WS-9659 A AND B, NOVEL TESTOSTERONE 5α-REDUCTASE INHIBITORS ISOLATED FROM A *STREPTOMYCES*

II. STRUCTURAL ELUCIDATION OF WS-9659 A AND B

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On the basis of spectroscopic and chemical evidence, the structures of WS-9659 A and B isolated as inhibitors of testosterone 5α-reductase from a *Streptomyces* have been established as **1** and **2**, respectively. The reductase inhibitory activities of the derivatives **5** and **6**, and degradation products **3** and **8** were considerably less active and substantially inactive, respectively.

Inhibition of the enzyme testosterone 5α-reductase represents a new pharmacological approach toward the treatment of benign prostate hyperplasia which appears to be mediated by 5α-dihydrotestosterone[1]. As part of a program aimed at the discovery of inhibitors of this enzyme, we isolated WS-9659 A (**1**) as a potent and specific inhibitor, together with WS-9659 B (**2**) as a minor product, from *Streptomyces* sp. No. 9659[2]. Herein we report the structural elucidation of these new natural products. The biological activities of the derivatives and degradation products obtained from **1** during the elucidation are also described.

The major species WS-9659 A (**1**) was isolated as deep blue prisms (mp 161~162°C). The molecular formula ($C_{22}H_{24}N_2O$) was established by elementary analysis and fast atom bombardment (FAB)-MS. The ^{13}C NMR spectrum ($CDCl_3$) of **1** showed 22 carbon signals, of which 14 carbons were observed in the sp^2 region (δ 179.7 (s), 147.4 (s), 143.2 (d), 135.8 (s), 135.1 (s), 134.6 (d), 133.9 (d), 133.7 (s), 131.0 (s), 124.4 (d), 121.3 (s), 117.2 (d), 113.3 (d), 91.5 (d)) and the remainder (eight carbons) in the sp^3 region (δ 49.6 (t), 46.3 (t), 34.9 (t), 28.9 (s), 28.0 (q, 2×C), 22.8 (t), 19.4 (q)).

The ^1H NMR analysis ($CDCl_3$) of **1**, together with the ^1H-^1H correlation spectroscopy (COSY) experiment, revealed two sets of serially connected aromatic protons (four protons at δ 8.33, 7.46, 7.73, 7.46; three protons at δ 6.55, 7.56, 5.86) (Table 1).

The ^1H NMR spectrum further showed three methyls at δ 0.82 (2×CH_3) and 1.89 (1×CH_3) and four methylenes at δ 1.20, 1.55, 1.89 and 5.03.

On treatment with 1 N NaOH (room temperature, 30 minutes), **1** was easily decomposed to two products **3** and **4**†. The former was identified to be 1-hydroxyphenazine by inspection of its physical data (see Experimental section) and finally by comparison with an authentic sample. The other product was found to be a tetra-substituted, α,β-unsaturated aldehyde (IR ($CHCl_3$) cm^{-1} 1660; δ_c 191.0 (s),

† In the alkaline treatment, **1** suffered an air oxidation, yielding **3** and **4**. In the literature[3], a similar oxidation had also been reported on pyocyanine (**9**), which gave 1-hydroxyphenazine and formic acid upon treatment with alkaline.

Table 1. ^{1}H NMR (400 MHz, $CDCl_3$) chemical shifts, multiplicities and coupling constants (J, Hz) for WS-9659 A (**1**), B (**2**) and **5**.

