Circular Dichroism Spectroscopy as a Probe for the Stereochemistry of Aziridine Cleavage Reactions of Mitomycin C. Application to Adducts of Mitomycin with DNA Constituents

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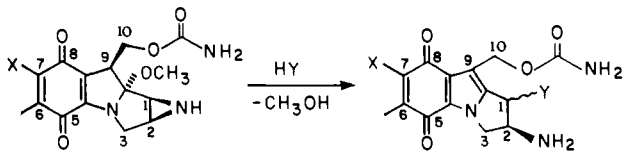
Abstract: Four diastereomeric pairs of 1,2-cis/trans isomers in the general class of 1-substituted 2-aminomitosenes (derivatives of mitomycin antibiotics) were separated and the individual isomers were chemically correlated with 1,2-*cis*- or 1,2-*trans*-1-hydroxy-2,7-diaminomitosene, the only members of the series with previously established stereochemistry. Analysis of the weak circular dichroism (CD) Cotton effect displayed by these substances in the 530-nm region shows that a positive Cotton effect is associated with a 1β configuration while a negative one is associated with a 1α configuration. Also, when C-1 is unsubstituted, as in 2,7-diaminomitosene, the 530-nm Cotton effect is absent, indicating that C-2 exerts no influence. These results establish that observation of the sign of the 530-nm Cotton effect constitutes a nonambiguous method for deducing the C-1 configuration of mitosenes, for which no definitive solution was available. This CD method was used to establish the stereochemistry of a new pair of mitosenes **14** and **15** derived from the acid-catalyzed solvolysis of porfiromycin (*N*-1a-methylmitomycin C). In addition, the method was applied to a minor mitosene–deoxyguanosine adduct formed upon reductive alkylation of d(GpC) by mitomycin C (MC), and it was concluded that the adduct is 1,2-*cis*-1-[O^6-(2′-deoxyguanosyl)]-2,7-diaminomitosene (**16**), i.e., the 1,2-cis isomer of the previously characterized major adduct **17** formed in the reaction (Tomasz, M.; Lipman, R.; Snyder, J. K.; Nakanishi, K. *J. Am. Chem. Soc.* **1983**, *105*, 2059–2063). The CD method is thus shown to be applicable to mitosene products derived from metabolic reactions of MC as well as nucleoside–mitosene adducts. The method should prove to be very useful for structural characterization of these types of substances formed as a result of interaction of MC with cellular constituents. The ratio of cis to trans products of the acid-catalyzed hydrolytic cleavage of the aziridine ring is ca. 3:1 in the case of both MC and 10-decarbamoylmitomycin C, and ca. 4:1 in the case of porfiromycin. No ^2H is incorporated into products when the hydrolysis is conducted in ^2H_2O. These and other data lead to the conclusion that the aziridine cleavage of MC probably takes place by an S_N1-type mechanism involving a carbocation at C-1.

Mitomycin C (**1**, X = NH_2, MC), the potent antibiotic and clinically used antitumor drug,[1] has been postulated to act as a bioreductive alkylating agent of DNA.[2-4] Recently, the bioreductive alkylating capacity of MC was conclusively demonstrated by isolation and characterization of model alkylation products formed in a rat liver microsomal system.[5] Formation of adducts between reductively activated MC and DNA constituents have also been reported: the fully characterized adduct **17** was isolated from alkylation of the dinucleoside phosphate, d(GpC),[6] while a similar adduct, with a 5′-phosphate group but unspecified stereochemistry at C-1, was obtained from alkylation of DNA along with two others in which MC was linked to N-2 of guanine and N^6 of adenine, respectively.[7,8]

The alkylating function of MC in these reactions involves cleavage of the 1,2-aziridine ring by HY, with concomitant elimination of methanol to yield "mitosene"[9] derivatives **2** (Scheme I). The derivatives can be formed by either of the two following routes. At neutral pH it is necessary to catalyze the aziridine ring-opening process by reduction of the quinone ring;[2,5] however, the isolated products are "mitosene" derivatives, due to facile reoxidation to the quinone upon exposure to air. On the other hand, at low pH values, reducing conditions are not required to induce mitosene formation; hydrolytic and phosphorolytic ring opening, for example, take place readily at or below pH 4.[11,18,19] In either of the two activation pathways, the overall ring-cleavage reaction can be illustrated as shown in Scheme I.

