Communications to the Editor

WS9761 A AND B: NEW NON-STEROIDAL ANDROGEN-RECEPTOR ANTAGONISTS PRODUCED BY A *Streptomyces*

Sir:

We have recently reported WB2838, 3-chloro-4-(2-amino-3-chlorophenyl)-pyrrole, as a non-steroidal androgen-receptor antagonist[1]. In the course of our continuing search for non-steroidal androgen-receptor antagonists from microbial products, strain No. 9761 was found to produce two novel androgen-receptor antagonists, named WS9761 A and B (Fig. 1). In this paper, we describe the fermentation, isolation, physico-chemical properties, structure determination and biological activities of WS9761 A and B.

Strain No. 9761 was isolated from a soil sample collected at Lake Kawaguchi, Yamanashi Prefecture, Japan and was taxonomically designated *Streptomyces* sp. No. 9761. Fermentation of *Streptomyces* sp. No. 9761 were carried out in 30-liter jar fermentors containing 20 liters of a production medium consisting of sucrose 3%, corn starch 1%, Pharmamedia 1%, soybean flour 1%, corn steep liquor 0.5%, $CaCO_3$ 0.3%, Adekanol LG-109 0.05% and Silicone KM-70 0.05% (pH 7.0) at 30°C under aeration of 20 liters/minute and agitation of 200 rpm for 4 days.

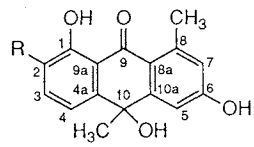
The cultured broth (40 liters) was filtered and the mycelial cake was extracted with 20 liters of acetone. The acetone extract was concentrated under reduced pressure to give 1.5 liters of aqueous solution, adjusted to pH 7.0 and extracted twice with 1.5 liters of ethyl acetate. The ethyl acetate extract was concentrated to give an oily residue, mixed with 200 ml of silica gel and applied to a silica gel (300 ml) column pre-packed with *n*-hexane. After the column was washed with *n*-hexane (1 liter) and *n*-hexane-ethyl acetate (3 : 1, 2 liters), the fractions containing WS9761 A were eluted with *n*-hexane-ethyl acetate (3 : 1, 2 liters). The column was further washed with *n*-hexane-ethyl acetate (1 : 1, 2 liters) and the fractions containing WS9761 B were eluted with ethyl acetate (1 liter). The fractions containing WS9761 A were concentrated to give yellow powders. These yellow powders were recrystallized from *n*-hexane-ethyl acetate to give WS9761 A as yellow crystals (490 mg). The fractions containing WS9761 B were concentrated to give an oily residue, dissolved in 5 ml of dichloromethane-methanol (20 : 1) and applied to a silica gel (120 ml) column pre-packed with dichloromethane-methanol (20 : 1). The column was washed with 360 ml of dichloromethane-methanol (20 : 1) and the fractions containing WS9761 B were eluted with 120 ml of the same solvent system. The fractions containing WS9761 B were concentrated to give yellow powders. These yellow powders were recrystallized from *n*-hexane-ether to give WS9761 B as yellow crystals (99 mg).

WS9761 A and B were soluble in methanol, ethanol, acetone, ethyl acetate, chloroform, dichloromethane and dimethyl sulfoxide, but insoluble in *n*-hexane and water. They showed positive color reactions to iodine vapor, ceric sulfate, sulfuric acid, ferric chloride and Ehrlich reagents, but negative to Ninhydrin, Molisch and Dragendorff reagents. The Rf values of WS9761 A and B on silica gel TLC developed with dichloromethane-methanol (10 : 1) were 0.79 and 0.56, respectively.

WS9761 A (**1**): MP 194°C; $[\alpha]_D^{20}$ −5.5° (*c* 0.25, DMSO); FAB-MS *m/z* 285 $(M+H)^+$; elemental analysis (%) C 72.07, H 5.84, O 22.09 (C 71.82, H 5.67, O 22.51, Calcd for $C_{17}H_{16}O_4$); UV λ_{max}^{MeOH} nm (log ε) 209 (4.38), 220 (sh, 4.30), 230 (sh, 4.10), 247 (3.97), 270 (3.82), 317 (4.17), 340 (4.04); IR ν_{max}^{KBr} cm^{-1} 3270, 1610, 1570, 1465, 1425, 1330, 1270, 1160, 1110, 1060, 1030, 890, 830, 800.

