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NOTE

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A New 5-Alkenylresorcinol Sch 725681 from *Aspergillus* sp.

Shu-Wei Yang, Tze-Ming Chan, Joseph Terracciano,[†] David Loebenberg, Mahesh Patel,[†††] Vincent Gullo,[††] Min Chu[†]

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Abstract A new 5-alkenylresorcinol Sch725681 (**1**) was isolated and identified from the culture of an *Aspergillus* sp. The structure elucidation of **1** was accomplished based on extensive NMR spectroscopic analyses. Compound **1** showed inhibitory activity against *Saccharomyces cerevisiae* (PM503) and *Candida albicans* (C43) with MICs of 16 and 64 μg/ml, respectively.

Keywords antifungal, antimicrobial, structure elucidation

A number of resorcinols (1,3-dihydroxyalkylbenzenes) have been isolated from plants [1], marine organisms [2], and microorganisms [3~6]. Many of them have been described to exhibit activities against various therapeutic targets, such as cleavage of DNA [1] and inhibition of glycosidase [4], HIV protease [6] and glycerol-3-phosphate dehydrogenase [3]. Some resorcinols have also been reported to display antifungal [7, 8], antibacterial [7], and cytotoxic activities [9].

In the course of our continuing search for novel antimicrobial agents [10], we have isolated a novel antimicrobial 5-alkenylresorcinol Sch 725681 (**1**), from an *Aspergillus* sp. culture (SPRI-0784). Sch725681 was identified as a new 5-alkenylresorcinol based on extensive NMR spectroscopic analyses. In this paper, we report the isolation, structure elucidation and antimicrobial activity of **1**.

Fermentation studies of *Aspergillus* sp. culture SPRI-0784 were conducted in shake flasks. Stock cultures were maintained as frozen whole broths at −80°C in a final concentration of 10% glycerol. The germination medium contained Proteus Peptone (5.0 g/liter), NaCl (5.0 g/liter), KH_2PO_4 (5.0 g/liter), Yeast Extract (3.0 g/liter), Cerelose (20.0 g/liter), and Soybean Grits (5.0 g/liter). The pH was adjusted to 7.0 prior to autoclaving. Each 250 ml Erlenmeyer flask containing 70 ml of this medium was inoculated with 2 ml of the stock culture. The flasks were incubated at 24°C on a rotary shaker at 250 rpm for 96 hours. This seed culture (2.5 ml) was used to inoculate a second stage seed in 250 ml Erlenmeyer flasks, each containing 70 ml of the same seed medium and the flasks were incubated as above for 96 hours.

This second stage seed was then used to inoculate the fermentation medium at 5% v/v. The fermentation was carried out in 500 ml Erlenmeyer flasks, each containing 100 ml of the fermentation medium, containing Neopeptone (10.0 g/liter) and Cerelose (40.0 g/liter). The pH was adjusted to 7.4 and $CaCO_3$, 4.0 g/liter was added. The flasks were incubated at 24°C on a rotary shaker at 250 rpm for 168 hours.

The harvested fermentation broth (1.0 liter) was stirred with 0.2 kg of NaCl and 2.0 liters of acetonitrile (MeCN) for 15 minutes. The organic layer was separated and concentrated to a slurry *in vacuo*. The slurry material was absorbed onto the polymeric resin, CG161 (~100 ml, Tosoh Biosep LLC, Montgomeryville, PA, USA) and the

S.-W. Yang (Corresponding author), **T.-M. Cjan, J. Terracciano, D. Loebenberg, M. Patel, V. Gullo, M. Chu:** Schering-Plough Research Institute, 2015 Galloping Hill Road, Kenilworth, NJ 07033, USA, E-mail: shu-wei.yang@spcorp.com

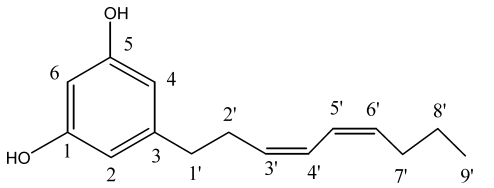
[†] Present address: Cubist Pharmaceuticals, Inc. 65 Hayden Ave. Lexington, MA 02421, USA

[††] Present address: Cetek Corporation, 260 Cedar Hill St. Marlborough, MA 01752, USA

