SYNTHESIS OF DERIVATIVES OF OLEANDOMYCIN AMINOHYDRIN

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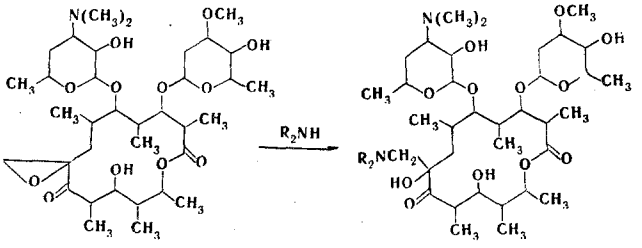
UDC 615.33:542.958:543.422.4.6

Reaction of oleandomycin with secondary amines proceeds at the epoxy group of the antibiotic in conformity with the Krasuskii rule to give biologically active derivatives of oleandomycin aminohydrin.

The constantly developing resistance of microorganisms requires constant renovation as well as improvement in the antibacterial, pharmacological, and physicochemical properties of the existing antibiotics. This can be achieved by chemical transformation of the latter.

The present paper is devoted to a study of the reaction of oleandomycin (I) with some aliphatic and heterocyclic secondary amines. Nucleophilic addition at the epoxy group of the antibiotic proceeds under relatively mild conditions (30-40°C in 15-30 h). Practically individual addition products II-VIII (Table 1) were obtained when the reaction was carried out in ether; other products of unestablished structure are formed in small amounts along with II-VIII when the reaction is carried out in alcohol. In conformity with the Krasuskii rule, one might have expected reaction to favor the formation of derivatives of oleandomycin aminohydrin* containing a hydroxyl group attached to the less hydrogenated carbon atom.



I II-VIII

II $NR_2=N(CH_3)_2$; III $NR_2=N(C_2H_5)_2$; IV NR_2= pyrrolidino V NR_2= piperidino
VI NR_2= morpholino VII NR_2= ethylenimino VIII NR_2= hexamethylenimino

The IR spectra of II-VIII show that the lactone ring (1080, 1710-1730 cm^{-1}) and the carbonyl group of the oleandolide ring (1690 cm^{-1}) are retained in them and that they contain hydroxyl groups. In addition, the absorption at 3075 cm^{-1} for VII is evidence for the presence of an ethyleneimine function.

The UV spectra of II-VIII, which, like the oleandomycin bases, contain a low-intensity or concealed maximum at 280-295 nm, indicate that they cannot be the anhydro forms: in the opposite case, we would have observed a bathochromic shift of the maximum to 310 nm and the appearance of a new intense maximum at about 240 nm, as occurs in the case of anhydrooleandomycin.

The protons of the epoxy group in the PMR spectrum of base I give a singlet at 8.23 τ. This singlet disappears in the spectrum of the product of reaction of oleandomycin with dimethylamine, while a new singlet at 7.93 τ and the characteristic AB quartet of the anisochronic protons of the $N-CH_2$ group at 7.3-7.8 τ (J = 14.5 Hz) appear, and the remaining spectral ranges are unchanged. These data are in conformity with structure II.

*In analogy with the names "oleandomycin chlorohydrin and glycol" [1-3].

Institute of Organic Synthesis, Academy of Sciences of the Latvian SSR, Riga. Translated from Khimiya Geterotsiklicheskikh Soedinenii, No. 2, pp. 168-171, February, 1974. Original article submitted February 27, 1973.

