[Chem. Pharm. Bull. 31(2) 490—498 (1983)]

Chaetoglobosins, Cytotoxic 10-(Indol-3-yl)-[13]cytochalasans from *Chaetomium* spp. IV.[1] ^{13}C-Nuclear Magnetic Resonance Spectra and Their Application to a Biosynthetic Study

SETSUKO SEKITA,* KUNITOSHI YOSHIHIRA, and SHINSAKU NATORI[2]

National Institute of Hygienic Sciences, Kamiyoga-1-chome, Setagaya-ku, Tokyo 158, Japan

(Received July 30, 1982)

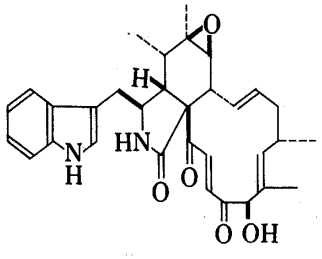
Assignments of the ^{13}C-nuclear magnetic resonance signals of eight naturally occurring chaetoglobosins were established. Incorporations of [1-^{13}C]-, [2-^{13}C]-, and [1,2-$^{13}C_2$]-acetate, [^{13}C-methyl]-L-methionine, and [1-^{13}C]- and [2-^{13}C]-DL-tryptophan into chaetoglobosins A and C by *Chaetomium globosum* demonstrated that the molecules were formed from nine units of acetate/malonate, three C_1 units, and one unit of tryptophan.

Keywords——*Chaetomium globosum*; chaetoglobosin; ^{13}C-NMR; biosynthesis; 10-(indol-3-yl)-[13]cytochalasan; cytochalasin; mycotoxin

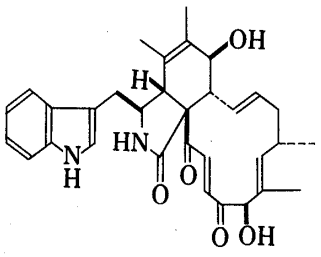
Chaetoglbosins (Chart 1),[3,4] metabolites of the *Chaetomium globosum* group,[5] belong to members of the cytochalasans,[6] which affect the structure and the function of mammalian cells.[6,7] Among thirty-nine members so far known,[8] biosynthetic studies have been reported only for cytochalasin B, a 10-phenyl-24-oxa-[14]cytochalasan, and cytochalasin D, a 10-phenyl-[11]cytochalasan.[9,10] The results demonstrated biogenetic pathways from phenylalanine and a nona- or octa-ketide-derived chain with two and three C_1-units introduced, respectively (Chart 2). Although the structural similarity of cytochalasans so far known led to the suggestion of a common biogenetic scheme for all of them,[6,9,10] some abnormal features such as the direct incorporation of the C(1)-atom of the amino acid at C(4), the head-to-head condensation at C(8)-C(9), and the tail-to-tail condensation at C(4)-C(5) were pointed out, and biosynthetic study of cytochalasans derived from amino acids other than phenylalanine seemed desirable.[10] Thus chaetoglobosins, 10-(indol-3-yl)-[13]cytochalasans probably derived from tryptophan, seemed to be worthy of study.

At the time of the structural elucidations of chaetoglobosins,[3] ^{13}C-nuclear magnetic resonance (NMR) data were not available in our laboratory. In recent publications on cytochalasans, several ^{13}C-NMR studies have been reported[8,9c,9d] but systematic data are still insufficient. In the present work, assignments of the ^{13}C-NMR signals of eight naturally occurring chaetoglobosins were carried out before the biosynthetic study. To perform the determination under the same condition, $^2H_6$-dimethyl sulfoxide (DMSO-d_6) was employed as the solvent, since all the chaetoglobosins were readily soluble in it.

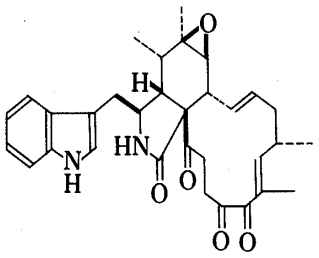
The proton noise-decoupled spectrum (complete decoupling, COM) of chaetoglobosin A is shown in Fig. 1(a). Comparison of the spectrum with those obtained under off-resonance decoupling (OFR) and gated decoupling with NOE revealed the nature of all the thirty-two carbons in the molecule: four methyl carbons, two methylene carbons, seven methine carbons, and two quaternary carbons in the sp^3 region; carbons showing ten doublets and four singlets under OFR in the sp^2 region; and three carbonyl carbons. Among these signals, eight aromatic carbons (five doublets and three singlets under OFR) were identified by comparison with the data for 3-methylindole[11] and the remaining singlet in the region (δ 131.5 ppm) was assigned to C(18). One of the three carbonyl carbons (δ 172.5 ppm) was assigned to C(1) on the basis of the chemical shifts value.



