ISOLATION AND CHARACTERIZATION OF A NEW ANTIBIOTIC, MALOLACTOMYCIN A

Sir:

A new 40-membered ring macrolide antibiotic, malolactomycin A (Fig. 1) which shows preventive effect to fungal infection of plants in a pot test, has been isolated from a culture broth of *Streptomyces* sp. 83-634. The strain was isolated from a soil sample collected in Nago city, Okinawa Islands. The strain was deposited at the National Institute of Bioscience and Human-technology (formerly the Fermentation Research Institute), Agency of Industrial Science and Technology, under the accession No. FERM P-10771. The strain was cultured at 28°C for 72 hours in 500-ml shaking flasks containing 70 ml of the following medium; glucose 2%, soluble starch 1%, meat extract 0.1%, dried yeast 0.4%, soybean flour 2.5%, NaCl 0.2% and K_2HPO_4 0.005% (pH 7.0).

Isolation of malolactomycin A was carried out by monitoring the antifungal activity *in vitro*. Malolactomycin A was extracted from the mycelia of 5-liters of fermentation broth with 80% aqueous acetone. After removal of acetone *in vacuo*, the residual solution was extracted with butanol. The butanol extract was concentrated *in vacuo* and the syrupy residue was applied to a silica gel column equilibrated with dichloromethane. The column was eluted stepwise with dichloromethane-methanol mixtures (30:1, 20:1, and 15:1). The active fractions were concentrated *in vacuo* and chromatographed on a silica gel column prepared with butanol-methanol-water mixtures (8:1:1). The elution was made stepwise with butanol-methanol-water (8:1:1, 5:1:1, and 4:1:2). Active fractions were further purified by chromatography on a Sephadex LH-20 column with methanol to yield a colorless powder (105 mg). Final purification was achieved by preparative HPLC on a Nucleosil $_5C_{18}$ column (20 × 300 mm) with 82% methanol, affording 43 mg of malolactomycin A as a white powder.

The antibiotic has a decomposition point of 129~131°C and a titrable group with a p*Ka'* 4.9 in 70% methyl cellosolve. It moved slightly toward a cathode at pH 3.0 but remained at an original point at pH 8.8 on high-voltage paper electrophoresis (2,000 V, 15 minutes). It is optically active with $[\alpha]_D^{20}$ +15.1° (*c* 0.34, methanol). It is soluble in lower alcohols, sparingly soluble in acetone, but hardly soluble in ethyl acetate, chloroform and water. It gave positive reactions to permanganate, periodate-benzidine and Sakaguchi test. The high resolution fast atom bombardment (HRFAB)-MS gave $(M+H)^+$ *m/z* 1,230.7890 (Calcd. for $(C_{63}H_{111}N_3O_{20}+H)^+$ 1,230.7833). *Anal*; Found C 61.28, H 9.15, N 3.37; Calcd. for $C_{63}H_{111}N_3O_{20}$

Fig. 1. Structure of malolactomycin A.

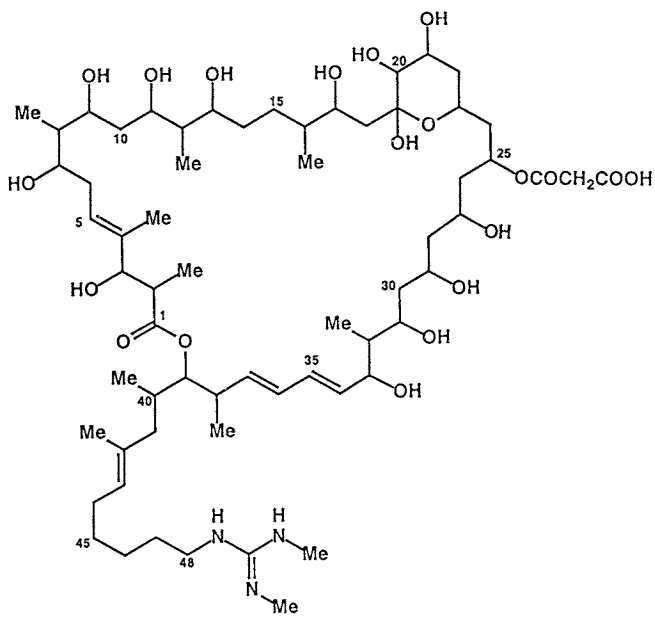


Fig. 2. IR spectrum of malolactomycin A in KBr.

