122. New Spirostaphylotrichins from the Mutant Strain *P 84* of *Staphylotrichum coccosporum*

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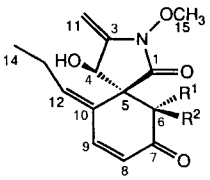
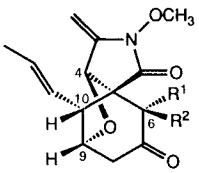
From a mutant strain of *S. coccosporum*, the new spirostaphylotrichins E (**2**), F (**3**), G (**4** or **5**), H (**5** or **4**), I (**6**), K (**7**), L (**8**), M (**9**), and S (**10**) have been isolated. Their structures have been elucidated by spectroscopic methods (UV, IR, ^{1}H-NMR, ^{13}C-NMR, and MS), chemical transformations, and X-ray analysis (**3** and **7**).

Introduction. – The spirostaphylotrichins belong to a new family of microbial secondary metabolites whose first member, spirostaphylotrichin A (**1**), has been isolated by *Peter* and *Auden* from cultures of *Staphylotrichum coccosporum* [1]. They possess a highly substituted spirocyclic system which contains a γ-lactam moiety. In connection with biosynthetic studies [2], we have isolated additional spirostaphylotrichins from the wild type of *S. coccosporum* [3]. Having established acetate, a C_4-dicarboxylic acid of the citric-acid cycle, most probably L-aspartate, and the methyl group of L-methionine as basic building blocks of **1**, it was desirable to identify the more advanced precursors of the biogenetic pathway. Therefore, we have carried out a screening for mutants of *S. coccosporum* which were blocked in the production of **1**, hoping to detect such intermediates. We now report on the isolation and structure elucidation of several new spirostaphylotrichins from the strain *P84* of *S. coccosporum*.

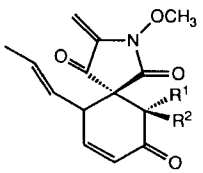
Results. – Mutants of *S. coccosporum* were obtained by UV irradiation [4]. Spores were reported to occur in *S. coccosporum*, but we never had been able to detect them. Variation of the nutrition source, in particular the C-atom sources [5], strain maintenance on straw, and irradiation of cultures by light [6] never induced sporulation. Therefore, we had to use the mycelium for the UV irradiation. The mycelium was ground in a phosphate buffer with a mixer in the presence of quartz sand. The suspension was then irradiated with UV light and incubated on agar plates. After some days, new agar plates were inoculated by colonies from cells that had survived after the UV irradiation. After a period of 7 days, the colonies on these plates were examined for the presence of **1**. Extracts of these colonies were analyzed by TLC [7] and compared with those of the wild type of *S. coccosporum*. By this procedure, 35 out of 635 strains (rate of surviving the UV irradiation was 0.2%) were obtained which did not produce any **1**. In the extracts of the cultures of three out of these mutant strains (*P84*, *P303*, and *P649*), substances which differed from **1** were detected by TLC. Producing cultures of the mutant strain *P303* yielded only very small amounts of crude extracts. The mutant strains *P84* and *P649* were subjected to further examination. The results obtained with *P649* will be reported in a subsequent paper.



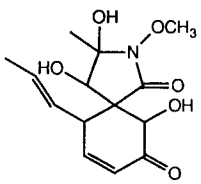
1 $R^1 = OH$, $R^2 = H$, spirostaphylotrichin A
12 $R^1 = H$, $R^2 = OH$, spirostaphylotrichin B



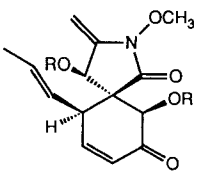
2 $R^1 = OH$, $R^2 = H$, spirostaphylotrichin E
19 $R^1 = OAc$, $R^2 = H$
21 $R^1 = OMs$, $R^2 = H$
3 $R^1 = H$, $R^2 = OH$, spirostaphylotrichin F
17 $R^1 = H$, $R^2 = OAc$



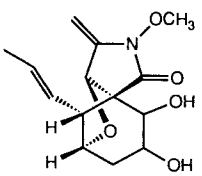
4 $R^1 = OH$, $R^2 = H$, spirostaphylotrichin G[a])
5 $R^1 = H$, $R^2 = OH$, spirostaphylotrichin H[a])



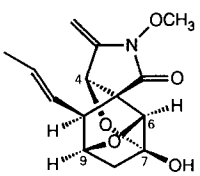
6 spirostaphylotrichin I



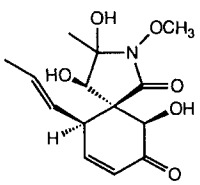
7 R = H, spirostaphylotrichin K
11 R = Ac



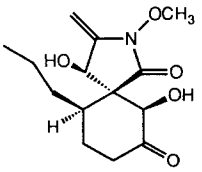
8 spirostaphylotrichin L



9 spirostaphylotrichin M



10 spirostaphylotrichin S



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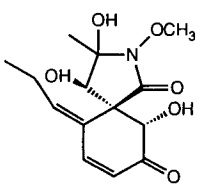
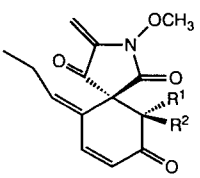
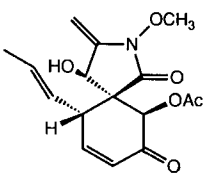


14 spirostaphylotrichin R



15 $R^1 = OH$, $R^2 = H$, spirostaphylotrichin C[a])
16 $R^1 = H$, $R^2 = OH$, spirostaphylotrichin D[a])



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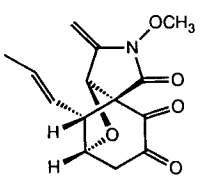

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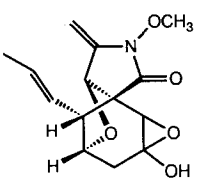
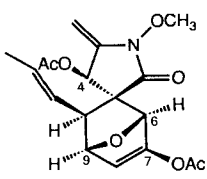


22



23

[a]) May be interchanged.

Table 1. *Spirostaphylotrichins Isolated from Cultures of the Mutant Strain* P84 *of* Staphylotrichum coccosporum

Spirostaphylotrichin	Soya medium [mg/l]	Minimal medium [mg/l]
E (**2**)	48	64
F (**3**)	14	79
G (**4** or **5**)	26	7
H (**5** or **4**)	2	traces
I (**6**)	–	1
K (**7**)	35	–
L (**8**)	–	24
M (**9**)	–	36
S (**10**)	5	–

The mutant strain *P84* was cultivated in 11-litre fermentations using two different media. One of them contained D-mannitol and soya meal (soya medium), the other D-glucose, NH_4NO_3, $MgHPO_4$, $MgSO_4$, NaCl, $CaCl_2$, a phosphate buffer, some vitamins, and trace elements (minimal medium). HPLC analysis of the culture broths of both media revealed a different pattern of metabolites. This observation was confirmed by the later isolation of the metabolites. Extraction of the culture broths with CH_2Cl_2 and repeated middle-pressure chromatography on silica gel with different solvents (see *Exper. Part*) yielded the spirostaphylotrichins E (**2**), F (**3**), G (**4** or **5**), H (**5** or **4**), I (**6**), K (**7**), L (**8**), M (**9**), and S (**10**). The isolated amounts and their distribution in the two media are listed in *Table 1*.

The same C-skeleton was established for all spirostaphylotrichins which were isolated from the mutant strain *P84*. However, all compounds contained a (*E*)-prop-1-enyl side chain in place of the propylidene side chain with (*Z*)-configuration which is characteristic for the metabolites of the wild-type strain [3], *e.g.* **1**. The ^{1}H-NMR and ^{13}C-NMR data of the spirostaphylotrichins isolated from the mutant strain *P84* are summarized in *Tables 2* and *3*.

Spirostaphylotrichin K (**7**). The EI-MS of **7** showed the M^+ ion at *m/z* 279 as observed for **1**. The CI-MS revealed $[M + 1]^+$ at *m/z* 280, corresponding to the molecular formula $C_{14}H_{17}NO_5$. Treatment of **7** with Ac_2O in pyridine at r.t. gave the di-*O*-acetyl derivative **11**. The structure of **7** was established by comparison with data of **1**, **3**, and **12**, and conversion of **7** and **12** to the same tetrahydro derivative **13** [3].