[†††] Present address: SMP International LLC, 42 Brentwood Drive, Verona, NJ 07044, USA

salts and hydrophilic substances were removed by washing with 1 liter of water. Then, the absorbed organic material was eluted with 85% aq. MeOH (1 liter) to yield ~0.8 g of dried material after removing solvent *in vacuo*. This organic material was purified on a semi-preparative ODS-A HPLC column (YMC, 120 Å, S-7, 20 mm×250 mm). The column was eluted with a gradient of MeCN - H_2O: 5~100% MeCN in 35 minutes, and then isocratically with 100% MeCN for another 15 minutes, with a flow rate of 15 ml/minute and the eluate was collected in 13 ml fractions. An enriched complex containing **1** (~10 mg) was obtained with two injections of 40 mg each of the crude material. The complex was further purified using another HPLC C-18 column (YMC Pro C18, 3 μm, 4.6×50 mm). The column was eluted with a isocratic 10% aq. MeCN solution for 2 minutes followed by a gradient of 10~100% MeCN solution in 13 minutes with a flow rate of 1 ml/minute and the desired peak containing pure **1** was collected. Combined pure **1** (~3 mg) was obtained at a retention time ~10 minutes with five injections of ~2 mg each of the enriched material.

The structure of **1** was mainly elucidated by extensive 1D and 2D NMR analyses. In the ^{1}H-NMR spectra, 18 proton signals were observed. Four aliphatic methylene and one methyl signals were observed in the upfield region, and six resonances with a total intensity indicating seven protons were observed in the olefinic/aromatic region. In the ^{13}C NMR spectrum, only thirteen carbon signals were observed. However, two signals (δ 108.1 and δ 159.5) had approximately twice the intensity of the other signals, indicating a symmetric portion in the structure of the molecule. The coupling patterns of the two signals (δ 6.12, 2H, d, J=2.2 Hz and δ 6.07, 1H, t, J=2.2 Hz) in the most downfield region of ^{1}H-NMR represented a pattern typical of a 1,3-dihydroxy-5-alkylbenzene moiety. The carbon chemical shift at δ 159.5 (2×C) represented two hydroxyl-substituted aromatic carbons (C-1 and C-5). Therefore, the basic skeleton was proposed as 5-alkyl- or 5-alkenyl-resorcinol. The molecular formula of **1** was calculated as $C_{15}H_{20}O_2$ which is consistent with a negative ESI-MS measurement (m/z 231, $[M-H]^-$). Excluding the six aromatic carbons, there were four olefinic protonated carbon signals remaining in the downfield region in the ^{13}C



Sch 725681(**1**)

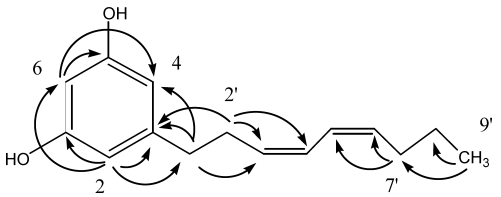


Fig. 1 Key HMBC correlations of **1**.

Table 1 NMR spectral data for compound **1** in CD_3OD[a]

C/H no.	^{1}H (δ)	^{13}C (δ)	HSQC-TOCSY
1, 5		159.5 s	
2, 4	6.12, 2H, d, J=2.2	108.1 d	C-6[b]
3		145.6 s	
6	6.07, 1H, t, J=2.2	101.3 d	C-2[b], C-4[b]
1′	2.50, 2H, t, J=7.3	37.3 t	C-2′
2′	2.29, 2H, q, J=7.3	35.6 t	C-1′, C-3′
3′	5.56, 1H, m, J=10.8, 7.3	132.3 d	C-2′, C-4′
4′	6.00, 1H, br dd, J=10.8, 10.5	132.3 d	C-2′[b], C-3′, C-5′
5′	5.97, 1H, br dd, J=10.8, 10.5	132.1 d	C-4′, C-6′
6′	5.53, 1H, m, J=10.8, 7.3	133.3 d	C-5′, C-7′
7′	2.01, 2H, q, J=7.3	35.9 t	C-6′, C-8′, C-9′[b]
8′	1.39, 2H, hex, J=7.3	23.8 t	C-7′, C-9′
9′	0.89, 3H, t, J=7.3	14.1 q	C-8′, C-7′[b]

[a] Recorded on a Varian Unity 500 NMR instrument at 500 MHz for ^{1}H and 125 MHz for ^{13}C, using standard Varian pulse sequence programs (VNMR Version 6.1 Software). δ in ppm; J in Hz.

[b] Three-bond correlation.

