start of a 90-min operant conditioning task. The tone reoccurred every 5 min until the monkey was awakened and a lever press occurred. This lever press was immediately reinforced with a banana-flavored pellet and also initiated a DRL (differential reinforcement of low rate of responding) 60-s schedule. According to the contingencies of the DRL-60, each lever press reset a timer to zero. However, if 60 s elapsed between lever presses, the monkey was automatically reinforced with a banana-flavored pellet. The number of reinforcements were recorded, and interresponse time, i.e., the times between lever presses, were counted.

Each animal served as its own control, with each parameter of sleep and performance being statistically compared to the last 15 control days. Each control night, the subjects were dosed intragastrically with a carboxymethylcellulose placebo suspension. Drugs were administered per os. Computerized statistical readouts were obtained for all control sessions in order to determine any drug rebound and/or carry-over effects on sleep patterns and to ensure control stability between drug administration.

Effects on Experimentally Induced Conflict in Rats. In the Geller conflict test[12] brief sessions of an approach–avoidance paradigm (conflict) were interposed upon a food-reinforced behavioral schedule (variable interval, VI). The primary activity of antianxiety drugs was demonstrable during the conflict trials as an increase in responding. Secondary activities, or side effects, were demonstrable as disruptions of the VI portion of the schedule.

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Acknowledgment. We are grateful to Dr. S. Barcza and his associates for instrumental determinations and to W. Bonkoski for the microanalyses.

Registry No. 1, 93040-58-5; 2, 93040-59-6; 3, 93040-60-9; 4, 93040-61-0; 5, 93040-62-1; 6, 93040-63-2; 7, 93040-64-3; 8, 93040-65-4; 9, 93040-66-5; 10, 56948-75-5; 11, 56948-73-3; 12, 93040-67-6; 13, 56948-74-4; 14, 93061-24-6; 15, 93040-68-7; 16, 93040-69-8; 17, 93040-70-1; 18, 93061-41-7; 19, 93040-71-2; 20, 93040-72-3; 21, 93040-73-4; 22, 23132-30-1; 23, 93040-74-5; 24, 59618-42-7; *m*-(trifluoromethyl)cinnamoyl chloride, 60689-14-7; *N*-*n*-butylsuccinimide, 3470-96-0; *N*-ethylsuccinimide, 2314-78-5; *N*-propylsuccinimide, 3470-97-1; *N*-isobutylsuccinimide, 13916-45-5; *m*-bromobenzotrifluoride, 401-78-5; 4-bromobenzotrifluoride, 402-43-7; bromobenzene, 108-86-1; 3-bromochlorobenzene, 108-37-2; 3-bromotoluene, 591-17-3; 3-[*m*-(trifluoromethyl)benzoyl]-propionic acid, 56948-76-6; 2-aminoethanol, 141-43-5; 2-hydroxybutylamine, 13552-21-1; *N*-methylpiperazine, 109-01-3; succinic acid, 110-15-6.

Lack of Influence of the Carbamoyl Group on the Stereochemistry of the Acid-Catalyzed Opening of the Aziridine Ring of the Mitomycins and of Congeners

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The acid-catalyzed opening of the aziridine ring of mitomycins A and C is known to occur predominantly with cis stereochemistry. We have observed that the presence or absence of a carbamoyl group at C-10 of mitomycin C and in certain of its analogues does not have a significant influence on the stereochemistry of the opening of this ring. The trans product obtained from mitomycin C was shown to be stable when treated with acid under the conditions of its formation. Mitomycin B was also shown to yield predominantly the cis product when it was subjected to acid-catalyzed opening of its aziridine ring. The ^{1}H NMR spectra of acetate derivatives prepared from mitomycin B show two sets of signals that are due to two populations of rotamers. The analysis of these spectra has substantiated several previous spectral assignments. This paper also presents some thoughts on acid-catalyzed bifunctional DNA alkylation by mitomycins and 10-decarbamoyloxy-9-dehydromitomycins.

The mitomycins[1-3] (**1a–d**, Chart I), the 10-decarbamoyloxy-9-dehydromitomycins such as **2a–b**,[4] and several derivatives of mitomycin C[5] are powerful antitumor antibiotics among which mitomycin C (**1c**) is currently being used for the mainly palliative treatment of clinical cancers.[6] The aziridine ring of this antibiotic is known to be involved in DNA alkylation reactions after reductive activation,[7] and it has been suggested that C-10 of mitomycin C may also be activated by reduction[8,9] and then may contribute a second binding site that may be involved in the formation of DNA cross-links. There exist two related reductive chemical model reactions that support this proposal,[10,11] but there exists presently no evidence that this second site is of biological significance.[7,12] Acid-catalyzed activation of mitomycins B and C was also shown to yield cross-linked DNA in vitro,[13,14] and it is likely that C-1 of mitomycin C after opening of the aziridine ring is involved in the DNA binding; however, hardly any information is presently available on any second alkylation site that could account for cross-link formation by mitomycins activated by mild acid treatment.

