Available online at www.sciencedirect.com
ScienceDirect
Chinese Chemical Letters 21 (2010) 1037–1040
CHINESE CHEMICAL LETTERS
www.elsevier.com/locate/cclet

Synthesis and antifungal activity of trichodermin derivatives

Jing Li Cheng, Yong Zhou, Jin Hao Zhao, Chulong Zhang, Fu Cheng Lin *

Ministry of Agriculture Key Laboratory of Molecular Biology of Crop Pathogens and Insects, Institute of Pesticide and Environmental Toxicology, Zhejiang University, Hangzhou 310029, China

Received 22 December 2009

Abstract

A series of derivatives were synthesized from trichodermin (**1**) which was an antifungal metabolite produced by *Trichoderma taxi* sp. nov. Their structures were confirmed by ^{1}H NMR, MS spectrum. Their antifungal activities were evaluated *in vitro*. The preliminary structure activity relationships (SAR) results indicated that the double bond, epoxide moiety and ester group were main pharmacophore elements, the stereochemistry of C4 position played a key role as well, and the compounds **1e**–**1g** displayed stronger antifungal activity against *Magnaporthe grisea* than **1**.

Keywords: Trichodermin derivatives; Synthesis; Antifungal activity

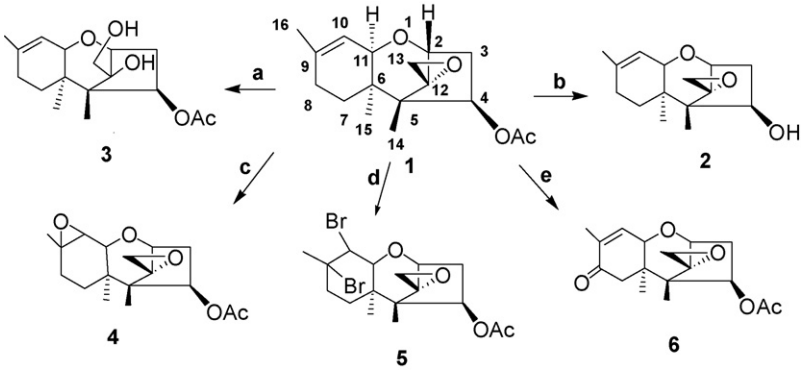
Trichothecenes, particularly those produced by species of *Trichoderma* spp. such as trichodermin (**1**), have been reported to show cytotoxicities against tumor cells [1–4]. Furthermore, trichodermin exhibited highly antifungal activities [5–7]. In our previous research, *Trichoderma taxi* sp. nov. (ZJUF0986) was isolated as an endophytic fungus of *Taxus mairei* growing at the Guanshan Nature Reserve of Jiangxi province, China [8]. Trichodermin was isolated from the metabolites and its stereochemistry was established by single crystal analysis [9]. The antifungal activity studies showed that trichodermin was a broad-spectrum antifungal compound [10]. So far, the fermentation potency of strain endophytic fungus *T. taxi* ZJUF0986 in 0.1 m^3 fermentor has reached 500 mg/L by using of a highly effective medium [11].

The modification of **1** at C4 position had been reported to form certain compounds [12]. However, their bioactivities, especially antifungal activities against plant pathogens have not been mentioned yet in detail. In order to develop novel, safe and potential microbial pesticides, some new derivatives of trichodermin (**2**–**9**, **1a**–**1r**) were synthesized by modifying its C-4, C-8, Δ-9, 10 and epoxide moiety, and their antifungal activities against five plant pathogens *in vitro* were also evaluated.

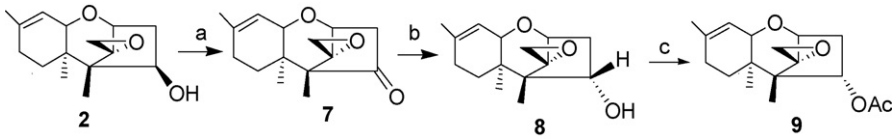
As shown in Scheme 1, **1** was hydrolyzed to **2** by coupling with 2 mol/L NaOH. And when **1** was treated with 15% (v/v) hydrochloric acid, the epoxide moiety was hydrolyzed to dihydroxy compound **3**. Oxidation of **1** with *m*-chloroperoxybenzoic acid led to compound **4** [13]. Bromination of Δ-9, 10 of **1** was also converted to **5**. **6** was obtained by oxidizing **1** with CrO_3 under the protection of nitrogen [14].

* Corresponding author.
E-mail address: fuchenglin@zj.com (F.C. Lin).

