MARIDOMYCIN, A NEW MACROLIDE ANTIBIOTIC. II

ISOLATION AND CHARACTERIZATION

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A new group of macrolide antibiotics was obtained from the fermentation broth of *Streptomyces hygroscopicus* No. B-5050. Isolation of six components, maridomycins I, II, III, IV, V and VI, has been accomplished by silica gel adsorption or partition chromatography. They show only end absorption in UV-spectra and are classified as new macrolide antibiotics from their physicochemical, chemical and biological properties.

A novel antibiotic complex, maridomycin, which is a lipophilic basic substance and mainly active against gram-positive bacteria, was obtained from the fermentation broth of *Streptomyces hygroscopicus* No. B-5050.[1] Maridomycin was found to consist of six components from its chromatogram on paper or silica gel thin-layer. They were separated by silica gel chromatography and designated as maridomycins I, II, III, IV, V and VI, in the order of their decreasing Rf values on thin-layer chromatogram. Their physicochemical properties suggest that maridomycin belongs to a new group of macrolide antibiotics. This paper deals with the isolation and characterization of maridomycin.

Isolation

The isolation of maridomycin (MDM) complex was carried out by the general procedure for lipophilic basic substances. MDM complex was extracted from a filtered broth of *Streptomyces hygroscopicus* with ethyl acetate at pH 8 and transferred to water at pH 3, and again into ethyl acetate at pH 8. This ethyl acetate extract was concentrated to give a crude material by addition of *n*-hexane. Crystallization of this crude material from benzene gave colorless crystals of MDM complex.

The MDM complex was roughly fractionated by partition between ethyl acetate and SÖRENSEN citrate buffer of pH 4.16. MDM III, IV, V and VI were mainly transferred to the aqueous layer, while I and II remained in ethyl acetate layer. The organic solvent layer (Fraction 1) was washed with water, evaporated to dryness and crystallized from benzene to give a mixture of MDM I and II. The aqueous layer was made slightly basic with aqueous sodium hydroxide solution and reextracted with ethyl acetate. The extract was evaporated and the residue was crystallized from benzene to give a mixture of MDM III, IV, V and VI (Fraction 2).

MDM I and II were isolated from Fraction 1 by the column chromatography on silica gel developed with benzene-ethyl acetate (2:3) or benzene-acetone (5:1). MDM III, IV, V and VI were isolated from Fraction 2 by the column chromatography on silica gel developed with the upper layer of ethyl acetate-*n*-hexane-ethanol-water (1:1:1:2), and obtained as colorless crystals, respectively.

