Grandisines C−G, Indolizidine Alkaloids from the Australian Rainforest Tree *Elaeocarpus grandis*

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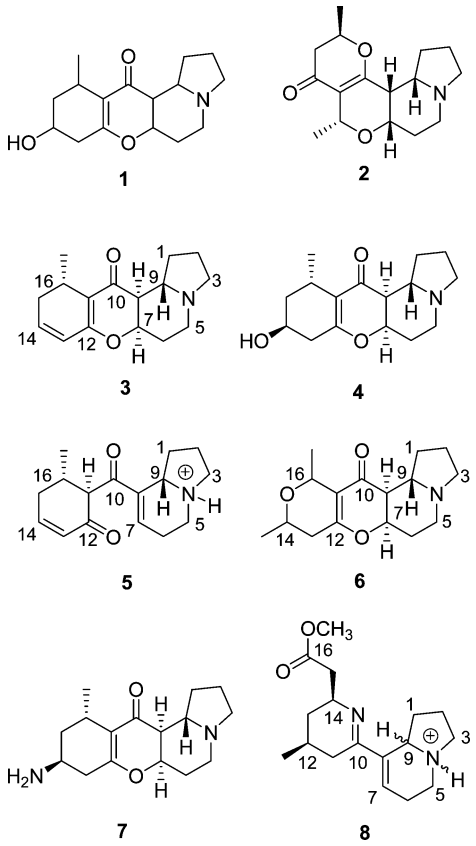
Five new indolizidine alkaloids, grandisines C, D, E, F, and G (**4**−**8**), and one known indolizidine alkaloid, (−)-isoelaeocarpiline (**3**), were isolated from the leaves of *Elaeocarpus grandis* and their structures determined by 1D and 2D NMR spectroscopy. Grandisine C (**4**) is isomeric with the known compound rudrakine (**1**). The absolute configuration of grandisine D (**5**) was deduced by its conversion to (−)-isoelaeocarpiline. Grandisine E (**6**) contains a novel tetracyclic ring system. Grandisine F (**7**) is the 14-amino analogue of grandisine C. Grandisine G (**8**) contains the novel combination of a piperidine attached to an indolizidine. Grandisines C, D, F, and G and (−)-isoelaeocarpiline showed receptor binding affinity for the human δ-opioid receptor with IC_{50} values of 14.6, 1.65, 1.55, 75.4, and 9.9 μM, respectively.

Plants from the family Elaeocarpaceae are found in tropical and temperate regions of Asia, Australasia, and South America. The genera *Aristotelia* and *Peripentadenia* have been investigated chemically, and well over 70 indole and pyrrolidine alkaloids have been reported.[1] Chemical studies in the 1970s on five species from the genus *Elaeocarpus* collected in Papua New Guinea showed that these plants produce a rich array of indolizidine alkaloids.[2] Subsequently *E. ganitrus* from India was reported to contain the indolizidine alkaloids elaeocarpine, isoelaeocarpine, and rudrakine (**1**).[3] The genus *Elaeocarpus* is well represented in Australia, particularly in tropical and subtropical rainforests along the east coast.[8] There are 27 species in Queensland,[9] and only three of these species were tested for alkaloid content as part of the Australian phytochemical survey in the 1950−1960s; all gave negative results.[2a] Until recently no chemical studies had been undertaken on any Australian species. As part of a high-throughput screening campaign to identify potential drug leads, we recently identified two novel indolizidine alkaloids, grandisines A (**2**) and B (**11**), in the Australian rainforest tree *Elaeocarpus grandis*.[10] We demonstrated that these alkaloids had affinity for the human δ-opioid receptor, a receptor associated with the prevention of chronic pain. This discovery prompted us to undertake a comprehensive survey of plants from the genus *Elaeocarpus* that we have in our extensive collection of plants from Queensland, Papua New Guinea (PNG), and southwest China. A total of 339 samples were investigated, and many PNG and Australian species proved positive for alkaloids. Among these alkaloid-positive samples was another collection of leaf material from *E. grandis* from South East Queensland. This paper reports on the isolation, structure determination, and human δ-opioid receptor binding affinities of indolizidine alkaloids from the leaf extracts of *E. grandis*. (−)-Isoelaeocarpiline (**3**) and five new compounds, grandisines C (**4**), D (**5**) E (**6**) F (**7**), and G (**8**), were isolated.



