The Biocatalytic Transformation of Furan to Amide in the Bioactive Marine Natural Product Palinurin

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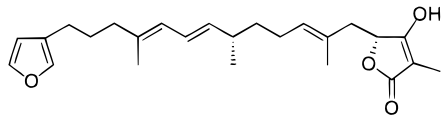
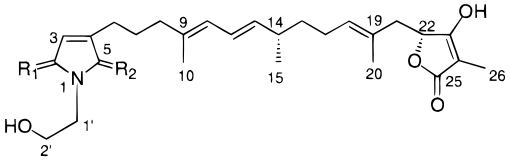
Introduction

Furanosesterpenes are frequently isolated from marine sponges of the genera *Ircinia*,[1−10] *Spongia*,[11−13] *Spongionella*,[14,15] *Cacospongia*,[16] *Dysidea*,[17] *Sarcotragus*,[18] *Amphimedon*,[19] and *Hippospongia*.[20] They display a wide range of bioactivity including antibacterial,[3,4,18,19] antiviral,[3] fertilized starfish eggs cell division inhibitory,[14−16] protein phosphatase inhibitory,[17] antispasmodic,[20] and antiinflammatory activities.[21] The use of microbial transformations to modify marine natural products and generate bioactive lead derivatives with minimal toxicity was recently reported.[22] Palinurin (**1**) is a linear furanoseterterpene previously reported from the Mediterranean sponge *Ircinia variabilis* and was reisolated in gram quantities from a Red Sea sponge *Ircinia echinata*.[5] In this paper, we describe two novel microbial metabolites of **1**, palinurine A (**2**) and B (**3**), produced by the fungus *Cunninghamella* sp. NRRL 5695. This reaction may be of significant value in the field of synthetic organic chemistry. The possible transformation of furans to amides (dehydro-3-enepyrrolidin-2-or 5-one) through a biomimetic reaction is certain to have applications in synthetic chemistry.



Palinurin (**1**)



	R_1	R_2
Palinurine A (**2**)	H_2	O
Palinurine B (**3**)	O	H_2

Results and Discussion

Bioconversion Studies and Structure Elucidation. Palinurin (**1**) was isolated from the lipophilic extract of the Red Sea sponge *I. echinata* in a 1% yield based on wet weight. Thirty growing cultures of bacteria and fungi were screened for potential to bioconvert **1** to active metabolites. Few cultures were able to transform **1** to more polar metabolites. Of these, *Cunninghamella* sp. NRRL 5695 was selected for preparative-scale fermentation.

Cunninghamella sp. NRRL 5695 was able to convert **1** to two more polar compounds (**2** and **3**). The NMR spectra of **2** and **3** indicated cleavage of the furan function with the addition of one ethanolamine molecule. The HRFTMS electrospray ionization data of **2** and **3** displayed molecular ion peaks at 480.2727 and 480.2752, respectively, suggesting the molecular formula $C_{27}H_{39}O_5N$ $(M + Na)^+$ and nine degrees of unsaturation. The IR spectra (KBr) of **2** and **3** showed absorption bands at 3429 and 3421 cm^{-1}, respectively, suggesting the presence of hydroxyl functionality. It also showed absorption bands at 1720, 1690 and 1725, 1686 cm^{-1}, respectively, suggesting the presence of α,β-unsaturated lactone functionality. The ^1H and ^{13}C NMR spectra of **2** (Table 1) indicated the lack of furan and the presence of a new *N*-2′-hydroxyethyldehydro-3-enepyrrolidin-5-one group, while the tetronic acid moiety and the linear chain remained intact. The broad proton singlet (2H) at δ 4.08 (Table 1) correlated to the methylene carbon resonating at δ 53.7 and was assigned H_2-2. This proton signal showed $^1H-^1H$ COSY coupling to the broad proton singlet resonating at δ 6.86, which correlated to the methine

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Table 1. ^{13}C, ^{15}N, and ^{1}H NMR Data of Compounds 2 and 3[a]

