199. Chaetoglobosin M, a New Metabolite of a Mutant of *Diplodia macrospora*, Belonging to the Family of (1*H*-Indol-3-yl)-Substituted 10,11-Diethyl-10,11-dinorcytochalasans

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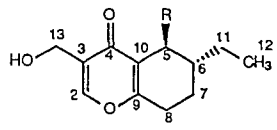
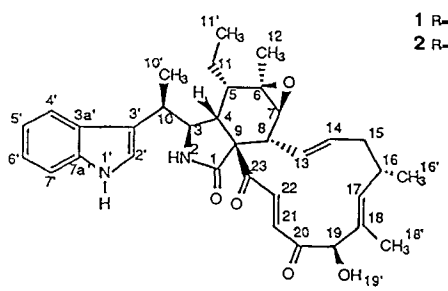
From a mutant of *Diplodia macrospora*, chaetoglobosin M (**5**), ergosterol, β-sitosterol, and a third sterol, most likely stigma-5,7,22-trien-3β-ol, were isolated. Metabolite **5** is a new member of the (1*H*-indol-3-yl)-substituted 10,11-diethyl-10,11-dinorcytochalasans. Its structure was determined by ^{1}H- and ^{13}C-NMR and mass spectrometry.

Diplodia macrospora produces four known secondary metabolites, diplosporin (**1**) [1], 5-deoxydiplosporin (**2**) [2], and the chaetoglobosins K (**3**) [3] and L (**4**) [4], the two latter belonging to the group of 10,11-diethyl-10,11-dinorchaetoglobosins. A common biogenetic building block of diplosporin (**1**) and the chaetoglobosins, namely a pentaketide, methylated in two positions, suggested a close relationship between these metabolites [4]. In order to test this hypothesis which at the same time would allow more detailed insight into the biogenetic pathway of the chaetoglobosins, mutants of *Diplodia macrospora* which were blocked in diplosporin biosynthesis, were searched. The influence of these mutations on the production of the chaetoglobosins K (**3**) and L (**4**) and the formation of possible intermediates in the biogenetic pathway were examined. Beyond the proper purpose of these investigations, examination of the secondary metabolites of these mutants led to the isolation of three known sterols and of a new metabolite, named chaetoglobosin M (**5**), belonging to the class of the 10,11-diethyl-10,11-dinorchaetoglobosins.

Mutants of *Diplodia macrospora* which were blocked in diplosporin biosynthesis were obtained by UV irradiation. To prepare spore suspensions, cultures on a solid corn medium were irradiated with visible light during growth. Microscopic inspection of the suspended mycelial pads revealed long bipartite spores. Spore suspensions which were stirred with constant speed were irradiated with UV light and incubated on agar plates containing a mixture glycerol/lactose/potassium citrate 1:1:1 as carbon source which effected stationary growth. The colonies were transferred to an agar complex medium. For the selection of the strains which produced no more diplosporin (**1**), extracts of these cultures were examinated by TLC and compared with those of the wild type of *Diplodia macrospora*. By this procedure, 10 (DM 2–DM 11) out of 700 strains analyzed were obtained which did not produce diplosporin (**1**) any more. These 10 strains were incubated on a solid wheat medium in standing cultures. The crude extracts of these cultures were tested by TLC and HPLC analyses. Whereas nine of these ten strains (DM 2–DM 10) produced still the chaetoglobosins K (**3**) and L (**4**), one produced 5-deoxydiplosporin



1 R=OH
2 R=H



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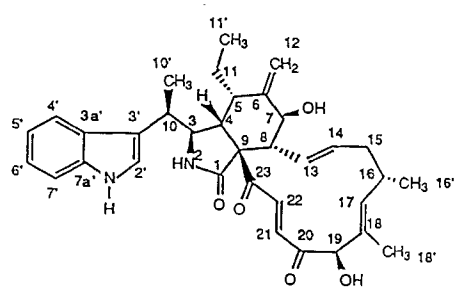

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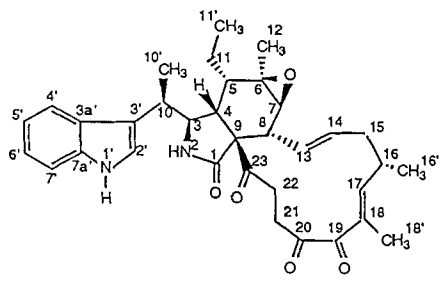

