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Metabolites from the Endophytic Fungus *Nodulisporium* sp. from *Juniperus cedre*[‡]

Jingqiu Dai,[a] Karsten Krohn,*[a] Ulrich Flörke,[a] Siegfried Draeger,[b] Barbara Schulz,[b] Attila Kiss-Szikszai,[c] Sándor Antus,[d] Tibor Kurtán,[d] and Teunis van Ree[e]

Dedicated to Prof. Hartmut Laatsch on the occasion of his 60th birthday

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Seven new metabolites, 3-hydroxy-1-(2,6-dihydroxyphenyl)-butan-1-one (**1**), 1-(2-hydroxy-6-methoxyphenyl)butan-1-one (**3**), 2,3-dihydro-5-methoxy-2-methylchromen-4-one (**6**), the dimeric naphthalenes nodulisporin A (**9**) and B (**10**), and the first naturally occurring dimeric indanone, nodulisporin C (**12**), as well as (4*E*,6*E*)-2,4,6-trimethylocta-4,6-dien-3-one (**13**) were isolated together with ten known compounds (**2**, **4**, **5**, **7**, **8**, **11**, **14**–**17**) from the culture extract of the endophytic fungus *Nodulisporium* sp. from *Juniperus cedre* from Gomera Island. The structure of dictafolin-A, previously erroneously assigned as structure **6**, is not identical with 2,3-dihydro-5-methoxy-2-methylchromen-4-one, isolated in this investigation. The structures of new compounds were determined by spectroscopic methods (mainly extensive 1D and 2D NMR experiments and mass spectral measurements) and X-ray single crystal analysis. All but one of the thirteen tested compounds exhibit herbicidal, antifungal and/or antibacterial activities.

Introduction

Endophytic fungi have been proven to be a rich source of new biologically active natural products because a) as a group they represent a relatively untapped ecological source and b) because their secondary metabolism is particularly active due to their metabolic interactions with their hosts.[1]

In the course of our search for biologically active compounds from endophytic fungi, we found that the crude ethyl acetate culture extract of *Nodulisporium* sp. (internal strain No. 7080) showed antifungal, antibacterial, and algicidal activities. In a further bioassay guided fractionation of the ethyl acetate fraction, we isolated seven new compounds and ten known metabolites (Figure 1). In this paper, we describe their isolation, structure elucidation by spectroscopic methods, and their antifungal, antibacterial and algicidal bioactivities.

Results and Discussion

The structures of compounds **2**, **4**, **5**, **7**, **8**, **11**, and **14**–**17** isolated from *Nodulisporium* sp. 7080 were identified by comparing their NMR spectroscopic data with those reported in the literature: 1-(2,6-dihydroxyphenyl)butan-1-one (**2**),[2,3] 5-hydroxy-2-methyl-4*H*-chromen-4-one (**4**),[2] 2,3-dihydro-5-hydroxy-2-methylchromen-4-one (**5**),[4] 8-methoxynaphthalen-1-ol (**7**),[5] 1,8-dimethoxynaphthalene (**8**),[5] daldinol (**11**),[6] (4*R*,5*S*,6*S*)-6-[(*E*)-but-2-en-2-yl]-tetrahydro-4-hydroxy-3,3,5-trimethylpyran-2-one (helicascolide A, **14**),[7] the eudesmane derivative (1*S*,4*S*,4a*S*,6*R*,8a*R*)-decahydro-1,4-dimethyl-6-(prop-1-en-2-yl)naphthalen-1-ol (**15**), previously isolated from unripe fruits of *Ocotea corymbosa*,[8] ergosterol (**16**),[9] and 5α,8α-epidioxyergosterol (**17**).[10]

The optically active new compound **1** ($[\alpha]_D^{25}$ = –12.0) was obtained as colorless crystals with the molecular formula $C_{10}H_{12}O_4$, as deduced from HREIMS spectral and ^{13}C NMR spectroscopic data. The IR spectra show strong absorptions for hydroxy groups (3405 cm^{-1}), while the ^1H NMR spectrum (Table 1) exhibits the presence of one methyl group (δ = 1.26 ppm), one proton on oxygenated C

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[a] Department of Chemistry, University of Paderborn, Warburger Strasse 100, 33098 Paderborn, Germany
Fax: +49-5251-60-3245
E-Mail: karsten.krohn@uni-paderborn.de
[b] Institut für Mikrobiologie, Technische Universität Braunschweig, Spielmannstraße 7, 31806 Braunschweig, Germany
[c] Research Group for Carbohydrates of the Hungarian Academy of Sciences, P. O. Box 55, 4010 Debrecen, Hungary
[d] Department of Organic Chemistry, University of Debrecen, Egyetem tér 1, 4010 Debrecen, Hungary
[e] Department of Chemistry, University of Venda, Thohoyandou, 0950 South Africa

