ERYTHROMYCIN SERIES

XII. ANTIBACTERIAL *IN VITRO* EVALUATION OF 10-DIHYDRO-10-DEOXO-11-AZAERYTHROMYCIN A: SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIP OF ITS ACYL DERIVATIVES

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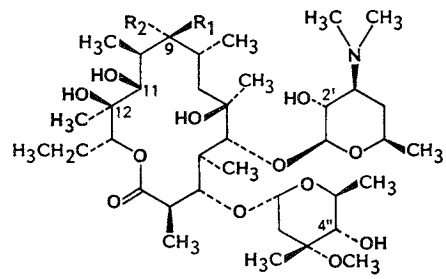
(Received for publication January 6, 1987)

Antibacterial *in vitro* evaluation of 10-dihydro-10-deoxo-11-azaerythromycin A† (**5**), the new 15-membered semi-synthetic macrolide antibiotic with nitrogen as additional atom in the aglycone ring of erythromycin A (**1**), was reported. Although amine (**5**) and its 13,14-cyclic carbonate (**14**) were less active than **1** against erythromycin-sensitive *Staphylococcus aureus* strains they showed advantageous properties against Gram-negative test organisms and clinical isolates. Also, a large number of acyl derivatives of **5** were synthesized and evaluated. N-11 monoacyl compounds exhibited 2 to 50 times lower *in vitro* antibacterial efficacy than the parent amine (**5**).

Erythromycin A (**1**)[1] is a well known antibiotic that is clinically useful against infections of Gram-positive bacteria and mycoplasmas. However, it is weakly active against Gram-negative bacteria and is particularly unstable toward acids. Efforts to improve on the latter have led to oximation of **1** with hydroxylamine to afford C-9 oxime (**2**)[2,3] which on hydrogenation yields 9(*S*)-erythromycylamine (**3**) as the major component and the minor 9(*R*)-epimer (**4**)[4]. Compounds **2** and **3** exhibited about 50% of the biological potency of **1**[2,4], but amine (**3**) indicates a trend toward higher Gram-negative *in vitro* antibacterial activity (Table 4). Kinetic study of acid-catalyzed hydrolysis of **2** and **3** revealed better acid stability than **1**[5].

With the view of further improvement of antibacterial and physico-chemical properties, we studied the Beckmann rearrangement of **2**[6] and its acid addition salts, especially erythromycin A oxime mono- and dihydrochlorides[7]. The rearrangement with *para*-substituted arene sulfonyl chlorides in aqueous acetone gave erythromycin A-imino ether which on reduction yielded 10-dihydro-10-deoxo-11-azaerythromycin A (**5**)[8]. Contrary to the other erythromycin derivatives which all possess a 14-membered aglycone, amine (**5**) contains an unusual ring-expanded aglycone with endocyclic nitrogen as an additional atom.



1 $R_1 = R_2 = =O$
2 $R_1 = R_2 = =NOH$
3 $R_1 = NH_2$ $R_2 = H$ (9*S*)
4 $R_1 = H$ $R_2 = NH_2$ (9*R*)

† This name is equivalent to 9-deoxo-9a-aza-9a-homoerythromycin (IUPAC: "Nomenclature of Organic Chemistry," 1979 Ed., *Eds.*, J. RIGAUDY & S. P. KLESNEY, Pergamon Press).

Table 1. Physico-chemical data of the new 10-dihydro-10-deoxo-11-azaerythromycins (**6**~**15**).

Compound No.	R_1	R_2	R_3	R_4	R_5	IR ν_{max}^{KBr} cm^{-1}	pK^a	Rf[b]	^1H NMR ($CDCl_3$, δ)			
									11-*N*-Acyl,	2′-*O*-Acyl,	4″-*O*-Acyl,	13-*O*-Acyl
5	H	H	H	H	H	1725, 1640	8.6	0.108				
6	Fr	H	H	H	H	1712, 1645, 1170	8.5	0.292	7.981			
7	Fr	Fr	H	H	H	1720, 1648, 1175	7.2	0.433	7.971,	8.199		
8	Ac	H	H	H	H	1718, 1610, 1220	8.6	0.341	2.108			
9	Ac	Ac	H	H	H	1740, 1620, 1240	7.1	0.567	2.078,	2.027		
10	Ac	Ac	Ac	H	H	1745, 1630, 1242	6.5	0.704	2.078,	2.029,	2.103	
11	Ac	Ac	Ac	Ac	H	1735, 1625, 1240	6.4	0.725	2.078,	2.028,	2.144,	2.060
12	Propionyl	H	H	H	H	1735, 1625, 1170	8.5	0.383	1.4, superimposed			
13	Propionyl	Propionyl	H	H	H	1725, 1615, 1175	7.2	0.662	1.4, superimposed			
14	H	H	H	>C=O		1790, 1730	8.4	0.275				
15	Ac	Ac	Ac	>C=O		1800, 1730, 1630, 1240	6.5	0.758	2.095,	2.053,	2.108	

