87. New Spirostaphylotrichins from *Staphylotrichum coccosporum*

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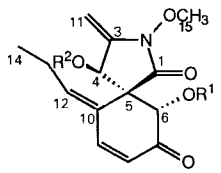
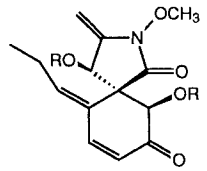
The isolation and structure elucidation of the new spirostaphylotrichins B (**2**), C and D (**3**/**4**), F (**5**), Q (**6**), and R (**7**) are described. Compounds **2** and **3** are artefacts formed during the isolation of the metabolites from the cultures. The absolute configuration of spirostaphylotrichin A (**1**) has been determined by CD spectroscopy.

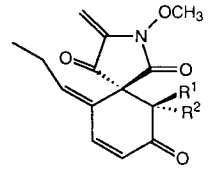
Introduction. – Spirostaphylotrichin A (**1**) is a secondary metabolite which has been isolated by *Peter* and *Auden* [1] from cultures of the fungus *Staphylotrichum coccosporum*. Its structure and relative configuration were established by an X-ray analysis. The compound represents a novel structural type of natural products. In the course of our investigations on the biosynthesis of **1** [2], we have isolated six new metabolites, named



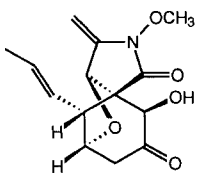
1 $R^1 = R^2 = H$, spirostaphylotrichin A
10 $R^1 = R^2 = Ac$
14 $R^1 = 4\text{-}R'\text{-}C_6H_4CO$, $R^2 = H$
16 $R^1 = R^2 = 4\text{-}CH_3O\text{-}C_6H_4CO$



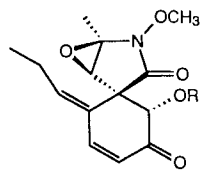
2 R = H, spirostaphylotrichin B
9 R = Ac
17 $R = 4\text{-}CH_3O\text{-}C_6H_4CO$



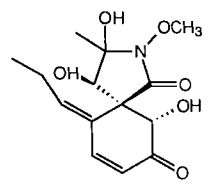
3 $R^1 = H$, $R^2 = OH$, spirostaphylotrichin C[2])
4 $R^1 = OH$, $R^2 = H$, spirostaphylotrichin D[2])



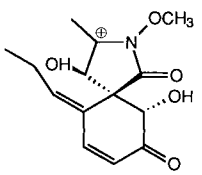
5 spirostaphylotrichin F



6 R = H, spirostaphylotrichin Q
15 $R = 4\text{-}CH_3O\text{-}C_6H_4CO$



7 spirostaphylotrichin R



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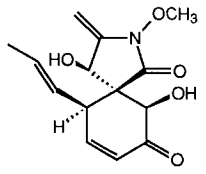
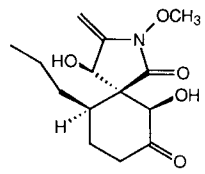


11 spirostaphylotrichin K



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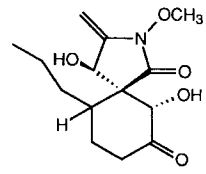

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spirostaphylotrichin B (**2**), C and D (**3**/**4**), F (**5**), Q (**6**) and R (**7**) from the culture broth of *S. coccosporum*. In the present paper, the isolation and structural determination of the new spirostaphylotrichins are described[1]).

Results. – *Spirostaphylotrichins Q* (**6**) *and R* (**7**). In the course of the biosynthetic studies on spirostaphylotrichin A (**1**), two additional substances were detected on TLC in variable amounts. Both compounds, spirostaphylotrichin Q and R, were separated from **1** by chromatography on silica gel. Structures **6** and **7** were assigned on the basis of the following evidence. The molecular formulae $C_{14}H_{17}NO_5$ for **6** and $C_{14}H_{19}NO_6$ for **7** were deduced from MS and ^{1}H-NMR *(Table 1)*. ^{13}C-NMR *(Table 2)* and elemental analysis were used only for **6**. In addition, **6** and **7** were correlated with **1** by chemical transformation.

Both compounds showed similar ^{1}H-NMR spectra than **1**. The difference only concerned the lactame ring, where an additional Me group appeared in place of the exocyclic double bond. In the less polar **6**, only 1 H-atom was exchanged by a D-atom on treatment with D_2O. These observations can be explained by structure **6**. The observed chemical shift of 97.8 ppm for C(3) is in agreement with this structure. In **7**, 3 exchangeable hydroxy protons were present, compatible with the proposed structure. The observation that **1** was partially converted to **6** in a $CDCl_3$ solution established for **6** the same configuration as for **1** and led to the suspicion that **6** is an artefact. Although the initial presence of small amounts of **6** could not be excluded as demonstrated by the HPLC analysis of the culture broth, it was obvious that the HPLC signal corresponding to **6** in the crude extract was increased after workup of the culture broth. The same was the case for **7**. Both **1** and **6** could be converted to **7** by treatment with dilute mineral acids. Most probably, the reaction proceeded *via* the carbenium ion **8**. Therefore, also **7** possesses the same configuration as **1**; only the configuration at C(3) remains unknown. Examination of the mother liquor of **7** by ^{1}H-NMR revealed the presence of a second similar compound which presumably is the 3-epimer of spirostaphylotrichin R (**7**). However, it was not possible to carry out further investigations on this compound.

