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Maridomycin, a New Macrolide Antibiotic. XI.[1] The Structures of Maridomycin Components

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Structures of five members of maridomycins have been determined from chemical and spectroscopic evidences. All the six components of maridomycin have been found to be constituted of the same sixteen membered lactone, D-mycaminose and 4-O-acyl-L-mycaroses. Furthermore, since maridomycin I was chemically interrelated with maridomycin II, the configurations have been elucidated to be the same in both components. Further chemical evidence for the other components established all of their configurations to be identical, and the absolute stereochemistries of six natural maridomycins have been finally determined as shown in Chart 11. Stereochemistry of the aglycone portion in solution is also discussed.

In the preceding paper,[1] the structure of maridomycin II (MDM II) has been elucidated to be a sixteen membered macrolide as shown in Chart 1. Other members of maridomycin, MDM I (**1**), III (**3**), IV (**4**), V (**5**) and VI (**6**) coproduced by *Streptomyces hygroscopicus* No. B-5050 have very similar physicochemical properties and their structures seem to be related to that of MDM II (**2**). In this paper, the structural determination of five components of maridomycins are presented.[3]

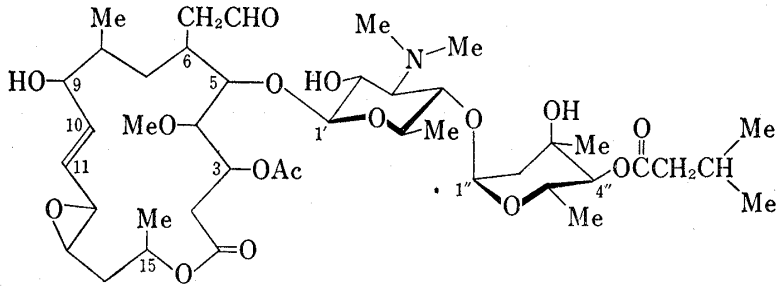


Chart 1

Maridomycin I (MDM I) (**1**), mp 129—132°, $[\alpha]_D^{23}$ −72.3° (EtOH) has a weak basic nature of pK_a' 6.7 (50% EtOH) like MDM II. Its mass spectrum gave a molecular ion peak at *m/e* 857, and the molecular formula, $C_{43}H_{71}O_{16}N \cdot H_2O$ was in good agreement with the analytical data and water content determination (Karl Fisher method), *i.e.*, one methylene greater than MDM II. The infrared (IR) spectrum indicated the presence of lactone and/or ester carbonyl (1720—1740 cm^{-1}) and aldehyde groups (2725 cm^{-1}), and difference between MDM I and MDM II was observed at the acetate band of 1235 cm^{-1}. The nuclear magnetic resonance (NMR) spectrum of MDM I revealed the common functional groups except acetyl group at δ 2.25 to MDM II as shown in Table I.

The signals of N-dimethyl group (δ 2.54, 6H, s), O-methyl (δ 3.55, 3H, s), two olefinic protons (δ 5.69 and 6.09, each 1H, dd) and aldehydic proton (δ 9.63, 1H, s) were almost identical

1) Part X: M. Muroi, M. Izawa, and T. Kishi, *Chem. Pharm. Bull.* (Tokyo), **24**, 450 (1976).
2) Location: *17–85, Jusohonmachi 2-chome, Yodogawa-ku, Osaka 532, Japan.*
3) A part of this work was reported as a preliminary communication; M. Muroi, M. Izawa, and T. Kishi, *Experientia*, **28**, 129 (1972).

Table I. Chemical Shifts of MDM I, II, and III (100 MHz, in $CDCl_3$, δ ppm)

	sec. CH_3	C_3–OAc	$-N(CH_3)_2$	$-OCH_3$	C_5–H	C_9–H	$C_{1'}$–H	$C_{5''}$–H
MDM I	0.99[a]	—	2.54	3.55	3.91	4.03	4.45	4.45
MDM II	1.00[a]	2.25	2.54	3.56	3.92	4.03	4.46	4.45
MDM III	1.05[b]	—	2.52	3.53	3.89	4.02	4.43	4.45

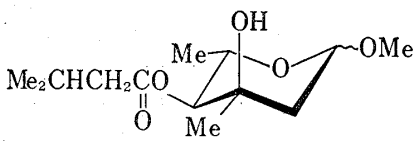
	$C_{4''}$–H	C_{15}–H	$C_{1''}$–H	C_3–H	C_{11}–H	C_{10}–H	–CHO
MDM I	4.63	4.95	5.06	5.16	5.69	6.09	9.63
MDM II	4.64	4.95	5.08	5.15	5.66	6.10	9.65
MDM III	4.61	4.95	5.08	5.15	5.67	6.08	9.62

a) Isovaleryl $CH_3+C_8-CH_3$
b) C_8-CH_3

with those of MDM II (**2**). Further, the signals of the methine protons attached to oxygen function at δ 3.91, 4.03, 4.45, 4.63 and 5.03 were quite similar to those of **2**. These signals were assigned as shown in Table I, and indicated that the partial structure deduced from the NMR, is very similar to MDM II. In addition, the mass spectrum of **1** suggested the presence of mycaminose moiety (*m/e* 173, 174 and 190) and O-isovaleryl mycarose moiety (*m/e* 229). The presence of two secondary hydroxyl groups was proved by the formation of diacetate (**7**), $C_{47}H_{75}O_{18}N$, $[\alpha]_D^{25}$ −80.4° (EtOH) upon treatment with acetic anhydride in pyridine at room temperature, its NMR spectrum exhibiting two new acetyl signals at δ 2.02.

Catalytic hydrogenation of MDM I over palladium on charcoal in EtOH led to absorption of two moles of hydrogen to afford tetrahydro MDM I (**8**) $C_{43}H_{75}O_{16}N$, $[\alpha]_D^{27}$ −59.6° (EtOH), suggesting the presence of a labile functional group besides one double bond. As in the case of MDM II, an epoxide group was suggested by formation of MDM I diol (**9**), $C_{43}H_{73}O_{17}N$, which gave MDM I diol tetraacetate (**10**), $C_{51}H_{81}O_{21}N$, (M^+, *m/e* 1043).

Saponification of **1** gave one mole each of isovaleric acid and propionic acid[4] identified with authentic sample, indicating that the ester groups were present as isovalerate and propionate. Methanolysis of MDM I (**1**) gave a pair of isomers of neutral sugar which were identified with authentic sample of α- and β-methyl 4-O-isovaleryl-L-mycarosides[5] (**11a** and **11b**).



11a,b

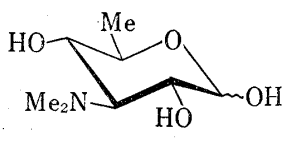


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Chart 2

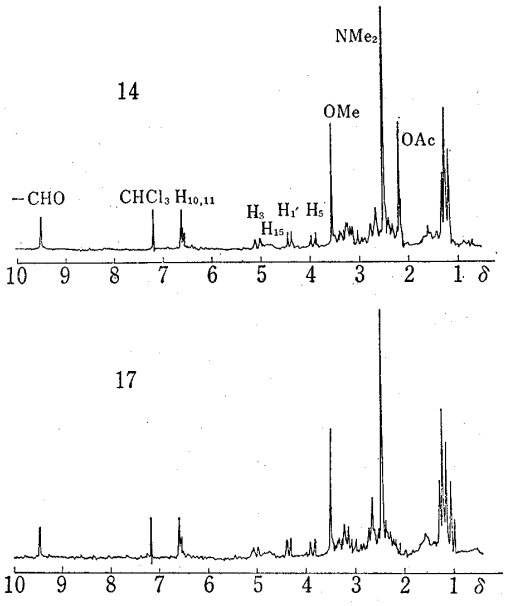


Fig. 1. NMR Spectra of **14** and **17** (in $CDCl_3$)

Vigorous acid hydrolysis of basic complex obtained from methanolysis of MDM I as above afforded D-mycaminose (**12**),[6] $C_8H_{17}O_4N \cdot HCl$.

These facts indicated that MDM I contained the same basic and neutral sugar moiety, and differed in the aglycone portion. In mass spectrum of **1**, the fragment peak due to the aglycone moiety was found to be one methylene (*m/e* 14) larger than that of **2**. It was assumed that the difference between **1** and **2** is ascribed to acyl group at the C-3 position because both of them showed the same elimination fragment of fatty acid in aglycone moiety. (*cf.* Chart 6 and Table IV). Therefore, the aglycone part of MDM I (**1**) is supposed to be by one methylene greater than that of MDM II, suggesting that **1** contains propionyl group instead of acetyl at C-3 position in MDM II.

Since the intact aglycone part of MDM I was difficult to obtain by direct hydrolysis,[7] for comparison of aglycone part, MDM I was oxidized with Sarret reagent,[8] giving dehydro MDM I (**15**), $C_{43}H_{69}O_{16}N$, mp 198—199°, $[\alpha]_D^{32}$ −55.0° ($CHCl_3$), along with N-formyl dehydro MDM I (**16**), $C_{43}H_{67}O_{17}N$. Dehydro MDM I (**15**) was further treated with 0.5N HCl at room temperature. Demycarosyl[9] dehydro MDM I (**17**), $C_{31}H_{49}O_{12}N$, mp 188—189°, obtained from the basic fraction besides 4-O-isovaleryl-L-mycarose (**18**) as a neutral sugar on this acid treatment, showed almost identical UV maximum at 240 nm (ε 13300) with **15** and demycarosyl 9-dehydro MDM II (**14**) obtained from 9-dehydro MDM II (**13**). The mass spectrum of the compound (**17**) showed a molecular ion peak at *m/e* 627 which is by 14 mass unit larger than that of **14** (M^+, *m/e* 613). Along with the lack of acetyl signal in the NMR of compound **17**, it is concluded that the propionate is located at the position C-3 of aglycone instead of the acetate. The signal of C-3 methine proton in the NMR of **17** ($CDCl_3$) was observed at δ 5.13 which is superimposable to that of **14**.