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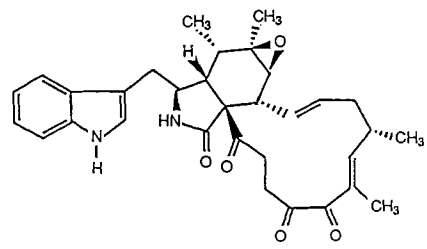

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(**2**), which was previously not detected in cultures of the wild type growing on this medium, and still a small quantity of diplosporin (**1**), but no chaetoglobosins K (**3**) and L (**4**). In the extracts of strain DM 7, four additional metabolites were detected. Three of them were identified as sterols, namely ergosterol, β-sitosterol, and a compound which is very likely to be identical with stigma-5,7,22-trien-3β-ol. The ^{1}H- and ^{13}C-NMR spectra of the fourth substance revealed a close relationship to the chaetoglobosins K (**3**) and L (**4**) (*Tables 1* and *2*). It turned out to be a new metabolite to which we assigned structure **5** and the name chaetoglobosin M. Thus, **5** is a homologue of chaetoglobosin C (**6**) [5].

The NMR data of **3** were assigned by a H,C-correlated spectrum and ^{1}H-double resonance [6], in some cases differing from former assignments [3]. The assignments of **4** resulted from a comparison with those of **3** [6]. The chemical shifts of the majority of the signals in the ^{1}H- and ^{13}C-NMR of **5** were almost identical with those of the chaetoglobosins **3** and **4** (*Tables 1* and *2*). But in the ^{1}H-NMR, the signals for H–C(21) and H–C(22) as well as that for H–C(19), present in the spectrum of chaetoglobosin K (**3**), were absent. At 2–3 ppm, new signals appeared. The signal of one Me group was shifted downfield to 1.82 ppm. In the ^{13}C-NMR, two signals from the olefinic range are shifted to the aliphatic range. An additional signal of a C=O group appears at 197 ppm. The fragmentation pattern of the MS of **5** did not exhibit any significant difference to that of chaetoglobosin K (**3**).

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Table 1. *^{13}C-NMR Data of Chaetoglobosin K* (**3**), *L* (**4**), *and M* (**5**). **3** and **5**: 90.5 MHz; **4**: 100.6 MHz (broad-band decoupled); in $CDCl_3$.

Atom	3	4	5	Atom	3	4	5
C(1)	173.5	172.6	175.5	C(19)	81.7	81.7	196.8
C(3)	56.8	56.1	57.8	C(20)	201.8	201.6	205.6
C(4)	44.1	39.8[a])	46.6[c])	C(21)	131.2	131.5	32.6[d])
C(5)	44.1	41.0[a])	44.4[c])	C(22)	136.6	137.0	38.3[d])
C(6)	57.4	146.8	56.7	C(23)	197.9	197.7	208.3
C(7)	61.7	71.1	60.6	C(2′)	121.9	121.4	122.5
C(8)	49.3	50.6	49.6	C(3′)	116.7	117.7	115.9
C(9)	63.9	62.2	63.5	C(4′)	118.6	118.7	118.5
C(10)	36.3	35.0	36.3	C(5′)	122.5	122.5	122.5
C(11)	21.8	21.4	21.5	C(6′)	120.0	119.8	120.0
C(12)	19.7	114.3	19.4	C(7′)	111.7	111.6	111.6
C(13)	128.4	128.5	126.7	C(10′)	13.8	12.9[b])	16.2[e])
C(14)	133.5	136.0	134.5	C(11)	12.7	12.1[b])	12.6[e])
C(15)	42.0	42.0	40.2	C(16′)	21.0	21.1	19.4
C(16)	32.0	32.1	33.4	C(18′)	10.6	10.7	10.3
C(17)	140.4	140.3	156.5	C(3′a)	126.1	126.0	126.7
C(18)	132.4	132.7	132.3	C(7′a)	136.6	136.6	136.1

[a])[b])[c])[d])[e]) Attribution can be exchanged.

Table 2. *^{1}H-NMR Data of Chaetoglobosin K* (**3**), *L* (**4**), *and M* (**5**). **3** and **5**: 360 MHz; **4**: 400 MHz; in $CDCl_3$.

