Arch. Pharm. Res. Vol.20, No.4, pp. 346-350, 1997

Synthesis and Activity of a Potent α-Glucosidase Inhibitor, (1*R*, 6*R*, 8*S*)-*cis*-1,6-Dihydroxypyrrolizidine, and Its Isomer

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(Received May 27, 1997)

The synthesis of *cis*- and trans-1,6-dihydroxypyrrolizidine starting from *trans*-4-hydroxy-L-proline and their evaluation as glycosidase inhibitors are reported. The *cis*-isomer was found to be a potent inhibitor against α-glucosidase and showed weak inhibitory effect against other glycosidases. The *trans*-isomer exhibited weak inhibitions of b-glucosidase and amyloglucosidase and poor inhibition of other glycosidases.

Key words : a-glucosidase inhibitor, swainsonine, pyrrolizidine derivatives

INTRODUCTION

Glycosidases process various oligosaccharides-containing glycoproteins and glycolipids and are important enzymes for the normal growth and development of all organisms. The profound impact of these enzymes on life processes has prompted the design and the synthesis of a number of glycosidase inhibitors (Elbein *et al.*, 1987). The discovery of nitrogen-containing natural products which resemble monosaccharides has contributed to the development of these inhibitors (Michael, 1995; Robins, 1995).

Swainsonine **1** (Dorling *et al.*, 1980; Kang *et al.*, 1983) and castanospermine 2 (Pan *et al.*, 1983) (Fig. 1) are polyhydroxyindolizidine alkaloids isolated from *Swainsona canescenes* and *Castanosperum* australe respectively. Swainsonine was found to be a very potent and specific inhibitor of α-mannosidase and castanospermine is a good inhibitor of α- and β-glucosidases.

Australin **3** (Molyneux *et al.*, 1988), a tetrahydroxypyrrolizidine alkaloid has been isolated from *C. australe* seed and it was found to be a potent inhibitor of amyloglucosidase. Many isomers and analogues of indolizidine and pyrrolizidine alkaloids were designed and prepared as inhibitors of glycosidases (Michael, 1995; Robins, 1995).

The mechanisms of glycosidase reactions which is the hydrolysis of monosaccharide from oligosaccharide and glycoprotein have been the object of much research. It has been postulated that the hydrolysis proceeds through a half-chair (or twist-boat) transition state with substantial sp^2 character at the anomeric carbon (Sinnot, 1990; Kuroki *et al.*, 1993). However, the precise mechanisms of inhibition by swainsonine and other inhibitors are still unknown.

Various combinational modifications of functional groups have been hypothesized as being necessary for inhibitory activity. Among them were the number, position and stereochemistry of the hydroxyl group and the presence of a 6-membered ring in bicyclic system (Elbein *et al.*, 1987; Molybneux *et al.*, 1986; Tropea *et al.*, 1989). However, none of these requirements suggests a mode of action which fits all of the SAR studies.

We have planned to synthesize various polyhydroxyindolizidines and pyrrolizidines as glycosidase inhibitors to improve the potency and selectivity of their biological activities.

Two dihydroxypyrrolizidines, (1*R*, 6*R*, 8*S*)-*cis*-1,6-dihydroxypyrrolizidine **5** and (1*S*, 6*R*, 8*S*)-*trans*-1,6-dihy-

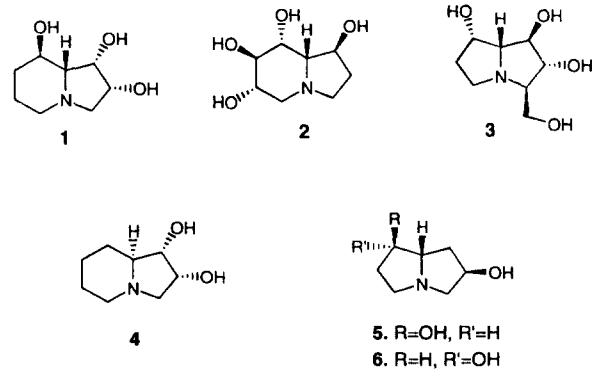

Fig. 1. Indolizidines and pyrrolizidines.