The ^{1}H-NMR and ^{13}C-NMR of **7** indicated structural similarity with **1**, the differences being explained by shifting the 10,12-double bond to the 12,13-position. In the off-resonance ^{13}C-NMR spectrum, C(10) appeared at 40.5 ppm as a *d*. H–C(10) gave rise to a *m* at 3.82 ppm which coupled with the olefinic protons at C(9) (J = 2.2 Hz), C(8) (J = 3.2 Hz), and C(12) (J = 7 Hz). CH_3(14) was found as a *dt* at 1.55 ppm. It showed vicinal and allylic coupling with the olefinic protons at C(13) and C(12), respectively. In the UV, only one maximum at 226 nm appeared, thus confirming that the keto group is not conjugated with two double bonds as it is the case in **1**. Concerning the configuration at C(4) and C(6) relative to C(5), the comparison of the chemical shifts of H–C(4) and H–C(6) allowed a better correlation between **7** and **12** as between **7** and **1**. From wild-type cultures using the soya medium, only **1** had been isolated, but no **12**. The fact that **7** was isolated only from the minimal-medium cultures further favoured the assignement of the same configuration at C(4) and C(6) as in **12**. Catalytic hydrogenation of **7** and **12** led to tetrahydro-spirostaphylotrichin **13** in both cases [3]. Assuming that the addition of H_2 to the 10,12-double bond of **12** had taken place from the sterically less hindered side, the configuration at C(10) was expected to be the inverse of **7** and to be the same as spirostaphylotrichin F (**3**), as demonstrated by X-ray diffraction (see below). But the large NOE[1]) between H–C(6) and H–C(10) and between H–C(4) and H–C(6) of **7** can only be explained by the proposed structure. Thus, H_2 addition did not occur from the expected side.

[1]) We thank Prof. Dr. *H. Fritz* and Dr. *H. Rumpel, Ciba-Geigy AG*, Basel, for providing these spectra.

Table 2. *400-MHz ^{1}H-NMR Data* ((D_6)DMSO) *of the Spirostaphylotrichins E* (**2**), *F* (**3**), *G* (**4** or **5**), *H* (**5** or **4**), *I* (**6**), *K* (**7**), *L* (**8**), *M* (**9**), *and S* (**10**) *and of* **23**[a])

H-Atom	Spirostaphylotrichin				
	E (**2**)	F (**3**)	G (**4** or **5**)	H (**5** or **4**)	I (**6**)[b])
OH–C(3)	–	–	–		5.83 (*d*, J = 1.3)[d])
H–C(4)	4.66 (*t*, J = 1.8)[c])	4.53 (*t*, J = 1.8)	–		4.09 (*d*, J = 6.0; with D_2O, *s*)
OH–C(4)	–		–		4.43 (*d*, J = 5.9)[d])
H–C(6)	4.49 (*d*, J = 5.5; with D_2O, *s*)	4.18 (br. *s*; with D_2O, sharper)	4.51 (*d*, J = 3.8; with D_2O, *s*)	4.55 (*d*, J = 2.9; with D_2O, *s*)	4.56 (*d*, J = 2.1; with D_2O, *s*)
OH–C(6)	6.02 (*d*, J = 5)[d])	6.55 (br. *d*, J = 3.8)[d])	6.18 (*d*, J = 4.1)[d])	6.17 (*d*, J = 3.7)[d])	5.15 (*d*, J = 2.1)[d])
H–C(7)		–	–	–	–
OH–C(7)		–	–	–	–
H–C(8)	2.74 (*ddd*, $J \approx$ 1, 3, 16.3)	2.80 (*dd*, J = 2.5, 17.5)	6.08 (*dd*, J = 3.1, 10.1)	6.06 (*dd*, J = 2.8, 10.0)	6.26 (*dd*, J = 0.5, 9.8)
H–C(8)	2.50 (*dd*, J = 2.6, 17.6)	2.39 (*ddd*, $J \approx$ 1, 3, 17.5)		–	–
H–C(9)	4.41 (*t*, J = 3)	4.39 (*t*, J = 2.7)	6.68 (*dd*, J = 2.2, 10.1)	6.64 (*dd*, J = 2.2, 10.2)	6.93 (*dd*, J = 6.0, 9.8)
H–C(10)	3.30 (*d*, J = 8.8)	3.59 (*d*, J = 9.2)	3.8 (*m*)	3.8 (*m*)	3.64 (br. *t*, J = 5.9)
H–C(11)	4.55 (*t*, J = 1.9)[c])	4.50 (*t*, J = 1.9)	5.06 (*d*, J = 2.2)	5.17 (*d*, J = 2.2)	1.57 (*d*, J = 1.2, 3 H; with D_2O, *s*)
H–C(11)	4.40 (*t*, J = 2)[c])	4.34 (*t*, J = 1.7)	4.85 (*d*, J = 2.1)	4.88 (*d*, J = 2.3)	
H–C(12)	5.27 (*ddq*, J = 9.0, 15.3, 1.7)	5.28 (*ddq*, J = 9.2, 15.4, 1.7)	5.13 (*ddq*, J = 8.8, 15.0, 1.5)	5.24 (*ddq*, J = 8.8, 15.3, 1.5)	5.8 (*m*)
H–C(13)	5.66 (*dq*, J = 15.5, 6.5)	5.66 (*dq*, J = 15.4, 6.6)	5.69 (*dq*, J = 15.1, 6.2)	5.58 (*dq*, J = 15.2, 6.5)	5.8 (*m*)
CH_3(14)	1.59 (*dd*, J = 1.6, 6.5)	1.58 (*dd*, J = 1.5, 6.5)	1.58 (*dd*, J = 1, 6.2)	1.58 (*d*, J = 6.4)	1.76 (*dd*, J = 0.6, 5.0)
CH_3(15)	3.70 (*s*)	3.69 (*s*)	3.85 (*s*)	3.81 (*s*)	3.87 (*s*)

Table 2 (cont.)

H-Atom	Spirostaphylotrichin				
	K (**7**)	L (**8**)	M (**9**)	23[c])	S (**10**)
OH–C(3)	–		–	–	5.29 (*s*)[d])
H–C(4)	4.96 (*dt*, *J* = 5, 1; with D_2O, *t*, *J* = 1)	5.31 (br. *s*)	5.13 (*t*, *J* = 1.5)	4.66 (*t*, *J* = 1.7)[e])	4.28 (*d*, *J* = 6.2; with D_2O, *s*)
OH–C(4)	6.35 (*d*, *J* = 5.1)[d])		–	–	5.84 (*d*, *J* = 6.4)
H–C(6)	4.18 (*d*, *J* = 3.7; with D_2O, *s*)	3.93 (br. *d*; with D_2O, br. *s*)	3.87 (*s*)	5.84 (*s*)	4.12 (*d*, *J* = 3.7; with D_2O, *s*)
OH–C(6)	5.61 (*d*, *J* = 3.7)[d])	5.10 (*d*, *J* = 4.3)[d])	–	–	5.42 (*d*, *J* = 3.7)[d])
H–C(7)	–	3.64 (*m*; with D_2O, br. *d*, *J* = 5.4)	–	–	–
OH–C(7)	–	4.99 (*d*, *J* = 2.5)[d])	6.45 (*s*)[d])		–
H–C(8)	6.01 (*dd*, *J* = 3.2, 10.2)	1.83 (*ddd*, *J* = 1.8, 5.9, 15.0)	2.37 (*dd*, *J* = 2.8, 14.8)	6.05 (*d*, *J* = 6.4)	5.98 (*dd*, *J* = 3.0, 10.3)
H–C(8)	–	1.76 (*dd*, *J* = 3.2, 15.0)	1.87 (*dd*, *J* = 1.6, 14.4)	–	–
H–C(9)	6.70 (*dd*, *J* = 2.2, 10.2)	4.18 (br. *s*)	4.20 (br. *t*, *J* ≈ 2.5)	4.56 (*d*, *J* = 6.4)	6.68 (*dd*, *J* = 2.4, 10.3)
H–C(10)	3.82 (*m*)	3.17 (*d*, *J* = 9.2)	3.07 (*d*, *J* = 9.2)	3.12 (*d*, *J* = 9.2)	3.7[f])
H–C(11)	4.41 (*dd*, *J* = 1, 1)	4.43 (br. *s*)	4.43 (*t*, *J* = 1.5)	4.59 (*t*, *J* = 1.9)[e])	1.39 (*s*, 3 H)
H–C(11)	4.34 (*dd*, *J* = 1, 1)	4.30 (br. *s*)	4.31 (*t*, *J* = 1.5)	4.45 (*t*, *J* = 1.7)[e])	
H–C(12)	5.40 (*ddq*, *J* = 7, 16, 1)	5.27 (*ddq*, *J* = 9.2, 15.6, 1.5)	5.24 (*ddq*, *J* = 9.2, 15.3, 1.6)	5.19 (*ddq*, *J* = 9.3, 15.4, 1.7)	5.73 (*ddq*, *J* = 7.2, 15.7, 1.6)
H–C(13)	5.51 (*dq*, *J* = 16, 5)	5.52 (*dq*, *J* = 15.4, 6.5)	5.54 (*dq*, *J* = 15.0, 6.6)	5.66 (*ddq*, *J* = 15.4, *ca.* 0.7, 6.5)	5.44 (*dq*, *J* = 15.7, 6.4)
CH_3(14)	1.55 (*dd*, *J* = 1, 5)	1.54 (br. *d*, *J* = 6.2)	1.54 (*dd*, *J* = 1.3, 6.5)	1.57 (*dd*, *J* = 1.6, 6.5)	1.61 (*d*, *J* = 7.0)
CH_3(15)	3.65 (*s*)	3.68 (*s*)	3.68 (*s*)	3.69 (*s*)	3.70 (*s*)

[a]) For all compounds, the numbering according to **1** is used.
[b]) At 90 MHz in $CDCl_3$/(D_6)DMSO 20:1.
[c]) 2 CH_3CO: 2.087 and 2.093 (2 *s*).
[d]) Exchangeable with D_2O.
[e]) Signals may be interchanged.
[f]) Submerged by the CH_3(15) signal.

