Louisianins A, B, C and D: Non-steroidal Growth Inhibitors of Testosterone-responsive SC 115 Cells

II. Physico-chemical Properties and Structural Elucidation

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(Received for publication April 24, 1995)

New non-steroidal growth inhibitors of testosterone-responsive SC 115 cells, louisianins A (MW: 189; $C_{11}H_{11}NO_2$), B (MW: 191; $C_{11}H_{13}NO_2$), C (MW: 173; $C_{11}H_{11}NO$) and D (MW: 173; $C_{11}H_{11}NO$) were isolated from the cultured broth of *Streptomyces* sp. WK-4028. Their structures were determined on the basis of spectroscopic data. The structure of louisianin A in particular was confirmed by X-ray crystallographic analysis. The four compounds commonly possess a unique pyrindine skeleton in the molecule.

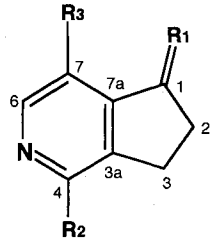
Androgens play an important role in the normal and abnormal differentiation and growth of the mammalian prostate. Abnormal growth can be manifested as benign prostatic hyperplasia or prostatic cancer. Steroidal antiandrogens have been used to atandrogen-dependent disease states, but the significant side effects of theses drugs pose serious problems. Therefore, more effective non-steroidal antiandrogens need to be developed. In the course of a search for non-steroidal antiandrogens of microbial origin, we have reported a testosterone 5α-reductase inhibitor, 8′,9′-dehydroascochlorine, discovered from the cultured mycelium of *Verticillium* sp. FO-2787. During the course of new screening utilizing testosterone-responsive mouse mammary carcinoma SC 115 cells, we isolated louisianins A (**1**), B (**2**), C (**3**) and D (**4**) from the cultured broth of *Streptomyces* sp. WK-4028, which had been isolated from a soil sample collected in the state of Louisiana, U.S.A. (Fig. 1). Taxonomic studies of the producing strain, the isolation procedure and the biological characteristics of **1**～**4** were reported in a previous paper[2]. This paper describes the determination of the structure of **1**～**4**.

Results and Discussion

Structure of 1

The molecular formula of **1** was established as $C_{11}H_{11}NO_2$ by HR FAB-MS (Table 1). The IR absorptions at 3200 cm^{-1}, 1716 cm^{-1} and 1657 cm^{-1} of **1** showed the presence of hydroxy, carbonyl and imino groups, respectively. The ^1H-^1H COSY spectrum of **1** indicated a sequence of H-10a, 10b (δ 5.11, 5.05), H-9 (δ 5.92), H-8 (δ 3.55), and H-6 (δ 7.20) which indicated the presence of an allyl group in the molecule (Figs. 2 and 3a). The presence of the neighboring methylene group (H-2: δ 2.71, H-3: δ 3.00) was also clarified by the ^1H-^1H COSY spectrum as shown in Figs. 2 and 3b. The HMBC experiments (8.0 Hz) revealed that **1** was composed of pyrindine skeleton with carbonyl, hydroxy and allyl groups substituted at the C-1, C-4 and C-7 positions, respectively (Fig. 4). Therefore, the structure of **1** was anticipated to be 7-allyl-5-aza-4-hydroxy-

Fig. 1. Structures of **1**～**4**.



	R_1	R_2	R_3
1	O	OH	$-{}^{8}CH_2-{}^{9}CH={}^{10}CH_2$
2	OH, H	OH	$-CH_2-CH=CH_2$
3	O	H	$-CH_2-CH=CH_2$
4	O	H	$-CH=CH-CH_3$ (Δ^8: *trans*)

Table 1. Physico-chemical properties of **1**~**4**.

