Phytochemistry xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem

Terezine derivatives from the fungus *Phoma herbarum* PSU-H256

Athip Maha [a], Vatcharin Rukachaisirikul [a,*], Saowanit Saithong [a], Souwalak Phongpaichit [b], Wimarak Poonsuwan [b], Jariya Sakayaroj [c], Patchreenart Saparpakorn [d], Supa Hannongbua [d]

[a] Department of Chemistry and Center of Excellence for Innovation in Chemistry, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand
[b] Natural Products Research Center of Excellence and Department of Microbiology, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand
[c] National Center for Genetic Engineering and Biotechnology (BIOTEC), Thailand Science Park, Klong Luang, Pathumthani 12120, Thailand
[d] Department of Chemistry, Faculty of Science, Kasetsart University, Chatuchak, Bangkok 10900, Thailand

ARTICLE INFO

Article history:
Received 30 April 2015
Received in revised form 14 November 2015
Accepted 19 November 2015
Available online xxxx

Keywords:
Phoma herbarum
Hevea brasiliensis
Euphorbiaceae
Pyrazinone
Pyrazine

ABSTRACT

Investigation of the fungus *Phoma herbarum* PSU-H256 isolated from a leaf of *Hevea brasiliensis* resulted in the isolation of eight terezine derivatives (E–L) together with four known compounds. Their structures were established by analysis of spectroscopic evidence. For terezines E and H, their structures were confirmed by single-crystal X-ray diffraction crystallography. In addition, the absolute configuration at C-7 in terezine E was established by Mosher's method. Terezines K and L were tested for antibacterial, antimalarial, antimycobacterial and cytotoxic activities, but were inactive.

1. Introduction

Endophytic fungi are known as important sources of compounds with high diversity not only in their chemical structures, but also their biological activities. Fungi in the genus *Phoma* produce a wide range of biologically active secondary metabolites including antimicrobial pyrenophorol derivatives (Zhang et al., 2008), the antifungal agent (+)-flavipucine (Loesgen et al., 2011) and cytotoxic metabolites, including phomazine B, epicorazine A and exserohilone A (Kong et al., 2014). In an effort to search for bioactive compounds from fungi, the fungus *Phoma herbarum* PSU-H256 isolated from a leaf of *Hevea brasiliensis* collected in Songkhla Province, Thailand, was studied. The crude EtOAc extract from the culture broth exhibited very weak antibacterial activity against *Staphylococcus aureus* ATCC25923 and methicillin-resistant *S. aureus* SK1 with equal MIC values of 200 μg/mL. Herein, reported are the isolation and characterization of seven new pyrazinone derivatives, terezines E–K (**1**–**7**), and one new pyrazine, terezine L (**8**), along with four known compounds including three phenols, (*E*)-4-hydroxybenzaldehyde *O*-methyloxime (Hioki et al., 2012), 4-hydroxybenzoic acid and 4-hydroxybenzaldehyde (Yoshioka et al., 2004), and one amide derivative, (*S*)-2-hydroxy-3-methylbutanamide (Klaiklay et al., 2012). (*E*)-4-Hydroxybenzaldehyde *O*-methyloxime is reported as a natural product for the first time.

2. Results and discussion

All compounds (**1**–**8**) (Fig. 1) were purified using chromatographic techniques and their structures were elucidated by analysis of spectroscopic data, including IR, UV, NMR and MS. The relative configurations of the new compounds were established using NOEDIFF data, while those of **1** and **4** were confirmed by X-ray data. For the known compounds, the structures were confirmed by comparison of ^{1}H and ^{13}C NMR spectroscopic data with those previously reported. In addition, the ^{1}H and ^{13}C NMR spectroscopic data of **10** are reported for the first time.

Terezine E (**1**) was isolated as colorless crystals. Its molecular formula $C_{16}H_{22}N_2O_6$ was deduced from HRESIMS, and indicated that it contained seven degrees of unsaturation. It exhibited UV absorption bands at 226 and 275 nm, indicating the presence of a benzene chromophore, while IR absorption bands were observed at 3368 and 1684 cm^{-1} for hydroxy and amide carbonyl groups, respectively. The ^{1}H NMR spectrum (Table 1) displayed characteristic signals for two hydroxy protons, an oxygen substituted 1,4-disubstituted benzene, one amide proton, one oxymethine proton, an isopropyl group, one methoxy group and an additional methyl group at δ_H 2.09. The latter methyl signal was ultimately assigned as a second methoxy group based on the ^{13}C chemical shift

* Corresponding author.
E-mail address: vatcharin.r@psu.ac.th (V. Rukachaisirikul).

http://dx.doi.org/10.1016/j.phytochem.2015.11.009
0031-9422/

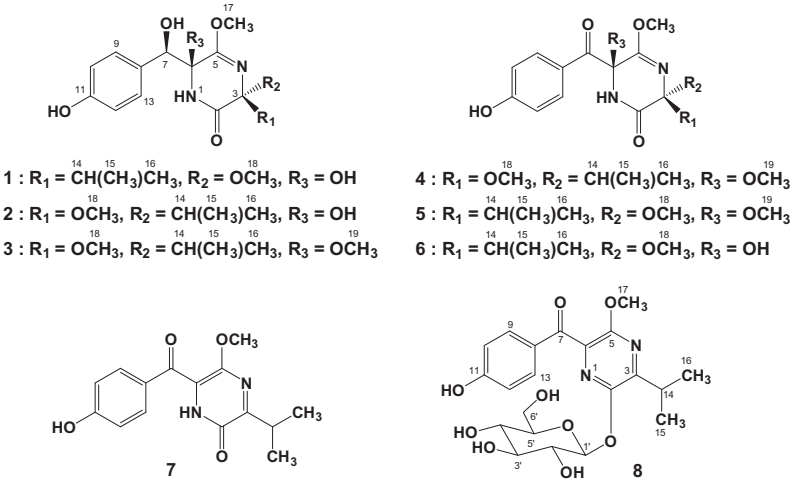

Fig. 1. Compounds **1**–**8** isolated from the fungus *Phoma herbarum* PSU-H256.

