100. New Spirostaphylotrichins from the Mutant Strain *P 649* of *Staphylotrichum coccosporum:* the Biogenetic Interrelationship of the Known Spirostaphylotrichins

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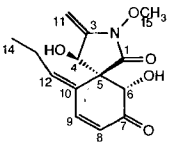
The isolation and structure elucidation of the new spirostaphylotrichins N (**2**), O (**3**), P (**4**), and T (**7**) from a mutant strain of *S. coccosporum* are described. The biogenetic relationship of the known spirostaphylotrichins is discussed.

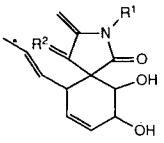
Introduction. – We have recently reported on studies regarding the biosynthesis of spirostaphylotrichin A (**1**) [1], a secondary fungal metabolite, which had been isolated by *Peter* and *Auden* [2] from cultures of *Staphylotrichum coccosporum.* In the course of further studies, we have isolated and determined the structures of a series of new spirostaphylotrichins either from cultures of the wild type or the mutant strain *P 84* which is blocked in the production of **1** [4]. Blocked mutants were searched in order to gain more insight into the biogenesis of **1**. We now communicate the results on the new spirostaphylotrichins N (**2**), O (**3**), P (**4**), and T (**7**) which were isolated from another blocked mutant of *S. coccosporum.* Finally, we shall discuss the biogenetic interrelationship of the various known spirostaphylotrichins.

Results. – Mutant *P 649* of *S. coccosporum* was obtained as described earlier [4]. Fermentation of *P 649* using the soya medium did not reveal relevant amounts of secondary metabolites. Only using the minimal medium, production of metabolites was achieved. By repeated middle-pressure chromatography on silica gel of a culture broth (11 l) of this mutant, the spirostaphylotrichins N (**2**), O (**3**), and P (**4**) were isolated. Their molecular formula and structure could be deduced from their MS, ^{1}H-NMR *(Table 1)*, and ^{13}C-NMR *(Table 2)* spectra (**2**: $C_{14}H_{17}O_5N$, **3**: $C_{14}H_{19}O_5N$, **4**: $C_{13}H_{15}O_4N$) and by comparison with the known spirostaphylotrichins [1] [3] [4]. They possess the same C-skeleton except for the missing MeO group in **4**, and they are characterized by the prop-1-enyl side chain as found in the spirostaphylotrichins from the mutant *P 84*. It replaces the propylidene side chain in the spirostaphylotrichins isolated from the wild-type strain. The chemical-shift differences of H–C(9) and H–C(8) and C(9) and C(8) relative to spirostaphylotrichin K (**5**), whose structure was elucidated by an X-ray analysis [4], indicate that an OH instead of a carbonyl function is present at C(7)[1]). These findings were confirmed by the coupling of H–C(8) with H–C(7). The latter is coupled further with an OH proton. From the coupling pattern and the chemical shift, substructure **6** was deduced for the three new compounds. Structure **6** was also suggested by a

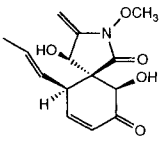
[1]) Numbering according to **1**, systematic names in the *Exper. Part.*



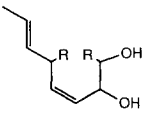
1 Spirostaphylotrichin A



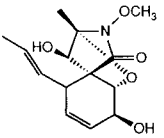
2 R^1 = MeO, R^2 = O
Spirostaphylotrichin N
3 R^1 = MeO, R^2 = OH,H
Spirostaphylotrichin O
4 R^1 = H, R^2 = O
Spirostaphylotrichin P



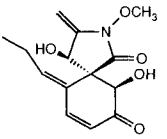
5 Spirostaphylotrychin K



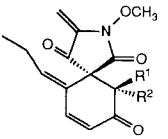
6



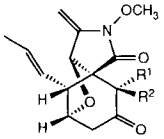
7 Spirostaphylotrichin T



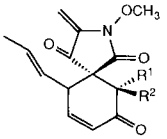
8 Spirostaphylotrichin B



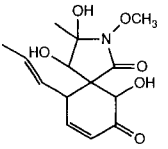
9 R^1 = H, R^2 = OH
Spirostaphylotrichin C [2]
10 R^1 = OH, R^2 = H
Spirostaphylotrichin D [2]



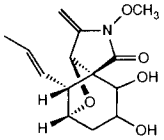
11 R^1 = OH, R^2 = H
Spirostaphylotrichin E
12 R^1 = H, R^2 = OH
Spirostaphylotrichin F



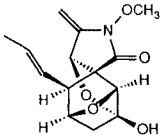
13 R^1 = OH, R^2 = H
Spirostaphylotrichin G [2]
14 R^1 = H, R^2 = OH
Spirostaphylotrichin H [2]



