Notes

6-DEOXY-8-*O*-METHYLRABELOMYCIN AND 8-*O*-METHYLRABELOMYCIN FROM A *STREPTOMYCES* SPECIES

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In the course of our screening for new antibiotic, new members of benz[a]anthraquinone antibiotics (**I** and **II**), which showed antibacterial activity against Gram-positive bacteria were isolated from a culture broth of *Streptomyces tsusimaensis* MI310-38F7. This strain produced nonactin and a minor yellow compound (**III**) which was identical with X-14881 E[1]. This report describes the isolation, characterization and structure determination of these compounds, named 6-deoxy-8-*O*-methylrabelomycin (**I**) and 8-*O*-methylrabelomycin (**II**).

The producing strain MI310-38F7 was cultured at 27°C for 4 days on a rotary shaker (180 rpm) in a 500-ml baffled Erlenmeyer flask containing 110 ml of a production medium consisting of potato starch 3.0%, soybean meal 1.5%, corn steep liquor 0.5%, yeast extract 0.2%, NaCl 0.3%, $MgSO_4 \cdot 7H_2O$ 0.05%, $CaCO_3$ 0.3% and $CoCl_2 \cdot 6H_2O$ 0.001% (pH 7.2 before sterilization). The antibiotic activity in the culture broth was determined by a cylinder plate method using *Micrococcus luteus* IFO 3333 and *Mycobacterium smegmatis* ATCC 607 as the test organism. The isolation procedure is shown in Scheme 2. Further purification was achieved by preparative TLC on silica gel developed with $CHCl_3$ to give **III** (1.0 mg) and $CHCl_3$ - MeOH (10 : 1) to give **I** (13.3 mg) and **II** (14.0 mg).

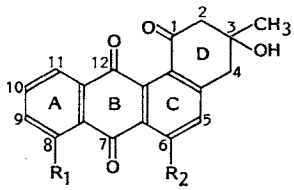
Physico-chemical properties of **I**, **II** and **III** are summarized in Table 1. The IR spectra of these compounds indicate the presence of ketone carbonyl (**I** 1703 cm^{-1}, **II** 1704 cm^{-1}), non-chelated quinone carbonyl (**I** 1674 cm^{-1}, **II** 1680 cm^{-1}, **III** 1660 cm^{-1}) and chelated carbonyl (**II** 1642 cm^{-1})[2]. These spectra are shown in Figs. 1, 2 and 3. These three antibiotics were soluble in $CHCl_3$, EtOAc and MeOH, but insoluble in water and *n*-hexane.

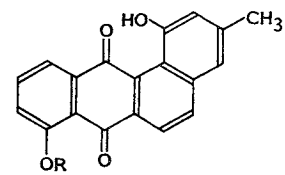
The ^1H NMR data of **I**, **II** and **III** are shown in Table 2. The ^{13}C NMR spectra of **I** and **II** are assigned as shown in Table 3.

The NMR data indicate that **I** is the methyl ether of tetrangomycin[2,3]. The position of methoxy group was determined from the results of nuclear Overhauser effect (NOE) experiments and the long-range ^{13}C-^1H correlation spectroscopy (COSY) spectrum as shown in Fig. 4. Its negative optical rotation was consistent with that of rabelomycin (literature[4], $-102° \pm 10°$). The absolute configuration at ring D in **I** was deduced to be the same as rabelomycin. On the basis of these results the structure of antibiotic **I** was determined to be 6-deoxy-8-*O*-methylrabelomycin.

The molecular formula of **II** differs from that

Scheme 1.





6-Deoxy-8-*O*-methylrabelomycin (**I**)	$R_1=OCH_3$	$R_2=H$	X-14881 E (**III**)	$R=CH_3$
8-*O*-Methylrabelomycin (**II**)	$R_1=OCH_3$	$R_2=OH$	Tetrangulol	$R=H$
Rabelomycin	$R_1=R_2=OH$			

Scheme 2.

