Bioactive Metabolites from a Marine-Derived Strain of the Fungus *Emericella variecolor*

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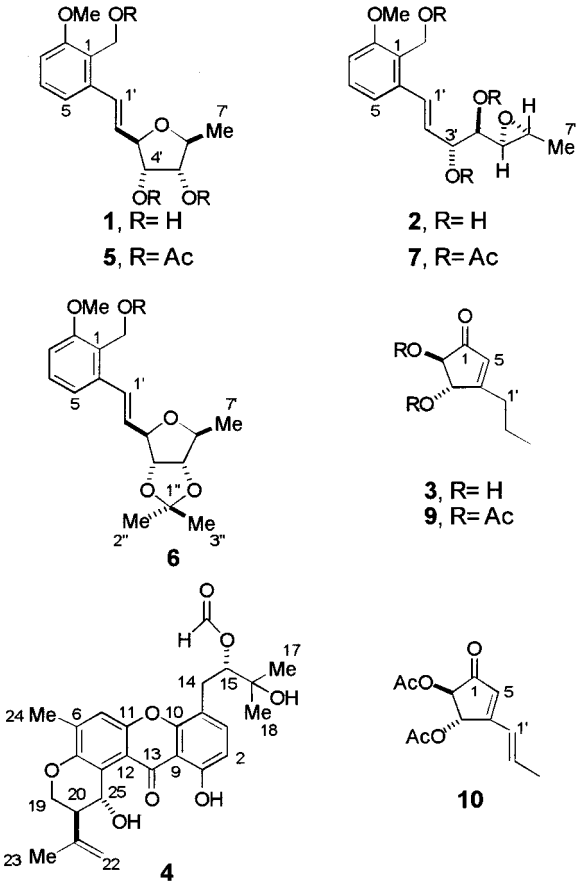
Received June 29, 2001

From a marine-derived strain of the fungus *Emericella variecolor*, varitriol (**1**), varioxirane (**2**), dihydroterrein (**3**), and varixanthone (**4**), besides the known mold metabolites ergosterol, terrein, shamixanthone, and tajixanthone hydrate, were identified. The chemical structures of **1**−**4** were established by means of spectroscopic techniques and some chemical transformations. In the NCI's 60-cell panel, varitriol (**1**) displayed increased potency toward selected renal, CNS, and breast cancer cell lines. Varixanthone (**4**) showed antimicrobial activity.

Recently, increased interest in the chemistry of fungi isolated from the marine environment has been documented.[1] Marine fungi are interesting organisms from an ecological point of view, because they are serious pathogens in the marine environment.[2] Furthermore, since many can be cultured, they represent an important biomedical resource. During our studies on the chemistry and biology of fungi,[3,4] we have investigated a marine strain[5] (named M75-2) of the fungus *Emericella variecolor*, isolated from a sponge collected in Venezuelan waters of the Caribbean Sea.[6] *E. variecolor* Berk and Br. is the perfect state of *Aspergillus variecolor* (Berk and Br.) Thom and Raper.[7] From different terrestrial strains of *A. variecolor* terrein,[8] 2-methoxy-6-(3,4-dihydroxyhepta-1,5-dienyl)benzyl alcohol,[9] 4,7-dimethoxy-5-methylcoumarin,[10] a dihydroisocoumarin,[11] and two sesterterpenoids (variecolin[12] and asterellatol[13]), as well as numerous xanthones[14] and meroterpenoids[15] of mixed polyketide and terpenoid origins, have been isolated. Moreover, in a terrestrial strain of *E. variecolor*, asteltoxin has been found.[16,17] This report deals with the chemical analysis and biological screening of the metabolites from the first marine strain of *E. variecolor*.

Chart 1. Chemical Structures of the Metabolites **1**−**4** from *E. variecolor* and the Derivatives **5**−**7**, **9**, and **10**



Results and Discussion

From a static culture of the fungus the new compounds **1**−**4** (Chart 1) were identified, in addition to the known mold metabolites ergosterol,[18] terrein,[19] shamixanthone,[20] and tajixanthone hydrate.[21]

