MARIDOMYCIN, A NEW MACROLIDE ANTIBIOTIC. VIII

ISOLATION AND STRUCTURES OF METABOLITES OF 9-PROPIONYLMARIDOMYCIN

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9-Propionylmaridomycin (PMDM) and maridomycin (MDM) were transformed into 4''-deacyl-9-propionylmaridomycin (PMDM-M) and 4''-deacylmaridomycin (MDM-M), respectively, by incubation with rat liver homogenates in pH 7.2 buffer solution. The structures of these products were elucidated from their physicochemical properties and the result of chemical conversions into the known derivatives of MDM. Furthermore, PMDM-M was isolated as a main metabolite from the urine of rats treated with PMDM by oral route. On the other hand, from human urine after oral administration of PMDM, three metabolites were recovered and identified as PMDM-M, MDM and MDM-M by direct comparison.

9-Propionylmaridomycin[1], which was synthesized by selective propionylation of maridomycin[2], is a novel therapeutically effective macrolide antibiotic with good oral absorption, better tissue distribution than blood level, increased stability, and enhancement of taste properties. As for the metabolism of basic macrolide antibiotics in animals, demethylation of dimethylamino group, deacylation of ester and hydroxylation of macrolactone moiety are known[3~7]. The present report deals with the isolation and structure elucidation of the transformation products obtained by incubation of PMDM or MDM with rat liver homogenate, and of the metabolite obtained by oral administration of PMDM in rat or human being.

Preparation of PMDM-M and MDM-M by Incubation with Rat Liver Homogenates

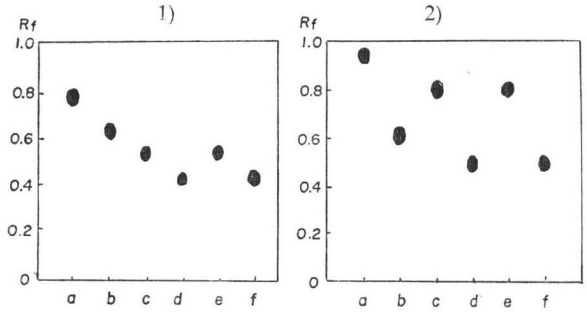
When PMDM III and MDM III were incubated with a rat liver homogenate at pH 7.2 and 37°C, new bioactive substances with lower Rf values on silica-gel thin-layer chromatograms than those of starting materials were observed, respectively (Fig. 1). These substances had the same Rf values on thin-layer chromatograms with the metabolites in rat plasma after oral administration of PMDM III and MDM III and designated as PMDM III-M and MDM III-M, respectively. Isolation of these substances was carried out by the following procedure. The reaction mixture after incubation with rat liver homogenates was precipitated with addition of acetone. After removal of the precipitate, the filtrate was extracted with AcOEt at pH 8.5 and transferred into aqueous solution at pH 3.0. The aqueous layer was reextracted with AcOEt at pH 8 and the AcOEt extract was concentrated to give crude PMDM III-M and MDM III-M. Purification by silica gel column chromatography afforded pure PMDM III-M and MDM III-M.

By similar procedures as described above, PMDM VI and MDM VI were transformed into PMDM VI-M and MDM VI-M and each metabolite was isolated by silica gel chromatography.

Physicochemical Properties and Structures of Metabolites

The physicochemical properties of PMDM III-M, PMDM VI-M, MDM III-M and MDM

Fig. 1. Thin-layer chromatograms of metabolites of PMDM and MDM
1) Support: Silica gel (Spotfilm, Tokyo Kasei Co.)
Solvent: C_6H_6-Me_2CO (1:1)
Detection: Conc. H_2SO_4
2) Support: Silica gel (Spotfilm, Tokyo Kasei Co.)
Solvent: $CHCl_3$-MeOH-NH_4OH (40:3:20, lower layer)
Detection: Conc. H_2SO_4



(a) PMDM III; (b) MDM III; (c) PMDM III-M; (d) MDM III-M; (e) metabolite in rat plasma after oral administration of PMDM III; (f) metabolite in rat plasma after oral administration of MDM III

Table 1. Physicochemical properties of the metabolites

	PMDM III-M	PMDM VI-M	MDM III-M	MDM VI-M
Mp (°C)	142~143	147~148	157~158	145~146
$[\alpha]_D$ (EtOH)	−64.4°	−68.3°	−75.9°	−78.4°
Molecular weight Mass $M^+(m/e)$	829	815	773	759
Analysis Found: C	59.02	58.17	57.92	57.36
H	8.22	8.05	8.30	8.09
N	1.57	1.88	1.78	1.77
Calcd. for	$C_{41}H_{67}NO_{16}$	$C_{40}H_{65}NO_{16}$	$C_{35}H_{63}NO_{15}\cdot H_2O$	$C_{37}H_{61}NO_{15}\cdot H_2O$
C	59.33	58.88	57.63	57.13
H	8.14	8.03	8.28	8.16
N	1.69	1.72	1.77	1.80
IR (KBr) ν C-O-Ac (cm^{-1})	—	1240	—	1245

VI-M are presented in Table 1, and their ir, nmr and mass spectral data are shown in Fig. 2, Table 2 and Table 3, respectively.

