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Destruction of Aflatoxin B_1 with Sodium Bisulfite: Isolation of the Major Product Aflatoxin B_1S

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ABSTRACT

The reaction between aflatoxin B_1 and sodium bisulfite yielded almost quantitatively a light yellow, highly fluorescent water-soluble product, aflatoxin B_1S. Reverse-phase (C_{18}) high pressure liquid chromatography (with and without the paired-ion technique), and normal- and reverse-phase thin layer chromatography revealed the ionic product at greater than 98% yield. The product's intense fluorescence, identical in color to that of aflatoxins B_1 and B_2, supported the conclusion that the lactone of aflatoxin B_1 was not opened by bisulfite and that the reaction took place at some other location on the aflatoxin B_1 molecule. The UV spectrum of aflatoxin B_1S exhibited identical absorbance maxima to aflatoxin B_1 and these maxima exhibited no bathochromic shift in alkaline solution. Aflatoxin B_1S exhibited change to bright yellow fluorescence under 365 nm UV light after being sprayed with 20% sulfuric acid in methanol. The IR spectrum of aflatoxin B_1S indicated the vinylene group of the furofuran ring system of aflatoxin B_1 was missing from aflatoxin B_1S, whereas the coumarin-cyclopentenone ring system present in aflatoxin B_1 was intact in aflatoxin B_1S. Furthermore, the IR spectrum suggested the presence of a sulfonate moiety in B_1S. Proton NMR spectra of aflatoxin B_1S also indicated that the cyclopentenone region was intact and that the furofuran had undergone reaction with bisulfite. Integration of the NMR spectrum of aflatoxin B_1S revealed 12.75 to 13.25 protons, consistent with a bisulfite addition across the double bond of aflatoxin B_1 to form aflatoxin B_1S. Aflatoxins B_2 and G_2 were not susceptible to the action of bisulfite, again supporting the conclusion that bisulfite acts at the unsaturation in the furofuran ring system of aflatoxin B_1. There was no evidence that aflatoxin B_2 was produced in the degradation of aflatoxin B_1 by bisulfite under these conditions. The fact that previously reported chemical modifications of this furofuran ring system resulted in reduced toxicity support the potential utility of bisulfite as a means of detoxification of aflatoxin-contaminated commodities.

Aflatoxins are a group of mycotoxins produced by *Aspergillus flavus* Link ex Fries and *Aspergillus parasiticus* Speare in corn, cottonseed, peanuts and other commodities in the field and storage. Aside from their much feared carcinogenic potential, aflatoxins cause serious economic loss to the nation's agriculture every year. The majority of the corn produced in North Carolina since 1976, when systematic surveys were initiated, has been contaminated with aflatoxin when it was offered for sale. In 1980, North Carolina agriculture lost an estimated $200,000,000 because of aflatoxin-induced losses to corn growers, corn dealers and animal producers (T. E. Nichols, personal communication). This recurring situation has motivated research in the prevention, elimination and detoxification of aflatoxin in farm commodities. Undoubtedly, prevention is the best method for controlling mycotoxins in feeds (*16*), but, in spite of all efforts, contamination is unavoidable at the present time. Consequently, much effort has been expended in developing methods for the detoxification of aflatoxin-contaminated materials. The most satisfactory method to date has been ammoniation (*16,25*) which has been commercialized for corn and cottonseed meal. Nevertheless, the ammoniation process is unsatisfactory in many regards because of its comparatively high cost, its potential toxicity and explosiveness, and the brown color and poor handling characteristics of the treated product which is marketed at a discount.

A potentially great improvement in detoxification of aflatoxin was reported by Doyle and Marth (*11-13*) who found that bisulfite degraded aflatoxins B_1 and G_1 in solution. Later, Moerck et al. (*27*) reported that sodium bisulfite was more effective in destroying aflatoxin in corn than were sodium hydroxide or aqueous ammonia. In addition to its apparent effectiveness, bisulfite is an acceptable food additive (*15,20,26,30,36*) which is commonly added to wines, fruit juices, jams, dried fruits and several other products where bisulfite inhibits both enzymatic and non-enzymatic browning, acts as an antioxidant and a reducing agent, and effectively controls the growth of microorganisms. These reports were particularly encouraging in view of an earlier report by Trager and Stoloff (*37*) that solutions of bisulfite and sulfite were not promising for destruction of the four primary aflatoxins even though there were reports of potassium sulfite inhibiting the biosynthesis of aflatoxins by mold mycelia (*8,17*).

