Secondary Metabolites by Chemical Screening, 42[‡] Cladospirones B to I from *Sphaeropsidales* sp. F-24′707 by Variation of Culture Conditions

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Variation of the culture conditions – static surface cultures in particular – of the fungus *Sphaeropsidales* sp. (strain F-24′707), which produces cladospirone bisepoxide (**1**), led to the isolation of eight new spirobisnaphthalenes – the cladospirones B to I (**8**–**15**) – together with seven known representatives of this class of secondary metabolites. Cladospirones C (**9**) and D (**11**) show antibiotic activity against bacteria and algae. The structures of cladospirone B (**8**) and E (**12**) were confirmed by X-ray structure analysis. Cladospirones C (**9**) and G to I (**10**, **14**–**15**) represent new members of the spirobisnaphthalene family, thanks to their hydroxylation patterns. Moreover, they underline the extraordinary status of this interesting class of compounds as the most diverse secondary metabolites, allowing for their small number of carbon atoms, described to date. Almost all possible permutations of stereochemistry and oxygen substitution pattern on the C_{10} skeleton are produced by different fungi.

Introduction

The antibiotic properties of cladospirone bisepoxide (**1**) from *Sphaeropsidales* sp. (strain F-24′707)[2–4] encouraged us to carry out a detailed chemical screening analysis of the secondary metabolite pattern of this strain under different culture conditions.[5] In HPTLC analysis, at least 25 compounds were detectable on TLC plates by visual detection, UV absorption (254 and 366 nm) and/or colour reactions. Applying the OSMAC (one strain/many compounds) method[6,7] to strain F-24′707, we isolated the major metabolites in yields of 5 mg/l to 2.6 g/l. Eight of the 15 isolated compounds proved to be new. These metabolites belong to the growing class of the spirobisnaphthalenes, which are known for various biological activities.[8–11] When we started our work with *Sphaeropsidales* sp. F-24′707, compound **1** was thought to be the only secondary metabolite produced by this strain. For the first time, an extended study is reported in which it has proved possible to direct the production of various members of the spirobisnaphthalene family: both in yield and in kind of compounds produced. We were able to show that cladospirone bisepoxide (**1**) is the main metabolite under oxygen-rich conditions with long incubation times, whereas palmarumycin C_3 (**6**) is produced in high quantities under oxygen-limiting conditions. This work therefore demonstrates possible means of direction of biosynthetic pathways to obtain interesting compounds that can be used directly for biological testing or as precursors for semisynthetic approaches.

Producer Organism and Isolation of Secondary Metabolites

Sphaeropsidales sp. (strain F-24′707), the fungus that produces cladospirone bisepoxide (**1**), has been described previously.[2] To examine the secondary metabolite pattern (Table 1), fermentations of the strain were carried out in two different media, using different culture vessels, following the OSMAC method.[6,7]

Table 1. Yields of spirobisnaphthalenes from strain F-24′707, resulting from altered cultivation conditions: I: 350-mL Erlenmeyer flasks with three intrusions, rotary shaker, medium A; II: 1-L Erlenmeyer flasks, unidirectional shaker, medium B; III: static surface culture (4-L P-flask), medium A; IV: static surface culture (4-L P-flask), containing 200 g of oat grains

Compound	I[a]	II[a]	III[a]	IV[b]
Cladospirone bisepoxide (**1**)	120	–	70	570
Diepoxin σ (**2**)	–	–	–	9
Diepoxin δ (**3**)	20	–	–	51
Diepoxin η (**4**)	–	–	–	+[c]
Palmarumycin C_2 (**5**)	–	–	30	3
Palmarumycin C_3 (**6**)	–	117	2600	2
Palmarumycin C_{12} (**7**)	–	18	70	–
Cladospirone B (**8**)	–	8	30	–
Cladospirone C (**9**)	–	–	–	9
Cladospirone G (**10**)	–	–	–	33
Cladospirone D (**11**)	–	–	–	20
Cladospirone E (**12**)	–	–	–	10
Cladospirone F (**13**)	–	5	–	–
Cladospirone H (**14**)	–	–	–	65
Cladospirone I (**15**)	–	–	–	25

[a] mg/L. – [b] mg/P-flask. – [c] In a mixture with **1**.

After addition of an equivalent quantity of ethyl acetate, the culture broth was homogenised using a blender and centrifuged to remove insoluble material. The phases were separated and the aqueous phase was extracted with ethyl acetate. The combined organic phases were dried with an-

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hydrous sodium sulfate and concentrated in vacuo to yield oily residues, which were subjected to repeated column chromatography on silica gel and Sephadex LH-20. Figure 1 demonstrates the workup procedure for the solid-phase cultivation, using wet oat grains as single substrate. From these experiments we were able to isolate the new cladospirones B to I (**8**−**15**), as well as the known compounds diepoxins σ (**2**) and δ (**3**),[9,10] and palmarumycin C_2 (**5**), C_3 (**6**) and C_{12} (**7**)[8]. Diepoxin η[9,10] (**4**) was isolated in small amounts from the oat-grain experiment as a mixture with **1** (Table 1, IV). The R_f values of the isolated metabolites and their colour reactions on TLC plates with different staining reagents are given in Table 2.

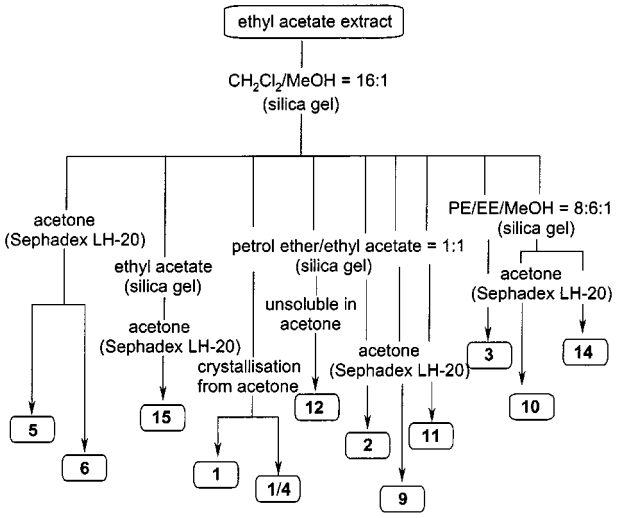


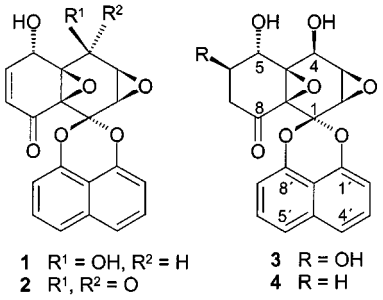
Figure 1. Workup procedure for experiment IV (solid surface cultivation)

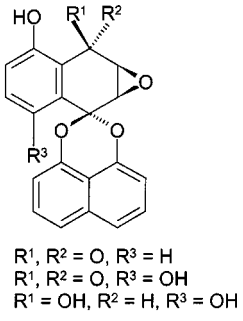
Table 2. R_f values and colour reactions of the isolated compounds on silica gel TLC plates: solvent systems: I: chloroform/methanol (9:1); II: ethyl acetate/cyclohexane (3:2); staining reagents: A: vanillin/H_2SO_4; B: anisaldehyde/H_2SO_4; n.d. = not determined; after spraying, the plates were heated at 120 °C for 5 min

Compound	I	II	A	B
1	0.31	0.22	black	grey-brown
2	0.66	0.24	blue-grey	grey
3	0.14	0.06	violet	brown
4	0.31	0.22	n.d.	n.d.
5	0.79	0.84	blue-violet	green-grey
6	0.78	0.84	dark blue	red-brown
7	0.60	0.66	brown	grey-green
8	0.68	0.75	blue	blue
9	0.63	0.29	blue-green	grey
10	0.21	0.17	turquoise	turquoise
11	0.52	0.32	green-brown	brown
12	0.41	0.32	blue-green	grey-blue
13	0.40	0.43	grey	grey
14	0.13	0.07	blue-green	grey
15	0.10	0.07	dark blue	violet

