MODIFICATION OF THE MACROLIDE ANTIBIOTIC MIDECAMYCIN

III. FORMATION OF NEOISOMIDECAMYCIN

SHOJI OMOTO, HIROKO OGINO, KATSUYOSHI IWAMATSU and SHIGEHARU INOUYE

Central Research Laboratories, Meiji Seika Kaisha Ltd.
Morooka-cho, Kohoku-ku, Yokohama 222, Japan

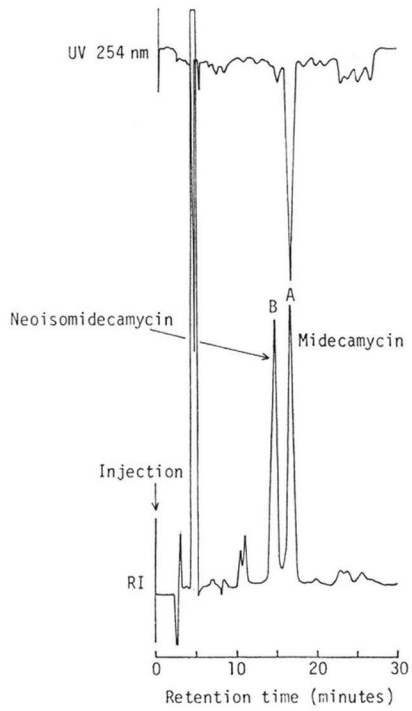
(Received for publication August 6, 1982)

Treatment of 9-trichloroacetylmidecamycin with aqueous alkali gave an isomer of midecamycin (neoisomidecamycin), in which one double bond underwent allylic rearrangement. The formation of neoisomacrolides was dependent on the nature of protective groups and conditions of deprotective reaction. Treatment of 9-trichloroethoxycarbonylmidecamycin with Zn/acetic acid resulted in no allylic rearrangement.

In an attempt to replace the C-4″ propionyl group of midecamycin (**I**) with other acyl groups, the C-9 hydroxyl group of 4″-depropionylmidecamycin[1] was protected with a trichloroacetyl group followed by acylation of the C-2′ and C-4″ hydroxyl groups. After deprotection of C-9, the products isolated turned out to be composed of an almost equal mixture of two components. One was the product expected and the other was found to be a product unexpectedly formed *via* allylic rearrangement involving one double bond. This type of allylic rearrangement has not been observed in the macrolide series so far, therefore we proposed the name "neoisomidecamycin". This paper deals with the preparation, physico-chemical properties and biological properties of neoisomidecamycin (II). The effects of various protective groups and/or deprotecting agents on the formation of neoisomidecamycin are also discussed.

Fig. 1. HPLC of the product obtained by alkali treatment of **Ia**.

Column: Develosil ODS-7 (4.6 mm×250 mm). Mobile phase: CH_3CN - CH_3OH - 0.2 M CH_3COO-NH_4 - H_2O, 45: 15: 5: 35. Flow rate: 1.0 ml/minute.



Preparation, Isolation and Properties of Neoisomidecamycin

First, the C-9 hydroxyl group of **I** was acylated with trichloroacetyl chloride which was chosen because of its easy introduction and smooth removal without affecting other sensitive parts of the molecule. After treatment with mild alkali, products were isolated by the use of silica gel column chromatography. They were seemingly homogeneous on TLC (SiO_2, benzene - acetone, 2: 1), but the PMR spectrum of the product showed two methoxy signals closely spaced at

3.55 and 3.57 ppm, with almost equal intensity. HPLC analysis of the product (Fig. 1) showed practically a single peak A (**I**) when detected with a UV detector at 254 nm, but an RI detector clearly revealed the presence of a second component B as well as midecamycin. Separation of the two components from each other was rather difficult, but was accomplished by silica gel LC coupled with semi-preparative HPLC on Nucleosil $5C_{18}$. The component A was identified as midecamycin from its retention time and physico-chemical properties.

Structure of Neoisomidecamycin

Compound B showed a molecular weight and molecular formula identical with those of midecamycin. Further, properties of B (Table 1) were very similar to those of midecamycin with the exception that component B showed end absorption in the UV spectrum. The only major difference in the PMR spectrum between midecamycin and B appeared in the region of olefinic protons, as shown in Fig. 2. These results suggested that compound B was an isomer of midecamycin involving a change in its diene system. Physico-chemical properties of 4″-acetyl derivatives, **Ig** and **IIb** are also shown in Table 1.

