Rare Sesquiterpenes from the Algicolous Fungus *Drechslera dematioidea*

Claudia Osterhage,[†] Gabriele M. König,[†,‡] Ulrich Höller,[‡] and Anthony D. Wright*[,†,‡]

Institute for Pharmaceutical Biology, Technical University of Braunschweig, Mendelssohnstrasse 1, 38106 Braunschweig, Germany

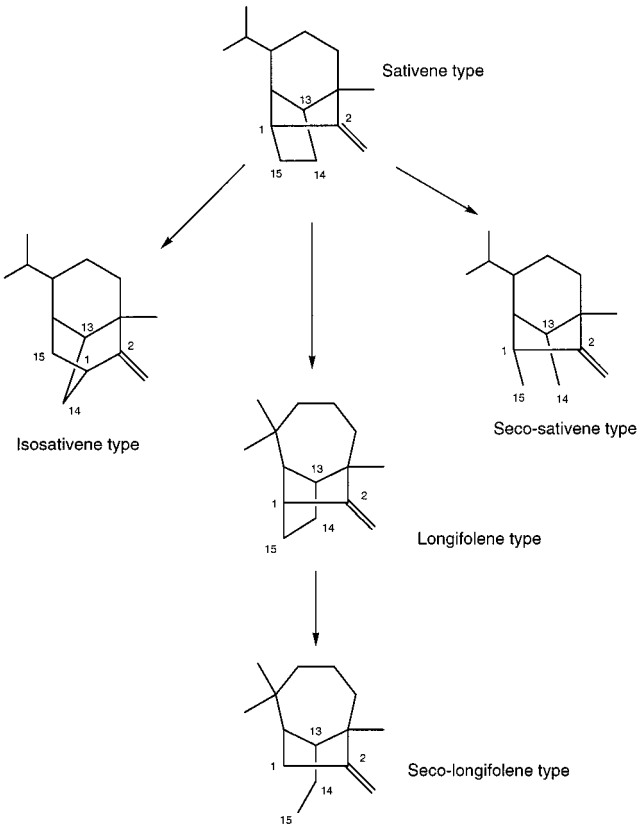
Received February 23, 2001

From the inner tissue of the marine red alga *Liagora viscida* the fungus *Drechslera dematioidea* was isolated. After mass cultivation, the fungus was investigated for its secondary metabolite content, and 10 new sesquiterpenoids [isosativenetriol (**1**), drechslerines A (**2**) and B (**3**), 9-hydroxyhelminthosporol (**5**), drechslerines C−G (**6**−**10**), and sativene epoxide (**12**)] were isolated. Compounds **8** and **10** exhibited antiplasmodial activity against *Plasmodium falciparum* strains K1 and NF54. The known compounds helminthosporol (**4**), *cis*-sativenediol (**11**), isocochlioquinone A (**14**), isocochlioquinone C (**15**), and cochlioquinone B (**16**) were also isolated. All structures were elucidated using spectroscopic methods, mainly 1D and 2D NMR and MS.

Marine-derived fungi represent a valuable resource in the search for secondary metabolites with potential therapeutic importance.[1,2] Almost one-third of all known higher filamentous marine fungi are associated with algae.[3] These plants are thus an interesting source for the isolation of fungal strains. Fungi living in algae have to deal with their host organisms; thus they might be especially creative in producing biologically active compounds in a fashion similar to their terrestrial counterparts,[4] those found predominantly in higher plants. If this proposal is correct, fungal isolates from algae will yield many new and biologically active natural products. To some extent this is already reflected by the increasing number of reports of new secondary metabolites from this field, e.g., communesins,[5] leptosins,[6−8] penochalasins,[9] penostasins,[10,11] and pyrenocines D and E.[12]

The genus *Drechslera* (*Helminthosporium*) contains many terrestrial species that have been investigated for their natural products content. Ophiobolins,[13,14] triticones A and B,[15] and eremophilane sesquiterpenes[16] have all been isolated from *Drechslera* species. During our investigations into fungal strains associated with marine algae the phytopathogenic fungus *Drechslera dematioidea* (Bubak and Wroblewski) Subram. and Jain, 1966, was obtained from the marine red alga *Liagora viscida* (Forskkal) C. Agardh, collected from the Mediterranean Sea, Moraira, Spain. The antimicrobial activity of the EtOAc extract of this fungus led us to the current investigation. The majority of compounds isolated in this study belong to four classes of unusual irregular terpenoids, i.e., isosativene (**1**), helminthosporene or seco-sativene (**2**−**10**), sativene (**11** and **12**), and secolongifolene (**13**) (Scheme 1).

Scheme 1. Relationship between the Four Main Structural Classes Represented by Compounds **1**−**13**



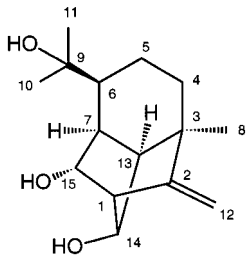
Results and Discussion

The fungus *D. dematioidea* was cultivated on a solid biomalt medium. Successive fractionation of the ethyl acetate (EtOAc) extract by vacuum-liquid chromatography (VLC) and normal- and reversed- (RP-C_{18}) phase HPLC yielded 16 compounds (**1**−**16**), 13 of which (**1**−**13**) are sesquiterpenoids, the remaining three (**14**−**16**) being of mixed biosynthetic origin.

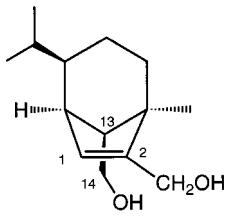
Isosativenetriol (**1**) has the molecular formula $C_{15}H_{24}O_3$ as deduced by FABMS, GC−MS analysis of a TMS derivative, and accurate mass measurement. From its ^{13}C NMR spectroscopic data it was evident that one of the four elements of unsaturation, indicated by the molecular formula of **1**, could be attributed to an *exo*-methylene group [δ 105.5 (C-12, t), 156.9 (C-2, s)] as the only multiple bond within the molecule; isosativenetriol is thus tricyclic. The ^1H and ^{13}C NMR spectra showed the presence of two further methylene groups, six methine groups, two of them attached to oxygen, and three methyl groups, all adjacent to quaternary carbons [δ 41.5 (C-3, s), 74.0 (C-9, s)], one of which is bonded to oxygen. These data also showed that all but three of the hydrogens were bonded directly to

* To whom correspondence should be addressed.
[†] Technical University of Braunschweig.
[‡] Current address: Institute for Pharmaceutical Biology, University of Bonn, Nussallee 6, 53115 Bonn, Germany. Tel: +49 228 73 3198. Fax: +49 228 73 3250. E-mail: a.wright@uni-bonn.de. Internet: http://www.uni-bonn.de/pharmbio/queen/GAWK.html.

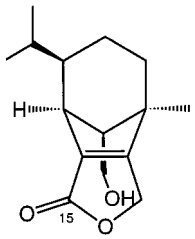
10.1021/np010092l CCC: $22.00
Published on Web 02/14/2002



