FLUVIRUCINS A_1, A_2, B_1, B_2, B_3, B_4 AND B_5, NEW ANTIBIOTICS ACTIVE AGAINST INFLUENZA A VIRUS

II. STRUCTURE DETERMINATION

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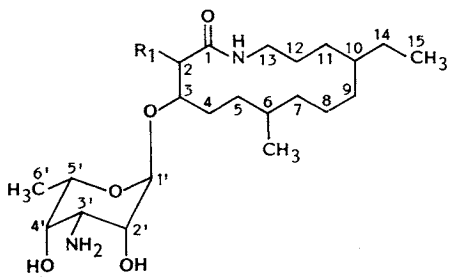
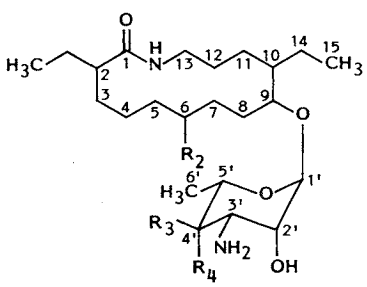
A series of structurally related antiviral antibiotics, fluvirucins A_1, A_2, B_1, B_2, B_3, B_4 and B_5 have been isolated from the fermentation broths of five unidentified actinomycete isolates. Based on spectroscopic analysis, partial degradation experiments and ^{13}C-enriched acetic acid-fed biosynthetic studies, their structures were elucidated to be 2,6,10-trialkyl-3(or 9)-aminoglycosyl-13-tridecanelactams.

In the course of our fermentation screening for agents with activity against influenza A virus, five soil actinomycete strains were discovered to produce active agents with closely related structures. These were designated fluvirucins A_1 (**1**), A_2 (**2**), B_1 (**3**), B_2 (**4**), B_3 (**5**), B_4 (**6**) and B_5 (**7**). In the preceding paper[1], the preliminary taxonomical study of the producing strains and production, isolation, physico-chemical

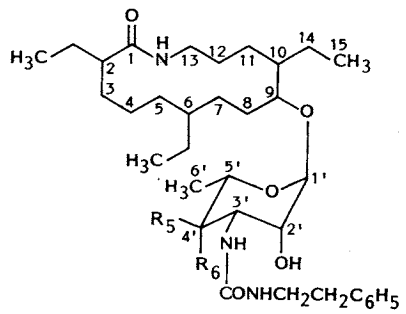
Fig. 1. Structures of fluvirucins.



Fluvirucin A_1 $R_1=CH_3$
Fluvirucin A_2 $R_1=CHOHCH_3$

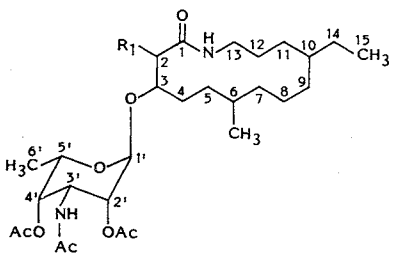


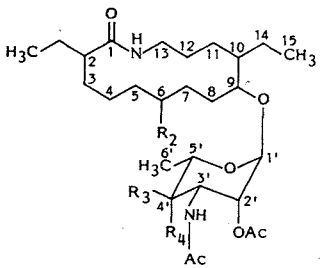
Fluvirucin B_1 $R_2=CH_3$ $R_3=H$ $R_4=OH$
Fluvirucin B_2 $R_2=C_2H_5$ $R_3=OH$ $R_4=H$
Fluvirucin B_3 $R_2=C_2H_5$ $R_3=H$ $R_4=OH$

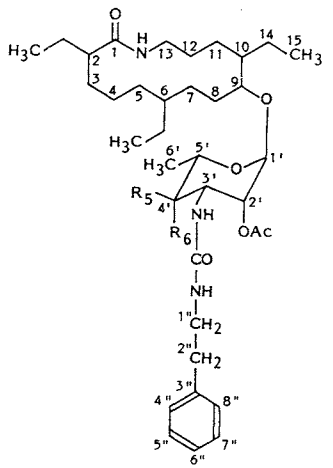


Fluvirucin B_4 $R_5=OH$ $R_6=H$
Fluvirucin B_5 $R_5=H$ $R_6=OH$

Table 1. ^{13}C NMR data (100 MHz in $CDCl_3$).







	Triacetylfluvirucin A_1 (**8**)	Tetraacetylfluvirucin A_2 (**14**)	Triacetylfluvirucins			Diacetylfluvirucins	
			B_1 (**17**)	B_2 (**25**)	B_3 (**29**)	B_4 (**30**)	B_5 (**31**)
	$R_1=CH_3$	$R_1=CH(OAc)CH_3$	$R_2=CH_3$ $R_3=H$ $R_4=OAc$	$R_2=CH_2CH_3$ $R_3=OAc$ $R_4=H$	$R_2=CH_2CH_3$ $R_3=H$ $R_4=OAc$	$R_5=OAc$ $R_6=H$	$R_5=H$ $R_6=OAc$
1	174.1	170.8	176.0	175.9	176.0	176.0	176.1
2	45.0	54.8	50.7	50.5	50.5	50.5	50.5
2-CH_3	15.8	2-$\underline{CH}$(OAc)CH_3 68.5 2-CH(OAc)$\underline{CH_3}$ 17.8	2-$\underline{CH_2}CH_3$ 26.4 2-$CH_2\underline{CH_3}$ 12.2	26.9 12.1	27.0 12.2	27.1 12.2	27.1 12.2

3	82.8	79.3	33.4	31.5	31.6	31.6	31.6
4	25.7	26.4	24.5	24.8	24.87	24.7	24.7
5	26.4	26.9	34.1	33.3	33.4	33.4	33.4
6	30.9	30.9	30.9	38.3	38.4	38.5	38.6
6-CH_3	20.5	20.8	20.8	6-$\underline{CH_2}CH_3$ 22.8	22.9	22.8	22.8
				6-$CH_2\underline{CH_3}$ 12.3	12.3	12.3	12.4
7	33.9	33.6	24.9	24.8	24.91	25.1	25.1
8	22.9	21.8	21.4	22.3	22.3	23.3	23.3
9	32.0	31.7	77.9	78.4	78.3	78.4	78.6
10	37.9	37.6	40.5	40.6	40.6	41.1	41.1
11	27.01	27.4	25.2	26.3	26.4	26.5	26.5
12	23.5	23.8	28.1	28.1	28.0	28.4	28.2
13	39.2	39.6	38.6	38.5	38.6	38.6	38.7
14	26.95	27.2	21.1	21.3	21.3	21.9	21.9
15	11.8	11.8	8.6	8.6	8.7	9.1	9.1
1′	99.0	98.4	94.6	94.0	95.0	94.4	95.4
2′	69.3	69.9	70.7	72.1	70.0	72.6	70.5
3′	44.8	45.2	44.9	48.3	45.0	49.3	45.9
4′	70.1	70.0	70.2	72.9	70.2	73.6	70.9
5′	65.4	65.8	65.4	66.5	65.4	66.8	65.6
6′	16.3	16.4	16.4	17.3	16.3	17.5	16.5
1″						41.6	41.7
2″						36.3	36.2
3″						139.0	139.1
4″, 8″						128.7	128.9
5″, 7″						128.5	128.5
6″						126.4	126.3
$\begin{matrix}NH\\NH\end{matrix}\!>\!C=O$						156.9	156.7

properties and biological activities of the seven components were described. Here we report the structure determination of the fluvirucins based on the spectroscopic data and results of chemical degradation and biosynthesis experiments by feeding [1-^{13}C]-, [2-^{13}C]- and [1,2-$^{13}C_2$]acetates, and [1-^{13}C]propionate.

Since fluvirucin A_1 (**1**) was available in the largest quantities, structural studies were first focused on this compound. The structures of the other components were determined on the basis of spectroscopic and degradation correlation to fluvirucin A_1.

