14-HYDROXY-6-*O*-METHYLERYTHROMYCINS A, ACTIVE METABOLITES OF 6-*O*-METHYLERYTHROMYCIN A IN HUMAN

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(14*R*)-14-Hydroxy-6-*O*-methylerythromycin A and (14*S*)-epimer have been isolated as active metabolites from human urine after oral administration of 6-*O*-methylerythromycin A (TE-031, A-56268). The structures of these metabolites were determined by means of mass, ^{1}H and ^{13}C NMR spectroscopies. Antimicrobial activities of (14*R*)-14-hydroxy-6-*O*-methylerythromycin A, a major metabolite, were comparable to those of the parent drug TE-031, whereas (14*S*)-14-hydroxy-6-*O*-methylerythromycin A was 4- to 8-fold less active than TE-031 against bacterial standard strains.

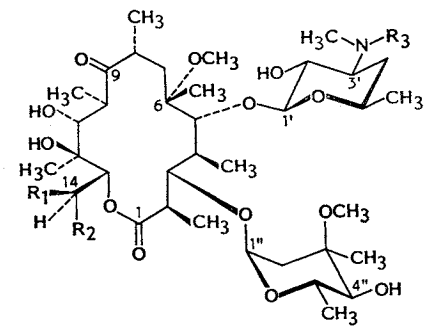
Erythromycin A (EM-A) has been one of the most useful macrolide antibiotics for the past three decades. In recent years, many derivatives of EM-A have been synthesized in order to improve antimicrobial activities and pharmacokinetics and also to reduce toxicities.

6-*O*-Methylerythromycin A (TE-031), a new semisynthesized macrolide antibiotic, shows similar antimicrobial spectrum to EM-A, and *in vitro* activities of TE-031 are comparable or superior to those of EM-A. Furthermore, TE-031 exhibits excellent antimicrobial activities *in vivo*, because of its outstanding stability in acidic conditions and superior pharmacokinetic properties[1~3].

In the course of studying metabolism of TE-031 in human, several urinary metabolites have been isolated[4]. Among them, three active metabolites, so-called M-5, M-6 and M-1, were determined as (14*R*)-14-hydroxy-6-*O*-methylerythromycin A, (14*S*)-14-hydroxy-6-*O*-methylerythromycin A and *N*-demethyl-6-*O*-methylerythromycin A, respectively, as shown in Fig. 1.

Herein we wish to describe the structure determination of 14-hydroxy-6-*O*-methylerythromycins A (M-5 and M-6) and their antimicrobial activities.

Fig. 1. Structures of TE-031, M-5, M-6 and M-1.



TE-031	$R_1=H$	$R_2=R_3=CH_3$	
M-5	$R_1=OH$	$R_2=R_3=CH_3$	
M-6	$R_1=CH_3$	$R_2=OH$	$R_3=CH_3$
M-1	$R_1=H$	$R_2=CH_3$	$R_3=H$

Experimental

Instruments

MP's were measured using a Yanaco micro mp apparatus and were uncorrected. IR spectra were recorded on a Jasco DS-701G spectrometer. UV spectra were recorded on a Shimadzu UV-240 spectrophotometer. Optical rotations were measured with a Jasco DIP-181 digital polarimeter. Mass spectra were obtained with a Jeol JMS-DX303 spectrometer. ^{1}H and ^{13}C NMR

spectra were recorded on a Jeol JNM-GX400 (^{1}H 400 MHz, ^{13}C 100.4 MHz) spectrometer. The signals of ^{1}H and ^{13}C NMR spectra were assigned by correlated homonuclear ^{1}H-^{1}H and heteronuclear ^{1}H-^{13}C 2D NMR spectroscopies.

Source of Metabolites

TE-031 was administered orally at 300 mg×2/day for 7.5 days to eight healthy volunteers. Approximately, 80 liters of urine were collected and used as the source of metabolites.

Thin-layer Bioautogram

Approximately, 30 and 1 μg of the crude residue, obtained by the EtOAc extraction of the urine, were applied to TLC plates (Silica gel 60 F_{254}, 5×20 cm, thickness 0.25 mm, Merck) and developed in a mobile phase composed of $CHCl_3$, MeOH and ammonium hydroxide (9 : 1 : 0.1). The air-dried TLC plates were laid on agar plates for 2 minutes which were inoculated with *Micrococcus luteus* ATCC 9341. The agar plates were incubated at 30°C for 18 hours for the detection of the active metabolites of TE-031.

Antimicrobial Activity

The MICs were determined by means of an agar dilution method, using sensitivity test agar plates containing a series of 2-fold dilutions of sample. Overnight cultures in sensitivity test broth were used for precultures of tested strains. The MICs were determined after overnight incubation at 37°C, with an inoculum equivalent to a 10^{-2} dilution of an 18 hours culture in sensitivity test broth (about 10^6 cfu/ml). Each inoculum was seeded onto agar plates using an inoculum-replicating apparatus.

