Synthesis of Some Polyhydroxylated Pyrrolidine Derivatives

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(Received February 21, 1987)

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. 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. This has prompted us to explore related pyrrolidine and piperidine alkaloids. In the preceding paper, 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.

During the course of our studies on the total synthesis of swainsonine, 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 NaIO4 in aqueous tetrahydrofuran (THF) and subsequently reduced with NaBH4 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.

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.\textsuperscript{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)\textsubscript{2}O in THF in the presence of Et\textsubscript{3}N 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\textsubscript{4} in aqueous THF, and the intermediary aldehyde was subjected to reduction with NaBH\textsubscript{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
capacity to restore the depression of mitogenic responses of mouse spleen cells by immuno-
suppressive 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.  

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 2R derivatives 15 and 18 are more active than the 2S 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 (1H-NMR) spectra were recorded on a JEOL FX-270 spectrometer using tetramethylsilane (TMS) or 3-(trimethylsilyl)propionic acid-d4 sodium salt (TSP-d4) 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.

(1R,5S,6S,4'S)-7-Benzylloxycarbonyl-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 H2O, the reaction mixture was extracted with AcOEt and the extract was washed with H2O and brine and dried over MgSO4. 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.  

1H-NMR (CDCl3) 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 C20H27N06 378.1917 (M + H), obsd. 378.1951 (M + H).

(2S,3S,4R,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 H2 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 6N HCl (10 ml) and the mixture was stirred at room temperature overnight. After evaporation of the mixture, the residue was dissolved in H2O and passed through a column of Amberlite IRA-400 (OH⁻) with H2O. The eluate was evaporated to dryness and the residue was dissolved in H2O and the solution was chromatographed on silica gel (n-hexane–AcOEt 1:1) to give 7 (65.5 mg, 80%) as a pale yellow syrup. IR (neat): 3300, 1345, 1110, 1065 cm⁻¹.  

1H-NMR (D2O): 2.80 (1H, dd, J = 3.5, 12 Hz), 3.5-4.4 (6H, m), 4.8 (2H, m), 4.5-5.0 (2H, m). [c]D20 = -40 ° (c = 0.2, H2O). MS m/z: Calcd for C6H13NO4 164.092 (M + H), obsd. 164.0918 (M + H).

(1R,5S,6S,1'S)-7-Benzylloxycarbonyl-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·H2O (220 mg, 1.16 mmol) in a mixture of MeOH (44 ml) and H2O (4.9 ml) was stirred at room temperature overnight. 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.  

1H-NMR (CDCl3) δ: 1.34 (3H, s), 1.41 (3H, s), 3.4-4.3 (8H, m), 4.80 (2H, m), 5.21

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<td>15</td>
<td>12.5</td>
</tr>
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TABLE I. Competitive Effect (MEC, µg/ml) against Immunosuppressive Factors Obtained from Tumor-Bearing Mouse Serum in Con A-Induced Stimulation of [3H]Thymidine Incorporation by Mouse Spleen Cells

MEC, minimal effective concentration.
crude oil. Di-tert-butyldicarbonate (Boc₂O) (1.04 ml, 4.53 mmol) and Et₃N (0.63 ml, 4.53 mmol) were added to a 

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**Acknowledgement**

We are grateful to Dr. H. Terano and his colleagues (Fujisawa) for the biological assays.

**References**