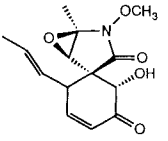
15 Spirostaphylotrichin I



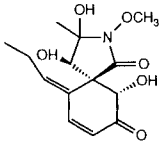
16 Spirostaphylotrichin L



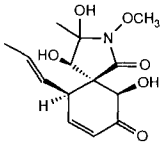
17 Spirostaphylotrichin M



18 Spirostaphylotrichin Q



19 Spirostaphylotrichin R



20 Spirostaphylotrichin S

2D-COSY experiment of **2**. In **3**, three OH groups were exchangeable by deuterium on treatment with D_2O. In the ^{13}C-NMR, only the carbonyl group corresponding to the lactam at 169.2 ppm was present. Thus, structure **3** was assigned to spirostaphylotrichin O which differs from spirostaphylotrichin K (**5**) only by having an OH group in place of a ketone functionality at C(7). In **2**, the downfield shift of CH_2(11) relative to **3** and **5**

[2] May be interchanged.

Table 1. *^{1}H-NMR (400 MHz) Data for the Spirostaphylotrichins N (**2**), O (**3**), P (**4**), and T (**7**) in (D_6)DMSO*

	2[1])	**3**[1])	**4**[a])[1])	**7**[1])
H–C(4)		5.07 (*m*, with D_2O br. *s*)		3.82 (br. *s*, with D_2O sharper)
OH–C(4)		4.92 (*d*, *J* = 6.2)[b])		5.92 (br. *d*, *J* = 3)[b])
H–C(6)	3.93 (*t*, *J* = 5.6)	3.65 (*t*, *J* = 5.5, with D_2O *d*, *J* = 5.1)	3.92 (*t*[c]) *J* = 5.0 with D_2O *d*[c]), *J* = 4.8)	3.69 (*d*, *J* = 8.8)
OH–C(6)	5.37 (*d*, *J* = 5.8)[b])	6.26 (*d*, *J* = 5.5)[b])	5.21 (*d*, *J* = 5.1)[c])	
H–C(7)	4.02 (*m*)	3.91 (*dt*, *J* = 11.7, 4.8, with D_2O *t*, *J* = 4.5)	3.88 (*m*, with D_2O br. *t*[b]), *J* = 4.8)	3.53 (*m*, with D_2O *dt*, *J* = 8.8, *ca.* 1.5)
OH–C(7)	3.63 (*d*, *J* = 8.8)[b])	4.64 (*d*, *J* = 11.6)[b])	4.12 (*d*, *J* = 11.1)[b])	5.28 (*d*, *J* = 5.9)[b])
H–C(8)	5.92 (*ddd*, *J* = 3.0, 4.8, 10.0)	5.84 (*ddd*, *J* = 3.0, 4.8, 10.0)	5.89 (*ddd*, *J* = 3.0, 4.8, 10.1)	5.40 (br. *d*, *J* = 10.1)
H–C(9)	5.58 (*m*)	5.56 (*dd*, *J* = 2.1, 10.1)	5.52 (*dd*, *J* = 1.8, 10.0)	5.54 (*ddd*, *J* = 2.0, 4.2, 10.1)
H–C(10)	3.20 (br. *d*, *J* = 9.2)	3.39 (*m*)	3.07 (br. *d*, *J* = 8.8)	3.15 (br. *s*)
H–C(11)	5.05 (*d*, *J* = 2.1)	4.47 (*t*, *J* = 1.2)	4.96 (*d*, *J* = 1.3)	1.47 (*s*, 3H)
H–C(11)	4.82 (*d*, *J* = 2.0)	4.38 (*t*, *J* = 1.1)	4.61 (*d*, *J* = 1.4)	
H–C(12)	5.04 (*ddq*, *J* = 9.2, 15.2, 1.7)	5.26 (*dd*, *J* = 7.4, 15.5)	5.12 (*ddq*, *J* = 9.1, 15.3, 1.6)	5.48 (*m*)
H–C(13)	5.54 (*dq*, *J* = 14.8, 6.6)	5.48 (*dq*, *J* = 15.5, 6.4)	5.44 (*dq*, *J* = 15.2, 6.2)	5.48 (*m*)
CH_3(14)	1.54 (*dd*, *J* = 1.6, 6.5)	1.52 (*d*, *J* = 6.2)	1.54 (*dd*, *J* = 1.5, 6.4)	1.67 (*d*, *J* = 3.7)
CH_3(15)	3.84 (*s*)	3.69 (*s*)		3.72 (*s*)

[a]) NH, 11.3 ppm (br. *s*, exchangeable with D_2O).
[b]) Exchangeable with D_2O.
[c]) Unsymmetrical.

