in *n*-hexane as a mobile phase. High-R_f enantiomer: mp 70.0–73.0 °C; $[\alpha]_D$ −35.4° (*c* 0.54, CH_2Cl_2). Low-R_f enantiomer: mp 71.0–73.5 °C; $[\alpha]_D$ +39.0° (*c* 0.8, CH_2Cl_2).

Acknowledgment. This work has been supported by a grant from the National Science Foundation.

Registry No. (*S*)-**2** (Ar = phenyl; R = CH_3; Y = H), 19145-06-3; (*S*)-**2** (Ar = phenyl; R = C_2H_5; Y = H), 87858-37-5; (*S*)-**2** (Ar = phenyl; R = *i*-C_3H_7; Y = H), 87858-38-6; (*S*)-**2** (Ar = phenyl; R = CH_3; Y = CH_3), 19144-86-6; (*S*)-**2** (Ar = phenyl; R = C_2H_5; Y = CH_3), 20306-86-9; (*S*)-**2** (Ar = phenyl; R = *i*-C_3H_7; Y = CH_3), 62474-74-2; (*S*)-**2** (Ar = *p*-anisyl; R = CH_3; Y= CH_3), 82776-14-5; (*S*)-**2** (Ar = 1-naphthyl; R = CH_3; Y = CH_3), 82796-68-7; (*S*)-**2** (Ar = 1-naphthyl; R = CH_3; Y = C_2H_5), 87782-87-4; (*S*)-**2** (Ar = 1-naphthyl; R = CH_3; Y = *n*-C_3H_7), 87782-88-5; (*S*)-**2** (Ar = 1-naphthyl; R = CH_3; Y = *n*-C_6H_{13}), 87782-89-6; (*S*)-**2** (Ar = 1-naphthyl; R = CH_3; Y = *n*-$C_{11}H_{23}$), 61761-45-3; (*S*)-**2** (Ar = 1-naphthyl; R = CH_3; Y = OCH_3), 87782-90-9; (*S*)-**2** (Ar = 1-napthyl; R = CH_3; Y = OC_2H_5), 87782-91-0; (*S*)-**2** (Ar = 1-naphthyl; R = CH_3; Y = $NHCH_3$), 87782-92-1; (*S*)-**2** (Ar = 1-naphthyl; R = CH_3; Y = NH-*n*-C_4H_9), 87801-35-2; (*S*)-**2** (Ar = 1-naphthyl; R = CH_3; Y = CF_3), 66964-33-8; (−)-**2** (Ar = 1-naphthyl; R = *i*-C_3H_7; Y = CH_3), 87782-93-2; (−)-**2** (Ar = 1-naphthyl; R = *i*-C_3H_7; Y = *n*-C_3H_7), 87782-94-3; (−)-**2** (Ar = 1-naphthyl; R = *i*-C_3H_7; Y = *n*-$C_{11}H_{23}$), 87782-95-4; (−)-**2** (Ar = 1-naphthyl; R = *i*-C_3H_7; Y = OCH_3), 87782-96-5; (−)-**2** (Ar = 1-naphthyl; R = *i*-C_3H_7; Y = OC_2H_5), 87782-97-6; (−)-**2** (Ar = 1-naphthyl; R *i*-C_3H_7; Y = $NHCH_3$), 87782-98-7; (−)-**2** (Ar = 1-naphthyl; R = *i*-C_3H_7; Y = NH-*n*-C_4H_9), 87782-99-8; (−)-**2** (Ar = 1-naphtyl; R = *i*-C_3H_7; Y = CF_3), 87783-00-4; (*S*)-**2** (Ar = 2-naphthyl; R = CH_3; Y = CH_3), 87783-01-5; (*S*)-**2** (Ar = 2-naphthyl; R = CH_3; Y = *n*-C_3H_7), 87783-02-6; (*S*)-**2** (Ar = 2-naphthyl; R = CH_3; Y = *n*-$C_{11}H_{23}$), 87783-03-7; (−)-**2** (Ar = 6,7-dimethyl-1-naphthyl; R = CH_3; Y = CH_3), 87783-04-8; (−)-**2** (Ar = 6,7-dimethyl-1-naphthyl; R CH_3; Y = *n*-C_3H_7), 87783-05-9; (−)-**2** (Ar = 6,7-dimethyl-1-naphthyl; R = CH_3; Y = *n*-$C_{11}H_{23}$), 87783-06-0; (+)-**2** (Ar = 6,7-dimethyl-1-naphthyl; R = *i*-C_3H_7; Y = CH_3), 87783-07-1; (+)-**2** (Ar = 6,7-dimethyl-1-naphthyl; R = *i*-C_3H_7; Y = *n*-$C_{11}H_{23}$), 87783-08-2; (+)-**2** (Ar = 6,7-dimethyl-1-naphthyl; R = *c*-C_6H_{11}; Y = CH_3), 87783-09-3; (+)-**2** (Ar = 6,7-dimethyl-1-naphthyl; R = *c*-C_6H_{11}; Y = *n*-C_3H_7), 87783-10-6; (+)-**2** (Ar = 6,7-dimethyl-1-naphthyl; R = *c*-C_6H_{11}; Y = *n*-$C_{11}H_{23}$), 87783-11-7; (−)-**2** (Ar = 6,7-dimethyl-2-naphthyl; R = *i*-C_3H_7; Y = CH_3), 87783-12-8; (−)-**2** (Ar = 6,7-dimethyl-2-naphthyl; R = *i*-C_3H_7; Y = *n*-$C_{11}H_{23}$), 87783-13-9; isobutyryl chloride, 79-30-1; 2,3-dimethylnaphthalene, 581-40-8; 6,7-dimethyl-1-naphthyl isopropyl ketone, 87783-14-0; 6,7-dimethyl-2-naphthyl isopropyl ketone, 87783-15-1; 6,7-dimethyl-1-naphthyl cyclohexyl ketone, 41284-79-1; α-(6,7-dimethyl-1-naphthyl)isobutylamine, 87783-16-2; α-(6,7-dimethyl-2-naphthyl)isobutylamine, 87783-17-3; cyclohexyl(6,7-dimethyl-1-naphthyl)methylamine, 87783-18-4; *N*-(10-undecenoyl)-α-(6,7-dimethyl-1-naphthyl)isobutylamine, 87783-19-5; 10-undecenoyl chloride, 38460-95-6.

