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(13) The final basis sets follow: for Zn, 12s, 6p, 4d → 6s, 4p, 3d; for the first row atoms, 7s, 4p → 3s, 3p; and for hydrogen, 4s → 2s. With the program IBMOL H a single computation on the complex $ImH-Zn^{2+}$ takes 34 min of CPU time on an IBM 370/168.
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Structures of 7,12-Dimethylbenz[*a*]anthracene 5,6-Oxide Derivatives Linked to the Ribose Moiety of Guanosine

Sir:

7,12-Dimethylbenz[*a*]anthracene (DMBA) is one of the most potent carcinogens which requires cell mediated activation before it can react with cellular macromolecules.[1] The K-region oxide (DMBA 5,6-oxide) has been implicated as one of the possible intermediates in the carcinogenic and/or mutagenic process.[2,3] Since chromatographic mobilities of the four known guanosine-DMBA 5,6-oxide adducts[4] formed under pH 5-6 did not coincide with the rat liver tissue culture products, we have prepared six other adducts G*-**1a**, -**1b**, and II-V by reacting the oxide with guanosine in acetone-water (2:1) at pH 9.5. About 15% of the guanosine reacted to yield in decreasing amounts G*-**1a**, III, II, G*-**1b**, IV, and V, which were isolated and purified on a Sephadex LH-20 column followed by HPLC.[4,5] Comparisons of the chromatographic behavior of the products with those isolated from the RNA of rat liver cells treated with $[^3H]$-DMBA showed that three of the products, G*-**1a**, -**1b** and II, which constituted less than ~10% of the total nucleoside-$[^3H]$-DMBA adduct, were detected in the cell culture.[5]

Structural studies of G*-**1a** and -**1b** (carried out on ~1 mg of each) showed that they can be expressed by **1** (or **3**) and **2** (or **4**), which, unlike other arene oxide and diol epoxide-nucleic acid base adducts identified so far,[4,6-8] are characterized by a unique ribose-DMBA link. Moschel et al.[9] have recently

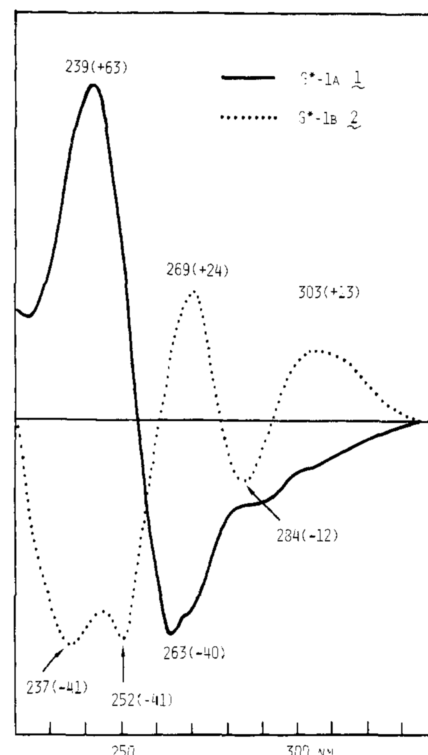


Figure 1. CD in 5% aqueous MeOH, extrema in nanometers (Δε).

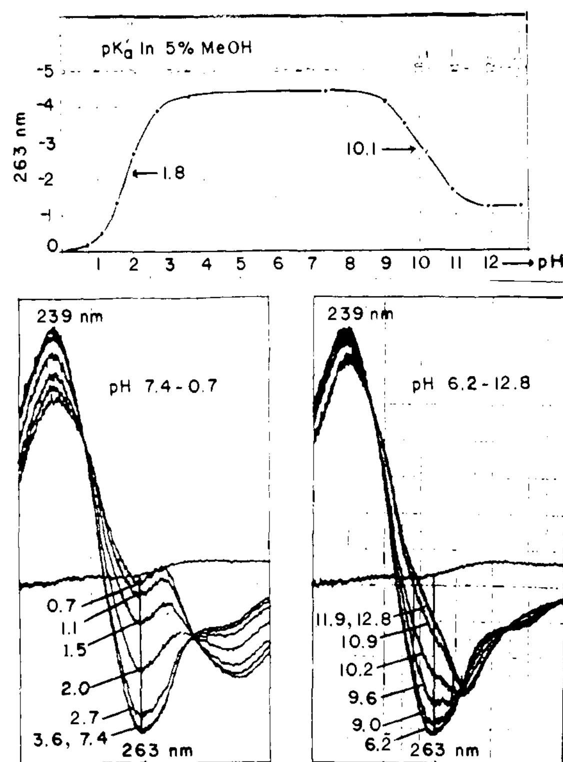


Figure 2. Change in CD of adduct **1** and plot of CD $\Delta\epsilon_{239}$ with pH, in 5% aqueous methanol. Owing to the instability of **1** to acidic conditions, the initial solution was neutralized to pH 7.4 and acidified to pH 0.7. A JASCO J-40 instrument was used.

