STRUCTURAL REDETERMINATION OF NEW ERYTHROMYCIN DERIVATIVE : 2',4"-*O*-DIMETHYLERYTHROMYCIN A USING 2D(^{13}C-^{1}H)-NMR SPECTROMETRIC ANALYSIS

DETERMINASI KEMBALI STRUKTUR TURUNAN ERITROMISIN BARU: 2',4"-O-DIMETILERITROMISIN A DENGAN MENGGUNAKAN ANALISIS SPEKTROMETRIK-NMR 2D (^{13}C-^{1}H)

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ABSTRACT

Redetermination of the chemical structure of the sample E-1 has been carried out using 2D(^{13}C-^{1}H) NMR spectrometric approach. The appearance of cross-peaks at δ_H; δ_C (3.28;50.1) and (3.33;50.0) strongly suggested the present of two methoxy groups at C-2' of the desosamine and C-4" of the cladinose respectively. This spectrometric analysis confirmed that the E-1 chemical structure is 2',4"-*O*-Dimethylerythromycin A.

Key-words : 2D(^{13}C-^{1}H) NMR spectrometric approach.

ABSTRAK

Penetapan kembali struktur kimia sampel E-1 telah dilakukan dengan menggunakan pendekatan spektrometrik NMR 2D(^{13}C-^{1}H). Munculnya *cross-peaks* pada δ_H; δ_C (3,28;50,1) dan (3,33;50,0) memberikan dugaan kuat akan adanya dua gugus metoksi berturut-turut pada C-2' dari desosamin dan C-4" dari kladinosa. Analisis spektrometrik ini memberikan keyakinan bahwa struktur kimia E-1 adalah 2',4"-*O*-Dimetileritromisin A.

Kata-kunci: Pendekatan spektrometrik 2D(^{13}C-^{1}H).

INTRODUCTION

Two new erythromycin A derivatives have been produced by direct methylation of erythromycin A using ethereal solution of diazomethane (Mun'im, 1997). The two derivatives havE different R_f value on the thin layer chromatographic analysis. The lower R_f –derivative was designated as E-1; where as higher R_f –derivative was E-2. The E-2 derivative was structurally elucidated as 4"-*O*-methylerythromycin A (4"-MER) by using ^{1}H-, ^{13}C-, and 2D(^{13}C-^{1}H)-NMR spectroscopic analyses (Mun'im, 1997; Jenie, 1997).

The other derivative, E-1, was assigned by Mun'im (1997) as 2'-*O*-methyl-erythromycin A (2'-MER) based on ^{1}H- and ^{13}C-NMR spestroscopic data. This assignment however is casting some doubts. Mun'im (1997) proposed that ^{13}C-chemical shift (δ_C) at 72.674 ppm was due to the

resonance of methoxy carbon at C-2' of desosamine, 2'-OMe. According to Gharbi-Benarous *et al.*, (1993) the δ_C of the methoxy carbon appears at around 48 ppm. Moreover Mun'im (1997) proposed that the proton chemical shift (δ_H) at 3.330 ppm was methoxy protons of the C-2' of desosamine. Actually this δ_H of 3.330 ppm could also be assigned as methoxy protons at C-4" of cladinose. This structural uncertainty has been clarified using 2D(^{13}C-^{1}H)-NMR spectroscopic approach.

METHODOLOGY

Materials: The E-1 derivative was obtained from Mun'im (1997). **Instrument:** NMR Varian XL-600 Spectrometer with magnetic strength of 600 MHz for the ^{1}H, and 150.8 MHz for the ^{13}C. **Experiment:** ^{13}C-NMR spectrum of E-1 was obtained using broadband decoupling experiment. 2D(^{13}C-^{1}H)-NMR spectral data were obtained using COSY-HSQC pattern with the sweep width of 40651.41 Hz and 32768 data points for ^{13}C-NMR spectra, and sweep width of 12004.80 Hz and 16384 data points for ^{1}H-NMR spectra. The E-1 derivative was dissolved in deuterated methanol (CD_3OD) in a concentration of 5.10^{-2} mol.dm^{-3} , and using TMS as the internal standard. Assignment of the 2D(^{13}C-^{1}H)-spectral data of the E-1 derivative was carried out by comparing with that of the erythromycin A assigned by Watanabe *et al.* (1992) and Gharbi-Benarous *et al.*(1993).

