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Grandisine A and B, Novel Indolizidine Alkaloids with Human δ-Opioid Receptor Binding Affinity from the Leaves of the Australian Rainforest Tree *Elaeocarpus grandis*

Anthony R. Carroll,*,† Garrie Arumugan,† Ronald J. Quinn,† Joanne Redburn,† Gordon Guymer,‡ and Paul Grimshaw‡

Natural Product Discovery, Eskitis Institute, Griffith University, Brisbane, Queensland 4111, Australia and Queensland Herbarium, Brisbane Botanic Gardens, Toowong, Brisbane, Queensland, Australia 4006

a.carroll@griffith.edu.au

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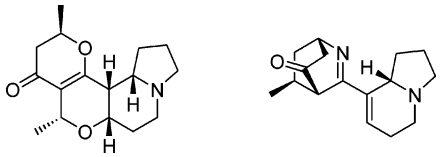
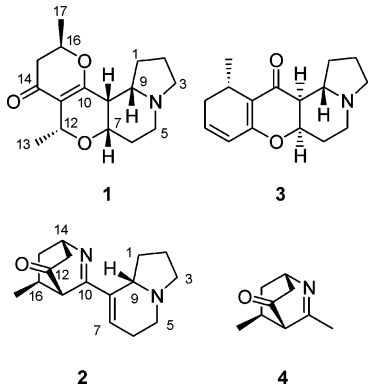


Two novel indolizidine alkaloids, grandisine A (**1**) and B (**2**), and the known alkaloid (−) isoelaeocarpiline (**3**) were isolated from the leaves of *Elaeocarpus grandis* and their structures determined by 1D and 2D NMR spectroscopy. The compounds showed affinity for the human δ-opioid receptor. Grandisine A contains a unique tetracyclic skeleton, while grandisine B possesses the unique combination of isoquinuclidinone and indolizidine groups in one molecule.

Agonists and antagonists of opioid receptors have long been known to modulate pain. The opioid receptors are G-protein coupled receptors that have been classified into three subtypes μ, κ, and δ. The occurrence of major side effects including dependence, respiratory depression, and muscle rigidity associated with drugs which modulate the μ-opioid receptor have led to the search for analgesics which act selectively at the δ-opioid receptor. Agonists of the δ-opioid receptor have been shown in animal models to provide antinocicepton without these undesirable side effects.[1] As part of our continuing high throughput screening (HTS) drug discovery program, an extract from the leaves of the Australian rainforest tree, *Elaeocarpus grandis*, was targeted for chemical evaluation as it displayed human δ opioid receptor affinity. The plant family Eleaocarpaceae has been recognized as a major alkaloid producer for several decades.[2,3] While the genus *Aristotelia* from Australia, New Zealand, and South America has yielded many novel indole alkaloids and the Australian genus *Peripentadenia* has furnished some rather simple pyrolidine alkaloids, it is members of the genus *Elaeocarpus*, almost exclusively collected from Papua New Guinea, that have provided a rich variety of novel indolizidine alkaloids.[2,3] This paper describes the bioassay guided isolation, structure determination, and human δ-opioid receptor affinity of two novel indolizidine alkaloids, grandisine A (**1**) and B (**2**), along with the known indolizidine alkaloid, (−) isoelaeocarpiline (**3**),[4] which have been identified as the bioactive components from *Elaeocarpus grandis*. Grandisine A (**1**) is a structurally unique pyrano[2′,3′:4,5]pyrano[2,3-*g*]indolizin-4-one alkaloid. Grandisine B (**2**) is the only alkaloid isolated to date that contains both an indolizidine and an isoquinuclidinone moiety. The isoquinuclidine moiety is a partial structure common to both the ibogaine alkaloids and alkaloids isolated from the genus *Daphniphyllum*. Isoquinuclidinone alkaloids are however very rare. Mearsine (**4**), an alkaloid isolated from *Peripentadania mearsii*,[5] together with grandisine B (**2**) are the only representative alkaloids of this class. This is the first reported example of an Australian *Elaeocarpus* species to produce alkaloids.



