Antifungal Substances Against Pathogenic Fungi, Talaroconvolutins, from *Talaromyces convolutus*

Shohei Suzuki,[†] Tomoo Hosoe,[†] Koohei Nozawa,[†] Ken-ichi Kawai,*[,†] Takashi Yaguchi,[‡] and Shun-ichi Udagawa[§]

Faculty of Pharmaceutical Sciences, Hoshi University, Ebara 2-4-41, Shinagawa-ku, Tokyo 142-8501, Japan, Pharmaceutical Research Center, Meiji Seika Kaisha, Ltd., Morooka-cho, Kohoku-ku, Yokohama 222-8567, Japan, and Nodai Research Institute, Tokyo University of Agriculture, Sakuragaoka 1-1-1, Setagaya-ku, Tokyo 156-8502, Japan

Received July 30, 1999

The dichloromethane extract of *Talaromyces convolutus* cultivated on barley exhibited antifungal activity against *Candida albicans*. In the course of a search for the active compounds, four new tetramic acid derivatives, talaroconvolutins A (**1**), B (**2**), C (**3**), and D (**4**), were isolated along with ZG-1494α (**5**), and mitorubrin derivatives. The structures of talaroconvolutins A−D (**1**−**4**) were established on the basis of spectroscopic and chemical investigations and chemical correlations. The antifungal activity of the talaroconvolutins against the pathogenic fungi *Aspergillus fumigates*, *Aspergillus niger*, *C. albicans*, and *Cryptococcus neoformans* was determined.

In our continuing investigation of the yellow pigments of the ascomata of *Talaromyces* spp., we recently isolated azaphilone derivatives, mitorubrin, mitorubrinol, mitorubrinal, and mitorubrinic acid, all of the 7*R*-configuration, along with pre-anthraquinone derivatives, anhydroflavomannin-9,10-quinone-6,6′-di-*O*-methyl ether and flavomannin-6,6′-di-*O*-methyl ether from the CH_2Cl_2 extract of the ascomata of *T. convolutus* Udagawa strain NE76-1.[1] On our further search for yellow pigments, a new compound, talaroconvolutin A (**1**), was isolated from the dichloromethane extract and shown to possess antifungal activity against *Candida albicans* (Robin) Berkhout, of the above fungus cultivated on barley. Talaroconvolutin A (**1**) shows a characteristic reddish coloration by phosphomolybdic acid−seric sulfate reagent[2] on TLC. A further search for compounds showing similar reactivity with this reagent led to the isolation and characterization of three new tetramic acid derivatives, talaroconvolutins B (**2**), C (**3**), and D (**4**), along with ZG-1494α (**5**), which was first reported from *Penicillium rubrum* Stoll as a novel acetyltransferase inhibitor of platelet activating factor.[3] We now report the structure determination of talaroconvolutins A (**1**), B (**2**), C (**3**), and D (**4**), as well as the antifungal activity of **1**−**5** (Chart 1).

Results and Discussion

Structure of Talaroconvolutin A (1). The molecular formula of talaroconvolutin A (**1**) was confirmed to be $C_{32}H_{41}NO_3$ by elemental analysis and HREIMS. The ^{1}H NMR spectrum of **1** was similar to that of ZG-1494α (**5**) (Table 1), except for the appearance of a vinylic proton at δ 6.33 in **1** instead of methylene protons (δ 2.88 and 3.01) observed in **5**. The ^{13}C NMR signals at δ 42.8 assigned to a methylene carbon and at δ 86.0 for a carbon bearing a tertiary alcohol in **5** shifted downfield to δ 121.5 and 132.8 in **1**. The absorption maximum (414 nm) of the UV spectrum of **1** shifted bathochromic compared with that of **5** (270 nm). From the above results and the molecular formulas of **1** and **5**, we assumed that **1** should be a dehydrate of a tertiary alcohol in **5**. From the detailed analyses of the ^{1}H−^{1}H COSY, HMQC, and HMBC spectra, the partial structure of the Decalin moiety was determined to be the same as that of **5**. The other part of **1** was determined from analysis of the HMBC correlation (Figure 1). From the result of the differential nuclear Overhauser enhancement (NOE) experiments (Figure 1) and the large coupling constant (11.0 Hz) between 14-H (δ 3.93) and 22-H (δ 1.88), the relative configuration at C-14, C-15, C-19, C-22, and C-23 in **1** was determined to be the same as that of **5**. The stereochemistry of the double bonds at C-5 to C-6 and at C-24 to C-25 was determined to be of the *Z*- and *E*-configuration, respectively, because 7.0% of a NOE was observed on the vinylic proton at C-6 (δ 6.33) when the proton at C-4 (δ 7.62) was irradiated, and a NOE (9.2%) was observed on the proton at C-15 (δ 3.21) when the proton at C-25 (δ 4.74) was irradiated. The structure of talaroconvolutin A was thus confirmed to be as shown in **1**, except for the stereochemistry at C-26, which has not been established. The assignments of the ^{1}H and ^{13}C NMR signals of **1** are summarized in Table 1.

