A New Tyrosine Kinase Inhibitor from a Marine Isolate of *Ulocladium botrytis* and New Metabolites from the Marine Fungi *Asteromyces cruciatus* and *Varicosporina ramulosa*

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Keywords: Marine fungi / Tyrosine kinase / Natural products / Macrodiolides / Polyketides

The sponge-derived fungi *Ulocladium botrytis* and *Asteromyces cruciatus*, and the algal-derived fungus *Varicosporina ramulosa*, were isolated and extracts from cultures investigated for their metabolite production. Investigations of the extract of the culture of *U. botrytis* guided by bioassay yielded the new tyrosine kinase ($p56^{lck}$) inhibitor ulocladol (**1**) together with 1-hydroxy-6-methyl-8-(hydroxymethyl)xanthone (**3**), which showed antifungal activity. The extract of the culture medium of *A. cruciatus* yielded the new metabolite (+)-2,4-dimethyl-4,5-dihydrofuran-3-carbaldehyde (**4**) together with the known compounds (3*S*,5*R*)-dimethyldihydrofuran-2-one (**5**) and tri-*O*-acetyl glycerol. From *V. ramulosa* the five macrodiolides grahamimycin A_1 (**6**), colletoketol (**7**), (6*R*,11*R*,12*R*,14*R*)-colletodiol (**8**), 9,10-dihydro-(6*R*,11*S*,12*S*,14*R*)-colletodiol (**9**) and 9,10-dihydro-(6*R*,11*R*,12*R*,14*R*)-colletodiol (**10**) together with ergosterol were obtained, **9** and **10** being new fungal metabolites.

Introduction

Marine-derived fungi are a source of novel and biologically active secondary metabolites, which have only recently received broader interest.[1][2] Of the reports concerning these fungi, less than one third relate to metabolites from obligate marine fungi. Most of the higher filamentous obligate marine fungi belong to the Ascomycota, while the number of mitosporic fungi is considerably lower.[3] Genera of mitosporic fungi with exclusively marine species comprise *Asteromyces* and *Varicosporina*, which may thus be especially interesting for chemical investigations. From these two genera only gliovictin, a metabolite of *A. cruciatus* has been reported.[4]

In our search for new and biologically active natural products from marine organisms, we have isolated more than 1000 fungal strains from marine sponges and algae, the great majority being isolates of genera well-known from terrestrial environments, e.g., *Coniothyrium*, *Fusarium* and *Phoma*. Screening of culture extracts of selected isolates led to the discovery of several strains which showed significant activity in agar diffusion assays for antimicrobial activity and ELISA-based test systems.[5] Previous work resulted in the isolation of three new metabolites from a *Coniothyrium* and a *Microsphaeropsis* strain.[6] Here the isolation of a new tyrosine kinase (TK, $p56^{lck}$) inhibitor from *Ulocladium botrytis*, and the results of the chemical investigations of the obligate marine mitosporic fungi *Asteromyces cruciatus* and *Varicosporina ramulosa* are reported.

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Results and Discussion

The fungus *U. botrytis* (strain no. 193A4) was isolated from the marine sponge *Callyspongia vaginalis*, collected from Dominica, Caribbean. The ethyl acetate (EtOAc) extract of the fungal culture (Biomalt agar) showed TK inhibitory activity (97% inhibition of enzyme activity at 200 μg/mL), and antimicrobial activity. Bioassay-guided work up by successive normal-phase VLC and HPLC led to the isolation of **1**, and the antifungal metabolite **3**.

The molecular formula of **1** was determined as $C_{16}H_{14}O_7$ by accurate mass measurement, indicating 10 elements of unsaturation within the molecule (see Figure 1). From the ^{1}H-NMR spectrum of **1** signals for two methoxyl groups (δ = 3.87, s, OCH_3-2 and δ = 3.95, s, OCH_3-9), two *meta*-coupled aromatic protons (δ = 6.55, d, J = 2.5 Hz, 3-H and δ = 6.92, d, J = 2.5 Hz, 1-H), one aromatic proton, not coupled further (δ = 6.57, s, H-8), three phenolic hydroxyl groups (δ = 5.68 and 5.77, each br. s, OH-10/OH-11) including one (δ = 10.29, s, OH-4) deshielded due to hydrogen bonding with a carbonyl moiety, and one methylene group attached to oxygen (δ = 4.79 and 5.11, each 1 H, d, J = 11.7 Hz, 7a,7b-H), could be deduced. The ^{13}C-NMR spectrum revealed signals for 13 sp^2-hybridized carbon atoms (six C=C bonds, one C=O bond), accounting for 7 of the 10 elements of unsaturation, and suggesting the presence of two separate aromatic rings. The third ring thus had to be a lactone due to signals in the IR spectrum at 1650 and 1660 cm^{-1}, and had to involve the only methylene group. The three hydroxyl groups, two methoxyl groups, and one lactone moiety together with three olefinic hydrogens accounted for 10 of the possible 12 substituents on the aromatic rings; thus, both rings had to be directly connected via a carbon−carbon bond. After correlating all car-

  1434−193X/99/1111−2949 $ 17.50+.50/0

bons with their directly bonded hydrogens via a $^1H-^{13}C$ shift correlated NMR measurement (HMQC), the structure was further elaborated by interpretation of the $^1H-^{13}C$ long-range (HMBC) correlations. Long-range correlations between each 1-H, 3-H and OCH_3-2 to C-2 connected C-1, C-3 and the methoxyl group to C-2. Furthermore, O*H*-4 showed long-range correlations to C-3, C-4 and C-4a establishing the molecular fragment from C-1 to C-4a. The signal of O*H*-4 appeared as a singlet at δ = 10.29, deshielded due to a hydrogen bond to a carbonyl moiety; thus the carbon of the lactone moiety C-5 had to be connected to C-4a. Long-range correlations between OCH_2-7 and C-5, C-7a, C-8 and C-11a established a further major part of the molecule, revealing that the two aromatic rings had to be connected via the two quaternary carbon atoms C-11a and C-11b. The remaining two hydroxyl groups, and one methoxyl group still had to be placed. The regiochemistry of the molecular fragment from C-8 to C-11 was established by difference NOE measurements. Irradiation at the resonance frequencies of OCH_3-9 (δ = 3.95, s) and OCH_2-7 (δ = 4.79 and 5.11, each 1 H, d, J = 11.7 Hz) respectively, both enhanced the signal of 8-H (δ = 6.57, s). Thus, OCH_2-7 and OCH_3-9 had to be attached to carbon atoms adjacent to C-8, leaving the two hydroxyl groups to be positioned at C-10 and C-11. Compound **1** is the new 4,10,11-trihydroxy-2,9-dimethoxy-7*H*-dibenzo[*c,e*]oxepin-5-one for which we propose the trivial name ulocladol.

