DEEPOXIDATION OF 16-MEMBERED EPOXYENONE MACROLIDE ANTIBIOTICS

II. CHEMICAL DEEPOXIDATION BY DISSOLVING METAL REDUCTION

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16-Membered epoxyenone macrolide antibiotics were reductively deepoxidized with dissolving metals such as zinc. Angolamycin and rosamicin which have a methyl substituent at C-12 in the epoxyenone structure were deepoxidized, but not isomerized further to the geometric isomers P2 and P3.

In the preceding paper[1] we described the microbial deepoxidation and subsequent isomerization of 16-membered epoxyenone macrolide antibiotics. Considering from a plausible reaction mechanism of the microbial deepoxidation, it seemed feasible to carry out the same type of reductive epoxide-ring cleavage by a chemical method.

This paper describes the chemical deepoxidation of 16-membered epoxyenone macrolides by the action of dissolving metals. The spontaneous isomerization of P1 to the geometric isomers P2 and P3 was suppressed in angolamycin and rosamicin which possess a methyl group at C-12 in the epoxyenone structure.

Materials and Methods

Antibiotics

Carbomycin A was prepared as reported in a previous paper[2]. 4″-Phenylacetyldeltamycin was synthesized from deltamycin X (4″-deacyldeltamycin) by acylation with phenylacetyl chloride[3]. Angolamycin was produced by fermentation of *Streptomyces eurythermus* IFO 12764 and purified in our laboratories, as detailed in a previous paper[4]. Rosamicin was the kind gift of Dr. T. FURUMAI, Tanabe Seiyaku Co., Ltd. (present address: Nippon Roche Research Center).

Deepoxidation of Carbomycin A with Zinc

Carbomycin A (500 mg) was dissolved in 10 ml of tetrahydrofuran and mixed with 1,500 mg of ammonium chloride in 20 ml of water. Under vigorous agitation at room temperature, 1,500 mg of zinc powder was added slowly in small portions and the reaction mixture was kept agitated for 2 hours. After the zinc powder was removed by filtration, 20 ml of saturated sodium chloride solution was added to the filtrate. Macrolide compounds were extracted twice at pH 7.5 from the solution with 20 ml each of benzene. The benzene extracts were combined, rinsed with water and concentrated to dryness under reduced pressure. The evaporation residue was dissolved in a small volume of methanol and charged on a Sephadex LH-20 column (35×450 mm). Under monitoring by silica gel thin-layer chromatography, carbomycin A P1 was eluted from the Sephadex LH-20 column with methanol. Active

fractions were collected on a fraction collector, combined and concentrated to dryness *in vacuo* to give a white powder of carbomycin A P1. The powder was dissolved in 1 ml of benzene and forced to precipitate by addition of 20 ml *n*-hexane. The yield of carbomycin A P1 was 230 mg (more than 98% pure).

Silica Gel Thin-layer Chromatography and General Methodology

They were as explained in the preceding paper[1].

Results and Discussion

Some Conditions for Chemical Deepoxidation of Carbomycin A with Dissolving Metals

pH

Treatment with zinc powder at a pH in the range of 2~5 gave a poor yield (less than 40%) on account of non-specific breakdown. The practically useful reaction pH is in the range of 5~6.5 in the presence of ammonium chloride.

Temperature

On treatment with zinc powder in the presence of ammonium chloride, carbomycin A gave a far better yield of the deepoxidation product at room temperature (20°C, 70%) than at 60°C (10%).

Dissolving Metal

When 2 mg of carbomycin A was treated at 20°C for 3 hours with 10 mg of a metal in the presence of 10 mg of ammonium chloride in 1 ml of a tetrahydrofuran - water mixture (1: 1), magnesium yielded no carbomycin A P1, while zinc, tin, chromium, iron and copper were active. Among the five metals, zinc was the most satisfactory with respect to the high yield of carbomycin A P1 and the least extent of non-specific breakdown.

Chemical Deepoxidation of 4″-Phenylacetyldeltamycin and Maridomycin

The dissolving metal reduction with zinc converted 4″-phenylacetyldeltamycin (PAD) to P1, P2 and P3. The physico-chemical properties of the substrate and the three deepoxidation products are listed in Table 1. The validity of the structures of the deepoxidation products was supported by spectrometric analysis.

As is the case in the microbial deepoxidation[1], maridomycins which belong to the epoxyenol

Table 1. Physico-chemical properties of 4″-phenylacetyldeltamycins P1, P2 and P3.

		PAD	PAD P1	PAD P2	PAD P3
Formula		$C_{45}H_{65}NO_{16}$	$C_{45}H_{67}NO_{16}$	$C_{45}H_{67}NO_{16}$	$C_{45}H_{67}NO_{16}$
Elementary analysis (%)					
Found (calcd)	C		61.52 (61.56)	61.39 (61.56)	61.50 (61.56)
	H		7.53 (7.69)	7.74 (7.69)	7.71 (7.69)
	N		1.38 (1.60)	1.49 (1.60)	1.39 (1.60)
MW (MS)		875	877	877	877
Mp (°C)		188~190	104~107	106~109	116~119
$[\alpha]_D^{20}$ ($CHCl_3$)		−60.9° (*c* 0.5)	−29.1° (*c* 0.1)	−49.6° (*c* 0.1)	+10.1° (*c* 0.1)
λ_{max}^{MeOH} nm (log ε)		240 (4.19)	End absorption	230 (4.10)	233 (4.02)
Color on H_2SO_4 treatment		Blueish purple	Brownish purple	Yellow	Yellow
Rf (SiO_2 TLC)					
Benzene - acetone, 2: 3		0.67	0.57	0.59	0.63
$CHCl_3$ - MeOH, 20: 1		0.70	0.38	0.51	0.65

PAD=4″-phenylacetyldeltamycin.

Table 2. Physico-chemical properties of angolamycin P1.

	Angolamycin P1	Angolamycin
Formula	$C_{46}H_{79}NO_{17}$	$C_{46}H_{77}NO_{17}$
MW (MS)	917	915
Mp	110~120°C	165~168°C
$[\alpha]_D^{24}$ (c 0.1, $CHCl_3$)	−69.7°	−64°
λ_{max}^{MeOH} nm (log ε)	End absorption	240 (4.16)
Color on H_2SO_4 visualization	Light brown	Brown
Rf (SiO_2 TLC)		
Benzene - acetone, 1: 2	0.19	0.25
$CHCl_3$ - MeOH, 4: 1	0.75	0.80

family of macrolides were resistant to reductive deepoxidation with zinc.

Chemical Deepoxidation of Angolamycin and Rosamicin

Angolamycin and rosamicin have a methyl group at C-12 in the epoxyenone structure, while carbomycin A and deltamycins have no substituent at C-12. Angolamycin was subjected to the dissolving metal reduction with zinc, giving P1. However angolamycin P1 could not be isomerized to its geometric isomers P2 and P3, probably because of the presence of the methyl group, or, the absence of the hydrogen atom, at C-12. Table 2 shows the physico-chemical properties of the substrate and angolamycin P1.

Rosamicin was also converted by dissolving zinc powder to rosamicin P1 (mp 115~125°C; no characteristic UV absorption maximum; Rf value on a silica gel thin-layer chromatographic plate 0.03 in benzene - acetone (1: 4) (rosamicin 0.07); 0.18 in chloroform - methanol (1: 1) (rosamicin 0.25)). Triethylamine treatment of rosamicin P1 provided no rosamicins P2 and P3.

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