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X-Ray Analysis of 9-Propionylmaridomycin III Iodo Adduct

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The complete three dimensional structure of 9-propionylmaridomycin III (PMDM III) has been determined by the X-ray analysis of its iodo adduct. This is the first case that the structure of the intact 16-membered ring marcrolide has been determined by the X-ray analysis.

Hydrophilic substituent groups of the 16-membered macrocyclic ring of PMDM III lie on one side of the ring, while the hydrophobic substituents are directed to the other side of the ring. This characteristic arrangement of the substituents gives the macrocyclic ring of PMDM III two physicochemically different faces, which have previously been pointed out in the case of the 14-membered ring of erythromycin A. Similar spatial arrangement of substituents on the macro ring is also found in the conformation of demycarosyl isoleucomycin A_3 elucidated by X-ray analysis of its hydrobromide. The spatial interrelation of 6-formylmethyl, 2′-hydroxyl and 3′-dimethylamino groups, which have been known most important substituents for the biological activities of the 16-membered ring macrolide antibiotics, is strikingly similar between PMDM III and demycarosyl isoleucomycin A_3.

Keywords——maridomycin; macrolide antibiotics; crystal structure; conformation; absolute configuration; X-ray analysis

Maridomycin (MDM, **1**), consisting of 7 components (maridomycins I, II, III, IV, V, VI, and VII), has been isolated from the culture filtrate of *Streptomyces hygroscopicus* No. B-5050.[2] They were classified as macrolide antibiotics from their physico-chemical and chemical properties. Selective propionylation at the hydroxyl group in the macrocyclic ring of MDM gave 9-propionylmaridomycin (PMDM), which showed improved oral absorption and increased stability.[3] Structures of MDM and PMDM were deduced by Muroi *et al.*[4] from spectroscopic evidence and chemical conversion into carbomycin A (**6**). We have undertaken the X-ray analysis for the confirmation of the structure and the establishment of the absolute configuration and conformation of the molecule in the crystal. Among a variety of heavy-atom derivatives, especially those of 9-propionylmaridomycin III (PMDM III, **2**), I_3 adduct of **2**, PMDM III–I_3 $C_{44}H_{71}I_3NO_{17}$, was found to give the most promising crystals.

Experimental

Single crystals of PMDM III-I_3 were obtained in brown prisms by recrystallization from a mixture of tetrachloromethane and 1,2-dichloroethylene. Thermal and elemental analyses suggested that the crystal contains 1 mol each of tetrachloromethane and 1,2-dichloroethylene as solvents of crystallization. To prevent the crystals from decomposition due to vaporization of these labile solvents of crystallization, speci-

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[1] Location: *Jusohonmachi, Yodogawa-ku, Osaka 532, Japan.*

[2] H. Ono, T. Hasegawa, E. Higashide, and M. Shibata, *J. Antibiot.* (Tokyo), *Ser. A*, **26**, 191 (1973); M. Muroi, M. Izawa, M. Asai, T. Kishi, and K. Mizuno, *ibid.*, **26**, 199 (1973).

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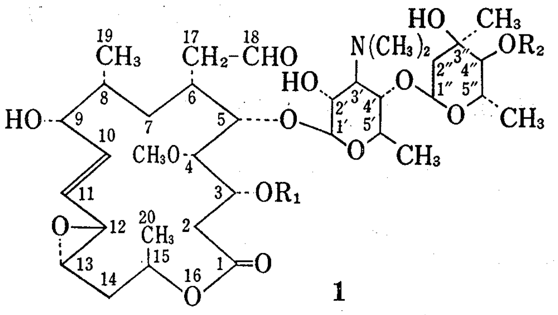

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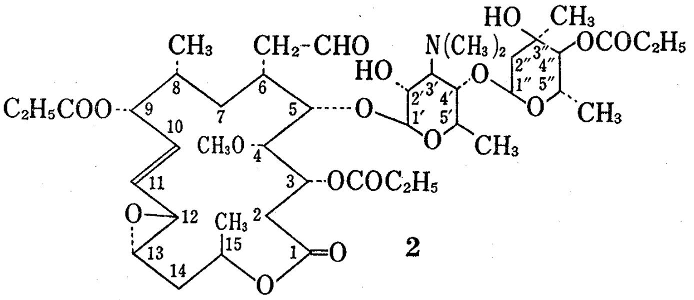
		R_1	R_2
MDM	I	$COCH_2CH_3$	$COCH_2CH(CH_3)_2$
	II	$COCH_3$	$COCH_2CH(CH_3)_2$
	III	$COCH_2CH_3$	$COCH_2CH_3$
	IV	$COCH_3$	$COCH_2CH_3$
	V	$COCH_2CH_3$	$COCH_3$
	VI	$COCH_3$	$COCH_3$
	VII	$COCH_2CH_3$	$COCH_2CH_2CH_3$



