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Phytomedicine 13 (2006) 576–583
Phytomedicine
www.elsevier.de/phymed

Native Kenyan plants as possible alternatives to methyl bromide in soil fumigation

J.K. Rugutt[a,*], A.N. Ngigi[b], K.J. Rugutt[c], P.K. Ndalut[b]

[a]*Department of Chemistry, Massachusetts College of Liberal Arts, 375 Church Street, North Adams, MA 0147, USA*
[b]*Department of Chemistry, Moi University, P.O. Box 1125, Eldoret, Kenya*
[c]*Department of Education, Illinois State University, Normal, IL 61790-2200, USA*

Received 3 September 2001; accepted 6 August 2003

Abstract

Methyl bromide (CH_3Br) is a biocidal fumigant used widely in crop production and commodity preservation worldwide. CH_3Br escapes to the stratosphere and releases bromine atom (Br), which contributes to significant destruction of the ozone (O_3). It is therefore necessary to explore alternatives to CH_3Br that are environmentally safe and suitable for resource-poor African farmers. We present here the results of a study on the inhibitory activity of crude extracts from Kenyan medicinal plants against three soil pathogens, *Fusarium oxysporum*, *Alternaria passiflorae*, and *Aspergillus niger*. Crude organic extracts of *Warburgia ugandensis* Sprague, *Azadirachta indica* A. Juss, *Tagetes minuta* and *Urtica massaica* were active against all soil pathogens, while those from *U. massaica* were not. Chromatographic purification of the crude extract of *W. ugandensis* provided two pure compounds, muzigadial (**1**) and muzigadiolide (**5**). The minimum inhibitory concentration (MIC value) for muzigadial (**1**) ranged from 5 to 100 μg/ml. Muzigadiolide (**5**) was not active. Greenhouse tests of *W. ugandensis* extracts against *F. oxysporium* pathogen showed the most effective inhibitory concentration to be at least 5 mg/ml. Quantitative structure–activity relationship (QSAR) models were used to rationalize the variation in biological activities of muzigadial (**1**), warburganal (**2**), polygodial (**3**), ugandensidial (**4**), muzigadiolide (**5**), azadirachtin (**6**), and CH_3Br. The models were based on several molecular descriptors including LogP, van der Waals surface area (VDW_A), van der Waals volume (VDW_v), dipole moment, total energy, polarizability, and differences between the highest occupied molecular orbital and the lowest unoccupied molecular orbital (HOMO–LUMO gap).

Keywords: Native Kenyan plants; Crude extracts; Muzigadial; Muzigadiolide; Soil pathogens; QSAR; Molecular descriptors

Introduction

Methyl bromide (CH_3Br) has been used as a fumigant for over 60 years. An important valuable property of CH_3Br is the broad spectrum of activity against several pests. The largest single global use is as a soil fumigant (Wang et al., 1997). For example, it is used as a fumigant against pathogens (fungi-, bacteria- and soil-borne viruses), insects, mites, nematodes and rodents (Puckhaber et al., 1998). These pests may be in the soil, in durable or perishable commodities, and in structures and transportation vehicles. The ease of application of

*Corresponding author. Tel.: +1 413 662 5451; fax: +1 413 662 5010.
E-mail address: jrugutt@mcla.mass.edu (J.K. Rugutt).

0944-7113/$ - see front matter © 2005 Published by Elsevier GmbH.
doi:10.1016/j.phymed.2003.08.005

CH_3Br, along with its reliability and speed of action, has led to its widespread use in agricultural systems producing economically important crops. Although CH_3Br is a most useful soil fumigant in specific instances, there are a number of technical and legislative limitations that have led to restrictions on its use.

