ANTICANDIDAL ACTIVITY OF ANTIBIOTIC A 19009 AND ITS ISOMER

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The antibiotic A 19009 i.e. N-(N³-fumaramoyl-L-2,3-diaminopropanoyl)-L-alanine (1) and its structural isomer (2) were synthesized and their antifungal activity in vitro against Candida albicans has been evaluated. The results demonstrate that these peptides inhibit the growth of C. albicans with minimum inhibitory concentrations ranging from 1.8 to 31 µg/ml.

Antibiotic A 19009 (1), originally isolated from a strain Streptomyces colinus Lindenbein¹), has been reported to be active against some fungi. However, no biological data concerning antifungal activity have been presented. Recently, van der Baan and coworkers²) have synthesized the antibiotic A 19009 (1) and its structural isomer (2) but little biological activity data were provided. According to their observations, dipeptide 1 showed a distinct activity against Trichomonas vaginalis, in contrast to compound 2 which had a very low activity.

In this paper we wish to report the anticandidal activity of both compounds 1 and 2. The general procedure used for the preparation of 1 and 2 is outlined in Scheme 1. N²-tert-Butoxycarbonyl-L-2,3-diaminopropanoic acid (3)³) was acylated with N-succinimidoyl ester (4) in H₂O in MeOH to afford N²-tert-butoxy carbonyl, N³-fumaramoyl-L-2,3-diaminopropanoic acid (5) in 82% yield, which was converted to its N-succinimidoyl active ester (6) with the aid of dicyclohexylcarbodiimide (DCC) and N-hydroxysuccinimide (HOSu)⁴). Coupling of this active ester 6 with L-alanine yielded the protected dipeptide 7 in 95% yield. Deprotection of the terminal amino function by means of 2 N HCl in dioxane, followed by purification using Dowex 1X2 (AcO⁻) anion exchange resin, furnished the antibiotic A 19009 (1) in 79% yield.

The dipeptide 2 was prepared by the similar reactions sequence. In this approach, N-tert-butoxy carbonyl-L-alanine N-succinimidoyl ester (8)⁵) was coupled with N³-benzyloxy carbonyl-L-2,3-diaminopropanoic acid (9)⁶) to give N³-(N-tert-butoxy carbonyl-L-alanyl)-N³-benzyloxy carbonyl-L-2,3-diaminopropanoic acid (10) in 96% yield. The resulting dipeptide 10 was hydrogenolyzed in the presence of 10% Pd-C catalyst, then acylated with N-succinimidoyl ester of fumaramic acid (4) to obtain the protected dipeptide 12 in 80% yield. Removal of the tert-butoxy carbonyl protecting group in 12 and purification of the final compound 2 was accomplished in the same way as described for the preparation of dipeptide 1. Compound 2 was obtained in 84% yield.

Both dipeptides, with N³-fumaramoyl-L-2,3-diaminopropanoyl residue in either the amino-terminal (1) or the carboxy-terminal position (2) show substantial antifungal activity against seven selected strains of C. albicans (Table 1). Antibiotic A 19009 (1) exhibited stronger antifungal activity than its isomer (2) against all C. albicans strains tested. However it is not clear why peptide 1 displayed stronger activity than 2. We assume that both peptides are transported into the cells by the same dipeptide permeases, but with different rates of peptide transport.

Table 1. In vitro activity of antibiotic A 19009 (1) and its structural isomer (2).

<table>
<thead>
<tr>
<th>Test organism</th>
<th>MIC (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Antibiotic A 19009 (1)</td>
</tr>
<tr>
<td>Candida albicans SR 30</td>
<td>7.5</td>
</tr>
<tr>
<td>C. albicans AMB 25</td>
<td>3.75</td>
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<tr>
<td>C. albicans ATCC 26278</td>
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</tr>
<tr>
<td>C. albicans 884</td>
<td>1.8</td>
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<tr>
<td>C. albicans 886</td>
<td>1.8</td>
</tr>
<tr>
<td>C. albicans clinical strain</td>
<td>1.8</td>
</tr>
<tr>
<td>C. albicans 4477</td>
<td>1.8</td>
</tr>
</tbody>
</table>
Experimental

Melting points are uncorrected. $^1$H NMR spectra were recorded at 80 MHz with a Tesla BS-487 spectrometer with hexamethyldisiloxane as an internal standard. Optical rotations were measured with a Hilger Watts (London) polarimeter. Purity of the synthetic compounds was confirmed by thin-layer chromatography using Kieselgel 60 F$_{254}$ plates (Merck).

