

## Structural identification of the major DNA adduct formed by aflatoxin B<sub>1</sub> *in vitro*

(chemical carcinogens/nucleic acids/high-pressure liquid chromatography/field-desorption mass spectrometry)

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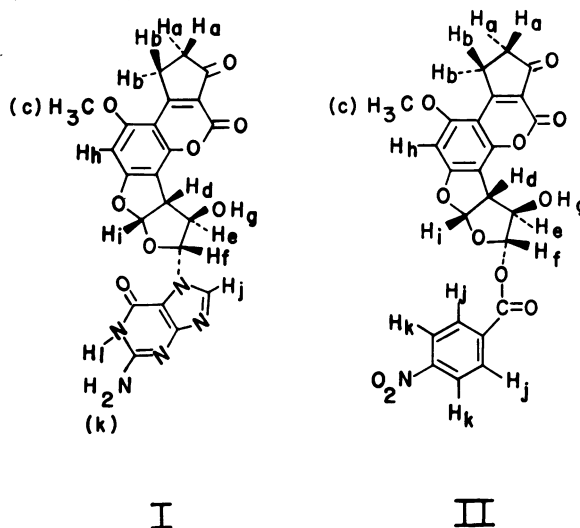
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**ABSTRACT** The covalent binding of the hepatocarcinogen aflatoxin B<sub>1</sub> by rat liver microsomes to calf thymus DNA resulted in a binding level equal to one aflatoxin residue per 60 DNA nucleotides. An aflatoxin derivative-guanine adduct was efficiently liberated from DNA with formic acid. Analytical reversed-phase high-pressure liquid chromatography of the DNA hydrolysate revealed that approximately 90% of the carcinogen bound to DNA could be accounted for as a single component. Preparative high-pressure liquid chromatography was used to isolate sufficient quantities of the adduct for structural analysis from large quantities (340 mg) of DNA. A combination of spectral and chemical data indicates that the major product of the interaction of metabolically activated aflatoxin B<sub>1</sub> and DNA is 2,3-dihydro-2-(N<sup>7</sup>-guanyl)-3-hydroxyaflatoxin B<sub>1</sub> with the guanine and hydroxyl functions possessing a *trans* configuration. The structural data support the hypothesis that the putative 2,3-oxide of aflatoxin B<sub>1</sub> is quantitatively important as an intermediate in the binding of aflatoxin B<sub>1</sub> to nucleic acids.

Structural modifications of cellular macromolecules by chemical carcinogens may represent early and requisite events in neoplastic transformation (1, 2). Through interactions of this nature, qualitative changes could be induced in informational macromolecules such as DNA and RNA, and these lesions could provide a molecular basis for alteration of gene expression in carcinogenesis. Identification of the products of these reactions (herein referred to as adducts) is essential in order to: (i) gain insights into mechanisms of carcinogen activation; (ii) determine the reactive centers in these macromolecules; (iii) follow the kinetics of appearance and disappearance of adducts in the cell; and (iv) relate specific patterns of macromolecule modification with the ultimate development of tumors in target organs of susceptible species.

Aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) is a very potent liver carcinogen in several animal species (3), and epidemiologic evidence indicates that it is also an important factor in the etiology of human liver cancer in certain sections of the world (4). AFB<sub>1</sub> binds covalently to cellular macromolecules, including DNA, *in vivo* (5-7) and *in vitro* after metabolic activation (8-10). The relationship of this type of interaction to its mechanism of action has been emphasized (11). Strong indirect evidence has indicated the production of AFB<sub>1</sub>-2,3-oxide as a major activated metabolite responsible for macromolecular binding *in vitro* and *in vivo* (5-7, 9, 12), but structures of specific adducts formed with nucleic acids or proteins have not been determined. The purpose of the research reported here was to determine the structure of the major adduct formed with DNA by AFB<sub>1</sub> activated

metabolically *in vitro*. The results indicate that approximately 90% of the binding *in vitro* can be attributed to a single adduct, which was isolated in sufficient quantity for structural analysis and identified as 2,3-dihydro-2-(N<sup>7</sup>-guanyl)-3-hydroxyaflatoxin B<sub>1</sub> (structure I).