Table 3. *^{13}C-NMR Data* ((D_6)DMSO) *of the Spirostaphylotrichins E* (**2**), *F* (**3**), *K* (**7**), *L* (**8**), *M* (**9**), *and S* (**10**) *and of* **23**[a])

C-Atom	Spirostaphylotrichin						
	E (**2**)	F (**3**)	K (**7**)	L (**8**)	M (**9**)	**23**[b])	S (**10**)
C(1)	163.5 (*s*)	164.0 (*s*)	165.9 (*s*)	166.3 (*s*)	163.9 (*s*)	162.9 (*s*)	165.0 (*s*)
C(3)	141.3 (*s*)	141.2 (*s*)	144.7 (*s*)	142.9 (*s*)	142.1 (*s*)	145.6 (*s*)	85.5 (*s*)
C(4)	74.1 (*d*)[c])	74.9 (*d*)[c])	65.5 (*d*)[c])	73.1 (*d*)[c])	74.6 (*d*)	69.0 (*d*)[c])	70.5 (*d*)[c])
C(5)	59.4 (*s*)	57.0 (*s*)	59.1 (*s*)	56.9 (*s*)	55.1 (*s*)	55.2 (*s*)	56.9 (*s*)
C(6)	75.1 (*d*)[c])	75.3 (*d*)[c])	72.2 (*d*)[c])	73.7 (*d*)[c])	70.0 (*d*)	73.6 (*d*)[c])	73.0 (*d*)[c])
C(7)	207.7 (*s*)	205.3 (*s*)	197.0 (*s*)	69.7 (*d*)[c])	95.3 (*s*)	139.7 (*s*)	197.3 (*s*)
C(8)	47.4 (*t*)	45.5 (*t*)	127.4 (*d*)[d])	36.4 (*t*)	43.7 (*t*)	120.0 (*d*)	127.4 (*d*)[d])
C(9)	82.1 (*d*)	81.9 (*d*)	148.1 (*d*)	82.5 (*d*)	81.2 (*d*)	77.3 (*d*)[c])	148.7 (*d*)
C(10)	52.0 (*d*)	46.8 (*d*)	40.5 (*d*)	47.1 (*d*)	46.9 (*d*)	48.4 (*d*)	39.9 (*d*)
C(11)	84.5 (*t*)	83.9 (*t*)	81.7 (*t*)	82.6 (*t*)	82.5 (*t*)	85.1 (*t*)	24.9 (*q*)
C(12)	125.6 (*d*)[d])	126.2 (*d*)[d])	126.4 (*d*)[d])	127.5 (*d*)[d])	126.7 (*d*)	125.3 (*d*)[d])	125.9 (*d*)[d])
C(13)	129.5 (*d*)[d])	129.3 (*d*)[d])	129.1 (*d*)[d])	128.2 (*d*)[d])	128.8 (*d*)	130.0 (*d*)[d])	130.3 (*d*)[d])
C(14)	17.6 (*q*)	17.7 (*q*)	17.7 (*q*)	17.6 (*q*)	17.6 (*q*)	17.7 (*q*)	17.8 (*q*)
C(15)	62.1 (*q*)	62.0 (*q*)	61.9 (*q*)	61.9 (*q*)	61.8 (*q*)	62.0 (*q*)	64.1 (*q*)

[a]) For all compounds, the numbering according to **1** is used.
[b]) 20.48 and 20.53 (2 *q*, 2 CH_3CO); 168.5 and 169.4 (2 *s*, 2 CH_3CO).
[c])[d]) May be interchanged.

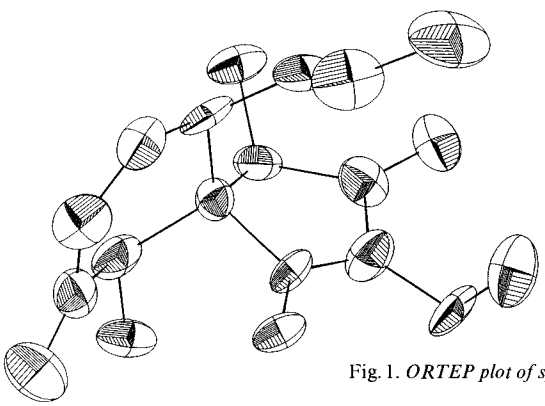


Fig. 1. *ORTEP plot of spirostaphylotrichin K* (**7**)

To clarify the astonishing situation that the two related metabolites **7** and **3** from the same fungus differed in the configuration at C(10), an X-ray analysis of **7** was performed. The required crystal was obtained by recrystallization from MeOH. *Fig. 1* shows the ORTEP plot of the X-ray analysis[2]) of **7** which clearly confirmed the configuration assigned on the basis of the NOE measurements.

Spirostaphylotrichin S (**10**). Comparison of the spectral data of **10** with those of **7** revealed structural agreement between the two compounds, with the exception that the exocyclic double bond is absent in **10**. Instead, in the ^{1}H-NMR, a new signal for a Me group appeared at 1.39 ppm (*s*) and, in the ^{13}C-NMR, at 24.9 ppm, corresponding to CH_3(11), C(3) was found at 85.5 ppm, and a third OH group was detected in the

[2]) We thank PD Dr. *M. Zehnder* and Dr. *A. Riesen*, Institut für Anorganische Chemie der Universität, Basel, for this measurement.

^{1}H-NMR. The MS indicated the M^+ at m/z 297 corresponding to the molecular formula $C_{14}H_{19}NO_6$ which readily can be accommodated with structure **10**.

From the wild-type strain of *S. coccosporum*, we isolated spirostaphylotrichin R (**14**) as an artefact, formed from **1** by acid-catalyzed addition of H_2O to the exocyclic double bond [3]. Similarly, when **7** was dissolved in a moist mixture of $CHCl_3$/DMSO 5:1, a crystalline product was isolated after standing for 4 days at r.t. (**7** decomposed slowly in $CHCl_3$) whose MS agreed well with that of **10**. The ^{1}H-NMR revealed this product to be a 1:1 mixture of **10** and its 3-epimer, thus indicating that **10** is derived from **7**.

Spirostaphylotrichin I (**8**). This metabolite was isolated only in small amounts. Its spectroscopic data indicated the same constitution as found for spirostaphylotrichin S (**10**). Unfortunately, the ^{1}H-NMR spectra of **8** – the first spirostaphylotrichin isolated from a mutant strain – was measured in $CDCl_3$/(D_6)DMSO 20:1. A reexamination of the spectra in (D_6)DMSO was obscured by the partial decomposition of the material. However, it is very likely that **8** is not an artefact derived from **7** because it is not identical with the 3-epimer of spirostaphylotrichin S (**10**) obtained from **7** (see above). Probably, **8** is an artefact derived from a compound which possesses the same constitution as **7**, but which is epimeric at C(10).

Spirostaphylotrichins G and H (**4** and **5** or **5** and **4**, resp.). The ^{1}H-NMR of both **4** and **5** were very similar to that of **7**. The main difference concerned the downfield shift of CH_2(11) and the missing of H–C(4) and OH–C(4). The MS indicated for both an M^+ at m/z 277, *i.e.* 2 amu below that of **7**. These findings were compatible with structures **4** and **5**. The resemblance of **4** and **5** and the isolation of the 6-epimers spirostaphylotrichins A (**1**) and B (**12**) as well as C and D (**15** and **16**) from wild-type cultures of *S. coccosporum* [3] indicated that the spirostaphylotrichins G and H were also epimeric at C(6). The present data do not allow a final assignment of structures **4** and **5**; they still can be interchanged.

Spirostaphylotrichin F (**3**). The MS of **3** suggested the same molecular formula $C_{14}H_{17}NO_5$ as found for **7**. Treatment of **3** with Ac_2O/pyridine at r.t. gave the monoacetyl derivative **17** which, in (D_6)DMSO, was partially converted to **18** by an elimination reaction. Only 1 proton of **3** was exchangeable with D_2O (^{1}H-NMR).

All signals in the ^{1}H-NMR and ^{13}C-NMR of **3** for the lactam ring with the *N*-methoxy group and the exocyclic double bond were assigned in accordance with the other spirostaphylotrichins. H–C(4) showed no coupling with an OH group although an O-atom must be attached to C(4) which would explain its chemical shift of 74.9 ppm. All signals corresponding to the prop-1-enyl side chain were observed as in **7**. H–C(10) appeared as a *d* coupling only with H–C(12). The 8-double bond was absent and the 7-carbonyl group shifted considerably to lower field indicating a non-conjugated keto function. Instead of the 2 olefinic protons, 3 new protons were observed in the ^{1}H-NMR: 2 geminal protons at 2.39 and 2.80 ppm with J_{gem} = 17.5 Hz assigned to CH_2(8) and 1 proton at 4.39 ppm (H–C(9)) as a pseudo-*t* coupling with the 2 other protons with coupling constants of about the same range. The proton at 2.39 ppm showed additional small coupling ($J \approx 1$ Hz), probably to H–C(6). The latter was observed as a broadened *s* (sharper after addition of D_2O). An O-atom has to be attached to C(9) in order to explain its chemical shift, as for C(4). Therefore, the presence of an O-bridge from C(4) to C(9) is a reasonable assumption.

