Note

Carquinostatin B, a New Neuronal Cell-protecting Substance Produced by *Streptomyces exfoliatus*

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Received April 18, 1997

Brain ischemia injury is elicited by the excitotoxicity of L-glutamate. Carquinostatin B was isolated from *Streptomyces exfoliatus* 2419-SVT2 as a potent neuroprotective substance which protects neuronal hybridoma N18-RE-105 cells from L-glutamate toxicity. The structure of carquinostatin B was established principally by NMR studies to be a carbazole derivative with an ortho quinone function.

Key words: ischemia injury; L-glutamate toxicity; neuroprotection; carquinostatin B; antioxidative substance

It is well accepted that L-glutamate, which acts as a neurotransmitter in the major part of the central nervous system, induces delayed neuronal cell death that follows an ischemic attack.1,2) Thus, substances which inhibit L-glutamate toxicity can be expected to prevent or ameliorate brain damage caused by brain ischemia.

In the course of our screening for substances which protect neuronal cells from L-glutamate toxicity in neuronal hybridoma N18-RE-105 cells, we isolated several active compounds.3–9) Further screening has resulted in the isolation of carquinostatin B from *Streptomyces exfoliatus* 2419-SVT2 as a minor congener of carquinostatin A.3) We describe here the structural elucidation and brief biological activities of carquinostatin B.

The carquinostatin B producing organism, which was identified as *Streptomyces exfoliatus* 2419-SVT2, was cultivated in a 50-liter jar fermenter containing 30 liters of a medium consisting of 2.5% dextran, 2.3% soybean meal, 0.2% dry yeast and 0.4% CaCO₃ at 27°C for 3 days. The mycelial acetone extract was concentrated to a small volume, and the aqueous residue was extracted with EtOAc. The EtOAc layer was dried over Na₂SO₄ and concentrated to give an oily residue. This material was washed with n-hexane, and the remaining residue was treated with a silica gel column packed with CHCl₃-MeOH (20:1). After washing with the same solvent system, the active material was eluted with CHCl₃-MeOH (10:1). Further purification of the active eluate by Sephadex LH-20 with CHCl₃-MeOH (1:1) gave a pure sample of carquinostatin B as a reddish brown powder (14.5 mg).

The molecular formula of carquinostatin B was established as C₂₄H₂₄NO₄ by HRFAB-MS data [m/z, (M+H)⁺]: found 354.1740; calc'd. for C₂₄H₂₄NO₄, 354.1705. The UV and visible spectra of carquinostatin B,10) which are identical to those of carquinostatin A and carbaquinostatins,11) indicate that the chromophore of carquinostatin B consisted of a carbazole nucleus with an ortho quinone function.

The ¹H- and ¹³C-NMR spectral data are summarized in Table 1. The sequence from C-10 to C-12 was revealed by analyzing the DQF-COSY spectrum of carquinostatin B as shown in Fig. 2. Together with these NMR data, the ¹H-¹³C long-range couplings revealed by HMBC experiments proved the structure of carquinostatin B to be 10-hydroxycarquinostatin A as shown in Fig. 2. The absolute stereochemistry at the C-11 position of carquinostatin A had already been established to be R by the

Table 1 ¹H- and ¹³C-NMR Spectral Data for Carquinostatin B

<table>
<thead>
<tr>
<th>No.</th>
<th>δC</th>
<th>δH</th>
<th>δC</th>
<th>δH</th>
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<tr>
<td>1</td>
<td>142.1</td>
<td>9a</td>
<td>144.5</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>132.5</td>
<td>10</td>
<td>73.9</td>
<td>4.70 (d, J=7 Hz)</td>
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<tr>
<td>3</td>
<td>184.0</td>
<td>11</td>
<td>69.3</td>
<td>3.95 (m, J=7, 5 Hz)</td>
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<tr>
<td>4</td>
<td>172.4</td>
<td>12</td>
<td>19.6</td>
<td>1.15 (d, J=5 Hz)</td>
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<tr>
<td>4a</td>
<td>111.3</td>
<td>13</td>
<td>12.2</td>
<td>1.93 (s)</td>
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<tr>
<td>4b</td>
<td>125.3</td>
<td>14</td>
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<tr>
<td>5</td>
<td>119.1</td>
<td>15</td>
<td>123.8</td>
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<tr>
<td>6</td>
<td>137.3</td>
<td>16</td>
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<td></td>
</tr>
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<td>7</td>
<td>124.9</td>
<td>17</td>
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<td>1.70 (s)</td>
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<td>18</td>
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<td>8a</td>
<td>135.6</td>
<td>9-NH</td>
<td>9.9</td>
<td>(br.s)</td>
</tr>
</tbody>
</table>

