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Synthesis of Some Polyhydroxylated Pyrrolidine Derivatives

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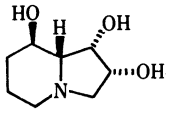
Polyhydroxylated pyrrolidine derivatives, **7**, **11**, **15**, and **18**, were synthesized from **4**, a key intermediate for our total synthesis of swainsonine (**1**). The immunostimulating activities of these new derivatives were found to be moderate and less than that of swainsonine.

Keywords——swainsonine; azamannofuranose; enantiospecific synthesis; D-mannose; immunostimulating activity

Polyhydroxylated pyrrolidine, piperidine, and indolizidine alkaloids are of great interest because of their specific glycosidase inhibitory activity.[1] Moreover, it has recently been found in our laboratories that swainsonine (**1**), a representative of the polyhydroxylated indolizidine alkaloids, has an immunostimulating activity, possibly as a result of its glycosidase inhibitory activity.[2] This has prompted us to explore related pyrrolidine and piperidine alkaloids. In the preceding paper,[3] we reported the syntheses of two piperidine alkaloids, deoxynojirimycin (**2**) and deoxymannojirimycin (**3**). Herein we report the syntheses and biological activity of some pyrrolidine derivatives **7**, **11**, **15**, and **18**.



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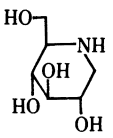

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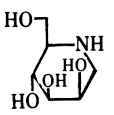

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Chart 1

During the course of our studies on the total synthesis of swainsonine,[4] we found that the intermediates **4** and **5** derived from D-mannose could be conveniently adopted for the synthesis of such pyrrolidine derivatives. For example, pyrrolidine ring formation starting from **4** and **5** would give compounds **6** and **12**, respectively, from which the pyrrolidine derivatives **7**, **11**, **15**, and **18** could be prepared.

For the cyclization of **4** to the pyrrolidine derivatives, **4** was treated with NaH in *N*,*N*-dimethylformamide (DMF) to give compound **6** in 54% yield. The protecting groups in **6** were removed by catalytic hydrogenation on 10% Pd–C in EtOH and subsequent acid treatment (6 N HCl) to give **7** in 80% yield. On the other hand, partial hydrolysis of the acetonide protecting groups in **6** with TsOH in aqueous MeOH gave a 68% yield of the diol **8**, which was then oxidized with $NaIO_4$ in aqueous tetrahydrofuran (THF) and subsequently reducted with $NaBH_4$ in MeOH to afford, *via* the aldehyde **9**, the alcohol **10** in 84% yield. Catalytic hydrogenation of **10** on 10% Pd–C in EtOH, followed by treatment with 6 N HCl gave the

trihydroxypyrrolidine **11** in 71% yield.

