7 and 194.3 ppm.[8,17]

In neither reaction was evidence found for oxygen substitution. These results suggested that in the reactions with mitomycin C (**1**), potassium thiobenzoate (**6c**) should react initially through sulfur.

Mitomycin C (1) Studies. The reactions were conducted according to the previously reported reductive procedure (sodium dithionite) for the room temperature reaction of mitomycin C (**1**) with potassium ethyl monothiocarbonate (**6b**).[8] The pH of the solution remained at 6 during the reaction. The reaction was quenched with oxygen and then extracted with ethyl acetate. TLC analysis of the ethyl acetate extract immediately after its concentration in vacuo indicated the presence of at least seven violet to red-violet compounds: a major compound (**12**), three minor adducts (**13–15**), and three trace compounds (**16–18**) (Chart I). These products were isolated by thick-layer chromatography. Compounds **12–15** were

Chart I. Structures of Mitomycin C-Thiobenzoate Adducts



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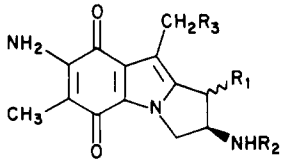
(17) Stothers, J. B. "Carbon-13 NMR Spectroscopy"; Academic Press: New York, 1972.

Table I. ^{1}H NMR Assignments of Mitomycin C-Thiobenzoate Adducts[a,b]



no.	12[c]	15[c]	19[c,d]	14[c]	17[c]	13[e]	16[e]	18[e]
R^1	SCOPh	SCOPh	SCOPh	SCOPh	SCOPh	SCOPh	SH	SCOPh
R^2	H	COPh	$COOCH_2CH_3$	H	COPh	COPh	COPh	COPh
R^3	$OCONH_2$	$OCONH_2$	$OCONH_2$	SCOPh	SCOPh	$OCONH_2$	$OCONH_2$	SCOPh
C_1H	4.93; d; 1	5.38; d; 3.4	5.22; d; 3.7	5.01; d; 2	5.49; d; 3.7	5.70; d; 7.3	5.27; d; 6.5	5.78;[f,g] br s
C_2H	4.76; br d; 5.1	5.32–5.36; m	4.87–4.91; m	4.80; br d; 5.4	5.31–5.38; m	5.74–5.82; m	5.52–5.63; m	5.78;[e] br s
C_3H_α	4.49; d, d; 5.1, 13.0	4.79; d, d; 6.9, 13.3	4.66; d, d; 6.7, 13.1	4.53; d, d; 5.4, 12.9	4.78; d, d; 6.9, 13.0	4.80; d, d; 7.8, 12.7	4.66; d, d; 8.1, 12.5	4.80; d, d; 7.3, 12.5
C_3H_β	4.11; d, d; 1, 13.0	4.42; d, d; 3.5, 13.3	4.29; d, d; 4.0, 13.1	4.11; d, d; 2, 12.9	4.40; d, d; 3.7, 13.0	4.30; d, d; 7.4, 12.7	4.23; d, d; 9.6, 12.5	4.34; d, d; 7.3, 12.5
$C_{10}H_2$	5.17; d, AB q; 13.3; 1 H	5.14; d, AB q; 12.5; 1 H	5.11; d, AB q; 12.5; 1 H	4.53; d, ABq; 13.6; 1 H	4.37; d, AB q; 13.3; 1 H	5.15; d, AB q; 13.0; 1 H	5.28; d, AB q; 12.9; 1 H	4.42; d, AB q; 13.4; 1 H
	5.21; d, AB q; 13.3; 1 H	5.18; d, AB q; 12.5; 1 H	5.17; d, AB q; 12.5; 1 H	4.56; d, AB q; 13.6; 1 H	4.58; d, AB q; 13.3; 1 H	5.23; d, AB q; 13.0; 1 H	5.39; d, AB q; 12.9; 1 H	4.58; d, AB q; 13.4; 1 H
C_6CH_3	1.79; s	1.84; s	1.84; s	1.84; s	1.83; s	1.85; s	1.80; s	1.85; s
C_2NH		5.97; br s	5.97; br s		5.99; br s	5.95; br s	5.96; br s	5.99;[f,g] br s