Recently the 4-methyl ether of **1** has been reported from the mycobiont of the lichen *Graphis scripta* var. *pulverulenta*.[7] It is interesting to note that a related compound, botrallin (**2**), formally derived from a common polyketide precursor by closure of the lactone at different sites, has been reported from *Botrytis allii*.[8] It was tentatively identified during the current investigation by ^1H-NMR spectroscopy and EIMS, but material was too scarce to enable unequivocal characterisation.

Compound **3** (see Figure 1) was identified as 1-hydroxy-6-methyl-8-(hydroxymethyl)xanthone, first isolated from the Basidiomycete *Cyathus intermedius*,[9] and recently reported also from the coprophilous fungus *Ascodesmis sphaerospora*,[10] by mass spectrometry and comparison of its NMR spectroscopic data, as well as the melting point with published values.

The fungus *Asteromyces cruciatus* (strain no. H5−81) was isolated from the marine sponge *Myxilla incrustans*, collected at Helgoland, Germany. Successive workup of the EtOAc extract of the medium by VLC and HPLC yielded the new metabolite (+)-2,4-dimethyl-4,5-dihydrofuran-3-carbaldehyde (**4**) (see Figure 1) together with the known compounds (3*S*,5*R*)-dimethyldihydrofuran-2-one (**5**) (Figure 1) and tri-*O*-acetyl glycerol, while the extract of the mycelium contained D-arabitol, D-mannitol, and D-trehalose. Gliovictin, previously reported from *A. cruciatus*,[4] was not obtained.

Compound **4** was obtained as a clear oil, and proved to be too volatile for direct EIMS analysis. GC−EIMS however, resulted in the detection of its molecular ion $[M]^+$ at *m/z* 126. The ^{13}C-NMR spectra of **4** (^1H decoupled and DEPT) revealed signals for seven carbon atoms, including an α,β-unsaturated aldehyde moiety (d, δ = 185.0, CHO-3), two quaternary sp^2-hybridized carbon atoms (s, δ = 120.5, C-3; s, δ = 174.6, C-2), two methyl groups (q, δ = 12.5, CH_3-2; q, δ = 19.3, CH_3-4), one methylene group adjacent to an oxygen (t, δ = 79.3, C-5), and one methine group (d, δ = 34.8, C-4). From the ^1H-NMR spectrum the molecular fragment from CH_3-4 (d, δ = 1.21, J = 6.6 Hz) to H-4 (m, δ = 3.34) and H_2-5 (dd, δ = 4.06, J = 5.1 Hz, J = 9.2 Hz and dd, δ = 4.54, J = 9.2 Hz, J = 9.7 Hz) could be established. Thus, CH_3-2, CHO-3, C-4 and OCH_2-5 (via the oxygen) had to be connected to the carbon−carbon double bond. $^1H-^{13}C$ long-range (HMBC) correlations between C*H*O-3, and both C-3 and C-4, and from both CH_3-4 and H-4 to C-3, respectively, connected CHO and C-4 to

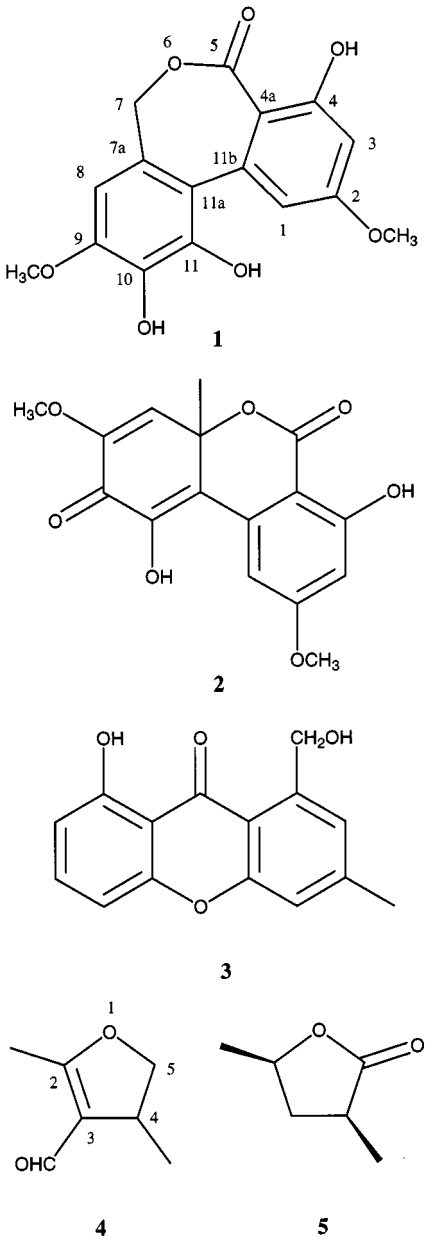

Figure 1. Metabolites of *Ulocladium botrytis* and *Asteromyces cruciatus*

C-3. CH_3-2 and the oxomethylene group therefore had to be the other substituents on the double bond. **4** is the new compound (+)-2,4-dimethyl-4,5-dihydrofuran-3-carbaldehyde. Compound **5** was found to be (3*S*,5*R*)-dimethyldihydrofuran-2-one by comparison of its NMR data and optical rotation with published values.[11][12] To our knowledge this is the first report of **5** as a natural product. Tri-*O*-acetylglycerol was also found and identified making use of its ^{1}H- and ^{13}C-NMR data.[13] Investigations of the mycelium extract yielded a high content of D-arabitol, D-mannitol and D-trehalose. Amounts were estimated to be 3.7 g, 2.8 g, and 10.8 g, respectively, per 100 g of freeze-dried mycelium. All compounds were identified by comparing their spectroscopic data with those of authentic samples. D-arabitol and D-mannitol have been reported to be accumulated in the mycelium of marine fungi in order to balance the osmotic pressure of the salt water.[14]

