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Structures of the Ozonolysis Products and Ozonolysis Pathway of Aflatoxin B_1 in Acetonitrile Solution

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ABSTRACT: The ozonolysis of aflatoxin B_1 (400 μg/mL) in acetonitrile solution was conducted with an ozone concentration of 6.28 mg/L at the flow rate of 60 mL/min for different times. The results showed that ozone was an effective detoxification agent because of its powerful oxidative role. Thin-layer chromatography and liquid chromatography−quadrupole time-of-flight mass spectra were applied to confirm and identify the ozonolysis products of aflatoxin B_1. A total of 13 products were identified, and 6 of them were main products. The structural identification of these products provided effective information for understanding the ozonolysis pathway of aflatoxin B_1. Two ozonolysis pathways were proposed on the basis of the accurate mass and molecular formulas of these product ions. Nine ozonolysis products came from the first oxidative pathway based on the Criegee mechanism, and the other four products were produced from the second pathway based on the oxidative and electrophilic reactions of ozone. According to the toxicity mechanism of aflatoxin B_1 to animals, the toxicity of aflatoxin B_1 was significantly reduced because of the disappearance of the double bond on the terminal furan ring or the lactone moiety on the benzene ring.

KEYWORDS: *Aflatoxin B_1, mechanism, ozone, ozonolysis products, structure*

INTRODUCTION

Aflatoxins are toxic metabolites mainly produced by the fungal species *Aspergillus flavus* and *Aspergillus parasiticus* through a polyketide pathway.[1−3] At least 18 different types of aflatoxins are produced in nature. Among various aflatoxins, aflatoxin B_1 is considered the most potent teratogen, mutagen, and hepatocarcinogen.[4−7] Many agricultural products, especially peanut and corn, exposed to a high humidity and temperature environment, are often contaminated by aflatoxins before harvest or during storage. Aflatoxin contamination of agricultural products is one of the most important factors determining the quality of them and has caused significant financial losses for producing and exporting countries.[8,9] It is not always possible to prevent agricultural products from being contaminated by toxigenic fungi; therefore, physical, chemical, and biological methods have been developed to remove or degrade aflatoxins in contaminated products.[10−12] Among these methods, ozone, as a powerful oxidative agent, has been studied and widely used to decompose aflatoxins in foods because of its safety and higher efficiency in the degradation of aflatoxins.[5,13−16]

Few literature reports have studied the ozonolysis products of aflatoxin B_1 in different conditions. McKenzie et al.[17] had used radiolabeled aflatoxin B_1 to follow the ozone degradation products. A primary product (3-keto derivative of aflatoxin B_1) and some final products (organic acids, volatile compounds, or mineralization products) were inferred, but a detailed degradation pathway and intermediate reaction products of aflatoxin B_1 were not provided because of the limited test conditions. Prudente[18] revealed seven intermediate reaction products from the reaction of ozone with aflatoxin B_1. However, the same deficiency existed in this research; i.e., the structures of seven intermediate products and a detailed degradation pathway were not presented.

Degradation products of aflatoxin B_1 are different because of different degradation methods. Liu et al.[19,20] and Wang et al.[21] have identified the photodegradation and radiolytic products of aflatoxin B_1 by liquid chromatography−quadrupole time-of-flight mass spectrometry (LC−QTOF/MS), respectively. Accurate mass measurements from TOF generate the elemental composition of ions (molecules and fragments). Moreover, tandem mass spectrometry (MS/MS) provides complementary structural information through in-source fragmentation using collision-induced dissociation (CID). LC−QTOF/MS has been successfully used to analyze and identify metabolites and degradation products of food contaminants by other groups.[22−24] Therefore, it was proven to be qualified for the identification of unknown compounds. On the basis of previous research, this study was designed to analyze and identify the structures of ozonolysis products of aflatoxin B_1 in acetonitrile solution and propose a detailed ozonolysis pathway of aflatoxin B_1 based on these results.

MATERIALS AND METHODS

Chemicals and Reagents. Aflatoxin B_1 (2,3,6a,9a-tetrahydro-4-methoxy-cyclopenta[*c*]furo[2′,3′:4,5]furo[2,3-*h*]chromene-1,11-dione; $C_{17}H_{12}O_6$; purity > 98%) and high-performance liquid chromatog-