The mechanism of bisulfite degradation of aflatoxin B_1 is unknown, but Doyle and Marth (*11-13*) reported that, in experiments using radio-labeled aflatoxin B_1, about 91% of the total aflatoxin B_1 was converted to unrecovered water-soluble form(s) which could not be partitioned into chloroform. They also found at least two products representing the remaining 9% of the total aflatoxin which were detected in the chloroform extracts of the reaction mixtures. The highly polar nature of the major product(s), and the report by Dey and Row (*9*) that unsaturated coumarins react readily with bisulfite in aqueous solution resulting in cleavage of the lactone ring and addition of sulfonic acid across the double bond, suggested that aflatoxins B_1 and G_1, which have the same unsaturated coumarin moiety, might react similarly with bisulfite to form a sulfonic acid addition product (*11-13*). However, Dey and Row (*9*) also reported that substituted coumarins had low or no reactivity to bisulfite depending on the size of the substituent groups. Since aflatoxins are substituted coumarins, they would not be expected to react unless some other portion of the molecule was susceptible. In addition, Doyle and Marth (*11-13*) proposed, on the basis of an oxygen-induced, anti-Markovnikov addition of bisulfite to alkenes (*31*) and of the presence of a vinylene in the furofuran moiety of aflatoxins B_1 and G_1, that a bisulfite radical may react at this site rather than at the unsaturated lactone to form a sulfonate addition product. An alternate mechanism of aflatoxin destruction was suggested by Moerck et al. (*27*) which involved nucleophilic attack by bisulfite on the carbonyl carbon of the cyclopentenone ring of aflatoxin B_1 to form a bisulfite addition product.

The purpose of the present communication is to report the occurrence of a single major product in the bisulfite degradation of aflatoxin B_1, its isolation, some of its properties and its tentative identification.

MATERIALS AND METHODS

Crystalline aflatoxins (B_1, B_2, G_1 and G_2) were purchased from Calbiochem-Behring Corp. (LaJolla, CA). Solvents, including water, were high pressure liquid chromatography (HPLC)-grade and reagents were ACS-grade (Fisher Scientific, Raleigh, NC). Toxins were dissolved in methanol or chlorofrom and quantified spectrophotometrically at 361 nm (*28*). Aflatoxins used for reactions were dissolved in methanol at the desired concentrations then diluted 1:9 with a 10% solution of freshly prepared sodium bisulfite solution, pH approx. 5.5. Progress of reactions was monitored by HPLC and/or thin layer chromatography (TLC). In preliminary studies, microgram quantities of aflatoxins were treated with bisulfite at ambient temperature by this method. Aflatoxin B_1S for isolation was prepared by dissolving 200 mg of aflatoxin B_1 in 100 ml of methanol and diluting 1:9 with a 10% aqueous solution of sodium bisulfite and incubating overnight at 100°C in a foil-capped 1-L Erlenmeyer flask before evaporation of methanol and isolation of aflatoxin B_1S with C_{18} SepPak cartridges (Water Associates, Milford, MA). Final purification was by HPLC.

HPLC was accomplished using a Waters Associates system including: two 6000A pumps, Wisp 710B automatic injector, a system controller, a data module dual pen recorder-integrator, 440 absorbance detector (operated at 254 or 365 nm), or a Kratos/Schoeffel FS 950 fluorescence detector equipped with 360-nm excitation and 418-nm emission filters (Kratos, Westwood, NJ). Column was a 10-μ particle size C_{18} reverse-phase cartridge in a Waters RCM-100 radial compression module. For paired-ion chromatography, tetrabutyl ammonium phosphate (Waters PIC Reagent A) was used in the mobile phase at 0.005 M over a linear gradient from 100% water to 80% methanol over a 10-min period. A linear solvent gradient (10 min) from 100% water to 100% methanol at a flow rate of 3 ml/min was used without ion pairing. There was a 5-min hold at the end of the solvent programs. Aflatoxin B_1S was first isolated by collection from HPLC column effluent. Effluent was lyophilized and the dry, light yellow powder redissolved in methanol. The use of C_{18} SepPak cartridges expedited isolation with methanol-free reaction mixtures being passed through cartridges which had been activated with methanol then washed with water. Cartridges were then washed with 5 ml of water to remove residual bisulfite and then aflatoxin B_1S was eluted with methanol. Aflatoxin B_1S was isolated by removal of methanol or by addition of ethyl ether to solution until aflatoxin B_1S precipitated.

