Med Chem Res (2014) 23:4627–4630
DOI 10.1007/s00044-014-1026-9

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Zeaoxazolinone, a new antifungal agent from *Zea mays* roots

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Received: 5 January 2014 / Accepted: 1 May 2014 / Published online: 22 May 2014

Abstract A new 7-methoxy-2-benzoxazolinone dimer named zeaoxazolinone (**2**), together with four known compounds; 9-Z-hexadecenoic acid (**1**), 6-methoxy-benzoxazolinone (**3**), gallic acid (**4**), and β-sitosterol-3-*O*-β-D-glucopyranoside (**5**) were isolated from *Zea mays* L. roots. The structural elucidation of isolated metabolites was established on the basis of UV, IR, 1D, 2D NMR, and MS spectral analyses. Compound **2** exhibited a potent antifungal activity against *Aspergillus flavus*, *Fusarium oxysporum*, and *Candida albicans*.

Keywords *Zea mays* · Gramineae · Benzoxazolinone · Zeaoxazolinone · Antifungal

Electronic supplementary material The online version of this article (doi:10.1007/s00044-014-1026-9) contains supplementary material, which is available to authorized users.

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Introduction

Zea mays L. (Gramineae) is a small tree. It grows in the tropical and sub-tropical Asian and American countries. Currently, it was cultivated in sub-tropical countries of the world and in warm climates (Hossain *et al*., 2006a, b). It employed direct chemical defenses against fungal pathogens by production of benzoxazolinones (Huffaker *et al*., 2011; Niemeyer, 2009; Sicker *et al*., 2000). It is commonly used in the control of soil-borne diseases (Park *et al*., 2004). Corn silk of *Z. mays* is medicinally used as stimulant, diuretic, and demulcent. Also, it is used in treatment of urethritis, cystitis, nephritis, lithiasis, prostatitis, and gonorrhoea (Nessa *et al*., 2012; Ren *et al*., 2009). It possesses antioxidant (Alam, 2011), anti-diabetic, antibiotic, and antitumour activities (Nessa *et al*., 2012). Previous phytochemical studies of *Z. mays* revealed the isolation of phenolic compounds (Ramos-Escudero *et al*., 2012; Hossain *et al*., 2006a, b; Nessa *et al*., 2012; Ren *et al*., 2009), benzoxazolinone alkaloids (Park *et al*., 2004; Suzuki *et al*., 2007), diterepenes (Hossain *et al*., 2006a, b), sterols (Suzuki *et al*., 2007), and saponins (Sosa *et al*., 1997). In the present study, investigation of the EtOAc fraction of *Z. mays* roots led to the isolation of one new benzoxazolinone alkaloid, in addition to four known compounds (Fig. 1). Their structures were verified by various spectroscopic methods. The new compound was evaluated for its antifungal activity.

Materials and methods

General

UV spectra were recorded in MeOH on a Shimadzu 1601 UV/VIS spectrophotometer. Melting point was measured on an

