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# Planar Structure and Antibacterial Activity of Korormicin Derivatives Isolated from *Pseudoalteromonas* sp. F-420

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Korormicin (**1**, Fig. 1) is an antibiotic produced by a marine bacterium, *Pseudoalteromonas* sp. F-420, collected from the Palauan sea.[1] Total synthesis studies by two independent laboratories have described the stereochemistry of the compound as 5*S*,3′*R*,9′*S*,10′*R*.[2,3] Compound **1** was first characterized by its specific inhibitory activity against Gram-negative marine bacteria.[1,4] The target molecule of **1** was found to be $Na^+$-translocating NADH-quinone reductase (Nqr).[4,5] Nqr is widely distributed among Gram-negative marine bacteria, and functions as a primary $Na^+$ pump.[6] Compound **1** acts as a noncompetitive inhibitor for a substrate ubiquinone-1, with the inhibition constant of 82 pM.[5] By using a korormicin-resistant mutant of *Vibrio alginolyticus*, KR2, Gly140 on NqrB, one of the subunits of the Nqr complex, was shown to be essential to the binding of **1**.[7] This amino acid residue Gly140 is well conserved among Gram-negative pathogens on which the *nqr* operon is found in the genome sequence. Korormicin could be used as an antibiotic not only against marine bacteria, but against any bacterium using the primary sodium pump to maintain life.

During a fermentation study of the strain F-420, we found some minor compounds detected in the HPLC chromatogram in a step of the purification of **1**. In this report, we describe our isolation of some compounds that were derivatives of **1**.

When a subculture of *Pseudoalteromonas* sp. F-420 (deposited as FERM P-16084), 0.2 ml, had an $OD_{650}$ of 0.5, it was transferred to 100 ml of Marine Broth 2216 (Becton, Dickinson Co., Franklin Lakes, NJ, USA) culture medium supplemented with 1% (w/v) maltose and 0.16% $NaHCO_3$ in a 1-liter Elrenmeyer flask with baffles. Incubation was done on a rotary shaker (110 rpm) at 30°C for 20 hours. Cells were collected from the 9.9-liter culture and were extracted with 500 ml of aqueous ethanol by using an ultrasonic cell disrupter. Then the extract was concentrated to dryness and was resolved in an aliquot of ethyl acetate. Solvent extraction, silica gel column chromatography, and size-exclusion chromatography for the separation of the antibacterial compounds were by the methods described in our previous paper.[1] A bioactive fraction was then further purified by using a reversed-phase HPLC (Capcell Pak C-18, Shiseido, Tokyo, Japan: 15 mm×250 mm column, 5 ml/minute flow rate), eluting with 80% methanol at ambient temperature. Under these conditions, **1** (163.83 mg in total) was eluted at 23.1 minutes when monitored with $A_{233}$. Compounds, **1a** (7.30 mg), **1b** (13.25 mg), **1c** (3.89 mg), **2** (10.69 mg), and **3** (12.78 mg) were isolated, eluting at 13.4, 17.3, 31.8, 23.8, and 21.9 minutes, respectively.

Their MS were recorded with a JEOL JMS-SX102 mass spectrometer. The NMR spectra were measured by using a Varian Unity 500 NMR spectrometer, and the data were recorded in DMSO-$d_6$ at 500 MHz for $^1H$, and at 125 MHz for $^{13}C$. The IR spectrum was obtained from a KBr pellet by using a JASCO FT-IR 7000 spectrophotometer. Optical rotations were found by using a Horiba SEPA-300 polarimeter.

Compound **1a**. A colorless oil; FAB-MS *m/z* 406 $(M+H)^+$, 428 $(M+Na)^+$; HR-MS *m/z* $(M+H)^+$: Calcd for $C_{23}H_{36}NO_5$: 406.2593, Found, 406.2596; $[\alpha]_D^{26}$ −42.4 (*c* 0.19, EtOH); IR $\nu_{max}$ (KBr) $cm^{-1}$ 3450, 2930, 2860, 1765, 1695, 1655, 1540, 1460, 1380, 1325, 1205, 1110, 1050, 995, 945; UV $\lambda_{max}^{EtOH}$ nm (log $\varepsilon$) 232 (4.55).

Compound **1b**. A colorless oil; FAB-MS *m/z* 420 $(M+H)^+$, 442 $(M+Na)^+$; HR-MS *m/z* $(M+Na)^+$: Calcd for $C_{24}H_{37}NO_5Na$: 442.2569, Found, 442.2571; $[\alpha]_D^{26}$ −24.1 (*c* 0.67, EtOH); IR $\nu_{max}$ (KBr) $cm^{-1}$ 3455, 2930, 2860, 1765, 1695, 1655, 1540, 1460, 1380, 1325, 1205, 1110, 1050, 995, 945; UV $\lambda_{max}^{EtOH}$ nm (log $\varepsilon$) 232 (4.59).

