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

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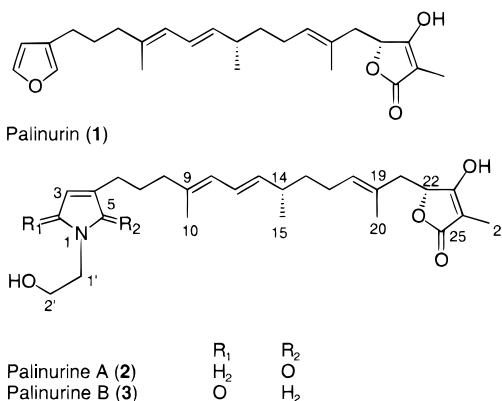
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## Introduction

Furanososterpenes are frequently isolated from marine sponges of the genera *Ircinia*,<sup>1–10</sup> *Spongia*,<sup>11–13</sup> *Spongionella*,<sup>14,15</sup> *Cacospongia*,<sup>16</sup> *Dysidea*,<sup>17</sup> *Sarcotragus*,<sup>18</sup> *Amphimedon*,<sup>19</sup> and *Hippospongia*.<sup>20</sup> They display a wide range of bioactivity including antibacterial,<sup>3,4,18,19</sup> antiviral,<sup>3</sup> fertilized starfish eggs cell division inhibitory,<sup>14–16</sup> protein phosphatase inhibitory,<sup>17</sup> antispasmodic,<sup>20</sup> and antiinflammatory activities.<sup>21</sup> The use of microbial transformations to modify marine natural products and generate bioactive lead derivatives with minimal toxicity was recently reported.<sup>22</sup> Palinurine (**1**) is a linear furanoses-

terterpene previously reported from the Mediterranean sponge *Ircinia variabilis* and was reisolated in gram quantities from a Red Sea sponge *Ircinia echinata*.<sup>5</sup> 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.



## Results and Discussion

**Bioconversion Studies and Structure Elucidation.** Palinurine (**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<sub>27</sub>H<sub>39</sub>O<sub>5</sub>N (M + Na)<sup>+</sup> and nine degrees of unsaturation. The IR spectra (KBr) of **2** and **3** showed absorption bands at 3429 and 3421 cm<sup>-1</sup>, respectively, suggesting the presence of hydroxyl functionality. It also showed absorption bands at 1720, 1690 and 1725, 1686 cm<sup>-1</sup>, respectively, suggesting the presence of α,β-unsaturated lactone functionality. The <sup>1</sup>H and <sup>13</sup>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<sub>2</sub>-2. This proton signal showed <sup>1</sup>H-<sup>1</sup>H COSY coupling to the broad proton singlet resonating at δ 6.86, which correlated to the methine

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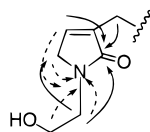
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**Table 1.**  $^{13}\text{C}$ ,  $^{15}\text{N}$ , and  $^1\text{H}$  NMR Data of Compounds **2** and **3**<sup>a</sup>

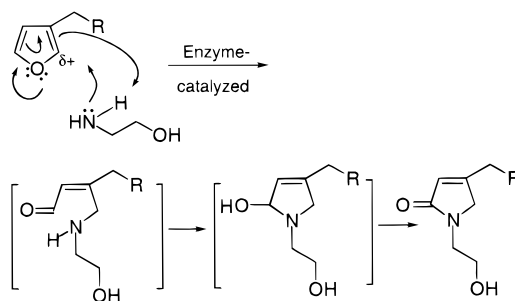
| no. | <b>2</b>                           |                                  | <b>3</b>                           |                                  |
|-----|------------------------------------|----------------------------------|------------------------------------|----------------------------------|
|     | $^{13}\text{C}$ or $^{15}\text{N}$ | $^1\text{H}$                     | $^{13}\text{C}$ or $^{15}\text{N}$ | $^1\text{H}$                     |
| 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);<br>2.14, m | 36.1, t                            | 2.52, dd (11.0, 2.1);<br>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)                |