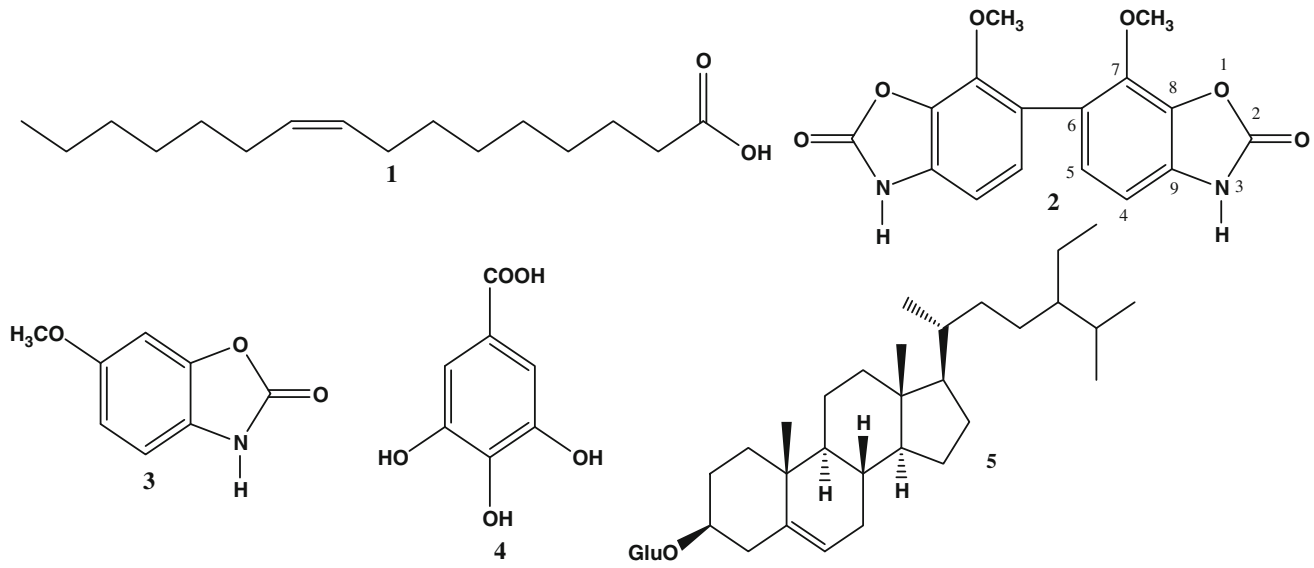

Fig. 1 Structures of the isolated compounds **1–5**

Electrothermal 9100 Digital Melting Point apparatus (Electrothermal Engineering Ltd, Essex, England). IR spectrum was measured on Schimadzu Infrared-400 spectrophotometer (Japan). LRESIMS and HRESIMS spectra were recorded on a LTQ Orbitrap and an API 2000 (ThermoFinnigan, Bremen, Germany) mass spectrometers. 1D and 2D NMR spectra were measured on Bruker DRX 500 spectrometer (Bruker, Rheinstetten, Germany). Vacuum liquid chromatography (VLC) was carried out on silica gel 60 (0.04–0.063 mm, Merck). Column chromatographic separations were performed over silica gel 60 (0.040–0.063 mm, Merck). TLC analyses were carried out on pre-coated silica gel F_{254} aluminium sheets. Compounds were detected by UV absorption at λ_{max} 254 and 366 nm followed by spraying with *p*-anisaldehyde/H_2SO_4 reagent and heating at 110 °C for 1–2 min. The solvent systems used for TLC analyses were $CHCl_3$:MeOH (95:5, S_1), $CHCl_3$:MeOH (90:10, S_2), $CHCl_3$:MeOH (85:15, S_3) and *n*-BuOH:acetone:formic acid:H_2O (60:17:8:15, S_4). Authentic sterol was obtained from Pharmacognosy Department, Faculty of Pharmacy, Assiut University, Assiut, Egypt.

Plant material

Zea mays L. roots were collected during August 2010 from plants cultivated in El-Galaa Village, Samalout, Minia, Egypt. The plant was kindly identified by Prof. Mohamed A. Farghali, Professor of Horticulture (Vegetable Crops), Faculty of Agriculture, Assiut University, Assiut, Egypt.

Antifungal assay

The procedure was determined as previously described (Kima *et al*., 2006). The antifungal activity was evaluated using the agar plate diffusion assay. Susceptibility discs (5.5 mm) were impregnated with compound **2** at concentration 10 µg/disc. The discs were dried and placed on agar plates inoculated with the tested fungal strains: *Candida albicans* (AUMC No. 418), *Geotrichum candidum* (AUMC No. 226), *Trichophyton rubrum* (AUMC No. 1804), *Fusarium oxysporum* (AUMC No. 5119), *Scopulariopsis brevicaulis* (AUMC No. 729), and *Aspergillus flavus* (AUMC No. 1276), all fungal strains were obtained from Assiut University Mycology center (http://www.aun.edu.eg/aumc/Catalog.htm). Each plate was inoculated with a single organism and the test was run in triplicate. The plates were incubated at 37 °C and checked for inhibition zones after 48 h. Clotrimazole (10 µg/disc) was used as positive reference standard for antifungal activity.

Chemistry

Extraction and isolation

The air-dried powdered roots of *Z. mays* L. (0.75 kg) were extracted with MeOH (3 L × 4) at room temperature. The combined extract was evaporated under reduced pressure to afford a dark brown residue (20.4 g). The latter was suspended in distilled H_2O (100 mL) then partitioned between *n*-hexane, EtOAc, and *n*-BuOH, successively. Each fraction was concentrated under reduced pressure to give *n*-hexane (5.9 g), EtOAc (4.1 g), *n*-BuOH (3.5 g), and aqueous (5.3 g) fractions. The EtOAc fraction was subjected to vacuum liquid chromatography (VLC) using *n*-hexane:EtOAc and EtOAc:MeOH gradients to obtain four subfractions: ZME-1 (1.2 g, *n*-hexane:EtOAc 50:50),

Table 1 NMR spectral data of **2** (DMSO-d_6, 500 and 125 MHz)