As for the isomerization of the diene alcohol system in 16-membered macrolides, isomacrolides such as isoleucomycin are already known to exist[2], in which two conjugated double bonds have undergone allylic rearrangement. Neoisomidecamycin could readily be differentiated from isomidecamycin, be-

Table 1. Physico-chemical properties of **I**, **IIa**, **Ig** and **IIb**.

	I	**IIa**	**Ig**	**IIb**
Appearance	Crystal	Powder	Crystal	Powder
mp	153~158°C	114~116°C	142~146°C	125~131°C
UV (EtOH)	λ_{max} 232 nm (ε 26,400)	End absorption	λ_{max} 232 nm (ε 27,000)	End absorption
IR (KBr)	Very similar		Very similar	
MS	Identical (M^+: 813)		Identical (M^+: 799)	
$[\alpha]_D^{22}$ (c 1.0, EtOH)	−67°	−53°	−63°	−54°
Rf: Silica gel TLC ($CHCl_3$ - MeOH, 15: 1)	0.45	0.47	0.65	0.67
PMR (100 MHz, ppm in $CDCl_3$)	-NMe: 2.54 -OMe: 3.55 -CHO: 9.64	-NMe: 2.54 -OMe: 3.57 -CHO: 9.62	-NMe: 2.54 -OMe: 3.55 -OAc: 2.17 -CHO: 9.64	-NMe: 2.54 -OMe: 3.57 -OAc: 2.16 -CHO: 9.64

Fig. 2. PMR spectra of **I** and **IIa** (100 MHz, $CDCl_3$).

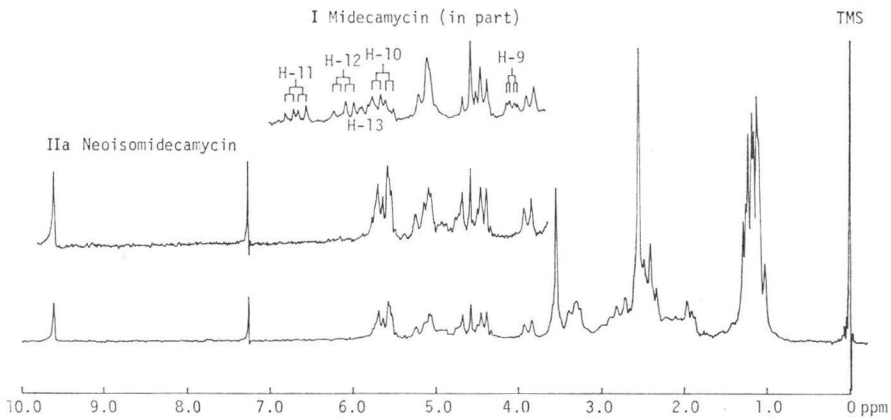
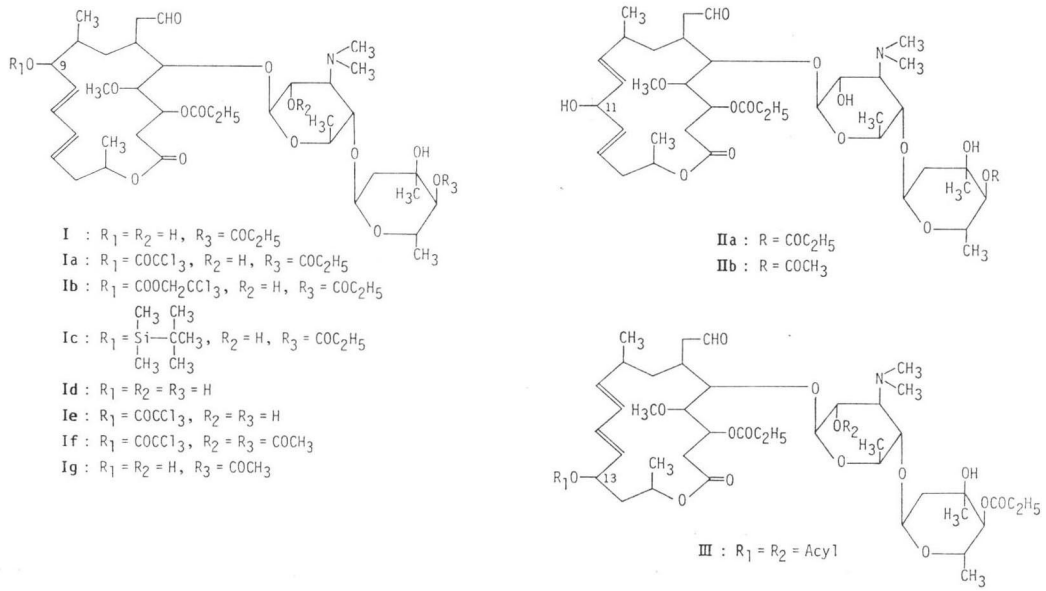


