SYNTHESIS AND PROPERTIES OF 5-NITROFUROATE AND FUROATE MONOESTERS OF ERYTHROMYCIN

Ya. A. Kastron, V. V. Novikova,
É. É. Liepin'sh, S. A. Giller,*
D. A. Kulikova, T. P. Radkevich,
V. P. Yakovlev, and É. A. Rudzit

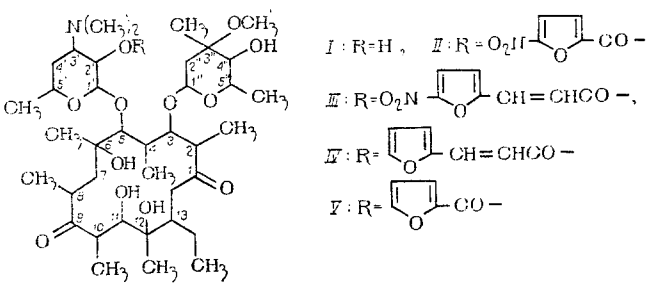
UDC 615.332(Erytromycinum).012.1

The chemistry of macrolide antibiotics (especially erythromycin I) has developed particularly intensively in recent years with the object of improving their antibacterial, pharmacological, and physicochemical properties and of solving several important problems in molecular biology [1].

There are few data in the literature on the furan derivatives of I, although a lot of work has been done on the transformation of this antibiotic. Several communications have been devoted to the synthesis of erythromycin esters and to the description of their physicochemical and biological properties [2-7]. In [6], among 20 new esters of I, its furoate is mentioned, although the empirical formula of the latter and its carbon analysis data are given erroneously.

The present work is devoted to the preparation and study of the properties of the 5-nitrofuroate and furoate monoesters of erythromycin, since it is known that many compounds containing nitrofuran groups have a broad activity spectrum, high acid stability, and resistance to them develops slowly.

The acylation of I was carried out with the acid chlorides of 5-nitro-2-furan- and 2-furancarboxylic acid, β-(5-nitro-2-furyl)acrylic acid, and β-(2-furyl)acrylic acid, in acetone with a reactant ratio of 1:1 in the presence of a fivefold amount of sodium bicarbonate. This gave 65-85% yields of the practically pure reaction products II-V:



Compounds II-V are optically active and are weaker bases (pK_a 6.7-7.1) than the starting erythromycin, the pK_a of which is 8.6. They readily form water-soluble phosphates and hydrochlorides (Table 1).

Acylation of I with the above carbonyl chlorides in the absence of sodium bicarbonate leads to the formation of a precipitate which, according to chromatographic analysis data, consists of a mixture of the hydrochloride of I, one of esters II-V, and its hydrochloride.

The UV spectra of compounds II-V confirm the presence of residues of the corresponding furan- and 5-nitrofurancarboxylic acids (see Fig. 1). In the IR spectra, the absorption

*Deceased.

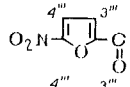
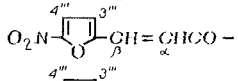
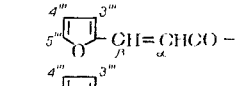
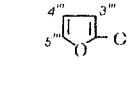
Institute of Organic Synthesis, Academy of Sciences of the Latvian SSR, Riga; Scientific-Research Institute for Biological Testing of Chemical Compounds, Kupavna, Moscow Oblast. Translated from Khimiko-Farmatsevticheskii Zhurnal, Vol. 10, No. 5, pp. 29-34, May, 1976. Original article submitted August 6, 1975.

TABLE 1. Physicochemical Properties of Erythromycin Monoesters

Compound	Melting point (deg)	Yield (%)	Found (%)			Empirical formula	Calculated (%)			$[\alpha]_D^{20}$* (deg)	pK_a†	R_f		Phosphate	
			C	H	N		C	H	N			system 1	system 2	melting point (deg)	$[\alpha]_D^{20}$‡ (deg)
II	197—8	88	57,56	8,09	3,15	$C_{42}H_{68}N_2O_{17}$	57,78	7,85	3,21	—62,8	6,65	0,76	0,64	142—4	—25,3
III	148—50	77	58,68	7,83	3,01	$C_{44}H_{70}N_2O_{17}$	58,78	7,85	3,12	—100,0	6,75	0,82	0,57	135—9	—50,2
IV	143—5	76	62,15	8,49	1,49	$C_{44}H_{71}NO_{15}$	61,88	8,38	1,64	—107,1	7,10	0,85	0,49	133—6	—43,2
V	174—6	85	61,04	8,48	1,53	$C_{42}H_{69}NO_{15}$	60,92	8,40	1,69	—75,3	6,85	0,81	0,52	130—3	—30,5

*c — 2, methanol.
†in 66% aqueous dimethylformamide solution.
‡c — 2, water.