1



2



3

carbons, indicating the remaining three to be present as part of hydroxyl functions. After assignment of all protons to their directly bonded carbons via a one-bond $^1H-^{13}C$ shift correlated 2D NMR measurement (HMQC), it was possible to deduce from the $^1H-^1H$ COSY spectrum of **1** a $^1H-^1H$ spin-system from H_2-4 to H-7, H-7 further couples with H-13, which also couples with H-14, and H-14 couples in turn with H-1, which further couples with H-15, which in turn couples with H-7. These couplings indicated C−C bonds to occur between C-4 and C-5, C-5 and C-6, C-6 and C-7, C-7 and C-13, C-13 and C-14, C-14 and C-1, C-1 and C-15, and C-15 and C-7, and completed the first two rings within the molecule. Diagnostic long-range $^1H-^{13}C$ HMBC correlations observed from the resonance of H_3-8 to those of C-2, C-3, C-4, and C-13 showed C-8 to bond to the quaternary carbon C-3, which further bonded to C-2, C-4, and C-13. The long-range correlations observed between the resonances for H_2-12 and that of C-1 indicated C-1 and C-2 to bond directly and enabled the third ring within **1** to be completed. Further long-range correlations, this time observed between the resonances for H_3-10 and H_3-11 and those of C-6 and C-9, showed the two methyl groups to reside on the quaternary carbon C-9, which is further bonded to C-6. Remaining to be incorporated into the planar structure were the hydroxyl functions. On the basis of the ^{13}C NMR chemical shifts of C-9 (δ 74.0, s), C-14 (δ 78.6, d), and C-15 (δ 76.0, d) it was evident that the hydroxyl functions must be located at C-9, C-14, and C-15. As one of the two protons located at C-5 (H-5ax) had three large coupling constants (>10 Hz) and one small one (<5 Hz), it had to have an axial orientation. This deduction also meant that H-6 had to be axial and on the opposite side of the molecule from H-5, and thus gave the six-membered ring a chair conformation. As H-6 has only one coupling constant indicative of an axial−axial interaction, with H-5, H-7 was given an equatorial orientation, and in so doing placed the C-7−C-15−C-1−C-2−C3 part of **1** on the upper face of the molecule. This deduction also meant the methyl group at C-3 had to be equatorial and H-13 had to be axial. The relative configuration at C-15 was deduced to be as shown in **1** based on $J_{H-7,H-15} < 2$ Hz, which meant these two protons must be at almost 90° to one another. The deduced relative stereochemistry of **1** and the shown configuration at C-14 were further supported by NOE difference measurements. Thus, irradiation at the resonance frequency of H-1 caused enhancement of the resonance of H-14, one part of H_2-12 and H-15. Irradiation at the resonance frequency of H-7 gave enhancement of the resonances of H-13 and H-6. Irradiation at the resonance frequency of H_3-10 and H_3-11 caused enhancement of the resonance for H-15. Finally, irradiation at the resonance frequency of H_3-8 gave enhancement of one of the resonances associated with H_2-12, one of the H_2-4 resonances, and the resonances associated with H-13 and H-14. Thus, the relative stereochemistry for isosativenetriol (**1**) is best described as being 1*R**, 3*S**, 6*R**, 7*S**, 13*R**, 14*R**, *15S**. Isosativenetriol seems to be identical in structure to the triol reported by Dorn and Arigoni.[17] The lack of any spectroscopic data in the work by these authors, however, makes any reasonable comparison between the two structures impossible. The two compounds also have significantly different optical rotations (−2.0° for **1** as compared to +11° for the compound reported by Dorn and Arigoni).

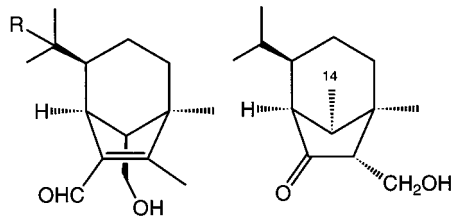
From accurate mass measurement drechslerine A (**2**) was found to have the molecular formula $C_{14}H_{24}O_2$. Its ^{13}C NMR spectrum contained 14 signals (see Table 1). These data also indicated the molecule to have only one multiple bond, a carbon−carbon double bond [δ 124.2 (C-1, d), 147.1 (C-2, s)], and showed the two remaining elements of unsaturation in the molecule to be in the form of rings. As all but two protons could be associated with directly bonded carbon atoms via an HMQC measurement, it was evident that the oxygen atoms within **2** must be present in the form of two hydroxyl functions. The ^1H and ^{13}C NMR spectra further revealed the presence of four methylene groups, two of them attached to the hydroxyl groups [δ 59.9 (C-12, t), 62.7 (C-14, t), (3300 cm^{-1})], four methine groups, three methyl groups, and a further quaternary carbon. From the $^1H-^1H$ COSY it was possible to deduce the majority of the planar structure of compound **2**. Thus, $^1H-^1H$ COSY cross-peaks revealed a continuous chain of coupling from H_3-10 and H_3-11, via H_2-5 to H_2-4, and via H-7 to H-13, H_2-14, and H-1. Diagnostic long-range $^1H-^{13}C$ 2D NMR correlations between the resonances of H_2-12 and those of C-1 and C-2 showed C-12 to bond to C-2. Further long-range correlations observed between the resonance of H_3-8 and those of C-2, C-3, C-4, and C-13 clearly positioned CH_3-8 at C-3 and showed C-3 to also bond with C-2, C-4, and C-13 and in so doing enabled the two rings within **2** to be completed. The two primary hydroxyl functions were thus CH_2OH-12 and CH_2OH-14, a deduction supported also by their ^1H and ^{13}C NMR chemical shifts. With the planar structure of **2** established the relative configuration at the four chiral centers within the molecule remained to be determined primarily from the results of NOE difference measurements. Thus, irradiation at the resonance frequency of H_3-11 caused enhancement of the resonances for H-1 and H-7. NOEs were also detected between H-7 and H-1, and H-7 and H_2-14. These NOE data, and the fact that the coupling constant between H-7 and H-13 is less than 3 Hz and that H-1 and H-13 "W" couple, are consistent with the relative configuration of **2** being described as 3*R**, 6*R**, 7*R**, 13*S**.

Table 1. ^{13}C NMR Data (ppm) for Compounds **1**−**13**[a]

position	**1**[c]	**2**[b]	**3**[c]	**4**[c]	**5**[c]	**6**[c]	**7**[c]	**8**[c]	**9**[b]	**10**[c]	**11**[d]	**12**[b]	**13**[b]
1	59.6 d[e]	124.2 d	134.9 s	137.3 s	139.1 s	n.o.[f]	38.2 d	36.8 d	51.9 d	56.6 d	55.8 d	52.9 d	128.3 d
2	156.9 s	147.1 s	180.9 s	165.9 s	170.7 s	55.0 d	50.5 d	52.3 d	51.6 d	89.0 s	159.3 s	68.4 s	147.9 s
3	41.5 s	47.7 s	47.8 s	50.8 s	52.2 s	41.9 s	45.1 s	44.2 s	42.2 s	49.4 s	44.0 s	39.9 s	51.4 s
4	39.1 t	35.8 t	35.6 t	34.2 t	34.9 t	36.2 t	41.3 t	41.7 t	37.7 t	30.5 t	41.2 t	37.3 t	45.7 t
5	20.7 t	26.4 t	27.0 t	25.2 t	21.8 t	26.0 t	21.1 t	21.5 t	26.0 t	24.1 t	26.8 t	26.0 t	22.1 t
6	48.8 d	45.4 d	45.3 d	44.9 d	49.8 d	50.2 d	49.8 d	50.9 d	47.0 d	44.9 d	43.7 d	43.4 d	42.7 t
7	46.3 d	43.9 d	41.3 d	41.3 d	40.7 d	51.4 d	52.5 d	50.5 d	41.1 d	39.5 d	43.0 d	42.5 d	36.7 s
8	28.8 q	19.0 q	17.8 q	18.4 q	18.6 q	22.0 q	19.8 q	20.2 q	18.8 q	20.9 q	21.1 q	19.6 q	21.8 q
9	74.0 s	33.8 d	33.7 d	31.8 d	72.9 s	30.0 d	72.6 s	73.2 s	32.2 d	30.6 d	34.3 d	34.5 d	58.9 d
10	28.9 q	21.6 q	21.8 q	21.7 q	28.8 q	21.5 q	29.3 q	28.9 q	21.4 q	20.0 q	21.5 q	21.5 q	32.5 q
11	27.1 q	21.3 q	21.0 q	20.7 q	28.3 q	20.4 q	28.6 q	28.8 q	21.2 q	18.1 q	21.3 q	21.3 q	27.9 q
12	105.5 t	59.9 t	69.3 t	10.6 q	11.0 q	62.1 t	69.5 t	64.9 t	71.6 t	61.6 t	103.3 t	51.1 t	59.7 t
13	56.6 d	63.9 d	68.1 d	61.3 d	64.5 d	50.7 d	152.6 s	157.8 s	67.8 s	52.5 d	59.7 d	59.6 d	47.0 d
14	78.6 d	62.7 t	61.9 t	62.5 t	61.8 t	6.3 q	105.8 t	100.9 t	51.0 t	69.0 t	76.0 d	69.9 d	35.2 t
15	76.0 d		173.8 s	188.2 d	192.5 d		171.6 s	103.8 d	174.5 s	203.3 d	70.0 d	73.2 d	62.1 t
16								54.7 q					

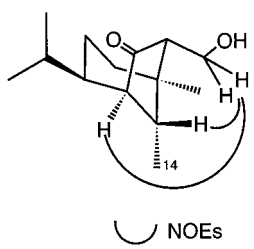
[a] Compounds **1**−**3**, **5**, **9**, and **11**−**13** were measured in CD_3OD, and compounds **4**, **6**−**8**, and **10** in $CDCl_3$. All assignments are based on extensive 1D and 2D NMR measurements (COSY, HMQC, HMBC). [b] Spectra were recorded at 75.5 MHz. [c] Spectra were recorded at 100 MHz. [d] Spectra were recorded at 150 MHz. [e] Implied multiplicity by DEPT (C = s, CH = d, CH_2 = t, CH_3 = q). [f] n.o. = not observed.