Table 2. *^{13}C-NMR Data for the Spirostaphylotrichins N (**2**), O (**3**), and P (**4**) in (D_6)DMSO*

	2[1])	**3**	**4**
C(1)	167.0(*s*)	169.2(*s*)	174.8(*s*)
C(3)	137.9(*s*)	145.3(*s*)	140.8(*s*)
C(4)	195.5(*s*)	65.1(*d*)[a])	200.9(*s*)
C(5)	59.6(*s*)	55.2(*s*)	58.6(*s*)
C(6)	70.4(*d*)[a])	68.1(*d*)[a])	70.9(*d*)[a])
C(7)	64.1(*d*)[a])	64.7(*d*)[a])	64.8(*d*)[a])
C(8)	130.2(*d*)[b])	129.1(*d*)[b])	129.5(*d*)[b])
C(9)	127.9(*d*)[b])	128.0(*d*)[b])	128.2(*d*)[b])
C(10)	44.0(*d*)	39.3(*d*)	45.1(*d*)
C(11)	89.4(*t*)	82.7(*t*)	90.7(*t*)
C(12)	128.8(*d*)[a])	128.6(*d*)[b])	128.5(*d*)[b])
C(13)	126.5(*d*)[a])	128.0(*d*)[b])	127.0(*d*)[b])
C(14)	17.4(*d*)	17.6(*d*)	17.4(*d*)
C(15)	62.7(*d*)	62.1(*d*)	

[a])[b]) May be interchanged.

revealed a ketone function at C(4), as it had been established earlier for the spirostaphylotrichins C and D (**9**/**10**[2]) [3] and G and H (**13**/**14**[2])) [4]. This observation was confirmed by the ^{13}C-NMR where, besides the lactam carbonyl C-atom at 167.0 ppm, a further CO group at 195.5 ppm was present. These results allowed to propose structure **2** for spirostaphylotrichin N. It differs from **3** only in the oxidation state at C(4). The

spectra of spirostaphylotrichin P resembled very much to those of **2**. However, the MeO group was absent in **4**. In place of this functionality, a broad signal exchanging with D_2O was observed in the ^{1}H-NMR. Based on these observation, structure **4** was assigned to spirostaphylotrichin P.

An additional compound, spirostaphylotrichin T, was isolated from the culture extract of a pilot fermentation in *Erlenmeyer* flasks by chromatography on silica gel. Structure **7** was assigned tentatively to this metabolite.

The EI-MS of **7** showed two weak signals at *m/z* 281 and 282. In the CI-MS (NH_3), the base peak was observed at *m/z* 282 ($[M + 1]^+$), and a weak signal appeared at *m/z* 298 ($[M + NH_4]^+$). These data are compatible with the molecular formula $C_{14}H_{19}O_5N$. In the ^{1}H-NMR, CH_3 (14) was observed as a *d* at 1.67 ppm. H–C(12) and H–C(13) were assigned to the *m* at 5.48 ppm and H–C(10) to the broad signal at 3.15 ppm ($w_{1/2} \approx 10$ Hz). Due to the overlapping of the olefinic protons, the configuration at the C(12)=C(13) bond could not be determined from the coupling constant. But it is very likely that **7** possesses (*E*) configuration as all other spirostaphylotrichins containing a prop-1-enyl side chain. As a further structural element, the presence of the C(8)=C(9) bond (H–C(9): 5.54 ppm; H–C(8): 5.40 ppm) was established to be adjacent to H–C(7) (3.53 ppm). The latter showed further coupling with the OH proton at 5.28 ppm and with H–C(6) at 3.69 ppm. The protons of the exocyclic double bond were missing. Instead of them, a Me group at 1.47 ppm was observed as a *s*, as it had been the case in the spirostaphylotrichins I (**15**), S (**20**) [4], R (**19**), and Q (**18**) [3]. The UV spectrum of **7** showed a maximum at 202 nm ($\varepsilon = 7400$) and is, thus, the only spirostaphylotrichin exhibiting a maximum at such a short wave length. It is due to the fact that no enone system is present in the cyclohexane moiety and that the lactam chromophore is changed, too. The broadened *s* at 3.82 ppm was assigned to H–C(4). It became smaller after addition of D_2O. Therefore, the OH proton at 5.92 ppm was assigned to the 4-OH group. Whereas an OH group at C(3) was present in the spirostaphylotrichins I (**15**), S (**20**), and R (**19**), in **7** no further additional proton was observed in the ^{1}H-NMR. The presence of an epoxide as in **18** was ruled out too, because OH–C(4) was observed. The fact that H–C(6) showed no coupling with an OH proton led to the tentative structure **7** with an O-bridge from C(6) to C(3). The configuration at C(3) is defined by the ring connectivities, and the configuration at C(6) leads to the less strained ring system and is, therefore, favoured over the inverse configuration. The large coupling constant observed between H–C(7) and H–C(6) ($J = 8.8$ Hz) established a diaxial position for these two protons. C(4) is shown with the same configuration as found in those spirostaphylotrichins in which it had been established beyond any doubt. But the definitive structure of spirostaphylotrichin T remains to be proven.

