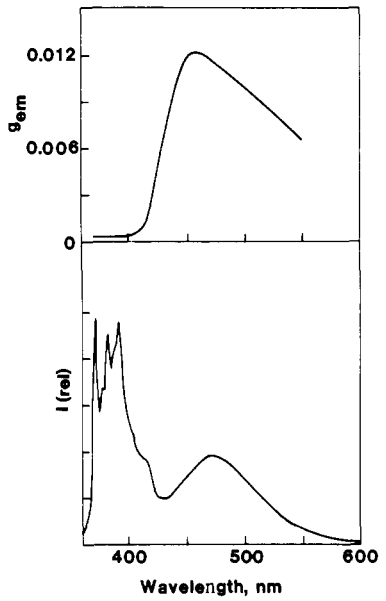

Figure 1. Fluorescence (bottom) and CPF (top) spectra of pyrene (2×10^{-6} M) in aqueous γ-CDx (1×10^{-2} M) solution at 25 °C.

toluene and dimethylformamide.[22] This also suggests that the large g_{em} in the present system is neither due to an artifact caused by a linear polarization nor due to an electronic perturbation by the γ-CDx cavity on an achiral excimer. The electronically induced CPF has been reported for the systems of an achiral fluorescer (fluorescein) in a chiral solvent (phenetylamine)[23] and a 1:1 inclusion complex of fluorescein and β-CDx.[24] The $|g_{em}|$ values for the electronically induced CPF, however, are significantly smaller (10^{-4}-10^{-3}) than that for the present system (0.0071-0.012). Consequently, it can be concluded that the pyrene molecules in the γ-CDx cavity form the excimer having an asymmetrically twisted configuration.

The intramolecular excimer systems can be regarded as the model guests for studing the 2:1 = guest:CDx complexes.[25-27] We used 1,3-bis(1-pyrenyl)propane (P(3)P). Since P(3)P is completely insoluble in water, a dilute aqueous dispersion (10^{-6} M) shows only excimerlike fluorescence centered at 478 nm due to the P(3)P aggregates, which shows a multiexponential fluorescence decay.[28] In the presence of γ-CDx (10^{-2} M), however, the fluorescence from the locally excited state of P(3)P was observed along with the intramolecular excimer fluorescence with a maximum intensity at 490 nm. The fluorescence decay of the P(3)P excimer was single exponential (τ_f = 159 ns) in the presence of γ-CDx and no rise of the excimer was observed, suggesting that the intramolecular dimer state is formed in the γ-CDx cavity in the ground state. A relatively weak CPF signal was observed for the P(3)P-γ-CDx system, g_{em} being ca. +0.003 at the excimer region.

Recently, an induced circular dichroism (ICD) of the pyrene dimer formed in the γ-CDx cavity has been reported.[18] A very small absorption dissymmetry factor of ICD (ca. 6×10^{-5} at the ^1L_a band)[29] indicates a weak asymmetric nature of the pyrene dimer in the γ-CDx cavity in the ground state. Upon excitation of a pyrene molecule, the reorientation of two pyrene molecules may occur in the γ-CDx cavity to form thermodynamically metastable excimer state. In fluid media, the pyrene excimer takes a sandwichlike configuration. In the γ-CDx cavity, however, the sterically restricted environment may not allow formation of a symmetric sandwich excimer. An asymmetrically twisted configuration seems to be taken under the conditions. In the case of P(3)P, an alkyl chain, which links two pyrenyl groups, seems to prohibit the configurational change of P(3)P included in the γ-CDx cavity. This may cause the smaller g_{em} value and the absence of the rise of the intramolecular excimer fluorescence. No wavelength dependence of the g_{em} value over the whole excimer region was observed for the P(3)P-γ-CDx system, suggesting only one fluorescent state which shows CPF. As Figure 1 shows, however, the g_{em} value for the pyrene-γ-CDx system decreases at longer wavelength. There may be two fluorescent states, one is the excimer state and another is the dimer excited state, whose configuration is almost the same as that of the ground-state dimer of pyrene in the γ-CDx cavity. The rise of the pyrene excimer fluorescence observed in the decay curve may be ascribed to the dynamic formation of the excimer state in the γ-CDx cavity which seems to be a considerably tight environment for two pyrene molecules.