1 **3**

2 **4**

High-throughput screening of approximately 40 000 extracts from Australian plants and marine organisms identified CH_2Cl_2 and MeOH extracts from the leaves of *E. grandis* to inhibit the binding of iodinated deltorphin to the human δ-opioid receptor at 50 μg/mL. These extracts were combined and the alkaloids separated from the neutral and acid components by partitioning between CH_2Cl_2 and H_2SO_4 followed by basification of the aqueous layer with NH_3 and partitioning back into CH_2Cl_2. The alkaloids were chromatographed on amino bonded silica with a stepped gradient from CH_2Cl_2 to MeOH. The known alkaloid, (−) isoeleaocarpiline (**3**), which was mildly active, eluted first. A later eluting more active fraction was further purified by HPLC on amino bonded silica, elution with hexane/isopropyl alcohol/NH_3 yielding grandisine A (**1**) and B (**2**) as the active components.

* To whom correspondence should be addressed. Tel: 61 7 38756015. Fax: 61 7 3875 6001.
† Griffith University.
‡ Queensland Herbarium.

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TABLE 1. ^{1}H (600 MHz), ^{13}C (150 MHz), and HMBC NMR Data for Grandisine A (1) in C_6D_6

atom	δ^{13}C (mult)[a]	δ ^{1}H mult, J (Hz)	HMBC ^{1}H to ^{13}C[b]
1α	25.6 (t)	1.31 m	C-2, C-3, C-8, C-9
1β		2.13 ddd, 6.0, 12.0, 16.8	C-2, C-3, C-8, C-9
2α	22.4 (t)	1.31 m	C-1, C-3, C-9
2β		1.75 m	C-1, C-3, C-9
3α	55.8 (t)	2.79 ddd, 9.0, 9.0, 12.0	C-1, C-2, C-9
		2.63 ddd, 2.4,9.0, 12.0	C-2, C-5, C-9
5α	42.5 (t)	2.21 ddd, 3.0, 4.2, 10.8	C-3, C-6, C-7, C-9
5β		2.67 ddd, 3.0, 10.8, 13.8	C-3, C-6, C-7, C-9
6α	30.9 (t)	1.75	C-5, C-8, C-7
6β		1.74	C-5, C-8, C-7
7	68.6 (d)	3.27 ddd, 3.0, 3.0, 3.0	C-5, C-6, C-9, C-12
8	38.8 (d)	2.57 ddd, 1.8, 3.0, 6.6	C-1, C-9, C-10
9	60.1 (d)	3.20 ddd, 6.6, 6.6, 12.0	C-1, C-2, C-5, C-7, C-8
10	170.6 (s)		
11	116.5 (s)		
12	70.6 (s)	4.63 dq, 1.8, 6.0	C-10, C-11, C-13
13	22.0 (q)	1.79 d, 6.0	C-11, C-12
14	189.7 (s)	-	-
15α	44.5 (t)	2.04 dd, 13.2, 16.8,	C-14, C-16, C-17
15β		1.98 dd, 4.2, 16.8	C-11, C-14, C-16, C-17
16	75.2 (t)	3.74 dqd, 4.2, 6.0, 13.2	-
17	20.6 (q)	0.91 d, 6.0	C-15, C-16

[a] Implied multiplicity determined by DEPT (s = C, d = CH, t = CH_2, q = CH_3). [b] Carbons that correlate to the proton resonance.

