Full Structure of a Mitomycin C Dinucleoside Phosphate Adduct. Use of Differential FT-IR Spectroscopy in Microscale Structural Studies

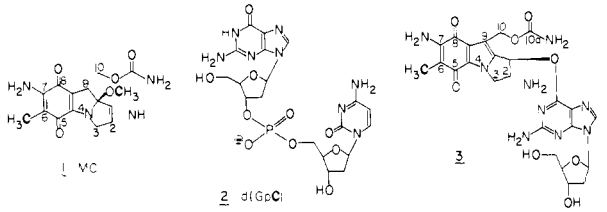
Maria Tomasz,*† Roselyn Lipman,† John K. Snyder,‡ and Koji Nakanishi*‡

Contribution from the Department of Chemistry, Hunter College, City University of New York, New York, New York 10021, and Department of Chemistry, Columbia University, New York, New York 10027. *Received August 6, 1982*

Abstract: Upon reductive activation by H_2/PtO_2 or microsomes/NADPH, mitomycin C reacts with d(GpC) to form several covalent adducts, each having a 1:1 molar ratio of drug to d(GpC). Enzymatic degradation of the mixture of mitomycin–d(GpC) adducts by snake venom diesterase yielded one major and several minor mitomycin–deoxyguanosine adducts. Their ultraviolet spectra were composites of 7-aminomitosene and guanine-type chromophores. The major adduct **3** was obtained in 500-μg yield in pure form. A combination of ^1H NMR, Fourier transform infrared (FT-IR), and circular dichroism (CD) spectroscopic techniques has established its structure as 1,2-*trans*-1-[O^6-(2′-deoxyguanosyl)]-2,7-diaminomitosene (**3**), indicating the aziridine-1 position of mitomycin C as the reactive electrophilic center. Application of the FT-IR difference method in this work demonstrates its value for microscale structure determination of DNA adducts, while the CD analysis offers a novel probe for assignment of the 1,2 stereochemistry of mitosenes in general. Mitomycin C provides the first example of a more complex drug selecting the O^6 position of guanine as its target in nucleic acid derivatives. The isolation and characterization of the adducts should facilitate analysis of mitomycin adducts formed directly with DNA in vitro and in vivo.

Mitomycin C (MC, **1**), an antibiotic and clinically used antitumor agent, interacts with DNA in vivo and in vitro, resulting in covalent linkage of the drug to DNA ("monofunctional binding") as well as in the formation of covalent cross-links between the two complementary strands.[1] These modifications of DNA are generally thought to be primary events in the antibiotic and antitumor activities of MC.[1] The drug requires acidic[2,3] or reductive[4] activation and has been termed the prototype of "bioreductive alkylating agents".[5] Despite its importance, the chemical nature of the modifications has not been previously determined.[6] It is known that high guanine and cytosine content of DNA promotes cross-link formation[2,4] and the monofunctional binding of MC to DNA and RNA is highly guanine specific,[8,9] indicating that guanine is the major or only linkage point of the drug to DNA. Adducts in which mitomycin C is linked to low-molecular-weight DNA constituents suitable for structure determination have not been isolated, with the exception of nucleotide adducts bound at the phosphate moiety.[3,6,10,11]

We report here the isolation and full structure of the major product **3**, which results from the reaction of mitomycin C (**1**)

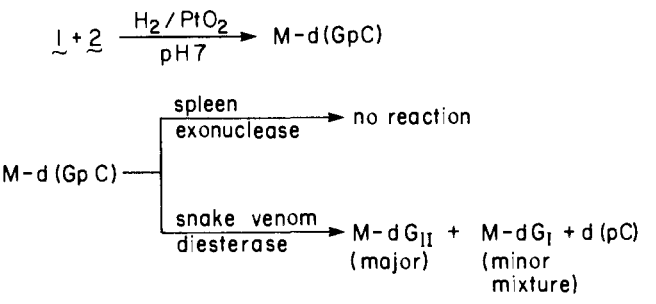


with d(GpC) (**2**) in vitro under reductive activation conditions and subsequent treatment of the adduct M–d(GpC) with snake venom diesterase (Scheme I). We chose this dinucleoside phosphate as the simplest model for a polynucleotide chain inasmuch as MC binding to DNA increases with increasing G–C base pair content and as a model suitable for observing the monofunctional binding of MC to guanine selectively.