The FAB-MS (*m/z* 429, (M+H)) and elemental analysis of **1** established its molecular formula as $C_{23}H_{44}N_2O_5$. This assignment was corroborated by its triacetate (**8**: $C_{29}H_{50}N_2O_8$ *m/z* 554, M) obtained by acetylation of **1** in pyridine. The ^{13}C NMR spectrum of **8** displayed 29 signals (Table 1) including 4 methyl, 9 methylene, 9 methine and one carbonyl carbon in addition to 6 carbons attributable to three acetyl groups (δ 20.8, 21.1, 23.2, 169.5, 170.5 and 170.7). The carbonyl group of **8** was considered to be an amide function from the ^{13}C NMR chemical shift (δ 174.1) and the IR absorption (1635 and 1540 cm^{-1}) of **1**. The presence of a sugar moiety was suggested by the ^{1}H NMR spectra of **1** and **8** (anomeric proton: δ 4.87, d, J=1.3 Hz and δ 4.96, br s, respectively) and ^{13}C NMR spectrum of **8** (anomeric carbon: δ 99.0).

Structure of Fluvirucinine A_1 (9), the Aglycone of Fluvirucin A_1

When heated with 5 N methanolic hydrogen chloride, **1** afforded a crystalline aglycone (fluvirucinine A_1: **9**) and a methyl glycoside (**10**). **9** possessed the IR absorption at 1640 and 1545 observed for **1** and was assigned a molecular formula of $C_{17}H_{33}NO_2$ by MS (*m/z* 283, M) and microanalysis. Upon acetylation **9** gave a mono-*O*-acetate (**11**: *m/z* 325, M, $\nu_{C=O}$ cm^{-1} 1730) and on pyridinium dichromate oxidation a keto derivative (**12**: *m/z* 281, M, $\nu_{C=O}$ cm^{-1} 1720). The ^{13}C and ^{1}H NMR spectra of **11** showed 4 methyl, 9 methylene, 4 methine and 2 carbonyl groups. Most of them were analyzed as the partial structure illustrated in Fig. 2 by ^{1}H NMR and ^{1}H-^{1}H 2D-COSY spectra. In order to further expand the carbon skeleton sequence of **9** by 2D-incredible natural abundance double quantum transfer experiment (INADEQUATE), it was converted to a more soluble *N,O*-dimethyl derivative (**13**: $C_{19}H_{37}NO_2$ *m/z* 311, M) by HAKOMORI's permethylation method[2]. Starting from the C-1 carbonyl carbon signal at δ 174.5 the ^{13}C-^{13}C connectivities were established up to the C-11 methylene carbon at δ 27.1, including the three side chains at C-2 (methyl), C-6 (methyl) and C-10 (ethyl). On the other hand, the amide nitrogen-bearing methylene carbon at δ 48.0 (C-13) was proved to be adjacent to the methylene carbon at δ 23.3 (C-12). The results are also summarized in Fig. 2. From this experiment, 2,6-dimethyl-10-ethyl-3-hydroxy-13-tridecanelactam structure for **9** became reasonable by linking C-11 and C-12 methylenes, and the carbonyl and nitrogen although a direct correlation between them was not observed in the spectra.

Structure of Sugar 10

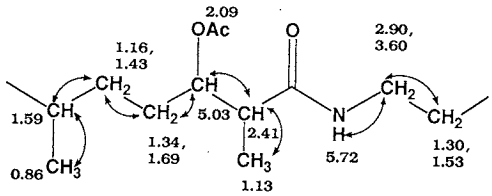
Methyl glycoside **10** was separated into the major α-methyl glycoside (**10a**) and minor β-methyl glycoside (**10b**) by preparative TLC followed by ion exchange chromatography. The ammonia-added EI-MS (*m/z* 178, (M+H)) and microanalysis established their molecular formula as $C_7H_{15}NO_4$. Their ^{1}H NMR and 2D-COSY spectra allowed the assignment of methyl α- and β-3-amino-3,6-dideoxytalopyranosides to **10a** and **10b**, respectively.

Although this sugar has been chemically synthesized[3], its isolation from natural sources has not been reported. By comparison of the optical rotational value of **10b** (+35° in H_2O) with literature data, the sugar was established as the L-series.

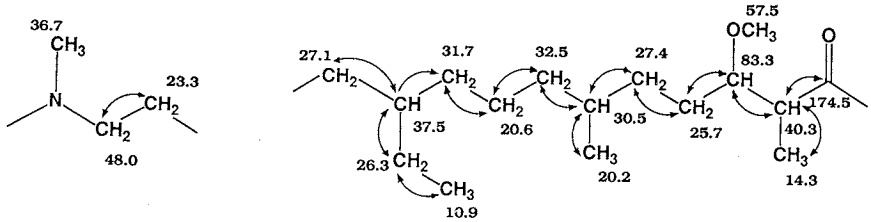
For confirmation of the structure, methyl 3-amino-3,6-dideoxy-α-D-talopyranoside was synthesized

Fig. 2. Partial structures of fluvirucinine A_1.

2D-COSY of acetylfluvirucinine A_1:



2D-INADEQUATE of *N*,*O*-dimethylfluvirucinine A_1:



from methyl α-mycosaminide obtained by acid methanolysis of amphotericin B[4,5]. Methyl α-mycosaminide was *N*-acetylated, *O*-mesylated, and then heated with sodium acetate in 2-methoxyethanol to convert its 4-hydroxyl from *trans* to *cis* with respect to 3-amino group[6,7]. Re-*O*-mesylation of the product yielded methyl 3-acetamido-3,6-dideoxy-2,4-di-*O*-mesyl-α-D-talopyranoside which was identical with the *N*-acetyl-*O*,*O*-dimesyl derivative of **10a** in chemical and spectral data. CD spectra of these two sugar derivatives, however, exhibited opposite Cotton curves indicating that **10a** is L.

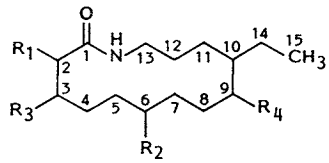
Structure of Fluvirucin A_1 (**1**)

Fluvirucinine A_1 (**9**) has only one hydroxyl group at C-3 and therefore sugar **10** should be liked to the C-3 hydroxyl group by a glycoside linkage in **1**. In the ^{13}C NMR spectrum, C-3 carbon of **8** exhibited significant downfield shift, and C-2 and C-4 carbons upfield shifts compared to those of **9** supporting the assumption. In the ^1H NMR spectra, the anomeric protons of both **10a** and **10b** appeared as narrow doublets ($J=1.1$ Hz) indicating that the bond angle between the anomeric proton and C-2 proton is about 60° in both anomers. The anomeric protons of **1** and **8** were also observed as a narrow doublet ($J=1.3$ Hz) and a broad singlet, respectively, which precluded application of the general rule of proton splitting for the assignment of sugar configuration. The anomeric protons of **1** and **8**, however, resonated at relatively low field (δ 4.87 and 4.96, respectively) suggesting the α-configuration. In the ^{13}C NMR spectrum, the anomeric carbon of **8** was observed at δ 99.0, nearly identical with that of **10a** and its ^{13}C-^1H coupling constant value measured by insensitive nuclear enhanced polarization transfer (INEPT) was $J=170$ Hz. These results clearly show that sugar **10** has the α-pyranoside configuration in **1**. Thus the whole structure of fluvirucin A_1 was established as shown in Fig. 1. This structure was confirmed by X-ray crystallographic analysis of **8** as described in the following paper[8].

Structure of Fluvirucin A_2 (**2**)

The molecular formula $C_{24}H_{46}N_2O_6$ assigned for **2** suggested that it had a CH_2O unit larger than **1**. This was substantiated by production of an *N*,*O*,*O*,*O*-tetraacetyl derivative (**14**: *m*/*z* 626, M) by acetyla-

Table 2. ^{1}H NMR data for acetylfluvirucinines.