Results

Extraction and Isolation of Metabolites

The extraction and isolation procedure of the metabolites from urine are shown in Fig. 2.

Urine was adjusted to pH 10 with aqueous sodium bicarbonate solution and extracted with EtOAc. The organic layer was filtered to remove the precipitates, washed with aqueous saturated sodium chloride solution and dried over magnesium sulfate. Evaporation of the solvent afforded a crude residue (28.7 g).

Thin-layer bioautogram of the residue is shown in Fig. 3. Considerably active compounds, M-5 and the parent drug TE-031, were detected. In addition to these compounds, M-6 and M-1 were also detected.

By combination of silica gel column chromatography and crystallization, M-5 (2.39 g) was isolated as a major metabolite. M-6 (31 mg) and M-1 (174 mg) were also obtained as minors.

Physico-chemical Properties of M-5 and M-6

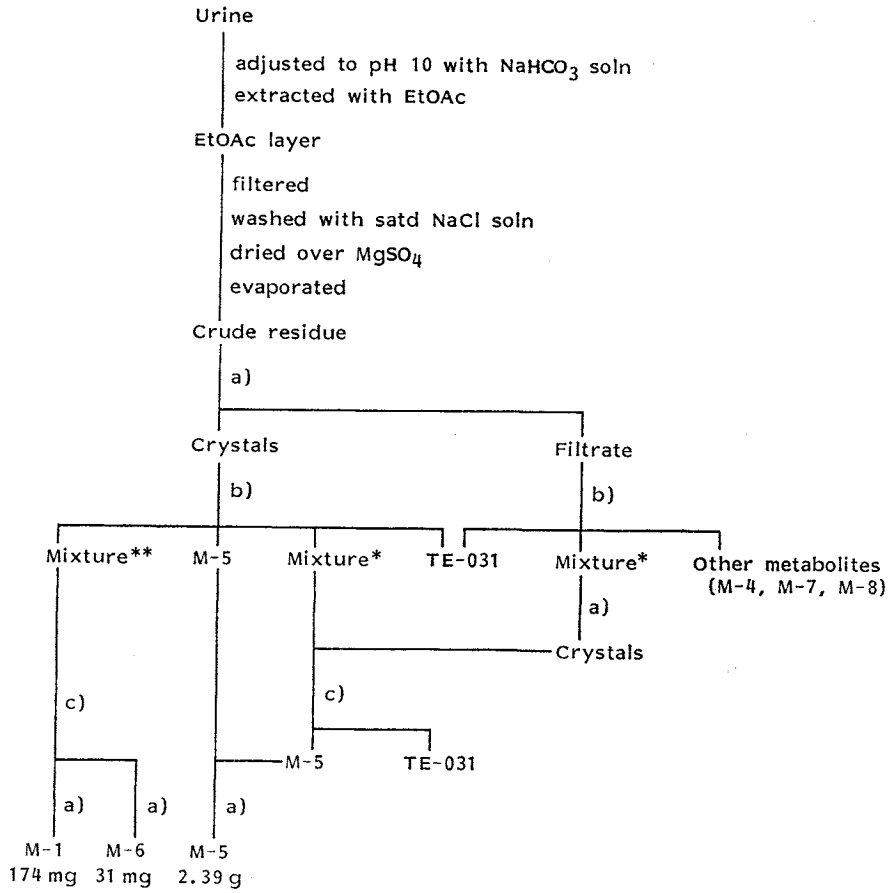
Physico-chemical properties of M-5 and M-6 are shown in Table 1. The ^{1}H and ^{13}C NMR spectral data are listed in Tables 2, 3 and 4.

Structure Determination of the Metabolites of TE-031

The molecular formula of M-5 was determined as $C_{38}H_{69}NO_{14}$ from the elemental analysis, fast atom bombardment (FAB)-MS and ^{13}C NMR spectra, indicating the introduction of one oxygen atom to TE-031.

Fig. 4 shows the ^{1}H NMR spectrum of M-5, which is almost similar to that of TE-031. As shown in Table 2, the signal of 14-H in M-5 was observed at 4.13 ppm, which was considerably in lower field than that of TE-031. The peak of 15-H in M-5 was observed at 1.10 ppm, which was in lower field by 0.25 ppm as compared with that in TE-031. The splitting patterns for the signals of 13-H, 14-H

Fig. 2. Extraction and isolation of the metabolites of TE-031.

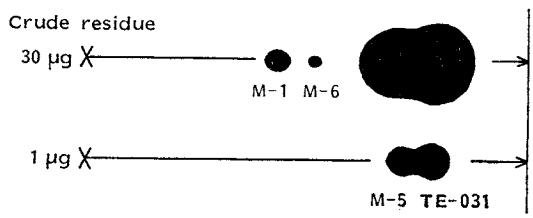


a): Crystallized from EtOH.
b): Silica gel column chromatography, Silica gel 60 (Merck), $CHCl_3$ - MeOH - NH_4OH, 20 : 1 : 0.1 and 10 : 1 : 0.1.
c): Silica gel column chromatography, LiChroprep Si 60 (Merck), $CHCl_3$ - MeOH - NH_4OH (20 : 1 : 0.1).
* Mixture of TE-031 and M-5.
** Mixture of M-6 and M-1.

and 15-H in M-5 were doublet, doublet of quartet and doublet, respectively, designating that C-14 was a methine carbon. The above results indicate that M-5 is 14-hydroxy TE-031.

