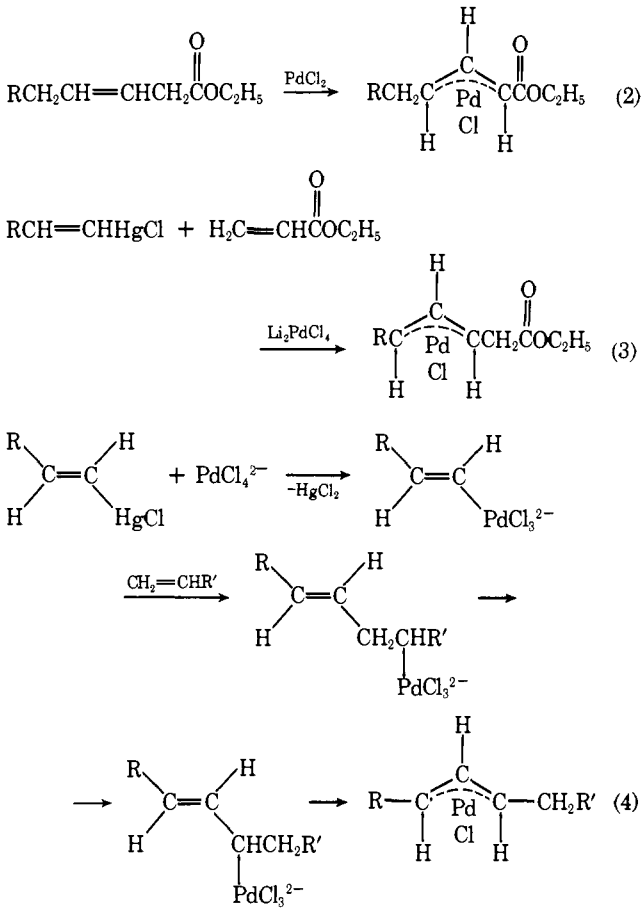
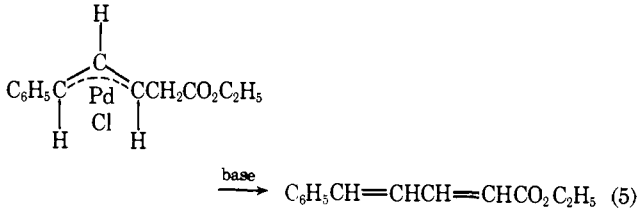


palladium species to the olefin, followed by a palladium hydride rearrangement as suggested below (eq 4).

The formation of π-allylpalladium compounds in these reactions is at first surprising in view of previous reports that vinylboronic acids and palladium acetate, as well as vinyl halides and palladium catalysts, react with olefins in the presence of organic bases to give 1,3-dienes.[7] However, it has been suggested previously that π-allylpalladium compounds may be involved in these reactions.[7] Indeed, we have observed that π-allylpalladium compounds possessing neighboring electron withdrawing groups like those generated in our reactions readily react with bases to give 1,3-dienes (eq 5). We are



presently examining this approach to unsymmetrical 1,3-dienes from vinylmercurials, as well as the possibility that other π-allyl transition metal complexes may be prepared in this same manner.

Acknowledgments. We gratefully acknowledge the donors of the Petroleum Research Fund, administered by the American Chemical Society, the Research Corporation, and the National Science Foundation (NSF-URP Grant No. EPP75-04944) for generous financial support, and Matthey Bishop, Inc. for a large loan of palladium chloride.

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(10) DuPont Young Faculty Scholar, 1975–1976.
(11) NSF-URP participant, summer 1975.

