mixture was stirred for 18 h at room temperature. The solvent was then evaporated and the residue was dissolved in CH_2Cl_2 and washed with brine. The residue obtained after drying ($MgSO_4$) and evaporating CH_2Cl_2 was chromatographed on silica gel (1% CH_3OH–CH_2Cl_2) to give 190 mg of **1** and 100 mg (29%) of the title compound: mp 138–140 °C; NMR ($CDCl_3$) δ 0.88 (s, 3 H), 1.73 (s, 3 H), 2.0 (m, 4 H), 2.08 (s, 3 H), 2.78 (d, 1 H, J = 4 Hz), 3.06 (d, 1 H, J = 4 Hz), 3.64 (d, 1 H, J = 5 Hz), 3.90 (d, 1 H, J = 13 Hz), 4.19 (d, 1 H, J = 13 Hz), 4.28 (m, 2 H), 5.50 (br d, 1 H, J = 6 Hz); IR (KBr) 3480, 1715, 1390, 1260, 1040 cm^{-1}. Anal. ($C_{19}H_{29}NO_5$) H, N; C: calcd, 64.93; found, 64.47.

4β,15-Diacetoxy-3α-morpholinotrichothec-9-ene (9): amorphous solid, mp 73–75 °C; yield 33%; NMR ($CDCl_3$) δ 0.83 (s, 3 H), 1.72 (s, 3 H), 2.00 (m, 4 H), 2.06 (s, 3 H), 2.16 (s, 3 H), 2.43–2.69 (m, 4 H), 2.77 (d, 1 H, J = 4 Hz), 3.06 (d, 1 H, J = 4 Hz), 3.61–3.80 (m, 4 H), 3.90–4.21 (m, 3 H), 5.14, (d, 1 H, J = 3 Hz), 5.53 (br d, 1 H, J = 6 Hz); IR (KBr) 1742, 1403, 1245, 1119 cm^{-1}. Anal. ($C_{23}H_{33}NO_7 \cdot 0.5H_2O$) C, H, N.

15-Acetoxy-3α-(methylamino)-4β-hydroxytrichothec-9-ene (10): amorphous solid, mp 92–94 °C; yield 27%; NMR ($CDCl_3$) δ 0.85 (s, 3 H), 1.74 (s, 3 H), 2.11 (s, 3 H), 2.62 (s, 3 H), 2.78 (d, 1 H, J = 4 Hz), 3.08 (d, 1 H, J = 4 Hz), 3.70 (d, 1 H, J = 5 Hz), 3.80–4.34 (m, 4 H), 5.48 (d, 1 H, J = 4 Hz). Anal. ($C_{18}H_{27}NO_5 \cdot 0.5H_2O$) C, H, N.

An earlier fraction in the silica gel chromatography produced 260 mg (24%) of 15-acetoxy-3-cyano-3-(methylamino)-4β-hydroxytrichothec-9-ene (**13**): mp 92–94 °C; NMR ($CDCl_3$) δ 0.88 (s, 3 H), 1.72 (s, 3 H), 2.00 (m, 4 H), 2.07 (s, 3 H), 2.64 (s, 3 H), 2.79 (d, 1 H, J = 4 Hz), 3.12 (d, 1 H, J = 4 Hz), 3.88 (d, 1 H, J = 12 Hz), 3.89 (s, 1 H), 4.11 (d, 1 H, J = 12 Hz), 4.29 (s, 1 H), 4.41 (d, 1 H, J = 4 Hz), 5.43 (d, 1 H, J = 5 Hz); IR (KBr) 3450, 2230, 1740, 1450, 1417, 1242, 1095, 1042 cm^{-1}; MS (CI), m/e 362 (M^+, 9%), 335 (M_+ – HCN, 100%). Anal. ($C_{19}H_{25}N_2O_5 \cdot 0.25H_2O$) C, H, N.