[a] Additional ^{1}H NMR spectral data for these adducts may be found in the Ph.D. dissertation of May Bean (University of Houston, 1983). [b] 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, followed by the coupling constant(s) in hertz. In select cases the number of hydrogens per signal is indicated. All spectra were recorded at 400.1 MHz, and the solvent used was acetone-d_6. [c] The values reported are for trans derivatives. [d] The signals for the ethyl carbamate methyl and methylene protons were located at δ 1.18 (t, J = 7.0 Hz) and 4.06 (q, J = 7.0 Hz), respectively. [e] The values reported are for the cis derivatives. [f] The half-height width of this signal was ~16 Hz. [g] The assignments for these protons may be interchanged.

characterized by IR, UV, and high-field ^{1}H NMR (Table I), and in some cases thermospray-ionization[18] or field-desorption spectroscopy. Homonuclear proton–proton decoupling experiments aided the structural elucidations. Characterization of **16–18** was by high-field ^{1}H NMR (Table I) and double irradiation experiments. Field-desorption mass spectrometry also contributed to the elucidation of **17** and **18**.

Compound **12** was the major product in the ethyl acetate extract and was formed in 13% yield while yields of **13–18** were no greater than 3%. The mass spectrum of **12** showed no molecular ion by thermospray ionization; however, a peak at *m/e* 397 was assigned to the protonated M – $CONH_2$ fragment. Observation of absorptions at 1720 and 1665 cm^{-1} in the infrared spectrum supported the presence of both a carbamate[6-8] and a thiobenzoate moiety, respectively, in **12**. In the ^{1}H NMR spectrum, a doublet (J = 1 Hz) at δ 4.93 was assigned to the carbon-1 methine proton, indicating the presence of a sulfur-bound moiety at this position.[6-8] A broad doublet at δ 4.76 was assigned to the carbon-2 methine proton. In related compounds containing an unsubstituted amino group the corresponding carbon-2 methine proton appeared at δ 4.17–4.20,[6-8,19] The lower field chemical shift observed for **12** was of note since this resonance appeared close to the value observed for the carbon-2 methine proton in a mitosene adduct with an acylated amine group (δ 4.76–4.79).[8] One explanation for this downfield shift in the carbon-2 hydrogen signal is that the thiobenzoate moiety at carbon-1 may deshield the carbon-2 hydrogen.

The time-averaged conformation as well as the stereochemistry present in the pyrrolidine ring of mitosene **12** was assigned by a Karplus analysis similar to that outlined in a preceding paper.[7] The small coupling interactions between C_2H–C_1H (1 Hz) and C_2H–C_3H_β (1 Hz) and large coupling constant between C_2H–C_3H_α (5.1 Hz) suggested

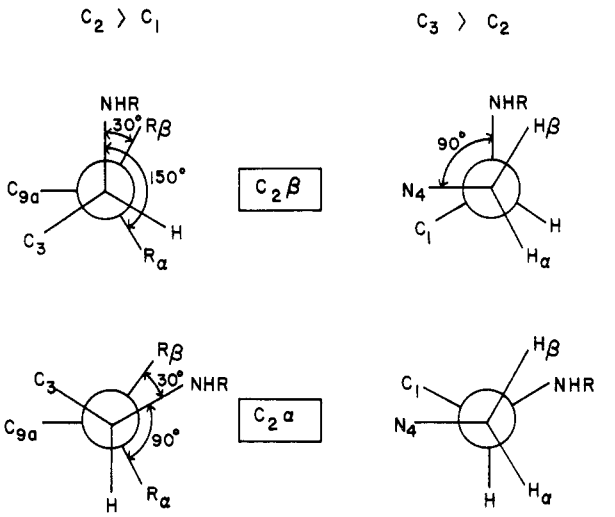


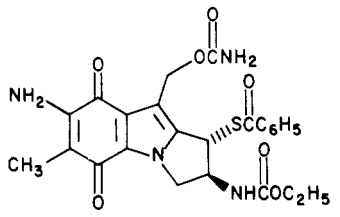
Figure 1. Spatial relationships of the two extreme conformations of the pyrrolidine ring in ring-opened mitosenes.