Discussion of the Biogenetic Relationship of the Known Spirostaphylotrichins. – So far, 19 spirostaphylotrichins have been isolated and their structures elucidated (see *Table 3*). The spirostaphylotrichins Q (**18**) and R (**19**) are artefacts formed from spirostaphylotrichin A (**1**) in the course of workup. The same is probably true for the spirostaphylotrichins I (**15**) and S (**20**); **20** is formed from spirostaphylotrichin K (**5**), and **15** must be derived from a diastereoisomer **IV** of spirostaphylotrichin K (**5**), though its configuration has not yet been elucidated. The hypothetical biogenetic relationship of the different spirostaphylotrichins is presented in *Scheme 1*. In order to arrive at structure **VI** (wild-type spirostaphylotrichins A (**1**) and B (**8**)) from **IV** (see **5**), only a shift of the C(12)=C(13) bond into the 10,12-position is required. The same is true for the spirostaphylotrichins G and H (**V**) and C and D (**VII**), respectively. This step appears to be blocked in mutant *P 84* leading to the accumulation of the products possessing the C(12)=C(13) bond as intermediates. The conversions **IV** → **VI** and **V** → **VII** remain to be proven. The fact that considerable amounts of the probable intermediates were produced by *P 84*, which had not been present – or at least not in relevant amounts – in wild-type cultures indicates that most likely the migration of the double bond is the last step in the biosynthesis of the wild-type spirostaphylotrichins.

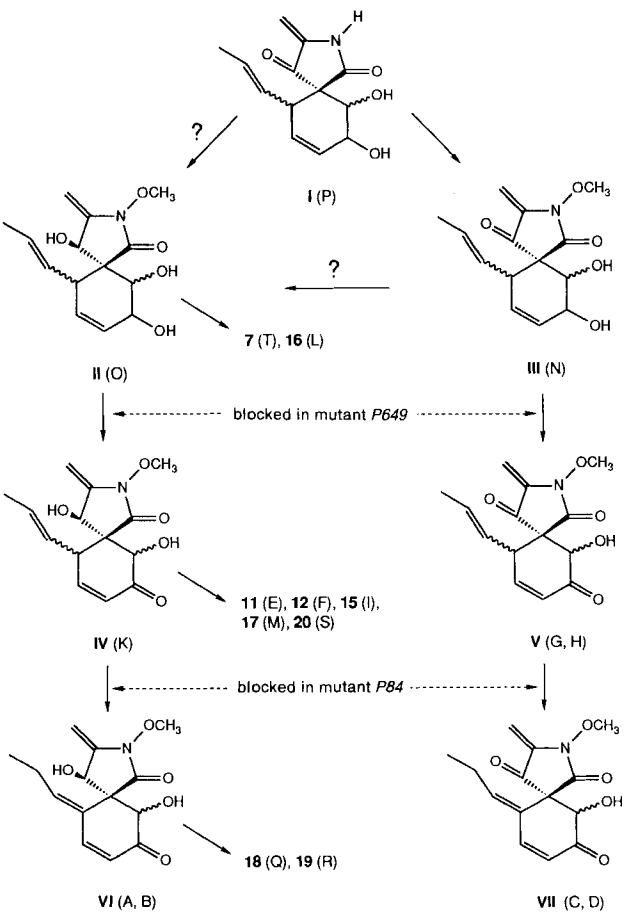
Spirostaphylotrichin M (**17**) is likely to be derived from **5**, while for the spirostaphylotrichins E (**11**) and F (**12**), intermediates of structure **IV** which differ from **5** in their

Table 3. *The Known Spirostaphylotrichins*

Spirostaphylotrichin	Source[a])	Ref.	Spirostaphylotrichin	Source[a])	Ref.
A(**1**)	WT	[1][2]	L(**16**)	*P84*	[4]
B(**8**)	WT	[3]	M(**17**)	*P84*	[4]
C(**9** or **10**)	WT	[3]	N(**2**)	*P649*	[b])
D(**9** or **10**)	WT	[3]	O(**3**)	*P649*	[b])
E(**11**)	*P84*	[4]	P(**4**)	*P649*	[b])
F(**12**)	*P84*	[4]	Q(**18**)	WT	[3]
G(**13** or **14**)	*P84*	[4]	R(**19**)	WT	[3]
H(**13** or **14**)	*P84*	[4]	S(**20**)	*P84*	[4]
I(**15**)	*P84*	[4]	T(**7**)	*P649*	[b])
K(**5**)	*P84*	[4]			

[a]) WT = wild-type strain of *S. coccosporum DSM 2602*; *P84* and *P649* = mutant strains.
[b]) See this paper.

