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Structural elucidation of $\Delta^{6,7}$-anhydroerythromycin D using ^{1}H-NMR-spectrometer

Umar A. Jenie[1], Retno S. Sudibyo[1], and Winarto Haryadi[2]

1) IUC-Biotechnology, Gadjah Mada University, Yogyakarta, Indonesia

2) Department of Chemistry Faculty of Science and Mathematics, Gadjah Mada University, Yogyakarta, Indonesia

Abstract

^{1}H-NMR-spectrometric analysis was carried out to samples I and 2 which were isolated respectively from Saccharopolyspora erythraea *ATCC 11912 with and without additional isonicotinic hydrzide (INH). The sample I was confirmed as erythromycin D due to the proton appearance of 12-H at δ 2.317[5] (dq) of the macrolactone, and the absence of 3"-methoxy proton at 3.31 (s) of its neutral sugar. The sample 2 was confirmed as $\Delta^{6,7}$-anhydroerythromycin D due to the presence of 7-H(-C=C-) at δ 5. 343 (dq) instead of the methylene proton at C_7 and the presence of proton 12-H at δ 2.318 (dq) of the macrolactone.*

Keywords: *$\Delta^{6,7}$-anhydroerythromycin D – NMR-spectroscopic analysis.*

Introduction

Production of erythromycin derivative which is stable in acidic condition has been carried out by conversion of C_6-hydroxy group of the erythromycin into its $\Delta^{6,7}$(C_6=C_7) group. FT-IR spectrometric analysis of this compound showed a stretching vibration at wave number of 1604 cm^{-1} which indicated the presence of -C=C- double bond. Moreover, the IR spectrum also showed a wave number at 3111 cm^{-1} which due to stretching vibration of Sp^2 C-H group (Sudibyo *et al.*, 1999).

To confirm the structure of this new erythromycin derivative, a ^{1}H-NMR spectrometric analysis was carried out.

Methodology

Material: $\Delta^{6,7}$-anhydroerythromycin derivative which was produced by fermentation of mutant *Saccharopolyspora erythraea* ATCC 11912 added with 0.1% isonicotinic hydrazide (INH).

Instrument: FT-^{1}HNMR Varian XL600 Spectrometer.

NMR experiment: The NMR analysis of the $\Delta^{6,7}$-anhydroerythromycin derivative is carried out in deuterated chloroform ($CDCl_3$). The ^{1}H-NMR data was obtained by using sweep frequency of 599.970 MHz and sweep widths of 10,000 Hz for 64,000 data points. Assignment of the ^{1}H-NMR spectra of the erythromycin D (produced by mutant

Sac. erythraea ATCC 11912 without addition of INH) and the $\Delta^{6,7}$-anhydroerythromycin derivative (produced by mutant *Sac. erythraea* ATCC 11912 with addition of INH) was carried out by comparing with spectrum data of the erythromycin A done previously by Everett and Taylor (1985), and Gharbi-Benarous *et al.* (1993).

Table 1. Proton chemical shifts of erythromycin D, $\Delta^{6,7}$-anhydroerythromycin D, and erythromycin A

Protons	Erythromycin D produced by *Sac. erythaea* without INH addition (Sample 1). (δ, ppm.)	$\Delta^{6,7}$-Anhydroerythromycin D produced by *Sac. erythaea* due to the INH addition (Sample 2). (δ, ppm.)	Erythromycin A (Everett and Taylor, 1985) (δ, ppm.)	Erythromycin A (Gharbi-Benarous *et al.*,1993) (δ, ppm.)
Macrolide:				
Methyl Groups				
2-CH_3	1.074 *d*	1.078, broadening	1.175, *d*, broadening	1.18
4-CH_3	0.922 *d*	0.922[5] *d*	1.10, *d*	1.10
6-CH_3	1.575 overlap	1.595 *s*, overlap	1.46,*s*, broadening	1.46
8-CH_3	1.015[5] *d*	1.017, broadening	1.155, *d*	1.16
10-CH_3	1.058[5] *d*	0.962, broadening	1.135, *d*	1.14
12-CH_3	1.016 *s*	1.052, broadening	1.12, *s*, broadening	1.12
15-CH_3	0.888 *t*	0.889 *t*	0.84, *t*	0.84
Lacton protons				
2-H	2.773[5] *dq*	2.777 *q*, broadening	2.87, *dq*	2.87
3-H	3.660 *t*	3.648 *dd*	3.99, *dd*	3.99
4-H	1.903 *m*, overlap	1.983 *mt*	1.97, *ddq*	1.97
5-H	3.602[5] *d*, overlap	3.600 *d*, broadening	3.56, *d*	3.56
7-H *axial*	1.916 *d*, broadening	-	1.93, *dd*	1.93
7-H *equatorial*	1.740 overlap	-	1.74, *ddd*	1.74
7-H (-C=C-)	-	5.343 *dq*	-	-
8-H	2.636 *m*	2.540[5] *dq*	2.68, *ddq*	2.68
10-H	3.567[5] *dq*, overlap	3.549 *dm*	3.08, *dq*	3.08
11-H	3.612 *d*	4.153 *dd*	3.82, *d*, broadening	3.82
11-OH	3.952 *s*, broadening	3.950 *s*, broadening	3.95, *s*, very broadening	3.95
12-H	2.317[5] *dq*	2.318 *dq*	-	-
13-H	4.250 *td*	4.301[5] *dd*	5.03, *dd*	5.03
14-H *axial*	1.438[5] *d*, broadening, overlap	1.561 overlap	1.475, *ddq*	1.48 1.91
14-H *equatorial*	1.926 *d*, broadening, overlap	1.923 *dq*	1.91, *ddq*	
Desosamine:				
1'-H	4.030 *dd*	4.056 *d*, overlap	4.40, *d*	4.40
2'-H	2.773[5] *dd*, overlap	2.764 *d*, broadening	3.21, *dd*	3.21
3'-H	2.360 *t*, broadening	2.364 *t*, broadening	2.43, *ddd*	2.43
3'-$N(CH_3)_2$	2.182 *s*	2.182 *s*	2.29, *s*	2.29
4'-H *axial*	1.262 overlap	1.263 overlap	1.22, *ddd*	1.22
4'-H *equatorial*	1.575 overlap	1.561 overlap	1.665, *ddd*	1.67
5'-H	3.567[5] *dq*, overlap	3.565 *tq*, overlap	3.48, *ddq*	3.48
5'-CH_3	1.262 overlap	1.526[5] *d*, overlap	1.22, *d*	1.22
Cladinose/mycarose:				
1''-H	4.094 *dt*	4.062 *t*	4.88, *d*, broadening	4.88
2''-H *axial*	1.575 overlap	1.560 *dd*, overlap	1.56, *dd*	1.56
2''-H *equatorial*	2.330 *d*, overlap	2.314 *dd*	2.35, *dd*	2.35
3''-CH_3	1.262 overlap	1.561 *s*, overlap	1.23, *s*	1.23
3''-OCH_3	-	-	3.31, *s*	3.31
4''-H	2.766 *d*, overlap	2.777 *d*, broadening	3.00, *dd*	3.00
4''-OH	2.108 *s*	2.111 *s*, overlap	2.23, *d*	2.23
5''-H	3.989 *q*, broadening	4.227[5] *dq*	3.99, *dq*	3.99
5''-CH_3	1.262 overlap	1.526 *s*, overlap	1.27, *d*	1.27