Drechslerine B (**3**) analyzed for $C_{15}H_{22}O_3$ by accurate mass measurement. The ^{13}C NMR spectral data of **3** were very similar (see Table 1) to those found for **2**; it was thus concluded that the two compounds were closely related. Careful examination of all of the spectroscopic data of **2** and **3** indicated the differences between the two data sets to arise from the presence of an extra carbon (C-15) in **3**, which was assigned to an α,β-unsaturated lactone function (δ 173.8, s), a deduction that was also supported by both the IR (1775 cm^{-1}) and UV (λ_{max} 235 nm) data of **3**. The electron-withdrawing effects of the lactone function significantly influenced the chemical shifts of C-1 (δ 134.9, s), C-2 (δ 180.9, s), and C-12 (δ 69.3, t), relative to the equivalent centers in **2** (δ 124.2, d, 147.1, s, 59.9, t, respectively). These data also showed the ester function to be between C-1 and C-12. NOE difference measurements similar to those made for **2**, as well as coupling constant analyses, revealed **2** and **3** to have identical relative stereochemistries.

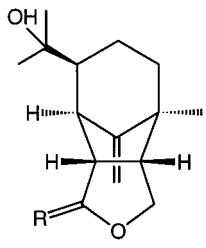


4 R = H
5 R = OH

6 Planar view



6 3D view



7 R = O
8 R = α H, β OCH_3

Accurate mass measurement showed **4** to have the molecular formula $C_{15}H_{24}O_2$. Comparison of its ^1H NMR, UV, and IR data and optical rotation with published values enabled **4** to be identified as the known compound helminthosporol or a stereoisomer thereof.[18] As helminthosporol was only partially characterized in the literature, 1D and 2D NMR experiments together with NOE difference measurements allowed the complete structure to be deduced as shown in **4**. Since the optical rotations of our metabolite and the one reported for helminthosporol[18] are essentially identical, it was concluded that **4** is indeed helminthosporol. The first reported isolation of helminthosporol was from the culture broth of *Helminthosporium sativum*.[18] This compound is probably most noted for its ability to inhibit Acyl-CoA cholesterol acyltransferase (ACAT).[19]

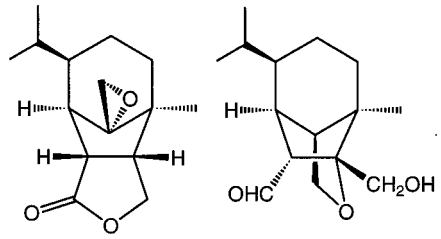
A compound similar in structure to **4** was also obtained from the EtOAc extract of *D. dematioidea*. This compound, 9-hydroxyhelminthosporol (**5**), differs from **4** by the presence of a hydroxyl group at C-9, as evidenced by the ^{13}C chemical shift and multiplicity of C-9 (δ 72.9, s) when compared to the corresponding carbon in **4** (δ 31.8, d). All other NMR data for the two compounds were in good agreement with each other. In a relative sense, the two molecules were deduced to have the same stereochemistry on the basis of the similarities between the two NMR data sets, in particular the fact that all of the couplings associated with H-7 are between 0 and 2 Hz.

Drechslerine C (**6**) was found to have the molecular formula $C_{14}H_{24}O_2$ by accurate mass measurement. Its ^{13}C NMR spectrum contained only 13 resonances (see Table 1). The resonance for the carbonyl group, C-1, was not observed, but this functionality was clearly present, as evidenced by the strong 1730 cm^{-1} absorption in the IR spectrum of **6**. From all the spectroscopic data of **6** only one multiple bond, the C═O group, was evident, indicating the molecule to be bicyclic and the remaining oxygen within the molecule to be present in the form of an OH group. The ^1H and ^{13}C NMR spectra further revealed the presence of three methylene groups, one of them attached to the hydroxyl group, five methine groups, and four methyl groups, one of them attached to a quaternary carbon. From the $^1H-^1H$ COSY spectrum of **6** coupling between H_3-10, H_3-11, and H-9, and from there via H-6 to H_3-14, was observed and showed C-10 and C-11 to be bonded to C-9, C-9 to C-6, C-6 to C-7, C-7 to C-13, and C-13 to C-14. In the $^1H-^1H$ COSY spectrum coupling was also seen between H-6 and H_2-5, and H_2-5 and H_2-4, and revealed C-6 to bond to C-5, and the latter to also bond to C-4. A second fragment of **6** was deduced from the $^1H-^1H$ coupling of H-2 to H_2-12, which revealed C-2 to be bonded to C-12. From

diagnostic $^1H-^{13}C$ long-range correlations (HMBCs) between the resonance for H_3-8 and those of C-2, C-3, C-4, and C-13 it was evident that the methyl group CH_3-8 bonded to C-3, which further bonded to C-2, C-4, and C-13, thus completing one of the two rings within **6**. This only left the carbonyl group to be attached to C-2 and C-7, which completed the second ring within the molecule and also its planar structure and left the relative configurations at C-2, C-6, C-7, and C-13 to be established. As all of the coupling constants associated with H-7 were less than 2 Hz, it was evident that H-7 must be almost at 90° to both H-6 and H-13, giving the molecule the same relative configurations at C-3, C-6, and C-7 as those shown for compounds **2**−**5**. NOE interactions between the protons at C-12 and H-13 and H-7 enabled the relative stereochemistries at C-2 and C-13 to be resolved as shown in **6**.

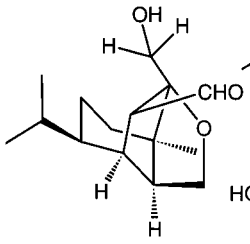
Mass spectral analysis of **7**, drechslerine D, indicated it to have the molecular formula $C_{15}H_{22}O_3$. Its ^{13}C NMR spectrum contained 15 resonances (see Table 1), of which three were associated with multiple bonds—an ester carbonyl function, which was also seen in the IR spectrum (1730 cm^{-1}), and an *exo*-methylene group [δ 152.6 (C-13, s), 105.8 (C-14, t)]—the molecule is thus tricyclic. The ^1H and ^{13}C NMR spectra together revealed the presence of three further methylene groups [δ 21.1 (C-5, t), 41.3 (C-4, t)], one of them attached to oxygen, four methine groups, three methyl groups, and two further quaternary carbons, one of them attached to oxygen. The remaining oxygen in the molecule is in the form of a hydroxyl function. In the $^1H-^1H$ COSY spectrum cross-peaks between the resonances for H_2-12 and that of H-2, and between those of H-2 and H-1, H-1 and H-7, H-7 and H-6, H-6 and H_2-5, and H_2-5 and H_2-4, were observed and enabled the C-4 to C-12, via C-7 and C-2, part of the molecule to be deduced. Long-range $^1H-^{13}C$ correlations from the resonances for H_2-12 to that for C-15 enabled the lactone to be positioned between C-12 and C-15. Further long-range $^1H-^{13}C$ correlations from the resonance for H-7 to those of C-13 and C-15, and from the resonances for H_2-14 to those of C-3 and C-7, showed C-13 to bond to C-3 and C-7, and C-1 to bond to C-15, and so completed the two remaining rings within the molecule. Further, the isopropyl group was identified and positioned; the resonances for the methyl groups H_3-10 and H_3-11 showed HMBC correlations to the resonances for C-9 and C-6. As both methyl groups are tertiary, they must bond to C-9. The chemical shift of C-9 (δ 72.6) which bonds to C-6 showed the hydroxyl group also to reside at C-9. Further long-range correlations from the resonance for H_3-8 to those for C-2, C-3, C-4, and C-13 enabled the connection from C-8 to C-3, which is further bonded to C-2, C-4, and C-13, to be established, and hence the basic structure of the molecule to be completed. The results of NOE difference measurements and coupling pattern analyses then allowed the relative configuration of the molecule to be deduced as shown in **7**. Thus, irradiation at the resonance frequency of H-1 caused enhancement of the resonance for H-2, and vice versa, and showed both protons to be positioned on the same side of the molecule. As irradiation at the resonance frequency of H-1 also caused enhancement of the resonances for H-5, H-7, H_3-10, and H_3-11, it was evident that H-1, H-2, and the isopropanoyl group had to be β-oriented and that the C-7, C-13, C-3 bridge was α-oriented.