	2		3	
no.	^{13}C or ^{15}N	^{1}H	^{13}C or ^{15}N	^{1}H
1	122.3, S		120.3, S	
2	53.7, t	4.08, 2H, brs	164.1, s	
3	138.2, d	6.86, brs	121.0, d	5.82, brs
4	140.3, s		139.0, s	
5	174.2, s		57.3, t	4.13, 2H, s
6	26.5, t	2.22, 2H, m	29.9, t	2.37, dd (7.4, 7.1)
7	26.9, t	1.66,2H, m	27.0, t	2.15,2H, m
8	40.5, t	2.08, 2H, t (7.3)	40.3, t	2.09, 2H, m
9	136.5, s		135.9, s	
10	16.6, q	1.71, 3H, s	16.4, q	1.71, 3H, brs
11	126.8, d	5.77, d (10.8)	127.1, d	5.77, d (10.4)
12	126.6, d	6.18, dd (15.0, 10.8)	126.4, d	6.18, dd (15.0, 10.8)
13	139.5, d	5.39, dd (15.0, 8.0)	139.6, d	5.40, dd (15.0, 8.3)
14	38.1, d	2.14, m	38.0, d	2.11, m
15	21.6, q	0.99, d (6.7)	21.4, q	0.98, d (6.9)
16	38.6, t	1.33, 2H, q (7.1)	38.5, t	1.33, 2H, q (7.1)
17	27.0, t	2.00, 2H, q (7.4)	26.9, t	2.00, 2H, q (7.8)
18	129.3, d	5.24, dd (7.0, 6.8)	128.9, d	5.24, dd (7.5, 6.8)
19	132.5, s		132.6, s	
20	24.5, q	1.77, 3H, s	24.3, q	1.76, 3H, brs
21	36.1, t	2.59, dd (11.0, 2.1); 2.14, m	36.1, t	2.52, dd (11.0, 2.1); 2.14, m
22	81.1, d	4.42, dd (10.3, 2.5)	81.3, d	4.33, dd (10.0, 2.5)
23	190.7, s		193.1, s	
24	89.5, s		89.5, s	
25	182.8, s		184.0, s	
26	6.2, q	1.56, 3H, s	6.0, q	1.53, 3H, s
1′	46.2, t	3.57, 2H, t (5.3)	45.7, t	3.51, 2H, t (5.4)
2′	61.4, t	3.71, 2H, t (5.3)	61.3, t	3.69, 2H, t (5.4)

[a] In CD_3OD, 400 MHz for ^{1}H-, 100 MHz for ^{13}C-, and 50 MHz for ^{15}N NMR. Carbon multiplicities were determined by DEPT 135° experiments. s = quaternary, d = methine, t = methylene carbons. Coupling constants (*J*) are in Hz. Nitromethane was used as an external standard for ^{15}N NMR. S = quaternary nitrogen.

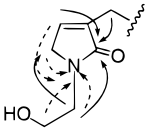

Figure 1. Important $^{1}H-^{13}C$ GHMBC (solid line) and $^{1}H-^{15}N$ GHMBC (dotted line) correlations of **2**.

carbon at δ 138.2, and was assigned H-3. Both H_2-2 and H-3 display ^{3}J-HMBC correlation to the quaternary carbonyl carbon resonating at δ 174.2 and assigned C-5 (Figure 1). The two $^{1}H-^{1}H$ COSY-coupled methylene signals resonating at δ 3.57 and 3.71, which correlated to the methylene carbons at δ 46.2 and 61.4, were assigned H_2-1′ and -2′, respectively. The proton triplet H-1′ also displayed a ^{3}J-HMBC correlation to the C-5 carbonyl group (Figure 1). Further evidence for the location of C5-carbonyl group was obtained from the ^{3}J-HMBC correlation with the H-7 methylene protons resonating at δ 2.22 (Figure 1). The $^{1}H-^{15}N$ GHMBC spectrum provides additional support for the proposed structure. The nitrogen N-1 resonating at δ 122.3 (Table 1) shows ^{3}J coupling with H-3 and H_2-2′. The nitrogen N-1 also shows ^{2}J coupling with H-2 and H_2-1′.

The ^{1}H and ^{13}C NMR spectra of **3** (Table 1) indicate close structural homology with **1** and **2**. The methylene singlet resonating at δ 4.13, which correlated to the methylene carbon at δ 57.3, is assigned H_2-5. Protons H_2-5 showed ^{3}J-HMBC correlations to the methylene carbons at δ 29.9 (C-6), 45.7 (C-1′) and to the quaternary carbonyl carbon at δ 164.1 (C-2). The protons H_2-5

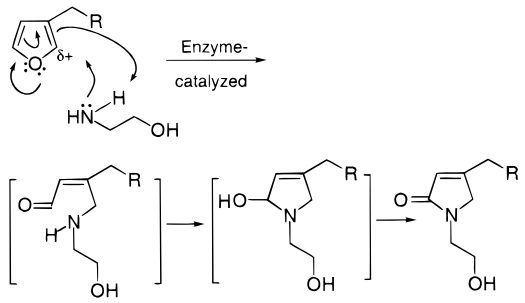

Figure 2. Plausible mechanism of formation of **3**.

displayed a ^{2}J-$^{1}H-^{15}N$-GHMBC correlation to the nitrogen N-1 resonating at δ 120.3 (Table 1), which further supported structure **3**. The proposed mechanism of formation of palinurine B is illustrated in Figure 2. In an enzyme-catalyzed reaction, the electrophilic attack of ethanolamine to C-5 of the furan ring would be followed by furan ring fission and formation of an intermediate with an aldehyde at C-2. Subsequent cyclization and oxidation would result in the formation of the more stable *N*-2′-hydroxyethyldehydro-3-enepyrrolidin-2-one derivative (palinurine B, **3**).

Conclusion

Cunninghamella sp. NRRL 5695 was able to transform the furan ring of palinurin into *N*-2′-hydroxyethyldehydro-3-enepyrrolidin-2-or 5-one. This unique biotransformation reaction indicates that a comparable synthetic route to transform furans to amides should be possible. A biomimetic transformation is certain to have applications in synthetic organic chemistry.