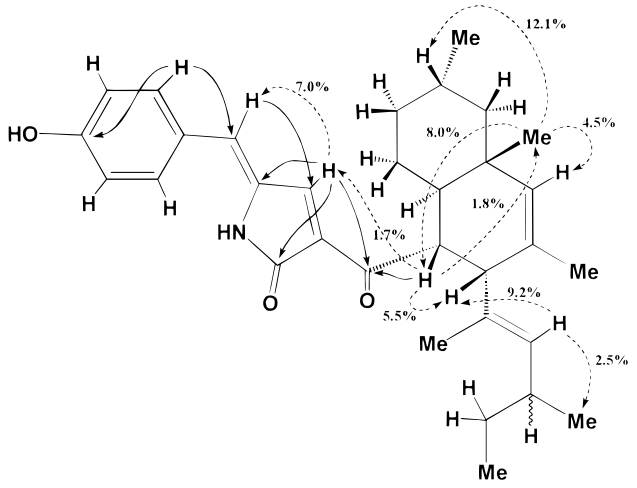


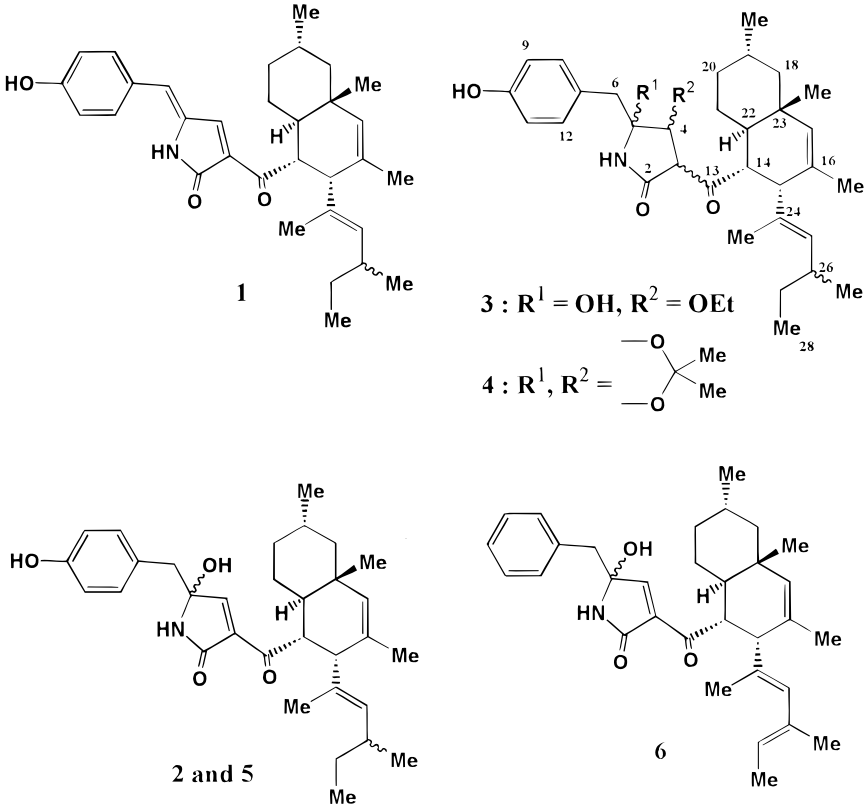
Figure 1. Correlations in HMBC spectrum and differential NOE observations of talaroconvolutin A (**1**). Arrow indicates the HMBC correlation from proton H_A to carbon C_B. Arrow with dotted line indicates the NOE on proton H_B irradiated at proton H_A.

Structure of Talaroconvolutin B (2). The molecular formula of talaroconvolutin B (**2**) was determined to be

* To whom correspondence should be addressed. Tel.: +81-3-5498-5788. Fax: +81-3-3787-0036. E-mail: kawai@hoshi.ac.jp.
† Hoshi University.
‡ Pharmaceutical Research Center.
§ Nodai Research Institute.