Experimental Section

Materials. The materials used and their sources are as follows: mitomycin C, Bristol Laboratories, Syracuse, NY; 2′-deoxyguanylyl-(3′→5′)-2′-deoxycytidine (d(GpC)), Collaborative Research, Waltham, MA; exonuclease II (spleen diesterase) and snake venom diesterase, Worthington, Bedford, MA; Sephadex G-25 (fine) gel, Pharmacia, Piscataway, NJ; NADPH, Boehringer Mannheim, Indianapolis, IN; rat liver microsomes, a gift from Dr. Arthur Cederbaum, Mt. Sinai School of Medicine, New York, prepared and stored as described previously.[11]

Scheme I



Reaction of MC with d(GpC) under Reductive Activating Conditions. A. Catalytic Hydrogenation.[11] In a typical experiment, a mixture of MC (63 μmol), d(GpC) (89 μmol), and solid PtO_2 (130 μg/μmol MC) in 7 mL of 0.05 M Tris pH 7.5 buffer at room temperature was deaerated by bubbling helium, followed by reduction by bubbling hydrogen for 5 min and then helium again for 5 min. As a result, the blue color of MC changed to dark violet in the solution. The mixture was exposed to air, filtered, and chromatographed (see below).

B. Microsomal Reductions.[11] A mixture of MC and d(GpC) (same concentrations as above) and NADPH (1.05 μmol/μmol MC) in the above buffer was deaerated by helium and then rat liver microsomes (40 μg protein/μmol MC) were added, followed by incubation under helium

† Hunter College.
‡ Columbia University.

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(6) While this manuscript was in preparation, work concerning the nature of mitomycin C adducts with calf thymus DNA appeared.[7] Three adducts were isolated that showed alkylation occurring at the N^6 of adenosine and the O^6 and N-2 of guanosine.
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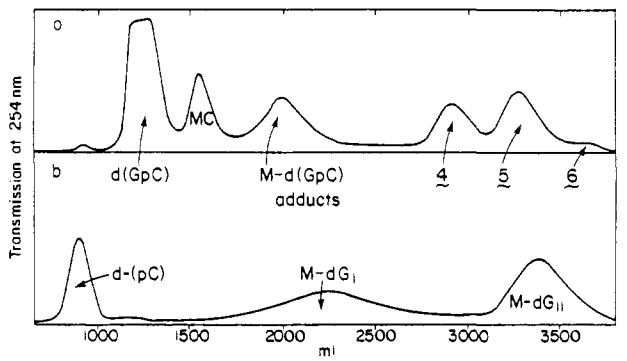


Figure 1. Sephadex G-25 column chromatography of (a) the reaction mixture of d(GpC) and MC reduced with H_2/PtO_2 and (b) the snake venom diesterase digest of M-d(GpC).

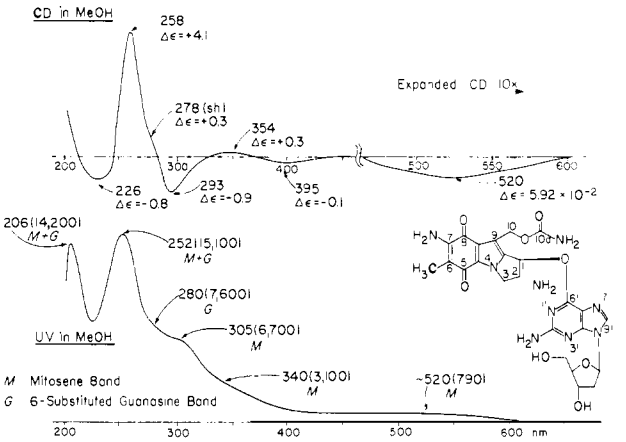


Figure 2. Ultraviolet/visible and circular dichroism spectra of **3** in methanol: (M) 7-aminomitosene band; (G) guanosine band.

for 15 min at 37 °C. After exposure to air, the mixture was filtered and immediately chromatographed as described below.

Separation of Products. Column chromatography was carried out with Sephadex G-25 (fine) gel and 0.02 M NH_4HCO_3 as eluant. This method has been shown to separate MC derivatives with good selectivity.[3]

HPLC separations utilized a Beckman Model 330 system, using reverse-phase Ultrasphere-ODS columns (0.46 × 25 cm for analytical and 1.0 × 25 cm for semipreparative purposes). Adduct **3** was purified on a semipreparative HPLC column (12.5:87.5 CH_3CN/0.03 M aqueous ammonium formate) followed by desalting on a short Sephadex G-25 column with 0.02 M NH_4HCO_3 as eluant and removal of the NH_4HCO_3 by lyophilization. The purple solid (**3**, 500 μg) was homogeneous on HPLC in three solvent systems (12.5:87.5 CH_3CN/0.02 M aqueous potassium phosphate, pH 5.1; same solvents, 8:92, respectively; 12.5:87.5 CH_3CN/0.03 M aqueous ammonium formate, pH 6.4).

