Habbemines A and B, Pyrrolidine Alkaloids with Human δ-Opioid Receptor Binding Affinity from the Leaves of *Elaeocarpus habbemensis*

Peter L. Katavic,[†] Debra A. Venables,[†] Topul Rali,[‡] and Anthony R. Carroll*,[†]

Natural Product Discovery, Eskitis Institute, Griffith University, Brisbane, Queensland, Australia 4111, and Biodiversity Research Ltd., Port Moresby, Papua New Guinea

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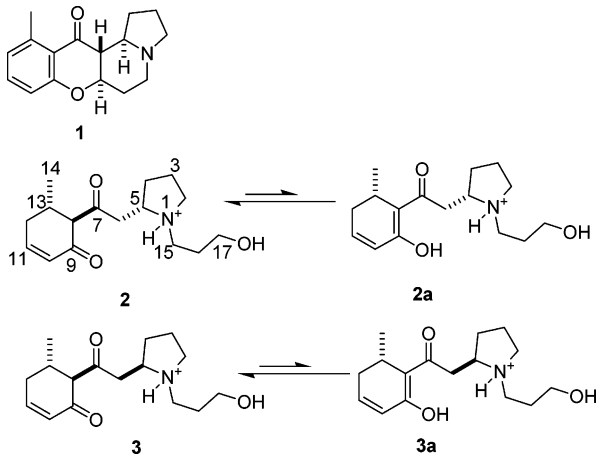
The first phytochemical investigation of the Papua New Guinean plant *Elaeocarpus habbemensis* resulted in the isolation of two new pyrrolidine alkaloids, habbemines A (**2**) and B (**3**), as a 1:1 mixture of inseparable diastereomers. The structures of these compounds and their relative configurations were determined by spectroscopic means. An equimolar mixture of habbemines A and B showed human δ-opioid receptor binding affinity with an IC_{50} of 32.1 μM.

Previous chemical studies of plants from the genus *Elaeocarpus* (Elaeocarpaceae) have been limited to five species from Papua New Guinea (PNG) and one from India.[1−6] These plants were identified to contain indolizidine alkaloids such as elaeocarpine (**1**) after giving strong positive results in an alkaloid field test.[2] Recently, we have reported on the human δ-opioid receptor binding affinity of a number of indolizidine alkaloids that were isolated from the Australian rainforest tree *E. grandis*.[7,8] In an attempt to extend structure−activity relationships within the indolizidine series we have undertaken a survey of all species of *Elaeocarpus* native to Queensland and a number of species collected from PNG. Leaf extracts from a previously uninvestigated species, *E. habbemensis* (Frodin, 1970), a plant found in the Manegegilli village swamp forest near Ialibu in the Southern Highlands province of PNG, gave a positive alkaloid test to Dragendorff's reagent and showed a strong ion at *m*/*z* 280 in the (+) ESIMS. In this paper, we report the isolation, structure elucidation, and δ-opioid binding affinity of habbemine A (**2**) and habbemine B (**3**), new pyrrolidine alkaloids from the leaves of *E. habbemensis*.

The dried, ground leaves were extracted exhaustively with MeOH, and the extract was filtered through polyamide gel to remove tannins and then evaporated. The residue was dissolved in H_2O and partitioned with DCM. The aqueous layer, which gave a positive Dragendorff test and a mass ion peak at *m*/*z* 280 in the (+) ESIMS, was filtered through strongly acidic ion-exchange resin (SCX). The resin was washed with a large volume of H_2O, and then an alkaloid-rich fraction was eluted from the resin with a 1 M solution of NaCl. The alkaloid fraction was evaporated under vacuum and the residue suspended in a 1:1 mixture of $CHCl_3$−MeOH and filtered to remove NaCl. The filtrate was purified on Sephadex LH-20, eluting with MeOH. Fractions were analyzed by (+) ESIMS and ^{1}H NMR spectroscopy, and the alkaloid-containing fractions were rechromatographed on Sephadex LH-20 to afford a fraction (7.7 mg, 0.0096%) showing a pseudomolecular ion in the (+) ESIMS at *m*/*z* 280. The ^{1}H NMR spectrum of this fraction in $CDCl_3$ was quite complex, most noticeably in the olefinic region (δ 5.8−7.2), where a ratio of 1:4 was observed between signals at δ 5.96 and 6.06 and between signals at δ 7.14 and 6.67. This suggested that the fraction was a 1:4 mixture of two compounds. There was no change in the intensity of these signals on heating the sample to 50 °C; however the ratio of these signals changed when the ^{1}H NMR spectra were acquired in different deuterated solvents (CD_3OD, CD_3CN, d_6-DMSO, and d_6-DMSO containing a drop of TFA). This indicated that the fraction was more likely a mixture of tautomers in solution. Acquisition in d_6-DMSO containing a drop of TFA gave the least complex spectrum with the two tautomers being observed in a ratio of 10:1. However, the ^{13}C NMR spectrum of this fraction in d_6-DMSO containing a drop of TFA was even more complex, with 25 distinct carbon signals, seven of which were double the intensity of the other signals. This suggested that the fraction was a 1:1 mixture of two closely related compounds, each of which tautomerizes in solution, since the mass of 25 carbons was greater than the mass observed in the mass spectrum. Exhaustive attempts to separate the mixture by C_{18} silica gel HPLC, using isocratic conditions of 84:15:1 H_2O−MeOH−TFA, 89:10:1 H_2O−ACN−TFA, and 85:7:7:1 H_2O−MeOH−ACN−TFA, were unsuccessful. An attempted separation using reversed-phase HPLC on phenyl-bonded silica gel and isocratic elution with 94:5:1 H_2O−MeOH−TFA also proved unsuccessful. To gain a better understanding of the reasons that these two compounds could not be separated, a structure determination was carried out on the mixture. The two compounds were named habbemines A (**2**) and B (**3**) and were isolated as their hydrochloride salts.



