Covalent Binding of Mitomycin C to Nucleosides under Reductive Conditions

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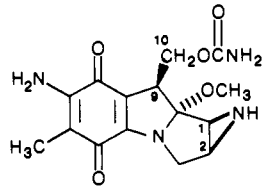
Quinone-containing substrates are among the most potent clinically useful antineoplastic antibiotic drugs.[1] One of these, mitomycin C (**1**), is considered to be the prototype of a class of



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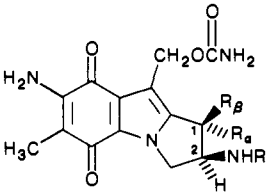
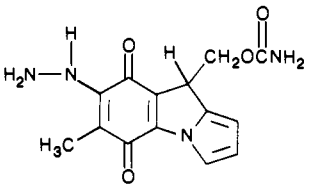
compounds termed bioreductive alkylating agents.[2] Conversion of the quinone ring in **1** to either the corresponding semiquinone or the hydroquinone species in vivo is believed to initiate the drug activation process leading to the binding of mitomycin C to DNA.[3-6] Historically, studies concerned with elucidating the mode of action of **1** have been hampered by the inability to efficiently activate the drug in the presence of biological nucleophiles.[7-12] In this paper, we describe a novel reductive technique for the binding of mitomycin C to simple mononucleosides.

Treatment of a deaerated 1.5 mM aqueous, buffered (Tris-acetic acid) solution of mitomycin C (**1**) with 440 equiv of dimethylhydrazine for 14 h led to the formation of **2**-**4**[11,13] along with trace amounts of *trans*-**5** and *cis*-**6** 1-hydroxy-2,7-diaminomitosenes and *cis*-2-acetamido-1-hydroxy-7-aminomitosene (**7**)[13,14] after oxidative workup. Correspondingly, when hydrazine (12-50 equiv) was employed in place of dimethylhydrazine, the principal products isolated were **5**-**7** along with a trace amount of the novel adduct **8**,[15] an unidentified compound, and unreacted mitomycin C. At very high hydrazine concentrations (1200 equiv) the only product obtained was **8**. The composite findings of these studies indicated that both dimethylhydrazine and hydrazine served as efficient reducing agents for mitomycin C.[16]



2 (OH^-), $R_\alpha = -N^+(CH_3)_2NH_2$, $R_\beta = H$, R = H
3 (OH^-), $R_\alpha = H$, $R_\beta = -N^+(CH_3)_2NH_2$, R = H
4. $R_\alpha = NHN(CH_3)_2$, $R_\beta = H$, R = H
5. $R_\alpha = OH$, $R_\beta = R = H$
6. $R_\alpha = H$, $R_\beta = OH$, R = H
7. $R_\alpha = H$, $R_\beta = OH$, R = Ac



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Hydrazine-mediated reduction (50 equiv) of mitomycin C in unbuffered aqueous solutions in the presence of 2′,3′-*O*-isopropylideneguanosine (**9**) led to the formation of two mitomycin C-nucleoside adducts, **5** and **6**, and several minor products (HPLC analysis). The reaction mixture was separated into its component parts by G25F Sephadex column chromatography and then the new guanosyl-mitomycin C products were further purified by semipreparative reverse-phase HPLC. One of these adducts has been tentatively identified as 1,2-*cis*-[O^6-(2′,3′-*O*-isopropylideneguanosyl)]-2,7-diaminomitosene (**10**) on the basis of the observed ^1H NMR and COSY spectra.[19-23] Several factors

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(15) The proposed ring substitution pattern in **8** other than for the C^7 substitution is supported by extensive NMR spectral studies, including ^1H NMR, two-dimensional proton-correlated COSY, ^{13}C NMR and ^1H-^{13}C correlated NMR experiments. Select data for **8**: ^1H NMR (300.1 MHz, CD_3OD) δ 1.76 (s, 3 H, C_6CH_3), 4.41-4.51 (m, 3 H, C_9H, $C_{10}H_2$), 5.99 (dd, 1 H, J = 2.7, 3.5 Hz, C_2H), 6.04 (dd, 1 H, J = 1.6, 3.5 Hz, C_1H), 6.65 (dd, 1 H, J = 1.6, 2.7 Hz, C_3H); ^{13}C NMR (75.5 MHz, CD_3OD) 8.4, 35.5, 65.9, 103.5, 105.6, 106.3, 108.2, 118.7, 130.9, 151.1, 151.3, 159.9, 179.8, 180.0 ppm; UV (H_2O) λ_{max} 210, 332, 500 nm. The assignment of the hydrazyl group rather than an amino moiety at C-7 is supported by mass and ^1H NMR spectral studies and is tentative.