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Use of Differential Second-Derivative UV and FTIR Spectroscopy in Structural Studies of Multichromophoric Compounds

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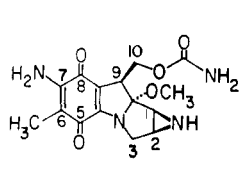
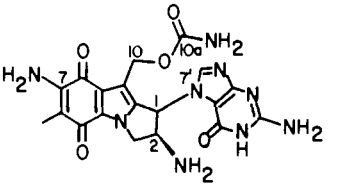
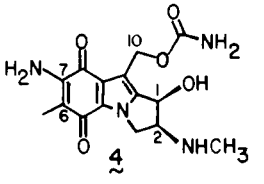
Mathematical second derivatization of absorption spectra has primarily been used as a tool for enhancing resolution or clarifying maxima in strongly scattering samples. However, the technique can be exploited to a much greater extent, especially when coupled with subtractive methods. This is shown by the usage of differential second-derivative UV (differential SEDUV) and FTIR (differential SEDIR) spectroscopy in the microgram-scale structure determination of complex molecules exemplified by the mitomycin C (MC, **1**)/guanine adducts **2** and **3**. The method



MC **1**



2 M-guanine A, Iα-
3 M-guanine B, Iβ-



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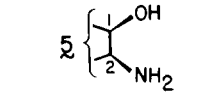

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described can be performed on most computerized UV and IR spectrometers without weighing of samples and should be generally applicable to a wide variety of problems.

Treatment of MC with the dinucleoside phosphate d(GpC) under acidic conditions yields a complex mixture from which were isolated two major adducts M-guanines A **2** and B **3**[1] (M denotes

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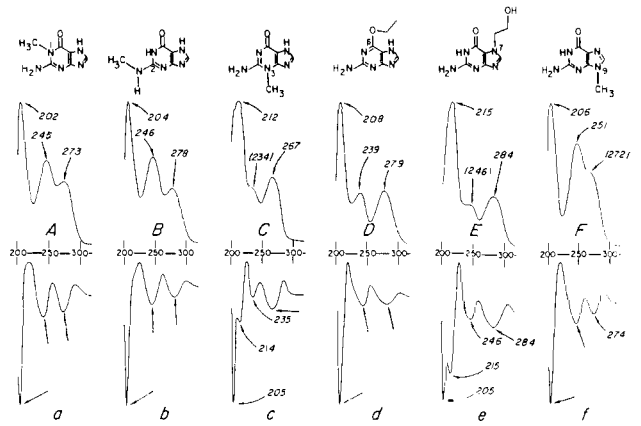

Figure 1. UV of guanines, 0.1 M K phosphate, pH 7.0. Arrows denote peak positions in nanometers; parenthesized numerals denote shoulders: (A–F) absorption spectra; (a–f) second-derivative (SEDUV) spectra.

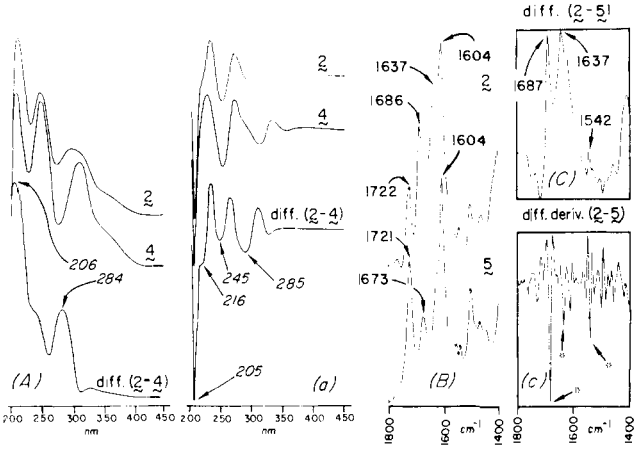

Figure 2. (A) UV of adduct **2** and mitosene **4** and differential spectrum of (**2** – **4**). (a) SEDUV of **2** and **4** and differential SEDUV of (**2** – **4**). (B) FTIR of adduct **2** and mitosene **5**. (C) Differential FTIR of (**2** – **5**). (c) Differential SEDIR of (**2** – **5**). Asterisked peaks in (c) correspond to labeled peaks in (C).