Results and Discussion

A MeOH extract of the leaves of *E. grandis* was filtered through strongly acidic ion-exchange resin. After washing the resin with MeOH and H_2O, the alkaloids were eluted with 1 M NaCl. The eluant was filtered through C18, and the alkaloids eluted with MeOH after the C18 were washed with copious amounts of H_2O. The alkaloids were purified by repeated preparative C18 HPLC, yielding grandisine C (0.00092%), grandisine E (0.00027%), (−)-isoelaeocarpiline (0.0012%), and grandisine D (0.0011%), respectively. All compounds were isolated as their TFA salts. A re-collection of *E. grandis* leaves was obtained to provide more of the indolizidine alkaloids grandisine D and isoelaeocarpiline for chemical modifications. This second batch of leaves was extracted with an ammonia solution followed by DCM. The extracts were combined and partitioned. The DCM layer was partitioned with aqueous H_2SO_4 and the aqueous layer basified with ammonia and partitioned with DCM to yield an alkaloid fraction. HPLC separation of the alkaloid fraction on C18 silica gel using a gradient from 1% TFA in H_2O to MeOH containing 1% TFA yielded grandsine D (**5**) (0.0037%), isoelaeocarpiline (**3**) (0.0086%), and grandisine C (**4**) (0.00039%) as well as two additional new alkaloids, grandisine F (**7**) (0.00029%) and grandisine G (**8**) (0.00021%).

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Table 1. ^{13}C NMR (125 MHz) Data for Isoelaeocarpiline (**3**) and Grandisines C (**4**), D (**5**), E (**6**), F (**7**), and G (**8**)

	3[a]	**4**[a]	**5**[b]	**6**[a]	**7**[c]	**8**[b]
position	δ_C, mult.	δ_C, mult.	δ_C, mult.	δ_C, mult.	δ_C, mult.	δ_C, mult.
1	29.2, CH_2	28.4, CH_2	28.2, CH_2	29.5, CH_2	28.9, CH_2	28.5, CH_2
2	21.3, CH_2	21.2, CH_2	20.3, CH_2	21.2, CH_2	21.2, CH_2	20.1, CH_2
3	54.0, CH_2	53.8, CH_2	52.9, CH_2	54.1, CH_2	53.0, CH_2	52.9, CH_2
5	47.7, CH_2	47.6, CH_2	43.2, CH_2	47.4, CH_2	47.8, CH_2	43.6, CH_2
6	30.3, CH_2	30.0, CH_2	23.0, CH_2	30.9, CH_2	31.1, CH_2	22.3, CH_2
7	76.4, CH	75.5, CH	140.1, CH	75.8, CH	76.2, CH	127.0, CH
8	52.8, CH	52.2, CH	137.7, qC	51.7, CH	52.6, CH	135.2, qC
9	60.5, CH	60.0, CH	58.0, CH	60.1, CH	60.4, CH	59.5, CH
10	192.5, qC	192.7, qC	198.3, qC	191.2, qC	193.1, qC	163.0, qC
11	112.8, qC	116.3, qC	59.3, CH	113.7, qC	115.6, qC	32.7, CH_2
12	164.3, qC	168.1, qC	196.6, qC	167.8, qC	169.0, qC	25.6, CH
13	122.5, CH	38.2, CH_2	128.4, CH	35.6, CH_2	39.5, CH_2	35.4, CH_2
14	139.7, CH	63.8, CH	151.6, CH	62.4, CH	43.3, CH	56.7, CH
15	31.9, CH_2	39.4, CH_2	32.6, CH_2		40.7, CH_2	41.8, CH_2
16	22.5, CH	25.4, CH	33.0, CH	66.8, CH	26.4, CH	172.0, qC
14-CH3				21.5, CH_3		
16-CH3	18.6, CH_3	20.2, CH_3	19.1, CH_3	18.8, CH_3	20.4, CH_3	
12-CH3						21.8, CH_3
16-OCH3						51.2, CH_3

[a] $CDCl_3$. [b] DMSO-d_6. [c] CD_2Cl_2

(−)-Isoelaeocarpiline (**3**) was converted to its free base by partitioning between $CHCl_3$ and ammonia solution. The structure of (−)-isoelaeocarpiline (**3**) was established from 2D NMR analysis (COSY, HSQC, HMBC, and ROESY) and comparison with literature spectroscopic data.[2] Full ^1H and ^{13}C NMR assignment of isoelaeocarpiline is reported here for the first time (Table 1 and 2). The specific rotation of (−)-isoelaeocarpiline was similar to that previously reported, indicating that it was isolated as the 7*R*,8*S*,9*S*,-16*S* isomer. The 2D NMR analysis of **3** proved crucial for the structure elucidation of the five new compounds.