(16) The mechanism of this transformation has not been elucidated. A variety of attractive pathways exist. These include both direct electron(s) transfer from the hydrazine to **1** as well as the initial formation of a hydrazine-mitomycin C adduct followed by electron(s) transfer. Previous investigations using inorganic substrates have demonstrated that hydrazines can function both as two- and as one-electron reductants.[17] A pronounced ESR signal was observed upon treatment of **1** with hydrazine (1200 equiv). A considerably weaker signal was also detected when either hydrazine (50 equiv) or dimethylhydrazine (440 equiv) served as the reducing agent. The g value (2.0046) calculated for the ESR signals corresponded to the previous number for both the mitomycin C and the corresponding aziridinomitosene semiquinone species.[3,18]

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(19) Alternative structures are conceivable; for a description of other mitomycin C-DNA based adducts, see ref 11, 12, 20-22. Compound **10**: ^1H NMR (500 MHz, Me_2SO-d_6) δ 1.30 (s, 3 H, iso-CH_3), 1.50 (s, 3 H, iso-CH_3), 1.73 (s, 3 H, C_6CH_3), 3.48 (m, 1 H, $C_{5'}H$), 3.96 (dd, 1 H, J = 5.3, 12.8 Hz, C_3H_β), 4.05 (m, 1 H, C_2H), 4.36 (dd, 1 H, J = 6.5, 12.8 Hz, C_3H_2), 4.90 (dd, 1 H, J = 3.0, 6.4 Hz, $C_{3'}H$), 4.94 (d, ABq, 1 H, J = 11.4 Hz, $C_{10}H_2$), 4.98 (d, ABq, 1 H, J = 11.4 Hz, $C_{10}H_{2'}$), 5.24 (dd, 1 H, J = 2.6, 6.4 Hz, $C_{2'}H$), 5.32 (d, 1 H, J = 7.0 Hz, C_1H), 5.93 (d, 1 H, J = 2.6 Hz, $C_{1'}H$), 6.26 (br s, 2 H), 6.52 (br s, 2 H), 6.57 (br s, 2 H), 7.92 (s, 1 H, C_8H). An unidentified peak obscured the $C_{4'}H$ signal.

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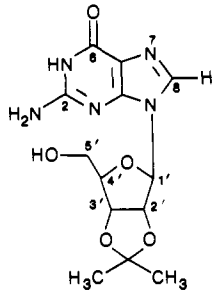

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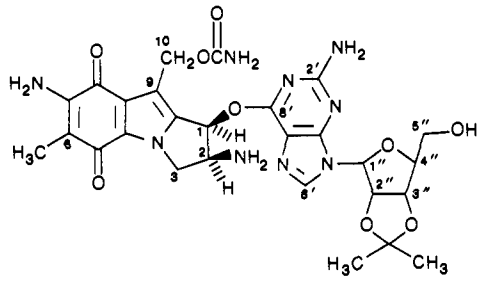

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supported the proposed *cis*-O^6-guanosyl-1-mitosene assignment. First, the mitosene chemical shift values agreed with expectation.[5,24] The resonance noted for the carbon-1 mitosene proton (δ 5.32) was notably downfield from that recorded for *cis*-1-methoxy-2,7-diaminomitosene[5] (δ 4.42) and is consistent with the proposed O^6-guanosyl substitution. A similar deshielding effect was noted in comparing the methoxy chemical shift value for dimethyl ether (δ 3.24) vs. 2-*N*-acetyl-O^6-methyl-2′-deoxyguanosine (δ 4.10).[25,26] Second, three broad singlets were observed in the ^{1}H NMR spectrum of **10** between δ 6.23 and 6.60 and have been attributed to the N-H protons at the C_{10}-carbamate, C_7-amino, and 2′-amino groups.[4] In agreement with this assignment, no signals were detected between δ 10 and 11, a region considered diagnostic for the guanosyl N-1 proton.[27]