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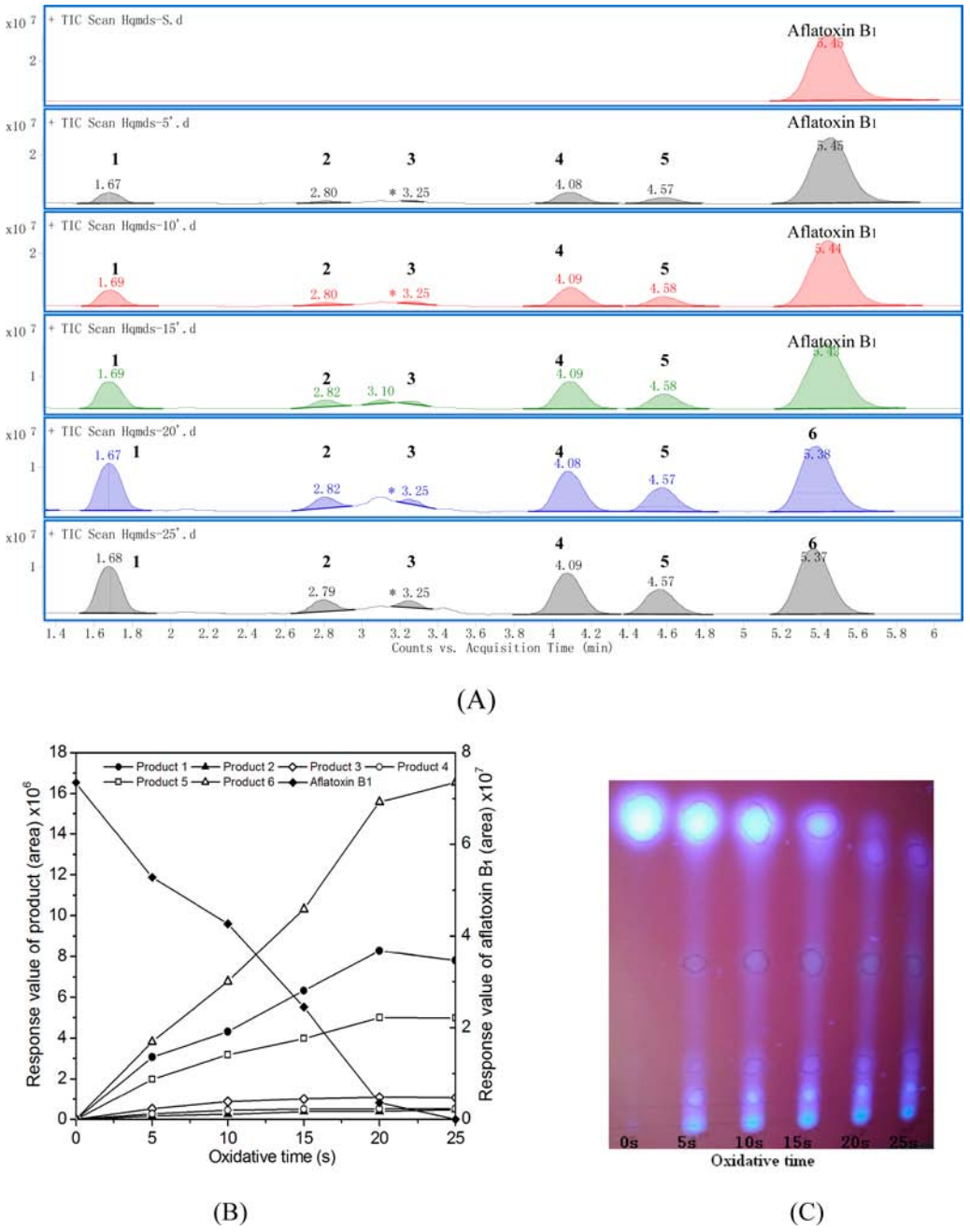

Figure 1. Changes of aflatoxin B_1 and ozonolysis products (**1**−**6**) in acetonitrile solution with the increasing oxidative time: (A) total ion chromatograms, (B) response value, and (C) TLC.

raphy (HPLC)-grade acetonitrile were purchased from Sangon Biotech (Shanghai, China).

Standard stock solutions (1.25 mg/mL) of aflatoxin B_1 were prepared in HPLC-grade acetonitrile and stored at 4 ± 2 °C in a refrigerated dark room. A total of 320 μL of the standard stock solution was placed in a disposable plastic tube (5 mL). The working solution (400 μg/mL, 1 mL) of aflatoxin B_1 was prepared by adding 680 μL of HPLC-grade acetonitrile in the disposable plastic tube.

Ozonolysis of Aflatoxin B_1. Ozone was generated using a model HLK-20 ozone generator (Hailin Technologies, Ji'nan, China). The ozone concentration (6.28 mg/L) was controlled by adjusting the flow rate of oxygen and voltage of the ozone generator. The ozone concentration was recorded using a model YX-303B3 ozone gas analyzer (Yuxiang Electronic Co., Ltd., Beijing, China). For the ozonolysis experiment, the working solutions of aflatoxin B_1 in disposable plastic tubes were treated by ozone at room temperature with a constant flow rate of 60 mL/min for 0, 5, 10, 15, 20, and 25 s. The experiment was performed in triplicate for each sample.

Thin-Layer Chromatography (TLC). After treatment of the working solutions by ozone, 10 μL of each sample was spotted on a 10 × 20 cm general purpose silica gel plate (Jiyida Silica Reagent Factory, Qingdao, China) at 1.5 cm intervals. The plate was developed with ether/methanol/water (96:3:1) and viewed in an ultraviolet (UV) cabinet at 365 nm, and then the R_f values were obtained.

LC−QTOF/MS. Operational procedures for LC−QTOF/MS were as follows: Ozonolysis products of aflatoxin B_1 were first separated by the separation column, and the separated components were ionized by the MS ion source. Through the first quadruple, the TOF was reached by a mass analyzer without CID. The CID mode was used for MS/MS analysis. The parent ions were fragmented in the cell of CID, and the fragmentation pathways were obtained.

