Acta Biologica Hungarica 67(2), pp. 220–224 (2016)
DOI: 10.1556/018.67.2016.2.10

CHLORINATED ORGANIC COMPOUNDS PRODUCED BY *FUSARIUM GRAMINEARUM*

SHORT COMMUNICATION

KHAYALETHU NTUSHELO*

Department of Agriculture and Animal Health, College of Agriculture and Animal Health,
University of South Africa, Science Campus,
Corner Christiaan De Wet and Pioneer Avenue, Florida, 1710. Gauteng, South Africa

(Received: October 6, 2015; accepted: October 20, 2015)

Fusarium graminearum, a pathogen of wheat and maize, not only reduces grain yield and degrades quality but also produces mycotoxins in the infected grain. Focus has been on mycotoxins because of the human and animal health hazards associated with them. In addition to work done on mycotoxins, chemical profiling of *F. graminearum* to identify other compounds produced by this fungus remains critical. With chemical profiling of *F. graminearum* the entire chemistry of this fungus can be understood. The focus of this work was to identify chlorinated compounds produced by *F. graminearum*. Various chlorinated compounds were detected and their role in *F. graminearum* is yet to be understood.

Keywords: Chemical profiling – chlorinated compounds – *Fusarium graminearum* – ^{1}H NMR

Fusarium graminearum Schwabe (teleomorph *Gibberella zeae* (Schwein) Petch), primarily a pathogen of wheat and maize, poses health hazards to humans and animals which consume grain infected by this pathogen. During its proliferation in the wheat and maize grains, *F. graminearum* produces fumonisins, a group of mycotoxins which causes oesophageal cancer in humans [12] and leukoencephalomacia in equine species and rabbits [2, 3, 9] among other serious illnesses. Realising the seriousness of *F. graminearum*, researchers focussed their attention of mycotoxins without profiling the entire chemical spectra of *F. graminearum*. It remains imperative to identify other compounds which are produced by *F. graminearum* and determine if they have harmful effects on either their plant hosts or the consumers of affected grain. It may also be possible to identify beneficial chemical compounds produced by this fungus. With nuclear magnetic resonance spectroscopy it is possible to capture a snapshot of the metabolites of an organism under any set of environmental conditions. The purpose of this work was to identify compounds produced by *F. graminearum* at moderate conditions of growth assuming that the same compounds are produced by this pathogen during its normal infestation of grains. Attention was paid only on chlorinated compounds. Detection of these compounds on its own has little value if no work is done to delineate their role in the interaction between *F. graminearum* and its hosts and the effect of these compounds on people and animals which consume infected

* E-mail address: ntushk@unisa.ac.za

grain. More work needs to be done in this area as well as further delineation of the chemical arsenal of *F. graminearum*.

Strain G44A of *F. graminearum* isolated from wheat was cultured on potato dextrose agar at 25 °C. Mycelia of five day old cultures were scrapped from the agar plates and ground with liquid nitrogen using a mortar and pestle. Into four 2 ml epperndoff tubes containing the ground mycelia weighing 0.10 grams, extraction reagents, 750 µL methanol-D_4, and 750 µL deuterium oxide were added. Both extraction reagents were supplied by Sigma-Aldrich® Corporation. Each of the four fractions represented a technical replication. The mixture of the ground mycelia with the extraction reagents was vortexed, sonicated for 20 minutes and centrifuged briefly at room temperature. The supernatant which contained secondary metabolites was dispensed into nuclear magnetic resonance (NMR) tubes for analysis. Nuclear magnetic resonance spectroscopic analysis was done using a 600 MHz Varian NMR instrument to obtain spectra for each of the four replications. Phaseline and baseline correction of resultant spectra were done using ACD/NMR Processor [1] and the represented compounds were identified using NMRShiftDB [10]. The NMRShiftDB queries returned 300 identities as compounds represented by each of the spectra. From the compounds represented by the spectra, known chlorinated compounds were selected and presented in Table 1 below.

