Notes

ISOLATION AND STRUCTURE OF A NEW PHENOXAZINE ANTIBIOTIC, EXFOLIAZONE, PRODUCED BY *STREPTOMYCES EXFOLIATUS*

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During the course of our screening program for new antibiotics active against *Valsa ceratosperma*, the causative fungus of the apple canker disease, we found that *Streptomyces exfoliatus* BT-38 produced a new phenoxazine antibiotic, exfoliazone (**I**). In this paper, we report the isolation and structure of **I**.

S. exfoliatus BT-38, which was isolated from Matsumoto city, Nagano Prefecture, Japan, was cultivated at 27°C in a 60-liter jar fermenter containing 30 liters of a medium with agitation rate of 400 rpm and air flow of 30 liters/minute. The medium consisted of glucose 2.5%, soybean meal 1.5% dry yeast 0.2% and $CaCO_3$ 0.4%; pH was adjusted to 6.2. After fermentation for 90 hours, the culture broth was separated into filtrate and mycelium by centrifugation. The flow diagram for the isolation of **I** is shown in Fig. 1. The culture filtrate was adsorbed on Diaion HP-20 (10 liters, batch treatment), which was washed with water and then eluted with MeOH. The active eluate was concentrated under reduced pressure to 5 liters and the aqueous residue was extracted with $CHCl_3$. Further purification was made by silica gel column chromatography, Toyopearl HW-40 column chromatography and preparative HPLC. Crystallization from hot $CHCl_3$ gave pure **I** (30.8 mg) as orange needles.

The physico-chemical properties of **I** were as follows: MP 294~296°C; IR ν_{max} (KBr) cm^{-1} 3310, 1700, 1620; UV λ_{max} nm (ε) 238 (75,300), 400 (42,300). The HREI-MS of **I** showed a molecular ion peak at *m/z* 284.0825, indicating its molecular formula to be $C_{15}H_{12}O_4N_2$ (Calcd 284.0853); ^1H NMR (500 MHz, DMSO-d_6) δ 2.22 (3H, s), 4.59 (2H, s), 6.46 (1H, s), 7.51 (1H, d, J=8.5 Hz), 7.56 (1H, dd, J=2.0 and 8.5 Hz), 7.75 (1H, d, J=2.0 Hz), 8.25 (1H, s) and 9.68 (1H, s, exchangeable).

These spectral data were very similar to those of *N*-acetylquestiomycin A[1], suggesting that **I** belongs to the group of phenoxazine antibiotics (Fig. 2). The ^{13}C NMR spectral data of **I** and *N*-acetylquestiomycin A are summarized in Table 1.

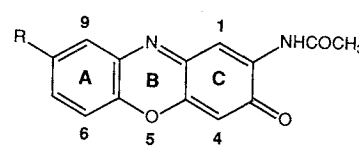
Comparison of ^{13}C NMR spectral data of these two compounds enabled us to assign 13 out of the 15 signals of **I**. The spectral differences between them were that the aromatic methine observed at δ_C 130.0 in *N*-acetylquestiomycin A was replaced by a quaternary aromatic carbon at δ_C 140.0 in **I** with appearance of a new oxymethylene signal at δ_C 63.4 in the latter. From the molecular formula of **I**, this new functional group was ascribed to a hydroxymethyl group.

These results suggested that **I** was a hydroxy-

Fig. 1. Isolation procedure of exfoliazone.

Culture filtrate (60 liters)
↓
Diaion HP-20
↓ eluted with MeOH, concd *in vacuo*, extracted with $CHCl_3$
Silica gel
↓ eluted with $CHCl_3$-MeOH (40:1)
Toyopearl HW-40
↓ eluted with $CHCl_3$-MeOH (1:1)
HPLC (YMC SH-343-5)
↓ eluted with 40% MeCN
Crystallized from $CHCl_3$ (30.8 mg)

Fig. 2. Structures of exfoliazone and *N*-acetylquestiomycin A.



Exfoliazone R=CH_2OH
N-Acetylquestiomycin A R=H

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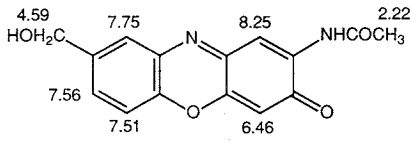
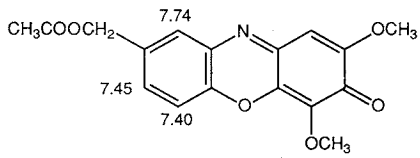
Fig. 3. Structures of exfoliazone and acetylmichigazone.



Exfoliazone



Acetylmichigazone

Table 1. ^{13}C NMR spectral data of exfoliazone and *N*-acetylquestiomycin A.

Functional group	Exfoliazone	*N*-Acetylquestiomycin A
CH_3	24.7	24.7
CH=	104.3	104.0
CH=	114.3	113.9
CH=	116.4	116.1
CH=	127.8	125.7
CH=	131.1	131.9
CH=	—	130.0
C=	133.9	133.9
C=	137.4	137.0
C=	142.7	143.2
C=	148.9	148.7
C=	149.9	149.4
N−C=O	170.6	169.4
C=O	180.1	179.7
CH_2−O−	63.4	—
C=	140.0	—

Measured at 125 MHz in $CDCl_3 + CH_3OD$ (8:2).

methyl derivative of *N*-acetylquestiomycin A. Comparison of ^1H NMR spectral data of the two antibiotics revealed that the two aromatic singlet protons on ring C remained unchanged in **I** (δ_H 8.25 and 6.46. *cf.* δ_H 8.37 and 6.41 in *N*-acetylquestiomycin A). Therefore, the hydroxymethyl group must be located on ring A. The aromatic ring protons on ring A were observed at δ_H 7.51 (J=8.5 Hz), 7.56 (J= 8.5 and 2.0 Hz) and 7.75 (J=2.0 Hz). This ABM type pattern suggested that the position of the hydroxymethyl group must be either C-7 or C-8. The C-8 position was favored, because it allowed the lowest field ring A hydrogen to be located at C-9, *peri* to the nitrogen at position 10[2]. The proposed structure of **I** was supported by the close similarity of the chemical shifts of the ring A protons between **I** and an analogous metabolite, acetylmichigazone[3] (Fig. 3).

I showed antifungal activity only against *V. ceratosperma*. The dosage for 50% inhibition of mycelial growth (ED_{50}) was 70 μg/ml. Tested so far, it was inactive against Gram-positive and Gram-negative bacteria, yeasts and other fungi. Detailed biological activities of **I** will be reported elsewhere.

References

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