Spirostaphylotrichin B (**2**). When **1** was produced in the minimal medium [2], a crystalline mixture contained, besides **1**, a very similar compound named spirostaphylotrichin B. It could be separated from **1** by HPLC. The production of **2** could be reduced by preventing the pH of the medium falling below 5.0. It is safe to say that the new metabolite is not an artefact derived from **1**, because the incorporation rates of various radioactive labelled precursors into **2** were significantly different from those into **1** [2]. Spirostaphylotrichin B was not produced in the soya medium. Structure **2** was assigned on the basis of the following data. The UV spectra of **2** and **1** were practically superimposable. The MS showed similar fragmentation patterns, and **2** had the same molecular formula $C_{14}H_{17}NO_5$ as **1** as shown by the elemental analysis. Compound **2** gave the diacetate **9** in the same way as the diacetate **10** was obtained from **1**. Comparison of the ^{13}C-NMR *(Table 2)* revealed a correspondence within ±2 ppm; only for C(4), the deviation was 5 ppm. Also the ^{1}H-NMR *(Table 1)* generally agreed well with that of **1**. However, H–C(4) was shifted by 0.4 ppm downfield and H–C(6) by 0.5 ppm upfield. These data indicate that **2** is a diastereoisomer of **1** differing only in the configuration of the OH groups. The occurrence of the spirostaphylotrichins C and D (**3**/**4**) which differ

[1]) Structure elucidation of F (**5**), see subsequent paper [3].

Table 1. *^{1}H-NMR Data* ($CDCl_3$) *for the Spirostaphylotrichins A* (**1**), *B* (**2**), *C and D* (**3**/**4**), *Q* (**6**), *and R* (**7**)[a])

H-Atom	Spirostaphylotrichin					
	A (**1**)	B (**2**)	C (**3**)[b])	D (**4**)[b])	Q (**6**)	R (**7**)[c])
H–C(4)	4.64 (*dt*, *J* = 6.2, 1.5; with D_2O, *t*, *J* = 1.5)	5.06 (*dt*, *J* = 6.6, 1.8; with D_2O, *t*, *J* = 1.8)			4.02 (*s*)	4.03 (*d*, *J* = 7)
OH–C(4)	2.63 (*d*, *J* = 6.2)[d])	1.83 (*d*, *J* = 6.5)[d])				4.43 (*d*, *J* = 8)[d])
H–C(6)	4.76 (*d*, *J* = 2.1)[e])	4.25 (*d*, *J* = 2.4)[e])	4.74 (*d*, *J* = 2.1)[e])	4.65 (br. *s*)[e])	3.99 (br. *s*)[e])	4.74 (*d*, *J* = 2.1)[e])
OH–C(6)	3.80 (*d*, *J* = 2.1)[d])	3.97 (*d*, *J* = 2.8)[d])	3.71 (*d*, *J* = 2.2)[d])	4.0 (br. *s*)[d])	4.40 (br. *s*)[d])	2.2 (br. *s*)[d])
H–C(8)	5.93 (*d*, *J* = 9.8)	5.99 (*d*, *J* = 10.1)	6.07 (*d*, *J* = 10)	6.07 (*d*, *J* = 10)	5.79 (*d*, *J* = 9.8)	5.91 (*d*, *J* = 9.8)
H–C(9)	7.07 (*d*, *J* = 10.1)	7.07 (*d*, *J* = 10.1)	7.05 (*d*, *J* = 10.1)	7.05 (*d*, *J* = 10)	7.06 (*d*, *J* = 10.4)	7.04 (*d*, *J* = 10.0)
H–C(11)	4.72 (*t*, *J* = 1.8)	4.78 (*t*, *J* = 2.1)	5.27 (*d*, *J* = 1.9)	5.41 (*d*, *J* = 2.1)	1.64 (*s*, 3 H)	1.63 (*s*, 3 H)
H–C(11)	4.56 (*t*, *J* = 1.7)	4.63 (*t*, *J* = 1.8)	4.91 (*d*, *J* = 1.9)	4.95 (*d*, *J* = 2.1)		
H–C(12)	6.27 (*t*, *J* = 7.4)	6.20 (*t*, *J* = 7.9)	6.05 (*t*, *J* = 7)	6.05 (*t*, *J* = 7)	6.35 (*t*, *J* = 7.9)	6.14 (*t*, *J* = 7.5)
2 H–C(13)	2.1 (*m*)	2.2 (*m*)	2.1 (*m*)	2.1 (*m*)	2.36 (*m*)	2.3 (*m*)
CH_3(14)	1.05 (*t*, *J* = 7.3)	1.02 (*t*, *J* = 7.3)	1.03 (*t*, *J* = 7.4)	0.99 (*t*, *J* = 7.3)	1.05 (*t*, *J* = 7.3)	1.04 (*t*, *J* = 7.3)
CH_3(15)	3.92 (*s*)	3.88 (*s*)	4.06 (*s*)	3.96 (*s*)	3.86 (*s*)	3.99 (*s*)

[a]) Numbering of side chains arbitrary.
[b]) Data from the mixture **3**/**4**.
[c]) OH–C(3) at 5.54 (*s*).
[d]) Exchangeable with D_2O.
[e]) With D_2O, *s*.

Table 2. *^{13}C-NMR Data of the Spirostaphylotrichins A* (**1**), *B* (**2**), *and Q* (**6**)[a])

C-Atom	δ(C)		
	6 ($CDCl_3$)	**1** ($CDCl_3$)[b])	**2** (CD_3OD/(D_6)DMSO)
C(1)	171.2 (*s*)	167.5 (169.8) (*s*)	167.8 (*s*)
C(3)	97.8 (*s*)	143.9 (145.5) (*s*)	145.4 (*s*)
C(4)	77.6 (*d*)[c])	64.7 (65.5) (*d*)	69.7 (*d*)
C(5)	59.9 (*s*)	57.3 (58.7) (*s*)	58.3 (*s*)
C(6)	76.2 (*d*)[c])	73.8 (74.9) (*d*)	74.9 (*d*)
C(7)	192.3 (*s*)	197.0 (198.5) (*s*)	197.4 (*s*)
C(8)	121.2 (*d*)	120.6 (122.1) (*d*)	121.4 (*d*)
C(9)	152.1 (*d*)	152.5 (152.9) (*d*)	153.5 (*d*)
C(10)	124.3 (*s*)	128.3 (130.2) (*s*)	129.3 (*s*)
C(11)	12.6 (*q*)[d])	86.3 (86.5) (*t*)	86.1 (*t*)
C(12)	150.8 (*d*)	150.9 (150.2) (*d*)	151.8 (*d*)
C(13)	24.7 (*t*)	23.1 (24.0) (*t*)	25.6 (*t*)
C(14)	13.4 (*q*)[d])	13.3 (13.6) (*q*)	13.4 (*q*)
C(15)	64.5 (*q*)	62.2 (62.6) (*q*)	62.8 (*q*)

