New Antioxidant Hydroquinone Derivatives from the Algicolous Marine Fungus *Acremonium* sp.

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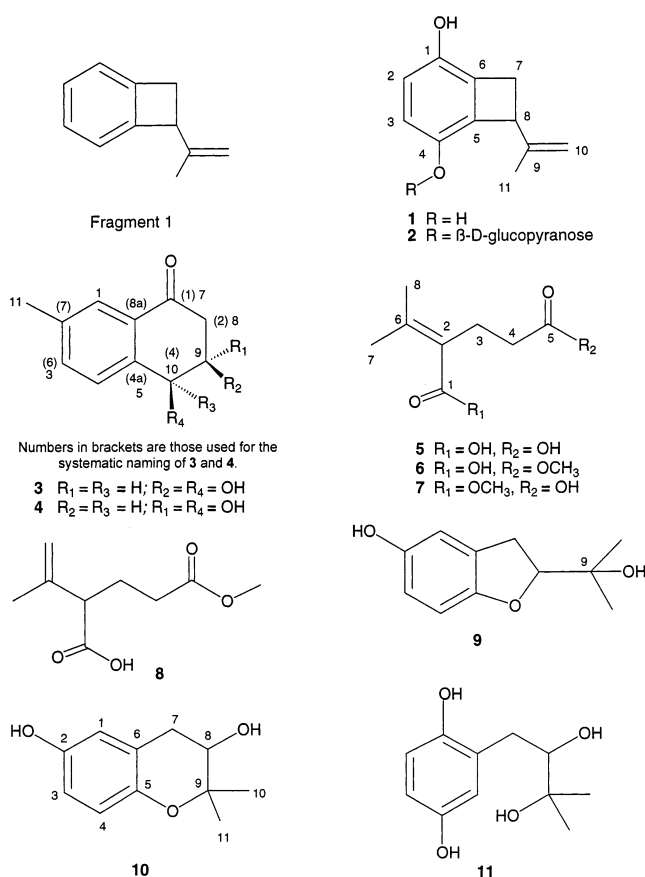
A marine fungal isolate, identified as *Acremonium* sp., was mass cultivated and found to produce two novel hydroquinone derivatives, 7-isopropenylbicyclo[4.2.0]octa-1,3,5-triene-2,5-diol (**1**) and 7-isopropenylbicyclo-[4.2.0]octa-1,3,5-triene-2,5-diol-5-β-D-glucopyranoside (**2**). Compound **1** and its glucoside **2** possess a most unusual ring system. The new natural products (3R*,4S*)-3,4-dihydroxy-7-methyl-3,4-dihydro-1(2H)-naphthalenone (**3**) and (3S*,4S*)-3,4-dihydroxy-7-methyl-3,4-dihydro-1(2H)-naphthalenone (**4**) were obtained as a 1:0.8 mixture. 2-(1-Methylethylidene)pentanedioic acid (**5**) was isolated for the first time as a natural product and its structure proven by X-ray analysis. In addition to these compounds an inseparable mixture of three new isomeric compounds, pentanedioic acid 2-(1-methylethylidene)-5-methyl ester (**6**), pentanedioic acid 2-(1-methylethylidene)-1-methyl ester (**7**), and pentanedioic acid 2-(1-methylethenyl)-5-methyl ester (**8**), was also obtained. Isolated together with the new compounds were three known hydroquinone derivatives, **9**, **10**, and **11**. The structures of all compounds were determined by interpretation of their spectroscopic data (1D and 2D NMR, MS, UV, and IR). Each isolate was tested for its antioxidant properties, and compounds **1** and **9**−**11** were found to have significant activity.

Marine microorganisms, particularly marine fungi, have recently gained prominence as an important source of biologically active secondary metabolites.[1] Among marine fungi, those living in association with marine algae are a particularly promising source of novel natural products due to the special ecological niche in which they exist. The association between algae and fungi appears to be highly developed since nearly one-third of all higher marine fungi described are so-called algicolous or algae-associated organisms.[2] Recently, marine-derived fungi have yielded some unique biologically active metabolites, such as ascosalipyrrolidinone and ascosalipyrone,[3] microsphaeropsisin,[4] and mactanamide,[5] further supporting the idea that these organisms are truly valuable producers of potential therapeutic agents.

Natural antioxidants[6] are known to be produced by *Penicillium roquefortii*,[7] *Aspergillus candidus*,[8−10] *Mortierella* sp.,[11] and *Emericella falconensis*[12] and by fungi of the genus *Acremonium*.[13] *Acremonium* sp., are noted for their secondary metabolite content, with around 90 compounds having been reported from fungi of this genus, including the halymecins,[14] oxepinamides A−C,[15] acremolactone,[16] and orbuticin.[17] In the current paper, the isolation and structure elucidation of two novel hydroquinone derivatives (**1** and **2**), two new dihydronaphthalenones (**3** and **4**), four new acyclic carboxylic acid derivatives (**5**−**8**), and three known fungal metabolites (**9**−**11**) are reported.[18]

The fungus *Acremonium* sp., closely related to *A. roseogriseum* (S. B. Saksena) W. Gams on the basis of morphological characteristics, was isolated from tissues of the brown alga *Cladostephus spongius* (Hudson) C. Agardh, collected at the Spanish coast (Moraira, Mediterranean Sea), as described by Osterhage et al.[3] The fungus was

Fragment 1

1 R = H
2 R = ß-D-glucopyranose

Numbers in brackets are those used for the systematic naming of **3** and **4**.

3 $R_1 = R_3 = H; R_2 = R_4 = OH$
4 $R_2 = R_3 = H; R_1 = R_4 = OH$

5 $R_1 = OH, R_2 = OH$
6 $R_1 = OH, R_2 = OCH_3$
7 $R_1 = OCH_3, R_2 = OH$

8

9

10

11

cultivated on a solid biomalt medium with added artificial sea salt. Successive fractionation of the EtOAc extract by vacuum liquid chromatography (VLC) over silica followed by normal- (NP) and reversed-phase (RP-C_{18}) HPLC yielded compounds **1**−**11**.

The molecular formula of **1** was deduced by accurate mass measurement to be $C_{11}H_{12}O_2$. In the ^{13}C NMR

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Table 1. ^{1}H [(CD$_3$)$_2$CO, 300 MHz] and ^{13}C NMR [(CD$_3$)$_2$CO, 75.5 MHz] Spectral Data for Compounds **1** and **2**[a] and ^{13}C NMR Data for Arbutin

