Inhibitory Effects on HIV-1 Protease of Tri-\(p\)-coumaroylspermidine from *Artemisia caruifolia* and Related Amides

Chao-mei Ma, Norio Nakamura, and Masao Hattori*

Institute of Natural Medicine, Toyama Medical and Pharmaceutical University, 2630 Sugitani, Toyama 930–0194, Japan.

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During the replication of AIDS virus (HIV), the viral polyprotein must be cleaved by viral protease (PR) to generate essential viral enzymes, such as reverse transcriptase, integrase and PR itself, as well as the viral structural proteins. HIV PR has been proved to be one of the therapeutic targets and some peptide mimetic compounds targeting the active site of HIV PR have been synthesized as potent anti-HIV drugs able to be used clinically. However, high dosages of these drugs are usually needed, which has led to severe side effects and thus long term administration is difficult for AIDS patients to adhere to. This means that novel types of anti-HIV agents are urgently needed.

In the present paper, we report the isolation of \(N^1,N^5,N^{10}\)-tri-\(p\)-coumaroylspermidine as an HIV-1 PR inhibitor from *Artemisia caruifolia* and the synthesis and inhibitory activity of some related amides.

**Results and Discussion**

In a preliminary screening, a methanol extract of *Artemisia caruifolia*, which showed a moderate inhibitory activity on HIV-1 protease in a preliminary screening, \(N^1,N^5,N^{10}\)-tri-\(p\)-coumaroylspermidine and three dicaffeoylquinic acids were isolated. The former compound was found to appreciably inhibit HIV-1 protease. Of related amides which were chemically synthesized, \(N^1,N^5,N^{10}\)-tetra-\(p\)-coumaroylspermine and \(N^1,N^4,N^7,N^{10}\)-penta-\(p\)-coumaroyltetraethylenepentamine inhibited HIV-1 protease more potently than \(N^1,N^5,N^{10}\)-tri-\(p\)-coumaroylspermidine.

**Key words** *Artemisia caruifolia*; tri-\(p\)-coumaroylspermidine; tetra-\(p\)-coumaroylspermine; penta-\(p\)-coumaroyltetraethylenepentamine; dicaffeoylquinic acid; human immunodeficiency virus type 1 protease inhibitor

From a methanol extract of *Artemisia caruifolia*, which showed a moderate inhibitory activity on HIV-1 protease in a preliminary screening, \(N^1,N^5,N^{10}\)-tri-\(p\)-coumaroylspermidine and three dicaffeoylquinic acids were isolated. The former compound was found to appreciably inhibit HIV-1 protease. Of related amides which were chemically synthesized, \(N^1,N^5,N^{10}\)-tetra-\(p\)-coumaroylspermine and \(N^1,N^4,N^7,N^{10}\)-penta-\(p\)-coumaroyltetraethylenepentamine inhibited HIV-1 protease more potently than \(N^1,N^5,N^{10}\)-tri-\(p\)-coumaroylspermidine.

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*To whom correspondence should be addressed. e-mail: saibo421@ms.toyama-mpu.ac.jp © 2001 Pharmaceutical Society of Japan

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Table 1. Inhibitory Activity of Compounds Isolated from the EtOAc Extract of *A. caroufola* and Related Amides on HIV PR

<table>
<thead>
<tr>
<th>Compound</th>
<th>% Inhibition (100 μg/ml)</th>
<th>IC₅₀ (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>70.2 ± 1.4</td>
<td>53</td>
</tr>
<tr>
<td>2</td>
<td>30.1 ± 7.8</td>
<td>&gt;100</td>
</tr>
<tr>
<td>3</td>
<td>0.0 ± 3.2</td>
<td>&gt;100</td>
</tr>
<tr>
<td>4</td>
<td>2.5 ± 1.4</td>
<td>&gt;100</td>
</tr>
<tr>
<td>5</td>
<td>2.7 ± 0.6</td>
<td>&gt;100</td>
</tr>
<tr>
<td>6</td>
<td>4.0 ± 4.3</td>
<td>&gt;100</td>
</tr>
<tr>
<td>7</td>
<td>99.5 ± 0.8</td>
<td>27</td>
</tr>
<tr>
<td>8</td>
<td>97.3 ± 1.7</td>
<td>30</td>
</tr>
</tbody>
</table>

Experimental

Apparatus ¹H- and ¹³C-NMR spectra were measured with a Varian Gemini 300 (¹H, 300 MHz; ¹³C, 75 MHz) or Varian UNITY 500 (¹H, 500 MHz; ¹³C, 125 MHz) or JEOL JNM-LA 400WB-FT (¹H, 400 MHz; ¹³C, 100 MHz) spectrometer, the chemical shifts being represented as ppm with tetramethylsilane as an internal standard. ESI-MS spectra were measured with a Perkin-Elmer SCIEX API-III biomolecular mass analyzer. FAB-MS and high resolution (HR)-FAB-MS were measured with a JEOL JMS-700T mass spectrometer, and m-nitrobenzyl alcohol was used as a matrix.

