Novel linear polyene antibiotics: linearmycins

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Two novel linear polyene antibiotics, termed linearmycin A 1 and B 2, have been isolated from the mycelial extracts of *Streptomyces* sp. no. 30 and characterized as compounds 1 and 2, respectively. These compounds have similar, long linear structures terminating with amino and carboxylic acid groups, and show antifungal and antibacterial activity. The incorporation experiments using ^{13}C-labelled acetate and propionate suggest that 1 is biosynthesized from one γ-aminobutyric acid, 24 acetate and 4 propionate molecules, by a polyketide pathway.

In the course of our screening search for new antifungal agents, using a spheroplast regeneration system of *Candida albicans*, mycelial extracts of *Streptomyces* sp. no. 30 were found to greatly inhibit the cell-wall regeneration process. This finding prompted us to isolate and characterize the active principle, which led to a discovery of novel linear polyene antibiotics, termed linearmycin A 1 and B 2. We have already reported the preliminary elucidation of the structure and biosynthesis of linearmycin A.[1] In this paper, we describe the isolation, detailed structural elucidation and biosynthesis as well as antimicrobial activity of linearmycins.

Results and discussion

Production and isolation of linearmycins

Streptomyces sp. no. 30 was cultured, in the dark, in a 500 cm^3 Erlenmeyer flask containing Bennet medium (100 cm^3). Cultivation in the dark was essential for the production of linearmycins by this strain. Linearmycin A production started at around the 24th hour of cultivation at the exponential growth phase, the onset of linearmycin B production occurring a day later than that of linearmycin A.

Because of their instability to neutral or, especially, acidic conditions, linearmycins were isolated under basic conditions. The mycelia of *Streptomyces* sp. no. 30 were extracted with ammoniacal aqueous methanol, and the extract was applied to an HP-20 column. Linearmycins were eluted from the column with a mixture of methanol and aqueous ammonia (99.5:0.5), and the eluate was finally purified by reversed-phase HPLC under basic conditions to afford linearmycin A and B. That alkaline hydrolytic ring cleavage might result in the linear structure during the purification procedure was disproved by the fact that mycelial extraction with methanol alone and subsequent rapid HPLC analysis also gave linearmycin A and B.

Structural elucidation of linearmycins

Linearmycin A 1 was isolated as a yellow powder after lyophilization. The pseudo-molecular ion of $(M + H)^+$ was observed at m/z 1140 in the FAB-MS spectrum of 1, and the molecular formula of 1 was indicated as $C_{64}H_{101}NO_{16}$ by its high-resolution spectrum. The absorbance maxima at 317, 330 and 348 nm observed in the UV spectrum of 1 suggested the presence of a pentaene system. The ^{1}H and ^{13}C NMR spectra

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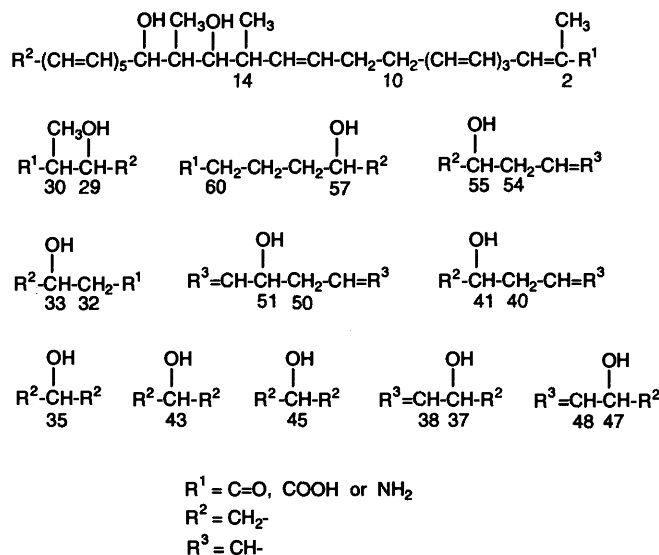

