STUDIES ON NEW ANTIFUNGAL ANTIBIOTICS, GUANIDYLFUNGINS A AND B

II. STRUCTURE ELUCIDATION AND BIOSYNTHESIS

KAZUTOH TAKESAKO* and TERUHIKO BEPPU

Department of Agricultural Chemistry, Faculty of Agriculture, The University of Tokyo, Yayoi, Bunkyo-ku, Tokyo 113, Japan

(Received for publication April 9, 1984)

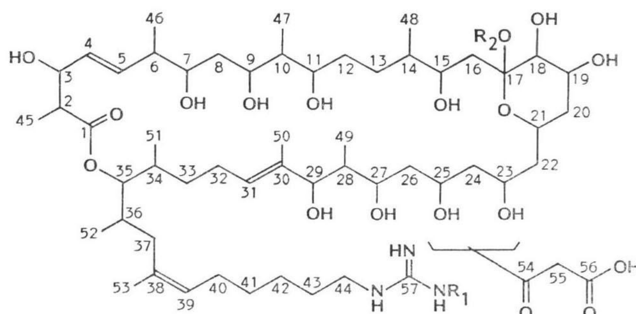
The structures of guanidylfungins A and B were elucidated from the physico-chemical properties of these compounds and the structures of the degradation products by ozonolysis and periodate oxidation. The guanidylfungins consist of a 36-membered polyhydroxyl lactone ring, a guanidine and a monoester of malonic acid. The labelling experiments with sodium [1-^{13}C]acetate and sodium [1-^{13}C]propionate revealed that twelve units of acetate and nine of propionate were incorporated into the molecule of guanidylfungin A.

In the preceding paper[1] we reported the production, isolation, and characterization of guanidylfungins A and B, together with the taxonomy of the producing organism, *Streptomyces hygroscopicus* No. 662.

This report presents the structural elucidation and biosynthesis of the guanidylfungins. The structures of guanidylfungins A (**1**) and B (**2**) containing a 36-membered polyhydroxyl lactone ring, a guanidine, an intramolecular hemiketal ring and a monoester of malonic acid (Fig. 1) were elucidated from the physico-chemical properties of **1**, **2** and methylguanidylfungin A (**3**), a methylated derivative of **1**, and from the structures of the degradation products by ozonolysis and periodate oxidation of **1** and **3**.

In order to confirm the structures of **1**, **2** and **3**, incorporation patterns of [1-^{13}C]acetate and [1-^{13}C]-propionate into guanidylfungin A were studied using ^{13}C NMR spectroscopy. The result revealed that twelve units of acetate and nine of propionate were incorporated into the molecule of guanidylfungin A, which was consistent with the pattern estimated from the structure in Fig. 1.

Fig. 1. Structures of guanidylfungins A (**1**), B (**2**) and methylguanidylfungin A (**3**).



Guanidylfungin A (**1**) $R_1 = CH_3$, $R_2 = H$

Guanidylfungin B (**2**) $R_1 = R_2 = H$

Methylguanidylfungin A (**3**) $R_1 = R_2 = CH_3$

Table 1. ^{13}C NMR of guanidylfungins A (**1**), B (**2**) and methylguanidylfungin A (**3**).

δ, multiplicity			Assignment	δ, multiplicity			Assignment
1[a,c]	**2**[a,c]	**3**[b,d]		**1**	**2**	**3**	
174.5, s	174.2, s	176.9, s	C-1	46.1	46.4	48.0, d	
170.2, s	169.9, s	173.7, s	C-56	45.4	45.5	45.4, t	
169.0, s	169.0, s	171.6, s	C-54	43.8	43.6	44.9, d	$-\overset{\vert}{C}HCH_3$ ×7
				42.7	42.8	44.8, t	
156.4, s	157.2, s	158.2, s	C-57			43.1, t	and
						42.9, d	
137.0, s	137.0, s	137.6, s	C-30			46.6, ttt	$-CH_2-$ ×18
134.1, d	134.1, d	136.5, d	C-5	e	e	42.2, t	
132.5, s	132.6, s	134.0, s	C-38			41.9, d	
131.5, d	131.2, d	132.3, d	C-4			41.0, t	
126.2, d	126.2, d	128.6, d	C-31[f]			40.4, d	
125.6, d	125.6, d	127.9, d	C-39[f]			39.3, t	
						35.9, t	
98.3, s	98.4, s	102.7, s	C-17	33.1	33.4	35.4, d	
				31.5	31.6	33.2, dt	
78.6, dd	78.8, d	81.2, dd				32.9, t	
	78.7, d		$-\overset{\vert}{C}HOH$			30.3, t	
75.7, d	76.3, d	76.7, d	or	28.8, t	28.8, t	29.9, t	
74.0, d	73.7, d	76.0, dd	$-\overset{\vert}{C}HOCO-$	28.4, t	28.4, t	29.8, t	
72.8, d	72.5, d		×13	27.3, t	27.3, t	28.6, t	
71.7, d		74.9, d		25.6, t	25.7, t	27.2, t	
69.8, dd	69.9, ddd	72.2, d		23.2, t	23.6, t	25.3, t	
		72.1, d					
69.3, d		71.6, d		27.6, q	—	28.4, q	NCH_3
67.4, d	67.5, d	69.0, dd					
66.1, d	66.5, d			16.7, q	16.5, q	16.9, q	
64.4, d	64.3, d	67.1, d		15.5, q	15.6, qq	16.2, q	
63.5, d	63.9, d	65.7, d		15.4, q		16.1, q	$-\overset{\vert}{C}HCH_3$ ×7
				14.6, q	14.9, q	14.9, qq	and
—	—	48.1, q	OCH_3	14.0, q	13.7, q		$-\overset{\vert}{C}=CH_3$ ×2
				12.6, q	13.2, q	13.9, q	
				10.3, q	10.8, q	11.7, q	
				10.1, q	10.5, q	11.3, q	
				9.8, q	10.0, q	10.7, q	

[a] 25 MHz in $(CD_3)_2SO$. [b] 100 MHz in CD_3OD. [c] Multiplicity in off-resonance spectrum; s=singlet, d=doublet, t=triplet, q=quartet. [d] Multiplicity determined by INEPT technique. [e] The signals from 36 to 41 ppm were masked by the $(CD_3)_2SO$ peak. [f] Assignments could be interchanged.

Physico-chemical Properties of Guanidylfungin A and Methylguanidylfungin A (3)

The molecular formula of guanidylfungin A was found to be $C_{58}H_{103}N_3O_{18}$ from the results of the ^1H, ^{13}C NMR, secondary ion mass spectrum (SIMS) and elemental analysis[1]. The ^{13}C NMR spectrum (Table 1) revealed the presence of three carbonyl carbons, one guanidyl carbon, six olefinic carbons, one hemiketal carbon, more than ten carbons bearing hydroxyl or acyloxy groups, ten methyl carbons and others.

Guanidylfungin A was treated with methanolic hydrochloride to give its methylated derivative (**3**), named methylguanidylfungin A. The molecular formula of **3** was found to be $C_{59}H_{105}N_3O_{18}$ from the

Table 2. ^1H NMR of guanidylfungin A (**1**) and methylguanidylfungin A (**3**) (400 MHz in CD_3OD).

δ, intensity, multiplicity,[a] J (Hz)		Assignment
1	**3**	
5.72, 1H, dd, $J_{5,4}$=15, $J_{5,6}$=8	5.72, 1H, dd, J=15, 8	H-5
5.44, 1H, dd, $J_{4,5}$=15, $J_{4,3}$=8	5.44, 1H, dd, J=15, 8	H-4
5.33, 1H, br t, $J_{31,32}$=7	5.36, 1H, br t, J=7	H-31[b]
5.17, 1H, m	5.25, 1H, m	*H*-C-O-malonyl
5.17, 1H, br t, $J_{39,40}$=7	5.17, 1H, br t, J=7	H-39[b]
4.73, 1H, dd	4.73, 1H, dd	H-35
4.18, 1H, d, J=9	4.19, 1H, d, J=9	
4.13, 1H, dd, $J_{3,4}$=8, $J_{3,2}$=8	4.12, 1H, dd, J=8, 8	H-3
4.09, 1H, m		
3.92~3.83, 5H	3.95~3.84, 4H	H-19 and others
3.81~3.73, 2H	3.84~3.71, 4H	H-7 and others
3.33, 1H, d, $J_{18,19}$=8	3.49, 1H, d, J=8	H-18
3.22, 2H	3.22, 2H	H-55
—	3.20, 3H, s	OCH_3
3.16, 2H, t, J=7	3.15, 2H, t, J=7	H-44
2.84, 3H, s	2.83, 3H, s	H-58
2.49, 1H, m, $J_{2,3}$=8	2.48, 1H, m	H-2
2.33, 1H, m	2.32, 1H, m	H-6
2.16, 1H, m	2.16, 1H, m	
2.1~1.2, 34H	2.1~1.2, 34H	
1.58, 6H, s×2	1.58, 6H, s×2	H-50 and 53
1.09, 3H, d, J=7	1.09, 3H, d, J=7	H-46
1.08, 3H, d, J=7	1.08, 3H, d, J=7	H-45
0.93, 3H, d, J=7	0.92, 6H, d×2, J=7	
0.91, 3H, d, J=7		
0.89, 3H, d, J=7	0.87, 3H, d, J=7	
0.87, 3H, d, J=7	0.86, 3H, d, J=7	
0.70, 3H, d, J=7	0.69, 3H, d, J=7	

[a] Multiplicity: s=singlet, d=doublet, t=triplet, br t=broad triplet, q=quartet, m=multiplet.
[b] The assignments could be interchanged.

results of the ^{13}C, ^1H NMR, SIMS (M+H, *m/z* 1,144) and elemental analysis. The ^{13}C NMR spectrum of **3** (Table 1) showed the presence of three carbonyl carbons, one guanidyl carbon, six olefinic carbons, one hemiketal carbon, thirteen carbons bearing hydroxyl or acyloxy groups, seven methine carbons, seventeen methylene carbons and eleven methyl carbons involving one *N*-methyl and one *O*-methyl groups. The ^1H NMR of **3** (Table 2) showed a very similar spectrum to that of guanidylfungin A except for the presence of an *O*-methyl signal at δ_H 3.20. It was supposed that the methylation occurred at the hemiketal position, which was supported from the downfield shift ($\Delta\delta_C$ 2.8) of the hemiketal carbon of **3** (δ_C 101.1 in $(CD_3)_2SO$) in comparison with the corresponding signal of guanidylfungin A (δ_C 98.3).

