New Pentanorlanostane Derivatives, Cladosporide B~D, as Characteristic Antifungal Agents Against *Aspergillus fumigatus*, Isolated from *Cladosporium* sp.

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In the previous paper,[1] we reported the new pentanorlanostane derivative, cladosporide A (**1**), which showed the characteristic inhibition of the growth of *Aspergillus fumigatus* FRESENIUS, one of the major opportunistic human pathogens causing aspergillosis, along with nonactive 23,24,25,26,27-pentanorlanost-8-ene-3β,22-diol (**5**) from the $CHCl_3$-MeOH (1:1) extract of *Cladosporium* sp. IFM 49189. Further investigation of the above fungus brought us the isolation of three new pentanorlanostane derivatives, cladosporides B (**2**)~D (**4**). The structure elucidation of **2**~**4** and the antifungal activity of these compounds against *A. fumigatus* are discussed in this paper.

The fungus *Cladosporium* sp. IFM 49189 was cultivated on rice (1500 g) as described in the previous paper.[1] After 21 days of cultivation at 25°C, the cultivated rice was extracted with $CHCl_3$-MeOH (1:1) and the extract (24 g) was partitioned with water and ethyl acetate and the organic layer was evaporated *in vacuo*. The evaporated residue (14.6 g) was chromatographed on silica gel with $CHCl_3$-EtOH (50:1) followed by further purification with LPLC on silica gel [CH_2Cl_2-acetone (20:1)], LPLC on ODS (90% MeOH), and finally HPLC on silica gel [$CHCl_3$-EtOH (50:1)] to give cladosporide B (**2**) (2 mg), cladosporide C (**3**) (2.5 mg), and cladosporide D (**4**) (1 mg) along with cladosporide A (**1**) (13 mg) and 23,24,25,26,27-pentanorlanost-8-ene-3β,22-diol (**5**) (3 mg).

The molecular formulae of cladosporides B (**2**), C (**3**), and D (**4**) were confirmed as $C_{25}H_{38}O_3$, $C_{25}H_{40}O_3$, and $C_{25}H_{38}O_3$, respectively, by high resolution electron-impact ionization mass spectrometry (HREI-MS). The physicochemical properties of **1**~**4** are shown in Table 1 and the ^1H and ^{13}C NMR data of **1**~**4** are summarized in Table 2. From the analysis of the HMQC and HMBC spectra of cladosporide C (**3**), it is clear that **3** is the stereoisomer of cladosporide A (**1**). The ^{13}C NMR spectrum of **3** was similar to that of **1**, except for the difference of the carbons at C-1 (δ 30.2 in **3**; δ 35.3 in **1**), C-2 (δ 26.5 in **3**; δ 28.4 in **1**), C-3 (δ 69.1 in **3**; δ 77.0 in **1**), C-5 (δ 45.2 in **3**; δ 52.0 in **1**), and C-19 (δ 22.4 in **3**; δ 18.3 in **1**). The large difference between **3** and **1** in the ^1H NMR spectrum was observed the proton signal at C-3 (δ 4.14 in **3**; δ 3.18 in **1**), including the coupling patterns. Therefore, the structure of cladosporide C (**3**) was confirmed as 3α,22-dihydroxy-23,24,25,26,27-pentanorlanost-8-en-30-al, *i.e.*, the 3-epimer of **1**. This result was also consistent from the analysis of the NOESY spectrum.

Cladosporides B (**2**) and D (**4**) were the didehydro derivatives of cladosporides A (**1**) and C (**3**), respectively. Compounds **2** and **4** showed the presence of four sp^2 carbons (δ 143.4, 143.0, 119.4, and 118.1 for **2**; δ 143.6, 142.9, 119.4, and 117.7 for **4**) instead of two sp^2 carbons in **1** and **3** (δ 134.4 and 133.2 for **1**; δ 134.5 and 133.7 for **3**), and two vinylic protons at δ 5.43 (br d) and 5.55 (br d), and those at δ 5.43 (br d) and 5.52 (br d) were newly observed in the ^1H NMR spectra of **2** and **4**, respectively. The other differences in the ^{13}C NMR signals of **2** and **4** from those of **1** and **3** were observed at C-6, C-12, C-19, C-28, and C-30 (Table 2). The UV absorption maxima at 236, 243, and 251 nm showed the presence of the conjugated diene system in both of **2** and **4**. From these results and the analysis of the HMBC and NOESY spectra of **2** and **4**, the structures of cladosporides B (**2**) and D (**4**) were determined as 3β,22-dihydroxy-23,24,25,26,27-pentanorlanosta-7,9(11)-dien-30-al and 3α,22-dihydroxy-23,24,25,26,27-pentanorlanosta-7,9(11)-dien-30-al, respectively, *i.e.*, 3-epimers of the conjugated diene

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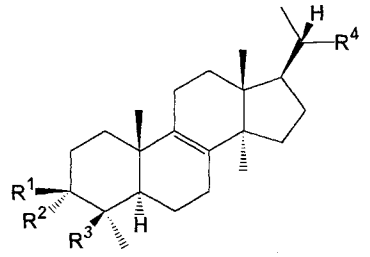
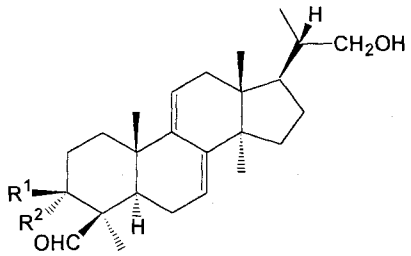
Fig. 1.