The HRMS of varitriol (**1**) indicated a $C_{15}H_{20}O_5$ molecular formula, corresponding to six elements of unsaturation. The main feature of the IR spectrum was a very strong absorption band centered at 3338 cm^{-1}, suggesting the presence of more than one hydroxyl group. On the other hand, no carbonyl bands were present. The ^1H NMR spectrum showed signals at δ 7.22 (t, J = 8 Hz, H-4), 7.13 (dd, J = 8.0, 1.0 Hz, H-5), and 6.89 ppm (dd, J = 8.0, 1.0 Hz, H-3), which were assigned to three adjacent aromatic protons. Additionally, the signals of two olefinic hydrogen atoms appeared at δ 7.14 (br d, J = 15.8 Hz, H-1′) and 6.19 (dd, J = 15.8, 6.7 Hz, H-2′), with chemical shifts and coupling constant values indicative of a trans-disubstituted double bond conjugated with the aromatic ring. The presence of the vinyl benzene conjugated system was confirmed by the UV spectrum (absorption bands at 260 and 296 nm). Moreover, apart from a methyl doublet (δ 1.27, J = 6.2 Hz, H-7′), the ^1H NMR spectrum also exhibited signals assigned to an oxygenated benzylic methylene (δ 4.72, br s) and four oxygenated methines (δ 4.32, br t, H-3′; δ 3.94, br q, H-4′; δ 3.74, br q, H-5′; δ 3.84, quintuplet, H-6′). A signal for a methoxy group appeared at δ 3.82 (s), suggesting the methoxy was directly attached to the aromatic ring. Three broad signals, roughly centered at δ 4.62, 4.41, and 3.75,

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10.1021/np0103214 CCC: $22.00
Published on Web 03/01/2002

Table 1. 2D NMR Data for Varitriol (**1**) (400 MHz, CD_3COCD_3) and NOEs Observed for Derivatives **5** and **6** (400 MHz, CD_3Cl)

proton	COSY[a] (**1**)	HMBC[b] (**1**)	NOE[c] (**5**)	NOE[c] (**6**)
H-3	4	1, 5		
H-4	3, 5	2, 6		
H-5	4	1, 3		
H-1′	2′	5, 3′		
H-2′	1′, 3′	6, 3′	5, 3′, 4′	3′, 4′
H-3′	2′, 4′		1′, 2′, 6′	1′, 2′, 6′, 2″
H-4′	3′, 5′, O*H*		2′, 5′	2′, 5′, 3″
H-5′	4′, 6′, O*H*		4′, 7′	4′, 7′, 3″
H-6′	5′, 7′		3′, 7′	3′, 7′, 2″
H_3-7′	6′	5′, 6′		
OCH_3		2		
OCH_2-C-1	O*H*	1, 2, 6		
H_3-2″				3′, 6′

[a] Numbers represent hydrogen atoms that were observed to couple with the proton(s) associated with this row. [b] Numbers refer to carbon atoms that were observed to long-range CH couple with the proton(s) corresponding with this row. [c] Enhanced proton signals observed by NOE difference experiments.

were assigned to three hydroxyl groups since they exchanged with D_2O. The ^{13}C NMR spectrum showed 15 signals, eight of which corresponded to the vinyl benzene system, which is responsible for five of the six unsaturation elements demanded by the molecular formula. Since no other double bond was observed, compound **1** must have a second ring, presumably a cyclic ether according to the number of oxygen atoms and oxygenated carbons (apart from those of the CH_3O−C-2 system, four methines and a benzylic methylene). At this point, 2D NMR experiments (Table 1) served to establish the connections between the different structural elements of **1**. Thus, diagnostic HMBC correlations were observed between the resonance for the hydrogens of the methoxy group and that for the aromatic C-2, between the resonance for the olefinic H-2′ and that for the aromatic C-6, and between the resonance for the hydrogens of the benzylic methylene and those for the aromatic carbon atoms C-1, C-2, and C-6, indicating that the benzylic methylene was located on the aromatic ring between the vinyl and the methoxy groups. In the COSY spectrum, $^1H-^1H$ couplings were observed between H-2′ and H-3′, between H-3′ and H-4′, between H-4′ and H-5′, between H-5′ and H-6′, and between H-6′ and H_3-7′; establishing the structure of the backbone of the side chain of **1**, from C-1′ to C-7′. Additionally, cross-peaks between the resonances for the OH groups and those for H-4′, H-5′, and the protons of the methylene group indicated that the hydroxyl groups were attached to C-4′, to C-5′, and to the benzylic methylene. Thus, the position of the oxygenated bridge between C-3′ and C-6′ could be deduced. Peracetylation of **1** gave the triacetate **5**, confirming the number and localization of the OH groups of varitriol. Once the planar structure of **1** was established, the determination of the relative configuration of the four stereogenic centers of the tetrahydrofuran ring unit was attempted. The triacetate **5** was subject to NOE experiments (Table 1), because the differences among the chemical shifts of protons H-3′, H-4′, H-5′, and H-6′ (higher than in the natural compound **1**) warranted more accurate measurements. The NOEs observed between H-2′ and H-4′, between H-4′ and H-5′, between H-5′ and H_3-7′, and between H-3′ and H-6′ indicated that while C-2′, H-4′, H-5′, and C-7′ were located at one side of the tetrahydrofuran ring, H-3′, H-6′, and two hydroxyl groups were located on the opposite side. Subsequently, acetonide **6** was prepared and additional NOE difference measurements were performed (Table 1), confirming the relative stereochemistry of the tetrahydrofuran moiety. Therefore, the structure (3′*R**,4′*R**,5′*S**,6′*S**)-2-methoxy-6-(3′,6′-epoxy-4′,5′-dihydroxyheptenyl)benzyl alcohol (**1**) is assigned to varitriol.