position	**1** δ ^{13}C	δ ^{1}H	HMBC	arbutin[b] δ ^{13}C	**2** δ ^{13}C	δ ^{1}H	HMBC
1	145.9 (s)[c]*			153.7 (s)	146.6 (s)*		
2	116.0 (d)	6.51 (1H, d, 8.8[d])	C-1, C-4, C-6	119.3 (d)	116.1 (d)	6.57 (1H, d, 8.8)	C-1, C-4, C-6
3	115.9 (d)	6.54 (1H, d, 8.8)	C-1, C-4, C-5, C-8	116.6 (d)	117.8 (d)	6.74 (1H, d, 8.8)	C-1, C-4, C-5, C-8
4	145.8 (s)*			152.4 (s)	147.9 (s)*		
5	132.3 (s)			116.6 (d)	134.4 (s)		
6	128.8 (s)			119.3 (d)	128.9 (s)		
7	34.7 (t)	3.26 (1H, dd, 13.2, 5.5)	C-1, C-2, C-5, C-6, C-8, C-9		34.5 (t)	3.28 (1H, dd, 13.2, 5.5)	C-1, C-2, C-5, C-6, C-8, C-9
		2.70 (1H, dd, 13.2, 2.6)				2.77 (1H, dd, 13.2, 2.6)	
8	48.9 (d)	3.94 (1H, brd, 5.5)	C-3, C-5, C-6, C-7, C-9, C-10, C-11		49.1 (d)	4.06 (1H, dd, 2.6, 5.5)	C-5, C-6, C-7, C-9, C-10, C-11
9	146.4 (s)				146.4 (s)		
10	110.4 (t)	4.76 (1H, d, 0.7)	C-8, C-9, C-11		111.6 (t)	4.78 (1H, d, 1.5)	C-8, C-9, C-11
		4.86 (1H, d, 0.7)	C-8, C-9, C-11			4.92 (1H, d, 1.5)	
11	20.3 (q)	1.76 (3H, s)	C-8, C-9, C-10		19.7 (q)	1.74 (3H, s)	C-8, C-9, C-10
OH-1		7.64 (1H, brs)				8.10 (1H, s)	
OH-4		7.64 (1H, brs)					
1'				103.6 (d)	102.7 (d)	4.81 (1H, d, 7.7)	C-3'
2'				74.9 (d)	74.9 (d)	3.39 (1H, m)	C-3', C-4'
3'				77.9 (d)	77.8 (d)	3.39 (1H, m)	C-2', C-4'
4'				71.4 (d)	71.3 (d)	3.45 (1H, m)	C-2', C-3'
5'				78.0 (d)	77.9 (d)	3.36 (1H, m)	C-2', C-3', C-4'
6'				62.6 (t)	62.8 (t)	3.70 (1H, m),	C-5', C-4'
						3.81 (1H, m)	

[a] All assignments are based on 1D and 2D NMR measurements (HMBC, HMQC, COSY). [b] Data obtained by measuring an authentic sample. [c] Implied multiplicities as determined by DEPT (C = s, CH = d, CH$_2$ = t, CH$_4$ = q). [d] J in Hz. *Assignments may be interchanged.

spectrum of **1**, 11 resonances, attributable to 1 × CH$_3$, 2 × CH$_2$, 3 × CH, and 5 × C groups (Table 1), were evident. It was also clear from these data that since four of the six degrees of unsaturation within **1** were due to multiple bonds (4 × C=C), the molecule was bicyclic. The ^{1}H and ^{13}C NMR data enabled all but two hydrogen atoms of **1** to be accounted for; thus, it was evident that the remaining two must be present in OH groups, a deduction supported by IR (ν_{max} 3314 cm^{-1}) and ^{1}H NMR (δ 7.64 brs, CD$_3$OD exchangeable) data. Also evident from the ^{1}H and ^{13}C NMR data of **1** were resonances consistent with the presence of a tetrasubstituted aromatic ring [δ 6.51 (d, J = 8.8, H-2); 6.54 (d, J = 8.8, H-3)], a deduction supported by the maximum at 286 nm in the UV spectrum of **1**, an allylic methyl, an exomethylene, a benzylic methylene, and an allylic-benzylic methine group. Accounting for all ^{1}H and ^{13}C NMR resonances associated with C−H one-bond interactions and from cross-peaks seen in the ^{1}H−^{13}C 2D NMR shift-correlated (HMQC) spectrum of **1**, it was possible to deduce the planar structure of **1** by interpretation of its ^{1}H−^{1}H COSY and HMBC spectra. From the ^{1}H−^{1}H COSY spectrum of **1**, it was evident that H$_2$-7 coupled to H-8 and that H$_3$-11 had an allylic coupling to H$_2$-10. Cross-peaks in the HMBC spectrum of **1** between H$_2$-10 and C-8, C-9, and C-11, between H$_2$-7 and C-2, C-5, and C-6, and between H-8 and C-3, C-4, C-5, and C-6 enabled fragment 1 to be developed. The similarity in the ^{13}C NMR chemical shifts of the resonances associated with C-1 and C-4 (δ 145.9 s, 145.8 s) and C-2 and C-3 (116.0 d, 115.9 d) showed the aromatic ring of **1** to be pseudo-symmetrically substituted, as shown in **1**. The absolute stereochemistry at C-8 was not determined. For **1**, a new hydroquinone derivative, the trivial name acremonin A is proposed.

The positive FABMS and NMR data of **2** showed it to have the molecular formula C$_{17}$H$_{22}$O$_7$. Comparison of the ^{1}H and ^{13}C NMR data of **2** with those of **1**, and with the ^{13}C NMR data of arbutin (see Table 1), revealed it to be a glucopyranose derivative of **1**. The chemical shift and coupling constant [4.81 d (J = 7.7. Hz)] attributable to the

anomeric proton of the glucose showed it to be β-linked to the rest of the molecule. Acid hydrolysis of **2** yielded free glucose, to which the D-configuration was assigned on the basis of its optical rotation (see Experimental Section). The point of attachment of the sugar to the aglycone was concluded to be at C-4 on the basis of the deshielded nature of the resonances associated with C-3, C-4, C-5, and that of H-3, relative to the equivalent resonances found in **1**. This conclusion was supported by cross-peaks in the NOESY spectrum of **2** between H-1' and H-8, and one of the exomethylene protons. For **2** the trivial name acremonin A glucoside is proposed.

The stereoisomers **3** and **4** (**3/4**), which proved to be inseparable by HPLC, analyzed for C$_{11}$H$_{12}$O$_3$ by accurate mass measurement. The ^{13}C NMR spectrum of the mixture contained 11 carbon resonances for each compound that were attributable in each case to 1 × CH$_3$, 1 × CH$_2$, 5 × CH, and 4 × C groups (see Table 2). It was also evident from these data that four of the six degrees of unsaturation within the molecules were due to multiple bonds, 3 × C=C and 1 × C=O; the molecules are thus bicyclic. As the ^{13}C NMR and ^{1}H NMR spectral data accounted for all but two of the hydrogen atoms within **3** and **4**, it was evident that the remaining two (in each compound) must be present as parts of OH groups. In the ^{1}H NMR spectrum of **3/4** the presence of three aromatic protons that were *meta* (δ 7.68), *ortho* and *meta* (δ 7.43, 7.45), and *ortho* coupled (δ 7.55, 7.60), respectively, revealed the presence of a 1, 3, 4 substituted aromatic ring in each molecule. The UV data of **3/4** (λ_{max} 251 and 296 nm) supported the presence of this group and showed it to be further conjugated with the keto-function (δ 196.4 and 196.2 s). HMBC correlations between the resonance of H$_3$-11 and those of C-1, C-2, C-3, and C-6 showed the methyl group to reside at C-2, leaving the keto-function to be bonded to either C-5 or C-6. Further HMBC correlation, this time between the resonance for H-4 and those for C-2, C-3, C-5, C-6, and C-10, and the resonance for H-1 and those of C-2, C-3, C-7, and C-11, clearly revealed C-6 to bond with C-7, and C-5 to bond with C-10.