Scheme 1. *Biogenetic Relationship of the Known Spirostaphylotrichins.* ~~~~~ means that both epimers occur at some stage. Capitals in parentheses reflect the isolated spirostaphylotrichins with the corresponding constitution.



configuration at C(10) have to be postulated. Both **5** and the corresponding epimer at C(10) from which **12** is derived would lead to spirostaphylotrichin B (**8**). The observation that no metabolites of type **IV** were accumulated in wild-type cultures – which might be the case if only one of the metabolites epimeric at C(10) had been converted to the wild-type spirostaphylotrichin – may be an indication that both epimers were transformed. However, these astonishing findings remain to be examined in detail. Spirostaphylotrichin L (**16**) is probably derived from **II**. Oxidation of the OH group at C(7) in intermediates such as **16** would open an alternative route to **11** and **12**.

The characteristic feature of the spirostaphylotrichins from mutant *P 649* is the replacement of the 7-keto group by an OH group. Oxidation at C(7) is obviously blocked in *P 649* and may occur as second last step in the biosynthetic sequence. The isolation of spirostaphylotrichin P (**4**) which corresponds to **I** in *Scheme 1* provides further insight into the biosynthesis of the spirostaphylotrichins. The hypothesis that *N*-oxidation and methylation occur already at the stage of aspartic acid, which is a precursor of **1** (see below), can be ruled out. Probably these two steps take place at the stage of **I** leading to **III**. Reduction of the keto group at C(4) would lead to the spirostaphylotrichins possessing an OH group at C(4). Weather this reduction is performed before or after the introduction of the *N*-MeO group is unknown. The occurrence of the spirostaphylotrichins which are epimeric at C(6) and C(10) requires the formation of all 4 different diastereoisomers of **I** as intermediates. An accurate investigation of culture extracts of strain *P 649* would certainly reveal a series of additional spirostaphylotrichins.

In the following, the results from incorporation experiments with **1** [1] are discussed in the light of the new results. The biosynthetic origin of the C-atoms of **1** are summarized in the *Figure* [1]. Various hypotheses for the formation of **I** from the basic precursors are possible *(Scheme 2)*. Acetyl-coenzyme A acts as starter unit. It is condensed in four successive cycles with malonyl-coenzyme A to form the C_{10}-polyketide **21**. It can be assumed that these reactions proceed in analogy to the biosynthesis of the fatty acids [5]. In a subsequent step, **21** may undergo cyclization by which a benzene ring is formed. The thioester **22** which is obtained, undergoes further condensation with aspartic acid to yield the amide **23**. *Claisen* condensation of its tautomer **24** leads to the spirocyclic γ-lactam **25**. Decarboxylation produces the exocyclic olefinic double bond. After further transformations within the six-membered ring, the hexadienol **26** is obtained. To accomplish the subsequent decarboxylation, leading to the exocyclic vinyl group, previous conversion of **26** either to the iminium ion **27** or to the hydroxylated intermediate **28** is required. Selective epoxidation of the product **29** as well as reduction of the keto group of the side chain and subsequent dehydration produce the epoxy-diene **30**. It possesses the spirostaphylotrichin skeleton. Attack of a hydride ion at C(10) which is followed by a

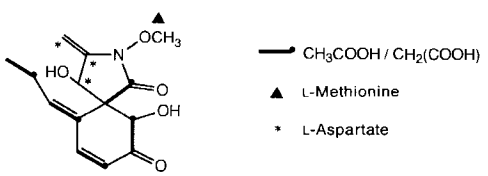
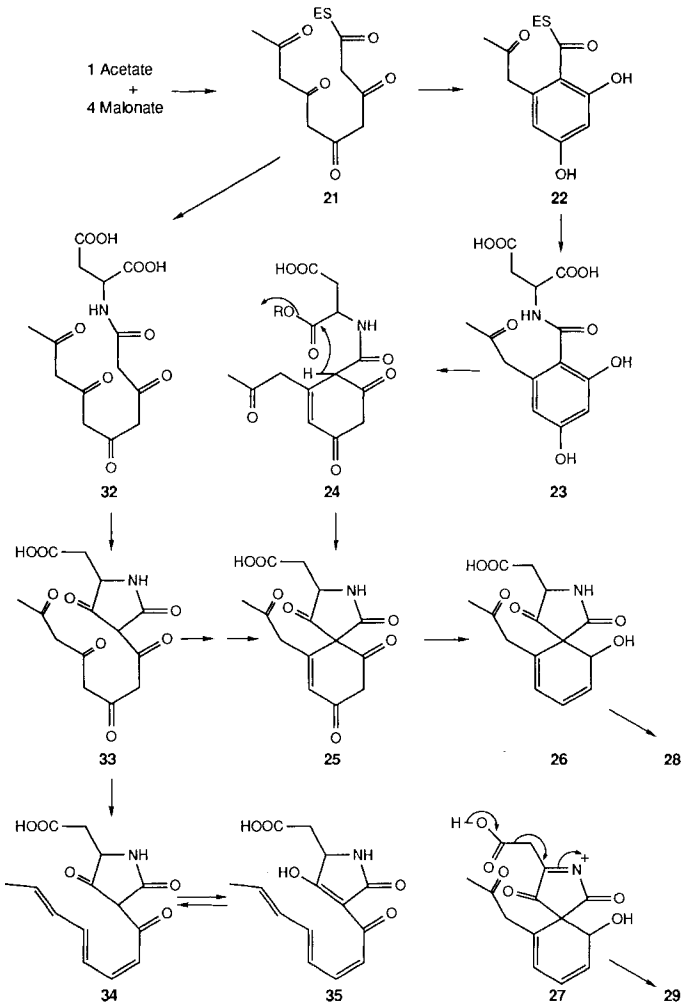