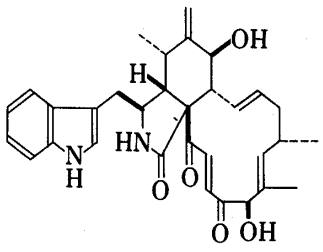
chaetoglobosin A



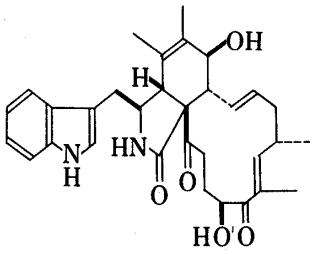
chaetoglobosin B



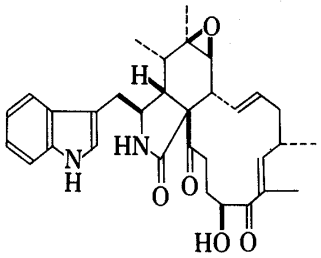
chaetoglobosin C



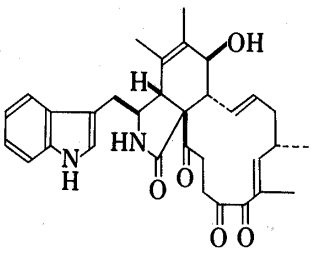
chaetoglobosin D



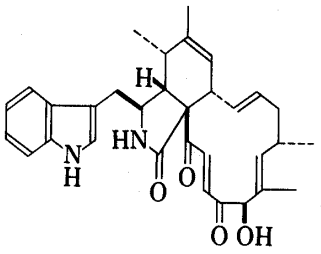
chaetoglobosin E



chaetoglobosin F



chaetoglobosin G



chaetoglobosin J

Chart 1. Structures of Chaetoglobosins

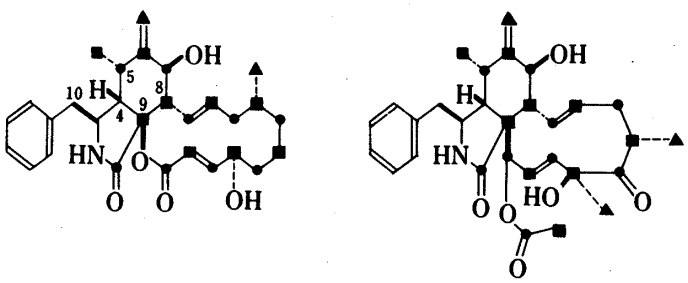


Chart 2. Biosynthesis of Cytochalasins B and D

●, ■, ▲: indicate carbon atoms derived from the C(1) and C(2) atoms of acetate and the *S*-methyl moiety of methionine, respectively.

Comparison of the chemical shifts of carbon atoms of chaetoglobosin A with those of the other seven congeners, taking account of the difference in their stuructures (Chart 1), allowed the assignments of several of the signals, *e.g.*, the signal at δ 35.3 ppm in chaetoglobosin A was assigned to C(5), δ 135.3 ppm to C(21) or C(22), and δ 81.5 ppm to C(19), while the signals at δ 125.2, 133.6, and 67.6 ppm in chaetoglobosin B were assigned to C(5), C(6), and C(7), respectively; those at δ 150.8 and 111.5 ppm in chaetoglobosin D were assigned to C(6) and C(12), respectively, and those at δ 33.0 and 36.6 in chaetoglobosin E and at δ 32.7 and 36.4 in chaetoglobosin F to C(21) and C(22), respectively (Table I).

By making use of the wide range of proton signals of chaetoglobosin A,[4b] various single-frequency irradiations ($^{13}C\{^1H\}$) (selective decouplings, SEL) were performed. Before the irradiation experiments, the assignments of ^{1}H-NMR signals in DMSO-d_6 were established as shown in Table II by comparison with the data in $CDCl_3$ reported in the previous papers[4b,c] as well as decoupling experiments. Thus, the four methyl carbons at C(11), C(12), 16-methyl, and 18-methyl in chaetoglobosin A were distinguished by SEL. In the same manner, C(21) and C(22) were distinguished[12] and C(3), C(5), C(7), C(13), C(14), C(17), and C(19) were unambigously identified. However, C(10)/C(15), C(4)/C(8)/C(16), C(6)/C(9), and C(20)/C(23) could not be differentiated due to the proximity of the signals of the protons attached to the carbons and final decision was made after the determination of samples of ^{13}C-enriched chaetoglobosin A derived from [1-^{13}C]-, [2-^{13}C]-, and [1,2-$^{13}C_2$]-acetate. Based on the

TABLE I. ^{13}C Chemical Shifts of Chaetoglobosins (in DMSO-d_6)