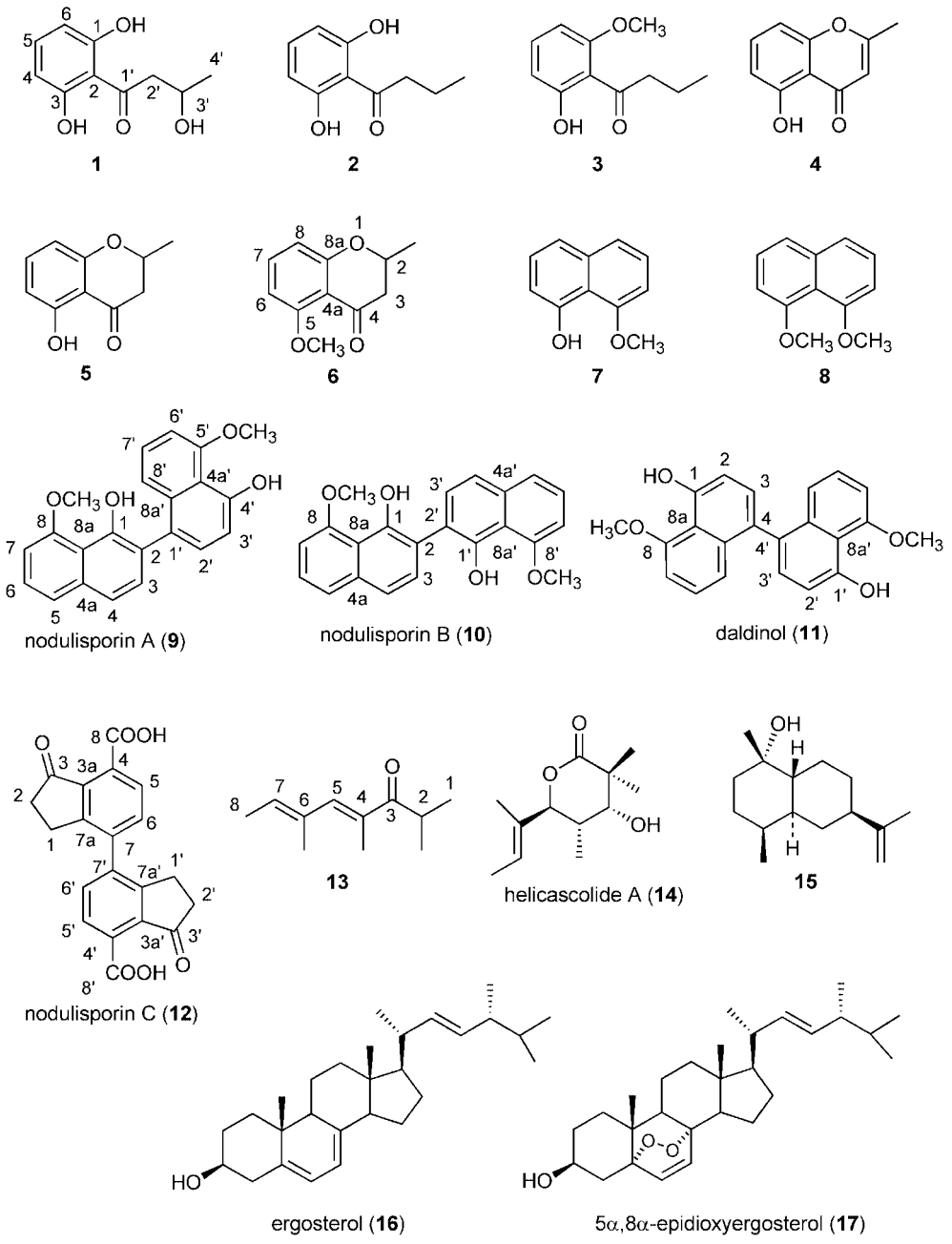


Figure 1. Structures of compounds isolated from the culture extract of *Nodulisporium* sp.

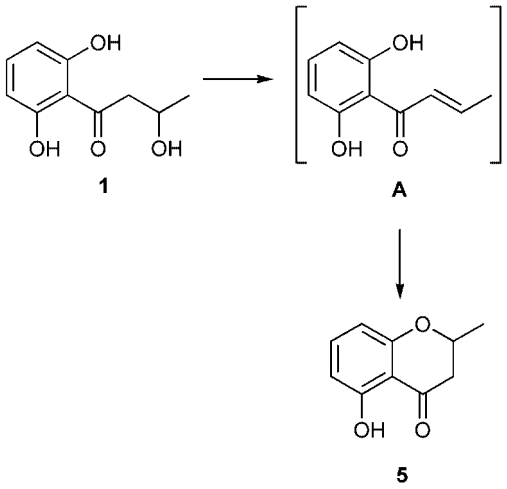
atoms (δ = 4.40, m), and three aromatic methines [δ = 6.36 (2 H), 7.20], respectively. The ^{13}C NMR spectrum of **1** (Table 1) shows signals for 10 C atoms, and the DEPT spectrum indicates the presence of one methyl, one methylene, four methines and four quaternary carbon atoms. Analysis of the coupling pattern of the ^{1}H NMR spectrum shows the presence of three vicinal protons on a benzene nucleus. The remaining substituents are supposed to be in a symmetrical arrangement because the usual *meta* coupling is missing (see Table 1). The low-field signal at (δ = 205.4 ppm) can be assigned to the carbon atom of a carbonyl group and the signal at δ = 4.40 indicates the presence of an oxygen bound to a carbon atom. This, in conjunction with the HMBC correlations, enabled the construction of the 3-hydroxybutan-1-one side chain attached to the aromatic nucleus. The remaining substituents are assumed to be hydroxy groups based on the chemical shift of the relevant carbon atom in the ^{13}C NMR spectrum. The symmetric substitution pattern enabled the structure elucidation of compound **1** as 3-hydroxy-1-(2,6-dihydroxyphenyl)butan-1-one (Figure 1). This compound is new as a natural product, although the absolute configuration of the stereogenic center in the side chain has not been established yet.

The optically inactive compound **3**, isolated as a colorless gum, has the molecular formula $C_{11}H_{14}O_3$, as deduced from HREIMS and ^{13}C NMR spectroscopic data. The 1D (^{1}H,^{13}C/DEPT) NMR spectra of **3** (Table 1) are closely related to those of **2** except for the appearance of a C-1-meth-

Table 1. ^1H and ^{13}C NMR assignments of compounds **1**–**3**[a] (125 and 500 MHz, $CDCl_3$).

Position	**1**		**2**		**3**	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	162.1		161.7		165.1	
2	110.3		110.5		111.6	
3	162.1		161.7		161.7	
4	107.1	6.36 (d, 8.2)	108.8	6.43 (d, 8.1)	111.2	6.61 (dd, 8.3, 0.8)
5	135.8	7.20 (t, 8.2)	136.3	7.26 (t, 8.1)	136.0	7.36 (t, 8.3)
6	107.1	6.36 (d, 8.2)	108.8	6.43 (d, 8.1)	101.6	6.43 (dd, 8.3, 0.8)
1′	205.4		208.7		208.2	
2′	53.3	3.30 m	47.1	3.17 (t, 7.3)	47.3	3.07 (t, 7.2)
3′	63.9	4.40 m	18.3	1.79 (q, 7.3)	18.3	1.77 m
4′	22.2	1.26 (d, 6.3)	14.3	1.04 (t, 7.3)	14.3	1.02 (t, 7.3)
1-OCH_3					56.0	3.90 s

[a] Chemical shift values are given in ppm, and *J* values (in Hz) are presented in parentheses.



Scheme 1. Biosynthetic connection of the open-chain form **1** and the cyclic form **5** via elimination product **A**.

oxy signal and the presence of a small *meta* coupling of the aromatic protons, indicating the loss of symmetrical substitution. Compound **3** can be derived from the dihydroxy derivative **2** by monomethylation and thus can be assigned to 1-(2-hydroxy-6- methoxyphenyl)butan-1-one, previously known only as a synthetic derivative.[2] The open-chain new benzophenone derivatives **1**–**3** are closely related to the known chromenone **4**[2] or chromanone **5**.[4] They might be biosynthetic precursors or shunt products via the enone **A** of their cyclic analogues **4** and **5** as depicted in Scheme 1.