Experimental Section

General Experimental Procedure. The ^{1}H and ^{13}C NMR spectra were recorded in CD_3OD on NMR spectrometers operating at 400 or 500 MHz for ^{1}H and 100 or 125 MHz for ^{13}C NMR. The HRMS spectra were measured on an FTMS with electrospray ionization mass spectrometer. TLC analyses were carried out on precoated silica gel G_{254} 500 μm, with the following developing system: EtOAc−MeOH−H_2O (100:16.5:13.5), upper organic phase. For column chromatography, Si gel 60, 40 μm was used.

^{15}N NMR. Inverse detected ^{15}N spectra were recorded using a 500 MHz NMR spectrometer equipped with a 3 mm inverse-detected gradient probe. A gradient HMBC pulse sequence with 1 ms Gaussian *Z*-axis gradient pulses (70:30:50) was used. Referencing of the indirectly detected ^{15}N dimension was accomplished using nitromethane as an external standard. A GHMBC experiment was performed on nitromethane and the ^{15}N correlation was calibrated to 380.2. This same calibration value was then used for the palinurines. The acquisition time was 24 h for each compound.

Sample Material. The sponge was collected in December 1997, 10 miles northeast of Hurghada, Egypt, from a depth of −15 m. The sponge forms a massive sphere 40 cm long, 20 cm wide, and 10 cm high and was found on vertical coral faces. The surface is covered with regularly spaced tough conules 3−5 mm high. The sponge is tough, compressible, and elastic. The color in life is light yellowish brown and darker brown in shaded areas. The skeleton consists of very robust golden ladder-like fiber fascicles with embedded sand grains, and fine collagen fibrils permeate the choanosome. The sponge is closely comparable to *I. echinata* Keller 1889 (order Dictyoceratida, family Irciniidae). A voucher specimen has been deposited at the Natural History Museum, London (BMNH 1998.8.11.1).

Organisms. Microbial metabolism studies were conducted as previously reported.[22,23] Thirty microbial cultures, obtained from the University of Mississippi, Department of Pharmacognosy culture collection, were used for screening. These microbes were the same as those previously reported,[22] in addition to: *Aspergillus alliaceus* NRRL315, *Aspergillus flavipes* ATCC 16795, *Cunninghamella echinulata* NRRL 3655, *Cunninghamella* sp. NRRL 5695, *Debaryomyces polymorphus* ATCC 20280, *Gongronella butleri* ATCC 22822, *Lipomyces lipofer* ATCC 10742, *Lodderomyces elongisporus* ATCC 22688, *Mucor mucedo* UI 4605, *Mucor ramannianus* 1839 (sih), *Mycobacterium cuneatum* ATCC 21498, *Nocardia restricta* ATCC 14887, *Penicillium claviforme* MR 376, *Penicillium frequentans* ATCC 10444, *Streptomyces griseus* L-103, *Streptomyces griseus* ATCC 13968.

Microbial Metabolism of Palinurin (1). *Cunninghamella* species NRRL 5695 was grown in 18 1 L culture flasks, each containing 300 mL of compound medium α. A total of 720.0 mg of **1** was mixed with 2.3 mL of EtOH and evenly distributed among the stage II (24 h) cultures at a concentration of 40 mg/1 L flask. After 14 days, the incubation mixtures were pooled and filtered. The filtrate (2.9 L) was exhaustively extracted with EtOAc (3 × 500 mL) followed by BuOH (2 × 500 mL), which was then dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The mixed residue (2.1 g) was flash chromatographed over 100 g of Si gel 60 using hexane, gradient elution with increasing proportions of ethyl acetate, and finally MeOH. The polar fractions were subjected to repeated preparative TLC on Si gel G (EtOAc−MeOH−H_2O, upper organic phase, 100:16.5:13.5) which afforded **2** (4.8 mg, R_f 0.67) and **3** (2.4 mg, R_f 0.60).

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Palinurine A (**2**): yellowish oil; $[\alpha]^{25}_D$ +69.9 (*c* 0.25, $CHCl_3$); UV λ_{max} (log ϵ) (MeOH) 246 (2.87), 268 (2.67) nm; IR υ_{max} ($CHCl_3$) 3429 (OH), 2950−2856, 1720 (C═O), 1690 (C═O), 1225 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; HRFTMS *m*/*z* calcd for $C_{27}H_{39}O_5N$ $(M + Na)^+$ 480.2726, found 480.2727.

Palinurine B (**3**): yellowish oil; $[\alpha]^{25}_D$ +58.5 (*c* 0.38, $CHCl_3$); UV λ_{max} (log ϵ) (MeOH) 246 (2.87), 268 (2.67) nm; IR υ_{max} ($CHCl_3$) 3421 (OH), 2970−2865, 1725 (C═O) 1686 (C═O), 1230 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; HRFTMS *m*/*z* calcd for $C_{27}H_{39}O_5N$ $(M + Na)^+$ 480.2726, found 480.2752.

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Supporting Information Available: The antibacterial and antiinflammatory activity of **1**−**3** and ^1H and ^{13}C NMR, DEPT 135°, HMQC, HMBC, and $^1H-^{15}N$ HMBC spectra of **2** and **3**. This material is available free of charge via the Internet at http://pubs.acs.org.

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