# 151. Chaetoglobosin L, a New Metabolite of *Diplodia macrospora*

by **Alessandro Probst** and **Christoph Tamm**[1])

Institut für Organische Chemie der Universität Basel, St. Johanns-Ring 19, CH-4056 Basel

(21.V.82)

*Summary*

From cultures of *Diplodia macrospora* a new metabolite of the cytochalasan family, chaetoglobosin L (**3**), has been isolated and its structure elucidated together with the known compounds diplosporin (**1**) and chaetoglobosin K (**2**).

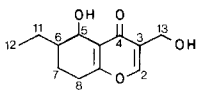
Chaetoglobosin K (**2**) is a fungal metabolite belonging to the family of the cytochalasans, and has been isolated recently from cultures of *Diplodia macrospora* (Strain ATCC No. 36'896) by *Cutler et al.* [1] who determined its structure by single crystal X-ray analysis [2]. The new compound differs from the known cytochalasans by the presence of an ethyl instead of a methyl group at C(5) and of an additional methyl group at C(10). These unique features raise an interesting biogenetic problem. While investigating the biogenetic origin of these two methyl groups we have isolated and identified two additional metabolites from cultures of the same microorganism.

The cultivation of *Diplodia macrospora* (ATCC No. 36'896) was carried out as described [1]. The mycelium and substrate were macerated with a homogenizer after addition of acetone, the homogenate filtered through a layer of *Celite*, and the filtrate extracted with methylene chloride after removal of the acetone. Thin layer chromatography (TLC.) of the crude product exhibited several spots which showed a reaction characteristic of phenols and indoles on subsequent spraying with 5%-solutions of $(NH_4)_2Ce(NO_3)_6$ in acetone and of $NH_2OH \cdot HCl$ in aqueous acetone [3]. After purification by chromatography on silica gel and further separation by preparative TLC., three pure compounds were obtained. The main product proved to be identical with diplosporin (**1**), a metabolite which has been isolated earlier from *Diplodia macrospora* [4] [5]. The second substance was recognized as chaetoglobosin K (**2**) by its very characteristic $^1$H-NMR. spectrum. The third compound, named chaetoglobosin L, was unknown. Its close relationship to chaetoglobosin K (**2**) was revealed by the $^1$H-NMR. spectrum in which the majority of the chemical shifts were practically identical. However, the singlet of the methyl group at C(6) was absent. It was replaced by two broad singlets at 5.33 and 5.18 ppm. The signal of H–C(7) was shifted downfield and showed a $^3J(7,8)$ coupling constant of 10 Hz

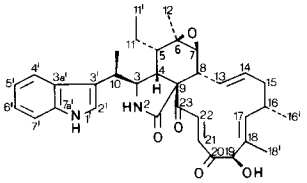
[1]) Author to whom correspondence should be addressed.

0018-019X/82/5/1543-04$01.00/0 

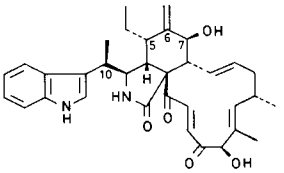
*Scheme*



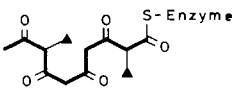
Diplosporin (1)



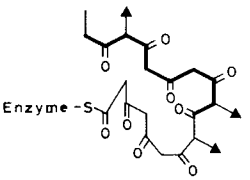
Chaetoglobosin K (2)



Chaetoglobosin L (3)



4



5

indicating a *trans* diaxial arrangement of the coupled protons. In metabolite **2** the $^3J(7,8)$ coupling constant is only 5 Hz. Finally, the chemical shifts and coupling constants of H–C(3), H–C(4), H–C(5) and H–C(8) of chaetoglobosin K (**2**) and the new compound show the same characteristic differences previously observed for 19-*O*-acetylchaetoglobosin A and D [4]. These observations permit the assignment of structure **3** to chaetoglobosin L.

Further evidence for the structure of the new metabolite was provided by its IR. and mass spectra. In the mass spectrum of **3** the molecular ion appears at *m/z* 556 indicating the same molecular formula ($C_{34}H_{40}N_2O_5$) as **2**. The base peak *m/z* 144 corresponds to the fragment 3-ethylindole thus verifying the presence of a methyl group at C(10). The IR. spectrum of the new compound exhibits a broad absorption between 3620 and 3360 $cm^{-1}$, indicating the presence of hydroxyl groups. Additional bands appear between 1720 and 1650 $cm^{-1}$ (lactam and conjugated carbonyl groups), at 970 $cm^{-1}$ ((*E*)-substituted double bonds), and at 900 $cm^{-1}$ (terminal double bond).