Reactions of Mitomycin C with Potassium Ethyl Xanthate in Neutral Aqueous Solution

Ulfert Hornemann,*[1] Kazuo Iguchi,[2] Paul J. Keller,[3] Huynh M. Vu,[1] John F. Kozlowski, and Harold Kohn[†]

Department of Medicinal Chemistry and Pharmacognosy, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, Indiana 47907, and Department of Chemistry, University of Houston, Central Campus, Houston, Texas 77004

Received March 24, 1983

The reaction of the antitumor antibiotic mitomycin C with potassium ethyl xanthate was investigated in neutral aqueous solution in the presence and absence of the reducing agent sodium dithionite. The reductive reaction afforded after reoxidation several lipophilic mitosene derivatives. Information on the isolation and structure elucidation of two of these derivatives which were 1,2-trans-disubstituted mitosenes was given earlier (*J. Am. Chem. Soc.* **1979**, *101*, 7121–7124). The present paper reports the isolation of several 1,2-cis-disubstituted-10-(ethylxanthyl)-7-aminodecarbamoylmitosenes and the structure elucidation of two of them. The total yields of the 1,2-trans- and of the 1,2-cis-substituted mitosenes were nearly equal in the reductive reaction, in marked contrast to acid-catalyzed reactions leading to opening of the aziridine ring of mitomycin C which yield cis/trans product ratios in excess of 3. Incubation of mitomycin C with potassium ethyl xanthate and sodium sulfite in the absence of sodium dithionite at 5 °C for 100 h in neutral aqueous medium afforded an aziridinothiourethane. This compound was chemically converted into a mitosene derivative that contained a 1,2-cis-fused thiazoline ring. In the course of high-field ^1H NMR studies of the new mitosene derivatives and of other known mitosenes, a framework for the determination, in favorable cases, of relative stereochemistry at C_1 and C_2 was developed.

Mitomycin C,[4] **1** (Chart I), is a bioreductively[5] activated or acid-activated[6] antitumor antibiotic that is clinically useful for the palliative treatment of various neoplasms.[7] The chemical reactivity of this antibiotic has been the subject of several recent investigations.[5,6,8–16] These investigations and earlier studies summarized in ref 5 and 7 have shown that while mitomycin C is fairly stable when kept at the oxidation level of a quinone and when kept at near neutral pH, its reduction or its exposure to acidic pH leads to changes in the molecule. Reduction of the quinone chromophore leads to loss of methanol from C_9 and C_{9a},[5,10,17] opening of the aziridine ring with participation of a nucleophile,[14,16] and, in some cases, loss and displacement by sulfur nucleophiles of the carbamoyl group.[14]

[†] University of Houston.

(1) Address correspondence to School of Pharmacy, University of Wisconsin, Madison, WI 53706.

(2) Permanent address: Tokyo College of Pharmacy, 1432-1 Horinouchi, Hachioji, Tokyo 192-03, Japan.

(3) Department of Chemistry, The Ohio State University, Columbus, OH 43210.

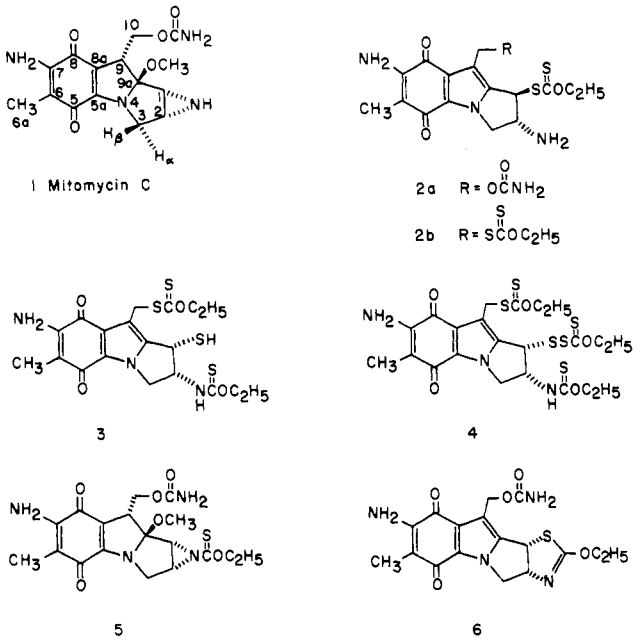
(4) Webb, J. S.; Cosulich, D. B.; Mowat, J. H.; Patrick, J. B.; Broschard, R. W.; Meyer, W. E.; Williams, R. P.; Wolf, C. F.; Fulmor, W.; Pidacks, V.; Lancaster, J. E. *J. Am. Chem. Soc.* **1962**, *84*, 3187.

(5) Szybalski, W.; Iyer, V. N. In "Antibiotics. Mechanism of Action"; Gottlieb, D., Shaw, P. D., Eds.; Springer: New York, 1967; Vol. 1, pp 211–245.

(6) Lown, J. W.; Begleiter, A.; Johnson, D.; Morgan, A. R. *Can. J. Biochem.* **1976**, *54*, 110–117.

0022-3263/83/1948-5026$01.50/0

Chart I. Structures of Mitomycin C and Ethyl Xanthate Adducts



At pH values lower than 4.0 the antibiotic decomposes by expulsion of methanol from C_9 and C_{9a} and opening of the aziridine ring which is accompanied by introduction of a new substituent exclusively at C_1.[9] The resulting stereochemistry is invariably such that cis products predominate over trans products by a ratio of 3:1 or more.[9,11,13]

Mitomycin C forms covalent bonds with biological macromolecules, notably with DNA upon reductive[5,8] or acid-catalyzed activation.[6] Direct evidence for its binding to DNA has been provided by Hashimoto et al.[18] who showed by analysis of alkylated nucleotides liberated by P1 nuclease digestion that C_1 of mitomycin C can be bound efficiently to O^6 of deoxyguanosine and N^6 of deoxyadenosine and less efficiently to N^2 of deoxyguanosine. Bond formation between C_1 of reductively activated mitomycin C and O^6 of guanine in the deoxydinucleotide d(GpC) has also been reported by Tomasz and co-workers.[19] Several authors have suggested that DNA can be covalently cross-linked by activated mitomycin. The chemical nature of the covalent cross-link, however, has remained elusive. It was postulated by Iyer and Szybalski in 1964 that C_1 and C_{10} of reductively activated mitomycin C could form a cross-link via two guanosine residues on opposite strands by attachment to O^6 of the guanine bases. No proposal exists presently concerning the possible chemical nature of a DNA cross-link formed by acid-activated mitomycin.

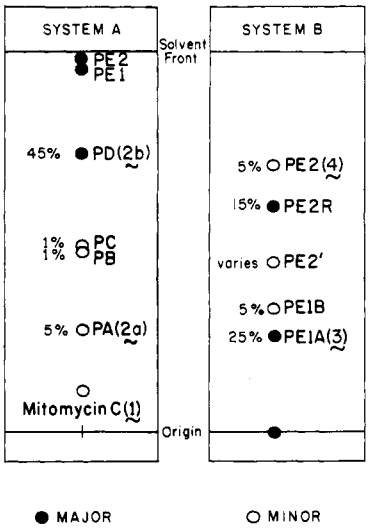


Figure 1.

