PF1092A, B and C, New Nonsteroidal Progesterone Receptor Ligands Produced by *Penicillium oblatum*

II. Physico-chemical Properties and Structure Elucidation

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The structures of PF1092A (**1**), B (**2**) and C (**3**), new nonsteroidal progesterone receptor ligands produced by *Penicillium oblatum*, were elucidated by spectroscopic analyses. These compounds possess an eremophilane-type sesquiterpene carbon skeleton and differ only in that **1** and **2** are different monoacetates of **3**. The absolute configurations of **1**~**3** were determined by single crystal X-ray diffraction analysis of the 4-bromobenzoyl ester of PF1092A and by measuring the optical rotations of the acetylation products of these compounds.

PF1092A (**1**), B (**2**) and C (**3**) were isolated as new progesterone receptor ligands from the mycelia of cultured *Penicillium oblatum* PF1092. These compounds inhibit [^{3}H]-progesterone binding to the progesterone receptor. In the preceding paper, we described the taxonomy and the fermentation of the producing strain and isolation and the biological activities of these compounds[1]. The physico-chemical properties and elucidation of structures of **1**, **2** and **3** are presented in this paper.

Results

Physico-chemical Properties

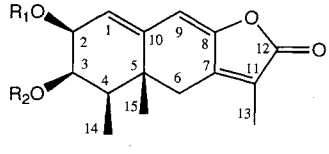
The physico-chemical properties of PF1092A (**1**), B (**2**) and C (**3**) are summarized in Table 1. The HR-FAB-MS and NMR data established the molecular formulae of **1**, **2** and **3** as $C_{17}H_{20}O_5$, $C_{17}H_{20}O_5$ and $C_{15}H_{18}O_4$, respectively. Each compound exhibited IR absorption peaks characteristic of hydroxyl (A and C; 3476 cm^{-1} or B; 3537 cm^{-1}) and lactone or ester carbonyl (A; 1765, 1736 and 1726 cm^{-1}, B; 1765, 1747 and 1732 cm^{-1} and C; 1765 and 1716 cm^{-1}). The UV spectra of these compounds indicated the existence of the same conjugated system in all three molecules.

Structure of PF1092A (1)

The ^{13}C and ^{1}H NMR spectra of **1** revealed the presence of four methyl groups, one methylene, one methine, two oxymethine, one quaternary carbon, six olefinic carbons and two carbonyl carbons (Table 2). The signals of six olefinic carbons between δ 107.7~149.3 indicated the presence of three double bonds in **1**. These and the two carbonyl carbons account for five degrees of unsaturation, so the remaining three degrees of unsaturation should be due to the presence of three rings in the molecule.

Detailed 1D and 2D-NMR studies led to the establishment of the connectivity of the above-mentioned structural fragment and functional groups. A C-H COSY experiment revealed all one-bond ^{1}H-^{13}C connectivities as shown in Table 2. A COSY experiment indicated the partial structure illustrated by a bold line in Fig. 2. The

Fig. 1. Structures of PF1092A (**1**), B (**2**), C (**3**) and their derivatives (**4** and **5**).



	R_1	R_2
1	H	$COCH_3$
2	$COCH_3$	H
3	H	H
4	$COCH_3$	$COCH_3$
5	4-Bromobenzoyl	$COCH_3$

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Table 1. Physico-chemical properties of PF1092A (**1**), B (**2**) and C (**3**).

	PF1092A (1)	PF1092B (2)	PF1092C (3)
Appearance	Colorless needles	Colorless needles	Colorless needles
MP	192-194℃ (decomposition)	176-177℃ (decomposition)	171-172℃ (decomposition)
$[\alpha]_D^{24}$ (c 0.5, $CHCl_3$)	−10.86°	−110.22°	−96.36°
Molecular fomula	$C_{17}H_{20}O_5$	$C_{17}H_{20}O_5$	$C_{15}H_{18}O_4$
HR-FAB-MS (m/z) $(M+H)^+$			
Found:	305.1361	305.1368	263.1258
Calcd:	305.1389	305.1389	263.1283
UV λ_{max} nm (ε)	265 (7,100, sh), 322 (17,500)	265 (6,500, sh), 320 (15,100)	265 (6,300, sh), 324 (14,900)
IR (KBr) ν_{max} cm^{-1}	3476, 1765, 1736, 1726	3537, 1765, 1747, 1732	3476, 1765, 1716
Solubility	Soluble in DMSO, acetone, EtOAc, MeOH, $CHCl_3$		
	Insoluble in H_2O, hexane		
TLC[a] (Rf)	0.25	0.42	0.20
HPLC[b] (Rt)	3.9	4.9	3.3

a: Merck art. No.5715 (hexane-EtOAc, 1:1)

b: Capcell pak C_{18} (Shiseido, Co.Ltd.), 50% aq CH_3CN, 0.8 ml/min., detection: 320 nm (UV)