10.1021/np990371x CCC: $19.00
Published on Web 04/19/2000

Chart 1



$C_{32}H_{43}NO_4$, the same as ZG-1494α (**5**), by HREIMS. The ^{1}H NMR spectrum of **2** is similar to that of **5** (Table 1). The ^{13}C NMR signals of **2** are also almost superimposable to **5**, except for the carbon chemical shifts at C-4 (δ 154.5 for **2** and δ 156.0 for **5**), suggesting that **2** is a stereoisomer of **5**. Careful analyses of the ^{1}H−^{1}H COSY, HMQC, and HMBC spectra revealed that **2** has the same planar structure as **5**. Based on differential NOE experiments (Figure 2) and the large coupling constant (12.2 Hz) between 14-H (δ 3.73) and 22-H (δ 1.70), the relative configuration at C-14, C-15, C-19, C-22, and C-23 and the configuration of the double bond between C-24 and C-25 in **2** were confirmed to be the same as those of **5**. Thus, the structure of talaroconvolutin B (**2**) is proposed as the stereoisomer of **5**, at C-5 and/or C-26. Because the stereochemistry at C-5 and C-26 has not yet been determined for either **2** or **5**, talaroconvolutin B may be a stereoisomer at either position or both. The assignments of the ^{1}H and ^{13}C NMR signals of **2** are summarized in Table 1.

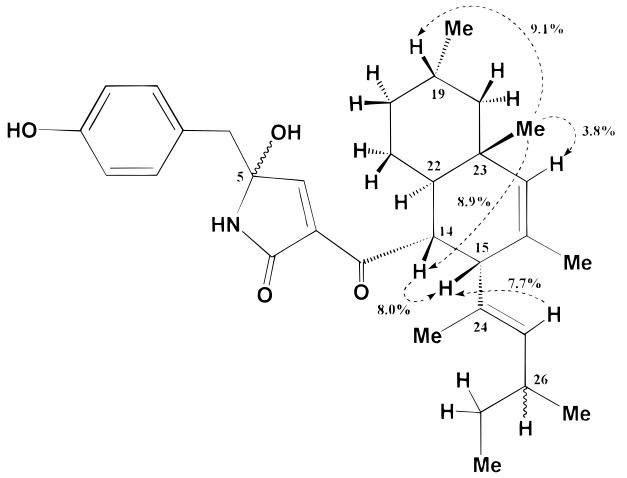


Figure 2. Differential NOE observations of talaroconvolutin B (**2**). Arrow with dotted line indicates the NOE on proton H_B irradiated at proton H_A.

Structure of Talaroconvolutin C (3). Talaroconvolutin C (**3**), $C_{34}H_{49}NO_5$, exhibits a ^{1}H NMR spectrum similar to that of ZG-1494α (**5**) (Table 1), except for the appearance of two adjacent protons at δ 3.71 and 4.41 and an ethoxy group [δ 0.92 (3H, t), 3.22 (1H, qd), and 3.30 (1H, qd)] in **3** instead of a vinylic proton (δ 7.46) in **5**. The ^{13}C NMR signals at δ 135.5 and 156.0 assigned to the double bond carbons in **5** shifted upfield to δ 64.5 and 78.8 in **3**. From the above results and the molecular formulas of **3** and **5**, it appeared that **3** is an ethanol adduct of **5** at the double bond on C-3 and C-4. Talaroconvolutin C (**3**) was easily converted to **5**, which was identical with the naturally occurring ZG-1494α, by dissolving in dimethyl sulfoxide, whereas **5** was also partially converted to **3** when dissolved in ethanol. Therefore, the relative structure of talaroconvolutin C was confirmed to be as shown in **3**. The configuration at C-3, C-4, C-5, and C-26 has not yet been determined. Compound **3** may be an artifact produced during extraction or purification, inasmuch as **3** and **5** were easily converted to each other in the solution. The assignments of the ^{1}H and ^{13}C NMR signals of **3** were determined from analysis of the various 2D NMR, as shown in Table 1.

Structure of Talaroconvolutin D (4). The molecular formula of talaroconvolutin D (**4**) was confirmed by HREIMS to be $C_{35}H_{49}NO_5$, which corresponds to the addition of C_3H_6O (acetone) to ZG-1494α (**5**). The ^{1}H NMR spectrum was similar to that of **5** (Table 1), except for the appearance of two adjacent protons at δ 3.92 and 4.65 and two quaternary methyl groups (δ 0.82 and 1.36) in **4** instead of the vinylic proton (δ 7.46) observed in **5**. The ^{13}C NMR signals at δ 135.5 and 156.0 assigned to the double-bond carbons in **5** shifted upfield to δ 63.5 and 77.7 in **4**. The ^{13}C NMR signals at δ 26.4 and 28.0 correspond to the above

Table 1. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) Spectral Data for Talaroconvolutins in $CDCl_3$