Whatman $KC_{18}F$ reverse-phase, 20×20-cm thin layer (0.2 mm) chromatography plates (Bodman Chemicals, Doraville, GA) spotted with aflatoxin and reaction mixtures were chromatographed (equilibrated tank) in a solvent system of ethanol:water (80:20, vol/vol, containing 0.5 M NaCl). Normal phase TLC was done on 20×20-cm, Silica Gel 60 thin layer (0.24 mm) plates (E. Merck, Darmstadt, West Germany) in chloroform:methanol (97:3, vol/vol) or methanol. Plates were examined under long (356 nm) and short wave (254 nm) ultraviolet light (*10*), sprayed with 20% sulfuric acid, and re-examined. Finally, plates were heated for 20 min at 120°C and examined for charred spots.

Ultraviolet spectra (UV) of aflatoxins B_1 and B_1S (400 to 210 nm) in 95% methanol were obtained on a Perkin-Elmer III (Coleman) UV-VIS spectrophotometer (Hitachi, Ltd., Tokyo, Japan). Extinction coefficients ($E^{1\%}_{1cm}$) were also determined in 95% methanol. A 1% solution of aflatoxin B_1S in 95% methanol was diluted 1:14 with 30% ammonium hydroxide and the UV spectrum examined for a bathochromic shift (*6,18,24*). Infrared spectra (IR) of aflatoxins B_1, B_2 and B_1S were obtained from KBr pellets or Nujol mulls (*14*) on a Perkin-Elmer 501 IR spectrometer (Perkin-Elmer Corporation, Norwalk, CT). Proton nuclear magnetic resonance (NMR) spectra of aflatoxins B_1, B_2, G_1, G_2 and B_1S were obtained using a Varian EM390 NMR 90MHz spectrometer (Varian Associates, Palo Alto, CA). Compounds were dissolved in deuterated dimethyl sulfoxide, deuterated methanol, deuterated chloroform, deuterated chloroform containing a few drops of trifluoroacetic acid, or trifluoroacetic acid for NMR analysis.

Chemical confirmation of retention of the cyclopentenone moiety in aflatoxin B_1S by formation of the 2,4-dinitrophenylhydrazone (*7,23*) was done on a TLC plate spotted with aflatoxins B_1 and B_1S and benzene sulfonic acid following differential development in chlorofrom:methanol (97:3, vol/vol) to move aflatoxins B_1 and G_2 and benzenesulfonic acid, and then 100% methanol to move aflatoxin B_1S.

RESULTS

Normal phase TLC of bisulfite reaction mixtures revealed two major fluorescent spots, the residual B_1 and the major spot (called aflatoxin B_1S) which remained at the origin in normal phase TLC with the relatively nonpolar solvent systems customarily used for aflatoxin analysis (*18*). Aflatoxin B_1S would migrate, however, when the developing solvent was 100% methanol. A very minor spot with an R_f similar to that of aflatoxin B_2 was sometimes seen in TLC of mixtures in which all detectable aflatoxin B_1 has been converted to aflatoxin B_1S.