that the time-averaged conformation of the constrained pyrrolidine ring approached the $C_2\beta$ extreme and that a trans relationship existed between the protons at carbons-1 and -2. The $C_2\beta$ designation denotes an extreme conformation for this molecule in which the C_2 atom is positioned ~30° above the plane generated by the two rings of the aromatic indoloquinone system and their one-bond substituents (Figure 1). The alternate extreme conformation for this molecule has been designated as $C_2\alpha$ and refers to the situation in which the C_2 atom is positioned ~30° below the plane of the indoloquinone system.[20]

(18) Blakely, C. R.; Vestal, M. L. *Anal. Chem.* **1983**, *55*, 750.
(19) (a) Taylor, W. G.; Remers, W. A. *J. Med. Chem.* **1975**, *18*, 307–331. (b) Cheng, L.; Remers, W. A. *J. Med. Chem.* **1977**, *20*, 767–770.

(20) The recent reassignment of the absolute configuration of mitomycin C by Shirahata and Hirayama[21] makes the $C_2\beta$ conformation the conformation that allows the trans compounds to be assigned rather than the $C_2\alpha$ conformation as reported in ref 7 and 8. This structural reassignment also necessitated the interchange of the ^{1}H NMR assignments listed for the C_3H_α and C_3H_β protons in the mitomycin adducts reported in those studies.
(21) Shirahata, K.; Hirayama, N. *J. Am. Chem. Soc.* **1983**, *105*, 7199–7200.

To insure that carbon-2 of **12** possessed a free amino group, compound **12** was treated with ethyl chloroformate and triethylamine to yield **19**. Both the slightly downfield



19

proton chemical shift value for the carbon-2 methine hydrogen in **19** (Table I) as well as the multiplicity of this signal indicated that the reaction had proceeded with acylation at the carbon-2 amino group. Of note, the C_1H–C_2H and C_2H–C_3H_β coupling constants were considerably larger in **19** than in **12**.

The compound isolated in the second highest yield (3%) was identified as **13**. No molecular ion was observed in the field desorption mass spectrum, but a peak at m/e 485 was assigned to the protonated M – $OCONH_2$ fragment. However, an absorption at 1720 cm^{-1} in the IR spectrum indicated that the carbamate moiety had not been displaced. Acylation of the carbon-2 amino group was implied by the relatively low field chemical shift (δ 5.74–5.82) observed for the carbon-2 methine proton in the ^1H NMR spectrum. The methine proton of carbon-1 appeared as a doublet (J = 7.3 Hz) at δ 5.70. Although this signal was downfield from the signals of corresponding protons in other sulfur-substituted compounds,[6-8] the difference was not sufficient to suggest oxygen substitution.[19] Since two large coupling constants ($J_{C_2H-C_3H_\beta}$ = 7.8 Hz, $J_{C_2H-C_3H_\beta}$ = 7.4 Hz) denoted the time-averaged $C_2\alpha$ conformation for the pyrrolidine ring of **13**, the stereochemistry of the C_1H–C_2H relationship could not be assigned on the basis of the large 7.3-Hz coupling constant observed for these protons.[7] However, evidence to be discussed later supported the cis geometry.

Compound **14** was the major (1.5%) carbon-1,carbon-10 disubstituted adduct isolated. Accordingly, a peak at m/e 518 for the protonated molecular ion ($C_{27}H_{24}N_3O_4S_2$) was observed in the thermospray-ionization mass spectrum. The thiobenzoate carbonyl stretching vibration was detected at 1660 cm^{-1} in the IR spectrum. In agreement with the proposed sulfur substitution at carbon-10 was the absence of the carbon-10 carbamate stretching frequency in the IR spectrum[6-8] and the upfield chemical shift (δ 4.50) value observed for the AB quartet of the carbon-10 methylene protons in the ^1H NMR spectrum. The carbon-1 and -2 methine protons were assigned to a doublet (J = 2 Hz) located at δ 5.01 and the broad doublet at δ 4.80, respectively. On the basis of the proton–proton coupling interactions in the pyrrolidine ring we have assigned a trans stereochemical relationship for the protons at carbons-1 and -2.

