Chem. Pharm. Bull. 24(3) 450—462 (1976)

UDC 547.898.02 : 615.282.011.5

Maridomycin, a New Macrolide Antibiotic. X.[1] The Structure of Maridomycin II

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(Received June 30, 1975)

The structure of maridomycin II was elucidated as a sixteenmembered macrolide constituted of 4-O-isovaleryl-L-mycarose, D-mycaminose and macrocyclic aglycone from chemical studies and spectroscopic data. Total structure of maridomycin II was determined as shown from the confirmatory evidence that 9-dehydro maridomycin II was identified with carbomycin A.

Maridomycin is a novel group of antibiotic produced by *Streptomyces hygroscopicus* No. B-5050,[3] and effective against gram positive bacteria and mycoplasma *in vitro* and *in vivo*.[4] Maridomycin was found to be comprised of six components, maridomycin I-VI (compound No. (1)—(6)) and details of the isolation and characterization were described.[5] In preliminary communication,[6] structure of maridomycin II has been reported. This paper describes the details on the structural elucidation of maridomycin II.

Maridomycin II (MDM II) was obtained as colorless needles of mp 134—136° from benzene or ether and as colorless prisms of mp 196—199° from acetone–*n*-hexane. It shows weak basicity of p*Ka'* 6.9 in 50% EtOH. Molecular weight measured by vapor pressure osmometry in AcOEt was 881 and its mass spectrum showed a molecular ion peak at *m/e* 843 ($C_{42}H_{69}O_{16}N$). From analytical data and water content determined by Karl Fisher method, the empirical formula $C_{42}H_{69}O_{16}N \cdot H_2O$ was given to MDM II (2).

It shows no characteristic absorption maxima in the ultraviolet (UV) or visible range. The infrared spectrum (IR) showed the presence of hydroxyl (3470 and 3600 cm^{-1}), ester and/or lactone (1730—1740 cm^{-1}), an acetate (1235 cm^{-1}), ether (1050—1200 cm^{-1}) and aldehyde (2725 cm^{-1}) groups. In color tests, positive molisch reaction suggested sugar moiety and positive Dragendorff but negative ninhydrin test indicated a tertiary amine group.

The nuclear magnetic resonance (NMR) spectrum (in $CDCl_3$) showed the presence of three secondary methyl (δ 1.01, 9H, d), an acetyl (δ 2.25, 3H, s), a dimethyl amino (δ 2.54, 6H, s), a methoxy (δ 3.56, 3H, s), two olefinic protons (δ 5.66, 6.10, each 1H, dd) and an aldehyde group (δ 9.65, 1H, s).

The presence of the aldehyde was also ascertained by the formation of thiosemicarbazone (7), $C_{43}H_{72}O_{15}N_4S$, $[\alpha]_D^{32}$ −106.7° ($CHCl_3$), which has no aldehyde proton, but a signal attributable to –CH=N– at δ 7.44 (1H, t, J=5 Hz) in the NMR spectrum. This fact suggests the presence of a methylene adjacent to an aldehyde group.

Reduction of MDM II (2) with $NaBH_4$ gave dihydro-MDM II (8) $C_{42}H_{71}O_{16}N$, $[\alpha]_D^{32}$ −76.7° (EtOH), in which the aldehyde proton has disappeared, Acetylation of MDM II (2) with acetic anhydride in pyridine at room temperature gave a diacetate (9), $C_{46}H_{73}O_{18}N$, $[\alpha]_D^{29}$ −81.4° (EtOH), which showed a molecular ion peak at *m/e* 927, and slightly weak basicity with p*Ka'*

1) Part IX: T. Matsuzawa, T. Kondo, and Y. Kita, *Antimicrob. Ag. Chemother.*, **6**, 685 (1974).
2) Location: *17–85, Jusohonmachi 2-chome, Yodogawa-ku, Osaka, 532, Japan.*
3) H. Ono, T. Hasegawa, E. Higashide, and M. Shibata, *J. Antibiotics*, **26**, 191 (1973).
4) M. Kondo, T. Oishi, K. Ishifuji, and K. Tsuchiya, *J. Antibiotics*, **26**, 206 (1973).
5) M. Muroi, M. Izawa, M. Asai, T. Kishi, and K. Mizuno, *J. Antibiotics*, **26**, 199 (1973).
6) M. Muroi, M. Izawa, H. Ono, E. Higashide, and T. Kishi, *Experientia*, **28**, 878 (1972).

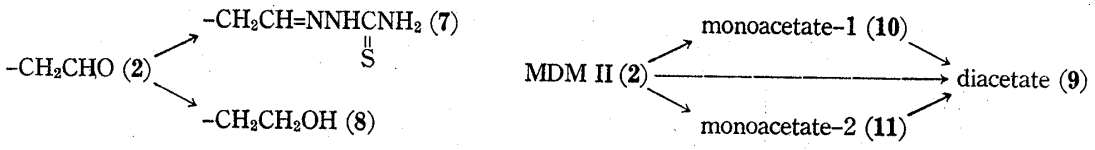


Chart 1

4.7. Its NMR spectrum showed two more acetyl signals (δ 2.02, 2.04) than that of **2**, indicating a diacetyl derivative. In addition, while the signal due to $-N(CH_3)_2$ is shifted to higher field (δ 2.43), two methine protons attaching to hydroxyl groups in MDM II at δ 3.5 and 4.03 are shifted to lower field (*ca.* 5.0) and another methine proton is also slightly shifted to lower field (δ 4.4 to 4.6). The presence of a band at 3480 cm^{-1} in the IR spectrum ($CHCl_3$ solution) indicated that one or more additional hydroxyl (tertiary or hindered secondary) remained without acetylation under usual conditions.

Under the selective conditions, two kinds of monoacetates were formed. Monoacetate-1 (**10**), $C_{44}H_{71}O_{17}N$, mp 134—135°, $[\alpha]_D^{26}$ −87.8° (EtOH), was obtained by acetylation with acetic anhydride in neutral organic solvents such as acetone or AcOEt without basic catalysts. The mass spectrum of **10** gave a molecular peak at *m/e* 885, and showed a weakened basicity (p*Ka*′ 4.7), indicating that the introduced acetyl group affects the basicity.

In the NMR spectrum of **10** ($CDCl_3$), one more acetyl signal was observed at δ 2.06, and a proton at δ 3.53 in **2** was shifted to lower field (δ *ca.* 5.0) and a doublet at δ 4.16 in **2** was slightly moved to δ 4.62. The higher field shift of $-N(CH_3)_2$ signal (δ 2.44, 6H, s) of **10** indicated that the introduced acetyl is located in the vicinity to $-N(CH_3)_2$ group. On the other hand, another monoacetate-2 (**11**), $C_{44}H_{71}O_{17}N$, mp 190—191°, $[\alpha]_D^{25}$ −66.9° ($CHCl_3$) obtained by acetylation with acetylchloride in pyridine at low temperature has a similar basicity with p*Ka*′ of 6.6 and showed no higher field shift of $-N(CH_3)_2$ in NMR spectrum, suggesting that the latter acetyl is not located closely to $-N(CH_3)_2$ group. In the NMR spectrum of **11**, a quartet at δ 4.03 in **2** was shifted to δ 5.0 and the introduced acetyl signal was observed at δ 2.01. Monoacetate-1 (**10**) or monoacetate-2 (**11**) gave the identical diacetate (**9**) by acetylation with acetic anhydride in pyridine at room temperature.

Since the presence of double bonds in MDM II (**2**) was suggested from the signals at δ 5.56 (1H, dd) and 6.10 (1H, dd), **2** was hydrogenated over Pd-charcoal as a catalyst. MDM II (**2**) readily absorbed 2 moles of hydrogen to afford a tetrahydro compound (**12**), $C_{42}H_{73}O_{16}N$, $[\alpha]_D^{22}$ −62.1°(EtOH). In the NMR spectrum of **12**, the signals of two olefinic protons have disappeared but an aldehyde proton still remained, showing that two olefinic bonds were present in **2**. However, when the tetrahydro compound (**12**) was acetylated with acetic anhydride in pyridine at room temperature, triacetate (**13**), $C_{48}H_{79}O_{19}N$, (M^+, *m/e* 973), mp 116—117°, $[\alpha]_D^{23}$ −76.4° ($CHCl_3$) [NMR; δ 2.00, 2.02, 2.06 (each 3H, 3X-$OCOCH_3$)] was obtained, while under the same condition MDM II gave a diacetate (**9**). This fact indicates that one additional secondary hydroxy group was formed in the stage of hydrogenation. Accordingly, it is clear that one of the two molar hydrogen was consumed for hydrogenolysis of a labile C–O–C bond.