Compound **1c**. A colorless oil; FAB-MS *m/z* 448 $(M+H)^+$, 470 $(M+Na)^+$; HR-MS *m/z* $(M+Na)^+$: Calcd for $C_{26}H_{41}NO_5Na$: 470.2882, Found, 470.2876; $[\alpha]_D^{26}$ −28.2 (*c* 0.25, EtOH); IR $\nu_{max}$ (KBr) $cm^{-1}$ 3470, 2930, 2860, 1740, 1655, 1540, 1460, 1400, 1055; UV $\lambda_{max}^{EtOH}$ nm (log $\varepsilon$) 233 (4.43).

Compound **2**. A colorless oil; FAB-MS *m/z* 513, 515

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Table 1-1. NMR chemical shifts for compounds **1**, **1a**~**c**, **2**, and **3**.

(1) $^1$H-NMR

| Compound / Position | 1[a] | 1a | 1b | 1c | 2 | 3 |
|---|---|---|---|---|---|---|
| 4 | 7.26 (1H, s) | 7.37 (1H, s) | 7.37 (1H, s) | 7.37 (1H, s) | 7.38 (1H, s) | 7.45 (1H, s) |
| 6 | 1.76 (2H, q) | 1.75 (2H, q) | 1.75 (2H, q) | 1.75 (2H, q) | 1.74 (2H, q) | 1.420 (3H, s)[g] |
| 7 | 0.74 (3H, t) | 0.75 (3H, t) | 0.75 (3H, t) | 0.75 (3H, t) | 0.75 (3H, t) | 1.423 (3H, s)[g] |
| 8 | 1.37 (3H, s) | 1.40 (3H, s) | 1.40 (3H, s) | 1.40 (3H, s) | 1.40 (3H, s) | |
| NH | 9.83 (1H, s) | 9.86 (1H, s) | 9.86 (1H, s) | 9.86 (1H, s) | 9.83 (1H, s) | 9.83 (1H, s) |
| 2a' | 2.39 (1H, dd) | 2.40 (1H, dd) | 2.39 (1H, dd) | 2.40 (1H, dd) | 2.40 (1H, dd) | 2.39 (1H, dd) |
| 2b' | 2.59 (1H, dd) | 2.61 (1H, dd) | 2.61 (1H, dd) | 2.61 (1H, dd) | 2.60 (1H, dd) | 2.61 (1H, dd) |
| 3' | 4.84 (1H, ddd) | 4.84 (1H, ddd) | 4.84 (1H, ddd) | 4.84 (1H, ddd) | 4.83 (1H, ddd) | 4.83 (1H, ddd) |
| 3'-OH | 5.09 (1H, d) | 5.11 (1H, d) | 5.11 (1H, d) | 5.11 (1H, d) | 5.10 (1H, br) | 5.10 (1H, d) |
| 4' | 5.30 (1H, dd) | 5.31 (1H, dd) | 5.31 (1H, dd) | 5.31 (1H, dd) | 5.26 (1H, d) | 5.31 (1H, dd) |
| 5' | 5.92 (1H, dd) | 5.93 (1H, dd) | 5.93 (1H, dd) | 5.93 (1H, dd) | 5.91 (1H, dd) | 5.93 (1H, dd) |
| 6' | 6.46 (1H, dd) | 6.47 (1H, dd) | 6.47 (1H, dd) | 6.47 (1H, dd) | 6.42 (1H, dd) | 6.47 (1H, dd) |
| 7' | 5.70 (1H, dt) | 5.71 (1H, dt) | 5.71 (1H, dt) | 5.71 (1H, dt) | 5.71 (1H, dt) | 5.71 (1H, dt) |
| 8' | 2.26 (2H, dd) | 2.28 (2H, dd) | 2.28 (2H, dd) | 2.27 (2H, dd) | 2.29 (2H, dd) | 2.28 (2H, dd) |
| 9' | 2.90 (1H, dt) | 2.91 (1H, dt) | 2.91 (1H, dt) | 2.91 (1H, dt) | 3.50 (1H, m) | 2.91 (1H, dt) |
| 9'-OH | | | | | 5.10 (1H, br) | |
| 10' | 2.87 (1H, ddd) | 2.88 (1H, ddd) | 2.88 (1H, ddd) | 2.88 (1H, ddd) | 4.05 (1H, m) | 2.88 (1H, ddd) |
| 11' | 1.48 (2H, m) | 1.47 (2H, m) | 1.47 (2H, m) | 1.47 (2H, m) | 1.80 (2H, m) | 1.47 (2H, m) |
| 12' | 1.38 (2H, m) | 1.2-1.4 (2H, m)[c] | 1.2-1.4 (2H, m)[d] | 1.2-1.4 (2H, m)[e] | 1.46 (2H, m) | 1.2-1.4 (2H, m)[h] |
| 13' | 1.2-1.4 (2H, m)[b] | 1.2-1.4 (2H, m)[c] | 1.2-1.4 (2H, m)[d] | 1.2-1.4 (2H, m)[e] | 1.2-1.4 (2H, m)[f] | 1.2-1.4 (2H, m)[h] |
| 14' | 1.2-1.4 (2H, m)[b] | 1.2-1.4 (2H, m)[c] | 1.2-1.4 (2H, m)[d] | 1.2-1.4 (2H, m)[e] | 1.2-1.4 (2H, m)[f] | 1.2-1.4 (2H, m)[h] |
| 15' | 1.2-1.4 (2H, m)[b] | 1.2-1.4 (2H, m)[c] | 1.2-1.4 (2H, m)[d] | 1.2-1.4 (2H, m)[e] | 1.2-1.4 (2H, m)[f] | 1.2-1.4 (2H, m)[h] |
| 16' | 1.22 (2H, m) | 0.85 (3H, t) | 1.2-1.4 (2H, m)[d] | 1.2-1.4 (2H, m)[e] | 1.24 (2H, m) | 1.2-1.4 (2H, m)[h] |
| 17' | 1.24 (2H, m) | | 0.85 (3H, t) | 1.2-1.4 (2H, m)[e] | 1.26 (2H, m) | 1.2-1.4 (2H, m)[h] |
| 18' | 0.83 (3H, t) | | | 1.2-1.4 (2H, m)[e] | 0.84 (3H, t) | 0.84 (3H, t) |
| 19' | | | | 0.85 (3H, t) | | |