Carbon atom	Chaetoglobosins							
	A	B	C	D	E	F	G	J
1	172.50, s	172.44, s	173.79, s	171.91, s	174.04, s	173.55, s	173.93, s	172.00, s
3	52.13, d	50.11, d	52.25, d	51.70, d	51.00, d	52.08, d	52.08, d	53.16, d
4	45.90, d	57.52, d	48.35, d	44.75, d	57.31, d	46.82, d	57.45, d	46.30, d
5	35.32, d	125.18, s	36.10, d	31.14, d	125.60, s	35.61, d	125.57, s	34.12, d
6	57.02, s	133.62, s	56.87, s	150.83, s	133.55, s	56.84, s	133.52, s	138.45, s
7	61.25, d	67.60, d	60.28, d	70.10, d	67.82, d	61.19, d	67.21, d	125.25, d
8	46.04, d	46.99, d	48.24, d	48.05, d	49.28, d	47.90, d	50.07, d	47.61, d
9	62.88, s	60.67, s	62.25, s	61.22, s	61.77, s	63.64, s	60.87, s	65.72, s
10	32.64, t	31.60, t	31.88, t	32.20, t	31.85, t	29.83, t	31.79,[b] t	32.55, t
11	12.11, q	14.47, q	12.30, q	13.13, q	14.62, q	12.02, q	14.30, q	13.45, q
12	19.02, q	16.88, q	18.99, q	111.53, t	16.92, q	19.03, q	16.72, q	19.61, q
13	127.24, d	126.76, d	127.04, d	127.68, d	128.46, d	128.49, d	127.59, d	130.51,[d] d
14	133.20, d	133.40, d	133.11, d	134.07, d	133.55, d	132.73, d	133.34, d	130.65,[d] d
15	40.16, t	40.10, t	39.47, t	40.75, t	40.90, t	40.91, t	41.10, t	41.16, t
16	31.67, d	31.84, d	32.50, d	31.67, d	31.65, d	32.72, d	32.69, d	31.38, d
16-CH_3	20.82, q	20.89, q	19.28, q	20.84, q	19.82, q	19.52, q	19.01, q	20.75, q
17	137.55, d	137.90, d	155.56, d	137.98, d	148.35, d	147.56, d	156.44, d	136.03,[e] d
18	131.54, s	131.33, s	130.95, s	131.62, s	135.09, s	134.86, s	131.38, s	131.15, s
18-CH_3	10.48, q	10.53, q	9.93, q	10.47, q	12.05, q	11.87, q	9.86, q	10.47, q
19	81.46, d	81.78, d	208.00, s	81.34, d	210.22, s	208.91, s	196.79,[c] s	80.87, d
20	200.26, s	200.00, s	205.15,[a] s	200.18, s	70.33, d	70.30, d	205.90,[c] s	200.38, s
21	135.34, d	136.25, d	31.88, t	134.48, d	32.98, t	32.72, t	32.25,[b] t	135.88, d
22	133.74, d	134.11, d	37.04, t	133.08, d	36.55, t	36.37, t	36.87, t	132.41,[e] d
23	199.20, s	200.00, s	196.04,[a] s	199.04, s	204.06, s	203.89, s	208.68,[c] s	198.51, s
2′	120.72, d	120.85, d	120.86, d	120.67, d	121.11, d	120.76, d	120.84, d	120.61, d
3′	109.05, s	109.78, s	108.02, s	109.16, s	110.04, s	108.99, s	109.31, s	108.96, s
3′a	127.24, s	127.10, s	127.63, s	127.36, s	127.18, s	127.44, s	126.95, s	127.44, s
4′	117.95, d	117.88, d	118.25, d	117.92, d	118.01, d	117.84, d	117.81, d	118.22, d
5′	123.94, d	123.48, d	125.02, d	123.88, d	123.59, d	124.11, d	123.82, d	124.14, d
6′	118.34, d	118.34, d	118.25, d	118.25, d	118.54, d	118.33, d	118.33, d	118.23, d
7′	111.29, d	111.36, d	111.22, d	111.24, d	111.53, d	111.35, d	111.33, d	111.15, d
1′a	136.03, s	136.05, s	135.81, s	136.00, s	136.23, s	135.97, s	136.00, s	135.88, s

a—*e*) Assignments may be interchanged.

TABLE II. ^{1}H and ^{13}C NMR Data for Chaetoglobosin A

	^{1}H				^{13}H				
	In $CDCl_3$	In DMSO-d_6			In $CDCl_3$	In DMSO-d_6			
	δ(ppm)	δ(ppm)	Coupling pattern	J(Hz)	δ(ppm)	δ(ppm)	Multiplicity under OFR	$^{1}J_{C-H}$ (Hz)	$^{1}J_{C-C}$[a] (Hz)
1′(NH)	8.21	10.8	br s		—	—			
2′	6.94	7.02	d	1.4	122.5	120.7	d	150	—
3′	—	—			110.5	109.0	s	—	—
3′a	—	—			127.5	127.2	s	—	—
4′	7.1—7.5	6.9—7.4	m		118.3	117.9	d	136	—
5′					123.1	123.9	d	172	—
6′					120.0	118.3	d	147	—
7′					111.5	111.2	d	160	—
1′a	—	—			136.3	136.0	s	—	—
10	2.95	2.58	br dd	14.6, 7.6[b]	34.4	32.6	t	125	—
	2.63	2.77	br dd	14.6, 5.1[b]					
2(NH)	5.85	7.92	[d]	1.0[b]	—	—			
3	3.81	3.67	dddd	7.6,[b] 5.1,[b] 3.0,[c] 1.0[b]	52.7	52.1	d	144	—
4	3.03	[d]		[c]	47.2	45.9	d	132	—
11	1.24	0.77	d	7.3[e]	13.5	12.1	q	126	35.1
5	1.85	1.72	br qd	7.3,[e] 5.6[c]	36.2	35.3	d	128	36.0
12	1.29	1.14	s		19.8	19.0	q	126	—
6	—	—			57.9	57.0	s	—	23.4
7	2.78	2.69	d	5.4[f]	62.4	61.2	d	175	25.0
8	2.14	2.16	dd	8.1,[g] 5.4[f]	48.0	46.0	d	132	43.9
13	6.05	6.07	ddd	15.6, 8.1,[g] 1.5	128.2	127.2	d	150	43.9
14	5.20	5.13	ddd	15.6, 9.7,[h] 3.8[h]	133.7	133.2	d	153	52.7
15	1.8—2.4	1.8—2.4	m	[h],[i]	41.8	40.1	t	[j]	[j]
16	2.42	[j]		[i]	32.1	31.6	d	127	43.9
16-CH_3	1.00	0.96	d	6.6[i]	20.9	20.8	q	126	—
17	5.57	5.39	dd	9.0,[i] 1.2	140.3	137.5	d	152	43.9
18	—	—			131.9	131.5	s	—	43.4
18-CH_3	1.31	1.34	d	1.2	10.6	10.4	q	126	—
19	5.01	4.87	br s		81.8	81.4	d	145	43.9
19-OH	3.84	[d]			—	—			
20(C=O)	—	—			201.5	200.2	s	—	53.0
21[k]	6.50	7.33	d	16.4	131.7	135.3	d	160	54.2
22[k]	7.72	6.55	d	16.4	136.3	133.7	d	163	43.9
23(C=O)	—	—			196.7	199.2	s	—	45.0
9	—	—			63.3	62.8	s	—	48.3
1(C=O)	—	—			173.0	172.5	s	—	48.3

