79953-13-2; **14** isomer 2, 79953-14-3; **15**, 30454-96-7; **16a**, 74614-90-7; **16b**, 74614-91-8; **17**, 79953-15-4; **18**, 74614-94-1; **19**, 79953-16-5; **20**, 79953-17-6; **21**, 74614-75-2; **22**, 79953-18-7; **23**, 74614-96-3; **24**, 74614-97-4; **25**, 74614-98-5; **26**, 74614-99-6; **27**, 79953-19-8; **29**, 74615-01-3; **30**, 74615-02-4; **31**, 74615-03-5; **32**, 74615-04-6; **33**, 74615-05-7; **34**, 74615-06-8; **35**, 79953-20-1; **36**, 79953-21-2; **37**, 74615-09-1; **38**, 74615-10-4; **39**, 74615-11-5; **40**, 74615-12-6; **41**, 79953-22-3; **42**, 79953-23-4; **43**, 74615-13-7; **44**, 3398-48-9; vinyl bromide, 593-60-2; ethyltriphenylphosphonium bromide, 1530-32-1; *N*-(cyanomethyl)pyrrolidine, 29134-29-0; dimethyl methlphosphonate, 756-79-6.

Photochemical Epoxidation of Aflatoxin B_1 and Sterigmatocystin: Synthesis of Guanine-Containing Adducts

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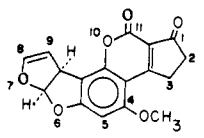
Abstract: Benzil-sensitized photoepoxidation of the mycotoxins aflatoxin B_1 and sterigmatocystin provided the presumed reactive epoxides which have been implicated in mutagenesis and carcinogenesis. These intermediates, after trapping with 3′,5′-di-*O*-butyryldeoxyguanosine and hydrolysis with aqueous acid, led to the N^7-guanine adducts identical with the in vivo natural products. This method provides an alternative to the more complex procedures of organ perfusion and whole-body dosing.

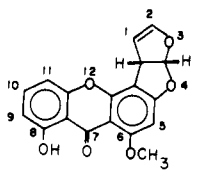
The formation of chemical carcinogen–DNA component adducts leads to the creation of mutations through the misrepair or misreplication of lesions, according to current theory. Because of DNA's central role as the repository of cellular genetic information, the fixation of mutations may represent a crucial factor in neoplastic transformation. As a result, the modes of formation and the structure of DNA adducts have been subjected to increasing scrutiny. The structures of the major adducts of aflatoxin B_1,[2] sterigmatocystin,[3] benzo[*a*]pyrene,[4] and acetylaminofluorene[5] have been identified, but a host of minor unidentified adducts are also formed, in part as the result of the complexity of the metabolic conversion of carcinogens to a variety of reactive species and the multiplicity of binding sites available in DNA. It is not possible currently to state the correlation between an adduct's structure and its efficiency in inducing a mutagenic transformation (if such a correlation indeed exists). Major adducts, in other words, may not necessarily present the highest risk of mutation to a cell if they are more easily excised from DNA than minor adducts or if they do not alter template function. This indeed is the case with alkylating agents, where quantitatively minor adducts are believed to be more significant in causing mutations than relatively more abundant lesions.[6]

The present line of investigation was initiated with the immediate objective of synthesizing adducts of known structure for comparison with the natural products, which often are available in quantities insufficient for full structure elucidation. As an extension of these studies, we have begun to chemically build carcinogens into specific genetic loci of biologically active DNA molecules in order to correlate adduct structure with mutagenic potential.[7]

In this paper we describe a simple synthesis of the major adducts of aflatoxin B_1 (AFB_1) (**1**) and sterigmatocystin (ST) (**2**) with DNA, viz., the guanine adducts **3** and **4**. AFB_1 and ST, like many



1



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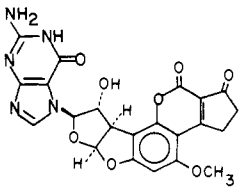
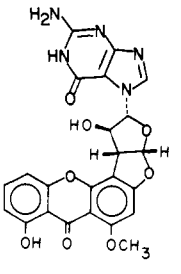


3



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other carcinogens, must be metabolically activated in order to exert their toxic effects, in this case principally by epoxidation of the terminal dihydrofuran double bond.[8] Although these highly reactive intermediates have not been isolated, the stereochemistry of the adducts **3** and **4** indicates that epoxidation occurs on the convex face of the molecules prior to concave face nucleophilic attack at the carbons adjacent to the tetrahydrofuryl oxygens.[2] The reactivity of the putative epoxides makes their generation for adduct synthesis by methods such as peroxyacid oxidation[2,9,10]

(1) NIH Postdoctoral Trainee, 1978–1981.