Structures of PMDM III-M and PMDM VI-M

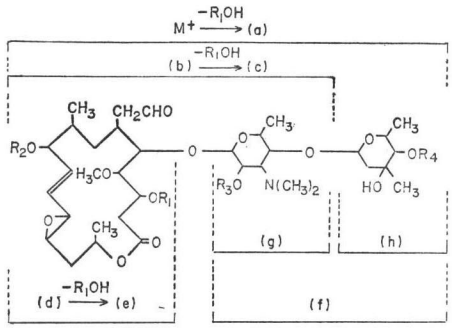
PMDM III-M shows no characteristic absorption maxima in the uv spectrum. The ir spectrum of PMDM III-M has intense bands at 1720~1740 cm^{-1} (C=O), 1050~1200 cm^{-1} (C-O-C) and a band at 2725 cm^{-1} (-CHO), similar to that of PMDM III. The nmr spectrum shows the presence of $-N(CH_3)_2$ at δ 2.51 (6H, s), $-OCH_3$ at δ 3.54 (3H, s), $C_{1'}$ proton at δ 4.42 (1H, d, J=7Hz), olefine protons at δ 5.78 (1H, dd) and 6.06 (1H, dd), and aldehyde proton at δ 9.64 (1H, s) (Table 2, Fig. 3). Chemical shifts and coupling constants of these signals are almost

Table 2. Chemical shifts in NMR spectra of the metabolites and related compounds
(100 MHz, in $CDCl_3$)

	PMDM III	PMDM III-M	PMDM VI	PMDM VI-M	MDM III	MDM III-M	MDM VI	MDM VI-M
$C_{4''}$-OAc (s)	—	—	2.16	—	—	—	2.17	—
C_3-OAc (s)	—	—	2.26	2.25	—	—	2.26	2.22
$-N(CH_3)_2$ (s)	2.53	2.51	2.53	2.50	2.52	2.50	2.55	2.48
$-OCH_3$ (s)	3.54	3.54	3.54	3.53	3.53	3.54	3.56	3.52
$C_{5''}$-H (m)	4.48	4.07	4.47	4.07	4.47	4.04	4.46	4.04
$C_{1'}$-H (d)	4.42	4.42	4.42	4.41	4.43	4.43	4.46	4.43
$C_{4''}$-H (d)	4.62	2.95	4.62	2.95	4.62	2.92	4.63	2.90
$C_{1''}$-H (q)	5.07	5.06	5.07	5.06	5.05	5.06	5.08	5.06
C_9-H (q)	5.07	5.08	5.06	5.05	4.02	4.04	4.03	4.02
C_{11}-H (dd)	5.76	5.78	5.74	5.73	5.67	5.69	5.67	5.64
C_{10}-H (dd)	6.04	6.06	6.04	6.04	6.08	6.09	6.10	6.07
-CHO (s)	9.66	4.64	9.65	9.66	9.62	9.63	9.64	9.63

Abbreviation: s, singlet; d, doublet; q, quartet; m, multiplet; dd, doublet of doublets.

