(9) R. Gordon, S. Spector, A. Sjoerdsma, and S. Udenfriend, *J. Pharmacol. Exp. Ther.*, **153**, 440 (1966).
(10) R. W. Fuller and H. Snoddy, *J. Pharm. Pharmacol.*, **20**, 156 (1968).
(11) L. Weintraub, S. R. Oles, and N. Kalis, *J. Org. Chem.*, **33**, 1679 (1968).
(12) J. Short and T. Darby, *J. Med. Chem.*, **10**, 833 (1967).
(13) C. Braun, *J. Amer. Chem. Soc.*, **55**, 1280 (1933).
(14) R. W. Fuller and J. M. Hunt, *Anal. Biochem.*, **16**, 349 (1966).
(15) P. A. Shore and J. S. Olin, *J. Pharmacol. Exp. Ther.*, **122**, 295 (1958).
(16) C. C. Chang, *Int. J. Neuropharmacol.*, **3**, 643 (1964).

Structure and Stereochemistry of Some 1,2-Disubstituted Mitosenes from Solvolysis of Mitomycin C and Mitomycin A[1]

Wesley G. Taylor and William A. Remers*

Department of Medicinal Chemistry and Pharmacognosy, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, Indiana 47907. *Received September 30, 1974*

Starting with mitomycin C (1), a number of solvolytic reactions were investigated and were found to result in opening of the aziridine ring with loss or migration of the 9a-methoxy group. A careful examination of the resulting 1,2-disubstituted 7-aminomitosenes indicated that there was a strong tendency for the aziridine ring on opening to furnish mainly one stereoisomer, always with the oxygen atom at C-1 and the nitrogen atom at C-2. Thus the hydrolysis of 1 with dilute aqueous hydrochloric acid gave mostly *cis*-2,7-diamino-1-hydroxymitosene (3) in addition to small amounts of the *trans*-aminohydrin (10). Mitomycin A (2) behaved analogously. Both 1 and 2 generated a *cis*-1-acetoxy-2-acetamide when they were allowed to react with acetic anhydride. Acetolysis of mitomycin C was found to give the *cis*-1-hydroxy-2-acetamide (5), the *trans*-1-acetoxy-2-amine (14), and a cis–trans mixture of 1-acetoxy-2-acetamides (4 and 11, respectively). Routes to *cis*-1-methoxy-2-acetamide (9) were possible through the methanolysis of 1 or through the methylation of 5. For comparison, the *trans*-1-methoxy-2-acetamide (16) was obtained through a known resin-catalyzed methoxy migration from C-9a to C-1 in mitomycin C. The use of ^{1}H nmr spectroscopy to assign configurations to 1,2-disubstituted mitosenes is discussed.

During the studies on structure elucidation of the mitomycins a variety of solvolysis products were obtained.[2-4] These products generally were 1,2-disubstituted mitosenes (*e.g.*, 3) in which the aziridine ring had been opened and the elements of methanol (water from mitomycin B) eliminated. More vigorous hydrolysis resulted in cleavage of the 7-substituent and the carbamate group. A careful kinetic study of these hydrolytic processes was made with porfiromycin.[5]

Despite the significance of mitosenes to the mitomycin structure elucidation studies, almost nothing was reported about their stereochemistry. Subsequent reports[6,7] on the antibacterial and antitumor activities of mitosenes such as 6 and its *N*-acetyl derivative made a knowledge of their stereochemistry even more important. These considerations, combined with an urgent need to establish unambiguously the stereochemistry of 1,2-disubstituted mitosenes resulting from our mitomycin synthesis program, led us to reinvestigate the mitomycin solvolysis products.

The only mitosene whose structure and stereochemistry had been assigned in the literature was *trans*-2,7-diamino-1-methoxymitosene (15).[3] This compound was claimed to be formed by treating mitomycin C with Dowex resin and methanol, with glacial acetic acid, or with methanol and acetic acid. The stereochemical assignment was based upon a probable migration of the 9a-methoxy group, especially in acetic acid solvent where recapture of the eliminated methoxy group was considered unlikely. However, acetylation of 15 was reported to give a derivative (16) which was identical with the compound prepared by O-methylation of a second product (1-hydroxy-2-acetamide, 5) isolated from the acetic acid reaction and claimed to have been formed by O → N acetyl migration.[3] Since such a migration requires cis stereochemistry the two pieces of evidence were in apparent conflict. However, the basis upon which that identity had been established was not given.

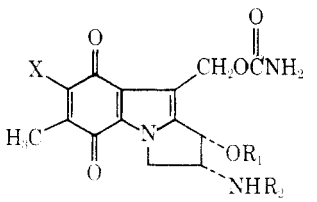
Another mitosene for which much evidence had been published was apomitomycin A.[2,8] This compound underwent semipinacolic deamination to 7-methoxymitosen-1-one upon treatment with nitrous acid, and it afforded a 1,2-cyclic carbamate with phosgene. Although not explicitly stated in the literature, these data are conclusive in establishing apomitomycin A to be *cis*-2-amino-1-hydroxy-7-methoxymitosene (6).

Since it seemed likely that mitosenes 15 and 6 represented examples of trans and cis stereochemistry, respectively, we sought to link them by suitable chemical transformations into a pair of compounds differing only by being epimeric at C-1 and examine the nmr spectra of these epimers. Although it appeared unlikely that these compounds would follow the Karplus equation we hoped for some characteristic differences in their spectra which might aid in stereochemical assignments for related compounds.