$N$-Succinimidoyl Ester of Fumaramic Acid (4)

Fumaramic acid$^{6}$ (0.575 g, 5 mmol) and $N$-hydroxysuccinimide (0.575 g, 5 mmol) were dissolved in dry DMF (15 ml), cooled to 5°C and DCC (1.13 g, 5.5 mmol) was added. After 20 hours the urea was filtered off, and the filtrate evaporated to dryness leaving a crystalline residue, which was crystallized from THF - hexane to yield 4 (0.96 g, 91% yield), mp 164~166°C (dec).

$N$-tert-Butoxycarbonyl-$N$-fumaramoyl-L-2,3-diaminopropanoic Acid (5)

To a solution of $N$-tert-butoxycarbonyl-$N$-fumaramoyl-L-2,3-diaminopropanoic acid (3, 0.612 g, 3 mmol) and Et$_3$N (0.4 ml, 3 mmol) in H$_2$O (5 ml) and MeOH (10 ml), active ester 4 (0.636 g, 3 mmol) in MeOH (5 ml) was added with stirring at 0°C. After 4 hours, the solvents were evaporated to a small volume (5 ml) and the residue was passed through a column of Dowex 1×2 (AcO$^-$). The column was washed with 40% MeOH, then 1 N AcOH in 40% MeOH. Fractions containing 5 were collected, evaporated to dryness in vacuo, and crystallized from MeOH - diethyl ether yielding 5 (0.74 g, 82% yield) with mp 240~242°C (dec).

Anal Calcd for C$_{12}$H$_{19}$N$_3$O$_6$: C 45.28, H 3.80, N 13.20.

Found: C 45.15, H 3.75, N 13.05.

$N^2$-tert-Butoxycarbonyl-$N^3$-fumaramoyl-L-2,3-diaminopropanoic Acid (5)
N-Succinimidoyl Ester of N-tert-Butoxycarbonyl-N-fumaramoyl-L-2,3-diaminopropanoic Acid (6)

Protected amino acid 5 (0.602 g, 2 mmol) and N-hydroxysuccinimide (0.23 g, 2 mmol) were dissolved in dry DMF (10 ml) at 0°C, and DCC (0.453 g, 2 mmol) were dissolved in dry DMF (10 ml) at 0°C, and DCC (0.453 g, 2.2 mmol) was added with stirring. After 24 hours, the precipitate was filtered off, the filtrate evaporated to dryness in vacuo and the residue triturated with EtOAc to afford 6 (0.73 g, 91% yield) as an amorphous powder.

Anal Calcd for C_{16}H_{22}N_{4}O_{3}: C 48.23, H 5.57, N 14.06.
Found: C 48.02, H 5.48, N 13.85.

N-(N-tert-Butoxycarbonyl-L-alanyl)-N-fumaramoyl-L-2,3-diaminopropanoyl-L-alanine (7)

To a solution of L-alanine (0.107 g, 12 mmol) and Et$_3$N (0.16 ml, 1.2 mmol) in H$_2$O (5 ml) and MeOH (5 ml), 6 (0.438 g, 1.1 mmol) was added at 0°C. After stirring overnight the reaction mixture was concentrated to a volume of 5 ml and passed through a column of Dowex IX2 (AcO$^-$). The peptide 7 was purified as described for the corresponding peptide 5 to give 7 (0.39 g, 95% yield), mp 255~258°C (dec).

Anal Calcd for C$_{15}$H$_{24}$N$_{4}$O$_{7}$: C 48.37, H 6.49, N 15.05.

N-(N-fumaramoyl-L-2,3-diaminopropanoyl)-L-alanine (1)

Peptide 7 (0.25 g, 0.67 mmol) was treated with 2N HCl in dry dioxane (10 ml) for 2 hours at 0°C. Evaporation to dryness in vacuo and trituration with diethyl ether gave 1 as its hydrochloride (0.21 g, 97% yield) which was dissolved in a small volume of H$_2$O (3 ml) passed through a column of Dowex 1X2 (AcO$^-$). The solution was evaporated to dryness and crystallized from H$_2$O - MeOH to give 1 (0.144 g, 79% yield), mp 288~292°C (dec), $[\alpha]_D^{20} = -6.7^\circ$ (c 1.0, H$_2$O), $^1$H NMR (D$_2$O) $\delta$ 1.20 (d, 3H, CH$_3$), 3.40~3.60 (m, 2H, CH$_2$), 3.90~4.10 (m, 2H, 2 $\times$ CH), 6.65 (s, 2H, CH = CH).  

