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Identification and Structure Elucidation of a *p*-Phenoxybenzaldehyde in Bamboo Shoots by HPLC-ESI/MS/MS and NMR

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ABSTRACT: In this study, a derivative of *p*-phenoxybenzaldehyde in bamboo shoots was investigated. Bamboo shoots were ground and extracted with water, and an aqueous suspension was purified by SPE using Oasis HLB cartridges. After the SPE procedure, the analytes were analyzed by HPLC with refractive index detection (HPLC-RI). In the HPLC-RI analysis for sucralose, a putative sucralose was detected. In the subsequent HPLC-PDA analysis, the suspicious peak showed a unique UV absorption spectrum with the maximum wavelength at 285 nm indicating the existence of an aromatic ring. The contents of the unknown compound in bamboo shoot products ranged from 0.01 to 0.15 mg/g. The identity of the unknown compound was further confirmed by HPLC-ESI/MS/MS. The molecular weight of the unknown compound was determined to be 244. The chemical structure of the unknown compound was elucidated on the basis of NMR spectroscopic analyses (^{1}H, ^{13}C, DEPT, COSY, HMQC, and HMBC). Finally, the structure of the unknown compound was characterized as 4-(4-dihydroxymethylphenoxy)benzaldehyde.

KEYWORDS: bamboo shoot, *p*-phenoxybenzaldehyde, SPE, HPLC-ESI/MS/MS, NMR

INTRODUCTION

Bamboo shoots are edible young growth of bamboo species including *Bambusa vulgaris* and *Phyllostachys edulis*.[1] They are sold in various processed shapes and available in fresh, dried, and canned forms. They have a tough exterior, but the inner core is soft and has a somewhat sweet flavor. Bamboo shoots have been widely used in the cuisine of many Asian nations because of their properties of low fat, high dietary fiber, and rich mineral content.[2,3] On the other hand, they also contain potentially toxic compounds such as cyanogenic glycosides and cyanide.[4,5]

In previous works, it has been noted that the bamboo plant has unusually high levels of acetylcholine, especially in the upper part of the bamboo shoot.[6] In addition, bamboo leaves, obtained from the common tall bamboos, have recently been utilized as a source of flavonoids such as vitexin and orientin. The flavonoids may reduce inflammation, promote circulation, and inhibit allergy reactions.[7]

In this study, a derivative of *p*-phenoxybenzaldehyde in bamboo shoots was investigated. Actually, the compound was detected following the legal requirements of analyzing added sweeteners including sucralose in bamboo shoots. *p*-Phenoxybenzaldehyde has two distinct functional groups. One of them, benzaldehyde, belongs to the family of aromatic aldehydes. It is a colorless liquid aldehyde with a characteristic almond odor. It is used chiefly in the synthesis of other organic compounds, ranging from pharmaceuticals to plastic additives. Also, benzaldehyde is an important intermediate for the processing of perfume and flavoring compounds and in the preparation of certain aniline dyes.[8] In the case of the phenoxy group, "phenoxy" is a prefix to indicate the presence of the group $-OC_6H_5$, composed of phenyl and an atom of oxygen.[9] The phenoxy compounds are precursors of antibiotics, especially penicillins, plant growth regulators, and herbicides. They are used as intermediates for manufacturing dyes, pharmaceuticals, pesticides, fungicides, and flavoring agents. Especially, *m*-phenoxybenzaldehyde is used as an intermediate of pesticides (i.e., synthetic pyrethroids such as cypermethrin and fenvalerate) and pharmaceuticals. The acute oral toxicity (LD_{50}) of *m*-phenoxybenzaldehyde was reported as 1222 mg/kg in rat.[10,11]

