CCA-481

615.779:547.29.024
Original Scientific Paper

Erythromycin Series. II.* Acylation of Erythromycin oxime and 9-Amino-3-*O*-cladinosyl-5-*O*-desosaminyl-6,11,12-trihydroxy-2,4,6,8,10,12-hexamethylpentadecane-13-olide with Ester-chlorides of Dicarboxylic Acids**

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Received July 12, 1967

The acylation of erythromycin oxime and 9-amino-3-*O*-cladinosyl-5-*O*-desosaminyl-6,11,12 - trihydroxy-2,4,6,8,10,12 - hexamethylpentadecane-13-olide (erythromycyl amine) was performed by means of dicarboxylic acid chloride esters and the corresponding *O*- and *N*-acyl derivatives were obtained. A comparison of the physical properties and antibiotic activity of these compounds with those of corresponding erythromycin esters shows that erythromycin oxime and erythromycyl amine are very similar to erythromycin itself in their chemical and antimicrobial behaviour.

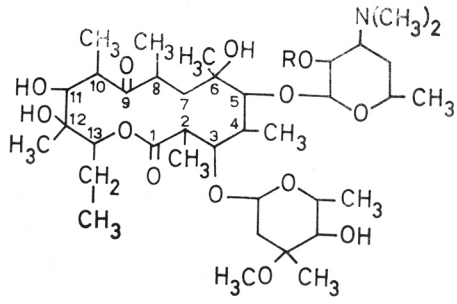
Acylation of erythromycin with different acid chlorides or acid anhydrides yielded a variety of products with a single acyl grouping bound to the hydroxyl group of desosaminyl rest (I). The sole exception was when acetanhydride was used as an acylating agent to yield erythromycin diacetate[1]. A large number of erythromycin esters with aliphatic mono- and dicarboxylic acids, with monoesters of aliphatic dicarboxylic acids, and with aromatic acids respectively has been prepared and described[1–9]. Several of these esters *e. g.* propionate, ethylcarbonate, ethylsuccinate, have been recommended in therapeutic praxis owing to their advantages over erythromycin itself.

In continuation of our experiments on structural modifications in the erythromycin molecule[10] we have performed a series of acylations of erythromycin oxime and erythromycyl amine with methyl- or ethylesterchlorides of succinic resp. adipic acid. By choosing proper reaction conditions it was possible to introduce either one or two acyl groups in the molecule. The purity of the products was controlled by thin-layer chromatography on Silicagel G (Merck) using a methylene chloride—methanol—benzene—formamide (20 : 5 : 5 : :0.5) mixture as the developing solvent system. The infrared absorption spectra of the prepared acyl derivatives were in good agreement with the infrared absorption spectra of erythromycin esters[2].

The formulas, molecular weights and physical properties for the new compounds are presented in Table I along with antibiotic activities found by the plate bioassay method. The specific rotations of the acyl derivatives

* Part I.: S. Djokić and Z. Tamburašev, *Tetrahedron Letters* No. 17 (1967) 1645.

** Presented in part at the 2nd *Yugoslav Congress for Pure and Applied Chemistry*, Beograd, June 16, 1966. Abstract of Papers, p. 195.



I
R = X—CO—

differ noticably from group to group (monoacyl-bisacyl-oxime-amine) but not significantly within the group. Monoacyl derivatives of erythromycin oxime resp. erythromycyl amine show only a slight decrease in their *pK*'a values when compared to the starting compounds. Erythromycin esters on the other hand have markedly decreased *pK*'a values[2]. The *pK*'a values of bis acyl derivatives of erythromycin oxime resp. erythromycyl amine are also significantly lower. The hydroxyl group of the desosaminyl rest has already been established as the site where acylation occurs. It can be suggested that monoacylation takes place exclusively at the amino resp. oximino group of erythromycyl amine resp. erythromycin oxime. The second acylation then occurs at the hydroxyl group of desosaminyl rest.

By comparison of the values obtained by the antibiotic assays of the compounds described in this paper with those of simple erythromycin esters and with starting materials (see Table I and II) the folowing characteristic features can be envisaged: the carbethoxy derivatives possess lower activities than corresponding carbomethoxy derivatives; introduction of the second acyl group in the molecule does not substantially change the activity of the parent compounds; the activities of carbometoxy derivatives do not differ from those of starting materials. Erythromycin oxime and erythromycyl amine behave very similarly to erythromycin itself in biological tests as well as towards acylating agents.

EXPERIMENTAL

General Procedure for the Preparation of Monoacyl Derivatives of Erythromycin oxime and Erythromycyl amine

To a solution of erythromycin oxime or erythromycyl amine (6.7 mmoles) in dry acetone (100 ml.) dry sodium hydrogen carbonate (2.5 g.) was added. Then a solution of one equivalent of the corresponding ester-chloride in dry acetone (25 ml.) was added dropwise with stirring over the period of one hour. After stirring for one additional hour the reaction mixture was filtered, the filtrate diluted with a solution of 2.5 g. of sodium hydrogen carbonate in 130 ml. water and extracted with one 100 ml. and two 50 ml. portions of ether. The combined ether extracts were dried over anhydrous sodium sulphate filtered and freed from ether *in vacuo.* The crude crystalline product was purified by dissolution in acetone and addition of water until persistent turbidity. After standing for three hours crystallization sat in. Generally one such crystallization was sufficient to obtain an analytically pure product in 70—80% yield.

