Tetrahedron 66 (2010) 9340–9347

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Synthesis of polyhydroxylated pyrrolizidine and indolizidine compounds and their glycosidase inhibitory activities

Thunwadee Ritthiwigrom [a], Robert J. Nash [b], Stephen G. Pyne [a,*]

[a] School of Chemistry, University of Wollongong, Wollongong, New South Wales 2522, Australia
[b] Phytoquest Limited, IBERS, Plas Gogerddan, Aberystwyth, Ceredigion, SY23 3EB, UK

ARTICLE INFO

Article history:
Received 17 July 2010
Received in revised form 17 September 2010
Accepted 4 October 2010

ABSTRACT

The synthesis of the natural product 3-*epi*-casuarine and two tricyclic ether bridged analogues, plus 7-deoxy-3,6-di*epi*-casuarine, 7-*epi*-australine, 1-*epi*-castanospermine and 1,6-di*epi*-castanospermine is described. The glycosidase inhibitory activities of these compounds, along with that of uniflorine A and other polyhydroxylated pyrrolizidines and indolizidines that we have published before, are reported.

1. Introduction

The polyhydroxylated 3-hydroxymethylpyrrolizidine group of natural products includes, uniflorine A **1**,[1,2,3] casuarine **2**,[4] australine **3**,[5] 3-*epi*-australine **4**[6,7] (Fig. 1), alexine[8] (7a-*epi*-australine), several other *epi*-australines (1-*epi*-australine, 3-*epi*-australine, 2,3-di*epi*-australine, 2,3,7-tri-*epi*-australine),[9] 1-*epi*-australine-2-*O*-β-glucoside, 3-*epi*-casuarine,[10] and casuarine-6-*O*-α-glucoside.[11] The hyacinthacine alkaloids are a more recent addition to this group of which nineteen novel compounds have been identified.[12] This group, along with the polyhydroxylated pyrrolidine, piperidine, indolizidine and nortropane alkaloids, have glycosidase inhibitory activities and thus have potential utility as antiviral, anticancer, antidiabetic and antiobesity drugs.[7] Three structurally related synthetic compounds have been marketed as antidiabetic drugs to treat type-2 diabetes based on their potent α-glucosidase inhibitory activities while others have been identified as candidates for therapeutics to treat type-1 Gaucher disease.[7] These potentially useful biological activities along with the stereochemical richness of these alkaloids have made these compounds attractive and important synthetic targets.[13]

We recently reported the synthesis of uniflorine A **1** from the chiral 2-substituted-2,5-dihydropyrrole **5** (Scheme 1), which is readily prepared in four synthetic steps from L-xylose.[13] We also demonstrated that compound **5** is a versatile precursor for the diastereoselective synthesis of the alkaloids casuarine **2**, australine **3** and 3-*epi*-australine **4** and the unnatural epimer, 3,7-*epi*-australine.[13]

In this paper we describe our attempts to prepare the natural product 3-*epi*-casuarine **7**[10] from the epoxide **6** that we have synthesized previously in seven steps from **5**.[13] This synthesis also resulted in the formation of two tricyclic bridged ether analogues of 3-*epi*-casuarine. The synthesis of 7-deoxy-3,6-di*epi*-casuarine, 7-*epi*-australine, 1-*epi*-castanospermine and 1,6-di*epi*-castanospermine is also described. The glycosidase inhibitory activities of these compounds, along with that of uniflorine A **1** and other polyhydroxylated pyrrolizidine and indolizidines that we have published before, are reported.

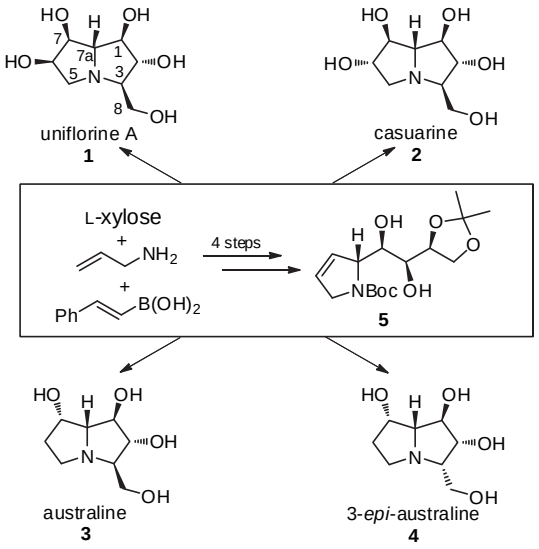
2. Results and discussion

Our anticipated synthesis of 3-*epi*-casuarine **7** from the epoxide **6** (Scheme 2) involved a regioselective ring-opening reaction of the epoxide moiety of **6** with $NaHSO_4$,[14] a reagent that we had successfully applied to the synthesis of casuarine **2** from 3-*epi*-**6**.[13]

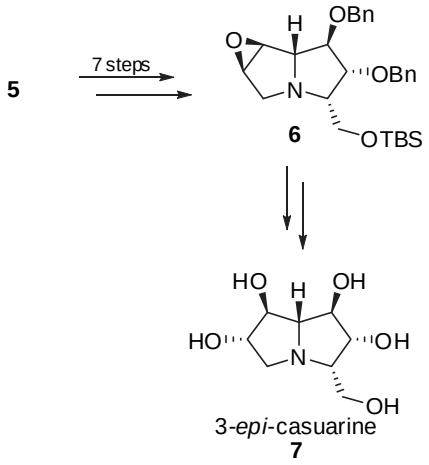
The epoxy-pyrrolizidine **6** was treated with $NaHSO_4/CH_2Cl_2$ under the conditions that we described before[13] except that heating at 50 °C was continued for 7 days (Scheme 3). We attribute the slower rate of epoxide ring-opening of **6**, when compared to that of 3-*epi*-**6**, to the increased steric hindrance of the α-face of the epoxide moiety due to the 3-α-CH_2OTBS substituent. Because of the slow reaction rate hydrolytic cleavage of the OTBS group also occurred resulting in a mixture of products that was difficult to separate. Acetylation of this mixture and then separation by column chromatography gave the desired acetylated product **8** (7% yield), the epoxide **9** in 9% yield and the undesired tricyclic bridged ether products **10** (8%) and **11** (17%) (Scheme 3). The isolation of epoxide **9** indicated that OTBS cleavage was faster than either the intermolecular or intramolecular (leading to tricyclic bridged ether products) epoxide ring-opening reactions. Unfortunately, several attempts to improve the yield of the desired compound **8** were not successful. While the use of a more stable protecting group for the primary alcohol group in **6** may have been more efficient this variation was not examined.

* Corresponding author. E-mail address: spyne@uow.edu.au (S.G. Pyne).

0040-4020/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2010.10.008



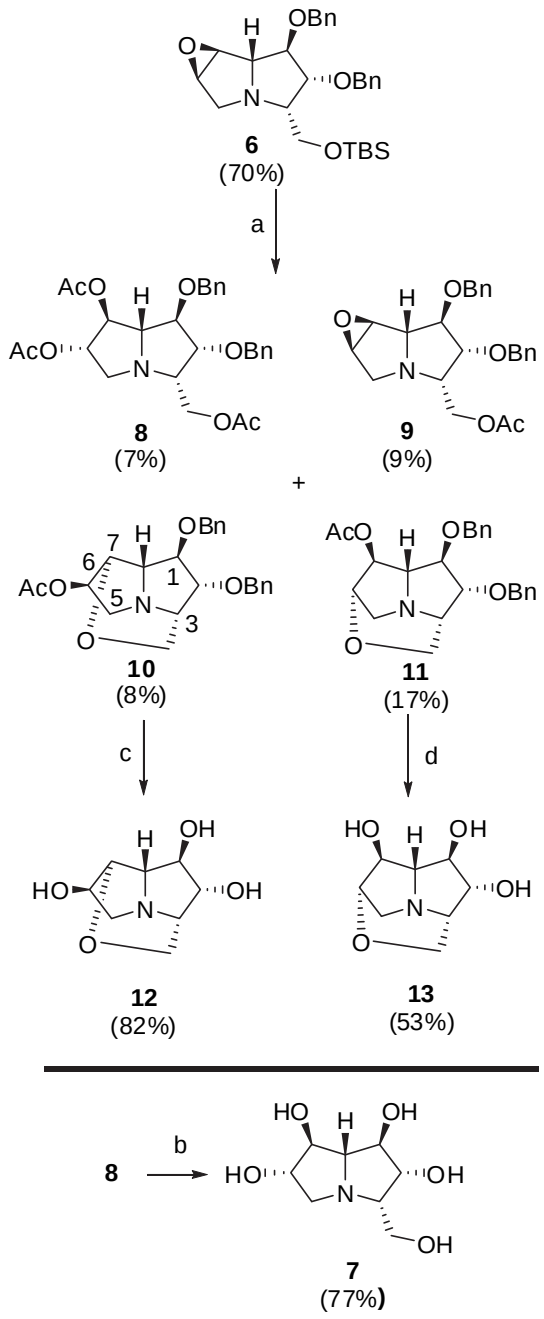
Scheme 1.



Scheme 2.

