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Isolation, biological activities, and synthesis of the natural casuarines

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Abstract

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ISOLATION, BIOLOGICAL ACTIVITIES AND SYNTHESIS OF THE NATURAL CASUARINES

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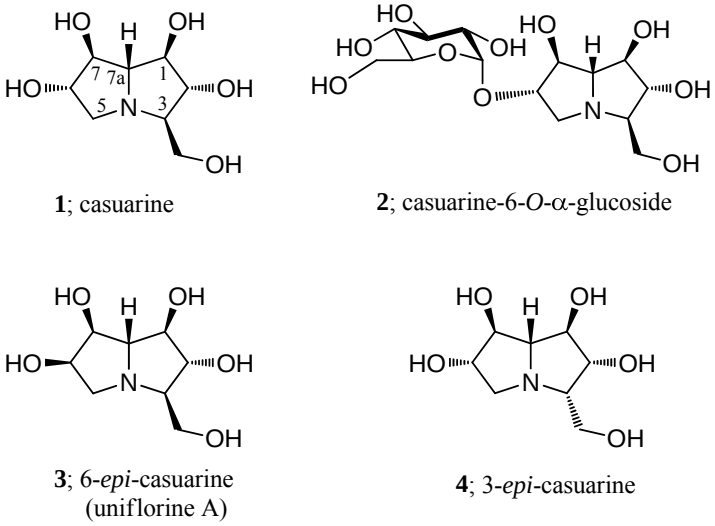
ABSTRACT: This chapter describes the isolation, structure elucidation, glycosidase inhibitory activities and the synthesis of the four naturally occurring casuarines. These are casuarine, casuarine-6-*O*-α-D-glucoside, 6-*epi*-casuarine (uniflorine A) and 3-*epi*-casuarine.

INTRODUCTION

Casuarine **1** [1], casuarine-6-*O*-α-glucoside **2** [2], 6-*epi*-casuarine **3** (uniflorine A) [3-5], and 3-*epi*-casuarine **4** [6] are members of the expanding group of polyhydroxylated 3-hydroxymethylpyrrolizidine natural products (Fig. (**1**)) [7]. This group also includes, australine [8], alexine [9] (7a-*epi*-australine), several other *epi*-australines (1-*epi*-australine, 3-*epi*-australine [10], 2,3-di*epi*-australine, 2,3,7-tri-*epi*-australine) [11], 1-*epi*-australine-2-*O*-α-glucoside and the more recently isolated hyacinthacine alkaloids 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-II 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, (uniflorine A and casuarine have six contiguous stereogenic carbons) has made these compounds attractive and important synthetic targets [13]. This chapter describes the isolation, structure elucidation, glycosidase inhibitory activities and the synthesis of the four naturally occurring casuarines.



1; casuarine

2; casuarine-6-*O*-α-glucoside

3; 6-*epi*-casuarine (uniflorine A)

4; 3-*epi*-casuarine

Fig. (1). Structures of casuarine **1**, casuarine-6-*O*-α-glucoside **2**, 6-*epi*-casuarine **3** (uniflorine A) and 3-*epi*-casuarine **4**

Isolation of the Natural Casuarines

Casuarina equisetifolia L., or commonly called, Australian pine, Filao or beach she oak is a plant in the family *Casuarinaceae*, native to South

East Asia, islands of the western Pacific Ocean (including French Polynesia, New Caledonia, Vanuatu), Australia (Northern Territory, north and east Queensland, and northeastern New South Wales) and West Africa. It is an evergreen tree that grows to over 6-35 m in height [14]

The first pentahydroxylated pyrrolizidine alkaloid, with six contiguous stereogenic centres and functional groups on all of the eight carbon atoms, was isolated in 1994 from the bark of *Casuarina equisetifolia L.* [1]. This bark was prescribed as a remedy to treat breast cancer in Western Somoa [2]. Extracts of the wood, bark and leaves of this plant have also been claimed to be useful for the treatment of diarrhoea, dysentery and colic [1]. This alkaloid was named casuarine **1**, (1*R*,2*R*,3*R*,6*S*,7*R*,7a*R*)-3-(hydroxymethyl)-1,2,6,7 tetrahydroxypyrrolizidine), by Nash *et al*. [1]. This investigation started with a GC-MS analysis of the per-trimethylsilylated bark extract which revealed a pentahydroxylated pyrrolizidine alkaloid and its glycoside as the major alkaloid components. The 75% aqueous ethanol bark extract was purified by ion-exchange column chromatography with Amberlite CG 120 (NH_4^+ form) which was eluted with 0.1 M NH_4OH to afford first the glycoside of casuarine **2** and then casuarine **1** itself (Fig. (**1**)). Both alkaloids were isolated in approximately the same amounts with latter in 0.013% yield based on the weight of the dried ethanol extract [1]. The absolute configuration of casuarine **1** was established by X-ray crystallographic analysis [1].

Eugenia jambolana is a plant in the family Myrtaceae, native to Bangladesh, India, Nepal, Pakistan and Indonesia. An evergreen tree it grows to 30 m in height. The extracts of the fruit pulp from *E.*

jambolana have been reported to have anti-diabetic properties, although this has been questioned in a more recent study [15]. In 1996, Wormald *et al.* [2] isolated casuarine **1** and its glucoside **2** from the leaves and the seeds of *Eugenia jambolana* using Amberlite CG 120 (NH_4^+ form) ion exchange chromatography. From 630 g of air dried leaves they isolated 140 mg of casuarine **1** and 15 mg of the glucoside **2**.

Eugenia uniflora, Surinam cherry, Brazilian cherry, or Cayenne cherry is a plant in the family Myrtaceae, native to tropical America and widely distributed in Paraguay, Uruguay, Argentina, and Brazil [3]. Decoctions of the leaves of this small tree are used as traditional medicines for a number of ailments, including use as an antidiabetic preparation. A number of studies have been made on the biological activities of the leaf extracts [16-18].

The water-soluble extracts of the leaves of *Eugenia uniflora* L. have been used as an antidiabetic agent in Paraguayan traditional medicine [3]. In 2000, Arisawa *et al.* [3] reported the isolation of uniflorine A and B from the leaves of this tree. The water-soluble extract was purified twice on Amberlite ion-exchange resins and then on silica gel and finally HPLC to give samples of uniflorine A, uniflorine B and (+)(3α, 4α, 5β)-1-methylpiperidine-3,4,5-triol in undisclosed amounts. The structures of the alkaloids uniflorine A and uniflorine B were deduced from NMR analysis to be that of the pentahydroxyindolizidine structures **3a** and **1a**, respectively (Fig. (**5**)).

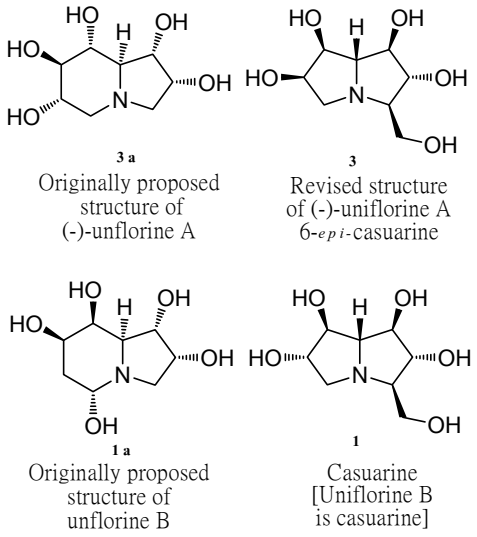


Fig. (5). Originally proposed and the revised structures of uniflorine A and B.

In 2004, Pyne and Davis [19] synthesised the proposed structure of uniflorine A however the NMR spectral data for synthetic **3a**, did not match with those reported for uniflorine A [3]. The structure of their synthetic **3a** was unequivocally established by a single-crystal X-ray crystallographic study of its pentaacetate derivative. The Wollongong researchers therefore concluded that the structure originally assigned to uniflorine A was not correct [19]. The initial thoughts of several researchers were that uniflorine A was a diastereoisomer of **3a**. In 2006, Dhavale *et al*. [20], in their paper of partial title, "Attempts To Find the Correct Structure of Uniflorine A.", reported the second synthesis of compound **3a**. Their sample of **3a** had NMR spectral data identical to those of **3a** that was earlier synthesised by Pyne *et al*. [20]. This paper also reported the synthesis of two diastereomers of **3a**, 8a-*epi*-**3a** and 1,2,8a-tri-*epi*-**3a**. In 2005 Mariano *et al.* [21] reported the synthesis of 1-

epi-**3a**, while that of 1,2-di-*epi*-**3a** had been reported by Fleet *et al.* in 1996 [22], before uniflorine A was even isolated, and later by Mariano *et al.* [21] and by Pyne *et al.* in 2008 [4]. In 2008 Pyne *et al.* reported the synthesis of 2-*epi*-**3a** (Fig. (**6**)) [4]. Despite these synthetic chemistry efforts these 1,2,6,7,8-pentahydroxyindolizidine molecules also had NMR spectral data significantly different to those of uniflorine A.

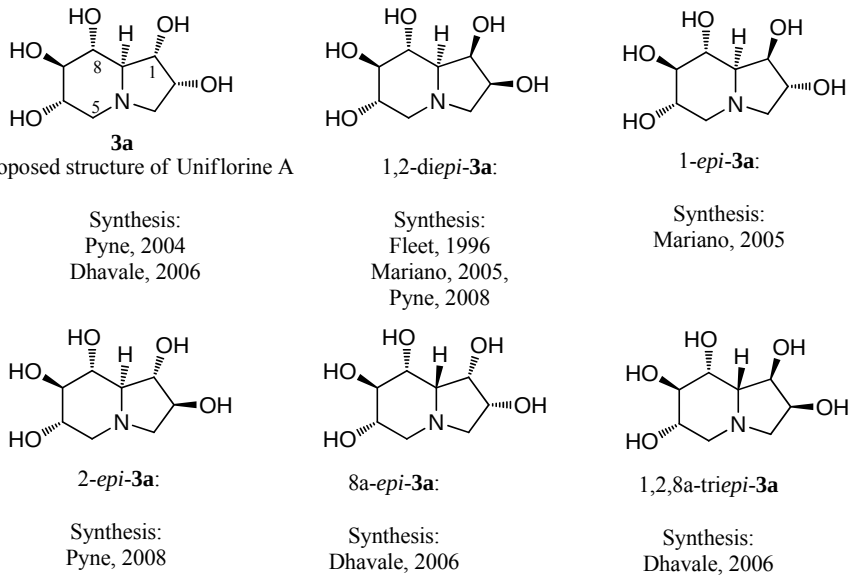


Fig. (6). Synthesis of diastereomers of structure **3a**.

