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Erythromycin Series. IX. Acid Solvolysis of *N*-(4-Substituted-Benzenesulfonyl)Erythromycylamines

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Degradation of new erythromycin derivatives, *N*-(4-substituted-benzenesulfonyl)erythromycylamines, at pH = 1.2 was studied both at 26 °C and 36 °C. Degradation products were isolated and characterized.

Under the conditions examined hydrolysis occurred at the C-3 glycosidic linkage, and N-(4-substituted-benzenesulfonyl)-3--des-cladinosyl-erythromycylamines and sugar cladinose were isolated and identified as degradation products by IR, NMR and mass spectroscopy.

Hydrolytic products were separated by TLC from the parent compounds and quantitatively analysed by densitometry. The pseudo first-order rate constants of hydroslysis were evaluated.

INTRODUCTION

The synthesis of *N*-(4-substituted-benzenesulfonyl)-erythromycylamines was reported in a previous paper[1]. Only limited information is available on their stability in acidic solutions[1], while the kinetics of acid hydrolysis and hydrolytic pathway have not been studied previously.

The purpose of this investigation was to elucidate the hydrolytic pathway of acid hydrolysis of *N*-(4-substituted-benzenesulfonyl)erythromycylamines in water or methanol at pH 1.2, at 26 °C and 36 °C. Degradation products were isolated and characterised by IR, NMR and mass spectroscopy, TLC and elemental analysis.

In order to study the kinetics of hydrolysis a quantitative analytical method for the simultaneous determination of parent compounds and their degradation products was developed.

RESULTS AND DISCUSSION

Hydrolytic Pathway and Structure Determination of the Degradation Products

This class of compounds (Scheme 1.) is susceptible to acid-catalysed hydrolysis at three potential sites of scission: C-3 and C-5 glycosidic and C-9 sulfonamide bonds. The sulfonamide[2] and C-5 glycosidic[3] bonds hydrolyse in

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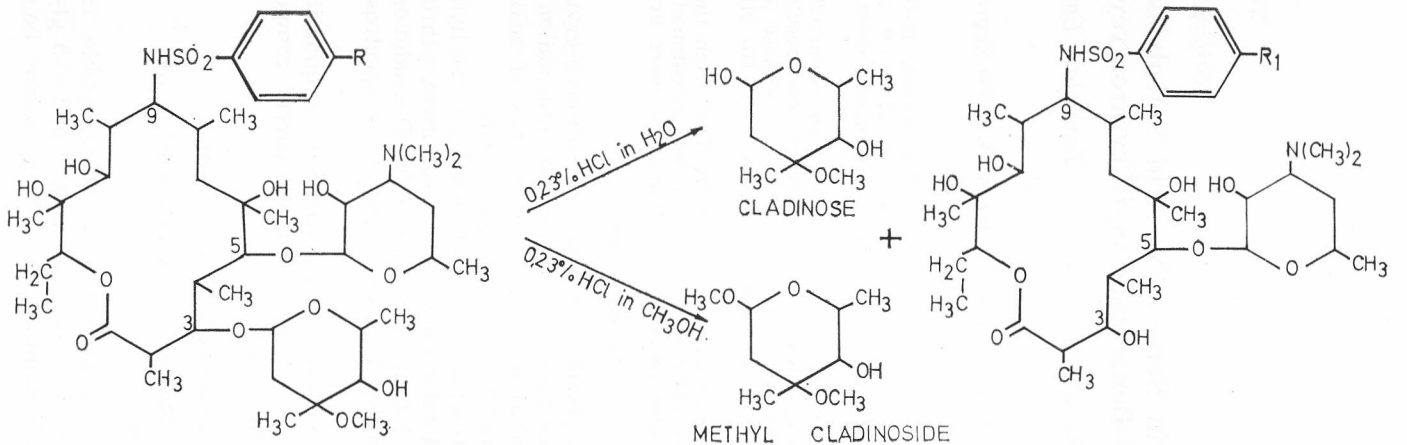
Scheme 1. Solvolysis of N-(4-substituted-benzenesulfonyl)erythromycylamines in 0,23% acid solutions



I	R = – Cl
II	R = – Br
III	R = – F
IV	R = – J
V	R = – CH_3
VI	R = – C_2H_5
VII	R = – $NHCOCH_3$
VIII	R = – $NHCOC_2H_5$

I a	R_1 = – Cl
II a	R_1 = – Br
III a	R_1 = – F
IV a	R_1 = – J
V a	R_1 = – CH_3
VI a	R_1 = – C_2H_5
VII a	R_1 = – NH_2

acidic solution under more vigorous conditions than the C-3 glycosidic bond. According to previously reported results for the degradation of erythromycin oxime and erythromycylamine under similar conditions[4] only rupture of the C-3 glycosidic bond and formation of cladinose would be expected (Sheme 1.).

