L-ASPARTATE OF ERYTHROMYCIN A CYCLIC 11,12-CARBONATE, A NEW SEMISYNTHETIC ERYTHROMYCIN DERIVATIVE

+H. BOJARSKA-DAHLIG, W. SŁAWIŃSKI, D. ROŚLIK-KAMIŃSKA, A. SCHAEFFER,*
A. SIPAK-KRZYSIAK,* E. DZILIŃSKI, I. SKIBIŃSKA, T. PRUSSAK-WIĘCKOWSKA,
W. KOTULA, R. KADŁUBOWSKI** and A. KURNATOWSKA

Institute of Pharmaceutical Industry, Warszawa, Poland
*Tarchomin Pharmaceutical Works "Polfa", Warszawa, Poland
**Institute of Biology and Morphology of the Medical Academy, Łódź, Poland

(Received for publication March 15, 1976)

Erythromycin A cyclic 11,12-carbonate, a compound with high antibacterial activity, forms with L-aspartic acid a salt possessing valuable properties as a potential chemotherapeutic agent. The L-aspartate of erythromycin A cyclic 11,12-carbonate exhibits strong antibacterial activity, especially against Gram-positive bacteria and shows low toxicity. The serum and the lung tissue levels of the discussed salt after a single dose administration to a rat were measured in comparison with those of erythromycin, its L-aspartate, erythromycin cyclic 11,12-carbonate and its L-glutamate. The new erythromycin derivative showed definitely superior characteristics to those of the other substances tested. The activity of the L-aspartate of erythromycin A cyclic 11,12-carbonate in chemotherapy of experimental staphylococcal infection and experimental pneumococcal bronchopneumonia in mice is superior to that of the parent carbonate and erythromycin itself.

For the past several years we have studied the synthesis and biological properties of a number of erythromycin derivatives modified in the aglycone ring, among them erythromycin A cyclic carbonate, a compound formed by the reaction of erythromycin A with ethylene carbonate.[1,2] Although this substance was supposed to be the 9,11-carbonate of the 6,9-hemiacetal form of the parent antibiotic[1,3], we considered that the structure might as well be the 11,12-carbonate[2]. Our investigations have demonstrated that this hypothesis was right[4,5]. MCALPINE and coworkers obtained similar results[6].

Using ^{13}C NMR spectroscopy we have found that erythromycin A cyclic 11,12-carbonate shows hydroxy ketone-hemiacetal tautomerism and in the solid state exists as the hemiacetal, while in water and methanol solutions it exists as a mixture of the tautomers[4].

Erythromycin A cyclic 11,12-carbonate is a substance with high biological potency, namely amounting to *ca.* 2,500 u/mg (980 u/mg for the erythromycin standard, cylinder-plate method, *Bacillus pumilus* NCTC 8241). It is clinically effective in the chemotherapy of infections with Gram-positive bacteria. Among the salts tested in our screening program, especially advantageous properties were shown by the salt of erythromycin A cyclic 11,12-carbonate with L-aspartic acid.

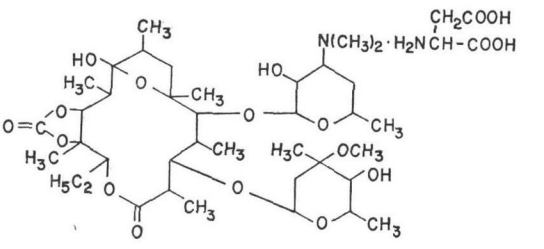
This paper deals with chemical characteristics, antibacterial activity, toxicity, comparative absorption studies and experimental chemotherapy with this new erythromycin derivative.