Table 3. Partial mass fragmentations of the metabolites and related compounds

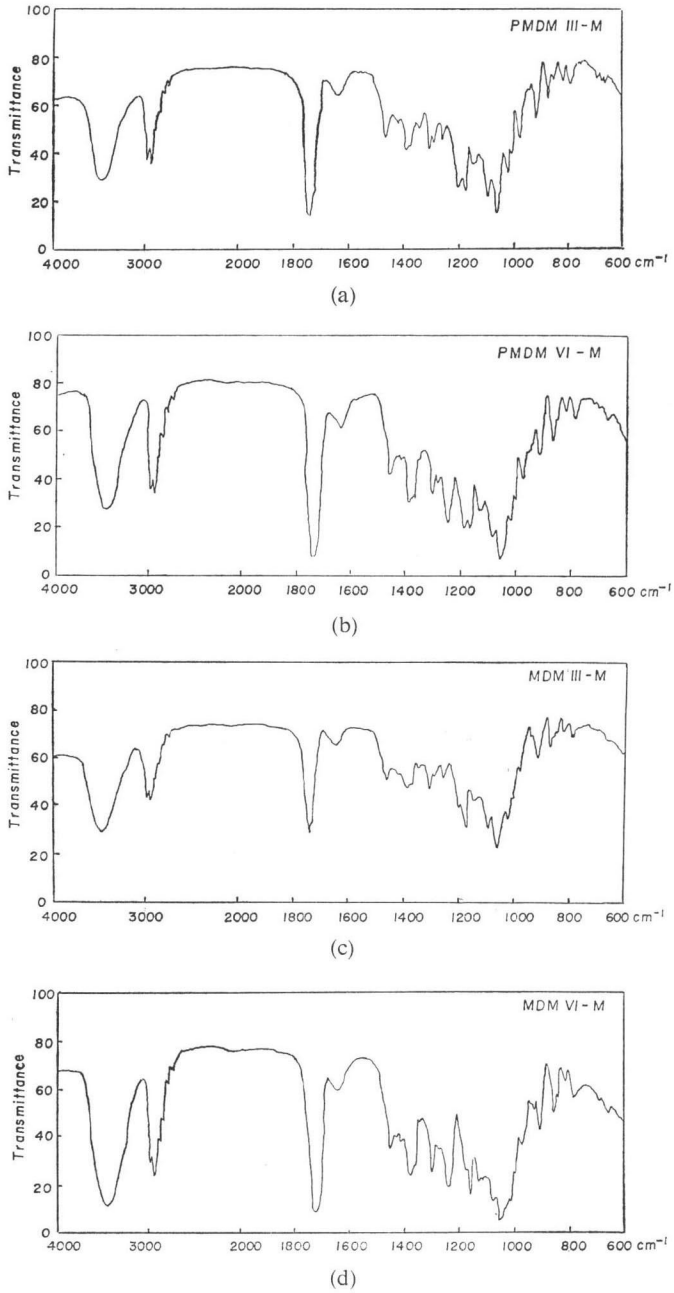


Compound	Origin of peaks								
	M^+	(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)
PMDM III	885	811	684	610	495	421	374	173	201
PMDM III-M	829	755	684	610	495	421	318	173	145
PMDM VI	857	797	670	610	481	421	360	173	187
PMDM VI-M	815	755	670	610	481	421	318	173	145
MDM III	829	755	628	554	439	365	374	173	201
MDM III-M	773	699	628	554	439	365	318	173	145
MDM VI	801	741	614	554	425	365	360	173	187
MDM VI-M	759	699	614	554	425	365	318	173	145
PMDM V-a	913	839	726	652	(495)	421	402	215	187

The substituents, R_1, R_2, R_3 and R_4, are shown in Fig. 4.

identical with those of PMDM III. The nmr spectra of both compounds differ mainly in two regions in which the signals due to $C_{4''}$ proton and $C_{5''}$ proton observed at δ 4.62 and 4.48 in PMDM III shift to the higher field at δ 2.95 and 4.07 in PMDM III-M. respectively. In addi-

Fig. 2. IR spectra of the metabolites (KBr)



tion, protons ascribed to one of three propionyl groups (CH_3–CH_2–C=O) in PMDM III disappeared in PMDM III-M. These data suggest that PMDM III-M is 4″-depropionyl derivative of PMDM III. Furthermore, the mass-spectrum of PMDM III-M also supports this structure as shown in Table 3. PMDM III-M showed molecular ion peak at *m/e* 829 and fragment ion peak due to M^+–74 (elimination of propionic acid) at *m/e* 755. Fragment ion peaks observed at *m/e* 684 and 610 are assigned to ions due to mycaminosyl macrolactone moiety and resulting from eli-

Fig. 3. NMR spectrum of PMDM III-M (in $CDCl_3$)

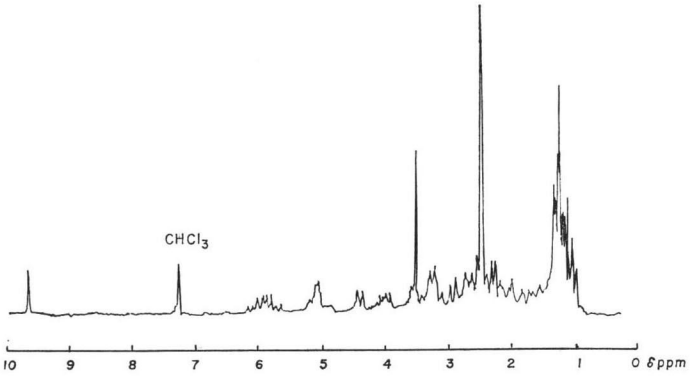
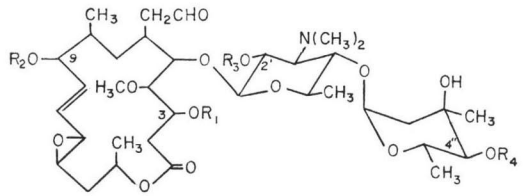