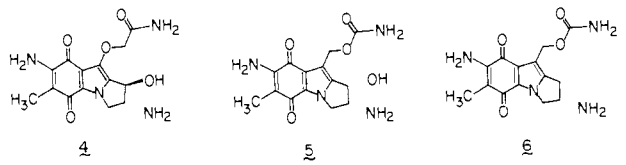
Quantitation of known nucleotide and MC derivatives were based on UV spectrophotometry as described previously.[3] Quantitation of nucleotide–MC adduct-type substances is based on ϵ_{312} 6030 (0.1 M potassium phosphate, pH 7.4), determined for purified **3**. This value was also used as a reasonable approximation for other adducts in this work that had the identical characteristic 7-aminomitosene chromophore above 300 nm.[12,13]

Enzymatic Digestions of the Adduct Fraction M–d(GpC). (a) Spleen exonuclease (0.10 unit) and M–d(GpC) (4 A_{310} units) in 1 mL of 0.01 M Tris, pH 6.7, were incubated at 37 °C for 2 h. (b) Snake venom diesterase (8 units) and M–d(GpC) (8 A_{310} units) were incubated in 1.3 mL of 0.005 M Tris–0.001 M $MgCl_2$, pH 8.5, at 37 °C for 3 h (Scheme I).

Spectroscopic Techniques. Proton nuclear magnetic resonance spectra were obtained with a Brüker WM-250 spectrometer. The spectra of adduct **3** were obtained by using 200 μg (0.35 μmol) in 0.3 mL of appropriate deuterated solvent (Me_2SO-d_6 and D_2O). The spectra of model aminomitosenes **4** and **5** were obtained with 320 μg (1 μmol) and 260 μg (0.81 μmol), respectively. The protonation of **3**, **4**, and **5** in the NMR tube (Figure 3) was carried out by adding appropriate amounts of 0.100 N HCl with a microliter pipet to a total of 2.1 equiv of acid (7.4 μL in the case of **3**). Two drops of D_2O were subsequently added to exchange the protons. Each spectrum is the Fourier transform of 4000 accumulations. Fourier transform infrared spectra and FT-IR difference spectra were obtained with an IBM IR/85 spectrometer. Circular dichroism spectra were obtained with a JASCO J-40 spectrometer. Intermolecular interactions in the FT-IR spectra were eliminated by dissolving the compounds (20–30 μg) with KBr in 20 mL of water followed by lyophilization to remove the water prior to making the pellet. This method had the effect of creating a solid solution to eliminate intermolecular interactions.[14] All FT-IR spectra are the Fourier transform of 100 accumulations. Subtraction spectra could not be obtained in the transmittance mode and were measured only in the absorbance mode. The coefficient (0.5) to weight the spectrum of **5** for the subtraction was optimized by nulling the mitosene band at 1500 cm^{-1}. This coefficient compared favorably with the molar ratio of **3**/**5** (namely, 0.47; Figure 4a, 25 μg of 0.044 μmol of **3**; Figure 4b, 30 μg or 0.094 μmol of **5**). Cary 219 and Gilford 250 spectrophotometers were used for ultraviolet spectra and absorbance determinations, respectively.

Results and Discussion

Isolation and Properties of Adducts. Reductive activation of MC by H_2/PtO_2[11] in the presence of d(GpC) resulted in the formation of M–d(GpC) adducts. This was evident from the analysis of the various fractions obtained upon Sephadex G-25 column chromatography of the reaction mixture (Figure 1a). These fractions were identified as follows: unreacted starting materials d(GpC) and mitomycin C, known conversion products **4**, **5**, and **6**[11] of mitomycin C formed during its reduction–oxidation



4 **5** **6**

cycle, and a new peak consisting of the M–D(GpC) adducts (see Figure 1a). The latter was resistant to spleen exonuclease, but after incubation with snake venom diesterase, it yielded deoxycytidine-5′-phosphate (d(pC)) and two other bands, M–dG_I and M–dG_{II} upon chromatography (Figure 1b, Scheme I). These last two had very similar ultraviolet spectra that appeared to be composites of the 7-aminomitosene[13] (e.g., as in **4** and **5**) and a guanine-type chromophore (Figure 2). Isolation of the d(pC) and mitomycin–dG fractions by G-25 chromatography demonstrates that mitomycin is linked to the deoxyguanosine moiety (Scheme I). The observed resistance of M–d(GpC) to spleen exonuclease but not to snake venom diesterase is consistent with this conclusion because the former enzyme recognizes the d(Gp-) portion while the latter recognizes the d(-pC) part. Selective resistance to the former therefore indicates alteration in the d(Gp-) moiety.

