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Chaetoglobosins, Cytotoxic 10-(Indol-3-yl)-[13]cytochalasans from *Chaetomium* spp. I. Production, Isolation and Some Cytological Effects of Chaetoglobosins A—J

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Bioproduction of chaetoglobosins, novel type cytochalasans, by *Chaetomium* spp. was examined. Details of the culture conditions and methods for separation of the metabolites of *Chaetomium globosum* are presented. Some cytological effects of chaetoglobosins were also mentioned.

Keywords——*Chaetomium globosum*; chaetoglobosins A—G and J; culture conditions; cytotoxicity; mammalian cells; HeLa cells; rat muscle cells; cytochalasans; cytochalasins; mycotoxins

In the course of screening for mycotoxin production by food-borne fungi using HeLa cells and mice,[1-4] several isolates of *Chaetomium cochliodes* Palliser and *C. globosum* Kunze *ex* Fries showed toxicity; the extracts of the mycelium and the culture filtrate exhibited acute toxicity to mice[1,2,4] and were cytotoxic to HeLa cells, producing peculiar morphological changes, *i.e.* inhibition of cytoplasmic division without interfering with nuclear division, resulting in the formation of multinuclear cells.[1-3] The causative agents, the major metabolite chaetoglobosin A and the minor metabolites, chaetoglobosins B—G and J, were then isolated and their structures were elucidated as perhydroisoindol-1-one derivatives bearing indol-3-ylmethyl groups at the 3-position and 13-membered rings at the 8—9 positions, as reported in the preliminary communications.[5-8] In this paper experimental details of the production and isolation of the metabolites and some cytological effects are presented.

Initially, selection of the strains was conducted on the basis of the cytotoxicity test. Among the isolates from foodstuffs collected in Japan[1-3,9] and in Malaysia,[10] extracts of two strains of *C. cochliodes*, eleven strains of *C. globosum*, and three strains of *Chaetomium* sp. were found to produce the characteristic cytotoxic agent(s). A strain (68-SA-2) of *C. globosum* obtained from soybeans collected at Saku City, Nagano Prefecture, was selected as the best producer. Recently, three additional species, *C. mollipilium* Ames, *C. globosum* var. *rectum* (Serg.) Dreyfuss, and *C. subaffine* Serg., were found to produce the same toxins.[11] The production of chaetoglobosins by three strains of *C. globosum* was also reported by Canadian workers.[12] All these five species belong to *C. globosum* group sensu Dreyfuss.[13]

Next culture conditions were examined and stationary liquid culture on potato–glucose-peptone medium at 25°C for three weeks was found to be suitable for the production of the cytotoxic agent(s). Several separation methods were examined for the extract of the mycelium and the filtrate using the cytotoxicity test as a criterion, and silica gel thin-layer chromatography (TLC) of the chloroform extract of the mycelium revealed the presence of three bands able to cause the characteristic cytological change (Fig. 1). Finally chaetoglobosins A (**A**), mp 168—170°C, B (**B**), mp 186—187°C, and C (**C**), mp 259—261°C, corresponding to fractions 6, 5, and 4, respectively, in Fig. 1, were isolated by silica gel column chromatography. They have the same molecular formula, $C_{32}H_{36}O_5N_2$ (by high resolution mass spectrometry and elemental analyses).[5]

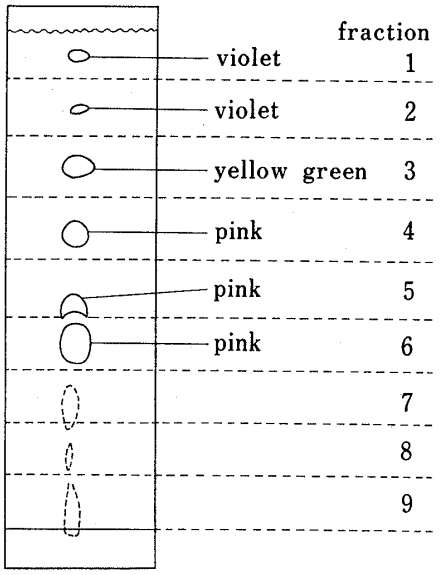


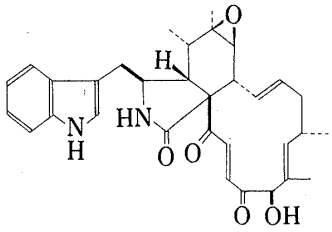
Fig. 1. Thin-layer Chromatogram and Cytotoxicity of Chloroform Extract of the Mycelium of *C. globosum*