Table II. Chemical Shifts of Compounds **14** and **17** (100 MHz, in $CDCl_3$, δ ppm)

	$-CH_2CH_3$ (3H, t)	*sec.* CH_3 (3H, d)	*sec.* CH_3 (3H, d)	$-OCOCH_3$ (3H, s)	$-N(CH_3)_2$ (6H, s)	$-OCH_3$ (3H, s)
14	—	1.20	1.24	2.20	2.52	3.58
17	1.10	1.20	1.24	—	2.51	3.55

	C_5–H (1H, dd)	$C_{1'}$–H (1H, d)	C_{15}–H (1H, m)	C_3–H (1H, dq)	C_{10}–H & C_{11}–H (2H, m)	–CHO (1H, s)
14	3.88	4.46	4.86	5.10	6.68	9.58
17	3.95	4.44	4.80	5.11	6.65	9.55

4) Isovaleric acid and propionic acid were quantitatively estimated using columns of Porapak Q and Chromosorb 101, respectively. Fatty acids liberated by saponification of other components were determined using Chromosorb 101 in the same manner.
5) S. Omura, M. Katagiri, H. Ogura, and T. Hata, *Chem. Pharm. Bull.* (Tokyo), **16**, 1167 (1968).
6) F.A. Hochstein and P.P. Regna, *J. Am. Chem. Soc.*, **77**, 3353 (1955); T. Watanabe, *Bull. Chem. Soc. Japan.*, **34**, 15 (1961).
7) Acid hydrolysis of MDM I with 0.5N HCl at room temperature as in the case of MDM II afforded a mixture of complicated products, some of which showed intensive absorption maxima at 280 nm, indicating the formation of dienone type chromophore. One of these rearrangement products were presumed to be a type of carbomycin B. Direct comparison of the rearrangement product from MDM II and demycarosyl carbomycin B confirmed the identity of both compounds. On methanolysis were formed rearrangement products with the same chromophore showing UV maxima at 280 nm. Similar reactions occurred also in other components of maridomycin under acidic conditions.
8) G.I. Poos, G.E. Arth, R.E. Beyler, and L.H. Sarret, *J. Am. Chem. Soc.*, **75**, 422 (1953).
9) The designation, "Demycarosyl" was used as an abbreviation of the basic compounds after cleavage of mycarose portion.

Further direct evidence for the structural interrelationship between MDM I and MDM II was attained by mild alkaline treatment which brought about newly introduced α,β-unsaturated lactone function through selective β-elimination. When MDM I was treated with a slightly excess of potassium hydroxide at low temperature, a compound with α,β-unsaturated lactone (Δ^2-MDN I) (**19**) was formed in almost quantitative yield. The compound (**19**) was found to be identical with Δ^2-MDM II in NMR, IR, UV and mass spectra, and optical rotations. Therefore, the structure of MDM I is defined as the compound with a propionyloxy group at the C-3 position instead of acetoxy group in MDM II.

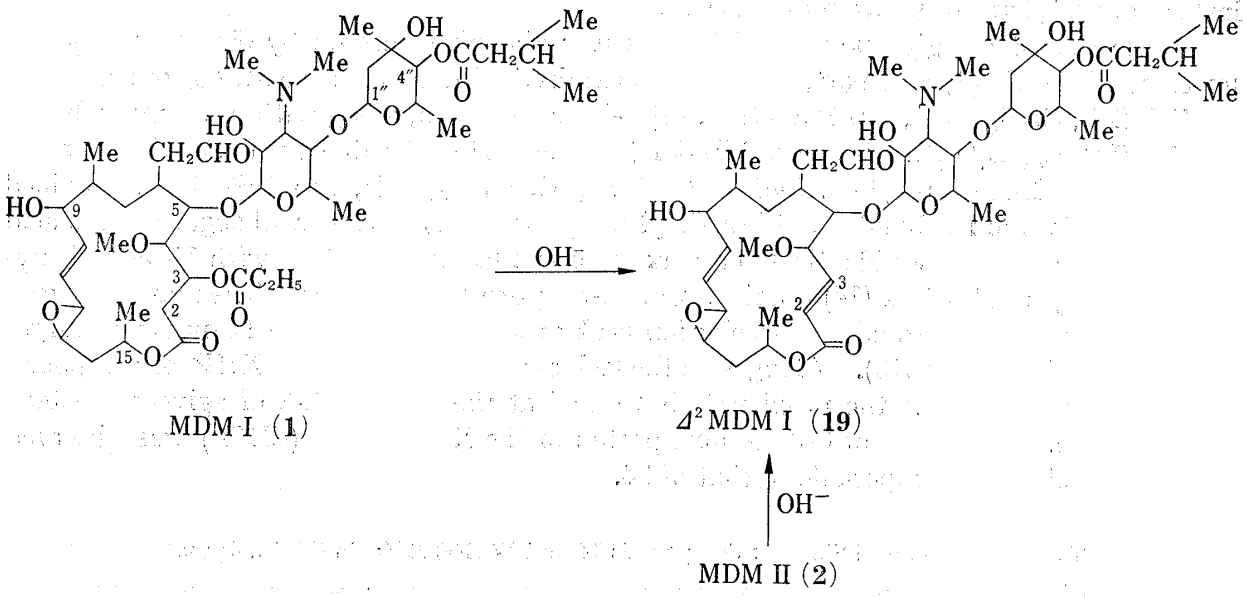


Chart 3

As, under this condition, selective β-elimination in the aglycone portion occurred without any changes at other functional groups, the configuration of MDM I was clarified except for C-3 and C-9 positions. As for the C-3 position, comparison of C-3 methine proton in NMR between MDM I and II or between their derivatives such as 9-dehydro-, tetrahydro-, *etc.*, indicates that the stereochemistry of MDM I is the same with MDM II. Easy elimination[10] of acyloxy groups at the C-3 position under the same condition suggests that both components exist in similar conformation in solutions in spite of difference of acyl group and the leaving groups at C-2 and C-3 positions have diaxial *trans*-relationship, with the C-1, C-2 bond and C-3, C-4 bond being antiperiplanar around the C-2, C-3 bond.

The structure of maridomycin III (MDM III, **3**) was analogously elucidated as a homologue of maridomycin II. MDM III was isolated as colorless needles of mp 135—138°, $[\alpha]_D^{23}$ −76.0° (EtOH), from maridomycin complex. MDM III gave a molecular ion peak at *m/e* 829 ($C_{41}H_{67}O_{16}N$), and the analytical data and water content determination were in good agreement with a monohydrate. The molecular formula was also confirmed by the molecular ion peaks at *m/e* 871 in mass spectra of two kinds of monoacetates, $C_{43}H_{67}O_{17}N$ (**20**), (**21**) which were obtained by acetylation with acetic anhydride in acetone or acetylchloride in pyridine, respectively.

The pK_a' value (7.1) of (**3**) indicated the presence of one basic group as in the case of MDM II (**2**). The IR spectrum of (**3**) as a whole showed the similarity to MDM I and II, *i.e.*, intense bands at 1720—1740 cm^{-1} (C=O) and 1050—1200 cm^{-1} (C–O–C), and a band at

10) D.J. Cram, "Olefine forming elimination reaction" in "Steric effects in organic chemistry," ed. by M.S. Newman, John Wiley & Sons, Inc., New York, 1956, p. 304; C.K. Ingold, "Structure and mechanism in organic chemistry," 2nd ed. Cornell University Press, Ithaca, 1969, p. 649.

2720 cm^{-1} characteristic of aldehyde function. Since the ultraviolet (UV) spectrum of (**3**) showed only end absorption, (**3**) was found not to contain any conjugated systems such as diene or enone. In addition, NMR spectrum in $CDCl_3$ of MDM III exhibited quite similarity in the region δ 3.5—10.0 ppm as shown in Table I. From these data, it is suggested that MDM III might be a homologue of MDM II. Although the signal of $-N(CH_3)_2$(6H, s) was found in the same position as MDM II, the O-acetyl signal was not observed in MDM III. Because saponification of **3** gave two moles of propionic acid,[4] the main difference was considered to be ascribed to acyl groups.

Then, MDM III was subjected to methanolysis under the same condition as in the case of MDM II, and from the neutral portion were isolated two methyl glycosides, one of which (**22a**), $C_{11}H_{20}O_5$ (M^+, *m/e* 232), $[\alpha]_D^{22}$ −145.2° ($CHCl_3$) was very similar to methyl 4-O-isovaleryl-α-L-mycaroside (**11a**) in the NMR and mass spectrum. The difference between them was found to be due to acyl group at the C-4 position, *i.e.*, isovaleryl and propionyl. All other physical data were in good agreement with those of 4-O-propionate of methyl-α-L-mycaroside and those reported.[11] Another methyl glycoside (**22b**) was analogously determined to be methyl 4-O-propionyl-β-L-mycaroside (not 3-O-propionate). The basic portion of methanolysis was not fully characterized due to the complexity.[7] And the aglycone part of MDM III could not be obtained through acid hydrolysis[7] of MDM III.

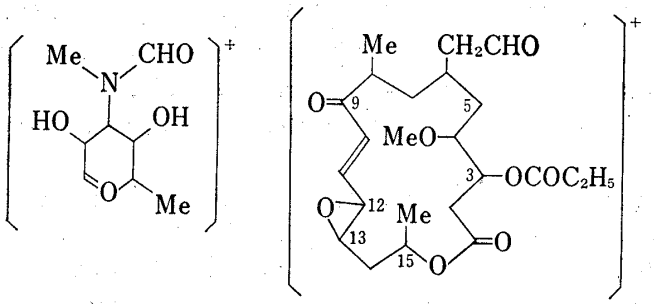
Then as a result of several attempts to verify the stereochemical inter-relationship among MDM I, II and III, mild alkaline treatment of MDM III under controlled condition afforded α,β-unsaturated lactone compounds (**23**) and (**24**) which had lost acyl functions. The compound (**23**), $C_{35}H_{57}O_{13}N$ (M^+, *m/e* 699) showed new signals at δ 5.99 (1H, d) and 6.63 (1H, dd) due to olefine group conjugated to carbonyl function. Both compounds **23** and **24** showed intensive absorption at 210 nm in the order of ε 17500. Another α,β-unsaturated lactone (**24**) appeared to have the same aglycone moiety from UV, NMR, and mass spectra. The compound (**24**) is found to be an elimination product of propionic acid from **3** in the aglycone moiety by the mass spectral comparison of **24** and **3**. The difference between **23** and **24** was recognized in the mass spectra in which the latter showed a prominent ion peak at *m/e* 201 due to propionylmycarose, while the former did not show such ion peak. These facts further support that one of the propionyl group in **3** was located at the C-4 position of mycarose, and the other at the β position of lactone carbonyl. Consequently, the positions of acyl functions were found to be the same as MDM I and II. Since the presence of allylic hydroxyl group was suggested, MDM III was oxidized with CrO_3-pyridine under the mild condition to give mainly two kinds of dehydro compounds which were separated by silica gel chromatography. One of them (**25**) $C_{41}H_{65}O_{16}N$, was obtained as colorless needles, mp 204—205°, $[\alpha]_D^{25}$ −60.1° ($CHCl_3$), and showed a UV maximum at 240 nm (ε 14400). The compound (**25**) was subsequently hydrolyzed with 0.5N HCl at room temperature to give a basic compound showing a UV maximum at 240 nm (ε 13300) along with a neutral sugar which was identified as 4-O-propionyl-L-mycarose (**26**) in comparison with the physical data (NMR, mass, IR, $[\alpha]_D$) of 4-O-isovaleryl-L-mycarose and methyl 4-O-propionyl-L-mycarosides.[11]

The basic compound was identified with demycarosyl 9-dehydro MDM I (**17**) by direct comparison. Therefore, it is evident that the aglycone and basic sugar moiety of MDM III and I is identical, including the absolute configuration. The mycarose unit of **3** should be linked at C-2 or C-4 position of mycaminose and furthermore the similarities of anomeric protons in the NMR spectra of MDM III and II, and their down field shifts of anomeric protons and C-2 methine protons by acylation of free hydroxyl group of mycaminose suggest that mycarose is linked at the C-4 position of mycaminose.

