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Isolation and Characterization of a New Less-Toxic Derivative of the *Fusarium* Mycotoxin Diacetoxyscirpenol after Thermal Treatment

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ABSTRACT: Trichothecenes are an important class of mycotoxins that act as potent protein synthesis inhibitors in eukaryotic organisms. The compound 4,15-diacetoxyscirpenol is highly toxic for plants and animals. Potatoes are especially prone to be contaminated with 4,15-diacetoxyscirpenol after infection with *Fusarium sambucinum*. In the current study, the reduction of 4,15-diacetoxyscirpenol during thermal treatment in aqueous solution was monitored. A new derivative was detected and named DAS-M1. After isolation, DAS-M1 was characterized with LC−HR-MS and LC−MS/MS and structurally elucidated with ^{1}H, ^{13}C, and 2D NMR. Potatoes were inoculated with *F. sambucinum*, and the infected potatoes were cooked at 100 or 121 °C, respectively. A reduction of 4,15-diacetoxyscirpenol from about 26% (1 h at 100 °C) to 100% (4 h at 121 °C) was detected by means of LC−MS/MS analysis. The effects of different pH values on the reduction of 4,15-diacetoxyscirpenol were investigated, showing higher conversion rates at more acidic pH values. In addition, the toxicity of 4,15-diacetoxyscirpenol and DAS-M1 was compared in vitro using a wheat germ transcription/translation assay and in vivo on *Saccharomyces cerevisiae*. The results show that the inhibitory effect of DAS-M1 on yeast growth is about 50 times lower and inhibition of protein synthesis is about 100 times lower than that of 4,15-diacetoxyscirpenol.

KEYWORDS: diacetoxyscirpenol, trichothecene, mycotoxin, detoxification, potatoes, *Fusarium*

INTRODUCTION

Fungal species of the genus *Fusarium* have been indicated as widely distributed plant pathogens and not only cause yield and quality losses but also lead to formation of mycotoxins that are hazardous to animals and humans. The prevention of mycotoxin contamination is of major importance for agricultural and food industries. However, the contamination of various commodities, like barley, wheat, maize, or rice, with *Fusarium* mycotoxins is often unavoidable.[1] Also, *Fusarium* strains isolated from potato tubers prevalently produce mycotoxins.[2]

Trichothecenes are an important class of mycotoxins and share a tricyclic nucleus, containing an epoxide group at C-12 and C-13, which is essential for their toxicity.[3] Trichothecenes act as potent protein synthesis inhibitors in eukaryotic organisms.[4] At least 182 different trichothecenes have been isolated from natural sources.[5] Trichothecenes can be classified into types A-, B-, C-, and D-trichothecenes, according to structural differences. Among the most commonly occurring trichothecenes are the type B trichothecene deoxynivalenol and the type A trichothecenes T-2 toxin and its hydrolyzed derivative HT-2 toxin. Europe, in comparison to the other continents of the world, has one of the most explicit regulations for mycotoxins in food and feed. In the EU, maximum tolerated limits have been established for several mycotoxin−food combinations.[6] A sum parameter for T-2 and HT-2 toxin in cereals and cereal products is considered but not yet in force.

Scirpentriol (3α,4β,15-trihydroxy-12,13-epoxytrichothec-9-ene) has three hydroxyl groups that can be partly or fully acetylated, forming three monoacetoxyscirpenols, three diacetoxyscirpenols, and triacetoxyscirpenol.[7] Members of the scirpentriol family, particularly 4,15-diacetoxyscirpenol (**1**) (Figure 1, DAS), were described to be toxic against plants[8,9] and animals.[10] 4,15-Diacetoxyscirpenol was detected and isolated from cultures of *Fusarium equiseti* (*Gibberella intricans*), and its chemical properties and structure have been characterized.[11] 4,15-Diacetoxyscirpenol can also be produced by a number of other *Fusarium* species, including *Gibberella pulicaris* (asexual stage: *Fusarium sambucinum*), which is the major causative agent of potato tuber dry-rot.[12,13] This important storage disease of potato tubers is of economic significance worldwide.[14] Susceptibility of potato cultivars toward *F. sambucinum* correlates highly with the concentration of 4,15-diacetoxyscirpenol in infected tubers.[15] The production of trichothecenes may occur in potatoes