In the food industry, various food additives could be added for the purposes of preservation, increasing flavor, and improving food quality. Although most of the sweeteners are generally recognized as safe, the use of individual sweeteners is regulated by governments according to food types. Especially, sucralose is a non-nutritive, high-intensity sweetener made by a process that begins with sucrose.[11] It is a water-soluble, white crystalline powder that is, on average, 600 times sweeter than sugar. In addition, sucralose is highly stable at the elevated temperatures that are often used in food, beverage, and drug manufacturing processes.[12,13] Especially, the use of sucralose in bamboo shoot products is allowed at 0.58 g/kg in Korea. In this regard, it is necessary to monitor the correct use of the allowed food additives including sucralose in foods. To quantify the usage of artificial sweeteners, generally HPLC-PDA is used. However, in the case of sucralose, HPLC-RI or HPLC-ELSD is used because of its weak UV absorption (<200 nm).[14,15]

Prior to HPLC analyses, bamboo shoot extracts were purified according to a solid-phase extraction (SPE) procedure using Oasis HLB cartridges.[16] After the SPE procedure, the analytes were analyzed by HPLC with refractive index detection (HPLC-RI). In the HPLC-RI analysis, a putative sucralose was detected at almost the same retention time as sucralose. The identity of the peaks was further confirmed by HPLC-ESI/MS/MS. In addition, HPLC-PDA analysis was performed to confirm the UV absorption spectrum of the unknown compound. Subsequently, the

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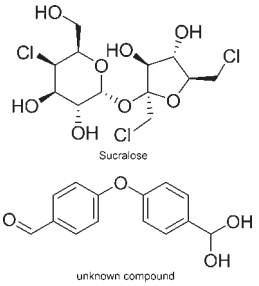

Figure 1. Chemical structures of sucralose and the unknown compound.

structure of the unknown compound was elucidated by 1D and 2D NMR spectroscopy. Finally, the chemical structure of the unknown compound was characterized as 4-(4-dihydroxymethylphenoxy)benzaldehyde (Figure 1).

MATERIALS AND METHODS

Chemicals and Materials. The standard of sucralose and methanol-d_4 were supplied by Sigma-Aldrich (St. Louis, MO). All of the solvents used in the analyses were of HPLC grade. The SPE cartridges Oasis HLB (200 mg, 6 cm^3) were obtained from Waters (Milford, MA).

Sample Preparation. Bamboo shoot samples imported from China were used for the study. Samples were ground using a laboratory mixer. Then, a portion of the sample (10 g) was suspended in 50 mL of distilled water and homogenized using a mechanical shaker for 5 min. The aqueous suspension was centrifuged at 3000 rpm and 5 °C for 20 min. Then, the clear supernatant was used for the SPE cleanup process. The Oasis HLB (200 mg, 6 cm^3) cartridges were previously activated with 1 mL of methanol and 2 mL of water. After the conditioning step, an aliquot of the clear supernatant (10 mL) was passed through the cartridge and then rinsed with 2 mL of water prior to elution. Subsequently, elution was accomplished with 5 mL of methanol.[17] The eluant was filtered through a 0.22 μm filter membrane. The filtered solutions were directly used for HPLC-RI, HPLC-PDA, and HPLC-ESI/MS/MS analyses. For the NMR experiments, the SPE procedure was repeated and the filtered solutions were dried using nitrogen purging.

Preparation of Standard Solutions. An accurately weighed solid portion of sucralose was dissolved in water to prepare stock solution (1000 μg/mL). Then, the stock solution was used for preparation of working standard solution (100 μg/mL). The standard solution was stored at 4 °C before use.

HPLC-RI Analysis. The analyses of sucralose and the unknown compound found in bamboo shoot were carried out on a Waters Alliance system with a 2414 RI detector. The analytes were separated using a Capcell-Pak C_{18} UG 120 column (5 μm, 150 mm × 4.6 mm i.d., Shiseido, Japan) by isocratic elution with methanol/water (30:70, v/v). The two compounds showed almost the same retention time at around 4.6 min.