Proton		Acetylfluvirucinine A_1 (**11**)	Acetylfluvirucinine A_2 (**16**)	Acetylfluvirucinine B_1 (**19**)	Acetylfluvirucinine B_2 (**28**)
	R_1=	CH_3	CH(OAc)CH_3	CH_2CH_3	CH_2CH_3
	R_2=	CH_3	CH_3	CH_3	CH_2CH_3
	R_3=	OAc	OAc	H	H
	R_4=	H	H	OAc	OAc
2-R_1		1.13[a], d[b], 7.0[c]	1.24, d, 6.4	0.88, t, 7.3	0.88, t, 7.5
6-R_2		0.86, d, 7.0	0.86, t, 6.8	0.85, d, 7.0	0.82, t, 7.5
15-H_3		0.84, t, 7.1	0.85, t, 7.7	0.82, t, 7.5	0.81, t, 7.5
2-H		2.41, dq, 9.4, 7.0	2.56, dd, 10.6, 3.4	1.95, m	1.95, m
3-H or 9-H[d]		5.03, dt, 2.9, 9.4	5.10, dt, 1.9, 10.6	4.81, ddd, 9.5, 5.5, 4.0	4.81, dt, 9.0, 4.7
13-H_2		2.96, m	2.89, dddd, 13.7, 5.7, 5.1, 1.3	2.89, m	2.93, m
		3.69, ddt, 13.6, 1.8, 8.1	3.70, ddt, 13.7, 3.0, 5.7	3.76, ddt, 13.9, 2.2, 8.1	3.74, ddt, 13.7, 2.1, 8.6
13-NH		5.72, br s	6.04, br t, 5.7	5.59, br s	5.58, br s
Ac		2.09, s	2.05, s	2.03, s	2.03, s
Ac			2.09, s		

[a] Chemical shifts in ppm relative to $CDCl_3$ as solvent reference (7.26 ppm).
[b] Multiplicity.
[c] J in Hz.
[d] Acetylfluvirucinines A_1 and A_2: 3-H, B_1 and B_2: 9-H.

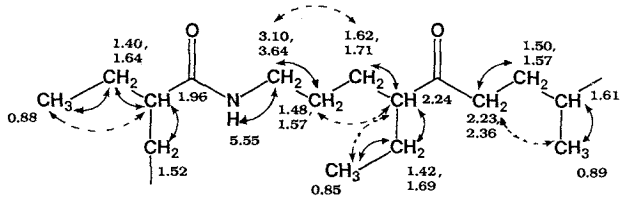
tion of **2**. Acid methanolysis of **2** yielded a mixture of **10a** and **10b** and fluvirucinine A_2 (**15**: $C_{18}H_{35}NO_3$). When acetylated in pyridine, **15** afforded a di-*O*-acetyl derivative (**16**), whose NMR spectra including 2D-COSY disclosed that a hydroxyl and a α-hydroxyethyl are at C-3 and C-2, respectively. Other spectral data of **16**, **14** and **2** corresponded well with those of **11**, **8** and **1**. (Tables 1 and 2)

Structure of Fluvirucin B_1 (**3**)

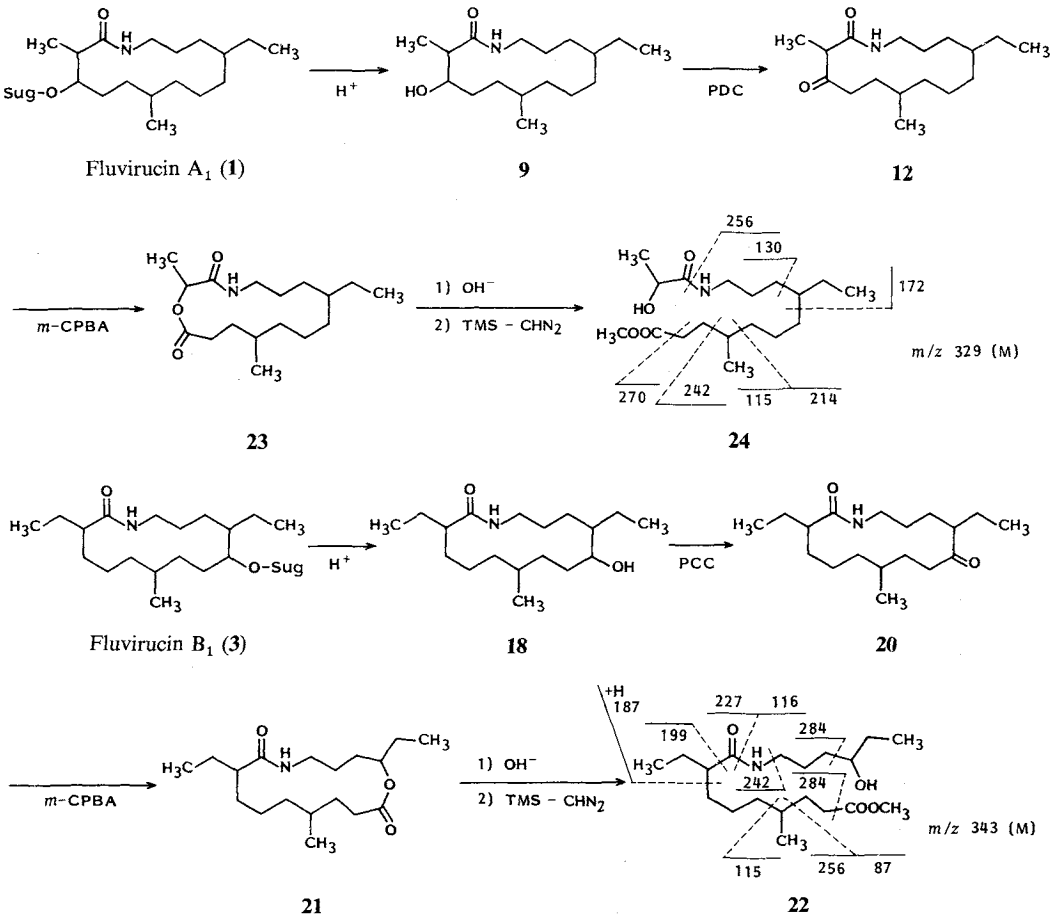
As discussed in the previous paper, though the seven fluvirucin components are quite similar to each other, the physico-chemical data and biological activity of **3** differ significantly from those of **1** and **2**, and much resemble those of **4** and **5**. The molecular formula of **3** ($C_{24}H_{46}N_2O_5$) is larger than that of **1** by CH_2. Two triplet methyls and two doublet methyls were observed in the ^{1}H NMR spectrum of **3** in contrast to one triplet methyl and three doublet methyls in that of **1**. Acetylation of **3** gave colorless needles of *N*,*O*,*O*-triacetylfluvirucin B_1 (**17**: *m*/*z* 568, M). Upon methanolysis, **3** afforded an anomeric mixture of methyl 3-amino-3,6-dideoxy-L-talopyranosides (**10a** and **10b**) and crystalline fluvirucinine B_1 (**18**: $C_{18}H_{35}NO_2$). Acetylation of **18** gave *O*-acetylfluvirucinine B_1 (**19**) which was shown to have an ethyl group at C-2 of the lactam ring instead of the methyl group in **11** by 2D-COSY. Unlike the spectrum of **11**, the COSY spectrum of **19** showed no correlation between the C-2 methine proton (δ 1.95, m) and the acetoxyl methine proton (δ 4.81, ddd), though the multiplicity of the latter proton indicated that it was adjacent to a methine and a methylene as in the case of **11**. The NOESY spectrum of **19** supported that the latter methine was also substituted by an another ethyl group. To clarify the position of the acetoxyl

Fig. 3. Partial structure of oxofluvirucinine B_1 (**20**).