Compound **15** was isolated in 2% yield. This adduct also appeared in pure, dry samples of **12** after standing for several days (TLC analysis). Consistent with the proposed acylation of the carbon-2 amino group in **15** was the downfield shift of the methine proton of carbon-2 from δ 4.76 in **12** to δ 5.32–5.36. Assignment of the trans stereochemistry of the carbon-1 and -2 hydrogens was made on the basis of a small C_1H–C_2H coupling (J = 3.4 Hz) and the large C_2H–C_3H_α (J = 6.9 Hz) and small C_2H–C_3H_β (J = 3.5 Hz) coupling constants. Since **15** was isomeric with **13**, cis geometry was indicated for the corresponding relationship in the latter adduct.

A small amount (0.3% yield) of a compound tentatively identified as **16** was isolated from the reaction mixture. Sulfur substitution and acylation were implied by the chemical shifts of the carbon-1 (δ 5.27) and -2 (δ 5.52–5.63) methine protons. Finally, since the conformation of **16** approaches $C_2\alpha$, the large C_1H–C_2H coupling constant could not be used in the assignment of stereochemistry. However, a cis relationship for the carbon-1 and -2 methine protons would facilitate the apparent migration of the benzoyl moiety.[6-8]

The structures of the trisubstituted adducts **17** and **18** are tentative. A molecular ion peak at m/e 621 was observed in the mass spectrum of each compound. The chemical shifts of the carbon-10 methylene and carbon-2 methine protons of both **17** and **18** indicated displacement of the carbamate moiety by sulfur and acylation of the amino group, respectively. Sulfur substitution at carbon-1 of **17** was consistent with the observed chemical shift of δ 5.49 for the proton at this site. The resonance of the carbon-1 methine proton of **18** was tentatively assigned to a broad singlet at δ 5.78. The observed C_2H–C_3H_2 coupling constants of **17** implied the time-averaged $C_2\beta$ conformation; therefore, the relatively small C_1H–C_2H coupling constant (J = 3.7 Hz) was indicative of a trans relationship for these protons. Since **17** and **18** are isomeric, a cis configurational relationship of the protons at carbons-1 and -2 was assigned to adduct **18**.

Many of the compounds formed in the reaction of the thiobenzoate anion and mitomycin C (**1**) underwent inter- and/or intramolecular reactions (i.e., **12** → **15**, **14** → **17**) both in the solid state and in solution due to the known lability of thio ester bonds. The trisubstituted adducts were found to be relatively stable over several months, a property previously noted for the trisubstituted compounds formed in the reaction of mitomycin C (**1**) with potassium ethyl monothiocarbonate (**6b**).[8]

Discussion

Spectral Properties and Structure. Detailed analysis of the high-field ^1H NMR data for mitosenes **12–19** as well as related compounds[6-8] has revealed several noteworthy trends. First, the time-averaged conformation of all 1,2-trans adducts isolated approached the $C_2\beta$ conformational extreme while a time-averaged $C_2\alpha$ conformation was indicated for all 1,2-cis noncyclic adducts. A potential explanation of this conformatinal preference may be offered. To a first approximation, this hypothesis is based simply on a consideration of the potentially adverse steric and electronic interactions present in the pyrrolidine ring system. The time-averaged $C_2\beta$ conformation shold predominate in the trans adducts since it allows the carbon-1 and -2 substituents (Figure 1, R_α and NHR, respectively) to be 150° apart as opposed to only 90° in the $C_2\alpha$ conformation. However, in the cis series, simple analysis of the dihedral angle between these substituents (Figure 1, R_β and NHR) does not suffice. In both extreme conformations, the dihedral angle between the carbon-1 and -2 substituents is 30°. Consideration of the only other potentially large steric and electronic interaction, the carbon-2 amino group substituent (NHR) and nitrogen-4, suggests that the $C_2\alpha$ conformation may be favored since it minimzies this interaction (dihedral angle 150° for $C_2\alpha$ vs. 90° for $C_2\beta$). This hypothesis assumes that the steric and electronic interactions between the substituents at carbons-1 and -2 are generally more significant than those between C_2NHR and nitrogen-4. Moreover, additional effects (i.e., H bonding) in select derivatives may reinforce or counteract these steric and electronic interactions.