In order to contribute to a better understanding of the mode of action of mitomycin C, we have investigated the reactivity of the reduced antibiotic toward nucleophiles in aqueous solution, to determine which sites are subject to nucleophilic attack under these conditions. In the present work and in the previously communicated studies[14] aqueous solutions of **1** were reduced at 0–5 °C with sodium dithionite for 10 min in the presence of a nucleophile, followed by reoxidation with oxygen. Potassium ethyl xanthate proved to be a very suitable nucleophile which afforded high yields of nonpolar products, whereas earlier studies with $NaHSO_3$ as a nucleophile had yielded highly polar reaction products.[10] Several other nucleophiles including potassium phthalimide, NaN_3, morpholine, Meldrum's acid (sodium salt), and sodium diethyl phosphate, as a dinucleotide model were also tested but were found to be unreactive. Only KCN, KCNO, and KCNS yielded products albeit in low yield. From the reaction mixture containing potassium ethyl xanthate two 1,2-trans-disubstituted mitosenes **2a** and **2b** were isolated and structurally elucidated as described previously.[14] Compound **2b** constituted the first report of a mitosene containing a new substituent at C_{10} which arose in a redox reaction of mitomycin C. The ratio of the total 1,2-trans products to the total 1,2-cis products, compounds **3** and **4** and their relatives (compounds PE2R and PE1B), was approximately 1:1.

While investigating the possible displacement of sulfonate groups located on mitosenes by a reductive reaction in the presence of potassium ethyl xanthate we observed the formation of the mitosane **5**. It subsequently became apparent that this mitosane arose from excess free mitomycin C present in the reaction mixture and that its formation did not require any reductive activation of the antibiotic. Compound **5** was converted into compound **6** which was shown to contain a 1,2-cis-fused thiazoline ring. During the course of our investigations on the structures of the movel mitosenes a theoretical framework for the determination of the conformation of the saturated ring of these compounds was developed on the basis of high field ^1H NMR data. It was found that in favorable cases reliable determinations of the relative stereochemistry of substituents of this ring are possible through consideration of the couplings between vicinal protons. Stereochemical assignments of 1,2-disubstituted mitosenes are also possible by the use of CD spectroscopic analysis as reported very

(7) Carter, S. K.; Crooke, S. T. "Mitomycin C - Current Status and New Developments"; Academic Press: New York, 1979.
(8) Tomasz, M.; Mercado, C. M.; Olsen, J.; Chatterjie, N.; *Biochemistry* **1974**, *13*, 4878–4887.
(9) Taylor, W. G.; Remers, W. A. *J. Med. Chem.* **1975**, *18*, 307–311.
(10) Hornemann, U.; Ho, Y. K.; Mackey, J. K.; Srivastava, S. C. *J. Am. Chem. Soc.* **1976**, *98*, 7069–7074.
(11) Cheng, L.; Remers, W. A. *J. Med. Chem.* **1977**, *20*, 767–770.
(12) Lown, J. W.; Weir, G. L. *Can. J. Chem.* **1978**, *56*, 249–257.
(13) Tomasz, M.; Lipman, R. *J. Am. Chem. Soc.*, **1979**, *101*, 6063–6067.
(14) Hornemann, U.; Keller, P. J.; Kozlowski, J. F. *J. Am. Chem. Soc.* **1979**, *101*, 7121–7124.
(15) Hashimoto, Y.; Shudo, K.; Okamoto, T. *Chem. Pharm. Bull.* **1980**, *28*, 1961–1963.
(16) Tomasz, M.; Lipman, R. *Biochemistry* **1981**, *20*, 5056–5061.
(17) Patrick, J. B.; Williams, R. P.; Meyer, W. E.; Fulmor, W.; Cosulich, D. B.; Broschard, R. W. *J. Am. Chem. Soc.* **1964**, *86*, 1889–1890.
(18) Hashimoto, Y.; Shudo, K.; Okamoto, T. *Tetrahedron Lett.* **1982**, *23*, 677–680.
(19) Tomasz, M.; Lipman, R.; Snyder, J. K.; Nakanishi, K. *J. Am. Chem. Soc.* **1983**, *105*, 2059–2063.

recently by Tomasz and co-workers.[19]

Results

Mitomycin C–Potassium Ethyl Xanthate Redox Reaction Products. Reduction of deoxygenated solutions of mitomycin C with sodium dithionite and with palladium on charcoal and hydrogen gas in the presence of potassium ethyl xanthate yielded colorless and purple reaction mixtures, respectively. Upon termination of the reactions by bubbling oxygen gas violet-red precipitates formed which adhered to the walls of the reaction vessel and which were readily soluble in ethyl acetate. TLC analysis of the ethyl acetate solution using mainly systems A and B (Experimental Section) indicated the presence of 9 violet-red compounds as shown in Figure 1 which were obtained in the relative yields indicated next to the designation of the compounds. The isolation and purification of a majority of these compounds was readily possible by a combination of high-performance–low-pressure liquid chromatography (HPLPLC),[20] reverse phase HPLPLC, and preparative TLC. The structures of compounds **3** and **4** (Chart I) designated PE1A and PE2, respectively, on the chromatograms represented in Figure 1 are reported below, while as indicated above the structures of compounds **2a** (PA) and **2b** (PD) have already been communicated.[14] The structure of compound PE2R appears to be very closely related to **3** and **4** but a complete structural assignment awaits to be accomplished. Compounds PB and PC are produced usually in low yield, the formation of PE2′ was erratic and PE1B proved to be very unstable, therefore no attempts were made to elucidate their structures.

Control studies and examination of the influence of reaction variables revealed that none of the violet-red colored compounds were formed when either the potassium ethyl xanthate or the reducing agent was omitted from the reaction mixture. The same family of products, but in different ratios, were formed when catalytic reduction by hydrogen with palladium on carbon was used in place of sodium dithionite. The catalytic reduction favors the formation of the more polar products, thus increased yields of PA, PB, and PC and decreased yields of the PE compounds are observed. However, in preparative scale reactions using 50 mg of mitomycin C, large amounts of uncharacterized polymeric material form in this catalytic reduction reaction, hence the utility of the catalytic procedure is limited. Under the standared dithionite-mediated redox conditions, the pH of the reaction mixture remains between 7.5–8.0 throughout the course of the reaction. Most of the reaction products were also observed when the reaction was allowed to proceed for only 30 s but no detailed kinetic studies have been carried out.

The structural studies on **3**, **4**, and PE2R relied on high-field ^1H and ^{13}C NMR data, UV spectroscopy, field-desorption mass spectra (FD-MS), and chemical interconversions. The UV spectra of these three compounds (see Experimental Section) are similar to one another and are compatible with those of mitosenes. Furthermore they indicate the presence of xanthate by absorptions at ca. 270 nm. FD-MS (Experimental Section) yielded molecular ions for **3** and **4**, and the observed fragmentations were apparently largely due to losses from the xanthate residues. Features common to the 360-MHz ^1H NMR spectra of the compounds (Table I) include a pentet signal for the C_2 proton, which arises due to nearly equal vicinal couplings from C_1H, C_2NH, C_3H_α, and C_3H_β. The integral and coupling pattern for the C_2NH signal demonstrate further

(20) Michel, K.; Miller, R. U.S. Patent 4 131 347, 1978. Equipment and additional information are available from Ace Glass Co., Vineland, NJ.

