Indolizidine Alkaloids with δ-Opioid Receptor Binding Affinity from the Leaves of *Elaeocarpus fuscoides*

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 Ltd., P.O. Box 24, University P.O. NCD, Papua New Guinea

Received December 6, 2006

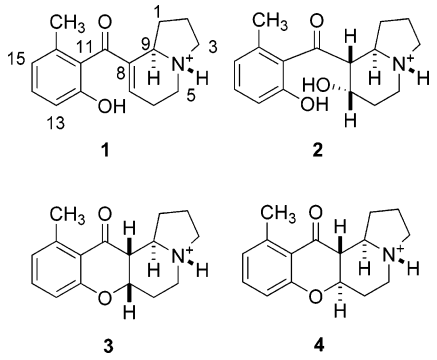
In the first chemical investigation of the Papua New Guinean plant *Elaeocarpus fuscoides*, one new indolizidine alkaloid, elaeocarpenine (**1**), and three known alkaloids, isoelaeocarpicine (**2**), isoelaeocarpine (**3**), and elaeocarpine (**4**), were isolated from the leaves. Their structures were determined by 1D and 2D NMR spectroscopy. Since treatment of elaeocarpenine (**1**) with ammonia produced a 1:1 mixture of the diastereomers **3** and **4**, we propose that elaeocarpenine (**1**) is the biogenetic precursor of isoelaeocarpine (**3**) and elaeocarpine (**4**). Compounds **1**−**4** demonstrated binding affinity for the human δ-opioid receptor with IC_{50} values of 2.7, 35.1, 13.6, and 86.4 μM, respectively.

The family Elaeocarpaceae is a major producer of plant alkaloids.[1−11] Early investigations of the genus *Elaeocarpus* uncovered a new class of alkaloids, the indolizidines.[1−9] Indolizidine alkaloids have been identified in seven species of *Elaeocarpus*. In a high-throughput screening campaign employing a receptor binding assay to identify natural products with affinity for the δ-opioid receptor, an assay that could ultimately provide novel drug leads to treat chronic pain, we found that extracts from the Australian species *E. grandis* contained the indolizidine alkaloids (−)-isoelaeocarpiline and grandisines A and B. Two of these compounds demonstrated moderate affinity for the human δ-opioid receptor.[11] Further studies have elucidated the structures of five new indolizidine alkaloids, grandisines C−G, from *E. grandis*.[12] Grandisines D and F also showed moderate δ-opioid receptor binding affinity (1.65 and 1.55 μM, respectively). These results prompted us to conduct a survey of plants from the family Elaeocarpaceae to identify new biologically active alkaloids. The survey represented 85 species from five genera collected from Queensland, Papua New Guinea, and China and included 339 distinct plant parts. This survey has identified *Elaeocarpus fuscoides* Knuth. (Elaeocarpaceae) as a new alkaloid-producing species. We report herein the isolation, structure determination, and human δ-opioid receptor binding affinity of a new indolizidine alkaloid, elaeocarpenine (**1**), together with the known compounds isoelaeocarpicine (**2**), isoelaeocarpine (**3**), and elaeocarpine (**4**),[5] from *E. fuscoides* Knuth., a rainforest tree collected from the Kirene Forest, Ialibu, in the Southern Highlands Province of PNG. 2D NMR assignment of the new compound and the three known compounds (**2**−**4**) is reported here for the first time.

Elaeocarpenine, isoelaeocarpine, and elaeocarpine were demonstrated to be racemic, while **2** was isolated as (+)-isoelaeocarpicine. In their original isolation studies on *E. polydactylus*, Johns et al. reported the isolation of **3** and **4** as racemates.[3,5]