Structure Elucidation

Cladospirone bisepoxide[2−4] (**1**, = diepoxin ζ,[9,10] palmarumycin C_{13}[8]) and the already known spirobisnaphthalenes diepoxin σ (**2**), δ (**3**) and η[9,10] (**4**, = palmarumycin C_{14}),[8] and palmarumycin C_2 (**5**), C_3 (**6**) and C_{12} (**7**)[8] were identified by a combination of ^1H and ^{13}C NMR spectroscopy and mass spectrometry, by comparison with data given in the literature. The molecular formulae of the new metabolites were determined by high-resolution mass spectra (HREI MS), and their structures were elucidated by analysis of the ^1H and ^{13}C NMR, ^1H-^1H correlation (COSY) and ^1H-^{13}C-shift correlation (HMQC, HMBC) data. X-ray analysis of cladospirone B (**8**) and cladospirone E (**12**), and NOESY spectroscopy on acetylated derivatives of cladospirone G (**10**) and H (**14**), provided the stereochemical information. The stereochemistry of the epoxides and epoxide-derived hydroxy groups was deduced by analogy with **1** and other spirobisnaphthalenes with known absolute configuration, confirming that all compounds so far investigated bear the same stereochemistry.





The isolated spirobisnaphthalenes exhibit typical signals for the different spectroscopic methods. In their EI mass spectra, members of this group of compounds can be easily identified from their fragment at $m/z = 160$ [$C_{10}H_6(OH)_2^+$], resulting from the 1,8-dihydroxynaphthalene (DHN) moiety. The IR spectra show absorption bands between 1400 and 600 cm^{-1}, similar to those in 1,8-dihydroxynaphthalene; the UV spectra exhibit strong absorption bands around 225, 300, 318 and 330 nm resulting from this chromophore, confirming the presence of the DHN residue. As expected, the ^1H NMR spectra show two ABC spin systems (2′-H/3′-H/4′-H and 5′-H/6′-H/7′-H) for the DHN moiety and, besides six proton-attached carbon atoms, ^{13}C NMR spectra show four signals at $\delta_C \approx 114$, 135, 147 and 148 for the quaternary carbon atoms C-8a′, C-4a′, C-1′ and C-8′. The DHN moiety is connected through the spiroketal carbon atom C-1 ($\delta_C \approx 100$) with the second part of the molecule. The spectroscopic data of the DHN moiety and the

bridging carbon atom C-1 of all cladospirones described in this publication are nearly identical, and so we shall only comment in the following structure elucidations on variations in the remaining nine carbon atoms of the second, modified naphthalene portion.

Cladospirone B (8)

The molecular formula $C_{20}H_{14}O_6$ follows from the HREI mass spectrum (m/z = 350.0790, M^+). Characteristically, fragmentation gives a peak at m/z = 332 ($M^+ - H_2O$). The IR spectrum shows absorption bands at $\tilde{\nu}$ = 3472, 1650 and 1611 cm^{-1}, indicating the presence of hydroxy and α,β-unsaturated carbonyl groups. As expected from the mass spectrum, the ^1H NMR spectrum ([D_6]acetone, 500 MHz) of **8** shows 14 proton signals, of which the signals at δ_H = 5.03, 8.08 and 12.27 are readily exchangeable with D_2O, indicating OH groups. An ABX spin system (δ_H = 2.74, 3.26 and 4.54) represents the only signals in the aliphatic area. The rest of the spectrum is very similar to the ^1H NMR spectrum of palmarumycin C_3 (**6**), with nearly identical NMR data for the DHN moiety and the hydroquinone chromophore. Proton-proton connectivities were obtained from both a comparison of coupling constants and a ^1H-^1H COSY experiment. The ^{13}C NMR spectrum (Table 3) shows the signals of twenty carbon atoms. Besides the characteristic signals of the DHN moiety with the spiroketal, the signals of a hydroquinone (δ_C = 114.5, 119.3, 121.8, 129.4, 150.1 and 156.8) and a ketone at δ_C = 202.1 are observable. Proton and carbon assignments were inferred from a 2D ^1H-^{13}C correlation spectrum. From a detailed analysis of the spectroscopic data it was deduced that cladospirone B has the constitution depicted for **8**.

This result was confirmed by an X-ray analysis (Figure 2) of a crystal obtained from an acetone/cyclohexane solution. The depicted absolute stereochemistry is postulated on the assumption that **8** is derived from **6** by reductive opening of the epoxide.

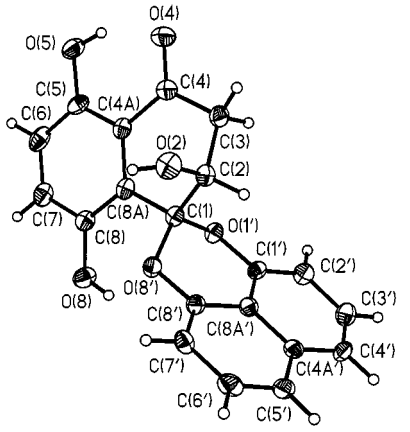


Figure 2. Perspective view of cladospirone B (**8**)

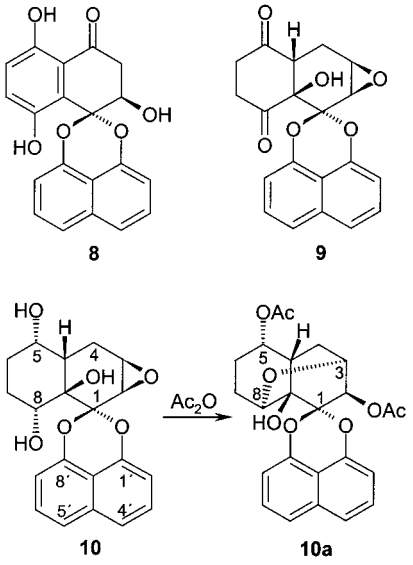


Table 3. ^{13}C NMR signals of cladospirones B to I (**8**–**15**)

C atom	**8**[a]	**9**[b]	**10**[c]	**11**[b]	**12**[d]	**13**[a]	**14**[d]	**15**[a]
1	102.8 (s)	97.5 (s)	102.9 (s)	93.7 (s)	99.1 (s)	103.4 (s)	99.6 (s)	102.6 (s)
2	66.4 (d)	51.8 (d)	76.1 (d)	54.9 (d)	64.2 (d)	33.0 (t)	70.9 (d)	77.2 (d)
3	43.0 (t)	52.9 (d)	71.6 (d)	58.0 (d)	133.6 (d)	63.6 (d)	71.5 (d)	71.3 (d)
4	202.1 (s)	18.4 (t)	21.0 (t)	197.8 (s)	127.1 (d)	66.8 (d)	26.2 (t)	124.8 (d)
4a	114.5 (s)	51.2 (d)	36.0 (d)	67.8 (s)	64.8 (s)	118.5 (s)	44.8 (d)	142.6 (s)
5	156.8 (s)	203.9 (s)	67.6 (d)	194.9 (s)	65.3 (d)	149.3 (s)	69.4 (d)	67.0 (d)
6	121.8 (d)	36.2 (t)	24.3 (t)	32.3 (t)	25.2 (t)	118.8 (d)	35.5 (t)	36.0 (t)
7	129.4 (d)	36.6 (t)	25.7 (t)	22.5 (t)	32.8 (t)	119.1 (d)	37.2 (t)	36.9 (t)
8	150. 1 (s)	204.7 (s)	69.9 (d)	62.5 (d)	199.9 (s)	151.3 (s)	205.9 (s)	205.8 (s)
8a	119.3 (s)	77.9 (s)	73.7 (s)	66.1 (s)	64.8 (s)	114.6 (s)	79.5 (s)	82.7 (s)
1′/8′	146.3 (s)/ 148.3 (s)	145.0 (s)/ 145.4 (s)	148.4 (s)/ 151.3 (s)	144.9 (s)/ 144.9 (s)	145.9 (s)/ 146.5 (s)	147.6 (s)/ 148.8 (s)	147.6 (s)/ 148.2 (s)	149.7 (s)/ 149.8 (s)
2′/7′	110.0 (d)/ 110.8 (d)	109.2 (d)/ 110.7 (s)	109.2 (d)/ 110.2 (d)	109.2 (d)/ 109.9 (d)	108.5 (d)/ 108.8 (d)	109.6 (d)/ 111.0 (d)	110.0 (d)/ 110.1 (d)	107.8 (d)/ 108.1 (d)
3′/6′	128.4 (d)/ 128.4 (d)	127.1 (d)/ 127.9 (d)	128.0 (d)/ 128.6 (d)	127.3 (d)/ 127.7 (d)	127.5 (d)/ 127.8 (d)	128.3 (d)/ 128.4 (d)	128.4 (d)/ 128.4 (d)	127.8 (d)/ 128.0 (d)
4′/5′	121.7 (d)/ 121.9 (d)	121.3 (d)/ 121.8 (d)	120.5 (d)/ 121.7 (d)	121.2 (d)/ 121.3 (d)	119.9 (d)/ 120.2 (d)	121.2 (d)/ 121.7 (d)	121.0 (d)/ 121.1 (d)	120.0 (d)/ 120.0 (d)
4a′	134.9 (s)	134.0 (s)	135.8 (s)	134.1 (s)	133.6 (s)	134.9 (s)	135.5 (s)	134.5 (s)
8a′	115.7 (s)	112.7 (s)	115.1 (s)	111.9 (s)	112.3 (s)	114.6 (s)	114.5 (s)	113.7 (s)

