UNUSUAL CIS STEREOSELECTIVITY IN AN AZIRIDINE CLEAVAGE REACTION OF MITOMYCIN C†

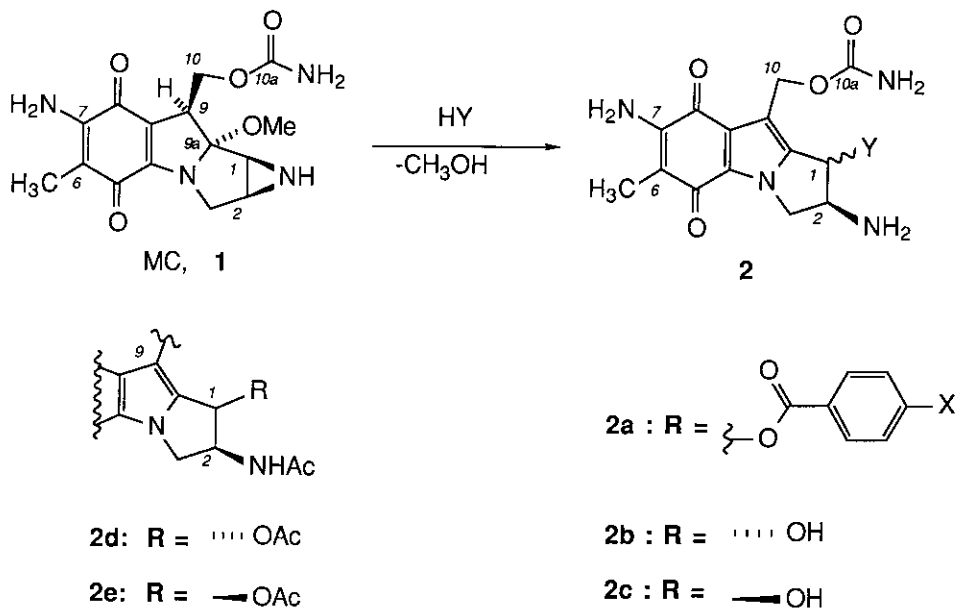
Gregory L. Verdine, Brian F. McGuinness, and Koji Nakanishi*
Department of Chemistry, Columbia University, New York, NY 10027, U.S.A.
Maria Tomasz*
Department of Chemistry, Hunter College, City University of New York, New York, NY 10021, U.S.A.

Abstract - Mitomycin C (1) undergoes a stereoselective aziridine ring-opening reaction upon treatment with trifluoroacetic acid in aprotic media to give 1β-hydroxy-2β-trifluoroacetamido-7-aminomitosene (3). Its formation is postulated to involve acid-catalyzed degradation of MC to the intermediate carbocation 7, which captures trifluoroacetate ion predominantly from the β-face at the 1-position to yield trifluoroacetate ester 8. Internal trans-acylation yields the observed product 3. A high yield synthesis of 3 is presented as well as the implications of this reaction for the occurrence of acidic activation of MC in vivo. The synthetic utility of 3 is also mentioned.

Introduction

The antibiotic mitomycin C (MC, 1) is a prominent member of the class of organic molecules which alkylate deoxyribonucleic acid (DNA) in vivo and in vitro. Exposure of cells to MC causes profound inhibition of DNA replication and cell growth; MC has therefore found extensive usage in the chemotherapeutic treatment of solid tumors, particularly those of the brain, breast, and gastrointestinal tract. MC is known to require activation by chemical/enzymatic reduction[1,2] or acid pH[3,4] for alkylating activity to be manifest. Thus, in the presence of the appropriate activating agents, MC undergoes a sequence of metabolic reaction steps involving expulsion of methanol from the 9/9a positions and stereorandom capture of nucleophiles at the 1-position to give rise to "mitosene" derivatives 2 (Scheme I).[2,3] While reductive and acidic activation follow mechanistically divergent pathways, isolated MC reaction products in both cases bear the common structure 2.