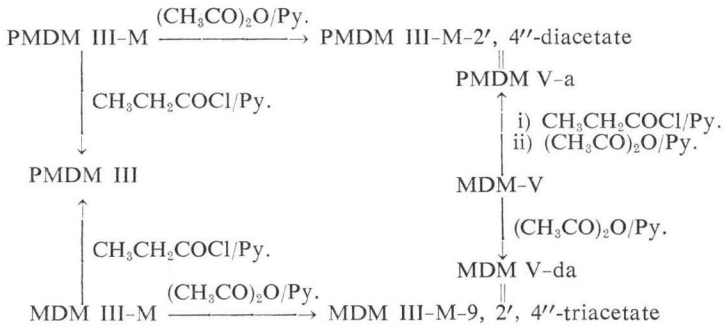
Fig. 4. Structures of PMDM metabolites and related compounds



Compound	Substituent			
	R_1	R_2	R_3	R_4
PMDM III	$-COCH_2CH_3$	$-COCH_2CH_3$	-H	$-COCH_2CH_3$
PMDM VI	$-COCH_3$	$-COCH_2CH_3$	-H	$-COCH_3$
PMDM III-M	$-COCH_2CH_3$	$-COCH_2CH_3$	-H	-H
PMDM VI-M	$-COCH_3$	$-COCH_2CH_3$	-H	-H
MDM III	$-COCH_2CH_3$	-H	-H	$-COCH_2CH_3$
MDM VI	$-COCH_3$	-H	-H	$-COCH_3$
MDM III-M	$-COCH_2CH_3$	-H	-H	-H
MDM VI-M	$-COCH_3$	-H	-H	-H
MDM V	$-COCH_2CH_3$	-H	-H	$-COCH_3$
MDM V-da	$-COCH_2CH_3$	$-COCH_3$	$-COCH_3$	$-COCH_3$
PMDM V-a	$-COCH_2CH_3$	$-COCH_2CH_3$	$-COCH_3$	$-COCH_3$

mination of propionic acid at the C_3 position, respectively. And 9-propionylmacrolactone (aglycone moiety) ion peak appears at *m/e* 495 and peaks due to sugar moiety are observed as follows: mycarosyl-mycaminose at *m/e* 318; deoxymycaminose at *m/e* 173 and 174; mycarose at *m/e* 145. Thus, the mass-spectral evidence in addition to nmr, ir, uv and $[\alpha]_D$ confirms the structure of PMDM III-M as 4''-depropionyl PMDM III (Fig. 4). The structure of PMDM III-M was finally established by chemical conversions as shown in Chart 1. Propionylation of PMDM III-M with propionylchloride in the presence of pyridine at low temperature afforded a monopropionyl PMDM III-M which was identical with PMDM III[1] in the Rf values on tlc, ir, nmr and mass-spectra. And acetylation of PMDM III-M with acetic anhydride in pyridine converted it to a diacetate which was identical with PMDM V-2'-acetate (PMDM V-a) in the Rf

Chart 1



values on tlc, ir, nmr and mass spectra. The structure of PMDM VI-M was determined in the same manner as described above. IR spectrum of PMDM VI-M is very similar to PMDM III-M except for the absorption band at 1240 cm^{-1} (ν_{C-O-Ac}). Although one of the two acetyl signals of PMDM VI has disappeared in PMDM VI-M, one acetyl signal is observed at δ 2.21 (3H, s) which is due to acetyl group at C_3 position. Mass-spectral fragmentation pattern of PMDM VI-M is quite similar to that of PMDM III-M (Table 3). In the mass-spectrum of PMDM VI-M, molecular peak at *m/e* 815, fragment peaks at *m/e* 670 (mycaminosyl macrolactone) and at *m/e* 481 (macrolactone) are by one methylene (14 mass units) smaller than the corresponding peaks of PMDM III-M, the same fragment peaks with PMDM III-M are observed at *m/e* 755 (M^+-AcOH), *m/e* 610 (670-AcOH) and *m/e* 421 (481-AcOH) all of which result from elimination of acetic acid between C_2 and C_3 of the macrolactone. In addition, no fragment peaks ascribed to acetylmycarose are detected. These data clearly indicate that deacetylation occurred at $C_{4''}$ position of mycarose, not at C_3 position of macrolactone.

