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# Australine and related alkaloids: easy structural confirmation by $^{13}C$ NMR spectral data and biological activities

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**Abstract**—The first polyhydroxylated pyrrolizidine alkaloid with a hydroxymethyl group at C-3 was isolated from pods of *Alexa leiopetala* (Leguminosae) and designated alexine **1**. The Australian legume *Castanospermum australe* is also known to produce the same structural type of pyrrolizidines. There are reports of the isolation of australine (7a-*epi*-alexine) **2**, 1-*epi*-australine **3**, 3-*epi*-australine **4**, and 7-*epi*-australine **5** from this plant to date. Their unambiguous syntheses established that the natural product reported as **5** is **2** and the published NMR data for **2** are those of **3**. These confusions prompted us to unambiguously confirm the structures and biological activities of australine isomers and related alkaloids. A careful search for polyhydroxylated pyrrolizidines in seeds of *C. australe* led to the discovery of three new alkaloids, 2,3-di*epi*-australine **6**, 2,3,7-tri*epi*-australine **7**, and the 2-*O*-β-D-glucopyranoside of **3** (**8**). Herein, we report the full $^{13}C$ NMR assignment of alkaloids **1**–**8** and the glycosidase inhibitory activities of alkaloids **1**–**8** together with the highly oxygenated pyrrolizidine, casuarine **9**, and its 6-*O*-α-D-glucopyranoside **10**. 

## 1. Introduction

In 1988, alexine **1**, a polyhydroxylated pyrrolizidine alkaloid, was isolated from pods of the legume *Alexa leiopetala*.[1] Although the broad class of pyrrolizidine alkaloids bear a carbon substituent at C-1,[2,3] alexine is the first example of a pyrrolizidine alkaloid with a carbon substituent at C-3. At about the same time, australine **2** was isolated from seeds of the Australian legume *Castanospermum australe* and found to be 7a-*epi*-alexine from X-ray crystallographic analysis.[4] The isolation of 1-*epi*-australine **3**,[5,6] 3-*epi*-australine **4**,[7] and 7-*epi*-australine **5**[6] from the same plant was later reported. The structure of **3** was firmly established by X-ray crystallographic analysis of the corresponding 1,7-isopropylidene derivative,[6] and the absolute configurations of **4** were also identified by X-ray crystal structure analysis.[6] Alkaloid **5** was tentatively assigned as 7-*epi*-australine based on the difference between its NMR parameters and those reported for **2**.[7] Recently, Denmark et al. unambiguously synthesized **2**,[8] **3**,[9] **4**,[8] and **5**.[10,11] Surprisingly, the $^1H$ NMR spectral data of the synthetic material of **5** did not match those reported for the natural product. Extensive NMR studies on the natural and synthetic isomers of **2** by Wormald et al.[12] and Denmark et al.[11] and personal communication with the authors of the original papers[4,6] established that the published NMR data for **2** are those of **3** and the natural product reported as **5** is really **2**. This means that 7-*epi*-austaline **5** has not yet been found as a natural product and the reported biological data[6,13] for **5** are erroneous. Hence, it is very important to reinvestigate the biological activities of the natural and synthetic isomers of **2** and the natural occurrence of **5** in *C. australe*. Our careful search for polyhydroxylated pyrrolizidines in seeds of this plant led to the discovery of three new alkaloids, 2,3-di*epi*-australine **6**, 2,3,7-

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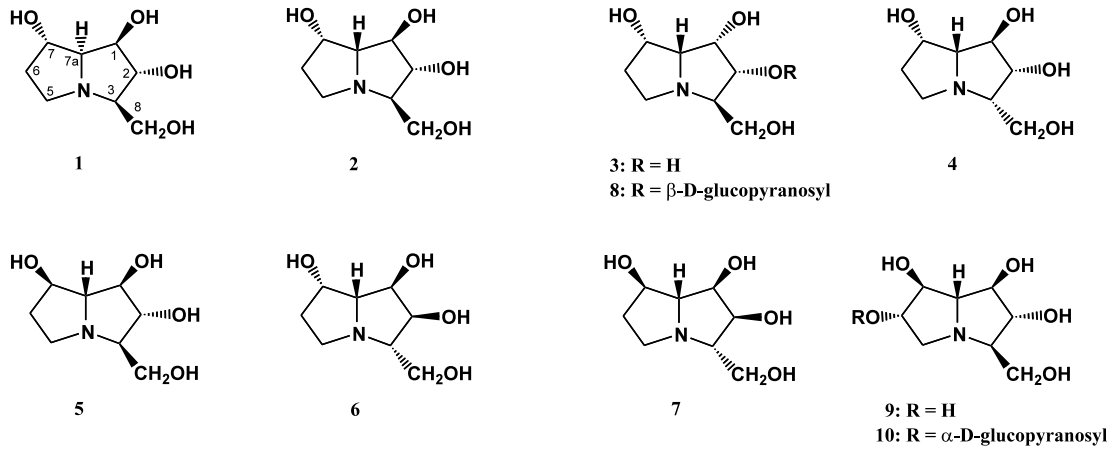


