[Chem. Pharm. Bull. 15(5) 682~684 (1967)]

UDC 615.779.925-011

85. Haruo Ogura,*1 Akiko Otagoshi,*1 Yoshimoto Sano,*1,2 and Toju Hata*1,2 : Structure of Amaromycin.

(*College of Pharmaceutical Sciences, Kitasato University,*1 and Kitasato Institute*2*)

Amaromycin is the same compound as pikromycin, which fact was proved hydrolytic studies of amaromycin and their direct comparison.

Stereochemistry of amaromycin was studied by nuclear magnetic resonance spectra and it was concluded that desosamine linkage has a β-configuration, and there is *trans*-olefinic structure in the lactone.

(Received November 1, 1966)

Amaromycin is an antibiotic produced by a strain of *Streptomyces flavochromogenes* by Hata, *et al.*[1] and a brief description of some of its properties has been given as a macrolide like erythromycin. In the present series of experiment, amaromycin was hydrolyzed by acid and yielded desosamine and aglycones (I and II), and chemical and spectroscopic interrelation with pikromycin are attempted, which led to an evidence that amaromycin is the same compound as pikromycin. The chemistry of pikromycin was reported already by Brockmann[2,3] and Anliker,[4] and the character of pikromycin is summarized and compared with that of amaromycin in Table I. The reported molecular formula of amaromycin, $C_{25}H_{39}O_7N$,[1] should be corrected to $C_{25}H_{43}O_7N$ from its elemental analyses.

Table I. Comparison with Amaromycin and Pikromycin

	Amaromycin	Pikromycin
Molecular formulae	$C_{25}H_{43}O_7N$ ($C_{25}H_{39}O_7N$)[1]	$C_{25}H_{43}O_7N$[2,4]
m.p. °C	164.5~165	169~170,[2] 169.5~170[4]
Optical rotations	$[\alpha]_D^{25}$ +6.19°(EtOH)	$[\alpha]_D^{24}$ +8.2°(EtOH)[2]
	$[\alpha]_D^{20}$ −48.8°($CHCl_3$)	$[\alpha]_D^{24}$ −50.2°($CHCl_3$)[2,4]
		$[\alpha]_D^{20}$ −33.5°(〃)[3]
UV λ_{max}^{EtOH} mμ (log ε)	225(4.06)	225(3.98)[4]
		222(4.02)(ether)[2]

Amaromycin (I) was treated under the same condition as used by Brockmann and others,[3] at pH 6.5 for 90~150 hrs. on a water bath at 60°. After the reaction colorless crystals separated on a wall of the reaction flask, and recrystallization from methanol gave the aglycone-I (II) as colorless prisms, m.p. 171~172°. Brockmann[3] and Anliker[4] obtained kromycin with similar properties from pikromycin (Table II). The filtrate was evaporated to dryness and extracted with acetone to obtain desosamine hydrochloride[5] (cited below). After evaporation of the mother liquor of desosamine hydrochloride (III), the remaining residue was treated with 5*N* hydrochloric acid at 100° to give aglycone-II (cited below) and additional desosamine hydrochloride (III).

*1,2 Shiba Shirogane Sankocho, Minato-ku, Tokyo (小倉治夫, 太田越朗子, 佐野敬元, 秦 藤樹).

1) T. Hata, Y. Sano, H. Tatsuta, R. Sugawara, A. Matsumae, K. Kanamori : J. Antibiotics (Japan), Ser. A, **8**, 9 (1955).
2) H. Brockmann, W. Henkel : Ber., **84**, 284 (1951).
3) H. Brockmann, R. Oster : *Ibid.*, **90**, 605 (1957).
4) R. Anliker, K. Gubler : Helv. Chim. Acta, **40**, 119 (1957).
5) H. Brockmann, H-B. König, R. Oster : Ber., **87**, 856 (1954).

Table II. Comparison with Aglycone-I and Kromycin

	Aglycone-I	Kromycin	
		Brockmann[3]	Anliker[4]
m.p. °C	171~172 164~165 (sealed tube)	172	168~170
$[\alpha]_D^{20}$ ($CHCl_3$)	−40.0°	−27.9°	−23.3°
UV λ_{max}^{EtOH} mμ (log ε)	226.5 (4.56)	223 (4.22) (ether)	226.5 (4.22)
IR cm^{-1} (KBr)	3480, 1724, 1674, 1639	3300, 1724, 1670	3510, 1730, 1680, 1645

Treatment of amaromycin under a more vigorous condition of 5*N* hydrochloric acid gave amaromycin aglycone-II as colorless needles, m.p. 200.5~202°, $[\alpha]_D^{26}$ +80.6°, IR ν_{max}^{KBr} cm^{-1} : 3040 (hydroxy), 1735, 1700 (lactone and carbonyl), and 1625 (unsaturated carbonyl). Brockmann[3] reported that pikromycin, under the same conditions, yielded kromin, m.p. 212°, $[\alpha]_D$ +85°, IR ν_{max}^{KBr} cm^{-1} : 3449, 1738, and 1709, molecular formula $C_{19}H_{28}O_5$. From