The UV spectra of guanidylfungin A and **3** showed the absence of a conjugated structure in their molecules.

Partial Structures of Guanidylfungin A and Methylguanidylfungin A (3)

The following partial structures A to E (Fig. 2) were elucidated from the spectroscopic data of guanidylfungin A (Tables 1 and 2).

Structure A: The structure of C-2 to C-7 was revealed by proton decoupling experiments. The (*E*)-orientation of C(4)=C(5) bond was elucidated from the large coupling constant (15 Hz) between

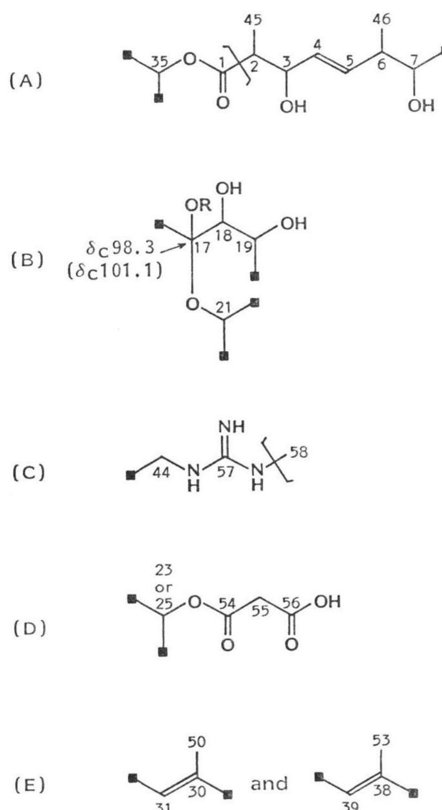
H-4 and H-5. The coupling pattern and the chemical shift of H-2 suggested the connection of C-2 to C-1 carbonyl carbon.

Structure B: The one proton at δ_H 3.33 could be assigned to H-18 which was on a carbon vicinal to the hemiketal position (C-17) by comparison with the H-18 signal (δ_H 3.34) of azalomycin F_{4a}[2]. The coupling between the signals at δ_H 3.33 and 3.87 (H-19) was revealed by proton decoupling experiments.

Structure C: As reported in the preceding paper[1], guanidylfungin A has a di-substituted guanidine. Signals of an *N*-methyl (δ_H 2.84, δ_C 27.6) and an *N*-methylene (δ_H 3.16) indicated the presence of structure C.

Structure D: Methylene protons appearing at δ_H 3.23 were found to be easily deutrated in CD_3OD. The ^{13}C and ^1H NMR data indicated the presence of two acyl carbonyl carbons (δ_C 170.2 and 169.0) and an acyloxy methine proton (δ_H 5.17), besides the acyl carbonyl carbon (δ_C 174.5) and the acyloxy methine proton (δ_H 4.73) shown in the structure A. These results suggested the presence of structure D, which was supported by comparison with the spectral data of the same moiety present in the structures of azalomycin F_{4a}[2] and copiamycin[3].

Fig. 2. Partial structures of guanidylfungin A (R=H) and methylguanidylfungin A (R=CH_3).



Structure E: The NMR data revealed the presence of structure E.

The spectroscopic data of **3** (Tables 1 and 2) showed that it also had structures A, C, D, E along with methylated B (R=CH_3).

Results of Degradation Experiments

Guanidylfungin A and methylguanidylfungin A were found to have three olefinic bonds shown in the partial structures A and E as well as 1,2-diol and 1,2-ketol moieties shown in the partial structure B. Then the oxidative procedures, *i. e.*, ozonolysis and periodate oxidation, were employed to obtain degradation products which might facilitate the structural study of guanidylfungin A.

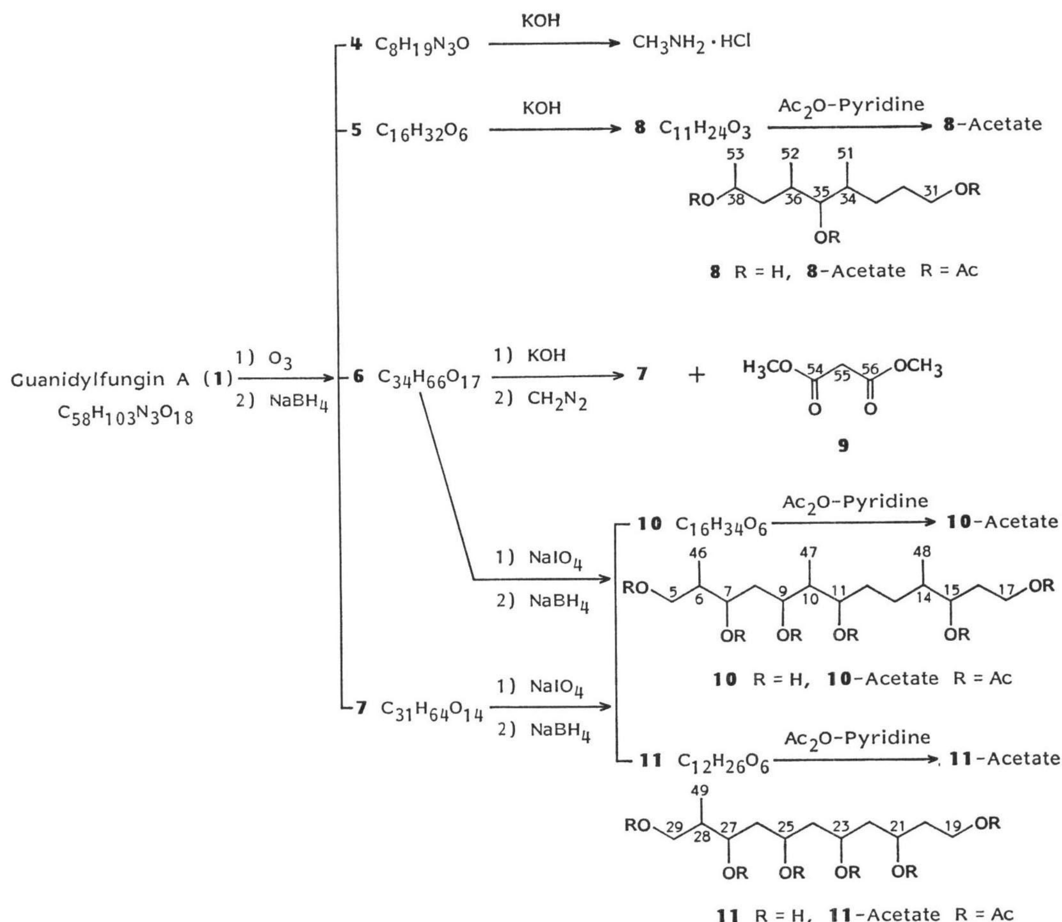
Ozonolysis of Guanidylfungin A

Guanidylfungin A was decomposed by ozonolysis in methanol - water (9: 1) at $-78°C$ followed by reduction with sodium borohydride ($NaBH_4$) to give degradation products **4**, **5**, **6** and **7**.

Alkaline hydrolysis of **4** afforded methylamine (trapped as its hydrochloride). Compound **5** gave **8** on alkaline hydrolysis. Compound **6** gave **7** and **9** on alkaline hydrolysis followed by methylation with diazomethane.

Compounds **4**, **5**, **7** and **8** were treated with acetic anhydride (Ac_2O) - pyridine in the presence of 4-dimethylaminopyridine (DMAP) to give their respective acetates. **8**-Acetate was separated into **8**-

Scheme 1. Degradation by O_3-$NaBH_4$-$NaIO_4$-$NaBH_4$ of guanidylfungin A.



acetate(α) and 8-acetate(β) by reversed phase high performance liquid chromatography (HPLC).

Periodate Oxidation of **6** and **7**

Compound **6** was oxidized with sodium periodate ($NaIO_4$) and then reduced with $NaBH_4$ to give **10** and **11**. Compound **7** was similarly treated with $NaIO_4$ and $NaBH_4$ to give **10** and **11**.

Ozonolysis after Periodate Oxidation of Guanidylfungin A

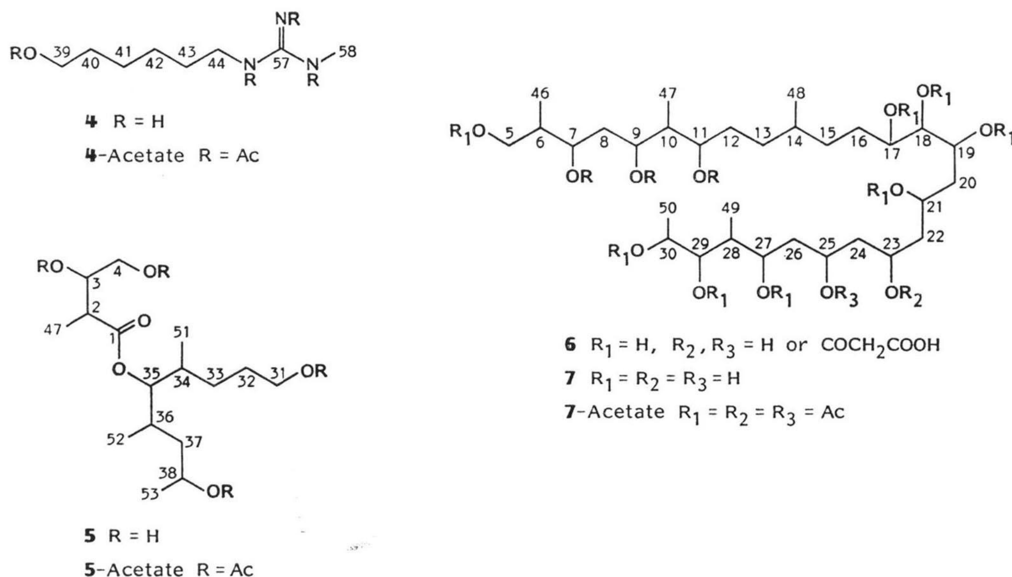
The $NaIO_4$ oxidation of guanidylfungin A for two hours was followed by ozonolysis and $NaBH_4$ reduction to give **4**, **5**, **12**, **13**, **14**, **15** and **16**.