Fig. 1 Partial structures deduced from COSY spectra

of 1 showed that it has 64 carbons comprising: C=O × 2, CH= × 25, C= × 1, CH–O × 13, CH × 3, CH_2 × 16 and CH_3 × 4. The degree of unsaturation of 1 was 15, which was satisfied by the presence of 2 carbonyl groups and 13 double bonds; this evidence indicated the absence of a ring system in 1. The above functional groups contained in total 85 hydrogen atoms, each of which was linked to a carbon atom. Thus, to fulfil the number of hydrogen atoms involved in the molecule of 1, an additional 16 hydrogen atoms, which should be linked to heteroatoms, were necessary. A simple calculation, based on this number together with the number of carbonyl groups as well as the molecular formula of 1, showed that the molecule contained 14 OH and 1 NH_2 groups. The 2 carbonyl and 14 OH groups accounted for the oxygen atoms present in 1; 1 OH group would then be part of a C(O)OH group (δ_C 175.8) and the 13 others would be CH–O groups since there were no other carbons in 1 having δ_C values to which an OH group could be attached. From these considerations, it was concluded that all the CH–O groups are free OH groups, and that, in addition, 1 contains 1 CO_2H and 1 NH_2 group.

Because 1 has only 3 quaternary carbons, most of the structure could be deduced from ^{1}H–^{1}H COSY (correlated spectroscopy) spectra of 1, which were measured in three different solvents ([^{2}H$_4$]MeOH, [^{2}H$_5$]pyridine and [^{2}H$_6$]-DMSO) to overcome the signal overlapping. By analysis of the spectra, 12 partial structures (Fig. 1), which contain all the

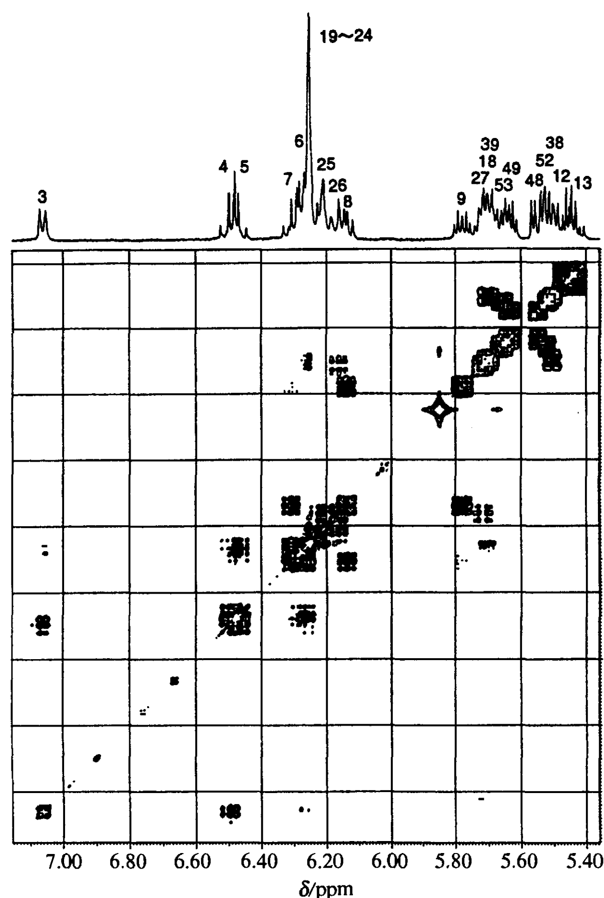

Fig. 2 COSY spectrum of **1** (600 MHz, in $[^2H_4]$MeOH). Numbers on the ^{1}H NMR spectrum indicate proton positions assigned.