Richard C. Larock,*[10] Mark A. Mitchell[11]
Department of Chemistry, Iowa State University
Ames, Iowa 50011
Received June 1, 1976

Alkylation of Polyguanylic Acid at the 2-Amino Group and Phosphate by the Potent Mutagen (±)-7β,8α-Dihydroxy-9β,10β-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene

Sir:

We recently described the stereoselective synthesis of (±)-7β,8α-dihydroxy-9β,10β-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (**1**) and established that anchimeric assistance by the benzylic 7-hydroxy group greatly enhances its reactivity toward nucleophiles[1,2] when compared to the isomeric 9α,10α-epoxide (**2**).[3,4] Both diol expoxides (**1** and **2**) are formed on metabolism of the environmental carcinogen benzo[*a*]pyrene (BP) via oxidation of the 7,8-dihydrodiol.[5] The remarkably high mutagenicity of **1** and **2** toward bacterial and mammalian cells,[6] the indications that **1** and **2** are responsible for most of the binding of metabolities of BP to DNA,[3,7] and the high carcinogenicity of benzo[*a*]pyrene 7,8-oxide[8] (precursor of **1** and **2**) suggest **1** and **2** as ultimate carcinogens from BP. Since the guanine base (**3**, R = H) in DNA is generally the best nucleophile toward alkylating agents,[9] we have established the structures of the products formed when the highly reactive **1** ($t_{1/2} \sim 30$ s at pH 7, 37 °C; solvolysis to tetraols by cis and trans addition of water at C-10)[5,10] covalently binds to polyguanylic acid (poly G).

In a typical binding experiment, tritiated **1** (0.4 mg/ml)[5] was added to a solution of poly G (1.7 mg/ml) in 50% aqueous acetone at 37 °C, Tetraols resulting from solvolysis of **1** were completely removed by three extractions with ethyl acetate. Precipitation of the polymer with ethanol followed by further extraction of aqueous solutions of the precipitate failed to release any of the bound hydrocarbon. Examination of the extent of binding as a function of pH and time established that optimum modification (10%) occurred near pH 6 and was complete within 1 h. The rate of binding was at least 30 times faster at pH 4 compared to pH 7, which required 2 h to reach completion. The uv spectrum of the modified poly G (Figure 1) showed the characteristic pyrene absorption pattern near 340 nm with a bathochromic shift which is typical of stacked chromophores.[11]

Acid hydrolysis of the modified poly G in ^{18}O-enriched (18%) 0.1 N HCl at 100 °C for 1 h released essentially all of the hydrocarbon as tetraols of **1** which had incorporated 0.96 atom % solvent water. The experiment provides little structural information since the tetraols incorporate 0.86 atom % solvent water under these conditions. Two classes of polymer-adducts were, however, identified by their differences in chemical stability. The minor and chemically labile products (type I adducts), 10–15% of the bound hydrocarbon, were released as tetraols with 0.95 atom % incorporation of solvent water on heating of modified polymer at 85 °C for 15 min at pH 7.0, conditions under which tetraols do not exchange.[12] Alkaline hydrolysis of the modified polymer (1 N KOH, 24 h, 37 °C)

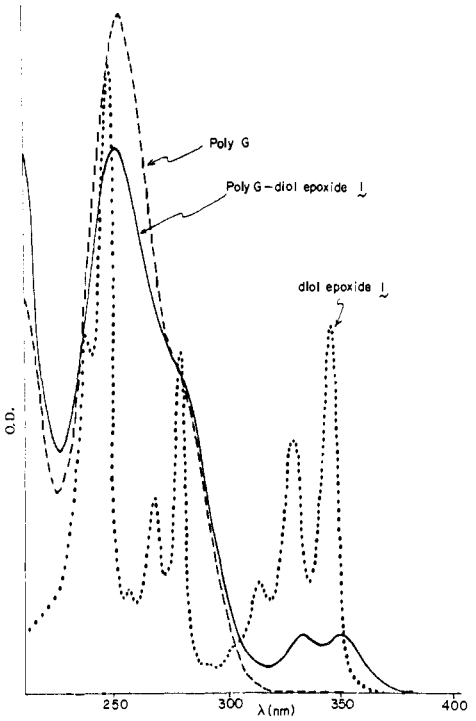


Figure 1. Ultraviolet spectra of poly G (water), poly G-diol epoxide **1** (water), and diol epoxide **1** (dry tetrahydrofuran); ϵ_{343} ~55 000 for diol epoxide **1** and ϵ_{254} ~10 000 for poly G. Spectra are not corrected for differences in intensities.

to nucleotides also releases 10–15% of the hydrocarbon as tetraols.[13] Alkaline phosphatase (10 units/35 OD_{254nm}, pH 8.4, 37 °C, 24 h) hydrolyzed this nucleotide mixture to guanosine and two pairs of diastereomeric guanosine–hydrocarbon adducts as established by their circular dichroism spectra[14] (type II adducts). The type I and II adducts account for >95% of the binding of **1** to poly G.