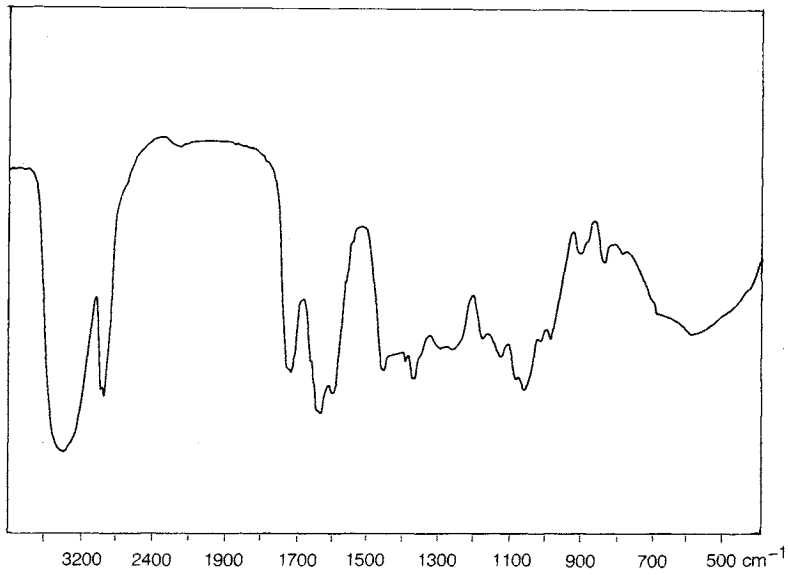
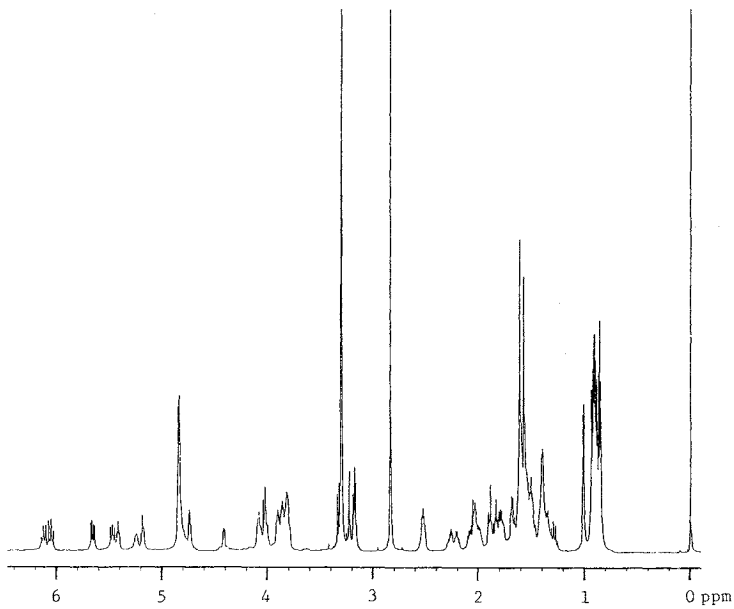


Fig. 3. ^{1}H NMR of malolactomycin A in methanol-d_4 at 600 MHz.



(C 61.49, H 9.09, N 3.41). The UV absorption spectrum in MeOH was observed as follows, λ_{max} nm (ε); 227 (sh, 28,000), 232 (29,600), 240 (sh, 20,600). The IR, ^{1}H and ^{13}C NMR spectra of malolactomycin A are shown in Figs. 2, 3 and 4, respectively.

All ^{13}C and ^{1}H signals of malolactomycin A were completely assigned by detailed NMR studies including 2D experiments of ^{1}H-^{1}H and ^{1}H-^{13}C COSY, HOHAHA, HMBC and 1D NOE difference spectra. The carbon sequence in malolactomycin A from the lactonic carbonyl carbon at 177.14 ppm to the terminal *N,N'*-dimethyl guanidine group (157.34 and 28.35 ppm) was determined by careful analyses of NMR data. The 40-membered macrocyclic structure was established by the correlation

Fig. 4. ^{13}C NMR of malolactomycin A in methanol-d_4 at 150 MHz.