carbon atoms except for 2 carbonyl carbons, were clarified. To determine the connection of these fragments, a detailed analysis of critical regions was performed with high-resolution COSY spectra (see Fig. 2), which could clearly confirm each connection of the olefinic protons except for the pentaene moiety. From these spectra, the following carbon connections were verified: C-27-28-29, C-33-34-35, C-35-36-37, C-38-39-40-41, C-41-42-43, C-43-44-45, C-45-46-47, C-48-49-50-51 and C-52-53-54-55. Although the connection C-55-56-57 could not be clarified from the sprctra because of overlapping of the methine proton signals at C-55 and 57, it was clear that C-55 and 57 were linked by the remaining methylene carbon. These linkages between C-55 and 56, and C-56 and 57 were further confirmed by analysis of INADEQUATE (incredible natural abundance double quantum transfer experiment) spectra, which were measured with the samples obtained from the later mentioned feeding experiments of $[1,2\text{-}^{13}C_2]$- and $[2\text{-}^{13}C]$-acetate, respectively. From these results, two large partial structures, carbon connections from C-2 to C-30 and from C-32 to C-60, were clarified. The presence of a methyl group at C-2 was revealed by the long-range couplings between H-3 and the methyl protons at C-2 in the COSY spectrum and between C-2 and the methyl protons at C-2 in the later mentioned COLOC (correlation spectroscopy *via* long-range couplings) spectrum.

To determine the positions of the remaining C=O, COOH and NH_2 groups, COLOC spectra of **1** were measured. In the spectra, significant long-range couplings were observed (see Fig. 3), and established each connection between COOH and C-2, C=O and C-30, and C=O and C-32. The linkage between C=O and C-32 was further confirmed by a pair of $^{13}C–^{13}C$ coupling constants (Table 3), which were observed in the ^{13}C NMR spectrum of **1** obtained from the later mentioned feeding experiment of $[1,2\text{-}^{13}C_2]$acetate. From the above results, all carbon connections

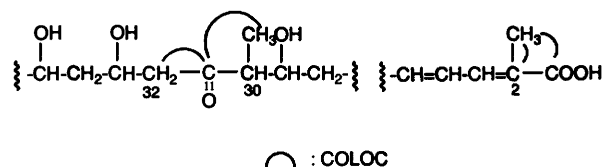

Fig. 3 COLOC correlations in **1**

Table 1 ^{1}H NMR assignment of **1** [a]

Assignment	δ_H	Assignment	δ_H
2-CH$_3$	1.93 d (1)	30-CH$_3$	1.09 d (7)
3-H	7.06 br d (10)	32-H	2.71 dd (17, 8)
4-H	6.50 dd (15, 10)		2.65 dd (17, 4)
5-H	6.46 dd (15, 9)	33-H	4.22
6-H	6.26 dd (15, 9)	34-H	1.62
7-H	6.31 dd (15, 10)	35-H	3.86
8-H	6.14 dd (15, 10)	36-H	1.61, 1.67
9-H	5.78 dt (15, 7)	37-H	4.23
10-H	2.21	38-H	5.50 dd (15, 7)
11-H	2.14	39-H	5.70 dt (15, 7)
12-H	5.48 dt (15, 6)	40-H	2.22
13-H	5.43 dd (15, 7)	41-H	3.88
14-H	2.34	42-H	1.47, 1.62
14-CH$_3$	0.97 d (7)	43-H	4.07
15-H	3.39 t (6)	44-H	1.58
16-H	1.63	45-H	4.04
16-CH$_3$	0.93 d (7)	46-H	1.58
17-H	4.25	47-H	4.26
18-H	5.70	48-H	5.55 dd (15, 6)
19-H	6.25 [b]	49-H	5.64 dt (15, 7)
20-H	6.25 [b]	50-H	2.25
21-H	6.25 [b]	51-H	4.05
22-H	6.25	52-H	5.52 dd (15, 7)
23-H	6.25	53-H	5.65 dt (15, 7)
24-H	6.25	54-H	2.22
25-H	6.21	55-H	3.80
26-H	6.17	56-H	1.56
27-H	5.71 dt (15, 8)	57-H	3.80
28-H	2.27	58-H	1.45, 1.57
29-H	3.95 q (6)	59-H	1.77, 1.79
30-H	2.64 dq (6, 7)	60-H	2.94