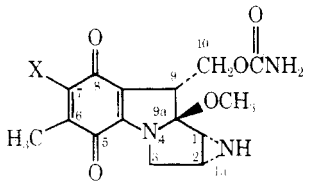
Hydrolysis of mitomycin C by 0.05 *N* hydrochloric acid according to Stevens procedure[3] gave in addition to the previously reported 2,7-diamino-1-hydroxymitosene (the cis isomer 3 as shown below) a second mitosene (3.5% yield) which was isomeric with 3 according to the analytical data. As described below this is the previously unknown trans isomer 10. Mitomycin A was hydrolyzed with 0.05 *N* hydrochloric acid[2] and again two mitosenes were obtained. One of these was the previously reported apomitomycin A, which was confirmed to be *cis*-2-amino-1-hydroxy-7-methoxymitosene (6). The other mitosene, called isoapomitomycin A, had been assigned the structure 1-amino-2-hydroxy-7-methoxymitosene (stereochemistry not specified).[8] This structure actually should be *trans*-2-amino-1-hydroxy-7-methoxymitosene (13) as shown below. Treatment of 6 and 13 with methanolic ammonia gave the corresponding 7-aminomitosenes 3 and 10, respectively, which interrelated the hydrolysis products from mitomycin A and mitomycin C.

Assignment of the 2-amino-1-hydroxy structural feature to mitosenes 3 and 6 was based upon spin decoupling experiments on their corresponding diacetates 4 and 7, respectively. Thus irradiation of the signals containing the

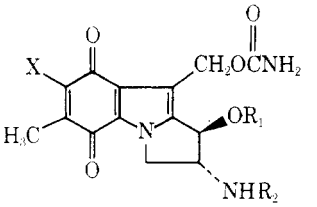
Scheme I



3. X = NH_2; $R_1 = R_2 = H$
4. X = NH_2; $R_1 = R_2 = COCH_3$
5. X = NH_2; $R_1 = H$; $R_2 = COCH_3$
6. X = OCH_3; $R_1 = R_2 = H$
7. X = OCH_3; $R_1 = R_2 = COCH_3$
8. X = NH_2; $R_1 = CH_3$; $R_2 = H$
9. X = NH_2; $R_1 = CH_3$; $R_2 = COCH_3$



1. X = NH_2
2. X = OCH_3



10. X = NH_2; $R_1 = R_2 = H$
11. X = NH_2; $R_1 = R_2 = COCH_3$
12. X = NH_2; $R_1 = H$; $R_2 = COCH_3$
13. X = OCH_3; $R_1 = R_2 = H$
14. X = NH_2; $R_1 = COCH_3$; $R_2 = H$
15. X = NH_2; $R_1 = CH_3$; $R_2 = H$
16. X = NH_2; $R_1 = CH_3$; $R_2 = COCH_3$

C-2 proton (δ 5.04 in both **4** and **7**) collapsed the doublets for the C-1 (δ 6.07) and amide protons to singlets and the doublet of doublets for one of the C-3 protons sharpened to a doublet. These experiments proved that the nitrogen atom was at C-2 in both diacetates. Diacetates **4** and **7** were prepared both by acetylation of aminohydrins **3** and **6** and by treatment of mitomycins C and A with acetic anhydride, and identities of the products formed by the two different routes were established by their nmr and ir spectra. Previously the samples of **4** had been shown to have the same R_f values on tlc.[3] Samples of **7** prepared by these two routes had not been established as identical, although this was suggested by nearly equal melting points for the two samples.[8] Interestingly, the reactions of acetic anhydride with mitomycins C and A gave no traces of *trans*-diacetates.

The preceding experiments, together with the previously published cyclic carbamate formation and semipinacolic deamination of **6**,[8] establish the structures and stereochemistry of **3**, **4**, **6**, and **7** to be *cis*-2-amino-1-hydroxymitosene derivatives as depicted in Scheme I. Structures of the isomeric compounds **10** and **13** were established as follows. A mixture of **3** and **10** from hydrolysis of mitomycin C was converted into diacetates **4** and **11** by acetic anhydride. Spin decoupling of the C-2 proton signals (centered at δ 5.04) in this mixture sharpened to singlets the C-1 proton (δ 5.94 for **11**) and amide proton of both compounds, indicating that **11** also had the nitrogen at C-2. Confirmation of this assignment was obtained by preparing 1-hydroxy-2-acetamides **5** and **12**, corresponding to **3** and **10**, and treating each hydroxyacetamide with manganese dioxide. The products of both reactions gave identical R_f values on tlc, which showed that they resulted from oxidation of the 1-hydroxy group to the same ketone. Facile manganese dioxide oxidation of a 1-hydroxymitosene to the corresponding ketone had been reported previously.[9] *cis*-Hydroxyacetamide **5** was prepared by hydrolysis of diacetate **4** with methanolic ammonium hydroxide. Treatment of aminohydrin **10** with acetic anhydride in methanol afforded *trans*-hydroxyacetamide **12**. These experiments show that **10** and **13** (which was converted to **10**) are 2-amino-1-hydroxymitosenes, rather than 1-amino-2-hydroxymitosenes and, therefore, they must be the trans isomers. This assignment of stereochemistry is supported by the previously reported nitrous acid deamination of **13** (incorrectly assigned the 1-amino-2-hydroxymitosene structure) wherein the product was a 1,2-diol and not a ketone.[8] With respect to the structure assignment for **13**, we wish to report that Dr. J. S. Webb informed us prior to the completion of this study (Feb 1974) of unpublished results on the hydrolysis of mitomycin B followed by nitrosation in which a pair of *cis*- and *trans*-1-hydroxy-7-methoxy-2-methylamino-*N*-nitrosomitosenes was isolated. He suggested that **13** (isoapomitomycin A) also might be a trans isomer.[10]