^1H-^1H COSY (⟷) and relayed ^1H-^1H COSY (←→) of oxofluvirucinine B_1.



Scheme 1. Derivatization of fluvirucins A_1 (**1**) and B_1 (**3**).



group on the lactam ring, **18** was oxidized to a ketone derivative (**20**). Analysis of the 2D-COSY and relayed 2D-COSY spectra of **20** revealed the spin systems shown in Fig. 3. The spectra could not define the positions of remaining two methylenes. **20** was further converted into lactone **21** by Baeyer-Villiger oxidation, and then hydrolyzed with alkali and methylated with trimethylsilyldiazomethane to afford ester **22** (Scheme 1). A similar oxidation of **12** to lactone **23**, followed by alkali cleavage and methylation yielded ester derivative **24** of fluvirucin A_1. The EI-MS fragmentation patterns of **22** unambiguously established that fluvirucin B_1 had a methyl at C-6, an ethyl each at C-2 and C-10 and hydroxyl at C-9. The MS of

24 confirmed the substitutions on the lactam ring of fluvirucin A_1. α-Glyosidic linkage of 3-amino-3,6-dideoxy-L-talopyranoside to the C-9 hydroxyl was evidenced by the NMR spectrum of **17** (C-9: δ_H 3.59 and δ_C 77.9, C-1′: δ_C 94.6).

Structure of Fluvirucin B_2 (4)

The physico-chemical and biological properties of **4** were similar to those of **3** and the molecular formula of **4** ($C_{25}H_{48}N_2O_5$) was a CH_2 more than that of **3**. This, combined with the ^1H NMR spectrum of its triacetyl derivative **25** which exhibited one doublet methyl and three triplet methyls, suggested substitution of an ethyl group at C-6 on the core ring in **4** instead of the methyl group in **3**. In the ^{13}C NMR spectra of **17** and **25**, the C-6 carbon of **25** was observed at 7.4 ppm lower field than that of **17**, supporting the assigned C-6 ethyl substitution, and the other parts of the aglycone were identical. **4** was hydrolyzed in methanolic hydrogen chloride to yield fluvirucinine B_2 (**26**: $C_{19}H_{37}NO_2$) and α- and β-methyl glycosides of a sugar (**27a** and **27b**). The ^1H NMR spectrum of monoacetylfluvirucinine B_2 (**28**) displayed three triplet methyls (δ 0.81, 0.82 and 0.88), and the chemical shifts of the other protons of **28** agreed with those of **19** (Table 2). Sugar **27a** was identical with methyl α-D-mycosaminide[5] in physico-chemical and spectral properties, but its optical rotational value (**27a**: $[\alpha]_D$ −47°) was opposite in sign to that of the latter ($[\alpha]_D$ +54°)[5]. Thus, the sugar of **4** was determined to be L-mycosamine. The α-pyranoside structure of the sugar was established by ^1H and ^{13}C NMR analysis of **25** including an INEPT spectrum for analysis of the anomeric configuration (169 Hz).

Structures of Fluvirucins B_3 (5), B_4 (6) and B_5 (7)

The ^1H NMR spectra of **5**, **6** and **7** and their acetates (**29**, **30** and **31**, respectively) and the ^{13}C NMR spectra of these acetates indicated that the three components had the same aglycone (fluvirucinine B_2, **26**) as **4** and acid methanolysis of them in fact produced **26** in quantitative yield.

5 was identical in molecular formula ($C_{25}H_{48}N_2O_5$) to **4**, and upon methanolysis, yielded an anomeric mixture of methyl 3-amino-3,6-dideoxy-L-talopyranoside (**10a** and **10b**) in addition to **26**. These data coupled with the NMR data allowed us to assign 4′-*epi*-fluvirucin B_2 structure to **5**.

6 and **7** showed the same molecular formula ($C_{34}H_{57}N_3O_6$) and, unlike other components, they were negative to ninhydrin test. Their IR spectra indicated amide carbonyl absorptions ($\nu_{C=O}$ cm^{-1} 1640 and 1560) and their UV spectra (λ_{max} nm 246, 252, 258, 264 and 268) revealed a phenyl chromophore. ^1H and ^{13}C NMR (Table 1) and 2D-COSY spectra of **30** and **31** revealed that a 2-phenethylureide was attached to the 3′-amino group of the sugar moiety. Actually, 2-phenethylamine (**32**) was recovered from the total acid hydrolysate of **6** and **7**. Furthermore, acid methanolysis of **6** yielded an anomeric mixture of methyl 3-*N*-(2-phenethylaminocarbonyl)-L-mycosaminides (**33**) in addition to **26**, whereas **7** gave the 3-*N*-(2-phenethylaminocarbonyl) derivatives of methyl α- and β-3-amino-3,6-dideoxy-L-talopyranoside mixture (**34**) together with **26** by the hydrolysis. Interpretation of these spectral data and degradation results led to the complete structures of **6** and **7** as in Fig. 1.

Biosynthetic Study of Fluvirucin A_1 (1)

In order to confirm the assigned 14-membered lactam structure and to elucidate the biosynthetic pathway of **1**, incorporation experiments with [1-^{13}C]-, [2-^{13}C]- and [1,2-$^{13}C_2$]acetates and [1-^{13}C]propionate were carried out. The ^{13}C-enriched samples of **1** were isolated from the fermentation broth[1] and converted to the triacetates (**8**) which were analyzed by proton noise-decoupled ^{13}C NMR

Table 3. ^{13}C Enrichments in triacetylfluvirucin A_1 (**8**) derived from ^{13}C-labeled precursors.

Carbon No.	δ (ppm)	Enrichment factor[a]			Coupling constant (Hz)
		[1-^{13}C]Acetate	[2-^{13}C]Acetate	[1-^{13}C]Propionate	[1,2-$^{13}C_2$]Acetate
1	174.1			19	
2	45.0				
2-CH_3	15.8				
3	82.8	48			38.2
4	25.7		74		38.2
5	26.4			23	
6	30.9				
6-CH_3	20.5				
7	33.9	42			33.7
8	22.9		75		33.7
9	32.0	28			33.7
10	37.9		23		33.7
11	27.01	20			36.7
12	23.5		49		36.7[b]
13	39.2		36		36.7[b]
14	26.95	26			35.2
15	11.8		34		35.2
1′	99.0				
2′	69.3				
3′	44.8				
4′	70.1				
5′	65.4				
6′	16.3				
CH$_3$CO	20.8				
CH$_3$CO	21.1				
CH$_3$CO	23.2				
CH$_3$*CO*	169.5	1	1	1	
CH$_3$*CO*	170.5				
CH$_3$*CO*	170.7				

[a] Ratio of carbon signal intensities for enriched and natural abundance samples measured under identical conditions; for normalization the acetoxy carbon signal at 169.5 ppm was used as reference.

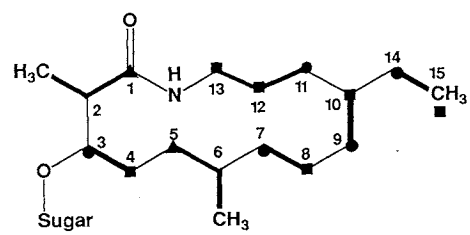
[b] This coupling was also observed in the case of [2-^{13}C]acetate enrichment.

spectroscopy. Table 3 shows the enriched carbons in **8** produced by feeding of [1-^{13}C]- and [2-^{13}C]-acetates and [1-^{13}C]propionate, and ^{13}C-^{13}C coupling constants for [1,2-$^{13}C_2$]acetate-enriched **8**.