### MATERIALS AND METHODS

Liver microsomes used for metabolic activation of AFB<sub>1</sub> were prepared from phenobarbital-treated male Fischer rats (13) by the procedure of Kinoshita *et al.* (14). The incubation mixture (400 ml) for the binding of AFB<sub>1</sub> to DNA included Tris-HCl (pH 7.5, 45 mM), MgCl<sub>2</sub> (3 mM), glucose-6-phosphate (5 mM), NADP (0.8 mM, Sigma Chemical Co.), glucose-6-phosphate dehydrogenase (0.4 unit/ml, Sigma Chemical Co.), approximately 1 mg of microsomal protein per ml, calf thymus DNA (20 A<sub>260</sub> units/ml or a total of 340 mg; type I, Sigma Chemical Co.), AFB<sub>1</sub> [224 μM added in dimethyl sulfoxide; isolated and purified by the method of Asao *et al.* (15)], and [<sup>3</sup>H]AFB<sub>1</sub> (0.4 mCi, Moravsek Biochemicals). The incubation mixture was shaken at 37° for 90 min, and the reaction was terminated by addition of 32 ml of 4 M NaCl/80 ml of 0.17 M sodium dodecyl sulfate and, after 1 min, 512 ml of phenol/chloroform/isoamyl alcohol (50:50:1, wt/vol/vol). After shaking, the emulsion was broken by centrifugation, and DNA was recovered from the aqueous epiphase by ethanol precipitation (0°). The DNA fibers were spooled onto glass rods and were rinsed twice with benzene, once with chloroform, once with ethanol, and twice with diethyl ether.

Bound adduct was removed from 340 mg of DNA by incubation in 20 ml of formic acid (88%) at ambient temperature

Abbreviations: AFB<sub>1</sub>, aflatoxin B<sub>1</sub>; I, 2,3-dihydro-2-(N<sup>7</sup>-guanyl)-3-hydroxyaflatoxin B<sub>1</sub>; II, 2,3-dihydro-3-hydroxy-2-(4-nitrobenzoxy)-aflatoxin B<sub>1</sub>; HPLC, high-pressure liquid chromatography; NMR, nuclear magnetic resonance; FD, field-desorption mass spectrometry; EI, electron-impact mass spectrometry.

for 60 min. After addition of 80 ml of water, the solution was lyophilized at 0.1 torr (13 Pa). The sample was reconstituted to 100 ml with 10% methanol and held at 0° for 4 hr before centrifugation at  $13,000 \times g$  for 15 min. Portions of hydrolysate supernatant were submitted to both analytical and preparative reversed-phase high-pressure liquid chromatography (HPLC), using a Micromeritics model 7000 chromatograph equipped with a Waters Associates model 440 detector (254 and 365 nm). Analytical chromatography of DNA hydrolysates (Fig. 1) was performed on a  $\mu$ Bondapak C<sub>18</sub> column (Waters Associates) eluted at 50° and 1 ml/min with a water/methanol gradient ranging from 10 to 80% methanol over 40 min. During preparative chromatography, large volumes of DNA hydrolysates (e.g., 30 ml) containing liberated adduct were placed directly onto a 70  $\times$  0.8 cm column packed with C<sub>18</sub>-Corasil B (Waters Associates) through the pumping head of a positive displacement pump (Milton Roy Co., model 196-31) at 0.8 ml/min. After the sample was loaded, the column was connected to the Micromeritics pumping system and eluted at 3 ml/min with 10% methanol until material absorbing at 254 nm was completely removed. The adduct was eluted from the column by programming the eluant composition to 80% methanol over 40 min. The adduct eluted at 30 min and was crystallized from the column effluent (approximately 60% methanol) at 4°.

UV spectra of the adduct (I) were obtained on a Cary model 14 recording spectrophotometer: UV max (0.1 M HCl, pH 1.25) 238, 263, 364 nm ( $\epsilon$  17,700, 14,100, 16,100). Because of difficulty in obtaining completely dry material for weighing, there may be some error associated with the absolute values of molar absorptivities. The absorbance maxima remained unaltered in alkaline solution (pH 10).

A portion of the adduct was methylated as part of the structural analysis. The adduct (1 mg) was treated with 66 mg of dimethyl sulfate in *N,N*-dimethylacetamide for 6 hr at 25° by a method similar to that of Jones and Robins for methylation of guanosine (16). The methylated adduct was purified by preparative reversed-phase HPLC. After removal of solvent, the product was treated with 0.1 ml of perchloric acid (72%) at 100° for 60 min. (Adduct not subjected to methylation was hydrolyzed under the same conditions to liberate guanine.) The solution was neutralized with 0.47 ml of 3 M aqueous KOH and adjusted to pH 4 with formic acid (88%). After filtration, portions of hydrolysates were submitted to base analysis by cation-exchange HPLC (see Fig. 3) on a 25  $\times$  0.22 cm Durrum DC-4A column eluted isocratically with 0.1 M ammonium formate (pH 4.0) at 0.6 ml/min at 62°.