The chemical lability and incorporation of solvent water of the type I adducts suggest alkylation of the phosphodiester linkages of the poly G to initially produce labile phosphotriesters.[15] When tritiated **1** (0.4 mg/ml) was mixed with 0.05 M phosphate buffer at pH 7, >15% of the hydrocarbon became nonextractable into ethyl acetate, presumably due to alkylation of phosphate. After heating the "hydrocarbon-phosphate" adduct (pH 7, 85 °C, 30 min), >99% of the hydrocarbon became extractable as tetraols.

The two pairs of diastereoisomeric nucleoside type II adducts arise from cis and trans addition of the amino group at C-2 of the guanine base to the 10-position of **1**. The usual criterion for assignment of position of alkylation on guanine, pH dependence of the uv spectrum,[9] cannot be applied because the hydrocarbon residue greatly dominates the spectrum. Alkylation of [8-^{3}H]poly G at N-7 or C-8 by **1** was excluded by a lack of tritium release (no tritium release above the blank of 0.5% with 10% modification) under conditions where complete exchange would be expected.[16,17] Isolated nucleoside adducts from the modified polymer had >80% of the original specific activity. Alkylation at O-6, N-1, or N-3 would lead to products which lack the normal acidic (pK_a = 9.2) proton at N-1.[9] The pK_a of the mixture of nucleoside adducts was readily estimated from the change in partition coefficient for the adducts between aqueous and organic solvent as a function of pH (Figure 2). Since both acidic and basic ionizations of the type II adducts were observed, alkylation at O-6, N-1, or N-3 can be elminated. The presence of a substituent at N-2 was directly indicated by the failure of the type II adducts to change pK_a on treatment with nitrous acid (30% acetic acid, pH 3.5, 2 h at 37 °C), conditions which deaminate guanosine at N-2 and cause the pK_a to change to 5.7[18,19]

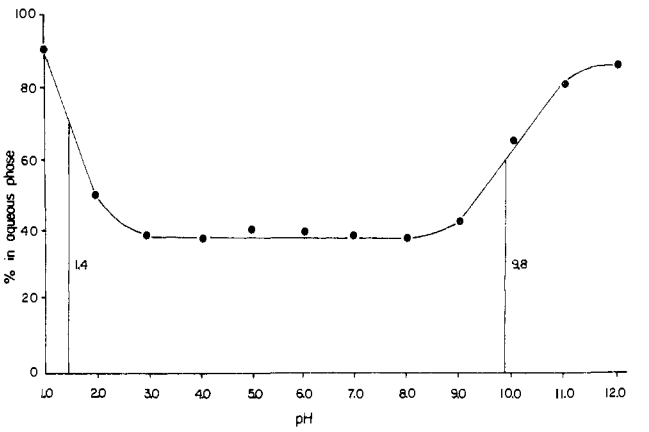
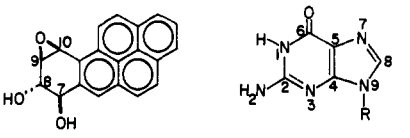


Figure 2. Estimation of pK_a via change in partition coefficient. Type II adducts (~15 nmol) were partitioned between equal volumes (0.4 ml) of 0.05 M buffers (except pH points 1–3 where phosphoric acid was added to obtain correct pH) and 20% 1-butanol in ethyl acetate. Distribution between the two phases was determined spectrophotometrically (A_{344}) and radiochemically.