This is also supported by ^{13}C NMR spectroscopic studies. The correlated heteronuclear ^{1}H-^{13}C 2D NMR spectroscopy of M-5 is shown in Fig. 5. As shown in Table 4, the chemical shifts of M-5 were also similar to those of TE-031 except for the signals of C-14 and C-15. In M-5 the observed downfield shifts of C-14 (+45.4 ppm) and C-15 (+9.2 ppm) with respect to TE-

Fig. 3. Thin-layer bioautogram of the crude residue.



TLC plate: Silica gel 60 F_{254} (Merck).
Solvent: $CHCl_3$ - MeOH - NH_4OH (9 : 1 : 0.1).
Detection: *Micrococcus luteus* ATCC 9341.

Table 1. Physico-chemical properties of M-5 and M-6.

	M-5	M-6
Appearance	Colorless needles	Colorless needles
MP (°C)	214.5~216.5	194~196
Molecular formula	$C_{38}H_{69}NO_{14}$	$C_{38}H_{69}NO_{14}$
FAB-MS (*m/z*)	764 (MH^+), 606 (MH^+-158)	764 (MH^+), 606 (MH^+-158)
Elemental analysis		
Calcd for:	$C_{38}H_{69}NO_{14}$	
	C 59.74, H 9.10, N 1.83.	
Found:	C 59.58, H 9.32, N 1.71.	
UV λ_{max}^{EtOH} nm (ε)	288 (43.1)	288 (39.1)
Specific rotation	$[\alpha]_D^{25}$ −96.0° (*c* 0.5, EtOH)	$[\alpha]_D^{24}$ −100.4° (*c* 0.25, EtOH)
IR ν_{max} cm^{-1}	3470, 1734, 1688 (KBr)	3470, 1730, 1683 ($CHCl_3$)

Table 2. ^1H NMR chemical shifts[a] of M-5, M-6 and TE-031[b].

Proton No.	Multiplicity	Chemical shifts (ppm)		
		M-5	M-6	TE-031
2-H	(dq)	2.84	2.99	2.89
3-H	(dd)	3.77	3.79	3.77
4-H	(ddq)	1.85	1.91	1.92
5-H	(d)	3.65	3.67	3.67
7-H_{eq}	(dd)	1.73	1.73	1.72
7-H_{ax}	(dd)	1.83	1.85	1.85
8-H	(ddq)	2.59	2.58	2.59
10-H	(dq)	2.98	nd	3.00
11-H	(d)	3.75	3.70	3.76
13-H	(d)	4.94	5.06	5.05[c]
14-H	(dq)	4.13	4.40	1.48[d], 1.92[d]
15-H (14-CH_3)	(d)	1.10	1.13	0.85[e]
16-H (2-CH_3)	(d)	1.18	1.23	1.20
17-H (4-CH_3)	(d)	1.08	1.10	1.10
18-H (6-CH_3)	(s)	1.41	1.41	1.41
19-H (8-CH_3)	(d)	1.13	1.13	1.14
20-H (10-CH_3)	(d)	1.11	1.12	1.13
21-H (12-CH_3)	(s)	1.27	1.36	1.12
6-OCH_3	(s)	3.02	3.03	3.04
1′-H	(d)	4.43	4.45	4.44
2′-H	(dd)	3.18	3.19	3.19
3′-H	(ddd)	2.41	2.41	2.41
4′-H_{ax}	(ddd)	nd	nd	1.21
4′-H_{eq}	(ddd)	1.66	1.66	1.66
5′-H	(ddq)	3.48	3.49	3.48
6′-H (5′-CH_3)	(d)	1.23	1.23	1.23
3′-$N(CH_3)_2$	(s)	2.28	2.28	2.28
1″-H	(dd)	4.91	4.93	4.93
2″-H_{ax}	(dd)	1.59	1.59	1.59
2″-H_{eq}	(dd)	2.36	2.37	2.37
4″-H	(dd)	3.03	nd	3.02
5″-H	(dq)	4.00	4.01	4.01
6″-H (5″-CH_3)	(d)	1.30	1.31	1.30
7″-H (3″-CH_3)	(s)	1.25	1.26	1.25
3″-OCH_3	(s)	3.33	3.33	3.33
11-OH		3.93	4.05	3.98
12-OH		4.23	nd	3.18
14-OH		4.49	nd	
2′-OH		3.44~3.48	3.45	3.45
4″-OH	(d)	2.19	2.19	2.18

[a] δ values in ppm from $(CH_3)_4Si$, measured in $CDCl_3$ at 400 MHz; as determined from a 2D homonuclear shift correlated experiments.