15-Acetoxy-3α-[(2-hydroxyethyl)amino]-4β-hydroxytrichothec-9-ene (11): amorphous solid, mp 54–56 °C; yield 49%; NMR ($CDCl_3$) δ 0.85 (s, 3 H), 1.73 (s, 3 H), 1.95 (m, 4 H), 2.08 (s, 3 H), 2.85 (d, 1 H, J = 4 Hz), 2.96 (t, 2 H, J = 5 Hz), 3.06 (d, 1 H, J = 4 Hz), 3.16 (dd, 1 H, J = 5, 4 Hz), 3.54–3.78 (m, 3 H), 3.86 (d, 1 H, J = 13 Hz), 3.98 (d, 1 H, J = 5 Hz), 4.16 (d, 1 H, J = 4 Hz), 4.18 (d, 1 H, J = 13 Hz), 5.45 (br d, 1 H, J = 6 Hz); IR (KBr) 3440, 3300, 1740, 1447, 1366, 1245, 1070, 1045 cm^{-1}. Anal. ($C_{19}H_{29}NO_6 \cdot 0.5H_2O$) C, H, N.

15-Acetoxy-3α-(propargylamino)-4β-hydroxytrichothec-9-ene (12): amorphous solid, mp 59–61 °C; yield 43%; NMR ($CDCl_3$) δ 0.86 (s, 3 H), 1.72 (s, 3 H), 1.80–2.10 (m, 4 H), 2.08 (s, 3 H), 2.25 (t, 1 H, J = 2 Hz), 2.67 (d, 1 H, J = 4 Hz), 3.06 (d, 1 H, J = 4 Hz), 3.35 (dd, 1 H, J = 5, 4 Hz), 3.61 (d, 2 H, J = 2 Hz), 3.65 (d, 1 H, J = 5 Hz), 3.86 (d, 1 H, J = 13 Hz), 4.03 (br d, 1 H, J = 5 Hz), 4.16 (d, 1 H, J = 13 Hz), 4.17 (d, 1 H, J = 4 Hz), 5.45 (br d, 1 H, J = 5 Hz); IR (KBr) 3460, 3300, 2430, 1742, 1454, 1370, 1250, 1070 cm^{-1}. Anal. ($C_{20}H_{27}NO_5 \cdot 0.5H_2O$) C, H, N.

Registry No. **1**, 2270-40-8; **2**, 2269-44-5; **5**, 79320-86-8; **7**, 79320-89-1; **8**, 96110-50-8; **9**, 96110-51-9; **10**, 96110-52-0; **11**, 96110-53-1; **12**, 96110-54-2; **13**, 96110-55-3; $HNMe_2$, 124-40-3; NH_2Me, 74-89-5; $NH_2(CH_2)_2OH$, 141-43-5; $NH_2CH_2C{\equiv}CH$, 2450-71-7; morpholine, 110-91-8.

4-Deoxypyrido[1′,2′:1,2]imidazo[5,4-*c*]rifamycin SV Derivatives. A New Series of Semisynthetic Rifamycins with High Antibacterial Activity and Low Gastroenteric Absorption

Egidio Marchi,*† Laura Montecchi,† Anna Paola Venturini,† Giuseppe Mascellani,‡ Mario Brufani,§ and Luciano Cellai⊥

Alfa Farmaceutici S.p.A., Via Ragazzi del '99 n. 5, 40133 Bologna, Opocrin S.p.A., 41040 Corlo, Modena, Gruppo di Chimica Biologica e Strutturistica Chimica, Università di Roma, 00185 Rome, and Istituto di Strutturistica Chimica "Giordano Giacomello", C.N.R., 00016 Monterotondo Stazione, Rome, Italy. *Received February 13, 1984*

A series of 4-deoxypyrido[1′,2′:1,2]imidazo[5,4-*c*]rifamycin SV derivatives (**6–11**) were prepared that demonstrated high antibacterial activity suitable for an intestinal disinfectant. These compounds are zwitterionic in nature and are poorly absorbed through the gastroenteric tract but maintain the ability to cross the bacterial cell wall. X-ray crystallographic data are presented to demonstrate the zwitterionic nature of these compounds. The structure–activity relationship of this novel series of antibiotics is discussed and the derivative with the highest ratio between subcutaneous and oral activity (**6**) was selected for clinical development. At the outset of this work several 3-(quaternary ammonium bromides) (**1–5**) were prepared and tested for antibacterial activity. These compounds were demonstrated to be too polar to even cross the bacterial cell wall but led to the synthesis of **6–11**.