RESULT AND DISCUSSION

The 2D(^{13}C-^{1}H)-NMR spectrum was presented in figures 1, 3, and 3. Figure 1 shows a limited window of the 2D-spectrum which is ranging from δ_H 0.8 – 2.0 ppm, and δ_C 10 – 30 ppm. Figure 1 shows cross-peaks of the aglycone methyl groups of the sample E-1. Figure 2 also a limited window of the 2D-spectrum which is ranging from δ_H 1.0 – 3.5 ppm, and δ_C 10 – 50 ppm. In addition to theses methyl groups, the figure 2 shows cross-peaks of protons and carbons of the macrolactone, the desosamine, and the methoxy groups. Figure 3 gives also a limited window of the 2D-spectrum ranging from δ_H 2.5 – 5.0 ppm, and δ_C 70 – 110 ppm. This figure shows crosspeaks of the cladinose, desosamine, and macrolactone. All the cross-peaks are tabulated in Table I.

The ^{1}H-NMR spectrum of sample E-1 (Mun'im, 1997) showed that integration of the resonance peak δ_H 3.300 ppm corresponds to six protons. That means that this resonance peak was due to two methoxy groups, in which one methoxy comes from the 3"-OMe of the cladinose, and the other one comes from the 2'-OMe of the desosamine. This assignment was confirmed by 2D(^{13}C-^{1}H)-NMR spectrum of the sample E-1 (see table I). The 2D-NMR spectrum shows cross-peaks at δ_H; δ_C (3.25; 49.0) and (3.28; 50.1) which were due to the methoxy protons and carbons at C-3" of cladinose and C-2' of desosamine respectively.