A new compound, 2,3-dihydro-3-hydroxy-2-(4-nitrobenzoxy)afatoxin B<sub>1</sub> (II) was synthesized as a model for the adduct by treatment of natural AFB<sub>1</sub> (62 mg) with *p*-nitroperoxybenzoic acid (37 mg) in dry dichloromethane. After 6 hr at 25°, the crude nitrobenzoate was collected by filtration, washed with ether, then dried (yield, 54 mg, 65%). Recrystallization (three times) from dichloromethane/methanol/ether gave II as a light yellow solid, which decomposed without melting at 292°: UV max (CH<sub>3</sub>CN) 223, 241, 257, 264, 352 nm ( $\epsilon$  25,420, 18,420, 19,050, 20,590, 20,730); infrared (Nujol) 3280, 1772, 1766, 1745 cm<sup>-1</sup>; mass spectrum *m/e* (relative intensity, %) 495 (0.5, M<sup>+</sup>), 328(3), 312(1), 271(5), 270(22), 242(5), 214(16), 199(6), 171(5), 167(7), 137(19), 121(6), 120(23), 115(5), 92(6), 65(100). The nuclear magnetic resonance (NMR) spectral data (Table 1) were consistent with the structure assigned.

Field-desorption mass spectra were obtained on a Varian model 731 spectrometer with a field desorption/field ionization/electron impact combined source. Samples were applied by the dipping technique (17) to a 10- $\mu$ m tungsten wire that

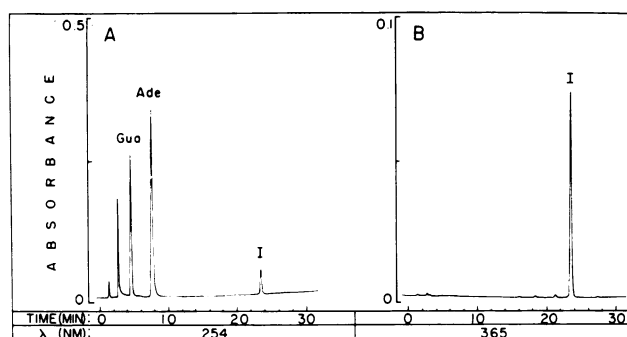


FIG. 1. Analytical HPLC of an aflatoxin-bound DNA hydrolysate. Chromatogram of a formic-acid hydrolysate representing approximately 18  $\mu$ g of calf thymus DNA incubated *in vitro* in the presence of AFB<sub>1</sub> and rat liver microsomes. The analytical reversed-phase HPLC column was eluted with a water/methanol gradient as described in *Materials and Methods*, and the detection system monitored absorbance at 254 nm (A) and 365 nm (B). The components Ade, Gua, and I are adenine, guanine, and the adduct, 2,3-dihydro-2-(N<sup>7</sup>-guanyl)-3-hydroxyafatoxin B<sub>1</sub>, respectively.

was conditioned (benzonitrile) at 40 mA. The anode heating current was 22 mA. Electron-impact mass spectra were obtained on a DuPont CEC-110 photoplate instrument using evaporated AgBr plates (Ionomet). The resolution obtained was 18,000 (at half peak width), and the average accuracy in the mass determination was  $\pm 2$  millimass units.

The 270 MHz <sup>1</sup>H-NMR spectra were obtained on approximately 0.5 mg of adduct (I) dissolved in deuterodimethyl sulfoxide or in deuterium chloride/deuterium oxide using a Bruker model HX-270 spectrometer operating in the Fourier-transform mode and equipped with an internal heteronuclear deuterium lock. The 90 MHz NMR spectrum of compound II was obtained on a Hitachi Perkin-Elmer R-22 spectrometer.