New derivatives of rifamycin SV[1] (Figure 1), with a pharmacokinetic behavior different from that of rifamycin SV itself and from that of rifampicin[2] (Figure 1), were sought with the aim of exploring new specific applications of this type of antibiotics. As references, rifamycin SV undergoes rapid elimination via the biliary route,[3] while rifampicin is not used for urinary or gastroenteric infections because of its low urinary elimination and good oral absorption.[4] Therefore it was planned to synthesize new, highly active, broad-spectrum rifamycin SV derivatives that are able to cross the bacterial cell wall but are not absorbed at the gastroenteric level and are thus suitable for the oral therapy of bacterial intestinal infections.

While structure–activity relationships (SAR) are well defined for both intrinsic and antibacterial activity,[5] no detailed study on the transport of rifamycins across the gastroenteric membranes is yet available. Nonetheless, it

† Alfa Farmaceutici S.p.A.
‡ Opocrin S.p.A.
§ Università di Roma.
⊥ Istituto di Strutturistica Chimica "Giordano Giacomello".

(1) Sensi, P.; Timbal, M. T.; Maffii, G. *Experientia* **1960**, *16*, 412.
(2) Maggi, G.; Pasqualucci, C. R.; Ballotta, R.; Sensi, P. *Chemotherapia* **1966**, *11*, 285.
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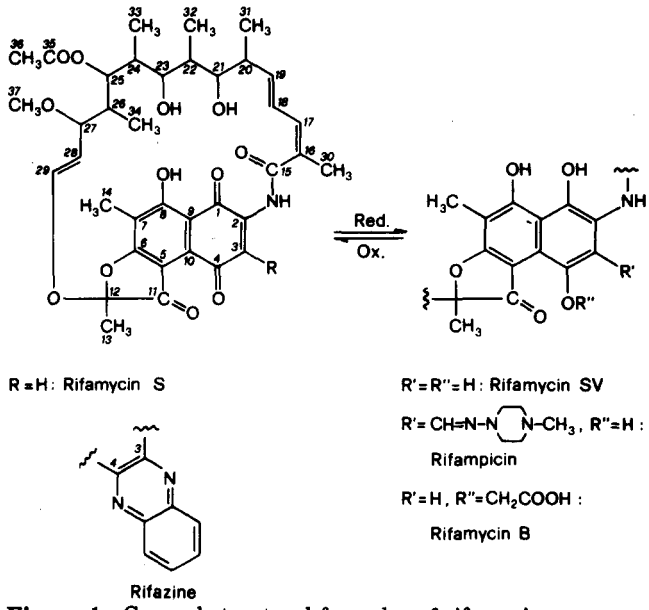


Figure 1. General structural formulas of rifamycins.

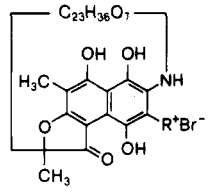
can be assumed that their absorption takes place by passive diffusion across the lipoid membranes and is therefore regulated by the distribution ratio between hydrophilic and lypophilic phases.[6] A modulated enhancement in the polarity, i.e., hydrophilicity, of these molecules appeared thus to be an appropriate way of suitably changing their pharmacokinetic behavior. This enhancement could be achieved, for example, either by increasing the dissociation of the phenolic hydroxyl at C_8 or by introducing acidic groups as in rifamycin B[7] (Figure 1) or by introducing charged groups at C_3 and/or C_4. The first two ways were rejected, since it was already known that rifamycin B, which bears a glycolic acid residue at C_4 (Figure 1), is virtually inactive in vitro[8] and that a series of derivatives of rifamycin S bearing electron-withdrawing substituents at C_3, which cause a net increase of the acidity of the phenolic hydroxyl, was also found to be inactive.[9]

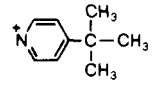
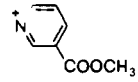
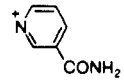
In both cases inactivity was attributed to the too highly polar nature of the molecules, which impaired their ability to penetrate the bacterial cell wall. The third way was therefore chosen.