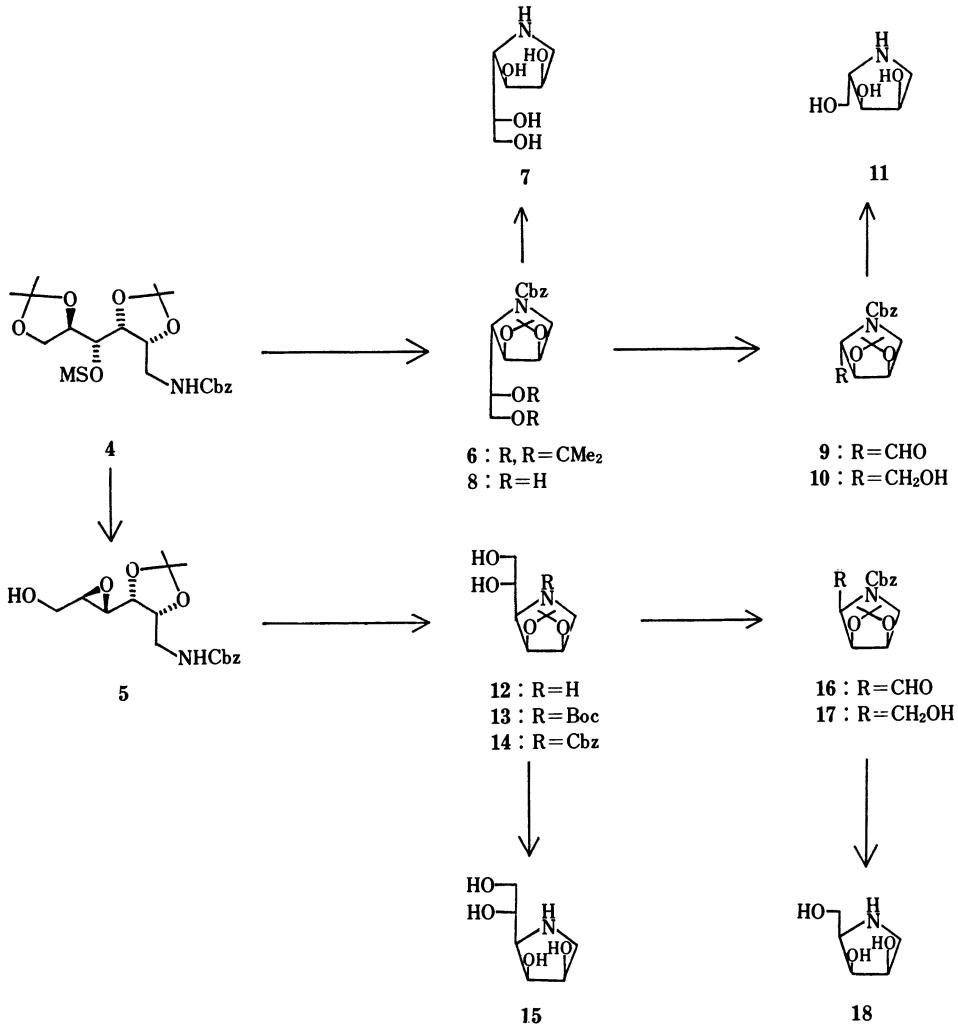

Chart 2

For the preparation of the stereoisomers **15** and **18**, the mesylate **4** was converted to the epoxy-alcohol **5** as described in the preceding paper.[4] Catalytic reduction of **5** as described above for the removal of the Cbz group directly gave the cyclized product **12**, which was successively converted to the Boc derivative **13** by treatment with $(Boc)_2O$ in THF in the presence of Et_3N and purified by silica gel chromatography (81% from **5**). The pure **13** was *then deprotected by treatment with 6 N HCl in THF to afford* **15** *in 78% yield. The crude* amine **12**, on the other hand, was acylated with CbzCl in aqueous THF to give a 68% yield of the Cbz derivative **14**, which in turn was oxidized with $NaIO_4$ in aqueous THF, and the intermediary aldehyde was subjected to reduction with $NaBH_4$ in MeOH to provide the alcohol **17** in 90% yield. The Cbz group in **17** was removed by catalytic hydrogenation as described above and then the acetonide group was removed by treatment with 6 N HCl to afford **18** in 95% yield.

The immunostimulating activity of **7**, **11**, **15**, and **18** was determined in terms of the

TABLE I. Competitive Effect (MEC, μg/ml) against Immunosuppressive Factors Obtained from Tumor-Bearing Mouse Serum in Con A-Induced Stimulation of [^{3}H]Thymidine Incorporation by Mouse Spleen Cells

Compound	MEC (μg/ml)
1	0.01
7	16
11	16
15	12.5
18	4

MEC, minimal effective concentration.

capacity to restore the depression of mitogenic responses of mouse spleen cells by immunosuppressive factors in tumor-bearing mouse serum.[2] The data are summarized in Table I. All the new derivatives showed moderate activity but were considerably less active than swainsonine (**1**). It was found, however, that all the new pyrrolidine derivatives were more active than the piperidine derivatives **2** and **3**.[3] These results suggested that the pyrrolidine derivatives corresponding to the five-membered part of swainsonine are more effective than the piperidine compounds. Moreover, the configuration at the 2 position in the pyrrolidine compounds seemed to be important for the immunostimulant activity. Thus, the 2*R* derivatives **15** and **18** are more active than the 2*S* counterparts **7** and **11** as shown in the table. This result suggests that the *R* configuration at C-8a of swainsonine is important for the activity.