Grandisine C (**4**) was also converted to its free base by partitioning between $CHCl_3$ and ammonia solution. Grandisine C (**4**) was assigned a molecular formula $C_{16}H_{23}NO_3$ by high-resolution positive ESI mass measurement of the $[M + H]^+$ ion (Δ −1.2 ppm). The ^1H NMR (Table 2) spectrum of **4** showed many similarities to that of (−)-isoelaeocarpiline. In particular, the protons H-1−H-9 of the indolizidine were almost identical in both spectra, suggesting that **4** had the same configuration about the indolizidine as (−)-isoelaeocarpiline (**3**). This was confirmed by 2D NMR analysis. ROESY correlations were observed between H-7 and H-8, between H-5β and H-9, and between H-6α and H-8. The major difference between **3** and **4** was the absence of the olefinic proton resonances observed in the ^1H NMR spectrum of (−)-isoelaeocarpiline (**3**), which had been replaced in **4** by an oxygenated methine and methylene proton resonances. That grandisine C (**4**) was 18 Da heavier than (−)-isoelaeocarpiline (**3**) suggested that the C-13−C-14 double bond in **3** had been hydrated. Correlations in the COSY spectrum of **4** from H-14 to H-13α, H-13β, H-15α, and H-15β indicated that the oxygenated methine H-14 was flanked by two methylenes, 13-CH_2 and 15-CH_2. The methylene protons H-15α and H-15 in turn showed correlations to a methine, H-16, which also correlated to secondary methyl protons (16-CH_3). The 16-CH_3 protons showed HMBC correlations to C-15, C-16, and an olefinic carbon C-11. H-16 also showed HMBC correlations to the olefinic carbon C-11 as well as to the oxygenated C-12 olefinic quaternary carbon. The 13-CH_2 protons also correlated to C-12 and C-11 in the HMBC spectrum. Grandisine C (**4**) therefore contained a 3-methyl-5-hydroxycyclohexene, confirming the C-14 hydroxylation site. The two-dimensional structure of grandsine C (**4**) was the same as rudrakine (**1**) previously isolated from *E. ganitrus*; however the spectroscopic properties for these two compounds were quite different, and this suggested that grandisine C (**4**) was a configurational isomer of rudrakine (**1**). ROESY correlations between H-14 and 16-CH_3 indicated that they were 1,3-diaxial and the C-14 hydroxyl was equatorial. As the rest of the spectroscopic properties for grandisine C (**4**) were so similar to those of (−)-isoelaeocarpiline, the molecular structure of grandisine C was assigned as **4**. Positive Cotton effects at 266 nm ([θ] 16507) and 273 nm ([θ] 12129) and negative Cotton effects at 341.6, 218 nm ([θ] −15411, −18700) and 351.6, 221 ([θ] −29587, −25009) in the CD spectra of grandisine C and (−)-isoelaeocarpiline, respectively, implied that grandisine C possessed the same absolute configuration about the indolizine moiety as (−)-isoelaeocarpiline. Grandisine C was therefore assigned a 7*R*, 8*S*, 9*S*, 14*S*, 16*S* configuration.

Grandisine D trifluoroacetate (**5**) was assigned the molecular formula $C_{16}H_{22}NO_2$ by mass measurement of the $[M + H]^+$ ion in the positive HRESIMS. An IR absorption band at 1652 cm^{-1} suggested that grandisine D contained an α,β-unsaturated ketone functionality, and this was corroborated by two ketone carbonyl carbons being observed in the ^{13}C NMR spectrum (Table 1) at δ 196.6 and 198.3. All 16 carbons were visible in the ^{13}C NMR spectrum, and gHSQC data established the presence of 21 carbon-bound protons (^1H NMR data in Table 2, one methyl, six methylenes, three methines, and three olefinic protons). Partial structures CH═$CHCH_2CH(CH_3)CH$, $CH_2CH_2CH_2CH$, and C═$CHCH_2CH_2$ were established from gCOSY data. A gHMBC experiment was used to establish the connection of the partial structures in **5**. The 5-methylcyclohexenone partial structure was secured by correlations from H-14 to C-12 and H-16 to C-12. The downfield chemical shifts of C-14 and H-14 were indicative of this olefinic carbon being β to one of the ketone functionalities. The indolizidine moiety was deduced from HMBC correlations between H-3β and C-9 and C-5, H-5α and C-9, and H-7 and C-9. The downfield chemical shift of the olefinic proton and carbon C/H-7 (δ 140.1/7.30) suggested that it was also β to a ketone carbonyl, and this was confirmed by the observation of a gHMBC correlation from H-7 to the C-10 carbonyl carbon (δ 198.3 ppm). The cyclohexenone was connected to the indolizidine at C-10 since H-11 also showed an HMBC correlation to C-10. The orientation of the 16-methyl and 11-ketone substituents of the cyclohexenone were established as equatorial since a large diaxial coupling was observed between H-11 and H-16. The orientation of H-9 relative to the cyclohexenone substituents could not be determined by NMR techniques. Subsequently grandisine D cyclized to form (−)-isoelaeocarpiline (**3**) on standing in DMSO. The formation of (−)-isoelaeocarpiline could be explained by Michael addition of the hydroxyl formed upon enolization of the C-12 ketone in **5** to C-7 from the face opposing the C-9−C-1 bond. This transformation provided evidence to assign the absolute configuration of grandisine D (**5**) as 9*S*, 11*R*, 16*S*. In a recent paper we postulated that a molecule like grandisine D could be a biosynthetic precursor to

Table 2. ^{1}H NMR (600 MHz) Data for Isoelaeocarpiline (**3**) and Grandisines C (**4**), D (**5**) E (**6**), F (**7**), and G (**8**)

