Isolation and Characterization of a Major Adduct between Mitomycin C and DNA

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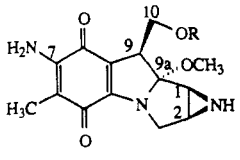
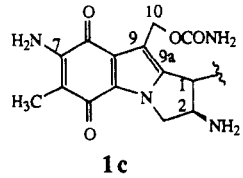
Abstract: Mitomycin C (MC; **1a**) has been shown previously to bind covalently to DNA upon reductive activation, forming three different major adducts with deoxyguanosine residues. Two of these adducts have been fully characterized before as products of monofunctional and bifunctional alkylation of N^2-positions of guanines by MC. The third, unknown adduct was now isolated in sufficient quantities for determination of its structure. Reaction of *Micrococcus luteus* DNA with either MC or 10-decarbamoyl-MC (DMC; **1c**) in the presence of $Na_2S_2O_4$ followed by enzymatic digestion of the DNA-drug complex released this adduct. Its structure was shown to be N^2-(10″-decarbamoyl-2″β,7″-diaminomitosen-1″α-yl)-2′-deoxyguanosine (**3a**) by using microscale spectroscopic techniques previously developed for the study of adducts in this series, featuring second derivative Fourier transform infrared spectroscopy, circular dichroism for assessing the C-1″ stereochemistry, and proton NMR for detection of coupling between the N^2-proton of guanine and the 1″-proton of the mitosene residue. Independent proof was obtained by converting the known 10″-carbamoyl homologue to **7**, the acetylation product of **3a**, by an authentic route. This completes the identification of the three major adducts of reduced MC and DNA. In contrast to MC, the biologically active analogue DMC yields **3a**, but no bifunctional alkylation adduct ("DNA cross-link"), as its sole product with DNA.

The antibiotic mitomycin C (**1a**; MC) is useful in the treatment of numerous types of cancers.[1] Alkylation of deoxyribonucleic acid (DNA) by this chemotherapeutic agent has been implicated in its ability to limit tumor cell growth. Iyer and Szybalski first demonstrated the production of both monofunctional lesions and lethal cross-links in the cellular DNA of bacteria upon treatment with MC.[2,3] These effects are dependent on reduction of the drug and may be mimicked in vitro by addition of enzymatic or chemical reducing agents[4-7] to MC-DNA mixtures. Model studies have demonstrated that this reductive activation serves to unmask the C-1 and C-10 alkylation sites.[6,9-15] Recently, we described the isolation and characterization of **4**, a mitosene[16] derivative covalently attached to two deoxyguanosine residues.[17] This provided definitive proof that the C-1 and C-10 of MC act as the alkylating sites responsible for MC's ability to cross-link DNA. Isolation of **4** was facilitated by the fast reduction kinetics of $Na_2S_2O_4$, which allow activation of both the C-1 and C-10 positions of the drug. In contrast, under kinetically slow reductive conditions (H_2/catalytic PtO_2), monoadducts **2a** and **2b** were the major products isolated (Figure 1a).[18,19] Like the cross-link **4**, the yield of an additional adduct **3a** was greatly enhanced upon sodium dithionite reduction (Figure 1b). It was therefore proposed that **3a** was similarly a product of bisactivation and was postulated to be the 10″-decarbamoyl analogue of **2a**.[19] This structure was further suggested by the finding that reduction of decarbamoyl-MC (**1b**; DMC) in the presence of DNA produced a sole adduct whose HPLC retention time was identical to that of **3a** (Figure 1c).[19] We presently describe the structural elucidation of this substance, showing its identity as N^2-(10″-decarbamoyl-2″β,7″-diaminomitosen[16]-1″α-yl)-2′-deoxyguanosine (**3a**).

This work receives special importance from the recent finding that **3a** was formed, along with cross-link **4**, in Chinese hamster ovary cells upon treatment with MC in culture.[20] Thus, **3a** is apparently one of the major intracellular adducts of MC with DNA, and as such, it may play a major role in the biological effects of this well-known clinically useful antitumor antibiotic.



1a (MC); R = $CONH_2$
1b (DMC); R = OH



1c

Experimental Section

Materials. *Micrococcus luteus* (*M. luteus*) DNA, calf thymus DNA (type I; sonicated before use), and bacterial alkaline phosphatase (type III-R) were obtained from Sigma. DNase I and phosphodiesterase I (SVD) were from Cooper Biomedical (Malvern, PA). Decarbamoyl mitomycin C was synthesized by a published procedure.[21]

Spectroscopic Techniques. Proton nuclear magnetic resonance spectra were measured on a Bruker WM-250 spectrometer, in Me_2SO-d_6. Chemical shifts are reported (in ppm) from an external tetramethylsilane standard. Gaussian multiplications of the free induction decay were