Scheme I. Formation of Mitosenes



Recent work conducted in our laboratories has led to a unified view of the DNA-binding reactions of MC *in vitro* under both reductive and acidic activation. Under reductive catalysis, MC binds to the N^2-position of guanine (G) residues with overwhelming 1,2-*trans* stereoselectivity ($\geq$ 95% of total adduct pool);[6] under acidic conditions, MC shows almost equal affinity for the N^2- and N7-positions of G;[7] in the latter case, >95% 1,2-*trans* stereoselectivity is also observed. We have recently elucidated the full structure of a MC-DNA bis-adduct,[8] the characterization of which unequivocally demonstrates for the first time the existence of a bifunctional mode of DNA-alkylation by MC; this previously putative crosslinking activity has long been believed to be the most biologically significant lesion produced in cellular genome by the action of MC.[1] Owing to the inherent difficulty of *in vivo* studies on DNA-alkylating agents, the biologically operative MC activation mechanism (acidic and/or reductive) continues to elude definition.

During the course of our studies on the acidic activation mechanism of MC[4,7] we observed an interesting degradation reaction of 1 which occurs under acidic conditions in aprotic media. In view of the importance of the activation chemistry of MC, our results on this reaction are reported. The interest in such acidic aprotic reactions stemmed from efforts to synthesize mitosenes bearing chromophoric esters at C-1 (Scheme 1; 2a) by treatment of MC with *p*-substituted benzoic acids in the presence of trifluoroacetic acid (TFA). The usage of an aprotic solvent such

as dioxane or acetonitrile was intended to prevent formation of the MC-aziridine hydrolysis products 2b and 2c commonly observed in acqueous media. Under all reaction conditions attempted, it was not possible to obtain the desired benzoates 2a; instead, a single non-polar mitosene of previously unknown structure was isolated in low yield (ca. 33%) from these reactions. The structure of this new mitosene (TFA mitosene) has been determined as 3; its spectroscopic properties and proposed mechanism of formation are reported here. Further studies of the reaction of 1 with TFA showed that: (i) removal of the chromophoric acid from the reaction mixture had no effect on the yield of 3; (ii) addition of 1.1 equivalents of ethyl carbamate dramatically increased the yield of 3 to 90-95%. A minor product isolated from this reaction was identified as the known mitosene 2b.

The TFA mitosene arises from a highly stereoselective aziridine ring-opening reaction of MC which involves initial capture of trifluoroacetate ion at the 1-position, followed by 1,2-acyl migration. In contrast to the 1,2-*cis*/*trans* product ratio of ~3:1 (i.e., 2c/2b) which is generally observed upon aqueous acidic activation,[9,10] the current aprotic acidic activation exhibits a stereochemical selectivity of 19:1 (i.e., ratio 3/2b). A high-yield preparation of 3 is described here because of the possible usage of 3 as a versatile synthetic precursor to more highly functionalized mitosenes. The mechanistic implications of the reaction reported here have potential relevance to the putative *in vivo* acidic activation of MC.

Structural studies of 3

^{1}H-NMR:[11] The overlapping signals of 10-H_2 and 1-H in $(CD_3)_2SO$ could be separated by addition of 50% C_6D_6. Presence of the exchangeable two-proton signals at 6.87 and 6.78 ppm due to 10a-NH_2 and 7-NH_2 indicates that the reaction does not involve C-10 or C-7. Two additional exchangeable one-proton signals are evident at 9.89 and 6.04 ppm; the former is coupled to 2-H (4.94 ppm) while the latter is coupled to 1-H (5.35 ppm); the observed intensities, chemical shifts, and coupling patterns[11] are consistent with the attachment of an amide-type N-H group at C-2 and a hydroxyl group at C-1. Several other features of the spectrum warrant further comment: (i) The 2-H and 3-H_2 signals are shifted downfield relative to values generally observed in mitosenes bearing an ammonium functional group at C-2; (ii) the 10-H_2 signal is a singlet[11] suggesting free rotation about the carbamate single-bonds; whereas in most mitosenes, free rotation is restricted.

