DOI: 10.1002/ejoc.200500425

Spirangien A and B, Highly Cytotoxic and Antifungal Spiroketals from the Myxobacterium *Sorangium cellulosum*: Isolation, Structure Elucidation and Chemical Modifications[‡]

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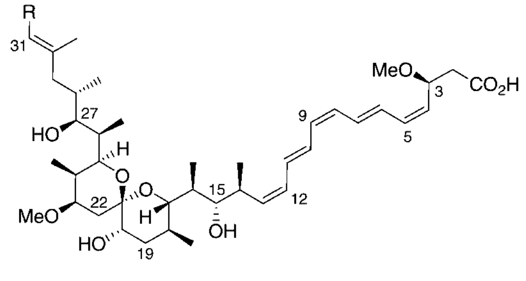
Keywords: Natural products / Myxobacteria / Structure elucidation / Spiro compounds / Ozonolysis / Olefin metathesis

Two novel highly cytotoxic metabolites, spirangien A (**1**) and B (**2**), were isolated from the myxobacterium *Sorangium cellulosum* (strain So ce90). The structures were elucidated by detailed NMR spectroscopic analysis. The previously unknown molecular framework common to spirangien A and B includes a highly functionalized spiroketal core structure, a side chain bearing a pentaene chromophore and a terminal carboxyl group, and a total of thirteen stereocenters. The absolute configuration at C-3 was determined by degradation and subsequent fragment analysis by GC. The relative stereochemistry of the spiroketal structure was proposed on the basis of vicinal proton couplings and ROESY data for hydroperoxide derivative **4**, obtained by ozonolysis of spirangien A, and for 1,3-diene **5**, obtained by cross-metathesis with ethylene. X-ray crystal structure analysis of **5** confirmed its structure and unambiguously provided the complete relative stereochemistry of all twelve stereocenters. The 1,3-diene derivative **5** still shows strong cytotoxic activity.

Introduction

Myxobacteria have proven to be a rich source of structurally diverse secondary metabolites exhibiting a variety of biological activities.[2] From culture extracts of the epothilone-producing[3] *Sorangium cellulosum* strain So ce90 we isolated a novel group of antifungal and highly cytotoxic compounds named spirangien A and B.[4] In this paper we describe the isolation, structure elucidation and chemical modification of spirangien A by ozonolysis and cross-metathesis reactions. As a characteristic structural feature, spirangien A and B each contain a spiroketal core structure. Natural products containing spiroketals and displaying various biological activities also include, for example, macrolide antibiotics from marine sponges such as spongistatin 1,[5] cinachyrolide A[6] and spirastrellolide A[7] or spiroketal antibiotics isolated from *Streptomyces* sp., such as the avermectins,[8] salinomycin[9] and reveromycin A.[10]



Spirangien A (**1**): R = CH_3
Spirangien B (**2**): R = CH_2CH_3
(relative configuration for C-14 to C-28)

Results and Discussion

Structure Elucidation

Spirangien A (**1**) and B (**2**) were isolated as amorphous solids with molecular formulas of $C_{41}H_{66}O_9$ and $C_{42}H_{68}O_9$, respectively, as established by HR-MS and ^{13}C NMR data. The signals in the ^{1}H and ^{13}C NMR spectra were assigned by ^{1}H,^{1}H-COSY, direct ^{1}H,^{13}C correlation (HMQC) and long-range ^{1}H,^{13}C-HMBC correlation spectroscopy (Table 1). The two compounds showed identical UV spectra with characteristic UV absorption maxima at 351, 333, 318 and 303 nm, suggesting the presence of a polyene system. The molecular formula of spirangien B differs from that of spirangien A only by an additional CH_2 group, and their close relationship was evident from comparison of their ^{1}H

[‡] Antibiotics from Gliding Bacteria, 103. Part 102: Ref.[1]