TABLE I

The Physical Properties and Plate Bioassay of O- and N-acyl Derivatives[a]

No.	Compound	Formula Mol. weight	m. p.[b] °C	$[\alpha]_D^{20}$[c]	pK[d]	Plate bioassay[e] u/mg.
II	Erythromycin oxime methyl succinate	$C_{42}H_{74}N_2O_{16}$ 863.06	108—112	— 125.7	8.31	400—450
III	Erythromycin oxime ethyl succinate	$C_{43}H_{76}N_2O_{16}$ 877.09	99—102	— 124.3	8.18	250—300
IV	Erythromycin oxime bis-methyl succinate	$C_{47}H_{80}N_2O_{19}$ 977.16	173—176	— 109.7	6.7	400—450
V	Erythromycin oxime bis-ethyl succinate	$C_{49}H_{84}N_2O_{19}$ 1005.21	160—162	— 109.8	6.61	200—250
VI	Erythromycin oxime methyl adipate	$C_{44}H_{78}N_2O_{16}$ 891.12	89—94	— 109.3	8.3	400—450
VII	Erythromycin oxime ethyl adipate	$C_{45}H_{80}N_2O_{16}$ 905.14	82—86	— 110.0	8.18	350—400
VIII	Erythromycin oxime bis-methyl adipate	$C_{51}H_{88}N_2O_{19}$ 1033.27	78—83	— 99.0	6.57	400—450
IX	Erythromycin oxime bis-ethyl adipate	$C_{53}H_{92}N_2O_{19}$ 1061.32	72—76	— 104.7	6.56	200—250
X	Erythromycyl amine methyl succinate	$C_{42}H_{76}N_2O_{15}$ 848.06	114—119	— 120.7	8.43	400—450
XI	Erythromycyl amine ethyl succinate	$C_{43}H_{78}N_2O_{15}$ 863.01	103—105	— 122.4	8.39	250—300
XII	Erythromycyl amine bis-methyl succinate	$C_{47}H_{82}N_2O_{18}$ 963.17	95—101	— 108.3	6.8	450—500
XIII	Erythromycyl amine bis-ethyl succinate	$C_{49}H_{86}N_2O_{18}$ 991.23	160—162	— 110.1	6.62	200—250
XIV	Erythromycyl amine methyl adipate	$C_{44}H_{80}N_2O_{15}$ 877.13	102—105	— 115.1	8.4	400—450
XV	Erythromycyl amine ethyl adipate	$C_{45}H_{82}N_2O_{15}$ 891.16	91—95	— 114.8	8.21	350—400
XVI	Erythromycyl amine bis-methyl adipate	$C_{51}H_{90}N_2O_{18}$ 1019.28	88—90	— 91.1	6.9	400—450
XVII	Erythromycyl amine bis-ethyl adipate	$C_{53}H_{94}N_2O_{18}$ 1047.34	69—71	— 99.9	6.61	200—250

[a] All compounds gave satisfactory C, H and N analyses; [b] Fischer-Jones apparatus; [c] 2% in acetone; [d] 66% dimethylformamide-water; [e] *British Pharmacopoeia* 1963, p. 1102.

General Procedure for the Preparation of Bis-acyl Derivatives of Erythromycin oxime and Erythromycyl amine

To a solution of erythromycin oxime or erythromycyl amine (6.7 mmoles) in dry acetone (100 ml.) dry sodium hydrogen carbonate (5 g.) was added. Then a solution of the corresponding ester-chloride (16.1 mmoles) in dry acetone (50 ml.) was added dropwise with stirring over the period of one hour. The stirring was continued for additional 8 hours whilst heating the reaction mixture under reflux. After cooling to room temperature and filtration, the clear filtrate was diluted with a solution of 5 g. sodium hydrogen carbonate in 250 ml. water and extracted with one 100 ml. and two 50 ml. portions of ether. The isolation of the product was performed in the same manner as for the mono-acyl derivatives. Yields 70—80%.

Acknowledgment. We are grateful to Mr. J. Hranilović and his staff for microanalyses, the infrared spectra and pK measurements and to Mrs. Poić for the microbiological assay.

TABLE II

The Physical Properties and Plate Bioassay of Erythromycin, Oxime, Amino Compound and of some Erythromycin Esters

Compound	Ref.	m. p. °C	$[\alpha]_D^{20}$	*pK*	Plate bioassay u/mg.
Erythromycin	1	135—140	— 73.5	8.6	
	11		— 78.0		
	12				949
Erythromycin oxime	10	184—189	— 73.5	—	520
Erythromycyl amine	10	145—148	— 50.0	8.4	480
Erythromycin methyl succinate	6	116—118	— 55.0	—	820
Erythromycin ethyl succinate	6	109—110	— 42.5	—	712
Erythromycin ethyl adipate*	6	113—115	— 70.0	—	710

* Erythromycin methyl adipate was not prepared.

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IZVOD

Studije u redu eritromicina. II. Aciliranje eritromicin oksima i 9-amino-3-*O*-kladinozil-5-*O*-desozaminil-6,11,12-trihidroksi-2,4,6,8,10,12-heksametilpentadekan-13-olida sa kloridima estera dikarbonskih kiselina

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Reakcijom eritromicin oksima i 9-amino-3-*O*-kladinozil-5-*O*-desozaminil-6,11-12-trihidroksi-2,4,6,8,10,12-heksametilpentadekan-13-olida (eritromicil amina) s kloridima metil i etil estera jantarne i adipinske kiseline pripravljeni su odgovarajući mono- i bis-acil derivati (II—XVII). Uspoređenje fizikalnih svojstava i antibiotskog aktiviteta ovih spojeva (Tabela I) s istim vrijednostima odgovarajućih estera eritromicina i njihovih matičnih supstancija (Tabela II) pokazuje da se eritromicin oksim i eritromicil amin ponašaju vrlo slično kao eritromicin.

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Primljeno 12. srpnja 1967.