^{13}C-NMR: Full ^{13}C-NMR assignments for the mitosene skeleton have not previously been published to our knowledge. Peak assignments[12] for TFA mitosene 3 are based on comparisons with other mitosenes and long-range C-H couplings. The chemical shifts (δ, ppm), in $(CD_3)_2SO$, are as follows: 6-methyl C, 7.7 ppm; C-3, 47.7; C-1, 54.8; C-10, 56.6; C-2, 63.4; C-6, 105.0; trifluoromethyl C, 108.8, 113.4, 118.0, 122.5 ($J_{C,F}$ = 287.0 Hz); C-8a, 112.9; C-9, 120.8; C-5a, 128.0; C-9a,

140.4; C-7, 146.7; trifluoromethyl acetamide carbonyl C, 155.3, 155.9, 156.5, 157.1 ($J_{C,F}$ = 37.7 Hz); C-10a, 156.4; C-5, 176.2; C-8, 178.1.

FTIR (Fig. 1): The FTIR solution spectrum, 1800-1400 cm^{-1} region, of 3 is shown. Absorptions of 3 at 1672, 1619, 1605, 1573, and 1499 cm^{-1} (Fig. 1) are in agreement with values obtained for similar mitosenes.[13,14] However, intensity of the 1718 cm^{-1} band is much greater in 3 than in simple mitosenes (e.g. 2b and 2c) owing to overlap of the 1722 cm^{-1} carbamate and 1718 cm^{-1} trifluoroacetamide carbonyl absorptions.

UV: The UV of 3 in 0.1 M potassium phosphate buffer (pH 7.0) shows peaks at 206, 249, 310, 352, and 535 nm; all are characteristic of 7-aminomitosene derivatives.

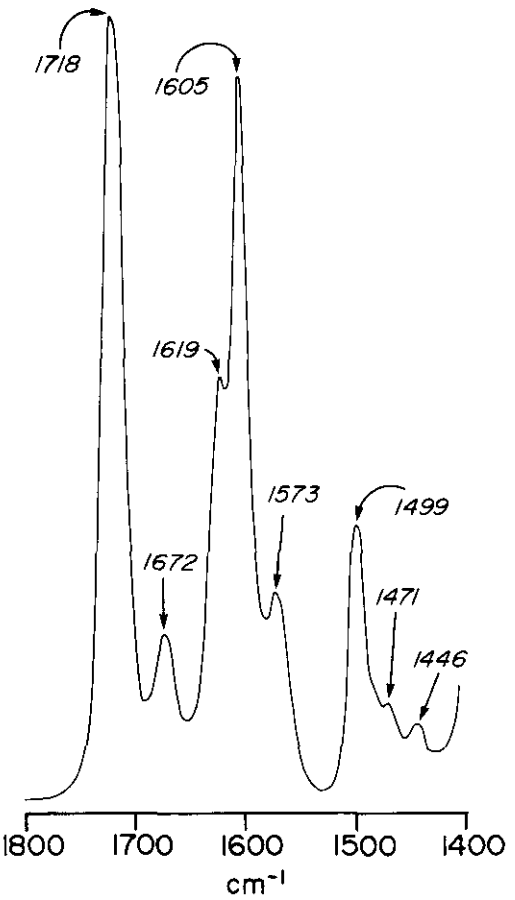


Figure 1. FTIR spectrum of TFA Mitosene 3, 1800-1400 cm^{-1} region, $(CD_3)_2SO$ solution, internal reflectance microcell.

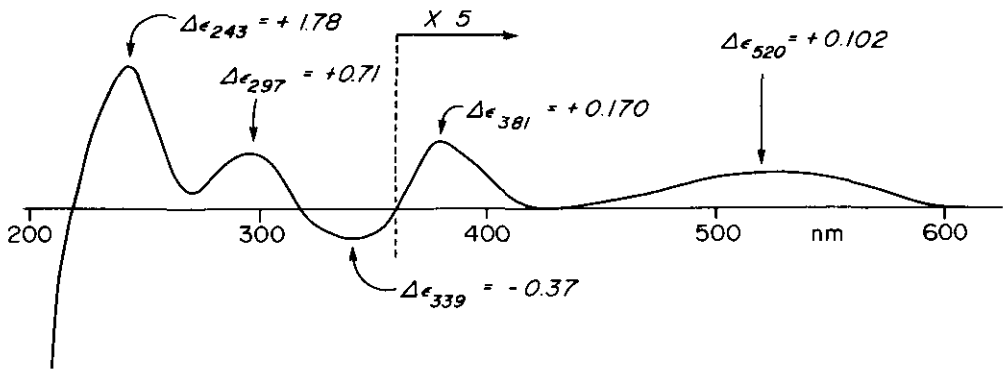


Figure 2. CD spectrum of TFA Mitosene 3 in MeCN.