Accurate mass measurement of the pseudomolecular ion in the (+) HRESIMS at *m*/*z* 280.18939 allowed a molecular formula of $C_{16}H_{26}NO_3$ to be assigned to habbemines A and B. Infrared absorption bands at 1712 and 1674 cm^{-1} suggested the presence of a ketone and an α,β-unsaturated ketone, and this was supported by a UV absorbance at 270 nm. Although 25 carbon signals were visible in the ^{13}C NMR spectrum (Table 1), these carbons could be assigned to two closely related molecules each containing 16 carbons since seven of the carbon signals were double the intensity of the remaining 18. These 18 carbon signals could each be grouped

* To whom correspondence should be addressed. Tel: 61 7 3735 6015. Fax: 61 7 3735 6001. E-mail: A.Carroll@griffith.edu.au.
[†] Griffith University.
[‡] Biodiversity Research Ltd, Port Morsby.

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Table 1. ^{1}H (600 MHz) and ^{13}C (125 MHz) NMR Spectroscopic Data for Habbemines A (**2**) and B (**3**) in d_6-DMSO Containing a Drop of TFA

	habbemine A (**2**)		habbemine B (**3**)	
position	δ_C, mult.	δ_H (*J* in Hz)	δ_C, mult.	δ_H (*J* in Hz)
1		10.35 bs		10.35 bs
2	52.4, CH_2	3.07 m	52.3, CH_2	3.07 m
		3.54 m		3.54 m
3	21.2, CH_2	1.87 ddd (9.0, 14.4, 15.2)	21.2, CH_2	1.87 ddd (9.0, 14.4, 15.2)
		1.96 m		1.96 m
4	29.4, CH_2	1.61 ddd (9.0, 9.0, 9.0)	29.4, CH_2	1.61 ddd (9.0, 9.0, 9.0)
		2.27 ddd (9.0, 9.0, 9.0)		2.27 ddd (9.0, 9.0, 9.0)
5	62.6, CH	3.65 bddd (4.2, 8.4, 9.0)	62.5, CH	3.65 bddd (4.2, 8.4, 9.0)
6	44.2, CH_2	3.02 dd (8.4, 18.0)	44.9, CH_2	3.15 dd (8.4, 18.6)
		3.32 dd (4.2, 18.0)		3.28 dd (4.2, 18.6)
7	205.8, qC		205.8, qC	
8	66.1, CH	3.58 d (11.4)	65.5, CH	3.56 d (11.4)
9	196.5, qC		196.4, qC	
10	128.0, CH	5.95 d (10.2)	128.0, CH	5.97 d (10.2)
11	152.3, CH	7.14 dd (6.6, 10.2)	152.2, CH	7.19 dd (6.6, 10.2)
12	31.3, CH_2	2.17 dd (8.4, 11.4)	31.6, CH_2	2.14 dd (8.4, 11.4)
		2.49 m		2.49 m
13	32.4, CH	2.49 m	32.4, CH	2.47 m
14	19.2, CH_3	0.93 d (6)	19.1, CH_3	0.94 d (6)
15	50.5, CH_2	2.96 m	50.4, CH_2	2.96 m
		3.39 td (6, 13)		3.39 td (6, 13)
16	28.1, CH_2	1.81 m	28.1, CH_2	1.81 m
17	57.9, CH_2	3.46 d (6.0)	57.9, CH_2	3.46 d (6.0)