Preparation and Physicochemical Properties

The L-aspartate salt of erythromycin A cyclic 11,12-carbonate is prepared by reacting 1 mole of erythromycin A 11,12-carbonate with 1 mole of L-aspartic acid[7]. The substance can be represented by

+ To whom correspondence should be addressed.

a structural formula given below:



The L-aspartate of erythromycin A 11,12-carbonate is soluble in water, 50% methanol, 50% ethanol and 50% acetone. It is insoluble in diethyl ether and hydrocarbons. Its apparent partition coefficient in an *n*-octanol-water system was estimated as 109 in comparison with 345.6 for erythromycin A cyclic 11,12-carbonate, 28.2 for erythromycin A and 33.6 for erythromycin of pharmaceutical grade of purity. Melting point (uncorrected) of the monohydrate of the L-aspartate of erythromycin A cyclic carbonate is 131~142°C and its elemental analysis supports the formula $C_{42}H_{72}N_2O_{18}\cdot H_2O$ (calcd: C 55.38, H 8.19, N 3.07, O 33.38; found: C 55.57, H 8.24, N 2.97, O 33.40). The specific rotation was measured as $[\alpha]_D^{21}$ −36° (*c* 1, 50% acetone). The infrared spectrum (KBr disc, Zeiss UR-10 spectrometer) shows absorption at 1820 cm^{-1} (assigned to the cyclic carbonate moiety) and at 1750 cm^{-1} (lactone carbonyl of the aglycone ring). The ^{1}H-NMR spectrum (diluted deuteriomethanol solution, Jeol JNM C 60 spectrometer) shows a signal at δ 2.86 ppm (singlet, 6 H, assigned to the protons on the N,N-dimethylamine moiety on C-3').

Table 1. Stability in artificial gastric juice

Substance	Activities remained			
	5 min.	15 min.	30 min.	60 min.
L-Aspartate of erythromycin A 11,12-carbonate	22.1*	13.2	4.2	3.3
Erythromycin A	2.4*	1.2	1.0	0.5

* The activity of substance was measured after 5, 15, 30 and 60 minutes in artificial gastric juice and given as percentage of initial activity taken as 100%.

The L-aspartate of erythromycin A cyclic 11,12-carbonate is more stable in acidic medium at pH 1.1~1.2 (gastric juice) than erythromycin* (Table 1).

Biological Properties

1. Antibacterial Activity

The biological potency of an anhydrous preparation of the L-aspartate of erythromycin A 11,12-carbonate is as high as 1,985 u/mg (980 u/mg erythromycin standard, cylinder-plate method, *Bacillus pumilus* NCTC 8241). The antibacterial activity *in vitro* of the L-aspartate of erythromycin A 11,12-carbonate shown as the minimal inhibitory concentrations (MIC) in comparison with erythromycin and its carbonate against a selected group of Gram-positive and Gram-negative bacteria is given in Table 2.

The L-aspartate of erythromycin A cyclic 11,12-carbonate is extremely active against Gram-positive microorganisms, especially staphylococci and diplococci and also shows activity against some Gram-negative microorganisms. As for strains isolated from pathological material it may be observed that the L-aspartate of erythromycin A 11,12-carbonate is more active against staphylococci than erythromycin itself.

2. Tonxicity

The species of animals were as follows: conventional strain BALB/c mice and conventional stock

* In all experiments erythromycin serving as the reference substance was the commercial preparation of "Polfa" of pharmaceutical grade of purity.

Table 2. Antibacterial spectra of erythromycin (Er), erythromycin cyclic 11,12-carbonate (CEr) and L-aspartate of erythromycin cyclic 11,12-carbonate (L-Asp. CEr) by the agar dilution method