CD (Circular dichroism) (Fig. 2): The CD spectrum of 3 is quite similar to that of 1,2-cis-mitosene 2c.[15] The characteristic feature of Fig. 2 is the presence of a 520 nm Cotton effect (CE), $\Delta\varepsilon$ = +0.102. It has previously been shown that the sign of the mitosene ~520 nm CE is dependent on the configuration at C-1: a positive ~520 nm CE denotes 1ß-stereochemistry (1,2-cis) while a negative ~520 nm CE denotes 1α-stereochemistry (1,2-trans).[10,15] The positive 520 nm CE of 3 leads to a 1ß-configuration. Since the C-2 configuration is retained in all mitosenes upon aziridine cleavage, the 1,2-vicinal stereochemistry in 3 is thus cis.

Fast Atom Bombardment Mass Spectrometry: The molecular weight of 3 is 416 (molecular formula $C_{16}H_{15}F_3N_4O_6$), agreeing well with the observed ions at: m/e 417 $[M+H]^+$, 439 $[M+Na]^+$, and 356 (major fragment ion; $[M-carbamate]^+$).

Deacylation of 3 to 2c: The trifluoroacetamide moiety of 3 was removed by treatment with NaOMe in 60/40 dry MeOH/benzene,[16] thus affording the known mitosene 2c[17,18] in 80% yield. Acetylation of 2c (excess Ac_2O/pyr/DMAP) yielded known diacetate 2e.[17]

Acetylation of 3: Attempts to protect the 1-OH function of 3 by conversion to the tert-butyldimethylsilyl (TBDMS) ether (TBDMS-Cl/DMF/imidazole, rt, 24h) were unsuccessful. The mono-acetate derivative 4[19] was, however, readily formed upon treatment with Ac_2O/pyr/DMAP.

Structure of the minor product 2b and its diacetate 2d: The minor product arising during the synthesis of 3 was identified as 2b by conversion to the corresponding diacetate 2d[20] and comparison with a sample of authentic 2d obtained by acetylation (vide supra) of known 2b.[17,18] The full ^{1}H-NMR spectrum of diacetate 2d[20] has, to our knowledge, not been reported previously.

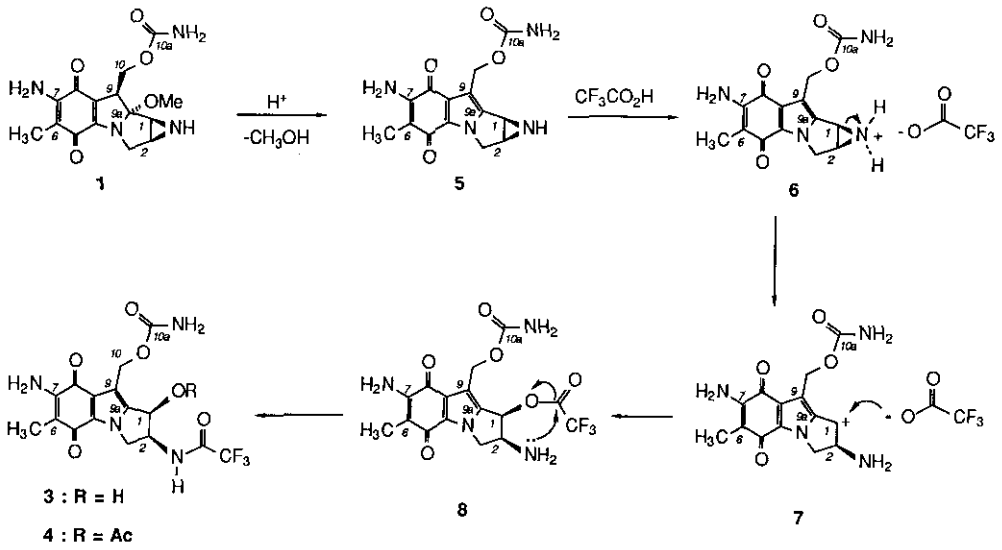
Synthetic utility of 3: Treatment of 1 with TFA typically yields 3 in >90% yield. Owing to the fact that 3 possesses a 2-amino function which is protected by a readily removable trifluoroacetamide group, it provides a rapid entry into mitosenes which carry various leaving groups at C-1. All known monofunctional DNA-alkylation reactions of MC proceed through mitosene intermediates bearing a potent electrophile at C-1; thus, mitosenes which possess good leaving groups at C-1 are very attractive candidates for design of new antitumor agents. The high-yield conversion of 1 to 3 reported here is carried out in organic solvents, thus allowing one to avoid the tedious lyophilization/HPLC isolation and purification procedures which are required by the previously employed aqueous acidic activation reactions of MC.

