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Structure of Malolactomycins A and B, Novel 40-Membered Macrolide Antibiotics

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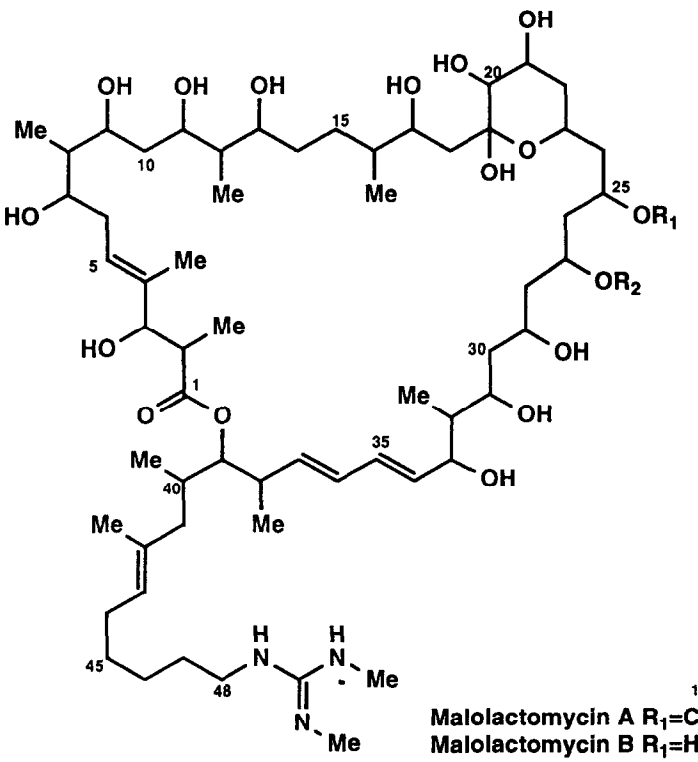
Abstract: Malolactomycins A and B are novel 40-membered polyol macrolide antifungal antibiotics which have hemiacetal, malonate and dimethylguanidyl groups Malolactomycins A and B are positional isomers of malonate and interconvertible each other by transesterification The structures of malolactomycins A and B have been determined by spectroscopic analyses, especially NMR for the parent antibiotics and MS for the oxidative product

Malolactomycins A and B are novel 40-membered macrolide antibiotics, produced by *Streptomyces* sp. 83-634, which have potent antifungal activity. The isolation, physico-chemical properties and biological activities will be reported in elsewhere.[1] In this paper, we report the structural determination of malolactomycins A and B, by spectroscopic analyses, especially NMR studies for the parent antibiotics including complete NMR assignments, and MS study for the oxidative product of malolactomycin A. We also discuss about the structural homology among malolactomycins A, B and related 32- or 36-membered macrolides, i.e amycins[2], azalomycins[3], copiamycins[4], guanidolides[5], guanidylfungins[6], niphimycin (scopafungin)[7]

Malolactomycin A(**1**) have a molecular formula $C_{63}H_{111}N_3O_{20}$ (M.W. = 1229), which was determined by high resolution FAB-MS (m/z 1230.7890 M^+) and elemental analysis. The UV spectrum of **1** showed absorption of diene chromophore at 227(sh), 232 and 240(sh) nm. The ^{13}C NMR spectrum of **1** (Table 1) contains 61 carbon signals (two overlapped peaks are present), including one guanidino carbon (157.34 ppm), three carboxylate carbons, eight olefinic carbons, indicating that **1** possesses two rings in the molecule because ten degrees of unsaturation are required from its molecular formula. Analyses of DEPT and ^1H-^{13}C COSY spectra revealed that the ^{13}C NMR spectrum contains 11 methyl carbons including two equivalent carbons at 28.35 ppm, 17 methylene carbons, and 28 methine carbons including 6 olefinic and 15 oxygenated methine carbons and one quaternary hemiacetal carbon at 99.77 ppm. A peak at 41.17 ppm is overlapped with signals of one methine and one methylene, therefore the number of carbon signals is in agreement with the required carbon number 63

Structural subunits of **1** were constructed using 1D and 2D NMR techniques: NOE difference spectra, ^1H-^1H COSY, ^1H-^{13}C COSY, 1D and 2D ^1H HOHAHA(TOCSY), 2D ^1H J-resolved spectra, ^1H-^{13}C HOHAHA and HMBC spectra, measured in deuterated methanol. In the HMBC spectrum summarized in Fig.