[a] In [D_6]acetone, 125.7 MHz. – [b] In $CDCl_3$, 75.5 MHz. – [c] In CD_3OD, 125.7 MHz. – [d] In [D_6]DMSO, 75.5 MHz.

Cladospirone C (9)

In the high-field portion of the ^{1}H NMR spectrum (300 MHz, $CDCl_3$) of Cladospirone C (**9**, $C_{20}H_{16}O_6$), three methylene groups (δ_H = 2.24, 2.70−2.90 and 2.95−3.14) and three methine signals (δ_H = 3.15, 3.23 and 3.41) are observable. The ^{13}C NMR spectrum (Table 3) shows the signals of 20 carbon atoms; these were assignable to proton-attached carbon atoms, carbon atoms in the DHN moiety, the spiroketal and three quaternary carbon atom signals at δ = 203.9, 204.7 and 77.9. Analysis of a ^{1}H-^{1}H COSY experiment suggested two aliphatic fragments that could be assigned (by HMBC correlation) to the quaternary carbon atoms and the DHN portion (Figure 3). The stereochemistry depicted in **9** was deduced in analogy to **1** and **10**.

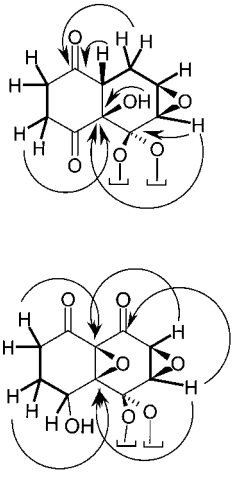


Figure 3. Substructures of cladospirones C (**9**) and D (**11**), derived from ^{1}H-^{1}H COSY (bold lines) and HMBC experiments (arrows)

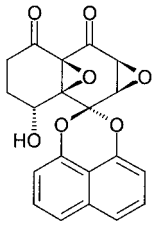
Cladospirone G (10)

Cladospirone G (**10**, $C_{20}H_{20}O_6$) shows at least one hydroxy group ($\tilde{\nu}$ = 3428 cm^{-1}). In the ^{1}H NMR spectrum (300 MHz, CD_3OD), four methine protons adjoining oxygen atoms (δ_H = 3.61, 3.98, 4.18 and 4.28) indicate the high degree of OH functionality. In addition, the spectrum shows the presence of three methylene groups (δ_H = 1.65/1.75, 1.70/1.75 and 2.02/2.12) and one methine proton (δ_H = 2.76). The ^{13}C NMR spectrum shows the signals of 20 carbon atoms: characteristic signals from the DHN moiety and C-1, of three methylene groups (δ_C = 21.0, 24.4 and 25.7), of five methine carbon atoms (δ_C = 36.0, 67.6, 69.9, 71.6 and 76.1) and one quaternary carbon atom signal at δ_C = 73.7 (Table 3). Proton and carbon atom signal assignments followed from a 2D ^{1}H-^{13}C correlation spectrum. A ^{1}H-^{1}H COSY experiment suggested two fragments, both of which could be associated (by HMBC experimentation) with the quaternary carbon atoms, leading to the constitution depicted for **10**. In order to obtain information concerning the stereochemistry of cladospirone G, it was intended to perform acetylation with acetic anhydride/pyridine, followed by detailed NOESY spectroscopy. However, the reaction resulted in the formation of the unexpected 2,5-di-*O*-acetyl-cladospirone G derivative depicted as **10a**.

The structure of **10a** was arrived at from HREI MS and detailed NMR spectroscopy and confirmed the stereochemistry of **10**. An HMBC experiment shows a correlation signal between the proton 3-H and the C-8 carbon atom, due to the new C-3/C-8 ether bridge. The nucleophilic attack of 8-OH onto the epoxide at C-3 of cladospirone G is only possible in the boat conformation resulting from the epoxide at C-2/C-3 and the *cis* connection of the rings at C-4a/C-8a. By analogy with the epoxide stereochemistry of cladospirone bisepoxide (**1**), **10** and **10a** must have the absolute stereochemistry shown.

Cladospirone D (11)

The HREI mass spectrum of cladospirone D (**11**, $C_{20}H_{14}O_7$) indicates the presence of an additional oxygen atom, compared to cladospirone B (**8**). The IR spectrum shows strong absorption bands at $\tilde{\nu}$ = 3426 (OH), 1720 (C=O) and 1610 cm^{-1} (naphthalene). The ^{1}H NMR spectrum shows two methylene group signals at δ_H = 1.85−2.10 and 2.40/2.65, and three methine proton signals at δ_H = 3.50, 3.80 and 4.93 for the modified C_{10} unit. In the ^{13}C NMR spectrum (Table 3), the signals of two carbonyl carbon atoms (δ_C = 197.8 and 194.9) and two quaternary carbon atoms (δ_C = 67.8 and 66.1) are observed, besides those of the proton-attached carbon atoms. Proton and carbon atom signal assignments were arrived at from an HMQC experiment. The signals at δ_C = 58.0 and 54.9 show correlations to δ_H = 3.80 and 3.50, pointing to an epoxide functionality. The total analysis of the 2D NMR spectra suggested the structure depicted in **11** for cladospirone D; Figure 3 shows important ^{1}H-^{1}H COSY and HMBC correlations. The analogous stereochemistry of both epoxides compared to **1** is shown. Because of the small $^3J_{H,H}$ coupling constants ($^3J_{7a,8}$ = $^3J_{7b,8}$ = 3.0 Hz) and the pseudo-boat conformation of the ring, an axial position was assigned to the hydroxy group, resulting in the stereochemistry shown.