The colors of the fluorescent spots seen on TLC were blue like that of aflatoxin B_1, although the intensity of aflatoxin B_1S was considerably brighter than the aflatoxin B_1 in controls. On spraying with 20% sulfuric acid in methanol, the fluorescence of the B_1S spot changed to yellow in the fashion of aflatoxin B_1. When the plates spotted with B_1 and B_1S were charred, only spots corresponding to these could be detected. After spraying TLC plates with the 2,4-dinitrophenylhydrazine reagent, aflatoxins B_1 and

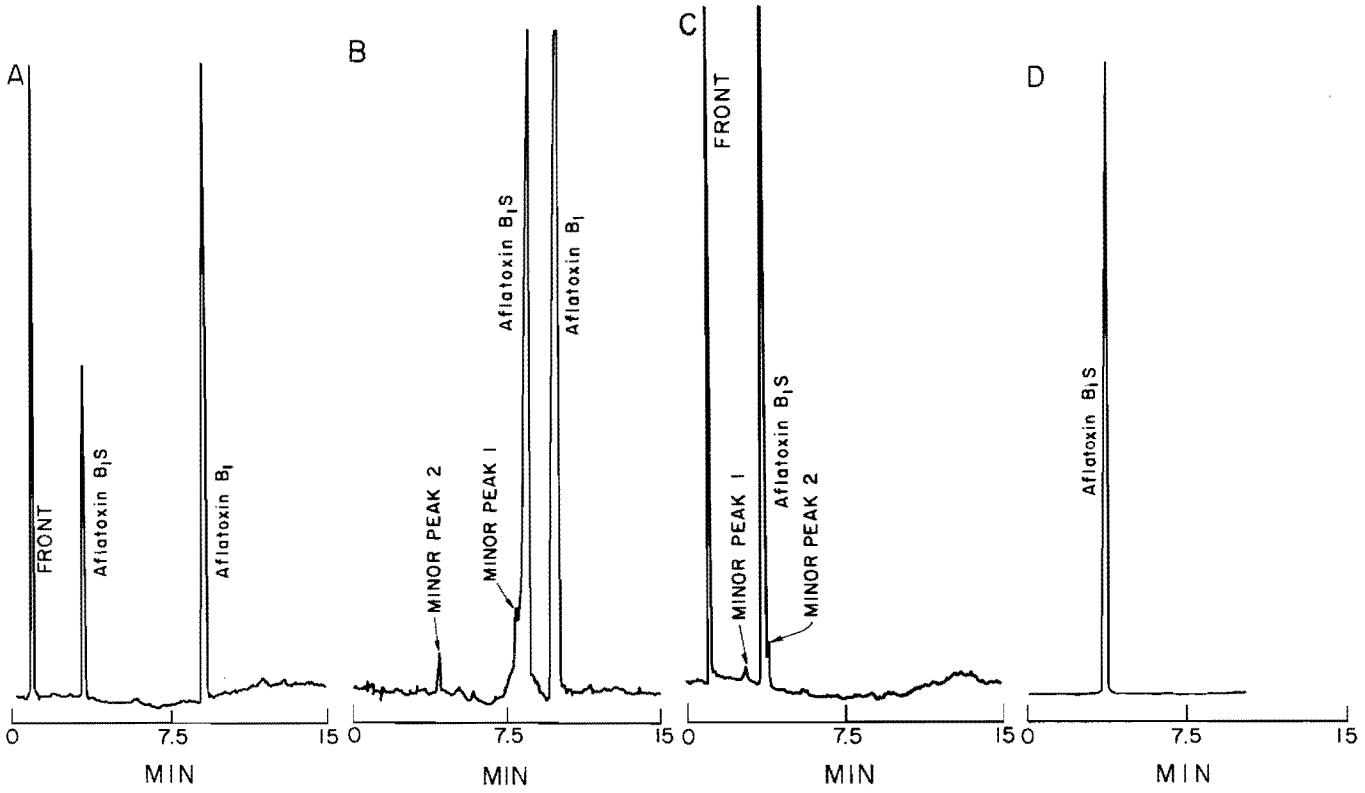

Figure 1. *HPLC chromatograms of aflatoxin B_1 destruction by sodium bisulfite (detector operated at 365 nm and 0.02 AUFS).*
(A) Chromatogram showing partial conversion of aflatoxin B_1 to B_1S.
(B) Paired ion chromatography of partially reacted mixture.
(C) Off-scale chromatogram of final reaction mixture showing minor products.
(D) Chromatogram of pure aflatoxin B_1S.

B_1S turned orange, whereas aflatoxin G_1 and benezensulfonic acid did not, indicating the cyclopentenone moiety was still intact in aflatoxin B_1S (*22,23*).