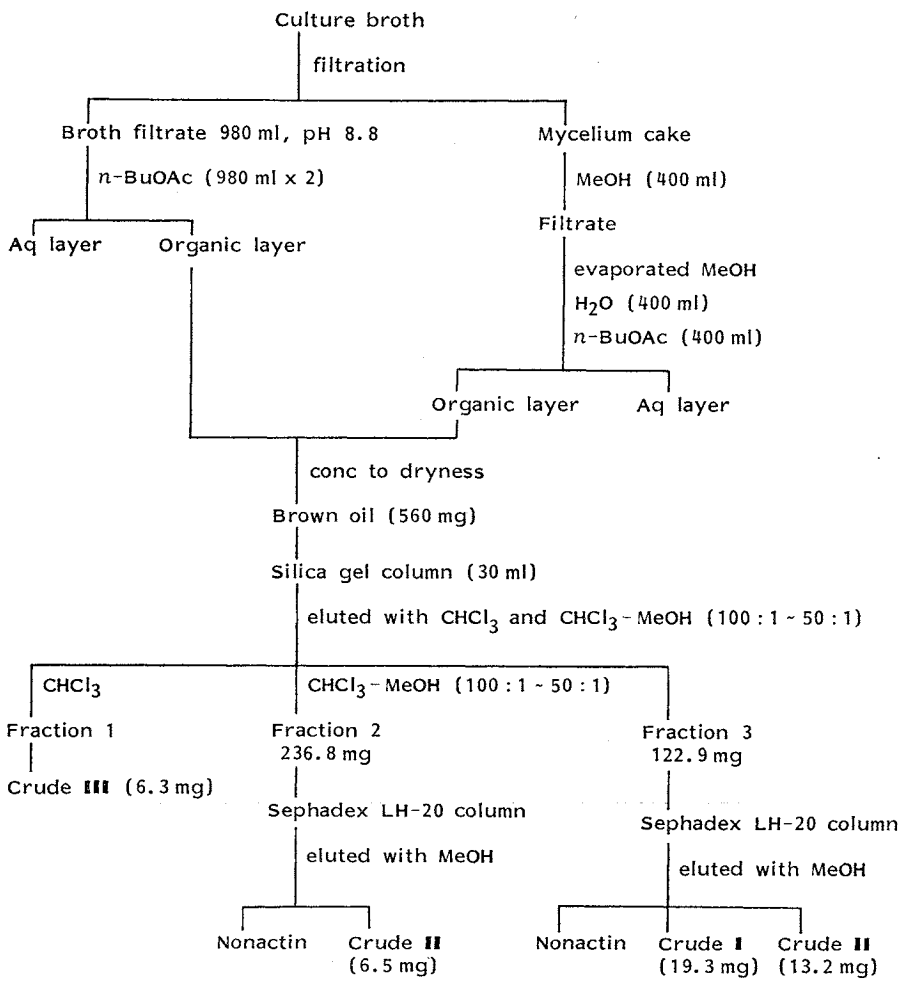


Table 1. Physico-chemical properties of I, II and III.

	I	II	III
Appearance	Yellow needle	Yellow needle	Brown needle
MP (°C)	117~173	191~193	194
Molecular formula	$C_{20}H_{16}O_5$	$C_{20}H_{16}O_6$	$C_{20}H_{14}O_4$
HR-MS (*m/z*) Found:	336.0992	352.0944	318.0865
Calcd:	336.0996	352.0945	318.0880
$[\alpha]_D^{28}$	−106.8° (*c* 0.5, MeOH)	−118.0° (*c* 0.5, MeOH)	0
UV λ_{max}^{MeOH} nm (ε)	214 (25,000), 265 (32,900), 375 (5,000)	224 (23,800), 263 (25,300), 405 (6,000)	223 (73,000), 308 (35,000), 402 (9,000)
$\lambda_{max}^{MeOH-NaOH}$ nm (ε)	214 (36,700), 265 (32,000), 375 (5,000)	254 (22,800), 316 (8,600), 465 (4,000)	228 (56,000), 334 (26,000), 540 (1,700)
IR ν_{max}^{KBr} cm^{-1}	3440, 1703, 1674	3340, 1704, 1680, 1642	3450, 1660
TLC Silica gel (Rf)			
$CHCl_3$ - MeOH (10 : 1)	0.61	0.68	—
$CHCl_3$	—	—	0.60

HR-MS: High-resolution mass spectra.

Fig. 1. IR spectrum of I (KBr).