Another dehydro compound of MDM III, (**27**), obtained as a byproduct of oxidation with CrO_3–pyridine showed the same UV maximum at 240 nm, but in the NMR spectrum $-N(CH_3)_2$

11) S. Omura, M. Katagiri, and T. Hata, *J. Antibiotics*, **21A**, 272 (1968).

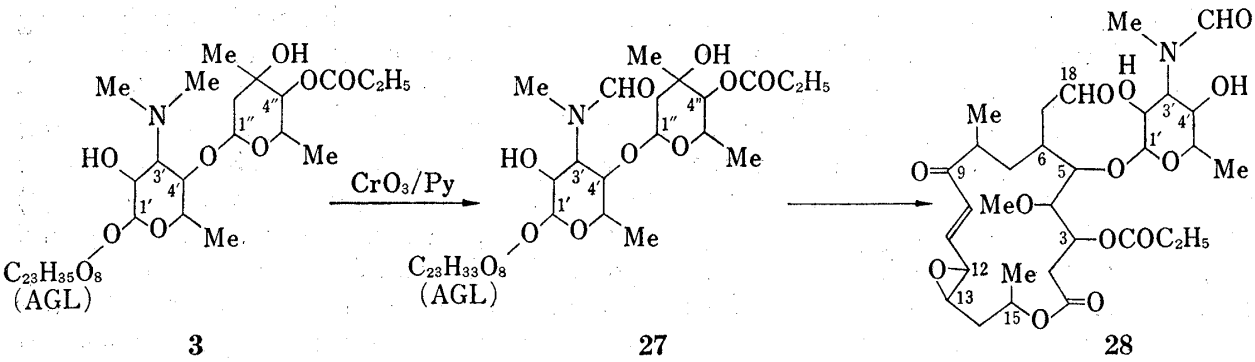
signal disappeared. Subsequent acid hydrolysis of **27** yielded 4-O-propionyl-L-mycarose (**26**) and demycarosyl compound (**28**), mp 161—162°, $C_{31}H_{47}O_{13}N$. In the NMR spectrum of (**28**), the signal of $-N-CH_3$ (3H, s) was observed at δ 2.78 instead of $-N(CH_3)_2$ signal, and in addition its mass spectrum showed prominent peak (**a**) due to N-demethyl-N-formyl-mycaminose at *m/e* 188 instead of deoxymycaminose ion peaks (*m/e* 173, 174). Fragment ion peaks due to the aglycone moiety were observed at *m/e* 437 (**b**) and 363 (**b**-C_2H_5COOH) like the compound (**17**).



a **b**

Chart 4

Accordingly, the structure of **27** was defined as a oxidized one of $-N-(CH_3)_2$ to N-formyl group as shown in Chart 5. Similar reaction product was reported in the case of oxidation of methymycin in which $-N(CH_3)_2$ of desosamine was found to be oxidized to N-formyl group.[12]



3 **27** **28**

AGL=aglycone
Py=pyridine

Chart 5

Further stereochemical evidence was offered from the following direct interrelationship between MDM I and III. When MDM I was subjected to mild alkaline treatment for prolonged time, was isolated 4″-deisovaleryl-Δ^2-MDM I. In comparison of this compound with **23** from MDM III both were found to be identical from IR, UV and NMR spectra and mixed melting point. This fact finally confirmed that MDM I and III have the same basic structure from the standpoint of stereochemistry. The interrelationship between MDM I and III are presented in Chart 6.

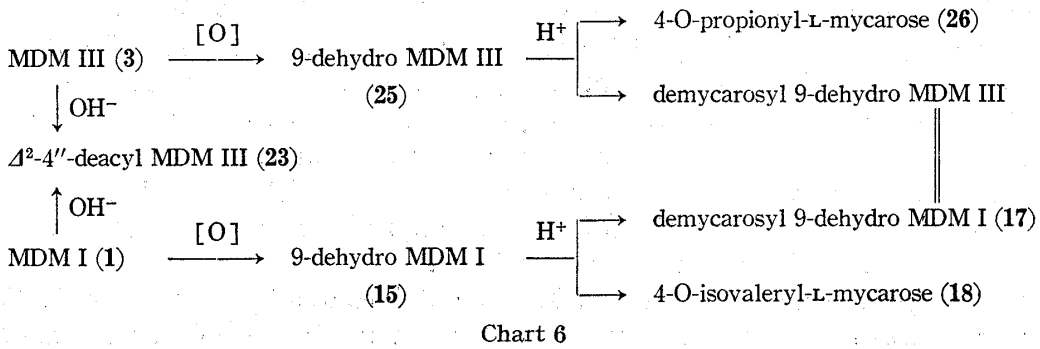


Chart 6

12) C. Djerassi, A. Bowers, and H.N. Khastgir, *J. Am. Chem. Soc.*, **78**, 1729 (1956).

The structures of other components, MDM IV, V and VI were analogously elucidated as follows. The physicochemical properties of these components were quite similar to those of MDM I, II and III. All of them exhibited no maxima in the UV region, and similar basicities with pK_a' 6.7—7.1. The IR spectra of MDM IV, V and VI revealed the common functional groups such as ester and/or lactone (1720—1740 cm^{-1}), aldehyde (2720—2730 cm^{-1}) and acetate (1239—1245 cm^{-1}) in addition to intense absorption bands due to ether linkages in the region from 1000 cm^{-1} to 1200 cm^{-1}. The NMR spectra of these components as shown in Table III revealed the common signals of $-N(CH_3)_2$ (6H, s), $-OCH_3$ (3H, s) eight methine protons attached to the oxygen functions in the region of δ 3.5—5.3, two olefinic protons and an aldehyde (1H, s). The chemical shifts and coupling constants of these signals are superimposable among these components, suggesting the existence of the same partial structures postulated from the assignments of these signals in comparison with those of MDM I, II and III. In addition to these signals, **4** and **5** show each one acetyl signal at the different chemical shift, while **6** revealed the presence of two acetyl groups.

Table III. Chemical Shifts of MDM IV, V, and VI (100 MHz, in $CDCl_3$, δ ppm)

	C_8–CH_3	C_4''–OAc	C_3–OAc	$-N(CH_3)_2$	$-OCH_3$	C_5–H	C_9–H	C_1'–H
MDM IV	1.04	—	2.24	2.54	3.55	3.91	4.02	4.45
MDM V	1.03	2.16	—	2.55	3.55	3.91	4.04	4.45
MDM VI	1.01	2.17	2.26	2.55	3.56	3.93	4.03	4.46

	C_5''–H	C_4''–H	C_{15}–H	C_1''–H	C_3–H	C_{11}–H	C_{10}–H	–CHO
MDM IV	4.45	4.62	4.95	5.06	5.14	5.65	6.08	9.64
MDM V	4.45	4.62	4.95	5.07	5.16	5.69	6.09	9.64
MDM VI	4.44	4.63	4.94	5.08	5.15	5.67	6.10	9.64

Mass spectra of MDM IV, V and VI and their esters served to clarify the structures of these components by comparison with those of MDM I, II and III, and their derivatives whose structures had been already elucidated. Fragmentation patterns of MDM II can be assigned as illustrated in Chart 7 from comparison of mass spectra of MDM II and their derivatives such as 9-monoesters (acetate, propionate), 2'-monoesters, 9,2'-diesters, 9-dehydro compounds, Δ^2-compounds and demycarosyl compounds, *etc.*, described in the preceding paper[1] as well as MDM I and III, and their corresponding derivatives. An increase of intensity of aglycone ion derived from fission at C-5 in Δ^2-compounds provided further evidence in confirmation of the structures of aglycone moiety. The components, MDM IV, V and VI showed relatively weak molecular ion peaks. As shown in Table IV, prominent fragment ion peaks due to sugar moieties such as O-acyl mycarosyl mycaminose (i), O-acyl mycarose (g), and mycaminose (f) were always observed and these fragments indicate that **4**, **5** and **6** also contain mycaminose and O-acylmycarose moieties. Although **4** and **5** exhibited the same molecular ion peak (*m/e* 815), different peaks due to fragment (**g**) and (**i**) were useful for direct assignment of acyl residues in mycarose portion. While in the high mass region, the component (**4**) revealed relatively intensive fragment peak (**a**) $[M^+-C_2H_5COO]$ at *m/e* 742 resulting from cleavage of acyloxy radical, the component, (**5**), showed the fragment peak (**a**), $[M^+-CH_3COO]$ at *m/e* 756. The components, **4** and **6** showed the same peak (**e**) derived from the aglycone as MDM II at *m/e* 425, on the other hand, MDM V exhibited the fragment ion peak (e) ascribed to the aglycone at *m/e* 439, which was the same with **1** and **3**.

On the basis of these spectroscopic data, structures of MDM IV, V and VI are presumed to be analogous to MDM I, II and III. Further chemical evidences confirmed this presumption. On saponification, **4** and **5** gave 1 mole each of propionic acid and acetic acid, respec-

Table IV. Characteristic Fragment Peaks in the Mass Spectra of Maridomycin Components and Their Derivatives

Compound[a]	Origin of peaks[b]														
	M	(a)	(b)	(c)	(d)	(a′)	(b′)	(c′)	(d′)	(a″)	(e)	(f)	(g)	(h)	(i)
1	857	756	658	628	613	682	584	554	539	738	439	174	229	300	402
2	843	742	644	614	599	682	584	554	539	724	425	174	229	300	402
3	829	756	658	628	613	682	584	554	539	738	439	174	201	300	374
4	815	742	644	614	599	682	584	554	539	724	425	174	201	300	374
5	815	756	658	628	613	682	584	554	539	738	439	174	187	300	360
6	801	742	644	614	599	682	584	554	539	724	425	174	187	300	360
29	927	826	728	698	683	766	668	638	623	808	467	216	229	342	444
30	885	784	686	656	641	724	626	596	581	766	425	216	229	342	444
31	885	784	686	656	641	724	626	596	581	766	467	174	229	300	402
13	841	740	642	612	597	680	582	552	537	722	437	174	229	300	402
10	941	840	742	712	697	766	688	638	623	822	481	216	229	342	444
32	969	868	770	740	725	794	696	666	651	850	495	230	229	356	458

a) **29**=MDM II 9,2′-diacetate, **30**=MDM II 2′-monoacetate, **31**=MDM II 9-monoacetate, **32**=MDM I 9,2′-dipropionate
b) Fragment peaks (a)—(i) (*m/e*) correspond to those of Chart 7.