The enriched factors observed for C-3～C-4, C-7～C-8, C-9～C-10 and C-14～C-15 of [1-^{13}C]- and [2-^{13}C]acetate-enriched **8** correlated well with ^{13}C-^{13}C coupling constants of [1,2-$^{13}C_2$]acetate-enriched **8** indicating that those carbons were derived from acetates. Effective incorporation of ^{13}C only at C-1 and C-5 of **1** by [1-^{13}C]propionate-enriched **8** revealed that C-1～2-CH_3 and C-5～6-CH_3 were produced by propionate.

Fig. 4. Labeling pattern of fluvirucin A_1 by [1-^{13}C]acetate, [2-^{13}C]acetate and [1-^{13}C]propionate; solid bars indicate intact transfer of acetates or propionates.

● [1-^{13}C]Acetate, ■ [2-^{13}C]acetate, ▲ [1-^{13}C]propionate.



The feeding experiments further demonstrated that an acetate was also incorporated at C-11～C-12 but only [2-^{13}C]acetate-labeled C-13 of **8**. Since [2-^{13}C]acetate enriched C-12 and C-13 and [1-^{13}C]acetate

only C-11, this C-3 unit was likely to be derived from an intermediate in the KREB's cycle produced by decarboxylation of succinate or its equivalent. There have been reported several examples of incorporation of the advanced precursors into polyketide biosynthesis[9] and fluvirucin is considered to be an additional example. The labeling pattern resulting from these experiments is shown in Fig. 4. Fluvirucinines A_2, B_1 and B_2 differ structurally from fluvirucinine A_1 at C-1~2-CH_3 and C-5~6-CH_3 parts. This shows these moieties are the variable parts in the biosynthesis and can be replaced by other units (butyrates *etc.*) instead of propionates of fluvirucin A_1. The biosynthetic experiments fully supported the 2,6,10-trialkyltridecanelactam structures assigned for fluvirucins.

Discussion

The structures of fluvirucins A_1, A_2, B_1, B_2, B_3, B_4 and B_5 were shown by chemical, spectral and biosynthetic experiments to be a unique 2,6,10-trialkyl-3 (or 9)-hydroxy-13-tridecanelactam core substituted with L-mycosamine or 3-amino-3,6-dideoxy-L-talose at the C-3 or C-9 hydroxyl. Fluvirucin components differ from each other in the alkyl substituents at C-2 or C-6, the position of the hydroxyl and/or the aminosugar. Fluvirucins B_4 and B_5 have an unusual 2-phenethylaminocarbonyl substitution at the amino group of the sugar.

The four aglycones of fluvirucins retain the anti-influenza A virus activity with potency being one-fourth to one-eighth that of the original antibiotics. The *N*-acylated representatives, fluvirucins B_4 and B_5 were bioinactive and *N*-acetylation of other components also resulted in bio-inactive derivatives. Therefore, the free amino group of the antibiotics is considered to be crucial for expression of the antiviral activity.

After we completed the structural studies on fluvirucins, a closely related antibiotic Sch 38516 was reported[10]. Although we have not yet determined the stereochemistry of the lactam ring of fluvirucin B series, fluvirucin B_1 is identical with Sch 38516 in 2D structure.

Experimental

General

TLC was performed on precoated Silica gel 60 F_{254} plates (E. Merck, Darmstadt, No. 5715 or 5744). The IR spectra were determined on a Jasco IR-810 and the UV spectra on a UVIDEC-610C spectrometer. The ^1H NMR and ^{13}C NMR spectra were recorded on a Jeol JNM-GX 400. The mass spectra were obtained with a Hitachi M-80B (EI) or a Jeol JMS-AX 505H (EI and FAB). Optical rotations were determined with a Jasco model DIP140. The mp's were recorded on Yanagimoto MP-2S and are uncorrected.

Acetylation of Fluvirucin A_1 (1)

1 (118 mg) was stirred with acetic anhydride (2 ml) in anhydrous pyridine (3 ml) for 16 hours at room temperature. To the reaction mixture, ethyl acetate (70 ml) and ice-water were added and the mixture was stirred for 1 hour. After washing with 1 M aqueous $CuSO_4$ (50 ml) and water, the organic layer was concentrated to give a pale yellow solid. This was charged on a column of Wakogel C-200 (50 ml) which was eluted with toluene - methanol (19 : 1). The fractions containing the desired acetate were collected and evaporated under reduced pressure to give a white powder of the triacetate (**8**, 149 mg). It was crystallized as colorless rods from acetone.

8: MP 259~261°C; MS *m/z* 554 (M), 539 (M$-CH_3$), 525 (M$-CH_2CH_3$); IR ν_{max} (KBr) cm^{-1} 1740, 1690, 1520; ^1H NMR (400 MHz, $CDCl_3$) δ 0.85 (3H, t, 15-H_3), 0.87 (3H, d, 6-CH_3), 1.13 (3H, d, 6'-H_3), 1.22 (3H, d, 2-CH_3), 1.97 (3H, s, Ac), 2.16 (3H, s, Ac), 2.18 (3H, s, Ac), 2.42 (1H, br dq, 2-H), 2.59 (1H, m, 13-H), 3.83 (1H, m, 3-H), 3.89 (1H, m, 13-H), 4.20 (1H, q, 5'-H), 4.66 (1H, dt, 3'-H), 4.81 (1H, br d, 2'-H), 4.96 (1H, br s, 1'-H), 5.10 (1H, br d, 4'-H), 5.73 (1H, d, 3'-NH), 5.88 (1H, br dd, 13-NH).

In an analogous way, **2**, **3**, **4**, **5**, **6** and **7** gave the corresponding acetates.

Tetraacetylfluvirucin A_2 (**14**): Colorless prisms; mp 125~129°C; MS *m/z* 626 (M).

Triacetylfluvirucin B_1 (**17**): Colorless needles; mp 213~216°C; MS *m/z* 568 (M); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.81 (3H, t, 15-H_3), 0.87 (3H, t, 2-CH_2CH_3), 0.88 (3H, d, 6-CH_3), 1.13 (3H, d, 6'-H_3), 1.89 (1H, m, 2-H), 1.97 (3H, s, Ac), 2.15 (3H, s, Ac), 2.18 (3H, s, Ac), 3.00 (1H, br d, 13-H), 3.59 (1H, m, 9-H), 3.73 (1H, br dd, 13-H), 4.17 (1H, q, 5'-H), 4.66 (1H, m, 3'-H), 4.68 (1H, d, 2'-H), 4.94 (1H, br s, 1'-H), 5.10 (1H, br s, 4'-H), 5.57 (1H, br s, 13-NH), 5.70 (1H, d, 3'-NH).

Triacetylfluvirucin B_2 (**25**): Colorless needles; mp 238~240°C; MS *m/z* 582 (M).

Triacetylfluvirucin B_3 (**29**): Colorless plates; mp 196~197°C; MS *m/z* 582 (M).

Triacetylfluvirucin B_4 (**30**): Colorless needles; mp 226~228°C; MS *m/z* 6.87 (M); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.83 (3H, t, 15-H_3), 0.85 (3H, t, 6-CH_2CH_3), 0.88 (3H, t, 2-CH_2CH_3), 1.16 (3H, d, 6'-H_3), 1.91 (1H, m, 2-H), 2.05 (3H, s, Ac), 2.12 (3H, s, Ac), 2.78 (2H, br t, 2''-H_2), 2.99 (1H, m, 13-H), 3.40 (2H, br t, 1''-H_2), 3.59 (1H, m, 9-H), 3.74 (1H, m, 13-H), 3.98 (1H, dq, 5'-H), 4.29 (1H, br s, 1''-NH), 4.41 (1H, d, 3'-NH), 4.46 (1H, dt, 3'-H), 4.75 (1H, t, 4'-H), 4.84 (1H, br s, 1'-H), 4.87 (1H, br s, 2'-H), 5.65 (1H, m, 13-NH), 7.17 (2H, dd, 4''-H, 8''-H), 7.23 (1H, dd, 6''-H), 7.30 (2H, t, 5''-H, 7''-H).