[a] Spectrum was obtained in $[^2H_4]$MeOH. [b] $J_{19,20}$ = 15 Hz and $J_{20,21}$ = 15 Hz (δ_H 6.77, 6.52 and 6.40 for 19-H, 20-H and 21-H) were observed in the spectrum obtained in $[^2H_5]$pyridine. Coupling constants in Hz are given in parentheses.

from C-1 to C-60 were established. Thus, the remaining NH_2 group would need to be attached to C-60; this evidence led to the assignment of structure **1**, a planar structure, to linearmycin A (Fig. 4). The geometry of each double bond was established as *E* except for C-22/C-23 and C-24/C-25 through NOE enhancement between H-4 and the methyl protons at C-2, and coupling constants (Table 1). The assignments of protons and carbons in the NMR spectra of **1** are summarized in Tables 1 and 2.

The molecular formula of linearmycin B **2** was indicated as $C_{66}H_{103}NO_{16}$ from its MS data, which was larger than **1** by a C_2H_2 unit. Its UV spectrum had four absorbance maxima at 317, 331, 349 and 365 nm. Three of them (317, 331 and 349 nm) suggested the presence of a pentaene system in **2**, similar to that in **1**; the remaining absorption maximum (365 nm) suggested that **2** had an additional conjugated system. The ^{1}H and ^{13}C NMR spectra of **2** (Table 2) were almost the same as those of **1**, only one difference being observed between the signals of the tetraenoic acid moiety of **1** and those of the corresponding part of **2**; additional signals for two CH= units were observed in the spectrum of **2**. Finally, by analysing the COSY spectrum of **2**, it was shown that the compound has a pentaenoic acid moiety rather than a tetraenoic acid system as in **1**. From these results, the structure of linearmycin B was assigned as **2** (Fig. 4). The geometrical isomerism of the double bonds in **2** was each assigned as *E*, except for C-22/C-23,

Table 2 ^{13}C NMR[a] assignments of **1** and **2**

1		**2**[b]		**1**		**2**	
C-No.	δ_C	C-No.	δ_C	C-No.	δ_C	C-No.	δ_C
1	175.8	1	—[c]	29	72.5	31	72.6
2	132.5	2	—	30	53.2	32	53.2
2-CH_3	13.9	2-CH_3	14.0	30-CH_3	11.0	32-CH_3	11.0
3	136.7	3	136.7[e]	31	214.4	33	214.4
4	129.2	4	129.7[e]	32	50.1	34	50.1
5	138.9	5	138.8[e]	33	67.2	35	67.3
6	132.1	6	132.3[e]	34	45.3	36	45.2
7	136.5	7	136.5[e]	35	69.2	37	69.2
8	132.3	8	132.5[e]	36	45.2	38	45.2
9	137.2	9	137.3[e]	37	72.0	39	72.0
		10	133.7[e]	38	136.5	40	136.5
		11	135.7[e]	39	129.1	41	129.1
10	34.0	12	34.1	40	41.6[g]	42	41.7[i]
11	33.4	13	33.4	41	69.0	43	69.1
12	131.4	14	131.5	42	45.4	44	45.4
13	134.5	15	134.5	43	66.2[h]	45	66.3
14	41.6	16	41.5	44	46.6	46	46.6
14-CH_3	18.5	16-CH_3	18.6	45	66.3[h]	47	66.3
15	77.8	17	77.8	46	46.1	48	46.1
16	42.4	18	42.5	47	70.3	49	70.3
16-CH_3	8.8	18-CH_3	8.9	48	137.2	50	137.3
17	75.1	19	74.7	49	127.5	51	127.6
18	137.1	20	137.1	50	41.4	52	41.4
19	131.8[d]	21	131.7[f]	51	73.3	53	73.3
20	133.0[d]	22	133.0[f]	52	136.5	54	136.5
21	134.0[d]	23	134.0[f]	53	128.6	55	128.6
22	133.6[d]	24	133.6[f]	54	41.9[g]	56	42.0[i]
23	134.4[d]	25	134.3[f]	55	71.0	57	71.1
24	133.9[d]	26	133.9[f]	56	44.2	58	44.2
25	134.2	27	134.2	57	70.7	59	70.7
26	134.4	28	134.4	58	35.0	60	35.0
27	132.0	29	132.1	59	24.9	61	25.0
28	39.7	30	39.8	60	40.8	62	40.9

[a] Spectra were obtained in $[^2H_4]$MeOH. [b] Based on the assignments of **1**. [c] Not detected because of signal broadening. [d,e,f,g,h,i] May be interchanged.