Results and Discussion

A series of 3-(quaternary ammonium bromide) derivatives of rifamycin SV was synthesized by reacting 3-bromorifamycin S[10] with tertiary amines and subsequently reducing to the hydroquinone SV form with ascorbic acid (Table I, 1–5). These compounds were tested in vitro on a few bacterial strains, but all displayed a poor spectrum of antibacterial activity (Table I). Evidently the derivatization had not lowered the intrinsic activity, since one of the derivatives, **2**, was as active as rifampicin against isolated DNA-dependent RNA polymerase[11] from *Escherichia coli* (DDRP). It was therefore concluded that

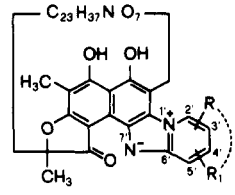
Table I. Structural Formulas and Activity of Compounds 1–5



	R+	MIC[a] μg/mL A	B	C	D	E	ED_{50}^{b} mg/Kg p.o.
1	$\overset{+}{N}(C_2H_5)_3$	0.005–0.01	>50	~50	25–50	12–25	>20
2		0.005–0.01	>50	>50	~50	12–25	>20
3		~0.01	>50	>50	>50	>50	n.e.[c]
4		~0.07	>50	>50	25–50	>50	n.e.[c]
5		0.1–0.3	>50	>50	>50	>50	>20

[a] A = *Staphylococcus aureus* 209 P (FDA), B = *Escherichia coli* ML/35, C = *Klebsiella pneumoniae* Ottaviani, D = *Salmonella paratyphi* B 0248 K (Sclavo), E = *Psuedomonas aeruginosa* ATCC 10145. [b] Mouse infection due to *Staphylococcus aureus*. [c] Not evaluated.

Table II. Structural Formulas and Activity of Compounds 6–11



	R	R_1	MIC[a] μg/mL A	B	C	D	E	ED_{50}^{b} mg/Kg p.o.	s.c.
6	4'-CH_3	H	<0.005	~6	~25	~6	~1.5	>10	0.46
7	3'-CH_3	H	<0.005	6–12	12–25	6–12	3–6	>5	>2.5
8	5'-CH_3	H	<0.005	6–12	6–12	6–12	0.7–1.5	>10	0.47
9	3'-$OCH_2C_6H_5$	H	<0.005	~50	>50	>50	~50	n.e.[c]	n.e.[c]
10	[4',5'-a] benzo		<0.005	12–25	12–25	~3	0.7–1.5	2.22	0.81
11	H	H	<0.005	3–6	6–12	3–6	6–12	10.3	0.21
	Rifampicin[d]		<0.005	6	25	~12	12–25	0.14	0.14

[a] A = *Staphylococcus aureus* 209 P (FDA), B = *Escherichia coli* ML/35, C = *Klebsiella pneumoniae* Ottaviani, D = *Salmonella paratyphi* B 0248 K (Sclavo), E = *Pseudomonas aeruginosa* ATCC 10145. [b] Mouse infection due to *Staphylococcus aureus*. [c] Not evaluated. [d] See Figure 1.

the introduction of quaternary ammonium groups at C_3 in rifamycin SV gives derivatives that are too highly polar and unable to penetrate the bacterial cell wall, especially in the case of Gram-negative bacteria. However, a few of these compounds were tested in mice against the experimental infection by *Staphylococcus aureus*, the bacterial organism that resulted to be most sensitive to them, and yielded a very high ED_{50} po (Table I), thus indicating a low level of absorption.

A further attempt was made by reacting in a similar manner as above 3-bromorifamycin S with 2-aminopyridines, a reaction that on similar naphthoquinonic

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(7) Oppolzer, W.; Prelog, V.; Sensi, P. *Experientia* **1964**, *20*, 336.
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(11) Chamberlin, M. In "The Enzymes. Bacterial DNA-dependent RNA polymerase"; Boyer, P. D., Ed.; Academic Press: New York, 1974; Vol. 10, p 333.