In the case of simple acid-catalyzed solvolysis reactions, **2** is usually a mixture of the 1,2-cis and -trans isomers, e.g., **3** and **4** (Table I), with cis predominating.[11,12] When the cleavage is initiated by reduction of the quinone ring instead of acid catalysis, the predominance of the cis products diminishes in a few known cases.[5-8,13] However, studies of this important ring-cleavage reaction of mitomycins have been hindered by the lack of a simple,

Scheme I. Formation of Mitosenes



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general method for determining the stereochemistry of the substitution products **2**.[11,12] It is well-known that the ^1H NMR $J_{1,2}$

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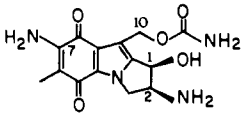
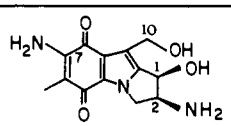
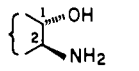
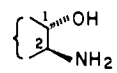
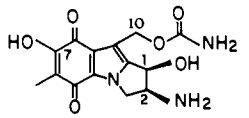
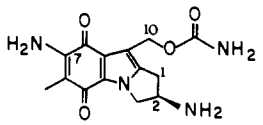
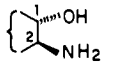
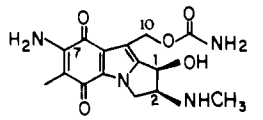
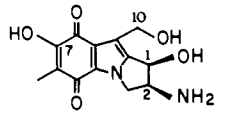
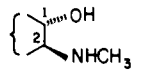
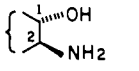
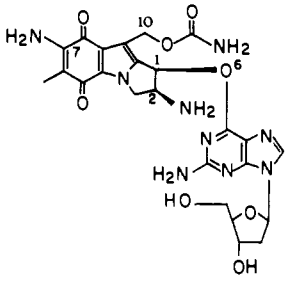
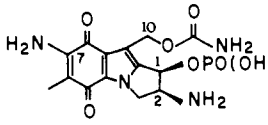
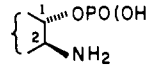
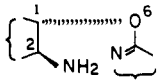
(1) Szybalski, W.; Iyer, V. N. In "Antibiotics, I. Mechanism of Actions"; Gottlieb, D., Shaw, P. D., Eds.; Springer-Verlag: New York, 1967; pp 211–245.
(2) Iyer, V. N.; Szybalski, W. *Science (Washington, D.C.)* **1964**, *145*, 55.
(3) Lin, A. J.; Cosby, L. A.; Sartorelli, A. C. *ACS Symp. Ser.* **1976**, *No. 30*, 71.
(4) Moore, H. W. *Med. Res. Rev.* **1981**, *1*, 249.
(5) Tomasz, M.; Lipman, R. *Biochemistry* **1981**, *20*, 5056.
(6) Tomasz, M.; Lipman, R.; Synder, J. K.; Nakanishi, K. *J. Am. Chem. Soc.* **1983**, *105*, 2059.
(7) Hashimoto, Y.; Shudo, K.; Okamoto, T. *Tetrahedron Lett.* **1982**, 677.
(8) Hashimoto, Y.; Shudo, K.; Okamoto, T. *Chem. Pharm. Bull.* **1983**, *31*, 861.
(9) The term "mitosene" refers to the structure as in **2** without substituents in the 1, 2, and 7 positions.[10]
(10) Webb, J. S.; Cosulich, D. B.; Mowat, J. H.; Patrick, J. B.; Broschard, R. W.; Meyer, W. E.; Williams, R. P.; Wolf, C. F.; Fulmore, W.; Pidacks, C.; Lancaster, J. E. *J. Am. Chem. Soc.* **1962**, *84*, 3185.
(11) Taylor, W. G.; Remers, W. A. *J. Med. Chem.* **1975**, *18*, 307.
(12) Cheng, L.; Remers, W. A. *J. Med. Chem.* **1977**, *20*, 767.
(13) Hornemann, U.; Iguchi, K.; Keller, P. J.; Vu, H. M.; Kozlowski, J. F.; Kohn, H. *J. Org. Chem.* **1983**, *48*, 5026. Bean, M.; Kohn, H. *Ibid.* **1983**, *48*, 5033.
(14) Hirayama, N.; Shirahata, K. *J. Am. Chem. Soc.* **1983**, *105*, 7199.
(15) Harada, N.; Nakanishi, K. "Circular Dichroic Spectroscopy—Exciton Coupling in Organic Stereochemistry"; University Science Books: Mill Valley, CA, 1983.

