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Note

Erythromycin series

IV. Thin-layer chromatography of erythromycin, erythromycin oxime, erythromycylamine and their acyl derivatives

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A large number of erythromycin esters prepared from aliphatic mono- and dicarboxylic acids have been described, the ester group being formed through the reaction of the basic sugar desosamine at the 2′-position[1–9] (Fig. 1, I). Jones *et al.*[10] reported the synthesis of mono-, di- and tri-substituted alkyl esters of erythromycin A and B by selective esterification and hydrolysis. The preparation of erythromycin oxime and erythromycylamine[11,12] made possible reactions on the oximino and amino groups (Fig. 1, II and III), and hence the preparation of their mono- and bisacyl derivatives (Fig. 1, IV–VII)[13,14].

Although numerous chromatographic investigations of erythromycin and its derivatives have been described, they were carried out for identification purposes or for the determination of their hydrolysis products[15–19].

In our work we tried not only to identify, but also to test and confirm by thin-layer chromatography, our assumptions concerning the position of the acyl group in mono- and bisacyl derivatives of erythromycin oxime and erythromycylamine. By choosing suitable reaction conditions, the derivatives were prepared by the reaction of oxime or amine with mono- and diacyl chlorides[13,14] and their behaviour on thin-layer chromatographic (TLC) plates was investigated. After having established previously that they behaved in a similar manner on the chromatograms, we limited our investigations to monopropionyl and monosuccinyl derivatives [Fig. 1, IV and V, where R_1, R_2 and R_3 are $-OCCH_2CH_3$ and $-OC(CH_2)_2COOH_3$, respectively] and dipropionyl and disuccinyl derivatives [Fig. 1, VI and VII, where R_1, R_2 and R_3 are $-OCCH_2CH_3$ and $-OC(CH_2)_2COOCH_3$, respectively.

The first problem was to find a system that would permit the separation of the substances being investigated. A series of solvents were tested, and the best results were obtained with those listed in Table I.

The R_F values of the substances investigated for these solvent systems are listed in Tables II and III and their physical constants are presented in Table IV.

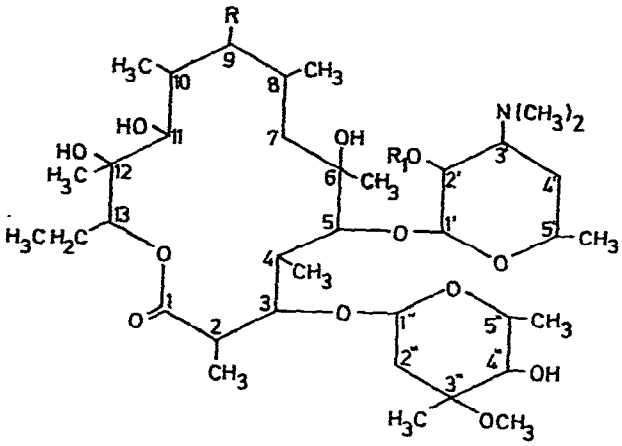

Fig. 1. Structures of compounds investigated. I: R = =O, R_1 = H. II: R = =NOH, R_1 = H. III: R = $-NH_2$, R_1 = H. IV: R = =NOR_2, R_1 = H or R = =NOH, R_1 = acyl. V: R = $-NHR_3$ R_1 = H or R = $-NH_2$, R_1 = acyl. VI: R = =NOR_2, R_1 = R_2. VII: R = $-NHR_3$, R_1 = R_3.

TABLE I

OPTIMAL SOLVENTS FOR SEPARATION OF ERYTHROMYCIN DERIVATIVES

Solvent system	*Component*			
	CH_2Cl_2	*$CHCl_3$*	*CH_3OH*	*$HCONH_2$*
A	80	20	20	—
B	100	—	20	2
C	100	—	20	—
D	—	20	100	—

TABLE II

R_F VALUES FOR MONO- AND DIPROPIONATES TESTED

E = Erythromycin; EO = erythromycin oxime; EA = erythromycylamine; EPr = E propionate; EOMPr = EO monopropionate; EAMPr = EA monopropionyl derivative; EODPr = EO dipropionate; EADPr = EA dipropionyl derivative. For solvent systems A–D, see Table I.