Table 2. ^{1}H [(CD$_3$)$_2$CO, 300 MHz] and ^{13}C NMR [(CD$_3$)$_2$CO, 75.5 MHz] Spectral Data for Compounds **3** and **4**[a]

	3		**4**		**3** and **4**
position	δ ^{13}C	δ ^{1}H	δ ^{13}C	δ ^{1}H	HMBC
1	135.2 (d)[b]	7.68 (1H, brs)	135.4 (d)	7.68 (1H, brs)	C-2, C-3, C-7, C-11
2	142.2 (s)		142.7 (s)		
3	126.6 (d)	7.43 (1H, dd, 7.7, 1.5[c])	126.7 (d)	7.45 (1H, d, 7.7, 1.5)	
4	129.4 (d)	7.55 (1H, d, 7.7)	128.9 (d)	7.60 (1H, d, 7.7)	C-6, C-2, C-3, C-5, C-10
5	138.0 (s)		138.1 (s)		
6	132.2 (s)		132.2 (s)		
7	196.4 (s)		196.2 (s)		
8	44.3 (t)	2.79 (1H, dd, 16.7, 3.4)	44.9 (t)	2.60 (1H, dd, 16.7, 9.3)	C-6, C-7, C-9, C-10
		2.86 (1H, dd, 16.7, 6.5)		2.95 (1H, dd, 16.7, 4.1)	
9	70.7 (d)	4.35 (1H, ddd, 2.9, 3.4, 6.5)	72.4 (d)	4.05 (1H, ddd, 9.3, 7.0, 4.1)	C-7, C-8
10	70.5 (d)	4.92 (1H, d, 2.9)	73.4 (d)	4.68 (1H, d, 7.0)	C-4, C-8
11	20.9 (q)	2.38 (3H, s)	21.0 (q)	2.38 (3H, s)	C-1, C-2, C-3, C-6

[a] All assignments are based on 1D and 2D measurements (HMBC, HMQC, and COSY). [b] Implied multiplicities as determined by DEPT (C = s, CH = d, CH$_2$ = t, CH$_4$ = q). [c] *J* in Hz.

Table 3. ^{1}H [(CD$_3$)$_2$CO, 300 MHz] and ^{13}C NMR [(CD$_3$)$_2$CO, 75.5 MHz] Spectral Data for Compound **5**[a]

position	δ ^{13}C	δ ^{1}H	HMBC
1	170.0 (s)[b]		
2	126.2 (s)		
3	26.1 (t)	2.40 (2H, m)	C-2, C-4, C-5
4	33.6 (t)	2.60 (2H, m)	C-1, C-2, C-3, C-5, C-6
5	174.2 (s)		
6	145.2 (s)		
7	22.2 (q)	1.86 (3H, s)	C-1, C-2, C-3, C-6
8	23.1 (q)	1.86 (3H, s)	C-1, C-2, C-3, C-6
2 OH		10.80 (2H, brs)	

[a] All assignments are based on 1D and 2D measurements (HMBC, HMQC, COSY). [b] Implied multiplicities were determined by DEPT (C = s, CH = d, CH$_2$ = t, CH$_4$ = q).

From the ^{1}H−^{1}H COSY spectrum of **3/4** it was evident that H-10 coupled with H-9, which further coupled with H$_2$-8, leaving C-8 to bond directly with the keto-function and in so doing complete the planar structure of **3/4**. The ^{1}H−^{1}H coupling constant associated with the resonances for H-9 in **3/4** shows H-9 in **4** to have an axial orientation, as must H-10. In **3**, the coupling constants associated with the resonance for H-9 are 2.9, 3.4, and 6.5 Hz and show this proton to have no diaxial coupling interactions; it must thus be equatorial. Accordingly, $J_{H-9,H-10}$ in **3** is noticeably smaller, 2.9 Hz, indicating it now to have an axial−equatorial interaction with H-9. The two new natural products **3** and **4** are best named systematically as (3*R**,4*S**)-3,4-dihydroxy-7-methyl-3,4-dihydro-1(2*H*)-naphthalenone and (3*S**,4*S**)-3,4-dihydroxy-7-methyl-3,4-dihydro-1(2*H*)-naphthalenone, respectively.

FABMS and NMR analysis of **5** showed it to have the molecular formula C$_8$H$_{12}$O$_4$. Its ^{13}C NMR spectrum contained eight carbon resonances attributable to 4 × C, 2 × CH$_2$, and 2 × CH$_3$ groups (Table 3). It was also evident from these data that the three degrees of unsaturation within **5** were due to a C=C double bond, and two carbonyl groups as parts of either ester or carboxyl functions, one of which was α,β-unsaturated; the molecule was acyclic. The ^{1}H and ^{13}C NMR data enabled all but two of the hydrogen atoms within **5** to be assigned to directly bonded C atoms. The two remaining hydrogens were therefore present as parts of two carboxylic functions, a deduction that was supported by the δ 10.80 (2H) ^{1}H NMR resonance and the IR data (2922 cm^{-1}) of **5**. With all ^{1}H and ^{13}C NMR resonances assigned in the HMQC spectrum of **5**, it was possible to deduce the structure of **5** by interpretation of its ^{1}H−^{1}H COSY and HMBC spectra. Compound **5** was crystalline, and X-ray diffraction studies were undertaken, the results of which are shown in Figures 1 and 2 and in the Experimental Section. As such, **5** is not a new chemical
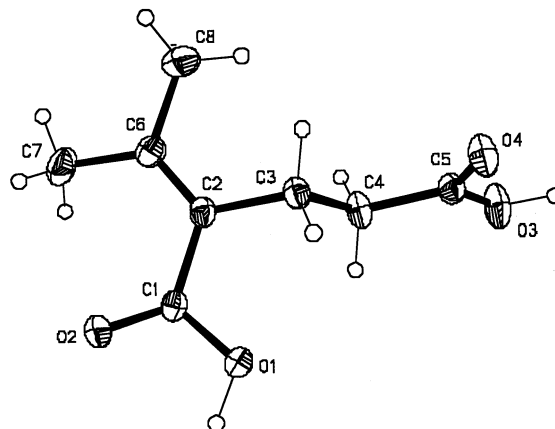
Figure 1. Structure of compound **5** in the crystal. Ellipsoids represent 50% probability levels.
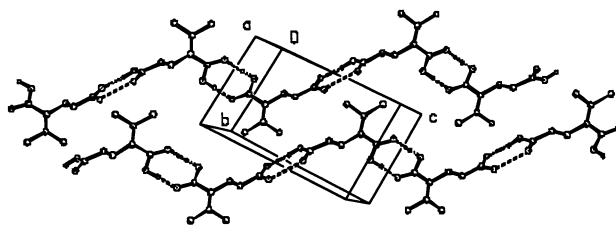
Figure 2. Packing diagram of compound **5**, showing the chains of molecules associated by hydrogen bonds (dashed). Only those hydrogen atoms involved in hydrogen bonding are shown. Radii are arbitrary.

entity since it is known synthetically.[19,20] The current report is, however, the first of it from a natural source and with complete spectroscopic and X-ray data.