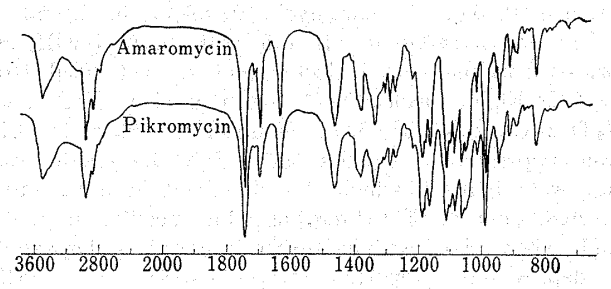

Fig. 1. IR Spectra of Amaromycin and Pikromycin (KBr)

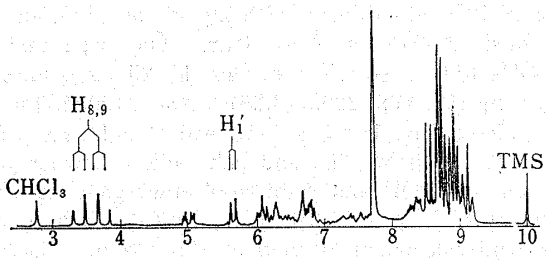

Fig. 2. NMR Spectrum of Amaromycin ($CDCl_3$, 100 mc.)

the mother liquor of aglycone-II, desosamine hydrochloride (III) was obtained in a good yield. Desosamine hydrochloride and O-diacetyldesosamine hydrochloride were identical with authentic samples obtained from erythromycin.[6]

According to these results, amaromycin is identical to pikromycin. Although we cannot compare directly aglycone-I and II with kromycin and kromin, but amaromycin was compared with pikromycin (Lot. 3608B-122D from American Cyanamid Co.) by mixed melting point and infrared spectra (Fig. 1). Albomycetin, $C_{32}H_{54}O_9N$, m.p. 166~167°, which had been reported by Takahashi,[7] was also compared directly with amaromycin by mixed melting point and infrared absorption spectra, and it was concluded that it was also the same compound as pikromycin.

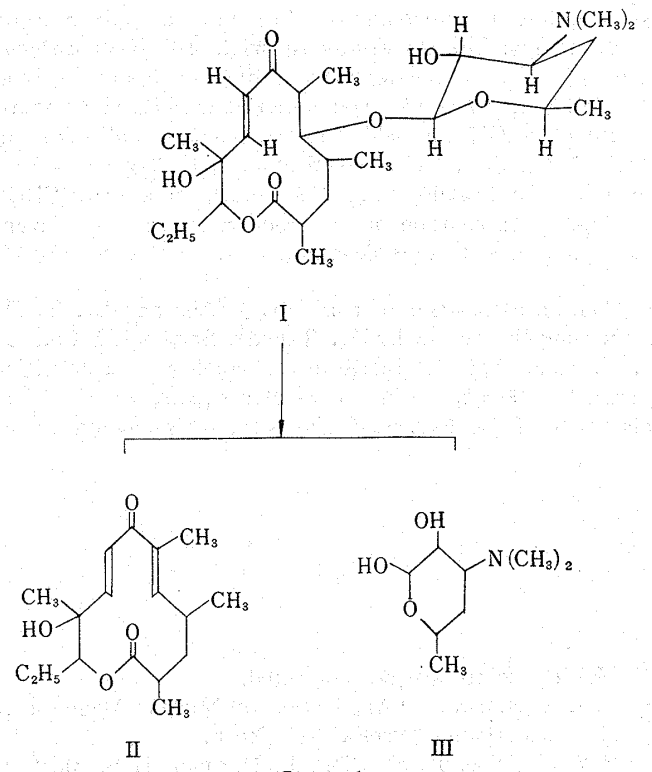

Chart 1.

6) E. H. Flynn, M. V. Sigal, Jr., P. F. Wiley, K. Gerzon : J. Am. Chem. Soc., **76**, 3121 (1954).
7) B. Takahashi : J. Antibiotics (Japan), Ser. A **7**, 149 (1954).

A nuclear magnetic resonance spectrum (Fig. 2) of amaromycin shows a quartet of lines centered at 3.58τ which may be attributed to 8-H and 9-H with J=15.6 c.p.s. This signal suggests a *trans*-olefinic coupling constant.[8] On the other hand, the doublet centered at 5.66τ should be attributed to the anomer proton[9] (1'-H) (J=7.02 c.p.s.) and this suggests a β-configuration of desosamine linkage.[10]

In conclusion, the structure of amaromycin could be proposed as shown on Chart 1.