Figure. *Biogenetic building blocks of spirostaphylotrichin A* (**1**)

Scheme 2

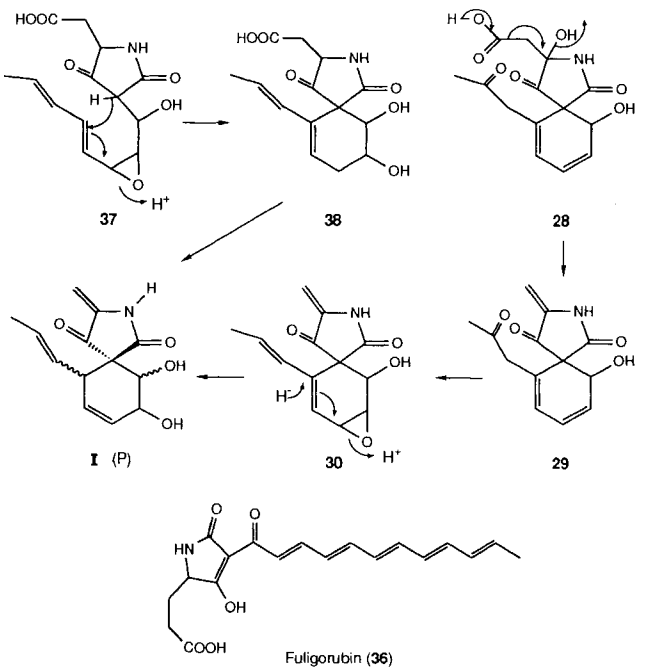


shift of the olefinic double bond and opening of the epoxy group leads to structure **I**. By this sequence of reactions, the OH group at C(7) is introduced which does not originate from the polyketide chain. The formation of both configurations at C(10) may be readily explained, because the attack of the hydride ion is possible from either side of the spirocyclic system. The individual steps of the postulated sequence outlined can be interchanged as well.

In an alternative biogenetic pathway, at first, formation of the γ-lactam takes place. The polyketide **21** reacts with aspartic acid in an early stage, thus, forming the acylic amide **32**. Condensation of the latter leads to the γ-lactam **33**. Similar steps have been postulated for the biosynthetic pathways of the cytochalasans [6] and the pseurotins [7]. A

second cyclization of **33** would lead to the spirocyclic system **25** which had been an intermediate also in the preceding pathway. Another alternative offers the transformation of **33** by a series of subsequent reductions and dehydrations leading to the trienone **34**. It is of course possible that these reactions occur in an earlier stage in a similar manner as in the course of the biosynthesis of the fatty acids and polyenes. Intermediate **34** is a tetramic acid. Its tautomer **35** exhibits remarkable structural similarity with the red pigment fuligurubin (**36**, *Scheme 3*) of *Fuligoseptica* [8]. Selective epoxidation of **30** yields the epoxy-diene **37**. It can undergo cyclization, thus, forming the spirocyclic system **38**, a reaction well known to occur in vinyl epoxides [9]. Finally, oxidation and subsequent

Scheme 3



decarboxylation, proceeding in an analogous manner as the conversion of **26** to **27**, results in the formation of structure **I**. Again, the latter reaction could proceed in an earlier stage of the biogenetic sequence. It is quite clear that the proposed biogenetic pathways are still hypothetical. However, they may serve as a basis for further experimental investigations.