Triacetylfluvirucin B_5 (**31**): Colorless prisms; mp 196~200°C; MS *m/z* 687 (M).

Acid Methanolysis of Fluvirucin A_1 (**1**)

1 (400 mg) was heated at 90°C for 3 hours with 5 N anhydrous methanolic HCl (70 ml) in a sealed tube. The colorless needles deposited in the tube were collected by filtration and washed with water (220 mg).

9: MP 230~240°C; MS *m/z* 283 (M); IR ν_{max} (KBr) cm^{-1} 1640, 1545; ^{13}C NMR (100 MHz, $CDCl_3$-CD_3OD, 1:1) δ 177.1 (C-1), 49.0 (C-2), 15.1 (2-CH_3), 74.1 (C-3), 32.6 (C-4), 31.1 (C-5), 31.8 (C-6), 21.4 (6-CH_3), 33.0 (C-7), 19.9 (C-8), 32.3 (C-9), 37.9 (C-10), 28.0 (C-11), 26.5 (C-12), 39.4 (C-13), 27.2 (C-14), 11.5 (C-15).

Anal Calcd for $C_{17}H_{33}NO_2$: C 72.04, H 11.73, N 4.94.
Found: C 72.32, H 11.87, N 4.93.

The filtrate and the wash were combined, diluted with water to 400 ml and then washed with butanol (200 ml). The aqueous layer was concentrated to 100 ml *in vacuo*, neutralized with Amberlite IRA-400 (OH^-) and charged on a column of Amberlite CG-50 (NH_4^+). Upon elution with 0.2 N NH_4OH, the fractions which showed a positive ninhydrin test were collected and concentrated to give a white solid (145 mg) of **10**. **10** was separated to the major methyl α-glycoside (**10a**) and minor β-anomer (**10b**) by preparative TLC developed by chloroform-ethanol-28% NH_4OH (4:7:2). After Amberlite CG-50 chromatography, homogeneous **10a** (106 mg) and **10b** (20 mg) were obtained as white solids.

10a: MP 140~142.5°C; $[\alpha]_D^{27}$ −95° (*c* 1.0, H_2O); ^{1}H NMR (400 MHz, D_2O) δ 3.41 (3H, s, 1-OCH_3), 4.80 (1H, d, J=1.1 Hz, 1-H), 3.72 (1H, ddd, J=3.3, 1.5 and 1.1 Hz, 2-H), 3.09 (1H, br dd, J=3.3 and 1.5 Hz, 3-H), 3.63 (1H, br t, J=1.5 Hz, 4-H), 4.02 (1H, q, J=6.6 Hz, 5-H), 1.26 (3H, d, J=6.6 Hz, 6-H_3).

Anal Calcd for $C_7H_{15}NO_4$: C 47.45, H 8.53, N 7.90.
Found: C 46.97, H 8.47, N 7.82.

10b: MP 117~120°C; $[\alpha]_D^{25}$ +35° (*c* 0.6, H_2O); ^{1}H NMR (400 MHz, D_2O) δ 3.55 (3H, s, 1-OCH_3), 4.49 (1H, d, J=1.1 Hz, 1-H), 3.81 (1H, br d, J=3.3 Hz, 2-H), 2.98 (1H, br t, J=3.3 Hz, 3-H), 3.42 (1H, br d, J=3.3 Hz, 4-H), 3.74 (1H, q, J=6.6 Hz, 5-H), 1.29 (3H, d, J=6.6 Hz, 6-H_3).

Anal Calcd for $C_7H_{15}NO_4 \cdot \frac{1}{4}H_2O$: C 46.27, H 8.60, N 7.71.
Found: C 46.49, H 8.38, N 7.65.

By an analogous method, **2** and **3**, were hydrolyzed to yield their aglycones **15** and **18**, respectively, and methyl glycosides **10a** and **10b**.

15: Colorless needles; mp 184~186°C; MS *m/z* 313 (M).

Anal Calcd for $C_{18}H_{35}NO_3 \cdot \frac{1}{4}H_2O$: C 67.99, H 11.25, N 4.40.
Found: C 67.73, H 11.15, N 4.39.

18: Colorless needles; mp 235~245°C; MS *m/z* 297 (M).

Anal Calcd for $C_{18}H_{35}NO_2$: C 72.68, H 11.86, N 4.71.
Found: C 72.21, H 11.90, N 4.68.

Acid Methanolysis of Fluvirucin B_2 (**4**)

4 was hydrolyzed with methanolic hydrogen chloride and the products were worked up as described for **1** to yield aglycone **26** and methyl L-mycosaminide mixture **27a** and **27b**.

26: Colorless needles; mp 235~245°C; MS *m/z* 311 (M).

Anal Calcd for $C_{19}H_{37}NO_2$: C 73.26, H 11.97, N 4.50.
Found: C 72.94, H 12.05, N 4.46.

27a: White sticky solid; mp 85~87°C; $[\alpha]_D^{27}$ −47° (*c* 0.2, MeOH); ^{1}H NMR (D_2O) δ 3.39 (3H, s, 1-OCH_3), 4.66 (1H, d, *J*=1.3 Hz, 1-H), 3.88 (1H, dd, *J*=3.0 and 1.3 Hz, 2-H), 2.92 (1H, dd, *J*=9.8 and 3.0 Hz, 3-H), 3.29 (1H, t, *J*=9.8 Hz, 4-H), 3.67 (1H, dq, *J*=9.8 and 6.4 Hz, 5-H), 1.28 (3H, d, *J*=6.4 Hz, 6-H_3).

Anal Calcd for $C_7H_{15}NO_4 \cdot H_2O$: C 43.07, H 8.78, N 7.16.
Found: C 43.30, H 8.90, N 6.82.

27b: Colorless syrup; ^{1}H NMR (D_2O) δ 3.51 (3H, s, 1-OCH_3), 4.56 (1H, d, *J*=1.1 Hz, 1-H), 3.92 (1H, br d, *J*=3.0 Hz, 2-H), 2.77 (1H, dd, *J*=9.8 and 3.0 Hz, 3-H), 3.21 (1H, t, *J*=9.8, 4-H), 3.41 (1H, dq, *J*=9.8 and 6.4 Hz, 5-H), 1.30 (3H, d, *J*=6.4 Hz, 6-H_3).

In similar fashion, **5** yielded aglycone **26** and sugars **10a** and **10b**.

Acid Methanolysis of Fluvirucins B_4 (**6**) and B_5 (**7**)

Acid methanolysis of **6** and **7** by the procedure described above gave methyl glycosides **33** and **34**, respectively, and aglycone **26**.

33: White solid; mp 141~146°C; $[\alpha]_D^{26}$ −43° (*c* 0.7, EtOH); MS *m/z* 324 (M); ^{1}H NMR ($CDCl_3$) δ 3.37 (3H, s, 1-OCH_3), 4.56 (1H, br s, 1-H), 3.73 (1H, br s, 2-H), 3.83 (1H, br d, *J*=9.4 Hz, 3-H), 3.27 (1H, t, *J*=9.4 Hz, 4-H), 3.65 (1H, dq, *J*=9.4 and 6.4 Hz, 5-H), 1.29 (3H, d, *J*=6.4 Hz, 6-H_3), 4.95, 5.38 (1H each, br s, NH×2), 3.42 (2H, t, *J*=7.0 Hz, 1′-H_2), 2.79 (2H, t, *J*=7.0 Hz, 2′-H_2), 7.18 (2H, dd, *J*=7.3 and 1.5 Hz, 4′-H, 8′-H), 7.30 (2H, t, *J*=7.3 Hz, 5′-H, 7′-H), 7.23 (1H, dd, *J*=7.3 and 1.5 Hz, 6′-H). The ^{1}H NMR spectrum showed the presence of a minor amount of β-anomer (ratio: *ca.* 1/12, 1-OCH_3 δ 3.53). HR-MS Calcd for $C_{16}H_{24}N_2O_5$: *m/z* 324.1685, Found: *m/z* 324.1686.

