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.

(20) Reed, L. J.; Muench, H. *Am. J. Hygiene* **1938**, *27*, 493.

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

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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

(1) Garrett, E. R. *J. Med. Chem.* **1963**, *6*, 488.
(2) Underberg, W. J. M.; Lingeman, H. *J. Pharm. Sci.* **1983**, *72*, 549.
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(4) Tomasz, M.; Lipman, R.; Snyder, J. K.; Nakaniskhi, K. *J. Am. Chem. Soc.* **1983**, 105, 2059.
(5) Hashimoto, Y.; Shudo, K.; Okamoto, T. *Chem. Pharm. Bull.* **1983**, *31*, 861.
(6) 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, C.; Lancaster, J. E. *J. Am. Chem. Soc.* **1962**, *84*, 3186.
(7) Stevens, C. L.; Taylor, K. G.; Munk, M. E.; Marshall, W. S.; Noll, K.; Shah, G. D.; Shah, L. G.; Uzu, K. *J. Med. Chem.* **1964**, *8*, 1.
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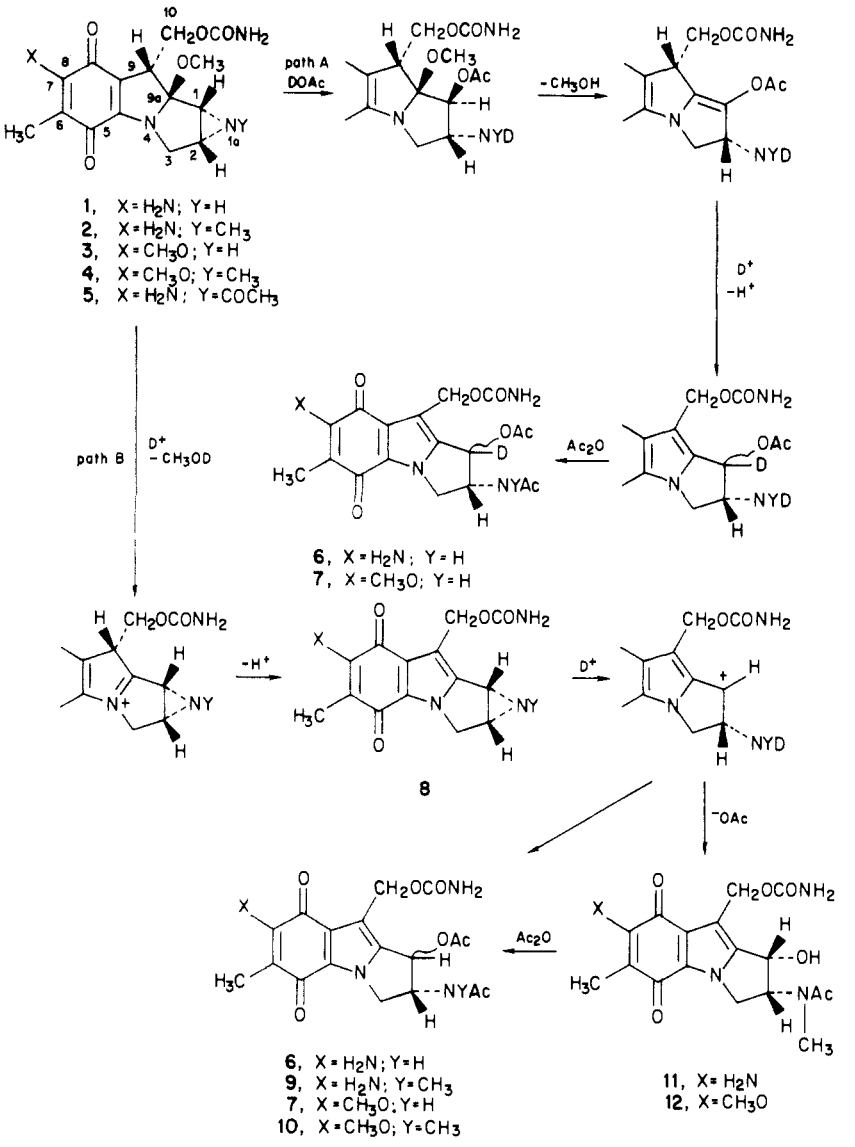
(9) Cheng, L.; Remers, W. A. *J. Med. Chem.* **1977**, *20*, 767.
(10) Tomasz, M.; Lipman, R. *J. Am. Chem. Soc.* **1979**, *101*, 6063.
(11) Hornemann, U.; Takeda, K. "Abstracts of Papers", 182nd National Meeting of the American Chemical Society, New York, NY, Aug 1981; American Chemical Society: Washington, DC, 1981; MEDI 89.