0002-7863/84/1506-7367$01.50/0

Table I. CD Data in the 530-nm Region

Entry	Structure	CD, Δε(nm), MeOH	Entry	Structure	CD, Δε(nm), MeOH
3		+0.065 (520)[6]	11		+0.091 (530)
4		−0.045 (520)[6]	12		−0.082 (530)
5		+0.050 (540)	13		NO C.E.
6		−0.017 (540)	14		+0.084 (520)
7		+0.013 (550)	15		−0.035 (520)
8		−0.004 (550)	16		+0.104 (535)
9		+0.035 (535)			
10		−0.035 (535)	17		−0.059 (535[a])[6]

[a] In ref 6 the broad Cotton effect on the long-wavelength band was read as 520 nm; however, 535 nm appears to be a more accurate reading.

values are not generally reliable for deducing the stereochemistry of these centers.[6,11]

We report here that circular dichroism (CD) spectroscopy provides a direct and general solution to this problem. Four diastereomeric pairs of 1,2-cis/trans isomers **5/6**, **7/8**, **9/10**, and **11/12** (Table I) were separated and chemically correlated with **3** and **4**. The 1,2-stereochemistry of the latter two has previously been established by formation of a 1,2-cyclocarbamate from **2** (X = OMe, Y = β-OH), the ammonolysis of which yielded **3**.[11] Analysis of the weak CD Cotton effect (CE) in the 530-nm region has shown that a positive CE is associated with a 1β-configuration, while a negative CE is associated with a 1α-configuration (Figure 1). When C-1 is unsubstituted as in **13**, the 530-nm CE is absent. This method has then been used to establish the stereochemistry of a new mitosene pair **14/15** derived from acidic solvolysis of porfiromycin (*N*-1a-MeMC). It has also been applied to determine the C-1 configuration of a minor mitosene–deoxyguanosine adduct **16** which was formed along with the major adduct **17**[6] upon reductive alkylation of d(GpC) by MC.

Recent X-ray studies carried out by N. Hirayama et al. have shown that the absolute configuration of MC should be reversed;[14] we have also secured independent CD results based on the nonempirical coupled oscillator theory[15] which necessitate revision of MC absolute configuration.[16] The CD method discussed in this paper, on the other hand, is an empirical one which correlates the sign of the 530-nm Cotton effect to the configuration at C-1 with use of mitosenes **3** and **4** as reference compounds. Therefore the current CD treatment for determination of stereochemistry at C-1 is not affected by reversal of absolute configuration except that now positive and negative Cotton effects at 530 nm are associated with 1β- and 1α-configurations, respectively (rather than 1α- and 1β-configurations[6]).

Table II. HPLC Data of Mitosene Derivatives

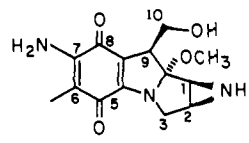
compd	elution time, min	compd	elution time, min
3	40	**10**	9.5
4	30	**11**	25
5	22[b]	**12**	19
6	17[b]	**14**	14[c]
7	10[b]	**15**	12[c]
8	9[b]	**16**	102
9	13	**17**	40

[a] Flow rate 2.0 mL/min. Other conditions are listed in the Experimental Section, unless otherwise noted. [b] The pH of the phosphate buffer was 7.4 rather than 5.0. All 7-OH mitosenes give extremely broad peaks at pH 5. [c] 15% acetonitrile rather than 8% was used.