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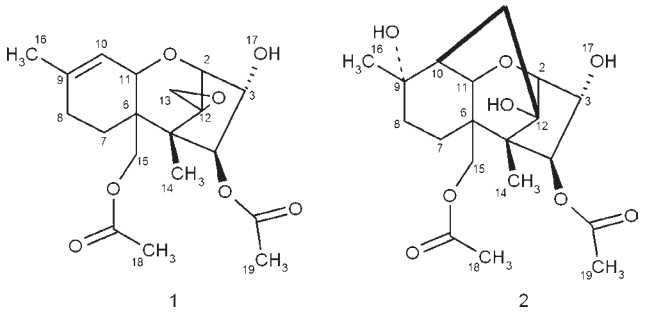

Figure 1. Chemical structures of 4,15-diacetoxyscirpenol (**1**) and DAS-M1 (**2**).

artificially and naturally infected with this fungus either in the field or during storage.[16,17] LD_{50} values of 4,15-diacetoxyscirpenol have been investigated in different animal species and found to be 23 mg/kg in mouse (intraperitoneal), 7.3 mg/kg in rat (oral), 1.0 mg/kg in rabbit (intravenous), and 0.37 mg/kg in swine (intravenous).[18]

In the current study, the reduction of 4,15-diacetoxyscirpenol due to thermal treatment in aqueous solution was monitored, detecting a new major derivative. In addition, this conversion was monitored by cooking potatoes inoculated with *Fusarium sambucinum* (synonym: *F. sulphureum*) strain MRC514.[19] After isolation, the structure of this derivative, termed DAS-M1 (**2**) (Figure 1), was elucidated with NMR. The toxicity of DAS-M1, in comparison to 4,15-diacetoxyscirpenol, has been examined *in vitro* by a wheat germ extract transcription/translation system and *in vivo* on *Saccharomyces cerevisiae*.

MATERIALS AND METHODS

Purification of 4,15-Diacetoxyscirpenol and DAS-M1. Strain MRC514,[19] which is a prolific producer of 4,15-diacetoxyscirpenol in liquid medium, was sporulated in mung bean broth. Liquid Czapek−Dox medium containing 2% peptone was inoculated with 10^5 spores/25 mL in magenta boxes. The still cultures were incubated in a plant growth chamber (16 h light/8 h dark) at 22 °C. After 3 weeks of incubation the mycelia were removed from the cultures by suction filtration and the filtrates were extracted with 1 volume of ethyl acetate. The pooled ethyl acetate extracts were used for silica gel normal-phase flash chromatography using gradient elution with ethyl acetate/methanol (20% → 100% methanol). The fractions were collected and analyzed using a QTrap LC−MS/MS instrument (AB Sciex, Foster City, CA) equipped with a heated nebulizer (APCI) source. The appropriate fractions were used for the purification of 4,15-diacetoxyscirpenol by an 1100 series preparative HPLC system (Agilent Technologies, Waldbronn, Germany). Separation was achieved at 25 °C using a 250 mm × 10 mm i.d., 10 μm, Luna RP-C18(2) semipreparative column (Phenomenex, Aschaffenburg, Germany). An injection volume of 500 μL and a flow rate of 16 mL/min were chosen. Mobile phases were Milli-Q water (Millipore, Molsheim, France) and LC grade methanol (Merck, Darmstadt, Germany). After an initial hold time of 2 min with 20% methanol, a linear gradient was applied which reached 100% methanol after 10 min. A 2 min hold time at the same composition was followed by a rapid change to the starting conditions, which were held for another 3 min to re-equilibrate the column. DAS-M1 was produced from an aqueous solution of 3.8 mg of 4,15-diacetoxyscirpenol after treatment for 72 h at 100 °C under reflux. An amount of 2.96 mg of DAS-M1 was purified from this solution by preparative HPLC system with the conditions shown above. Time based fractionation was used. 4,15-Diacetoxyscirpenol eluted at 9.30 min, while the more polar DAS-M1 eluted at 7.90 min.