mitosene[2]). ^{1}H NMR clarified the structure of the mitosene moiety but gave very limited information on the guanine portion due to the lack of usable proton signals other than 8′-H. The usage of UV in determining the point of attachment of the mitosene to the guanine moiety was therefore explored. As shown in Figure 1, the UV spectra of alkylated guanines do differ according to the substitution site but are not sufficiently characteristic to serve as fingerprints, especially when subtraction of strongly absorbing chromophores are involved (see below). In contrast, the SEDUV spectra (Figure 1a–f)[3,4] show greatly enhanced resolution as seen by clarification of shoulders (compare Figure 1, curves C/c, E/e, F/f) and resolution of overlapping bands into two peaks (Figure 1, curves C/c, E/e). Increase in derivative order leads to further enhanced resolution but also to reduced S/N and hence the second derivative is the best compromise in most cases.

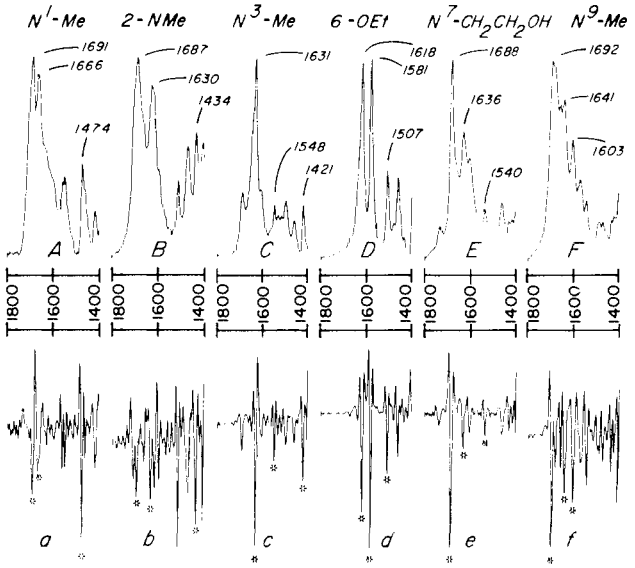

Figure 3. FTIR of guanines, Me_2SO-d_6. The three major characteristic frequencies are shown in reciprocal centimeters (A–F) and by asterisks (a–f): (A–F) absorbance spectra; (a–f) second derivative (SEDIR) spectra.

The absorption spectrum of M-guanine A adduct **2** (Figure 2A) is a summation of mitosene bands at 245 (ϵ 14 400), 309 (10 000), 350 (3200), and 530 nm (700) and substituted guanine bands at 204–215 (ϵ 15 000), 234–246 (10 000), and 267–284 nm (8000). Computer-assisted subtraction of the spectrum of model mitosene **4** from adduct **2** using the mitosene 350-nm absorbance as the normalizing wavelength leads to two (**2** – **4**) difference spectra, Figure 2A in the normal mode and Figure 2a in the SEDUV mode. Comparison of the difference spectra with authentic sets Figure 1, curves A–F and a–f, shows they match the spectra of *N*7-(hydroxyethyl)guanine (Figure 1E/e). However, the agreement between the difference curve in Figure 2A with maxima at 206/284 nm and reference curve Figure 1E with maxima at 215/284 nm is not convincing. In contrast, agreement between the SEDUV set is excellent: 205/216/245/285 nm (Figure 2a) vs. 205/215/246/284 nm (Figure 1e). Analysis of spectra of adduct M-guanine B **3** leads to the same conclusion. The subtractive technique where difference curve (**2** – **4**) is compared with the spectra of reference guanines is valid since (i) the exciton coupling between two identical chromophores shifts the peaks maximally by 6 nm;[6] in adducts **2**/**3** the shift is much less because the two chromophores are different, and (ii) the ionic conditions of measurements preclude the possibility of guanine peaks in adducts **2**/**3** being shifted by an intramolecular H bond. The 1α- and 1β-configurations were determined by the CD method, **2**, 525 nm ($\Delta\epsilon$ −0.081) and, **3**, 520 nm ($\Delta\epsilon$ +0.112).[7]

