J. Antibiot. 58(8): 535–538, 2005

NOTE

THE JOURNAL OF ANTIBIOTICS

Structure Elucidation of Sch 725674 from *Aspergillus* sp.

Shu-Wei Yang, Tze-Ming Chan, Joseph Terracciano, David Loebenberg, Mahesh Patel, Min Chu

Received: June 11, 2005 / Accepted: August 12, 2005

Abstract A new macrolide Sch725674 (**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 8 and 32 μg/ml, respectively.

Keywords antifungal, antimicrobial, structure elucidation, Sch 725674

In the course of our continuing search for novel antimicrobial agents [1~8], we have isolated a novel antifungal macrolide, Sch 725674 (**1**), from an *Aspergillus* sp. culture (SPRI-0836). Sch725674 was identified as a new 14-membered macrocyclic lactone based on extensive NMR spectroscopic analyses. In this paper, we describe the isolation and the structure elucidation of **1**. The antimicrobial activity of **1** against fungal pathogens is also reported.

Fermentation studies of the *Aspergillus* sp. culture SPRI-0836 were conducted in shake flasks. Stock cultures were maintained as frozen whole broths at −80°C in a final concentration of 10% glycerol. The inoculum medium contained Proteus Peptone (5 g/liter), NaCl (5 g/liter), KH_2PO_4 (5 g/liter), yeast extract (3 g/liter), cerelose (20 g/liter), soybean grits (5 g/liter), antifoam (1 ml/liter), and distilled H_2O (1 liter). The pH was adjusted to 7.2 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 2 liter Erlenmeyer flasks, each containing 350 ml of the fermentation medium, containing oat flour (20 g/liter), soy flour (20 g/liter), yeast extract (2 g/liter), corn steep powder (5 g/liter), K_2HPO_4 (11 g/liter), KH_2PO_4 (4 g/liter), and distilled H_2O (1 liter). The flasks were incubated at 24°C on a rotary shaker at 250 rpm for 120 hours.

The harvested fermentation broth (10 liter) was stirred with 2 kg of NaCl and 20 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 (~200 ml, Tosoh Biosep LLC, Montgomeryville, PA, USA) and the salts and small hydrophilics were washed out with 20 liters of water. Then, the absorbed organic material was eluted with 85% aq. MeOH (4 liter) to yield ~1.5 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

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

Present address: **M. Chu, J. Terracciano:** Cubist Pharmaceuticals, Inc. 65 Hayden Ave. Lexington, MA 02421, USA

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

C/H no.	^1H (δ)	^{13}C (δ)	HSQC-TOCSY
1		168.4 s	
2	6.07, dd, 15.8, 1.6	123.1 d	C-3
3	6.86, dd, 15.8, 6.0	149.3 d	C-2, C-4
4	4.48, ddd, 6.0, 3.0, 1.6	76.0 d	C-2[b], C-5
5	3.84, ddd, 6.0, 4.7, 3.0	72.9 d	C-6
6	1.82, ddd, 14.7, 6.5, 6.0	38.3 t	C-5, C-7
	1.65, m		
7	3.98, q, 6.5	69.5 d	C-6, C-8
8	1.36, m	36.8 t	
9	1.19, m; 1.37, m	25.8 t	C-8, C-10
10	1.15, m; 1.40, m	29.5 t	
11	1.19, m; 1.45, m	27.0 t	C12
12	1.54, m; 1.70, m	34.1 t	C10[b], C-11, C-13
13	4.94, dddd, 9.8, 7.5, 5.0, 2.2	77.6 d	C-12, C-14
14	1.57, m; 1.61, m	36.5 t	C-13, C-15, C-16[b]
15	1.32, m	26.4 t	C-13[b]
16	1.30, m	32.9 t	
17	1.31, m	23.8 t	C-18
18	0.89, t, 6.8	14.5 q	C-16[b], C-17
NH			
OH			

[a] Recorded on a Varian Unity 500 NMR instrument at 500 MHz for ^1H 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

15 ml/minute and the eluate was collected in 13 ml fractions. An enriched complex containing **1** (~7 mg) was obtained with three injections of 40 mg each of the crude material. The complex was further purified through another HPLC ODS-H80 column, (YMC J'sphere, 4 μm, 15×100 mm). The column was eluted with a two-step gradient of MeCN - H_2O: 3~50% MeCN in 40 minutes, and then 50~100% MeCN over another 10 minutes, with a flow rate of 3 ml/minute and ~2 ml fractions were collected for each fraction. Pure **1** (~1.5 mg) was obtained, at retention time ~41 minutes with two injections of ~3.5 mg each of the enriched material.

