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# Two Cyclic Peptides Produced by the Endophytic Fungus # 2221 from *Castaniopsis fissa* on the South China Sea Coast

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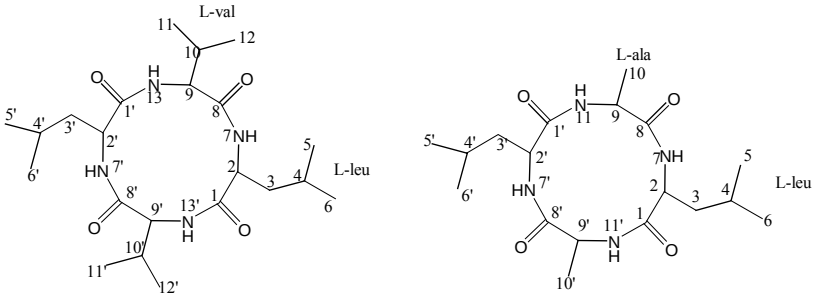
**Abstract:** New cyclic peptides **1** and **2** were isolated from the endophytic fungus #2221 from *Castaniopsis fissa* on the south China sea coast. By 2D NMR methods and chiral HPLC technique, their structures were elucidated as cyclo (L-Val-L-Leu-L-Val-L-Leu) and cyclo(L-Leu-L-Ala-L-Leu-L-Ala), respectively.

**Keywords:** Endophytic fungus, cyclic peptides, metabolite.

Cyclic peptides have a very wide distribution in the nature, existing in plants, animals, microorganisms, bacteria and fungi. Many of them have potent bioactivity[1].

Recently, we have embarked on a study of the metabolites of marine fungi from the south China sea and have isolated a number of interesting compounds[2-4]. We have reported three new cyclotetrapeptides from the mangrove fungus #2516[5]. As a part of our

**Figure 1** The structure of compound **1** and **2**




**1** **2**

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continuous studies, we investigated the EtOAc extract of the culture filtrate of the fungus #2221. Two cyclotetrapeptides, **1** and **2**, have been isolated and their structures were elucidated by analysis of spectroscopic data and chiral HPLC experiments. **1** is a new compound, whil **2** was a known one.

A 100 L culture filtrate was concentrated and extracted with EtOAc. The extract was repeatedly chromatographed on silica gel columns. Compound **1** was obtained as a white solid, subliming at 230℃. It had the molecular formula $C_{22}H_{40}N_4O_4$, as established by FABMS at *m/z* 425$[M+H]^+$. In the infrared spectrum, the bands at 3193 $cm^{-1}$ and 1663 $cm^{-1}$ were characteristic absorptions of amide NH and amide carbonyl groups, respectively. The $^1H$ and $^{13}C$-NMR spectra (**Table 1**) showed the signals of four methine groups ($\delta_c$ 61.0, 53.9, 45.2, and 32.7, $\delta_H$ 3.77, 3.93, 1.95 and 2.26), one methylene, four methyl groups, two amide protons ($\delta_H$ 7.00 and 7.16) and two amide carbonyl groups ($\delta_C$ 170.0 and 173.8). The elemental analysis ([found: C 62.34, H 9.52,N 13.08, calcd. for $C_{11}H_{22}N_2O_2$ C 62.26, H 9.43, N 13.21]) and NMR spectroscopic data indicated that **1** should be two times of the mass of $C_{11}H_{20}N_2O_2$. The above evidence suggested that compound **1** was a cyclic tetrapeptide. The individual amino acid residues were established to be two valine and two leucine residues by analysis of $^1H$-$^1H$COSY and the linkages of these amino acid residues were confirmed by the HMBC correlations between each amide NH and amide C=O of the neighboring amino acid. In the HMBC spectrum, the correlations between $\delta_C$170.0 ($Leu_{CO}$) and $\delta_H$3.93 ($Leu_{CH}$), $\delta_H$7.16 ($Val_{NH}$), $\delta_C$173.8 ($Val_{CO}$) and $\delta_H$3.77 ($Val_{CH}$), $\delta_H$ 7.00 (-$Leu_{NH}$) suggested the presence of two peptide fragments (–Val-Leu-), only one linkage is possible that is to form cyclo (-Val-Leu-Val-Leu-). The absolute configuration of the leucine and valine residues of compound **1** was determined through comparing their retention times of the hydrolytic products of **1** with the standard amino acids in the chiral HPLC experiment. The results showed that their retention times are identical to those of the standard sample L-leucine and L-valine respectively (**Table 2**), then we concluded that they were the L-formed amino acids.