LC−MS(/MS) Measurements. A QTrap LC−MS/MS system with an APCI source (see above) was used to characterize the purified compounds. Chromatographic separation was achieved on an Agilent 1100 HPLC system using a 150 mm × 4.6 mm i.d., 5 μm, Gemini RP-C18 column (Phenomenex) at 25 °C. A linear gradient from 20% to 90% aqueous methanol, containing 5 mM ammonium acetate, was applied. An amount of 25 μL of each sample was injected into a flow rate of 1 mL/min. The source was used solely in the positive ionization mode at 450 °C. MS parameters were the following: curtain gas 35 psi (241 kPa), nebulizer gas 60 psi (414 kPa), auxiliary gas 15 psi (103 kPa), declustering potential 21 V, entrance potential 5.5 V. For characterization experiments, 10 mg/L solutions of 4,15-diacetoxyscirpenol and DAS-M1 were prepared in 50% aqueous methanol and injected by HPLC. First, Q3 full scans were acquired in positive mode from m/z 200−700 within 1 s. The enhanced product ion (EPI) scan mode was used to acquire MS/MS spectra between m/z 150 and 450. For the EPI scans, the following further parameters were set: scan rate 1000 Da/s, Q0 trapping on, linear ion trap fill time 20 ms, collision energy 20 eV.

The SRM mode was used for quantitation of 4,15-diacetoxyscirpenol and DAS-M1. Selection of the quantifier and qualifier transitions was based on transitions from the $[M + NH_4]^+$ ions to the most and second-most predominant fragment ions and were as follows: 4,15-diacetoxyscirpenol (quantifier) m/z 384.3 > 307.2; 4,15-diacetoxyscirpenol (qualifier) m/z 384.3 > 349.2; DAS-M1 (quantifier) m/z 402.3 > 325.2; DAS-M1 (qualifier) m/z 402.3 > 367.2. For all transitions, optimum declustering potentials of 21 V and collision energies of 15 eV were evaluated. The dwell time was set to 50 ms per transition. Analyst, version 1.5, was used for instrument control and data evaluation.

For HR-MS experiments a LTQ Orbitrap XL high resolution mass spectrometer (Thermo Fisher Scientific, Waltham, MA) was used. A solution containing both 1 mg/L 4,15-diacetoxyscirpenol and DAS-M1 in 50% aqueous methanol together with 5 mM ammonium acetate was infused in the ion source using a syringe pump at a flow rate of 5 μL/min. Ionization was performed in electrospray positive mode with the following settings: ion transfer tube 300 °C, sheath gas flow 45 (arbitrary units), auxiliary gas flow 5 (arbitrary units), source voltage 4 kV, capillary voltage 25 V, tube lens 125 V. FTMS data were acquired at m/z 200−700 with a resolution of 60.000. The ammonium adduct of 4,15-diacetoxyscirpenol was used for internal mass calibration (m/z 384.2017). Possible elemental formulas were generated with the following restrictions: maximum mass deviation 2 ppm, number of carbon atoms 0−50, number of hydrogen atoms 0−100, number of oxygen atoms 0−30, number of nitrogen atoms 0−10. The nitrogen rule was applied, ruling out elemental formulas with an odd number of nitrogen atoms. The instrument was controlled with Xcalibur 2.0.7.

NMR Spectroscopy. NMR spectra were recorded on a Varian INOVA 600 MHz spectrometer (Palo Alto, CA). A concentrated solution of DAS-M1 in DMSO-d_6 in a 5 mm tube was used for structural determination. The 2D experiments were conducted with the default parameter set up. g-COSY (1024 × 256 data points) was used for scalar coupling correlation, and ROESY (1024 × 256 data points) was used for dipolar coupling, using a 200 ms spin-lock time. The assignments were also confirmed by matching of the corresponding C−H correlations obtained by HMQC spectra (with j1xh = 140 Hz average scalar coupling constant). NMR spectra were processed with MestReC software (Mestrelab Research SL, Santiago de Compostela, Spain).

