Determination of the pK_a Values for the Mitomycin C Redox Couple by Titration, pH Rate Profiles, and Nernst–Clark Fits. Studies of Methanol Elimination, Carbocation Formation, and the Carbocation/Quinone Methide Equilibrium

Romesh C. Boruah and Edward B. Skibo*

Department of Chemistry and Biochemistry, Arizona State University, Tempe, Arizona 85287-1604

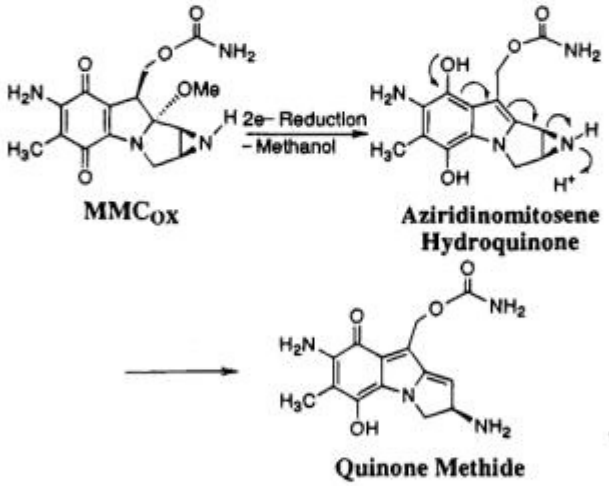
Received October 7, 1994 (Revised Manuscript Received January 25, 1995®)

This report provides the results of a study of the mitomycin C (MMC) redox couple pK_a values employing spectrophotometric titration, pH rate profiles, and Nernst–Clark plots. Oxidized mitomycin C (MMC_{ox}) has three acid dissociations: pK_{a1} = −1.2 for the protonated quinone, pK_{a2} = 2.7 for the protonated indoline nitrogen, and pK_{a3} = 7.6 for the protonated aziridino nitrogen. Two-electron reduction to MMC_{red} results in a shift to higher pK_a values: pK_{a1} = 1.4 for the protonated 7-amino group, pK_{a2} = 5.1 to 6.1 for the protonated indoline nitrogen, and pK_{a3} = 9.1 for the protonated aziridino nitrogen. These pK_a values were successfully integrated into previous mechanistic studies. The general conclusions are that indoline nitrogen protonation prevents methanol elimination, that methanol elimination from MMC_{ox} and MMC_{red} are both specific acid catalyzed, and that there is a 100000-fold increase in the second-order rate constant for specific acid-catalyzed elimination of methanol upon reduction of MMC_{ox} to MMC_{red}. Finally, the formation and fate of the mitosene hydroquinone carbocation was studied in a mitosene model bearing an acetate leaving group. The carbocation species undergoes acid dissociation to the quinone methide (pK_a = 7.1) resulting in a pH dependence for product formation. Below pH 7.1 the carbocation species traps water and above this pH the quinone methide species traps both water and a proton.

Introduction

Mitomycin C is an antitumor antibiotic that is activated as an alkylating agent by reduction to the hydroquinone derivative.[1] Reduction is followed by elimination of methanol to afford the aromatized indole (aziridinomitosene hydroquinone) derivative, which is converted to an alkylating quinone methide species by aziridino ring opening (Scheme 1).[2] The importance of mitomycin C as an antitumor agent has prompted detailed studies of the mechanism of reductive alkylation of DNA.[3] An important requirement for a mechanistic understanding of mitomycin C is a knowledge of all the relevant pK_a values for both the oxidized (MMC_{ox}) and two-electron reduced (MMC_{red}) species. Thus far, only a pK_a in the range of 2.7 to 3 for MMC_{ox}[4–6] and a pK_a of 5.1[7] for MMC_{red} have been determined. These pK_a values have been assigned to acid dissociation from the protonated aziridino nitrogen of MMC_{ox} and MMC_{red}, respectively. Queries posed at the outset of the present study dealt with the identity of the other pK_a values of the MMC redox couple as well as how these pK_a values relate to previous mechanistic studies.

Scheme 1



Provided in this report are the results of a study of the MMC pK_a values employing spectrophotometric titration, pH rate profiles, and Nernst–Clark plots.

The pK_a values thus determined are summarized in Scheme 2 for successive acid dissociation from triprotonated MMC species. The pK_a in the range of 2.7 to 3 is assigned to the protonated indoline nitrogen of MMC_{ox} rather than to the N-protonated aziridino group, which

* Abstract published in *Advance ACS Abstracts*, March 15, 1995.

(1) For reviews, see: (a) Remers, W. A. *The Chemistry of Antitumor Antibiotics*; Wiley: New York, 1979; Vol. I, pp 221–276. (b) Fisher, J. F.; Aristoff, P. A. *Prog. Drug Res.* **1988**, *32*, 411–498. (c) Franck, R. W.; Tomasz, M. In *The Chemistry of Antitumor Agents*; Wilman, D. E. V., Ed.; Blackie and Sons, Ltd.: Glasgow, Scotland, 1990; pp 379–394.

(2) Moore, H. W.; Czerniak, R. *Med. Res. Rev.* **1981**, *1*, 249–280.

(3) (a) Tomasz, M.; Lipman, R. *Biochemistry* **1981**, *20*, 5056–5061. (b) Tomasz, M.; Lipman, R.; Showdary, D.; Pawlak, J.; Verdine, G. L.; Nakanishi, K. *Science* **1987**, *235*, 1204–1208. (c) Tomasz, M.; Lipman, R.; McGuinness, B. F.; Nakanishi, K. *J. Am. Chem. Soc.* **1988**, *110*, 5892–5896. (d) Hopkins, P. B; Millard, J. T.; Woo, J.; Weidner, M. F.; Kirchner, J. J.; Sigurdsson, S. T.; Raucher, S. *Tetrahedron* **1991**, *47*, 2475–2489. (e) For a recent monograph, see: *Bioreductive Activation, DNA Interaction, and Antitumor Activity of Mitosene Compounds*; Malliepaard, M., Ed.; Offsetdrukkerij Ridderprint B. V: Ridderkerk, 1994.

(4) Stevens, C. L.; Taylor, K. G.; Munk, M. E.; Marshall, W. S.; Noll, K.; Shah, G. D.; Shah, L. G.; Uzu, K. *J. Med. Chem.* **1964**, *8*, 1–10.

(5) McClelland, R. A.; Lam, K. *J. Am. Chem. Soc.* **1985**, *107*, 5182–5186.

(6) Similar pK_a values were also measured for mitomycins A and B: Beijnen, J. H.; van der Houwen, O. A. G. J.; Rosing, H.; Underberg, W. J. M. *Chem. Pharm. Bull.* **1986**, *34*, 2900–2913. See also: Beijmen, J. H.; van der Heuwen, O. A. G. J.; Paaij, J. D. F.; Paalman, A. C. A.; Underberg, W. J. M. *Int. J. Pharm.* **1988**, *45*, 189–196.

(7) Hoey, B. M.; Butler, J.; Swallow, A. J. *Biochemistry* **1988**, *27*, 2608–2614.

0022-3263/95/1960-2232$09.00/0