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(1) Carter, S. K.; Crooke, S.T. *Mitomycin C. Current Status and New Developments*; Academic: New York, 1979.
(2) Iyer, V. N.; Szybalski, W. *Proc. Natl. Acad. Sci. U.S.A.* **1963**, *50*, 355.
(3) Szybalski, W.; Iyer, V. N. *Microb. Genet. Bull.* **1964**, 16.
(4) Iyer, V. N.; Szybalski, W. *Science (Washington, D.C.)* **1964**, *145*, 55.
(5) Tomasz, M.; Mercado, C. M.; Olson, J.; Chatterjie, N. *Biochemistry* **1974**, *13*, 4878.
(6) Tomasz, M.; Lipman, R. *Biochemistry* **1981**, *20*, 5056.
(7) Pan, S.-S.; Tracki, T.; Bachur, N. R. *Mol. Pharmacol.* **1986**, *29*, 622.
(8) Tomasz, M.; Lipman, R.; Snyder, J. K.; Nakanishi, K. *J. Am. Chem. Soc.* **1983**, *105*, 2059.
(9) Hornemann, U.; Keller, P. J.; Kozlowski, J. F. *J. Am. Chem. Soc.* **1979**, *101*, 7121.
(10) Hornemann, U.; Iguchi, K.; Keller, P. J.; Vu, H. M.; Kozlowski, J. F.; Kohn, H. *J. Org. Chem.* **1983**, *48*, 5026.
(11) Bean, M.; Kohn, H. *J. Org. Chem.* **1983**, *48*, 5033.
(12) Bean, M.; Kohn, H. *J. Org. Chem.* **1985**, *50*, 293.
(13) Kohn, H.; Zein, N. *J. Am. Soc. Chem.* **1983**, *105*, 4105.
(14) Kohn, H.; Zein, N.; Lin, X. Q.; Ding, J.-Q.; Kadish, K. M. *J. Am. Chem. Soc.* **1987**, *109*, 1833.
(15) Andrews, P. A.; Pan, S.-S.; Bachur, N. R. *J. Am. Chem. Soc.* **1986**, *108*, 4158.
(16) The term "mitosene" refers to the structure as in **1c**, without substituents at the 1-,2-, and 7-positions: Webb, J. S.; Cosulich, D. B.; Mowat, J. H.; Patrick, J. B.; Broschard, R. W.; Meyer, W. E.; Williams, R. P.; Wolf, C. F.; Fulmor, W.; Pidacks, C.; Lancaster, J. E. *J. Am. Chem. Soc.* **1962**, *84*, 3185.
(17) Tomasz, M.; Lipman, R.; Chowdary, D.; Pawlak, J.; Verdine, G. L.; Nakanishi, K. *Science (Washington, D.C.)* **1987**, *235*, 1204.
(18) Tomasz, M.; Lipman, R.; Verdine, G. L.; Nakanishi, K. *Biochemistry* **1986**, *25*, 4337.
(19) Tomasz, M.; Chowdary, D.; Lipman, R.; Shimotakahara, S.; Vero, D.; Walker, V.; Verdine, G. L. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 6702.
(20) Chowdary, D.; Tomasz, M. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1987**, *46*, 2037.
(21) Kinoshita, S.; Uzu, K.; Nakano, K.; Takahashi, T. *J. Med. Chem.* **1971**, *14*, 109.

0002-7863/88/1510-5892$01.50/0

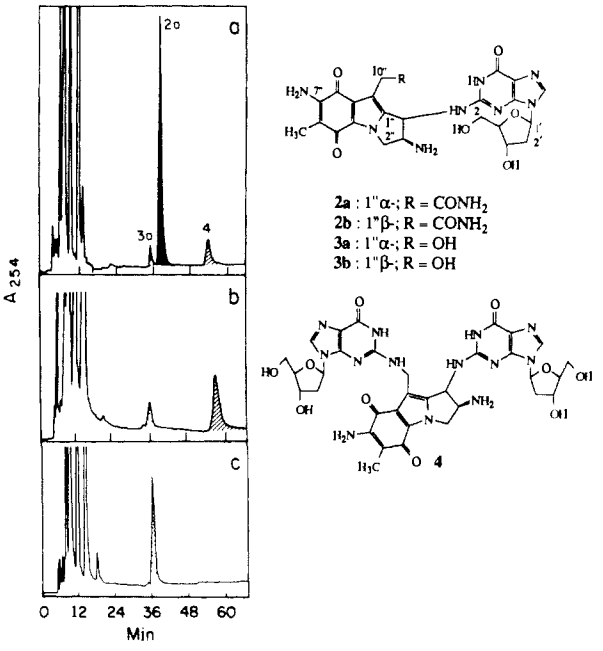


Figure 1. HPLC traces from nuclease digests of MC- and DMC-*M. luteus* DNA complexes formed under various reductive activation conditions: (a) digest of MC-DNA complex, H_2/PtO_2 activation; (b) digest of MC-DNA complex, $Na_2S_2O_4$ activation; (c) digest of DMC-DNA complex, H_2/PtO_2 activation. Formation of a DMC-DNA complex using $Na_2S_2O_4$, and subsequent digestion, produces an identical pattern. Segments a and b are reproduced from an earlier publication.[17] Beckman Ultrasphere ODS column, 1.0 × 25 cm; eluant, 8:92 (v/v) CH_3CN/0.02 M potassium phosphate, pH 5.0; flow rate 2.0 mL/min; detection 254 nm.