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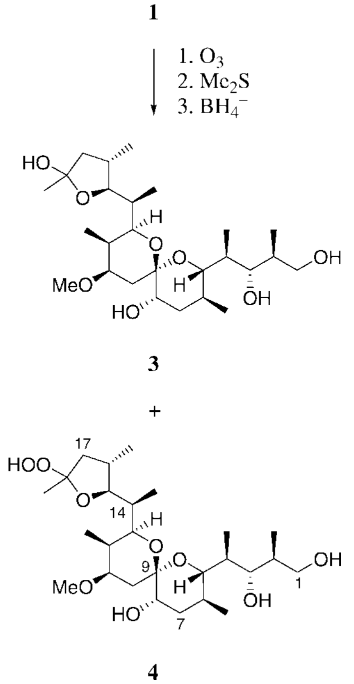
and ^{13}C NMR spectroscopic data. The only significant difference appeared in the chemical shifts of C-30 (δ = 134.6 ppm), C-31 (δ = 129.4 ppm) and C-32 (sp^3-methylenic carbon atom: δ = 22.0 ppm) and in the presence of an additional methyl group signal (δ_H = 0.99 ppm; δ_C = 14.7 ppm), indicating that the C-32 methyl group in spirangien A is replaced by an ethyl group (C-32 to C-33) in spirangien B. The presence of a conjugated pentaene side chain was indicated by the ten sp^2-methine protons 4-H to 13-H (δ_H = 5.35–6.90 ppm; δ_C = 129.2–134.8 ppm). The configurations of the double bonds of the pentaene were assigned on the basis of the vicinal coupling constants, indicating (*Z*) configurations of the $\Delta^{4,5}$, $\Delta^{8,9}$ and $\Delta^{12,13}$ double bonds, whilst the configurations of the $\Delta^{6,7}$ and $\Delta^{10,11}$ double bonds were assigned as (*E*) on the basis of their vicinal coupling constants of 14.5 Hz. The spiroketal structure from C-17 to C-25 was deduced from HMBC correlations. The signal of the quaternary carbon atom at δ = 99.5 (C-21) suggested the presence of a 6,6-spiroketal moiety.[11] The ether linkages between C-17 and C-21 and between C-21 and C-25 were deduced from long-range $^1H,^{13}C$ correlations of 17-H (δ = 3.63 ppm) and 25-H (δ = 3.73 ppm) to C-21. For the determination of the absolute configuration at C-3 by gas chromatographic analysis, degradation of spirangien A by ozonolysis was performed, followed by oxidative workup. Esterification of the resulting 2-methoxysuccinic acid with diazomethane and comparison with GC standards obtained from (*S*)-(–)-hydroxysuccinic acid and (±)-hydroxysuccinic acid established the (3*S*) configuration in spirangien A. The relative stereochemistry of

Table 1. ^{13}C and ^1H NMR chemical shifts of **1** measured in MeOD at 600 MHz.

Pos.	δ_C [ppm]	δ_H [ppm]	Multiplicity	*J* [Hz]	HMBC
1	174.8	–	–		
2	42.0	2.60 (a)	dd	8.1, 15.1	C-1
		2.43 (b)	dd	5.2, 15.1	C-1
3	74.8	4.66	td	5.2, 8.3, 9.2	3-OMe, C-5
4	131.4	5.35	dd	10.1, 10.5	C-2
5	133.8	6.40	dd	11.0, 11.3	C-3
6	129.2	6.69	dd	11.4, 14.5	
7	131.9	6.90	dd	11.1, 14.5	
8	130.3	6.14	dd	11.1, 11.1	
9	132.2	6.20	dd	11.1, 11.1	
10	129.5	6.83	dd	11.1, 14.5	
11	130.9	6.64	dd	11.2, 14.5	
12	130.0	6.22	dd	11.2 11.2	C-14
13	134.8	5.68	dd	10.8, 10.8	
14	36.2	2.99	m		
15	76.6	3.60	dd	2.5, 9.7	C-13
16	40.0	1.63	m		
17	75.2	3.63	dd	1.2, 10.5	C-19, C-21
18	25.6	1.97	m		
19	37.3	1.77 (a)	m		
		1.71 (b)	dt	3.2, 3.2, 13.4	
20	71.0	3.43	t	3.0	C-21
21	99.5	–	–		
22	34.0	2.07 (a)	dd	4.8, 13.0	C-21
		1.40 (b)	dd	12.4, 12.8	C-21
23	78.8	3.68	m		
24	33.0	2.16	m		
25	72.5	3.73	dd	1.9, 10.2	C-21
26	37.8	1.87	m		
27	76.1	3.70	m		C-29
28	35.4	1.75	m		
29	46.0	2.69 (a)	d (br.)	12.3	30-CH_3
		1.63 (b)	m		
30	136.0	–	–		
31	121.1	5.25	q	6.6	C-29, 30-CH_3
32	13.5	1.63	m		
33	–	–	–		
14-CH_3	19.5	1.16	d	7.0	
16-CH_3	7.9	0.84	d	7.1	
18-CH_3	18.0	0.79	d	6.6	C-19
24-CH_3	4.1	0.81	d	6.8	C-23
26-CH_3	9.4	0.85	d	6.9	
28-CH_3	15.6	0.75	d	6.6	C-29
30-CH_3	15.8	1.64	m		C-30
3-OMe	56.6	3.29	s		C-3
23-OMe	55.4	3.38	s		C-23