Dragendorff's reagent in conjunction with positive electrospray MS was used to identify alkaloids in extracts from plants from the Elaeocarpaceae. The leaves, roots, and bark of *E. fuscoides* were surveyed, but only the leaves returned a positive test for alkaloids. Johns et al. employed a general acid−base extraction protocol (H_2SO_4/NH_3) in the original isolation of compounds **2**, **3**, and **4** from *E. polydactylus*.[5] Our concerns for the stability of some *Elaeocarpus* alkaloids under basic conditions have led us to purify the alkaloids from *E. fuscoides* using strongly acidic ion exchange (SCX), followed by RP-HPLC isolation methods. An MeOH extract of the leaves of *E. fuscoides* was filtered through strongly acidic ion exchange (SCX) resin. The resin was washed sequentially with MeOH and H_2O prior to elution of the alkaloids with 1 M NaCl. Desalting of the alkaloid fraction was achieved by C_{18} RP flash vacuum chromatography. Subsequent fractionation by C_{18} silica gel RP-HPLC, employing a linear gradient from H_2O to MeOH (1% TFA), yielded pure elaeocarpenine (**1**).

Further purification by C_{18} RP-HPLC afforded the indolizidine alkaloids **2**−**4**. All four compounds were isolated as their TFA salts.



Elaeocarpenine (**1**) was assigned the molecular formula $C_{16}H_{19}NO_2$ by high-resolution positive electrospray mass measurement of the $[M + H]^+$ ion (Δ + 0.1 ppm). The ^{13}C NMR spectrum (Table 1) revealed 16 unique carbons, and gHSQC data established the presence of 18 carbon-bound protons (one methyl, five methylene, one methine, and four olefinic protons). Relevant features of the ^{13}C NMR spectrum include the presence of an oxygenated aromatic carbon and an α,β-unsaturated ketone at δ 154.2 and 196.7, respectively. This was confirmed by an IR absorption band at 1683 cm^{-1}. A further seven carbons resonated in the downfield region of the ^{13}C NMR spectrum. Carbons at δ 43.4, 52.7, and 58.1 were observed and suggested that these carbons were bound to heteroatoms. The remaining resonances comprised a methyl at δ 18.4 and three aliphatic methylenes at δ 20.6, 22.5, and 28.2.

Inspection of the ^1H NMR data (Table 2) revealed the presence of three aromatic protons. gCOSY correlations confirmed the 1,2,3-trisubstituted aromatic system. The partial structures $-CHCH_2CH_2-CH_2-$ and $-CH_2-CH_2-CH{=}C-$ were established from gCOSY correlations. Correlations observed in a gHMBC experiment established the connection of the partial structures.

Correlations from the methylene H-3 to C-5, from H-3 and H-5 to C-9, from H-9 to C-5 and C-7, and from H-1 and H-7 to C-8

* Corresponding author. Tel: 61 7 3735 6015. Fax: 61 7 3735 6001. E-mail: A.Carroll@griffith.edu.au.
[†] Griffith Univeristy.
[‡] Biodiversity Ltd.

10.1021/np060607e CCC: $37.00
Published on Web 04/24/2007

Table 1. ^{13}C NMR (125 MHz) Data for Elaeocarpenine (**1**), Isoelaeocarpicine (**2**), Isoelaeocarpine (**3**), and Elaeocarpine (**4**)

	1[a]	**2**[a]	**3**[a]	**4**[a]
position	δ_C, mult.	δ_C, mult.	δ_C, mult.	δ_C, mult.
1	28.2 CH_2	26.6 CH_2	28.7 CH_2	29.7 CH_2
2	20.6 CH_2	19.2 CH_2	21.0 CH_2	22.1 CH_2
3	52.7 CH_2	51.3 CH_2	54.0 CH_2	52.5 CH_2
4				
5	43.4 CH_2	44.8 CH_2	47.4 CH_2	48.8 CH_2
6	22.5 CH_2	30.9 CH_2	30.2 CH_2	30.3 CH_2
7	140.4 CH	63.1 CH	74.5 CH	77.8 CH
8	135.5 qC	56.2 qC	54.0 qC	52.8 qC
9	58.1 CH	60.4 CH	59.6 CH	63.7 CH
10	196.7 qC	203.0 qC	194.6 qC	193.4 qC
11	126.0 qC	125.7 qC	119.1 qC	120.0 qC
12	154.2 qC	154.8 qC	162.9 qC	162.3 qC
13	113.0 CH	113.3 CH	116.3 CH	116.0 CH
14	129.9 CH	130.6 CH	135.2 CH	135.2 CH
15	120.5 CH	121.6 CH	125.1 CH	125.2 CH
16	136.5 qC	136.5 qC	142.9 qC	142.3 qC
17	18.4 CH_3	19.2 CH_3	23.1 CH_3	22.7 CH_3