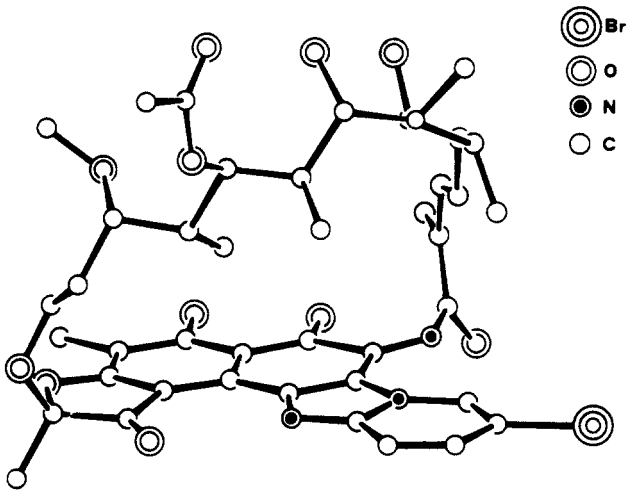

Figure 2. Molecular structure of 4-deoxy-3′-bromopyrido-[1′,2′:1,2]imidazo[5,4-*c*]rifamycin S. Courtesy of S. Cerrini.

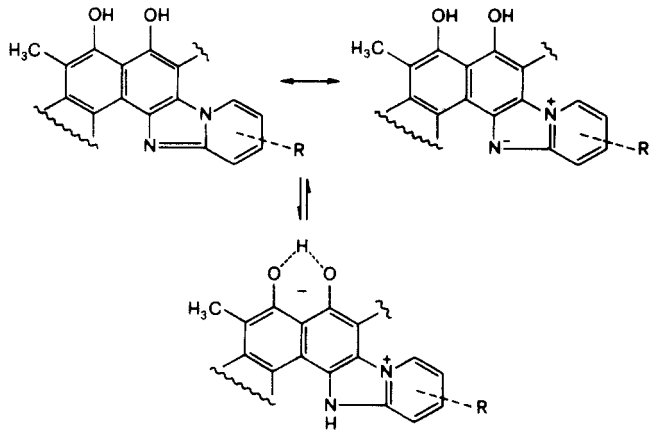

Figure 3. Resonance and tautomeric equilibria in 4-deoxypyrido[1′,2′:1,2]imidazo[5,4-*c*]rifamycin SV derivatives.

compounds leads to the formation of charged condensed polycyclic systems.[12] A new series of compounds (Table II, **6–11**) was thus synthesized and their chemical identity was unambiguously proved by NMR analysis and by single-crystal X-ray analysis on a closely related derivative[13] (Figure 2). In detail, these rifamycin SV derivatives bear a pyrido[1,2-*a*]imidazo system condensed to C_3–C_4. The polycyclic system is entirely planar and therefore aromatic, with a high contribution by charged forms to the resonance structure (Figure 3), as confirmed both by X-ray analysis and by electron spectroscopy for chemical analysis measurements,[13] showing the presence in these molecules of two oppositely charged nitrogen atoms.

Almost all the compounds (**6–11**) were highly active in vitro and gave good levels of protection in the experimental infection in mice when administered parenterally, while their systemic therapeutic efficacy was much lower in oral administration (Table II). This difference in activity between the two ways of administration suggested that these drugs either, as expected, are poorly absorbed when given po or else undergo inactivating transformations in the gastroenteric tract. Therefore, compound **6**,[14] chosen among those giving the highest ratio between subcutaneous and oral activity (Table II), was tested pharmacokinetically.

A first study[15] on urinary and fecal recovery in rats of **6** given po yielded 53% of the unaltered drug in feces and about 0.3% in urine, as measured by microbiological and chemical methods. Analogous measurements performed on **6** on serum and tissue levels in rats and on serum levels in dogs showed absorption levels lower than 1% of the oral dose. Furthermore, **6** was stable in gastric juice for 24 h. Recovery of about 97% of radioactivity[17] from feces was observed after oral administration to rats of ^{3}H-labeled **6**.[16]

These data indicate that our initial hypothesis was justified, and compound **6** is now undergoing clinical trials.