1 : R^1 = OH, R^2 = H, R^3 = CHO, R^4 = CH_2OH
3 : R^1 = H, R^2 = OH, R^3 = CHO, R^4 = CH_2OH
5 : R^1 = OH, R^2 = H, R^3 = CH_3, R^4 = CH_2OH
6 : R^1 = OH, R^2 = H, R^3 = CH_2OH, R^4 = CH_2OH
7 : R^1, R^2 = O, R^3 = CHO, R^4 = CO_2H



2 : R^1 = OH, R^2 = H
4 : R^1 = H, R^2 = OH

Table 1. Physico-chemical properties of cladosporides A (**1**)~D (**4**).

	1	2	3	4
Appearance	colorless plates (from MeOH)	Colorless crystals (from CH_2Cl_2-MeOH)	Colorless crystals (from CH_2Cl_2-MeOH)	Colorless crystals (from CH_2Cl_2-MeOH)
Melting point (°C)	206-209	212-215	219-221	209-211
Molecular formula	$C_{25}H_{40}O_3$	$C_{25}H_{38}O_3$	$C_{25}H_{40}O_3$	$C_{25}H_{38}O_3$
HREI-MS (M+)	388.2962 (Calcd. 388.2977)	386.2832 (Calcd. 386.2821)	388.2975 (Calcd. 388.2977)	386.2806 (Calcd. 386.2821)
IR ν^{KBr} cm^{-1}	3400 (OH) 1715 (CO)	3370 (OH) 1710 (CO)	3385 (OH) 1720 (CO)	3380 (OH) 1715 (CO)
UV λ^{MeOH} nm (log ε)	284 (2.12)	236 (4.15) 243 (4.20) 251 (4.03)	—	236 (4.22) 243 (4.28) 251 (4.03)
CD nm (Δε)	300 (-0.55)	304 (-1.23)	296 (-0.57)	301 (-1.63)

Table 2. ^{1}H and ^{13}C NMR spectral data of cladosporides A (**1**)~D (**4**) in $CDCl_3$.

Carbon	1		2		3		4	
No.	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
1	35.3	1.25 ddd (13,13, 5)	35.7	1.55 m	30.2	1.52 m	29.9	1.68 m
		1.83 m		2.05 m		1.52 m		1.76 m
2	28.4	1.83 m	28.6	1.92 m	26.5	1.65 m	26.4	1.76 m
		1.93 m		1. 94 m		2.08 m		1.76 m
3	77.0	3.18 m	77.0	3.23 dd (7,7)	69.1	4.14 brs	69.3	4.12 brs
4	52.4		52.4		52.4		51.9	
5	52.0	1.32 dd (15, 2)	50.6	1.40 dd (13,4)	45.2	1.83 m	43.8	1.85 dd (13,4)
6	18.1	1.69 m	22.6	2.27 brdd (13,13)	17.8	1.83 m	21.9	2.40 brdd (17,13)
		2.01 m		2.40 ddd (13,6,4)		1.92 m		2.29 ddd (17,6,4)
7	26.4	2.12 m	119.4	5.55 brd (6)	26.4	2.10 m	119.4	5.52 brd (6)
		2.12 m				2.10 m		
8	134.4		143.4		133.7		143.6	
9	133.2		143.0		134.5		142.9	
10	37.0		37.3		37.3		37.3	
11	21.1	2.02 m	118.1	5.43 brd (6)	21.2	1.95 m	117.7	5.43 brd (6)
		2.10 m				2.13 m		
12	30.5	1.71 m	37.7	2.10 dd (13,6)	30.8	1.68 m	37.7	2.10 dd (18,6)
		1.78 m		2.24 brd (13)		1.78 m		2.26 brd (18)
13	44.3		43.8		44.6		43.8	
14	49.3		50.0		49.7		50.1	
15	30.7	1.23 ddd (12,12,2)	31.6	1.42 m	31.0	1.23 m	31.6	1.42 m
		1.60 m		1.60 m		1.65 m		1.62 m
16	27.3	1.40 m	27.3	1.38 m	27.6	1.38 m	27.3	1.38 m
		1.93 m		1.98 m		1.94 m		2.00 m
17	46.4	1.58 m	47.2	1.65 m	46.7	1.58 m	47.2	1.62 m
18	15.7	0.72 s	15.8	0.59 s	15.9	0.72 s	15.8	0.59 s
19	18.3	0.93 s	22.4	0.91 s	18.1	0.88 s	21.8	0.87 s
20	39.2	1.58 m	39.2	1.58 m	39.5	1.58 m	39.2	1.58 m
21	16.5	1.03 d (6)	16.6	1.02 d (6)	16.8	1.03 d (6)	16.6	1.02 d (6)
22	67.9	3.36 dd (10,6)	68.1	3.38 dd (10,6)	68.2	3.37 dd (10,5)	68.1	3.38 dd (10,7)
		3.66 dd (10,2)		3.67 dd (10,2)		3.66 brd (10)		3.67 dd (10,3)
28	24.1	0.90 d (1)	25.4	0.89 s	24.3	0.90 s	25.4	0.91 s
29	18.7	1.31 s	19.4	1.30 d (2)	19.6	1.14 s	19.8	1.15 s
30	208.1	9.78 d (2)	208.1	9.91 d (2)	204.9	9.76 s	205.0	9.88 s