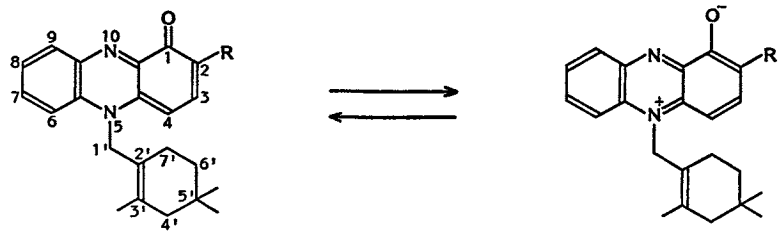
	1	2	5
2-H	6.55 (d, 9.2) (or 5.86)	——	6.85 (d, 8.5)
3-H	7.56 (dd, 9.2, 7.8)	7.75 (d, 8.0)	7.20 (t, 8.0)
4-H	5.86 (d, 7.8) (or 6.55)	5.89 (d, 8.0)	6.81 (d, 8.5)
6-H	7.46 (m) (or 8.33)	7.58 (d, 8.0)	6.91 (d, 8.0)
7-H	7.73 (ddd, 8.8, 7.2, 1.5) (or 7.46)	7.83 (t, 8.0)	7.18 (t, 7.5)
8-H	7.46 (m) (or 7.73)	7.55 (t, 8.0)	7.04 (t, 7.5)
9-H	8.33 (dd, 8.5, 1.5) (or 7.46)	8.40 (d, 8.0)	7.34 (br s)
1′-H	5.03 (2H, br s)	5.12 (2H, br s)	4.49 (2H, br s)
4′-H	1.89 (2H, br s)	1.51 (2H, br s)	1.81 (br s)
6′-H	1.20 (2H, t, 6.5)	1.22 (2H, t, 6.5)	1.23 (2H, t, 6.2)
7′-H	1.55 (2H, unresolved)	1.90 (2H, br s)	1.81 (2H, br s)
3′-CH_3	1.89 (3H, s)	1.93 (3H, s)	1.85 (3H, s)
5′-CH_3	0.82 (6H, s)	0.85 (6H, s)	0.81 (6H, s)
10-$COCH_3$	——	——	2.13 (3H, s), 2.35 (3H, s)

154.7 (s), 132.3 (s); δ_H 10.17 (1H, s). The NMR spectra further revealed the presence of two geminal methyls (δ_c 28.0 (q, 2×C); δ_H 0.92 (6H, s)), one methyl linked to an olefine (δ_c 18.4 (q); δ_H 2.12 (3H, s)), and three methylenes (δ_c 48.1 (t), 34.4 (t), 20.0 (t); δ_H 2.23 (2H, br s), 2.00 (2H, br s), 1.38 (2H, t, J=6.7 Hz)). These data were identical with those of β-cyclolavandulal (**4**)[4]. The aldehyde was thus determined to be **4**.

The problem remaining for the full structure of **1** is to link 1-hydroxyphenazine (**3**) and β-cyclolavandulal (**4**). This was accomplished by nuclear Overhauser effect (NOE) studies on the derivatives of **1** as follows. Acetylation of **1** with Ac_2O in pyridine gave diacetates **5** and **6**, together with β-cyclolavandulal (**4**), 1-acetoxyphenazine (**7**) and 5,10-dihydro-1-hydroxyphenazine diacetate (**8**) (**4** and **8** were probably formed by hydrolysis of **6** during the workup). In the ^{1}H NMR spectrum ($CDCl_3$) of **5**, three aromatic protons adjacent to each other were observed at δ 6.81, 7.20 and 6.85 (Table 1). Irradiation of the methylene protons (δ 4.49) assignable to 1′-H (2H) enhanced the intensities of the signal at δ 6.81 (which was thereby assigned to 4-H; hence δ 7.20 to 3-H and δ 6.85 to 2-H), indicating that the β-cyclolavandulal substituent is linked to N-5. The aromatic proton at δ 6.91, which is one of the four serially connected protons (the others: δ 7.18 (7-H), 7.04 (8-H) and 7.34 (9-H)), was also increased in intensity on irradiation of 1′-H, being assigned to 6-H. A NOE experiment was also carried out on compound **6** and the result showed NOE's between 1′-H (δ 6.24, s) and 4-H (δ 6.85, d, J=8.2 Hz), and 1′-H and 6-H (δ 6.96, d, J=8.2 Hz), leading to the same conclusion as described above. In this latter experiment, a NOE was also observed between 1′-H and 3′-CH_3 (δ 2.06, s), revealing that the 1′,2′-double bond in **6** is *E*. The structure of WS-9659 A was thus assigned to be **1**.