The yield of the crude M–d(GpC) adducts was 11–13%, based on original mitomycin. Snake venom diesterase treatment yielded approximately 50% M–dG_{II} and a much smaller amount (5–10%) of the M–dG_I fraction (Figure 1b). HPLC analysis of these crude M–dG adducts (12.5:87.5 CH_3CN/0.02 M KH_2PO_4, pH 5.1; flow rate 1.5 mL/min) indicated that M–dG_I is a mixture of several closely eluting components with similar UV spectra (not shown) while the M–dG_{II} fraction is more than 90% homogenous (retention time 3.6 min, adduct **3**), containing a small amount of another adduct (retention time 6.4 min) having a very similar UV spectrum.

The hydrolytic stability of M–dG_{II} was tested by heating at 100 °C for 20 min in aqueous buffer at pH 7.0. Approximately 80% was recovered unchanged as analyzed by Sephadex G-25 chromatography.

Mitomycin C was likewise activated by a rat liver microsomal NADPH system to yield a similar M–d(GpC) adduct mixture. Again, **3** was the major product after snake venom diesterase treatment, and its identity was rigorously established by mixed–

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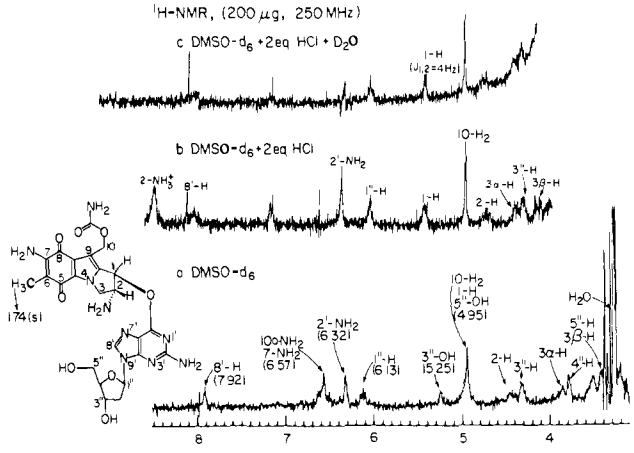

Figure 3. Proton magnetic resonance spectra of **3** (200 μg): (a) in Me_2SO-d_6; (b) after titration with 2.1 equiv of HCl (0.10 N, 7.4 μL); (c) after addition of 2 drops of D_2O.

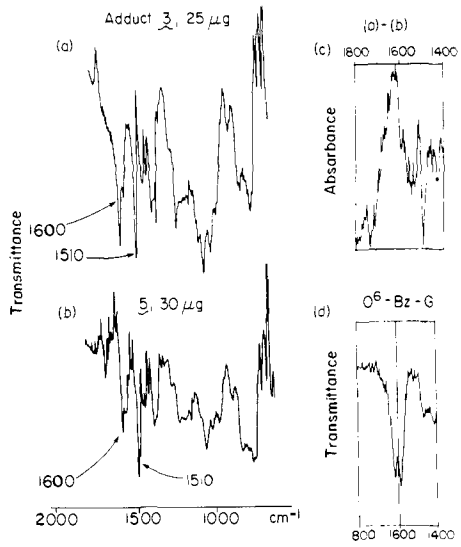

Figure 4. FT-IR spectra of (a) **3** and (b) **5** in KBr pellets prepared by lyophilization of an aqueous solution of **3** and **5** with KBr to remove crystalline effect in the solid state[14] (each spectrum is the Fourier transform of 100 accumulations); (c) their subtraction spectrum, 1800–1400-cm^{-1} region, in the absorbance mode (subtraction spectra could not be obtained in the transmittance mode); (d) FT-IR spectrum of O^6-benzylguanosine in transmittance mode[14] for comparison with (c). Differential IR measurements were carried out in detail with **5** (cis) rather than **4** (trans) since it was originally thought that the 1,2 stereochemistry was cis. Subsequent subtraction results with **4** in the 1600-cm^{-1} region are identical with that shown in this figure.