H-Atom	3	4	5
H–C(3)	3.83 (*m*)	3.55 (*m*)	3.78 (*m*)
H–C(4)	1.50 (*m*)	2.55 (*m*)	1.5–1.7
H–C(7)	2.75 (*d*, *J* = 6 Hz)	3.90 (*d*, *J* = 10 Hz)	2.65 (*d*, *J* = 7 Hz)
H–C(8)	2.13 (*m*)	2.39 (*m*)	2.28 (*m*)
H–C(10)	3.10 (*m*)	3.10 (*m*)	3.12 (*m*)
H–C(11)	1.80 (*m*), 1.50 (*m*)	1.57 (*m*), 1.88 (*m*)	1.5–1.7 (*m*), 1.7–1.8 (*m*)
H–C(12)	1.30 (*s*)	5.29 (*s*), 5.33 (*s*)	1.22 (*s*)
H–C(13)	6.10 (*dd*)	5.99 (*dd*)	6.14 (*dd*)
H–C(14)	5.23 (*m*)	5.37 (*m*)	5.10 (*m*)
H–C(15)	2.23 (*m*), 2.02 (*m*)	2.03 (*m*), 2.28 (*m*)	1.88 (*m*)[a]), 2.35 (*m*)[a])
H–C(16)	2.42 (*m*)	2.47 (*m*)	2.14–2.4 or 2.6–2.8
H–C(17)	5.58 (*d*, *J* = 9 Hz)	5.61 (*d*, *J* = 9 Hz)	6.04 (*d*, *J* = 9 Hz)
H–C(19)	5.02 (*d*, *J* = 5 Hz)	5.08 (*d*, *J* ≈ 1 Hz)	
H–C(21)	6.50 (*d*, *J* = 18 Hz)	6.63 (*d*, *J* = 18 Hz)	2.1–2.4[a])
H–C(22)	7.69 (*d*, *J* = 18 Hz)	7.94 (*d*, *J* = 18 Hz)	2.6–2.8[a])
H–C(2′)	6.90 (*d*, *J* ≈ 1 Hz)	6.90 (*d*, *J* ≈ 1 Hz)	6.90 (*d*, *J* ≈ 2 Hz)
H–C(4′)	7.50 (*d*, *J* = 7 Hz)	7.50 (*d*, *J* = 7 Hz)	7.55 (*d*, *J* = 7 Hz)
H–C(5′)	7.15	7.10–7.22 (*m*)	7.13–7.25
H–C(6′)	7.15 (*m*)	7.10–7.22 (*m*)	7.13–7.25
H–C(7′)	7.32 (*d*, *J* = 7 Hz)	7.37 (*d*, *J* = 7 Hz)	7.35 (*d*, *J* = 7 Hz)
H–C(10′)	1.14 (*d*, *J* = 7 Hz)	1.10–1.15	1.30 (*d*, *J* = 7 Hz)[b])
H–C(11′)	1.10 (*d*, *J* = 7 Hz)	1.10–1.15	0.97 (*d*, *J* = 7 Hz)
H–C(16′)	1.00 (*d*, *J* = 7 Hz)	1.00 (*d*, *J* = 7 Hz)	1.05 (*d*, *J* = 7 Hz)[b])
H–C(18′)	1.31 (*d*, *J* ≈ 1 Hz)	1.32 (*s*)	1.82 (*d*, *J* ≈ 1 Hz)
HO–C(7′)		2.00	
HO–C(19)	3.88 (*d*, *J* = 5 Hz)	3.90	
H–N(1)	6.02 (*s*)	5.61	7.05 (*s*)
H–N(1′)	8.38 (*s*)	8.35 (*s*)	8.55 (*s*)

[a])[b]) *Attribution can be exchanged.*

Experimental Part

General. $[\alpha]_D$: *Perkin-Elmer-141* polarimeter. UV spectra (λ_{max} (log ε) in nm): *Beckman* spectrometer. IR spectra (in cm^{-1}): *Perkin-Elmer-177* spectrometer. ^1H- and ^{13}C-NMR spectra: *Bruker* instrument (360 MHz (^1H), 90.5 MHz (^{13}C)) or *Varian* spectrometer (400 MHz (^1H), 100.6 MHz (^{13}C)); δ in ppm rel. to Me_4Si, *J* in Hz. MS: *VG-70-250* spectrometer. GC/MS: *HP-5790-B* instrument.

