# STRUCTURE OF AN ANTITUMOR ANTIBIOTIC, SPERGUALIN

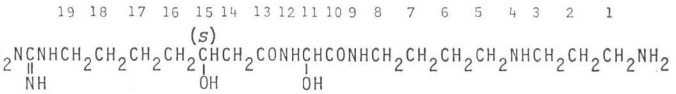
Sir:

As reported in the preceding paper[1], in the study of antitumor antibiotics, an antibiotic which exhibited a strong inhibition against experimental mouse tumors has been discovered in bacterial culture filtrates and named spergualin. In this paper, the structural elucidation of this antibiotic is described.

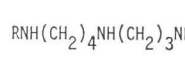
Spergualin (**1**) trihydrochloride was obtained from culture filtrates of the bacterial strain BMG162-aF2 by the processes described in the preceding paper. It shows no definite melting point and $[\alpha]_D^{24}$ $-11°$ (*c* 1, $H_2O$). *Anal.* Calcd. for $C_{17}H_{37}N_7O_4 \cdot 3HCl \cdot \frac{1}{2}H_2O$: C 39.12, H 7.92, N 18.79, Cl 20.38. Found: C 39.00, H 8.02, N 17.40, Cl 19.65. UV ($H_2O$); end absorption, IR (KBr); 3400, 1660, 1540, 1470, 1170, 1115, 1090 and 1020 $cm^{-1}$. $^1H$ NMR ($D_2O$); $\delta$ 1.8~2.3 ($CH_2 \times 5$), 2.57 (2-$CH_2$), 2.95 (14-$CH_2$, $J$=1, 3.5, 8 Hz), 3.5~3.8 ($NCH_2 \times 5$), 4.55 (15-CH), 5.98 (11-CH), $^{15}N$ NMR ($D_2O$, ppm relative to external $NO_3^-$); -240 (CONH), -257 (CONH), -290 (NH), -304 ($NH=C-NH_2$), -330 (NH), -343 ($NH_2$). The chemical shifts of the $^{13}C$ NMR spectra are shown in Table 1. The antibiotic (**1**) gives positive ninhydrin, RYDON-SMITH and SAKAGUCHI reactions. It is easily soluble in water and methanol, but slightly or not in ethanol, ethyl acetate, acetone, cyclohexane and other organic solvents. The thin-layer chromatography on a cellulose plate (Avicel) developed with 1-butanol - pyridine - acetic acid - water (6: 4: 1: 3) and with 1-butanol - ethanol - water (4: 1: 2) showed Rf 0.14 and 0.20, respectively. By high-voltage paper electrophoresis with 3,500 V for 15 minutes in formic acid - acetic acid - water (1: 3: 36), **1** moved to the cathode with Rm (relative mobility to alanine) 1.6.

The crystalline tripicrate of **1** was obtained from the aqueous ethanol solution, mp 62~78°C (decomp.). *Anal.* Calcd. for $C_{17}H_{37}N_7O_4 \cdot 3C_6H_3N_3O_7$: C 38.54, H 4.25, N 20.54, picric acid 63.01. Found: C 38.76, H 4.71, N 18.31, picric acid (UV) 60.0.

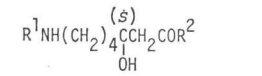
Acid hydrolysis of **1** (trihydrochloride, 74.8mg)



**1**



**2**: R = H
**5**: R = $(HO)_2CHCO$



**3**: $R^1 = H_2NC=NH$, $R^2 = OH$
**4**: $R^1 = H$, $R^2 = OH$
**6**: $R^1 = H_2NC=NH$, $R^2 = NH_2$

Table 1. $^{13}C$ Chemical shifts (ppm) of spergualin (**1**) and compounds **3**, **5** and **6**.

| Carbon | **1**·3HCl | **3**·HCl | **5**·2HCl | **6**·HCl | Carbon | **1**·3HCl | **3**·HCl | **5**·2HCl | **6**·HCl |
|---|---|---|---|---|---|---|---|---|---|
| 13 | 174.0 s | 178.0 | | 173.3 | 1, 8 | 38.1 t<br>36.2 t | | 37.8<br>36.3 | |
| 10 | 170.6 s | | 171.7 | | | | | | |
| C=N | 156.2 s | 157.5 | | 156.5 | 16 | 35.0 t | 36.2 | | 36.7 |
| 11 | 71.4 d | | 86.3 | | 18 | 27.2 t | 28.5 | | 28.3 |
| 15 | 67.9 d | 69.0 | | 67.0 | 2 | 25.0 t | | 25.0 | |
| 3, 5 | 46.2 t<br>44.0 t | | 46.9<br>44.0 | | 6, 7 | 23.2 t<br>22.4 t | | 23.3<br>22.4 | |
| 19 | 42.8 t | 42.2 | | 43.0 | 17 | 21.4 t | 22.7 | | 22.1 |
| 14 | 40.6 t | 41.8 | | 41.7 | | | | | |

