# Isolation and Structure of an Antimitotic Cyclic Peptide, Ustiloxin F: Chemical Interrelation with a Homologous Peptide, Ustiloxin B

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Ustiloxin F, a microtubule inhibitor, was isolated as a minor metabolite of *Ustilaginoidea virens*. The structure was determined from the spectral data and by chemical interrelation to ustiloxin B through reductive removal of the sulfoxide-containing side chain of ustiloxin B to give ustiloxin F. Ustiloxin F inhibited microtubule assembly with an $IC_{50}$ value of 10.3 $\mu$M.

Ustiloxins A~D (**1**~**4**), which are strong inhibitors of microtubule assembly, have been isolated from the water extract of false smut balls on rice panicles caused by the fungus *Ustilaginoidea virens*,[1~3] and their structures were determined to be as shown in Figure 1.[4] During our extensive search for unknown active principles in the water extract of false smut balls, ustiloxin F (**5**) was isolated as a new minor component. The structure of ustiloxin F was determined by spectroscopic analyses and by chemical interrelation with ustiloxin B (**2**).

This paper deals with isolation, structure determination and anti-tubulin activity of ustiloxin F (**5**), as well as the chemical conversions of ustiloxin A (**1**) to D (**4**) and ustiloxin B (**2**) to F (**5**) through a reductive cleavage of the sulfoxide-containing side chain on the aromatic ring.

## Results and Discussion

The isolation procedure for ustiloxin F (**5**) is shown in Figure 2. The water extract of false smut balls was subjected to ODS column chromatography, and fractions containing ustiloxins B, D and F were combined. Ustiloxin F (**5**) was purified from the combined fraction by successive column chromatographies using Diaion CHP 20P, silica gel, ODS, and finally ODS-HPLC. The overall yield of **5** was less than 0.5 mg starting from 500 g of false smut balls.

The molecular formula ($C_{21}H_{30}N_4O_8$) of **5** determined by FAB-MS and HRFAB-MS was $C_5H_9NO_4S$ less than **2** and $C_2H_4$ less than **4** (Table 1). Its $^1H$ and $^{13}C$ NMR data[6] suggested that compound **5** lacks the side chain on the aromatic ring, like ustiloxin D (**4**), and has the same cyclic peptide skeleton as ustiloxin B (**2**) (Tables 2 and 3). Combining these spectroscopic data, the structure of **5** was elucidated and was proved by direct conversion of ustiloxin B (**2**) to **5** *via* reductive removal of the sulfoxide-containing side chain of **2** by $PtO_2$ treatment in water.

In order to optimize the reaction conditions for cleavage of the sulfur-containing side chain, conversion of ustiloxin A (**1**), the most abundant homolog of this family, to ustiloxin D (**4**) was attempted under a variety of reduction conditions. The optimal result so far attained involved stirring a solution of **1** in $H_2O$ for 3 hours in the presence of $PtO_2$ (15-fold molar excess) under atmospheric pressure of $H_2$ (Figure 3). The yield of **4** was 58 to 67% with 14% recovery of **1**. In acetic acid with $PtO_2$, the yield of **4** after 5 hours was only 25 to 40% with the recovery of 30% of **1**. In ethanol with $PtO_2$, the initially formed **4** was *N*-ethylated by acetaldehyde generated *in situ* to give *N*-ethylustiloxin D.[5] In the case of the reaction with Raney Ni, **4** could not be isolated.