	1	**2**	**3**	**4**
Appearance	Colorless needles	Colorless plates	Dark brown oil	Colorless plates
M.p.	189 ~ 191℃	168 ~ 170℃	—	165 ~ 169℃
M. W.	189	191	173	173
Molecular formula	$C_{11}H_{11}NO_2$	$C_{11}H_{13}NO_2$	$C_{11}H_{11}NO$	$C_{11}H_{11}NO$
UV λ_{max}^{MeOH} nm (log ε)	205 sh. (4.13) 222 (4.23) 246 sh. (3.69) 346 (3.74)	205 (4.06) 235 (3.63) 301 (3.54)	205 (3.98) 231 (3.57) 296 (3.33)	203 (3.74) 219 (3.64) 240 (3.49) 262 (3.46) 267 (3.43) 324 (3.31)
IR ν_{max}^{KBr} cm^{-1}	3200, 1716, 1657, 1612, 1425, 1120	3250, 1651, 1608, 1554, 1421, 1255	1718, 1419, 1286, 1190	1716, 1458, 1294, 1194
Pos. FAB-MS (*m/z*)	190 $(M+H)^+$	192 $(M+H)^+$	174 $(M+H)^+$	174 $(M+H)^+$
HR Pos. FAB-MS (*m/z*)				
Obsd.	190.0879	192.1029	174.0909	174.0921
Calcd.	190.0864	192.1024	174.0919	174.0919
Color reaction				
Positive	50% H_2SO_4 + Δ Ehrlich's reagent + Δ Iodine	50% H_2SO_4 + Δ Ehrlich's reagent + Δ Iodine	50% H_2SO_4 + Δ Ehrlich's reagent + Δ Iodine	50% H_2SO_4 + Δ Ehrlich's reagent + Δ Iodine
Negative	Dragendorff's reagent Ninhydrin reagent	Dragendorff's reagent Ninhydrin reagent	Dragendorff's reagent Ninhydrin reagent	Dragendorff's reagent Ninhydrin reagent

Fig. 2. The ^1H-^1H COSY spectrum of **1**.

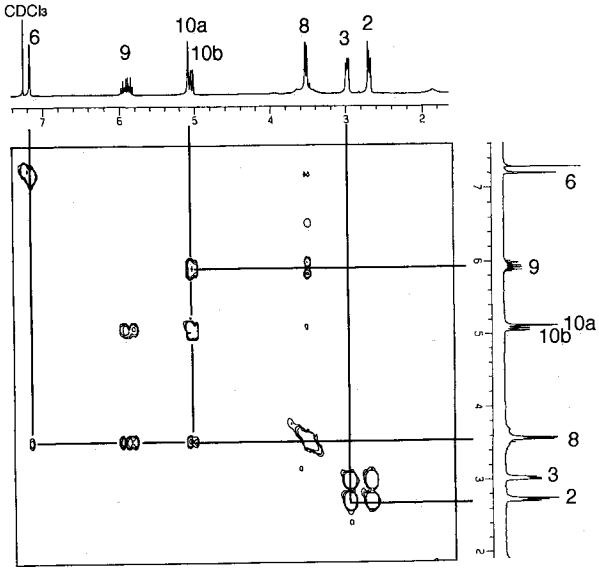
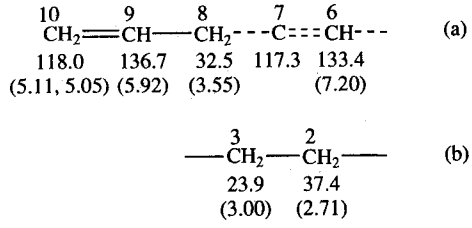


Fig. 3. Partial structures of **1** elucidated by ^1H-^1H COSY.



Values indicate ^{13}C NMR chemical shifts (^1H NMR chemical shifts).

indan-1-one as shown in Fig. 1.

Furthermore, a unique skeleton of **1** was confirmed by X-ray diffraction analysis. The structure was determined by direct methods (MITHRIL)[3] and an ORTEP drawing of **1** is shown in Fig. 5. However, it is possible that this compound exists as the lactam-form in consideration of the intramolecular distances, torsion or conformation angles in the crystalline state of **1**.