Second, the chemical shift values observed for the carbon-1 and -2 protons of the 1,2-cis isomers (i.e., **13**, **18**) are 0.29–0.32 ppm and 0.33–0.44 ppm, respectively, downfield from the values of corresponding protons in the 1,2-trans isomers (i.e., **15**, **17**). This trend may be attributable in part to the differences in the proposed time averaged conformations for the cis and trans series of compounds ($C_2\alpha$ and $C_2\beta$, respectively). An analogous NMR pattern has been observed in simple five-membered ring compounds.[22] In these monocyclic systems, the downfield shift in the ^1H NMR spectra for the cis vs. the trans isomers (Δ ppm = 0.32–0.55) has been explained in terms of an unfavorable gauche γ-interaction[23] experienced in the cis series. The downfield shift stems from electron density changes caused by sterically induced polarization of the C–H bonds. The juxtaposition of the two substituents on the same side of the ring leads to this steric interaction. A similar explanation may be applicable in this study. Adverse steric interactions are expected at carbons-1 and -2 in the cis adducts possessing the time-averaged $C_2\alpha$ conformation. Correspondingly, these interactions are minimized in trans compounds having a time-averaged $C_2\beta$ conformation. (The bond angle between carbon-1 and -2 substituents is 150° for trans adducts in the $C_2\beta$ conformation and only 30° for cis adducts in the $C_2\alpha$ conformation.)

Third, an increase in the C_1H–C_2H coupling constants in the trans isomers was observed when the amino group at carbon-2 was acylated. Although the increase was small (~1 Hz) in the ethyl monothiocarbonate series,[8] a larger increase (1.7–2.7 Hz) was detected in this coupling constant in the thiobenzoate series (i.e., **12** → **19**, **12** → **15**, **14** → **17**). This change may be indicative of an alteration in the preferred conformation of the pyrrolidine ring from the extreme $C_2\beta$ form to a time-averaged conformation intermediate between $C_2\beta$ and $C_2\alpha$. Consistent with this hypothesis are the changes in the coupling constants observed for the C_1H–C_2H, C_2H–C_3H_α, and C_2H–C_3H_β interactions of the thiobenzoate adducts. As the conformation changes from $C_2\beta$ to $C_2\alpha$, the dihedral angles between C_1H and C_2H and C_2H and C_3H_β progressively increase from 90° to 150° while the dihedral angle between C_2H and C_3H_α changes from −30° to 0° to 30°. Application of a Karplus analysis to these dihedral angle changes predicts that the proposed conformational change should be accompanied by an increase in $J_{C_2H-C_3H_\beta}$, and a slight increase followed by a slight decrease in $J_{C_2H-C_3H_\alpha}$ as the $C_2\alpha$ extreme is approached.[7] Increases in each of these coupling constants are observed in the C_2 amino acylated derivatives of the thiobenzoate series. At the present time, the reason for the suggested alteration in the time-averaged conformation is unknown. However, such a deviation from the extreme $C_2\beta$ conformation to a more intermediate one may increase the possibility of intramolecular hydrogen-bonding between the substituents at carbons-1 and -2.

Mode of Action. Evaluation of the lipophilic adducts (**12** → **18**) isolated from the ethyl acetate extract permitted several tentative conclusions to be reached concerning the mode of action of mitomycin C. The findings of this study are in accord with the concepts of bioreductive alkylation[4] (Scheme I). A particularly interesting aspect of this reaction was the high ratio (>9:1) of trans (**12**, **14**, **15**, and **17**) to cis (**13**, **16**, and **18**) products. This ratio differed considerably from the results obtained with potassium ethyl monothiocarbonate[8] (**6b**) and potassium ethyl xanthate[6,7] (**6a**), where both the trans and the cis products were formed in nearly equal amounts. Despite the high percentage of trans products from **6c**, a departure from the previously proposed S_N1 mechanism (Scheme I) to a bimolecular process is considered unlikely in view of the decreased nucleophilicity of **6c**. Moreover, small amounts of cis adducts (i.e., **13**, **16**, and **18**) were isolated in this study. Rather, the stereochemistry of carbon-1 functionalized mitosene adducts may be a function of a complex interplay of several factors: the preferred conformation of the proposed reactive intermediate[4] **3**, the lifetime of this species under the experimental conditions employed, and the reactivity and concentration of the nucleophile present in the reaction medium.[24]