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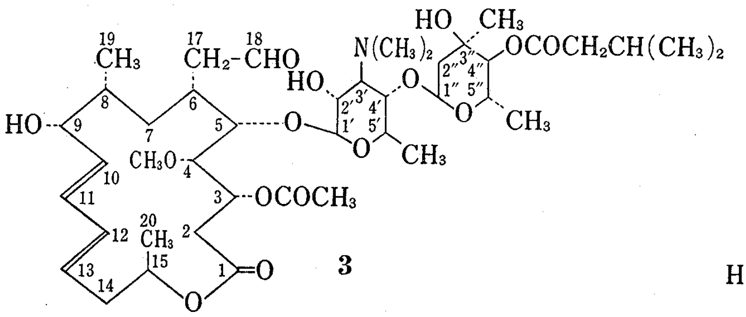

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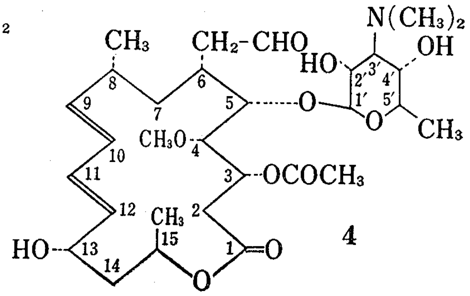

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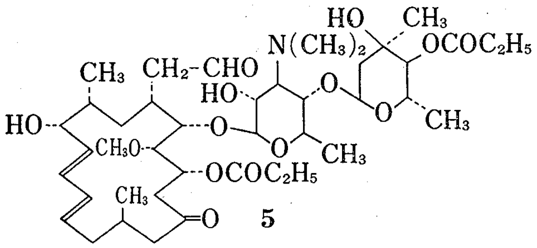
Chart 1



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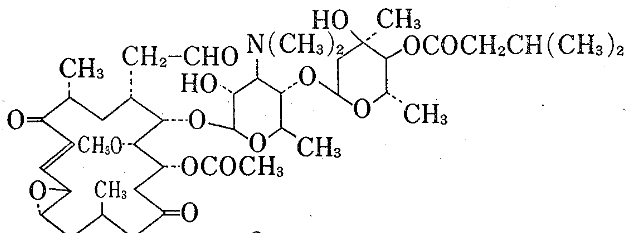

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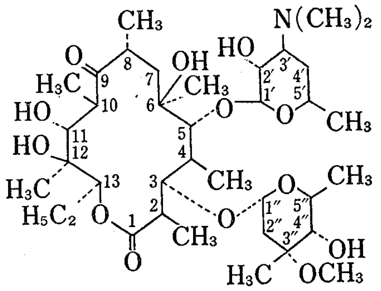

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Chart 2

mens used for the analysis were sealed in capillary tubes containing a small amount of the mother liquor at their one end.

Data for determination of the space group and cell dimensions were obtained using a Hilger and Watt's linear diffractometer (MoKα; λ=0.7107 Å). The crystal data are summarized in Table I. Intensities of 3500 reflexions were measured on the diffractometer about the *b* axis, up to the 12th layer. The orientation of crystals in the capillary tube made it impossible to obtain intensity data about the other axes. After the usual Lorentz and polarization corrections, the data were brought to an absolute scale by the method of Wilson. Altogether, 2921 non-zero F^2 values were derived and were used for the subsequent structure analysis.

Table I. Crystal Data

Crystal system	Monoclinic
Space group	$P2_1$
Cell dimensions	$a = 17.44$ Å
	$b = 20.90$ Å
	$c = 9.86$ Å
	$\beta = 105.5°$
Cell volume	$V = 3463$ Å^3
Number of molecules in the unit cell	$z = 2$
Composition of asymmetric unit	$C_{44}H_{71}I_3NO_3 \cdot CCl_4 \cdot C_2H_4Cl_2$
Formula weight	1519.5
Calculated density	1.46 g cm^{-3}

Determination of the Structure——Atomic co-ordinates of I_3^- ion in the asymmetric unit were determined without anbiguity from a three-dimensional Patterson synthesis. Starting with these co-ordinates, the structure of PMDM III-I_3 was easily determined by our usual procedures. The atomic co-ordinates and isotropic temperature factors were refined by the least-squares method to an R value of 0.18. Positions of solvent molecules were not definitely determined even in the difference electron density map obtained at this refined stage. The atomic parameters of the PMDM III-I_3 are listed in Table II. Perspective view of the molecule, bond distances and angles can be seen in Fig. 1, 2 and 3, respectively.