The minor spot which co-chromatographed with aflatoxin B_2 on TLC apparently was another compound, as indicated by the failure to find an aflatoxin B_2 peak on HPLC which was extremely sensitive for aflatoxins B_2 and G_2 under the conditions used. Aflatoxin B_2 itself was completely refractory, as was aflatoxin G_2, to sodium bisulfite under reaction conditions which gave essentially 100% destruction of aflatoxin B_1. Traces of other blue-fluorescent compounds, probably present in the commercial aflatoxin B_1 starting material, were sometimes noted on TLC.

Reverse-phase HPLC of reaction mixtures disclosed three major peaks whose retention times coincided with aflatoxin B_1, the solvent front containing bisulfite and an unknown peak whose thin-layer chromatographic behavior was identical to that of the spot named aflatoxin B_1S (Fig. 1A). In addition, there were two very minor peaks in the chromatograms whose identities are uncertain (Fig. 1B,C). The retention times of aflatoxins B_1, B_2 and B_1S with and without the presence of the paired-ion reagent in reverse-phase HPLC are given in Table 1. Based on retention times, reaction mixtures contained aflatoxin B_1S, B_1, a minor material that had a shorter retention time than aflatoxin B_1S and a minor material with a slightly longer retention time. There was a peak in the column void volume (Table 1, Fig. 1A) which was due to sodium bisulfite. When the reaction mixture was chromatographed by HPLC in the presence of the tetrabutyl ammonium counter ion, only aflatoxin B_1S of the major peaks was delayed on the column, moving with a retention time of 7.85 min instead of the normal 3.37 min (Fig. 1B). The peak in the column void volume disappeared in the paired-ion chromatography. It should be emphasized that there was no indication

TABLE 1. *Retention times of components of reaction mixtures in reverse-phase HPLC.*[a,b]

Component	Retention time (min)	
	Without paired ion reagent[c]	With paired ion reagent
Solvent front	0.95	---
Aflatoxin B_1	9.10	9.17
Aflatoxin B_2	9.00	9.00
Aflatoxin B_1S	3.37	7.85
Minor Peak 1	2.50	7.75
Minor Peak 2	3.41	3.41

[a]Absorbance detector was operated at 365 or 254 nm and the fluorescence detector operated at 360 nm excitation and 418 nm emission. The absorbance and fluorescence peaks coincided.
[b]C_{18} column with solvent programming from 0 to 80% methanol at a flow-rate of 3.0 ml/min over a 10-min period with a 5-min hold at 80% methanol.
[c]Paired ion reagent was tetrabutylammonium phosphate at 0.005 M.

TABLE 2. *UV absorbance maxima and extinction coefficients of a 1% solution of aflatoxin B_1S in 95% methanol.*[a]

Wavelength (nm)	$E^{1\%}_{1cm}$	Coefficient of variation %
361	367 ± 4.6	2.18
264	201 ± 7.6	6.57
219	287 ± 11.3	6.79

[a]Values are means of three replicates ± standard errors.

in either reverse-phase or paired-ion chromatography on HPLC of the presence of aflatoxin B_2.

Aflatoxin B_1S was isolated by collecting from C_{18} Sep-Pak cartridges and from HPLC effluents. The chromatographic purity of aflatoxin B_1S after isolation is shown in Fig. 1D. Operation of the absorbance detector at 254 nm revealed no additional components. Aflatoxin B_1S was light yellow, freely soluble in water, dimethyl sulfoxide and trifluoroacetic acid, fairly soluble in methanol, and insoluble in chloroform. The UV absorbance characteristics of aflatoxin B_1S are shown in Table 2. Aflatoxin B_1S was essentially identical in absorption maxima and similar in extinction coefficients to aflatoxin B_1 (*4,18,28,32*). This indicated the reaction between bisulfite and aflatoxin B_1 had no great effect on the chromophore. As an example, reduction of the cyclopentenone moiety of aflatoxin B_1 to yield aflatoxicol shifts the 361 to 362 nm maximum to about 325 nm (*5,18,21,34,35*). In addition, the identity of the spectra for B_1 and B_1S indicates that the coumarin system of B_1 was not disturbed in the reaction (*6,21,23,38,40*). No change in the UV spectrum of aflatoxin B_1S was induced by addition of ammonium hydroxide to the 45% methanol solution, though the maximum at 219 nm was obscured by end absorbance. This treatment opens the furofuran rings of aflatoxin B_{2a}, the hydration product of aflatoxin B_1 (*6,18*). The fluorescence properties of B_1S were practically identical to aflatoxins B_1 and B_2 except for an apparent intensification of the fluorescence. From the extinction coefficients (Table 2) and HPLC, the yield of aflatoxin B_1S from aflatoxin B_1 was calculated to be about 98%. Any other products were minor since they constituted a total of less than 2% of the starting material.