Chart 1. Procedure for the isolation of maridomycin components

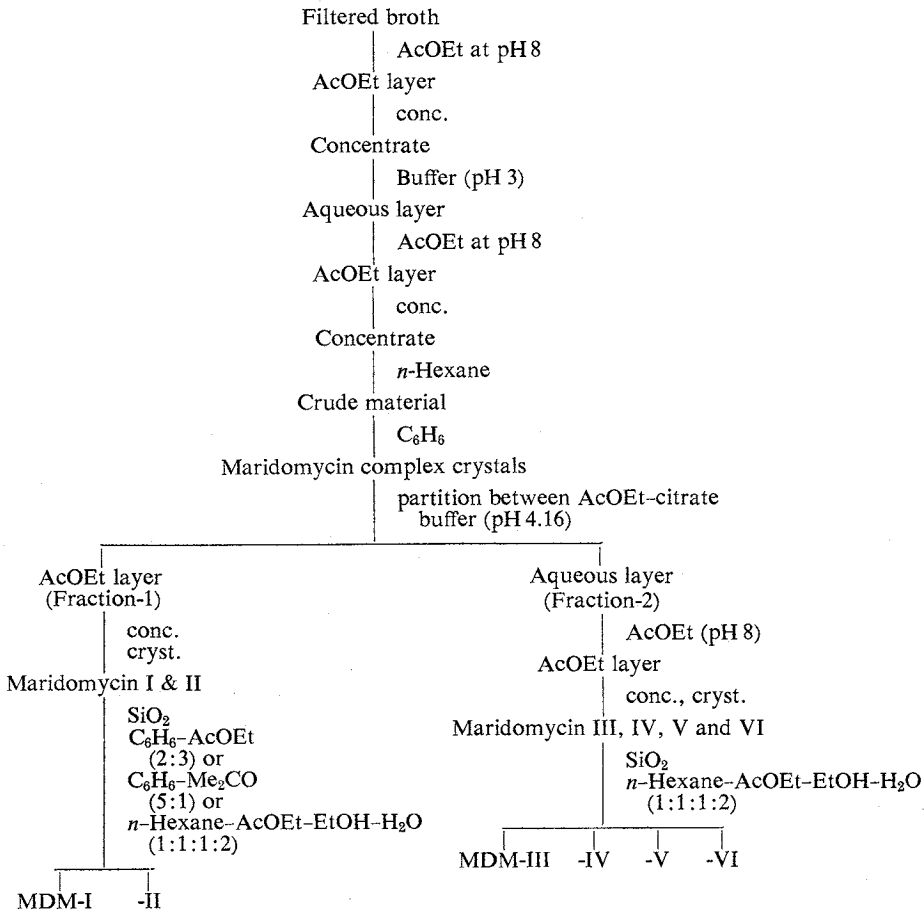


Table 1. Physicochemical properties of maridomycins

MDM		I	II	III	IV	V	VI
m.p. (°C) (decomp.)		129~132	134~136	135~138	143~146	144~149	149~154
$[\alpha]_D$ (*c* 1, EtOH)		−72.3°	−71.9°	−76.0°	−76.2°	−73.2°	−77.7°
Molecular weight							
Mass M^+ (*m/e*)		857	843	829	815	815	801
V.P.O.* (AcOEt)		910	856	830	830	889	864
Analysis							
Found	C	59.08	58.62	57.93	57.62	57.76	57.00
	H	8.41	8.22	8.18	8.09	8.13	7.86
	N	1.59	1.49	1.69	1.76	1.63	1.75
Calcd. for		$C_{43}H_{71}NO_{16}\cdot H_2O$	$C_{42}H_{69}NO_{16}\cdot H_2O$	$C_{41}H_{67}NO_{16}\cdot H_2O$	$C_{40}H_{65}NO_{16}\cdot H_2O$	$C_{40}H_{65}NO_{16}\cdot H_2O$	$C_{39}H_{63}NO_{16}\cdot H_2O$
	C	58.95	58.52	58.07	57.61	57.61	57.13
	H	8.40	8.30	8.20	8.10	8.10	7.99
	N	1.60	1.62	1.65	1.68	1.68	1.71
pKa′ (50%EtOH)		6.7	6.8	7.1	6.9	7.1	7.1

* V.P.O. = Vapour Pressure Osmometry.

Each component of MDM was also isolated by partition column chromatography on Celite impregnated with the lower layer of *n*-hexane-ethylenedichloride-methanol-water (120:80:30:6), and developed with the upper layer of this solvent system.

Physicochemical Properties

All the six components of maridomycin have very similar physicochemical properties. They are soluble in methanol, ethanol, acetone, ethyl acetate, *n*-butyl acetate and chloroform; slightly soluble in ethyl ether and benzene; hardly soluble in *n*-hexane, petroleum ether and water. The physicochemical properties of six components of MDM are summarized in Table 1. They give the

Fig. 1a. IR spectra of maridomycins (KBr)