From a re-examination of the original NMR data Pyne, Davis and Ritthiwigrom reassigned uniflorine B as the known pyrrolizidine alkaloid casuarine **1**, while the structure of (-)-uniflorine A was suggested to be that of 6-*epi*-casusarine **3** (Fig. (**5**)) [4]. The structure of uniflorine A was unequivocally established to be that of 6-*epi*-casuarine **3** by its total synthesis (see synthesis section) [5, 23, 24].

Myrtus communis L., commonly known as Myrtle or True Myrtle, belongs to the family Myrtaceae. It originates from the Mediterranean, North African and Western Asia regions. Casuarine **1** and 3-*epi*-casuarine **4** were isolated *M. communis* L. growing in the grounds of the Institute of Grassland and Environmental Research in Aberystwyth, UK. The isolation was conducted using ion exchange chromatography. Casuarine **1** was the major alkaloid present, which eluted first with water from the anion exchange resin Dowex 1 (OH form) followed by 3-*epi*-casuarine **4** ((1*R*,2*R*,3*S*,6*S*,7*R*,7a*R*)-3-(hydroxymethyl)-1,2,6,7-tetrahydroxypyrrolizidine (Fig. (1)) No other epimer of **1** was isolated. Casuarine **1** and 3-*epi*-casuarine **4** were crystallized from warm 95% aqueous ethanol by layering with acetone. The absolute configuration of 3-*epi*-casuarine **4** was established by X-ray crystallographic analysis [6].

Glycosidase Inhibitory Activities of the Natural Casuarines

The inhibitory activities of casuarine **1** and casuarine-6-*O*-α-glucoside **2** against a panel of 14 glycosidases were examined. Casuarine **2** was a much more potent inhibitor of α-D-glucosidases (for example, rice α-D-glucosidase (IC_{50} 1.2 μM) and rat intestinal maltase (IC_{50} 0.7 μM)) than casuarine-6-*O*-α-glucoside **2** (for example, rice α-D-glucosidase (IC_{50} 440 μM) and rat intestinal maltase (IC_{50} 260 μM)) [11]. In contrast, casuarine-*O*-α-glucoside **2** was a more active inhibitor of β-D-glucosidase from almond (IC_{50} 7.0 μM). Both compounds **1** and **2** were potent inhibitors of amyloglucosidase from *Aspergillis niger*, with IC_{50} values of 0.7 μM and 1.1 μM, respectively [11].

Casuarine **1** and casuarine-6-*O*-α-glucoside **2** were found to be inhibitors of the human *N*-terminal subunit of maltase-glucoamylase (NtMGAM) and *Escherichia coli* trehalose (Tre37A). Casuarine **1** and casuarine-6-*O*-α-glucoside **2** had *Ki* values of 0.45 μM and 280 μM, respectively, against human NtMGAM and *Ki* values of 17 μM and 12 nM, respectively against Tre37A [25]. The high potency of casuarine-6-*O*-α-glucoside **2** against Tre37A is most significant. These studies confirmed an earlier study that showed casuarine **1** and casuarine-6-*O*-α-glucoside **2** were active inhibitors of trehalase from porcine kidney with IC_{50} values of 12 μM and 0.34 μM, respectively [11].

There is current interest in inhibitors of these enzymes. Human maltase-glucoamylase is one enzyme involved in the digestion of starch to glucose. Inhibitors of this enzyme can be used to control the rate of glucose production and thus potentially aid in the treatment of type-II diabetes [26]. Trehalase is found mainly in the midgut of insects and converts trehalose, the major sugar in the blood of insects, to glucose which is vital for insect flight. Thus inhibitors of this enzyme may have potential as insecticides [26, 27]. X-ray crystal structures of the complexes of casuarine **1** with human NtMGAM and casuarine-*O*-α-glucoside **2** with Tre37A were determined and revealed similarities in the catalytic sites of these unrelated enzymes [25]. Computer-aided docking studies of casuarine **1** into the active site of NtMGAM were consistent with the X-ray crystal structure and both studies indicated that all 5 hydroxyl groups of casuarine **1** are involved in H-bonding to amino acid residues in the active site and the protonated nitrogen atom of casuarine **1** forms a salt bridge with Asp443 [28].

In another study, casuarine-6-*O*-α-glucoside **2** was found to be an nM inhibitor of trehaloses from midge larvae (*Chironomus riparius*), mammalian pig kidney and *E. coli*. Significantly, casuarine-6-*O*-α-glucoside **2** and two of its analogues were 10 or more times more potent on the insect trehalase than the other two enzymes indicating their potential as selective insecticides [29]. Other studies showed that casuarine **1** inhibited a membrane-bound trehalase from midge larvae (*C. riparius*) with an IC_{50} of 250 nM [30].

Uniflorine A and B were found to be inhibitors of the α-glucosidases, rat intestinal maltase (IC_{50} values of 12 and 4.0 μM, respectively) and sucrase (IC_{50} values 3.1 and 1.8 μM, respectively) [3]. The biological activity of the leaf extracts may be a result of the glycosidase inhibition activities of the natural product components, including the alkaloids uniflorine A and B [3]. The structures of these two alkaloids were later revised to be that of 6-*epi*-casuarine **3** and casuarine **1**, respectively [5, 23, 24]. In 2010, the results of the glycosidase inhibitory testing of 6-*epi*-casuarine **3** at 143 μg/mL showed 94-97% inhibition against the α-D-glucosidases of *Saccharomyces cerevisiae* and *Bacillus sterothermophilus* and against the amyloglucosidase of *Aspergillus 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 [31]. In the same assays, casuarine **1** had IC_{50} values of 139 μM and 5.6 μM, respectively [32].

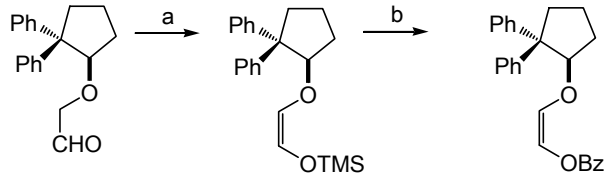
In contrast to casuarine **1**, 3-*epi*-casuarine **4** showed weak activity against three α-D-glucosidases (from yeast, rice and *Bacillus*) and was more active against β-D-glucosidase from almond (IC_{50} *ca*. 700

μM). In the same assay, casuarine **1** showed 0% inhibition of this latter enzyme at a concentration of 700 μM, while castanospermine ("the bench mark for β-D-glucosidase inhibition") had an IC_{50} of 20 μM [6].

Synthesis of the Natural Casuarines

Synthesis of casuarine **1**

The first synthesis of casuarine **1** was achieved by Denmark *et al.* [33a,b] in four additional steps from a key tandem [4+2]/[3+2] nitroalkene cycloaddition reaction in 20% overall yield. The synthesis commenced with the preparation of the enantiomerically enriched (98% ee) vinyl ether **7** (Scheme **1**). The chiral, alkoxy aldehyde **5** [33c] was converted to the silyl enol ether **6** in 99% yield as a 10:1 (*Z*/*E*) mixture. *O*-Benzoylation of **6** with benzoyl fluoride (BzF) and a catalytic amount of tetrabutylammonium fluoride (TBAF) (2 mol%) provided **7** as a mixture of *Z* and *E* vinyl ethers, which were separated by silica gel chromatography in yields of 81% and 6%, respectively (Scheme **1**).

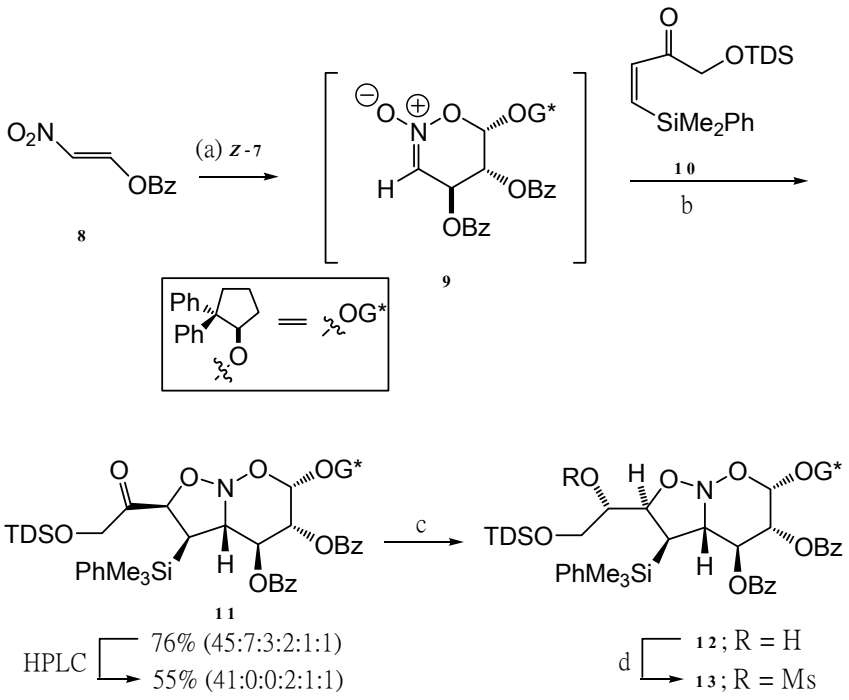


5 **6** **7**

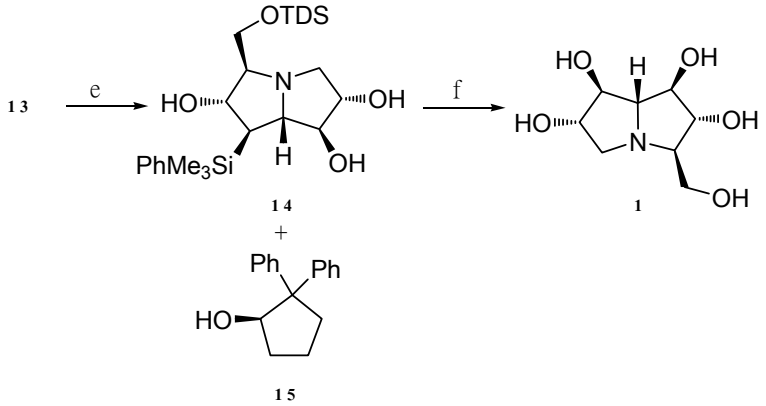
Scheme 1. Synthesis of the chiral vinyl ether **7**. *Reagents and conditions:* (a) TMSCl, Et_3N, CH_3CN, 81 °C, 99%; (b) BzF, TBAF, THF, 0 °C, 2 h, (81% *Z*: 6% *E*).