Grandisine A (**1**) was assigned the molecular formula $C_{16}H_{23}NO_3$ by high resolution ESI mass measurement of the $[M+H]^+$ ion. An IR absorption band at 1665 cm^{-1} indicated that the molecule contained an α,β-unsaturated ketone. Signals for all 16 carbons were visible in the ^{13}C NMR spectrum (Table 1), and gHSQC spectral data established the presence of 23 carbon-bound protons (two methyls, six methylenes, and five methines). The remaining three non-protonated sp^2 carbons were observed downfield of 115 ppm and were assigned to a tetrasubstituted β-oxygenated α,β-unsaturated ketone. A gCOSY experiment established partial structures $NCH_2CH_2CH_2$-$CH(N)CHCH(O)CH_2CH_2N$, CH_3CHO-, and $CH_2CH(O)$-CH_3. The connection of these partial structures in **1** was established from correlations observed in a gHMBC experiment. The indolizidine moiety was established from correlations between H-3β and C-5 and between H-9 and C-5. An ether linkage between C-7 and C-12 was established from a correlation between H-7 and C-12. The tetrasubstituted double bond was attached to C-12 since correlations were observed between H-13 and H-12 and the olefinic carbon, C-11, and between H-12 and the downfield olefinic carbon C-10. A correlation between H-8 and C-10 indicated that C-8 of the indolizidine was attached directly to the double bond at C10 forming a pyran. Correlations were also observed between H-15β and both C-11 and the ketone carbon, C-14. To complete the structural assignment the molecular formula dictated that the molecule must have an ether linkage between C-16 and C-10 to create a 2,3-dihydro-γ-pyrone however no HMBC correlation was observed between H-16 and C-10. With the gross structure of grandisine A established, the relative stereochemistry of the molecule (Figure 1) was deduced from a combination of proton coupling constants and ROESY correlations. Small couplings and ROESY correlations between H-7 and H-8 and between H-8 and H-9 indicated that the three protons were cis to each other. Furthermore, small couplings between H-7 and both H-6β and H-6α suggested that H-7 was equatorial. The 1,3-diaxial relationship of H-8 to H-6α was supported by ROESY correlations between the two protons and this was in agreement with the piperidine ring adopting a chair conformation. A large coupling between H-5β and H-6α supported this conclusion. A ROESY correlation between H-7 and H-12 indicated that these protons were 1,3-diaxial. A ROESY correlation between H-9 and H-1α and H-3α together with a large diaxial coupling between H-9 and H-1β indicated that C-1 was axial which dictated that the lone pair of electrons on the nitrogen of the indolizidine must be cis to H-9. The methine proton, H-16, showed a large diaxial coupling to H-15α indicating that the methyl, C-17, was equatorial. A ROESY correlation between H-1α and H-16 indicated that H-16 was situated on the β face of the dihydropyrone. Grandisine A (**1**) possesses a pyrano[2′,3′:4,5]pyrano[2,3-*g*]indolizin-4-one skeleton a ring system which has not been described previously.

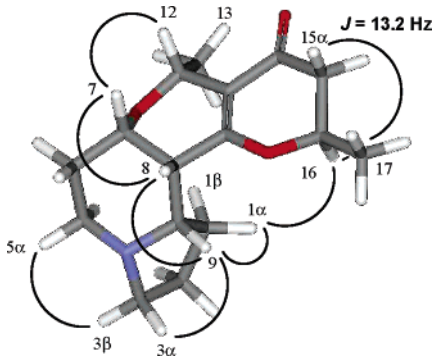

FIGURE 1. Relative stereochemistry of grandisine A (**1**) with key ROESY correlations and the H15α-H16 coupling constant.

Grandisine B (**2**) was assigned a molecular formula $C_{16}H_{22}N_2O$ by high resolution ESI mass measurement of the $[M+H]^+$ ion. All 16 carbons were visible in the ^{13}C NMR spectrum (Table 2), and gHSQC spectral data established the presence of 22 carbon-bound protons (1 methyl, 7 methylenes, and 5 methines). Correlations observed in gCOSY and gHMBC spectra indicated that grandisine B contained an indolizidine unsaturated at C7−C8. The presence of an isoquinuclidine was also established from a combination of gCOSY and gHMBC correlations. In particular, the proton spin system C-13−C-14−C-15−C-16(CH_3)C-11 was readily defined from COSY correlations. C-11 and C-13 were bridged by a carbonyl carbon C-12 since correlations were observed between H-11, H-13a and H-13b, and C-12. C-11 was adjacent to an imine carbon since correlations were observed between H-16 and H-11 and C-10. A nitrogen bridge was found between C-14 and C-10 since a correlation was observed between H-14 and C-10. A weak four bond correlation was also observed between H-11 and C-14. The indolizidine and the isoquinuclidine were joined at C-8-C-10 since a correlation was observed between H-7 and C-10. Grandisine B was therefore

