Alkaloids with Human δ-Opioid Receptor Binding Affinity from the Australian Rainforest Tree *Peripentadenia mearsii*

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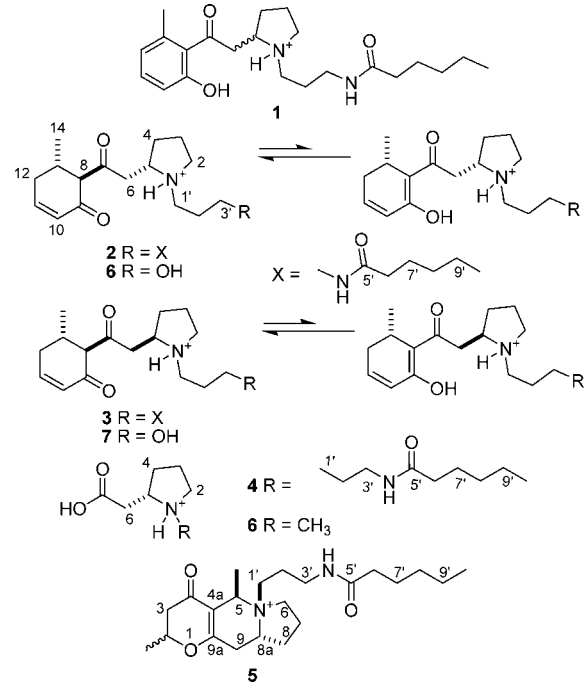
Three new pyrrolidine alkaloids, peripentonine A–C (**2**−**4**), one known pyrrolidine alkaloid, peripentadenine (**1**), and one novel indolizidine alkaloid, mearsamine (**5**), were isolated from the leaves of *Peripentadenia mearsii* and their structures determined by 1D and 2D NMR spectroscopy. Peripentonines A (**2**) and B (**3**) were isolated as a 1:1 mixture of inseparable diastereomers. Mearsamine (**5**) contains a novel tricyclic ring system. Peripentadenine and peripentonines A/B and C showed receptor binding affinity for the human δ-opioid receptor with IC_{50} values of 11.4, 69.2, and 30.9 μM, respectively. Mearsamine did not bind to the δ-opioid receptor.

We recently reported on the indolizidine and pyrrolidine alkaloid constituents of three species from the genus *Elaeocarpus*, and several of the 14 alkaloids isolated have shown moderate activity in a δ-opioid receptor binding assay.[1−4] These results prompted us to conduct a survey of plants from the family Elaeocarpaceae to identify additional biologically active alkaloids.[4] Eighty-five species from the Elaeocarpaceae collected from Australia, Papua New Guinea, and China were surveyed, and leaf, seed, and bark extracts from *Peripentadenia mearsii* (C.T. White) L.S.Sm. proved to be alkaloid positive. The genus *Peripentadenia* is comprised of two species, *P. mearsii* and *P. phelpsii*, both of which are rainforest trees endemic to North Queensland.[5] The bark and leaves of *P. mearsii* collected from Boonjie have been the focus of previous chemical investigations,[6,7] and the pyrrolidine alkaloid peripentadenine (**1**) was identified as the major alkaloid constituent of bark extracts and the only alkaloid constituent of leaf extracts.[7] A further four minor alkaloids, dinorperipentadenine, peripentamine, anhydroperipentamine, and mearsine, were also isolated from the bark.[7] We report herein the isolation, structure determination, and human δ-opioid receptor binding affinity of three new pyrrolidine alkaloids, peripentonines A (**2**), B (**3**), and C (**4**), a new indolizidine alkaloid, mearsamine (**5**), and the known alkaloid peripentadenine (**1**) from *P. mearsii*, collected from Wooroonooran National Park in North Queensland. All compounds were isolated as their TFA salts.



Results and Discussion

Dragendorff's reagent was used to identify alkaloid-positive extracts. False positive results to Dragendorff's reagent have been reported for phenolic compounds,[8] and since plants from the Elaeocarpaceae contain high concentrations of tannins,[9] a backup test was required to confirm the positive Dragendorff's reagent result. We have observed that plants containing even low concentrations (>0.01% yield dry weight) of alkaloids produce intense ions by (+) ESIMS, and so only extracts that showed a positive result from the Dragendorff's reagent test and that produced strong ions by (+) ESIMS were considered to contain alkaloids. The leaves, seeds, and bark of *P. mearsii* were surveyed, and all three returned positive tests for alkaloids. The (+) ESIMS of the leaf extract showed ions at *m*/*z* 285, 377, and 395, the seed extract showed an ion at *m*/*z* 377, and the bark extract showed an ion at *m*/*z* 375.

