Modeling of the Electrophilic Activation of Mitomycins: Chemical Evidence for the Intermediacy of a Mitosene Semiquinone as the Active Electrophile

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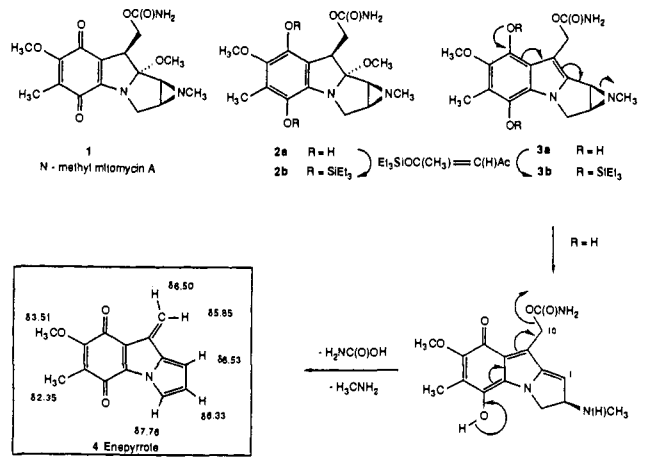
Mitomycin C (MMC) is becoming increasingly useful in a variety of cancer chemotherapeutic regimens.[1] In model biological systems, and presumably in vivo, reductive priming is necessary for MMC to alkylate and cross-link double-helical DNA.[2] Our approach toward gaining insight into the mode of action of mitomycins has been to synthesize and characterize the likely intermediates in the reductive activation process and then to explore their characteristics.[3a,b] An understanding of the issues pertinent to the "small molecule" part of the problem would provide a sounder basis from which reactions of mitomycins with DNA could be understood.[4a-c]

N-Methylmitomycin A (*N*-MeMMA) has served as a convenient substrate for this study.[5] The leucomitomycin **2a**[3a] and the leucoaziridinomitosene **3a**[3b] have been synthesized and characterized for the first time. A high yielding route to the aziridinomitosene (**7**) from *N*-MeMMA has been accomplished. The intermediacy of semiquinone **5** in the process leading from **1** to **7** has been inferred.[2,3b] Similar conclusions were drawn by Bachur and by Kohn[6a-c] through a detailed electrochemical study, which showed that one-electron reduction led to products in which the aziridine linkage was solvolyzed.

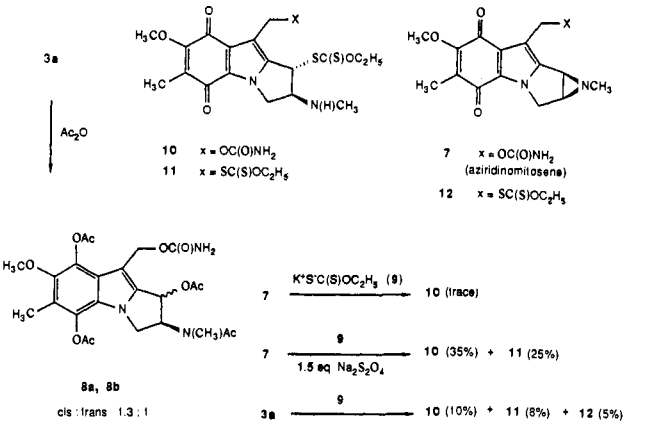
In this paper we report (i) the first preparation of stabilized versions of the leuco compounds with the aziridine rings intact (see compounds **2b** and **3b**), (ii) the remarkable "unimolecular" chemistry of compound **3a** (see formation of ene pyrrole **4**)[7] and (iii) the first report of bimolecular reactions starting with discretely identified intermediates of the presumed activation cascade. In light of these findings, a pathway for the reductive priming of mitomycins can be advanced with considerable confidence (see Scheme III).