[a] In d_6-DMSO.

secured the structure of the indolizidine moiety. The methylphenol moiety was constructed from correlations of H-17_{Me} to C-15, H-14 to C-16, H-15, H-13, and H-17_{Me} to C-11, and H-14 to the phenolic carbon C-12. The indolizidine and methylphenol moieties were connected by correlations from H-7 to the C-10 carbonyl, and four-bond correlations from H-13 and H-15 to C-10. Thus the structure of the TFA salt of elaeocarpenine (**1**) was established.

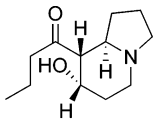
The relative *trans*-diaxial arrangement of H-9 and H-4 was supported by the large coupling constants observed for H-9 (J = 9.6, 9.6). An analogous observation has been made by Johns et al., who have noted that other protonated indolizidines possess a *trans*-diaxial arrangement of the proton alpha to the nitrogen and the protonated nitrogen in other indolizidine[5] structures. Therefore, the same *trans*-diaxial arrangment exists in elaeocarpenine trifluoroacetate (**1**).

Elaeocarpenine (**1**) was isolated as a racemate, on the basis of a measured optical rotation of 0°.

Compound **2** was identified as isoelaeocarpicine by 2D NMR analysis (COSY, HSQC, HMBC, and ROESY) and comparison with literature data.[5] The positive ESI mass spectrum of isoelaeocarpicine (**2**) showed a dehydration product that could be elaeocarpenine (**1**). The reported mass spectrum also showed a dehydration product.[5]

The relative configuration of isoelaeocarpicine (**2**) was determined from analysis of gCOSY and ROESY data. The *trans*-diaxial arrangment of H-8 relative to H-9 was established from the large coupling constant (10.8 Hz) (lit. 11.0 Hz).[5] The relationship between H-4 and H-9 is also proposed to be axial−axial, as a strong COSY correlation was observed. A ROESY correlation between H-7 and H-8 indicated a *cis*-arrangement, suggesting that H-7 was equatorial. The relative configuration determined for isoelaeocarpicine (**2**) is therefore consistent with the literature. The reported specific rotation for **2** was +29, and the measured specific rotation of isoelaeocarpicine trifluoroacetate was +9.25. The specific rotation determined in this study was on the TFA salt in MeOH compared to the reported rotation of the free base in $CHCl_3$. In the work of Johns et al., only the relative configuration of (+)-isoelaeocarpicine was reported. However, the relationship between the specific rotation and absolute configuration of elaeokanine C (**5**) has been established by synthesis.[13] Elaeokanine C (**5**) possesses the same 7-hydroxy-8-ketoindolizidine group as isoelaeocarpicine (**2**). The specific rotation of synthetic 7*S*,8*R*,9*R*-elaeokanine C was +36.9, and this indicated that (+)-isoelaeocarpicine was obtained as the 7*S*,8*R*,9*R*-isomer.

The structures of compounds **3** and **4** were determined as isoelaeocarpine and elaeocarpine, respectively, from 2D NMR



5

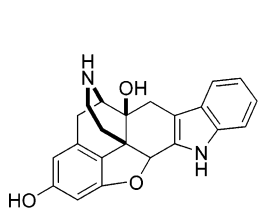
analysis (COSY, HSQC, HMBC, and ROESY) and comparison with literature data.[5]

The conversion of elaeocarpenine (**1**) to isoelaeocarpine (**3**) and elaeocarpine (**4**) was observed upon reacting **1** (5.2 mg) with NH_3 in MeOH. The reaction mixture was separated by reversed-phase semipreparative HPLC, employing a gradient from 3:1 to 1:3 H_2O/MeOH over 10 min. This afforded isoelaeocarpine (**3**) (1.2 mg) and elaeocarpine (**4**) (1.2 mg).