HPLC analysis. These results are consistent with our previous conclusion that the two activation conditions generate the same reactive form of the drug.[11] They show also, importantly, that **3** represents a product of biochemically activated mitomycin.

Structure of 3. The following structural studies were carried out on the 500 μg of sample **3** isolated as described in the Experimental Section. As shown in Figure 2, the UV/visible spectrum of **3** is a composite of 7-aminomitosene and substituted guanosine chromophores. Mitomycin C adducts are all known to be linked through the 1-position. Adduct **3** conforms to this well-known chemistry as shown by the ^1H NMR spectrum (250 MHz, 200 μg of sample in Me_2SO-d_6, Figure 3) in which the 1-H proton appears at δ 4.95 (overlapped by 10-methylene protons and the 5''-OH proton) and the 2-H proton appears as a broad multiplet at δ ~4.45.[15] These assignments were confirmed by the observed changes in the chemical shifts of 1–H and 2–H upon protonation at the 2-amino group to δ 5.43 (d, 4 Hz) and δ ~4.75 (m), respectively.

The ^1H NMR spectrum also readily showed that both the carbamate moiety at C-10, the site invoked to account for DNA cross-linking by mitomycin C[4,16,17] and the 7-amino group, which hydrolyzes to a hydroxyl function under certain aqueous conditions,[13] remained intact. Thus, these four amino protons appeared as two broad overlapping singlets at δ 6.57, a shift that compared well with the corresponding amino proton signals at δ 6.53 for both the trans and cis mitosenes, **4** and **5**. The appearance of the 6-methyl peak at δ 1.74, identical with the shifts of the 6-methyl groups in both **4** and **5**, further corroborates the presence of the 7-amino group.

The anomeric proton 1–H appeared as a broad[18] triplet at δ 6.13, and hence deribosylation had not occurred. Moreover, the following evidence reveals that the ribose 3''-OH of 5''-OH are both unsubstituted. The 3''-OH should be free because it was phosphorylated in the original adduct M–d(GpC), and the 3''-OH proton appeared at δ ~5.25 in the ^1H NMR spectrum. The 5''-methylene protons appeared as a multiplet at δ ~3.55 in the ^1H NMR spectrum taken in D_2O, a position unshifted from that in 2'-deoxyguanosine. In Figure 3, the 5''-OH appears at δ ~4.95 overlapped by the 10-methylene and 1-H signals while the 5''-methylene protons appear at δ ~3.55 in Me_2SO-d_6 (overlapped with H_2O peak). The 3''- and 5''-OH peaks of the deoxyribose moiety of 2'-deoxyguanosine in Me_2SO-d_6 appeared at 5.24 and 4.93, respectively.

The mitosene moiety must therefore be linked to the purine ring. The 8'-H signal at δ 7.92 and the 2'-NH_2 signal at δ 6.32 showed that these two positions are unsubstituted; furthermore, the N-7' position is also unsubstituted because the 8'-H signal is unshifted from that of 2'-deoxyguanosine (δ 7.92).[19] These observations indicate that the MC binding occurs through the O^6, N-1, or N-3 positions of guanine. Of these, N-3 was ruled out by the observed thermal hydrolytic stability of M–dG_{II} since N-3-alkylation would result in depurination under such conditions.[20] As the pK_a values of O^6- and N-1-substituted guanosines are nearly the same, i.e., 2.4 and 2.6, respectively, spectrophotometric titration monitored by either UV or circular dichroism spectroscopy[14] would not be expected to distinguish between these two alternatives.