[b] ^1H NMR spectra of TE-031 had been recorded at 200 MHz in ref 1. The present data of TE-031 were newly taken at 400 MHz. [c] (dd), [d] (ddq), [e] (t).

nd: Not determined because of the complexities of the spectra.

Table 3. Coupling constants (J=Hz)[a] of M-5, M-6 and TE-031.

H-H	M-5	M-6	TE-031	H-H	M-5	M-6	TE-031
2-3	9.0	9.0	9.0	1′-2′	7.3	7.3	7.2
3-4	1.0	1.0	1	2′-3′	10.3	10.4	10.2
4-5	7.5	7.5	7.4	$3'$-$4'_{ax}$	12.0	12.9	12.0
7_{eq}-7_{ax}	14.8	15.0	14.8	$3'$-$4'_{eq}$	3.7	3.7	3.0
7_{eq}-8	2.0	2.0	1.8	$4'_{ax}$-$4'_{eq}$	12.5	13.0	12.6
7_{ax}-8	11.4	11.5	11.5	$4'_{ax}$-5′	10.6	11	12.0
10-11	1.4	1.3	1.0	$4'_{eq}$-5′	2.0	2	2.0
13-14	9.2	2.4	11.0, 2.2	5′-6′ (5′-5′CH_3)	5.9	6.0	6.0
				$1''$-$2''_{ax}$	4.5	4.5	4.6
14-15	6.1	6.5	7.3	$1''$-$2''_{eq}$	0	0	1
16-2 (2CH_3-2)	7.3	8.0	7.2	$2''_{ax}$-$2''_{eq}$	15.3	15.2	15.0
17-4 (4CH_3-4)	5.9	5.9	6.0	4″-5″	9.3	9.3	9.4
19-8 (8CH_3-8)	6.5	7.0	7.0	5″-6″ (5″-5″CH_3)	6.3	6.3	6.4
20-10 (10CH_3-10)	6.7	6.7	7.0	4″-4″OH	10.0	10.5	10.3

[a] Coupling constants (J=Hz) were measured in $CDCl_3$ at 400 MHz.

Table 4. ^{13}C NMR chemical shifts[a] of M-5, M-6 and TE-031[b].

Carbon No.	Chemical shifts (ppm)			Carbon No.	Chemical shifts (ppm)		
	M-5	M-6	TE-031		M-5	M-6	TE-031
C-1	175.1	175.7	175.9	C-20 (10-CH_3)	12.3	12.2	12.3
C-2	45.0	44.9	45.1	C-21 (12-CH_3)	16.3	17.9	16.0
C-3	78.2	78.5	78.5	6-OCH_3	50.6	50.7	50.7
C-4	39.5	39.2	39.3	C-1′	102.8	102.8	102.9
C-5	80.6	80.8	80.8	C-2′	70.9	71.0	71.0
C-6	78.4	78.5	78.5	C-3′	65.6	65.6	65.6
C-7	39.5	39.5	39.4	C-4′	28.5	28.6	28.6
C-8	45.2	45.3	45.3	C-5′	68.8	68.8	68.8
C-9	220.8	221.3	221.1	C-6′ (5′-CH_3)	21.4	21.5	21.5
C-10	36.9	36.5	37.3	3′-$N(CH_3)_2$	40.2	40.3	40.3
C-11	68.9	69.9	69.1	C-1″	96.1	96.1	96.1
C-12	76.8	74.8	74.3	C-2″	34.8	34.9	34.9
C-13	74.5	75.8	76.7	C-3″	72.7	72.7	72.7
C-14	66.5	66.2	21.1	C-4″	77.9	78.0	78.0
C-15 (14-CH_3)	19.8	20.9	10.6	C-5″	65.7	65.8	65.8
C-16 (2-CH_3)	15.5	16.0	16.0	C-6″ (5″-CH_3)	18.7	18.7	18.7
C-17 (4-CH_3)	8.9	9.1	9.1	C-7″ (3″-CH_3)	21.4	21.5	21.5
C-18 (6-CH_3)	19.7	19.8	19.8	3″-OCH_3	49.5	49.5	49.5
C-19 (8-CH_3)	17.9	18.1	18.0				

[a] δ values in ppm from $(CH_3)_4Si$, measured in $CDCl_3$ at 100.4 MHz; as determined from a 2D heteronuclear shift correlated experiments.

[b] ^{13}C NMR spectra of TE-031 had been recorded at 50.3 MHz in ref 1. The present data of TE-031 were newly taken at 100.4 MHz.

031 clearly pointed to C-14 as the site of hydroxylation, which were in good agreement with known substituent effects[5,6].

The stereochemistry at C-14 in M-5 has been established to be *R* configuration. On the basis of the long range coupling experiment between C-21 (12-CH_3) and 12-OH by correlated heteronuclear ^1H-^{13}C 2D techniques, the hydroxy protons at 4.23 ppm (sharp) and 4.49 ppm (br) in M-5 were assigned to 12-OH and 14-OH, respectively, whereas 12-OH in TE-031 was observed at 3.18 ppm. The

Fig. 4. ^1H NMR spectrum of M-5 in $CDCl_3$ (400 MHz).