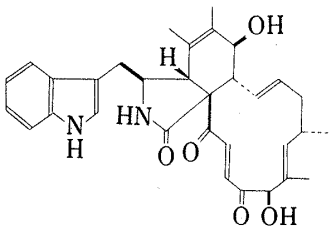
For the cytotoxicity scoring, see Experimental.
Plate: Silica gel $60F_{254}$; solvent: benzene–ethyl acetate (2: 3).

TABLE I. Cytotoxicity of the Metabolites formed in Shaking Culture

Medium[a]	Incubation time (d)	Weight of CH_2Cl_2 extract (g/l)		Cytotoxicity of the extract to HeLa cells[b]	
		Filtrate	Mycelium	Filtrate	Mycelium
PG	4	0.18	0.12	±	±
	6	0.15	0.18	+	+
	8	0.27	0.29	+	+
A	4	0.16	0.14	−	−
	6	0.19	0.21	±	−
	8	0.10	0.24	±	±
B	4	0.05	0.27	±	±
	6	0.07	0.27	+	+
	8	0.07	0.26	+	±
C	4	0.02	0.25	±	−
	6	0.07	0.36	+	±
	8	0.04	0.27	++	+
D	4	0.04	0.21	±	−
	6	0.11	1.42	±	±
	8	0.13	1.78	+	±
E	4	0.02	0.29	±	−
	6	0.04	0.40	±	−
	8	0.06	0.47	±	±

a) See Experimental.
b) Criteria for estimating the degree of toxicity; ±: cytotoxicity over 2 at 100 μg/ml of the extract, +: over 2 at 32 μg/ml, and ++: over 2 at 10 μg/ml.


chaetoglobosin A (**A**)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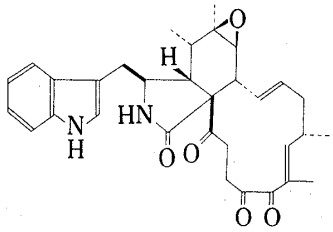
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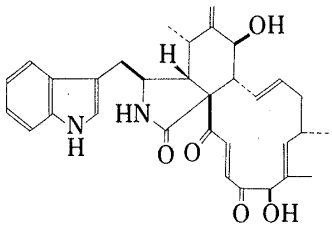
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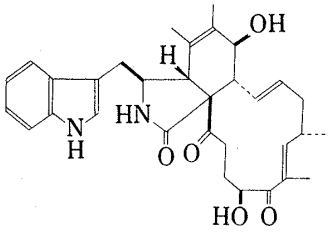
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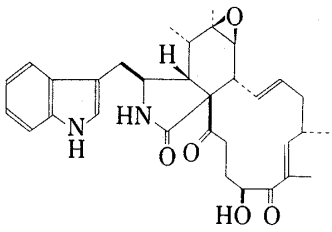
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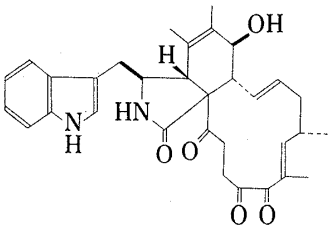
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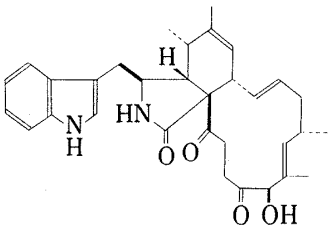
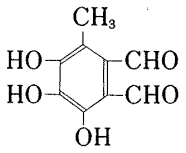
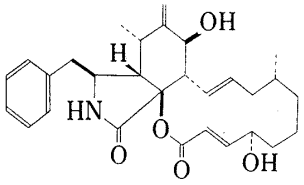

chaetoglobosin J (**J**)


flavipin (**1**)


cytochalasin A (**2**)