0022-2623/85/1828-0963$01.50/0

Scheme I



product. This mechanism differs from the one in path A by the initial site of protonation, the 9a-methoxy group vs. the aziridino nitrogen. Another difference is that it would not show any incorporation of deuterium at C-1.

It seemed to us that both of these mechanisms might be operating in acid hydrolyses of mitomycins and that the partial deuterium incorporation at C-1 indicated that about 40% of the reaction went by path A and 60% by path B in the specific case of mitomycin C in CH_3CO_2D. If this were true, an useful test might be to examine under the same conditions a mitomycin whose structure would favor a shift from one mechanism to the other. Porfiromycin (2), the 1a-*N*-methyl analogue of mitomycin C, appeared to be a good compound for this purpose because the lower pK_a of its conjugate acid (0.91 vs. 1.19 for mitomycin C)[2] might decrease the proportion of the reaction going by path A and, consequently, decrease the amount of deuterium exchange at C-1. To perform this test we repeated with slight modification the reaction of mitomycin C in CH_3CO_2D described by Stevens and co-workers,[7] converting the initial product to the *N,O*-diacetyl derivative 6 before determining the deuterium incorporation at C-1 by ^1H NMR spectroscopy. Our result (Experimental Section) shows no loss of hydrogen at C-1, which is in contrast with the previous result. The experiment (and others described below) was run three times and no deuterium exchange

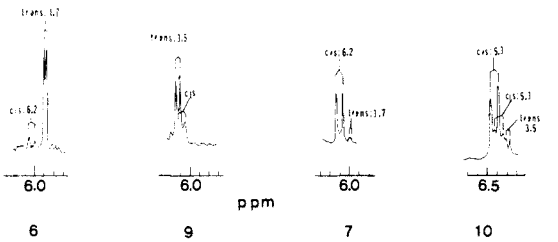


Figure 1. ^1H NMR spectra for the C-1 proton of diacetates derived from the mitomycins. Coupling constants for the C-1 and C-2 protons ($J_{1,2}$) in hertz. Compounds **6**, **9**, and **7** were taken in Me_2SO-d_6 and **10** was taken in $CDCl_3$.

was found in any run. Interestingly, the ^1H NMR spectrum showed a strong predominance of *trans*-diacetate (Figure 1). A ratio of 4:1 trans/cis was determined from the peaks at δ 5.94 (d, $J_{1,2}$ = 1.7 Hz) and 6.2 (d, $J_{1,2}$ = 6.2 Hz).

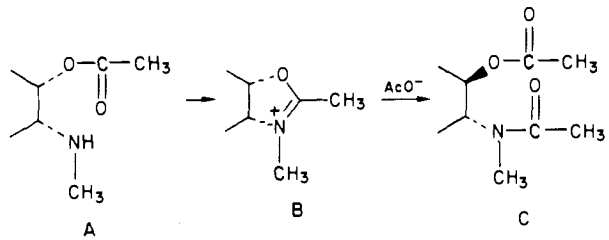
Treatment of porfiromycin under the same conditions used for mitomycin C also resulted in no exchange of deuterium for hydrogen at C-1. It was not possible to make a precise determination of the cis/trans ratio of the diacetates **9** because the C-1 protons in these epimers partially overlapped. However, it is clear from the ^1H NMR spectrum (Figure 1) that the trans form ($J_{1,2}$ = 3.5 Hz) predominates. The immediate products from the treat-

ment of porfiromycin and mitomycin F with acetic had not been isolated previously. Therefore, we repeated the experiments in acetic acid but isolated the mixture and resolved it by thin-layer chromatography without treating it with acetic anhydride. Porfiromycin gave a mixture of *cis*- and *trans*-diacetates **9**, *cis*-2-(acetylmethylamino)-7-amino-1-hydroxymitosene (**11**), showing a coupling constant of 5.8 Hz, and a trace of a third component that could not be isolated. In order to obtain better information on the relative amounts of diacetates **9** and monoacetate **11**, the crude product from a reaction run the same way as just described was resolved by thin-layer chromatography, and the spots were scraped off, extracted with methanol, and examined by UV absorption spectroscopy. This method showed a ratio of 59:41 for diacetate mixture **9** to **11**.