Since NMR spectra of the acetylated type II adducts were somewhat complicated due to the overlap of signals in the 5–6 ppm region, a ribose-free derivative of the type II adducts was sought. Methylation at N-7 of the modified polymer (or mixture of nucleoside adducts) with four aliquots of dimethyl sulfate at 30-min intervals (pH 7, 25 °C), followed by brief heating (pH 6, 100 °C), cleaved the hydrocarbon-7-methylguanine from the ribose. After acetylation and purification, two main products were isolated[20] which had mass spectra indicative of 7-methylguanyltriacetoxytetrahydrobenzo[*a*]pyrene adducts **4**.[21] The major and minor adducts were assigned as trans (**4a**) and cis (**4b**) addition products, respectively, to the C-10 position of the epoxide in **1** based on comparison of their NMR spectra with model compounds.[22]



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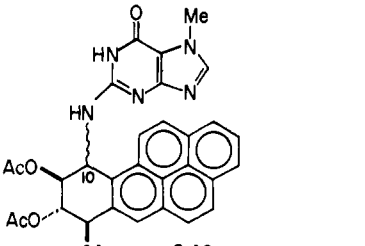

4 a 9,10-trans adduct
b 9,10-cis adduct

The reaction of **1** with poly G is unique in that selective alkylation occurs at two sites which rarely suffer extensive modification by other alkylating agents.[9,23] Furthermore, the extent of alkylation exceeds that typically observed for arene oxides (cf. ref 24). Alkylation of guanine bases in DNA at N-2 and alkylation of the ribose-phosphate backbone[25] with subsequent implied strand breaks is unusual for alkylating agents and may be responsible for the high mutagenic and potentially carcinogenic activity of diol epoxide **1**. The release of tritiated adducts at the N-2 position of guanine in DNA by [^{14}C] di-

methyl sulfate should provide a highly sensitive technique for detection of this type of alkylation of DNA.

Note Added in Proof. Since submission of this manuscript, Professor K. Nakanishi of Columbia University kindly provided us with preprints of studies on the binding of the isomeric 9α,10α-epoxide (**2**) to poly G: I. B. Weinstein, A. M. Jeffrey, K. W. Jennette, S. H. Blobstein, R. G. Harvey, H. Kasai, and K. Nakanishi, *Science*, **193**, 592 (1976), and A. M. Jeffrey, K. W. Jennette, S. H. Blobstein, I. B. Weinstein, F. A. Beland, R. G. Harvey, H. Kasai, I. Miura, and K. Nakanishi, *J. Am. Chem. Soc.*, **98**, 5714 (1976). These studies showed that the 2-amino group of quanine adds to **2** to form a trans adduct as well as other unidentified products. In our hands, diol epoxide **2** behaves much like diol epoxide **1** in that alkylation of phosphate also occurs with this diastereomer of BP 7,8-diol-9,10-epoxide. In addition, 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), have shown that DMBA 5,6-oxide alkylates the N-2 amino group of quanine in poly G.