Table I. 360-MHz ^1H NMR Data

assignment[a]	3[b]	PE2R[b]	4[b]	5[b]	6[c]
C_1H	5.06, d, 6.3[d]	4.93, d, 9.0	5.10, d, 7.2	3.56, d, 4.6	5.36, d, 7.4
C_2H	5.63, p, 7–9	5.50, p, 7–9	5.73, p, 7–9	3.42, dd, 4.6, 2.0	5.46, ddd, 7.4, 7.0, 2.7
C_2NH	7.64, d, br, 8	7.18, d, br, 8.0	7.41, d, br, 9.0		
C_3H_α	4.14, dd, 12.6, 9.0	3.87, dd, 13.0, 9.0	3.84, dd, 12.6, 9.0	3.53, dd, 13.5, 2.0	4.22, dd, 13.2, 2.6
C_3H_β	4.74, dd, 12.6, 8.1	4.83, dd, 13.0, 8.1	4.80, dd, 12.6, 8.1	4.54, d, 13.5	4.53, dd, 13.2, 7.0
C_6CH_3	1.81, s	1.81, s	1.83, s	1.76, s	1.73, s
C_7NH_2	4.97, br	4.92, br	4.96, br	5.19, br	6.59, br
OCH_3				3.19, s	
OCH_2CH_3	1.33, t, 7.2	1.35, t, 7.2	1.38, t, 7.2	1.32, t, 7.1	1.21, t, 7.1
OCH_2CH_3	1.36, t, 7.2	1.41, t, 7.2	1.40, t, 7.2		
OCH_2CH_3			1.46, t, 7.2		
OCH_2CH_3	4.51, dq, 7.1, 1.8	4.51, q, 7.2	4.56, q, 7.2	4.46, q, 7.1	4.25, m
OCH_2CH_3	4.58, dq, 7.2, 4.2	4.65, q, 7.2	4.67, q, 7.2		
OCH_2CH_3			4.70, dq, 7.2		
$C_{10}H_2$	4.59, ABq, 13.5	4.57, ABq, 13.5	4.59, ABq, 13.5	4.31, t, 10.9	5.04, s
				4.91, dd, 10.9, 4.7	
C_9H				3.67, dd, 10.9, 4.7	

[a] Satisfactory integrations were obtained for all signals. Assignments are supported by homonuclear decoupling experiments; s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, br = broad, m = multiplet. [b] Solvent = $CDCl_3$. [c] Solvent = Me_2SO-d_6. Chemical shift in ppm from Me_4Si, multiplicity, coupling constant (s) in hertz.

Table II. 50.3-MHz ^{13}C NMR Data[a-d]

assignment	3	4	5
C_1	d, 48.3[a]	49.1[a]	d, 41.8[a]
C_2	d, 59.2[a]	59.2[a]	d, 47.6[a]
C_3	t, 48.3	48.5	t, 48.7
C_{5a}	s, 146.1[b]	145.9[b]	s, 154.1[b]
C_5	s, 177.8[c]	178.1[c]	s, 178.4[c]
C_6	s, 107.6[b]	107.7[b]	s, 105.8[d]
C_{6a}	q, 8.2	8.1	q, 7.9
C_7	s, 135.7[b]	135.8[b]	s, 147.0[b]
C_8	s, 177.1[c]	177.1[c]	s, 175.9[c]
C_{8a}	s, 115.4[b]	114.6[b]	s, 110.4[d]
C_9	s, 121.8[b]	121.9[b]	d, 44.8[a]
C_{9a}	s, 129.6[b]	129.4[b]	s, 105.2[d]
C_{9a}-OCH_3			q, 49.7
C_{10}	t, 30.6	30.6	t, 62.1
C_{10a}			s, 156.3
C_{10}-C=S	s, 214.3	214.4	
OCH_2CH_3	t, 67.3	67.4	t, 69.3
OCH_2CH_3	t, 70.2	70.2	
OCH_2CH_3		72.3	
OCH_2CH_3	q, 13.9	13.9	q, 13.6
OCH_2CH_3	q, 14.4	14.2	
OCH_2CH_3		13.7	
-NHC=(S)O-	s, 191.2	190.9	
-SSC=(S)O-		210.4	
>NC=(S)O-			s, 201.6

[a-d] Assignments may be interchanged within these classes. Solvent = $CDCl_3$.

substitution of this nitrogen. Homonuclear decoupling experiments support these assignments. Distinction between the C_3H_α and C_3H_β resonances rests on the large chemical shift differences of these signals in mitosenes and their correlation with those signals in ^1H NMR spectra of **2a** and **2b**, which as outlined below, assume conformations which allow relative stereochemical determinations to be made. Interesting resonances in the ^{13}C NMR spectrum of **3** (Table II) include the low-field singlets for the C_{10} thiocarbonyl at δ 214.3 and the thiourethane carbon at δ 191.2. These assignments are based in part on the observation that the thiocarbonyls of the model compounds *S*-ethyl *O*-ethyl xanthate and *S*-benzyl *O*-ethyl xanthate show ^{13}C NMR signals at δ 214 and 213, respectively, and that the model compound morpholinothiourethane shows an absorption at δ 188.3. Resonances appearing in the ^{13}C NMR spectrum of **4** include signals equivalent to those discussed for **3** as well as an additional low field signal at δ 210.4 corresponding to a third thiocarbonyl. The assignment of this signal is based on the observation that the model compound diethyl dixanthogenate (CH_3CH_2O-C(=S)SSC(=S)OCH_2CH_3) shows a thiocarbonyl absorption at δ 207.2. The presence of thiols in **3** and PE2R was indicated by the observation that chloroform solutions of both compounds gave a violet colored precipitate upon addition of ethanolic $HgCl_2$, while **4** does not. Compound PE2R is most likely derived from **3** and presumably carries a substituent on the sulfur atom of the thiol group of **3** which however still affords reactivity with $HgCl_2$ for reasons which require further experimental studies.

The forgoing information in concert with the information available on compounds **2a** and **2b** is compatible with the following mechanistic proposals. Initial attack of the xanthate anion at C_1 of reductively activated mitomycin C leads to both trans and cis substituted adducts. In the case of the cis adduct, however, acyl migration occurs yielding the amide thiol **3**. Compound **3** subsequently gives rise to the other cis compounds of this series, compounds **4**, PE2R, and PE1B. Absolute stereochemical relationships for **3**, **4**, and PE2R were assigned on the basis of the known configuration of mitomycin C and the likely assumption[9] of an α orientation for the C_2 amino group.

Compounds **3**, **4**, and PE2R are readily interconvertible. An additional unstable, and incompletely characterized red-violet colored product of the initial mitomycin C–ethyl xanthate redox reaction, which is denoted as PE1B, also participates in these interconversion reactions. Compound PE1B has properties which are very similar to those of compounds **3** and PE2R. However, in the field desorption mass spectrum there appear besides a strong signal at m/z 485 three signals (485 + 32, 485 + 64, and 485 + 96) indicating the presence of additional surfur possibly due to formation of a polysulfide. PE1B gives rise to **3** upon heating or treatment with mild base, which also converts PE2R into **3**. Treatment of **3** with H_2S, used with the intent of providing a mildly acidic environment, mediates the formation of PE1B and PE2R. Compound **4** gives **3**, PE2R, and PE1B after selective reduction of the disulfide with dithiothreitol. Conversely, treatment of **3**, PE2R, and PE1B with potassium ethyl xanthate under oxidizing conditions forms **4**.