The presence of an epoxide as a labile ether linkage was shown by the following results. When **2** was subjected to a mild acid treatment (0.05N HCl, room temperature), complex of diol compounds (**14**), $C_{42}H_{71}O_{17}N$, $[\alpha]_D^{22}$ −52.1° (EtOH), which gave a tetraacetate (**15**), $C_{50}H_{79}O_{21}N$, (M^+, *m/e* 1029); NMR, four additional acetyl signals at δ 2.02 and 2.06, *i.e.*, the presence of two more secondary hydroxy groups than **2** was obtained. The diol (**15**) consists of some isomers resulting from allylic rearrangement and from mode of ring opening under acidic conditions.

When tetrahydro MDM II was hydrolyzed under more acidic conditions than that described above, a basic substance (**16**), $C_{30}H_{53}O_{12}N$, (M^+, *m/e* 619), $[\alpha]_D^{23}$ −20.5° (EtOH), p*Ka*′ 8.2, was obtained together with a neutral sugar (**17**). Compound (**16**) showed the presence of $-N(CH_3)_2$ at δ 2.53 (6H, s), $-OCOCH_3$ at δ 2.33 (3H, s), $-OCH_3$ at δ 3.59 (3H, s) and an

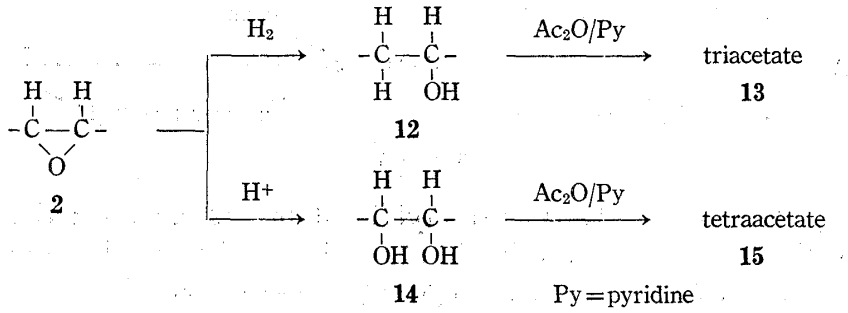


Chart 2

aldehyde group at δ 9.65 (1H, s). Acetylation of **16** with acetic anhydride in pyridine at room temperature gave a tetraacetyl derivative (**18**), $C_{38}H_{61}O_{16}N$ which showed M^+ at *m/e* 787 and in the NMR spectrum, the signal of three $OCOCH_3$ at δ 2.01 (9H, s) and one $OCOCH_3$ at δ 2.04 (3H, s), in addition to the acetyl signal present in **16**. The tetraacetate (**18**) exhibited no free hydroxyl absorption in the IR spectrum.

The neutral sugar (**17**) [$C_{12}H_{22}O_5$, $[\alpha]_D^{22}$ $-75.3°$ ($CHCl_3$)] showed a molecular ion peak at *m/e* 246, and in the NMR spectrum (in $CDCl_3$) revealed the signals of two secondary methyl at δ 1.03 (6H, d), one tertiary methyl at δ 1.19 (3H, s), one secondary methyl at δ 1.20 (3H, d), methylene at δ 2.29 (2H) and three methine protons attached to oxygen functions at δ 4.22 (1H, m), 4.68 (1H, d) and 5.20 (1H, brs or dd). These data suggested that **17** is a derivative of mycarose, and by direct comparison, **17** was identified with 4-O-isovaleryl-L-mycarose[7,8] obtained from leucomycin A_3.

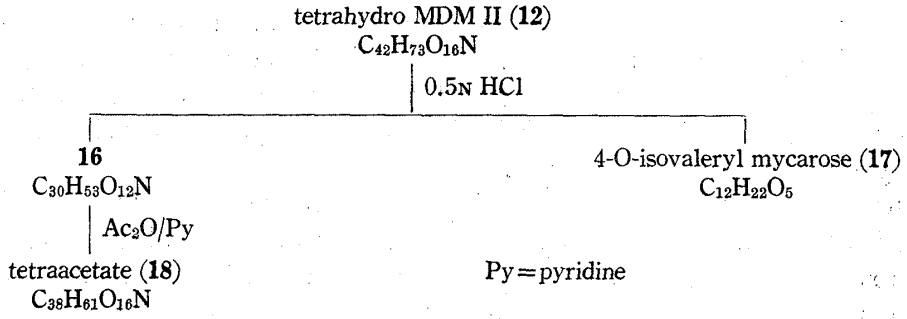
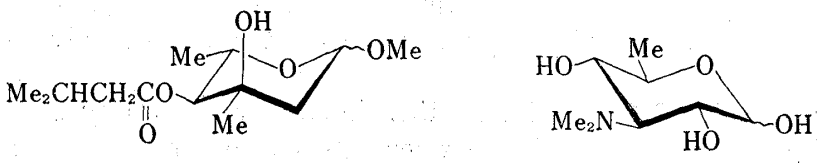


Chart 3

In addition, methanolysis of MDM II (**2**) with HCl–MeOH gave methyl 4-O-isovaleryl-L-mycarosides (α & β) (**19a, b**) which were identified with the authentic samples obtained from leucomycin A_3.[9] Besides (**19a**) and (**19b**) mixtures of basic compounds (**20**) were obtained



19a, b **21**

Chart 4

7) T. Watanabe, T. Fujii, and K. Satake, *J. Biochem.*, **50**, 197 (1961).
8) R.L. Wagner, F.A. Hochstein, K. Murai, and P.P. Regna, *J. Am. Chem. Soc.*, **75**, 4684 (1953).
9) *a*) P.P. Regna, F.A. Hochstein, R.L. Wagner, and R.B. Woodward, *J. Am. Chem. Soc.*, **75**, 4625 (1953); *b*) T. Watanabe, H. Nishida, and K. Satake, *Bull. Chem. Soc. Jap.*, **34**, 1285 (1961); *c*) S. Omura, H. Ogura, and T. Hata, *Tetrahedron Letters*, **1967**, 609; *d*) S. Omura, M. Katagiri, H. Ogura, and T. Hata, *Chem. Pharm. Bull.* (Tokyo), **16**, 1167 (1968).

from lipophilic fraction. The basic mixture (**20**) obtained by methanolysis was hydrolyzed under more drastic conditions (2N HCl, reflux), giving a basic sugar which was identified with D-mycaminose hydrochloride[10] (**21**) prepared from leucomycins. The compound (**21**) was also obtained by vigorous acid hydrolysis of basic substance (**16**).

Saponification of MDM II (**2**) with 1N KOH liberated two moles of fatty acids which were estimated as one mole each of acetic acid and isovaleric acid by gas liquid chromatography.[11] It is obvious that isovaleric acid is derived from mycarose portion while acetic acid comes from aglycone portion.

The presence of macrolactone moiety was suggested from the following data. Mild alkaline treatment of MDM II at room temperature gave a carboxylic acid derivative (**22**) with an absorption at 1580 cm^{-1} (COO^-), indicating an amphoteric property. It can be presumed that the carboxylic acid group was formed by cleavage of lactone moiety with aq. alkali. Thus, 15 out of 16 oxygen functions were disclosed except for one labile oxygen function, as shown in Chart 5. Besides above, –CH=CH– (*trans*) are apparent as another functional group not containing oxygen.

$-CH_2CHO$, $-OCH_3$, –OH (secondary), $-OCOCH_3$, –OCO–, (–C–O–C–),

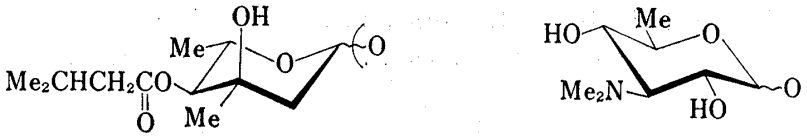
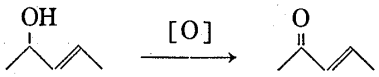
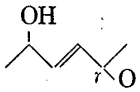


Chart 5

The presence of an allylic alcohol became evident from the fact that oxidation of MDM II under mild conditions with active MnO_2[12] in $CHCl_3$ or Sarret reagent[13] (CrO_3–pyridine) gave dehydro compounds with newly introduced intensive absorption maximum at 240 nm in UV region. As active MnO_2 and CrO_3-pyridine are known to oxidize allylic hydroxyl groups, the formation of α,β-unsaturated carbonyl reflected by UV absorption maximum at 240 nm suggests the presence of an allylic hydroxyl group in MDM II.



Furthermore, catalytic hydrogenation of MDM II over Pd-charcoal readily gave an additional secondary alcohol by hydrogenolysis, showing that a labile oxygen function is present in the allylic position of the double bond. That is to say, an oxygen function is located at the γ position of the allylic hydroxyl group.