Another method to distinguish the two substitution sites, an FT-IR microscale subtractive technique, was therefore explored.[14] The idea is to subtract the FT-IR spectrum of **4** or **5** from that of adduct **3**; the difference spectrum thus obtained should correspond to that of a specifically substituted guanosine. Both the mitosene and guanosine moieties are polar, and accordingly, the 2000–900-cm^{-1} region consists of strong absorptions due to both halves of the adduct (Figure 4, a and b). When the IR spectrum of mitosene **5** (Figure 4b, 30 μg or 0.094 μmol) was computer-substracted from the IR spectrum of adduct **3** (Figure 4a, 25 μg or 0.044 μmol), the difference IR showed a single broad band around 1600 cm^{-1} (Figure 4c, absorbance mode) that is characteristic of O^{16}-substituted guanosines (Figure 4d, transmittance mode).[14] The subtraction was carried out by estimating the subtraction weighting factor of the spectrum of **5** from the molar quantities of compounds **3** and **5** contained in the two KBr pellets (see Experimental Section). This subtraction gave the difference spectrum (Figure 4c) in which the strong 1510-cm^{-1} band of mitosene **5** had been more or less nullified. It should be noted that despite the presence of a 1600-cm^{-1} band in both adduct **3** and compound **5** (Figure 4, a and b), the difference spectrum (Figure 4c) still shows a strong absorption at ca. 1600 cm^{-1} that is diagnostic for the O^6-substituted guanosine moiety. The point of linkage on the purine ring is thus at the O^6 position.

The remaining point to be clarified in the structure of adduct **3** is the stereochemistry at C-1. It was originally hoped that the

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(16) Moore, H. W. *Science (Washington, D.C.)* **1977**, *197*, 527.
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(18) In Me_2SO-d_6, all signals were broadened.
(19) The H-8 signal of 7-methylguanosine is shifted downfield to 9.14 (in Me_2SO-d_6) due to N-7 quaternization.
(20) Lawley, P. D.; Shah, S. A. *Chem.-Biol. Interact.* **1973**, *7*, 115.

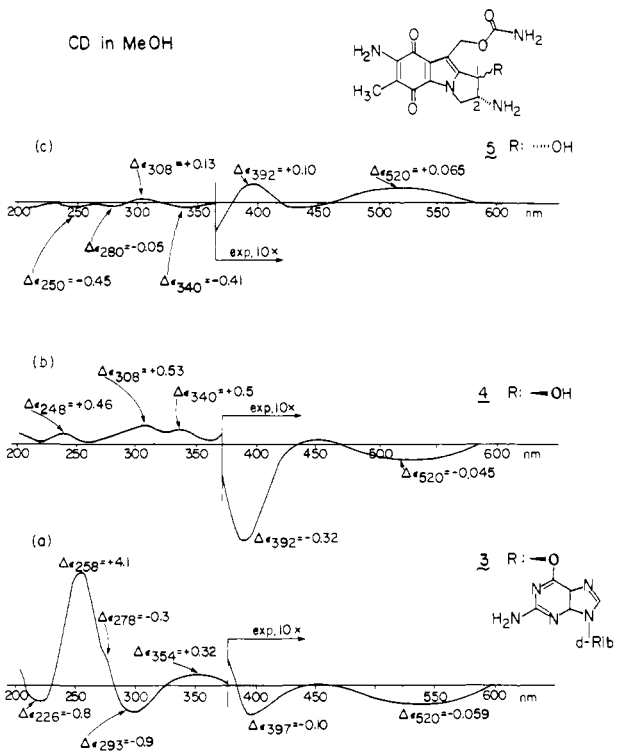

Figure 5. Circular dichroism spectra of (a) **3**, (b) **4**, and (c) **5** in methanol.

displacement of the 1-H NMR signal upon protonation of the 2-NH_2 group would establish the stereochemistry by comparing the displacements observed for models **4** and **5**. Thus, a titration of the adduct to a total of 2 equiv of 0.10 N HCl in the NMR tube was undertaken.[21] Downfield shifts of the 1-H proton signal of δ 0.45 for **3**, δ 0.47 for the trans compound **4**, and δ 0.52 for the cis compound **5** were observed. Although this displacement comparison may favor a trans stereochemistry, the overlap of the 1-H with the 10-H_2 and 5''-OH protons in the original adduct spectrum (Figure 3a, broad peak at δ 4.95) and the unknown anisotropic effect of the guanosine moiety render this approach unsuitable. Elucidation of the C-1 configuration was then sought from $J_{1,2}$ coupling constants. A $J_{1,2}$ value of 4 Hz could be measured from the spectrum shown in Figure 3c obtained upon further addition of 2 drops of D_2O (this had a peak-sharpening effect). However, the general range of $J_{1,2}$ coupling constants observed for trans-1,2-mitosene derivatives is only ca. 1 Hz less than that observed for the corresponding cis-1,2 compounds;[15] furthermore, the danger of using $J_{1,2}$ values in assigning the C-1 stereochemistry of 2-aminomitosene derivatives[15] and in 1,2-disubstituted indans[22] has already been noted. In conclusion, the C-1 configuration could not be determined from NMR data.