**Figure 1.** Structures of polyhydroxylated pyrrolizidines **1**–**10**.

tri*epi*-australine **7**, and 1-*epi*-australine-2-*O*-β-D-glucopyranoside **8**. We now report the full $^{13}C$ NMR assignment of alkaloids **1**–**8** and glycosidase inhibitory activities of **1**–**8** and highly oxygenated pyrrolizidines, casuarine **9**,[14] and its 6-*O*-α-D-glucopyranoside **10** (Fig. 1).[15]

## 2. Results and discussion

A 50% MeOH extract of the seeds (850 g) of *C. australe* was chromatographed with various ion-exchange resins to give **2** (1.43 g), **3** (2.54 g), **4** (317 mg), **6** (39 mg), **7** (27 mg), and **8** (17 mg), together with castanospermine (9.1 g), 6-*epi*-castanospermine (330 mg), 8-*O*-β-D-glucopyranosyl-castanospermine (196 mg), fagomine (350 mg), 3-*epi*-fagomine (8.5 mg), and (2*R*,3*S*)-3-hydroxy-2-hydroxymethylpyrrolidine (CYB-3) (1.3 g). Although 8-*O*-β-D-glucopyranosyl-castanospermine has been chemically synthesized,[16] this is the first report of its natural occurrence. Alkaloids **6**, **7**, and **8** are new compounds obtained in this work (Fig. 2).

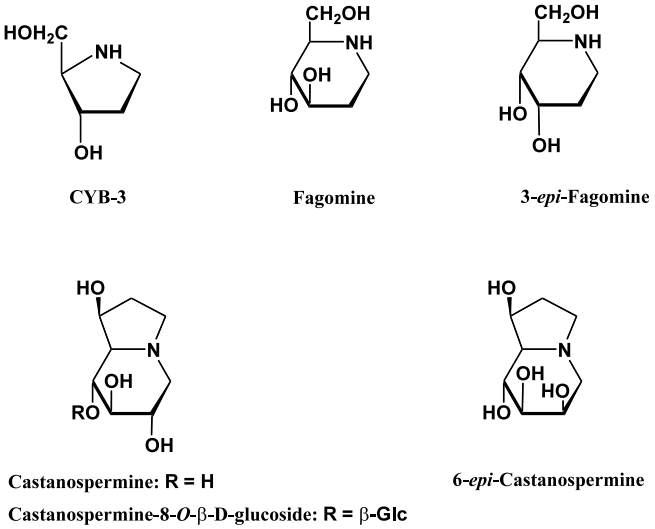


**Figure 2.** Structures of alkaloids other than pyrrolizidines isolated from *Castanospermum australe*.

Alkaloid **6** was determined to have the molecular formula $C_8H_{15}NO_4$ by HRFABMS. The $^{13}C$ NMR spectroscopic data of **6** were closely related to those of 3-*epi*-australine **4**, as seen in Table 1. The complete connectivity of the carbon and hydrogen atoms was defined from $^1H$–$^1H$ COSY, $^1H$–$^{13}C$ COSY, and HMBC spectroscopic data. The NOE interactions are shown in Fig. 3. The definite NOE between H-7a and H-3 indicates that H-7a and H-3 are on the same side of the ring. Irradiation of H-5α enhanced the NOE signal intensity of H-2 and the C-8 ($CH_2OH$) protons. The couplings of 4.6 Hz for H-7a to H-7 and 5.1 Hz for H-1 to H-2 indicate that these proton pairs are in *cis* arrangements. These results show that H-3, H-7, and H-7a are on the same side of the ring, and H-1 and H-2 are on the opposite site. Thus, alkaloid **6** was determined to be 2,3-di*epi*-australine.