The MS and ^1H and ^{13}C NMR chemical shift data of drechslerine E (**8**) showed it to be the 15-acetal derivative of **7**. The orientation of the methoxyl group was deduced as being β on the basis of the NOE interaction observed between H-7 and H-15.

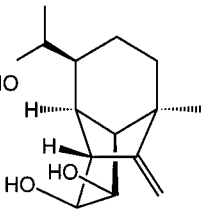


9

10 Planar view



10 3D view



11

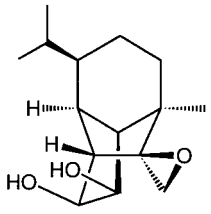
The spectroscopic data of **9** (drechslerine F) indicated it to have a structure very similar to those of **7** and **8**. The major differences between the three data sets are attributable to an *exo*-epoxy group in **9** [δ 67.8 (C-13, s), 51.0 (C-14, t)], instead of the *exo*-methylene group present in **7** and **8**, and the absence, in **9**, of a hydroxyl group at C-9. Low-power irradiation at the resonance frequencies of H_3-10 and H_3-11 caused enhancement of the resonances of H-1, H_2-5, and H-7 and showed the isopropyl group, H-1, and H-7 to have the same relative orientation as in **7** and **8**. Low-power irradiation at the resonance frequency for H_3-8 caused enhancement of the resonances of H-2, H_2-4, H-12 (δ 4.49), and H_2-14 (δ 2.92) and showed the epoxy group to have the orientation as shown in **9**. The $^1H-^1H$ coupling information also supported these deductions.

Drechslerine G (**10**) analyzed for $C_{15}H_{24}O_3$ by accurate mass measurement. Of the four elements of unsaturation indicated by the molecular formula of **10**, one was attributed to an aldehyde group [δ 203.3 (d, C-15)], which also accounted for all of the multiple bonds within the molecule; **10** is thus tricyclic. From its IR spectrum the presence of the aldehyde group (1715 cm^{-1}) was supported and a hydroxyl functionality (3440 cm^{-1}) was evident. The remaining oxygen in the molecule had, thus, to be present as an ether function. In the $^1H-^1H$ COSY spectrum cross-peaks observed between the resonances for H_3-10 and H_3-11 and that for H-9, between that for H-9 and that for H-6, between that for H-6 and those for H_2-5, and between those for H_2-5 and those for H_2-4 showed C-10 and C-11 to be bonded to C-9, which is further bonded to C-6, C-6 to C-5, and C-5 to C-4. Further $^1H-^1H$ COSY cross-peaks between the resonance for H-6 to that for H-7, between that for H-7 to that for H-1, and between that for H-1 to that for H-15 revealed C-6 to bond also to C-7, C-7 to C-1, and C-1 to the aldehyde carbon, C-15. Additionally, $^1H-^1H$ COSY cross-peaks were present which showed H-7 to couple with H-13, and H-13 with H_2-14, and revealed C-7 to bond also to C-13, and C-13 to further bond with C-14. The hydroxyl group proton (δ 2.24 brm) couples with H_2-12 (δ 3.88) and clearly showed this function to reside at C-12. Consequently, C-2 (δ 89.0, s) and C-14 (δ 69.0, t), which are both attached to oxygen, as evidenced by their ^{13}C NMR chemical shifts, are both bonded to the oxygen of the ether moiety. Diagnostic

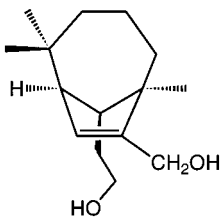
long-range $^1H-^{13}C$ 2D NMR correlations observed between the resonances for H_3-8 and those for C-2, C-3, C-4, and C-13 showed C-8 to be bonded to C-3, which is further bonded to C-2, C-4, and C-13, and in so doing completed two of the three rings within the molecule. Further long-range correlations, this time seen between the resonances for H_2-12 and those for C-1 and C-2, showed C-12 to bond to C-2, which further bonded to C-1, and thus completed the third and final ring and the planar structure of **10**. On the basis of the magnitude of $^1H-^1H$ coupling constants, particularly those between H-7 and H-1, H-7 and H-6, and H-7 and H-13 (<2 Hz), and also the rigidity of the molecule, the relative configuration for **10** is proposed as that shown.

Comparison of the ^1H NMR data and optical rotation for **11** (see Table 2) with those published for *cis*-sativenediol,[20] a plant growth promotor, showed the two compounds to be identical. This deduction was also supported by the ^{13}C NMR (see Table 1) and MS data of **11**.

MS analysis of sativene epoxide (**12**) showed it to have the molecular formula $C_{15}H_{24}O_3$. Comparison of its ^1H and ^{13}C NMR spectral data with that of **11** revealed the two compounds to be structurally very similar. Evident from the ^1H and ^{13}C NMR data of **12** was the absence of any resonances associated with a 2,12 *exo*-methylene group and the presence of resonances attributable to an *exo*-epoxy function in its place. All of the remaining physical and spectroscopic data showed the remaining structural features of the two molecules to be identical, in a relative sense. The relative configuration at C-2 was deduced as being as shown in **12** on the basis of the NOEs observed between H_3-8 and the epoxy proton (δ 2.79) resonance, and between the other epoxy proton (δ 2.74) and H-15. These NOE interactions also further supported the overall relative configurations shown for **11** and **12**.



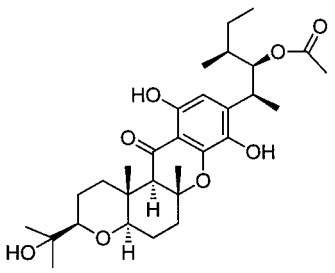
12



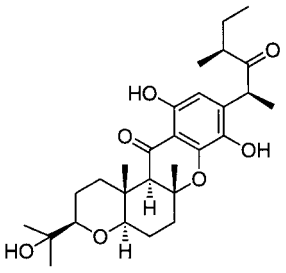
13

Mass spectral analysis of **13** [(+)-secolongifolene diol] showed it to have the molecular formula $C_{15}H_{26}O_2$. All of the remaining data for this compound (^1H and ^{13}C NMR both 1D and 2D) clearly showed **13** to be a compound identical to the one reported by Dorn and Arigoni[21] and the optical antipode of the one synthesized by Yadav et al.[22]

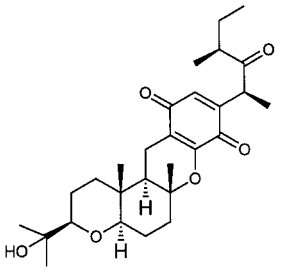
Together with compounds **1**−**13**, isocochlioquinones A and C (**14** and **15**) and cochlioquinone B (**16**) were also isolated from the EtOAc extract of *D. dematioidea*. Isocochlioquinone A was first isolated from a culture of *Bipolaris bicolor* El-1, a fungal pathogen of gramineous plants.[23] Isocochlioquinone C, also a plant fungal pathogen, was also first isolated from a culture of *Bipolaris*, this time *B. cynodontis* cynA.[24] Culture filtrates of the perfect stage of *B. oryzae*, *Cochliobolus miyabeanus*, were the original source of cochlioquinone B,[25,26] which has also been isolated from *B. bicolor*.[23] Studies into the biosynthetic origin of the carbon skeleton of the cochlioquinones showed their mixed biosynthesis occurred by the introduction of a farnesyl unit onto an aromatic precursor whose secondary methyl groups derived from methionine. All three cochlioquinone derivates inhibit the root growth of gramineous plants and electron



14



15



16

transfer in the mitochondrial respiratory system.[27] It appears unusual that the irregular terpenoids **1**−**13** and the merosesquiterpenes **14** and **15** co-occur in the same organism.