The opening of the aziridine ring of mitomycin C in acid-catalyzed reactions occurs predominantly with cis

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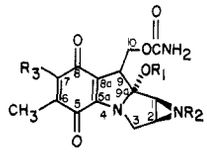
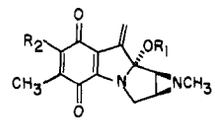
0022-2623/85/1828-0031$01.50/0

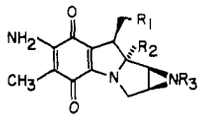
Chart I



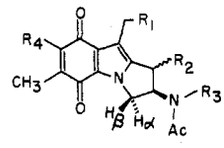
	R_1	R_2	R_3	CONFIGURATION AT C9
1a	CH_3	H	OCH_3	β
b	H	CH_3	OCH_3	α
c	CH_3	H	NH_2	β
d	CH_3	CH_3	OCH_3	β



	R_1	R_2
2a	H	OCH_3
b	CH_3	NH_2



	R_1	R_2	R_3
3a	OH	OCH_3	H
b	OAc	OCH_3	Ac
c	$OCONH_2$	SO_3Na	H
d	OH	SO_3Na	H



	R_1	R_2 cis/trans	R_3	R_4
4a	$OCONH_2$	OAc	H	OCH_3
b	$OCONH_2$	OAc	H	NH_2
c	$OCONH_2$	OH	H	NH_2
d	OAc	OAc	H	NH_2
e	$OCONH_2$	OAc	CH_3	OCH_3
f	OAc	OAc	H	OAc

stereochemistry[15-17] in contrast to the trans stereochemistry that is usually observed in the acid-catalyzed opening of simple aziridines.[18] Taylor and Remers[16] subjected mitomycin A (**1a**) and C to hydrolysis with 0.05 N HCl and subsequently acetylated the initial reaction products and obtained and characterized the acetates **4a** and **4b**. They observed cis and trans ratios of 5.2:1 and 6.6:1, respectively. Other workers found similar cis/trans isomer ratios in related reactions.[19] Cheng and Remers[17] hydrolyzed both *N*-methylmitomycin A (**1d**) and 7-methoxy-1,2-(*N*-methylaziridino)mitosene (**6a**, $R_1 = OCONH_2$) with 0.05 N HCl and after acetylation obtained and characterized the acetates **4e**. The cis/trans isomer ratios were 3:1 and 4:1, respectively. The authors concluded that, in this series, the 9a-methoxy group does not have an influence on the stereochemistry of aziridine ring opening, and they speculated that the carbamoyl group might have such an influence.

We report studies that examine this possible involvement of the carbamoyl group in determining the stereochemical course of aziridine ring opening. We used decarbamoylmitomycin C[20] (**3a**) and its diacetate (1a,10-diacetyl-10-decarbamoylmitomycin C, **3b**) to examine the role of the carbamoyl group and its amino group directly. Mitomycin B (**1b**) was included since in contrast to mitomycins A and C its carbamoyloxymethylene group has an orientation opposite to that of the aziridine ring and hence was not expected a priori to have a directing influence on the opening of the aziridine ring. Sodium decarbamoyl-7-aminomitosane-9a-sulfonate (**3d**) and its parent compound sodium 7-aminomitosane-9a-sulfonate (**3c**) were studied as they provided another pair of compounds like **1c** and **3a** that differ only by a carbamoyl group. Furthermore, they were of interest because of their increased acid stability,[21] which is presumably due to the presence of the 9a-sulfonate, and thus were expected to provide additional information on the possible role of substitution at C-9a on acid-catalyzed aziridine ring opening.

Results

The mitomycin analogues used in these studies were all prepared from mitomycin C. Compound **3a** was obtained by treating mitomycin C with $NaOCH_3$, which removes the carbamoyl group.[20] The resulting material was used to prepare the diacetate **3b** with acetic anhydride. Compound **3c** was obtained by reduction/reoxidation of mitomycin C with $Na_2S_2O_4$ as the reductant, which itself is converted into $NaHSO_3$, which then forms a bisulfite adduct with the activated antibiotic.[21] Compound **3d** was prepared from compound **3c** by treatment with $NaOCH_3$. The high-resolution ^1H NMR spectra of compounds **3a–c** were recorded and are presented in Table I. In addition, the ^{13}C NMR spectra of compounds **3a** and **3c** were measured. These NMR spectral data are fully in accord with the assigned structures.