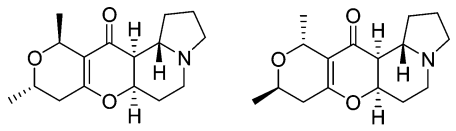
	3[a]	**4**[a]	**5**[b]	**6**[a]	**7**[c]	**8**[b]
position	δ_H (*J* in Hz)	δ_H (*J* in Hz)	δ_H (*J* in Hz)	δ_H (*J* in Hz)	δ_H (*J* in Hz)	δ_H (*J* in Hz)
1α	1.68, m	1.66, m	1.62, dddd (8.4, 9.6, 9.6, 9.6)	1.67, m	1.56, m	1.59 m
1*β*	1.72, m	1.66, m	2.40, dddd (8.4, 9.6, 9.6, 9.6)	1.67, m	1.63, m	2.49 m
2*β*	1.66, m	1.66, m	2.00, t (7.8)	1.67, m	1.63, m	1.95 ddd (3.0, 9.6, 10.2)
2α	1.82, bdd (8.4, 16.0 ,8.0)	1.82, bd (9.0)	2.00, t (7.8)	1.81, bdd (9.0, 16.8)	1.74, m	2.00, ddd (3.0, 4.2, 10.2)
3α	2.16, m	2.26, d (9.6)	3.28, m	2.13, m	2.13, m	3.30 m
3*β*	3.08, dd (8.4, 8.4)	3.11, dd (9.0, 9.0)	3.48, ddd (10.8, 10.8, 10.8)	3.09, dd (7.8, 7.8)	3.02, bdd (9.0, 9.0)	3.52 m
4			10.25, bs			10.01, bs
5*β*	2.48, m	2.47, dd (12.0, 12.0)	3.09, ddd (6.6, 6.6, 12.6)	2.45, m	2.40, ddd (3.0, 11.4, 15.0)	3.07, ddd (5.0, 5.0, 12.0)
5α	3.00, ddd (2.4, 4.8, 11.4)	3.01, ddd (2.4, 2.4, 12.0)	3.28, m	3.00, dd (5.4, 10.8)	2.95, ddd (1.2, 4.8, 11.4)	3.30, m
6α	1.94, dddd (5.4, 5.4, 13.8, 13.8)	1.91, dddd (2.0, 4.8, 12.0, 14.4)	2.60, m	1.93, dddd (3.6, 3.6, 12.0, 15.0)	1.85, dddd (3.0, 5.4, 13.8, 15.0)	2.49, m
6*β*	2.07, m	2.07, ddd (2.4, 2.4, 14.4)	2.60, m	2.06, bddd (2.4, 2.4, 15.0)	1.99, dddd (1.2, 3.0, 3.0, 13.8)	2.49, m
7	4.58, bs	4.56, bs	7.30, bdd (4.0, 4.0)	4.59, bdd (2.4, 2.4)	4.51, ddd (2.4, 3.0, 3.0)	6.55 dd (4.2, 4.2)
8	2.19, m	2.16, m		2.20, m	2.10, m	
9	2.10, m	2.10, m	4.32, dd (8.4, 8.4)	2.20, m	2.10, m	4.32, dd (10.8, 10.8)
11			4.29, d (11.4)			1.61, dd (2.4, 14.6)
11b						2.65, dd (6.0, 14.6)
12						1.67, m
13α	5.94, d (9.6)	2.24, dd (10.2, 18.0)	5.94, d (9.6)	2.20, m	1.99, dd (9.6, 18.0)	0.79, ddd (11.4, 11.4, 11.4)
13*β*		2.66, dd (6.0, 18.0)		2.20, m	2.49, dd (5.4, 18.0)	1.75, ddd (3.0, 3.0, 11.4)
14	6.32, bdd (9.6, 9.6)	4.17, m	7.13, ddd (5.4, 9.6, 9.6)	4.03, ddq (6.6, 6.6, 6.6)	3.19, m	3.82, dddd (4.0, 6.6, 9.0, 11.4)
15α	2.17, m	1.60, m	2.20, dd (11.4, 19.8)		1.39, ddd (6.0, 12.6, 12.6)	2.37, dd (9.0, 15.0)
15*β*	2.45, m	1.82, m	2.45, m		1.63, m	2.54, dd (6.6, 15.0)
16	3.05, dq (6.6, 6.6)	3.01, m	2.40, ddq (6.6, 6.6, 11.4)	4.83, q (6.6)	2.89, dq (6.0, 6.0)	
14-CH_3				1.27, d (5.4)		
16-CH_3	0.88, d (6.6)	1.04, d (6.6)	0.84, d (6.6)	1.33, d (6.6)	0.98, d (6.0)	
12-CH_3						0.94, d (6.6)
16-OCH_3						3.61, s

[a] $CDCl_3$. [b] DMSO-d_6. [c] CD_2Cl_2.

isoelaeocarpiline and grandisine B. The isolation of grandisine D in the current study lends support for that hypothesis.[10]