Structures of MDM III-M and MDM VI-M

The structure of MDM III-M was elucidated as follows: MDM III-M exhibits no absorption maxima in the uv spectrum (210~320 nm). The ir spectrum shows strong bands at 1720 ~1740 cm^{-1} (C=O), 1050~1200 cm^{-1} (C-O-C) and a band at 2725 cm^{-1} (-CHO). MDM III-M reveals the signals of $-N(CH_3)_2$ at δ 2.50 (6H, s), $-OCH_3$ at 3.54 (3H, s), $C_{1'}$ proton at 4.43 (1H, d), olefine protons at 5.69 (1H, dd) and 6.09 (1H, dd), and aldehyde proton at 9.63 (1H, s) in the nmr spectrum which is almost identical with that of MDM III. Both compounds differ in that the signals of $C_{4''}$ proton (δ 2.92) and $C_{5''}$ proton (4.04) of MDM III-M appear at higher field than those of MDM III. Furthermore, protons due to one propionyl ($\underline{CH_3}$-$\underline{CH_2}$-CO) group have disappeared in MDM III-M. These data along with uv and ir spectral data suggest 4″-depropionyl derivative of MDM III for MDM III-M. The mass-spectrum of MDM III-M quite agrees with the proposed structure. The molecular peak appears at *m/e* 773 and a fairly intense peak at *m/e* 699 is assigned to fragment resulting from the pyrolytic β-elimination of propionic acid at the C_2-C_3 position of macrolactone. Other fragment peaks reasonable for the structure are observed at *m/e* 439 (macrolactone), *m/e* 318 (mycarosyl mycaminose), *m/e* 173 and 174 (mycaminose) and at *m/e* 145 (mycarose). No propionylmycarosyl mycaminose peaks appearing in MDM III are observed in MDM III-M.

The structure of MDM III-M was finally established in the following manner (Chart 1).

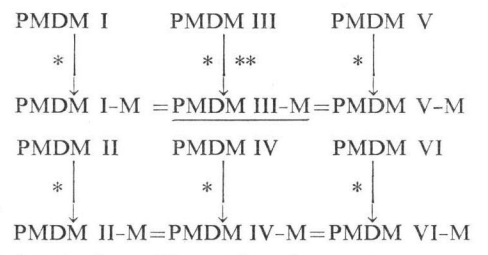
MDM III-M was converted into its dipropionate which was identical with PMDM III in the Rf values on tlc, ir, nmr, and mass spectra, by propionylation with propionyl chloride in the presence of pyridine at low temperature. Furthermore, acetylation of MDM III-M with acetic anhydride in pyridine afforded its triacetate that was identical with MDM V-diacetate (MDM V-da) prepared from MDM V in all respects.

The structure of MDM VI-M was analogously established from comparison of ir, uv, nmr and mass spectra of MDM VI-M with those of MDM VI, PMDM VI-M and MDM III-M.

Isolation of PMDM III-M from Rat Urine

PMDM III-M was recovered from the urine of rats treated with PMDM III by oral route. Rat urine was extracted with AcOEt at pH 8 and the extract was transferred into buffer solution at pH 5.3. The aqueous layer was reextracted with AcOEt at pH 9, and the AcOEt layer was washed with buffer solution at pH 5.8. Concentration of the organic layer gave a mixture of metabolites which was purified by silica gel preparative thin-layer chromatography to yield a pure substance, identified as PMDM III-M in the Rf values on tlc, nmr and mass spectra. The metabolite was acetylated to its diacetate which was identical with PMDM V-2′-acetate in the nmr and mass-spectra.

Chart 2



* incubation with rat liver homogenates
** metabolite in rat plasma after oral administration

Isolation of Three Metabolites from Human Urine

Urine of adults who had received a single oral dose of 1 g/man of PMDM (containing predominantly PMDM III) was extracted with AcOEt at pH 9 (Fig. 5) and the AcOEt solution was extracted with phosphate buffer at pH 5.3. Metabolites were separated into two fractions, AcOEt layer (Fraction 1) and aqueous layer (Fraction 2) (Fig. 6). From Fraction 1, PMDM, PMDM-M and MDM were isolated by repeating preparative tlc with two solvent systems, C_6H_6-Me_2CO (1:1) and $CHCl_3$-MeOH-NH_4OH (40:3:20, lower layer). On the other hand, purification of Fraction 2 by similar preparative tlc as used for Fraction 1, afforded MDM-M. The metabolites thus obtained were identical with the authentic samples in the Rf values on tlc, ir, nmr and mass spectra.