Table 1

List of chlorinated organic compounds detected in *Fusarium graminearum* isolate G44A using ^{1}H nuclear magnetic resonance spectroscopy

Technical replicate	Compounds identified by NMR
1	1-{[chloro(dimethyl)silyl]methyl}-2,6-piperidinedione
	trimethyl-[(2E)-2-(9-propan-2-yl-9-borabicyclo[3.3.2]decan-10-ylidene)-2-trimethylstannylethoxy]stannane
	(4R,5R)-4,5-bis[methoxy(diphenyl)methyl]-2-[(1R,2S)-2-(methoxymethoxymethyl) cyclopropyl]-1,3,2-dioxaborolane
	methoxy-dimethyl-(trichlorogermylmethyl)silane
	benzyl-(chloromethyl)-methoxy-methylsilane
	Chloromethylcyclopropane
	[(1R,2S)-2-[(4R,5R)-4,5-bis[methoxy(diphenyl)methyl]-1,3,2-dioxaborolan-2-yl]cyclopropyl]methanol
	(4R,5R)-4,5-bis[methoxy(diphenyl)methyl]-2-[(1S,2R)-2-(methoxymethoxymethyl) cyclopropyl]-1,3,2-dioxaborolane
	[(1S,2R)-2-[(4R,5R)-4,5-bis[methoxy(diphenyl)methyl]-1,3,2-dioxaborolan-2-yl]cyclopropyl]methanol
	[tert-butylperoxy-(chloromethyl)-methylsilyl] acetate
2	1-{[chloro(dimethyl)silyl]methyl}-2,6-piperidinedione
	trimethyl-[(2E)-2-(9-propan-2-yl-9-borabicyclo[3.3.2]decan-10-ylidene)-2-trimethylstannylethoxy]stannane
	(4R,5R)-4,5-bis[methoxy(diphenyl)methyl]-2-[(1R,2S)-2-(methoxymethoxymethyl) cyclopropyl]-1,3,2-dioxaborolane

Table 1 (cont.)

Technical replicate	Compounds identified by NMR
2	methoxy-dimethyl-(trichlorogermylmethyl)silane
	benzyl-(chloromethyl)-methoxy-methylsilane
	Chloromethylcyclopropane
	[(1R,2S)-2-[(4R,5R)-4,5-bis[methoxy(diphenyl)methyl]-1,3,2-dioxaborolan-2-yl]cyclopropyl]methanol
	(4R,5R)-4,5-bis[methoxy(diphenyl)methyl]-2-[(1S,2R)-2-(methoxymethoxymethyl) cyclopropyl]-1,3,2-dioxaborolane
	[(1S,2R)-2-[(4R,5R)-4,5-bis[methoxy(diphenyl)methyl]-1,3,2-dioxaborolan-2-yl]cyclopropyl]methanol
	[tert-butylperoxy-(chloromethyl)-methylsilyl] acetate
3	1-{[chloro(dimethyl)silyl]methyl}-2,6-piperidinedione
	trimethyl-[(2E)-2-(9-propan-2-yl-9-borabicyclo[3.3.2]decan-10-ylidene)-2-trimethylstannylethoxy]stannane
	(4R,5R)-4,5-bis[methoxy(diphenyl)methyl]-2-[(1R,2S)-2-(methoxymethoxymethyl) cyclopropyl]-1,3,2-dioxaborolane
	methoxy-dimethyl-(trichlorogermylmethyl)silane
	benzyl-(chloromethyl)-methoxy-methylsilane
	Chloromethylcyclopropane
	[(1R,2S)-2-[(4R,5R)-4,5-bis[methoxy(diphenyl)methyl]-1,3,2-dioxaborolan-2-yl]cyclopropyl]methanol
	(4R,5R)-4,5-bis[methoxy(diphenyl)methyl]-2-[(1S,2R)-2-(methoxymethoxymethyl) cyclopropyl]-1,3,2-dioxaborolane
	[(1S,2R)-2-[(4R,5R)-4,5-bis[methoxy(diphenyl)methyl]-1,3,2-dioxaborolan-2-yl]cyclopropyl]methanol
	[tert-butylperoxy-(chloromethyl)-methylsilyl] acetate
4	1-{[chloro(dimethyl)silyl]methyl}-2,6-piperidinedione
	trimethyl-[(2E)-2-(9-propan-2-yl-9-borabicyclo[3.3.2]decan-10-ylidene)-2-trimethylstannylethoxy]stannane
	(4R,5R)-4,5-bis[methoxy(diphenyl)methyl]-2-[(1R,2S)-2-(methoxymethoxymethyl) cyclopropyl]-1,3,2-dioxaborolane
	methoxy-dimethyl-(trichlorogermylmethyl)silane
	benzyl-(chloromethyl)-methoxy-methylsilane
	Chloromethylcyclopropane
	[(1R,2S)-2-[(4R,5R)-4,5-bis[methoxy(diphenyl)methyl]-1,3,2-dioxaborolan-2-yl]cyclopropyl]methanol
	(4R,5R)-4,5-bis[methoxy(diphenyl)methyl]-2-[(1S,2R)-2-(methoxymethoxymethyl) cyclopropyl]-1,3,2-dioxaborolane
	[(1S,2R)-2-[(4R,5R)-4,5-bis[methoxy(diphenyl)methyl]-1,3,2-dioxaborolan-2-yl]cyclopropyl]methanol
	[tert-butylperoxy-(chloromethyl)-methylsilyl] acetate