The leaves of *P. mearsii* were extracted with MeOH, and the extract was filtered through strongly acidic ion-exchange resin (SCX) under vacuum and washed sequentially with MeOH and H_2O. Alkaloids were eluted from the SCX with 1 M NaCl. The separation of NaCl from the alkaloids was facilitated by filtration through C_{18} Si gel. The NaCl was removed by extensive washing of the C_{18} Si gel with H_2O, and an alkaloid fraction was obtained by elution with 1% TFA/MeOH. Mass ion peaks at *m*/*z* 285, 377, and 395 were detected in this fraction by (+) ESIMS. Gradient C_{18} HPLC separation yielded peripentonine C (**4**) (11.2 mg, 0.022%), mearsamine (**5**) (13.2 mg, 0.026%), and peripentonines A (**2**) and B (**3**) as an inseparable 1:1 mixture (33.2 mg, 0.636%). The bark and seeds of *P. mearsii* were each extracted and purified using a protocol similar to that used for the leaves. The bark yielded peripentadenine (**1**) (19.0 mg, 0.013%), and a 1:1 mixture of peripentonines A (**2**) and B (**3**) (18.7 mg, 0.047%) was isolated from the seeds. The structure of **1** was confirmed by analysis of 2D NMR data (Table 1) and by comparison with published results. Compound **1** was isolated as a racemate.

Peripentonines A (**2**) and B (**3**) were both assigned the molecular formula $C_{22}H_{36}N_2O_3$ by (+) HRESI mass measurement of the [M

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Table 1. ^{1}H (600 MHz) and ^{13}C (125 MHz) NMR Spectroscopic Data for Compounds **1**−**4**

	1[a]		**2**[b]		**3**[b]		**4**[a]	
position	δ_C	δ_H (*J*, Hz)	δ_C	δ_H (*J*, Hz)	δ_C	δ_H (*J*, Hz)	δ_C	δ_H (*J*, Hz)
1		9.73 bs		9.69 bs		9.69 bs		9.48 bs
2	52.6	3.09 m	53.0[c]	3.05 m	53.1[c]	3.05 m	52.9	3.08 m
		3.54 bdd (5.4, 12.6)		3.65 bdd (6.6, 13.8)		3.65 bdd (6.6, 13.8)		3.55 bdd (4.8, 9.6)
3	21.3	1.91 dddd (2.4, 7.2, 7.2, 13.2)	22.3	2.06 m	22.3	2.06 m	21.4	1.89 dddd (7.2, 7.2, 7.2, 15.6)
		2.00 m						1.97 dddd (5.4, 5.4, 5.4, 13.2)
4	29.4	1.68 bdd (8.4, 16.4)	29.8	1.81 ddd (2.4, 7.7, 15.6)	29.8	1.81 ddd (2.4, 7.7, 15.6)	29.3	1.66 ddd (7.8, 7.8, 15.4)
		2.32 bdd (6.6, 13.8)		2.36 bdd (6.6, 13.8)		2.36 bdd (6.6, 13.8)		2.25 ddd (6.6, 6.6, 14.4)
5	63.4	3.79 bdd (9.0, 9.0)	64.2[c]	3.78 bdd (7.2, 13.8)	64.0[c]	3.78 bdd (7.2, 13.8)	64.0	3.62 bs
6	44.7	3.30 dd (9.6, 18.0)	44.9	3.00 dd (6.0, 14.4)	44.8	3.18 dd (6.0, 14.4)	35.4	2.62 dd (9.0, 16.8)
		3.49 dd (3.6, 18.0)		3.18 dd (7.8, 14.4)		3.30 dd (7.8, 14.4)		2.94 dd (4.8, 16.8)
7	203.4		206.0[c]		206.1[c]		171.2	
8	127.7		66.5	3.46 d (9.6)	66.5	3.45 d (9.6)		
9	154.7		197.2		197.2			
10	113.5	6.77 d (7.8)	128.3	5.97 d (10.2)	128.3	5.97 d (10.2)		
11	130.6	7.13 dd (7.8, 7.8)	152.4[c]	7.11 ddd (4.6, 7.2, 9.6)	152.2[c]	7.11 ddd (4.6, 7.2, 9.6)		
12	121.1	6.68 d (7.8)	32.9	2.16 m	33.0	2.16 m		
				2.55 m		2.52 m		
13	135.8		31.7[c]	2.55 m	31.9[c]	2.55 m		
14	19.0	2.14 s	19.0[c]	0.99 d (6.6)	19.1[c]	0.99 d (6.6)		
1′	50.8	2.99 ddd (6.6, 12.6, 12.6)	51.2[c]	2.93 ddd (6.0, 6.0, 13.2)	51.5[c]	2.93 ddd (6.0, 6.0, 13.2)	51.2	2.97 m
		3.33 m		3.23 ddd (5.4, 5.4, 13.8)		3.23 ddd (5.4, 5.4, 13.8)		3.29 ddd (2.4, 9.6, 9.6)
2′	25.5	1.77 t (7.2)	25.8[c]	1.93 m	25.7[c]	1.93 m	25.5	1.76 bt (7.2)
3′	35.6	3.09 q (7.2)	35.9[c]	3.30 m	36.0[c]	3.30 m	35.6	3.08 t (7.2)
4′		7.92 bt (7.2)		7.23 bt (7.2)		7.23 bt (7.2)		7.90 bt (7.2)
5′	172.5		176.2[c]		176.3[c]		172.5	
6′	35.3	2.04 t (7.2)	35.7	2.21 m	35.7	2.21 m	35.3	2.05 t (7.2)
7′	24.8	1.47 t (7.2)	25.4	1.57 m	25.3	1.58 m	24.8	1.48 t (7.2)
8′	30.8	1.21 (7.2)	31.7	1.30 m	31.7	1.30 m	30.9	1.22 t (7.2)
9′	21.8	1.24 t (7.2)	21.5[c]	1.30 m	21.7[c]	1.30 m	22.0	1.26 t (7.2)
10′	13.7	0.82 t (7.2)	13.4	0.89 bt (7.2)	13.4	0.89 bt (7.2)	13.8	0.85 t (7.2)

