or appetizers and in sauces. The dark green leafy vegetables, small amounts of which are used in almost every dish, also represent a valuable source of the vitamin. Soybean is a moderate source (1.3 ± 0.17). α-Tocopherol is not, however, its principal tocopherol, but γ-tocopherol (Bauerfeind, 1980), which has 3–20% the anti-erythrocyte haemolysis activity of α-tocopherol in the rat (Machlin and Brin, 1980), is. Its third most abundant tocopherol, δ-tocopherol, has a very low anti-haemolysis activity; the fourth most abundant, α-tocotrienol, has an activity similar to that of γ-tocopherol. It may be, therefore, that the total vitamin E activity of soybean is very roughly double the α-tocopherol content.

It was found previously the mean value for serum vitamin E in adults in Singapore is 0.84 mg/100 mL (4.9 μg/mg of cholesterol) (Candlish, 1981), and so the figures in the table increase the probability that vitamin E nutrition in the region is adequate.

ACKNOWLEDGMENT

Thanks are extended to Lim Beng Gek for the assays and to Drs. Weber and Leuenberger of Hoffmann-La Roche (Basel) for their gift of the standards.

Registry No. α-Tocopherol, 59-02-9.

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Received for review November 20, 1981. Accepted August 19, 1982.

Fast Atom Bombardment Mass Spectrometry of Aflatoxins and Reaction Products of Sodium Bisulfite with Aflatoxins

Aflatoxins B_1S and G_1S, products of reaction of aflatoxins B_1 and G_1 with sodium bisulfite, were isolated by HPLC and analyzed by fast atom bombardment mass spectrometry (FAB-MS) using direct analysis and metal exchange reactions within the mass spectrometer. Molecular weight determinations and confirmation of identity of B_1S and G_1S were done by introduction of the compounds individually into the mass spectrometer or in the presence of LiCl, KCl, or NaCl to induce metal exchange reactions. B_1S and G_1S were found to be the respective sodium sulfonates of B_1 and G_1 with molecular weights of 416 and 432 amu. The parent aflatoxins and aflatoxins B_2 and G_2 yielded strong $(M + 1)^+$ fragments on direct analysis by FAB-MS and the corresponding metal-containing fragments when ionized in the presence of other metal salts used.

Aflatoxins are toxic, mutagenic, teratogenic, and carcinogenic furocoumarin secondary metabolites produced by the fungi *Aspergillus flavus* link ex. Fries and *Aspergillus parasiticus* Speare. These mycotoxins are frequently found as natural contaminants of corn as well as in peanuts, Brazil nuts, pistachio nuts, copra, cotton seed, and occasionally small grains and other commodities.

The potent carcinogenic activity of aflatoxins and the high incidence of aflatoxins in commodities destined for feed and foodstuffs as well as the potential for transmission of active residues or metabolites of aflatoxin into animal products such as milk, eggs, or edible tissues makes them of concern to human as well as animal health (Rodricks and Stoloff, 1977). This concern has been strengthened by epidemiological evidence associating the aflatoxin in human diets in Africa and Southeast Asia with liver cancer and acute aflatoxicosis in humans (Van Rensburg, 1977).

While prevention of aflatoxin contamination is the preferred method of avoiding contamination of feeds and foods (Goldblatt and Dollear, 1979), this is not possible at the present time. Therefore, detoxification of feeds and foods containing aflatoxin or other mycotoxins appears to be desirable. Of the many possible detoxification methods (Goldblatt and Dollear, 1979; Doyle and Marth, 1978a,b), ammoniation has been best developed (Heathcote and Hibbert, 1978; Bagley, 1979). Another promising experimental process is treatment of aflatoxin-contaminated substrates with bisulfite. Doyle and Marth (1978a,b) observed that potassium bisulfite solutions buffered at pH 5.5 degraded pure aflatoxins B_1 and G_1. Moerck et al. (1980) tested bisulfite for its ability to destroy aflatoxins B_1 and B_2 in naturally contaminated corn and presented data indicating destruction of aflatoxins B_1 and B_2 equal to or better than that achieved with ammonium hydroxide or sodium hydroxide. Hagler et al. (1982) have shown that bisulfite destroys aflatoxin B_1 in naturally contaminated corn but generally not aflatoxin B_2. Using pure aflatoxins, the authors found that aflatoxin B_1 was quantitatively converted to a yellow, water-soluble derivative, aflatoxin B_1S, similar in spectral characteristics to the aflatoxin B_1 starting material. IR spectra suggested that the product was a sulfonate but definitive data were lacking.