Varioxirane (**2**) could be isolated as its peracetylated derivative **7**. The HRMS of **7** indicated a $C_{21}H_{26}O_8$ molecular formula, isomeric with that of triacetylvaritriol (**5**). The ^1H NMR spectrum of **7** showed several signals (H-3, H-4, H-5, H-1′, H-2′, H-4′, H_3-7′, and three acetate groups) closely related to those of **5**, but in the case of **7**, the chemical shift and coupling constant values of the signals at δ 3.02 (dq, $J = 5.2$, 2.1 Hz, H-6′) and 2.85 (dd, $J = 6.0$, 2.1 Hz, H-5′) revealed a trans-disubstituted oxirane ring involving the C-5 and C-6 carbon atoms. As in **5**, two acetate groups were connected to the benzylic methylene and C-4′, but in **7**, the third acetate group was attached to C-3′, as indicated by the chemical shift of H-3′ (5.70 ppm). The ^{13}C NMR spectrum of derivative **7** confirmed the chemical structure, and thus, the structure of natural metabolite **2** (Chart 1) could be inferred. We have no direct evidence of the stereochemistry around C-3′ and C-4′ of **2**, but based on a hypothetical biogenetic relationship between **2** and **1**, the 3′*R**,4′*R**,5′*S**,6′*R** relative configuration is proposed. It is noteworthy that a structurally related metabolite, 2-methoxy-6-(3,4-dihydroxyhepta-1,5-dienyl)-benzyl alcohol (**8**), has previously been found in *Aspergillus variecolor* (the imperfect state of *Emericella variecolor*).[9] Although the relative configuration at C-3′ and C-4′ of **8** was not established by Dunn and Johnstone,[9] the biosynthesis of varitriol (**1**) from **8**, via varioxirane (**2**), in *E. variecolor* cannot be ruled out. Enzymatic epoxidation of the 5′, 6′ double bond of **8** would yield **2** and enzyme-catalyzed S_N2 reaction at C-6′ in **2** by the 3′-OH would yield **1**.

Dihydroterrein (**3**) was isolated as the diacetyl derivative **9** (Chart 1). The HRMS of **9** indicated a $C_{12}H_{16}O_5$ molecular formula. The ^1H NMR spectrum of **9** showed signals (H-2, H-4, H-5, and two acetate groups) closely related to those of diacetylterrein (**10**) obtained from terrein by conventional acetylation using acetic anhydride and pyridine. However, in the ^1H NMR spectrum of **9**, the signals corresponding to the propenyl moiety of **10** were replaced by two multiplets, centered at 2.34 (2H, H-1′) and 1.62 ppm (2H, H-2′), as well as a triplet at 0.98 ppm (3H, $J = 6.4$ Hz, H-3′), which were assigned to a saturated *n*-propyl side chain. In the ^{13}C NMR spectrum of **9**, the signals at 31.8 (t, C-1′), 20.0 (t, C-2′), and 13.8 ppm (q, C-3′) confirmed the presence of the saturated side chain of derivative **9**, and thus, the structure of natural metabolite **3** could be inferred. Presumably, **3** is synthesized by the enzymatic hydrogenation of terrein in *E. variecolor*.

The HRMS of varixanthone (**4**) indicated a $C_{26}H_{28}O_8$ molecular formula. Its ^1H and ^{13}C NMR spectra were closely related to those of tajixanthone hydrate.[21,22] However, the ^1H NMR spectrum of **4** showed significant differences; the signal corresponding to H-15 was displaced to 5.18 ppm and a new signal appeared at 7.87 ppm, which were assigned to the presence of a formate group connected to C-15. Despite the different chemical shifts, the multiplicity and coupling constant values for H-14a, H-14b, and H-15 of **4** were very similar to those of tajixanthone hydrate, suggesting the same relative stereochemistry for both compounds. Subsequent hydrolysis of **4** gave tajixanthone hydrate with spectroscopic properties, including optical rotation, in agreement with those previously described.[21,22] Thus, the structure and relative stereochemistry of **4** were confirmed. Furthermore, the absolute configuration (depicted in Chart 1) could be established,

because that of tajixanthone hydrate is known.[21] Since the extraction was performed with a solvent mixture containing formic acid (1%), the possibility of varixanthone (**4**) representing an artifact from tajixanthone hydrate was considered. However, tajixanthone hydrate, subject to the extraction conditions, was stable and no formation of varixanthone was detected. Accordingly we believe that **4** is a true natural product.