[a]) Numbering of side chains arbitrary.
[b]) Values in parenthesis in CD_3OD/(D_6)DMSO.
[c])[d]) May be interchanged.

from each other only in the configuration at C(6) suggest the same difference as for **2** and **1**. Final proof of structure **2** was made possible after the structure elucidation of spirostaphylotrichin K (**11**) by X-ray crystallography which was isolated from a mutant strain of *S. coccosporum* [3]. Selective hydrogenation of the propylidene and C(8)=C(9) bond of **2** or of the prop-1-enyl and C(8)=C(9) bond in **11** both yielded the same tetrahydrospirostaphylotrichin **12**, whereas the tetrahydrospirostaphylotrichin **13** was obtained from **1**.

Spirostaphylotrichin C and D (**3**/**4**[2]). On TLC, extracts of cultures of *S. coccosporum* revealed a characteristic spot which was less polar than that of **1**. It became green on standing. From the crude extract of a 11-litre fermentation of *S. coccosporum* using the soya medium, **1** was separated by chromatography on silica gel. The less polar fractions containing the unstable substance gaving the green spot on TLC were pooled and further separated by droplet counter current chromatography (DCCC) [4]. After evaporation and recrystallization, a new compound, named spirostaphylotrichin C (**3**[2]) was obtained. Its slightly yellow crystals were not very stable. Spectroscopic examination of the mother liquors showed that they consisted of a mixture of **3** and a very similar substance designated as spirostaphylotrichin D (**4**[2]). All attempts to separate **4** from **3** on a preparative scale were unsuccessful. Therefore, the characterization of **4** is only based on the ^{1}H-NMR data of the mixture. The UV spectrum of **3**/**4** was very similar to that of **1**. The MS showed the molecular ion at *m/z* 277 indicating the molecular formula $C_{14}H_{15}NO_5$ which could be confirmed by elemental analysis. The ^{1}H-NMR data of **3**/**4** *(Table 1)* resembled that of **1**, but H–C(4) and OH–C(4) were missing. The protons CH_2=C(3) (= 2 H–C(11)) appeared as *ds* instead of *triplets* and were shifted to lower field. These observations can be explained by assuming a carbonyl function at C(4) instead of the OH function in **1**, leading to the observed downfield shift for CH_2=C(3) [5].

[2]) The structures of spirostaphylotrichin C (**3**) and D (**4**) may be interchanged. The data of **4** were determined from the mixture **3**/**4**.

Because of the good correlation of the ^{1}H-NMR data, **3** and **4** must be epimeric at C(6). A definite assignment is not possible on the basis of the above data. Further structural studies are handicapped by the instability of **3** and **4** and the lack of a method for their separation.

Spirostaphylotrichin F (**5**). A further compound was obtained after prep. HPLC (silica gel) of fractions eluted later in the course of the DCCC mentioned above. It proved to be identical (IR, MS, ^{1}H-NMR) with spirostaphylotrichin F (**5**) which was isolated from a mutant strain of *S. coccosporum* [3].

Absolute Configuration of Spirostaphylotrichin A (**1**). The X-ray diffraction of compounds like **1** revealed only the relative but not the absolute configuration. To solve this