## RESULTS

### Modification of DNA with AFB<sub>1</sub> and Adduct Isolation.

The binding *in vitro* of AFB<sub>1</sub> to DNA by rat live microsomes resulted in a level of modification equal to one AFB<sub>1</sub> moiety per 60 DNA nucleotides, a level comparable to those obtained with this carcinogen in other systems (8, 10). When microsomal preparations were denatured by heating at 100° for 3 min, no significant binding of AFB<sub>1</sub> to DNA occurred. Treatment of unadducted DNA with formic acid at 25° was found to release 66% of the constituent purine bases. The adduct (I), however, was almost completely liberated from adduct-bound DNA by identical treatment, presumably due to destabilization of the glycosidic bond as is characteristic of 7-substituted guanine deoxyribonucleosides (16, 18–20). Analytical HPLC (Fig. 1) revealed that approximately 90% of the radioactivity from the carcinogen originally incorporated into DNA eluted with the adduct peak.

Procedures were developed for isolation of sufficient quantities of purified adduct for structure analysis, including large-scale incubations, which provided quantities of adduct in excess of 1 mg. Isolation by analytical HPLC of the adduct from hydrolysates of over 300 mg of DNA was impractical. Consequently, a preparative HPLC system was constructed. In contrast to unmodified nucleic acid components, the adduct is lipophilic and does not migrate significantly when the reversed-phase column is eluted with 10% methanol. Therefore, lyophilized DNA hydrolysates were dissolved in this solvent and large volumes (e.g., 30 ml or the equivalent of over 90 mg of

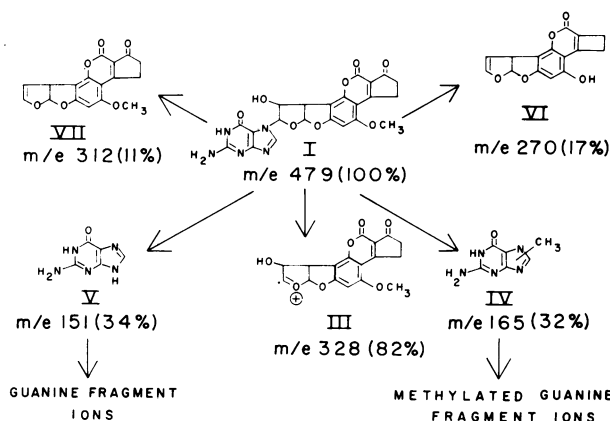


FIG. 2. Proposed structures and fragment ions observed in the field-desorption mass spectrum of adduct (I). The molecular ion at  $m/e$  479 was the base peak; numbers in parentheses are relative abundances of fragment ions. The ions III, IV, V, VI, and VII gave compositions by high-resolution electron-impact mass spectrometry that were attributed to  $C_{17}H_{12}O_7$ ,  $C_6H_7N_5O$ ,  $C_5H_5N_5O$ ,  $C_{15}H_{10}O_5$ , and  $C_{17}H_{12}O_6$ , respectively.

DNA) were delivered onto the column. The adduct accumulated on the column, while more polar components (adenine, guanine, and partially degraded DNA) were eluted. After removal of the adduct from the column with a water/methanol gradient, crystalline material was obtained from the appropriate column effluent fraction by precipitation at 4°. Approximately 5 mg of white crystals were recovered from 340 mg of DNA, and these were pure with respect to other 254 and 365 nm-absorbing components by analytical HPLC.

**Structure Analysis.** The structure of the adduct (I) was deduced from a combination of chemical, NMR, UV, and mass spectral evidence. Analysis of the adduct by high-resolution electron-impact (EI) and low-resolution field-desorption (FD) mass spectrometry provided significant structural information (Fig. 2). FD of the adduct resulted in a single molecular ion at  $m/e$  479 which, at higher emitter currents, yielded three additional prominent fragment ions at  $m/e$  328, 165, and 151. Although the molecular ion at  $m/e$  479 was not detected in the EI mode, the latter three ions were measured by high resolution and gave compositions  $C_{17}H_{12}O_7$ ,  $C_6H_7N_5O$ , and  $C_5H_5N_5O$ , respectively. These can be accounted for as ions III, IV, and V in Fig. 2. Additional fragments observed in the FD spectrum gave compositions determined by EI as  $C_{17}H_{12}O_6$  (VII) and  $C_{15}H_{10}O_5$  (VI), which are attributable to AFB<sub>1</sub> ( $m/e$  312) and the product of *O*-dealkylation and loss of carbon monoxide from the cyclopentenone ring of AFB<sub>1</sub> ( $m/e$  270). The major ion at  $m/e$  328 (III) had the composition of an oxygenated AFB<sub>1</sub> produced by the facile cleavage of the adduct bond. The compositions of several other ions measured are similar to those observed (21) in the fragmentation of guanine (V,  $m/e$  151) and a methylated guanine (IV,  $m/e$  165). The partial alkylation of guanine would appear to result from intramolecular transfer following ionization, for no alkylated products were detected at higher mass than the molecular ion at  $m/e$  479. Further evidence for the presence of guanine in the adduct was obtained by chemical cleavage of the adduct bond with perchloric acid. The base released was indistinguishable from authentic guanine by cation-exchange HPLC, UV, and mass spectral characteristics.