Mechanism of formation of 3: As mentioned above, treatment of MC with TFA in the presence of p-substituted benzoic acids afforded 3. Omission of the p-substituted benzoic acids from the reaction mixture had no effect on the yield of 3; neither had changing the solvent from dioxane to MeCN. These reactions, however, gave significant amounts (>60%) of polar mitosene products; while these products were not subjected to further analysis, it seemed possible that they could have been formed by acid-catalyzed cleavage of the 10-carbamate function to yield mixtures of 10-decarbamoylmitosenes. This postulate was checked by running experiments in which 1.1 equivalents of ethyl carbamate was added to the solution of 1 prior to the addition of TFA; in these cases the yield of 3 increased dramatically to ~95%. While the role of ethyl carbamate is not entirely clear, likely functions are: (i) buffering of the acidity of the reaction; (ii) providing an external source of isocyanide (i.e., carbamate to replace the lost 10-$CONH_2$ group).

The formation of 3 (Scheme II) is presumed to take the following course. Acid-catalyzed expulsion of methanol from 1[5] gives rise to the aziridinomitosene 5, which undergoes rapid protonation at the aziridine nitrogen by TFA to generate intermediate salt 6. Heterolytic cleavage of the C-1/N bond in 6 yields the ring-opened carbocation 7, which immediately captures trifluoroacetate ion from the β-face to give TFA ester 8. Intramolecular O to N acyl migration leads to the observed product 3.

In addition to ~95% 3, ~5% of a minor product 2b is obtained in the reaction. Presumably, 2b is formed by a reaction in which carbocation 7 traps TFA anion from the α-face, giving rise to the 1α-epimer of TFA ester 8. Apparently, 1,2-acyl migration of the TFA ester in 1α-8 does not occur owing to its 1,2-trans stereochemistry; rather, the TFA ester moiety is presumably cleaved to yield 2b during the workup procedure. The overall stereochemical outcome of the 1-TFA reaction, then, is 95% 1,2-cis (3)/5% 1,2-trans (2b); this corresponds to a 19:1 cis/trans ratio. The MC aziridine ring opening reported here is thus overwhelmingly cis-stereospecific; the present result is in stark contrast with the poor (~3:1) cis/trans stereochemical selectivity which is observed upon MC

Scheme II. Formation of TFA mitosene 3



aziridine hydrolysis in aqueous solution under acidic activation.[17,18]

The likely origin of the high cis stereochemical preference demonstrated in this reaction lies in the kinetically-controlled capture of trifluoroacetate ion by intermediate mitosene carbocation 7. Apparently, 7 is very unstable in organic solvents, thus reacting with trifluoroacetate ion before the anion can undergo appreciable diffusion to the α-face of the molecule. Thus, protonation of the aziridine nitrogen in 5 triggers a sequence in which C-1/N bond fission occurs with rapid nucleophilic substitution at C-1.