Hydrogenolysis of **8** over $PdCl_2/H_2$ in MeOH for 4 days gave diastereomerically pure 3-*epi*-casuarine **7** ($[\alpha]_D^{25}$ +2.0 (*c* 0.4, H_2O), lit.[10] $[\alpha]_D^{23}$ +5.7 (*c* 0.5, H_2O)) in 77% yield after purification by ion-exchange chromatography (Scheme 3). The ^1H NMR spectroscopic data (D_2O) of **7** and those of the natural product were essentially identical ($\Delta\delta_H$=0.01–0.02 ppm) as were the ^{13}C NMR chemical shifts ($\Delta\delta_C$=0.0–0.1 ppm).[10] The reasons for the difference in the magnitude of the optical rotation between our synthetic **7** and the natural isolate were perhaps due to the relatively small amount of sample we had, compared to that obtained from the natural source, which may have lead to difficulties in drying (slightly hydroscopic) and weighing our sample.

The relative ratio of **10** and **11** (ca. 1:2) was consistent with their relative heats of formation as calculated using PC Spartan (AM1). The calculated heat of formation of **10** (−122.3 kcal/mol) was more than that of **11** (−129.8 kcal/mol) suggesting **10** had more ring strain than **11**. This energy difference would be expected to be reflected in the differences in transition state energies leading to these regioisomeric products. The identities of **10** and **11** were established by 1D and 2D NMR analysis. The ^1H NMR spectrum of **10** showed that the most downfield proton (H-6) at 5.24 (dd, 1H, *J*=6.4, 2.8 Hz) ppm was coupled to two of the diastereotopic H-5 protons at 3.65 (dd, 1H, *J*=14.4, 6.4 Hz, H-5α) and 2.85 (dd, 1H, *J*=14.4, 2.8 Hz, H-5β) ppm. The H-5α and H-5β protons were



Scheme 3. Reagents and conditions: (a) (i) $NaHSO_4$, CH_2Cl_2, 50 °C, 7 days; (ii) Ac_2O, py, DMAP, 24 h; (b) $PdCl_2$, H_2 (1 atm), MeOH, 4 days; ion-exchange, 77%; (c) Amberlyst OH, MeOH, rt, 12 h; $PdCl_2$, H_2 (1 atm), MeOH, 24 h; ion-exchange, 82%; (d) Amberlyst (OH^-), MeOH, rt, 16 h; $PdCl_2$, H_2 (1 atm), MeOH, 12 h; ion-exchange, 53%.

individually assigned based upon their vicinal coupling constants to H-6 and from NOESY NMR spectra. Using PC Spartan (AM1) the dihedral angle (φ) between H-6 and H-5β was 120.3° and between H-6 and H-5α was 0.2°. Based on the Karplus equation[15] $J_{5\beta,6}$ should be 3–6 Hz and $J_{5\alpha,6}$ 8.5–12.5 Hz. The larger observed vicinal coupling constant (6.4 Hz) between H-6 and the two H-5 protons was therefore assigned to $J_{5\alpha,6}$ and the smaller one (2.8 Hz) to $J_{5\beta,6}$. While the most downfield proton in the ^1H NMR spectrum of **11** (H-7) resonated at 4.97 (br s, $W_{1/2}$=1 Hz, 1H) ppm and showed a weak COSY cross peak to H-5α (W-coupling). The calculated dihedral angle from PC Spartan (AM1) between H-7 and H-6 was 99.2° and between H-7 and H-7a was 128.8°. From the Karplus equation $J_{6,7}$ should be 0–3 Hz and $J_{7,7a}$ 3–7 Hz. The coupling constants observed for H-7 were consistent with the calculated *J* values and the

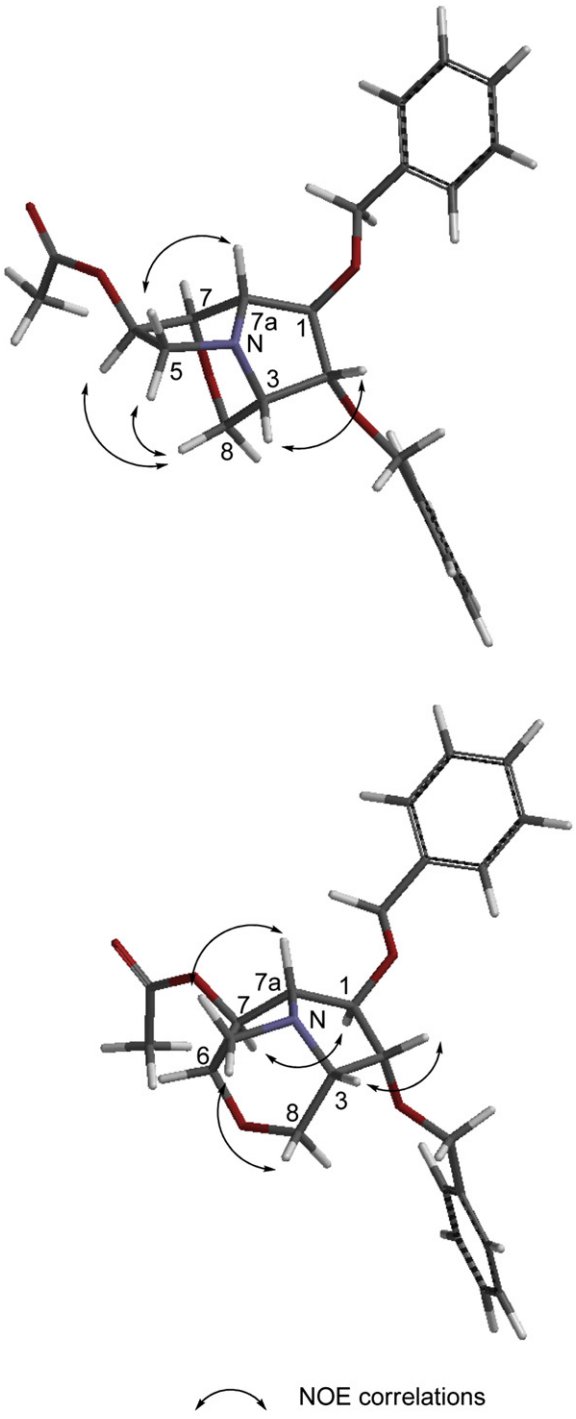


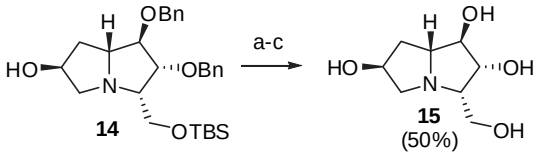
Fig. 1. Structures of **10** and **11** from PC Spartan (AM1).

structure of **11**. The structures and relative configurations of **10** and **11** were further supported by NOESY NMR experiments (Fig. 1).

The NOESY correlation between H-7a and H-5β in **10** indicated that these protons were on the same face of the ring. The correlation between H-5α and H-8 indicated that H-3 was on the opposite face to H-5α. The NOESY correlation between H-3 and H-2 indicated that they were on the same face of the ring. Furthermore NOESY correlations between H-6 and H-8 further supported the structure of **10** as shown in Fig. 1. NOESY experiments on **11** showed correlations between H-7 and H-1, H-7a and H-5β and H-2 and H-3. H-5α showed a weak correlation to H-8. These correlations further supported the structure assigned to **11**.

The acetate group of **10** was first removed using Amberlyst (OH^- form) in MeOH and then hydrogenolysis over $PdCl_2/H_2$ in MeOH for 1 day gave the deprotected tricyclic bridged ether derivative **12** $[\alpha]_D^{23}$ +8.6 (*c* 0.5, MeOH), in 82% yield after ion-exchange chromatography (Scheme 3). Following the same two-step deprotection method described above, compound **11** was converted to the tricyclic derivative **13** $[\alpha]_D^{23}$ +7.6 (*c* 0.34, MeOH), in 53% yield after purification by ion-exchange chromatography (Scheme 3).

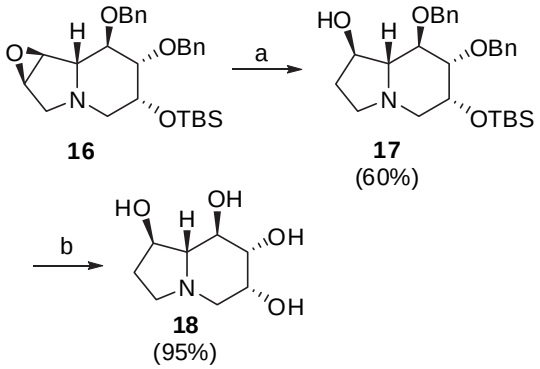
In order to prepare further polyhydroxylated pyrrolizidine and indolizidine derivatives for testing as glycosidase inhibitors several compounds that we obtained as byproducts from our earlier studies[13] were deprotected and/or modified as indicated in the following discussion. The alcohol **14**,[13] which we had obtained as a minor regioisomeric product from the reductive ring-opening reaction of **6** with $LiAlH_4$, underwent hydrogenolysis over $PdCl_2/H_2$ to provide a product that was difficult to purify. This material was subsequently acetylated by treatment with Ac_2O in pyridine and the peracetylated product was readily purified. Following deacetylation using Amberlyst A-26 (OH^-) in MeOH then pure 7-deoxy-3,6-di*epi*-casuarine **15** ($[\alpha]_D^{23}$ +28.6 (*c* 0.23, MeOH)) was obtained in 50% yield over the three steps (Scheme 4).