**Table 1** The NMR data of compound **1** (acetone-$d_6$, TMS δ ppm)

| | $^{13}C$ | $^1H$ | $^1H$-$^1H$COSY | HMBC |
|---|---|---|---|---|
| Leu | | | | |
| 1 | 170.0(C) | | | H-2, 3, 13′ |
| 2 | 53.9(CH) | 3.93 (m) | H-3, 7 | H-3, 4, 7 |
| 3 | 24.8($CH_2$) | 1.78(ddd, 14.0, 8.5, 5.0 Hz) | H-2, 4 | H-2, 4, 5, 6 |
| | | 1.60(ddd, 14.0, 8.5, 5.5 Hz) | | |
| 4 | 45.2(CH) | 1.95 (m) | H-3, 5, 6 | H-2, 3, 5, 6 |
| 5 | 23.4($CH_3$) | 0.95 (d, 6.5 Hz) | H-4 | H-3, 4, 6 |
| 6 | 21.9($CH_3$) | 0.93 (d, 6.5 Hz) | H-4 | H-3, 4, 5 |
| 7 | NH | 7.00 (brs) | H-2 | |
| Val | | | | |
| 8 | 173.8(C) | | | H-7, 9 |
| 9 | 61.0(CH) | 3.77(dd, 4.0, 3.0 Hz) | H-10, 13 | H-10, 11, 12, 13 |
| 10 | 32.7(CH) | 2.26 (dq, 7.0, 4.0 Hz) | H-9, 11, 12 | H-9, 11, 12 |
| 11 | 19.1($CH_3$) | 1.06(d, 7.0 Hz) | H-10 | H-10, 12 |
| 12 | 17.6($CH_3$) | 0.96(d, 7.0 Hz) | H-10 | H-10, 11 |
| 13 | NH | 7.16 (brs) | H-9 | |

**Table 2** The chiral HPLC retention time of the hydrolytic samples of **1** and the standard amino acids

| Sample | DL-Val | L-Val | DL-Leu | L-Leu | Hydrolytic sample of **1** |
|---|---|---|---|---|---|
| Retention | 2.994(D) | | 6.596(D) | | 3.377 |
| time (min) | 3.322(L) | 3.240 | 11.135(L) | 10.609 | 11.382 |

**Table 3** The NMR data of compound **2** (DMSO-$d_6$, TMS δ ppm)

| | $^{13}$C | $^{1}$H | $^{1}$H-$^{1}$HCOSY | HMBC |
|---|---|---|---|---|
| Leu | | | | |
| 1 | 174.9(C) | | | H-2, 3, 11′ |
| 2 | 59.1(CH) | 3.77(ddd, 8.0, 5.0 Hz) | H-3, 7 | H-3, 4, 7 |
| 3 | 49.1($CH_2$) | 1.62 (ddd, 13.5, 8.5, 5.0, 2.5 Hz) | H-2, 4 | H-2, 4, 5, 6 |
| | | 1.48 (ddd, 13.5, 8.0, 5.5 Hz) | | |
| 4 | 30.1(CH) | 1.82(ddq, 8.5, 6.5, 5.5 Hz) | H-3, 5, 6 | H-2, 3, 5, 6 |
| 5 | 29.5($CH_3$) | 0.89 (d, 6.5 Hz) | H-4 | H-3, 4, 6 |
| 6 | 28.4($CH_3$) | 0.87 (d, 6.5 Hz) | H-4 | H-3, 4, 5 |
| 7 | NH | 8.04 (brs) | H-2 | |
| Ala | | | | |
| 8 | 175.4(C) | | | H-7, 9, 10 |
| | 56.4(CH) | 3.86(dq, 6.5, 1.0 Hz) | H-10, 11 | H-10, 11 |
| 10 | 26.1($CH_3$) | 1.28(d, 6.5 Hz) | H-9 | H-9 |
| 11 | NH | 8.05 (brs) | H-9 | |

**Table 4** The chiral HPLC retention time of the hydrolytic samples of **2** and standard amino acids

| Sample | DL-Ala | L-Ala | DL-Leu | L-Leu | Hydrolysis sample of **2** |
|---|---|---|---|---|---|
| Retention | 2.087(D) | | 6.596(D) | | 3.092 |
| time (min) | 2.900(L) | 2.819 | 11.135(L) | 10.609 | 10.919 |

Compound **2** was obtained as a white solid. Its molecular formula $C_{18}H_{32}N_4O_4$( Ω =5) was derived from the combination of FABMS at *m/z* 369[M+H]$^+$, $^{13}$C-NMR and DEPT spectra. The $^{1}$H and $^{13}$C-NMR spectra (**Table 3**) exhibited the signals of four methine, one methylene, three methyl groups, two amide protons and two amide carbonyl groups, respectively. The composition was $C_9H_{16}N_2O_2$, which was just a half molecule according to the FABMS at *m/z* 369[M+H]$^+$. The above data suggested that compound **2** was also a cyclic tetrapeptide, which consists of two amino acid residues linked alternatively with two others such as compound **1**. In the HMBC spectrum, the correlative signals between δ175.4($Ala_{CO}$) and δ3.86 ($Ala_{CH}$), δ8.04 (-$Leu_{NH}$), and between δ174.9 ($Leu_{CO}$) and δ3.77 (-$Leu_{CH}$), δ8.05 ($Ala_{NH}$) showed the sequence of individual amino acid residues. Thus, the structure of compound **2** was identified as cyclo (-Leu-Ala-Leu-Ala-). The chiral HPLC analysis of the hydrolytic products of **2** showed that alanine and leucine residues of compound **2** were all in the L configuration (**Table 4**).

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