In Vivo Toxicity Test. The trichothecene sensitive *Saccharomyces cerevisiae* strain YZAS107 [20] was used to investigate growth inhibition upon toxin addition in liquid cultures. An amount of 100 μL of exponentially growing yeast cells (OD_{600} = 0.05) in YPD medium was combined with 100 μL of 4,15-diacetoxyscirpenol or DAS-M1 solutions in 10 mL glass tubes in triplicate. 4,15-Diacetoxyscirpenol solutions were

in the range 0−20 mg/L (0−54.6 μM), while DAS-M1 concentrations ranged from 0 to 500 mg/L (1302 μM) in water. Tubes were incubated at 30 °C for 24 h on a shaker (200 rpm). Then an amount of 800 μL of water was added to the tubes and the mixed contents were transferred into cuvettes. OD_{600} was measured using a DU-640 spectrophotometer (Beckman, Brea, CA). The absorbance of the initial cells at the beginning of the experiment was subtracted from all measured values. For the calculation of the percent growth, the average OD_{600} of the no toxin control was set to 100%.

In Vitro Toxicity Test. The toxic effect of trichothecenes is caused by the inhibition of protein synthesis. To analyze and compare the toxicities of DAS-M1 and 4,15-diacetoxyscirpenol in a cell-free system, an in vitro assay, based on inhibition of protein synthesis using translation of firefly luciferase in wheat germ extract (Promega-TNT wheat germ extract, Mannheim, Germany), was performed according to the manufacturer's instructions. After 30 min (still in the linear range of product formation) the reactions containing variable concentrations of toxin were stopped by addition of 1 μL of 20 mM cycloheximide solution. The reaction mixtures were transferred into a 96-well black plate, and an amount of 20 μL of luciferase assay reagent (Promega, Mannheim, Germany) was added. The amount of light produced by the formed luciferase was measured in a multichannel luminometer (Wallac 1420 VICTOR2, Perkin-Elmer, San Jose, CA).

Thermal Treatment of 4,15-Diacetoxyscirpenol and *F. sambucinum* Inoculated Potatoes. The thermal treatment tests were performed at two different conditions. The first experiment was conducted with pure 4,15-diacetoxyscirpenol standards in H_2O. In order to examine the effects of pH on the conversion of 4,15-diacetoxyscirpenol to DAS-M1, the standard solutions (27.2 μM) were prepared at three different pH values of 3.0, 5.0, and 7.0 in respective buffer solutions. The cooking tests were performed at three different temperatures: at 80 °C (using a drying chamber), 100 °C (under reflux) for 1, 4, and 8 h, respectively, and also at 121 °C by using a pressure cooker (2 bar) for 1 and 4 h. All tests were carried out in triplicate.

For the second set of thermal treatment tests, 25 potatoes of the cultivar Tosca with similar sizes were washed and weighed. Surfaces were sterilized by 10% chlorine bleach for 5−10 min, and afterward the potatoes were washed 3 times with sterile water for 5 min. A 3 mm deep cut was made in the middle of each potato by using a 9 mm cork borer. Afterward, a 9 mm agar block from 3 d old PDA plates inoculated with spores of the *Fusarium sambucinum* strain MRC514 was inserted into the wound. The potatoes were transferred into magenta boxes containing 50 mL of water−agar (2% agar in H_2O) and incubated for 14 d at 20 °C in the dark. Five potatoes were chosen randomly as controls (uncooked), and five potatoes from each treatment were cooked or autoclaved for 1 or 4 h, respectively. For cooking, each potato was put into a 400 mL beaker filled with 200 mL of boiling doubly distilled water. The beakers were covered by aluminum foil, the water levels were marked on the glass, and the beakers were put on a heat plate for 1 and 4 h, respectively. The water level was kept constant during the experiment by successively adding water as required. The pressure cooking experiments were carried out by autoclaving five potatoes each for 1 or 4 h, respectively, at 121 °C, 2 bar. For this experiment the potatoes were also kept in 200 mL of water in 400 mL beakers, which were covered with aluminum foil. Water was added after autoclaving to compensate for evaporation losses. For the control, five potatoes were put into five 400 mL beakers. Each tuber was cut into small pieces, and an amount of 200 mL of doubly distilled water was added. The contents of the beakers (from all experiments) were shredded with an Ultra-Turrax T25 (IKA, Staufen, Germany) for 5 min. The homogenized samples were transferred into 600 mL beakers, and an amount of 200 mL of acetonitrile was added to each sample. A magnetic stirrer was used to mix the contents for 10 min. Afterward, the extracts were decanted over folded paper filters into centrifuge bottles. The extracts were centrifuged for 10 min at 3500 rpm. The 2 mL aliquots of each sample were evaporated to dryness under a gentle stream of nitrogen, and the residues were dissolved in 100 μL of 50% aqueous methanol. The concentrations of 4,15-diacetoxyscirpenol and DAS-M1 were determined by LC−MS/MS in SRM mode.