No.	Microorganism	Minimal inhibitory concentration (MIC, μg/ml, after 24-hour incubation at 37°C)			No.	Microorganism	Minimal inhibitory concentration (MIC, μg/ml, after 24-hour incubation at 37°C)		
		Er	CEr	L-Asp. CEr			Er	CEr	L-Asp. CEr
1	*Staphylococcus epidermidis* 12228 ATCC	0.78	0.19	0.39	39	*K. pneumoniae*	100	25	50
					40	*K. pneumoniae*	100	50	100
2	*Staphylococcus aureus* 209 P	0.19	0.09	0.19	41	*Enterobacter aerogenes* 1053 ATCC	100	25	50
3	*S. aureus*	0.78	0.19	0.19	42	*Shigella flexneri*	50	12.5	25
4	*S.aureus* Smith 1	0.2	0.1	0.1	43	*Shigella flexneri*	12.5	3.12	6.25
5	*S. aureus* Selim 2	0.2	0.2	0.1	44	*Shigella flexneri*	50	25	50
6	*S.aureus* Furans 3	0.1	0.2	0.1	45	*Bacillus cereus* 11778 ATCC	0.09	0.04	0.09
7	*S. aureus* Mendida	0.2	0.1	0.1	46	*Bacillus subtilis* 8236 NTCT	0.19	0.09	0.09
8	*S. aureus* 8	0.1	0.1	0.1	47	*B. subtilis* 6633 ATCC	0.1	0.1	0.05
9	*Staphylococcus*	0.39	0.19	0.19	48	*Proteus mirabilis*	100	100	100
10	*Staphylococcus*	0.19	0.19	0.19	49	*Proteus vulgaris*	100	100	100
11	*Staphylococcus*	0.09	0.09	0.04	50	*Bordetella bronchiseptica* 4617 ATCC	0.02	0.02	1.56
12	*Staphylococcus*	0.39	0.09	0.19	51～52	*Listeria monocytogenes*	0.19	0.09	0.78
13	*Staphylococcus*	1.56	3.12	1.56	53	*Streptococcus faecalis* DManhattan 39457-4/49, 971	1.25	0.078	0.078
14～18	*Staphylococcus*	>100	>100	>100					
19	*Diplococcus pneumoniae* Pn. Type 3 No. 2, encapsulated 992	0.312	0.078	0.0185	54	*S.faecalis* DF 87	1.25	0.078	0.078
20	*Diplococcus pneumoniae* Pn. R36-A, nonencapsulated 937	0.312	0.078	0.078	55	*S. faecalis*	100	100	100
21～25	*Pseudomonas*	>100	>100	>100	56	*Streptococcus pyogenes*	0.19	0.04	0.04
26	*Escherichia coli* 308	25	12.5	25	57	*Streptococcus viridans*	0.02	0.02	0.09
27	*E. coli* 10536 ATCC	6.25	6.25	3.12	58	*Streptococcus* gr. B	1.56	0.39	0.78
28	*E. coli*	>100	50	50	59～61	*Streptococcus*	0.19	0.09	0.78
29～30	*E. coli*	25	12.5	25	62～63	*Streptococcus*	1.56	0.39	0.78
31～33	*E. coli*	100	25	25	64	*Streptococcus*	100	100	100
34	*Salmonella enteritidis*	100	25	50	65	*Streptococcus*	0.39	0.19	0.04
35	*Salmonella typhi*	25	12.5	25	66	*Streptococcus*	0.39	0.09	0.78
36	*Salmonella typhi*	100	50	50	67	*Streptococcus*	0.39	0.19	0.78
37	*S. typhi* 4	25	6.25	6.25	68	*Streptococcus*	0.09	0.09	0.78
38	*Klebsiella pneumoniae* 10031 ATCC	3.12	1.56	12.5					

Wistar rats.

On acute toxicity determination an oral administration in mice of doses ranging from 2 g/kg to 5 g/kg did not result in any toxic manifestations. In rats treated by the same route LD_{50} was 5.05 g/kg. The analogous data for the parent carbonate, erythromycin, its L-aspartate[8] and L-glutamate of erythro-

mycin A 11,12-carbonate[2] given in Table 3 reveal that the L-aspartate of erythromycin A 11,12-carbonate is one of the least toxic compounds in this group of derivatives.

Table 3. Acute toxicity

No.	Substance	LD_{50} g/kg	
		Mouse	Rat
1	Erythromycin	2.58[10]	4.60[10]
2	Erythromycin A 11,12-carbonate	4.05	5.80
3	L-Aspartate of erythromycin	2.65	3.15
4	L-Aspartate of erythromycin A 11,12-carbonate	> 5.0	5.05
5	L-Glutamate of erythromycin A 11,12-carbonate	4.5	—

Safety test performed according to the Code of Federal Regulations[9] done as with erythromycin, with the exception that 50 mg per mouse was used instead of 30 mg showed that the preparation of the L-aspartate of erythromycin A 11, 12-carbonate was not toxic.

Oral administration for 6 weeks at daily doses of 75 mg/kg caused no abnormalities in rats. Haematological and biochemical testing of blood, urine analysis and histological examination of organs at autopsy were performed.