Mitomycin F (*N*-methylmitomycin A (**4**)) also showed no deuterium exchange at C-1 when treated with deuterioacetic acid. A quantitative determination of the cis/trans ratio of the derived diacetates could not be made because the peaks overlapped (Figure 1), but there is clearly a predominance of the cis isomer. The ^{1}H NMR spectrum of the region δ 6.5–6.2 actually shows two doublets ($J_{1,2}$ = 5.7 Hz) for the cis isomer and a small doublet ($J_{1,2}$ = 3.5 Hz), at δ 6.3, for the trans isomer. It is uncertain why two doublets are seen for the cis isomer, but restricted rotation of the 2-substituent might be involved. The TLC plate showed only two spots. Isolation of a before treatment with acetic anhydride gave a TLC pattern similar to that obtained from acetolysis of porfiromycin, except that the slower moving spot predominated. The faster moving spot was shown to be a mixture of diacetates by its ^{1}H NMR spectrum, whereas the slower moving spot was shown to be *cis*-2-acetylmethylamino)-1-hydroxy-7-methoxymitosene (**12**) by its ^{1}H NMR spectrum and conversion into porfiromycin acetolysis product **11** upon treatment with ammonia.

Treatment of mitomycin A (**3**) with acetic acid-*d* and then acetic anhydride also gave a diacetate mixture that had no deuterium exchange at C-1. The ^{1}H NMR spectrum (Figure 1) showed that 90% of the product mixture was cis isomer (δ 6.18 $J_{1,2}$ = 6.2 Hz) and 10% was the trans isomer (δ 6.0).

At this point in the study it was apparent that little or no deuterium exchange occurred in the aziridine ring opening of mitomycins. This results favors the mechanism proposed by Underberg and Lingeman (path B). It also shows that our original premise about the effect of an *N*-methyl group on the deuterium exchange was not needed. However, the experiments suggested by this premise proved valuable in settling the deuterium exchange question and in raising two new ones. One question is how to account for the remarkable variation in cis/trans ratio of the diacetates: from 1:4 from mitomycin C to 10:1 from mitomycin A. A long-range effect of the 7-substituent must be involved because of the apparent preference of trans isomer from 7-amino compounds (mitomycin C and porfiromycin) and cis isomer preference from the 7-methoxy compounds. Rebek and co-workers have discussed the importance of solvent effects and strength of the nucleophile in determining the stereochemical course of aziridine ring opening.[12] These effects are clearly different in acetic acid solution than in aqueous acid, which gives a rather uniform preference for cis stereochemistry. The other question of importance is why are *O*,*N*-diacetates formed in glacial acetic acid. This reaction is the predominant process in the case of porfiromycin. It is possible to write reaction sequences that give diacetates with trans stereochemistry, for example, A → B → C, but supporting evidence for this sequence or others is lacking.



Because an aziridinomitosene intermediate (**8**) was proposed in path B, we thought it appropriate to show that such an intermediate, under the experimental conditions described above, would give the same product as obtained from the corresponding mitomycin. The aziridinomitosene **8** (X = CH_3O, Y = CH_3)[9] prepared from mitomycin F was used for this purpose. It gave **10** with no incorporation of deuterium at C-1.

Another consideration that seemed important was a comparison between the products obtained from the acetic acid experiments described above and the products that would be formed by opening the aziridine ring of mitomycin C in aqueous acid. For this purpose, mitomycin C was treated with 0.05 N DCl in D_2O and the product mixture was neutralized with sodium acetate, concentrated, and treated with acetic anhydride. The crude diacetate mixture was purified by silica gel chromatography, which showed one main purple band. Much decomposed material remained at the origin. The low yield of mitosenes obtained from acid hydrolysis of mitomycin C was noted previously.[8] The ^{1}H NMR spectrum of the product showed the same diacetate mixture that was obtained from the reaction in acetic acid-*d*; however, the cis isomer predominated to the extent of about 90% to 10% for the trans isomer. There was an appreciable loss of hydrogen for deuterium at C-1, estimated to be 45%. It is risky to make firm conclusions about the sereochemistry and deuterium exchange in the aqueous acid hydrolysis of mitomycin C because of the poor material balance. However, the results appear to be quite distinct from the ones obtained in acetic acid. It is not surprising that a different cis/trans ratio is obtained in aqueous acid, because the ion pair produced on aziridine ring opening would be in a much different environment than one formed in acetic acid. The significant deuterium exchange might be the result of product instability in the DCl.

