Useful Approach to Find the Plausible Biosynthetic Precursors of Secondary Metabolites using *P*-450 Inhibitors: Postulated Intermediates of Chaetoglobosin A[1]

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Treatment of *Chaetomium subaffine* with specific cytochrome *P*-450 inhibitors resulted in a new generation of plausible precursors of chaetoglobosin A **1**, which we have named prochaetoglobosins I **2**, II **3**, III **4** and IV **5**, whose structures were determined by spectroscopic analysis. HPLC analysis of mycelial extract treated with the inhibitors suggest that the accumulated metabolites are precursors in the biosynthesis of compound **1**. During this study, new less oxidized analogues, prochaetoglobosin III$_{ed}$ **6** and isochaetoglobosin J **7**, were also isolated, and their structures were elucidated in a similar way.

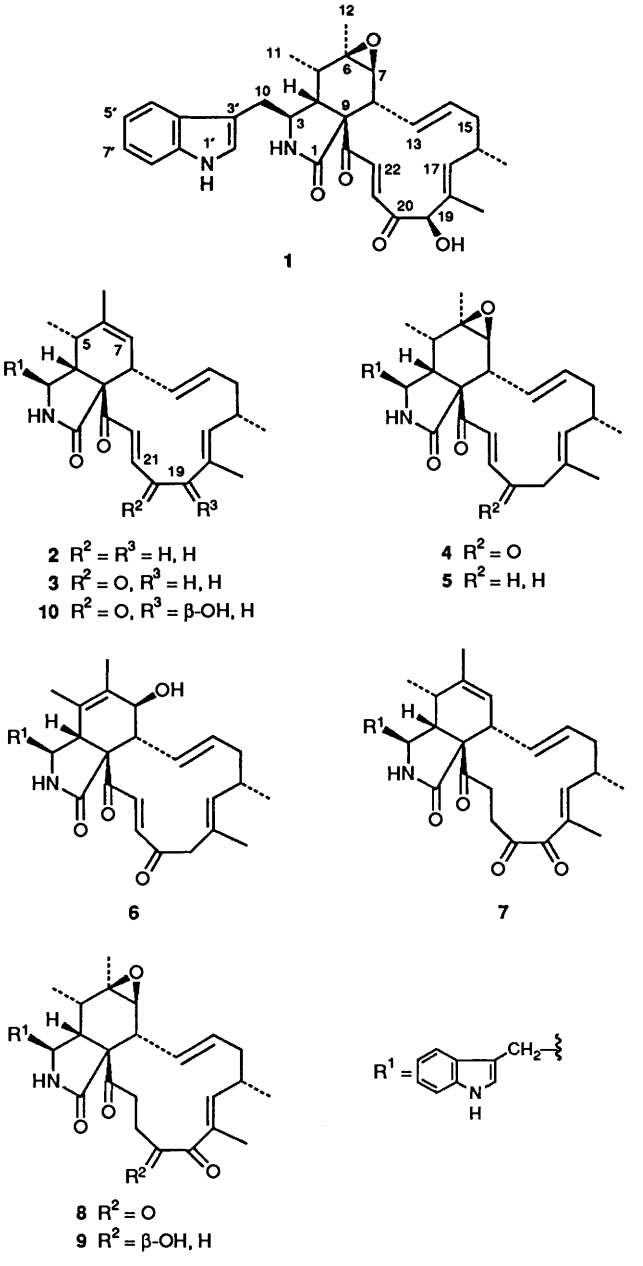
Cytochrome *P*-450 is a well known oxidative enzyme present in numerous organisms, from bacteria to mammals,[2] and plays an important role in the biosynthesis of sterols[2b] and the plant hormone gibberellin.[3] In order to control the biological activity of steroidal hormones or to cause damage to mycelial membrane when acting as antifungal agent, a variety of *P*-450 inhibitors have been developed.[4] In 1986, VanMiddlesworth *et al.* discovered that a less oxidized intermediate, trichodiene, was accumulated when the inhibitors were added directly to the fermentation medium of the strain producing the mycotoxin diacetoxyscirpenol.[5] This result suggests that it is possible to use the inhibitors to obtain a biosynthetic precursor without serious damage to the mycelial steroid metabolism which is essential to the maintenance of the cytoplasmic membrane. Recently, we have applied this methodology to biosynthetic studies of microbial secondary metabolite groups such as the terpenoid aphidicolin,[6] and the polyketides betaenone B[7] and nigericin.[8] In this paper, we extend this methodology to an investigation of the fungal metabolite chaetoglobosin A **1**.[9]