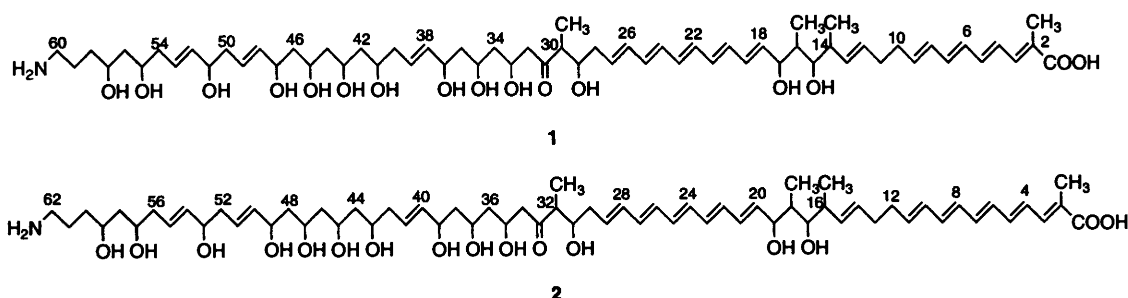

Fig. 4 Structures of linearmycin A **1** and B **2**. The configurations around C-22/C-23 and C-24/C-25 in **1** and **2**, and that around C-6/C-7 in **2** have not been established.

C-24/C-25 and C-6/C-7, through coupling constants and by comparison of the NMR spectra of **2** with those of **1**.

Biosynthesis of linearmycin A

To investigate the biogenesis of **1**, feeding experiments using $[1\text{-}^{13}C]$-, $[2\text{-}^{13}C]$- and $[1,2\text{-}^{13}C_2]$-acetate, and $[1\text{-}^{13}C]$propionate were carried out. A labelled precursor was added, in one portion, to the culture after the 24 h of cultivation at the exponential growth phase, around which time production of **1** started. The ^{13}C NMR spectrum of labelled **1** obtained was measured to determine the carbon enrichment. Table 3 shows the results of the feeding experiments.

A high level of ^{13}C incorporation was observed in the feeding experiment of $[1\text{-}^{13}C]$acetate with the incorporation at carbons 3, 5, 11, 17, 25, 27, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55 and 60 clearly detected. However, because of signal overlapping, it was not possible to determine which positions of the pentaene moiety (C-19–C-24) were enriched; there was a similar problem with the carbons at 7, 9, 18, 38 and 52. On the other hand, in the feeding experiment with $[2\text{-}^{13}C]$acetate, enrichment was observed not only at the expected carbon positions, but also at the positions which had been shown to be enriched by the feeding of $[1\text{-}^{13}C]$acetate; this was due to a rapid turnover of labelled acetate at the exponential growth phase. By comparing the results of the experiments of $[1\text{-}^{13}C]$- and $[2\text{-}^{13}C]$-acetate, the carbon positions originating from the C-2 carbon of acetate were determined. In this case, because of signal overlapping, enrichment of carbons 4, 7, 14, 19–24, 38, 39, 40 and 52 was not clear. The ^{13}C NMR spectrum of **1** obtained by the feeding of $[1,2\text{-}^{13}C_2]$acetate also showed complex coupled signals because of the rapid turnover of acetate mentioned above. Accordingly, crucial signal overlappings were observed in the spectrum. The $^1J_{CC}$ values, which could be deduced from the spectrum, are listed in Table 3. In the feeding experiment with $[1\text{-}^{13}C]$propionate, a high level of ^{13}C incorporation into four carbons (C-1, 13, 15 and 29) was observed.