The chiral nitronate **9**, the 1,3-dipolar component of the [3+2] cycloaddition, was prepared from an *endo*-diastereoselective [4+2]-

cycloaddition reaction of the nitroalkene **8** and the chiral vinyl ether **7** in the presence of 2.5 equiv of $SnCl_4$ in toluene at -78 °C (Scheme **2**). The nitronate **9** was not stable and was immediately treated with the 1,1,2-trimethylpropylsilyl (TDS) protected β-phenyldimethylsilyl enone **10** to give a 45:7:3:2:1:1 mixture of six isomeric cycloadducts in 76% yield. Purification by HPLC afforded the desired nitroso acetal **11** in 55% overall yield. Reduction of the ketone group of **11** with L-selectride at -78 °C led to a 10:1 mixture of epimeric alcohols **12** in 87% yield. Mesylation of the secondary alcohol **12** gave the mesylate **13** in 97% yield. This mesylate was converted to the pyrrolizidine **14** in 64% yield *via* hydrogenolysis over Raney nickel and then hydrolysis of both benzoate esters under basic conditions. The final step was transformation of the C-1 silyl group to the final hydroxyl substituent (Tamao-Fleming reaction) by dearylation of the phenyldimethylsilyl group with mercuric trifluoroacetate in trifluoroacetic acid, followed by oxidation with peracetic acid to afford pure casuarine **1** in 84% yield after ion-exchange column chromatography. Since aldehyde **5** can be prepared from diphenylacetonitrile in six overall steps [33c] the total steps in this synthesis of casuarine **1** are 14 (or 13 if you count the one-pot reaction going from **8** to **11**).

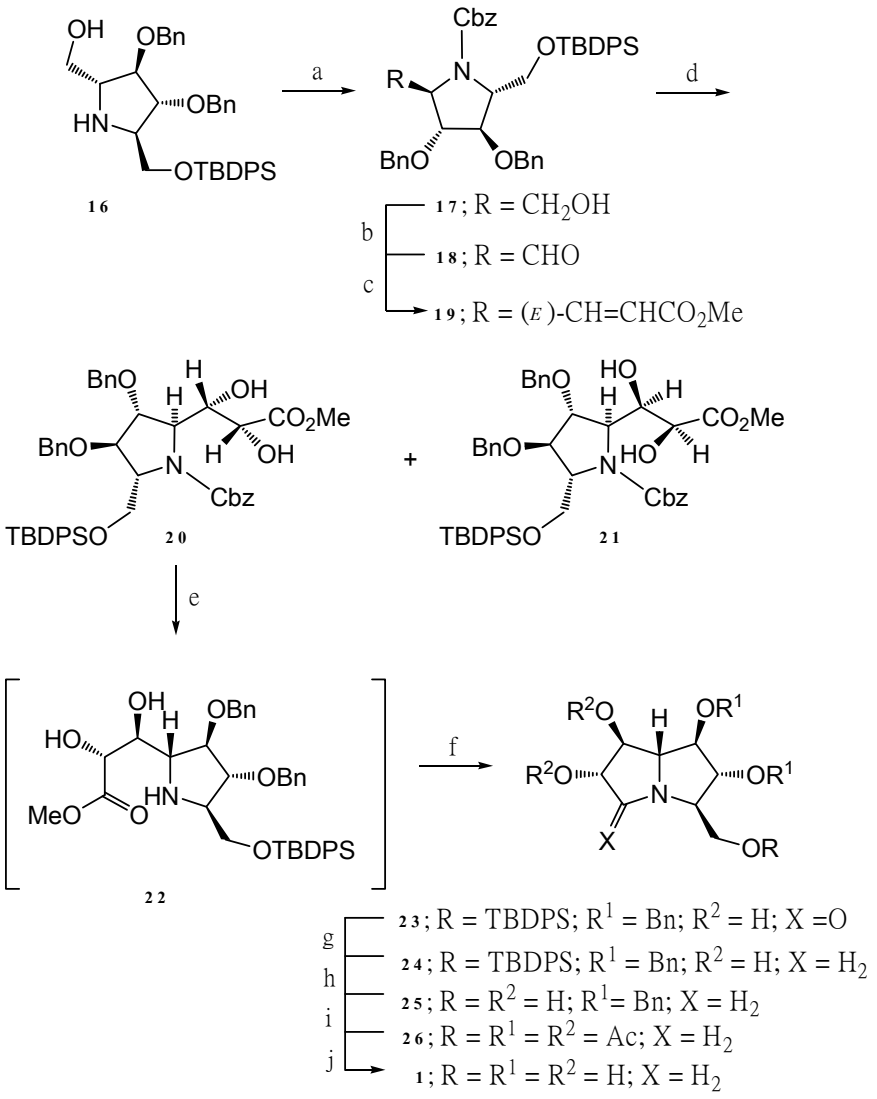


Scheme 2. Total synthesis of casuarine **1** by Denmark *et al.* [33a,b]. *Reagents and conditions:* (a) *Z*-**7**, $SnCl_4$, toluene, -78 °C (b) **10**, $CHCl_3$; (c) L-Selectride, THF, -78 °C, 87% (10:1); (d) Ms_2O, py, 1 h, 97%.



Scheme 2. (Continued) Total synthesis of casuarine **1** by Denmark *et al.* [33a,b]. *Reagents and conditions:* (e) i: Raney Nickel, MeOH, 260 psi H_2; ii: K_2CO_3, MeOH, rt, 64%; (f) $Hg(OTFA)_2$, TFA, HOAc, AcO_2H, 84%.

A synthesis of casuarine **1** and its 6,7-diepimer, in a stereocontrolled manner, was reported by Izquierdo *et al.* [34a]. The synthesis of casuarine **1** began with *N*-Cbz protection of the DMDP derivative **16** [34b] that gave the Cbz compound **17** in 93% yield (Scheme **3**). Primary alcohol oxidation and then a Wittig reaction of **18** gave the pyrrolidinic propenoate **19** (in 2 steps). Dihydroxylation of **19** using osmium tetraoxide and NMO in the presence of *O*-(4-chlorobenzoyl)hydroquinine (DHQ-CLB) as a chiral ligand gave a mixture of **20** and **21** in yields of 58% and 27%, respectively. The configuration of both diol products could not be determined at this stage. After two more steps, an NOE experiment confirmed that **20** was the desired intermediate to make casuarine **1**. *N*-deprotection of **20** under hydrogenolysis reaction conditions provided pyrrolidine **22** which was subsequently transformed to **23** by heating a methanol solution at reflux in the presence of a catalytic amount of NaOMe.

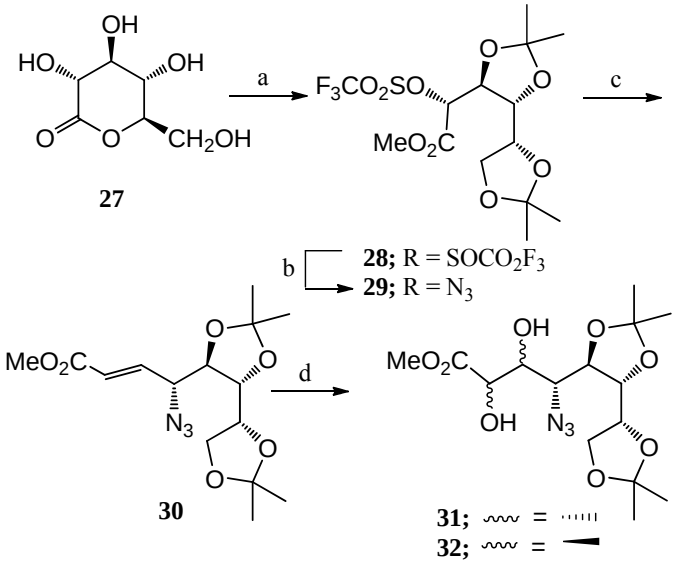


Scheme 3. Total synthesis of casuarine **1** by Izquierdo *et al.* [34a]. *Reagents and conditions:* (a) CbzCl, Me_2CO, K_2CO_3, rt, 93%; (b) TPAP, NMO, 4Å MS, CH_2Cl_2; (c) $Ph_3P{=}CHCO_2Me$, CH_2Cl_2, rt, 85% (from **17**); (d) OsO_4, NMO, DHQ-CLB, acetone/H_2O, rt, 2 d, (**20**:**21** = 58%:27%); (e) H_2, 10% Pd-C, MeOH; (f) cat. MeONa, MeOH, rt, 85%; (g) $BH_3{\cdot}SMe_2$, THF, then MeOH, Δ, 89%; (h) *n*-$Bu_4N^+F^-{\cdot}3H_2O$, THF, rt, 95%; (i) i: H_2, 10% Pd-C, MeOH, then Amberlite IRA-400 (OH^- form), ii: Ac_2O, py, DMAP, 41%; (j) cat. NaOMe, MeOH, rt, 93%.