1001-8417/$ – see front matter
doi:10.1016/j.cclet.2010.04.033



Scheme 1. Reagents and conditions: (a) 15% HCl, methanol, rt, 6 h, 85%; (b) 2 mol/L NaOH, methanol, 5 h, 89%; (c) *m*-chloroperoxybenzoic acid, CH_2Cl_2, 25 °C, 65%; (d) Br_2, C_5H_5N, 0 °C, 4 h, 88%; (e) $CrO_3{\cdot}Py_2$, CH_2Cl_2, refluxed, 12 h, 30%.



Scheme 2. Reagents and conditions: (a) CrO_3, pyridine, 0–5 °C, 5 h, 65%; (b) $NaBH_4$, CH_3OH/THF, rt, 1 h, 60%; (c) $(CH_3CO)_2O$, C_5H_5N, rt, 1 h, 90%.

2 was oxidated and reduced to obtain **8** [15,16], and **8** reacted with $(CH_3CO)_2O$ in C_5H_5N giving the *S*-trichodermin **9** in 90% yield (Scheme 2).

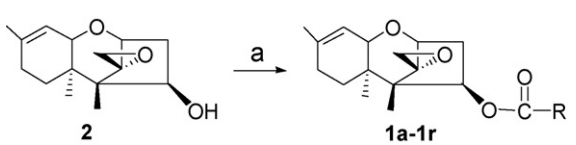
A series of new derivatives **1a**–**1r** from **2** was prepared by reacting with the appropriate alkane acyl chloride and aromatic acyl chloride (Scheme 3) [17].

The antifungal activities of novel compounds *in vitro* were shown in Table 1. It was measured in a bioassay consisting of 3-mm diameter mycelial plugs of gray mold (*Botrytis cinerea*), *peronophythora litchii* (*Peronophythora litchii* Chen ex Ko *et al.*), rice sheath blight (*Rhizoctonia solani*), rice false smut (*Ustilaginoidea virens*) and rice blast (*Magnaporthe grisea*) incubated in 50 μg/mL purified products in Tinline minimal medium agar [18]. And their inhibitory rates were obtained by means of standard two-fold serial dilution method using agar media [19]. All assays were performed in triplicate.

The results showed the antifungal activity only reached 70% relative inhibitory rates against the tested pathogens when the acetyl ester at C4 of **1** was hydrolyzed to **2**. Obviously, the activity of the compound was dramatically reduced when epoxide moiety in **1** was destroyed by the hydrochloric acid solution. Interestingly, although addition of bromine at the Δ-9, 10 led to the absolutely loss in antifungal activity, the epoxidation of the double bond at the Δ-9, 10 (**4**) only result in some decreases in activity. In addition, oxidation at the C-8 (**6**) also led to decrease in activity.

Otherwise, compound **9** [20], which was the *trans*-isomers of **1**, exhibited no activity on all the tested pathogens strains at concentration up to 50 μg/mL. That was to say the stereochemistry at C4 position in **1** was very important for the antifungal activity.

Among C4-ester derivatives of **1**, the compounds **1e**–**1g** exhibited stronger activity against *M. gri.* than **1**, especially compound **1e** gave an inhibition rate up to 100% and the bioactivity of the **1** was only 67.6%, at the concentration of 50 μg/mL.



Scheme 3. Reagents and conditions: (a) R–C(=O)–Cl, DMAP, $N(CH_2CH_3)_3$, CH_2Cl_2.

Table 1
The structures and antifungal data of compound 1 and new derivatives **2–9**, **1a–1r**.

No.	R	Inhibitory rate (50 μg/mL)				
		B. cin.	*P.L.C.*	*R. sol.*	*S. scl.*	*M. gri.*
1	CH_3	100	100	100	84.9	67.6
1a	CF_3	25.84	24.19	77.6	67.5	10.76
1b	CCl_3	79.63	25.50	8.0	46.8	24.32
1c	CH_2Br	67.76	23.53	36.3	44.5	35.14
1d	CH_3CHBr	33.90	40.68	56.2	36.6	25.59
1e	$CH_2{=}CH$	100	49.02	95.5	87.7	100
1f	CH_3–CH=CH	100	38.89	92.5	78.6	90
1g	CH_3O-CH=CH	69.77	38.98	86	67.8	74.26
1h	$CH_3CH_2CH_2CH_2$	60.30	48.39	46.6	56.3	47.69
1i		81.32	56.90	52.0	55.7	31.3
1j		17.79	-22	44.7	43.4	8.11
1l	Ph	50.80	45.16	5.6	26.7	15.38
1m	2-CH_3-Ph	52.95	41.43	0	32.7	28.57
1n	2-Cl-Ph	65.86	74.28	6.4	57.5	25.71
1o	2-OCH_3-Ph	47.38	51.62	0	23.9	23.08
1p	4-CH_3-Ph	72.12	80.71	5.5	3.40	17.14
1q	4-F-Ph	43.07	29.03	2.43	3.5	1.53
1r	4-Cl-Ph	48.70	62.70	1.56	6.9	21.62
2	–	78	75	67	56	58
3	–	0	0	0	0	0
4	–	15	12	10	6	5
5	–	0	0	0	0	0
6	–	15	12	5	15	6
9	–	0	0	0	0	0