It was found that the incubation of each component of PMDM or MDM with a rat liver homogenate afforded PMDM (I~VI)-M or MDM (I~VI)-M in good yield through selective hydrolysis of acyl group at $C_{4''}$ position of mycarose moiety.

PMDM I, III and V were transformed into the same metabolite which has a hydroxy group at $C_{4''}$ position and a propionyloxy group at C_3 position. And the transformation product of PMDM II, IV or VI having acetoxy group at C_3 position was found to be identical with each other. The interrelationship is summarized in Chart 2 and the same transformations were also observed in the case of MDM component.

These facts indicate that all the components of PMDM and MDM were selectively hydrolyzed at $C_{4''}$ position by rat liver esterase, but not at C_3 and/or C_9 position.

The other enzymatic reaction was not observed from the spectral data of the transforma-

Fig. 5. Bioautogram of PMDM metabolites extracted from human urine
Support: Silica gel (Spotfilm, Tokyo Kasei Co.)
Solvent: $CHCl_3$-MeOH-NH_4OH (40:3:20, lower layer)
Detection: Bioautography with *Sarcina lutea*

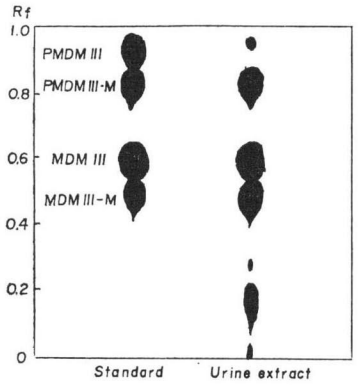
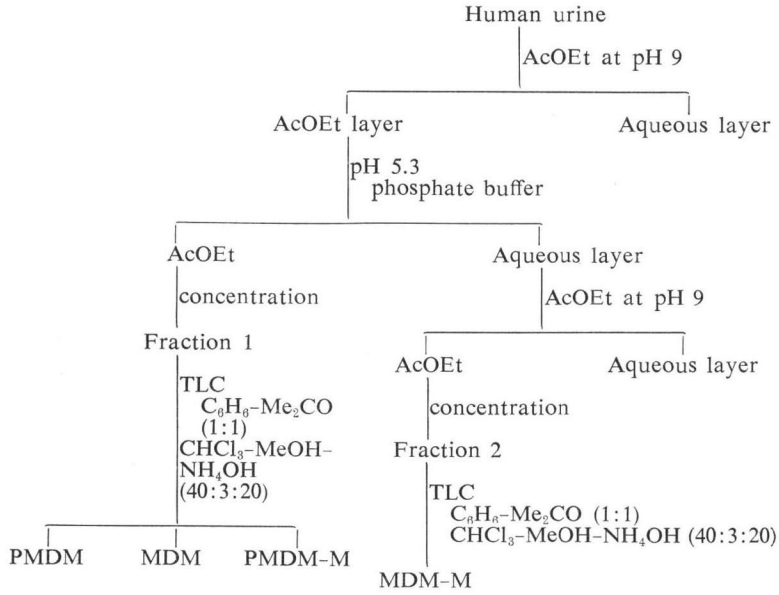


Fig. 6. Isolation of PMDM metabolites from human urine



tion product. PMDM III-M was also isolated as a main metabolite from the urine of rats after oral administration of PMDM III. On the other hand, three metabolites were obtained from human urine after ingestion of PMDM and identified as PMDM-M, MDM and MDM-M. They were produced by hydrolysis of acyl group at C_9 and/or $C_{4''}$ position of PMDM in man.

As for the metabolism of PMDM in human beings and rats, other reports have been presented elsewhere[8,9,10,11].

Experimental

Preparation of PMDM III-M, PMDM VI-M, MDM III-M and MDM VI-M by Incubation with a Rat Liver Homogenate

PMDM III-M

Female Sprague-Dawley rats weighing 150~200 g were sacrificed by cervical dislocation.

Livers were excised and homogenized with a Teflon homogenizer in ten volumes of the 1/15 M KH_2PO_4–Na_2HPO_4 buffer solution at pH 7.2. To 450 ml of the liver homogenate thus prepared was added PMDM III (900 mg) in glycofurol (30 ml) and incubated for 4 hours at 37°C. At the end of the incubation, 1 liter of Me_2CO was added to the reaction mixture and after centrifugation the supernatant was concentrated *in vacuo*. The resulting aqueous solution was adjusted to pH 8~9 and extracted twice with AcOEt (500 ml). The extract was transferred into aqueous layer at pH 3 and the aqueous solution was reextracted twice with 100 ml of AcOEt at pH 9. The extract was concentrated and purified by column chromatography on silica gel (Merck, 350 g) using C_6H_6–Me_2CO (2:1) as the solvent system. The fractions containing PMDM III-M were collected and concentrated to give pure PMDM III-M (700 mg) which was recrystallized from AcOEt - *n*-hexane, mp 142~143°C. $[\alpha]_D^{22}$ −63.9° (*c* 0.5, EtOH); IR ν_{max}^{KBr} cm^{-1} 3470 (OH), 1735 (C=O).