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Table 1. Mass Accuracy Measurement of Aflatoxin B_1[a] and Ozonolysis[b] Products in Acetonitrile Solution Using LC−QTOF/MS

proposed products	retention time (min)	molecular formula	observed mass[c] (m/z)	calculated mass[c] (m/z)	mass difference (ppm)	DBE[d]	score (%)
1	1.676	$C_{17}H_{14}O_{10}$[e]	379.0658	379.0660	0.53	11	100
	2.076	$C_{17}H_{12}O_9$	361.0557	361.0554	−0.83	12	100
	2.090	$C_{16}H_{12}O_8$	333.0611	333.0605	−1.8	11	100
	2.793	$C_{16}H_{10}O_8$	331.0449	331.0448	−0.30	12	100
2	2.824	$C_{18}H_{16}O_{10}$[e]	393.0822	393.0816	−1.53	11	100
	2.918	$C_{15}H_{10}O_5$	271.0607	271.0601	−2.21	11	100
	3.118	$C_{15}H_{10}O_6$	287.0545	287.0550	1.74	11	100
	3.243	$C_{16}H_{10}O_7$	315.0502	315.0499	−0.95	12	100
3	3.251	$C_{16}H_{10}O_6$[e]	299.0546	299.0550	1.34	12	100
	3.437	$C_{17}H_{12}O_8$	345.0603	345.0605	0.58	12	100
4	4.085	$C_{19}H_{15}NO_9$[e]	402.0827	402.0820	0.58	13	100
5	4.583	$C_{17}H_{12}O_9$[e]	361.0561	361.0554	−1.74	12	100
6	5.372	$C_{17}H_{12}O_9$[e]	361.0554	361.0554	0.01	12	100
aflatoxin B_1	5.454	$C_{17}H_{12}O_6$	313.0710	313.0707	−0.96	12	100

[a]Initial concentration of aflatoxin B_1 = 400 μg/mL. [b]Ozonolysis conditions: ozone concentration, 6.28 mg/L; flow rate, 60 mL/min; and exposure time, 25 s. [c]The m/z in the table is the m/z of $[M + H]^+$. [d]DBE = double bond equivalents. [e]Main products.