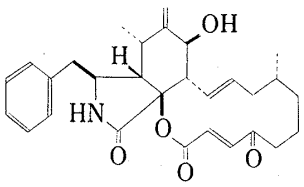

cytochalasin B (**3**)

Chart 1

When the chemical nature of the compounds was partly unveiled, detection of chaetoglobosins became easier and Ehrlich's reagent was applied for detection on TLC. Examination of the production of these compounds using solid substrates was also carried out, since culture on cereals often results in good yields of metabolites.[14] Indeed, culture on polished rice was found to give better yields of the chaetoglobosins; the yield of chaetoglobosin A, the major metabolite, reached 0.25 g per kg rice and new congeners, chaetoglobosins D (**D**), mp 216°C, E (**E**), mp 276—280°C, F (**F**), mp 177—178°C, and G (**G**), mp 251—253°C, were isolated besides **B** and **C**. Chaetoglobosins D and G are isomers of **A**—**G**, while **E** and **F** have the molecular formula, $C_{32}H_{38}O_5N_2$, corresponding to the dihydro compounds.

The conditions for submerged culture were also examined for large-scale production. In the preliminary test (see Experimenatl), medium C of the six tested media gave the best results and the yield of the metabolites was found to reach the maximum after 8 days' shaking culture (Table I). Submerged culture in jar fermenters and in tanks was also performed on medium C and the formation of the metabolites was pursued by TLC and cytotoxicity testing. From the mycelial extract, **A** was obtained in a yield of 0.15 g/l, accompanied by

B—**F** and a new congener, chaetoglobosin J (**J**), mp 149—151°C, $C_{32}H_{36}O_4N_2$, corresponding to a deoxy compound of **A**—**D**.

In the case of submerged cultures, a yellow pigment was formed in the culture filtrate and was identified as flavipin (**1**), a phenolic compound first isolated from *Aspergillus terreus* Thom.[15]

Although several metabolites of *C. globosum* and *C. cochliodes* such as chetomin[16] and cochliodinol[17] are known,[11,12] chaetoglobosins are new compounds, and structure studies were performed as preliminarily reported.[5-8] The details are presented in the accompanying papers.

The estabilished structures of chaetoglobosins (Chart 1) clearly indicated that they were novel type cytochalasans.[18-21] The cytochalasans are a group of mold secondary metabolites affecting the structure and functions of mammalian cells; they cause inhibition of cellular movements including cell division, motility, secretion and phagocytosis, and also cause changes in cell shape. In chaetoglobosins, the phenyl group in the first members of the group such as cytochalasins A (**2**) and B (**3**) (dehydrophomin and phomin) is replaced by an indol-3-yl group, *i.e.* the phenylalanine unit is replaced by tryptophan.

Table II. Cytotoxicity of Chaetoglosins to HeLa Cells

	32 (μg/ml)	10 (μg/ml)	3.2 (μg/ml)	1.0 (μg/ml)	0.32 (μg/ml)
Chaetoglobosin A	4	4	1	0	
Chaetoglobosin B	4	3—4	0—1	0	
Chaetoglobosin C	3—4	1	0—1	0	
Cheatoglobosin D	4	4	2	0	
Chaetoglobosin E	4	4	2	0	
Chaetoglobosin F	4	2	1	0	
Chaetoglobosin G	4	3	1	0	
Chaetoglobosin J	4	4	3	1	
Isochaetoglobosin D[a]	4	3	2	0	
Chaetoglobosin A monoacetate[a]	4	2	1	0	
Chaetoglobosin B diacetate[a]	4	2	0—1	0	
Chaetoglobosin E diacetate[a]	4	1	0	0	
Chaetoglobosin F monoacetate[a]	4	3	2	0	
Chaetoglobosin G monoacetate[a]	4	0	0	0	
Isochaetoglobosin D monoacetate[a]	4	3	0	0	
Cytochalasin A (dehydrophomin)	4	4	2	0—1	0
Cytochalasin B (phomin)	4	3—4	2—3	1	0
Cytochalasin C	4	3—4	3—4	3	2
Cytochalasin D (zygosporin A)	4	3	3	2—3	1

a) See parts II and III of this series.
For details of the cytotoxicity scoring, see Experimental.