The solvolysis reactions utilized 0.05 N HCl for 25 min for compounds **1b**, **1d**, **3a**, **3b**, and **4c** and 0.1 N HCl for 4 days for compounds **3c** and **3d** at room temperature to open the aziridine ring. The higher concentration of acid was required for compounds **3c** and **3d** because of their increased acid stability.[21] This higher concentration of the acid leads to substitution of the C-7 amino group by an OH group and simultaneously to hydrolysis of the carbamate. Reaction products were correlated with known compounds by suitable reactions. Thus mitomycin C was first treated with acid, as reported by Taylor and Remers[16] and by Stevens et al.[22] The resulting mixture of mitosenes was subjected to treatment with $NaOCH_3$ to remove the carbamoyl group and was then acetylated. Likewise for the solvolysis reaction involving mitomycin B (**1b**), reference *N*-methylmitomycin A (**1d**) was also solvolyzed as reported,[17] and the respective products were acetylated, analyzed, and compared. Mitomycin C was treated with 0.1 N HCl, and the mixture of mitosenes was subsequently acetylated to yield tetraacetates **4f**, which were used in turn to identify the final reaction products arising from the solvolysis and subsequent acetylation reactions of compounds **3c** and **3d**.

The acetylation reactions yielded nonpolar derivatives of the compounds of interest that could be analyzed more readily by TLC than the initially obtained reaction products. Generally, we obtained good to excellent overall yields of the pairs of acetates of interest; however, an exact materials balance was not established for any of the reactions. Cis and trans reaction products were largely

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Table I. 470-MHz ^{1}H NMR Data

assignment[a]	**3a**[b]	**3b**[b]	**3d**[c]	**4d trans**[b]	**4d cis**[b]	**4e-trans**,[b] predominant rotamer (90%)	**4e-trans**,[b,d] minor rotamer (10%)	**4e-cis**,[b] predominant rotamer (70%)	**4e-cis**,[b,d] minor rotamer (30%)
C_1 H	2.85, br s	3.41, d, 4.6	3.15, br s	6.00, d, 2.8	6.03, d, 5.7	6.35, d, 3.3	6.28	6.48, d, 6.4	6.43
C_2 H	2.85, br s	3.19, m	2.96, br s	4.82, ddd, 2.8, 3.5, 6.5	5.24, m	5.17, ddd, 3.3, 4.9, 8.4	4.93	5.75, q, 6.0–8.5	5.15
C_2 NH				6.10, br d	5.88, br d, 9.2				
C_2 NCH_3						2.97, s	2.72	3.04, s	2.84
C_3 H_α	3.52, d, 13.0	3.53, dd, 1.9, 13.4	3.69, dd, 1.9, 12.5	4.68, dd, 6.5, 13.4	4.75, dd, 7.8, 12.5	4.61, dd, 8.4, 13.3	4.63	4.47, dd, 8.3, 12.8	4.55
C_3 H_β	4.21, d, 13.0	4.41, d, 13.4	4.06, d, 12.5	4.15, dd, 3.5, 13.4	3.88, dd, 8.7, 12.5	4.15, dd, 4.9, 13.3	4.22	4.37, dd, 7.8, 12.9	4.45
C_6 CH_3	1.74, s	1.76, s	1.65, s	1.81, s	1.82, s	1.93, s	1.95	1.94, s	1.95
C_7 NH_2	5.24, br	5.23, br		4.93, br s	4.94, br s				
C_7 OCH_3						4.00, s	4.03	4.01, s	4.03
C_9 H	3.38, t, 5.8	3.64, dd, 4.8, 11.1	3.93, dd, 4.3, 10.6						
C_{9a} OCH_3	3.19, s	3.19, s							
$NCOCH_3$		2.06, s[e]		1.99, s[e]	2.03, s[e]	2.10, s[e]	2.18	2.13, s[e]	2.20
$OCOCH_3$		2.14, s[e]		2.02, s[e]	2.05, s[e]	2.10, s[e]	2.13	2.07, s[e]	2.10
$OCOCH_3$				2.09, s[e]	2.09, s[e]				
C_{10} H_2	4.00, m	4.00, t, 11.2	4.24, t, 10.8	5.22, d, AB q, 12.7	5.24, d, AB q, 12.7	5.22, d, AB q, 13.8	5.20	5.20, d, AB q, 14.4	5.24
		4.77, dd, 4.8, 11.1	4.48, dd, 4.3, 10.8	5.26, d, AB q, 12.7	5.28, d, AB q, 12.7	5.33, d, AB q, 13.8	5.37	5.38, d, AB q, 14.4	5.34

[a] Satisfactory integrations were obtained for all signals. [b] Solvent = $CDCl_3$. [c] Solvent = D_2O. [d] Multiplicities and coupling constants were identical with those given in the previous column for the respective signals. [e] Assignments may be interchanged.

identified by R_f value comparison with reference products reported by Remers and co-workers[16,17] and by Stevens et al.[22] Cis and trans acetates were scraped separately off the respective TLC plate, eluted from the layer material, and subjected to quantitative analysis by determination of the UV absorbance at an appropriate wavelength as described also by Remers and co-workers.[16,17] Separate experiments showed that the elution of the respective acetates was possible in nearly quantitative yield for compounds **4d** and **4e** and was achieved with 70% recovery for both cis and trans acetates **4f**.