CH_3Br can have adverse effects on a number of commodities; it is phytotoxic and causes taint and odors. Repeated fumigation with CH_3Br may result in the production of bromide ion (Br^-) residues that accumulate rapidly. Some European countries are concerned about the toxicity of CH_3Br in ground water and its ozone-depleting potential. In November 1992, CH_3Br was listed as an ozone-depleting substance by the fourth meeting of the parties to the Montreal Protocol on Substances that Deplete the Ozone Layer in Copenhagen (Abritton and Watson, 1992). Since then, plant health services throughout the world have been advocating the phasing out of CH_3Br. The technical availability of chemical and non-chemical alternatives to CH_3Br has been proposed by the Methyl Bromide Technical Options Committee (Yuen et al., 1991). Non-chemical alternatives include cultural practices, biological control, organic alterations and physical methods (Watson et al., 1992). Chemical alternatives can be either fumigants or non-fumigants. Fumigants include methyl isothiocyanate (MITC), MITC generators, metasodium, dazomet and halogenated hydrocarbons (e.g. 1,3-dichloropropene, chloropicrin (trichloronitromethane) and ethylene dibromide). All these chemicals have major drawbacks: phytotoxicity, skin and eye irritation, sensitization, genotoxicity, and carcinogenicity. Non-fumigant nematicides such as organophosphates or carbamates are neurotoxins (cholinesterase inhibitors) and do not exhibit broad-range disinfestation properties typical of CH_3Br. These compounds are therefore not attractive candidates for soil fumigation because they are either decomposed by soil microflora or the soil pests develop resistance easily (Shorter et al., 1995). Encouraged by the recent use of crude plant extracts in combating Striga weeds in Nigerian soils (Rugutt and Berner, 1998), the present study examined the activity of native Kenyan plant extracts against three economically important soil pathogens.

Experimental

Plant collection

All plant materials were collected in Kenya and identified at the Department of Botany, Moi University; voucher specimens have been deposited at the Herbarium there. Leaves of *Warburgia ugandensis* were collected at Moi University Forestry farm and Nursery in September of 1997. Stem barks were collected in Kerio Valley, Keiyo District in May 1998. Leaves of *Azadirachta indica* and *Urtica massaica* were collected in August 1997 in Mombasa and Molo, Nakuru District, respectively. The aerial parts of *Tagetes minuta* were collected at Chepkoilel Campus, Moi University.

Plant extraction

Wet stem bark (3 kg) of *W. ugandensis* was extracted using methanol. The resulting crude extract was concentrated in a rotary evaporator in vacuo; water bath temperature was set at 40 °C. The concentrated crude extract was then partitioned into water and chloroform. Separation of the water–chloroform phases afforded 95 g of crude extract in the chloroform fraction. A portion (15 g) of the concentrated chloroform fraction was filtered (in vacuo) through TLC-grade Merck silica gel using ethyl acetate (EtOAc). The filtrate was concentrated, packed in a pre-packed Merck silica gel column (40–63 mm) and then subjected to flash chromatography. The column was first eluted with neat hexane followed by hexane containing increasing amounts of EtOAc. The fractions eluted were monitored by TLC; the developing solvent was 30% EtOAc in hexane. The compounds on developed TLC plates were visualized either by observation under an ultraviolet lamp or by spraying with concentrated sulfuric acid and then heating at 110 °C on a hot plate for about 1 min. The crude extract from the bark of *W. ugandensis* yielded two compounds, muzigadial (**1**) and warburganal (**2**). Crude extracts from *A. indica*, *T. minuta*, and *U. massaica* plant materials were obtained following a procedure similar to that described by Rugutt et al. (1999).

Structural elucidation

The IR data of muzigadial (**1**) and warburganal (**2**) were obtained from their spectra run using a Shimagzu-IR408 spectrophotometer. Melting point data were obtained on a Reichet Thermovar apparatus. All 1*D* (^1H, ^{13}C) and 2*D* (^1H–^1H COSY, ^1H–^1H NOESY, HMQC, and HMBC) NMR experiments were recorded at 298 K on a Bruker AMX 400 MHz spectrometer using the pulse sequences described by Rugutt et al. (1999). About 0.75 ml of deuterated chloroform ($CDCl_3$; δ_H 7.24 ppm, δ_C 77.0 ppm) was added to 5 mg of pure compounds (**1**–**6**) contained in 5-mm outer diameter NMR tubes. Chemical shifts are expressed in δ (ppm) scale downfield from TMS (internal reference standard). ^1H–^1H COSY was performed using the following: acquisition parameters: recycling delay (*D*1), 1.5 s; dummy scans (DS) = 2; number of scans (NS) = 32; *D*0 increment, 3.0 μs. The ^1H–^1H NOESY spectra were