The presence of an epoxide is suggested by the fact that while MDM II gave a diacetate (**9**), tetrahydro MDM II (**12**) afforded a triacetate (**13**) under the same condition, indicating the hydrogenolysis of an epoxide group. In addition, treatment of the crude dehydro compounds of MDM II (obtained by mild oxidation) with potassium iodide in acetic acid[14] resulted in the formation of $\alpha,\beta,\gamma,\delta$-unsaturated ketone which showed an intensive absorption at 280 nm.

10) F.A. Hochstein and P.P. Regna, *J. Am. Chem. Soc.*, **77**, 3353 (1955); T. Watanabe, *Bull. Chem. Soc. Jap.*, **34**, 15 (1961).
11) Acetic acid and isovaleric acid were quantitatively determined using columns of Chromosorb 101 and Porapak Q, respectively.
12) J. Attenburrow, A.F.B. Cameron, J.H. Chapman, R.M. Evans, B.A. Hems, A.B.A. Jansen, and T. Walker, *J. Chem. Soc.*, **1952**, 1094.
13) G.I. Poos, G.E. Arth, R.E. Beyler, and L.H. Sarret, *J. Am. Chem. Soc.*, **75**, 422 (1953).
14) S. Bodforss, *Chem. Ber.*, **49**, 2795 (1916).

In this way, it is clear that the epoxide is located allylic to the α,β-ene-ol. Finally, the presence of the α,β-ene-γ,δ-epoxy-ol system was ascertained by the NMR decoupling studies (Fig. 1). In d_6-acetone, irradiation of the proton attached to the carbon carrying OH group at δ 3.96 collapsed the olefinic proton at δ 6.04 (1H, dd) to doublet (J=16 Hz) and in turn the former (δ 3.96, 1H, dd) was decoupled by irradiation at the latter proton (δ 6.04), and furthermore another olefinic proton (δ 5.49) was decoupled at the same time. Irradiation of the signal at δ 3.10 where an epoxide proton is expected to appear collapsed the olefinic proton signal at δ 5.49 (dd) into a doublet (J=16 Hz). On reverse irradiation at δ 5.49, the epoxide signal was decoupled. Consequently, the system shown above was confirmed.

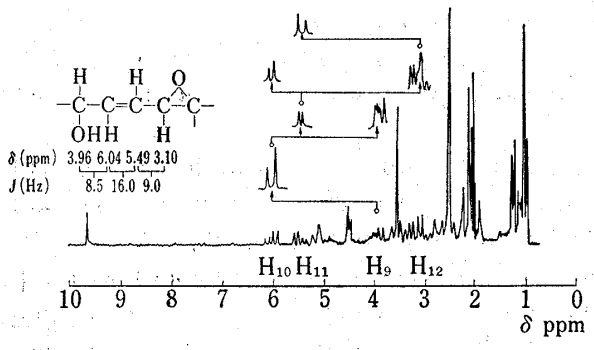
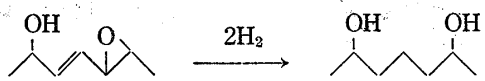


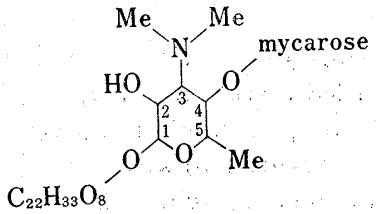
Fig. 1. NMR Spectrum of Maridomycin II (in d_6-acetone)

Further, tetrahydro derivatives (**13**) and (**16**) of MDM II are considered as a product hydrogenolysed at the epoxide as illustrated below.[15] On acid hydrolysis at room temperature



(0.5N HCl), the tetrahydro compound (**13**) gave a basic substance (**16**) which showed more basic property (pKa′ 8.2) than the tetrahydro compound (**13**) (pKa′ 7.1), together with the neutral sugar (**17**). The compound (**16**) afforded a tetraacetate with acetic anhydride in pyridine, showing one additional hydroxyl group formed on acid hydrolysis. The increase of basicity in **16** suggested that hydrolyzed mycarose was linked to a basic mycaminose portion in the vicinity of $-N(CH_3)_2$ group, *i.e.*, C-2 or C-4 position of mycaminose. Furthermore, as the basicity of diacetate (**10**) of MDM II was decreased to pKa′ 4.7, one of two secondary hydroxyl was located in the vicinity of $-N(CH_3)_2$ of mycaminose. This is also evident from the fact that $-N(CH_3)_2$ signal (6H, s) was shifted to higher field in diacetate (**10**). Therefore, it follows that anomeric hydroxyl group of mycaminose was glycosidically linked to the aglycone part.

The anomeric proton of mycaminose portion and methine proton (H-2′) coupling with the anomeric proton in monoacetate-1 (**10**) were shifted from δ 4.46 to 4.62 and from 3.53 to *ca.* 5.0, respectively, indicating that C-2′ OH is free and C-4′ OH further attached to mycarose, *i.e.*, linear disaccharide type as shown below;



As a result, in the aglycone portion $C_{22}H_{33}O_8$, the following functional groups were clarified; $-CH_2-CHO$, $-OCH_3$, $-OCO-CH_3$, lactone ($-O-\overset{\overset{O}{\|}}{C}-$), and α,β-ene-γ,δ-epoxy-ol. In addition, as NMR spectrum of a basic compound (**16**) showed three secondary methyl signals, one of which was assigned to that of mycaminose portion, the other two secondary methyl signals were

15) *a*) P. Heinänen, *Chem. Abstr.*, **39**, 4051 (1945); *b*) D.H. Kelly, *Dissertation Abstr.*, **24**, 974 (1963).

ascribed to the aglycone part. In order to clarify the position of oxygen functions, hexahydro MDM II (**23**) which was obtained by reduction of **16** with $NaBH_4$, was treated with hot sodium hydroxide solution, resulting in the formation of intensive absorption at 267 nm in the UV spectrum together with a volatile basic substance. This absorption maximum shifted to longer wave length at 277 nm on acidification, indicating the formation of $\alpha,\beta,\gamma,\delta$-unsaturated carboxylic acid-like system. Therefore, it is suggested that at the β and γ-position were located some oxygen functions which could be easily eliminated under alkaline conditions.

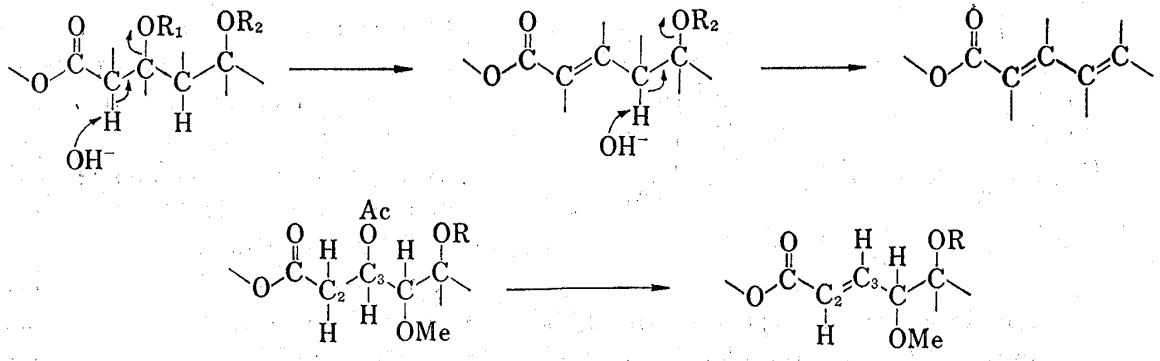
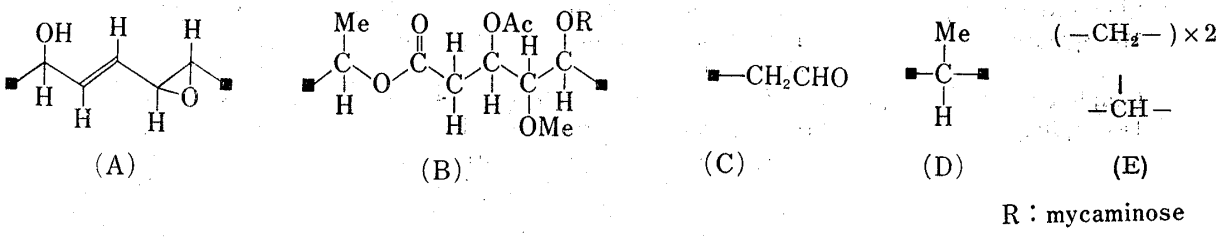


Chart 6

Furthermore, on mild alkali treatment of MDM II, an α,β-unsaturated lactone (**25**), showing strong absorption at 210 nm was generated. The NMR spectrum of **25** revealed no acetyl signal, indicative of β-elimination of an acetoxyl group. This suggests the location of acetoxyl group at the C-3 position of the lactone. By spin-decoupling studies on the compound (**25**), β-olefinic proton was found to be coupled to a methine proton attached to oxygen function, *i.e.*, –OMe from consideration of remaining oxygen function.