Experimental Section

Materials and Methods. Mitomycin C was obtained from Bristol Laboratories, Syracuse, NY, and porfiromycin from the Upjohn Co., Kalamazoo, MI. 10-Decarbamoylmitomycin C (**18**) was prepared and purified by published procedures.[5,17] 1,2-*cis*- and 1,2-*trans*-1-hydroxy-2,7-diaminomitosene (**3** and **4**, respectively) were prepared as a mixture by mild acid hydrolysis of MC[18] and separated into the two isomers[11] by

(16) Verdine, G. et al., manuscript in preparation.
(17) Kinoshita, S.; Uzu, K.; Nakano, K.; Takashi, T. *J. Med. Chem.* **1970**, *14*, 109.
(18) Stevens, C. L.; Taylor, K. G.; Mink, M. E.; Marshall, W. S.; Noll, K.; Shah, G. D.; Uzu, K. *J. Med. Chem.* **1965**, *8*, 1.
(19) Tomasz, M.; Lipman, R. *J. Am. Chem. Soc.* **1979**, *101*, 6063.



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a Sephadex G-25 (fine) column (5.0 × 56 cm) with 0.02 M NH_4HCO_3 as eluent. The two fractions (elution volumes 3230 and 2840 mL) were lyophilized to remove the water and NH_4HCO_3, yielding homogeneous **3** and **4**, respectively, as tested by HPLC. Sephadex G-25 column chromatography was carried out generally as previously described.[5,19] HPLC was conducted on a Beckman Model 330 system with a reverse-phase (Ultrasphere ODS) column (1.0 × 25 cm), and 8% CH_3CN–92% 0.02 M K-phosphate, pH 5.0, as eluent. Elution times are listed in Table II. Purity of all compounds was tested by HPLC. In case of impurity, preparative HPLC purification was carried out followed by a desalting procedure described previously.[6] Quantification of all substances was routinely based on UV spectrophotometry, using a Gilford 250 spectrophotometer, as previously described.[5,6,19] The extinction coefficients used for individual cis and trans isomers are those reported originally for their mixtures[10,18] unless noted otherwise. Comparison of a sample with an authentic standard was based on three criteria: (1) elution volume on a standard Sephadex G-25 column (data are given in the text for all compounds); (2) mixed run on HPLC (Table II); and (3) ultraviolet spectra at neutral and acid pH.[5,19]

Circular dichroism spectra were obtained in methanol solution with a JASCO J-40 spectrophotometer and ultraviolet spectra with a Cary 219 spectrophotometer.

Origin, Separation, and Isomer Assignment of Cis/Trans Pairs of Various Mitosene Derivatives. The pairs **3**/**4**, **5**/**6**, and **7**/**8** were generated by acid-catalyzed hydrolysis of MC under three increasingly stronger conditions, respectively.[18] These structures, except for the stereochemistry at positions 1 and 2, have been known.[10,18]

Separation and assignments of stereoisomers were only recently accomplished for the **3**/**4**[11] and the **5**/**6**[19] mixtures. We have now also separated the **7**/**8** pair, by Sephadex G-25 column chromatography. The first component to be eluted (elution volume 290 mL; 2.5 × 55 cm column) was identified as the trans isomer **8**, since it proved to be identical with the hydrolysis product of **4**, an authentic trans starting material (Scheme II). The second component (elution volume 315 mL) was the cis isomer **7**, since it was identical with authentic **7** produced by similar hydrolysis of **3**, a pure cis starting material (Scheme II).

The 1-phosphate pair **9** and **10** was prepared by the reaction of inorganic phosphate with MC at acid pH.[19] The resulting cis/trans mixture was separated on a Sephadex G-25 column (2.5 × 56 cm). The earlier fraction (elution volume 500 mL) was identified as **10** by its conversion to authentic **4** upon alkaline phosphatase treatment while the later fraction (elution volume 526 mL) was identified as **9** by its conversion upon similar treatment to authentic **3** (Scheme II).