Based on the above result, hydrogenolysis of **2** was

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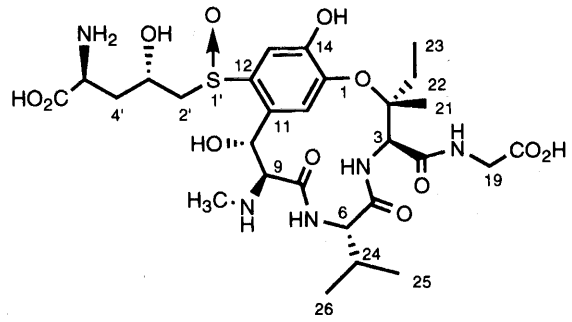
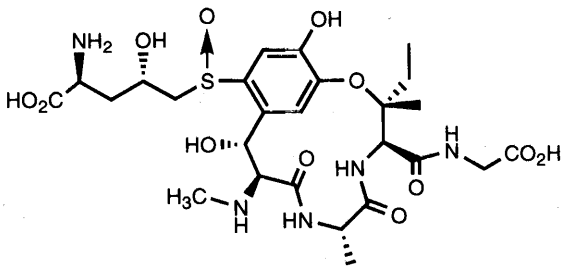
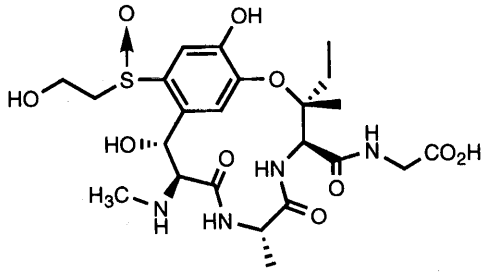
Fig. 1. Structures of ustiloxins.



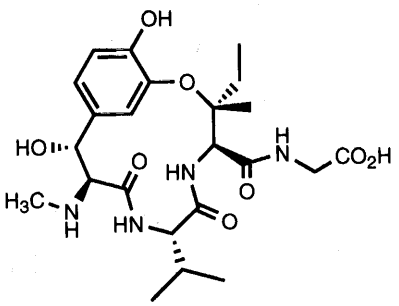
Ustiloxin A (**1**)[1,2]



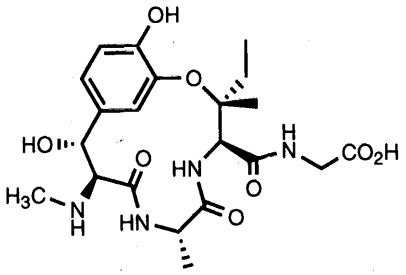
Ustiloxin B (**2**)[2]



Ustiloxin C (**3**)[2]



Ustiloxin D (**4**)[2]



Ustiloxin F (**5**)

carried out with $PtO_2$ ($\times$ 15 molar) in $H_2O$ for 4 hours to give 44% of **5** along with 22% recovery of **2**. The synthetic **5** obtained from **2** was identical with natural **5** in its $^1H$ NMR, FAB-MS and HPLC retention time. The signals in the NMR spectra of **4** and **5** were fully assigned with the aid of $^1H$, $^{13}C$, COSY (**4** only), C-H COSY and HMBC experiments (Tables 2 and 3).[6] Isopropyl signals of **4** at positions 24, 25 and 26 appear at 1.80, 0.71, 0.80 ppm ($\delta_H$) and 31.6, 21.3, 20.8 ppm ($\delta_C$), respectively, while the methyl signal of **5** at position 24 appears at 1.05 ppm ($\delta_H$) and 15.8 ppm ($\delta_C$).

The inhibitory activity of **5** against tubulin polymerization was evaluated. The $IC_{50}$ value, *i.e.*, the concentration required for 50% inhibition of polymerization, was determined to be 10.3 $\mu$M (Figure 4).

The $IC_{50}$ values of ustiloxin A (**1**), B (**2**), D (**4**) and

Fig. 2. Isolation of ustiloxin F from water extract of false smut balls.