Chart 1. Structures of midecamycin (**I**), neoisomidecamycin (**II**) and isomacrolide (**III**).



cause isomidecamycin should show a UV absorption similar to that of **I**. Since compound B showed end absorption in UV spectrum with retention of two double bonds, the most probable structure of component B satisfying the above properties was structure **IIa** possessing 9,12-diene-11-ol instead of 10,12-diene-9-ol system which could be formed *via* allylic rearrangement involving only one double bond as shown in Chart 1.

Neoisomidecamycin (**II**) was the first sixteen membered macrolide possessing a non-conjugated diene system, although allylic rearrangement itself has been known to occur often in allylic esters[3].

Effect of Protective Groups on Formation of Neoisomidecamycin

Protection and deprotection of allylic hydroxyl group of macrolide antibiotics was thought to be one of the fundamental processes in their chemical modification. In order to get high yields, it was necessary to find the effect of various protective groups and/or deprotecting agents. Several protective groups were selected and tested to determine if allylic rearrangement occurred during deprotection of midecamycin derivatives. The ratio of a normal product, midecamycin, and a rearranged neoisomidecamycin was determined by densitometry on TLC (SiO_2, chloroform - methanol, 15: 1, without UV indicator) and the results are listed in Table 3. It was surprising that neoisomidecamycin was rather readily formed either by aqueous alkali, acid or even silica gel. However formation of neoisomidecamycin alone has not been recognized so far. Easy formation of neoisomidecamycin during silica gel column chromatography made the purification of the C-9 acylated or silylated derivatives rather difficult. On the other hand, deprotection by methanolic ammonia or 10% aqueous pyridine did not give any neoiso-isomer. Trichloroethoxycarbonyl derivative gave also the normal product when treated with Zn/acetic acid, but it did give neoiso-isomer when treated with aqueous alkali or even after silica gel column chromatography. Of particular interest was the fact that neoiso-isomer was formed also by the treatment of 9-(*t*-butyldimethylsilyl) derivative with acetic acid.

Table 2. Antibacterial spectra of **I**, **IIa**, **Ig** and **IIb**.

Test organisms	MIC (μg/ml)			
	I	**IIa**	**Ig**	**IIb**
Staphylococcus aureus 209P JC-1	0.39	0.39	3.13	3.13
Staphylococcus aureus Smith (1)	0.78	0.78	6.25	6.25
Staphylococcus aureus No. 26	1.56	1.56	6.25	3.13
Staphylococcus epidermidis ATCC 14990	0.78	0.78	3.13	3.13
Staphylococcus epidermidis N-0026	1.56	1.56	1.56	0.78
Streptococcus faecalis ATCC 8043	0.78	0.78	3.13	1.56
Bacillus anthracis No. 119	0.78	0.39	0.39	1.56
Bacillus subtilis ATCC 6633	0.78	0.39	1.56	3.13
Salmonella typhi O-901-W	>100	>100	>100	>100
Salmonella enteritidis No. 11	25	25	25	25

Inoculum size: 10^6 CFU/ml
Medium: Nutrient agar (Difco)

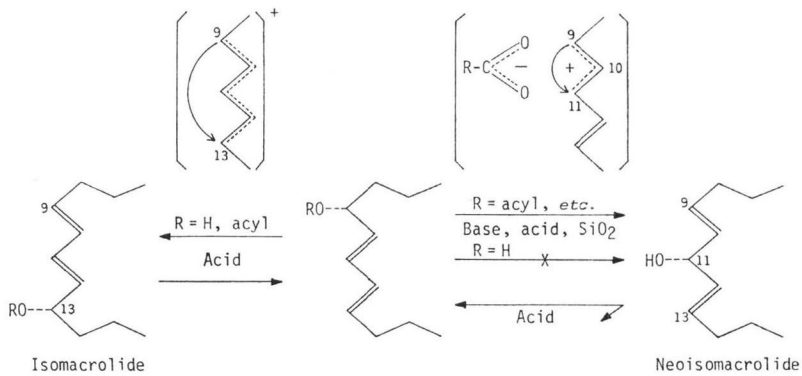
Biological Properties of Neoisomidecamycin (**IIa**)

The antibacterial spectrum of **IIa** is listed in Table 2 with that of 4″-acetyl analog (**IIb**). The activity of **IIa** was essentially identical to that of midecamycin. This indicates that the position of a double bond in the lactone ring did not effect bioactivity.

