JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

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# Toxic Ipomeamarone Accumulation in Healthy Parts of Sweetpotato (*Ipomoea batatas* L. Lam) Storage Roots upon Infection by *Rhizopus stolonifer*

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Supporting Information

**ABSTRACT:** Furanoterpenoid accumulation in response to microbial attack in rotting sweetpotatoes has long been linked to deaths and lung edema of cattle in the world. However, it is not known whether furanoterpenoid ipomeamarone accumulates in the healthy-looking parts of infected sweetpotato storage roots. This is critical for effective utilization as animal feed and assessment of the potential negative impact on human health. Therefore, we first identified the fungus from infected sweetpotatoes as a *Rhizopus stolonifer* strain and then used it to infect healthy sweetpotato storage roots for characterization of furanoterpenoid content. Ipomeamarone and its precursor, dehydroipomeamarone, were identified through spectroscopic analyses, and detected in all samples and controls at varying concentrations. Ipomeamarone concentration was at toxic levels in healthy-looking parts of some samples. Our study provides fundamental information on furanoterpenoids in relation to high levels reported that could subsequently affect cattle on consumption and high ipomeamarone levels in healthy-looking parts.

**KEYWORDS:** *sweetpotato, furanoterpenoids, ipomeamarone, animal and human health safety, Rhizopus stolonifer*

## INTRODUCTION

Despite the prevalence of respiratory infections and deaths relating to furanoterpenoids on global health of cattle and possibly humans, the concentration levels of these compounds in diseased and healthy-looking parts of infected sweetpotatoes is poorly understood and insufficiently documented. Phytoalexins are inducible metabolites elicited by biotic and abiotic factors. Sweetpotato contains several phytoalexins, collectively known as furanoterpenoids, including ipomeamarone, **1**; dehydroipomeamarone, **2**; 4-ipomeanol, **3**; and 1,4-ipomeadiol, **4** (Figure 1).[1,2] Biotic factors such as fungi are reported to elicit varying levels of furanoterpenoids,[2] which cause hepatoxicity, pneumonia, lung edema,[3−5] and cattle deaths.[6] Although information on furanoterpenoids is available concerning isolation methods[7] and presence in sweetpotatoes,[2,8] there is no documented or accessible information on variation of the concentrations from inoculation point on toward healthy-looking parts of an infected sample. This is important because in sub-Saharan Africa (SSA), sweetpotato is a food crop for many rural poor families whereby both healthy and infected sweetpotato storage roots are harvested together. The infected parts are usually removed and fed to farm animals, while the remaining apparently healthy parts are consumed by the farm households, typically in western Kenya (Dr. Robert Mwanga, sweetpotato breeder, personal communication).

Sweetpotato is ranked the third most important root and tuber crop after potato and cassava in SSA. As a food crop, sweetpotato is used by many poor families because of the higher yield in dry matter content per unit area compared to cereal cultivation on an equivalent piece of land.[9] In addition, sweetpotatoes are particularly important during dry periods of the year when cereal crops are unavailable.[9] Unfortunately, it is during the same dry period that the crop is attacked by insects and microbes.[10,11] It is also known that farmers in SSA practice piece-meal harvesting, which exposes the storage roots left in the soil during dry seasons to insects and microbes.[12] Farmers who harvest sweetpotato storage roots for sale are faced with postharvest infection because microbes attack the storage roots owing to the favorable warm conditions and bruising caused by handling.[13] Storage roots respond to attack by eliciting production of furanoterpenoids to fight off the infection within the sweetpotato tissues. The total furanoterpenoid level of three compounds was documented between 25 and 67,000 mg/kg in harvested storage root samples irrespective of the microbe that elicited their accumulation.[1,2] The use of radioactivity to analyze ipomeamarone levels has been done, but more sensitive

Received: May 6, 2014
Revised: November 13, 2014
Accepted: November 24, 2014
Published: November 24, 2014

  

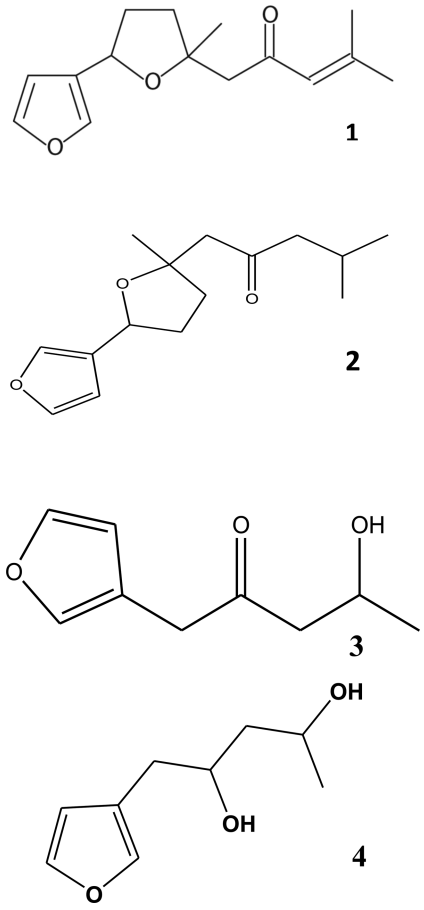


**Figure 1.** Furanoterpenoids in sweetpotatoes: ipomeamarone, **1**; dehydroipomeamarone, **2**; 4-ipomeanol, **3**; and 1,4-ipomeadiol, **4**.

techniques, such as coupled liquid chromatography-quadruple time-of-flight mass spectrometry (LC−QToF−MS) and coupled gas chromatography−mass spectrometry (GC−MS), are needed.[14]

It is not known how far into the healthy-looking storage root samples that these furanoterpenoids are found and at what levels. Since this could potentially expose both humans and farm animals to either chronic or acute toxicity, we (i) isolated and identified a fungus from field samples that elicited furanoterpenoid production; (ii) characterized the furanoterpenoids using GC−MS and LC−QToF−MS; (iii) evaluated the furanoterpenoid concentration levels produced for different sweetpotato cultivars after infection by the isolated fungus; and (iv) determined the furanoterpenoid levels within the apparently healthy part of infected storage roots.