Additional evidence for structure I was gained from UV and high-resolution NMR spectra. A 270 MHz Fourier transform-NMR spectrum of I (deuterodimethyl sulfoxide) was quite similar to that of AFB<sub>1</sub>, except for added resonances at  $\delta$  10.75

Table 1. NMR data for compounds I and II

Proton	Chemical shift ( $\delta$ )* (relative no. protons, multiplicity, coupling)	
	I	II
a	[2.57 (2 H, m)] <sup>†</sup>	2.58 (2 H, m)
b	[3.30 (2 H, m)] <sup>†</sup>	3.22 (2 H, m)
c	3.90 (3 H, s)	3.90 (3 H, s)
d	4.15 (1 H, d, $J$ = 5.6)	4.18 (1 H, d, $J$ = 6)
e	5.23 (1 H, d, $J$ = 4.4) <sup>‡</sup>	4.70 (1 H, br s) <sup>‡</sup>
f	6.24 (1 H, s)	6.38 (1 H, s)
g	6.38 (1 H, d, $J$ = 4.4) <sup>§</sup>	6.38 (1 H, br s) <sup>§</sup>
h	6.62 (1 H, s)	6.69 (1 H, s)
i	6.87 (1 H, d, $J$ = 5.6)	6.89 (1 H, d, $J$ = 6)
j	7.33 (1 H, s)	7.50 (2 H, m, A <sub>2</sub> of A <sub>2</sub> B <sub>2</sub> )
k	6.10 (2 H, br s) <sup>§</sup>	8.15 (2 H, m, B <sub>2</sub> of A <sub>2</sub> B <sub>2</sub> )
l	10.75 (1 H, br s) <sup>§</sup>	— — —

\* Chemical shifts are given in parts per million downfield from internal tetramethylsilane in deuterodimethyl sulfoxide. Abbreviations used are: s, singlet; d, doublet; m, multiplet; br, broad;  $J$ , coupling constant in Hertz.

<sup>†</sup> Chemical shifts observed in deuterium oxide-deuterium chloride solution.

<sup>‡</sup> Resonance collapses to a sharp singlet upon addition of deuterium oxide.

<sup>§</sup> Resonance disappears upon addition of deuterium oxide.

(H<sub>i</sub>), 7.33 (H<sub>j</sub>), and 6.10 (H<sub>k</sub>) which were attributed to the guanine portion (22). Comparison of chemical shifts for the adduct (Table 1) and AFB<sub>1</sub> (15) revealed that chemical modification had occurred in the terminal dihydrofuran ring. The presence of a secondary hydroxyl group was evident from the pair of coupled doublets (4.4 Hz) at  $\delta$  6.38 (H<sub>g</sub>) and 5.23 (H<sub>e</sub>) which collapsed to a sharp singlet ( $\delta$  5.26, H<sub>e</sub>) upon exchange of the hydroxyl proton with deuterium oxide. The singlet at  $\delta$  6.24 was assigned to aminal proton, H<sub>f</sub>.

Assignment of the hydroxyl function to C-3 of I is based on chemical shift data and on the absence of a base-induced bathochromic shift in the UV spectrum of I. Such base-induced shifts are quite indicative of AFB<sub>1</sub> derivatives bearing a hydroxyl function at C-2 (23).

The absence of spin-spin coupling (24) between proton pairs H<sub>d</sub>, H<sub>e</sub> and H<sub>e</sub>, H<sub>f</sub> and the observation of coupling (5.6 Hz) between H<sub>d</sub> and H<sub>i</sub>, protons of known *cis* configuration (25), establishes a *trans-trans* relationship for H<sub>d</sub>, H<sub>e</sub>, and H<sub>f</sub>. This permits assignment of absolute stereochemistry to I, because that of AFB<sub>1</sub> is known (25).