Discussion

Stereochemistry of the 11-hydroxyl group in **II** was not known, but it was likely to be on the same side of the lactone ring as original compound because allylic rearrangement was considered to take place *via* carbonium ion pair system followed by hydrolysis[4]. Neoisomacrolide (**II**) was structurally an intermediate between normal macrolide **I** and isomacrolide **III**. But upon treatment with dilute hydrochloric acid, **II** was smoothly converted to **I**, but not to **III**. Therefore, neoisomacrolide and isomacrolide seemed to be thermodynamically unstable. Isomerization of the allylic system is summarized in Fig. 3. Since **I**, with free C-9 hydroxyl group, did not give **II** under the same basic condition, removal of the protective group was considered to play an important role in the formation of neoisomacrolide. In view of our results, one must carefully analyze the deprotective reaction of allylic alcohol, because HPLC and TLC analyses based on the UV absorption could possibly miss the formation of neoiso-isomer.

Fig. 3. Isomerization of allylic system.



Experimental

Selective Protection of the C-9 Hydroxyl Group of Midecamycin (**I**) or 4″-Depropionylmidecamycin (**Id**)

Midecamycin (**I**, 1 mmole) was dissolved in 20 ml of benzene containing 2 ml of pyridine. To this was added dropwise acyl chloride or alkylsilyl chloride (1.1~1.5 mmole) dissolved in benzene (2 ml) and the mixture was stirred for 15~30 minutes at room temperature. The reaction was monitored by TLC. After usual work-up, 9-acylated or 9-silylated product (**Ia~Ic**) was obtained in a practically homogeneous state (~90%). Following compounds were prepared:

9-Trichloroacetylmidecamycin (**Ia**): Glass, mp 123~125°C (dec.), $[\alpha]_D^{22}$ −80° (*c* 0.5, EtOH), *Anal.* Found: C 53.58, H 7.20, N 1.24%, Calcd. for $C_{43}H_{66}NO_{16}Cl_3$: C 53.82, H 6.93, N 1.46%.

9-Trichloroethoxycarbonylmidecamycin (**Ib**): Glass, mp 125~129°C, $[\alpha]_D^{25}$ −80° (*c* 0.5, EtOH), *Anal.* Found: C 53.05, H 6.81, N 1.18%, Calcd. for $C_{44}H_{68}NO_{17}Cl_3$: C 53.41, H 6.93, N 1.41%.

9-*t*-Butyldimethylsilylmidecamycin (**Ic**): Glass, mp 125~129°C, $[\alpha]_D^{23}$ −75° (*c* 0.5, EtOH), EI-MS (*m/z*) 927, *Anal.* Found: C 69.50, H 9.12, N 1.39%, Calcd. for $C_{47}H_{81}NO_{15}Si$: C 60.81, H 8.80, N 1.51%.

9-*p*-Nitrophenyloxycarbonylmidecamycin was also prepared by treating **Ia** with *p*-nitrophenylchloroformate in pyridine. The product showed M+1 peak at *m/z* 798 in FD-MS, but it could not be purified further because of its instability.

4″-Depropionylmidecamycin[1] (**Id**) (1 mmole) was dissolved in 20 ml of benzene containing 2 ml of pyridine. To this was added dropwise trichloroacetyl chloride (1.1~1.5 mmole) in benzene (2 ml) and the mixture was stirred for 15 minutes. After usual work-up, 9-trichloroacetyl-4″-depropionylmidecamycin (**Ie**) was obtained in practically homogeneous state. **Ie** was acetylated in a usual manner to give 2′,4″-diacetyl derivative of **Ie** almost quantitatively. Following compounds were prepared:

9-Trichloroacetyl-4″-depropionylmidecamycin (**Ie**): Glass, mp 123~135°C (dec.), $[\alpha]_D^{22}$ −69° (*c* 0.86, EtOH), *Anal.* Found: C 53.18, H 6.78, N 1.26, Cl 11.42%, Calcd. for $C_{40}H_{62}NO_{15}Cl_3$: C 53.18, H 6.92, N 1.55, Cl 11.79%.