Compounds **6**−**8** are structural isomers that proved to be inseparable by HPLC. GC−MS analysis and accurate mass measurement of the mixture **6**−**8** (see Experimental Section) showed the three compounds present to have the identical molecular formula C$_9$H$_{14}$O$_4$. A comparison of the MS and NMR spectral data of **6** and **7** with those of **5** (see Tables 3 and 4) indicated **6** and **7** to be monomethoxyl derivatives of **5**. In each case the position of the methoxyl group was established from HMBC data. For **6**, an HMBC correlation between the resonance for H$_3$-9 and that of C-5 clearly made the C-9 methyl group part of a methyl ester function and revealed the molecule to be pentanedioic acid 2-(1-methylethylidene)-5-methyl ester. In the case of **7**, the diagnostic HMBC correlation was between H$_3$-9 and C-1 and showed the molecule to be pentanedioic acid 2-(1-methylethylidene)-1-methyl ester.

Even though the molecular formula of **8** (C$_9$H$_{14}$O$_4$) was identical to those of **6** and **7**, the NMR data were notably

Table 4. ^{1}H (C$_6$D$_6$, 500 MHz) and ^{13}C NMR (C$_6$D$_6$, 125.5 MHz) Spectral Data for Compounds **6**−**8**[a]

	6			7			8		
position	δ ^{13}C	δ ^{1}H	HMBC	δ ^{13}C	δ ^{1}H	HMBC	δ ^{13}C	δ ^{1}H	HMBC
1	174.2 (s)[b]			168.7 (s)			179.0 (s)		
2	125.3 (s)			125.3 (s)			52.1 (d)	3.08 (1H, dd, 7.6)	C-1, C-3, C-4, C-7, C-9
3	25.6 (t)	2.70 (2H, t, 7.9[c])	C-1, C-2, C-4, C-6	25.3 (t)	2.65 (2H, t, 7.9)	C-1, C-2, C-4, C-6	25.2 (t)	2.18 (1H, m)	C-2, C-4, C-6
								1.90 (1H, m)	C-2, C-4, C-6
4	33.6 (t)	2.41 (2H, t, 7.9)	C-3, C-5	33.5 (t)	2.40 (2H, t, 7.9)	C-3, C-5	31.6 (t)	2.15 (2H, m)	C-2, C-3, C-5
5	172.8 (s)			179.2 (s)			172.9 (s)		
6	150.2 (s)			145.4 (s)			141.6 (s)		
7	23.1 (q)	1.97 (3H, s)	C-2, C-6	23.1 (q)	1.94 (3H, s)	C-2, C-6	20.0 (q)	1.56 (3H, s)	C-1, C-2, C-6, C-8
8	22.8 (q)	1.51 (3H, s)	C-2, C-6	21.8 (q)	1.49 (3H, s)	C-2, C-6	115.0 (t)	4.88 (1H, s)	C-1, C-2, C-6, C-7
								4.83 (1H, s)	C-1, C-2, C-6, C-7
9	51.1 (q)	3.36 (3H, s)	C-5	50.7 (q)	3.42 (3H, s)	C-1	51.0 (q)	3.37 (3H, s)	C-5
OH		8.53 (1H, brs)			8.53 (1H, brs)			8.53 (1H, brs)	

[a] All assignments are based on 1D and 2D NMR measurements (HMBC, HMQC, COSY). [b] Implied multiplicities as determined by DEPT (C = s, CH = d, CH$_2$ = t, CH$_4$ = q). [c] J in Hz.

different (see Table 4). As for **6** and **7**, the ^{13}C NMR spectrum contained nine carbon resonances attributable to 1 × CH$_3$, 1 × OCH$_3$, 3 × CH$_2$, 1 × CH, and 3 × C, groups. It was also evident from these data that one of the three degrees of unsaturation within **8** was present as an exomethylene group, in contrast to the endo-carbon−carbon double bond found in **6** and **7**, and that the two carbonyl functions present in **5**−**7** were also to be found in **8**. Interpretation of the HMQC, ^{1}H−^{1}H COSY, and HMBC spectral data of **8** (Table 4) enabled its planar structure to be deduced. Thus, cross-peaks in the ^{1}H−^{1}H COSY spectrum of **8** showed H$_3$-7 to long-range couple with H$_2$-8 and H-2; H-2 also coupled with H$_2$-3, which further coupled with H$_2$-4, clearly characterizing the C-8 to C-4 part of the molecule. The HMBC spectral data of **8** showed correlations between the resonances for H$_2$-8 and those for C-1, C-2, C-6, and C-7; between that for H-2 and those for C-1, C-3, C-6, C-7, and C-8; and between those for H$_2$-3 and C-2, C-4, C-5, and C-6, and between that for H$_3$-9 and that for C-5. These data revealed C-1 to bond with C-2, C-4 with C-5, and the methoxyl group to be part of the methyl ester function at C-5. Accordingly, **8** is most appropriately named pentanedioic acid 2-(1-methylethenyl)-5-methyl ester.

Together with the new compounds the known compounds, **9**−**11**, were identified by comparison of their spectroscopic data and optical rotations with published values.[18] Biosynthetically, **1**, **2**, and **9**−**11** are probably related (see Figure 3a). All of these compounds possess an aromatic moiety, possibly of polyketide origin, substituted with a hemiterpene unit. Different cyclization reactions would then yield the three types shown.

The most unusual bicyclo[4.2.0]octa-1,3,5-triene ring system found in **1** and **2** can be generated synthetically by the action of UV light (245 nm) on a butane solution of 3-methyl-1,2-dihydronaphthalene (3-MDHN) (see Figure 3b);[21] the mode of cyclization used in this *Acremonium* sp. is, however, not known.

Compounds **1**, **2**, **5**, **9**, and **10** were tested in ELISA-based assays for their HIV-1 reverse transcriptase and tyrosine kinase p56^{lck} inhibitory activities and found to be inactive. The antioxidative properties of all compounds were assessed using DPPH radical and TBARS assays. The results of these assays are shown in Tables 5 and 6 and Figure 4. Compounds **1**, **2**, and **9**−**11** have significant DPPH radical scavenging effects (85.5, 17.5, 85.8, 72.9, 90.2%, respectively, at 25.0 μg/mL), with **1**, **9**, **10**, and **11** being also able to inhibit peroxidation of linolenic acid (35.5, 15.9, 9.2, and 16.6%, respectively, at 37.0 μg/mL) (Figure 4). The results
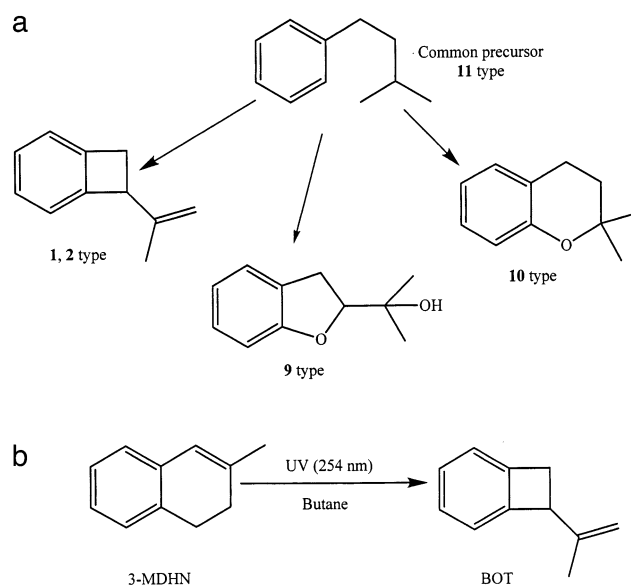
Figure 3. (a) Proposed generalized biosynthetic precursor (compound type **11**) for compound types **1**, **2**, **9**, and **10**. (b) Phytolytic generation of bicyclo[4.2.0]octa-1,3,5-triene (BOT) containing compounds from 3-methyl-1,2-dihydronaphthalene (3-MDHN).