The fungus *Varicosporina ramulosa* (strain no. 195−31) was isolated from an alga of the genus *Cytoseira* collected from Tenerife, Spain. It was cultured on a seawater and malt extract-containing medium. Purification of its EtOAc extract by VLC and normal-phase HPLC yielded compounds **6**−**10** and ergosterol (Figure 2). Cultures of the fungus in liquid sea water media containing either chitin from crab shells or freeze-dried *Fucus vesicolosus* as the only nutrient source, did not lead to the production of metabolites **6**−**10**, as deduced by ^{1}H-NMR spectroscopy of the culture extract fractionated by VLC.

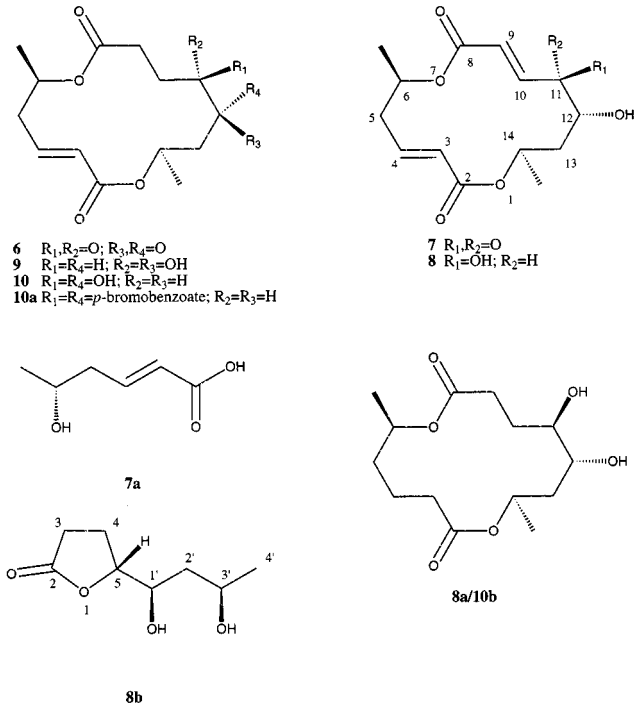

Figure 2. Macrodiolides and their degradation products

Compounds **6**, **8** and **9** were identified by comparing their spectroscopic data and optical rotations with published values as grahamimycin A_1 (**6**),[15] (6*R*,11*R*,12*R*,14*R*)-colletodiol (**8**),[16−18] and 9,10-dihydro-(6*R*,11*S*,12*S*,14*R*)-colletodiol (**9**).[19] Compound **7** was found to have the same basic structure as both colletoketol[16] and grahamimycin A[20][21] on the ground of extensive 1D- and 2D-NMR studies. The configuration of colletoketol is 6*R*,12*R*,14*R*, that of grahamimycin A 6*R*,12*R*,14*S*.[21] The ^{13}C-NMR data for **7** were found to be in good agreement with values published for grahamimycin A, but the ^{1}H-NMR data differed.[21] There appeared to be no ^{13}C-NMR data reported for colletoketol, and other physical properties such as melting point and optical rotation proved to be insufficient to determine unambiguously if **7** was identical with one of the two compounds in question. To resolve these issues a single crystal X-ray crystallographic analyses of **7** was performed, and established its relative stereochemistry.[22] The absolute configuration at C-6 was determined as *R* by alkaline hydrolysis of **7** resulting in (−)-5-hydroxy-(2*E*)-hexenoic acid (**7a**) of known absolute configuration as one product.[16] Thus, **7** could be shown to have the 6*R*,12*R*,14*R*-configuration and is identical with colletoketol.

By comparing their mass-,^{1}H- and ^{13}C-NMR spectroscopic data, compound **10** appeared to be similar to **9**. After assigning all hydrogens to their directly bonded carbons via a ^{1}H−^{13}C shift correlated NMR measurement (HMQC), **10** was shown to be a stereoisomer of **9**, differing in configuration at one or more of the four chiral centres. The conformational flexibility of these 14-membered rings usually means the stereochemistry of each individual chiral centre has to be determined separately, e.g., by degradation studies. Attempts to produce crystals of **10** suitable for a X-ray crystallographic analysis proved unsuccessful. Therefore, the 11,12-di-(*p*-bromobenzoate)-derivative of **10** (**10a**) was synthesized; unfortunately this compound was a clear oil. Its CD-spectrum showed for the two *p*-bromobenzoate units a negative ($\Delta\varepsilon_{255.2\,nm} = -8.4$), and a positive ($\Delta\varepsilon_{241.2\,nm} = +7.8$) Cotton effect implying a negative chirality. MacMillan and Simpson reported a CD analysis of the dibenzoate-derivative of **8** and, after establishing the stereochemistry at C-11 as *S* from degradation studies, determined the absolute stereochemistry of colletodiol as 11*S*,12*R*.[16] Although this assignment was later revised to 11*R*,12*R* after a X-ray crystallographic analysis,[17] both the 11*R*,12*R* and 11*S*,12*R* configurations are in agreement with the observed cotton effect of the two benzoate units. This finding suggested one of these configurations also for **10**. As all the other macrodiolides isolated (**6**−**9**) possess the *R* configuration at C-6 and C-14, **10** was likely also to have the 6*R*,14*R* configuration. Taking this possibility into account, **10** was then possibly the 9,10-dihydro derivative of (6*R*,11*R*,12*R*,14*R*)-colletodiol (**8**). Hydrogenation of **8** and **10** should then lead to identical products. The 3,4,9,10-tetrahydro derivative of **8** (**8a**) was known and readily accessible by hydrogenation with H_2 over Pd/charcoal.[16] The 9,10-dihydro-derivative of **10** (**10b**) was prepared analogously. The hydrogenation products of both reactions proved to be unstable, and their spontanous decomposition/rearrangement started during the reaction and continued in the $CDCl_3$ solution during NMR measurements. This was