The cytotoxicity of chaetoglobosins was precisely examined, as reported previously.[22,23] After 3 days' treatment, HeLa cells were lethally affected and the IC_{50} value of chaetoglobosins were about 3.2—10 μg/ml except for C, IC_{50} of which was about 20 μg/ml (Table II). Chaetoglobosins induced polynucleation in HeLa cells and other cultured cells (Fig. 2a). Application of A to primary cell culture from rat muscle induced inhibition of myotube formation. When the toxin was applied to the culture after myotube formation, large, long and slender myotubes immediately became shortened and changed to spindle-shaped or round giant cells (Fig. 2b—d). These findings indicated that chaetoglobosins affected contractile proteins in the cells. This view is supported by the findings that cytochalasans, including chaetoglobosins A, B, C, E, F and J, block the salt-induced polymerization of G-actin prepared from skeletal muscle.[24]

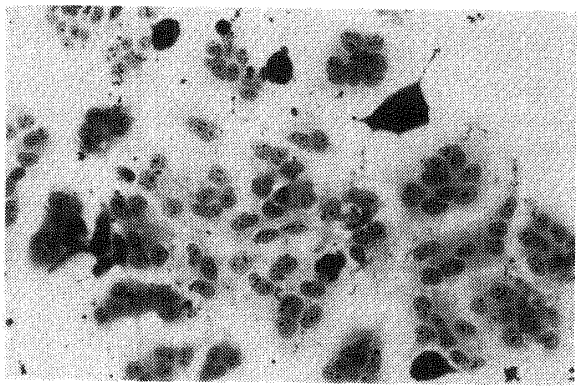


Fig. 2a. HeLa Cells treated with 3.2 μg/ml of Chaetoglobosin A

Note polynucleated cells and multipolar divisions.

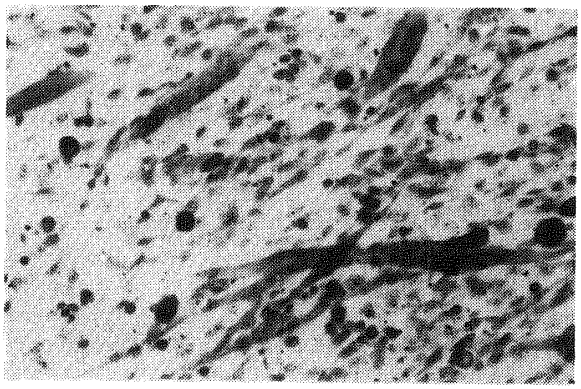


Fig. 2b. Control Rat Muscle Culture incubated for 5 d

Note the formation of multinucleated myotubes.

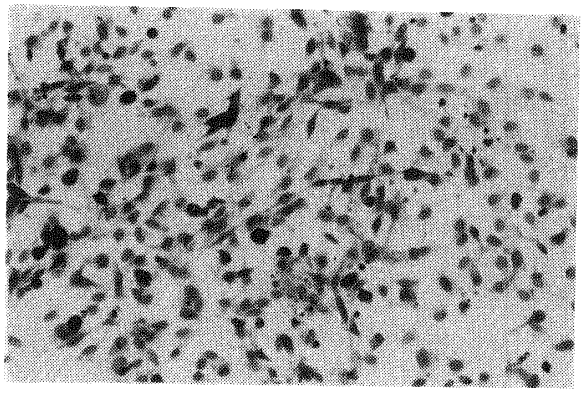


Fig. 2c. Rat Muscle Culture with 3.2 μg/ml of Chaetoglobosin A added on Day 2 and further incubated until Day 5

Note little formation of myotubes.

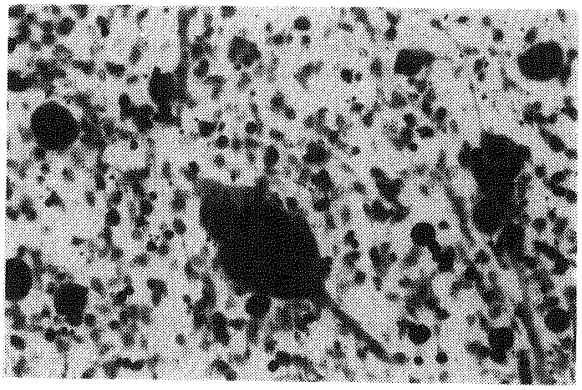


Fig. 2d. Rat Muscle Culture with 3.2 μg/ml of Chaetoglobosin A added on Day 5 and further incubated until Day 8

Note the shortening of the preformed myotubes.