Although the isolation and identification of *trans*-aminohydrins **10** and **13** provided, along with **3** and **6**, cis–trans pairs of compounds to serve as a basis for assignment of stereochemistry to synthetic 1,2-disubstituted mitosenes, it still was important to prepare the reputed *trans*-methoxyamine **15** and confirm its stereochemistry. The literature procedure by which **15** was prepared from mitomycin C and Dowex resin in methanol[3] afforded a product that showed four spots on tlc. However, acetylation of this mixture followed by crystallization gave a pure methoxyacetamide (shown below to be **16**) which agreed in melting point with the literature compound.[3] When mitomycin C was treated with methanol and a small amount of glacial acetic acid, we obtained **15** and a new compound, isomeric with **15**, which proved to be *cis*-2,7-diamino-1-methoxymitosene (**8**). Only compound **15** was reported previously as the product of this reaction.[3] Treatment of **8** with acetic anhydride gave the *N*-acetyl derivative **9**, in which form it was characterized. Methylation of *cis*-2-acetamido-7-amino-1-hydroxymitosene (**5**) afforded a 1-methoxy derivative **9** which was identical by tlc in two systems with either of the isomeric methoxyacetamides prepared from methoxyamines **15** and **8**, but whose infrared spectrum was superimposable only with that of the methoxyacetamide **9** prepared from **8**. This result shows that **8** is the *cis*-methoxyamine and **15**, as reported previously,[3] is the *trans*-methoxyamine. For the methylation of **5** we found it essential to use methyl iodide in combination with silver oxide and dimethylformamide. The literature method, involving methyl iodide and potassium carbonate,[3] gave only starting material in our laboratory.

Finally, we investigated the solvolysis of mitomycin C in glacial acetic acid. This reaction had been reported previously to give a mixture containing a small amount of *trans*-methoxyamine **15**, mainly a 1-hydroxy-2-acetamide (**5**) considered to be cis on the basis of a probable O → N acetyl migration, and a trace of an unidentified substance.[3] Following the reported conditions we obtained a mixture of four products which included **5** as the main component, but

which contained no **15**. The other components were *cis*- and *trans*-diacetates, shown to be identical with **4** and **11**, and *trans*-1-acetoxy-2,7-diaminomitosene (**14**). This experiment was repeated four times with no variation in the product composition. Treatment of **14** with methanolic ammonium hydroxide gave *trans*-aminohydrin **10**, which helped to confirm its structure.

Thus the structures and relative stereochemistry of a variety of mitomycin solvolysis products are established. Biologically active mitosenes in the 7-methoxymitosene series, such as **6** and its *N*-acetyl derivative, have cis stereochemistry. Of the corresponding trans isomers only **13** was prepared in our study and it was in insufficient quantity for biological testing. A previous report, however, had indicated that **13** and the *cis*-diacetate **7** were somewhat less active than **6** when they were tested against bacteria in cultures.[8] Since the 7-aminomitosenes, for example **3**[11] and **8**,[3] showed almost no antibacterial activity, comparisons in this series were impractical.

Structures of various 7-hydroxymitosenes and mitomycinones, obtained by more vigorous hydrolysis of the mitomycins, follow from their previously established relationships to the corresponding mitosenes.[2-4] The absolute stereochemistry of the mitosenes, depicted in Scheme I, follows from that of the mitomycins[12] since it is unlikely that the 2-amino substituent would undergo epimerization upon ring opening. This means that the trans isomers are 1*S*,2*R* and the cis isomers are 1*R*,2*R*.

Concerning the use of nmr spectra for the assignment of stereochemistry to new mitosenes, it appears that reliance on vicinal coupling constants ($J_{1,2}$) alone for differentiating between isomeric mitosenes is very risky. In DMSO-d_6 a doublet for H-1 was seen in the spectra of *cis*-aminohydrins **3** and **6** ($J_{1,2}$ = 5.0 Hz) and their diacetates ($J_{1,2}$ = 6.0 Hz), whereas certain *trans*-mitosenes gave a broadened H-1 singlet from which half-bandwidth measurements (4–5 Hz) showed J_{cis} to be about 1 Hz greater than J_{trans}. However, when the C-1 substituent was methoxy, as in compounds **15** and **16**, chemical shift differences were so small that the H-1, H-2, and H-3 signals overlapped and vicinal coupling constants could not be obtained by inspection. Thus, the nmr method appears to be useful for assigning configurations to 1-*O*-acyl-2-aminomitosenes, but the differences in J_{cis} and J_{trans} are not sufficiently characteristic to be singularly applicable to all 1,2-disubstituted mitosenes. In a previous study on 1,2-disubstituted indans, $J_{1,2}$ was shown to be unreliable for assigning configurations where J_{cis} was greater than J_{trans} in approximately one-half of a series of isomeric 2-amino-1-indanols.[13]

Experimental Section

Melting points were taken on a Mel-Temp apparatus and are uncorrected. The designated thin-layer chromatography systems include A (precoated silica gel F-254 glass plates, 0.25 mm thickness, 5 × 20 cm, E. Merck, with an MeCN–*n*-BuOH–Me_2CO 3:1:1 solvent system), B (as for A with solvent system of MeOH), C (as for A, with hexane–*i*-PrOH–EtOAc 2:3:2 as solvent mixture), and D (precoated polyamide 11 F-254 aluminum sheets, 0.15 mm thick, 5 × 20 cm, E. Merck, with an $MeNO_2$–*n*-BuOH–MeCOEt–MeOH 3:1:1:1 solvent system). Preparative layer chromatography was carried out in the solvent systems indicated above using precoated silica gel F-254 glass plates, 2.0 mm thickness, 20 × 20 cm, E. Merck. Elemental analyses were carried out by Dr. C. S. Yeh, Department of Chemistry, Purdue University, and by Galbraith Laboratories, Inc., Knoxville, Tenn. Ultraviolet spectra were run on a Cary Model 17 instrument. Nuclear magnetic resonance spectra were obtained on a Varian XL-100 spectrometer using the deuterium of DMSO-d_6 as a field-frequency lock. Chemical shifts are expressed in parts per million downfield from tetramethylsilane. Infrared spectra were recorded on a Perkin-Elmer 237 B spectrophotometer and, where frequencies are given, the band intensities are designated as strong (s), medium (m), or weak (w). The adsorbents for column chromatography were Silic AR CC-7 (60–200 mesh) or 100–200 mesh Florisil (supplied by Fischer Scientific Co.) as indicated.