The stereochemistry of the adduct was finally determined to be trans from analyses of the CD spectra of models **4**/**5** and adduct **3** (Figure 5). The CD curve of adduct **3** shows a straightforward correlation with either that of **4** or **5** in the UV region due to dominance of the strong Cotton effect at 258 nm. As shown in Figure 2, the mitosene and guanine chromophores both have absorptions in the 200–300-nm region, and hence the intense CD Cotton effects in adduct **3** in the region below 400 nm can certainly be ascribed to coupled-oscillator-type interactions.[23] However, since the direction of electric transition moments for the two interacting chromophores are unknown and the conformation of the guanosine moiety relative to the mitosene chromophore is not clear, the coupled CD extrema cannot be used for absolute configurational studies.

Figure 1 shows that there is a weak but distinct absorption around 520 nm (ϵ 790) that is responsible for the purple color of the adduct and that is accompanied by a CD Cotton effect; this arises from the mitosene chromophore. Although absorption bands as far apart as 120 nm can interact to give rise to coupled-oscillator-type CD Cotton effects,[23] the 520-nm mitosene transition is too far removed to be coupled to other guanosine transitions. Furthermore, Figure 5, b and c show that in *trans*-**4** and *cis*-**5** the two CD bands at 520 nm are of similar amplitudes but of opposite signs; since the only difference between **4** and **5** is the configuration of the 2-hydroxyl group, it can be concluded that *the sign of the 520-nm CD curve represents the C-1 configuration.* In adductive **3** this CD (Figure 5a) is *negative* and again its amplitude is of the same order of magnitude as those of **4** and **5**, i.e.; it is not coupled to guanosine transitions. This establishes the C-1 configuration and structure of **3** as represented by the trans structure. This analysis also demonstrates that the 520-nm CD sign of mitosene derivatives represents a general method for determining the configuration at C-1 for which there previously has been no definitive solution.

Significance

Application of the FT-IR difference method (Figure 4) could prove to be a valuable tool for microscale structure determination of molecules exemplified by DNA adducts. Namely, this method is applicable to molecules of the type A–B, where B, such as the guanosine portion in **3**, has several sites that could be involved in its bonding to A. A comparison of the FT-IR difference spectrum resulting from the subtraction of A from adduct A–B with authentic IR spectra of B derivatized at different sites can then lead to determination of the linkage site; it should be particularly advantageous in cases where ^{1}H NMR studies are restricted either due to scarcity of material or due to lack of analyzable protons in moiety B.

The CD analysis in the 520-nm region of mitosene derivatives has shown that the C-1 configuration is β (or *S*, as in **4**) when the sign is negative and α (or *R*, as in **5**) when it is positive (Figure 5). This solves the historically nettlesome problem of assigning C-1 stereochemistry in mitosene derivatives from $J_{1,2}$ values, which have continued to be unreliable.

The O^6 position of guanine as the major target of alkylation of d(GpC) by activated mitomycin C is most interesting. This position is not appreciably reactive under physiological conditions in nucleic acids toward the more common alkylating agents, for example, dimethyl sulfate, alkyl methanesulfonates, sulfur and nitogen mustards, or ethyleneimine. It is alkylated, however, by *N*-alkyl-*N*-nitroso compounds,[24] e.g., *N*-methyl(ethyl)-*N*-nitrosourea[25] or *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine,[26] which are broadly categorized as S_N1-type alkylating agents.[27] This analogy suggests that the C-1 center possesses carbonium ion type characteristics in the reactive intermediate formed upon the reduction of mitomycin.

Although most of the more complex drugs that bind covalently to DNA select guanine as their preferred target, mitomycin C provides the first example of this group where reactivity toward the O^6 position has been detected.[7] Thus, for example, aflatoxin[28-30] binds at N-7, anthramycin,[31] polynuclear aromatic hydrocarbon epoxides,[32,33] at N-2, and *N*-acetylacetoxyamino-

(21) One equivalent of HCl would have sufficed to selectively protonate the 2-NH_2 group, but this resulted in broadening of the proton signals; addition of 2 equiv sharpened the H-1 signal.

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fluorene[34] at C-8. The O^6 position of guanine as the main point of linkage of mitomycin C to nucleic acids has long been a subject of speculation, based on various indirect evidence.[1,8] It has now been shown that the O^6-substituted adduct is the major product with the dinucleoside phosphate d(GpC). Its method of isolation and characterization will facilitate the search for this and other mitomycin adducts in nucleic acids in vitro and in vivo. It is notable that **3** represents a product of monofunctional alkylation by activated mitomycin at its aziridine function. The nature of a second minor alkylating function, assumed because of the presence of cross-links in mitomycin-treated DNA,[4] remains unknown.