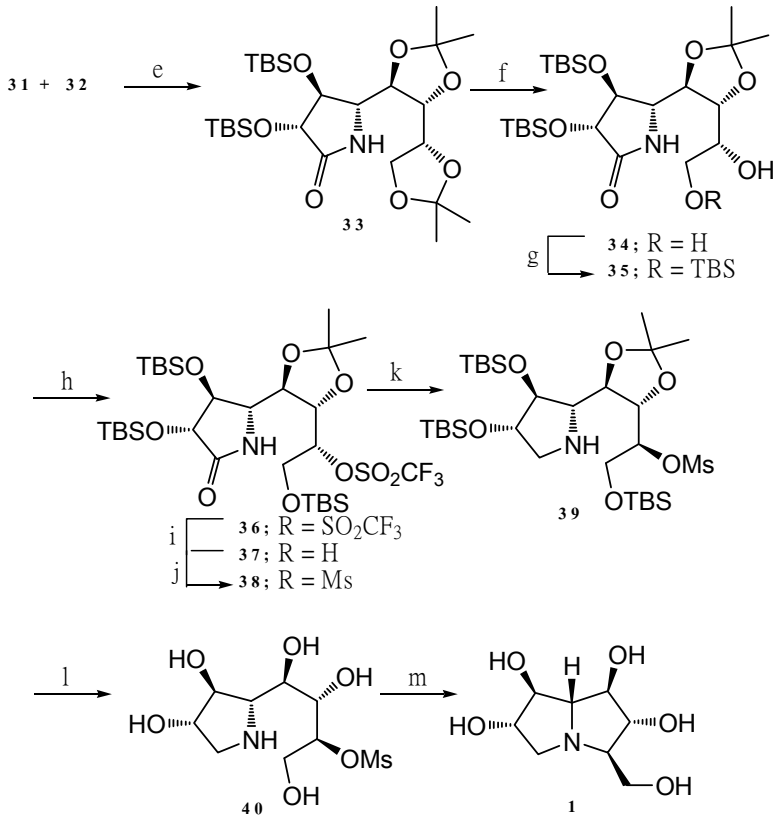
Reduction of the lactam carbonyl group of **23** using $BH_3 \cdot SMe_2$ complex gave **24** in 89% yield. *O*-TBDPS deprotection and then debenzylation of **24** gave **25** in 95% yield. Hydrogenolysis then gave an impure sample of **1**. This sample was further purified by peracetylation which gave **26** in 41% yield. Base catalysed deacetylation of **26** afforded casuarine **1** in 93% yield. This synthesis was achieved in 8 steps from the DMDP derivative **16** in 13 % overall yield. DMDP **16** was prepared in 5 steps from a compound derived from D-fructose [34b], thus the total number of synthetic steps using this route is more than 13.

In 2006, Fleet *et al.* [6] published the synthesis of casuarine **1** from D-gluconolactone **27** (Scheme **4**). D-Gluconolactone **27** was reacted with 2,2-dimethoxypropane and the open chain diacetonide, was subsequently esterified with trifluoromethanesulfonic anhydride to afford the triflate **28** in 72% yield. The trifate group of **28** was displaced with sodium azide in DMF to give the azide **29** in 97% yield. The unsaturated ester **30** was obtained from reduction of the azidoester **29** with DIBAL, followed by treatment of the resulting aldehyde with the Wittig reagent, $Ph_3P{=}CHCO_2Me$, in 75% yield over the two steps. The unsaturated ester **30** had an *E:Z* ratio of 10:1. After isolation of the pure *E* isomer of compound **30** it was converted to a mixture (1:4) of the diols **31** and **32**, using an OsO_4 catalysed dihydroxylation reaction, in 72% yield. Hydrogenation of this mixture gave a mixture of amines which cyclized to the pyrrolizidine framework upon heating in toluene. Finally, after treatment of the reaction mixture with TBSCl, the lactam **33** was separated in 70% yield over the 3 steps. The terminal acetonide of **33** was removed by acid hydrolysis to afford the diol **34** in 69% yield. Selective protection of the primary hydroxyl group of diol **34** with

TBSCl gave the secondary alcohol **35** in 81% yield. The remaining secondary hydroxyl group of **35** required inversion of its configuration. This was achieved by treatment with triflic anhydride to afford the unstable triflate **36** which was reacted with caesium trifluoroacetate. Base hydrolysis of the resulting trifluoroacetate gave the inverted alcohol **37** in 20% yield over the 3 steps. The desired lactam mesylate **38** was obtained in 90% yield by treating **37** with methanesulfonyl chloride. Reduction of the lactam carbonyl group of **38** with $BH_3 \cdot THF$ gave the amine **39** (57% yield). Finally pure casuarine **1** was obtained after 2 more steps, *O*-silyl group hydrolysis with TFA and then cyclization by treatment with sodium acetate (91% yield over the two steps). Overall the total number of synthetic steps was 13 starting from D-gluconolactone **27**.



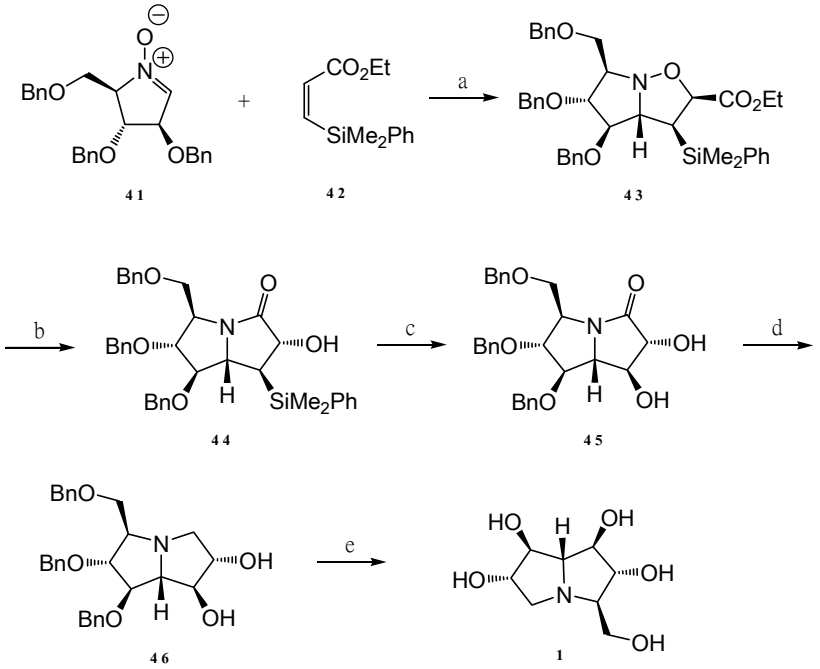
Scheme 4. Total synthesis of casuarine **1** by Fleet *et al.* [6]. *Reagents and conditions:* (a) $Me_2C(OMe)_2$, *p*-TsOH, MeOH; then $(CF_3SO_2)_2O$, py, CH_2Cl_2, 72%; (b) NaN_3, DMF, 97%; (c) *t*-Bu_2AlH, -78 °C; then $Ph_3P{=}CHCO_2Me$, toluene (75% over two steps); (d) cat. OsO_4, NMO, *t*-BuOH/H_2O, 72%.



Scheme 4. (Continued) Total synthesis of casuarine **1** by Fleet *et al.* [6]. *Reagents and conditions:* (e) H_2, Pd/C, THF; then toluene, Δ; then *t*-$BuMe_2SiCl$, imidazole, THF (70% over three steps); (f) 60% HOAc, H_2O/MeOH, 69%; (g) *t*-$BuMe_2SiCl$, py, 81%; (h) $(CF_3SO_2)_2O$, py, CH_2Cl_2; (i) CF_3CO_2Cs, 2-butanone; then K_2CO_3, MeOH (20% from **35**); (j) CH_3SO_2Cl, Et_3N, CH_2Cl_2, 90%; (k) BH_3·THF, THF, 57%; (l) 90% CF_3CO_2H, H_2O; (m) NaOAc, H_2O (91% over two steps).

In 2009, Goti *et al.* [25a] published the synthesis of casuarine **1** and the first total synthesis of its 6-*O*-α-glucoside **2**. Their key steps were a 1,3-dipolar cycloaddition reaction, a Tamao-Fleming reaction and a Mitsunobu reaction. Their total synthesis (Scheme **5**) began with a stereoselective cycloaddition reaction of the nitrone **41** with the alkene **42** in CH_2Cl_2 to give the isoxazolidine **43.** N-O bond cleavage of **43** with

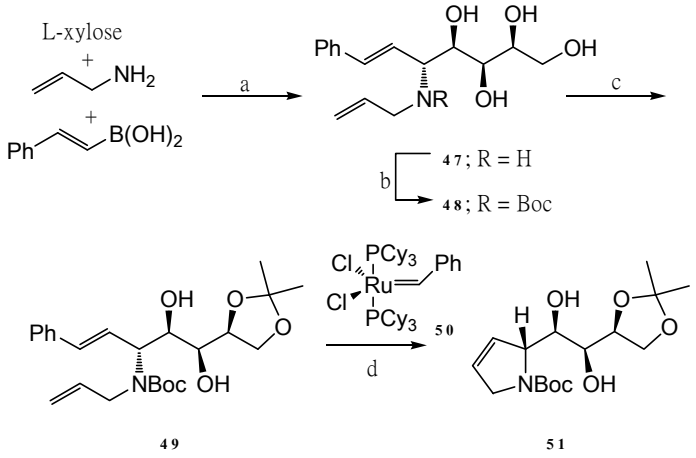
Zn/HOAc then attack of the amine on to the ester carbonyl group resulted in the lactam **44**. This compound was converted to **45** using the Tamao-Fleming reaction similar to that employed by Denmark (Scheme **2**) [33a,b]. Reduction of lactam **45** with $LiAlH_4$ gave **46** in 76% yield, which was debenzylated under standard hydrogenolysis reaction conditions to give pure casuarine **1** in five steps and 44% overall yield from the nitrone **41**. This latter compound is prepared in seven steps from L-xylose or D-arabinose [25b] making the total number of synthetic steps for the synthesis of casuarine **1** as 12 and the total overall yield 19%.