SCHEME 1. Biogenetic Pathway Proposed for Grandisine A (1), B (2) and Isoelaeocarpiline (3)

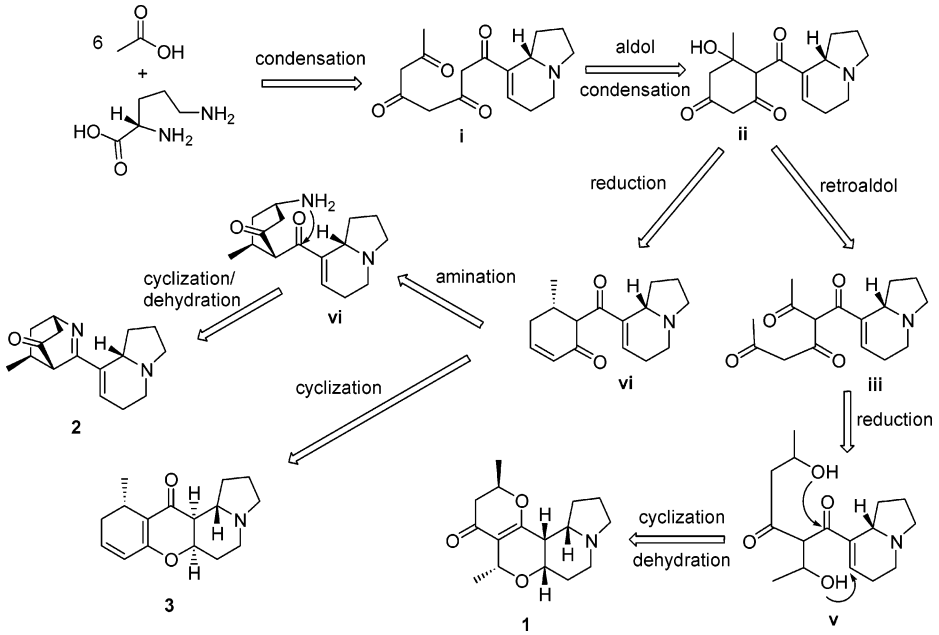

TABLE 2. ^{1}H (600 MHz), ^{13}C (150 MHz), and HMBC NMR Data for Grandisine B (2) in $CDCl_3$

atom	δ^{13}C (mult)[a]	δ^1H (mult, *J* Hz)	HMBC ^{1}H to ^{13}C[b]
1a	29.0 (t)	1.35 dddd, 9.6, 9.6, 9.6, 9.6	C-2, C-3, C-8, C-9
1b		2.30 m	
2a	21.5 (t)	1.76 m	C-1, C-3
2b		1.80 m	C-1, C-3
3a	51.5 (t)	2.78 ddd, 9.0, 9.0, 12.0	C-1, C-2, C-9
3b		2.92 ddd, 5.4, 7.2, 12.0	C-1, C-9, C-2
5a	44.1 (t)	2.64 ddd, 5.4, 5,4, 12.0	C-6, C-9
5b		2.87 ddd, 6.0, 6.0, 12.0	C-6, C-7, C-9
6a	24.0 (t)	2.30 m	
6b		2.40 m	C-7, C-8
7	129.4 (d)	6.40 s	C-5, C-6, C-8, C-9, C-10
8	138.0 (s)		
9	58.1 (d)	3.67 dd, 7.8, 9.6	C-8
10	170.3 (s)		
11	57.0 (d)	3.54 d, 2.4	C-8, C-10, C-12, C-13, C-14, C-15
12	207.8 (s)		
13a	39.2 (t)	2.05 d, 19.2	C-12
13b		2.16 bd, 19.2	C-12
14	54.9 (d)	4.62 s	
15a	31.4 (t)	1.26 dd, 3.0, 12.0	C-11, C-13, C-14, C-16, C-17
15b		1.95 bdd, 12.0, 12.0	
16	28.9 (d)	1.78 m	C-10
17	19.8 (q)	1.06 d, 7.2	C-11, C-15, C-16