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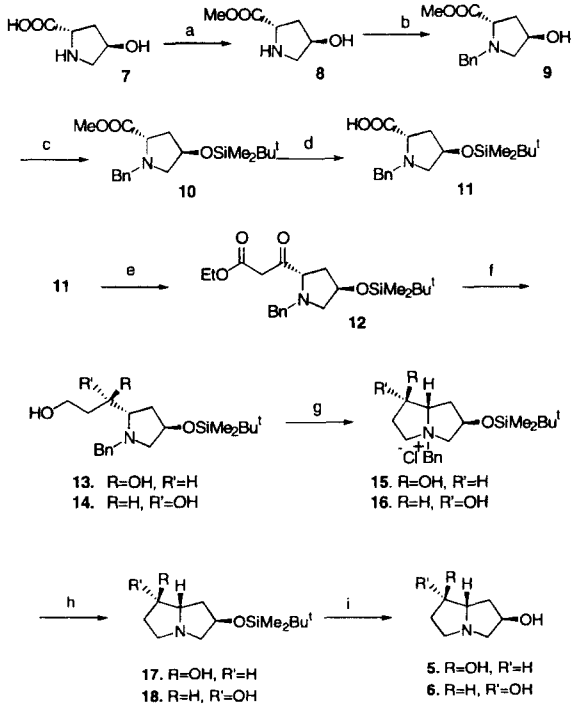
droxypyrrolizidines **6** were synthesized. Few studies have been done for dihydroxypyrrolizidine (or indolizidine) alkaloids since only tetra or trihydroxy alkaloids were considered to have inhibitory activity against glycosidases. However, the natural product, lentiginosine **4**, isolated from *Astragalus lentiginosine var. dipbysus*, showed strong inhibition of amyloglucosidase (Pastuszak *et al.*, 1990).

MATERIALS AND METHODS

Chemistry

As shown in Scheme I, our approach to the synthesis of dihydroxypyrrolizidines **5** and **6** started with *trans*-4-hydroxy-L-proline **7**, which is commercially available. The reflux of **7** with acetyl chloride in methanol produced the methyl ester salt **8**. The secondary amine moiety of **8** was then protected by a benzyl group to give **9**. Silylation of alcohol **9** with TBDMSCl and subsequent treatment of Claisen alkali provided acid **11**. The reaction of acid **11** with malonic acid monoester in the presence of anhydrous magnesium chloride and triethylamine provided β-ketoester **12**.

Reduction of both ketone and ester functional groups of **12** was accomplished by using sodium borohydride in methanol and THF to give the 1:1.7 mixture of **13** and **14**. After the separation by chromatography,



Scheme I. a. MeOH, CH_3COCl. b. BnBr, K_2CO_3, CH_3CN. c. TBDMSCl, imidazole, DMF. d. Claisen alkali. e. $CH_2(COOH)(COOEt)$, $MgCl_2$, Et_3N. (65%) f. $NaBH_4$, MeOH, THF. (**13**: **14**=1:1.7, 61%) g. MsCl, Et_3N, CH_2Cl_2. h. Pd/C, H_2. (crude yield: 84%) i. TBAF, THF.

each of diol **13** and **14** was treated with 1 mol equivalent of mesyl chloride and triethylamine to give the cyclized product **15** and **16** respectively. Debenzylation by using palladium/charcoal in methanol and the deprotection of TBDMS group by using TBAF gave pyrrolizidines **5** and **6**.

The stereochemistry of **5** and **6** was determined by ^{1}H-NMR experiments, 2D-COSY and 2D-NOESY using Bruker DMX 600 spectrometer. The NMR data for other compounds were obtained using Bruker 300 spectrometer.

Synthesis

***Trans*-4-hydroxy-*L*-proline methyl ester. HCl (8):** *Trans*-4-hydroxy-*L*-proline (30 g, 0.23 mol) was dissolved in MeOH (200 mL) slowly and acetyl chloride (22.3 mL, 0.32 mol) was added dropwise to this solution. The reaction mixture was refluxed for 7 hrs and cooled to room temperature. Ether (1 L) was added and the resulting white crystals were obtained by filteration. (39. 4 g, 96%) mp: 170°C. ^{1}H NMR ($CDCl_3$) δ 2.03, 2.38 (2H, m, C_3-H), 3.06, 3.38 (2H, m C_5-H), 3. 74 (3H, s, O-CH_3), 4.38, 4.47 (2H, m, C_2-H, C_4-H). IR (KBr) 3376 (OH), 1742 (C=O) cm^{-1}.