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(4) (a) Weinstein, I. B.; Jeffrey, A. M.; Jennette, K. W.; Blobstein, S. H.; Harvey, R. G.; Harris, C.; Autrup, H.; Kasai, H.; Nakanishi, K. *Science (Washington, D.C.)* **1976**, *193*, 592. (b) Jeffrey, A. M.; Jennette, K. W.; Blobstein, S. H.; Weinstein, I. B.; Beland, F. A.; Harvey, R. G.; Kasai, H.; Miura, I.; Nakanishi, K. *J. Am. Chem. Soc.* **1976**, *98*, 5714–5. (c) Koreeda, M.; Moore, P. D.; Yagi, H.; Yeh, H. J. C.; Jerina, D. M. *Ibid.* **1976**, *98*, 6720–2. (d) Blobstein, S. H.; Weinstein, I. B.; Grunberger, D.; Weisgras, J.; Harvey, R. G. *Biochemistry* **1975**, *14*, 3451.

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(8) Swenson, D. H.; Lin, H.-K.; Miller, E. C.; Miller J. A. *Cancer Res.* **1977**, *37*, 172–81.

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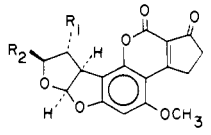
impractical because of competing nucleophilic attack of the remaining reduced reagent. We therefore have exploited Bartlett's procedure[11] of α-diketone-sensitized photochemical epoxidation to generate the desired epoxides in the presence of a protected deoxyguanosine.

Reaction Conditions. Photolyses were carried out in an oven-dried small apparatus made from an 11 × 110 mm glass tube equipped with fritted filter to introduce dry oxygen into the reaction solution and a serum stopper inlet to introduce dry solutions of reagents by syringe. Typically, a solution of AFB_1, 2'-deoxyguanosine 3',5'-dibutyrate (**5**), and benzil in *o*-dichloro-

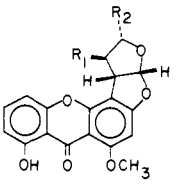


6a. R_1, R_2 = OH

b. R_1, R_2 = OAc

c. R_1 = H, R_2 = OH

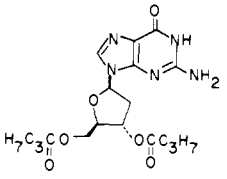
d. R = H, R = OAc



7a, R_1, R_2 = OH

b. R_1, R_2 = OAc

c. R_1 = H, R_2 = OH



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benzene was irradiated with a 450-W medium-pressure mercury lamp at 0 °C for 15 min as O_2 was continuously bubbled through the solution. Longer photolysis times appeared to destroy the product. The irradiated solution was then stirred vigorously at 100 °C for 1 h in the presence of 0.1 N HCl to cleave the glycosyl bond (an amount sufficient to dissolve a 100% yield of adduct assuming a solubility of 0.1 mg/mL). The acid layer was submitted to analysis by analytical reversed-phase HPLC (Figure 1), and the yield of adduct was determined by comparison with a solution of authentic material of known concentration. In preparative runs, the acid solution could be neutralized, concentrated, and desalted by using reversed-phase cartridges[12] at the end of a syringe (see Experimental Section), purified by preparative HPLC, and crystallized as previously reported.[2,3]

Formation of AFB_1-N^7-Guanine Adducts 3. The yield of adduct **3** did not vary significantly with changes in the molar ratio of dibutyrate **5** to AFB_1 since 5 equiv of **5** did not trap the presumed epoxide (15% yield) any better than 1 equiv (14%). However, the overall concentration of AFB_1 seemed to have some influence on yield, for solutions of approximately 10 mM concentration gave higher yields (17–19%) than those of 6 mM (9–11%) or 1 mM (1%).

2'-Deoxyguanosine 3',5'-dibutyrate (**5**) crystallized from ethanol as the hemihydrate and was used as such in reactions since material dried by repeated evaporation with pyridine did not improve the yield of adduct. In the absence of benzil, no adduct was