the spiroketal was deduced from ^{1}H-^{1}H coupling constants and NOE correlations of hydroperoxide **4**, obtained by ozonolysis of spirangien A followed by reductive workup (see below, Scheme 1 and Figure 1). The complete relative configuration for C-14 to C-28 of spirangien A and B was finally derived from the X-ray crystal structure analysis of 1,3-diene **5**, obtained by ethylene cross-metathesis of spirangien A (see below, Scheme 2 and Figure 2).



Scheme 1. Ozonolysis of spirangien A (**1**); relative configuration for C-2 to C-16.

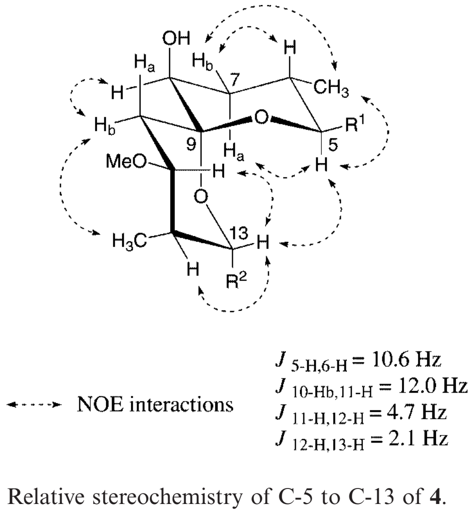
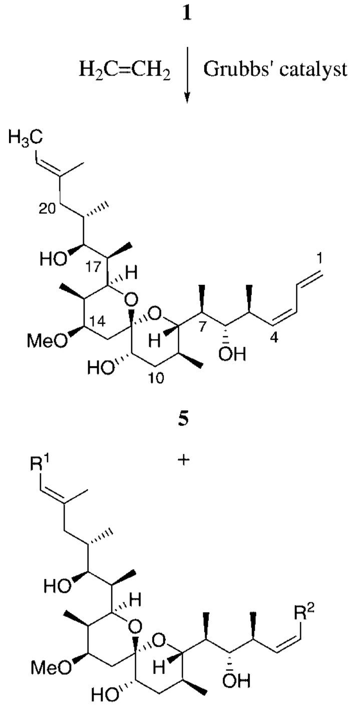


Figure 1. Relative stereochemistry of C-5 to C-13 of **4**.

Chemical Modifications

Chemical derivatives of spirangien A were prepared by fragmentation, both in order to determine characteristic structural features essential for biological activity and, on the other hand, to provide compounds including the spiroacetal core, which might be more suitable either for crystallization and the determination of the relative stereochemistry by X-ray analysis or for further derivatization.



6: $R^1 = CH_3$, $R^2 = H$
7: $R^1 = H$, $R^2 = H$
8: $R^1 = H$, $R^2 = CH{=}CH_2$
9: $R^1 = CH_3$, $R^2 = CH{=}CH_2\text{-}CH{=}CH_2$

Scheme 2. Olefin metathesis of spirangien A (**1**); relative configuration.

Cleavage of the double bonds of spirangien A (**1**) by ozonolysis (O_3, Me_2S), followed by reductive workup with polymer-supported borohydride, afforded derivatives **3** and **4** in a nearly 1:1 mixture, and these were separated by preparative HPLC (Scheme 1). Compound **3**, with a molecular mass of $M = 488$, turned out to be the expected product from the ozonolysis reaction and subsequent hemiacetal formation (molecular formula: $C_{26}H_{48}O_8$). The second product, with $M = 504$, was identified as the corresponding hydroperoxide **4** ($C_{26}H_{48}O_9$; positive peroxide test) formed by nucleophilic addition of the C-15 hydroxy group to an intermediate C-18 carbonyl oxide. The NMR spectra revealed that **3** was isolated as a 2:1 mixture of diastereoisomers, whereas hydroperoxide **4** was a single diastereomer of unknown configuration. The relative stereochemistry of the C-5 to C-13 spiroketal moiety of **4** was elucidated from ROESY and coupling constant data (Figure 1). Because of the presence of overlapping multiplets in the ^{1}H NMR spectrum, additional spin-spin coupling information was obtained from a ^{2}D *J*-resolved ^{1}H NMR experiment ("^{1}H broad-band-decoupled" proton spectrum). The coupling constant of $J = 10.6$ Hz between 5-H and 6-H indicates that 5-H and 6-H are both axial and that the substituent at C-5