Haematological tests included: haemoglobin estimation, total red cell counts, color index determination, reticulocyte counts, total white cell counts and white blood cell differential counts. Biochemical testing of blood included: estimation of blood glucose, one-stage prothrombin time, serum glutamic-oxaloacetic transaminase activity, serum total protein, serum albumin/globulin ratio, blood urea nitro-

Table 4. Oral rat blood levels of erythromycin derivatives (mean of 5 animals±SE)

No.	Substance	Antibiotic concentration, μg/ml							
		15 min.	30 min.	1 hr.	2 hrs.	3 hrs.	5 hrs.	7 hrs.	24 hrs.
1	Erythromycin (standard)	0.93 ±0.22	1.29 ±0.35	1.5 ±0.20	2.09 ±0.29	1.86 ±0.28	1.49 ±0.43	0.56 ±0.46	0
2	Erythromycin A cyclic 11, 12-carbonate	0.81 ±0.07	1.85 ±0.06	2.43 ±0.32	2.91 ±0.19	2.84 ±0.20	1.74 ±0.11	1.3 ±0.12	0
3	L-Aspartate of erythromycin	1.32 ±0.19	1.97 ±0.35	3.64 ±0.61	2.28 ±0.34	1.25 ±0.53	0.37 ±0.39	0.32 ±0.09	0
4	L-Aspartate of erythromycin A cyclic 11,12-carbonate	1.43 ±0.07	4.13 ±0.23	4.88 ±0.57	4.63 ±0.28	3.21 ±0.40	2.07 ±0.10	1.1 ±0.09	traces (*ca.* 01)
5	L-Glutamate of erythromycin A cyclic 11,12-carbonate	1.04 ±0.24	1.99 ±0.16	2.07 ±0.32	2.92 ±0.29	1.78 ±0.21	1.41 ±0.24	0.86 ±0.09	traces (*ca.* 01)

Table 5. Oral rat lung tissue levels of erythromycin derivatives (mean of 3 animals±SE)

No.	Substance	Antibiotic concentration, μg/mg							
		15 min.	30 min.	1 hr.	2 hrs.	3 hrs.	5 hrs.	7 hrs.	24 hrs.
1	Erythromycin (standard)	2.26 ±0.23	4.15 ±1.30	5.18 ±0.94	10.2 ±1.15	11.4 ±1.99	7.83 ±0.61	6.83 ±0.22	1.56 ±0.02
2	Erythromycin A cyclic 11,12-carbonate	1.38 ±0.17	7.66 ±0.33	22.6 ±0.56	33.5 ±2.44	41.8 ±1.47	47.3 ±0.9	22.8 ±0.22	0.47 ±0.10
3	L-Aspartate of erythromycin	2.94 ±0.31	8.09 ±1.13	12.5 ±3.31	20.6 ±5.0	11.4 ±3.2	11.11 ±1.15	6.49 ±0.64	1.55 ±0.17
4	L-Aspartate of erythromycin A cyclic 11,12-carbonate	9.13 ±2.36	34.0 ±2.68	42.4 ±1.20	108.0 ±27.72	133.9 ±11.06	12.5 ±8.03	102.2 ±19.45	7.97 ±0.47
5	L-Glutamate of erythromycin A cyclic 11,12-carbonate	4.27 ±0.86	16.1 ±3.20	53.2 ±7.87	80.1 ±3.94	77.3 ±6.90	62.6 ±3.00	26.9 ±2.05	5.01 ±1.03

gen, serum sodium, serum potassium and serum chlorides. Urine analyses included: determination of glucose, protein, pH, blood, sodium, potassium, 24-hour volume and microscopic examination of centrifugated sediment. Post mortem dissections were performed in animals after 6 weeks. Organs (liver, spleen, kidneys, heart, lungs, pancreas, gonads and adrenals) were weighed and taken for further study. Tissues from these organs were fixed in NEWCOMER's fluid, embedded in paraffin, sectioned, stained with hematoxylin and eosin and evaluated histologically.

3. Absorption

During preliminary experiments in animals the L-aspartate of erythromycin A 11,12-carbonate was found to be absorbed more readily than erythromycin and its carbonate itself, yielding high concentration in blood, penetrating into organs and reaching especially high levels in lungs, much higher than those found at the same time in the blood. Owing to this observation the oral rat serum and lung tissue levels of the L-aspartate of erythromycin A cyclic 11,12-carbonate after single dose administration were studied in comparison with erythromycin, its L-aspartate, erythromycin 11,12-carbonate and its L-glutamate.