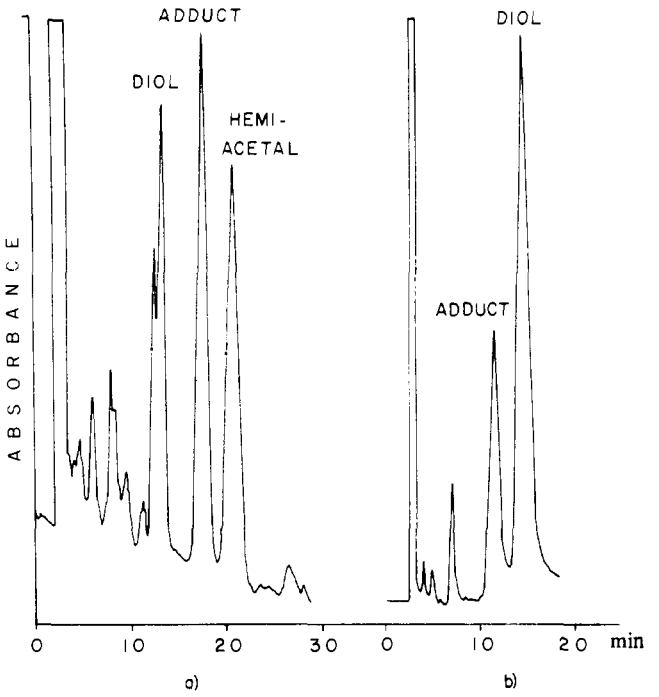

Figure 1. HPLC analysis of reaction mixture hydrolysates from the photolysis of (a) aflatoxin B_1 (20% EtOH/10 mM KOAc, pH 4.7, μ-Bondapak C_{18} column, 1 mL/min at 254 nm) and (b) sterigmatocystin (32% EtOH).

produced, indicating that the aflatoxin itself cannot sensitize the epoxide formation.

The crystalline adduct obtained proved to be identical with authentic in vivo adduct by ^1H NMR and quantitative UV spectroscopy, HPLC retention time, CD spectroscopy, and chemical methylation. In the last experiment, previously reported by Essigmann et al.,[2] the adduct is treated with dimethyl sulfate followed by acid to effect glycosyl bond cleavage. 9-Methylguanine occurs as the major purine product and the absence of any detectable 7-methylguanine confirms that the adduct is a 7-alkylated guanine.

^1H NMR analysis reveals that the stereochemistry about the terminal tetrahydrofuran ring is the same as in the natural product since coupling is observed only between protons 6a and 9a and not between 9a and 9 or 8 and 9. The protons 8, 9, and 9a are thus in a trans–trans relationship, and the epoxide therefore forms photochemically, as it does biologically, on the less hindered convex face of the molecule.[13] The circular dichroic spectra of both natural and synthetic products exhibit a positive maximum at 263 nm, indicating the same enantiomer, but the low solubility of the adduct in 0.1 N HCL prevents accurate determination of ellipticity. The ultraviolet spectrum of synthetic **3** is identical with that of the natural material and also shows no bathochromic shift in base as would be expected were the hydroxyl and guanyl substituents to be reversed (8-hydroxylaflatoxin derivatives undergo furan ring openings in base to give phenoxide intermediates).[14] Consistent with previous observations,[2] a high-resolution electron-impact mass spectrum did not show the parent molecular ion but did show the fragments $C_{17}H_{12}O_7$ (loss of guanine), methylated guanines, and $C_{15}H_{10}O_5$ (loss of methyl and CO from the cyclopentenone ring).

(9) (a) Gorst-Allman, C. P.; Steyn, P. S.; Wessels, P. L. *J. Chem. Soc., Perkin Trans. 1*, **1977**, 1360–4. (b) Coles, B. F.; Lindsay Smith, J. R.; Garner, R. C. *Ibid.* **1979**, 2664–71. (c) Coles, B. F.; Welch, A. M.; Hertzog, P. J.; Lindsay Smith, J. R.; Garner, R. C. *Carcinogenesis* **1980**, *1*, 79–80.

(10) We allowed AFB to react with MCPBA and dibutyrate **5** but obtained only small amounts of presumed adduct and low conversion of AFB_1 even after several days and excess MCPBA. cf. Martin, C. N. and Garner R. C., *Nature (London)* **1977**, *267*, 863–5.

(11) (a) Shimizu, N.; Bartlett, P. D. *J. Am. Chem. Soc.* **1976**, *98*, 4193–200. (b) Bartlett, P. D. In "Organic Free Radicals"; Pryor, W. A., Ed.; American Chemical Society: Washington, DC, 1978; *ACS Symp. Ser.* **1978**, *No. 69*, Chapter 2.

(12) Sep-Pak, available from Waters Associates, Milford, MA. See Experimental Section for detailed procedure.

(13) In Chemical Abstracts nomenclature the stereochemistry about the terminal ring in **3** and **6b** is given as 6aα,8β,9α,9aα. Proton 6a, the lowest numbered chiral center, is labeled α as a reference. Similarly, the stereochemistry of **4** and **7b** is given as 1α,2β,3aα,12cα. The α and β designations should not be confused with those in the steroid series. We thank Dr. Kurt Loening of Chemical Abstracts Service for assistance in the naming of compounds.