Plant Material The aerial part of *Artemisia caroufola* Buch-Ham. ex Roos. was purchased from Yoaogaoyongzhang of Huhiot, Inner Mongolia of the People’s Republic of China in September of 1998. The plant material was identified by Dr. Katsuko Komatsu of the Analytical Research Center for Ethnomedicines, Institute of Natural Medicine, Toyama Medical and Pharmaceutical University. A voucher specimen (TMPW No. 19154) is stored at the Museum of Materia Medica, Toyama Medical and Pharmaceutical University, Japan.

Extraction and Isolation The aerial part of *A. caroufola* (3.0 kg) was extracted with MeOH under reflux (201×3, each 2 h). The combined MeOH solutions were evaporated to give a residue (190 g). The residue was suspended in water and extracted with CHCl₃, AcOEt, and BuOH to give the respective fractions in yields of 96, 11 and 21 g, together with the residual water-soluble fraction (62 g). The AcOEt-soluble fraction was chromatographed on an ODS column eluted with increasing amounts of MeOH in CHCl₃. (40% MeOH eluate, 5.1 g) showed no inhibitory activity against HIV-1 PR. Further repeated chromatography of the second eluate on silica gel with CHCl₃–MeOH (8:2) and Sephadex LH-20 with 20—100% MeOH afforded compounds 1—4 (30, 40, 30 and 50 mg, respectively).

N₃,N₃,N₅,N₅-Tri-p-coumaroylamipereidine (1): White powder, IR (KBr): νₑᵥₑᵣₑᵣₑ = 3300 (OH), 1660 (CONH–), 1610, 1580, 1520, 1450 (benezene ring), 1220, 1160, 980, 830, 520 cm⁻¹. Negative ESI-MS: m/z 582 ([M–1]¹, 70), 462 (50), 342 (45), 205 (30), 115 (100). Positive ESI-MS: m/z 607 (M⁺Na⁺), 584 ([M⁺H⁺]¹, 35), 438 (70), 294 (70), 147 (100). ¹H-NMR (500 MHz, CD₂OD): δ 1.60 (2H, m, H–8), 1.67 (2H, m, H–7), 1.85 (quin, J = 7.0 Hz) (H–9), 3.33 (4H, m, H–2, 9), 3.52 (4H, m, H–4, 6), 6.37 (d, J = 15.0 Hz) (H–6) (overlapped), 6.42 (d, J = 15.5 Hz) (H–8), 6.71 (d, J = 9.0 Hz) (H–7), 6.77 (overlapped) (H–6), 6.87 (overlapped) (H–3, 5), 7.19 (overlapped) (H–7, 5), 7.43 (overlapped) (H–5, 7), 7.47 (d, J = 15.5 Hz) (H–7), 7.50 (d, J = 15.5 Hz) (H–5), 7.60 (d, J = 15.5 Hz) (H–7). ¹³C-NMR (100 MHz, CD₂OD): δ 26.3 (C–7), 27.8 (C–8) (C–9), 28.9 (C–6), 37.9 (C–2), 39.9 (C–1, C–4), 45.7 (C–4), 47.6 (C–9), 114.9 (C–7), 116.8 (C–5, 3, 3′), 117.5 (C–5), 118.2 (C–8), 118.5 (C–8), 127.1 (C–1′, 1′′), 127.9 (C–1′′), 130.6 (C–2′, 6′, 2′′, 6′′), 130.9 (C–2′, 6′′), 141.8 (C–7′′, 7″), 144.4 (C–7), 145.9 (C–3′, 3′′), 160.3 (C–9′, 3′′), 160.9 (C–9, C–9′, C–9′′).

Methyl 3,5-Dicaffeoylquinate (2): White powder, positive ESI-MS m/z: 553 ([M⁺Na⁺]¹, 100), 385 (40). Negative ESI-MS m/z: 529 ([M–1]¹, 100), 367 (80), 179 (77). ¹H-NMR (500 MHz, CD₂OD): δ 2.14 (dd, J = 13.5, 8.5 Hz, Ha–2), 2.18 (dd, J = 13.5, 3.5 Hz, Ha–6), 2.29 (dd, J = 13.5, 6.5 Hz, Hb–6), 2.32 (dd, J = 13.5, 4.0 Hz, Hb–2), 3.68 (3H, –OCH₃), 3.98 (dd, J = 6.5, 3.0 Hz, H–9), 5.31 (m, H–5), 5.39 (dt, J = 8.5, 4.0 Hz, H–3), 6.21 (d, J = 16.0 Hz, H–8), 6.34 (d, J = 16.0 Hz, H–8), 6.77 (d, J = 8.5 Hz, H–5), 6.78 (d, J = 8.5 Hz, H–5), 6.85 (dd, J = 8.5, 2.5 Hz, H–6), 6.90 (dd, J = 8.5 Hz, H–5), 7.05 (d, J = 2.5 Hz, H–2), 7.54 (d, J = 16.0 Hz, H–7), 7.61 (d, J = 16.0 Hz, H–7), (*, **: Assignments may be exchangeable).