Structure of **2**

The molecular formula of **2** was assigned as $C_{11}H_{13}NO_2$ based on the HR FAB-MS (Table 1). The peak at *m/z* 173 in the EI-MS corresponds to the fragment loosing 18 mass units from M^+, which is the characteristic fragmentation pattern of the dehydration peak. The IR spectrum of **2** (Table 1) did not show the carbonyl band observed in that of **1**. In the ^{13}C NMR spectrum of **2** (Table 2), the signals were very similar to those of **1**, except for the carbon signal at C-1 (δ 77.1, d). In the ^1H-^1H COSY spectrum of **2**, the connections between H-1 (δ 4.89) and H-3 (δ 2.56) *via* H-2 (δ 2.05, 1.59) were clarified. From these NMR data, compound **2** was assumed to bear a hydroxy group at the C-1 position instead of the carbonyl group in compound **1**.

Fig. 4. Key ^{1}H-^{13}C long range couplings detected by HMBC experiments of **1**~**4**.

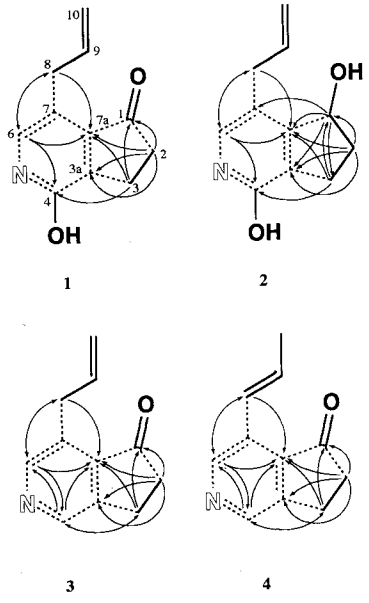
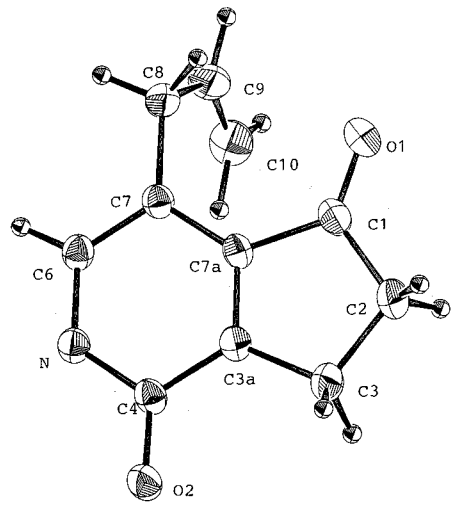


Fig. 5. Molecular structure of **1** in the crystalline state.



Final elucidation of the structure of **2** was performed using HMBC experiments as shown in Fig. 4. The structure of **2** was determined to be 7-allyl-5-aza-1,4-dihydroxy-indan as shown in Fig. 1.

Structures of 3 and 4

The molecular formula of **3** was established to be $C_{11}H_{11}NO$ by HR FAB-MS. The IR spectrum of **3** (Table 1) showed no absorption associated with a hydroxy group. In the ^{1}H NMR spectrum of **3** (Table 3), the proton signal at H-4 (δ 8.75) newly appeared compared with **1**. Other proton data were very similar to those of **1**. Finally, the structure of **3** was confirmed by HMBC experiments. In the HMBC spectrum of **3** (Fig. 4), the cross peaks between the olefinic proton at H-4 (δ 8.75) and the methylene at C-3 (δ 23.1, t), an olefinic carbon at C-6 (δ 149.1, d), and a quaternary carbon at C-7a (δ 139.6, s) were observed. Therefore, the structure of **3** was determined to be 7-allyl-5-aza-indan-

Table 2. ^{13}C NMR chemical shifts of **1**~**4**.