HPLC-PDA Analysis. The HPLC-PDA analysis was carried out on a Waters Alliance system with a 2996 PDA detector (Waters). The mobile phase and analytical column for the HPLC-PDA analysis were identical to those for the HPLC-RI analysis. In the PDA analysis, sucralose did not show any UV absorption spectrum, but the unknown compound showed a unique UV absorption spectrum with maximum wavelength at 285 nm.

HPLC-ESI/MS/MS Analysis. For the HPLC-ESI/MS/MS analysis, an Acquity UPLC system coupled to a Quattro premier XE mass spectrometer (Waters, Manchester, U.K.) equipped with a Z-Spray ESI source was used. The unknown compound was analyzed using a BEH C_{18} column (1.7 μm, 50 mm × 2.1 mm i.d., Waters) with the mobile phase of methanol/water (50:50, v/v). The flow rate was 0.3 mL/min, and the volume of sample injected was 3 μL. The electrospray source was operated in negative mode. The capillary potential was set at 3.0 kV, desolvation temperature at 350 °C, source temperature at 120 °C, desolvation gas (N_2) flow at 650 L/h;, cone gas (N_2) flow at 50 L/h, cone voltage at 10 V, and collision energy at 5 eV. The molecular ion of the unknown compound was determined using MS scan experiments, and the fragments of molecular ion were analyzed by MS/MS experiments using daughter scan mode.

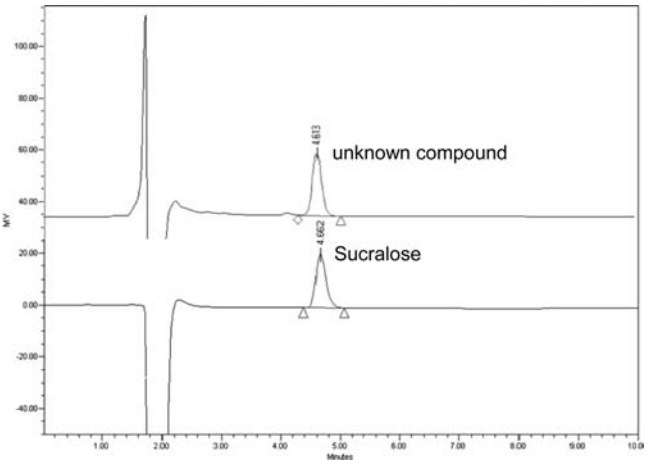

Figure 2. HPLC-RI chromatograms of sucralose and the unknown compound.

NMR Spectroscopy. All NMR experiments were performed on a Bruker Avance 400 spectrometer (9.4 T, Karlsruhe, Germany) at a temperature of 298 K. The NMR spectra were collected in methanol-d_4. For ^{1}H NMR analysis, 16 transients were acquired with a 1 s relaxation delay using 32K data points.[18] The 90° pulse was 9.7 s with a spectral width of 3306 Hz. ^{13}C NMR and distortionless enhancement by polarization transfer spectra (DEPT) were obtained for a spectral width of 23148 Hz, collecting 64K data points. The DEPT was used for distinguishing between a CH_3 group, a CH_2 group, and a CH group. The 90° pulse was 9.8 s. Correlation spectroscopy (COSY), heteronuclear multiple-quantum coherence (HMQC), and heteronuclear multiple-bond coherence (HMBC) spectra were acquired with 2048 data points for t2 increments and with 256 data points for t1 increments. The long-range coupling constant for the HMBC experiments was 60 ms.[19]

RESULTS AND DISCUSSION

To ensure food safety, various food additives have been analyzed by governments. Of those, sucralose has been analyzed in processed foods including bamboo shoot products. In the HPLC-RI analysis for sucralose, a putative sucralose was detected (Figure 2). In general, when compounds have similar retention times in HPLC analysis, they can be separated or further confirmed by changing the chromatographic conditions, such as mobile phase, column, or temperature. However, in the case of food analysis, it is important to identify the suspicious or unknown peaks as well as to separate them because the unknown compound may be a hazardous substance such as melamine contained in infant foods.[20]