Compound	*A*	*B*	*C*	*D*
E	0.26	0.48	0.25	0.28
EO	0.23	0.48	0.28	0.27
EA	0.05	0.07	0.06	0.07
EPr	0.76	0.93	0.81	0.80
EOMPr	0.22	0.51	0.28	0.27
EAMPr	0.11	0.23	0.12	0.25
EODPr	0.81	0.94	0.81	0.77
EADPr	0.60	0.85	0.70	0.78

EXPERIMENTAL

Preparation of TLC plates

TLC plates (20 × 20 cm, 0.25 mm layer thickness) were prepared with standard equipment using a slurry of commercial silica gel G (Merck, Darmstadt, G.F.R.) in phosphate buffer (pH 8).

TABLE III

R_F VALUES FOR MONO- AND DIMETHYLSUCCINATES TESTED

E = Erythromycin; EO = erythromycin oxime; EA = erythromycylamine; EES = E ethyl succinate; EOMMS = EO monomethylsuccinate; EAMMS = EA monomethylsuccinyl derivative; ECDMS = EO dimethylsuccinate; EADMS = EA dimethylsuccinyl derivative. For solvent systems A–D, see Table I.

Compound	*A*	*B*	*C*	*D*
E	0.18	0.33	0.14	0.22
EO	0.16	0.32	0 13	0.21
EA	0.04	0.06	0.03	0.05
EES	0.51	0.81	0.66	0.69
EOMMS	0.21	0.39	0.17	0.22
EAMMS	0.07	0.18	0.07	0.19
EODMS	0.51	0.90	0.70	0.70
EADMS	0.04	0.72	0.55	0.68

TABLE IV

PHYSICAL CONSTANTS OF SUBSTANCES INVESTIGATED

Compound	*Melting point (°C)* *	$[\alpha]_D^{20}$ **	pK_a ***
E	135–140	−73.5	8.6
EO	152–157	−70.5	8.4
EA	122–126	−50.0	8.4
EES	116–118	−42.5	6.7
EPr	122–126	−81.6	6.9
EOMPr	126–129	−120.0	8.2
EAMPr	139–145	−50.5 §	8.5
EODPr	196–202	−133.0	5.9
EADPr	135–142	−46.5 §	6.7
EOMMS	108–112	−125.7	8.31
EAMMS	117–123	−45.1	9.0
EODMS	173–176	−109.7	6.7
EADMS	101–106	−33.0	6.5

* Fischer–Jones apparatus.
** 2% in acetone.
*** 66% in dimethylformamide–water.
§ 1% in chloroform.

Reagents

The spray phenol–sulphuric acid reagent was prepared from 3 g of phenol, 95 ml of absolute ethanol and 5 ml of concentrated sulphuric acid.

Solutions for spotting were prepared by dissolving each substance investigated in methanol (10 mg/ml).

Chromatographic procedure

Volumes of 1 μl of the methanolic solutions of each compound were applied to the plate by means of micropipettes and the plates were inserted into a chromatographic chamber, lined with filter-paper, that had previously been saturated with the vapour of the corresponding solvent system. The plates were developed to a distance