[a]Data from reference 1).

b-h, interchangeable.

$(M+H)^+$; HR-MS *m/z* $(M+H)^+$: Calcd. for $C_{25}H_{41}{}^{81}BrNO_5$: 516.2168, Found, 516.2141; $[\alpha]_D^{26}$ −36.1 (*c* 0.45, EtOH); IR $\nu_{max}$ (KBr) $cm^{-1}$ 3448, 2928, 2858, 1763, 1694, 1653, 1541, 1458, 1383, 1328, 1207, 1110, 1031, 948; UV $\lambda_{max}^{EtOH}$ nm (log $\varepsilon$) 234 (4.47).

Compound **3**. A colorless oil; FAB-MS *m/z* 420 $(M+H)^+$, 442 $(M+Na)^+$; HR-MS *m/z* $(M+H)^+$: Calcd for $C_{24}H_{38}NO_5$: 420.2750, Found, 420.2742; $[\alpha]_D^{26}$ +42.3 (*c* 0.21, EtOH); IR $\nu_{max}$ (KBr) $cm^{-1}$ 3440, 2930, 2860, 1765, 1695, 1655, 1540, 1460, 1380, 1325, 1205, 1110, 1050, 950; UV $\lambda_{max}^{EtOH}$ nm (log $\varepsilon$) 232 (4.53).

The $^1$H and $^{13}$C NMR spectra for these compounds (Table 1) suggested that their planar structures were very similar to that of **1**.[1] Their IR and UV spectra were substantially identical to those of **1**. From HMBC and the result of the FAB-MS analysis, **1a**~**c** were deduced to be

Table 1-2. Continued.

(2) $^{13}$C-NMR[a]