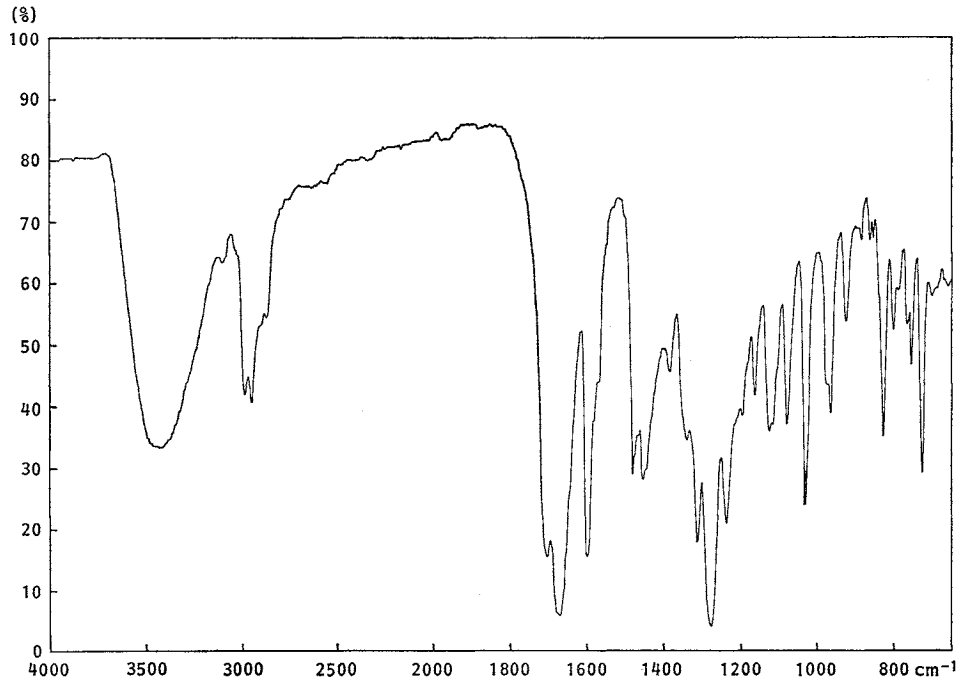
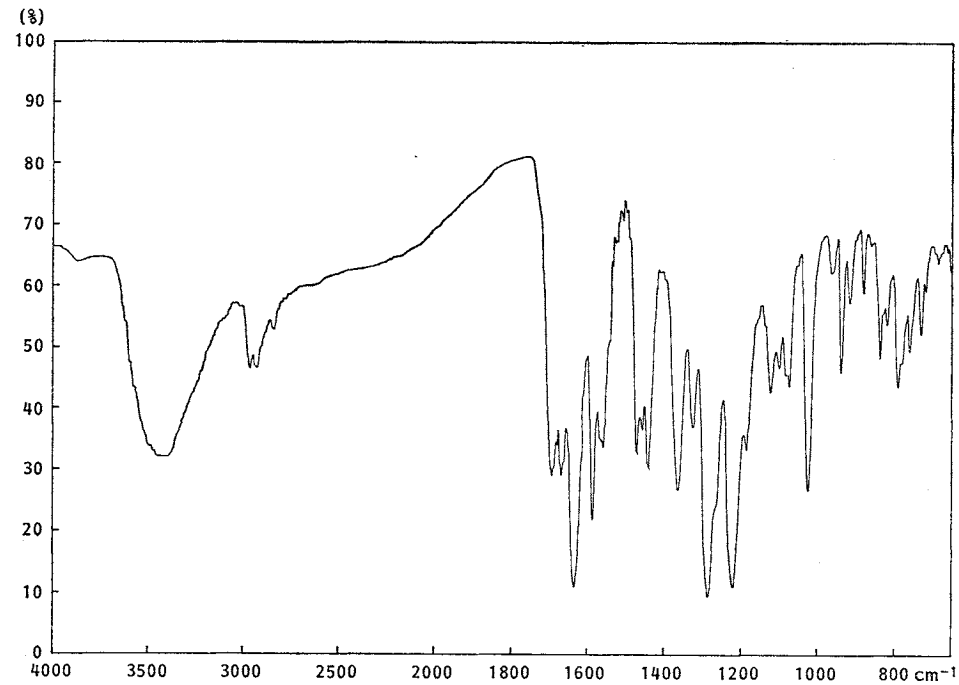