Information concerning the generality of the mitomycin C alkylation process was secured by examining the reactivity of **1** with the 2′,3′-*O*-isopropylidene derivatives of adenosine, cytidine, and uridine in the presence of hydrazine (50 equiv). In each case, no noticeable amounts of nucleoside-mitomycin C products were detected (HPLC analysis).[28] Significantly, the base preference noted in this preliminary study mirrors the high guanine specificity reported for the treatment of polynucleotides with mitomycin C under reductive conditions.[8] This suggests that the observed selectivity is a reflection of the reactivity differences which exist at the monomeric nucleoside level for reductively activated mitomycin C. These results imply that prior association (i.e., intercalation) of **1** with DNA may not necessarily be a prerequisite for covalent binding. We note that both the base specificity and the proposed guanosine alkylation site are in agreement with the prescient thesis by Szybalski and Iyer concerning the primary drug binding site on DNA.[2f]

The beneficial properties observed for the hydrazine-mediated reduction of mitomycin C strongly argue for the implementation of this technique in future mitomycin C studies. Moreover, the elucidation of the mode of interaction of the drug with simple nucleosides should serve as a touchstone for understanding the antineoplastic activity of mitomycin C. Additional studies in progress are aimed at determining the generality of this reaction and the structures of the adducts, as well as factors that govern the selectivity of the alkylation process.

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Supplementary Material Available: Experimental section and table of spectral data for compounds **2-4**, **8**, and **10** and a ^{1}H NMR spectrum of **10** (3 pages). Ordering information is given on any current masthead page.

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(23) The following ^{1}H NMR spectral properties have been secured for the second guanosyl-mitomycin C adduct: ^{1}H NMR (300.1 MHz, Me_2SO-d_6) δ 1.27 (s, 3 H), 1.44 (s, 3 H), 1.74 (s, 3 H), 3.66 (m, 2 H), 4.12-4.21 (m, 2 H), 4.41-4.43 (m, 1 H), 4.73 (dd, 1 H, J = 8.8, 13.0 Hz), 4.88 (dd, 1 H, J = 3.0, 6.2 Hz), 4.98 (s, 1 H), 5.01 (d, AB_q, 1 H, J = 12.0 Hz), 5.08 (d, AB_q, 1 H, J = 12.0 Hz), 5.17 (dd, 1 H, J = 3.3, 6.2 Hz), 5.73 (br s, 1 H), 6.39 (br s, 2 H), 6.66 (br s, 2 H), 6.68 (br s, 2 H), 8.01 (s, 1 H).
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Photoinduced Electrocyclic Rearrangements of Allyl Phosphites via Possible Phosphoranyl 1,3-Biradicals

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Both singlet and triplet excited states of alkenes participate in H-abstraction reactions (eq 1) which are analogous to those of

$$>C{=}CH_2 \xrightarrow{h\nu} [>C{=}CH_2]^* \xrightarrow[S_1 \text{ or } T_1]{RH} >\dot{C}\text{-}CH_2\text{-}H + R^\bullet \quad (1)$$

alkyl radicals.[1,2] It is known that methyl and ethyl radicals react with trialkyl phosphites to yield the product of a free radical Arbuzov process when the radical formed on β-scission (eq 2) is

$$Et^\bullet + PhCH_2OP(OEt)_2 \rightleftharpoons \underset{\mathbf{1}}{Et\dot{P}(OEt)_2(OCH_2Ph)} \xrightarrow{\beta\text{-scission}} EtP(O)(OEt)_2 + PhCH_2^\bullet \quad (2)$$

(1) For reviews of the photochemistry of alkenes, see: Kropp, P. J. *Org. Photochem.* **1979**, *4*, 1. Kropp, P. J. *Mol. Photochem.* **1978/1979**, *9*, 39.
(2) Indeed, intramolecular hydrogen abstraction to give a 1,4-biradical leading to a Norrish II like cleavage occurs on triplet-sensitized photoreaction of $PhCHOHCHCH_2CPh{=}CH_2$. (Hornback, J. M.; Proehl, G. S. *J. Am. Chem. Soc.* **1979**, *101*, 7367.) Both singlet and triplet excited states of alkenes are capable of H-abstraction, see: Kropp, P. J. *J. Am. Chem. Soc.* **1969**, *91*, 5783. Scully, F.; Morrison, H. *J. Chem. Soc., Chem. Commun.* **1973**, 529. Kropp, P. J.; Tise, F. P. *J. Am. Chem. Soc.* **1981**, *103*, 7293.

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