Hydrolysis of Mitomycin C.[3] A solution of **1** (750 mg, 2.2 mmol) in 0.05 *N* HCl (150 ml) was stirred at 5° for 5 min and then at room temperature for 35 min. The purple mixture was cooled externally in ice and the pH was adjusted to 10 with aqueous K_2CO_3. After 6 hr, 1 ml of aqueous NH_4OH was added and after another 16 hr at room temperature the neutralized mixture was cooled in an ice bath for 1.5 hr. A deep purple hydrolysate was collected by filtration to give 900 mg of air-dried product. The crude material was dissolved in MeOH, evaporated to dryness *in vacuo*, applied to a column of silica gel (containing 15% by weight of H_2O), and eluted with MeOH. The first fraction gave *trans*-2,7-diamino-1-hydroxymitosene (**10**, 23.5 mg, 3.5%) as purple plates: R_f 0.08 (A), 0.30 (B). Two recrystallizations from MeCN gave an analytical sample with no melting point: ir (KBr) 1600 (s, C=C), 1660 (m, C=O), 1715 (s, C=O), and 3300–3400 cm^{-1} (s, OH, NH). *Anal.* ($C_{14}H_{16}N_4O_5$) C, H, N.

The last main fraction from the column showed some **10** and was rechromatographed through silica gel with EtOAc, MeCN, and MeCN–Me_2CO mixtures. On evaporation *in vacuo*, there was obtained 114.5 mg (21%) of *cis*-2,7-diamino-1-hydroxymitosene (**3**)[3] as reddish-brown crystals: R_f 0.22 (A), 0.21 (B). Fine purple needles crystallized from EtOH: nmr (DMSO-d_6) δ 6.52 (br s, 4, C_7NH_2, carbamate NH_2, exchanged with D_2O), 5.04 (ragged s, 2, $C_{10}H_2$), 4.70 (d, 1, J = 5 Hz, C_1H), 4.34 (m, 1, C_2H), 4.14–2.84 (m, 5, C_3H_2, C_1OH, C_2NH_2, latter 3 protons exchanged with D_2O), and 1.76 ppm (s, 3, C_6CH_3).

Fractions from the two previous columns which gave mixtures of **3** and **10** were dried and combined and the resulting 71 mg was acetylated with Ac_2O (1 ml) in pyridine (3 ml) for 1 hr at 5° and for 15 hr at room temperature. After adding 95% EtOH (20 ml) and evaporating to dryness *in vacuo* with the last traces of pyridine removed by azeotroping with toluene, the mixture was passed through a silica gel column. One main red band (41.5 mg) was obtained on eluting with $CHCl_3$, EtOAc, EtOAc–MeCN, and MeCN. The nmr spectrum (DMSO-d_6) gave signals at δ 8.53 (d, J = 7 Hz, C_2NH of diacetate **11**), 8.34 (d, J = 8 Hz, C_2NH of diacetate **4**), 6.07 (d, J = 6 Hz, C_1H of **4**), and 5.94 ppm (s, half-bandwidth 4 Hz, C_1H of **11**). Irradiating the C_2H centered at δ 5.04 collapsed the above lines to sharp singlets thus confirming that N was at C-2 in **3** and **10**.

Reaction of Mitomycin C with Acetic Anhydride.[3] A suspension of mitomycin C (100 mg, 0.3 mmol) in Ac_2O (1 ml) was heated at 125° (oil bath) for 5 min and stirred for 2 hr at room temperature. After adding 10 ml of 95% EtOH, the mixture was evaporated to dryness *in vacuo*. The resulting solid was chromatographed on a silica gel column. Elution with EtOAc and EtOAc–MeCN mixtures gave, after recrystallization from *i*-PrOH, about 1 mg of deep purple crystals, R_f 0.72 (A), mp 145–147°, which showed uv maxima (MeOH) at 247.5 and 307 nm. Continued elution with MeCN and MeCN–Me_2CO mixtures gave *cis*-2-acetamido-1-acetoxy-7-aminomitosene (**4**, 57 mg, 47%) as red needles from MeOH: no melting point; R_f 0.55 (A), 0.19 (C), 0.84 (D); nmr (DMSO-d_6) δ 8.38 (d, 1, J = 8 Hz, C_2NH), 6.68–6.42 (two s, 4, C_7NH_2, carbamate NH_2, exchanged with D_2O), 6.07 (d, 1, J = 6 Hz, C_1H), 5.04 (s, 3, $C_{10}H_2$, C_2H), 4.55 (dd, 1, J = 8, 13 Hz, C_3H), 3.88 (dd, 1, J = 8.5, 12 Hz, C_3H), 2.04 (s, 3, $OCOCH_3$), 1.84 (s, 3, $NCOCH_3$), and 1.74 ppm (s, 3, C_6CH_3).