Anal. Calcd. for $C_{41}H_{67}NO_{16}$: C, 59.33; H, 8.14; N, 1.69.
Found: C, 59.02; H, 8.22; N, 1.57.

PMDM VI-M

In the same manner as described in PMDM III-M, incubation of PMDM VI (300 mg) afforded PMDM VI-M (230 mg) as a white powder. $[\alpha]_D^{22}$ −68.3° (*c* 0.5, EtOH). IR ν_{max}^{KBr} cm^{-1}: 3470 (OH), 1740 (C=O).

Anal. Calcd. for $C_{40}H_{65}NO_{16}$: C, 58.88; H, 8.03; N, 1.72.
Found: C, 58.17; H, 8.05; N, 1.88.

MDM III-M

Incubation of MDM III (950 mg) in glycofurol (15 ml) with a rat liver homogenate (350 ml) in the same manner as described in the case of PMDM III-M, afforded MDM III-M (700 mg) as a white powder which was crystallized from AcOEt - *n*-hexane, mp 157~158°C, $[\alpha]_D^{21}$ −75.9° (*c* 0.98, EtOH).

Anal. Calcd. for $C_{38}H_{63}NO_{15}\cdot H_2O$: C, 57.63; H, 8.28; N, 1.77.
Found: C, 57.92: H, 8.30; N, 1.78.

MDM VI-M

Incubation of MDM VI (1,000 mg) in glycofurol (15 ml) with a rat liver homogenate (350 ml) gave MDM VI-M (700 mg) as a white powder. $[\alpha]_D^{22}$ −78.4° (*c* 1.05, EtOH).

Anal. Calcd. for $C_{37}H_{61}NO_{15}\cdot H_2O$: C, 57.13; H, 8.16; N, 1.80.
Found: C, 57.36; H, 8.09, N, 1.77.

Isolation of PMDM Metabolite from Rat Urine

To 65 Sprague-Dawley male rats weighing 250~300 g was orally administered PMDM III suspended in 5% aqueous arabic gum at a dose of 1 g/kg body weight. The urine samples (873 ml) collected for 8 hours after ingestion were extracted three times with one half volume of AcOEt and the combined AcOEt extracts were transferred twice into 1/2 volume of phosphate buffer solution at pH 5.3. The aqueous layer adjusted to pH 9.0 was reextracted two times with a half volume of AcOEt and the organic extracts were successively washed with phosphate buffer solution at pH 5.8 and H_2O. Concentration of the extract gave 240 mg of crude metabolites which were purified by silica gel preparative tlc (Merck HF_{254}) with $CHCl_3$–MeOH–NH_4OH (40:3:20, lower layer) as the solvent system. A main band corresponding to PMDM III-M was extracted with AcOEt saturated with H_2O, and the extract was concentrated to a small volume, giving a white powder of PMDM III-M (110 mg) by addition of *n*-hexane. PMDM III-M thus obtained from rat urine showed the same Rf values on silica gel tlc and identical ir, nmr and mass spectra with the authentic sample.

Anal. Calcd. for $C_{41}H_{67}NO_{16}\cdot H_2O$: C, 58.07; H, 8.20; N, 1.65.
Found: C, 58.28; H, 8.12; N, 1.57.

PMDM III-M thus obtained (10 mg) was dissolved in a mixture of pyridine (0.4 ml) and acetic anhydride (0.2 ml), and the reaction mixture was kept at room temperature for 5 days. After the acetylation, 5 ml of water was added to the mixture under ice cooling and the

mixture was extracted twice with 5 ml of AcOEt. The extract was washed with H_2O and concentrated to dryness. Thus PMDM III-M-diacetate (6 mg) was obtained; mass spectrum, M^+ *m/e* 913 ($C_{45}H_{71}NO_{18}$); nmr, two new acetyl groups were observed at 2.02 (3H, s) and 2.14 (3H, s).