shown by fluorescence methods that the binding of DMBA to DNA mostly occurs after metabolic activation in the angular ring rather than the K region (C-5, C-6). The differences could possibly be attributed to different experimental procedures (e.g., RNA vs. DNA) and also to the fact that the present

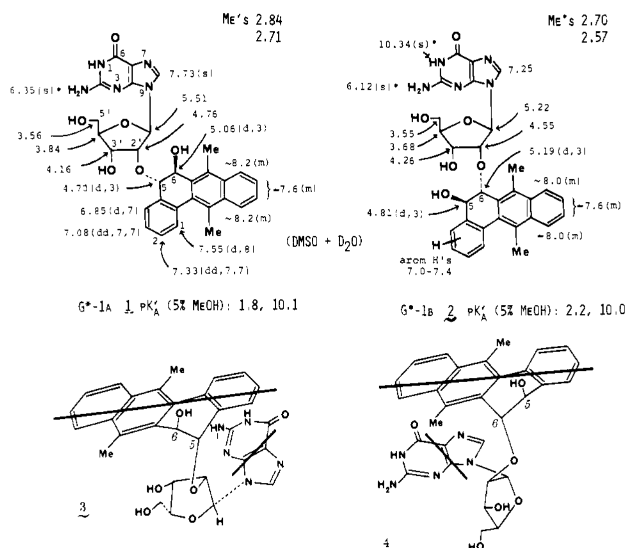
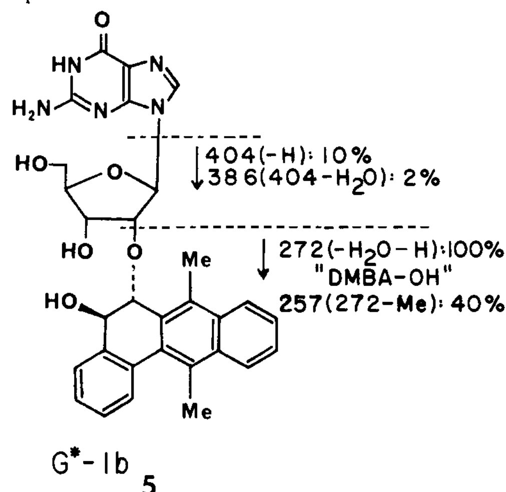


Figure 3. ^{1}H NMR data of **1** and **2** in Me_2SO-d_6 and 1 drop D_2O, 220 MHz. Peaks with * are for dry solvent; they disappear with addition of D_2O.

adducts represent only the minor products. The biological implification of these adducts in carcinogenesis will be discussed later.[5]

The UV spectra of **1**, 253 nm (sh, ϵ 39 000), 262 (51 000), 270 (52 000), 305 (8500), 318 (sh, 6900), and **2**, 252 nm (ϵ 52 000), 262 (54 000), 270 (52 000), 303 (sh, 12 000), 316 (sh, 9700), were dominated by the DMBA moiety. Therefore the UV spectra could not be used for determination of pK_a's, the numbers and values of which are diagnostic for the substitution on the guanosine moiety.[10] The extrema of CD spectra (Figure 1) also reflect the UV maxima of the DMBA moiety and do not reflect those of the purine portion. However, since the extrema mainly arise from the coupled interaction between the two aromatic chromophores,[4,7,8b] they should be influenced by the electric charge of the guanine moiety. This indeed is the case for the adducts formed between polyaromatic hydrocarbons and nucleic acid bases so that a plot of the pH against changes in CD $\Delta\epsilon$ values affords a sensitive method for determining pK_a' values.[11] Both **1** and **2** showed the presence of *two* pK_a's in the range of pH 1 to 11 (see Figure 2 and structures **1** and **2** for data) and hence the DMBA portion had to be attached to C-8, 2-NH_2, or the ribose. The 1800–1400-cm^{-1} region of the Fourier transform IR (FTIR) spectra is another sensitive method for characterizing the substitution on purine nucleosides.[11] This method showed that the guanine portion of **1** and **2** was substituted neither at O-6 nor at N-1.