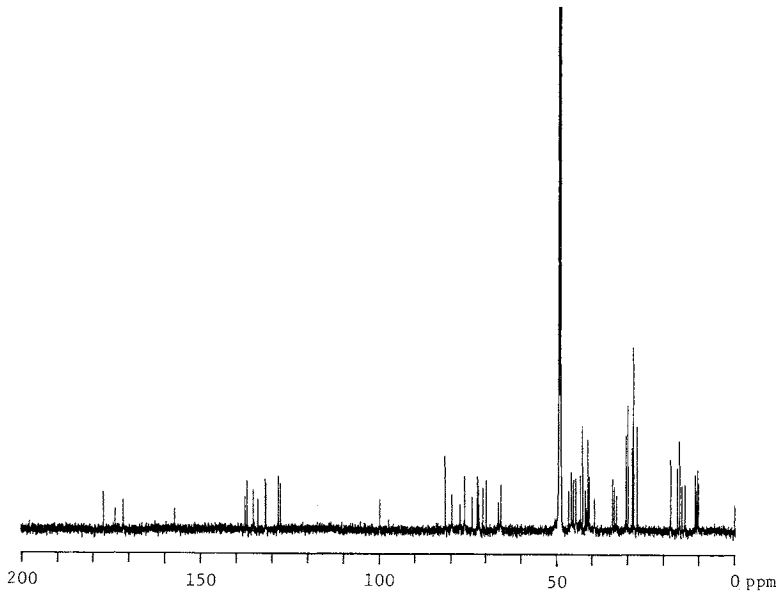


Table 1. *In vitro* antimicrobial activity of malolactomycin A.

Test organism	MIC (μg/ml)
Staphylococcus aureus FDA 209P	12.5
Bacillus subtilis NIHJ PCI 219	12.5
Escherichia coli K-12 IFO 3301	>100
Salmonella typhimurium TV 119	>100
Cochliobolus miyabeanus IFO 5277	3.2
Alternaria mail IFO 8984	12.5
Botryotinia fuckeliana IFO 5365	1.6
Colletotrichum lagenarium IFO 7513	1.6
Pellicularia filamentosa sp. *sasakii* IFO 6258	1.6
Glomerella cingulata IFO 9767	25
Pyricularia oryzae IFO 5994	6.25
Trichophyton mentagrophytes IFO 6202	6.25
Aspergillus oryzae IFO 5239	25
Saccharomyces cerevisiae IFO 0304	1.6
Candida albicans IFO 1594	1.6

Minimal inhibitory concentrations (MICs) were determined by the conventional agar dilution method. Potato sucrose agar medium was used for yeasts and fungi, and nutrient agar medium was used for bacteria.

Table 2. Preventive effects (%) of malolactomycin A against some plant diseases.

Malolactomycin A (μg/ml)	Gray mold on cucumber	Anthracnose on cucumber	Brown spot on rice plant
100	97	82	81
75	92	77	79
50	88	60	75
25	0	43	64

Botritis cinerea, *Colletotrichum lagenarium*, and *Cochlibolus miyabeanus* are pathogenic fungi of gray mold, anthracnose and brown spot diseases, respectively. Cucumber (*cv.* Sagamihanjiro) and rice plant (*cv.* Aichi-asahi) were sprayed with 1-ml of malolactomycin A solution at the concentrations indicated prior to inoculation of pathogenic fungi on a leaf surface. Plants infected with pathogenic fungi were incubated for 4 days at suitable conditions. The size and number of rotten lesions was measured to calculate the preventive value (%).

from the oxymethine proton H-39 at 4.74 ppm to the carbonyl C-1 in the HMBC spectrum. The position of malonate was determined to C-25 which proton appeared at 5.24 ppm. The structure was also supported by mass spectral data for malolactomycin A and its $NaIO_4$ oxidative product. The structural determination of malolactomycin A will be reported in a separate paper[1].

Malolactomycin A is a new antibiotic having a 40-membered ring, a malonate and a methylguanidino group. Azalomycins F_{3a}, F_{4a} and F_{5a}[2~4], copiamycin[5], neocopiamycin A[6], guanidolide A[7], guanidylfungins A and B[8], niphithricin[9] and niphimycin (nifimycin)[10~12] have some structural similarity possessing a macrocyclic lactone and a guanidino residue, however, all antibiotics are different from malolactomycin A in their molecular

weight and formula. Amycin A[13] is the most similar to malolactomycin A, however, the former has a 36-membered ring and the latter has a 40-membered ring. Moreover, amycin A has two malonyl and one *N*-methyl residue in a structure, on the contrary, malolactomycin A has one malonyl and two *N*-methyl residues in a structure.

Malolactomycin A showed broad antimicrobial activity against various bacteria, fungi and yeasts *in vitro* (Table 1). The preventive effect of the antibiotic against plant diseases was tested in pot tests (Table 2). Malolactomycin A was effective in the prevention of gray mold disease but not so effective against anthracnose and brown spot diseases. The acute toxicity (LD_{50}) of malolactomycin A was 6.7 mg/kg in mice by intraperitoneal injection. The mechanism of action of the antibiotic should be elucidated.

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