The formation of **3** may be rationalized by the attack of the 4-OH group at the 8-double bond in a compound similar to **7**. In **3**, the configuration at C(9) is fixed by the connection to the lactam ring. The fact that no coupling between H–C(9) and H–C(10) was observed suggests for C(10) the configuration shown in formula **3**, where the dihedral angle H–C(9)–C(10)–H is *ca.* 90°. In the inverse configuration, an angle of *ca.* 40° is expected. In the later case, coupling should be observed with certainty. The proposed

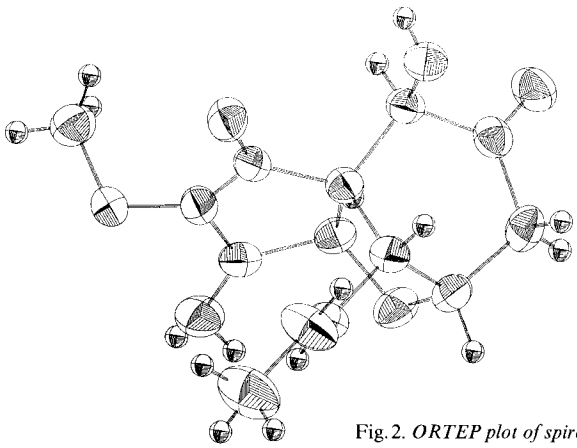


Fig. 2. *ORTEP plot of spirostaphylotrichin F* (**3**).

structure was proven by X-ray diffraction. *Fig. 2* shows an ORTEP plot of **3**. From this analysis, the dihedral angle H–C(9)–C(10)–H was found to be 86°.

Spirostaphylotrichin E (**2**). Treatment of **2** with Ac_2O in pyridine at r.t. gave the *O*-acetyl derivative **19**. Comparison of the data of **2** with those of **3** revealed great similarity of the two metabolites and suggested an identical constitution. The configuration at C(9) and C(10) relative to C(5) in **2** and the configuration at C(4) must be the same as in **3**. Inverse configuration at C(4) would imply two *trans*-fused five-membered rings, an arrangement with strong ring strain, and thus C(1), C(3), C(11), and N(2) would not lie in the same plane. This situation would lead to lower intensity of the UV absorption of this chromophor [8]. But the UV and CD spectra of **2** were in good agreement with those of **3**. Consequently **2** and **3** must differ from each other by the configuration at C(6) (*cf.* the 6-epimers **1**/**12**, **4**/**5**, and **15**/**16**). X-Ray analysis of **3** revealed for the 6-OH group an axial orientation, hence an equatorial arrangement was expected for **2**.

On the basis of the ^{1}H-NMR data of **3**, the expected chemical shifts of **2** were estimated from literature data [9]; they were in good agreement with the observed ones (*Table 4*). For H–C(6), the shift of 0.31 ppm to lower field in going from **3** to **2** was in line with the observed shift of 0.51 ppm in going from **1** to **12**. In a similar way, the influence of the inversion at C(6) on the chemical shift of the different C-atoms was estimated from the known data for substituted cyclohexanes [10]; again they were in good agreement with the observed chemical-shift differences (*Table 5*). The strong NOE (in CD_3CN) between H–C(6) and H–C(10) was considered to be a further proof for **2**.

Table 4. *Selected Chemical Shifts* (ppm) *of* **3** *and* **2** *and Estimated Values of* **2**

H-Atom	**3**	**2**	
		estimated	observed
H_{ax}–C(8)	2.80[a])	2.41	2.50
H_{eq}–C(8)	2.39[a])	2.67	2.74
H–C(9)	4.39	4.53	4.41
H–C(10)	3.59	3.20	3.30

[a]) Assignment based on NOE observed at 2.80 on irradiation at H–C(10).

Table 5. *Estimated and Observed* ^{13}*C-NMR Chemical-Shift Differences* (ppm) *of* **2** *and* **3**

C-Atom	$\Delta(\delta(\mathbf{2})-\delta(\mathbf{3}))$	
	estimated	observed
5	2.4	2.4
7	2.4	2.4
8	4.7	1.9
9	0.9	0.3
10	4.7	5.2

Chemical correlation of **3** and **2** was unsuccessful. Oxidation of the 6-OH group in both metabolites should lead to the same ketone **20**; but pyridinium-chlorochromate oxidation [11] of **3** failed. Inversion of the activated alcohol function in **3** by using acetate as a nucleophile should lead to the acetate **19**; however, no stable product could be obtained by treating **3** with MsCl or TsOH in pyridine. Although **2** gave the mesylate **21** (unstable in solution), its conversion with CsOAc in toluene [12] with or without addition of [18]crown-6 gave only very polar decomposition products. The use of Et_4NOAc in acetone [13] was equally unsuccessful.

Spirostaphylotrichin L (**8**). The EI-MS of **8** showed M^+ at *m/z* 281 and the CI-MS $[M+1]^+$ at 282. These data as well as the NMR established the molecular formula $C_{14}H_{17}NO_5$. Comparison of the ^{1}H-NMR and ^{13}C-NMR data of **8** with those of **2** and **3** revealed fair agreement. Finally, structure **8**, with a 7-OH rather than a C(7)=O group, was confirmed by a detailed ^{1}H-NMR analysis.

The ^{1}H-NMR signals of CH_2(11), H–C(3), and H–C(9) of **8** were observed as broadened *s* at 400 MHz but are in fact *dd*. At 90 MHz, CH_2(11) was observed as pseudo-*t* with $J = 1.8$ Hz; however, the corresponding signal of H–C(4) was overlapped by the olefinic protons of the side chain (it might be a *t* with $J = 1.7$ Hz). The signal of H–C(9) appeared as a broadened *t* with $J = 2.4$ Hz. One important difference between **8** and **3** as well as **2** was observed: the C(7) signal at *ca.* 205 ppm was absent, and a new signal at *ca.* 70 ppm was observed. H–C(8) at 1.83 ppm coupled with H–C(7) which underwent further coupling with the OH proton at 4.99 ppm. H–C(6) at 3.93 ppm coupled with the OH proton at 5.10 ppm. Coupling between H–C(6) and H–C(7) was not resolved, but their signals were broadened *d* and *s*, respectively, after addition of D_2O. At 90 MHz, $J = 0.7$ Hz was observed for H–C(6); H–C(7) was overlapped by CH_3(15). Further proof for **8** was provided by a COSY spectrum, showing a weak cross peak for H–C(6) and H–C(7) and revealing the segments CH_3–CH=CH–CH, CH–CH_2–CH(OH)–CH(OH), and CH–CH=CH_2.

Spirostaphylotrichin M (**9**). The EI-MS of **9** showed M^+ at 279 and the CI-MS $[M+1]^+$ at 280 (molecular formula, $C_{14}H_{17}NO_5$). The UV spectrum exhibited the maximum at 230 nm, *i.e.* at a slightly longer wavelength when compared to **2** and **3**, with an ε value (= 5200) lower than half that of **2** and **3**. It should be noted that **9** is the only spirostaphylotrichin with a strong IR absorption at 1070 cm^{-1} ($\tilde{\nu}$(C–O)). Detailed NMR analyses clearly established structure **9** and excluded the inverse configuration at C(4).

The assignments in the NMR spectra are supported by a 2D ^{13}C,^{1}H shift correlation experiment (HETCOR) *via* ^1J(C,H). Two-dimensional heteronuclear correlation *via* long-range coupling was employed to detect ^2J(C,H) and ^3J(C,H) coupling constants of the magnitude of 7 Hz (see *Exper. Part* for connectivities). In the NMR spectra, all signals of the lactam ring with the exocyclic double bond and the MeO group and of the side chain with H–C(10) were present and comparable to **2**, **3**, and **8**. In the ^{13}C-NMR, the signal at 95 ppm (quarternary C-atom) represents a new feature in the spirostaphylotrichin series. The proton at 6.54 ppm, the only one exchangeable with D_2O, showed long-range couplings with C(6), C(7), and C(8). This observation in combination with the coupling between H–C(9) and CH_2(8) established the structural fragment CH(9)–CH_2(8)–(OH)C(7)–CH(6). The signal of H–C(9) appeared at 4.20 ppm as a broadened, poorly resolved *t* (should be *dd*). The fact that H–C(4) did not show any coupling with an OH proton led to the conclusion that C(4) participates in an ether bridge as in **2**, **3**, and **8**; H–C(10) of **9** also appeared only as a *d*. But the same connection of C(4) and C(9) *via* an O-atom did not lead to a chemically reasonable structure (**22** would, however, explain approximately the observed chemical shifts). In the proposed structure **9**, an O-bridge from C(4) to C(7) implies a hemiacetal function. A further ring is formed by an O-bridge between C(6) and C(9).