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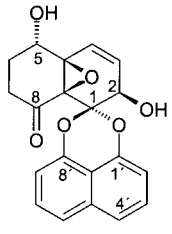

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Cladospirone E (12)

Cladospirone E (**12**) precipitated as a white, amorphous solid from an enriched fraction, after addition of acetone. The molecular formula $C_{20}H_{16}O_6$ was deduced from an HREI mass spectrum (*m*/*z* = 352.0946, M$^+$). The ^{1}H NMR spectrum (300 MHz, [D_6]DMSO) of **12** shows the signals of a C−C double bond at δ_H = 6.14 and 6.35, of two methylene groups at δ_H = 1.80/2.15 and 2.48 and of two methine protons at δ_H = 3.96 and 4.52. Signals at δ_H =

4.73 and 5.84 are readily exchangeable with D_2O, indicating two OH groups. With the signals of two methine and two quaternary carbon atoms between δ_C = 63 and 68, of one carbonyl carbon atom at δ_C = 199.9 and of the typical spiroketal at δ_C = 99.1, the ^{13}C NMR spectrum (Table 3) reflects the high degree of oxygenation. Two aliphatic fragments could be deduced from a COSY experiment: one containing the double bond next to a secondary alcohol, the second containing both methylene groups next to each other and a further secondary alcohol. The positions of the hydroxy groups at C-2 and C-5 were established by the same experiment. The fragments derived from the ^1H-^1H COSY experiment were associated with the remaining quaternary carbon atoms and the DHN moiety, resulting in proposed structure **12**. In order to prove this conclusion and to obtain information concerning the stereochemistry of cladospirone E, an X-ray analysis was performed, using a crystal obtained from dimethyl sulfoxide. The crystal structure is shown in Figure 4, including the postulated absolute stereochemistry deduced by analogy from other cladospirones.

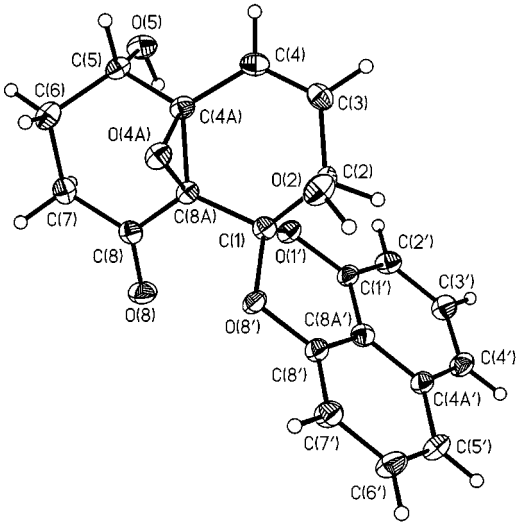
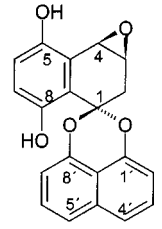


Figure 4. Perspective view of cladospirone E (**12**)

Cladospirone F (13)

From the HREI mass spectra results, component F (**13**, $C_{20}H_{14}O_5$) has the lowest number of oxygen atoms of all cladospirones so far, and contains no carbonyl groups. In the ^1H NMR spectrum (500 MHz, [D_6]acetone), close analogies to **7** are observable (e.g. the signals of the DHN and hydroquinone moieties). The main difference is the ABX spin system (δ_H = 4.19, 2.34 and 2.25), next to an additional methine proton signal at δ_H = 5.15, compared with three neighbouring methine protons in **7**. As expected, in the ^{13}C NMR spectrum (Table 3) signals of one methylene group at δ_C = 33.0 and of two methine groups at δ_C = 63.6 and 66.8 are observed. A detailed analysis of 2D NMR spectra gave evidence for the structure depicted in **13** for cladospirone F.



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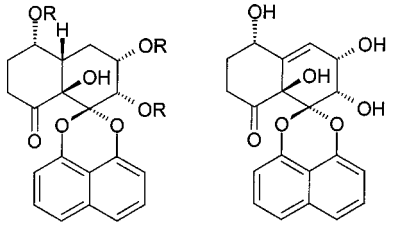

14 R = H
14a R = Ac

15

Cladospirone H (14)

Cladospirone H (**14**, $C_{20}H_{20}O_7$) exhibits one more oxygen atom than cladospirone G (**10**) and the IR spectrum shows an additional carbonyl group absorption band at $\tilde{\nu}$ = 1720 cm^{-1}. The ^1H NMR spectrum (500 MHz, CD_3OD) shows signals for 16 protons, indicating the presence of four exchangeable OH groups. Four methine protons at δ_H = 2.59, 3.79, 3.92 and 4.04 show the close similarity to cladospirone G (**10**). Proton connectivities were arrived at from a comparison of coupling constants and a ^1H-^1H COSY experiment. In the ^{13}C NMR spectrum, besides the expected signals of the DHN residue, the spiroketal and the proton-attached carbon atoms, the signals of two quaternary carbon atoms are observable at δ_C = 79.5 and 205.9 (Table 3). Detailed 2D NMR spectroscopy led us to propose the constitution for cladospirone H depicted for **14**. The differences with **10** are the ketone at C-8 and the OH groups at C-2 and C-3 instead of the epoxide. From the ^3J coupling constant between 2-H and 3-H ($^3J_{2,3}$ = 3.0 Hz), a *cis* configuration of the two OH groups was deduced. The stereochemistry of **14** was established from a NOESY experiment on 2,3,5-tri-*O*-acetylcladospirone H (**14a**), obtained after a standard acetylation procedure. Cross peaks were observed between the protons 2-H/3-H, 3-H/4a-H, 4a-H/5-H and the proton 8a-OH and 2-H, 3-H and 4a-H, confirming the positions of these protons on the same side of the decalin moiety (Figure 5).

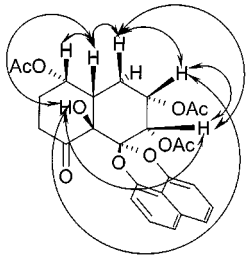

Figure 5. Important NOESY data for **14a**

Cladospirone I (15)

Cladospirone I (**15**, $C_{20}H_{18}O_7$) has two fewer protons than cladospirone H (**14**), and a high degree of similarity between both compounds was deduced by IR and NMR spectroscopy. The ^1H NMR spectrum (500 MHz, [D_6]acetone) shows signals of three methine protons (δ_H = 4.34, 4.44, 4.84) and of one substituted olefinic double bond (δ_H = 5.97). In agreement with these observations, the ^{13}C NMR spectrum (Table 3) shows signals of three methine carbon atoms at δ_C = 67.0, 71.3, and 77.2, and of two olefinic carbon atoms at δ_C = 124.8 and 142.6 (quaternary). Besides the expected signals for the DHN moiety and C-1, signals of two methylene carbon atoms (δ_C = 36.0 and 36.9) and two quaternary carbon atoms at δ_C = 82.7 and 205.8 complete the ^{13}C NMR spectrum. Detailed analysis of 2D NMR spectra gave evidence for the constitution of cladospirone I as depicted for **15**. The stereochemistry shown is in analogy to cladospirone bisepoxide (**1**) and cladospirone H (**14**). The observed $^3J_{H-H}$ coupling constant between 2-H and 3-H, of 7.0 Hz (compared to the $^3J_{H-H}$ coupling constant of 3.0 Hz in the cyclohexane ring of **14**), might result from axial-equatorial positioning of both OH groups resulting from the pseudo-chair conformation in the cyclohexene ring.

Biological Activity

The cladospirones C (**9**) and D (**11**) show good antibacterial activity in the agar diffusion assay against *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis*, but no activity against *Candida albicans* and *Mucor hiemalis*. In addition, though, **11** is active against *Chlorella vulgaris* and *Chlorella sorokiniana* (Table 4). The cladospirones B (**8**) and E-I (**10**, **12**−**15**) show no antibacterial or antifungal activity in the agar diffusion assay. The biological activity of the known compounds **1**−**7** has been described elsewhere.

Table 4. Antibacterial, antifungal and algicidal activity of **9** and **11** in agar diffusion assay (c = 1 mg/mL); test organisms: Bacteria (E.c.: *Escherichia coli*; S.a.: *Staphylococcus aureus*; B.s.: *Bacillus subtilis*); fungi (M.h.: *Mucor hiemalis*; C.a.: *Candida albicans*); algae (C.v.: *Chlorella vulgaris*; C.s.: *Chlorella sorokiniana*; S.c.: *Scenedesmus subspicatus*)

Compound	Radius of inhibition [mm] E.c.	S.a.	B.c.	M.h.	C.a.	C.v.	C.s.	S.c.
9	16	11	15	–	–	–	–	–
11	32	22	32	–	–	17	22	–

Discussion

The cladospirones B to I (**8**−**15**) represent new members of the spirobisnaphthalene family, produced by the fungus *Sphaeropsidales* sp. (strain F-24′707). The compounds bear a decalin moiety with different oxygenation patterns, connected to a 1,8-dihydroxynaphthalene residue through a spiroketal bridge. The cladospirones C (**9**) and D (**11**) show good antibiotic activity against gram-positive and gram-negative bacteria and different algae. Most of the known spiobisnaphthalenes exhibit various biological activities; e.g., **1** shows antifungal and antibacterial activity[2] and **2** inhibits phospholipase D, a putative antitumor target.[11] Because of this broad spectrum of biological activities and the complex stereochemistry, synthetic chemists have focused on this interesting class of natural products.[12−15] Here, we present the production of various members of this class of compounds, in yields of up to 2.6 g/L, that might be useful precursors for semisynthetic approaches.

