85. Haruo Ogura,*1 Akiko Otagoshi,*1 Yoshimoto Sano,*1,2 and Tojo Hata*5,1 : Structure of Amaromycin.

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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.

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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 Brockmann5,3 and Anliker,9) and the character of pikromycin is summarized and compared with that of amaromycin in Table I. The reported molecular formula of amaromycin, C23H30O7N3,1) should be corrected to C24H30O7N3 from its elemental analyses.

Table I. Comparison with Amaromycin and Pikromycin

<table>
<thead>
<tr>
<th></th>
<th>Amaromycin</th>
<th>Pikromycin</th>
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<tbody>
<tr>
<td>Molecular formulae</td>
<td>C23H30O7N3</td>
<td>C24H30O7N3</td>
</tr>
<tr>
<td>m.p. °C</td>
<td>164.5~165</td>
<td>169<del>170,2) 169.5</del>170(4)</td>
</tr>
<tr>
<td>Optical rotations</td>
<td>[α]D +6.19°(EtOH)</td>
<td>[α]D +8.2°(EtOH)</td>
</tr>
<tr>
<td></td>
<td>[α]D +48.8°(CHCl3)</td>
<td>[α]D -50.2°(CHCl3)</td>
</tr>
<tr>
<td></td>
<td>(α)D -33.3°(ν)</td>
<td>(α)D -33.5°(ν)</td>
</tr>
<tr>
<td>UV λmax mp (log ε)</td>
<td>225(4.06)</td>
<td>225(3.98)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>222(4.02)(ether)</td>
</tr>
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</table>

Amaromycin (I) was treated under the same condition as used by Brockmann and others,5) 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°. Brockmann5) and Anliker9) obtained kromycin with similar properties from pikromycin (Table II). The filtrate was evaporated to dryness and extracted with acetone to obtain desosamine hydrochloride9) (cited below). After evaporation of the mother liquor of desosamine hydrochloride (III), the remaining residue was treated with 5N hydrochloric acid at 100° to give aglycone–II (cited below) and additional desosamine hydrochloride (III).

*1,3 Shiba Shirogane Sankocho, Minato-ku, Tokyo (小倉清友, 太田望子, 佐野敬元, 奈 藤樹).
2) H. Brockmann, W. Henkel : Ber., 84, 284 (1951).
### Table II. Comparison with Aglycone-I and Kromycin

<table>
<thead>
<tr>
<th></th>
<th>Aglycone-I</th>
<th>Kromycin Brockmann&lt;sup&gt;4&lt;/sup&gt;</th>
<th>Kromycin Anliker&lt;sup&gt;4&lt;/sup&gt;</th>
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<tr>
<td>m.p. °C</td>
<td>171~172</td>
<td>172</td>
<td>168~170</td>
</tr>
<tr>
<td>CHCl&lt;sub&gt;3&lt;/sub&gt;</td>
<td>164~165 (sealed tube)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[α]&lt;sub&gt;20&lt;/sub&gt;</td>
<td>-40.0&lt;sup&gt;o&lt;/sup&gt;</td>
<td>-27.9&lt;sup&gt;o&lt;/sup&gt;</td>
<td>-23.3&lt;sup&gt;o&lt;/sup&gt;</td>
</tr>
<tr>
<td>UV&lt;sub&gt;λ&lt;/sub&gt; max (log ε)</td>
<td>226.5 (4.56)</td>
<td>223 (4.22) (ether)</td>
<td>226.5 (4.22)</td>
</tr>
<tr>
<td>IR cm&lt;sup&gt;-1&lt;/sup&gt; (KBr)</td>
<td>3480, 1724, 1674, 1639</td>
<td>3300, 1724, 1670</td>
<td>3510, 1730, 1680, 1645</td>
</tr>
</tbody>
</table>

Treatment of amaromycin under a more vigorous condition of 5N hydrochloric acid gave amaromycin aglycone-II as colorless needles, m.p. 200.5~202<sup>o</sup>, [α]<sub>20</sub> +80.6<sup>o</sup>, IR ν<sub>max</sub> cm<sup>-1</sup>: 3040 (hydroxy), 1735, 1700 (lactone and carbonyl), and 1625 (unsaturated carbonyl). Brockmann<sup>3</sup> reported that pikromycin, under the same conditions, yielded kromin, m.p. 212<sup>o</sup>, [α]<sub>20</sub> +85<sup>o</sup>, IR ν<sub>max</sub> cm<sup>-1</sup>: 3449, 1738, and 1709, molecular formula C<sub>19</sub>H<sub>25</sub>O<sub>6</sub>. From 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.<sup>5</sup>

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<sub>24</sub>H<sub>34</sub>O<sub>6</sub>N, m.p. 166~167<sup>o</sup>, which had been reported by Takahashi<sup>7</sup>, 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.

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A nuclear magnetic resonance spectrum (Fig. 2) of amaromycin shows a quartet of lines centered at 3.58, 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. On the other hand, the doublet centered at 5.66 should be attributed to the anomer proton (1'-H) (J=7.02 c.p.s.) and this suggests a β-configuration of desosamine linkage.

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

**Experimental**

**Amaromycin**—Amaromycin was recrystallized from MeOH to give colorless prisms, m.p. 163-164°, ($\alpha$)$_D$ = -48.8°(c=2.0, CHCl$_3$). *Anal. Calcd.* for C$_{23}$H$_{38}$O$_7$N: 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.

Albomycin, 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$_2$O, the solution was adjusted to pH 6.5 with 0.1N NaOH, and warmed at 60° for 90-150 hr. The separated colorless crystals were collected and recrystallized from MeOH-H$_2$O to colorless needles (II) (25 mg.), m.p. 171-172° (Koffler block), ($\alpha$)$_D$ = -40.0°(c=1.0, CHCl$_3$), UV $\lambda_{max}$ mp (log $\epsilon$): 226.5,5 (4.36). *Anal. Calcd.* for C$_{20}$H$_{32}$O$_{12}$: C, 69.56; 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$_2$CO, and pale yellow powder (380 mg.) was obtained, which was dissolved in a minimum amount of EtOH and addition of ether gave desosamine hydrochloride (II) (31 mg.) as white needles, m.p. 172° (decomp.), ($\alpha$)$_D$ = +19.2°(c=1.0, EtOH). This compound, when mixed with an authentic sample of desosamine hydrochloride obtained from erythromycin, showed no depression. Reported, m.p. 183-184°, ($\alpha$)$_D$ = +54.5° (1%; EtOH), m.p. 187-189° (decomp.), ($\alpha$)$_D$ = +50.5°(H$_2$O), m.p. 189-191° (decomp.)(Koffler block), ($\alpha$)$_D$ = +49.5°(c=10.0, H$_2$O), ($\alpha$)$_D$ = +53.4°(c=2.1, EtOH), and ($\alpha$)$_D$ = +19°(c=2.0, MeOH).

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.), m.p. 194-195° (decomp.).

**Hydrolysis of Amaromycin with 5N Hydrochloric Acid**—A mixture of 0.50 g. of amaromycin and 15 ml. of 5N 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$_2$O and dried. Minimum amount of MeOH was added to the residue, and aglycone—II (30.3 mg.) was obtained as colorless needles, m.p. 200.5-202°(subl.), ($\alpha$)$_D$ = +80.6°(c=1.0, CHCl$_3$), mixed m.p. with aglycone—I (II) (cited above) was 150-160°.

Reported 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 (II) (140 mg.).

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83 Melting points are not corrected.