Products of the Nonreductive Reaction of Mitomycin C with Potassium Ethyl Xanthate. Mitomycin C in Tris·HCl buffer (pH 7.4) was treated with excess potassium ethyl xanthate in the presence of excess sodium sulfite at 5 °C for 100 h. The aqueous reaction mixture was extracted with ethyl acetate and TLC analysis in system C (Experimental Section) showed the presence of a blue compound as the major product (R_f 0.34) besides minor products having green (R_f 0.11), blue (R_f 0.44), and red (R_f 0.60) colors, respectively, and unreacted starting material (R_f 0.03). The major product was isolated by HPLPLC in 37% yield. Spectroscopic studies and chemical interconversions, which are presented below, have shown that the major product possesses structure **5**. A tenfold excess of the reagents over mitomycin C appeared to be required for the reaction to proceed at a significant rate. The formation of **5** was dependent on both potassium ethyl xanthate and sodium sulfite and shorter reaction times clearly led to reduced yields of products. Replacement of sodium sulfite by sodium thiosulfate or sodium chloride led to a dramatic reduction in the amount of **5** and the other blue compounds, while the amounts of the red and green compounds increased. Purging the reaction mixture with oxygen prior to incubation in a sealed container yielded a greater proportion of **5** than an analogous reaction in which nitrogen was used. Incubation of **5** under conditions that led to its formation yielded all of the minor products with the exception of the green compound. The structures of these other compounds have not yet been elucidated.

The FD-MS of **5** showed a strong peak at m/z 422 as well as satellite peak intensities compatible with a molecular formula $C_{18}H_{22}N_4O_6S$. The sulfur atom in the molecule is presumably derived from a xanthate anion. The UV spectrum gave absorption maxima at 214 and 356 nm, which are very similar to those seen in the UV spectrum of mitomycin C,[4] as well as an absorption at 253 nm, which is attributed to a thiocarbonyl bound to the aziridine nitrogen atom. The ^1H NMR spectrum (Table I) showed the presence of a single ethoxy substituent, as well as retention of the methoxy group at C_{9a} and the carbamate amino group. No signal was observed for C_2 amino protons. Eighteen resonances which displayed multiplicities compatible with structure **5** were seen in the proton coupled ^{13}C NMR spectrum (Table II), including a singlet at δ 201.6 which was assigned to the thiocarbonyl carbon. Reduction of **5** with hydrogen over palladium on carbon resulted in the elimination of methanol and the formation

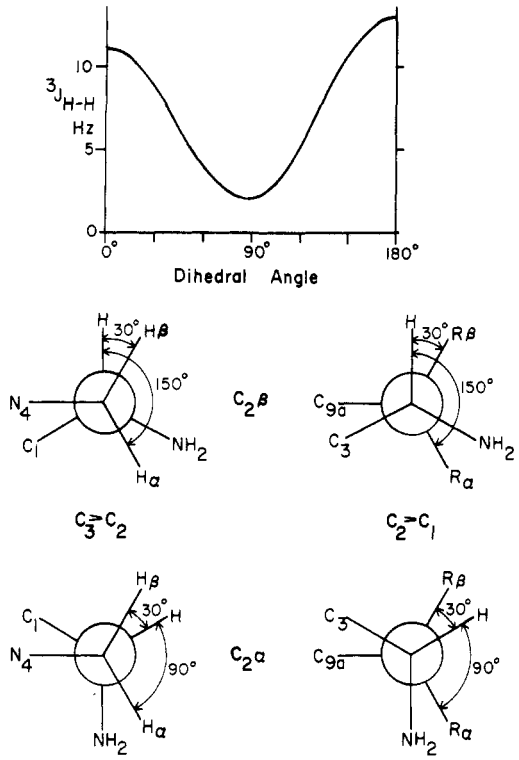


Figure 2. Vicinal proton relationships of mitosenes.

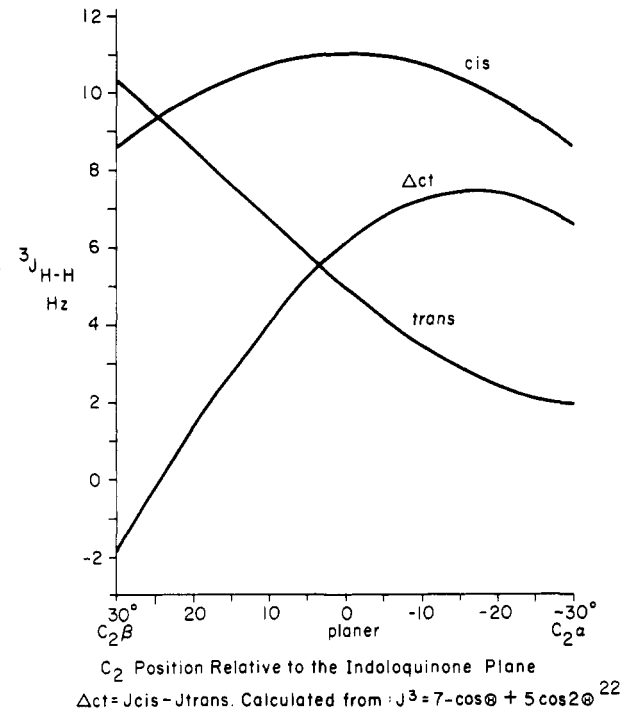


Figure 3. Vicinal proton couplings predicted for intermediate mitosene conformations.

of a thiazoline ring to yield mitosene **6**. The spectral properties of **6** are summarized in the Experimental Section and Table I. A small amount of **6** was also formed when **5** was reduced with $Na_2S_2O_4$ in the presence of potassium ethyl xanthate. This reaction, however, yielded predominantly other compounds. It is suggested in analogy to the predominance of formation of **2b** relative to **2a** that a majority of the reaction products arose by displacement of the carbamoyl group by a xanthate residue; however, this has not been experimentally verified.

Karplussian Analysis of Vicinal Proton Couplings of Mitosenes. It was stated by Taylor and Remers[9] in 1975 that for 1,2-disubstituted mitosenes and related compounds, the H_1–N_2 vicinal couplings are generally not reliable indicators of the relative stereochemistry of the carbons of the saturated five-membered rings bearing these protons, and the applicability of Karplussian analysis in five-membered rings of this kind was questioned. Similar considerations have been reported for disubstituted indans.[21] New information from high-field ^1H NMR spectra of mitosenes that has become available in the course of the present work appears to have changed this outlook. In conjunction with an examination of molecular models of mitosenes this new information leads to the recognition that the vicinal coupling data can be rationalized with the Karplus relationship on the basis of conformational effects in the saturated ring, and that, in favorable cases, relative stereochemical assignments are indeed possible.