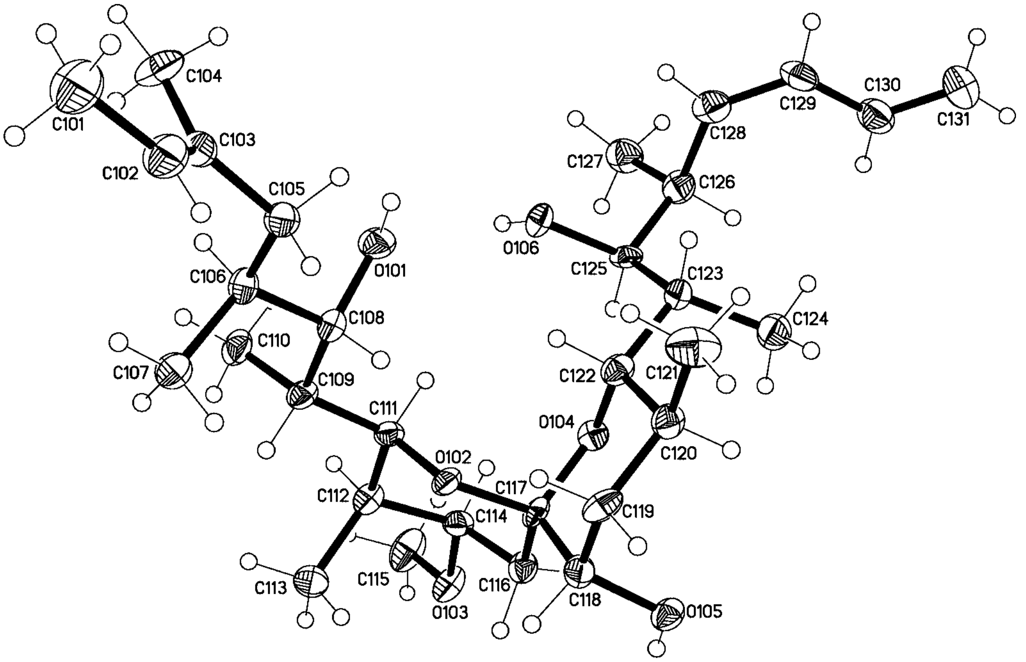


Figure 2. Molecular structure of **5** (molecule 1; molecule 2 follows labelling scheme 2); displacement ellipsoids shown at the 50% probability level.

must be equatorial. NOE correlations between 5-H and 7-H$_a$ and a large coupling constant of J = 13.3 Hz between 6-H and 7-H$_a$ confirmed that the C-5 to C-9 tetrahydropyran ring was in a chair conformation. The NOE enhancement observed between 8-H and both 7-H$_a$ and 7-H$_b$, together with a small coupling constant of J = 2.7 Hz between 8-H and 7-H$_a$, established that 8-H was equatorial. The relative configuration at the spiroketal carbon atom C-9 was defined by an NOE correlation between 8-H and 10-H$_b$, together with an NOE enhancement observed between 13-H and 5-H. The large coupling constant between 10-H$_b$ and 11-H of J = 12.0 Hz, together with an NOE correlation between 11-H and 13-H, showed that the C-9 to C-13 tetrahydropyran ring was also in a chair conformation, with 10-H$_b$, 11-H and 13-H all axial and with equatorial orientation of the substituent at C-13. The coupling constant (J = 4.7 Hz) between 11-H and 12-H and NOE correlations between 12-H and both 11-H and 13-H, together with an observed NOE between 10-H$_b$ and 12-CH$_3$, indicated that the CH$_3$ group at C-12 was axial.