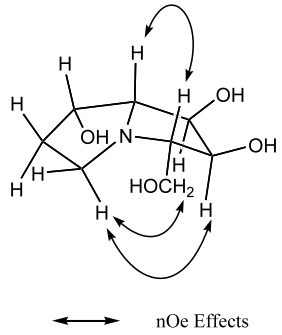


**Figure 3.** NOE interactions for 2,3-di*epi*-australine, **6**.

Alkaloid **7** was determined to have the molecular formula $C_8H_{15}NO_4$ by HRFABMS. The $^{13}C$ NMR spectroscopic features were similar to those of **6** (Table 1). The $^1H$–$^1H$ COSY, $^1H$–$^{13}C$ COSY, and HMBC spectroscopic data indicated that alkaloid **7** was an isomer of **6**. The relative configurations at the stereogenic centers in **7** were determined from the NOE effects (Fig. 4) and $^3J_{H,H}$ coupling constants. Irradiation of H-1 enhanced the NOE signal intensity of H-7, and irradiation of H-5α enhanced the NOE signals of H-2 and the C-8

**Table 1.** $^{13}C$ NMR chemical shifts[a] for pyrrolizidines **1**–**7** and **9**

| Carbon | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 9 |
|---|---|---|---|---|---|---|---|---|
| 1 | 76.0 | 75.9 | 75.1 | 77.0 | 77.5 | 72.6 | 76.4 | 81.0 |
| 2 | 77.4 | 73.5 | 77.5 | 81.6 | 75.9 | 75.1 | 73.8 | 79.9 |
| 3 | 68.8 | 73.3 | 73.0 | 66.3 | 67.9 | 66.2 | 67.4 | 73.2 |
| 5 | 50.2 | 54.6 | 55.0 | 47.7 | 51.4 | 47.3 | 47.7 | 61.3 |
| 6 | 36.3 | 38.0 | 38.2 | 37.8 | 33.1 | 37.3 | 36.3 | 80.7 |
| 7 | 71.9 | 81.8 | 76.0 | 72.6 | 74.8 | 72.2 | 77.0 | 82.0 |
| 7a | 75.4 | 72.3 | 69.1 | 77.6 | 73.5 | 76.9 | 78.7 | 75.4 |
| 8 | 59.9 | 65.5 | 65.8 | 60.0 | 62.3 | 62.1 | 61.8 | 65.6 |

[a] Chemical shifts are expressed in ppm downfield from sodium 3-(trimethylsilyl)propionate (TSP) as an internal standard in $D_2O$.

($CH_2OH$) protons. Furthermore, the NOE between H-7a and H-6β was also observed. The coupling constant of 8.0 Hz indicates a *trans* arrangement for H-6β and H-7. These results indicate that H-1, H-2, and H-7 are on the same side of the ring, and H-3 and H-7a are on the opposite site. Hence, alkaloid **7** was determined to be 2,3,7-tri*epi*-australine.

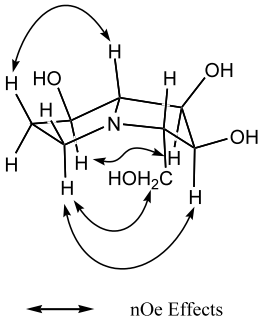


**Figure 4.** NOE interactions for 2,3,7-tri*epi*-australine, **7**.

Alkaloid **8** was determined to have the molecular formula $C_{14}H_{25}NO_9$ by HRFABMS. The response to the naphthoresorcinol–sulfuric acid reagent and the characteristic carbon (C-1′, $\delta$ 106.4) signal in the $^{13}C$ NMR suggested that **8** was a glycoside of an alkaloid. A small amount of this glycoside was subjected to acid hydrolysis (100°C, 8 h) using Dowex 50W-X2 ($H^+$ form) resin. After washing the resin with water, the aglycone part was displaced from the resin with 0.5 M $NH_4OH$, concentrated to dryness, and confirmed as 1-*epi*-australine **3** from the $^{13}C$ NMR spectroscopic data. The COSY and HMBC spectra of **8** elucidated that the carbon signals of $\delta$ 63.4, 72.3, 75.9, 78.5, 78.7 and 106.4 are derived from the sugar part, and these carbon chemical shifts were in good accord with those of β-D-glucopyranoside. The HMBC spectrum showed a correlation peak between the anomeric proton and the aglycone C-2 carbon, defining the linkage site, which was also supported by the 9.0 ppm downfield shift for C-2 and the 1.8 upfield shift for C-3. Thus, the structure of **8** was determined to be 1-*epi*-australine-2-*O*-β-D-glucopyranoside.