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(12) When the polymer was heated for an additional 4 h, no significant further release of hydrocarbon was observed. After removal of initially released tetraols, an equivalent amount of tetraols was added to the modified poly G solution in ^{18}O enriched water. No incorporation of solvent water into the tetraols was detected after identical heating and recovery. Identification of the tetraols was as described.[5,10]
(13) When the modified polymer was first heated in water and the resulting tetraols removed by extraction, no further amount of tetraols was observed upon alkaline hydrolysis of the polymer.
(14) The four nucleoside–hydrocarbon adducts (uv spectra similar to diol epoxide **1**, Figure 1) were separated by high pressure liquid chromatography on a DuPont 5μ, ODS column (7.8 mm × 0.25 m) eluted with 65% methanol in water at a constant flow of 1.6 ml/min; retention times were 13.7, 15.2, 16.8, and 21.2 min. When the poly G was modified at pH 7, the nucleoside adducts were formed in a ratio of 1:2:1:2, respectively, based on absorption at 344 nm. This ratio approached 1:1:1:1 as the pH of the binding experiment was decreased. The first and third compounds ($\Delta\epsilon_{250}$ −90 and +90, respectively) and the second and fourth compounds ($\Delta\epsilon_{250}$ −92 and +92, respectively) to elute from the column constitute two diasteriomeric pairs. Calculations were based on an extinction coefficient (344 nm) of 55 000 (see Figure 1). Mirror image CD spectra among the two pairs indicate that the absolute stereochemistry of the tetrahydro benzo[a]pyrene moiety plays a predominant role in the CD spectra presumably through its chiral interaction with the guanine base.
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(17) Reaction of **2** with DNA containing [8-^{3}H]guanine also failed to cause a release of tritium.[7d] Further chemical evidence for the structure of the adduct(s) was not presented.
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(20) Conditions for HPLC were the same as in note 14 except that the column was eluted with 80% methanol in water. Retention times of 17 min for the major and 22 min for the minor product were observed. A small uncharacterized peak was also observed at 11 min.
(21) Spectra were run on a Finnigan 1015D mass spectrometer by C.I. with CH_4 gas. Major fragments observed were *m/e* 534 ($M^+ + 1 - 60$), 474 ($M^+ + 1 - 2 \times 60$), and 332 (474 − 42). Tetraacetates of the tetraols show a similar pattern of fragmentation.
(22) The Fourier transform NMR spectra (220 MHz, $CDCl_3$) of **4a** and **4b** were compared with the spectra of the acetates of the trans-aniline adduct (10α-NHC_6H_5 in **4**) and the cis-phenol adduct (10β-OC_6H_5 in **4**) of diol epoxide **1**.[10] The coupling constants for the methyl guanine adducts, **4a** and **4b**, were within 1 Hz of those for the model compounds: **4a** (trans adduct) H_7 δ 6.71, H_8 5.52, H_9 5.58, H_{10} 6.14, O-Ac 1.98, 2.04, and 2.25, N_7-Me 3.38, guanine H_8 and aromatic hydrogens 7.95–8.28 with $^3J_{7eq,8eq}$ = 5.2, $^3J_{8eq,9eq}$ = 5.4, $^3J_{9eq,10eq}$ = 2.6 Hz; **4b** (cis adduct) H_7 δ 6.93, H_8 6.18, H_9 5.61, H_{10} 6.29, O-Ac 1.95, 2.00, and 2.13, N_7-Me 3.93, guanine H_6 7.95, aromatic hydrogens 8.0–8.4 with $^3J_{7ax,8ax}$ = 8.0, $^3J_{8ax,9ax}$ = 12.0, $^3J_{9ax,10eq}$ = 4.0 Hz.
(23) Although not a potential metabolite, A. Dipple, P. Brookes, D. S. Macintosh, and M. P. Rayman, *Biochemistry*, **10**, 4323 (1971), suggested that 7-bromomethylbenzo[a]anthracene alkylates guanine at N-2. In contrast, S. H. Blobstein, I. B. Weinstein, D. Grunberger, J. Weisgras, and R. G. Harvey, *ibid.*, **14**, 3451 (1975), suggested that 7,12-dimethylbenzo[a]anthracene 5,6-oxide alkylates guanine on the imidazole ring of GMP.
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Masato Koreeda,* Patrick D. Moore
Department of Chemistry, The Johns Hopkins University Baltimore, Maryland 21218

Haruhiko Yagi, Herman J. C. Yeh, Donald M. Jerina*
Laboratory of Chemistry National Institute of Arthritis, Metabolism and Digestive Diseases, National Institutes of Health Bethesda, Maryland 20014

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A Total Synthesis of *d,l*-Luciduline by a Regioselective Intramolecular Addition of an *N*-Alkenylnitrone

Sir:

Although several studies have been made of intramolecular thermal additions of *C*-alkenylnitrones[1] the corresponding reaction of *N*-alkenylnitrones has received only scant attention.[2] We now wish to report an application of the unexplored thermal reaction of an *N*-alkenylhydroxylamine, A, with an aldehyde (Scheme I)[3] to afford a simple total synthesis of racemic luciduline (**9**). The natural *d*-alkaloid, isolated from *Lycopodium lucidulum*, has been shown by chemical and x-ray evidence[4] to have structure **9**. Its racemate was synthesized recently by a multistep approach involving an internal Mannich reaction.[5]

Scheme I