Cross-metathesis of spirangien A in the presence of ethylene and Grubbs' second-generation catalyst was applied to convert internal olefin bonds into terminal olefins (Scheme 2). HPLC-MS analysis of the reaction mixture after 24, 48 and 72 h revealed the formation of a complex mixture of ethylene cross-metathesis products. Reactions at the pentaene side chain resulted in 1-ene (**6**, **7**), 1,3-diene (**5**, **8**) and 1,3,5-triene (**9**) derivatives, including in part additional conversion of the trisubstituted $\Delta^{21,22}$ double bond (**7**, **8**). Attempts to achieve a more complete conversion failed even with prolonged reaction times, which might be due to the formation of an equilibrium mixture. The 1,3-diene **6**, with a UV absorption maximum at 228 nm, could be isolated as the main product in 15% yield and was characterized by ^{1}H and ^{13}C NMR spectroscopy. The relative stereochemistry of the C-8 to C-16 spiroketal moiety of **5** was elucidated from ROESY and coupling constant data, and the results were consistent with those obtained for hydroperoxide **4**. In order to provide more material for crystallization and derivatization of **5**, a larger scale reaction was performed with refined material containing spirangien A and B. After isolation by MPLC and HPLC, an additional purification step by preparative TLC on silica gel was required. 1,3-Diene **5** crystallized as colourless needles from methanol. The relative configuration of all twelve stereocenters in **5** was provided by X-ray crystal structure analysis, as illustrated in Figure 2, and confirmed the proposed relative configuration suggested by our NMR studies for the spiroketal moiety. However, determination of the absolute configuration was not possible (see Exp. Sect. for details). Attempts to determine the absolute stereochemistry by synthesis of the 2-methoxy-2-phenyl-2-(trifluoromethyl)-acetic acid (MTPA) esters of **5** in order to apply Mosher's method[12] were not successful, as the synthesis of defined MTPA esters failed, so the assignment of the absolute stereochemistry of 1,3-diene **5** and correspondingly for C-14 to C-28 of spirangien A and B so far remains unresolved.

Biological Activity

Spirangien A (**1**) was tested for biological activity in agar diffusion assays against a broad spectrum of bacteria, yeasts and fungi and showed activity against selected yeasts and fungi (e.g., diameters of inhibition zones: *Pichia membranaefaciens* 24 mm, *Rhodotorula glutins* 19 mm, *Botrytis* *cinerea* 11 mm). High cytotoxicity was observed for spirangien A (**1**) in assays with an L929 mouse fibroblast cell line (IC_{50} = 0.7 ng mL^{-1}). Whilst spiroketal derivatives **3** and **4** showed no cytotoxic activity (MIC > 5 µg mL^{-1}), 1,3-diene **5** was found to be still very active, with an IC_{50} value of 7 ng mL^{-1}, thus retaining one tenth of the activity of spirangien A (**1**).

Experimental Section

General Procedures: Analytical TLC: silica gel (Si 60 F_{254}) on aluminium sheets (Merck); detection by UV absorption at 254 nm and/or by staining with cerium(IV) sulfate/phosphomolybdic acid in sulfuric acid followed by charring. Precoated silica gel (Si 60 F_{254}) plates of 0.25 mm layer thickness were used for preparative TLC. Analytical HPLC: 125 × 2 mm Nucleosil 100-7 C-18 with a pre-column of 11 mm (Macherey–Nagel) with UV detection at 320 nm and/or 230 nm and diode array detection; eluent A: H_2O/CH_3CN (95:5), 5 mM NH_4OAc, pH = 5.5; eluent B: CH_3CN/H_2O (95:5), 5 mM NH_4OAc, pH = 5.5; gradient: 10% B to 100% B in 30 min; flow: 0.3 mL min^{-1}. LH-20 chromatography: Sephadex LH-20 (Pharmacia) column (10 × 80 cm; eluent: methanol; flow rate 25 mL min^{-1}). MPLC: 7 × 50 cm HD-Sil Labogel 18-30-60, 20–45 µ (Kronlab). Preparative HPLC: 16 × 250 mm Nucleosil 100-7 C-18 (Macherey–Nagel) column. UV: Shimadzu UV-2102 PC UV/Vis scanning spectrometer; solvent: methanol [Uvasol (Merck)]. IR: Nicolet 20DXB FT-IR spectrometer. Optical rotations: Perkin–Elmer 245 polarimeter. NMR: Bruker ARX 600 spectrometer (^{1}H: 600 MHz) and Bruker AM 300 spectrometer (^{13}C: 75.5 MHz); internal standard was the solvent signal. ESI-HPLC-MS: ET 125/2 Nucleosil 120-5 C-18 Macherey–Nagel, PE Sciex Api-2000 LC/MS/MS. Mass spectra: Micromass ESI-QTOF spectrometer. GC-analysis: Shimadzu GC-17 A gas chromatograph with FID detection; capillary column HYDRODEX® *β*-PM [heptakis(2,6-di-*O*-methyl-3-*O*-pentyl)-*β*-cyclodextrin], 25 m × 0.25 mm ID; temperature gradient: 5 min 80 °C, 6 °C min^{-1} to 180 °C. GC standards were obtained from (*S*)-(–)-hydroxysuccinic acid and (±)-hydroxysuccinic acid by esterification with diazomethane, followed by treatment with methyl iodide/silver oxide. Ozonolysis: Sander Labor Ozonisator. Polymer-supported borohydride on Amberlite® IRA-400 (ca. 2.5 mmol BH_4^- per g of resin) and Hoveyda–Grubbs' catalyst 2nd generation (1,3-bis(2,4,6-trimethylphenyl)-2-imidazolidinylidene) dichloro(*o*-isopropoxyphenylmethylene)ruthenium were from Aldrich.