	1		**2**		**3**		**4**		**5**[a]	
carbon no.	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1-NH		8.89 br s		8.58 br s		6.61 br s		8.82 br s		8.55 br s
2	170.2		167.3		169.2		167.5		167.2	
3	131.4		136.3		64.5	3.71 d	63.5	3.92 br s	135.5	
4	144.8	7.62 d	154.5	7.25 s	78.8	4.41 d	77.7	4.65 br s	156.0	7.46 s
5	132.8		85.9		87.4		96.6		86.0	
5-OH				6.08 br s		6.72 br s				6.06 br s
6	121.5	6.33 s	42.9	2.93 d	45.3	2.93 br s	40.7	2.91 d	42.8	2.88 d
				3.05 d		2.93 br s		3.05 d		3.01 d
7	126.4		125.3		127.3		125.1		125.3	
8 and 12	131.7	7.42 d	130.9	7.03 d	132.7	7.29 d	131.7	7.15 d	131.2	6.99 d
9 and 11	116.8	6.95 d	114.5	6.64 d	115.9	6.79 d	114.3	6.70 d	114.4	6.57 d
10	158.0		155.9		156.6		155.9		155.8	
10-OH		9.25 br s		9.08 br s		12.36 br s		9.11 br s		9.12 br s
13	196.6		197.4		204.6		204.7		196.6	
14	49.6	3.93 dd	48.8	3.73 dd	51.5	4.10 dd	51.0	3.56 dd	48.4	3.50 dd
15	50.9	3.21 d	50.2	2.80 d	52.2	2.88 d	49.7	3.26 d	50.5	2.75 d
16	130.1		129.7		130.4		129.2		129.7	
16-Me	22.3	1.52 s	21.7	1.48 s	22.8	1.57 s	21.5	1.52 s	21.9	1.44 s
17	136.5	5.41 s	135.4	5.33 s	137.4	5.40 s	135.8	5.42 s	135.5	5.29 s
18	48.5	0.92 m	47.9	0.83 m	49.1	0.97 m	47.8	0.88 m	48.0	0.80 m
		1.47 m		1.48 m		1.43 m		1.52 m		1.44 m
19	27.4	1.69 m	26.7	1.68 m	28.0	1.60 m	26.7	1.68 m	26.8	1.65 m
19-Me	22.8	0.86 d	22.5	0.87 d	23.4	0.81 d	22.4	0.88 d	22.7	0.82 d
20	35.8	0.97 m	35.4	0.87 m	36.6	0.97 m	34.7	0.89 m	35.5	0.79 m
		1.71 m		1.68 m		1.70 m		1.72 m		1.65 m
21	24.4	0.97 m	23.1	0.87 m	24.5	1.28 m	23.0	0.91 m	23.2	1.38 m
		1.71 m		1.43 m		1.78 m		1.45 m		1.38 m
22	40.1	1.88 m	39.2	1.70 m	40.8	2.02 m	39.6	1.70 m	39.9	1.64 m
23	35.2		35.0		36.6		35.2		35.0	
23-Me	20.5	0.91 s	20.1	0.90 s	21.0	1.11 s	20.1	0.91 s	20.3	0.85 s
24	133.7		132.8		135.0		133.8		132.0	
24-Me	14.0	1.47 s	13.9	1.35 s	15.7	1.65 s	13.5	1.47 s	14.0	1.27 s
25	136.6	4.74 d	135.1	4.73 d	136.5	4.87 d	135.4	5.03 d	135.6	4.47 d
26	34.0	2.13 m	33.4	2.16 m	34.7	2.20 m	33.2	2.19 m	33.5	2.00 m
26-Me	20.6	0.68 d	20.4	0.82 d	21.4	0.89 d	19.5	0.75 d	20.2	0.66 d
27	30.3	1.05 m	29.7	1.19 m	31.2	1.15 m	29.7	1.15 m	29.7	1.06 m
		1.21 m		1.32 m		1.27 m		1.52 m		1.18 m
28	12.0	0.75 t	11.6	0.86 t	12.6	0.86 t	11.5	0.83 t	11.9	0.74 t
−O−C−O−							110.6			
Me							26.4	0.82 s		
							28.0	1.36 s		
$-OCH_2-$					67.6	3.22 dq				
						3.30 dq				
Me					15.5	0.92 t				

[a] This compound was measured in $(CD_3)_2SO$.

two methyl protons, respectively. The carbon observed at δ 110.6 is characteristic of a hemiketal or a ketal carbon. From the above results, in addition to the consideration of the molecular formulas of **4** and **5**, compound **4** was proposed to be an acetonide on C-4 and C-5. The planar structure of **4** was confirmed from detailed analyses of the ^{1}H−^{1}H COSY, HMQC, and HMBC spectra.