It appears reasonable to assume that the two rings of the aromatic indoloquinone system and their one-bond substituents define a plane, which restricts the saturated ring to a single degree of conformational freedom. This freedom is expressed by the puckering of this ring caused by the movement of the C_2 atom out of the plane defined by the remainder of the molecule. Model building studies indicate two extreme conformations denoted as $C_2\alpha$ and $C_2\beta$, in which the C_2 atom is positioned 30° below or above the plane of the indoloquinone system, respectively (see Figure 2). Karplussian analysis of the $C_2\beta$ conformation leads to the expectation of large coupling values (ca. 70% of maximum) for all three vicinal relationships ($^3J_{H1-H2}$, $^3J_{H2-H3\alpha}$, and $^3J_{H2-H3\beta}$) regardless of the stereochemistry of C_1 relative to C_2. Similar analysis of the $C_2\alpha$ conformation reveals that 1,2-trans disubstitution leads to a small value for $^3J_{H1-H2}$, while the cis geometry predicts a large value for this coupling. Furthermore, one large and one small coupling are anticipated for the interactions of the C_2 proton with the two C_3 protons. The analysis of intermediate conformations gives the data depicted in Figure 3, where a maximum for the difference between cis and trans vicinal couplings (Δct) occurs approximately halfway between the coplanar and the $C_2\alpha$ conformations. The values given in this figure for $^3J_{H-H}$ are not expected to strictly correspond with those observed for mitosenes since steric and electronic effects have not been accounted for, but rather this figure is intended to qualitatively show the relationship between conformation and cis and trans vicinal couplings in these systems.

It follows that a conformation which approaches the $C_2\alpha$ extreme would be expected to yield ^1H NMR data that are indicative of cis or trans 1,2-disubstitution, while the $C_2\beta$ conformer would give ambiguous information concerning this geometry. From an experimental point of view, the foregoing arguments imply that observation of a small vicinal coupling for one of the C_3 protons and a large vicinal coupling for the other would allow the conclusion that the time averaged conformation of the mitosene saturated ring approaches the $C_2\alpha$ conformational extreme, and that a large value of $^3J_{H1-H2}$ is a true indicator of cis stereochemistry, while a small value of $^3J_{H1-H2}$ reflects trans disubstitution. In the other situation, where two large vicinal couplings are observed for the C_3 protons, a time averaged $C_2\beta$ conformation would be indicated, but no conclusions could be drawn concerning the relative orientation of the C_1 and C_2 substituents.

Assuming that the above considerations are valid, it is possible to draw the following conclusions for the com-

(21) Huebner, C. F.; Donoghue, E. M.; Novak, C. J.; Dorfman, L.; Wenkert, E. *J. Org. Chem.* **1970**, *35*, 1149–1154.
(22) Bothner-By, A. A. *Adv. Magn. Reson.* **1965**, *1*, 195–316.

Table III. Vicinal Proton Coupling Constants of Mitomycin C-Ethyl Xanthate Redox Products

compd	$^3J_{H1-H2}$[a]	$^3J_{H2-H3\beta}$[a]	$^3J_{H2-H3\alpha}$[a]	conformation of C_2	C1-C2 geometry
2a	0	5	0	α	trans
2b	1.5	5	2	α	trans
3	6	8	9	β	cis[b]
PE2R	9	8	9	β	cis[b]
4	7	8	9	β	cis[b]
6	7	7	2.6	α	cis

[a] J values are in hertz. [b] Deduced from chemical reactions.

pounds listed in Table III: (a) Compounds **2a** and **2b** favor the $C_2\alpha$ conformer; thus the relative geometry at C_1 and C_2 can be deduced from their spectra as trans. (b) Compounds **3**, **4**, and PE2R display coupling data which are compatible with a $C_2\beta$ conformation, and therefore relative stereochemistry cannot be assigned to them on this basis. (c) Compound **6** gives an example of a $C_2\alpha$ conformation for which $^3J_{H1-H2}$ is representative of cis- 1,2-disubstitution. It is expected that substituent effects on the vicinal coupling constants, which are generally manifested as vertical displacements of the Karplus curve, should not vary considerably within the series thus far presented.

Activities against P388 Mouse Leukemia. Compounds **3**, **4**, and **5** have been tested for anticancer activity in the P388 system at Bristol Laboratories, Syracuse, NY. Compound **5** gave a mean survival time which was 144% that of saline controls at a dosage of 25.6 mg/kg, ip. Compounds **3** and **4** were inactive and **4** proved to be toxic to mice. This lack of activity is paralleled by our finding that **2a** bearing a ^{14}C label at the xanthate methylene, did not lose any radioactivity upon its reductive conversion into **2b** in the presence of a large excess of unlabeled potassium ethyl xanthate (data not shown). This result indicates that a xanthate at C_1 of a mitosene cannot be displaced under redox conditions, and it can be assumed that under physiological conditions a xanthate at C_{10} would also be stable.

Discussion

In accord with the results of previous studies reported from this laboratory,[14] it is shown in the present investigation that the reduction of mitomycin C with $Na_2S_2O_4$ in aqueous solution at near neutral pH in the presence of the strong nucleophile potassium ethyl xanthate yields reaction products which demonstrate that C_1 and C_{10} of the antibiotic are reaction centers under these conditions. Compounds known to carry substituents at these two centers amount to approximately 90% of the total reaction products, and thus the present model system affords strong, albeit indirect support for the suggestion by Iyer and Szybalski[5] that C_1 and C_{10} of reduced mitomycin may be reactive centers involved in the alkylation and cross-linking of DNA. Two mitosenes PE2R and PE1B which constitute approximately 15% and 5%, respectively, of the total reaction products await to be structurally elucidated although it is clear that they are very closely related to **3** and **4**. In addition several minor reaction products, all of them probably mitosenes such as the compounds PB, PC, and PE2', remain to be structurally elucidated to examine whether they conform to the C_1 and C_{10} reactivity pattern, or whether they represent structures indicative of possibly different modes of activation of the antibiotic.