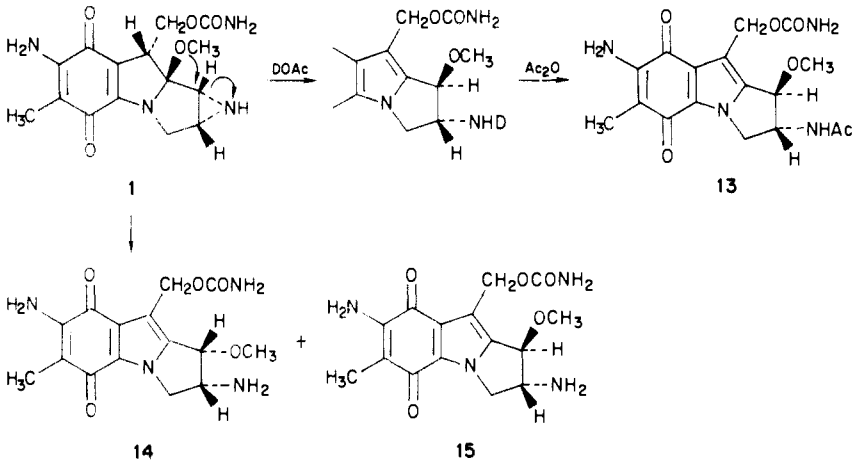
Another point of interest was the effect of electron-withdrawing groups on the aziridine nitrogen in determining stereochemistry and deuterium exchange in the ring-opening process. For this purpose, we prepared the known[13] *N*-acetyl derivative **5** and subjected it to acetic-*d* acid. The resulting diacetate mixture **6** showed no deuterium incorporation at C-1 and a cis/tans ratio of 4:1. Thus, the *N*-acetyl group is able to overcome the effect of the 7-amino group in enhancing the trans product in acetic acid. Of course, this might be explained by a change in mechanism caused by the nonbasic acetylaziridine.

One novel proposal for a mitomycin to mitosene mechanism (Scheme II) resulted from the observation by Stevens and co-workers that the reaction of mitomycin C with CH_3CO_2D followed by *N*-acetylation gave a minor product

(12) Rebek, J.; Jr.; Shaber, S.; Shue, Y. K.; Gehret, J. C.; Zimmerman, S. *J. Org. Chem.*, in press.

(13) Kinoshita, S.; Uzu, K.; Nakano, K.; Shimizu, M.; Takahashi, T.; Matsui, M. *J. Med. Chem.* **1971**, *14*, 103.

Scheme II



(13, 10% yield) that had a 1-methoxy group and no incorporation of deuterium at C-1. This product was viewed as resulting from an intramolecular migration of the 9a-methoxy group to C-1 as the aziridine ring opened (Scheme II).[7] Such a process requires trans stereochemistry in the product. We have been unable to find this product despite many attempts to repeat the reaction of mitomycin C in CH_3CO_2D.[8] However, we were able to prepare a compound with the same properties (after acetylation) by their method of treating mitomycin C with methanol and a small amount of acetic acid.[7] We also obtained a small amount of an isomeric 1-methoxy compound, which we assigned cis stereochemistry.[8] The recent publication by Rebek and Shaber of the stereospecific total synthesis of both of these isomers[15] has shown that our compounds were assigned the wrong stereochemistry and that they must be revised. Thus, the major product from mitomycin C in methanol is the cis isomer **14** and the minor product is the trans isomer **15**. This was evident from the direct comparison of one of our isomers with the trans isomer of Rebek and Shaber and supported further by their 360-MHz ^1H NMR spectra. By implication, the structures proposed by Stevens and co-workers might have cis stereochemistry, but we cannot say that this is certain, because we were unable to prepare a 1-methoxy compound from the CH_3CO_2D reaction.