possibly due to traces of HCl in the NMR solvent. Nevertheless, **8a** and **10b** were shown to have identical ^{1}H-NMR spectra, indicating the configurations of **10** at C-6, C-11, C-12 and C-14 to be either all *R* as in **8**, or all *S*. The *R* configuration, however, would be in accord with the negative chirality observed for **10a**. The *R* configurations could be further substantiated by closer inspection of one major decomposition product of **8a**, which was shown to be (5*R*)-[(1′*R*,3′*R*)-dihydroxybutyl]dihydrofuran-2-one (**8b**).[16] The identical compound was obtained from the decomposition of **10b**. Thus, **10** is 9,10-dihydro-(6*R*,11*R*,12*R*,14*R*)-colletodiol.

The common fungal sterol ergosterol was identified by NMR and mass spectrometry as being present in the extract of this fungus.[23] Three of the five macrodiolides obtained from *V. ramulosa* were first reported from terrestrial fungi, grahamimycin A_1 (**6**) from an isolate of the genus *Cytospora*,[20] and colletoketol (**7**)[16] and (6*R*,11*R*,12*R*,14*R*)-colletodiol (**8**), from the plant pathogen *Colletotrichum capsici*.[16][24] 9,10-Dihydro-(6*R*,11*S*,12*S*,14*R*)-colletodiol (**9**), and 9,10-dihydro-(6*R*,11*R*,12*R*,14*R*)-colletodiol (**10**) are new fungal metabolites, **9** being first reported as an intermediate in the total synthesis of **6**.[19] In contrast to the prominent antimicrobial activity of **6** and **7** (as grahamimycin A),[20] no biological activities have been reported for (6*R*,11*R*,12*R*,14*R*)-colletodiol. Its abundant production by the fungus suggests this compound to have a biological function yet to be discovered.

Compounds **1**, **3** and **6**−**10** were tested for antimicrobial activity in agar diffusion assays and in ELISA-based assays for HIV-1 reverse transcriptase and tyrosine kinase ($p56^{lck}$) inhibitory activity. Ulocladol (**1**) inhibited tyrosine kinase ($p56^{lck}$). Enzyme activity was reduced to 7% at 0.02 μg/μL. Test organisms for the agar diffusion assays (50 μg compound per test disk) were the bacteria *Escherichia coli*, *Bacillus megaterium*, the fungi *Ustilago violacea*, *Eurotium repens*, *Mycotypha microspora*, *Fusarium oxysporum* and the alga *Chlorella fusca*. Compounds **3**, **9** and **10** inhibited the growth of *E. repens* with inhibiton zones of 2, 2 and 1 mm respectively, and **3** additionally inhibited *U. violacea* (inhibition zone 2 mm). Compound **7** also showed antifungal activity against *E. repens* and *U. violacea* (inhibition zones 2 mm each). These findings correlate with the reported antimicrobial activity of grahamimycin A.[20]

Experimental Section

General: The general experimental procedures were carried out as previously described.[25] − The CD spectrum of **10a** was recorded with a Jasco J-715 spectropolarimeter using EtOH as solvent. − NMR spectra were referenced to solvent peaks ($CHCl_3$ in $CDCl_3$: δ = 7.26; $CDCl_3$: δ = 77.00).

Isolation and Taxonomy of Fungal Strains: Fungal strains were isolated by inoculating small pieces of the inner tissue of the sponges on glucose peptone yeast extract agar (GPY) at room temperature (room temp.): Glucose·H_2O 1 g/L, peptone from soymeal 0.5 g/L, yeast extract 0.1 g/L, benzylpenicillin 250 mg/L, streptomycin sulfate 250 mg/L, agar 15 g/L, artificial sea water (ASW) 800 mL/L. ASW contained the following salts [g/L]: KBr 0.1, NaCl 23.48, MgCl2·6 H_2O 10.61, CaCl2·2 H_2O 1.47, KCl 0.66, SrCl2·6 H_2O 0.04, Na_2SO_4 3.92, $NaHCO_3$ 0.19, H_3BO_3 0.03. − Strain 193A4 was isolated from the sponge *Callyspongia vaginalis*. The sponge was collected by divers using SCUBA from the waters around the Caribbean island of Dominica. The fungus was identified as *Ulocladium botrytis* Preuss 1851. − Strain H5−81 was isolated from the sponge *Myxilla incrustans*. The sponge was collected by divers using SCUBA from the waters around Helgoland, German Bight. The fungus was identified as *Asteromyces cruciatus* Moreau *et* Moreau *ex* Hennebert 1962. − Strain 195-31 was isolated from an unidentified species of brown alga of the genus *Cytoseira* collected from Tenerife, Spain, by inoculating small pieces of the alga on GPY agar made with ASW. It was identified as *Varicosporina ramulosa* Meyers *et* Kohlmeyer 1965. − All fungal strains were taxonomically identified by Dr. S. Draeger, Institute for Microbiology, Technical University of Braunschweig, and are deposited at the fungal culture collection of the Institute.

Table 1. ^{1}H- and ^{13}C-NMR spectral data for ulocladol (**1**)[a]

Position	δ_H	δ_C[b]
1	6.92 (d, *J* = 2.5 Hz)	110.2 (d)
2	−	163.2 (s)
3	6.55 (d, *J* = 2.5 Hz)	100.8 (d)
4	OH 10.29 (s)	163.0 (s)
4a	−	106.9 (s)
5	−	172.5 (s)
7	4.79 (d, *J* = 11.7 Hz)	70.3 (t)
	5.11 (d, *J* = 11.7 Hz)	
7a	−	127.1 (s)
8	6.57 (s)	103.1 (d)
9	−	146.3 (s)
10	−	133.8 (s)
11	−	135.9 (s)[c]
11a	−	118.8 (s)
11b	−	142.1 (s)[c]
OCH_3-2	3.87 (s)	55.5 (q)
OCH_3-9	3.95 (s)	56.3 (q)
OH-10 and OH-11	5.68 (br. s), 5.77 (br. s)	

[a] $CDCl_3$, 400 MHz (^{1}H), 100 MHz (^{13}C). − [b] Multiplicity deduced by DEPT (s = C, d = CH, t = CH_2, q = CH_3). − [c] Assignments may be interchanged.