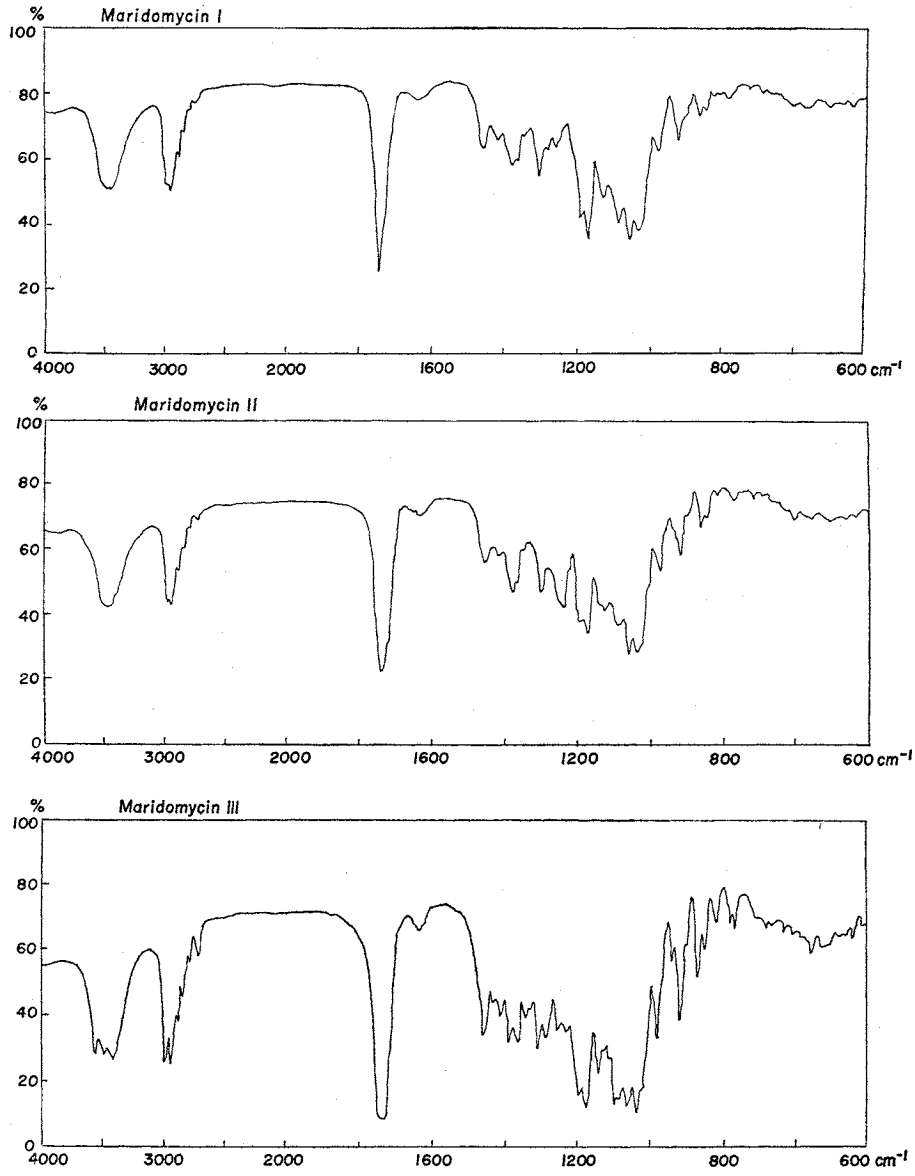