More than 220 organochlorines are produced naturally by the environment [4, 5]. It has previously been found that fungal parasites of plants produce organically bound chlorine during substrate degradation. Öberg et al. [11] found that white-rot fungi produce organically-bound chlorine during degradation of wood. Although the organically-bound chlorine which was detected in this study was during degradation of PDA it can be assumed that *F. grameanium* produces these chlorinated organic compounds during its degradation of similar substrates in the plant medium.

In all four technical replications the same compounds were consistently detected. From the output of NMRShiftDB ten chlorinated compounds were selected in each technical replication. Chlorination of organic compounds brings about a structural change which can change the reactivity of the resultant compound. Although the role of chlorinated organic compounds in *F. graminearum* is unknown, the introduction of chlorine to organic compounds is assumed to result into more chemical and biological activity, but toxicity resulting from chlorination of methane derivatives is documented while chlorination of ethane derivatives increases the anesthetic effects of ethane [6] just like does the chlorination of ethene derivatives. The chlorination of ethyne derivatives may result into chlorinated molecules which are neurotoxic, with this having been demonstrated with dichloroacetylene. Chlorination of propene derivatives, butane derivatives, butane derivatives and phenol derivatives also results in the increased toxicity of these groups of compounds, while the chlorination of benzene derivatives results in both increase and decrease in toxicity [7]. Benzyl-(chloromethyl)-methoxy-methylsilane, [(1R,2S)-2-[(4R,5R)-4,5-bis[methoxy(diphenyl)methyl]-1,3,2-dioxaborolan-2-yl]cyclopropyl]methanol, (4R,5R)-4,5-bis[methoxy(diphenyl) methyl]-2-[(1S,2R)-2-(methoxymethoxymethyl)cyclopropyl]-1,3,2-dioxaborolane and [(1S,2R)-2-[(4R,5R)-4,5-bis[methoxy(diphenyl)methyl]-1,3,2-dioxaborolan-2-yl]cyclopropyl]methanol are chlorinated benzene derivatives which were detected in this study. Overwhelmingly, the chlorination of organic compounds results in increased toxicity [7]. Presently, it may be assumed that the chlorination reactions that have not been studied also result in increased toxicity of the resultant compound. Because *F. graminearum* produces mycotoxins, it is easy to assume that the toxicoses which result from the consumption of foods from crops infected with *F. graminearum* is mainly caused by the known mycotoxins when such compounds as chlorinated organic compounds may be playing a significant role. Most of the chlorinated organic compounds detected from the mycelia of *F. graminearum* had the C-Cl bond which changes the toxicological behaviour of organic compounds due to electron withdrawing effects. This chlorination also increases the lipophilicity of organic compounds and its effects also influence neighbouring C-C sequences with further increases in reactivity.

