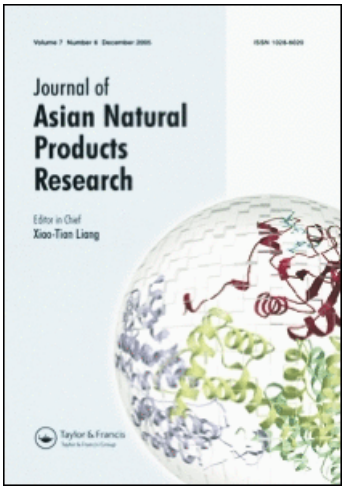


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# CYTOCHALASIN D FROM *HYPOCRELLA BAMBUSAE*

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Cytochalasin D which shows marked cytotoxic effects on multi-tumor cells was newly isolated at high content(5.28 mg/g, dry weight) from Fungus *Hypocrella bambusae*(*B.et Br.*) *Sacc.* Its structure was elucidated by spectroscopic methods. Two-dimensional NMR techniques were applied to make complete assignment for the $^{1}$H- and $^{13}$C-NMR chemical shifts of this compound.

*Keywords*: Cytochalasin D; *Hypocrella bambusae*; Cytotoxic effect; Structure elucidation

## INTRODUCTION

Cytochalasin D, first isolated and characterized by Aldridge and Turner [1] as one of a group of toxic fungal metabolites from Fungus *Metarrhizium anisopliae* and *Hypoxylon terricola*, showed significant cytostatic effects on mammalian cells in tissue culture. During our studies on cytotoxic constituents from Fungi, the ethanolic extract of *Hypocrella bambusae* (*B.et Br.*) *Sacc* was found to be very toxic towards 4 tumor cell lines, namely A549, A2780, KB and HCT-8. Column chromatography of the ethanolic extract with petroleum ether and ethyl acetate as eluants gave six fractions (GX-3-1 to GX-3-6). Further purification of bioactive fractions GX-3-1 and GX-3-2 led to the isolation of cytochalasin D as the major active constituent

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TABLE I Effect of different fractions of *Hypocrella bambusae*(*B.et Br.*) *Sacc* on the proliferation of tumor cells

| *Sample* | *A2780* $EC_{50}$ (*ug/ml*) | *HCT-8* $EC_{50}$ (*ug/ml*) |
|---|---|---|
| GX-3-1 | 0.936 | 0.445 |
| GX-3-2 | 0.357 | 0.0740 |
| GX-3-3 | 1.70 | 5.38 |
| GX-3-4 | > 50 | 22.70 |
| GX-3-5 | 5.15 | 20.16 |
| GX-3-6 | 5.698 | 16.75 |

TABLE II Effect of cytochalasin D on the proliferation of tumor cells

| *Cell lines* | $EC_{50}$ (*ug/ml*) |
|---|---|
| A549 | 0.062 |
| KB | 0.18 |
| HCT-8 | 0.018 |

(5.28 mg/per gram of fruitbodies) (Tabs. I and II). The structure of this compound was determined by spectral methods. Furthermore, the 2D NMR ($^1$H–$^1$H COSY, $^{13}$C–$^1$H COSY, HMBC) data enable us to make complete assignments for $^1$H- and $^{13}$C-NMR chemical shifts of this compound.

## RESULTS AND DISCUSSION

Compound GX-4-3 was obtained as a white powder (mp 266–268°C). $[\alpha]_D^{25}$ – 7.3 (c, 0.55 in dioxan). The molecular formula $C_{30}H_{37}NO_6$ of this compound was derived from the data of FAB-MS (*m/z* 508[M+H]$^+$) and its $^1$H, $^{13}$C (DEPT) NMR. It can be reasoned that it possesses a tricyclic skeleton after deduction of 10 double-bond equivalents (DBEs), attributed to 1 phenyl, 3 carbonyl groups and 3 pair of double bonds, from 13 DBEs of this molecule.

