SYNTHESIS OF (−)-15-DEOXYSPERGUALIN AND (−)-SPERGUALIN-15-PHOSPHATE

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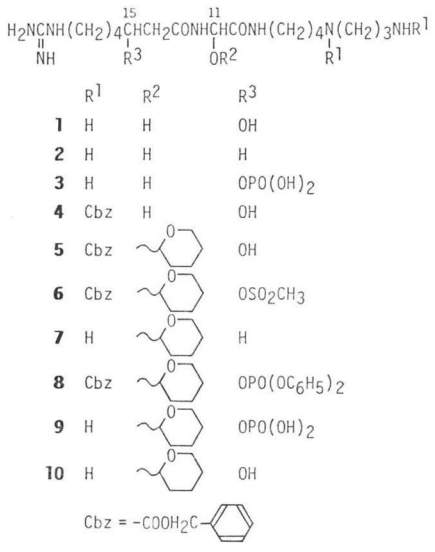
The method for chemical modification of spergualin with retention of the configuration at the C-11 has been achieved by the use of tetrahydropyranyl group for protection of the 11-hydroxyl group. (−)-15-Deoxyspergualin (**2**), which shows about eight times stronger inhibition against mouse leukemia L-1210 than the natural (−)-spergualin (**1**), and (−)-spergualin-15-phosphate (**3**) possessing a good antitumor activity have been synthesized starting from **1**.

In our studies on new antitumor antibiotics, spergualin was discovered in culture filtrates of a bacterial strain BMG162-aF2 which is related to *Bacillus laterosporus*, and the structure was determined by us to be (−)-(15*S*)-1-amino-19-guanidino-11,15-dihydroxy-4,9,12-triazanonadecane-10,13-dione.[1,2] But, only the C-11 stereochemistry remained undefined. The total synthesis was accomplished by the acid-catalyzed condensation of 11-amino-1,1-dihydroxy-3,8-diazaundecan-2-one (a hydrate of glyoxylylspermidine) with 7-guanidino-3-hydroxyheptanamide followed by the separation of the 11-epimeric mixture.[3] Antibacterial or antitumor activity of the epimeric mixture of spergualin was about a half of that of the natural (−)-spergualin.[4] It suggests that the C-11 stereochemistry plays a role in the biological action of spergualin.

Therefore, in addition to the synthesis of spergualin and its related compounds by the acid-catalyzed condensation, we studied the method for the derivation of (−)-spergualin (**1**) with retention of the configuration at C-11. A facile method has been achieved for protection of the 11-hydroxyl group by the use of tetrahydropyranyl group. In this paper, we report the synthesis of (−)-15-deoxyspergualin (**2**) and (−)-spergualin-15-phosphate (**3**).



	R^1	R^2	R^3
1	H	H	OH
2	H	H	H
3	H	H	$OPO(OH)_2$
4	Cbz	H	OH
5	Cbz		OH
6	Cbz		OSO_2CH_3
7	H		H
8	Cbz		$OPO(OC_6H_5)_2$
9	H		$OPO(OH)_2$
10	H		OH

Cbz =

Protection of the primary and secondary amines of **1** with *N*-(benzyloxycarbonyloxy)succinimide gave a bis-*N*,*N*′-benzyloxycarbonyl derivative **4** in 91% yield. Compound **4** was treated with 3,4-dihydro-2*H*-pyran in the presence of *p*-toluenesulfonic acid in anhydrous *N*,*N*-dimethylformamide for 7 hours at room temperature to yield a key compound, 11-*O*-tetrahydropyranyl derivative **5** in 26% yield. It was confirmed that the stereochemistry at the C-11 was retained during protection and deprotection of the amine and hydroxyl groups, since deprotection of *N*-benzyloxycarbonyl and *O*-tetrahydro-

Table 1. Antitumor effects of spergualin and its derivatives on mouse leukemia L-1210.

Dose (mg/kg/day)	1 ($3HCl \cdot 1/2H_2O$)		2 ($3HCl \cdot 2H_2O$)		3 ($3HCl \cdot Na \cdot 1/2H_2O$)	
	T/C(%)	Survivor	T/C(%)	Survivor	T/C(%)	Survivor
50	295	0/8			444	0/8
25	334	0/8			674	8/8
12.5	586	4/8			674	8/8
6.25	732	8/8	408	0/8	624	4/8
3.13	441	3/8	526	2/8	129	0/8
1.56	301	1/8	493	2/8	107	0/8
0.78	107	0/8	789	8/8		
0.39			629	6/8		
0.20			664	6/8		
0.10			138	0/8		
0.05			129	0/8		

Inoculation: 10^5 cells/CDF_1 mouse i.p., administration: i.p. daily from day 1 to day 9, evaluation: at day 60, prolongation rate (T/C, %): mean survival period of treated/mean survival period of controls.

pyranyl groups in **5** by catalytic hydrogenation and mild acid hydrolysis as described later gave the optically pure **1**.