<sup>a</sup> In  $\text{CD}_3\text{OD}$ , 400 MHz for  $^1\text{H}$ -, 100 MHz for  $^{13}\text{C}$ -, and 50 MHz for  $^{15}\text{N}$  NMR. Carbon multiplicities were determined by DEPT  $^{135}^\circ$  experiments. s = quaternary, d = methine, t = methylene carbons. Coupling constants ( $J$ ) are in Hz. Nitromethane was used as an external standard for  $^{15}\text{N}$  NMR. S = quaternary nitrogen.

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

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

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

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

displayed a  $^2J$ - $^1\text{H}$ – $^{15}\text{N}$ -GHMBC correlation to the nitrogen N-1 resonating at  $\delta$  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 palinurine 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  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were recorded in  $\text{CD}_3\text{OD}$  on NMR spectrometers operating at 400 or 500 MHz for  $^1\text{H}$  and 100 or 125 MHz for  $^{13}\text{C}$  NMR. The HRMS spectra were measured on an FTMS with electrospray ionization mass spectrometer. TLC analyses were carried out on precoated silica gel G<sub>254</sub> 500  $\mu\text{m}$ , with the following developing system: EtOAc–MeOH–H<sub>2</sub>O (100:16.5:13.5), upper organic phase. For column chromatography, Si gel 60, 40  $\mu\text{m}$  was used.

**$^{15}\text{N}$  NMR.** Inverse detected  $^{15}\text{N}$  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  $^{15}\text{N}$  dimension was accomplished using nitromethane as an external standard. A GHMBC experiment was performed on nitromethane and the  $^{15}\text{N}$  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.<sup>22,23</sup> 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,<sup>22</sup> 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 Palinurine (1).** *Cunninghamella* species NRRL 5695 was grown in 18 1 L culture flasks, each containing 300 mL of compound medium  $\alpha$ . 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  $\times$  500 mL) followed by BuOH (2  $\times$  500 mL), which was then dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> 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<sub>2</sub>O, upper organic phase, 100:16.5:13.5) which afforded **2** (4.8 mg, *R<sub>f</sub>* 0.67) and **3** (2.4 mg, *R<sub>f</sub>* 0.60).

Palinurine A (**2**): yellowish oil; [ $\alpha$ ]<sub>D</sub><sup>25</sup> +69.9 (*c* 0.25, CHCl<sub>3</sub>); UV  $\lambda_{\max}$  (log  $\epsilon$ ) (MeOH) 246 (2.87), 268 (2.67) nm; IR  $\nu_{\max}$  (CHCl<sub>3</sub>) 3429 (OH), 2950–2856, 1720 (C=O), 1690 (C=O), 1225 cm<sup>-1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR, see Table 1; HRFTMS *m/z* calcd for C<sub>27</sub>H<sub>39</sub>O<sub>5</sub>N (M + Na)<sup>+</sup> 480.2726, found 480.2727.

Palinurine B (**3**): yellowish oil; [ $\alpha$ ]<sub>D</sub><sup>25</sup> +58.5 (*c* 0.38, CHCl<sub>3</sub>); UV  $\lambda_{\max}$  (log  $\epsilon$ ) (MeOH) 246 (2.87), 268 (2.67) nm; IR  $\nu_{\max}$  (CHCl<sub>3</sub>) 3421 (OH), 2970–2865, 1725 (C=O) 1686 (C=O), 1230 cm<sup>-1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR, see Table 1; HRFTMS *m/z* calcd for C<sub>27</sub>H<sub>39</sub>O<sub>5</sub>N (M + Na)<sup>+</sup> 480.2726, found 480.2752.

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**Supporting Information Available:** The antibacterial and antiinflammatory activity of **1–3** and <sup>1</sup>H and <sup>13</sup>C NMR, DEPT 135°, HMQC, HMBC, and <sup>1</sup>H–<sup>15</sup>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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