Compound **5** is known as a natural product but insufficiently characterized.[4] It was originally isolated as a semisolid substance, crystallisation from petroleum ether at low temperatures affords long colorless needles with m.p. 33–34 °C. The pure compound has a small positive optical rotation value of $[\alpha]_D^{25} = +6$. The absolute configuration was determined in the context of the methyl ether **6** (see below).

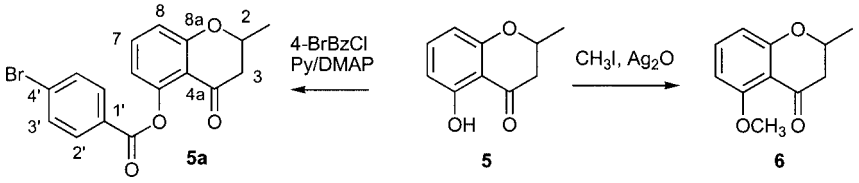
The optically active compound **6** has the molecular formula $C_{11}H_{12}O_3$ as deduced from HREIMS and ^{13}C NMR spectroscopic data. The 1D (^1H,^{13}C/DEPT) NMR spectra of **6** (Table 2) are similar to those of the known 2,3-dihydro-5-hydroxy-2-methylchromen-4-one (**5**),[4] except for the appearance of the signal of an additional methoxy group. The 2D (COSY, HMQC, HMBC) NMR spectra (in particular the HMBC correlations, see Table 2) allow the unambiguous assignment of the position of the methyl group to the phenol methyl ether function at C-5 and also the assembly of the other carbon atoms as shown in structure **6** (Table 2). Therefore, compound **6** is identical with 2,3-dihydro-5-methoxy-2-methylchromen-4-one. Additional proof of the structure came from a methylation experiment of phenol **5** that afforded the methyl ether **6** almost quantitatively, identical in all respects with the compound isolated from the cultivation broth (Scheme 2). Similarly, the phenol was benzoylated to give **5a**, initially with the intention to determine the absolute configuration of the chromanone. Surprisingly, after two recrystallizations, the 4-bromobenzoate **5a** proved to be racemic. The X-ray data gave valuable indications about the conformation of the pyranone ring (see Figure 2).

A literature survey revealed that structure **6** can be assigned to dictafolin-A, a compound isolated from the root bark of *Dictamnus angustifolius*.[11] However, the published NMR spectroscopic data, measured in $CDCl_3$, did not match those of our compound **6** (see comparison in Table 2, the strongly deviating chemical shift values are printed in

Table 2. NMR spectroscopic data for compound **6** (125 and 500 MHz, $CDCl_3$)[a] in comparison with those published for dictafolin-A (100 and 400 MHz).[11]

Position	**6**, δ_C	HMBC	Dictafolin-A, δ_C	**6**, δ_H	Dictafolin-A, δ_H
2	73.7		74.2	4.57 m	4.50 (dqd, 12.8, 6.4, 3.2)
2-Me	20.8		20.7	1.50 (d, 6.3)	1.39 (d, 6.4)
3a	46.0		*36.1*	2.68 (dd, 17.1, 12.0)	*2.27* (dd, 16.4, 12.8)
3b				2.65 (dd, 17.1, 3.7)	*2.95* (dd, 16.4, 3.2)
4	191.2	H-2, H-3	186.8		
4a	111.2	H-3, H-6, H-8	113.8		
5	163.3	5-OMe, H-6, H-7	161.2		
5-OMe	56.2		56.2	3.92 (s)	3.86 (s)
6	110.0		110.9	6.58 (dd, 8.3, 0.7)	6.88 (d, 7.2)
7	135.8		134.3	7.39 (t, 8.3)	7.49 (dd, 8.8, 7.2)
8	103.7		*119.1*	6.52 (dd, 8.3, 0.7)	*7.04* (d, 8.8)
8a	160.7	H-7, H-8	*141.9*		

[a] Chemical shift values are in ppm, and *J* values (in Hz) are presented in parentheses. Strongly deviating chemical shifts are printed in *italics*.



Scheme 2. Benzoylation of phenol **5** to the 4-bromobenzoate **5a** and methylation to the naturally occurring methyl ether **6**.

italics). The data suggest that the structure of dictafolin-A needs to be reassigned. With the exception of one signal for the carbonyl group (δ = 186.8 ppm), all the other ^{13}C NMR spectroscopic data reported for dictafolin-A are in very close agreement with those of (–)-mellein methyl ether [or its enantiomer (+)-mellein methyl ether[12] also named (+)-ochracin[7]], in particular those strongly deviating from those recorded for **6** (see Table 2) are in complete agreement (reported data: δ = 36.1, 119.1, 141.9;[11] ref. data: δ = 36.1, 119.1, 141.8;[11] δ = 36.1, 119.1, 141.9;[12]). Our suggestion is that dictafolin-A, the structure of which is also incorrectly reported in the Chapman and Hall database, is identical with the (*S*)-(+)-ochracin[7] methyl ether because of the positive rotation reported for dictafolin-A.[11]

A CD study of **5** and **6** revealed that these compounds are not enantiopure and thus the separations of their enantiomers were carried out with HPLC by using a chiral stationary phase. The LC/CD and LC/UV chromatograms of compound **5** (Figure 3) monitored at 340 nm showed that the first eluted peak has only 6% enantiomeric excess. Similar results were obtained for compound **6**. Then their LC/CD spectra (Figure 4) were recorded on-line which allowed their configurational assignment on the basis of their high wavelength n→π* transition. Snatzke[13] established a relationship between the chirality of aryl ketones and their high wavelength n→π* Cotton effects (CEs) which was extended to flavanones[14] and 2-alkylchromanones.[15] According to this rule, 2-alkylchromanones of *P*-helicity (dihedral angle $\omega_{C8a,O1,C2,C3}$ is positive) or of the conformation shown in Figure 5 exhibit a positive n→π* CE (and a negative π→π* CE in the case of flavanones). Since the first-eluted enantiomer of both **5** and **6** has positive n→π* and negative π→π* CEs around 340 and 310 nm, respectively, their heterorings adopt *P*-helicity which implies *R* absolute configuration, provided that the methyl group is equatorially oriented. In fact, the X-ray data of the *p*-bromobenzoate of **5a** showed that the chromanone heteroring has an envelope conformation with torsion angles $\omega_{C8a,\ O1,C2,C3}$ +44.9(7)° and $\omega_{C5,C4a,C4,O}$ –1.3(7)°. The equatorial position of the methyl group is further in agreement with the large coupling constant of J = 12.8 Hz observed for the transdiaxial protons 2a-H and 3a-H (see Figure 5 and Table 2).