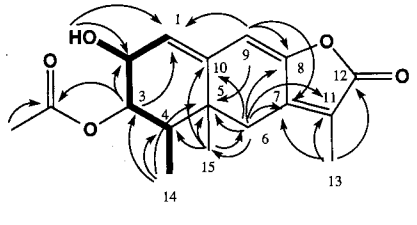
Table 2. ^1H and ^{13}C NMR chemical shifts of PF1092A (**1**), B (**2**) and C (**3**) in $CDCl_3$.

	PF1092A (1)		PF1092B (2)		PF1092C (3)	
Position	^{13}C	^1H	^{13}C	^1H	^{13}C	^1H
1	128.7	5.66	124.5	5.57	129.1	5.64
2	68.2	4.53	72.5	5.46	69.0	4.37
2-OH	-	2.05	-	-	-	2.45
2-CO-CH_3	-	-	170.2	-	-	-
2-CO-CH_3	-	-	21.4	2.15	-	-
3	73.6	5.28	70.5	4.05	72.4	3.93
3-OH	-	-	-	1.97	-	2.39
3-CO-CH_3	171.8	-	-	-	-	-
3-CO-CH_3	20.9	2.19	-	-	-	-
4	40.2	1.97	41.0	1.83	41.3	1.78
5	37.8	-	38.1	-	37.9	-
6	35.6	2.18, 2.83	35.8	2.18, 2.84	35.8	2.17, 2.83
7	146.3	-	146.7	-	146.7	-
8	149.3	-	149.5	-	149.3	-
9	107.7	5.97	107.4	5.95	107.8	5.96
10	140.4	-	142.4	-	141.0	-
11	122.3	-	122.4	-	122.1	-
12	170.9	-	170.9	-	171.0	-
13	8.6	1.91	8.6	1.91	8.5	1.91
14	12.7	1.10	12.8	1.23	13.0	1.24
15	21.0	1.15	21.2	1.22	21.5	1.20

proton signal (2-OH, δ_H 2.05) had no one-bond ^1H-^{13}C correlation. The hydroxyl group was shown to be linked to the C-2 position by the coupling of the proton (2-OH) with the H-2 methine proton (δ_H 4.53). This was confirmed by the following experiment. Treatment of **1** with acetic anhydride gave an acetyl derivative (**4**). In the ^1H NMR spectrum of **4**, an additional acetyl methyl singlet was observed, and the H-2 oxymethine signal (δ_H 5.56) was shifted downfield.

The heteronuclear multiple-bond correlation (HMBC)[2] spectrum of **1** revealed almost all the ^{13}C and ^1H long-

Fig. 2. COSY and HMBC correlations for PF1092A (**1**).