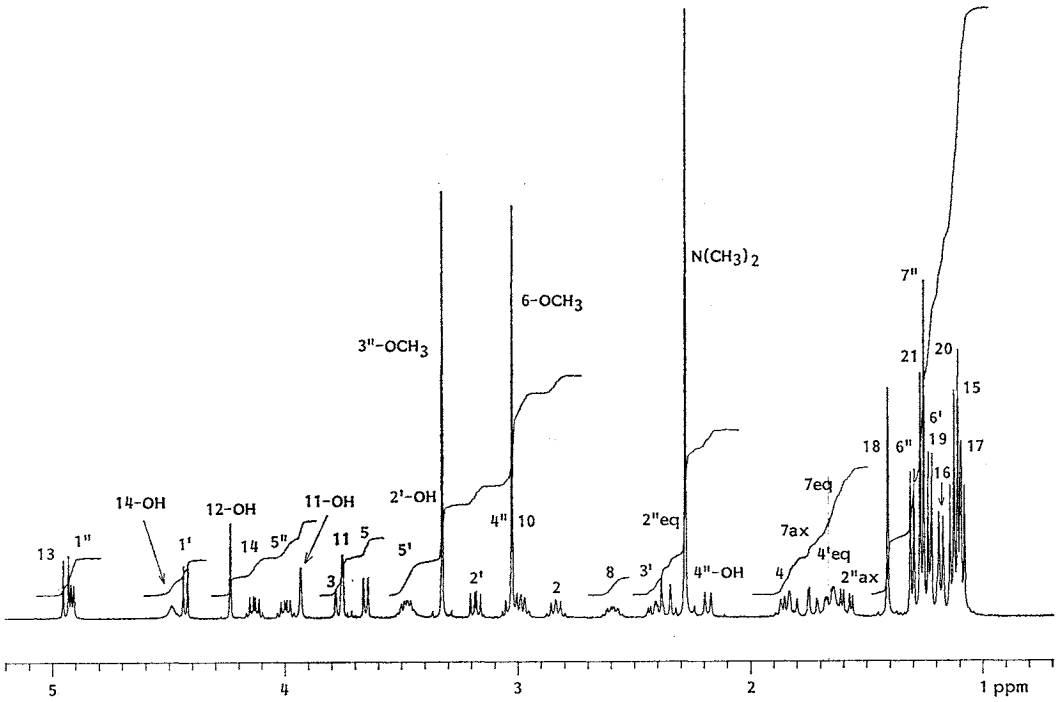


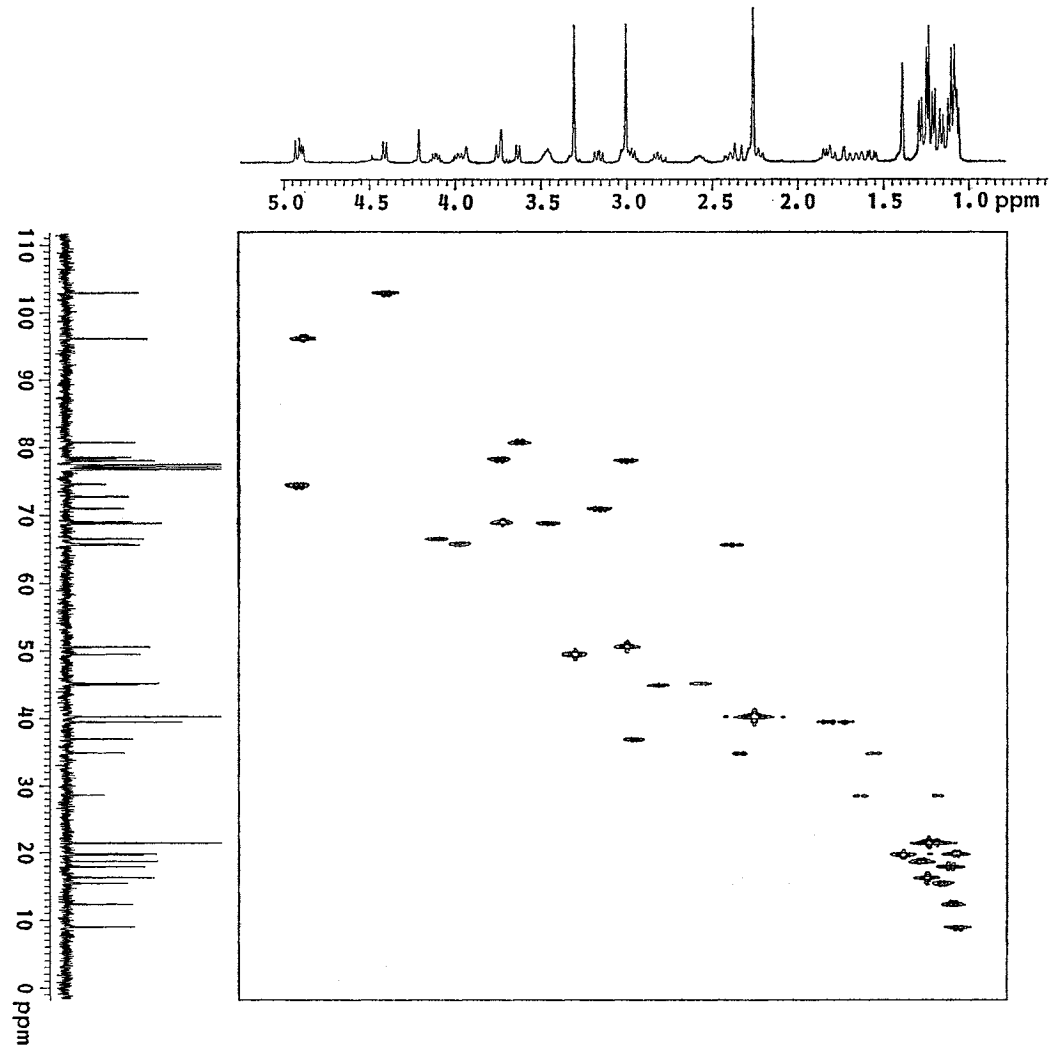
Table 5. Antimicrobial activities of metabolites of TE-031.