The minor species WS-9659 B (**2**) was isolated as deep blue prisms (mp 152~153°C). The molecular formula, $C_{22}H_{23}N_2OCl$ (elemental analysis and FAB-MS), showed that **2** is a chloro derivative of **1**. The ^{1}H NMR spectrum ($CDCl_3$) of **2** showed a signal pattern similar to that of **1** (Table 1). An exception is that the proton signal corresponding to that at δ 6.55 (probably 2-H) in **1** was not observed in **2**. A NOE study on **2** showed that irradiation of 1′-H (δ 5.12) enhanced the intensities of the protons at δ 5.89 (4-H) and 7.58 (6-H) as in the case of **5**. These facts suggested that the chlorine is located at the 2-position in **2**. The structure of WS-9659 B was thus presumed to be **2**. These natural products, WS-9659 A and B may exist as a mixture of tautomers **a** and **b** as depicted in Fig. 1.

Fig. 1. The numbering of **1** and **2**.



a b

1 R=H
2 R=Cl

Each of the compounds derived from **1** was evaluated *in vitro* for inhibition of testosterone 5α-reductase and the results were given in comparison with those of WS-9659 A (**1**), B (**2**) and pyocyanine (**9**) in Table 2. All the derivatives and degradation products were found to be considerably less potent or substantially inactive. It is notable, however, that pyocyanine showed an activity of the same order as **1**.

Table 2. Inhibition of testosterone 5α-reductase.

Compound	IC_{50} (M)	Compound	IC_{50} (M)
3	1×10^{-3}	**8**	$>1\times10^{-3}$
4	$>1\times10^{-3}$	**1**	5×10^{-7}
5	6×10^{-5}	**2**	1×10^{-5}
6	2×10^{-5}	**9**	5×10^{-7}

Experimental

IR spectra were recorded on a Jasco A-102 spectrophotometer. ^{1}H and ^{13}C NMR spectra were measured on a Bruker AM400WB spectrometer. The chemical shifts are given in ppm (δ) relative to internal TMS. UV spectra were measured on a Hitachi 220A spectrophotometer. Electron impact (EI)- and FAB-MS were recorded using a VG ZAB-SE mass spectrometer. MP's were measured with a Yanagimoto microscope hot-stage apparatus and are uncorrected. Preparative TLC (PTLC) was performed on pre-coated Silica gel 60 F_{254} plates (E. Merck). Biological assay was carried out according to the method described in the preceding paper of this series.

WS-9659 A (**1**): Deep blue prisms; mp 161~162°C.

Anal Calcd for $C_{22}H_{24}N_2O$: C 79.48, H 7.28, N 8.43.
Found: C 79.13, H 7.13, N 8.40.

FAB-MS: *m/z* 333 (M+H); IR ($CHCl_3$) cm^{-1} 1665, 1630, 1600, 1560; UV λ_{max}^{MeOH} nm 238, 320; ^{13}C NMR ($CDCl_3$) δ 179.7 (s), 147.4 (s), 143.2 (d), 135.8 (s), 135.1 (s), 134.6 (d), 133.9 (d), 133.7 (s), 131.0 (s), 124.4 (d), 121.3 (s), 117.2 (d), 113.3 (d), 91.5 (d), 49.6 (t), 46.3 (t), 34.9 (t), 28.9 (s), 28.0 (q, 2×C), 22.8 (t), 19.4 (q).

WS-9659 B (**2**): Deep blue prisms; mp 152~153°C.

Anal Calcd for $C_{22}H_{23}N_2OCl$: C 72.02, H 6.32, N 7.64, Cl 9.66.
Found: C 71.82, H 6.37, N 7.86, Cl 9.60.