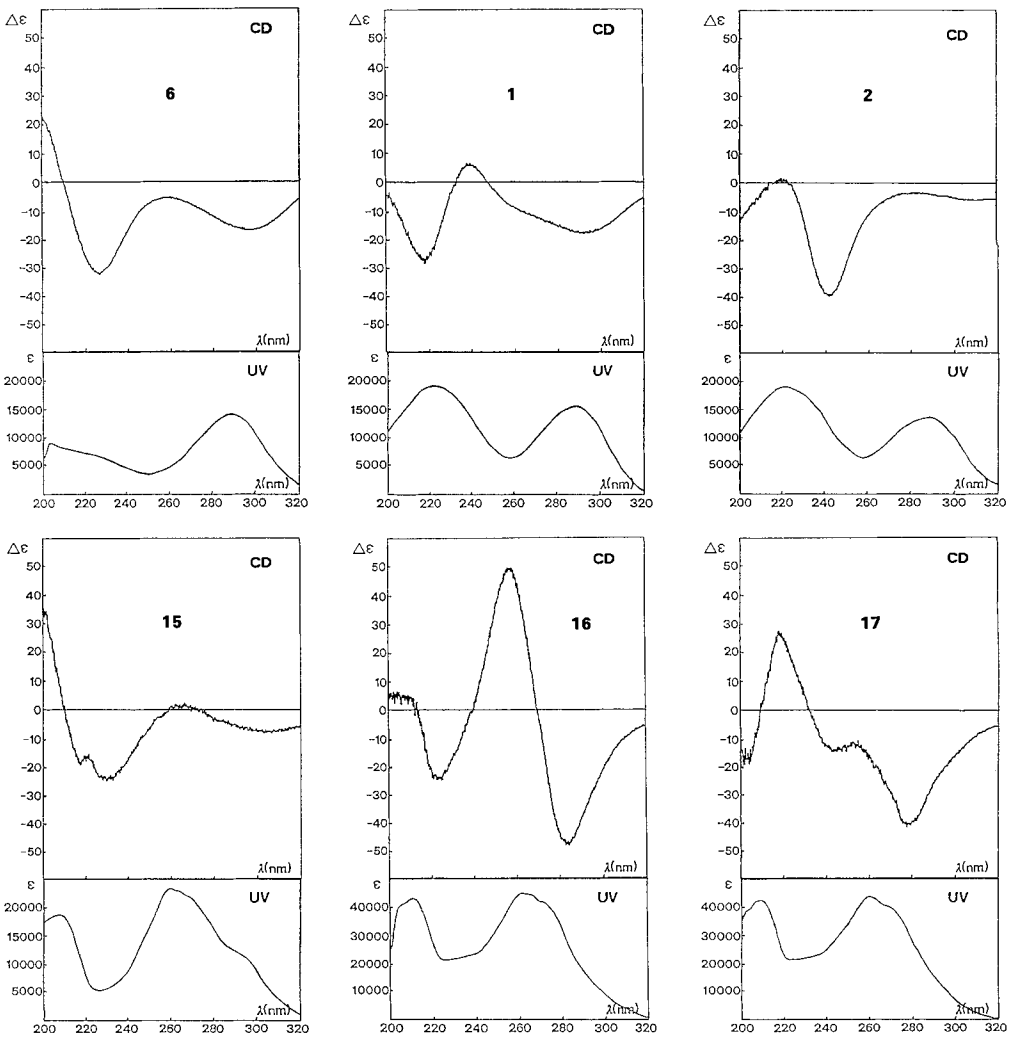

Figure. *CD curves and UV spectra of the spirostaphylotrichins A* (**1**), *B* (**2**), *Q* (**6**), *and of their 4-methoxybenzoyl derivatives* **16**, **17**, *and* **15**, *respectively*

problem, we decided to use circular dichroism (CD). Our studies are based on a suggestion made by *Nakanishi* [6]: in a benzoate of **1**, *e.g.* **14**, a split *Cotton* effect (CE) with a negative sign for the CE at longer wavelengths is expected due to the interaction of the benzoate with the dienone chromophore. On the other hand, a positive sign for this CE is expected for the enantiomer of **14**. In order to prevent additional complication by a second benzoate chromophore at C(4), it was desirable to introduce only one benzoyl group selectively in the 6-position, a goal that is not easy to achieve for **1**. However, in **6**, the 4-OH group is absent due to the formation of an ether bridge. In its 4-methoxybenzoate **15**, no expected CE was observed (see the *Fig.*). Obviously, the 4-methoxybenzoyl group has an angle of *ca.* 180° to the dienone chromophore, a possibility that already *Nakanishi* had not excluded. The CD curve of the bis(4-methoxybenzoate) **16** of **1** is also shown in the *Figure*. As in **15**, no CE was expected for the interaction of the 6-benzoate and the dienone. A *Cotton* split from the interaction of the two benzoates would be expected around 257 nm, where the absorption of the two chromophores reaches its maximum. The observed *Cotton* split around 270 nm can not be caused by such an interaction. But the 4-benzoate and the dienone share an angle of *ca.* 90° where a maximal CE is expected. The observed *Cotton* split of **16** is in agreement with the expected one between the 4-benzoate and the dienone if the compound possesses the absolute configuration as shown in formula **16**. Therefore, it is also possible to assign the absolute configuration (4*R*,5*S*,6*S*) as depicted in formula **1** for spirostaphylotrichin A.

Less unequivocal is the situation in the bis(4-methoxybenzoate) **17** of **2**. The CD curve of **17** *(Fig.)* is more complex, and its interpretation is not yet clear.

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

Experimental Part

General. Fermenter: *New Brunswick Scientific Co.*, model *MF 114*. The org. extracts were dried (Na_2SO_4) and evaporated under reduced pressure below 40°. Column chromatography: silica gel *60* (63–200 μm, *Merck*), TLC: silica gel *60* F_{254} *(Merck)*, detection with UV, I_2, $KMnO_4$, or H_2SO_4. HPLC from culture broths and from crude extracts: *Nucleosil-*C_8 (10 μm, 4.5 × 250 mm, *Macherey-Nagel*) using H_2O/CH_3CN 7:3; *μBondapak* C_{18} (5 μm, 3.9 × 300 mm, *Waters*) using H_2O/CH_3CN 85:15. Droplet counter current chromatography (DCCC): *Büchi B-670* DCC chromatograph. M.p.: Kofler block; corrected; large intervals may arise because of the thermal instability of the spirostaphylotrichins. NMR: *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 in ppm relative to internal Me_4Si and J in Hz. IR: *Perkin-Elmer-781* spectrometer. UV: *Beckman* spectrophotometer, model *25*. MS: *VG-70-250* instrument. CD: *Mark-V* circulardichrometer *(Jobin Yvon)*, concentration range $2 \cdot 10^{-4}$ to $1 \cdot 10^{-3}$ mmol/ml in abs. EtOH *(Fluka, puriss.)*.

Isolation of the Spirostaphylotrichins. Fermentation of *S. coccosporum* was performed as described in [2] using the soya medium. Culture broths were extracted by CH_2Cl_2. On chromatographing a culture extract on silica gel with pentane/Et_2O, **6** was eluted after **1**. Crystallization from CH_2Cl_2/Et_2O gave pure **6**. The subsequent fractions gave a single spot on TLC. From these fractions **7** was crystallized. For the isolation of **2**, see [2]. The crude extract from a 11-litre fermentation was chromatographed on silica gel with pentane/Et_2O. The fractions containing the substances giving a green spot on TLC on standing for some h were pooled and further chromatographed by DCCC [4] with hexane/Et_2O/propanol/EtOH/H_2O 4:8:3:5:8 using the aq. phase as stationary phase. Evaporation and recrystallization of the crude crystals from CH_2Cl_2/Et_2O yielded **3**/**4** as TLC-pure, slightly yellow coloured crystals. Subsequent fractions contained **5** which was subjected to prep. HPLC (*Lichrosorb Si60*, 7 μm, 16 × 250 mm, *Knauer*, 1% MeOH in CH_2Cl_2) giving, after crystallization from Et_2O, TLC-pure **5**, which was identical (IR, MS, ^1H-NMR) with **5** isolated from the mutant strain *P84* of *S. coccosporum* [3].