accomplished with a line broadening of −3.0 and Gaussian broadening of 0.3. Fourier transform infrared spectra were recorded on a Perkin-Elmer Model 1800 spectrophotometer in Me_2SO-d_6 solution using a cylindrical internal reflectance (ClRcle) microcell (Spectra Tech, Inc.; ZnSe crystal). All measurements were recorded at 2-cm^{-1} resolution using a mercury-cadmium-telluride (MCT) detector. The spectra are the Fourier transform of 2500 transients measured in the single-ratio mode against an argon charged cell background. Absorbances due to the solvent (Me_2SO-d_6) and water vapor were eliminated by subtraction of the appropriate spectra in the interactive difference mode. Second derivative spectra were obtained by using the "width" software parameter 19. Circular dichroism spectra were measured on a Jasco J500A spectropolarimeter in methanol. Ultraviolet (UV) spectra were obtained on a Perkin-Elmer Model 320 spectrophotometer in methanol. Fast atom bombardment (FAB) mass spectra were obtained on a VG 70EQ spectrometer using xenon as the ionization gas and a glycerol matrix.

Preparation of a MC-*M. luteus* DNA Complex. Formation of the MC-*M. luteus* DNA complex under sodium dithionite activation conditions, digestion with DNAse 1/snake venom diesterase/alkaline phosphatase, and isolation of the major adducts **3a** and **4** from the digest by Sephadex G-25 chromatography were already described.[17]

Preparation of DMC-Calf Thymus DNA Complexes. (i) H_2/PtO_2 activation: DNA (0.67 μmol/mL), DMC (1.33 μmol/mL), and PtO_2 catalyst (135 μg/μmol DMC) were mixed in 0.015 M Tris-HCl buffer (pH 7.4) and hydrogenated by a published procedure[8] to yield a DMC-DNA complex with a binding ratio of 0.1. (ii) $Na_2S_2O_4$ activation: DNA (0.67 μmol/mL) and DMC (0.355 μmol/mL) in 0.015 M Tris-HCl buffer (pH 7.4) were treated with 0.06 M $Na_2S_2O_4$ (1.5 mol/mol DMC) to yield a DMC-DNA complex with a binding ratio of 0.074. Anaerobic conditions were maintained as described previously for the analogous procedure using MC instead of DMC. Isolation and determination of the binding ratio of the resulting DMC-DNA complexes [both (i) and (ii)] were accomplished as described previously for MC-DNA complexes.[5]

Digestion of the DMC-DNA complexes by DNAse 1/snake venom diesterase/alkaline phosphatase was accomplished by the same protocol described previously for MC-DNA complexes.[19] HPLC analysis of the resultant adduct mixtures was carried out on a Beckman Ultrasphere ODS column: 8:92 CH_3CN/0.02 M potassium phosphate, pH 5.0; flow rate 2.0 mL/min.

Large-Scale Preparation of the Major Adduct of a DMC-Calf Thymus DNA Complex. A DMC-calf thymus DNA complex, from 93.2 mg (320 μmol) of DMC and 51.2 mg (160 μmol) of calf thymus DNA, was prepared by the H_2/PtO_2 procedure (see above) and isolated in four batches on Sephadex G-100 chromatography (5 × 56 cm column; 0.04 M NH_4HCO_3 as eluant).[22] After lyophilization, the four eluted fractions that contained complex were digested by the above procedure, and the digest was chromatographed on a Sephadex G-25 (superfine) column (5 × 56 cm column; 0.04 M NH_4HCO_3; in four equal batches). After the two major UV-absorbing peaks (dT plus dC, elution volume 1055 mL; dA plus dG, elution volume 1438 mL), a last, smaller peak **3a** (elution volume 2467 mL) was collected. Lyophilization resulted in 585 μg of a purple powder.

Acetylation of 3a. Separate acetylations of **3a** obtained from the two sources described above (MC-DNA, DMC-DNA) were carried out in a sonication bath for 1 h using 8 equiv of acetic anhydride and a crystal of DMAP in 2 mL of dry pyridine. After removal of the pyridine in vacuo, the product of each reaction was isolated by preparative TLC (Analtech preadsorbent silica gel 500 μm; 90:10 $CHCl_3$/MeOH). Further purification was completed with reverse-phase HPLC chromatography (IBM semipreparative ODS column: 60:40 H_2O/MeCN; flow rate 3.0 mL/min; retention time 4.6 min.)

Preparation of 7 from 5. The fully characterized adduct **2a** was available in its peracetylated form **5** from a previous study.[18] Triacetate **5** (3.0 mg, 9.0 μmol) was subjected to conditions known to affect the removal of carbamate groups from simple mitosene derivatives[21] as follows. Triacetate **5** was dissolved in a mixture of 500 μL of dry methanol and 400 μL of dry benzene. After addition of NaOMe (60 mg), the reaction was stirred for 24 h. Subsequently, the reaction was quenched with dry ice, filtered, and concentrated in vacuo. The resultant **6** was isolated by preparative TLC (Analtech preadsorbent silica gel 500 μm; 80:20 $CHCl_3$/MeOH; R_f = 0.1). The UV and CD spectra of **6** were identical to those of the starting material **5**. After characterization by NMR and MS analysis, the collected material **6** was acetylated in a sonication bath as described above. Preparative TLC (90:10 $CHCl_3$/MeOH) yielded 200 μg of adduct **7**.