Table 5. Antioxidative Effects (TBARS Assay)[a] of the EtOAc Extract of *Acremonium* sp. and Compounds **1**−**11**

	% inhibition[a]	
item tested	7.4 μg/mL	37.0 μg/mL
EtOAc extract	−27.2	−55.7
1	13.9	35.5
2	−10.6	−10.6
3 and **4**	−22.5	−18.5
5	−9.0	−18.1
6−**8**	−8.6	−16.3
9	−11.8	15.9
10	−9.8	9.2
11	2.0	16.6
BHT[c]	36.2	43.9
arbutin	−27.6	−25.2

[a] TBARS = thiobarbituric acid method. [b] % inhibition = 100 − (A sample[d] − A sample blank) × 100/(A control − A blank). [c] BHT = butylated hydroxytoluene. [d] A = absorbance of test blank and control solutions are measured at both 532 and 600 nm.

of the assays were not totally surprising since it is well known that the phenolic compounds have antioxidant properties,[22] the activity being dependent on the number and the location of the hydroxyl groups.

Table 6. DPPH[a] Radical Scavenging Effects of the EtOAc Extract of *Acremonium* sp. and Compounds **1**−**11**

item tested	% scavenging[b]				
	5[c]	25	50	100	500
EtOAc extract	nt[d]	nt	2.0	4.8	29.3
1	26.0	85.5	90.0	90.0	90.1
2	5.6	17.5	27.7	43.0	79.9
3 and **4**	nt	nt	−1.6	2.8	12.1
5	nt	nt	−0.5	3.8	15.3
6−**8**	nt	nt	3.4	10.3	35.1
9	35.3	85.8	89.1	90.2	90.4
10	25.7	72.9	85.1	89.1	89.9
11	nt	90.2	90.7	89.9	82.9
BHT[e]	6.0	24.2	41.1	63.8	89.8
arbutin	1.9	18.3	23.5	52.6	78.6

[a] DPPH = α,α-diphenyl-β-picrylhydrazyl. [b] Scavenging % = 100 − (A sample[f] × 100/A control[f]). [c] Concentrations in μg/mL. [d] nt = not tested. [e] BHT = butylated hydroxytoluene. [f] Absorbance of sample and control measured at 517 nm.
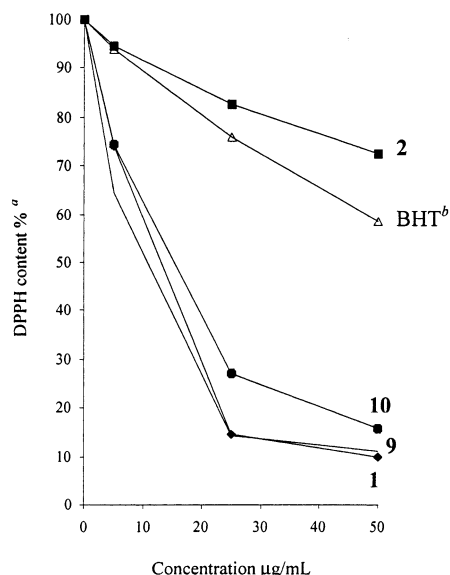

Figure 4. DPPH[c] radical scavenging activity of compounds **1**, **2**, **9**, and **10**. [a]Remaining concentration of DPPH radical. [b]BHT = butylated hydroxytoluene. [c]DPPH = α,α-diphenyl-β-picrylhydrazy1.

Experimental Section

General Experimental Procedures. HPLC was carried out using a Merck-Hitachi system consisting of a L-6200A pump, a L-4500 photodiode array detector, and a D-6000 interface. All NMR spectra were recorded on a Bruker Avance 300 DPX spectrometer. Spectra were referenced to residual solvent signals with resonances at $\delta_{H/C}$ 2.04/29.8 ([CD$_3$]$_2$CO), 3.35/49.0 (CD$_3$OD), and 7.20/128.0 (C$_6$D$_6$), respectively. UV and IR spectra were obtained using Perkin-Elmer Lambda 40 and Perkin-Elmer Spectrum BX instruments, respectively. Optical rotations were recorded on a Jasco DIP 140 polarimeter. FAB and EIMS were measured on a Kratos MS 50 spectrometer. GC−MS spectra were obtained using a Perkin-Elmer (Auto system XL) gas chromatograph coupled with a Perkin-Elmer-Turbomass spectrometer.

Isolation and Taxonomy of the Fungal Strain. Algal material was collected by divers from waters around Spain (Moraira, Mediterranean Sea). After sterilization with 70% ethanol, algal samples were rinsed with sterile water and pressed onto agar plates to detect any residual fungal spores on the surface. Sterilized algae were then cut into pieces and placed on agar plates containing isolation medium: 15 g/L agar, 1 L of seawater from the sample-collecting site, benzyl penicillin, and streptomycin sulfate (250 mg/L). Fungal colonies growing out of the algal tissue were transferred onto medium for sporulation: 1.0 g/L glucose, 0.1 g/L yeast extract,

0.5 g/L peptone from meat, enzymatic digest, 15 g/L agar, 1 L of artificial seawater; the pH was adjusted at 8. The identification of strain M1-11-1 was carried out by Dr. R. A. Samson (Centraalbureau Voor Schimmel Cultures, Baarn, The Netherlands) and was assigned registry No. 157. Mi-11-1 is an *Acremonium* sp., most similar to *A. roseogriseum*.

Cultivation. The fungus was cultivated at room temperature for two months in 7 L (14 Fernbach flasks) of solid biomalt agar containing 50 g of Biomalt (Villa Natura Gesundheitsprodukte GmbH, Germany), 15 g of agar (Fluka Chemie AG), and 1 L of artificial seawater [(g/L): KBr (0.1), NaCl (23.48), MgCl$_2$·6H$_2$O (10.61), CaCl$_2$·6H$_2$O (1.47), KCl (0.66), SrCl$_2$·6H$_2$O (0.04), Na$_2$SO$_4$ (3.92), NaHCO$_3$ (0.19), H$_3$BO$_3$ (0.03)].[4] Each Fernbach flask was inoculated with 10 mL of 10-day-old cultures (room temperature) grown in biomalt media without agar.

