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Preparation of Fortamine and 6-*epi*-Purpurosamine B from Fortimicin B

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Synopsis. Benzyl alcoholysis and hydrolysis of tetrakis-*N*-(benzyloxycarbonyl)fortimicin B and further transformation of the solvolysis products are described. Several derivatives of 6-*epi*-purpurosamine B and fortamine potentially useful for the synthesis of fortimicin analogs are presented.

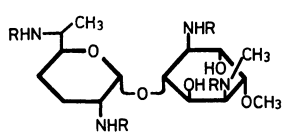
Fortimicins A and B were found by Nara and his coworkers in the culture broth of *micromonospora* sp. MK-70.[1] It was also found that fortimicin B is obtained by hydrolysis of fortimicin A.[2] Both antibiotics belong to a class of pseudodisaccharide composed of 6-*epi*-purpurosamine B and fortamine which is a novel diaminocyclitol. In the course of the synthetic studies of fortimicins and their analogs through coupling reaction of these two components, it will be useful to obtain them from the natural products as their suitable protected derivatives. The present paper reports the preparation of fortamine and 6-*epi*-purpurosamine B by solvolysis of protected fortimicin B and their derivatives.

Fortimicin B was benzyloxycarbonylated to 1,4,2',6'-tetrakis-*N*-(benzyloxycarbonyl)fortimicin B (**1**). Upon benzyl alcoholysis, **1** afforded α- and β-benzyl glycosides of 2,6-bis-*N*-(benzyloxycarbonyl)-6-*epi*-purpurosamine B (**2** and **3**) and 1,4-bis-*N*-(benzyloxycarbonyl)fortamine (**4**) after separation by silica gel chromatography. Each structure was confirmed by the PMR spectra.

When **2** was hydrogenolyzed with a palladium catalyst α-benzyl glycoside (**5**) was obtained. It should be noted that the 1-*O*-benzyl group resisted even to prolonged hydrogenolysis.

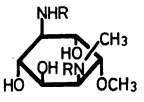
Upon hydrolysis of **1** with 2 M hydrochloric acid, 2,6-bis-*N*-(benzyloxycarbonyl)-6-*epi*-purpurosamine B (**6**) and **4** were obtained. Hydrogenolysis of **6** and hydrolysis of **4** afforded 6-*epi*-purpurosamine B (**7**) and fortamine (**8**) respectively as their hydrochloride.

Acetylation of **6** gave crystalline product of acetate (**9**) and when it was hydrogenolyzed 1-*O*-acetyl-6-*epi*-α-purpurosamine B (**10**) was obtained. **4** and **10** are important intermediates for fortimicin analog syntheses.



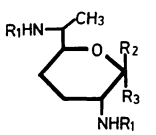
Fortimicin B R = H

1 R = Z



4 R = Z

8 R = H



2 R_1 = Z R_2 = H R_3 = OCH_2Ph

3 R_1 = Z R_2 = OCH_2Ph R_3 = H

5 R_1 = H R_2 = H R_3 = OCH_2Ph

6 R_1 = Z R_2,R_3 = H,OH

7 R_1 = H R_2,R_3 = H,OH

9 R_1 = Z R_2 = H R_3 = OAc

10 R_1 = H R_2 = H R_3 = OAc

Z = CO_2CH_2Ph

Experimental

TLC was carried out on 7.5×2.5 cm slides coated with silica gel (Wakogel B-5, Wako Pure Chemicals Co., Ltd., Osaka) for protected compounds and 20×5 cm plates of cellulose (Avicel SF, Funakoshi Pharmaceutical Co., Ltd., Tokyo) for free compounds. Silica-gel column chromatography was performed with Kiesel gel 60 (E. Merck, Darmstadt, Germany). Melting point was not corrected.