The configuration at C(6), C(7), and C(9) relative to C(5) is defined by the ring connections. The fact that no coupling between H–C(9) and H–C(10) was observed led to the proposed configuration at C(10), with a dihedral angle H–C(9)–C(10)–H of *ca.* 80–85°. The inverse configuration would give rise to an angle of *ca.* 50° and coupling should be observed. The dihedral angles were confirmed by calculating the various structures using the MM2 program [14] in which the CH_3O–N group was replaced by the CH_3CH_2–N group. However, the hypothetic structure corresponding to the 4-epimer of **9** is less strained and should be favoured. Indeed, the two *trans*-fused

5-membered rings in structure **9** are rather disfavoured in view of the stability of the hemiacetal function. But the C(6)–O–C(9) bridge fixes the cyclohexane ring in a boat conformation which might improve the stability. Finally, NOE measurements (DMSO, 35°) established the C(4) configuration of **9**. Irradiation at H–C(10) led only to enhancement at H–C(13) (3.3%) and H–C(9) (2.3%). Irradiation at H–C(6) produced enhancement at OH–C(7) (2.3%) and a H–C(4) (3.8%) and irradiation at H–C(4) at H–C(6), (2.4%).

It was intended to provide further proof for structure **9** by preparing suitable derivatives. Opening of the hemiacetal would prove that O–C(4) is part of this functionality because in the open form H–C(4) should couple with OH–C(4). Unfortunately, this conversion failed due to the instability of **9** under acidic or basic conditions. Treatment of **9** with Ac_2O in pyridine led to derivative **23** by acetylation of the hemiacetal OH followed by base-catalyzed elimination and subsequent acetylation of the formed 4-OH group. Thus, the diacetate **23** provides additional confirmation of structure **9** for spirostaphylotrichin M.

The M^+ of **23** in the EI-MS at *m/z* 363 clearly showed that diacetylation had taken place. The UV spectrum, comparable to those of **3** and **2**, exhibited the maximum at 226 nm (ε = 10900). The increase of the ε-value as compared to **9** may be due to the removal of the distortion of the chromophore in the lactam ring. In the ^{1}H-NMR *(Table 2)* and ^{13}C-NMR *(Table 3)* of **23**, the signals for the side chain and the lactam ring appeared at the same positions as for the other spirostaphylotrichins, besides 2 Ac signals. In the ^{13}C-NMR, the signals of C(6) and C(9) gave rise to shifts comparable to those of **9**. Only the signals for C(8) and C(7) at 44 and 85 ppm, respectively, were absent and replaced by 2 new signals at 120 (*d*) and 140 ppm (*s*), compatible with a trisubstituted C=C bond. In the ^{1}H-NMR, the corresponding proton (H–C(8)) was found at 6.05 ppm (*d*, *J* = 6.4 Hz) with coupling to H–C(9) at 4.56 ppm (*d*, *J* = 6.4 Hz). The chemical shifts observed for the C-atoms of the double bond are compatible with an enol-acetate group [10]. The strong shift of H–C(6) of **23** to lower field as compared to **9** can only be explained by deshielding by magnetic anisotropy [15] of the lactam and the carbonyl moiety of the 7-AcO group.

Financial support of these investigations by the *Swiss National Science Foundation* and *Ciba-Geigy, AG*, Basel, is gratefully acknowledged.

Experimental Part

General. See [2] [3]. The spirostaphylotrichins are not very stable and can only be stored in the refrigerator without decomposition, In soln., decomposition was observed. So NMR spectra has to be recorded immediately. NMR spectra have been recorded by *K. Aegerter* and MS by Dr. *H. Nadig*.

Mutagen Treatment of the Microorganism. Cell suspension suitable for mutagen treatment were prepared from fresh cultures of *Staphylotrichum coccosporum DSM 2602* on potato dextrose agar (25 ml; *Difco*) in 100-ml *Erlenmeyer* flasks by suspending the mycelial pads in 40 ml of 0.1M phosphate buffer (pH 7) containing 0.1% *Tween 80 (Merck)*. To reduce the mycelium to smaller pieces, this suspension was ground with a mixer under addition of 5 g of sterile quartz sand and filtered through glass wool. Then, 10 ml of the suspension containing *ca.* 20000 cells capable of germination were exposed to UV irradiation (*Philips TUV*, 15 watt, 254 nm, preheated) of 2 mW/cm^2 in a petri disk (∅ 50 mm) under constant stirring. The time of irradiation to get a surviving rate of 0.5% maximal lied between 90 and 120 s. Defined volumes of the suspension were plated on *Mycological Agar® (Difco)* and incubated at 27° for 4 days.

Selection of Mutants Lacking Production of **1**. Each colony was transferred to a *Mycological Agar®* plate and incubated for 7 days. After storage on separate plates, from each colony, an agar cylinder of 10-mm diameter was stamped out, transferred to a test tube, and left for 2 h after addition of 0.3 ml of CH_2Cl_2. The org. extracts were examined by TLC using Et_2O and CH_2Cl_2/MeOH 9:1 as solvents (**1** was detected by UV light). Strains lacking production of **1** were reexamined from new cultures on agar plates. Mycelium from the interesting strains was then suspended in 0.1M phosphate buffer (pH 7) containing 0.1% *Tween 80*, filtered, and plated on agar. In all, 10 clones per mutant were examined for production of **1** which was in each case absent.

Isolation of the Spirostaphylotrichins. All chromatographic steps were carried out by middle-pressure chromatography using a *Büchi 681* pump, a glass column 26 × 460 mm, a *Isco* type *6* optical unit (254 nm) with a 2-mm cell and a *Isco UA5* absorption monitor. Fermentation of *S. coccosporum* was run according to [1] [2] with slight modifications concerning the incubation times. The culture broths (11 l) were filtered and extracted 3 times with CH_2Cl_2.

The crude extract obtained from the soya medium was digested with pentane to remove the lipids. From CH_2Cl_2, crude **7** was crystallized and recrystallized from MeOH. The mother liquor was chromatographed with CH_2Cl_2 containing 0–10% MeOH to give 5 fractions. *Fr. 2* contained mainly spirostaphylotrichins G and H (**4** and **5** or **5** and **4**, resp.) and was further chromatographed with pentane/Et_2O, where first spirostaphylotrichin H was eluted (this compound was subjected to a further chromatography with Et_2O). Then spirostaphylotrichin G was eluted and crystallized from Et_2O. *Fr. 3* was further chromatographed with pentane/AcOEt to give **3** and **2** which could be crystallized from CH_2Cl_2/Et_2O. *Fr. 4* gave further **2**. *Fr. 5* yielded **10**, after chromatography with AcOEt.

The crude extract from the minimal medium was chromatographed with a pentane/Et_2O/acetone/MeOH gradient. From *Fr. 2*, spirostaphylotrichin G (**4** or **5**) was crystallized. Spirostaphylotrichin H (**5** or **4**) was detected as trace by TLC, but was not isolated. *Fr. 3* was rechromatographed with pentane/AcOEt where **3** was eluted first, followed by **2**. In the same way, *Fr. 4* was rechromatographed giving **8**, after crystallization from Et_2O. *Fr. 5* gave **6** and *Fr. 7* **9**.

Spirostaphylotrichin E (= *(3aR*,5S*,8S*,8aS*,9R*)-3,3a,5,6,-Tetrahydro-8-hydroxy-2-methoxy-3-methylidene-9-[(E)-prop-1-enyl]-5,8a-methanooxepino[2,3-c]pyrrole-1,7(2H,8H)-dione;* **2**[3]). M.p. 107–109°. UV (EtOH): 225 (12100). CD (EtOH): 195 (–5), 204 (0), 219 (+17), 229 (0), 240 (–16). IR (KBr): 3400 (br., sh), 3350 (br., OH), 3020*w*, 2980*m*, 2950*m*, 2920*m*, 2860*w*, 1745*s*, 1735*s*, 1670*m*, 1440*m*, 1380*m*, 1280*m*, 1145*m*, 1025*m*, 970*m*, 910*m*, 855*m*, 695*m*, 680*m*. ^{1}H-NMR (400 MHz, (D_6)DMSO): *Table 2*. ^{1}H-NMR (400 MHz, CD_3CN): 1.62 (*dd*, *J* = 6.6, 1.4, CH_3(14)); 2.67 (*m*, CH_2(8)); 3.20 (*d*, *J* = 9.2, H–C(10)); 3.75 (*s*, CH_3(15)); 4.03 (br., OH–C(6)); 4.43 (*t*, *J* = 2.7, H–C(9)); 4.45 (*t*, *J* = 1.8, H–C(11 or 4)); 4.54 (*s*, H–C(6)); 4.60 (*t*, *J* = 2, H–C(11 or 4)); 4.66 (*t*, *J* = 2.1, H–C(11 or 4)); 5.35 (*ddq*, *J* = 9.0, 15.3, 1.7, H–C(12)); 5.73 (*ddq*, *J* = 15.3, ⩽1, 6.6, H–C(13)). ^{13}C-NMR (22.6 MHz, (D_6)DMSO): *Table 3*. EI-MS (70 eV, 200°): 279 (M^+), 248 ($[M - CH_3O]^+$), 222, 220, 208, 192, 178, 161, 154, 133, 124, 105, 81 (100). CI-MS (NH_3, 200°): 297 ($[M + NH_4]^+$), 280 (100, $[M + 1]^+$), 264, 250, 234.