The $^{13}C$ FT NMR spectra were taken with a Varian XL-100 spectrometer in $D_2O$. Multiplicities (s, d, and t) were shown by off-resonance experiment.

with 1 N HCl in a sealed tube at 105°C for 3 hours followed by column chromatography on Sephadex LH-20 developed with methanol gave a polyamine **2** as the hydrochloride (40.9 mg), which was identical with authentic spermidine trihydrochloride in all respects, and a hydrochloride of a new SAKAGUCHI-positive compound **3** (28.7 mg), $[\alpha]_D^{23}+2°$ (*c* 2.1, 1 N HCl), $C_8H_{17}N_3O_3\cdot HCl$, FD-MS; *m/z* 204 ($MH^+$), $^1H$ NMR ($D_2O$); $\delta$ 1.8~2.3 (4-, 5-, 6-$CH_2$), 2.95 (2-$CH_2$, *J*=2, 7 Hz), 3.66 (7-$CH_2$, t, *J*=7 Hz), 4.5 (3-CH). The structure of **3** was determined to be 7-guanidino-3-hydroxyheptanoic acid by spectral data of $^1H$ and $^{13}C$ NMR (Table 1). Hydrolysis of **3** with $Ba(OH)_2$-saturated aqueous solution gave a deguanidino compound **4** as the hydrochloride, $[\alpha]_D^{22}+3°$ (*c* 0.95, 1 N HCl). The compound **4** hydrochloride was identical with the hydrochloride of (*S*)-7-amino-3-hydroxyheptanoic acid which was obtained by chemical synthesis starting from L-lysine[2]. Therefore, the C-3 of **3** has *S*-configuration.

Mild acid hydrolysis of **1** (trihydrochloride, 49.0 mg) with 1 M acetic acid for 1.5 hours under refluxing followed by column chromatography on Sephadex LH-20 developed with methanol gave a ninhydrin-positive compound **5** (hydrochloride, 31.3 mg) $C_9H_{21}N_3O_3\cdot 2HCl$, $^1H$ NMR ($D_2O$); $\delta$ 2.1 (6-, 7-$CH_2$), 2.4~2.7 (2-$CH_2$), 3.5~3.8 (1-, 3-, 5-, 8-$CH_2$), 5.77 (11-CH, s) and a SAKAGUCHI-positive compound **6** (hydrochloride, 19.7 mg), $[\alpha]_D^{26}-3°$ (*c* 1, $H_2O$), $C_8H_{18}N_4O_2\cdot HCl$, FD-MS; *m/z* 203 ($MH^+$), $^1H$ NMR ($D_2O$); $\delta$ 1.8~2.3 (4-, 5-, 6-$CH_2$), 2.95 (2-$CH_2$, *J*=2, 6 Hz), 3.69 (7-$CH_2$, t, *J*=7 Hz), 4.5 (3-CH), which was shown to be an amide of **3**. Treatment of **5** (hydrochloride, 24.7 mg) with 2,4-dinitrophenylhydrazine (25 mg) in 1 N HCl under refluxing for 1.5 hours followed by extraction with ethyl acetate gave yellow needles which was identified to be glyoxylic acid 2,4-dinitrophenylhydrazone (2.4 mg) by comparison with an authentic sample[3] (IR and TLC). From the aqueous layer of the ethyl acetate extraction, 2,4-dinitrophenylhydrazone of **5** (28.7 mg) was obtained as its dihydrochloride, $C_{15}H_{23}N_7O_5\cdot 2HCl\cdot H_2O$, FD-MS; *m/z* 382 ($MH^+$). By deamination of the hydrazone with $NaNO_2$ in acetic acid followed by acid hydrolysis with 6 N HCl, *N*-(3-hydroxypropyl)-1,4-butanediamine hydrochloride was obtained and identified with a synthetic sample[4] by their $^1H$ NMR spectra. Therefore, the structure of **5** was determined to be 11-amino-1,1-dihydroxy-3,8-diazaundecan-2-one (a hydrate of glyoxylylspermidine).

From the foregoing results and $^{13}C$ NMR data (Table 1), the structure of spergualin (**1**) can be proposed to be (−)-(15*S*)-1-amino-19-guanidino-11,15-dihydroxy-4,9,12-triazanonadecane-10,13-dione. The total synthesis of **1** including the synthesis of **4** will be reported in the next paper[2].

Among known antibiotics, laterosporamine[5] is similar to spergualin in its optical rotation, molecular formula ($C_{17}H_{35}N_7O_4$), color reactions and weak antibacterial activities. However, no antitumor activity has been reported for laterosporamine. Although it was reported that the hydrolysis of laterosporamine gave spermidine and a SAKAGUCHI-positive substance of $C_6H_{13}N_3O$, the hydrolysis of spergualin did not give such a $C_6$-compound. Moreover, the description on laterosporamine suggests that laterosporamine hydrochloride has a stronger activity against *Staphylococcus aureus* FDA 209P than spergualin trihydrochloride[1] and also is more soluble in ethanol.

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