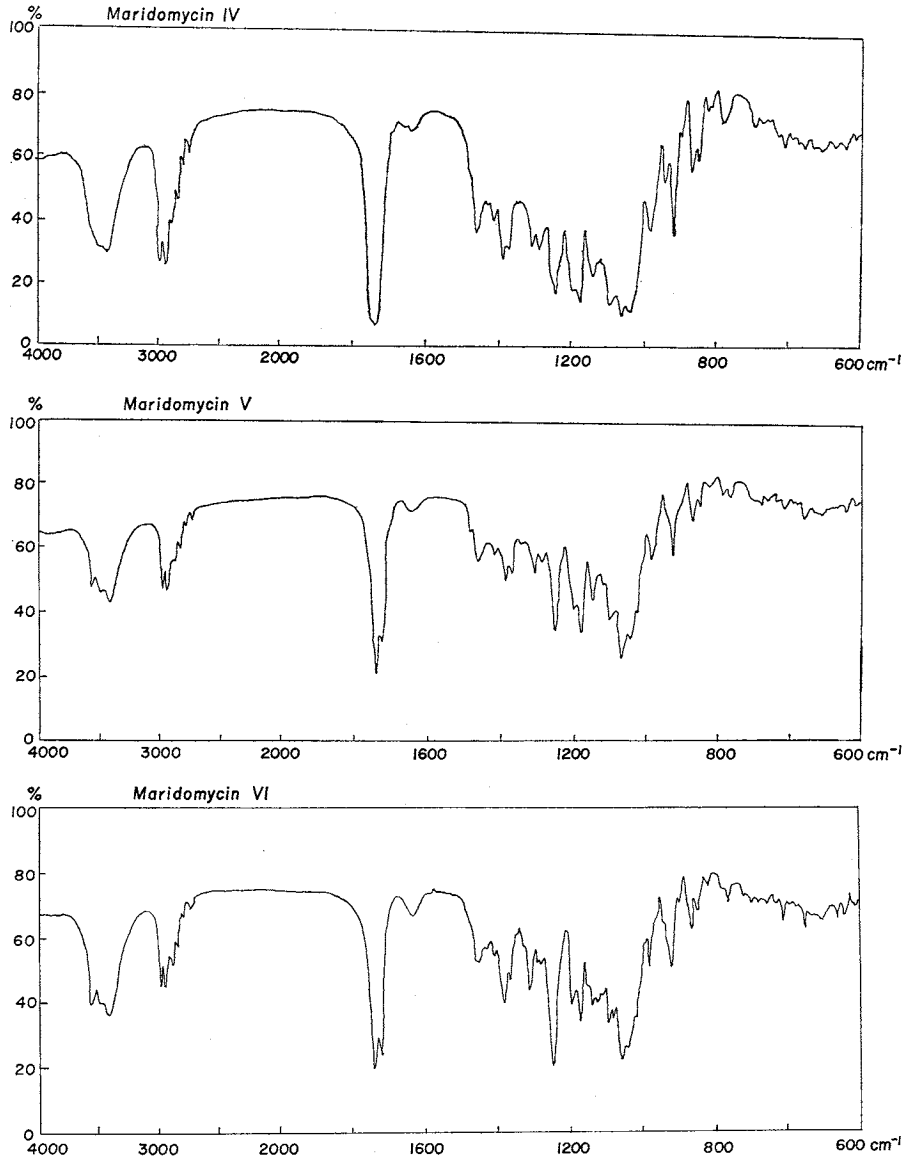