No.	δ_H [mult., J (Hz)]	δ_C (mult.)	HMBC
2, 2′	–	156.1 C	–
4[a]	6.71 d (8.5)	119.5 CH	5, 6 8, 9
5[a]	6.98 d (8.5)	126.1 CH	4, 6, 7′, 9, 6′
6, 6′	–	122.3 C	–
7, 7′	–	155.7 C	–
8, 8′	–	133.6 C	–
9, 9′	–	135.7 C	–
4′[a]	6.75 d (8.5)	119.5 CH	5′, 6′, 8′, 9′
5′[a]	7.02 d (8.5)	126.1 CH	6, 4′, 6′, 7′, 9′
7-OCH_3[a]	3.74 s	55.7 CH_3	7
7′-OCH_3[a]	3.77 s	55.7 CH_3	7′
3, 3′	9.67 s	–	2, 2′ 9, 9′

[a] may be interchangeable

ZME-2 (0.6 g, 100 % EtOAc), ZME-3 (0.8 g, EtOAc:-MeOH 50:50), and ZME-4 (1.0 g, 100 % MeOH). Subfraction ZME-1 was subjected to silica gel column chromatography (100 g, 50 × 2 cm) using *n*-hexane: EtOAc gradient to afford compound **1** (7 mg, colourless oil). Subfraction ZME-2 was chromatographed over silica gel column chromatography (100 g, 50 × 2 cm) using $CHCl_3$:MeOH gradient to give **2** (5 mg, yellowish white crystals) and **3** (13 mg, yellowish white crystals). Silica gel column chromatography of subfraction ZME-3 (100 g, 50 × 2 cm) using $CHCl_3$:MeOH gradient elution afforded compounds **4** (17 mg, brown residue) and **5** (42 mg, white amorphous powder).

Zeaoxazolinone (**2**): Yellowish white crystals (acetone). $R_f = 0.81$ (S_1). m.p 298–299 °C. UV λ_{max} (MeOH) nm: 270, 355. HRESIMS *m/z*: 329.0687 (Calcd. for $C_{16}H_{13}N_2O_6$ 329.0695) $[M+H]^+$, 657.1372 $[2M+H]^+$, 299.5459 $[M+H-2CH_3]^+$, 150.0176 $[M/2+H-CH_3]^+$, 105.1237, 91.0459, 77.3259. ^1H and ^{13}C NMR (see Table 1).

Results and discussion

The MeOH extract of *Z. mays* roots was subjected to solvent/solvent partition between *n*-hexane, EtOAc, *n*-BuOH, and H_2O. The EtOAc fraction was successively chromatographed over (VLC) and normal silica gel column to afford a new benzoxazolinone dimer named zeaoxazolinone (**2**) and four known compounds (**1** and **3**–**5**).

Compound **2** was isolated as yellowish white crystals. It gave positive reactions with ninhydrin reagent, indicating its nitrogenous nature. The HRESIMS exhibited a pseudomolecular ion peaks at *m/z* 329.0687 $[M+H]^+$ and 657.1372 $[2M+H]^+$, corresponding to a molecular formula of $C_{16}H_{12}N_2O_6$, requiring 12 degrees of unsaturation. The fragment ion peaks at *m/z* 299.5459 $[M+H-2CH_3]^+$ and 150.0176 $[M/2+H-CH_3]^+$ indicated that **2** is a symmetric dimer (D′Souzan *et al*., 1997). Also, it showed characteristic mass fragments at *m/z* 105.1237, 91.0459, and 77.3259, suggesting the presence of 2-benzoxazolinone (Amer *et al*., 2004) (Fig. 2). IR spectrum showed characteristic absorption bands at 3320 (NH), 1665 (amide carbonyl), 1485, 1390, 1240 (C–O–C), and 1052 cm^{-1}. The ^1H, ^{13}C NMR, and HSQC spectra of **2** revealed the presence of eight carbon resonances: an amide carbonyl, two methines, four quaternary carbons, and one methoxy group, which were the characteristic signals of methoxy-benzoxazolinone (D'Souzan *et al*., 1997). The ^1H NMR spectrum showed two methoxy signals at δ_H 3.77 (7′-OCH_3) and 3.74 (7-OCH_3), two pairs of *ortho*-coupled protons at δ_H 6.71 (1H, d, $J = 8.5$ Hz, H-4), 6.98 (1H, d, $J = 8.5$ Hz, H-5) and 6.75 (1H, d, $J = 8.5$ Hz, H-4′), 7.02 (1H, d, $J = 8.5$ Hz, H-5′), correlated to the carbons resonating at δ_C 55.7 (7, 7′-OCH_3), 119.5 (C-4, 4′), and 126.1 (C-5, 5′) in the HSQC spectrum, respectively (Table 1). The ^1H NMR spectrum showed two nitrogen-bearing protons at δ_H 9.67 (2H, s) assignable to H-3 and H-3′. They showed ^2J HMBC cross peaks to C-2, 2′ (δ_C 156.1) and C-9, 9′ (δ_C 135.7).