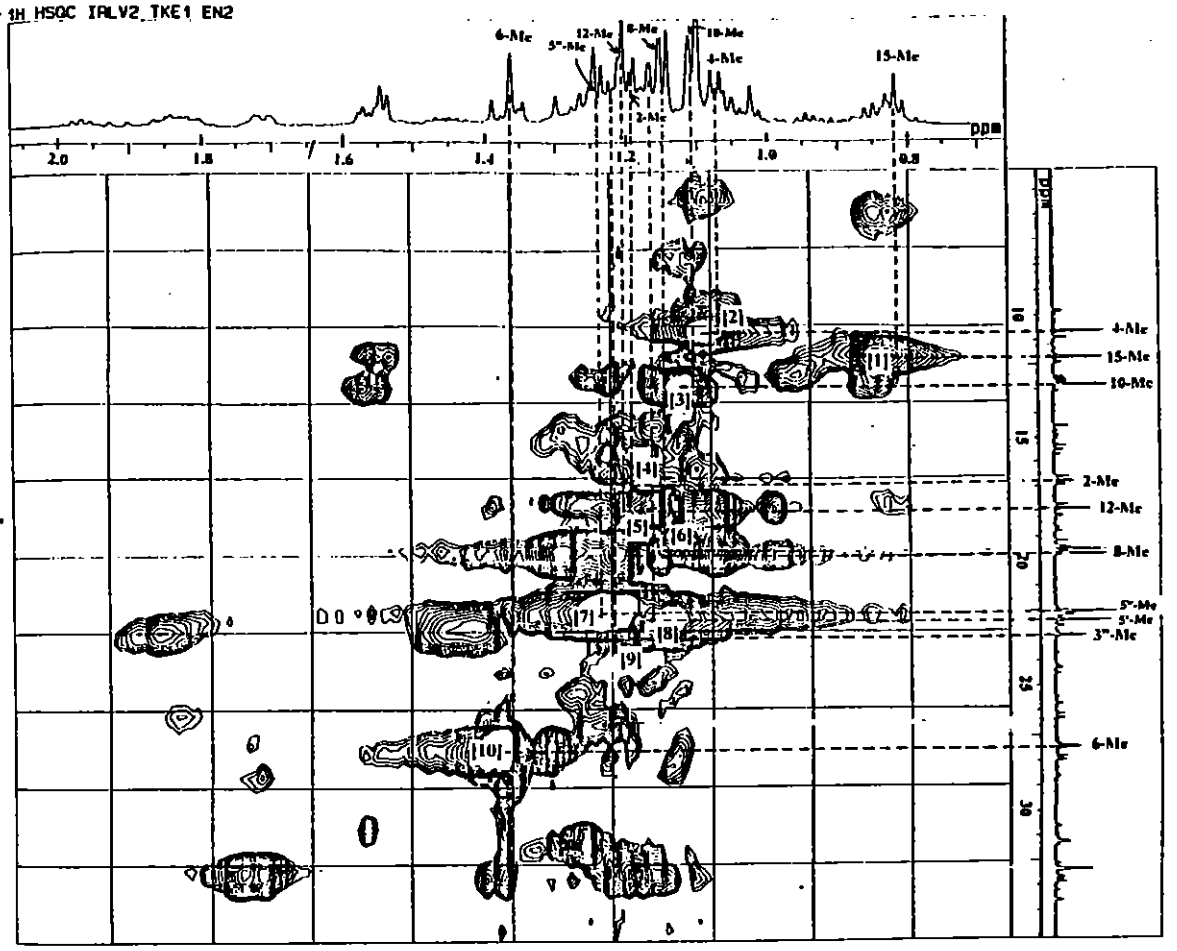

Figure 1 : Limited window of 2D-spectrum of 2',4"-*O*-dimethyl-erythromycin A (δ_H 0.8 – 2.0 ppm, and δ_C 10 – 30 ppm).

In addition there was a cross-peak at (3.33; 50.0) which was due to the methoxy protons and carbon at C-4" of the cladinose. Based on the assignment, the chemical structure of the sample

E-1 is 2',4"-*O*-dimethylerythromycin A (see figure 4). The previous assignment by Mun'im (1997) was then corrected.

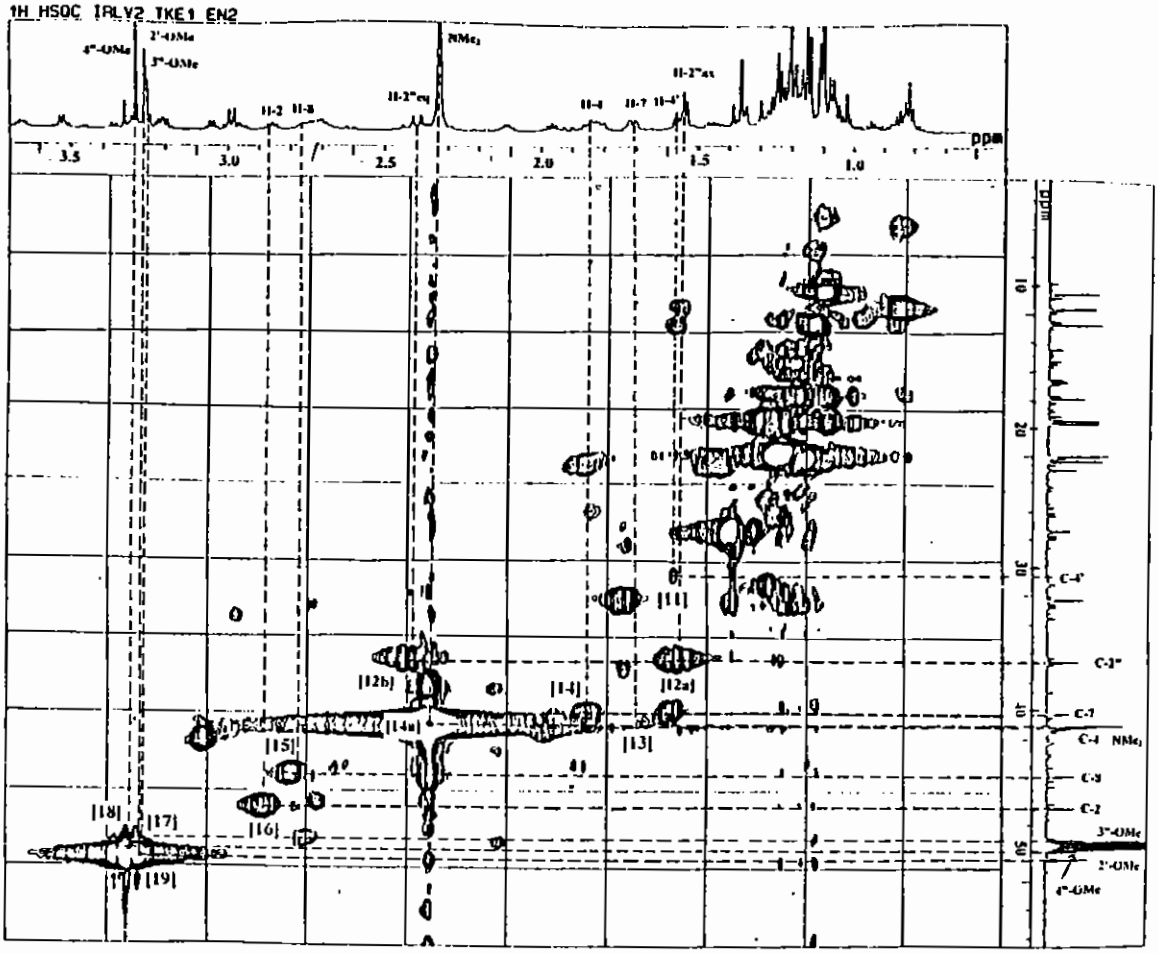

Figure 2 : Limited window of 2D-spectrum of 2',4"-*O*-dimethyl-erythromycin A (δ_H 1.0 – 3.5 ppm, and δ_C 10 – 50 ppm).