Accordingly, to identify the putative sucralose more exactly, HPLC-ESI/MS/MS experiments were carried out. In the MS experiments, original sucralose showed a molecular ion $[M - H]^-$ at m/z 395, but the putative sucralose showed a molecular ion $[M - H]^-$ at m/z 243 (Figure 3). The MS results confirmed that

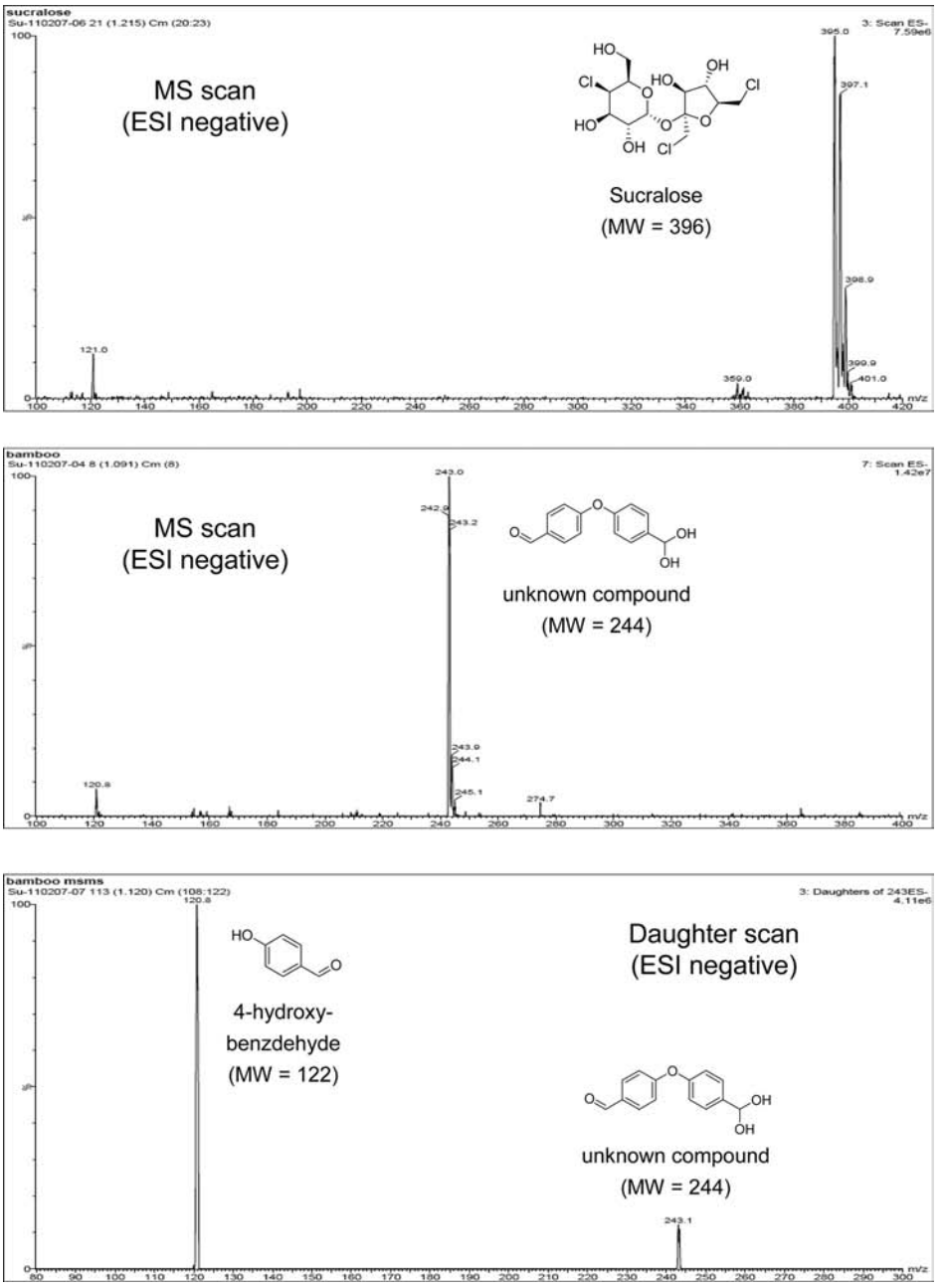

Figure 3. HPLC-ESI/MS/MS spectra of sucralose and the unknown compound.

the putative sucralose was not sucralose. Additionally, HPLC-PDA analysis was performed to confirm the UV absorption spectrum of the unknown compound. In the PDA analysis, the unknown compound showed a unique UV spectrum with a maximum wavelength at 285 nm indicating the existence of an aromatic moiety (Figure 4).

Content of the Unknown Compound. Twenty bamboo shoot products were analyzed. Among them, five samples contained the unknown compound. By measuring the dried weight of the unknown compound, total contents of the unknown compound in samples were determined as 0.01−0.15 mg/g. The ingredient lists of the five samples indicated no additives, the ingredients being only bamboo shoots and water. Therefore, it could be considered that the compound occurred naturally in bamboo shoot. However, there are possibilities of contamination or mislabeling so that further studies will be carried out for other bamboo shoot products.

Structure Elucidation of the Unknown Compound. A derivative of *p*-phenoxybenzaldehyhe was isolated and identified during the analysis of sucralose in bamboo shoot products. The unknown compound is a yellowish solid and soluble in water and methanol. Sucralose is a white powder and soluble in water and methanol. The overall structure of the unknown compound is similar to that of sucralose. Both sucralose and the unknown compound are composed of two ring moieties, which are linked with one oxygen atom. However, the types of ring moieties are quite different. The chemical structures of sucralose and the unknown compound are shown in Figure 1. In the 2D PDA spectrum of the SPE extract, no interferences were observed (Figure 4). Hence, it

