J Chin Med 13(4): 209-219, 2002

CYCLODESIPEPTIDE AND DIOXOMORPHOLINE DERIVATIVES ISOLATED FROM THE INSECT-BODY PORTION OF THE FUNGUS *CORDYCEPS CICADAE*

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(*Received 19th September 2002, revised Ms received 4th October 2002, accepted 11th October 2002*)

Seven compounds have been isolated from the methanol extract of the insect-body portions of Chan-hua, and namely ergosterol (**1**), ergosterol peroxide (**2**), bassiatin (**3**), bassiatin A (**4**), beauvericin (**5**), beauvericin A (**6**), and beauvericin B (**7**). The structures of **1**, **2** and **7** were determined by spectral analyses and by comparison with existing data from literatures. Compounds **3**, **4**, **5** and **6** were elucidated by spectral analysis and further confirmed by X-ray crystallography. Among them, three cyclodesipeptides (**5**, **6** and **7**) and two dioxomorpholines (**3**, **4**) derivatives were not previously reported from Chan-hua. Bassiatin A is first isolated from nature.

Key words: *Cordyceps cicadae*, Fungi, Sterol, Cyclodesipeptide, Dioxomorpholine.

INTRODUCTION

*Cordyceps cicada*e Shing is a parasitic fungus on the larvae of *Cicada flammata* Dist. Both the ascocarps and the insect-body portions are named Chan-hua, and have been used as a drug for childhood convulsion, palpitation, and sedation.[1] In the previous work, there have been a considerable number of papers on the antitumor activity of various polysaccharides in Chan-hua.[2-7] However, there has been a relative scarcity of papers on the immuno-pharmacological activity of the ascocarps and the insect-body portion of Chan-hua. In the course of our search for physiologically active substances in nature, we found that methanol extract of the insect-body portion of Chan-hua

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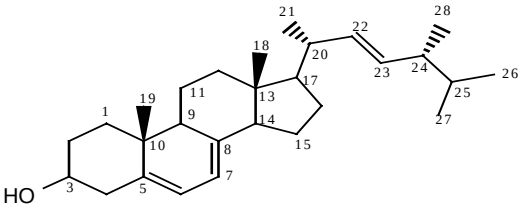
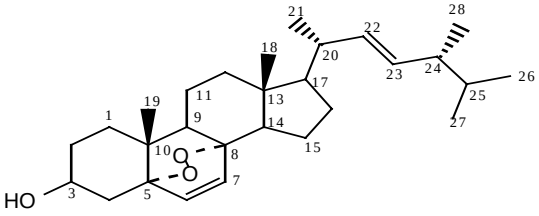
suppressed proliferation in human mononuclear cells (HMNC) activated by phytohemagglutinin (PHA). The inhibitory activity of the extract was concentration dependent. The IC_{50} of the extract on PHA-treated HMNC was 32.5 µg/ml. Similarly, interleukin-2 (IL-2) in activated HMNC was also inhibition by the treatment of the extract. The inhibition of IL-2 was concentration dependent.[8] This observation led us to investigate the potential biologically active substances of Chan-hua. In the present paper, we describe the extraction, isolation, purification and identification of three cyclodesipeptide and two dioxo-morpholine derivatives. They were not previously reported from this fungus, namely one novel substance, bassiatin A (**4**), as well as two known sterols.

RESULTS AND DISCUSSION

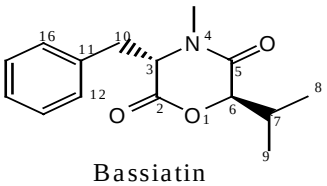
The methanol extract of the insect-body portions of *C. cicadae* was partitioned between water and ethyl acetate. Column chromatography of the ethyl acetate soluble fraction gave seven compounds. They are ergosterol (**1**),[9] ergosterol peroxide (**2**),[10,11] bassiatin (**3**),[12] bassiatin A (**4**), beauvericin (**5**),[13] beauvericin A (**6**)[13] and beauvericin B (**7**).[13] The structures of these compounds, except **4**, were identified by the spectral analyses and by comparison with existing data from the literatures. Compounds **3**, **5** and **6** were further confirmed by X-raystallographic analysis. The novel substance **4**, was elucidated by the spectral analyses and confirmed by X-ray crystallographic analysis. ORTEP