[a] 66% DMF - H_2O.
[b] $CHCl_3$ - MeOH - $HCONH_2$ (100 : 20 : 2).
Fr: Formyl.

In this paper, we report the antimicrobial evaluation of **5**. The synthesis, structure elucidation and *in vitro* antibacterial activities of its *N*-, *O*- and *N,O*-acyl derivatives are also discussed.

Chemistry

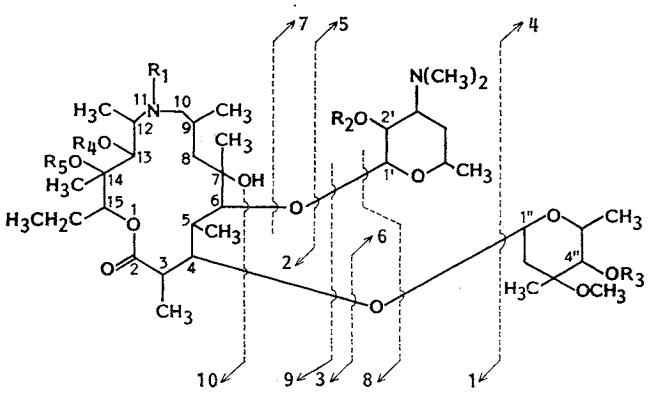
Treatment of amine (**5**) with acetic anhydride in pyridine at room temperature for 30 minutes, similar to the conditions used for the synthesis of 2'-esters of **1**[9~12], gave the diacetyl derivative (**9**)

Table 2. ^{13}C NMR chemical shift data of some new 10-dihydro-10-deoxo-11-azaerythromycins.