HPLC of Acetylated Adducts. The acetylated adducts **7** (derived from three sources: MC-DNA complex, DMC-DNA complex, and **5**) were analyzed by HPLC (Perkin-Elmer short C-18 column) coinjection in two solvent systems: 23:67 MeCN/H_2O, flow rate 0.4 mL/min, retention time 6.8 min.; 40:60 MeOH/H_2O, flow rate 0.4 mL/min, retention time 6.4 min.

Results and Discussion

A large-scale preparation of the putative **3a** was undertaken in order to completely characterize it by spectroscopy. Basing our strategy on the assumption outlined in the introduction (namely, that **3a** was the 10″-decarbamoyl derivative of **2a**), we chose to expose DNA to reduced DMC, instead of MC, thus avoiding formation of adducts **2a**, **2b**, and **4**. DMC reduced under mild conditions (H_2/PtO_2)[23] in the presence of calf thymus DNA produced a purple DMC-DNA complex in a yield that was dramatically lower than yields observed with large-scale preparations of **2a** and **4**.[17-19] This may be partly due to the complex's limited solubility and its aggregation during chromatography. Enzymatic digestion of the DMC-DNA complex and separation of the digest into its components by chromatography (Sephadex G-25) yielded a purple solid. HPLC analysis of this product indicated >95% purity of the main substance, eluted at 36 min. A minor component was eluted at 95 min (<5% of the major peak). This was not removed from the bulk of the product for the structural investigation.

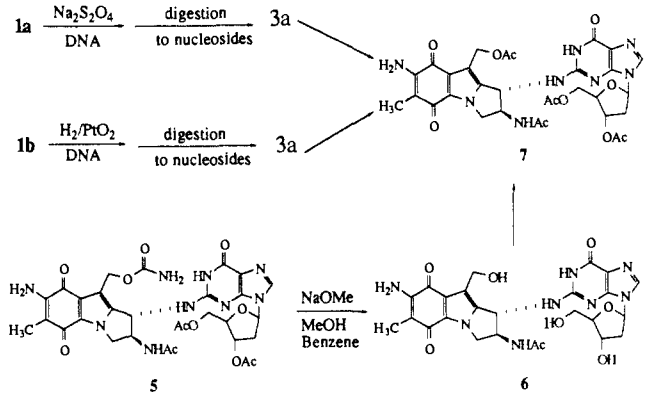
Ultraviolet (UV), second derivative UV, and circular dichroism (CD) spectra of the substance were identical to those of adduct **3a**, previously isolated from a reaction of MC with *M. luteus* DNA. A weak negative Cotton effect at 520 nm in the CD spectrum of **3a** indicated α linkage at C-1″.[24] Although the associated 95-min minor component was not analyzed separately, it is assumed to be the 1″-β isomer of **3a** (i.e., **3b**) by analogy to

(22) On Sephadex G-100 chromatography, the DMC-DNA complex eluted with the void volume. Simple mitosenes, produced by the attack of water on the 1″-position of reduced DMC, were the side products eluting from the column after the DMC-DNA complex.

(23) Since *DMC* was used as the starting material in the large-scale preparation, the choice of reductant (H_2/PtO_2, $Na_2S_2O_4$) was arbitrary. Both reaction conditions have been shown to produce **3a** as the major product in similar yield.[28]

(24) Tomasz, M.; Jung, M.; Verdine, G. L.; Nakanishi, K. *J. Am. Chem. Soc.* **1984**, *106*, 7367.

Scheme I



the adduct pair **2a**/**2b** (retention times and product ratio comparison).

In order to confirm the absence of carbamate from adduct **3a**, an authentic sample was prepared from the fully characterized **2a** (Scheme I). The triacetate of **2a** (**5**) was subjected to conditions known to remove carbamate groups from simple mitomycin derivatives (NaOMe/MeOH/benzene).[21] Quenching the reaction with CO_2 produced $NaHCO_3$, to which the product adhered, thus leading to substantial losses. Proton NMR of the purified compound confirmed a loss of two *O*-acetates under these conditions. A number of criteria led to assignment of structure **6** to the compound, including absence of a 10″-NH_2 resonance at 6.5 ppm as well as a 0.5 ppm upfield shift of the 10″-H_2 protons in its ^1H NMR spectrum. The FAB mass spectrum provided the expected molecular weight (M + H at 569). This authentic substance **6** was acetylated to yield **7**, which then served as the standard for direct comparision with the peracetate of the adduct in question, **3a**. For this purpose, both samples of **3a** described above (one obtained from a MC–DNA complex and the other from a DMC–DNA complex) were separately acetylated in a sonication bath and submitted to tests of identity as well as further spectroscopic characterization. The products of each reaction (**7**) were indistinguishable by HPLC coinjection under two distinct solvent systems (40:60 MeOH/H_2O, retention time 6.4 min; 23:77 MeCN/H_2O, retention time 6.8 min). The second derivative UV and CD spectra (Figure 2) of all three compounds were identical. (The origin of **7** from the three separate routes is summarized in Scheme I).