¹H-NMR and ¹3C-NMR spectra were taken in DMSO-d₆ at 500 MHz and 125 MHz, respectively.

Fig. 1. Structure of Carquinostatin B.

Fig. 2. NMR Analyses of Carquinostatin B.

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modified Mosher method. To elucidate the absolute configuration at the C-10 position, carquinoestatin B dimethylketal was prepared by using 2,2-dimethoxypropane and pyridinium p-toluene sulfonate in CH₂Cl₂. By assuming the identical stereochemistry of C-11 for both carquinoestatins A and B, the absolute stereochemistry at the C-10 position is concluded to have been S, based on the observation of strong NOE between 10-H and 11-H, and its coupling constant (J₁₀₋₁₁ = 9 Hz) as shown in Fig. 3.

With the evaluation system we employed, carquinoestatin B inhibited the l-glutamate toxicity in N18-RE-105 cells with an EC₅₀ value of 72 nm which is similar to that of vitamin E (EC₅₀ value: 57 nm). In contrast, carquinoestatin A suppressed this toxicity more effectively with an EC₅₀ value of 3.1 nm. The l-glutamate toxicity in N18-RE-105 cells has been reported to be mediated by the inhibition of cystine uptake, which consequently suppressed the synthesis of the intracellular reducing agent, glutathione. Thus, antioxidants such as vitamin E suppress the l-glutamate toxicity in N18-RE-105 cells by scavenging oxygen radicals in place of glutathione. To prove the antioxidative activity of carquinoestatin B, we further evaluated the inhibitory effect against buthionine sulfoximine (BSO), which is an inhibitor of glutathione synthesis. Carquinoestatin B prevented cell death from BSO toxicity with an EC₅₀ value of 110 nm, whereas the values for carquinoestatin A and vitamin E were 2.3 nm and 31 nm, respectively. Since the reason for the difference in activity between carquinoestatins A and B is unknown, studies on the detailed biological activity are now underway.

Acknowledgments. This work was supported in part by a Grant-in-Aid for Scientific Research (C) to K.S. and by Research for the Future, Japan Society for the Promotion of Science to H.S.

References and Notes
10. mp 142–144°C; UV am (ε) in MeOH: 230 (32,200), 267 (29,700), 425 (5,400); in MeOH + NaOH: 242 (30,400), 287 (28,000), 470 (8,100); IR (KBr): 3450, 3220, 1660 (sh), 1640 (sh), 1615 cm⁻¹; [α]D could not be measured since the visual absorption was too great.
14. C₈H₁₂NO₂: HRFB-MS ([M+H]+, m/z: 394.1970 (→4.8 mmu error)); °H-NMR (CDCl₃: δ: 10.0 (9-NH, br.s), 7.92 (5-H, br.s), 7.20 (8-H, d, 8 Hz), 7.08 (7-H, dd, 8, 1 Hz), 5.33 (15-H, br.t, 7.5 Hz), 4.93 (10-H, d, 9 Hz), 4.15 (11-H, dq, 9, 6 Hz), 3.43 (14-H, d, 7.5 Hz), 2.07 (13-H, s), 1.77 (methyl proton assignable to dimethylketal, s), 1.74 (17, 18-H, 6H, br.s), 1.58 (methyl proton assignable to dimethylketal, a), 1.39 (12-H, d, 6 Hz).