Carbon	**5**	**6**	**7**	**8**	**9**	**12**	**13**	**14**
2	178.5	176.6	176.7	175.1	175.7	174.8	175.6	176.5 s
3	45.3	45.3	45.1	45.9	44.9	44.6	44.9	43.8 d
4	78.1	78.9	79.0	78.9	78.8	78.4	79.0	79.4 d
5	42.1	41.5	41.2	40.6	40.6	40.2	40.7	43.1 d
6	83.4	86.0	84.9	87.2	84.4	84.1	85.0	85.1 d
7	73.7	75.7	75.9	75.1	75.6	75.5	75.7	75.0 s
8	42.2	35.1	35.9	34.9	34.4	35.5	34.9	41.1 t
9	29.9	26.4	26.9	28.0	27.2	27.8	27.7	30.5 d
10	57.3	52.0	53.5	49.8	51.5	50.0	49.7	55.8 t
12	56.7	51.2	51.6	51.1	51.1	50.3	49.4	53.0 d
13	73.2	73.7	75.7	73.9	75.5	74.6	74.7	76.7 d
14	73.8	75.1	74.9	74.0	73.5	73.4	75.1	85.0 s
15	77.2	77.2	77.0	77.5	77.2	77.2	77.0	77.0 d
13, 14 CO								152.8 s
3-CH_3	15.0	15.1	15.4	14.7	16.0	15.6	16.0	14.2 q
5-CH_3	9.4	9.6	9.5	10.4	9.7	9.7	9.8	10.0 q
7-CH_3	27.4	27.3	27.4	27.9	27.2	26.9	27.1	24.8 q
9-CH_3	14.0	11.9	12.0	12.1	12.5	12.9	12.6	12.4 q
12-CH_3	21.9	21.2	21.2	23.5	23.4	20.2	20.7	24.8 q
14-CH_3	16.2	17.5	17.7	17.9	17.6	18.3	17.8	16.7 q
15-CH_2	21.1	21.1	22.0	22.3	22.0	21.7	22.0	21.9 t
15-CH_3	11.2	11.2	11.1	11.4	10.9	11.1	10.8	10.3 q
1'	103.1	103.7	102.5	104.1	99.9	101.7	100.1	101.4 d
2'	70.9	70.7	70.7	70.6	71.6	70.7	71.4	70.6 d
3'	65.3	65.0	62.8	64.7	62.9	64.5	63.5	66.1 d
4'	28.7	29.0	29.3	29.1	31.2	29.5	31.2	28.6 t
5'	68.8	69.0	68.1	69.2	68.0	66.9	68.0	69.0 d
5'-CH_3	21.3	21.2	21.2	21.1	21.1	21.3	21.2	21.1 q
3'-$N(CH_3)_2$	40.3	40.3	40.3	40.4	40.6	40.3	40.7	40.0 q
1''	94.9	95.9	96.8	96.5	95.3	95.1	95.2	95.3 d
2''	34.8	34.2	35.9	34.9	34.8	34.7	34.2	34.7 t
3''	72.9	72.5	72.4	72.6	72.6	72.4	72.6	72.5 s
4''	77.9	77.6	77.9	78.2	77.7	78.4	77.7	77.8 d
5''	65.7	65.5	64.8	65.8	66.2	64.7	65.5	65.1 d
3''-CH_3	21.6	21.5	21.3	21.5	21.4	20.9	21.5	21.6 q
5''-CH_3	18.3	18.2	17.7	17.9	18.4	18.5	18.4	18.3 q
3''-OCH_3	49.4	49.3	49.5	49.3	49.3	48.7	49.3	49.2 q
11-*N*-Acyl		164.7	164.7	172.9	171.6	172.7	174.6	s
						26.9	28.7	t
				21.1	21.4	9.0	9.1	q
2'-*O*-Acyl			160.2		169.4		172.6	s
							27.7	t
					20.7		8.9	q

The ^{13}C NMR spectra were taken with Jeol 90 Q spectrometer. Samples were dissolved in $CDCl_3$ or DMSO containing TMS as an internal standard.

Table 3. Diagnostic mass fragmentations (*m/z*) for 10-dihydro-10-deoxo-11-azaerythromycin A (**5**) and its acetyls (**8**~**11**).

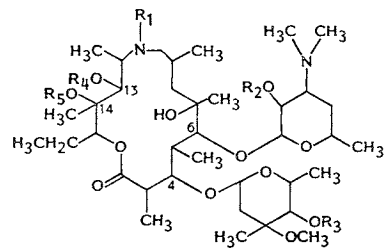


5 $R_1 = R_2 = R_3 = R_4 = R_5 = H$
8 $R_1 = Ac$ $R_2 = R_3 = R_4 = R_5 = H$
9 $R_1 = R_2 = Ac$ $R_3 = R_4 = R_5 = H$
10 $R_1 = R_2 = R_3 = Ac$ $R_4 = R_5 = H$
11 $R_1 = R_2 = R_3 = R_4 = Ac$ $R_5 = H$

	5	8	9	10	11
M^+	734	776	818	860	902
1		617	659	659	701
2	576	618	618	660	
3	+H 560	601	643	643	685
4	159	159	159	201	201
5	158	158	200	200	200
6	175	175	175	217	217
7	174	174	216	216	216
8 $M^+-(4+5)$		+H 460	+H 460	+H 460	+H 502
9 $M^+-(5+6)$	−H 402	+H 444	443	443	+H 486
10 $M^+-(6+7)$	385	427	−H 426	−H 426	−H 468

with two indicative chemical shift assignments in the ^1H NMR spectrum (Table 1) and two new carbonyl resonances at 169.4 and 171.6 ppm in the ^1H decoupled ^{13}C NMR experiment (Table 2). The IR spectrum showed an amide carbonyl band at 1620 cm^{-1} indicating that one of the acetyl groups was located at N-11. The electron impact mass spectra (EI-MS) (Table 3) gave the molecular ion at *m/z* 818. Prominent fragments at *m/z* 659 and 618 formed by glycosidic cleavage of cladinose (*m/z* 159) and desosamine (*m/z* 200) suggested that the other acyl group was at 2′-position[13]. Finally, the downfield chemical shifts of C-2′ and C-4′ and an upfield shift of C-3′ carbon were compatible with the known data for 2′-acylation of erythromycins[14].