TABLE 1. N,N-Disubstituted Derivatives of Oleandomycin Aminohydrin

Compound	mp, °C	Empirical formula	Found, %			Calc., %			$[\alpha]_D^{20}$ (c 2, methanol)	Dihydrochloride		
			C	H	N	C	H	N		mp, °C	$[\alpha]_D^{20}$ (c 2, water)	
II	98—102	$C_{37}H_{68}N_2O_{12}$	60,7	9,6	3,6	60,3	9,3	3,8	−78,9°	120—123	−61,5°	97
III	93—96	$C_{39}H_{72}N_2O_{12}$	61,5	9,7	3,9	61,6	9,5	3,7	−87,5	142—145	−62,0	97
IV	98—101	$C_{39}H_{70}N_2O_{12}$	61,6	9,6	3,6	61,7	9,3	3,7	−74,2	126—130	−44,4	85
V	151—153*	$C_{40}H_{72}N_2O_{12}$	61,9	9,6	3,7	62,1	9,4	3,6	−81,6	133—137	−37,8	80
VI	205—206	$C_{39}H_{70}N_2O_{13}$	60,4	9,3	3,6	60,4	9,1	3,6	−80,2	128—130	−67,8	75
VII	118—121	$C_{37}H_{66}N_2O_{12}$	60,5	9,1	3,7	60,8	9,1	3,8	−73,6	—	—	92
VIII	152—154	$C_{41}H_{74}N_2O_{12}$	62,4	9,4	3,6	62,6	9,5	3,5	−85,2	140—142	−67,7	80

TABLE 2. Ionization Constants of N,N-Disubstituted Oleandomycin Aminohydrins (in aqueous ethanol at 20°C)

Compound	Computational method				Noyes method	
	pK_{a_1}	pK_{a_2}	$pK_{a_1}{}_T$	$pK_{a_2}{}_T$	pK_{a_1}	pK_{a_2}
II	8,74±0,10	7,34±0,03	8,72±0,09	7,19±0,06	8,74±0,06	7,51±0,03
III	9,10±0,09	7,82±0,05	9,10±0,06	7,68±0,05	8,95±0,06	7,93±0,04
IV	9,45±0,07	7,93±0,08	9,43±0,02	7,79±0,06	9,28±0,05	8,14±0,06
V	9,49±0,06	7,85±0,05	9,46±0,03	7,74±0,05	9,38±0,06	7,98±0,03
VIII	9,48±0,10	7,89±0,06	9,45±0,10	7,75±0,06	9,26±0,06	8,14±0,03
VI	8,57±0,02	4,61±0,02	8,54±0,02	4,48±0,02	8,50*	4,60*
VII	8,52±0,05	5,09±0,06	8,50±0,04	4,97±0,04	8,60*	5,25*
I	8,47±0,02	—	—	—	8,50*	—

*Graphical method (the pH at the half-neutralization point).

The ionization constants of each amino group were determined for the oleandomycin derivatives obtained (II-VIII) (Table 2). The thermodynamic ionization constants (pK_a^t) [4] were also calculated. The basicity of the dimethylamino group of the desosamine function of the aminohydrins is characterized by pK_{a_1}, the value of which depends on the structure of the N,N-disubstituted residue of the aminohydrin. The pK_{a_2} value reflects protonation of the latter. In this case, correlation between the pK_a values of the secondary amines [5-7] * used in the preparation of II-VIII and the pK_{a_1} and pK_{a_2} values is observed for II-V and VIII. The higher the basicity of the secondary amines, the higher the pK_{a_1} and pK_{a_2} values of the substituted aminohydrins.

In the case of VI, we demonstrated that N,N-disubstituted aminohydrins of oleandomycin form N-oxides at both amino groups in a dilute methanol solution of hydrogen peroxide. N-Oxide IX was isolated as a stable solvate with chloroform. This is also indicated by the PMR spectrum of IX (in hexadeuteroacetone): a singlet, which can be assigned to the proton of solvated chloroform, is observed at 2.1 τ. The formation of solvates with chloroform and other chlorinated hydrocarbons is a characteristic property of oleandomycin [2].

According to preliminary data, II-VIII display activity with respect to Gram-positive microorganisms. Prior to this, only one biologically active derivative of oleandomycin with an open epoxide ring – oleandomycin chlorohydrin [8] – was known.

