Celite and rinsed with 1.0 mL of dichloromethane, 1.0 mL of ethyl acetate, 12 mL of dichloromethane with 2% acetic acid, and 10 mL of ethyl acetate with 1% acetic acid. The filtrate was concentrated in vacuo to give a brown solid. The residue was chromatographed over 0.6 g of silica gel (eluted with ethyl acetate–hexane, 1:5 progressing to 1:1, and then with ethyl acetate–hexane–acetic acid, 66:31:2) to give 2.9 mg (32%) of pleurotin (**1**) as a pale yellow solid: mp 205–208 °C; IR ($CHCl_3$) 3030, 2870, 1790, 1670 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.93 (d, J = 6.9 Hz, 3 H, CH_3), 1.17 (td, J = 11.6, 6.3 Hz, 1 H, CH), 1.38 (qd, J = 12, 4 Hz, 1 H, C*H*H), 1.55 (ddd, J = 12.8, 6.1, 3.7 Hz, 1 H, C*H*H), 1.70 (qd, J = 13.2, 4.0 Hz, 1 H, C*H*H), 1.72–1.98 (m, 4 H, CH and CH_2 manifold), 2.06 (ddd, J = 12.1, 8.8, 3.2 Hz, 1 H, C*H*H), 2.12 (dd, J = 7.7, 6.7 Hz, 1 H, CH), 2.24, 2.78 (AB, then doublet, J_{AB} = 7.0 Hz, J_d = 3.4 Hz, 2 H, CH_2), 2.36 (dd, J = 14.3, 6.8 Hz, 1 H, O=CCH), 3.35 (dd, J = 12.1, 8.8 Hz, 1 H, OC*H*H), 4.02 (dd, J = 12.1, 8.5 Hz, 1 H, OC*H*H), 4.49 (d, J = 1.3 Hz, 1 H, OCH), 5.46 (dd, J = 6.8, 1.7 Hz, 1 H, O=COCH), 6.76, 6.79 (AB q, J = 10.2 Hz, 2 H, CH=CH); ^{13}C NMR ($CDCl_3$, broad band) δ 20.17, 22.50, 23.94, 24.83, 30.56, 32.73, 39.57, 44.61, 47.23, 48.77, 52.09, 69.44, 71.37, 73.81, 136.07 (two peaks), 137.96, 143.81, 174.58, 185.32, 186.74; mass spectrum, m/e (relative intensity) 356 (M^+ + 2, 31), 354 (M^+, 60), 336 (21), 325 (16), 310 (19), 296 (24), 295 (28), 294 (28), 267 (32), 251 (48), 250 (39), 249 (56), 237 (33), 223 (38), 211 (39), 210 (30), 197 (29), 128 (40), 119 (69), 117 (36), 115 (48), 93 (32), 91 (100), 79 (48), 77 (54), 67 (40), 65 (36), 55 (48), 55 (55), 44 (15), 41 (90); exact mass calcd for $C_{21}H_{22}O_5$ m/e 354.1468, found m/e 354.1492. The IR, ^1H NMR, ^{13}C NMR (broad band), and mass spectra, as well as TLC behavior of the synthetic pleurotin (**1**) were indistinguishable from those of the natural product. Further elution gave 3.0 mg (33%) of quinone acid **2** as a pale yellow solid.

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Supplementary Material Available: Experimental procedures for all reactions not presented in the Experimental Section (18 pages). Ordering information is given on any current masthead page.

The Macrolactins, a Novel Class of Antiviral and Cytotoxic Macrolides from a Deep-Sea Marine Bacterium

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Contribution from the Scripps Institution of Oceanography, University of California, San Diego, La Jolla, California 92093-0228. *Received April 24, 1989*

Abstract: Eight new secondary metabolites, macrolactins A–F (**1–6**) and macrolactinic and isomacrolactinic acids (**7** and **8**), of an unprecedented C_{24} linear acetogenin origin, have been isolated from the culture broth of an apparently taxonomically unclassifiable marine bacterium. The new compounds include 24-membered ring lactones and related glucose β-pyranosides and open-chain acids. Macrolactin A (**1**), the parent aglycon, shows selective antibacterial activity and inhibits B16-F10 murine melanoma cancer cells in vitro assays. Macrolactin A also shows significant inhibition of mammalian *Herpes simplex* viruses (types I and II) and protects T-lymphoblast cells against human HIV viral replication.

Studies of the natural-products chemistry of terrestrial microorganisms, initiated at least 4 decades ago, have illustrated that bacteria and fungi of largely soil origin are prolific sources for structurally unique, highly bioactive, and biomedically utilitarian secondary metabolites. Of the over 10 000 compounds described from terrestrial microorganisms, at least 100 have proven effective as chemotherapeutic agents in the treatment of human and animal diseases. The fact that there is a continuing international focus on microbial products by the pharmaceutical and agrichemical industries points to their recognized importance in the development of new therapeutic agents. Although both shallow and deep marine habitats have been observed to contain taxonomically diverse microorganisms, progress in the isolation and mass culture of these organisms has been slow. Several studies of marine-derived actinomycetes[1] and of bacteria isolated from seawater[2] have, however, illustrated that unique metabolites may be produced.

As part of a new program to explore methods for the successful culture and chemical evaluation of marine microorganisms, we have focused our attention on those microorganisms that are adapted to saline environments, living within marine sediments or in close association with other marine organisms. An unusual Gram-positive bacterium, which could not be readily identified by using normal taxonomic methods,[3] was obtained from a deep-sea sediment core[4] and subsequently grown in liquid culture. This bacterial isolate was found to produce a novel family of antiviral and cytotoxic macrocyclic lactones. One of these macrolides is active against several pathogenic viruses, including the human immunosuppressive virus (HIV), the causative agent of AIDS.[5] In this paper, we report the structural elucidation and preliminary biological properties of these novel bacterial metabolites.

Cultures of the unicellular bacterium were grown for periods of 5–15 days at 20 °C, and the whole-cell suspension was extracted

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(2) (a) Wratten, S. J.; Wolfe, M. S.; Andersen, R. J.; Faulkner, D. J. *Antimicrob. Agents Chemother.* **1977**, *11*, 411–414. (b) Burkholder, P. R.; Pfister, R. M.; Leitz, F. H. *Appl. Microbiol.* **1966**, *14*, 649–653. (c) Umezawa, H.; Okami, Y.; Kurasawa, S.; Ohnuki, T.; Ishizuka, M.; Takeuchi, T.; Shiio, T.; Yugari, Y. *J. Antibiot.* **1983**, *36*, 471–477.