41 42 43 44 45 46 1

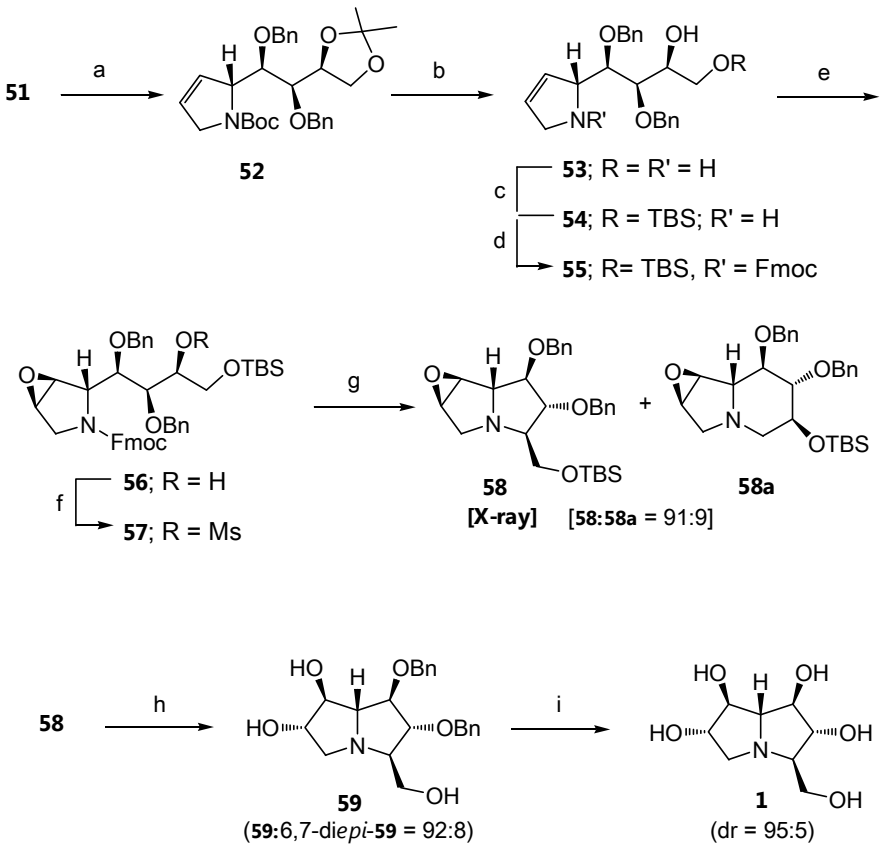
Scheme 5. Total synthesis of casuarine **1** by Goti *et al.* [25a]. *Reagents and conditions:* (a) CH_2Cl_2, rt, 36 h, 79%; (b) Zn, AcOH/H_2O, 60-65 °C, 5 h, 93%; (c) $Hg(CF_3CO_2)_2$, TFA, AcOH, AcOOH, $CHCl_3$, 76%; (d) $LiAlH_4$, THF, reflux, 78%; (e) H_2, Pd/C, MeOH, HCl, 100%.

The total synthesis of casuarine by Ritthiwigrom and Pyne was completed in 13 steps and 8% overall yield (Schemes **6** and **7**) [23]. The 1,2-*anti* aminoalcohol **47** was obtained from the boronic acid-Mannich reaction (Petasis reaction) of L-xylose, allylamine, and (*E*)-styrene boronic acid [19, 35] in 92% yield as a single diastereomer after purification by ion-exchange chromatography. The amino-tetraol **47** was converted to its *N*-Boc derivative **48** (80% yield) and then the terminal diol functionality of **48** was selectively protected as the acetonide derivative **49** under standard conditions. A ring-closing metathesis reaction of the diene **49** using Grubbs' first-generation ruthenium catalyst **50** provided the 2,5-dihydropyrrole **51** in 97% yield (Scheme **6**).



Scheme 6. Synthesis of **51**. [23] *Reagents and conditions:* (a) (*E*) PhCH=CHB(OH)$_2$, allyl amine, EtOH, rt, 3 d; ion-exchange, 92%; (b) (Boc)$_2$O, Et$_3$N, MeOH, rt, 3 d, 80%; (c) DMP, PPTS, acetone, rt, 20 h, 64%; (d) Grubbs' I **50**, CH_2Cl_2, 50 ^oC, 18 h, 97%.

The synthesis of casuarine **1** from the chiral 2,5-dihydropyrrole **51** is shown in Scheme **7**. To secure the 6α,7β-configuration of the target molecule the synthetic plan involved a regioselective ring-opening reaction of the epoxide **58** with an oxygen nucleophile (Scheme **7**).

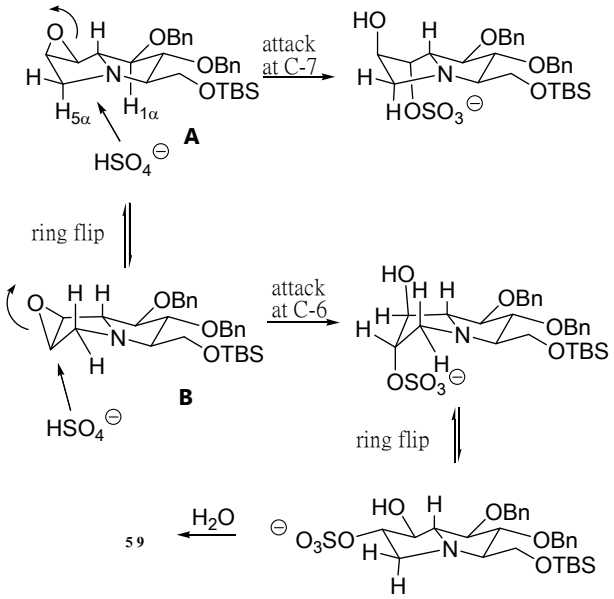


Scheme 7. Total synthesis of casuarine **1** from the precursor **51** by Ritthiwigrom and Pyne [23]. *Reagents and conditions:* (a) NaH, BnBr, *n*-Bu_4NI, THF, 18 h, 92%; (b) HCl/MeOH, rt, 30 h, 76%; (c) TBSCl, DMAP, imidazole, THF, rt, 1 d, 81%; (d) FmocCl, THF, sat. Na_2CO_3, 0 °C, 3 h, 94%; (e) CF_3COCH_3, oxone, $NaHCO_3$, $MeCN/H_2O$, 0 °C, 2 h, 81%; (f) MsCl, Et_3N, CH_2Cl_2, N_2, 0 °C, 3 h, 94%; (g) piperidine, MeCN, rt, 15 h, 96%; (h) $NaHSO_4$, CH_2Cl_2, reflux, 2 d; water, rt, 1 h, 51%; (i) $PdCl_2$, H_2 (1 atm), MeOH, rt, 1.5 h; ion-exchange, 93%.

To obtain the key epoxide **58**, the two unprotected secondary hydroxyl groups in **51** were protected as their *O*-benzyl ethers and the resulting dibenzyl ether **52** (92% yield) was treated under acidic conditions to effect hydrolysis of both the acetonide and *N*-Boc

protecting groups and to provide amino diol **53** in 76% yield. Regioselective *O*-silylation of **53** at the primary hydroxyl group gave the TBS ether **54** (81% yield) which was efficiently *N*-protected as its Fmoc derivative **55** in 94% yield. Epoxidation of the alkene moiety of **55** using 1,1,1-trifluoroacetone and oxone [36] provided the epoxide **56** in 81% yield as a single diastereomer. *O*-mesylation of the free secondary hydroxyl of **56** followed by treatment of the mesylate **57** (94% yield) with piperidine resulted in smooth *N*-Fmoc deprotection and then cyclization of the free cyclic secondary amine to give in 96% yield a 91:9 mixture of the desired pyrrolizidine **58** and the undesired indolizidine **58a**, respectively. It was assumed that **58** arose from *O*-TBS migration under the basic conditions of the *O*-mesylation reaction. Fortunately, pure **58** could be obtained by further separation of the mixture by column chromatography. The structure of the epoxide **58** was confirmed by a single crystal X-ray analysis. Several attempts in our laboratory to ring-open the epoxide group of compounds related to **58** using aqueous acid conditions (for example, H_2SO_4/water) led to complex mixtures and low yields of diol products. However, when **58** was treated under the conditions reported by Saracoglu [37], using $NaHSO_4$ as both the acid catalyst and the nucleophilic species in dichloromethane at reflux, followed by the addition of water to hydrolyze the intermediate sulfate, then the desired diol **59** was obtained as an 86:14 mixture of regioisomers. Purification of this mixture by column chromatography gave a 92:8 mixture of the diastereomeric diols **59** and 6,7-di-*epi*-**59**, respectively in 51% yield. The regiochemistry of this ring-opening reaction was consistent with that reported on related epoxy-pyrrolizidines [38] and was expected from stereoelectronic

considerations as shown in Scheme **8**. For *trans*-1,2-diaxial ring opening of epoxide **58** by HSO_4^-, the two reactive conformations, **A** and **B** are possible. Attack on conformation **A** at C-7 is inhibited by 1,3-diaxial interactions between the nucleophile (HSO_4^-) and the pseudo-axial protons H-1α and H-5α and thus addition to conformation **B** at C-6 predominates resulting in **59** as the major regioisomeric product. Hydrogenolysis of **59** over $PdCl_2/H_2$ gave casuarine **1**, in 93% yield after purification by ion-exchange chromatography. The diastereomeric purity of **1** was 95:5 from ^1H NMR spectroscopic analysis.