Spirostaphylotrichin F (= *(3aR*,5S*,8R*,8aS*,9R*)-3,3a,5,6,-Tetrahydro-8-hydroxy-2-methoxy-3-methylidene-9-[(E)-prop-1-enyl]-5,8a-methanooxepino[2,3-c]pyrrole-1,7(2H,8H)-dione;* **3**). M.p. 114–116°. UV (EtOH): 225 (12200). CD (EtOH): 194 (–11), 205 (0), 223 (+17), 233 (0), 244 (–13). IR (KBr): 3420 (br., OH), 3020*w*, 2970*m*, 2940*w*, 2920*w*, 1730*s*, 1720*s*, 1695*m*, 1660*s*, 1445*m*, 1270*m*, 1210*m*, 1115*m*, 1035*m*, 975*m*, 850*m*, 840*m*, 835*m*. ^{1}H-NMR (400 MHz, (D_6)DMSO): *Table 2*. ^{1}H-NMR (90 MHz, $CDCl_3$): 1.65 (*dd*, *J* = 1.3, 6.2, CH_3(14)); 2.71 ('*d*', '*J*' = 2.9, *AB* of *ABX*, CH_2(8)); 3.65 (*d*, *J* = 8.7, H–C(10)); 3.67 (br. *s*, exchangeable with D_2O, OH–C(6)); 3.83 (*s*, CH_3(15)); 4.26 (br. *s*, with D_2O sharper, H–C(6)); 4.49 (*t*, *J* = 2.8, *X* of *ABX*, H–C(9)); 4.60 (*m*, 2 H–C(11) or H–C(11) and H–C(4)); 4.73 (*t*, *J* = 2.1, H–C(4 or 11)); 5.42 (*ddq*, *J* = 8.7, 15, 1.4, H–C(12)); 5.84 (*dq*, *J* = 15, 6.0, H–C(13)). ^{13}C-NMR (22.6 MHz, (D_6)DMSO): *Table 3*. EI-MS (70 eV, 150°): 279 (M^+), 251, 222, 208, 179, 154 (100). CI-MS (NH_3, 175°): 297 ($[M + NH_4]^+$), 280 (100, $[M + 1]^+$), 267, 264, 250, 234.

Spirostaphylotrichin G (= *(5R*,6S*)-6-Hydroxy-2-methoxy-3-methylidene-10-[(E)-prop-1-enyl]-2-azaspiro[4.5]dec-8-en-1,4,7-trione;* **4** *or* **5**). M.p. 160–176°. UV (EtOH): 279 (3200), 232 (10000), 202 (10800). CD (EtOH): 195 (+18), ~206 (sh, +11), 220 (0), 243 (–21). IR (KBr): 3450 (sh), 3410 (br., OH), 3010*w*, 2970*w*, 2860*w*, 1760*m*, 1725*s*, 1700*s*, 1650*m*, 1445*m*, 1315*m*, 1280*m*, 1120*m*, 970*m*, 960*m*, 905*m*, 865*m*. ^{1}H-NMR (400 MHz, (D_6)DMSO): *Table 2*. EI-MS (70 eV, 200°): 277 (M^+), 218, 170, 149, 138, 121, 108 (100). CI-MS (NH_3, 250°): 295 ($[M + NH_4]^+$), 278 ($[M + 1]^+$), 265.

Spirostaphylotrichin H (= *(5R*,6R*)-6-Hydroxy-2-methoxy-3-methyliden-10-[(E)-prop-1-enyl]-2-azaspiro[4.5]dec-8-en-1,4,7-trione;* **5** *or* **4**). UV (qual., EtOH): max. < 200, *ca.* 230 (sh), 280 (sh). CD (qual., EtOH): 192 (+), 214 (0), 242 (–). IR (KBr): 3470 (br., OH), 2940*m*, 2880*m*, 1770*m*, *ca.* 1730*s* (several), 1650*m*, 1310*m*, 1280*m*, 1120*m*, 980*m*. ^{1}H-NMR (400 MHz, (D_6)DMSO): *Table 2*. EI-MS (70 eV, 150°): 277 (M^+), 194, 170, 108 (100).

Spirostaphylotrichin I (= *3,4,6-Trihydroxy-2-methoxy-3-methyl-10-[(E)-prop-1-enyl]-2-azaspiro[4.5]dec-8-en-1,7-dione;* **6**). M.p. 167–176°. UV (EtOH): max. < 200, *ca.* 220 (sh, 7600), *ca.* 240 (sh, 5500). IR (KBr): 3480 (br.), 3340 (br., OH), 2940*w*, 2920*w*, 1695*s*, 1470*m*, 1240*m*, 1165*m*, 1095*m*, 960*m*. ^{1}H-NMR (90 MHz, $CDCl_3$/(D_6)DMSO 20:1): *Table 2*. EI-MS (70 eV, 250°): 297 (M^+), 279 ($[M - H_2O]^+$), 254, 248 ($[M - H_2O - CH_3O]^+$), 226, 208, 108 (100). CI-MS (NH_3, 350°): 315 ($[M + NH_4]^+$), 298 ($[M + 1]^+$), 280 ($[M + 1 - H_2O]^+$), 268, 250.

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[3]) For all spirostaphylotrichins, assignments of relative configurations according to the IUPAC-conform numbering.

Spirostaphylotrichin K (= *(4R*,5S*,6R*,10R*)-4,6-Dihydroxy-2-methoxy-3-methyliden-10-[(E)-prop-1-enyl]-2-azaspiro[4.5]dec-8-en-1,7-dione;* **7**). M.p. 200–205°. UV (EtOH): 226 (15100). CD (EtOH): 194 (+28), 208 (0), 219 (−11), 226 (−0), 244 (−19). IR (KBr): 3420 (br., OH), 3260 (br., OH), 2945*w*, 2920*w*, 1715*s*, 1700*s*, 1675*s*, 1440*m*, 1275*m*, 1160*m*, 1110*m*, 1100*m*, 1080*m*, 995*m*, 975*m*, 870*m*. ^{1}H-NMR (400 MHz, (D$_6$)DMSO): *Table 2*. ^{13}C-NMR (22.6 MHz, (D$_6$)DMSO): *Table 3*. EI-MS (70 eV, 300°): 279 (M^+), 248 ([M − CH_3O]$^+$), 230 ([M − CH_3O − H_2O]$^+$), 172, 161, 133, 108 (100). CI-MS (NH_3, 300°): 297 ([M + NH_4]$^+$), 280 ([M + 1]$^+$), 267.

Spirostaphylotrichin L (= *(3aR*,5S*,7ξ,8ξ,8aS*,9R*)-3,3a,5,6,7,8-Hexahydro-7,8-dihydroxy-2-methoxy-3-methylidene-9-[(E)-prop-1-enyl]-5,8a-methanooxepino[2,3-c]pyrrole-1(2H)-one;* **8**). M.p. 131–137°. UV (EtOH): 228 (9500). IR (KBr): 3440 (br., OH), 2950*m*, 1725*s*, 1695*m*, 1665*s*, 1270*m*, 1060*m*, 1025*m*, 975*m*. ^{1}H-NMR (400 MHz, (D$_6$)DMSO): *Table 2*. ^{13}C-NMR (22.6 MHz, (D$_6$)DMSO): *Table 3*. EI-MS (70 eV, 200°): 281 (M^+), 250 ([M − CH_3O]$^+$), 232 ([M − CH_3O − H_2O]$^+$), 222, 154 (100). CI-MS (NH_3, 200°): 299 (weak, [M + NH_4]$^+$), 282 (100), [M + 1]$^+$), 252.

Spirostaphylotrichin M (= *(2R*,3aS*,4aS*,7aS*,7bS*,8R*)-4a,5,6,7-Tetrahydro-3a-hydroxy-6-methoxy-5-methylidene-8-[(E)-prop-1-enyl]-2H,3H-2,7a-methanofuro[2',3':4,5]furo[2,3-c]pyrrol-7(3aH)-one;* **9**). M.p. 187–190°. UV (EtOH): 230 (5200). IR (KBr): 3400 (br., OH), 2950*m*, 1730*s*, 1690*s*, 1670*s*, 1275*m*, 1120*m*, 1070*s*, 980*m*, 970*m*, 860*m*. ^{1}H-NMR (400 MHz, (D$_6$)DMSO): *Table 2*. ^{13}C-NMR (22.6 MHz, (D$_6$)DMSO): *Table 3*. HETCOR shift correlation: C(1)/*H*−C(6); C(3)/*H*−C(4),CH_2(11); C(4)/H_{cis}−C(11); C(5)/*H*−C(4), *H*−C(6), *H*−C(9), *H*−C(10); C(6)/*H*−C(4), O*H*−C(7), *H*−C(10); C(7)/*H*−C(6), O*H*−C(7), CH_2(8), *H*−C(9); C(8)/*H*−C(6), O*H*−C(7); C(9)/*H*−C(8); C(11)/*H*−C(4); C(12)/*H*−C(10), CH_3(14); C(13)/*H*−C(10), CH_3(14); C(14)/*H*−C(12). EI-MS (70 eV, 350°): 279 (M^+), 251 ([M − CO]$^+$), 222, 208, 179, 154 (100). CI-MS (NH_3, 400°): 297 ([M + NH_4]$^+$), 280 ([M + 1]$^+$). 264, 250, 234.