The binding sites for cytochalasans, including chaetoglobosins, were also studied using human erythrocyte membrane.[25] Quite recently the correlation between the effects of **A**—**F** and **J** and their acetates, along with other 12 cytochalasans, on cellular structures and cellular events and their effects on actin polymerization was precisely examined.[26]

The acute toxicity to mice and rats of **A** was examined (LD_{50}(mice) ♂ 6.5 mg/kg, ♀ 17.5 mg/kg (*s.c.*)).[27] Chaetoglobosins did not show significant mutagenicity[28] but **A** shows teratogenicity to mice.[29] Since the toxicity by oral administration is low (LD_{50} (mice and rat) >400 mg/kg),[27] chaetoglobosins may not be acutely toxic to humans and animals as a result of possible ingestion through foods and feeds, but they do have effects on mammalian cells and subcellular organelles, as already reported,[23-26] and further biological studies are in progress.

Experimental

Selection of Strains Producing Chaetoglobosins——The strains of *Chaetomium* spp. and allied genera were cultivated on potato–glucose medium (potato 300 g, glucose 50 g, water to 1 l) for 3 weeks at 25°C. The dried mycelia were extracted with $CHCl_3$ twice and the filtrate was extracted with EtOAc. After removal of the solvents by evaporation, the extracts were tested by means of the cytotoxicity test and thin-layer chromatography (TLC).[1-3] The reported screening procedure was used for the culture on rice.[11]

TLC was carried out using precoated silica gel 60 F_{254} plates. The following developing solvent systems were used: solvent A, benzene–EtOAc (2: 3), and solvent B, $CHCl_3$–MeOH (9: 1). For detection, Ehrlich's reagent was used.

Cytotoxicity Test using HeLa Cells——HeLa cells were grown in Eagle's minimum essential medium supplemented with 10% calf serum. For testing toxicity and morphological changes, a modified panel method was employed. Each cup of the panels contained a round cover-glass on which cells were grown, and the cells were treated with test samples for 3 d. These cover-glasses were then fixed with Carnoy's fixative and stained with hematoxylin and eosin. Cell injuries were scored as 0 through 4, where 0 indicates no appreciable effect, 4 lethal effect and 1, 2, 3, grades of injuries between 0 and 4.

Application to Primary Prepared Rat Muscle Cell Culture——Thigh muscle tissue from 2- to 5-d-old Sprague-Dawley rats was dissociated using 0.25% trypsin. The obtained cell suspension was poured into culture dishes with cover-glasses. The medium consisted of Eagle's minimum essential medium supplemented with 10% fetal bovine serum. Myotubes were formed by the fusion of myoblasts usually after cultivation for several days. Chaetoglobosin A was applied to the culture before or after the myotube formation. Cells on the cover-glasses were fixed, stained and examined microscopically.

Production and Isolation of Chaetoglobosins——a) Media: For stationary liquid culture,

Potato–glucose (PG) medium: potato 300 g, glucose 50 g, made up to 1000 ml with demineralized water.

Potato–glucose–peptone (PGP) medium: potato 300 g, glucose 50 g, peptone 10 g, made up to 1000 ml with demineralized water.

For stationary solid culture,

Rice medium: see below.

For submerged culture,

PG medium: same as the medium for stationary culture.

Medium A: $NaNO_3$ 3 g, K_2HPO_4 1 g, KCl 0.5 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, $FeSO_4$ 0.01 g, glucose 20 g, peptone 10 g, corn steep liquor 3 g, demineralized water to 1000 ml, adjusted to pH 6—6.5.

Medium B: sucrose 20 g, peptone 5 g, K_2HPO_4 5 g, $MgSO_4 \cdot 7H_2O$ 2.5 g, potato 100 g, demineralized water to 1000 ml, adjusted to pH 5.5.

Medium C: glucose 30 g, soybean cake 10 g, K_2HPO_4 3 g, corn steep luquor 3 g, demineralized water to 1000 ml, adjusted to pH 6.0.