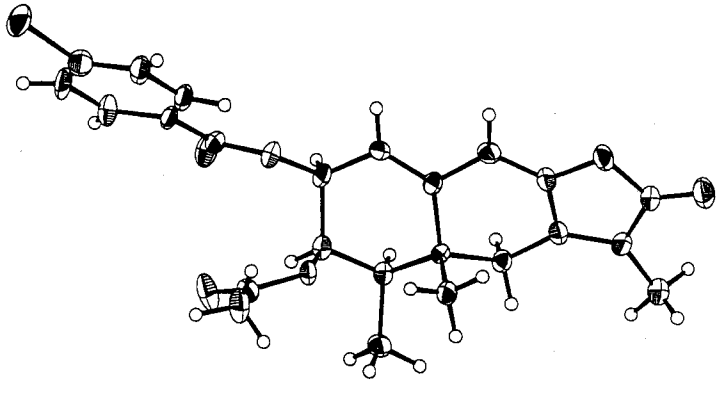
range correlations, which are summarized in Fig. 2. In spite of the presence of a number of quaternary carbons, the connectivity of the molecule was elucidated by analyses of the above-mentioned correlations. A sharp methyl singlet at δ_H 2.19 showed long-range coupling to the carbonyl carbon at δ_C 171.8, which indicated the presence of an acetyl group in **1**. The attachment of the acetyl group to the oxy-methine carbon C-3 (δ_C 73.6) to form an acetyl ester was indicated by the long-range correlation between the H-3 oxymethine proton at δ_H 5.28 and the acetyl carbonyl carbon. The doublet methyl proton H-14 at δ_H 1.10 showed long-range coupling with the quaternary carbon C-5 at δ_C 37.8, the oxymethine C-3 and C-4 at δ_C 40.2, which revealed the attachment of C-4 and C-5. The methyl proton H-15 at δ_H 1.15 had long-range correlations with four carbons (C-4, C-5, C-6 at δ_C 35.6 and C-10 at δ_C 140.4). Considering the chemical shift value and proton-proton coupling pattern of H-15 ($J=0.8$ Hz with H-6b), the C-15 methyl group must be connected to the aliphatic quaternary carbon C-5. The remaining methyl proton (H-13 at δ_H 1.91) showed long-range couplings with two olefinic carbons (C-7 at δ_C 146.3 and C-11 at δ_C 122.3) and one carbonyl carbon (C-12 at δ_C 170.9). The attachment of the methyl group C-13 to the olefinic quaternary carbon C-11 was indicated by the above observation and the proton-proton long-range correlation between H-13 ($J=2$ Hz) and H-6b (δ_H 2.18). The methylene group (C-6, δ_C 35.6) was placed between C-5 and the olefinic carbon (C-7, δ_C 146.3), because the 6-Ha signal at δ_H 2.83 showed long-range correlations to six carbon signals (C-5, C-15, C-10 at δ_C 140.4, C-8 at δ_C 149.3, C-7 and C-11 at δ_C 122.3). The COSY spectrum of **1** showed long-range coupling between two olefinic protons (H-1 at δ_H 5.66 and H-9 at δ_H 5.97) and the HMBC spectrum of **1** indicated long-range coupling between H-9 and four carbon signals (C-1, C-5, C-8 and C-7). The above observation suggested the connection of C-1 with C-9 *via* the olefinic carbon C-10 to form a decalin ring structure in **1**. The remaining bond of the quaternary carbon C-8 must be connected to the carbonyl carbon C-12 to form a γ-lactone ring. Thus, the gross structure of PF1092A (**1**) was deduced to be 3-acetoxy-2-hydroxyeremophil-1(10), 7(11), 8(9)-triene-12(8)-olide.

The absolute configuration of **1** was determined by a single X-ray diffraction analysis of the 4-bromobenzoyl ester of PF1092A (**5**). Treatment of **1** with 4-bromobenzoyl chloride followed by crystallization of the product from MeOH solution gave colorless prisms which were suitable for X-ray diffraction analysis. The molecular structure of **5** calculated from the crystallographic measurements is shown in Fig. 3. The absolute configuration of **1** was determined to be 3β-acetoxy-2β-hydroxyeremophil-1(10), 7 (11), 8 (9)-triene-12 (8)-olide, as shown in Fig. 1.

Structure of PF1092B (2)

The physico-chemical properties of **1** and **2** were almost identical. These two compounds have the same molecular formula. Slight differences were observed in the NMR spectra of **1** and **2**. The downfield shift of H-2 (δ_H 5.46) and upfield shift of H-3 (δ_H 4.05) indicated the presence of an acetyl group connected to the oxymethine carbon C-2 (δ_C 72.5) and the hydroxyl group attached to C-3 (δ_C 70.5) is free. Thus, the structure of PF1092B (**2**) was determined to be 2-acetoxy-3-hydroxyeremophil-1(10),7(11),8(9)-triene-12(8)-olide. This was confirmed by acetylation of **2**. Treatment of **2** with acetic anhydride and dimethylaminopyridine gave the acetyl derivative (**4**), and the physico-chemical properties of **4** were identical with those of the acetylation product of PF1092A (**1**), including specific rotation ($[\alpha]_D^{24}$ (c 0.4, $CHCl_3$) acetate

Fig. 3. Molecular structure of the 4-bromobenzoyl ester of PF1092A.



of **2** $-23.8°$, acetate of **1** $-24.0°$). Thus, the structure and absolute configuration of PF1092B was determined to be 2β-acetoxy-3β-hydroxyeremophil-1(10),7(11),8(9)-triene-12(8)-olide as shown in Fig. 1.