into nine pairs with no more than a 0.7 ppm difference in chemical shift between each signal within a pair. This observation provided evidence that habbemines A and B are diastereomeric. The two ketone carbonyl carbon resonances at δ 196.5/196.4 and 206.6 and olefinic carbons signals at δ 128.0 and 152.3/152.2 supported the assignment that each isomer contained an α,β-unsaturated ketone and a saturated ketone. Analysis of correlations obtained from a gHSQC spectrum established the presence of one methyl, eight methylenes (two of which were attached to nitrogen and one attached to oxygen), three methines, and two olefinic carbons in each of the isomers. Interestingly, the proton chemical shifts observed for most of the correlations between proton carbon pairs for the two isomers in this spectrum were very similar, with the only striking difference being observed for the proton signals that correlated to the methylene carbons C-6, at δ 44.2 and 44.9.

Since most of the proton chemical shifts were identical between the two isomers, analysis of a gCOSY spectrum was used to establish the partial structures $CH{=}CHCH_2CH(CH_3)CH$, $CH_2CH(N)CH_2CH_2CH_2(N)$, and $(N)CH_2CH_2CH_2(O)$ in both isomers. The connection of these partial structures was established from interpretation of correlations observed in a gHMBC experiment. The pyrrolidine functionality was established from a correlation between the H-2a aminomethylene proton at δ 3.07 and C-5 (δ 62.5/62.6). The methylcyclohexenone partial structure was determined from correlations observed from the H-11 olefinic proton at δ 7.14/7.19 and the H-8 methine proton at δ 3.58/3.56 to the C-9 ketone carbon at δ 196.5/196.4. The second ketone carbon was vicinal to both C-8 and C-6 since correlations were observed from the H-6 methylene protons and H-8 to the C-7 ketone carbon at δ 205.8. This indicated that habbemines A (**2**) and B (**3**) both contained a 1,3-diketone, a functionality known to undergo tautomerism in solution. Strong correlations from the H-6 methylene protons to C-5 (δ 62.6/62.5) and C-4 (δ 29.4) indicated that the diketone moiety was attached to the pyrrolidine via a methylene bridge. A correlation from the H-15 aminomethylene proton at δ 3.39 to C-5 indicated that a propyl side chain was attached to the pyrrolidine nitrogen. The ^{1}H and ^{13}C chemical shifts observed for the C-17 methylene at δ 3.46/57.9, and consideration of the molecular formula obtained from HRESIMS, dictated that C-17 is a primary alcohol. The molecular structures of habbemines A and B were therefore established. As the gross structure for the two compounds contained three chiral centers, it was safe to assume that habbemines A and B differ from each other only in the configuration at either one or two of these chiral centers. Correlations observed in a ROESY spectrum between H-14 and H-8 demonstrated that the H-14 methyl protons and the H-8 were on the same face of the cyclohexenone ring in both isomers. This observation was supported by a large diaxial coupling (11.4 Hz) between H-8 and H-13 in both isomers. The two diastereomers therefore differed in the configuration at either C-5 or both C-8 and C-13. Since it has been reported previously that 2-substituted pyrrolidines can easily racemize,[9] habbemines A (**2**) and B (**3**) are most likely epimers at C-5, and this may also explain why it proved impossible to separate the two diastereomers. The minor tautomers observed in solution were likely to be molecules in which the C-7 ketone is in conjugation with cyclohexadienols.

Habbemines A (**2**) and B (**3**) are similar in structure to peripentadenine and norperipentadenine, two pyrrolidine alkaloids that have previously been isolated as racemates from the related plant *Peripentadenia mearsii* (Elaeocarpaceae).[9] The major differences between the habbemines and the peripentadenines is truncation of the side chain, replacement of the amino group by an alcohol, and oxidation of the 3-methylphenol to 3-methylcyclohexenone. The habbemines could also be considered to be biosynthetic precursors to all of the *Elaeocarpus* indolizidine alkaloids since oxidation of the primary alcohol in habbemines A and B to an aldehyde (**4**) followed by an aldol condensation at C-6 would lead to the indolizidine alkaloid grandisine D (**5**), which has been postulated to be a precursor to the more complex tetracyclic indolizidines (Figure 1).[7,8]