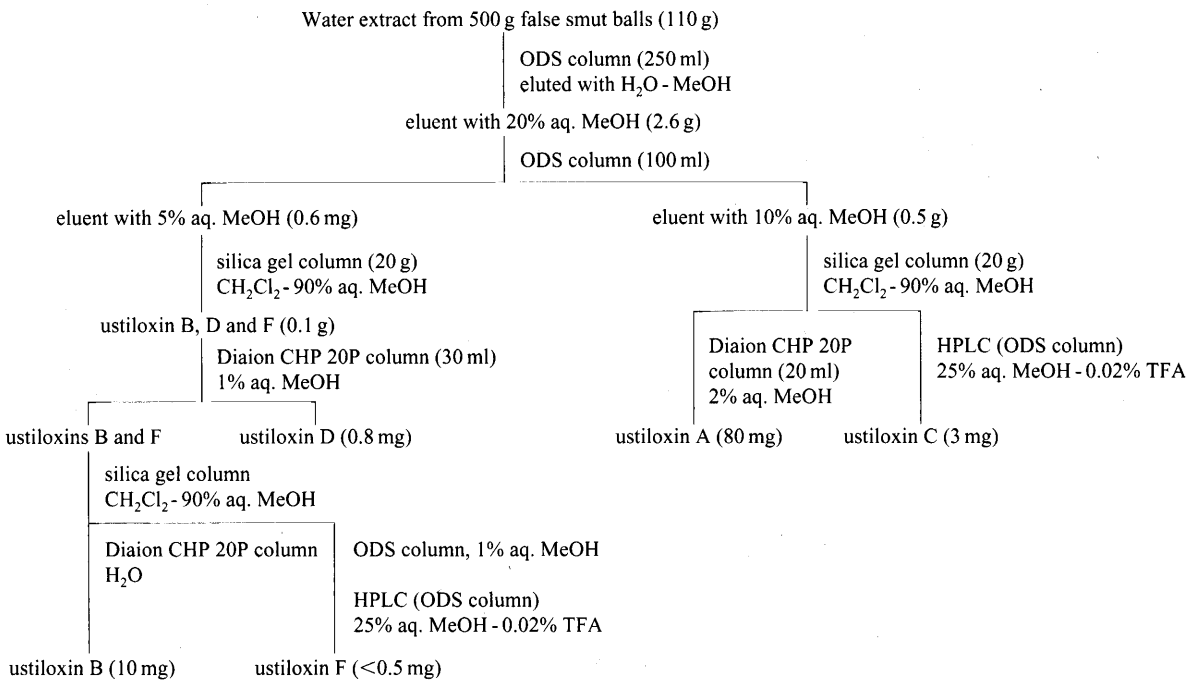
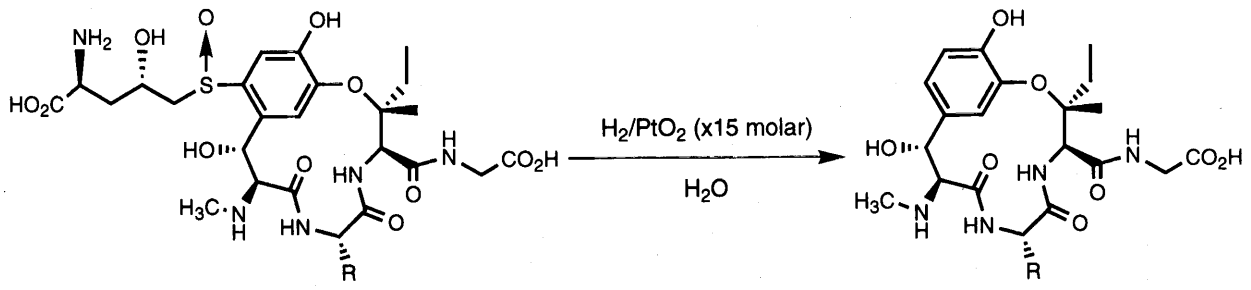



Table 1. Physico-chemical properties of ustiloxins B (**2**), D (**4**) and F (**5**).

| | Ustiloxin B[2)] | Ustiloxin D | Ustiloxin F |
|---|---|---|---|
| Molecular formula | $C_{26}H_{39}N_5O_{12}S$ | $C_{23}H_{34}N_4O_8$ | $C_{21}H_{30}N_4O_8$ |
| Appearance | Colorless powder | Colorless powder | Colorless powder |
| UV $\lambda_{max}^{H_2O}$ nm ($\varepsilon$) | 252 (5,000), 290 (2,500) | 227 (6,200), 280 (1,600) | 227 (4,500), 280 (1,100) |
| FAB-MS (*m/z*) (M+H) | 646 | 495 | 467 |
| HRFAB-MS (*m/z*) (M+H) | | | |
| Calcd | 646.2394 | 495.2455 | 467.2142 |
| Found | 646.2390 | 495.2401 | 467.2186 |
| $[\alpha]_D$ | +14.1° (*c* 0.5, $H_2O$) | −48.8° (*c* 0.5, $H_2O$) | −68.0° (*c* 0.5, $H_2O$) |

Fig. 3. Hydrogenolysis of ustiloxin A (**1**) and ustiloxin B (**2**).