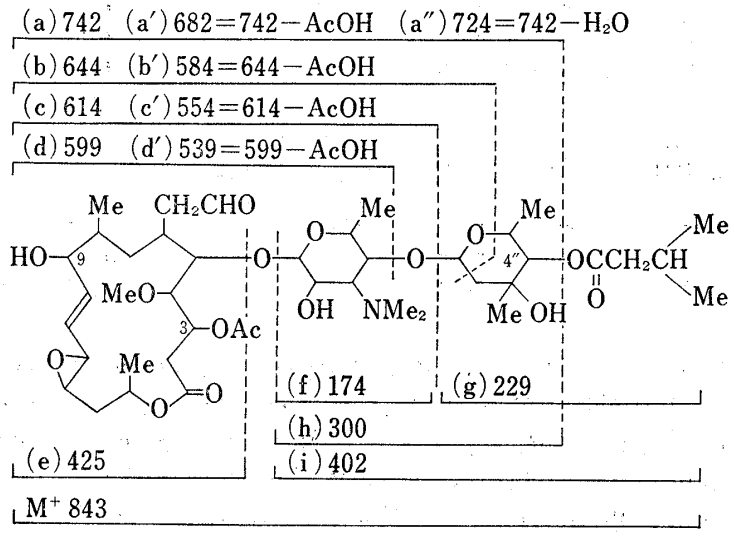


Chart 7. Mass Fragmentations of Maridomycin II

tively, while **6** did 2 moles of acetic acid.[4] Methanolysis of (**4**) afforded α- and β-methyl 4-O-propionyl-L-mycarosides (**22**) which were identified with those from MDM III, while **5** and **6** yielded α- and β-methyl 4-O-acetyl-L-mycarosides (**33a**, **33b**), $C_{10}H_{18}O_5$. Their structures were determined in comparison with other methyl 4-O-acyl-L-mycarosides obtained from MDM II and III, and physical data were in good agreement with those reported.[11]

Since the direct chemical evidence for aglycone parts of **4**, **5** and **6** were difficult to obtain by acid hydrolysis, they were analogously oxidized with CrO_3–pyridine under mild conditions to give dehydro MDM IV (**34**), $C_{40}H_{63}O_{16}N$, mp 195—196°, $[\alpha]_D^{26}$ −62.7° ($CHCl_3$) dehydro MDM V (**35**), $C_{40}H_{63}O_{16}N$, mp 207—208°, $[\alpha]_D^{25}$ −59.4° ($CHCl_3$), and dehydro MDM VI (**36**), $C_{39}H_{61}O_{16}N$, mp 205—207°, $[\alpha]_D^{23}$ −62.2° ($CHCl_3$). Subsequently, these dehydro compound (**34**), (**35**) and (**36**) were hydrolyzed under acidic conditions at room temperature, giving basic compounds and acyl mycaroses as shown in Chart 8.

Dehydro MDM IV and VI gave the same basic compound (demycarosyl compound) which was identified with that from dehydro MDM II (=carimbose A). On the other hand, dehydro MDM V gave the identical compound with demycarosyl dehydro MDM I (**17**) by acid hydrolysis.

Therefore, the aglycone part and basic sugar moiety of MDM IV and VI were confirmed to be identical with those of MDM II including their stereochemistries. Those of MDM V were also confirmed as the same demycarosyl compound with MDM III. These interrelationships of all the components I—VI are illustrated in the following Chart 9.

The position of the anomeric linkage could be assigned as in the case of MDM II and configuration at the anomeric carbon atom of mycarose in MDM IV, V and VI is estimated

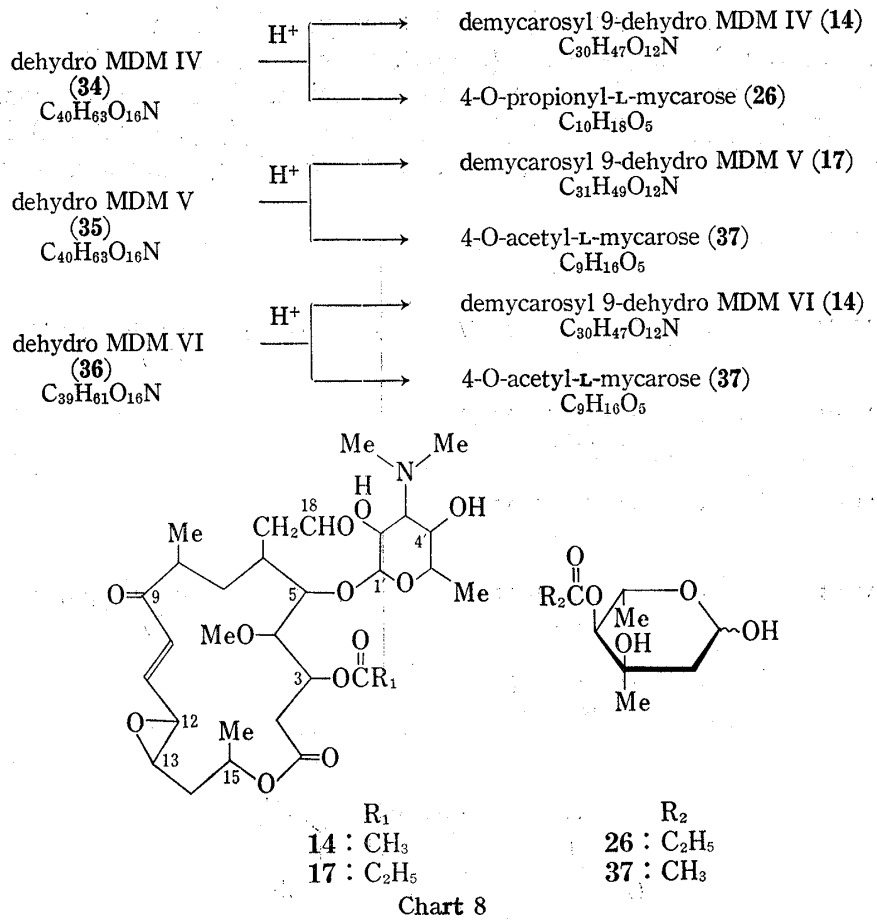


Chart 8

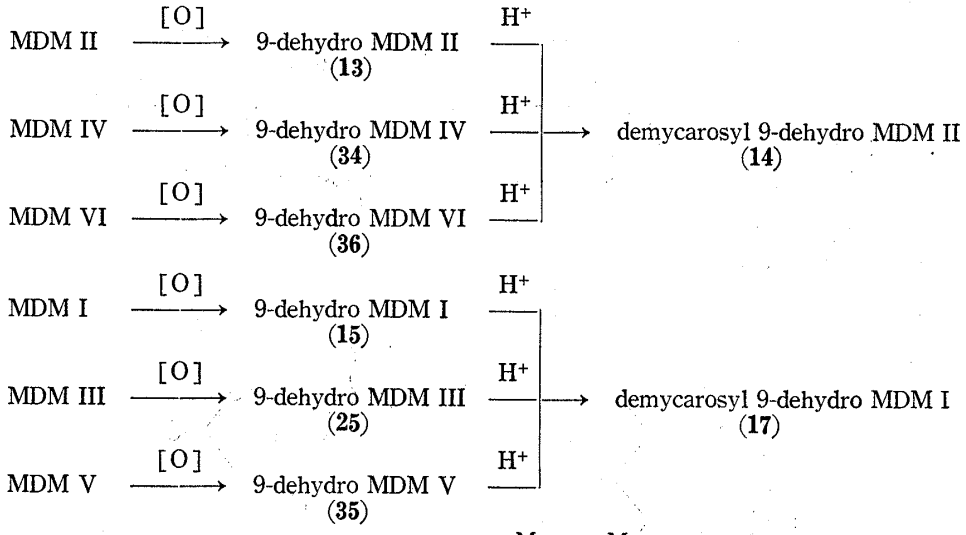
to be the same (α-L) from NMR ($J_{1'',2''}$=3.5/1.0 Hz) and molecular rotation differences[13] between 9-dehydro compounds (**34**, **35** and **36**) and their demycarosyl compounds (**14**, **17**) (Δ[M]; −388.6∼−404.5°). In addition, unambiguous evidences were obtained by the following facts as shown in the Chart 10. When MDM IV were subjected to mild alkaline hydrolysis, it yielded the same two compounds with those from MDM III, *i.e.*, Δ^2-MDM III (**24**) and 4″-depropionyl Δ^2-MDM III (**23**). Analogously, MDM V and VI gave the same α,β-unsaturated lactones (**23**) and (**38**), the former of which was identical with that from MDM III. Identities of those compounds were confirmed by NMR, IR, UV and mass spectra, optical rotation and mixed melting point.[14]

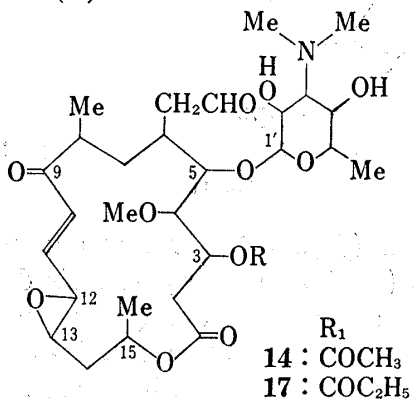
Accordingly, stereochemistry of the glycosidic linkages of mycarose and mycaminose was determined to be the same in all the six components of maridomycin. These results also indicate that the configuration at the C-9 position is the same. In this way, since the interrelationships of the six components have been clarified, it is concluded that the absolute configurations at all of the asymmetric centers are the same in the series of maridomycins. As stated in the preceding paper on MDM II,[1] since MDM II was chemically interrelated with carbomycin A and B, the stereochemistry of MDM II was determined to be the same with carbomycin A except for C-9 position. Consequently, it follows that in other components of maridomycin all of the configurations at asymmetric centers except for C-9 have been defined in an absolute sence.