As described above, we isolated two new isomers of australine **2** and a new glucoside of 1-*epi*-australine **3**, together with known pyrrolizidine alkaloids **2**, **3**, and **4** from seeds of *C. australe*. However, despite our careful search, we were not able to find out 7-*epi*-australine **5** in the seeds. Casuarine **9**, which is α-6-hydroxy-7-*epi*-australine, has been isolated from *Casuarina equisetifolia* (Casuarinaceae)[14] and also unambiguously synthesized.[17,18] We re-examined the $^{13}C$ NMR spectral data in $D_2O$ of the natural products **1**, **2**, **3**, **4**, **6**, and **7** and the synthetic samples **5** and **9**, based on their COSY spectroscopic data. Their full $^{13}C$ NMR assignments are summarized in Table 1.

Australine **2** can be regarded as a derivative of (2*R*,5*R*)-bis(hydroxymethyl) - (3*R*,4*R*) - dihydroxypyrrolidine (DMDP) with an ethylene bridge between the hydroxymethyl group and the ring nitrogen. DMDP is a potent inhibitor of yeast α-glucosidase,[19] mammalian digestive β-glucosidase and β-galactosidase, with $IC_{50}$ values in the micromolar range.[20] On the other hand, australine is a good inhibitor of amyloglucosidase ($IC_{50}=5.8$ μM), but it does not inhibit β-glucosidase and β-galactosidase.[21] Australine also inhibited the glycoprotein processing glucosidase I (50% inhibition at approximately 20 μM), but had only slight activity toward processing glucosidase II.[21] Australine is the first example of a polyhydroxylated pyrrolizidine inhibiting processing glucosidase I. Alexine **1** is a poor inhibitor of mammalian digestive β-glucosidase (less than 50% inhibition at 330 μM) and β-galactosidase ($IC_{50}=150$ μM) in comparison to DMDP.[1] 1-*epi*-Australine **3** and 3-*epi*-australine **4** have been reported to be potent inhibitors of amyloglucosidases.[6] The glucosidase I inhibitory activity and antiviral activity[13] reported for 7-*epi*-australine **5** are erroneous since this compound had not been present at the time as a natural or a synthetic compound. Casuarine **9** is a potent inhibitor of processing glucosidase I (72% inhibition at 5 μg/mL) but has no effect on glycoprotein processing in culture cells.[17] With respect to casurine-6-*O*-α-D-glucoside **10**, its biological activity has not yet been reported.

We extensively investigated the inhibitory activity of polyhydroxylated pyrrolizidines **1**–**10** toward various glycosidases. The results are shown in Table 2. Although alexine **1** had no significant inhibitory activity toward mammalian digestive α-glucosidases, it was a moderate inhibitor of porcine trehalase. Australine **2** showed a moderate inhibitory activity toward rice α-glucosidase, rat intestinal maltase, and fungal amyloglucosidase, but it potently inhibited rat intestinal

**Table 2.** Concentration of pyrrolizidine alkaloids giving 50% inhibition of glycosidase activities

| Enzyme | $IC_{50}$ (μM) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** |
| α-Glucosidase | | | | | | | | | | |
| Rice | 250 | 21 | 280 | – | 350 | – | 420 | 1.8 | 1.2 | 440 |
| Yeast | –[a] | – | – | – | 95 | – | – | 1.6 | 570 | – |
| Rat intestinal maltase | 540 | 24 | – | – | 310 | – | 130 | 41 | 0.7 | 260 |
| Rat intestinal isomaltase | – | 97 | – | – | 350 | – | 69 | 2.9 | 3.9 | – |
| Rat intestinal sucrase | – | 4.6 | 470 | | 100 | | 35 | | 305 | – |
| β-Glucosidase | | | | | | | | | | |
| Almond | – | – | – | – | – | – | – | – | – | 7.0 |
| Trehalase | | | | | | | | | | |
| Porcine kidney | 55 | 160 | – | – | 310 | – | 310 | 450 | 12 | 0.34 |
| α-L-Fucosidase | | | | | | | | | | |
| Human placenta | – | – | – | 240 | – | 0.67 | 38 | – | – | – |
| Amyloglucosidase | | | | | | | | | | |
| *Aspergillus niger* | – | 28 | 300 | 420 | 92 | – | – | – | 0.7 | 1.1 |