From the results obtained, we may illustrate the biosynthetic origin of **1** as shown in Fig. 5. The origin of a small number of

Table 3 ^{13}C Abundances and ^{13}C–^{13}C couplings in **1** obtained from feeding experiments with ^{13}C-labelled precursors

C-No.	Relative ^{13}C abundance[a] in **1** biosynthesized from: [1-^{13}C]acetate	[2-^{13}C]acetate	[1-^{13}C]propionate	$^1J_{cc}$[b] (Hz)
1	1.6	1.1	35.9	
2	1.9	1.5		
2-CH_3	1.3	1.1		
3	10.3	2.3		*i*
4	1.3	*f*		57.4
5	9.9	1.7		56.6
6	1.5	3.8		56.9
7	*c*	*g*		*i*
8	1.2	3.5		60.0
9	*d*	2.2		*i*
10	1.0	3.1		43.5
11	13.9	1.8		43.3
12	1.1	3.2		43.1
13	2.8[e]	1.3	33.5	
14	1.4	*h*		
14-CH_3	1.5	1.1		
15	2.1	1.3	44.3	
16	0.9	1.1		
16-CH_3	1.4	1.1		
17	11.0	1.8		48.5
18	*d*	3.9		*i*
19	15.2[j]	1.9[k]		*i*
20	1.1[j]	3.4[k]		*i*
21	12.8[j]	2.4[k]		*i*
22	3.9[e,j]	3.7[k]		*i*
23	15.9[j]	2.1[k]		*i*
24	0.9[j]	3.5[k]		*i*
25	16.4	2.1		*i*
26	3.7[e]	3.2		*i*
27	18.0	2.5		43.8
28	1.2	3.7		43.9
29	2.1	1.1	45.1	
30	1.0	1.0		
30-CH_3	1.3	1.1		
31	18.1	2.1		39.0
32	1.1	3.1		39.0
33	14.1	1.8		38.5
34	1.2	3.6		*i*
35	15.2	1.8		38.2
36	1.0	3.4		*i*
37	14.2	1.9		47.5
38	*c*	*g*		*i*
39	12.5	*f*		43.2
40	1.3	*h*		43.3
41	13.7	1.8		38.7
42	1.2	3.5		38.6
43	14.6	1.8		38.4
44	1.2	3.5		38.7
45	12.9	1.6		38.3
46	0.9	3.3		38.6
47	14.2	1.9		47.4
48	1.4	3.4		*i*
49	13.8	2.0		43.3
50	1.1	3.5		43.4
51	13.0	1.9		47.8
52	*c*	*g*		*i*
53	13.6	2.0		43.0
54	1.1	3.6		43.4
55	14.1	1.8		38.5
56	1.2	3.6		38.6
57	1.3	2.1		
58	1.2	2.1		
59	1.1	2.8		35.7
60	12.1	1.8		35.7

[a] Peak height ratio of ^{13}C enriched to natural abundance. [b] In **1** from [1,2-$^{13}C_2$]acetate. [c,d] Signal overlappings. Enrichment was observed. [e] Shoulder peaks. [f,g,h] Signal overlappings. Enrichment was observed. [i] Cannot be determined because of signal overlapping. [j,k] May be interchanged.

carbon atoms involved in the portion C-1 to C-56 still remained unclear, but the ^{13}C-labelled patterns obtained strongly suggested that each of the C_2 and C_3 units involved in the portion originated, by a simple polyketide pathway, from an acetic acid and a propionic acid molecule, respectively. The labelled pattern of the 4 carbon atoms, from C-57 to 60, suggests that this C_4 unit probably came from a γ-aminobutyric acid molecule, which was biosynthesized from ornithine. Therefore, each molecule of **1** would be synthesized from γ-aminobutyric acid as a starter, and 24 acetate and 4 propionate molecules, by polyketide biosynthesis.