In addition to making stereochemical assignments via the prepared relay compounds, it was possible in the case of several of the resulting acetates to independently assign trans stereochemistry to one member of certain pairs of reaction products. The assignment was achieved by the use of high-field ^{1}H NMR following a published procedure initially developed by Keller and Hornemann,[10] which relys on an interpretation of vicinal proton relationships in the respective mitosenes. Briefly, if C-2 is in a β orientation as judged by the presence of one large and one small coupling constant $^3J_{H_2-H_{3\alpha}}$ and $^3J_{H_2-H_{3\beta}}$, respectively, then observing a small coupling constant $^3J_{H_1-H_2}$ indicates the presence of a trans compound while a large coupling constant $^3J_{H_1-H_2}$ would indicate a cis compound. This analysis, however, does not yield an answer if C-2 is in the α configuration, which entails large coupling constants $^3J_{H_2-H_{3\alpha}}$ and $^3J_{H_2-H_{3\beta}}$ since then the coupling constants $^3J_{H_1-H_2}$ for both the cis and the trans isomers are large.[23] Presumably for steric reasons, cis compounds adopt the α configuration and hence cannot be assigned by this method. Examples that illustrate these relationships are given in Table I. Confirmatory evidence for the assignment of **4d-cis** was obtained by a difference NOE experiment. Upon irradiation of C_2 H of **4d-cis**, a 15% NOE was observed for C_1 H. In contrast irradiation of C_2H of **4d-trans** yielded only a 1–2% NOE. In addition, the difference NOE experiment with **4d-cis** revealed a 3–5% NOE on the downfield C_3H, thus confirming its assignment as C_3 H_α.

In the high-field, ^{1}H NMR spectra of analytically pure samples of the acetates **4e-trans** and **4e-cis**, we noted the occurrence of an unexpected second set of signals that accompanied all peaks at a constant intensity ratio while exhibiting more or less pronounced differences in chemical shift values. The coupling constants, however, were exactly the same in the second set of signals as in the major set of signals. These secondary peaks amounted to approximately 10% and 30% of the total signal intensity in the spectra of **4e-trans** and **4e-cis**, respectively. They are due to the presence of two conformers that are most likely the result of hindered rotation around the acetamide bond. Proof for the presence of two rotamers was provided by a saturation transfer experiment using **4e-cis**. Irradiation of the C_1H signal of the predominant roatmer at very low decoupler power ($\gamma_{H_2}/2\pi$ = 1.8 Hz) caused the selective diminution of the intensity of the C_1 H signal of the minor conformer.

The most notable differences in the chemical shift values between the major and the minor set of peaks are upfield shifts of C_2 H of **4e-cis** and to a lesser extent of C_2 H of **4e-trans** as well as upfield shifts of the NCH_3 signals of both compounds. In the absence of additional experimental data, it is not readily possible to rationalize why all four mentioned signals are shifted upfield. In *N*-acetyl-3-nitropyrrole and in *N*-acetylproline, which may be regarded as reasonably closely related model compounds, both upfield and downfield shifts were reported for protons in close proximity to the acetyl groups in the respective rotamers.[24,25] In the present case, however, the

(23) It should be noted that the reassignment of the absolute configuration of mitomycin C (note 2, ref 3) makes the C_2 β conformation the conformation that allows trans compounds to be assigned rather than the C_2 α conformation as stated in ref 10. It also becomes necessary to switch the ^{1}H NMR assignments for C_3 H_α and C_3 H_β published in ref 10 for several mitosenes.

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Table II. Cis/Trans Isomer Ratio of Mitosenes Obtained by Acid-Catalyzed Opening of the Aziridine Ring of Mitomycins B and C of *N*-Methylmitomycin A and of Derivatives of Mitomycin C

starting material	acetylated product	R_f values (system)		cis/trans isomer ratio		ref
		cis	trans	observed	reported	
3a	**4d**	0.34	0.47 (A)	2.7:1 (3.4:1)		
1c via **4c**	**4d**	0.34	0.47 (A)	2.6:1[a]	5.2:1	15
1b	**4e**	0.45	0.52 (B)	2.6:1		
1d	**4e**	0.45	0.52 (B)	3.0:1[b]	4.0:1	16
3b	**4f**	0.78	0.81 (B)	5.6:1		
3c	**4f**	0.78	0.81 (B)	3.0:1		
3d	**4f**	0.78	0.81 (B)	1.8:1		

[a] Determined by weighing isolated samples. [b] Estimated by visual inspection.