Finally, some commentary on the coupling constants between the protons on C-1 and C-2 in the various cis and trans mitosenes seems appropriate. Hornemann and Kohn and their co-workers have analyzed this problem in detail and concluded that, as the saturated ring approaches the C_2 conformational extreme, a large value $J_{1,2}$ indicates cis stereochemistry and a small $J_{1,2}$ value indicates trans stereochemistry.[14] Our value for the diacetates **6** and **7** from mitomycin C and mitomycin A are in good agreement with this analysis, with cis $J_{1,2}$ = 6.2 Hz and trans $J_{1,2}$ = 1.7 Hz, almost identical with their reference structures. However, the corresponding diacetates with *N*-methyl groups (**9** from porfiromycin and **10** from mitomycin F) experience small changes in coupling constants (to 5.7 and 3.2 Hz), which indicates deviation from the conformational extreme. This probably is caused by steric crowding. The presence of two doublets for the C-1 proton of **10** also suggests steric crowding and conformational restriction. The C-1 proton for the corresponding cis isomer **9** from porfiromycin was too much overlapped by the trans isomer to tell with certainty that there were two conformers for the cis isomer. However, the width of the C-1 proton signal suggests that this is likely.

(14) Hornemann, U.; Iguchi, K.; Keller, P. J.; Vu, H. M.; Kozlowski, J.; Kohn, H. *J. Org. Chem.* **1983**, *48*, 5026.
(15) Rebek, J., Jr.; Shaber, S. *Heterocycles* **1981**, *16*, 1173.

Experimental Section

Melting points were recorder on a Mel-Temp melting pont apparatus and are uncorrected. Proton magnetic resonance (^1H NMR) spectra were recorded on a JEOL FX90Q spectrometer with tetramethylsilane as the standard. Ultraviolet–visible spectra were recorded on a Beckman DU-8 spectrophotometer in methanol. Elemental analyses were performed at the Analytical Center, University of Arizona, Tucson, AZ. Products were purified by preparative thin-layer chromatography (TLC) on precoated silica gel plates (20 × 20 cm, 2 mm thick) with $CH_3OH/CHCl_3$ (2:8 by volume) as the developing solvent.

Preparation of the Diacetates 6–10. The diacetates **6–10** were obtained from mitomycins by slightly modifying the procedure described by Stevens.[7] A solution of the mitomycin 1–4 (10 mg) or aziridinomitosene **8** in 1 mL of CH_3CO_2D (98.0% D, obtained from Aldrich) was stirred for 1 h under nitrogen. The reaction mixtures turned purple (from **1** and **2**) or orange (from **3** and **4**). The solvent was removed by evaporation under reduced pressure. The residue was dissolved in pyridine (1 mL) and the solution was stirred with acetic anhydride (1 mL) for 1 h under nitrogen. The solvent was removed by evaporation under reduced pressure, followed by azeotroping with toluene to remove traces of pyridine, and the crude product was purified by preparative TLC. The ^1H NMR spectra of the diacetate **6** obtained from mitomycin C and **9** from porfiromycin were taken with use of Me_2SO-d_6 solvent. The integration of the C-1 proton (5.9–6.2 ppm) was compared with the integrations of the 10-CH_2 (5.01 ppm), $OCOCH_3$, $NCOCH_3$, and CH_3 (2.03 and 1.77 ppm) in order to calculate the percent D incorporated at C-1. The ^1H NMR spectra of the diacetate **7** obtained from mitomycin A and **10** from mitomycin F or the corresponding aziridinomitosene **8** were taken in Me_2SO-d_6 or $CDCl_3$, respectively, and analyzed in the same manner as **6** and **9**.

These experiments were repeated three times with the same results. In no case was any diminution of the C-1 H integration observed.

Compound **9**, a mixture of *cis*- and *trans*-diacetates, was obtained as a purple solid: mp 162–166 °C dec; NMR (Me_2SO-d_6) δ 1.75 (3 H, s), 2.0–2.1 (6 H, overlapped singlets), 2.90 (3 H, s), 3.3–3.5 (1 H, m), 4.10–4.60 (2 H, m), 5.03 (2 H, s), 6.1–6.3 (1 H, d), 6.50 (2 H, br s), 6.70 (2 H, br s); IR (KBr) 3200–3440, 1720, 1700, 1600 cm^{-1}. Anal. ($C_{19}H_{22}N_4O_7 \cdot CH_3OH$) C, H, N.