a) Observed in the [1,2-$^{13}C_2$]acetate-derived sample.
b, c, e–i) Mutual couplings confirmed by double irradiations.
d) Overlapping with H_2O signal.
j) Overlapping with the solvent signal.
k) See note 12).

assignments made for chaetoglobosins A and C after the ^{13}C-acetate incorporation experiments, other congeners were assigned by the comparison of the chemical shifts, as shown in Table I.

As a preliminary experiment for the biosynthetic studies, a time-coure study of the production of chaetoglobosin A was carried out using a thin-layer chromatography-densitometry method. In submerged culture employing the 68-SA-2 strain of *Chaetomium globosum* in potato-glucose-peptone medium,[4a] a rapid increase in the amount of chaetoglobosin A was observed on the 6th day after inoculation and the amount reached to the maximum on the 9th day.

For the biosynthetic studies, sodium [1-^{13}C]-, [2-^{13}C]-, and [1,2-$^{13}C_2$]-acetate, [1-^{13}C]- and [2-^{13}C]-DL-tryptophan, and [^{13}C-methyl]-L-methionine were administered to growing cultures of the strain. Using the ^{13}C-enriched samples of chaetoglobosins A and C, ^{13}C-NMR determinations were performed under identical conditions as described in "Experimental."

In [1-^{13}C]-acetate-derived chaetoglobosin A (Fig. 1(b)), the intensities of the signals due to C(1), C(5), C(7), C(13), C(15), C(17), C(19), C(21), and C(23) were enhanced relative of the natural abundance, while in [2-^{13}C]-acetate-derived chaetoglobosin A, those due to C(6), C(8), C(9), C(11), C(14), C(16), C(18), C(20), and C(22) were enhanced (Fig. 1(c)).

The spectrum of chaetoglobosin A derived from [1,2-$^{13}C_2$]-acetate was complicated by satellite signals due to couplings between enriched carbon atoms from different acetate units in a multiply labelled molecule.[13] This was circumvented by pulse feeding of the precursor. Thus the spectrum (COM) of [1,2-$^{13}C_2$]-acetate-enriched chaetoglobosin A showed doublets due to ^{13}C-^{13}C couplings in the doubly labelled acetate units besides singlets of natural abundance ^{13}C, as shown in Fig. 2 and Table II.

The results were consistent with biosynthesis from nine acetate/malonate units and, at the same time, the assignments of all the carbon atoms in chaetoglobosin A were decisively confirmed.