The absolute configuration of the molecule was determined by the anomalous dispersion method. The dispersion terms of the iodine atom for MoKα radiation were assumed to be $\Delta f' = -0.6$ and $\Delta f'' = 2.2$. Structure factors of Friedel pairs were calculated and intensities of the pairs ($I(hkl)$ and $I(h\bar{k}l)$) were compared visually on the 1st layer precession photograph (c axis). The result is shown in Table III. All the data were consistent with the calculated differences based on the right handed set of atomic co-ordinates of Table II. The absolute configuration of PMDM III was therefore established as shown in Fig. 1.

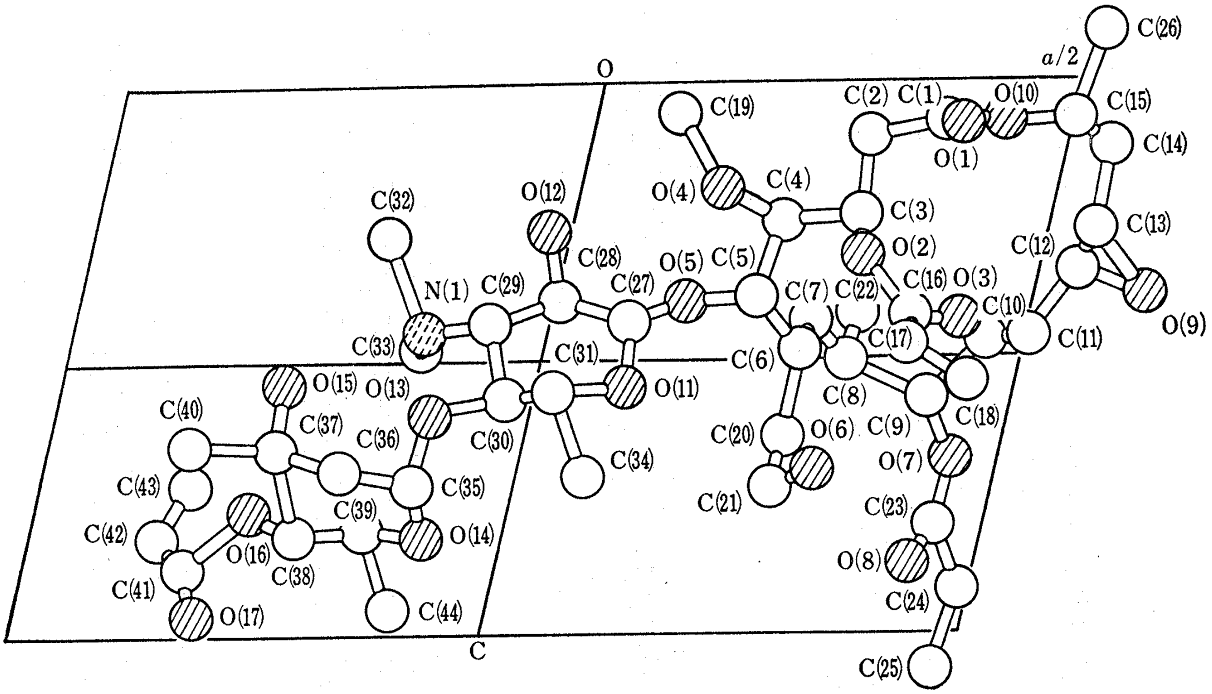

Fig. 1. PMDM III Molecule Viewed along the b Axis

Table II. Atomic Coordinates and Temperature Factors with Their Standard Deviations in Parentheses