## MATERIALS AND METHODS

**Plant Materials.** Two types of sweetpotato (*Ipomoea batatas* L. Lam) samples were used to analyze for ipomeamarone concentrations: weevil-infected sweetpotatoes for fungal isolation and healthy sweetpotatoes for inoculation experiments with the isolated fungus. Healthy sweetpotatoes were purchased from markets in Nairobi, Kenya and were classified by the vendors as Kemb, Naspot, Bungoma, and Nyawo cultivars. Weevil-infested sweetpotato storage roots were sampled from a farm at the Kenya Agricultural Research Institute (KARI), Marigat, Kenya during the dry season when infestation was high. Sampled storage roots were carefully placed in covered but aerated plastic jars (20.5 cm × 10.5 cm) immediately after harvest and then incubated at 25−28 °C for 5 days to promote growth of microbes.

**Isolation and Identification of Fungus from Weevil-Infested Sweetpotatoes.** Isolation was carried out from fully infected storage roots in jars by using a pin and plated on potato dextrose agar (PDA) (Oxoid, Hampshire, England) media supplemented with 50 μg/mL streptomycin sulfate (Sigma, St. Louis, MO) and chloramphenicol (25 μg/mL) (Sigma, St. Louis, MO) to inhibit bacterial growth. Plates were incubated overnight at 25 °C during which several colonies emerged. Single colonies were isolated and subcultured on PDA without antibiotics. There was a colony that was consistently found on all the PDA plates, and then named Marigat isolate-1 (MI-1). It was identified using both morphological and molecular characterization. For morphological identification, a fungal isolation technique previously described was used.[15] The PDA plate was inoculated using a sterile needle. A sterile glass coverslip was placed in an oblique position on the culture, and plates were incubated at 25 °C for 4−6 days. From day two onward, coverslips were removed daily and mounted on a slide stained with lactophenol aniline blue (Sigma-Aldrich, St. Louis, MO). The coverslips were examined using a light microscope (Leica, Wetzlar, Germany) at the magnifications ×8 and ×40.

Molecular characterization of MI-1 was initiated by isolation of genomic DNA using CTAB method from mycelia grown on PDA plates.[16] Polymerase chain reaction (PCR) was conducted using internal transcribed spacers (ITS) primer pairs.[17] Nonspecific PCR products generated were further eliminated by band-stab PCR.[18] The PCR comprised a 25 μL reaction that consisted of PCR buffer (1*x*), ITS1 primer (0.2 μM), ITS4 primer (0.2 μM), dNTPs (0.06 mM), $MgCl_2$ (2 mM), and Taq polymerase (0.5 unit) (Thermo Scientific, Wyman, MA). The primer sequences for ITS1 and ITS4 were 5′ TCCGTAGGTGAACCTGCGG 3′ and 5′ TCCTCCGCTTATTGATATGC 3′, respectively.[17] This reaction mix was subjected to the following PCR program: initial denaturation 94 °C for 5 min, followed by 40 cycles of denaturation 94 °C for 45 s, annealing temperature 60 °C for 30 s, elongation 72 °C for 90 s, and a final elongation 72 °C for 6 min. PCR was conducted on several other microbes to verify that the amplicon generated from MI-1 was not present in other microbes or if present, it was not of a similar size. The microbes included were as follows: MI-1; *Metarhizium anisopliae* IC30 from ICIPE; *Ceratocystis fimbriata* f. sp. *platani* (CBS 127659); *Clavibacter michiganensis* ssp. *michiganensis*; *Catharanthus roseus* containing Udinese-Stolbur phytoplasma from CBS-KNAW Fungal Biodiversity Centre (Utrecht, The Netherlands); and an unknown fungus culture collected from potato fields and were grown on PDA media containing streptomycin sulfate. PCR products were resolved on 1% agarose gel in TBE for 1 h at 100 V. PCR products were purified using QIAquick PCR Purification kit (Qiagen, Hilden, Germany) and Gel Extraction kit (Qiagen, Hilden, Germany). Purified PCR products were sequenced using GS-FLX 454 platform (454 Life Sciences/Roche, Bradford, CT).

**Extraction and Identification of Furanoterpenoids from Fungal-Infected Sweetpotatoes.** The storage root samples were prepared using a previously described method[2] with few modifications. Healthy storage roots from sweetpotato cultivars namely Kemb, Naspot, Nyawo, and Bungoma were washed, surface sterilized for 5 min using 0.5% sodium hypochlorite, and rinsed three times in sterile distilled water in a laminar flow hood. In the first experiment, the storage root samples were cut into halves and placed in clean sterile plastic containers and then inoculated with actively growing fungal isolate (MI-1) from agar plugs, while the controls were not inoculated. The sweetpotato samples (both inoculated and noninoculated) were replicated three times for each cultivar. The second experiment consisted of inoculating Kemb and Naspot cultivars using MI-1, and storage roots tied with polythene and elastic bags to restrict growth of the fungus. The storage roots were incubated at 25 °C for 7−14 days for infection. This second experiment consisted of two cultivars, Kemb and Naspot, with seven samples. Each sample was chopped into 1 cm slices to analyze furanoterpenoid levels from infection point to the healthy-looking tissue. Differences between means were estimated using a Student−Newman−Keuls test.