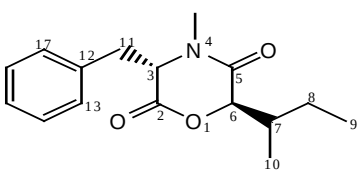
Ergosterol



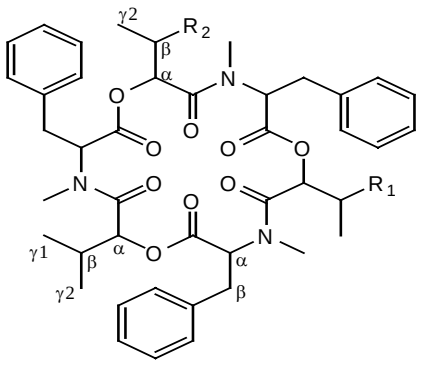
Ergosterol peroxide



Bassiatin

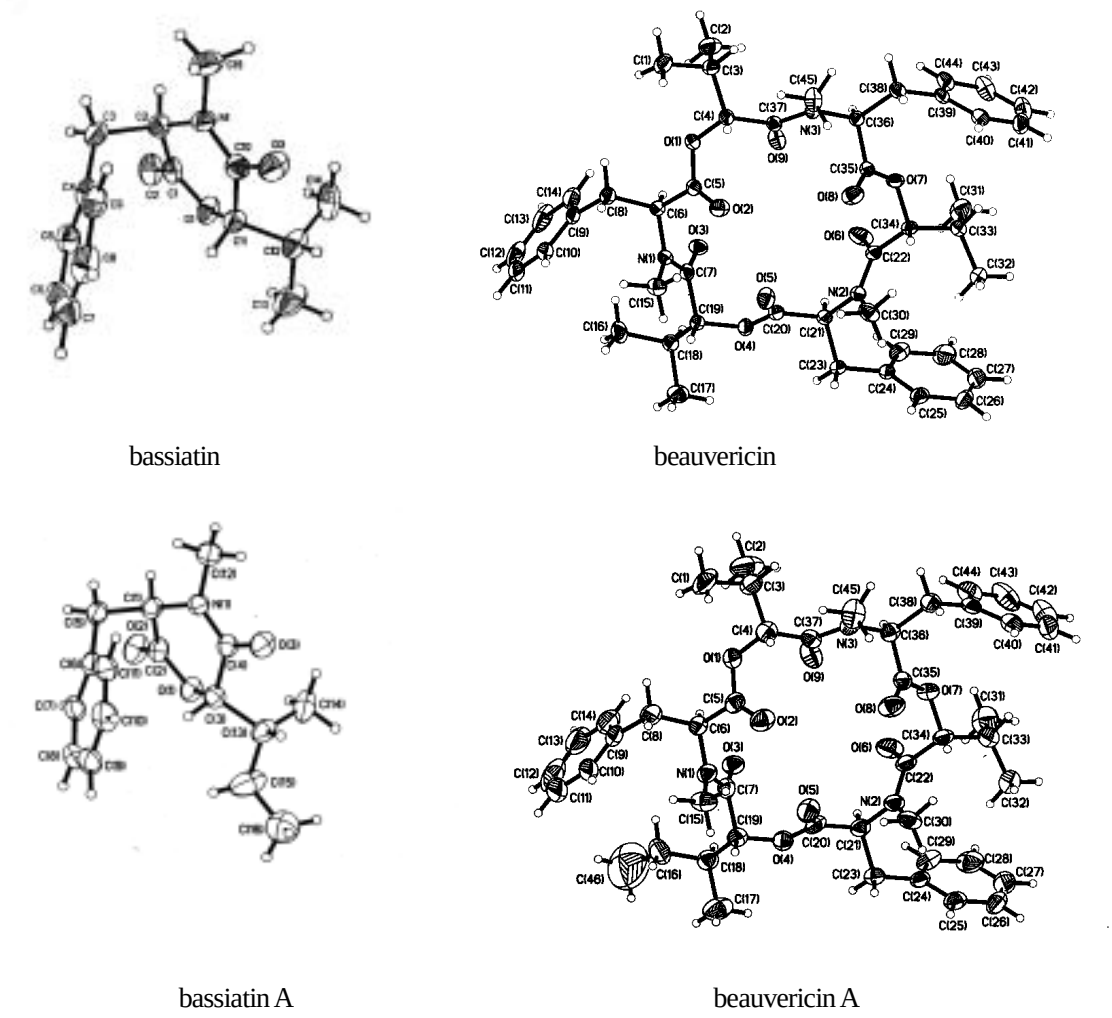


Bassiatin A



Beauvericin, $R_1=R_2=CH_3$
Beauvericin A, $R_1=CH_3$, $R_2=CH_2(\gamma 1)CH_3(\delta)$
Beauvericin B, $R_1=R_2=CH_2CH_3$

Fig. 1. Structures isolated from Cordyceps cicadae



bassiatin beauvericin

bassiatin A beauvericin A

Fig. 2. Perspective structures of bassiatin (3) ,bassiatin A (4) ,beauvericin (5), and beauvericin A (6) [atom numbering of this figure does not correspond to the line drawing of structures]

drawing of **3**, **4**, **5** and **6** were shown in Fig. 2.