Methyl-(2*S*,4*R*)-4-hydroxy-*N*-benzylpyrrolidine carboxylate (9): Potassium carbonate (81.9 g, 0.57 mol) and benzyl bromide (23.76 mL, 0.16 mol) were added to compound **8** (36 g, 0.19 mol) in acetonitrile. After adding potassium iodide (360 mg, 0.0021 mol) as a catalyst the reaction mixture was refluxed for 6 hrs and cooled to room temperature. After evaporation of acetonitrile under reduced pressure, methylene chloride and water were added and the organic layer was dried with sodium sulfate. Evaporation of solvent and column chromatography (ethyl acetate) produced 39.6 g of yellow oil product (yield : 74 %).

^{1}H NMR ($CDCl_3$) δ 2.46, 3.31 (2H, m, C_5-H), 2.22, 2.08 (2H, m, C_3-H), 3.63 (1H, m, C_2-H), 65 (3H, s, O-CH_3), 3.89, 3.65 (2H, dd, J=12.75 Hz, N-CH_2-arom), 4.43 (1H, m, C_4-1H), 7.27 (5H, m, aromatic-H) ^{13}C NMR ($CDCl_3$) δ 39.59, 51.77, 58.18, 61.14, 63.67, 70. 24, 127.26, 128.29, 129.10, 138.06, 174.04. IR (KBr) 3404 (OH), 3062 (aromatic), 2810 (aliphatic), 1732 (C=O) cm^{-1}.

Methyl-(2*S*,4*R*)-4-*tert*-buthyldimethylsiloxy-*N*-benzylpyrrolidine-2-carboxylate (10): Imidazole (18.7 g, 0.275 mol) and tert-buthyldimethylsilylchloride (24.8 g, 0.7 mol) were stirred with **10** (39.6 g, 0.11 mol) in DMF (100 mL) for 12 hrs. Ethyl acetate and water were added and the organic layer was washed with 5% aqueous sodium bicarbonate solution and water and dried. Evaporation of solvent and column chromatography (ethyl acetate:hexane=1:5) gave 42 g of **10** (yield:72 %).

^{1}H NMR ($CDCl_3$) d 0.05 (6H, s, Si-CH_3), 0.87 (9H, s,

tert-butyl), 2.21, 2.06 (2H, m, C_3-H), 2.46, 3.31 (2H, m, C_5-H), 3.62 (1H, m, C_2-H), 3.65 (3H, s, O-CH_3) 3.89, 3.65 (2H, dd, J=12.7 Hz, N-CH_2-arom), 4.43 (1H, m, C_4-H), 7.27 (5H, m, aromatic-H). IR (KBr) 3028 (aromatic), 2952 (aliphatic), 1750 (C=O) cm^{-1}.

(2*R*,4*S*)-*N*-benzyl-4-*tert*-buthyldimethylsiloxyproline (11): Compound **10** (42 g, 0.12 mol) was dissolved to 420 mL of Claisen alkali (CH_3OH, KOH, H_2O) and the reaction mixture was stirred for 30 min in an ice bath. After water was added the reaction solution was evaporated under reduced pressure and washed with dichloromethane. The organic layer was removed and the water layer was neutralized to pH 7 with conc. HCl in an ice bath. The reaction solution was extracted with dichloromethane and the organic layer was dried with sodium sulfate. Evaporation of solvent and column chromatography (CH_3Cl:MeOH=3:1) gave 40 g of **11** (yield: 70%).