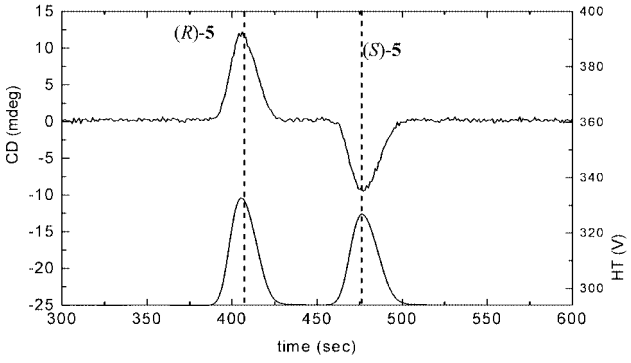


Figure 3. LC/CD (upper curve) and LC/UV (lower curve) chromatograms of chromanone **5** monitored at 340 nm [separation factor (α) 1.43 and resolution (R_s) 2.42 for (*S*)-**5**, *ee* = 6% for (*R*)-**5**]. For chromatographic protocols, see Exp. Sect.

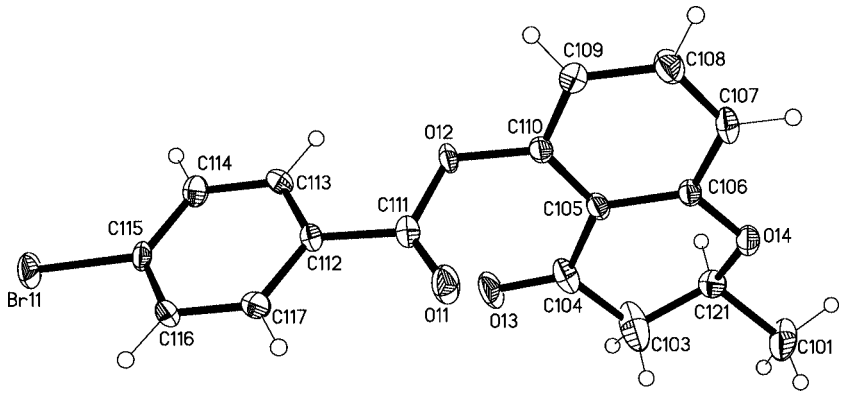


Figure 2. The molecular structure of **5a** showing one of the two independent molecules; the second molecule has identical geometry; only main the part of the disordered chromanone ring is shown (see Exp. Sect.). Displacement ellipsoids are shown at the 50% probability level.

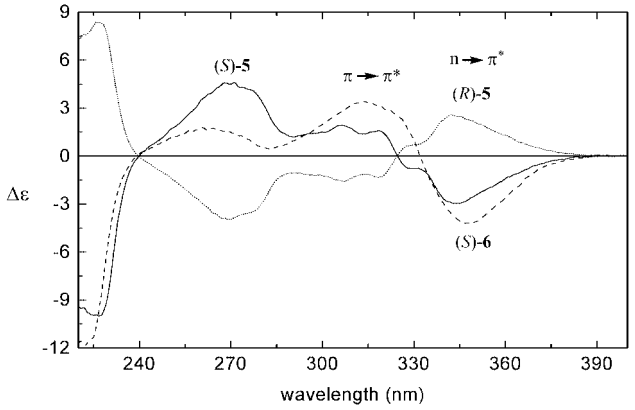


Figure 4. CD spectra of (*S*)-**5** (solid line), (*R*)-**5** (dotted line), and (*S*)-**6** (dashed line) in hexane/2-propanol, 9:1.

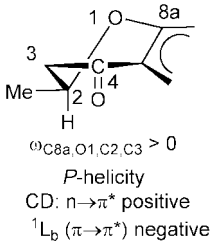


Figure 5. Preferred conformation of the heteroring of (*R*)-**5** and (*R*)-**6**.

The low enantiomeric excess of both compounds **5** and **6** demands an explanation. As suggested in Scheme 1, the cyclization of the open chain precursor **1** could occur via the enone **A**, using a Michael-type reaction. In principle, this reaction could happen non-enzymatically and the low enantioselectivity might be a result of chiral induction by the chiral environment in the cell.

The optically inactive compound **9** has the molecular formula $C_{22}H_{18}O_4$, as deduced from EIMS and ^{13}C NMR spectroscopic data. The 1D (^1H,^{13}C/DEPT) (Table 3) and 2D (COSY, HMQC, HMBC) NMR spectra of **9** are similar to those of **11** (see below) and a comparison with the data of the known 1-hydroxy-8-methoxynaphthalene (**7**)[5] suggests the presence of a dimeric naphthalene structure. Based on this assumption, the spectroscopic data were analyzed with respect to the location of the functional hydroxy and methoxy groups and the connection site of the two naphthalene rings. The HMBC spectrum of **9** shows ^{13}C-^1H long-range correlation signals between C-8 and H-7, H-6, and 8-OCH_3, C-5′ and H-6′, and H-7′ and 5′-OCH_3. This observation allows to locate the positions of the respective methoxy groups at C-8 and C-5′ and indirectly those of the remaining hydroxy groups at C-1 and C-4′. The position of the carbon–carbon linkage of the two naphthalene units was determined to be C-2 and C-1′ as seen from the HMBC correlation of C-2 with H-2′ and C-1′ with H-3. Therefore, the structure of a dimer as shown in formula **9** is assumed. Compound **9** is named nodulisporin A with reference to the producing fungus. The *ortho-meta* coupling with respect to the hydroxy groups of the subunit **7** is unambiguously confirmed by X-ray diffraction analysis of a single crystal of **9** as shown in Figure 6.