Compound **12** was treated with Ac_2O - pyridine in the presence of DMAP and then methylated with diazomethane to give **12′**. Compounds **13**, **14** and **15** were acetylated with Ac_2O - pyridine in the presence of DMAP to give their respective acetates. **15**-Acetate was separated into **15**-acetate(α) and **15**-acetate(β) by silica gel HPLC. Compound **16** was methylated with diazomethane and acetylated with Ac_2O - pyridine to give **16′**, which was separated into **16′**(α) and **16′**(β) by silica gel HPLC.

Ozonolysis of Methylguanidylfungin A (**3**)

Ozonolysis of **3** was carried out in methanol at $-78°C$ and the resultant ozonide was reduced with $NaBH_4$ to give **4**, **5**, **17** and **18**.

Fig. 3. Structures of the degradation products by O_3-$NaBH_4$ of guanidylfungin A.



Compound **17** gave **9** on alkaline hydrolysis followed by methylation with diazomethane. Compound **18** was acetylated with Ac_2O - pyridine in the presence of DMAP to give **18′**, which was separated into **18′**(α) and **18′**(β) by silica gel HPLC.

Periodate Oxidation of 17 and 18

The $NaIO_4$ oxidation of **17** was followed by reduction with $NaBH_4$ to give **10**, **11**, **12**, **13** and **14**. Compound **18** was similarly treated with $NaIO_4$ and $NaBH_4$ to give **10**, **11** and **12**.

Structures of Degradation Products

The molecular formula of **4**, a basic product, was determined to be $C_8H_{19}N_3O$ from the SIMS (M+H, *m/z* 174) and ^{13}C NMR. The structure of **4** (Fig. 3) was deduced from the ^{13}C and ^1H NMR of **4** and **4**-acetate considering the partial structure C (Fig. 2) and was confirmed by the result that **4** gave methylamine on alkaline hydrolysis.

Compound **5** showed an [M+H] ion peak at *m/z* 321 in its field desorption (FD) MS. The ^{13}C NMR of **5** (Table 3) revealed the presence of sixteen carbons, which contained seven carbons split into a doublet because of the stereoisomerism at C-38. The ^{13}C NMR and IR spectra revealed the presence of an ester bond (δ_C 176.7, IR 1710 cm^{-1}). From these results the molecular formula of **5** was established to be $C_{16}H_{32}O_6$ (MW

Table 3. ^{13}C NMR of **5** and **8** (25 MHz in CD_3OD).

δ, multiplicity[a]				Assignment
5		**8**		
176.7,	s	—		C-1
82.4 (80.1)[b],	d	79.5 (78.5),	d	C-38
74.7,	d	—		C-3
65.8 (65.7),	d	65.8 (65.5),	d	C-35
64.7,	d	—		C-4
63.1,	t	62.9,	t	C-31
44.8,	d	—		C-2
44.7 (44.1),	t	44.5,	t	C-32[c]
35.5,	d	36.3 (36.2),	d	C-34[d]
31.8,	d	32.2,	d	C-36[d]
30.5,	t	30.2,	t	C-33[c]
29.5 (29.1),	t	29.4 (29.1),	t	C-37[c]
24.4 (24.0),	q	24.3 (23.7),	q	C-53
16.3 (16.2),	q	16.1,	q	C-52[e]
14.6 (13.3),	q	12.6,	q	C-51[e]
14.3,	q	—		C-45

[a] Multiplicity determined by INEPT technique.
[b] The signals shown in parentheses were splitted because of the stereoisomerism at C-38.
[c,d,e] Assignments could be interchanged.

Table 4. ^{1}H NMR of **5**-acetate and **8**-acetate(α) (400 MHz in $CDCl_3$).

δ, intensity, multiplicity, J (Hz)			Assignment
5-Acetate		**8**-Acetate(α)	
5.27,	1H, m	—	H-3
5.00 (5.07)*,	1H, m	4.99, 1H, m	H-38
4.80 (4.72),	1H, dd, J=8, 5 (9, 3)	4.68, 1H, dd, J=8, 5	H-35
4.45,	1H, dd, J=12, 3	—	H-4a
4.14,	1H, dd, J=12, 5	—	H-4b
4.03,	2H, m	4.03, 2H, m	H-31
2.90,	1H, dq, J=8	—	H-2
2.09～2.00, 12H, 4×s		2.07, 3H, s 2.04, 3H, s 2.02, 3H, s	Acetyl methyls
1.94～1.3,	7H	1.84, 1H, m	H-36
		1.76, 1H, m	H-34
		1.73, 1H, m	H-32a
		1.63, 1H, m	H-37a
		1.53, 1H, m	H-32b
		1.42, 1H, m	H-33a
		1.20, 1H, m	H-37b
1.24,	3H, d, J=7	—	H-45
1.21 (1.18),	3H, d, J=6	1.21, 3H, d, J=6	H-53
1.12,	1H, m	1.11, 1H, m	H-33b
0.92 (0.90),	3H, d, J=7	0.88, 3H, d, J=7	H-52
0.88 (0.87),	3H, d, J=7	0.87, 3H, d, J=7	H-51

* The signals shown in parentheses were splitted because of the stereoisomerism at H-38.

320). The ^{1}H NMR of **5**-acetate (Table 4) revealed the partial structure of $-OCOCH(CH_3)CH(OAc)-CH_2OAc$ by proton decoupling experiments.

The molecular formula of **8**, an alkaline hydrolysis product of **5**, was established to be $C_{11}H_{24}O_3$ from the results of the SIMS (M^+, m/z 204) and ^{13}C NMR (Table 3). The structure of **8** (Scheme 1) was elucidated by proton decoupling experiments in the ^{1}H NMR of **8**-acetate(α) (Table 4) and **8**-acetate(β), which were epimers at H-38.

The molecular formula of **6**, a mixture of stereoisomers at C-17 and C-30, was determined to be $C_{34}H_{66}O_{17}$ (MW 746) from the SIMS (M+Na+H, m/z 770) and ^{13}C NMR. Compound **6** gave **7** and **9**, which was identified to be dimethyl malonate by the ^{1}H NMR, IR and gas chromatography (GC) MS, on alkaline hydrolysis followed by methylation with diazomethane. The ^{13}C NMR of **7** showed a similar spectrum to that of **6** except the absence of two carbonyl carbons (δ_C 174.7 and 171.9). From these results the molecular formula of **7** was established to be $C_{31}H_{64}O_{14}$ (MW 660), which was supported by the finding that the SIMS of **7**-acetate showed an [M+H] ion peak at m/z 1,249, indicating the incorporation of 14 acetyl groups. The ^{1}H NMR of **7**-acetate revealed the presence of the partial structures $-CH(CH_3)OAc$ and $-CH(CH_3)CH_2OAc$ as the terminal moieties, but was still too complex for complete spectroscopic analysis. The whole structures of **6** and **7** were elucidated from the structures of **10** and **11**, which were degradation products by $NaIO_4$ oxidation and $NaBH_4$ reduction of **6** and **7**, and **12**, **13**, **14**, **15** and **16**, which were degradation products by $NaIO_4$ oxidation and ozonolysis of guanidylfungin A, as discussed later.

The molecular formula of **10**, $C_{16}H_{34}O_6$, was established by its SIMS (M+H, m/z 323) and ^{13}C NMR (Table 5). The structure of **10**-acetate (Scheme 1) was elucidated by proton decoupling experiments in

its ^{1}H NMR, and was confirmed by comparison of the spectral data with those of the degradation products **16a**[4], **9a**[3] and **23**[5], which were obtained from azalomycin F_{4a}, copiamycin and niphimycin, respectively.

Proton decoupling experiments in the ^{1}H NMR of **11**-acetate (Table 6) revealed the presence of partial structures $-CH_2CH(OAc)CH(CH_3)CH_2OAc$ and $-CH(OAc)CH_2CH_2OAc$ as terminal moieties. The fact that **11** was obtained by periodate oxidation indicated the absence of 1,2-diol structures in it. Hence the structures of **11** and **11**-acetate were elucidated as shown in Scheme 1, and were confirmed by comparison of the spectral data with those of the degradation products of niphimycin, **20** and **22**[5], and the degradation product of copiamycin, **8a**[3].

Comparison of the ^{13}C NMR spectra of **12**, **13** and **14** with that of **10** (Table 5) revealed the

Table 5. ^{13}C NMR of **10**, **12**, **13** and **14** (δ and multiplicities determined by INEPT technique, 25 MHz in CD_3OD).