derivatives of **1** and **3**, respectively.

Cladosporide A (**1**) (1.5 mg) was reduced by $NaBH_4$ (20 mg) in MeOH (1 ml) at room temperature. After extraction with $CHCl_3$, the evaporated residue was purified by LPLC using the solvent system of $CHCl_3$-MeOH (40 : 1) to give dihydrocladosporide A (**6**) (1.2 mg). The physico-chemical properties of **6** were as follows: Colorless amorphous. EI-MS *m/z* (%): 400 (M^+, 61), 375 ($M-CH_3$, 100). ^{1}H NMR ($CDCl_3$) δ: 0.70 (3H, s, 18-H_3), 0.88 (3H, s, 28-H_3), 0.94 (3H, s, 19-H_3), 1.02 (3H, d, J=5.8 Hz, 21-H_3), 1.25 (3H, s, 29-H_3), 3.36 (2H, m, 3-H, 30-H), 3.47 (1H, dd, J=11.0, 5.0 Hz, 22-H), 3.66 (1H, dd, J=11.0, 2.5 Hz, 22-H), 4.25 (1H, d, J=11.0 Hz, 30-H). 3,30-Dioxo-23,24,25,26,27-pentanorlanost-8-en-22-oic acid (**7**) was prepared from the oxidation of **1** with pyridium dichromate in dimethylformamide at 40°C as described in the previous paper.[1)]

The antifungal activity of **1** against 7 strains of *A.*

Table 3. Antifungal activity of pentanorlanostane derivatives (**1**~**7**) against *A. fumigatus*.

Compound (μg/disc)	1	2	3	4	5	6	7
100	15	17	—	—	—	±	10
50	15	16	—	—	—	—	9
25	15	14	—	—	—	—	±
12.5	13	13	—	—	—	—	—
6	13	13	—	—	—	—	—
3	11	12	—	—	—	—	—
1.5	—	11	—	—	—	—	—

The diameter of inhibitory zone was measured in mm.
The minus (—) means no inhibition.

fumigatus (IFM 4942, IFM 40819, IFM 41375, IFM 41382, IFM 46075, IFM 47064, IFM 47078) was already determined as IC_{80} of 0.5~4.0 μg/ml, whereas no activity was observed against other filamentous fungi, *A. flavus* LINK: FRIES and *A. niger* VAN TIEGHEM, and pathogenic yeasts, *Candida albicans* (ROBEN) BERKHOUT, *C. dubliniensis* D. JSULLIVAN, *C. guilliermondii* (CASTELLANI) LANGERON & GUERRA, *C. kefyr* (BEIJERINCK) VAN UDEN & BUCKLEY, *C. stellatoidea* (JONES & MARTIN) LANGERON, *C. tropicalis* (CASTELLANI) BERKHOUT, and *Cryptococcus neoformans* (SANFELICE) VUILLEMIN up to the concentration of 128 μg/ml[1]. Since **5**, the 4β-methyl derivative of **1**, showed no inhibition against all above fungi, the 4β-aldehyde seems to be essential for this antifungal activity against *A. fumigatus*.[1]

The antifungal activity of **2**~**7** was determined by the paper disc method against *A. fumigatus* IFM 41243, *A. niger* H7160B, *C. albicans* IFM40009, and *C. neoformans* ATCC 90112, as test organisms, as described in the previous paper.[1] The results are summarized in Table 3. Compounds **1**~**7** showed entirely no antifungal activity against *A. niger*, *C. albicans*, and *C. neoformans*. Cladosporides B (**2**) and A (**1**) strongly inhibited the growth of *A. fumigatus* (1.5 and 3.0 μg/disc, respectively), whereas cladosporides C (**3**) and D (**4**) showed no antifungal activity. On the other hand, **6** and **7** still retained weak inhibitory activity against *A. fumigatus*. These results indicated that 3β-hydroxyl group (at least oxygen function at C-3, but not effective 3α-hydroxyl) was essential as well as 4β-aldehyde in this characteristic antifungal activity. It is very interesting that the antifungal activity was typically changed by the configuration of the hydroxyl group at C-3.

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