Mass spectrometry has been performed successfully on aflatoxins by using various ionization modes, including electron impact (EI) (Haddon et al., 1971, 1977), field desorption (FD) (Sphon et al., 1977), and both positive chemical ionization (PCI) (McFadden et al., 1977) and negative chemical ionization (NCI) (Brumley et al., 1981), coupled with a separation technique such as gas chromatography (GC) (Freidli, 1981) or liquid chromatography

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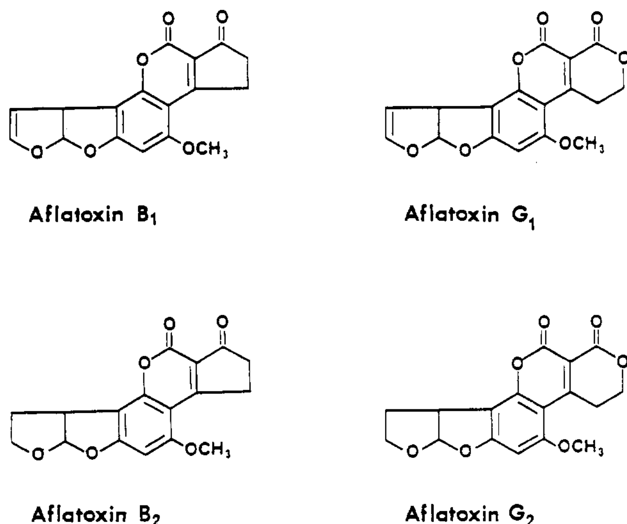

Figure 1. Structures of aflatoxins B_1, B_2, G_1, and G_2.

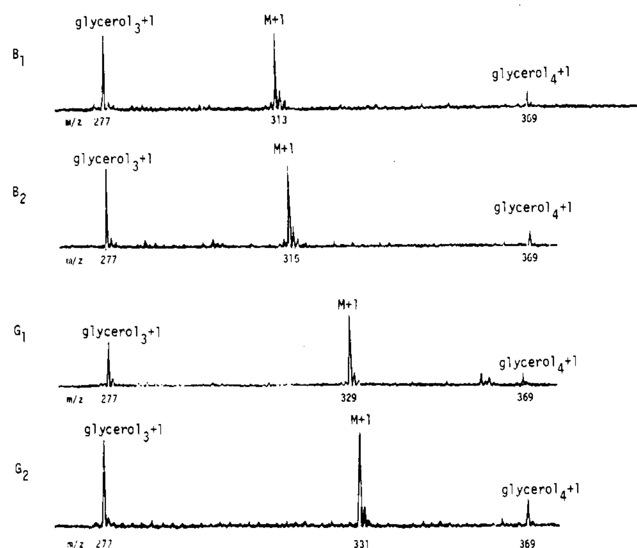

Figure 2. FAB mass spectra of aflatoxins B_1, B_2, G_1, and G_2.

(LC) Brumley et al., 1981). There have been many problems including nonreproducibility between laboratories and lack of sensitivity or nonquantitative analysis, however.

Electron impact mass spectra generally show strong molecular ions and fairly extensive fragmentation, including the loss of CO and $COCH_3$. Methane positive chemical ionization (MPCI) shows $(M + 1)^+$ ions, which were detected by selected ion monitoring (McFadden et al., 1977). Negative ion chemical ionization gives spectra showing M^-, $(M - 1)^-$, and $(M - CH_3)^-$, with a strong temperature dependence of the relative intensities observed because more fragmentation occurred at higher source temperatures (Brumley et al., 1981). We are reporting the use of fast atom bombardment (FAB) mass spectrometry (Barber et al., 1981) in identification and characterization of aflatoxins B_1, B_2, G_1, and G_2, as well as aflatoxins B_2S and G_1S, which are not amenable to other ionization techniques used in mass spectrometry.

EXPERIMENTAL SECTION

Solvents and Standards. Crystalline preparations of aflatoxins B_1, B_2, G_1, and G_2, stated to be pure by TLC, were purchased from Calbiochem-Behring Corp. (San Diego, CA) and were used as received. Aflatoxins B_1S and G_1S (the respective sodium bisulfite reaction products of B_1 and G_1) were prepared as described by Hagler et al. (1982) and purified by HPLC.