We previously showed that differential FTIR using a lyophilized KBr technique offered a powerful method for determining substitution positions on purine bases.[7a,8] As demonstrated below, resolution is greatly enhanced and shoulders became clear bands when solution spectra taken in an attenuated total reflectance CIRCLE microcell[9] are recorded as second derivatives. Figure 3A–F shows regular absorbance spectra[10] of substituted guanines whereas Figure 3a–f shows the corresponding second-derivative spectra.

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(2) "Mitosene" refers to the structure as in **4** without substituents in 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.; Fulmore, W.; Pidacks, C.; Lancaster, J. E. *J. Am. Chem. Soc.* **1962**, *84*, 3185.

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(5) If the spectra measured at pH 7 are not sufficiently different to be of diagnostic use, Figure 1 curves A/a vs. B/b, differentiation can be achieved by measurements at different pH's.

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(10) IBM IR-85 equipped with an MCT detector, 2-cm^{-1} resolution, in Me_2SO-d_6, using an ATR CIRCLE microcell[9] fitted with a ZnSe rod, and corrected for solvent and H_2O vapor. Each spectrum is the Fourier transform of 2500 accumulations. SEDIR spectra were calculated on the digitized absorbance files using a smoothing factor of −7.

The 1800–1400 cm^{-1} region FTIR of adduct **2** (Figure 2B, curve **2**) consists of bands due to the mitosene and guanine moieties. The mitosenes exemplified by **5** exhibit a ca. 1722-cm^{-1} band due to the carbamate group (Figure 2B, curve **5**) but this region is completely transparent in guanines. Weighted subtraction of the spectrum of **5** from that of **2** using the 1722-cm^{-1} band as subtraction marker thus produces the difference spectrum Figure 2C which is composed of guanine-related bands. The peaks in curve C of Figure 2 compare most closely with the FTIR 3 *N*7-(hydroxyethyl)guanine,[11] curve E, Figure 3, but the conclusion is not unambiguous. In contrast, a comparison of differential SEDIR curve in Figure 2c with curves in Figure 3a–f shows clearly that the three asterisked peaks together with a few others in curve c in Figure 2 only match those of Figure 3e.

The above-mentioned examples demonstrate that diff. SEDUV and differential SEDIR offer extremely powerful micromethods for structural studies of molecules containing two (or more) nonconjugated moieties. While the present example is rather specific, we believe that the additional information gained from second derivatization should make it an attractive technique in a broad range of investigations.

Acknowledgment. We are grateful to Prof. M. Tomasz, Hunter College, CUNY, New York, for a supply of samples and discussions, Dr. Reiji Takeda, Suntory Foundation for Bioorganic Research, Osaka, for discussions, and NIH Grant GM 34509.

Supplementary Material Available: Expanded scale FTIR and SEDIR spectra for all compounds; FTIR and SEDIR spectra of M–guanine B (9 pages). Ordering information is given on any current masthead page.

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Nature of the Destruction of Deoxyguanosine Residues by Mitomycin C Activated by Mild Acid pH

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Alkylation of genomic deoxyguanosine residues at the 7-position has been implicated as the primary event in the chemically induced carcinogenesis/mutagenesis resulting from exposure to agents such as aflatoxin B_1,[1,2] N-mustards, and dimethyl sulfate.[1] The modified deoxyguanosine residues produced by 7-alkylation decompose readily via (i) loss of the ribofuranoside moiety to form apurinic DNA sites or (ii) imidazolium-ring scission to generate *N*-formamidopyrimidine (NFP) DNA bases (see below). With respect to the latter case, while numerous studies have been conducted on NFP-ribosides, including DNA, their exact structures have continued to elude definition.[1,2]