[a] No inhibition (less than 50% inhibition at 1000 μM).

sucrase ($IC_{50}=4.6$ μM). Although 1-*epi*-australine **3** and 3-*epi*-australine **4** have been reported as potent inhibitors of fungal amyloglucosidase when potato amylose was used as a substrate,[5] in our present study they were found to be very weak inhibitors of the same enzyme when maltose was used as substrate. The epimerization at C-7 in **2** to give **5** lowered its inhibition toward rice and mammalian α-glucosidases and amyloglucosidase. However, the introduction of the OH group to C-6α in **5** to give casuarine **9** significantly enhanced its inhibition toward rice α-glucosidase ($IC_{50}=1.2$ μM), rat intestinal maltase ($IC_{50}=0.7$ μM), rat intestinal isomaltase ($IC_{50}=3.9$ μM), and amyloglucosidase ($IC_{50}=0.7$ μM). The mode of inhibition of amyloglucosidase by **9** was a competitive manner with a $K_i$ value of 0.44 μM. Interestingly, 2,3-di*epi*-australine **6** was proven to be a very specific inhibitor ($IC_{50}=0.67$ μM) of human α-L-fucosidase without inhibition toward other glycosidases tested. Although epimerization at C-7 of **6** to give **7** resulted in weak inhibition of α-glucosidases, it markedly lowered its inhibition toward α-L-fucosidase. Significantly, the introduction of the β-D-glucosyl group to C-2 in **3** generated strong inhibition toward rice α-glucosidase ($IC_{50}=1.8$ μM; $K_i=0.22$ μM, in a competitive manner), rat intestinal maltase ($IC_{50}=1.8$ μM), and rat intestinal sucrase ($IC_{50}=2.9$ μM) although the aglycone **3** has no appreciable inhibitory activity toward such α-glucosidases. On the other hand, the 6-*O*-α-D-glucoside of **9** retained the potency toward amyloglucosidase and further generated inhibitory activity toward β-glucosidase, but markedly lowered or abolished the inhibition toward α-glucosidases. The most noteworthy activity of **10** is the inhibition of porcine kidney trehalase. All powerful trehalase inhibitors reported to date are a pseudodisaccharide type of inhibitors (Fig. 5), such as validoxylamine A,[22] MDL 25637 (7-*O*-β-D-glucopyranosyl-α-homonojirimycin),[23] trehazolin,[24] and salbostatin.[25] These compounds are powerful competitive inhibitors of porcine kidney trehalase.[25–27] We have previously proven the presence of two subsites, one for catalysis and one for recognition, on the active center of porcine kidney trehalase.[28] The extremely high affinity of a pseudodisaccharide inhibitors derives from the synergistic interactions of an alkaloid unit and a sugar (or cyclitol) unit with two subsites.[28] It was predicted that casurine-6-*O*-α-D-glucoside **10** may have the ability to inhibit trehalase since it can be regarded as a pseudodisaccharide comprised of an α-glucosidase-inhibiting alkaloid unit and a sugar unit, similar to MDL 25637. This glucoside in fact was a very potent inhibitor of porcine kidney trehalase with an $IC_{50}$ value of 0.34 μM and inhibited the enzyme in a competitive manner, with a $K_i$ value of 0.018 μM. The structural basis for the inhibition of trehalase by the glucoside **10** has thus become further obvious.

In conclusion, this study made it possible to easily identify naturally occurring australine isomers from *C. australe* and other plants, and secured the biological properties of natural and synthetic australine isomers and related alkaloids which will provide useful information for the design of new pyrrolizidine alkaloids.