Treatment of compound **5** with methanesulfonyl chloride in anhydrous pyridine afforded a mesylate **6** in 54% yield. Halogenation of **6** with sodium iodide in anhydrous *N,N*-dimethylformamide followed by hydrogenation with a palladium catalyst gave (−)-15-deoxy-11-*O*-tetrahydropyranylspergualin (**7**) in 18% yield. The tetrahydropyranyl group of **7** (trihydrochloride) was hydrolyzed with one tenth molar amount of *p*-toluenesulfonic acid in water under ice-cooling for 7 hours to yield levorotatory (−)-15-deoxyspergualin (**2**) in 78% yield. Compound **2** was about eight times more potent than **1** against mouse leukemia L-1210, as shown in Table 1. The acute LD_{50} of **2** trihydrochloride to mice by intravenous or intraperitoneal injection was 35~40 mg/kg.

Phosphorylation of **5** with diphenyl phosphorochloridate in anhydrous pyridine afforded the 15-diphenyl phosphate (**8**) in 53% yield. Hydrogenation of **8** with a platinum catalyst (48% yield) followed by mild hydrolysis of **9** with *p*-toluenesulfonic acid in water (62% yield) gave (−)-spergualin-15-phosphate (**3**). Compound **3** showed about half the antitumor activity compared with **1** (Table 1).

(−)-11-*O*-Tetrahydropyranylspergualin (**10**) prepared by catalytic hydrogenation of **5** was almost inactive against mouse leukemia L-1210 in the dose range of 0.78~25 mg/kg/day, intraperitoneally. The daily administration of 12.5 mg/kg or more caused death of mice.

Experimental

General

All compounds described in this paper did not show definite melting points. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. The ^1H NMR spectra were recorded with Varian XL-100 and EM-390 spectrometers. The chemical shifts in CD_3OD or D_2O refer to an external standard of tetramethylsilane ($\delta=0$), TLC was performed on a plate of Silica gel 60 (E. Merck) developed with a mixture (3: 2: 2: 1) of 1-butanol, pyridine, water and acetic acid; zones were detected by ninhydrin spray; spergualin showed Rf 0.13. Antibacterial activities were determined by the cylinder-plate method against *Bacillus subtilis* PCI219 with pure spergualin trihydrochloride as the assay standard.

(−)-1-*N*,4-Bis(benzyloxycarbonyl)spergualin (**4**)

To a solution of **1** trihydrochloride (3.0 g, 5.85 mmole) in methanol was added triethylamine (7.2 ml, 17.6 mmole) and a solution of *N*-(benzyloxycarbonyloxy)succinimide (3.21 g, 12.9 mmole) in dioxane at room temperature. After stirring for 3 hours, the mixture was concentrated to dryness. The residue was dissolved in 0.1 M NaCl (50 ml) and the solution was adjusted to pH 6.5 with 2 M HCl. The product in the solution was adsorbed on a column of CM-Sephadex C-25 (200 ml, equilibrated with 0.1 M NaCl) and eluted with a linear gradient of 0.1 M to 0.5 M NaCl (each 1,000 ml). The eluate was cut into 20-ml fractions. Fractions (Nos. 34～80) were combined and concentrated to dryness. The residue was extracted with methanol (10 ml×3). The product in the methanol solution was purified by a column of Sephadex LH-20 (200 ml) eluted with 90% aqueous methanol (2-ml fractions). Fractions (Nos. 51～63) were combined and concentrated to give a colorless syrup of **4** hydrochloride (3.8 g, 91%). $[\alpha]_D^{21}$ −11° (*c* 1, water), ^{1}H NMR (CD_3OD) δ 5.06 and 5.09 (benzyl CH_2, s), 7.33 (10H, aromatic).

Anal. Calcd. for $C_{33}H_{49}N_7O_9 \cdot HCl \cdot 1/2H_2O$: C 55.26, H 7.17, N 13.67, Cl 4.94.
Found: C 54.95, H 7.25, N 13.83, Cl 5.06.