Spirostaphylotrichin S (= *(3ξ,4R*,5S*,6R*,10R*)-3,4,6-Trihydroxy-2-methoxy-3-methyl-6-[(E)-prop-1-enyl]-2-azaspiro[4.5]dec-8-en-1,7-dione;* **10**). M.p. 166–173°. UV (EtOH): max. < 200, 210–220 (sh, 7000), 230–240 (sh, 5000). IR (KBr): 3350 (br., OH), 2950*w*, 1700*s*, 1410*m*, 1225*m*, 1100*m*. ^{1}H-NMR (400 MHz, (D$_6$)DMSO): *Table 2*. ^{13}C-NMR (22.6 MHz, (D$_6$)DMSO): *Table 3*. EI-MS (70 eV, 250°): 297 (M^+), 279 ([M − H_2O]$^+$), 266 ([M − CH_3O]$^+$), 254 ([M − C_2H_3O]$^+$), 248 ([M − H_2O − CH_3O]$^+$), 224, 196, 172, 108 (100). CI-MS (NH_3, 400°): 315 ([M + NH_4]$^+$), 298 ([M + 1]$^+$), 280 (100, [M + 1 − H_2O]$^+$).

4,6-Di-O-acetylspirostaphylotrichin K (**11**). A soln. of **7** (14 mg, 0.05 mmol) in dry pyridine (0.1 ml) and Ac_2O (0.05 ml) was allowed to stand at r.t. for 2 h. The mixture was evaporated, and the resulting yellow crystals were washed with Et_2O to give slightly yellow crystals of **11** (12 mg). M.p. 176–184°. IR (KBr): 1760*s*, 1735*s*, 1700*s*, 1670*m*, 1370*m*, 1230*s* (C−O), 1095*m*, 1070*m*. ^{1}H-NMR (400 MHz, (D$_6$)DMSO): 1.61 (*dd*, *J* = 6.5, 1.5, CH_3(14)); 2.13, 2.22 (2*s*, 2 CH_3CO); 3.73 (*s*, CH_3(15)); 3.95 (*m*, H−C(10)); 4.40 (*t*, *J* = 2.0, H−C(11)); 4.59 (*t*, *J* = 2.2, H−C(11)); 5.37 (*ddq*, *J* = 7.9, 15.7, 1.6, H−C(12)); 5.62 (*s*, H−C(6)); 5.67 (*dq*, *J* = 15.6, 6.5, H−C(13)); 5.80 (*t*, *J* = 2.1, H−C(4)); 6.08 (*dd*, *J* = 3.1, 10.3, H−C(8)); 6.72 (*dd*, *J* = 2.3, 10.3, H−C(9)). EI-MS (70 eV, 300°): 363 (M^+), 321 ([M − C_2H_2O]$^+$), 303 ([M − C_3H_2O − H_2O]$^+$), 272, 261, 230, 213, 108, 43 (100). CI-MS (NH_3, 300°): 381 (100, [M + NH_4]$^+$), 364 ([M + 1]$^+$).

Spirostaphylotrichin S (**10**) *from Spirostaphylotrichin K* (**7**). A soln. of **7** (25 mg, 0.09 mmol) in DMSO (0.2 ml) and $CHCl_3$ (1 ml) and a trace of H_2O was allowed to stand at r.t. for 4 days when, on TLC, practically no **7** was detectable. After evaporation, the mixture was chromatographed on silica gel with pentane/Et_2O to give a pure (TLC) product (5 mg) with m.p. 164–167°. ^{1}H-NMR (400 MHz, (D$_6$)DMSO): *ca.* 1:1 mixture of **10** and a closely related compound; a.o. 1.33 (*s*); 1.39 (*s*); 1.61 (*d*, *J* = 6.2); 1.64 (*dd*, *J* = 5.9, *ca.* 1); 4.11 (*d*, *J* = 2.4; with D_2O, *s*); 4.12 (*d*, *J* = 3.5; with D_2O, *s*); 4.28 (*d*, *J* = 5.6; with D_2O, *s*); 4.38 (*d*, *J* = 5.5; with D_2O, *s*); 5.30 (*s*, exchangeable with D_2O); 5.43 (*d*, *J* = 3.6; exchangeable with D_2O); 5.45 (*d*, *J* = 3.7; exchangeable with D_2O); 5.84 (br. *d*, *J* = 5.2, exchangeable with D_2O); 6.56 (*dd*, *J* = 2.5, 10.1); 6.68 (*dd*, *J* = 2.5, 10.2). EI-MS (70 eV, 200°): 297 (M^+), 279 ([M − H_2O]$^+$), 266 ([M − CH_3O]$^+$), 254 ([M − C_2H_3O]$^+$), 248 ([M − H_2O − CH_3O]$^+$), 224, 196, 172, 108 (100). CI-MS (NH_3, 300°): 315 ([M + NH_4]$^+$), 298 ([M + 1]$^+$), 280 (100, [M + 1 − H_2O]$^+$).

6-O-Acetylspirostaphylotrichin E (**19**). A soln. of **2** (51 mg, 0.18 mmol) in dry pyridine (0.5 ml) and Ac_2O (0.5 ml) was allowed to stand at r.t. for 70 min. After evaporation, the mixture was chromatographed on silica gel with Et_2O to give pure (TLC), crystalline **19** (55 mg). M.p. 182–184°. IR (KBr): 2940*w*, 1760*s*, 1740 (sh), 1735*s*, 1675*s*, 1445*m*, 1370*m*, 1360*m*, 1270*m*, 1230*s*, 1140*m*, 1060*m*, 975*m*. EI-MS (70 eV, 200°): 321 (M^+), 279 ([M − C_2H_2O]$^+$), 261 ([M − C_2H_2O − H_2O]$^+$), 230, 170, 43 (100). CI-MS (NH_3, 300°): 359 ([M + NH_4]$^+$), 322 (100, [M + 1]$^+$), 280. ^{1}H-NMR (400 MHz, (D$_6$)DMSO): 1.60 (*dd*, *J* = 1.6, 6.5, CH_3(14)); 2.06 (*s*, CH_3CO); 2.58 (*dd*, *J* = 2.8, 17.4, H−C(8)); 2.88 (*dd*, *J* = 2.6, 17.2, H−C(8)); 3.65 (*d*, *J* = 9.2, H−C(10)); 3.71 (*s*, CH_3(5)); 4.49 (*t*, *J* = 1.8, H−C(11 or 4)); 4.51 (*t*, *J* = 2.7, H−C(9)); 4.63 (*t*, *J* = 2.0, H−C(11 or 4)); 4.71 (*t*, *J* = 1.8, H−C(4 or 11)); 5.29 (*ddq*, *J* = 9.1, 15.3, 1.7, H−C(12)); 5.70 (*dq*, *J* = 15.5, 6.4, H−C(13)); 5.91 (*s*, H−C(6)). ^{13}C-NMR (101 MHz, (D$_6$)DMSO): 17.7

(*q*, C(14)); 20.0 (*q*, CH_3CO); 47.4 (*t*, C(8)); 52.1 (*d*, C(10)); 57.5 (*s*, C(5)); 62.3 (*q*, C(15)); 74.8 (*d*, C(4 or 6)); 75.2 (*d*, C(4 or 6)); 82.1 (*d*, C(9)); 85.7 (*t*, C(11)); 124.8 (*d*, C(13)); 130.4 (*d*, C(12)); 140.3 (*s*, C(3)); 162.2 (*s*, C(1)); 168.6 (*s*, CH_3CO), 201.6 (*s*, C(7)).

Acetylation of Spirostaphylotrichin F (**3**). A soln. of **3** (50 mg, 0.18 mmol) in dry pyridine (0.5 ml) and Ac_2O (0.4 ml) was allowed to stand at r.t. for 60 min. After evaporation, the mixture was chromatographed on silica gel with pentane/Et_2O. Crystallization from Et_2O gave pure (TLC) **17** (46 mg). NMR spectra: 4:5 mixture **17**/**18**, the latter being formed in the NMR soln. Isolation of **18** by chromatography on silica gel with pentane/AcOEt was not successful; only **17** was eluted. The attempt to acetylate the arising **18** *in situ* by dissolving **17** in DMSO and adding pyridine/Ac_2O failed. No stable product could be isolated.