A combination of difference and second derivative solution Fourier transform infrared spectroscopy (FTIR) may be used effectively to distinguish guanine substitution patterns.[18,25,26] By the usual protocol, subtraction of the mitosene[16] portion of the spectrum (using model mitosenes) from the adduct spectrum unveils the guanine bands. In addition, application of this difference technique to second derivative spectra enhances the information obtained. In order to apply this technique to adduct **7** (Figure 3), a band due to the mitosene moiety must be chosen as a subtraction marker. In the past, the unique carbamate 1722-cm^{-1} band has been useful in these subtractions.[18] In this case, the 1722-cm^{-1} carbamate band is conspicuously absent, and hence, the spectrum must be searched for another nullification site. Subtraction of the mitosene-dominated 1675–1625-cm^{-1} region was useful in determining the substitution pattern on the 10″-guanine moiety of the cross-link adduct **4**.[17] The analogous difference spectrum (obtained by subtraction of the second derivative FTIR spectrum of the mitosene model 1,10-diacetoxy-2-acetamino-7-aminomitosene from the second derivative FTIR spectrum of adduct **7**) exhibits a prominent 1696-cm^{-1} band. This band correlates well with the characteristic 1691-cm^{-1} carbonyl stretch of model N^2-substituted guanosines[18] and rules out the O^6-position of guanine as the point of alkylation. Unfortunately,

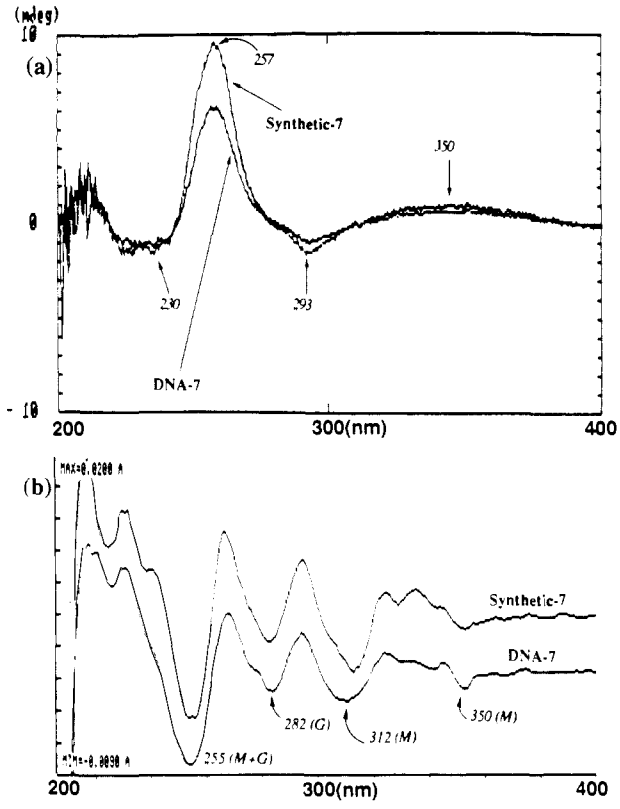


Figure 2. Comparison of the 200–400-nm region of the (a) CD spectra and (b) second derivative UV spectra of **7** obtained from two distinct sources: (i) acetylation of the MC–DNA digest product **3a** and (ii) derivatization of the fully characterized **5** (authentic synthetic material.)

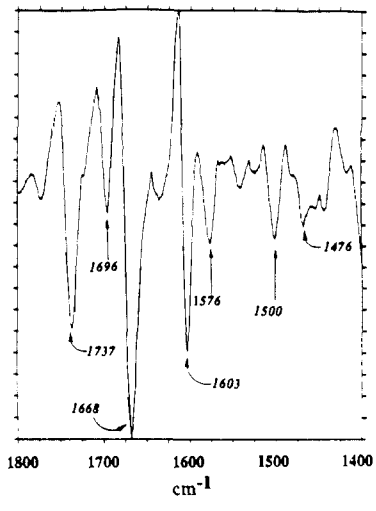


Figure 3. Second derivative solution FTIR spectrum of **7**. Bands at 1696 and 1576 cm^{-1} correspond to characteristic bands at 1691 and 1580 cm^{-1} in model N^2-substituted guanosines. As explained in the text, the 1696-cm^{-1} band becomes prominent upon subtraction of the mitosene portion of the spectrum (not shown).

much of the additional detail of this difference spectrum is lost under the strong absorption of the 1601-cm^{-1} quinone carbonyl band of the model mitosene.

The ^1H NMR spectrum of **7** (Figure 4) clearly exhibited four acetate resonances and, as in **6**, the absence of 10″-NH_2 protons. The spectra were fully assignable via a series of decoupling experiments.[27] Finally, independent proof that the mitosene moiety was attached to the N^2 of deoxyguanosine was obtained by decoupling of the broad N^2-proton at 7.3 ppm sharpening the 1″-H

(25) Tomasz, M.; Lipman, R.; Lee, M. S.; Verdine, G. L.; Nakanishi, K. *Biochemistry*, **1987**, *26*, 2010.
(26) Verdine, G. L.; Nakanishi, K. *J. Am. Chem. Soc.* **1985**, *107*, 6118.