Medium D: glucose 50 g, peptone 5 g, pharma media 5 g, corn steep liquor 1 g, demineralized water to 1000 ml, adjusted to pH 6.0.

Medium E: glucose 30 g, wheat bran 5 g, corn steep liquor 2 g, demineralized water to 1000 ml, adjusted to pH 6.0.

b) Stationary Liquid Culture on PGP Medium: The strain (68-SA-2) was grown in fifty 1000 ml Roux flasks containing 200 ml each of PGP medium at 25°C for 3 weeks. After incubation, the mycelia were separated from the broth by filtration and dried at 60° for 2—3 d. Dried mycelium (144 g) was extracted with benzene twice. The benzene extract (1.0 g) was applied to a silica gel (Mallinckrodt) (43 g) column and eluted with a gradient system of benzene–ethyl ether. From the fraction eluted with benzene–ethyl ether (7: 3), **C** (10 mg) was obtained. From the fraction eluted with benzene–ethyl ether (2: 1), **A** (116 mg) and **B** (12 mg) were obtained by fractional recrystallization.

c) Stationary Solid Culture on Polished Rice: Polished rice (brand: Sasanishiki)[30] (200 g) was soaked in tap water and then placed in a 1000-ml Roux flask. Sufficient water (about 20 ml) was added to just moisten the rice. After sterilization, the strain (68-SA-2) was grown in 100 Roux flasks at 25°C for 3 weeks. The flaks were agitated by hand daily. The moldy rice was extracted with $CHCl_3$ twice at room temperature. The combined extracts were evaporated to dryness.

The dark brown residue (55 g) was chromatographed over silica gel (2.2 kg) and eluted with a gradient system of $CHCl_3$–acetone. Fractions of 2 l were collected. Fraction 19 ($CHCl_3$) afforded ergosterol (50 mg). Fractions 21—26 ($CHCl_3$) gave a colorless powder, and recrystallization from acetone afforded **C** (1.08 g). Fractions 31—42 ($CHCl_3$) gave a yellowish powder and recrystallization from CH_2Cl_2 afforded **A** (4.95 g). The mother liquor was evaporated to dryness and recrystallization of the residue from benzene gave **B** (160 mg). Fraction 44 ($CHCl_3$–acetone, 20: 1) gave a colorless powder, which was recrystallized from MeOH to give **G** (10 mg). Fractions 46—50 ($CHCl_3$–acetone, 20: 1) gave an orange powder, and recrystallization from CH_2Cl_2 afforded **D** (340 mg). Fractions 51—55 ($CHCl_3$–acetone, 9: 1) gave a colorless sticky mass, and recrystallization from MeOH afforded **E** (400 mg). The mother liquor of the fractions 44—55 was rechromatographed and fractions of 500 ml were collected. Fraction 9 ($CHCl_3$) gave a colorless powder and recrystallization from benzene afforded **F** (1.30 g). Fraction 11 ($CHCl_3$–acetone, 20: 1) gave an orange powder, which was recrystallized from CH_2Cl_2 to give **D** (490 mg).

d) Submerged Culture: As a preliminary test, each medium (100 ml) was placed into a Sakaguchi flask, autoclaved, and inoculated with small amount of spore suspension of the strain (68-SA-2). For foam control, 0.01 g of an antifoaming agent (CB-422) was added to each flassk. The flasks were incubated for 8d at 25°C on a reciprocatory shaker (145 rev/min). As a result, medium C was selected for the subsequent large-scale fermentation. The strain (68-SA-2) was preincubated under submerged conditions (aeration, 1.0vvm at a pressure of 1.0 kg/cm^2) for 1 d at 25°C in medium C in a tank (100 l) with stirring at 260 rev/min.