Chaetoglobosin A **1** belongs to the mycotoxin cytochalasans[10] which are acutely toxic to mammals and are cytotoxic to HeLa cells.[11] Their peculiar biological activities: inhibition of cell movement, cytoplasmic division, and causation of multinucleation, make them potentially useful agents for the study of fundamental cellular processes.[11] Although biosynthetic studies of chaetoglobosin building units have been carried out,[12] the late biosynthetic route has not been investigated. For the purposes of this investigation, we describe herein inhibitor experiments carried out to obtain pertinent data.

Results and Discussion

The inhibitor metyrapone **11**[4b] (1 mmol/flask) was added at the fifth day after inoculation to the culture of *Chaetomium subaffine*[13] which produces chaetoglobosins A **1**, C **8**[14] and F **9**[14] as major components. After an additional 9 days of fermentation, the mycotoxins were extracted from the mycelium. Compared with non-treated samples, the accumulation of four new metabolites was recognized in a less polar band on TLC in the extract. These metabolites were isolated by repeated chromatography to provide four constituents, **2**, **3**, **4** and **5**, which we have named as prochaeoglobosins I, II, III and IV, respectively.

From EI-HRMS, molecular formulae of the four components **2**, **3**, **4** and **5** were determined as $C_{32}H_{38}N_2O_2$, $C_{32}H_{36}N_2O_3$, $C_{32}H_{36}N_2O_4$ and $C_{32}H_{38}N_2O_3$, respectively. Their UV spectra and characteristic mass fragment at *m/z* 130 (indolylmethyl)



The structures of chaetoglobosins and prochaetoglobosins

Table 1 ^{1}H NMR data (500 MHz; $CDCl_3$) of chaetoglobosin A **1**, prochaetoglobosins I **2**, II **3**, III **4**, IV **5**, III_{ed} **6**, and isochaetoglobosin J **7**

(i) Chemical shifts

	1[a]	**2**	**3**	**4**	**5**	**6**	**7**
2-NH	5.78	5.44	5.47	5.80	5.73	5.64	6.01
10-H[a]	2.96	3.02	3.13	2.93	2.81	2.87	3.04
10-H[b]	2.63	2.57	2.58	2.64	2.72	2.67	2.70
3-H	3.81	3.30	3.30	3.79	3.72	3.53–3.58	3.40
4-H	3.03	3.10	3.15	3.01	2.69	3.40	2.44–2.53
5-H	1.85	2.47–2.53	2.43–2.53	1.86	1.91		2.44–2.53
11(Me)	1.24	1.34	1.43	1.23	1.10	1.64	1.30
12(Me)	1.29	1.78	1.60	1.28	1.17	1.73	1.74
7-H	2.80	5.37	5.30	2.80	2.89	3.93	5.42
8-H	2.14	2.65	2.56	2.16	2.39	2.10	3.00–3.09
13-H	6.06	6.11	5.91	6.08	6.53	6.19	6.08
14-H	5.23	5.12	5.19	5.22	5.17	5.35	5.04
15-H[a]	2.04	2.25	2.27	2.26	2.16–2.33	2.31	2.34
15-H[b]	2.27	1.94	2.0	2.17	1.88	2.06	1.93
16-H	2.45	2.40–2.47	2.43–2.53	2.40–2.50	2.43–2.53	2.46–2.56	2.66–2.75
16-Me	1.00	0.92	0.96	0.95	0.93	0.98	1.03
17-H	5.61	4.97	5.37	5.26	4.72	5.26	6.10
18-Me	1.32	1.52	1.49	1.49	1.56	1.55	1.65
19-H[a]	5.01	2.37–2.43	3.72	3.56	2.16–2.33	3.57	
19-H[b]		2.11–2.18	3.05	3.00	2.16–2.33	3.02	
20-H[a]		2.37–2.43			2.23–2.33		
20-H[b]		2.37–2.43			2.35–2.45		
21-H	6.49	6.79	6.45	6.44	6.82	6.72	2.90–3.04
22-H	7.75	7.09	8.20	7.65	6.35	7.62	1.99–2.10