(−)-1-*N*,4-Bis(benzyloxycarbonyl)-11-*O*-tetrahydropyranylspergualin (**5**)

To a solution of **4** hydrochloride (3.45 g, 4.81 mmole) in *N*,*N*-dimethylformamide (30 ml) was added 3,4-dihydro-2*H*-pyran (0.63 ml, 9.75 mmole) and *p*-toluenesulfonic acid hydrate (464 mg, 2.44 mmole) and the solution was stirred for 7 hours at room temperature. The reaction mixture was treated with triethylamine (0.33 ml, 2.44 mmole) and concentrated to dryness. The residue was purified by column chromatography of silica gel (Wakogel C-200, 300 g) developed with a mixture (240: 40: 4: 1) of chloroform, methanol, pyridine and 50% aqueous acetic acid (20-ml fractions). Fractions (Nos. 66～78) were combined and concentrated to give a colorless syrup of **5** as the acetate salt (1.07 g, 26%), $[\alpha]_D^{22}$ −13° (*c* 1, methanol), ^{1}H NMR (CD_3OD) δ 3.7～4.1 (tetrahydropyranyl 6-CH_2, m), 5.07 and 5.09 (benzyl CH_2, s), 7.30 (10H, aromatic).

Anal. Calcd. for $C_{38}H_{57}N_7O_9 \cdot CH_3COOH \cdot 3/2H_2O$: C 56.99, H 7.65, N 11.63.
Found: C 56.65, H 7.76, N 11.57.

Fraction Nos. 85～92 were combined and concentrated to recover **4** as the acetate salt (362 mg, 10%).

(−)-1-*N*,4-Bis(benzyloxycarbonyl)-15-*O*-methanesulfonyl-11-*O*-tetrahydropyranylspergualin (**6**)

To a solution of **5** (acetate, 950 mg, 1.13 mmole) in anhydrous pyridine (10 ml) was added methanesulfonyl chloride (0.13 ml, 1.74 mmole) under ice-cooling. After stirring for 3 hours, water (0.2 ml) was added to the solution and the solution was concentrated to give a syrup. The syrup was purified by column chromatography of silica gel (Wakogel C-200, 100 g) with a mixture (320: 40: 4: 1) of chloroform, methanol, pyridine and 50% aqueous acetic acid to give **6** as the acetate salt (598 mg, 54%).

(−)-15-Deoxy-11-*O*-tetrahydropyranylspergualin (**7**)

To a solution of **6** (acetate, 591 mg, 0.699 mmole) in anhydrous *N*,*N*-dimethylformamide was added NaI (5.02 g, 33.5 mmole). After stirring at 90°C for 15 hours, the solution was concentrated to dryness. The residue was dissolved in ethyl acetate (30 ml). The solution was washed with 20% aqueous $Na_2S_2O_3$ solution (30 ml) and saturated-NaCl solution (30 ml), successively. The organic layer was dried over anhydrous Na_2SO_4 and concentrated to give a syrup. The syrup was purified by column chromatography of silica gel (Wakogel C-200, 50 g) with a mixture (320: 40: 4: 1) of chloroform, methanol, pyridine and 50% aqueous acetic acid (10-ml fractions). Fractions (Nos. 7～28) were combined and concentrated to give a 15-iodo derivative as the acetate salt (204 mg, 33%).

The 15-iodo derivative (acetate, 198 mg, 0.214 mmole) in 80% aqueous methanol (20 ml) was hydrogenated with 5% palladium on $BaCO_3$ (40 mg) under a hydrogen stream at atmospheric pressure for 10 hours at room temperature. The catalyst was removed by filtration and the filtrate was concentrated to give a syrup. The syrup was dissolved in 0.1 M NaCl (30 ml) and the solution was adjusted to pH 6.5 with 1 M HCl. The product in the solution was adsorbed on a column of CM-Sephadex C-25 (40 ml, equilibrated with 0.1 M NaCl) and eluted with a linear gradient of 0.1 M to 0.8 M NaCl (each 200 ml). The eluate was cut into 4-ml fractions. Fractions (Nos. 67～77) were combined and concentrated to dryness. The residue was extracted with methanol (5 ml×3). The product in the methanol solution

was purified by a column of Sephadex LH-20 (100 ml) eluted with 90% aqueous methanol (1-ml fractions). Fractions (Nos. 36~47) were combined and concentrated to give a colorless syrup of **7** trihydrochloride (62.9 mg, 54% from the 15-iodo derivative), $[\alpha]_D^{26}$ −16° (*c* 1, water), ^{1}H NMR (CD_3OD) δ 3.7~4.1 (tetrahydropyranyl 6-CH_2, m), 5.56 (11-CH, s).