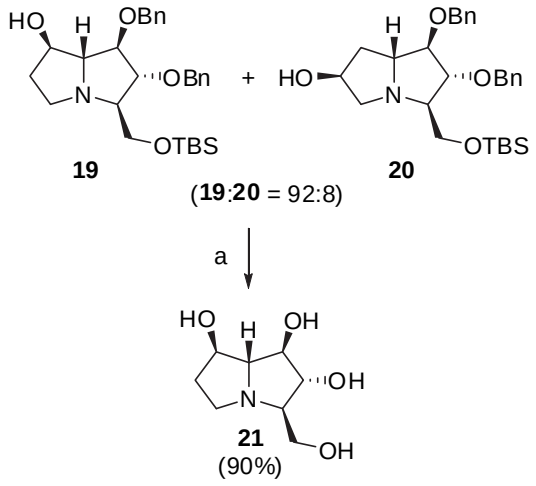
Scheme 4. Reagents and conditions: (a) $PdCl_2$, H_2 (1 atm), MeOH, rt, 3 h; then concd HCl five drops; (b) Ac_2O, py, DMAP, rt, 24 h; (c): Amberlyst A-26 (OH^-), MeOH, 3 h, 50% (three steps).

The previously prepared indolizidine **16**[13] was treated with $LiAlH_4$ in THF at rt for 20 h to provide the indolizidine **17** as the only regioisomeric product in 60% yield. Hydrogenolysis of **17** under acidic conditions with $PdCl_2/H_2$ afforded 1,6-di*epi*-castanospermine **18** $[\alpha]_D^{25}$ −74.2 (*c* 1.5, MeOH), lit.[16] $[\alpha]_D^{24}$ −72.0 (*c* 0.7, MeOH) in 95% yield and 95% purity (Scheme 5). The ^1H NMR spectroscopic data (D_2O) of **18** were consistently downfield ($\Delta\delta_H$=0.11–0.15 ppm) from those reported for the synthetic material prepared by Fleet et al.[16] The ^{13}C NMR spectroscopic data (in D_2O with MeCN as an internal reference at δ 1.47) of **18** and that reported in the literature for 1,6-di*epi*-castanospermine were all consistently 0–0.2 ppm downfield.

Hydrogenolysis of a mixture of **19** and **20** (dr 92:8)[13] over $PdCl_2/H_2$ followed by purification and neutralized by ion-exchange chromatography gave 7-*epi*-australine **21** in 90% yield (Scheme 6). ^1H NMR analysis suggested that **21** was of 92% purity ($[\alpha]_D^{23}$ −13.2 (*c* 1.2, H_2O), lit.[17] $[\alpha]_D^{25}$ −13.04 (*c* 0.55, H_2O, pH 8.37)). The ^1H NMR spectroscopic data (D_2O) of **21** were all consistently downfield



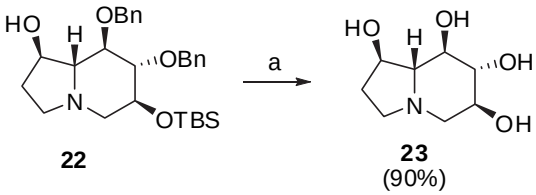
Scheme 5. Reagents and conditions: (a) $LiAlH_4$, THF, rt, 20 h, 60% (b) $PdCl_2$, H_2 (1 atm), MeOH, rt, 4 days; ion-exchange, 95%.



Scheme 6. Reagents and conditions: (a) $PdCl_2$, H_2 (1 atm), MeOH, rt, 3 h, then concd HCl rt, 17 h, ion-exchange, 90%.

($\Delta\delta_H$=0.16–0.18 ppm) of those of the synthetic compound prepared by Denmark and Martinborough.[17] The ^{13}C NMR signals of **21** (in D_2O with MeCN as an internal reference at δ 1.47) were consistently 1.0–1.1 ppm downfield of those reported earlier.[17]

Hydrogenolysis of **22**[13] over $PdCl_2/H_2$ gave diastereomerically pure 1-*epi*-castanospermine **23** ($[\alpha]_D^{22}$ +3.3 (*c* 0.3, MeOH)), lit.[18] $[\alpha]_D^{26}$ +3.8 (*c* 0.5, MeOH), in 94% yield after ion-exchange chromatography (Scheme 7). The ^1H NMR spectroscopic data (D_2O) of **23** and those of the synthetic compound from Murphy[19] were essentially identical ($\Delta\delta_H$=0.02–0.03 ppm). The ^{13}C NMR signals of **23** (in D_2O with MeCN as an internal reference at 1.47 ppm) however, were all consistently 1.7–1.8 ppm downfield of those reported previously. However the internal reference used in the published ^{13}C NMR spectrum was not reported.[19]



Scheme 7. Reagents and conditions: (a) $PdCl_2$, H_2 (1 atm), MeOH, rt, 3 h, then concd HCl rt, 21 h, ion-exchange, 94%.

A comparative study of the inhibitory activities of alexine, australine, casuarine and some of their epimers and *O*-α- and *O*-β-glucoside derivatives against a panel of glycosidase enzymes revealed that casuarine and 1-*epi*-australine-2-*O*-β-glucoside were the most potent compounds.[9] These alkaloids showed low μM activities against several α-glucosidases. For example, casuarine had an IC_{50} value of 0.7 μM against rat intestinal maltase and of 0.7 μM against amyloglucosidase from *Aspergillus niger*. In a separate study, uniflorine A **1** was shown to have moderate inhibitory activity against the α-glucosidases, rat intestinal maltase and sucrase, with IC_{50} values of 12 and 3.1 μM, respectively.[1] The results of our glycosidase inhibitor testing for synthetic uniflorine A **1**[13] are shown in Table 1. At 143 μg/mL uniflorine A **1** showed 94–97% inhibition against the α-D-glucosidases of *Saccharomyces cerevisiae* and *Bacillus stearothermophilus* and against amyloglucosidase of *A. niger*. The IC_{50} values were only determined for the two aforementioned α-D-glucosidases and were found to be modest at 34 and 28 μM, respectively.

Compounds **12**, **13**, **15**, **18** and **23** along with the pyrrolizidine **24**[13] and the indolizidines **25**,[20] **26**[2] and **27**[2] were screened against 10 different glycosidases at 143 μg/mL. None showed strong inhibition with only compounds **13**, **18**, **23**, **25** and **27** showing approximately 40–50% inhibition against the α-D-glucosidase from *B. stearothermophilus* at this relatively high concentration (Table 2). 1,6-Di*epi*-castanospermine showed similar activity against almond β-D-glucosidase and α-D-galactosidase (coffee bean).

Table 1
The glycosidase inhibition of uniflorine A **1** (Mean % Inhibition at 143 μg/mL)

Enzyme	Source	pH	% Inhibitions
α-D-Glucosidase	*S. cerevisiae*	6.8	94 IC_{50} 34 μM
α-D-Glucosidase	*B. stearothermophilus*	6.8	97 IC_{50} 28 μM
α-D-Glucosidase	Rice	4	ND
β-D-Glucosidase	Almond (*Prunus* sp.)	5	5
α-D-Galactosidase	Green coffee bean (*Coffea* sp.)	6.5	5
β-D-Galactosidase	Bovine liver	7.3	0
α-L-Fucosidase	Bovine kidney	5.5	ND
α-D-Mannosidase	Jack bean (*Canavalia ensiformis*)	4.5	5
β-D-Mannosidase	*Cellullomonas fimi*	6.5	2
Naringinase	*Penicillium decumbens*	4	ND
N-Acetyl-β-D-glucosaminidase	Bovine kidney	4.25	13
N-Acetyl-β-D-glucosaminidase	Jack bean	5	5
N-Acetyl-β-D-hexosaminidase	*Aspergillus oryzae*	5	ND
Amyloglucosidase	*A. niger*	4.5	97
β-Glucuronidase	Bovine liver	5	0

ND=No determined.

3. Conclusions

The total synthesis of naturally occurring 3-*epi*-casuarine **8** was achieved in 13 steps and in 0.4% overall yield from L-xylose. The low overall yield was due to a poor yielding epoxide ring-opening reaction due to a competing intramolecular epoxide ring-opening reaction involving the 3-α-hydroxymethyl substituent. We also report the synthesis of two novel tricyclic ether bridged analogues of 3-*epi*-casuarine, plus 7-deoxy-3,6-di*epi*-casuarine, 7-*epi*-australine, 1-*epi*-castanospermine and 1,6-di*epi*-castanospermine. The glycosidase inhibitory activities of these compounds, along with that of uniflorine A and other polyhydroxylated pyrrolizidine and indolizidines that we have published before, are reported. Uniflorine A showed moderate activity against the α-D-glucosidases from *S. cerevisiae* and *B. stearothermophilus* (IC_{50} 34 and 28 μM, respectively). None of the other compounds tested showed any significant inhibitory activities.