(ii) Coupling constants (Hz)

	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{7,8}$	$J_{8,13}$	$J_{13,14}$	$J_{14,15a}$	$J_{14,15b}$	$J_{15a,16}$	$J_{15b,16}$	$J_{16,17}$	$J_{21,22}$
1[a]	<2	*b*	3.3	5.1	10.2	15.7	4.2	10.7	*b*	*b*	9.5	16.9
2	<2	3.9	3.9	<2	9.9	14.8	2.9	10.7	5.9	10.7	8.6	15.5
3	<2	5.0	5.0	1.5	10.5	14.9	3.6	11.1	1.9	11.1	9.4	16.5
4	<2	4.0	5.7	4.9	10.0	15.1	3.9	11.0	*b*	11.1	10.3	16.3
5	<2	<2	6.3	5.5	9.7	15.1	2.8	10.2	*b*	10.2	9.6	15.3
6	2	<2		8.9	10.1	15.3	3.4	10.4	*b*	10.4	9.7	*b*
7	<2	3.8	*b*	<2	10.3	14.4	2.5	11.1	*b*	11.1	9.9	*b*

[a] The data were obtained by measurement of compound at 500 MHz in $CDCl_3$. [b] The *J*-value was not obtained due to signal overlapping or broadening.

showed the presence of an indole group. The accumulated compounds were assumed to be less oxidized precursors of compound **1** considering that the differences between chaetoglobosin A **1** and new metabolites were the number of hydrogen and oxygen atoms. By comparison of the ^{13}C NMR spectrum of chaetoglobosin A **1**[12] with those of the accumulated compounds (Table 2), it was noted that the differences between the structures were concentrated in the areas around C-6,C-7 and C-19,C-20. Thus, the C-19 atoms of all new metabolites were present as methylene groups (**2**, δ_C 36.0; **3**, 53.1; **4**, 53.3; **5**, 37.8). Similarly to compound **1**, the epoxy group remained intact in compounds **4** [δ 58.0 (C), 62.5 (CH)] and **5** [δ 57.4 (C), 62.2 (CH)], while being replaced by olefin carbons in intermediates **2** [δ_C 139.8 (C), 126.7 (CH)] and in **3** [δ_C 140.7 (C), 125.7 (CH)]. Similarly, as in compound **1**, the carbonyl group at C-20 remained intact in intermediates **3** (δ 201.6) and **4** (δ 201.3), but was changed to a methylene group in compounds **2** (δ_C 28.7) and **5** (δ_C 30.0). In the H–H COSY spectrum of intermediate **2**, the correlation of an olefin proton at δ_H 5.37 (br s) with a methine at δ_H 2.65 (br d) which further gave a cross-peak with 13-H at δ_H 6.11 (ddd), clearly suggested the presence of a double bond between C-6 and C-7. This spectrum also indicated that a double triplet (21-H) at δ_H 6.79 was coupled with a methylene at δ_H 2.37–2.43. Analogously, a vinylic proton at δ_H 5.30 in intermediate **3** shows the presence of a double bond between C-6 and C-7, while a characteristic doublet at δ_H 2.80 and 2.89 in intermediates **4** and **5**, respectively, exhibited the presence of an epoxide. Moreover, the isolated geminally coupled signals for intermediates **3** [δ_H 3.05 and 3.72] and **4** [δ_H 3.00 and 3.56] supported the presence of a methylene group at C-19 in these two metabolites. Fortunately, the good separation of the signals in ^{1}H NMR spectra of the new metabolities **2**–**5** enabled us to assign all proton signals using homo decoupling and COSY experiments as shown in Table 1. Furthermore, the protonated carbon signals in the ^{13}C NMR of spectra of compounds **2**, **4** and **5** were assigned by C–H COSY and C–H HOHAHA[15] spectra, and the remaining quaternary carbon signals were assigned by consideration of the chemical shifts and comparison with the data of other chaetoglobosins in Table 2.[12] The above data allowed us to determine the structures of intermediates **2**–**5** as shown.