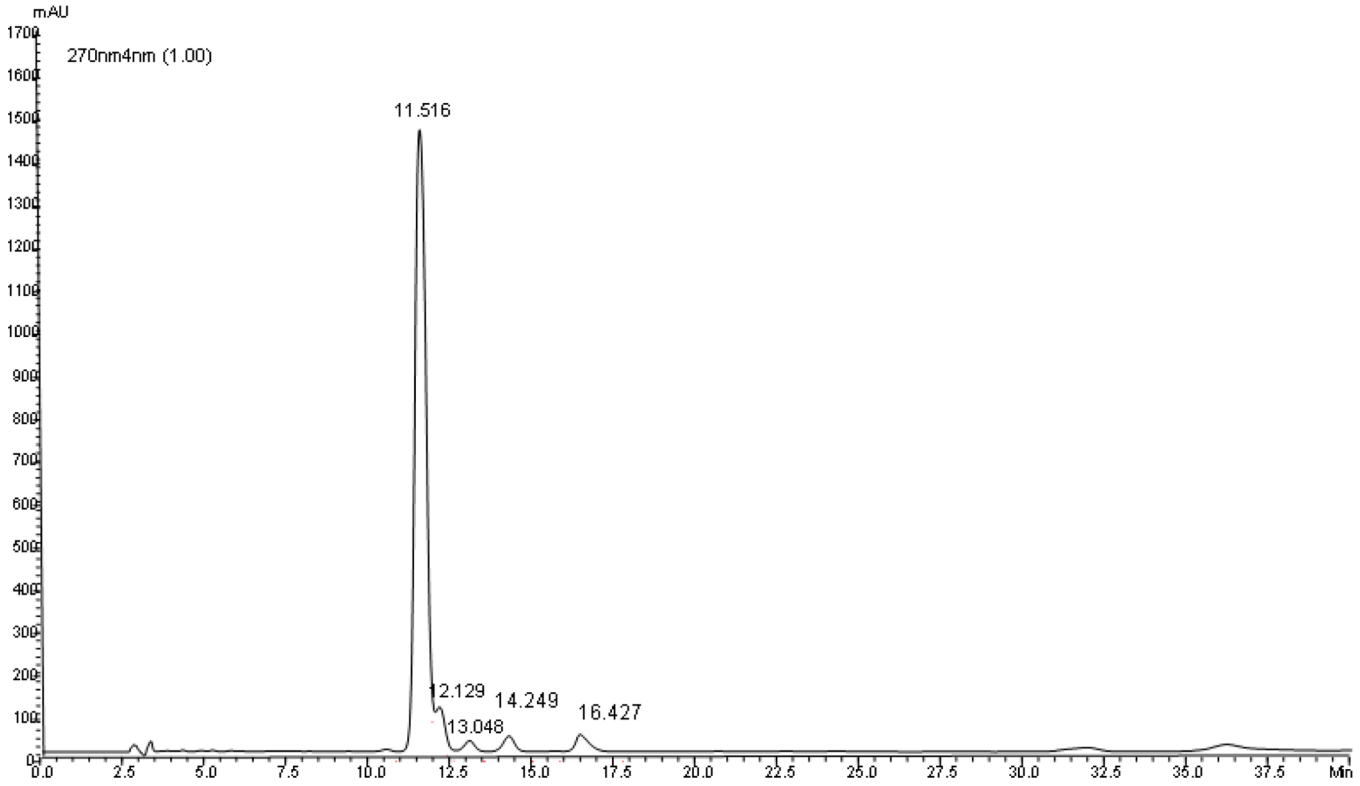


**Figure 2.** HPLC profile of the furanoterpenoid mix isolated from column chromatography with ipomeamarone and dehydroipomeamarone indicated as 16.43 min and 11.52 min, respectively.

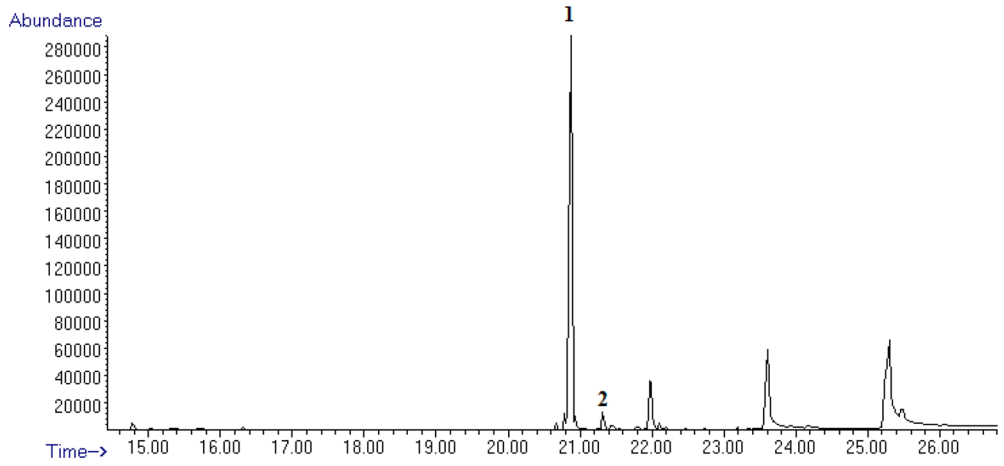


**Figure 3.** Representative GC−MS total ion chromatogram of purified furanoterpenoids: ipomeamarone, **1**; and dehydroipomeamarone, **2**.