Extraction and Isolation. The mass cultivated fungus, mycelia and medium, was diluted with water (100 mL/L) and homogenized using a Waring blender. The resulting mixture was exhaustively extracted with EtOAc (3 × 10 L) to yield 4.5 g of a highly viscous brownish black gum. The extract (4.3 g) was fractionated by VLC (Si gel 60, 0.063−0.200 mm, Merck) employing gradient elution from petroleum ether to EtOAc to MeOH, to yield 8 fractions (200 mL each). VLC fraction 3 (1 g) was further fractionated by VLC using RP-C$_{18}$ material (Polyprep 60-50 C18, Machery-Nagel 71150) and gradient elution from H$_2$O to MeOH, to yield 15 fractions (50 mL each), which were combined, on the basis of ^{1}H NMR spectral similarities, to give nine fractions. Fraction 3 appeared most promising on the basis of several ^{1}H NMR resonances in the δ 6−9 region and was further fractionated by normal-phase HPLC (Eurospher Si, 5 μm, 250 × 8 i.d., Knauer) with 8:2 cyclohexane/(CH$_3$)$_2$CO as eluent to yield six compounds: **1** (14 mg or 2 mg/L), **6**−**8** (14 mg or 2 mg/L), **9** (8 mg or 1.1 mg/L), and **10** (10 mg or 1.4 mg/L). Fraction 4 (1.1 g) was also further fractionated by VLC using normal-phase silica and gradient elution from hexane to EtOAc to MeOH, to yield 15 fractions (50 mL each), which were combined, again on the basis of their ^{1}H NMR spectral data, into six fractions. Of these, fraction 5 was purified using HPLC with RP-C$_{18}$ (Eurospher-100, 5 μm, 250 × 8 mm i.d., Knauer) and gradient elution from 3:1 H$_2$O/MeOH to MeOH in 25 min, 2 mL/min, to yield **11** (25 mg or 3.5 mg/L). From fraction 4 crystals of **5** (6 mg or 0.85 mg/L) were obtained by recrystallization from CH$_2$Cl$_2$/acetone (3:7); the mother liquor was purified by RP-C$_{18}$ HPLC employing gradient elution from 4:1 H$_2$O/MeCN to MeCN in 40 min, 2 mL/min, to yield **3** and **4** (2 mg or 0.28 mg/L) and **10** (3 mg). VLC fraction 6 (0.6 g) was further fractionated by VLC over normal-phase HPLC (230−400 mesh ASTM, Merck), and 15 fractions were collected (50 mL each) and combined into seven pools according to ^{1}H NMR spectral data. Pool 3 (0.1 g) was the most promising one and fractionated over normal-phase HPLC (Eurospher Si, 5μ, 250 × 8 i.d., Knauer) using a gradient from CH$_2$Cl$_2$ to MeOH, in 25 min, 2 mL/min, to afford **2** (20 mg, 2.8 mg/L). All other experimental details were as previously reported.[23]

Bioassays. HIV-1 reverse transcriptase and tyrosine kinase p56^{lck} inhibitory activity were measured as described by Kirsch et al.[24]

Antioxidative Activity. Thiobarbituric Acid Reactive Substances Method (TBARS Assay). The method used was adapted from Wallin et al.[25] and modified as follows: Assays were performed in flat-bottom polystyrene 96-well microtiter plates. The final volume of the reaction mixture in the oxidation step of linolenic acid methyl ester was 70 μL/well. The solution was made of 40 μL of 50 mM phosphate buffer (pH = 7.2), 10 μL of 10% (w/v) sodium dodecyl sulfate, 5 μL of sample in ethanol (1 and 0.2 mg/mL), 5 μL of linolenic acid methyl ester 70 mM in ethanol, and finally 10 μL of 0.08% (w/v) FeSO$_4$·7H$_2$O in 0.025 M HCl. Plates were placed in a thermomixer and incubated at 50 °C and 500 rpm for 30 min. At the end of this period 5 μL of butylated hydroxytoluene (BHT) 15 mM in ethanol was added immediately so as to prevent further oxidation. To each well 20 μL of 50% (w/v) trichloroacetic acid and 40 μL of 0.88% (w/v) thiobarbituric

acid, dissolved in 0.3% (w/v) NaOH, were added. The plate was then covered with a micromat and incubated at 60 °C and 500 rpm for a further 30 min. At the end of this period the plate was maintained at room temperature for 4 min and the absorbances were then determined at 532 nm, less the background absorbance at 600 nm, using a SLT Spectral Rainbow microtiter plate reader.

α,α-Diphenyl-β-picrylhydrazyl (DPPH) Radical Scavenging Effects. Assays were performed in flat bottom polystyrene 96-well microtiter plates. The DPPH radical scavenging effects of the EtOAc extract of *Acremonium* sp. and compounds **1**−**11** were undertaken using a modified previously established methodology.[26,27] To 100 μL of each sample (1 mg/mL) in EtOH was added 25 μL of DPPH (1 mM) in EtOH and 75 μL of EtOH to give a final volume of 200 μL. The resultant mixture was briefly shaken and maintained at room temperature in the dark for 30 min. At the end of this period the absorbance of the mixture was measured at 517 nm, using a SLT Spectral Rainbow microtiter plate reader.

7-Isopropenylbicyclo[4.2.0]octa-1,3,5-triene-2,5-diol (acremonin A, 1): (14.0 mg) yellowish brown viscous oil; $[\alpha]^{23}_D$ +93.0° (*c* 1.4, $[CH_3]_2CO$); UV λ_{max} (MeOH) (log ϵ) 204.9 (4.4), 286 (3.6) nm; IR (film) ν_{max} 3314, 2931, 1495, 1453, 1239 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; EIMS *m/z* (% rel int) 176 [M$^+$] (100), 147 (33), 115 (23), 91 (20); HREIMS *m/z* 176.0837, calcd for $C_{11}H_{12}O_2$ 176.0837.

7-Isopropenylbicyclo[4.2.0]octa-1,3,5-triene-2,5-diol, 5-β-D-glucopyranoside (acremonin A glucoside, 2): (20.0 mg) amorphous powder; $[\alpha]^{23}_D$ +4.3° (*c* 2.0, $[CH_3]_2CO$); UV λ_{max} (MeOH) (log ϵ) 204.9 (4.3), 280 (3.2) nm; IR (film) ν_{max} 3330, 2921, 1490, 1448, 1242 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; FABMS, *m/z* 361 [M + Na]$^+$.

Acid Hydrolysis of 2. A solution of **2** (5 mg in 1 mL of EtOH) and 10 mL of 7% HCl/EtOH (3:7) was refluxed for 4 h. At the end of this period the mixture was extracted with EtOAc, and the aqueous layer neutralized with 7% KOH. After evaporation of the solvent, the residue was extracted with C_6H_5N. HPLC purification of the C_6H_5N extract (Lichrosorb-100 NH$_2$, 5 μ, 250 × 8 mm i.d., Knauer) using 8:2 MeCN/H$_2$O as eluent yielded glucose (1 mg), $[\alpha]^{23}_D$ +20.7° (*c* 0.08, H$_2$O), lit.[28] $[\alpha]^{23}_D$ +18.7° − +52.7° (*c* 10.0, H$_2$O).

(3*R**,4*S**)-3,4-Dihydroxy-7-methyl-3,4-dihydro-1(2*H*)-naphthalenone (3) and (3*S**,4*S**)-3,4-dihydroxy-7-methyl-3,4-dihydro-1(2*H*)-naphthalenone (4): (2.0 mg) amorphous white powder; UV λ_{max} (MeOH) (log ϵ) 251 (3.6), 296 (2.9) nm; ^1H and ^{13}C NMR data, see Table 3; EIMS *m/z* (% rel int) 192 [M$^+$] (10), 174 (30), 148 (50), 119 (100); HREIMS *m/z* 192.0799, calcd for $C_{11}H_{12}O_3$ 192.0786.

