# ANTICANDIDAL ACTIVITY OF ANTIBIOTIC A 19009 AND ITS ISOMER

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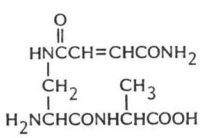
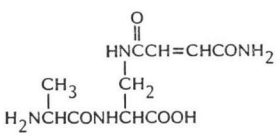
The antibiotic A 19009 *i.e.* *N*-($N^3$-fumaramoyl-L-2,3-diaminopropanoyl)-L-alanine (**1**) and its structural isomer (**2**) were synthesized and their antifungal activity *in vitro* against *Candida albicans* has been evaluated. The results demonstrate that these peptides inhibit the growth of *C.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

Antibiotic A 19009 (**1**), originally isolated from a strain *Streptomyces collinus* Lindenbein[1], has been reported to be active against some fungi. However, no biological data concerning anticandidal activity have been presented.



**1**



**2**

Recently, VAN DER BAAN and coworkers[2] 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 the both compounds **1** and **2**.

The general procedure used for the preparation of **1** and **2** is outlined in Scheme 1. $N^2$-*tert*-Butoxycarbonyl-L-2,3-diaminopropanoic acid (**3**)[3] was acylated with *N*-succinimidoyl ester of fumaramic acid (**4**) in a $H_2O$ - MeOH solution to afford $N^2$-*tert*-butoxycarbonyl, $N^3$-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.

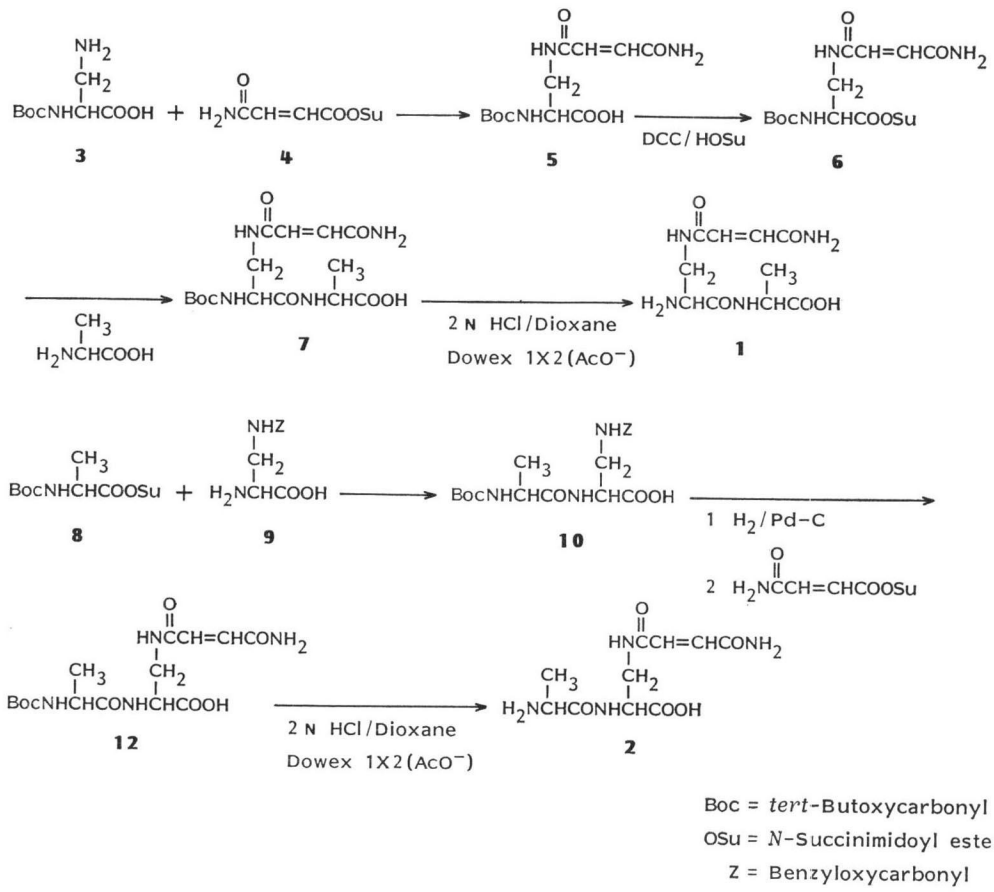
The dipeptide **2** was prepared by the similar reactions sequence. In this approach, *N*-*tert*-butoxycarbonyl-L-alanine *N*-succinimidoyl ester (**8**)[4] was coupled with $N^3$-benzyloxycarbonyl-L-2,3-diaminopropanoic acid (**9**)[5] to give $N^2$-(*N*-*tert*-butoxycarbonyl-L-alanyl)-$N^3$-benzyloxycarbonyl-L-2,3-diaminopropanoic 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*-butoxycarbonyl 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 $N^3$-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 anticandidal 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**).