Varitriol (**1**) was tested in the National Cancer Institute (NCI) 60-cell-line in vitro panel. It showed a more than 100-fold increased potency (over the mean toxicity) toward the RXF 393 (renal cancer, $GI_{50} = 1.63 \times 10^{-7}$ M), T-47D (breast cancer, $GI_{50} = 2.10 \times 10^{-7}$ M), and SNB-75 (CNS cancer, $GI_{50} = 2.44 \times 10^{-7}$ M) cell lines and lower potency against the DU-145 (prostate cancer, $GI_{50} = 1.10 \times 10^{-6}$ M), HL-60 (TB) (leukemia, $GI_{50} = 2.52 \times 10^{-5}$ M), CCRF-CEM (leukemia, $GI_{50} = 2.60 \times 10^{-5}$ M), OVCAR-5 (ovarian cancer, $GI_{50} = 6.82 \times 10^{-5}$ M), SNB-19 (CNS cancer, $GI_{50} = 9.13 \times 10^{-5}$ M), and COLO 205 (colon cancer, $GI_{50} = 9.59 \times 10^{-5}$ M) cell lines. Varitriol was inactive against the remaining cell lines at a concentration of 10^{-4} M. When tested in an antimicrobial assay,[23] varitriol (**1**) did not inhibit growth of bacteria and yeast at 100 μg/mL.

Varixanthone (**4**), tested against three cell lines (see Experimental Section) did not show any cytotoxic activity at 1 μg/mL. However, varixanthone displayed antimicrobial potency, against Gram positive and Gram negative bacteria, higher than terrein, shamixanthone, and tajixanthone hydrate.

Experimental Section

General Experimental Procedures. NMR spectra were recorded in $CDCl_3$ or acetone-d_6 on a Bruker AMX 300 or a Bruker ARX 400. UV spectra were recorded in EtOH on a Hewlett-Packard 8453 diode array spectrophotometer. IR spectra were recorded on a Nicolet 20SXB spectrometer. Optical rotations were measured on a Perkin-Elmer model 124 polarimeter. Melting points were measured on a Reichert instrument and are uncorrected.

Collection, Isolation, and Fermentation. *Emericella variecolor* (strain M75-2) was isolated from a sponge (Porifera) collected in the Caribbean waters of the Mochima Bay (Mochima National Park, Sucre State, Venezuela) in January 1997.[6] A voucher specimen of the fungal strain is kept at the Department of Biotechnology, The Technical University of Denmark. For metabolite production, the fungus was grown on 200 Petri dishes containing solid YES medium for a period of 14 days at 25 °C.

Extraction and Isolation. Mycelium and agar were harvested and extracted with a mixture of ethyl acetate/chloroform/methanol (3:2:1) containing 1% formic acid in a stomacher bag. The dried extract (10.6 g) was split in two fractions by partition between methanol/water (80:20) and hexane. From the less polar fraction, shamixanthone[20] (hexane/t-BuOMe, 8:2) and ergosterol[18] (hexane/t-BuOMe, 1:1) were isolated by column chromatography on Si gel. From the most polar fraction, varixanthone (**4**) (methylene chloride/acetone, 95:5), tajixanthone hydrate[21] (methylene chloride/acetone, 8:2), terrein[19] (methylene chloride/acetone, 7:3), varitriol (**1**) (methylene chloride/acetone, 6:4), a mixture of **1** plus varioxirane (**2**) (methylene chloride/acetone, 6:4), and a mixture of dihydroterrein (**3**), terrein, and **1** (EtOH) were isolated by the same way. To the mixture of **1** plus **2**, were added pyridine and Ac_2O. The solution was stirred for 3 h, and after usual workup, the crude residue was chromatographed by semipreparative TLC (methylene chloride/t-BuOMe, 95:5) to give triacetylvaritriol (**5**) and triacetylvarioxirane (**7**). To the mixture of dihydroterrein, terrein, and **1** were added pyridine and Ac_2O. The solution was stirred for 3 h, and the crude residue was chromatographed by semipreparative TLC (6:4 hexane/t-BuOMe, four developments), obtaining diacetyldihydroterrein (**9**), diacetylterrein (**10**), and **5**.