It is therefore recommended that the metabolome of *F. graminearum* be studied towards understanding the toxic chemistry of this fungus. 1-{[chloro(dimethyl)silyl] methyl}-2,6-piperidinedione is similar to 4-(2-hydroxy-5,7-dimethyl-4-oxo-6,8-nonadienyl)-2,6-piperidinedione which was found to be an antifungal agent produced by *Micromonospora coerulea* strain Ao58 [8]. The production of 1-{[chloro(dimethyl)silyl]methyl}-2,6-piperidinedione by *F. graminearum* may

imply that this compound is used by this fungus to inhibit the growth of other fungal competitors. The purpose for the production of other compounds by *F. graminearum* could not be ascertained. In conclusion, it is recommended that the metabolome of *F. graminearum* be rigorously studied in an attempt to reveal chemical compounds which may contribute towards the pathogenicity and the harmful effects of this fungus on humans and animals. Moreover, ^{1}H NMR is not sufficient for identification of chemical compounds and therefore additional chemical analytical methods are recommended for further identification of chlorinated organic compounds found in *F. graminearum*.

ACKNOWLEDGEMENTS

I thank Ms. Cathy De Villiers of the Agricultural Research Council – Small Grain Institute, South Africa for providing the G44A isolate.

REFERENCES

1. ACD/Structure Elucidator (2014). version 12.01, Advanced Chemistry Development, Inc., Toronto, ON, Canada, www.acdlabs.com.
2. Bucci, T., Hansen, D. K., Laborde, J. B. (1996) Leucoencephalomacia and hemorrhage in the brain of rabbits gavaged with mycotoxin fumonisin B1. *Nat. Toxins*. *4*, 51–52.
3. Fandohan, P., Hell, K., Marasas, W. F. O., Wingfield, M. J. (2003) Infection of maize by Fusarium species and contamination with fumonisin in Africa. *Afr. J. Biotechnol*. *2*, 570–579.
4. Gribble, G. W. (2009) Naturally Occurring Organohalogen Compounds: A Comprehensive Update, Springer.
5. Gribble, G. W. (2003) The diversity of naturally produced organohalogens. *Chemosphere 52*, 289–297.
6. Henschler, D. (1994) Toxicologie chlororganischer Verbindungen. – Der Einfluß von Chlorsubstituenten auf die Toxizität organischer Moleküle, VCH, Weinheim.
7. Henschler, D. (1996) Halogenated alkanes, alkenes and alkynes: in Bioactivation mechanisms (Ed.: Anders, M. W.). Academic Press, New York.
8. Kim, B. S., Moon, S. S., Hwang, B. K. (1999) Isolation, Antifungal Activity, and Structure Elucidation of the Glutarimide Antibiotic, Streptimidone, Produced by *Micromonospora coerulea*. *J. Agric. Food Chem*. *47,* 3372–3380.
9. Marasas, W. F. O., Kellerman, T. S., Gelderblom, W. C. A., Coetzer, J. A. W., Thiel, P. G., Van Der Lugt, J. J. (1988) Leukoencephalomalacia in horse induced by fumonisin B1 isolated from *Fusarium moniliforme*. *Onderstepoort J. Vet. Res. 55,* 197–203.
10. NMRShiftDB Servers, (2014). NMRShiftDB, Ref type: online source; Available from: http://nmrshiftdb.nmr.uni-koeln.de/.
11. Öberg, G., Brunberg, H., Hjelm, O. (1997) Production of organically-bound chlorine during degradation of birch wood by common white-rot fungi. *Soil Biol. Biochem. 29,* 191–197.
12. Peraica, M., Radic, B., Lucic, A., Pavlovic, M. (1999) Toxic effects of mycotoxins in humans. *Bull World Health Organ. 77,* 754–766.