Compound **4** was obtained as colorless crystals. The molecular formula of **4** assigned as $C_{16}H_{21}NO_3$ by ^{13}C NMR and DEPT, as well as EI-mass spectrum. As cited in Table 1, the ^{13}C NMR and DEPT shown two carbonyl carbons at δ 167.3 and 165.9, suggesting that these two carbons were connected oxygen and/or nitrogen. The ^{1}H NMR shown the signals of an oxymethine proton at δ 3.06 (*d*, *J* = 1.9 Hz), a sp^3 methine proton at δ 4.39 (*t*, *J* = 4.5 Hz) and one *N*-methyl proton at δ 3.03 (s). On the basis of above data, **4** suggested having a skeleton of *N*-methyl-dioxomorpholine. Five aromatic protons at δ 7.28-7.31 (3H, *m*), 7.09 - 7.13 (2H, *m*) were correlated to the carbon signals at δ 129.2, 128.2 and 129.8, respectively, in its C-H COSY spectrum, and one quaternary carbon at δ 134.1 was observed in ^{13}C NMR and DEPT, indicating the presence of a non-substitute benzyl group. Furthermore, the proton signals at δ 3.06 (1H), 1.98 (1H), 1.20 (2H), 0.65 (3H) and 0.71 (3H) were correlated to the carbon signals at

Table 1. ^{13}C and ^{1}H NMR spectral data of 3 and 4 in $CDCl_3$ [a]

Compound					
3			**4**		
Atom	δC	δH	Atom	δC	δH
2	167.2 (*s*)		2	167.3 (*s*)	
3	62.7 (*d*)	4.38 (*t* , *J* , 43 Hz)	3	62.7 (*d*)	4.39 (*t* , *J*, 4.5 Hz)
5	165.5 (*s*)		5	165.9 (*s*)	
6	81.2 (*d*)	2.99 (*d* , *J*, 2.1 Hz)	6	79.0 (*d*)	3.06 (*d* , *J*, 1.9 Hz)
7	29.6 (*d*)	2.28 (*m*)	7	36.1 (*d*)	1.98 (*m*)
8	18.5 (*q*)	0.82 (*d* , *J*, 7.0 Hz)	8	25.4 (*t*)	1.20 (*m*)
9	15.1 (*q*)	0.74 (*d* , *J*, 6.8 Hz)	9	11.5 (*q*)	0.65 (*t*, *J*, 7.3)
10	37.1 (*t*)	3.26 (*dd* , *J*, 4.1, 14.0 Hz)	10	13.3 (*q*)	0.71 (*d*, *J*, 6.9 Hz)
		3.16 (*dd* , *J*, 4.4, 14.0 Hz)	11	37.0 (*t*)	3.14 (*dd*, *J*, 4.6 , 13.9 Hz)
11	134.1 (*s*)				3.26 (*dd*, *J*, 4.0 , 13.9 Hz)
12, 16	129.7 (*d*)	7.09 ~ 7.12 (*m*)	12	134.1 (*s*)	
13, 15	129.2 (*d*)	7.29 ~ 7.34 (*m*)	13, 17	129.8 (*d*)	7.09 ~ 7.13 (*m*)
14	128.2 (*d*)	7.29 ~ 7.34 (*m*)	14,1 6	129.2 (*d*)	7.28 ~ 7.31 (*m*)
N-CH_3	32.4(*q*)	3.00 (*s*)	15	128.2 (*d*)	7.28 ~ 7.31 (*m*)
			N-CH_3	32.4 (*q*)	3.03 (*s*)

[a] Assignments were based on ^{13}C-DEPT, ^{13}C-^{1}H COSY and ^{1}H-^{1}H COSY spectra.

δ 79.0, 36.1, 25.4, 11.5 and 13.3, respectively, in its C-H COSY spectrum. These data together with two spin coupling systems, HC-6/HC-7/H_2C-8/H_3C-9 and HC-7/H_3C-10, were obtained in the ^{1}H-^{1}H COSY spectrum, indicating the presence of a 1-methylpropyl moiety in the structure of **4**. Comparison of the ^{1}H and ^{13}C spectra data of **4** with those of **3**, except the 1-methylethyl moiety is re-placed by the 1-methylpropyl moiety located at C-6 position, were shown similarities. On the basis of above analyses, the structure of **4** was elucidated as 4-methyl-6-(1-methylpropyl)-3-phenylmethyl-1,4-perhydrooxazine-2,5-dione.