Reaction of 1a-Acetylmitomycin C with CH_3CO_2D. A solution of 1a-acetyl-mitomycin C[13] (10 mg) in 1 mL of CH_3CO_2D was stirred under nitrogen. The solvent was removed under reduced pressure and the purple residue was purified by preparative TLC, which showed one spot with an R_f value identical with that of the diacetate mixture obtained from mitomycin C. The ^1H NMR spectrum in Me_2SO-d_6 was analyzed in the same way as described above for the diacetates **9**. It showed no loss of the C-1 proton. However, the integral for the C-1 proton in the cis

isomer (6.15 ppm, $J_{1,2}$ = 6.2 Hz) was 4 times as large as that from the trans isomer (5.90 ppm, $J_{1,2}$ = 1.7 Hz).

Reaction of Porfiromycin with CH_3CO_2H. A solution of porfiromycin (30 mg) in 2 mL of CH_3CO_2H was stirred for 3 h. The solvent was removed under reduced pressure and the purple residue was divided into two parts: a 5-mg part to be used for UV analysis and the main part. The main part was purified by preparative TLC with ethyl acetate/acetone (8:2) as solvent. Two main spots and a trace of a third spot with intermediate R_f were observed. The spot with highest R_f gave 8.6 mg of purple solid, which had a ^1H NMR spectrum identical with that of the mixture of *cis*- and *trans*-diacetates **9** described above. The R_f of this spot also was identical with that of the diacetates. From the spot with lowest R_f was obtained 6.5 mg of a new purple compound, which was shown to be *cis*-2-(acetylmethylamino)-7-amino-1-hydroxymitosene (**11**) by its conversion into the *trans*-diacetate on acetylation with acetic anhydride and by the following eivdence: IR (KBr) 3240–3440, 1725, 1700, 1600 cm^{-1}; NMR (Me_2SO-d_6) δ 1.76 (s, 3 H), 2.05 (s, 3 H), 2.7–2.9 (d, 3 H), 3.3–3.5 (m, 1 H), 4.10–4.60 (m, 2 H), 5.03 (s, 2 H), 4.9–5.4 (m, 1 H), 5.5 (d, 1 H), 6.5 (s, 2 H), 6.6 (s, 2 H) ppm. Anal. ($C_{17}H_{20}N_4O_6 \cdot 1.5CH_3OH$) H; C: calcd, 52.36; found, 52.80. N: calcd, 13.19; found, 12.49.

The 5-mg portion of crude product was dissolved in methanol and a 0.1-mL aliquot was placed on a silica gel plate (5 × 20 cm). After the place dried it was developed with ethyl acetate/acetone (8:2). The purple spots were scraped off the plate and extracted with 25 mL of CH_3OH, each, and the UV absorption spectra of these extracts were measured. They showed a ratio of diacetates **9**/**11** of 59:41 at both 255 and 305 nm.

Reaction of Mitomycin F with CH_3CO_2H. This reaction was carried out in exactly the same way as described for porfiromycin, except that it was done on a one-third scale. Two strong and one very faint orange spots were seen on preparative TLC. The spot with highest R_f was identical in R_f with the *cis*- and *trans*-diacetate mixture **10** obtained from mitomycin F. The spot with lowest R_f gave on treatment with 0.5 N NH_4OH in methanol a product identical in R_f and infrared spectrum with compound **11** obtained from porfiromycin. Therefore, the spot with lowest R_f must have contained *cis*-2-(acetylmethylamino)-1-hydroxy-7-methoxymitosane (**12**).

Chromatography of the small portion of the crude product, followed by UV absorption analysis, showed a ratio of 28.5:71.5 for the diacetates **10**/**12**.