O-acetyl groups present in both **4e-trans** and in **4e-cis** may have an influence on the observed large upfield shifts in addition to having an influence on the C_{10} protons to which they are close. Model building studies have indicated that C_3 H_β of **4e-trans** should be influenced more than C_3 H_β of **4e-cis** and the respective C_3 H_α signals in both compounds by rotation of the acetyl group of the *N*-acetamido group. This prediction is borne out by the data presented in Table I, thus lending further support to our proposal for the existence of C_2 α and C_2 β conformers in cis and trans mitosenes, respectively.

The results obtained in our solvolysis studies are given in Table II. In all cases where we duplicated experiments such as the experiment using **3a** as a starting material or experiments reported by Remers and co-workers, we obtained essentially the same results. Therefore, we feel justified to use the cis/trans ratio information obtained for acetates arising from previously uninvestigated starting materials as fully representative of the stereochemical course of the reactions even though we did not establish materials balances for these reactions. It is apparent from the results shown in the table that in all cases examined, the cis and trans ratio is always greater than 2 and is in most cases approximately 3. These ratios except the ratio obtained for the products arising from **3b** are lower than those reported by Remers and co-workers; however, this difference, although not readily explained, is probably not very significant since the overall findings that cis products predominate over trans products are the same. It is thus clear that the presence in compounds **1c** and **3c** and the absence in compounds **3a** and **3d** of a carbamoyl group and its presence in an orientation opposite to the orientation of the aziridine ring in mitomycin B do not influence the stereochemistry of the solvolysis reaction. Treatment of **3a** with acetic anhydride afforded exclusively **4d-cis**, similar to the report by Taylor and Remers that similar treatment of **1c** with acetic anhydride yields exclusively **4b-cis**. It is of particular interest to note that the cis/trans ratio of products arising from **3c** and **3d** which require strong acid for ring opening is comparable to the ratio found for compounds **1c** and **3a** which require less rigorous conditions.

In order to examine the possibility that unexpectedly initially isolated cis and trans products are in slow equilibrium under acidic conditions, we treated purified compound **4d-trans** with acid under the exact conditions that had led to its formation. Subsequent analysis by TLC revealed that no change had occurred, indicating that the initial reaction products are stable and are not in a dynamic equilibrium after they are formed.

Discussion

Since in our experiments we did not observe any substantial difference with regard to the stereochemistry of aziridine opening between mitosanes possessing the $OCONH_2$ group and those not possessing it, we can conclude that anchimeric assistance does not play any major role in the stereochemistry of ring opening. Thus it appears likely that the following mechanism that is entirely independent of any carbamoyl group involvement can satisfactorily account for the observed results. The first event in the acid-catalyzed activation and decomposition of the mitomycins and related mitosanes is most likely removal of the 9a-substituent, presumably generating a carbocation at 9a which is stabilized by proton abstraction from C-9. The next step is opening of the aziridine ring after protonation of the aziridine nitrogen atom. The resulting presence of a 9,9a-double bond imparts benzylic character to the C-1 position and attack at this position by nucleophiles would then explain the fact that all known mitomycin conversion products obtained by acid catalysis always carry the substituent at this position. It is attractive to speculate that the OH group that is to become the new substituent at C-1 associates initially via hydrogen bond formation with the NH_2 group at C-2 and perhaps more weakly also with the "indolic" nitrogen atom before attacking the C-1 carbocation. This kind of association would greatly favor cis adduct formation. It may be assumed that any trans product arises by attack via an S_N2 mechanism on the aziridine ring or via attack on an entirely free carbocation from the side opposite to that of the amino group.

The mechanistic interpretation of the assistance in the ring-opening reaction by the amino group is influenced by pertinent considerations by Dolbier.[26] Similar interpretations can also account for the observations of Chiu and Kohn[27] that opening of the aziridine ring of indano[1,2-*b*]aziridine which they synthesized occurs predominantly with cis stereochemistry. These authors undertook their study on the indano[1,2-*b*]aziridine and other model compounds in an attempt to shed light on the mechanism of opening of the mitomycin aziridine ring. They reached similar conclusions to those presented in the present work.