The relative stereochemistry of **4** was determined by X-ray crystallographic analysis. A single crystal having an approximate dimension of 0.40 × 0.25 × 0.10 mm was obtained from methanol-water system and used for X-ray craystallographic analysis. An ORTEP drawing of the molecular structure of **4** is shown in Fig. 2. It indicated that the relative stereochemistry at C-3 and C-6 could be either S/R or R/S. Comparing the optical rotations of **4** ($[\alpha]_D$ + 187.5° (c 0.038, dichloromethane)) with that of bassiatin [(3S, 6R)-4-methyl-6-(1-methylethyl)-3-phenylmethyl-1,4-perhydrooxazine-2,5-dione] ($[\alpha]_D$ + 181.05° (c 0.024, chloroform)) and other three isomer - (3R, 6S)-, (3R, 6R)-, (3S, 6S)-compounds. [12] The structure of **4** was determined to be (3S, 6R)-4-methyl-6-(1-methylpropyl)-3-phenylmethyl-1,4-perhy-drooxazine-2,5-dione, and named as bassiatin A.

In the ^{1}H NMR spectrum of **4**, the signal at δ 3.06 assigned to H-6 is unusually in high field as an oxymethine proton neighboring to the 5-carbonyl group. This phenomenon is similar to the proton signal displayed at δ 2.99 of H-6 of bassiatin.[2] Therefore, judging from the conformation of **4**, the unusual chemical shift of H-6 can be attributed to the strong shielding effect from the benzyl group at C-3.

EXPERIMENTAL

General Experiment Procedures

Melting points were determined with a Yanaco MP-13 micro-melting point apparatus and are uncorrected. IR spectra were obtained as KBr pellets on a Nicolet Avatar 320 FT-IR spectrometer. Optical rotations were measured on a JASCO DIP-370 polarimeter. HRFABMS spectra were recorded in the positive ion mode on a JEOL JMS-HX 110 spectrometer. EIMS spectra were recorded on a Finnigan GCQ GC/MS spectrometer. ^{1}H, ^{13}C-, and 2D-NMR spectra were recorded on a Bruker ACP-300 spectrometer and a Varian INOVA 500MHz NMR spectrometer. Chemical shifts are shown in δ values (ppm) with deuterated solvents as internal standard. Column chromatography was performed on silica gel 60 (70 ~ 230 mesh, Merck) using a solvent mixture system of ethyl acetate and hexane. HPLC was performed on a preparative reverse phase column and eluted with water-methanol system.

Materials

Cordyceps cicadae (Chan-hua) was purchased from a Chinese medicinal store in Taipei, and identified by Dr. Tun-Tschu Chang of the Division of Forest Protection, Taiwan Forestry Research Institute. A voucher specimen (No. TFRIA46) was deposited in the herbarium of Taiwan Forestry Research Institute, Taipei, Taiwan.

Extraction and Isolation

The dried Chan-hua (fungus: *C. cicadae*) was divided into two portions (the ascocarps and the insect-body). The insect-body of Chan-hua (9.7 Kg) was extracted with methanol at 60 °C over night for three times. The extracts were filtered and vacuum evaporated. The concentrate (1.5 Kg) was suspended in 3L distilled water and partitioned between ethyl acetate and water (1:1 v/v) to give ethyl acetate, water and suspended fractions. Ethyl acetate soluble fraction (535 g) was carried out on silica gel column chromatography with a gradient of ethyl acetate in hexane, and fourteen fractions (1-14) were collected. The fractions were collected in 450 mL portions and pooled according to their TLC profile in hexane-ethyl acetate (90:10, or 80:20 v/v). Fraction 5 was dissolved in methanol and gave crystalline ergosterol (**1**). Fraction 6 was further purified by silica gel MPLC (ethyl acetate gradient in hexane) to give ergosterol peroxide (**2**). After chromatographic separation of fraction 8 on silica gel MPLC (ethyl acetate gradient in hexane), sub-fraction (8-4) was further purified by preparative HPLC with Cosmosil $5C_{18}$-AR-II column (20 × 250 mm) using a gradient of methanol in water system to give bassiatin (**3**) and bassiatin A (**4**). Fraction 10 was separated and purified as above treatment to afford beauvericin (**5**), beauvericin A (**6**) and beauvericin B (**7**).