All compounds, with the exceptions of **6**, **9**, and **11**, were tested for their antimicrobial and antialgal activities in agar diffusion assays, in ELISA-based assays in order to evaluate their HIV-1 reverse transcriptase and tyrosine kinase ($p56^{lck}$) inhibitory activity, and in assays to investigate their activities against brine shrimps, nematodes, and *Mycobacterium tuberculosis* as well as for their antiplasmodial activity (Table 3). Five of the compounds, **4**, **8**, **10**, **14**, and **16**, inhibited the growth of the malaria-causing protozoan of *Plasmodium falciparum* to a significant extent (IC_{50}s $\leq$ 5.1 μg/mL). The quinoid-containing compound (**16**) markedly reduced the activity of the enzyme tyrosine kinase $p56^{lck}$. The majority of compounds demonstrated moderate antifungal activity. In the assays that investigated activity against brine shrimp, nematodes, and *M. tuberculosis* all of the tested compounds were inactive.

Experimental Section

General Experimental Procedures. The general experimental procedures were carried out as previously described.[28]

Isolation and Taxonomy. Algal material was collected from the Mediterranean Sea, Moraira, Spain. After surface sterilization with 70% ethanol for 40 s algal samples were rinsed with sterile seawater. Sterilized algae were then cut into small pieces and placed on agar plates containing isolation medium (15 g of agar in seawater from the sample collecting site and 250 mg of each of the antibiotics benzylpenicillin and streptomycin sulfate). Fungal colonies growing out of the algal tissue were transferred to medium for sporulation (1.0 g of glucose, 0.1 g of yeast extract, 0.5 g of peptone from meat, enzymatic digest, 15 g of agar, and seawater, pH 8) in order to enable isolates to be taxonomically identified. The fungal strain, *Drechslera dematioidea*,[29] voucher number MOR5 test 2-1, was identified by Dr. S. Draeger, Institute for Microbiology, Technical University of Braunschweig.

Cultivation. The fungus was cultured at 20 °C for 30 days in 13.5 L of solid medium containing 20 g/L biomalt extract, 6.8 g/L agar, and demineralized water.

Biological Activity. Activity of extracts, VLC and HPLC fractions, and pure compounds was tested in agar diffusion assays against the bacteria *Bacillus megaterium*, *Escherichia coli*, the fungi *Microbotryum violaceum*, *Eurotium repens*,

Table 2. ^{1}H NMR Data [δ (multiplicity, J in Hz)] for Compounds **1**−**13**[a]

position	**1**[c]	**2**[b]	**3**[d]	**4**[d]	**5**[b]	**6**[c]
1	2.68 (brs)	5.59 (brd, 1.5)				
2						1.69 (dd, 5.1, 8.1)
4	1.38 (ddd, 1.5, 13.0, 13.2) 1.75 (3.1, 3.1, 13.2)	1.43 (m), 1.29 (m)	1.60 (m), 1.63 (m)	1.41 (m)	1.55 (m)	1.41 (ddd, 5.6, 5.9, 13.7), 1.65 (ddd, 1.5, 7.6, 13.7)
5	1.19 (dddd, 1.5, 3.1, 3.1, 13.0) 1.64 (dddd, 3.1, 12.8, 13.0, 13.0)	1.68 (m) 1.24 (m)	1.94 (dddd, 3.5, 3.5, 3.6, 13.3) 0.92 (m)	1.75 (m) 0.88 (m)	1.76 (m) 0.97 (m)	1.79 (dddd, 1.5, 5.6, 5.6, 14.2) 0.86 (dddd, 5.9, 7.6, 13.6, 14.2)
6	1.51 (ddd, 3.1, 3.1, 12.8)	1.09 (m)	1.20 (m)	0.99 (m)	1.54 (m)	1.31 (dddd, 2.5, 5.6, 10.7, 13.6)
7	2.62 (brm)	2.80 (brs)	3.20 (brs)	3.19 (brs)	3.27 (brs)	2.68 (brs)
8	1.04 (s)	1.00 (s)	1.20 (s)	1.04 (s)	1.09 (s)	1.08 (s)
9		1.28 (m)	1.20 (m)	0.98 (brs)		1.49 (m)
10	1.33 (s)	0.99 (d, 6.8)	1.10 (d, 6.1)	1.08 (d, 5.4)	1.34 (s)	1.01 (d, 6.6)
11	1.27 (s)	0.89 (d, 6.8)	0.90 (d, 6.1)	0.77 (d, 5.4)	1.16 (s)	0.81 (d, 6.6)
12	5.03 (s) 4.77 (s)	4.04 (dd, 1.5, 14.3) 4.12 (ddd, 1.0, 1.1, 14.3)	4.97 (dd, 1.3, 18.3) 4.90 (d, 18.3)	2.02 (s)	2.12 (s)	3.84 (dd, 5.1, 10.7) 3.49 (dd, 8.1, 10.7)
13	1.78 (m)[e]	1.58 (dd, 5.3, 9.4)	2.05 (dd, 5.1, 9.7)	1.68 (dd, 5.4, 8.7)	1.77 (m)	2.09 (brq, 7.1)
14	4.31 (m)	3.68 (dd, 5.3, 10.6) 3.41 (dd, 9.4, 10.6)	3.76 (dd, 5.1, 11.2) 3.39 (dd, 9.7, 11.2)	3.65 (dd, 5.4, 10.8) 3.33 (dd, 8.7, 10.8)	3.64 (dd, 4.6, 10.7) 3.23 (dd, 9.7, 10.7)	0.95 (d, 7.1)
15	3.75 (brs)			10.0 (s)	9.98 (s)	

position	**7**[c]	**8**[d]	**9**[d]	**10**[c]	**11**[d]	**12**[b]	**13**[b]
1	2.61 (brs)	2.73 (brs)	2.49 (d, 2.5)	2.84 (brd, 1.5)	2.62 (brs)	1.69 (brs)	5.75 (brs)
2	1.77 (brdd, 1.7, 4.5)	1.31 (brd, 1.5)	2.08 (dd, 2.5, 4.5)				
4	1.58 (m), 1.53 (m)	1.47 (m)	1.67 (m), 1.49 (m)	1.20 (m)[e]	1.55 (m), 1.38 (m)	1.49 (m), 1.40 (m)	1.71 (m), 1.42 (m)
5	1.69 (m), 1.52 (m)	1.59 (m), 1.47 (m)	1.89 (m), 1.52 (m)	1.15 (m)[e], 1.67 (m)[e]	1.64 (m)	1.70 (m), 1.65 (m)	1.65 (m), 1.39 (m)
6	1.66 (m)	1.45 (m)	1.51 (m)	1.23 (m)[e]	1.36 (m)	1.50 (m)	1.42 (m)
7	3.80 (brs)	2.95 (brs)	2.56 (brs)	2.66 (brs)	2.54 (brs)	2.58 (brs)	
8	1.23 (s)	1.21 (s)	0.98 (s)	0.97 (s)	1.09 (s)	0.85 (s)	1.06 (s)
9			1.46 (m)	1.63 (m)[e]	1.39 (m)	1.45 (m)	1.99 (brs)
10	1.27 (s)	1.25 (s)	1.01 (d, 2.3)	0.81 (d, 6.1)	1.00 (d, 6.2)	1.01 (d, 6.5)	0.97 (s)
11	1.24 (s)	1.21 (s)	0.99 (d, 2.3)	0.89 (d, 6.1)	0.93 (d, 6.2)	0.97 (d, 6.5)	0.98 (s)
12	4.42 (dd, 0.5, 11.7) 4.25 (dd, 4.7, 11.7)	3.77 (dd, 1.5, 11.1) 3.74 (dd, 2.0, 11.1)	4.59 (d, 11.9) 4.39 (dd, 4.5, 11.9)	3.88 (m)[e]	4.95 (s) 4.65 (s)	2.79 (d, 4.6) 2.74 (d, 4.6)	4.00 (ddd, 1.5, 1.7, 14.9) 4.07 (ddd, 1.7, 1.9, 14.9)
13				1.63 (m)[e]	1.56 (brs)	1.62 (brs)	2.02 (m)
14	5.20 (dd, 0.8, 1.0) 4.86 (dd, 1.0, 1.0)	4.83 (brs) 4.73 (brs)	2.92 (d, 4.5) 2.74 (d, 4.5)	3.87 (d, 7.6) 3.46 (d, 7.6)	3.57 (dd, 0.9, 6.2)	4.15 (d, 6.5)	1.76 (m) 1.32 (m)
15		4.36 (d, 3.6)		9.95 (d, 1.5)	3.99 (dd, 0.9, 6.2)	3.94 (d, 6.5)	3.65 (m), 3.56 (m)
other		3.36 (s, OCH_3-16)		2.24 (br, 12-OH)			