For the assignment of the absolute configuration of C-9 hydroxy group, the benzoate sector rule[15] is considered to be applicable in the case of maridomycins. MDM III was

13) W. Klyne, *Biochem. J.*, **47**, xli (1950).
14) All of Δ^2 compounds have *trans* geometries at the C-2, C-3 double bond from the coupling constants ($J_{2,3}$= 15.5—16.0 Hz).
15) N. Harada, M. Ohashi, and K. Nakanishi, *J. Am. Chem. Soc.*, **90**, 7349 (1968).

converted to 9-benzoate by benzoylation with benzoyl chloride in pyridine. MDM III 9-benzoate (**39**), $C_{48}H_{71}O_{17}N$, $[\alpha]_D^{25}$ −96.1° (EtOH), thus obtained exhibited similar coupling constants for the ring protons from C-8 to C-12 portion. The coupling constants of $J_{8,9}$ (3.5 Hz), $J_{9,10}$ (8.5 Hz), $J_{10,11}$ (15.5 Hz), $J_{11,12}$ (8.5 Hz) and $J_{12,13}$ (≦2.0 Hz) in **39** indicate the *trans* arrangements[16] of H-9, H-10, H-11, H-12 and H-13, and maintenance of natural





R_1
14 : $COCH_3$
17 : COC_2H_5

Chart 9

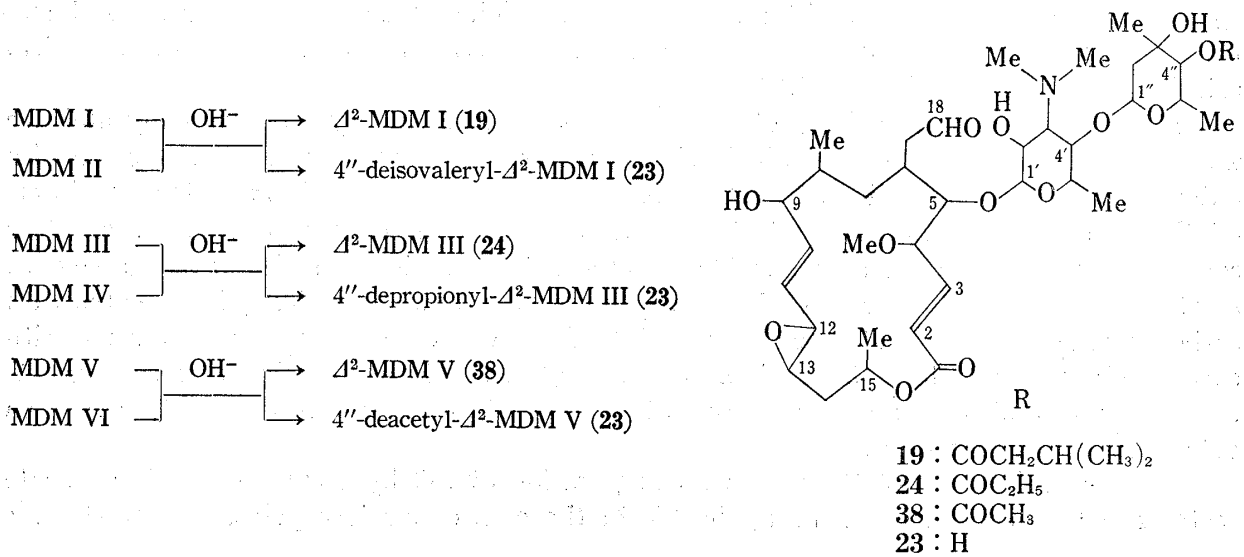


Chart 10

16) *a*) E.W. Garbish, *J. Am. Chem. Soc.*, **86**, 5561 (1964); *b*) A.A. Bothner-By, S. Castellano, S.J. Ebersole, and H. Gunter, *J. Am. Chem. Soc.*, **88**, 2466 (1966).

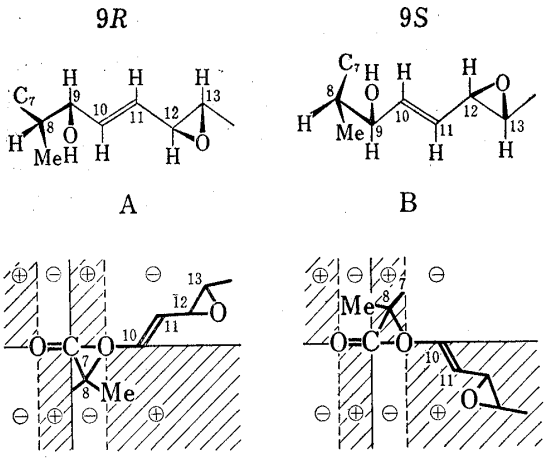
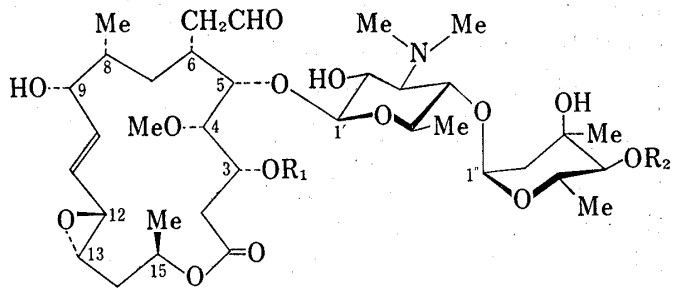


Fig. 2



maridomycin	R_1	R_2
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:	$COCH_3$	$COCH_3$

Chart 11

stereochemistry. Two kinds of the conformations (**A**) and (**B**) are probable, depending on the configuration of C-9 as shown in Fig. 2.

Circular dichroism (CD) spectrum of MDM III 9-benzoate (**39**) exhibited a negative Cotton effect at 230 nm with $[\theta]^{32}$—31100. Since the contribution of the C-10, C-11 double bond would be larger, the configuration at C-9 was assigned as *R* which was consistent with the X-ray analysis.

Recent X-ray determination[17] on propionylmaridomycin III finally confirmed the structure previously announced[3,18] and established the total absolute stereochemistry including the configuration of C-9 position. In the course of acylation of MDM III to 9-propionyl maridomycin III, it is evident that inversion of configuration at this asymmetric center and stereochemical changes of other portions do not occur,[19] thereby establishing the absolute stereochemistry of natural maridomycins. Finally, the absolute configurations in maridomycin I—VI are represented as follows; 3*R*; 4*S*; 5*S*; 6*R*; 8*R*; 9*R*; 12*S*; 13*S*; 15*R*; 1′*R*; 2′*R*; 3′*R*; 4′*S*; 5′*R*; 1″*R*; 3″*R*; 4″*R*; 5″*S*; 10*E*.

Since the configurations in six components of maridomycin have been determined and maridomycin II was chemically interrelated with carbomycin A and B,[1] it follows that the stereochemistries of carbomycin A and B have been unambiguously established.[20]

As concerns the conformation of maridomycins in solution, several intersting results were observed. NMR spectral analysis of maridomycins indicates that they might adopt very similar conformations in solution. The coupling data of the macroring protons listed

17) K. Kamiya, *et al.*, paper in preparation.
18) M. Muroi, M. Izawa, H. Ono, E. Higashide, and T. Kishi, *Experientia*, **28**, 878 (1972).
19) Neither epimerization nor rearrangement occurred in propionylation of MDM III to 9-propionate. Although the signal of C-9 methine proton was overlapped with other ones, aldehyde derivatives of 9-propionate such as thiosemicarbazone and dimethylhydrazone showed separate signals at δ 5.32 and 5.33, respectively. The coupling constants, $J_{8,9}$ (3.5—4.0) and $J_{9,10}$ (8.5—9.0), are nearly the same with those of natural maridomycins. In addition, 9-propionylmaridomycin III was converted into 4″-depropionyl Δ^2 MDM III (**23**) which was obtained from MDM III (**3**), by mild alkali treatment.
20) Although two different results[21] were reported, the stereochemistries of natural carbomycin A and B have been finally determined from the interrelationships of MDM II, carbomycin A and B: (12*S*, 13*S*) in MDM II and carbomycin A, (10*E*, 12*E*) in carbomycin B. In addition, *trans* geometry of C-12, C-13 epoxide is reasonable from the coupling constants ($J_{12,13} \leqq 2.0$[22]) in the series of maridomycins.
21) *a*) W.D. Celmer, *J. Am. Chem. Soc.*, **88**, 5028 (1967); *b*) S. Omura, A. Nakagawa, M. Otani, T. Hata, H. Ogura, and K. Furuhata, *J. Am. Chem. Soc.*, **91**, 3401 (1969).
22) H. Booth, "Applications of ^{1}H Nuclear Magnetic Resonance Spectroscopy to the Conformational Analysis of Cyclic Compounds" in "Progress in NMR Spectroscopy," Vol. 5, ed. by J.W. Emsley, J. Feaney, and L.H. Sutcliffe, Pergamon Press, Oxford, 1969, p. 180.

in Table V shows that the changes of C-3 acyloxy group of the components have nearly no effect on the ring conformation and furthermore no significant change in the coupling constants for the ring protons was observed in the series of maridomycins and their derivatives. Since the magnitudes of the coupling constants of the above ring protons in solvents of different polarities ($CDCl_3$, C_6D_6, CD_3COCD_3, CD_3OD, d_5-pyridine) are nearly the same, it is suggested that the macroring portion is conformationally homogenous in the maridomycin homologs. Comparison of chemical shifts of maridomycins and some derivatives as shown in the Table VI, allows the following conclusions to be drawn;

Table V. Coupling Constants for Ring Protons of MDM III and VI
(100 MHz, in $CDCl_3$, J=Hz)

	MDM III	MDM VI		MDM III	MDM VI
$J_{2a,2b}$	14.0	14.0	$J_{8,9}$	3.5	3.5
$J_{2a,3}$	10.0	10.0	$J_{9,10}$	9.0	9.0
$J_{2b,3}$	≦2.0	≦2.0	$J_{10,11}$	15.5	15.0
$J_{3,4}$	≦2.0	≦2.0	$J_{11,12}$	8.5	9.0
$J_{4,5}$	10.5	10.0	$J_{12,13}$	≦2.0	≦2.0
$J_{5,6}$	≦2.0	≦2.0			

Table VI. Chemical Shifts of Maridomycins and Their Derivatives
(100 MHz, in $CDCl_3$)