The stereochemistry of these new metabolites **2** and **4** was determined by NOESY and NOED experiments. Taking NOE data into consideration, MM2 calculations[16] for the preferred conformation of intermediate **2** were performed. The energy-minimized conformation is shown in Fig. 1. Since most of the observable NOEs between the corresponding protons situated separately within 3.5 Å of each other were detected, the conformation of intermediate **2**, especially the macrocyclic part, is well established. In addition, the observed conformation was similar to that of chaetoglobosin A **1** obtained from X-ray analysis.[17] An analogous observation was noted in the previous study[18] on the torsionally constrained 13-membered macrocyclic compound 7-oxobrefeldin A. The conformation of this compound has been studied by both molecular mechanics calculations and analysis of NOE data, and the conformations obtained from both methods were closely related to the X-ray

Table 2 ^{13}C NMR (125 MHz; $CDCl_3$) data of chaetoglobosin A **1**, prochaetoglobosin I **2**, II **3**, III **4**, IV **5**, III_{ed} **6**, and isochaetoglobosin J **7**

	1[a]	2	3[b]	4	5	6[c]	7[d]
C-1	173.2	173.9	172.6	173.3	174.5	173.1	175.2
C-3	52.8	53.6	53.7	52.6	52.7	52.4	54.2
C-4	46.8	50.2	49.8	47.4	48.2	58.0	51.7
C-5	36.3	34.8	34.6	36.2	36.6	126.1	35.0
C-6	58.0	139.8	140.0	58.0	57.4	135.4	138.7
C-7	62.4	126.7	125.7	62.5	62.2	68.6	126.2
C-8	48.8	47.2	46.7	47.8	47.2	47.9	46.2
C-9	63.4	66.0	66.0	63.4	61.2	61.2	66.0
C-10	33.7	35.0	34.5	34.5	34.1	33.1	33.6
C-11	13.2	14.3	14.9	13.5	12.8	14.0	13.9
C-12	19.7	20.1	20.4	19.6	19.4	17.9	19.5
C-13	128.3	129.5	130.7	127.9	125.8	127.4	129.0
C-14	133.1	132.7	132.0	134.0	136.0	134.5	132.6
C-15	41.7	41.2	41.7	41.8	40.1	41.4	39.6
C-16	32.0	32.4	32.3	32.3	32.9	32.5	33.5
16-Me	20.9	21.6	21.3	21.2	21.6	21.3	20.1
C-17	139.9	139.9	138.0	138.3	132.6	138.2	155.9
C-18	132.3	130.6	128.4	128.2	130.5	128.1	131.9
18-Me	10.5	15.3	15.4	16.0	16.6	16.3	10.5
C-19	81.7	36.0	53.1	53.3	37.8	53.5	196.2
C-20	201.4	28.7	201.6	201.3	30.0	200.7	204.9
C-21	131.6	146.5	136.7	135.5	149.2	137.5	32.5
C-22	136.1	128.2	134.4	134.9	126.4	136.9	37.8
C-23	197.1	197.6	197.8	197.3	196.9	197.9	208.3

[a] Ref. 9*b*. [b–d] Assignments were made by comparison of those of compounds **2** and **4** and ref. 12.