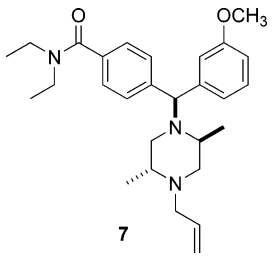
This observation suggests that NH_3 promotes ring closure by acting as a sponge to deprotonate the oxonium-type intermediate formed upon 1,4-Michael addition of the phenolic hydroxyl group to form a pyran. The NH_3 thus drives the reaction to completion. The phenolic hydroxy could attack from either side of the indolizidine ring, resulting in both epimers at position C-7 being obtained. This provides justification for employing an NH_3-free extraction process.

Biological Activity. Elaeocarpenine (**1**), isoelaeocarpicine (**2**), isoelaeocarpine (**3**), and elaeocarpine (**4**) inhibited the total binding of [^{125}I]-deltorphin II to HEK cell membranes expressing recombinant human δ-opioid receptor with IC_{50} values of 2.74, 35.1, 13.6, and 86.4 μM, respectively. IC_{50} values for the positive controls DPDPE and naloxone were 1.12 and 138 nM, respectively.

We have observed previously that the orientation of the heterocyclic rings attached to the indolizidine core is a determining factor in the δ-opioid receptor binding of the indolizidine alkaloids.[11] Receptor binding affinity for the human δ-opioid receptor has now been reported for 13 indolizidine alkaloids.[11,12] In all cases we observe that in the more active compounds orientation of the heterocyclic ring attached via C-8 is orthogonal to the piperidine ring. Comparatively, in the less active compounds modeling indicates that the piperidine is in the same plane as the attached heterocycle. Computational studies carried out in the mid 1990s led to a three-dimensional pharmacophore model of the active site of the δ-opioid receptor.[14] The pharmacophore consists of a protonated amine binding site, an aromatic ring, and a hydrophobic interaction site. The potent nonpeptide agonists of the δ opioid receptor, nor-OMI[15] (**6**) and SNC-80[16] (**7**), both possess piperidines with orthogonal aromatic groups and a third group that can bind in a hydrophobic pocket. The indolizidine alkaloids by comparison are significantly less potent, which suggests that only two points of interaction are found within these compounds.



6



7

Experimental Section

General Experimental Procedures. All solvents used were Omnisolv HPLC grade. Millipore Milli-Q PF filtered water was used. Optical rotations were measured on a JASCO P-1020 polarimeter (23 °C, 10 cm cell). UV spectra were recorded on a CAMSPEC M501 UV/vis spectrophotometer and IR spectra on a Thermo Nicolet NEXUS FT IR spectrometer. NMR spectra were recorded on Varian Inova 600 MHz and 500 MHz NMR spectrometers. Samples were dissolved in either d_6-DMSO or CD_2Cl_2, and chemical shifts were calculated relative to the dimethylsulfoxide solvent peak (^{1}H δ 2.49 and ^{13}C δ 39.5 ppm).

Table 2. ^{1}H NMR (600 MHz) Data for Elaeocarpenine (**1**), Isoelaeocarpicine (**2**), Isoelaeocarpine (**3**), and Elaeocarpine (**4**)