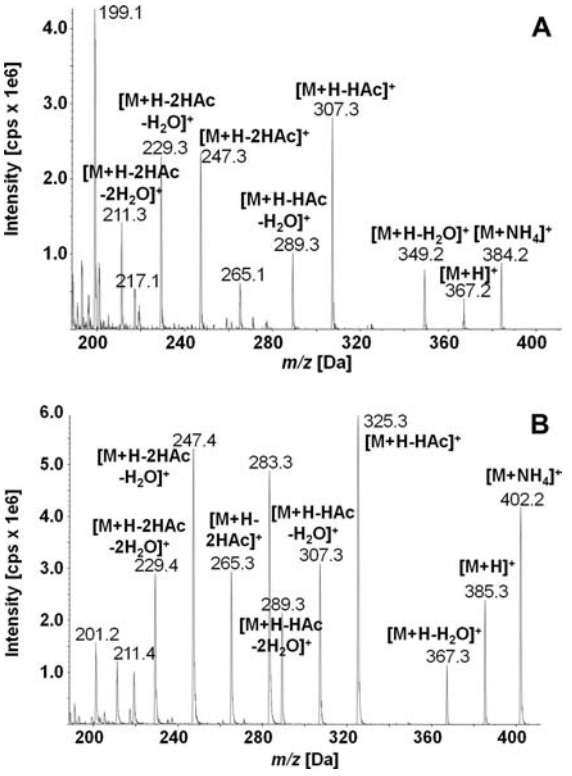

Figure 2. MS/MS spectra of 4,15-diacetoxyscirpenol (A) and DAS-M1 (B).

RESULTS AND DISCUSSION

LC−MS(/MS) Characterization of DAS-M1. A reduction of the 4,15-diacetoxyscirpenol concentrations in repeatedly heat treated 4,15-diacetoxyscirpenol stock solutions was noted. The investigation of this unexpected effect led to the identification of a putative major degradation product, named DAS-M1, which was purified by preparative HPLC for further characterization. For comparison of collision induced dissociation fragmentation patterns, enhanced product ion (EPI) mass spectra of 4,15-diacetoxyscirpenol (Figure 2A) and purified DAS-M1 (Figure 2B) were recorded at equal concentrations (2.7 μM). The MS/MS scans were performed in the positive ionization mode in the range of m/z 150−450 with collision energies of 20 eV using the ammoniated ions as precursors. Despite the mass difference of 18 Da of the precursor ions, very similar MS/MS spectra, corresponding to neutral losses of multiple water and acetic acid molecules, were gained for both compounds. The elemental formula of DAS-M1 was obtained by high resolution mass spectrometry using a LTQ-Orbitrap and applying internal mass calibration. Two major ions were visible in the spectrum at m/z 402.2124 and m/z 407.1677, showing the $[M + NH_4]^+$ and $[M + Na]^+$ adducts, respectively. The sole possible elemental formula for DAS-M1 was $C_{19}H_{28}O_8$. The difference between the theoretical mass and the measured mass of the $[NH_4]^+$ and $[Na]^+$ adducts of DAS-M1 was +0.4 and +0.1 ppm, respectively.