Methanolysis of diacetyl (**9**) developed for the removal of 2′-*O*-acetyls[15,16], followed by silica gel column chromatography, produced



5 $R_1 = H$ $R_2 = H$ $R_3 = H$ $R_4 = H$ $R_5 = H$
6 $R_1 = CHO$ $R_2 = H$ $R_3 = H$ $R_4 = H$ $R_5 = H$
7 $R_1 = CHO$ $R_2 = CHO$ $R_3 = H$ $R_4 = H$ $R_5 = H$
8 $R_1 = Ac$ $R_2 = H$ $R_3 = H$ $R_4 = H$ $R_5 = H$
9 $R_1 = Ac$ $R_2 = Ac$ $R_3 = H$ $R_4 = H$ $R_5 = H$
10 $R_1 = Ac$ $R_2 = Ac$ $R_3 = Ac$ $R_4 = H$ $R_5 = H$
11 $R_1 = Ac$ $R_2 = Ac$ $R_3 = Ac$ $R_4 = Ac$ $R_5 = H$
12 $R_1 =$ Propionyl $R_2 = H$ $R_3 = H$ $R_4 = H$ $R_5 = H$
13 $R_1 =$ Propionyl $R_2 =$ Propionyl $R_3 = H$ $R_4 = H$ $R_5 = H$
14 $R_1 = H$ $R_2 = H$ $R_3 = H$ $R_4, R_5 = >CO$
15 $R_1 = Ac$ $R_2 = Ac$ $R_3 = Ac$ $R_4, R_5 = >CO$

Table 4. Primary *in vitro* activity of 10-dihydro-10-deoxo-11-azaerythromycin A (**5**) and its 13,14-cyclic carbonate (**14**).

Organism	MIC (μg/ml)				
	1	**2**	**3**	**5**	**14**
Staphylococcus aureus 209P JC-1	0.2	0.2	0.39	0.39	0.78
S. aureus Smith	0.2	0.2	0.39	0.78	0.78
S. aureus No. 50774	0.2	0.2	0.39	1.56	1.56
S. aureus No. 80	200	200	200	200	200
Corynebacterium pyogenes C-21	0.0125	0.05	0.05	0.05	0.05
Escherichia coli NIHJ JC-2	50	50	25	12.5	12.5
E. coli P-5101	100	50	25	12.5	12.5
E. coli No. 33	50	50	25	12.5	12.5
E. coli 2259	25	25	12.5	6.25	3.13
E. coli 2296	50	50	12.5	6.25	3.13
Salmonella typhimurium S-9	50	25	6.25	3.13	3.13
S. enteritidis No. 1891	25	25	6.25	3.13	3.13
Shigella flexneri 2a EW-10	3.13	3.13	12.5	3.13	3.13
S. flexneri 4a P-330	50	100	50	25	6.25
Klebsiella pneumoniae No. 13	100	100	50	25	12.5
Enterobacter cloacae P-2540	200	200	50	50	25
E. aerogenes 3046	100	100	100	50	25
Pseudomonas aeruginosa Tsuchijima	50	50	100	50	25
P. aeruginosa No. 12	200	200	200	200	200
P. aeruginosa Ky-32	200	200	200	200	200
P. aeruginosa Ky-39	200	200	200	200	200
Serratia marcescens IFO 3736	100	100	100	50	25
S. marcescens M-6	100	100	100	50	25
Morganella morganii Kano	200	200	200	100	25
Proteus vulgaris 336	200	200	200	200	25
P. mirabilis	200	200	200	200	25

Medium: Heart infusion, pH 7.2.
Inoculum: One loopful of bacterial suspension (about 10^8 cells per 1 ml).
Incubation: 20 hours at 37°C.

homogeneous monoacetyl (**8**). The strong band at 1610 cm^{-1} in the IR spectrum and increased *pK*-value (8.6) in comparison with **9** (*pK* 7.1)[15] indicated **8** to be 11-*N*-acetyl-10-dihydro-10-deoxo-11-azaerythromycin A. Furthermore, comparison of ^{13}C NMR spectra of amine (**5**) and monoacetyl (**8**) showed that the chemical shifts assigned to desosamine moiety were nearly identical.