Grandisine E (**6**) was converted to its free base by partitioning between $CHCl_3$ and ammonia solution and was obtained as a colorless gum. A pseudomolecular ion in the positive HRESIMS at *m*/*z* 278.17578 allowed a molecular formula $C_{16}H_{23}NO_3$ to be assigned to **6**. A strong absorption band at 1648 cm^{-1} in the IR spectrum, in combination with a weak absorption maxima at 272 nm in the UV spectrum, suggested that **6** contained an α,*β*-unsaturated ketone functionality. All 16 carbons were visible in the ^{13}C NMR spectrum (Table 1) and analysis of the gHSQC spectral data established the presence of 23 carbon-bound protons, two methyls, six methylenes, and five methines. Correlations observed in a gCOSY spectrum established partial structures CH_2-CH(O)CH_3, CH(O)CH_3, and $NCH_2CH_2CH(O)CHCHCH_2CH_2$-$CH_2N$. Correlations observed in a gHMBC spectrum allowed these partial structures to be connected. The indolizidine moiety was deduced from correlations between H-3*β* and C-9 and C-5 and between H-5α and C-9. HMBC correlations from H-16 and 13-CH_2 to the C-12 oxygenated olefinic quaternary carbon and the C-11 upfield quaternary olefinic carbon indicated that the two remaining partial structures established from COSY correlations could be connected at either end of a tetrasubstituted double bond. A correlation between H-16 and C-14 established an ether linkage between C-14 and C-16, and this indicated that the molecule contained a dihydropyran moiety. The indolizidine proton H-8 showed correlations to the C-10 ketone carbonyl and to the upfield C-11 olefinic carbon, indicating that the dihydropyran was connected to the indolizidine by a ketone bridge between C-8 and C-10. The double-bond equivalents deduced from the molecular formula dictated that the molecule contain another ring, and this could be assigned to an ether linkage between C-7 and C-12, although no HMBC correlation was observed between H-7 and C-12. The chemical shifts of the indolizidine protons (H-1 to H-9) and carbons (C-1 to C-9) were almost identical with those observed for isoelaeocarpiline (**3**). This suggested that the relative configurations at C-7, C-8, and C-9 in **6** were the same as in (−)-isoelaeocarpiline (**3**). ROESY correlations were in agreement with this conclusion, although unambiguous assignment was not possible because several crucial proton signals (H-8 and H-9) were overlapped. The relative configuration at C-14 and C-16 in grandisine E (**6**) was established from ROESY correlations. A strong ROESY correlation between H-14 and 16-CH_3 indicated that they were 1,3-diaxial. However, the distance between any of the protons in the dihyropyran ring and the indolizidine protons was greater than 4 Å, and so there was no possibility of observing ROE correlations between protons

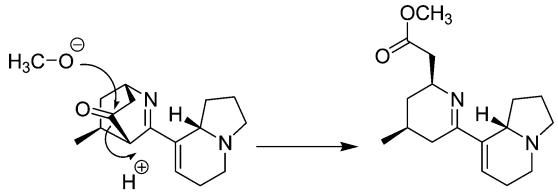
from the two distinct partial structures. It was therefore impossible to relate the regiochemistry of the indolizidine protons to the dihydropyran protons by NMR techniques. There were therefore two possible isomers for grandisine E, **9** or **10**, and the correct configuration remains to be assigned.



9 **10**

Grandisine F (**7**) was converted to its free base by partitioning between $CHCl_3$ and ammonia solution and was obtained as a gum. High-resolution ESI mass measurement of the pseudomolecular ion at *m*/*z* 277.190345 allowed the molecular formula $C_{16}H_{24}N_2O_2$ to be assigned to grandisine F. Strong infrared absorption bands at 2929 and 1654 cm^{-1} suggested that the molecule contained exchangeable hydrogens and α,β-unsaturated ketone functionalities. An absorption band at 273 nm in the UV spectrum was also indicative of an α,β-unsaturated ketone moiety. The ^1H NMR and ^{13}C NMR spectra of grandisine F were very similar to that of grandisine C. The major difference was associated with chemical shifts observed for H-14 and C-14, which had shifted upfield 1 and 19 ppm, respectively, compared to grandisine C. This suggested that the hydroxyl group in grandisine C was replaced in grandisine F by an amino group. NMR analysis (gCOSY, gHSQC, gHMBC, and ROESY) of grandisine F confirmed this assignment and indicated that grandisine F possessed the same relative configuration as grandisine C. The CD spectra of grandisines C and F were very similar, and this indicated that grandisine F possessed a 7*R*, 8*S*, 9*S*, 14*S*, 16*S* absolute configuration. Since grandisine F was isolated only when ammonia was used during the extraction process, there is a possibility that this compound may be an artifact.