Spirostaphylotrichin B (= *(4R*,5S*,6R*)-4,6-Dihydroxy-2-methoxy-3-methylidene-10-[(Z)-propylidene]-2-azaspiro[4.5]dec-8-ene-1,7-dione;* **2**). M.p. 162–176°. UV (EtOH; see the *Fig.*): 290 (13600), 223 (19000). CD (EtOH): *Figure.* IR (KBr): 3410 (br., OH), 3320 (sh, OH), 1720*s*, 1670*s*, 1650*s*, 1620*s*, 1575*m*, 1260*m*, 1075*m*. ^{1}H-NMR (90 MHz, $CDCl_3$): *Table 1.* ^{1}H-NMR (90 MHz, $CD_3OD/(D_6)DMSO$ 20:1): 0.97 (*t*, *J* = 7.4, CH_3(14)); 2.20 (*m*, CH_2(13)); 3.83 (*s*, CH_3(15)); 4.16 (*s*, H–C(6)); 4.59 (*t*, *J* = 1.6, 1 H–C(11)); 4.72 (partially hidden by the HOD signal, 1 H–C(11)); 4.91 (*t*, *J* = 1.8, H–C(4)); 5.83 (*d*, *J* = 10.0, H–C(8)); 6.22 (*t*, *J* = 7.9, H–C(12)); 7.16 (*d*, *J* = 10.1, H–C(9)). ^{13}C-NMR (22.6 MHz, $CD_3OD/(D_6)DMSO$ 20:1): *Table 2.* EI-MS (70 eV, 160°): 279 (M^+), 247 ($[M - CH_3OH]^+$), 230 ($[M - H_2O - CH_3O]^+$), 218, 205, 176, 158, 44 (100). Anal. calc. for $C_{14}H_{17}NO_5$ (279.30): C 60.20, H 6.14, N 5.02; found: C 60.02, H 6.30, N 4.97.

Spirostaphylotrichin C (= *(5R*,6S*)-6-Hydroxy-2-methoxy-3-methylidene-10-[(Z)-propylidene]-2-azaspiro[4.5]dec-8-ene-1,4,7-trione;* **3**)[2]). M.p. 133–138°. UV (EtOH): 284 (14300), 222 (13700). IR (KBr): 3440*s* (OH), 2980*w*, 1765*m*, 1735*s*, 1685*s*, 1645*m*, 1620*m*, 1285*m*, 1110*m*, 955*m*. ^{1}H-NMR (90 MHz, $CDCl_3$): *Table 1.* ^{1}H-NMR (400 MHz, $(D_6)DMSO$, mixture with **4**): 0.93 (*t*, *J* = 7.4, CH_3(14)); 1.90, 2.02 (2 *m*, CH_2(13)); 3.94 (*s*, CH_3(15)); 4.59 (*d*, *J* = 3.4, *s* with D_2O, H–C(6)); 5.00 (*d*, *J* = 2.3, 1 H–C(11)); 5.19 (*d*, *J* = 2.3, 1 H–C(11)); 5.93 (*d*, *J* = 10.2, H–C(8)); 6.18 (*t*, *J* = 7.8, H–C(12)); 6.37 (*d*, *J* = 3.7, exchangeable with D_2O, OH–C(6)); 7.19 (*d*, *J* = 10.2, H–C(9)). EI-MS (70 eV, 110°): 277 (100, M^+), 246 ($[M - CH_3O]^+$), 218, 200, 190, 177, 161, 149. Anal. calc. for $C_{14}H_{15}NO_5$ (277.28): C 60.64, H 5.45, N 5.05; found: C 60.41, H 5.63, N 5.05.

Spirostaphylotrichin D (= *(5R*,6R*)-(6-Hydroxy-2-methoxy-3-methylidene-10-[(Z)-propylidene]-2-azaspiro[4.5]dec-8-ene-1,4,7-trione;* **4**)[2]). ^{1}H-NMR (90 MHz, $CDCl_3$): *Table 1.* ^{1}H-NMR (400 MHz, $(D_6)DMSO$): 0.89 (*t*, *J* = 7.3, CH_3(14)); 1.79 (*m*, CH_2(13)); 3.86 (*s*, CH_3(15)); 4.56 (*d*, *J* = 3.7, H–C(6)); 5.02 (*d*, *J* = 2.3, 1 H–C(11)); 5.31 (*d*, *J* = 2.2, 1 H–C(11)); 5.92 (*d*, *J* = 9.9, H–C(8)); 6.19 (*t*, *J* = 8.1, H–C(12)); 6.33 (*d*, *J* = 3.9, exchangeable with D_2O, OH–C(6)); 7.18 (*d*, *J* = 10.1, H–C(9)).

Spirostaphylotrichin Q (= *(3R*,4R*,5S*,6S*)-3,4-Epoxy-6-hydroxy-2-methoxy-3-methyl-10-[(Z)-propylidene]-2-azaspiro[4.5]dec-8-ene-1,7-dione;* **6**). M.p. 163–169°. UV (EtOH; see the *Fig.*): 291 (14200), 205 (8900). CD (EtOH): *Figure.* IR (KBr): 3400*s* (OH), 3000*w*, 2980*w*, 2940*w*, 1720*s*, 1680*s*, 1630*m*, 1585*m*, 1235*m*, 1115*m*, 980*m*. ^{1}H-NMR (90 MHz, $CDCl_3$): *Table 1.* ^{13}C-NMR (22.6 MHz, $CDCl_3$): *Table 2.* EI-MS (70 eV, 160°): 279 (M^+), 250, 207 (100), 206, 176, 161, 149.