| Test organism | MIC ($\mu$g/ml) | |
|---|---|---|
| | Antibiotic A 19009 (**1**) | Isomer of A 19009 (**2**) |
| *Candida albicans* SR 30 | 7.5 | 15 |
| *C. albicans* AMB 25 | 3.75 | 7.5 |
| *C. albicans* ATCC 26278 | 1.8 | 3.75 |
| *C. albicans* 884 | 1.8 | 3.75 |
| *C. albicans* 886 | 1.8 | 3.75 |
| *C. albicans* clinical strain | 1.8 | 31 |
| *C. albicans* 4477 | 1.8 | 15 |

Scheme 1.




Boc = *tert*-Butoxycarbonyl
OSu = *N*-Succinimidoyl ester
Z = Benzyloxycarbonyl

## 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).

*Anal* Calcd for $C_8H_8N_2O_5$:
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**)

To a solution of $N^3$-*tert*-butoxycarbonyl-L-2,3-diaminopropanoic acid (**3**, 0.612 g, 3 mmol) and $Et_3N$ (0.4 ml, 3 mmol) in $H_2O$ (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 1X2 ($AcO^-$). 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 $C_{12}H_{19}N_3O_6$:

C 47.83, H 6.36, N 13.95.
Found: C 47.58, H 6.32, N 13.82.

*N*-Succinimidoyl Ester of $N^2$-*tert*-Butoxycarbonyl-$N^3$-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 $C_{16}H_{22}N_4O_8$:
C 48.23, H 5.57, N 14.06.
Found: C 48.02, H 5.48, N 13.85.

*N*-($N^2$-*tert*-Butoxycarbonyl-$N^3$-fumaramoyl-L-2,3-diaminopropanoyl)-L-alanine (**7**)

To a solution of L-alanine (0.107 g, 1.2 mmol) and $Et_3N$ (0.16 ml, 1.2 mmol) in $H_2O$ (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 1X2 ($AcO^-$). 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 $C_{15}H_{24}N_4O_7$:
C 48.37, H 6.49, N 15.05.
Found: C 48.19, H 6.42, N 14.85.

*N*-($N^3$-Fumaramoyl-L-2,3-diaminopropanoyl)-L-alanine (**1**)

Peptide **7** (0.25 g, 0.67 mmol) was treated with 2 N 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 $H_2O$ (3 ml) passed through a column of Dowex 1X2 ($AcO^-$) and eluted with $H_2O$. Fractions containing **1** were collected, evaporated to dryness and crystallized from $H_2O$ - MeOH to give **1** (0.144 g, 79% yield), mp 288~292°C (dec), $[\alpha]_D^{25}$ −6.7° (*c* 1.0, $H_2O$), $^1H$ NMR ($D_2O$) $\delta$ 1.20 (d, 3H, $CH_3$), 3.40~360 (m, 2H, $CH_2$), 3.90~4.10 (m, 2H, 2×CH), 6.65 (s, 2H, CH=CH).

*Anal* Calcd for $C_{10}H_{16}N_4O_5$:
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)-$N^3$-benzyloxycarbonyl-L-2,3-diaminopropanoic Acid (**10**)

To a solution of $N^3$-benzyloxycarbonyl-L-2,3-diaminopropanoic acid (**9**, 0.476 g, 2 mmol) and $NaHCO_3$ (0.168 g, 2 mmol) in $H_2O$ (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 $H_2O$ (5 ml). The $H_2O$ layer was acidified with 10% citric acid and extracted with EtOAc (50 ml). The EtOAc solution was washed with $H_2O$, 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 $C_{19}H_{27}N_3O_7$:
C 55.73, H 6.64, N 10.26.
Found: C 55.52, H 6.52, N 10.14.

$N^2$-(*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 $C_{11}H_{21}N_3O_5$:
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 $H_2O$ (10 ml) with heating. The solution was cooled to room temp and $Et_3N$ (0.2 ml, 1.5 mmol) was added. Then *N*-succinimidoyl ester of fumaramic 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 $C_{15}H_{24}N_4O_7$:
C 48.37, H 6.49, N 15.05.
Found: C 48.22, H 6.41, N 14.97.

$N^2$-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° (*c* 0.25, $H_2O$), $^1H$ NMR ($D_2O$) $\delta$ 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 $C_{10}H_{16}N_4O_5$:
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[7].

## Acknowledgment

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