NMR ($CDCl_3$) δ 0.05 (6H, s, Si-CH_3), 0. 87 (9H, s, *tert*-butyl), 2.24, 2.34 (2H, m, C_4-H), 1H 2.96, 3.88 (2H, m, C_3-H), 4.07 (1H, t, C_4-H), 4.60, 4.16 (2H, dd, J=3.8 Hz, N-CH_2-arom), 4.45 (1H, m, C_4-H), 7.29-7.43 (5H, m, aromatic-H). ^{13}C NMR ($CDCl_3$) δ 2.19, 17.80, 25.68, 39.23, 60.00, 61.14, 67.68, 70.82, 129.18, 130.25, 131.67, 138.60, 170.99. IR (KBr) 3355 (OH), 2954 (aliphatic), 1634 (C=O, conjugated) cm^{-1}.

(2*S*,4*R*)-*N*-benzyl-4-(*tert*-butyldimethylsiloxy)-2-(2-carboxylethyl-1-oxo)-ethylpyrrolidine (12): Carbodiimidazole (6.8 g, 44 mmol) was stirred with **11** (10 g, 0.03 mol) in THF (50 mL) for 2 hrs at room temperature giving imidazolide product. To another flask, monoethyl malonate (13.2 g, 0.120 mol) was dissolved in THF (120 mL) and magnesium chloride (8.56 g, 90 mmol) and triethylamine (12.64 mL, 90 mmol) were added drowise. The reaction mixture was stirred for 3 hrs at room temperature to give magnesium salt product. Magnesium salts solution was added to imidazolide solution and this reaction mixture was stirred for 12 hrs at room temperature and washed with aqueous ammonium sulfate solution and extracted with ethyl acetate. The organic layer was dried and concentrated. Column chromatography (hexane:ethyl acetate=9:1) of residue gave 10.12 g of 12 (yield:83%).

^1H NMR ($CDCl_3$) δ 0.06 (6H, s, Si-CH_3), 0.82 (9H, s, *tert*-butyl), 1.11, 1.18 (3H, m, O-CH_2-CH_3), 2.01, 2.08 (2H, m, C_3-H), 2.41, 3.22 (2H, m, C_5-H), 3.42 (1H, t, C_2-H), 3.42, 3.85 (2H, d, N-CH_2-aromatic), 3.60 (2H, m, CO-CH_2-), 4.12, 4.22 (2H, qr, O-$\underline{CH_2}$-CH_3), 4.37 (1H, m, C_4-1H), 7.21-7.43 (5H, m, aromatic-H). IR (KBr) 3390 (OH, enol), 2930 (aliphatic), 1732 (C=O), 1652 (C=O, enol) cm^{-1}.

(2*S*,4*R*)-*N*-benzyl-4-(tert-butyldimethylsiloxy)-2-(1(*R*/*S*)-1,3-dihydroxypropyl) pyrrolidine (13,14): $NaBH_4$ (2.358 g, 0.062 mol) and methanol (25 mL) were added to **12** (10.12 g, 0.025 mol) in THF (300 mL) and the reaction mixture was stirred for 2 hrs at room temperature. Aqueous ammonium sulfate solution was added and ethyl acetate was used for extraction. The organic layer was dried and concentrated. Column chromatography (hexane: ethyl acetate=1:1) of residue produced 5.58 g of dihydroxypyrrolidines **13** and **14** (**13**:**14**=1:1.7, yield:61%).

13: ^1H NMR ($CDCl_3$) δ: 0.01 (6H, s, Si-CH_3), 0.87 (9H, s, *tert*-butyl), 1.54, 1.66 (2H, m, CH-$\underline{CH_2}$-CH_2-OH), 1.88, 1.99 (2H, m, C_3-H), 2.37, 2.85 ($\underline{2H}$, m, C_5-H), 2.71 (1H, m, C_2-H), , 4.8 (2H, m, J=13.12 Hz, N-$\underline{CH_2}$-arom), 3.86 (2H, m, $\underline{CH_2}$-OH), 3.98 (1H, m, CH-$\underline{OH}$), 4.24 (1H, m, C_4-H), 7.28 (5H, m, aromatic). IR (KBr) 3424 (OH), 2928 (aliphatic) cm^{-1}.