Preparation of 2-Acetamido-7-amino-1-hydroxymitosene (5). The diacetate **4** (15 mg, 0.037 mmol) was dissolved in MeOH (18 ml) and 0.2 ml of NH_4OH was added. After refluxing on a steam bath for 15 min, the mixture was cooled and evaporated to dryness *in vacuo*. Recrystallization from MeOH gave the desired hydroxyacetamide **5** (11 mg, 82%) as deep purple crystals: noticable decomposition (250–300°) without melting up to 375° in agreement with Stevens, *et al.*,[3] who quote no melting point below 300°. Kinoshita, *et al.*,[6] quote mp 174–178° for **5** which was prepared by treatment of 2-acetamido-1-acetoxy-7-methoxymitosene (prepared from mitomycin A and Ac_2O) with methanolic NH_3: R_f 0.45 (A), 0.73 (D); nmr (DMSO-d_6) δ 8.11 (d, 1, J = 7 Hz, C_2NH, slowly exchanged with D_2O), 6.54 (s, 4, carbamate NH_2, C_7NH_2, exchanged with D_2O), 5.55 (d, 1, J = 5 Hz, C_1OH, exchanged with D_2O), 5.06 (s, 2, $C_{10}H_2$), 4.92 (m, 1, C_1H), 4.76–4.26 (m, 2, C_2H, C_3H), 3.78 (dd, 1, J = 8, 12 Hz), 1.92 (s, 3, $NCOCH_3$), and 1.75 ppm (s, 3, C_6CH_3).

Reaction of Mitomycin C with Glacial Acetic Acid.[3] A solution of mitomycin C (600 mg, 1.8 mmol) in 30 ml of glacial HOAc

was stirred occasionally at room temperature for 2.5 hr. The solvent was removed by evaporation *in vacuo* and tlc (A) showed three components. The mixture was separated by column chromatography on silica gel with $CHCl_3$, EtOAc–MeCN, and finally MeCN–Me_2CO mixtures as the elution solvents. The second red band represented the major component and gave, after rechromatography on silica gel of the fractions containing this material, a total of 172 mg (26%) of *cis*-2-acetamido-7-amino-1-hydroxymitosene (**5**) (as purple needles from MeOH). This hydroxyacetamide was identical by ir, nmr, and tlc (A, B, D) with **5** prepared by deacetylating the diacetate **4**.

The first band off the column yielded 32 mg (4.4% based on mol wt of 404) of approximately a 1:2 mixture of *cis*- (**4**) and *trans*-(**11**) diacetates (as brown flakes, mp 155–165° with decomposition, from H_2O). This mixture was identical with the mixture of diacetates from acetylating the mixture of amino alcohols **3** and **10** by nmr and tlc (A, D) analysis. A small amount was allowed to react with Ac_2O in pyridine (2 hr at 5° and 3 hr at room temperature) which showed no change by tlc (A, C, D). After adding 95% EtOH and evaporating *in vacuo*, the mixture was deacetylated (60 min on steam bath) by NH_4OH–MeOH to a mixture of hydroxyacetamides as judged by tlc (**5** and **12** had identical R_f values in all tlc solvent systems examined).

The last fraction off the column was rechromatographed with silica gel and recrystallized from *i*-PrOH to give 26 mg (4% based on mol wt of 362) of a purple solid **14** with no melting point below 390°: R_f 0.36 (A); nmr (DMSO-d_6) δ 6.58–6.40 (two br s, 4, C_7NH_2 and carbamate NH_2, the lower field signal underwent complete exchange with D_2O; the δ 6.40 peak underwent slow D_2O exchange), 5.62 (m, 1, C_1H, half-bandwidth 5 Hz), 5.00 (m, 2, $C_{10}H_2$), 4.52–3.72 (m, 3, C_2H, C_3H_2), 3.20 (br s, C_2NH_2, exchanged with D_2O), 2.03 (s, 3, $OCOCH_3$), and 1.74 ppm (s, 3, C_6CH_3).

A 10-mg sample was recrystallized again from *i*-PrOH: ir (KBr) 1615 (s, C=C), 1670 (w, C=O), 1740 (s, C=O), and 3300–3400 cm^{-1} (NH). Correct microanalyses were never obtained for **14** because of its instability.

Samples of **14** were combined and the available 20 mg was dissolved in absolute MeOH (30 ml). NH_4OH (1 ml) was added and the mixture was heated under reflux for 1.6 hr. After cooling and adding Me_2CO, the solution was evaporated to dryness *in vacuo* and passed through a small column of Florisil. Elution with $CHCl_3$, EtOAc, EtOAc–MeCN, and MeCN–Me_2CO gave a first red band of **5** [tlc (A) evidence]. The main purple band was eluted with Me_2CO and was further purified by plc (B). There was obtained 7 mg of product which was identical by tlc (A, B) to **10** obtained from the hydrolysis of mitomycin C.

Preparation of 2-Acetamido-7-amino-1-acetoxymitosene (4). Apomitomycin C (**3**, 100 mg, 0.31 mmol), purified by dual silica gel chromatography, was dissolved in anhydrous pyridine (4 ml) and cooled at 5°, and Ac_2O (1.5 ml) was added. After stirring in ice for 1 hr and at room temperature for 17 hr, 95% EtOH (20 ml) was added and the mixture was evaporated to dryness *in vacuo* using toluene (twice) and MeOH (twice). Tlc (A) showed traces of a more mobile component (R_f 0.595) in addition to the desired diacetate (R_f 0.55). The solid was chromatographed with silica gel to remove impurities and the main red band was eluted with EtOAc–MeCN and MeCN–Me_2CO mixtures. There was obtained 34 mg (27%) of the *cis*-diacetate **4** as determined by its nmr spectrum (DMSO-d_6) being superimposable with **4** obtained by the reaction of mitomycin C with Ac_2O.