Isolation of Spirangien A and B: The crude extract (120 g) obtained from 750 L of fermentation broth of *Sorangium cellulosum*, strain So ce90, by MeOH elution of XAD-16 adsorber resin present during fermentation, was extracted with ethyl acetate and, after evaporation of the organic solvent, was partitioned between MeOH and heptane. The MeOH layer was concentrated in vacuo to give the extract (73 g). Subsequent LH-20 column chromatography of a portion (43 g) of the MeOH extract gave refined material (17.5 g). Fractionation of a sample (270 mg) was performed by MPLC [eluent: MeOH/H_2O (85:15), 50 mM K_2HPO_4/NaH_2PO_4, pH = 7; flow rate 12 mL min^{-1}; detection by UV absorption at 320 nm]. The organic solvent was removed in vacuo and the aqueous layer was extracted three times with ethyl acetate. The combined organic phases were dried with sodium sulfate and concentrated to dryness. The obtained spirangien fractions were further purified by HPLC [eluent: MeOH/H_2O (85:15), 50 mM K_2HPO_4/NaH_2PO_4, pH = 7] to give spirangien A (**1**, 9.2 mg) and spirangien B (**2**, 2.3 mg) as amorphous solids.

Spirangien A (1): R_t = 23.6 min (analytical HPLC). (–)-ESI-HPLC-MS: 701.3. $[\alpha]_D^{20}$ = –19.4 (*c* = 1.0, MeOH). ^{1}H and ^{13}C NMR: see Table 1. IR (KBr): $\tilde{\nu}_{max}$ = 3439, 2963, 2925, 2850, 1715, 1457, 1382, 1103, 1029, 995, 972 cm^{-1}. UV (MeOH): λ_{max} (lg *ε*) = 351 (4.68), 333 (4.72), 318 (4.53), 303 (4.24) nm. ESI-MS: *m*/*z* (%) = 725.5 (100) $[M + Na]^+$. HRMS (ESI) for $C_{41}H_{66}O_9$: calcd. 725.460; found 725.460.

Spirangien B (2): R_t = 24.9 min (analytical HPLC). (–)-ESI-HPLC-MS: 715.3. $[\alpha]_D^{20}$ = –8.8 (*c* = 0.4, MeOH). ^{1}H and ^{13}C NMR spectra obtained in MeOD at 600 MHz; only data differing from those for spirangien A are reported: ^{1}H NMR: δ = 5.20 (31-H), 2.07 (32-H_2), 0.99 (33-H_3) ppm. ^{13}C NMR: δ = 134.6 (C-30), 129.4 (C-31), 22.0 (C-32), 14.7 (C-33) ppm. ESI-MS: *m*/*z* (%) = 739.5 (60) $[M + Na]^+$. HRMS (ESI) for $C_{42}H_{68}O_9$: calcd. 739.476; found 739.477.