Atom	x/a	y/b	z/c	B
I (1)	0.0859 (05)	0.0031 (06)	0.1022 (08)	8.6 (0.2)
(2)	0.0985 (06)	0.0533 (06)	0.8295 (11)	12.7 (0.3)
(3)	0.0659 (04)	0.9603 (05)	0.3685 (08)	8.8 (0.2)
N (1)	0.8672 (32)	0.1594 (33)	0.4470 (60)	3.9 (1.4)
O (1)	1.3853 (39)	0.3063 (37)	0.0774 (70)	8.7 (1.9)
(2)	1.3093 (26)	0.2553 (26)	0.3152 (48)	4.3 (1.1)
(3)	1.4331 (34)	0.2559 (33)	0.4300 (62)	7.0 (1.6)
(4)	1.1495 (23)	0.2282 (24)	0.1850 (43)	3.2 (1.0)
(5)	1.1387 (33)	0.1518 (36)	0.3929 (62)	2.4 (1.3)
(6)	1.3052 (34)	0.2391 (33)	0.6988 (60)	6.8 (1.5)
(7)	1.4530 (29)	−0.0151 (29)	0.6760 (53)	5.9 (1.4)
(8)	1.4308 (36)	0.0349 (34)	0.8689 (65)	7.9 (1.7)
(9)	1.6164 (31)	0.1330 (31)	0.3887 (57)	6.2 (1.4)
(10)	1.4270 (27)	0.2059 (27)	0.0849 (49)	4.5 (1.2)
(11)	1.0945 (20)	0.2327 (21)	0.5531 (37)	1.9 (0.8)
(12)	0.9745 (18)	0.1200 (20)	0.2642 (36)	1.3 (0.8)
(13)	0.8972 (30)	0.2586 (29)	0.5907 (54)	5.6 (1.3)
(14)	0.9109 (23)	0.2346 (24)	0.8262 (42)	3.1 (1.0)
(15)	0.7395 (28)	0.2067 (28)	0.5404 (52)	5.1 (1.2)
(16)	0.7263 (32)	0.1381 (32)	0.7706 (58)	6.6 (1.5)
(17)	0.6965 (37)	0.1658 (36)	0.9612 (67)	8.4 (1.8)
C (1)	1.3745 (54)	0.2485 (52)	0.0793 (98)	7.2 (2.5)
(2)	1.2909 (47)	0.2209 (47)	0.0952 (86)	5.5 (2.0)
(3)	1.3036 (57)	0.1968 (56)	0.2413 (105)	7.8 (2.8)
(4)	1.2180 (28)	0.1814 (31)	0.2491 (53)	0.8 (1.1)
(5)	1.2100 (33)	0.1775 (36)	0.3838 (62)	2.4 (1.3)
(6)	1.2733 (36)	0.1381 (38)	0.4847 (67)	3.3 (1.5)
(7)	1.2796 (47)	0.0771 (45)	0.4276 (86)	5.5 (2.1)
(8)	1.3249 (32)	0.0216 (33)	0.5048 (61)	2.1 (1.3)
(9)	1.4117 (36)	0.0384 (38)	0.5717 (66)	2.8 (1.5)
(10)	1.4615 (34)	0.0499 (38)	0.4738 (64)	2.8 (1.4)
(11)	1.5031 (35)	0.1039 (37)	0.4610 (65)	2.9 (1.4)
(12)	1.5426 (34)	0.1065 (37)	0.3387 (65)	2.8 (1.4)
(13)	1.5547 (56)	0.1714 (56)	0.2770 (102)	8.0 (2.7)
(14)	1.5476 (58)	0.1720 (57)	0.1192 (106)	8.0 (2.7)
(15)	1.5028 (55)	0.2309 (53)	0.0658 (92)	7.0 (2.5)
(16)	1.3820 (44)	0.2940 (44)	0.4287 (81)	4.9 (1.9)
(17)	1.3835 (56)	0.3587 (55)	0.4735 (101)	7.7 (2.6)
(18)	1.4548 (58)	0.3693 (58)	0.5425 (106)	8.3 (2.8)
(19)	1.0874 (48)	0.1932 (49)	0.0675 (89)	6.3 (2.2)
(20)	1.2699 (41)	0.1337 (43)	0.6453 (76)	4.3 (1.7)
(21)	1.2726 (48)	0.2026 (48)	0.7260 (90)	6.1 (2.2)
(22)	1.3232 (43)	−0.0300 (46)	0.4177 (78)	4.9 (2.0)
(23)	1.4503 (49)	−0.0034 (52)	0.8090 (88)	6.8 (2.3)
(24)	1.4962 (73)	−0.0592 (68)	0.9208 (124)	11.7 (3.8)
(25)	1.4798 (78)	−0.0725 (71)	1.0556 (135)	12.1 (4.2)
(26)	1.5057 (62)	0.2234 (60)	−0.0813 (106)	8.7 (3.0)
(27)	1.0812 (40)	0.1960 (41)	0.4360 (74)	4.2 (1.7)
(28)	1.0047 (27)	0.1441 (31)	0.3949 (51)	0.7 (1.1)
(29)	0.9377 (28)	0.1781 (32)	0.4428 (55)	1.1 (1.1)
(30)	0.9683 (36)	0.2093 (38)	0.5655 (67)	3.0 (1.5)
(31)	1.0244 (41)	0.2628 (43)	0.5552 (75)	4.5 (1.8)
(32)	0.8103 (38)	0.1485 (39)	0.2791 (71)	3.7 (1.6)
(33)	0.8720 (29)	0.0848 (31)	0.4806 (55)	1.2 (1.2)
(34)	1.0766 (30)	0.2843 (33)	0.7129 (57)	1.8 (1.2)
(35)	0.8933 (37)	0.2841 (38)	0.7269 (69)	3.1 (1.5)
(36)	0.8063 (27)	0.3041 (30)	0.6979 (52)	0.9 (1.1)
(37)	0.7421 (78)	0.2435 (72)	0.6639 (141)	13.6 (4.1)
(38)	0.7836 (48)	0.1980 (48)	0.8101 (90)	6.4 (2.2)
(39)	0.8551 (35)	0.1653 (38)	0.8065 (67)	2.9 (1.5)
(40)	0.6515 (45)	0.2743 (45)	0.6491 (83)	5.1 (2.0)
(41)	0.6758 (60)	0.1124 (60)	0.8787 (110)	9.2 (3.0)
(42)	0.6397 (54)	0.0581 (54)	0.8209 (96)	6.9 (2.5)
(43)	0.6621 (50)	0.0217 (48)	0.7231 (92)	6.1 (2.3)
(44)	0.8926 (46)	0.1358 (47)	0.9479 (85)	5.8 (2.1)