C	1 *	2 **	3 *	4 *
1.	208.6 (s)	77.1 (d)	207.3 (s)	207.5 (s)
2.	37.4 (t)	35.2 (t)	36.4 (t)	36.5 (t)
3.	23.9 (t)	28.5 (t)	23.1 (t)	23.0 (t)
3a.	150.3 (s)	133.8 (s)	148.1 (s)	147.5 (s)
4.	165.2 (s)	163.9 (s)	147.1 (d)	147.0 (d)
6.	133.4 (d)	133.2 (d)	149.1 (d)	145.0 (d)
7.	117.3 (s)	119.7 (s)	133.0 (s)	131.0 (s)
7a.	145.7 (s)	158.5 (s)	139.6 (s)	137.1 (s)
8.	32.5 (t)	34.0 (t)	32.6 (t)	123.7 (d)
9.	136.7 (d)	137.9 (d)	135.4 (d)	132.3 (d)
10.	118.0 (t)	117.3 (t)	116.8 (t)	19.0 (q)

*: in $CDCl_3$, **: in CD_3OD, ( ): multiplicity.

Table 3. ^{1}H NMR chemical shifts of **1**~**4**.

H	1* δ_H (J/Hz)	2** δ_H (J/Hz)	3* δ_H (J/Hz)	4* δ_H (J/Hz)
1	—	4.89 (1H, dd, 7.3, 3.0)	—	—
2a	2.71 (2H, ddd, 6.3, 3.3, 1.0)	2.05 (1H, m)	2.72 (2H, ddd, 6.3, 4.3, 2.6)	2.72 (2H, ddd, 6.3, 4.0, 2.6)
2b	—	1.59 (1H, m)	—	
3a	3.00 (2H, dt, 6.6, 2.0)	2.56 (1H, ddd, 13.5, 8.6, 4.6)	3.15 (2H, dd, 6.3, 2.3)	3.14 (2H, t, 6.3)
3b	—	2.30 (1H, ddd, 13.5, 8.9, 4.6)	—	—
4	—	—	8.75 (1H, s)	8.65 (1H, s)
6	7.20 (1H, s)	6.73 (1H, s)	8.45 (1H, s)	8.75 (1H, s)
8a	3.55 (2H, dd, 6.6, 1.0)	3.10 (1H, dd, 15.8, 1.7)	3.80 (2H, br d, 6.6)	7.42 (1H, dd, 15.8, 1.7)
8b	—	2.95 (1H, ddd, 2.95, 15.8, 5.9)	—	—
9	5.92 (1H, dddd, 17.2, 10.6, 6.6, 2.6)	5.62 (1H, dm, 15.8)	6.00 (1H, ddd, 16.2, 9.5, 6.6, 3.6)	6.55 (1H, dq, 15.8, 6.9)
10a	5.11 (1H, dd, 10.6, 1.7)	4.73 (2H, dm, 15.8)	5.06 (1H, dd, 9.5, 1.7)	1.98 (3H, dd, 6.9, 2.0)
10b	5.05 (dd, 17.2, 1.7)	4.73 (2H, dm, 15.8)	5.11 (1H, dd, 16.2, 1.7)	—
4-OH	13.1 (1H, br s)	—	—	—

*: in $CDCl_3$, **: in CD_3OD.

1-one as shown Fig. 1.

The molecular formula of 4 was observed to be the same as that of **3**. In the ^{13}C NMR spectrum of **4** (Table 2), the chemical shifts were similar to those of **3**, except for the signals of the side chain substituted at the C-7 position. In the ^{1}H NMR spectrum of **4** (Table 3), a set of the signals of olefinic protons were observed at δ 7.42 (dd, $J=15.8$, 1.7, H-8) and δ 6.55 (dq, $J=15.8$, 6.9 Hz, H-9). The connection between the methyl signal at C-10 and the olefinic signal at C-8 was demonstrated by the ^{1}H-^{1}H COSY spectrum. Accordingly, compound **4** was assumed to possess a *trans* propenyl moiety attached to the C-7 position. Finally, the structure of **4** was determined to be 5-aza-7-(*trans*-propenyl)-indan-1-one on the basis of HMBC experiments as shown in Fig. 4.

