AN ANALYSIS OF THE ^{1}H AND ^{13}C NMR SPECTRA OF THE MACROLIDE ANTIBIOTIC, NEOISOMIDECAMYCIN

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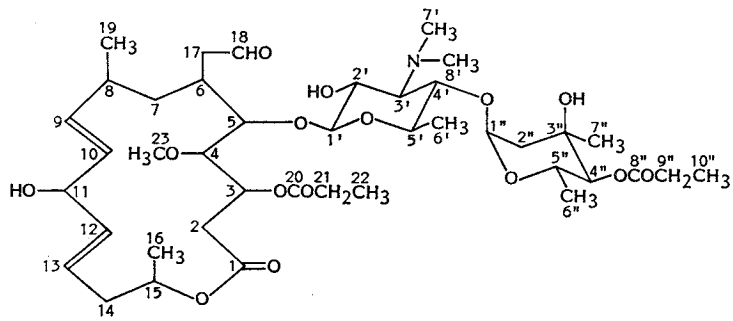
Neoisomidecamycin (**1**) is an isomer of midecamycin (**2**), a clinically important 16-membered macrolide antibiotic[1,2].

In the previous paper, we reported that neoisomidecamycin was prepared by treatment of 9-trichloroacetylmidecamycin with aqueous alkali[3]. We now complete the structural analysis of **1** by 2D ^{1}H and ^{13}C NMR experiments††.

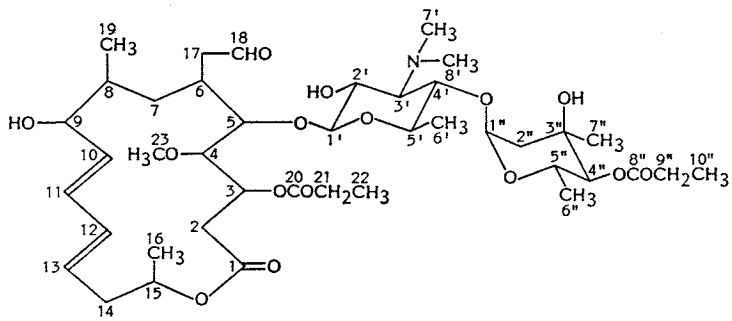
First, in order to analyze the NMR spectra of **1**, the ^{1}H and ^{13}C NMR chemical shifts of **2** were obtained using 2D NMR techniques. Several protons, such as the olefinic, anomeric and aldehyde protons, and two methyl singlet protons of dimethylamino group and methoxy group, were assigned readily from the 400 MHz ^{1}H NMR spectrum of **2**, but the other protons were overlapped and could not be analyzed directly with certainty. The ^{13}C NMR spectrum of **2** showed all 40 signals anticipated including the dimethylamino resonance of the equivalent C-7′ and C-8′ carbons of mycaminose.

All of the ^{1}H and ^{13}C NMR chemical shifts for **2** were obtained by the use of 2D NMR

Fig. 1. Structures of neoisomidecamycin (**1**) and midecamycin (**2**).



Neoisomidecamycin (**1**)



Midecamycin (**2**)

†† A part of this work was presented at the 104th Annual Meeting of Pharmaceutical Society of Japan, Sendai, Miyagi, 1984.

Table 1. ^{1}H NMR chemical shifts[a] and ^{1}H-^{1}H coupling constants for **1** and **2**.