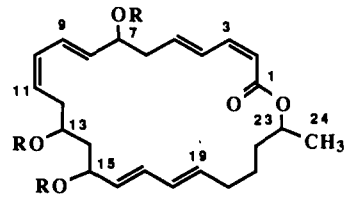
(3) The microorganism in question, isolate C-237, is a motile, Gram-positive, oxidase and catalase positive, unicellular bacterium with a strong salt requirement for growth. With use of biochemical methods, isolate C-237 could not be placed within any previously defined bacterial class. Further studies including rRNA sequence measurements are planned. We gratefully acknowledge Professor Kenneth Nealson, University of Wisconsin—Milwaukee, for his assistance with this preliminary taxonomic investigation.

(4) The sediment core sample was collected as part of the "Deep Sea Drilling Program" in the North Pacific (37° 12.4′ N, 123° 04.6′ W) at a depth of −980 m on June 21, 1970.

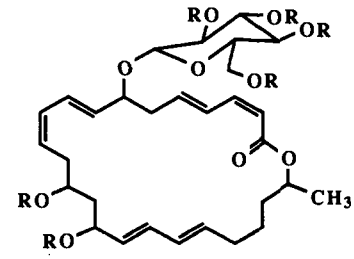
(5) Gallo, R. C.; Sarin, P. S.; Gelmann, E. P.; Robert-Guroff, M.; Richardson, E.; Kalyanaraman, V. S.; Mann, D. L.; Sidhu, G.; Stahl, R. E.; Zolla-Pazner, S.; Liebowitch, J.; Popovic, M. *Science* **1983**, *220*, 865–867. Barre'-Sinoussi, F.; Chermann, J. C.; Rey, R.; Nugeyre, M. T.; Chamaret, S.; Gruest, J.; Dauguet, C.; Axler-Blin, C.; Vezinet-Brun, F.; Rouzioux, C.; Rozenbaum, W.; Montagnier, L. *Science* **1983**, *220*, 868–871. Coffin, J.; Haase, A.; Levy, J. A.; Montaginer, L.; Oroszlan, S.; Teich, N.; Temin, H.; Toyoshima, K.; Varmus, H.; Vogt, P.; Weiss, R. *Science* **1986**, *232*, 697.

0002-7863/89/1511-7519$01.50/0

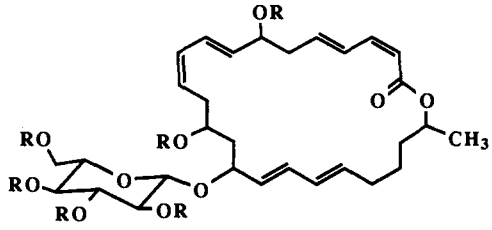
Chart I. The Macrolactins



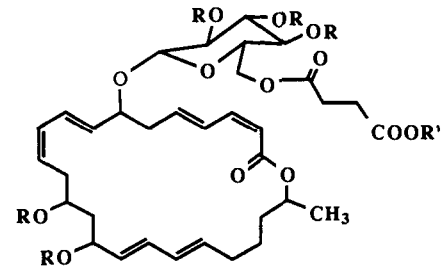
1, R=H, Macrolactin-A
9, R=Ac, Macrolactin-A Triacetate



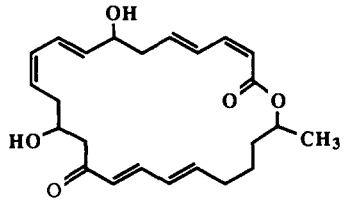
2, R=H, Macrolactin-B
10, R=Ac, Macrolactin-B Hexaacetate



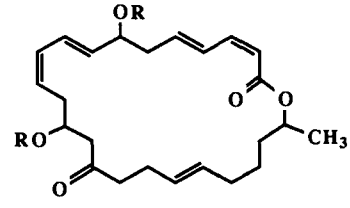
3, R=H, Macrolactin-C
11, R=Ac, Macrolactin-C Hexaacetate



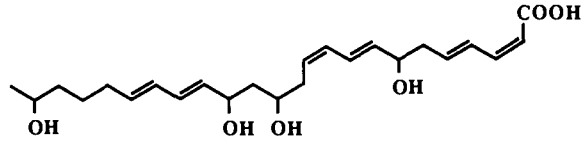
4, R=R'=H, Macrolactin-D
12, R=Ac, R'=Me, Methyl Macrolactin-D Pentaacetate
13, R=H, R'=Me, Methyl Macrolactin-D



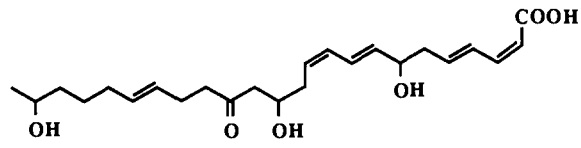
5, Macrolactin-E



6, R=H, Macrolactin-F
14, R=Ac, Macrolactin-F Diacetate



7, Macrolactinic Acid



8, Isomacrolactinic Acid

with ethyl acetate. Concentration of the ethyl acetate extracts produced a strong-smelling residue that was rich in butyric, 2-methylbutyric, and 3-methylbutyric acids. The ethyl acetate soluble material was initially fractionated by Sephadex LH-20 chromatography using 2:5:1 hexane/dichloromethane/methanol. Early eluting fractions consisted primarily of the butyric acid derivatives, while later fractions contained macrolactins A–F (**1**–**6**), macrolactinic acid (**7**), and isomacrolactinic acid (**8**, Chart I). The composition and relative abundance of the mixture of macrolactin metabolites obtained from individual cultures were extremely variable and seemed highly dependent upon the specific conditions of culture.

The aglycon, macrolactin A (**1**), was successfully purified by C-18 reversed-phase HPLC using 80:20 MeOH/H_2O and analyzed for $C_{24}H_{34}O_5$ by high-resolution FABMS. The infrared spectrum of **1** ($CHCl_3$) showed absorbance bands at 3550–3200, 1695, 1680, and 1640 cm^{-1}, indicative of hydroxyl, ester, and olefinic functionalities. Ultraviolet absorbances at 261 (ϵ = 18 700) and 227 nm (ϵ = 49 200) were assigned to a chromophore with extended conjugation. Analysis of the ^{13}C NMR spectrum (Table I) showed an ester carbonyl resonance at 166.3 ppm and 12 methine carbons between 143.7 and 117.8 ppm assigned to six double bonds. These accounted for seven of the eight degrees of unsaturation required by the molecular formula, therefore illus-