34: Colorless syrup; $[\alpha]_D^{26}$ −75° (*c* 0.2, EtOH); MS *m/z* 324 (M); ^{1}H NMR ($CDCl_3$) δ 3.36 (3H, s, 1-OCH_3), 4.66 (1H, br s, 1-H), 3.65 (1H, br s, 2-H), 3.95 (1H, br s, 3-H), 3.61 (1H, br s, 4-H), 3.92 (1H, q, *J*=6.4 Hz, 5-H), 1.24 (3H, d, *J*=6.4 Hz, 6-H_3), 5.54 (2H, br s, NH×2), 3.41 (3H, t, *J*=7.0 Hz, 1′-H_2), 2.79 (3H, t, *J*=7.0 Hz, 2′-H_2), 7.19 (2H, dd, *J*=7.3 and 1.5 Hz, 4′-H, 8′-H), 7.29 (2H, t, *J*=7.3 Hz, 5′-H, 7′-H), 7.22 (1H, dd, *J*=7.3 and 1.5 Hz, 6′-H). This ^{1}H NMR spectrum also indicated a minor anomer (1-OCH_3, δ 3.55). HR-MS Calcd for $C_{16}H_{24}N_2O_5$: *m/z* 324.1685, Found: *m/z* 324.1693.

Acetylation of Fluvirucinine A_1 (**9**)

9 (43 mg) was reacted with acetic anhydride (1 ml) in anhydrous pyridine (3 ml) for 16 hours at room temperature. The crude product was purified by column chromatography on Wakogel C-200 using a solvent system of toluene-methanol (49:1). The relevant fractions were collected and concentrated to afford a white powder (50 mg) of the acetate (**11**). This was crystallized from methanol to give colorless needles.

11: MP 230~235°C; MS *m/z* 325 (M), 296 (M−CH_2CH_3), 282 (M−Ac), 265 (M−AcOH); IR ν_{max} (KBr) cm^{-1} 1730, 1250.

In an analogous manner, **15**, **18** and **26** were acetylated to acetates **16**, **19** and **28**, respectively.

Oxidation of Fluvirucinine A_1 (**9**)

9 (64.5 mg) and pyridinium dichromate (PDC, 100 mg) were suspended in dichloromethane (100 ml) and stirred for 32 hours at room temperature. After removal of the insolubles by filtration, the filtrate was concentrated under reduced pressure to give a brownish solid. This product was chromatographed on Wakogel C-200 using toluene-methanol (49:1) as eluent. After TLC examination, the relevant fractions were concentrated to afford a white solid (32 mg) of **12**. Some starting material (16 mg) was recovered from the following eluate. **12** was crystallized from methanol as colorless needles.

12: MP 198~200°C; MS *m/z* 281 (M), 266 (M−CH_3), 252 (M−CH_2CH_3); IR ν_{max} (KBr) cm^{-1} 1720; ^{1}H NMR ($CDCl_3$) δ 0.83 (3H, t, *J*=7.3 Hz, 15-H_3), 0.89 (3H, d, *J*=6.0 Hz, 6-CH_3), 1.33 (3H, d, *J*=7.3 Hz, 2-CH_3), 2.44, 2.63 (1H each, m, 4-H_2), 3.16, 3.34 (1H each, m, 13-H_2), 3.44 (1H, q, *J*=7.3 Hz, 2-H), 5.90 (1H, br s, 13-NH).

Anal Calcd for $C_{17}H_{31}NO_2$: C 72.55, H 11.10, N 4.98.
Found: C 72.77, H 11.21, N 4.91.

Methylation of Fluvirucinine A_1 (**9**)

To a solution of sodium dimethylsulfinyl anion prepared from NaH (20 g of 50% oil suspension) and DMSO (200 ml), **9** (890 mg) and CH_3I (90 ml) were added and the mixture was stirred in argon stream for 1 hour at room temperature. After dilution with water, the methylated product was extracted with chloroform. The extract was concentrated *in vacuo* and the residue subjected to medium pressure column chromatography (E. Merck, TLC Silica gel 60 H, No. 11695) eluted with hexane-acetone (19:1). A white solid of **13** (727 mg) was obtained by evaporation of the appropriate fractions.

13: MP 51.5~52°C; MS *m/z* 311 (M); ^{1}H NMR ($CDCl_3$) δ 0.84 (3H, t, J=7.3 Hz, 15-H_3), 0.90 (3H, d, J=6.9 Hz, 6-CH_3), 1.21 (3H, d, J=6.9 Hz, 2-CH_3), 3.07 (3H, s, N–CH_3), 3.41 (3H, s, O–CH_3), 2.47 (1H, ddd, J=13.3, 6.9 and 1.2 Hz, 13-H), 2.82 (1H, dq, J=9.7 and 6.9 Hz, 2-H), 3.41 (1H, m, 13-H), 4.32 (1H, m, 3-H).

Anal Calcd for $C_{19}H_{37}NO_2$: C 73.26, H 11.97, N 4.50.
Found: C 73.86, H 12.21, N 4.50.

Methyl α- and β-D-Mycosaminides from Amphotericin B

Amphotericin B (Makor Chemicals Ltd., Israel, 1 g) was hydrolyzed under the reported conditions to afford methyl α-D-mycosaminide (79 mg) and its β-anomer (21 mg).

Methyl α-D-mycosaminide: $[\alpha]_D^{27}$ +61° (*c* 0.5, MeOH); ^{1}H NMR spectrum (D_2O) was identical with that of **27a**.

Methyl β-D-mycosaminide: ^{1}H NMR spectrum (D_2O) was identical with that of **27b**.

Preparation of Methyl 3-Acetamido-3,6-dideoxy-2,4-di-*O*-methanesulfonyl-α-D-talopyranoside

Methyl α-D-mycosaminide (79 mg) was acetylated in anhydrous methanol (2 ml) to give a syrup of its *N*-acetate (97 mg)[5]: $[\alpha]_D^{22.5}$ +46° (*c* 1.0, EtOH); MS *m/z* 220 (M+H); ^{1}H NMR (CD_3OD) δ 3.36 (3H, s, 1-OCH_3), 4.51 (1H, d, J=1.7 Hz, 1-H), 3.73 (1H, dd, J=3.0 and 1.7 Hz, 2-H), 4.07 (1H, dd, J=9.8 and 3.0 Hz, 3-H), 3.34 (1H, t, J=9.8 Hz, 4-H), 3.63 (1H, dq, J=9.8 and 6.4 Hz, 5-H), 1.26 (1H, d, J=6.4 Hz, 6-H_3), 1.99 (3H, s, NAc). It (61 mg) was reacted with methanesulfonyl chloride (0.25 ml) in anhydrous pyridine (2 ml) at room temperature. The reaction mixture was diluted with ice-water and extracted with ethyl acetate. After washing successively with 1 N HCl, aqueous $NaHCO_3$, and water, the extract was evaporated to dryness to afford the crude mesylate. This was purified by preparative TLC developed with chloroform-methanol (9:1) followed by Sephadex LH-20 chromatography and then by crystallization from acetone-*n*-hexane to give colorless rods of the mesylate (58 mg): MP 149~150°C; $[\alpha]_D^{23}$ +18.6° (*c* 0.5, EtOH).

Anal Calcd for $C_{11}H_{21}NO_9S_2$: C 35.19, H 5.64, N 3.73, S 17.08.
Found: C 35.68, H 5.67, N 3.73, S 17.09.