Ergosterol (3ß-hydroxyergosta-5, 7, 22-triene) (1) [9]

Colorless plates; mp 150-152°C; IR (KBr) ν max cm^{-1} 3420, 2880, 1650, 1460, 1390, 1380, 1060, 1040; ^1H NMR ($CDCl_3$): δ 3.62 (1H, *m*, H-3), 5.55 (1H, *m*, H-6), 5.35 (1H, *m*, H-7), 0.61 (3H, *s*, C$\underline{H}_3$-18), 0.92 (3H, *s*, C$\underline{H}_3$-19), 1.01 (3H, *d*, *J* = 6.6Hz, C$\underline{H}_3$-21), 5.18 (1H, *m*, H-22), 5.18 (1H, *m*, H-23), 0.78 (3H, *d*, *J* = 6.8Hz, C$\underline{H}_3$-26), 0.82 (3H, *d*, *J* = 6.8Hz, C$\underline{H}_3$-27), 0.90 (3H, *d*, *J* = 6.8Hz, C$\underline{H}_3$-28); ^{13}C NMR ($CDCl_3$): δ 38.4 (*t*, C-1), 32.0 (*t*, C-2), 70.5 (*d*, C-3), 40.8 (*t*, C-4), 141.3 (*s*, C-5), 119.6 (*d*, C-6), 116.3 (*d*, C-7), 139.8 (*s*, C-8), 46.3 (*d*, C-9), 37.0 (*s*, C-10), 21.1 (*t*, C-11), 39.1 (*t*, C-12), 42.8 (*s*, C-13), 54.6 (*d*, C-14), 23.0 (*t*, C-15), 28.7 (*t*, C-16), 55.7 (*d*, C17), 12.0 (*q*, C-18), 16.3 (*q*, C-19), 40.4 (*d*, C-20), 19.6 (*q*, C-21), 132.0 (*d*, C-22), 135.6 (*d*, C-23), 42.8 (*d*, C-24), 33.1 (*d*, C-25), 19.9 (*q*, C-26), 21.1 (*q*, C-27), 17.6 (*q*, C-28); EIMS: *m/z* 396 $[M]^+$.

Ergosterol peroxide (3ß-hydroxy-5, 8-epidioxyergosta-6, 22-diene) (2) [10,11]

Colorless needles; mp 180-182 °C; ^1H NMR ($CDCl_3$): δ 3.94 (1H, *m*, H-3), 6.20 (1H, *d*, *J* = 8.4Hz, H-6), 6.47 (1H, *d*, *J* = 8.5Hz, H-7), 0.79 (3H, *s*, C$\underline{H}_3$-18), 0.86 (3H, *s*, C$\underline{H}_3$-19), 0.97 (3H, *d*, *J* = 6.5Hz, C$\underline{H}_3$-21), 5.20 (1H, *dd*, *J* = 7.5, 15.4Hz, H-22), 5.11 (1H, *dd*, *J* = 8.5, 15.4Hz, H-23), 0.79 (3H, *d*, *J* = 6.7Hz, C$\underline{H}_3$-26), 0.80 (3H, *d*, *J* = 6.6 Hz,C$\underline{H}_3$-27), 0.88 (3H, *d*, *J* = 6.5Hz, C$\underline{H}_3$-28); ^{13}C NMR ($CDCl_3$): δ 34.7 (*t*, C-1), 30.3 (*t*, C-2), 66.4 (*d*, C-3), 36.9 (*t*, C-4), 82.2 (*s*, C-5), 135.4 (*d*, C-6), 130.9 (*d*, C-7), 79.4 (*s*, C-8), 51.1 (*d*, C-9), 36.9 (*s*, C-10), 20.6 (*t*, C-11), 39.3 (*t*, C-12), 44.5 (*s*, C-13), 51.6 (*d*, C-14), 23.4 (*t*, C-15), 28.6 (*t*, C-16), 56.2 (*d*, C-17), 12.8 (*q*, C-18), 18.1 (*q*, C-19), 39.7 (*d*, C-20), 20.9 (*q*, C-21), 135.2 (*d*, C-22), 132.3 (*d*, C-23), 42.7 (*d*, C-24), 33.0 (*d*, C-25), 19.9 (*q*, C-26), 19.6 (*q*, C-27), 17.5 (*q*, C-28); EIMS: *m/z* 428 $[M]^+$.