Table I. ^{13}C NMR (50 MHz) Data for Macrolactins A–F (**1**–**6**), Macrolactinic Acid (**7**), and Isomacrolactinic Acid (**8**)

carbon[a]	1[c]	2[c]	3[b]	4[b]	5[d]	6[d]	7[b]	8[d]
1	166.3 (0)	166.2 (0)	168.0 (0)	168.0 (0)	166.2 (0)	166.3 (0)	169.0 (0)	171.2 (0)[c]
2	117.8 (1)	117.7 (1)	118.0 (1)	117.8 (1)	118.1 (1)	117.8 (1)	118.4 (1)	116.0 (1)
3	143.7 (1)	144.0 (1)	145.0 (1)	145.2 (1)	144.7 (1)	143.2 (1)	144.5 (1)	146.1 (1)
4	129.4 (1)	129.2 (1)[e]	130.2 (1)[e]	129.8 (1)[e]	129.1 (1)[e]	128.7 (1)[e]	129.5 (1)[e]	128.5 (1)[e]
5	142.4 (1)	141.5 (1)	142.1 (1)	142.3 (1)	142.2 (1)	139.4 (1)	141.3 (1)	140.6 (1)
6	42.8 (2)	41.0 (2)	42.8 (2)	41.3 (2)	41.6 (2)	41.2 (2)	42.1 (2)	40.6 (2)
7	71.2 (1)	72.0 (1)	72.6 (1)	72.1 (1)	71.5 (1)	71.3 (1)	71.5 (1)	71.6 (1)
8	138.3 (1)	133.3 (1)[f]	137.4 (1)[f]	134.9 (1)[f]	139.5 (1)[f]	136.2 (1)	138.0 (1)	136.0 (1)
9	124.9 (1)	129.2 (1)[e]	126.4 (1)	128.8 (1)[e]	125.2 (1)	125.3 (1)	125.6 (1)	125.9 (1)
10	130.6 (1)	130.5 (1)[e]	131.3 (1)[e]	131.3 (1)[e]	129.7 (1)[e]	130.8 (1)[e]	130.1 (1)[e]	130.4 (1)[e]
11	128.6 (1)	128.5 (1)[e]	128.9 (1)[e]	129.1 (1)[e]	127.7 (1)	127.3 (1)	129.1 (1)[e]	127.6 (1)
12	36.7 (2)	36.9 (2)	36.1 (2)	36.5 (2)	35.2 (2)	35.1 (2)	39.6 (2)	38.5 (2)
13	68.8 (1)	68.5 (1)	69.0 (1)	69.2 (1)	68.1 (1)	67.9 (1)	68.4 (1)	68.1 (1)
14	43.9 (2)	43.7 (2)	41.3 (2)	43.6 (2)	43.4 (2)	43.8 (2)	45.1 (2)	43.3 (2)
15	69.2 (1)	69.0 (1)	72.2 (1)	69.8 (1)	201.6 (0)	211.8 (0)	69.2 (1)	212.0 (0)[c]
16	136.6 (1)	136.9 (1)[f]	135.1 (1)[f]	135.3 (1)[f]	130.7 (1)[e]	47.6 (2)	136.7 (1)	48.6 (2)
17	131.2 (1)	131.3 (1)[e]	131.8 (1)[e]	131.7 (1)[e]	145.8 (1)	26.8 (2)	130.8 (1)[e]	26.6 (2)
18	129.6 (1)	129.6 (1)[e]	130.9 (1)[e]	131.1 (1)[e]	129.4 (1)[e]	129.7 (1)[e]	129.7 (1)[e]	129.5 (1)[e]
19	133.7 (1)	133.6 (1)[f]	134.9 (1)[f]	134.0 (1)[f]	136.3 (1)[f]	131.0 (1)[e]	134.1 (1)	131.5 (1)[e]
20	32.3 (2)	32.1 (2)	32.9 (2)	32.9 (2)	32.4 (2)	31.9 (2)	33.0 (2)	32.3 (2)
21	25.0 (2)	24.9 (2)	25.6 (2)	25.5 (2)	24.5 (2)	24.9 (2)	26.1 (2)	25.3 (2)
22	35.3 (2)	35.3 (2)	35.1 (2)	36.0 (2)	34.7 (2)	34.7 (2)	37.3 (2)	34.8 (2)
23	70.8 (1)	70.5 (1)	71.7 (1)	71.8 (1)	70.5 (1)	70.8 (1)	66.9 (1)	67.6 (1)
24	19.9 (3)	20.0 (3)	20.1 (3)	20.1 (3)	20.0 (3)	20.0 (3)	24.3 (3)	23.2 (3)
1′		101.6 (1)	104.8 (1)	101.6 (1)				
2′		78.7 (1)[g]	78.5 (1)[g]	78.5 (1)[g]				
3′		78.5 (1)[g]	78.2 (1)[g]	78.1 (1)[g]				
4′		77.0 (1)[g]	77.9 (1)[g]	75.2 (1)[g]				
5′		75.3 (1)[g]	75.4 (1)[g]	75.1 (1)[g]				
6′		63.1 (2)	62.9 (2)	64.9 (2)				
7′				174.1 (0)				
8′				30.1 (2)[h]				
9′				29.9 (2)[h]				
10′				174.8 (0)[b]				

[a] The number of attached protons appears in parentheses and was determined by using the DEPT pulse sequence. Carbon assignments for macrolactin A (**1**) were provided by a 2D direct carbon–proton correlation (HETCOR) experiment. Assignments for the other compounds were based upon their analogy with macrolactin A and by comparison with model compounds. [b] CD_3OD. [c] Pyridine-d_5. [d] $CDCl_3$. [e,f,g,h] Assignments may be interchanged within a column.

trating macrolactin A to be monocyclic.

Macrolactin A was amenable to thorough ^1H NMR analysis (Table II) because it contained 23 contiguous protonated carbons and provided highly dispersed spectra in a variety of NMR solvents. In differing solvents it was possible to resolve the resonances of each protonated carbon and thus unambiguously define the structure of **1**. Through extensive decoupling and COSY[6] analysis, it was possible to identify the C-2 proton at 5.68 ppm ((1H, d, J = 11.2 Hz) and then sequentially determine the coupling to each adjacent protonated center in a stepwise fashion. Macrolactin A was thus demonstrated to be constructed of a 24-member lactone that incorporated six disubstituted olefins, four methine carbons bearing oxygen, and a single methyl substituent at C-23. The geometries of the carbon–carbon double bonds in the $\alpha,\beta,\gamma,\delta$-unsaturated ester (C-1 through C-5) and the two pairs of conjugated dienes (C-8 to C-11 and C-16 to C-19) were assigned on the basis of their readily measured ^1H coupling constants: 11.2 Hz for Z and 14.8–15.3 Hz for E.