10	12	13	14	Assignment
76.1, d	76.0, d	76.1, d	76.0, d	C-7
75.3, d	75.2, d	75.3, d	75.2, d	C-9
73.9, d	73.5, d	72.8, d	72.7, d	C-15
72.7, d	72.5, d	72.7, d	72.7, d	C-11
—	—	67.1, t	67.9, t	C-18
65.5, t	65.4, t	65.5, t	65.5, t	C-5
60.8, t	*	75.5, d	70.4, d	C-17
43.8, d	43.7, d	43.9, d	43.8, d	
42.4, d	42.2, d	42.4, d	42.4, d	
40.3, d	40.4, d	40.3, d	40.5, d	
38.5, t	38.4, t	38.6, t	38.5, t	
36.6, t	39.6, t	36.8, t	37.6, t	C-16
33.4, t	33.3, t	33.4, t	33.6, t	
29.9, t	29.8, t	29.7, t	29.9, t	
15.6, q	15.5, q	15.5, q	15.5, q	
13.6, q	13.5, q	13.6, q	13.5, q	
10.5, q	10.5, q	10.5, q	10.5, q	

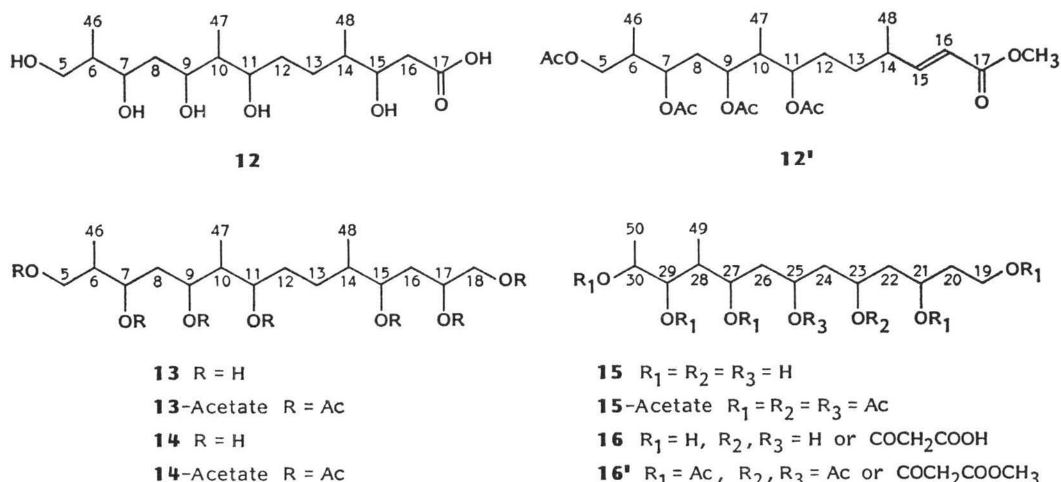
* The carboxylate carbon at C-17 of **12** were not observed.

Table 6. ^{13}C NMR and ^{1}H NMR of **11**-acetate and **16'**(α).

^{13}C NMR (25 MHz in $CDCl_3$)			^{1}H NMR (400 MHz in $CDCl_3$)		
δ, multiplicity		Assignment	δ, intensity, multiplicity, J (Hz)		Assignment
11-Acetate	**16'**(α)		**11**-Acetate	**16'**(α)	
170.9, s	170.4, s	Acetyl carbonyls	—	5.17, 1H, m	H-30
170.4, s	165.9, s		4.99, 3H, m	5.05, 1H, m	
—	74.8, d	C-30	—	5.00, 2H, m	
70.0, d	69.2, d		4.92, 1H, m	4.83, 1H, m	
—	69.0, d		—	4.80, 1H, dd	
67.4, d	67.5, d×2		4.08, 2H, t	4.08, 2H, t	H-19
66.7, d	66.8, d		4.01, 1H, dd, J=7, 11	—	H-29a[b]
65.7, t	—	C-29[a]	3.87, 1H, dd, J=7, 11	—	H-29b
60.6, t	60.6, t	C-19	—	3.75, 3H, s	OCH_3
—	52.5, q	OCH_3	—	3.37, 2H, s	H-55
—	41.4, t	C-55	2.06, 3H, s	2.12, 3H, s	Acetyl methyls
39.5, t	39.5, t		2.05, 3H, s	2.09, 3H, s	
39.1, t	39.2, t		2.04, 6H, s×2	2.05, 3H, s	
36.3, d	36.8, d	C-28	2.02, 6H, s×2	2.04, 3H, s	
36.1, t	36.8, t			2.03, 3H, s	
33.6, t	33.6, t			2.00, 3H, s	
21.0, q	21.0, q	Acetyl methyls	2.04, 1H	2.01, 1H	H-28
			1.89, 2H, dt, J=7, 7	1.90, 2H, dt	H-20
—	16.9, q	C-50	1.85~1.75, 5H	1.88~1.74, 6~7H	
11.7, q	10.1, q	C-49	—	1.15, 3H, d, J=7	H-50
			0.96, 3H, d, J=7	0.96, 3H, d, J=7	H-49

[a] The C-29 carbon of **16'**(α) was deduced to be the signal at δ_C 69.0 or 69.2.
[b] The H-29 proton of **16'**(α) was deduced to be the signal at δ_H 4.80.

Fig. 4. Structures of the degradation products by $NaIO_4$-O_3-$NaBH_4$ of guanidylfungin A.



overlapping of the carbon skeleton, C-5 to C-17, in these compounds, indicating that each of the carbon chains of **13** and **14** is longer than that of **10** or **12** by one carbon.

The structure of **12** (Fig. 4) was deduced by comparison of the ^{13}C NMR with that of **10** (Table 5), and was confirmed by the ^{1}H NMR and SIMS of **12′**, which was produced by *trans*-elimination dehydration at α,β-position of the carbonyl group at C-17 in acetylation of **12**.

The structure of **13** (Fig. 4) was also deduced by comparison of the ^{13}C NMR with that of **10** (Table 5), and was confirmed by the ^{1}H NMR and SIMS (M+H, *m/z* 647) of **13**-acetate. Compound **14** (Fig. 4) was concluded to be an epimer of **13** at C-17 by comparison of the ^{13}C NMR with that of **13** (Table 5) and from the result that **14-acetate** showed the same [M+H] ion peak as **13-acetate** in its SIMS.

The structures of **10**, **12**, **13** and **14** indicated that the hemiketal carbon should be located at C-17 in the molecule of guanidylfungin A.

Comparison of the ^{1}H and ^{13}C NMR spectra of **15**-acetate and **16′** with those of **11**-acetate (Table 6) revealed the overlapping of the carbon skeleton, C-19 to C-29, in these compounds.

Compound **15** was isolated as its acetates, **15**-acetate(α) and **15**-acetate(β), which were epimers at C-30. The structure of **15**-acetate was elucidated by proton decoupling experiments in the ^{1}H NMR of **15**-acetate(α) and by comparison of the spectral data with those of **11**-acetate.

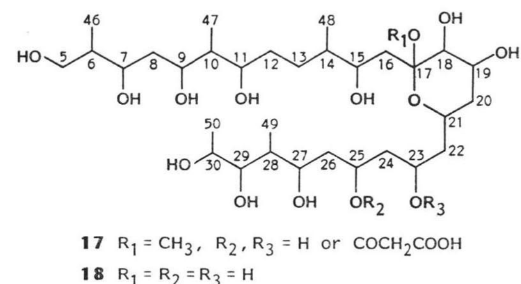
The ^{13}C NMR of **16**, a mixture of epimers at C-30, showed two carbonyl carbons at δ_C 174.8 and 172.0 assigned as a malonyl group. The structure of **16** (Fig. 4) was elucidated from the spectroscopic data of its methylated and acetylated derivatives, **16′**(α) and **16′**(β), which were epimers at C-30. The structure of **16′**(α) (Fig. 4) was elucidated from the ^{1}H NMR, ^{13}C NMR (Table 6) and EIMS (*m/z* 575, $M^+ - CH(CH_3)OAc$), and by comparison of the spectral data with those of **11**-acetate and **15**-acetate. The location of the malonyl monoester could not be determined by the spectral data of **16**, **16′**(α) and **16′**-(β), but was deduced to be at C-23 or C-25 from the biosynthetic studies of guanidylfungin A as discussed later.

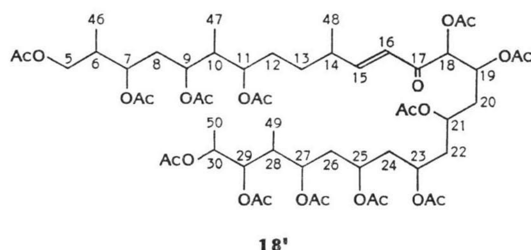
As described above, **7**-acetate possessed the partial structures of $-CH(CH_3)CH_2OAc$ and $-CH(CH_3)$-OAc as terminal moieties, which were also present in **10** (**12**, **13**, **14**) and **15** (**16**), respectively. This suggested the connection of C-18 and C-19 in **7**-acetate and the structure of **7** was elucidated as shown

in Fig. 3, which was supported by the fact that the number of carbon atoms of **7** coincides with the sum of those of **13** (**14**) and **15**. The structure of **6** was elucidated as shown in Fig. 3 from the result that **6** gave **7** and malonic acid on alkaline hydrolysis as described above. The structures of **6** and **7** were also supported by those of **17** and **18** as discussed below.

The ^{13}C NMR of **17**, a mixture of stereoisomers at C-17 and C-30, showed the presence of two carbonyl carbons at δ_C 174.7 and 171.9 assigned as a malonyl group, one ketal carbon at δ_C 99.8, one methoxy carbon at δ_C 53.4 and others. The ^{13}C NMR of **18** showed a similar spectrum to that of **17** except the absence of carbonyl carbons and a methoxy carbon. The ^{13}C NMR of **18′**(α) and **18′**(β), which were epimers at C-30, showed 31 carbons including one ketone carbon at δ_C 192.3 instead of a hemiketal carbon at δ_C 99.9 of **18**, two olefinic carbons at δ_C 153.5 and 123.2, twelve carbons bearing acyloxy groups, for methine carbons, seven methylene carbons and five methyl carbons in addition to the carbons assigned as *O*-acetyl groups. Compounds **18′**(α) and **18′**(β) were found to be produced by *trans*-elimination dehydration at α,β-position of the hemiketal group at C-17 in acetylation as well as **12′**. The molecular formula of **18** was established to be $C_{31}H_{62}O_{14}$ from the ^{13}C NMR of **18′**(α) and **18′**(β) described above and the SIMS of **18′**(α) and **18′**(β) (M+H, *m/z* 1,145), which indicated the incorporation of 14 acetyl groups. The structure of **18** was elucidated as shown in Fig. 5 from the structures of its degradation products, **10**, **11** and **12**, by $NaIO_4$ oxidation and $NaBH_4$ reduction and was supported by the spectral data of **18′**(α) and **18′**(β). The structure of **17** was elucidated as shown in Fig. 5 from the ^{13}C NMR and the structures of its degradation products, **10**, **11**, **12**, **13** and **14**, by $NaIO_4$ oxidation and $NaBH_4$ reduction.