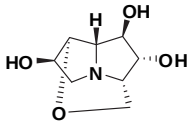
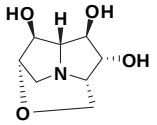
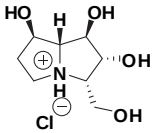
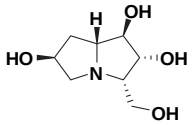
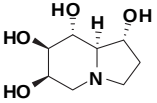
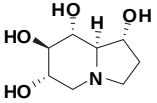
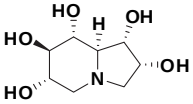
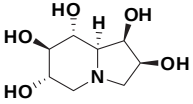
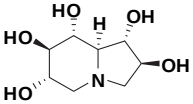
4. Experimental

4.1. General methods

All IR spectra were run as neat samples. All NMR spectra were run at 500 MHz (^1H NMR) or 125 MHz (^{13}C NMR) in solutions of $CDCl_3$ unless otherwise noted. NMR assignments were made on the basis of COSY, DEPT, HSQC and sometimes HMBC experiments. In the ^1H NMR assignments of some compounds the diastereotopic H-8 (pyrrolizidine compounds) and H-5 protons (indolizidine compounds) are referred to H-8a and H-8b and H-5a and H-5b, respectively where the most downfield proton is labelled as 'a'. Petrol refers to the hydrocarbon fraction of bp 40–60 °C. FCC is an abbreviation for flash column chromatography.

*4.1.1. (1S,2S,5S,6R,7R,7aR)-5-(Acetoxymethyl)-6,7-bis(benzyloxy)-1,2-diacetoxy-hexahy-dro-1H-pyrrolizine (**8**), (1aR,4S,5R,6R,6bS)-5,6-bis(benzyloxy)-4-(acetoxymethyl)hexahydro-1aH-oxireno[2,3-a]pyrrolizine (**9**) 1,2-bis(benzyloxy)-6-acetoxy-3-methyl-7,8-epoxyindolizidine (**10**) and 1,2-bis(benzyloxy)-7-acetoxy-3-methyl-6,8-epoxy-indolizidine*

Table 2
The glycosidase inhibition of synthesized compounds (Mean % Inhibition at 143 μg/mL)

Compound No.	Structures	α-D-Glucosidase		β-D-Glucosidase	α-D-Galactosidase	β-D-Galactosidase	α-D-Mannosidase	β-D-Mannosidase	*N*-acetyl-β-D-Glucosaminidase		β-Glucuronidase
		(Yeast)	(*Bacillus*)						Bovine kidney	Jack bean	Bovine liver
Tricyclic **12**		0	24	0	0	6	0	0	17	24	0
Tricyclic **13**		44	50	27	ND	19	-21	12	30	26	8
3,7-Di*epi*-australine·HCl **24**·HCl[13]		0	−9	0	−11	0	0	17	0	7	ND
7-Deoxy-3,6-di*epi*-casuarine **15**		−13	−16	−34	−38	40	−29	−17	0	0	ND
1,6-Di*epi*-castanospermine **18**		36	43	55	45	31	23	30	7	−12	ND
1-*epi*-Castanospermine **23**		14	43	24	0	25	−10	0	13	17	0
25[2]		0	42	16	0	21	0	−11	19	0	−7
26[2]		0	34	18	0	8	0	−14	25	0	0
27[2]		20	44	13	−7	7	−25	0	0	0	0

ND=No determined.

(***11***). To a solution of the epoxide **6** (100.0 mg, 0.208 mmol) in anhydrous CH_2Cl_2 (5 mL) was added $NaHSO_4$ (125 mg, 1.04 mmol). The reaction mixture was stirred and heated at reflux for 7 days under an atmosphere of N_2. The reaction was quenched by the addition of water (5 mL) and stirred for 1 h. The solvent was removed under reduced pressure and the residue was extracted with EtOAc (3×10 mL). TLC analysis showed four major products. The crude mixture was purified by FCC (100% EtOAc to 8.0:1.5:0.5 EtOAc/MeOH/NH_3). The fractions were not pure and they were combined, evaporated and then acetylated. To a solution of the crude product in pyridine (2.0 mL) was added acetic anhydride (0.184 mL, 1.95 mmol) and a crystal of 4-dimethylaminopyridine. The mixture was stirred at rt for 24 h. The reaction was quenched by the addition of satd $NaHCO_3$ solution, followed by removal of the solvent under reduced pressure. The residue was extracted with CH_2Cl_2 (3×10 mL). The combined CH_2Cl_2 extracts were washed with water (10 mL), dried (Na_2CO_3), filtered and then evaporated. The residue was purified by FCC (90:10 EtOAc/petrol to 8.5:1:0.5 EtOAc/MeOH/NH_3) to give **8** as a pale yellow oil (7.0 mg, 7%), **10** as a colourless oil (6.7 mg, 8%), **9** as a pale yellow oil (8.0 mg, 9%) and **11** as a pale yellow oil (14.0 mg, 17%).

Compound **8**: R_f 0.47 (50:50 EtOAc/petrol). $[\alpha]_D^{22}$ +22.3 (*c* 1.4, $CHCl_3$). MS (ESI +ve) *m/z* 512 ($M+H^+$, 100%). HRMS (ESI +ve) calculated for $C_{28}H_{34}NO_8$ ($M+H^+$) 512.2284, found 512.2277. IR ν_{max} (cm^{-1}): 2919, 1738, 1372, 1228, 1042. ^1H NMR δ 7.36–7.26 (m, 10H, Ar), 5.32 (app. dt, 1H, *J* 8.0, 6.7 Hz, H-6), 5.22 (app. t, 1H, *J* 6.7 Hz, H-7), 4.61 (d, 1H, *J* 12.3 Hz, C*H*HPh), 4.55 (d, 1H, *J* 11.5 Hz, C*H*HPh), 4.48 (d, 1H, *J* 11.5 Hz, C*H*HPh), 4.42 (dd, 1H, *J* 11.1, 6.7 Hz, H-8), 4.37 (d, 1H, *J* 12.3 Hz, C*H*HPh), 4.34 (dd, 1H, *J* 11.1, 7.8 Hz, H-8), 4.30 (br s, 1H, H-1), 3.95 (d, 1H, *J* 4.0 Hz, H-2), 3.49–3.45 (m, 1H, H-3), 3.40–3.30 (m, 1H, H-7a), 3.31 (app. t, 1H, *J* 7.8 Hz, H-5β), 3.13 (app. t, 1H, *J* 8.0 Hz, H-5α), 2.11 (s, 3H, CH_3), 2.05 (s, 3H, CH_3), 2.01 (s, 3H, CH_3). ^{13}C NMR δ 170.9 (CO), 170.8 (CO), 170.3 (CO), 137.7 (C), 137.6 (C), 128.5 (CH), 128.4 (CH), 127.9 (CH), 127.8 (CH), 127.7 (CH), 127.6 (CH), 85.7 (C-1), 85.6 (C-2), 79.0 (C-7), 76.5 (C-6), 73.8 (C-7a), 71.8 (CH_2), 71.7 (CH_2), 62.3 (C-3), 60.6 (C-8), 50.4 (C-5), 20.94 (CH_3), 20.9 (CH_3), 20.8 (CH_3).

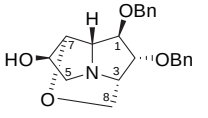
Compound **9**: (NMR assignments are given based on the numbering system of the parent pyrrolizine (compare with **8** above) and are not based on the systematic compound name give in the title). R_f 0.34 (100% EtOAc). MS (ESI +ve) *m/z* 410 ($M+H^+$, 100%). ^1H NMR δ 7.41–7.23 (m, 10H, Ar), 4.58 (d, 1H, *J* 12.0 Hz, C*H*HPh), 4.54 (d, 1H, *J* 11.5 Hz, C*H*HPh), 4.53 (d, 1H, *J* 11.5 Hz, C*H*HPh), 4.43 (dd, 1H, *J* 11.3, 6.5 Hz, H-8), 4.37 (dd, 1H, *J* 11.3, 4.5 Hz, H-8), 4.36 (d, 1H, *J* 11.5 Hz, C*H*HPh), 4.03 (d, 1H, *J* 4.0 Hz, H-2), 3.81 (d, 1H, *J* 4.0 Hz, H-1), 3.71 (d, 2H, *J* 5.0 Hz, H-7a and H-6 or H-7), 3.63 (d, 1H, *J* 3.0 Hz, H-6 or H-7), 3.57 (app. dd, 1H, *J* 11.0, 7.0 Hz, H-3), 3.25 (d, 1H, *J* 11.3 Hz, H-5), 3.15 (d, 1H, *J* 11.3 Hz, H-5), 2.03 (s, 3H, CH_3). ^{13}C NMR δ 170.9 (CO), 137.6 (C), 137.4 (C), 128.6 (CH), 128.5 (CH), 128.1 (CH), 127.9 (CH), 127.6 (CH), 127.5 (CH), 87.4 (C-2), 84.8 (C-1), 72.2 (C-7a), 71.8 (CH_2), 71.7 (CH_2), 62.4 (C-3), 60.3 (C-8), 57.4 (C-6 or C-7), 57.2 (C-6 or C-7), 48.1 (C-5), 20.9 (CH_3).