Chemical Modifications

1) Degradation of Spirangien A for GC Analysis: Spirangien A (1.2 mg, 0.0017 mmol) was dissolved in MeOH (1 mL), and ozone was introduced at –68 °C for 7 min. After the system had been purged with oxygen for 3 min, NaOH (1 N, 200 µL) and H_2O_2 (30%, 20 µL) were added and the reaction mixture was heated to 100 °C for 5 min. Excess H_2O_2 was destroyed with Pt/C, and the solution was diluted with HCl (1 N) and extracted three times with ethyl acetate. The combined organic phases were dried with Na_2SO_4 and the solvents were evaporated. The residue was dissolved in EtOH (200 µL) and treated with a solution of diazomethane in diethyl ether for 1.5 h. The solvent was removed with a flow of nitrogen, and the residue was dissolved in CH_2Cl_2 (50 µL) and analysed by GC.

2) Ozonolysis: Refined material (145 mg, containing approximately 22 mg spirangien A and 4 mg spirangien B) was dissolved in methanol (6.5 mL) and CH_2Cl_2 (2.25 mL), and ozone was introduced for 7 min at –65 °C. Dimethyl sulfide (500 µL) was added and the reaction mixture was stirred for 45 min at room temperature and was then evaporated in vacuo. The residue was dissolved in MeOH (30 mL) and treated with polymer-supported borohydride (1.5 g). After stirring for 3 h at room temperature, the reaction mixture was filtered and the solvent was removed in vacuo. Purification of the residue by preparative HPLC (MeOH/H_2O 75:25) gave **3** (11 mg) and **4** (13 mg).

Compound 3: R_f = 0.24 [CH_2Cl_2/MeOH (95:5)]. ^{1}H NMR: 2:1 mixture of diastereoisomers. ESI-MS: *m*/*z* (%) = 511.3 (90) $[M + Na]^+$. HRMS (ESI) for $C_{26}H_{48}O_8$: calcd. 511.325; found 511.325.

Compound 4: R_f = 0.21 [CH_2Cl_2/MeOH (95:5)]. $[\alpha]_D^{23}$ = +66.9 (*c* = 1.3 in MeOH). ^{1}H and ^{13}C NMR: see Table S1 (Supporting Information). ESI-MS: *m*/*z* (%) = 527.3 (100) $[M + Na]^+$. HRMS (ESI) for $C_{26}H_{48}O_9$: calcd. 527.319; found 527.319.

3) Olefin Metathesis

a) Grubbs' catalyst (10 mg, 0.016 mmol) was added to a solution of spirangien A (**1**, 14 mg, 0.020 mmol) in CH_2Cl_2 (25 mL) and the reaction flask was filled with ethylene (three vacuum/ethylene flush cycles). The reaction mixture was stirred for 24 h at room temperature under a slight overpressure of ethylene. A sample of the solution was analysed by analytical HPLC-MS. **7**: R_t = 25.1 min; *m*/*z* = 483 $[M + H]^+$; **8**: R_t = 26.2 min; *m*/*z* = 509 $[M + H]^+$; **6**: R_t = 26.5 min; *m*/*z* = 497 $[M + H]^+$; **5**: R_t = 27.7 min; *m*/*z* = 523 $[M + H]^+$; **9**: R_t = 28.9 min; *m*/*z* = 549 $[M + H]^+$. A second portion of Grubbs' catalyst (5 mg, 0.008 mmol) was added, and the flask was filled with ethylene as above and stirred at room temperature for 72 h. The solvent was removed in vacuo and the residue was purified by preparative HPLC [eluent: MeOH/H_2O (85:15), 50 mM

K_2HPO_4/NaH_2PO_4, pH = 7; detection by UV absorption at 230 nm]. The organic solvent was removed in vacuo and the aqueous layer was extracted three times with ethyl acetate. The combined organic phases were dried with sodium sulfate and concentrated to dryness to give **5** (1.6 mg, 15%) as the main product.

b) Refined material (508 mg, containing approximately 22 mg of spirangien A and 4 mg of spirangien B) was dissolved in CH_2Cl_2 (225 mL) and treated with Grubbs' catalyst (36 mg, 0.055 mmol, 15 mol-%). The reaction flask was filled with ethylene as described above and stirred under a slight overpressure of ethylene at room temperature for 24 h. After addition of Grubbs' catalyst (36 mg, 0.055 mmol), the reaction mixture was stirred for another 24 h under ethylene at room temperature. The solvent was removed in vacuo and the residue was purified by preparative MPLC [eluent: $MeOH/H_2O$ (85:15), 50 mM K_2HPO_4/NaH_2PO_4, pH = 7]. The organic solvent was removed in vacuo and the aqueous layer was extracted three times with ethyl acetate. The combined organic phases were dried with sodium sulfate and concentrated to dryness. A second portion of crude extract (515 mg) was treated in the same way and the combined MPLC product fractions of the two reactions were subsequently purified by preparative HPLC [eluent: $MeOH/H_2O$ (85:15), 50 mM K_2HPO_4/NaH_2PO_4, pH = 7] to give crude product (20.0 mg). Purification by preparative TLC [petroleum ether/diethyl ether (1:2)] yielded **5** (11 mg), which crystallized from MeOH as colourless needles.