1, long-range couplings are observed between the carbonyl carbon at 177.14 ppm (C-1) and protons of doublet methyl (Me-2) and two methines (H-2 and H-3) The allylic oxygenated methine (C-3, 81.57ppm) was correlated to an olefinic methine proton (H-5, 5 41ppm) and methyl protons (Me-4, 1.61ppm). Allylic methylene protons (H-6) were coupled to the olefinic proton (H-5) and an oxygenated methine (H-7, 4.02ppm) in the ^{1}H-^{1}H COSY spectrum Irradiation at 2.27 ppm (one of H-6) in NOE difference spectrum showed negative NOE between H-6 and Me-4 protons This NOE data suggested that the trisubstituted double bond at C-4 is ***E*** configuration. A vicinal coupling correlation between H-7 and a methine proton shift at 1.50 ppm (H-8) was not observed in ^{1}H-^{1}H COSY and 1D and 2D HOHAHA spectra, because the vicinal coupling constant is very small. The sequential assignments of C-7 to C-9 depended on the HMBC spectrum. Doublet methyl protons at 0.93 ppm (Me-8) was correlated to three methine carbons C-7 (71.92 ppm), C-8 (43.28 ppm) and C-9 (75.94 ppm). A three bond coupling between H-6 and C-8 also supported this sequential assignments. Proton chemical shift of H-9 (3.82 ppm) showed correlation to methylene protons (H-10 at 1 52 and 1.88 ppm) in ^{1}H homonuclear 2D spectra The methine proton signal of H-9 can not be distinguished from that of H-11(3.82 ppm), and carbon chemical shifts were very close (C-9, 75.94 ppm and C-11, 76 00 ppm). The assignments for partial structure C-9 to C-11 were difficult, and cross peak between H-10 (1.88 ppm) and C-9 and/or C-11 in the ^{1}H-^{13}C HOHAHA spectrum was not helpful. Chemical shift of the methylene C-10 was observed relatively upfield to other methylene carbons in 1,3-polyol system (see data of C-20 to C-33 segment in Table 1), presumably caused by double γ-effects of two methyl groups (Me-8 and Me-12) But these sequential assignments remain ambiguous, and this partial structure may be verified after all the remaining signal assignments are completed (*vide infra*). The assignments for C-12 to C-18 segments are based on ^{1}H-^{1}H spin connectivities and supported by the HMBC spectrum In the HMBC spectrum (Fig. 1), doublet methyl signal at 0 912 ppm (Me-12) was correlated with C-11, C-12 and C-13, and methyl signal at 0.907 ppm



Malolactomycin A R_1=$COCH_2COOH$, R_2=H
Malolactomycin B R_1=H, R_2=$COCH_2COOH$

(Me-16) was correlated with C-15, C-16 and C-17. Methylene protons of H-18 (1.77 and 1.88 ppm) were correlated to the methine carbon C-17 and to the hemiacetal quaternary carbon C-19 at 99.77 ppm by two bond coupling. Cross peaks between H-18 and a methine carbon C-20 were also observed in the HMBC spectrum. From a doublet methine proton signal of H-20 adjacent to the quaternary carbon C-19, the sequential assignments to an allylic oxygenated methine (C-33, 73.78 ppm and H-33, 4.42 ppm) were determined by careful analyses of ^{1}H-^{1}H COSY and HOHAHA including 1D HOHAHA differential spectra irradiated at 0.85 ppm (Me-32), 1.29 ppm (H-22), 4.07 ppm (H-23 and H-29) and 5.24 ppm (H-25). These assignments were supported by the HMBC (Fig. 1) and ^{1}H-^{13}C HOHAHA spectra. In the ^{1}H-^{13}C HOHAHA spectrum, the four partial sequences of C-20 - C-25, C-26 - C-27, C-28 - C-30 and C-32 - Me-32 were supported. Low field chemical shift of the oxygenated methine proton H-25 at 5 24 ppm suggested that this methine was acylated. Large coupling constants between H-20 and H-21 (J =9.1Hz), H-21 and H-22a at 1.29 ppm (J =12.0 Hz) and H-22a and H-23 (J =12 0 Hz) indicated the presence of a six membered hemiacetal ring between C-23 alcohol and C-19 ketone, and these three large coupling constants can be assigned to the diaxial coupling constants. The allylic oxymethine H-33 was connected to the diene portion (C-34 to C-37) by ^{1}H-^{1}H COSY analyses. Stereochemistry of the diene was determined as 34 ***E***, 36***E*** from large *trans* coupling constants J =15.0 Hz An allylic methine C-38 at 41 17 ppm overlapped to C-22, and its proton H-38 at 2.52 ppm also overlapped