In the NMR spectrum of the compound (**16**), one of three doublet methyl signals (δ 1.26) is coupled to a methine proton at δ 4.99 which appears to be attached to O-acyl or lactone function, indicating that the methyl (–CH–CH_3) is linked to the lactobe oxygen.

In this way, it is clear that partial structures (A), (B), (C), (D) and (E) were clarified in the aglycone portion of MDM II (**2**).



R : mycaminose

Chart 7

However, on alkali treatment at room temperature, MDM II (**2**) gave a compound (**22**) undergoing lactone cleavage, which did not show any intensive absorption at 210 nm in comparison with **2**, suggesting the lack of conjugated double bonds. On the other hand, dihydro MDM II (**8**) not containing aldehyde function, gave a similar amphoteric compound (**24**). The presence of conjugated double bond in **24** was evident from its intensive absorption at 210 nm ($\Delta\varepsilon$ between **24** and **22**; 10300). Therefore, these facts indicate that active methylene adjacent to aldehyde function are easily condensed intramolecularly with β carbon to carbonyl of the lactone or carboxylic acid by intramolecular Michael-type condensation.[16] Consequent-

16) The presence of aldehyde in compound (**22**) was ascertained by formation of thiosemicarbazone which showed λ_{max}^{MeOH} at 271 nm. Similar intramolecular Michael condensation occurred in the case of Δ^2-MDM II without lactone opening.

ly, the position of $-CH_2CHO$ was suggested by ease of intramolecular cyclization to form five- or six-membered ring, *i.e.*, the methylene adjacent to aldehyde is δ to ε position from C-3 position of the lactone.

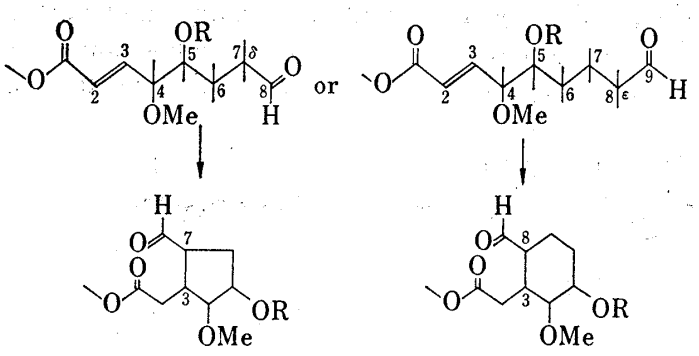
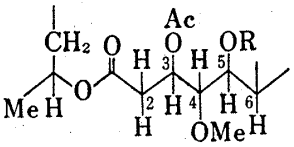


Chart 8

Furthermore, spin decoupling of the compound (**16**) verified the above partial structure (B) of the aglycone portion as shown in Table I and permitted expansion of partial structure(B) to (G). Irradiation at the frequency of H-2a collapsed the doublet of quartets of H-3 at δ 5.35 to a doublet of doublets. In turn, irradiation of H-3 converted a doublet of doublets (H-2a) at δ 2.76 to a doublet and a doublet of doublets of H-4 at δ 3.43 to a doublet. Conversely, irradiation of the latter (H-4) caused the signals of H-3 and H-5 to simultaneously change to a doublet of doublets and a doublet, respectively. Irradiation of H-5 collapsed the doublet of doublets of H-4 to a doublet. Although the signal of H-6 was difficult to assign, splitting pattern of H-5 indicates the presence of one methine proton at the C_6 position. On the other hand, since irradiation of the doublet methyl at δ 1.26 collapsed the multiplet of methine proton at δ 4.99 to a doublet of doublets, $(-CH-CH_3)$ is adjacent to methylene.

Table I. NMR Spectral Data of Compound 16 (in $CDCl_3$)



	Me	H	H_{2a}	H_3	H_4	H_5	H_6
Chemical shift (δ)	1.6	4.99	2.76	5.35	3.43	3.96	
Multiplicity	d	m	dd	dq	dd	dd	
Coupling constant (Hz)	6.5		9.5	≦2.0	9.5		<2.0
Decoupling	irr ⟶ dd		irr ⟶ dd				
	s ⟵ irr		d ⟵ irr	⟶ d			
				dd ⟵ irr	⟶ d		
					d ⟵ irr		

Abbreviations; irr=irradiation; d=doublet; m=multiplet; dq=doublet of quartets; dd=donblet of doublets.

Although the position of remaining secondary methyl which is overlapped with isovaleryl methyl in MDM II is not easy to assign, in compound (**8**), methine proton at δ 1.6 coupling with one of three secondary methyl in the high field region (not isovaleryl methyl) was found to be coupling to the methine proton attatched to allylic hydroxyl (δ 4.19). In this way, the partial structure, (F), (G) and one remaining methylene are given to the aglycone portion $C_{22}H_{33}O_8$ as shown in Chart 9.

Since the number of sites of unsaturation in the aglycone is six and partial structure represented above indicates five sites of unsaturation, the structure of the aglycone is monocyclic, *i.e.*, macrocyclic lactone consisting of the above partial structures.

Although MDM II has a remarkable characteristic that it shows no UV maxima, this macrocyclic structure with mycarosyl mycaminose moiety bounded at the C_5 position is very

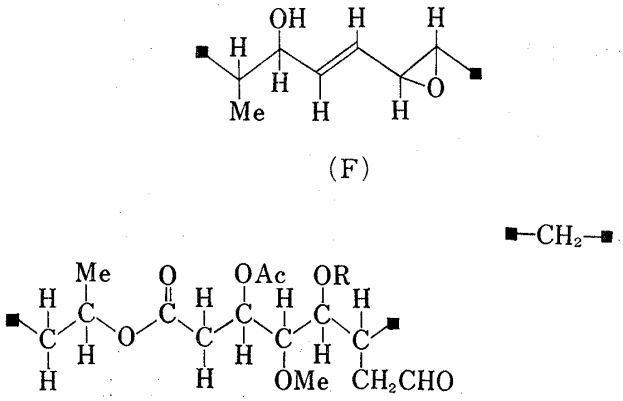


Chart 9

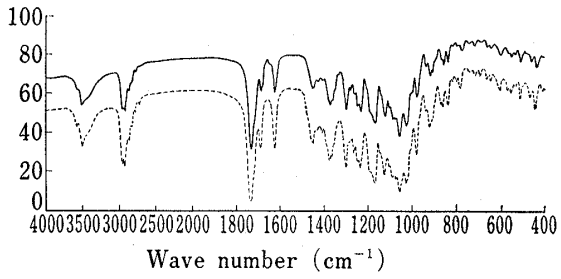


Fig. 2. IR Spectra of 9-Dehydro MDM II and Carbomycin A (KBr)

——: carbomycin A
·····: 9-dehydromaridomycin II

similar to the structure of carbomycin A.[17] Then, MDM II (2) was oxidized under mild conditions with Sarret reagent, giving mainly two substances, both of which showed intensive UV absorption at 240 nm. One of two compounds, dehydro MDM II-1 (**26**) which was obtained as colorless prisms, mp 208—209° showed molecular ion peak at *m/e* 841 ($C_{42}H_{67}O_{16}N$) in the mass spectrum, and on comparison with carbomycin A,[17] the both were found to be identical in the NMR, IR (Fig. 2), mass spectra and optical rotation. Identity was also confirmed by mixed mp (no depression, 208—209°).