We wish to report that mitomycin C[3] (MC, **1**), the clinically used antitumor antibiotic, alkylates d(GpC)[4] and DNA in vitro

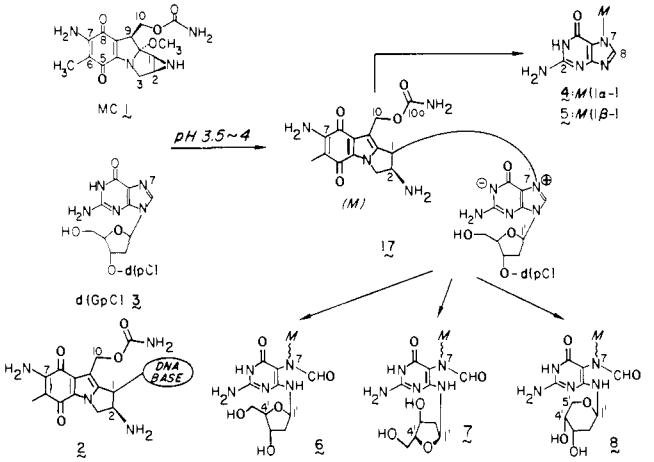

Figure 1. Products resulting from MC (**1**) and d(GpC) (**3**). Intermediate **17** has not been isolated. *M* in **4–8** denotes the mitosene moiety.

at the guanine 7-position under acidic activation.[5] This finding raises the possibility that MC activation in vivo may well be accomplished by protonation,[6] the other possibility being the well-known (in vitro) reductive activation of MC.[7-9] Furthermore, we report the full structures of the elusive imidazolium-cleaved NFP moieties generated from base-treated 7-ethylguanosine (**9**) (Figure 2). On the basis of the latter model studies, we have also characterized the NFP-deoxyribose–mitosene **6–8** system shown in Figure 1.

An aqueous solution of 18 μmol of d(GpC) (**3**) acidified to pH 3.5–4.0 with Bio-Rad AG 50-W-X-B beads was treated with MC (20 μmol) and the solution adjusted to 2.5 mL with water and maintained at pH 3.5–4.0 by additions of 0.01 N HCl. The solution was neutralized after 3.5 h, room temperature, and chromatographed on Sephadex G-25[9] to give adduct fractions designated M–guanines and M–d(GpC); digestion of M–d(GpC) with snake venom diesterase gave M–dG_I (major) and M–dG_{II} (minor). The latter, the major product under reductive conditions, consists of type **2** mitosene[10] 1α- and 1β-adducts to the O^6 of dG.[9]

The M–guanines were separated by HPLC, Ultrasphere ODS, MeCN/0.02 M aqueous KH_2PO_4 (pH 5.0), 8/92, into **4** and **5** (Figure 1). The ^1H NMR in Me_2SO-d_6 showed three exchangeable 2 H signals assigned as follows by comparison with 2β,7-diamino-1α-hydroxymitosene and guanine: 6.74/6.58 (**4**) and 6.52/6.36 ppm (**5**), 10a-/7-NH_2; 6.20 (**4**) and 6.18 ppm (**5**), guanine 2-NH_2 (mitosene 2-NH_2 signals are broad and rarely observed). The guanine 8-H's were observed at 7.91 (**4**) and 7.46 ppm (**5**). The rest of the spectra showed typical mitosene peaks;[9] **4** and **5** are thus type **2** adducts. The linkage of the mitosene group to N-7 was determined by differential second-derivative UV and FTIR spectroscopy.[11] Finally, the CD of **4**, $\Delta\epsilon$ −0.081 at 525 nm, and **5**, $\Delta\epsilon$ +0.112 at 520 nm show them to be 1α- and 1β-adducts, respectively.[9]

HPLC (conditions same as above) of 200 μg of M–dG_I gave an ill-resolved four-peak pattern which was reproduced upon reinjection of any single peak; M–dG_I is therefore a mixture of interconverting compounds. The ^1H NMR showed nonex-

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‡ Columbia University.

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