The fungus covering the sweetpotato was scraped off. The storage roots were then weighed and blended in 100 mL of methanol and 3 g of NaCl for 3 min. For isolation and purification of dehydroipomeamarone and ipomeamarone, 268 g of infected sweetpotato was extracted with 550 mL of methanol. The extracts were filtered using Whatman filter paper No. 4, poured in conical flasks, and then concentrated in a rotary evaporator to remove methanol and water. The volume of concentrated crude extract was estimated as 10 mL, and the same volume of 10 mL of dichloromethane (Sigma, St. Louis, MO) was added to effectively double the volume of the extract for each flask. The organic phases were combined, concentrated to dryness in a rotary evaporator to remove any remaining solvents from the crude extract, weighed, and then purified by chromatography.

Fifteen grams of the extracted materials was chromatographed on 32−63 $\mu$m silica gel (Riedel-de Haen, Seelze, Germany) using a hexane−ethyl acetate gradient. The furanoterpenoids fraction was eluted with 90% hexane:ethyl acetate, which was further cleaned by column chromatography using a hexane−acetone gradient to yield ipomeamarone mix (1 g). Further purification of the eluent was carried out on a Shimadzu VP series using a 205 mm × 10 mm i.d., 5 $\mu$M ACE Q C-18 column (ACE, Aberdeen, Scotland). The mobile phase used an isocratic program (A:B), 60:40 with a flow rate of 1 mL/min, and run time was 20 min. Detection was by UV absorption at 270 nm to obtain 18 mg of ipomeamarone and 15 mg of dehydroipomeamarone with percentage yield being 0.12% and 0.10%, respectively (Figure 2). Structures of the isolated furanoterpenoids were determined by means of chromatographic and spectroscopic techniques (LC−QToF−MS, GC−MS, and 1- and 2-D NMR) and by comparison with spectroscopic literature data.[1,19]

For GC−MS analyses, the furanoterpenoids fractions was analyzed by split/splitless injection using a model 7890 gas chromatograph coupled to a 5975C inert XL EI/CI mass spectrometer (Agilent


dx.doi.org/10.1021/jf504702z | J. Agric. Food Chem. 2015, 63, 335−342

Technologies, Palo Alto, CA) (GC−MS) equipped with a 30 m × 0.25 mm i.d. × 0.25 μm film thickness HP-5 column (El 70 eV) (Agilent Technologies, Palo Alto, CA). Helium was used as the carrier gas at a flow rate of 1.2 mL/min. The oven temperature was held at 35 °C for 3 min and then programmed to increase at 10 °C/min to 280 °C and maintain this temperature for 10 min. The target peaks were identified through comparison of their mass spectra with Adams2.L, Chemecol.L, and NIST05a.L library data (Figure 3).

For LC−QToF−MS analyses, the crude extract was concentrated in vacuo to dryness, redissolved in 3 mL of LC−MS grade CHROMASOLV methanol (Sigma-Aldrich, St. Louis, MO), and centrifuged at 14,000 rpm for 5 min, after which 0.5 μL was automatically injected into LC−QToF−MS. The chromatographic separation was achieved on a Waters ACQUITY UPLC (ultra-performance liquid chromatography) I-class system (Waters Corporation, Maple Street, MA) fitted with a 2.1 mm × 100 mm, 1.7-μm particle size Waters ACQUITY UPLC BEH C18 column (Waters Corporation, Dublin, Ireland) heated to 40 °C and an autosampler tray cooled to 15 °C. Mobile phases of water (A) and acetonitrile (B), each with 0.01% formic acid were employed. The following gradient was used: 0−1.5 min, 10% B; 1.5−2 min, 10−50% B; 2−6 min, 50−100% B; 6−9 min, 100% B; 9−10 min, 90−10% B; 10−12 min, 10% B. The flow rate was held constant at 0.4 mL/min. The UPLC system was interfaced by electrospray ionization (ESI) to a Waters Xevo QToF−MS operated in full scan $MS^E$ in positive mode. Data were acquired in resolution mode over the $m/z$ range of 100−1200 with a scan time of 1 s using a capillary voltage of 0.5 kV, sampling cone voltage of 40 V, source temperature of 100 °C, and desolvation temperature of 350 °C. The nitrogen desolvation flow rate was 500 L/h. For the high-energy scan function, a collision energy ramp of 25−45 eV was applied in the T-wave collision cell using ultrahigh purity argon (≥99.999%) as the collision gas. A continuous lock spray reference compound (leucine enkephalin; $[M + H]^+ = 556.2766$) was sampled at 10 s intervals for centroid data mass correction. The mass spectrometer was calibrated across the 50−1200 Da mass range using a 0.5 mM sodium formate solution prepared in 90:10 2-propanol/water (v/v). MassLynx version 4.1 SCN 712 (Waters Corporation, Maple Street, MA) was used for data acquisition and processing. The elemental composition was generated for every analyte. Potential assignments were calculated using monoisotopic masses with specifications of a tolerance of 10 ppm deviation and both odd- and even-electron states possible. The number and types of expected atoms were set as follows: carbon, ≤100; hydrogen, ≤100; oxygen, ≤50; nitrogen, ≤6; sulfur, ≤6. The LC−QToF−MS data acquisition and analysis were based on the following defined parameters: mass accuracy (ppm) = 1,000,000 × (calculated mass − accurate mass)/calculated mass; fit conf % is the confidence with which accurate mass (measured data) matches the theoretical isotope models of the elemental composition in the list; elemental composition is a suggested formula for the specified mass. This is a summation of the quantities of elements, isotopes, or superatoms that can compose the measured data, calculated using the following atomic masses of the most abundant isotope of the elements: C = 12.0000000, H = 1.0078250, N = 14.0030740, O = 15.9949146, and S = 31.9720718. The empirical formula generated was used to predict structures that were proposed based on the online database, fragmentation pattern, and literature.