Table III. MS, UV, and IR Data for Chaetoglobosins

	MS main peak (m/e)	UV λ_{max}^{EtOH} nm (log ε)	IR ν_{max}^{KBr} cm^{-1}
Chaetoglobosin A	510, 398, 149, 131,[b] 130	223, 274, 282, 291 (4.61, 3.82, 3.82, 3.73)	3438, 3259, 1689(br), 1615, 983, 969, 760
Chaetoglobosin B	510, 398, 149, 131,[b] 130	222, 274, 281, 290 (4.64, 3.90, 3.90, 3.83)	3440(br), 1690(br), 1621, 1153, 972, 746
Chaetoglobosin C	510, 398, 149, 131, 130[b]	222, 273, 281, 291[a] (4.56, 3.83, 3.83, 3.76)	3445, 3305, 1697(br), 1642, 986, 832, 745
Chaetoglobosin D	510, 398, 131,[b] 130	221, 273, 281, 290 (4.64, 3.96, 3.96, 3.88)	3421, 3280, 1686(br), 1606, 972, 908, 750
Chaetoglobosin E	445, 382, 131, 130[b]	221, 275, 281, 291 (4.75, 3.85, 3.85, 3.80)	3410(br), 1704, 1676, 1046, 746
Chaetoglobosin F	445, 382, 149, 131, 130[b]	222, 276, 283, 292 (4.68, 3.84, 3.83, 3.78)	3346(br), 1676(br), 1618, 979, 879, 740
Chaetoglobosin G	510, 398, 150, 131, 130[b]	222, 275, 282, 291 (4.51, 3.79, 3.79, 3.73)	3455, 3300, 1713, 1693, 1646, 1623, 987, 848, 741
Chaetoglobosin J	495, 382, 331, 131, 130[b]	224, 270, 280, 290 (4.68, 3.86, 3.86, 3.78)	3412, 3273, 1683, 1639, 1612, 1055, 982, 971, 925, 750

a) In MeOH. *b*) Base peak.

A part of the culture was used as the inoculum, and cultivation was conducted for 5 d under the same conditions in a tank (600 l) containing the same medium (310 l). The mycelium (28.0 kg) was separated by filtration and extracted with acetone (70 l×2). The acetone extract was concentrated to 35 l and then extracted with CH_2Cl_2 (50 l×2). The combined CH_2Cl_2 extract was evaporated to dryness at 65°C.

The dark brown residual solid (320 g) was chromatographed over silica gel (4.0 kg) and eluted with a gradient system of $CHCl_3$–acetone. Fractions of 1 l were collected. Fractions 7—8 ($CHCl_3$) gave a yellowish gum which was rechromatographed over silica gel (100 g). From the first fraction eluted with $CHCl_3$, ergosterol (20 mg) was obtained. From the following fractions, **J** (0.6 g), **C** (0.2 g), **A** (1.7 g), **B** (0.5 g), and **D** (8 mg) were obtained successively. From fractions 9—16 ($CHCl_3$), yellow crystals of **A** (5.52 g) separated out. The mother liquor was chromatographed over silica gel (100 g) and **J** (0.8 g) and **C** (0.08 g) were obtained. By the same procedure, **J** (1.8 g), **A** (22.4 g), and **B** (0.7 g) were obtained from fractions 17—20 ($CHCl_3$). Fractions 21—25 ($CHCl_3$) gave a yellowish gum, which was rechromatographed to afford **A** (30 g), **B** (0.6 g), and **D** (50 mg). Fractions 26—31 ($CHCl_3$) gave **D** (50 mg). The mother liquor after the removal of D was chromatographed over silica gel (50 g) to give flavipin (50 mg), **B** (0.2 g), and **D** (20 mg). Fractions 32—37 ($CHCl_3$) gave a crystalline mixture, from which **A** (10 mg), **D** (10 mg), and **E** (10 mg) were obtained by the same method. The mother liquor was chromatographed over silica gel (30 g) to give **D** (20 mg), **E** (10 mg), and **F** (20 mg). Fractions 38—40, 46—53, and 54—62 each gave a brown gum, exhibiting positive reactions to Ehrlich's reagent, but, due to poor yields, further separation was not attempted.

The culture filtrate (200 l) was extracted with EtOAc (210 l) and the organic layer was concentrated. The separated crystals (67 mg), identical with the authentic flavipin, were removed and the filtrate was subjected to column chromatography on silica gel (100 g) and eluted successively with benzene and benzene–EtOAc. From the fraction eluted with benzene–EtOAc (85: 15), flavipin (1.2 g) was isolated and identified. Although the presence of **A** and **C** was dectected by TLC, these compounds were not isolated because of the poor yields in the filtrate.