Methods

Studies were carried out in conventional animals. Female Wistar rats weighing 180~200 g were fasted overnight and given compounds under evaluation orally at a single dose of 80 mg/kg as a suspension. Blood samples were taken in the presence of heparin and immediately centrifugated. Lungs were kept in the frozen state (−20°C) until assayed.

Erythromycin and erythromycin A cyclic 11,12-carbonate in serum were determined by the usual diffusion cylinder-plate method (comparison with the standard curve) using *Bacillus pumilus* NCTC 8241 as a test strain.

For the assay of antibiotic in lung tissue the organs were homogenized, the homogenate centrifugated at 4,000 rpm for 15 minutes. Erythromycin or its carbonate in the supernatant was determined by the method used for blood assay.

Results

Serum and lung tissue concentrations of erythromycin after administration of this antibiotic as well as its L-aspartate, concentrations of erythromycin A 11,12-carbonate after its administration as well as after administration of L-aspartate and L-glutamate of this carbonate were assayed at intervals until

Fig. 1. Blood levels

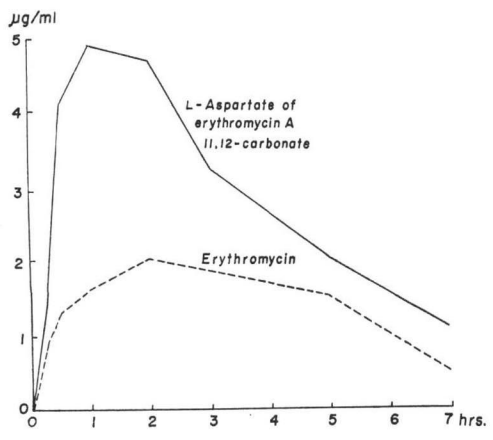
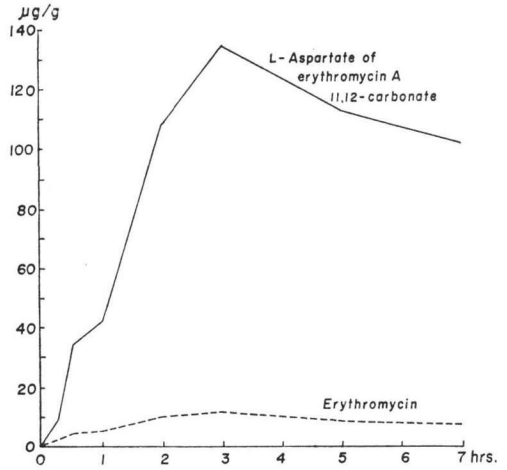


Fig. 2. Lung tissue levels



the 7th hour and at 24th hour. The results are summarized in Table 4 and Table 5.

Fig. 1 and Fig. 2 show the comparison of serum and lung tissue levels respectively for erythromycin and for L-aspartate of erythromycin cyclic 11,12-carbonate up to the 7th hour after administration.

The absorption of the L-aspartate of erythromycin A 11,12-carbonate in rats after oral administration is definitely superior to that of the other compounds tested. The single dose of 80 mg/kg gave the peak level of L-aspartate of erythromycin A 11,12-carbonate much higher and earlier in comparison with that of the reference antibiotic. The affinity to the lung tissue can be distinctly recognized.

4. Experimental Chemotherapy

The activity of the L-aspartate of erythromycin A cyclic 11,12-carbonate in chemotherapy of experimental staphylococcal infection and experimental pneumococcal bronchopneumonia was studied in mice.