Table 1
The ^{1}H and ^{13}C NMR spectroscopic data for **1** and **3** in acetone-d_6 and **2** in $CDCl_3$ + CD_3OD.

Position	**1**		**2**		**3**	
	δ_H, mult, *J* (Hz)	δ_C, type	δ_H, mult, *J* (Hz)	δ_C, type	δ_H, mult, *J* (Hz)	δ_C, type
1-NH	7.10, br s		6.90, br s		6.86, br s	
2		169.6, C		[a]		169.0, C
3		91.2, C		88.0, C		89.7, C
5		160.3, C		157.1, C		158.0, C
6		84.0, C		89.2, C		89.7, C
6-OH	5.67, br s					
7	5.14, s	77.1, CH	5.08, s	75.9, CH	5.05, d, 3.5	76.2, CH
7-OH					4.66, d, 3.5	
8		130.7, C		128.0, C		130.0, C
9, 13	7.23, d, 8.7	129.7, CH	7.12, d, 8.5	128.7, CH	7.16, d, 8.5	129.5, CH
10, 12	6.73, d, 8.7	115.4, CH	6.74, d, 8.5	115.3, CH	6.75, d, 8.5	115.5, CH
11		158.3, C		157.5, C		158.5, C
11-OH	8.34, br s				8.35, s	
14	2.04, sep, 6.9	37.8, CH	0.85, sep, 6.5	34.7, CH	0.52, m	36.3, CH
15	0.89, d, 6.9	16.3, CH_3	0.72, d, 6.5	15.8, CH_3	0.66, d, 6.5	16.7, CH_3
16	0.76, d, 6.9	17.5, CH_3	0.30, d, 6.5	15.7, CH_3	0.36, d, 6.5	16.5, CH_3
17	3.75, s	53.2, CH_3	3.85, s	53.2, CH_3	3.81, s	53.4, CH_3
18	2.09, s	50.5, CH_3	3.21, s	51.5, CH_3	3.07, s	51.6, CH_3
19					3.21, s	51.5, CH_3

[a] Not observed.

(δ_C 50.5). The ^{13}C NMR spectrum (Table 1) consisted of one amide carbonyl, five non-protonated carbons, and other signals consistent with the units listed above. The protons of the 1,4-disubstituted benzene resonating at δ_H 7.23 (H-9 and H-13) showed key HMBC correlations with the oxymethine C-7 and C-11 (Fig. 2). The oxymethine singlet (δ_H 5.14, H-7) exhibited HMBC cross peaks with C-5, C-6, C-8, C-9 and C-13. The chemical shifts of C-6, C-7 and C-11 indicated the substituents at these carbons to be hydroxy groups. The methoxy group at δ_H 3.75 (H_3-17) was attached at C-5 on the basis of an HMBC correlation with C-5. The methoxy protons at δ_H 2.09 (H_3-18) and H-14, H_3-15 and H_3-16 of the isopropyl group showed HMBC correlations with C-3 (δ_C 91.2), indicating that the methoxy and isopropyl groups were both located at C-3. The amide carbonyl carbon (δ_C 169.6) was attached at C-3 on the basis of an HMBC correlation from the amide proton (δ_H 7.10, 1-NH) to C-3. The 1-NH also showed an HMBC cross peak with C-5, thus linking the nitrogen of the amide group with C-6. Based on the numbers of unsaturation, the presence of two nitrogen atoms and the chemical shifts of C-3 and C-5, a pyrazinone ring was formed. The upfield shift of the methoxy group at δ_H 2.09 (H_3-18) suggested its orientation in the shielding region of the phenyl group (Wang et al., 1995). Irradiation of the H-9/H-13 signal in an NOEDIFF experiment (Fig. 2) affected the signal intensity of H_3-18, supporting this conclusion. The relative configuration of **1** was established by X-ray data (Fig. 3). The absolute configuration at C-7 in **1** was determined to be *R* by Mosher's method (Ohtani et al., 1991) (Fig. 4). The remaining chiral centers were then assigned as 3*R* and 6*S*. The calculated ECD spectrum for the 3*R*,6*S*,7*R* stereoisomer of **1** was in agreement with the experimental CD spectrum with the negative and positive bands at 230 and 245 nm, respectively (Fig. S10 and Table S1). Consequently, **1** was characterized as a new terezine derivative.

Terezine F (**2**) was obtained as a colorless gum and was deduced to be an isomer of **1** on the basis of HRESIMS data. The UV and IR data were similar to those of **1**, indicating the presence of identical chromophores. Comparison of the ^{1}H and ^{13}C NMR data of **2** (Table 1) with those of **1** indicated the same overall structure. Since the ^{1}H NMR spectrum was recorded in a mixture of $CDCl_3$ and CD_3OD, signals for the 6-OH and 11-OH were not observed. However, the chemical shifts of C-6 (δ_C 89.2) and C-11 (δ_C 157.5) confirmed oxygen substitution at these carbons. The most obvious difference in the ^{1}H NMR spectrum was the appearance of H_3-18

Please cite this article in press as: Maha, A., et al. Terezine derivatives from the fungus *Phoma herbarum* PSU-H256. Phytochemistry (2015), http://dx.doi.org/10.1016/j.phytochem.2015.11.009