## 3. Experimental

### 3.1. General

The purity of samples was checked by HPTLC on silica gel $60F_{254}$ (E. Merck) using the solvent system PrOH–AcOH–$H_2O$ (4:1:1), and a chlorine-*o*-tolidine reagent or iodine vapor was used for detection. Optical rotations were measured with a Jasco DIP-370 digital polarimeter. $^1H$ NMR (400 MHz) and $^{13}C$ NMR (100 MHz) spectra were recorded on a Jeol JNM-GX 400 spectrometer. Chemical shifts are expressed in ppm downfield from sodium 3-(trimethylsilyl)propionate (TSP) in $D_2O$ and $Me_4Si$ (TMS) in $CD_3OD$ as internal standards. MS were measured on a Jeol JMS-SX 102A spectrometer.

### 3.2. Preparation of pyrrolizidine alkaloids

7-*epi*-Australine **5** and casuarine **9** were synthesized according to the literature.[10,18] Alexine **1** and casuarine-6-*O*-α-D-glucopyranoside **10** were isolated from leaves of *Alexa* spp.[1] and leaves of the Indian medicinal plant *Eugenia jambolana*,[15] respectively, according to the literature. Other pyrrolizidine alkaloids were isolated from seeds of *C. australe* as described below.

### 3.3. Extraction and isolation

A 50% MeOH extract of seeds (850 g) of *C. australe* was applied to a column of Amberlite IR-120B (500 mL, $H^+$ form). The 0.5 M $NH_4OH$ eluate was concentrated to give a brown oil (35.6 g), which was chromatographed over a Dowex 1-X2 column (3.8×90 cm, $OH^-$ form) with $H_2O$ as eluant (fraction size 15 mL). The $H_2O$ eluate was divided into two pools: A (fractions 16–50, 20.6 g) and B (fractions 51–86, 3.78 g). Pool A was chromatographed on a Amberlite CG-50 column (3.8×90 cm, $NH_4^+$ form) with $H_2O$ as eluant (fraction size 15 mL) and the $H_2O$ eluate was divided into two parts, A-1 (fractions 12–30, 12 g) and A-2 (fractions 34–90). The 0.5 M $NH_4OH$ eluate from the same column was concentrated to give a colorless oil (2.7 g) and designated A-3. Concentration of A-2 gave fagomine (350 mg). A further chromatography of A-1 with a Dowex 1-X2 column (3.8×90 cm, $OH^-$ form) ($H_2O$ elution) gave 6-*epi*-castanospermine (330 mg), castanospermine (9.1 g), and australine **2** (1.43 g), in order of elution. The A-3 part was chromatographed over a CM-Sephadex C-25 column (1.9×92 cm, $NH_4^+$ form) to give 3-*epi*-fagomine (8.5 mg) by the water elution, 2,3-di*epi*-australine **6** (39 mg), 2,3,7-tri*epi*-australine **7** (27 mg), and 3-*epi*-australine **4** (317 mg) in order of elution by the 0.01 M $NH_4OH$ elution, and 2*R*-hydroxymethyl-3*S*-hydroxypyrrolidine (CYB-3) (1.3 g) by the 0.03 M $NH_4OH$ elution. Repeated chromatography of pool B with a Dowex 1-X2 column (3.8×90 cm, $OH^-$ form) using $H_2O$ as eluant gave castanospermine-8-*O*-β-D-glucopyranoside (196 mg), 1-*epi*-australine-2-*O*-β-D-glucopyranoside **8** (17 mg), and 1-*epi*-australine **3** (2.54 g), in order of elution.

### 3.4. Glycosidase inhibitory activities

The enzymes α-glucosidase (from rice and yeast), β-glucosidase (from almond), trehalase (from porcine kidney), α-L-fucosidase (from human placenta), amyloglucosidase (from *Aspergillus niger*), *p*-nitrophenyl glycosides and disaccharides were purchased from Sigma Chemical Co. Brush border membranes, prepared from the intestine of male Wister rats by the method of Kessler et al.,[29] were used as the source of rat intestinal glycosidases.

The activities of rice α-glucosidase, rat intestinal glycosidases, trehalase and amyloglucosidase were determined using the appropriate disaccharides as substrates at the optimum pH of each enzyme. The released D-glucose was determined colorimetrically using Glucose B-test Wako (Wako Pure Chemical Ind.). Other glycosidase activities were determined using an appropriate *p*-nitrophenyl glycoside as substrate at the optimum pH of each enzyme. The reaction was stopped by adding 400 mM $Na_2CO_3$. The released *p*-nitrophenol was measured spectrometrically at 400 nm.