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The aflatoxin dihydrodiol **6a** may result from the attack of water on the reactive epoxide or by cleavage of the guanine N^7–aflatoxin bond rather than the guanine N^9–deoxyribose bond in the positively charged alkylated imidazole intermediate which presumably forms from N^7 attack on the epoxide.[15] The diol cochromatographed with an authentic sample and was fully characterized as the diacetoxy derivative **6b**[16] after treatment with acetic anhydride. Assignment of the 8β,9α (8*S*,9*R*)[13] stereochemistry was supported by the appearance of acetate resonances at δ 1.77 and 2.20. The shielding of 8β-acetoxyl group parallels that observed for AFB_{2a} acetate **6d** (δ 1.74).[17] Also, the lack of vicinal coupling among protons 8, 9, and 9a confirms their mutual trans–trans relationship.

The later-eluting hemiacetal AFB_{2a} **6c** probably arises from acid-catalyzed hydration of unreacted AFB_1 during the hydrolysis step. It was converted to the known acetate **6d**[14] for positive identification.

Synthesis of ST–N^7-Guanine Adduct 4. In an experiment similar to that performed on AFB_1, photolysis of ST (**2**) in the presence of 2′-deoxyguanosine 3′,5′-dibutyrate **5**, benzil, and oxygen and acid hydrolysis of the reaction mixture (0.1 N HCl, 1 h, 100 °C) led to two major products (Figure 1), the ST–N^7-guanine adduct **4** and ST dihydrodiol **7a**. Purification of the mixture by preparative HPLC provided a 23% yield of adduct and neutralization of an acidic solution of adduct caused precipitation of pure material (8%) whose ^1H NMR and UV spectra and HPLC retention time were identical with those of an authentic sample.[3] As has been previously described, this adduct possesses the same stereochemistry about the terminal tetrahydrofuran ring as does aflatoxin adduct **3** and is therefore also the result of ring opening of a convex face epoxide.

The other major product of the reaction, the dihydrodiol **7a**[18] exhibits a bathochromic shift in base from 327 to 340 nm, which is characteristic of 2-hydroxysterigmatocysin derivatives. (ST hemiacetal **7c**[19] undergoes a shift from 326 to 356 nm). This is in contrast to the adduct **4** and ST (**2**) itself, which undergo larger shifts of 327–380 and 328–384 nm, respectively. Acetlyation of the diol provided the diacetoxy derivative **7b** which was by IR, ^1H NMR, MS, and UV spectra and TLC identical with an authentic sample produced by peracetic acid oxidation and acetylation of ST (see Experimental Section). Similar to aflatoxin diacetoxydiol **6b**, **7b** displays acetoxyl resonances at δ 1.71 and 2.22, corresponding to a 1α,2β (1*R*,2*S*) stereochemistry, and resonances demonstrating a trans–trans relationship among protons 12c, 1, and 2. Acid hydrolysis (THF, 0.1 N HCl, reflux 18 h, N_2) of diacetoxydiol **7b** obtained from peracid oxidation provided material which cochromatographed with photochemically produced ST dihydrodiol **7a**. Unlike the AFB_1 photolysis, no ST hemiacetal **7c** was detected in the photolysis reaction mixture.[19]

Conclusion. The photochemical epoxidation of AFB_1 and ST allows the rapid and simple production of milligram quantities of their guanine adducts **3** and **4** without the use of metabolic activating systems in vitro, organ perfusion, or whole animal dosing. This method makes possible the synthesis of other mycotoxin adducts which are present in small amounts in DNA but which maybe more significant in the etiology of neoplastic transformation. For example, evidence suggests that some aflatoxin adducts result from multiple metabolic events, e.g., adducts arising from epoxidation of demethylated and hydroxylated AFB_1 metabolites AFP_1 and AFM_1.[20] Futher, experiments have suggested that aflatoxin epoxide also binds to adenine.[21] The current method now allows the possible synthesis of such adducts and a means for introducing carcinogens into defined DNA sequences.

Experimental Section

FT NMR spectra were recorded on a Bruker HX-270, Perkin-Elmer R-22, or Jeol FX-60Q spectrometer. UV spectra were obtained on a Perkin-Elmer Model 200 UV–visible spectrophotometer. IR spectra were determined on a Perkin-Elmer 237B instrument. Low-resolution mass spectra were recorded on a Varian MAT-44 chemical ionization spectrometer at 70 eV. High-resolution EI mass spectra were obtained on a Du Pont CEC-110 photoplate instrument using evaporated AgBr plates (Ionomet). All aqueous solutions were prepared from reagent-grade water (Milli-Q-System, Millipore Corp.), and organic solvents were distilled in glass. Glassware and syringes for the photolysis were oven-dried, and O_2 was passed through P_2O_5 before entering the reaction. Melting points were corrected. Microanalysis was performed by Robertson Laboratory, Florham Park, NJ. Reversed-phase HPLC was performed with a μ-Bondapak C_{18} analytical column (Waters Associates) equipped with a Model 660 solvent programmer and two M-6000A pumps except as indicated. The effluent was analyzed by a 254 nm fixed wavelength detector or a variable wavelength detector set as 254 nm. Ion-exchange HPLC was performed as described in ref 2.