The chemical protection of compounds **2a** and **3a** was accomplished by application of the elegant methodology of Mitscher.[8] Thus, pyridine solutions of **2a** and **3a** prepared as previously described,[3a,b] were treated with the triethylsilyl enol ether derived from acetylacetone. Evaporation of all volatiles in vacuo afforded high yields of **2b** and **3b**, respectively. Compound **3b**, the first stable leucoaziridinomitosene, is now serving as a starting material for the preparation of novel mitomycin congeners.[9]

Scheme I



Scheme II



The chemistry of unprotected **3a**, a dilute solution in pyridine, was explored. Transformation of **3a** to a new product occurred slowly and could be followed by NMR spectroscopy. Formation of this product was accompanied by a change in the color of the solution from colorless to bright yellow. Characteristic quinone absorptions were observed in the IR spectrum. Although the instability of the unimolecular degradation product precludes its isolation, examination of its high-field NMR spectrum (250 MHz, pyridine-d_5) allows it to be provisionally formulated as the "ene pyrrole" **4**. A mechanistic pathway from **3a** to **4** is suggested in Scheme I (see arrows). This pathway embodies the essence of the reasoning of the bioreductive activation concepts articulated by Moore[10] and it is attractive to interpret the process in "even-electron" terms. However, the possibility that ene pyrrole **4** is produced from the hypothetical semiquinone **6** cannot be excluded and should be carefully considered in light of subsequent results of bimolecular processes (vide infra). Regardless of this uncertainty, the formation of ene pyrrole **4** provides an excellent model for the capacity of leucoaziridinomitosene (**3a**) or aziridinomitosene semiquinone (**6**) to cross-link double-helical DNA via reactions at C_1 and C_{10}.[11]

Reaction of **3a** with excess acetic anhydride afforded a 40% isolated yield (overall from **7**) of a 1.3:1 mixture of **8a** and **8b**. The stereochemical assignments of these compounds followed closely from spectral (NMR) comparisons with related compounds.[12] The lack of stereospecificity in the opening of the aziridine ring under reductive conditions is also well precedented.[13a,b] Clearly, the interpretation of this transformation is

(1) Carter, S. K.; Crooke, S. T. *Mitomycin C.; Current Status and New Developments*; Academic: New York, 1979.
(2) Tomasz, M.; Mercado, C. M.; Chatterjie, N. *Biochemistry* **1974**, *13*, 4878.
(3) (a) Danishefsky, S.; Cuifolini, M. *J. Am. Chem. Soc.* **1984**, *106*, 6424. (b) Danishefsky, S. J.; Egbertson, M. *Ibid.* **1986**, *108*, 4648.
(4) (a) Tomasz, M.; Lipman, R. *Biochemistry* **1981**, *20*, 5056. (b) Tomasz, M.; Lipman, R.; Snyder, J. K.; Nakanishi, K. *J. Am. Chem. Soc.* **1983**, *105*, 2059. (c) Tomasz, M.; Lipman, R.; Verdine, G.; Nakanishi, K. *Ibid.* **1985**, *107*, 6120.
(5) *N*-methylmitomycin A was first chosen for its solubility and its spectroscopic convenience (N–Me and N–OMe resonances). Another important advantage is that the aziridinomitosene (**7**) is a stable compound. In the mitomycin C series, the leucomitomycin analogues of **2a** and **2b** have been prepared. However, the mitomycin C analogue of **7** has not, in fact, been successfully prepared by our methodology. Accordingly, in the mitomycin C series we do not have access to the pure leucoaziridinomitosene analogue of **3a** and **3b**. It should be noted that compound **7** is quite active in certain antitumor screens. Iyengar, B. S.; Remers, W. A.; Bradner, W. T. *J. Med. Chem.* **1986**, *29*, 1864.
(6) (a) Andrews, P. A.; Bachur, N.; Glover, C. J.; Pan, S. S. *J. Biol. Chem.* **1984**, *259* (2), 959. (b) Andrews, P. A.; Bachur, N. R.; Pan S. *J. Am. Chem. Soc.* **1986**, *108*, 4158. (c) We would like to thank H. Kohn for providing us with these results.
(7) H. Kohn also informs us that he has encountered loss of the aziridine with concomitant formation of a pyrrole in a related substrate.
(8) Mitscher, C. A.; Veysoglu, T. *Tetrahedron Lett.* **1981**, 1303.
(9) Feigelson, G.; Egbertson, M., unpublished results.
(10) Moore, H. W. *Science* (*Washington*, D. C.) **1977**, *197*, 527.
(11) Tomasz, M.; Chowardy, D.; Lipman, R.; Shimotakahara, S.; Veiro, D.; Waller, V.; Verdine, G. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 6702.
(12) Iyengar, B. S.; Remers, W. A. *J. Med. Chem.* **1985**, *28*, 963.