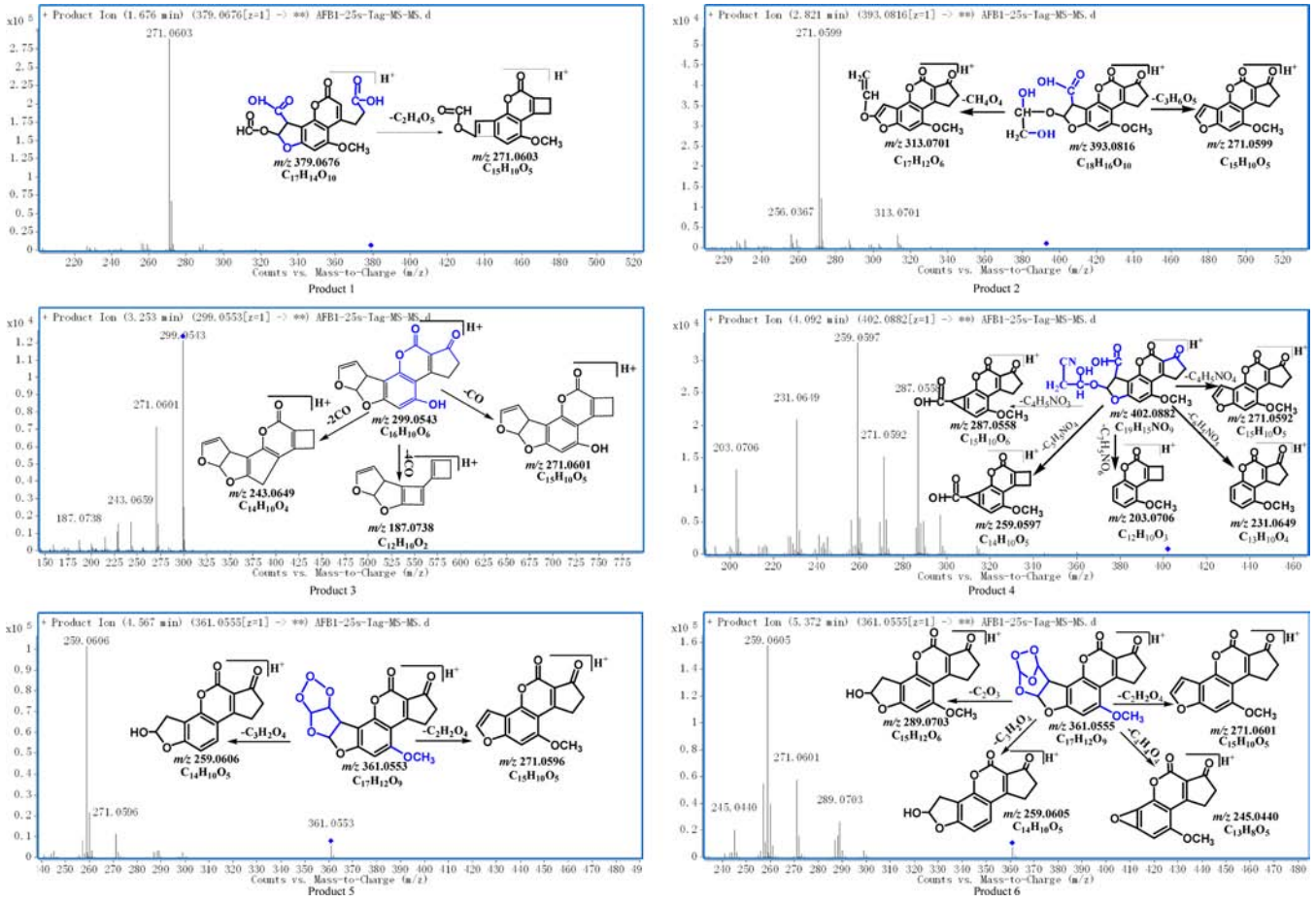

Figure 2. TOF/MS/MS spectra and proposed fragmentation (insets) of ozonolysis products of aflatoxin B_1 formed in the process of ozone treatment. Blue markers in the structure of aflatoxin B_1 and ozonolysis products are the sites of CID.

LC was performed on Agilent 1200 series HPLC (Agilent, Palo Alto, CA) equipped with an autoinjector and a quaternary HPLC pump. Chromatography was performed on a 2.1 × 150 mm inner dismeter, 5 μm, Agilent Plus C18 column. The injection volume was 2 μL. The mobile phase was acetonitrile and an aqueous solution containing 0.1% formic acid in 70:30 (v/v) solution. The total run time was 12 min, with a flow rate of 0.4 mL/min.

MS was performed with Agilent 6520 accurate-mass QTOF LC/MS (Agilent, Palo Alto, CA). The optimized conditions were as follows: Compounds were analyzed in positive-ion mode. Capillary and fragmentor voltages were 3500 and 175 V, respectively, and the skimmer voltage was 65.0 V. The flow rate of drying gas was 10.0 L/min, and nebulizer was 40 psi. Nitrogen was used as the collision gas. Mass spectra were acquired in a full-scan analysis within the range of m/z 100−1000 using an extended dynamic range and a scan rate of 1.4

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spectra/s and varying the collision energy with mass. The data station operating software used was the Mass Hunter Workstation software (version B.04.00). A reference mass solution containing reference ions 121.0508 and 922.0097 was used to maintain mass accuracy during the run time.

RESULTS AND DISCUSSION

Ozonolysis Products of Aflatoxin B_1 by Ozone Treatment at Different Exposure Times. Liu et al.[19,20]

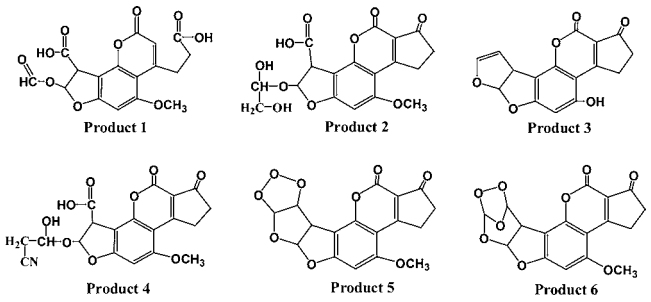

Figure 3. Structures of ozonolysis products of aflatoxin B_1 in acetonitrile solution.

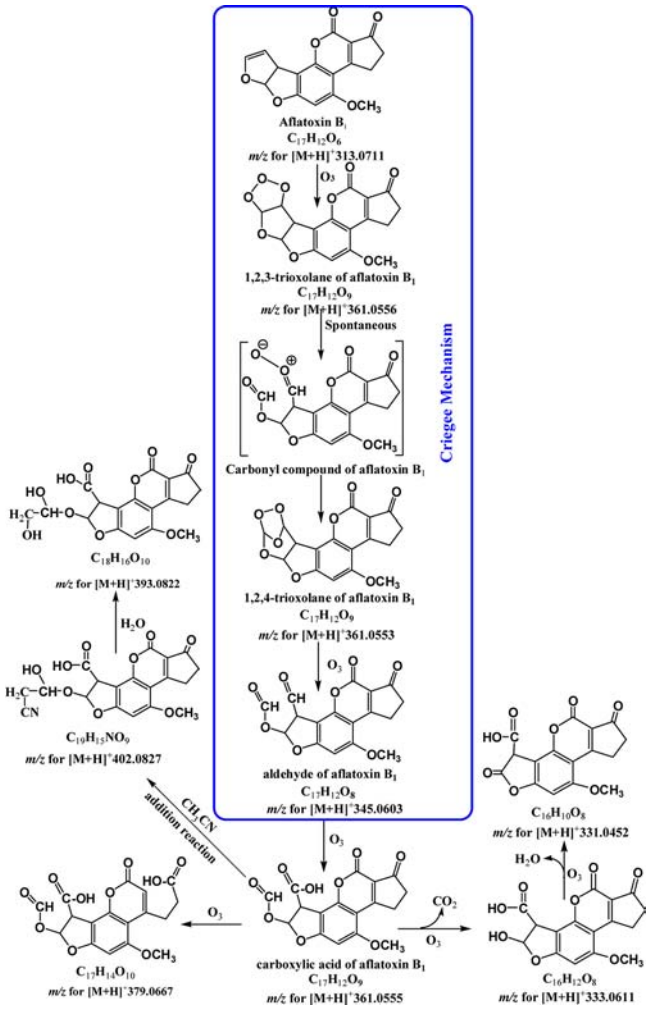

Figure 4. First ozonolysis pathway of aflatoxin B_1 in acetonitrile solution.