Discussion

Historically, the potent antitumor activity of MC has been attributed to its ability to bind covalently to DNA under the activating influence of biological reducing agents.[1] It has been known for over ten years, however, that MC also undergoes activation in the presence of acid catalysis; furthermore, it has been proposed that MC is capable of forming covalent crosslinks with DNA in acidic media in vitro.[21,22] Thus, the possiblity exists that bioreductive alkylation may not be the exclusive motif of MC activation employed in vivo. This speculation is lent further support by the recent finding that the toxicity and DNA-crosslinking activity of MC in anaerobic cultures EMT tumor cells increased dramatically upon controlled acidification of the extra- and intracellular growth medium.[23]

Recent studies conducted in our laboratories have demonstrated conclusively that MC binds covalently to both a model dinucleoside phosphate[4] and DNA[7] upon lowering the pH of the medium to

a value of ~4 or below. The relevance of such in vitro studies to the biological activity of MC rests in the assertion that the weakly acidic conditions used for acidic activation may mimic the low pH found inside gastric and solid tumors, for which MC is an effective treatment.[24] However, the actual intracellular pH of solid tumors is speculated to be ~5.2[24] and kinetic data shows that the binding (activation[5]) of MC to DNA in vitro at pH >5 is probably too slow to account for the in vivo activity of MC.[7] Thus, it is probable that acidic activation must require more than just a proton-rich ambient milieu in order for it to be a viable MC-activation motif in vivo. For these reasons, we have speculated that biologically-operative acidic activation may be accomplished by the assistance of enzymatic factors within the cell.[7] It is of relevance to note that recent work has demonstrated the efficient activation of MC in vitro by Lewis acidic *metal complexes;*[25] *enzymes which possess active-site Lewis acidic* (i.e., Zn) centers are well-known.[26] Such an MC-activating enzyme would presumably exclude water from its active site both in order to bind MC and in order to increase the acidity of its catalytically active residue(s). Thus the current aprotic acidic activation may actually have more biological relevance than simple aqueous MC solvolysis studies by virtue of allowing the activation cascade to proceed in a hydrophobic environment, in analogy to the hydrophobic active site of an enzyme.

As shown in Scheme II, acidic activation consists of two steps[5] which involve a protonation: (i) methanol elimination; and (ii) aziridine-opening with nucleophile-trapping. As discussed earlier, it appears on the basis of this study that step (ii) is kinetically controlled and rapid when proceeding in aprotic solvents. Step (i) is the one which requires a relatively strong acid, while step (ii) presumably requires only a weak acid. It is relevant to note that aziridinomitosene 5 has never been obtained from MC to date. Such a rapid nucleophile-trapping reaction suggests that upon enzymatic activation of MC, unstable intermediate 5 might undergo aziridine-substitution involving an acidic residue in the active site before it could diffuse out of the binding pocket. This scenario casts MC into the role of a potential enzyme suicide inactivator, a putative function which has not previously been considered. Such an activity, if existent, may have profound implications for the observed biological effects of the drug, including those which are undesirable. We are currently in the process of experimentally evaluating the validity of the foregoing speculations.

EXPERIMENTAL

Synthesis of 3 from mitomycin C and ethyl carbamate (urethane): 10 mg 1 was suspended in 2.0 ml dry MeCN at room temperature in a round-bottom flask equipped with a rubber septum and a magnetic stirrer, under argon. Ethyl carbamate (3.1 mg, 1.1 equivalent) was added as a solid to the MC suspension (MeCN solvent) prior to the addition of 1.1 equivalents of TFA (2.4 µl). Upon

addition of the TFA, an immediate color change from bluish-purple to pink ensued. After 1h, all starting 1 had been consumed, as judged by TLC analysis (10% MeOH/$CHCl_3$; MC elutes as a blue spot, r_f 0.48). The reaction mixture was applied directly to two 20 X 20 cm preparative silica gel TLC plates (Analtech pre-adsorbent, 500 μm) and eluted with 100% EtOAc. In this system, 3 elutes as a pink band at r_f 0.91 while 2b (or 1α-8; vide infra) elutes as a pink band at r_f 0.10. Virtually no material was observed at the origin of the TLC plate after the reaction; the isolated products account for >99% of the starting material 1. This reaction can be carried out on a 100 mg (MC) scale with similar results. Changing the solvent from dioxane to dry MeCN did not affect the yield of 3 or 2b.