Position	1			2		
	δ_H (ppm)	m[b]	J_{HH} (Hz)	δ_H (ppm)	m[b]	J_{HH} (Hz)
2-H_{ax}	2.71	dd	11.5, 12.0	2.72	dd	11.0, 13.5
2-H_{eq}	2.42*			2.24	dd	1.8, 13.5
3-H	5.19	dt	1.8, 11.5	5.11	dt	1.8, 11.0
4-H	3.35	dd	1.8, 9.2	3.21	dd	1.8, 9.5
5-H	3.89	dd	1.5, 9.2	3.85	dd	1.5, 9.5
6-H	2.05	m		2.13	m	
7-H_{eq}	1.42	dt	3.5, 13.5	1.42	dt	3.5, 13.5
7-H_{ax}	1.01	(dt)	13.5	0.92	dt	7.5, 13.5
8-H	2.13	m		1.88	m	
9-H	5.54	dd	6.0, 15.0	4.06	dd	4.5, 9.5
10-H	5.60	dd	4.5, 15.0	5.60	dd	9.5, 15.0
11-H	4.72	dd	4.5, 7.8	6.62	dd	10.5, 15.0
12-H	5.64	ddd	1.5, 7.8, 15.0	6.05	ddd	1.5, 10.5, 15.0
13-H	5.77	ddd	3.8, 9.5, 15.0	5.75	ddd	3.8, 11.3, 15.0
14-H_{eq}	2.41*			2.45*		
14-H_{ax}	2.30	dt	11.5, 13.5	2.12	dt	11.3, 13.5
15-H	4.92	ddq	3.5, 6.5, 11.5	5.01	ddq	3.5, 6.5, 11.3
16-H	1.26	d	6.5	1.24	d	6.5
17-H_{ax}	2.85	dd(d)	11.0, 18.0, (1.5)	2.80	ddd	1.5, 11.5, 18.0
17-H_{eq}	2.37	dd	(3.0), 18.0	2.33	dd	3.0, 18.0
18-H	9.63	s		9.62	s	
19-H	1.06	d	6.5	0.98	d	6.5
21-Ha	2.61	dq	7.5, 16.0	2.60	dq	7.5, 17.0
21-Hb	2.48*			2.48	dq	7.5, 17.0
22-H	1.16	t	7.5	1.19	t	7.5
23-H	3.55	s		3.51	s	
1′-H	4.42	d	7.8	4.39	d	7.5
2′-H	3.50	dd	7.8, 10.3	3.49	dd	7.5, 10.0
3′-H	2.51*			2.44	(dd)	7.5, 10.0
4′-H	3.29*	m		3.25	m	
5′-H	3.30*	m		3.27	m	
6′-H	1.20	d	6.0	1.17	br d	6.0
7′-H and 8′-H	2.53	s		2.49	s	
1″-H	5.07	br d	3.8	5.04	br d	3.8
2″-H_{eq}	2.02	dd	<1.0, 14.0	1.99	dd	1.0, 14.0
2″-H_{ax}	1.85	dd	3.8, 14.0	1.82	dd	3.8, 14.0
4″-H	4.62	d	10.5	4.60	d	10.5
5″-H	4.44	dq	6.0, 10.5	4.43	dq	6.0, 10.5
6″-H	1.14	d	6.0	1.11	d	6.0
7″-H	1.12	s		1.09	s	
9″-Ha	2.49*			2.44	dq	1.5, 7.5
9″-Hb	2.43*			2.39	dq	1.5, 7.5
10″-H	1.18	t	7.5	1.16	t	7.5

[a] ppm downfield from TMS in $CDCl_3$. [b] Multiplicity. * Not definited.

techniques, such as *J*-resolved, ^{1}H-^{1}H correlation spectroscopy (COSY), distortionless enhancement by polarization transfer (DEPT) and ^{13}C-^{1}H COSY experiments. The *J*-resolved projection was compared with the 1D spectrum and almost all of the chemical shifts were identified clearly by the cross-sections. The ^{1}H-^{1}H COSY spectrum was analyzed for coupled spin systems starting with the olefinic protons on the lactone ring successively and then the macrolide ring protons were assigned. The sugar part was analyzed similarly starting with each anomeric

proton. Both 4′-H and 5′-H protons of mycaminose had nearly identical shifts with each other. The signals of 3′-H proton of mycaminose and 14-H_{eq} proton on the lactone ring were buried at 2.45 ppm, but these assignments were possible from the DEPT and ^{13}C-^{1}H COSY experiments.

Table 2. ^{13}C NMR chemical shifts[a] of **1** and **2**.

Position	**1**		**2**	
	δ_c (ppm)	m[b]	δ_c (ppm)	m[b]
C-1	169.67	s	169.80	s
C-2	38.39	t	37.01	t
C-3	70.58	d	68.57	d
C-4	85.47	d	84.81	d
C-5	78.50	d	77.52	d
C-6	29.95	d	28.83	d
C-7	35.75	t	30.37	t
C-8	32.35	d	33.47	d
C-9	135.34	d	73.17	d
C-10	131.36	d	127.46	d
C-11	75.92	d	135.71	d
C-12	132.37	d	131.87	d
C-13	130.44	d	132.69	d
C-14	39.01	t	40.92	t
C-15	71.56	d	68.90	d
C-16	20.29	q	20.30	q
C-17	43.49	t	42.34	t
C-18	201.19	s	201.10	s
C-19	21.12	q	14.65	q
C-20	173.45 or 174.34	s	173.80 or 174.30	s
C-21	27.57	t	27.58 or 27.53	t
C-22	8.95	q	8.86	q
C-23	62.20	q	62.35	q
C-1′	103.61	d	103.68	d
C-2′	72.89	d	71.56	d
C-3′	68.70	d	68.64	d
C-4′	77.06	d	75.85	d
C-5′	73.59	d	72.89	d
C-6′	18.81	q	18.76	q
C-7′, 8′	41.88	q	41.89	q
C-1″	97.00	d	96.93	d
C-2″	41.65	t	41.62	t
C-3″	69.34	s	69.30	d
C-4″	77.20	d	77.05	d
C-5″	63.51	d	63.41	d
C-6″	17.72	q	17.71	q
C-7″	25.29	q	25.24	q
C-8″	174.34 or 173.45	s	174.30 or 173.80	s
C-9″	27.37	t	27.53 or 27.58	t
C-10″	9.30	q	9.92	q