14: ^1H NMR ($CDCl_3$) δ: 0.01 (6H, s, Si-CH_3), 0.87 (9H, s, *tert*-butyl), 1.57,1.69 (3H, m, C_3-H), 2.02 (1H, m, C_3-H), 2.35, 3.11 (2H, m, C_5-H), 2.93 (1H, m, C_2-H), 3.97 (1H, m, OH-C1H), 3.43, 4.03 (2H, dd, J=13. 12 Hz, N-CH_2-arom), 3.85 (2H, t, CH_2-OH) 4.22 (1H, m, C_4-1H), 7.26-7.33 (5H, m, aromatic). IR (KBr) 3422 (OH), 2954 (aliphatic) cm^{-1}.

(1*R*,6*R*,8*S*)-6-(*tert*-buthyldimethylsiloxy)-*N*-benzyl-1-hydroxypyrrolidinium-chloride (15): Triethylamine (1. 8 mL, 0.018 mol) and methanesulfonyl chloride (1.03 mL, 0.013 mol) were added to 13 (4.28 g, 0.012 mol) in dichloromethane (100 mL). The reaction mixture was stirred overnight at room temperature under nitrogen and washed with saturated ammonium sulfate solution. The water layer was extracted with dichloromethane and the organic layer was combined and dried. Evaporation of solvent and column chromatography gave **15** (3.8 g, yield:87%).

^1H NMR ($CDCl_3$) δ: 0.27 (6H, s, Si-CH_3), 0.92 (9H, s, tert-butyl), 1.68 (1H, m, C_1-H), 2.02 (1H, m, C_7-H), 2.42 (1H, m, C_2-H), 2. 65 (1H, m, C_2-H), 3.22 , 3. 52 (2H, m, C_5-H), 3.98 , 4.12 (2H, m, C_3-H), 4.31 (1H, m, C_7H-OH), 4.32 (1H, m, C^2-H), 4.61, 4.98 (2H, dd, J=1 4.12 Hz, N-CH_2-aromatic), 7. 43-7.72 (5H, m, aromatic)

(1*R*,6*S*,8*S*)-6-(*tert*-buthyldimethylsiloxy)-*N*-benzyl-1-hydroxypyrrolidinium-chloride (16): Same procedure of **15** was used to get product **16** (yield:76%) from **14**.

^1H NMR ($CDCl_3$) δ 0.23 (6H, s, Si-CH_3), 0.97 (9H, s, tert-butyl), 1.98 (1H, m, C_1-H), 2.17 (1H, m, C_1-H), 2.33 (1H, m, C_6-H), 2. 54 (1H, m, C_6-H), 3.22, 3.42 (2H, m, C_5-H), 4.02 , 4.06 (2H, m, C_3-H), 4.38 (1H, m, C_7H-OH), 4.54 (1H, t, C_2-H), 4.73, 4.96 (2H, dd, J= 12.79 Hz , N-CH_2-aromatic), 7. 53-7.59 (5H, m, aromatic).

(1*R*, 6*R*, 8*S*)-6-(*tert*-buthyldimethylsiloxy)-1-hydroxypyrrolizidine (17): Compound **15** (2.82 g, 0.07 mol) was hydrogenized with 10% Pd/C (8.8 g, 0.07 mol) in ethanol-HCl (20 mL) for 24 hrs at 50 psi. The reaction solution was filted with celite and evaporated. Column chromatography (CH_2Cl_2:CH_3OH=5:1) produced **17** (0.95 g, yield: 50%).

^{1}H NMR ($CDCl_3$) δ: 4.22 (1H, m, C_2-H), 3.82 (1H, m, C_7-H), 3.12 (1H, m, C_5-H), 3.00 (1H, m, C_3-H), 2.38 (1H, m, C_8-H), 2.15 (2H, m, C_5-H, C_3-H), 1.71 (2H, m, C_6-H, C_1-H), 1.61 (2H, m, C_6-H, C1-H), 0.92 (9H, s, *tert*-butyl), 0.13 (6H, s, Si-CH_3).

(1*R*, 6*S*, 8*S*)-6-(*tert*-buthyldimethylsiloxy)-1-hydroxy-pyrrolizidine (18): Same procedure of **17** was used to produce **18** (1.06 g, yield: 56%) from **16**.