To support the proposed hydrolytic pathway isolation and identification of degradation products were necessary. Thus, compounds I—VIII were left at room temperature in 0.23% methanolic hydrochloric acid for 24 hrs. The hydrolysis was monitored by TLC using methanol as solvent, and *N*-(4-substituted-benzenesulfonyl)erythromycylamines and methyl cladinoside were spotted as reference standards. In the course of time, a decrease in intensity of *N*-(4-substituted-benzenesulfonyl)erythromycylamines (lowest spot) and an increase in intensity at the upper (methyl cladinoside) and centre spots was observed (Figure 1.). According to Scheme 1., the centre spot was attributed

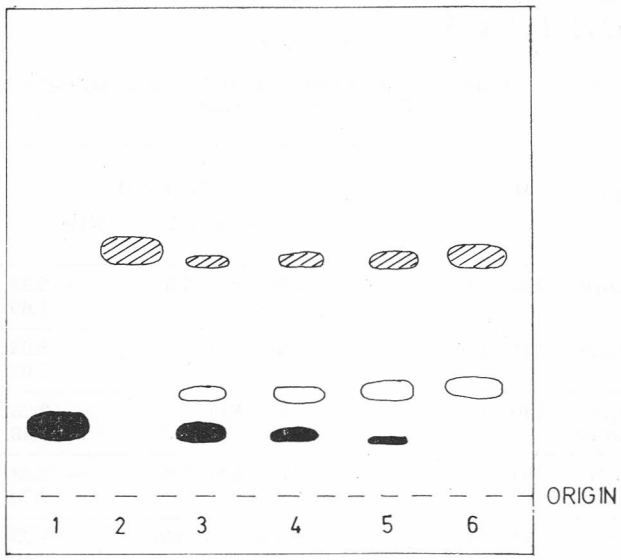


Figure 1. A thin layer chromatogram of a hydrolysis of *N*-(4-chloro-benzenesulfonil)-erythromycylamine (I) in 0.23% methanolic hydrochloric acid at room temperature for 24 hrs. 1 = STD (I); 2 = SDT methyl cladinoside; 3, 4, 5, 6 = samples at various time of hydrolysis.

to *N*-(4-substituted-benzenesulfonyl)-3-des-cladinosyl-erythromycylamines (Ia—VIIa). Its strong UV-absorbance suggested the existence of a sulfonamide bond. After 24 hrs, TLC analysis showed only the upper and central spots and in the next 48 hrs no further hydrolysis was detected.

Degradation products were isolated from the reaction mixture by chloroform extraction and recrystallization.

Structural assignment of the compound Ia was made as follows (the discussion can also be extended to compounds IIa—VIa):

The IR spectrum of Ia showed a close resemblance to the spectrum of the parent compound I, and the presence of bands at 1585, 825 and 750 cm^{-1} (p-C_6H_4) was indicative of an intact sulfonamide bond. ^{1}H-NMR spectrum of

Ia was similar to that of I, and differences were recognized in the disappearance of signals at δ 3.32 and 5.0 corresponding to the cladinose protons[5]. Further support was obtained from the chemical ionization mass spectrum which displayed the expected molecular ion peak at m/e 750.

Contrary to the above compounds, the IR spectra of *p*-acylamino derivatives VII and VIII differed from those of their hydrolytic products. The disappearance of infrared absorption at 1700 and 1500 cm^{-1} (-NHCO-) and the appearance of a new band at 1630 cm^{-1} ($-NH_2$) indicated that also deacylation took place under the hydrolysis conditions investigated. Thus, both VII and VIII derivatives gave the same hydrolytic product VIIa with the amino group in the para position. This was confirmed by the ^{1}H-NMR spectrum of VIIa (lack of a signal at δ 8.0 ppm, 1H, NHCO), and the mass spectrum which displayed a molecular ion peak at m/e 731.