Solvents used were HPLC-grade methanol (Fisher Scientific, Raleigh, NC) and deionized water (Darco Water Systems, Durham, NC). Reagent-grade inorganic salts used in FAB-MS were sodium chloride (Fisher Scientific), lithium chloride (Alfa-Ventron, Beverly, MA), and potassium chloride (J. T. Baker Chemical Co., Phillipsburgh, NJ). Glycerol (reagent-grade) for FAB-MS experiments was obtained from J. T. Baker Chemical Co.

Fast atom bombardment mass spectra were obtained on a VG 70/70 mass spectrometer (VG Analytical, Altrincham, England). The fast atom beam is produced with a saddle field ion source from Ion Tech, Ltd. (Middlesex, England), modified by VG for neutral beam production. The sample was dissolved in 0.5 μL of glycerol, applied on a metal probe, and bombarded with 7.0–7.5 kV xenon atoms at a source pressure of 6×10^{-6} torr. The addition of salt was achieved by dissolving the sample in 0.5 μL of a solution of glycerol and the appropriate salt (approximately 0.5 μg/μL). The spectra were obtained at 4 kV, resolution 700, over a mass range of 200–500 amu, at a scan speed of 5 s/decade.

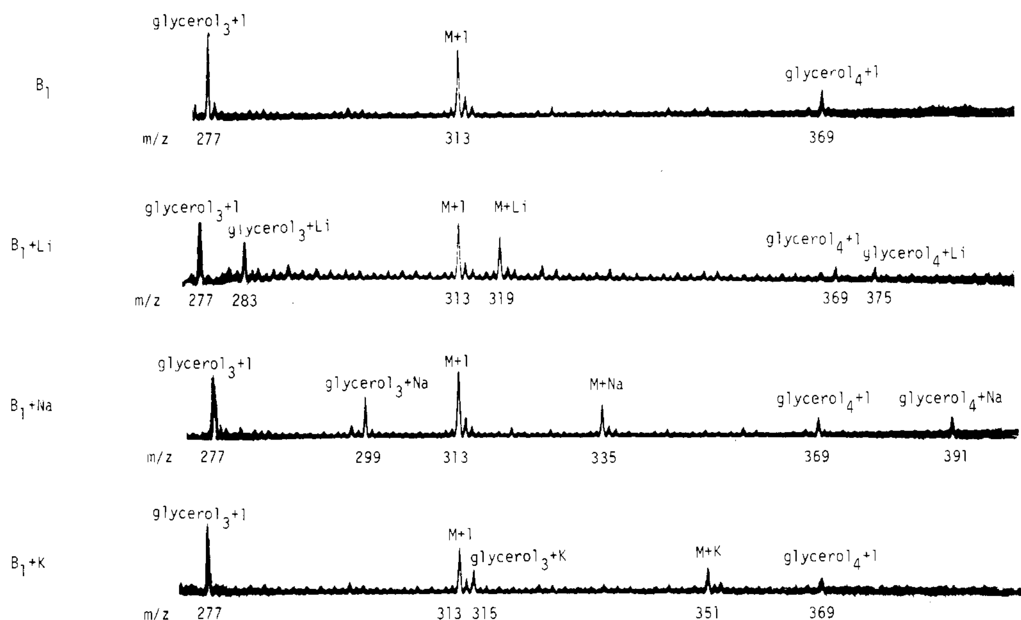

Figure 3. FAB mass spectra of aflatoxins B_1, with the addition of NaCl, LiCl, and KCl.

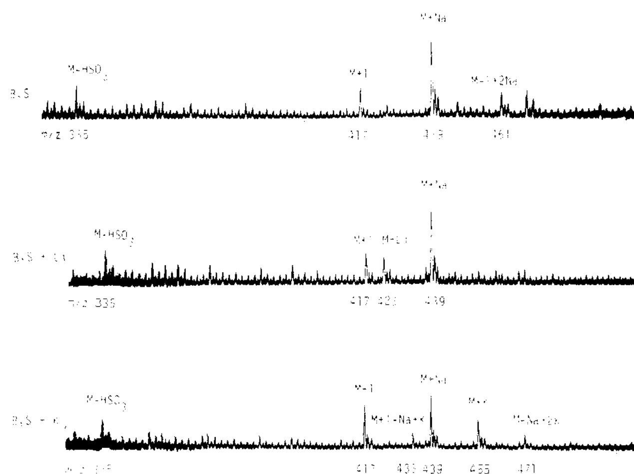

Figure 4. FAB mass spectra of aflatoxin B_1S, with the addition of LiCl and KCl.