A solution of the mesylate (56 mg) and anhydrous sodium acetate (100 mg) in 2-methoxyethanol (20 ml) was refluxed for 3 days. The solution was evaporated and the residue was partitioned between ethyl acetate and water (50 ml each). The organic layer was concentrated under reduced pressure. The residue was mesylated again with mesyl chloride in anhydrous pyridine. The crude product was purified by preparative TLC and Sephadex LH-20 chromatography to yield a white powder of methyl 3-acetamido-3,6-dideoxy-2,4-di-*O*-methansulfonyl-α-D-talopyranoside (17 mg, 31% from the starting mesylate): MP 167~169°C; $[\alpha]_D^{23}$ +58° (*c* 0.3, EtOH); CD (*c* 0.04, MeOH) $[\theta]^{rt}$ (nm) −2,800 (212) (negative maximum); MS *m/z* 344 (M−OCH_3); IR ν_{max} (KBr) cm^{-1} 1675, 1525, 1340, 1175; ^{1}H NMR ($CDCl_3$) δ 3.42 (3H, s, 1-OCH_3), 4.87 (1H, d, J=1.7 Hz, 1-H), 4.77 (1H, m, 2-H), 4.66 (1H, dt, J=8.1 and 3.2 Hz, 3-H), 4.72 (1H, m, 4-H), 4.17 (1H, dq, J=0.9 and 6.4 Hz, 5-H), 1.32 (3H, d, J=6.4 Hz, 6-H_3), 2.04 (3H, s, NAc), 3.13 (3H, s, SCH_3), 3.14 (3H, s, SCH_3), 6.58 (1H, br d, J=8.1 Hz, 3-NH).

Anal Calcd for $C_{11}H_{21}NO_9S_2$: C 35.19, H 5.64, N 3.73, S 17.08.
Found: C 35.85, H 5.73, N 3.72, S 16.42.

Preparation of *N*-Acetyl-di-*O*-methansulfonyl Derivative of **10a**

10a (125 mg) was *N*-acetylated with acetic anhydride (0.5 ml) in anhydrous methanol (4 ml) and then

O-mesylated as described above. The product was purified by preparative TLC and Sephadex LH-20 chromatography to afford a white powder of the desired product (110 mg): MP 168~169.5°C; $[\alpha]_D^{23}$ −57° (*c* 1.0, EtOH); CD (*c* 0.04, MeOH) $[\theta]^{rt}$ (nm) +2,800 (212) (positive maximum); MS *m/z* 344 (M−OCH_3).

Anal Calcd for $C_{11}H_{21}NO_9S_2$: C 35.19, H 5.64, N 3.73, S 17.08.
Found: C 35.77, H 5.72, N 3.66, S 16.28.

IR (KBr) and ^{1}H NMR ($CDCl_3$) spectra are identical with those of the synthetic methyl 3-acetamido-3,6-dideoxy-2,4-di-*O*-methansulfonyl-α-D-talopyranoside.

Oxidation of Fluvirucinine B_1 (**18**)

18 (5 mg) was oxidized with pyridinium chlorochromate (PCC, 5 mg) and **20** was obtained in quantitative yield (4.7 mg).

20: MP 222~226°C; MS *m/z* 295 (M), 280 (M−CH_3), 267, 238, 210, 128; IR ν_{max} (KBr) cm^{-1} 1700; ^{1}H NMR ($CDCl_3$) δ 0.85 (3H, t, *J*=7.4 Hz, 15-H_3), 0.88 (3H, t, *J*=7.4 Hz, 2-CH_2CH_3), 0.89 (3H, d, *J*=7.2 Hz, 6-CH_3), 1.95 (1H, m, 2-H), 2.23 (1H, ddd, *J*=16.4, 10 and 5.4 Hz, 8-H), 2.24 (1H, m, 10-H), 2.36 (1H, ddd, *J*=16.4, 10 and 5.4 Hz, 8-H), 3.10 (1H, dddd, *J*=14.0, 7.8, 6.9 and 2.5 Hz, 13-H), 3.64 (1H, dddd, *J*=14.0, 9.2, 7.9 and 2.5 Hz, 13-H), 5.55 (1H, br s, NH).

Baeyer-Villiger Reaction of **20**

20 (4 mg) and *m*-chloroperbenzoic acid (*m*-CPBA, 10 mg) were dissolved in dichloromethane (1 ml) and stirred for 7 days at room temperature. The mixture was diluted with 10 ml of dichloromethane and washed with 1 N NaOH and then water. After concentration of the organic layer under reduced pressure, the residue was purified by preparative TLC (toluene-MeOH, 9:1) and column chromatography on Sephadex LH-20 (MeOH) to afford 0.7 mg of lactone **21** (yield 17%) and the starting material (2.6 mg).

21: MP 166~169°C; MS *m/z* 311 (M), 294, 283, 169, 128, IR ν_{max} (KBr) cm^{-1} 1720, 1265.

23 (yield 34%) was prepared in a similar procedure from **12**.

23: MP 178~179°C; MS *m/z* 297 (M), 282 (M−CH_3), 268, 158, 118; IR ν_{max} (KBr) cm^{-1} 1740, 1200; ^{1}H NMR ($CDCl_3$) δ 0.85 (3H, t, *J*=6.8 Hz, 15-H_3), 0.91 (3H, d, *J*=6.4 Hz, 6-CH_3), 1.43 (3H, d, *J*=6.8 Hz, 2-CH_3), 3.15 (1H, m, 13-H), 3.32 (1H, q, *J*=7.3 Hz, 2-H), 3.43 (1H, ddt, *J*=11.8, 4.3 and 6.8 Hz, 13-H), 3.99 (1H, ddd, *J*=11.1, 8.4 and 3.6 Hz, 4-H), 4.46 (1H, ddd, *J*=11.1, 7.4 and 4 Hz, 4-H), 6.18 (1H, br s, NH), HR-MS Calcd for $C_{17}H_{31}NO_3$: *m/z* 297.2304, Found: *m/z* 297.2307.

Preparation of Ester **22**

21 (0.5 mg) was dissolved in 0.5 N methanolic KOH (0.2 ml) and heated at 70°C for 30 minutes in a sealed tube. To the reaction mixture 5 ml of water was added and the solution was adjusted to pH 5.0 and then extracted with butanol (5 ml). The organic layer, after washing with water, was concentrated to dryness *in vacuo*. The residue was dissolved in 0.5 ml of a mixture of methanol-benzene (1:1) and added 0.1 ml of 10% *n*-hexane solution of trimethylsilyldiazomethane (TMS-CHN_2) and then stirred for 30 minutes. Removal of the solvent yielded colorless oil of **22**.

22: IR ν_{max} (neat) cm^{-1} 1740, 1260, 1170.

24 was also prepared by a similar reaction of **23**.

24: IR ν_{max} (neat) cm^{-1} 1740, 1200, 1090.

^{13}C-Acetate-fed Fermentation

Fluvirucin A_1-producing strain Q464-31 was grown at 32°C for 4 days in a medium composed of soluble starch 2.0%, Pharmamedia 1.0%, $ZnSO_4$ 0.003% and $CaCO_3$ 0.4%. The culture was inoculated in 100 ml of the fresh medium in 500-ml Erlenmeyer flasks and fermented at 28°C on a rotary shaker at 180 rpm. The ^{13}C-enriched sodium acetate or propionate was dissolved in distilled water ([1-$^{13}C_2$]- and [2-^{13}C]acetates and [1-^{13}C]propionate: 1 g/6.3 ml and [1,2-$^{13}C_2$]acetate: 1 g/9.5 ml) and a 1-ml aliquot of the solution was added to each fermentation flask. The ^{13}C-sources were first added 48 hours after inoculation and then at 60 hours and 72 hours. The fermentation was continued another 24 hours after the final supply of the ^{13}C-sources. Fluvirucin A_1 production reached *ca.* 90 μg/ml at the end. The antibiotic activity was extracted and purified by the procedure previously reported. The sample was acetylated as described in the previous Experimental section for ^{13}C NMR measurement.

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