Physical and spectral data of compounds Ia—VIIa are given in Table I.

TABLE I

Physical and Spectral Data of N-(4-Substituted-benzenesulfonyl)-3-des-cladinosyl--Erythromycylamines

Compd. No.*	Formula (M^+)	M. p.** °C	pK_a***	IR (cm^{-1}) *p*-phenyl	IR (cm^{-1}) NH_2	Spectra data H^1-NMR δ ppm****
Ia	$C_{35}H_{59}ClN_2O_{11}S$ (750)	148—152	8.45	1585, 825, 750	—	2.23 (s, 6H, $-N(CH_3)_2$) 7.60 (q, 4H, arom.)
IIa	$C_{35}H_{59}BrN_2O_{11}S$ (794)	151—154	7.98	1600, 820, 735	—	2.23 (s, 6H, $-N(CH_3)_2$) 7.68 (q, 4H, arom.)
IIIa	$C_{35}H_{59}FN_2O_{11}S$ (not determined)	140—147	7.72	1595, 835	—	2.23 (s, 6H, $-N(CH_3)_2$) 7.48 (m, 4H, arom.)
IVa	$C_{35}H_{59}JN_2O_{11}S$ (not determined)	153—158	7.70	1570, 820, 730	—	2.23 (s, 6H, $-N(CH_3)_2$) 7.70 (q, 4H, arom.)
Va	$C_{36}H_{62}N_2O_{11}S$ (730)	141—145	7.94	1600, 810, 680	—	2.23 (s, 6H, $-N(CH_3)_2$) 2.40 (s, 3H, *p*-CH_3) 7.50 (q, 4H, arom.)
VIa	$C_{37}H_{64}N_2O_{11}S$ (744)	144—149	8.00	1600, 830	—	2.23 (s, 6H, $-N(CH_3)_2$) 2.70 (q, 3H, ethyl-CH_3) 7.48 (q, 4H, arom.)
VIIa	$C_{35}H_{61}N_3O_{11}S$ (731)	165—169	8.10	1600, 830	1630	2.23 (s, 6H, $-N(CH_3)_2$) 7.00 (q, 4H, arom.)

* Recrystallized from chloroform-petroleum ether; ** Fisher-Johns; *** In dimethylformamide — water; **** In $CDCl_3$.

Kinetics

Hydrolysis of compounds I—VIII was carried out in 0.23% aqueous hydrochloric acid at 26 °C and 36 °C, or in 0.23% methanolic hydrochloric acid at 26 °C. In both media three-component mixtures were generated, which corre-

sponded to the cleavage of cladinose (Scheme 1.) and under the conditions investigated no further hydrolysis occurred.

After TLC separation, spectrophotometric densitometry was used to quantify each of the three components in the hydrolysis mixture. The calibration curves (peak area against the spotted quantity) generated from experimental data by the least-squares linear regression method, were linear from 10 to 43 μg, with the value of the correlation coefficient greater than 0.995.

The reproducibility of the quantitative TLC analysis by densitometry was checked by applying ten samples of standard (IV), each of 25 μg, on the plate. Each spot was scanned five times. The relative standard deviation was 2.3%. To avoid variations caused by different plates, standards and samples were always run on the same plate.[6].

Since the semilogarithmic plot of the amount of *N*-(4-substituted-benzenesulfonyl)erythromycylamines vs, time gave a straight line, it was concluded that the hydrolytic degradation followed pseudo-first order kinetics. The first order rate constants are listed in Table II.

TABLE II

Rate Constants for Acid Solvolysis of N-(4-Substituted-benzenesulfonyl)-Erythromycylamines

Compd. No.	Hydrolysis 26 °C	Hydrolysis 36 °C	Methanolysis 26 °C
I	7.0×10^{-4}	2.0×10^{-3}	2.9×10^{-2}
II	7.0×10^{-4}	2.2×10^{-3}	2.8×10^{-2}
III	1.4×10^{-3}	3.3×10^{-3}	3.1×10^{-2}
IV	3.3×10^{-4}	1.3×10^{-3}	2.5×10^{-2}
V	1.5×10^{-3}	3.2×10^{-3}	3.7×10^{-2}
VI	8.1×10^{-4}	2.2×10^{-3}	3.2×10^{-2}
VII	1.3×10^{-3}	3.0×10^{-3}	2.9×10^{-2}
VIII	1.1×10^{-3}	3.1×10^{-3}	3.2×10^{-2}

The data show that different *p*-substituents on the aromatic ring do not significantly affect the rate of hydrolysis. Increasing the temperature from 26 °C to 36 °C the degradation rate increased about three times, as expected.