[a] Compounds **1**−**3**, **5**, **9**, and **11**−**13** were measured in CD_3OD, compounds **4**, **6**−**8**, and **10** in $CDCl_3$. All assignments are based on extensive 1D and 2D NMR measurements (COSY, HMQC, HMBC). [b] Spectra were recorded at 300 MHz. [c] Spectra were recorded at 400 MHz. [d] Spectra were recorded at 600 MHz. [e] Chemical shifts were obtained from HMQC spectrum.

Table 3. Antibacterial, Antifungal, Enzyme Inhibitory (Reverse Transcriptase, Tyrosine Kinase), and Antiplasmodial Activities of Compounds **1**−**16**

	antimicrobial activity		enzyme inhibitory activity			antiplasmodial activity (IC_{50})	
compound	bacteria[a] [50 μg/disc]	fungi[b] [50 μg/disc]	TK^{lck}activity[c] [200 μg/mL]	TK^{lck} activity[c] [40 μg/mL]	RT activity[d] [66 μg/mL]	K1[e] [ng/mL]	NF54[e] [ng/mL]
1	n.a.	n.a.	79.9		80.9	>10 000	>10 000
2	Ec 1 mm	Mv 3 mm; Er 2 mm	>100		93.9	>10 000	>10 000
3	n.a.	Mv 2 mm	>100		>100	>10 000	>10 000
4	Bm 1 mm	Mv 2 mm	58.2		>100	4711	6705
5	n.a.	Mv 1 mm	>100		80.9	>10 000	>10 000
6	n.a.	Mv 1 mm			90.2		
7	Ec 1 mm	Mv 2 mm	>100		95.3	>10 000	>10 000
8	n.a.	Mv 2 mm	>100		>100	5095	3651
9	n.a.	Mv 2 mm			>100		
10	Bm 2 mm	Mv 3 mm	72.7		>100	2904	4244
11			58.0		>100		
12	n.a.	Mv 3 mm	>100		89.6	>10 000	>10 000
13	n.a.	Mv 2 mm	>100		94.1	>10 000	>10 000
14	Bm 2 mm	Mv 1 mm	44.2		>100	1412	3303
15	n.a.	Mv 3 mm	>100		97.2	6945	9261
16	n.a.	Mv 5 mm	0.0	48.1	70.2	2611	3411
chloroquine						48	3.2

[a] Tested against *Bacillus megaterium* (Bm) and *Escherichia coli* (Ec), benzylpenicillin and streptomycin sulfate were positive controls: inhibition zones of benzyl penicillin 2 mm (Ec), 17 mm (Bm); inhibition zones of streptomycin sulfate 2 mm (Ec), 10 mm (Bm). Inhibition zones were measured from the edge of the filter disks. [b] Tested against *Eurotium repens* (Er), *Fusarium oxysporum* (Fo), *Microbotryum violaceum* (Mv), and *Mycotypha microspora* (Mm), miconazol was positive control: inhibition zone 25 mm (Mv), 25 mm (Er), 3 mm (Fo), and 7 mm (Mm), respectively. [c] Values are % residual tyrosine kinase (TK^{lck}) activity. Lck = lymphocytic kinase. [d] Values are % residual reverse transcriptase (HIV-1) activity. [e] Antiplasmodial activity was measured against two reference strains of *Plasmodium falciparum* K1 (Thailand; resistant to chloroquine and pyrimethamine) and NF 54 (an airport strain of unknown origin; susceptible to standard antimalarials). [f] n.a. = not active.

Fusarium oxysporum, *Mycotypha microspora*, and the green alga *Chlorella fusca*.[30] Assay systems with *Artemia salina* (brine shrimp) and *Caenorrhabditis elegans* (nematode), to investigate the lethality/toxicity of test substances, were also employed.[31] In ELISA-based test systems inhibitory activity against tyrosine kinase $p56^{lck}$ and reverse transcriptase HIV-1 was investigated.[32,33] Antiplasmodial activity was determined as described by Desjardins.[34] Activity against *Mycobacterium tuberculosis* was assessed as described by Collins et al.[35]

Extraction and Isolation. Prior to extraction with EtOAc (40 L) the solid medium and fungal mycelium were diluted with H_2O to enable them to be easily blended using an Ultra Turrax T 25 at 8000 min^{-1}. The resultant EtOAc extract (10.0 g) was purified employing a combination of chromatographic techniques. First, it was passed over normal-phase Si gel (vacuum liquid chromatography, VLC) using gradient elution from cyclohexane to EtOAc to MeOH to yield 15 fractions each of 250 mL. VLC fractions 3 and 4 (2.4 g, eluted with cyclohexane−EtOAc, 3:2), in which the antimicrobial and antialgal activity was concentrated, were combined and further subjected to normal-phase VLC employing a gradient elution from petroleum ether to EtOAc to MeOH. Fraction 4 (550 mg, eluted with petroleum ether−EtOAc, 2:3) from this separation was purified by HPLC (LiChrocart 5 μm, 7 mm × 25 cm) using petroleum ether−$(CH_3)_2CO$, 82:18, as eluent followed by RP-18 HPLC (Eurospher 100, 5 μm, 8 mm × 25 cm) with MeOH−H_2O, 76:24, as eluent to yield compounds **4**, **6**, and **14**−**16**. Fraction 3 [405 mg, eluted with petroleum ether−$(CH_3)_2CO$, 3:2] was further purified by HPLC (LiChrocart 5 μm, 7 mm × 25 cm) using petroleum ether−$(CH_3)_2CO$ (9:1) as eluent and RP-18 HPLC (Eurospher 100, 5 μm, 8 mm × 25 cm) using MeOH−H_2O (3:1) as eluent to yield compounds **1** and **13**.

VLC fractions 5 and 6 from the first VLC separation, which showed moderate antimicrobial activity, were combined (3.3 g, eluted with cyclohexane−EtOAc, 15:85) and passed over normal-phase Si gel (VLC) using gradient elution from petroleum ether to EtOAc to MeOH. Fraction 5 from this separation (1.2 g, eluted with petroleum ether−EtOAc, 3:7) was purified by HPLC (LiChrospher Si 60 5 μm, 7 mm × 25 cm) using petroleum ether−$(CH_3)_2O$−$(CH_3)_2CO$, 85:15, to yield compound **10**. VLC fractions 7, 8, and 9 were combined (2.9 g, eluted with EtOAc−MeOH, 4:1) on the basis of their similar TLC information and further investigated due to their interesting ^1H NMR data. They were subjected to further normal-phase VLC using a gradient elution from petroleum ether to EtOAc to MeOH. The third fraction from this separation (350 mg, eluted with 100% EtOAc) was further separated using normal-phase HPLC (LiChrospher Si 60 5 μm, 7 mm × 25 cm using petroleum ether−$(CH_3)CO$, 74:26, as eluent). Resultant fractions were further purified by RP-HPLC (Eurospher RP-18 100 5 μm, 8 mm × 25 cm using MeOH−H_2O, 7:3 or 65:35, as eluent), to yield compounds **2**, **3**, **5**, **7**−**9**, **11**, and **12**.