Spirostaphylotrichin R (= *(3ζ, 4R*,5S*,6S*)-3,4,6-Trihydroxy-2-methoxy-3-methyl-6-[(Z)-propylidene]-2-azaspiro[4.5]dec-8-ene-1,7-dione;* **7**). M.p. 157–166°. UV (EtOH): 291 (14100), 204 (9100). IR (KBr): 3480 (br., OH), 3360 (br., OH), 1700*s*, 1675*s*, 1615*m*, 1580*m*, 1410*m*, 1215*m*, 1185*m*, 1120*m*, 1105*m*, 1075*m*, 975*m*. ^{1}H-NMR (90 MHz, $CDCl_3$): *Table 1.* EI-MS (70 eV, 200°): 297 (M^+), 279 ($[M - H_2O]^+$), 250, 224, 207, 206, 177, 161, 149, 43 (100). CI-MS (NH_3): 298 ($[M + 1]^+$), 280 (100, $[M + 1 - H_2O]^+$).

Compound Observed in the Mother Liquor of **7**. ^{1}H-NMR (90 MHz, $CDCl_3$): 1.01 (*t*, *J* = 7, CH_3(14)); 1.54 (*s*, CH_3(11)); 2.2 (*m*, CH_2(13)); 3.95 (*s*, CH_3(15)); 4.6 (br., several OH, exchangeable with D_2O); 5.87 (*d*, *J* = 10, H–C(8)); 6.29 (*t*, *J* = 7, H–C(12)); 7.08 (*d*, *J* = 10, H–C(9)).

6 *from* **1**. A soln. of **1** (80 mg, 0.29 mmol) in $CDCl_3$ (1 ml; *Fluka*) was allowed to stand at r.t. for 3 days. After evaporation and crystallization from Et_2O, **6** (50 mg, 0.18 mmol) was obtained which was identical (TLC, IR, ^{1}H-NMR) with **6** isolated from culture extracts.

7 *from* **6** *and* **1**. A soln. of **6** (140 mg, 0.50 mmol) in 0.1N HCl (3 ml) was allowed to stand at r.t. for 1 h. After evaporation, the residue was extracted with acetone, filtered, and evaporated again. From CH_2Cl_2/Et_2O/hexane, 24 mg (0.08 mmol) of **7** could be crystallized which was identical (TLC, IR, ^{1}H-NMR) with **7** isolated from culture extracts. The same product was obtained in a similar manner from **1** (100 mg, 0.36 mmol): 6 mg (0.02 mmol) of **7**.

*4,6-Di-*O*-acetylspirostaphylotrichin B* (**9**). A soln. of **2** (51 mg, 0.18 mmol) in dry pyridine (0.3 ml) and Ac_2O (0.15 ml) was allowed to stand at r.t. for 120 min. The mixture was evaporated and chromatographed on silica gel with pentane/Et_2O to give **9** (60 mg, 0.17 mmol) as a colourless gum which decomposed on standing at r.t. or in soln. for NMR. IR (KBr): 2980*m*, 2940*m*, 1760*s*, 1745*s*, 1700*s*, 1675*s*, 1620*m*, 1375*m*, 1310*m*, 1220*s* (C–O), 1115*m*, 1070*m*. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.05 (*t*, *J* = 7.4, 3 H–C(14)); 2.05, 2.12 (2 *s*, 2 CH_3CO); *ca.* 2.3 (*m*, CH_2(13)); 3.91 (*s*, CH_3(15)); 4.65 (*t*, *J* = 1.7, 1 H–C(11)); 4.90 (*t*, *J* = 1.8, 1 H–C(11)); 5.51 (*t*, *J* = 1.3, H–C(4)); 5.56 (*s*, H–C(6)); 5.94 (*d*, *J* = 10.2, H–C(8)); 6.19 (*t*, *J* = 7.7, H–C(12)); 7.03 (*d*, *J* = 10.6, H–C(9)). ^{13}C-NMR (101 MHz, $CDCl_3$): 13.2 (*q*, C(14)); 20.6, 20.7 (2 *q*, 2 CH_3CO); 24.5 (*t*, C(13)); 53.1 (*s*, C(5)); 62.3 (*q*, C(15)); 68.7 (*d*, C(4)); 72.9 (*d*, C(6)); 89.9 (*t*, C(11)); 122.3 (*d*, C(8)); 126.3 (*s*, C(10)); 138.0 (*s*, C(3)); 150.3, 150.8 (2 *d*, C(9), C(12)); 164.1 (*s*, C(1)); 169.4, 169.5 (2 *s*, 2 CH_3CO); 188.9 (*s*, C(7)). EI-MS (70 eV, 300°): 363 (M^+), 321 ($[M - C_2H_2O]^+$), 290 ($[M - C_2H_2O - CH_3O]^+$), 279 ($[M - 2\,C_2H_2O]^+$), 248, 230, 177, 161, 43 (100).