Extended treatment of **5** with acetic anhydride in pyridine yielded a new ester (**10**) with three acetyl singlets at 2.078, 2.029 and 2.103 ppm in the ^1H NMR spectrum and the molecular ion at *m/z* 860 in the mass spectrum. Consistent with the location of the new acyl group at 4″-OH, the fragments due to the cleavage of cladinose with or without glycosidic oxygen at *m/z* 159 and 175 increased by 42 mass units leading to fragments *m/z* 201 and 217, respectively. Vigorous acetylation of amine (**5**) by heating in a pyridine solution with acetic anhydride at 70°C gave a tetraacetyl (**11**) with *m/z* 902 (M^+). 11-*N*-Propionyl- (**12**) and 11-*N*,2′-*O*-dipropionyl-10-dihydro-10-deoxo-11-azaerythromycin A (**13**) were synthesized in a similar manner using propionic anhydride. To prepare mono- (**6**) and diformate (**7**) formic acetic anhydride as formylating reagent was employed[16]. Their structures were ascertained principally by the combination of mass spectrometry and ^1H and ^{13}C NMR spectroscopy.

The reaction of **5** with ethylene carbonate[17,18] yielded 13,14-cyclic carbonate (**14**). The carbonate carbonyl band in the IR spectrum appeared at 1790 cm^{-1} and the mass spectrum gave a molecular ion

Table 5. *In vitro* antibacterial activity of amine (**5**) and erythromycin A (**1**) against clinically isolated Gram-negative bacteria.

Organism	Compound	No. tested	No. resistant	MIC (μg/ml)							
				0.5	1.0	2.0	4.0	8.0	16.0	32.0	64.0
Escherichia coli	**1**	100	22					2	28	40	8
	5		3	1		2	39	50	2	1	2
Klebsiella pneumoniae	**1**	9	4						1	2	2
	5		1				3	1	3	1	
K. aerogenes	**1**	10	7						1		2
	5		2					3	5		
Proteus mirabilis	**1**	16	3					13			
	5		10					2		3	1
Pseudomonas aeruginosa	**1**	10	9								1
	5		4					1		2	3
Enterobacter aerogenes	**1**	18	17								1
	5		1					1	5	7	4
Haemophilus influenzae	**1**	6		1		2	3				
	5			1	3	2					
Moraxella polymorpha	**1**	3	2						1		
	5									2	1
Herella	**1**	6						1	3	1	1
	5		1		1				1	3	
Total	**1**	178	64	1		2	3	16	34	43	15
	5		22	2	4	4	42	58	16	19	11

Medium: Mueller-Hinton agar.
Inoculum: One loopful of a bacterial suspension (about 1.5×10^8 cells/ml).
Incubation: 20 hours at 37°C.

at *m/z* 760. Comparing the ^{13}C NMR spectrum of **14** with that of **5**, C-13 and C-14 signals in **14** shifted downfield from the corresponding resonances in **5** (from 73.2 and 73.8 ppm to 76.7 and 85.0 ppm, respectively). The presence of a new carbonate carbonyl in **14** was indicated by a singlet at 152.8 ppm. The position of the cyclic carbonate ring in the aglycone was confirmed by treating **14** with acetic anhydride in pyridine under conditions necessary for the acylation of the C-13 hydroxyl group. ^1H NMR spectrum of **15** revealed only three acetyl methyls at 2.095, 2.053 and 2.108 ppm. Thus, engagement of the C-13 hydroxyl in the formation of the carbonate ring precludes formation of a tetraacetyl derivative in this case.

Antibacterial Activity

The ring-expansion of the 14-membered aglycone of **1** and the introduction of the secondary amino group leading to **5** resulted in decrease of *in vitro* biological potency. By a standard diffusion method using *Micrococcus luteus* ATCC 9341 as the test strain, the amine (**5**) exhibited about 67% (607 U/mg) of the activity of **1** but it was more potent in comparison with C-9 oxime (**2**). Contrary to the observations of BOJARSKA-DAHLING *et al.*[10], trans-esterification of ethylene carbonate with amine (**5**) leading to desired 13,14-cyclic carbonate (**14**) did not increase the activity (577 U/mg).