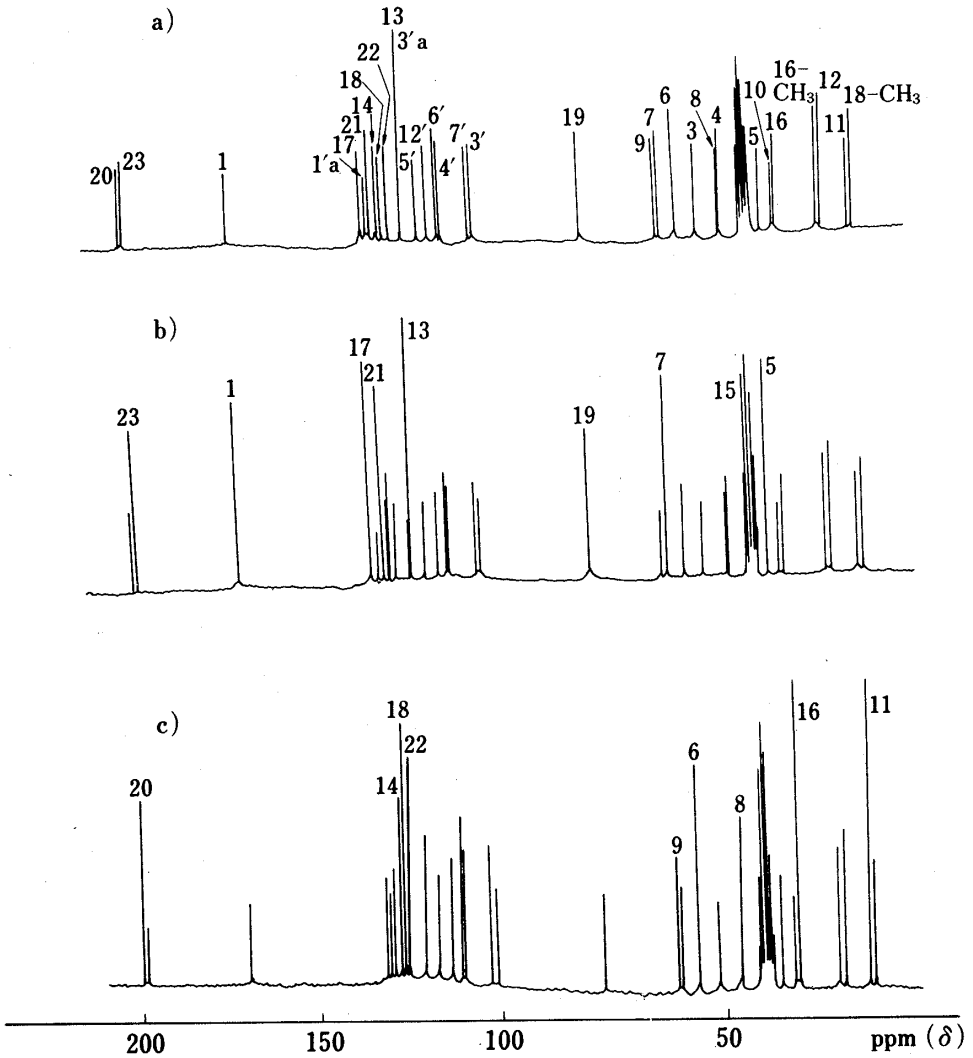


Fig. 1. ^{13}C-NMR Spectra (COM) of Chaetoglobosin A (in DMSO-d_6)

a) Natural abundance, b) [1-^{13}C]-acetate-derived, c) [2-^{13}C-]-acetate-derived.

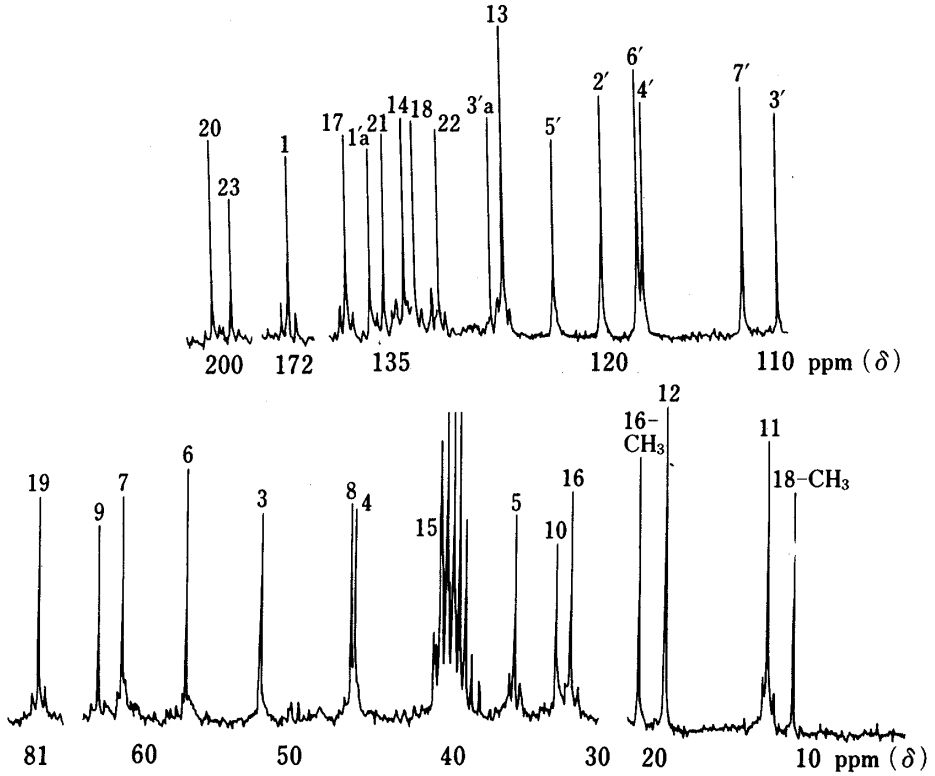


Fig. 2. ^{13}C-NMR Spectrun (COM) of Chaetoglobosin A derived from Sodium [1,2-$^{13}C_2$]Acetate (in DMSO-d_6)

In the feeding experiments of [1-^{13}C]- and [2-^{13}C]-DL-tryptophan, enrichments at C(4) and C(3), respectively, of chaetoglobosin A with high incorporation were observed (Figs. 3(a) and 3(b)). The results indicated the direct incorporation of tryptophan, presumably of naturally predominant L-tryptophan possessing the same (*S*)-configuration as C(3) of chaetoglobosin A.

Finally the feeding experiment with [^{13}C-methyl]-L-methionine clearly produced enhanced signal intensities of C(12) and the methyl carbons at C(16) and C(18) (Fig. 3(c)).

The results for chaetoglobosin C were exactly the same.

These incorporation experiments demonstrated that the biosynthetic pathways of chaetoglobosins are closely related to those of cytochalasins B and D;[9] the formation of chaetoglobosins from one unit of tryptophan, nine units of acetate/malonate, and three C_1-units was verified (Chart 3).