The structure of **1** was mainly elucidated by extensive 1D and 2D NMR data analyses. In the ^{13}C- and ^1H-NMR spectra, 18 carbon and 29 proton signals were observed, respectively (Table 1). The characteristics of the 18 carbon signals were identified as one methyl, one carbonyl, two olefinic methine, four oxygenated-methine, and ten aliphatic methylene carbons on the basis of analyses of ^1H- and ^{13}C-NMR, and APT and HSQC data. The molecular formula of **1** was therefore established as $C_{18}H_{32}O_5$ which is consistent with a positive ESI-MS measurement (m/z 329, $[M+H]^+$, performed on a Waters MicromassZQ mass spectrometer). Based on three degrees of unsaturation and only five oxygen atoms in the molecule, a cyclic ring structure through either an ether or ester linkage was proposed.

The multiplicity of the carbons was determined through an APT experiment, and the proton-attached carbon resonances were assigned to the corresponding proton signals by analysis of HSQC data. However proton signals were highly overlapped between δ 1.00 and 1.80 ppm in the ^1H NMR spectrum. These could be only interpreted through a two dimensional HSQC-TOCSY spectrum. The HSQC-TOCSY data shown in Table 1 strongly suggested a linear chain moiety based on the analysis of ^1H-^{13}C correlations from C-2 through C-18, which are all proton attached carbons. Thus the locations of double bond (Δ2, 3), four oxygen atoms (on C-4, C-5, C-7, and C-13), and the terminal methyl group (C-18) were determined. These assignments were confirmed by HMBC data analysis, as shown in Fig. 1. H-2 (δ 6.07, dd, J=15.8, 1.6 Hz) showed a simple coupling pattern in ^1H NMR and thus indicated that C-2 was adjacent to the remaining

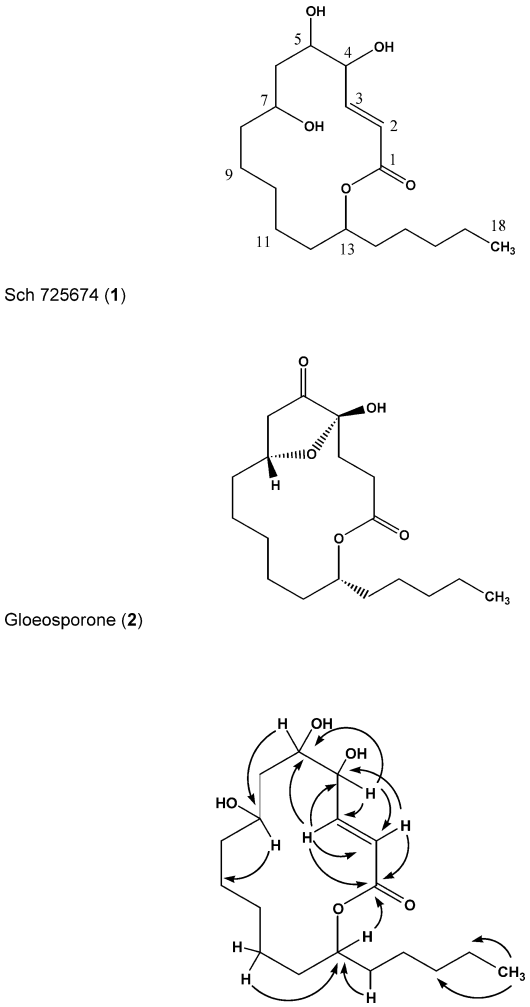

Fig. 1 Structures of **1**, **2** and key HMBC correlations of **1**.

carbonyl carbon C-1. This was confirmed by long-range correlations of H-2 (δ 6.07) and H-3 (δ 6.86) to C-1 (δ 168.4) on the basis of HMBC data analysis. Finally, the observation of the long-range correlation of H-13 (δ 4.94) and C-1 established the ester linkage of the 14-membered macrocyclic ring skeleton.

The coupling constant (J=15.8 Hz) between H-2 and H-3 established the *trans* configuration of Δ2, 3. Thus, the structural elucidation of **1** was completed. 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. The stereochemistry of the four oxygenated methines could not be established at this stage due to the limited amount of sample.

Compounds with fourteen-membered macrocyclic mono-lactone skeletons without additional methyl group substitution on the ring (erythromycin-like) are very rare in nature. Gloeosporone (**2**), the fungal germination self-inhibitor from the fungus *Colletotrichum gloeosporioides*, is the best known and studied in this class [9, 10]. Gloeosporone has shown antifungal activity [11]. Due to its interesting biological activity gloeosporone and its stereo isomers have been chemically synthesized through different routes [11～15]. To the best of our knowledge, compound **1** is the second member in this class without a highly branched skeleton chain.