Our studies reported here were not primarily concerned with reactions occurring at C-10 of mitomycins and of derivatives. Nevertheless, reactivity at this site is of great interest since Lown and co-workers[13,14] reported that cross-linking of DNA is observable after acid-catalyzed activation of the mitomycins. 10-Decarbamoyloxy-9-dehydromitomycin B (**2a**) and 10-decarbamoyloxy-9-dehydro-*N*-methylmitomycin C (**2b**) are of special interest in this regard. It is possible after acid-catalyzed removal of the OH group of **2a** or **2b**, which should yield intermediate **5**, that a nucleophile can add at C-10 as outlined in Scheme I. This addition would set up an aziridinomitosene structural element such as **6b** (R_1 = Nu), which would subsequently be available for alkylation at C-1 to yield a derivative of **4**. An important difference relative to reductive mitomycin activation can be suggested: re-

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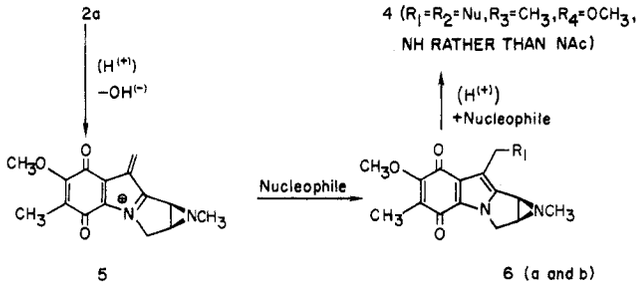
Scheme I



action of nucleophiles with compounds **2a** or **2b** should occur first at C-10 and only later at C-1, a reversal of the order of addition that possibly prevails during reductive activation conditions.[10,28]

Experimental Section

Melting points were determined in a Fisher-Johns melting point apparatus and are uncorrected. UV data were recorded on a Cary 17 or a Coleman 124 spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were measured on Nicolet instruments NT-200 and NT-470 on Varian instruments FT80A and XL200 and on a Bruker WM-300 instrument, respectively, accumulating 8K and 32K data sets, respectively, for each spectrum. Proton-decoupled ^{13}C NMR spectra were obtained by two-level noise decoupling. Chemical-ionization mass spectra were obtained on a Dupont 21-492B instrument. TLC systems used in the isolation and quantitation of acetates were (A) tetrahydrofuran:*n*-hexane:ethyl acetate = 8:3:2 and (B) acetone:*n*-hexane:benzene = 9:3:1. Supports were silica gel (Merck G60, 0.25 mm, without fluorescent indicator). Mitomycins A, B, and C were gifts from Dr. S. Wakaki, Kyowa Hakko Kogyo Co., Tokyo, Japan, and from Dr. W. Bradner, Bristol Laboratories, Syracuse, NY, respectively. They were used as received.

Preparation of Decarbamoylmitomycin C (3a). Mitomycin C (100 mg, 300 μmol) was dissolved in absolute methanol (10 mL) and dry benzene (17 mL), treated with $NaOCH_3$ (1 g in 7 mL of absolute methanol) at room temperature for 48 h with stirring and isolated as described.[20] **3a** was obtained in 90% yield. The CI-MS spectrum showed m/z 292 ($M^+ + 1$, 86%). TLC in acetone:chloroform (2:1): **1c**, R_f 0.21; **3a**, R_f 0.29. ^{13}C NMR (50.3 MHz, pyridine-d_5) δ 8.8 (C_6CH_3), 33.4 (C_2), 36.6 (C_1), 47.9 (C_9), 49.5 (OCH_3), 50.7 (C_3), 61.3 (C_{10}), 104.1 (C_6), 107.6 (C_{9a}), 113.0 (C_{8a}), 149.5 (C_7), 156.0 (C_{5a}), 177.2 (C_8), 178.5 (C_5). These assignments are based on the published ^{13}C NMR spectrum of mitomycin C.[29]

Preparation of Decarbamoylmitomycin C Diacetate (3b). Decarbamoylmitomycin C (2 mg, 6.9 μmol) was dissolved in dry pyridine (0.3 mL) and treated with acetic anhydride (0.4 mL) for 5 h at room temperature. After quenching with methanol and evaporation of the solvent, **3b** was isolated by preparative TLC in acetone:chloroform (2:1), R_f 0.77.

Preparation of Sodium Decarbamoyl-7-aminomitosane-9a-sulfonate (3d). Sodium 7-aminomitosene-9a-sulfonate (6 mg, 14.8 μmol) prepared from mitomycin C as described[21] was dissolved in absolute methanol (2 mL) and absolute benzene (2 mL). $NaOCH_3$ (200 mg) in absolute methanol (1 mL) was added, and the reaction mixture was stirred for 30 h at room temperature, neutralized by addition of excess dry ice, and filtered. The collected $NaHCO_3$ was washed with acetone until the residue was colorless. The combined filtrates were subjected to preparative TLC on silica gel in the system 2-propanol:ethyl acetate:methanol (6:3:2). One main band was observed and isolated by elution with methanol to yield 1.5 mg of a green solid. TLC in 2-propanol:water = 9:1: **3d**, R_f 0.3; **3c**, R_f 0.43. **3d**: UV $\lambda_{max}^{CH_3OH}$ nm, 262 (sh), 220; ^{13}C NMR (75.4 MHz, D_2O) δ 10.1 (C_6CH_3), 36.9 (C_2) 37.9 (C_1), 48.7 (C_9), 54.0 (C_3), 63.3 (C_{10}), 95.3 (C_6), 106.9 (C_{9a}), 114.2 (C_{8a}), 154.6 (C_7), 160.4 (C_{5a}), 178.4 (C_8), 179.9 (C_5). These assignments are based on the published ^{13}C NMR spectrum of mitomycin C.[29]