Overall, the IR spectra of aflatoxins B_1, B_2 and B_1S were closely related, though that of B_1S was more similar to the spectrum of aflatoxin B_2. The maxima at 1067 and 722 cm^{-1}, which have been attributed to the vinyl ether system in B_1, were not present in the spectra of B_2 and B_1S, whereas the bands at 1750, 1630 and 1590 cm^{-1} attributed to the coumarin portion of the molecule were in the spectra of all three compounds (*2,3,5,6,18,19,21, 23,34,38,40*). There was an intense absorbance in the IR spectrum of B_1S at 1210 cm^{-1} associated with a sulfonate moiety and a medium intensity band at 3460 cm^{-1} which was also consistent with the presence of a sulfonate moiety (*14*). Bands at 1685 and 1760 cm^{-1}, characteristic of a coumarin containing a cyclopentenone, were present in all three spectra (*1,2,5,19,23,35*).

The NMR spectra of aflatoxins B_1, B_2, G_1, G_2 and B_1S were closely related, and those of B_1, B_2, G_1 and G_2 agreed closely with published information (*1-3,5,21,33, 34,38*). The spectrum of B_1S was quite similar to the spectra of B_1 and B_2 in regard to the cyclopentenone protons and dissimilar to those of G_1 and G_2 in regard to the lactone protons of the latter compounds (Table 3). The chemical shifts associated with the *d*-position of the furofuran system, methoxy and aromatic protons were quite similar for all five compounds (*33*). The spectrum of B_1S contained signals in locations similar to those assigned to the protons of the furofuran system in the other aflatoxins. The B_1S spectrum revealed the signals from protons assigned to positions *a* and *c* ($\delta = 4.82$ to 5.04) were not completely resolved; this peak was integrated clearly for two protons. That the signal of the methoxy protons overlapped with the signal of position *b* was clear because the peak at $\delta = 4.07$ to 4.41 integrated for five protons. Note that the signals due to the *a*, *b* and *c* positions of the furofuran system were not shifted as far upfield in B_1S as in B_2 or G_2 when compared to B_1 or G_1. Repeated runs indicated between 12.75 and 13.25 protons for B_1S. Final assignments await decoupling experiments and ^{13}C-NMR analysis.

TABLE 3. *Proton NMR chemical shifts.*[a]

	Aflatoxin				
	B_1	B_2	G_1	G_2	B_1S
Protons	(δ = ppm relative to tetramethylsilane)				
Cyclopentenone	2.58; 3.36	3.02; 3.71	----	----	2.99; 3.72
Lactone	----	----	3.40; 4.36	3.46; 4.41	----
Methoxy	3.93	4.12	3.90	3.93	4.12
Aromatic	6.39	6.62	6.35	6.32	6.63
Furofuran					
a	6.43	4.39	6.42	4.18	4.82 - 5.04
b	5.42	2.49	5.40	2.30	4.07 - 4.41
c	4.72	3.89	4.72	3.67	4.82 - 5.04
d	6.79	6.73	6.75	6.46	6.82

[a]Spectra of aflatoxins B_1, B_2, G_1 and G_2 were obtained in deuterated chloroform, whereas spectra of B_1S were obtained in trifluoroacetic acid.