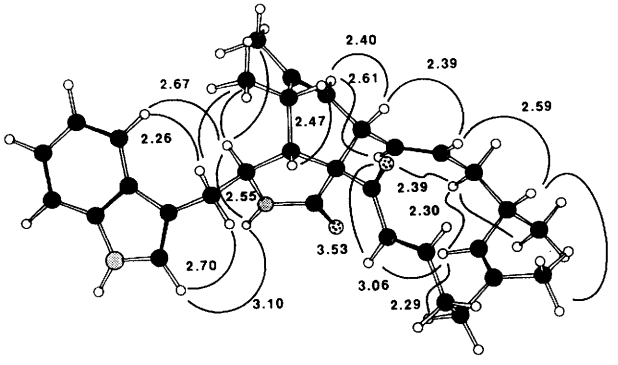


Fig. 1 The energy-minimized conformation of prochaetoglobosin I **2** obtained by considering the observed NOEs. The figures are the distances (Å) between the corresponding protons.

results.[18] Comparison of NMR data of intermediates **2** and **4** with those of intermediates **3** and **5** indicates that the stereochemistry of the latter compounds is as shown in their display structures.

In addition to prochaetoglobosins I **2**, II **3**, III **4** and IV **5**, two other metabolites, **6** and **7**, which we have named as prochaetoglobosin III_{ed}* and isochaetoglobosin J,* were also isolated from the inhibitor-treated mycelia. Their molecular formula, $C_{32}H_{36}N_2O_4$, obtained from EI-HRMS, suggested that they were isomers of chaetoglobosin J **10**[14] or prochaetoglobosin III **4**. In the ^1H NMR spectrum of compound **6**, two olefinic methyl groups and an allylic oxymethine resonated at δ_H 1.64, 1.73 and 3.93, the spectrum lacking signals for secondary and tertiary methyl groups and the epoxy methine

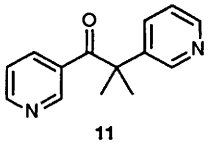
* Subscripted characters, 'ed' means that this compound is an *endo*-allyl alcohol isomer [5(6)-en-7β-ol] of the corresponding epoxide at C-6 and C-7, whereas the prefix, 'iso' means that this isomer possesses a 21,22-dihydro-19,20-diketo form replacing the 19-hydroxy-20-keto moiety.[14] To simplify the trivial names, we propose the above terminology for the new metabolites of this family.

Table 3 HPLC analytical data[a] of chaetoglobosins A **1**, C **8** and F **9** and prochaetoglobosins I **2**, II **3**, III **4** and IV **5** in the mycelial extracts treated with the *P*-450 inhibitor metyrapone **11**

Quantity (mmol)[b]	1	2	3	4	5	8	9
0.0	17.28	0.13	0.09	0.27	0.09	2.64	8.00
0.01	16.32	0.16	0.15	0.22	0.08	2.24	6.90
0.1	13.28	0.68	0.40	0.59	0.18	4.82	6.48
1.0	6.18	5.19	0.76	1.94	1.37	4.79	4.98

[a] The values are peak areas in HPLC analysis of compounds **1**–**5**, **8** and **9** monitored by UV absorption at 220 nm. [b] The quantity of inhibitor **11** per flask.

in compound **4**. These indicate that compound **6** is an *endo*-allyl alcohol isomer of **4**, similar to the relationship between chaetoglobosins A **1** and B.[9] Similarly, the presence of new ketone carbonyl signals at δ_C 196.2 and two methylene signals at δ_C 32.5 and 37.8 in the ^{13}C NMR spectrum of compound **7**, and the absence of signals for two olefinic protons at C-21 and C-22 and a secondary methyl at C-5 in its ^1H NMR spectrum, suggest that metabolite **7** is an isomer of compound **10** just as chaetoglobosin C **8**[12,14] is an isomer of compound **1**. The stereochemistry of metabolites **6** and **7** at C-3, C-16 and the cyclohexane part is the same as in the closely related analogues on the basis of coupling constants and chemical shifts.[9,12] In particular, large coupling constants (>9 Hz) of 8-H–13-H, 13-H–14-H, 14-H–15-Hb, 15-Hb–16-H and 16-H–17-H in both metabolites **6** and **7** show that this part is conformationally rigid and that these protons are in an *anti* relationship, as Natori and co-workers had proposed previously.[14] Owing to this and other NMR data in Tables 1 and 2, the assigned structure for compounds **6** and **7** are as depicted.