	1[a]	**2**[a]	**3**[a]	**4**[a]
position	δ_H (*J* in Hz)	δ_H (*J* in Hz)	δ_H (*J* in Hz)	δ_H (*J* in Hz)
1a	2.53, m	2.49, m	1.78, ddd (2.4, 10.8, 17.4)	1.77, ddd (1.8, 11.4, 18.0)
1b	1.81, ddd (6.0, 10.8, 15.6)	1.61, ddd (2.4, 10.8, 18.0)	1.90, m	2.68, br
2	2.07, t (8.4)	2.03, dddd (1.8, 6.0, 6.0, 15.6)	1.90, m	1.96, brdd (10.8, 10.8)
		1.84, dddd (2.4, 11.4, 11.4, 18.0)		2.09, br
3a	3.57, dd (2.4, 13.2)	3.59, ddd (2.4, 2.4, 9.6)	3.07, ddd (2.4, 9.6, 18.0)	2.95, brdd (9.6, 18.0)
3b	3.31, m	3.08, m	3.60, ddd (2.1, 2.1, 10.2)	3.50, m
4	10.82, s	9.56, br	10.23, brs	10.36, br
5a	3.16, br	3.41, dd (2.4, 12.6)	3.28, ddd (3.3, 12.0, 12.0)	3.03, ddd (3.0, 12.6, 18.0)
5b	3.31, m	3.08, m	3.65, brdd (2.4, 13.8)	3.69, brdd (4.8, 13.2)
6	2.53, m	1.87, t, 16.8	2.29, brdd (3.8, 15.6)	2.15, ddd (4.8, 12.0, 12.0)
			2.08, brdd (4.8, 15.6)	2.40, brdd (3.6, 13.8)
7	6.60, dd (4.8, 4.8)	4.12, br	4.91, brs	4.60, ddd (4.4, 12.0, 12.0)
8		3.82, brd (10.8)	2.82, brd (12.0)	3.21, dd (11.4, 11.4)
9	4.51, brdd (9.6, 9.6)	3.53, ddd (2.4, 11.4, 11.4)	3.45, ddd (6.0, 12.0, 12.0)	3.32, brdd (10.8, 10.8)
10				
11				
12				
13	6.72, d (9.6)	6.74, d (7.8)	6.96, d (7.8)	6.90, d (7.8)
14	7.12, dd (9.6, 9.0)	7.13, dd (7.8, 7.8)	7.47, dd (7.8, 7.8)	7.43, dd (7.8, 7.8)
15	6.68, d (9.0)	6.68, d (7.8)	6.91, d (7.8)	6.90, d (7.8)
16				
17	2.02, s	2.19, s	2.55, s	2.52, s
C7-OH		5.15, s		
C12-OH		10.13, s		

[a] In d_6-DMSO.

ESIMS and HRMS were measured on a Mariner Biospectrometry workstation using positive electrospray ionization, with a mobile phase of 1:1 MeOH/H_2O−0.1% formic acid. Dowex 50WX8-400 ion-exchange resin, strongly acidic cation exchange resin 200−400 mesh (Aldrich), and DAVISIL bonded C_{18} (30−40 μm) were used during separation. Further HPLC purifications were done using Hypersil BDS C_{18} preparative (150 × 21.2 mm, 5 μm) and semipreparative (250 × 10 mm, 5 μm) columns on a Waters 600 system. Concentrated NH_3 solution (32%) was used. [^{125}I]-Deltorphin II radioactive ligand, code IMQ1595, pack size 100 μCi, was purchased from Amersham Biosciences as a lyophilized solid. HEK cell membranes expressing recombinant human δ-opioid receptors were obtained from AstraZeneca Montreal. Data analysis of radioactive counts per minute was achieved by GraphPad Prism 4, San Diego, CA, to determine potency (IC_{50}) and hill slope (n_H).

Plant Material. Leaves of *E. fuscoides* Knuth. were collected and identified by Topul Rali (Biodiversity Ltd) on August 14, 1999 from Kirene Forest, Ialibu, Southern Highlands Province, Papua New Guinea. A voucher specimen, 1277, is deposited at Biodiversity Ltd., at the University of Papua New Guinea, Port Moresby.