*1,4,2',6'-Tetrakis-*N*-(benzyloxycarbonyl)fortimicin B (**1**).* To an ice-cooled suspension of fortimicin B (10.0 g) and anhydrous sodium carbonate (8.79 g) in aqueous acetone (1:1, 250 ml), benzyloxycarbonyl chloride (25.1 ml) was added and the mixture was stirred for 1 h in the ice bath and then for 2 h at room temperature. The reaction mixture was evaporated to remove acetone and extracted with chloroform. The chloroform solution was dried over anhydrous magnesium sulfate and evaporated to give a pale yellow sirup, which was washed with hexane to give a colorless powder, 24.8 g (96.6%); $[\alpha]_D^{26}$ +42° (*c* 1, MeOH); R_f 0.41 (benzene:ethanol=9:1); PMR (in CD_3OD): δ 2.96 (3H, s, NCH_3), 3.31 (3H, s, OCH_3).

Found: C, 63.68; H, 6.38; N, 6.06%. Calcd for $C_{47}H_{56}N_4O_{13}$: C, 63.78; H, 6.38; N, 6.33%.

*Benzyl Alcoholysis of **1**.* A solution of **1** (1.20 g) in 1 M hydrogen chloride–benzyl alcohol (60 ml) was heated at 50 °C for 4 h. Basic lead carbonate (7.45 g) was added and the mixture was stirred for several hours to render pH about 5. The mixture was centrifuged and the clear supernatant was evaporated under reduced pressure to give a yellow sirup, which was washed with hexane and chromatographed on a silica-gel column with benzene:ethyl methyl ketone (EMK) (25 : 1—4 : 1, gradually changed). Evaporation of the fractions in turn gave benzyl 2,6-bis-*N*-(benzyloxycarbonyl)-α-6-*epi*-purpurosaminide B (**2**), benzyl 2,6-bis-*N*-(benzyloxycarbonyl)-β-6-*epi*-purpurosaminide B (**3**) and 1,4-bis-*N*-(benzyloxycarbonyl)fortamine (**4**), respectively (cited in the order of elution).

*Benzyl 2,6-Bis-*N*-(benzyloxycarbonyl)-α-6-*epi*-purpurosaminide B (**2**).* Crystals (from ethanol), 397 mg (56%); mp 131.5—134.5 °C; $[\alpha]_D^{26}$ +78° (*c* 1, MeOH); R_f 0.49 (benzene:EMK=15:1); PMR (in $CDCl_3$): δ 1.16 (3H, d, *J*=6 Hz, 6-CH_3), 4.55 (2H, ABq, *J*=12 Hz, 1-O-$C\underline{H}_2Ph$), 4.83 (1H, d, *J*=3 Hz, H-1), 5.07, 5.10 (each 2H, s, $CO\cdot OC\underline{H}_2Ph$).

Found: C, 69.48; H, 6.61; N, 5.40%. Calcd for $C_{30}H_{34}N_2O_6$: C, 69.41; H, 6.74; N, 5.30%.

*Benzyl 2,6-Bis-*N*-(benzyloxycarbonyl)-β-6-*epi*-purpurosaminide B (**3**).* Crystals (from ethanol), 61 mg (9%); mp 180.5—183.5 °C; $[\alpha]_D^{27}$ −77° (*c* 0.6, $CHCl_3$); R_f 0.29 (benzene : EMK =15:1); PMR (in $CDCl_3$): δ 1.21 (3H, d, *J*=6 Hz, 6-CH_3), 4.27 (1H, d, *J*=8 Hz, H-1), 4.70 (2H, ABq, *J*=12 Hz, 1-O-$C\underline{H}_2Ph$), 5.11 (4H s, $CO\cdot OC\underline{H}_2Ph$).

Found: C, 69.28; H, 6.86; N, 5.28%.

*1,4-Bis-*N*-(benzyloxycarbonyl)fortamine (**4**).* Crystals (from ether), 340 mg (53%); mp 161—166 °C; $[\alpha]_D^{27}$ +47° (*c* 1, MeOH); R_f 0.41 (chloroform:methanol=24:1); PMR (in $CDCl_3$): δ 3.05 (3H, s, NCH_3), 3.29 (3H, s, OCH_3), 5.10, 5.16 (each 2H, s, $CO\cdot OC\underline{H}_2Ph$).