The stereochemistry of the double bond between C-24 and C-25 in **4** was determined as having *E*-configuration, the same as that of **5**, from the correlation peak between 15-H (δ 3.26) and 25-H (δ 5.03) in the NOESY spectrum (Figure 3). NOE correlation peaks were observed between the methyl protons at C-23 (δ 0.91) and 14-H (δ 3.56) and 19-H (δ 1.68). Correlation peaks were also observed between 3-H (δ 3.92) and 14-H and 15-H. These results, together with the large coupling constant (12.2 Hz) between 14-H (δ 3.56) and 22-H (δ 1.70), supported the relative configurations at C-14, C-15, C-19, C-22, and C-23. The structure of talaroconvolutin D was confirmed as shown in **4**. The stereochemistry at C-3, C-4, C-5, and C-26 has not yet been determined. Compound **4** may be an artifact of isolation, because we used acetone on column chromatography. The assignments of the ^{1}H and ^{13}C NMR signals of **4** are summarized in Table 1.

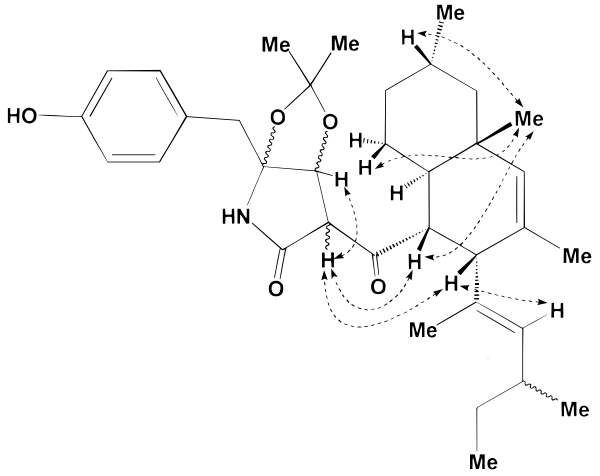


Figure 3. Correlations in NOESY spectra of talaroconvolutin D (**4**). Arrow with dotted line indicates the NOE correlation between proton H_A and proton H_B.

Antifungal Activity of Talaroconvolutins A−D (1−4) and ZG-1494α (5). The antifungal activities of talaroconvolutins compared with a known antifungal agent, amphotericin B, against the filamentous pathogenic fungi,

Table 2. Antifungal Activity of Talaroconvolutins[c]

compound		test organisms μg/disk			
		A. fumigatus	*A. niger*	*C. albicans*	*C. neoformans*
2	100	12[a]	11	15	*b*
	20	11	10	14	*b*
	5	10	9	13	*b*
	1	*b*	*b*	11	*b*
3	100	11	11	11	*b*
	20	11	10	10	*b*
	5	*b*	*b*	*b*	*b*
	1	*b*	*b*	*b*	*b*
5	100	17	16	20	*b*
	20	15	15	18	*b*
	5	14	13	17	*b*
	1	11	11	15	*b*
amphotericin B	100	17	25	20	19
	20	14	24	20	16
	5	12	20	18	13
	1	10	16	15	11

[a] The diameter of inhibitory zone was measured in mm. [b] No inhibition. [c]Compounds **1** and **4** gave no inhibitory zone for all tested organisms up to 100 μg/disk.

Aspergillus fumigatus Fresenius and *Aspergillus niger* van Tieghem, and the pathogenic yeasts, *C. albicans* and *Cryptococcus neoformans* (Sanfelice) Vuillemin, are summarized in Table 2. Talaroconvolutins B (**2**) and C (**3**) and ZG-1494α (**5**) inhibited the growth of *A. fumigatus*, *A. niger*, and *C. albicans*. Talaroconvolutin B (**2**) and ZG-1494α (**5**) showed measurable activity at 1−5 μg/disk against all test organisms, except for *Cr. neoformans*. The activity of compound **2** is slightly weaker than that of **5**, while compound **3**, the ethanol adduct of **5**, shows weak activity at 20 μg/disk. There is the possibility that the marginal activity of **3** may be due to its conversion to **5** under the assay condition.

ZG-1494α (**5**) was isolated as a novel acetyltransferase inhibitor of platelet activating factor from *P. rubrum*.[3] Oteromycin (**6**) was first isolated as a novel antagonist of the subtype ET_B of endothelin receptors (IC_{50} 2.5 mM) from unidentified fungi (ATCC 74201 and ATCC 74202).[4] Thus, talaroconvolutin B (**2**) and its diastereomer (**5**) exhibit antifungal activity against *A. fumigatus*, *A. niger*, and *C. albicans*, whereas talaroconvolutin C (**3**) shows significantly low activity.