Strains used for infection

Bacteriostatic activity on the *Staphylococcus aureus* 8 and *Diplococcus pneumoniae* Type 3 No. 2 encapsulated 992 strain from the collection of the State Institute of Hygiene in Warszawa was determined by the modified ROSE-MÜLLER plate method (10 mm holes, incubation at 37°C for 24 hours). The minimal inhibitory concentration of the L-aspartate of erythromycin A 11,12-carbonate (mol. weight 893), 11,12-carbonate of erythromycin A (mol. weight 760) and erythromycin (mol. weight 734) was calculated from the linear regression equation in half-logarithmic system on the basis of the dose-response curve[11]. As shown in Table 6 the MIC values of the L-aspartate of erythromycin A 11,12-carbonate and its parent carbonate against *Staphylococcus* strain are several times lower than that of erythromycin. The corresponding indices of both substances (relative activity) are 6.0 and 5.6. The

Table 6. Minimal bacteriostatic concentrations (MIC) of the tested antibiotics

	Parameter	L-Asp. CEr	CEr	Er
Staphylococcus aureus 8	MIC	0.0114 μg/cm^3 =0.0127 μmole	0.0105 μg/cm^3 =0.0138 μmole	0.0563 μg/cm^3 =0.0767 μmole
	Confidence limits of MIC P=95%	0.0092~0.0141 μg/cm^3 0.0103~0.0158 μmole	0.0070~0.0159 μg/cm^3 0.0092~0.0210 μmole	0.0442~0.0721 μg/cm^3 0.0602~0.0982 μmole
	Index of the relative activity	6.04	5.56	1.00
	Difference significance	P>99.9%	P>99.9%	—
Diplococcus pneumoniae Type 3 No. 2 encapsulated 992	MIC	0.0150 μg/cm^3 =0.0168 μmole	0.0197 μg/cm^3 =0.0259 μmole	0.0459 μg/cm^3 =0.0627 μmole
	Confidence limits of MIC P=95%	0.0130~0.0173 μg/cm^3 0.0146~0.0194 μmole	0.0127~0.0300 μg/cm^3 0.0167~0.0399 μmole	0.0338~0.0631 μg/cm^3 0.0461~0.0860 μmole
	Index of the relative activity	3.72	2.42	1.00
	Difference significance	P>99.9%	P>98%	—

similar results were obtained for *Diplococcus* strain, the relative activity index amounting to 3.7 and 2.4 respectively.

Infection, therapy and results

The white conventional mice (BALB/c strain, males, 16~18 g) were infected intraperitoneally by 0.2 cm^3 suspension of *Staphylococcus aureus* 8 24-hour culture and treated per os during 6 days with the L-aspartate of erythromycin A 11,12-carbonate, erythromycin A 11,12-carbonate and erythromycin, the 24 hour dosis being 6.3~242 mg/kg (Table 7). The survival of animals was defined after 7 days. To calculate the mean protective dose (PD_{50}) of the tested antibiotics the linear regression equation in half-logarithmic system was used[12]. According to the data given in Table 7 the L-aspartate of erythromycin A 11,12-carbonate is nearly four times more efficient in staphylococcal infection than

Table 7. Therapeutical efficacy and PD_{50} of the tested antibiotics in experimental infection in mice by *Staphylococcus aureus* 8

Parameter	L-Asp. CEr	CEr	Er
Survival, dosis mg/kg/24 hours			
242	8/8	10/10	10/10
106	10/10	9/13	6/9
46	7/8	7/12	3/10
30	9/12	3/8	2/9
15	4/12	3/10	0/9
6.3	0/9	0/9	0/8
PD_{50}	21.4 mg/kg =24.0 μmole/kg	35.1 mg/kg =46.2 μmole/kg	65.4 mg/kg =89.1 μmole/kg
Confidence limits of PD_{50} P=95%	16.1~28.5 mg/kg 18.0~32.0 μmole/kg	19.8~62.2 mg/kg 26.1~81.9 μmole/kg	45.5~94.2 mg/kg 62.0~128 μmole/kg
Index of the relative activity	3.71	1.92	1.00
Difference significance	P>99.9%	90<P<95%	—

Table 8. Therapeutical efficacy and PD_{50} of the tested antibiotics in experimental infection in mice by *Diplococcus pneumoniae* Type 3 No. 2 encapsulated 992

Parameter	L-Asp. CEr	CEr	Er
Survival, dosis mg/kg/24 hours			
312	8/8	8/8	10/10
184	10/10	10/10	8/10
63.6	7/9	5/7	4/10
25.5	7/10	5/9	1/9
10.4	2/10	1/9	0/9
3.2	0/9	0/9	0/8
PD_{50}	18.3 mg/kg =20.5 μmole/kg	30.2 mg/kg =39.7 μmole/kg	71.2 mg/kg =97.0 μmole/kg
Confidence limits of PD_{50}	11.4~29.6 mg/kg 12.7~33.1 μmole/kg	18.1~50.5 mg/kg 23.8~66.4 μmole/kg	38.1~133 mg/kg 51.9~181 μmole/kg
Index of the relative activity	4.73	2.44	1.00
Difference significance	P>99%	P>95%	—

erythromycin itself. This new semisynthetic derivative in the same experimental infection is twice as efficient as the parent carbonate, although both compounds do not differ in their antistaphylococcal activity *in vitro* (Table 6).