Found: C, 60.75; H, 6.37; N, 5.90%. Calcd for $C_{24}H_{30}$-

N_2O_8: C, 60.43; H, 6.17; N, 5.89%.

Benzyl α-6-epi-Purpurosaminide B Dihydrochloride (5). A solution of **2** (871 mg) in dioxane (23 ml) was hydrogenolyzed in the presence of 10% palladium on carbon for 24 h under 50 psi hydrogen atmosphere. After filtration the filtrate was neutralized with 2 M hydrochloric acid, and then evaporated under reduced pressure to give a solid, 351 mg (90%), $[\alpha]_D^{26}$ +78°→+80° (*c* 1, H_2O); R_f 0.23 (1-butanol : pyridine : water : acetic acid=6 : 4 : 3 : 1); PMR (in D_2O): δ 1.16 (3H, d, *J*=7 Hz, 6-CH_3), 4.62 (2H, s, 1-O-$C\underline{H}_2$Ph), 5.10 (1H, d, *J*=4 Hz, H-1), 7.37 (5H, s, C_6H_5).

Hydrolysis of 1. To a solution of **1** (1.00 g) in dioxane (30 ml), 4 M hydrochloric acid (30 ml) was added and the mixture was heated at 90 °C for 2 h. The solution was evaporated to 20 ml. Water (200 ml) was added and precipitates were filtered to give a solid, which was chromatographed on a silica-gel column with toluene: EMK(6 : 1—4 : 1, gradually changed). Evaporation of the fractions in turn gave, 2,6-bis-*N*-(benzyloxycarbonyl)-6-*epi*-purpurosamine B (**6**) (70 mg, 15%) and 1,4-bis-*N*-(benzyloxycarbonyl)-fortamine (**4**) (140 mg, 27%) in the order of elution.

2,6-Bis-N-(benzyloxycarbonyl)-6-epi-purpurosamine B (6). A solid; $[\alpha]_D^{26}$ +11° (*c* 1, MeOH); R_f 0.60 (toluene: EMK=3:2); PMR (in $CDCl_3$): δ 1.13 (d, *J*=6 Hz), 1.22 (d, *J*=6 Hz) (3H in total, 6-CH_3), 5.11 (4H, s, $C\underline{H}_2$Ph), 7.37 (10H, s, C_6H_5).

Found: C, 64.40; H, 6.60; N, 6.34%. Calcd for $C_{23}H_{28}N_2O_6$: C, 64.47; H, 6.59; N, 6.54%.

Fortamine Dihydrochloride (8). A suspension of **4** (100 mg) in 6 M hydrochloric acid (25 ml) was heated to reflux for 2 h. The resulting clear solution was evaporated to give a solid, which was dissolved in water and washed with ether. The aqueous layer separated was evaporated to give a residue, which was crystallized from ethanol to give needles, 54 mg (91%); mp 230—240 °C (dec); $[\alpha]_D^{25}$+4° (*c* 0.8, H_2O); R_f 0.27 (1-butanol : pyridine : water : acetic acid=6 : 4 : 3 : 1); PMR (in D_2O): δ 2.87 (3H, s, NCH_3), 3.53 (3H, s, OCH_3), 3.54 (1H, H-1), 3.77 (1H, q, H-4), 3.91 (1H, t, H-6), 4.03 (1H, q, H-3), 4.25 (1H, q, H-5), 4.26 (1H, q, H-2). $J_{1,2}$=8 Hz, $J_{2,3}$=3 Hz, $J_{3,4}$=6 Hz, $J_{5,6}$=8 Hz, $J_{1,6}$=8 Hz. Free base (in D_2O): δ 2.38 (3H, s, NCH_3), 2.83 (1H, t, H-1), 3.13 (1H, q, H-4), 3.37 (1H, t, H-6), 3.45 (3H, s, OCH_3), 3.64 (1H, q, H-2), 3.81 (1H, q, H-5). $J_{1,2}$=10 Hz, $J_{2,3}$=3 Hz, $J_{3,4}$=3 Hz, $J_{4,5}$=5 Hz, $J_{5,6}$=10 Hz, $J_{1,6}$=10 Hz.