The minimum inhibitory concentrations (MICs) of **5** and **14** against variety of Gram-positive and Gram-negative bacteria using erythromycin A (**1**), C-9 oxime (**2**) and 9(*S*)-erythromycylamine (**3**) as reference compounds are shown in Table 4. Against erythromycin-sensitive *Staphylococcus aureus* strains the *in vitro* activity of **5** and its 13,14-cyclic carbonate (**14**) was lower than that of **1**. However,

Table 6. *In vitro* antibacterial activity of some new acyl derivatives of 10-dihydro-10-deoxo-11-azaerythromycin A.

Organism	MIC (μg/ml)						
	5	**6**	**8**	**9**	**10**	**11**	**12**
Streptococcus faecium ATCC 8043	0.5	1.0	10	10	50	175	10
Staphylococcus epidermidis ATCC 12228	0.1	0.5	2.5	2.5	5	200	2.5
S. aureus ATCC 6538P	0.5	10	25	25	50	200	25
Micrococcus flavus ATCC 10240	0.1	0.5	2.5	2.5	10	175	2.5
M. luteus ATCC 9341	0.1	1.0	1.0	1.0	10	10	2.5
Bacillus cereus var. *mycoides* ATCC 11778	1.0	5	25	25	50	175	25
B. subtilis ATCC 6633	0.5	5	25	5	75	200	25

Medium: According to UHLIK[23].
Inoculum: One loopful of bacterial suspension (about 10^5 cells per 1 ml).
Incubation: 18 hours at 37°C.

both of the new semi-synthetic compounds showed an interesting improvement of MICs (μg/ml) over **1** against Gram-negative organisms. These results supported the determination of sensitivity of 178 clinically isolated Gram-negative bacteria (Table 5). In concentrations of 0.5 to 4.0 μg/ml amine (**5**) inhibited 52 (29%) strains, whereas the parent antibiotic (**1**) was active only against 6 (3.4%) clinical isolates. However, this substantial increase of *in vitro* efficacy of **5** was not enough to render it useful, *in vivo*[20].

Acylation of the secondary amine (**5**) decreased the *in vitro* activity. *N*-Monoacyl derivatives (**6**, **8** and **12**) exhibited antibacterial efficacy 2 to 50 times lower than that of **5** (Table 6). The introduction of the second acyl group, as demonstrated for the diacetyl (**9**) in comparison with *N*-acetyl derivative (**8**), resulted in little or no change of *in vitro* potency. Similar findings were previously reported for mono- and diacyl derivatives of C-9 oxime (**2**)[21]. Furthermore, the activity of multiesters decreased with increasing the chain length and the number of acyl groups.

In conclusion, the *in vitro* antibacterial activities of the new semi-synthetic macrolide antibiotics were somewhat less than those of erythromycin A (**1**) against Gram-positive bacteria. On the other hand, amine (**5**) and its 13,14-cyclic carbonate (**14**) showed somewhat better activity *in vitro* against Gram-negative strains, while *N*-acylation resulted in considerably less *in vitro* potency. From these findings we concluded that **5** was interesting enough for further investigations.

Encouraging results which have been obtained with *N*-alkyl derivatives of **5**[22] will be published in our succeeding paper. One of these, *N*-methyl derivative of **5** is under developement as DCH3, XZ-450 or CP-62,993.

Experimental

Melting points were taken using Fisher-Johns apparatus and are uncorrected. IR spectra were recorded using Perkin-Elmer 256 G spectrometer. Mass spectra were measured on a CEC 21~110 C spectrometer at 70 eV. ^{1}H NMR spectra were recorded with Jeol FX-100 spectrometer in $CDCl_3$ and chemical shifts are given in ppm relative to TMS as an internal standard. ^{13}C NMR spectra were determined with Jeol 90 Q spectrometer in $CDCl_3$ or DMSO solutions. Thin-layer chromatography (TLC) was performed on Merck Kieselgel 60 F_{254} with $CHCl_3$ - MeOH - $HCONH_2$ (100:20:2). Silica gel column chromatography was performed with Merck Kieselgel 60.