Spectroscopic measurements: NMR spectra were obtained on a Bruker WM 250 instrument (250 MHz) using 100 atom % d $(CD_3)_2SO$ and C_6D_6 as indicated in text. Chemical shifts are reported in ppm downfield from external TMS. FTIR spectra were recorded on an IBM IR85 instrument (MCT detector, 2.0 cm^{-1} resolution) operating in the single beam mode. Samples (~500 μg) were dissolved in 50μL $(CD_3)_2SO$ (100 atom % d) and charged into the cylindrical internal reflectance (CIRCLE) microcell (ZnSe rod, 25 μL capacity). The 2500 transient interferograms were Fourier transformed, converted to absorbance spectra by 1:1 subtraction of the single beam spectrum of the uncharged cell, and corrected for ambient H_2O vapor and CO_2 by absorbance-mode subtraction using the IBM SAM option. UV spectra were measured on a Perkin Elmer model 320 spectrophotometer in 0.1 M aqueous potassium phosphate buffer (pH 7.0) and are corrected for baseline absorbance. CD spectra were obtained on a Jasco J-40 instrument operating in the single beam mode and corrected for baseline errors. Fast Atom Bombardment mass spectra were obtained on a VG 70EQ instrument, 6kV ionization energy, Xenon ionization gas, glycerol matrix; and corrected for glycerol ions.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. J. Pawlak, Columbia University, for mass spectral measurements, and Dr. R. Takeda, Suntory Institute for Bio-organic Research (Osaka) for helpful discussions. This research was supported by NIH grants CA 11572 (to K.N.) and CA 28681 (to M.T.) and a PSC-CUNY Faculty Research Award #15 (to M.T.).

REFERENCES AND FOOTNOTES

† Dedicated to Professor Gilbert Stork on the occasion of his 65th birthday.

1. W. Szybalski and V.N. Iyer, in 'Antibiotics 1. Mechanism of Action', eds. D. Gottlieb and P.D. Shaw, Springer Verlag, New York, 1967, p. 211.
2. M. Tomasz and R. Lipman, Biochem. 1981, 20, 5056.
3. M. Tomasz and R. Lipman, J. Am. Chem. Soc., 1979, 101, 6063.

4. M. Tomasz, R. Lipman, G.L. Verdine, and K. Nakanishi, J. Am. Chem. Soc., 1985, 107, 6120.

5. R.A. McClelland and K. Lam, J. Am. Chem. Soc., 1985, 107, 5182.

6. M. Tomasz, D. Chowdary, R. Lipman, S. Shimotakehara, D. Veiro, V. Walker, and G.L. Verdine, Proc. Natl. Acad. Sci., 1986, in press.

7. M. Tomasz, R. Lipman, M.S. Lee, G.L. Verdine, and K. Nakanishi, manuscript submitted.

8. M. Tomasz, R. Lipman, G.L. Verdine, J. Pawlak, and K. Nakanishi, manuscript submitted.

9. L. Cheng and W.A. Remers, J. Med. Chem., 1977, 20, 767.

10. M. Tomasz, M. Jung, G.L. Verdine, and K. Nakanishi, J. Am. Chem. Soc., 1984, 106, 7367.

11. ^{1}H-NMR of 3. In 50/50 (v/v)C_6D_6/ $(CD_3)_2SO$: 9.89 ppm (1H; exch d; 7.3 Hz), N^2-H; 6.87 (2H; exch br s) and 6.78 (2H; exch br s), 10a-NH_2 and 7-NH_2; 6.04 (1H; exch d; 5.2 Hz), 1-OH; 5.43 (2H; s), 10-H_2; 5.35 (1H; dd; 5.2, 6.0 Hz), 1-H; 4.94 (1H; dddd; 7.9, 8.3, 7.3, 6.0 Hz), 2-H; 4.63 (1H; dd; 13.5, 7.9 Hz), 3-H_b; 4.47 (1H; dd; 13.5, 8.3 Hz), 3-H_a; 2.08 (3H; s), 6-CH_3. In $(CD_3)_2SO$: 9.67 ppm (1H, exch d), N^2-H; 6.62 (2H, exch br s), and 6.54 (2H, exch br s), 10a-NH_2 and 7-NH_2; 5.76 (1H, exch d), 1-OH; 5.15-5.0 (3H, m), 10-H_2 and 1-H; 4.79 (1H, br m), 2-H; 4.49 (1H,dd), 3-H_b; 4.15 (1H, dd), 3-H_a; 1.75 (3H, s), 6-CH_3.