Varitriol (1): 30 mg; colorless oil; $[\alpha]^{25}_D$ +18.5° (*c* 2.30, MeOH); UV (EtOH) λ_{max} (log ϵ) 204 (4.64) 260 (4.05) 296 (3.49) nm; IR (film) ν_{max} 3338, 1597, 1578, 1470, 1264, 1092 cm^{-1}; ^1H NMR (CD_3COCD_3, 300 MHz) δ 7.22 (1H, t, J = 8.0 Hz, H-4), 7.14 (1H, br d, J = 15.8 Hz, H-1′), 7.13 (1H, dd, J = 8.0, 1.0 Hz, H-5), 6.89 (1H, dd, J = 8.0, 1.0 Hz, H-3), 6.19 (1H, dd, J = 15.8, 6.7 Hz, H-2′), 4.72 (2H, br s, OC*H*$_2$-C-1), 4.62 (1H, br s, *H*O-C-4′, changed with D_2O), 4.41 (1H, br s, *H*O-C-5′, changed with D_2O), 4.32 (1H, br t, J = 6.4 Hz, H-3′), 3.94 (1H, br q, J = 5.3 Hz, H-4′), 3.84 (1H, quintuplet, J = 6.0 Hz, H-6′), 3.82 (3H, s, OC*H*$_3$), 3.80−3.70 (1H, br, *H*OCH$_2$-C-1, changed with D_2O) 3.74 (1H, br q, J = 5.4 Hz, H-5′), 1.27 (3H, d, J = 6.2 Hz, H-7′); ^{13}C NMR (CD_3COCD_3, 100 MHz) δ 158.9 (s, C-2), 139.0 (s, C-6), 132.3 (d, C-2′), 129.4 (d, C-1′), 129.3 (d, C-4), 127.9 (s, C-1), 119.3 (d, C-5), 110.7 (d, C-3), 85.2 (d, C-3′), 80.1 (d, C-6′), 77.2 (d, C-5′), 76.5 (d, C-4′), 56.0 (q, O*C*H$_3$), 55.5 (t, O*C*H$_2$-C-1), 19.5 (q, C-7′); NMR assignments have been made with the aid of 2D NMR experiments (COSY, HMQC, HMBC); HRCIMS *m/z* 281.1390 (calcd for $C_{15}H_{21}O_5$, 281.1388).

Varixanthone (4): 190 mg; yellow needless (95:5 methylene chloride/acetone); mp 125−127 °C; $[\alpha]^{25}_D$ +62.1° (*c* 1.13, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 392.5 (3.68) nm; IR (film) ν_{max} 3458; 1722 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 12.56 (1H, s, O*H*), 7.87 (1H, s, *H*CO$_2$), 7.35 (1H, d, J = 8.4 Hz, H-3), 7.18 (1H, s, H-5), 6.65 (1H, d, J = 8.4 Hz, H-2), 5.38 (1H, br d, J = 2.0 Hz, H-25), 5.18 (1H, dd, J = 10.5, 1.8 Hz, H-15), 5.10 (1H, br s, O*H*), 4.80 (1H, s, H-22a), 4.60 (1H, s, H-22b), 4.43 (1H, br dd, J = 10.8, 2.5 Hz, H-19a), 4.34 (1H, dd, J = 10.9, 3.0 Hz, H-19b), 3.32 (1H, dd, J = 14.3, 2.2 Hz, H-14a), 2.81 (1H, dd, J = 14.3, 10.6 Hz, H-14b), 2.73 (1H, br s, H-20), 2.33 (3H, s, H-24), 1.84 (3H, s, H-23), 1.38 (3H, s, H-17), 1.35 (3H, s, H-18); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 184.3 (s, C-13), 160.6 (s, C-10), 160.4 (d, H*C*OO), 153.1 (s, C-1), 151.9 (s, C-11), 149.6 (s, C-7), 142.4 (s, C-21), 138.6 (s, C-6), 138.0 (d, C-3), 121.0 (s, C-8), 119.2 (d, C-5), 116.9 (s, C-12), 114.6 (s, C-4), 112.5 (t, C-22), 109.9 (d, C-2), 109.1 (s, C-9), 78.9 (d, C-15), 72.1 (s, C-16), 64.6 (t, C-19), 63.3 (d, C-25), 44.9 (d, C-20), 29.7 (t, C-14), 25.6 (q, C-18), 25.4 (q, C-17), 22.5 (q, C-23), 17.4 (q, C-24); HRFABMS *m/z* 491.1679 (calcd for $C_{26}H_{28}O_8Na$, 491.1681).

Hydrolysis of varixanthone (**4**) was carried out as follows: DBU (0.035 mL) was added to a solution of **4** (50 mg) in 2 mL of wet THF. The solution was stirred for 42 h, and then tBuOMe (10 mL) was added. The ethereal solution was washed with 1 N HCl and water. The organic layer was dried with anhydrous Na_2SO_4 and the solvent was removed. Flash chromatography (methylene chloride/acetone, 8:2) of the residue gave tajixanthone hydrate (20 mg) as a yellow oil, $[\alpha]^{25}_D$ −97.3° (*c* 1.66, $CHCl_3$), (lit.[22] $[\alpha]^{25}_D$ −71.5°, *c* 2.3, $CHCl_3$); ^1H and ^{13}C NMR spectra matched those previously described.[21,22]