Oxidation of *cis*- and *trans*-Hydroxyacetamides with MnO_2. (**a**) The *cis*-hydroxyacetamide **5** (110 mg, 0.304 mmol) was dissolved in anhydrous CH_2Cl_2 (120 ml) with stirring. During 5 days there was added in portions a total of 3.0 g of MnO_2 (Attenburrow activity) and 220 ml of Me_2CO. The MnO_2 was removed by filtration, washed with hot Me_2CO, and extracted with Me_2CO in a Soxhlet thimble. After combining the fractions containing the desired compound [(R_f 0.62 (A)] and evaporating *in vacuo*, the mixture [showing unreacted starting material in addition to small amounts of a mobile R_f 0.80 (A) component] was chromatographed using silica gel. The main brown band was eluted with EtOAc–MeCN (9:1), evaporated *in vacuo*, and recrystallized from $CHCl_3$. Very fine brown crystals formed after 9 days which were collected (filter paper) and dried to give 1.5 mg (1.4% based on mol wt of 360.326): ir (KBr) 1605 (s, with shoulder peaks, C=C), 1730 (s, br C=O), and 3400 cm^{-1} (s, NH). The $CHCl_3$ mother liquors could not be manipulated due to decomposition.

(**b**) The *trans*-hydroxyacetamide **12** was obtained (2.7 mg, 17.7%) from a small scale N-acetylation of **10** (14 mg, 0.044 mmol) in cold MeOH (5 ml) and Ac_2O (0.3 ml). After 1 hr, excess MeOH (10 ml) was added and then the solution was evaporated to dryness *in vacuo* with temperature <60°. The red solid was passed down a small Florisil column using $CHCl_3$, MeCN–EtOAc, Me_2CO, and Me_2CO–MeOH. On adding anhydrous Et_2O to a cold MeCN solution, deep purple flakes of **12** [R_f 0.45 (A)] were obtained which showed no definite melting point. This product was dissolved in anhydrous CH_2Cl_2 (10 ml) and Me_2CO (10 ml) to which Attenburrow activity MnO_2 (200 mg) was added with stirring during 18 hr. After 22.5 hr, the oxidizing agent was removed by filtration and washed with hot Me_2CO and the evaporated filtrate was chromatographed through silica gel with MeOAc as the eluent. The brown band off the column was identical with the product isolated in (**a**) by tlc [R_f 0.62 (A), 0.70 (D)].

2-Amino-1-hydroxy-7-methoxymitosenes (6 and 13). An ice-cold solution of mitomycin A (250 mg, 0.715 mmol) in 0.05 *N* aqueous HCl (40 ml) was stirred occasionally for 1 hr and left at room temperature for 17 hr. On recooling with ice, the pH of the crude mixture was adjusted to 8.0–8.5 with anhydrous K_2CO_3. A brown solid was removed by filtration and the aqueous filtrate was extracted exhaustively with hot EtOAc. The yellow organic extract was washed with H_2O and evaporated *in vacuo*. Recrystallization of the concentrate from MeCN gave two crops of apomitomycin A (**6**) as orange needles (79 mg, 33%): R_f 0.31 (A); 0.25 (D); nmr (DMSO-d_6) δ 6.52 (s, 2, NH_2), 5.06 (s, 2, $C_{10}H_2$), 4.72 (d, 1, J = 5 Hz, C_1H), 4.34 (m, 1, C_2H), 3.90 (s, 3, C_7OCH_3), 3.82–3.50 (m, 2, C_3H_2), 3.42–2.90 (br s, 2), and 1.86 ppm (s, 3, C_6CH_3).

6 (2 mg) in 15 ml of anhydrous MeOH was cooled in an ice bath and NH_3 gas was bubbled through the yellow solution during 3 hr. The reaction was continued by stirring at room temperature for 73 hr and the resulting purple solution was evaporated to dryness *in vacuo*. The purple crystals which formed in Me_2CO were collected with the aid of dry ether (0.7 mg) and identified as apomitomycin C (**3**) by tlc (A, C, D).

The MeCN mother liquor from the hydrolysis reaction above showed another component [R_f 0.17 (A)] on tlc which was isolated by column chromatography on silica gel (eluted with $CHCl_3$, EtOAc, EtOAc–MeCN, MeCN, and MeCN–Me_2CO) and refiltered through a plug of Florisil. The 13 mg (5.4%) of orange semisolid **13** failed to crystallize from MeOH and was converted to the 7-amino analog **10** by passing NH_3 gas through a cold methanolic solution (15 ml) for 3 hr. On stirring the parafilm-sealed flask for 25.5 hr at room temperature, there was obtained 5.9 mg of the purple **10** [from combining a first crop from MeCN and by plc (B) on the MeCN mother liquor] as judged by tlc (A, B).

Preparation of 2-Acetamido-1-acetoxy-7-methoxymitosene (7). (**a**) Apomitomycin A (**6**, 39 mg, 0.116 mmol) in anhydrous pyridine (1 ml) was treated with Ac_2O (0.5 ml) as previously described. The product (36 mg, 74%) was collected by addition of cold anhydrous Et_2O. Recrystallization from MeCN gave the *cis*-diacetate **7** (18 mg) as deep orange flakes: mp 230–231° dec; lit.[2] mp 244–246.5°; nmr (DMSO-d_6) δ 8.34 (d, 1, J = 8 Hz, C_2NH, slow exchange with D_2O), 6.40 (ragged s, 2, carbamate NH_2, exchanged with D_2O), 6.07 (d, 1, J = 6 Hz, C_1H), 5.04 (ragged s, 3, $C_{10}H_2$, C_2H), 4.54 (dd, J = 8, 12 Hz, C_3H), 3.92 (m, 4, C_3H, C_7OCH_3), 2.04 (s, 3, $OCOCH_3$), and 1.87 ppm (s, 3, $NCOCH_3$). Decoupling the C_2H signal collapsed the C_2NH and C_1H doublets to singlets.

