

- (9) H. R. Luss and D. L. Smith, *Acta Crystallogr., Sect. B*, **28**, 884 (1972).  
 (10) Inclusion of the higher order coupling term in eq 3<sup>7</sup> is not justified in this system. It appears to contribute less than 1% to the values of  $T_1$ .  
 (11) These are average internuclear separations,  $r_{\alpha}$ , and are related to the equilibrium values,  $r_e$ , by  $r_{\alpha} = r_e + \langle \Delta z \rangle$  where  $\langle \Delta z \rangle$  is the anharmonicity correction.

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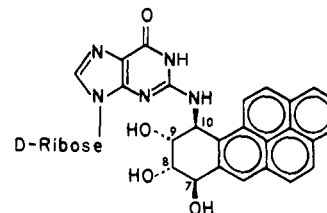
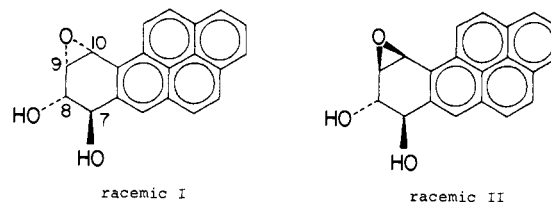
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### Benzo[a]pyrene-Nucleic Acid Derivative Found in Vivo: Structure of a Benzo[a]pyrenetetrahydrodiol Epoxide-Guanosine Adduct

Sir:

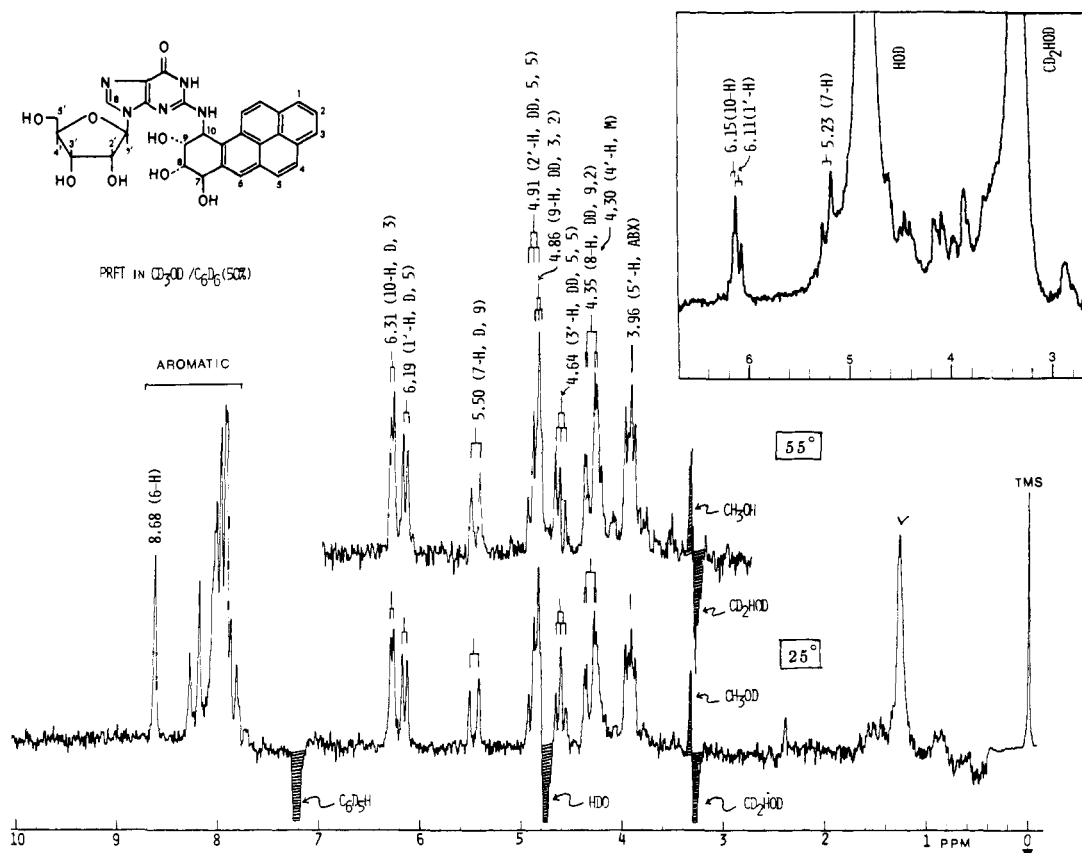
Benzo[a]pyrene (BP) and other polycyclic aromatic hydrocarbon carcinogens are known to bind covalently to cellular nucleic acid in vivo. Several lines of evidence suggest that a 7,8,9,10-tetrahydro-7,8-diol 9,10-epoxide is involved.<sup>1</sup> Two isomeric forms of this compound ( $\pm$ )-I and ( $\pm$ )-II yield covalent complexes with poly(G) in vitro.<sup>2</sup> We have also recently found<sup>2</sup> that at least one of the forms of BP which becomes bound to nucleic acid in bovine bronchial explants during incubation of this tissue with <sup>3</sup>H-BP, and the product of reaction of isomer I with poly(G), are identical when hydrolyzed to ribonucleoside derivatives. Further chromatography of these derivatives as their diacetone and diacetone diacetates confirmed their identity and distinguished these derivatives from those obtained by in vitro reaction of poly(G) with isomer II. The structure of the major guanosine adduct formed with isomer I in vivo is now reported.