The electron impact and chemical ionization mass spectra were obtained on a VG ZAB-2F mass spectrometer (VG analytical), coupled to a Finnigan/Incos 2300 data system (Finnigan Corp., Sunnyvale, CA). Electron impact spectra were obtained at 70-eV ionization energy, 100-μA trap current at 8-kV acceleration voltage. Chemical ionization spectra were obtained by using isobutane reagent gas at a 1×10^{-4} torr ion gauge pressure in the source, 0.0-V repellar voltage, 0.5-mA emission current, and a source temperature of 170 °C. Both EI and CI spectra were obtained by diret probe sample insertion.

The HPLC analyses and sample purification were performed on a system consisting of two Waters 600A pumps, a Waters 6000 solvent programmer, a Waters 440 UV detector, and a Waters data module (Waters Associates, Milford, MA). Methanol used for the HPLC analyses was filtered through a 0.5-μm Millipore filter, type FH (Millipore Corp., Bedford, MA); water used for the HPLC analyses was filtered through 0.45-μm Millipore filters, type HA. Separations were done on a Zorbax ODS column, 4.6 mm i.d. × 25 cm length, 5-μm particle size (Du Pont Instruments, Analytical Division, Wilmington, DE). A solvent consisting of 15:85 methanol–water was used to separate the products from the starting materials. Flow rates used were 1 mL/min, and UV detection was at 254 nm.

RESULTS AND DISCUSSION

Structures of the aflatoxins studied are shown in Figure 1. Fast atom bombardment mass spectra were obtained for aflatoxins B_1, G_1, B_2, and G_2 (Figure 2). These unreacted aflatoxins show $(M + 1)^+$ ions.

Figure 3 shows metal exchange reactions for aflatoxin B_1. Ions observed correspond to exchange of protons for metal ions in the aflatoxins and the glycerol substrate. Similar reactions for organic compounds have been observed in spark source mass spectrometry (Hass, 1972) and in field desorption mass spectrometry (Rollgen and Schulten, 1975).

While electron impact and chemical ionization have been successful for unreacted aflatoxins (Haddon et al., 1971 and 1977; Sphon et al., 1977; McFadden et al., 1977; Brumley et al., 1981), electron impact and isobutane positive chemical ionization mass spectra obtained on the B_1S and G_1S reaction products did not give molecular weight information, showing only low-mass fragment ions. Aflatoxins B_1S showed a weak ion for the parent aflatoxin, B_1, possibly due to decomposition of the reaction product on the probe.

The FAB spectrum of B_1S, the sodium bisulfite reaction product, is shown in Figure 4. B_1S appears to be the addition product of $NaHSO_3$ to aflatoxin B_1. This product would have a molecular weight of 416 amu ($M = B_1 + NaHSO_3$). The ions at m/z 417 and 439 correspond to $(M + 1)^+$ and $(M + Na)^+$, respectively.