Fig. 5. Structures of the degradation products by O_3-$NaBH_4$ of **3**.





Biosynthesis of Guanidylfungin A

Guanidylfungins are probably derived *via* so called polyketide biosynthetic pathway like azalomycin F_{4a}[6] and the polyene macrolide antibiotics. In order to elucidate the structure of guanidylfungin A, biosynthetic studies were performed using ^{13}C-labeled acetate and propionate.

S. hygroscopicus No. 662 was cultured in a 500-ml Erlenmeyer flask containing 100 ml of the medium composed of glucose 1.0%, Polypeptone 0.2%, beef extract 0.1% and yeast extract 0.1% (pH 7.2) on a rotary shaker.

Sodium [1-^{13}C]acetate (91 atom%, 10 mg) or sodium [1-^{13}C]propionate (92 atom%, 10 mg) was added to the fermentation broth at 50 and 65 hours after inoculation. Fermentation was stopped after further incubation for 24 hours, and ^{13}C-labelled guanidylfungin A, which contained a little of guanidylfungin B, was isolated from the mycelia. ^{13}C-Labeled guanidylfungin A (**1**) was treated with methanolic hydrochloride to give the labeled methylguanidylfungin A (**3**).

Fig. 6. ^{13}C NMR of methylguanidylfungin A; nonlabeled (a), ^{13}C-acetate labeled (b) and ^{13}C-propionate labeled (c) (25 MHz in CD_3OD).

The signal (*) was assigned as the carbon bearing the malonyl monoester.

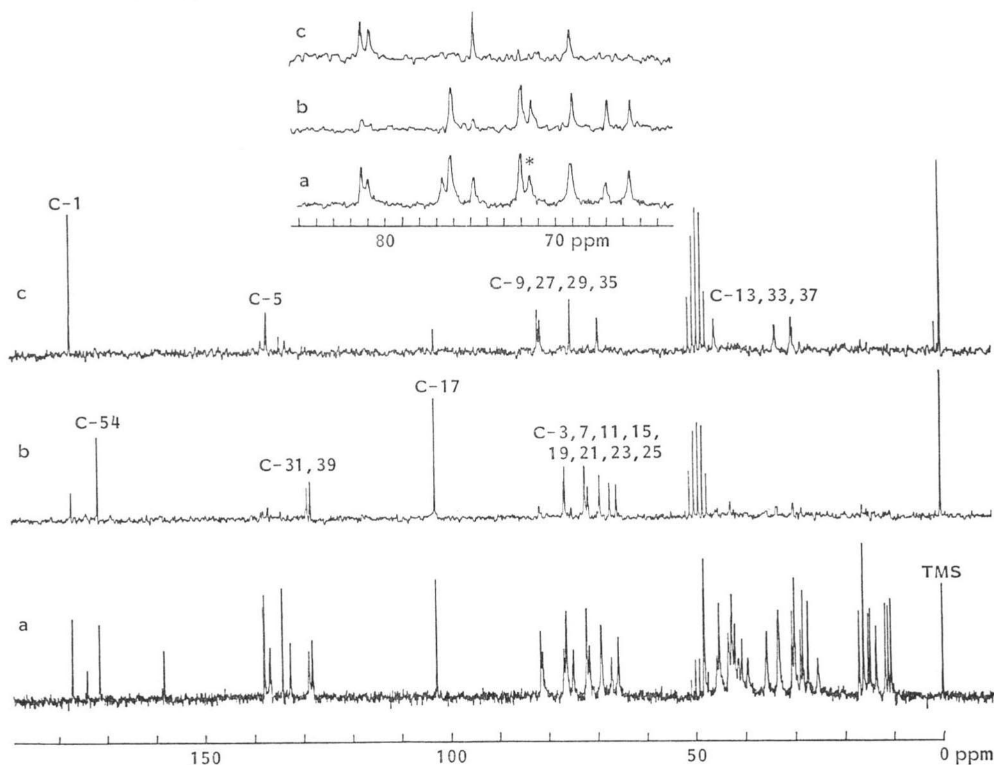
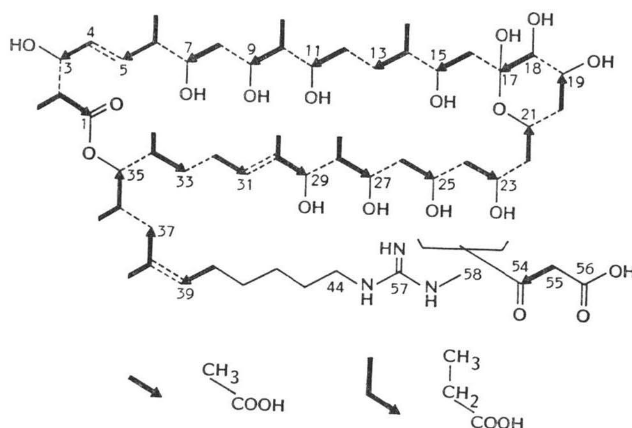


Fig. 7. Biosynthesis of guanidylfungin A (**1**).



The ^{13}C NMR spectrum of the labeled **3** (Fig. 6) as well as that of the labelled **1** (data not shown) revealed that twelve units of [1-^{13}C]acetate and nine of [1-^{13}C]propionate were incorporated into the molecule of **1** as shown in Fig. 7. [1-^{13}C]Acetate was incorporated into eight carbons bearing hydroxyl groups and the carbons at C-17, C-31, C-39 and C-54, whereas [1-^{13}C]propionate was incorporated into four carbons bearing hydroxyl or acyloxy groups and three methylene carbons as well as the carbons at C-1 and C-5 (Fig. 6 and Fig. 7).

Skeletal Structures of Guanidylfungins A and B

As described above, ozonolysis of guanidylfungin A ($C_{58}H_{103}N_3O_{18}$) followed by $NaBH_4$ reduction gave a mixture of degradation products which were isolated as **4** ($C_8H_{16}N_3O$), **5** ($C_{16}H_{30}O_6$) and **6** ($C_{34}H_{66}$-O_{17}). Similarly ozonolysis of **3** ($C_{59}H_{105}N_3O_{18}$) followed by $NaBH_4$ reduction gave a mixture of degradation products, **4**, **5** and **17** ($C_{35}H_{68}O_{18}$). The sum of the carbon atom numbers found in those degradation products coincides with those of guanidylfungin A or **3**. Since such ozonolysis products should, of course, be derived from the cleavage of three C=C double bonds present in guanidylfungin A or **3**, the elucidation of the skeletal structures of guanidylfungin A and **3** should be possible by determining the mode of connection of those degradation fragments.

The partial structures A and E (Fig. 2) suggested that **5** and **6** should be connected directly through two C=C double bonds between C-4 and C-5, and C-31 and C-30 in guanidylfungin A, as well as between **5** and **17** in **3**. Then the connection of **5** and **4** through a C=C double bond between C-38 and C-39 in guanidylfungin A and **3** was necessary, which was supported by the partial structure E. The hemiketal carbon at C-17 was deduced to constitute a six membered ring by linking with C-21.

In the ^{13}C NMR of **3** the carbon bearing the malonyl monoester was assigned to be the signal appeared at δ_C 71.4 by a selective proton spin decoupling experiment in which the proton of H-C-O-malonyl (δ_H 5.25) was irradiated. The fact that the signal at δ_C 71.4 was enriched by sodium [1-^{13}C]-acetate in the biosynthetic experiment of guanidylfungin A (Fig. 5) indicated the location of the malonyl monoester at C-23 or C-25.

In conclusion, the structures of guanidylfungin A (**1**) and methylguanidylfungin A (**3**) were elucidated as shown in Fig. 1. Guanidylfungin A (**1**) consists of a 36-membered macrocyclic lactone ring, an intramolecular hemiketal ring involving a keto group at C-17 and a hydroxyl group presumably at C-21, and a malonyl monoester at C-23 or C-25.

The structure of guanidylfungin B (**2**) was elucidated as shown in Fig. 1 from the results that the ^{13}C NMR of **2** showed a very similar spectrum to that of **1** except for the absence of an *N*-methyl group at δ_C 27.6 (Table 1) and that the SIMS of **2** showed an [M+H] ion peak at *m/z* 1,116 which is smaller by 14 mass units than that of **1**.

The structures of the guanidylfungins closely resemble to those of azalomycins F_{3a}, F_{4a} and F_{5a}[4,7], copiamycin[3], neocopiamycin A[8], niphimycin[5] and scopafungin[9]. These antibiotics all consist of a macrocyclic polyhydroxyl lactone ring with groups of malonyl monoester and intramolecular hemiketal, and a side chain with a mono-, di-, or tri-substituted guanidine as their terminal moiety.

Experimental

General

Melting points were measured using a Yazawa BY-1 and are uncorrected. UV spectra were measured on a Uvidec 610 spectrometer. IR spectra were recorded with a Jasco A-102 spectrometer. EIMS and GCMS were carried out on a Hitachi RMU-6L, FDMS on a Jeol DX-300, and SIMS on a Hitachi M-80A.

HPLC were carried out on a reversed phase column (Nucleosil ODS-5) or a silica gel column (LiChroprep 5), employing a Uvidec 100 spectrometer and a Shodex SE-30 differential refractometer as detectors.

^1H NMR and ^{13}C NMR spectra were recorded on Jeol JNM FX-100 (^1H 99.6 MHz, ^{13}C 25.1 MHz) and JNM FX-400 (^1H 400.5 MHz, ^{13}C 100.7 MHz) spectrometers; chemical shifts are given in ppm (in δ) relative to TMS (0 ppm) as an internal or external standard and coupling constants are recorded in

Hz (J).

Sodium [1-^{13}C]acetate and sodium [1-^{13}C]propionate were purchased from Prochem.