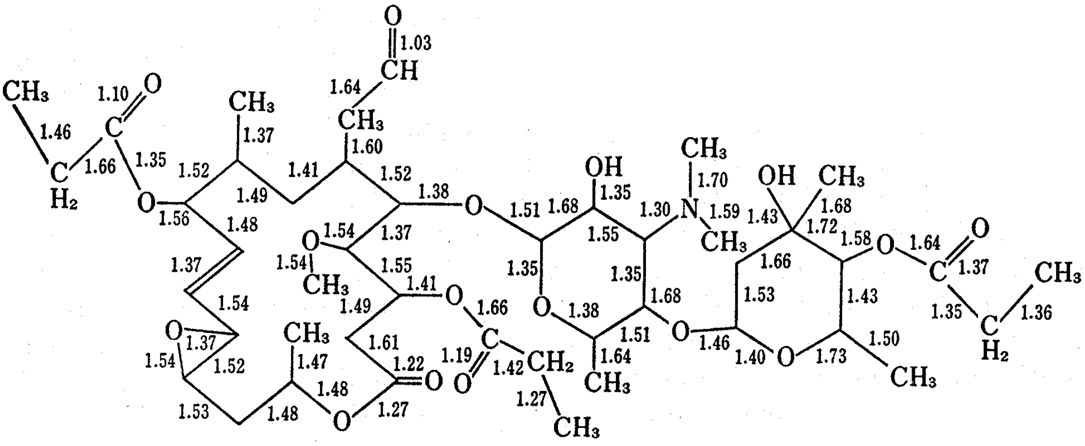

Fig. 2. Bond Lengths

Standard deviations range from 0.06 to 0.16 Å.