^{1}H NMR ($CDCl_3$) δ: 4.31 (1H, m, C_2-H), 3.79 (1H, m, C7-H), 3.32 (1H, m, C_5-H), 3.02 (1H, m, C_3-H), 2.43 (1H, m, C_8-H), 2.15 (2H, m, C_3-H, C_5-H), 1.81 (2H, m, C_1-H, C_6-H), 1.48 (2H, m, C_1-H, C_6-H), 0.97 (9H, s, tert-butyl), 0.23 (6H, s, Si-CH_3).

(1*R*, 6*R*, 8*S*)-1,6-*cis*-dihydroxy pyrrolizidine (5): Compound **17** (0.6 g, 2.28 mmol) was stirred with TBAF (6 mL, 12 mmol in 1 Mol THF) in THF (30 mL) at room temperature for 2 days under nitrogen. Evaporation of solvent and column chromatography (CH_2Cl_2:CH_3OH :NH_4OH=5:4:1) with the residue gave 5 (0.22g, yield: 67 %).

^{1}H NMR (D_2O) δ 4.42 (1H, m, C_6-H), 4.32 (1H, m, C_1-H), 3.44 (1H, dt, J=3.9, 8.0 Hz, C_8-H), (2H, m, C_3-H, C_5-H), 3.01 (1H, m, C_3-H), 2.72 (1H, dd, J=5.8, 11.2 Hz, C_5-H), 2.35 (2H, m, C_2-H, C_7-H), 1.83 (1H, m, C_2-H), 1.70 (1H, m, C_7-H) ^{13}C NMR (D_2O) δ 76.85, 71.54, 70.61, 60.21, 52.80, 37.15, 32.41. MS (EI) m/z 143, 99, 82. HRMS (EI) m/z 143.0946 calcd for $C_7H_{13}N_1O_2$, found 143.0940.

(1*R*, 6*S*, 8*S*)-1,6-*trans*-dihydroxy pyrrolizidine (6): Same procedure of **5** was used to get product **6** (yield: 62 %) from **18**.

^{1}H NMR (D_2O) δ 4.52 (1H, m, C_6-H), 4.17 (1H, m, C_1-H), 3.54 (1H, dt, C_8-H), 3.17 (1H, m, C_3-, 3.06 (1H, dd, J=11.7 Hz, C_5-H), 2.78 (2H, m, C_3-H, C_5-H), 2.11 (2H, m, C_2-H, C_7-H), 1.79 (2H, m, C_2-H, C_7-H). ^{13}C NMR (D_2O) δ 76.30, 72.38, 70.85, 61.60, 52.05, 37.48, 32.17. MS (EI) m/z 143, 99, 82. HRMS (EI) m/z 143.0946 calcd for $C_7H_{13}N_1O_2$, found 143.0956.

Biological test

Marterials and experimental procedure: The enzymes α-glucosidase (yeast), α-amyloglucosidase (Aspergillus niger), β-glucosidase (almonds), α-mannosidase (jack bean), α-galactosidase (green coffee bean), β-galactosidase (bovine liver) were purchased from Sigma Chemical Co. Assay was performed with *p*-nitrophenyl α (or β)-D-glycopyranosides as substrates at 37°C in 23 mM sodium-citrate buffers. Yeast α-glucosidase and amyloglucosidase were assayed at pH 5.0 and β-glucosidase and α- and β-galactosidase were assayed at pH 6.6. α-Mannosidase was assayed at pH 5.0 and 6.6. The typical assay mixture for these enzymes contained 5 μmol of the indicated *p*-nitrophenyl-glucoside and 11.5 μmol of sodium citrate buffer, and enzymes (α-mannosidase: 1.32 μg, α-glucosidase (yeast): 0.5 mg, amylo-glucosidase: 40 mg, β-glucosidase: 1 mg, α-galactosidase: 50 mg, β-galactosidase: 100 mg) in a final volume of 0.5 mL. The mixture was preincubated at 37°C for 5 min and the reaction started by the addition of substrates and incubated for 15 min. The reaction was stopped by the addition of 2.5 mL of 400 mM glycine buffer, pH 10.4. The *p*-nitrophenol liberated in the reaction was measured at 410 nm.