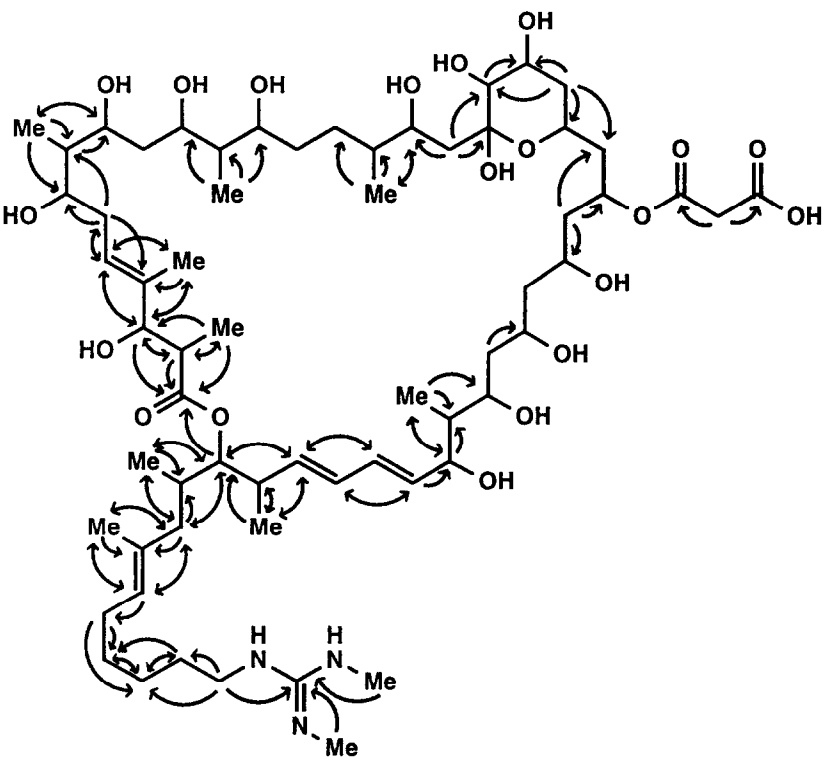


Fig. 1 ^{1}H-^{13}C Long range couplings observed in HMBC spectrum of malolactomycin A (1).

Table 1. ^{13}C NMR data (150MHz) of malolactomycin A(**1**) and B(**2**), and DIS data of **1**.*

No	**1** CD_3OD	**1** CD_3OH	**DIS**[a]	**2** CD_3OD	Δ(1-2)[b] CD_3OD
1	177 14	177.15	0 01	177.18	-0.04
2	45 38	45 45	0 07	45.50	-0 02
3	81.57	81 72	0.15	81.58	-0.01
4	137.53	137.58	0 05	137 48	0.05
5	127.58	127.63	0 05	127.64	-0.06
6	34.29	34.38	0.09	34 31	-0.02
7	71 92	72.06	0.14	71.95	-0.03
8	43 28	43.40	0.12	43.47	-0.19
9	75 94	76 06	0 14	75 95	-0.01
10	39.29	39.38	0 09	39.27	0.02
11	76 00	76 12	0 12	75 95	-0 01
12	44 50	44 63	0 13	44 58	-0.08
13	72.34	72.45	0 11	72.30	0.04
14	33 83	33 94	0.11	33.69	0.14
15	30 62	30.68	0.06	30.30	0.32
16	40.79	40 88	0.09	40 63	0 16
17	72 21	72 36	0 15	72 15	0 06
18	41.56	41.67	0.11	41.86	-0.30
19	99 77	99 88	0 11	99 84	-0 07
20	77.36	77.51	0 15	77.51	-0 15
21	69 70	69 89	0 19	69 76	-0.06
22	41 17	41 28	0 11	41 31	-0 14
23	65 52	65.53	0 01	66.14	-0 62
24	41.92	41.95	0 03	44 47	-2 55
25	70.67	70.71	0.04	65 51	5 16
26	44 60	44.68	0.13	44.66	-0.06
27	65 61	65.74	0.13	70.81	-5 20
28	46.64	46 80	0 16	44 01	2 63
29	66.25	66.35	0 10	65.51	0 74
30	43.20	43 36	0.16	43 34	-0.14
31	71.70	71.80	0.10	71.40	0.30
32	45 81	45 95	0 14	45.91	-0.10
33	73 78	73 90	0 12	73 68	0 10
34	135 25	135.34	0.09	135.16	0.09
35	131 61	131.62	0.01	131 61	0
36	131.90	131.95	0 05	131 89	0.01
37	136.98	137.02	0 04	136.95	0 03
38	41 17	41 22	0 05	41.19	-0 02
39	79.66	79 68	0.02	79.81	-0 15
40	33.15	33 16	0 01	33 20	-0.05
41	45 18	45.22	0 04	45 15	0.03
42	133.91	133 96	0.05	133.95	-0 04
43	128.20	128.25	0.05	128.20	0
44	28.71	28.75	0 04	28.73	-0.02
45	30 40	30 45	0.05	30.41	-0 01
46	27.32	27.37	0 05	27.33	-0 01
47	29.94	29 99	0 05	29 94	0
48	42 66	42.80	0 14	42 66	0
2Me	15 40	15 44	0 04	15.44	-0.04
4Me	10 74	10 77	0 03	10 76	-0 02
8Me	10.06	10.06	0	10 40	-0 34
12Me	10.39	10.39	0	10.54	-0 15
16Me	14.78	14 78	0	15.20	-0.42
32Me	11.08	11.08	0	11 08	0
38Me	17 93	17 94	0.01	18.04	-0 11
40Me	13.93	13.94	0.01	13.97	-0.04
42Me	16.02	16.05	0.03	16.06	-0.04
1'	171.66	171.61	-0.05	171.80	-0.14
2'	46.10	46.10	0	46 15	-0 05
3'	173.95	174 01	0.06	174.00	-0.05
$N{=}CN_2$	157 34	157 51	0.17	157.30	0 04
NMe	28.35	28.51	0.16	28.38	-0.03

* Chemical shifts are reported in ppm relative to TMS as internal standard.