In this way, the structure of MDM II was determined as shown in Chart 10 and to be the same with carbomycin A except for the C-9 position. Further attempt to deepoxidize 9-dehydro MDM II (**26**) with KI in AcOH resulted in the formation of 12,13-deepoxy-9-dehydro MDM II (**27**) which was identified with carbomycin B,[17a,18] thereby giving another support for the total structure of maridomycin II. The compound (**28**), $C_{30}H_{47}O_{12}N$, was obtained by mild acid hydrolysis of **26** and identified with carimbose A.[8]

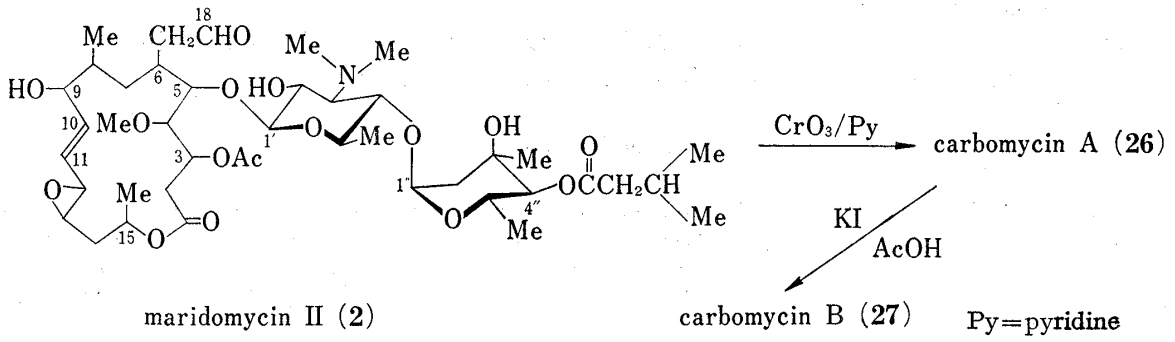
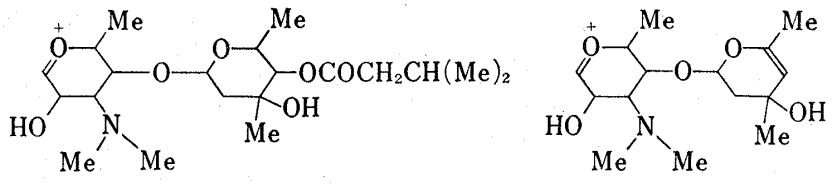


Chart 10

In addition, mass spectral data of MDM II and its derivatives are in agreement with the proposed structure.[19] From the fact that all of the compounds with isovaleryl mycarose moiety exhibited prominent peaks of isovaleryl mycarosyl deoxymycaminose ion, a (*m/e* 402), along with a fragment ion b (*m/e* 300) resulting from loss of isovaleric acid at the terminal neutral sugar, the antibiotic is not a bisglycoside contained in the other macrolide antibiotics

17) *a*) R.B. Woodward, *Angew. Chem.*, **69**, 50 (1957); *b*) M.E. Kuehne and B.W. Benson, *J. Am. Chem. Soc.*, **87**, 4660 (1965); *c*) R.B. Woodward, L.S. Weiler, and P.C. Dutta, *ibid.*, **87**, 4662 (1965); *d*) W.D. Celmer, *ibid.*, **88**, 5028 (1966).
18) F.A. Hochstein and K. Murai, *J. Am. Chem. Soc.*, **76**, 5080 (1954).
19) The details of fragmentation pattern of MDM II will be discussed in the following paper.



a b

m/e 402 *m/e* 300

Chart 11

such as erythromycin and oleandomycin, but a disaccharide derivative bounded in the only one position of aglycone. The mass spectra of MDM II (**2**) and its derivatives, **9**, **10**, **11**, *etc.*, indicate that any sugar moieties other than those described above are not bounded to the macrolactone moiety. This is different from other macrolides such as tylosine[20] and spiramycin.[21]

Configurations of anomeric centers in the sugar parts of MDM II were defined by NMR spectroscopy and application of Klyne's rule.[22] In all of MDM II and its derivatives, coupling constants of anomeric protons in the mycaminose moiety were nearly the same ($J_{1',2'}$=7.0—7.5 Hz) and this value indicated β-D configuration at this anomeric center. Although H-1″ signal of MDM II in $CDCl_3$ partly overlapped with other ones, the spectrum of **2** in d_5-pyridine showed a separate signal of H-1″ with coupling constants $J_{1'',2''}$=3.5/1.0 indicating the α-L-configuration. And Δ^8-MDM II (**25**) showed the signal of H-1″ separately with coupling constants ($J_{1''e,2''a}$=3.5, $J_{1''e,2''e}$=1.0 Hz) which are consistent with those of MDM II in d_5-pyridine.

Molecular rotation differences between the compounds **12** and **16** and between **26** and **28** were −406.0° and −374.2°, respectively, providing further evidence that the anomeric configuration in the mycarose moiety is α.

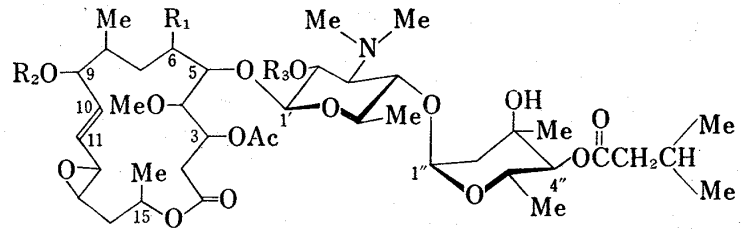
From these evidences, the total structure of maridomycin II was elucidated as 3-acetoxy-5-[3′,6′-dideoxy-4′-O-(2″,6″-dideoxy-4″-O-isovaleryl-3″-C-methyl-α-L-ribohexopyranosyl)-3′-dimethylamino-β-D-glucopyranosyloxy]-12,13-epoxy-6-formylmethyl-9-hydroxy-4-methoxy-8-methyl-10-hexadecen-15-olide.

Structures of related compounds of MDM II (**2**) are represented as shown in the Chart 12 and stereochemistry will be discussed in the following paper.

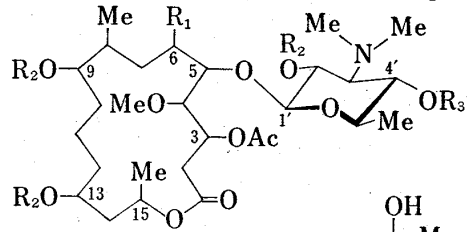
Experimental[23]

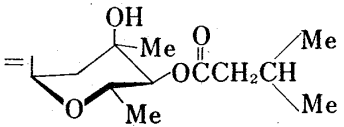
Isolation of Maridomycin II (**2**)——Maridomycin II was isolated from maridomycin complex by the procedures described in the previous report.[5] MDM II was obtained as colorless needles, mp 134—136°, from benzene or ether–*n*-hexane, while recrystallization from acetone–*n*-hexane gave colorless prisms of mp 196—199°. $[\alpha]_D^{23}$ −71.9° (c=1.0, EtOH). *Anal.* Calcd. for $C_{42}H_{69}O_{16}N \cdot H_2O$: C, 58.52; H, 8.30; N, 1.62. Found: C, 58.62; H, 8.24; N, 1.67.

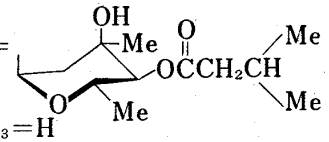
20) R.B. Morin, M. Gorman, R.L. Hamill, and P.V. Demarco, *Tetrahedron Letters*, **1970**, 4737.
21) S. Omura, A. Nakagawa, H. Ogura, and K. Furuhata, *J. Am. Chem. Soc.*, **91**, 3401 (1969).
22) *a*) W. Klyne, *Biochem. J.*, **47**, xli (1950); *b*) W.D. Celmer, "Biogenesis of Antibiotic Substances," ed. by Z. Vanek and Z. Hostalek, Academic Press, Inc., New York, 1965, p. 119.
23) Melting points were determined with a Mettler FP 5 apparatus. IR spectra were obtained with a Perkin Elmer model 21 or a Hitachi model EPI-G2 spectrometer. UV spectra were run with a Perkin-Elmer model 450 or a Hitachi EPS-3T spectrometer. NMR spectra were recorded on a Varian HA-100 spectrometer with tetramethylsilane as the internal standard. Chemical shifts are reported on the δ scale. Mass spectra were obtained with a JEOL JMS-OlSG mass spectrometer using a direct inlet system. Optical rotations were measured with a Perkin-Elmer model 141 polarimeter. Thin-layer chromatography (TLC) was performed on Silica gel spotfilm (Tokyo Kasei Co.), Silica gel G, or precoated Silica gel plates (Merck). Abbreviations are as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad.