(27) Absence of a resonance for the N^1-proton of the guanine moiety has been previously noted for similar adducts.[18]

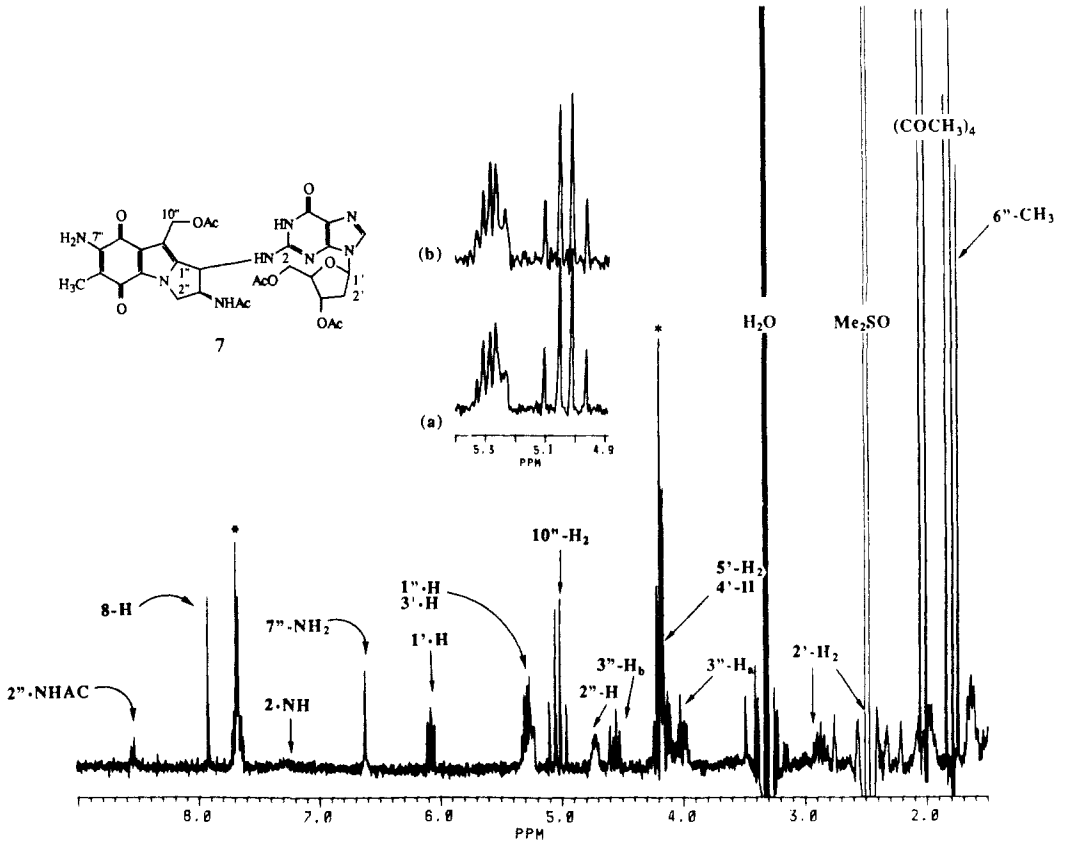
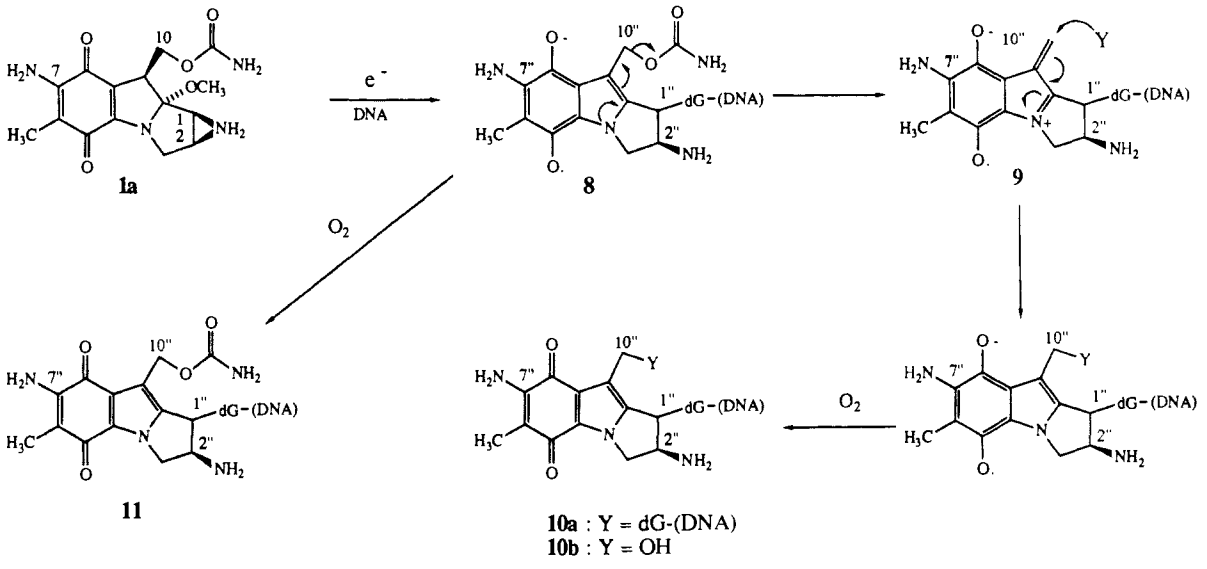