[a] DIS - differential isotope shift is the chemical shift difference in ppm between the ^{13}C chemical shift as observed in CD_3OH and in CD_3OD solutions

[b] Chemical shift difference between the values of malolactomycin A(**1**) and B(**2**) in ppm

Table 2. ^{1}H NMR data (600MHz) of malolactomycin A (**1**) and B (**2**).

No	1	2
2	2 52 m	2 53 m
3	4 03 d (9 9)	4 04 d (9 9)
5	5 41 br t (7.0)	5 43 br t (7 0)
6	2 20 ddd (14 0, 7.0, 7.0), 2 27 ddd (14.0, 7.0, 7.0)	2 22 m, 2 25 m
7	4 02 m	4 03 m
8	1 50 m	1 55 m
9	3.82 m	3 82 m
10	1 52 m, 1.88 m	1 53 m, 1 92 m
11	3 82 m	3.82 m
12	1.56 m	1 55 m
13	3 90 m	3.90 m
14	1.36 m, 1.62 m	1.35 m, 1 59 m
15	1.39 m	1 42 m
16	1.63 m	1 60 m
17	3.86 m	3.90 m
18	1.77 m, 1.88 m	1.76 m, 1.86 m
20	3.33 d (9 1)	3 34 d (9 2)
21	3 86 m	3 86 m
22	1 29 ddd (16 0, 12 0, 12.0), 1.90 m	1 32 m, 1 88 m
23	4 07 m	4 17 m
24	1 65 - 1 78 m	1 65 m
25	5 24 m	4 00 m
26	1 69 m	1 62 - 1.78 m
27	3 89 m	5 30 m
28	1 50 m	1 60 - 1 75 m
29	4 08 m	3.85 m
30	1 64 m	1.45 - 1.60 m
31	3 84 m	3 85 m
32	1.55 m	1 55 m
33	4 42 dd (6 6, 3 0)	4 43 dd (6 2,3.0)
34	5 66 dd (15 0, 6 6)	5 66 dd (15 0, 6.2)
35	6 12 dd (15 0, 10 2)	6 13 dd (15.0, 10 7)
36	6.05 dd (15 0, 10.2)	6 05 dd (15.0, 10.7)
37	5 47 dd (15.0, 9.2)	5 47 dd (15 0, 9 2)
38	2.52 m	2.53 m
39	4 74 dd (8 4, 3 3)	4.73 dd (8.1, 3.3)
40	1 99 m	2 00 m
41	1 83 m, 2.08 dl (13.0, 6 5)	1 83 m, 2.07 m
43	5 18 br t (7 0)	5 18 br t (7 0)
44	2 03 m	2.04 m
45	1.40 m	1 42 m
46	1.39 m	1 40 m
47	1.61 m	1.60 m
48	3 18 t (7 3)	3.18 t (7.3)
2Me	0.86 d (6 9)	0 86 d (7.0)
4Me	1 61 s	1 61 s
8Me	0 93 d (7 0)	0 93 d (7 0)
12Me	0 912 d (7 0)	0 91 d (7 0)
16Me	0 907 d (6 6)	0 88 d (7 0)
32Me	0 85 d (6 9)	0 84 d (7 0)
38Me	1 01 d (6 6)	1 01 d (6 9)
40Me	0 89 d (6.5)	0 89 d (6 5)
42Me	1 57 s	1 57 s
2'	3 24 m	3 25 m
NMe	2.84 s	2.84 s

* Recorded in CD_3OD solutions Chemical shifts are reported in ppm relative to TMS as internal standard Coupling constants (J values in Hz) are give in parentheses