Extraction and Isolation. The dried and ground leaves of *E. fuscoides* (120 g) were extracted with MeOH (3 L). The MeOH extract was filtered through SCX resin (50 g). The resin was washed sequentially with 1 L each of MeOH and H_2O, prior to elution of an alkaloid fraction with 1 M NaCl (1 L). The salt was removed from the alkaloid fraction by vacuum filtration through C_{18} RP silica gel chromatography. NaCl was removed by washing with copious amounts of H_2O (5 L) prior to elution of the alkaloids with 1% TFA/MeOH (500 mL). The alkaloid fraction was evaporated, and the residue (402.4 mg) was absorbed onto C_{18} RP silica gel (1.2 g), which was transferred into a metal HPLC column (10 × 15 mm). This column was connected to a preparative C_{18} RP-HPLC column, and the alkaloids were separated into 50 fractions using a linear gradient from H_2O to 1% TFA/MeOH over 100 min. Mass ion peaks of *m*/*z* 276 in fractions 13−20, and *m*/*z* 258 in fractions 21−24 and 26−31, were observed following analysis by +ESIMS. Semipure isoelaeocarpicine (**2**) was detected in fractions 13−20, pure elaeocarpenine (**1**) in fractions 21−24, and a mixture of two compounds in fractions 26−31. Fractions 21−24 were combined to yield elaeocarpenine (**1**) (94.7 mg, 0.079%). Fractions 13−20 were combined and separated by semipreparative C_{18} RP-HPLC employing a gradient of 3:1 H_2O/MeOH to 1% TFA/MeOH over 15 min. Fractions 29 to 31 were combined to yield isoelaeocarpicine (**2**) (4.0 mg, 0.0033%). Purification of the two compounds detected in fractions 26−31 (*m*/*z* 258) was done by C_{18} RP-HPLC, with a gradient of 1:1 H_2O/MeOH to 1% TFA/MeOH over 15 min. Analysis of the fractions afforded isoelaeocarpine (**3**) (38.7 mg, 0.032%) in fractions 22−24 and elaeocarpine (**4**) (17.6 mg, 0.011%) in fractions 27−31.

Elaeocarpenine trifluoroacetate (1): yellow gum; $[\alpha]_D^{23}$ 0 (*c* 0.067 and 0.1334, MeOH); UV (MeOH) λ_{max} (log ϵ) 223 (3.29) nm; IR (KBr) ν_{max} 3404 br, 1683, 1461, 1281, 1199, 1123, 720 cm^{-1}; ^{13}C NMR (125 MHz, d_6-DMSO) Table 1; ^{1}H NMR (600 MHz, d_6-DMSO) Table 2; (+)-LRESIMS *m*/*z* (rel int) 258 (100%) [MH^+, $C_{16}H_{20}NO_2]^+$; (+)-HRESIMS *m*/*z* 258.1490 $[M + H]^+$ (calcd for $C_{16}H_{20}NO_2$, 258.14886).

Isoelaeocarpicine trifluoroacetate (2): yellow gum; $[\alpha]_D^{23}$ +6.19 (*c* 0.067, MeOH), +9.25 (*c* 0.1334, MeOH) (lit.[5] +29, *c* 0.30, $CHCl_3$); ^{13}C NMR (125 MHz, d_6-DMSO) Table 1; ^{1}H NMR (600 MHz, d_6-DMSO) Table 2; (+)-LRESIMS *m*/*z* (rel int) 276 (75%) [MH^+, $C_{16}H_{22}NO_3]^+$, 258 (25%) [$MH^+ - H_2O]^+$.

Isoelaeocarpine trifluoroacetate (3): yellow gum; $[\alpha]_D^{23}$ 0 (*c* 0.067, MeOH); ^{13}C NMR (125 MHz, d_6-DMSO) Table 1; ^{1}H NMR (600 MHz, d_6-DMSO) Table 2; (+)-LRESIMS *m*/*z* (rel int) 258 (100%) [MH^+, $C_{16}H_{20}NO_2]^+$.

Elaeocarpine trifluoroacetate (4): yellow gum; $[\alpha]_D^{23}$ 0 (*c* 0.067, MeOH); ^{13}C NMR (125 MHz, d_6-DMSO) Table 1; ^{1}H NMR (600 MHz, d_6-DMSO) Table 2; (+)-LRESIMS *m*/*z* (rel int) 258 (100%) [MH^+, $C_{16}H_{20}NO_2]^+$.