The biosynthesis of eight members of chaetoglobosins by *Chaetomium* spp., based on the proposed biogenetic scheme for the cytochalasans,[9,10] is outlined in Chart 4.

Following the completion of this work[1] we became aware that the same conclusion had been reached for chaetoglobosin A and 10-*O*-acetylchaetoglobosin A using the strain Lederle H-124 of *Chaetomium globosum* by Probst and Tamm.[14] Further work using [2-^{13}C, 2-$^{2}H_3$]- and [1-$^{13}C^{18}O_2$]-acetate is in progress.

Experimental

General Methods——The spectra were run on a JEOL FX-200 machine (^{13}C, 50.1 MHz) using solutions (20 mg in 0.25 ml) in DMSO-d_6. The standard conditions for measurement were as follows: pulse width, 7 μs; pulse delay, 26.9 μs; acquisition time, 0.6820 s; spectral width, 12004 Hz; data points, 16384; accumulation of FID, 3×10^4 times. ^{13}C precursors were purchased from Daiichi Kagaku Co., Ltd. and were all of 90 atom% ^{13}C purity.

Culture Conditions——The culture of *Chaetomium globosum* (68-SA-2)[4a] was incubated at 25°C in Sakaguchi flasks each containing 100 ml of potato-glucose-peptone medium[4a] on a reciprocal shaker (145

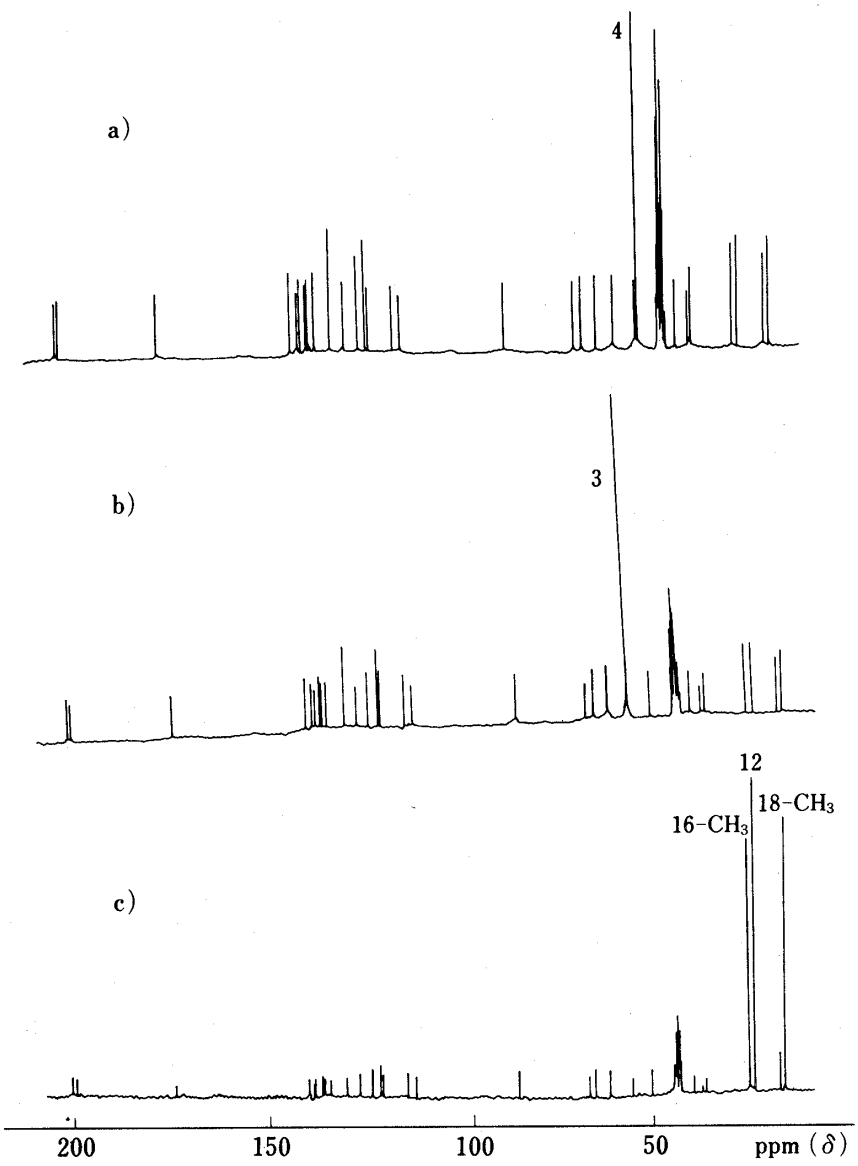


Fig. 3. ^{13}C-NMR Spectra (COM) of Chaetoglobosin A (in DMSO-d_6)

a) [1-^{13}C]-DL-Tryptophan-derived. b) [2-^{13}C]-DL-tryptophan-derived, c) [methyl-^{13}C]-L-methionine-derived.

rev/min).

The time-course study for the production of chaetoglobosins was carried out by examination of extracts of mycelia and filtrates by the thin-layer chromatography-densitometry method.

For each incorporation experiment ten flasks (1000 ml of medium) were used.

Incorporation of ^{13}C-Enriched Precursors——^{13}C-Labelled precursors were dissolved in sterilized water (50 ml) and 5 ml of the solution was added to each flask on the 6th day after inoculation (see Table III). In the case of [1,2-$^{13}C_2$]-acetate the solution was divided into three portions and given on the 6th and 7th days.