A detailed interpretation at the molecular level of the pharmacokinetic properties of these drugs is not yet possible. A chromatographic study on the partition behavior[18] of **6** showed that this compound is more lipophilic than either rifamycin SV (ΔR_m = 0.67) or rifampicin (ΔR_m = 0.49). One can only observe that rifamycin SV derivatives such as rifazine (Figure 1), which bears a benzophenazino system condensed to C_3–C_4, display a high antibacterial activity in vivo on oral administration.[19] The most relevant chemical difference between the benzophenazino and the pyridoimidazo rifamycins seems to be the fact that charged forms make a considerable contribution to the resonance structure only of the latter (Figure 3). Therefore, the peculiar pharmacokinetic properties of the pyridoimidazo rifamycin SV derivatives described in this paper seem due to their zwitterionic nature at physiological pH.

Experimental Section

Synthesis. Standard procedures were followed: 1 equivalent of 3-bromorifamycin S[10] was reacted with 5 equiv of amine for compounds **1–5** and with 2 equiv of amine for compounds **6–11**, in chloroform, with stirring for 4 h at reflux for compounds **1–5** and for 2 h at room temperature for compounds **6–11**. The reaction products were then reduced with ascorbic acid, and their purification was carried out by column chromatography on silica gel 60. Each compound was identified by elemental analysis and by ^{1}H NMR.

Antimicrobial Activity. MIC values were determined in a liquid medium by the serial dilution method in test tubes. The medium used was brain–heart infusion (BHI, Difco). The inoculum size was always 10^6 cells/mL. MIC was defined as the lowest antibiotic concentration able to prevent a visible growth after 24 h of incubation at 35 °C.

ED_{50} Determination. Mice were infected by an intraperitoneal route with 0.25 mL of a *Staphylococcus aureus*–hog gastric mucin suspension in broth culture, standardized overnight to give an infective inoculum of 10–100 lethal doses. The antibiotics were administered immediately after infection either by an oral or subcutaneous route. Groups of 10 mice were treated at each dosage and route of administration. The number of animals

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(19) (a) Sensi, P.; Greco, M. A.; Furesz, S.; Maffi, G. *Antimicrob. Agents Chemother.* **1966**, 699. (b) Bickel, H.; Knusel, F.; Kump, W.; Neipp, L.; Sackmann, W. *Ibid.* **1966**, 359.

surviving 4 days after infection were recorded and the ED_{50} value was calculated by the Reed and Muench method.[20]

Activity on Isolated DDRP. The inhibition tests of **2**, further purified by chromatography on a Merck Lobar prepacked column, LiChroprep RP-8, 40–63 μm, size A, eluted with acetonitrile–water (8:2), and rifampicin (Boehringer, Mannheim), as reference compound, on isolated DDRP from *E. coli* B (EC 2.7.7.6), were performed according to standard procedures.[11] A 20 nM concentration of enzyme and concentrations of the two antibiotics up to 100 nM were used. At 20 nM both compounds yielded about 95% inhibition.

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Partition measurements: $R_m = \log (1/R_f - 1)$ as measured by TLC on silica gel F plates impregnated with silicone DC 200 oil,[16] eluting with a mixture of phosphate buffer (25 mM, pH 7.3) and acetone (6:4). The color of the compounds allowed direct positioning of the spots on the plate.

Registry No. 1, 95863-72-2; 2, 95863-73-3; 3, 95836-34-3; 4, 95836-35-4; 5, 95836-36-5; 6, 80621-81-4; 7, 80621-83-6; 8, 80621-82-5; 9, 96165-99-0; 10, 80621-85-8; 11, 80621-86-9; 3-bromorifamycin S, 57375-25-4; triethylamine, 121-44-8; pyridine, 110-86-1; 4-*tert*-butylpyridine, 3978-81-2; methyl nicotinate, 93-60-7; nicotinamide, 98-92-0; 2-amino-4-methylpyridine, 695-34-1; 2-amino-5-methylpyridine, 1603-41-4; 2-amino-3-methylpyridine, 1603-40-3; 2-amino-5-benzyloxypyridine, 96166-00-6; 1-aminoisoquinoline, 1532-84-9; 2-aminopyridine, 504-29-0.