Cultivation: The isolate of *U. botrytis* was cultured on Biomalt agar (1 L, Biomalt, Vitaborn, Hameln, Germany, 20 g/L, agar 6.8 g/L) in Fernbach flasks (0.25 L each), for 14 days at room temp. The isolate of *A. cruciatus* was cultured on Biomalt agar (Biomalt 20 g/L, agar 20 g/L), and starch yeast extract agar (soluble starch 15 g/L, yeast extract 4 g/L, agar 20 g/L), both made up with 20% dist. H_2O and 80% ASW, (2.5 L each) in penicillium flasks (0.5 L each) at room temp. for three months. The isolate of *V. ramulosa* was cultured on Biomalt agar (Biomalt 20 g/L, agar 6.8 g/L) made up with 20% dist. H_2O and 80% ASW, total 6.5 L agar in penicillium flasks (0.5 L each) at room temp. for five weeks.

Biological Activity: Agar diffusion assays were performed according to Schulz et al.[26] Applied amounts were 250 μg for extracts or 50 μg for pure compounds per test disk. Tyrosine kinase ($p56^{lck}$) inhibitory activity was determined ELISA-based according to a commercial test kit (Fa. Boehringer Mannheim, cat. no. 1,534,505), modified by Ms. G. Matthée.[27] Compounds and extracts were dissolved in half of their weight (μL) dimethyl sulfoxide (DMSO) and diluted with water (1:1 v/v) to yield appropriate sample solutions. Inhibitory activity was expressed as remaining enzyme activity (%) relative to a negative control.

Extraction and Isolation: *U. botrytis*: Mycelium and medium were diluted with water (500 mL) and homogenised using a Waring blender. The resulting mixture was extracted with EtOAc (3 × 700 mL) to yield 366 mg of a brown oil. The extract was separated by Vacuum Liquid Chromatography (VLC) (TLC-Silica 60GF 15µm, Merck) using hexane containing increasing proportions of EtOAc (10% step gradient per 50 mL solvent) to give 6 fractions each of 100 mL. ELISAs showed that the tyrosine kinase inhibitory activity was concentrated in fractions 4 and 5. These fractions were further purified by normal-phase HPLC (LiChrosorb Si60, 5 µm, 250 × 8 mm; hexane/EtOAc, 75/25) to give **1** (5.4 mg; 5.4 mg/L medium), **3** (6.0 mg; 6.0 mg/L medium), and a sample presumably containing **2**.

***A. cruciatus*:** Mycelium and medium were separated. The media were combined and extracted as described for the *U. botrytis* strains, yielding 473 mg of a brown oil. Subsequent normal-phase VLC (gradient hexane-EtOAc-MeOH) yielded four fractions. Fraction 1 (352 mg), and 2 (31 mg) were further purified by normal-phase HPLC (hexane/EtOAc, 75/25) to give **4** (6.1 mg; 1.2 mg/L medium), **5** (5.3 mg; 1.1 mg/L medium), and tri-*O*-acetyl glycerol (6.1 mg; 1.2 mg/L medium). The mycelium was freeze-dried (23 g), crushed in a mortar and extracted with hexane (500 mL), and MeOH (3 × 500 mL). The MeOH extract was suspended in EtOAc and the residue subjected to VLC (RP-18) with MeOH to yield 4.09 g of a gum. Further VLC (Si-60) (EtOAc/MeOH/H_2O/HAc, 60/15/15/10) yielded D-arabitol, D-mannitol and D-trehalose. As not all of the extract was separated, total amounts were estimated to be 3.7 g D-arabitol, 2.8 g D-mannitol and 10.8 g D-trehalose per 100 g freeze-dried mycelium.

***V. ramulosa*:** Mycelium and medium were extracted as described for the *U. botrytis* strains, yielding 3.85 g of a yellow oil. The extract was subjected to VLC (gradient hexane/EtOAc) to give six fractions; fraction 5 contained pure **8** (680 mg; 105 mg/L). Fraction 3 (65 mg) was purified by normal-phase HPLC (hexane/EtOAc, 90/10) to yield 8 mg ergosterol (1.2 mg/L medium). Fraction 4 (380 mg) was purified by normal-phase HPLC (EtOAc/hexane, 25/75) to give **6** (24 mg; 3.7 mg/L medium), and **7** (168 mg; 26 mg/L medium). Fraction 6 was purified by normal-phase HPLC (EtOAc/hexane, 50/50) to give **9** (23 mg; 3.5 mg/L medium) and **10** (12.7 mg; 2.0 mg/L medium).

Ulocladol (1): White solid (5.4 mg). − M.p. 110−111°C. − UV (EtOH): λ_{max} (log ε) = 251 nm (4.11), 299 (3.54). − IR (film): $\tilde{\nu}$ = 3440 cm^{-1}, 2930, 1660, 1650, 1615, 1345, 1155, 1095. − ^1H and ^{13}C NMR data, see Table 1. − EI MS; *m/z*: $[M]^+$ 318 (100), 300 (70), 282 (20), 254 (30). − $C_{15}H_{14}O_7$: calcd. 318.074; found : 318.073 (HR-EIMS; *m/z*).