Fig. 1b. IR spectra of maridomycins (KBr)



same color reactions; positive DRAGENDORFF reaction and erythromycin test,[2] but negative ninhydrin reaction, ferric chloride reaction and carbomycin test.[2] MDM show only end absorption in UV spectra. MDM are weakly basic with pKa' 6.7~7.1 in 50% aqueous ethanol. As shown in Fig. 1, the IR spectra of these components are quite similar and exhibit strong absorption bands at about 3500 cm^{-1} (ν_{OH}), 1730 cm^{-1} ($\nu_{C=O}$), 1050~1200 cm^{-1} (ν_{C-O-C}). Except for MDM I and III, the other components, II, IV, V and VI, have a rather strong absorption band at 1230~1240 cm^{-1} which suggests the presence of acetate (ν_{C-O-Ac}) but different from each other in their finger print region. Six components of MDM are differentiated from one another on thin-layer chromatogram as shown in Fig. 2. The Rf values of these components on thin-layer chromatograms are also given in Table 3.

Table 2. Physicochemical properties of known macrolides

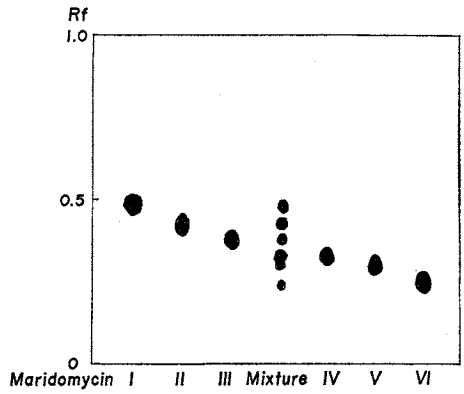
	Erythromycin	Erythromycin B	Erythromycin C	Oleandomycin
m.p. (°C)	135~140 190~193	202~203 198	121~125	120~121 110
$[\alpha]_D$	−73.5°	−78°		−65°
Molecular weight	738.1	730	730	688
Molecular formula	$C_{37}H_{67}NO_{13}$	$C_{37}H_{67}NO_{12}$	$C_{33}H_{65}NO_{13}$	$C_{35}H_{61}NO_{12}$
pKa′	8.6	8.8, 8.5	8.5	8.5
	Megalomicin A	**Megalomicin B**	**Megalomicin C_1**	**Megalomicin C_2**
m.p. (°C)	255~259	135~140	238~242	146~150
$[\alpha]_D$	−90°	−92°	−102°	−102°
Molecular weight	868		914	
Molecular formula	$C_{44}H_{80}N_2O_{15}$	$C_{45}H_{82}N_2O_{16}$	$C_{45}H_{84}N_2O_{17}$	$C_{49}H_{86}N_2O_{17}$
N content	3.31%	2.74%	2.90%	2.88%
pKa′	9.0			

Table 3. Rf values of maridomycins on thin-layer chromatograms (SiO_2)

MDM	Solvent system		
	a	b	c
I	0.48	0.68	0.71
II	0.42	0.63	0.66
III	0.37	0.57	0.61
IV	0.32	0.53	0.55
V	0.30	0.50	0.52
VI	0.27	0.43	0.48

Solvent: a; C_6H_6–Me_2CO (3:2) (Spotfilm, Tokyo-kasei)
b; C_6H_6–Me_2CO (3:2) (Kieselgel G, Merck)
c; C_6H_6–MeOH (10:1) repeated three times (Kieselgel G, Merck)
Detection: i) conc. H_2SO_4, ii) 5% I_2 in $CHCl_3$, iii) 5% phosphomolybdic acid in EtOH, iv) 5% ceric sulfate in N–H_2SO_4.

Fig. 2. Thin-layer chromatogram of maridomycin.
Adsorbent: SiO_2
Solvent: C_6H_6–Me_2CO (3:2)



Biological Properties

Each component of maridomycin shows strong activity against Gram-positive bacteria[3] and weak activity against an artificially induced oleandomycin-erythromycin resistant strain and mycobacteria.[1] The antibiotics have strong antimycoplasma activity.* Among the MDM components, MDM I is the strongest. The increase in antimicrobial activities of components is correlated with the increase in their molecular weights.

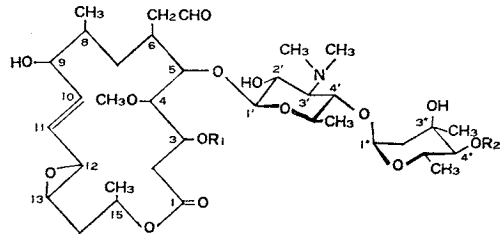
When MDM complex and each component were administered in ICR-JCL male mice by oral route, none of them showed any significant acute toxicity even at a dose of 10 g/kg. The acute toxicities (LD_{50}) of them administered intraperitoneally were more than 1,000 mg/kg.

* KANEKIYO, T.; I. OOE *et al.*: private communication.

Discussion

The physicochemical and biological properties described above clearly indicate that maridomycins are macrolide antibiotics. The characteristic property of MDM is that they do not exhibit any maxima in the UV region except for end absorption below 210 nm. From this point, MDM are readily distinguishable from the other macrolide antibiotics which have strong UV absorption maxima at about 225 nm (such as pikromycin), 232 nm (such as leucomycin), 240 nm (such as carbomycin A) or 280 nm (such as carbomycin B). Erythromycins,[4~8] oleandomycin[9] and megalomicins which show only weak absorption at 280~290 nm, also differ from MDM[10,11] in the IR spectra, molecular weights, molecular formulae, melting points, specific rotation, nitrogen content and pKa′, as shown in Table 2. From these findings, MDM I, II, III, IV V and VI are found to be a new group of macrolide antibiotics.[12]

Fig. 3. Structures of maridomycins.