	C-3 OAc	C-4 OMe	H-3	H-5	H-9	H-10	H-11
MDM II (**2**)	2.25	3.56	5.15	3.92	4.03	6.10	5.66
MDM IV (**4**)	2.24	3.55	5.14	3.91	4.02	6.08	5.65
MDM VI (**6**)	2.26	3.56	5.15	3.93	4.03	6.10	5.67
MDM II-9 Ac (**35**)	2.25	3.54	5.15	3.99	*ca.* 5.0	6.05	5.76
TH MDM II (**36**)[a]	2.32	3.58	5.37	3.92	NA[b]	NA	NA
DH MDM II (**37**)[c]	2.11	3.57	5.12	3.92	4.19	6.12	5.60
MDM II TSC (**38**)[d]	2.06	3.58	5.10	3.88	4.19	6.12	5.58
MDM II DMHZ (**39**)[e]	2.00	3.54	5.07	3.85	4.19	6.09	5.60
9-Dehydro MDM II (**40**)	2.16	3.56	5.11	3.95	—	*ca.* 6.70	
9-Dehydro MDM IV (**30**)	2.20	3.57	5.11	3.97	—	*ca.* 6.70	
Δ^2-MDM II (**19**)	—	3.26	6.63	3.79	3.97	5.99	5.37
DH Δ^2-MDM II (**42**)[f]	—	3.26	6.54	3.84	4.11	6.00	5.37

a) 10, 11, 12, 13-tetrahydro
b) no assignment
c) 18-dihydro
d) thiosemicarbazone
e) dimethylhydrazone
f) 18-dihydro

i) The –OAc signals of C-3 –OAc are shifted to higher field (Δ 0.14—0.25) in the 18-dihydro compound and other aldehyde derivatives. Therefore, the –OAc group of C-3 was under the anisotropic effect by the aldehyde C=O. At the same time, downfield shifts of the C-9 protons were observed in those aldehyde derivatives (Δ 0.17). This is also probably due to the loss of the anisotropic effect of aldehyde C=O. Accordingly, the C-9 protons of maridomycins are in the range of shielding effect by aldehyde C=O.

ii) The loss of C-3 O-acyloxy groups in the MDM (α,β-unsaturated lactone, compound (**19**) *etc.*) has caused upfield shift of C-11 protons (Δ 0.29), while the signals of C-15 proton have shifted to lower field (Δ *ca.* 0.4). It is suggested that the acyloxy C=O produced deshielding effect to the H-11 and shielding effect to the H-15.

iii) Since the chemical shift of H-3 is shifted to lower field from δ 5.15 in MDM II to δ 5.35 in tetrahydro MDM II (**36**) (Δ 0.22), the double bond of C-10, C-11 in maridomycins

(the same shifts also occurred in other components) is considered to give anisotropic effects on the methine proton at C-3 position.

It is interesting that the biologically important function, C-18 aldehyde is relatively in close proximity to C-3 acyloxy groups, and H-9 and they are located on the same side of the mean plane of macroring together with H-11 and H-15. Furthermore, the coupling constants of $J_{9,10}$=8.5—9.0 and $J_{11,12}$=8.5—9.0 in the series of natural maridomycins, acyl derivatives and aldehyde derivatives indicate that H-9 and H-10 as well as H-11 and H-12 are placed in *trans* relationships.[16] Since the geometry of the double bond is *trans* ($J_{10,11}$=15.5—16.0), the portion, C-9 to C-12 appears to be in nearly planar arrangement, which agrees with the conformation in the crystal state.

These results indicate that the overall conformation of the macroring portion in solution is very similar to that determined by X-ray analysis of propionylmaridomycin III.

Experimental[23]

Isolation of the Maridomycin Antibiotics——The maridomycin complex produced by *Streptomyces hygroscopicus* No. B-5050 was isolated and separated into six components as described previously.[24]

MDM I 9,2′-Diacetate (7)——Acetylation of 450 mg of MDM I with 3.6 ml of dry pyridine and 0.9 ml of acetic anhydride at room temperature overnight followed by usual work-up furnished 473 mg of (**7**) as a white powder which was crystallized from ether–*n*-hexane mp 149—150°, $[\alpha]_D^{25}$ −80.4° (c=1.0, EtOH). *Anal.* Calcd. for $C_{47}H_{75}O_{18}N$: C, 59.92; H, 8.02; N, 1.49. Found: C, 59.49; H, 8.21; N, 1.62.

Tetrahydro MDM II (8)——A solution of 460 mg of MDM I in 20 ml of EtOH was hydrogenated over 150 mg of 10% Pd-carbon at room temperature for 7 hr. Filtration followed by evaporation *in vacuo* gave a white residue which was chromatographed on a silica gel column. Elution with benzene–acetone (5: 1) furnished 250 mg of pure tetrahydro MDM I as a white amorphous powder. $[\alpha]_D^{27}$ −59.6° (c=0.51, EtOH). *Anal.* Calcd. for $C_{43}H_{75}O_{16}N\cdot H_2O$: C, 58.68; H, 8.82; N, 1.59. Found: C, 58.86; H, 8.73; N, 1.62. NMR ($CDCl_3$) δ: 2.54 (6H, s, $-N(CH_3)_2$), 3.57 (3H, s, $-OCH_3$), 9.62 (1H, s, -CHO), no olefinic protons.

MDM I-Diol (9)——A solution of MDM I (900 mg) in 0.05N HCl (26 ml) was allowed to stand at room temperature for 5 hr. The reaction mixture was diluted with H_2O and extracted with AcOEt three times at pH 9. The extract was washed with H_2O and concentrated to give a crude diol (811 mg), which was chromatographed on silica gel to afford 450 mg of MDM I diol (**9**) by elution with AcOEt. *Anal.* Calcd. for $C_{43}H_{73}O_{17}N\cdot H_2O$: C, 57.77; H, 8.46; N, 1.57. Found: C, 57.36; H, 8.83; N, 1.68.

MDM I-Diol Tetraacetate (10)——To a solution of MDM I diol (200 mg) in dry pyridine (2 ml) was added acetic anhydride (0.4 ml) under ice-cooling, and allowed to stand at room temperature overnight. The reaction mixture was poured into ice-water and the precipitate was washed with H_2O, giving 198 mg of tetraacetate (**10**). *Anal.* Calcd. for $C_{51}H_{81}O_{21}N$: C, 58.66; H, 7.82; N, 1.34. Found: C, 58.69; H, 7.84; N, 1.32. NMR ($CDCl_3$) δ: 2.03 (3H, s, $-OCOCH_3$), 2.05 (6H, s, $2\times-OCOCH_3$), 2.08 (3H, s, $-OCOCH_3$). Mass Spectrum m/e: 1043 (M^+).

Methanolysis of MDM I and Identification of Methyl 4-O-Isovaleryl-L-mycaroside (11) and D-Mycaminose (12)——A mixture of 910 mg of MDM I, 9 ml of MeOH and 0.45 ml of conc. HCl was allowed to stand at room temperature for 6 hr, poured into ice-water and extracted with AcOEt. Evaporation of the washed and dried organic extract afforded a syrupy residue (240 mg) which was identified α- and β- methyl 4-O-isovaleryl-L-mycarosides (**11a** and **11b**) from TLC behaviors, NMR spectra and optical rotations. The aqueous phase was extracted with AcOEt at pH 9 and the washed and dried extract was concentrated to give a mixture of basic substances. This material was subsequently hydrolyzed with 2N HCl as described in the case of MDM II, and the basic sugar obtained was identified with D-mycaminose (**12**), by direct comparison with an authentic sample in NMR and mass spectra, TLC and $[\alpha]_D$.

9-Dehydro MDM I (15)——To CrO_3-pyridine complex (prepared from 600 mg of CrO_3 and 6 ml of dry pyridine) was added a solution of MDM I (900 mg) in 9 ml of pyridine under ice-cooling, and the mixture was stirred at 15—16° for 30 min. The reaction mixture was poured into ice-water and extracted with AcOEt. The washed and dried extract was concentrated to yield pale brown crude material (759 mg), which was subjected to silica gel column chromatography. Elution with benzene–AcOEt (1: 1) gave 9-dehydro MDM I (**15**) (360 mg) which was crystallized from EtOH–H_2O as colorless prisms. mp 198—199°. $[\alpha]_D^{32}$ −55.0° (c=1.0, $CHCl_3$). *Anal.* Calcd for $C_{43}H_{69}O_{16}N$: C, 60.33; H, 8.12; N, 1.64. Found: C, 60.20; H, 8.32; N, 1.80. UV λ_{max}^{EtOH} nm (ε): 240.5 (13830). IR ν_{max}^{KBr} cm^{-1}: 1690 (conjugated C=O), 1625-(C=C). NMR ($CDCl_3$) δ: 0.98 (6H, d, $2\times$ *sec.* CH_3), 2.53 (6H, s,- $N(CH_3)_2$), 3.55 (3H, s, $-OCH_3$), 4.42 (1H, d, H-1′), 4.63 (1H, d, H-4″), 5.05

23) Instrumentations and abbreviations are the same with those of the preceding paper.[1]
24) M. Muroi, M. Izawa, M. Asai, T. Kishi, and K. Mizuno, *J. Antibiotics*, **26**, 199 (1973).

(1H, dd, H-1″), 5.10 (1H, d like, H-3), 9.55 (1H, s, –CHO). Mass Spectrum *m/e*: 855 (M^+). Further elution of the column with benzene–AcOEt (1: 3) afforded 216 mg of N-formyl 9-dehydro compound (**16**) as an amorphous powder. *Anal.* Calcd. for $C_{43}H_{67}O_{17}N \cdot H_2O$: C, 58.16; H, 7.83; N, 1.58. Found: C, 58.20; H, 7.75; N, 1.60. UV λ_{max}^{EtOH} nm (ε): 239.5 (13700).

Acid Hydrolysis of 9-Dehydro MDM I (15) (4-O-Isovaleryl-L-mycarose (18) and Demycarosyl 9-Dehydro MDM I (17))——A solution of 150 mg of 9-dehydro MDM I (**15**) in 0.5N HCl (3 ml) was allowed to stand at room temperature for 5 hr. The reaction mixture was extracted with ether at pH 2, and the washed and dried extract was evaporated to dryness, giving 4-O-isovaleryl-L-mycarose (**18**). The aqueous layer further extracted with $CHCl_3$ at pH 9 and the solvent was evaporated to 112 mg of demycarosyl compound (**17**) which was crystallized from acetone–*n*-hexane. mp 188—189°. *Anal.* Calcd. for $C_{31}H_{49}O_{12}N \cdot 1/2H_2O$: C, 58.47; H, 7.92; N, 2.20. Found: C, 58.50; H, 7.75; N, 2.03. UV λ_{max}^{EtOH} nm (ε): 240.5 (13300). IR ν_{max}^{KBr} cm^{-1}: 1690 (conjugated C=O), 1625 (C=C). NMR ($CDCl_3$) δ: 2.53 (6H, s, $-N(CH_3)_2$), 3.57 (3H, s, $-OCH_3$), 3.94 (1H, d, H-5), 4.44 (1H, d, H-1′), 9.57 (1H, s, –CHO).