and Wang et al.[21] have reported photodegradation products and radiolytic products of aflatoxin B_1 by UV and ^{60}Co irradiation that could cause rapid decomposition of aflatoxin B_1. For this experiment, ozone can decompose aflatoxin B_1 in acetonitrile solution rapidly (Figure 1A), which is consistent with the reported results.[17,18] Figure 1A shows the total ion chromatograms of aflatoxin B_1 and its ozonolysis products at different exposure times of ozone. As shown in Figure 1A, only one peak was observed for aflatoxin B_1 untreated with ozone and five to six bigger peaks for treated samples, which indicates that aflatoxin B_1 was decomposed through the oxidative role of ozone. The results of TLC for aflatoxin B_1 and its ozonolysis products further confirmed the above results (Figure 1C). The R_f value of aflatoxin B_1 was 0.58, and those of its ozonolysis products were all less. There were 13 ozonolysis products formed in the oxidative process of aflatoxin B_1 based on the total ion chromatograms, and 6 of them were the main products, especially products 1, 4, 5, and 6, whose concentrations increased obviously with the reduction of aflatoxin B_1 (panels A and B of Figure 1). On the basis of these results, under the constant concentration and flow rate of ozone, the exposure time of ozone strongly affects the degradation of aflatoxin B_1 in acetonitrile solution. Aflatoxin B_1 is decomposed completely within 25 s under the experimental conditions. Furthermore, Figure 1C shows at least five products formed (their R_f values being 0.004, 0.05, 0.12, 0.31, and 0.55, respectively) in this process, and aflatoxin B_1 is decomposed completely at the exposure time of 25 s, which are consistent with the results of QTOF/MS. Molecular formulas of ozonolysis products listed in Table 1 are calculated using the formula calculator based on m/z, a function of Agilent Mass Hunter Qualitative Analysis software. Table 1 lists the proposed products, the retention times, the deduced molecular formulas, the observed masses, the calculated masses, the differences between the observed and calculated masses (ppm), the double bond equivalents (DBE), and the scores (an overall score is 0−100%, with the score closer to 100% being better). For all of the ozonolysis products, the observed masses are consistent with the calculated masses (mass difference less than 5 ppm) and the scores are all 100%, which preliminarily confirms the ozonolysis products of aflatoxin B_1. In addition, the response values (area) of most products in the QTOF evidently increase with the rapid reduction of aflatoxin B_1 (panels A and B of Figure 1), whereas Wang et al.[19] reported that the responses of some radiolytic products are very weak and the scores are not high because of very low concentrations. In comparison to the DBE value (DBE = 12) of aflatoxin B_1, those of ozonolysis products (DBE = 11−13) are close in our experiment, which indicates little structural changes of ozonolysis products. In contrast, the DBE values of radiolytic products[21] are from 10 to 25, changes that are greater than our results because of different degradation conditions.

For the six main ozonolysis products, their concentrations gradually increase within 20 s of ozone treatment. After 20 s, the concentrations of some products (**1**, **3**, and **5**) begin to decline because of their oxidative decomposition through the role of ozone (Figure 1B).

According to the results of total ion chromatograms and LC−QTOF/MS analysis (Table 1 and Figure 1A), m/z 313.0711 is aflatoxin B_1 and products **1**−**6** are the six main ozonolysis products of aflatoxin B_1.

Structural Identification of Ozonolysis Products. To confirm the structures of ozonolysis products, it is important to further analyze the fragmentation patterns and their accurate masses. TOF/MS/MS of the ozonolysis products as precursor