12. G.L. Verdine et al., to be published.

13. M. Tomasz, R. Lipman, G.L. Verdine, and K. Nakanishi, Biochem., 1986, in press.

14. G.L. Verdine and K. Nakanishi, J. Am. Chem. Soc., 1985, 107, 6118.

15. M. Tomasz, R. Lipman, J.K. Snyder, and K. Nakanishi, J. Am. Chem. Soc., 1983, 105, 2059.

16. S. Kinoshita, K. Uzu, K. Nakano, and T. Takahashi, J. Med. Chem., 1971, 14, 109. This procedure is commonly used to effect cleavage of the C-10 carbamate function in mitosenes; however, brief treatment (2h) of 3 under the described conditions leads mainly to loss of its trifluoroacetamide moiety.

17. W.C. Taylor and W.A. Remers, J. Med. Chem., 1975, 18, 307.

18. L. Cheng and W.A. Remers, J. Med. Chem., 1977, 20, 767.

19. ^{1}H-NMR of 4 in $(CD_3)_2SO$: 9.89 ppm (1H; exch d; 7.8 Hz), N^2-H; 6.71 (2H; exch br s) and 6.43 (2H; exch br s), 10a-NH_2 and 7-NH_2; 6.14 (1H; d; 6.0 Hz), 1-H; 5.12 (1H; dddd; 7.8, 6.0, 8.2, 8.2 Hz), 2-H; 5.06 and 4.99 (2H, two d; 12.8 Hz), 10-H_2; 4.57 (1H; dd; 12.6, 8.2 Hz), 3-H_b; 4.16 (1H; dd; 12.6, 8.2 Hz), 3-H_a; 1.99 (3H, s), 1-acetate CH_3; 1.75 (3H; s), 6-CH_3.

20. ^{1}H-NMR of 5 in $(CD_3)_2SO$: 8.51 ppm (1H; exch d; 6.7 Hz), N^2-H; 6.68 (2H; exch br s) and 6.42 (2H, exch br s), 10a-NH_2 and 7-NH_2; 5.88 (1H; d; 1.9 Hz), 1-H; 5.25 and 4.93 (2H, two d, 12.4Hz); 4.64 (1H; dddd; 6.7, 6.6, 2.7, 1.9 Hz), 2-H; 4.47 (1H; dd; 13.2, 6.6 Hz), 3-H_b; 4.05 (1H; dd; 13.2, 2.7 Hz), 3-H_a; 2.02 (3H; s), 1-acetate-CH_3; 1.80 (3H; s), 2-acetamide-CH_3; 1.74 (3H; s), 6-CH_3.

21. J.W. Lown, A. Begleiter, D. Johnson, and A.R. Morgan, Can. J. Biochem., 1976, 54, 110.

22. J.W. Lown and G. Weir, Can. J. Biochem., 1978, 56, 296.

23. K.A. Kennedy, J.D. McGurl, L. Leondaridis, and O. Alabaster, Cancer Res., 1985, 45, 3541.

24. H.O. Douglass, P.T. Lavin, A. Goudsmit, D.J. Klassen, and A.R. Paul, J. Clin. Oncol., 1984, 2, 1372, and references cited therein.

25. B.S. Iyengar, T. Takahashi, W.A. Remers, and W.T. Bradner, J. Med. Chem., 1986, 29, 144.

26. For example, see B.L. Vallee in 'The Enzymes', P.D. Boyer, H. Lardy, and K. Myrback, eds., 2nd Ed., Vol. 3, Chapter 15, Academic Press, New York, 1960, pp. 225-276.

Received, 14th July, 1986