Hydrolysis of Decarbamoylmitomycin C (3a) and of Its Diacetate 3b. A solution of decarbamoylmitomycin C (11 mg, 37.8 μmol) in 0.05 N HCl (10 mL) was stirred for 25 min at room temperature, then cooled and neutralized (pH 8) with solid potassium carbonate, and concentrated in vacuo. The violet residue was treated with acetic anhydride (1.5 mL) in pyridine (3 mL) for 4 h at room temperature. After addition of methanol to destroy excess acetic anhydride, the mixture was evaporated to dryness with addition of toluene to assist in the complete removal of pyridine. The residue was taken up in ethyl acetate and water and the organic layer was washed with a 4% solution of $NaHCO_3$ and with a saturated NaCl solution. The organic layer was dried with Na_2SO_4 and a major portion was subjected to thin-layer chromatography in system A, which revealed two major products, which were recovered by elution from the gel with methanol. The more mobile component isolated as orange-red crystals (1.1 mg), mp 201–202 °C dec, was *trans*-2-acetamido-7-amino-1,10-diacetoxydecarbamoylmitosene (**4d-trans**): UV $\lambda_{max}^{CH_3OH}$ 333 nm (sh), 304, 252. The less mobile component also isolated as orange-red crystals (3.1 mg), mp 199–201 °C dec, was *cis*-2-acetamido-7-amino-1,10-diacetoxydecarbamoylmitosene (**4d-cis**): UV $\lambda_{max}^{CH_3OH}$ 338 nm (sh), 308, 253.

The ratio of the cis and trans products were determined by subjecting the remaining solution containing 213 μg of the products obtained after acetylation to TLC analysis in system A and by quantitatively eluting the bands of the cis and trans products with methanol. After evaporation of the solvent, the dried residues were dissolved in 10 mL each of methanol for a comparison of the peak heights at 252 nm. A ratio of 73.1:26.9 was observed. A repetition of this experiment under identical conditions gave very similar results (Table I). The reisolation yield from TLC plates was tested and was found to be 93% and 89% for **4c-trans** and **4c-cis**, respectively. 1a,10-Diacetyl-10-decarbamoylmitomycin C (**3d**; 1.5 mg, 4.1 μmol) was treated in essentially the same manner, yielding the same products **4c-cis** and **4c-trans** in a ratio of 84.8:15.2.

Hydrolysis of Mitomycins B (1b) and C (1c) and of *N*-Methylmitomycin A[17] (1d). The hydrolysis conditions were the same as above with the exception that 100 mg of **1c** was used and that the initial reaction product, a mixture of apomitomycins,[22] was isolated by filtration for further workup via the decarbamoylation reaction reported above and subsequent acetylation. The procedure for **1b** and **1d** differed in that TLC of the respective acetates **4e** was performed in system B in which in each case two major yellow bands were seen, which were recovered with methanol. The more mobile component isolated as a yellow powder (1 mg) was *trans*-1-acetoxy-7-methoxy-2-(methylacetamido)mitosene (**4e-trans**): UV $\lambda_{max}^{CH_3OH}$ 330 nm (sh), 288, 235. The less mobile component (3 mg), mp 215–220 °C dec, was *cis*-1-acetoxy-7-methoxy-2-(methylacetamido)mitosene (**4e-cis**): UV $\lambda_{max}^{CH_3OH}$ 330 nm (sh), 288, 235. The ratio of the cis and trans products determined by UV analysis of the peak heights at 287 nm of solutions of the respective compounds in 10 mL methanol was 72.5:27.5 for **1b** as starting material. The reisolation yield from TLC plates was found to be 82% and 96% for **4e-trans** and **4e-cis**, respectively.

Hydrolysis of Sodium 7-Aminomitosane-9a-sulfonate (3c) and Its Decarbamoyl Derivative 3d. A solution of sodium 7-aminomitosane-9a-sulfonate (10 mg, 24.6 μmol) in 0.1 N HCl (10 mL) was stirred for 4 days at room temperature. During this time, the color changed from blue to yellow-orange. The solution was cooled and neutralized (pH 8) with solid potassium carbonate, and the reaction products were acetylated and analyzed as described above by TLC in system B. Two major components were seen on the TLC plate and were recovered with methanol as yellow powders. The more mobile component (0.9 mg) was *trans*-2-acetamido-1,7,10-triacetoxydecarbamoylmitosene (**4f-trans**): UV $\lambda_{max}^{CH_3OH}$ 270 nm, 232. The less mobile component (2.5 mg) was *cis*-2-acetamido-1,7,10-triacetoxydecarbamoylmitosene (**4f-cis**):[22] UV $\lambda_{max}^{CH_3OH}$ 270 nm, 232. These compounds were identified by cochromatography with authentic **4f-trans** and **4f-cis** as prepared directly from **1c** by the procedure of Stevens et al.[22] Compound **3d** (1.5 mg, 4.1 μmol) was treated in essentially the same manner and yielded the same cis and trans compounds described above.