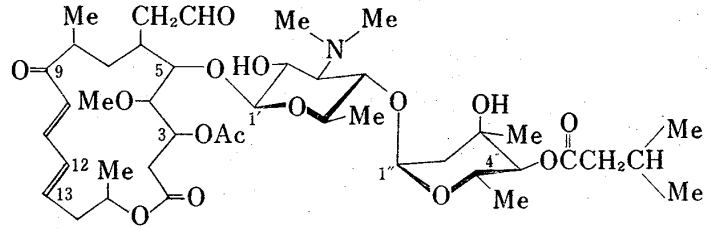
2 : $R_1 = -CH_2CHO$, $R_2 = H$, $R_3 = H$
7 : $R_1 = -CH_2CH{=}NNHC(=S)NH_2$, $R_2 = H$, $R_3 = H$
8 : $R_1 = -CH_2CH_2OH$, $R_2 = H$, $R_3 = H$
9 : $R_1 = -CH_2CHO$, $R_2 = Ac$, $R_3 = Ac$
10 : $R_1 = -CH_2CHO$, $R_2 = H$, $R_3 = Ac$
11 : $R_1 = -CH_2CHO$, $R_2 = Ac$, $R_3 = H$



12 : $R_1 = -CH_2CHO$, $R_2 = H$, $R_3 =$ 

13 : $R_1 = -CH_2CHO$, $R_2 = Ac$, $R_3 =$ 

16 : $R_1 = -CH_2CHO$, $R_2 = H$, $R_3 = H$
23 : $R_1 = -CH_2CH_2OH$, $R_2 = H$, $R_3 = H$



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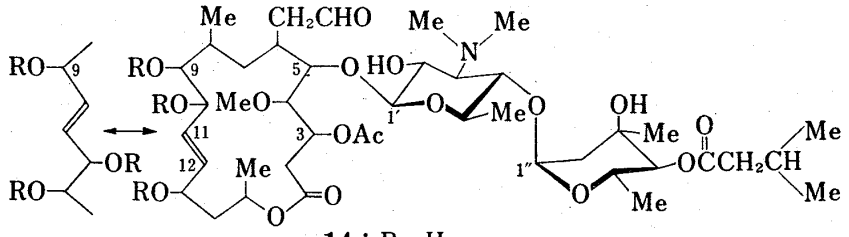
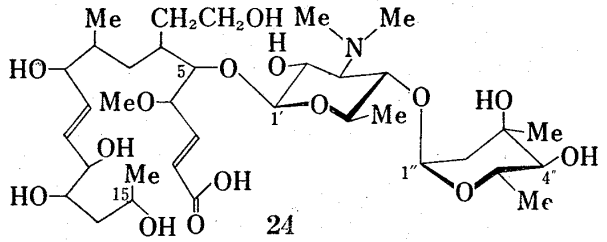


14 : R = H
15 : R = Ac



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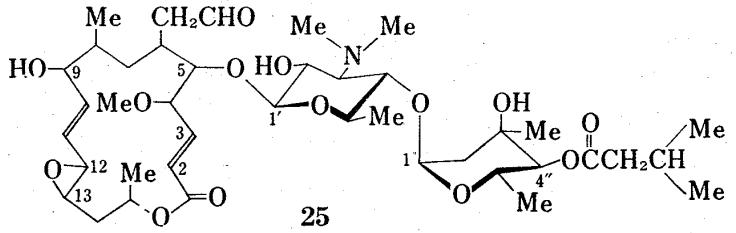

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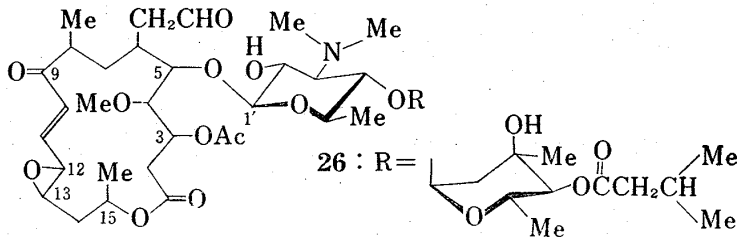

26 : R =

28 : R = H

Chart 12

MDM II 18-Thiosemicarbazone (7)——A mixture of MDM II (200 mg) and thiosemicarbazide (42 mg) in EtOH (8 ml) was refluxed for 4 hr. The reaction mixture was concentrated to dryness and the residue was extracted twice with 5 ml of $CHCl_3$. The substance after evaporation of solvent from the extract was chromatographed on a silica gel column using $CHCl_3$–MeOH (49: 1) as the solvent. The effluent was concentrated to yield 152 mg of thiosemicarbazone (**7**) as a white solid. $[\alpha]_D^{32}$ −106.7° (c=1.0, $CHCl_3$). *Anal.* Calcd. for $C_{43}H_{72}O_{15}N_4S\cdot H_2O$: C, 55.23; H, 7.98; N, 5.99; S, 3.43. Found: C, 55.12; H, 7.78; N, 5.87; S, 3.38. UV λ_{max}^{EtOH} nm (ε): 270 (23800). NMR ($CDCl_3$) δ: 2.07 (3H, s, $-OCOCH_3$), 2.55 (6H, s, $-N(CH_3)_2$), 3.57 (3H, s, $-OCH_3$), 7.45 (1H, t, J=5 Hz, $-CH_2CH=N-$).

18-Dihydro MDM II (8)——To a solution of MDM II (300 mg) in MeOH (8 ml) and H_2O (2 ml) was added 12.6 mg of $NaBH_4$ and stirred for 1 hr at room temperature. The reaction mixture was diluted with 1/15M phosphate buffer of pH 7.38 and extracted twice with AcOEt. After the washing with H_2O, the AcOEt solution was concentrated to dryness and the residue was dissolved in CH_2Cl_2. Addition of n-hexane to CH_2Cl_2 solution gave a white powder of 18-dihydro MDM II (**8**) (234 mg) which was recrystallized from isopropyl ether, mp 131—132°. $[\alpha]_D^{32}$ −76.7° (c=1.0, EtOH). *Anal.* Calcd. for $C_{42}H_{71}O_{16}N\cdot H_2O$: C, 58.38; H, 8.52; N, 1.62. Found: C, 58.38; H, 8.37; N, 1.64. NMR ($CDCl_3$) δ: 2.11 (3H, s, $-OCOCH_3$), 2.54 (6H, s, $-N(CH_3)_2$), 3.57 (3H, s, $-OCH_3$), 5.66 (1H, dd, olefinic H), 6.12 (1H, dd, olefinic H).

MDM II 9,2′-Diacetate (9)——To a solution of MDM II (100 mg) in dry pyridine (0.5 ml) was added acetic anhydride (0.2 ml) under ice cooling. After the reaction mixture was allowed to stand at room temperature overnight, it was poured into ice water and extracted with AcOEt. The extract was washed with H_2O, dried over Na_2SO_4, and evaporated to give MDM II 9,2′-diacetate (**9**) as a white powder, $[\alpha]_D^{29}$ −81.4° (c=0.5, EtOH). *Anal.* Calcd. for $C_{46}H_{72}O_{18}N$: C, 59.53; H, 7.93; N, 1.51. Found: C, 59.43; H, 7.98; N, 1.51. NMR ($CDCl_3$) δ: 2.02 (3H, s, $-OCOCH_3$), 2.04 (3H, s, $-OCOCH_3$), 2.43 (6H, s, $-N(CH_3)_2$), 4.61 (1H, d, $H_{1'}$, J=7 Hz). Mass Spectrum m/e: 927 (M^+).

MDM II 2′-Acetate (10)——To a solution of MDM II (200 mg) in acetone (1.2 ml) was added acetic anhydride (0.1 ml) and allowed to stand overnight in a refrigerator. The reaction mixture was poured into cold aqueous $NaHCO_3$ solution and extracted with AcOEt. The washed and dried extract was concentrated to give 162 mg of monoacetate-1 (**10**) which was crystallized from benzene–n–hexane, mp 134—135°. $[\alpha]_D^{26}$ −87.8° (c=1.0, EtOH). *Anal.* Calcd. for $C_{44}H_{71}O_{17}N\cdot 1/2H_2O$: C, 59.04; H, 8.11; N, 1.57. Found: C, 59.02; H, 8.14; N, 1.54. NMR ($CDCl_3$) δ: 2.05 (3H, s, $-OCOCH_3$), 2.26 (3H, s, $-OCOCH_3$), 2.44 (6H, s, $-N(CH_3)_2$), 4.62 (1H, d, anomeric H, J=7.5 Hz). Mass Spectrum m/e: 885 (M^+).

MDM II 9-Acetate (11)——To a solution of MDM II (600 mg) in dry pyridine (2.5 ml) was added dropwise acetyl chloride (0.2 ml) under ice cooling and further stirred at 0—5° for 2 hr. The reaction mixture was poured into ice water and extracted with AcOEt. After washing and drying, the extract was concentrated to dryness, and the residue was chromatographed on a silica gel column (15 g) using $CHCl_3$–MeOH (10: 1) as a solvent. Removal of the solvent from the eluate gave a monoacetate-2 (**11**) which was recrystallized from ether–n-hexane to give colorless prisms (303 mg), mp 190—191°. $[\alpha]_D^{25}$ −66.9° (c=1.0, $CHCl_3$). *Anal.* Calcd. for $C_{44}H_{71}O_{17}N$: C, 59.64; H, 8.08; N, 1.58. Found: C, 59.55; H, 8.15; N, 1.57. NMR ($CDCl_3$) δ: 2.01 (3H, s, $-OCOCH_3$), 2.25 (3H, s, $-OCOCH_3$), 2.53 (6H, s, $-N(CH_3)_2$), 4.42 (1H, d, anomeric H, J=7.5 Hz). Mass Spectrum m/e: 885 (M^+).