Ozonolysis of Guanidylfungin A

Guanidylfungin A (0.5 g) was ozonized in MeOH - H_2O (4: 1, 100 ml) at $-78°C$ and the resultant ozonide was decomposed by addition of $NaBH_4$ (0.5 g) in MeOH (5 ml) initially with stirring at 0°C and additional 0.5 g in MeOH (5 ml) after stirring for an hour at room temp. After an hour the reaction with $NaBH_4$ was stopped by neutralization with dilute aq HCl. The reaction solution was passed through a column of Amberlite CG-50 (H^+, 200 ml) to adsorb a basic product and the column was washed with MeOH - H_2O (2: 1, 400 ml) and H_2O (400 ml). Then the column was eluted with aq 1 N HCl (500 ml) and the eluate was neutralized, concentrated to dryness under reduced pressure and extracted with 1-BuOH after dissolution in H_2O. From the extract **4** (40 mg) was obtained by a preparative silica gel TLC with $CHCl_3$ - MeOH - H_2O (65: 25: 4). **4** (20 mg) was treated with Ac_2O - pyridine and DMAP to give its acetate, which was purified by a preparative TLC with benzene - acetone (3: 1) to obtain **4**-acetate (5 mg).

The washings of the Amberlite CG-50 column were combined with the effluent from the column and the solution was concentrated to dryness under reduced pressure. The residue was extracted with a small amount of MeOH and $CHCl_3$ - MeOH - H_2O (65: 25: 4) and the extract was chromatographed on a silica gel column with $CHCl_3$ - MeOH - H_2O (65: 25: 4) and MeOH to obtain **5** (152 mg), **6** (308 mg) and **7** (76 mg).

5 (25 mg) was treated with Ac_2O - pyridine and DMAP to give its acetate, which was purified by a silica gel column with benzene - acetone (9: 1) to obtain **5**-acetate (31 mg).

7 (51 mg) was similarly acetylated and purified by reversed phase HPLC with MeOH - H_2O (8: 2) to obtain **7**-acetate (41 mg).

6 failed to give its objective acetate by treatment with Ac_2O - pyridine and DMAP.

4 (as HCl): SIMS m/z 174 (M+H); UV λ_{max}^{MeOH} nm (ε) 208 (1,630); IR (neat) cm^{-1} 3600 ~ 3000, 2950, 2880, 1670 ~ 1630, 1460, 1060. ^1H NMR (100 MHz in CD_3OD) δ 3.52 (2H, t, H-39), 3.10 (2H, t, H-44), 2.86 (3H, s, H-58), 1.8 ~ 1.3 (8H); ^{13}C NMR (25 MHz in D_2O) δ 157.2 (s, C-57), 62.6 (t, C-39), 42.0 (t, C-44), 32.0 (t), 28.8 (t), 28.3 (q, C-58), 26.5 (t), 25.5 (t).

4-Acetate: SIMS m/z 342 (M+H); UV λ_{max}^{MeOH} nm (ε) 223 (12,200), 254 (16,700); ^1H NMR (100 MHz in $CDCl_3$) δ 4.05 (2H, t, H-39), 3.54 (2H, t, H-44), 3.11 (3H, s, H-58), 2.25, 2.23, 2.20 and 2.05 (12H, 4×s, acetyl methyls), 1.8 ~ 1.3 (8H); ^{13}C NMR (25 MHz in $CDCl_3$) δ 181.7, 171.2 and 171.0 (s, acetyl carbonyls), 146.2 (s, C-57), 64.3 (t, C-39), 47.3 (t, C-44), 35.1 (q, C-58), 28.6 (2×t), 26.7 (t), 25.6 (t), 25.5, 23.8, 23.1 and 21.0 (4×q, acetyl methyls).

5: FDMS m/z 321 (M+H), 343 (M+Na); IR (neat) cm^{-1} 3400, 2950, 2890, 1730, 1710, 1460, 1380, 1260, 1180, 1050; ^{13}C NMR see Table 3.

5-Acetate: EIMS m/z (relative intensity) 488 (7), 373 (12), 359 (28), 271 (10), 201 (85), 159 (100); ^{13}C NMR (25 MHz in $CDCl_3$) δ 173.1, 171.1, 170.7, 170.5 and 169.8 (s, C-1 and acetyl carbonyls), 81.3 (79.9)* (d, C-38), 72.4 (d, C-3), 69.0 (68.4) (d), 64.6 (t), 62.7 (t), 41.1 (d, C-2), 40.5 (40.3) (t), 34.1 (d), 31.0 (30.8) (d), 28.2 (27.7) (t), 25.8 (25.7) (t), 21.2, 20.9, 20.7 and 20.2 (q, acetyl methyls), 15.8 (15.6) (q), 13.8 (q), 13.4 (q); ^1H NMR see Table 4.

6: SIMS m/z 792 (M+2Na), 770 (M+Na+H), 683 (M+Na+H−$COCH_2COOH$), 665 (M+Na−$COCH_2COOH$−H_2O); ^{13}C NMR (25 MHz in D_2O) δ 174.7 (s), 171.9 (s), 77.3 (77.0)* (d), 75.5 (d), 74.5 (d), 73.8 (d), 72.1 (d), 68.7 (d), 68.1 (d), 65.6 (d), 65.4 (d), 64.4 (t), 45.5 (t), 42.8 (d, t), 41.1 (d×2), 38.5 (d), 37.6 (t), 32.6 (t), 28.1 (t), 19.8 (15.2) (q), 15.6 (q), 13.5 (q), 10.3 (9.5) (q), 10.0 (q) and others.

7: ^{13}C NMR (25 MHz in acetone-d_6+D_2O) 77.5, 75.5, 74.9, 74.0, 68.1, 67.9, 65.8, 65.7, 64.5, 45.9, 43.0, 41.4, 40.4, 38.9, 37.6, 36.2, 28.5, 20.0, 15.6, 14.3, 13.5, 10.6, 10.3 and others.

7-Acetate: SIMS m/z 1,249 (M+H); ^1H NMR (100 MHz in C_6D_6) δ 5.14 (1H, H-30), 5.3 ~ 4.7 (10 ~ 12H), 3.96 (2H, d, H-5), 2.1 (1H, H-6), 2.4 ~ 1.4 (30 ~ 40H, acetyl methyls and others), 1.13 (3H, d, H-50), 1.05 ~ 0.7 (12 ~ 15H, H-46 and others); ^{13}C NMR (25 MHz in C_6D_6) δ 170.2, 170.1, 169.9 and 169.6 (s, acetyl carbonyls), 74.9 (d), 74.2 (d), 72.4 (d), 72.0 (d), 71.8 (d), 69.0 (d), 68.8 (d), 66.8 (d), 65.2 (t),

* The signals shown in parentheses were split because of the stereoisomerism at C-38 or C-30 and/or C-17.

40.1 (d), 40.0 (t×2), 37.1 (t), 37.0 (d), 36.4 (d×2), 36.2 (t), 34.1 (t), 32.1 (t), 30.3 (t), 28.1 (t), 21.0, 20.9, 20.7 and 20.5 (q, acetyl methyls), 17.1 (q), 14.9 (q), 14.2 (q), 13.7 (q), 10.0 (q) and others.

Alkaline Hydrolysis of **4**

A solution of **4** (5 mg) in 1 N aq KOH (5 ml) was heated to reflux for 4 hours under gentle bublling of nitrogen gas, and evolved gas was passed through 1 N aq HCl. The aq HCl was evaporated off to give methylamine hydrochloride (1 mg), which was identified by comparison of the TLC and ^{1}H NMR with those of a standard sample.

Alkaline Hydrolysis of **5**

To a suspension of **5** (18 mg) in MeOH (2 ml), 2 N aq KOH (2 ml) was added and the solution was allowed to stand at room temp overnight, neutralized with dilute aq HCl, and concentrated to dryness under reduced pressure. The residue was extracted with MeOH and from the extract **8** (8 mg) was obtained by a preparative silica gel TLC with EtOAc. **8** (8 mg) was treated with Ac_2O - pyridine and DMAP to give **8**-acetate (10 mg).

8: SIMS *m/z* 204 (M^+); ^{13}C NMR see Table 3.

8-Acetate: EIMS *m/z* 271 (M^+ −OAc), 227, 210, 201, 186, 173; ^{13}C NMR (25 MHz in $CDCl_3$) δ 171.1 and 170.9 (s, acetyl carbonyls), 80.7 (79.4)* (d, C-38), 68.7 (69.2) (d, C-35), 64.7 (t, C-31), 40.6 (40.3) (t), 34.1 (d), 30.7 (31.0) (d), 28.1 (28.4) (t), 25.9 (t), 21.2, 20.9 and 20.8 (q, acetyl methyls), 15.8 (q), 13.7 (13.0) (q).

8-Acetate (8 mg) was separated into **8**-acetate(α) (2 mg) and **8**-acetate(β) (2 mg) by reversed phase HPLC with MeOH - H_2O (7:3).

8-Acetate(α): ^{1}H NMR see Table 4.

8-Acetate(β): ^{1}H NMR (400 MHz in $CDCl_3$) δ 5.06 (1H, m, H-38), 4.76 (1H, dd, J=4, 3 Hz, H-35), 4.03 (2H, m, H-31), 2.07, 2.04 and 2.02 (9H, 3×s, acetyl methyls), 1.83 (1H, m, H-36), 1.75 (1H, m, H-34), 1.73 (1H, m, H-32a), 1.51 (1H, m, H-32b), 1.42 (1H, m, H-37a), 1.40 (1H, m, H-33a), 1.38 (1H, m, H-37b), 1.18 (3H, d, J=7 Hz, H-53), 1.12 (1H, m, H-33b), 0.89 (3H, d, J=7 Hz, H-51), 0.86 (3H, d, J=7 Hz, H-52).

Periodate Oxidation of **6** and **7**

A solution of **6** (84 mg) and $NaIO_4$ (240 mg) in H_2O (10 ml) was stirred for 2 hours at room temp. Excess $NaIO_4$ was decomposed with ethylene glycol (0.1 ml), and to the solution $NaBH_4$ (150 mg) in 0.1 N aq NaOH (1.5 ml) was added. The solution was stirred for an hour before neutralization with dilute aq HCl and concentrated to dryness under reduced pressure. The residue was extracted with MeOH, and the extract was chromatographed on a silica gel column with $CHCl_3$ - MeOH - H_2O (65:25:4) to obtain **10** (24 mg) and **11** (20 mg).