[a] In d_6-DMSO. [b] In CD_3CN. [c] Assignments within a row may be interchanged.

+ H]$^+$ ion (Δ −1.8 ppm). The ^{1}H NMR spectrum of the mixture of **2** and **3** in d_6-DMSO contained signals of varying intensity, most noticeably in the olefinic region (δ 5.8–7.2), where a ratio of 1:2 was observed between signals at δ_H 7.13 and 6.65 and between signals at δ_H 5.94 and 6.06. The ratio of these signals changed when the ^{1}H NMR spectra were acquired in different deuterated solvents (CD_3OD, CD_3CN, and d_5-pyridine). Acquisition of the ^{1}H NMR spectrum in CD_3CN with one drop of TFA (Table1) provided the best ratio of tautomers (6:1) and allowed the structures of peripentonines A (**2**) and B (**3**) to be determined. The chemical shifts of the olefinic protons from the major and minor tautomers of peripentonines A and B were almost identical to those observed for habbemines A (**6**) and B (**7**), alkaloids we had previously isolated from *Elaeocarpus habbemensis*.[3] This suggested that peripentonines A and B contained the same 6-ketocyclohex-2-enone moiety found in habbemines A (**6**) and B (**7**). Like habbemines A and B the ^{13}C NMR spectrum of peripentonines A and B (Table 1) contained more carbon resonance than the number of carbons determined by mass spectrometry. Although 36 carbon signals were visible in the ^{13}C NMR spectrum, these carbons could be assigned to two closely related molecules each containing 22 carbons since eight of the carbon signals were double the intensity of the remaining 28 carbons. These 28 carbon signals could each be grouped into 14 pairs with no more than a 0.3 ppm difference in chemical shift between each signal within a pair. Closer inspection of the ^{13}C NMR spectra of peripentonines A and B with that of habbemines A and B revealed a number of other similarities. In particular signals for a 6-(2-oxoethylpyrrolidinyl)cyclohex-2-enone moiety were almost identical between the compounds. This observation provided evidence that peripentonines A and B contained the same 6-(2-oxoethylpyrrolidinyl)cyclohex-2-enone moiety as that found in **6** and **7** and that doubling of the carbon signals was a result of epimerization at C-5, which yields a pair of diastereomers. Analysis of correlations observed in gCOSY, gHSQC, and gHMBC spectra confirmed that peripentonines A and B contained the 6-(2-oxoethylpyrrolidinyl)cyclohex-2-enone moiety. The remaining signals observed in the ^{1}H NMR spectrum of peripentonines A and B were 14 methylene protons between δ_H 1.30 and 3.30, a methyl triplet at δ_H 0.89, and an amide triplet at δ_H 7.23. Correlations observed in a gHSQC spectrum suggested that two of the methylenes were attached to nitrogen (δ_H/δ_C 2.93, 3.23/53.1, 3.30/36.0), while gCOSY correlations indicated that a 3-aminopropylamide and a pentyl group were present in the molecule. Correlations from δ_H 2.93 (H-1′) of the 3-aminopropylamide to δ_C 53.0/53.1 (C-2) and δ_C 64.0/64.2 (C-5) of the pyrrolidine in the gHMBC spectrum indicated that the propylamide group was attached to the pyrrolidine. Finally gHMBC correlations from δ_H 1.57/1.58 (H-7′) and 2.21 (H-6′) of the pentyl group and δ_H 3.30 (H-3′) of the propylamide to an amide carbonyl carbon at δ_C176.2/176.3 (C-5′) provided evidence that a hexamide group was attached to the propyl. Therefore the structures of peripentonines A (**2**) and B (**3**) were assigned, and they were diastereomeric tetrahydro derivatives of peripentadenine (**1**). A large coupling between H-8 and H-13 (J = 9.6 Hz) in both diastereomers indicated that these protons were diaxial. Dehydrogenation of the 1:1 mixture of peripentonines A (**2**) and B (**3**) with Pd/C in ethanol at 90 °C produced peripentadenine (**1**) in 63% yield. The ^{1}H NMR spectrum (d_6-DMSO) of the product was identical to that of the natural product (Table 1). Peripentadenine (**1**) obtained from dehydrogenation of **2** and **3** was racemic since it displayed no optical rotation, and this confirmed that **2** and **3** were epimeric at C-5.