It is very likely that compounds **3** and **4** represent 1,2-cis-disubstituted mitosenes. Since **3**, **4**, and PE2R amount to approximately 50% of the total products, while **2a** and **2b** together also account for approximately 50% of the products, cis and trans opening of the aziridine ring appears to occur with nearly equal likelihood upon reductive activation of mitomycin C under the reported experimental conditions. Similar observations were reported by Hashimoto et al.[15] and by Tomasz and Lipman.[16] These findings are in marked contrast to the acid-catalyzed opening of the aziridine ring of mitomycin C and its relatives, which yield cis-1,2-disubstituted mitosenes over trans-1,2-disubstituted mitosenes by a factor of 3 to 9:1, most often by a factor of 6:1.[9,11,13] The mechanistic reasons for the preponderance of cis products in the acid-catalyzed reaction and for the nearly equal cis/trans proportions in products arising from reductive activation of the mitomycins are presently unclear. A further distinction between reductive activation as observed in the present study and acid-catalyzed activation of the mitomycins lies in the fact that C_1 and C_{10} appear to be essentially equally reactive with xanthate, while C_1 is far more reactive than C_{10} under acidic conditions.[23]

The ideas concerning the assignment of stereochemistry of the saturated ring of mitosenes developed in this paper were subsequently used to analyze the stereochemistry of two other mitosenes, *cis*-2-acetamido-1-acetyl-7-aminomitosene[9] and 2,7-diaminomitosene,[16] for which sufficient coupling information has been reported and $C_2\beta$ conformations were deduced for both compounds. Additionally, high-field ^1H NMR data from ten other mitosenes, whose structures are not yet published,[24] show vicinal couplings which are compatible with the present arguments. From the currently available data, it appears that the $C_2\beta$ conformation is favored in the absence of steric or electronic influences; however the reasons for the observed conformational preferences are unclear. The benzaldehyde Schiff base adduct of **2b**[14] displays couplings indicative of a $C_2\alpha$ conformation as does **2b** itself, thus N_2 substitution alone does not force the time averaged conformation to change to $C_2\beta$. It will be of interest in future studies to examine whether conditions can be found which would permit the chemical conversion of mitosenes that are prone to assume the $C_2\beta$ conformation into derivatives that assume $C_2\alpha$ conformations, as this could allow assignment of stereochemistry. Alternatively, it will be of interest to examine whether these conformational effects exhibit a solvent dependency. To date, no X-ray crystallographic study of a mitosene has been reported, and thus independent verification of the validity of these ideas about conformations in these systems by this method is presently not possible.

The observed reactivity of both C_1 and C_{10} toward potassium ethyl xanthate differs from recent work reported by Tomasz and Lipman[16,19] and by Hashimoto et al.[15] These authors reduced mitomycin C with a rat liver microsomal system and by catalytic reduction with PtO_2 and Pt/C and hydrogen, respectively, and observed that the rat liver microsomal and the catalytic systems did not afford any products that represented reactivity at C_{10}, as all the compounds isolated still carried the C_{10} carbamoyl group. Since it was reported earlier that mitomycin C does cross-link DNA[5,6,8] and that both the aziridine ring and the carbamate are necessary structural components for cross-linking activity,[25] the possibilities exist that the

(23) Stevens, C. L.; Taylor, K. G.; Munk, M. E.; Marshall, W. S.; Knoll, K.; Shaw, G. D.; Shaw, L. G.; Uzu, K. *J. Med. Chem.* **1965**, *8*, 1-10.

(24) Hornemann, U.; Takeda, K. 182nd American Chemical Society National Meeting, Division of Medicinal Chemistry, New York, NY, Aug 23–28, 1981; American Chermical Society: Washington, DC; Abstr. No. 89.

(25) Otsuji, N.; Murayama, I. *J. Bacteriol.* **1972**, *109*, 475–483.

microsomal and catalytic systems used are not conducive to bifunctional reactivity or that only a minor fraction of the reaction products, as yet uninvestigated, contains compounds representative of cross-link formation.

It is of interest in this connection that novel mitomycins were recently reported which carry an exocyclic methylene group instead of a (carbamoyloxy)methyl group at C-9.[26,27] Some of these compounds show pronounced antitumor activity.[27] An exocyclic methylene group can conceivably impart on these derivatives a reactivity similar to that of the (carbamoyloxy)methyl group of mitomycin C. This would strengthen the assumption that C_{10} of the mitomycins may be involved in bifunctional alkylation.

The formation of compound **5** appears to be catalyzed by Na_2SO_3 but the mechanism of this catalysis is presently not known. It is likewise not understood why the presence of oxygen promotes the formation of **5**. The exclusive cis opening of the aziridine ring of **5** in the reaction yielding **6** is presumably related to a reaction reported by Taylor and Remers in which interaction of **1** and acetic anhydride yielded exclusively *cis*-1-acetoxy-2-acetamidomitosene.[9] While the cis stereochemistry of C is well supported by NMR data it is not clear why this compound assumes the $C_2\alpha$ conformation, likewise it is not clear why compounds **2a** and **2b** assume this conformation.

Results of related studies on the interaciton of reductively activated mitomycin C and potassium ethyl monothiocarbonate are reported in the following paper.[28]

Experimental Section

Analytical TLC Systems. A: silica gel, 1-octanol/acetone/ligroin (90–115 °C), 2:5:5. B: silica gel, $CHCl_3$/ethyl acetate, 4:1. C: silica gel, ethyl acetate/$CHCl_3$, 2:1. D: silica gel, ethyl acetate/hexanes, 1:2. E: silica gel, hexanes/$CHCl_3$/acetone, 2:3:1. F: C_{18} phase bonded silica, CH_3OH/H_2O, 9:1. G: silica gel, hexanes/ethyl acetate, 4:1. Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. UV data were recorded on a Cary 17 or a Coleman 124 spectrophotometer, and optical rotation data were determined with a Perkin-Elmer Model 241 polarimeter. ^1H NMR and ^{13}C NMR spectra were measured on Nicolet instruments NT-360 and NT-200, respectively, accumulating 32K data sets for each spectrum. Proton decoupled ^{13}C NMR spectra were obtained by two-level noise decoupling, while gated decoupling (decoupler off only during acquisition) yielded proton coupled spectra with NOE. Field desorption mass spectra were obtained on a Varian-MAT 731 instrument at Eli Lilly Co., Indianapolis, IN.

Redox Reaction of Mitomycin C. The redox reaction of mitomycin C in the presence of potassium ethyl xanthate was performed as previously described[14] except that the ethyl acetate extract of the reaction mixture was washed once with orthophosphate buffer (0.05 M, pH 7.5) in order to remove remaining ethyl xanthate. Initial TLC analysis of the dried and concentrated ethyl acetate extract in system A showed four major violet-red colored products: R_f 0.11 (**1**), 0.27 (**2a**), 0.74 (**2b**), 0.97 (**3** and PE1B), and 0.99 (**4** and PE2R). Further TLC in system B separated the high R_f material: R_f 0.13 (**2b**), 0.26 (**3**), 0.33 (PE1B), 0.60 (PE2R), and 0.71 (**4**). This was the best system found for the analytical TLC separations of these compounds. Their homogeneity was demonstrated in additional TLC systems: system D, R_f 0.06 (**3**), 0.07 (PE1B), 0.37 (PE2R), 0.04 (**4**); system E, R_f 0.30 (**3**), 0.35 (PE1B), 0.65 (PE2R), 0.73 (**4**); system F, R_f 0.24 (PE1B), 0.30 (**3**), PE2R and **4** decompose in this system; system G, R_f 0.15 (**4** and PE2R), this system provides good separation from more nonplar minor products.