Δ^2-MDM I (19)——To a solution of MDM I (910 mg) in MeOH (15 ml) was added 1N KOH in MeOH (1.8 ml) under ice-cooling, and allowed to stand in a refrigerator overnight. The reaction mixture was diluted with ice-water, adjusted to pH 8.5 with aq. AcOH and concentrated under reduced pressure. The resulting aqueous mixture was extracted with AcOEt. The washed and dried extract was evaporated to give crude α,β-unsaturated lactone (744 mg) which was chromatographed over silica gel. Pure Δ^2-MDM I (**19**) was obtained as a white solid. $[\alpha]_D^{22}$ −81.2° (c=1.0, EtOH). *Anal.* Calcd. for $C_{40}H_{65}O_{14}N \cdot H_2O$: C, 59.90; H, 8.42; N, 1.75. Found: C, 60.41; H, 8.26; N, 1.82. NMR ($CDCl_3$) δ: 1.00 (9H, s, 3×*sec.* CH_3), 2.52 (6H, s, $-N(CH_3)_2$), 3.24 (3H, s, $-OCH_3$), 3.97 (1H, dd, H-9), 4.62 (1H, d, H-4″), 5.06 (1H, dd, H-1″), 5.37 (1H, dd, H-11), 5.97 (1H, d, H-2), 5.99 (1H, dd, H-10), 6.63 (1H, dd, H-3), 9.73 (1H, s, –CHO). Mass Spectrum *m/e*: 783 (M^+). This compound was identical with Δ^2-MDM II in all respects.

Methanolysis of MDM III (Methyl 4-O-Propionyl-L-mycaroside (22a, 22b))——As in the case of MDM I, a mixture of 4.5 g of MDM III, 45 ml of MeOH and 2.5 ml of concd. HCl was allowed to stand at room temperature overnight, poured into ice-water and extracted with $CHCl_3$. Evaporation of the washed and dried extract yielded syrupy residue (1.22 g) which was chromatographed on a silica gel. Elution with benzene–acetone (19: 1) afforded methyl 4-O-propionyl-α-L-mycaroside (**22a**) and its β-anomer (**22b**), respectively. Methyl 4-O-propionyl-α-L-mycaroside: $[\alpha]_D^{22}$ −145.2° (c=0.5, $CHCl_3$). *Anal.* Calcd. for $C_{11}H_{20}O_5$: C, 56.88; H, 8.68. Found: C, 56.55; H, 8.63. NMR ($CDCl_3$) δ: 1.11 (3H, s, *tert.* CH_3), 3.38 (3H, s, $-OCH_3$), 3.96 (1H, s, OH), 4.0 (1H, oct., H-5), 4.64 (1H, d, H-4, J=10.0), 4.78 (1H, dd, H-1). Methyl 4-O-propionyl-β-L-mycaroside (**22b**): $[\alpha]_D^{22}$ +18.2° (c=1.5, $CHCl_3$). *Anal.* Calcd. for $C_{11}H_{20}O_5$: C, 56.88; H, 8.68. Found: C, 56.85; H, 8.72. NMR ($CDCl_3$) δ: 1.16 (3H, s, *tert.* CH_3), 1.82 (1H, s, OH), 3.48 (3H, s, $-OCH_3$), 3.95 (1H, oct., H-5), 4.62 (1H, d, H-4, J=10.0), 4.72 (1H, dd, H-1).

Alkaline Hydrolysis of MDM III (Δ^2-MDM III (25) and 4″-Depropionyl-Δ^2-MDM III (24))——To a solution of 3.5 g of MDM III in 70 ml of MeOH was added dropwise 17.5 ml of 1N KOH in 50% aq. MeOH and allowed to stand in a refrigerator overnight. The reaction mixture was poured into ice-water and extracted with AcOEt. The washed and dried extract was evaporated to give crude material (2.2 g) which was chromatographed on a column of silica gel. Elution with benzene–acetone (3: 1) gave 181 mg of compound (**24**), and further elution with the same solvent afforded 1.37 g of (**23**) as a white solid. Δ^2-MDM III (**24**): $[\alpha]_D^{25}$ −80.9° (c=1.0, EtOH). *Anal.* Calcd. for $C_{38}H_{61}O_{14}N \cdot H_2O$: C, 58.97; H, 8.14; N, 1.84. Found: C, 59.28; H, 8.14; N, 1.84. NMR ($CDCl_3$) δ: 1.0 (3H. d, *sec.* CH_3) 2.50 (6H, s, $-N(CH_3)_2$), 3.24 (3H, s, $-OCH_3$), 3.97 (1H, dd, H-9), 4.36 (1H, d, H-1′), 4.63 (1H, d, H-4″), 5.07 (1H, dd, H-1″), 5.40 (1H, dd, H-11), 5.99 (1H, d, H-2, J= 15.0 Hz), 6.01 (1H, dd, H-10), 6.64 (1H, dd, H-3), 9.74 (1H, s, –CHO). Mass Spectrum *m/e*: 755 (M^+). 4″-Depropionyl-Δ^2-MDM III (**23**): mp 144—145° (crystallized from EtOH) $[\alpha]_D^{22}$ −80.2° (c=1.0, EtOH). *Anal.* Calcd. for $C_{35}H_{57}O_{13}N \cdot H_2O$: C, 58.56; H, 8.28; N, 1.95. Found: C, 59.06; H, 8.28; N, 1.91. NMR ($CDCl_3$) δ: 1.0 (3H, d, CH_3–8), 2.50 (6H, s, $-N(CH_3)_2$), 2.93 (1H, d, H-4″, J=10.0 Hz), 3.25 (3H, s, $-OCH_3$), 3.97 (1H, dd, H-9), 4.05 (1H, m, H-5″), 4.35 (1H, d, H-1′).

9-Dehydro MDM III (25) and 9-Dehydro-N-formyl MDM III (27)——Oxidation of MDM III (1.8 g) with CrO_3–pyridine complex (prepared from 1.2 g of CrO_3 and 12 ml of dry pyridine) in the manner described in the previous section gave 290 mg of 9-dehydro MDM III (**25**) which had mp 204—205°, $[\alpha]_D^{25}$ −60.1° (c=1.0, $CHCl_3$) along with 678 mg of N-formyl compound (**27**). 9-Dehydro MDM III (**25**): *Anal.* Calcd. for $C_{41}H_{65}O_{16}N$: C, 59.48; H, 7.91; N, 1.69. Found: C, 59.56; H, 8.24; N, 1.69. UV λ_{max}^{EtOH} nm (ε): 240.5 (14400). IR ν_{max}^{KBr} cm^{-1}: 1690 (conjugated C=O), 1625 (C=C). NMR ($CDCl_3$) δ: 2.54 (6H, s, $-N(CH_3)_2$), 3.57 (3H, s, $-OCH_3$), 4.42 (1H, d, H-1′), 4.63 (1H, d, H-4″), 9-Dehydro-N-formyl MDM III (**27**): *Anal.* Calcd. for $C_{41}H_{63}O_{17}N \cdot H_2O$: C, 57.26; H, 7.62; N, 1.63. Found: C, 57.41; H, 7.60; N, 1.63. UV λ_{max}^{EtOH} nm (ε): 240.5 (13300).

Mild Acid Hydrolysis of (25) (4-O-Propionyl-L-mycarose (26) and Demycarosyl-9-dehydro MDM III (17))——A solution of dehydro MDM III (**25**) (400 mg) in 0.5N HCl (8 ml) was allowed to stand at room temperature for 6 hr. The reaction mixture was adjusted to pH 2.0, and extracted with ether. The washed and dried extract was evaporated to give 20 mg of 4-O-propionyl-L-mycarose (**26**). 4-O-Propionyl mycarose (**26**): mp 106—107° $[\alpha]_D^{23}$ −85.9° (c=0.98, $CHCl_3$, after 24 hr). *Anal.* Calcd. for $C_{10}H_{18}O_5$: C, 55.05, H, 8.26. Found: C, 54.75; H, 8.41. The aqueous solution after extraction was adjusted to pH 9.0 and extracted with $CHCl_3$. The washed and dried extract was concentrated to afford crystalline residue (320 mg) (**17**) which was recrys-

tallized from acetone–*n*-hexane as colorless plates, mp 188—189°. $[\alpha]_D^{25}$ −12.5° (c=1.0, $CHCl_3$). *Anal.* Calcd. for $C_{31}H_{49}O_{12}N \cdot 1/2H_2O$: C, 58.47; H, 7.92; N, 2.20. Found: C, 58.67; H, 7.84; N, 2.36. UV λ_{max}^{EtOH} (ε): 240.5 nm (14500). IR ν_{max}^{KBr} cm^{-1}: 1690 (conjugated C=O), 1630 (C=C). This compound was identified with that (**17**) from dehydro MDM I from comparison of NMR, IR and mass spectra and in addition both did not show any depression by mixture melting point.

Acid Hydrolysis of 9-Dehydro-N-formyl MDM III (27) (Demycarosyl-9-dehydro-N-formyl MDM III (28))——A mixture of the compound (**27**) (600 mg) and 0.5N HCl (20 ml) was stirred at room temperature for 7 hr. A solution after filtration of the mixture was extracted with AcOEt at pH 4.5 and evaporation of the washed and dried extract gave 436 mg of crude material, which was chromatographed on a column of silica gel (20 g). Elution with benzene–acetone (1: 1) afforded demycarosyl compound (**28**) (109 mg) which was crystallized from AcOEt–*n*-hexane as colorless prisms, mp 161—162°. *Anal.* Calcd. for $C_{31}H_{47}O_{13}N \cdot CH_3COOC_2H_5$: C, 57.60; H, 7.60; N, 1.92. Found: C, 57.84; H, 7.83; N, 1.83. IR ν_{max}^{KBr} cm^{-1}: 1680 (α,β-unsaturated C=O), 1660, 1650 (amide C=O), 1620 (C=C) NMR (d_6-acetone) δ: 2.78 (3H, s, –N–CH_3), 4.57 (1H, d, H-1′, J=7.0), 6.41 (1H, dd, H-11), 7.0 (1H, d, H-10), 7.94 (1H, s, –N–CHO), 9.58 (1H, s, –CHO). Mass Spectrum *m/e*: 613 (M^+ –CO), 567 (M^+ −74).