10 (20 mg) and **11** (20 mg) were treated with Ac_2O - pyridine and DMAP to give **10**-acetate and **11**-acetate, which were purified by silica gel column chromatographies with benzene - acetone (9:1) to obtain 24 mg and 26 mg, respectively.

10: SIMS *m/z* 323 (M+H), 345 (M+Na); ^{13}C NMR see Table 5.

10-Acetate: EIMS *m/z* (relative intensity) 515 (1), 473 (3), 413 (3), 352 (4), 334 (4), 259 (18), 176 (10), 157 (20), 108 (100); ^{13}C NMR (25 MHz in $CDCl_3$) δ 170.9, 170.6, 170.4 and 170.3 (s, acetyl carbonyls), 74.0 (d), 72.4 (d), 71.9 (2×d), 65.3 (t, C-5), 61.1 (t, C-17), 39.3 (d), 36.3 (d), 36.0 (d), 33.4 (t), 29.9 (t), 29.2 (t), 28.0 (t), 21.0 and 20.9 (q, acetyl methyls), 14.8 (q), 13.6 (q), 9.8 (q); ^{1}H NMR (400 MHz in $CDCl_3$) δ 5.00 (1H, H-11), 4.94 (1H, H-7), 4.89 (1H, H-15), 4.80 (1H, H-9), 4.07 (1H, H-17), 4.02 (1H, H-5a), 3.98 (1H, H-5b), 2.11 (1H, H-6), 2.06, 2.05, 2.04 and 2.01 (18H, 6×s, acetyl methyls), 2.00 (1H, H-8a), 1.83 (2H, H-8b and H-16), 1.72 (1H, H-12a), 1.40 (1H, H-12b), 1.32 (1H, H-13a), 1.10 (1H, H-13b), 0.97 (3H, d, H-47), 0.89 (3H, d, H-48).

11: ^{13}C NMR (25 MHz in CD_3OD) δ 69.8 (d), 67.0 (d), 66.6 (d), 66.4 (d), 66.1 (t, C-29), 60.3 (t, C-19), 46.8 (t), 43.5 (t), 42.6 (d, C-28), 41.5 (t), 11.4 (q, C-49).

11-Acetate: EIMS *m/z* (relative intensity) 417 (3), 356 (3), 296 (20), 255 (40), 236 (65), 176 (40), 135 (100); ^{1}H NMR and ^{13}C NMR see Table 6.

Ozonolysis after Periodate Oxidation of Guanidylfungin A

To a suspension of guanidylfungin A (0.5 g) in MeOH (40 ml) $NaIO_4$ (0.6 g) in H_2O (10 ml) was

added. After 2 hours of stirring at room temp, ethylene glycol (0.6 ml) was added to decompose excess $NaIO_4$. The reaction solution was then ozonized at $-78°C$, and treated with $NaBH_4$ (1 g) in MeOH (10 ml) for 2 hours at 0°C. Excess $NaBH_4$ was decomposed by neutralization with dilute aq HCl and the solution was passed through a column of Amberlite CG-50 (H^+, 200 ml). The column was washed with MeOH - H_2O (2: 1, 500 ml) and H_2O (500 ml), and eluted with aq 1 N HCl. The eluate was neutralized with dilute aq NaOH and concentrated to dryness under reduced pressure. The residue was extracted with MeOH to obtain **4** (41 mg). The washings of the Amberlite CG-50 column were combined with the effluent from the column, evaporated to remove MeOH and subjected to a HP-20 column (200 ml). The column was washed with H_2O and eluted with MeOH. The eluate was concentrated to dryness under reduced pressure and the residue was chromatographed on a silica gel column with $CHCl_3$ - MeOH - H_2O (65: 25: 4 and 6: 4: 1) and MeOH to obtain **5** (140 mg), **12** (76 mg), **13** (13 mg), **14** (40 mg), **16** (40 mg) and crude **15** (29 mg).

12 (10 mg) was treated with Ac_2O - pyridine and DMAP to obtain its acetate, which was then methylated with diazomethane in chloroform to give **12′** (4 mg).

13 (5 mg) and **14** (3 mg) were treated with Ac_2O - pyridine and DMAP to give **13**-acetate (2 mg) and **14**-acetate (1 mg), respectively.

12′,**13**-Acetate and **14**-acetate were purified by silica gel HPLC with benzene - acetone (20: 1).

The crude **15** was treated with Ac_2O - pyridine and DMAP, and the acetylated product was purified by silica gel HPLC with benzene - acetone (10: 1) to obtain **15**-acetate(α) (3 mg) and **15**-acetate(β) (3 mg).

16 was treated with diazomethane in MeOH and then acetylated with Ac_2O - pyridine to give **16′**. **16′** was purified by silica gel HPLC with benzene - acetone (9: 1) to obtain **16′**(α) (5 mg) and **16′**(β) (24 mg).

12: ^{13}C NMR see Table 5.

12′: SIMS m/z 501 (M+H), 441 (M−OAc), 381, 321; ^1H NMR (100 MHz in $CDCl_3$) δ 6.82 (1H, dd, J=8, 16 Hz, H-15), 5.78 (1H, d, J=16 Hz, H-16), 5.2~4.6 (3H, H-7, H-9 and H-11), 3.99 (2H, d, J=6 Hz, H-5), 3.73 (2H, d, J=6 Hz, H-5), 3.73 (3H, s, OCH_3), 2.25 (1H, m, H-14), 2.09 (1H, m, H-6), 2.06, 2.04 and 2.02 (4×s, acetyl methyls), 2.0~1.1 (5~10H), 1.05 (3H, d, J=7 Hz, H-48), 0.95 (6H, 2×d, J=7 Hz, H-46 and H-47).

13: ^{13}C NMR see Table 5.

13-Acetate: SIMS m/z 647 (M+H), 587 (M−OAc), 527, 467, 407, 347; ^1H NMR (100 MHz in $CDCl_3$) δ 5.05 (1H, m, H-17), 5.2~4.6 (4H, H-7, H-9, H-11 and H-15), 4.18 (2H, dd, J=5, 10 Hz, H-18), 4.00 (2H, d, J=6 Hz, H-5), 2.1 (1H, H-6), 2.07, 2.05, 2.03 and 2.01 (acetyl methyls), 2.1~1.1 (8~12H), 0.95 (6H, 2×d, J=7 Hz, H-46 and H-47), 0.88 (3H, d, J=7 Hz, H-48).

14: ^{13}C NMR see Table 5.

14-Acetate: SIMS m/z 647 (M+H), 587 (M−OAc), 527, 467, 407, 347.

15-Acetate(α): ^1H NMR (100 MHz in $CDCl_3$) δ 5.13 (1H, m, H-30), 5.2~4.8 (5H, H-21, H-23, H-25, H-27 and H-29), 4.08 (2H, t, H-19), 2.07, 2.04, 2.03, 2.01 and 1.99 (acetyl methyls), 2.1~1.6 (8~10H), 1.19 (3H, d, J=6 Hz, H-50), 1.02 (3H, d, J=7 Hz, H-49).

15-Acetate(β): ^1H NMR (100 MHz in $CDCl_3$) δ 5.2~4.7 (6H, H-21, H-23, H-25, H-27, H-29 and H-30), 4.08 (2H, t, H-19), 2.07, 2.04, 2.03, 2.01 and 1.99 (acetyl methyls), 2.1~1.6 (8~10H), 1.15 (3H, d, J=6 Hz, H-50), 0.94 (3H, d, J=6 Hz, H-49)

16: ^{13}C NMR (25 MHz in D_2O) δ 174.8 (s), 172.0 (s), 77.4 (77.1)* (d, C-30), 71.0 (71.4) (d), 68.7 (d), 68.1 (d), 65.8 (66.0) (d), 65.6 (65.3) (d), 59.5 (t), 45.0 (45.3) (t), 43.0 (42.8) (t), 42.5 (t), 42.0 (t), 41.1 (40.4) (d, C-28), 40.0 (t), 19.9 (15.9) (q, C-50), 10.3 (9.6) (q, C-49).

16′(α): EIMS m/z 575 (M^+ −CH (CH_3)OAc), 475, 473, 415, 413, 382, 355, 322; ^1H NMR and ^{13}C NMR see Table 6.

16′(β): ^{13}C NMR (25 MHz in $CDCl_3$) δ 170.4 and 165.8 (s, C-54, C-56 and acetyl carbonyls), 73.3 (d, C-30), 69.4 (d), 68.6 (d), 68.3 (d), 67.4 (2×d), 60.6 (t, C-19), 52.4 (q, OCH_3), 41.4 (t, C-55), 39.5 (t), 39.2 (t), 37.3 (d, C-28), 36.6 (t), 33.6 (t), 21.1 and 21.0 (q, acetyl methyls), 13.3 (q, C-50), 10.2 (q, C-49).

Preparation of Methylguanidylfungin A (3) from Guanidylfungin A

The powder of guanidylfungin A (0.6 g) was solubilized in 0.5 N methanolic hydrochloride (4 ml),

and the solution was allowed to stand for 20~30 minutes at room temp and subjected to preparative liquid chromatography on PrepPak 500/C_{18} with MeOH - 0.01 M aq NH_4OAc (76: 24). The fractions containing **3** were collected, concentrated to dryness under reduced pressure and crystallized from hot aq acetone to obtain fine needles of **3** (0.3 g).

3: MP 144~146°C; SIMS *m/z* 1,144 (M+H); *Anal* Cacld for $C_{59}H_{105}N_3O_{18}$: C 61.94, H 9.19, N 3.67, O 25.20. Found: 61.43, H 9.24, N 3.55, O 25.25; ^{1}H NMR see Table 2; ^{13}C NMR see Table 1.