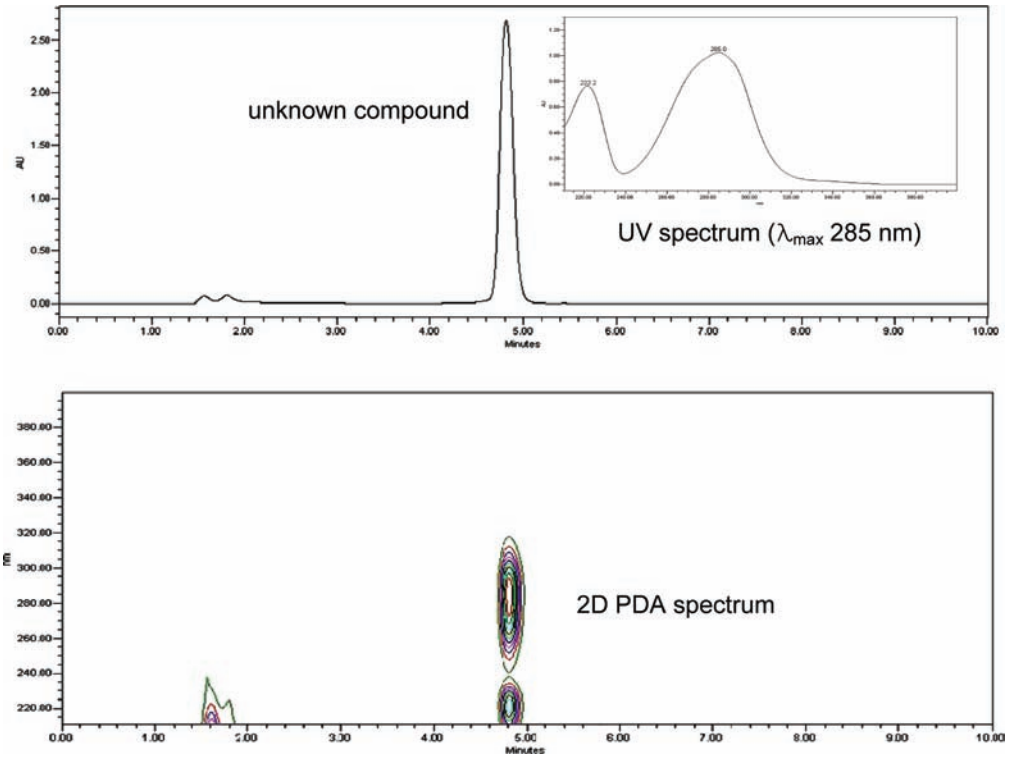

Figure 4. HPLC-PDA chromatogram and spectrum of the unknown compound.

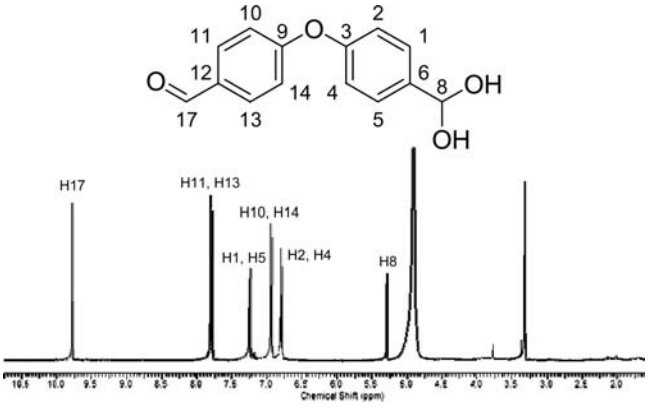

Figure 5. ^{1}H NMR spectrum of the unknown compound.

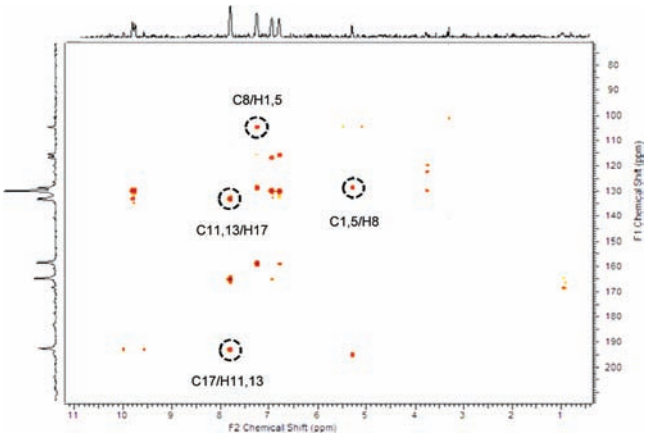

Figure 6. 2D HMBC spectrum of the unknown compound and key correlations.

could be considered that the purity of the SPE extract was suitable for NMR experiments.

In the ^{13}C NMR spectrum, 14 peaks were observed and their multiplicities were determined by the DEPT experiments. There were 10 methine groups and 4 quaternary carbons. On the basis of the interpretation of HMQC, four methine carbons between 115.8 and 133.6 ppm were connected to the peaks at around 7 ppm in the ^{1}H NMR spectrum (Figure 5). Double intensities of the ^{13}C peaks at 115.8, 116.9, 129.0, and 133.6 ppm were observed. According to the observations, the existence of two phenyl rings could be considered. The ^{13}C peak at 192.9 ppm was assigned to carbonyl carbon. In the HMQC experiment, the ^{13}C peak at 192.9 ppm was connected to the ^{1}H peak at 9.76 ppm. In the HMBC experiment, the carbonyl carbon was connected to ^{1}H peaks at 7.78 ppm of a phenyl ring. According to the interpretation of COSY, the ^{1}H peaks of two phenyl rings could be assigned. As a result, it could be considered that there are two phenyl groups and one of them is a benzaldehyde group. However, the connectivity of the two phenyl rings was not observed in the HMBC experiment. It was considered that there there were heteroatoms such as oxygen and nitrogen between the two phenyl rings. In the HMBC experiments, a quaternary carbon at 165.2 ppm was correlated with the ^{1}H peaks at 7.23 ppm. Therefore, the quaternary carbon was assigned to the ^{13}C peak at the para-position of a benzaldehyde. In addition, because the chemical shift was observed at 165.2 ppm, it could be considered that an oxygen atom was adjacent to the para-position carbon. Because another phenyl ring had a similar quaternary carbon peak at 158.8 ppm, the entire structure was considered to be a derivative of benzaldehyhe with a phenoxy group at the para-position.