The 90 MHz NMR spectrum of synthetic 2,3-dihydro-3-hydroxy-2-(4-nitrobenzoxy)AFB<sub>1</sub> (II) provided additional chemical shift data in support of structure I. Compound II, obtained by treatment of natural AFB<sub>1</sub> with 4-nitroperoxybenzoic acid, had almost identical chemical shifts to those of the aflatoxin portion of I. The absence of coupling in II between protons H<sub>d</sub>, H<sub>e</sub>, and H<sub>f</sub> confirms the *trans-trans* orientation of these protons. This result is consistent with the expected attack of the peroxyacid on AFB<sub>1</sub> from the more sterically accessible convex face to provide the extremely reactive, probably transient  $\beta$ -2,3-epoxide, which suffers facile epoxide ring opening to *trans* addition product II in the presence of 4-nitrobenzoic acid.

The positions on guanine where substitution by the AFB<sub>1</sub> moiety could occur are N<sup>1</sup>, N<sup>2</sup>, N<sup>3</sup>, O<sup>6</sup>, N<sup>7</sup>, and C-8. The following lines of evidence based on spectral and other data indicate N<sup>7</sup> to be the site of attachment. The presence of a broad

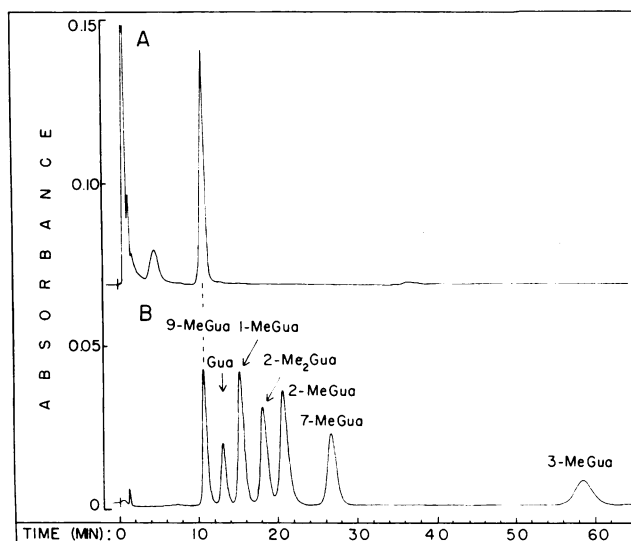


FIG. 3. Determination of the methylated guanine released by hydrolysis of a methylated derivative of the adduct (I). The adduct was methylated with dimethyl sulfate in *N,N*-dimethylacetamide. The product was purified by preparative reversed-phase HPLC and was hydrolyzed with perchloric acid to cleave the bond between the aflatoxin moiety and the methylated base. The hydrolysate was analyzed by cation-exchange HPLC (A) and the retention time of the major product (11 min) was compared to retention times of other methylated guanines (B).  $N^7,N^9$ -Dimethylguanine (not shown) eluted at 106 min under these conditions and was not detected in the experiment shown in A. Chromatographic conditions are given in *Materials and Methods*. 1-MeGua,  $N^1$ -methylguanine; 2-MeGua,  $N^2$ -methylguanine; 2-Me<sub>2</sub>Gua,  $N^2,N^2$ -dimethylguanine; 3-MeGua,  $N^3$ -methylguanine; 7-MeGua,  $N^7$ -methylguanine; 9-MeGua,  $N^9$ -methylguanine.

two-proton singlet in the NMR spectrum at  $\delta$  6.10 suggested a free amino group at  $N^2$  of guanine and rendered linkage at  $N^2$  unlikely. The mass spectral data support this contention, in that no AFB<sub>1</sub> fragments were found containing nitrogen. Such fragments would be expected if the adduct were linked through the  $N^2$  of guanine (26–28). Attachment at C-8 is ruled out by the presence of a one-proton singlet in the adduct NMR spectrum at  $\delta$  7.33 (attributed to H<sub>j</sub> of I). As expected, this proton shifted downfield (to  $\delta$  8.40) in acidic medium. Observation of the amide proton at  $\delta$  10.75 effectively eliminated the possibility of substitution at  $N^1$  and O<sup>6</sup> (29) and made substitution at  $N^3$  of guanine unlikely (30). Taken together, these spectral data prompted consideration of  $N^7$  as the most chemically reasonable site of attachment, especially in view of the ease with which the adduct was removed from DNA and the accessibility of this position in the DNA molecule to attack by electrophilic reagents (31).