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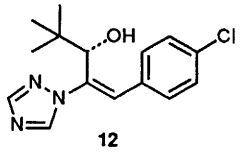

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The dose effect of the *P*-450 inhibitor metyrapone **11** was then investigated. Since the ε-values of a UV absorption at 220 nm in the chaetoglobosins (CGs) and prochaetoglobosins (PCGs) are nearly the same (4.5–4.7),[11b] the total mount of CGs and PCGs can be estimated by the summation of peak areas in HPLC analysis. The mycelia treated with metyrapone **11** were obtained as described earlier. The mycelial extracts were roughly separated by preparative TLC (PLC) to eliminate a large amount of inhibitor, and each fraction was subjected to HPLC analysis. The results in Table 3 showed that treatment of inhibitor at high concentration (1.0 mmol) did not cause a serious decrease in the total amount of CGs and PCGs, but did affect the metabolite pattern depending on concentration. Thus, the sum of the normal CGs (A, C and F) was dominant in control experiments without inhibitor (98%) but this was decreased to 63% at 1.0 mmol and the remainder was replaced by PCGs. Among the accumulated PCGs, more than half was compound **2** and the amounts of PCGs **2**–**5** were increased at 1.0 mmol 7–40 times over that of control experiments without inhibitor. After the introduction of inhibitor, the increase of chaetoglobosin C **8** was assumed to be due to an isomerization of A **1** owing to the basicity of metyrapone **11**. It has been reported that conversion from chaetoglobosin A **1** in compound **8** occurs easily with a weak base.[9] These observations suggest that *P*-450 inhibitors block hydroxylations at C-19 and C-20, and possibly epoxidation as well. The type of inhibitors was also an important factor for the effective accumulation of less

oxidized analogues. Metyrapone **11** was 25 times more effective than S-3370D **12** for the preparation of compound **2**.[1]

We would like to point out that most of the metabolites obtained by inhibitor experiments are non-oxidized forms of the final products such as trichodiene,[5] trideoxyaphidicolin,[6] probetaenone I,[7] prochaetoglobosin I **2**, *ent*-kaurene,[3,4b] and lanosterol.[4b] If the accumulation of these compounds is caused by the specific inhibition of *P*-450, these observations indicate that cytochrome *P*-450 frequently involves the initial oxidation of secondary metabolites.

Since the inhibitors do not block each oxidation completely, the information for the sequence of the oxidations can be provided. Therefore, inhibitor experimentation might be effective in biosynthetic studies of the metabolites that involve sequential oxidations. Finally, the experiment described here provides not only information for the timing of oxidation but also the isotopically labelled precursors[6,7] which are usually difficult to prepare from terminal metabolities by chemical deoxygenation. The biosynthetic pathway at late stages of chaetoglobosin A **1** will be discussed in the following paper.

Experimental

M.p.s were determined on a Yanaco Micro-melting Point Apparatus MP-30, UV spectra on a Hitachi U-3210 spectrophotometer, IR spectra on Hitachi 285 and Perkin-Elmer 1720 spectrophotometers, ^1H and ^{13}C NMR spectra on a Bruker AM-500 spectrometer for solutions of $CDCl_3$, mass spectra on JEOL DX-300 and 01SG-2 spectrometers, and optical rotations on a Jasco DIP-4 polarimeter. MM2 calculations were performed by Cambridge Scientific Computing Chem 3D Plus. Column chromatography used Merck Kieselgel 60 (0.04–0.063 mm) and Wakogel C-200 (0.075–0.15 mm) and TLC was performed on Merck Kieselgel 60 F_{254}. HPLC was performed with Waters 600E and 741 data modules or a Hitachi 635 and a GL Science reversed-phase column (Inertsil ODS-2, 5 μm, 4.6 × 250 mm, or 10 μm, 20 × 250 mm). Metyrapone **11** was purchased from Aldrich.