Caution. All manipulations of mycotoxins and their crystalline derivatives were carried out in a well-ventilated hood by using disposable vinyl gloves. All contaminated equipment was soaked in bleach before washing.

2′-Deoxyguanosine 3′,5′-Dibutyrate (5). A suspension of dry 2′-deoxyguanosine (267 mg, 1 mmol) in a mixture of 5 mL of dimethylacetamide, 2 mL of pyridine, and 1 mL (6 mmol) of butyric anhydride was stirred under N_2 at 40 °C for 15 h. Removal of volatiles under reduced pressure gave a semisolid syrup which was taken up in 35 mL of $CHCl_3$ and washed with water (15 mL), 5% aqueous $NaHCO_3$ (15 mL), and water (20 mL). After the mixture was dried ($MgSO_4$), the chloroform was removed to provide an off-white solid which was recrystallized from EtOH (charcoal treatment) to give **5** as white crystals (171 mg, 42%): mp 191–193 °C; UV max (EtOH) 255 nm (ε 14 700), 270 (sh, 10 600); IR ($CHCl_3$) 3105, 2960, 1736, 1696, 1608, 1370, 1251, 1169, 1088 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.93 (t, 3, *J* = 7 Hz, CH_3C), 0.97 (t, 3, *J* = 7 Hz, CH_3C), 1.67 (m, 4, $MeCH_2C$), 2.35 (t, 4, CH_2CO), 2.2–3.2 (br m, 2, H-2′), 4.38 (br s, 3, H-3′, H-5′), 5.40 (br s, 1, H-4′), 6.31 (t, 1, *J* = 7 Hz, H-1′), 6.75 (br s, 2, NH_2, exchanges with D_2O), 7.88 (s, 1, H-8 of guanine), 12.44 (br s, 1, NH, exchanges with D_2O); mass spectrum, *m/e* (relative intensity) 407 (0.6,M^+), 319 (5), 257 (2), 248 (1), 232 (4), 231 (4), 221 (5), 218 (2), 215 (2), 206 (1), 199 (2), 190 (2), 189 (6), 188 (8), 187 (2), 168 (9), 151 (90), 81 (100), 71 (94). Anal. ($C_{18}H_{25}N_5O_6 \cdot {}^1/_2\ H_2O$): C, H, N.

[6a*S*-(6aα,8β,9α,9aα)]-8-(2-Amino-1,6-dihydro-6-oxo-7*H*-purin-7-yl)-2,3,6a,8,9,9a-hexahydro-9-hydroxy-4-methoxycyclopenta[*c*]furo[3′,2′:4,5]furo[2,3-*h*][1]benzopyran-1,11-dione (AFB_1–N^7-Guanine, 3). Aflatoxin B_1 (12.8 mg, 41 μmol), benzil (8.3 mg, 39 μmol), and 2′-deoxyguanosine di-*n*-butyrate (35.8 mg, 88 μmol) were dissolved in 4 mL of dry *o*-dichlorobenzene (distilled from CaH_2) and placed via syringe into a small Pyrex photolysis tube equipped with a fritted filter stick inlet to bubble in O_2 and a serum stopper inlet. The apparatus was affixed to the outside of a lamp cooling well and was submerged in an ice–water bath. Irradiation with a 450-W medium-pressure Hg lamp proceeded for 15 min, and the solution was stirred vigorously with 200 mL of 1 N HCl at 100 °C for 1 h. Quantitative analytical HPLC analysis indicated a 19% absolute yield of adduct **3** in addition to dihydrodiol **6a** and hemiacetal **6c**. The acidic aqueous solution was neutralized to pH 5 with 2.7 N KOH and adsorbed onto an activated C_{18} reversed-phase cartridge (Sep-Pak, Waters Assoc.) in two portions. Each adsorbed portion was washed with 10 mL of 20% MeOH/H_2O; then the major products were eluted with 50% MeOH (20-mL total). The combined product-containing fractions were evaporated to dryness, and the light yellow residue was dissolved in 7.5 mL of 0.1 N HCl with gentle warming. The solution was neutralized with KOH to pH 7 and centrifuged. The supernatant was loaded in three separate runs onto a μ-Bondapak C_{18} HPLC column using a Milton-Roy pump, and the column was washed at low pressure with 2% EtOH. The system was then immediately pressurized to 750 psi with 12% EtOH/10 mM KOAc (pH 5.1). Three major fractions eluting at 37 min (dihydrodiol), 50 min (adduct), and 60 min (hemiacetal) were collected. Each fraction was pure by analytical HPLC.