Grandisine G trifluoroacetate (**8**) was isolated as a colorless oil. The positive ESIMS displayed a pseudomolecular ion at *m*/*z* 291. However, grandisine G decomposed before a high-resolution mass spectrum could be acquired. The combined NMR and MS data suggested that the molecular formula $C_{17}H_{26}N_2O_2$ could be assigned to **8**. The ^1H NMR spectrum of grandisine G (**8**) (Table 2) displayed resonances typical of an olefinic proton at δ 6.55, methyl ester or ether protons at δ 3.61, and aliphatic secondary methyl protons at δ 0.94. The ^{13}C NMR spectrum of grandisine G (Table 1) displayed signals assignable to an ester carbonyl carbon (δ 172.0), a trisubstituted double bond (δ 135.2 and 127.0), an sp^2-hybridized quaternary carbon (δ 163.0), and a methyl ester (δ 51.2). Of the remaining 12 carbons visible in the ^{13}C NMR spectrum (Table 1) of grandisine G, the chemical shifts of carbons C-1 to C-9 were almost identical to those observed in the spectrum of grandisine D (**5**), suggesting that grandisine G also contained an indolizidine functionality unsaturated at C-7−C-8. Correlations in the gCOSY and gHSQC spectra confirmed the presence of this unsaturated indolizidine group as well as a second spin system, $CH_2CH(CH_3)CH_2CHCH_2$. HMBC correlations from H-15α and H-15β of this second partial structure as well as from 16-OCH_3 to the C-16 ester carbonyl carbon indicated methyl ester substitution at C-15. The methylhexanoate partial structure could be extended further to include a quaternary carbon attached to C-11 since a gHMBC correlation was observed between H-11b and the downfield C-10 quaternary carbon at δ 163.0. This carbon was attached directly to C-8 of the indolizidine since H-11b correlated to the C-8 olefinic quaternary carbon (δ 135.2) and H-7 correlated to C-10 in the gHMBC spectrum. The molecular formula for this partial structure accounted for all but one nitrogen atom, as determined by mass spectrometry. The chemical shifts of H/C-14 (δ 3.82/56.7 ppm) and C-10 (163.0 ppm) suggested that the missing nitrogen was an imine nitrogen, which bridged C-10 and C-14, forming a piperidine; however, no gHMBC correlation was observed between H-14 and C-10. The relative configuration about the piperidine was established from ROESY correlations and inspection of H−H coupling constants. Two large couplings (11.4 Hz) from H-13α to H-12 and H-14 indicated that these three protons were all axial. This was corroborated by a ROESY correlation between H-12 and H-14, which supported their 1,3-diaxial orientation. In this conformation the torsion angle between H-14 and C-10 was ~90°, and this was consistent with a very small or no coupling between these atoms and therefore the lack of any observable HMBC correlation between these atoms. The configuration at C-9 relative to C-12 and C-14 could not be determined by NMR. Grandisine G (**8**) could possibly be a methanolysis product of grandisine B (**11**), an alkaloid that we have previously reported from *E. grandis*.[10] Methoxide attack at the ketone carbonyl of **11** followed by ring opening of the isoquinuclidinone would yield grandisine G. It is therefore possible that grandisine G could possess the same relative stereochemistry as that assigned to grandisine B.



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Biological Activity. Compounds **3**−**5**, **7**, and **8** inhibited the binding of [^{125}I]deltorphin II to HEK cell membranes expressing recombinant human δ-opioid receptors with IC_{50} values of 9.9, 14.6, 1.65, 1.55, and 75.4 μM, respectively. IC_{50} values for the positive controls DPDPE and naloxone were 1.12 and 138 nM, respectively.

Experimental Section

General Experimental Procedures. All solvents used were Omnisolv HPLC grade. Optical rotations were measured on a JASCO P-1020 polarimeter (23 °C, 10 cm cell). UV spectra were recorded on a CAMSPEC M501 and GBC 916 UV/vis spectrophotometer, and IR spectra were recorded on a Nicolet NEXUS FT IR spectrometer. CD spectra were recorded in methanol on a JASCO J-715 spectrophotometer. NMR spectra were recorded on Varian Inova 600 and 500 MHz NMR spectrometers. Samples were dissolved in either DMSO-d_6, $CDCl_3$, or CD_2Cl_2, and chemical shifts were calculated relative to the DMSO solvent peak (^1H δ 2.49 and ^{13}C δ 39.5), $CHCl_3$ solvent peak (^1H δ 7.26 and ^{13}C δ 77.0), or DCM solvent peak (^1H δ 5.32 and ^{13}C δ 54.0). ESIMS and HRESIMS were measured on a Mariner Biospectrometry TOF workstation using positive electrospray ionization, mobile phase 1:1 MeOH/H_2O containing 0.1% formic acid. Dowex 50WX8-400 strongly acidic ion-exchange resin (SCX), 200−400 mesh (Aldrich), and Davisil bonded C18 (30−40 μm) were used during purification. HPLC purifications were achieved using Hypersil BDS C18 preparative (150 × 21.2 mm, 5 μm) and semipreparative (250 × 10 mm, 5 μm) columns.

Plant Material. Leaves of *E. grandis* were collected in December 2001 from Mt. Tamborine in South East Queensland, Australia, by one of the authors (A.R.C.). A second collection of the leaves of *E. grandis* was undertaken in January 2004 by P.I.F. in State Forest 185, Danbulla in North Queensland. A voucher specimen, PIF27569, is housed at the Queensland Herbarium.