A pertinent finding was that the CD spectra of the two major in vitro components<sup>3</sup> were almost equal in shape but opposite in sign<sup>4</sup> and hence, except for the  $\beta$ -D-ribose moiety, the compounds were enantiomeric. The evidence presented below allows us to now assign structures 1 and 3 to these two compounds; the radioactive in vivo product corresponds to 3.<sup>2</sup> Determination of the absolute configuration requires further studies.



1 (retention time, 21 min) and  
 3 (34 min)<sup>3</sup> (enantiomeric at 7,  
 8, 9 and 10)

Both 1 and 3 gave similar <sup>1</sup>H NMR spectra. However, the crucial regions of the original spectra (1 shown in Figure 1 inset) were heavily overlaid by the solvent signals. Partially relaxed Fourier transform (PRFT) <sup>1</sup>H NMR measurements led to considerable improvement since the peaks due to protons with neighboring deuteriums, i.e., solvent signals, became in-



**Figure 1.** Inset: <sup>1</sup>H NMR of 1 in CD<sub>3</sub>OD. Bottom and middle spectra: Partially relaxed Fourier transform spectra of 1 in CD<sub>3</sub>OD:C<sub>6</sub>D<sub>6</sub> (1:1) as measured by the inversion recovery method  $(180-\tau-90-T)_n$ , where  $\tau = 2.2$  s and  $T = 6.1$  s, 4096 scans, JEOL PS-100. The sharp positive peaks at 3.35 ppm are due to CH<sub>3</sub>OH present in the deuterated solvents.

verted negative peaks. The region 4–7 ppm was still too congested and hence 50% C<sub>6</sub>D<sub>6</sub> was added to spread out the spectrum (Figure 1, bottom run). For further improvement, the 4.75 ppm HDO peak was moved upfield out of the range of overlap by warming the sample to 55 °C. As shown in Figure 1 (top run), the resulting spectrum was a dramatic improvement over the original (inset), and revealed almost all chemical shifts and coupling constants. This assignment is consistent with that of the trans opening of isomer I by guanine when compared with the cis and trans hydration products from isomer I<sup>5</sup> and was confirmed by showing that the 7-H doublet at 5.23 ppm (see insert, Figure 1) moves downfield to 6.60 ppm  $J_{7,8} = 9$  Hz in the per-*O*-acetate derivative (acetic anhydride–pyridine, room temperature 12 h).

The point of attachment of the guanosine moiety was proved as follows. When measured in Me<sub>2</sub>SO-*d*<sub>6</sub> the <sup>1</sup>H NMR spectrum of **1** showed a conspicuous doublet at 6.92 ppm ( $J = 8$  Hz, N<sup>2</sup>-H of guanine), which collapsed to a singlet upon irradiation of the 10-proton at 5.93 ppm (overlapping with the 1'-H) and disappeared upon addition of D<sub>2</sub>O. Such observations are only consistent with substitution of the N<sup>2</sup> of guanine at the 10-position of isomer I. The high resolution mass spectrum of the 5',7-diacetate 2',3',8,9-diacetonide of **3**<sup>6,7</sup> also indicated that substitution had occurred through the N<sup>2</sup> of guanine. An ion at  $m/e$  342.1152 (1.9%, C<sub>22</sub>H<sub>16</sub>NO<sub>3</sub> = 342.1129) corresponds to cleavage between the C-2 and N<sup>2</sup> positions of guanine and loss of acetone from the BP moiety. An additional loss of acetic acid from this ion was also observed:  $m/e$  282.0930 (4.5% C<sub>20</sub>H<sub>12</sub>NO = 282.0919).