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UV analysis at 270 nm of the acetates **4f** dissolved in 10 mL of methanol after TLC separation in system B yielded cis/trans ratios of 75.1:24.9 (**3c** as starting material) and 64.7:35.3 (**3d** as starting material), respectively.

Acid Treatment of 4c-trans. **4c-trans** (0.4 mg) was dissolved in 0.05 N HCl (1 mL) and stirred for 25 min at room temperature. After neutralization with $KHCO_3$, an aliquot was chromatographed in acetone:chloroform (2:1) together with reference compounds; **4c-trans**, R_f 0.11; **4c-cis**, R_f 0.35. No trace of the cis compound was detected. It should be noted that the TLC system used gives a lower R_f for the trans isomer **4c** while the trans compound in the acetate series **4e** has the higher R_f value. An analogous reversal of chromatographic mobilities of a related series of compounds was noted by Bachur and co-workers.[30]

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Registry No. **1b**, 4055-40-7; **1c**, 50-07-7; **1d**, 18209-14-8; **3a**, 26909-37-5; **3b**, 26909-43-3; **3c**, 61070-23-3; **3d**, 92762-77-1; **4d-trans**, 92842-90-5; **4d-cis**, 92842-91-6; **4e-trans**, 92762-78-2; **4e-cis**, 92934-66-2; **4f-trans**, 92842-92-7; **4f-cis**, 92842-93-8; mitomycin, 1404-00-8.

Inhibition of Liver Alcohol Dehydrogenase and Ethanol Metabolism by 3-Substituted Thiolane 1-Oxides

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3-Substituted thiolane 1-oxides (methyl, *n*-butyl, *n*-hexyl, and phenyl) were prepared and tested as inhibitors of horse, monkey, and rat liver alcohol dehydrogenases and of ethanol metabolism in rats. These compounds inhibit alcohol oxidation in an uncompetitive manner with respect to ethanol as a varied substrate. Lengthening the alkyl substituent increased the inhibitory potency because of tighter binding in the hydrophobic substrate binding pocket of the alcohol dehydrogenases. Thus, the 3-hexyl derivative was the most potent inhibitor of the purified rat liver alcohol dehydrogenase, with a K_{ii} value of 0.13 μM. The 3-butyl derivative was the best inhibitor of ethanol metabolism in rats, with a K_{ii} value of 11 μmol/kg. The acute toxicity in mice of the butyl derivative was 1.4 mmol/kg. Since high concentrations of alcohol do not prevent the inhibitory effects of these compounds, they may be particularly useful for preventing poisoning by methanol or ethylene glycol.

Alcohols, such as methanol, ethanol, and ethylene glycol, are metabolized in man primarily through liver alcohol dehydrogenase.[1] At least in rats, the enzyme activity is a major rate-limiting factor in ethanol metabolism.[2-4] Thus, specific inhibitors of the dehydrogenase would be useful for studying the physiological role of the enzyme and perhaps for therapeutically preventing poisoning by methanol[5,6] and ethylene glycol.[7,8] These compounds become more toxic when they are oxidized to the corresponding acids.[7,9] The utility of 4-methylpyrazole as an inhibitor of alcohol dehydrogenase and of methanol and ethylene glycol metabolism has been demonstrated in monkeys,[6,10] but it is a competitive inhibitor of alcohol dehydrogenase and may not be the most efficacious compound to use in man.[11]

We prepared and evaluated a series of sulfoxides as inhibitors of alcohol dehydrogenase in vitro and in vivo and found that thiolane 1-oxide (tetramethylene sulfoxide) was an exceptionally potent inhibitor.[12] This compound is an uncompetitive inhibitor of ethanol metabolism in rats, which is advantageous as compared to competitive inhibitors in that the inhibition is not prevented when the concentration of alcohol is increased to levels that saturate the alcohol dehydrogenase. Since the active site of horse liver alcohol dehydrogenase has a large hydrophobic pocket,[13,14] alkyl or aryl substitutents at the 3-position of the five-membered ring in thiolane 1-oxide should enhance the inhibitory potency.

Biological Results and Discussion

Table I shows that increasing the size of the alkyl substituent increases the inhibitory potency of the thiolane

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