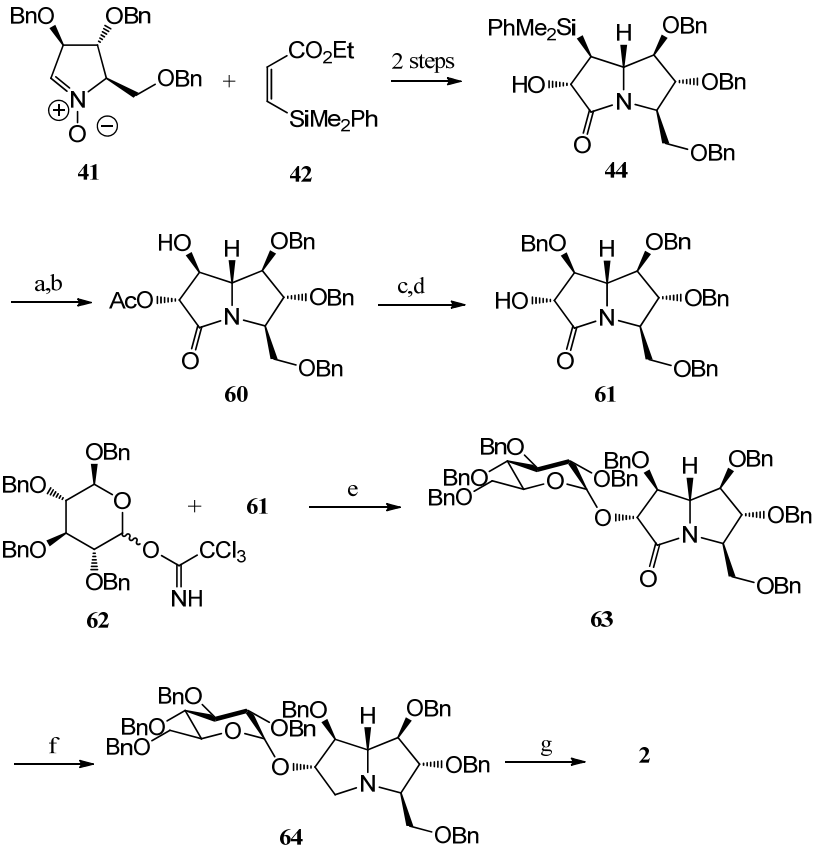
Scheme 8. Ring-opening reactions of epoxide **58** via conformations **A** and **B** [23].

Thus there have been five total syntheses of casuarine **1**, with that of Goti being the shortest and most efficient with a total of 12 synthetic steps from D-arabinose in an overall yield of 19%. The other four syntheses involve 13 or more steps, and unlike that of Goti, include the

separation of unwanted regioisomers, diastereomers or (*E*) and (*Z*) isomers. Further the Goti synthesis allows for the ready preparation of casuarine-6-*O*-α-D-glucoside **2** (Scheme **9**).

Synthesis of casuarine-O-α-glucoside **2**

In 2009, Goti *et al*. [25a] reported the synthesis of casuarine-6-*O*-α-D-glucoside **2** in the same publication in which they reported the synthesis of casuarine **1**. The synthesis of casuarine-6-*O*-α-D-glucoside **2** started with the same precursor **44**, followed by acetylation and the Tamao-Fleming reaction for the oxidation of the C-Si bond to afford the alcohol **60** (Scheme **9**).



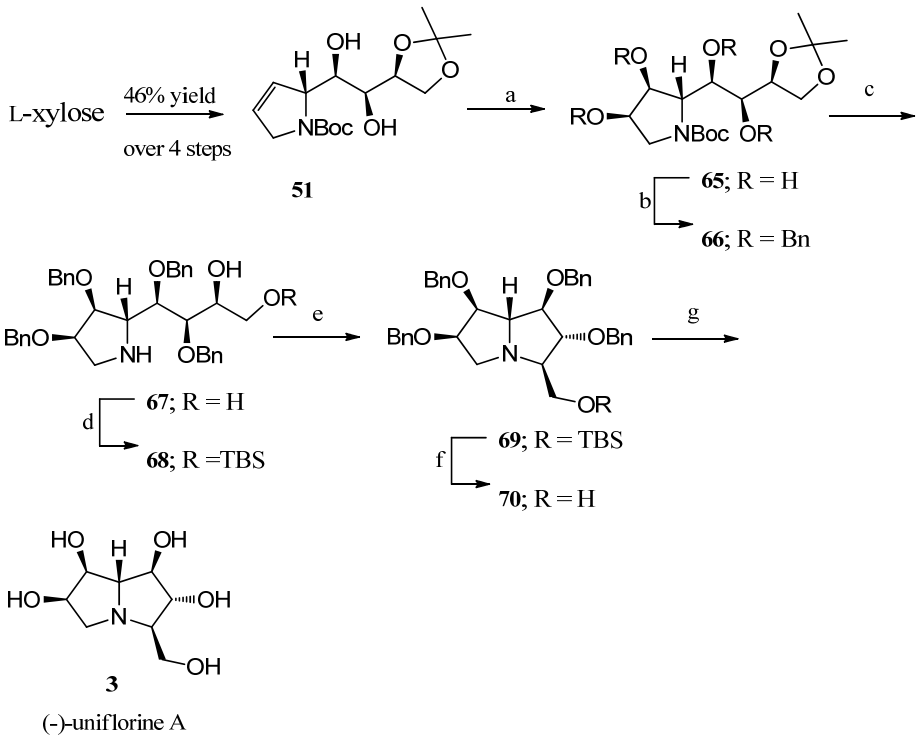
Scheme 9. Total synthesis of casuarine-6-*O*-α-D-glucoside **2** from the precursor **44** by Goti *et al.* [25]. *Reagents and conditions:* (a) Ac_2O, pyridine, rt, 15 h, 100%; (b) $Hg(CF_3CO_2)_2$, TFA, AcOH, AcOOH, $CHCl_3$, 82%; (c) $BnOC(=NH)CCl_3$, CF_3SO_3H, Et_2O, rt, 3 h; (d) Ambersep 900 OH, MeOH, rt, 15 h, 75% (2 steps); (e) TMSOTf, Et_2O, -20 °C, 40 min, 72%; (f) $LiBH_4$, BH_3·THF, THF, 23°C, 3 d, 68%; (g) H_2, Pd/C, MeOH, HCl, 77%.

Then, protection of secondary alcohol of **60** followed by an acetyl group deprotection sequence gave **61** in 75% yield over the 2 steps. Compound **63** was prepared by a coupling reaction between the alcohol **61** and the trichloroacetimidate **62**. The glucoside alkaloid **2** was obtained by reduction of the lactam carbonyl of **63** and then deprotection of **64** by hydrogenolysis over Pd/C.

Synthesis of 6-epi-casuarine (uniflorine A) **3**

The structural reassignment of uniflorine A to that of 6-*epi*-casuarine **3** was unequivocally confirmed in 2008 by Ritthiwigrom and Pyne on the basis of the total synthesis of *ent*-6-*epi*-casuarine **3** [(+)-uniflorine A] from D-xylose in 11 synthetic steps [5]. The NMR spectral data of the synthetic compound matched almost perfectly with that of the natural product. The specific rotation of synthetic (+)-uniflorine A (*ent*-6-*epi*-casuarine **3**) ($[\alpha]^{22}_{D}$ + 6.6 (*c* 0.35, H_2O) was essentially equal in magnitude and opposite in sign to that of the natural product (-)-uniflorine A, ($[\alpha]_D$ -4.4 (*c* 1.2, H_2O)). In 2010 these workers reported the total synthesis of the correct enantiomer of natural uniflorine A in 11 steps and 13% overall yield. This synthesis was the same as the previous one except that the starting material was D-xylose rather than L-xylose [23]. The synthesis of (-)-uniflorine A **3** from the chiral 2,5-dihydropyrrole **51** is shown in Scheme **10**. This intermediate was readily prepared on a 4 g scale from L-xylose in 4 steps and in 46% overall yield. The 2,5-dihydropyrrole **51** underwent an osmium(VIII)-catalyzed *syn*-dihydroxylation (DH) reaction to furnish the tetrol **65** as a single diastereomer in 72% yield (Scheme **10**). The stereochemical outcome of this DH reaction was expected due to the stereodirecting effect of the C-2 pyrrolidine substituent in **51** [4, 19, 39, 40]. The configuration of this diol was established by ROESY NMR studies on the final product **3**. The tetrol **65** was readily converted to its per-*O*-benzyl protected derivative **66** in 96% yield using standard reaction conditions. [19] Treatment of **66** under acidic conditions (HCl/MeOH) resulted in *N*-Boc and acetonide hydrolysis and gave the amino diol **67** in 81% yield.

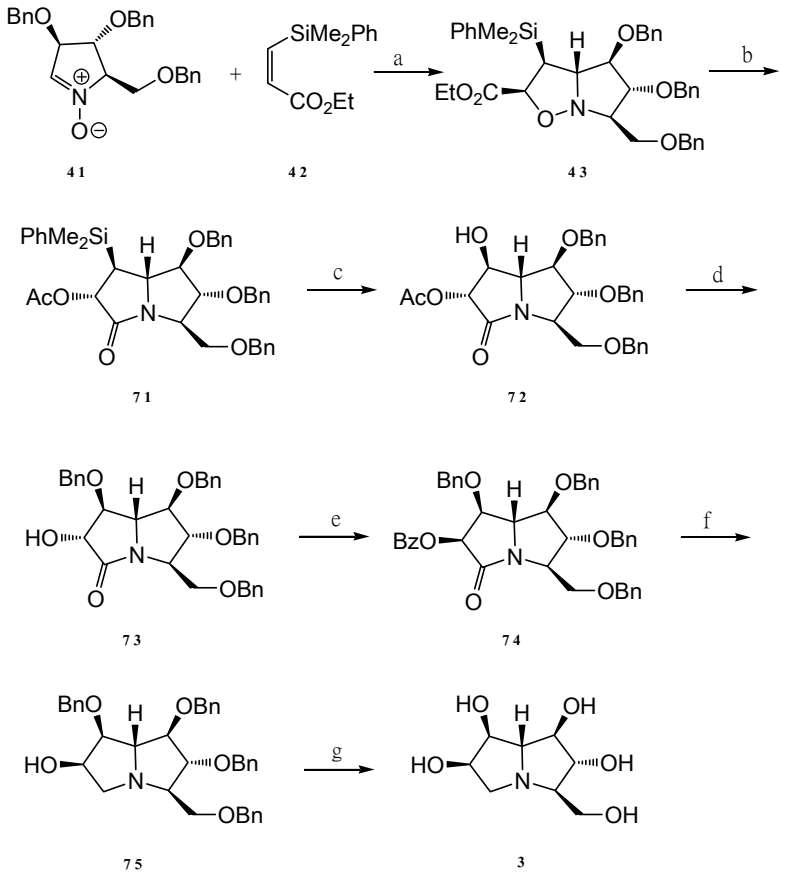
Regioselective *O*-silylation of **67** with TBSCl/imidazole/DMAP gave the primary silyl ether **68** in 85% yield. In the earlier synthesis of (+)-uniflorine A, compound *ent*-**68** underwent cyclization under Mitsunobu reaction conditions using pyridine [4, 41-43] as the solvent to give a mixture (*ca* 4:1) of the desired pyrrolizidine *ent*-**69** and an indolizidine product (structure not shown) in a combined yield of about 30% after purification of the crude reaction mixture by column chromatography. The undesired indolizidine product arose from first base catalyzed *O*-TBS migration to the secondary hydroxyl group in *ent*-**68** followed by Mitsunobu cyclization onto the primary carbon of the butyl side chain. It was found that by buffering the reaction mixture with $Et_3N{\cdot}HCl$ [44] that the yield of **69** could be dramatically improved to 76% with little or no formation of the undesired product. Acid hydrolysis of **69** gave the primary alcohol **70** in 90% yield which upon hydrogenolysis using $PdCl_2/H_2$ gave uniflorine A **3** in 87% yield after ion-exchange chromatography in a total of 11 synthetic steps and 13% overall yield from L-xylose.



