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New Naphthalene-Chroman Coupling Products from the Endophytic Fungus, *Nodulisporium* sp. from *Erica arborea*[‡]

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Dedicated to Professor Dr. Axel Zeeck on the occasion of his 70th birthday

Keywords: Natural products / Fungal secondary metabolites / Nodulisporins D–F / *Nodulisporium* sp. / Structural elucidation / Bioactivity

Six novel metabolites, nodulisporins D–F (**1**–**3**), (3*S*,4*S*,5*R*)-2,4,6-trimethyloct-6-ene-3,5-diol (**4**), 5-hydroxy-2-hydroxymethyl-4*H*-chromen-4-one (**5**) and 3-(2,3-dihydroxyphenoxy)-butanoic acid (**6**) were isolated together with seven known compounds (**7**–**13**) from the culture extract of the endophytic fungus *Nodulisporium* sp. The relative configurations of these new compounds were determined by means of spectroscopic data, including HREIMS, ^{1}H NMR, ^{13}C NMR and 2D NMR (HMQC, HMBC and NOESY). The novel compounds exhibit antifungal and antialgal activities and most of them were also antibacterial.

Introduction

An endophytic fungus is a bacterial or fungal microorganism, which spends the whole or part of its life cycle by colonizing the healthy tissues of its host plant, typically causing no apparent disease symptoms.[2] This growth *in planta* necessitates continual metabolic interactions with the host, which may explain why these organisms produce a high proportion of novel metabolites.[3,4] Thus, this group of organisms is a promising and relatively untapped source of novel and biologically active compounds with huge medicinal and agricultural potential.[4–10] In our ongoing search for novel, bioactive compounds from endophytic fungi,[11] we investigated the metabolites synthesized by the endophytic fungus, *Nodulisporium* sp., internal strain

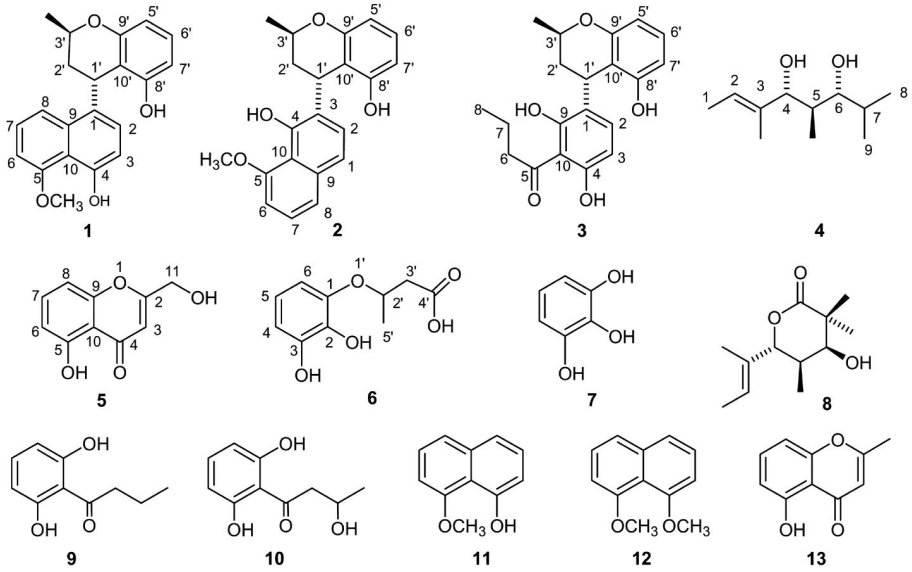

Figure 1. Compounds isolated from the culture extract of the endophytic fungus *Nodulisporium* sp.

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number 7093, which had been isolated from the plant *Erica arborea* from Gomera. Structural elucidation of the ethyl acetate extract from a culture of this endophyte yielded six novel metabolites (**1**–**6**), along with seven known compounds (**7**–**13**, see Figure 1). Here we describe the isolation and structural elucidation of these compounds and their biological activity.