Triacetylvaritriol (5): 4 mg; colorless oil; $[\alpha]^{25}_D$ +16.9° (*c* 0.33, $CHCl_3$); IR (film) ν_{max} 1741, 1246 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.30 (1H, t, J = 8.0 Hz, H-4), 7.13 (1H, br d, J = 7.9 Hz, H-5), 6.98 (1H, br d, J = 15.7 Hz, H-1′), 6.85 (1H, br d, J = 8.2 Hz, H-3), 6.13 (1H, dd, J = 15.7, 6.8 Hz, H-2′), 5.25 (2H, d, J = 1.9 Hz, OC*H*$_2$−C-1), 5.08 (1H, t, J = 5.8 Hz, H-4′), 4.93 (1H, t, J = 5.5 Hz, H-5′), 4.53 (1H, br t, J = 6 Hz, H-3′), 4.13 (1H, quintuplet, J = 6.2 Hz, H-6′), 3.84 (3H, s, OC*H*$_3$), 2.10 (3H, s, C*H*$_3$CO$_2$), 2.08 (3H, s, C*H*$_3$CO$_2$), 2.06 (3H, s, C*H*$_3$-CO$_2$), 1.38 (3H, d, J = 6.4 Hz, H-7′); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.2 (s, CH$_3$*C*O$_2$), 169.9 (s, CH$_3$*C*O$_2$), 169.8 (s, CH$_3$*C*O$_2$), 158.9 (s, C-2), 138.5 (s, C-6), 130.5 (s, C-1), 130.2 (d, C-2′), 130.0 (d, C-1′), 129.9 (d, C-4), 118.9 (d, C-5), 110.4 (d, C-3), 81.5 (d, C-3′), 77.7 (d, C-6′), 75.7 (d, C-5′), 74.9 (d, C-4′), 57.7 (t, OCH$_2$−C-1), 55.9 (q, O*C*H$_3$), 21.1 (q, *C*H$_3$CO$_2$), 20.8 (q, *C*H$_3$CO$_2$), 20.7 (q, *C*H$_3$CO$_2$), 19.2 (q, C-7′).

Isopropilidenevaritriol (6). PPTS (5 mg) and anhydrous $CuSO_4$ (10 mg) were added to a solution of 20 mg of **1** in 5 mL of dry acetone. The solution was stirred for 24 h and filtered, and the solvent was removed in vacuo. Flash chromatography (methylene chloride/acetone, 92:8) of the residue gave **6** (8 mg) as a colorless oil; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.23 (1H, t, J = 8.1 Hz, H-4), 7.08 (1H, br d, J = 8.0 Hz, H-5), 7.06 (1H, br d, J = 15.7 Hz, H-1′), 6.82 (1H, br d, J = 8.2 Hz, H-3), 6.14

(1H, dd, J = 15.7, 6.6 Hz, H-2′), 4.78 (2H, s, OCH_2-C-1), 4.53 (1H, dd, J = 7.0, 5.0 Hz, H-4′), 4.44 (1H, ddd, J = 6.6, 5.0, 1.2 Hz, H-3′), 4.33 (1H, dd, J = 7.0, 4.7 Hz, H-5′), 4.03 (1H, dq, J = 6.4, 4.7 Hz, H-6′), 3.85 (3H, s, OCH_3), 1.57 (3H, s, H-2″), 1.35 (3H, d, J = 6.4 Hz, H-7′), 1.34 (3H, s, H-3″); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 158.2 (s, C-2), 137.4 (s, C-6), 130.9 (d, C-2′), 129.5 (d, C-1′), 128.9 (d, C-4), 126.5 (s, C-1), 119.4 (d, C-5), 115.2 (s, C-1″), 109.8 (d, C-3), 86.4 (d, C-3′), 85.7 (d, C-5′), 84.9 (d, C-4′), 80.4 (d, C-6′), 57.0 (t, OCH_2−C-1), 55.7 (q, OCH_3), 27.5 (q, C-2″), 25.6 (q, C-3″), 19.2 (q, C-7′).