Chart I. Pertinent mass spectral peaks were obtained by EI/MS. Relative percent refers to low-resolution data.



The ^{1}H NMR spectrum of **1** (Figures 3 and 4) showed the sharp 8-H singlet at 7.73 ppm,[12] and a two-proton intensity singlet at 6.35 ppm (2-NH_2 in dry Me_2SO-d_6) which disappeared upon addition of D_2O. The DMBA moiety is therefore attached to the ribose. Since adduct **1** is also formed in tissue culture,[5] the 3'- and 5'-hydroxyls are blocked in RNA; it follows that the DMBA moiety is linked through the 2'-OH. The above arguments on pK (see **2** for data), FTIR, ^{1}H NMR, and point of linkage hold similarly for adduct G*-**1b** (**2**). Structures **1** and **2** are corroborated by the mass spectral (MS) data as exemplified for G*-**1b** in structure **5** (Chart I):[13] calcd for $C_{20}H_{16}O$ 272.1199, obsd 272.1192; calcd for $C_{19}H_{13}O$ 257.0964, obsd 257.0954. The peaks with m/e 404 and 386 uniquely show the loss of the purine nucleus. It is to be noted that in agreement with their structures with a 2'-substitution, the ribose moiety of adducts **1** and **2** resisted cleavage by periodate and lysine,[14] conditions under which other DMBA-nucleoside adducts containing a free ribose readily lose the sugar.[4]

A comparison of the ^{1}H NMR data of **1** and **2** show that (i) in **1** the phenyl protons are characteristically spread out (Figure 4) and are at higher fields; (ii) in contrast, in **2** the naphthyl protons,methyl groups, and the guanine 8-H signal are at higher fields. These differences indicate that the guanine nucleus is stacked with the phenyl ring in **1** (**3**), whereas in **2** (**4**) it is stacked with the naphthyl ring. It is reasonable to assume that, since G*-**1a** and **1b** are also produced by reaction of (±)-DMBA 5,6-oxide with guanosine in the relatively high pH media of 9.5, the oxirane has undergone a trans opening.

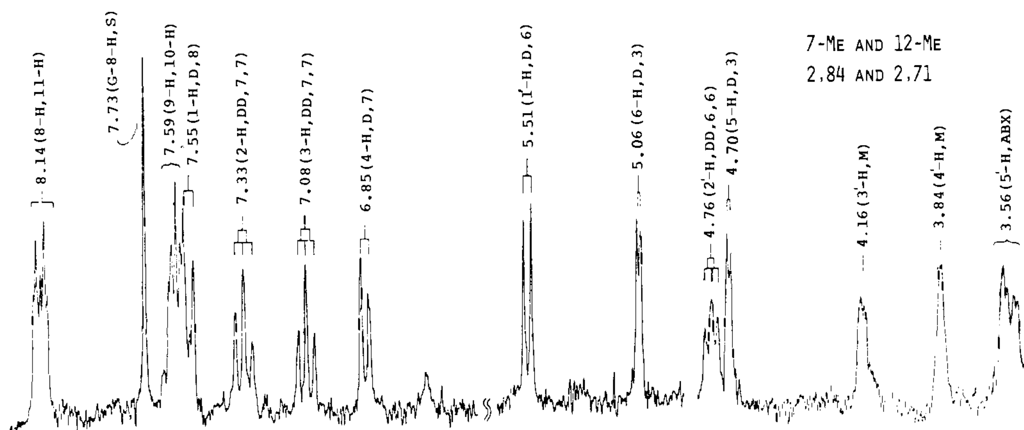


Figure 4. ^{1}H NMR of G*-**1a** in Me_2SO-d_6 and 1 drop D_2O, 220 MHz.

The ^{1}H NMR $J_{5,6}$ values of 3 Hz for both **1** and **2** therefore show that the guanosine moiety is axially connected to DMBA. *Molecular models reveal that these features then uniquely define the absolute configurations as well as the point of attachment of the guanosine moiety* of **1** and **2** (see **3** and **4**).[15] This leads to configurations $5S,6S$ for **1** and $5R,6R$ for **2**; i.e., both are products from the $5R,6S$ epoxide.[16] Although the direction of electric transition moments of the guanine nucleus is still not established,[17] it is gratifying to note that, as shown by the solid lines in **3** and **4**, the chirality between the chromophoric axes of *structures derived independently of the CD* are indeed "antipodal" and are in agreement with the "antipodal" CD data (Figure 1).