The simultaneous occurrence of diplosporin (**1**), chaetoglobosin K (**2**) and chaetoglobosin L (**3**) is interesting from a biogenetic view point. The building blocks of both types of metabolites are the same. In both cases branched polyketide structures, **4** and **5**, are formed. The skeleton **4** of diplosporin (**1**) is likely to be identical with part of the polyketide structure of the chaetoglobosins as indicated in formula **5**. Studies are being carried out in order to test this hypothesis.

The support of these investigations by the *Schweizerischer Nationalfonds zur Förderung der wissenschaftlichen Forschung* is gratefully acknowledged.

## Experimental Part

*General methods.* See [6].

*Medium and isolation of the metabolites.* The microorganism was grown in standing cultures in ten 1-l-*Erlenmeyer* flasks. Each flask contained 40 g of shredded wheat, 80 ml of *Difco* mycological broth (pH 4.8), 16 g of sucrose and 1.6 g of yeast extract [1]. The flasks were sterilized in a pressure autoclave during 1 h. After the medium had cooled to r.t. and solidified, it was inoculated with a suspension of spores of *Diplodia macrospora* (ATCC No. 36'896) and incubated at 27° for 22 days. To each *Erlenmeyer* flask 150 ml of acetone was added. After transferring mycelium and substrate into 6-l-beaker, 1.5 l of 70% aqueous acetone was added and the suspension macerated with a homogenizer and filtered through a layer of *Celite*. After washing the residue with acetone, the organic solvent of the filtrate was removed *in vacuo*. The remaining aqueous phase was extracted 4 times, each time with an equal volume of $CH_2Cl_2$. The dried extracts ($Na_2SO_4$) were evaporated *in vacuo* and the resulting crude product (red-brown oil) was dissolved in 90% aqueous methanol and extracted 3 times, each with an equal volume of petroleum ether. The combined petroleum ether extracts were dried ($Na_2SO_4$) and evaporated *in vacuo*. The remaining water-methanol phase was diluted with water, the methanol removed *in vacuo* and the aqueous phase extracted 3 times, each with an equal volume of $CH_2Cl_2$, and 3 times, each with an equal volume of ethyl acetate. The extracts were dried separately ($Na_2SO_4$) and evaporated *in vacuo*. TLC. of the 3 extracts with $CH_2Cl_2$/methanol 95:5 as solvent showed that only the $CH_2Cl_2$ extract contained compounds with a positive reaction for phenols and indoles after spraying with 5%-solutions of $(NH_4)_2Ce(NO_3)_6$ in acetone and of $NH_2OH \cdot HCl$ in 80% aqueous acetone [3]. The petroleum ether and ethyl acetate extracts were discarded. The $CH_2Cl_2$ extract (*ca.* 2 g of red-brown oil) was chromatographed on 200 g of silica gel using increasing amounts of methanol in $CH_2Cl_2$. The fractions eluted with $CH_2Cl_2$/methanol 97:3 contained chaetoglobosin K (**2**). In the following fractions ($CH_2Cl_2$/methanol 95:5) chaetoglobosin L (**3**) and diplosporin (**1**) were present. The last fractions ($CH_2Cl_2$/methanol 9:1) contained mainly diplosporin (**1**). For the separation of the compounds on TLC. $CH_2Cl_2$/methanol 95:5 and toluene/ethyl acetate/formic acid 5:4:1 served as solvents. The fractions containing the chaetoglobosins K (**2**) and L (**3**) were purified by preparative TLC. on silica gel with toluene/ethyl acetate/formic acid 5:4:1. The isolated yields were 1.5 g[2]) for diplosporin (**1**), 30 mg for chaetoglobosin K (**2**), and 10 mg for chaetoglobosin L (**3**).

*Diplosporin* (**1**) crystallized from benzene/petroleum ether; colourless crystals, m.p. 82.5–84°. – $^1$H-NMR. (60 MHz; $CDCl_3/D_2O$): 7.8 (*s*, 1 H, H–C(2)); 4.5 (*d*, $J$ = 4, 1 H, H–C(5)); 4.35 (*s*, 2 H, H–C(13)); 2.8–2.3 (*m*, 2 H, H–C(8)); 2.3–1.2 (*m*, 5 H, H–C(6), 2 H–C(7), 2 H–C(11)); 1.0 (*t*, $J$ = 5, 3 H, 3 H–C(12)), *cf.* [4].