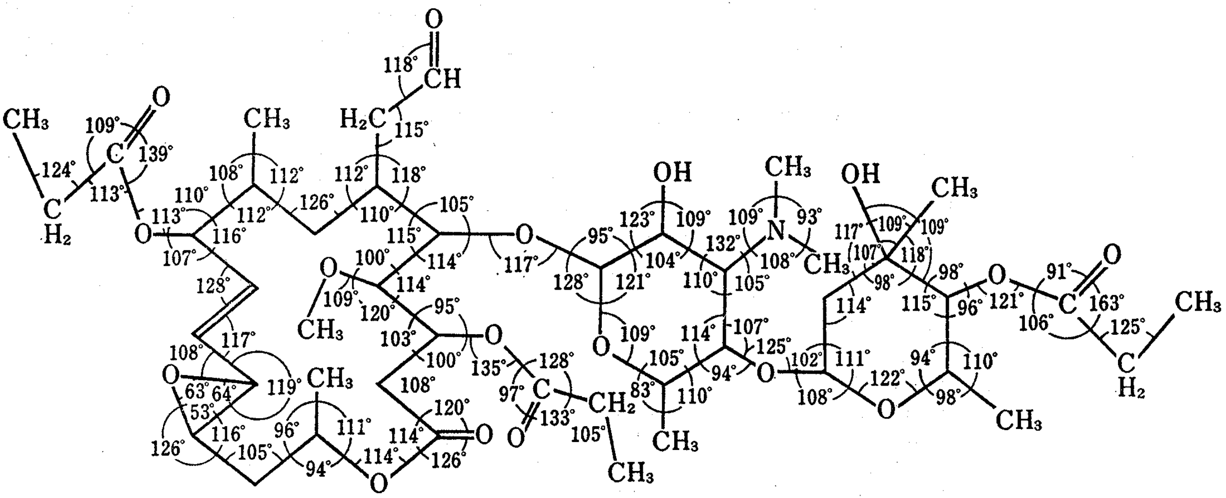

Fig. 3. Bond Angles

Standard deviations range from 2 to 9°.

Table III. Comparison of the Observed and Calculated Intensity Differences Used for the Establishment of the Absolute Configuration

h	k	l	$Fc^2(hkl)/Fc^2(h\bar{k}l)$	$I_0(hkl)/I_0(h\bar{k}l)$
−8	1	1	1.22	>1
−1	3	1	1.43	>1
−4	3	1	0.82	<1
11	3	1	1.32	>1
5	4	1	0.80	<1
9	4	1	0.80	<1
12	4	1	0.66	<1
−12	6	1	1.21	>1
−5	7	1	1.33	>1
6	7	1	0.68	<1

Discussion

Absolute Configuration

The structure of PMDM III was determined as **2** by the present analysis. From the previous works of Muroi *et al.*[4] together with the present work, the structures of MDM were confirmed to be **1**.

MDM is closely related to leucomycins,[5] and carbomycins.[6] These basic macrolide antibiotics are composed of a 16-membered macrocyclic lactone, a dimethylamino sugar and a neutral sugar, and they are convertible to each other by simple chemical reactions. Thus, MDM II was oxidized to carbomycin A (**6**) with CrO_3 in pyridine. Oxidation of leucomycin A_3 (**3**) with MnO_2 gave carbomycin B.[6] Epoxidation of 18-dihydroleucomycin A_3 and midecamycin[7] (**5**) gave 18-dihydromaridomycin II and MDM III, respectively. The stereochemical identity of MDM has been ascertained by interconversion reactions which are not accompanied with any configurational inversion. There will remain little doubt, therefore, that the stereochemical interrelationship, with respect to corresponding asymmetric carbons, of leucomycins, carbomycins and MDM is identical. Among these antibiotics, the absolute configuration of carbomycin A (**6**) was studied by Celmer[6] and 3-(*R*), 4-(*S*), 5-(*S*), 6-(*R*), 8-(*R*), 12-(*R*),[8] 13-(*S*) and 15-(*R*) configurations were assumed to this antibiotic. The result of the X-ray analysis of demycarosyl isoleucomycin A_3 (**4**) hydrobromide[10] ascertained the Celmer's assumption about the stereochemistry at 3-, 4-, 5-, 6-, 8- and 15-positions and the absolute configurations of these asymmetric carbons were definitely determined. In regard to the absolute configuration at 9-position of leucomycins, the above X-ray analysis did not provide any information. Later, it has been established as 9-(*R*) by the X-ray analysis of diacetyl-3,

Table IV. The Conformation of the 16-Membered Ring

Bond	Angle[a]		Δ Angle[b]
	PMDM III (°)	**4** (°)	(°)
C (1)-C (2)	68	65	3
C (2)-C (3)	159	177	18
C (3)-C (4)	−160	−170	10
C (4)-C (5)	−50	−55	5
C (5)-C (6)	−53	−61	8
C (6)-C (7)	−169	156	35
C (7)-C (8)	−56	−61	5
C (8)-C (9)	−67	−90	23
C (9)-C (10)	122	174	52
C (10)-C (11)	−173	180	7
C (11)-C (12)	151	180	29
C (12)-C (13)	−146	−74	72
C (13)-C (14)	138	78	60
C (14)-C (15)	−70	−84	14
C (15)-O (10)	165	170	5
O (10)-C (1)	178	−173	9

a) This is the azimuthal angle which, in the part -X-A-B-Y- of the ring, X-A and B-Y make around A-B. The positive sign indicates that the angle is measured in the right way, while the negative sign denotes the measurement in the other way.

b) Differences between PMDM III and demycarosyl isoleucomycin A_3 (**4**).