Compound **10** has the molecular formula $C_{22}H_{18}O_4$, as deduced from EIMS and ^{13}C NMR spectroscopic data, which are the same as found for **9**, suggesting an isomeric structure of **9**. The IR and ^1H NMR spectra also indicate the presence of a hydrogen-bonded phenolic hydroxy [3405 cm^{-1}; δ_H 9.77 (s)] and a methoxy [δ_H 4.06 (s)] group

Table 3. NMR data for compounds **9** and **10** (125 and 500 MHz, $CDCl_3$).[a]

	9			**10**		
Position	δ_C	δ_H	HMBC	δ_C	δ_H	HMBC
1	151.5		H-3	151.2		H-3
2	122.4		H-2′, H-3, H-4	120.4		H-3, H-4
3	131.2	7.28 (d, 8.4)		130.8	7.52 (d, 8.3)	
4	118.5	7.42 (d, 8.4)		118.4	7.41 (d, 8.3)	
5	121.9	7.51 (d, 7.7)		121.9	7.49 (d, 7.7)	
6	126.7	7.40 (t, 7.7)		125.5	7.35 (t, 7.7)	
7	104.2	6.86 (d, 7.7)		104.2	6.80 (d, 7.7)	
8	156.4		H-6, H-7,8-OCH_3	156.4		H-7, H-6, 8-OCH_3
4a	136.3		H-3, H-4, H-5, H-6	136.3		H-3, H-4, H-5, H-6
8a	115.2		H-4, H-5, H-7	115.3		H-4, H-5, H-7
8-OCH_3	56.1	4.06 (s)		56.1	4.06 (s)	
1′	127.8		H-3, H-2′, H-3′, H-8′			
2′	129.6	7.34 (d, 7.9)				
3′	110.3	7.01 (d, 7.9)				
4′	154.1		H-2′, H-3′			
5′	156.4		H-6′, H-7′, 5′-OCH_3			
6′	104.3	6.83 (d, 7.5)				
7′	125.4	7.25 (t, 7.5)				
8′	120.7	7.42 (d, 7.5)				
4a′	115.0		H-3′, H-6′, H-8′			
8a′	135.0		H-2′, H-7′, H-8′			
5′-OCH_3	56.1	4.06 s				

[a] Chemical shift values are in ppm, and *J* values (in Hz) are presented in parentheses.

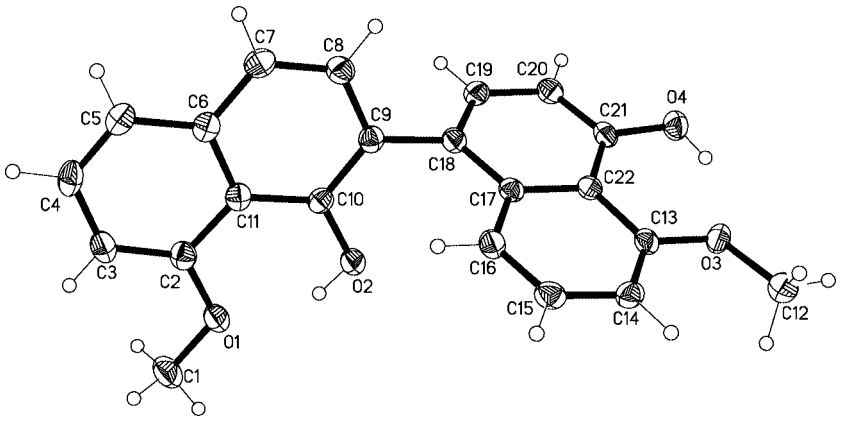


Figure 6. The molecule of nodulisporin A (**9**) in the crystal. Displacement ellipsoids are shown at the 50% probability level.

(see Table 3). Both the ^{1}H NMR and the ^{13}C NMR spectra show good agreement with the first set of signals for dimer **9** (Table 3). However, in contrast to the spectrum of **9**, there is only one set of signals; this observation supports the assumption of a symmetric structure. In the HMBC spectrum of **10**, ^{13}C-^{1}H long-range correlation signals appear between C-8 and H-7, H-6, and 8-OCH$_3$; between C-1 and 1-OH and H-3, as well as between C-4a and H-4 and H-5. These findings confirm the position of the substituents. With the same type of substitution as in **7** and **9**, it had to be decided whether the hydroxy groups were in *ortho* or *para* position with respect to the coupling site. The ^{1}H-^{1}H COSY and HMQC spectra of **10** prove the presence of the coupled proton systems CH(3)–CH(4)- and CH(5)–CH(6)–CH(7) and consequently confirm the positions of the substituents at C-1 and C-8. In conjunction with the symmetric structure an *ortho* coupling of the two monomeric naphthalenes **7** is assumed. The dimer **10** is identical with nodulisporin B as shown in Figure 1.

For compound **11** we also propose a dimeric structure of monomer **7** which is deduced from NMR spectra (only one set of signals) and the mass spectrum. This assignment was performed in analogy as described for **10**, resulting in the elucidation of a *para*-*para* coupling product **11**. A literature survey revealed that this dimer is a known natural product, named daldinol.[6,16] Interestingly, in both papers, only this *para*-*para* coupling product **11** is described to occur in the Ascomycete *Daldinia concentrica*, whereas the other possible *ortho*-*para* (**9**) and *ortho*-*ortho* (**10**) combinations are only produced by the currently investigated endophytic fungus *Nodulisporium* sp.

Compound **12** was obtained as an amorphous powder with the molecular formula $C_{20}H_{14}O_6$, as deduced from the mass spectrum with m/z = 350 [M$^+$] and the ^{13}C NMR spectroscopic data. The ^{1}H NMR spectrum (Table 4) shows signals of two olefinic protons (δ = 8.71 and 7.32 ppm, d, J = 9.2 Hz). The ^{13}C NMR spectrum of **12** (Table 4) shows signals for 10 C atoms, and the DEPT spectra indicate the presence of two methylene, two methine groups and six quaternary carbon atoms. This is only half of the number of the carbon signals expected from the molecular formula and again indicates a symmetrically substituted dimer, similar to that found for **10** and **11**. In the HMBC spectrum of **12**, ^{13}C-^{1}H long-range correlation signals are visible between C-3 and H-2 and H-1, between C-4 and H-5, H-6, between C-7 and H-1, H-5, H-6, as well as between C-8 and H-5. These data in conjunction with the *ortho*-coupling of H-5 and H-6 (J = 9.2 Hz) allow to determine the connection of the two aromatic rings between C-7 and C-7′ and the monomeric units as being an indanone system (see structure **12** in Figure 1).

Table 4. NMR spectroscopic data for compound **12** (125 and 500 MHz, $CDCl_3$).

Position	δ_C	HMBC	δ_H
1	24.7		3.46 m
2	36.6		3.04 m
3	203.8	H-1, H-2	
3a	130.2	H-1, H-2, H-5	
4	111.2	H-5, H-6	
5	131.8		7.32 (d, 9.2)
6	118.9		8.71 (d, 9.2)
7	121.9	H-1, H-5, H-6	
7a	128.8	H-1, H-2, H-6	
8	162.4	H-5	
COOH			13.2 s

The unusual high-field chemical shift of the signal due to the carboxylic group at C-8 is explained by the strong chelation with the neighboring C-3 carbonyl group. The new natural product is named nodulisporin C. This is the first time that dimeric symmetrical indanone systems are found in natural products. Their biosynthesis has not yet been elucidated, but may be connected with that of the dimeric naphthalenes **9**–**11** by an oxidative-type ring contraction.