FAB-MS *m/z* 367 (M+H); IR ($CHCl_3$) cm^{-1} 1663, 1630, 1605, 1575, 1565; UV λ_{max}^{MeOH} nm 241, 328.

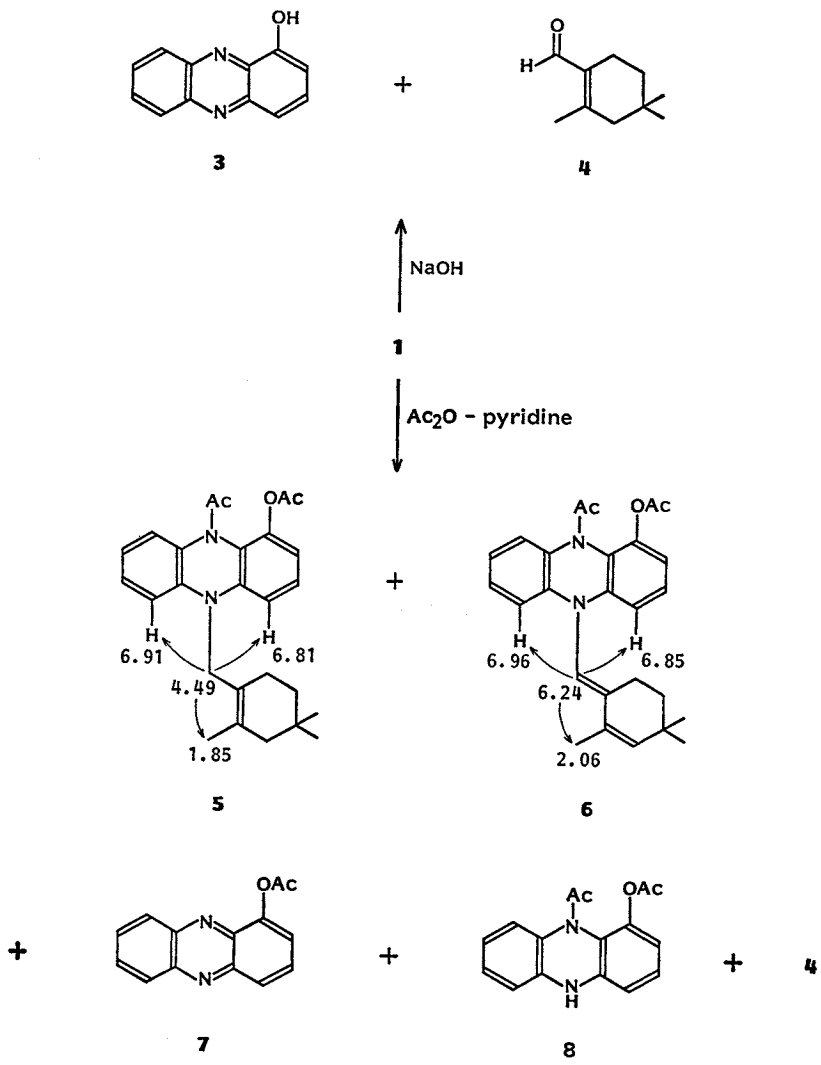
Treatment of **1** with 1 N NaOH

To a solution of **1** (50 mg) in MeOH (2 ml) was added 1 N NaOH (1 ml) and the mixture was stirred for 30 minutes at room temperature. After acidification to pH 1 with 1 N HCl, the mixture was extracted with EtOAc, dried over $MgSO_4$ and evaporated *in vacuo* to give an oily residue, which was purified by PTLC developing with $CHCl_3$ to give **3** (15 mg) and **4** (7.3 mg).

1-Hydroxyphenazine (**3**): MP 159~160°C; FAB-MS; *m/z* 197 (M+H); ^{1}H NMR ($CDCl_3$) δ 7.24 (1H, dd, J=6 and 2 Hz), 7.79 (2H, m), 7.86 (2H, m), 8.25 (2H, m); IR ($CHCl_3$) cm^{-1} 1640, 1560, 1520.