Peripentonine C (**4**) was obtained as a gum. A molecular formula, $C_{15}H_{28}N_2O_3$, was assigned from analysis of the pseudomolecular ion (Δ −4.9 ppm) in the (+) HRESIMS. Peaks at 3404 and 1683 cm^{-1} in the IR spectrum of **4** indicated the presence of hydroxyl and carbonyl bonds, respectively. The ^{1}H NMR spectrum of peripentonine C (Table 1) was very similar to the spectra of peripentonines A (**2**) and B (**3**) and peripentadenine (**1**) but was much simpler. In particular, signals for the pyrrolidine and propylhexanamide moieties were still present; however, signals for olefinic or aromatic protons and a C-14 methyl doublet or singlet were absent. An amide signal was observed at δ_H 7.90, and an

exchangeable proton signal was observed at δ_H 9.48. The ^{13}C NMR spectrum of peripentonine C (**4**) (Table 1) contained 15 carbon signals. Two carbonyl carbons were observed at δ_C 172.5 and 171.2, and one of these was assigned to the amide carbonyl carbon of the hexanamide group. The chemical shifts of most carbons were identical to the pyrrolidine-*N*-propylhexanamide carbons of peripentadenine (**1**). The only difference in the ^{13}C NMR spectrum of peripentonine C, excluding the absence of aromatic carbons, was the upfield shift of C-6 from δ_C 44.7 in **1** to δ_C 35.4 in **4**. Analysis of gHSQC spectroscopic data established the presence of 26 carbon-bound protons including one methyl, 11 methylenes, and one methine. Correlations observed in a gCOSY spectrum demonstrated the presence of pentyl, propylamide, and 2-methylenepyrrolidinyl moieties. The pyrrolidine and propylamide fragments could be linked since a COSY correlation was observed from the exchangeable proton at δ_H 9.48 (1-NH) to δ_H 3.62 (H-5), 3.08 (H-2a), and 2.97 (H-1′a). Correlations observed in a gHMBC spectrum from H-3′, H-4′, H-7′, and H-8′ to the carbonyl carbon at δ_C 172.5 confirmed that a propylhexanamide was present in the molecule. HMBC correlations from the methylene protons at δ_H 3.29 (H-1′b) and 2.97 (H-1′a) to δ_C 52.9 (C-2) and 64.0 (C-5) and from δ_H 3.08 (H-2a) and 3.55 (H-2b) to δ_C 51.2 (C-1′) and C-5 confirmed the connection of a propyl group to the nitrogen of the pyrrolidine. HMBC correlations from H-6a, H-6b, and H-5 to a carbon at δ_C 171.2 (C-7) indicated that the second carbonyl carbon was vicinal to C-6. This carbon was assigned to a carboxylic acid since an oxygen and hydrogen were the only two atoms unaccounted for from the molecular formula. The relationship between specific rotation and absolute configuration of 4*S*-(1-methylpyrrolidin-2-yl)acetic acid (**6**) has been established by synthesis.[10] Since both **6** and **4** possess negative specific rotations ($[\alpha]_D$ −10 for **6** and $[\alpha]_D$ −5.8 for **4**), the chirality at C-5 of the pyrrolidin-2-yl-acetic acid in peripentonine C (**4**) was also assigned the *S* configuration. The structure of the TFA salt of peripentonine C was therefore established as **4**.