Current investigations are directed towards the absolute configurations of **1** and **3** and other derivatives formed with RNA and DNA during metabolism of BP.<sup>8</sup>

## References and Notes

- (1) A. Borgen, H. Darvey, N. Castagnoli, T. T. Crocker, R. E. Rasmussen, and I. Y. Wang, *J. Med. Chem.*, **16**, 502 (1973); P. Sims, P. L. Grover, A. Swaisland, K. Pal, and A. Hewer, *Nature (London)*, **252**, 326 (1974); P. Daudel, M. Duquesne, P. Vigny, P. L. Grover, and P. Sims, *FEBS Lett.*, **57**, 250 (1975).
- (2) I. B. Weinstein, A. M. Jeffrey, K. W. Jennette, S. H. Blobstein, R. G. Harvey, H. Kasai, and K. Nakanishi, *Science*, **193**, 592 (1976).
- (3) The adducts were prepared by reacting isomer (±)-I with poly(G) (both at 1 mg/ml) in 2:1 acetone–water for 24 h at 37 °C. The unbound derivatives were removed by extraction with ethyl acetate and 1-butanol followed by precipitation of the poly(G) with ethanol. The modified polymer was hydrolyzed for 18 h at 37 °C with 0.3 N NaOH. The nucleotides were applied to a LH-20 Sephadex column (1.8 × 66 cm), eluting first with 20 mM NH<sub>4</sub>HCO<sub>3</sub>, pH 8.5, to remove GMP and then a gradient of 20–80% methanol which separated guanosine derivatives **1** and **3** as their monophosphates. The latter were treated with alkaline phosphatase and the nucleosides (**1** and **3**) re-purified by LH-20 Sephadex chromatography. Analysis of these derivatives by HPLC (Dupont Zorbax ODS column, 0.25 m × 6.4 mm; 2500 psi; 50 °C; 40% methanol in water) showed **1** (21 min) to be essentially pure and **3** (34 min) to be contaminated by about 10% with a third component **2** (25 min) (structure unknown). For detailed methods see S. H. Blobstein, I. B. Weinstein, D. Grunberger, J. Weisgras, and R. G. Harvey, *Biochemistry*, **14**, 3451 (1975), and A. M. Jeffrey, S. H. Blobstein, I. B. Weinstein, and R. G. Harvey, *Anal. Biochem.*, **73**, 378 (1976).
- (4) The CD spectra of **3** and **1** (50% water/methanol, Cary 60, JASCO J-40 instruments) showed weak extrema of same intensities but of opposite signs in the region 350–290 nm. Only the strong Cotton effects are listed. Compound **3** had the following  $\Delta\epsilon$  at the indicated wavelengths: 280 nm, +31; 275 nm, -16; 249 nm, +115; and 240 nm, -53; Compound **1**: 280 nm, -34; 273 nm, +4; 249 nm, -56; 240 nm, +25. The 5',7-diacetate-2',3',8,9-diacetonides of **3** and **1** showed the following CD spectra **3**: 280 nm, +37; 275 nm, -26; 249 nm, +125; 236 nm, -63, **1**: 280 nm, -38; 275 nm, +34; 249 nm, -121; 236 nm, +61.
- (5) J. Keller, PhD Dissertation, Department of Chemistry, University of Wisconsin, Madison, Wis., 1976.
- (6) The mass spectrum (Jeol JMS-01SG-2, EI mode, 70 eV, probe 290 °C, source 250 °C) showed ions mainly resulting from losses of acetic acid and/or acetone from the molecular ion  $m/e$  749.2704 (1.1%, C<sub>40</sub>H<sub>39</sub>N<sub>5</sub>O<sub>10</sub> = 749.2697); 384.1365 (5.9%, C<sub>25</sub>H<sub>20</sub>O<sub>4</sub> = 384.1362); 350.1099 (4.6%, C<sub>14</sub>H<sub>15</sub>N<sub>5</sub>O<sub>8</sub> = 350.1100); 324.1158 (16%, C<sub>23</sub>H<sub>16</sub>O<sub>2</sub> = 324.1150); 284.0869 (30%, C<sub>20</sub>H<sub>12</sub>O<sub>2</sub> = 284.0837); 268.0876 (12% C<sub>20</sub>H<sub>12</sub> = 268.0888); 256.0879 (12%, C<sub>19</sub>H<sub>12</sub>O = 256.0888); 255.0825 (14%, C<sub>19</sub>H<sub>11</sub>O = 255.0810); 239.0833 (13% C<sub>19</sub>H<sub>11</sub> = 239.0860); 43 (100%).
- (7) The authors wish to thank Dr. P. Roller, NCI, NIH, for the thorough analysis of the high resolution mass spectrum of the diacetate, diacetonide of **3**. This work was supported by NIH Grants CA-02332 and CA-11572, NCI Contracts E-72-3234 and CP-033365, and American Cancer Society Grant BC-132.
- (8) Note Added in Proof: When reacted with poly(G), 7,12-dimethylbenz[*a*]-