Fig. 2. IR spectrum of II (KBr).



of **I** by the presence of one additional oxygen atom. The ^{1}H NMR spectrum of **II** showed four proton signals in aromatic region, which were attributable to 5-H, 9-H, 10-H and 11-H. The singlet signal of 5-H (δ 6.92) shifted to higher field than that of **I** (δ 7.50, d, J=8.0 Hz) and a

Fig. 3. IR spectrum of III (KBr).

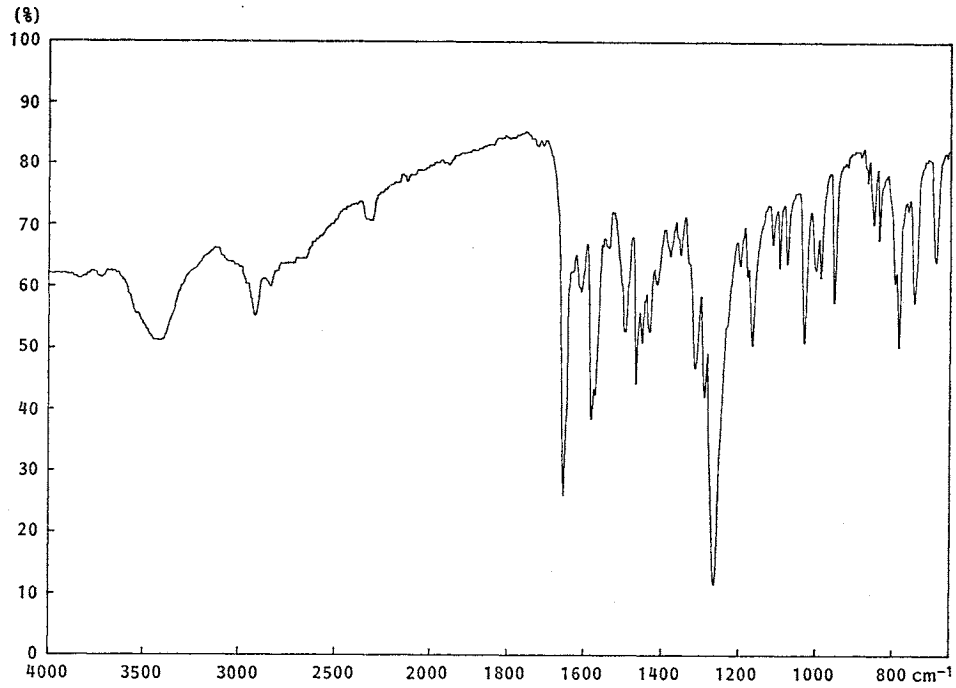


Table 2. ^{1}H NMR spectrum of I, II and III in $CDCl_3$ at 400 MHz.

Position	I	II	III
1-OH	—	—	11.15 (s)
2-H_2	3.03 (ABq, J=15.0 Hz)	2.94 (ABq, J=15.0 Hz)	—
2-H	—	—	7.15 (d, J=1.5 Hz)
3-CH_3	1.49 (s)	1.45 (s)	2.49 (s)
3-OH	2.29 (s)	2.51 (s)	—
4-H_2	3.16 (s)	3.05 (s)	—
4-H	—	—	7.26 (d, J=1.5 Hz)
5-H	7.50 (d, J=8.0 Hz)	6.92 (s)	8.13 (d, J=8.0 Hz)
6-H	8.26 (d, J=8.0 Hz)	—	8.31 (d, J=8.0 Hz)
6-OH	—	13.03 (s)	—
8-OCH_3	4.03 (s)	4.02 (s)	4.08 (s)
9-H	7.29 (dd, J=1.5, 8.0 Hz)	7.30 (dd, J=1.5, 8.0 Hz)	7.36 (dd, J=1.5, 8.0 Hz)
10-H	7.70 (t, J=8.0 Hz)	7.72 (t, J=8.0 Hz)	7.75 (t, J=8.0 Hz)
11-H	7.74 (dd, J=1.5, 8.0 Hz)	7.69 (dd, J=1.5, 8.0 Hz)	7.95 (dd, J=1.5, 8.0 Hz)