Adduct 3. The combined 50-min. fractions in the 12% EtOH buffer eluant were diluted to 3% EtOH and adsorbed in two separate portions

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(16) This compound was mentioned previously but not fully characterized: Swenson, D. H.; Miller, J. A.; Miller, E. C. *Biochem. Biophys. Res. Commun.* **1973**, *53*, 1260–7.

(17) (a) Wiley, M.; Waiss, A. C., Jr.; Bennett, N. *J. Assoc. Off. Anal. Chem.*, **1969**, *52*, 75–6. (b) Dutton, M. F.; Heathcote, J. G. *Chem. Ind. (London)* **1968**, 418.

(18) The 2-*m*-chlorobenzoyloxy-1-hydroxy derivative is known.[9a]

(19) Chen, P. N.; Kingston, D. G. I.; Vercelotti, J. R. *J. Org. Chem.* **1977**, *42*, 3599–605. Our sample was prepared by F_3CCO_2H-catalyzed addition of wate.

(20) Essigmann, J. M.; Donahue, P. R.; Story, D. L.; Wogan, G. N.; Brunengraber, H. *Cancer Res.* **1980**, *40*, 4085–91.

(21) D'Andrea, A. D.; Haseltine, W. A. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 4120–4.

onto a C_{18} cartridge (which had been washed with 90% MeOH, 10% MeOH, and water). The cartridge was washed with 4 mL of water and then with 5 mL of 90% MeOH to elute the adduct. Careful rotary evaporation of about half the MeOH and cooling at −5 °C overnight gave short white needles. The slurry was centrifuged, the needles were washed twice with cold absolute EtOH, the solvent was carefully removed under a stream of N_2, and the crystals were dried in vacuo to provide 1.9 mg of adduct[2] (10%, based on starting aflatoxin): ^{1}H NMR (Me_2SO-d_6) δ 3.92 (s, 3), 4.17 (d, 1, J = 5.9 Hz), 5.25 (d, 1, J = 3.9 Hz, collapses to singlet with D_2O), 6.25 (s, 1), 6.36 (d, 1, J = 3.9 Hz, exchanges with D_2O), 6.62 (s, 1), 6.87 (d, 1, J = 5.9 Hz), 7.34 (s, 1), 6.08 (br s, 2, exchanges with D_2O), 10.73 (br s, 1, exchanges with D_2O); UV max (0.1 N HCl) 364 nm (ϵ 16 200), 263, (13 800), 237 (18 000), no bathochromic shift in base; CD max (0.1 N HCl) 263 nm; high-resolution mass spectrum, m/e 328.0581 ($C_{17}H_{12}O_7$, loss of guanine), 270.0512 ($C_{15}H_{10}O_5$, loss of guanine and cyclopentenone carbonyl), 214.0649 ($C_{13}H_{10}O_3$), 193.0978 ($C_8H_{11}N_5O$, trimethylguanine), 179.0835 ($C_7H_9N_5O$, dimethylguanine), 166.0695 ($C_6H_7N_5O$-^{13}C, methylguanine), 165.0677 ($C_6H_7N_5O$, methylguanine), 151.0494 (guanine).

Methylation of adduct **3** according to the published procedure[2] with dimethyl sulfate gave 9-methylguanine, identical with authentic material by ion-exchange[2] HPLC and UV spectroscopy, as the major product. No traces of 7-methyl- or 7,9-dimethylguanine were detected.

Hemiacetal 6c. The 60-min-eluting peak was purified by using a C_{18} cartridge as described above. The 90% MeOH eluate was evaporated to provide 2.4 mg of yellow solid: UV max (EtOH) 403 nm, 290, 265 (phenoxide form). Freeze drying of a 0.1 N HCl solution of the substance gave 2.0 mg (14%) racemic blue fluorescent hemiacetal as a white solid, which was by TLC and IR and UV spectra identical with authentic material.[14a] Acetylation [AcOH, $(F_3CCO)_2O$] provided the known hemiacetal acetate (TLC, IR).[14a]