Results and Discussion

Compound **1** was obtained as a colorless resin with the molecular formula $C_{21}H_{20}O_4$, as deduced from high-resolution mass spectroscopic data. Its IR spectrum showed strong absorptions for hydroxy groups at 3405 cm^{-1}. The ^1H NMR spectrum (Table 1) exhibited the presence of two methyl groups at δ = 1.30 ppm (d, J = 6.2 Hz) and δ = 4.12 ppm (s), one proton on an oxygenated carbon at δ = 4.01 (m), and eight aromatic methines. The ^{13}C NMR spectrum of **1** showed signals for 21 carbons, and the DEPT spectrum indicated the presence of two methyls, one methylene, ten methines and eight quaternary carbon atoms. The NMR spectroscopic data indicated the presence of a structure with two units, one of which is identical with 8-methoxynaphthalen-1-ol (**11**), isolated from the same source. Substitution at C-4 was indicated from the absence of an aromatic signal at δ = 7.30 ppm of **11**.

The second part showed signals corresponding to 2-methylchroman with the substitution at C-4. Elucidation of the ^1H-^1H COSY and HMQC spectra of **1** (Figure 2) enabled the deduction of the fragments –CH(1′)–CH_2(2′)–CH(3′)–CH_3; –CH(5′)–CH(6′)–CH(7′)–; –CH(2)–CH(3)– and –CH(6)–CH(7)–CH(8)–. In the HMBC spectrum of **1**, ^{13}C-^1H long-range correlation signals were found between C-4 and H-2 and 3; between C-5 and H-6,7 and 5-OCH_3; between C-9 and H-2,7 and 8; between C-10 and H-3,6 and 8; between C-8′ and H-1′,6′ and 7′; between C-9′ and H-5′ and 6′; as well as between C-10′ and H-1′,2′ and 7′, which enabled the establishment of two units. Connection of the two fragments was established through $^{2,3}J_{C,H}$ correlations from C-1 to H-2,3,8,1′ and H-2′, confirming its planar structure.

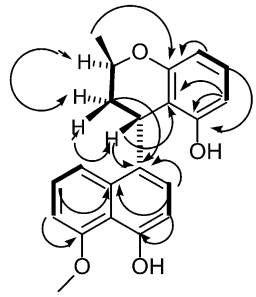


Figure 2. Selected ^1H-^1H COSY (–), HMBC (H→C) and NOESY (H↔H) correlations of nodulisporin D (**1**).

The relative configurations of the C-1′ and C-3′ asymmetric centers were deduced from the coupling constants ($J_{1',2'\beta}$ = 5.8 Hz, $J_{2',3'\beta}$ = 11.3 Hz), which indicated a $H_{1'\beta,3'\alpha}$ configuration. The absence of any 1,2-*trans*-diaxial relationship in the couplings of the 1′ and 2′β hydrogen atoms suggests an axial position of the naphthalene substituent at C-1′, probably due to severe steric interaction with 8′-OH preventing any equatorial orientation. By contrast, the coupling for $J_{2',3'\beta}$ = 11.3 Hz indicates the axial orientation of 3′-H and thus the equatorial position of the 3′-methyl group as shown in Figure 1. In addition, the NOESY spectrum of **1** shows correlations between H-1′ and H-2′β, as well as between H-3′ and H-2′α. All these data are in agreement with the relative configuration of structure **1**. Consequently the compound was named nodulisporin D in order to document the production by the fungus *Nodulisporium* sp. and in continuation of previously isolated similar coupling products named nodulisporins A–C.[11]

The structure of **1** in which a chroman ring system is coupled to a naphthalene moiety, is unique and has previously not been encountered in natural products. Nodulisporin D is a further extension of the ever-growing group in

Table 1. ^1H NMR spectral data of compounds **1**–**3**[a] (500 MHz, $CDCl_3$).