Strain	MICs (μg/ml)		
	M-5	M-6	TE-031
Staphylococcus aureus 209P-JC	0.10	0.78	0.10
S. aureus BB	0.20	0.78	0.10
S. aureus Smith 4	0.20	0.78	0.10
S. aureus J-109	>100	>100	>100
S. aureus B1	>100	>100	>100
S. aureus C1	1.56	12.5	0.78
S. epidermidis sp-al-1	0.20	1.56	0.10
Enterococcus faecalis ATCC 8043	0.05	0.20	0.025
Bacillus subtilis ATCC 6633	0.10	0.39	0.05
Micrococcus luteus NIHJ	0.05	0.10	0.025
M. luteus ATCC 9341	0.025	0.05	0.012
Escherichia coli NIHJ JC-2	50	>100	100
E. coli K-12	12.5	25	12.5
Klebsiella pneumoniae IFO 3317	25	100	50
Branhamella catarrhalis NNBr-1	0.10	0.39	0.10

Inoculum size: 10^6 cfu/ml.
Medium: Sensitivity Test Agar (Eiken).

downfield shift of 12-OH in M-5 suggested an intramolecular hydrogen bond between 12-OH and 14-OH. The coupling constant value between 13-H and 14-H in M-5 was J=9.2 Hz, indicating that 13-H was in relation to an *anti*-staggered conformation with 14-H. Furthermore, nuclear Overhauser effect (NOE) enhancement was observed with 14-H on irradiation of 21-H (12-CH_3). From the above results, the partial conformation around C-12~C-15~C-1 in M-5 is illustrated in Fig. 6, and which

Fig. 5. ^{1}H-^{13}C 2D NMR spectrum of M-5.



closely resembles a comparable portion of the Perun conformation, model III[7]. Therefore, M-5 was determined as (14*R*)-14-hydroxy-6-*O*-methylerythromycin A.

On the bases of FAB-MS and ^{13}C NMR spectra, the molecular formula of M-6 was determined as $C_{38}H_{69}NO_{14}$, which was the same as that of M-5.

Fig. 6. Stereochemistry of M-5.