Anal Calcd for C$_{18}$H$_{26}$N$_{4}$O$_{5}$: C 44.11, H 5.92, N 20.58.
Found: C 43.95, H 5.82, N 20.45.

N$_2$-(tert-Butoxycarbonyl-L-alanyl)-N$_2$-fumaramoyl-L-2,3-diaminopropanoic Acid (10)

To a solution of N$_2$-benzoylcarbonyl-L-2,3-diaminopropanoic acid (9, 0.476 g, 2 mmol) and NaHCO$_3$ (0.168 g, 2 mmol) in H$_2$O (5 ml), N-tert-butoxycarbonyl-L-alanine N-succinimidoyl ester (8, 0.577 g, 2 mmol) dissolved in MeOH (5 ml) was added with stirring at 0°C. After being stirred overnight at room temp the solvent was removed in vacuo and the residue was dissolved in a small amount of H$_2$O (5 ml). The H$_2$O layer was acidified with 10% citric acid and extracted with EtOAc (50 ml). The EtOAc solution was washed with H$_2$O, dried over MgSO$_4$ and evaporated in vacuo. The residue was crystallized from EtOAc-hexane to yield 10 (0.785 g, 96% yield), mp 72~74°C.

Anal Calcd for C$_{19}$H$_{27}$N$_{4}$O$_{7}$: C 55.73, H 6.64, N 10.26.

N$_2$-(tert-Butoxycarbonyl-L-alanyl)-L-2,3-diaminopropanoic Acid (11)

A solution of 10 (0.614 g, 1.5 mmol) in MeOH (20 ml) was stirred with Pd-C 10% (50 mg) and hydrogenated at atmospheric pressure for 2 hours. The catalyst was filtered off, the filtrate evaporated in vacuo left a colorless solid, which was crystallized from MeOH - diethyl ether yielding 11 (0.43 g, 97% yield), mp 168~170°C (dec).

Anal Calcd for C$_{20}$H$_{24}$N$_{3}$O$_{5}$: C 47.98, H 7.68, N 15.26.
Found: C 47.65, H 7.70, N 15.20.

N$_2$-(tert-Butoxycarbonyl-L-alanyl)-N$_3$-fumaramoyl-L-2,3-diaminopropanoic Acid (12)

Peptide 11 (0.375 g, 1.36 mmol) was dissolved in H$_2$O (10 ml) with heating. The solution was cooled to room temp and Et$_3$N (0.2 ml, 1.5 mmol) was added. Then N-succinimidoyl ester of fumaric acid (0.29 g, 1.5 mmol) in MeOH (5 ml) was added with stirring and the solution was left to stand for 4 hours. The reaction mixture was concentrated to a volume of 5 ml and passed through a column of Dowex 1X2 (AcO$^-$). Compound 12 was purified as described for compound 5, evaporated to dryness yielding 12 as an amorphous powder (0.401 g, 80% yield).

Anal Calcd for C$_{23}$H$_{28}$N$_{5}$O$_{7}$: C 48.37, H 6.49, N 15.05.

N$_2$-L-Alanyl-N$_3$-fumaramoyl-L-2,3-diaminopropanoic Acid (2)

From the protected dipeptide 12 (0.3 g, 0.8
mmol), compound 2 (0.185 g, 84% yield) was obtained in the same way as described for 1, mp 292 ~ 295°C, $[\alpha]_D^{25} -3.7^o$ (c 0.25, H$_2$O). $^1$H NMR (D$_2$O) $\delta$ 1.25 (d, 3H, CH$_3$), 3.30 ~ 3.60 (m, 2H, CH$_2$), 3.80 (q, 1H, CH), 4.15 (m, 1H, CH), 6.67 (s, 2H, CH=CH).

**Anal Calcd for C$_{10}$H$_{16}$N$_1$O$_5$:**

- C 44.11, H 5.92, N 20.58.
- Found: C 44.01, H 5.84, N 20.42.

### Biological Assays

The minimum inhibitory concentrations (MIC) of antibiotic A 19009 (1) and its structural isomer (2) were determined on MA medium for strains of *C. albicans* using the previously described method$^1$.

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### References