Proton	1	2	3
1		7.22 (d, 7.8)	
2	6.93 (d, 7.8)	7.01 (d, 7.8)	6.95 (d, 7.8)
3	6.76 (d, 7.8)		6.15 (d, 7.8)
6	6.95 (d, 8.0)	6.84 (d, 8.0)	3.10 (t, 7.2)
7	7.51 (t, 8.0)	7.31 (t, 8.0)	1.62 (m)
8	7.85 (d, 8.0)	7.39 (d, 8.0)	0.91 (t, 6.7)
1′	4.83 (d, 5.8)	4.76 (d, 5.8)	4.62 (d, 5.9)
2′α	2.02 (dt, 13.4, 2)	2.17 (dt, 13.4, 2)	1.75 (m)
2′β	2.12 (ddd, 13.4, 11.3, 5.8)	2.08 (ddd, 13.4, 12.2, 5.8)	2.32 (ddd, 14.5, 11.3, 5.9)
3′	4.01 (dq, 11.3, 6.2)	4.09 (dq, 12.2, 6.2)	4.11 (m)
5′	6.62 (d, 8.1)	6.60 (d, 8.0)	6.55 (d, 8.1)
6′	7.13 (t, 8.1)	7.11 (t, 8.0)	7.10 (t, 8.1)
7′	6.45 (d, 8.1)	6.44 (d, 8.0)	6.40 (d, 8.1)
5-OCH_3	4.12 (s)	4.11 (s)	
3′-CH_3	1.30 (d, 6.2)	1.34 (d, 6.2)	1.29 (d, 6.2)

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

which two bicyclic fragments are coupled by C–C and/or C–O bonds as found in the spiroxines,[12] palmarumycins,[13,14] or preussomerins[15,16] (see review article in ref.[17]).

The empirical formula of compound **2** was determined as $C_{21}H_{20}O_4$ by HREIMS and ^{13}C NMR spectroscopic data, which is the same as that found for compound **1**. The ^1H, ^{13}C, and 2D NMR spectroscopic data of **2** were quite similar to those of **1**, except for significant differences in some chemical shifts (Table 1). The ^1H-^1H COSY and HMQC spectra of **2** suggested the presence of the fragments –CH(1′)–CH_2(2′)–CH(3′)–CH_3, –CH(5′)–CH(6′)–CH(7′)–, –CH(1)–CH(2)–, and CH(6)–CH(7)–CH(8)–. In the HMBC spectrum of **2**, ^{13}C-^1H long-range correlation signals were found between C-3 and H-2,1,1′ and 2′; between C-4 and H-2; between C-5 and H-6,7 and 5-OCH_3; between C-9 and H-1,2,7 and 8; between C-10 and H-1,6 and 8; between C-8′ and H-1′,6′ and 7′; between C-9′ and H-5′ and 6′; as well as between C-10′ and H-1′,2′, and 7′, which enabled the establishment of the same two subunits as present in **1**. The connection of the two fragments was established through $^{2,3}J_{C,H}$ correlations between C-3 and H-2,1,1′ and 2′. Thus, whereas the coupling of the two bicyclic fragments in nodulisporin D (**1**) occurred in *para*-position to the phenol group (C-1–C-1′), the chroman moiety was linked to the *ortho*-position of the naphthol system in nodulisporin E (**2**). The coupling constants in the ^1H NMR of the relevant protons 1′–3′ were similar to those observed for **1** (Table 1). The NOESY spectrum exhibited clear correlation between H-1′ and H-2′β; H-3′ and H-2′α, showing that these protons are on the same side of the molecule confirming the relative configuration as shown in Figure 1.