Isosativenetriol (1): white amorphous powder (0.7 mg/L); $[\alpha]_D^{22}$ −2.0° (*c* 0.10, EtOH); IR (film) ν_{max} 3580, 3270, 2920, 2315 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; FABMS *m/z* 275 $[M + Na]^+$; EIMS *m/z* 234 $[M - H_2O]^+$ (22), 219 (18), 188 (42), 173 (62), 145 (100), 133 (48), 119 (54), 105 (68), 91 (62); HREIMS *m/z* 234.1614 (calcd for $C_{15}H_{22}O_2$ $[M - H_2O]^+$, 234.1614).

Drechslerine A (2): white needles (1.2 mg/L); mp 125.0 °C, $[\alpha]_D^{22}$ −25.0° (*c* 0.10, EtOH); IR (film) ν_{max} 3270, 2950, 1455 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* 224 $[M]^+$ (10), 206 (42), 193 (38), 181 (24), 175 (68), 163 (24), 139 (72), 133 (100), 119 (42), 105 (74); HREIMS *m/z* 206.1664 (calcd for $C_{14}H_{22}O$ $[M - H_2O]^+$, 206.1665).

Drechslerine B (3): colorless oil (0.4 mg/L); $[\alpha]_D^{22}$ −42.0° (*c* 0.10, EtOH); UV (EtOH) λ_{max} (log ϵ) 235 (3.63), IR (film) ν_{max} 3420, 2925, 2870, 1745 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* 250 $[M]^+$ (22), 232 (28), 207 (60), 189 (100), 177 (58), 164 (52), 150 (76), 133 (38), 119 (58), 105 (52); HREIMS *m/z* 250.1563 (calcd for $C_{15}H_{22}O_3$, 250.1563).

Helminthosporol (4): colorless oil (9.3 mg/L); $[\alpha]_D^{22}$ −25.0° (*c* 0.08, EtOH); lit.[18] $[\alpha]_D$ −28.7° (*c* 1.93, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 263 (3.72); lit.[18] UV (EtOH) λ_{max} (log ϵ) 267 (3.99); IR (film) ν_{max} 3420, 2920, 1650, 1470 cm^{-1}; lit.[18] IR (Nujol) ν_{max} 3440, 1645, 1610 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* 236 $[M^+]$ (72), 218 (38), 205 (34), 189 (26), 175 (24), 154 (30), 147 (34), 133 (46), 123 (65), 107 (86), 91 (100); HREIMS *m/z* 236.1770 (calcd for $C_{15}H_{24}O_2$, 236.1770).

9-Hydroxyhelminthosporol (5): amorphous, white powder (0.5 mg/L); $[\alpha]_D^{22}$ +50.0° (*c* 0.40, EtOH); UV (EtOH) λ_{max} (log ϵ) 268 (3.66); IR (film) ν_{max} 3415, 2935, 1730, 1640 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* 252 $[M]^+$ (2), 194 (26), 176 (100), 161 (50), 147 (48), 135 (62), 119 (32), 107 (53), 91 (53).

Drechslerine C (6): white amorphous powder (0.4 mg/L); $[\alpha]_D^{22}$ −13.0° (*c* 0.15, EtOH); UV (EtOH) λ_{max} (log ϵ) 220 (3.08); IR (film) ν_{max} 2950, 2920, 2865, 1730, 1685, 1460, 1030 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* 224 $[M]^+$ (65), 209 (2), 193 (28), 181 (22), 175 (8), 163 (4), 153 (15), 140 (100), 123 (27), 107 (38); HREIMS *m/z* 224.1770 (calcd for $C_{14}H_{24}O_2$, 224.1770).

Drechslerine D (7): colorless oil (0.5 mg/L); $[\alpha]_D^{22}$ −90.0° (*c* 0.40, EtOH); UV (EtOH) λ_{max} (log ϵ) 217 (3.10); IR (film) ν_{max} 3455, 2930, 1730, 1455 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* 250 $[M^+]$ (4), 235 (8), 217 (4), 192 (78), 173 (32), 147 (20), 125 (100), 105 (42), 91 (38); HREIMS *m/z* 250.1563 (calcd for $C_{15}H_{22}O_3$, 250.1563).

Drechslerine E (8): amorphous white powder (1.1 mg/L); $[\alpha]_D^{22}$ +12.0° (*c* 0.40, EtOH); UV (EtOH) λ_{max} (log ϵ) 264 (2.43); IR (film) ν_{max} 3450, 2960, 2870, 1725, 1655 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* 266 $[M]^+$ (12), 252 (4), 235 (18), 219 (12), 191 (18), 188 (32), 173 (47), 163 (26), 148 (70), 145 (46), 119 (82), 107 (57), 91 (42); HREIMS *m/z* 266.1874 (calcd for $C_{16}H_{26}O_3$, 266.1875).

Drechslerine F (9): white amorphous powder (0.2 mg/L); $[\alpha]_D^{22}$ −35.5° (*c* 0.20, EtOH); UV (EtOH) λ_{max} (log ϵ) 226 (3.14); IR (film) ν_{max} 2960, 2930, 2875, 1735, 1460 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* 250 $[M]^+$ (18), 235 (6), 221 (3), 207 (18), 191 (5), 177 (24), 163 (15), 151 (32), 137 (62), 121 (47), 107 (100), 91 (95); HREIMS *m/z* 250.1563 (calcd for $C_{15}H_{22}O_3$, 250.1563).

Drechslerine G (10): yellowish oil (1.2 mg/L); $[\alpha]_D^{22}$ −7.2° (*c* 0.47, EtOH); UV (EtOH) λ_{max} (log ϵ) 264 (2.98); IR (film) ν_{max} 3430, 2935, 2875, 1715 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* 252 $[M]^+$ (8), 232 (70), 224 (18), 206 (100), 193 (42), 175 (56), 163 (32), 139 (68), 107 (82), 91 (74); HREIMS *m/z* 252.1719 (calcd for $C_{15}H_{24}O_3$, 252.1719).

***cis*-Sativenediol (11):** a clear oil (0.5 mg/L); $[\alpha]_D^{22}$ −103.8° (*c* 0.13, EtOH); lit.[20] $[\alpha]_D$ −119° (*c* 0.94, $CHCl_3$); ^1H and ^{13}C NMR data, see Tables 1 and 2; HREIMS *m/z* 236.1770 (calcd for $C_{15}H_{24}O_2$, 236.1770).

Sativene epoxide (12): white amorphous powder (0.2 mg/L); $[\alpha]_D^{22}$ −39.0° (*c* 0.10, EtOH); IR (film) ν_{max} 3550, 2925, 2870, 1650 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* 252 $[M]^+$ (44), 234 (6), 221 (15), 209 (12), 193 (34), 180 (32), 161 (54), 149 (46), 137 (44), 121 (46), 105 (66), 91 (100); HREIMS *m/z* 252.1719 (calcd for $C_{15}H_{24}O_3$, 252.1719).

(+)-Secolongifolene diol (13): amorphous white powder (0.5 mg/L); $[\alpha]_D^{22}$ +7.0° (*c* 0.08, EtOH); lit.[22] $[\alpha]_D$ −23 ± 2° (*c* 1.06%, $CHCl_3$) for (−)-secolongifolene diol; IR (film) ν_{max} 3330, 2925, 1730, 1460 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* 238 $[M]^+$ (20), 220 (40), 205 (56), 193 (28), 189 (57), 175 (64), 153 (38), 138 (82), 133 (59), 119 (72), 107 (100), 91 (74); HREIMS *m/z* 220.1821 (calcd for $C_{15}H_{24}O$ $[M - H_2O]^+$, 220.1821).