After 8 days of incubation, cultures were filtered and the mycelia were dried at 60°C for 12 h.

Isolation of ^{13}C-Enriched Chaetoglobosins——The dried mycelia (from ten flasks for each experiment) were extracted

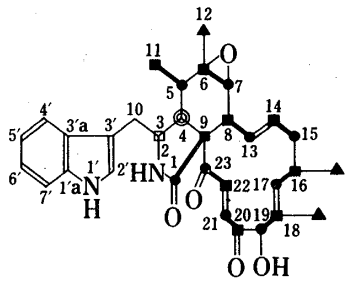


Chart 3. Labelling Pattern of Chaetoglobosin A

●, [1-^{13}C]acetate;
■, [2-^{13}C]acetate;
◎, [1-^{13}C]-DL-tryptophan;
□, [2-^{13}C]-DL-tryptophan;
▲, [methyl-^{13}C]-L-methionine;
●—■, [1.2-$^{13}C^2$]acetate.

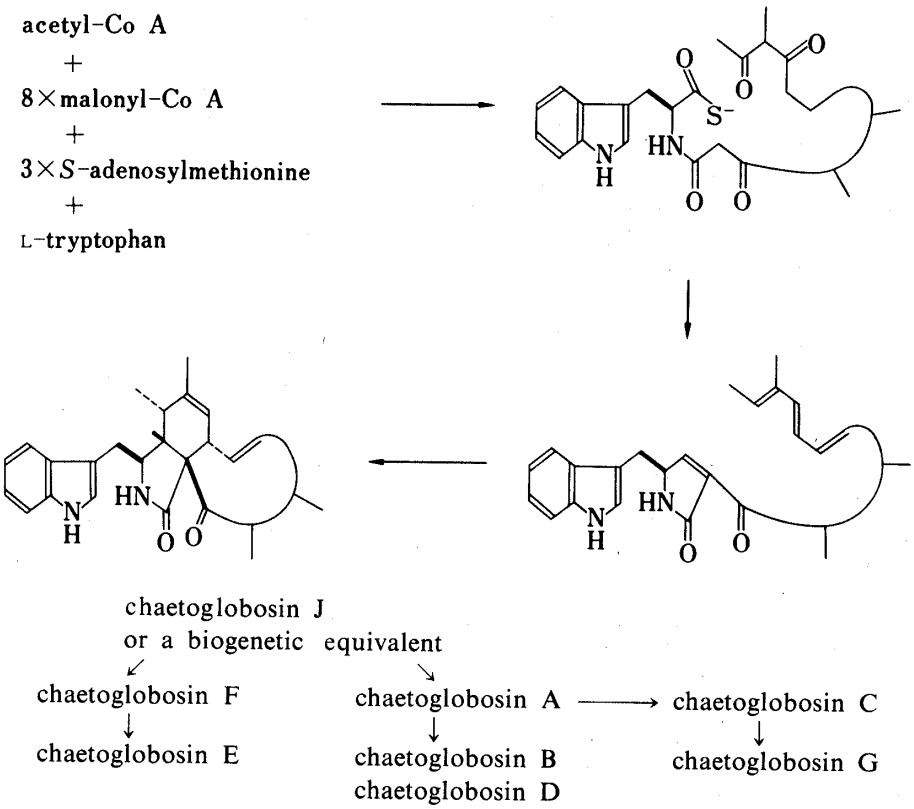


Chart 4. Biosynthesis of Chaetoglobosins

TABLE III. Incorporation of ^{13}C-Precursors

	Precursors		Chaetoglobosins A and C					
	Amount added (mg/l)	Isotopic purity (%)	Yield (mg/l)		Incorporation ratio[a] (%)		Signal enhancement[b]	
			A	C	A	C	A	C
Sodium[1-^{13}C]acetate	400	90	145	23	0.2	0.1	1.7	1.7
Sodium[2-^{13}C]acetate	400	90	151	43	0.7	0.1	2.2	2.4
Sodium[1,2-$^{13}C_2$]acetate	100	90	135	[c]	[d]	–	0.4	—
[1-^{13}C]-DL-tryptophan	45	90	111	84	9.2	11.0	5.0	5.1
[2-^{13}C]-DL-tryptophan	45	90	86	25	2.8	10.1	7.3	7.2
[^{13}C-Methyl]-L-methionine	200	90	54	[c]	1.3	—	8.8	—

a) Determined by the mass spectrometric method.[9c]
b) Ratio between peak heights of the observed resonances of a ^{13}C-enriched sample (the mean of the enhanced signals using C(7') as the standard signal) and of a natural-abundance sample.
c) Not isolated.
d) Not determined.

twice with CH_2Cl_2 (500 ml) for 2 days at room temperature. The combined extracts were dried over Na_2SO_4, concentrated to 50 ml, and applied to a silica gel (Merck, Kieselgel 60) column. Elution with benzene–AcOEt (4:1) gave chaetoglobosin C and elution with benzene–AcOEt (7:3) gave chaetoglobosin A. They were recrystallized from MeOH and CH_2Cl_2, and the physical properties of the products coincided well with those given in the previous papers.[4] The yields and the incorporations of the precursors are shown in Table III.