NMR spectrum, indicating two double bonds in the chain moiety. The HSQC-TOCSY data listed in Table 1 strongly suggested a linear chain moiety based on the analysis of ^{1}H-^{13}C correlations from C-1′ through C-9′, which are all proton attached carbons. The location of the two double bonds and the straight chain structure were thus determined. The coupling constants (J=10.8 Hz) between H-3′ and H-4′ and between H-5′ and H-6′ established the *cis* configuration of both Δ3′,4′ and Δ5′,6′. The connectivity of the alkenyl chain to aromatic ring was confirmed based on the long-range heteronuclear shift correlations of H-1′ (δ 2.50) to C-2 (δ 108.1), C-3 (δ 145.6) and C-4 (δ 108.1) observed in the HMBC spectrum. Finally, the proposed structure was confirmed by HMBC data as shown in Fig. 1. Thus compound **1** was determined to be 5-[(3*Z*,5*Z*)-3,5-nonadienyl]-1,3-benzenediol. Unambiguous assignment of the proton and carbon chemical shifts was achieved based on 2D NMR data analyses including HSQC, HSQC-TOCSY, and HMBC as detailed in Table 1.

Previously reported 5-alkenylresorcinols usually have isolated double bond(s) in the chain portion of the molecule [1, 2, 5]. Conjugated double bonds in the chain moiety for resorcinols are unusual. Sch725681 (**1**) represents a unique example of a 5-alkenylresorcinol having conjugated double bonds both with *cis* configurations in the chain moiety.

Sch725681 (**1**) displayed antifungal activity against *Saccharomyces cerevisiae* (PM503) [11] and *Candida albicans* (C43) with MICs of 16 and 64 μg/ml, respectively. Compound **1** also showed antibacterial activity against *Staphylococcus aureus* at 64 μg/ml. This result indicated that compound **1** does not have a significant selective antimicrobial profile.

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References

1. Related references were cited here: Lytollis W, Scannell RT, An H, Murty VS, Reddy KS, Barr JR, Hecht SM. 5-Alkylresorcinols from *Hakea trifurcate* that cleave DNA. J Am Chem Soc 117: 12683–12690 (1995)
2. Barrow RA, Capon RJ. Alkyl and alkenyl resorcinols from an Australian marine sponge, *Haliclona* sp. (Haplosclerida: Haliclonidae). Aust J Chem 44: 1393–1405 (1991)
3. Tsuge N, Mizokami M, Imai S, Shimazu A, Seto H. Adipostatins A and B, new inhibitors of glycerol-3-phosphate dehydrogenase. J Antibiot 45: 886–891 (1992)
4. Yamada H, Shiomi K, Xu Q, Nagai T, Shibata M, Oya I, Takahashi Y, Ōmura S. New Glycosidases inhibitors, panosialins D and wD produced by *Streptomyces* sp. OH-5186. J Antibiot 48: 205–210 (1995)
5. Matsuzaki K, Tahara H, Inokoshi J, Tanaka H, Masuma R, Ōmura S. New brominated and halogen-less derivatives and structure-activity relationship of azaphilones inhibiting gp120-CD4 binding. J Antibiot 51: 1004–1011 (1998)
6. Dolak LA, Seest EP, Cialdella JI, Li GP, Bohanon MJ. Compounds used for the inhibition of HIV-protease. PCT WO 93/04055A2. March 4, 1993
7. Orabi KY, Mossa JS, El-Feraly FS. Isolation and characterization of two antimicrobial agents from mace (*Myristica fragrans*). J Nat Prod 54: 856–859 (1991)
8. Garcia S, Garcia C, Heinzen H, Moyna P. Chemical basis of the resistance of barley seeds to pathogenic fungi. Phytochemistry 44: 415–418 (1997)
9. Filip P, Anke T, Sterner O. 5-(2′-Oxoheptadecyl)-resorcinol and 5-(2′-oxononadecyl)-resorcinol, cytotoxic metabolites from a wood-inhabiting basidiomycete. Zeitschrift fur Naturforschung Section C 57: 1004–1008 (2002)
10. Related references were cited here: Yang SW, Chan TM, Terracciano J, Loebenberg D, Patel M, Chu M. Structure elucidation of Sch 725674 from *Aspergillus* sp. J Antibiot 58: 535–538 (2005)
11. Yang SW, Chan TM, Pomponi SA, Chen G, Loebenberg D, Wright A, Patel M, Gullo V, Pramanik B, Chu M. Structure elucidation of a new antifungal sterol sulfate, Sch 575867, from a deep-water marine sponge (Family: Astroscleridae). J Antibiot 56: 186–189 (2003)