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6) R.B. Woodward, *Angew. Chem.*, **69**, 50 (1957); W.D. Celmer, *J. Am. Chem. Soc.*, **88**, 5028 (1966); R.B. Woodward, L.S. Weiler, and P.C. Dutta, *ibid.*, **87**, 4662 (1965).

7) S. Inoue, T. Tsuruoka, T. Shomura, S. Omoto, and T. Niida, *J. Antibiot.* (Tokyo), *Ser. A*, **24**, 460 (1971).

8) Although the configuration at C_{12} of Carbomycin A has been revised as (*S*) by Omura *et al.*,[9] no definite proofs have been presented.

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10) M. Hiramatsu, A. Furusaki, T. Noda, K. Naya, Y. Tomiie, I. Nitta, T. Watanabe, T. Take, J. Abe, S. Omura, and T. Hata, *Bull. Chem. Soc. Jpn.*, **43**, 1966 (1970).

6-bicyclo-leuconolide A_3,[11] a derivative of aglycone of leucomycin A_3. Our present study clearly revealed 12-(*S*), 13-(*S*) configurations and α linkage of mycarose in MDM and the absolute configurations of these 16-membered ring macrolides have been established without any ambiguity.

This is the first case that the structure of the intact 16-membered macrolide has been determined by the X-ray analysis.

Conformation

Comparison is made between the conformational structures in crystals of two 16-membered macrolides, **2** and **4** determined by X-ray analysis. Table IV shows torsion angles of the 16 bonds constituting the macrocyclic lactone ring. Aside from dissimilarities arising from the structural difference between $-\underset{\diagdown O \diagup}{C_{12}-C_{13}}-$ in **2** and $-C_9=C_{10}-C_{11}=C_{12}-$ in **4**, the torsion angles are similar between the two antibiotics, which show that overall conformation of their 16-membered rings resembles with each other. Similarity is also seen at spatial arrangement between the macrolide ring and mycaminose, the common amino sugar in both antibiotics. The interplanar angles between the least-squares planes of the 16-membered rings and those of the 6-membered rings of mycaminose are almost the same (85° in **2** and 86° in **4**).

According to the X-ray analysis and NMR study of erythromycin A (**7**),[12] its macrocyclic ring has been reported to possess a hydrophilic face and a hydrophobic face.[13] The former face is comprised of the lactone carbonyl and hydroxyl groups at C_6 and C_{11} positions, while the hydrophobic face is made of methyls on C_4, C_8 and C_{12} as well as hydrogens on C_2, C_7, C_{10}, and the ethyl group at C_{13}. Hydrophilic substituents of desosamine, 3′-dimethylamino