Compound **10**: R_f 0.50 (100% EtOAc). $[\alpha]_D^{25}$ −14.9 (*c* 0.7, $CHCl_3$). MS (ESI +ve) *m/z* 410 ($M+H^+$, 100%). HRMS (ESI +ve) calculated for $C_{24}H_{28}NO_5$ ($M+H^+$) 410.1967, found 410.1984. IR ν_{max} (cm^{-1}): 2929, 2868, 1737, 1237, 1102, 1046. ^1H NMR δ 7.38–7.26 (m, 10H, Ar), 5.24 (dd, 1H, *J* 6.4, 2.8 Hz, H-6), 4.62 (d, 1H, *J* 12.0 Hz, C*H*HPh), 4.59 (d, 2H, *J* 12.0 Hz, 2x*CH*HPh), 4.55 (d, 1H, *J* 12.0 Hz, C*H*HPh), 4.37 (d, 1H, *J* 4.0 Hz, H-1), 4.30 (dd, 1H, *J* 5.5, 4.0 Hz, H-2), 3.95 (d, 1H, *J* 2.0 Hz, H-7), 3.73 (d, 1H, *J* 12.0 Hz, H-8), 3.65 (dd, 1H, *J* 14.4, 6.4 Hz, H-5), 3.59 (dd, 1H, *J* 12.0, 1.8 Hz, H-8), 3.10 (d, 1H, *J* 2.0 Hz, H-7a), 3.07 (dd, 1H, *J* 5.0, 1.8 Hz, H-3), 2.85 (dd, 1H, *J* 14.4, 2.8 Hz, H-5), 2.03 (s, 3H, CH_3). ^{13}C NMR δ 170.0 (CO), 138.0 (C), 137.8 (C), 128.4 (CH), 128.3 (CH), 127.9 (CH), 127.87 (CH), 127.8 (CH), 127.7 (CH), 85.5 (C-2), 81.9 (C-1), 78.7 (C-7), 77.1 (C-6), 72.2 (CH_2), 71.8 (CH_2), 68.6 (C-7a), 62.3 (C-3), 59.8 (C-8), 56.1 (C-5), 20.9 (CH_3).

Compound **11**: R_f 0.32 (100% EtOAc). MS (ESI +ve) *m/z* 410 ($M+H^+$, 100%). $[\alpha]_D^{25}$ −16.7 (*c* 1.6, $CHCl_3$). HRMS (ESI +ve) calculated for $C_{24}H_{28}NO_5$ ($M+H^+$) 410.1967, found 410.1974. IR ν_{max} (cm^{-1}): 2929, 2873, 1735, 1378, 1237, 1120. ^1H NMR δ 7.41–7.26 (m, 10H, Ar), 4.97 (br s, 1H, H-7), 4.75 (d, 1H, *J* 12.0 Hz, C*H*HPh), 4.66 (d, 1H, *J* 12.5 Hz, C*H*HPh), 4.61 (d, 1H, *J* 12.0 Hz, C*H*HPh), 4.48 (d, 1H, *J* 12.0 Hz, C*H*HPh), 4.24–4.23 (m, 2H, H-1 and H-2), 4.17 (s, 1H, H-6), 3.71 (d, 1H, *J* 10.5 Hz, H-8), 3.56–3.53 (m, 2H, H-3 and H-8), 3.47 (d, 1H, *J* 13.0 Hz, H-5α), 3.25 (br s, 1H, H-7a), 2.72 (dd, 1H, *J* 13.0, 1.0 Hz, H-5β), 2.05 (s, 3H, CH_3). ^{13}C NMR δ 170.0 (CO), 138.2 (C), 138.0 (C), 128.4 (CH), 128.3 (CH), 127.9 (CH), 127.8 (CH), 127.7 (CH), 127.6 (CH), 86.0 (C-1), 84.7 (C-2), 81.8 (C-7), 75.3 (C-6), 74.2 (C-7a), 72.5 (CH_2), 72.1 (CH_2), 62.9 (C-3), 55.7 (C-8), 49.4 (C-5), 21.0 (CH_3).

*4.1.2. (1R,2R,3S,6S,7S,7aR)-3-(Hydroxymethyl)-hexahydro-1H-pyrrolizine-1,2,6,7-tetraol (3-epi-casuarine (**7**)).* To a solution of **8** (14 mg, 0.027 mmol) in MeOH (1 mL) was added $PdCl_2$ (9.7 mg, 0.055 mmol). The mixture was stirred at rt under an atmosphere of H_2 (balloon) for 4 days. The mixture was filtered through a Celite pad and the solids were washed with MeOH. The combined filtrates were evaporated in vacuo and the residue was dissolved in water (1 mL) and applied to a column of Amberlyst A-26 (OH^-) resin (3 cm). Elution with water followed by evaporation in vacuo gave 3-*epi*-casuarine **7** as a white solid (4.3 mg, 77%). $[\alpha]_D^{25}$ +2.0 (*c* 0.04, H_2O), lit.[10] $[\alpha]_D^{23}$ +5.7 (*c* 0.5, H_2O). MS (ESI +ve) *m/z* 206 ($M+H^+$, 100%). HRMS (ESI +ve) calculated for $C_8H_{16}NO_5$ ($M+H^+$) 206.1028, found 206.1024. IR ν_{max} (cm^{-1}): 3300, 2924, 2901, 1361, 1027. ^1H NMR (D_2O) δ 4.28 (br s, 1H, H-1), 4.19 (dd, 1H, $J_{2,3}$=3.5, $J_{1,2}$=1.5 Hz, H-2), 4.11 (dt, 1H, $J_{5,6}$=7.8, $J_{5,6}$=$J_{6,7}$=8.0 Hz, H-6), 4.05 (t, 1H, $J_{6,7}$=$J_{7,7a}$=8.0 Hz, H-7), 4.00 (dd, 1H, $J_{8,8}$=11.7, $J_{3,8a}$=6.6 Hz, H-8a), 3.94 (dd, 1H, $J_{8,8}$=11.7, $J_{3,8b}$=7.0 Hz, H-8b), 3.27 (ddd, 1H, $J_{3,8a}$=6.6, $J_{3,8b}$=7.0, $J_{2,3}$=3.5 Hz, H-3), 3.10 (d, 3H, $J_{7,7a}$=8.0 Hz, 2×H-5 and H-7a). ^{13}C NMR (D_2O) δ 80.4 (C-1), 79.7 (C-2), 79.2 (C-7), 75.9 (C-6), 75.5 (C-7a), 64.9 (C-3), 57.4 (C-8), 51.6 (C-5).

*4.1.3. 1,2-Bis(benzyloxy)-6-hydroxyl-3-methyl-7,8-epoxyindolizidine (**10a**) and 3-methyl 1,2,6-trihydroxyl-7,8-epoxyindolizidine (**12**).*



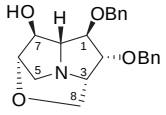
10a

To a solution of **10** (13.4 mg, 0.033 mmol) in MeOH (1 mL) was added Amberlyst A-26 (OH^-) resin (40 mg). The reaction was stirred for 12 h at rt then filtered through a Celite pad and the solids were washed with MeOH (10 mL). The filtrate was evaporated in vacuo to give **10a** as a pale yellow oil that was used to next step without purification. MS (ESI +ve) *m/z* 368 ($M+H^+$, 100%). HRMS (ESI +ve) calculated for $C_{22}H_{26}NO_4$ ($M+H^+$) 368.1844, found 368.1862. IR ν_{max} (cm^{-1}): 3385, 2925, 2863, 1454, 1362, 1099, 1070. ^1H NMR δ 7.37–7.26 (m, 10H, Ar), 4.63 (d, 1H, *J* 12.0 Hz, C*H*HPh), 4.59 (d, 1H, *J* 11.5 Hz, C*H*HPh), 4.57 (d, 1H, *J* 11.5 Hz, C*H*HPh), 4.54 (d, 1H, *J* 12.0 Hz, C*H*HPh), 4.49 (dd, 1H, *J* 6.2, 2.5 Hz, H-6), 4.36 (d, 1H, *J* 4.0 Hz, H-1), 4.33 (dd, 1H, *J* 5.5, 4.0 Hz, H-2), 3.80 (d, 1H, *J* 2.0 Hz, H-7), 3.68 (d, 1H, *J* 12.0 Hz, H-8), 3.53 (dd, 1H, *J* 13.0, 6.2 Hz, H-5), 3.50 (dd, 1H, *J* 12.0, 2.5 Hz, H-8), 3.24 (br s, 1H, H-7a), 3.02 (br d, 1H, *J* 3.0 Hz, H-3), 2.86 (dd, 1H, *J* 13.0, 2.5 Hz, H-5). ^{13}C NMR δ 138.0 (C), 137.9 (C), 128.3 (CH), 127.9 (CH), 127.8 (CH), 127.7 (CH), 127.6 (CH), 85.5 (C-2), 82.0 (C-1), 81.5 (C-7), 75.1 (C-6), 72.2 (CH_2), 71.7 (CH_2), 67.8 (C-7a), 62.3 (C-3), 59.8 (C-8), 57.9 (C-5).