The pair **11** and **12** was generated by mild acid hydrolysis of **18**.[5] Separation of the reaction mixture was unsuccessful previously,[5] but Sephadex G-25 chromatography (5 × 56 cm column) with 0.001 M NH_4HCO_3 as eluent now achieved separation, elution volumes being 2516 and 2151 mL, respectively. The faster and slower eluting components were identified as **12** and **11**, respectively, by their conversions to authentic **8** and **7** upon hydrolysis (Scheme II).

The pair **16** and **17** arise from the mild acid hydrolysis of porfiromycin (*N*-1a-MeMC) which was carried out under the same conditions as those used to produce **3** and **4** from MC.[18] The two isomers were separated by Sephadex G-25 chromatography (elution volumes 2672 and 2370 mL, respectively). The products were assigned structures **14** and **15** on the basis of very close similarity to the ^1H NMR, UV, and (800–200-nm range) CD spectra of **3** and **4**, respectively.

A minor deoxyguanosine–mitomycin adduct which was formed in addition to the major adduct **17** upon reductive alkylation of d(GpC) by MC was briefly noted.[6] Its structure **16** has since been elucidated by microspectroscopic techniques including subtractive FTIR and subtractive UV methods and has been found to have the same adduct linkage as that in **17**:[6] C-1 of the mitosene is connected to O^6 of deoxyguanosine and therefore this adduct is the 1-epimer of **17** (details to be published elsewhere). This result is confirmed by the present CD analysis (Table I and Discussion), thus leading to structure **16** for the minor product.

Ratios of Cis/Trans Products in Acid-Catalyzed Aziridine Ring-Opening Reactions of MC, 10-Decarbamoylmitomycin C, and Porfiromycin. These ratios were determined by direct HPLC analysis of the reaction mixtures and measurements of the areas under the individual isomer peaks. The following ratios were obtained: hydrolysis of MC to **3** and **4**, **3**:**4** = 3.0:1; phosphorolysis of MC to **9** and **10**, **9**:**10** = 3.2:1;[20] hydrolysis of 10-decarbamoylmitomycin C (**18**) to **11** and **12**, **11**:**12** = 3.0:1; hydrolysis of porfiromycin (*N*-1a-MeMC) to *N*-2-methyl analogues of **3** and **4**. **14**(cis):**15**(trans) = 4.0:1.

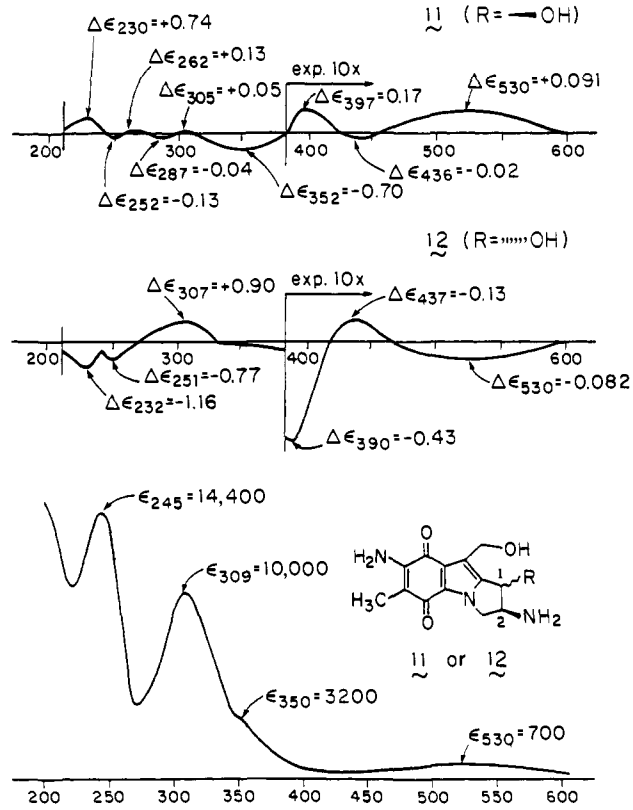
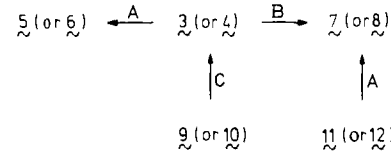


Figure 1. (a) CD of **11**; (b) CD of **12**; (c) UV of **11** or **12** (identical). All spectra are recorded in MeOH.