The point of cyclization of the ester in macrolactin A was indicated by the low-field shift of the C-23 proton at 5.10 ppm, which was also clearly coupled (J = 6.5 Hz) to the C-23 methyl group. Acetylation produced the triacetate derivative **9** in which the C-7, C-13, and C-15 proton resonances shifted downfield by approximately 1 ppm. As expected, the C-23 proton band remained essentially unchanged in this derivative. This confirmed the presence of three secondary hydroxyl groups at C-7, C-13, and C-15 and established that the C-23 oxygen atom was part of the lactone linkage. A direct carbon–proton heteronuclear correlation (HETCOR)[7] experiment allowed assignment of the complete ^{13}C NMR spectrum of macrolactin A and the correlations observed in a long-range (two and three bond) heteronuclear correlation (COLOC)[8] experiment were also fully consistent with the proposed structure for **1**.

Macrolactin B (**2**), isolated by C-8 reversed-phase HPLC with 70:30 $MeOH/H_2O$, had ultraviolet absorbances at 261 (ϵ = 20 800) and 229 nm (ϵ = 55 900) and infrared bands at 3550–3200, 1695, and 1638 cm^{-1} characteristic of the aglycon **1**. A molecular formula of $C_{30}H_{44}O_{10}$, determined by high-resolution FABMS, suggested that **2** also contained a hexose sugar substituent. Resonances in the ^{13}C and ^1H NMR spectra of macrolactin B confirmed the presence of the macrocyclic lactone functionality found in **1**. In addition, a C-6 pyranose glycoside substituent was established by an acetal carbon resonance at 101.6 ppm, four methine signals between 78.7 and 75.3 ppm, and a methylene carbon at 63.1 ppm. Appropriate ^1H NMR resonances for a β-pyranose sugar, including the anomeric (axial) proton at 4.33 ppm (1 H, d, J = 7.6 Hz), were also observed. Homonuclear ^1H decoupling and COSY experiments with **2** and the peracetylated derivative **10** confirmed both the overall macrolide structure and the presence of the sugar moiety. Coupling constant analysis revealed diaxial couplings ranging from 7.6 to 9.0 Hz between all of the glycoside ring protons, thus defining the sugar as glucose. Assignment of its position of attachment at C-7 on the lactone ring was based upon the small upfield shift observed for the C-7 methine proton (+0.26 ppm) of the peracetylated derivative **10** and the large downfield shift observed for the C-13 and C-15 methine protons (−1.10 and −1.02 ppm, respectively). Thus, glucose was confirmed to be positioned as a β-glycoside at the

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Table II. ^{1}H NMR (360 MHz) Assignments for Compounds **1–8**[a]

carbon	**1**[b]	**2**[c]	**3**[c]	**4**[c]	**5**[b]	**6**[d]	**7**[c]	**8**[d]
2	5.68 (d, 11.2)	5.54 (d, 11.5)	5.54 (d, 11.2)	5.54 (d, 11.5)	5.65 (d, 11.5)	5.59 (d, 11.5)	5.57 (d, 10.2)	5.62 (d, 11.2)
3	6.29 (dd, 11.2, 11.5)	6.63 (dd, 11.5, 11.5)	6.63 (dd, 11.2, 11.5)	6.63 (dd, 11.5, 11.5)	6.25 (dd, 11.2, 11.5)	6.54 (dd, 11.2, 11.5)	6.61 (dd, 10.2, 10.2)	6.63 (dd, 11.2, 11.2)
4	7.48 (dd, 11.5, 14.9)	7.1. (dd, 11.5, 14.9)	7.21 (dd, 11.5, 15.1)	7.21 (dd, 11.5, 14.8)	7.51 (dd, 11.2, 14.4)	7.29 (dd, 11.2, 15.1)	7.38 (dd, 10.2, 14.8)	7.42 (dd, 11.2, 15.1)
5	5.85 (ddd, 7.2, 7.2, 14.9)	6.24 (ddd, 5.4, 8.3, 14.9)	6.18[g]	6.41 (dt, 6.8, 14.8)[e]	5.88[g]	6.06 (dt, 7.6, 15.1)	6.09[g]	6.11 (dt, 7.5, 15.1)
6	2.30 (m)	2.48 (m)	2.42 (2 H, m)	2.40 (m)	2.13 (m)	2.48 (2 H, m)	2.43 (2 H, t, 6.8)	2.50 (2 H, m)
	2.35 (m)	2.59 (m)		2.56 (m)	2.19 (m)			
7	4.15 (m)	4.49 (ddd, 5.8, 6.3, 7.6)	4.23 (dt, 6.1, 6.5)	4.41 (m)	3.91 (m)	4.34 (dt, 5.8, 6.1)	4.22 (m)	4.35 (dt, 6.1, 6.1)
8	5.58 (dd, 4.7, 15.0)	5.62 (dd, 7.6, 15.1)	5.73 (dd, 6.5, 15.0)	5.65 (dd, 5.8, 15.1)	5.46 (dd, 5.8, 14.8)	5.77 (dd, 6.1, 15.1)	5.70 (dd, 6.1, 15.1)	5.74 (dd, 6.1, 15.1)
9	6.73 (dd, 11.5, 15.0)	6.71 (dd, 11.1, 15.1)	6.54 (dd, 10.8, 15.0)	6.72 (dd, 10.4, 15.1)	6.39 (dd, 11.1, 14.8)	6.49 (dd, 11.1, 15.1)	6.55 (dd, 11.5, 15.1)	6.54 (dd, 11.2, 15.1)
10	6.04 (dd, 11.2, 11.5)	6.14[g]	6.10 (dd, 10.4, 10.8)	6.22 (dd, 10.4, 11.1)[e]	6.01 (t, 11.1)	6.14 (dd, 10.8, 11.1)	6.12[g]	6.13 (dd, 11.2, 11.2)
11	5.42 (m)	5.59[g]	5.57[g]	5.60[g]	5.54 (dt, 8.6, 11.1)	5.51 (dt, 8.3, 10.8)	5.51[g]	5.48 (dt, 8.0, 11.2)
12	2.43 (m)	2.44 (2 H, m)	2.55 (m)	2.48 (m)	2.38 (m)	2.46 (2 H, m)	2.37 (2 H, t, 6.8)	2.50 (2 H, m)
	2.70 (m)		2.61 (m)	2.60 (m)	2.51 (m)			
13	4.03 (m)	3.92 (m)	3.99 (m)	3.90 (m)	4.19 (m)	4.06 (m)	3.86 (m)	4.13 (m)
14	1.74 (m)	1.61 (2 H, m)	1.65 (m)	1.61 (2 H, m)	2.54 (2 H, m)	2.53 (dd, 7.6, 17.0)	1.58 (2 H, m)	2.56 (dd, 8.6, 17.6)
	1.83 (m)		1.72 (m)			2.61 (dd, 4.0, 17.0)		2.63 (dd, 3.6, 17.6)
15	4.62 (m)	4.29 (m)	4.41 (m)	4.32 (m)			4.32 (m)	
16	5.70[g]	5.56[g]	5.62[g]	5.60[g]	5.95 (d, 15.8)	2.43 (2 H, m)	5.65[g]	2.35 (m)
17	6.35 (dd, 10.6, 15.3)	6.17[g]	6.15[g]	6.54 (dd, 10.6, 15.1)[e]	6.95 (dd, 10.1, 15.8)	2.26 (2 H, m)	6.18 (dd, 10.4, 15.1)	2.27 (2 H, m)
18	6.08 (dd, 10.6, 14.8)	6.03 (dd, 10.4, 14.8)	6.04 (dd, 10.4, 14.8)	6.06 (dd, 10.6, 15.1)	5.85[g]	5.39[g]	6.04 (dd, 10.4, 15.4)	5.38[g]
19	5.70[g]	5.58[g]	5.56[g]	5.61[g]	5.83[g]	5.41[g]	6.09[g]	5.40[g]
20	1.91 (m)	2.12 (m)	2.10 (m)	2.11 (m)	1.70 (m)	1.93 (m)	2.09 (2 H, m)	1.99 (2 H, m)
	2.06 (m)	2.20 (m)	2.19 (m)	2.19 (m)	2.00 (m)	2.01 (m)		
21	1.32 (2 H, m)	1.50 (2 H, m)	1.52 (2 H, m)	1.51 (2 H, m)	1.24 (2 H, m)	1.45 (2 H, m)	1.42 (2 H, m)	1.42 (2 H, m)
22	1.32 (m)	1.58 (2 H, m)	1.49 (m)	1.50 (m)	1.15 (m)	1.40 (m)	1.38 (m)	1.39 (2 H, m)
	1.49 (m)		1.57 (m)	1.62 (m)	1.44 (m)	1.63 (m)	1.57 (m)	
23	5.10 (m)	5.02 (m)	5.01 (m)	5.02 (m)	5.06 (m)	5.00 (m)	3.70 (m)	3.79 (m)
24	1.09 (3 H, d, 6.5)	1.25 (3 H, d, 6.5)	1.25 (3 H, d, 6.5)	1.24 (3 H, d, 6.5)	1.07 (3 H, d, 6.1)	1.25 (3 H, d, 6.1)	1.13 (3 H, d, 6.5)	1.17 (3 H, d, 6.1)
1′		4.33 (d, 7.6)	4.42 (d, 7.9)	4.36 (d, 7.9)[f]				
2′		4.10 (dd, 7.6, 8.2)[e]	3.19 (dd, 7.9, 8.3)	3.27 (dd, 7.9, 8.6)[f]				
3′		3.28[g]	3.30[g]	3.41 (dd, 8.6, 9.0)[f,h]				
4′		3.34[g]	3.30[g]	3.36 (dd, 8.6, 9.0)[f,h]				
5′		3.22[g]	3.35[g]	3.46 (m)[f]				
6′		3.66 (dd, 5.8, 11.9)	3.68 (dd, 4.7, 11.9)	4.22 (dd, 6.5, 11.5)[f]				
		3.87 (dd, 1.8, 11.9)	3.85 (dd, 1.5, 11.9)	4.42 (dd, 0.8, 11.5)[f]				
8′				2.61[g]				
9′				2.61[g]				