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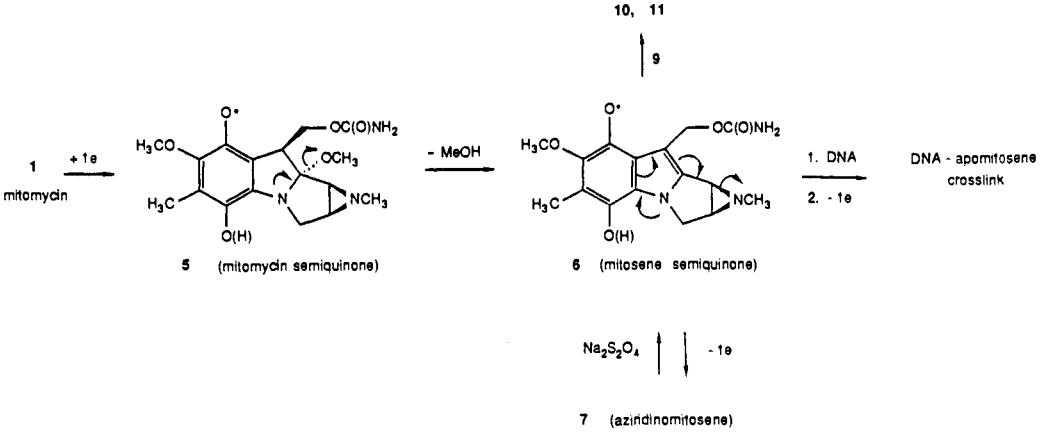
Scheme III. Summary of the Reductive Activation–Alkylation Reactions of *N*-Methylmitomycin A



complicated by uncertainties in the sequence of the various acylations.

Hornemann and Kohn had previously examined the reaction of MMC with potassium ethyl xanthate (**9**) under reductive ($Na_2S_2O_4$) conditions.[14] Apomitosenes, arising from the nonstereospecific ring opening of the aziridine by nucleophile at C_1, were encountered. It was implicitly presumed that reaction had occurred via the MMC derived version of **3a**. In the *N*-MeMMA series, we could evaluate the reactivity of the aziridine-containing compounds **7** and **3a**, generated as discrete entities by our methodology.[3a,b] Reactions were conducted in aqueous pyridine. From this series of reactions, three products could be obtained and identified. These were the C_1 and C_{10} xanthates **10** and **12**, as well as the dixanthate **11**. When reaction was conducted with **3a** (10 min, 0 °C) followed by subsequent air oxidation, a 20% yield of the three products in the indicated ratio was obtained. When reaction was conducted on **7**, a trace of **10** (ca. 5%) could be detected and ca. 95% of **7** was recovered.