To a solution of crude **10a** in MeOH (1 mL) was added $PdCl_2$ (8.71 mg, 0.05 mmol). The mixture was stirred at rt under an atmosphere of H_2 (balloon) for 24 h The mixture was filtered through a Celite pad and the solids were washed with MeOH. The combined filtrates were evaporated in vacuo and the residue was dissolved in water (1 mL) and applied to a column of Amberlyst A-26 (OH^-)

resin (3 cm). Elution with water followed by evaporation in vacuo gave **12** as a pale yellow solid (5.0 mg, 82%). $[\alpha]_D^{23}$ +8.6 (*c* 0.5, H_2O). MS (ESI +ve) *m/z* 188 ($M+H^+$, 100%). HRMS (ESI +ve) calculated for $C_8H_{14}NO_4$ ($M+H^+$) 188.0923, found 188.0928. IR ν_{max} (cm^{-1}): 3395, 2965, 2934, 1316, 1033. ^1H NMR (D_2O) δ 4.41 (dd, 1H, $J_{5,6}$=6.0, 2.3 Hz, H-6), 4.27 (br d, 1H, $J_{1,2}$=4.3 Hz, H-1), 4.20 (br dd, 1H, $J_{2,3}$=5.3, $J_{1,2}$=4.3 Hz, H-2), 3.87 (br s, 1H, H-7), 3.62–3.54 (m, 3H, 2×H-8 and H-5α), 2.96 (br s, 1H, H-7a), 2.94 (br d, 1H, $J_{2,3}$=5.3 Hz, H-3), 2.63 (dd, 1H, $J_{5,5}$=14.5, $J_{5,6}$=2.3 Hz, H-5β). ^{13}C NMR (D_2O) δ 81.4 (C-7), 79.9 (C-2), 75.8 (C-1), 74.4 (C-6), 70.6 (C-7a), 63.7 (C-3), 59.5 (C-8), 56.8 (C-5).

*4.1.4. 1,2-Bis(benzyloxy)-7-hydroxyl-3-methyl-6,8-epoxyindolizidine (**11a**) and 3-methyl-1,2,7-trihydroxyl-6,8-epoxyindolizidine (**13**).*



11a

To a solution of **11** (14.0 mg, 0.034 mmol) in MeOH (1 mL) was added Amberlyst A-26 (OH^-) resin (42 mg). The reaction was stirred for 16 h at rt, the mixture was filtered through a Celite pad and the solids were washed with MeOH (10 mL). The filtrate was evaporated in vacuo to give **11a** as a pale yellow oil that was used to next step without purification. MS (ESI +ve) *m/z* 368 ($M+H^+$, 100%). HRMS (ESI +ve) calculated for $C_{22}H_{26}NO_4$ ($M+H^+$) 368.1844, found 368.1862. IR ν_{max} (cm^{-1}): 3365, 2919, 2863, 1440, 1115, 1014. ^1H NMR δ 7.42–7.26 (m, 10H, Ar), 4.73 (d, 1H, *J* 12.0 Hz, C*H*HPh), 4.61 (d, 1H, *J* 12.0 Hz, C*H*HPh), 4.56 (d, 1H, *J* 12.0 Hz, C*H*HPh), 4.49 (d, 1H, *J* 12.0 Hz, C*H*HPh), 4.24 (app. t, 1H, *J* 6.3 Hz, H-2), 4.07 (dd, 1H, *J* 7.0, 3.8 Hz, H-1), 3.98 (s, 1H, H-6), 3.91 (s, 1H, H-7), 3.66 (app d, 1H, *J* 10.5 Hz, H-8), 3.54–3.51 (m, 2H, H-3 and H-8), 3.40 (d, 1H, *J* 13.0 Hz, H-5), 3.07 (d, 1H, *J* 3.8 Hz, H-7a), 2.77 (d, 1H, *J* 13.0 Hz, H-5). ^{13}C NMR δ 138.3 (C), 138.0 (C), 128.5 (CH), 128.4 (CH), 128.2 (CH), 127.9 (CH), 127.7 (CH), 127.6 (CH), 85.6 (C-1), 84.7 (C-2), 81.0 (C-7), 77.3 (C-6), 76.0 (C-7a), 72.6 (CH_2), 72.5 (CH_2), 62.7 (C-3), 55.4 (C-8), 48.6 (C-5).

To a solution of crude **11a** in MeOH (1 mL) was added $PdCl_2$ (9.1 mg, 0.051 mmol). The mixture was stirred at rt under an atmosphere of H_2 (balloon) for 24 h. The mixture was filtered through a Celite pad and the solids were washed with MeOH. The filtrate was evaporated in vacuo and the residue was dissolved in water (1 mL) and applied to a column of Amberlyst A-26 (OH^-) resin (3 cm). Elution with water followed by evaporation in vacuo gave **13** as a yellow solid (3.4 mg, 53%). $[\alpha]_D^{23}$ +7.6 (*c* 0.34, H_2O). MS (ESI +ve) *m/z* 188 ($M+H^+$, 100%). HRMS (ESI +ve) calculated for $C_8H_{14}NO_4$ (M^+) 188.0923, found 188.0928. IR ν_{max} (cm^{-1}): 3380, 2919, 1114, 1054, 1033. ^1H NMR (D_2O) δ 4.25 (t, 1H, $J_{1,2}$=$J_{2,3}$=7.3 Hz, H-2), 4.22 (s, 2H, H-6 and H-7), 4.16 (dd, 1H, $J_{1,2}$=7.3, $J_{1,7a}$=4.3 Hz, H-1), 3.76 (dd, 1H, $J_{8a,8b}$=12.5, $J_{3,8a}$=7.3 Hz, H-8a), 3.69 (d, 1H, $J_{8a,8b}$=12.5 Hz, H-8b), 3.63 (d, 1H, $J_{5,5}$=14.0 Hz, H-5), 3.54 (t, 1H, $J_{2,3}$=$J_{3,8a}$=7.3 Hz, H-3), 3.08 (d, 1H, $J_{1,7a}$=4.3 Hz, H-7a), 2.80 (d, 1H, $J_{5,5}$=14.0 Hz, H-5). ^{13}C NMR (D_2O) δ 80.2 (C-6), 79.5 (C-1), 77.9 (C-7), 77.7 (C-2), 76.5 (C-7a), 64.3 (C-3), 54.9 (C-8), 47.7 (C-5).

*4.1.5. (1R,2R,3S,6S,7aR)-3-(Hydroxymethyl)hexahydro-1H-pyrrolizine-1,2,6-triol (**15**).* To a solution of **14** (12 mg, 0.025 mmol) in MeOH (1 mL) was added $PdCl_2$ (6.6 mg, 0.037 mmol). The mixture was stirred at rt under an atmosphere of H_2 (balloon) for 3 h, followed by the dropwise addition of concd HCl (five drops). The mixture was stirred at rt for 21 h. The mixture was filtered through a Celite pad and the solids were washed with MeOH. The combined filtrates were evaporated in vacuo and the residue was dissolved in water (1 mL) and applied to a column of Amberlyst A-26 (OH^-) resin (3 cm). Elution with water followed by evaporation in vacuo gave product as a white solid (4.0 mg). However, the ^1H NMR spectrum of this compound showed an impure product and therefore this compound was acetylated. To a solution of the above product in pyridine (0.5 mL) was added acetic anhydride (20 μL, 0.254 mmol) and a crystal of 4-dimethylaminopyridine. The mixture was stirred at rt for 24 h. The reaction was quenched by the addition of satd $NaHCO_3$ solution, followed by removal of the solvent under reduced pressure and the residue was extracted with CH_2Cl_2 (3×5 mL). The combined CH_2Cl_2 extracts were washed with brine, dried (Na_2CO_3) and evaporated. The crude product was purified by FCC (70:30 EtOAc/petrol to 8.5:1:0.5 EtOAc/MeOH/NH_3) to give the tetraacetate derivative of **15** as a pale yellow oil (3.6 mg). R_f 0.49 (90:10 EtOAc/MeOH). MS (ESI +ve) *m/z* 358 ($M+H^+$, 100%). $[\alpha]_D^{23}$ −10.6 (*c* 0.3, $CHCl_3$). IR ν_{max} (cm^{-1}): 2924, 2825, 1736, 1372, 1221, 1039. ^1H NMR δ 5.40 (app. t, 1H, *J* 4.5 Hz, H-6), 5.35 (dd, 1H, *J* 5.0, 1.5 Hz, H-2), 4.83 (br d, 1H, *J* 1.5 Hz, H-1), 4.38 (dd, 1H, *J* 12.0, 6.5 Hz, H-8), 4.23 (dd, 1H, *J* 12.0, 7.0 Hz, H-8), 3.75 (app. dt, 1H, *J* 6.3, 5.0 Hz, H-3), 3.59–3.55 (ddd, 1H, *J* 7.5, 3.0, 3.0 Hz, H-7a), 3.27 (dd, 1H, *J* 10.5, 4.0 Hz, H-5), 2.99 (d, 1H, *J* 10.5 Hz, H-5), 2.35 (dd, 1H, *J* 14.3, 7.8 Hz, H-7), 2.13–2.05 (m, 13H, 4×CH_3 and H-7). ^{13}C NMR δ 170.8 (CO), 170.6 (CO), 170.1 (CO), 169.3 (CO), 82.7 (C-1), 79.8 (C-2), 75.6 (C-6), 69.3 (C-7a), 61.0 (C-3), 59.6 (C-8), 53.6 (C-5), 36.3 (C-7), 21.3 (CH_3), 20.9 (2×CH_3), 20.8 (CH_3).