The adduct methylation experiment provided further evidence in support of  $N^7$  as the attachment site. The adduct was treated with dimethyl sulfate under conditions that are selective for methylation of the imidazole ring nitrogens (16). A major methylation product (presumed to be  $N^9$ -methyl-I) was obtained and purified by preparative HPLC. Hydrolysis of the methylated adduct with perchloric acid gave a single methylated guanine, having an HPLC retention time identical to that of authentic  $N^9$ -methylguanine (Fig. 3A and clearly distinct from other possible methylated guanines that could have been formed under these conditions (Fig. 3B). The identity of  $N^9$ -methylguanine was further established by isolation (HPLC) and determination of its UV spectrum, which was found to be indistinguishable from that of authentic  $N^9$ -methylguanine.

Isolation of  $N^9$ -methylguanine and the absence of detectable methylation at  $N^7$  implies an adduct structure in which the guanine base is substituted at  $N^7$ . A structure with the aflatoxin at any other position (e.g.,  $N^3$ ,  $N^2$ , etc.) would be expected to give significant quantities of  $N^7$ -methylguanine and, possibly, higher alkylated products under the above methylation and hydrolysis conditions (16, 32). The possibility that  $N^7,N^9$ -dimethylguanine was a significant methylation product was also eliminated by the absence of a peak at the retention time of this base in the methylated adduct hydrolysate chromatogram (not shown in Fig. 3B, because this base eluted at 106 min).

## DISCUSSION

The adduct identified in this study as 2,3-dihydro-2-( $N^7$ -guanyl)-3-hydroxyaflatoxin B<sub>1</sub> (I) represents the major product formed by the interaction of metabolically activated AFB<sub>1</sub> with DNA *in vitro*. This work extends that of other workers (5–7, 9, 12) in supporting the hypothesis that the proposed AFB<sub>1</sub>-2,3-oxide is quantitatively important as an intermediate in the binding of the carcinogen to nucleic acids. We have recently found that the *in vitro* adduct identified in this study is identical chromatographically and in certain spectral characteristics to the major adduct produced in liver DNA when AFB<sub>1</sub> is administered to rats (R. G. Croy, unpublished data). This may indicate a functional significance for I, in view of the fact that tissue susceptibility to tumor formation in the rat seems to correlate with the extent of total covalent binding of AFB<sub>1</sub> to DNA (7). Alternatively, the situation could be analogous to modification of DNA by alkylating agents, where the predominant modified bases, 7-alkylguanines, are believed to be of lesser importance to tumorigenesis than less abundant products (29, 33). It is noteworthy that the adduct, like the 7-alkylguanines, has a positively charged imidazole ring system in DNA, and it is possible that apurinic sites could be formed spontaneously in the polynucleotide chain as a result of labilization of the glycosidic bond. Additional distortions of the structure of DNA could result from the perturbations by non-polar aflatoxin residues of normal stacking of bases within the double helix. While these factors possibly contribute to a complex alteration of DNA structure, it is difficult to assess their significance as initiating events for carcinogenicity or toxicity, because very little is known about the ways that structural modifications alter the functional properties of nucleic acids.

While a sizeable literature exists on the incorporation of alkyl residues (primarily methyl and ethyl groups) into nucleic acids by alkylating agents (20), comparatively few data exist on the interactions of larger functional groups derived from other classes of carcinogens. Weinstein and coworkers have recently identified the products of the reaction of a synthetic 7,12-dimethylbenz[*a*]anthracene epoxide and a synthetic benzo[*a*]pyrene diol epoxide with poly(G), and both compounds were found to bind to the  $N^2$  of guanine (26–28). One of the benzo[*a*]pyrene adduct isomers has identical properties to those of an adduct formed in RNA when this carcinogen is incubated with bovine bronchial explants (27). The carcinogenic arylamine and arylamide, *N*-methyl-4-aminoazobenzene and 2-acetylaminofluorene, respectively, bind to hepatic nucleic acids predominantly at C-8 of guanine (1, 34, 35), though a minor product recently has been tentatively identified for the latter carcinogen which, like the polycyclic aromatic hydrocarbons, is bound to the  $N^2$  of guanine (36).

Further studies on identifying the products of interactions of chemical carcinogens with macromolecules should help to clarify the extent to which this phenomenon is related to car-

cinogenesis. The present research represents the initial results of investigations of the specific products formed when metabolically activated AFB<sub>1</sub> reacts with DNA.

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