[a] Assignments were based upon chemical shifts, coupling constants and couplings as determined by homonuclear decoupling and COSY experiments. [b] C_6D_6. [c] CD_3OD. [d] $CDCl_3$. [e] Pyridine-d_5. [f] Acetone-d_6. [g] Overlapping signal. [h] Assignments may be reversed.

C-7 hydroxyl of the lactone ring.

Macrolactin C (**3**) also had a molecular formula establish by HR FABMS as $C_{30}H_{44}O_{10}$, which suggested that it was a positional isomer of **2**. The ultraviolet, infrared, and ^{13}C and ^{1}H NMR spectra of **3** were almost identical with those of **2**. Proton NMR single-frequency decoupling and COSY analyses again defined the lactone ring and sugar components. In this case, however, acetylation caused the C-7 and C-13 methine protons in derivative **11** to shift below 5.0 ppm while the C-15 methine proton remained unchanged. Macrolactin C is hence a glucose positional isomer of **2** with the β-glucoside positioned at C-15.

The polar nature of macrolactin D (**4**) and its poor solubility in most solvents made purification difficult. Repeated C-8 reversed-phase HPLC using 65:35 MeOH/H_2O eventually provided a pure compound with a molecular formula established by high-resolution FABMS as $C_{34}H_{48}O_{13}$. The infrared spectrum, obtained as a thin film, exhibited broad and intense absorption from 3600 to 2800 cm^{-1} indicative of both hydroxyl and carboxylic acid functionalities. Additional bands at 1733, 1705, 1695, and 1640 cm^{-1} supported the presence of carboxylic acid, ester, and olefinic groups. Ultraviolet absorbances at 261 (ϵ = 19 200) and 228 nm (ϵ = 43 500) were analogous to those of the aglycon **1**. The ^{13}C and ^{1}H NMR spectra of **4** were also characteristic of both the macrolide and sugar moieties observed in glycoside **2**. Numerous proton homonuclear decoupling and COSY experiments performed with macrolactin D and the peracetyl methyl ester derivative **12** confirmed that the lactone ring was intact and that glucose was present as a β-glycoside at the C-7 hydroxyl position. The macrolide and glucose components accounted for all but a $C_4H_4O_3$ fragment, which by consideration of unassigned ^{13}C NMR resonances at 174.8, 174.1, 30.1, and 29.9 ppm must be assigned to a succinic acid half-ester functionality. This assignment was confirmed by the presence of a four-proton multiplet at 2.61 ppm in the ^{1}H NMR spectrum of **4** and formation of the corresponding methyl ester derivative **13** following treatment with diazomethane. The downfield chemical shifts of the C-6′ protons in **4** (4.41 and 4.25 ppm) relative to the analogous shifts of the protons in **2** (3.85 and 3.68 ppm) indicated that the succinic acid group was esterified to glucose at the C-6′ hydroxyl group. The chemical shifts of the C-6′ protons remained virtually unchanged in the fully acetylated derivative **12**, while the C-2′, C-3′, and C-4′ protons all exhibited a downfield shift of at least 0.9 ppm relative to their observed chemical shifts in **4**. This firmly established that the glucose moiety was substituted at the C-6′ position with a succinic acid half-ester, and hence macrolactin D was assigned structure **4**.