Acknowledgment. We are grateful to Professor R. G. Harvey, University of Chicago, for the supply of DMBA 5,6-oxide, to S. Traiman, Hoffmann-La Roche Inc., for FTIR measurements, to I. Miura and V. Parmakovich, Columbia University, for NMR and MS measurements, and to Dr. T. Wachs, Cornell University, for high-resolution MS data.[18]

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(13) The low-resolution MS were run on a computerized Finnigan 3300 system. The high-resolution MS data were obtained on an MS-902 instrument, VG Datasystem 2020, Cornell NIH. The m/e 404 and 386 peak intensities were too weak for high-resolution analyses owing to sample scarcity.
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(16) The diastereomers of **1** and **2** were not isolated from the in vitro experiments; in spite of the fact that (±)-DMBA 5,6-oxide was employed, they are either not formed or formed in too minute quantities. However, since the oxirane is attacked by the ribose 2′-OH group which is attached to a chiral center, it is reasonable that only one of the diastereomers is formed preponderantly.
(17) Footnote in ref 4, p 2314.
(18) The studies were supported by DHEW CA 11572, NSF CHE76-18435 (to K.N.) and CA 21111 (to D.G.).

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Binuclear Cryptates. Binuclear Copper(I) and Copper(II) Inclusion Complexes of Polythia Cylindrical Macrotricyclic Ligands

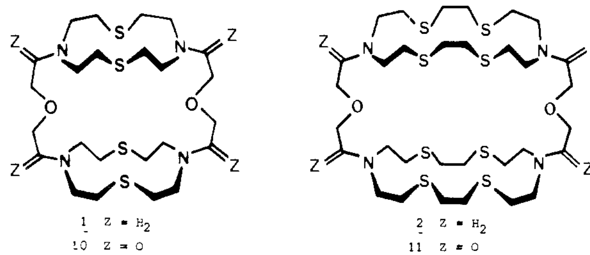
Sir:

Macro*poly*cyclic ligands may form *polynuclear cryptate complexes* by inclusion of two or more metal cations into the intramolecular cavities; distance and arrangement of the metal cations may be regulated via ligand structure. Such systems present much interest as models of polynuclear biological complexes or as polynuclear catalysts, especially if *cascade complexes*[1-3] may be formed by inclusion of substrate molecules between the cations.

We have previously described two types of macropolycyclic structures which may present such properties: cylindrical macrotricyclic ligands containing "face-to-face" macrocyclic subunits[3-5,6] and bis(tren) macrobicyclic molecules incorporating two coaxially aligned tripodal subunits.[1] The cylindrical macrotricycles have a particularly attractive topology[2-5,7,8] since the lateral macrocycles may serve to select and hold the cations while the central cavity is available for substrate inclusion (Figure 1).

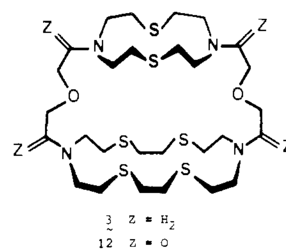
The previous macrotricycles were designed for the study of binuclear alkali and alkaline-earth complexes.[2-5] We now report (i) a general synthetic method for the construction of cylindrical macrotricycles which contain *different* macrocyclic subunits, and which may therefore complex two different cations or stabilize different oxidation states; (ii) the synthesis of the new macrotricyclic ligands **1–3** bearing nitrogen and sulfur binding sites, and (iii) preliminary complexation experiments which yield binuclear copper complexes and relate to the biologically important copper proteins, subject to much current interest.[9-14]

The synthetic strategy allows the incorporation of different macrocycles, whereas the earlier method[3,4] may introduce different bridges linking the macrocycles. It involves (a) attachment of two appendages at diagonally opposed positions

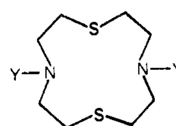


1 Z = H_2
10 Z = O

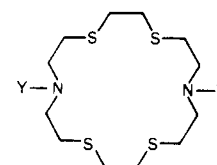
2 Z = H_2
11 Z = O



3 Z = H_2
12 Z = O



4a Y = H
4b Y = CH_3
5 Y = $COCH_2OCH_2COOH$
6 Y = $COCH_2OCH_2COO$-p-nitrophenyl



7a Y = H
7b Y = CH_3
8 Y = $COCH_2OCH_2COOH$
9 Y = $COCH_2OCH_2COO$-p-nitrophenyl