DISCUSSION

Several properties of aflatoxin B_1S, the major product of degradation of aflatoxin B_1 by sodium bisulfite, suggested that the compound was a sulfonate. The very polar nature of aflatoxin B_1S in both HPLC and TLC suggested the introduction of a group much more polar than a hydroxyl group. The solubility of the isolated B_1S in water but not in chloroform, which is directly opposite the solubility of aflatoxin B_1, also indicated drastic change in the polarity of the product such as would be caused by the introduction of a sulfonate group. These solubilities agreed with the report of Doyle and Marth (*12,13*) that the product could not be extracted from water with chloroform. The ionic nature of the product was indicated by its behavior in counter-ion chromatography. The intense IR absorbances of aflatoxin B_1S at 1210 cm^{-1} and a medium intensity absorbance at 3460 cm^{-1} are also consistent with the presence of a sulfonate moiety. Finally, there is precedent for the formation of sulfonates when sodium bisulfite is reacted with compounds of the nature of aflatoxin (*9,31*).

Consideration of the literature and the structure of aflatoxin B_1 suggests three possible sites where bisulfite could have attacked the aflatoxin molecule, specifically at the cyclopentenone, at the unsaturated lactone, and at the vinylene group of the furofuran portion (Fig. 2). The addition of bisulfite at the cyclopentenone group as suggested by Moerck et al. (*27*) is ruled out by the ability of aflatoxin B_1S to form its 2,4-dinitrophenylhydrazone, by the retention of the UV absorption spectrum identical to aflatoxin B_1, by the presence of infrared absorption bands at 1685 and 1760 cm^{-1} characteristic of coumarin containing cyclopentenone, by the failure of aflatoxin B_2, which has a cyclopentenone moiety, to react with bisulfite, and by the ability of aflatoxin G_1, which does not contain a cyclopentenone moiety, to react with bisulfite. These data argue against the mechanism proposed by Moerck et al. (*27*) which invoked a nucleophilic attack by bisulfite on the carbonyl carbon of the cyclopentenone ring. The reaction of bisulfite with the unsaturated coumarin moiety of aflatoxin B_1 does not seem likely, despite reports by Dey and Row (*9*) that unsaturated coumarins react readily with bisulfite in aqueous solution resulting in cleavage of the lactone ring and addition of sulfonic acid across the double bond, because such reaction is greatly retarded or prohibited in substituted coumarins such as aflatoxin. The retention of essentially unaltered UV absorption and fluorescence by aflatoxin B_1S indicated that the lactone ring was intact because Lee et al. (*22,23*) reported that the cleavage of the lactone ring of aflatoxin B_1 resulted in a non-fluroescent product. The retention of the unaltered fluorescence and absorption also suggested that the conjugation between the carbonyl group of the lactone and the benzenoid ring of the coumarin moiety remained intact. Additional evidence suggesting that bisulfite did not attack this portion of the aflatoxin B_1 molecule was the failure of aflatoxins B_2 and G_2 to react despite being identical to aflatoxins B_1 and G_1 in this portion of the molecule. Therefore, the absence of evidence for these two possibilities implies that the third possibility is most likely.

The addition of bisulfite to the vinylene portion of the furofuran moiety of aflatoxin B_1 to form a sulfonate was proposed by Doyle and Marth (*12,13*) on the precedent of oxygen-induced, anti-Markovnikov addition of bisulfite to alkenes (*31*). They pointed out the similarity of this proposed reaction to the formation of the hydrated forms, B_{2a}, and G_{2a}, from aflatoxins B_1 and G_1 by addition of H_2O across the same double bond under mildly acidic conditions (*29*). Additional proof for this site of bisulfite addition to aflatoxin B_1 lies in the failure of aflatoxins B_2 and G_2, which do not possess a vinylene group in the furofuran rings, to form bisulfite addition products. IR absorption bands at 1067 and 722 cm^{-1}, which have been attributed to the vinyl ether system in aflatoxin B_1, were not present in the spectra of aflatoxin B_2 or B_1S. This absence in B_1S strongly suggests that the vinyl ether system is the site of attack. As noted earlier, the proton NMR spectra were not only consistent with the formation of a sulfonate at this position, but could not be explained in another fashion. The major remaining problem in establishing the identity of aflatoxin B_1S was deciding to which of the two possible positions the bisulfite added. On the basis of the NMR spectra of B_1S, specifically on the unambigous integration of the peak at $\delta = 4.82$ to 5.04 as two protons and the overlap of the signal accounting for two protons at $\delta = 4.07$ to 4.41 with the methoxy peak ($\delta = 4.12$), we concluded that addition of a sulfonyl moiety across the furofuran double bond at *a* in a manner analogous to hemiacetal formation with B_1 was most likely. The proposed structure of B_1S is shown in Fig. 2. Fast atom bombardment mass spectrometry of aflatoxin B_1S revealed a molecular weight of 416 a.m.u. which is identical to that calculated for the proposed structure of B_1S (*39*).