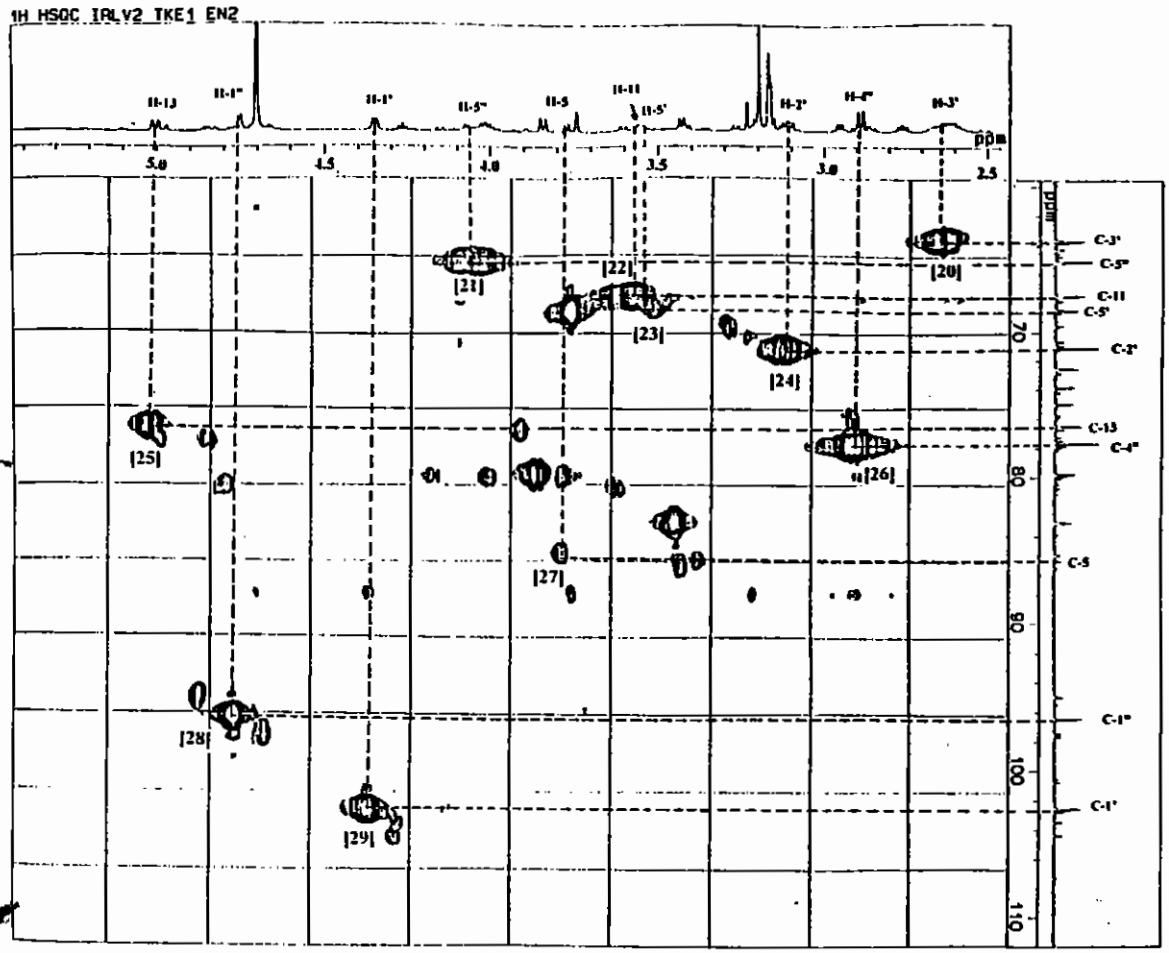

Figure 3 : Limited window of 2D-spectrum of 2',4"-*O*-dimethyl-erythromycin A (δ_H 2.5 – 5.0 ppm, and δ_C 70 – 110 pp

Table 1.: Proton, ^{13}carbon shifts (δ_H and δ_C), and cross peaks (δ_H, δ_C) of 2D(^{13}C-^{1}H) of 2',4"-*O*-Dimethylerythromycin A (in CD_3OD).

