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A New Hydrogenated Azaphilone Sch 725680 from *Aspergillus* sp.

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Abstract A new hydrogenated azaphilone Sch725680 (**1**) was isolated and identified from the culture of an *Aspergillus* sp. The structure elucidation of **1** was achieved based on extensive NMR spectroscopic analyses. Compound **1** showed inhibitory activity against *Saccharomyces cerevisiae* (PM503) and *Candida albicans* (C43) with MICs of 8 and 64 μg/ml, respectively.

Keywords azaphilone, antifungal, antimicrobial, structure elucidation

A number of azaphilones and hydrogenated azaphilones have been isolated from various microorganisms, mainly fungal species, such as *Emericella falconensis* [1~3], *Penicillium multicolor* [4], *Penicillium funiculosum* [5], and *Penicillium sclerotiorum* [6]. Many of them have been described to exhibit inhibitory activities against various therapeutic targets, such as inhibition of acyl-CoA: cholesterol acyltransferase [4], cholesteryl ester transfer protein [7], platelet-derived growth factor [8], endothelin receptor [6], gp120-CD4 [9], monoamine oxidase [10], and phospholipase A_2 [11]. Some azaphilones have also been reported to display inhibitory activity of tumor promotion [12].

In the course of our continuing search for novel antimicrobial agents [13, 14], we have isolated and identified a novel hydrogenated azaphilone Sch 725680 (**1**), from an *Aspergillus* sp. culture (SPRI-0814). In this paper, we report the fermentation, isolation, structure elucidation and antimicrobial activity of **1** (Fig. 1).

Fermentation studies of *Aspergillus* sp. culture SPRI-0814 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

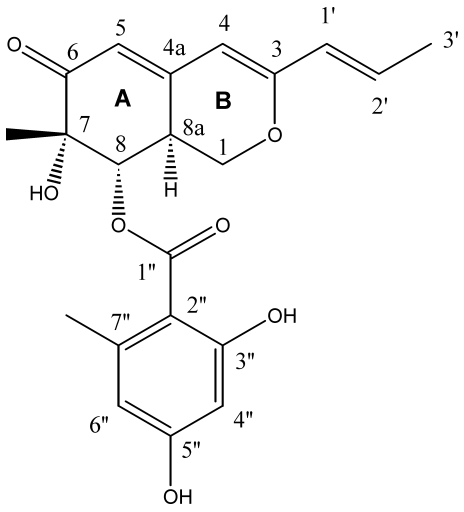

Fig. 1 Structure of Sch 725680 (**1**)

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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), and soybean grits (5 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, which consisted of neopeptone (10 g/liter) and cerelose (40 g/liter). The pH was adjusted to 7.4 and $CaCO_3$ (4 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 (10 liters) 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 hydrophilic substances were removed by washing with 20 liters of water. Then, the absorbed organic material was eluted with 85% aq. MeOH (4 liters) to yield ~2.4 g of dried material after removing solvent *in vacuo*. Part of this organic material was purified on a semi-preparative ODS-A HPLC column (YMC, 120 Å, S-7, 20×250 mm). The column was eluted with a gradient of MeCN - H_2O: 5~100% MeCN in 50 minutes, and then held isocratically with 100% MeCN for additional 15 minutes with a flow rate of 15 ml/minute. The eluate was collected in 13 ml for each fraction. An enriched complex containing **1** (~20 mg) was obtained with three injections of 40 mg each of the crude material. The complex was further purified using another HPLC C-18 column (YMC ODS-A, 3 μm, 4.6×100 mm). The column was eluted with a gradient of MeCN - H_2O 5~50% in 20 minutes followed by the second gradient of 50~100% MeCN solution in 6 minutes with a flow rate of 0.8 ml/minute and the desired peak containing pure **1** was collected. Combined pure **1** (~6 mg) was obtained with four injections of ~5 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 spectrum, a total of 19 carbon-attached protons were counted. Three methyl, one methylene, and two methine signals were observed in the aliphatic region, and six resonances were

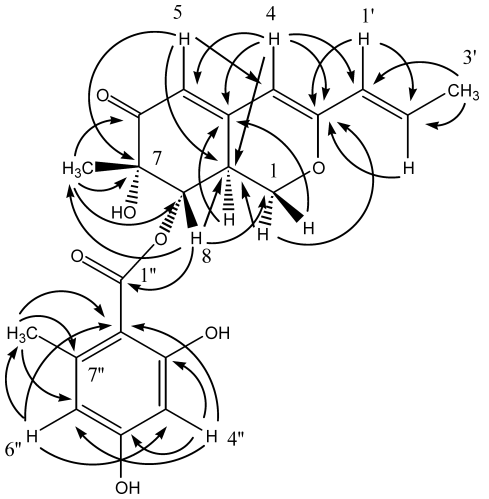

Fig. 2 HMBC correlations of **1**.