Found: C, 34.42; H, 7.22; N, 10.04%. Calcd for $C_8H_{18}N_2O_4 \cdot 2HCl$: C, 34.53; H, 7.39; N, 9.72%.

6-epi-Purpurosamine B Dihydrochloride (7). To a solution of **6** (5.10 g) in dioxane (250 ml), 2 M hydrochloric acid (11.7 ml) was added and hydrogen was bubbled through the solution in the presence of palladium–carbon. After 2 h water (100 ml) was added and the reaction was continued for further 1 h. The solution was filtered and evaporated to give a solid, 2.60 g (93%), which was crystallized from ethanol–ether to give colorless crystals, mp 186—191 °C (dec), $[\alpha]_D^{24}$ +6°→+23° (*c* 0.6, water), R_f 0.26 (1-butanol: pyridine : water : acetic acid=6 : 4 : 3 : 1), PMR (in D_2O): δ 1.29 (d, *J*=7 Hz), 1.30 (d, *J*=7Hz) (total 3H, 6-CH_3), 4.80 (more intense, d, *J*=8.5 Hz), 5.38 (d, 3Hz) (total 3H, H-1).

Found: C, 36.43; H, 7.29; N, 11.65%. Calcd for $C_7H_{16}N_2O_2 \cdot 2HCl$: C, 35.72; H, 6.85; N, 11.42%.

1-O-Acetyl-2,6-bis-N-(benzyloxycarbonyl)-α-6-epi-purpurosamine B (9). To a solution of **6** (2.0 g) in pyridine (55 ml), acetic anhydride (1.5 ml) was added and the solution was allowed to stand at room temperature overnight. After a drop of water was added, the solution was evaporated and the residue was dissolved in chloroform. The solution was washed with water and evaporated to give a solid, which was crystallized from toluene to give needles of **9**, 1.81 g (79%), mp 153.5—156.5 °C, $[\alpha]_D^{26}$ +41° (*c* 1, MeOH), R_f 0.52 (toluene : EMK=3 : 1), PMR (in $CDCl_3$): δ 1.12 (3H, d, *J*=7Hz, 6-CH_3), 2.10 (3H, s, Ac), 5.09 (4H, s, $C\underline{H}_2$Ph), 6.08 (1H, d, *J*=3Hz, H-1), 7.33 (10H, s, C_6H_5).

Found: C, 63.95; H, 6.45; N, 5.85%. Calcd for $C_{25}H_{30}N_2O_7$: C, 63.82; H, 6.43; N, 5.95%.

1-O-Acetyl-α-6-epi-purpurosamine B Dihydrochloride (10). Hydrogen was bubbled through a solution of **9** (600 mg) in dioxane (26 ml) in the presence of palladium–carbon. During the course of the reaction pH was maintained at 3—4 with 2 M hydrochloric acid, and water (0.2 ml×3) was added at 30 min intervals. The solution was filtered and evaporated to give a solid, 357 mg (99%), $[\alpha]_D^{24}$ +40° (*c* 1, MeOH), R_f 0.25 (1-butanol : pyridine : water : acetic acid=6 : 4 : 3 : 1), IR: 1730 (ester) cm^{-1}, PMR (in CD_3OD): δ 1.32 (3H, d, *J*=6Hz, 6-CH_3), 2.25 (3H, s, Ac), 6.30 (1H, bs, H-1).

Found: C, 32.87; H, 7.78; N, 8.22%. Calcd for $C_9H_{18}N_2O_3 \cdot 2HCl \cdot 3H_2O$: C, 32.83; H, 7.96; N, 8.50%.

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