(**b**) Mitomycin A (160 mg, 0.46 mmol) was dissolved in Ac_2O (10 ml) and the ice-cold solution was stirred for 2 hr and then left at room temperature for 3 hr. 95% EtOH (30 ml) was added with stirring and removal of solvents *in vacuo* gave a wine semisolid which showed spots on tlc (A) with R_f values of 0.69 and 0.48. The more mobile material was obtained by eluting a silica gel column with $CHCl_3$–EtOAc mixtures. A portion of this material was washed with petroleum ether (bp 30–60°) and dried in a vacuum desiccator, and the resulting wine powder gave uv max (MeOH) at 321 nm (ϵ ~11,780) and 513 (~806), consistent with the uv spectra[10] of *N*-acetylmitomycin A (mol wt 391.38). Further treatment of the *N*-acetylmitomycin A with Ac_2O (10 ml) and glacial HOAc (3 ml) for 2 hr at 0° and stirring at room temperature for 6.5 days gave, after evaporating to dryness *in vacuo* using toluene as a chaser, the diacetate **7** (50 mg) from 20 ml of absolute MeOH. Optimizing the yield by evaporation of the MeOH mother liquor and recrystallizing both crops from MeCN gave 55 mg (28.6%) of orange crystals, identical with **7** prepared by route (**a**) above by nmr, ir, and tlc [R_f 0.60 (A), R_f 0.71 (B), R_f 0.87 (C)] analyses.

Preparation of 2,7-Diamino-1-methoxymitosene (15). To a slurry of a cation exchange resin (Dowex AG 50W-X2, 100–200 mesh, 85 ml, Bio-Rad), previously washed thoroughly with MeOH, in 30 ml of MeOH was added a solution of mitomycin C (250 mg, 0.75 mmol) dissolved in warm MeOH (65 ml). After a 30-min addi-

tion, stirring was continued for another 30 min at room temperature. The product was desorbed by addition of 3% (by weight) NH_3 in 190 ml of MeOH while the reaction flask was kept at ≤0°. After removing the resin by filtration, a crude product was obtained on evaporation *in vacuo* which showed four spots on tlc [R_f values (A) of 0.075, 0.12, 0.22, 0.33]. A crude brown material (32.5 mg), representing the desired methoxyamine **15** (R_f 0.075), and detectable amounts of the R_f 0.12 and 0.22 components were obtained after two recrystallizations from H_2O. The mother liquors were evaporated *in vacuo* and chromatographed on Florisil with EtOAc–MeCN, MeCN, MeCN–Me_2CO, Me_2CO, and Me_2CO–MeOH as the elution solvents, and the appropriate fraction was combined with the 32.5-mg sample above and recrystallized from H_2O to give 9.1 mg of chromatographically homogeneous **15**. All remaining fractions containing **15**, including 37 mg of crystals from the second crop before chromatography and middle bands from the first column, were rechromatographed on a 1.2 × 112.5 cm Florisil column to give an additional 39.5 mg of **15** after recrystallization from H_2O (total yield of 48.6 mg, 19.5%). Another recrystallization from H_2O gave deep red crystals which showed no definite melting point (lit.[3] mp 204–205.5°).

In another methoxy migration experiment with Dowex 50 X2 (150 ml, 100–200 mesh) and mitomycin C (500 mg, 1.5 mmol), the same four components above were observed [tlc (A)] on the crude product. The mixture (0.461 g) was dissolved in MeOH (120 ml) and cooled. Ac_2O (6 ml) was added and the reaction mixture was stirred for 1.5 hr followed by an additional room temperature stir for 2 hr. On evaporating *in vacuo* and crystallizing the residue from H_2O, 121 mg (21.5%) of the *trans*-methoxyacetamide **16** was obtained. [This acetate was identical by tlc [R_f 0.47 (A), 0.81 (D)] to the methoxyacetamide obtained by a tlc scale acetylation of **15** (isolated above) with Ac_2O in MeOH.] A further recrystallization from H_2O gave deep red flakes: mp 225° dec (lit.[3] mp 230–231° dec); nmr (DMSO-d_6) δ 8.27 (d, 1, J = 7 Hz, C_2NH, slowly exchanged with D_2O), 6.60 (br s, 4, carbamate NH_2, C_7NH_2, exchanged with D_2O), 5.11 (s, 2, $C_{10}H_2$), 4.90–4.30 (m, 3, C_1H, C_2H, C_3H), 3.88 (m, 1, C_3H), 3.32 (s, 3, C_1OCH_3), 1.92 (s, 3, $NCOCH_3$), and 1.75 ppm (s, 3, C_6CH_3). *Anal.* ($C_{17}H_{20}N_4O_6$) C, H, N.

Reaction of Mitomycin C with Methanol and Glacial Acetic Acid. A mixture of mitomycin C (400 mg, 1.2 mmol), MeOH (80 ml), and glacial HOAc (0.7 ml) was heated for 5 hr at 70° according to the literature procedure.[3] Tlc (A) showed five spots with the three most mobile components corresponding to the mitomycin C glacial HOAc experiment. A 100-mg portion of the crude solid was removed and the remainder was subjected to column chromatography on silica gel. Elution with EtOAc, EtOAc–MeCN, MeCN, and MeCN–Me_2CO gave the three mobile components in addition to *cis*-methoxyamine **8** [R_f 0.17 (A), 43.5 mg, 13.6%] and the *trans*-methoxyamine **15** [R_f 0.075 (A), 55 mg, 17.2%].