[a] Implied multiplicity determined by DEPT (s = C, d = CH, t = CH_2, q = CH_3). [b] Carbons that correlate to the proton resonance.

assigned structure **2**. The relative stereochemistry of the isoquinuclidine was determined from coupling constant analysis. A large coupling between H-16 and H-15b (12 Hz) indicated that these protons were eclipsed while H-15b also showed a w-coupling to H-13b (observed in the COSY spectrum). These observed coupling were consistent only with a structure that has the methyl group, C-17, on the same face as the ketone (Figure 2). The stereochemistry C-9 relative to C-11, C-14, and C-16 could not be determined from NMR analysis.

Johns *et al.* have proposed that the *Elaeocarpus* alkaloids are probably biosynthesized by condentation of a polyketomethylene chain derived from six acetates with ornithine.[4] Given this hypothesis it seems possible that grandisines A (**1**) and B (**2**) and (-) isoelaeocarpiline (**3**) could be derived from a common precursor (**ii**). A plausible biogenetic pathway is outlined in Scheme 1. Aldol condensation of **i** to generate the cyclohexanone (**ii**) could be followed either by a retroaldol reaction to yield **iii**, a precursor to **1** or reduction to yield **iv**, a precursor to **2** and **3**. Reduction of the two methyl ketones in **iii** to yield **v**, followed by cyclization and dehydration would generate grandisine A (**1**). β-amination of the α,β-unsaturated ketone in **iv** to yield **vi**, followed by attack of the ketone by the amino group and dehydration would yield grandisine B (**2**). Conversion of **iv** to its 12-enol tautomer and nucleophilic attack of the C-7−C-8 double bond by the enol oxygen would yield isoelaeocarpiline (**3**). The close

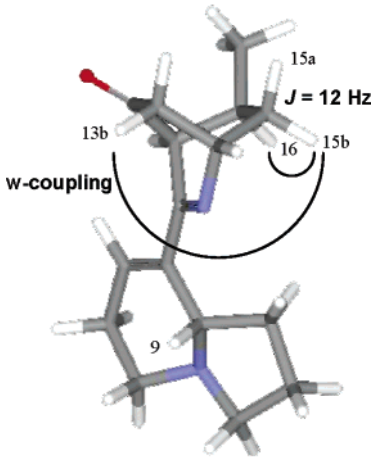

FIGURE 2. Relative stereochemistry of the isoquinuclidine moiety in grandisine B (**2**) as defined by coupling constants.

biogenetic relationship of **2** to **3** might indicate that the absolute stereochemistry at C-9 and C-16 for **2** is the same as that of the known compound **3**. Likewise the absolute stereochemistry at C-9 for grandisine A (**1**) may be the same that found in (-) isoelaeocarpiline (**3**).

Many alkaloids isolated from plants from the families Apocynaceae and Daphniphyllaceae have been reported to contain the fully saturated isoquinuclidine group as part of more complex polycyclic systems. However, only one other natural product that contains an isoquinuclidinone moiety, mearsine (**4**) an alkaloid isolated from *P. mearsii*,[5] has been reported previously. Grandisine B is the only alkaloid isolated to date that contains both an isoquinuclidine and an indolizidine moiety.

Compounds **1**−**3** inhibited the binding of [^{125}I]-deltorphin II to HEK cell membranes expressing recombinant human δ-opioid receptors with IC_{50} values of 2.7 μM, 52.2 μM and 11μM, respectively. It has been recognized for some time that a basic nitrogen is an important feature of δ-opioid receptor binders and that active molecules containing piperidine groups with aromatic substituents are commonly encountered.[6] Although compounds **1**−**3** all contain piperidine moieties none have aromatic substitution. It appears that the orientation of the heterocyclic rings attached to the indolizidines is important for binding to the receptor. In the two more active compounds **1** and **3** the relatively flat pyran ring attached at C7−C8 is directed in an orthogonal orientation to the piperidine whereas in **2** the bulky isoquinuclidine group is orientated into the same plain as the piperidine.