Conversion of 1 to 3 and 4. The conversion of elaeocarpenine (**1**) to isoelaeocarpine (**3**) and elaeocarpine (**4**) was observed upon reacting **1** (5.2 mg) with an excess of NH_3 in MeOH (2 mL). The reaction mixture was evaporated and separated by reversed-phase semipreparative HPLC, employing a gradient from 3:1 to 1:3 H_2O/MeOH over 10 min. This afforded isoelaeocarpine (**3**) (1.2 mg, 23%) and elaeocarpine (**4**) (1.2 mg, 23%).

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. Compounds **1**−**4** were screened in triplicate 11-point CRC in a 96-well format. [D-pen^2,D-pen^5]enkephalin (DPDPE) and naloxone were used as positive controls and were screened in duplicate. Compounds were screened at a final concentration of 2% DMSO. Plates were sealed and shaken at room temperature (~23 °C) for 1 h. Plates were harvested using a Brandel harvester. Filtermats used for harvesting were presoaked in 0.1% PEI (polyethylenimine) and were allowed to dry at 50 °C for 30 min after harvest. Filtermats were sealed in plastic with scintillant (BetaScint) and counted for 1 min per well on a Wallac Microbeta Trilux.

Acknowledgment. We thank P. Baron (Natural Product Discovery, Griffith University) for obtaining accurate mass measurements. We

thank Dr. G. Fechner for help with biological assays. One of us (P.L.K) acknowledges the support of the Australian Research Council in the form of an Australian Postgraduate Award.

References and Notes

(1) Johns, S. R.; Lamberton, J. A.; Sioumis, A. A. *Chem. Commun.* **1968**, 410.
(2) Johns, S. R.; Lamberton, J. A.; Sioumis, A. A. *Chem. Commun.* **1968**, 1324−1325.
(3) Johns, S. R.; Lamberton, J. A.; Sioumis, A. A.; Wunderlich, J. A. *Chem. Commun.* **1968**, 290−291.
(4) Johns, S. R.; Lamberton, J. A.; Sioumis, A. A. *Aust. J. Chem.* **1969**, *22*, 793−800.
(5) Johns, S. R.; Lamberton, J. A.; Sioumis, A. A.; Willing, R. I. *Aust. J. Chem.* **1969**, *22*, 775−792.
(6) Johns, S. R.; Lamberton, J. A.; Sioumis, A. A. *Aust. J. Chem.* **1969**, *22*, 801−806.
(7) Johns, S. R.; Lamberton, J. A.; Sioumis, A. A.; Suares, H.; Willing, R. I. *Chem. Commun.* **1970**, 804−805.
(8) Johns, S. R.; Lamberton, J. A.; Sioumis, A. A.; Suares, H.; Willing, R. I. *Aust. J. Chem.* **1971**, *24*, 1679−1694.
(9) Hart, N. K.; Johns, S. R.; Lamberton, J. A. *Aust. J. Chem.* **1972**, *25*, 817−835.
(10) Ray, A. B.; Chand, L.; Pandey, V. B. *Phytochemistry* **1979**, *18*, 700−701.
(11) Carroll, A. R.; Arumugan, G.; Quinn, R. J.; Redburn, J.; Guymer, G.; Grimshaw, P. *J. Org. Chem.* **2005**, *70*, 1889−1892.
(12) Katavic, P. L.; Venables, D. A.; Carroll, A. R. *J. Nat. Prod.* **2006**, *69*, 1295−1299.
(13) Arai, Y.; Kontani, T.; Koizumi, T. *Tetrahedron Asymmetry* **1992**, *3*, 535−538.
(14) Loew, G. H. *Mod. Drug Discovery* **1999**, *2*, 24−26.
(15) Portoghese, P. S.; Larson, D. L.; Sultana, M.; Takemori, A. E. *J. Med. Chem.* **1992**, *35*, 4325−4329.
(16) Bilsky, E. J.; Calderon, S. N.; Wang, T.; Bernstein, R. N.; Davis, P.; Hruby, V. J.; McNutt, R. W.; Rothman, R. B.; Rice, K. C.; Porreca, F. *J. Pharmacol. Exp. Ther.* **1995**, *273*, 359−366.

NP060607E