EXPERIMENTAL

The homogeneity of the oleandomycin aminohydrin derivatives was monitored by thin-layer chromatography (TLC) on Silufol in butanol–water–acetone–ammonia (200 : 150 : 25 : 25) and butanol–acetic acid–water (3 : 1 : 1) systems; the chromatograms were developed with "aldehyde sulfuric acid." The PMR spectra of I and II (20% solutions in C_6D_6), N-oxide IX [in $(CD_3)_2CO$], and amine VI (in $CDCl_3$) were recorded with a Perkin–Elmer R12A spectrometer. The internal standard was tetramethylsilane. The basicity of the amines were measured with a Seibold DVN potentiometer with glass and silver chloride electrodes. A 0.5 mmole sample of II-V and VIII was dissolved in 10 ml of ethanol, and 75 ml of water was added (VI and VII were dissolved in 5 ml of methanol, and 80 ml of water was added); the solutions were titrated under nitrogen with 0.1 N hydrochloric acid at 20 ± 1°. The pK_a values were determined both by a graphical method from the titration curves [9] and by the usually employed computational method [4].

*We determined the basicity of hexamethyleneimine (pK_a 11.20) by potentiometry at 20°.

Oleandomycin N,N-Dimethylaminohydrin (II, Table 1). A. A mixture of 6.87 g (0.01 mole) of oleandomycin in 100 ml of absolute ether and 51 ml of 1.97 N dimethylamine in ether was held at 30° in a sealed ampule for 20 h. Filtration of the mixture, removal of the solvent by distillation, and drying of the residue gave 7.13 g (97%) of chromatographically homogeneous II, which was quite soluble in ether, benzene, methanol, and chloroform but only slightly soluble in water. IR spectrum (in Nujol): 3480 (OH), 1730 (lactone C=O), 1690 (shoulder, C=O of the keto group of the oleandolide ring), 1080 cm^{-1} (lactone ring). UV spectrum in 3% aqueous ethanol: inflection at 280-295 nm, $\varepsilon \sim 80$-115. The dihydrochloride was prepared as follows. A total of 4.38 ml of a 0.915 N ether solution of hydrogen chloride was added to a solution of 1.446 g (0.002 mole) of amino alcohol II in 250 ml of absolute ether, and the mixture was allowed to stand overnight in a refrigerator. The gelatinous precipitate was removed by filtration, washed with ether, and dried over phosphorus pentoxide to give 1.38 g of the dihydrochloride as a hygroscopic powder with mp 120-123° and $[\alpha]_D^{20} = -61.5°$ (c 2, water). Found, %: C 55.0; H 8.8; N 3.3; Cl 9.1. $C_{37}H_{68}N_2O_{12} \cdot 2HCl$. Calculated, %: C 55.1; H 8.8; N 3.5; Cl 8.8. The diphosphate was prepared as follows. A 0.244 g (0.002 mole) sample of 88% phosphoric acid in 150 ml of absolute ether was added to a solution of 0.72 g (0.001 mole) of amino alcohol II in 100 ml of absolute ether. The solution was stored in a refrigerator for 24 h. The gelatinous precipitate was separated and dried in vacuo to give 0.71 g (76%) of the diphosphate as a powder with mp 142-146° and $[\alpha]_D^{20} = -58.1°$ (c 2, water). Found, %: C 47.7; H 7.9; N 3.0; P 6.9. $C_{37}H_{68}N_2O_{12} \cdot 2H_3PO_4$. The methiodide was prepared as follows. A 0.5 ml sample of methyl iodide was added to a solution of 0.50 g of amino alcohol II in 50 ml of absolute ether, and the mixture was allowed to stand in the dark for 24 h. The light-yellow precipitate was removed by filtration, washed with ether, and dried to give 0.30 g of the methiodide with mp 170-175° (dec.). Reprecipitation from alcohol solution by the addition of ether gave a product with mp 175-177° (dec.). Found, %: C 51.9; H 8.1; N 3.2. $C_{38}H_{71}N_2O_{12}$. Calculated, %: C 52.2; H 8.2; N 3.2.