Its IR spectrum showed the presence of hydroxyl (3419, 3241 cm$^{-1}$), carbonyl (1741, 1702, 1691 cm$^{-1}$) and double bond (1458, 962, 908 cm$^{-1}$) groups. The peak at *m/z* 490 ([M$^+$ + H-18]) also revealed the presence of at least one hydroxyl group. In $^{13}$C-NMR and DEPT spectra, 4 primary, 3 secondary, 16 tertiary and 7 quaternary carbons (Tab. III) were observed. Together with its $^1$H NMR, two *trans*-disubstituted (H-13/H-14 and H-19/H-20) and 1 exocyclic ($H_2$-12, and C-6/C-12) double bonds, 1 phenyl, 1 ketonic (C-17) and 1 acetoxy, and 1 amido groups were identified. Some substructures (C-10/C-3 to C-5, C-7/C-8/C-13 to C-17 and C-19 to

TABLE III $^{1}H$ (500 MHz), $^{13}C$ (125 MHz) NMR and 2D NMR data for cytochalasin D

| *Proton* | *δ ppm. mult., J(Hz), int.* | *$^{1}H$-$^{1}HCOSY$* | *Observedlong-range correlations(HMBC-spectrum)* | *Carbon* | *C-H(δ c) $CDCl_3$ solution* | *C-H(δ c) pyridine solution* |
|---|---|---|---|---|---|---|
| | | | | C-1 | 173.64 | 175.23 |
| H-3 α | 3.22, m, 4.5, 4.0, 1H | H-10, H-4 | | C-3 | 53.53 | 54.51 |
| H-4 | 2.84, m, 5, 2, 1H | H-3 α, H-5 β | C-5, C-3, C-9, C-1 | C-4 | 46.96 | 48.02 |
| H-5 β | 2.14, t, 5, 1H | H-4, H-3 α | C-6 | C-5 | 49.96 | 50.26 |
| | | | | C-6 | 147.46 | 151.76 |
| H-7 α | 3.80, d, 10.5, 1H | H-8 β | C-8, C-6, C-12, C-14 | C-7 | 69.80 | 71.39 |
| H-8 β | 2.83, m, 10.5, 5, 2, 1H | H-7 α, H-14, H-13 | C-7, C-13, C-14, C-9 | C-8 | 32.63 | 33.31 |
| | | | | C-9 | 53.24 | 54.14 |
| H-10 α | 2.65, dd, 13.5, 9.5, 1H | H-10 β, H-3 α | C-3, C-4 | C-10 | 45.28 | 45.66 |
| H-10 β | 2.83, m, 13.5, 10, 2.5, 1H | H-10 α, H-3 α | | | | |
| H-11 | 0.95, d, 7, 3H | H-5 | C-5, C-6 | C-11 | 13.63 | 13.75 |
| H-12 | 5.09, s, 5.29, s, 2H | | C-6, C-7, C-5 | C-12 | 114.50 | 112.30 |
| H-13 | 5.35, m, 15.5, 10, 5, 1H | H-14, H-8 β | C-7, C-8 | C-13 | 134.11 | 133.97 |
| H-14 | 5.65, dd, 15.5, 10, 1H | H-13, H-15 | C-15 | C-14 | 130.59 | 132.35 |
| H-15 α | 2.02, dd, 13.5, 5, 1H | H-15 β, H-14 | C-14, C-13, C-16, C-17 | C-15 | 37.70 | 38.70 |
| H-15 β | 2.51, dd, 13.5, 11, 1H | H-15 α, H-16 β | | | | |
| H-16 β | 2.73, m, 11, 4.5, 1H | H-15 β, H-22 | C-22 | C-16 | 42.29 | 42.60 |
| | | | | C-17 | 210.23 | 211.02 |
| | | | | C-18 | 77.66 | 78.51 |
| H-19 | 5.15, dd, 16, 2.5, 1H | H-20, H-21 | C-20, C-18 | C-19 | 127.08 | 126.95 |
| H-20 | 6.11, dd, 16, 2.5, 1H | H-19, H-21 | C-21, C-19 | C-20 | 132.26 | 132.91 |
| H-21 α | 5.63, dd, 2.5, 1H | H-20, H-19 | C-20, C-19, C-9, C-4, O=C(OAc) | C-21 | 77.26 | 78.15 |
| H-22 | 1.20, d, 7, 3H | H-16 β | C-16, C-17, C-15 | C-22 | 19.39 | 19.49 |
| H-23 | 1.51, s, 3H | | C-18, C-19, C-17 | C-23 | 24.16 | 24.75 |
| Ph-H | 7.25, m, 15, 7, 1.5, 5H | | C-10 | C-1′ | 137.21 | 138.60 |
| | | | | C-2′, C-6′ | 129.06 | 130.04 |
| | | | | C-3′, C-5′ | 128.92 | 128.94 |
| | | | | C-4′ | 127.57 | 127,98 |
| OAc | 2,26, s, 3H | | | $OCOCH_3$ | 169.69 | 170.58 |
| | | | | $OCOCH_3$ | 20.84 | 20.65 |
| NH | 5.53, brs, 1H | | | | | |