anthracene-5,6-oxide has also been shown to link covalently via the 2-amino group of guanosine: A. M. Jeffrey, S. H. Blobstein, I. B. Weinstein, F. A. Beland, R. G. Harvey, H. Kasai, and K. Nakanishi, *Proc. Natl. Acad. Sci., U.S.A.*, **73**, 2311 (1976).

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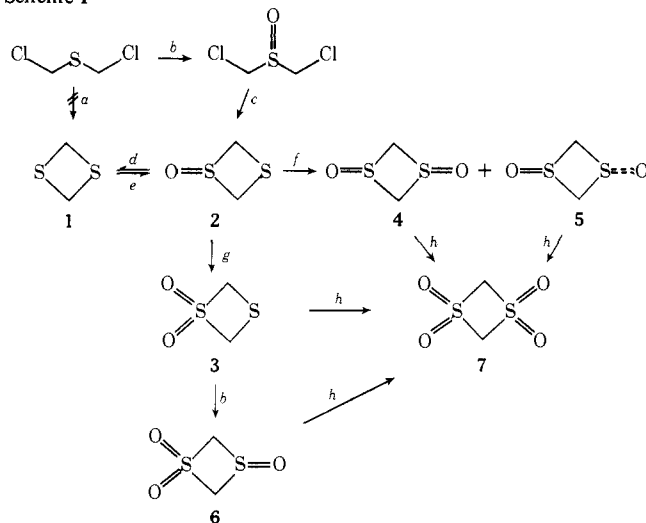
## 1,3-Dithietane

Sir:

Although derivatives of 1,3-dithietane (**1**) have been known for over 100 years,<sup>1</sup> the parent compound has until now remained unknown. We describe herein a simple synthesis of **1** from readily available starting materials. We also report the preparation of the previously unknown *S*-oxides of **1**, 1,3-dithietane 1-oxide (**2**), 1,3-dithietane 1,1-dioxide (**3**), *cis*- and *trans*-1,3-dithietane 1,3-dioxide (**4** and **5**, respectively), and 1,3-dithietane 1,1,3-trioxide (**6**), and the conversion in high yield of several of these compounds (**3**, **4**, **5**, **6**) to the previously described sulfene dimer, 1,3-dithietane 1,1,3,3-tetraoxide (**7**).<sup>2</sup> We have initiated a detailed investigation of the reactions and structural features of these interesting heterocycles. Novel structural features of two of the above compounds are described in this communication while one aspect of the chemistry of **2**, namely, its facile pyrolytic conversion into sulfine and thioformaldehyde, is reported elsewhere.<sup>3</sup>

While bis(chloromethyl) sulfide fails to give monomeric product with sodium sulfide,<sup>4</sup> presumably due to the high reactivity of the former compound in displacement processes,

## Scheme I



<sup>a</sup> Na<sub>2</sub>S·9H<sub>2</sub>O, DMF. <sup>b</sup> CH<sub>3</sub>CO<sub>3</sub>H, CHCl<sub>3</sub>, 0 °C. <sup>c</sup> Na<sub>2</sub>S·9H<sub>2</sub>O, 0.3 equiv of "Aliquat 336", H<sub>2</sub>O, vigorously stirred. <sup>d</sup> 2 mol equiv of 1 M THF–BH<sub>3</sub>, 24 h at 25 °C. <sup>e</sup> PhICl<sub>2</sub>, CH<sub>3</sub>CN, Et<sub>3</sub>N, H<sub>2</sub>O (or H<sub>2</sub><sup>18</sup>O). <sup>f</sup> PhICl<sub>2</sub>, C<sub>6</sub>H<sub>5</sub>N, H<sub>2</sub>O, -30 °C or *m*-ClC<sub>6</sub>H<sub>4</sub>CO<sub>2</sub>H, CH<sub>2</sub>Cl<sub>2</sub>, 0 °C. <sup>g</sup> 0.67 mol equiv of KMnO<sub>4</sub>, MgSO<sub>4</sub>, acetone, -30 °C. <sup>h</sup> 30 mol equiv of CH<sub>3</sub>CO<sub>3</sub>H, 100 °C, 4 h.