Scheme II. Chemical Correlation of Mitosenes[a]



[a] A: 0.1 N HCl, room temperature, 15 h.[10] B: 2 N HCl, 55 °C, 2 h.[17] C: Alkaline phosphatase, 37 °C, 1.5 h.[18]

Discussion

The assignment of the stereochemistry of 1,2-disubstituted mitosenes **2** poses a problem for which no definitive solution exists by NMR as concluded in a systematic study by Taylor and Remers.[11] They established the stereochemistry of a number of solvolysis products of mitomycins A (**1**, X = OMe) and C (**1**, X = NH_2), respectively, by relating each one chemically to apomitomycin A (**2**, X = OCH_3, Y = β-OH), the only compound in the series with previously established stereochemistry.[21] The NMR analysis of these substances indicated that differences in $J_{1,2}$ for the cis and trans isomers are too small in many cases, making this method unreliable for assigning the 1,2-stereochemistry.

On the other hand, during structural studies of the nucleoside–MC adduct **17**,[6] we discovered that the CD Cotton effects

(20) In a previous paper[19] we reported a much higher (>10:1) cis/trans ratio for the formation of the phosphates **9** and **10**. The present ratio is more accurate since it was determined by direct HPLC analysis of the phosphate mixture itself; in the earlier work the ratio was measured indirectly after alkaline phosphatase treatment to **3** and **4**. An error in previous results presumably could have been caused by undetected precipitation of the trans compound from solution at some stage of the analysis.

(21) This was determined by the formation of a 1,2-cyclic carbamate and by semipinacolic deamination to the 1-one.[22]

at 530 nm of the cis/trans pair **3** and **4** were of similar amplitudes but of opposite sign, thus indicating that the sign of the 530-nm CD band is dependent on the C-1 configuration.

It appeared important that the generality of this CD method in mitomycin chemistry be demonstrated by a study of additional authentic mitosene pairs. Since all known aziridine ring-opening reactions of the mitomycins result in the NH_2 group being at C-2 with β-configuration,[5,23,24] all eight new compounds employed in this study (**5–12**) are represented by the cis or trans form of **2** or of 10-decarbamoylated **2**. These simple hydrolysis products of mitomycins should also prove to be useful as reference compounds for structural studies of complex mitomycin derivatives.

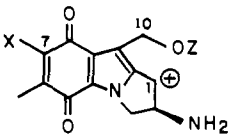
As shown in Table I, in all five pairs with established configuration, **3–12**, *the 1β- and 1α-configurations lead respectively to positive and negative CE's at ca. 530 nm* associated with the weak mitosene band around 525 nm (ϵ ca. 800) which is responsible for the purple color. Although the CE is broad and weak (e.g., Figure 1, a and b), $\Delta\epsilon$ being in the range of +0.013 to +0.091 for 1β- and −0.004 to −0.082 for 1α-compounds, the sign is dependent only on the configuration and is not affected by the substituents at C-1 (OH or OPO_3H_2), C-7 (NH_2 or OH), or C-10 (OH or $OCONH_2$).

Also, in compound **13** where C-1 carries no substituent the 530-nm CE is absent. It thus appears that the chiral center at C-2 is too remote from the mitosene chromophore to perturb the weak 530-nm absorption band.