	R_1	R_2
Maridomycin I	$COCH_2CH_3$	$COCH_2CH(CH_3)_2$
II	$COCH_3$	$COCH_2CH(CH_3)_2$
III	$COCH_2CH_3$	$COCH_2CH_3$
IV	$COCH_3$	$COCH_2CH_3$
V	$COCH_2CH_3$	$COCH_3$
VI	$COCH_3$	$COCH_3$

The structure of MDM II was determined from the fact that the 9-dehydro derivative of MDM II was identified with carbomycin A.[13] The structures of other components were elucidated as shown in Fig. 3.[14] Recently SUZUKI *et al.* reported the isolation and structural elucidation of antibiotic YL-704C_1, which was to be identical with MDM-III.[15]

Experimental

Separation of maridomycin complex

The broth filtrate (4,000 liters) of *Streptomyces hygroscopicus* No. B-5050 was adjusted to pH 8, and extracted with one third of AcOEt. The extract was concentrated *in vacuo* to 100 liters and after washing with water, the concentrate was extracted three times with 50 liters of M/3 KH_2PO_4 solution adjusted to pH 3.0 with aqueous phosphoric acid. The acidic aqueous solutions were combined and reextracted with 75 liters of AcOEt at pH 9. The extract was washed with water and concentrated to 1.5 liters. Thirty liters of *n*-hexane was added into the concentrate to yield 522 g of crude powder. Crystallization of this material from benzene gave 272 g of maridomycin complex as colorless needles. m.p. 137~141°C (dec.), $[\alpha]_D$—76.4° (*c* 1, EtOH), pKa′ 6.8 (in 50% EtOH). Anal. found: C 58.07, H 8.20, N 1.65. M.W. (V.P.O., in AcOEt) 880, UV end absorption, IR (KBr) 1735 cm^{-1} ($\nu_{C=O}$), 1200~1050 cm^{-1} (ν_{C-O-C}).

Isolation of maridomycin components

(1) A solution of MDM complex (40 g) in AcOEt (4 liters) was extracted twice with 2 liters of M/10 citrate buffer of pH 4.16. The upper layer was washed with H_2O and concentrated to dryness. Crystallization of the residue from benzene gave a mixture consisting mainly of MDM I and II (Fraction 1, yield; 9.4 g). The aqueous solutions were combined and reextracted with AcOEt at pH 8. The organic layer was evaporated to give a mixture consisting mainly of MDM III, IV, V and VI (Fraction 2, yield; 26.8 g).

The Fraction 1 (2 g) was chromatographed on 250 g of silica gel (E. Merck, 0.05~0.2 mm). The column was developed first with a mixture of AcOEt and benzene (1:1, 200 ml), then with a mixture of AcOEt-benzene (3:2), monitored by TLC of each fraction (20 ml) with the solvent system composed of benzene-acetone (3:2). Fractions giving a single spot were combined and evaporated. Thus, 435 mg of pure MDM I and 185 mg of II were obtained as colorless crystals.

The Fraction 2 (5 g) was subjected to a column prepared from 500 g of silica gel which was saturated in advance with the upper layer of a mixture composed of *n*-hexane–AcOEt–EtOH–H_2O (1:1:1:2). The column was developed with the same solvent, monitored by the TLC method described above and 20 ml portions were collected. Fractions giving a single spot of each component were pooled and concentrated to dryness. Crystallization of each residue afforded crystals of MDM III (1.64 g), IV (450 mg), V (300 mg) and VI (220 mg), respectively.

(2) A column for partition chromatography prepared with *n*-hexane (120 ml), ethylene dichloride (80 ml), methanol (30 ml), H_2O (6 ml), Celite 535 (Johns-Manville, 5 g) and chlorophenol red (1 mg) was adjusted with hydrogen chloride until the color of the indicator just regained its yellow color. MDM complex (25 mg) was dissolved in the upper layer (0.4 ml) and the solution was applied to the column, then elution was carried out slowly with the upper layer. Separation of each component was observed in order of MDM I, II, III, IV, V and VI as purple bands. MDM I (4 mg), II (3 mg), III (5 mg), a mixture of IV and V (4 mg), and VI (2 mg) were obtained from each band by evaporation of the eluates.

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