Growth of Chaetomium subaffine *Sergejeva IFO8361* (*ATCC22132*).—The culture medium in 500 cm^3 flasks was prepared by autoclaving water (10 cm^3) and pre-soaked polished rice (100 g). After inoculation, the organism was grown at 25 °C for three weeks.

Treatment of Ch. subaffine *with Cytochrome* P-*450 Inhibitors*.—Metyrapone **11** (226.3 mg) was dissolved in EtOH (2 cm^3) and the solution was adjusted to 100 cm^3 with sterilized water. At the fifth day after inoculation, this solution was distributed equally into ten flasks (inhibitor, 1 mmol/flask). These flasks were shaken manually. After a further 9 days of fermentation, CH_2Cl_2 (~150 cm^3) was directly poured into each flask and extracted twice, then solvent was replaced to EtOAc and each flask was again extracted twice. The combined extracts were dried over Na_2SO_4 and concentrated under reduced pressure. The concentrate was chromatographed over SiO_2 column with sequential elution with C_6H_6–EtOAc [4:1 (fr. 1), 2:1 (fr. 2), 1:2 (fr. 3)], and fr. 1 was further eluted with C_6H_6–EtOAc (6:1) to give fr. 1–1. These fractions were separated by HPLC with the following solvent systems (MeOH–water) (i) 90:10 for fr. 1–1 and fr. 3; (ii) 85:15 for fr. 2] to yield prochaetoglobosin I **2** (11.7 mg) and II **3** (3.0 mg) from fr. 1–1, prochaetoglobosin III **4** (10.5 mg), III_{ed} **6** (2.6 mg), and isochaetoglobosin J **7** (2.1 mg) from fr. 2, and prochaetoglobosin IV **5** (2.6 mg) and compound **10** (3.3 mg) from fr. 3. Compound **10** was identical with chaetoglobosin J in its ^1H NMR (500 MHz) and MS spectra.

Compound **2**: crystals, m.p. 214–216 °C (from MeOH); $[\alpha]_D$ -101.3×10^{-1} deg cm^2 g^{-1} (*c* 0.31 in MeOH); ν_{max}(NaCl)/cm^{-1} 3333, 2962, 1693, 1619, 1457 and 1453; λ_{max}(MeOH)/nm ($\log_{10}$ ε) 223 (4.45) and 291 (3.50); δ_H(500 MHz; $CDCl_3$) see Table 1; δ_C(125 MHz; $CDCl_3$) see Table 2; *m/z* 482.2932 (M^+. $C_{32}H_{38}N_2O_2$ requires M, 482.2935); EIMS *m/z* 482 (M^+), 352, 211 and 130.

Compound **3**: pale yellow crystals, m.p. 187–189 °C (from MeOH); $[\alpha]_D$ −204.2 (*c* 0.38 in MeOH); ν_{max}(NaCl)/cm^{-1} 3300, 2926, 1683, 1457 and 1453; λ_{max}(MeOH)/nm ($\log_{10}$ ε) 221 (4.45) and 273 (3.59); δ_H(500 MHz; $CDCl_3$) see Table 1; δ_C(125 MHz; $CDCl_3$) see Table 2; *m/z* 496.2741 (M^+. $C_{32}H_{36}N_2O_3$ requires M, 496.2729); EIMS *m/z* 496 (M^+), 212 and 130.

Compound **4**: pale yellow crystals, m.p. 137–139 °C (from 10% aq. MeOH); $[\alpha]_D$ −210.4 (*c* 0.23 in MeOH); ν_{max}(NaCl)/cm^{-1} 3352, 2962, 2926, 1691, 1457 and 1252; λ_{max}(MeOH)/nm ($\log_{10}$ ε) 221 (4.70), 280 (3.87) and 290 (3.79); δ_H(500 MHz; $CDCl_3$) see Table 1; δ_C(125 MHz; $CDCl_3$) see Table 2; *m/z* 512.2671 (M^+. $C_{32}H_{36}N_2O_4$ requires M, 512.2677); EIMS *m/z* 512 (M^+), 494, 382 and 130.