Macrolactin E (**5**) analyzed for $C_{24}H_{32}O_5$ by desorption chemical ionization MS and showed spectral features that closely matched those of aglycon **1**. However, an additional infrared band at 1705 cm^{-1}, a ^{13}C NMR signal at 201.6 ppm, and an increase in intensity of the ultraviolet absorbance at 261 (ϵ = 23 700) relative to 234 nm (ϵ = 19 000) suggested that macrolactin E contained an additional enone functionality. The absence of a C-15 proton resonance, the appearance of the C-16 proton as a clean doublet at 5.95 ppm (J = 15.8 Hz), and a downfield shift of the C-14 protons to 2.54 ppm (2 H, m) in the ^{1}H NMR spectrum in benzene-d_6 revealed that the allylic alcohol at C-15 had been oxidized to a ketone. Confirmation of the location of the ketone was seen in the ^{1}H NMR spectrum in $CDCl_3$ in which the C-14 protons appeared as a pair of well-resolved doubled doublets at 2.81 (J = 3.2 and 16.9 Hz) and 2.66 ppm (J = 8.3 and 16.9 Hz), each of which were vicinally coupled to the C-13 carbinol proton. Comparison of the ^{13}C NMR spectra of **5** and **1** suggested that they differed only by the presence in **5** of a ketone. Extensive proton decoupling and COSY experiments were in full agreement with this assignment and helped define the structure of macrolactin E as **5**.

Macrolactin F (**6**) analyzed for $C_{24}H_{34}O_5$ by desorption chemical ionization MS and showed ultraviolet absorbances at 261 (ϵ = 15 400) and 234 nm (ϵ = 16 600). The loss of two olefinic methine carbons and the appearance of two additional aliphatic methylenes in the ^{13}C NMR spectrum of **6** established that one of the olefins had been saturated. A downfield shift of the ketone carbonyl to 211.8 ppm indicated that the ketone was no longer conjugated and that it was the C-16, C-17 olefin that had been reduced. Proton NMR analysis confirmed the position of the ketone at C-15, since the C-14 protons appeared as a pair of double doublets at 2.661 (J = 4.0 and 17.0 Hz) and 2.53 ppm (J = 7.6 and 17.0 Hz), while the C-16 protons were an unresolved peak at 2.43 ppm (2 H, m). The remainder of the structure was defined by proton decoupling and COSY experiments with **6** and with the diacetate derivative **14**. Further structural evidence was obtained from NMR shift reagent studies of **6** and **14**, employing $Eu(fod)_3$, which allowed unambiguous assignment of all proton signals. The resonances of the C-18 and C-19 olefin protons could not be shifted far enough apart, however, to accurately measure their coupling constants. Hence, difference NOE experiments were performed to determine the geometry of the C-18, C-19 double bond in **6**. Irradiation of the C-20 allylic methylene protons resulted in an enhancement of the C-18 olefin proton but did not enhance the C-17 protons. Conversely, irradiation of the C-17 protons caused an enhancement of the C-19 olefin proton but no enhancement of the C-20 protons. The geometry of the olefin was thus confirmed to be *E* and the structure of macrolactin F was fully assigned as **6**.

Macrolactinic acid (**7**) analyzed for $C_{24}H_{36}O_6$ by FABMS and had a broad infrared band at 3650–2500 cm^{-1} and a ^{13}C NMR signal at 169.8 ppm, indicative of an unsaturated carboxylic acid. The acid carbonyl and six carbon–carbon double bonds seen in the ^{13}C spectrum accounted for all seven of the unsaturation equivalents required by the molecular formula. These data illustrated that **7** was acyclic. The ^{1}H and ^{13}C NMR spectra of **7** corresponded very closely to those of macrolide **1**. Macrolactinic acid was apparently the linear hydrolysis product of the ester in **1**. This was confirmed when treatment of **1** with methanolic KOH provided material that by ^{1}H and ^{13}C NMR was identical with **7**.

Isomacrolactinic acid (**8**) analyzed for $C_{24}H_{36}O_6$ by high-resolution FABMS and had infrared bands at 3500–2700, 1705, and 1695 cm^{-1} and ^{13}C NMR signals at 212.0 and 171.2 ppm, diagnostic of the presence of both a conjugated carboxylic acid and an isolated ketone. Five carbon–carbon double bonds were apparent in the ^{13}C spectrum; therefore, **8** was also acyclic. Ultraviolet absorbances at 261 (ϵ = 18 300) and 234 nm (ϵ = 25 300) and ^{1}H and ^{13}C NMR features for **8** closely matched those of **6**. Keto acid **8** was evidently derived from keto lactone **6** by a similar hydrolysis of the lactone. Comprehensive proton NMR decoupling and COSY analyses fully confirmed structure **8** for this metabolite.

In an effort to address the relative and absolute stereochemistry of the four chiral centers in the lactone ring of the macrolactins, crystals of suitable derivatives were sought. On a single occasion, macrolactin A (mp 75–78 °C) crystallized from a 85:15 EtOAc/isooctane solution. Unfortunately, these crystals were not suitable for X-ray crystallographic analysis. Derivatives of **1** which might provide superior crystals were also prepared. Catalytic hydrogenation with PtO_2 provided the perhydro derivative **15** (mp 78–82 °C), which was a solid but not sufficiently crystalline to be utilized for X-ray diffraction. Derivatives of **1** and **15**, including the *p*-bromophenylurethane, *p*-bromobenzoate, *p*-nitrobenzoate, and tosylates were also prepared, but none provided suitable crystals for X-ray analysis.

Several macrolactins were found to possess interesting pharmacological properties. Macrolactin A (**1**), for example, inhibited the bacteria *Bacillus subtilis* and *Staphylococcus aureus*, by using standard agar plate-assay disk methods, at concentrations of 5 and 20 μg/disk. More importantly, macrolactin A was found to possess significant antiviral and cancer cell cytotoxic properties. Lactone **1** inhibited B16-F10 murine melanoma cell replication with in vitro IC_{50} values of 3.5 μg/mL. Further, lactone **1** was a potent inhibitor of *Herpes simplex* type I virus (strain LL), as well as type II virus (strain G) with IC_{50} values of 5.0 and 8.3 μg/mL, respectively. In these latter assays, comparison of its cytotoxicity against the carrier cell lines Hep-2 and MA-104 indicated a potential therapeutic index range of 10–100. Macrolactin A was also tested by the National Cancer Institute for

its potential utility in controlling human HIV replication (using human T-lymphoblast cells). Antiviral effects were recorded with maximum protection observed at concentrations of 10 μg/mL. The antiproliferative properties of the macrolactins are currently under more intense investigation.