with H-2 proton signal. But the sequential assignments from C-33 to C-41 were easily established by correlations from two doublet methyl protons Me-38 (1.01 ppm) and Me-40 (0.89 ppm) in the HMBC spectrum (Fig. 1). In the HMBC spectrum, the oxymethine proton H-39 at 4.74 ppm was coupled by the carbonyl carbon C-1 at 177.14 ppm, suggesting that malolactomycinA (**1**) possesses 40-membered macrolide structure. Presence of a double bond at C-42 was confirmed by the cross peaks observed in the HMBC spectrum from the methylene protons at 1.83 and 2.08 ppm (H-41) to olefinic carbons at 133.91 ppm (C-42) and 128.20 ppm (C-43) and olefinic methyl carbon at 16.02 ppm (Me-42). Stereochemistry of the double bond at C-42 was determined as 42*E* by NOE differential spectra. NOEs were observed from an allylic methylene protons H-44 at 2.03 ppm to the methyl protons Me-42 at 1.57 ppm, and from the olefinic proton H-43 at 5.18 ppm to the protons H-41 Segment from C-43 to C-48 containing five methylene groups was determined by analyses of the ^{1}H-^{13}C HOHAHA spectrum. The HMBC spectral data also supported these sequential assignments and showed correlation between the methylene protons H-48 to guanidyl carbon at 157.34 ppm. Chemical shift of the methylene protons H-48 (3.18 ppm) and that of C-48 (42.66 ppm) suggested that this methylene group was connected to the guanidyl group. Two equivalent methyl groups whose signal appeared at 2 84 ppm (6H, s) in the ^{1}H NMR and appeared at 28 35 ppm in the ^{13}C NMR were determined as *N*-methyl groups. *N*-alkyl, *N'*-, *N''*-dimethyl trisubstituted guanidine group was assigned from the chemical shift of *N*-methyl groups.[3d] No correlation between two equivalent *N*-methyl groups was observed in the HMBC spectra, and this evidence eliminates the possibility of the presence of *N*-alkyl, *N'*-dimethylguanidine group. One remaining isolated methylene group at 3.24 ppm in the ^{1}H NMR and at 46.10 ppm in the ^{13}C NMR was assigned to a malonyl group based on the evidence of the HMBC data and the results of deuterium exchange experiments. In the HMBC spectrum, the methylene protons H-2' were correlated to two carbonyl carbons at 171.61 ppm (C-1') and 174.01 ppm (C-3'). The methylene protons H-2' were gradually (in 1 or 2 days) exchanged by deuterium in CD_3OD solution.[8] Completely deuterated methylene was re-exchanged with protons by dissolving in CD_3OH solution. In the above discussion, all signals of the carbons and protons of **1**, including the segment C-9 to C-11, were completely assigned and summarized in Tables 1 and 2 To confirm the presence of the hemiacetal six membered ring and twelve free secondary hydroxyl groups, deuterium induced differential isotope shift (DIS) in the ^{13}C NMR (CD_3OD vs. CD_3OH environments) was applied.[9] The large upfield shifts due to deuterium substitutions of hydroxyl groups were observed in the range from 0.19 to 0.10 ppm for twelve oxymethines; C-3, 7, 9, 11, 13, 17, 20, 21, 27, 29, 31 and 33, which were assigned to hydroxy methine carbons. No induced upfield shift was observed for the oxymethines C-23, 25 and 39. The results strongly suggested that the hydroxyl group at C-23 formed the six membered hemiacetal ring with C-19 carbonyl group, and in the case of C-25 and C-39, the hydroxyl groups were acylated Some methylene carbons in the 1,3-polyol system shifted to upfield due to γ-effects of the deuterium isotope This evidence indicates that application of DIS methodology to the modified 1,3-polyol system such as in the malolactomycinA molecule is very useful, but some difficulty and complication of analysis to the 1,2-polyol system are suspected.[9] DIS were also observed on the nitrogen bearing carbons C-48 and *N*-methyl carbons Additionally in the ^{1}H NMR spectrum of **1** in CD_3OH solution, broad signals of NH protons were observed at 7.20 - 7.30 (2H) ppm. Based on the results of the NMR studies described above, the planar structure of malolactomycin A (**1**) was determined as shown.

FAB-MS spectral data of **1** gave some structural information supporting the proposed structure (Fig. 2) Fragment ions at m/z 1144 and 1126 were assigned to demalonylated ions. Small fragment ions derived from side chain portion were observed at m/z 114, 142, 210 and 238. More sequential information were obtained from analyses of the derivative **1a** prepared from **1** by $NaIO_4$ oxidative cleavage of hemiacetal ring and demalonylation by alkaline treatment(0.1*N* NaOH). In MeOH solution, **1a** takes hemiacetal form(**1b**) which is a ca 1·1 equilibrium of α- and β-anomers. The hemiacetal methine (C-21) signals were observed at 95.3 and 93 4 ppm in the ^{13}C NMR and at 5.32(*br.d*, *J* =2 Hz) and 4.67(*br.d*, *J* =8 Hz) in the ^{1}H NMR. In the SI-MS spectrum of **1a** (Fig 3), sequential fragment ions derived from C-1 - C-19 portion were observed together with fragment ions from C-21- C-39 portion. These mass spectral data supported the proposed structure of malolactomycin A(**1**)