(−)-15-Deoxyspergualin (**2**)

To a solution of **7** (trihydrochloride, 61 mg, 0.112 mmole) in water (3 ml) was added *p*-toluenesulfonic acid hydrate (2.1 mg, 0.011 mmole) under ice-cooling. After stirring for 7 hours, the solution was adjusted to pH 6.5 with 1 M NH_4OH and added 0.4 M NaCl (10 ml). Compound **2** in the solution was adsorbed on a column of CM-Sephadex C-25 (25 ml, equilibrated with 0.4 M NaCl) and eluted with a linear gradient of 0.4 M to 1.0 M NaCl (each 100 ml). The eluate was cut into 2-ml fractions. Fractions (Nos. 73~81) were combined and concentrated to dryness. The residue was extracted with methanol (5 ml×3). Compound **2** in the methanol solution was purified by a column of Sephadex LH-20 (100 ml) eluted with 90% aqueous methanol (1-ml fractions). Fractions (Nos. 38~48) were combined and concentrated to give a colorless syrup of **2** trihydrochloride (46.6 mg, 78%), $[\alpha]_D^{25}$ −7.3° (*c* 1, water), ^{1}H NMR (D_2O) δ 1.7~2.3 (CH_2×6, m), 2.5~2.9 (2-CH_2 and 14-CH_2, m), 3.5~3.9 (NCH_2×5, m), 5.96 (11-CH, s), TLC Rf 0.17, antibacterial activity, 134% of **1**.

Anal. Calcd. for $C_{17}H_{37}N_7O_3 \cdot 3HCl \cdot 2H_2O$: C 38.31, H 8.32, N 18.40, Cl 19.96.
Found: C 38.45, H 8.08, N 18.61, Cl 20.26.

(−)-1-*N*,4-Bis(benzyloxycarbonyl)-11-*O*-tetrahydropyranylspergualin-15-diphenyl phosphate (**8**)

To a solution of **5** (acetate, 663 mg, 0.786 mmole) in anhydrous pyridine (10 ml) was added diphenyl phosphorochloridate (0.34 ml, 1.63 mmole) under ice-cooling and the mixture was stirred for 4 hours. After addition of water (0.5 ml), the reaction mixture was concentrated to dryness. A solution of the residue in ethyl acetate (20 ml) was washed with water (20 ml). The organic layer was dried over anhydrous Na_2SO_4 and concentrated to give a syrup. The syrup was purified by column chromatography of silica gel (Wakogel C-200, 50 g) developed with a mixture (320: 40: 4: 1) of chloroform, methanol, pyridine and 50% aqueous acetic acid (10-ml fractions). Fractions (Nos. 30~91) were combined and concentrated to give a colorless syrup of **8** as the acetate salt (437 mg, 51%), ^{1}H NMR (CD_3OD) δ 3.7~4.1 (tetrahydropyranyl 6-CH_2, m), 5.04 and 5.08 (benzyl CH_2, s), 5.3 (15-CH, m), 7.20 and 7.31 (10H, aromatic).

(−)-11-*O*-Tetrahydropyranylspergualin-15-phosphate (**9**)

Compound **8** (acetate, 435 mg, 0.415 mmole) in 80% aqueous methanol (10 ml) was hydrogenated with PtO_2 catalyst (30 mg) under a hydrogen stream at atmospheric pressure for 7 hours at room temperature. The catalyst was removed by filtration and the filtrate was concentrated to dryness. The residue was dissolved in 0.1 M NaCl (20 ml) and the solution was adjusted to pH 6.4 with 1 M HCl. The product in the solution was adsorbed on a column of CM-Sephadex C-25 (20 ml, equilibrated with 0.1 M NaCl) and eluted with a linear gradient of 0.1 M to 1.0 M NaCl (each 100 ml). The eluate was cut into 2-ml fractions. Fractions (Nos. 36~44) were combined and concentrated to dryness. The residue was extracted with methanol (5 ml×3). The product in the methanol solution was purified by a column of Sephadex LH-20 (100 ml) eluted with 90% aqueous methanol (1-ml fractions). Fractions (Nos. 38~45) were combined and concentrated to give a colorless syrup of **9** trihydrochloride (128 mg, 48%), ^{1}H NMR (CD_3OD) δ 3.8~4.2 (tetrahydropyranyl 6-CH_2, m), 5.2 (15-CH, m).