Experimental

All melting points are uncorrected. The proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on a JEOL FX-270 spectrometer using tetramethylsilane (TMS) or 3-(trimethylsilyl)propionic acid-d_4 sodium salt (TSP-d_4) as an internal reference. The infrared (IR) spectra were taken with a JASCO A-102 spectrometer. The optical rotations were measured with JASCO automatic polarimeter. The fast atom bombardment (FAB) high-resolution mass spectra (MS) were recorded on a VG ZAB spectrometer.

(1*R*,5*S*,6*S*,4′*S*)-7-Benzyloxycarbonyl-3,3-dimethyl-6-4′-2′,2′-dimethyl-1′,3′-dioxanyl-2,4-dioxa-7-azabicyclo-[3.3.0]octane (6)——NaH (60 mg, 60% in oil) was added to a solution of **4** (440 mg, 0.93 mmol) in DMF (15 ml), and the mixture was heated at 70 °C for 6 h. After quenching with H_2O, the reaction mixture was extracted with AcOEt and the extract was washed with H_2O and brine and dried over $MgSO_4$. The solvent was removed by evaporation to give an oil, which was chromatographed on silica gel (*n*-hexane–AcOEt 1 : 1) to give **6** (206 mg, 54%) as a pale yellow oil. IR (neat): 1695, 1365 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.32 (9H, s), 1.50 (3H, s), 3.5—4.4 (6H, m), 4.6—4.8 (2H, m), 5.18 (2H, s), 7.37 (5H, s). MS *m/z*: Calcd for $C_{20}H_{27}NO_6$ 378.1917 (M+H), obsd. 378.1951 (M+H).

(2*S*,3*S*,4*R*,1′*S*)-3,4-Dihydroxy-2-1′,2′-dihydroxyethylpyrrolidine (7)——A solution of **6** (189 mg, 0.50 mmol) in EtOH (12 ml) was shaken under H_2 in the presence of 10% Pd–C (30 mg) at room temperature for 4 h. After removal of the catalyst by filtration, the filtrate was added to 6 N HCl (10 ml) and the mixture was stirred at room temperature overnight. After evaporation of the mixture, the residue was dissolved in H_2O and passed through a column of Amberlite IRA-400 (OH^-) with H_2O. The eluate was evaporated to give **7** (65.5 mg, 80%) as a pale yellow syrup. IR (neat): 3300, 1345, 1110, 1065 cm^{-1}. ^{1}H-NMR (D_2O) δ: 2.80 (1H, dd, J=3.5, 12 Hz), 2.96 (1H, dd, J=4, 7.5 Hz), 3.21 (1H, dd, J=5, 12 Hz), 3.60 (1H, dd, J=8, 12 Hz), 3.71 (1H, dd, J=4, 12 Hz), 3.82 (1H, dt, J=4, 8 Hz), 3.97 (1H, dd, J=5, 8 Hz), 4.14 (1H, dt, J=3.5, 5 Hz). $[\alpha]_D^{20}$ −40° (c=0.2, H_2O). MS *m/z*: Calcd for $C_6H_{13}NO_4$ 164.0924 (M+H), obsd. 164.0918 (M+H).

(1*R*,5*S*,6*S*,1′*S*)-7-Benzyloxycarbonyl-6-1′,2′-dihydroxyethyl-3,3-dimethyl-2,4-dioxa-7-azabicyclo[3.3.0]octane (8)——A mixture of **6** (4.40 g, 11.6 mmol) and $TsOH \cdot H_2O$ (220 mg, 1.16 mmol) in a mixture of MeOH (44 ml) and H_2O (4.9 ml) was stirred at room temperature overnight. The mixture was treated with Amberlite IRA-400 (OH^-) and after removal of the resin by filtration, the filtrate was concentrated to give a crude oil, which was purified by chromatography on silica gel. Elution with *n*-hexane–AcOEt (1 : 1) gave **8** (2.57 g, 68%) as an oil. IR (neat): 3420, 1665, 1420, 1370, 1235 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.34 (3H, s), 1.41 (3H, s), 3.4—4.3 (8H, m), 4.80 (2H, m), 5.21

(2H, s), 7.40 (5H, s). MS m/z: Calcd for $C_{17}H_{23}NO_6$ 338.1604 (M+H), obsd. 338.1638 (M+H).