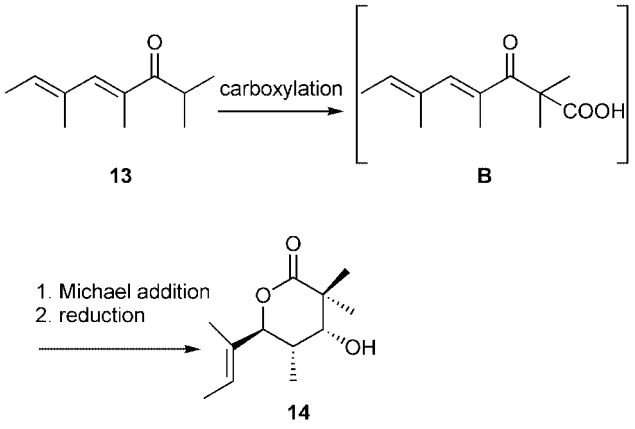
The optically inactive compound **13** was isolated as a colorless gum and the molecular formula $C_{11}H_{18}O$ was deduced from the combined HREIMS and ^{13}C NMR spectroscopic data. The ^{1}H NMR spectrum shows the presence of five methyl groups, two of which cause singlets (δ = 1.90 and 1.97) and three give doublets [δ = 1.13 (d, J = 6.8 Hz, 2 H) and 1.80 (d, J = 6.9 Hz)] (Table 5). Two alkene protons resonate at δ = 5.78 (q) and 6.98 (s). The ^{13}C NMR spectrum of **13** shows signals for 11 carbon atoms, and the

DEPT spectrum indicates the presence of five methyl, three methines and three quaternary carbon atoms. These data, in combination with the HMBC spectrum, are compatible with 2,4,6-trimethylocta-4,6-dien-3-one (**13**), a new natural product, shown in Figure 1. Again, it is interesting to see the biogenetic connection of the dienone **13** and helicascolide A (**14**),[7] fortunately isolated from the same culture broth. Simple carboxylation at the activated site α to the carbonyl group of **13** followed by Michael-type addition and ketone reduction would convert the open-chain dienone to the cyclic helicascolide A (**14**) as shown in Scheme 3.

Table 5. NMR data for compound **13** (125 and 500 MHz, $CDCl_3$).[a]

Position	δ_C	HMBC	δ_H
1	20.1		1.13 (d, 6.8)
2	34.2		3.38 m
3	207.6	H-1, H-2, H-5, 2-Me, 4-Me	
4	133.8	H-2, H-5, 4-Me	
5	142.9		6.98 s
6	133.6	H-5, H-7, H-8, 6-Me	
7	131.4		5.78 (q, 6.9)
8	13.8		1.80 (d, 6.9)
6-Me	14.5		1.90 s
4-Me	16.6		1.97 s
2-Me	20.1		1.13 (d, 6.8)

[a] Chemical shift values are in ppm, and *J* values (in Hz) are presented in parentheses.



Scheme 3. Hypothetical conversion of the open chain dienone **13** to the cyclic helicascolide A (**14**).

In our studies, metabolites **2**–**13**, particularly compounds **2** and **3**, were biologically active against some or all of the test organisms at a concentration of 0.25 mg/filter disc: the gram-positive bacterium, *Bacillus megaterium*, the fungi *Microbotryum violaceum* and *Septoria tritici*, and the green alga, *Chlorella fusca*, as shown in Table 6.

In summary, our attempt to identify the entire set of compounds from the fungal culture broth, including minor constituents, revealed the close biosynthetic connection of the open-chain (**1**–**3**, **13**) and cyclic derivatives (**4**–**6**), as well as diversification via dimerization of the monomer **7** to the three isomeric dimers **9**–**11**, and chemical modification to the structurally unique indanone dimer **12**.

Table 6. Biological activities of compounds **1**–**13** in agar diffusion tests; radius of zone of inhibition in mm. Bm = *Bacillus megaterium*, Mb = *Microbotryum violaceum*, St = *Septoria tritici*, Cf = *Chlorella fusca*. PI = partial inhibition within the cleared zone.

Code No	Bm	Mb	St	Cf
1	0	0	0	0
2	15	3	17	20
3	0	15	6	11
4	0	1	7	7
5	0	12	0	1
6	0	10 PI	7 PI	12
7	0	6	0	5
8	0	5	5	6
9	0	7	0	0
10	0	6	0	6
11	0	0	0	5
12	0	5	0	0
13	0	8	7 PI	7

Experimental Section

General Experimental Procedures: For general methods and instrumentation see ref.[17], and for microbiological methods and conditions of culture see ref.[18]. Melting points were determined on a Gallenkamp micro-melting point apparatus and are uncorrected. NMR spectra were run on Bruker-200 and -500 NMR spectrometers with TMS as internal standard. EIMS were obtained on a MAT 8200 mass spectrometer.

LC/CD Analysis: HPLC separation was carried out with a Chiralcel OD column (5 μm; 250 × 4.6 mm) for the enantiomers of **5** [separation factor (α) 1.43, resolution (R_s) 2.42 and retention time (t_r) 8.00 min for (*S*)-**5**, t_r 6.80 min for (*R*)-**5**] and with Chiralpak AS-H column (5 μm, 150 × 4.6 mm) for the enantiomers of **6** [separation factor (α) 1.20, resolution (R_s) 1.78 and retention time (t_r) 9.80 min for (*S*)-**6**, t_r 8.60 min for (*R*)-**5**], eluted at 0.8 mL/ min with hexane/ 2-propanol, 9:1. The LC/CD and LC/UV traces were recorded on-line at 340 nm with a Jasco J-810 CD spectropolarimeter equipped with an HPLC flow cell. The on-line CD and UV spectra (220–400 nm) were recorded simultaneously at the maxima of the UV peaks where the flow was stopped.

Tests for Biological Activity: Compounds **1**–**13** were dissolved in methanol/acetone (1:1) at a concentration of 5 mg /mL. 50 μL of the solution were pipetted onto a sterile filter disc (Schleicher & Schuell, 9 mm), which was placed onto an appropriate agar growth medium and subsequently sprayed with a suspension of the respective test organism. The test organisms were the bacterium *Bacillus megaterium* (NB medium), the fungi *Microbotryum violaceum* and *Septoria tritici* (MPY medium) and the alga *Chlorella fusca* (MPY).