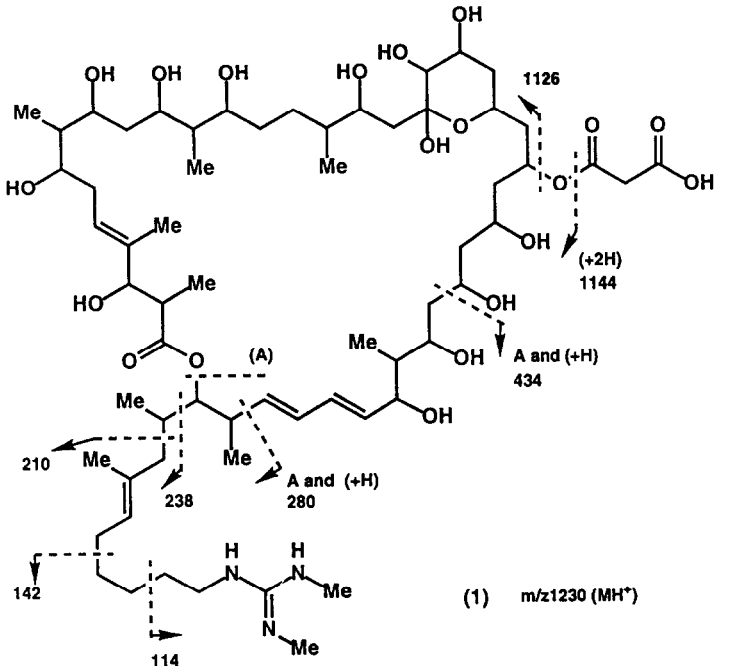


Fig. 2 FAB-MS fragmentations of malolactomycin (1).

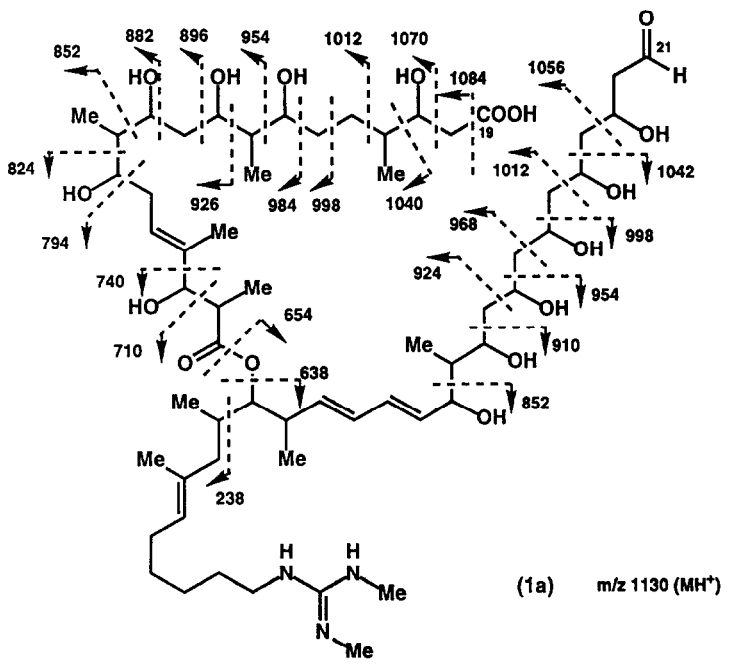
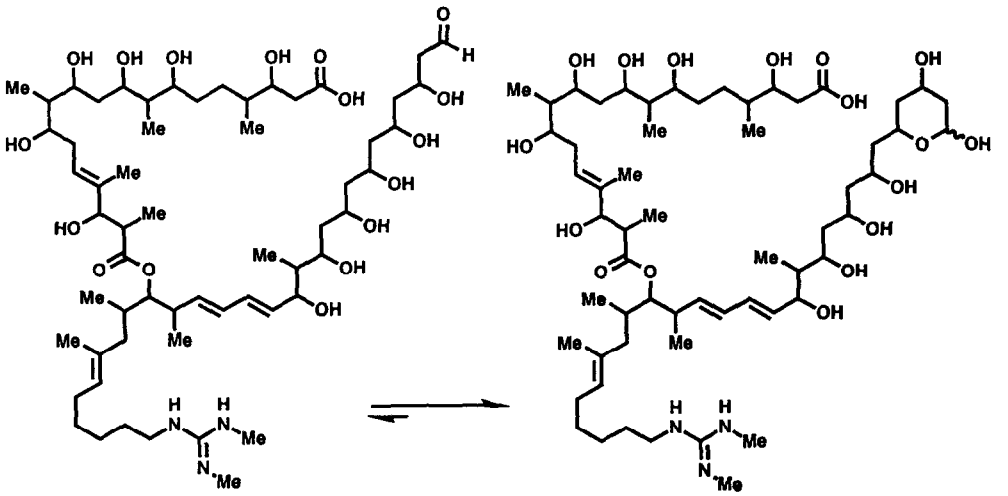


Fig. 3 SI-MS fragmentations of oxidative derivative (1a) of malolactomycin A



(1a) (1b) (α:β = 1:1)