This investigation also provided useful information concerning the reactivity of carbon-1 vs. carbon-10. In previous studies with sulfur-based reagents[6–8] (**6a** and **6b**) discrimination between these two alkylation sites was difficult. Use of potassium thiobenzoate (**6c**) led to little carbon-10 substitution at room temperature. The ratio of carbon-1 monosubstituted adducts (**12**, **13**, **15**, and **16**) vs. carbons-1,10 disubstituted adducts (**14**, **17**, and **18**) was greater than 10:1. This ratio corresponded well with the high percentage of monosubstituted vs. disubstituted adducts observed with DNA.[2,3] In light of the similar reductive conditions utilized in all the sulfur-based nucleophilic studies,[6–8] the selectivity of the potassium thiobenzoate (**6c**) reactions has been attributed in part to the decreased nucleophilicity of this anion and the enhanced reactivity of carbon-1 vs. carbon-10 in **1**.

Experimental Section

General Methods. The methods employed in this study are the same as those described in ref 8. All high-field ^1H NMR spectra were taken by Ms. Helga Cohen at the Department of Chemistry, University of South Carolina, on a Bruker WH-400 instrument. Field-desorption mass spectra were run by Dr. Robert Cotter at the Department of Pharmacology, The Johns Hopkins University, and by Dr. J. L. Occolowitz of the Eli Lilly Co., Indianapolis, IN. Solvent systems available for thin-layer analysis and preparative chromatography were A, 1-octanol/acetone/ligroin (90–100 °C) (2:5:5); B, 2-propanol/ethyl acetate/hexanes (2:5:5); C, 2-propanol/ethyl acetate/hexanes (1:5:5); and D, 2-propanol/ethyl acetate/hexanes (1:10:10).

Preparation of Potassium Thiobenzoate (6c). An aqueous solution (10 mL) of KOH (1.52 g, 27 mmol) was treated with 4 mL (4.70 g, 34 mmol) of thiobenzoic acid and vigorously stirred at room temperature (5 min). The reaction mixture was then extracted with CH_2Cl_2 (3 × 10 mL) to remove unreacted starting material. Evaporation of the aqueous layer in vacuo afforded 4.10 g (86%) of yellow, crystalline **6c**: mp 225–229 °C dec (lit.[14a] mp >230 °C dec): IR (KBr) 3060, 1590, 1520, 1485, 1300, 1205 cm^{-1}; ^1H NMR (80 MHz, D_2O) δ 7.18–7.39 (m, 3 H), 7.89–8.02 (m, 2 H); ^{13}C NMR (20 MHz, D_2O) 127.33, 127.41, 130.69, 142.98, 213.75 ppm.

Reaction of 6c with Et_3OBF_4 (7). A CH_2Cl_2 (50 mL) slurry of **6c** (0.80 g, 4.5 mmol) and **7** (0.86 g, 4.5 mmol) was stirred at room temperature (24 h). The reaction mixture was filtered, and the filtrate was evaporated in vacuo. Bulb-to-bulb distillation of the residue at 31 °C (external temperature, 0.04 torr) yielded 0.67 g (89%) of a yellow liquid identified as **8**: IR (neat, NaCl) 3060, 2965, 2930, 1655, 1595, 1580 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ 1.32 (t, 3 H, J = 7.4 Hz), 3.05 (q, 2 H, J = 7.4 Hz), 7.37–7.63 (m, 3 H), 7.89–8.05 (m, 2 H); ^{13}C NMR (20 MHz, $CDCl_3$) 14.57 (q, J = 128 Hz), 23.21 (t, J = 141 Hz), 126.94 (d, J = 161 Hz),

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128.34 (d, J = 161 Hz), 132.99 (d, J = 161 Hz), 137.06 (s), 191.70 (s) ppm; mass spectrum, m/e (relative intensity) 166 (5), 106 (8), 105 (100), 77 (30), 51 (8); high-resolution mass spectrum, calcd for $C_9H_{10}OS$ m/e 166.0452, found 166.0456.