Triacetylvarioxirane (**7**): 4 mg; colorless oil; $[\alpha]^{25}_D$ −28.0° (c 0.31, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 212 (5.5), 294 (4.4) nm; IR (film) ν_{max} 1739, 1236 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.31 (1H, t, J = 8.0 Hz, H-4), 7.11 (1H, br d, J = 8 Hz, H-5), 6.98 (1H, br d, J = 15.8 Hz, H-1′), 6.86 (1H, dd, J = 8.0, 1.0 Hz, H-3), 6.14 (1H, dd, J = 15.8, 7.5 Hz, H-2′), 5.70 (1H, ddd, J = 7.5, 3.7, 1.2 Hz, H-3′), 5.26 (2H, d, J = 1.2 Hz, OCH_2-C-1), 4.89 (1H, dd, J = 6.0, 3.7 Hz, H-4′), 3.84 (3H, s, OCH_3), 3.02 (1H, dq, J = 5.2, 2.1 Hz, H-6′), 2.85 (1H, dd, J = 6.0, 2.1 Hz, H-5′), 2.12 (3H, s, CH_3CO_2), 2.09 (3H, s, CH_3CO_2), 2.06 (3H, s, CH_3CO_2), 1.29 (3H, d, J = 5.2 Hz, H-7′); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.1 (s, CH_3CO_2), 169.9 (s, CH_3CO_2), 169.8 (s, CH_3CO_2), 158.6 (s, C-2), 138.4 (s, C-6), 132.0 (d, C-1′), 131.9 (s, C-1), 130.0 (d, C-4), 126.0 (d, C-2′), 119.0 (d, C-5), 110.6 (d, C-3), 73.8 (d, C-3′), 73.2 (d, C-4′), 57.6 (t, OCH_2-C-1), 56.0 (q, OCH_3), 53.0 (d, C-5′), 27.1 (d, C-6′), 21.1 (q, CH_3CO_2), 21.0 (q, CH_3CO_2), 20.9 (q, CH_3CO_2), 17.2 (q, C-7′); HRCIMS m/z 407.1708 (calcd for $C_{21}H_{27}O_8$, 407.1706).

Diacetyldihydroterrein (**9**): 15 mg; colorless oil; $[\alpha]^{25}_D$ +6.4° (c 1.15, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 216.0 (4.67) nm; IR (film) ν_{max} 1746, 1730, 1223, 1038 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.14 (1H, q, J = 1.3 Hz, H-2), 5.91 (1H, dd, J = 2.9, 1.1 Hz, H-4), 5.19 (1H, d, J = 2.9 Hz, H-5), 2.34 (2H, m, H-1′), 2.15 (6H, s, 2 CH_3CO_2), 1.62 (2H, m, H-2′), 0.98 (3H, t, J = 6.4 Hz, H-3′); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 196.7 (s, C-1), 174.3 (s, C-3), 170.32 (s, CH_3CO_2), 170.28 (s, CH_3CO_2), 129.9 (d, C-2), 78.3 (d, C-5), 76.1 (d, C-4), 31.8 (t, C-1′), 20.0 (t, C-2′), 13.8 (q, C-3′); HRFABMS m/z 263.0897 (calcd for $C_{12}H_{16}O_5$-Na, 263.0895).

Diacetylterrein (10): 9 mg; oil; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.37 (1H, dq, J = 15.5, 6.5 Hz, H-2′), 6.29 (1H, d, J = 15.5 Hz, H-1′), 6.18 (1H, br s, H-2), 6.06 (1H, br d, J = 2.9 Hz, H-4), 5.20 (1H, d, J = 2.9 Hz, H-5), 2.15 (3H, s, CH_3CO_2), 2.13 (3H, s, CH_3CO_2), 1.92 (3H, d, J = 6.5 Hz, H-3′).

Cytotoxicity Assays. Varitriol (**1**) was tested in the NCI 60-cell screen, using the standard NCI protocol. The NCI's mean graphs of **1** are included in the Supporting Information. Varixanthone (**4**) was tested toward P388 (mouse lymphoma), A549 (human lung carcinoma), and HT29 (human colon carcinoma) cell lines, following a previously reported procedure,[24] by the Biomar Institute.

Antimicrobial Tests. The antimicrobial activities of **1**, **4**, shamixantone, tajixanthone hydrate, and terrein were tested against Gram positive (*Enterococcus faecalis*, *Bacillus subtilis*, *Staphylococus aureus*) and Gram negative bacteria (*Salmonella tiphymurium*, *Escherichia coli*, *Proteus* sp.) as well as against the yeast *Candida albicans*, following a previously established procedure.[23] Varitriol (**1**) did not show antimicrobial activity at concentrations of 100 μg/mL. Varixanthone (**4**) was active against *E. coli*, *Proteus* sp., *B. subtillis*, and *S. aureus*, showing a minimal inhibitory concentration (MIC) of 12.5 μg/mL in all these cases. Varixanthone showed lower potency against *E. faecalis* (MIC = 50 μg/mL). Shamixanthone, tajixanthone hydrate, and terrein showed activity against *E. coli*, *E. faecalis*, *B. subtillis*, and *S. aureus*, with MIC = 50 μg/mL in all cases.

Acknowledgment. This research was financed by the Danish National Science Research support and the Spanish DGICYT (Poject No. PB 98-1365). We thank Dr. J. C. Frisvad (Technical University of Denmark) for the fungal identification, Dr. M. Maqueda (University of Granada, Spain) for providing us Gram positive and Gram negative bacteria, Dr. F. Uruburu (CECT, University of Valencia, Spain) for the yeast *Candida albicans*, Ms. A. Tate for her comments and revision of our English text, and the Biomar Institute (León, Spain) and the NCI (Bethesda, MD) for the cytotoxicity assays. Two of us (J.J. and A.R.) thank the Spanish Ministries of "Ciencia y Tecnología" and "Educación y Cultura", respectively, for their grants.