Reaction of Mitomycin C with DCl. A solution of mitomycin C (20 mg) in 3.2 mL of D_2O (99.8%, Aldrich) containing 0.05 N DCl was stirred under N_2 for 40 min. The resulting purple solution was cooled in an ice bath and treated with aqueous sodium acetate until the pH was 9.5. Three extractions with 50-mL portions of ethyl acetate were made and the combined extracts were dried (Na_2SO_4) and concentrated. The residual solid was treated with 3 mL of pyridine and 1 mL of acetic anhydride. After 16 h the mixture was worked up as described above and the crude product was purified by TLC on silica gel with CH_3OH–$CHCl_3$ (2:8) as solvent. Considerable material remained at the origin. The main purple band was scraped off the plate and extracted with methanol. Concentration of this extract gave about 3 mg of purple solid. A ^1H NMR spectrum of this solid (Me_2SO-d_6) showed it to be the diacetate **6**, with the C-1 protons occurring in a ratio of about 9:1 cis (6.1 ppm, $J_{1,2}$ = 6.2 Hz) to trans (5.9 ppm, $J_{1,2}$ = 1.7 Hz). The ratio of these combined peak integrals to those of the three methyl groups showed that about 55% of the theoretical amount of H-1 was still present.

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Registry No. 1, 50-07-7; 1 (1a-acetyl), 1102-95-0; **2**, 801-52-5; **3**, 4055-39-4; **4**, 18209-14-8; **6** (isomer 1), 96412-15-6; **6** (isomer 2), 96479-82-2; **7** (isomer 1), 96412-16-7; **7** (isomer 2), 96479-83-3; **8** (X = CH_3O, Y = CH_3), 15973-07-6; **9** (isomer 1), 96412-17-8; **9** (isomer 2), 96479-44-6; **10** (isomer 1), 96479-45-7; **10** (isomer 2), 96479-46-8; 11, 96412-18-9; **12**, 96412-19-0.

Improved Antagonists of Luteinizing Hormone-Releasing Hormone Modified in Position 7[1]

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The structure–activity relationship of position 7 in the antagonist [N-Ac-D-Nal[1],D-pClPhe[2],D-Trp[3],D-Arg[6],D-Ala[10]]-LH-RH has been investigated by the incorporation of a series of amino acids at this position. The analogues were prepared by solid-phase peptide synthesis. All purifications were performed in two stages: gel permeation followed by preparative reversed-phase high-performance liquid chromatography. The analogues were assayed in the standard rat antiovulatory assay using a 40% propylene glycol–saline vehicle. The results demonstrated that position 7 requires a hydrophobic aromatic amino acid for greatest antiovulatory activity. The compound [N-Ac-D-Nal[1],D-pClPhe[2],D-Trp[3],D-Arg[6],Phe[7],D-Ala[10]]-LH-RH caused 65% blockade of ovulation at the 500-ng dose and is approximately twice as active as the parent analogue in this assay system. The enhanced activity may indicate the stabilization of the active conformation via intramolecular hydrophobic or π–π interactions.

One aim for the synthesis of antagonists of luteinizing hormone-releasing hormone (LH-RH), a decapeptide with the sequence Glp-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2, is the control of conception by the effective blockade of ovulation. Toward this goal, over 1000 analogues have been synthesized internationally in the last several years in the search for ever more potent antagonists. Currently, the most active analogues are characterized by distinct hydrophobic and hydrophilic regions and are typified by the antagonist [N-Ac-D-Nal[1],D-pClPhe[2],D-Trp[3],D-Arg[6],D-Ala[10]]-LH-RH.[2] The incorporation of the bulky, hydrophobic residue D-Nal[1] in position 1 resulted in a pronounced increase in antiovulatory activity over the previous halogenated phenylalanines, when combined with D-Arg in position 6.[3] However, past experience has dem-

(1) Abbreviations used in this paper for amino acids, protecting groups, and peptides follow the recommendations of the IUPAC–IUB Commission on Biochemical Nomenclature and Symbols as described in *Eur. J. Biochem.* **1972**, *27*, 201 and *J. Biol. Chem.* **1975**, *250*, 3215; D-Nal, 3-(2-naphthyl)-D-alanine; D-pClPhe, 4-chlorophenyl-D-alanine; D-Abu, D-2-aminobutanoic acid; D-3-Pal, 3-pyridyl-D-alanine; Glp, pyroglutamic acid; NMePhe, *N*-methylphenylalanine; pFPhe, 4-fluorophenylalanine; pMePhe, 4-methylphenylalanine; F_5Phe, 2,3,4,5,6-pentafluorophenylalanine.

(2) Horvath, A.; Coy, D. H.; Nekola, M. V.; Coy, E. J.; Schally, A. V.; Teplan, I. *Peptides* **1982**, *3*, 969.

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