Bassiatin; (3S, 6R-4-methyl-6-(1-methylethyl)-3-phenylmethyl-1, 4-perhydro-oxazine-2, 5-dione (3) [12]

Colorless crystals; mp 146-149°C; IR (KBr) ν max cm^{-1} 2960, 2920, 2895, 2860, 1745, 1645, 1490, 1450, 1440, 1405, 1360, 1330, 1320, 1250, 1030, 770, 700; $[\alpha]_D$ +176° (c 0.5, chloroform); EIMS m/z : 261 $[M]^+$; HREIMS *m/z*: 261.1355 (calcd for $C_{15}H_{19}NO_3$, 261.1356)

X-ray Crystal Structure Analysis of Bassiatin (3)

A colorless orthorhombic crystal of **4** with dimensions 0.10 × 0.25 × 0.35mm was obtained by re-crystallization from ethyl acetate-hexane system and selected for X-ray analysis. The crystallographic data were collected on a Bruker Smart CCD diffractometer using graphite-monochromated Mo Kα radiation. A structure analysis was made using the SHELXTL program on PC.[14] The compound crystallized in the space group $P2_1$, a = 10.0092(4) Å, b = 10.8521(4) Å, c = 13.1658(5) Å, monoclinic, *V* = 1430.08(9) $Å^3$, Z = 4, Decal = 1.214 Mg/m^3, λ = 0.71073 Å, μ(Mo Kα) = 0.084 mm^{-1}, F(000) = 560, and *T* = 296(2) K. A total of 10120 reflections were collected in the range of 2.43° ≤ θ ≤ 27.49°, of which

only 3275 unique reflections with $I > 2\sigma(I)$ were corrected for the analysis. The structure was solved using direct methods and refined by full-matrix-least-squares on F^2 values. Non-hydrogen atoms were refines anisotropically. Hydrogen atoms were fixed at calculated positions and refined using a riding mode. The final indices were $R = 0.0690$, $R_w = 0.1567$ with goodness-of-fit = 0.985. Scattering factors were taken from International Tables for X-ray Crystallography.[15]

Bassiatin A; (3S, 6R)-4-methyl-6-(1-methylpropyl)-3-phenylmethyl-1,4-perhydrooxazine-2,5-dione (4)

Colorless crystals; mp 145-147°C; $[\alpha]_D$ +187.5° (c 0.038, dichloromethane); EIMS *m/z*: 275 $[M]^{+\cdot}$.

X-ray Crystal Structure Analysis of Bassiatin A (4)

A colorless monoclinic crystal of **4** with dimensions 0.40 × 0.25 × 0.10mm was obtained by re-crystallization from methanol-water system and selected for X-ray analysis. The crystallographic data were collected on a Bruker Smart CCD diffractometer using graphite-monochromated Mo Kα radiation. A structure analysis was made using the SHELXTL program on PC.[14] The compound crystallized in the space group $P2_1$, a = 6.7580(3) Å, b = 13.9359(6) Å, c = 8.1205(4) Å, β = 93.9460(10)°, monoclinic, $V = 762.97(6)$ Å^3, Z = Q, Decal =1.199 Mg/m^3, λ =0.71073 Å, μ(Mo Kα) = 0.082 mm^{-1}, F (000) = 296, and $T = 295(2)$ K. A total of 8037 reflections were collected in the range of $2.51° \leq \theta \leq 27.50°$, of which only 3490 unique reflections with $I > 2\sigma(I)$ were corrected for the analysis. The structure was solved using direct methods and refined by full-matrix-least-squares on F^2 values. Non-hydrogen atoms were refines anisotropically. Hydrogen atoms were fixed at calculated positions and refined using a riding mode. The final indices were $R = 0.0401$, $R_w = 0.1170$ with goodness-of-fit =1.001. Scattering factors were taken from International Tables for X-ray Crystallography.[15]

Beauvericin (5) [13]

Colorless crystal; ^{1}H NMR ($CDCl_3$): δ 0.37 (3H, *d*, *J* = 6.6Hz, γ1-CH_3, Hiv), 0.76 (3H, *d*, *J* = 6.6Hz, γ2- CH_3, Hiv), 1.97 (1H, *m*, β-CH, Hiv), 2.93 (1H, *dd*, *J* = 11.9, 14.5Hz, β-CH(*H*), Phe), 2.98 (3H, *s*, N- CH_3), 3.34 (1H, *dd*, *J* = 4.9, 14.5Hz, β-CH(*H*), Phe), 4.89 (1H, *d*, *J* = 8.5Hz, α-CH, Hiv), 5.51 (1H, *dd*, *J* = 4.8, 11.8Hz, α-CH, Phe), 7.20(5H, ar CH, Phe); ^{13}C NMR ($CDCl_3$): δ 17.4 (*q*, γ1-CH_3, Hiv), 18.2 (*q*, γ2- CH_3, Hiv), 29.6 (*d*, β-CH, Hiv), 32.2 (*q*, N- N- CH_3), 34.7 (*t*, β- CH_2, Phe), 57.2 (*d*, α-CH, Phe), 75.4 (*d*, α-CH, Hiv), 126.7 (*d*, ar CH, Phe), 128.4 (*d*, ar CH, Phe), 128.8 (*d*, ar CH, Phe), 136.6 (*s*, ar C, Phe), 169.3 (*s*, CO), 169.8 (s, CO); HRFABMS *m/z*: $[M+H]^+$ 784.4164 (calcd for $C_{45}H_{58}N_3O_9$, 784.4173).