We were able to isolate eight new compounds, together with seven known spirobisnaphthalenes. Variation of the culture conditions turned out to be a useful tool for altering the metabolic profile of different strains. We have named this way of enhancing metabolite diversity the OSMAC (one strain/many compounds) method[6,7] and it provides a detailed insight into the biosynthetic capacities of microorganisms, information on the regulation of biosynthetic pathways and access to new structures. Static surface cultivation, using wet oat grain as single substrate, resulted in the production of at least six new spirobisnaphthalenes. One reason for this unexpected result could be the different aeration conditions, compared to fermentations in liquid media.[16] Further evidence for this hypothesis is the production of reduced spirobisnaphthalenes − e.g. palmarumycin C_3 (**6**) −in high yield from static surface cultures with liquid medium. In this case, the oxygen concentration is limited by diffusion into the medium. The fact that reduced or oxidised spirobisnaphthalenes are produced in different shaking flasks indicates the sensitivity of this regulation. Different time courses of fermentations in shaking flasks and fermenters (data not shown) and biogenetic studies[17] provided evidence for the biosynthesis of cladospirone bisepoxide (**1**) via different palmarumycin intermediates, starting from 1,8-dihydroxynaphthalene as the pentaketide precursor first postulated by Krohn et al.[8] Epoxidation of the first spirobisnaphthalene palmarumycin CP_1[18] would result in the formation of palmarumycin C_2 (**5**), which would be further oxygenated to give palmarumycin C_3 (**6**). Reduction of **6** would generate palmarumycin C_{12} (**7**), which after a second epoxidation would yield cladospirone bisepoxide (**1**).

The spirobisnaphthalenes, at least those of the early stages of biosynthesis, are modulated by oxygen in regio- and stereochemically controlled pathways. Considering the fact that almost all the carbon atoms of the upper decalin moiety are involved in these alterations, the large number of similar metabolites can easily be explained. Assuming that, in spite of the vast number of spirobisnaphthalenes described so far, some members of this family still need to be isolated, the spirobisnaphthalenes may be considered the most diverse secondary metabolites, allowing for the small number of carbon atoms available to host these variations. Almost all stereochemical and oxygen substitution pattern permutations possible for a C_{10} skeleton have been found in only a small number of different fungi. The highly oxy-

genated cladospirones C to E (**9**, **11**−**12**) and G to I (**10**, **14**−**15**) were produced only in static surface cultures, in low yields compared to **1**, over 28 d of incubation. This fact supports the hypothesis that these compounds are shunt products of cladospirone bisepoxide biosynthesis, probably arising from enzymatic reactions. Further investigations, using UV mutants and enzyme inhibitors, into elucidating the late biosynthesis of this interesting class of natural compounds have been published elsewhere.[19]

Experimental Section

General Remarks: See ref.[1]

Fermentation: *Sphaeropsidales* sp. F-24′707 was maintained as a stock culture in liquid nitrogen. A 1-cm^2 piece of agar from a 10 d old culture (grown on LCSB agar) was used to inoculate 100 mL of medium A [glucose 2%, oatmeal 2%, degreased soy meal 2%, deionised water]. These pre-cultures were cultivated in a rotary shaker (250 rpm) at 28 °C, and 2.5−5% were used to inoculate the main cultures using medium A, B [glycerol 2%, malt extract 1%, yeast extract 0.4%, deionised water] or oat grain. Fermentations in shaking flasks were carried out at 28 °C for 3 d, either in 300-mL Erlenmeyer flasks with three intrusions on a rotary shaker (250 rpm) using 100 mL of medium A, or in 1000-mL Erlenmeyer flasks on a linear shaker (100 spm) using 150 mL of medium B.

Fermentation in Static (not Shaken) Cultures: Liquid cultures: 50 mL of a 3 d old pre-culture was used to inoculate 1000 mL of medium A in 4-L P-flasks (P = *Penicillium*). Oat-grain surface cultures: in a 4-L P-flask, 250 g of oat grain soaked in deionised water for 24 h and autoclaved twice were inoculated with 5 mL of pre-culture. The flasks were cultivated at 28 °C for 28 d.

Workup Procedure: The harvested cultures were homogenised with an equal quantity of ethyl acetate, using a blender (Janke & Kunkel KG). After centrifugation to remove insoluble material and separation of the phases, the aqueous phase was extracted twice with ethyl acetate. The combined organic phases were dried with anhydrous Na_2SO_4 and concentrated to furnish the crude products.

Experiment I (see Table 1): The crude extract (2.24 g from 1 L culture broth) was chromatographed on silica gel [column 4 × 30 cm, petroleum ether/ethyl acetate (2:1)]. Further purification of **1** and **3** was carried out by gel permeation chromatography on Sephadex LH-20 (column 2.5 × 100 cm, acetone), yielding the pure compounds.

Experiment II (see Table 1): Palmarumycin C_3 (**6**) was crystallised from a solution of the ethyl acetate extract [25 g from 5 P-flasks (1 L medium per flask)] in acetone. The residue was dissolved in CH_2Cl_2 and filtered using silica gel (column 6 × 15 cm, CH_2Cl_2); dichloromethane-insoluble material was eluted from the column with ethyl acetate. The dichloromethane eluate was chromatographed on silica gel [column 1.5 × 30 cm, ethyl acetate/petroleum ether (1:5)] to yield pure palmarumycin C_2 (**5**), which was crystallised from acetone. The ethyl acetate eluate was chromatographed on silica gel [column 2.5 × 30 cm, ethyl acetate/petroleum ether (1:2)] and the enriched fractions were further purified by gel permeation chromatography on Sephadex LH-20 (column 2.5 × 100 cm, acetone) to yield pure cladospirone bisepoxide (**1**), cladospirone B (**8**) and palmarumycin C_{12} (**7**).

Experiment III (see Table 1): The crude extract (3.3 g from 3 L culture broth) was chromatographed on silica gel (column 3 × 30 cm, CH_2Cl_2); two main fraction were obtained. Chromatography of the polar fraction on silica gel [column 2.5 × 30 cm, CH_2Cl_2/MeOH (20:1)], followed by gel permeation chromatography (column 2.5 × 100 cm, acetone) of the enriched fractions, led to the isolation of pure cladospirone B (**8**), cladospirone F (**13**) and palmarumycin C_{12} (**7**). Crystallisation of the less polar fraction from acetone yielded pure palmarumycin C_3 (**6**).

Experiment IV (see Table 1): The crude extract was further purified by column chromatography as shown in Figure 1. The isolated amounts of the natural products are given in Table 1.