Mearsamine (**5**) was obtained as a yellow gum. The (+) LRESIMS exhibited ions at *m/z* 377 M^+ and 395 $[M^+ + H_2O]^+$. Accurate mass measurement of the $[M^+ + H_2O]^+$ ion in the (+) HRESIMS at *m/z* 395.2906 allowed the molecular formula $C_{22}H_{37}N_2O_3$ to be assigned to mearsamine (**5**). Absorption bands at 3457, 3288, and 1677 cm^{-1} in the IR spectrum of **5** indicated the presence of an −NH and carbonyl bonds, respectively. The ^1H NMR spectrum of mearsamine (**5**) (Table 2) contained characteristic signals for a propylhexanamide chain; however the downfield region of the spectrum was unique when compared to the other *Peripentadenina* alkaloids. Two additional methyl doublets at δ_H 1.38 and 1.42, 10 methylene protons at δ_H 1.81, 2.03 (2×H), 2.27, 2.47, 2.49, 2.60, 3.15, 3.17, and 3.53, and three methine protons at δ_H 4.10, 4.37, and 4.60 were observed. The ^{13}C NMR spectrum of mearsamine (**5**) (Table 2) contained signals for seven carbons, which were almost identical to the chemical shifts of C-3′ and C-5′ to C-10′ in peripentadenine (**1**) and peripentonine C (**4**). Six additional methylene carbons were observed between δ_C 20 and 56 and two additional methyl signals were observed at δ_C 16.4 and 19.9. Carbon signals at δ_C 55.8, 56.8, 58.6, 66.7, and 75.9 suggested these carbons were attached to heteroatoms. In addition to the amide carbonyl carbon at δ_C 172.5 three downfield signals were observed at δ_C 108.7, 168.0, and 188.4. The signal at δ_C 188.4 was indicative of an α,β-unsaturated ketone carbon, while the carbons at δ_C 108.7 and 168.0 could be assigned to the carbons α and β to the ketone, respectively. The β-carbon was shifted downfield, relative to the typical chemical shift for such carbons, and this suggested an electron-withdrawing atom, possibly an oxygen, was attached to the β-carbon. HSQC correlations established the presence of 36 carbon-bound protons including three methyls, 12 methylenes, and three methines. Correlations observed in a gCOSY spectrum allowed three partial structures in addition to the aminopropylhexamide to be assigned. An aliphatic fragment was assembled from correlations between the methylene protons at δ_H 2.03 (7-CH_2) and the methylene protons at δ_H 3.53 (H-6α), 3.17 (H-6β), 2.27 (H-8β), and 1.81 (H-8α). COSY correlations from H-8β and H-8α to the proton at δ_H 4.10 (H-8a) indicated that this methine was adjacent to C-8. COSY correlations from the methylene protons at δ_H 3.15 (H-9β) and 2.49 (H-9α) to H-8a demonstrated that these protons were also vicinal to H-8a. The remaining two partial structures were assigned from correlations from the methyl protons at δ_H 1.42 (5-CH_3) to the methine proton at δ_H 4.37 (H-5) and from correlations observed from the proton of an oxygenated methine at δ_H 4.60 (H-2) to the protons of a methyl group at δ_H 1.38 (2-CH_3) and methylene protons at δ_H 2.60 (H-3b) and 2.47 (H-3a). The proton at δ 4.60 was bound to a carbon at δ_C 75.9, and this chemical shift was characteristic of an ether carbon. Only two HMBC correlations were observed from the methyl protons at δ_H 1.42 (5-CH_3) to the methine carbon at δ_C 56.8 (C-5) and the quaternary olefinic carbon at δ_C 108.7 (C-4a). The proton at δ_H 4.37 (H-5) exhibited $^3J_{CH}$ correlations to the ketone carbon at δ_C 188.4 (C-4), the olefinic carbon at δ_C 168.0 (C-9a), the heterosubstituted methine carbon at δ_C 66.7 (C-8a), and the aminomethylene carbon at δ_C 58.6 (C-6). This confirmed that C-5 was attached to C-4a, the α-carbon of the α,β-unstaturated ketone. The correlations from H-5 to C-8a and C-6 suggested that a nitrogen atom could be inserted between C-5, C-6, and C-8a to form a pyrrolidine. This was corroborated by correlations observed between H-1′ and C-6 and C-8a, between H-8a and C-6, and between H-6α and H-6β and C-8a and C-1′. This partial structure could be expanded to an indolizidine since a $^3J_{CH}$ correlation was observed between H-8a, H-9α, and H-9β and C-9a, while H-9α and H-9β also correlated to C-4a. The methylene protons at δ_H 2.60 (H-3b) and 2.47 (H-3a) displayed $^2J_{CH}$ correlations to the ketone carbon at δ_C 188.4 (C-4), and this indicated that the $-CH_2-CH(CH_3)-O-$ partial structure was adjacent to the ketone. A weak $^4J_{CH}$ correlation from the methyl protons at δ_H 1.38 (2-CH_3) to C-9a as well as to C-2 and C-3 indicated that a pyran ring could be formed from insertion of an oxygen atom between C-2 and C-9a. The planar structure of mearsamine was therefore established as **5**. Hydration and ring opening of the quaternary ammonium ion (N-5a) could explain the observation of