Sch725674 (**1**) displayed antifungal activity against *Saccharomyces cerevisiae* (PM503) [16] and *Candida albicans* (C43) with MICs 8 and 32 μg/ml, respectively. Compound **1** did not show antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* at levels up to 256 μg/ml.

Acknowledgement The authors are grateful to Mr. Lewis B. Fan for extract preparation.

References

1. Yang SW, Buevich A, Chan TM, Terracciano J, Chen G, Loebenberg D, Patel M, Boehm E, Gullo V, Pramanik B, Chu M. A new antifungal sterol sulfate, Sch 601324, from *Chrysosporium* sp. J Antibiot 56: 419–422 (2003)
2. Yang SW, Chan TM, Pomponi SA, Gonsiorek W, Chen G, Wright AE, Hipkin W, Patel M, Gullo V, Pramanik B, Zavodny P, Chu M. A new sesterterpene, Sch 599473, from a marine sponge, *Ircinia* sp. J Antibiot 56: 783–786 (2003)
3. Yang SW, Chan TM, Pomponi SA, Chen G, Wright AE, Patel M, Gullo V, Pramanik B, Chu M. A new bicyclic guanidine alkaloid, Sch 575948, from a marine sponge, *Ptilocaulis spiculifer*. J Antibiot 56: 970–972 (2003)
4. Yang SW, Chan TM, Terracciano J, Loebenberg D, Chen G, Patel M, Gullo V, Pramanik B, Chu M. Structure elucidation of a new diketopiperazine Sch 725418 from *Micromonospora* sp. J Antibiot 57: 345–347 (2004)
5. Yang SW, Chan TM, Terracciano J, Patel R, Loebenberg D, Chen G, Patel M, Gullo V, Pramanik B, Chu M. A new anthracycline antibiotic micromonomycin from *Micromonospora* sp. J Antibiot 57: 601–604 (2004)
6. Yang SW, Xu L, Mierzwa R, He L, Terracciano J, Patel M, Gullo V, Black T, Zhao W, Chan TM, Chu M. Two novel antibiotics, Sch 419558 and Sch 419559, produced by *Pseudomonas fluorescens*: effect on activity by overexpression of RpoE. Bioorg Med Chem 12: 3333–3338 (2004)
7. Yang SW, Chan TM, Patel R, Terracciano J, Loebenberg D, Patel M, Chu M. A new antimicrobial dibenzofuran Sch 725421 from an unidentified fungus. J Antibiot 57: 465–467 (2004)
8. Yang SW, Chan TM, Terracciano J, Patel R, Loebenberg D, Chen G, Patel M, Gullo V, Pramanik B, Chu M. New

antibiotic Sch 725424 and its dehydration product Sch 725428 from *Kitasatosporia* sp. J Antibiot 192–195 (2005)
9. Meyer WL, Lax AR, Templeton GE, Brannon MJ. The structure of gloeosporone, a novel germination self-inhibitor from conidia of Colletotrichum gloeosporioides. Tetrahedron Lett 24: 5059–5062 (1983)
10. Meyer W, Schweizer WB, Beck AK, Scheifele W, Seebach D, Schreiber SL, Kelly SE. 31. Revised structure of the fungal germination self-inhibitor gloeosporone. Helv Chim Acta 70: 281–291 (1987)
11. Seebach D, Adam G, Zibuck R, Simon W, Rouilly M, Meyer WL, Hinton JF, Privett TA, Templeton GE, Heiny DK, Gisi U, Binder H. Gloeosporone–a macrolide fungal germination self-inhibitor total synthesis and activity. Liebigs Ann Chem 1233–1240 (1989)
12. Matsushita M, Yoshida M, Zhang Y, Miyashita M, Irie H, Ueno T, Tsurushima T. Synthesis of a germination self-inhibitor, (−)-gloeosporone, and related compounds and evaluation of their activities. Chem Pharm Bull 40: 524–527 (1992)
13. Schreiber SL, Kelly SE, Porco JA Jr., Sammakia T, Suh EM. Structural and synthetic studies of the spore germination autoinhibitor gloeosporone. J Am Chem Soc 110: 6210–6218 (1988)
14. Adam G, Zibuck R, Seebach D. Total synthesis of (+)-gloeosporone: assignment of absolute configuration. J Am Chem Soc 109: 6176–6177 (1987)
15. Furstner A, Langemann K. Total syntheses of (+)-ricinelaidic acid lactone and of (−)-gloeosporone based on transition-metal-catalyzed C–C bond formation. J Am Chem Soc 119: 9130–9136 (1997)
16. 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)