B. Performance of the reaction in ethanol gave 7.27 g of chromatographically nonhomogeneous product, containing two components, with mp 78-83°. Treatment with ether gave 1.19 g of ether-insoluble material with mp 179-181°. Compound II, with mp 98-102°, was isolated by passing the ether extract through finely grained powdered KSK silica gel.

Oleandomycin N,N-Diethylaminohydrin (III, Table 1). A. A total of 14.66 g (97%) of analytically pure powdered III with mp 88-94° was obtained from 13.74 g (0.02 mole) of epoxide I and 21.5 ml (0.21 mole) of diethylamine in 200 ml of absolute ether (at 30° for 23 h). Chromatographically pure amino alcohol III, with mp 93-96°, was obtained after treatment with KSK silica gel.

B. A two-component mixture was obtained when the reaction was carried out in ethanol. Treatment of the mixture with ether gave 13.80 g of amino alcohol III. The residue contained 0.66 g of a product of unestablished structure with mp 186-188° (from 50% ethanol) and $[\alpha]_D^{20} = -52.6°$ (c 2, methanol).

Aminohydrins IV-VI (Table 1). The reaction of epoxides I with pyrrolidine, piperidine, and morpholine was carried out in ethanol at 40° for 13-16 h. Reaction products IV-VI were isolated and purified as in the preparation of amino alcohol III. Compound VI was recrystallized from ether to give fine needles.

Oleandomycin Ethyleneiminohydrin (VII). A 14 ml (0.27 mole) sample of freshly distilled ethyleneimine was added to a solution of 6.87 g (0.01 mole) of oleandomycin in 150 ml of absolute ether, and the mixture was held at 30° for 34 h. The solvent was removed by distillation, and the residue was dried thoroughly in vacuo over KOH and P_2O_5. It was then dissolved in 200 ml of dry ether, and the solution was filtered through a dense filter. Crystallization commenced when the solution was carefully evaporated with a rotary evaporator. The solid material was removed by filtration, washed with dry ether, and dried to give 6.69 g (92%) of chromatographically pure amino alcohol VII with mp 118-121°. Quantitative determination of the ethyleneimine ring by means of potassium thiocyanate [10] in acidic media showed that its percentage was 95%. IR spectra (in Nujol): 3520, 3075 (asymmetrical vibrations of the aziridine ring), 1720, 1680, 1080 cm^{-1}. UV spectrum (in 3% aqueous ethanol): λ_{max} 288 nm, ε 59. In ethanol, this reaction gave a mixture of two products of unestablished structure: one, with mp 58-60°, was soluble in ether, and the other (mp 139-140°) was insoluble in ether. Both products gave a qualitative reaction for a primary amino group with dibepine [11].

Oleandomycin N,N-Hexamethyleneaminohydrin (VIII). This compound was obtained by the method used to prepare VII by heating the reaction mixture for 10 h. The product was purified by recrystallization from ether.

N,N-Dioxide of Amine VI (IX). Water (8 ml) and 1.30 ml (12.8 mmole) of 30% hydrogen peroxide were added to a solution of 0.5 g (0.64 mmole) of amine VI in 18 ml of methanol, and the mixture was allowed to stand for 24 h. The methanol was then removed by vacuum distillation, and the residue was extracted several times with chloroform to give 0.29 g of a white, powdery, chromatographically homogeneous solvate of N-oxide IX with chloroform, with mp 111-115° (dec.). IR spectrum (in Nujol): 950 cm^{-1} (N → O). A pronounced shift to weak field of the singlet of the dimethylamino group as compared with the starting amine (7.72 τ in VI and 6.80 τ in IX) was observed in the PMR spectrum. Found, %: C 51.6; H 7.8; N 3.1. $C_{39}H_{70}N_2O_{15} \cdot CHCl_3$. Calculated, %: C 51.9; H 7.7; N 3.0.

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