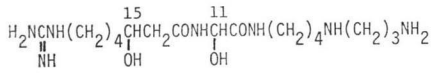
THE TOTAL SYNTHESIS OF SPERGUALIN, AN ANTITUMOR ANTIBIOTIC

Sir:

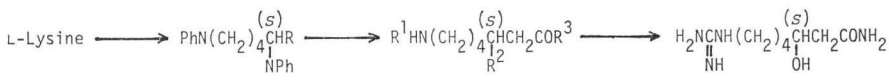
During our studies of antitumor antibiotics, spergualin was discovered in culture filtrates of a *Bacillus* strain.[1] Spergualin showed a marked inhibition against experimental mouse tumors, and the structure was determined to be (−)-(15*S*)-1-amino-19-guanidino-11,15-dihydroxy-4,9,12-triazanonadecane-10,13-dione[2]. In this communication, we report the synthesis of epimeric spergualin (**1**) and epimeric (15*R*)-spergualin (**2**), and separation of **1** into natural (−)-spergualin (**1a**) and unnatural (+)-spergualin (**1b**).* The total synthesis of **1** has been accomplished by acid-catalyzed condensation of (*S*)-7-guanidino-3-hydroxyheptanamide (**3**) and 11-amino-1,1-dihydroxy-3,8-diazaundecan-2-one (**4**, a hydrate of glyoxylylspermidine).



1: 15*S* **2**: 15*R*

By homologation of L-lysine according to the ARNDT-EISTERT method[3], (*S*)-3,7-diaminoheptanoic acid (**9**, colorless syrup, $[\alpha]_D^{21}$ +29° (*c* 1, water) 27% yield) was obtained through compounds **5**~**8** (Scheme 1). The selective ω-amino protection[4] of **9** with an equimolar amount of *N*-benzyloxycarbonyloxysuccinimide in a mixture of pyridine, water and triethylamine (10: 10: 1) at room temperature for 5 hours gave (*S*)-3-amino-7-benzyloxycarbonylaminoheptanoic acid (**10**, mp 143~147°C, $[\alpha]_D^{22}$ +14° (*c* 1, methanol), 48% yield). In this step, unreacted **9** was recovered in 24% yield by column chromatography on Amberlite CG-50 (80% NH_4^+). Deamination of **10** with sodium nitrite in 33% aqueous acetic acid overnight at 5°C followed by silica gel (Wakogel C-200) column chromatography (chloroform - methanol - 28% ammonia, 30: 10: 1) afforded (*S*)-7-benzyloxycarbonylamino-3-hydroxyheptanoic acid (**11**, mp 115~117°C, $[\alpha]_D^{23}$ +3° (*c* 2, methanol), 17% yield) with retention of configuration. It was identical with the compound derived from natural spergualin. Esterification of **11** with diazomethane in 1,2-dimethoxyethane followed by amidation with anhydrous ammonia in methanol at room temperature for 3 days in a sealed tube gave (*S*)-7-benzyloxycarbonylamino-3-hydroxyheptanamide (**12**, mp 126~127°C, $[\alpha]_D^{22}$ −3° (*c* 5, methanol), 85% yield). Hydrogenolysis of **12** with 5% palladium on carbon in a mixture of methanol, water and acetic acid (9: 1: 0.01) followed by column chromatography on Dowex 50W-x4 (H^+) resin eluted with 0.5 M ammonia afforded (*S*)-7-amino-3-hydroxyheptanamide (**13**, $[\alpha]_D^{22}$ −2° (*c* 2, water), 96% yield). Treatment of **13** with an equimolar amount of 2-methyl-1-nitrosourea[5] and sodium hydroxide in methanol at 5°C for 5.5 hours followed by hydrogenolysis with 5% palladium on carbon in a mixture of methanol, water and acetic acid (2: 2: 1) for 1 hour yielded the hydrochloride of **3** ($[\alpha]_D^{22}$

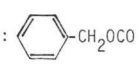
Scheme 1.



3

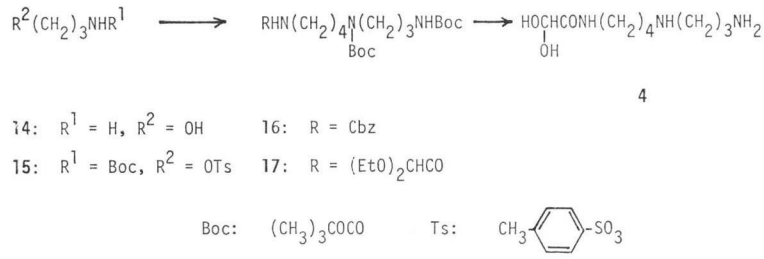
5: R = COOH
6: R = COCl
7: R = $COCHN_2$
8: R = CH_2COOCH_3

9: R^1 = H, R^2 = NH_2, R^3 = OH
10: R^1 = Cbz, R^2 = NH_2, R^3 = OH
11: R^1 = Cbz, R^2 = OH, R^3 = OH
12: R^1 = Cbz, R^2 = OH, R^3 = NH_2
13: R^1 = H, R^2 = OH, R^3 = NH_2

Ph:  Cbz: 

* Satisfactory results of elemental analyses and NMR spectroscopy were obtained for all compounds described in this communication.