Malolactomycin B(**2**) has the same molecular formula $C_{63}H_{111}N_3O_{20}$ as that of malolactomycin A (**1**), determined by HR-FAB-MS. The UV, FAB-MS and NMR spectra of **2** were similar to those of **1**. Purified **2** slowly changed to the mixture of **1** and **2**, and the ratio of the mixture reached to ca. 1:1 by about 1 month in MeOH solution at room temperature. MalolactomycinA (**1**) also changed to the mixture in a similar mode. The ratio was checked by HPLC and ^{1}H NMR. These evidences suggested that **1** and **2** are interconvertible isomers.[3a] Analyses of 2D NMR data, homo and hetero COSY and HOHAHA, and HMBC of **2** revealed that **2** is a positional isomer of **1** in the malonate linkage position. The position of the malonate for **2** was determined to be C-27 by the sequential assignments from the doublet methine proton (H-20) at 3.34 ppm based on the ^{1}H connectivity. NMR assignments were summarized in Table 1 and 2. One oxymethine proton signal at 4.17 ppm was separated in chemical shift and was assigned to H-23. In the 1D HOHAHA experiments, spectral data obtained by irradiation of protons H-23 and the malonylated oxymethine proton at 5.30 ppm suggested that the methine proton at 4.00 ppm is H-25 and the methine proton at 5.30 ppm is H-27. The carbon chemical shifts of **2** in the ^{13}C NMR assigned by 2D NMR data were compared by those of **1**. The values of the chemical shift differences between **1** and **2** are summarized in Table 1. Large chemical shift differences were observed on the carbon signals of C-23 to C-29 and the α, β and γ -effects of the malonyl substitution reasonably explain the shift differences. Based on the these spectral evidences the structure of malolactomycin B(**2**) was determined as 25-*O*-demalonyl-27-*O*-malonyl malolactomycin A.

Some structural homologies are observed in comparison of the structures between malolactomycin A and some related 36-membered macrolides, guanidylfungin(**3**), niphimycin I(**4**) ,azalomycin F_{4a}(**5**) and 32-membered copiamycin (**6**). The segment of C-8 to C-29 in malolactomycin A around the hemiacetal portion has good homology with these macrolides. The positions of the ketone group masked as a hemiacetal, C-19 in the case of malolactomycin A, are (*n*/2 -1) position in *n*-membered macrocyclic lactones. Two types of side chains containing guanidyl group exist. One type is seen in malolactomycinA and **3** which contains five linked methylenes and a trisubstituted double bond. Another is longer and contains a *trans* disubstituted double bond as seen in the molecules of **4**, **5** and **6**. In the case of **5**, one C3 unit originated from propionate is absent.[7,10] Some variation occured on the segments around the carbonyl group and ring oxygen of the lactone. **3**, **4**, **5** and **6** have *N*-monomethylguanidyl group *N*, *N'*-dimethyl guanidyl group like malolactomycins A and B is seen in azalomycin F_{5a}.[3d] The most characteristic structural feature of malolactomycins A and B is that **1** and **2** have the largest 40-membered lactone ring