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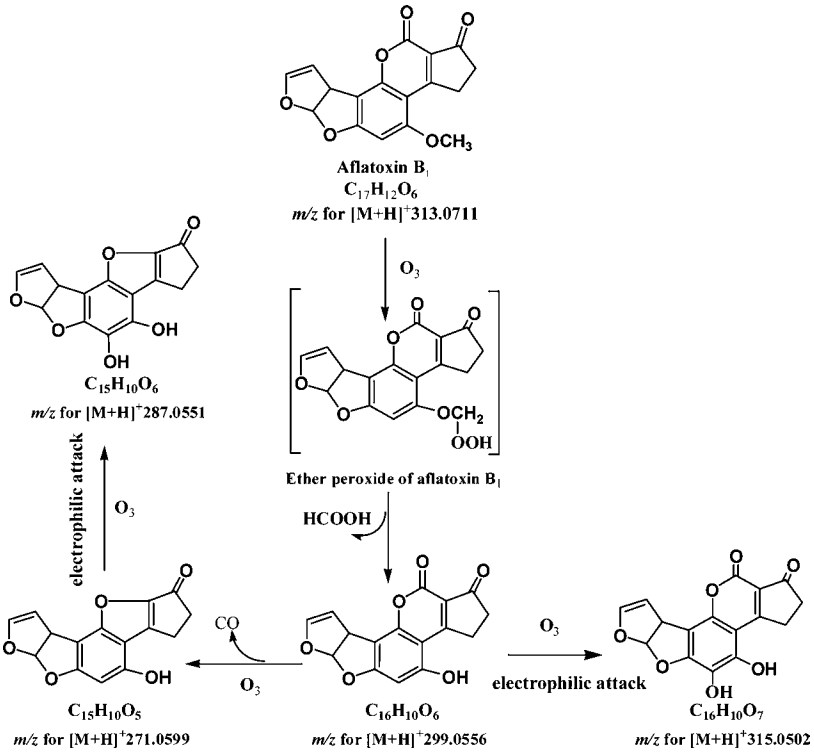

Figure 5. Second ozonolysis pathway of aflatoxin B_1 in acetonitrile solution.

ions was performed to pass to the collision cell using the ion-filtering function of QTOF. MS/MS spectra and fragmentation of six main precursor compounds are shown in Figure 2. The accurate masses and molecular formulas of these product ions (Table 1) were obtained by use of the Agilent Mass Hunter Qualitative Analysis software.

On the basis of the accurate masses of the parent ions and the fragments obtained from TOF/MS/MS experiments (Figure 2), the structures of six main products are deduced as follows (Figure 3). As shown in Figure 3, the structures of six main products are similar to that of aflatoxin B_1. The double bonds on the furan ring of five main products (**1**, **2**, **4**, **5**, and **6**) are cleaved through the role of ozone and transformed into trioxolane, alcohol, aldehyde, or carbonyl acid of aflatoxin B_1. However, the product **3** (m/z 299.0556) is a product of aflatoxin B_1, losing a methylene group from the methoxy group on the benzene ring, whose double bond on the furan ring is not destroyed by ozone.

Ozonolysis Pathway of Aflatoxin B_1 in Acetonitrile Solution. According to the structural identification of ozonolysis products of aflatoxin B_1 in acetonitrile solution, two ozonolysis pathways for aflatoxin B_1 in acetonitrile solution are proposed. The first pathway is from aflatoxin B_1 to $C_{16}H_{10}O_8$ (m/z 331.0452), $C_{17}H_{14}O_{10}$ (m/z 379.0667), and $C_{18}H_{16}O_{10}$ (m/z 393.0822). The second pathway is from aflatoxin B_1 to $C_{16}H_{10}O_7$ (m/z 315.0502) and $C_5H_{10}O_6$ (m/z 287.0551) (Figure 4).

For the first pathway, ozonolysis cleaves alkene double bonds by the Criegee mechanism (blue box in Figure 4). The first step is a 1,3-dipolar cycloaddition of ozone to the alkene, leading to the primary ozonide (molozonide, 1,2,3-trioxolane, or Criegee intermediate product), which decomposes to a carbonyl oxide and a carbonyl compound. For the ozonolysis of aflatoxin B_1, a 1,3-dipolar cycloaddition of ozone is formed at the C8−C9 double bond in aflatoxin B_1,[17] which is an unstable molozonide, and then spontaneously decomposes to form a carbonyl compound of aflatoxin B_1.

The carbonyl oxides are similar to ozone in being 1,3-dipolar compounds, and a secondary ozonide (1,2,4-trioxolane) undergoes 1,3-dipolar cycloaddition to the carbonyl compounds with the reverse regiochemistry. Most of the secondary ozonides are alcohol, ketone, and acid; therefore, they are more stable than the primary ozonide. 1,2,4-Trioxolane of aflatoxin B_1 is further oxidized by ozone and leads to a carboxylic acid (Figure 4) of aflatoxin B_1 ($C_{17}H_{12}O_9$, m/z 361.0555). The next step is complicated in that this carboxylic acid has three possible branched pathways.

The first branch is the oxidation of the formyl group on the furan ring of $C_{17}H_{12}O_9$ (m/z 361.0555) formed from the above steps. The formyl group can be oxidized by ozone to produce $C_{16}H_{12}O_8$ (m/z 333.0611) and release CO_2. In this process, ozone transforms the formyl group to the hydroxyl group of $C_{16}H_{12}O_8$ (m/z 333.0611) because of its powerful oxidative role. The hydroxyl group of $C_{16}H_{12}O_8$ can be further oxidized to the carbonyl group and form a new lactone ring on the basis of the furan ring. The oxidative product is $C_{16}H_{10}O_8$ (m/z 311.0452) by elimination of H_2O (Figure 4).

The second branch is the addition reaction of acetonitrile and aldehyde group of formic ether on the furan ring. Formic ether of the furan ring on the left side of carboxylic acid of aflatoxin B_1 (m/z 361.0555) carries out the nucleophilic addition reaction with acetonitrile to form $C_{19}H_{15}NO_9$ (m/z 402.0827), which could be further hydrolyzed to produce $C_{18}H_{16}O_{10}$ (m/z 393.0822) by replacing the nitrile group with a hydroxyl group (Figure 4).