To verify the identity of these peaks, nuclear magnetic resonance (NMR) was done. The isolated and purified ipomeamarone and dehydroipomeamarone samples (5 mg each) were each dissolved in $CDCl_3$ (Cambridge Isotope Laboratories, Tewksbury, MA) and placed in 2.5 mm × 100 mm MATCH NMR tubes (Norell, Landisville, NJ). 1D and 2D $^1H$ and $^{13}C$ NMR spectroscopy, including correlation spectroscopy (COSY), heteronuclear single-quantum coherence (HSQC), and heteronuclear multiple-bond correlation (HMBC) spectroscopy, were used for verification. For the dehydroipomeamarone, 2D nuclear Overhauser effect spectroscopy (NOESY) data were collected and analyzed for additional structural verification (see Supporting Information). All NMR spectra were acquired at 22 °C using a 5 mm TXI CryoProbe (Bruker Corporation, Billerica, MA) and a Bruker Avance II 600 console (600 MHz for $^1H$ and 151 MHz for $^{13}C$) except for 2D NOESY data, which was collected at 25 °C. Residual $CHCl_3$ was used to reference chemical shifts to $\delta$ 7.26 ppm for $^1H$, and $\delta$ C1 of ipomeamarone is referenced to 72.6 ppm for $^{13}C$ in the HSQC spectrum by a previous report for consistency with the literature.[20] We also checked the residual $CHCl_3$ in $CDCl_3$ for $^{13}C$ in the HSQC spectra to confirm that it was properly referenced according to a previous study.[21] NMR spectra were processed using Bruker Topspin 2.0 and MestReNova (Mestrelab Research) software packages.

**Quantitation of Furanoterpenoids in Sweetpotato Samples.** LC−QToF−MS in full scan $MS^E$ in positive mode was used to detect furanoterpenoids in extracts based on accurate mass measurement, retention time, fragmentation pattern, and reference spectra database published online of isolated dehydroipomeamarone and ipomeamarone (standards).[22] Dehydroipomeamarone and ipomeamarone were quantitated using generated standard calibration curves prepared from the isolated compounds. Serially diluted solutions of isolated standards (0.01−200 μg/μL) were analyzed by LC−QToF−MS to generate linear calibration curves (peak area vs concentration) for ipomeamarone [$y = 583{,}064x + 642{,}221$ ($R^2 = 0.9991$)] and dehydroipomeamarone [$y = 324{,}980x + 177{,}617$ ($R^2 = 0.9995$)], which served as the bases for external quantitation.

## RESULTS AND DISCUSSION

**Identification of MI-1 as *Rhizopus stolonifer*.** Identification of MI-1 fungus isolated from the field-collected sweetpotato storage roots was conducted because different microbes elicit varying furanoterpenoid responses.[2] On the basis of morphological features (sporangia, sporangiophores, spores, collumella, rhizoids, and stolons); sporangiophores that arise from intersections with rhizoids and stolons; the dome-shaped columella; and not falling off when the sporangium dried out, MI-1 was identified as belonging to the genus *Rhizopus*. Morphological features of MI-1 had similarities to previously reported *R. stolonifer*.[23,24] The major finding was the collumella being dome-shaped, which differed from other *Rhizopus* species as previously reported as follows: *R. oryzae* has an ellipsoidal collumella; *R. sexualis*, a conical-cylindrical shape; and *R. microspores*, a subglobose to conical shape.[25] From these observations, it was appropriate to suggest that MI-1 fungus was *R. stolonifer*.

The morphological features of *Rhizopus* further confirmed by molecular evidence enabled us identify the species. The ITS primer pair generated an amplicon of 950 bp for MI-1 fungus; none of the other species in this study had a similar band size to MI-1. *Metarhizium anisopliae* IC30 had a band of 600 bp. Sequencing generated a number of reads, but searches were conducted using sequences longer than 200 bp, based on nonredundant database of NCBI, and produced 100 hits. These hits were mainly ITS1, ITS2, 5.8S, 18S, and 28S fragments with partial or complete length sequences; the top 19 hits based on *e*-value were *Rhizopus stolonifer*. Sequence similarity was of 91−99% with *e*-values between 0 and $9 \times 10^{-162}$. The best alignment to MI-1 was isolate AM933546.1, which had a sequence identity of 98% and an *e*-level of 0. The *R. stolonifer* isolate had a partial sequence of 18S rRNA gene, ITS1; 5.8S rRNA gene, ITS2; and partial sequence of 28S rRNA gene. Molecular characterization using ITS primers confirmed that MI-1 fungus was *R. stolonifer*; such primers have previously been used in *R. stolonifer* identification.[16]