2′,4″-Diacetyl-9-trichloroacetyl-4″-depropionylmidecamycin (**If**): Glass, mp 127~136°C (dec.), $[\alpha]_D^{22}$ −75° (*c* 0.6, EtOH), *Anal.* Found: C 53.14, H 6.85, N 1.28, Cl 10.35%, Calcd. for $C_{44}H_{66}NO_{17}Cl_3$: C 53.51, H 6.74, N 1.42, Cl 10.79%.

Deprotection of Protective Groups

Deprotection of C-9 protective groups was effected by following conventional methods using aqueous alkali as listed in Table 3.

Preparation of Neoisomidecamycin

Two grams of a crude mixture of **I** and **IIa**, which was obtained from 9-trichloroacetyl derivative by

Table 3. Properties of various protective groups and deprotecting reagents.

Protective group	Deprotecting reagent	I : IIa
CH_3CO–	Phosphate buffer pH 7.0, 50°C	*ca.* 50 : 50
Cl_3CCO–	5% Na_2CO_3(2)/acetone(8), room temperature, 18 hours	50 : 50
	NH_3/MeOH(10 minutes) or 90% pyridine (18 hours)	>98 : <2
O_2N–C_6H_4–OCO–	Aqueous K_2CO_3 or imidazole	50 : 50
	NH_3/MeOH	>98 : <2
H_3C–C(CH$_3$)(CH$_3$)–Si(CH$_3$)(CH$_3$)–	60% Acetic acid	70 : 30
Cl_3CCH_2OCO–	Aqueous K_2CO_3 or SiO_2	40 : 60
	Zn/aqueous acetic acid	>98 : <2

treating **Ia** with a mixture of acetone (8 parts) and 5% Na_2CO_3 (2 parts) at room temperature overnight, were chromatographed on a silica gel column (2.0 cm×120 cm, Wako-gel C-300, $CHCl_3$ - MeOH, 30: 1, ~15 kg/cm²) to give partially purified samples of **I** (700 mg) and **IIa** (780 mg). Final purification was carried out by HPLC using Nucleosil $5C_{18}$ (10 mm×250 mm) (MeOH - 0.1 M acetate buffer (pH 4.7) - CH_3CN, 40: 15: 45) to give pure samples of **I** (300 mg) and **IIa** (350 mg).

Isolation of C-4″ acetyl analog was effected by the same procedure. A mixture of **Ig** and **IIb** was obtained from 2′,4″-diacetyl-9-trichloroacetyl-4″-depropionyl derivative by treatment with aqueous 80% methanol at room temperature overnight. Final purification was carried out by HPLC using Nucleosil $5C_{18}$ (10 mm×250 mm) (MeOH - 0.2 M NH_4OAc (pH 7.0) - CH_3CN - H_2O, 15: 5: 45: 35) to give pure samples of **Ig** (200 mg) and **IIb** (320 mg), respectively.

Acknowledgment

The authors wish to thank Dr. M. NATSUME, Itsuu Institute, Tokyo, for his kind suggestion on the studies of neoisomidecamycin.

References

1) TSURUOKA, T.; S. INOUYE, T. SHŌMURA, N. EZAKI & T. NIIDA: Studies on antibiotic SF-837, a new antibiotic. IV. Structures of antibiotics SF-837 A_2, A_3 and A_4. J. Antibiotics 24: 526~536, 1971
2) OMOTO, S.; S. INOUYE & T. NIIDA: Monoesters of antibiotic SF-837 and leucomycin A_3. German Patent 2,230,729 (1973); Japan Kokai Patent 48-13,380 [C. A. 78: 84771 (1973)]
3) ŌMURA, S.; A. NAKAGAWA, M. KATAGIRI, T. HATA, M. HIRAMATSU, T. KIMURA & K. NAYA: Chemistry of leucomycins. VIII. Absolute configuration of leucomycin and isoleucomycin. Chem. Pharm. Bull. (Tokyo) 18: 1501~1508, 1970
4) P. DE MAYO: Molecular Rearrangement. John Wiley & Sons, New York, 1963
5) GOERING, H. L. & M. PONBO: Stereochemistry of allylic rearrangements. X. The isomeric rearrangement and solvolysis of *trans* α,γ-dimethylallyl-*p*-nitrobenzoate in aqueous acetone. J. Amer. Chem. Soc. 82: 2515~2521, 1960