Reaction of 6c with Cyclopentene Oxide (10). Cyclopentene oxide (**10**) (0.68 g, 8.1 mmol) was added to an ethanol solution (100 mL) of **6c** (1.04 g, 5.9 mmol) and anhydrous citric acid (0.51 g, 2.7 mmol). The reaction mixture was stirred at room temperature (3 days) and then filtered. Evaporation of the filtrate in vacuo gave a yellow, semisolid material which was triturated with CH_2Cl_2 (3 × 10 mL). The CH_2Cl_2 soluble fractions were combined and evaporated in vacuo. Distillation of the remaining viscous oil afforded 0.40 g (31%) of yellow liquid **11**: bp 32–34 °C (external temperature, 0.05 torr): IR (neat, NaCl) 3420, 2960, 1655, 1595, 1580 cm^{-1}; ^{1}H NMR (80 MHz, $CDCl_3$) δ 1.36–2.58 (m, 6 H), 3.26 (br s, 1 H), 3.64–3.94 (m, 1 H), 4.17–4.38 (m, 1 H), 7.48–7.66 (m, 3 H), 7.88–8.08 (m, 2 H); ^{13}C NMR (20 MHz, $CDCl_3$) 23.15 (t, J = 136 Hz), 30.23 (t, J = 134 Hz), 33.74 (t, J = 131 Hz), 50.90 (d, J = 145 Hz), 80.64 (d, J = 152 Hz), 127.24 (d, J = 161 Hz), 128.57 (d, J = 162 Hz), 133.48 (d, J = 161 Hz), 136.80 (s), 194.28 (s) ppm; mass spectrum, m/e (relative intensity) 222 (2), 105 (100), 86 (60), 84 (98), 77 (34), 51 (35); high-resolution mass spectrum, calcd for $C_{12}H_{14}O_2S$ m/e 222.0714, found 222.0722.

Competition Study of Potassium Ethyl Monothiocarbonate (6b) and Potassium Thiobenzoate (6c). A vigorously stirred CH_2Cl_2 (50 mL) slurry of **6b**[8,25] (0.97 g, 6.5 mmol) and **6c** (1.14 g, 6.5 mmol) was cooled to 0–5 °C. To this slurry was added **7** (0.75 g, 4.6 mmol). After 15 min the reaction mixture was allowed to warm to room temperature and was then maintained at this temperature for 18 h. Filtration of the reaction mixture followed by evaporation of the filtrate in vacuo at 20 °C afforded a 70/30 mixture (^{1}H NMR analysis) of **9**[8] and **8**, respectively.

Reactions of Mitomycin C (1) with Potassium Thiobenzoate (6c). In a typical reaction, an aqueous solution (200 mL) of **1** (200 mg, 0.60 mmol) was purged with N_2 (10 min) at room temperature. The N_2 bubbling was continued until the reaction's termination. To this solution were added **6c** (0.636 g, 3.61 mmol) and then $Na_2S_2O_4$ (0.627 g, 3.60 mmol) freshly dissolved in H_2O (10 mL). After the reaction mixture was stirred at the desired temperature (10 min), the reaction was terminated by bubbling O_2 through the solution. During the reaction the pH of the solution remained at 6. The reaction mixture was extracted with ethyl acetate (3 × 100 mL), and the combined organic layers were dried (Na_2SO_4) and then evaporated to dryness in vacuo. TLC analysis of the ethyl acetate extract in system A indicated the presence of at least seven violet to red-violet compounds: a major compound (R_f 0.24), three minor compounds (R_fs 0.43, 0.48, and 0.55) and three trace compounds (R_fs 0.07, 0.72, and 0.76). An increase in the relative quantities of the R_f 0.43, 0.48, and 0.76 compounds was observed if TLC analysis was repeated after several hours. For chromatographic purposes, the residue remaining after evaporation of the ethyl acetate extract in vacuo was dissolved in acetone. A small portion of the residue that was not readily soluble was set aside. Preparative thick-layer chromatography in system C separated the acetone solution into four zones. TLC analysis of these zones in system A revealed the following: (1) zone 1 consisted of the R_f 0.07 and 0.24 compounds, (2) zone 2 contained the R_f 0.43 and 0.55 compounds, (3) zone 3 corresponded to a compound with the R_f value of 0.48, and (4) zone 4 consisted of the R_f 0.72 and 0.76 compounds.