The third branch is the oxidation of the carbonyl group on cyclopentanone on the right of carboxylic acid of aflatoxin B_1 (m/z 361.0555), which is cleaved at the lactone−cyclopentanone ring junction by oxidation of ozone, and forms

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$C_{17}H_{14}O_{10}$ (m/z 379.0667), a compound containing two carboxyl groups (Figure 4).

For the second ozonolysis pathway of aflatoxin B_1, the benzene ring methoxy group is oxidized to form an ether peroxide, which is a very unstable compound, and transforms into $C_{16}H_{10}O_6$ (m/z 299.0556) by releasing formic acid (HCOOH). $C_{16}H_{10}O_6$ divides into two branches for further ozonolysis. The first branch is to produce $C_{16}H_{10}O_7$ (m/z 315.0502) through the electrophilic attack of ozone on the benzene ring. The second branch is to oxidize and generate $C_{15}H_{10}O_5$ (m/z 271.0599) with the cleavage of the carbonyl group on the lactone ring, coupled with the release of CO_2. The product $C_{15}H_{10}O_5$ continues to be oxidized through the electrophilic attack of ozone on the benzene ring and forms $C_{15}H_{10}O_6$ (m/z 287.0551), which is similar to $C_{16}H_{10}O_7$ in structure (Figure 5).

Our study shows that aflatoxin B_1 can be completely decomposed to other products within 25 s at the given conditions, which is confirmed by both TLC and LC−QTOF/MS analysis. Seen from experimental results, all ozonolysis products of aflatoxin B_1 are similar to aflatoxin B_1 in structure, which can be further confirmed from the close DBE values (from 11 to 13) (Table 1). Molecular weights or m/z values of most ozonolysis products (∼77%) are greater than that of aflatoxin B_1, which illustrates that aflatoxin B_1 is primarily decomposed by ozone within 25 s. A higher concentration or longer exposure to ozone must be necessary to completely decompose aflatoxin B_1, namely, into CO_2 and H_2O. It has been reported that the furan ring on the left and the lactone ring on the right of aflatoxin B_1 are essential for its toxic and carcinogenic activity. Especially, the double bond on the terminal furan ring is an important determinant of toxicity.[25−27] The structure analysis shows that the double bond on the terminal furan ring is destroyed in all nine ozonolysis products and the lactone ring is destroyed in two products. The remaining two products lose a methoxyl group from the benzene ring, which is replaced by a hydroxyl group. For the six main products, the double bonds on the terminal furan ring of five products have disappeared. Therefore, the toxicity of most ozonolysis products is reduced in comparison to that of aflatoxin B_1. For the product **3** (m/z 299.0556), whose double bond on the furan ring is not destroyed, its toxicity still exists. To reduce the toxicity of aflatoxin B_1 and its ozonolysis products, it is necessary to further decompose them by increasing the exposure time of ozone or its concentration. The structures of these degradation products are speculative, and complete characterization will require their isolation and analysis by NMR spectroscopy in future work.

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Notes

The authors declare no competing financial interest.

REFERENCES

(1) Juan, C.; Zinedine, A.; Molto, J. C.; Idrissi, L.; Manes, J. Aflatoxin levels in dried fruits and nuts from Rabat-Sale, Morocco. *Food Control* **2008**, *19*, 849−853.

(2) Turner, N.; Subrahmanyam, S.; Piletsky, S. Analytical methods for determination of mycotoxins: A review. *Anal. Chim. Acta* **2009**, *632*, 168−180.

(3) Arzandeh, S.; Jinap, S.; Lioe, H. Aflatoxin in raw peanut kernels marketed in Malaysia. *J. Food Drug Anal.* **2010**, *18*, 44−50.

(4) Shenasi, M.; Aidoo, K. E.; Candlish, A. A. G. Microflora of date fruits and production of aflatoxins at various stages of maturation. *Int. J. Food Microbiol.* **2002**, *79*, 113−119.

(5) Proctor, A. D.; Ahmedna, M.; Kumar, J. V.; Goktepe, I. Degradation of aflatoxins in peanut kernels/flour by gaseous ozonation and mild heat treatment. *Food Addit. Contam.* **2004**, *21*, 786−793.

(6) Nakai, V.; de Oliveira Rocha, L.; Goncalez, E.; Fonseca, H.; Ortega, E.; Corrêa, B. Distribution of fungi and toxins in a stored peanut variety. *Food Chem.* **2008**, *106*, 285−290.

(7) Bhat, R.; Rai, R. V.; Karim, A. A. Mycotoxins in food and feed: Present status and future concerns. *Compr. Rev. Food Sci. Food Saf.* **2010**, *9*, 57−81.

(8) Misra, J. B. A mathematical approach to comprehensive evaluation of quality in groundnut. *J. Food Compos. Anal.* **2004**, *17*, 69−79.

(9) Burlingame, B.; Pineiro, M. The essential balance: Risks and benefits in food safety and quality. *J. Food Compos. Anal.* **2007**, *20*, 139−146.