Cladospirone B (8): $C_{20}H_{14}O_6$ (350.33); m. p. 230 °C (decomp.). − IR (KBr): $\tilde{\nu}$ = 3472 cm^{-1}, 1650, 1611, 1475, 1412, 1379, 1315, 1268, 1211, 1162, 1100, 1029, 967, 880, 756. − UV (MeOH): λ_{max} (lg ε) = 226 nm (4.73), 260 (3.70), 297 (3.83), 312 (3.71), 326 (3.68), 366 (3.71). − MeOH/NaOH: λ_{max} = 226 nm (4.75), 299 (3.89), 328 (3.71), 376 (3.56). − $[\alpha]_D^{20}$ = −270 (*c* = 0.31, $CHCl_3$). − CD (MeOH): $\lambda_{extr.}$ ($[\Theta]^{22}$) = 210 nm (80600), 230 (−168500), 318 (−1600), 365 (−6900). − ^{1}H NMR (500 MHz, [D$_6$]acetone): δ = 2.74 (dd, $J_{3a,3b}$ = 17.0, $J_{3a,2}$ = 4.0 Hz, 3-H$_a$), 3.26 (dd, $J_{3a,3b}$ = 17.0 Hz, $J_{3b,2}$ = 3.0 Hz, 3-H$_b$), 4.54 (m, 2-H), 5.03 (br., 3-OH), 7.03/7.16 (dd, *J* = 7.5, 1.0 Hz, 2′-H/7′-H), 7.05 (d, $J_{6,7}$ = 9.0 Hz, 6-H), 7.27 (d, $J_{6,7}$ = 9.0 Hz, 7-H), 7.51/7.55 (dd, *J* = 8.5, 7.5 Hz, 3′-H/6′-H), 7.61/7.65 (dd, *J* = 8.5, 1.0 Hz, 4′-H/5′-H), 8.08 (br., 8-OH), 12.27 (s, 5-OH). − ^{13}C NMR (125.7 MHz, [D$_6$]acetone): see Table 3. − EI MS (70 eV): *m*/*z* (%) = 350 (100) [M$^+$] (high resolution calcd. for $C_{20}H_{14}O_6$ 350.0790, found 350.0790), 332 (14) [M$^+$ − H_2O], 303 (6), 160 (28) [$C_{10}H_6(OH)_2^+$], 115 (7).

X-ray Crystal Structure Analysis of 8:[20] Compound **8** (empirical formula: $C_{20}H_{14}O_6$, M_r = 350.31) was crystallised by concentrating a saturated solution of **8** in a mixture of acetone/cyclohexane (2:1) at 7 °C. Crystal size 0.07 × 0.4 × 0.4 mm, monoclinic, space group $P2_1$, *a* = 755.3(2), *b* = 1970.2(3), *c* = 1281.3(2) pm, β = 91.22(1)°; *V* = 1.5192(5) nm^3, *Z* = 4, $D_{calcd.}$ = 1.532 g/cm^3, μ = 0.114 mm^{-1}, Stoe-Siemens-Huber diffractometer coupled to a Siemens CCD-area detector with graphite-monochromated Mo-K_α radiation (λ = 0.71073), −140 °C, Θ range = 3−50°, 31779 reflections measured, 5364 unique. Structure solved by direct methods using SHELXS-97[21] and refined against F^2 on all data by full-matrix least squares with SHELXL-97.[22] A riding model with idealised hydrogen geometry was employed; the anisotropic refinement converged at R_1 = 0.0448 for $F > 2\sigma(F)$ and $wR_2(F^2)$ = 0.0941 for all reflections.

Cladospirone C (9): $C_{20}H_{16}O_6$ (352.35); m. p. 164 °C. − IR (KBr): $\tilde{\nu}$ = 3430 cm^{-1}, 1718, 1609. − UV (MeOH): λ_{max} (lg ε) = 225 nm (4.66), 297 (3.81), 312 (3.66), 327 (3.52). − MeOH/NaOH: λ_{max} = 226 nm (4.70), 299 (4.03), 327 (3.73), 3.68 (3.50), 374 (3.51). − $[\alpha]_D^{20}$ = −35 (*c* = 1.0 in $CHCl_3$). − CD (MeOH): $\lambda_{extr.}$ ($[\Theta]^{22}$) = 221 nm (−25345), 294 (1707), 326 (−854). − ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.24 (dd, *J* = 16.0, 6.0 Hz, 4-H$_a$), 2.70−2.90 (m, 4-H$_b$, 7-H$_a$), 2.95−3.14 (m, 6-H$_2$, 7-H$_b$), 3.15 (br.d, *J* = 4.0 Hz, 4a-H), 3.23 (d, $J_{2,3}$ = 4.0 Hz, 2-H), 3.41 (t, $J_{3,2}$ = $J_{3,4}$ = 4.0 Hz, 3-H), 4.40 (br., 8a-OH), 6.86/7.11 (dd, *J* = 7.0, 1.0 Hz, 2′-H/7′-H), 7.36/7.42 (dd, *J* = 8.0, 7.5 Hz, 3′-H/6′-H), 7.49 (m, 4′-H, 5′-H). − ^{13}C NMR (75.5 MHz, $CDCl_3$): see Table 3. − EI MS (70 eV): *m*/*z* (%) = 352 (58) [M$^+$] (high resolution calcd. for $C_{20}H_{16}O_6$ 352.0946, found 352.0946), 334 (16) [M$^+$ − H_2O], 200 (100), 160 (14) [$C_{10}H_6(OH)_2^+$], 115 (18).

Cladospirone G (10): $C_{20}H_{20}O_6$ (356.38); m. p. 135 °C. − IR (KBr): $\tilde{\nu}$ = 3428 cm^{-1}, 1636, 1608. − UV (MeOH): λ_{max} (lg ε) = 226 nm (4.64), 299 (3.81), 313 (3.69), 327 (3.58). − $[\alpha]_D^{20}$ = +5.0 (*c* = 0.24

in MeOH). – CD (MeOH): $\lambda_{extr.}$ ([Θ]^{22}) = 205 nm (−10176), 211 (−17521), 227 (17237), 250 (−1094), 265 (163), 296 (−1958). – ^{1}H NMR (500 MHz, CD_3OD): δ = 1.61−1.67 (m, 6-H_a), 1.67−1.73 (m, 7-H_b), 1.79−1.91 (m, 6-H_b, 7-H_b), 2.02 (dm, $J_{4a,4b}$ = 14.5 Hz, 4-H_a), 2.12 (ddd, $J_{4a,4b}$ = 14.5, J = 11.0, 1.0 Hz, 4-H_b), 2.76 (br. d, J = 11.0 Hz, 4a-H), 3.61 (t, $J_{3,2}$ = 3.5 Hz, 3-H), 3.98 (dd, $J_{2,3}$ = 4.0, $J_{2,4}$ = 1.5 Hz, 2-H), 4.18 (m, 5-H), 4.28 (t, $J_{8,7}$ = 1.5 Hz, 8-H), 6.95/6.96 (d, J = 5.5 Hz, 2′-H/7′-H), 7.38−7.44 (m, 3′-H, 6′-H, 4′-H or 5′-H), 7.48 (dd, J = 8.0, 1.0 Hz, 4′-H or 5′-H). – ^{13}C NMR (125.7 MHz, CD_3OD): see Table 3. – EI MS (70 eV): *m/z* (%) = 334 (88) [M$^+$] (high resolution calcd. for $C_{20}H_{14}O_6$ 334.0841, found 334.0841), 316 (74) [M$^+$ − H_2O], 191 (12), 175 (30), 160 (100) [$C_{10}H_6(OH)_2^+$], 115 (30), 44 (61).