X-ray Crystal Structure Analysis of Beauvericin (5)

A colorless monoclinic crystal of **5** with dimensions 0.45 × 0.40 × 0.40mm was obtained by recry-stallization from methynol-water system and selected for X-ray analysis. The crystallographic data were collected on a Bruker

Smart CCD diffractometer using graphite-monochromated Mo Kα radiation. A structure analysis was made using the SHELXTL program on PC. [14] The compound crystallized in the space group $P2_1$, a = 8.3591(3) Å, b = 17.1798(7) Å, c = 15.9151(6) Å, β = 93.084(1)°, monoclinic, V = 2282.22(15) Å^3, Z = Q, Dcalc = 1.240 Mg/m^3, λ = 0.71073 Å, μ (Mo Kα) = 0.089mm^{-1}, F (000) = 916, and T = 150 K. A total of 24247 reflections was collected in the range of 1.28° ≤ θ ≤ 27.50°, of which only 10466 unique reflections with $I > 2\sigma(I)$ were corrected for the analysis. The structure was solved using direct methods and refined by full-matrix-least-squares on F^2 values. Non-hydrogen atoms were refines anisotropically. Hydrogen atoms were fixed at calculated positions and refined using a riding mode. The final indices were R = 0.045, R_w = 0.122 with goodness-of-fit = 1.046. Scattering factors were taken from International Tables for X-ray Crystallography.[15]

Beauvericin A (6) [13]

Colorless crystal; ^{1}H NMR ($CDCl_3$): δ 0.39 (3H, *d*, *J* = 6.7Hz, γ1-CH_3, Hiv), 0.40 (3H, *d*, *J* = 6.8Hz, γ1-CH_3, Hiv), 0.65 (3H, *m*, δ-CH_3, Hmp), 0.70 (2H, *m*, γ1- CH_2, Hmp), 0.74 (3H, *d*, *J* = 6.5Hz, γ2- CH_3, Hiv), 0.76 (3H, *d*, *J* = 6.7Hz, γ2- CH_3, Hiv), 0.78 (3H, *d*, *J* = 6.9Hz, γ2- CH_3, Hmp), 1.77 (1H, *m*, β-CH, Hmp), 2.02 (2H, *m*, β-CH, Hiv), 2.95 (3H, *s*, N- CH_3), 2.99 (6H, *s*, N- CH_3), 2.90~2.99 (3H, β-CH(*H*), Phe), 3.34 (3H, β-CH(*H*), Phe), 4.87 (1H, *d*, *J* = 8.6Hz, α-CH, Hiv), 4.89 (1H, *d*, *J* = 8.4Hz, α-CH, Hiv), 4.98 (1H, *d*, *J* = 7.7Hz, α-CH, Hmp), 5.50 (3H, *m*, α-CH, Phe), 7.20 (15H, *m*, ar CH, Phe); ^{13}C NMR ($CDCl_3$): δ 11.3 (δ- CH_3, Hmp), 14.4 (γ2- CH_3, Hmp), 17.4 (γ1-CH_3, Hiv), 18.3 (γ2- CH_3, Hiv), 24.3 (γ1- CH_2, Hmp), 29.7 (β-CH, Hiv), 32.1 (N- CH_3), 32.1 (N- CH_3), 34.6 (β- CH_2, Phe), 34.8 (β- CH_2, Phe), 35.9 (β-CH ,Hmp), 57.1 (α-CH, Phe), 57.2 (α-CH, Phe), 74.3 (α-CH, Hmp), 75.5 (α-CH, Hiv), 126.7 (ar CH, Phe), 128.4 (ar CH, Phe), 128.8 (ar CH, Phe), 136.6 (ar C, Phe), 169.5 (CO), 169.9 (CO); HRFABMS m/z $[M+H]^+$ 798.4332 (calcd for $C_{46}H_{60}N_3O_9$, 784.4330).