1-Hydroxy-6-methyl-8-(hydroxymethyl)xanthone (3): White crystals (6.0 mg), m.p. 176−178°C. − Melting point, EI MS, and ^1H NMR data are as published.[9][10]

(+)-2,4-Dimethyl-4,5-dihydrofuran-3-carbaldehyde (4): Colorless oil (6.1 mg), $[\alpha]_D^{20}$ = +38.9 (*c* = 0.61, $CHCl_3$). − UV (EtOH): λ_{max} (log ε) = 272 (3.41) nm. − IR (film) $\tilde{\nu}$ = 2930 cm^{-1}, 2740, 2730, 1730, 1635, 1625, 1455, 1395, 1240, 1120, 1035. − ^1H NMR (400 MHz, $CDCl_3$): δ = 1.21 (3 H, d, *J* = 6.6 Hz, CH_3-4), 2.16 (br. s, 3 H, CH_3-2), 3.34 (m,1 H, H-4), 4.06 (dd, 1 H, *J* = 5.1 Hz, *J* = 9.2 Hz, H-5), 4.54 (dd, 1 H, *J* = 9.2 Hz, *J* = 9.7 Hz, H-5), 9.71 (s, 1 H, CHO). − ^{13}C NMR (100 MHz, $CDCl_3$): δ = 12.5 (q, CH_3-2), 19.3 (q, CH_3-4), 34.8 (d, C-4), 79.3 (t, C-5), 120.5 (s, C-3), 174.6 (s, C-2), 185.0 (d, CHO). − GC-EIMS; *m/z*: $[M]^+$ 126 (100), 111 (80), 83 (97), 55 (68), 43 (98). − HRMS, sample decomposed prior to this measurement.

(3*S*,5*R*)-Dihydrodimethylfuran-2-one (5): Colorless oil (5.3 mg). − $[\alpha]_D^{20}$ = +3.8 (*c* = 0.53, $CHCl_3$); ref.[12] $[\alpha]_D^{20}$ = +1.4 (*c* = 1, CH_2Cl_2).

Grahamimycin A_1 (6): Yellow solid (24 mg). − $[\alpha]_D^{20}$ = −5.5 (*c* = 0.5, $CHCl_3$); ref.[15] $[\alpha]_D^{20}$ = −14.2 (*c* = 0.45, $CHCl_3$).

Colletoketol (7): Light yellow crystals (EtOAc, 168 mg), mp. 142−143° C. − $[\alpha]_D^{20}$ = −24.0 (*c* = 0.68, $CHCl_3$). − ^1H NMR (300 MHz, $CDCl_3$): δ = 1.29 (d, 3 H, *J* = 6.4 Hz, CH_3-14), 1.38 (d, 3 H, *J* = 6.4 Hz, CH_3-6), 1.85 (ddd, 1 H, *J* = 3.0 Hz, *J* = 7.2 Hz, *J* = 15.6 Hz, H-13), 2.07 (ddd, 1 H, *J* = 2.6 Hz,*J* = 5.7 Hz, *J* = 15.6 Hz, H-13), 2.27 (ddd, 1 H, *J* = 10.9 Hz, *J* = 11.5 Hz, *J* = 12.8 Hz, H-5), 2.49 (dddd, 1 H, *J* = 1.1, *J* = 3.0, *J* = 5.1, 12.8 Hz, H-5), 3.70 (1 H, br. s, O*H*-12), 4.46 (1 H, dd, *J* = 3.0, 5.7 Hz, H-12), 5.09 (1 H, ddq, *J* = 3.0 Hz, *J* = 11.5 Hz, *J* = 6.4 Hz, H-6), 5.31 (ddq, 1 H, *J* = 2.6 Hz, *J* = 7.2 Hz, *J* = 6.4 Hz, H-14), 5.76 (br. d,1 H, *J* = 15.8 Hz, H-3), 6.67 (1 H, d, *J* = 15.8 Hz, H-9), 6.68 (1 H, ddd, *J* = 5.1, 10.9, 15.8 Hz, H-4), 7.12 (1 H, d, *J* = 15.8 Hz, H-10). − ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 18.8 (q, CH_3-14), 20.3 (q, CH_3-6), 40.2 (t, C-13), 40.3 (t, C-5), 66.6 (d, C-14), 70.6 (d, C-6), 73.0 (d, C-12), 126.8 (d, C-3), 132.2 (d, C-9), 134.2 (d, C-10), 143.7 (d, C-4), 165.4 and 165.8 (s, C-2/C-8), 201.3 (s, C-11). − All other properties as previously published.[16]

(2*E*,5*R*)-Hydroxy-2-hexenoic Acid (7a): 43 mg of **7** were hydrolysed with NaOH to yield **7a**:[16] clear oil (10.5 mg). − $[\alpha]_D^{20}$ = −11.6 (*c* = 1.05, EtOH); ref.[16] $[\alpha]_D^{20}$ = −108 (*c* = 1.0, EtOH). − ^1H NMR (300 MHz, $CDCl_3$): δ = 1.25 (d, 3 H, *J* = 6.2 Hz, H-6), 2.40 (ddt, 2 H, *J* = 1.1, *J* = 6.2 Hz, *J* = 7.5 Hz, H-4), 3.99 (tq, 1 H, *J* = 6.2 Hz, *J* = 6.2 Hz, H-5), 5.91 (dt, 1 H, *J* = 15.7 Hz, *J* = 1.1 Hz, H-2), 5.51 (br. s, 2 H, O*H*-5, COO*H*-1), 7.07 (dt, 1 H, *J* = 15.7 Hz, *J* = 7.5 Hz, H-3). − ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 23.2 (q, C-6), 41.8 (t, C-4), 66.8 (d, C-5), 123.2 (d, C-2), 147.7 (d, C-3), 170.8 (s, C 1). − FAB MS (glycerol, negative mode); *m/z*: $[M - H]^-$ 129 (100). − All other data as previously published.[16][24]

(6*R*,11*R*,12*R*,14*R*)-Colletodiol (8): Colorless crystals (680 mg), m.p. 164°C, ref.[16] m.p. 162−164°C. − $[\alpha]_D^{20}$ = +27.3 (*c* = 0.41, $CHCl_3$); ref.[18] $[\alpha]_D^{20}$ = +36.9 ($CHCl_3$).

Hydrogenation of (6*R*,11*R*,12*R*,14*R*)-Colletodiol (8): 31 mg of **8** in EtOAc (5 mL) was hydrogenated with H_2 and 5 mg 10% Pd/C. After stirring for 2 h the resulting mixture was purified by normal-phase (Si-60) VLC (acetone), followed by normal-phase (Si-60) HPLC (acetone/petroleum ether 50/50) to give **8a** and **8b**.