Both **8** and **15** were acetylated with Ac_2O (1 ml) in cold MeOH (20–25 ml) for 1 hr and then 1 hr at room temperature followed by evaporation *in vacuo* and storing at −15°. With **8**, a deep red oil [R_f 0.47 (A), 0.77 (D)] was obtained after azeotropically removing traces of HOAc with benzene. The unstable product was stored at −15° and then was passed through a silica gel column with $CHCl_3$, EtOAc, EtOAc, and MeCN to give, after evaporation of the solvents at ≤50°, 20.7 mg (10.3% based on mitomycin C) of the pure *cis*-methoxyacetamide (**9**) with no melting point below 390°: ir (KBr) 1610 (s, C=C), 1670 (m, C=O), 1740 (m, C=O), and 3450 cm^{-1} (s, NH); nmr (DMSO-d_6) δ 8.36 (d, 1, J = 7 Hz, C_2NH, slowly exchanged with D_2O), 6.64 (ragged s, 4, carbamate NH_2, C_7NH_2, exchanged with D_2O), 5.09 (s, 2, $C_{10}H_2$), 4.74–3.92 (m, 4, C_1H, C_2H, C_3H_2), 3.32 (s, 3, C_1OCH_3), 1.90 (s, 3, $NCOCH_3$), and 1.76 ppm (s, 3, C_6CH_3). *Anal.* ($C_{17}H_{20}N_4O_6$) C, H, N.

The methoxyamine **15** gave an acetate derivative [R_f 0.47 (A), 0.81 (D)] as a wine semisolid after azeotroping traces of HOAc with trichloroethylene. After cooling, the product crystallized from H_2O to give the *trans*-methoxyacetamide **16** (24.5 mg, 12.2% based on mitomycin C) as determined by nmr spectral comparison to **16** prepared by the cation resin experiment.

Methylation of *cis*-1-Hydroxy-2-acetamido-7-aminomitosene (5). To an ice-cold solution of **5** (109 mg, 0.3 mmol) in DMF (3 ml) was added with stirring K_2CO_3 (100 mg), methyl iodide (0.06 ml, 1 mmol), and silver oxide (70 mg, 3 mmol). After the reaction had come to room temperature for 6 hr, tlc (A) showed mostly starting alcohol and the reaction was continued by adding excess methyl iodide (0.90 ml), silver oxide (140 mg), and DMF (1 ml) in portions during 7 days followed by 28 hr of heating at 55°. A precipitate was removed by filtration and washed with DMF. The combined filtrate was concentrated *in vacuo*, suspended in H_2O, filtered, and washed the grey precipitate with EtOAc (100 ml). The aqueous layer was separated and washed with EtOAc (4 × 40 ml), and the combined EtOAc layers were washed with H_2O (2 × 50 ml). The organic extract was dried (Na_2SO_4) and evaporated to dryness *in vacuo*, and the resulting unstable red oil was chromatographed through silica gel. Elution with $CHCl_3$ gave a mobile component [R_f = 0.80 (A)] and the main purple band was eluted with $CHCl_3$–MeCN and EtOAc–MeCN mixtures. Later fractions showed traces of **5** which were removed by plc (A). On combining all fractions and passing through a small column of silica gel, there was obtained 2 mg (1.6%) of the desired *cis*-methoxyacetamide **9** which was identical with **9** isolated in the methanolysis experiment by ir and tlc (A, D).

Acknowledgments. This research was supported by Public Health Service Grant No. CA 11686. We thank Bristol Laboratories Division, Bristol-Meyers Co., for a generous supply of mitomycin C and Kyowa Hakko Kogyo Co. for a sample of mitomycin A. We also thank Dr. D. L. Fost and Miss C. Schlusemeyer for experimental assistance, Dr. J. S. Webb of Lederle Laboratories for helpful discussions, and the Purdue University Department of Chemistry for help in obtaining nmr spectra.

References and Notes

(1) Presented in part at the American Pharmaceutical Association Meeting, Chicago, Ill., August 1974. A part was communicated previously in *Tetrahedron Lett.*, 3483 (1974).
(2) J. S. Webb, D. B. Cosulich, J. H. Mowat, J. B. Patrick, R. W. Broschard, W. E. Meyer, R. P. Williams, C. F. Wolf, W. Fulmor, C. Pidacks, and J. E. Lancaster, *J. Amer. Chem. Soc.*, **84**, 3185, 3186 (1962).
(3) C. L. Stevens, K. G. Taylor, M. E. Munk, W. S. Marshall, K. Noll, G. D. Shah, L. G. Shah, and K. Uzu, *J. Med. Chem.*, **8**, 1 (1965).
(4) K. Uzu, Y. Harada, and S. Wakaki, *Agr. Biol. Chem.*, **28**, 388 (1964).
(5) E. R. Garrett, *J. Med. Chem.*, **6**, 488 (1963).
(6) S. Kinoshita, K. Uzu, K. Nakano, M. Shimizu, and T. Takahashi, *J. Med. Chem.*, **14**, 103 (1971).
(7) S. Oboshi, M. Matsui, S. Ishii, N. Masago, S. Wakaki, and K. Uzu, *Gann*, **58**, 315 (1967).
(8) J. H. Mowat, J. B. Patrick, R. P. Williams, D. B. Cosulich, and J. S. Webb, U.S. Patent 3,179,671 (April 20, 1965).
(9) D. L. Fost, N. N. Ekwuribe, and W. A. Remers, *Tetrahedron Lett.*, 134 (1973).
(10) J. S. Webb, private communication.
(11) W. A. Remers and C. S. Schepman, *J. Med. Chem.*, **17**, 729 (1974).
(12) A. Tulinsky and J. H. van den Hende, *J. Amer. Chem. Soc.*, **89**, 2905 (1967).
(13) C. F. Huebner, E. M. Donoghue, C. J. Novak, L. Dorfman, and E. Wenkert, *J. Org. Chem.*, **35**, 1149 (1970).