Ustiloxin A (**1**) R=CH(CH$_3$)$_2$
Ustiloxin B (**2**) R=CH$_3$

Ustiloxin D (**4**) R=CH(CH$_3$)$_2$
Ustiloxin F (**5**) R=CH$_3$

Table 2. $^1$H NMR chemical shifts ($\delta_H$, ppm relative to water: $\delta_H$ 4.65 ppm), multiplicities,[a] and coupling constants ($J$, Hz) of ustiloxins B (**2**), D (**4**) and F (**5**) and FGHMBC correlation of **4** and **5** in $D_2O$.

| H No. | 2[2] | 4 | | 5 | |
|---|---|---|---|---|---|
| | $^1$H NMR | $^1$H NMR | FGHMBC (C No.) | $^1$H NMR | FGHMBC (C No.) |
| 3 | 4.55 s | 4.81 s | 2, 17 | 4.58 s | 2, 5, 17, 21 |
| 6 | 4.27 q, 7.0 | 3.96 d, 10.0 | 5, 8, 24, 25, 26 | 4.24 q, 7.0 | 5, 8, 24 |
| 9 | 4.00 d, 10.0 | 3.34 d, 9.0 | 8, 10, N-$CH_3$ | 3.26 d, 9.0 | 8, 10, 11, N-$CH_3$ |
| 10 | 4.70 d, 10.0 | 4.50 d, 9.0 | 9, 11, 12, 16 | 4.51 d, 9.0 | 8, 9, 11, 12, 16 |
| 12 | — | 7.09 dd, 9.0, 2.0 | 10, 14, 16 | 7.04 dd, 8.3, 2.0 | 10, 14, 16 |
| 13 | 7.37 s | 6.98 d, 9.0 | 11, 14, 15 | 6.89 d, 8.3 | 11, 14, 15 |
| 16 | 7.18 s | 6.89 d, 2.0 | 10, 12, 14, 15 | 7.07 br s | 12, 14, 15 |
| 19 | 3.60 d, 17.0 | 3.61 s | 17, 20 | 3.66 d, 17.0 | 17, 20 |
| | 3.76 d, 17.0 | | | 3.71 d, 17.0 | 17, 20 |
| 21 | 1.58 s | 1.63 s | 2, 3, 22 | 1.54 s | 2, 3, 22 |
| 22a | 1.53 dq, 14.0, 7.2 | 1.65 dq, 15.0, 7.5 | 23 | 1.58 m[b] | 2, 3, 21, 23 |
| 22b | 1.95 dq, 14.0, 7.2 | 2.08 dq, 15.0, 7.5 | 2, 3, 23 | 1.91 dq, 14.0, 7.0 | 2, 3, 21, 23 |
| 23 | 0.82 t, 7.2 | 1.04 t, 7.5 | 2, 22 | 0.85 t 7.0 | 2, 22 |
| 24 | 1.04 d, 7.0 | 1.80 m | | 1.05 d, 7.0 | 5, 6 |
| 25 | — | 0.71 d, 6.5 | 6, 24, 26 | — | |
| 26 | — | 0.80 d, 6.5 | 6, 24, 25 | — | |
| N-$CH_3$ | 2.50 s | 2.34 s | 9 | 2.28 s | 9 |
| 2′a | 2.86 dd, 13.4, 2.8 | — | | — | |
| 2′b | 3.20 dd, 13.4, 10.0 | — | | — | |
| 3′ | 4.19 dddd, 2.8, 3.0, | — | | — | |
| | 10.0, 10.0 | | | | |
| 4′a | 1.91 ddd, 3.0, 8.0, 15.0 | — | | — | |
| 4′b | 2.02 ddd, 4.0, 10.0, 15.0 | — | | — | |
| 5′ | 3.80 dd, 4.0, 8.0 | — | | — | |

[a] Multiplicities, s: singlet, d: doublet, t: triplet, q: quartet, m: multiplet.
[b] Overlapped with other signals.