The molecular formula of compound **3** was determined as $C_{20}H_{22}O_5$ by HREIMS and ^{13}C NMR spectroscopic data. The ^1H NMR spectrum (Table 1) exhibited the presence of two methyl groups at δ = 1.29 ppm (d, J = 6.2 Hz) and δ = 0.91 ppm (t, J = 6.7), one proton on an oxygenated carbon at δ = 4.11 ppm (m), and five aromatic methines. The ^{13}C NMR spectrum of **3** showed signals for 20 carbons, and the DEPT spectrum indicated the presence of two methyls, three methylenes, seven methines and eight quaternary carbon atoms. The above NMR spectroscopic data indicated the presence of a structure similar to compound **1** with two subunits. The second part was similar to compound **9**, isolated from the same source. The first part showed signals corresponding to the naphthalene part of compounds **1** and **2**. The location of connection between the two units at C-1/C-1′ were evidenced from HMBC correlations between C-1 and H-2,3,1′ and 2′; between C-9 and H-1′, as well as between C-10′ and H-2,1′,5′ and 7′. The NOESY spectrum exhibited clear correlations between H-1′ and H-2′β as well as between H-3′ and H-2α′. Based on the above data, the chroman part of compound **3**, named nodulisporin F, was linked to a 1-(2,6-dihydroxyphenyl)-butan-1-one moiety in *para*-position to the phenol group (C-1–C-1′). Interestingly, the coupling fragment also occurred as a monomer **9** in the culture medium (see below). The relative configuration was assumed to be related to that of compounds **1** and **2**, based on the coupling pattern of the relevant protons (Table 1).

Compound **4** was obtained as a colorless gum with the molecular formula $C_{11}H_{22}O_2$, as deduced from HREIMS and ^{13}C NMR spectroscopic data. Its IR spectra shows strong absorptions for hydroxy groups at 3405 cm^{-1}, while the ^1H NMR spectrum exhibits the presence of five methyl groups, of which one is a singlet (δ = 1.58) and four are doublets (δ = 0.83 ppm, d, J = 6.3 Hz; δ = 0.92 ppm, d, J = 6.7 Hz; δ = 1.02 ppm, d, J = 6.7 Hz; δ = 1.67 ppm, d, J = 6.8 Hz;), two protons of oxygenated carbons at δ = 3.46 ppm (dd, J = 9.5, 1.5 Hz) and δ = 4.03 ppm (d, J = 5.8 Hz), and one alkene proton at δ = 5.61 ppm (br. q, J = 6.8 Hz). The ^{13}C NMR spectrum of **4** shows signals for 11 carbon atoms, and the DEPT spectrum indicates the presence of five methyls, five methines and one quaternary carbon atom. The ^1H-^1H COSY and HMQC spectra of **4** suggest the presence of the fragments –CH(4)–CH(5)-[CH_3(11)]–CH(6)–CH(7)[CH_3(8)]–CH_3(9)– and –CH_3(1)–CH(2)–. In the HMBC spectrum of **4**, ^{13}C-^1H long-range correlation signals were found between C-3 and H-2,1,11,4 and 5, which enabled establishment of its planar structural skeleton. A comparison of the NMR spectroscopic data of **4** with those of compound **8** showed the absence of a CO_2 fragment. Evidently, the open-chain fragment was a decarboxylation product of helicascolide A (**8**) with known stereochemistry.[18] Based on this biogenetic correlation and the similarity of relevant signal patterns in the ^1H NMR spectra, compound **4** was assigned to be (3*R*,4*R*,5*R*)-2,4,6-trimethyloct-6-ene-3,5-diol with the relative stereochemistry shown in Figure 1.

The HREIMS of compound **5** shows a molecular ion signal at *m*/*z* 192.04033 corresponding to the molecular formula $C_{10}H_8O_4$ (calcd. 192.04227). Analysis of the ^1H NMR spectroscopic data of **5** revealed the presence of an olefinic methine unit, two protons on oxygenated carbon atoms [δ = 4.53 ppm (s)], and three aromatic methines. The ^{13}C NMR spectrum of **5** shows signals of 10 carbon atoms, and the DEPT spectrum indicates the presence of one methylene, four methines and five quaternary carbon atoms. The ^1H-^1H COSY and HMQC spectra of **5** suggest the presence of the fragment –CH(6)–CH(7)–CH(8)–. In the HMBC spectrum of **5**, ^{13}C-^1H long-range correlations were found between C-2 and H-3 and H-11, between C-4 and H-3 and H-11, between C-5 and H-6 and H-7, as well as between C-9 and H-7 and H-8. The NMR spectra of **5** were similar to those of **13**, while the methyl group (H-11) in **13** was replaced by a new oxygenated methylene group (δ_H = 4.53, s). This information led to identification of the structure of **5** as 5-hydroxy-2-hydroxymethyl-4*H*-chromen-4-one.