(10) Netke, S. P.; Roomi, M. W.; Tsao, C.; Niedwiecki, A. Ascorbic acid protects Guinea pigs from acute aflatoxin toxicity. *Toxicol. Appl. Pharmacol.* **1997**, *143*, 429−435.

(11) Das, C.; Mishra, H. N. Effect of aflatoxin B_1 detoxification on the physicochemical properties and quality of ground nut meal. *Food Chem.* **2000**, *70*, 483−487.

(12) Haskard, C.; Binnion, C.; Ahokas, J. Factors affecting the sequestration of aflatoxin by *Lactobacillus rhamnosus* strain GG. *Chem.-Biol. Interact.* **2000**, *128*, 39−49.

(13) Prudente, A. D.; King, J. M. Efficacy and safety evaluation of ozonation to degrade aflatoxin in corn. *J. Food Sci.* **2002**, *67*, 2866−2872.

(14) Akbas, M. Y.; Ozdemir, M. Effect of different ozone treatments on aflatoxin degradation and physicochemical properties of pistachios. *J. Sci. Food Agric.* **2006**, *86*, 2099−2104.

(15) Zorlugenç, B.; Zorlugenç, F. K.; Öztekin, S.; Evliya, I. B. The influence of gaseous ozone and ozonated water on microbial flora and degradation of aflatoxin B_1 in dried figs. *Food Chem. Toxicol.* **2008**, *46*, 3593−3597.

(16) Alencar, E. R.; Faroni, L. R. D.; Soares, N. F. F.; Silva, W. A.; Carvalho, M. C. S. Efficacy of ozone as a fungicidal and detoxifying agent of aflatoxins in peanuts. *J. Sci. Food Agric.* **2011**, *92*, 899−905.

(17) McKenzie, K. S.; Sarr, A. B.; Mayura, K.; Bailey, R. H.; Miller, D. R.; Rogers, T. D.; Norred, W. P.; Voss, K. A.; Plattner, R. D.; Kubena, L. F.; Phillips, T. D. Oxidative degradation and detoxification of mycotoxins using a novel source of ozone. *Food Chem. Toxicol.* **1997**, *35*, 807−820.

(18) Prudente, A. D. Evaluation of aflatoxin-related products from ozonated corn. Doctoral Dissertation, Louisiana State University, Baton Rouge, LA, 2008; p 99.

(19) Liu, R. J.; Jin, Q. Z.; Tao, G. J.; Shan, L.; Huang, J. H.; Liu, Y. F.; Wang, X. G.; Mao, W. Y.; Wang, S. S. Photodegradation kinetics and byproducts identification of the aflatoxin B_1 in aqueous medium by ultra-performance liquid chromatography−quadrupole time-of-flight mass spectrometry. *J. Mass Spectrom.* **2010**, *45*, 553−559.

(20) Liu, R. J; Jin, Q. Z.; Tao, G. J.; Shan, L.; Liu, Y. F.; Wang, X. G. LC−MS and UPLC−quadrupole time-of-flight MS for identification of photodegradation products of aflatoxin B_1. *Chromatography* **2010**, *71*, 107−112.

(21) Wang, F.; Xie, F.; Xue, X. F.; Wang, Z. D.; Fan, B.; Ha, Y. M. Structure elucidation and toxicity analyses of the radiolytic products of

aflatoxin B_1 in methanol−water solution. *J. Hazard. Mater.* **2011**, *192*, 1192−1202.
(22) Mortishire-Smith, R. J.; O'Connor, D.; Castro-Perez, J. M.; Kirby, J. Accelerated throughput metabolic route screening in early drug discovery using high-resolution liquid chromatography/quadrupole time-of-flight mass spectrometry and automated data analysis. *Rapid Commun. Mass Spectrom.* **2005**, *19*, 2659−2670.
(23) Bateman, K. P.; Castro-Perez, J.; Wrona, M.; Shockor, J. P.; Yu, K.; Oballa, R.; Nicoll-Griffith, D. A. MS^E with mass defect filtering for *in vitro* and *in vivo* metabolite identification. *Rapid Commun. Mass Spectrom.* **2007**, *21*, 1485−1496.
(24) Lin, Z. S.; Le, J.; Hong, Z. Y. Liquid chromatography−quadrupole-time-of-fight mass spectrometry and its application in the metabolism (metabolomics) research of traditional Chinese medicines. *Chin. Pharm. J. (Taipei, Taiwan)* **2012**, *47*, 401−405.
(25) Wogan, G. N.; Edwards, G. S.; Newberne, P. M. Structure−activity relationships in toxicity and carcinogenicity of aflatoxins and analogs. *Cancer Res.* **1971**, *31*, 1936−1942.
(26) O'Brien, K.; Moss, E.; Judah, D.; Neal, G. Metabolic basis of the species difference to aflatoxin B_1 induced hepatotoxicity. *Biochem. Biophys. Res. Commun.* **1983**, *114*, 813−821.
(27) Verma, R. J. Aflatoxin cause DNA damage. *Int. J. Hum. Genet.* **2004**, *4*, 231−236.