| Compound / Position | 1[b] | 1a | 1b | 1c | 2 | 3 |
|---|---|---|---|---|---|---|
| 2 | 168.4 (s) | 168.1 (s) | 168.1 (s) | 168.1 (s) | 168.2 (s) | 168.0 (s) |
| 3 | 125.1 (s) | 124.8 (s) | 124.8 (s) | 124.8 (s) | 124.9 (s) | 124.1 (s) |
| 4 | 133.9 (d) | 133.7 (d) | 133.6 (d) | 133.6 (d) | 133.8 (d) | 135.1 (d) |
| 5 | 87.0 (s) | 86.9 (s) | 86.9 (s) | 86.9 (s) | 87.0 (s) | 84.5 (s) |
| 6 | 31.2 (t) | 31.02 (t) | 31.03 (t) | 31.0 (t) | 31.16 (t) | 25.8 (q)[h] |
| 7 | 8.0 (q) | 7.8 (q) | 7.8 (q) | 7.8 (q) | 7.9 (q) | 25.7 (q)[h] |
| 8 | 24.1 (q) | 24.0 (q) | 24.0 (q) | 24.0 (q) | 24.1 (q) | |
| 1' | 170.1 (s) | 169.7 (s) | 169.7 (s) | 169.7 (s) | 169.9 (s) | 169.7 (s) |
| 2' | 44.0 (t) | 43.8 (t) | 43.8 (t) | 43.8 (t) | 43.9 (t) | 43.8 (t) |
| 3' | 63.0 (d) | 63.6 (d) | 63.6 (d) | 63.6 (d) | 63.7 (d) | 63.6 (d) |
| 4' | 132.7 (d) | 132.4 (d) | 132.4 (d) | 132.4 (d) | 132.07 (d) | 132.5 (d) |
| 5' | 128.0 (d) | 127.9 (d) | 127.9 (d) | 127.9 (d) | 128.3 (d) | 127.9 (d) |
| 6' | 127.6 (d) | 127.3 (d) | 127.3 (d) | 127.3 (d) | 127.3 (d) | 127.3 (d) |
| 7' | 130.9 (d) | 130.6 (d) | 130.6 (d) | 130.6 (d) | 132.14 (d) | 130.6 (d) |
| 8' | 30.9 (t) | 30.8 (t) | 30.8 (t) | 30.8 (t) | 38.1 (t) | 30.8 (t) |
| 9' | 55.1 (d) | 54.9 (d) | 54.9 (d) | 54.9 (d) | 71.9 (d) | 54.9 (d) |
| 10' | 56.0 (d) | 55.8 (d) | 55.8 (d) | 55.8 (d) | 63.9 (d) | 55.8 (d) |
| 11' | 27.2 (t) | 28.4 (t)[d] | 28.7 (t)[e] | 28.80 (t)[f] | 34.5 (t) | 28.8 (t)[i] |
| 12' | 26.1 (t) | 27.0 (t)[d] | 28.4 (t)[e] | 28.75 (t)[f] | 27.3 (t) | 28.7 (t)[i] |
| 13' | 28.92 (t)[c] | 25.9 (t)[d] | 27.0 (t)[e] | 28.72 (t)[f] | 28.8 (t)[g] | 28.4 (t)[i] |
| 14' | 28.89 (t)[c] | 31.04 (t) | 26.0 (t)[e] | 28.5 (t)[f] | 28.5 (t)[g] | 27.0 (t)[i] |
| 15' | 28.6 (t)[c] | 21.9 (t) | 31.04 (t) | 27.0 (t)[f] | 28.4 (t)[g] | 26.0 (t)[i] |
| 16' | 31.2 (t) | 13.8 (q) | 21.9 (t) | 26.0 (t)[f] | 31.18 (t) | 31.1 (t) |
| 17' | 22.1 (t) | | 13.8 (q) | 31.1 (t) | 22.0 (t) | 21.9 (t) |
| 18' | 13.9 (q) | | | 21.9 (t) | 13.9 (q) | 13.8 (q) |
| 19' | | | | 13.8 (q) | | |

[a]Multiplicity in parenthesis was determined by DEPT.

[b]Data from reference 1).

c-i, interchangeable.

derivatives of **1**, with different alkyl chain lengths at their amide moiety (Fig. 1).

Compound **2** had the molecular formula of $C_{25}H_{40}BrNO_5$. The chemical shifts of H-9′ ($\delta_H$ 3.50) and H-10′ ($\delta_H$ 4.05) on **2** were lower than those on **1** ($\delta_H$ 2.90 and 2.87, respectively). This suggests the absence of the epoxide ring in compound **2**. COSY indicated that a hydroxyl group was correlated with H-9′, but not with H-10′. The chemical shift of C-10′ on **2** ($\delta_C$ 63.9) was lower than the corresponding carbon on **1** ($\delta_C$ 56.0), suggesting

Fig. 1. Planar structures of korormicin (**1**) and the derivatives.