Experimental Section

General Experimental Procedures. Optical rotations were determined with JASCO DIP-1000 polarimeter. UV and IR spectra were recorded on a Hitachi U-3210 spectrophotometer and a JASCO IR-810 spectrophotometer, respectively. ^1H and ^{13}C NMR spectra were recorded on a JEOL Lambda-500 (^1H, 500.00 MHz; ^{13}C, 125.43 MHz) spectrometer, using tetramethylsilane as an internal standard. CD curves were determined on a JASCO J-600 spectropolarimeter. EIMS were obtained with a JEOL JMS-MS600W spectrometer, whereas FABMS was obtained with a JEOL JMS−SX-102A spectrometer. Column chromatography was performed using Kieselgel 60 (Art. 7734, Merck). HPLC was performed with a Senshu SSC-3160 pump (flow rate, 7 mL/min) and a YMC-Pack SIL-06 pre-packed column (250 × 20 mm), equipped with a Shimamura YAD-883 RI detector. TLC was conducted on precoated Kieselgel 60 F_{254} plates (Art. 5715, Merck). Spots on TLC were detected by UV light on 254 nm and/or by spraying with phosphomolybdic acid (5%)−ceric acid (trace) in 5% H_2SO_4 and then heating.[2]

Isolation of Talaroconvolutins from *T. convolutus*. *T. convolutus*, strain NE 76-1, was cultivated at 25 °C for 21 days in two Roux flasks containing a total of 200 g of moistened barley grains. The barley culture was extracted with CH_2Cl_2, and the organic layer was evaporated in vacuo. The resultant extract (4.2 g) was chromatographed on Si gel with $CHCl_3$−acetone (20:1), $CHCl_3$−acetone (10:1), $CHCl_3$−acetone (5:1), and acetone in turn. The first fraction eluting with $CHCl_3$−acetone (20:1) was further purified by repeated HPLC using benzene−EtOAc (10:1) and hexane−EtOAc (2:1) to give talaroconvolutin D (**4**) (9 mg). Talaroconvolutin A (**1**) (72 mg) was obtained by recrystallization (cyclohexane) from the fraction eluted by $CHCl_3$−acetone (10:1). The fraction eluting with $CHCl_3$−acetone (5:1) was chromatographed on Si gel with benzene−EtOAc (6:1) to give two fractions. The first fraction was further purified by repeated HPLC benzene−EtOAc (3:1) to afford talaroconvolutin C (**3**) (4 mg), whereas the second fraction was purified by repeated HPLC using benzene−EtOAc (2:1) to give talaroconvolutin B (**2**) (6 mg) and ZG-1494α (**5**) (27 mg).

Talaroconvolutin A (1): yellow prisms (cyclohexane); mp 142−144° (dec); $[\alpha]^{20}_D$ −111° (*c* 0.45, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 255 (4.01), 414 (4.44) nm; IR ν_{max} (KBr) 3400 (OH), 1710, 1690 (−CO−, −CONH−) cm^{-1}; CD (EtOH) $\Delta\epsilon$ (nm) −4.5 (240), −4.3 (250), +0.4 (350), −2.3 (416); ^1H NMR ($CDCl_3$, 500 MHz) δ 0.68 (3H, d, J = 6.7 Hz, Me), 0.75 (3H, t, J = 7.3 Hz, Me), 0.86 (3H, d, J = 6.1 Hz, Me), 0.91 (3H, s, Me), 0.92 (1H, m), 0.97 (2H, m), 1.05 (1H, m), 1.21 (1H, m), 1.47 (3H, s, Me), 1.47 (1H, m), 1.52 (3H, s, Me), 1.69 (1H, m), 1.71 (2H, m), 1.88 (1H, m), 2.13 (1H, m), 3.21 (1H, d, J = 7.5 Hz), 3.93 (1H, dd, J = 11.0, 7.5 Hz), 4.74 (1H, d, J = 9.1 Hz), 5.41 (1H, s), 6.33 (1H, s), 6.95 (2H, d, J = 8.5 Hz), 7.42 (2H, d, J = 8.5 Hz), 7.62 (1H, d, J = 1.8 Hz), 8.89 (1H, br s, NH). ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 1; EIMS *m/z* 487 $[M]^+$ (26), 469 $[M - H_2O]^+$ (38), 440 (7), 430 (15), 412 (50), 398 (48), 384 (14), 214 (74); HREIMS *m/z* $[M]^+$ 487.3092 (calcd for $C_{32}H_{41}NO_3$, 487.3086); anal. C, 79.63%; H, 9.45%; N, 2.68% (calcd for $C_{32}H_{41}NO_3 \cdot C_6H_{12}$, C, 79.82%; H, 9.34%; N, 2.44%).