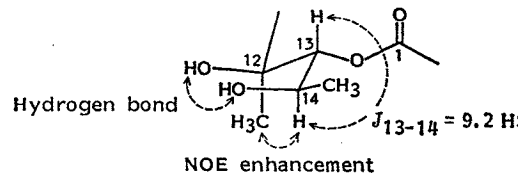
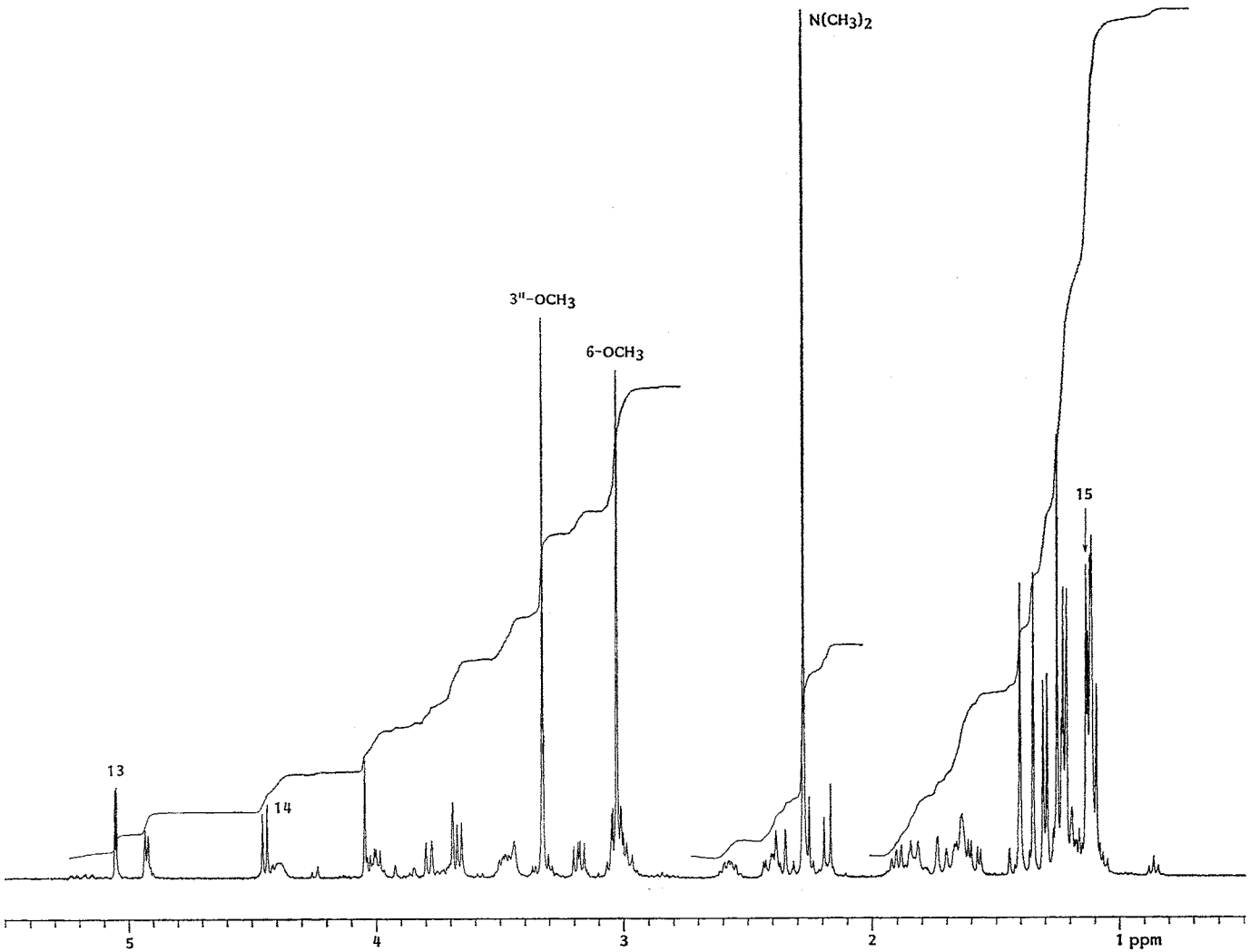


Fig. 7 shows the ^{1}H NMR spectra of M-6. The signals of 14-H, 15-H, C-14 and C-15 in M-6 were similar to those in M-5. The splitting patterns of 13-H, 14-H and 15-H in M-6 were identical with those in M-5. However, J_{13-14} was 2.4 Hz in M-6, a typical difference between M-5 and M-6, indicating that the stereochemistry of C-14 in M-6 was different from that in M-5. Therefore, M-6 was determined as (14*S*)-14-hydroxy-6-*O*-methylerythromycin A, an epimer of M-5 at C-14.

Fig. 7. ^{1}H NMR spectrum of M-6 in $CDCl_3$ (400 MHz).



Antimicrobial Activity

The *in vitro* antimicrobial activities of M-5 and M-6 against various standard strains are shown in Table 5. M-5 was equal to or 2-fold less active than the parent drug (TE-031). M-6 was 4- to 8-fold less active than TE-031.

Discussion

As the macrolide antibiotics have polyfunctional structures, various types of metabolism have been reported. Concerning the metabolism of EM-A, *N*-demethylation, cleavage of cladionose moiety and the formation of 6,9;9,12-spiroketal have been reported[8~13]. However, these metabolisms cause the inactivation of EM-A.

We isolated (14*R*)- and (14*S*)-14-hydroxy-6-*O*-methylerythromycin A (M-5 and M-6) as active metabolites of TE-031 from urine of human. These structures were determined by means of mass, ^{1}H and ^{13}C NMR spectroscopies. The ^{1}H and ^{13}C NMR assignments on M-5 and M-6 were made unambiguosly by homonuclear ^{1}H-^{1}H and heteronuclear ^{1}H-^{13}C 2D techniques, which were consistent with those reported on EM-A in $CDCl_3$[14]. In Fisher projection, 14*R* (M-5) and 14*S* (M-6) are equivalent to 14D and 14L, respectively. Hydroxylation at C-14 is the first to be discovered in the metabolism of 14-membered macrolide antibiotics.

The metabolism of TE-031 in human is considerably different from that in animals. *N*-Demethylation is a major metabolic pathway in rat and dog[15], whereas 14-hydroxylation is a major one in human. From the viewpoint of antimicrobial activity, M-5 was equal to or 2-fold less active than TE-031 *in vitro*. Furthermore, M-5 was more effective than TE-031 against systemic infections in mice[16]. Because of the formation of M-5 in significant quantity in human and the excellent antimicrobial activities, M-5 gives distinctive features to TE-031.