Compound **6** was analyzed for $C_{10}H_{12}O_5$ by CIMS. The ^1H NMR spectrum exhibits the presence of three aromatic methines and one methyl group. The ^{13}C NMR spectrum of **6** shows signals for 10 carbon atoms, and the DEPT spectrum indicates the presence of one methyl, one methylene, four methines and four quaternary carbon atoms. Analysis of the COSY and HMQC spectra of **6** enabled the deduction of the fragments –CH_3(5′)–CH(2′)–CH_2(3′)– and

–CH(4)–CH(5)–CH(6)–. In the HMBC spectrum of **6**, ^{13}C-^{1}H long range correlation signals were found between C-1 and H-2′, H-5 and H-6; between C-3 and H-4 and H-5; between C-2 and H-4 and H-6; as well as between C-4′ and H-2′, H-3′ and H-5′. Therefore, the structure of **6** was determined as 3-(2,3-dihydroxyphenoxy)butanoic acid, a new fungal metabolite.

The known compounds **7**–**13** were identified as benzene-1,2,3-triol (**7**), a decarboxylation product of gallic acid,[19] helicascolide A (**8**),[18] 1-(2,6-dihydroxyphenyl)butan-1-one (**9**), 1-(2,6-dihydroxyphenyl)-3-hydroxybutan-1-one (**10**),[11] 8-methoxynaphthalen-1-ol (**11**),[11,20] 1,8-dimethoxynaphthalene (**12**),[11,20] 5-hydroxy-2-methyl-4*H*-chromen-4-one[11,21] by comparison of their physical and spectroscopic data with those reported in the literature and previously isolated fungal metabolites.[11]

Biological Activity

The antibacterial, fungicidal, and algicidal properties of the six novel substances as well as 7 were tested in an agar diffusion assay in comparison to four standard antibiotics (Table 2). All the substances were antifungal and antialgal, and **1**–**3** and **7** were also antibacterial. The strongest inhibitions were caused by **3**.

Table 2. Biological activity of metabolites **1**–**7** and control metabolites against microbial test organisms in an agar diffusion assay; results in mm of radius of zone of inhibition.[a]

Substance	*Bacillus megaterium*	*Microbotryum violaceum*	*Chlorella fusca*
1	8	7	8 g.i.
2	7	7	5 g.i.
3	8	10	8 g.i.
4	0	8	6 g.i.
5	0	6 g.i.	6 g.i.
6	0	6 g.i.	7 g.i.
7	6 g.i.	10	7
Penicillin	18	0	0
Tetracycline	18	0	10 g.i.
Nystatin	0	20	0
Actidione	0	50	35
Acetone	0	0	0

[a] g.i. = growth inhibition, i.e. there was some growth within the zone of inhibition; concentrations of 0.05 mg/filter disc.