Extraction and Isolation: The fungus *Nodulisporium* sp. 7080, belonging to the *Xylariaceae*, was isolated from twigs of the plant *Juniperus cedre* and was cultivated at room temperature for 21 days on biomalt solid agar media. Twelve liters of culture medium including fungus were then extracted with ethyl acetate to afford 6 g of a residue on removal of solvent under reduced pressure. The extract was separated into three fractions by column chromatography on silica gel (350 g), using gradients of dichloromethane/ethyl acetate (85:15, 50:50, 0:100). The gummy crude extract containing compounds **3**, **4**, **5**, **6**, **7**, **8**, **13**, **15** and **16** was obtained from the fraction eluted with dichloromethane/ethyl acetate (85:15), and **1**, **2**, **9**, **10**, **11**, **12**, **14** and **17** from the fraction eluted with dichloromethane/ethyl acetate (50:50). Gel filtration (Sephadex, LH-20) followed by silica gel column chromatography with hexane/ethyl ace-

tate (1:1) gave 15 mg of **1**, 10 mg of **2**, 18 mg of **3**, 10 mg of **4**, 40 mg of **5**, 20 mg of **6**, 20 mg of **7**, 32 mg of **8**, 10 mg of **9**, 15 mg of **10**, 11 mg of **11**, 1.5 mg of **12**, 13 mg of **13**, 15 mg of **14**, 10 mg of **15**, 30 mg of **16**, and 20 mg of **17**.

1-(2,6-Dihydroxyphenyl)-3-hydroxybutan-1-one (1): Colorless crystals, m.p. 150 °C. $[\alpha]_D^{25}$ = –12.0 (c = 0.005, CH_2Cl_2). IR (KBr, film): $\tilde{\nu}_{max}$ = 3405, 1625, 668 cm^{-1}. UV ($CHCl_3$): λ_{max} = 275 nm. For ^{1}H and ^{13}C NMR spectroscopic data see Table 1. EIMS: *m*/*z* 196 [M$^+$] (65), 163 (100), 137 (60), 108 (20), 81 (10). HREIMS: *m*/*z* 196.0725 (calcd. for $C_{10}H_{12}O_4$ 196.0735).

1-(2,6-Dihydroxyphenyl)propan-1-one (2): Amorphous powder. IR (KBr, film): $\tilde{\nu}_{max}$ = 3265, 1631, 1237 cm^{-1}. For ^{1}H and ^{13}C NMR spectroscopic data see Table 1. EIMS: *m*/*z* 180 [M$^+$] (40), 137(10), 97 (15), 81 (30), 28 (100).

1-(2-Methoxyphenyl)butan-1-one (3): Colorless gum. IR (KBr, film): $\tilde{\nu}_{max}$ = 1755, 1625, 1243 cm^{-1}. For ^{1}H and ^{13}C NMR spectroscopic data see Table 1. EIMS: *m*/*z* 194 [M$^+$] (100), 151 (30), 108 (25). HREIMS: *m*/*z* 194.0942 (calcd. for $C_{11}H_{14}O_3$ 194.0943).

5-Hydroxy-2-methyl-2,3-dihydrochromen-4-one (5): M.p. 33–34 °C from petroleum ether. $[\alpha]_D^{25}$ = +6 [c = 0.19, CH_2Cl_2, 6% *ee* for (*R*)-enantiomer as determined by HPLC]. UV (MeCN): λ_{max} (ε 10^4) = 346 (0.30), 269 (0.97), 220 sh (1.47), 203 nm sh (2.58). CD data recorded on-line in hexane/2-propanol 9:1 for (*R*)-**5**: λ_{max} ($\Delta\varepsilon$) = 360 sh (1.27), 342 (2.57), 327 sh (0.61), 318 (–1.35), 307 (–1.58), 277 sh (–3.49), 269 (–3.96), 226 nm (8.38).

(*S*)-5: 364 sh (–1.09), 343 (–2.96), 328 sh (–0.76), 319 (1.56), 306 (1.93), 277 sh (4.05), 271 (4.61), 227 nm (–9.99).

2,3-Dihydro-5-methoxy-2-methylchromen-4-one (6): M.p. 89–90 °C. $[\alpha]_D^{25}$ = +6 [c = 0.44, CH_2Cl_2, 6% *ee* for (*R*)-enantiomer as determined by HPLC]. UV (MeCN): λ_{max} (ε 10^4) = 326 (0.38), 271 sh (0.76), 263 (0.93), 215 (2.19), 192 nm (3.97). IR (KBr, film): $\tilde{\nu}_{max}$ = 2924, 2365, 1744, 1268, 756 cm^{-1}. For ^{1}H and ^{13}C NMR spectroscopic data see Table 2. EIMS: *m*/*z* 192 [M$^+$] (90), 163 (50), 150 (100), 107 (60), 57 (50). HREIMS: *m*/*z* 192.0786 (calcd. for $C_{11}H_{12}O_3$ 192.0786). CD data recorded on-line in hexane/2-propanol 9:1 for (*S*)-**6**: λ_{max} ($\Delta\varepsilon$) = 365 sh (–1.62), 347 (–4.21), 322 sh (2.85), 314 (3.38), 260 (1.79), 219 nm (–11.89).

Methylation of 5 to 6: A solution of phenol **5** (89 mg, 0.5 mmol) in dichloromethane (2 mL) and methyl iodide (1 mL) was treated with freshly precipitated silver oxide (232 mg, 1 mmol) and the mixture was stirred for 8 h at 21 °C. The suspension was filtered, the solvent removed at reduced pressure, and the residue crystallized from pentane to afford 88 mg (92%) of methyl ether **6**, m.p. 89–90 °C (for other data see above).