RESULTS AND DISCUSSION

Both compounds **5** and **6** were tested as possible inhibitors of glycosidases using α-mannosidase, α- and β-glucosidases, and α- and β-galactosidases. (Table I) To test the effect of compound **5** and **6** on α-glucosidases, yeast α-glucosidase and amyloglucosidase (exo-1,4-α-glucosidase) were chosen. The *trans*-isomer exhibited weak inhibition of β-glucosidase (50% inhibition with 170 μg/mL) and amyloglucosidase (50% inhibition with 50 μg/mL) and it did not show any inhibition of yeast α-glucosidase at high concentration (1.4 mM). Both compounds showed weak inhibition of α-galactosidase and very poor inhibition of β-galactosidase. Castanospermine[4] was reported to be a very sensitive inhibitor of amyloglucosidase but did not show any inhibition of yeast α-glucosidase. However, **5** exhibited strong inhibition of yeast α-glucosidase and weak inhibition of amyloglucosidase. The IC_{50} of **5** in yeast α-glucosidase was only about 9 mg/mL at pH 5.0.

Table I. Inhibitions of glycosidase activity by compound **5** and **6**. Concentration of inhibitor required to show enzyme-catalyzed hydrolysis of corresponding nitrophenylglycopyranoside by 50% under standard conditions

Enzyme	Inhibitor			
	Castanospermine	Swainsonine	**5**	**6**
α-Glucosidase (yeast)	NI*	NI	9 μg/mL	NI
α-Mannosidase (jack bean)	NI	200 ng/mL	NI	8% inhibition at 200 μg/mL
Amyloglucosidase (fungal)	1-2 μg/mL	NI	80 μg/mL	50 μg/mL
β-Glucosidase (almonds)	5-20 μg/mL	NI	260 μg/mL	170 μg/mL
α-Galactosidase (green coffee bean)	NI	NI	200 μg/mL	10% inhibition at 200 μg/mL
β-Galactosidase (bovine liver)	NI	NI	8% inhibition at 200 μg/mL	9% inhibition at 200 μg/mL

*NI: no inhibiton

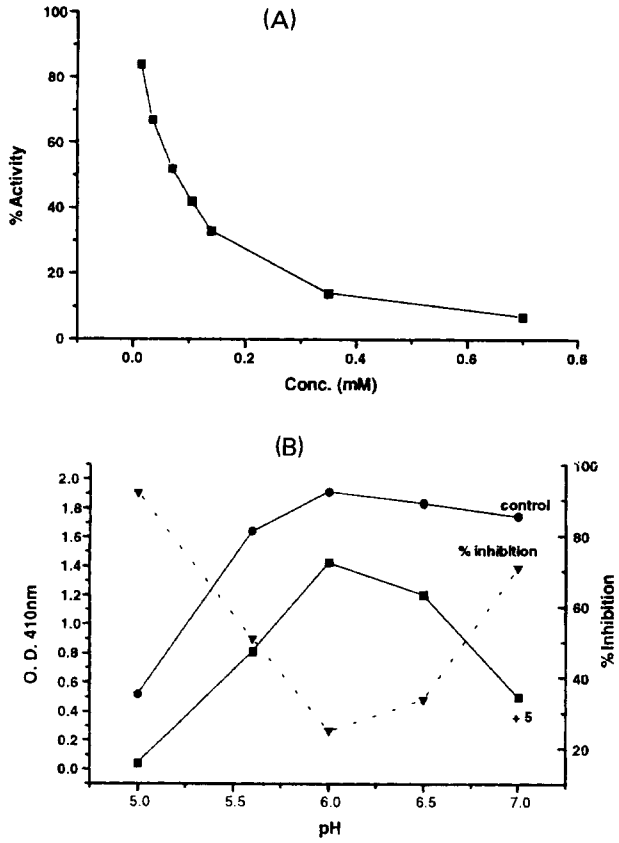


Fig. 2. (A) Effect of compound 5 concentration on yeast a-glucosidase activity at pH 5.0. Incubations were described in the experimental section. (B) pH Curve of yeast α-glucosidase activity in the absence of (●) and presence of (■) 0.7 mM (50 mg/mL) of compound 5.