Compound 5: M.p. 73 °C (from MeOH). $R_f = 0.53$ [CH_2Cl_2/MeOH (95:5)]. $R_t = 27.7$ min (analytical HPLC). (+)-ESI-HPLC-MS: $m/z = 523.3$. (–)-ESI-HPLC-MS: $m/z = 521.3$. $[\alpha]_D^{20} = +33.1$ ($c = 1.0$, MeOH). ^1H and ^{13}C NMR: see Table S2 (Supporting Information). UV (MeOH): λ_{max} (lg ε) = 228 (4.71) nm. ESI-MS: m/z (%) = 545.4 (50) $[M + Na]^+$. HRMS (ESI) for $C_{31}H_{54}O_6$: calcd. 545.381; found 545.380.

Crystal Structure Determination of 5:[13] $C_{31}H_{54}O_6\cdot 2CH_3OH$; $M = 586.8$, triclinic, space group $P1$ (no. 1), $a = 7.819(2)$, $b = 10.252(3)$, $c = 22.437(6)$ Å, $\alpha = 77.031(7)$, $\beta = 81.186(7)$, $\gamma = 78.893(6)°$, $V = 1708.5(8)$ Å^3, $Z = 2$, $D_X = 1.141$ g cm^{-3}, $F(000) = 648$, $T = 120(2)$ K. Bruker-AXS SMART APEX,[14] graphite monochromator, $\lambda(\text{Mo-}K_\alpha) = 0.71073$ Å, $\mu = 0.079$ mm^{-1}, crystal size $0.50 \times 0.2 \times 0.20$ mm, 17436 intensities collected $1.9° < \theta < 28.1°$, $-10 < h < 10$, $-13 < k < 13$, $-29 < l < 27$. Structure solved by direct methods,[14] full-matrix, least-squares refinement[14] based on F^2 and 751 parameters, all but H atoms refined anisotropically, H atoms refined with riding model in idealized positions with $U = 1.5\cdot U_{iso}$(methyl-C and OH) or $1.2\cdot U_{iso}$(C). The title compound crystallizes in the noncentrosymmetric space group $P1$; in the absence of significant anomalous scattering effects, however, the Flack parameter is essentially meaningless. Accordingly, Friedel pairs were merged. There are two independent but geometrically identical molecules (1 and 2) in the unit cell, together with four methanol solvent molecules. Because of severe disorder, one of these CH_3OH moieties could not be refined properly, but treatment with the SQUEEZE facility of PLATON[15] resulted in smooth refinement, which converged at $R1(F) = 0.064$, $wR_2(F^2$, all data) = 0.148, $S = 0.914$, max(δ/σ) < 0.001, min./max. height in final ΔF map –0.26/0.32 e Å^{-3}. Figure 2 shows the molecular structure.

Biological Assays: Spirangien A (**1**) was tested in agar diffusion assays against a broad spectrum of bacteria, yeasts and fungi on paper discs of 6 mm diameter with 10 µg of spirangien A. Organisms were grown on standard agar (1.5%). The cytotoxicity of spirangien A (**1**) and derivatives **3**, **4** and **5** was determined with L929 mouse fibroblast cell culture by the MTT assay.[16]

Supporting Information Available (see footnote on the first page of this article): Spectroscopic data of compounds **4** and **5**.

Acknowledgments

We thank A. Ritter for skilled assistance, S. Reinecke for HPLC-MS analysis, A. Roß, H. Schüler, R. Krützfeld and co-workers for large-scale fermentation, C. Kakoschke and B. Jaschok-Kentner for recording the NMR spectra, V. Wray and H.-J. Hecht for helpful discussions, U. Felgenträger for measuring the mass spectra, and F. Sasse and B. Hinkelmann for the cytotoxicity assays.

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Received: June 13, 2005
Published Online: October 12, 2005