The mitosene chromophore has relatively strong absorptions at 200–400 nm. The fact that the weak CD extrema in this region (Figure 1, also see Figure 5 in ref 6) do not show an enantiomeric relation is due to the diastereomeric rather than enantiomeric nature of the respective pairs. The case of the nucleoside–mitosene adduct pair **16**/**17** is especially significant since it shows that the CD method is applicable to MC–DNA adducts, substances of great current interest[6-8] since their formation in vivo is believed to be related to the biological activity of MC. The guanosine chromophore attached to C-1 absorbs at 250–280 nm. Although the direction of electric transition moments of neither the mitosene nor the purine moieties are known, as expected the two chromophores constitute a coupled oscillator[14] and give rise to coupled CD in the 200–400-nm region, the CE's of which are much more intense[25] than those due to the isolated mitosene moiety (Figure 1, a and b).

Fortunately, the 540-nm maximum is not only weak but also too far removed from the purine maxima[6] so that the bands cannot efficiently couple;[26] hence this longest wavelength CE is dominated by the C-2 configuration of the mitosene moiety. The present CD method thus provides a simple means for determining the C-1 configurations of various mitomycin metabolites.

As mentioned in the Experimental Section, acid hydrolysis or phosphorolysis opens the aziridine ring (Scheme II) to give a ca. 3:1 mixture of the 1,2-cis and -trans compounds[20] in agreement with a related study of Cheng and Remers.[12] Acid hydrolysis of MC carried out in D_2O gave the mitosenes **3** and **4** containing no ^2H (by NMR). Therefore cis–trans isomerization involving an intermediate such as **19** is ruled out. Furthermore, the cis and trans isomers are not interconverted under conditions of their formation.[27] These facts indicate that the stereochemistry of substitution at C-1 is kinetically determined. Since absence of the carbamoyl group does not influence the cis–trans ratio (**11** and **12**), there appears to be no participation from the side chain. It thus can be concluded that aziridine cleavage presumably takes place by an S_N1-type mechanism involving a carbocation after loss of methanol, i.e., species **20**. This conclusion is supported by results of a recent study of the hydrolysis of model aziridines mimicking various structural features of the aziridine ring of MC.[28]



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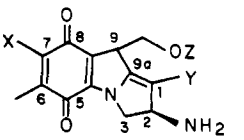

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Registry No. 1 (X = NH_2), 50-07-7; **5**, 92761-56-3; **6**, 92841-57-1; **7**, 92761-57-4; **8**, 92761-58-5; **9**, 72091-35-1; **10**, 92761-59-6; **11**, 92761-60-9; **12**, 92761-61-0; **13**, 92695-32-4; **14**, 92761-62-1; **15**, 92761-63-2; **18**, 26909-37-5; 1-[O^6-(2′-deoxyguanosyl)]-2,7-diaminomitosene, 92761-64-3; porfiromycin, 801-52-5.

(22) Mowat, J. H.; Patrick, J. B.; Williams, R. P.; Cosulich, D. B.; Webb, J. S. U.S. Patent 3 179 671, 1965.

(23) Remers, W. A. "The Chemistry of Antitumor Antibiotics"; Wiley: New York, 1978; Vol. 1, pp 221–276.

(24) Hornemann, U.; Keller, P. J. Kozlowski, J. F. *J. Am. Chem. Soc.* **1979**, *101*, 7121.

(25) See Figure 2 in ref 6 which shows the full CD spectrum of adduct **17**.

(26) Two chromophores can still be coupled when separation of the absorption maxima is around 100 nm provided the extinction coefficients are large. See ref 15, pp 85, 265.

(27) Treatment of either **3** or **4** in 0.05 N HCl for 1 h (conditions used for hydrolysis of MC to a mixture of **3** and **4**[18]) resulted in quantitative recovery of stereochemically pure starting material. Furthermore, the experiments illustrated in Scheme II show that even much stronger acid conditions (0.1 N HCl/room temperature/15 h and 2 N HCl/55 °C/2 h) leave the C-1 stereochemistry unaffected while causing changes elsewhere in the molecule.

(28) Kohn, H.; Chiu, I. C. *J. Org. Chem.* **1983**, *48*, 2857.