11-*N*-Formyl-10-dihydro-10-deoxo-11-azaerythromycin A (**6**)

To a suspension of K_2CO_3 (1 g, 7.2 mmol) in a soln of 1.83 g (2.5 mmol) of 10-dihydro-10-deoxo-

11-azaerythromycin A (**5**) dissolved in Et_2O (50 ml) was slowly added (30 minutes) at 0~4°C formic acetic anhydride (2.64 ml, 28 mmol). After stirring at 25°C for 3 hours the mixture was poured over ice, the Et_2O layer separated and the aq layer extracted with $CHCl_3$. The combined $CHCl_3$ extracts were dried (K_2CO_3) and evaporated to give a colorless residue, which was purified by column chromatography ($CHCl_3$ - MeOH, 7:3) to afford 11-*N*-formyl-10-dihydro-10-deoxo-11-azaerythromycin A (**6**) 1.23 g (65.1%) as a white powder: MP 234~236°C; TLC Rf 0.292 (Table 1); *Anal* ($C_{38}H_{70}N_2O_{13}$) C, H, N.

11-*N*,2'-*O*-Diformyl-10-dihydro-10-deoxo-11-azaerythromycin A (**7**)

To a soln of 2.45 g (3.3 mmol) of **5** in pyridine (25 ml) was added at 0~4°C formic acetic anhydride (10 ml, 0.113 mol). After 30 minutes the reaction mixture was diluted with cold 5% $NaHCO_3$ soln and extracted with $CHCl_3$. The organic layer was dried over K_2CO_3 and evaporated to give 2.69 g of a residue which was chromatographed on a silica gel column with $CHCl_3$ - MeOH (7:3) to give **7**, 1.86 g (70.7%): MP 252~256°C; TLC Rf 0.433; *Anal* ($C_{39}H_{70}N_2O_{14}$) C, H, N.

11-*N*,2'-*O*-Diacetyl-10-dihydro-10-deoxo-11-azaerythromycin A (**9**)

To a soln of **5** (4 g, 5.4 mmol) in pyridine (20 ml), acetic anhydride (10 ml, 0.106 mol) was added and the reaction mixture allowed to stand at 25°C for 30 minutes. The soln was poured over ice, the pH of the reaction mixture adjusted with 20% NaOH to 9 and extracted with $CHCl_3$. The $CHCl_3$ layer was washed with 5% $NaHCO_3$ (50 ml) and H_2O (25 ml). After drying (K_2CO_3), $CHCl_3$ was evaporated to yield 4.7 g of a glassy solid which was chromatographed over silica gel column with $CHCl_3$ - MeOH - NH_4OH (6:1:0.1) to give **9**, 3.24 g (72.7%): MP 132~134°C; TLC Rf 0.567; *Anal* ($C_{41}H_{74}N_2O_{14}$) C, H, N.

11-*N*-Acetyl-10-dihydro-10-deoxo-11-azaerythromycin A (**8**)

The diacetyl derivative (**9**) (1.8 g, 2.2 mmol) was dissolved in MeOH (30 ml) and the hydrolysis followed by TLC. After 7 days MeOH was evaporated to yield 1.6 g of a residue which was chromatographed over silica gel column with $CHCl_3$ - MeOH (7:3) to yield 0.94 g (49.4%) of 11-*N*-acetyl (**8**): MP 248~252°C; TLC Rf 0.341; *Anal* ($C_{39}H_{72}N_2O_{13}$) C, H, N.

11-*N*,2',4''-*O*-Triacetyl-10-dihydro-10-deoxo-11-azaerythromycin A (**10**)

To a soln of **5** (4 g, 5.4 mmol) in pyridine (20 ml), acetic anhydride (20 ml, 0.212 mol) was added and the reaction mixture allowed to stand at room temp for 3 days. The soln was poured onto ice and isolated as described for **9** to afford 3.9 g of a glassy solid. The material obtained was precipitated from $CHCl_3$ - petroleum ether (1:10) yielding triacetyl (**10**) 3.1 g (66.2%): MP 138~140°C; TLC Rf 0.704; *Anal* ($C_{43}H_{76}N_2O_{15}$) C, H, N.