Talaroconvolutin B (2): colorless microcrystals (cyclohexane); mp 112−114°; $[\alpha]^{20}_D$ −57° (*c* 0.11, EtOH); UV (EtOH) λ_{max} (log ϵ) 226 (3.84), 273 (3.09) nm; IR ν_{max} (KBr) 3400 (OH), 1720, 1710 (−CO−, −CONH−) cm^{-1}; CD (EtOH) $\Delta\epsilon$ (nm) +1.8 (231), +1.3 (283); ^1H NMR ($CDCl_3$, 500 MHz) δ 0.82 (3H, d, J = 7.0 Hz, Me), 0.83 (1H, m), 0.86 (3H, t, J = 5.2 Hz, Me), 0.87 (3H, d, J = 7.0 Hz, Me), 0.87 (2H, m), 0.90 (3H, s, Me), 1.19 (1H, m), 1.32 (1H, m), 1.35 (3H, s, Me), 1.43 (1H, m), 1.48 (3H, s, Me), 1.48 (1H, m), 1.68 (2H, m), 1.70 (1H, m), 2.16 (1H, m), 2.80 (1H, d, J = 6.7 Hz), 2.93 (1H, d, J = 13.7 Hz), 3.05 (1H, d, J = 13.7 Hz), 3.73 (1H, dd, J = 12.2, 6.7 Hz), 4.73 (1H, d, J = 9.5 Hz), 5.33 (1H, s), 6.08 (1H, br s, OH), 6.64 (2H, d, J = 8.2 Hz), 7.03 (2H, d, J = 8.2 Hz), 7.25 (1H, s), 8.58 (1H, br s, NH), 9.08 (1H, br s, OH); ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 1; EIMS *m/z* 505 $[M]^+$ (100), 488 (16), 472 (13), 434 (31), 409 (19), 399 (39), 391 (45); HREIMS *m/z* 505.3198 $[M]^+$ (calcd for $C_{32}H_{43}NO_4$, 505.3194).

Talaroconvolutin C (3): colorless amorphous powder; $[\alpha]^{20}_D$ −56° (0.028, $CHCl_3$); UV (cyclohexane) λ_{max} (log ϵ) 224 (3.87), 262 (3.52) nm; IR ν_{max} (KBr) 3400 (OH), 1720, 1700 (−CO−, −CONH−) nm; CD (cyclohexane) $\Delta\epsilon$ (nm) +1.6 (237), −0.3 (261), +2.0 (298); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.81 (3H, d, J = 6.4 Hz, Me), 0.86 (3H, t, J = 7.3 Hz, Me), 0.89 (3H, d, J = 6.7 Hz, Me), 0.92 (3H, t, J = 7.0 Hz, Me), 0.97 (2H, m), 1.11 (3H, s, Me), 1.15 (1H, m), 1.27 (1H, m), 1.28 (1H, m), 1.43 (1H, m), 1.57 (3H, s, Me), 1.60 (1H, m), 1.65 (3H, s, Me), 1.70 (1H, m), 1.78 (1H, m), 2.02 (1H, m), 2.20 (1H, m), 2.88 (1H, d, J = 6.1 Hz), 2.93 (2H, br s), 3.22 (1H, qd, J = 7.0, 9.1 Hz), 3.30 (1H, qd, J = 7.0, 9.1 Hz), 3.71 (1H, d, J = 2.4 Hz), 4.10 (1H, dd, J = 12.2, 6.1 Hz), 4.41 (1H, d, J = 2.4 Hz), 4.87 (1H, d, J = 8.9 Hz), 5.40 (1H, s), 6.61 (1H, br s, NH), 6.72 (1H, br s, OH), 6.79 (2H, d, J = 8.4 Hz), 7.29 (2H, d, J = 8.4 Hz), 12.36 (1H, br s, OH); ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 1; EIMS m/z 533 $[M - H_2O]^+$ (1), 505 $[M - EtOH]^+$ (68), 488 (11), 472 (10), 434 (22), 409 (24), 399 (23), 391 (21), 301 (24), 107 (67), 97 (100); FABMS (*m*-nitrobenzyl alcohol as matrix) m/z 552 $[M + 1]^+$ (86), 534 $[M - H_2O + 1]^+$ (100).