Preparative separation of the ethyl acetate extract of the redox reaction mixture was performed in three chromatographic steps. An initial separation was obtained by HPLPLC. The sample (6 mL) was loaded onto a column (350 × 37 mm; silica gel, Whatman LPS-1) and run with hexanes/ethyl adcetate, 2:1 (30 psi, 10 mL/min); after bands containing **4** and PE2R began to separate, the amount of ethyl acetate in the eluting solvent was gradually increased until the band of **3** and PE1B had eluted. Washing the column with acetone then eluted the 1,2-trans mitosenes (**2a** and **2b**) and unreacted **1**. The pooled fractions containing mostly **4** were reapplied to the top of the same column and eluted with hexanes/ethyl acetate 3:1, thus removing most of the residual PE2R which was combined with the pooled fractions containing mostly PE2R from the first pass separation. The PE2R enriched solution was then separated in the same fashion as described for **4**. The final purifications of **4** and PE2R were accomplished by preparative TLC (Analtech silica gel, 20 × 20 × 0.1 cm; $CHCl_3$/ethyl acetate, 6:1). Compounds **3** and PE1B were not resolved in the initial separation and fractions containing these components were evaporated and redissolved in $CH_3OH/CH_3CN/H_2O$ 7:2:1 and applied to the top of an HPLPLC column (350 × 37 mm, C_{18} phase bonded silica gel, 10% carbon load on LPS-1, prepared according to Kingston and Gerhart[29]). The column was eluted with $CH_3OH/CH_3CN/H_2O$ 7:2:1 (45 psi, 10 mL/min), which resulted in good separation of **3** and PE1B, while residual amounts of **4** and PE2R decompose in this system and tend to remain bound to the solid support. Fractions containing **3** were concentrated to a small volume, diluted with water, and extracted with ethyl acetate. For the final purification of **3**, the dried (Na_2SO_4) and concentrated organic extract was subjected to preparative TLC (Analtech silica gel, 20 × 20 × 0.1 cm; $CHCl_3$/ethyl acetate 5:1). Compounds **3**, **4**, and PE2R were microcrystalline.

3: mp 152–154 °C, $[\alpha]^{20}_D$ −300° (*c* 0.0083, CH_3OH); FD-MS, *m/z* (% of base) likely ion 485 (100.0) M, 486 (27.0) MH, 487 (23.0) MH_2, 484 (29.0) M − H, 483 (10.6) M − H_2, 452 (11.0) M − SH, 427 (13.0), 395 (32.0) M − C_3H_5OS, 363 (8.0) M − ethyl xanthic acid, 361 (12.0) M − H − ethyl xanthic acid. All other peaks <8% of base. UV $\lambda_{max}^{CH_3OH}$ nm (ε) sh = shoulder 252 (28 200), 271 (29 500), 310 sh (12 000), 355 (5500).

PE2R: mp 92–94 °C, $[\alpha]^{20}_D$ −36° (*c* 0.0083, CH_3OH); FD-MS; *m/z* (% of base) 573 (3.0), 485 (100.0), 486 (36.0), 487 (27.5) 364 (2.0). All other peaks <3% of base. UV $\lambda_{max}^{CH_3OH}$ nm (ε) sh = shoulder 250 (30 900), 270 (20 000), 280 sh (19 500), 310 sh (13 200), 355 (5 000).

4: mp 85–87 °C, $[\alpha]^{20}_D$ −40° (*c* 0.0125, CH_3OH); FD-MS, *m/z* (% of base) likely ion 606 (4.3) MH, 605 (2.9) M, 573 (2.5) M − S, 483 (100.0) M − ethyl xanthic acid, 484 (32.8), 485 (32.9), 452 (8.6) *m/z* 484 − S, 451 (8.6) *m/z* 483 − S, 362 (7.5) M − 2 ethyl xanthates, 242 (1.3) M − 3 xanthates, 122 (3.4) ethyl xanthic acid. All other peaks are <2% of base, UV $\lambda_{max}^{CH_3OH}$ nm (ε) sh = shoulder 250 (24 500), 270 (26 300), 297 sh (18 600), 355 (4 700).

Conversion of 4 into 3, PE2R, and PE1B. A solution of dithiothreitol (100 μL, 20 mM in $CHCl_3$) was added to compound **4** (1 mg, 1.7 μmol in 5 mL of $CHCl_3$). After 4 h at room temperature, TLC analysis in system B showed the presence of **3** (~20%), PE2R (~20%), PE1B (~20%), as well as **4** (~10%).

Conversion of PE2R into 3 and PE1B. A solution of triethylamine (10 μL, 1:100 in $CHCl_3$) was added to PE2R (0.5 mg in 2 mL of $CHCl_3$). After 4 h at room temperature, most of the chloroform was evaporated and TLC analysis in system B demonstrated the presence of **3** (~30%), PE1B (~30%), and PE2R (~20%).

Conversion of 3, PE2R, and PE1B into 4. A potassium ethyl xanthate solution (2 mL, 0.5 M in orthophosphate buffer 0.05 M, pH 6.5) was added to ethanolic solutions of **3**, PE2R, and PE1B, respectively, (5 mg in 20 mL) and oxygen was slowly bubbled through the respective mixtures for 3 h. Most of the ethanol was removed by rotary evaporation and then water (100 mL) was added, followed by extraction with ethyl acetate. The organic extract was dried (Na_2SO_4) and concentrated for TLC analysis in systems B and G. Yields of **4** varied from 60 to 80%.

Conversion of 3 into PE2R and PE1B. Through a dilute solution of **3** (0.1 mg/mL of ethanol) H_2S, used here as a mild

(26) Urakawa, C.; Tsuchiya, H.; Nakano, K.-I. *J. Antibiot.* **1981**, *34*, 243–244.
(27) Urakawa, C.; Tsuchiya, H.; Nakano, K.-I.; Nakamura, N. *J. Antibiot.* **1981**, *34*, 1152–1156.
(28) Bean, M.; Kohn, H. *J. Org. Chem.*, following article in this issue.
(29) Kingston, D. G. I.; Gerhart, B. B. *J. Chromatogr.* **1976**, *116*, 182–183.

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

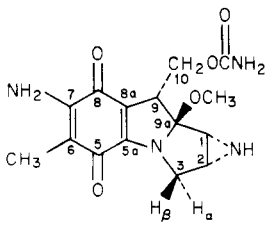
Mary Bean[1a] and Harold Kohn*[1b]

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

Received March 24, 1983

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



1

(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.

(2) Carter, S. K.; Crooke, S. T. "Mitomycin C. Current Status and New Developments"; Academic Press: New York, 1979.

(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.

(4) (a) Iyer, V. N.; Szybalski, W. *Science* **1964**, *145*, 55–58. (b) Szybalski, W.; Iyer, V. N. In "Antibiotics. Mechanism of Action"; Gottlieb, D., Shaw, P. D., Eds.; Springer: New York, 1967; Vol. 1, pp 211–245. (c) Patrick, J. B.; Williams, R. P.; Meyer, W. E.; Fulmor, W.; Cosulich, D. B.; Broschard, R. W. *J. Am. Chem. Soc.* **1964**, *86*, 1889–1990. (d) Moore, H. W.; Czerniak, R. *Med. Res. Rev.* **1981**, *1*, 249–280. (e) Moore, H. W. *Science* **1977**, *197*, 527–532.

0022-3263/83/1948-5033$01.50/0