Acknowledgment. We thank Dr. W. T. Bradner, Bristol Laboratories, Syracuse, NY, for a gift of mitomycin C, Dr. Arthur I. Cederbaum, Mt. Sinai School of Medicine, for supply of rat liver microsomes, and Dr. D. Cozart for assistance in FT-IR difference measurements. This work was supported by a PSU–CUNY Faculty Research Award and grants from the National Cancer Institute, CA 28681 (to M.T.) and CA 11572 (to K.N.).

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A Catechol Receptor Model by Macrocyclic Polyamines

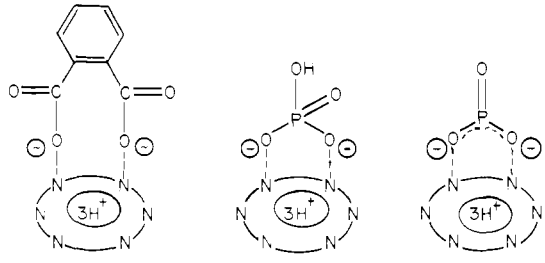
Eiichi Kimura,*[1a] Asuka Watanabe,[1a] and Mutsuo Kodama[1b]

Contribution from the Department of Medicinal Chemistry, Hiroshima University School of Medicine, Kasumi, Hiroshima 734, Japan, and the Department of Chemistry, College of General Education, Hirosaki University, Bunkyo, Hirosaki 036, Japan. *Received July 14, 1982*

Abstract: 18-Azacrown-6 (L) (as triprotonated species) has been shown to form stable 1:1 complexes with catechol (**1**) and its biological derivatives **2–8** in neutral pH solutions. Their stability constants β_L were determined polarographically. The catechol receptor model L interacts with Dopa and dopamine with a similar order of β_L to indicate that the residual donor groups on the catecholamines do not significantly contribute to the complex formation. L has similar affinities to methyl ether derivatives of catechols. Further, L associates with an adrenergic blocking agent, dichloroisoproterenol (**10**). It also recognizes other drugs or drug functions such as resorcinol (**9**), tropolone (**11**), salicylic acid (**12**), *p*-aminosalicylic acid (**13**), and α-picolinic acid (**14**).

The catechol group is essential for biological activities in number of biogenic amines and their relevant drugs. The pharmacological studies have undeniably proved the presence of the catechol recognition and binding sites in biological systems. However, entities of the catechol receptors (like those of other receptors) remain almost unknown. So far few pictures have been proposed concerning the chemistry of the catechol recognition. Small molecular compounds having the efficient and selective catechol receptor functions would be very useful not only in the chemical elucidations but also in biological and medicinal applications. However, without precedent there had been no way of designing them.

We have shown that highly protonated macromonocyclic polyamines such as 18-azacrown-6 (as $3H^+$ species) make good



anion receptors (at neutral pH) for organic and inorganic oxygen anions, including polycarboxylates (e.g., citrate, succinate, *o*-phthalate, etc.),[2] phosphates (inorganic phosphate, AMP, ADP, and ATP),[3] and carbonate.[4] These polyoxyanions probably associate by ionic hydrogen bonds with the polyamine protons packed in the macrocyclic cavities, yielding highly stable and selective 1:1 anion complexes in aqueous solutions as postulated below.

Then, it occurred to us that the *o*-dihydroxy group of catecholamines might be a good donor to macrocyclic polyamine cations. The interaction has been examined with various polyamines by the anodic polarographic technique that we had previously used for the study of the macrocyclic polyamine complexes with polyoxyanions.[2-4] We have now found that 18-azacrown-6 (L) is indeed a strong receptor of catechol (**1**) and catecholamine derivatives **2–4** (see Figure 1). Our receptor model L moreover works on O-methylated catechols **5–8**. Interestingly, L further binds with drugs or drug functions **9–14**, some of which are recognized by biological catecholamine receptors.

Experimental Section

Materials. The catechol derivatives and other chemicals purchased were purified or were reagent grade. The macrocyclic hexaamine 18-azacrown-6 (L) was synthesized and purified as a 6HCl salt.[5] The purity was checked by TLC and gas chromatography techniques.[6]

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