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Experimental Part

General. Stock cultures of *S. coccosporum* strain *DSM 2601* were maintained on *Mycophil Agar* (*Baltimore Biological Laboratories*) or *Mycological Agar* (*Difco*) and stored at 4°. For mutagen treatment of *S. coccosporum DSM 2602* and selection of mutants lacking in production of **1**, see [4]. Fermenter: *New Brunswick Scientific Co.*, Model *MF 114*. The org. extracts were dried (Na_2SO_4) and evaporated under reduced pressure below 40°. Spirostaphylotrichins are relatively little stable and can only be stored in the refrigerator without dec. In soln. dcc. was observed. Column chromatography: silica gel *60* (63–200 μm, *Merck*), TLC: silica gel $60F_{254}$ (*Merck*), detection with UV, I_2, $KMnO_4$, or H_2SO_4. HPLC of culture broths and of crude extracts: *Nucleosil-C_8* (10 μm, 4.5 × 250 mm, *Macherey-Nagel*) using H_2O/MeCN 7:3; *μBondapak C_{18}* (5μ, 3.9 × 300 mm, *Waters*) using H_2O/MeCN 85: 15. M.p.: *Kofler Block*; corrected; large intervals may arise because of the thermal instability of the spirostaphylotrichins. UV: *Beckman* spectrometer model *25*, in nm (*E*). IR: *Perkin Elmer 781*, in cm^{-1}. NMR (*K. Aegerter*): *Bruker-WH-90* spectrometer with *Fourier* transform (^1H, 90 MHz; ^{13}C, 22.63 MHz) and *Varian-VXR-400* spectrometer with *Fourier* transform (^1H, 400 MHz; ^{13}C, 101 MHz): δ(ppm) relative to internal Me_4Si and *J* in Hz. MS (Dr. *H. Nadig*): *VG-70-250* instrument, in *m/z*.

Isolation of the Spirostaphylotrichins N (**2**), *O* (**3**), *P* (**4**), *and T* (**7**). Fermentations of *S. coccosporum* mutant *P 649* were run according to [1] [2] using the minimal medium with slight modifications concerning the incubation times. The culture broth of an 11-litre fermentation was filtered and extracted 3 times with CH_2Cl_2. Evaporation after drying gave 7.8 g of brown extract which was chromatographed on silica gel with CH_2Cl_2/MeOH. Two fractions were further used. The first fraction was chromatographed twice with pentane/AcOEt and gave, after crystallization from Et_2O, 184 mg of **2**. From the second fraction, **3** could be crystallized from Et_2O. The mother liquor was further chromatographed with pentane/AcOEt. There, **4** was eluated first and could be crystallized from Et_2O after a second similar chromatography (270 mg). After **4**, an additional amount of **3** was eluted (total yield of **3**, 452 mg).

The culture broth (1.2 l) of a fermentation in 8 *Erlenmeyer* flasks was filtered and extracted with Et_2O in a *Kutscher-Steudel* apparatus. After evaporation, the crude extract was subsequently chromatographed on silica gel with pentane/Et_2O and pentane/AcOEt, respectively, yielding 17 mg of crystalline (Et_2O) **7**.

Spirostaphylotrichin N (**2**; *= 9,10-Dihydroxy-2-methoxy-3-methylidene-6-[(E)-prop-1-enyl]-2-azaspiro-[4.5]dec-7-ene-1,4-dione*). M.p. 92–95°. UV (EtOH): 275 (4300), 225 (7800). IR (KBr): 3520*m*, 3400 (br., OH), 3050*w*, 2975*w*, 2905*w*, 1750*s*, 1650*s*, 1320*m*, 1290*m*, 1110*m*, 970*m*, 900*m*. ^1H-NMR (400 MHz, (D_6)DMSO): *Table 1*. ^1H-NMR[1]) (90 MHz, $CDCl_3$): 1.59 (*dd*, *J* = 6.3, 1.5, H–C(14)); 3.03 (*d*, *J* = 8.5, exchangeable with D_2O); 3.12 (*d*, *J* = 3.5, exchangeable with D_2O); 3.33 (br. *d*, *J* = 9, H–C(10)); 3.96 (*s*, CH_3(15)); 4.15 (*m*, with D_2O br. *s*, H–C(7), H–C(6)); 4.80 (*d*, *J* = 1.9, H–C(11)); 5.07 (*ddq*, *J* = 15, 9, 1.5, H–C(12)); 5.17 (*d*, *J* = 1.9 H–C(11)); 5.67 (*m*, H–C(9), H–C(13)); 6.07 (*ddd*, *J* = 10.4, 3, H–C(8)). ^{13}C-NMR (22.6 MHz, (D_6)DMSO): *Table 2*. EI-MS (70 eV, 180°): 279 (M^+), 230 ($[M - H_2O - MeO]^+$), 202, 194, 181, 170, 110 (100). CI-MS (NH_3, 150°): 297 ($[M + NH_4]^+$), 280 ($[M + 1]^+$), 262 (100 $[M + 1 - H_2O]^+$).