Experimental Section

Plant Material. The leaves of *E. grandis* F. Muell. (Elaeocarpaceae) were collected from the Indooroopilly Agricultural Research Laboratories Rainforest plot, Brisbane, Australia, in October 1993. A voucher specimen (AQ 600168) has been deposited in the Queensland Herbarium, Brisbane. The plant was identified by Paul Grimshaw from the Queensland Herbarium.

Extraction and Fractionation. The air-dried and milled leaves of *E. grandis* (100 g) were extracted exhaustively with CH_2Cl_2 (2 × 300 mL) and MeOH (3 × 300 mL). The CH_2Cl_2 and MeOH extracts were combined yielding a dark green gum (34 g). This extract was partitioned between CH_2Cl_2 (300 mL) and 2 M H_2SO_4 (2 × 200 mL). The aqueous layer (400 mL) was basified with 27% NH_4OH (200 mL) and partitioned with CH_2Cl_2 (3 × 300 mL). Bioactivity was located in the second CH_2Cl_2 fraction (light brown gum, 0.65 g), and this was chromatographed on amino-bonded silica (50 g) with a stepped gradient from CH_2Cl_2 to MeOH. Twenty-three fractions were collected, and bioactivity was found in fractions 11, 16, and 17. Fraction 11 was pure (−) isoelaeocarpiline (**3**) (95 mg, 0.95%).[4] Fractions 16 and 17 were combined (124 mg) and chromatographed on amino bonded silica elution with hexane/isopropyl alcohol/NH_3 (85:14:1). Two bioactive compounds were isolated, grandisine A (**1**) (20 mg, 0.02%) and grandisine B (**2**) (35 mg, 0.035%).

Grandisine A (1): yellow gum (20 mg, 0.02%); $[\alpha]_D$ = + 38.6 (*c* 0.1, CH_2Cl_2); UV (MeOH) λ_{max} 270 nm (log ϵ 2.1); IR (film) ν_{max} 1665 cm^{-1}; ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR Table 1; (+)-LRESMS *m/z* (rel int) 278 (100) [MH, $C_{16}H_{24}NO_3]^+$; (+)-HRESMS *m/z* 278.1755 (calcd for $C_{16}H_{24}NO_3$ $[M+H]^+$, 278.1751).

Grandisine B (2): yellow gum (35 mg, 0.035%); $[\alpha]_D$ = +11.0 (*c* 0.1 CH_2Cl_2); UV (MeOH) λ_{max} 275 nm (log ϵ 2.9); IR (film) ν_{max} 1722, 1651 cm^{-1}; ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR Table 2; (+)-LRESMS *m/z* (rel int) 259 (100) [MH^+, $C_{16}H_{23}N_2O]^+$; (+)-HRESMS *m/z* 259.1816 (calcd for $C_{16}H_{23}N_2O$ $[M+H]^+$, 259.1805).

***δ*-Opioid Receptor Binding Assay.** Assays were performed in 50 mM Tris containing 3 mM $MgCl_2$, 1 mg/mL of BSA, pH 7.4 with HEK cell membranes expressing recombinant human δ-opioid receptors (2 μg/well), [^{125}I]-deltorphin II (56 pM), and SPA beads (700 μg/well) in a total volume of 200 μL. Controls included 10 μM naloxone (for nonspecific binding) and 1 nM DPDPE (for reference). Extracts were tested at a final concentration of 2% DMSO. Microplates were shaken for 1 h at ambient temperature (~23 °C) and then left to reach steady state for 4 h. Microplates were counted for 1 min/per well.

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Supporting Information Available: General experimental procedures, ^{1}H and ^{13}C NMR, and two-dimensional NMR spectra of grandisine A (**1**) and B (**2**). This material is available free of charge via the Internet at http://pubs.acs.org.

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