To a solution of the above tetraacetate (3.6 mg, 0.010 mmol) in MeOH (1 mL) was added Amberlyst A-26 (OH^-) resin (15 mg). The reaction was stirred for 3 h at rt, the mixture was then filtered through a Celite pad and the solids were washed with MeOH (10 mL). The combined filtrates were evaporated in vacuo to give 7-deoxy-3,6-di*epi*-casuarine **15** as a pale yellow solid (2.3 mg, 50%). $[\alpha]_D^{23}$ +28.6 (*c* 0.23, MeOH). MS (ESI +ve) *m/z* 190 ($M+H^+$, 100%). HRMS (ESI +ve) calculated for $C_8H_{16}NO_4$ ($M+H^+$) 190.1079, found 190.1074. IR ν_{max} (cm^{-1}): 3400, 2923, 2852, 1460, 1053, 1038. ^1H NMR (D_2O) δ 4.57 (br s, 1H, H-6), 4.22 (br s, 1H, H-2), 4.09 (br s, 1H, H-1), 4.00 (dd, 1H, $J_{8a,8a}$=11.4 Hz, $J_{3,8a}$=6.7 Hz, H-8a), 3.92 (dd, 1H, $J_{8a,8b}$=11.4, $J_{3,8b}$=6.7 Hz, H-8b), 3.59 (t, 1H, $J_{1,7a}$=$J_{7,7a}$=7.8 Hz, H-7a), 3.41 (br s, 1H, H-3), 3.31 (dd, 1H, $J_{5,5}$=10.5, $J_{5,6}$=2.5, H-5), 2.92 (d, 1H, $J_{5,5}$=10.5 Hz, H-5), 2.22–2.17 (m, 1H, H-7), 2.04–2.00 (m, 1H, H-7). ^{13}C NMR (D_2O) δ 81.1 (C-1), 79.7 (C-2), 72.9 (C-6), 71.4 (C-7a), 65.0 (C-3), 57.5 (C-8), 56.2 (C-5), 38.0 (C-7).

*4.1.6. (1R,6R,7S,8R,8aR)-7,8-Bis(benzyloxy)-6-(tert-butyldimethylsilyloxy)octahydroindolizin-1-ol (**17**).* To a solution of the epoxide **16**[13] (20.0 mg, 0.042 mmol) in anhydrous THF (1 mL) was added dropwise a 1 M solution of lithium aluminium hydride in THF (62.4 μL, 0.062 mmol). The reaction was stirring for 20 h at rt and then evaporated to leave a residue, which was chromatographed on silica gel by FCC (20:80 EtOAc/petrol to 100% EtOAc) to give **17** as a yellow viscous oil (12 mg, 60%). R_f 0.59 (5:95 MeOH/EtOAc).$[\alpha]_D^{25}$ −31.1 (*c* 1.8, $CHCl_3$). MS (ESI +ve) *m/z* 484 ($M+H^+$, 100%). HRMS (ESI +ve) calculated for $C_{28}H_{42}NO_4Si$ ($M+H^+$) 484.2883, found 484.2871.IR ν_{max} (cm^{-1}): 3390, 3030, 2922, 2850, 1248, 1092. ^1H NMR δ 7.40–7.25 (m, 10H, Ar), 4.96 (d, 1H, *J* 12.0 Hz, C*H*HPh), 4.73 (d, 1H, *J* 12.0 Hz, C*H*HPh), 4.70 (d, 1H, *J* 12.0 Hz, C*H*HPh), 4.60 (d, 1H, *J* 12.0 Hz, C*H*HPh), 4.15 (br s, 1H, H-6), 4.01 (app. dt, 1H, *J* 9.4, 4.8 Hz, H-1), 3.80 (app. t, 1H, *J* 9.2 Hz, H-8), 3.35 (dd, 1H, *J* 9.2, 3.0 Hz, H-7), 2.94–2.90 (m, 2H, H-5 and H-3), 2.40 (app. dt, 1H, *J* 8.8, 8.0 Hz, H-3), 2.22–2.14 (m, 2H, H-5 and H-2), 1.95 (dd, 1H, *J* 9.4, 5.8 Hz, H-8a), 1.59–1.53 (m, 1H, H-2), 0.90 (s, 9H, *t*-Bu), 0.08 (s, 3H, CH_3), 0.04 (s, 3H, CH_3). ^{13}C NMR δ 138.6 (C), 138.5 (C), 128.5 (CH), 128.4 (CH), 128.3 (CH), 127.8 (CH), 127.5 (CH), 127.4 (CH), 85.2 (C-7), 78.5 (C-8), 75.2 (C-1), 74.7 (CH_2), 73.4 (C-8a), 71.7 (CH_2), 68.2 (C-6), 56.3 (C-5), 51.5 (C-3), 31.8 (C-2), 25.8 (C(CH_3)$_3$), 18.2 (C), −4.5 (CH_3), −4.53 (CH_3).

*4.1.7. (1R,6R,7R,8R,8aR)-Octahydroindolizine-1,6,7,8-tetraol (1,6-diepi-castanospermine (**18**)).* To a solution of **17** (37.9 mg, 0.079 mmol) in MeOH (2 mL) was added $PdCl_2$ (20.9 mg, 0.112 mmol). The mixture was stirred at rt under an atmosphere of H_2 (balloon) for 12 h,

followed by the dropwise addition of concd HCl (10 drops), The mixture was stirred at rt for 3 days. The mixture was filtered through a Celite pad and the solids were washed with MeOH. The filtrate was evaporated in vacuo and the residue was dissolved in water (1.5 mL) and applied to a column of Amberlyst A-26 (OH^-) resin (3 cm). Elution with water followed by evaporation in vacuo gave 1,6 di*epi*-castanospermine **18** in 95% purity, as a yellow viscous oil (14 mg). $[\alpha]_D^{25}$ −74.2 (*c* 1.5, MeOH), lit.[16] $[\alpha]_D^{24}$ −72.0 (*c* 0.7, MeOH). MS (ESI +ve) *m/z* 190 ($M+H^+$, 100%). HRMS (ESI +ve) calculated for $C_8H_{16}NO_4$ ($M+H^+$) 190.1079, found 190.1072. IR ν_{max} (cm^{-1}): 3365, 3308, 2914, 2888, 1444, 1202, 1060. ^1H NMR (D_2O) δ 4.29–4.25 (m, 1H, H-1), 4.03 (br s, 1H, H-6), 3.68 (t, 1H, $J_{7,8}$=$J_{8,8a}$=9.4 Hz, H-8), 3.53 (dd, 1H, $J_{7,8}$=9.4, $J_{6,7}$=3.5 Hz, H-7), 3.05 (dd, 1H, $J_{5a,5b}$=12.7, $J_{5a,6}$=2.5 Hz, H-5a), 2.92 (app. t, 1H, *J*=8.5 Hz, H-3), 2.48 (dt, 1H, $J_{2,3}$=$J_{3,3}$=9.3, $J_{2,3}$=9.0 Hz, H-3), 2.42 (d, 1H, $J_{5a,5b}$=12.7 Hz, H-5b), 2.34–2.25 (m, 1H, H-2), 1.99 (dd, 1H, $J_{8,8a}$=9.4, $J_{1,8a}$=6.5 Hz, H-8a), 1.67–1.62 (m, 1H, H-2). ^{13}C NMR (D_2O) δ 75.8 (C-7), 74.6 (C-1), 74.0 (C-8a), 72.2 (C-8), 69.3 (C-6), 55.7 (C-5), 51.7 (C-3), 32.9 (C-2).

*4.1.8. (1R,2R,3R,7R,7aR)-3-Hydroxymethyl-hexahydro-pyrrolizine-1,2,7-triol (7-epi-australine (**21**)).* To a solution of a 92:8 mixture of **19** and **20** (37.1 mg, 0.077 mmol) MeOH (2 mL) was added $PdCl_2$ (20.4 mg, 0.115 mmol). The mixture was stirred at rt under an atmosphere of H_2 (balloon) for 3 h, followed by the dropwise addition of concd HCl (eight drops). The mixture was stirred at rt for 17 h and was then filtered through a Celite pad and the solids were washed with MeOH. The combined filtrates were evaporated in vacuo and the residue was dissolved in water (1.5 mL) and applied to a column of Amberlyst A-26 (OH^-) resin (3 cm). Elution with water followed by evaporation in vacuo gave 7-*epi*-australine **21** (dr 92:8) as a pale yellow solid (13.0 mg, 90%). $[\alpha]_D^{23}$ −13.2 (*c* 1.2, H_2O), lit.[17] $[\alpha]_D^{25}$ −13.04 (*c* 0.55, H_2O, pH 8.37). MS (ESI +ve) *m/z* 190 ($M+H^+$, 100%). HRMS (EI +ve) calculated for $C_8H_{15}NO_4$ (M^+) 189.1001, found 189.0999. IR ν_{max} (cm^{-1}): 3358, 2980, 1340, 1126, 1027. ^1H NMR (D_2O) δ 4.34 (br s, 1H, H-7), 3.77 (dd, 1H, $J_{8a,8b}$=12.0, $J_{3,8a}$=4.5 Hz, H-8a), 3.76 (t, 1H, $J_{1,2}$=$J_{2,3}$=8.5 Hz, H-2), 3.71 (t, 1H, $J_{1,2}$=$J_{1,7a}$=8.0 Hz, H-1), 3.64 (dd, 1H, $J_{8a,8}$=11.8, $J_{3,8b}$=5.8 Hz, H-8b), 3.08 (dd, 1H, $J_{5,5}$=$J_{5,6}$ 10.8, $J_{5,6}$=6.0 Hz, H-5), 3.01 (br d, 1H, $J_{1,7a}$=8.0 Hz, H-7a), 2.89–2.84 (m, 1H, H-5), 2.69–2.64 (m, 1H, H-3), 2.11–2.04 (m, 1H, H-6), 1.80–1.74 (m, 1H, H-6). ^{13}C NMR (D_2O) δ 78.6 (C-1), 77.0 (C-2), 75.8 (C-7), 74.5 (C-7a), 69.0 (C-3), 63.4 (C-8), 52.4 (C-5), 32.3 (C-6).