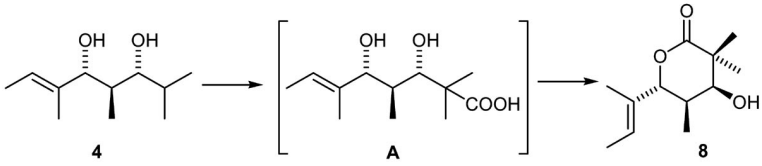
Summary and Discussion

The natural products isolated from cultures of the endophytic fungus *Nodulisporium* sp. present another example of the chemical diversity of secondary metabolites that fungi are able to generate. The coupling of identical, similar, or even chemically quite different subunits is a very common motive to increase the chemical diversity. In these coupling reactions, the products increase in molecular weight and chemical shape in only one single step and thus also improve the quality of chemical diversity.[22] In the present example, the naphthalene part **11** is coupled at different sites with a chroman part similar to **13**, producing the title compounds nodulisporin D (**1**) and E (**2**). In a third example, the chroman moiety is linked to the fragment **9**, also occurring as a monomer in the fungus. Although comparatively simple in structure, the unique skeletons of **1**–**3** are found for the first time as natural products. Interestingly, in another *Nodulisporium* sp., the coupling products formed were homodimers of naphthalene **11**, although the fungi also produced some identical compounds (**8**, **11**–**13**).[11] Diversification can also happen by production of open chain and cyclic products. For instance, at first glace, the diol **4** has quite a different shape than that found in helicascolide A (**8**).[17] However, closer inspection shows that helicascolide A is just the lactone derived from the carboxylation product **A** (Scheme 1).

Experimental Section

General Procedures: For general methods and instrumentation see ref.[11] and for further microbiological methods and conditions of culture see ref.[23] Melting points were determined on a Gallenkamp micro-melting point apparatus and are uncorrected. NMR spectra were run on a Bruker Avance –500 NMR spectrometer with TMS as internal standard. EIMS were obtained on a MAT 8200 mass spectrometer.

Cultivation, Extraction and Isolation: The endophytic fungus *Nodulisporium sp.* was isolated from the plant *Erica arborea*, from Gomera. It was cultivated at room temperature for 28 d on biomalt solid agar medium. The culture media were then extracted with ethyl acetate to afford 9.6 g of a residue after removal of the solvent under reduced pressure. The extract was separated into three fractions by column chromatography (CC) on silica gel (200 g), using gradients of dichloromethane/ethyl acetate (85:15, 50:50, 0:100). The less polar fraction 1 (2.8 g) contained mainly fatty acids and lipids. The remaining two fractions were each further purified by silica gel column chromatography (CC), preparative TLC and Sephadex (LH-20). The next fraction (3.0 g) was separated by CC over 100 g silica gel with hexane/ethyl acetate (10:1, 1000 mL, 5:1, 1000 mL) to give two subfractions A and B. Fraction A (500 mg) was separated by CC over 10 g silica gel with hexane/ethyl acetate (7:1, 550 mL) to give crude **1**, **2**, **8** and **9**. Fraction B (750 mg) was separated by CC over 12 g silica gel with hexane/ethyl acetate (5:1, 450 mL) to give crude **3**, **4**, **11**, **12** and **13**. Subsequently, each crude fraction was further purified by preparative TLC chromatography



Scheme 1. Formal biosynthetic connection of **4** and **8** via the acid **A**.

on silica gel (1 mm, Macherey–Nagel & Co.) and Sephadex (LH-20) to give compounds **1** (5 mg), **2** (3 mg), **3** (4 mg), **4** (15 mg), **8** (10 mg), **9** (10 mg), **11** (15 mg), **12** (17 mg) and **13** (20 mg). The more polar fractions (1.5 g) were separated by silica gel column chromatography eluted with dichloromethane/ethyl acetate (4:1, 980 mL) to give crude gum of **5**, **6** and **7**, successively. The samples were further subjected to silica gel column chromatography eluted with dichloromethane/methanol (15:1, 580 mL) to give pure compounds **5** (1.5 mg), **6** (5 mg) and **7** (10 mg).