Spirostaphylotrichin O (**3**; *= 4,9,10-Trihydroxy-2-methoxy-3-methylidene-6-[(E)-prop-1-enyl]-2-azaspiro-[4.5]dec-7-en-1-one*). M.p. 147–151°. UV (EtOH): 228 (11200), 202 (11200). IR (KBr): 3420 (br., OH), 3040*w*, 2950*w*, 1665*s*, 1440*m*, 1280*m*, 1170*m*, 1090*m*, 1060*m*, 985*m*. ^1H-NMR (400 MHz, (D_6)DMSO) *Table 1*. ^1H-NMR[1]) (90 MHz, $CDCl_3$): a.o. signals at 1.62 (*d*, *J* = 6.3, CH_3(14)); 2.30 (*d*, *J* = 9, exchangeable with D_2O); 3.30 (*d*, *J* = 8, exchangeable with D_2O); 3.45 (br. *d*, *J* = 7, H–C(10)); 3.84 (*s*, CH_3(15)); 4.52 (*t*, *J* = 2.0, H–C(11)); 4.63 (*t*, *J* = 2.2 H–C(11)); 6.02 (*ddd*, *J* = 4.5, 3.0, 10.0, H–C(8)). ^{13}C-NMR (22.6 MHz, (D_6)DMSO): *Tab. 2*. EI-MS (70 eV, 200°): 281 (M^+), 263 ($[M - H_2O]^+$), 250 ($[M - MeO]^+$), 234, 232 ($[M - H_2O - MeO]^+$), 214, 196, 172, 161, 154, 110 (100). CI-MS (NH_3, 300°): 300 ($[M + NH_4 + 1]^+$), 282 ($[M + 1]^+$), 264 ($[M + 1 - H_2O]^+$).

Spirostaphylotrichin P (**4**; *= 9,10-Dihydroxy-3-methylidene-6-[(E)-prop-1-enyl]-2-azaspiro[4.5]dec-7-ene-1,4-dione*). M.p. 141–144°. UV (EtOH): 275 (4000), 222 (8800). IR (KBr): 3500–3250 (several, br., OH, NH), 2975*w*, 2965*w*, 1760*m*, 1700*s*, 1660*s*, 1300*m*, 1085*m*, 970*m*, 880*m*. ^1H-NMR[1]) (400 MHz, (D_6)DMSO): *Table 1*. ^1H-NMR (90 MHz, $CDCl_3$): 1.60 (*dd*, *J* = 6.2, 1.2, CH_3(14)); 3.21 (br. *d*, *J* = 9, H–C(10)); 3.33 (*d*, *J* = 7.6, exchangeable with D_2O); 4.0–4.2 (*m*, 3H, with D_2O 2H, H–C(7), H–C(6)); 4.68 (*d*, *J* = 2,2, H–C(11)); 5.25 (*d*, *J* = 2.2, H–C(11)); 5.0–5.8 (*m*, H–C(9), H–C(12), H–C(13)); 6.07 (*ddd*, *J* = 10, 3.5, 2.8, H–C(8)); 8.11 (br., 1H, exchangeable with D_2O, NH). ^{13}C-NMR (22.6 MHz, (D_6)DMSO): *Table 2*. EI-MS (70 eV, 200°): 249 (very weak, M^+), 231 ($[M - H_2O]^+$), 202, 188, 164, 140, 110 (100). CI-MS (NH_3, 300°): 267 ($[M + NH_4]^+$), 250 ($[M + 1]^+$), 232 ($[M + 1 - H_2O]^+$).

Spirostaphylotrichin T (**7**; *= 3,10-Epoxy-4,9-dihydroxy-2-methoxy-3-methyl-6-[(E)-prop-1-enyl]-2-azaspiro-[4.5]dec-7-en-1-one*). M.p. 152–159°. UV (EtOH): 202 (7400). IR (KBr): 3480 (br., OH), 3270 (br., OH), 3040*m*, 2950*m*, 1730*s*, 1190*m*, 1095*m*, 1065*m*, 995*m*. ^1H-NMR (400 MHz, (D_6)DMSO): *Table 1*. EI-MS (70 eV, 300°): 282

(0.4, $[M + 1]^+$), 281 (0.4, M^+), 250 (23, $[M - MeO]^+$), 238 (3), 210 (15), 192 (10), 175 (20), 172 (20), 154 (26), 147 (37), 145 (37), 121 (63), 43 (100). CI-MS (NH_3, 900°): 299 (0.4, $[M + NH_4]^+$), 298 (0.15), 282 (100, $[M + 1]^+$).

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