*4.1.9. (1R,6S,7R,8R,8aR)-Octahydroindolizine-1,6,7,8-tetraol (1-epi-castanospermine (**23**)).* To a solution of **22** (9.0 mg, 0.019 mmol) MeOH (1 mL) was added $PdCl_2$ (6.6 mg, 0.037 mmol). The mixture was stirred at rt under an atmosphere of H_2 (balloon) for 3 h, followed by the dropwise addition concd HCl (four drops). The mixture was stirred at rt for 21 h and was then filtered through a Celite pad and the solids were washed with MeOH. The filtrate was evaporated in vacuo and the residue was dissolved in water (1 mL) and applied to a column of Amberlyst A-26 (OH^-) resin (3 cm). Elution with water followed by evaporation in vacuo gave 1-*epi*-castanospermine **23** as a colourless solid (3.3 mg, 94%). $[\alpha]_D^{22}$ +3.3 (*c* 0.3, MeOH), lit.[18] $[\alpha]_D^{26}$ +3.8 (*c* 0.5, MeOH). MS (ESI +ve) *m/z* 190 ($M+H^+$, 100%). HRMS (ESI +ve) calculated for $C_8H_{16}NO_4$ ($M+H^+$) 190.1079, found 190.1088. IR ν_{max} (cm^{-1}): 3330, 2924, 2822, 1444, 1312, 1086. ^1H NMR (D_2O) δ 4.26–4.22 (m, 1H, H-1), 3.61 (ddd, 1H, $J_{5a,6}$=10.8, $J_{6,7}$=9.3, $J_{5b,6}$=5.7 Hz, H-6), 3.39 (app. t, 1H, $J_{7,8}$=$J_{8,8a}$=9.3 Hz, H-8), 3.33 (app. t, 1H, $J_{6,7}$=$J_{7,8}$=9.3 Hz, H-7), 3.16 (dd, 1H, $J_{5a,5b}$=10.8, $J_{5a,6}$=5.7 Hz, H-5a), 2.95 (ddd, 1H, $J_{3,3}$=9.4, $J_{2,3}$=8.0, 1.5 Hz, H-3), 2.57 (dt, 1H, $J_{3,3}$=9.4, $J_{2,3}$=8.5 Hz, H-3), 2.34–2.28 (m, 1H, H-2), 2.24 (t, 1H, $J_{5a,5b}$=$J_{5b,6}$=10.8 Hz, H-5b), 2.12 (dd, 1H, $J_{8,8a}$=9.3, $J_{1,8a}$=6.5 Hz, H-8a), 1.70 (app. t, 1H, *J*=11.5 Hz, H-2). ^{13}C NMR (D_2O) δ 79.3 (C-7), 74.3 (C-1), 74.0 (C-8), 73.4 (C-8a), 70.5 (C-6), 55.5 (C-5), 51.4 (C-3), 33.0 (C-2).

Acknowledgements

We thank the Australian Research Council and the University of Wollongong for financial support and Chiang Mai University and the Thai Government for a Ph.D. scholarship to T.R.

Supplementary data

Supplementary data associated with this article can be found in online version at doi:10.1016/j.tet.2010.10.008.

References and notes

1. Matsumura, T.; Kasai, M.; Hayashi, T.; Arisawa, M.; Momose, Y.; Arai, I.; Amagaya, S.; Komatsu, Y. *Pharm. Biol.* **2000**, *38*, 302–307.
2. Davis, A. D.; Ritthiwigrom, T.; Pyne, S. G. *Tetrahedron* **2008**, *64*, 4868–4879.
3. Ritthiwigrom, T.; Pyne, S. G. *Org. Lett.* **2008**, *10*, 2769–2771.
4. Nash, R. J.; Thomas, P. I.; Waigh, R. D.; Fleet, G. W. J.; Wormald, M. R.; de Q. Lilley, P. M.; Watkin, D. J. *Tetrahedron Lett.* **1994**, *35*, 7849–7852.
5. Molyneux, R. J.; Benson, M.; Wong, R. Y.; Tropea, J. E.; Elbein, A. D. *J. Nat. Prod.* **1988**, *51*, 1198–1206.
6. Nash, R. J.; Fellows, L. E.; Plant, A. C.; Fleet, G. W. J.; Derome, A. E.; Baird, P. D.; Hegarty, M. P.; Scofield, A. M. *Tetrahedron* **1988**, *44*, 5959–5964.
7. Asano, N. In *Modern Alkaloids: Structure, Isolation, Synthesis and Biology*; Fattorusso, E., Taglialatela-Scafati, O., Eds.; Wiley-VCH: Weinheim, 2008; pp 111–138.
8. Nash, R. J.; Fellows, L. E.; Dring, J. V.; Fleet, G. W. J.; Derome, A. E.; Hamor, T. A.; Scofield, A. M.; Watkin, D. J. *Tetrahedron Lett.* **1988**, *29*, 2487–2490.
9. Kato, A.; Kano, E.; Adachi, I.; Molyneux, R. J.; Watson, A. A.; Nash, R. J.; Fleet, G. W. J.; Wormald, M. R.; Kizu, H.; Ikeda, K.; Asano, N. *Tetrahedron: Asymmetry* **2003**, *14*, 325–331.
10. Van Ameijde, J.; Horne, G.; Wormald, M. R.; Dwek, R. A.; Nash, R. J.; Jones, P. W.; Evinson, E. L.; Fleet, G. W. J. *Tetrahedron: Asymmetry* **2006**, *17*, 2702–2712.
11. Wormald, M. R.; Nash, R. J.; Watson, A. A.; Bhadoria, B. K.; Langford, R.; Sims, M.; Fleet, G. W. J. *Carbohydr. Lett.* **1996**, *2*, 169–174.
12. Kato, A.; Adachi, I.; Miyauchi, M.; Ikeda, K.; Komae, T.; Kizu, H.; Kameda, Y.; Watson, A. A.; Nash, R. J.; Wormald, M. R.; Fleet, G. W. J.; Asano, N. *Carbohydr. Res.* **1999**, *316*, 95–103; Asano, N.; Kuroi, H.; Ikeda, K.; Kizu, H.; Kameda, Y.; Kato, A.; Adachi, I.; Watson, A. A.; Nash, R. J.; Fleet, G. W. J. *Tetrahedron: Asymmetry* **2000**, *11*, 1–8; Yamashita, T.; Yasuda, K.; Kizu, H.; Kameda, Y.; Watson, A. A.; Nash, R. J.; Fleet, G. W. J.; Asano, N. *J. Nat. Prod.* **2002**, *65*, 1875–1881; Kato, A.; Kato, N.; Adachi, I.; Hollinshead, J.; Fleet, G. W. J.; Kuriyama, C.; Ikeda, K.; Asano, N.; Nash, R. J. *J. Nat. Prod.* **2007**, *70*, 993–997; AsanoN.; IkedaK.; KasaharaM.; AraiY.; KizuH.. *J. Nat. Prod.* **2004**, *67*, 846–850.
13. Ritthiwigrom, T.; Willis, A. C.; Pyne, S. G. *J. Org. Chem.* **2010**, *75*, 815–824 and references cited therein.
14. Cavdar, H.; Saracoglu, N. *Tetrahedron* **2009**, *65*, 985–989.
15. Pavia, D. L.; Lampman, G. M.; Kriz, G. S.; Vyvyan, J. R. *Introduction to Spectroscopy*, 4th ed.; Brooks/Cole: Belmont CA, 2009; p 242.
16. Fleet, G. W. J.; Ramsden, N. G.; Nash, R. J.; Fellows, L. E.; Jacob, G. S.; Molyneux, R. J.; di Bello, I. C.; Winchester, B. *Carbohydr. Res.* **1990**, *205*, 269–282.
17. Denmark, S. E.; Martinborough, E. A. *J. Am. Chem. Soc.* **1999**, *121*, 3046–3056.
18. Ina, H.; Kibayashi, C. *J. Org. Chem.* **1993**, *58*, 52–61.
19. Cronin, L.; Murphy, P. V. *Org. Lett.* **2005**, *7*, 2691–2693.
20. Davis, A. S.; Pyne, S. G.; Skelton, B. W.; White, A. H. *J. Org. Chem.* **2004**, *69*, 3139–3143.