observed in the olefinic/aromatic region. In the ^{13}C NMR spectrum, twenty one carbon signals were detected, in which a conjugated ketone functionality (C-6, δ 197.5) was identified. The molecular ion *m/z* 387, $[M+H]^+$ was observed on an ESI MS instrument, and therefore the molecular formula of **1** was calculated as $C_{21}H_{22}O_7$. Thus three hydroxyl groups were proposed to be present in the molecule. The hydrogenated azaphilone skeleton was mainly determined by ^{1}H-^{13}C long range correlations measured in an HMBC experiment. The methyl group showing a doublet-doublet resonance (H_3-3′, δ 1.84, dd, J=7.0, 1.7 Hz) in ^{1}H NMR was determined to be adjacent to a double bond consist of two olefinic methine carbons (C-1′ and C-2′) due to the observation of the correlations between H_3-3′ and C-1′ (δ 126.7) and C-2′ (δ 135.0). This double bond was conjugated to additional two double bonds and further extended to the ketone (C-6) at the other end on the basis of the following correlations: H-1′ to C-3; H-2′ to C-3; H-4 to C-3, C-4a, C-5, C-8a, and C-1′; H-5 to C-4, C-7 and C-8a (Fig. 2). The second methyl group (7-CH_3, δ 2.57, s) substituted on a oxygenated quaternary carbon (C-7) was located two bonds away from the conjugated ketone (C-6) and an aliphatic oxygenated methine carbon (C-8, δ 76.7) due to the significant coupling of CH_3-7 and C-7, C-6, and C-8. An aliphatic methine carbon (C-8a, δ 36.4) showing long-range correlations to H-4, H-5, H-8, and H-1 was determined to be adjacent to C-4a, C-8 and C-1. Therefore, the first six-member ring A was constructed based on these evidences. The oxygenated methylene proton signals (H-1α and H-1β) indicated a three-bond correlation to the olefinic carbon C-3 (δ 162.2) through an oxygen atom to form the second six-member ring B identified as a dihydro-pyran moiety. Thus the 6,7,8,8a-

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

C/H no.	^1H (δ)	^{13}C (δ)	^1H-^1H COSY
1α	4.44, 1H, dd, *J*=10.8, 5.2	69.4 t	H-1β, H-8a
1β	3.83, 1H, dd, *J*=13.6, 10.8		H-1α, H-8a
3		162.2 s	
4	5.71, 1H, s	103.9 d	
4a		153.7 s	
5	5.75, 1H, d, *J*=2.1	117.0 d	
6		197.5 s	
7		75.2 s	
7-Me	1.30, 3H, s	19.9 q	
8	5.27, 1H, d, *J*=10	76.7 d	H-8a
8a	3.42, 1H, dddd, *J*=13.6, 10, 5.2, 2.1	36.4 d	H-1α, H-1β, H-8
1′	5.99, 1H, dq, *J*=15.4, 1.7, 1.7, 1.7	126.7 d	H-2′
2′	6.45, 1H, dq, *J*=15.4, 7.0, 7.0, 7.0	135.0 d	H-1′, H-3′
3′	1.84, 3H, dd, *J*=7.0, 1.7	18.6 q	H-2′
1″		172.1 s	
2″		105.7 s	
3″		166.2 s	
4″	6.19, 1H, d, *J*=2.5	102.0 d	H-6″
5″		164.4 s	
6″	6.26, 1H, d, *J*=2.5	112.9 d	H-4″
7″		145.1 s	
7″-Me	2.57, 3H, s	24.8 q	

[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.

tetrahydro-1*H*-isochromene ring skeleton was determined.

The remaining seven carbons was constructed to represent a 2,4-dihydroxy-6-methyl-benzoyl moiety based on the following observation: correlations H-4″ to C-2″, C-3″, C-5″ and C-6″; H-6″ to C-2″, C-4″, C-5″, and CH_3-7″; CH_3-7″ to C-2″, C-6″, and C-7″. The ^{13}C chemical shifts of the highly substituted benzoyl moiety well matched the data of the same benzoyl moiety reported in the literature [15]. The benzoyl group was unambiguously assigned to 8-*O* position based on the correlation from H-8 to C-1″ (δ 172.1). Thus the two dimensional structure of **1** was determined, and the unambiguous assignment of the ^1H and ^{13}C chemical shifts was achieved based on 2D NMR data analyses including ^1H-^1H COSY, HSQC, and HMBC as detailed in Table 1.

The stereochemistry of **1** was established by analyses of ^1H-^1H coupling constants and NOESY. The double bond Δ1′,2′ was determined as *trans* due to the typical large coupling constant (*J*=15.4 Hz). NOE correlation between H-1′ and H-4 confirmed the proposed linear conjugation of these double bonds (Fig. 3). The relative stereochemistry on C-1, C-8, and C-8a was assigned on the basis of the

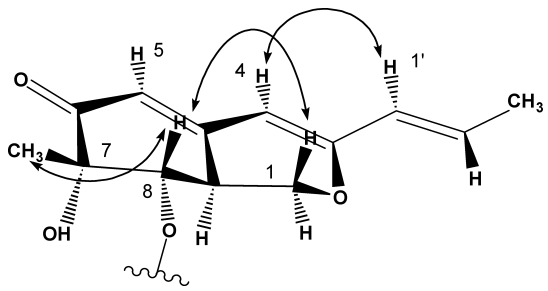

Fig. 3 The relative configuration of **1** and NOE correlations observed in NOESY spectrum (represented by double arrows).

NOE correlations between H-1β and H-8 and between H-8 and CH_3-7 as shown in Fig. 3. Thus H-8a was assigned on pseudo-axial position and α orientation, the same as two oxygen atoms (8-*O* and 7-*O*). The large coupling constants measured for H-8a (*J*=13.6 and 10 Hz) were consistent with the stereochemistry assignment.

Most of the previously reported azaphilones have benzoyl substitution on C-7 position [1～3]. Benzoyl

substitution on C-8 for hydrogenated azaphilone is rare [5]. To our best knowledge, Sch725680 (**1**) represents the second example of a C-8 benzoyl substituted hydrogenated azaphilone.

Sch725680 (**1**) displayed antifungal activity against *Saccharomyces cerevisiae* (PM503) [16] and *Candida albicans* (C43) with MICs of 8 and 64 μg/ml, respectively. Compound **1** also showed weak antibacterial activity against *Staphylococcus aureus* at 64 μg/ml.

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