Scheme 10. Synthetic route for (-) uniflorine A **3** by Ritthiwigrom and Pyne [23]. *Reagents and conditions*: (a) $K_2OsO_4 \cdot H_2O$, NMO, acetone/H_2O, rt, 18 h, 72%; (b) NaH, BnBr, *n*-Bu_4NI, THF, 24 h, 96%; (c) HCl/MeOH, rt, 18 h, 81%; (d) TBSCl, DMAP, imidazole, CH_2Cl_2, rt, 48 h, 85%; (e) DIAD, Ph_3P, Et_3NHCl, py, rt, 3 d, 76%; (f) HCl/MeOH, rt, 18 h, 90%; (g) $PdCl_2$, H_2 (1 atm), MeOH, rt, 24 h; ion-exchange, 87%.

In 2009, Goti *et al*. [24] reported the total synthesis of (-)-uniflorine A **3** in 9 steps and 11% overall yield from **41** or 16 steps from L-xylose or D-arabinose [25b] (Scheme **11**). Their syntheses started with the 1,3-dipolar cycloaddition reaction product **43** that they used earlier to prepare casuarine (Scheme **5**)**.** The lactam **71** was obtained from cleavage of the *N*-*O* bond in **43** with Zn/HOAc followed by attack of the resulting amine onto the ester carbonyl group to form a hydroxy lactam. This intermediate was then acetylated to give the lactam **71** in 93%

yield. The Tamao-Fleming reaction ($Hg(CF_3CO_2)_2$, TFA, AcOH, AcOOH) was used to convert the silyl group in **71** to the hydroxyl group in lactam **72** with retention of configuration. Benzylation of this hydroxyl group with $BnOC(=NH)CCl_3$, and CF_3SO_3H in Et_2O, also resulted in deprotection of the acetyl group at C-6, and provided compound **73** in 75% yield. The pyrrolizidine **75** was obtained by inversion of the stereochemistry at the C-6 position of compound **73** by a Mitsunobu reaction with BzOH, PPh_3, DIAD in THF that gave **74** in 75% yield. This was then followed by reduction of the lactam carbonyl group and deprotection of the benzoylated group with $LiAlH_4$. Debenzylation of the pyrrolizidine **75** using standard hydrogenolysis conditions gave (-) uniflorine A **3** in 71% yield after purification by ion-exchange chromatography with Dowex 50WX8 resin. The ^1H NMR and ^{13}C NMR spectroscopic data was identical with those from our previous synthesis [5]. This synthetic material **3** had a mp 117-180 °C and an $[\alpha]_D^{21}$-6.9 (*c* 0.42, H_2O).

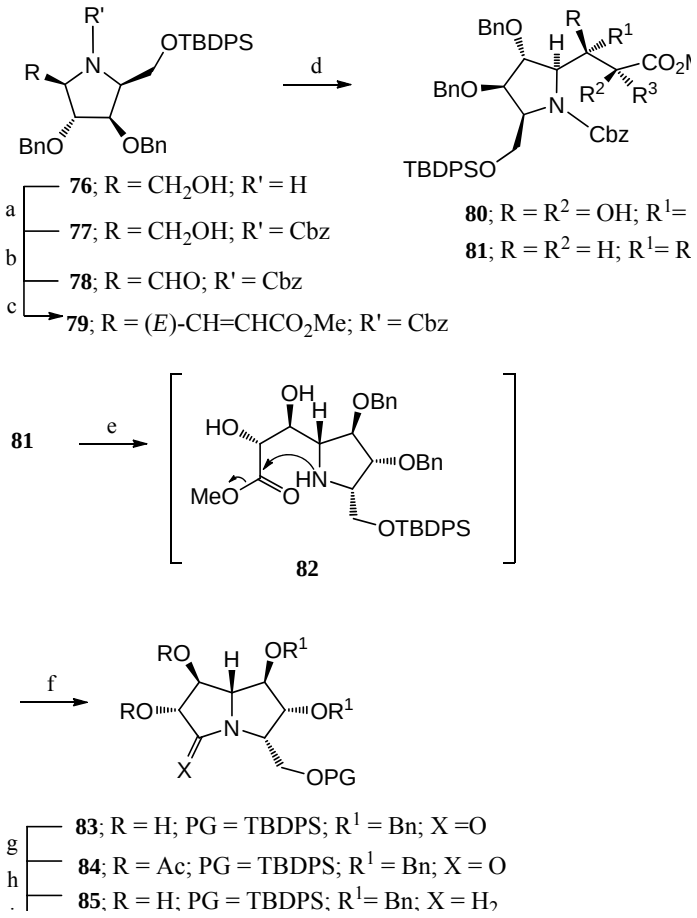


Scheme 11. Total synthesis (-)-uniflorine A **3** by Goti *et al*. [24]. *Reagents and conditions*: (a) CH_2Cl_2, rt, 36 h, 79%; (b) i: Zn, AcOH/H_2O, 60–65 °C, 5 h, 93%; ii: Ac_2O, py, rt,15 h, 100%; (c) $Hg(CF_3CO_2)_2$, TFA, AcOH, AcOOH, $CHCl_3$, 82%; (d) i: $BnOC(=NH)CCl_3$, CF_3SO_3H, Et_2O, rt, 3 h; ii: Ambersep 900 OH, MeOH, rt, 15 h, 75% (2 steps); (e) BzOH, PPh_3, DIAD, THF, rt, 75%; (f) $LiAlH_4$, THF, reflux, 45%; (g) H_2, 10% Pd/C, MeOH, HCl, rt, then Dowex 50WX8, 6% NH_4OH, 71%.

Synthesis of 3-epi-casuarine **4**

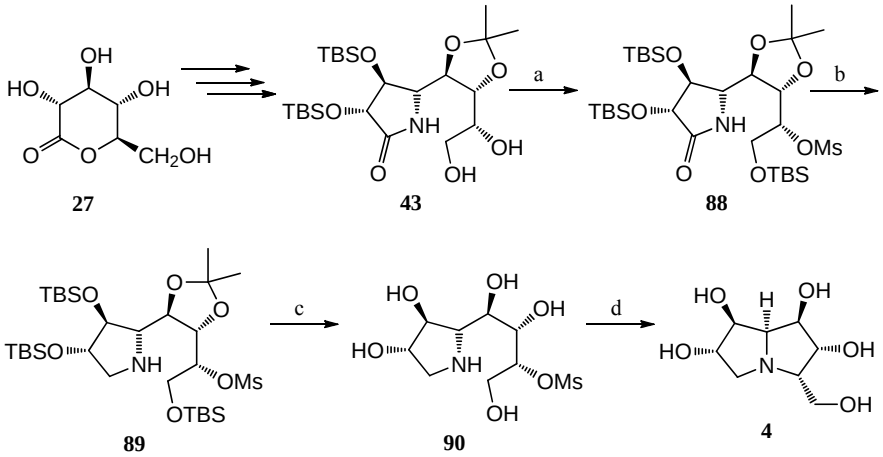
In 2006, Izquierdo *et al.* [45] published the synthesis of 3-*epi*-casuarine **4** in the same year that Fleet *et al.*[6] reported its isolation as a natural product and also its synthesis. The synthesis of 3-*epi*-casuarine **4** by

Izquierdo *et al.* [45] involved the same methodology that they used for the synthesis of casuarine **1** (Scheme **3**) except using the pyrrolidine **76** as the starting material. *N*-Cbz protection of **76** gave the Cbz carbamate **77** in only 25% yield (Scheme **12**). The primary alcohol of **77** was oxidized using TPAP and NMO to afford the aldehyde **78** which after a Wittig reaction gave the (*E*)-pyrrolidinic propenoate **79** (93% yield). A *cis*-DH reaction of **79** using osmium tetraoxide and NMO in the presence of *O*-(4-chlorobenzoyl)hydroquinine (DHQ-CLB) as a chiral ligand gave the diols **80** (13% yield) and **81** (84% yield). The configuration of these diol products could not be determined at this stage. After two more synthetic steps an NOE experiment confirmed that **82** was the desired intermediate to prepare 3-*epi*-casuarine **4**. *N*-deprotection of **81** using catalytic hydrogenolysis provided pyrrolidine **82** which was subsequently transformed to **83** by refluxing in methanol in the presence of a catalytic amount of NaOMe. Acetylation under standard reaction conditions then produced the acetate derivative **84** in an 88% yield. Reduction of the lactam carbonyl group of **84** using $BH_3 \cdot SMe_2$ complex in THF gave **85** in 96% yield. *O*-TBDPS deprotection and then debenzylation provided **86** in 73% yield. Hydrogenolysis then gave the final compound, however it was not pure. The product was further purified by per-acetylation that gave **87** in 88% yield. Base catalysed deacetylation of **87** afforded 3-*epi*-casuarine **4** in 66% yield. This synthesis was achieved in 12 steps from the pyrrolidine derivative **76** in an overall yield of 2.2%.