TABLE 2. PMR Parameters of Erythromycin Esters

Compound	R	Chemical shifts (ppm)											Spin—spin coupling constants* (Hz)				
		NCH_3	OCH_3	$C_1'=H$	$C_2'=H$	$C_1''=H$	$C_{13}=H$	R: H_3'''	R: H_4'''	R: H_5'''	R: H_α	R: H_β	$^3I_{1'2'}$	$^3I_{\alpha\beta}$	$^3I_{3'''4'''}$	$^3I_{4'''5'''}$	$^4I_{3'''5'''}$
I	H	7,75	6,71	5,63	6,3	5,1	5,0	—	—	—	—		6,7	—	—	—	—
II		7,78	6,55	5,26	5,1	5,1	5,0	2,77	2,69	—	—	—	6,9	—	3,3	—	—
III		7,77	6,64	5,55	5,1	6,1	5,0	3,30	2,70	—	2,64	3,44	6,9	15,5	3,2	—	—
IV		7,79	6,69	5,59	5,1	5,1	5,0	3,45	3,59	2,56	2,63	3,76	6,9	16,0	3,1	1,7	0,7
V		7,74	6,61	5,37	5,1	5,1	5,0	2,95	3,53	2,45	—		6,9	—	3,1	1,5	0,7

*$^3IC_{13}H=CH_2$=10 and 2 Hz; $^3IC_1''H=C_2H_2$=4 and 1 Hz.

TABLE 3. Antimicrobial Activity of Erythromycin 5-Nitrofuroate and Furoate Esters, Their Mean Therapeutic Doses (TD_{50}) and Mean Lethal Doses (LD_{50})

Compound	Minimum inhibitory concentration (μg/ml)											TD_{50} (mg/kg)			LD^{*}_{50} (mg/kg)
	staph. aureus 209 P	strept. hemolyticus 295	C. diphtheriae PW-8	B. anthracoides 1312	E. coli 675	Salm. typhosa 1196	B. dysenteriae 2a-516	proteus vulgaris 5(28-III)	B. pyocyaneus 165	candida albicans 67/846	trichophyton gypseum 4/3	staphylococcal infection	streptococcal infection	pneumococcal infection	
I	0,08	0,08	0,08	0,62	50	200	50	>200	200	>1000	>1000	90	25	100	650 (524–806)
II	0,08	0,08	0,04	0,62	50	100	50	>200	>200	83,3	333	175	150	155	>6000
III	0,08	0,08	0,04	0,62	50	100	50	200	>>200	20,9	167	265	50	180	355 (298–422)
IV	0,16	0,62	0,08	2,5	100	200	100	>200	>>200	>>1000	500	210	170	165	2050 (1206–3485)
V	0,08	0,08	0,08	0,62	100	200	100	>200	>200	>1000	>1000	115	100	110	>10 000

*Intraperitoneal administration to white mice.

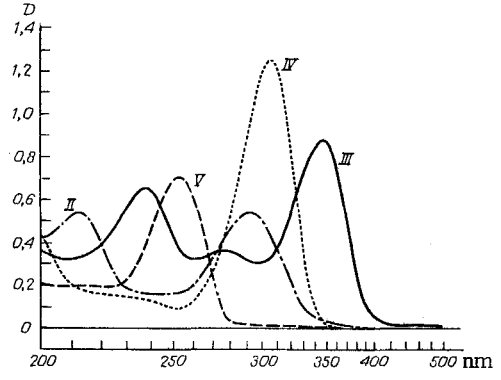


Fig. 1. UV spectra of erythromycin esters.

