

Structure Elucidation of Sch 725674 from *Aspergillus* sp.

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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 μ g/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), cerelese (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 \AA , S-7, $20 \text{ mm} \times 250 \text{ mm}$). 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

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Table 1 NMR spectral data for compound **1** in CD₃OD^a

C/H no.	¹ H (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 1.65, m	38.3 t	C-5, C-7
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 ¹³C, 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-H₂O: 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 ¹³C- 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 ¹³C-NMR, and APT and HSQC data. The molecular formula of **1** was therefore established as C₁₈H₃₂O₅ 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-¹³C 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

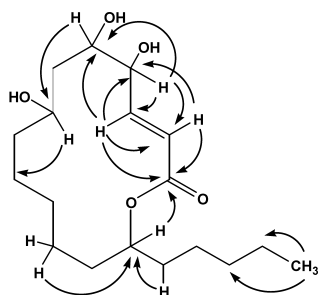
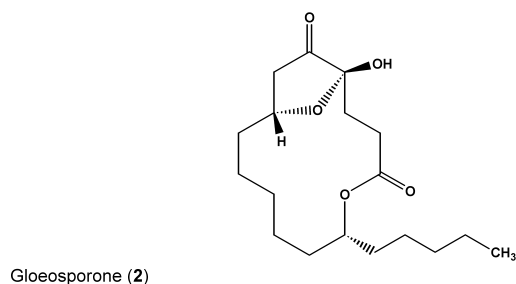
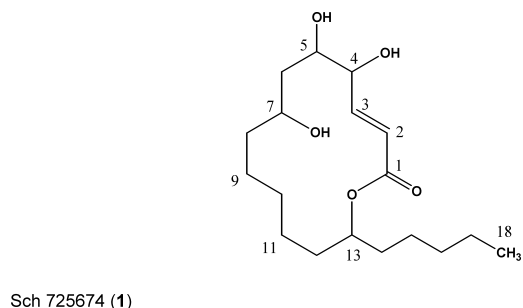


Fig. 1 Structures of **1**, **2** and key HMBC correlations of **1**.

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 $\Delta^{2,3}$. 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 $\mu\text{g/ml}$, respectively. Compound **1** did not show antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* at levels up to 256 $\mu\text{g/ml}$.

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