Table 1. NMR Spectroscopic Data for 4,15-Diacetoxyscirpenol and DAS-M1 in DMSO (600 MHz Proton, 150 MHz Carbon)[a]

	4,15-diacetoxyscirpenol				DAS-M1			
position	δ ^{1}H (ppm)	multipl[b]	J (Hz)	δ ^{13}C (ppm)	δ ^{1}H (ppm)	multipl[b]	J (Hz)	δ ^{13}C (ppm)
2	3.40	d	4.8	78.55	3.57	d	4.2	79.82
3	4.06	m	3.6−4.2	75.79	4.03	ddd	2.4, 3.6, 6.0	77.62
4	5.33	d	3.6	82.54	5.03	d	3.6	84.81
5				48.52				51.34[c]
6				43.62				43.40[c]
7a	1.69	m	nm[d]	20.38	1.77[e]	dd	3.6, 14.4	26.85
7b	1.86	m	nm[d]		1.44[e]	dt	5.4, 14.4	
8a	1.84	m	nm[d]	27.39	1.56[e]	dt	4.2, 13.8	30.70
8b	1.92	m	nm[d]		1.20[e]	dd	4.8, 13.8	
9				139.05				71.68[c]
10	5.37	d	5.4	118.79	1.95	dt	4.2, 10.8	43.44
11	3.95	d	5.4	66.97	3.48	d	4.2	68.54
12				62.96				76.33[c]
13a	2.98	d	4.2	46.15	1.30[e]	dd	5.4−13.8	29.20
13b	2.75	d	4.2		1.65[e]	dd	11.4−14.0	
14	0.62	s		6.43	0.84	s		9.66
15a	3.87[c]	d	12.0	64.37	3.63[c]	d	11.4	71.63
15b	4.12[c]	d	12.0		4.49[c]	d	11.4	
16	1.65	s		22.81	1.05	s		27.45
17	5.58	d	4.2		4.72	d	6.0	
18	2.06	s		20.65	2.00[c]	s		20.66[c]
19	1.92	s		20.71	1.99[c]	s		20.93[c]
20					4.28	s		
21					4.62	s		
C═O				170.35				170.62
C═O				170.04				169.85

[a] Assignments were made on the basis of g-COSY, ROESY, and HMQC spectra. [b] Multiplicity: s, singlet; d, doublet; dd, doublet of doublets; dt, doublet of triplets; ddd, doublet of doublets of doublets; m, multiplet. [c] Assignment not definitive, no direct evidence. [d] nm: not measurable. [e] Assignment based on ROESY correlation with other protons.

The obtained elemental formula corresponds to the sum of the 4,15-diacetoxyscirpenol formula ($C_{19}H_{26}O_7$) plus H_2O.

NMR Characterization. NMR measurements (Table 1) revealed the exact chemical structure of DAS-M1. First of all, two acetyl groups, whose ^{1}H and ^{13}C chemical shift are similar to those of 4,15-diacetoxyscirpenol,[21] were assigned. Furthermore, there are three hydroxy protons (identified by the lack of correlation in H−C heterocorrelate spectrum). One is a doublet (OH-17), and the other two are singlets. It is difficult to envisage some alternative structure in which two hydroxyl protons are not coupled. The proton and carbon signals for both the epoxide (^{1}H, 2.75 + 2.98 ppm; ^{13}C, 46.15 ppm) and the alkene (^{13}C for carbon 9, 139.05 ppm) in 4,15-diacetoxyscirpenol are not present in DAS-M1, showing the modification of these structural elements. However, since the hydrogen deficiency index is decreased by only one unit, either another double bond or another ring has to be formed. Since no signal consistent with an alkene proton or carbon is seen, we conclude that a new ring is formed. CH_3-16 is not coupled to any other proton, though it is no longer linked to an alkene. There are four CH_2−, five CH−, and four CH_3− groups in the spectra. Two proton signals appeared at 1.65 and 1.30 ppm, which could be due to the CH_2− group on carbon 13. However, the chemical shifts indicate that these protons are not on a carbon bearing oxygen. Most important, these protons are coupled to a proton (at 1.95 ppm, identified as 10), which in turn is coupled to proton on carbon 11 (3.48 ppm). The ROESY spectrum indicates the proximity of one −OH to CH_3-16 (1.05 ppm) and of the other to CH_3-14 (0.84 ppm). The indicated stereochemistry is inferred from the same ROESY spectrum. All other correlations can be easily explained according to the structure. A possible mechanism was proposed for other trichothecenes.[22] After protonation, an intramolecular attack by the π-bond acting as nucleophile occurs, favored by the formation of a tertiary carbonium ion. The latter is then able to react with water as nucleophile from the rear side of the ring. Deprotonation yields the final structure.