Preparative thick-layer chromatography of zone 1 in system B afforded 33.8 mg (13%) of red-violet **12** (R_f 0.24): mp 78–82 °C; IR (KBr) 1720, 1665, 1605, 1500, 1385, 1240 cm^{-1}; UV (MeOH) λ_{max} nm 203, 214, 248, 305, 343; thermospray-ionization mass spectrum, m/e (relative intensity) 397 (100), 382 (67), 244 (28), 234 (79), 233 (35), 229 (80). The quantity of the R_f 0.07 compound was too small to be isolated.

Compounds **13** and **14** were obtained in pure form by preparative thick-layer chromatography of zone 2 in system B. The red-violet **13** (R_f 0.43) was isolated in 3% (7.0 mg) yield: mp 137–140 °C; IR (KBr) 1720, 1665, 1605, 1490, 1470, 1385, 1325, 1270, 1240 cm^{-1}; UV (MeOH) λ_{max} nm 200, 249, 300, 345; field-desorption mass spectrum, m/e 485.

The red-violet **14** (R_f 0.55) was isolated in 1.5% (3.8 mg) yield: mp 74–77 °C; IR (KBr) 1660, 1600, 1470, 1425, 1385, 1355, 1280, 1235 cm^{-1}; UV (MeOH) λ_{max} nm 203, 247, 304, 355; thermospray-ionization mass spectrum, m/e (relative intensity) 518 (100), 244 (6), 229 (8).

Zone 3 was identified as compound **15** (R_f 0.48): yield 5.0 mg (2%); mp 97–100 °C; IR (KBr) 1715, 1660, 1600, 1485, 1450, 1380, 1320, 1235 cm^{-1}; UV (MeOH) λ_{max} nm 200, 248, 303, 345; thermospray-ionization mass spectrum, m/e (relative intensity) 302 (100), 301 (65), 299 (26).

Thick-layer chromatography in system D resolved zone 4 into its components. The R_f 0.72 (system A) compound was tentatively identified as **18**: yield 0.68 mg (0.2%); field-desorption mass spectrum, m/e 621.

The R_f 0.76 (system A) compound was identified as **17**: yield 0.80 mg (0.2%); field-desorption mass spectrum, m/e 621.

TLC analysis of the slightly acetone insoluble residue indicated the presence of the R_f 0.07 compound and small amounts of the higher R_f compounds. Thick-layer chromatography of this material in system B afforded 0.72 mg (0.3%) of a compound tentatively identified as **16**.

Reaction of 12 with Ethyl Chloroformate. To a THF solution (5 mL) of **12** (9.07 mg, 0.021 mmol) were added 3.8 μL (0.027 mmol) of Et_3N and 2.0 μL (0.021 mmol) of ethyl chloroformate. The reaction solution was stirred at room temperature (4 h) and then evaporated in vacuo. TLC analysis of the residue in system A revealed the presence of unreacted **12** and a new adduct (R_f 0.50). Preparative thick-layer chromatography of this mixture in system D afforded 1.4 mg (13.4%) of **19**.

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Registry No. 1, 50-07-7; **6b**, 35832-93-0; **6c**, 28170-13-0; **7**, 368-39-8; **8**, 1484-17-9; **9**, 3554-12-9; **10**, 285-67-6; **11**, 93604-41-2; **12**, 93644-77-0; **13**, 93604-42-3; **14**, 93604-43-4; **15**, 93712-79-9; **16**, 93604-44-5; **17**, 93644-78-1; **18**, 93778-93-9; **19**, 93604-45-6.

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