Experimental Section

Culturing, Extraction and Purification. The bacterium (isolate C-237) was initially isolated from a slurry of sterile seawater and sediment from a deep-sea sediment core that had been stored in the core locker of the Scripps Institution of Oceanography. The slurry was diluted 1:10000 with sterile seawater and applied to the surface of nutrient agar layered in a Petri dish. Serial transfers of one of the resulting bacterial colonies provided a pure strain that inhibited *Candida albicans* when the two microorganisms were grown in proximity on agar. Purified bacterial cultures were typically grown in liquid medium containing 75% autoclaved, charcoal-filtered seawater and 25% distilled water with 3.0 g of yeast extract (Diffco), 5.0 g of peptone (Diffco), and 3.0 mL of glycerol per liter of solution. Twenty-liter carboys containing 16 L of medium were inoculated with 500 mL of an actively growing bacterial culture and then vigorously aerated with filtered compressed air for the entire 5–15-day growth period. Cultures usually reached maximum cell density (senescence), as determined by direct cell-counting techniques, within 5 days of the initial inoculation. At the time of harvest, the pH of the cultures ranged from 4.8 to 8.3. Cultures were extracted at a variety of pH levels, but it was generally most efficient to adjust the pH to 7.0 prior to extraction. Each 16-L culture was extracted with EtOAc (2 × 5 L). Evaporation of the solvent under reduced pressure provided approximately 1.2 g of residue per 16 L of culture. Initial fractionation of the extract was achieved on a column of LH-20 (20 g of LH-20/g of extract) with 2:5:1 hexane/CH_2Cl_2/MeOH. The *Candida*-active material eluted rapidly from the column and consisted of butyric, 2-methylbutyric, and 3-methylbutyric acids. The later eluting fractions contained the macrolactins. These compounds were conspicuous on silica, C-18, and C-8 TLC due to their strong UV absorbance and characteristic color development when heated in the presence of H_2SO_4. Macrolactins A–F (**1–6**) all charred to give distinct blue/purple spots while compounds **7** and **8**, which lack the lactone ring, charred yellow/brown. Macrolactin A (**1**) was purified by C-18 HPLC with 80:20 MeOH/H_2O, while macrolactins E (**5**) and F (**6**) could be purified by silica HPLC with 80:20 EtOAc/isooctane. Final purifications of macrolactins B (**2**), C (**3**), and D (**4**), macrolactinic acid (**7**), and isomacrolactinic acid (**8**) were achieved only after repeated C-8 HPLC separations with 70:30 MeOH/H_2O. The overall yield and relative abundance of the different macrolactin metabolites showed significant variance from one culture to the next. No single culture was ever found to produce all eight of the macrolactin compounds; however, the aglycone macrolactin A (**1**) was the most abundant metabolite in each culture examined. Typical recoveries from a 16-L culture were 6–9 mg of macrolactin A (**1**) and 0 to 4 mg of the other macrolactins. It is interesting to note that compounds **5–8** were found only in cultures that were grown for an excess of 10 days.

Macrolactin A (1): White plates (EtOAc/isooctane); mp 75–78 °C $[\alpha]_D$ −9.6° (*c* 1.86, MeOH); UV (MeOH) 227 (ε = 49200), 261 nm (ε = 18700); IR ($CHCl_3$), 3550–3200, 1695, 1680, 1640 cm^{-1}; HR FAB mass spectrum, obsd 425.2307 (M^+ + Na), $C_{24}H_{34}O_5Na$ requires 425.2310.

Macrolactin A triacetate (9): Treatment of 10 mg of macrolactin A with acetic anhydride and pyridine under standard conditions followed by silica gel HPLC purification with 70:30 EtOAc/isooctane, provided 9 mg of the triacetate **9**: ^1H NMR ($CDCl_3$) δ 1.26 (3 H, d, *J* = 6.1 Hz), 2.00 (3 H, s), 2.03 (3 H, s), 2.09 (3 H, s), 5.00 (1 H, m), 5.04 (1 H, m), 5.34 (1 H, m), 5.39 (1 H, m).

Perhydromacrolactin A (15): A solution of 10 mg of macrolactin A in 2 mL of EtOAc with a catalytic amount of PtO_2 was stirred under a H_2 atmosphere for 12 h. The solution was filtered through silica gel and evaporated to an oil that was purified by silica HPLC with 80:20 EtOAc/isooctane to yield 9 mg of perhydromacrolactin A: white plates (EtOAc/isooctane); mp 78–82 °C; ^{13}C NMR ($CDCl_3$) δ 20.19, 25.07, 25.15, 25.26, 25.40, 25.47, 25.49, 25.62, 28.65, 28.97, 29.11, 29.20, 29.37, 34.91, 35.94, 36.54, 36.60, 36.97, 39.88, 69.30, 69.73, 70.71, 71.50, 173.52; HR FAB mass spectrum, obsd 415.3431 (M^+ + H^+), $C_{24}H_{47}O_5$ requires 415.3423.

Macrolactin B (2): $[\alpha]_D$ −42.0° (*c* 3.8, MeOH); UV (MeOH) 229 (ε = 55900), 261 nm (ε = 20800); IR (film) 3550–3200, 1695, 1638, 1602 cm^{-1}; HR FAB mass spectrum, obsd 587.2832 (M^+ + Na), $C_{30}H_{44}O_{10}Na$ requires 587.2832.

Macrolactin B Hexaacetate (10): Macrolactin B (7 mg) was treated with acetic anhydride and pyridine for 12 h. The mixture was concentrated and purified by C-18 reversed-phase HPLC with 85:15 MeOH/H_2O to provide 5 mg of the peracetylated derivative **10**: ^1H NMR (C_6D_6) δ 1.04 (3 H, d, *J* = 6.5), 1.67 (3 H, s), 1.70 (3 H, s), 1.71 (3 H, s), 1.80 (6 H, s), 1.82 (3 H, s), 3.12 (1 H, m), 4.08 (1 H, dd, *J* = 1.8 and 12.2 Hz), 4.23 (1 H, m), 4.24 (1 H, dd, *J* = 4.0 and 12.2 Hz), 4.49 (1 H, d, *J* = 7.65 Hz); HR FAB mass spectrum, obsd 839.3482 (M^+ + Na), $C_{42}H_{56}O_{16}Na$ requires 839.3466.

Macrolactin C (3): $[\alpha]_D$ −21.0° (*c* 0.87, MeOH); UV (MeOH) 228 (ε = 60800), 261 nm (ε = 23100); IR (film) 3550–3200, 1695, 1638, 1602 cm^{-1}; HR FAB mass spectrum, obsd 587.2881 (M^+ + Na), $C_{30}H_{44}O_{10}Na$ requires 587.2832.