Figure 4. Resolution-enhanced proton magnetic resonance spectrum of **7** (purified by preparative TLC) in Me_2SO-d_6, 250 MHz. The peaks marked * were present in a control NMR spectrum of the elutant from elsewhere on the TLC plate. Inset: Expanded view of overlapping 1″-H and 3-H resonances; (a) before and (b) after decoupling of the 2-NH.

Scheme II. Mechanism of DNA Alkylation by MC[a]



[a] dG-(DNA) designates a deoxyguanosine residue of DNA linked at its N^2-position to the mitosene.

peak at 5.3 ppm (Figure 4, inset). A similar coupling was previously noted for the adduct **5**.[18]

These results establish conclusively the structure of the adduct **3a**. This work completes the identification of all major adducts formed between reductively activated MC and DNA, observed by us in vitro[17-19] (**2a**, **3a**, **4**) and in vivo[17,20] (**3a**, **4**).

Isolation of decarbamoylated adduct **3a** adds support to the proposed mechanism of DNA alkylation by mitomycin C (Scheme II).[17,28] Following reductive activation, it is thought that C-1 of the drug binds covalently to N-2 of a guanine in the minor groove to yield **8**. At this point, two fates are possible; **8** is either reoxidized (by excess MC in vitro or oxygen in vivo) resulting in monofunctional adducts, or its carbamate group is expelled to produce **9**. Furthermore, if a guanine is present at a binding distance in the complementary strand (fulfilled at GpC or CpG sequences)[17] 10″ alkylation may result (**10a**), producing a cross-linked double helix. Intrastrand cross-links at a GpG sequence are also possible. In such a scheme, the bisactivated drug **9** is attacked by water in all other sequences to produce **10b**, leading to **3a** (or **3b**) upon enzymatic digestion.

It is notable that DMC, an analogue of MC possessing strong antibiotic and cytotoxic activities itself,[21,29] is shown here to alkylate

(28) Tomasz, M.; Chawla, A. K.; Lipman, R. *Biochemistry* **1988**, *27*, 3182.

DNA, giving the same monofunctional adduct **3a** as the parent drug MC. However, DMC, unlike the parent drug MC, is apparently not capable of formation of the cross-link adduct **4**. Evidently, expulsion of the C-10″ hydroxyl group of DMC, required for formation of **4**, via intermediate **9** would be much less favorable than expulsion of the C-10″ carbamate from MC (Scheme II). Lack of DNA-cross-linking ability of DMC has been noted by others previously in vitro[31] as well as in vivo.[29-31] It should be interesting to compare the conformational and functional effects of the two drugs on DNA, in light of their differential covalent effects as shown by the present report.

Acknowledgment. Supported by NIH Grants GM 34509 (K.N.) and CA 28681 (M.T.), a PSC-CUNY Faculty Research Award (M.T.), and a Research Centers for Minority Institutions award RR-03037 from NIH (M.T.). We thank T. Doyle of Bristol-Myers for the generous gift of MC, J. Pawlak for mass spectral measurements, and G. L. Verdine for helpful discussions.

(29) Otsuji, N.; Murayama, I. *J. Bacteriol.* **1972**, *109*, 475.
(30) Fujiwara, Y.; Tatsumi, M.; Sasaki, M. S. *J. Mol. Biol.* **1977**, *113*, 635.
(31) Carrano, A. V.; Thompson, L. H.; Stetka, D. G.; Minker, J. L.; Marrimas, J. A.; Fong, S. *Mutat. Res.* **1979**, *63*, 175.

Communications to the Editor

1,3-Bis(*m*-nitrophenyl)urea: An Exceptionally Good Complexing Agent for Proton Acceptors

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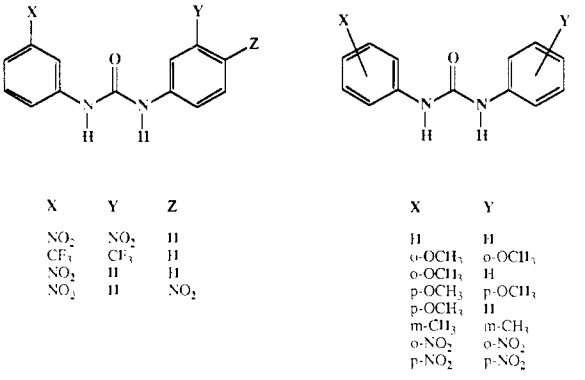
Received April 21, 1988

We have shown that intermolecular hydrogen bond patterns and molecular aggregate configurations of organic molecules can be controlled by the number and position of their hydrogen-bonding functional groups[1] since all the proton donors and acceptors are usually incorporated into the hydrogen bond network in the solid state.[2] Guest donors or acceptors are also incorporated if they compete with and displace the "normal" hydrogen bonding groups of the uncomplexed molecule. For example, triphenylphosphine oxide (TPPO), with one good proton acceptor (the phosphoryl group), complexes with amides, sulfonamides, and phenols and replaces the "normal" acceptor group of the host molecule.[3] This complexation process is a model for molecular recognition based on selective hydrogen bond interactions. It is also a useful way to design new solid-state materials since host molecules can be forced into many different solid-state environments by cocrystallizing them with different proton donor molecules.