GC−MS Analysis of 3 and 4. This analysis was carried out on a Perkin-Elmer (Auto system XL) gas chromatograph coupled with a Perkin-Elmer Turbomass spectrometer using a 30 m × 0.32 mm N931-6023 Pe-1 (film thickness of 0.25 μm) capillary column. The instrument was set to an initial temperature of 50 °C and maintained at this temperature for 10 min. At the end of this period the oven was heated at 10°/min up to 250 °C and kept there for 10 min. Injection port temperature was 220 °C, He flow rate was 2 mL/min, and samples were injected in the splitless mode. Mass spectral scan range was 35−650 Da. The GC of **3** and **4** showed two peaks. Retention times, relative intensities (%), and EIMS of the relevant peaks were as follows: peak A, 21.4 min, 100, EIMS *m/z* (% rel int), 192 [M$^+$] (<1), 174 (77), 159 (5), 145 (11), 119 (32), 90 (18); peak B, 24.2 min, 84, EIMS *m/z* (% rel int), 192 [M$^+$] (<1), 174 (77), 159 (5), 148 (46), 145 (13), 119 (100), 90 (20).

2-(1-Methylethylidene)pentanedioic acid (5): (6 mg) transparent crystalline solid; mp 131−132°; $[\alpha]^{23}_D$ +4.4° (*c* 0.8, $[CH_3]_2CO$); UV λ_{max} (MeOH) (log ϵ) 221 (3.8) nm; IR (film) ν_{max} 2922, 2604, 1688, 1655, 1615, 1411 cm^{-1}; ^1H and ^{13}C NMR data, see Table 4; FABMS *m/z* 173 [M + H]$^+$.

X-ray Structure Analysis of Compound 5. 2-(1-Methylethylidene)pentanedioic acid (**5**) was recrystallized from MeOH to yield transparent prismatic crystals. A crystal 0.34 × 0.15 × 0.11 mm^3 was selected for crystallographic measurement. It has molecular formula $C_9H_{12}O_4$, molecular mass 172.18 amu,

crystal system triclinic, space group $P\bar{1}$, unit cell dimensions $a = 5.0439$ Å, $b = 7.2708$ Å, $c = 12.3363(16)$ Å, $\alpha = 89.527(3)°$, $\beta = 84.456(3)°$, $\gamma = 74.754(3)°$, volume = 434.37(10) Å^3, $Z = 2$, density = 1.316 mg/m^3, $F(000) = 184$, absorption = 0.106 μ, λ(Mo Kα) = 0.71073 Å. The intensity data were collected at 133 K to θ(max) 30° on a Bruker SMART 1000 CCD diffractometer. A total of 5047 reflections were recorded, of which 2495 [R(int) = 0.0282] were unique. The structure was solved by direct methods and refined by full matrix least-squares methods on F^2 using the SHELXL97 program.[29] Acidic hydrogens were refined freely, methyls as rigid groups, and methylene hydrogens with a riding model. Final R indices: R1 [$I > 2\sigma(I)$] = 0.042, wR2 (all reflections) = 0.124; goodness of fit 1.047.[30] The molecule is shown in Figure 1. Its dimensions may be regarded as normal. The molecular conformation is illustrated by the torsion angles C2−C3−C4−C5 177.3(1)°, C3−C4−C5−O3 177.6(1)° (antiperiplanar) and C1−C2−C3−C4 85.0(1)°, C6−C2−C3−C4 93.2(1)° (orthogonal). The molecules are connected by hydrogen bonds of the "carboxylic acid dimer" type [O3−H03···O4′ and O1−H01···O2′] across inversion centers to form chains of molecules parallel to [11−1] (Figure 2).

Pentanedioic acid 2-(1-methylethylidene)-5-methyl ester (6), pentanedioic acid 2-(1-methylethylidene)-1-methyl ester (7), and pentanedioic acid 2-(1-methylethenyl)-5-methyl ester (8): (14 mg) brownish viscous oil; $[\alpha]^{23}_D$ (**8**) −28.4° (*c* 0.4, MeOH), based on **8** being approximately one-third of the mixture; UV λ_{max} (MeOH) (log ϵ) 220 (3.6) nm, based on **6** and **7** being approximately two-thirds of the mixture; IR (film) ν_{max} 2922, 2359, 1735, 1720, 1436, 1216 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 5, 6; EIMS data, see GC−MS analysis of compounds **6**−**8**; HREIMS *m/z* 186.0891 (all three compounds have the identical exact mass), calcd for $C_9H_{14}O_4$ 186.0892.

GC−MS Analysis of 6−8. The mixture of compounds **6**−**8** was analyzed by GC−MS in a manner identical to the analysis performed with **3** and **4**. Retention times, relative intensities, and EIMS of the relevant peaks were as follows: peak A (19.4 min, 92), EIMS *m/z* (% rel int), 186 (1), 168 (50), 140 (100), 81 (90), 67 (100); peak B (19.8 min, 72), EIMS *m/z* [M]$^+$ (% rel int), 186 [M$^+$] (3), 168 (10), 140 (33), 81 (40), 67 (100); peak C (20.2 min, 100), EIMS *m/z* (% rel int), 186 [M$^+$] (2), 168 (77), 140 (100), 81 (60), 67 (100).

2-(1-Hydroxy-1-methyl)-2,3-dihydrobenzofuran-5-ol (9): (8 mg) transparent crystals; $[\alpha]^{23}_D$ +13.0° (*c* 0.25, $[CH_3]_2CO$), lit.[18] $[\alpha]^{23}_D$ +49.8° (*c* 1.0, MeOH); ^1H NMR (300 MHz, $[CD_3]_2$-CO) δ 6.65 (1H, dd, $J = 0.7$, 2.2 Hz, H-1), 6.49 (1H, dd, $J = 2.2$, 8.8 Hz, H-3), 6.46 (1H, d, $J = 8.8$ Hz, H-4), 3.03 (1H, dd, $J = 9.5$, 16.1 Hz, H-7), 3.16 (1H, dd, $J = 8.8$, 16.1 Hz, H-7), 4.5 (1H, dd, $J = 8.8$, 9.5 Hz, H-8), 1.17 (3H, s, H$_3$-10), 1.20 (3H, s, H$_3$-10), 3.60 (1H, s, OH-9), 7.73 (1H, brs, OH-2); ^{13}C NMR (75.5 MHz[CD$_3$]$_2$CO) δ 112.8 (d, C-1), 152.0 (s, C-2), 114.3 (d, C-3), 109.3 (d, C-4), 154.1 (s, C-5), 129.2 (s, C-6), 31.6 (t, C-7), 90.0 (d, C-8), 71.4 (s, C-9), 25.5 (q, CH$_3$, C-10), 26.1 (q, CH$_3$, C-11); EIMS *m/z* (% rel int), 194 [M$^+$] (50), 161 (32), 136 (100), 123 (20), 107 (20).