As a result of screening using testosterone-responsive SC 115 cells, we isolated louisianins A, B, C and D, and their structures were determined to be 7-allyl-5-aza-4-hydroxy-indan-1-one (**1**), 7-allyl-5-aza-1,4-dihydroxy-indan (**2**), 7-allyl-5-aza-indan-1-one (**3**) and 5-aza-7-(*trans*-propenyl)-indan-1-one (**4**), respectively, by spectroscopic and X-ray crystallographic analyses. Louisianins have a unique pyrindine skeleton. A number of aza analogs prepared synthetically, which showed antiallergic activity assessed in the rat passive cutaneous anaphylaxis test, were reported[4]. However, to the best of our knowledge, this is the first natural product bearing a pyrindine skeleton. Recently, HORI *et al.* reported an androgen-receptor antagonist, WB2838 with pyrrole ring, which also inhibited the growth of androgen-responsive SC-3 cells[5]. Therefore, it is of interest to consider the relationship between alkaloidal structure and potential antiandrogen activity.

Experimental

UV spectra were recorded on a Shimadzu model UV-160A spectrophotometer. IR spectra were taken with a Horiba model Fourier transform infrared spectrophotometer. MS were obtained with a JEOL model JMS DX-300 mass spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL JNM-EX 270.

Single-Crystal X-Ray Diffraction Analysis of 1

A colorless needle crystal of $C_{11}H_{11}NO_2$ having approximate dimensions of $0.50 \times 0.200 \times 0.100$ mm was mounted on a glass fiber. All X-ray measurements were made on a Rigaku AFC5R diffractometer with graphite monochromated CuKα radiation and a 12 KW rotating anode generator. Cell constants and an orientation matrix for data collection were obtained from a least-squares refinement using the setting angles of 25 carefully centered reflections in the 2θ range of $47.84 \sim 49.83°$.

The crystal system was monoclinic and the space group was determined to be $p2_1/2$ (#14) with unit cell dimensions: $a=5.236(4)$ Å, $b=16.934(7)$ Å, $c=10.639(3)$ Å, $\beta=95.84(3)$ Å, $V=938.4(6)$ Å^3, $Z=4$. The data were collected at 23°C using the ω-2θ scan technique to a maximum 2θ value of 139.8°. Omega scans of several intense reflections, made prior to data collection, had an average width at half-height of 0.32° with a take-off angle of 6.0°. Scans of $(1.73+0.30\tan\theta)°$ were made at a speed of 32.0°/minute in omega. The weak reflections ($I<1.00\sigma$ (I)) were rescanned and the counts were accumulated to assure good counting statistics. Stationary background counts were recorded on each side of the 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 and the crystal to detector distance was 25.8 cm. Of the 1907 reflections which were collected, 1702 reflections were unique ($R_{int}=0.014$). The intensities of three representative reflections, which were measured after every 150 reflections, remained constant throughout data collection indicating crystal and electronic stability. The linear absorption coefficient for CuKα is 7.2 cm^{-1}. Azimuthal scans of several reflections indicated no need for an absorption correction. The data were corrected for Lorentz and polarization effects.

The structure was solved by direct methods[3]. The non-hydrogen atoms were refined anisotropically. The final cycle of full-matrix least-squares refinement was based on 1220 observed reflections ($I>3.00\sigma$ (I)) and 167 variable parameters, and converged with an R value of 0.051. The standard deviation of an observation of unit weight was 4.09. The weighting scheme was based on counting statistics and included a factor ($p=0.01$) to downweight the intense reflections. The maximum and minimum peaks on the final difference Fourier map corresponded to 0.47 and $-0.26 e^-/$Å^3, respectively.

Acknowledgments

The authors wish to thank Mr. S. KAI, School of Pharmaceutical Sciences, Kitasato University, for measurement and advice of X-ray diffraction. This work was supported in part by Grants-in Aid from the Ministry of Education, Science and Culture, Japan and funds from the Japan Keirin Association.

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