(6*R*,11*R*,12*R*,14*R*)-3,4,9,10-Tetrahydrocolletodiol (8a): White solid (8.0 mg). − ^1H NMR (300 MHz, $CDCl_3$): δ = 1.22 (d, 3 H, *J* = 6.4 Hz), 1.27 (d, 3 H, *J* = 6.4 Hz), 1.50−2.50 (m, 12 H), 3.58 (ddd, 1 H, *J* = 3.8 Hz, *J* = 4.9 Hz, *J* = 8.7 Hz), 3.72 (ddd, 1 H, *J* = 3.8 Hz, *J* = 3.8 Hz, *J* = 7.9 Hz), 5.04 (ddq, 1 H, *J* = 3.0 Hz, *J* = 12.4 Hz, *J* = 6.4 Hz), 5.15 (ddq, 1 H, *J* = 2.3 Hz, *J* = 13.2 Hz, *J* = 6.4 Hz).

(5*R*)-[(1′*R*,3′*R*)-Dihydroxybutyl]dihydrofuran-2-one (8b): Clear oil (8.9 mg). − $[\alpha]_D^{20}$ = −57.1 (*c* = 0.45, $CHCl_3$). − $[\alpha]_D^{20}$ = −45.5 (*c* = 0.1, EtOH); ref.[16] $[\alpha]_D^{20}$ = −42 (*c* = 0.7, EtOH). − IR (film): $\tilde{\nu}$ = 3440 cm^{-1}, 2965, 2920, 1765, 1645, 1455, 1420, 1375, 1195, 1135, 1035, 920. − ^1H NMR (300 MHz, $CDCl_3$): δ = 1.24 (d, 3 H, *J* = 6.4 Hz, H-4′), 1.59 (dt, 1 H, *J* = 14.5 Hz, *J* = 2.8 Hz, H-2′), 1.72 (dt, 1 H, *J* = 14.5 Hz, *J* = 10.0 Hz, H-2′), 2.13 (dddd, 1 H, *J* = 6.6 Hz, *J* = 7.9 Hz, *J* = 10.2 Hz, *J* = 13.0 Hz, H-4), 2.25 (dddd, 1 H, *J* = 6.0 Hz, *J* = 7.5 Hz, *J* = 9.8 Hz, *J* = 13.0 Hz, H-4), 2.50 (ddd, 1 H, *J* = 7.9 Hz, *J* = 9.8 Hz, *J* = 17.7 Hz, H-3), 2.64 (ddd, 1 H, *J* = 6.0 Hz, *J* = 10.2 Hz, *J* = 17.7 Hz, H-3), 3.04 (br. s, 2 H, O*H*), 3.89 (ddd, 1 H, *J* = 2.8 Hz, *J* = 4.3 Hz, *J* = 10.0 Hz, H-1′), 4.09 (ddd, 1 H, *J* = 2.8 Hz, *J* = 6.4 Hz, *J* = 10.0 Hz, H-3′),

4.42 (ddd, 1 H, J = 4.3 Hz, J = 6.6 Hz, J = 7.5 Hz, H-5). − ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 23.7 (t), 24.2 (q), 28.5 (t), 40.0 (t), 68.3 (d), 74.0 (d), 82.8 (d), 177.5 (s). − FAB MS (glycerol, negative mode); *m/z*:173 (100) $[M - H]^-$.

(6*R*,11*S*,12*S*,14*R*)-9,10-Dihydrocolletodiol (9): Colorless solid (23 mg). − $[\alpha]_D^{20}$ = −39.1 (c = 0.46, $CHCl_3$); ref.[19] $[\alpha]_D^{20}$ = −48 (c = 0.3, $CHCl_3$).

(6*R*,11*R*,12*R*,14*R*)-9,10-Dihydrocolletodiol (10): Colorless crystals (12.7 mg), m.p. 146−147°C. − $[\alpha]_D^{20}$ = −5.0 (c = 0.42, $CHCl_3$). − IR (film): $\tilde{\nu}$ = 3330 cm^{-1}, 2975, 2920, 1725, 1705, 1655, 1450, 1415, 1380, 1350, 1325, 1265, 1230, 1185, 1140, 1070, 1050, 1005 . − ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.28 (d, 3 H, J = 6.6 Hz, CH_3-14), 1.31 (d, 3 H, J = 6.1 Hz, CH_3-6), 1.61 (dddd, 1 H, J = 4.6 Hz, J = 5.6 Hz, J = 11.1 Hz, J = 13.7 Hz, H-10), 1.83 (ddd, 1 H, J = 3.1 Hz, J = 9.0 Hz, J = 15.3 Hz, H-13), 1.94 (ddd, 1 H, J = 2.1 Hz, J = 5.6 Hz, J = 15.3 Hz, H-13), 1.97 (dddd, 1 H, J = 4.8 Hz, J = 5.6 Hz, J = 7.4 Hz, J = 13.7 Hz, H-10), 2.28 (dddd, 1 H, J = 1.0 Hz, J = 8.1 Hz, J = 11.4 Hz, J = 13.2 Hz, H-5), 2.39 (ddd, 1 H, J = 4.6 Hz, J = 4.8 Hz, J = 13.2 Hz, H-9), 2.42 (ddd, 1 H, J = 3.0 Hz, J = 8.1 Hz, J = 13.2 Hz, H-5), 2.52 (ddd, 1 H, J = 5.6 Hz, J = 11.1 Hz, J = 13.2 Hz, H-9), 3.43 (ddd, 1 H, J = 4.6 Hz, J = 7.4 Hz, J = 10.7 Hz, H-11), 3.61 (ddd, 1 H, J = 3.1 Hz, J = 5.6 Hz, J = 10.7 Hz, H-12), 5.16 (ddq, 1 H, J = 3.0 Hz, J = 11.4 Hz, J = 6.1 Hz, H-6), 5.30 (ddq, 1 H, J = 2.1 Hz, J = 9.0 Hz, J = 6.6 Hz, H-14), 5.79 (br. d, 1 H, J = 15.8 Hz, H-3), 6.73 (ddd, 1 H, J = 8.1 Hz, J = 8.1 Hz, J = 15.8 Hz, H-4). − ^{13}C NMR (100 MHz, $CDCl_3$): δ = 20.8 (q, CH_3-14), 21.1 (q, CH_3-6), 28.0 (t, C-10), 30.4 (t, C-9), 37.6 (t, C-13), 39.0 (t, C-5), 66.7 (d, C-14), 69.2 (d, C-6), 70.5 (d, C-11), 71.7 (d, C-12), 125.0 (d, C-3), 143.4 (d, C-4), 165.3 (s, C-2), 173.1 (s, C-8). − EI MS; *m/z*: $[M]^+$ 286 (<1), 242 (5), 215 (10), 157 (15), 139 (20), 113 (100).