2,5-Di-*O*-acetylcladospirone G (10a): $C_{24}H_{24}O_8$ (440.45); compound **10** (30 mg) was dissolved in dry pyridine (2 mL), and DMAP (10 mg) and acetic anhydride (5 mL) were added to the stirred solution. After stirring for 4 h at ambient temperature, the reaction was quenched with 20 mL of H_2O. The reaction mixture was extracted twice with 20 mL of CH_2Cl_2; the combined organic layers were dried with anhydrous Na_2SO_4 and concentrated to dryness. The residue was purified by gel permeation chromatography on Sephadex LH-20 (column 2.5 × 100 cm, acetone), yielding 10 mg (26%) of **10a** as a colourless amorphous solid. – M.p. 68 °C. – IR (KBr): $\tilde{\nu}$ = 3435 cm^{-1}, 1732, 1610. – UV (MeOH): λ_{max} (lg ε) = 225 nm (4.66), 298 (3.76), 313 (3.61), 327 (3.49). – $[\alpha]_D^{20}$ = +6.5 (*c* = 0.93 in MeOH). – CD (MeOH): $\lambda_{extr.}$ ([Θ]^{22}) = 210 nm (−19724), 227 (36683), 296 (−5472). – ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.19 (s, 2-OAc-H_3), 1.80 (m, 7-H_a), 1.95 (m, 7-H_b), 2.05 (s, 5-OAc-H_3), 2.10 (m, 4-H_2), 2.99 (m, 4a-H), 3.73 (m, 3-H), 4.36 (m, 8-H), 5.22 (dd, J = 4.0, 1.5 Hz, 2-H), 5.35 (m, 5-H), 6.97/6.99 (dd, J = 7.5, 1.0 Hz, 2′-H/7′-H), 7.35−7.44 (m, 3′-H, 6′-H), 7.48 (d, J = 8.0 Hz, 4′-H, 5′-H). – ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 19.3 (q, 2-OAc−Me), 20.3 (t, C-6), 21.3 (q, 5-OAc−Me), 21.7 (t, C-4), 24.2 (t, C-7), 32.5 (d, C-4a), 67.1 (d, C-3), 68.5 (d, C-8), 70.4 (d, C-5), 72.6 (s, C-8a), 73.1 (d, C-2), 100.2 (C-1), 108.4/110.4 (d, C-2′/C-7′), 113.3 (s, C-8a′), 120.3/121.4 (d, C-4′/C-5′), 127.2/127.5 (d, C-3′/C-6′), 134.1 (s, C-4a′), 146.0/148.6 (s, C-1′/C-8′), 167.9 (s, 2-OAc−CO), 170.4 (s, 5-OAc−CO). – EI MS (70 eV): *m/z* (%) = 440 (100) [M$^+$] (high resolution calcd. for $C_{24}H_{24}O_8$ 440.1471, found 440.1471), 200 (18), 160 (41) [$C_{10}H_6(OH)_2^+$], 115 (7), 43 (36).

Cladospirone D (11): $C_{20}H_{14}O_7$ (366.33); m. p. 127 °C. – IR (KBr): $\tilde{\nu}$ = 3426 cm^{-1}, 1720, 1610. – UV (MeOH): λ_{max} (lg ε) = 225 nm (4.74), 298 (3.88), 313 (3.75), 327 (3.66). – $[\alpha]_D^{20}$ = 55 (*c* = 1.8 in $CHCl_3$). – CD (MeOH): $\lambda_{extr.}$ ([Θ]^{22}) = 225 nm (−18790), 299 (6912), 330 (2479). – ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.85−2.10 (m, 7-H_2), 2.40 (ddd, J = 19.0, 6.0, 2.0 Hz, 6-H_a), 2.65 (ddd, J = 19.0, 12.5, 7.0 Hz, 6-H_b), 3.50 (d, $J_{2,3}$ = 4.0 Hz, 2-H), 3.81 (d, $J_{2,3}$ = 4.0 Hz, 3-H), 4.93 (t, J = 3.0 Hz, 8-H), 6.96/7.15 (dd, J = 7.5, 1.0 Hz, 2′-H/7′-H), 7.40/7.47 (t, J = 8.0 Hz, 3′-H/6′-H), 7.52/7.53 (dd, J = 8.0, 1.0 Hz, 4′-H/5′-H). – ^{13}C NMR (75.5 MHz, $CDCl_3$): see Table 3. – EI MS (70 eV): *m/z* (%) = 366 (100) [M$^+$] (high resolution calcd. for $C_{20}H_{14}O_7$ 366.0739, found 366.0739), 211 (10), 160 (7) [$C_{10}H_6(OH)_2^+$], 115 (6).

Cladospirone E (12): $C_{20}H_{16}O_6$ (352.35); m. p. 236 °C (decomp.) – IR (KBr): $\tilde{\nu}$ = 3554 cm^{-1}, 3436, 1712, 1612. – UV (MeOH): λ_{max} (lg ε) = 225 nm (4.58), 299 (3.66), 313 (3.51), 327 (3.42). – $[\alpha]_D^{20}$ = −217 (*c* = 0.93 in MeOH). – CD (MeOH): $\lambda_{extr.}$ ([Θ]^{22}) = 225 nm (−30029), 271 (849), 301 (4634), 329 (1226). – ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.80 (m, 6-H_a), 2.15 (m, 6-H_b), 2.48 (m, 7-H_2), 3.96 (dd, $J_{2H,2OH}$ = 9.0, $J_{2,3}$ = 6.0 Hz, 2-H), 4.52 (m, 5-H), 4.73 (d, $J_{2H,2OH}$ = 9.0 Hz, 2-OH), 5.84 (d, $J_{5H,5OH}$ = 4.5 Hz, 5-OH), 6.14 (dd, $J_{3,4}$ = 10.0, $J_{2,3}$ = 6.0 Hz, 3-H), 6.35 (d, $J_{3,4}$ = 10.0 Hz, 4-H), 6.93/7.07 (d, J = 7.5 Hz, 2′-H/7′-H), 7.52/7.57 (t, J = 8.0 Hz, 3′-H/6′-H), 7.63 (m, 4′-H, 5′-H). – ^{13}C NMR (75.5 MHz, $CDCl_3$): see Table 3. – EI MS (70 eV): *m/z* (%) = 352 (100) [M$^+$] (high resolution calcd. for $C_{20}H_{16}O_6$ 352.0946, found 352.0946), 278 (21), 171, (22), 160 (50) [$C_{10}H_6(OH)_2^+$], 115 (24).

X-ray Crystal Structure Analysis of 12:[20] Compound **12** (empirical formula $C_{20}H_{16}O_6$, M_r = 352.33) was crystallised from [D$_6$]dimethyl sulfoxide at ambient temperature. Crystal size 0.4 × 0.2 × 0.2 mm, orthorhombic, space group $P2_12_12_1$, a = 603.86(4), b = 1173.10(7), c = 2143.50(8) pm, V = 1.51843(15) nm^3, Z = 4, $D_{calcd.}$ = 1.541 g/cm^3, μ = 0.115 mm^{-1}, Stoe-Siemens-Huber diffractometer coupled to a Siemens CCD area detector with graphite-monochromated Mo-K_α radiation (λ = 0.71073), −140 °C, Θ range = 5−50°, 18166 reflections measured, 2791 unique. Structure solved by direct methods using SHELXS-97[21] and refined against F^2 on all data by full-matrix least squares with SHELXL-97.[22] A riding model with idealised hydrogen geometry was employed; the anisotropic refinement converged at R_1 = 0.0330 for $F > 2\sigma(F)$ and $wR_2(F^2)$ = 0.0710 for all reflections.

Cladospirone F (13): $C_{20}H_{14}O_5$ (334.33); m. p. 140 °C (decomp.). – IR (KBr): $\tilde{\nu}$ = 3448 cm^{-1}, 1636, 1608. – UV (MeOH): λ_{max} (lg ε) = 206 nm (4.48), 226 (4.68), 300 (3.95). – MeOH/NaOH: λ_{max} = 225 nm (4.69), 299 (3.91), 3.27 (3.66). – $[\alpha]_D^{20}$ = −150 (*c* = 0.18 in $CHCl_3$). – CD (MeOH): $\lambda_{extr.}$ ([Θ]^{22}) = 207 nm (−10417), 210 (−9844), 229 (−41413), 255 (−30), 294 (−6628), 310 (−4866), 323 (−3325), 336 (1033). – ^{1}H NMR (500 MHz, [D$_6$]acetone): δ = 2.25 (ddd, $J_{2a,2b}$ = 14.5, $J_{2a,3}$ = 5.0, $J_{2a,4}$ = 3.0 Hz, 2-H_a), 2.34 (ddd, $J_{2a,2b}$ = 14.5, $J_{2b,3}$ = 5.0, $J_{2b,4}$ = 2.0 Hz, 2-H_b), 4.19 (dd, $J_{3,4}$ = 17.0, $J_{2b,3}$ = 2.0 Hz, 3-H), 5.15 (dd, $J_{3,4}$ = 5.0, $J_{2a,4}$ = 3.0 Hz, 4-H), 6.81 (d, $J_{6,7}$ = 8.5 Hz, 6-H), 6.90/7.07 (dd, J = 7.5, 1.0 Hz, 2′-H/7′-H), 6.94 (d, $J_{6,7}$ = 8.5 Hz, 7-H), 7.45/7.50 (dd, J = 8.5, 7.5 Hz, 3′-H/6′-H), 7.55/7.59 (dd, J = 8.5, 1.0 Hz, 4′-H/5′-H). – ^{13}C NMR (125.7 MHz, [D$_6$]acetone): see Table 3. – EI MS (70 eV): *m/z* (%) = 356 (100) [M$^+$] (high resolution calcd. for $C_{20}H_{20}O_6$ 356.1259, found 356.1259), 200 (10), 160 (62) [$C_{10}H_6(OH)_2^+$], 115 (16).