Maximum yield was realized from the reaction of **7** with $Na_2S_2O_4$ in the presence of **9**. Oxidation (air) after the 10-min incubation period afforded a 35% yield of **10** and a 25% yield of **11**. Thus the process of reductive priming of **7** with sodium dithionite gave a substantially higher yield than was realized from the two-electron reduction product (**3a**) itself. Furthermore, attempted reduction of **7** with dithionite (aqueous pyridine) in the absence of nucleophile **9** led to very slow reaction and the product was not **3a**, but rather the ene pyrrole **4** (NMR analysis). *The rate of formation of* ***3a*** *is too slow for it to be the primary alkylating agent.* Hence the formulation whereby the two electron reduction product, **3a**, acts as the active alkylating agent, producing **10**, **11**, and **12**, is untenable. The sequence embodied in Scheme III, wherein mitosene semiquinone **6** alkylates nucleophile **9**, accounts very well for the observed result. Further support for the proposal comes from the reaction of aziridinomitosene (**7**) with a catalytic amount (0.3 equiv) of $Na_2S_2O_4$ in the presence of nucleophile **9**. Workup after 35 min yielded an 80% combined yield of xanthate alkylated products.[15] *Thus the extent of alkylation substantially exceeds the availability of reducing agent.* These data in the aggregate point toward the intervention of a steady-state reactive intermediate (cf. semiquinone **6**) as the active electrophile.

A parallelism is noted between the intervention of semiquinone equivalent **6** in the xanthate alkylation reactions and the involvement of species **5** in the C_{9a} methoxy-ejection event (**1** → **7**).[3b] The rough vinylogy between the two processes is indicated (cf. arrows). Our data do not preclude significant alkylation properties for compound **3a**. They also do not define the precise species involved in the remarkable transformation of **3a** → **4**.[16] They do, however, provide a basis for proposing a very concise sequence for bioactivation of mitomycins, as shown in Scheme III. A natural consequence of these findings is that new departures in mitomycin drug development might well center on substitutions which will favor species generically related to **6**. This proposition will now be pursued.

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Supplementary Material Available: Experimental data for compounds **2a,b**, **3a,b**, **4**, **7**, **8a,c** and **10–12** (3 pages). Ordering information is given on any current masthead page.

(13) (a) Tomasz, M.; Lipman, R. *Biochemistry* **1986**, *25*, 4337. (b) Compounds **8a** and **8b** are briefly alluded to (stereochemistry not defined) by the following patent: Matsui, M.; Yamada, Y.; Uzu, K.; Hirata, T.; Wakaki, S. U.S. Patent 3 429 894, Feb. 25, 1969.

(14) Hornemann, U.; Iguchi, K.; Keller, P. J.; Huynh, M. V.; Kozlowski, J. F.; Kohn, H. *J. Org. Chem.* **1983**, *48*, 5026.

(15) Compounds **10**, **11**, and **12** were obtained in yields of 8%, 23%, and 33%, respectively. Twelve percent of the starting material (**7**) was recovered. Reaction of **7** and **9** for a similar time period resulted in a 15% yield of **10** and an 85% recovery of starting material.

(16) The similarities of "electron flow" inherent in the formation of xanthate adducts **10**, **11**, **12**, and of ene pyrrole **4** make tempting the possibility that semiquinone **6** is intervening in the formation of **4**.

Stereocontrolled Construction of Key Building Blocks for the Total Synthesis of Amphoteronolide B and Amphotericin B

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Amphotericin B[1] (I, Scheme I, with β-linked mycosamine at the C-19 hydroxyl), a clinically used antifungal agent isolated from *Streptomyces nodosus*, and its aglycon, amphoteronolide B[2] (I, Scheme I), are important synthetic targets of considerable current interest.[3] In this paper we describe stereocontrolled constructions

(1) Isolation: Vandeputte, J.; Watchtel, J. L.; Stiller, E. T. *Antibiot. Annu.* **1956**, 587. X-ray structural determination: Mechinski, W.; Shaffner, C. P.; Ganis, P.; Avitabile, G. *Tetrahedron Lett.* **1970**, 3873. Ganis, P.; Avitabile, G.; Mechinski, W.; Shaffner, C. P. *J. Am. Chem. Soc.* **1971**, *93*, 4560.

(2) Preparation from amphotericin B: Nicolaou, K. C.; Chakraborty, T. K.; Daines, R. A.; Ogawa, Y. *J. Chem. Soc., Chem. Commun.*, in press.

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