Supporting Information Available: NCI's mean graphs of varitriol (**1**). This information is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Faulkner, D. J. *Nat. Prod. Rep.* **2000**, *17*, 7−55.
(2) Fenical, W.; Jensen, P. R. In *Marine Biotechnology*; Attaway, D. H., Zaborsky, O. K., Eds.; Plenum Press: New York, 1993; Vol. 1, pp 419−457.
(3) Malmstroem, J.; Christophersen, C.; Frisvad, J. C. *Phytochemistry* **2000**, *54*, 301−309.
(4) Barrero, A. F.; Oltra, J. E.; Cerdá-Olmedo, E.; Ávalos, J.; Justicia, J. *J. Nat. Prod.* **2001**, *64*, 222−225.
(5) To adopt a pragmatic definition of "marine" microorganisms as those isolated on seawater media from marine organisms and sediments has been recently proposed (cf. Faulkner, D. J. *Nat. Prod. Rep.* **1999**, *16*, 155−198).
(6) For more details on the collection of marine samples and the preparation of this and other fungal isolates see: Christophersen, C.; Crescente, O.; Frisvad, J. C.; Gram, L.; Nielsen, J.; Nielsen, P. H.; Rahbaek, L. *Mycopathologia* **1999**, *143*, 135−138.
(7) Raper, K. B.; Fennel, D. I. *The Genus Aspergillus*; The Williams and Wilkins Company: Baltimore, 1965.
(8) Dunn, A. W.; Entwistle, I. D.; Johnstone, R. A. W. *Phytochemistry* **1975**, *14*, 2081−2082.
(9) Dunn A. W.; Johnstone, A. W. *J. Chem. Soc., Perkin Trans. 1* **1979**, 2122−2123.
(10) Chexal, K. K.; Fouweather, C.; Holker, J. S. E. *J. Chem. Soc., Perkin Trans. 1* **1975**, 554−556.
(11) Simpson, T. J. *J. Chem. Soc., Perkin Trans. 1* **1978**, 627−628.
(12) Hensens, O. D.; Zink, D.; Williamson, J. M.; Lotti, V. J.; Chang, R. S. L.; Goetz, M. A. *J. Org. Chem.* **1991**, *56*, 3399−3403.
(13) Simpson, T. J. *J. Chem. Soc., Perkin Trans. 1* **1994**, 3055−3056.
(14) Ahmed, S. A.; Bardshiri, E.; McIntyre, R.; Simpson, T. J. *Aust. J. Chem.* **1992**, *45*, 249−274.
(15) Simpson, T. J.; Ahmed, S. A.; McIntyre, C. R.; Scott, F. E.; Sadler, I. H. *Tetrahedron* **1997**, *53*, 4013−4034.
(16) Maebaxashi, Y.; Suzuki, S.; Horie, Y.; Murai, T.; Yamazaki, M. *Maikotokishin (Tokyo)* **1983**, *17*, 47−49; *Chem. Abstr.* **1983**, *99*, 103865j.
(17) Steyn, P. S.; Vleggaar, R. *J. Chem. Soc., Chem. Commun.* **1984**, 977−979.
(18) Barrero, A. F.; Oltra, J. E.; Poyatos, J. A.; Jiménez, D.; Oliver, E. *J. Nat. Prod.* **1998**, *61*, 1491−1496.
(19) Kolb, H. C.; Hoffmann, H. M. R. *Tetrahedron: Asymmetry*, **1990**, *1*, 237−250.
(20) Chexal, K. K.; Fouweather, C.; Holker, J. S. E.; Simpson, T.; Young, K. *J. Chem. Soc., Perkin Trans. 1* **1974**, 1584−1593.
(21) Chexal, K. K.; Holker, J. S. E.; Simpson, T. *J. Chem. Soc., Perkin Trans. 1* **1975**, 549−554.
(22) Holker, J. S. E., Lapper, R. D., Simpson, J. *J. Chem. Soc., Perkin Trans. 1* **1974**, 2135−2140.
(23) Barrero, A. F.; Oltra, J. E.; Herrador, M. M.; Cabrera, E.; Sánchez, J. F.; Quílez, J. F.; Rojas, F. J.; Reyes, J. F. *Tetrahedron* **1993**, *49*, 141−150.
(24) Berengeron, R. I.; Davanaugh, Jr., P. F.; Kline, S. J.; Hughes, R. G., Jr.; Elliot, G. T.; Porter, C. W. *Biochem. Biophys. Res. Commun.* **1984**, *121*, 848−854.

NP0103214