A Comparison of Mechanisms Proposed for the Conversion of Mitomycins into Mitosenes

Bhashyam S. Iyengar and William A. Remers*

Department of Pharmaceutical Sciences, College of Pharmacy, University of Arizona, Tucson, Arizona 85721.
Received December 15, 1983

Two mechanisms proposed for the acid-catalyzed conversions of mitomycins into mitosenes were investigated by deuterium incorporation methods. Four different mitomycins, an aziridinomitosene, and an *N*-acetylmitomycin all underwent the conversion in acetic acid-*d* with no incorporation of deuterium at C-1. This evidence suggests that the mechanism based on initial elimination of the elements of methanol to give an aziridinomitosene is more likely the correct one. The products of these reactions had considerable variation in the ratios of cis to trans isomers: 7-aminomitosanes gave a predominance of trans and 7-methoxymitosanes gave a predominance of cis. Treatment of mitomycin C with DCl in D_2O gave predominantly cis product with about 45% deuterium exchange at C-1. The isomeric 2,7-diamino-1-methoxymitosenes previously obtained by treating mitomycin C in methanol containing acetic acid were found to have stereochemistry opposite to that originally assigned by us.

The conversion of mitomycins into mitosenes is characterized by complex and rather unpredictable chemistry. However, it has important implications both in the stability of mitomycins[1,2] and in the mechanisms of their interactions with biopolymers. In particular, the alkylation of DNA by mitomycin C involves its conversion into a mitosene.[3-5]

Treatment of mitomycins with acids results in the formation of mitosenes in which the aziridine ring has been opened, with the nitrogen remaining bonded to C-2 and the nucleophile becoming bonded to C-1 (Scheme I).[6-8] The predominance of cis over trans stereochemistry in many of these products has been a subject of considerable interest.[8-11] In strong acids, the mitosenes can be degraded further by hydrolysis of the carbamate and 7-amino groups.[1,6,7] Two different mechanisms have been proposed for the conversion of mitomycins into 1,2-disubstituted mitosenes by acids and they both are attractive.[2,7] We thought that it might be useful to evaluate their relative importance. The first mechanism (Scheme I, path A) was proposed by Stevens and co-workers to account for their observation that at least 40.5% of the hydrogen at C-1 was exchanged for deuterium when mitomycin C (**I**) was treated with CH_3CO_2D for 15 min at room temperature.[7] Their mechansim begins with protonation of the aziridine nitrogen and ring opening with incorporation of a trans acetoxy group at C-1. The resulting intermediate then undergoes trans elimination of methanol (in a two-step reaction involving an imminium ion) from C-1 and C-9a to give the 1-acetoxy 1(9a)-ene, which adds deuterium at C-1 as it loses a proton from C-9 to form the mitosene structure. Although not suggested by the authors, this mechanism can explain the predominance of cis stereochemistry because the deuterium ion would prefer to approach the double bond from the side opposite to the 2-amino group, which should be protonated in acid ($pK_a \approx 7$). These authors noted that other mechanisms must be operating because much of the hydrogen at C-1 was not exchanged.

The second mechanism (path B) was proposed recently by Underberg and Lingeman.[2] It begins with protonation of the 9a-methoxy group, which leaves as methanol and forms an immonium ion. Loss of the hydrogen at C-9 then gives an aziridinomitosene (**8**). Protonation on the aziridine nitrogen results in ring opening to give a C-1 carbonium ion, stabilized by conjugation with the 9,9a-double bond. The nucleophile then adds to C-1, with possible hydrogen bonding between it and the 2-amino and 10-oxy groups serving to control the stereochemistry of the

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