F (**5**) were compared under identical experimental conditions, and were obtained as 1.0, 1.8, 2.5 and 10.3 $\mu$M, respectively. These values are somewhat different from those reported previously[2], and this may be partly due to inevitable slight differences in the experimental conditions and also in the purities of the samples used.

These values indicate that ustiloxin F, the smallest homolog of ustiloxins, retains the essential activity, but the functional groups of the side chains and the alkyl moiety on the cyclic skeleton enhance the binding affinity to tubulin.

## Experimental

### General

$^1$H and $^{13}$C NMR spectra were measured in $H_2O$ on a JEOL ALPHA-500 NMR spectrometer at 500 and 125 MHz, respectively. FAB-MS, HRFAB-MS and CAD spectra were measured on a JEOL JMS-HX110 instrument with the EBE arrangement. Optical rotations were measured on a JASCO DIP-1000 digital polarimeter. UV spectra were measured on a Shimadzu apparatus, model UV-2500. Thin layer chromatography was carried out on Merck Kieselgel 60F-254 plates, and HPLC was performed on a Shimadzu LC-10A apparatus.

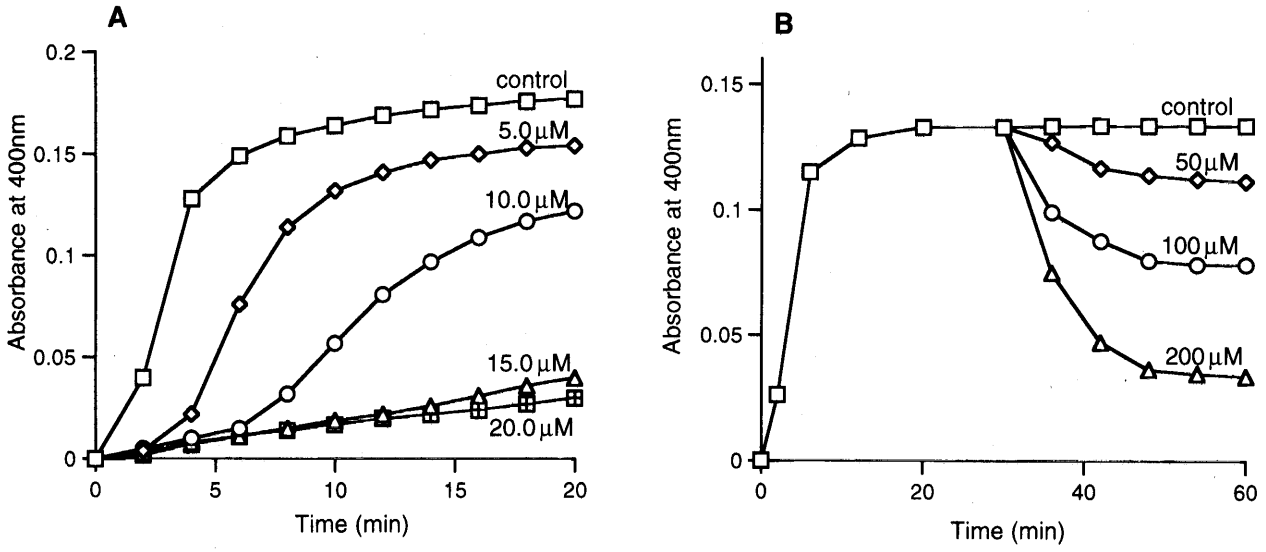
### Preparation of Microtubule Protein and Microtubule Assembly Assay

The procedures were the same as described in the previous papers.[2,3]

### Conversion of Ustiloxin A (**1**) to Ustiloxin D (**4**)

A mixture of 100 mg of compound **1** and $PtO_2$ (506 mg, × 15 molar) in water (10 ml) was stirred for 3 hours under an atmosphere of $H_2$. The catalyst dissolved during the reaction. After removal of the water from the black solution with a rotary evaporator at *ca.* 50°C, the products were extracted with 20 ml of acetic acid, with the aid of ultrasonication. The catalyst in the solution was separated by centrifugation, and the acetic acid was removed with a rotary evaporator. Compound **4** was purified by CHP 20P column chromatography (elution

Fig. 4. Effect of ustiloxin F on the polymerization and depolymerization of microtubule proteins.