Abbreviations: *B. cin.*, *Botrytis cinerea*; *P.L.C.*, *Peronophythora litchii* Chen ex Ko *et al.*; *R. sol.*, *Rhizoctonia solani*; *S. scl.*, *Sclerotonia sclerotiorum*; *M. gri.*, *Magnaporthe grisea.*

According to these preliminary structure activity relationships (SAR), it was concluded that the epoxide moiety, the double bond, ester group in the C4 side chain were main pharmacophore elements of **1**. And the stereochemistry of C4 position also played an important role. Furthermore, modification of **1** at the C3 and C8 site except for maintaining its skeleton may be necessary to find better antifungal compounds for crop protection. These experiments are currently being carried out in the laboratory.

Acknowledgments

This work is financially supported by National Natural Science Foundation of China (No. 30700532) and the Science and Technology Project of Zhejiang province (No. 2008C02007-3).

References

[1] C. Mekhancha-Dahel, C. Lafarge-Franssinet, A.M. Venuat, J. Cell. Pharmacol. 2 (1991) 343.
[2] R.N. Melmed, M.R. Ishai, B. Yagen, Biochem. Pharmacol. 34 (1985) 2809.
[3] Y. Rosenstein, F.C. Lafarge, Toxicol. Appl. Pharmacol. 70 (1983) 283.
[4] Y. Ueno, M. Nakajima, N.J. Shimada, Biochemistry 74 (1973) 285.
[5] G.E. Harman, C.P. Kubicek, Trichoderma and Gliocladium—Enzymes, Biological Control and Commercial Applications, vol. 2, Taylor and Francis Ltd., London, UK, 1998.
[6] W.M. Haggag, S.A. Abo-Sedera, Int. J. Agric. Biol. 7 (2005) 222.
[7] B.L. Bertagnolli, S. Daly, J.B. Sinclair, J. Phytopathol. 146 (1998) 131.
[8] C.L. Zhang, S.P. Liu, F.C. Lin, FEMS Microbiol. Lett. 270 (2007) 90.

[9] S.Y. Chen, C.L. Zhang, Y.Z. Chen, et al. Acta Cryst. E64 (2008) o702.
[10] C.L. Zhang, G.P. Wang, F.C. Lin, Mycosystema 27 (2008) 439.
[11] G.P. Wang, B.Q. Zheng, F.C. Lin, CN 101168757A.
[12] Fr Patent 1,508,066 (1968).
[13] G. Solladie, J.J. Hutt, Org. Chem. 52 (1987) 3560.
[14] W.S. Johnson, W.A. Vredenburgn, J.E. Pike, 1-Testosteronaned dl-Isotestosterone 82 (1960) 3409.
[15] G. Georges, B. Thierry, J. Catherine, Tetrahedron Lett. 21 (1980) 4653.
[16] J.L. Cheng, Y. Zhou, J.H. Zhao, Acta Cryst. E65 (2009) o2897.
[17] P. Coutrot, Synthesis 8 (1986) 661.
[18] W.S. Trahanovsky, P. Mullen, J. Am. Chem. Soc. 94 (1972) 5086.
[19] H. Chen, L.T. Janet, R.A. Suzanne, J. Bioorg. Med. Chem. Lett. 17 (2007) 1979.
[20] The data of selected compounds: (other spectroscopic data of derivatives were in the support information) **9**: yield 90%, [α] 20 *D* = 32.8267 (*c* 0.005, CH_3OH). ESI-MS: 293 $(M+H)^+$ (100%). ^1H NMR (500 MHz, $CDCl_3$)*:* δ 5.47–5.46 (m, 1H, H-4), 5.15–5.12 (m, 1H, H-10), 4.12 (d, 1H, *J* = 5.5 Hz, H-2), 3.71 (d, 1H, *J* = 5.5 Hz, H-11), 3.09 (d, 1H, *J* = 4.0 Hz, H-13), 2.83 (d, 1H, *J* = 4.0 Hz, H-13), 2.68–2.61 (m, 1H, H-3), 2.12 (s, 3H, CH_3COO–), 2.03–2.01 (m, 2H, H-8), 2.01–1.98 (m, 1H, H-3), 1.86 (dd, 1H, *J* = 5.0, 15.5 Hz, H-7), 1.73 (s, 3H, H-16), 1.36–1.33 (m, 1H, H-7), 1.02 (s, 3H, H-14), 0.85 (s, 3H, H-15).