Mutagen Treatment of the Microorganism. Diplodia macrospora was incubated in 100-ml *Erlenmeyer* flasks, each containing 6 g of corn and 10 ml of H_2O, and exposed to permanent light at 27°. After 15–16 days, the cultures were suspended in 18 ml of 0.1M phosphate buffer (pH 7.0) with 2 ml *Tween 80* (1% in H_2O) and filtered through glass wool. Then, 10 ml of the suspension, containing about 2000 spores/ml, were exposed to UV irradiation of 2 mW/cm^2 in a petri disk ($\varnothing$ = 5.5 cm) under constant stirring during 150 min. A surviving rate of 1% resulted. Defined volumes of the suspension were plated out on a medium, containing 10 g of glycerol, 10 g of lactose, 10 g of potassium citrate, 3 g of $NaNO_3$, 1.2 g of K_2HPO_4, 0.5 g of $MgSO_4 \cdot 7\,H_2O$, 0.01 g of $FeSO_4$, 0.01 g of $CaCl_2$, and 20 g of agar per 1000 ml of H_2O, and incubated at 27° at 100% air humidity during 14 days.

Selection. Each colony was transferred to a 3-ml flask, containing 24 g of potatoe dextrose broth *(Difco)*, 1 g of yeast extract *(Difco)*, and 20 g of agar per 1000 ml of H_2O, and incubated at 27° for 7 days. After storage on separate agar plates, the cultures were extracted with 1 ml of CH_2Cl_2 each. The extracts were examinated by TLC (AcOEt), diplosporin (**1**) was detected with UV light.

Medium and Extraction of Production Cultures. The microorganism was grown in standing cultures on a medium containing 400 g of shredded wheat, 150 g of sucrose, 50 g of mycological broth *(Difco)*, and 20 g of yeast extract *(Difco)* per 1000 ml of H_2O. After incubation for 27 days at 27°, the cultures were refluxed with $CHCl_3$ (250 ml/100 ml of medium). The mixture was filtered and the extraction repeated 4 times (100 ml of solvent/100 ml of medium each time). The combined extracts were dried (Na_2SO_4) and evaporated. The residue was washed with petroleum ether.

Examination of the Crude Extracts. The crude extracts were examinated by TLC, using 3 solvent systems (AcOEt, CH_2Cl_2/MeOH 95:5, $CHCl_3$/acetone 3:1) and UV light and H_2SO_4 for detection. For HPLC examination, a *Styragel*-100 Å column, $CHCl_3$ as solvent, and UV light at 254 nm for detection were used.

Isolation of Metabolites from the Strain DM 7. The crude extract was purified on a silica-gel column (increasing amounts of MeOH in CH_2Cl_2), yielding 150 mg/l of chaetoglobosin K (**3**) and 50 mg/l of chaetoglobosin L (**4**). Fractions containing a new substance (38 mg/l), according to TLC, were examined by GC/MS (dimethyl-silicone column *(Hewlett-Packard)*, 5°/min from 200 to 250°). Two components were identified as *ergosterol* (84%) and *β-sitosterol* (11%) by comparison of their MS with those of the *EPA/NIH Mass Spectral Data Base*, a third component was supposed to be *stigma-5,7,22-trien-3β-ol*, according to its MS. β-Sitosterol was additionally identified by coinjection with a reference sample. The chaetoglobosin M (**5**) containing fractions were purified on a silical-gel column a second time with the same solvent system, yielding 23 mg/l of **5** as a slightly yellow gum.

Chaetoglobosin K (**3**): Yellow prisms. M.p. 260–261°. NMR: *Tables 1* and *2*. MS: 556 (M^+), 538, 413, 412, 188, 179, 157, 144 (100), 130, 117.

Chaetoglobosin L (**4**): Yellow gum. NMR: *Tables 1* and *2*. MS: 556 (M^+), 423, 412, 157, 144 (100), 130, 117.

Chaetoglobosin M (*= 6,7-Epoxy-5-ethyl-3-[1-(1*H*-indol-3-yl)ethyl]-16,18-dimethyl-10,11-dinor[13]cytochalasa-13,17-diene-1,19,20,23-tetrone;* **5**). $[\alpha]_D^{22} = -18.3°$ (CH_2Cl_2, *c* = 0.92). UV (CH_2Cl_2): 230 (4.09), 290 (3.60). IR (film): 3350, 3030, 2970, 2930, 2880, 1695. NMR: *Tables 1* and *2*. MS: 556 (M^+), 538, 413, 412, 394, 214, 179, 144 (100), 130, 117.

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