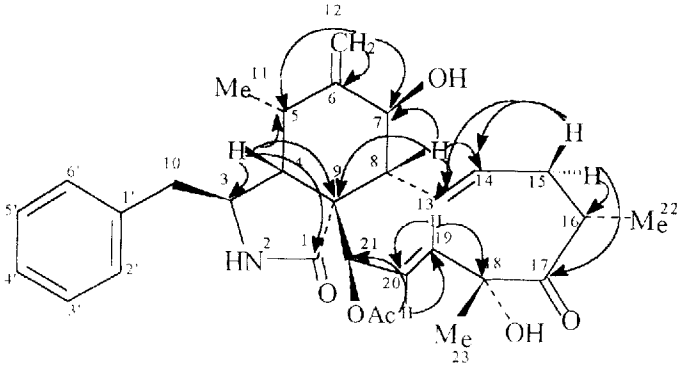

FIGURE 1 The structure of cytochalasin D.

C-21/C-9) can be constructed on the basis of cross peaks in $^{1}H-^{1}H$ and $^{13}C-^{1}H$ COSY experiments.

In HMBC spectrum some crucial crosspeaks were observed, which enable us to combine the above mentioned substructures and other parts into a complete molecular structure (Fig. 1). These crosspeaks include $\delta$ 2.14 t (H-5)/147.46 (C-6); 3.80 d (H-7)/C-6 and 114.50 (C-12) suggesting the connection of C-5 to C-6 to C-7; 1.51 s (Me-23)/77.66 (C-18); 210.23 (C-17) and 127.08 (C-19) in agreement with the connection of C-17 to C-18 to C-19; and both $\delta$ 5.63 dd (H-21) and 2.84 m (H-4)/53.24 (C-9) leading us to the connection of C-4 to C-9. Other meaningful connections based on HMBC data were illustrated in Figure 1 with arrows.

A detailed comparison of the spectral data of $^{13}C$-NMR (pyridine) [2] for this compound and cytochalasin D showed that they have the common signals. So the structure of compound GX-4-3 was confirmed as cytochalasin D (Zygosporin A).

Finally, complete assignments for $^{1}H$-and $^{13}C$-NMR chemical shifts of this compound were realized (Tab. III).

## EXPERIMENTAL SECTION

### General Experimental Procedures

NMR spectra were measured on Varian INOVA-500 spectrometer using TMS as internal standard; IR spectra were recorded on Nicolet-Impact 400 spectrometer; FAB-MS was taken by AutoSpec-Ultima mass spectrometer; Melting point was determined on XT4-100X apparatus and is uncorrected.

Silica gel (100 – 140 mesh) was used for column chromatography(CC), TLC plates were prepared with precoated si gel GF254, and spots visualized by spraying with 10%$H_2SO_4$ in water.

**Plant Material**

The fruiting body of *Hypocrella bambusae*(*B. Et Br.*) *Sacc* was collected from Li Jiang, Yunnan province of China in November, 1997, and identified by Prof. Y. H. Chen of Department of Botany of our Institute.

**Extraction and Isolation**

The dried and pulverized fruiting bodies (250 g) of *Hypocrella bambusae* (*B.et Br.*)*Sacc* were extracted with hot 95% EtOH. The ethanolic extract was concentrated under reduced pressure to give a black residue (64 g), 22 g of which was chromatographed on a silica gel column (400 g) with gradient petroleum ether-EtOAc elution. The eluent was monitored by TLC and combined to give 6 fractions. Fraction 1 (GX-3-1) and fraction 2 (GX-3-2) were combined (1.59 g) and chromatographed on a silica gel column (100 g) with gradient elution $CH_2Cl_2$-MeOH. The eluent was combined to give 8 fractions. Compound GX-4-3(220 mg) was obtained from fraction 3($CH_2Cl_2$-MeOH, 100 : 3) and were purified by re-crytallization from $CH_2Cl_2$.



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