Ozonolysis of Methylguanidylfungin A (3)

3 (1 g) in MeOH (100 ml) was ozonized at −78°C, and the resultant ozonide was decomposed by addition of $NaBH_4$ (1 g) in MeOH (10 ml) at 0°C. The reaction by $NaBH_4$ was stopped by neutralization with dilute aq HCl. The products by ozonolysis purified by a silica gel column chromatography with $CHCl_3$ - MeOH - H_2O (65: 25: 4) and MeOH to obtain **4** (61 mg), **5** (87 mg), **17** (113 mg) and **18** (275 mg).

18 (61 mg) was treated with Ac_2O - pyridine and DMAP to give **18′** (53 mg), which was separated into **18′**(*α*) (8 mg) and **18′**(*β*) (9 mg) by silica gel HPLC with benzene - acetone (5: 1).

17 failed to give its objective acetate by treatment with Ac_2O - pyridine and DMAP.

17: ^{13}C NMR (25 MHz in D_2O) δ 174.7 (s), 172.0 (171.7)* (s), 99.8 (102.2) (s, C-17), 77.0, 74.5, 73.8, 72.0, 69.0, 68.6, 68.0, 65.5, 64.4, 53.4 (q, OCH_3), 42.8, 41.1, 40.4, 39.8, 39.3, 37.7, 32.7, 28.8, 19.9, 15.3, 13.6, 10.4, 10.1, 9.6.

18: ^{13}C NMR (25 MHz in C_6D_6) δ 99.9 (101.5)* (s, C-17), 77.3, 74.6, 73.8, 72.0 (2×C), 69.0, 68.6, 66.4, 65.9 (2×C), 64.4, 45.5, 43.2, 42.9 (2×C), 41.4, 41.1 (2×C), 40.4, 39.8, 39.2, 37.6, 32.6, 28.9, 19.9 (16.0), 15.3, 13.6 (10.2), 10.4.

18′(*α*): ^{13}C NMR (25 MHz in C_6D_6) δ 192.3 (s, C-17), 170.1 and 169.8 (s, acetyl carbonyls), 153.5 (d, C-15), 125.2 (d, C-16), 77.8 (d, C-30), 75.0 (d), 72.4 (d), 72.0 (d), 71.7 (d), 69.2 (d), 68.9 (d), 68.1 (d), 67.7 (d), 66.9 (d), 66.6 (d), 65.2 (t, C-5), 39.9 (2×t), 39.0 (d), 37.2 (t), 37.0 (2×d), 36.5 (d), 35.8 (t), 34.3 (t), 32.2 (t), 30.2 (t), 20.8, 20.5 and 20.1 (q, acetyl methyls), 19.4 (q), 17.1 (q), 13.7 (q), 10.1 (q), 9.9 (q); SIMS *m/z* 1,145 (M+H).

18′(*β*): ^{13}C NMR (25 MHz in C_6D_6) δ 192.3 (s, C-17), 170.2 and 169.8 (s, acetyl carbonyls), 153.5 (d, C-15), 125.2 (d, C-16), 77.8 (d, C-30), 73.4 (d), 72.4 (d), 72.0 (d), 71.7 (d), 69.5 (d), 68.6 (d), 68.0 (d), 67.6 (d), 66.9 (d), 66.5 (d), 65.2 (t, C-5), 39.8 (2×t), 39.5 (d), 37.9 (d), 37.0 (t), 36.4 (d), 35.9 (t), 34.3 (t), 32.2 (t), 30.2 (t), 20.8, 20.7, 20.5 and 20.2 (q, acetyl methyls), 19.4 (q), 17.1 (q), 13.7 (q), 10.1 (q), 9.9 (q); SIMS *m/z* 1,145 (M+H).

Periodate Oxidation of **17** and **18**

A solution of **17** (95 mg) and $NaIO_4$ in H_2O (5.6 ml) was stirred at room temp for 3 hours. After excess $NaIO_4$ was decomposed by addition of ethylene glycol (0.1 ml), $NaBH_4$ (150 mg) in 0.1 N aq NaOH (1.5 ml) was added and the solution was stirred for 3 hours. The solution was chromatographed on a Diaion HP-20 column (200 ml) after neutralization with dilute aq HCl. The column was washed with H_2O (400 ml) and eluted with MeOH (400 ml). The eluate was subjected to preparative TLC with $CHCl_3$ - MeOH - H_2O (65: 25: 4) to obtain **10** (30 mg), **11** (6 mg), **12** (3 mg), **13** (5 mg) and **14** (3 mg).

18 was similarly treated with $NaIO_4$ and $NaBH_4$ to obtain the degradation products of **10**, **11** and **12** by preparative silica gel TLC.

Those degradation products were identified by silica gel TLC.

Alkaline Hydrolysis of Guanidylfungin A, **7**, **16** and **17**

To a suspension of guanidylfungin A (0.5 g) in MeOH (50 ml) 2 N aq KOH was added and the solution was allowed to stand at room temp overnight, neutralized with dilute aq HCl and concentrated to dryness under reduced pressure. The residue was extracted with ethyl ether, and the extract was methylated with diazomethane in ethyl ether. The reaction mixture was subjected to a silica gel column chromatography with $CHCl_3$ - EtOAc (10: 1) to isolate **9** (23 mg).

7, **16** and **17** were similarly hydrolyzed with KOH and then methylated with diazomethane to obtain **9**.

9: ^{1}H NMR (100 MHz in $CDCl_3$) 3.78 (6H, 2×s, OCH_3), 3.42 (2H, s, H-55); IR (neat) cm^{-1}

3700~3000, 2950, 2850, 1740, 1440, 1340, 1280, 1200, 1150, 1020.

9 was identified as dimethyl malonate by comparison with a standard sample on GC (ethylene glycol adipate 2%, 2 m, 140°C, He).

Purification of ^{13}C-Labeled **1** and Preparation of ^{13}C-Labeled **3**

The mycelial cake separated by centrifugation from the cultured broth (200 ml) was extracted with acetone - H_2O (7: 3). The extract was concentrated to a brownish paste under reduced pressure. The paste was subjected to a preparative silica gel TLC with $CHCl_3$ - MeOH - H_2O (65: 25: 4). The fractions containing **1** were collected and eluted with MeOH. The eluate was concentrated to dryness under reduced pressure and the residue was dissolved in hot aq acetone. The solution was allowed to stand at room temp overnight to obtain a white powder of ^{13}C-labeled **1** ([^{13}C]acetate labeled **1**: 40 mg, [^{13}C]-propionate labeled **1**: 40 mg). The labeled **1** (40 mg) was treated with 0.5 N methanolic hydrochloride at room temp for 20 minutes, and the solution was chromatographed on reversed phase HPLC with MeOH - 0.01 M aq NH_4OAc (80: 20) to obtain the labeled **3** ([^{13}C]acetate labeled **3**: 20 mg, [^{13}C]propionate labeled **3**: 5 mg).

Addendum in Proof

Scopafungin[9] was found to be identical with niphimycin I[5] and the structure of scopafungin was revised[10].

Acknowledgments

We wish to thank Dr. H. SETO, Mr. K. FURIHATA, Dr. M. NAMIKOSHI and Dr. A. ISOGAI of The University of Tokyo for valuable technical assistance and for helpful discussions.

References

1) TAKESAKO, K. & T. BEPPU: Studies on new antifungal antibiotics, guanidylfungins A and B. I. *Taxonomy*, fermentation, isolation and characterization. J. Antibiotics 37: 1161~1169, 1984
2) NAMIKOSHI, M.; K. SASAKI, Y. KOISO, K. FUKUSHIMA, S. IWASAKI, S. NOZOE & S. OKUDA: Studies on macrocyclic lactone antibiotics. I. Physicochemical properties of azalomycin F_{4a}. Chem. Pharm. Bull. 30: 1653~1657, 1982
3) FUKUSHIMA, K.; T. ARAI, S. IWASAKI, M. NAMIKOSHI & S. OKUDA: Studies on macrocyclic lactone antibiotics. VI. Skeletal structure of copiamycin. J. Antibiotics 35: 1480~1494, 1982
4) NAMIKOSHI, M.; S. IWASAKI, K. SASAKI, M. AMANO, K. FUKUSHIMA, S. NOZOE & S. OKUDA: Studies on macrocyclic lactone antibiotics. II. Partial structures of azalomycin F_{4a}. Chem. Pharm. Bull. 30: 1669~1673, 1982
5) KELLER-SCHIERLEIN, W.; B. JOOS, H. P. KAISER & P. GASSMANN: Versuche zur Strukturaufklärung von Niphimycin. 2. Die Konstitution von Desmalonylniphimycin I. Helv. Chim. Acta 66: 226~258, 1983
6) IWASAKI, S.; K. SASAKI, M. NAMIKOSHI & S. OKUDA: Studies on macrocyclic lactone antibiotics. IV. Biosynthetic studies on azalomycin F_{4a} using ^{13}C-labelled acetate and propionate. Heterocycles 17: 331~335, 1982
7) IWASAKI, S.; M. NAMIKOSHI, K. SASAKI, K. FUKUSHIMA & S. OKUDA: Studies on macrocyclic lactone antibiotics. V. The structures of azalomycins F_{3a} and F_{5a}. Chem. Pharm. Bull. 30: 4006~4014, 1982
8) ARAI, T.; J. UNO, I. HORIMI & K. FUKUSHIMA: Isolation of neocopiamycin A from *Streptomyces hygroscopicus* var. *crystallogenes*, the copiamycin source. J. Antibiotics 37: 103~109, 1984
9) SAMAIN, D.; C. COOK, Jr. & K. L. RINEHART, Jr.: Structure of scopafungin, a potent nonpolyene antibiotic. J. Am. Chem. Soc. 104: 4129~4141, 1982
10) GASSMANN, P.; L. HAGMANN, W. KELLER-SCHIERLEIN & D. SAMAIN: Versuche zur Strukturaufklärung von Niphimycin. 3. Identität von Scopafungin mit Niphimycin I und Lage des Malonylreste in Niphimycin und Copiamycin. Helv. Chim. Acta 67: 696~705, 1984.