bands in the 1735-1740, 1020-1180, and 1690 cm^{-1} regions indicate that the lactone ring at the erythronolide keto groups are retained [4, 8]. Since the ester carbonyl group also absorbs at 1735 cm^{-1}, its presence can only be established by analyzing the relative intensities of the corresponding bands. By means of PMR spectra we proved unequivocally that acylation proceeded through the hydroxyl group of the desosamine function. The resonance of the C_1' and especially the C_2' protons is shifted to low field compared with unsubstituted I. The huge paramagnetic shift in the absorption of the C_2' hydrogen atom ($\sim$ 1.2 ppm) is particularly important; it can arise only when the hydroxyl at C_2' is acylated (Table 2). This is in good agreement with the literature data concerning the higher reactivity of the hydroxyl group in the desosamine part of the antibiotic [4]. The ease with which esterification takes place in this position is explained by the proximity of the $N(CH_3)_2$ group, which acts as an intramolecular catalyst for O-acylation. It is known that the 2' esters of erythromycin are readily absorbed when administered *per os*, giving high concentrations in the blood serum. This is connected with two factors, viz., the increased lipophilicity of the ester and its reduced basicity compared with I. To manifest biological activity of this kind, the esters must be hydrolyzed to erythromycin in the organism [7]. If the degree of hydrolysis of the 2' ester is low, the observed higher concentration of the ester in the blood will be counteracted by the low concentration of the active base I.

As can be seen from Table 3, compounds II-V possess high activity against *Staph. aureus*, *Strept. hemolyticus*, *C. diphtheriae*, and *B. anthracoides*, and do not differ significantly from I in this respect. Only ester IV had a somewhat weaker action. Like the starting compound I, the preparations studied showed only insignificant activity, of no practical interest, against Gram-negative bacteria. Esters II and III had appreciable activity against pathogenic fungi.

In a study of the chemotherapeutic effectiveness of compounds II-V in experiments on infected mice, they displayed a less pronounced action than I. The activity of V does not differ significantly from that of I against staphylococcal and pneumococcal infections, but is considerably inferior against streptococcal sepsis. It should be noted that the toxicity of compounds II, IV, and V (see Table 3), as determined by intraperitoneal administration to white mice, is considerably lower than that of erythromycin.

Thus, except for a decrease in acute toxicity, we have found no advantages in compounds II-V over I with respect to antimicrobial activity, either *in vitro* or *in vivo*. However, the compounds studied are of theoretical interest as starting materials for the synthesis of new preparations of this type for potential use as drugs.

EXPERIMENTAL

The starting material needed for the work, i.e., erythromycin A, was recrystallized from chloroform; it was chromatographically pure and its physicochemical properties matched the literature data [9]. The purity of the erythromycin esters was monitored by thin-layer chromatography on Silufol using the butanol—water—acetone—ammonia (8:6:1:1) system (system 1) and the methylene chloride—benzene—methanol (16:2:3) system (system 2), and using "aldehyde—sulfuric acid" as developer. The IR spectra were recorded with a UR-20 spectrophotometer, and the UV spectra were recorded with a Specord spectrophotometer on 10^{-4} M alcohol solutions (l = 0.5 cm). The PMR spectra of compounds I-V were recorded with a Perkin—Elmer R-12A instrument using 15% solutions in deuterochloroform and deuteroacetone, with tetramethylsilane as internal standard. The basicity of esters II-V was measured potentiometrically using a Seibold GVN potentiometer with glass and silver chloride electrodes. Compounds II-V (0.5 mmole) were dissolved in 5 ml of dimethylformamide (DMF), treated with 80 ml of 66% aqueous DMF, and titrated with 0.1 N hydrochloric acid.

3-Cladinosyl-5-[2'-O-(5-nitro-2-furoyl)]desoaminylerythronolida A (II). Erythromycin (2.00 g, 2.72 mmole) was dissolved in 25 ml of acetone prepurified by the method described in [10]. The solution was treated with 1.01 g (12 mmole) of dry, finely divided sodium bicarbonate, and a solution containing 0.48 g (2.72 mmole) of 5-nitro-2-furoyl chloride in 6 ml of acetone was added dropwise over 20-30 min while stirring (room temperature, protection from atmospheric moisture). Stirring was continued for 6-7 h, and the mixture left overnight. The further treatment and isolation of the product were carried out as described in [6], to give 2.08 g (88%) of a lightly colored crystalline powder, mp 182-186°. This was dissolved in 40 ml of acetone, treated with OU-A carbon, and reprecipitated with water. The product was dried under vacuum over phosphorus pentoxide to give 1.40 g of chromatographi-