6-O-Acetylspirostaphylotrichin F (**17**). M.p. 159–162°. IR (KBr): 2940*m*, 1760*s*, 1740*s*, 1670*s*, 1375*m*, 1265*m*, 1230*m*, 1035*m*, 975*m*, 865*m*. ^{1}H-NMR (400 MHz, (D_6)DMSO; mixture with **18**): 1.59 (*dd*, *J* = 1.6, 6.5, CH_3(14)); 2.11 (*s*, CH_3CO); 2.59 (br. *d*, *J* = 19.3, H–C(8)); 2.85 (*dd*, *J* = 3.7, 19.0, H–C(8)); 3.34 (*d*, *J* = 8.8, H–C(10)); 3.69 (*s*, CH_3(15)); 4.42 (*t*, *J* = 1.7, H–C(11)); 4.47 (H–C(9), together with H–C(11) of **18**); 4.57 (*t*, *J* = 1.9, H–C(11)); 5.03 (*t*, *J* = 1.6, H–C(4)); 5.26 (*ddq*, *J* = 9.0, 15.3, 1.7, H–C(12)); 5.64 (*s*, H–C(6)); 5.68 (*dq*, *J* = 15.4, 6.6, H–C(13)). ^{13}C-NMR (101 MHz, (D_6)DMSO; mixture with **18**): 17.7 (*q*, C(14)); 20.4 (*q*, CH_3CO); 45.8 (*t*, C(8)); 48.4 (*d*, C(10)); 54.6 (*s*, C(5)); 62.0 (*q*, C(15)); 73.3 (*d*, C(4 or 6)); 74.7 (*d*, C(4 or 6)); 80.4 (*d*, C(9)); 84.8 (*t*, C(11)); 125.2 (*d*, C(13)); 130.1 (*d*, C(12)); 140.5 (*s*, C(3)); 162.8 (*s*, C(1)); 168.7 (*s*, CH_3CO); 202.3 (C(7)). EI-MS (70 eV, 200°): 321 (M^+), 279 ([$M - C_2H_2O$]$^+$), 251, 230, 208, 154, 43 (100). CI-MS (NH_3, 300°): 339 ([$M + NH_4$]$^+$), 322 (100, [$M + 1$]$^+$).

(4R,5S*,6R*,10S*)-4-Hydroxy-2-methoxy-3-methylidene-1,7-dioxo-10-[(E)-prop-1-enyl]-2-azaspiro[4.5]-dec-8-en-6-yl Acetate* (**18**). ^{1}H-NMR (400 MHz, (D_6)DMSO; mixture with **17**): 1.70 (br. *d*, *J* = 6.5, CH_3(14)); 2.01 (*s*, CH_3CO); 3.57 (br. *t*, *J* = 5.7, H–C(10)); 3.73 (*s*, CH_3(15)); 4.31 (*d*, *J* = 5.8, H–C(4)); 4.39 (br. *s*, H–C(11)); 4.47 (H–C(11), together with H–C(9) of **17**); 5.53 (*s*, H–C(6)); 5.59 (*ddq*, *J* = 15.8, 1.1, 6.5, H–C(13)); 5.92 (*ddq*, *J* = 6.6, 15.8, 1.6, H–C(12)); 6.11 (*d*, *J* = 10.3, H–C(8)); 6.16 (*d*, *J* = 5.8, OH–C(4)); 7.00 (*dd*, *J* = 5.6, 10.0, H–C(9)). ^{13}C-NMR (101 MHz, (D_6)DMSO; mixture with **17**): 18.0 (*q*, C(14)); 20.1 (*q*, CH_3CO); 40.3 (*d*, C(10)); 53.8 (*s*, C(5)); 61.7 (*q*, C(15)); 68.3 (*d*, C(4)); 73.1 (*d*, C(6)); 84.0 (*t*, C(11)); 126.3, 126.4, 129.8 (each *d*, C(8), C(12), C(13)); 143.9 (*s*, C(3)); 150.6 (*d*, C(9)); 166.2 (*s*, C(1)); 169.1 (*s*, CH_3CO); 190.2 (*s*, C(1)).

Acetylation of Spirostaphylotrichin M (**9**). Spirostaphylotrichin M (**9**), 100 mg, 0.36 mmol) was suspended in dry pyridine (0.6 ml), and Ac_2O (0.3 ml) was added. After 2 days standing at r.t., the mixture was diluted with AcOEt and evaporated. Chromatography on silica gel with pentane/Et_2O gave pure (TLC) *(4R*,5S*,6R*,9S*,10S*)-6,9-epoxy-2-methoxy-3-methylidene-1-oxo-10-[(E)-prop-1-enyl]-2-azaspiro[4.5]dec-7-ene-4,7-diyl diacetate* (**23**; 23 mg) as a colorless gum. UV (EtOH): 225 (10900). IR (KBr): 2950*w*, 1750*s* (br.), 1670*m*, 1375*m*, 1230 (*s*, C–O), 1180*m*, 1025*m*. ^{1}H-NMR (400 MHz, (D_6)DMSO): *Table 2*. ^{13}C-NMR (101 MHz, $CDCl_3$): *Table 3*. EI-MS (70 eV, 200°): 363 (M^+), 321 ([$M - C_2H_2O$]$^+$), 279 ([$M - 2 \times C_2H_2O$]$^+$), 261, 232, 161, 43 (100).

Oxidation of Spirostaphylotrichin F (**3**). Pyridinium chlorochromate (23 mg, 0.11 mmol) and NaOAc (4 mg) were suspended in CH_2Cl_2, then **3** (20 mg, 0.072 mmol) was added. Slowly, a black precipitate appeared, indicating that oxidation took place. After 3 h, the mixture was diluted with Et_2O and filtered over *Florisil*. The precipitate was exhaustively extracted with Et_2O. After evaporation, 12 mg of product were obtained, corresponding essentially to **3** by TLC. MS: good agreement with **3**. Neither by EI-MS (70 eV, 200°) nor by CI-MS (NH_3, 120° and 200°), traces of M^+ for **20** at *m/z* 277 or [$M + 1$] at *m/z* 278, resp., were detectable (detection limit < 0.01% of [$M + 1$]$^+$ of **3** in CI-MS).

6-O-(Methanesulfonyl)spirostaphylotrichin E (**21**). To **2** (70 mg, 0.25 mmol) in dry pyridine (1 ml), methanesulfonyl chloride (0.1 ml) was added. The mixture was allowed to stand at r.t. for 4 h. Then, 25 ml of moist AcOEt was added and evaporated. After chromatography on silica gel with pentane/AcOEt, almost pure (TLC) **21** (59 mg) was obtained as a colourless foam. ^{1}H-NMR (400 MHz, (D_6)DMSO): a.o. signals at 1.64 (*d*, *J* = 5.9, CH_3(14)); 2.59 (*d*, *J* = 18.2, H–C(8)); 2.83 (*dd*, *J* = 17.9, 2.9, H–C(8)); 3.19 (*s*, CH_3S); 3.47 (*d*, *J* = 7.3, H–C(10)); 3.75 (*s*, CH_3(15)); 5.25 (*ddq*, *J* = 15.4, 7, 1.5, H–C(12)); 5.68 (*dq*, *J* = 7, 15.4, H–C(13)). EI-MS (70 eV, 350°): 357 (M^+), 278 ([$M - SO_2CH_3$]$^+$), 248, 230, 161, 154, 81 (100).

Crystal-Structure Analysis of **7** *and* **3**. Details of crystal data and parameters of data collection are given in *Table 6*. Unit cell parameters were determined from accurate centering of 25 strong reflections by the least-squares method. Four standard reflections monitored every 3600 s showed no significant variation of the intensity. The raw data set was corrected for polarization effects, but no correction for absorbance was applied. The structures were solved by the direct methods using the programs SHELXS-76 [16] and SHELXS-86 [17] for **7** and **3**, respectively, and showed for **3** two independent molecules per unit cell. Equal bond lengths in the two molecules differed not

Table 6. *Crystal Data and Parameters of the Data Collection for the Spirostaphylotrichins K* (**7**) *and F* (**3**)

	Spirostaphylotrichin K (**7**)	Spirostaphylotrichin F (**3**)
Formula	$C_{14}H_{17}NO_5$	$C_{14}H_{17}NO_5$
Space group	monoclinic, $P2_1$	triclinic, $P1$
a [Å]	8.068 (2)	7.790 (6)
b [Å]	9.034 (4)	8.568 (2)
c [Å]	9.717 (3)	12.515 (2)
α [°]	90	91.50 (2)
β [°]	91.64 (2)	108.13 (2)
γ [°]	90	117.02 (3)
V [Å]	707.9	693.2
Z	2	2 (independent)
Temperature [K]	293	293
Θ_{max} [°]	26	25
Radiation	MoK_α ($\lambda = 0.71069$ Å)	MoK_α ($\lambda = 0.71069$ Å)
Scan type	$\omega/2\Theta$	$\omega/2\Theta$
Collected intensities	$\pm h, +k, +l$	$\pm h, \pm k, \pm l$
No. of ind. reflections	1476	4871
No. of refl. used in ref.	882 ($F > 2\sigma(F)$)	4601 ($F > 2\sigma(F)$)
No. of variables	189	488
Final R value	0.087	0.030

more than 0.01 Å. Anisotropic least-squares refinements were carried out on all non H-atoms. For **3**, H-atoms were localized from a final difference *Fourier* map. For **7**, all H-atoms were set in calculated positions. Scattering factors are from *Cromer et al.* [18], except those for H-atoms, which are from *Stewart et al.* [19]. *Figs. 1* and *2* show an ORTEP plot for **7** and **3**, resp. Fractional coordinates and supplementary material are deposited in the *Cambridge Crystallographic Data Base*.

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