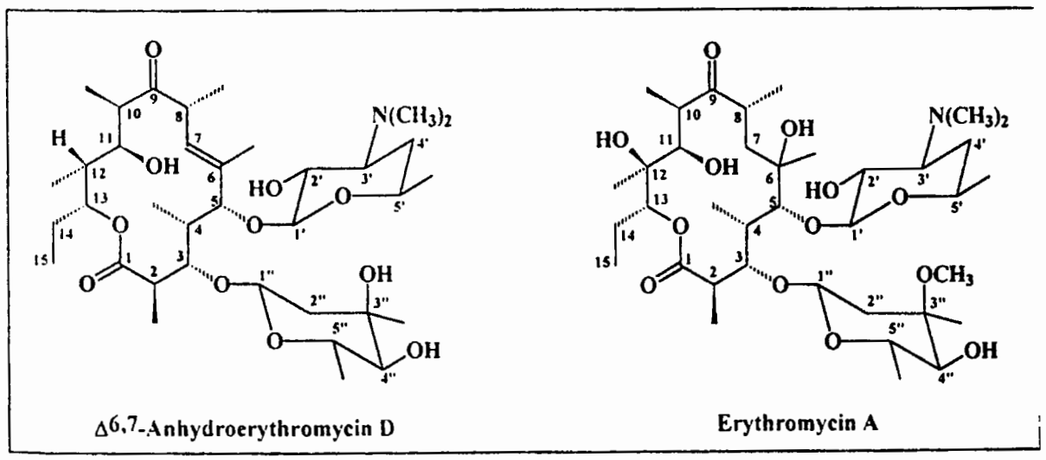
Results and Discussions

Erythromycin produced by *Sac. erythraea* 11912 without the addition of INH was designated as sample l; where as its derivative which was produced by this mutant with additional INH was called as sample 2. The proton chemical shifts of those erythromycin derivatives and the standard erythromycin A are shown in the Table 1.

The proton NMR spectrum of sample 1 showed similar chemical shift for all protons of the macrolacton ring system to those of the erythromycin A, except the appearance of a chemical shift at δ 2.317[5] ppm which having splitting pattern of *dq*. This chemical shift indicated the presence of a proton of 12-H. Since 12-H is still exist in the macrolacton ring system, therefore the aglycon of this erythromycin was erythronolide B instead of erythronolide A. This spectrum also showed identical chemical shifts for all protons of the glycons (the aminosugar and neutral sugar) to those of the standard erythromycin A, except that the neutral sugar showed no methoxy protons. These results confirmed that sample 1 was erythromycin D.

The proton NMR spectrum of sample 2 again showed similar chemical shift for all protons of the macrolacton ring system to those of the erythromycin A. However there were no chemical shifts at δ 1.916 and 1.74 of 7-H*ax* and 7-H*eq* respectively as in the sample 1 or standard erythromycin A. Instead, the chemical shift at δ 5.343 having splitting pattern of *dq* appeared to indicate one proton of H(C=C) in the macrolacton ring system. This H(C= C) assignment was based on Silverstein *et al.* (1991). Moreover the spectrum also gave chemical shift at δ 2.318 *(dq)* which indicated the presence of 12-H. It was confirmed that sample 2 consisted of **$\Delta^{6,7}$- anhydroerythronolide B.** The chemical shift of the all sugars' protons of sample 2, either the aminosugar or neutral sugar, were identical to those of sample l; which mean having glycons of desosamine and mycarose. It was concluded therefore that sample 2 was **$\Delta^{6,7}$-anhydroerythromycin D.**

The structural elucidation of samples 1 and 2 was in accord with the IR spectrometric analysis of the samples in the previous research (Sudibyo *et al.*, 1999).



Δ6,7-Anhydroerythromycin D Erythromycin A

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