Diaryl ureas contain one proton acceptor (the carbonyl group) and two N-H proton donors so their "normal" or expected hydrogen bond pattern is a chain of molecules with the urea carbonyl oxygen positioned between and bonding to both urea protons of a neighboring molecule, as found in the crystal structure of *N*,*N*′-diphenylurea.[4] We have found that guest acceptors will cocrystallize with 1,3-bis(*m*-nitrophenyl)urea, **1**, through hydrogen bonding between the N-H urea protons and the acceptor group of the guest which displaces the urea carbonyl groups in the hydrogen bond network. Even though urea carbonyl groups are reported to be reasonably good acceptors,[5] we find that nearly any acceptor is better than the carbonyl group in **1**. Compound **1** is a rare example of an organic molecule that behaves only as an intermolecular proton donor and not as an intermolecular proton acceptor.[6]



X	Y	Z
NO_2	NO_2	H
CF_3	CF_3	H
NO_2	H	H
NO_2	H	NO_2

X	Y
H	H
o-OCH_3	o-OCH_3
o-OCH_3	H
p-OCH_3	p-OCH_3
p-OCH_3	H
m-CH_3	m-CH_3
o-NO_2	o-NO_2
p-NO_2	p-NO_2

Figure 1. Diaryl ureas that form cocrystals with proton acceptors are shown in the left-hand column; those that do not, in the right-hand column.

Urea **1** has been reported previously to exist in three polymorphic forms (all obtained from ethanol solution).[7] When **1** is crystallized from solvents that are only proton acceptors, like acetone, THF, or DMSO, the crystals that form are cocrystal solvates of high quality, although they slowly turn cloudy when removed from the mother liquor. Likewise, when **1** is crystallized from solutions containing a third component which is a proton acceptor, like TPPO, *N*,*N*′-dimethyl-*p*-nitroaniline, or diethylene glycol, good quality cocrystals are also readily obtained.[8]

Through a combination of melting point, IR, NMR, and X-ray crystallographic techniques we have shown that **1** forms 1:1 cocrystals with a wide variety of proton acceptors to give complexes as shown.[9] The carbonyl stretching frequency of **1** varies from

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(1) (a) Etter, M. C. *Isr. J. Chem.* **1985**, *25*, 312-319. (b) Etter, M. C.; Urbanczyk-Lipkowska, Z.; Jahn, D. A.; Frye, J. S. *J. Am. Chem. Soc.* **1986**, *108*, 5871-5876. (c) Panunto, T. W.; Urbanczyk-Lipkowska, Z.; Johnson, R. B.; Etter, M. C. *J. Am. Chem. Soc.* **1987**, *109*, 7786-7797.
(2) Etter, M. C. *J. Am. Chem. Soc.* **1982**, *104*, 1095-1096.
(3) (a) Etter, M. C.; Rasmussen, J. K.; Gleason, W. B.; Kress, R. B.; Duerst, R. W.; Gillard, R. D. *J. Org. Chem.* **1986**, *51*, 5405-5408. (b) Etter, M. C.; Baures, P. W. *J. Am. Chem. Soc.* **1988**, *110*, 639-640. (c) Mori, K.; Stein, Z.; Goldberg, I. *J. Org. Chem.* **1988**, *53*, 308-312.
(4) Dannecker, W.; Kopf, J.; Rust, H. *Cryst. Struct. Commun.* **1979**, *8*, 429-432.
(5) Taft reports a value of 0.71 for β (the solvatochromic parameter for proton-accepting strength) compared to 0.55 for THF and 0.94 for triphenylphosphine oxide (Kamlet, M. J.; Abboud, J.-L. M.; Abraham, M. H.; Taft, R. W. *J. Org. Chem.* **1983**, *48*, 2877-2887).
(6) The nitro groups in **1** are potential proton acceptors (ref 1c), but they are not used as hydrogen bond acceptors in complexes we have studied. Other examples of compounds that are proton donors but not acceptors include the *m*-CF_3 and mono *m*-NO_2 analogues of **1**, 1,8-biphenylenediol (Hine, J.; Hahn, S.; Miles, D. E. *J. Org. Chem.* **1986**, *51*, 577-584), and fluorinated alcohols (ref 5).
(7) Groth, P. *An Introduction to Chemical Crystallography*; Wiley: New York, 1906; pp 29-30.
(8) The observation that crystal quality is better for cocrystals than for the pure substrate has been observed frequently in our lab (see, for example, ref 3b).

0002-7863/88/1510-5896$01.50/0