Table 2. ^1H (600 MHz) and ^{13}C (125 MHz) NMR Spectroscopic Data for Mearsamine (**5**) in d_6-DMSO

position	δ_C	δ_H (*J*, Hz)	HMBC
2	75.9	4.60 ddd (4.2, 7.2, 14.4)	
2-CH_3	19.9	1.38 d (7.2)	C-2, C-3, C-9a
3	41.8	2.47 dd (4.2, 15.6)	C-2, 2-CH_3, C-4, C-5
		2.60 dd (14.4, 15.6)	C-2, 2-CH_3, C-4, C-5
4	188.4		
4a	108.7		
5	56.8	4.37 q (6.6)	C-4, C-4a, 5-CH_3, C-6, C-8a, C-9a
5-CH_3	16.4	1.42 d (6.6)	C-4a, C-5
6α	58.6	3.53 ddd (5.4, 7.2, 12.6)	C-7, C-8, C-8a, C-1′
6β		3.17 ddd (2.4, 9.6, 12.6)	C-7, C-8, C-8a, C-1′
7	20.1	2.03 m	C-8a,
8α	29.6	1.81 ddd (6.6, 6.6, 15.0)	C-6, C-7, C-8a, C-9
8β		2.27 ddd (7.2, 7.2, 15.0)	C-6, C-7, C-9
8a	66.7	4.10 bs	C-6, C-9, C-7, C-9a,
9α	29.8	2.49 m	C-4a C-8, C-8a, C-9a
9β		3.15 m	C-4a C-8, C-8a, C-9a
9a	168.0		
1′	55.8	3.28 m	C-6, C-8a, C-2′, C-3′
2′	21.8	1.87 tt (7.2, 7.2)	C-1′, C-3′
3′	35.4	3.10 m	C-1′, C-2′, C-5′
4′		7.91 t (7.2)	C-3′, C-5′, C-6′
5′	172.5		
6′	35.6	2.03 t (7.2)	C-5′, C-7′, C-8′
7′	24.8	1.47 tt (7.2, 7.2)	C-5′,C-6′, C-8′, C-9′
8′	30.9	1.20 tt (7.2, 7.2)	C-6′, C-7′, C-9′, C-10′
9′	21.8	1.25 qt, (7.2, 7.2)	C-7′, C-8′, C-10′
10′	13.8	0.83 t (7.2)	C-9′, C-8′

a hydrated product in the (+) ESIMS. The relative configurations of three of the four stereogenic centers in **5** were deduced from correlations observed in a ROESY spectrum. Correlations between 5-CH_3, H-1′, and H-8a indicated that these protons were all on the same face of the molecule. This assignment was corroborated by correlations between H-5 and H-6β and between H-9β and H-8α since all of these protons were located on the opposite face of the molecule. H-2 exhibited one large (J = 14.4 Hz) and one small (J = 4.2 Hz) coupling to the protons attached to C-3, and this indicated that this proton was axial. The configuration at C-2 relative to C-5, N-5a, and C-8a could not be determined since the distance between any of the protons in the dihyropyranone 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 mearsamine, and the correct relative configuration at C-2 remains to be assigned. Mearsamine (**5**) contains a novel tricyclic 2,3,8,9,9a,10-hexahydro-5*H*-pyrano[3,2-*f*]indolizin-4-(7*H*)-one ring system.