Dihydrodiol 6a. The blue fluorescent material eluting at 37 min had the same reverse-phase HPLC retention time and TLC behavior as authentic dihydrodiol: UV max (EtOH) 360 nm, 264, 240 (sh); UV max (EtOH/NaOH) 400 nm, 290. About half of the material was purified with a C_{18} cartridge, giving 3.7 mg of yellow gum which was dissolved in 2 mL of acetic anhydride containing one crystal of *p*-toluenesulfonic acid. After 18 h the volatile components were removed in vacuo, and the residue (5.8 mg) was purified by preparative TLC (silica gel, 3% MeOH/$CHCl_3$). The band of R_f 0.26 was removed and rechromatographed (1% MeOH/$CHCl_3$, 3 developments) to give a trace of compound A (R_f 0.47) and 1.1 mg of compound B (R_f 0.40). Compound B was identical (TLC, MS, 270-MHz NMR) with the 8β,9α-diacetoxydiol **6b** obtained from peracetic acid oxidation of AFB_1 (vide infra). The compound A had acetate resonances at δ 2.20 and 2.10, suggesting the 8α,9α product. Compound A: mass spectrum, m/e (relative intensity) 430 (0.4), 370 (0.4), 328 (1.2), 299 (1.7), 271 (2.7), 91 (100), 43 (88.6). Compound B: 430 (0.6), 370 (3.5), 299 (4.8), 271 (7.4), 91 (81.6), 43 (100).

[6a*S*-(6aα,8β,9α,9aα)]-8,9-Bis(acetyloxy)-2,3,6a,8,9,9a-hexahydro-4-methoxycyclopenta[*c*]furo[3′,2′:4,5]furo[2,3-*h*][1]benzopyran-1,11-dione (Aflatoxin Diacetoxydiol 6b). A 1.03 M solution of anhydrous peracetic acid in benzene[22] (0.8 mL, 0.83 mmol) was added to a dry stirred solution of aflatoxin B_1 (120 mg, 0.385 mmol) in 3 mL of CH_2Cl_2. After 15 h at 25 °C, volatiles were removed in vacuo to give a light yellow residue which was treated with 2 mL of acetic anhydride in 4 mL of dry pyridine for 1.5 h. Removal of solvent gave a dark solid which was purified by preparative TLC (6% EtOH/benzene). The major component was recrystallized from EtOAc (charcoal) to provide 55 mg (34%) of **6b** as white crystals: mp 260–261 °C dec; UV max (CH_3CN) 350 nm (ϵ 20 100), 263 (12 100), 255 (sh, 8900), 221 (21 400); IR ($CHCl_3$) 2955, 1778, 1768, 1694, 1632, 1605, 1563, 1485, 1444, 1375, 1348, 1305, 1235, 1210, 1144, 1136, 1066, 1019, 988, 855 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.77 (s, 3, C-8 Ac), 2.20 (s, 3, C-9 Ac), 2.66 (m, 2) and 3.46 (m, 2, A_2B_2 of cyclopentenone ring), 4.02 (s, 3, $ArOCH_3$), 4.24 (d, 1, J = 5.5 Hz, H-9a), 5.40 (s, 1, H-9), 6.33 (s, 1, H-8), 6.44 (s, 1, ArH), 6.70 (d, 1, J = 5.5 Hz, H-6a); mass spectrum, m/e (relative intensity) 430 (5, M$^+$), 370 (4), 328 (39), 327 (13), 311 (12), 300 (17), 299 (54), 298 (32), 289 (18), 288 (11), 287 (8), 283 (20), 282 (10), 272 (20), 271 (66), 270 (34), 259 (10), 115 (11), 55 (10), 43 (100); high-resolution mass spectrum calcd for $C_{21}H_{18}O_{10}$, m/e 430.0900 (found m/e 430.0904).

[1*R*-(1α,2β,3aα,12cα)]-2-Amino-1,7-dihydro-7-(1,2,3a,12c-tetrahydro-1,8-dihydroxy-6-methoxy-7-oxo-7*H*-furo[3′,2′:4,5]furo[2,3-*c*]xanthen-2-yl)-6*H*-purin-6-one (ST-*N*^{7}-Guanine 4). Sterigmatocystin (10 mg, 31 μmol), benzil (8.5 mg, 40 μmol), and 2′-deoxyguanosine 3′,5′-di-*n*-butyrate (**5**) (13.6 mg, 33.4 μmol) were dissolved in 2.5 mL of dry *o*-dichlorobenzene, and the heterogeneous solution was irradiated as described above for 20 min and hydrolyzed with 80 mL of 0.1 N HCl at 100 °C for 1 h. Analytical HPLC (Figure 1) showed only two major products eluting at 12 and 15 min (32% EtOH, 10 mM KOAc, pH 4.7), the former and lesser of which cochromatographed with authentic adduct. Injection of authentic sterigmatocystin hemiacetal **7c**[17] showed that none had been produced. The acidic solution was neutralized to pH 6 with 3 N KOH and adsorbed onto a C_{18} cartridge in three separate portions. Each portion was washed with 5 mL each of water, 10% MeOH, 40% MeOH, and 90% MeOH, the major products being eluted in the last solvent. This solution was evaporated, and the residue was taken up in 5 mL of 0.1 N HCl (neutralization to pH 4.5 caused precipitation of product but no increase in purity, even upon repeated precipitation). Neutralization to pH 6 and preparative HPLC chromatography provided two fractions. Fraction I was evaporated after Sep-Pak purification (90% MeOH elution) to provide 3.4 mg of adduct **4** (23%). Solution of this product in 6.5 mL of 0.1 N HCl, neutralization to pH 4.5 with 3 N KOH, and cooling at −5 °C overnight provided 1.2 mg white crystalline adduct[3] (8%) which was isolated by centrifugation: UV max (0.1 N HCl) 326 nm (relative ϵ 0.43), 251 (1.0), 240 (1.80); UV max (base) 373 nm (relative ϵ 0.76), 316 (1.0), 266 (2.45); ^{1}H NMR (Me_2SO-d_6) δ 3.87 (s, 3, OCH_3), 4.26 (d, 1, J = 5.9 Hz, H-12c), 5.21 (s, 1, H-1, C*H*OH), 6.31 (s, 1, H-2, OCHN), 6.61 (s, 1, ArH), 6.74 (d, 1, J = 8.4 Hz, H-11), 6.92 (d, 1, J = 7.8 Hz, H-9), 6.89 (d, 1, J = 5.9 Hz, H-3a, OCHO), 6.46 (br s, 2, NH_2), 7.64 (t, 1, J = 8.4 Hz, H-10), 7.52 (br s, 1, H-8 of guanine),[23] 11.14 (br s, 1, NH), 13.30 (br s, 1, ArOH).