4-Bromobenzoylation of 5 to 5a: A solution of phenol **5** (89 mg, 0.5 mmol) in pyridine (2 mL) was treated with 4-bromobenzoyl chloride (228 mg, 1 mmol) and 4-(dimethylamino)pyridine (DMAP) (1 mg). The mixture was stirred for 3 h at 21 °C and then poured into cold 2 N HCl (5 mL) and stirred for 1 h to hydrolyze the excess 4-bromobenzoyl chloride. The aqueous phase was extracted three times with dichloromethane (5 mL), the organic phase was washed with 2 N sodium hydrogen carbonate solution (5 mL) to remove 4-bromobenzoic acid, and dried with Na_2SO_4. Optical rotation of crude product: $[\alpha]_D^{25}$ = +26.3 (c = 0.30, CH_2Cl_2). The solvent was removed at reduced pressure and the residue was crystallized twice from diethyl ether (1 mL) to afford colorless prisms (146 mg, 81%); m.p. 131.5–132.5. IR (KBr, film): $\tilde{\nu}_{max}$ = 2924, 2365, 1744, 1268, 756 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.27 (d, J = 7.8 Hz, 6 H, 2-CH_3), 2.59 (dd, J = 3.1, 16.6 Hz, 1 H, 3e-H), 2.68 (dd, 1 H, J = 12.4, 16.6 Hz, 2a-H), 4.65 (m, 1 H, 2-H), 6.80 (dd, J = 1.0, 8.2 Hz, 1 H, 6/8-H), 6.98 (dd, J = 1.0, 8.2 Hz, 1 H, 8/6-H), 7.52 (7, 1 H, J = 8.2 Hz 7-H), 7.68 (d, J = 8.7 Hz, 2 H, 3′, 5′-H) 8.10 (d, J = 8.7 Hz, 2 H, 2′, 6′-H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 20.8 (q, 2-*C*H$_3$), 45.2 (t, C-3), 74.1 (d, C-2), 113.7 (q, C-4a), 115.9 and 116.3 (each d, C-6 and C-8), 128.6 and/or 128.8 (each q, C-1′ and C-4′), 131.88 and 131.93 (each d, C-2′ and/or C-3′, each 2 carbons), 135.6 (d, C-7), 150.1 and 162.8 (8a and/or C-9), 164.6 (s, ester C=O), 190.5 (s, C-4) ppm. EIMS: *m*/*z* 362/361 [M$^+$] (46), 187/185 (100), 157/155 (46), 107 (18), 76 (30).

Crystal Structure Determination of *p*-Bromobenzoate (5a): [19] $C_{17}H_{13}BrO_4$, Mr = 361.2, monoclinic, space group P 2$_1$/c, a = 7.3735(9), b = 11.9277(14), c = 33.776(4) Å, β = 90.143(2)°, V = 2970.6(6) Å^3, Z = 8, D_x = 1.615 g/cm^3, F(000) = 1456, T = 120(2) K. Bruker-AXS SMART APEX,[20] graphite monochromator, λ(Mo-K_α) = 0.71073 Å, μ = 2.783 mm^{-1}, colorless crystal, size 0.42 × 0.21 × 0.19 mm^3, 29357 intensities collected 1.8 < θ < 28.1°, –9 < h < 9, –15 < k < 15, –41 < l < 44. Structure solved by direct methods,[20] full-matrix least-squares refinement[20] based on F^2 and 397 parameters, all but H atoms refined anisotropically, H atoms refined with riding model on idealized positions with U = 1.5 U_{iso}(methyl-C) or 1.2 U_{iso}(C). There are two independent but geometrically identical molecules A and B per asymmetric unit. Each of it shows disorder of the chromanone ring over two sites with occupation factors 0.63(1)/0.37(1) for C121/C122 of A and 0.35(1)/0.65(1) for C221/C222 of B. Refinement converged at $R_1[F > 4\sigma(F)]$ = 0.052, $wR_2(F^2$, all data) = 0.118, S = 0.940, max(δ/σ) < 0.001, min./max. height in final ΔF map –0.50/0.95 e/Å^3. Figure 2 shows the molecular structure.

2-(4-Hydroxy-5-methoxynaphthalen-1-yl)-8-methoxynaphthalen-1-ol [Nodulisporin A (9)]: Colorless crystals, m.p. 250 °C. IR (KBr, film): $\tilde{\nu}_{max}$ = 3379, 2934, 2841, 1750, 1408, 1253, 1077, 818 cm^{-1}. UV ($CHCl_3$): λ_{max} = 307, 323, 337 nm. For ^{1}H and ^{13}C NMR spectroscopic data see Table 3. EIMS: *m*/*z* 346 [M$^+$] (70), 176 (65), 149 (100), 71 (55), 57 (80).

Crystal Structure Determination of Nodulisporin A (9):[19] $C_{22}H_{18}O_4$, Mr = 346.4, orthorhombic, space group *Pbca*, a = 13.5817(7), b = 10.3048(5), c = 24.4013(11) Å, V = 3415.1(3) Å^3, Z = 8, D_x = 1.347 g/cm^3, F(000) = 1456, T = 120(2) K. Bruker-AXS SMART APEX,[20] graphite monochromator, λ(Mo-K_α) = 0.71073 Å, μ = 0.092 mm^{-1}, colorless crystal, size 0.30 × 0.20 × 0.03 mm^3, 27030 intensities collected 1.7 < θ < 28.0°, –17 < h < 17, –13 < k < 13, –32 < l < 24. Structure solved by direct methods,[20] full-matrix least-squares refinement[20] based on F^2 and 234 parameters, all but H atoms refined anisotropically, H atoms refined with riding model on idealized positions with U = 1.5 U_{iso}(methyl-C and OH) or 1.2 U_{iso}(C). Refinement converged at $R_1[F > 4\sigma(F)]$ = 0.044, $wR_2(F^2$, all data) = 0.086, S = 0.930, max(δ/σ) < 0.001, min./max. height in final ΔF map –0.28/0.23 e/Å^3. Figure 6 shows the molecular structure.

2-(1-Hydroxy-8-methoxynaphthalen-2-yl)-8-methoxynaphthalen-1-ol [Nodulisporin B (10)]: Yellow powder. $[\alpha]_D^{25}$ = +12.4 (c = 0.003, CH_2Cl_2). IR (KBr, film): $\tilde{\nu}_{max}$ = 3405, 1574, 1237, 1062, 751 cm^{-1}. UV ($CHCl_3$): λ_{max} = 275, 337 nm. For ^{1}H and ^{13}C NMR spectroscopic data see Table 3. EIMS: *m*/*z* 346 [M$^+$] (100), 173 (80), 144 (50).

Nodulisporin C (12): Amorphous powder. IR (KBr, film): $\tilde{\nu}_{max}$ = 2930, 2345, 1732, 1268 cm^{-1}; for ^{1}H and ^{13}C NMR spectroscopic data see Table 4. EIMS: *m*/*z* 350 [M$^+$] (10), 322 (20), 149 (50), 71 (70), 57 (100).

(4*E*,6*E*)-2,4,6-Trimethylocta-4,6-dien-3-one (13): Colorless gum. IR (KBr, film): $\tilde{\nu}_{max}$ = 2918, 2339, 1656, 1294, 767 cm^{-1}. For ^{1}H and

^{13}C NMR spectroscopic data see Table 5. EIMS: *m/z* 166 [M^+] (40), 151 (90), 123 (100), 67 (50), 55 (55), 43 (40). HREIMS: *m/z* 166.1339 (calcd. for $C_{11}H_{18}O$ 166.1357).

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