Linearmycin has a long carbon chain of either 60 or 62 carbons, in a linear structure with amino and carboxylic acid terminals. Its structural features resemble those of tetrafibricin,[2,3] a fibrinogen receptor antagonist having no antibacterial and antifungal activity and, basically, it resembles several polyene macrolide antibiotics, except for its linearity. The aglycon structure of lienomycin,[4,5] one of the pentaene macrolide antibiotics, partially resembles linearmycin A. Although enacyloxins are known as antibacterial antibiotics having linear structures,[6-8] their linear chains have only 23 carbons with their carboxylic termini esterified as cyclohexanecarboxylic acid derivatives. Linearmycin A showed both antibacterial and antifungal activity (see Table 4). Linearmycin is the first example of a simple linear polyene antibiotic with antifungal activity.

Table 4 Antimicrobial activity of linearmycin A

Microorganism	MID[a] (μg/paper disc)	MIC[b] (μg cm^{-3})
Staphylococcus aureus	3.1	6.3
Escherichia coli	1.6	1.6
Saccharomyces cerevisiae	0.1	50
Candida albicans	1.6	50
Aspergillus niger	0.2	100

[a] Minimum dose where inhibitory zone was observed. [b] Minimum inhibitory concentration.

Experimental

General procedures

NMR spectra were recorded on a Bruker AM-600 spectrometer and mass spectra were obtained on a JEOL JMS SX-102 spectrometer. Optical rotation values were measured on a Jasco DIP-181 polarimeter and are recorded in units of 10^{-1} deg cm^2 g^{-1} and an IR spectrum was recorded on a JEOL JIR-AQS20M spectrometer.

Screening for antifungal agents with a spheroplasts regeneration system

The preparation and regeneration of spheroplasts of *Candida albicans* ATCC 10231 was carried out according to the method of Surarit *et al.*[9] In brief, spheroplasts of *C. albicans* cells, which were cultured in a Sabouraud medium, were prepared with Zymolyase T-20 (Seikagaku Kogyo Co.) and Chitinase T-1 (Takara Shuzo Co.) in a buffer (0.1 mol dm^{-3} Tris/HCl, 0.5 mol dm^{-3} $MgSO_4$, pH 7.2). A test sample for screening was dissolved in water or ethanol and added to a regeneration medium (0.1 mol dm^{-3} Tris/HCl, 0.5 mol dm^{-3} $MgSO_4$, 5 mmol dm^{-3} glucose, pH 7.2) containing the spheroplasts. After carrying out the regeneration at 37 °C and 100 spm (strokes per minute) for 2 h on a reciprocating shaker, an ethanolic solution of Calcofluor white M2R (Sigma) was added to the culture for detection of chitin on a regenerating cell wall; the effect of a test sample on regeneration was observed through a fluorescence microscope.

Production and isolation of linearmycins

Streptomyces sp. no. 30 was one of the strains newly isolated from soil for screening. Spores of the strain maintained on