**Chromatographic and Spectroscopic Techniques Confirmed Presence of Furanoterpenoids.** The furanoterpenoids ipomeamarone and dehydroipomeamarone were successfully identified using HPLC, GC−MS, LC−QToF−MS,

**Table 1. Furanoterpenoids-Present and Concentrations in Sweetpotato Extracts from Four Cultivars for Both Inoculated Samples and Their Respective Controls[a]**

| cultivar samples inoculated with MI-1 and respective controls with no inoculation | phytolaexin present | mean concentration levels ($\mu$g/g) ± s.e. |
|---|---|---|
| kemb samples | ipomeamarone | 1476.2 ± 278.7 a |
| kemb samples | dehydroipomeamarone | 2914.2 ± 420.8 a |
| control | ipomeamarone | 95.9 ± 8.2 c |
| control | dehydroipomeamarone | 56.2 ± 9.3 c |
| nyawo samples | ipomeamarone | 1089.9 ± 269.5 ab |
| nyawo samples | dehydroipomeamarone | 1459.7 ± 339.9 b |
| control | ipomeamarone | 96.0 ± 8.2 c |
| control | dehydroipomeamarone | 61.8 ± 9.3 c |
| naspot samples | ipomeamarone | 833.7 ± 245.4 ab |
| naspot samples | dehydroipomeamarone | 1153.2 ± 223.9 b |
| control | ipomeamarone | 56.1 ± 8.2 c |
| control | dehydroipomeamarone | 61.8 ± 9.3 c |
| bungoma samples | ipomeamarone | 676.5 ± 132.2 bc |
| bungoma samples | dehydroipomeamarone | 910.0 ± 208.7 bc |
| control | ipomeamarone | 105.1 ± 8.2 c |
| control | dehydroipomeamarone | 92.6 ± 9.3 c |

[a]Means with the same letter are not significantly different from each other.

and 1D and 2D NMR techniques by comparing their resonances to published data.[20,21,26,27] The HPLC analysis (Figure 2) showed six peaks with ipomeamarone and dehydroipomeamarone eluting at 16.4 and 11.6 min, respectively. GC−MS analysis also revealed six peaks and tentatively identified two of these peaks as ipomeamarone, **1**, at a retention time ($R_t$) of 20.8 min and dehydroipomeamarone, **2**, at an $R_t$ of 21.4 min (Figure 3). LC−QToF−MS analysis also identified the two peaks as ipomeamarone (with elemental composition, $C_{15}H_{23}O_3$; $m/z$ 251.1647, 0.0 ppm error to theoretical value; and a fit conf % of 99.96) at a $R_t$ of 2.87 min and dehydroipomeamarone (with elemental composition, $C_{15}H_{21}O_3$; $m/z$ 249.1491, 2.0 ppm error to theoretical value; and a fit conf % of 98.78) at a $R_t$ of 3.04 min. These results were consistent with the GC−MS analysis.

The HPLC purified peaks analyzed by NMR confirmed the presence of ipomeamarone and dehydroipomeamarone. $^1$H chemical shifts of ipomeamarone were in agreement with literature, while dehydroipomeamarone was a mixture based on LC−QToF−MS and NMR data.[20,27] For dehydroipomeamarone, we observed the $^1$H chemical shifts at positions C-7 as 6.15 ppm (1H), C-9 as 2.13 ppm (3H), and C-10 as 1.85 ppm (3H), which were the only $^1$H chemical shifts as provided by previous workers.[27] These workers reported the $^1$H chemical shifts for positions C-7 as 6.11 ppm (1H), C-9 as 2.09 ppm (3H), C-10 as 1.81 ppm (3H), and all of the other $^1$H chemical shifts were similar to those of ipomeamarone.[27] Unfortunately, these three $^1$H chemical shifts differed 0.04 ppm between our spectrum and previously reported data.[27] Since the dehydroipomeamarone sample was a mixture, having just $^1$H 1D NMR was not satisfactory to confirm the presence of dehydroipomeamarone. Unfortunately, previous workers did not provide any information about NMR solvent or $^{13}$C chemical shift data for the synthetic dehydroipomeamarone.[27] Therefore, we acquired a 2D NMR (COSY, HSQC, HMBC, NOESY) spectrum for dehydroipomeamarone and ipomeamarone samples to compare the $^{13}$C chemical shifts, since dehydroipomeamarone is a derivative of ipomeamarone, which we confirmed using $^1$H NMR. Ipomeamarone 2D NMR data was used for comparison. Both $^1$H and $^{13}$C chemical shifts of dehydroipomeamarone were similar to ipomeamarone for carbon positions from 1′−4′ and from 1−6, but differed at positions 7, 8, 9, and 10: consistent with the structure. NOESY data for dehydroipomeamarone provided us additional evidence to confirm its correct identification.

**Variations in Ipomeamarone Concentrations between and within Cultivars.** The main furanoterpenoids produced were ipomeamarone, **1**, and dehydroipomeamarone, **2**, found in all samples and noninoculated controls. The concentration of furanoterpenoid ranged between 50.6 and 2,330 mg/kg for inoculated samples and 12.4−144.5 mg/kg for the controls. These high levels exceeded those reported previously for *R. stolonifer* of 200−1,100 mg/kg but were comparable to high ipomeamarone elicitors such as *C. fimbriata* and *Fusarium solani* with levels from 1,100−9,300 mg/kg.[2] Similar results have been reported on noninfected sweetpotatoes with low ipomeamarone concentrations between 40 and 325 mg/kg for cultivars in the United Kingdom.[8] Generally, mechanical injury or damaged but noninfected sweetpotato is reported to elicit low furanoterpenoid levels, while infected samples have high levels due to the increase in enzyme activity, which correlates to furanoterpenoid production.[28,29] The low ipomeamarone levels in storage roots of negative control samples from this study confirm that injury or bruising of sweetpotato roots occurs, but the ipomeamarone levels are low.