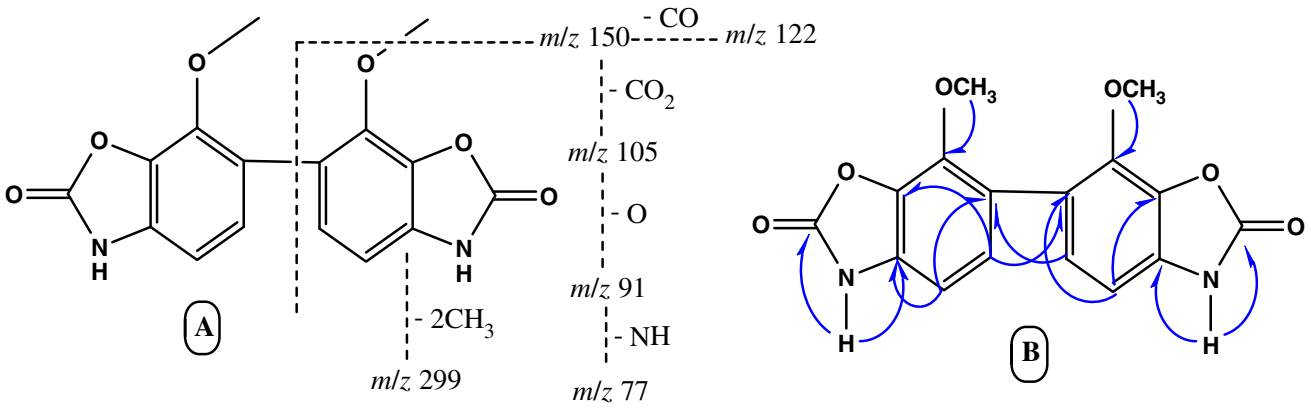

Fig. 2 Possible fragmentation pattern (**a**) and key HMBC correlations (**b**) of **2**

Table 2 Antifungal activity of **2**

Sample	Conc. (μg/disc)	Inhibition zone (mm)					
		A. flavus	*S. brevicaulis*	*F. oxysporum*	*T. rubrum*	*G. candidum*	*C. albicans*
2	10	31	21	21	13	18	23
Clotriamazole	10	32	30	18	28	23	21

The methoxy groups were attached to C-7 and C-7′ on the basis of their HMBC correlations to C-7 and C-7′ (δ_C 155.7) (Fig. 2). The connectivity of the dimer at C-6 was deduced from HMBC correlations of H-4′ and H-5 to C-6′ and H-4 and H-5′ to C-6. These spectral data indicated that compound **2** has a dimeric 7-methoxy-2-benzoxazolinone skeleton as part of its structure. On the basis of these data and by comparison with those reported in the literature for benzoxazolinone dimer (D′Souzan *et al*., 1997), the structure of **2** was unequivocally deduced and named zeaoxazolinone.

The known compounds were identified by analysis of the spectroscopic data (1D, 2D NMR and MS) and comparison of their data with the literature to be 9-*Z*-hexadecenoic acid (**1**) (Okuyama *et al*., 1997), 6-methoxybenzoxazolinone (**3**) (Amer *et al*., 2004), gallic acid (**4**) (Gerothanassis *et al*., 1998), and *β*-sitosterol-3-*O*-*β*-D-glucopyranoside (**5**) (Ibrahim *et al*., 2012).

Compound **2** was subjected to antifungal activity testing (Conc. 10 μg/disc). It showed activity against all tested fungal strains. It exhibited highest activity against *A. flavus*, *F. oxysporum,* and *C. albicans* (Table 2). According to the available literature, benzoxazolinones have been identified from several Graminea plants and used for host plant resistance (Park *et al*., 2004).

Supplementary Material

Supporting Information Available (Experimental procedures; 1D and 2D NMR spectra and MS data of **2** and **3**).

Acknowledgments The authors are grateful to the 'Research Center of the Female Scientific and Medical Colleges', Deanship of Scientific Research, King Saud University for the financial support and to Dr. Volker Brecht (Nuclear Magnetics Resonance, Institut fuer Pharmazeutische Wissenschaften, Albert-Ludwigs-Universität Freiburg, Germany) for HRESIMS measurements.

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