(A) Various concentrations of ustiloxin F were mixed with microtubule proteins (1.5 mg/ml) at 0°C and incubated at 37°C.

(B) Microtubule proteins (1.5 mg/ml) were incubated at 37°C. Various concentrations of ustiloxin F were added 30 minutes later.

Table 3. $^{13}C$ NMR chemical shifts ($\delta_C$, ppm relative to dioxane $\delta_C$ 67.5 ppm) of ustiloxins B (**2**), D (**4**) and F (**5**) in $D_2O$.

| C No. | 2[2] | 4[6] | 5 |
|---|---|---|---|
| 2 | 84.5 | 88.7 | 86.2 |
| 3 | 60.3 | 62.3 | 60.3 |
| 5 | 172.7 | 173.8 | 173.1 |
| 6 | 50.0 | 62.8 | 49.7 |
| 8 | 169.4 | 168.9 | 171.9 |
| 9 | 68.2 | 71.7 | 71.1 |
| 10 | 74.7 | 75.9 | 74.4 |
| 11 | 129.0 | 133.3 | 132.5 |
| 12 | 137.1 | 125.3 | 123.2 |
| 13 | 114.4 | 121.7 | 118.8 |
| 14 | 153.2 | 153.6 | 150.7 |
| 15 | 146.3 | 145.5 | 142.7 |
| 16 | 124.6 | 126.4 | 124.0 |
| 17 | 170.6 | 173.5 | 170.9 |
| 19 | 44.2 | 46.6 | 44.2 |
| 20 | 176.9 | 179.0 | 176.9 |
| 21 | 22.2 | 24.0 | 22.0 |
| 22 | 31.7 | 35.2 | 31.6 |
| 23 | 8.4 | 10.7 | 8.4 |
| 24 | 15.7 | 31.6 | 15.8 |
| 25 | — | 21.3 | — |
| 26 | — | 20.8 | — |
| N-$CH_3$ | 33.0 | 35.2 | 33.6 |
| 2′ | 65.1 | — | — |
| 3′ | 64.2 | — | — |
| 4′ | 37.1 | — | — |
| 5′ | 53.2 | — | — |
| 6′ | 174.8 | — | — |

with 20% aqueous MeOH). **4**: 40~47 mg (54~64%). Recovery of **1**: 14%.

Conversion of Ustiloxin B (**2**) to Ustiloxin F (**5**)

Compound **2** was transformed to **5** by the same procedure as used in the synthesis of **4** from **1**. The reaction rate of **2** to **5** was slower, and after 4 hours of reaction, compound **5** was obtained in 44% yield (32 mg) and the recovery of **2** was 22%. Compound **5** was purified by CHP 20P column chromatography (elution with water).

## References

1) Koiso, Y.; M. Natori, S. Iwasaki, S. Sato, R. Sonoda, Y. Fujita, H. Yaegashi & Z. Sato: Ustiloxin: A phytotoxin and a mycotoxin from false smut balls on rice panicles. Tetrahedron Letters 33: 4157~4160, 1992

2) Koiso, Y.; Y. Li, S. Iwasaki, K. Hanaoka, T. Kobayashi, R. Sonoda, Y. Fujita, H. Yaegashi & Z. Sato: Ustiloxins, antimitotic cyclic peptides from false smut balls on rice panicles caused by *Ustilaginoidea virens*. J. Antibiotics 47: 765~773, 1994

3) Li, Y.; Y. Koiso, H. Kobayashi, Y. Hashimoto & S. Iwasaki: Ustiloxins, new antimitotic cyclic peptides: Interaction with porcine brain tubulin. Biochem. Pharmacol. 49: 1367~1372, 1995

4) Ustiloxin E was assumed to be a degradation product of **1**. Its structure has not been determined (see ref. 2).

5) Schwoegler, E. J. & H. Adkins: Preparation of certain amines. J. Am. Chem. Soc. 61: 3499~3502, 1939

6) $^{13}C$ NMR data previously reported for ustiloxin D (**4**)[2] were revised.