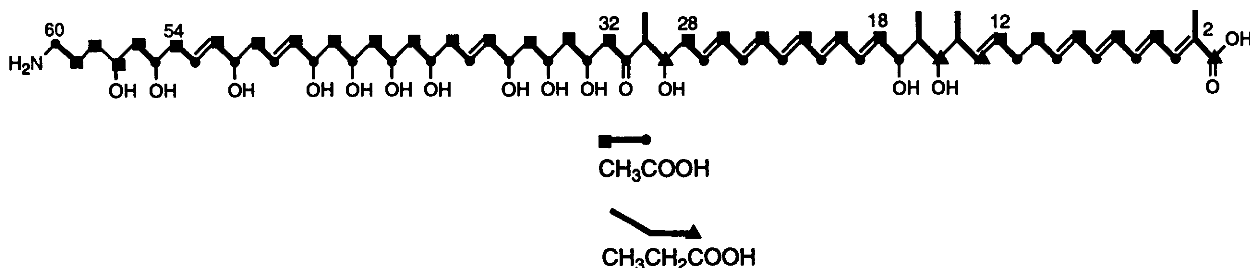

Fig. 5 Biosynthetic origin of linearmycin A

Bennet agar slants in the dark were inoculated into a Bennet medium (100 cm^3), which consisted of glucose 1%, peptone 0.2%, meat extract 0.1% and yeast extract 0.1%, pH 7.2 for preculture in a 500 cm^3 Erlenmeyer flask. The flask was incubated at 28 °C and 150 rpm on a rotary shaker for 48 h in the dark. This culture (2 cm^3) was transferred into the same medium (100 cm^3) for the main culture. Incubation was carried out for 96 h under the same conditions as that of preculture. The culture broth (1000 cm^3) was filtered and the mycelial cake obtained was extracted with a solution of methanol and 28% aqueous ammonia (99.5:0.5; 100 cm^3). The extract was concentrated under reduced pressure to 20 cm^3 and then applied onto an HP-20 column (3 × 75 cm), which had been equilibrated with 10 mmol dm^{-3} ammonium acetate buffer (pH 9.0). After being washed with 50% methanol in the same buffer (1000 cm^3), the column was successively eluted with 80% methanol in the same buffer (1000 cm^3) and a solution of methanol and 28% aqueous ammonia (99.5:0.5; 1000 cm^3). Linearmycins were eluted with the final solution, the fraction being evaporated and lyophilized. The residue was finally purified by HPLC column Capcell-Pak C_{18}, 4.6 × 250 mm, Shiseido; mobile phase: gradient elution of 30–80% CH_3CN in 10 mmol dm^{-3} $AcONH_4$–NH_4OH pH 8.9 in 30 min; flow rate: 1 cm^3 min^{-1}). After collecting each peak with a retention time of 12.3 and 13.1 min, CH_3CN was removed by evaporation and the remaining solution was lyophilized to afford linearmycin A **1** (9.0 mg) and B **2** (4.0 mg), respectively, as yellow powders. **1**: HR-FABMS (3-nitrobenzyl alcohol matrix) *m/z* 1140.7260 $(M + H)^+$ (Calc. for $C_{64}H_{102}NO_{16}$: 1140.7199); ν_{max}(Nujol)/cm^{-1} 1709 and 1643br; λ_{max}(MeOH)/nm (ε) 317 (119 000), 330 (145 000) and 348 (118 000); $[\alpha]_D^{32}$ −15.9 (*c* 0.115, MeOH). **2**: HR-FABMS (3-nitrobenzyl alcohol matrix) *m/z* 1166.7366 $(M + H)^+$ (Calc. for $C_{66}H_{104}NO_{16}$: 1166.7355); λ_{max}(MeOH)/nm (ε) 317 (58 000), 331 (91 000), 349 (85 000) and 365 (32 000); $[\alpha]_D^{29}$ −11.8 (*c* 0.115, MeOH); δ_H(600 MHz; $[^2H_4]$-MeOH) pentaenoic acid moiety of **2** 1.93 (3 H, br s, 2-CH_3), 7.08 (1 H, br d, *J* 10, 3-H), 6.53 (1 H, 4-H), 6.51 (1 H, *J* 15 and 10, 5-H), 6.36 (1 H, 6-H), 6.37 (1 H, 7-H), 6.21 (1 H, 8-H), 6.27 (1 H, dd, *J* 15 and 10, 9-H), 6.13 (1 H, dd, *J* 15 and 10, 10-H) and 5.75 (1 H, dt, *J* 15 and 7, 11-H).

Administration of labelled compounds

Labelled acetate, or propionate, was dissolved in water at a concentration of 50 mg cm^{-3} and each solution was autoclaved before administration. Portions (50 mg) of sodium [1-^{13}C]acetate, [2-^{13}C]acetate, [1,2-$^{13}C_2$]acetate or [1-^{13}C]propionate (each of 99 atom % ^{13}C, Aldrich) were added to each 500 cm^3 flask containing 100 cm^3 of the medium at the 24 th hour of cultivation. After further cultivation for 72 h and work-up, 11.2 mg, 13.9 mg, 8.2 mg and 17.3 mg of **1** were obtained from 10 × 100 cm^3 broths in the experiments with sodium [1-^{13}C]acetate, [2-^{13}C]acetate, [1,2-$^{13}C_2$]acetate and [1-^{13}C]propionate, respectively.

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