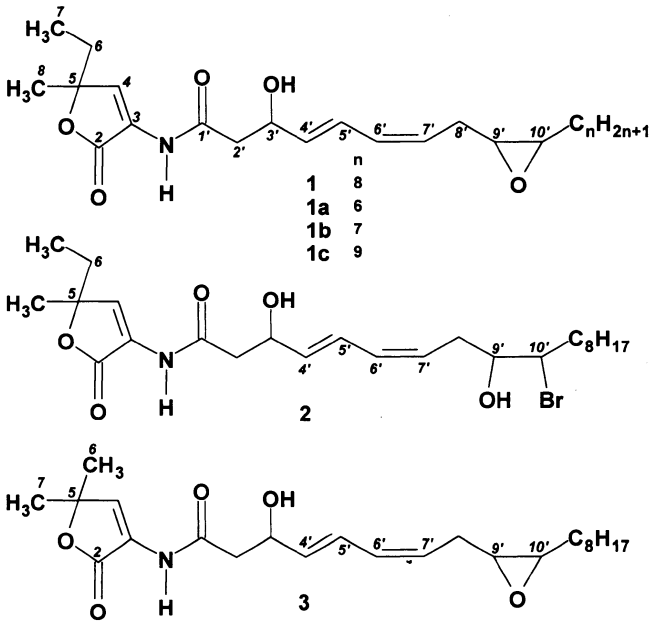


Table 2. Coupling constant $^3J_{H\text{-}H}$ of selected bondings (Hz) for compounds **1**, **1a**~**c**, **2**, and **3**.

| Compound Position | 1[a] | 1a | 1b | 1c | 2 | 3 |
|---|---|---|---|---|---|---|
| C-4'/C-5' | 10.9 | 10.8 | 10.7 | 10.9 | 10.9 | 10.9 |
| C-5'/C-6' | 11.2 | 11.0 | 11.2 | 11.3 | 11.3 | 11.2 |
| C-6'/C-7' | 15.1 | 14.8 | 14.9 | 14.8 | 15.0 | 14.8 |
| C-9'/C-10' | 4.2 | 4.2 | 4.2 | 4.2 | - | 4.2 |

[a] Data from reference 1).

Table 3. Antibacterial Activity of compounds **1**, **1a**~**c**, **2**, and **3**[a].

| Compound Strain[b] | Halophilicity[c] | 1 | 1a | 1b | 1c | 2 | 3 | B |
|---|---|---|---|---|---|---|---|---|
| *S. costicola* | (+) | 27 | 38 | 34 | 22 | 21 | 26 | 18 |
| *P. haloplanktis* | (+) | 26 | 27 | 24 | 19 | 18 | 21 | 21 |
| *E.coli* | (-) | - | - | - | - | - | - | 16 |

[a]Ten μg of each compound was used for a paper disk 8 mm in diameter, except the control inhibitor polymixin B (B, 5 μg was used). Values are diameters in mm of inhibition zone on the agar plate.

[b]Bacterial strains used were *Salinivibrio costicola* ATCC33508, *Pseudoalteromonas haloplanktis* ATCC14393, and *E. coli* IFO3301.

[c]*S. costicola* and *P. haloplanktis* are marine bacteria characterized by their halophilicity (designated as (+)).

the binding of bromine at this carbon. Fig. 1 shows the planar structure of **2** that was established.

Compound **3** had the molecular formula of $C_{24}H_{37}NO_5$, one methylene unit difference from **1**. HSQC data were shown to be almost identical to each other in the amide part of **1** and **3**. However, the lactone moiety lacked a secondary carbon ($\delta_C$ 31.2; C-6 in **1**). A methyl group with a higher chemical shift ($\delta_C$ 8.0 and $\delta_H$ 0.74 in **1**) also disappeared. Instead, two methyl groups were observed: $\delta_C$ 25.7 and 25.8 in $^{13}C$ NMR, and $\delta_H$ 1.420 and 1.423 in $^1H$ NMR, respectively. Fig. 1 shows the planar structure of **3** that was established.

The regiochemistry at the double bonds and the epoxide in compounds **1a**~**c**, **2**, and **3** was found from their coupling constants, $J_{4',5'}$, $J_{6',7'}$, and $J_{9',10'}$, respectively, recorded on $^1H$ NMR charts. All derivatives shared conformation with **1**: C-4′/C-5′, *Z*; C-6′/C-7′, *E*; C-9′/C-10′ (except **2**), *cis* (Table 2). The complete configuration for these new compounds is unknown now.

The strength of the antibacterial activity of these derivatives was compared by using the paper disk method.[1] The result (Table 3) indicates that all derivatives have antibacterial activity against *Salinivibrio costicola* and *Pseudoalteromonas haloplanktis*, but not against *Escherichia coli*. Specificity against Nqr in the Gram-negative marine bacteria for these derivatives is highly possible from their structural similarity to **1**, but a demonstrative study needs to be done.

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