While the elucidated structure of DAS-M1 is new, analogue reaction products have been reported in very early literature about 4,15-diacetoxyscirpenol.[3] Also for T-2 toxin, a similar reaction was found to form T-2 tetraol hydrates,[23] which proved to be nontoxic in a feed refusal assay with mice.

In Vivo Toxicity Test. As expected, 4,15-diacetoxyscirpenol strongly inhibited growth of the highly trichothecene sensitive yeast strain YZAS107.[20] 4,15-Diacetoxyscirpenol concentrations in the range of about 11 μM led to a growth reduction of >90% (Figure 3). In contrast, DAS-M1 did not show any reduction of growth at the same concentration. A reduction of growth of >90% was obtained only after adding about 600 μM DAS-M1 to

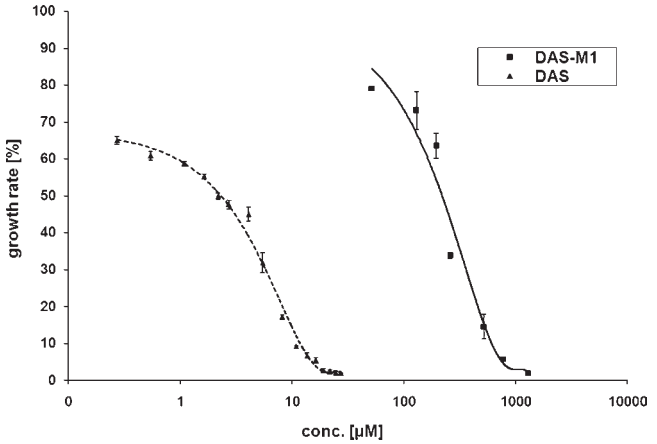

Figure 3. Yeast bioassay for testing the in vivo toxicity of 4,15-diacetoxyscirpenol and DAS-M1.

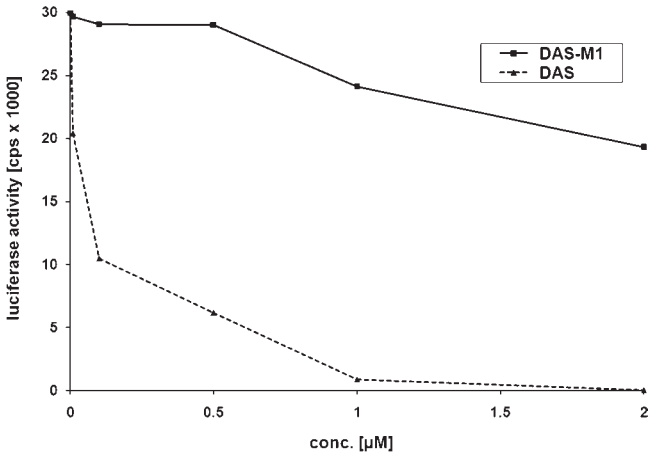

Figure 4. Inhibitory effects of 4,15-diacetoxyscirpenol and DAS-M1 on protein biosynthesis.

the growing yeast cells in liquid medium. We conclude that the in vivo toxicity of DAS-M1 to the indicator yeast is at about a factor of 50 lower than that of 4,15-diacetoxyscirpenol.