Isocochlioquinone A (14): yellow solid (0.7 mg/L); $[\alpha]_D^{22}$ +148.0° (*c* 0.21, EtOH); lit.[24] $[\alpha]_D$ +130.9° (*c* 0.1, EtOH); and with all remaining physical and spectroscopic data as previously published.[24]

Isocochlioquinone C (15): yellow solid (1.6 mg/L); $[\alpha]_D^{22}$ +155.5° (*c* 0.20, EtOH); lit.[24] $[\alpha]_D$ +287.4° (*c* 0.27, MeOH); and with all remaining physical and spectroscopic data as previously published.[24]

Cochlioquinone B (16): yellow solid (0.9 mg/L); $[\alpha]_D^{22}$ +111.5° (*c* 0.10, EtOH); lit.[24] $[\alpha]_D$ +108.4° (*c* 0.1, EtOH); and with all remaining physical and spectroscopic data as previously published.[24]

Acknowledgment. We thank Dr. S. Draeger, Institute for Microbiology, TU Braunschweig, for taxonomic identification of the investigated fungal strain. We also thank Dr. V. Wray and his group, GBF Braunschweig, for recording all NMR spectra, Dr. U. Papke, Dr. H.-M. Schiebel, and D. Döring, Department of Chemistry, TU Braunschweig, for mass spectral measurements, and Dr. L. Witte, Institute for Pharmaceutical Biology, TU Braunschweig, for GC−MS measurements. Thanks also go to Ms. I. Rahaus, Institute for Pharmaceutical Biology, TU Braunschweig, L. Peters and C. Dreikorn, Institute for Pharmaceutical Biology, University of Bonn, for performing the enzyme, agar diffusion, brine shrimp and nematode assays, Dr. A. Flores-Moya, Depatamento de Biologia Vegetal, Facultad de Ciencias, Universidad de Malaga, Spain, for the taxonomic identification of the investigated red alga, Mr. T. Osterhage, Braunschweig, Germany, for collecting the investigated alga, and Mr. and Mrs. Zöller, Moraira, Spain, for help organizing the summer 1997 collecting trip. Financial support from the Bundesministerium für Bildung und Forschung (BMBF) and Bayer AG, Leverkusen, is gratefully acknowledged.

References and Notes

(1) König, G. M.; Wright, A. D. *Planta Med.* **1996**, *62*, 193−209.
(2) Pietra, F. *Nat. Prod. Rep.* **1997**, *14*, 453−464.
(3) Kohlmeyer, J.; Kohlmeyer, E. Marine Mycology. *The Higher Fungi*; Academic Press: New York, 1979; pp 54−69.
(4) Schulz, B.; Römmert, A.-K.; Dammann, U.; Aust, H.-J.; Strack, D. *Mycol. Res.* **1999**, *10*, 1275−1283.
(5) Numata, A.; Takahashi, C.; Ito, Y.; Takada, T.; Kawai, K.; Usami, Y.; Matsumura, E.; Imachi, M.; Ito, T.; Hasegawa, T. *Tetrahedron Lett.* **1993**, *34*, 2355−2358.
(6) Takahashi, C.; Numata, A.; Ito, Y., Matsumura, E.; Araki, H.; Iwaki, H.; Kushida, K. *J. Chem. Soc., Perkin Trans. 1* **1994**, 1859−1864.
(7) Takahashi, C.; Takai, Y.; Kimura, Y.; Numata, A.; Shigematsu, N.; Tanaka, H. *Phytochemistry* **1995**, *38*, 155−158.
(8) Takahashi, C.; Numata, A.; Matsumura, E.; Minoura, K.; Eto, H.; Shingu, T.; Ito, T.; Hasegawa, T. *J. Antibiot.* **1994**, *47*, 1242−1249.
(9) Numata, A.; Takahashi, C.; Ito, Y.; Minoura, K.; Yamada, T.; Matsuda, C.; Nomoto, K. *J. Chem. Soc., Perkin Trans. 1* **1996**, 239−245.
(10) Takahashi, C.; Numata, A.; Yamada, T.; Minoura, K.; Enomoto, S.; Konishi, K.; Nakai, M.; Matsuda, C.; Nomoto, K. *Tetrahedron Lett.* **1996**, *37*, 655−658.
(11) Iwamoto, C.; Minoura, K.; Hagishita, S.; Nomoto, K.; Numata, A. *J. Chem. Soc., Perkin Trans. 1* **1998**, 449−456.
(12) Amagata, T.; Minoura, K.; Numata, A. *J. Antibiot.* **1998**, *51*, 432−434.
(13) Sugawara, F.; Takahashi, N.; Strobel, G.; Yun, C.-H.; Gray, G.; Fu, Y.; Clardy, J. *J. Org. Chem.* **1988**, *53*, 2170−2172.
(14) Sugawara, F.; Strobel, G.; Strange, R. N.; Siedow, J. N.; Van Duyne, G. D.; Clardy, J. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 3081−3085.
(15) Sugawara, F.; Takahashi, N.; Strobel, G. A.; Strobel, S. A.; Lu, H. S. M.; Clardy, J. *J. Am. Chem. Soc.* **1988**, *110*, 4086−4087.
(16) Sugawara, F.; Hallock, Y. F.; Bunkers, G. D.; Kenfield, D. S.; Strobel, G.; Yoshida, S. *Biosci. Biotech. Biochem.* **1993**, *57*, 236−239.
(17) Dorn, F.; Arigoni, D. *Experientia* **1975**, *31*, 753−754.
(18) Tamura, S.; Sakurai, A.; Kainuma, K.; Takai, M. *Agric. Biol. Chem.* **1963**, *27*, 738−739.
(19) Park, J. K.; Hasumi, K.; Endo, A. *J. Antibiot.* **1993**, *46*, 1303−1305.
(20) Nukina, M.; Hattori, H.; Marumo, S. *J. Am. Chem. Soc.* **1975**, *97*, 2542−2543.
(21) Dorn, F.; Arigoni, D. *Experientia* **1974**, *30*, 851−852.
(22) Yadav, J. S.; Chawla, H. P. S.; Dev, S. *Ind. J. Chem.* **1983**, *22B*, 212−214.
(23) Miyagawa, H.; Nagai, S.; Tsurushima, T.; Sato, M.; Ueno, T.; Fukami, H. *Biosci. Biotech. Biochem.* **1994**, *58*, 1143−1145.
(24) Lim, C.-H.; Miyagawa, H.; Akamatsu, M.; Nakagawa, Y.; Ueno, T. *J. Pesticide Sci.* **1998**, *23*, 281−288.
(25) Carruthers, J. R.; Cerrini, S.; Fedeli, W.; Casinovi, C. G.; Galeffi, C.; Torracca Vaccaro, A. M.; Scala, A. *Chem. Commun.* **1971**, 164−166.
(26) Canonica, L.; Casinovi. C. G.; Fiecchi, A.; Galeffi, C.; Marini Bettoldo, G. B.; Scala. A.; Torracca Vaccaro, A. M. *Gazz. Chim. Ital.* **1976**, *106*, 147−166.
(27) Canonica, L.; Beretta, M. G.; Colombo, L.; Gennari, C.; Ranzi, B. M.; Scolastico, C. *J. Chem. Soc., Perkin Trans. 1* **1980**, 2686−2690.
(28) Wright, A. D.; König, G. M.; Angerhofer, C. K.; Greenidge, P.; Linden A.; Desqueyroux-Faundez, R. *J. Nat. Prod.* **1996**, *59*, 710−716.
(29) Ellis, M. B. *Dematiaceous Hyphomycetes*; Commonwealth Agricultural Bureau, Commonwealth Mycological Institute: Kew, Surrey, England, 1971; pp 418−420.
(30) Schulz, B.; Sucker, J.; Aust, H.-J.; Krohn, K.; Ludewig, K.; Jones, P. G.: Döring, D. *Mycol. Res.* **1995**, *99*, 1007−1015.
(31) Peters, L.; König, G. M.; Wright, A. D. Unpublished data.
(32) Wessels, M.; König, G. M.; Wright, A. D. *J. Nat. Prod.* **1999**, *62*, 927−930.
(33) Höller, U.; Wright, A. D.; Matthee, G. F.; Konig, G. M.; Draeger, S.; Aust, H.-J.; Schulz, B. *Mycol. Res.* **2000**, *104*, 1354−1365.
(34) Desjardins, R. E.; Canfield, C. J.; Haynes, J. D.; Chulay, J. D. *Antimicrob. Agents Chemother.* **1979**, *16*, 710−718.
(35) Collins, L. A.; Franzblau, S. G. *Antimicrob. Agents Chemother.* **1997**, *41*, 1004−1009.

NP010092L