Fraction II was purified and concentrated by using a Sep-Pak and evaporated to provide 1.4 mg of diol **7a** as a yellow solid: UV max (32% EtOH, pH 4.7) 327 nm (relative ϵ 1), 267 (sh, 0.5), 248 (2), 232 (1.5); UV max (base) 340 nm, 263 (sh), 242 (sh); mass spectrum, m/e (relative intensity) 359 (1), 358 (1, M$^+$), 342 (1), 341 (5), 324 (4), 312 (3), 311 (3), 300 (3), 295 (5), 279 (18), 278 (43), 277 (31). Acetylation of the diol (Ac_2O, 50 °C, 18 h) and preparative TLC purification provided diacetoxydiol **7b**, which was by TLC and UV, IR, and ^{1}H NMR spectra identical with authentic material produced by acetic peracid oxidation and acetylation of sterigmatocystin (vide infra). Hydrolysis of a small amount of peracid-derived diacetate (THF/0.1 N HCl, reflux 18 h, N_2) provided dihydrodiol **7a** of the same HPLC retention time as photochemical material.

[1*R*-(1α,2β,3aα,12cα)]-1,2-Bis(acetyloxy)-1,2,3a,12c-tetrahydro-8-hydroxy-6-methoxy-7*H*-furo[3′,2′:4,5]furo[2,3-*c*]xanthen-7-one (ST Diacetoxydiol 7b). A solution of sterigmatocystin (16.3 mg, 50 μmol) in 1 mL of CH_2Cl_2 was treated with an excess of peracetic acid in CH_2Cl_2 in two portions over 4 days at 25°C. Volatiles were removed in vacuo and the residue was dissolved in 2 mL of Ac_2O and 10 drops of pyridine. After 2 days the yellow residue remaining after evaporation was purified by preparative TLC (2 dev, $CHCl_3$) to provide 21.8 mg (98%) of crude product. Recrystallization from CH_2Cl_2/ether gave a first crop of 13.8 mg of yellow-white crystals of **7b** (62%): mp 225 °C dec; UV max ($CHCl_3$) 245 nm (ϵ 35 300), 250 (sh, 34 100) 280 (3970), 319 (14 700), 360 (sh, 3480); IR ($CHCl_3$) 3000, 1740, 1640, 1622, 1614, 1590, 1490, 1460, 1200 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.71 (s, 3, C-2 Ac), 2.22 (s, 3, C-1 Ac), 4.01 (s, 3, OCH_3), 4.17 (d, 1, J = 6 Hz, H-12c), 5.56 (s, 1, H-1), 6.33 (s, 1, H-2), 6.38 (s, 1, aryl), 6.66 (d, 1, J = 6 Hz, H-3a), 6.78 (d, 1, J = 8 Hz, H-9 or -11), 6.97 (d, 1, J = 8 Hz, H-9 or -11), 7.53 (t, 1, J = 8 Hz, H-10); mass spectrum, m/e (relative intensity) 442 (3, M$^+$), 383 (0.5), 382 (0.8), 311 (13), 281 (21), 43 (100); high-resolution mass spectrum calcd for $C_{22}H_{18}O_{10}$, 442.0900 found, m/e 442.0876.

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(23) Chemical shift varies from sample to sample with water concentration.