[a] ppm downfield from TMS in $CDCl_3$.
[b] Multiplicity.

According to the ^{13}C NMR chemical shifts of the metabolites of 9,3″-diacetylmidecamycin[4] and the ^{13}C-^{1}H COSY spectral data of **2**, the methyl triplets due to 22-H and 10″-H of the two propionyl units were assigned as 1.19 ppm and 1.16 ppm, respectively. The carbonyl carbons (C-20 and C-8″) and the methylene carbons (C-21 and C-9″) of these propionyl groups were not able to be distingushed in the ^{13}C-^{1}H COSY spectrum.

The long range coupling (*i.e.*, $J<2$ Hz) between 7″-H and 2″-H_{ax} protons of mycarose could be observed, as well as 6″-H and 4″-H, 1″-H and 5″-H, 12-H and 14-H_{eq} and 23-H and 4-H in the ^{1}H-^{1}H COSY spectrum.

In this way, the ^{1}H and ^{13}C NMR chemical shifts of **2** were obtained, as listed in Tables 1 and 2, respectively. The ^{13}C NMR chemical shifts of **2** are almost in agreement with the literature values reported on leucomycins by ŌMURA *et al.*[5,6].

Next, the same experimental conditions were used to obtain the 2D NMR spectral data of **1**. The ^{1}H and ^{13}C NMR chemical shifts for **1** are also presented in Tables 1 and 2, respectively.

The NMR spectra were analyzed considering the migration of the hydroxyl group on the macrolide ring. Comparing with **2**, the 400 MHz ^{1}H NMR spectrum of **1** was more complex. Four olefinic protons led to nearly identical shifts at about 5.5 ppm. On the basis of the ^{13}C-^{1}H COSY spectrum, it was found that 8-H proton was observed as a broad multiplet at 2.13 ppm, which was correlated with C-8 methine carbon connected to methyl group (19-H). The 8-H proton signal shape in the ^{1}H NMR spectrum of **1** was similar to that of **2**. The ^{1}H-^{1}H COSY spectrum of **1** was analyzed for correlated protons starting with the 8-H proton in a similar manner. Almost all of the lactone ring protons were able to be assigned and were shifted a little lower except for 11-H, 12-H and 6-H methine protons. It could be presumed that the 11-H proton peak was shifted to high field at 4.72 ppm as a new doublet of doublets. Also, the 9-H proton signal was shifted to low field at 5.54 ppm as a doublet of doublets. Among the olefinic proton signals, 10-H and 13-H were not shifted, but 12-H was shifted

higher at 5.64 ppm.

The ^{13}C NMR spectrum of **1** showed 40 signals. In the ^{13}C-^{1}H COSY spectrum, C-11 carbon was observed as an oxymethine carbon at 75.92 ppm and the peak was shifted to high field compared with the C-11 olefinic carbon signal of **2** at 135.71 ppm. C-9 was responsible for an olefinic carbon signal at 135.34 ppm and was shifted to low field compared with the C-9 carbon signal of **2** connected the hydroxyl group at 73.17 ppm. The C-10 and C-12 olefinic methine, C-7 methylene and C-19 methyl carbon signals were shifted lower, and the C-8 and C-13 methine carbon signals were shifted slightly higher.

The result of these ^{1}H and ^{13}C NMR spectral analyses of **1** are consistent with the proposal that **1** has a 9,12-diene-11-ol system instead of a 10,12-diene-9-ol system. This could be formed by an allylic rearrangement including only one double bond so that the hydroxyl group is not at C-9 but rather is at C-11 on the macrolide ring.

Thus, we could confirm the structure of neoisomidecamycin as shown in Fig. 1. An almost complete analysis of the ^{1}H and ^{13}C NMR spectra of midecamycin has been made for the first time.

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