Nodulisporin D (1): Colorless gum. $[\alpha]_D^{25}$ = –4.3 (c = 0.02, MeOH). UV (MeOH): λ_{max} (log ε) = 275, 337 nm. IR (film): ν_{max} = 3405, 2924, 2365, 1744, 1574, 1268, 1237, 1062, 756 cm^{-1}. ^{1}H NMR spectroscopic data see Table 1. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 157.2 (s, C-5), 156.9 (s, C-9′), 154.3 (s, C-8′), 154.1 (s, C-4), 133.6 (s, C-9), 129.4 (s, C-1), 128.4 (d, C-2), 128.3 (d, C-6′), 126.4 (d, C-7), 116.9 (d, C-8), 115.9 (s, C-10), 110.8 (s, C-10′), 109.8 (d, C-3), 109.4 (d, C-5′), 107.6 (d, C-7′), 104.4 (d, C-6), 67.4 (d, C-3′), 56.3 (q, OCH_3), 36.1 (t, C-2′), 32.0 (d, C-1′), 21.1 (q, 3′-CH_3) ppm. EIMS: m/z (%) = 336 (30) [M$^+$], 293 (5), 185 (20), 174 (100), 91 (70). HREIMS: m/z = 336.13645, calcd. for $C_{21}H_{20}O_4$ 336.13617.

Nodulisporin E (2): Colorless gum. $[\alpha]_D^{25}$ = –4.5 (c = 0.02, CH_2Cl_2). UV ($CHCl_3$) λ_{max} (log ε) = 307, 323, 337 nm. IR (film): ν_{max} = 3379, 2934, 2841, 1750, 1408, 1253, 1077, 818 cm^{-1}. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 156.9 (s, C-9′), 156.2 (s, C-5), 154.4 (s, C-8′), 151.0 (s, C-4), 135.8 (s, C-9), 128.2 (d, C-6′), 128.0 (d, C-2), 125.5 (d, C-7), 124.4 (d, C-3), 121.9 (d, C-8), 118.5 (s, C-1), 114.9 (s, C-10), 110.6 (s, C-10′), 109.3 (d, C-5′), 107.3 (d, C-7′), 104.3 (d, C-6), 67.8 (d, C-3′), 56.2 (q, OCH_3), 35.7 (t, C-2′), 29.3 (d, C-1′), 21.3 (q, 3′-CH_3) ppm. ^{1}H NMR spectroscopic data see Table 1. EIMS: m/z (%) = 336 (30) [M$^+$], 279 (10), 174 (75), 149 (70), 83 (100), 48 (90). HREIMS: m/z = 336.13617, calcd. for $C_{21}H_{20}O_4$ 336.13617.

Nodulisporin F (3): Colorless gum. $[\alpha]_D^{25}$ = –5.2 (c = 0.2, CH_2Cl_2). IR (film): ν_{max} = 3265, 1631, 1237 cm^{-1}. EIMS: m/z (%) = 342 (60) [M$^+$], 162 (95), 174 (25), 149 (50), 97 (65), 57 (100). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 208.1 (s, C-5), 160.5 (s, C-9), 157.9 (s, C-4), 157.8 (s, C-9′), 154.8 (s, C-8′), 134.4 (d, C-2), 128.1 (d, C-6′), 122.4 (s, C-1), 112.1 (s, C-10′), 109.8 (s, C-10), 109.8 (d, C-5′), 108.7 (d, C-7′), 107.9 (d, C-3), 72.3 (d, C-3′), 46.8 (t, C-6), 39.3 (t, C-2′), 29.7 (d, C-1′), 21.1 (q, 3′-CH_3), 17.7 (t, C-7), 13.8 (q, C-8) ppm. ^{1}H NMR spectroscopic data see Table 1. HREIMS: m/z = 342.1471, calcd. for $C_{20}H_{22}O_5$ 342.1467.