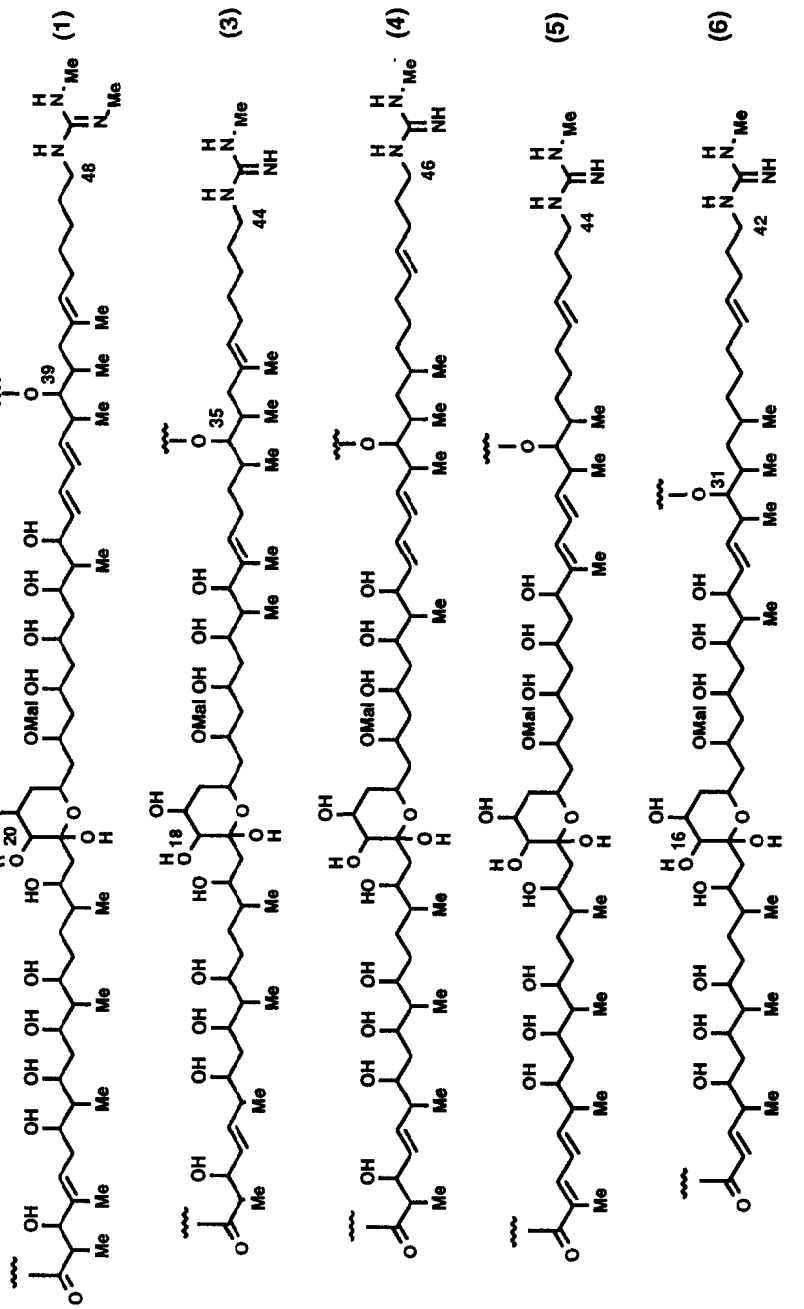


Fig. 4 Structures of malolactomycin A (1), guanidylfungin (3), niphimycin I (4), azalomycin F_{4a}(5), and copiamycin (6).

EXPERIMENTAL

General. NMR spectra including 2D experiments were measured by Jeol JNM-α600 spectrometer. DIS data were obtained by Jeol JNM α400 spectrometer Mass spectra were taken by Jeol JMS HX-110 and Hitachi M-80 mass spectrometers for FAB-MS and SI-MS, respectively

Preparation of 1a. $NaIO_4$ (6 mg) was added to a solution of **1**(12 mg) in MeOH-H_2O (1 ml, 1:1) After standing for 12 hr at room temperature, the solution was diluted with brine (5 ml) and extracted with 1-BuOH(5 ml , 3 times) The combined 1-BuOH extracts were washed with water and evaporated to dryness. The residue was dissolved in MeOH (1 ml) and added to a 0.1*N* aq. solution (1 ml). The reaction mixture was left for 12 hr at room temperature. The solution was neutralized with diluted HCl and washed with EtOAc (5 ml, 3 times) The aq. solution was extracted with 1-BuOH (5 ml, 3 times) and washed with brine (5 ml) and water (5 ml). After evaporation of the combined 1-BuOH extract, the residue was purified by HPLC (Capcell Pak C-18 column (Shiseido) , MeOH-H_2O , 7:3) to give **1a** (3 mg): SI-MS m/z 1130 (MH^+). ^{1}H-NMR (600MHz, in CD_3OD)· δ 6 18 (dd, H-35), 6.09 (dd, H-36), 5 67 (dd, H-34), 5.54 (dd, H-37), 5.44 (br.t, H-5), 5.32 and 4.67 (each br.d, H-21), 5.18 (br.t, H-43), 4 75 (dd, H-39), 4.43 (m, H-33), 4.20-3.60 (m, H-7, 9, 11, 13, 17, 23, 25, 27, 29 and 31), 4.03 (d, H-3), 3 18 (t, H-48), 2 83 (s, N-Me), 2.54(m, H-2 and 38), 2 50-1.10 (m, H-8, 10, 12, 14, 15, 16, 18, 22, 24, 26, 28, 30, 32, 40, 41, 44, 45, 46 and 47), 1.63, (s, Me-4), 1 57 (s, Me-42), 1.01 (d, Me-38), 0.95, 0.92, 0.91(x2), 0.85 and 0.83 (each d, Me-2, 8, 12, 16, 32 and 40) ^{13}C-NMR (150 MHz, in CD_3OD): δ 177.5 (C-1), 175 0 (C-19), 157.5 (N=CN_2), 137.8 (C-4), 136.4 (C-37), 134.8 (C-34), 134 0 (C-42), 131.9 (C-36), 131.5 (C-35), 128.1 (C-43), 127.0 (C-5), 95.3 and 93.4 (C-21), 81.5 (C-3), 80.2 (C-39), 75.9 and 75.8 (C-9 and 11), 73.7 (C-33), 74.0, 73.8, 72.6, 72.0, 71.5, 70.0, 68.6, 68 4, 66 3, 65 6 and 64.1(C-13, 17, 23, 25, 27, 29 and 31), 46.7, 46.8, 45.5, 45.4, 45.0, 43.8, 43.5, 43 3, 42 8, 41 8, 41 2, 40.9, 40.7, 40 3, 39 8, 39.5, 39 4 and 39.2 (C-2, 8, 10, 12, 16, 18, 22, 24, 26, 28, 30, 32, 38 and 41), 42.6 (C-48), 34.0 (C-6), 33.4 (C-14 and 40), 30.4 (C-45), 30.0 (C-15), 29.9 (C-47), 28 7 (C-44), 28 3 (NMe), 27.3 (C-46), 17 9 (Me-38), 15.9 (Me-42), 15.4 (Me-2), 14. 7 (Me-16), 14 2 (Me-40), 11.1 (Me-32), 10 9, 10 4 and 10.3 (Me-4, 8 and 12)

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