A mixture of habbemines A (**2**) and B (**3**) inhibited the binding of [^{125}I]-deltorphin II to HEK cell membranes expressing recombinant human δ-opioid receptors with an IC_{50} of 32.1 μM. IC_{50} values for the positive controls DPDPE and naloxone were 1.2 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 Nicolet NEXUS FT-IR spectrometer. NMR spectra were recorded on Varian Inova 600 and 500 MHz NMR spectrometers. Samples were dissolved in d_6-DMSO containing a drop

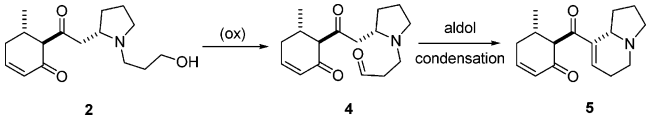

Figure 1. Possible biogenesis of the *Elaeocarpus* indolizidine alkaloid grandisine D (**5**) from habbemine A (**2**).

of TFA, and chemical shifts were calculated relative to the DMSO solvent peak (^{1}H δ 2.49 and ^{13}C δ 39.5). 2D NMR spectra were recorded at 30 °C using standard Varian pulse sequences gCOSY, gHSQC, gHMBC, and ROESY. 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 Sephadex LH-20 were used during purification. HPLC purifications were attempted using a Hypersil BDS C_{18} semipreparative (250 × 10 mm, 5 μm) column and a Hypersil BDS phenyl-bonded silica gel semipreparative (250 × 10 mm, 5 μm) column.

Plant Material. Leaves of *E. habbemensis* were collected by T.R. in January 1999 from the Manegegilli village swamp forest near Ialibu, in the southern highlands province of Papua New Guinea. A voucher specimen, 910, is deposited at Biodiversity Ltd., at the University of Papua New Guinea, Port Moresby.

Extraction and Isolation. The air-dried leaves of *E. habbemensis* (80.5 g) were ground and extracted with MeOH at room temperature. The MeOH extract was filtered through polyamide gel (50 g) under vacuum, and the filtrate was evaporated. The resulting residue (6.28 g) was dissolved in H_2O (500 mL) and partitioned with DCM (500 mL). The aqueous layer that gave a positive result in a Dragendorff's alkaloid test was filtered through SCX resin. The SCX resin was washed with H_2O (500 mL) before an alkaloid fraction was eluted with 1 M NaCl solution (500 mL). The alkaloid fraction was evaporated under vacuum, and the salt residue was suspended in a 1:1 $CHCl_3$−MeOH mixture (100 mL), which was filtered to remove NaCl. The filtrate (22.9 mg) was separated by Sephadex LH-20 column chromatography (60 × 2.5 cm) eluting with MeOH. Fractions containing an ion at *m*/*z* 280 in the (+) ESIMS were combined and rechromatographed on Sephadex LH-20 eluting with MeOH to afford habbemines A and B (**2** and **3**) (7.7 mg, 0.0096%). Attempts to separate the diastereomers by analytical C_{18} silica gel HPLC, using isocratic conditions of 84:15:1 H_2O−MeOH−TFA, 89:10:1 H_2O-ACN−TFA, and 85:7:7:1 H_2O−MeOH−ACN−TFA, were unsuccessful. An attempted separation by HPLC on phenyl-bonded silica gel and isocratic elution with 94:5:1 H_2O−MeOH−TFA also proved unsuccessful.

Habbemine A chloride (2) and habbemine B chloride (3): yellow gum; $[\alpha]_D^{23}$ +13.7 (*c* 0.13, MeOH); UV (MeOH) λ_{max} (log ϵ) 227 (3.35), 270 (2.85), 339 (2.70) nm; IR (KBr) ν_{max} 3400 br, 1712, 1674, 1201, 1127 cm^{-1}; ^{1}H (600 MHz, d_6-DMSO containing a drop of TFA) and ^{13}C NMR (125 MHz, d_6-DMSO containing a drop of TFA), Table 1; (+)-LRESIMS *m*/*z* 280 (100%) $[MH^+, C_{16}H_{26}NO_3]^+$; (+)-HRESIMS *m*/*z* 280.1894 $[M + H]^+$ (calcd for $C_{16}H_{26}NO_3$, 280.1907).

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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Supporting Information Available: ^{1}H, ^{13}C, gCOSY, gHSQC, and gHMBC spectra of habbemines A (**2**) and B (**3**). This material is available free of charge via the Internet at http://pubs.asc.org.

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