cally pure pale-yellow acicular crystals of monoester II, mp 197-196°. The compound had a slightly bitter taste, was readily soluble in acetone, ether, and DMF, but sparingly soluble in water and glycerol. IR spectrum (in nujol), cm^{-1}: 3450 (OH), 1735 (ester and lactone CO), 1690 (CO of erythronolide keto group), 1350, 1540 (CNO_2), 1020, 1060, 1080, 1115, 1135, 1170 (lactone C=O=C). UV spectrum in ethanol, λ_{max}, nm (ε): 213 (10,800), 293 (10,800); pK_a 6.65 (in 66% aqueous DMF).

Phosphate of II. A vigorously stirred solution of 1.00 g (1.17 mmole) of II in 180 ml of absolute ether was treated dropwise with 0.08 ml of 85% phosphoric acid in 60 ml of ether, and refrigerated for 20 min. The precipitate was filtered off, carefully washed with ether, and dried under vacuum over phosphorus pentoxide, to give 0.86 g (78%) of the phosphate in the form of a white powder, mp 142-144° (decomp.), readily soluble in water and acetone; $[\alpha]_D^{20} = -25.3°$ (c = 2, water). Found, %: N 2.83. $C_{42}H_{68}O_{11}N_2 \cdot H_3PO_4$. Calculated, %: N 2.89.

Hydrochloride of II. A stirred solution of 0.61 g (0.7 mmole) of II in 100 ml of absolute ether was treated dropwise with a solution of 0.7 mmole of hydrogen chloride in 50 ml of ether. The precipitate was rapidly separated, carefully washed with ether, and dried under vacuum over calcium chloride to give 0.47 g (73%) of the hydrochloride, mp 109-113° (decomp.). Found, %: C 55.54; H 7.64; N 3.04. $C_{42}H_{68}O_{17}N_2.HCl$. Calculated, %: C 55.47; H 7.65; N 3.08.

3-Cladinosyl-5-[2'-O-(5-nitro-2-furyl)acryloyl]desosaminylerythrnolide A (III), 3-Cladinosyl-5-[2'-O-(2-furyl)acryloyl]desosaminylerythronolida A (III), 3-Cladinosyl-5-[2'-O-(2-furoyl)]desosaminylerythronolide A (V). These were prepared by reacting I with the acid chlorides of β-(5-nitro-2-furyl)acrylic, β-(2-furyl)acrylic and 2-furancarboxylic acid, and were isolated and purified under the conditions described for monoester II. Ester V was then recrystallized from anhydrous acetone.

The antibacterial activity *in vitro* was studied by serial twofold dilution in a liquid nutrient medium. Tests were carried out on 11 strains, including 4 types of Gram-positive bacteria (*Staphylococcus, Streptococcus, B. anthracoides,* and *C. diphtheriae*) and 5 types of Gram-negative bacilli (*E. coli, Salm. typhosa, B. dysenteriae, B. pyocyaneus,* and *P. vulgaris*). The fungistatic activity of the compounds was determined against two strains of pathogenic fungi (*Candida albicans* 67/846 and *Trichophyton gypseum* 4/3) in a Saburo nutrient medium. The experiments with the *Streptococcus* were carried out using a meat–peptone broth containing 10% normal equine serum, and those with the other bacteria were carried out using a Höttinger broth.

Generalized infection in mice weighing 18-20 g was induced by intraperitoneal injection of the bacteria in a 0.25% agar solution. The challenge dose of "Zhaev" staphylococci was 400 million microbial cells, and that of the hemolytic streptococcus was 295-350 million microbial cells. Phenumococcal sepsis was produced by intraperitoneal injection of 0.5 ml of a one-day culture grown on a 10% serum meat–peptone broth and diluted to 10^{-7} with peptone water. In the case of the staphylococcal and streptococcal infections, the preparations were tested at a daily dose of 400 mg/kg or less, and in the case of pneumococcal sepsis the daily dose was 200 mg/kg or less. The preparations were administered internally twice a day for two days. The treatment commenced immediately after the challenge. The chemotherapeutic effectiveness of the preparations was assessed from the surviving animals. The mean therapeutic dose (TD_{50}) was calculated by the method of Reed and Muench [11].

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