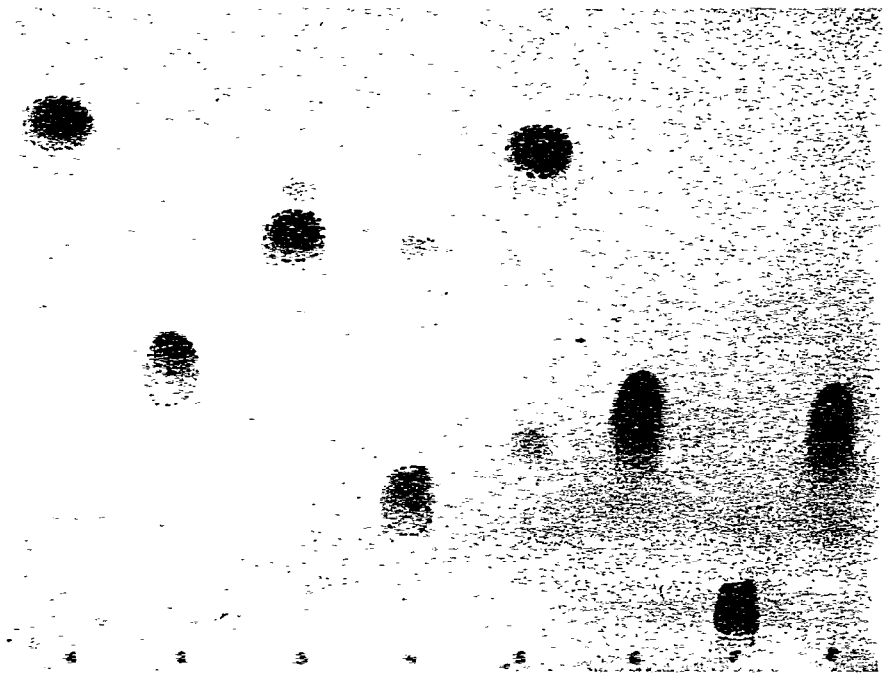

Fig. 2. Typical thin-layer chromatogram of mono- and dimethylsuccinyl derivatives. 1 = EODMS; 2 = EOMMS; 3 = EADMS; 4 = EAMMS; 5 = EES; 6 = E; 7 = EA; 8 = EO. Solvent system B.

of about 15 cm, removed from the tank, dried to remove the solvent, sprayed with the spray reagent and finally heated for 10 min at 110°. A typical chromatogram is shown in Fig. 2.

RESULTS AND DISCUSSION

On comparison of Tables II, III and IV it can be seen that there is a similar relationship between the R_F values of erythromycin and its esters and between erythromycin oxime and erythromycylamine and their diacyl derivatives, while the R_F values for their monoacyl derivatives are lower. The pK_a values of erythromycin esters, erythromycin oxime and erythromycylamine diacyl derivatives are much lower than the pK_a values of erythromycin, erythromycin oxime and erythromycyl amine, respectively. On the other hand, the pK_a values of the monoacyl derivatives of erythromycin oxime and erythromycylamine are virtually identical with those of erythromycin oxime and erythromycylamine, respectively.

Taking into consideration that the esterification of the hydroxyl group of desosamine in the erythromycin molecule decreases the pK_a values, it can be concluded that in the monoacyl derivatives of the oxime and amine the groups first acylated were the oximino and amino groups, respectively.

By calculating the ΔR_M values from the experimentally obtained data (Table V), it can immediately be observed that ΔR_M (2'-O-acyl) parameters for erythromycin are high and approximately of the same order as the ΔR_M values for erythromycin

TABLE V

ΔR_M VALUES FOR SOLVENT SYSTEMS TESTED

Compound	*A*	*B*	*C*	*D*
E/EPr	0.955	1.158	1.107	1.012
E/EES	0.676	0.929	1.076	0.897
EO/EOMPr	−0.025	0.052	0.0	0.0
EOMPr/EODPr	1.180	1.178	1.040	0.957
EA/EAMPr	0.399	0.598	0.330	0.648
EAMPr/EADPr	1.084	1.278	1.233	1.027
EO/EOMMS	0.118	0.133	0.137	0.0
EOMMS/EODMS	0.619	0.714	1.057	0.943
EA/EAMMS	0.257	0.536	0.378	0.649
EAMMS/EADMS	0.947	1.069	1.219	0.957

oxime and erythromycylamine diacyl derivatives, while the values for the corresponding mono-derivatives are relatively low.

It is known[20] that for a particular reaction on the same functional group, similar compounds give similar ΔR_M values for a particular chromatographic system, and our results confirm the above assumption that it was only in erythromycin oxime and erythromycylamine diacyl derivatives that the reaction of the hydroxyl group in the 2'-position occurred.

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