Scheme 2.



$-2°$ (c 2, water), 64% yield), which was purified by successive column chromatography on CM-Sephadex C-25 (Na^+) eluted with 0.5 M sodium chloride and on Sephadex LH-20 eluted with methanol (Scheme 1). The hydrochloride was identical with the compound derived from natural spergualin in all respects.

N-Protection of 3-amino-1-propanol (**14**) with *tert*-butyl *S*-4,6-dimethylpyrimid-2-ylthiocarbonate followed by *O*-tosylation with *p*-toluenesulfonyl chloride gave 3-*tert*-butoxycarbonylamino-1-tosyloxypropane (**15**) in 46% yield. Compound **15** was treated with lithium bromide in *N*,*N*-dimethylformamide, reacted with 4-benzyloxycarbonylaminobutanamine which was derived from 1,4-butanediamine, and then acylated with *tert*-butyl *S*-4,6-dimethylpyrimid-2-ylthiocarbonate, yielding the di-*N*-*tert*-butoxycarbonyl-mono-*N*-benzyloxycarbonylspermidine (**16**) in 52% yield. Compound **16** was hydrogenated with 5% palladium on barium carbonate and then coupled with 2,2-diethoxyacetic acid (derived from commercial ethyl diethoxyacetate) in the presence of 1-hydroxybenzotriazole and dicyclohexylcarbodiimide in ethyl acetate to give 3-*N*-*tert*-butoxycarbonyl-11-*tert*-butoxycarbonylamino-1,1-diethoxy-3,8-diazaundecan-2-one (**17**) in 79% yield. Deprotection of **17** in a mixture of 0.1 N HCl and dioxane (5: 2) at 100°C for 4 hours followed by neutralization with 0.2 N NaOH and Sephadex LH-20 column chromatography eluted with methanol afforded the dihydrochloride of **4** (46% yield), which was identical with the compound derived from natural spergualin (Scheme 2).

Treatment of the hydrochloride of **3** with the dihydrochloride of **4** (1.9 equiv.), glutaric acid (2.5 equiv.) and water (18.5 equiv.) at 60°C for 43 hours* followed by purification by column chromatography on CM-Sephadex C-25 eluted with a gradient of 0.4 M to 1.0 M NaCl and on Sephadex LH-20 eluted with methanol gave epimeric spergualin (**1**) trihydrochloride in 35% yield, $[\alpha]_D^{22}$ $-2°$ (c 2, water).

By high-performance liquid chromatography (HPLC) (Waters Associates, 6000A/UK6) on Nucleosil $5C_{18}$ (Macherey-Nagel, Germany) column (8×300 mm) eluted with a mixture of $(NH_4)H_2PO_4$ (1.16 g), PIC B-5 low UV (Waters Associates, 20 ml), methanol (150 ml) and water (850 ml) at a flow rate of 3 ml/minute, detected by absorption at 205 nm (spectrophotometric detector SPD-1, Shimadzu), the epimeric mixture (**1**) was separated into natural (−)-spergualin (**1a**) and unnatural (+)-spergualin (**1b**) in retention time at 16.9 and 16.0 minutes, respectively. By purification of each fraction on Sephadex LH-20 column, **1a** trihydrochloride which was identical with the natural one in all respects including optical rotation and antimicrobial activity[2], and **1b** trihydrochloride, $[\alpha]_D^{26}$ $+7.5°$ (c 0.2, water), containing 14% of **1a** were obtained. The latter showed 31% activity of **1a** by ordinary cylinder plate method using *Bacillus subtilis* PCI219 as the test organism.

Acid-catalyzed condensation of **4** with (*R*)-7-guanidino-3-hydroxyheptanamide, $[\alpha]_D^{24}$ $+2°$ (c 2, water), which was synthesized starting from D-lysine by the same procedure as in the synthesis of **3** afforded (15*R*)-spergualin (**2**) trihydrochloride, no definite mp, $[\alpha]_D^{24}$ $+1°$ (c 2, water). The trihydrochloride showed 19% activity of **1a** against *Bacillus subtilis* PCI219 and only weak antitumor activity against leukemia L-1210 in mice.

* This reaction condition in detail will be reported elsewhere.

Acknowledgments

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SHINICHI KONDO
HIROYUKI IWASAWA
DAISHIRO IKEDA
YOSHIHISA UMEDA
YOKO IKEDA
HIRONOBU IINUMA
HAMAO UMEZAWA

Institute of Microbial Chemistry
14–23 Kamiosaki 3-Chome,
Shinagawa-ku, Tokyo 141, Japan

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