Extraction and Isolation. First Batch of Leaves from South East Queensland. The dried and ground leaves (293.7 g) of *E. grandis* were extracted exhaustively with MeOH (3.0 L). The MeOH extract was filtered through 100 g of SCX resin under vacuum. The SCX resin was washed several times with MeOH (1.0 L) and then with H_2O (1.0 L) before the alkaloids were eluted using 1 M NaCl (500 mL). The alkaloid fraction was vacuum filtered through C18 (30 g). The C18 was washed with H_2O (3.5 L) to remove NaCl. The alkaloids were eluted off the C18 with 99% MeOH/1% TFA (400 mL) and evaporated to yield a yellow gum (180.3 mg). The alkaloid fraction was absorbed

onto C18 by evaporating a MeOH solution of the alkaloids into which a suspension of C18 (1.2 g) had been added. The alkaloid/C18 was loaded into an empty metal HPLC column (10 mm × 15 mm), which was then connected in series with a preparative C18 HPLC column. The alkaloids were separated with a gradient of 1% TFA/99% H_2O to 1% TFA/99% MeOH over 100 min followed by a 20 min wash with 1% TFA/99% MeOH. Sixty 2-min fractions were collected. Fractions were analyzed by +ESIMS and ^{1}H NMR. Impure grandisine C (**4**) eluted in fraction 14 and was further purified by C18 HPLC, eluted with 84.5:14.5:1 H_2O/MeOH/TFA. Fraction 18 contained impure grandisine E (**6**), and it was also purified using C18 HPLC, eluted with 84.5:14.5:1 H_2O/MeOH/TFA. Fractions 19 and 20 contained impure grandisine D (**5**), and these fractions were combined and purified by C18 HPLC, eluted with 79.5:19.5:1 H_2O/MeOH/TFA. Fraction 21 contained impure isoeleaocarpiline (**3**), and it was purified on C18 HPLC, eluted with 74.5:24.5:1 H_2O/MeOH/TFA.

Isoelaeocarpiline (3): yellow gum (3.4 mg, 0.00092%); $[\alpha]_D^{22}$ −354.5 (*c* 0.09, $CHCl_3$), (lit.[2] −400.0 (*c* 0.13, $CHCl_3$)); CD (MeOH) 221, 273, 351 nm, [θ] −25009, 12129, −29587; ^{1}H (600 MHz, $CDCl_3$) Table 2; ^{13}C NMR (125 MHz, $CDCl_3$) Table 1; (+)-LRESIMS *m*/*z* (rel int) 260 (100%) $[MH^+, C_{16}H_{22}NO_2]^+$.

Grandisine C (4): yellow gum (2.7 mg, 0.00092%); $[\alpha]_D^{22}$ −59.2 (*c* 0.15, MeOH); UV (MeOH) λ_{max} (log ϵ) 204 (3.39), 273 (3.56) nm; IR (NaCl) ν_{max} 3367, 2956, 1664, 1604, 1406, 1182, 1056, 754 cm^{-1}; CD (MeOH) 218, 266, 342 nm, [θ] −18700, 16507, −15411; ^{1}H (600 MHz, $CDCl_3$) Table 2; ^{13}C NMR (125 MHz, $CDCl_3$) Table 1; (+)-LRESIMS *m*/*z* (rel int): 278 (100%) $[MH^+, C_{16}H_{24}NO_3]^+$; (+)-HRESIMS *m*/*z* 278.14709 [M + H]$^+$ (calcd for $C_{16}H_{24}NO_3$, 278.17507).

Grandisine D trifluoroacetate (5): yellow gum (3.3 mg, 0.0011%); $[\alpha]_D^{23}$ +34.6 (*c* 0.09, MeOH); UV (MeOH) λ_{max} (log ϵ) 222 (3.28), 267 (2.80), 325 (2.43) nm; IR (KBr) ν_{max} 3419, 1652, 1208, 1125 cm^{-1}; CD (MeOH) 231, 267, 308 nm, [θ] 1676, 37271, −3237; ^{1}H (600 MHz, DMSO-d_6) Table 2; ^{13}C NMR (125 MHz, d_6-DMSO) Table 1; (+)-LRESIMS *m*/*z* (rel int): 260 (100%) $[MH^+, C_{16}H_{22}NO_2]^+$; (+)-HRESIMS *m*/*z* 260.16502 [M + H]$^+$ (calcd for $C_{16}H_{22}NO_2$, 260.16451).

Grandisine E (6): unstable colorless gum (0.8 mg, 0.00027%); UV (MeOH) λ_{max} (log ϵ) 272 (1.14) nm; IR (NaCl) ν_{max} 3419, 1646, 1318, 1318, 1203, 1134 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) Table 2; ^{13}C NMR NMR (125 MHz, $CDCl_3$) Table 1; (+)-LRESIMS *m*/*z* (rel int): 278 (100%) $[MH^+, C_{16}H_{24}NO_3]^+$; (+)-HRESIMS *m*/*z* 278.17578 [M + H]$^+$ (calcd for $C_{16}H_{24}NO_3$, 278.17507).