Macrolactin C hexaacetate (11): Macrolactin C was acetylated and purified in a procedure identical with that described for the production of **10** to yield the hexaacetate **11**: ^1H NMR (CD_3OD) δ 2.00 (3 H, s), 2.03 (3 H, s), 2.05 (3 H, s), 2.09 (9 H, s); FAB mass spectrum, obsd 839.3488 (M^+ + Na), $C_{42}H_{56}O_{16}Na$ requires 839.3466.

Macrolactin D (4): $[\alpha]_D$ −29.2° (*c* 0.96, MeOH); UV (MeOH) 228 (ε = 43500), 261 nm (ε = 19200); IR (film) 3600–2800, 1733, 1705, 1695, 1640 cm^{-1}; HR FAB mass spectrum, obsd 687.2937 (M^+ + Na), $C_{34}H_{48}O_{13}Na$ requires 687.2993.

Methyl Macrolactin D pentaacetate (12): Macrolactin D (3 mg) was treated with acetic anhydride and pyridine, and after 12 h MeOH was added to quench the reaction. Concentration of the resulting solution under reduced pressure and silica gel HPLC with EtOAc provided 2 mg of **12**: IR ($CHCl_3$) 2940, 1755, 1740, 1705, 1638 cm^{-1}; ^1H NMR (CD_3OD) δ 1.04 (3 H, d, *J* = 6.1 Hz), 1.66 (3 H, s), 1.69 (3 H, s), 1.79 (6 H, s), 1.82 (3 H, s), 3.12 (1 H, m), 3.34 (3 H, s), 4.11 (1 H, dd, *J* = 2.2 and 12.2 Hz), 4.18 (1 H, dd, *J* = 4.7 and 12.2 Hz), 4.28 (1 H, m), 4.47 (1 H, d, *J* = 7.2 Hz); HR FAB mass spectrum, obsd 911.3655 (M^+ + Na), $C_{45}H_{60}O_{18}Na$ requires 911.3677.

Macrolactin D methyl ester (13): Treatment of macrolactin D (3 mg) with excess diazomethane in Et_2O effected a quantitative conversion to the methyl ester derivative **13**: ^1H NMR (pyridine-d_5) δ 1.32 (3 H, d, *J* = 6.1 Hz), 3.60 (3 H, s); ^{13}C NMR (pyridine-d_5) δ 19.9 (3), 24.8 (2), 29.3 (2), 29.5 (2), 32.1 (2), 35.3 (2), 36.9 (2), 40.9 (2), 43.6 (2), 51.5 (3), 64.9 (2), 68.5 (1), 68.9 (1), 70.5 (1), 71.5 (1), 75.0 (1), 75.2 (1), 77.2 (1), 78.4 (1), 101.7 (1), 117.7 (1), 128.0 (1), 129.2 (1, two-carbon signal), 129.5 (1), 130.5 (1), 131.3 (1), 133.2 (1), 133.7 (1), 136.8 (1), 141.7 (1), 143.9 (1), 166.2 (0), 172.4 (0), 172.5 (0); HR FAB mass spectrum, obsd 701.3123 (M^+ + Na), $C_{35}H_{50}O_{13}Na$ requires 701.3149.

Macrolactin E (5): $[\alpha]_D$ +21.8° (*c* 0.44, MeOH); UV (MeOH) 234 (ε = 19000), 261 nm (ε = 23700); IR ($CHCl_3$) 3450 (br), 1705, 1695, 1638, 1600 cm^{-1}; HR desorption chemical ionization (NH_3) mass spectrum, obsd 418 (M^+ + NH_3) and obsd 401.2321 (M^+ + H), $C_{24}H_{33}O_5$ requires 401.2328.

Macrolactin F (6): $[\alpha]_D$ −30.1° (*c* 1.31, MeOH); UV (MeOH) 234 (ε = 16600), 261 nm (ε = 15400); IR (CH_2Cl_2) 3500 (br), 1705, 1695, 1638, 1602 cm^{-1}; HR desorption chemical ionization (NH_3) mass spectrum, obsd 420.2736 (M^+ + NH_3), $C_{24}H_{38}NO_5$ requires 420.2750.

Macrolactin diacetate (14): Treatment of macrolactin F (10 mg) with acetic anhydride and pyridine followed by silica HPLC with 35:65 EtOAc/isooctane provided 10 mg of the diacetate **14**: ^1H NMR ($CDCl_3$) δ 1.25 (3 H, d, *J* = 6.1 Hz), 2.02 (3 H, s), 2.08 (3 H, s), 5.02 (1 H, m), 5.60 (1 H, d, *J* = 11.5 Hz), 5.67 (1 H, dd, *J* = 6.8 and 15.1 Hz), 5.98 (1 H, dt, *J* = 7.2 and 15.1 Hz), 6.10 (1 H, t, *J* = 11.2 Hz), 6.44 (1 H, dd, *J* = 11.2 and 15.5 Hz), 6.52 (1 H, t, *J* = 11.5 Hz), 7.26 (1 H, dd, *J* = 11.2 and 15.1 Hz).

Macrolactinic acid (7): $[\alpha]_D$ −13.9° (*c* 0.58, MeOH); UV (MeOH) 230 (ε = 55400), 261 nm (ε = 28400); IR (film) 3650–2500, 1695, 1638, 1602 cm^{-1}; negative-ion FAB mass spectrum, obsd 419.2427 ($M - H^-$), $C_{24}H_{35}O_6$ requires 419.2434.

Conversion of macrolactin A (1) to macrolactinic acid (7): one milliliter of a 1.0 N KOH solution was added to 10 mg of macrolactin A in 2 mL of MeOH. The mixture was stirred at 40 °C for ca. 2 h and then acidified by the slow addition of 1.0 N HCl. After addition of 2 mL of H_2O, the solution was then extracted with EtOAc (2 × 5 mL). The EtOAc layer was dried over $MgSO_4$, filtered, concentrated, and purified by C-8 reversed-phase HPLC with 70:30 MeOH/H_2O to yield 6 mg of material that was identical with **7** by ^1H and ^{13}C NMR.

Isomacrolactinic acid (8): $[\alpha]_D$ −9.3° (*c* 0.22, MeOH); UV (MeOH) 234 (ε = 25300), 261 nm (ε = 18300); IR ($CHCl_3$) 3500–2700, 1705, 1695, 1638, 1602 cm^{-1}; HR negative-ion FAB mass spectrum, obsd 419.2423 ($M - H^-$), $C_{24}H_{35}O_6$ requires 419.2434.

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