Structure of PF1092C (**3**)

In the ^{1}H NMR spectrum of **3**, two hydroxyl proton signals (2-OH δ_H at 2.45 and 3-OH at δ_H 2.39) were observed, but no acetyl methyl signal was present. The oxymethine proton H-3 (δ_H 3.93) of **3** was shifted upfield compared to that of **1**. The other proton signals of **3** were almost identical with those of **1**. These observations indicated the absence of the acetyl group from the C-3 oxymethine carbon and the presence of a free hydroxyl group attached to the oxymethine in **3**. These results were confirmed by acetylation of **3**; the acetylation product of **3** was identical with that of **1**. Thus, the structure and absolute configuration of **3** was determined to be 2β,3β-dihydroxyeremophil-1(10),7(11),8(9)-triene-12(8)-olide as shown in Fig. 1.

Discussion

PF1092A, B and C are new progesterone receptor ligands isolated from mycelia of cultured *Penicillium oblatum*. They have a common eremophilane-type sesquiterpene carbon skeleton. Among the known eremophilenolides, the structure of ligularenolide[3], a sesquiterpene lactone isolated from roots of a Ligularia species, is closely related to that of PF1092A, B and C. Various microbial eremophilane-type sesquiterpenoids[4] have been reported, but as far as we know, PF1092A, B and C are the first eremophilenolides found to have progesterone receptor binding activity. Recently, a marine natural product, cyclocymopol monomethyl ether, was reported to be a nonsteroidal progesterone receptor antagonist[5]. However, PF1092A, B and C are the first progesterone receptor ligands found to be produced by a microorganism. Now lead optimization studies of PF1092 are in progress through total synthesis[6] and semisynthetic work in our research center.

Experimental

General

UV and IR spectra were recorded on Shimadzu UV-260 and Shimadzu FTIR-8100 spectrophotometers, respectively. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL JNM-GSX 400 spectrometer. SI and FD mass spectra were recorded with a Hitachi M-80B mass spectrometer, and HR-FAB mass spectra were measured on a JEOL JMS-700 mass spectrometer. Optical rotations were measured on a JASCO DIP-370 digital polarimeter using a 10 cm cell. Melting points were measured on a Yanaco MP-S3 micro melting point apparatus without correction. TLC was done on Silica gel 60F254 plates (Merck, Art. No. 5715).

Acetylation of PF1092A, B and C

Acetic anhydride (0.2 ml) was added to a solution of PF1092A (5.6 mg) in pyridine (0.2 ml) and the mixture was stirred for 1 hour at room temperature, then concentrated to dryness. The residual solid was purified by preparative TLC with a mixture of hexane and ethyl acetate (1 : 1) to give **4** as a colorless solid (5.6 mg): $[\alpha]_D^{24}$ (c 0.4, $CHCl_3$) $-24.0°$, SI-MS; m/z 347 $[M+1]^+$, UV (MeOH); λ_{max} (nm) (ε) 265 (sh, 10,000) and 319 (24,000), IR; (KBr) ν cm^{-1} 1759 and 1741, ^{1}H NMR ($CDCl_3$, δ_H); 5.97 (1H, br s), 5.59 (1H, m), 5.56 (1H, m), 5.36 (1H, m), 2.86 (1H, d, 16.2 Hz), 2.21 (1H, br d, 16.2 Hz), 2.10 (3H, s), 2.03 (1H, m), 2.02 (3H, s), 1.91 (3H, d, 1.8 Hz), 1.18 (3H, s) and 1.09 (3H, d, 7.2 Hz).

Acetic anhydride (0.2 ml) was added to a solution of PF1092B (5.1 mg) in pyridine (0.2 ml) and the mixture was stirred for 1 hour at room temperature. Paradimethylaminopyridine (DMAP, 4 mg) was added, and the reaction mixture was stirred for 1 hour, then concentrated to dryness. The residual solid was purified by preparative TLC with a mixture of hexane and ethyl acetate (1 : 1) to give **4** as a colorless solid (5.7 mg): $[\alpha]_D^{24}$ (c 0.4, $CHCl_3$) $-23.8°$, SI-MS and ^{1}H NMR data were identical with those of **4** from PF1092A.

