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分离自木霉 PT2的一个新倍半萜糖苷

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摘 要: 从海藻真菌木霉 *Trichoderma* sp. PT2菌体提取物中分离到一个新倍半萜糖苷类化合物 Trichodermoside (1)。并通过 1D-, 2D-NMR波谱数据鉴定了化合物的结构, 并应用 MTT法对化合物 1的细胞毒活性进行了研究。

关键词: 倍半萜糖苷; Trichodermoside; 木霉; 结构解析; 生物活性
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A Novel Sesquiterpene Glucoside from *Trichoderma* sp. PT2

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Abstract: A novel sesquiterpene glucoside, trichodermoside (1), was isolated from the mycelia extract of algicolous fungus *Trichoderma* sp. PT2. The structure was elucidated by spectroscopic analyses, including 1D-, 2D-NMR data and mass-spectrometric analyses. Compound 1 displayed weak growth inhibition against human HeLa cells.

Key words: sesquiterpene glucoside; Trichodermoside; *Trichoderma* sp.; structure determination; cytotoxicity

Introduction

The fungal genus *Trichoderma* is a widespread saprophyte that occurs almost ubiquitously. Because of the useful secondary metabolites produced by this genus, many strains have received considerable attention as biocontrol agents[1,2]. Some secondary metabolites, including N-acylated peptides, peptaibols[3,4], koninginins A-G[5-8], octaketide-derived compounds, two butenolide compounds[9,10], and demethylsorbicillin and oxosorbicillinol[11] were reported as antibiotics with various activities. During the course of examining fungi for biologically active natural products, the strain PT2 from *Blidingia minima* Kylin, collected at Wuyu Island, Zhangzhou of Fujian Province, was isolated. The ethyl acetate extract of the fermentation showed strong antitumor activities against KB and Raji cell lines[12]. Cyclopeptides were the active constituent[13]. Our further research on the chemical components of *Trichoderma* sp. PT2 yielded one new compound. Here the isolation, structure elucidation and bioactivity assay of this compound was described.

Results and Discussion

Fermentation (10 L) was carried out at 28 ℃ for 20 d without agitation. After filtration, the mycelia were extracted exhaustively with acetone. Acetone was evaporated *in vacuo* and the crude extract was partitioned between methanol and petroleum ether. The methanol solution was collected and evaporated to dryness *in vacuo* to afford 17 g extract. The extract was purified over RP-18, Sephadex LH-20, and silica gel columns to afford a novel sesquiterpene glucoside.

Trichodermoside (1) was obtained as a white powder with $[\alpha]_D^{20}$ + 5.76 (*c*, 0.26, MeOH). The molecular formula $C_{23}H_{39}NO_8$ was determined according to the

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qusi-molecular ion peaks at *m/z* 480.5 $[M + Na]^+$ and 937.3 $[2M + Na]^+$ in ESI-MS, and NMR data. The presence of a α-glucosyl N-acyl moiety was revealed by the ^1H NMR signals at δ 4.97 (d, 3.5, H-1′), 3.88 (dd, 10.2, 3.1, H-2′), 3.76 (dd, 13.4, 4.0, H-3′), 3.64 (t, 9.4, H-4′), 3.45 (t, 8.9, H-5′), 3.78 (d, 5.0, H-6′), 1.98 (s, 3H, H-8′), and the ^{13}C NMR signals at δ 98.8 (C-1′), 54.6 (C-2′), 72.0 (C-3′), 72.8 (C-4′), 71.0 (C-5′), 61.7 (C-6′), 172.9 (C-7′), 22.5 (C-8′) [14]. The HMBC correlations of H-1′/C-3′, H-2′/C-1′, C-3′ and C-7′, H-3′/C-4′ and C-5′, H-6′/C-5′, and H-8′/C-7′ confirmed this conclusion (Table 1).

Besides the α-glucosyl N-acyl moiety, the ^{13}C NMR spectra of 1 (Table 1) displayed three quaternary carbons, three methylenes (one oxygenated), five methines (two olefinic and two oxygenated), and four methyls, indicating the presence of a sesquiterpene moiety. The following HMBC correlations were observed: H-13(δ 0.97)/C-3, C-4, C-5 and C-14, H-14 (δ 0.70)/C-3, C-4, C-5 and C-13, H-12(δ 1.68)/C-5, C-6, and C-1. In combination with the ^1H, ^1H-COSY correlations between H-1/H-2 and H-2/H-3 the fragment b was established (Fig. 1). The fragment c could be also postulated from HMBC experiment. The HMBC correlations from H-8/C-5, C-7, C-9, C-10, and C-15

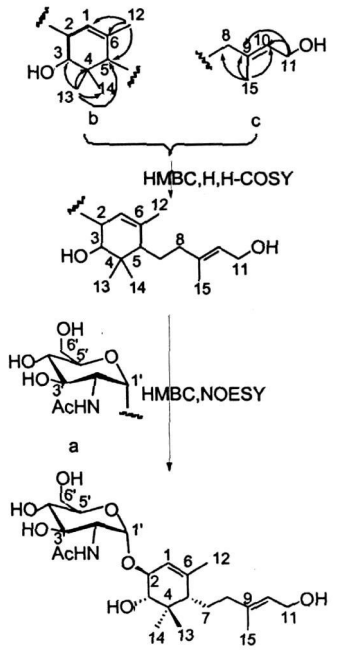

Fig. 1 The fragments a, b, and c of compound 1, and the selected HMBC correlations (H→C) and ^1H, ^1H-COSY correlations

revealed that fragments b and c were joined together. Therefore, the structure of the sesquiterpene moiety was determined. The glycosyl was located at C-2 in view of the HMBC correlation H-1′ (δ 4.97) / C-2 (δ 81.1). Thus, the structure of **1** was determined as shown in figure **1**. The relative configuration of 1 was determined by NOESY cross signals H-3 / H-5, H-3/H_3-13, H-2 /H-14