9-Dehydro MDM IV (34)——Oxidation of MDM IV (450 mg) in 4.5 ml of dry pyridine with CrO_3–pyridine complex (prepared from 300 mg of CrO_3 and 6 ml of dry pyridine) at 15—20° for 30 min in the same manner as described in the previous parts afforded after column chromatography over silica gel, 132 mg of (**34**) which was recrystallized from acetone–*n*-hexane as colorless prisms. mp 195—196°. $[\alpha]_D^{21}$ −62.7° (c=0.99, $CHCl_3$). *Anal.* Calcd. for $C_{40}H_{63}O_{16}N \cdot H_2O$: C, 57.74; H, 7.88; N, 1.68. Found: C, 58.17; H, 7.64; N, 1.47. IR ν_{max}^{KBr} cm^{-1}: 1690 (α,β-unsaturated C=O), 1625 (C=C), 1245 (C–O–Ac). NMR ($CDCl_3$) δ: 2.21 (3H, s, $-OCOCH_3$), 2.55 (6H, s, $-N(CH_3)_2$), 3.57 (3H, s, $-OCH_3$), 4.64 (1H, d, H-4″), *ca.* 6.7 (2H, m, H-10, H-11), 9.56 (1H, s, –CHO).

Mild Acid Hydrolysis of (34) (Demycarosyl 9-Dehydro MDM IV (14) and 4-O-Propionyl-L-Mycarose (26)——Dehydro MDM IV (**34**) (200 mg) dissolved in 4 ml of 0.5N HCl was kept at room temperature for 6 hr. The mixture was worked up as in the case of dehydro MDM I (**15**) to give demycarosyl compound (120 mg), identical (IR, NMR, mixed mp) with demycarosyl dehydro MDM II (**14**) obtained by acid hydrolysis of 9-dehydro MDM II (**13**). Demycarosyl 9-dehydro MDM IV: *Anal.* Calcd. for $C_{30}H_{47}O_{12}N \cdot 1/2H_2O$: C, 57.86; H, 7.77; N, 2.25. Found: C, 57.95; H, 7.60; N, 2.03. From neutral fraction, was obtained 4-O-propionyl-L-mycarose (**26**) (40 mg) identical with that from MDM III (IR, NMR).

Methanolysis of MDM V (5) (Methyl 4-O-acetyl-L-mycaroside (33a, 33b))——MDM V (2.48 g) was subjected to methanolysis in a similar manner as in the case of MDM III to give complicated mixtures of demycarosyl compounds (1.1 g) and, α and β- methyl 4-O-acetyl-L-mycarosides (**33a** and **33b**) (97 mg and 243 mg). Methyl 4-O-acetyl-α-L-mycaroside (**33a**): $[\alpha]_D^{25}$ −162.0° (c=0.6, $CHCl_3$). *Anal.* Calcd. for $C_{10}H_{18}O_5$: C, 55.03; H, 8.31. Found: C, 54.87; H, 8.12. Methyl 4-O-acetyl-β-L-mycaroside (**33b**): mp 66—67°. $[\alpha]_D^{22}$ +19.3° (c=1.5, $CHCl_3$). *Anal.* Found: C, 54.88; H, 8.24.

9-Dehydro MDM V (35)——MDM V (890 mg) was oxidized with CrO_3–pyridine complex (prepared from 600 mg, of CrO_3 and 12 ml of dry pyridine) at 15—20° for 30 min. The mixture was worked up as in the case of MDM I to give 312 mg of 9-dehydro MDM V (**35**) which was recrystallized from acetone–*n*-hexane as colorless prisms. mp 207—208°. $[\alpha]_D^{25}$ −59.4° (c=1.1, $CHCl_3$). *Anal.* Calcd. for $C_{40}H_{63}O_{16}N$: C, 59.02; H, 7.80; N, 1.72. Found: C, 58.66; H, 7.83; N, 1.72. IR ν_{max}^{KBr} cm^{-1}: 1685 (α,β-unsaturated C=O), 1620 (C=C), 1245 (C–O–Ac). NMR ($CDCl_3$) δ: 2.17 (3H, s, $-OCOCH_3$), 2.54 (6H, s, $-N(CH_3)_2$), 3.57 (3H, s, $-OCH_3$), 6.70 (2H, m, H-10, H-11), 9.55 (1H, s, –CHO).

Acid Hydrolysis of 9-Dehydro MDM V (35) (Demycarosyl-9-dehydro MDM V (17) and 4-O-Acetyl-L-mycarose (37))——9-Dehydro MDM V (**35**) (200 mg) was subjected to acid hydrolysis as in the case of other dehydro compounds to give demycarosyl compound identical with demycarosyl 9-dehydro MDM III (IR, NMR, mixed mp) from basic fraction. From neutral fraction was obtained 4-O-acetyl-L-mycarose (**37**) as needles. mp 98—100°. $[\alpha]_D^{23}$ −92.8° (c=1.0, $CHCl_3$, after 24 hr). *Anal.* Calcd. for $C_9H_{16}O_5$: C, 52.93; H, 7.90. Found: C, 53.46; H, 7.41. NMR ($CDCl_3$) δ: 1.15 (3H, s, *tert.* CH_3), 2.12 (3H, s, $-OCOCH_3$). Mass Spectrum *m/e*: 203 (M^+−1), 186 (M^+−18).

9-Dehydro MDM VI (36)——A solution of 1.8 g of MDM VI in 18 ml of dry pyridine was added to 1.2 g of CrO_3 in 24 ml of dry pyridine, and stirred at 15—20° for 30 min. Work up as in the case of oxidation of other components afforded 353 mg of the compound (**36**) which was recrystallized from acetone–*n*-hexane as colorless prisms. mp 205—207°. $[\alpha]_D^{23}$ −62.2° (c=1.0, $CHCl_3$). *Anal.* Calcd. for $C_{39}H_{61}O_{16}N$: C, 58.56; H, 7.69; N, 1.75. Found: C, 58.34; H, 7.80; N, 1.75.

Acid Hydrolysis of 9-Dehydro MDM VI (36) (Demycarosyl 9-Dehydro MDM VI (14) and 4-O-Acetyl-L-mycarose (37))——9-Dehydro MDM VI (**36**) was subjected to acid hydrolysis in a similar manner as described in the previous parts to give demycarosyl compound identical with the compound (**14**). As a neutral sugar, was obtained 4-O-acetyl-L-mycarose (**37**) identical with that from dehydro MDM V.

Alkaline Hydrolysis of MDM V and VI (Δ^2-MDM V (38) and 4″-Deacetyl-Δ^2-MDM V (23)——A solution of MDM V (850 mg) in MeOH (15 ml) and 1N KOH in MeOH (2 ml) was kept overnight in a refrigerator, poured into ice-water, concentrated at pH 8.0—9.0 *in vacuo*, diluted with H_2O and extracted with AcOEt. The washed and dried extract was evaporated, and the residue was subjected to column chromatography on

silica gel. The first eluate gave 102 mg of (**38**) as a white solid. $[\alpha]_D^{27}$ −81.2° (c=0.5, EtOH). *Anal.* Calcd. for $C_{37}H_{59}O_{14}N \cdot H_2O$: C, 58.48; H, 8.09; N, 1.84. Found: C, 58.86; H, 7.90; N, 1.65. IR ν_{max}^{KBr} cm^{-1}: 1720 (α,β-unsaturated lactone), 1250 (C–O–Ac). NMR ($CDCl_3$) δ: 2.12 (3H, s, $-OCOCH_3$), 5.98 (1H, d, H-2), 6.63 (1H, dd, H-3). The second eluate from the column gave α,β-unsaturated lactone (**23**) identical with that from MDM III (IR, UV, NMR, and $[\alpha]_D$). The compounds (**38**) and (**23**) were obtained from MDM VI in a similar manner to the case of MDM V.

MDM III 9-Benzoate (39)——MDM III (850 mg) in dry pyridine (6 ml) was acylated with benzoylchloride (0.4 ml) at 0—5°. After stirring for 7 hr under 5°, the reaction mixture was poured into cold $NaHCO_3$ solution, and extracted with AcOEt. The washed and dried extract was concentrated to give a mixture of 9-benzoate and starting material, which was applied to a column of silica gel. Pure 9-benzoate (**39**) (530 mg) was obtained as an amorphous powder. $[\alpha]_D^{25}$ −96.1° (c=1.1, EtOH). *Anal.* Calcd. for $C_{48}H_{71}O_{17}N \cdot H_2O$: C, 60.55; H, 7.73; N, 1.47. Found: C, 60.76; H, 7.54; N, 1.18. NMR ($CDCl_3$) δ: 2.53 (6H, s, $-N(CH_3)_2$), 3.55 (3H, s, $-OCH_3$), 4.64 (1H, d, H-4), 5.34 (1H, dd, H-9), 5.87 (1H, dd, H-11), 6.18 (1H, dd, H-10), 9.70 (1H, s, –CHO). CD[25] (c=0.01, MeOH) $[\theta]^{32}$ (nm): −31100 (230) (negative maximum).

MDM II Dimethylhydrazone (44)——To a solution of MDM II (430 mg) in 10 ml of MeOH was added 1mM of $NH_2N(CH_3)_2$, and the mixture was left to stand at room temperature overnight. The concentrate of reaction mixture was extracted with AcOEt and the washed and dried extract was again concentrated to give a white solid of dimethylhydrazone (**44**), which was crystallized from CCl_4–n-hexane. mp 114—116°. *Anal.* Calcd. for $C_{44}H_{75}O_5N_3 \cdot H_2O$: C, 58.45; H, 8.59; N, 4.65. Found: C, 58.52; H, 8.30; N, 4.64. UV λ_{max}^{MeOH} nm (ε): 243 (7300). NMR ($CDCl_3$) δ: 2.00 (3H, s, $-OCOCH_3$), 2.48 (6H, s, $-N(CH_3)_2$), 2.66 (6H, s, $-N(CH_3)_2$), 3.54 (3H, s, $-OCH_3$), 6.45 (1H, t, –CH=N–).

18-Dihydro Δ^2-MDM II (45)——Δ^2-MDM II (161 mg) in a mixture of MeOH (2 ml) and H_2O (1 ml) was reduced with $NaBH_4$ (11.7 mg) at room temperature for 1 hr. The washed and dried AcOEt extract from the reaction mixture was concentrated to give 149 mg of 18-dihydro Δ^2-MDM II as a white solid. $[\alpha]_D^{27}$ −80.3° (c=1.0, EtOH). *Anal.* Calcd. for $C_{40}H_{67}O_{14}N \cdot H_2O$: C, 59.75; H, 8.65; N, 1.74. Found: C, 60.15; H, 8.74; N, 1.78. NMR ($CDCl_3$) δ: 0.99 (9H, d, *sec.* CH_3), 2.52 (6H, s, $-N(CH_3)_2$), 3.26 (3H, s, $-OCH_3$), 5.37 (1H, dd, H-11), 6.0 (1H, dd, H-10), and disappearance of –CHO signal.

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25) Circular dichroism (CD) curves were recorded on a JASCO Model ORD/UV-5 spectropolarimeter.