***p*-Bromobenzoylation of 10:** 5 mg of **10**, 15 mg of *p*-bromobenzoyl chloride and 3 mg of 4-(dimethylamino)pyridine were dissolved in 2 mL CH_2Cl_2, and kept at 35°C in an ultrasonic bath for 4 h. The reaction mixture was then purified by normal-phase VLC (hexane/EtOAc, 50/50) and normal-phase HPLC (hexane/EtOAc, 80/20) to give **10a**.

(6*R*,14*R*,11*R*,12*R*)-11,12-bis(*p*-Bromobenzoate)-9,10-dihydro-6,14-colletodiol (10a): Colorless oil (8.8 mg). − $[\alpha]_D^{20}$ = +3.6 (c = 0.29, $CHCl_3$). − UV (EtOH): λ_{max} (log ε) = 245 (4.46), 280 (sh, 2.95) nm. − CD (EtOH): $\Delta\varepsilon_{255.2\,nm}$ = −8.4, $\Delta\varepsilon_{241.2\,nm}$ = +7.8. − IR (film): $\tilde{\nu}$ = 2955 cm^{-1}, 2925, 1725, 1590, 1260, 1170, 1120, 1100, 1010, 755. − ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.30 (d, 3 H, J = 6.4 Hz, CH_3-14), 1.30 (d, 3 H, J = 6.0 Hz, CH_3-6), 1.99 (m, 1 H, H-13), 2.03 (m, 2 H, H-10), 2.14 (m, 1 H, H-13), 2.33 (m, 1 H, H-5), 2.44 (m, 2 H, H-9), 2.49 (m, 1 H, H-5), 5.12 (m, 1 H, H-14), 5.15 (m, 1 H, H-6), 5.33 (ddd, 1 H, J = 4.9 Hz, J = 7.2 Hz, J = 7.2 Hz, H-11), 5.56 (ddd, 1 H, J = 4.5 Hz, J = 4.5 Hz, J = 7.2 Hz, H-12), 5.93 (d, 1 H, J = 15.8 Hz, H-3), 6.93 (ddd, 1 H, J = 7.2 Hz, J = 8.7 Hz, J = 15.8 Hz, H-4), 7.54 (m, 2 H), 7.57 (m, 2 H), 7.86 (m, 2 H), 7.87 (m, 2 H). − ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 20.2 (q, CH_3-14), 20.8 (q, CH_3-6), 25.7 (t, C-10), 29.5 (t, C-9), 35.9 (t, C-13), 39.3 (t, C-5), 67.2 (d, C-14), 69.5 (d, C-6), 70.7 (d, C-12), 71.6 (d, C-11), 124.4 (d, C-3), 128.2 (s), 128.4 (s), 128.6 (s), 128.7 (s), 131.1 (d, 2 C), 131.2 (d, 2 C), 131.7 (d, 2 C), 131.9 (d, 2 C), 144.8 (d, C-4), 164.5 (s), 165.0 (s), 165.2 (s), 171.1 (s, C-8). − EI MS; *m/z*: $[M]^+$ 654 (1), 652 (3), 650 (1), 610 (<1), 608 (1), 606 (<1), 543 (1), 541 (2), 539 (1), 469 (5), 467 (5), 452 (3), 450 (3), 350 (4), 348 (4), 341 (12), 339 (12), 297 (3), 295 (3), 185 (98), 183 (100). − $C_{28}H_{29}{}^{79}Br^{81}BrO_8$: calcd. 652.013; found 652.013 (HR-EIMS; *m/z*).

Hydrogenation of 10: 5.2 mg of **10** were hydrogenated analogously to **8**, for 30 min to yield 4.8 mg of the dihydro derivative **10b**. ^{1}H NMR data were identical with those of **8a**. The decomposition products of **10b** were separated by HPLC as described for **8a**. One product proved to be identical (^{1}H-NMR, optical rotation) with **8b**.

Acknowledgments

We thank Dr. V. Wray and his group, GBF Braunschweig, for recording all NMR spectra, Dr. H.-M. Schiebel and Dr. U. Papke, Department of Chemistry, and Dr. L. Witte, Institute for Pharmaceutical Biology, TU Braunschweig, for mass spectral measurements, Ms. H.-S. Cho, Institute for Pharmaceutical Technology, for recording the CD-spectrum of **10a**, Ms. B. Störmann for work on the cultures of *V. ramulosa* grown on chitin and alga medium, and Ms. G. Matthée, Mr. C. Dreikorn and Ms. I. Rahaus for performing all bioassays. Sponge samples were identified by Dr. R. Desqueroux-Faundez, Musée d'Histoire Naturelle Genève (*C. vaginalis*), and Dr. D. Barthel, Institut für Meereskunde, Universität Kiel (*M. incrustans*). We also thank Dr. C. Schütt and the scientific dive team of the Biologische Anstalt Helgoland for collection of the sponge sample from Helgoland, Dr. S. Draeger, Institute for Microbiology, TU Braunschweig, for taxonomic identification of the fungal strains and Dr. B. Schulz for indispensable help with some of the mycological work. A grant from the German BMBF together with Bayer AG Leverkusen is gratefully acknowledged. Finally, we would like to express our sincere thanks to the Ministry of Agriculture, Fisheries Development Division, Commonwealth of Dominica, for allowing us to collect and export a number of marine samples in 1992.

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Received March 11, 1999
[O99142]