Second Batch of Leaves from North Queensland. The ground, dried leaves of *E. grandis* (1.73 kg) were initially extracted with 32% NH_3 solution (500 mL) followed by an exhaustive extraction with DCM (10 L). The DCM extract was separated from the aqueous ammonia. The volume of the organic extract was reduced under vacuum and was subsequently partitioned with aqueous H_2SO_4 (1.5 L, pH 1). The organic layer from this partition was discarded (2 L). The aqueous layer was then basified with NH_3 to pH 10 and partitioned (×3) with DCM (2 L). The presence of alkaloids in the organic layer was determined by +ESIMS. This alkaloid fraction was evaporated to dryness. The residue (1.69 g) was absorbed onto 2 g of C18 silica gel and purified by MPLC on C18 silica gel (30 × 3.5 cm). The alkaloids were separated into 100 fractions using a gradient of 1% aqueous TFA to 1%TFA/MeOH over 200 min. Two sets of pooled fractions were obtained from this separation. Fractions 27 to 43 and 44 to 48 were combined on the basis of similar ^{1}H NMR spectra and mass ion peaks in the +ESIMS. The combined fraction of 27−43 contained mass ion peaks of *m*/*z* 277, 278, 291, and 296. A mass ion peak at *m*/*z* 260 was detected for the combined fractions 44−48. Analysis of this fraction by ^{1}H NMR indicated a mixture of grandisine D (**5**) and isoelaeocarpiline (**3**) was present. Reversed-phase HPLC on C18 silica gel using a gradient of 3:2 to 9:11 H_2O:1%TFA/MeOH over 15 min separated this fraction, yielding grandisine D (**5**) (63.3 mg, 0.0037%) and isoelaeocarpiline (**3**) (148 mg, 0.0086%). The pooled fraction 27−43 was separated into 50 fractions by preparative HPLC on C18 silica gel using a gradient of H_2O/1% TFA to 1:4 H_2O/1% TFA:1% TFA/MeOH over 100 min. Pure grandisine C (**4**) (6.8 mg, 0.00039%) was detected in fractions 10 to 17. Mass ion peaks at *m*/*z* 277 and 260 were detected in fractions 27 to 30 from the preparative HPLC separation. These fractions were combined and separated into 50 fractions by semipreparative C18 silica gel HPLC using a gradient of 4:1 to 2:3 H_2O/1% TFA:1% TFA/MeOH over 25 min. The mass ion peaks at *m*/*z* 277 and 260 were observed in fractions 14−24 and 32−34, respectively. Analysis by ^{1}H NMR revealed fractions 32 to 34 to be grandisine D (**5**) (5.3 mg, 0.00031%). Fractions 14−17 yielded grandisine F (**7**) (5.1 mg, 0.00029%). The mass ion peak of *m*/*z* 291 was detected by +ESIMS in fractions 31 to 36 from the preparative C18 HPLC separation. These fractions were combined and purified by semipreparative C18 silica gel HPLC using a gradient of 7:3 to 2:3 1% TFA/H_2O:1% TFA/MeOH over 20 min to yield grandisine G (**8**) (3.8 mg, 0.00022%)

Grandisine F (7): yellow gum (5.1 mg, 0.00029%); $[\alpha]_D^{23}$ −22.4 (*c* 0.09, MeOH); UV (MeOH) λ_{max} (log ϵ) 202 (3.20), 274 (3.50) nm; IR (NaCl) ν_{max} 3292, 2929, 2800, 1654, 1608, 1406, 1181, 752 cm^{-1}; CD (MeOH) 217, 270, 322 nm, [θ] 2056, 29915, −13835; ^{1}H (600 MHz, CD_2Cl_2) Table 2; ^{13}C NMR (125 MHz, CD_2Cl_2) Table 1; (+)-LRESIMS *m*/*z* (rel int) 277 (100%) $[MH^+, C_{16}H_{25}N_2O_2]^+$; (+)-HRESIMS *m*/*z* 277.190345 [M + H]$^+$ (calcd for $C_{16}H_{25}N_2O_2$, 277.191054).

Grandisine G trifluoroacetate (8): unstable yellow gum (3.8 mg, 0.00021%); UV (MeOH) λ_{max} (log ϵ) 209 (3.39), 271 (3.08), 308 (3.00) nm; IR (NaCl) ν_{max} 3400, 2957, 1681, 1415, 1200, 1130, 830, 799, 754, 720 cm^{-1}; ^{1}H (600 MHz, d_6-DMSO) Table 2; ^{13}C NMR (125 MHz, d_6-DMSO) Table 1; (+)-LRESIMS *m*/*z* (rel int) 291 (100%) $[MH^+, C_{17}H_{27}N_2O_2]^+$.

δ-Opioid Receptor Binding Assay. Assays were performed in 50 mM Tris containing 3 mM $MgCl_2$ and 1 mg/mL 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). Isolated compounds were tested at a final concentration of 2% DMSO. Microplates were shaken for 1 h at ambient temperature (~23 °C), then left to reach steady state for 4 h. Microplates were counted for 1 min/well.

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