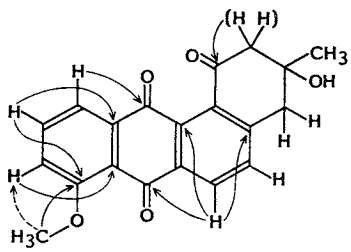
signal of hydrogen bonded hydroxyl group appeared at δ 13.03. These results indicate that 6-H in **I** is replaced by a hydroxyl group in **II**. NOE experiment indicated vicinal relationship between 9-H (δ 7.30) and 8-OCH_3 (δ 4.02). From the above mentioned results, antibiotic **II** was determined to be 8-*O*-methylrabelomycin. **II** must have the same configuration as rabelomycin since the negative value of optical rotation was almost the same as that of rabelomycin.

The NMR spectrum of **III** indicated that **III** was related to tetrangulol. The methylene signals at δ 2.94 (2H) and 3.05 (2H) which were observed in the ^{1}H NMR spectrum of **II** disappeared in the spectrum of **III**. The singlet signal of methyl group (δ 2.49) and two new aromatic protons [δ 7.15 (d, J=1.5 Hz), 7.26 (d, J=1.5 Hz)] appeared in the spectrum of **III**. The presence of the signal at δ 7.26 overlapping with solvent signal was confirmed by spin decoupling

Table 3. ^{13}C NMR chemical shifts of I and II (100 MHz, $CDCl_3$).

Position	I	II
C-1	196.9	195.8
C-2	53.8	53.6
C-3	72.5	71.9
C-4	44.0	44.1
C-4a	146.4	149.5
C-5	133.7	121.8
C-6	130.0	163.8
C-6a	135.1 (or 135.2)	117.6
C-7	181.4	188.1
C-7a	120.6	119.6
C-8	159.8	160.2
C-9	117.2	117.4
C-10	135.4	136.3
C-11	119.6	119.8
C-11a	137.7	137.7
C-12	184.6	184.6
C-12a	135.2 (or 135.1)	137.5
C-12b	134.2	127.0
3-CH_3	29.9	29.7
8-OCH_3	56.5	56.5

Fig. 4. The structure of I.



→: Part of $^3J_{C-H}$ and $^2J_{C-H}$ couplings observed in I by the long range ^{13}C-^1H COSY experiment.
⇢: The NOE arising from irradiation of OCH_3 at δ 4.03.

experiment. A signal of hydrogen bonded hydroxyl group was seen at δ 11.15 which was not observed in the spectrum of I. Therefore, the compound III was found to be X-14881 E[1] on the basis of spectroscopy. Treatment of I with 1 N NaOH gave a dehydrated product, which was identical with III by comparison of their TLC, UV and IR spectra. The presence of III in the culture broth was analyzed by TLC of BuOAc extract which was taken during the fermentation.

Table 4. The antibacterial spectrum on Mueller-Hinton agar.

Test organism	MIC (μg/ml)	
	I	II
Staphylococcus aureus Smith	100	12.5
S. aureus MS9610 (multi-resistant)	100	12.5
Micrococcus luteus PCI 1001	100	25
Bacillus subtilis NRRL B-558	100	12.5
Escherichia coli NIHJ	>100	50
Shigella dysenteriae JS11910	100	50
Salmonella typhi T-63	>100	>50
Proteus vulgaris OX19	>100	>50
Serratia marcescens	100	50
Pseudomonas aeruginosa A3	>100	>25
Klebsiella pneumoniae PCI 602	>100	>50
Mycobacterium smegmatis ATCC 607	>100	>50
Candida albicans 3147	>100	>50

The antibacterial activity of I and II were shown in the Table 4. III was less active than that of I against Gram-positive and Gram-negative bacteria. The acute toxicities (LD_{50}, ip) of I and II in mice were >100 mg/kg and >100 mg/kg, respectively.

We have isolated other *Streptomyces* strains which produced these benz[a]anthraquinone antibiotics: Strain MH897-SF5 produced I and II, strain MI165-34F3 produced only I, strain MI334-21F4 produced rabelomycin.

References

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