### 3.5. 2,3-Di*epi*-australine, 6

$[\alpha]_D$ +38.2 (*c* 0.51, $H_2O$); HRFABMS: *m*/*z* 190.1080 $[M+H]^+$ ($C_8H_{16}NO_4$ requires 190.1079); $\delta$ (400 MHz; $CD_3OD$) 1.84 (2H, m, H-6α, H-6β), 2.90 (1H, m,

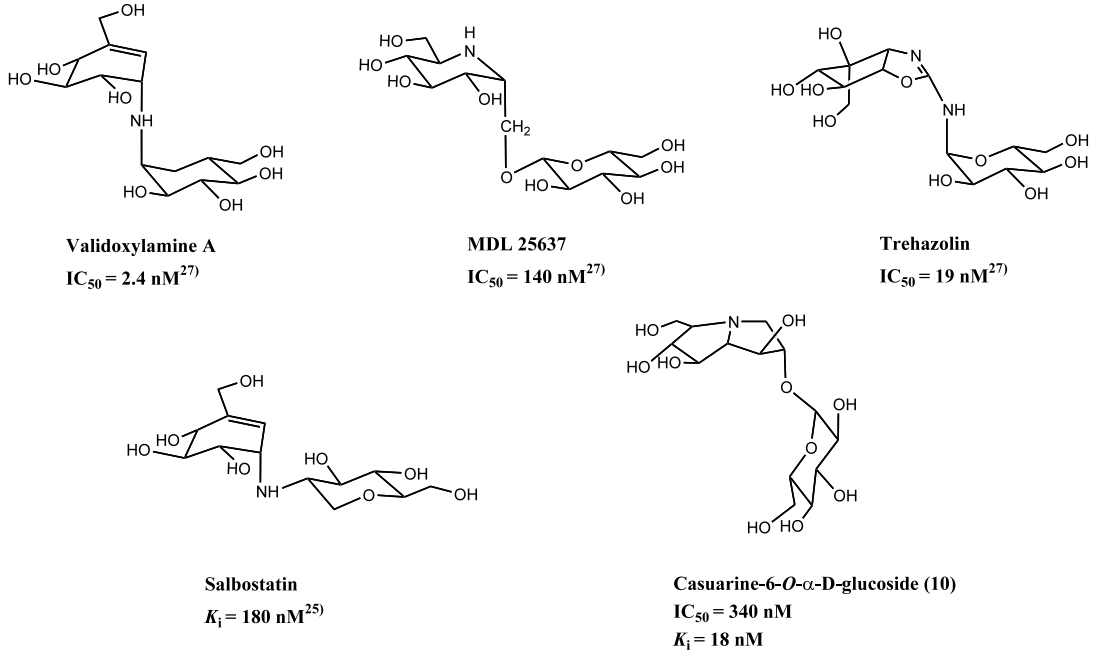


**Figure 5.** Structures of a disaccharide type of trehalase inhibitors and inhibitory activity toward porcine kidney trehalase.

H-5β), 3.03 (1H, m, H-5α), 3.22 (1H, ddd, $J=9.0$, 6.1, 4.2 Hz, H-3), 3.42 (1H, dd, $J=4.6$, 2.2 Hz, H-7a), 3.81 (1H, dd, $J=12.0$, 6.1 Hz, H-8), 3.82 (1H, dd, $J=9.0$, 5.1 Hz, H-2), 3.87 (1H, dd, $J=12.0$, 4.2 Hz, H-8′), 4.19 (1H, dd, $J=5.1$, 2.2 Hz, H-1), 4.29 (1H, m, H-7); $^{13}$C NMR data, see Table 1.

### 3.6. 2,3,7-Tri*epi*-australine, 7

$[\alpha]_D$ +59.7 (*c* 0.58, $H_2O$); HRFABMS: *m*/*z* 190.1080 $[M+H]^+$ ($C_8H_{16}NO_4$ requires 190.1079); $\delta$ (400 MHz; $D_2O$) 1.76 (1H, m, H-6β), 2.14 (1H, m, H-6α), 2.90 (1H, ddd, $J=10.6$, 10.3, 5.8 Hz, H-5α), 3.01 (1H, ddd, $J=10.3$, 6.8, 3.4 Hz, H-5β), 3.19 (1H, dd, $J=5.9$, 2.5 Hz, H-7a), 3.21 (1H, ddd, $J=9.3$, 6.0, 6.0 Hz, H-3), 3.87 (2H, d, H-8, H-8′), 3.95 (1H, dd, $J=9.3$, 5.3 Hz, H-2), 4.06 (1H, dd, $J=5.3$, 2.5 Hz, H-1), 4.14 (1H, ddd, $J=8.0$, 5.9, 5.9 Hz, H-7); $^{13}$C NMR data, see Table 1.