Acetic anhydride (0.3 ml) was added to a solution of PF1092C (20 mg) in pyridine (0.3 ml) and the mixture was stirred for 1 hour at room temperature. DMAP (4 mg) was added, and the reaction mixture was stirred for another one hour. It was purified by preparative TLC as above to give **4** as colorless needles (23.4 mg): $[\alpha]_D^{24}$ $-25.4°$, SI-MS and ^{1}H NMR data were identical with those of **4** from PF1092A.

4-Bromobenzoyl Ester of PF1092A (**5**)

4-Bromobenzoyl chloride (11 mg) was added to a solution of PF1092A (10 mg) in pyridine and the mixture was stirred for 2.5 hours at room temperature. Toluene (10 ml) was added and the whole was concentrated to dryness. The residual solid was purified by preparative TLC using a mixture of hexane and ethyl acetate (1 : 1). Crystallization of the product from MeOH solution gave colorless prismatic crystals (7.6 mg); SI-MS m/z 487 $[M+1]^+$; ^{1}H NMR ($CDCl_3$, δ_H) 7.84 (2H, d, 8.6 Hz), 7.58 (2H, d, 8.6 Hz), 6.00 (1H, br s), 5.80 (1H, m), 5.69 (1H, br s), 2.89 (1H d, 16.2 Hz), 2.25 (1H, br d, 16.2 Hz), 2.11 (1H, m), 2.07 (3H, s), 1.94 (3H, d, 1.5 Hz), 1.22 (3H, s) and 1.13 (3H, d, 6.9 Hz).

Single-crystal X-Ray Diffraction Analysis of the 4-Bromobenzoyl Ester of PF1092A (**5**)

A colorless prismatic crystal of $C_{24}H_{23}O_6Br$ having approximate dimensions of $0.2 \times 0.2 \times 0.15$ mm was mounted on a glass fiber. All measurements were made on a Rigaku AFC5R diffractometer with graphite-monochromated Cu-Kα radiation and a 3 kw rotating

anode generator. Cell constants and the orientation matrix for data collection were obtained from a least-squares refinement using the setting angles of 20 reflections in the 2θ range of $25\sim35°$.

The crystal system was monoclinic, space group $P2_1$(#4) with unit cell dimensions a=12.792(2) Å, b=6.632(1) Å, c=13.2103(6) Å, V=1082.1(2) Å^3, Z=2 and D_{calc}=1.496 g/cm^3. The data were collected using the ω-2θ scan mode to a maximum 2θ value of 120°. Scans of $(1.15+0.3\tan\theta)°$ were made at a speed of 16.0°/minute in omega. Stationary background counts were recorded on each side of every reflection. The ratio of peak counting time to background counting time was 2:1. The diameter of the incident beam collimator was 0.5 mm, the distance from the crystal to the detector was 285 mm and the detector aperture was 6.0×6.0 mm (horizontal×vertical).

A total of 1859 reflections were collected, of which 1775 were unique (R_{int}=0.033); equivalent reflections were merged. No decay correction was applied. The linear absorption coefficient, μ, for Cu-Kα radiation is 29.2 cm^{-1}. Azimuthal scans of several reflections indicated no need for an absorption correction.

The structure was solved by direct methods (SHELXS)[7] and expanded using Fourier techniques (DIRDIF)[8]. The non hydrogen atoms were refined anisotropically. Hydrogen atoms were included, but not refined. The final cycle of full-matrix least-squares refinement was based on 1566 observed reflections ($I>3\sigma(I)$) and 279 variable parameters and converged with unweighted and weighted agreement factors of R=0.062 and R_w=0.081. The maximum and minimum peaks on the final difference Fourier map corresponded to 0.83 and $-1.39e^-$/Å^3, respectively. Comparing $|Fobs(hkl)|-|Fobs(\bar{h}\bar{k}\bar{l})|$ and $|Fcalc(hkl)|-|Fcalc(\bar{h}\bar{k}\bar{l})|$ for the largest 15 Friedel pairs showed consistently the absolute configuration depicted in Fig. 3. The above-mentioned Friedel pairs were measured with a slow scan speed (4°/minute). All calculations were performed using the teXsan crystallographic software package of Molecular Structure Corporation.

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