Scheme 12. Total synthesis of 3-*epi*-casuarine **4** by Izquierdo *et al.* [45]. *Reagents and conditions:* (a) CbzCl, Me_2CO, K_2CO_3, rt, 25%; (b) TPAP, NMO, 4°A Ms, CH_2Cl_2, 64%; (c) $Ph_3P{=}CHCO_2Me$, CH_2Cl_2, rt, 93%; (d) OsO_4, NMO, DHQ-CLB, acetone/H_2O, rt, 2 d, (**80**:**81** = 13%:84%); (e) H_2, 10% Pd-C, MeOH; (f) cat. NaOMe, MeOH, rt, 63%; (g) Ac_2O, py, DMAP, 88%; (h) $BH_3{\cdot}SMe_2$, THF, then MeOH, Δ, 96%; (i) *n*-$Bu_4N^+F^-{\cdot}3H_2O$, THF, rt, 73%; (j) i: H_2, 10% Pd-C, MeOH, then Amberlite IRA-400 (OH^- form), ii: Ac_2O, py, DMAP, 70%; (k) cat. NaOMe, MeOH, rt, 66%.

In 2006, Fleet *et al.* [6] reported the synthesis of casuarine **1** (Scheme **4**) together with the synthesis of 3-*epi*-casuarine **4** from D-gluconolactone **27** in the same publication (Scheme **13**). [6] He followed the same methodology that he used to synthesize casuarine **1** up to the precursor **34**. Regioselective protection of the primary hydroxyl group of diol **34** with TBSCl and then reaction at the secondary hydroxyl group by treatment with methanesulfonyl chloride generated the mesylate **88** in 66% yield. Reduction of the lactam carbonyl group of **88** with $BH_3 \cdot THF$ gave the protected amine **89** (57% yield). Finally pure 3-*epi*-casuarine **4** was obtained after 2 more steps; (i) *O*-silyl group hydrolysis with TFA to produce **90**; and then (ii) cyclization by treatment with sodium acetate (89% yield over the two steps).

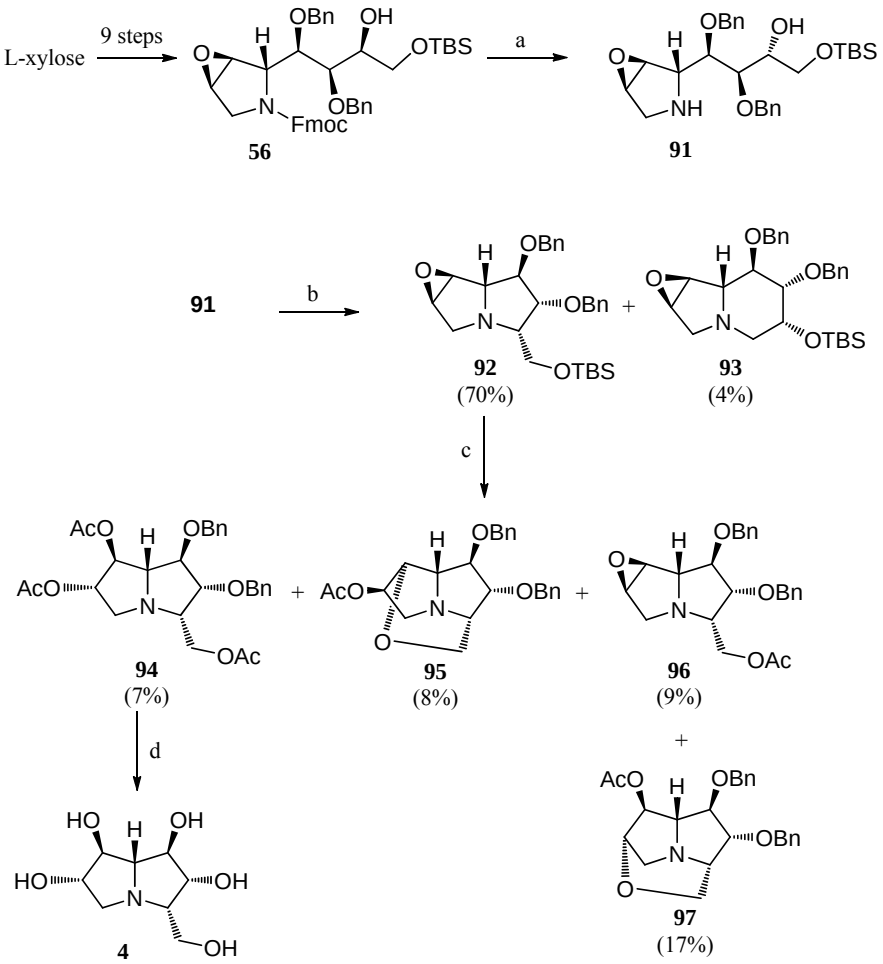


Scheme 13. Total synthesis of 3-*epi*-casuarine **4** by Fleet *et al.* [6]. *Reagents and conditions:* (a) *t*-$BuMe_2SiCl$, py; then CH_3SO_2Cl, Et_3N, CH_2Cl_2, 66%; (b) $BH_3 \cdot THF$, THF, 57%; (c) 90% CF_3CO_2H, H_2O; (d) NaOAc, H_2O (89% over two steps).

Ritthiwigrom and Pyne's synthesis of 3-*epi*-casuarine **4** [31] started with the precursor **56** which was prepared in 9 steps from L-xylose (Scheme **14**). Their syntheses required an inversion of the

configuration of the butyl side-chain secondary hydroxyl group in **56**. This was achieved by the Mitsunobu reaction of **56** using 4-nitrobenzoic acid [46]. Base treatment (K_2CO_3/MeOH, rt, 1 d) of the resulting secondary 4-nitrobenzoate ester resulted in benzoate hydrolysis. Cyclization under Mitsunobu reaction conditions, using toluene rather than pyridine as the solvent, gave a separable mixture the desired pyrrolizidine **92** in 70% yield and the indolizidine product **93** in 4% yield. The epoxy-pyrrolizidine **92** was treated with $NaHSO_4/CH_2Cl_2$ under the conditions that were described in Scheme **7** except that heating at 50 °C was continued for 7 days (Scheme **14**). The slower rate of epoxide ring-opening of **92**, when compared to that of 3-*epi*-**92**, was attributed 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 the mixture and then separation by column chromatography gave the desired acetylated product **94** (7% yield), the epoxide **96** in 9% yield and the undesired tricyclic bridged ether products **95** (8%) and **97** (17%) (Scheme **14**). The isolation of epoxide **96** 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 product **94** were not successful. While the use of a more stable protecting group for the primary alcohol group in **92** may have been more efficient, this variation was not examined. Hydrogenolysis of **94** over $PdCl_2/H_2$ in MeOH for 4

days gave diastereomerically pure 3-*epi*-casuarine **4** in 77% yield after purification by ion-exchange chromatography.



Scheme 14. Total synthesis of 3-*epi*-casuarine **4.** [31] *Reagents and conditions*: (a) i: *p*-NO_2ArCO_2H, PPh_3, toluene, 80 °C, 5 h; ii: K_2CO_3, MeOH, rt, 24 h, 50%; (b) DIAD, Ph_3P, toluene, 80 °C, 3 d; (c) i: $NaHSO_4$, CH_2Cl_2, 50 °C, 7 d; ii: Ac_2O, py, DMAP, 24 h; (d) $PdCl_2$, H_2 (1 atm), MeOH, 4 d; ion-exchange, 77%.

CONCLUSIONS

In summary, we have described the isolation, structure elucidation, glycosidase inhibitory activities and the synthesis of the four naturally occurring casuarines, namely, casuarine **1**, casuarine-6-*O*-α-D-glucoside **2**, 6-*epi*-casuarine **3** (uniflorine A) and 3-*epi*-casuarine **4**. Casuarine **1** is a potent inhibitor of α-D-glucosidases whereas casuarine-*O*-α-glucoside **2** was found to be a more active inhibitor of β-D-glucosidase. Both compounds **1** and **2** were potent inhibitors of amyloglucosidase from *Aspergillis niger*. Casuarine **1** was found to be a potent inhibitor of the human *N*-terminal subunit of maltase-glucoamylase (NtMGAM) which could potentially aid in the treatment of type-II diabetes. Casuarine-*O*-α-glucoside **2** was found to be a potent (nM) trehalase inhibitor with potential use as an insecticide. X-ray structural analysis and computer aided docking studies of these natural products bound to these target enzymes have been carried out. These studies may assist in the design of more potent inhibitors in future studies. The originally assigned structures of uniflorine A and B were found to be incorrect. Based on careful NMR analysis and the total synthesis of uniflorine A, their structures were concluded to be 6-*epi*-casuarine **3** and casuarine **1**, respectively. 6-*Epi*-casuarine **3** was found to be an inhibitor of α-glucosidases and amyloglucosidase from *Aspergillis niger*. In contrast to casuarine **1**, 3-*epi*-casuarine **4** showed weak activity against three α-D-glucosidases (from yeast, rice and *Bacillus*) and was more active against β-D-glucosidase from almond. Future phytochemical studies may unveil other new casuarine epimers or glycoside derivative natural products.

ABBREVIATIONS

[α]	= specific rotation
Ac	= acetyl
Boc	= *tert*-butoxycarbonyl
Bn	= benzyl
Bz	= benzoyl
Cbz	= benzyloxycarbonyl
DH	= dihydroxylation
DHQ-CLB	=*O*-(4-chlorobenzoyl)hydroquinine
DIBAL	= di-iso-butylaluminium hydride
DMAP	= 4-(*N*,*N*-dimethylamino)pyridine
DMDP	=2,5-dihydroxymethyl-3,4-dihydroxypyrrolidine
DMF	= dimethylformamide
Fmoc	= 9-fluorenylmethoxycarbonyl
IC_{50}	= 50% inhibitory concentration
K_i	= the dissociation constant for binding of inhibitor to enzyme
TDS	= 1,1,2-trimethylpropylsilyl
THF	= tetrahydrofuran
μM	= micro molar
NMO	= *N*-methylmorpholine *N*-oxide
NOE	= NOE nuclear Overhauser effect
nM	= nano molar

NtMGAM	= *N*-terminal subunit of maltase-glucoamylase
RCM	= ring-closing metathesis
ROESY	= rotating frame Overhauser effect spectroscopy
TBAF	= tetrabutylammonium fluoride
TBDPS	= *tert*-butyldiphenylsilyl
TBS	= *tert*-butyldimethylsilyl
TMS	= trimethylsilyl
TPAP	= tetrapropylammonium perruthenate
Tre37A	= *Escherichia coli* trehalose

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