11-*N*,2',4'',13-*O*-Tetraacetyl-10-dihydro-10-deoxo-11-azaerythromycin A (**11**)

To 1.5 g (2 mmol) of **5** dissolved in pyridine (30 ml) was added acetic anhydride (15 ml, 0.160 mol) and the soln heated at 70°C for 16 hours. The reaction mixture was concentrated *in vacuo*, the residue dissolved in $CHCl_3$ (25 ml) and washed with 5% $NaHCO_3$ soln. After drying (K_2CO_3), $CHCl_3$ was removed to give a solid residue with two spots faster moving than **5** (TLC). The analytical sample, mp 110~115°C, was obtained by preparative TLC using $CHCl_3$ - MeOH - $HCONH_2$ (100:20:2) as a solvent (Rf 0.725), *Anal* ($C_{45}H_{78}N_2O_{16}$) C, H, N.

11-*N*,2'-*O*-Dipropionyl-10-dihydro-10-deoxo-11-azaerythromycin A (**13**)

The dipropionyl derivative (**13**) was obtained from 2 g (2.7 mmol) of **5** and propionic anhydride (25 ml, 0.194 mol) in pyridine (40 ml) as described for **9**. The glassy solid (1.98 g) obtained was crystallized from Et_2O yielding 1.35 g (59.3%) of dipropionyl (**13**): MP 183~189°C; TLC Rf 0.662; *Anal* ($C_{43}H_{78}N_2O_{14}$) C, H, N.

11-*N*-Propionyl-10-dihydro-10-deoxo-11-azaerythromycin A (**12**)

To a stirred soln of 2.15 g (2.5 mmol) of dipropionyl derivative (**13**) in MeOH (45 ml), 5% $NaHCO_3$ soln (45 ml) was added and the reaction mixture allowed to stand at room temp for 7 days. MeOH was evaporated, the obtained residue extracted with $CHCl_3$, the combined organic extracts

washed with H_2O, dried (K_2CO_3) and concentrated *in vacuo* to yield 1.84 g (88.8%) of 11-*N*-monopropionyl derivative (**12**): MP 122~129°C; TLC Rf 0.383; *Anal* ($C_{40}H_{74}N_2O_{13}$) C, H, N.

10-Dihydro-10-deoxo-11-azaerythromycin A 13,14-Cyclic Carbonate (**14**)

In an EtOAc soln (240 ml) of **5** (24 g, 32.7 mmol), K_2CO_3 (6.4 g, 46.3 mmol) was added and then slowly during 30 minutes at reflux temp 16 g (182 mmol) of ethylene carbonate. After the addition was complete, stirring was continued for additional 24 hours at reflux temp, EtOAc evaporated *in vacuo* and the residue dissolved in $CHCl_3$ (200 ml). H_2O (200 ml) was added, the layers separated and the aq layer extracted with $CHCl_3$. The combined $CHCl_3$ extracts were washed with H_2O, dried ($MgSO_4$) and then purified by precipitation from Me_2CO - H_2O (1:3) (160 ml). The resulting suspension was stirred at room temp for 1 hour, the product filtered off to give 19.1 g (76.9%) of 13,14-cyclic carbonate (**14**): MP 125~129°C; TLC Rf 0.275; *Anal* ($C_{38}H_{68}N_2O_{13}$) C, H, N.

11-*N*,2',4''-*O*-Triacetyl-10-dihydro-10-deoxo-11-azaerythromycin A 13,14-Cyclic Carbonate (**15**)

To a soln of **14** (1 g, 1.3 mmol) in pyridine (10 ml), acetic anhydride (10 ml, 0.106 mol) was added and the reaction mixture left at room temp for 10 days. The soln was poured onto ice and isolated as described for **9** to yield 1.2 g of a glass which upon crystallization from Et_2O - petroleum ether gave 0.75 g (64.3%): MP 128~137°C; TLC Rf 0.758; *Anal* ($C_{44}H_{74}N_2O_{16}$) C, H, N.

Acknowledgment

The authors would like to thank Dainippon Pharmaceutical Co., Ltd., Osaka, Japan for performing the antimicrobial studies at initial stage of this project. Helpful discussions with Dr. S. YOSHIZAWA are greatly appreciated. We are also indebted to Dr. T. TAMBIĆ, General Hospital "Dr. J. KAJFEŠ", Zagreb as well as M. JAKOVINA and S. ŠIMAGA, Pliva, Pharmaceutical, Chemical, Food and Cosmetic Industry, Zagreb, for providing the *in vitro* data and the results of biological potency.

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