(3*S*,4*S*,5*R*)-2,4,6-Trimethyloct-6-ene-3,5-diol (4): Colorless powder. $[\alpha]_D^{25}$ = +7.7 (c = 0.05, CH_2Cl_2). IR (film): ν_{max} = 3515, 2918, 1755, 1243, 1015, 720 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.61 (q, J = 6.8 Hz, 1 H, 2-H), 4.03 (d, J = 5.8 Hz, 1 H, 4-H), 3.46 (dd, J = 9.5, 1.5 Hz, 1 H, 6-H), 1.88 (ddd, J = 7.1, 5.8, 1.5 Hz, 1 H, 5-H), 1.72 (m, 1 H, 7-H), 1.67 (d, J = 6.8 Hz, 3 H, 1-H), 1.58 (s, 3 H, 11-H), 1.02 (d, J = 6.7 Hz, 3 H, 9-H), 0.92 (d, J = 7.1 Hz, 3 H, 10-H), 0.83 (d, J = 6.7 Hz, 3 H, 8-H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 136.6 (s, C-3), 120.6 (d, C-2), 80.7 (d, C-4), 76.4 (d, C-6), 36.1 (d, C-5), 31.3 (d, C-7), 19.9 (q, C-9), 18.9 (q, C-8), 13.0 (q, C-1), 12.2 (q, C-11), 9.8 (q, C-10) ppm. EIMS: m/z (%) = 186 (10) [M$^+$], 177 (15), 169 (20), 168 (100), 153 (45), 85 (20).

5-Hydroxy-2-hydroxymethyl-4*H*-chromen-4-one (5): Amorphous powder. UV ($CHCl_3$) λ_{max} (log ε) = 275, 237 nm. IR (film): ν_{max} = 3405, 1625, 668 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.61 (t, J = 8.4 Hz, 1 H, 7-H), 6.98 (d, J = 8.4 Hz, 1 H, 6-H), 6.79 (d, J = 8.4 Hz, 1 H, 8-H), 6.43 (s, 1 H, 3-H), 4.53 (s, 2 H, 11-H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 183.8 (s, C-4), 171.3 (s, C-2), 160.5 (s, C-9), 156.6 (s, C-5), 135.5 (d, C-7), 110.8 (d, C-8), 110.3 (s, C-10), 106.8 (d, C-6), 105.8 (d, C-3), 60.0 (t, C-11) ppm. EIMS: m/z (%) = 192 (90) [M$^+$], 163 (50), 149 (20), 137 (15), 44 (95), 31 (100). HREIMS: m/z = 192.04226, calcd. for $C_{10}H_8O_4$ 192.04227.

3-(2,3-Dihydroxyphenoxy)butanoic Acid (6): Colorless gum. $[\alpha]_D^{25}$ = +32.5 (c = 0.02, CH_2Cl_2). UV ($CHCl_3$) λ_{max} (log ε) = 355, 270, 210 nm. IR (film): ν_{max} = 3410, 1765, 1450, 1382, 1227, 1010 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.50 (d, J = 7.8 Hz, 1 H, 6-H), 6.51 (t, J = 7.8 Hz, 1 H, 5-H), 6.45 (d, J = 7.8 Hz, 1 H, 4-H), 4.50 (m, 1 H, 2′-H), 2.65 (dd, J = 16.1, 8.0 Hz 1 H, 3′a-H), 2.52 (dd, J = 16.1, 4.2 Hz 1 H, 3′b-H), 1.32 (d, J = 6.0 Hz 3 H, 5′-H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 174.7 (s, C-4′), 145.4 (s, C-3), 144.7 (s, C-1), 136.3 (s, C-2), 118.5 (d, C-5), 110.2 (d, C-4), 110.2 (d, C-6), 72.8 (d, C-2′), 40.8 (t, C-3′), 19.0 (q, C-5′) ppm. HREIMS: m/z = 212.06847, calcd. for $C_{10}H_{12}O_5$ 212.06847.

Tests for Biological Activity: For the agar diffusion assay, the compounds were dissolved in acetone at a concentration of 1 μg/μL. Fifty microliters of the solution were pipetted onto a sterile filter disc (final concentrations of 0.05 mg/filter disc), which was placed onto an appropriate agar growth medium for the respective test organism (*Bacillus megaterium, Microbotryum violaceum* and *Chlorella fusca*) and subsequently sprayed with a suspension of the test organism.[24]

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