Concentration levels for ipomeamarone ranged between 50.6 and 2,126.7 mg/kg, while they ranged from 39.3−2,230.4 mg/kg for dehydroipomeamarone. The higher dehydroipomeamarone levels could possibly be because it is a precursor of ipomeamarone, which means that it was yet to be enzymatically converted to ipomeamarone, or dehydroipomeamarone might have inhibited production of ipomeamarone to some extent as reported previously.[1] In preliminary experiments (not shown), ipomeamarone levels were high in inoculated samples, probably due to long incubation time of 28 days compared to the current study where inoculation ranged between 7 and 14 days. More research needs to be conducted to verify this.

Variations in mean ipomeamarone levels by cultivar were as follows: Kemb had the highest mean of 1,476.2 mg/kg, followed by Nyawo with 1,089.9 mg/kg; Naspot had a mean of 833.7 mg/kg, while Bungoma had a mean of 676.5 mg/kg (Table 1). High concentrations of dehydroipomeamarone were


dx.doi.org/10.1021/jf504702z | J. Agric. Food Chem. 2015, 63, 335−342

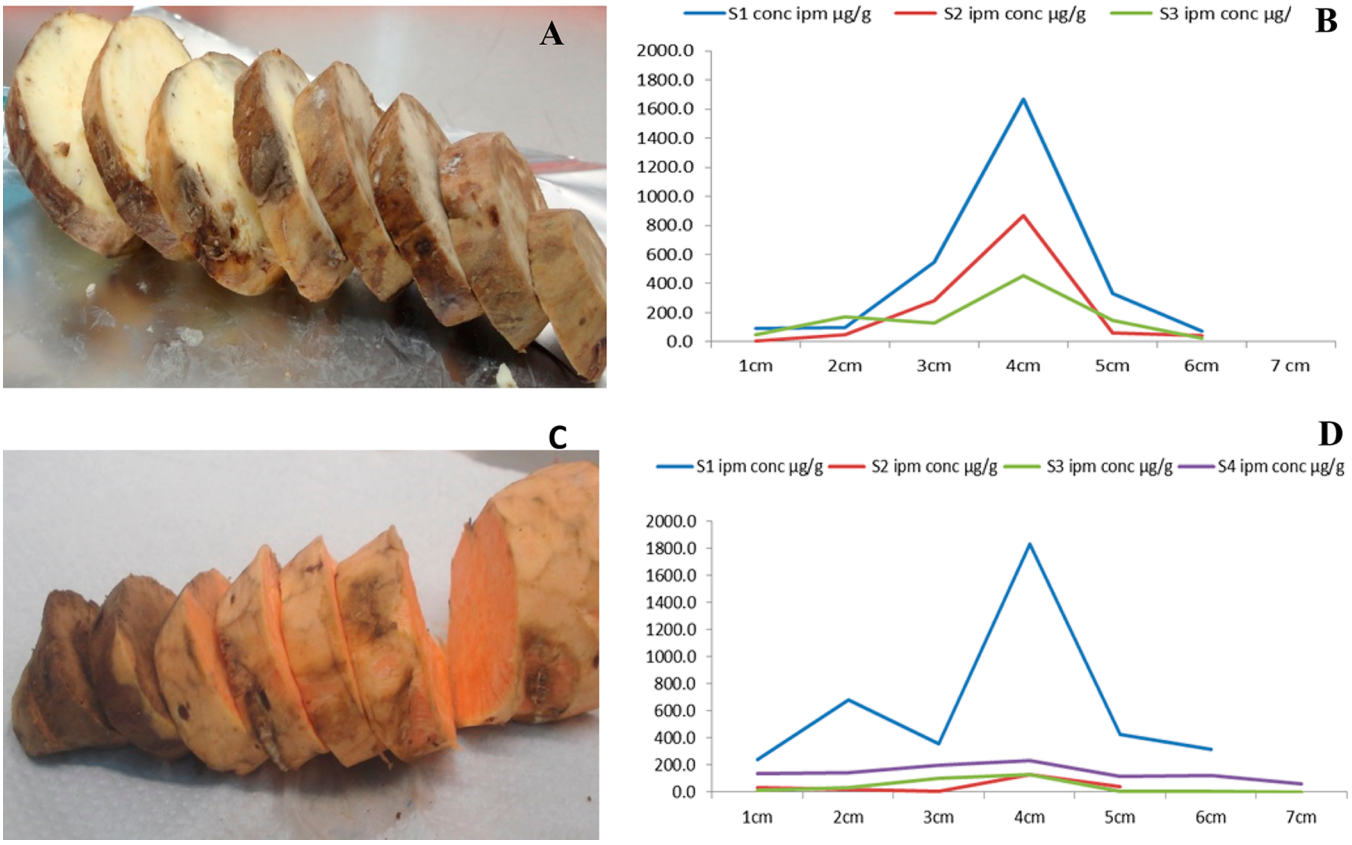


**Figure 4.** Controlled infection of sweetpotato samples by *Rhizopus stolonifer* that enabled ipomeamarone analysis of 1 cm slices for Kemb (A, B) and Naspot (C, D) cultivar samples.