Talaroconvolutin D (4): colorless amorphous powder; $[\alpha]^{20}_D$ −34° (*c* 0.043, EtOH); UV (EtOH) λ_{max} (log ϵ) 226 (3.92), 276 (3.44), 326 (2.79) nm; IR ν_{max} (KBr) 3300 (OH), 1710, 1700 (−CO−, −CONH−) cm^{-1}; CD (MeOH) $\Delta\epsilon$ (nm) −6.5 (226), +1.9 (293); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.75 (3H, d, J = 6.7 Hz, Me), 0.82 (3H, s, Me), 0.83 (3H, t, J = 7.3 Hz, Me), 0.88 (3H, d, J = 6.1 Hz, Me), 0.88 (1H, m), 0.89 (1H, m), 0.91 (3H, s, Me), 0.91 (1H, m), 1.15 (1H, m), 1.36 (3H, s, Me), 1.45 (1H, m), 1.47 (3H, s, Me), 1.52 (3H, s, Me), 1.52 (2H, m), 1.68 (1H, m), 1.70 (1H, m), 1.72 (1H, m), 2.19 (1H, m), 2.91 (1H, d, J = 14.0 Hz), 3.05 (1H, d, J = 14.0 Hz), 3.26 (1H, d, J = 7.3 Hz), 3.56 (1H, dd, J = 12.2, 7.3 Hz), 3.92 (1H, br s), 4.65 (1H, br s), 5.03 (1H, d, J = 8.9 Hz), 5.42 (1H, s), 6.70 (2H, d, J = 8.5 Hz), 7.15 (2H, d, J = 8.5 Hz), 8.82 (1H, br s, NH), 9.11 (1H, br s, OH); ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 1; EIMS m/z 563 $[M]^+$ (10), 548 $[M - CH_3]^+$ (3), 545 $[M - H_2O]^+$ (4), 530 (5), 505 $[M - (CH_3)_2CO]^+$ (82), 434 (27), 409 (30), 399 (24), 391 (20), 366 (9), 301 (25), 97 (100); HREIMS m/z $[M]^+$ 563.3581 (calcd for $C_{35}H_{49}NO_5$, 563.3610).

Conversion from Talaroconvolutin C (3) to ZG-1494α (5). Talaroconvolutin C (**3**) (10 mg) in dimethyl sulfoxide (5 mL) was kept at room temperature for 24 h. The reaction mixture was poured into water and extracted with CH_2Cl_2. After the removal of the solvent in vacuo, the residue was purified by HPLC using benzene−EtOAc (2:1) to afford ZG-1494α (**5**) (6 mg), which was identified by comparison of the UV, ^{1}H NMR, and CD spectra and the TLC behavior with that previously reported.[3]

A small amount of **5** could also be detected by TLC when talaroconvolutin C (**3**) (1 mg) was dissolved in either benzene or acetone (0.5 mL each) and kept at room temperature for 48 h.

Conversion from ZG-1494α (5) to Talaroconvolutin C (3). ZG-1494α (**5**) (20 mg) dissolved in ethanol (5 mL) was kept at room temperature for 40 h. After removal of the solvent in vacuo, the residue was purified by HPLC using $CHCl_3$−EtOAc (3:1) to afford talaroconvolutin C (**3**) (5 mg), which was identified by comparison of UV and ^{1}H NMR spectra and the TLC behavior. Unreacted **5** was also recovered (11 mg).

Antifungal Assay. The antifungal assay was performed by the paper-disk assay with *A. fumigatus* 167, *A. niger* H7160B, *C. albicans* 7N, and *Cr. neoformans* ATCC 90112 as test organisms. The extract and the isolated compounds were absorbed by the paper disks (8 mm diameter) and placed on the assay plates. The fungi were cultivated in Sabouraud's glucose broth agar at 25 °C. After 48−72 h incubation, zones of inhibition (mm in diameter) were recorded. Amphotericin B was used for positive control. The results are summarized in Table 2.

Acknowledgment. We are grateful to Dr. H. Kasai, Dr. M. Shirao, and Dr. S. Saitoh of Hoshi University for elemental analyses and NMR and mass measurements. This work was supported in part by a Grant-in-Aid for Scientific Research (no. 09672169) from the Ministry of Education, Science, Sports and Culture, Japan.

References and Notes

(1) Suzuki, S.; Hosoe, T.; Nozawa, K.; Yaguchi, T.; Udagawa, S.; Kawai, K. *J. Nat. Prod.* **1999**, *62*, 1328−1329.
(2) Ayer, W. A.; Hoyano, Y.; Pedras, M. S.; Clardy, J.; Arnold, E. *Can. J. Chem.* **1987**, *65*, 748−753.
(3) West, P. R.; van Ness, J.; Varming, A.-M.; Rassing, B.; Biggs, S.; Gasper, S.; McKernan, P. A.; Piggott, J. *J. Antibiot.* **1996**, *49*, 967−973.
(4) Singh, S. B.; Goetz, M. A.; Jones, E. T.; Bills, G. F.; Giacobbe, R. A.; Herranz, L.; Stevens-Miles, S.; Williams, D. L., Jr. *J. Org. Chem.* **1995**, *60*, 7040−7042.

NP990371X