Biological Activity. Compounds **1**, **2/3**, and **4** inhibited the binding of [^{125}I]-deltorphin II to HEK cell membranes expressing recombinant human δ-opioid receptors with IC_{50} values of 11.4, 69.2, and 30.9 μM, respectively, while mearsamine was inactive at 100 μM. IC_{50} values for the positive controls DPDPE and naloxone were 1.2 and 138 nM, respectively. When comparing the activity of **1** with **2/3**, it is clear that replacing the cyclohexenone with an aromatic group significantly improves the binding affinity. When one compares the activity of habbemines A and B (32.1 μM) with that of peripentonines A and B, replacement of the propyl alcohol with the propylhexamide leads to a halving of the binding affinity. Removing the cyclohexenone and replacing it with a carboxylic acid doubles the binding affinity. The observation that mearsamine shows no binding affinity for the δ-opioid receptor suggests that either conversion of the nitrogen of the pyrrolidine to a quaternary amine or steric crowding of the pyrrolidine nitrogen is not tolerated.

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 GBC 916 UV/vis spectrophotometer, and IR spectra were recorded on a Nicolet NEXUS FT IR spectrometer. NMR spectra were recorded on Varian Inova 600 and 500 MHz NMR spectrometers. Samples were dissolved in either d_6-DMSO or CD_3CN containing a drop of TFA, and chemical shifts were calculated relative to the DMSO solvent peak (^{1}H δ 2.49 and ^{13}C δ 39.5) or the CH_3CN solvent peak (^{1}H δ 1.94 and ^{13}C δ 118.7). 2D NMR spectra were recorded at 30 °C using standard Varian pulse sequences gCOSY, gHSQC, gHMBC, and ROESY. ESIMS were measured on a Mariner Biospectrometry TOF workstation using positive electrospray ionization, mobile phase 1:1 MeOH/H_2O. HRESIMS were recorded on a Bruker Daltonics APEX III 4.7e FT-MS equipped with an Apollo API ionization source. Dowex 50WX8-400 strongly acidic ion-exchange resin (SCX), 200–400 mesh (Aldrich), and Allech Davisil C_{18} bonded Si gel (end capped 35–70 μM) were used during purification. HPLC purifications were achieved using a Hypersil BDS C_{18} preparative (150 × 21.2 mm, 5 μm) column.

Plant Material. The leaves, seeds, and bark of *Peripentadenia mearsii* (Elaeocarpaceae) were collected in November 1998 by P.I.F. (Queensland Herbarium) from Wooroonooran National Park in North Queensland. A voucher specimen, PIF23959, is housed at the Queensland Herbarium

Extraction and Isolation. The dried and ground leaves of *P. mearsii* (50.79 g) were extracted with MeOH (3 L). The extract was filtered through SCX (45 g) under vacuum and washed sequentially with MeOH (1 L) and H_2O (1 L). Alkaloids were eluted from the SCX with 1 M NaCl (1 L). The alkaloid/NaCl solution was filtered through C_{18} Si gel (27 g) under vacuum. The NaCl was separated from the alkaloids by extensive washing of the C_{18} Si gel with H_2O (5 L), and an alkaloid fraction was eluted with 1% TFA/MeOH (500 mL). This alkaloid fraction was evaporated, and the residue (108.7 mg) was adsorbed onto C_{18} Si gel (1.2 g). The alkaloid-impregnated C_{18} Si gel was loaded into a metal HPLC column (10 × 15 mm), which was connected to a preparative C_{18} HPLC column. The alkaloids were separated into 50 fractions with a gradient from H_2O to 1% TFA/MeOH over 100 min. Fractions 22 and 23 were found to be pure peripentonine C (**4**) (11.2 mg, 0.022%), fraction 25 yielded pure mearsamine (**5**) (13.2 mg, 0.026%), and fractions 28−32 yielded pure peripentonines A (**2**) and B (**3**) (33.2 mg, 0.636%).

The dried and ground bark of *P. mearsii* (150 g) was extracted with MeOH (3 L). The extract was filtered through SCX (60 g) under vacuum and washed sequentially with MeOH (1 L) and H_2O (1 L). Alkaloids were eluted from the SCX resin with 1 M NaCl (1 L). The alkaloid/NaCl solution was filtered through C_{18} Si gel (30 g) under vacuum. The NaCl was separated from the alkaloids by extensive washing of the C_{18} Si gel with H_2O (5 L), and an alkaloid fraction was eluted with 1% TFA/MeOH (500 mL). This alkaloid fraction was evaporated, and the residue (42.6 mg) was adsorbed onto C_{18} Si gel (1.2 g). The alkaloid-impregnated C_{18} was loaded into a metal HPLC column (10 × 15 mm), which was connected to a preparative C_{18} HPLC column. The alkaloids were separated into 50 fractions with a gradient from H_2O to 1% TFA/MeOH over 100 min. Fractions 34 to 36 were found to be pure peripentadenine (**1**) (19.0 mg, 0.013%).