(1*R*,5*S*,6*S*)-7-Benzyloxycarbonyl-3,3-dimethyl-6-hydroxymethyl-2,4-dioxa-7-azabicyclo[3.3.0]octane (10)——A solution of $NaIO_4$ (3.20 g, 14.8 mmol) in H_2O (50 ml) was added dropwise to a solution of **7** (2.50 g, 7.41 mmol) in THF under ice-bath cooling. After being stirred at the same temperature for 1.5 h, the mixture was extracted with $CHCl_3$ and washed successively with H_2O, aqueous $NaHCO_3$ and brine. Drying over $MgSO_4$ and evaporation of the solvent gave **9** (2.26 g) as a crude oil. This oil was used for the next step without further purification.

A solution of **9** in MeOH (50 ml) was cooled in an ice bath and $NaBH_4$ (290 mg, 7.67 mmol) was added. The mixture was stirred at the same temperature for 30 min, then diluted with $CHCl_3$, washed with H_2O and brine, dried over $MgSO_4$, and evaporated to give an oil, which was purified by column chromatography on silica gel (*n*-hexane–AcOEt 1:1) to give **10** (2.06 g, 84% from **8**) as a colorless oil. IR (neat): 3450, 1685, 1425, 1125 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.30 (3H, s), 1.43 (3H, s), 3.57 (1H, dd, J=5, 12 Hz), 3.6—3.9 (4H, m), 4.17 (1H, m), 4.71 (2H, m), 5.14 (2H, s), 7.36 (5H, s). MS *m/z*: Calcd for $C_{16}H_{21}NO_5$ 308.1498 (M+H), obsd. 308.1489 (M+H).

(2*S*,3*S*,4*R*)-3,4-Dihydroxy-2-hydroxymethylpyrrolidine (11)——Compound **10** (900 mg, 2.93 mmol) was treated in a manner similar to that used for the preparation of **7** from **8** to give **11** (343 mg, 88%) as a pale yellow solid. mp 114—117 °C (EtOH–Et_2O). IR (Nujol): 3280, 1320, 1140, 1100, 1035 cm^{-1}. ^{1}H-NMR (D_2O) δ: 2.82 (1H, dd, J=4, 12 Hz), 3.06 (1H, ddd, J=4.5, 6, 7 Hz), 3.18 (1H, dd, J=5.5, 12 Hz), 3.63 (1H, dd, J=6, 11.5 Hz), 3.74 (1H, dd, J=4.5, 11.5 Hz), 3.87 (1H, dd, J=5.5, 7 Hz), 4.15 (1H, dt, J=4, 5.5 Hz). $[\alpha]_D^{22}$ −20.7° (*c*=0.5, H_2O). MS *m/z*: Calcd for $C_5H_{11}NO_3$ 134.0819 (M+H), obsd. 134.0811 (M+H).

(1*R*,5*S*,6*R*,1′*S*)-7-*tert*-Butoxycarbonyl-6-1′,2′-dihydroxyethyl-3,3-dimethyl-2,4-dioxa-7-azabicyclo[3.3.0]octane (13)——A solution of **5** (2.20 g, 6.52 mmol) in EtOH (60 ml) was shaken under H_2 (3 atm) in the presence of 10% Pd–C (300 mg) at room temperature for 5 h. After removal of the catalyst, the solvent was evaporated to give **12** as a crude oil. Di-*tert*-butyldicarbonate (Boc_2O) (1.04 ml, 4.53 mmol) and Et_3N (0.63 ml, 4.53 mmol) were added to a solution of **12** (760 mg) in a mixture of THF (20 ml) and H_2O (5 ml) under ice-bath cooling, and the mixture was stirred at room temperature for 3 h. THF was evaporated off and the resulting aqueous solution was extracted with $CHCl_3$. The organic layer was washed with H_2O, dried over $MgSO_4$, and evaporated to give an oil, which was purified by chromatography on silica gel (*n*-hexane–AcOEt 1:1) to give **13** (916 mg, 81% from **5**) as a pale yellow oil. IR (neat): 3420, 1670, 1400 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.34 (3H, s), 1.43 (9H, s), 1.53 (3H, s), 3.0—4.3 (8H, m), 4.6—5.0 (2H, m). MS *m/z*: Calcd for $C_{14}H_{25}NO_6$ 304.1760 (M+H), obsd. 304.1713 (M+H).