10,11,12,13-Tetrahydro MDM II (12)——MDM II (1.8 g) in 80 ml of EtOH was hydrogenated in the presence of 10% Pd-charcoal (500 mg) for 3 hr at room temperature. The catalyst was filtered off and evaporation of the solvent from the filtrate afforded a glassy solid, which was extracted with AcOEt. Addition of n-hexane to the extract gave a white powder of tetrahydro MDM II (**12**) (1.32 g). $[\alpha]_D^{22}$ −62.1° (c=0.94, EtOH). *Anal.* Calcd. for $C_{42}H_{73}O_{16}N\cdot H_2O$: C, 58.25; H, 8.73; N, 1.62. Found: C, 58.40; H, 8.76; N, 1.64.

10,11,12,13-Tetrahydro MDM II 9,13,2′-Triacetate (13)——To a solution of tetrahydro MDM II (**12**) (200 mg) in dry pyridine (1 ml) was added acetic anhydride (0.4 ml) under ice cooling and allowed to stand at room temperature overnight. The mixture was poured into ice water and extraction with AcOEt yielded, after washing and drying of the extract and evaporation *in vacuo*, a white solid which was crystallized from CH_2Cl_2–n-hexane as colorless needles (**13**) (148 mg), mp 116—117°, $[\alpha]_D^{23}$ −76.4° (c=1.0, $CHCl_3$). *Anal.* Calcd. for $C_{48}H_{79}O_{19}N\cdot H_2O$: C, 58.10; H, 8.23; N, 1.41. Found: C, 57.70; H, 8.15; N, 1.44. NMR ($CDCl_3$) δ: 1.99 (6H, s, 2×$-OCOCH_3$), 2.01 (3H, s, $-OCOCH_3$), 2.23 (3H, s, $-OCOCH_3$), 2.40 (6H, s, $-N(CH_3)_2$), 3.50 (3H, s, $-OCH_3$), 4.59 (1H, d, anomeric H, J=7 Hz), 9.64 (1H, s, $-CHO$).

MDM II Diol (14)——MDM II (900 mg) was treated with 26 ml of 0.05N HCl at room temperature for 6 hr, diluted with H_2O, and extracted with AcOEt at pH 8.5. The washed and dried extract was evaporated to give a white solid (782 mg) which was chromatographed on a silica gel column (25 g) with the solvent system of $CHCl_3$–MeOH. Elution with $CHCl_3$–MeOH (25: 1) gave MDM II diol (390 mg) composed of at least two isomers, and further elution gave another diol (189 mg) which was consisted of almost one compound. MDM II diol complex: $[\alpha]_D^{22}$ −52.1° (c=0.8, EtOH). *Anal.* Calcd. for $C_{42}H_{71}O_{17}N\cdot H_2O$: C, 57.32; H, 8.36; N, 1.59. Found: C, 57.31; H, 8.13; N, 1.63.

MDM II Diol Tetraacetate (15)——Acetylation of 150 mg of MDM II diol complex (**14**) with 1.5 ml of dry pyridine and 0.6 ml of acetic anhydride at room temperature overnight followed by dilution with ice water and extraction with AcOEt yielded, after washing and drying of the extract and evaporation, 177 mg of tetraacetate as a white solid. *Anal.* Calcd. for $C_{50}H_{79}O_{21}N$: C, 58.30; H, 7.73; N, 1.36. Found: C, 57.69; H, 7.58;

N, 1.31. NMR ($CDCl_3$) δ: 2.06 and 2.10 (12 H, 4×-$OCOCH_3$), 2.44 (6H, s, -$N(CH_3)_2$). Mass Spectrum *m/e*: 1029 (M^+).

Acidic Hydrolysis of 10,11,12,13-Tetrahydro MDM II (12)——A solution of 10,11,12,13-tetrahydro MDM II (**12**) (300 mg) in 0.5N HCl was allowed to stand at room temperature for 5 hr, adjusted to pH 2.5 with 2N NaOH, and extracted with ether. Evaporation of the washed and dried extract furnished 62 mg of (**17**) as colorless needles of mp 73—74°. $[\alpha]_D^{22}$ −75.3° (*c*=1.0, $CHCl_3$ after 1 hr) (lit.[8] −73°). *Anal.* Calcd. for $C_{12}H_{22}O_5$: C, 58.52; H, 9.01. Found: C, 58.50; H, 9.15. This compound was identified as 4-O-isovaleryl mycarose obtained from leucomycin A_3 by comparison of NMR, IR and mass spectra, and *Rf* values on TLC.

The aqueous layer after the above extraction of the hydrolyzate was readjusted to pH 8.0 and extracted with $CHCl_3$. Evaporation of the washed and dried extract gave a basic compound, demycarosyl[24] 10,11,12,-13-tetrahydro MDM II (**16**) as a white solid. $[\alpha]_D^{23}$ −20.5° (*c*=1.0, EtOH). *Anal.* Calcd. for $C_{30}H_{53}O_{12}N \cdot H_2O$: C, 56.50; H, 8.69; N, 2.20. Found: C, 57.30; H, 8.57; N, 2.23. NMR ($CDCl_3$) δ: 0.95 (3H, d, *sec.* CH_3), 1.24 (3H, d, *sec.* CH_3), 1.29 (3H, d, *sec.* CH_3), 2.33 (3H, s, -$OCOCH_3$), 2.53 (6H, s, -$N(CH_3)_2$), 3.59 (3H, s, -OCH_3), 4.47 (1H, d, anomeric H, *J*=7.0 Hz), 5.0 (1H, m), 5.34 (1H, br. d), 9.65 (1H, s, -CHO). Mass Spectrum *m/e*: 619 (M^+).

Demycarosyl 10,11,12,13-Tetrahydro MDM II 9,13,2′,4′-Tetraacetate (18)——Acetylation of the basic compound (**16**) (300 mg) with 1 ml of dry pyridine and 0.6 ml of acetic anhydride at room temperature overnight followed by usual work-up yielded tetraacetate (**18**) which was treated with ether–*n*-hexane, giving a white powder (238 mg) of **18**. *Anal.* Calcd. for $C_{38}H_{61}O_{16}N$: C, 57.93; H, 7.80; N, 1.78. Found: C, 57.93; H, 8.02; N, 1.62. NMR ($CDCl_3$) δ: 0.90 (3H, d, *sec.* CH_3), 1.10 (3H, d, *sec.* CH_3), 1.30 (3H, d, *sec.* CH_3), 2.04 (9H, s, 3×-$OCOCH_3$), 2.09 (3H, s, -$OCOCH_3$), 2.29 (3H, s, -$OCOCH_3$), 2.37 (6H, s, -$N(CH_3)_2$). Mass Spectrum *m/e*: 787 (M^+).

Methanolysis of MDM II (2)——A mixture of 1.9 g of MDM II, 19 ml of MeOH, and 0.95 ml of concd. HCl was allowed to stand at room temperature for 6 hr, poured into ice water and extracted with $CHCl_3$. Evaporation of the washed and dried extract gave syrupy residue (691 mg) which was chromatographed on a silica gel column. Elution with benzene–acetone (25: 1) gave 162 mg of α-anomer (**19a**) and further elution with benzene–acetone (20: 1) gave 317 mg of β-anomer of methyl 4-O-isovaleryl-L-mycaroside (**19b**). α-Methyl 4-O-isovaleryl-L-mycaroside (**19a**): $[\alpha]_D^{27}$ −151° (*c*=1.0, $CHCl_3$). *Anal.* Calcd. for $C_{13}H_{24}O_5$: C, 59.98; H, 9.27. Found: C, 60.12; H, 9.41. Mass Spectrum *m/e*: 260 (M^+). β-Methyl 4-O-isovaleryl-L-mycaroside (**19b**): $[\alpha]_D^{22}$ +8.6° (*c*=1.5, $CHCl_3$). *Anal.* Found: C, 59.55; H, 9.31. Mass Spectrum *m/e*: 260 (M^+). **19a** and **19b** were identified with authentic samples from leucomycin A_3 by direct comparison of *Rf* values on TLC, IR and NMR spectra.

The aqueous layer after extraction of **19a** and **19b** was further extracted with $CHCl_3$ at pH 8.5, and the washed and dried extract was concentrated to give a complicated mixture of basic substances (**20**). $[\alpha]_D^{24}$ −5.2° (*c*=1.0, EtOH). *Anal.* Found: C, 57.06; H, 8.71; N, 2.18.