The first order rate constants of hydrolysis at 26 °C increased markedly by changing the solvent from water to methanol. This indicates that the transition state has a less ionic character than the reactant, which is consistent with the reported mechanism of glycosidic hydrolysis.

EXPERIMENTAL

General

Melting points were determined by Fisher-Johns apparatus and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer spectrophotometer model 527 G. ^{1}H-NMR spectra were recorded on a Varian A 60 instrument with TMS as internal standard. Mass spectra were obtained on the CEC 21—110 C mass spectrometer at 70 eV. Analytical thin layer chromatography was performed on glass plates coated with a 0.25 mm layer of silica gel F_{254} (Kemika) using methanol as solvent. The compounds were detected either by UV light (254 nm) or spraying the plates with phenol-sulfuric acid and heating at 110 °C.

The fluorescent quenching measurements were made on the densitometer (Opton) at $\lambda = 254$ nm, using a slit 10×0.4 mm and scan speed 50/120 mm min^{-1}. Peak areas were determined by multiplying the peak height with peak width at half-height.

Materials

The standards, *N*-(4-substituted-benzenesulfonyl)-erythromycylamines (I—VIII), were synthesized by the reported method[1]. Other chemicals were of analytical grade.

Preparation of Degradation Products (Ia—VIIa)-General Procedure

A solution of *N*-(4-substituted-benzenesulfonyl)-erythromycylamines I—VIII (0.003 mol) in 1% methanolic hydrochloric acid (300 ml) was left at room temperature for 24 hrs, until TLC analysis indicated complete conversion.

The solution was evaporated in vacuo, the residue dissolved in chloroform (8 ml) and then added dropwise to a stirred mixture of saturated NaCl solution (12 ml), 20% Na_2CO_3 solution (20 ml) and saturated $NaHCO_3$ solution (12 ml). The separated aqueous layer was extracted with chloroform 3 times. The combined extracts were washed in succession with saturated $NaHCO_3$ (10 ml) and saturated NaCl solution (10 ml). After evaporation of the chloroform extract (dried over K_2CO_3), the residue was purified by precipitation from chloroform/petroleum ether and the purity checked by TLC. For all compounds the analyses of C, H, N and S were within $\pm$ 0.4% of the theoretical values.

Kinetics

Compounds I—VIII were hydrolysed at 26 °C and 36 °C in 0.23% aqueous or methanolic hydrochloric acid for 24 hrs. The concentration of the investigated substances in acid solutions was 2%. Samples (1 ml) were withdrawn at suitable time intervals, immediately neutralised by sodium carbonate and then 1 ml of methanol was added.

Sample solutions (10 mg/ml) were spotted (10 μl) on TLC plates in 1 cm long lines using a capillary pipet (Desaga). The calibration graphs were prepared by spotting alternately unknown samples and standards (10—43 μg). The plates were developed in methanol and spots quantified by densitometry.

For calculation of rate constants only those concentrations of compounds in hydrolysates were used which were within the linear portion of the calibration curve (10—43 μg). The sampling time intervals depended partly on the previously observed or expected degradation rate.

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SAŽETAK

Kisela hidroliza *N*-(4-supstituiranih-benzensulfonil)eritromicilamina

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Proučavana je kisela hidroliza (pH=1,2) N-(4-supstituiranih-benzensulfonil)eritromicilamina, novih derivata iz reda eritromicina, pri 26 °C i 36 °C, radi identifikacije svih produkata degradacije.

Ustanovljeno je da kod navedenih uvjeta puca glikozidna veza C-3, te nastaju N-(4-supstituirani-benzensulfonil)-3-des-kladinozil-eritromicilamini i šećer kladinoza, što je dokazano pomoću IR, NMR i masenih spektara.

Produkti hidrolize odijeljeni su od polaznih spojeva s pomoću tankoslojne kromatografije, kvantitativno su analizirani na denzitometru, a zatim su izračunane konstante brzine hidrolize pseudoprvog reda.