X-ray Crystal Structure Analysis of Beauvericin A (6)

A colorless monoclinic crystal of **6** with dimensions 0.45 × 0.40 × 0.13mm was obtained by re-crystallization from methanol-water system and selected for X-ray analysis. The crystallographic data were collected on a Bruker Smart CCD diffractometer using graphite-monochromated Mo Kα radiation. A structure analysis was made by using the SHELXTL program on PC.[14] The compound crystallized in the space group $P2_1$, a = 8.4078(4) Å, b = 17.0702(7) Å, c = 16.4296(10) Å, β = 92.947(1)°, monoclinic, V = 2341.60(19) Å^3, Z = Q, Dcalc = 1.228 Mg/m^3, λ = 0.71073 Å, μ (Mo Kα) = 0.088mm^{-1}, F (000) = 932, and T = 150 (2) K. A total of 20829 reflections was collected in the range of 1.25° ≤ θ ≤ 27.50°, of which only 9936 unique reflections with $I > 2\sigma(I)$ were corrected for the analysis. The structure was solved using direct methods and refined by full-matrix-least-squares on F^2 values. Non-hydrogen atoms were refines anisotropically. Hydrogen atoms were fixed at calculated positions and refined using a riding mode. The final

indices were R = 0.0614, R_w = 0.1362 with goodness-of-fit = 1.034. Scattering factors were taken from International Tables for X-ray Crystallography.[15]

Beauvericin B (7) [13]

Colorless crystal; ^{1}H NMR ($CDCl_3$): δ 0.38 (3H, *d*, *J* = 6.6Hz, γ1-CH_3, Hiv), 0.65 (6H, *m*, δ- CH_3, and 4H, *m*, γ1-CH_2, Hmp), 0.73 (3H, *d*, *J* = 6.7Hz, γ2- CH_3, Hiv), 0.75 (3H, *d*, *J* = 7.0Hz, γ2- CH_3, Hmp), 0.76 (3H, *d*, *J* = 6.5Hz, γ2-CH_3, Hiv), 1.62 (2H, *m*, β-CH, Hmp), 1.93 (1H, *m*, β-CH, Hiv), 2.96 (6H, *s*, N- CH_3), 2.99 (3H, *s*, N- CH_3), 2.87~3.02 (3H, *m*, β-CH(*H*), Phe), 3.37 (3H, *m*, β-CH(*H*), Phe), 4.84 (1H, *d*, *J* = 8.1Hz, α-CH, Hiv), 4.94 (1H, *d*, *J* = 7.5Hz, α-CH, Hmp), 4.95 (1H, *d*, *J* = 7.3Hz, α-CH, Hmp), 5.57 (3H, *m*, α-CH, Phe), 7.21(15H, *m*, ar CH, Phe); HRFABMS m/z $[M+H]^+$ 812.4492 (calcd for $C_{47}H_{62}N_3O_9$, 812.4486).

ACKNOWLEDGMENT

The authors thank the National Science Council of the Republic of China for financial support (NSC 89-2113-M-077-007), and Dr. Tun-Tschu Chang for the identification of *C. cicadae.*

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J Chin Med 13(4): 209-219, 2002

中藥蟬花活性成分之研究

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(2002 年 9 月 19 日受理，2002 年 10 月 4 日收校訂稿，2002 年 10 月 11 日接受刊載)

中藥蟬花是麥角菌科(Clavicipitaceae)大草蟬草菌(*Cordyceps cicadae* Shing)寄生在山蟬(*Cicada flammata* Dist)幼蟲的真菌，藥材包括其子實體及蟲體，是作為治療小兒驚厥，心悸，及鎮定的中藥。在我們擬由天然物中覓找生理活性物質的系列,發現其蟲體甲醇抽取物在植物凝集素（DHA）刺激下，呈現抑制人類單核細胞增生活性,其抑制度與濃度成比例關係，因此，進行其活性成分之探研，經本次實驗研究，蟬花蟲體部份離出麥角甾醇（ergosterol），過氧麥角甾醇（ergosterol peroxide），白僵菌酮（bassiatin）及白僵菌酮甲（bassiatin A），白僵菌素（beauvericin），白僵菌素甲（beauvericin A），白僵菌素乙（beauvericin B）,後五個成分環酯 抗生素及嗎 二酮，係首次從中藥蟬花獲得，其中白僵菌酮甲（bassiatin A）更是首次由天然物界發現。

關鍵詞：蟬花，真菌，甾醇，環酯 化合物，嗎 二酮化合物。

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