Isolation of D-Mycaminose (21) from Basic Mixtures (20) by Vigorous Acidic Hydrolysis——Hydrolysis of **20** (2.05 g) with 2N HCl (40 ml) under reflux for 3 hr, followed by filtration of the reaction mixture, extraction of the filtrate with $CHCl_3$ and *n*-BuOH left colored aqueous solution which was concentrated to dryness. The resulting pale brown syrup (500 mg) in 5 ml of H_2O was adsorbed on a column of Dowex 50W×2 (100—200 mesh, H form), which was eluted with 0.5N HCl. The fraction of positive Fehling reaction was concentrated to yield colorless syrup of basic sugar (**21**) which was crystallized from aq. isopropanol. mp 114—115°. $[\alpha]_D^{25}$ +32.6° (*c*=1.0, H_2O). *Anal.* Calcd. for $C_8H_{17}O_4N \cdot HCl \cdot H_2O$: C, 39.10; H, 8.20; N, 5.70. Found: C, 39.27; H, 8.10; N, 5.53. Mass Spectrum *m/e*: 191 (M^+), 174 (M^+-17), 173 (M^+-18). This compound was identical with D-mycaminose from leucomycins also in NMR and IR spectra, and *Rf* values on TLC.

Mild Alkaline Hydrolysis of MDM II——A mixture of 900 mg of MDM II, 10 ml of MeOH and 10 ml of 1N NaOH was allowed to stand at room temperature 4 hr, neutralized to pH 8.0 with 1N HCl, and concentrated to dryness. The residue was extracted three times with AcOEt and the extract was evaporated to give an amorphous solid (827 mg) which was subjected to a column of silica gel (25 g). Elution with AcOEt–MeOH (2: 1) afforded 528 mg of white solid (**22**). *Anal.* Calcd. for $C_{35}H_{63}O_{16}N$: C, 55.76; H, 8.42; N, 1.86. Found: C, 55.48; H, 8.15; N, 1.97. UV end absorption. IR ν_{max}^{KBr} cm^{-1}: 1580 (COO^-).

Reduction of (16) with $NaBH_4$——To a solution of 272 mg of **16** in 10 ml of 50% aqueous MeOH was added 37.8 mg of $NaBH_4$ and stirred at room temperature for 1 hr. The solution was treated with 400 mg of Dowex 50 (H form), which was washed with H_2O and stirred with 1N NH_4OH. The eluate was evaporated to give glassy solid (250 mg) which was extracted with $CHCl_3$. The extract was concentrated and treated with *n*-hexane, giving a white powder of 10,11,12,13,18-hexahydro compound (**23**). *Anal.* Calcd. for $C_{30}H_{55}O_{12}N$: C, 57.95; H, 8.92; N, 2.25. Found: C, 57.93; H, 8.86; N, 2.22. Mass Spectrum *m/e*: 621 (M^+), 603 (M^+-18), 561 (M^+-60).

Selective β-Elimination of Acetoxyl Group in MDM II——To a solution of 860 mg of MDM II in 15 ml of MeOH was added 3 ml of 0.5N methanolic KOH and the mixture was allowed to stand at 5° overnight. The solution was diluted with ice water, neutralized with 1N AcOH, and concentrated to remove MeOH. The

24) Designation "demycarosyl" was used for the compounds having lost mycarose moiety.

aqueous solution was extracted with AcOEt and the washed and dried extract was evaporated to give 677 mg of the compound Δ^2 MDM II (**25**). $[\alpha]_D^{22}$ −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. UV end absorption (ε at 210 nm; 17500). IR ν_{max}^{KBr} cm^{-1}: 1725 (conjugated lactone). NMR ($CDCl_3$) δ: 5.37 (1H, dd, olefinic H), 5.97 (1H, d, J=16 Hz, olefinic H), 5.99 (1H, dd, olefinic H), 6.63 (1H, dd, J=16 Hz, 9 Hz, olefinic H). Mass Spectrum m/e: 783 (M^+), 365 (aglycone).

Mild Alkaline Hydrolysis of 18-Dihydro MDM II (8)——To a solution of 440 mg of (**8**) in 67% aqueous MeOH (7.5 ml) was added 2.5 ml of 2N NaOH, and the mixture was left to stand at room temperature for 4 hr. The solution was neutralized with 1N HCl and evaporated to dryness. The resulting residue was extracted twice with acetone, and the extract was again concentrated to give 313 mg of white solid (**24**), which was purified by silica gel column chromatography using benzene–MeOH (2: 1) as the solvent system. $[\alpha]_D^{23}$ −63.8° (c=1.0, MeOH). *Anal.* Found: C, 55.48; H, 8.23; N, 1.82. IR ν_{max}^{KBr} cm^{-1}: 1565 (COO^-). UV λ_{end}^{MeOH} 210 nm (ε; 12600).

Oxidation of MDM II with CrO_3-Pyridine Complex——To CrO_3–pyridine complex (prepared from 200 mg of CrO_3 and 2 ml of dry pyridine) was added a solution of MDM II (300 mg) in 3 ml of dry pyridine under ice-cooling, and the mixture was stirred for 30 min at 15—20°. The reaction mixture was poured into ice-water and extracted with AcOEt. The washed and dried extract was concentrated to dryness to give 231 mg of pale brown residue, which was chromatographed on a column of silica gel (15 g). Elution with AcOEt–benzene (2: 3) gave 76 mg of 9-dehydro MDM II (**26**), which was crystallized from acetone–*n*-hexane or EtOH–H_2O as colorless prisms. mp 208—209°. The mixture melting point with carbonycin A[8] was not depressed. $[\alpha]_D^{22}$ −57.4° (c=0.94, $CHCl_3$). (lit.[8] −58.6°). *Anal.* Calcd. for $C_{42}H_{67}O_{16}N$: C, 59.91; H, 8.02; N, 1.66. Found: C, 59.43; H, 7.97; N, 1.63. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1695 (conjugated C=O), 1663 (C=C). UV λ_{max}^{EtOH} nm (ε): 240 (15000). NMR ($CDCl_3$) δ: 2.16 (3H, s, $-OCOCH_3$), 2.50 (6H, s, $-N(CH_3)_2$), 3.53 (3H, s, $-OCH_3$), 6.5—6.8 (2H, olefinic protons), 9.54 (1H, s, –CHO), and in d_6-acetone: 6.38 (1H, dd, olefinic H), 6.98 (1H, d, olefinic H, J=15 Hz).

Mild Acid Hydrolysis of 9-Dehydro MDM II (26)——A solution of 9-dehydro MDM II (**26**) (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 3.0 with 1N NaOH and extracted with AcOEt. The washed and dried extract was evaporated to dryness to give 95 mg of 4-O-isovaleryl mycarose (**17**). The aqueous solution remaining after AcOEt extraction was adjusted to pH 9 and extracted with $CHCl_3$. The washed and dried extract was concentrated to afford crystalline residue (**28**) (234 mg) which was recrystallized from acetone–*n*-hexane as colorless plates, mp 191—192° (lit.[8] 190—192°). $[\alpha]_D^{31}$ −17.7° (c=0.92, $CHCl_3$), (lit.[8] −16°). UV λ_{max}^{MeOH} nm (ε): 240.0 (14200). NMR ($CDCl_3$) δ: 2.20 (3H, s, $-OCOCH_3$), 2.52 (6H, s, $-N(CH_3)_2$, 2.68 (1H, s, OH), 3.58 (3H, s, $-OCH_3$), 6.68 (2H, m, olefinic protons), 9.58 (1H, s, –CHO).

9-Dehydro-12,13-deepoxy MDM II (27)——A mixture of 9-dehydro MDM II (149 mg), KI (300 mg), and AcOH (1 ml) was stirred on a steam bath for 45 min. The reaction mixture was poured into ice water, neutralized to pH 8, and extracted with AcOEt. The extract was washed with aq. $Na_2S_2O_3$ solution and H_2O, successively, and concentrated to give crude material (124 mg) which was chromatographed on a silica gel column. Elution with benzene–AcOEt (2: 1) gave 53 mg of deepoxy compound (**27**) and further elution afforded another deepoxy compound (41 mg) which had lost the neutral sugar portion. 9-Dehydro-12,13-deepoxy MDM II (**27**): $[\alpha]_D^{26}$ −33.7° (c=0.5, $CHCl_3$). IR ν_{max}^{KBr} 1680 (conjugated C=O), 1635 (C=C), 1595 (C=C), 1240 (C–O–Ac) cm^{-1}. UV λ_{max}^{EtOH} nm (ε): 279 (26000). The NMR (in $CDCl_3$ and d_6-acetone) and mass spectra were identical with those of carbomycin B.[18,25] Another deepoxy compound was characterized only by NMR and UV spectra and was not further pursued in this experiment.

Acknowledgement The authors are grateful to Dr. F.A. Hochstein of Pfizer Inc. for his generous gift of carbomycin A and B and to Dr. R. Takeda for his encouragement throughout this work. Thanks are also due to the members of Chemical Research Laboratories of this Division for physicochemical measurements.

25) The compound was directly compared with an authentic sample of carbomycin B.