### 3.7. 1-*epi*-Australine-2-*O*-β-D-glucopyranoside, 8

$[\alpha]_D$ +35.8 (*c* 0.42, $H_2O$); HRFABMS: *m*/*z* 352.1602 $[M+H]^+$ ($C_{14}H_{26}NO_9$ requires 352.1608); $\delta$ (400 MHz; $D_2O$) 2.03–2.18 (2H, m, H-6αH-6β), 3.01 (1H, m, H-5β), 3.25 (2H, H-3, H-5α), 3.43 (1H, dd, $J=9.3$, 7.8 Hz, H-2′), 3.50–3.61 (4H, H-7a, H-3′, H-4′, H-5′), 3.77 (1H, dd, $J=11.7$, 6.4 Hz, H-8), 3.85 (1H, dd, $J=12.2$, 4.6 Hz, H-6′), 3.94 (1H, dd, $J=11.7$, 3.4 Hz, H-8′), 3.99 (1H, dd, $J=12.2$, 2.0 Hz, H-6′), 4.10 (1H, dd, $J=9.8$, 4.2 Hz, H-2), 4.62 (1H, d, $J=7.8$ Hz, H-1′), 4.63 (1H, dd, $J=4.7$, 4.2 Hz, H-1), 4.68 (1H, m, H-7); $\delta$ (100 MHz; $D_2O$) 38.0 (C-6), 55.0 (C-5), 63.4 (C-6′), 65.2 (C-8), 68.6 (C-7a), 71.2 (C-3), 72.3 (C-4′), 75.2 (C-1), 75.9 (C-2′), 76.3 (C-7), 78.5 (C-3′), 78.7 (C-5′), 86.5 (C-2), 106.4 (C-1′).

### 3.8. Castanospermine-8-*O*-β-D-glucopyranoside

$[\alpha]_D$ +44.1 (*c* 0.65, $H_2O$); HRFABMS: *m*/*z* 352.1604 $[M+H]^+$ ($C_{14}H_{26}NO_9$ requires 352.1608); $\delta$ (400 MHz; $D_2O$) 1.74 (1H, m, H-2α or H-2β), 2.08 (1H, dd, $J=11.0$, 10.6 Hz, H-5ax), 2.18 (1H, dd, $J=10.0$, 4.4 Hz, H-8a), 2.25 (1H, m, H-3α or H-3β), 2.35 (1H, m, H-2β or H-2α), 3.11 (1H, m, H-3), 3.19 (1H, dd, $J=11.0$, 5.1 Hz, H-5eq), 3.32 (1H, dd, $J=9.0$, 7.8 Hz, H-2′), 3.42 (1H, dd, $J=9.5$, 9.0 Hz, H-4′), 3.47 (1H, dd, $J=9.0$, 9.0 Hz, H-7), 3.50 (1H, ddd, $J=9.5$, 5.7, 2.0 Hz, H-5′), 3.52 (1H, dd, $J=9.0$, 9.0 Hz, H-3′), 3.66 (1H, ddd, $J=10.5$, 9.3, 5.1 Hz, H-6), 3.74 (1H, dd, $J=12.4$, 5.7 Hz, H-6′a), 3.80 (1H, dd, $J=9.8$, 9.0 Hz, H-8), 3.93 (1H, dd, $J=12.4$, 2.0 Hz, H-6′b), 4.48 (1H, ddd, $J=6.8$, 4.4, 2.2 Hz, H-1), 4.77 (1H, d, $J=7.8$ Hz, H-1′); $\delta$ (100 MHz; $D_2O$) 35.6 (C-2), 54.3 (C-3), 57.9 (C-5), 63.5 (C-6′), 72.3 (C-1), 72.4 (C-4′), 72.8 (C-6), 73.1 (C-8a), 76.2 (C-2′), 78.5 (C-3′), 78.9 (C-5′), 80.2 (C-7), 81.7 (C-8), 105.3 (C-1′).

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