(−)-Spergualin-15-phosphate (**3**)

To a solution of **9** trihydrochloride (112 mg, 0.175 mmole) in water (5 ml) was added *p*-toluenesulfonic acid hydrate (3.3 mg, 0.017 mmole) under ice-cooling. After stirring for 8 hours, the solution was adjusted to pH 6.5 with 1 M NH_4OH and added 0.4 M NaCl (20 ml). Compound **3** in the solution was adsorbed on a column of CM-Sephadex C-25 (20 ml, equilibrated with 0.4 M NaCl) and eluted with a linear gradient of 0.4 M to 1.0 M NaCl (each 100 ml). The eluate was cut into 2-ml fractions. Fractions (Nos. 46~59) were combined and concentrated to dryness. The residue was extracted with methanol (5 ml×3). Compound **3** in the methanol solution was purified by a column of Sephadex LH-20 (100 ml) eluted with 90% aqueous methanol (1-ml fractions). Fractions (Nos. 41~52) were combined and concentrated to give a colorless syrup of **3** as the salt (67.7 mg, 62%), $[\alpha]_D^{22}$ −8.2° (*c* 1, water), ^{1}H NMR

(CD_3OD) δ 1.4~1.9 ($CH_2 \times 5$, m), 2.0~2.3 (2-CH_2, m), 2.4~2.7 (14-CH_2, m), 2.9~3.4 ($NCH_2 \times 5$, m), 5.3 (15-CH, m), 5.52 (11-CH, s), TLC Rf 0.08, antibacterial activity, 18% of **1**.

Anal. Calcd. for $C_{17}H_{37}N_2O_7P \cdot 3HCl \cdot Na \cdot 1/2H_2O$: C 32.73, H 6.62, N 15.72, Cl 17.05, P 4.96.
Found: C 32.85, H 6.86, N 15.71, Cl 16.87, P 5.20.

(−)-11-*O*-Tetrahydropyranylspergualin (**10**)

Compound **5** (acetate, 100 mg, 0.119 mmole) in 90% aqueous methanol (5 ml) was hydrogenated with 5% palladium on $BaCO_3$ (10 mg) under a hydrogen stream at atmospheric pressure for 3 hours at room temperature. The catalyst was removed by filtration and the solution was concentrated to give a syrup. The syrup was dissolved in 0.4 M NaCl (20 ml) and the solution was adjusted to pH 6.5 with 1 M HCl. The product in the solution was adsorbed on a column of CM-Sephadex C-25 (20 ml, equilibrated with 0.4 M NaCl) and eluted with a linear gradient of 0.4 M to 1.0 M NaCl (each 100 ml). The eluate was cut into 2-ml fractions. Fractions (Nos. 37~49) were combined and concentrated to dryness. The residue was extracted with methanol (5 ml × 3). The product in the methanol solution was purified by a column of Sephadex LH-20 (100 ml) eluted with 90% aqueous methanol (1-ml fractions). Fractions (Nos. 48~59) were combined and concentrated to give a colorless syrup of **10** trihydrochloride (46 mg, 65%), $[\alpha]_D^{22}$ −21° (*c* 3, water), ^1H NMR(CD_3OD) δ 3.7~4.1 (tetrahydropyranyl 6-CH_2, m), 5.67 (11-CH, s), antibacterial activity, 7% of **1**.

Anal. Calcd. for $C_{22}H_{45}N_7O_5 \cdot 3HCl$: C 44.26, H 8.10, N 16.42, Cl 17.81.
Found: C 44.43, H 8.21, N 16.20, Cl 18.13.

Deprotection of (−)-11-*O*-tetrahydropyranylspergualin (**10**)

By the similar method described in the synthesis of **2**, **10** (trihydrochloride, 50 mg, 0.089 mmole) was hydrolyzed with *p*-toluenesulfonic acid hydrate (1.7 mg, 0.009 mmole) in water (1 ml) under ice-cooling for 7 hours. After adjusting to pH 6.5 with 1 M NH_4OH, 0.4 M NaCl (5 ml) was added to the solution. The product in the solution was adsorbed on a column of CM-Sephadex C-25 (10 ml, equilibrated with 0.4 M NaCl) and eluted with a linear gradient of 0.4 M to 1.0 M NaCl (each 50 ml, 1-ml fractions). Fraction Nos. 43~51 were combined and concentrated to dryness. After extraction with methanol (2 ml × 3), the extract was passed through a column of Sephadex LH-20 (100 ml) and eluted with 90% aqueous methanol (1-ml fractions). Fraction Nos. 39~46 were combined and concentrated to give a colorless syrup of **1** trihydrochloride (29 mg, 64%), $[\alpha]_D^{26}$ −11° (*c* 2, water).

Acknowledgments

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