Compound **5**: crystals, m.p. 256–258 °C (from $CHCl_3$); $[\alpha]_D$ −54.5 (*c* 0.02 in MeOH); ν_{max}(NaCl)/cm^{-1} 3300, 2900, 2850, 1700, 1610 and 1060; λ_{max}(MeOH)/nm ($\log_{10}$ ε) 222 (4.45) and 291 (3.67); δ_H(500 MHz; $CDCl_3$) see Table 1; δ_C(125 MHz; $CDCl_3$) see Table 2; *m/z* 498.2880 (M^+. $C_{32}H_{38}N_2O_3$ requires M, 498.2884); EIMS *m/z* 498 (M^+), 480, 350, 131 and 130.

Compound **6**: pale yellow crystals, m.p. 153–155 °C (from $CHCl_3$); $[\alpha]_D$ −15.3 (*c* 0.065 in MeOH); ν_{max}(NaCl)/cm^{-1} 3300, 2900, 1670, 1240 and 1080; λ_{max}(MeOH)/nm ($\log_{10}$ ε) 222 (4.43), 282 (3.66) and 291 (3.60); δ_H(500 MHz; $CDCl_3$) see Table 1; δ_C(125 MHz; $CDCl_3$) see Table 2; *m/z* 512.2665 (M^+. $C_{32}H_{36}N_2O_4$ requires M, 512.2677); EIMS *m/z* 512 (M^+), 494, 290, 184 and 131.

Compound **7**: pale yellow crystals, m.p. 117–119 °C (from $CHCl_3$); $[\alpha]_D$ −20.0 (*c* 0.070 in MeOH); ν_{max}(NaCl)/cm^{-1} 3300, 2950, 2900, 1680 and 1080; λ_{max}(MeOH)/nm ($\log_{10}$ ε) 221 (4.59) and 291 (3.74); δ_H(500 MHz; $CDCl_3$) see Table 1; δ_C(125 MHz; $CDCl_3$) see Table 2; *m/z* 512.2703 (M^+. $C_{32}H_{36}N_2O_4$ requires M, 512.2677); EIMS *m/z* 512 (M^+), 494, 212 and 131.

Semi-quantitative HPLC Analysis of Chaetoglobosin Analogues obtained under Inhibitor Treatment.—Four 100 cm^3 flasks containing water (2.5 cm^3) and polished rice (25 g) were autoclaved. On the fifth day after inoculation a solution of metyrapone **11** [0.01 mmol in water (20 cm^3)] was added in equal portions to the cultures, and these were incubated for a further 9 days. Similarly, the experiments for the different inhibitor concentrations (0.1 and 1.0 mmol) were undertaken and that without the addition of the inhibitor was used as the control. The cultures were extracted with CH_2Cl_2 (50 cm^3 × 2) and EtOAc (50 cm^3 × 2). Then, portions (1/50) of the obtained extracts were separated by PLC [C_6H_6–$CHCl_3$–acetone (2:5:3)] into two fractions (A: R_f 0.17–0.68 containing compounds **2** and **3**; B: R_f 0.68–0.90 containing compounds **1**, **4**, **5**, **8** and **9**) and eluted with EtOAc. These fractions were individually taken up in MeOH (5 cm^3), and aliquots (50 mm^3) of each solution were subjected to analysis. Analytical conditions were as follows: UV 220 nm, flow rate 1 cm^3/min, fr. A: MeOH–water (85:15) [t_R(min) **2** 11.8, **3** 10.4]; fr. B: MeCN–water (55:45) [t_R(min) **1** 10.1, **4** 22.9, **5** 30.0, **8** 15.0, **9** 8.1].

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