also observed in almost all samples in this study: Kemb had the highest with 1,462.6 mg/kg, Nyawo had 1,459.7 mg/kg, Naspot had 1,153.2 mg/kg, and Bungoma had 910 mg/kg (Table 1). There were significant ($p < 0.05$) differences between cultivars. In addition, there were differences in ipomeamarone levels within the same cultivar, for example, Nyawo had 602 mg/kg in one sample, while the other had 2,126 mg/kg. Similar variations were observed for different cultivars for ipomeamarone in other studies ranging from 11−2,000 mg/kg.[2,8] Such variations have been speculated to be associated with moisture loss, root maturity, and susceptibility or resistance to fungal infection.[30] Other causes of variation include environmental factors such as postharvest practices by different farmers before arrival at the markets. These variations could have affected the concentrations in the current study. Differences in concentrations for these cultivars also suggest that they vary in resistance to *R. stolonifer* attack with Kemb being the most resistant, followed by Nyawo, Naspot, and Bungoma. These results concur with reports from other studies where the differences in ipomeamarone levels were attributed to *Rhizopus* soft rot resistance.[31]

**Higher Ipomeamarone Concentrations in Healthy Parts Away from the Infection Site.** We were successful in confining MI-1 fungus in the controlled infection experiment of Naspot and Kemb cultivars. We observed visually the infection to be high at the inoculation place while decreasing progressively with increased distance from the inoculation site (Figure 4). By contrast, evaluation of the 1 cm slices recorded high ipomeamarone levels for regions with visibly low infection, while low levels were observed for regions with visibly high infection (Figure 4). This trend was observed for all samples evaluated, which showed high ipomeamarone levels at the border between the visibly infected and the healthy-looking site. Similar results have been reported that phytoalexins are produced in healthy tissues surrounding wounded parts but terminate in the necrotic tissue.[32] The highest levels of ipomeamarone were observed in both Kemb and Naspot samples of 1,671.2 and 1,708.4 mg/kg, respectively, at the border between the visible infection and healthy-looking tissues (Figure 4). We also observed differences in ipomeamarone levels in some samples, which could have been due to variations in infection on the fungus along the storage root (Figure 4). There were significant ($p < 0.05$) differences between the samples. In another study, it was reported that elimination of 3−10 mm of disease portions beyond infected regions and also cooking removes most ipomeamarone.[33] This could be true for root samples with high levels only at the border, but in the current study, some samples had high levels even in subsequent layers. This suggests that removal of 1 cm from the infection area will still leave behind most of ipomeamarone depending on how much the subsequent layers had accumulated. In other studies, cooking was reported to destroy about 80% of ipomeamarone, but this depends on the initial concentration level found in the storage root before cooking.[34,35]

Since rural poor farmers in SSA keep 1−2 cows, and these farm animals contribute 30−80% of farm income,[36] if they feed them with diseased sweetpotatoes with exceeding toxicity, their fragile revenues will be at threatened by high costs of treatment or even total low of the intoxicated cow. This information is important for farmers' knowledge since they harvest infected roots, cut off the infected part to feed the farm animals, and then consume the healthy-looking parts (Dr. Robert Mwanga,


dx.doi.org/10.1021/jf504702z | J. Agric. Food Chem. 2015, 63, 335−342

sweetpotato breeder, personal communication). Although there are no reports on how far into the visibly healthy-looking part the farmers cut off these storage roots, either the farmers' household consume the parts with high ipomeamarone concentration, or the animals are fed with it. Results drawn from this study suggest that at least 2−3 cm away from the border between the healthy-looking and the infected part of the damaged sweetpotato storage root should be cut off and disposed of, and the infected part should not be fed to the farm animals because of the presence of toxic phytoalexins.

In conclusion, ipomeamarone accumulation in the apparently healthy parts of damaged sweetpotato storage roots should be considered as a potential economic problem and health threat to both the rural poor farm families and large-scale farmers worldwide. Our results reveal the importance of this problem, but more research should be directed to obtaining information on (i) furanoterpenoids present in infested and infected sweetpotatoes harvested from the sweetpotato fields; (ii) furanoterpenoid levels in sweetpotatoes from farmers' households; (iii) varietal response to fungal infection resulting in ipomeamarone accumulation; (iv) degradation of ipomeamarone after processing infested roots and accumulation in metabolism in animal and human organisms; and (v) management of the problem by the rural poor.

## ■ ASSOCIATED CONTENT

### Supporting Information

HMBC and NOESY NMR spectroscopic data in $CDCl_3$ for ipomeamarone and dehydroipomeamarone. This material is available free of charge via the Internet at http://pubs.acs.org.

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### Notes

The authors declare no competing financial interest.

## ■ ACKNOWLEDGMENTS

This study was financially supported by the Rockefeller Foundation, United States Agency for International Development, and the Bill and Melinda Gates Foundation. We are grateful to Daisy Salifu from ICIPE for guidance on statistical analysis. We are also grateful to George Michuki for the sequencing work using the 454-sequencer. NMR experiments were performed in the McKnight Brain Institute at the National High Magnetic Field Laboratory's AMRIS Facility, which is supported by National Science Foundation Cooperative Agreement No. DMR-1157490 and the State of Florida, in part by an NIH award No. S10RR031637, for magnetic resonance instrumentation; we thank J. Rocca for assistance. James Woodhall and Neil Boonham of the Food and Environment Research Agency (FERA), United Kingdom, gave advice on mycology, and Marcel Westenberg of the National Plant Protection Organisation, The Netherlands, provided the genomic DNA of some microbes, and we are thankful.

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