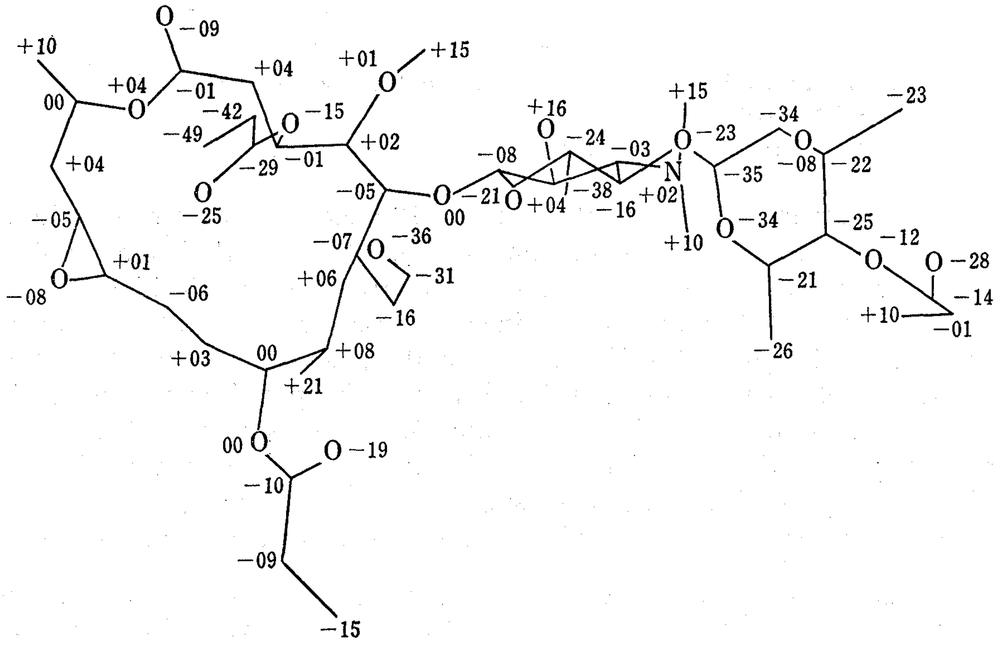

Fig. 4. Superposition of PMDM III Molecule along the Normal of the Mean Plane of the Macro Ring

Displacements of atoms from the plane are shown in 10 × Å units.

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and 2′-hydroxyl groups which are known to be indispensable for biological activity, situate rather in the hydrophobic region.[14]

The similar feature is also found in **2** and **4** . Projections of **2** and **4** molecules on each least-squares plane of the macro ring can be seen in Fig. 4 and 5, respectively. Interposing the least-squares plane of the macro ring of **2**, hydrophilic groups at C_1 (C=O), C_3 ($\mathrm{C_3{-}O{-}\underset{\underset{\displaystyle O}{\|}}{C}{-}R}$), C_6 ($\mathrm{C_6{-}CH_2{-}CHO}$) and $\mathrm{C_{12}\underset{\diagdown O \diagup}{-}C_{13}}$ lie on one side of the plane and hydrophobic methyl groups at C_4, C_8 and C_{15} lie on the other side of the plane. This orientation of the substituents gives the macro ring of **2** two physicochemically different faces. The substituent groups of mycaminose, especially the hydrophilic groups of $C_{2'}$ hydroxyl and its adjacent dimethylamino are oriented to the hydrophobic face of the macro ring. The similar spatial arrangement in **4** is also clear from Fig. 5.

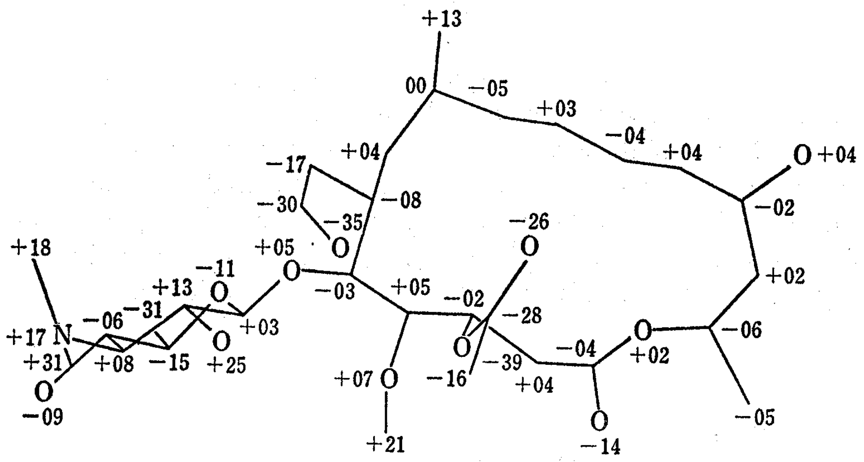

Fig. 5. Projection of Molecule **4** on the Least-squares Plane of the 16-Membered Ring

Calculated from data of reference 10.

A study on structure-activity relationship of 16-membered ring macrolide antibiotics showed that the removal or the chemical modification of 3′-dimethylamino, 2′-hydroxyl or 6-formylmethyl group caused complete loss or substantial decrease in biological activity.[14] Inter-atomic distances of hetero atoms contained in these three functional groups are nearly the same in **2** and **4**; $\mathrm{N(C_{3'})}$–$\mathrm{O(C_{2'})}$, $\mathrm{N(C_{3'})}$–$\mathrm{O(C_6)}$ and $\mathrm{O(C_{2'})}$–$\mathrm{O(C_6)}$ being 3.0, 7.6 and 6.7 Å in **2** and 3.0, 7.8 and 6.8 Å in **4**, respectively. Similarity in spatial arrangement of the three groups in **2** and **4** is also seen in Fig. 4 and 5. These structural features, especially of the three groups and the foregoing two different faces of the macro ring, commonly found by X-ray analyses of **2** and **4** may suggest that these arrangements are one of the important factors for the biological activity of the 16-membered ring macrolide antibiotics.

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