In Vitro Toxicity Test. A coupled in vitro transcription/translation system based on a wheat germ extract was used to express the reporter gene firefly luciferase, which oxidizes luciferin and releases light. The amount of light, which can be measured in a luminometer, is proportional to the amount of expressed enzyme. 4,15-Diacetoxyscirpenol inhibits protein synthesis, which is reflected in the concentration dependent decrease of luciferase activity (Figure 4). Average values of the duplicate measurements showed that the inhibitory effect of 4,15-diacetoxyscirpenol is much higher than of the same concentration of DAS-M1. The IC_{50} value could not be reached for DAS-M1 even at 2 μM. To compare the two substances with the available data, concentrations where luciferase activities are reduced to about $^2/_3$ of the starting values were used. 4,15-Diacetoxyscirpenol reached this value (actually 68%) at 0.01 μM, while 2 μM DAS-M1 was necessary to reach a similar value (64%). From these results, we infer that the inhibitory influence of 4,15-diacetoxyscirpenol on protein biosynthesis is about 100 times higher than that of DAS-M1.

Table 2. Average Conversion Rates of 4,15-Diacetoxyscirpenol in Aqueous Solution at Different pH Values (n = 3 Each)

temp [°C]	pH	time [h]	c(DAS) [μM]	c(DAS-M1) [μM]	DAS reduction (%)
80	3.0	0	27.2	0	0
		1	25.8	1.3	5
		4	24.9	2.5	8
		8	12.6	8.6	54
	5.0	0	27.2	0	0
		1	27.3	1.3	0
		4	25.9	2.7	5
		8	16.3	10.8	40
	7.0	0	27.2	0	0
		1	27.6	0.9	0
		4	27	1.5	1
		8	24.7	4.4	9
100	3.0	0	27.2	0	0
		1	25.8	2.7	5
		4	17	7.5	38
		8	0	26.1	100
	5.0	0	27.2	0	0
		1	26.9	2.5	1
		4	19.1	8.5	30
		8	0.2	21.1	99
	7.0	0	27.2	0	0
		1	25.6	1.5	6
		4	21.8	3.8	20
		8	5.1	16.7	81
121	3.0	0	27.2	0	0
		1	2.5	16	91
		4	0	18.4	100
	5.0	0	27.2	0	0
		1	4.2	17.6	85
		4	0	21	100
	7.0	0	27.2	0	0
		1	14.6	11.8	46
		4	1.7	21.5	94

Thermal Treatment of 4,15-Diacetoxyscirpenol and *F. sambucinum* Inoculated Potatoes. The thermal treatment of aqueous 4,15-diacetoxyscirpenol standards resulted in the formation of DAS-M1. The conversion was increased considerably by either higher temperatures or more acidic pH values (Table 2). For example, exposure to elevated temperatures for 4 h at pH 7.0 resulted in a decrease of 1%, 20%, or 46% 4,15-diacetoxyscirpenol at 80, 100, or 121 °C, respectively. At pH 3.0 (again for 4 h of treatment), already 8%, 38%, or 91% less 4,15-diacetoxyscirpenol was found at 80, 100, or 121 °C. Also, the results of cooking potatoes inoculated with *Fusarium sambucinum* showed that the amount of 4,15-diacetoxyscirpenol was the highest in the case of uncooked potatoes. Here, a reduction of 26% 4,15-diacetoxyscirpenol after 1 h of cooking at 100 °C and 81% after 4 h was observed. The transformation of 4,15-diacetoxyscirpenol to DAS-M1 was increased when a pressure cooker (121 °C) was used. 89% or even 100% of 4,15-diacetoxyscirpenol was turned over after cooking for 1 or 4 h, respectively, even at neutral pH.

This is particularly intriguing, as mycotoxins usually are considered to be thermally very stable. The long cooking times required to inactivate most of the toxin make it unlikely that thermal treatment can be exploited as the detoxification method for food or feed. Yet, possibly higher conversion rates due to slight acidification of the cooking water of potatoes (as seen with pure standards) might allow improvement of the process. From the viewpoint of analytical chemistry the generally assumed thermal stability of 4,15-diacetoxyscirpenol (and other trichothecenes) is relative. Extended heat treatment of biological samples containing 4,15-diacetoxyscirpenol (e.g., culture filtrates in order to inactivate *Fusarium*) or heating of analytical standards to increase the solubility should be avoided, since it might lead to significant losses and to formation of the rearranged reaction product DAS-M1.

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