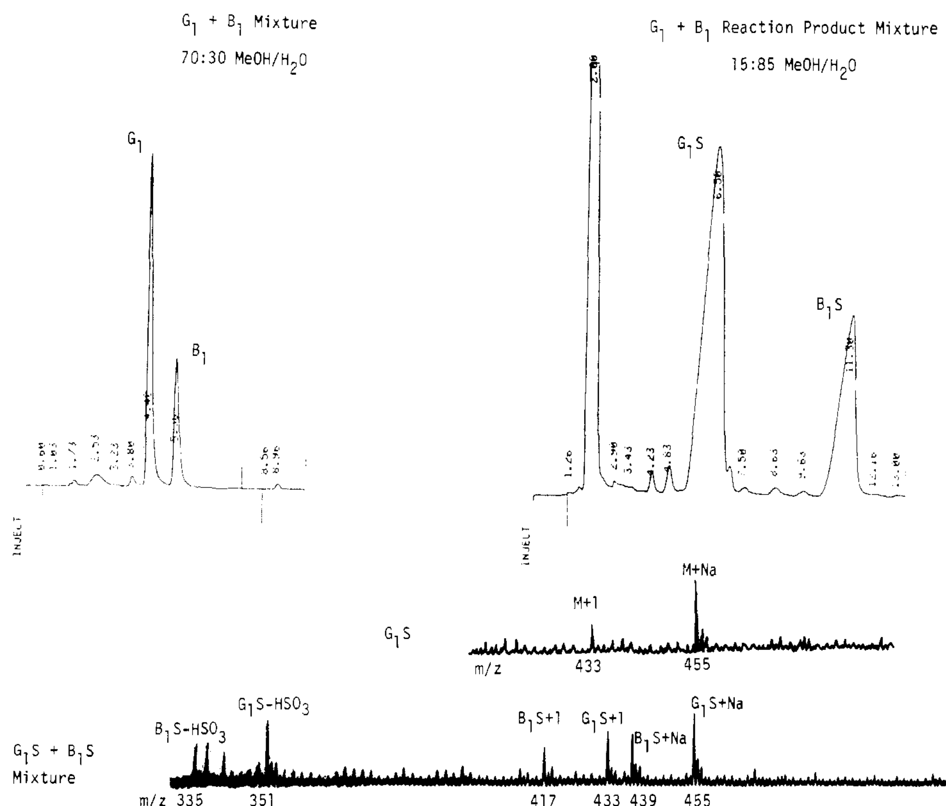

Figure 5. HPLC chromatograms of a mixture of aflatoxins B_1 and G_1, the products of the reaction of sodium bisulfite with this mixture, and the FAB mass spectra of aflatoxin G_1S and the mixture of B_1S and G_1S.

Metal addition reactions were performed to B_1S, giving partial exchange of Li and K for the Na. These spectra are shown in Figure 5. Since the reaction product was already a sodium salt, no change in the spectrum was observed on addition of NaCl.

The reaction product of G_1 with sodium bisulfite, G_1S, which would have a molecular weight of 432 amu shows ions at m/z 433 and 455, corresponding to $(M + 1)^+$ and $(M + Na)^+$, respectively (Figure 5). Figure 5 also shows an HPLC chromatogram of a mixture of aflatoxins, extracted from contaminated rice, and with peaks corresponding to aflatoxins B_1 and G_1. The HPLC chromatogram of the sodium bisulfite reaction product obtained from this extract shows peaks corresponding to B_1S and G_1S, in approximately the same ratio. A FAB mass spectrum of the reaction product mixture confirms the presence of B_1S and G_1S since peaks corresponding to $(M + 1)^+$ and $(M + Na)^+$ are found for both compounds.

CONCLUSION

Fast atom bombardment appears to be a useful technique for the identification of aflatoxins and aflatoxin bisulfite reaction products. By use of this technique, molecular weight information was obtained on the bisulfite reaction products that was not accessible by other ionization techniques (EI or CI). The metal addition reaction technique provides a convenient method for molecular weight confirmation. Further studies are in progress to characterize the bisulfite reaction products and to identify the site of bisulfite addition.

Registry No. Aflatoxin B_1, 1162-65-8; aflatoxin G_1, 1165-39-5; aflatoxin B_2, 7220-81-7; aflatoxin G_2, 7241-98-7; aflatoxin B_1 sodium sulfonate, 83219-44-7; aflatoxin G_1 sodium sulfonate, 83219-45-8.

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Received for review April 26, 1982. Accepted August 20, 1982. Paper No. 8291 of the Journal Series of the North Carolina Agricultural Research Service, Raleigh, NC. The use of trade names in this publication does not imply endorsement by the North Carolina Agricultural Research Service nor criticism of similar ones not mentioned.

Use of a Sulfuric Acid Cleanup Step in the Determination of 1,2-Dibromoethane Residues in Lemons, Oranges, and Grapefruits

After isolation of ethylene dibromide (EDB) from citrus rind or whole fruit samples by steam distillation via a benzene–water azeotrope, the dried benzene distillate was cleaned up by addition of silica gel (2 parts) impregnated with fuming sulfuric acid (1 part). This cleanup procedure allowed facile quantification of 5 ppb (nanograms per gram) of EDB by gas chromatography with electron-capture detection. Recovery of EDB from samples of rind and whole fruit fortified with 500, 50, and 5 ppb averaged 87 ± 6%. Hexane could be substituted for benzene if a dimethyl silicone defoaming agent was used. With the use of hexane, recovery of EDB from whole fruit samples fortified at 5 ppb of EDB was 102 ± 3%.

The capture in detection traps of two Mediterranean fruit flies, *Ceratitis capitata*, in northern California and one in southern California on June 5, 1980, created considerable concern in the agricultural community (Hagen et al., 1981). The California citrus industry was faced with the requirement by a number of domestic and foreign markets that fruit be fumigated with ethylene dibromide (1,2-dibromoethane, EDB) prior to its acceptance. In addition, EDB residues had to meet strict legal tolerance requirements. Therefore, a method to analyze for low levels of EDB in all major varieties of citrus fruits was needed. King et al. (1980) reported on a method for the

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