*4,6-Di-*O*-acetylspirostaphylotrichin A* (**10**). A soln. of **1** (507 mg, 1.82 mmol) in dry pyridine (10 ml) and Ac_2O (10 ml) was allowed to stand at r.t. for 80 min. After evaporation, the residue was dissolved in Et_2O. Crude **10** crystallized and was recrystallized from CH_2Cl_2/Et_2O: pure (TLC) **10** (613 mg, 1.69 mmol). M.p. 88–99°. IR (KBr): 2980*m*, 2940*m*, 1765–1730*s* (several C=O), 1705*s*, 1675*s*, 1620*m*, 1440*m*, 1370*m*, 1305*m*, 1210*s* (C–O), 1110*m*, 1035*m*, 965*m*, 860*m*, 840*m*. ^{1}H-NMR (90 MHz, $CDCl_3$): 1.08 (*t*, *J* = 7.3, CH_3(14)); 2.00, 2.12 (2 *s*, 2 CH_3CO); *ca.* 2.2 (*m*, CH_2(13)); 3.94 (*s*, CH_3(15)); 4.57 (*dd*, *J* = 2.0, 1.3, 1 H–C(11)); 4.80 (*t*, *J* = 1.7, 1 H–C(11)); *ca.* 5.9 (*m*, H–C(8), H–C(6)); 6.23 (*t*, *J* = 7.6, H–C(12)); 6.94 (*d*, *J* = 10.1, H–C(9)). ^{13}C-NMR (22.6 MHz, $CDCl_3$): 13.1 (*q*, C(14)); 20.2, 20.7 (2 *q*, 2 CH_3CO); 23.5 (*t*, C(13)); 54.5 (*s*, C(5)); 62.0 (*q*, C(15)); 65.9 (*d*, C(4)); 75.2 (*d*, C(6)); 88.3 (*t*, C(11)); 122.5 (*d*, C(8)); 127.6 (*s*, C(10)); 139.7 (*s*, C(3)); 149.2, 149.7 (2 *d*, C(9), C(12)); 166.0 (*s*, C(1)); 168.3, 168.8 (2 *s*, 2 CH_3CO); 189.2 (*s*, C(7)).

8,9,10,12-Tetrahydrospirostaphylotrichin B (*= (4*R*,*5*S*,*6*R*,*10*R*)*-4,6-Dihydroxy-2-methoxy-3-methylidene-10-propyl-2-azaspiro[4.5]decane-1,7-dione;* **12**). To a soln. of **2** (20 mg, 0.072 mmol) in EtOH (12 ml) 10% Pd/C (2 mg) was added, and the mixture was stirred under H_2 for 13 min, filtered, and evaporated. Crystallization from Et_2O gave 13 mg (0.046 mmol) of pure (TLC) **12**. M.p. 140–152°. IR (KBr): 3400 (br., OH), 2960*m*, 2940*m*, 1730*s*, 1690*m*, 1660*s*, 1120*m*, 1070*m*, 990*m*, 865*m*. ^{1}H-NMR (400 MHz, (D_6)DMSO): 0.81 (*t*, *J* = 7.2, CH_3(14)); 1.1–2.3 (*m*, CH_2(13), CH_2(12), CH_2(9), CH_2(8)); 2.47 (*m*, H–C(10)); 3.72 (*s*, CH_3(15)); 3.99 (*d*, *J* = 4.0, *s* with D_2O, H–C(6)); 4.33 (*t*, *J* = 1, 1 H–C(11)); 4.41 (*t*, *J* = 1, 1H–C(11)); 4.93 (*d*, *J* = 5.0, *s* with D_2O, H–C(4)); 5.25 (*d*, *J* = 4.3, exchangeable with D_2O, OH–C(6)); 6.17 (*d*, *J* = 5.5, exchangeable with D_2O, OH–C(4)). EI-MS (70 eV, 300°): 283 (M^+), 255, 222, 182, 172, 111, 74 (100).

8,9,12,13-Tetrahydrospirostaphylotrichin K (**12**). To a soln. of **11** (20 mg, 0.072 mmol) in MeOH (6 ml), 10% Pd/C (4 mg) was added, and the mixture was stirred under H_2 for 32 min, filtered, and evaporated. Chromatography on silica gel (Et_2O) yielded 14 mg (0.049 mmol) of pure (TLC) **12**. Crystallization from Et_2O gave colourless crystals with m.p. 140–155°, identical with **12** from **2** (TLC, IR, MS, ^{1}H-NMR).

8,9,10,12-Tetrahydrospirostaphylotrichin A (*= (4*R*,*5*S*,*6*S*)*-4,6-Dihydroxy-2-methoxy-3-methylidene-10-propyl-2-azaspiro[4.5]decane-1,9-dione;* **13**). To a soln. of **1** (300 mg, 1.08 mmol) in EtOH (12 ml), 10% Pd/C (50 mg) was added, and the mixture was stirred under H_2 for 3 min, filtered, and evaporated: 300 mg (1.06 mmol) of **13**, colourless gum. IR (KBr): 3430 (br., OH), 2960*m*, 2870*m*, 1720*s*, 1660*s*, 1265*m*, 1125*m*, 1080*m*. ^{1}H-NMR (400 MHz, (D_6)DMSO): 0.81 (*t*, *J* = 7.0, CH_3(14)); 1.11 (*m*, CH_2(13)); 1.3–2.3 (*m*, CH_2(12), CH_2(9), CH_2(8)); 2.61 (*m*, H–C(10)); 3.77 (*s*, CH_3(15)); 4.29 (*s*, 1 H–C(11)); 4.35 (*d*, *J* = 4.0, *s* with D_2O H–C(6)); 4.43 (*t*, *J* = 1, 1 H–C(11)); 4.74 (*d*, *J* = 5.9, *s* with D_2O H–C(4)); 5.43 (*d*, *J* = 4.1, exchangeable with D_2O, OH–C(6)); 5.87 (*d*, *J* = 6.1, exchangeable with D_2O, OH–C(4)). ^{13}C-NMR (101 MHz, $CDCl_3$): 14.0 (C(14)); 21.5 (C(13)); 28.4, 32.0 (C(9), C(12)); 38.0, 41.6 (C(10), C(8)); 59.2 (C(5)); 62.6 (C(15)); 65.5 (C(4)); 75.7 (C(6)); 84.2 (C(11)); 144.4 (C(3)); 168.1 (C(1)); 211.2 (C(7)). EI-MS (70 eV, 300°): 283 (M^+), 252 ([$M - OCH_3$]$^+$), 234, 222, 166, 155, 124, 111, 55 (100).