Chaetoglobosins——Chaetoglobosin A: mp 168—170°C, pale yellow prisms from CH_2Cl_2, $[\alpha]_D$ −270° (c=0.10, MeOH). MS m/e: 528.263 (M^+) (calcd for $C_{32}H_{36}N_2O_5$, 528.262). *Anal.* Calcd for $C_{32}H_{36}N_2O_5 \cdot H_2O$: C, 70.31; H, 7.01; N, 5.13. Found: C, 70.49; H, 7.08; N, 5.20.

Chaetoglobosin B: mp 186—187°C, pale yellow needles from benzene, $[\alpha]_D$ −176° (c=0.10, MeOH). MS m/e: 528.265 (M^+) (calcd for $C_{32}H_{36}N_2O_5$, 528.262). *Anal.* Calcd for $C_{32}H_{36}N_2O_5 \cdot H_2O$: C, 70.31; H, 7.01; N, 5.13. Found: C, 70.74; H, 6.92; N, 5.20.

Chaetoglobosin C: mp 259—261°C, colorless leaflets from acetone, $[\alpha]_D$ −30° (c=0.10, MeOH). MS m/e: 528.264 (calcd for $C_{32}H_{36}N_2O_5$, 528.262). *Anal.* Calcd for $C_{32}H_{36}N_2O_5$: C, 72.70; H, 6.86; N, 5.30. Found: C, 72.35; H, 6.89; N, 5.47.

Chaetoglobosin D: mp 216°C, pale yellow prisms from CH_2Cl_2, $[\alpha]_D$ −269° (c=0.10, MeOH). MS m/e: 528.261 (M^+) (calcd for $C_{32}H_{36}N_2O_5$, 528.262).

Chaetoglobosin E: mp 279—280°C, colorless needles from MeOH, $[\alpha]_D$ +158° (c=0.10, MeOH). MS m/e: 530.275 (M^+) (calcd for $C_{32}H_{38}N_2O_5$, 530.278). *Anal.* Calcd for $C_{32}H_{38}N_2O_5 \cdot H_2O$: C, 70.05; H, 7.35; N, 5.11. Found: C, 69.88; H, 7.32; N, 5.24.

Chaetoglobosin F: mp 177—178°C, colorless leaflets from benzene, $[\alpha]_D$ −69° (c=0.10, $CHCl_3$). MS m/e: 530.275 (M^+) (calcd for $C_{32}H_{38}N_2O_5$, 530.278).

Chaetoglobosin G: mp 251—253°C, colorless leaflets, from MeOH, $[\alpha]_D$ +89° (c=0.10, MeOH). MS m/e: 528.269 (M^+) (calcd for $C_{32}H_{36}N_2O_5$, 528.262).

Chaetoglobosin J: mp 149—151°C, pale yellow prisms from benzene, $[\alpha]_D$ +41° (c=0.10, MeOH). MS m/e: 512.258 (M^+) (calcd for $C_{32}H_{36}N_2O_4$, 512.250). *Anal.* Calcd for $C_{32}H_{36}N_2O_4$: C, 74.97; H, 7.08; N, 5.46. Found: C, 74.62; H, 7.02; N, 5.20.

The mass (MS), ultraviolet (UV), and infrared (IR) spectral data are given in Table III. The ^{1}H-nuclear magnetic resonance (^{1}H-NMR) data are given in the accompanying papers.

Flavipin——Yellow needles, mp 223—224°C (dec.), from $CHCl_3$. MS m/e: 196.034 (M^+) (calcd for $C_9H_8O_5$, 196.037), 178.030 (M^+-H_2O) (calcd for $C_9H_6O_4$, 178.027), 167.033 (M^+-CHO) (calcd for $C_8H_7O_4$, 167.034). IR ν_{max}^{Nujol} cm^{-1}: 3160, 1656, 1621, 1584, 1334, 1301, 1227, 1156, 1103, 934, 740. ^{1}H-NMR (in acetone-d_6) δ: 2.49 (3H, s), 10.40 (1H, s), 10.60 (1H, s). Comparison with an authentic sample of flavipin by IR and TLC established the identity of this product.

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