*Chaetoglobosin K* (**2**) gave yellow prisms from acetone, m.p. 235–240°. – $^1$H-NMR. (90 MHz; $CDCl_3/D_2O$): 8.23 (br., 1 H, H–N(1')); 7.73 (*d*, $J$(21,22) = 17, 1 H, H–C(21)); 7.60–7.10 (*m*, 4 H, H-indolyl); 6.93 (*d*, $J$ = 2, 1 H, H–C(2')); 6.50 (*d*, $J$(21,22) = 17, 1 H, H–C(22)); 6.12 (*d*×*d*, $J$(13,14) = 14, $J$(8,13) = 10, 1 H, H–C(13)); 5.85 (br., 1 H, H–N(2)); 5.65 (br. *d*, $J$(16,17) = 10, 1 H, H–C(17)); 5.50–5.00 (*m*, 1 H, H–C(14)); 5.03 (*s*, 1 H, H–C(19)); 3.84 (*m*, 1 H, H–C(3)); 3.35–3.00 (*m*, 2 H, H–C(10), H–C(4)); 2.75 (*d*, $J$(7,8) = 5, 1 H, H–C(7)); 2.50–1.50 (*m*, 7 H, H–C(5), H–C(8), 2 H–C(11), 2 H–C(15), H–C(16)); 1.33 (*d*, $J$(17,18 $CH_3$) = 2, 3 H, $H_3C$–C(18)); 1.29 (*s*, 3 H, H–C(12)); 1.22 (*d*, $J$(10,10 $CH_3$) = 6, 3 H, $H_3C$–C(10)); 1.13 (*d*, $J$(16,16 $CH_3$) = 7, 3 H, $H_3C$–C(16)); 1.04 (*t*, $J$(11,11 $CH_3$) = 7, 3 H, $H_3C$–C(11)).

*Chaetoglobosin L* (**3**) was obtained as a yellow gum which still contained traces of diplosporin (**1**). – IR. (KBr): 3400, 2930, 1720, 1640 *S*, 1360, 1220, 970, 900, 740. – $^1$H-NMR. (90 MHz; $CDCl_3/D_2O$): 8.20 (br., 1 H, H–N(1')); 7.95 (*d*, $J$(21,22) = 17, 1 H, H–C(21)); 7.60–6.90 (*m*, 5 H, H-indolyl); 6.62 (*d*, $J$(21,22) = 17, 1 H, H–C(22)); 6.03 (*d*×*d*, $J$(13,14) = 15, $J$(8,13) = 9, 1 H, H–C(13)); 5.80–5.55 (*m*, 1 H, H–C(17)); 5.60 (br., 1 H, H–N(2)); 5.45–5.25 (*m*, 1 H, H–C(14)); 5.33 (br. *s*, 1 H, H–C(12)); 5.18 (br. *s*, 1 H, H–C(12)); 5.09 (*s*, 1 H, H–C(19)); 3.93 (br. *d*, $J$(7,8) = 10, 1 H, H–C(7)); 3.75–3.50 (*m*, 1 H, H–C(3)); 3.36 (*t*, $J$(4,5) = 4, $J$(3,4) = 4, 1 H, H–C(4)); 3.12–3.02 (*m*, 1 H, H–C(10)); 2.70–1.90 (*m*, 5 H, H–C(5), H–C(8), 2 H–C(15), H–C(16)); 1.90–1.50 (*m*, 2 H, H–C(11)); 1.35 (*d*, $J$(18,18 $CH_3$) = 1, 3 H, $H_3C$–C(18)); 1.29 (*d*, $J$(10,10 $CH_3$) = 5, 3 H, $H_3C$–C(10)); 1.12 (*d*, $J$(16,16 $CH_3$) = 7, 3 H, $H_3C$–C(16)); 1.05 (*t*, $J$(11,11 $CH_3$) = 7, 3 H, $H_3C$–C(11)). – MS.: 556 ($M^+$), 412, 381, 352, 239, 144.

---

[2]) Further amounts of **1** were present in the discarded ethyl acetate extract.

## REFERENCES

[1] *H.G. Cutler, F.G. Crumley, R.H. Cox, R.J. Cole, J.W. Dorner, J.P. Springer, F.M. Latterell, J.E. Thean & A.E. Rossi*, J. Agric. Food Chem. *28*, 139 (1980).
[2] *J.P. Springer, R.H. Cox, H.G. Cutler & F.G. Crumley*, Tetrahedron Lett. *21*, 1905 (1980).
[3] *J. Crastil*, J. Chromatogr. *115*, 273 (1975).
[4] *A.A. Chalmers, C.P. Gorst-Allman, N.P.J. Kriek, W.F.O. Marasas, P.S. Steyn & R. Vleggaar*, South African J. Chem. *31*, 111 (1978).
[5] *A.A. Chalmers, C.P. Gorst-Allman, P.S. Steyn, R. Vleggaar & D.B. Scott*, J. Chem. Soc., Perkin I *1979*, 1481.
[6] *A. Probst & Ch. Tamm*, Helv. Chim. Acta *64*, 2056 (1981).