Fig. 2 (A) shows the amount of inhibition of yeast α-glucosidase versus the concentration of **5** at pH 5.0. The pH range for inhibition by **5** of yeast α-glucosidase was studied at various pH's. The pH profile of α-glucosidase inhibition showed that the inhibition by **5** was pH dependent, with maximum at pH 5.0 and almost no inhibition was observed at pH 6.0. (Fig. 2 (B))

It seems that the protonated form of **5** gives better interaction with the carboxyl group of the enzyme and resulted in higher inhibition. Because of the fact that **5** shows high inhibition against this enzyme but the other isomer (1-epimer) gives almost no inhibition, the stereochemistry of hydroxyl group at C1-position can be considered to be important for activity. The reason of drastic change in activity of **5** from pH 5 to pH 6 remains to be understood. For the other enzymes, these two isomers did not show significant difference in activity.

Recently, many glycosidase inhibitors have been reported but most of them are three or more hydroxylated indolizidines and pyrrolizidines. Lentiginosine was the only dihydroxyindolizidine potent as a glycosidase inhibitor (amyloglucosidase inhibitor).

Here we report the first pyrrolizidine glycosidase inhibitor, compound **5** which has only two hydroxyl groups.

ACKNOWLEDGEMENTS

The authors wish to thank Il Dong Pharm. Co. for its donation of chair Fund to KIST.

REFERENCES CITED

Dorling, P. R., Huxtable, C. R. and Colgate, S. M., Inhibition of lysosomal α-mannosidase by swainsonine, an indolizidine alkaloids isolated from *Swanisona canescens. Biochem. J.*, 191, 649 (1980).

Elbein, A. D., Inhibitors of the biosynthesis and processing of N-linked oligosaccharide chains. *Annu. Rev. Biochem.*, 56, 497 (1987).

Elbein, A. D. and Molyneux, R. J., in *Alkaloids; Chemical, Biological Perspectives*, Vol. 5; S. W. Pelletier, Eds.; Wiley-Inter Science; New York, 1987, p 1-54.

Kang, M. S. and Elbein A. D., Mechanism of inhibition of Jack bean α-mannosidase by swainsonine. *Plant Physiol.*, 71, 551 (1983).

Kuroki, R., Weaver, B. W. and Matthews, B. W., A Covalent enzyme-substrate intermediate with saccharide distortion in a mutant T4 lysozyme. *Science*, 262, 2030 (1993).

Michael, J. P., Indolizidine and qinolizidine alkaloids. *Nat. Product Rep.*, 535 (1995)

Molyneux, R. J., Roitman, J. N., Dunnhein, G., Szumilo, T. and Elbein A. D., 6-Epicastanospermine, a novel indolizidine alkaloid that inhibits α-glucosidase. *Arch. Biochem. Biophy.* 251, 450 (1986).

Molyneux, R. J., Benson, M., Wong, R. Y., Tropea, J. E. and Elbein, A. D., Australine, a, novel pyrrolizidine alkaloid glucosidase inhibotor from *Castanospermum australe. J. Nat. Prod.*, 51, 1198 (1988).

Pan, Y. T., Hori, H., Saul, R., Sanford, B. A., Molyneux, R. J. and Elbein, A.D., Castanospermine inhibits the processing of the oligosaccharide portion of the influence viral hemmaglutinin. *Biochemistry*, 22, 3975 (1983).

Pastuszak, I., Molyneux, R. J., James, L. F. and Elbein, A. D., *Biochemistry*, lentiginosine, a dihydroxyindolizidine alkaloid that inhibits amyloglucosidase. 29, 1886 (1990).

Robins, D. J., Pyrrolizidine alkaloids. *Nat. Product Rep.*, 413 (1995).

Sinnott, M. L., Catalytic mechanisms of enzymic glycosyl transfer. *Chem. Rev.*, 90, 1171 (1990).

Tropea, J. E., Molyneux, R. J., Kaushal, G. P., Pan, Y. T., Mitchell, M. and Elbein, A. D., Australine, a pyrrolizidine alkaloid that inhibits amyloglucosidase and glycoprotein processing. *Biochemistry*, 28, 2027 (1989).