No.	Aglycone	δ_H (ppm)	δ_C (ppm)	(δ_H, δ_C)	Cross-peak
1	C-1	-	177.229	-	-
2	(CH)-2	2.890	46.369	(2.86; 46.9)	[16]
3	2-Me	1.223	16.283	(1.17; 16.8)	[4]
4	(CH)-3	4.400	81.298	Not observed	Not observed
5	(CH)-4	1.860	40.632	(1.84; 41.0)	[14]
6	4-Me	1.070	10.134	(1.07; 10.6)	[2]
7	(CH)-5	3.97	84.635	(3.78; 85.8)	[27]
8	C-6	-	76.530	-	-
9	6-Me	1.396	26.937	(1.36; 27.4)	[10]
10	(CH)-7	1.750 *ax.*	39.793	(1.71; 40.2)	[13]
		1.740 *eq.*		Not observed	Not observed
11	(CH)-8	2.751	44.166	(2.77; 44.8)	[15]
12	8-Me	1.180	19.201	(1.15; 19.5)	[6]
13	C-9	-	221.9	-	-
14	(CH)-10	3.080	31.902	Not observed	Not observed
15	10-Me	1.140	12..321	(1.12; 12.9)	[3]
16	(CH)-11	3.560	69.049	(3.58; 67.5)	[22]
17	C-12	-	75.387	-	-
18	12-Me	1.240	17.426	(1.19; 17.9)	[5]
19	(CH)-13	5.130	78.059	(5.01; 76.6)	[25]
20	(CH)-14	1.480 *ax.*	Not	Not observed	Not observed
		2.000 *eq.*	Observed		
21	15-Me	0.850	11.203	(0.82; 11.6)	[1]
	Amino sugar				
22	(CH)-1'	4.470	104.101	(4.35; 102.8)	[29]
23	(CH)-2'	3.240	72.674	(3.11; 70.2)	[24]
24	(CH)-3'	2.425	65.268	(2.63; 63.8)	[20]
25	N-Me_2	2.355	40.171	(2.34; 40.8)	[14-a]
26	(CH)-4'	1.600	30.734	(1.58; 30.7)	[11]
27	(CH)-5'	3.550	70.077	(3.54; 68.8)	[23]
28	5'-Me	1.272	21.889	(1.17; 22.4)	[8]
29	2'-OMe	3.300	49.995	(3.28; 50.1)	[19]
	Neutral sugar				
30	(CH)-1"	4.890	97.977	(4.76; 96.7)	[28]
31	(CH)-2"	1.575 *ax.*	36.111	(1.55; 36.8)	[12a]
		2.425 *eq.*		(2.40; 36.8)	[12b]
32	C-3"	-	74.113	-	-
33	3"-OMe	3.300	48.466	(3.25; 49.0)	[17]
34	3"-Me	1.262	22.489	(1.22; 23.0)	[9]
35	(CH)-4"	3.020	79.210	(2.89; 77.8)	[26]
36	4"-OMe	3.330	49.419	(3.33; 50.0)	[18]
37	(CH)-5"	4.145	66.789	(4.06; 65.4)	[21]
38	5"-Me	1.200	21.518	(1.24; 22.0)	[7]

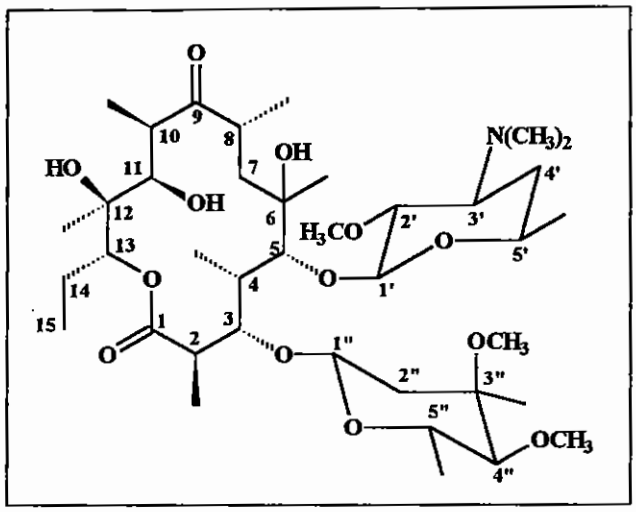

Figure 4 : Chemical structure of 2',4"-*O*-dimethylerythromycin A

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