Cladospirone H (14): $C_{20}H_{20}O_7$ (372.37); m. p. 156 °C. – IR (KBr): $\tilde{\nu}$ = 3429 cm^{-1}, 1720, 1637, 1609. – UV (MeOH): λ_{max} (lg ε) = 226 nm (4.71), 299 (3.80), 313 (3.62), 327 (3.49). – $[\alpha]_D^{20}$ = −22 (*c* = 0.22 in MeOH). – CD (MeOH): $\lambda_{extr.}$ ([Θ]^{22}) = 206 nm (−628), 210 (1620), 223 (−18129), 256 (986), 266 (−222), 303 (2523). – ^{1}H NMR (500 MHz, CD_3OD): δ = 1.76 (m, 6-H_a), 2.10−2.17 (m, 4-H_a/7-H_b), 2.17−2.23 (m, 4-H_b), 2.23−2.27 (m, 6-H_b), 2.59 (ddd, J = 13.0, 11.0, 1.0 Hz, 4a-H), 3.11 (dt, J = 14.0, 6.0 Hz, 7-H_a), 3.79 (d, J = 3.0 Hz, 2-H), 3.92 (dd, J = 6.0, 3.0 Hz, 3-H), 4.04 (ddd, J = 11.0, 11.0, 4.5 Hz, 5-H), 6.89/6.95 (d, J = 7.5 Hz, 2′-H/7′-H), 7.39/7.41 (t, J = 8.5 Hz, 3′-H/6′-H), 7.44/7.46 (d, J = 8.0 Hz, 4′-H/5′-H). – ^{13}C NMR (125.7 MHz, CD_3OD): see Table 3. – EI MS (70 eV): *m/z* (%) = 372 (96) [M$^+$] (high resolution calcd. for $C_{20}H_{20}O_7$ 372.1209, found 372.1209), 226 (100), 200 (82), 172 (32), 160 (72) [$C_{10}H_6(OH)_2^+$], 154 (33), 151 (22).

2,3,5-Tri-*O*-acetylcladospirone H (14a): $C_{26}H_{26}O_{10}$ (498.49); the reaction was carried out with 30 mg of **14** as described for **10a** and resulted in 30 mg (75%) of **14a**. M.p. 224 °C. – IR (KBr): $\tilde{\nu}$ = 3436 cm^{-1}, 1737, 1610. – UV (MeOH): λ_{max} (lg ε) = 225 nm (4.70), 299 (3.81), 312 (3.67), 327 (3.55). – $[\alpha]_D^{20}$ = −29.2 (*c* = 1.58 in MeOH). – CD (MeOH): $\lambda_{extr.}$ ([Θ]^{22}) = 209 nm (−5420), 225 (18730), 239 (−345), 297 (−2625), 326 (415). – ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.85 (m, 6-H_a), 1.90 (m, 4-H_a), 2.03 (s, 2-OAc-H_3), 2.09 (s, 3-

OAc−Me), 2.10 (s, 5-OAc−Me), 2.24 (m, 4-H_b), 2.30−2.45 (m, 6-H_b, 7-H_a), 2.85 (m, 4a-H), 3.14 (m, 7-H_b), 3.46 (br., 8a-OH), 4.94 (m, 3-H), 5.11 (dd, J = 3.0, 1.5 Hz, 2-H), 5.32 (dt, J = 11.0, 4.5 Hz, 5-H), 6.76/6.85 (dd, J = 8.0, 1.0 Hz, 2′-H/7′-H), 7.35/7.38 (t, J = 8.0 Hz, 3′-H/6′-H), 7.47 (m, 4′-H, 5′-H). − ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 20.4 (q, 2-OAc−Me), 21.0 (q, Me), 21.1 (q, Me), 23.0 t, C-4), 29.9 (t, C-6), 35.7 (t, C-7), 40.5 (d, C-4a), 66.7 (d, C-2), 68.2 (d, C-3), 70.4 (d, C-5), 76.8 (s, C-8a), 97.2 (s, C-1), 108.5/108.8 (d, C-2′/C-7′), 112.5 (s, C-8a′), 121.08/121.12 (d, C-4′/C-5′), 127.1/127.4 (d, C-3′/C-6′), 134.3 (s, C-4a′), 145.0/145.4 (s, C-1′/C-8′), 168.2 (s, 2-OAc−CO), 169.4 (s, 3-OAc−CO), 170.5 (s, 5-OAc−CO), 202.1 (s, C-8). − EI MS (70 eV): *m/z* (%) = 498 (55) [M^+] (high resolution calcd. for $C_{26}H_{26}O_{10}$ 498.1525, found 498.1525), 470 (2), 246 (43), 226 (100), 160 (15) [$C_{10}H_6(OH)_2^+$], 91 (100).

Cladospirone I (15): $C_{20}H_{18}O_7$ (370.36); m. p. 138 °C. − IR (KBr): $\tilde{\nu}$ = 3441 cm^{-1}, 1608. − UV (MeOH): λ_{max} (lg ε) = 226 nm (4.67), 299 (3.76), 313 (3.60), 327 (3.49). − $[\alpha]_D^{20}$ = +14.6 (*c* = 0.27 in MeOH). − CD (MeOH): $\lambda_{extr.}$ ($[\Theta]^{22}$) = 208 nm (−17521), 214 (−24508), 228 (38223), 243 (382), 278 (−57), 312 (2677). − ^{1}H NMR (500 MHz, [D_6]acetone): δ = 1.51, (m, 6-H_a), 2.24 (ddd, $J_{7a,7b}$ = 12.5, $J_{7a,6b}$ = 6.0, $J_{7a,6a}$ = 2.5 Hz, 7-H_a), 2.32 (dddd, $J_{6a,6b}$ = 12.0, $J_{6b,7b}$ = 7.0, $J_{7a,6b}$ = 6.0, $J_{6b,5}$ = 2.5 Hz, 6-H_b), 3.19 (dt, $J_{7a,7b}$ = 12.5, $J_{6b,7b}$ = 7.0 Hz, 7-H_b), 4.34 (d, $J_{2,3}$ = 7.0 Hz, 2-H), 4.34 (br, OH), 4.44 (dt, $J_{2,3}$ = 7.0 Hz, 3-H), 4.44 (br, OH), 4.84 (m, 5-H), 5.41 (s, 8a-OH), 5.97 (dd, $J_{3,4}$ = $J_{4,5}$ = 2.0 Hz, 4-H), 6.74/6.76 (dd, J = 6.5, 1.5 Hz, 2′-H/7′-H), 7.30−7.37 (m, 3′-H, 6′-H, 4′-H, 5′-H). − ^{13}C NMR (125.7 MHz, [D_6]acetone): see Table 3. − EI MS (70 eV): *m/z* (%) = 370 (36) [M^+] (high resolution calcd. for $C_{20}H_{18}O_7$ 370.1041, found 370.1041), 352 (20) [$M^+ - H_2O$], 334 (3)[352^+-H_2O], 200 (100), 160 (60) [$C_{10}H_6(OH)_2^+$], 115 (34).

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[20] Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publications no. CCDC-142793 (**8**) and -142794 (**12**). Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [Fax: (internat.) + 44-1223/336-033; E mail: deposit@ccdc.cam.ac.uk].
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