Isolation of PMDM-M, MDM and MDM-M from Human Urine

The urine (5.9 liters) from ten adults who had orally received PMDM (mainly consisting of PMDM III) at a single dose of 1 g/man was extracted twice with AcOEt (3 liters) at pH 9. The combined AcOEt extracts were shaken with phosphate buffer at pH 5.3 (1.5 liters, twice). AcOEt layer was washed with H_2O and concentrated to dryness (yield 78 mg, Fraction 1). On the other hand, aqueous layer was adjusted to pH 9 and reextracted with AcOEt (1.5 liters, twice). After washing with H_2O, AcOEt extracts were concentrated to dryness (yield12 5 mg, Fraction 2). Fraction 1 was separated by silica gel preparative tlc using C_6H_6-Me_2CO (1:1) as the solvent. Eight bands were observed in the above solvent system on tlc. The second band, fifth band and seventh band from the top, followed by AcOEt extraction yielded 3 mg of PMDM, and 28 mg of MDM, and 36 mg of PMDM-M, respectively. The material from fifth band in the above solvent system was further purified by preparative tlc using $CHCl_3$-MeOH-NH_4OH (40:3:20, lower layer) as the solvent to give 16 mg of pure MDM. The one from the seventh band was purified in the same solvent system to give PMDM-M (19 mg). Fraction 2 was separated by silica gel preparative tlc (Merck HF_{254}) using C_6H_6-Me_2CO (1 : 1) as the solvent. The band corresponding to MDM-M was extracted with AcOEt, and after washing with H_2O, the AcOEt extract was concentrated to dryness (yield 43 mg). This material was further purified by silica gel preparative tlc using $CHCl_3$-MeOH-NH_4OH (40:3:20, lower layer) as the solvent system. Pure MDM-M (24 mg) was obtained as a white powder. MDM obtained from human urine was identical with the authentic sample in ir, uv, nmr, mass spectra and Rf values on tlc. PMDM-M and MDM-M isolated from human urine were identical with those prepared by incubation with rat liver homogenates in all respects.

Preparation of PMDM III from PMDM III-M

To a stirred solution of PMDM III-M (80 mg) in dry pyridine (0.5 ml) was added 0.3 ml of propionylchloride-pyridine (1:9) solution under cooling at 0~5°C and allowed to stand for 2 hours at 5°C. At the end of the reaction, the mixture was poured into ice water (200 ml) and the resulting precipitate was filtered. The precipitate was purified by silica gel preparative tlc (Merck HF_{254}) using C_6H_6-Me_2CO (2:1) as the solvent system. The AcOEt extract from the main band afforded PMDM III (53 mg) as a white powder. This substance was identical with the authentic sample of PMDM III in ir, uv, nmr, mass spectra and Rf values on tlc. $[\alpha]_D^{22}$ -62.6° (*c* 0.99, $CHCl_3$).

Anal. Calcd. for $C_{44}H_{71}NO_{17}$: C, 59.64; H, 8.08; N, 1.58.
Found: C, 59.34; H, 8.23; N, 1.59.

Preparation of PMDM III from MDM III-M

MDM III-M was propionylated by the same procedure as described above and the product was identical with PMDM III in all respects. $[\alpha]_D^{22}$ -61.3° (*c* 1.0, $CHCl_3$).

Anal. Found: C, 59.17; H, 8.28; N, 1.73.

Preparation of MDM V-9, 2-diacetate from MDM III-M

To a stirred solution of MDM III-M (400 mg) in dry pyridine (3 ml) was added acetic anhydride (0.5 ml) under cooling and the mixture was allowed to stand for 48 hours at room temperature. At the end of the reaction the mixture was poured into ice water (200 ml) and extracted with AcOEt. AcOEt extract was washed with H_2O and concentrated to dryness. Crystallization of the residue from Et_2O gave colorless prisms of MDM III-M-triacetate (337 mg) which was identical with MDM V-diacetate prepared from MDM V in a usual procedure[1], mp 148~149°C. $[\alpha]_D^{23.5}$ -82.1° (*c* 1.04, $CHCl_3$).

Anal. Calcd. for $C_{44}H_{69}NO_{18}\cdot H_2O$: C, 57.56; H, 7.88; N, 1.53.
Found: C, 57.61; H, 7.75; N, 1.55.

Preparation of PMDM V-2′-Acetate from PMDM III-M

PMDM III-M (406 mg) was acetylated with acetic anhydride-pyridine by the same manner as described for acetylation of MDM III-M, followed by crystallization from Et_2O-*n*-hexane to give PMDM III-M-diacetate (317 mg) which was identical with PMDM V-2′-acetate prepared from PMDM V in all respects, mp 228~229°C (dec.). $[\alpha]_D^{22.5}$ -82.6° (*c* 1.0, $CHCl_3$).
Anal. Calcd. for $C_{45}H_{71}NO_{18}\cdot H_2O$: C, 57.99; H, 7.89; N, 1.59.
Found: C, 57.91; H, 7.71; N, 1.55.

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