Acknowledgement The authors thank Drs. H. Kurata, S. Udagawa, and F. Sakabe of this institute for their help in the mycological part of this work. The authors also thank Professor U. Sankawa, University of Tokyo, for his interest and valuable suggestions.

References and Notes

1) The content of this paper was presented at the Annual Meetings of the Pharmaceutical Society of Japan, Kumamoto, April 1981, and Osaka, April 1982; Part III, S. Sekita, K. Yoshihira, S. Natori, and H. Kuwano, *Chem. Pharm. Bull.*, **30**, 1629 (1982).
2) Present address: *Meiji College of Pharmacy, 1-22-1, Yato-cho, Tanashi-shi, Tokyo 188, Japan.*
3) Preliminary communications: *a*) S. Sekita, K. Yoshihira, S. Natori, and H. Kuwano, *Tetrahedron Lett.*, **1973**, 2109; *b*) J. V. Silverton, T. Akiyama, C. Kabuto, S. Sekita, K. Yoshihira, and S. Natori, *ibid.*, **1976**, 1349; *c*) S. Sekita, K. Yoshihisa, S. Natori, and H. Kuwano, *ibid.*, **1976**, 1351; *d*) *Idem, ibid.*, **1977**, 2771.
4) *a*) S. Sekita, K. Yoshihira, S. Natori, S. Udagawa, F. Sakabe, H. Kurata, and M. Umeda, *Chem. Pharm. Bull.*, **30**, 1609 (1982); *b*) S. Sekita, K. Yoshihira, S. Natori, and H. Kuwano, *ibid.*, **30**, 1618 (1982); *c*) *Idem, ibid.*, **30**, 1629 (1982).
5) S. Udagawa, T. Muroi, H. Kurata, S. Sekita, K. Yoshihira, S. Natori, and M. Umeda, *Can. J. Microbiol.*, **25**, 170 (1979); S. Sekita, K. Yoshihira, S. Natori, S. Udagawa, T. Muroi, Y. Sugiyama, H. Kurata, and M. Umeda, *ibid.*, **27**, 766 (1981).
6) M. Binder and Ch. Tamm, *Angew. Chem., Intern. Ed.*, **12**, 370 (1973); S. Natori, "Mycotoxins in Human and Animal Health," ed. by J. V. Rodricks, C. W. Hesseltine, and M. H. Mehlman, Pathotox Publishers, Inc., Illinois, 1977, pp. 559-581; S. W. Tanenbaum (ed.), "Cytochalasins, Biochemical and Cell Biological Aspects," North Holland Publishing Co., Amsterdam, 1978.
7) I. Yahara, H. Harada, S. Sekita, K. Yoshihira, and S. Natori, *J. Cell. Biol.*, **92**, 69 (1982).
8) For new additional members of the group, see T. Fex, *Tetrahedron Lett.*, **22**, 2703 (1981); A. Probst and Ch. Tamm, *Helv. Chim. Acta*, **64**, 2056 (1981); P. S. Steyn, F. R. van Heerden, and C. J. Rabie, *J. Chem. Soc., Perkin Trans, 1*, **1982**, 541; R.J. Cole, D.M. Wilson, J.L. Harper, R.H. Cox, T.W. Cochran, H.G. Cutler, and D.K. Bell, *J. Agr. Food Chem.*, **30**, 301 (1982).
9) *a*) M. Binder, J.-R. Kiechel, and Ch. Tamm, *Helv. Chim. Acta*, **53**, 1797 (1970); *b*) C.-R. Lebet and Ch. Tamm, *ibid.*, **57**, 1785 (1974); *c*) W. Graf, J.-L. Robert, J.C. Vederas, Ch. Tamm, P.H. Solomon, I. Miura, K. Nakanishi, *ibid.*, **57**, 1801 (1974); *d*) J.C. Vederas, W. Graf, L. David, and Ch. Tamm, *ibid.*, **58**, 1886 (1975); *e*) J.-L. Robert and Ch. Tamm, *ibid.*, **58**, 2501 (1975); *f*) J.C. Vederas and Ch. Tamm, *ibid.*, **59**, 558 (1976), *g*) J.C. Vederas, T.T. Nakashima, and J. Diakur, *Planta Med.* **39**, 201 (1980).
10) Ch. Tamm, "Biosynthesis of Mycotoxins," ed. by P.S. Steyn, Academic Press, New York, 1980, pp. 269—299.
11) J.F. Johnson and W.C. Jankowski, "Carbon-13 NMR Spectra," John Wiley & Sons Inc., N. Y., 1972 .
12) As was discussed in the preceding papers[4b,c] the assignments for the C(21) and C(22) protons in $CDCl_3$ were reversed from those reported in the preliminary communication.[3a] The assignments made for the two carbons by SEL were harmonious with the signal enhancements observed in the [1-^{13}C]- and [2-^{13}C]-acetate derived samples in $CDCl_3$. However, the determination of the samples in DMSO-d_6 revealed that the chemical shifts of the two carbons in the enriched samples were reversed, and SEL indicated that the chemical shifts of the protons were also reversed in the solvent (Table II). This result indicates a change of the conformation of this part of the molecule in the two solvents.
13) A. G. McInnes and J. L. C. Wright, *Acc. Chem. Res.*, **8**, 313 (1975).
14) A. Probst and Ch. Tamm, *Helv. Chim. Acta*, **64**, 2065 (1981).