(1*R*,5*S*,6*R*,1′*S*)-7-Benzyloxycarbonyl-6-1′,2′-dihydroxyethyl-3,3-dimethyl-2,4-dioxa-7-azabicyclo[3.3.0]octane (14)——A crude sample of **12** prepared from 6.20 g (18.4 mmol) of **5** was acylated in a manner similar to that used for the preparation of **13** to give **14** (3.17 g, 51%) as an oil. IR (neat): 3430, 1680, 1405, 1245 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.32 (3H, s), 1.53 (3H, s), 2.9—4.3 (8H, m), 4.82 (2H, m), 5.11 (2H, s), 7.30 (5H, s). MS *m/z*: Calcd for $C_{17}H_{23}NO_6$ 338.1604 (M+H), obsd. 338.1621 (M+H).

(2*R*,3*S*,4*R*,1′*S*)-3,4-Dihydroxy-2-1′,2′-dihydroxyethylpyrrolidine (15)——A solution of **13** (800 mg, 2.64 mmol) in a mixture of THF (20 ml) and 6 N HCl (20 ml) was stirred at room temperature overnight and the solvent was evaporated off to give an oil. This oil was treated in a manner similar to that used for **7** to give **15** (335 mg, 78%) as a pale yellow powder. mp 125—128 °C (EtOH–Et_2O). IR (Nujol): 3450, 3260, 3210, 1335, 1085 cm^{-1}. ^{1}H-NMR (D_2O) δ: 2.75 (1H, dd, J=7, 12 Hz), 3.09 (1H, dd, J=4, 10 Hz), 3.15 (1H, dd, J=8, 11.5 Hz), 3.55 (1H, dd, J=7, 12 Hz), 3.75 (1H, dd, J=3.5, 12 Hz), 3.85 (1H, ddd, J=3.5, 7, 10 Hz), 4.20 (1H, t, J=4 Hz), 4.32 (1H, dt, J=4, 8 Hz). $[\alpha]_D^{25}$ −12.4° (*c*=0.7, H_2O). [Lit.[1e] mp 137 °C. $[\alpha]_D^{20}$ −10.4° (*c*=0.12, H_2O)].

(1*R*,5*S*,6*R*)-7-Benzyloxycarbonyl-3,3-dimethyl-6-hydroxymethyl-2,4-dioxa-7-azabicyclo[3.3.0]octane (17)——Compound **13** (3.00 g, 8.89 mmol) was treated with $NaIO_4$ (3.24 g, 15.0 mmol) and then with $NaBH_4$ (336 mg, 8.89 mmol) in the same way as described for **6** to provide **17** (2.45 g, 90%) as an oil. IR (neat): 3420, 1685, 1415, 1080 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.33 (3H, s), 1.47 (3H, s), 3.66 (2H, d, J=2 Hz), 3.8—4.0 (4H, m), 4.75 (2H, m), 5.13 (2H, s), 7.31 (5H, s) MS *m/z*: Calcd for $C_{16}H_{21}NO_5$ 308.1498 (M+H), obsd. 308.1502 (M+H).

(2*R*,3*S*,4*R*)-3,4-Dihydroxy-2-hydroxymethylpyrrolidine (18)——A solution of **17** (1.09 g, 3.55 mmol) in EtOH (50 ml) was treated in the same manner as described for **10** to give **18** (450 mg, 95%) as an oil. IR (neat): 3300, 1415, 1125, 1055 cm^{-1}. ^{1}H-NMR (D_2O) δ: 2.77 (1H, dd, J=7, 12 Hz), 3.11 (1H, dd, J=7, 12 Hz), 3.25 (1H, dt, J=4, 7 Hz), 3.64 (1H, dd, J=7, 11 Hz), 3.79 (1H, dd, J=7, 11 Hz), 4.18 (1H, t, J=4 Hz), 4.28 (1H, dt, J=4, 7 Hz). $[\alpha]_D^{25}$ −9.3° (*c*=1.3, H_2O). MS *m/z*: Calcd for $C_5H_{11}NO_3$ 134.0819 (M+H), obsd. 138.0793 (M+H).

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