*6-*O*-(4-Methoxybenzoyl)spirostaphylotrichin Q* (**15**). A soln. of **6** (33 mg, 0.12 mmol) and 4-methoxybenzoyl chloride (60 mg, 0.34 mmol) in dry pyridine (1 ml) was allowed to stand at r.t. for 14 h. The excess of acyl chloride was decomposed by H_2O and the mixture evaporated and chromatographed on silica gel (Et_2O). After further purification by prep. HPLC (*Lichrosorb Si60*, 10 μm, 32 × 500 mm, *Knauer*, Et_2O), 10 mg (0.024 mmol) of **15** were obtained as a colourless gum. UV (EtOH; see the *Fig.*): 262 (23 100). CD (EtOH): *Figure*. IR (KBr): 2980*m*, 2940*m*, 1740 (sh), 1730*s*, 1690*s*, 1610*s*, 1510*m*, 1260*s*, 1170*s*, 1110*s*, 770*m*. ^{1}H-NMR (400 MHz, (D_6)DMSO): 0.72 (*t*, *J* = 7.5, CH_3(14)); 1.63 (*s*, CH_3(11)); 2.22, 2.35 (*m*, CH_2(13)); 3.84 (*s*, CH_3O); 3.86 (*s*, CH_3O); 4.50 (*s*, H–C(4)); 5.14 (*s*, H–C(6)); 5.98 (*d*, *J* = 10.3, H–C(8)); 6.09 (*t*, *J* = 7.8, H–C(12)); 7.09 (*m*, 2 arom. H); 7.64 (*d*, *J* = 10.3, H–C(9)); 7.89 (*m*, 2 arom. H). EI-MS (70 eV): 413 (M^+), 384, 232, 230, 135 (100, [$CH_3OC_6H_4CO$]$^+$). CI-MS (NH_3): 414 (100, [$M + 1$]$^+$), 384, 234, 218, 206, 191, 170, 152, 135.

*4,6-Bis-*O*-(4-methoxybenzoyl)spirostaphylotrichin A* (**16**). As for **15**, with **1** (200 mg, 0.72 mmol), 4-methoxybenzoyl chloride (490 mg, 2.9 mmol), and pyridine (3 ml; 18 h). Chromatography (silica gel, pentane/Et_2O) and crystallization from pentane/Et_2O/CH_2Cl_2 gave 215 mg (0.39 mmol) of **16**. M.p. 135–142°. UV (EtOH; see the *Fig.*): 262 (44 800). CD (EtOH): *Figure*. IR (KBr): 2980*w*, 2950*w*, 1740*s*, 1725*s*, 1695*m*, 1605*s*, 1510*m*, 1250*s*, 1170*s*, 1090*s*. ^{1}H-NMR (400 MHz, (D_6)DMSO): 0.99 (*t*, *J* = 7.4, CH_3(14)); 2.13 (*m*, CH_2(13)); 3.70 (*s*, CH_3O); 3.84 (*s*, CH_3O); 3.87 (*s*, CH_3O); 4.90 (*s*, 1 H–C(11)); 4.93 (*s*, 1 H–C(11)); 5.84 (*s*, H–C(4 or 6)); 5.95 (*d*, *J* = 10.0, H–C(8)); 6.15 (*s*, H–C(4 or 6)); 6.39 (*t*, *J* = 7.7, H–C(12)); 7.09 (*m*, 4 arom. H); 7.18 (*d*, *J* = 10.3, H–C(9)); 7.82 (*m*, 2 arom. H); 7.91 (*m*, 2 arom. H). EI-MS (70 eV): M^+ missing, 517, 365, 231, 213, 135 (100, [$CH_3OC_6H_4CO^+$]). CI-MS (NH_3): 565 ([$M + NH_4$]$^+$), 548 ([$M + 1$]$^+$), 398, 366, 170, 152, 135 (100, [$CH_3OC_6H_4CO$]$^+$).

*4,6-Bis-*O*-(4-methoxybenzoyl)spirostaphylotrichin B* (**17**). As for **15**, with **2** (10 mg, 0.036 mmol), 3 drops of 4-methoxybenzoyl chloride, and pyridine (0.15 ml; 2 h). Chromatography (twice; silica gel, pentane/Et_2O, then CH_2Cl_2/MeOH) gave 16 mg (0.029 mmol) of **17**, colourless gum. UV (EtOH; see the *Fig.*): 262 (43600). CD (EtOH): *Figure*. IR (KBr): 2940*w*, 1740*s*, 1700*s*, 1670*m*, 1605*s*, 1510*m*, 1250*s*, 1170*s*, 1090*s*, 760*m*. ^{1}H-NMR (400 MHz, (D_6)DMSO): 0.87 (*t*, *J* = 7.3, CH_3(14)); 2.13 (*m*, CH_2(13)); 3.85 (*s*, 2 CH_3O); 3.94 (*s*, CH_3O); 4.80 (*s*, 1 H–C(11)); 4.93 (*s*, 1 H–C(11)); 5.73 (*s*, H–C(4 or 6)); 5.97 (*d*, *J* = 10.0, H–C(8)); 6.20 (*t*, *J* = 8, H–C(12)); 6.31 (*s*, H–C(4 or 6)); 7.10 (*m*, 4 arom. H); 7.24 (*d*, *J* = 10.2, H–C(9)); 7.84 (*m*, 2 arom. H); 7.91 (*m*, 2 arom. H). EI-MS (70 eV): 547 (M^+), 517, 395, 365, 152, 135 (100, $[CH_3OC_6H_4CO]^+$). CI-MS (NH_3): 565 ($[M + NH_4]^+$), 548 ($[M + 1]^+$), 518, 398, 368, 135 (100, $[CH_3OC_6H_4CO]^+$).

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