The dried and ground seeds of *P. mearsii* (40 g) were extracted with MeOH (3 L). The extract was filtered through SCX (28 g) under vacuum and washed sequentially with MeOH (1 L) and H_2O (1 L). Alkaloids were eluted from the SCX resin with 1 M NaCl (1 L). The alkaloid/NaCl solution was filtered through C_{18} Si gel (22 g) under vacuum. The NaCl was separated from the alkaloids by extensive washing of the C_{18} Si gel with H_2O (5 L), and an alkaloid fraction was eluted with 1% TFA/MeOH (500 mL). This alkaloid fraction was evaporated, and the residue (38.4 mg) was adsorbed onto C_{18} Si gel (1.2 g). The alkaloid-impregnated C_{18} Si gel was loaded into a metal HPLC column (10 × 15 mm), which was connected to a preparative C_{18} HPLC column. The alkaloids were separated into 50 fractions with a gradient from H_2O to 1% TFA/MeOH over 100 min. Fractions 34 to 36 were found to be pure peripentonines A (**2**) and B (**3**) (18.7 mg, 0.468%).

Peripentonine A and B trifluoroacetate (2/3): yellow gum; $[\alpha]^{23}_D$ +19.0 (*c* 0.13, MeOH); UV (MeOH) λ_{max} (log ε) 226 (3.45), 339 (2.81) nm; IR (KBr) ν_{max} 3410, 3294 br, 2967, 2926, 1667, 1543, 1415, 1193, 1123, 715 cm^{-1}; ^{1}H (600 MHz, CD_3CN) and ^{13}C NMR (125 MHz, CD_3CN), Table 1; (+)-LRESIMS *m/z* (rel int) 377 (75%) [MH^+, $C_{22}H_{37}N_2O_3]^+$, 225 (25%) (+)-HRESIMS *m/z* 377.2792 [M + H]$^+$ (calcd for $C_{22}H_{37}N_2O_3$, 377.2799).

Peripentonine C trifluoroacetate (4): yellow gum; $[\alpha]^{23}_D$ −5.8 (*c* 0.07, MeOH); UV (MeOH) λ_{max} (log ε) 204 (2.62), 269 (2.05), 339 (1.73) nm; IR (KBr) ν_{max} 3404 br, 2955, 2920, 1683, 1438, 1199, 1152 cm^{-1}; ^{1}H (600 MHz, d_6-DMSO) and ^{13}C NMR (125 MHz, d_6-DMSO), Table 1; (+)-HRESIMS *m/z* 285.2159 [M + H]$^+$ (calcd for $C_{15}H_{29}N_2O_3$, 285.2173).

Mearsamine trifluoroacetate (5): yellow gum; $[\alpha]^{23}_D$ −1.5 (*c* 0.07, MeOH); UV (MeOH) λ_{max} (log ε) 202 (3.14), 265 (3.38) nm; IR (KBr) ν_{max} 3457 br, 3288 br, 2973, 2932, 2873, 1677, 1555, 1426, 1187, 1123 cm^{-1}; ^{1}H (600 MHz, d_6-DMSO) and ^{13}C NMR (125 MHz, d_6-DMSO), Table 2; (+)-LRESIMS *m/z* (rel int) 377 (100%) [M]$^+$, 395 (20%) [M^+ + H_2O]$^+$; (+)-HRESIMS *m/z* 395.2906 [M^+ + H_2O]$^+$ (calcd for $C_{22}H_{39}N_2O_4$, 395.2904).

Dehydrogenation of Peripentonines A (2) and B (3). Pd/C (23.2 mg) was added to a solution of peripentonines A (**2**) and B (**3**) (44.0 mg) in ethanol (5 mL), and the suspension was allowed to stir under reflux for 16 h at 90 °C. The reaction mixture was filtered through Celite to remove the Pd/C. The filtrate was separated by C_{18} HPLC using a gradient from 7:3 to 1:4 H_2O:1%TFA/MeOH to yield peripentadenine (**1**) (25.5 mg, 63%). The optical rotation of **1** from this reaction was 0.

***δ*-Opioid Receptor Binding Assay.** Assays were performed in 50 mM Tris containing 3 mM $MgCl_2$, 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). 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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