Some discrepancies between the present results and earlier reports deserve comment. Doyle and Marth (*12,13*) reported the formation of aflatoxin B_2 during bisulfite destruction of aflatoxin B_1 whereas Moerck et al. (*27*) found a decrease in the content of aflatoxin B_2 when corn contaminated with both B_1 and B_2 was treated with bisulfite. In the present study, we found neither formation nor destruction of aflatoxin B_2 when aflatoxin B_1 and aflatoxin B_2 were treated with sodium bisulfite. The importance of these observations lies in the fact that Doyle and Marth (*12,13*) used their observations to support the possibility that bisulfite attacks the double bond in the furofuran moiety, while Moerck et al. (*27*) used their observation of the non-accumulation and destruction of aflatoxin B_2 to support the idea that bisulfite adds to the cyclopentenone moiety through a nucleophilic attack on the carbonyl carbon. Regardless of which of the three reports is precisely correct, the observations we made on the pure compounds suggest that bisulfite attacks the double bond in the furofuran system of aflatoxin B_1 to form a sulfonate.

The toxicological properties of aflatoxin B_1S are unknown at present, but a substantial decrease in toxicity can be anticipated on the basis that changes in the furofuran

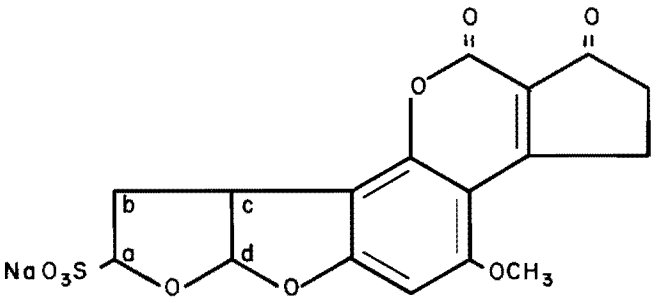

Figure 2. *Proposed sturcture of aflatoxin B_1S.*

moiety of aflatoxin B_1 generally lead to large decreases in toxicity. Lillehoj and Ciegler (*24*) reported approximately a 200-fold decrease in toxicity (ability to produce bile ductile hyperplasia in ducklings) when aflatoxin B_1 was hydrated to form aflatoxin B_{2a}. This decrease was so great that Ciegler and Peterson (*6*) proposed the conversion of aflatoxin B_1 to aflatoxin B_{2a} as a possible method for the detoxification of corn or other commodities contaminated with aflatoxin.

Our failure to find destruction of aflatoxin B_2 by bisulfite would not necessarily prevent the successful employment of a bisulfite detoxification process, assuming that aflatoxin B_1S is sufficiently less toxic than aflatoxin B_1, because aflatoxin B_2 customarily accounts for less than 10% of the total aflatoxin in contaminated materials and because it is several-fold less toxic than aflatoxin B_1. Aflatoxin B_1 is the major aflatoxin in corn which in turn is the major source of aflatoxin among grains. The other major aflatoxin is aflatoxin G_1 which was reported to be susceptible to bisulfite attack (*12*) and which we have confirmed (data not shown).

It is obvious that several research needs must be fulfilled before bisulfite degradation of aflatoxin B_1 and G_1 can be considered for adaptation as a practical process. The stereochemical characterization of aflatoxin B_1S must be completed, the toxicological evaluation of B_1S and of contaminated commodites treated with the bisulfite process must be done, and the bisulfite treatment process in contaminated corn or other commodities must be optimized. Despite these rather formidable tasks, the bisulfite process, which apparently can give essentially 100% degradation of aflatoxin B_1 to a single product, suggests a possible benefit over the ammoniation process which gives rise to several unknown products of unknown toxicology.

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