Table 1 The NMR data of compound 1 (CDCl, ppm, *J* in Hz)

No.	δ_H	δ_C	HMBC	^1H, ^1H-COSY
1	5.28 (s, 1H)	122.0 (d)	C-3, C-5, C-12	H-2
2	3.88 (dd, 10.2, 3.1, 1H)	81.1 (d)	C-1′	H-3, H-1
3	3.34 (d, 8.6, 1H)	78.7 (d)	C-2, C-4, C-5, C-13, C-14	H-2
4	–	40.0 (s)	–	–
5	1.73 (br s, 1H)	49.7 (d)	–	H-7
6	–	139.4 (s)	–	–
7	1.37 (m, 1H), 1.20 (m, 1H)	25.8 (t)	C-6, C-8	H-8
8	2.18 (m, 1H), 1.95 (m, 1H)	41.1 (t)	C-5, C-7, C-9, C-10, C-15	H-7
9	–	138.9 (s)	–	–
10	5.35 (t, 6.5, 1H)	123.9 (d)	C-8, C-11, C-15	H-11
11	4.09 (d, 6.7, 2H)	58.9 (t)	C-9, C-10	H-12
12	1.68 (s, 3H)	21.9 (q)	C-1, C-5, C-6	–

13	0.97 (s, 3H)	24.4 (q)	C-3, C-4, C-5, C-14	–
14	0.70 (s, 3H)	14.1 (q)	C-3, C-4, C-5, C-13	–
15	1.63 (s, 3H)	16.1 (q)	C-8, C-9, C-10	–
1′	4.97 (d, 3.5, 1H)	98.8 (d)	C-3′, C-1	H-2′
2′	3.88 (dd, 3.1, 10.2, 1H)	54.6 (d)	C-1′, C-3′, C-7′	H-1′, H-3′
3′	3.76 (dd, 13.4, 4.0, 1H)	72.0 (d)	C-4′, C-5′	H-2′
4′	3.64 (t, 9.4, 1H)	72.8 (d)	C-2′, C-5′	H-5′
5′	3.45 (t, 8.9, 1H)	71.0 (d)	C-3′, C-6′	H-4′
6′	3.78 (d, 5.0, 2H)	61.7 (t)	C-5′	H-5′
7′	–	172.9 (s)	–	–
8′	1.98 (s, 3H)	22.5 (q)	C-7′	–

Bioassays

The cytotoxic activity against the HeLa cell line of compound 1 was measured 72 h post treatment by the MTT method[15]. Compound 1 (10 μg/mL) displayed weak growth inhibition (12%).

Experimental

General

Precoated TLC plates were from *Qingdao Haiyang Chemical Factory*, Qingdao, P. R. China. For column chromatography (CC), silica gel (200-300, and 80-100 mesh; Qingdao), silica gel 60 (Merck), RP-18 (Merck), and Sephadex LH-20 gel (Amersham Biosciences) were used. Optical rotation was measured on a *Perkin-Elmer* 341 ploarimeter with $CHCl_3$ as solvent. NMR Spectra were recorded on a *Bruker DRX*-500 spectrometer (δ in ppm rel. to Me$_4$Si; *J* in Hz), and MS spectra on a Finnigan LCQ-Advantage and VG Auto-Spec-3000 mass spectrometers.

Fermentation of the Strain

The strain PT2 was identified as *Trichoderma* sp. according to its ITS sequence of rDNA (ITS1-5.8S-ITS2). The strain was incubated on slope of 50% sea water potato dextrose agar (PDA) media in a test tube at 28 ℃ for 7 d to afford seed cultures. Then the seed cultures were washed with sterile water, and the spores were transferred to 3000 mL Erlenmeyer flasks each containing 1000 mL of 50% seawater potato dextrose broth (PDB) for static incubation, room temperature, 20 d.

Extraction and isolation

The flask cultures were filtered and the mycelia were extracted with acetone exhaustively. The acetone solution was collected and evaporated in vacuo to afford a crude extract. Then dissolved in methanol and partitioned between petroleum ether and methanol. The brown oil (17 g) was obtained from the methanol phase.

The extract (17 g) was subjected to MPLC (170 g, RP-18) eluted with H_2O, 30%, 50%, 70%, and 100% methanol, respectively. Then five fractions (Fr. S1-S5) were obtained. Fr. S3 (98 mg) was subjected to CC (45 g Sephadex LH-20; MeOH). All fractions were analyzed by TLC (EtoAc/MeOH 1∶2), and pooled into 3 portions (Fr. S31-S33). Fr. S33 (74 mg) was further seperated over CC (silica gel, $CHCl_3$ / acetone 10∶1, 5∶1, 1∶1, 1∶2) to yield 1 (5 mg).

Trichodermoside [*N*-((2*S*, 3*S*, 4*R*, 5*S*)-2-((1*S*, 4*S*, 6*S*)-6-hydroxy-4-((*E*)-5-hydroxy-3-methylpent-3-enyl)-3, 5, 5-trimethylcyclohex-2-enyloxy)-tetrahydro-4, 5-dihydroxy-6-(hydroxymethyl)-2*H*-pyran-3-yl) acetamide, 1] $[\alpha]_D^{20}$ + 5.76 (*c*, 0.26, MeOH), ESI-MS *m*/*z* 480.5 $[M + Na]^+$ and 937.3 $[2M + Na]^+$, NMR data, see Table 1.

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