Table 1. ^{1}H and ^{13}C NMR Peak Assignments of the Unknown Compound

assignment	δ ^{13}C	CH_n	δ ^{1}H (J, Hz)	HMBC	COSY
8	104.8	d	5.28 (s)	C8/H1,5	
2,4	115.8	d	6.77 (d, 8.8)		H2,4/H1,5
10,14	116.9	d	6.92 (d, 8.8)		H10,14/H11,13
1,5	129.0	d	7.23 (d, 8.8)	C1,5/H8	H1,5/H2,4
12	130.3	s		C12/H10,14	
6	130.4	s		C6/H2,4	
11,13	133.6	d	7.78 (d, 8.8)	C11,13/H17	H11,13/H10,14
3	158.8	s		C3/H1,5	
				C3/H2,4	
9	165.2	s		C9/H11,13,	
				C9/H10,14	
17	192.9	d	9.76 (s)	C17/H11,13	

The methine ^{13}C peak at 104.8 ppm was connected to the ^{1}H peaks at 7.23 ppm according to the HMBC experiment (Figure 6). Finally, interpretation of COSY and HMBC experiments revealed the chemical structure of the unknown compound, determined as 4-(4-dihydroxymethylphenoxy)benzaldehyde. Complete assignments of the unknown compound are shown in Table 1.

The final structure of the unknown compound was consistent with the results of the HPLC-ESI/MS/MS experiments. In the ESI/MS experiment, the molecular weight was determined as 244, where the molecular ion $[M - H]^-$ was observed at m/z 243. In the ESI/MS/MS experiment, the molecular ion was fragmented to an ion at m/z 121, which was considered as a 4-hydroxybenzaldedyde ion. Finally, the unknown compound was characterized as 4-(4-dihydroxymethylphenoxy)benzaldehyde. The chemical structure was considered as a partially hydrated form of 4-(4-formylphenoxy)benzaldyhyde. The covalent hydration of benzaldehydes has been investigated in many studies.[21] For example, electrochemical studies indicated that in aqueous solutions of terephthalaldehyde one of the formyl groups is present in the partially hydrated form.[22,23] Currently, the chemical, physical, and toxicological properties of 4-(4-formylphenoxy)benzaldehyde have not been thoroughly investigated.[24]

In summary, a derivative of *p*-phenoxybenzaldehyhe was isolated and identified during the analysis of sucralose in bamboo shoot products. The chemical structure of the unknown compound was elucidated on the basis of NMR spectroscopic analyses. Also, the elucidated NMR structure was confirmed by HPLC-ESI/MS/MS experiments. Finally, the chemical structure of the unknown compound was characterized as 4-(4-dihydroxymethylphenoxy)benzaldehyde, and it was considered as a partially hydrated form of 4-(4-formylphenoxy)benzaldyhyde. Even though there was no evidence that the compound showed any toxic effects, the structural characterization might be valuable for the evaluation of food safety. Additionally, in future works, biological tests will be carried out to determine whether the compound shows any toxic effects to humans or not.

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