β-Cyclolavandulal (**4**): Oil; EI-MS *m/z* 152 (M^+); ^{1}H NMR ($CDCl_3$) δ 0.92 (6H, s), 1.38 (2H,

Fig. 2. Treatment of **1** with 1 N NaOH and acetylation of **1**.



t, J=6.7 Hz), 2.00 (2H, br s), 2.12 (3H, s), 2.23 (2H, br s), 10.17 (1H, s); ^{13}C NMR ($CDCl_3$) δ 191.0 (s), 154.7 (s), 132.3 (s), 48.1 (t), 34.4 (t), 28.7 (s), 28.0 (q, 2×C), 20.0 (t), 18.4 (q); IR ($CHCl_3$) cm^{-1} 1660, 1635.

Acetylation of **1**

To a solution of **1** (50 mg) in pyridine (1 ml) was added Ac_2O (100 μl) and the mixture was stirred for 60 hours at room temperature. After evaporation of the mixture *in vacuo*, the residue was purified by PTLC developing with hexane - $CHCl_3$ (1 : 1) to give **4** (7.4 mg) and **8** (8.3 mg). PTLC was repeated on the other fractions and developed with hexane - $CHCl_3$ - EtOAc (4 : 4 : 1) to give **7** (6.9 mg). The other fractions were further developed on TLC with CH_2Cl_2 to give **5** (9.7 mg) and **6** (3.9 mg).

Diacetate **5**: Oil; EI-MS m/z 418 (M^+); IR ($CHCl_3$) cm^{-1} 1765, 1670, 1610, 1585.

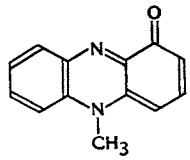
Diacetate **6**: Oil; EI-MS m/z 416 (M^+); ^1H NMR ($CDCl_3$) δ 1.04 (6H, s), 1.43 (2H, t, J=6.5 Hz), 2.06 (3H, s), 2.19 (3H, s), 2.27 (2H, t, J=6.5 Hz), 2.37 (3H, s), 5.60 (1H, s), 6.24 (1H, s), 6.85 (1H, d, J=8.2 Hz), 6.88 (1H, d, J=8.2 Hz), 6.96 (1H, d, J=8.2 Hz), 7.07 (1H, t, J=7.5 Hz), 7.19 (1H, t, J=8.2 Hz), 7.20 (1H, t, J=8.2 Hz), 7.38 (1H, br d, J=7.5 Hz); IR ($CHCl_3$) cm^{-1} 1765, 1670, 1605,

1590.

1-Acetoxyphenazine (**7**): Oil; EI-MS *m/z* 238 (M^+); ^1H NMR ($CDCl_3$) δ 2.61 (3H, s), 7.58 (1H, dd, J=7.5 and 1.5 Hz), 7.83 (3H, m), 8.17 (1H, dd, J=8.5 and 1.5 Hz), 8.26 (2H, m); IR ($CHCl_3$) cm^{-1} 1765, 1630, 1600.

5,10-Dihydro-1-hydroxyphenazine Diacetate (**8**): Oil; EI-MS *m/z* 282 (M^+); ^1H NMR ($CDCl_3$) δ 2.16 (3H, s), 2.32 (3H, s), 6.19 (1H, br s), 6.70 (1H, dd, J=8 and 1.5 Hz), 6.8 (2H, m), 7.0 (1H, dt, J=1.5 and 7 Hz), 7.11 (1H, dt, J=1.5 and 7 Hz), 7.13 (1H, t, J=8 Hz), 7.35 (1H, br d, J=8 Hz); IR ($CHCl_3$) cm^{-1} 1770, 1675, 1470.

Fig. 3. Structure of pyocyanine (**9**).



Addendum in Proof

WS-9659 A is identical to YP-0298L-C which was isolated independently by T. SATO, *et al.* (Jpn. Kokai 280,073 ('88), Nov. 17, 1988)

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