Experimental*3

Amaromycin——Amaromycin was recrystallized from MeOH to give colorless prisms, m.p. 163~164°, $[\alpha]_D^{20}$ −48.8°(c=2.0, $CHCl_3$). *Anal.* Calcd. for $C_{25}H_{43}O_7N$: C, 63.94; H, 9.23; N, 2.98. Found: C, 64.08; H, 9.14; N, 2.85.

Pikromycin (Lot. 3608B-122D, American Cyanamid Co.) was recrystallized from MeOH, m.p. 161~162°, mixed m.p. 161.5~162.5° with amaromycin.

Albomycetin,[7] m.p. 158~160°, alone and in admixture with amaromycin.

Hydrolysis of Amaromycin at pH 6.5——A solution of 0.50 g. of amaromycin dissolved in 2.0 ml. of 0.5 *N* HCl was diluted with 20 ml. of H_2O, the solution was adjusted to pH 6.5 with 0.1*N* NaOH, and warmed at 60° for 90~150 hr. The separated colorless crystals were collected and recrystallized from MeOH-H_2O to colorless needes (II) (25 mg.), m.p. 171~172° (Kofler block), $[\alpha]_D^{20}$ −40.0°(c=1.0, $CHCl_3$), UV λ_{max}^{EtOH} mμ (log ε): 226.5 (4.36). *Anal.* Calcd. for $C_{17}H_{26}O_4$: C, 69.36; H, 8.90. Found: C, 69.27; H, 8.78.

The filtrate left after removal of aglycone-I (II) was evaporated to dryness below 50°, the residue was extracted with Me_2CO, and pale yellow powder (380 mg.) was obtained, which was dissolved in a minimum amount of EtOH and addition of ether gave desosamine hydrochloride (III) (31 mg.) as white needles, m.p. 172° (decomp.), $[\alpha]_D^{20}$ +19.2°(c=1.0, EtOH). This compound, when mixed with an authentic sample of desosamine hydrochloride obtained from erythromycin,[6] showed no depression. Reported, m.p. 183~184°, $[\alpha]_D$ +54.5° (1%, EtOH),[11] m.p. 187~189°(decomp.), $[\alpha]_D$ +50.5°(H_2O),[6] m.p. 189~191°(decomp.) (Kofler block), $[\alpha]_D^{20}$ +49.5°(c=10.0, H_2O), $[\alpha]_D^{20}$ +53.4°(c=2.1, EtOH),[5] and $[\alpha]_D$ +19°(c=2.0, MeOH).[12]

Further identification of desosamine was made as its O-diacetyldesosamine hydrochloride obtained by the usual method of acetone and pyridine, m.p. and mixed m.p. 185°(decomp), reported,[13] m.p. 194~195°(decomp.).

Hydrolysis of Amaromycin with 5*N* Hydrochloric Acid——A mixture of 0.50 g. of amaromycin and 15 ml. of 5*N* HCl was warmed at 100° for 10 min. When cooled, the solution was extracted with $CHCl_3$. The $CHCl_3$ solution was evaporated at a reduced pressure after being washed with H_2O and dried. Minimum amount of MeOH was added to the residue, and aglycone-II (30.3 mg.) was obtained as coloress needles, m.p. 200.5~202°(subl.), $[\alpha]_D^{26}$ +80.6°(c=1.0, $CHCl_3$), mixed m.p. with aglycone-I (II) (cited above) was 150~160°. Reported[3] for kromin, m.p. 212°(subl.), $[\alpha]_D$ +85°($CHCl_3$).

The acidic solution of the reaction mixture was evaporated under reduced pressure, and the residue was treated as above to give desosamine hydrochloride (III) (140 mg.).

The authors wish to thank Dr. J. Abe and Dr. T. Watanabe, Toyo Jozo Co., Ltd., for their kind supply of amaromycin, and to Dr. K. Takeda, Shionogi & Co., Ltd., for erythromycin. The authors are grateful to Dr. T. Hino, National Institute of Radiological Science, for the measurement of the nuclear magnetic resonance spectra by 100 mc. Varian Associates spectrometer. The authors are also indebted to Mrs. A. Sano, Central Laboratory of the Kitasato University, for elemental analyses.

*3 Melting points are not corrected.

8) L. M. Jackman: "Application of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," 82 (1959), Pergamon Press, New York.
9) P. W. K. Woo, H. W. Dion, L. Durham, H. S. Mosher: Tetrahedron Letters, 735 (1962).
10) D. R. Harris, S. G. McGeachin, H. H. Mills: *Ibid.*, 679 (1965).
11) R. K. Clark, Jr.: Antibiotics and Chemotherapy, **3**, 663 (1953).
12) G. Els, W. D. Celmer, K. Murai: J. Am. Chem. Soc., **80**, 3777 (1958).
13) R. L. Whistler, M. L. Wolfrom: "Methods in Carbohydrate Chemistry," **I**, 258 (1964), Academic Press Inc., New York.