As it was shown before it is possible to obtain after the oral administration of the L-aspartate of erythromycin A cyclic 11,12-carbonate the antibiotic level in the rat lung much higher than that found in blood. In consequence of these findings we have to study the efficacy of this substance in experimental bronchopneumonia. The white conventional mice (BALB/c strain, males, 15~18 g) were infected by nasal application of 0.05 cm^3 of 24-hour culture of *Diplococcus pneumoniae* Type 3 No. 2 encapsulated 992 suspension and treated per os during 6 days with L-aspartate of erythromycin A cyclic 11,12-carbonate, the parent carbonate and erythromycin, the 24-hour dosis being 3.2~312 mg/kg (Table 8). The survival of animals was defined after 7 days.

According to the data given in Table 8 the efficacy of the L-aspartate of erythromycin A cyclic 11,12-carbonate in the therapy of experimental bronchopneumonia is nearly five times greater in comparison with the efficacy of erythromycin and about two times greater than that of the parent carbonate.

The discussed advantageous properties of the new semisynthetic erythromycin derivative stimulated us for further preclinical and clinical trials which are in progress.

References

1) MURPHY, H. W.; V. C. STEPHENS & J. W. CONINE: Erythromycin derivatives and the process of preparation thereof. U.S. Pat. 3,417,077, 1968
2) BOJARSKA-DAHLIG, H. & W. SŁAWIŃSKI: Pochodne erytromycyny. IV. Cykliczny węglan erytromycyny i jego pochodne. Roczniki Chem. 46: 2211~2222, 1972
3) LEO, A.; C. HANSCH & D. ELKINS: Partition coefficients and their uses. Chem. Rev. 71: 525~616, 1971
4) SŁAWIŃSKI, W.; H. BOJARSKA-DAHLIG, T. GŁĄBSKI, I. DZIĘGIELEWSKA, M. BIEDRZYCKI & S. NAPERTY: The structure of erythromycin A cyclic carbonate. Rec. Trav. Chim. 94: 236~238, 1975
5) HEMPEL, A.; M. BOGUCKA-LEDÓCHOWSKA, Z. DAUTER, E. BOROWSKI & Z. KOSTURKIEWICZ: The structure of anhydroerythromycin A carbonate. Tetrahedron Letters 1975: 1599~1602, 1975
6) MCALPINE, J. B.; J. A. FREIBERG, P. H. JONES, R. S. EGAN, W. H. WASHBURN & J. W. CORCORAN: The structure of erythromycin A carbonate. Communicate presented at 14th Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, 1974 (Abstract 304)
7) BOJARSKA-DAHLIG, H. & Z. SZYPKA: L-Aspartate of cyclic erythromycin A carbonate and process of preparing the same. U.S. Pat. 3,925,352, 1975
8) CASILLI, L.; C. VACCA, S. CASTALDI & R. A. CALIENDO: Ricerche sperimentali sull'aspartato di eritromicina. Boll. Chim. Farm. 111: 239~249, 1972
9) Code of Federal Regulations, Title 21, § 148 e.2
10) CHOJNOWSKI, W.; A. ZAREMBA & H. BOJARSKA-DAHLIG: Niektóre właściwości biologiczne cyklicznego węglanu erytromycyny A. Acta Polon. Pharm. 31: 241~244, 1974
11) KADŁUBOWSKI, R.: Einfaches Verfahren zur Ermittlung von mykostatischen Stoffkonzentrationen. Derm. Wschr. 159: 469, 1973
12) KADŁUBOWSKI, R.: Approximate methods in analysis of dose response curve. Communicate presented at 7th International Congress of Chemotherapy, Prague, 1971 (Abstract A-12/28)