References

1) MORIMOTO, S.; Y. TAKAHASHI, Y. WATANABE & S. ŌMURA: Chemical modification of erythromycins. I. Synthesis and antibacterial activity of 6-*O*-methylerythromycins A. J. Antibiotics 37: 187~189, 1984
2) MORIMOTO, S.; T. ADACHI, Y. TAKAHASHI, T. ASAKA, M. KASHIMURA, Y. WATANABE, S. OMURA & K. SOTA: A new macrolide antibiotic, TE-031 (A-56268); synthesis and biological properties. Program and Abstracts of the 26th Intersci. Conf. on Antimicrob. Agents Chemother., No. 409, p. 172, New Orleans, Sept. 28~Oct. 1, 1986
3) MITSUHASHI, S.; T. ONO, T. NAGATE, K. SUGITA & S. OMURA: A new macrolide antibiotic, TE-031 (A-56268); *in vitro* and *in vivo* antibacterial activities. Program and Abstracts of the 26th Intersci. Conf. on Antimicrob. Agents Chemother., No. 412, p. 172, New Orleans, Sept. 28~Oct. 1, 1986
4) ADACHI, T.; S. MORIMOTO, Y. WATANABE & K. SOTA: Isolation and identification of metabolites of TE-031 in human urine. Chemotherapy (Tokyo), in press
5) MORISHITA, M.; M. OHNO, K. SERIZAWA, T. FUJIWARA & H. SAKAKIBARA: Isolation and identification of metabolites of TMS-19-Q. Chemotherapy (Tokyo) 32(Suppl. 6): S85~S92, 1984
6) SHOMURA, T.; S. SOMEYA, S. MURATA, K. UMEMURA & M. NISHIO: Metabolism of 9,3″-diacetylmidecamycin. II. The structures of several metabolites of 9,3″-diacetylmidecamycin. Chem. Pharm. Bull. 29: 2413~2419, 1981
7) NAKAGAWA, A. & S. ŌMURA: V. Conformation of macrolide antibiotics. 2. Structure and stereochemistry of macrolides. *In* Macrolide Antibiotics. Chemistry, Biology, and Practice. *Ed.*, S. ŌMURA, pp. 75~84, Academic Press, Orlando, 1984
8) WELLES, J. S.; R. C. ANDERSON & K. K. CHEN: A metabolite of erythromycin. *In* Antibiotics Annual 1954-1955. *Eds.*, H. WELCH & F. MARTI-IBAÑEZ, pp. 291~294, Medical Encyclopedia, New York, 1955
9) LEE, C. C.; R. C. ANDERSON & K. K. CHEN: The excretery products of *N*-methyl-^{14}C-erythromycin in rats. J. Pharmacol. Exp. Ther. 117: 274~280, 1956
10) MAO, J. C. H. & P. L. TARDREW: Demethylation of erythromycins by rabbit tissues *in vitro*. Biochem. Pharmacol. 14: 1049~1058, 1965
11) FURUKAWA, H.: Studies on the metabolism of erythromycin base and its propionyl ester. Chemotherapy (Tokyo) 16: 799~804, 1968

12) MAJER, J.; C. RAYA & J. W. CORCORAN: Metabolites of erythromycin in man. Program and Abstracts of the 15th Intersci. Conf. on Antimicrob. Agents Chemother., No. 14, Washington, D.C., Sept. 24~26, 1975
13) MAJER, J.; R. S. STANASZEK, S. L. MUELLER & G. MARTI: *N*-Didemethyl-*N*-propionyl-6,9;9,12-erythromycin A-spiroketal, a new metabolite of erythromycin ethyl succinate in man. Drug Metab. Dispos. 6: 673~676, 1978
14) EVERETT, J. R. & J. W. TYLER: An analysis of the ^{1}H and ^{13}C n.m.r. spectra of erythromycin A using two-dimensional methods. J. Chem. Soc. Perkin Trans. I 1985: 2599~2603, 1985
15) SUWA, T.; H. YOSHIDA, S. YOSHITOMI & K. KAMEI: Metabolism of TE-031 (A-56268), a new macrolide antibiotic, in rat, dog and man. Program and Abstracts of the 26th Intersci. Conf. on Antimicrob. Agents Chemother., No. 414, p. 172, New Orleans, Sept. 28~Oct. 1, 1986
16) NAGATE, T.; T. ADACHI, T. OTAKE, H. YOSHIDA, J. MIYAJI, E. SEKIGUCHI, S. MORIMOTO & S. OMURA: A new macrolide antibiotic, TE-031 (A-56268); structures and activities of metabolites. Program and Abstracts of the 26th Intersci. Conf. on Antimicrob. Agents Chemother., No. 410, p. 172, New Orleans, Sept. 28~Oct. 1, 1986