^1H NMR spectrum of **1** (Table 1) revealed the presence of three isolated methyl groups [δ_H 1.45 (3H, s), 2.19 (3H, s) and 2.68 (3H, s)], two sets of coupling spin systems on the aromatic rings [*ortho*-coupling: δ_H 7.23 (1H, d, J=8 Hz) and 7.42 (1H, d, J=8 Hz); *meta*-coupling: δ_H 6.69 (1H, d, J=2 Hz) and 7.26 (1H, d, J=2 Hz)] and three D_2O-exchangeable hydroxyl groups [δ_H 6.02 (1H, s), 10.56 (1H, br s) and 13.45 (1H, s)]. ^{13}C NMR spectrum of **1** (Table 1) showed the presence of 17

Fig. 1. Structures of WS9761 A (**1**) and B (**2**).



WS9761 A (**1**) R=CH_3
WS9761 B (**2**) R=CH_2OH

Table 1. ^{1}H and ^{13}C NMR data for WS9761 A (**1**) and B (**2**) in DMSO-d_6.

Position	δ_H (400 MHz)		δ_C (100 MHz)	
	1	**2**	**1**	**2**
1			159.6 (s)	158.3 (s)
1-OH	13.45 (1H, s)	13.45 (1H, s)		
2			123.6 (s)	128.4 (s)
3	7.42 (1H, d, J=8 Hz)	7.64 (1H, d, J=8 Hz)	136.0 (d)	132.9 (d)
4	7.23 (1H, d, J=8 Hz)	7.33 (1H, d, J=8 Hz)	115.0 (d)	115.0 (d)
4a			148.2 (s)	148.9 (s)
5	7.26 (1H, d, J=2 Hz)	7.26 (1H, d, J=2 Hz)	110.6 (d)	110.6 (d)
6			162.0 (s)	162.0 (s)
6-OH	10.56 (1H, br s)	10.59 (1H, br s)		
7	6.69 (1H, d, J=2 Hz)	6.69 (1H, d, J=2 Hz)	118.6 (d)	118.6 (s)
8			144.1 (s)	144.1 (s)
8a			119.0 (s)	119.0 (s)
9			189.5 (s)	189.5 (s)
9a			113.9 (s)	113.7 (s)
10			69.6 (s)	69.6 (s)
10-OH	6.02 (1H, s)	6.07 (1H, s)		
10a			155.8 (s)	155.8 (s)
1′	2.19 (3H, s)	4.55 (2H, m)	15.0 (q)	57.2 (t)
1′-OH		5.08 (1H, br s)		
1″	1.45 (3H, s)	1.45 (3H, s)	39.4 (q)	39.3 (q)
1‴	2.68 (3H, s)	2.69 (3H, s)	24.0 (q)	24.1 (q)

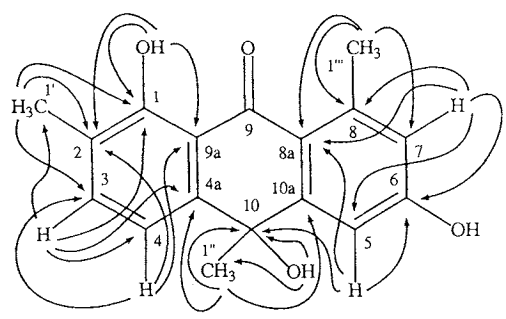
Chemical shifts are given in ppm. Proton intensities, multiplicity and coupling constants are given in parentheses.

carbon signals, including one carbonyl carbon [δ_C 189.5 (s)], 12 aromatic carbons [δ_C 110.6 (d), 113.9 (s), 115.0 (d), 118.6 (d), 119.0 (s), 123.6 (s), 136.0 (d), 144.1 (s), 148.2 (s), 155.8 (s), 159.6 (s) and 162.0 (s)], one quaternary carbon [δ_C 69.6 (s)] and three methyl carbons [δ_C 15.0 (q), 24.0 (q) and 39.4 (q)]. The connection of these partial structures mentioned above was clarified by analyses of long-range ^{13}C-^{1}H coupling relationships derived from COLOC (Fig. 2). The methyl protons at δ_H 1.45 (H-1″) were long-range coupled with the aromatic carbons both at δ_C 148.2 (C-4a) and 155.8 (C-10a). Further, the aromatic proton at δ_H 7.26 (H-5) was long-range coupled with the quaternary carbon at δ_C 69.6 (C-10). Consequently, the structure of WS9761 A (**1**) was determined to be 1,6,10-trihydroxy-2,8,10-trimethyl-10*H*-anthracen-9-one as shown in Fig. 1. The structure as well as ^{1}H NMR spectrum of WS9761 A was similar to those of the hydrogenolytic products of oxanthromicin[2].