1,3-Dicaffeoylquinic Acid (3): White powder, positive ESI-MS m/z: 539 ([M⁺Na⁺]¹, 100), 413 (80), 385 (50). ¹H-NMR (400 MHz, CD₂OD): δ: 2.02 (dd, J = 13.9, 8.9 Hz, Ha–2), 2.38 (2H, m, H–6), 2.52 (dd, J = 13.9, 3.8 Hz, H–2), 3.74 (dd, J = 8.3, 3.4 Hz, H–4), 4.25 (q, J = 4.3 Hz, H–5), 5.34 (td, J = 8.3, 3.6 Hz, H–3), 6.24 (d, J = 16.0 Hz, H–9), 6.74 (2H, J = 8.3 Hz, H–5, 5′), 6.92 (2H, dd, J = 8.3, 2.0 Hz, H–6, 6′), 7.01 (2H, dd, J = 2.0 Hz, H–2, 2′), 7.54 (2H, dd, J = 16.3 Hz, H–7, 7′) (*, **: Assignments may be exchangeable).

3,5-Dicaffeoylquinic Acid (4): White powder, positive ESI-MS m/z: 539 ([M⁺Na⁺]¹, 100), 413 (70). ¹H-NMR (300 MHz, CD₂OD): δ: 2.26 (4H, m, H–2, 6), 4.01 (dd, J = 7.5, 3.3 Hz, H–4), 5.46 (2H, m, H–3, 5), 6.30 (d, J = 16.0 Hz, H–9), 6.36 (d, J = 16.0 Hz, H–8), 6.82 (2H, dd, J = 16.0 Hz, H–5, 5′), 7.01 (2H, dd, J = 1.5 Hz, H–2, 2′), 7.62 (dd, J = 16.0 Hz, H–7), 7.66 (dd, J = 16.0 Hz, H–7) (*, **: Assignments may be exchangeable).

Synthesis of Polyacoumaroylamines A mixture of E-p-coumaric acid (1.0 g) and Ac₂O (10 ml) in 10 ml of pyridine was stirred overnight at room
yield, positive FAB-MS: m/z 7.11 (5H, m), 7.58 (9H, m) (aromatic and olefinic protons). HR-FAB-MS: J 3.31 (4H, overlapped, H-2, 9), 3.58 (4H, m, H-4, 6), 6.52 (d, 6H, m, H-2, 2, 15). 1H-NMR (300 MHz, CD3OD): δ: 1.64 (4H, m, H-7, 8), 1.84 (4H, m, H-3, 12), 2.31 (12H, s, CH3CO–), 3.42 (4H, overlapped with solvent, H-2, 13), 3.51 (8H, m, H-4, 6, 9, 11), 6.48 (2H, m), 6.81 (2H, m), 7.08 (8H, m), 7.55 (12H, m) (H-ac-cou).

N1,N2,N10,N14-Tetra-p-acetylcoumaroylspermine (7a): White powder, 70% yield, positive ESI-MS: m/z 798 ([M + Na]+), 100, 955 ([M + H]+), 75. 1H-NMR (300 MHz, CDCl3–CD3OD 2:1): δ: 1.68 (4H, m, H-7, 8), 1.84 (4H, m, H-3, 12), 2.31 (12H, s, CH3CO–), 3.42 (4H, overlapped with solvent, H-2, 13), 3.51 (8H, m, H-4, 6, 9, 11), 6.48 (2H, m), 6.81 (2H, m), 7.08 (8H, m), 7.55 (12H, m) (H-ac-cou).

N1,N2,N10,N14-Tetra-p-acetylcoumaroylspermine (7a): White powder, 91% yield, negative ESIMS: m/z 785 ([M - H]-), 7. 1H-NMR (300 MHz, CD3OD): δ: 1.69 (4H, m, H-7, 8), 1.86 (4H, m, H-3, 12), 3.31 (4H, overlapped with solvent, H-2, 13), 3.54 (8H, m, H-4, 6, 9, 11), 6.40 (2H, m), 6.79 (10H, m), 7.40 (12H, m) (H-cou).

N1,N2,N10,N14,Tetra-p-acetylcoumaroylspermine (7a): White powder, 75% yield, positive FAB-MS: m/z 1130 ([M + Na]+), 25, 307 (13). 1H-NMR (300 MHz, CDCl3): δ: 2.30 (15H, s, CH3CO–), 3.5–3.8 (16H, m, H-2, 3, 5, 6, 8, 9, 11, 12), 6.3–6.5 (2H, m), 6.9–7.7 (28H, m) (H-ac-cou). HR-FAB-MS: m/z 1130.4347 (Calcd for C63H64N5O15 [M+H]+, 1130.4400).

N1,N2,N10,N14-Penta(p-acetylcoumaroyl)tetrathylenepentamine (8a): White powder, 51% yield, positive FAB-MS: m/z 920 ([M1+1]+), 7, 307 (15), 289 (10). 1H-NMR (300 MHz, CD3OD): δ: 3.4–3.9 (16H, m, H-2, 3, 5, 6, 8, 9, 11, 12, 6.2–6.5 (2H, m), 6.6–7.0 (13H, m), 7.2–7.6 (15H, m) (H-ac-cou). HR-FAB-MS: m/z 920.3834 (Calcd for C63H64N5O15 [M+H]+, 920.3872).

References