2, 2-Dimethylchroman-3, 6-diol (10): (13 mg) transparent crystalline solid, mp 175 °C; $[\alpha]^{23}_D$ −41.0° (*c* 0.25, MeOH), lit.[18] $[\alpha]^{23}_D$ −20.4° (*c* 1.0, MeOH); ^1H NMR (300 MHz, $[CD_3]_2CO$) δ 6.56 (1H, d, $J = 1.8$ Hz, H-1), 6.52 (1H, m, H-3), 6.52 (1H, m, H-4), 2.90 (1H, dd, $J = 4.6$, 16.8 Hz, H-7), 2.63 (1H, dd, $J = 8.4$, 16.8 Hz, H-7), 3.73 (1H, d, $J = 8.4$ Hz, H-8), 1.18 (3H, s, H$_3$-10), 1.30 (3H, s, H$_3$-10), 4.10 (1H, d, $J = 4.7$, Hz, OH-8), 7.70 (1H, s, OH-2); ^{13}C NMR (75.5 MHz, $[CD_3]_2CO$) δ 116.1 (d, C-1), 147.0 (s, C-2), 117.1 (d, C-3), 115.1 (d, C-4), 151.4 (s, C-5), 121.1 (s, C-6), 32.4 (t, C-7), 70.1 (d, C-8), 77.1 (s, C-9), 20.1 (q, CH$_3$) 26.1 (q, CH$_3$); EIMS *m/z* (% rel int), 194 [M$^+$] (100), 161 (35), 136 (35), 123 (100), 71 (60).

2-(3-Dihydroxy-3-methylbutyl)benzene-1,4-diol (11): (25 mg) light brown oil; $[\alpha]^{23}_D$ +15.8° (*c* 0.15, MeOH); lit.[18] $[\alpha]^{23}_D$ +36.0° (*c* 0.2, MeOH); ^1H NMR (300 MHz, $[CD_3]_2CO$) δ 6.56 (1H, m, H-2), 6.56 (1H, m, H-2), 6.49 (1H, dd, $J = 2.9$, 0.3 Hz, H-5), 2.58 (1H, dd, $J = 9.9$, 14.0 Hz, H-7), 2.71 (1H, dd, $J = 1.9$, 14.0 Hz, H-7), 3.55 (1H, ddd, $J = 1.9$, 9.9, 14.0 Hz, H-8), 1.18 (3H, s, H$_3$-10), 1.18 (3H, s, H$_3$-10), 4.60 (1H, brs, OH-9),

7.65 (1H, brs, OH-4), 8.20 (1H, brs, OH-1), 4,79 (1H, brs, OH-8); ^{13}C NMR (75.5 MHz, [CD$_3$]$_2$CO) δ 148.8 (s, C-1), 117.0 (d, C-2), 116.5 (d, C-3), 150.3 (s, C-4), 113.7 (d, C-5), 128.0 (s, C-6), 33.6 (t, C-7), 80.4 (d, C-8), 72.0 (s, C-9), 24.6 (q, CH$_3$-10), 24.5 (q, CH$_3$-11).

Supporting Information Available: This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) König, G. M.; Wright, A. D. *Planta Med.* **1996**, *62*, 193−211.
(2) Kohlmeyer, J.; Kohlmeyer, E. In *Marine Mycology, The Higher Fungi*; Academic Press: New York, 1979; pp 54−69.
(3) Osterhage, C.; Kaminsky, R.; König, G. M.; Wright, A. D. *J. Org. Chem.* **2000**, *65*, 6412−6417.
(4) Höller, U.; König, G. M.; Wright, A. D. *J. Nat. Prod.* **1999**, *62*, 114−118.
(5) Lorenz, P.; Jensen, P. R.; Fenical, W. *Nat. Prod. Lett.* **1998**, *12*, 55−60.
(6) Yen, G. C.; Lee, C. A. *J. Food Prot.* **1996**, *59*, 1327−1330.
(7) Hayashi, K. I.; Suzuki, K.; Kawaguchi, M.; Nakajima, T.; Suzuki, T.; Numata, M.; Nakamura, R. *Biosci. Biotech. Biochem.* **1995**, *59*, 319−320.
(8) Yen, G. C.; Lee, C. E. *J. Sci. Food Agric.* **1997**, *75*, 326−332.
(9) Yen, G. C.; Chang, Y. C. *J. Food Prot.* **1999**, *62*, 657−661.
(10) Yen, G. C.; Chang, Y. C.; Sheu, F.; Chiang, H. C. *J. Agric. Food Chem.* **2001**, *49*, 1426−1431.
(11) Hirota, A.; Morimitsu, Y.; Hojo, H. *Biosci. Biotech. Biochem.* **1997**, *61*, 647−650.
(12) Takahashi, N.; Tamagawa, K.; Kawai, K. I.; Fukui, T. *Biol. Pharm. Bull.* **2000**, *23*, 989−994.
(13) Teshima, Y.; Shin-Ya, K.; Shimazu, A.; Furihata, K.; Chul, H. S.; Furihata, K.; Hayakawa, Y.; Nagai, K.; Seto, H. *J. Antibiot.* **1991**, *44*, 685−687.
(14) Chen, C.; Imamura, N.; Nishijima, M.; Adachi, K.; Sakai, M.; Sano, H. *J. Antibiot.* **1996**, *49*, 998−1005.
(15) Belofsky, N. G.; Anguera, M.; Jensen, R. P.; Fenical, W.; Köck, M. *Chem. Eur. J.* **2000**, *6*, 1355−1360.
(16) Sassa, T.; Kinoshita, H.; Nukina, M.; Sugiyama, T. *J. Antibiot.* **1998**, *51*, 967−969.
(17) Roy, K.; Chatterjee, S.; Deshmukh, S. K.; Vijayakumar, K. S. E.; Ganguli, N. B.; Fehlhaber, H. W. *J. Antibiot.* **1996**, *49*, 1186−1187.
(18) Tanaka, M.; Nara, F.; Yamasato, Y.; Ono, Y. Y.; Ogita, T. *J. Antibiot.* **1999**, *52*, 827−830.
(19) Fleury, J. P.; Lantz, A.; Lichtenberger, A. L. *J. Bull. Soc. Chim. Fr.* **1963**, 556−570.
(20) Puterbaugh, W. H. *J. Org. Chem.* **1962**, *27*, 4010−4015.
(21) Duguid, J. R.; Morrison, H. *J. Am. Chem. Soc.* **1991**, *113*, 1271−1281.
(22) Aoyama, T.; Nakakita, Y.; Nakajawa, M.; Sakai, H. *Agric. Biol. Chem.* **1982**, *46*, 2369−2371.
(23) Wright, A. D.; König, G. M.; Angerhofer, C. K.; Greenidge, P.; Linden, A.; Desqueyroux-Faúndez, R. *J. Nat. Prod.* **1996**, *59*, 710−716.
(24) Kirsch, G.; König, G. M.; Wright, A. D.; Kaminsky R. *J. Nat. Prod.* **2000**, *63*, 825−829.
(25) Wallin, B.; Rosengren, B.; Shertzer, H. G.; Camejo, G. *Anal. Biochem.* **1993**, *208*, 10−15.
(26) Blois, M. S. *Nature* **1958**, *181*, 1199−1200.
(27) Amarowicz. R.; Naczk, M.; Shahidi, F. *J. Agric. Food Chem.* **2000**, *48*, 2755−2759, and references therein.
(28) Merck Index online, http://www.ulb.uni-bonn.de/Cdroms/datenbanken.htm, monograph number 4467.
(29) Sheldrick, G. M. SHELX97 (*SHELXS and SHELXL*). *Manual of Programs for the Refinement of Crystal Structures*; University of Göttingen: Germany, 1997.
(30) The crystallographic data for **5** have been deposited at the Cambridge Crystallographic Data Center, 12 Union Road, Cambridge, CB2 1EZ, UK, and can be obtained free of charge on application to the Director (e-mail: deposit@ccdc.cam.ac.uk).

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