WS9761 B (**2**): FAB-MS m/z 301 $(M+H)^+$; elemental analysis (%) C 66.11, H 5.71, O 28.18 (C 66.01, H 5.54, O 28.45, Calcd for $C_{17}H_{16}O_5 \cdot \frac{1}{2}H_2O$).

The structure of **2** was elucidated by comparison of its physico-chemical properties and spectroscopic data with those of **1** (Table 1). The molecular formula of **2** suggested that **2** was the hydroxy

Fig. 2. The ^{13}C-^{1}H long-range coupling relationships from a COLOC spectrum of WS9761 A (**1**).



derivative of **1**. The ^{1}H NMR spectrum of **2** was superimposable on that of **1** except for the protons at δ_H 4.55 (2H, m) and 5.08 (1H, br s) in **2**, which corresponded to those at δ_H 2.19 (3H, s) in **1**. This result showed that 1′-methyl group in **1** was replaced with a hydroxymethyl group in **2**. Thus the structure of WS9761 B (**2**) was determined to be 1,6,10-trihydroxy-2-hydroxymethyl-8,10-dimethyl-10*H*-anthracen-9-one as shown in Fig. 1.

The androgen-receptor antagonistic activities of WS9761 A and B were examined according to the method described previously[1]. WS9761 A and B inhibited androgen-receptor binding in a dose dependent manner, with IC_{50} values of 8.6×10^{-7} M

Table 2. Inhibition of androgen- and estrogen-receptor binding by WS9761 A and WS9761 B.

Drugs	IC_{50} (M)	
	Androgen-receptor	Estrogen-receptor
WS9761 A	8.6×10^{-7}	1.2×10^{-5}
WS9761 B	4.5×10^{-7}	5.7×10^{-6}

and 4.5×10^{-7} M, respectively (Table 2). WS9761 A and B showed weak inhibitory activity against estrogen-receptor binding as shown in Table 2. The IC_{50} values of WS9761 A and B against androgen-receptor binding were 12- to 14-fold lower than those against estrogen-receptor binding. Lineweaver-Burk plot analysis for inhibition of androgen-receptor binding by WS9761 A suggested that it was a competitive inhibitor (data not shown).

The effects of WS9761 A and B on the growth of androgen-responsive mouse mammary carcinoma SC-3 cells *in vitro* were examined. WS9761 B slightly stimulated the growth of SC-3 cells in the absence of testosterone. WS9761 A and B inhibited the growth of SC-3 cells in the presence of 10^{-8} M testosterone in a dose dependent manner, with IC_{50} values of 1.9×10^{-6} M and 3.9×10^{-6} M, respectively (data not shown). Their inhibitory activities against the growth of SC-3 cells were moderately reversed in the presence of 10^{-5} M testosterone. These results suggested that WS9761 A and B were androgen-receptor antagonists.

Further studies on the androgen-receptor antagonistic activities of WS9761 A and B are in progress.

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References

1) HORI, Y.; Y. ABE, H. NAKAJIMA, S. TAKASE, T. FUJITA, T. GOTO, M. OKUHARA & M. KOHSAKA: WB2838 [3-chloro-4-(2-amino-3-chlorophenyl)-pyrrole]: Non-steroidal androgen-receptor antagonist produced by a *Pseudomonas*. J. Antibiotics 46: 1327~1333, 1993
2) WRIGHT, J. J. K.; Y. MERILL, M. S. PUAR & A. T. MCPHAIL: Structure of oxanthromicin (Antibiotic 16-550), a novel dimeric anthrone peroxide. J. Chem. Soc., Chem. Commun. 1984: 473~474, 1984