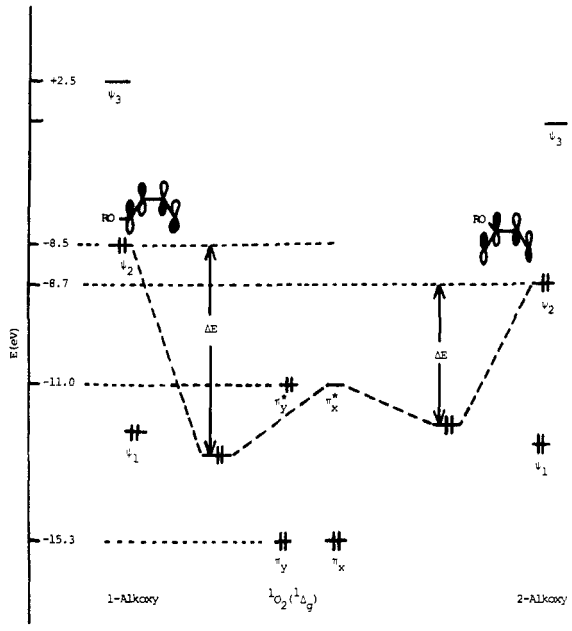

Figure 1. Molecular orbital diagram for the interaction of singlet oxygen with 1- and 2-alkoxy-substituted 1,3-butadienes.

substituents to enhance the singlet oxygen reactivity of 1,3-butadienes in comparison to 2,3-bis-alkoxy substituents is explicable in frontier molecular orbital terms as depicted in Figure 1.[14] The 1-substituent is more effective at raising the HOMO of the diene than is the 2-substituent,[15] leading to a larger stabilization energy and consequently a smaller activation barrier.

The poor Diels–Alder reactivity of diene 1 has been attributed[11] to a large separation between the terminal carbons in the 1,3-diene moiety (1,4-distance) and may be the underlying reason for the ability of the 2 + 2 cycloaddition to compete with the 4 + 2 cycloaddition of singlet oxygen.

Experimental Section

Preparation gas chromatographic separations were carried out on a Hewlett Packard 5710A gas chromatograph on a 20% Carbowax 20M/Chromsorb W NAW $^1/_4$ in. by 20 ft column. Proton and carbon NMR spectra were obtained on a JOEL FX 270 at 270 and 67.83 MHz, respectively. The proton and carbon spectra are referenced to tetramethylsilane. Mass spectra were obtained by electron impact on a VG-ZAB-1F. Infared spectra were obtained on a Beckman Microlab 600 spectrometer. Kinetic studies were completed on a Perkin-Elmer MPF-2A spectrofluorometer.

Photolysis Conditions. A mixture of 13–17 mg of 1 or 2 and 10 μL of a 10^{-3} M Rose Bengal stock solution was placed in 1 mL of acetone-d_6. Half of this solution was then placed in a 5-mm NMR tube and saturated with oxygen for 30 min. The irradiation was conducted with a WKO 750-W 120-V lamp through 1 cm of a 0.5% $K_2Cr_2O_7$ filter solution. The reaction was monitored by low-temperature NMR (−65 °C to −80 °C) after removing the oxygen by bubbling with argon for a half-hour.

(*Z*,*Z*)- and (*E*,*Z*)-4,5-Diethylidene-2,2-dimethyl-1,3-dioxolane (2). These compounds were synthesized by the method of Scharf[11] and were separated by preparative gas chromatography. The retention times were 19 min for 1 and 22 min for 2 when the helium flow rate was 120 mL/min, the injector temperature 200 °C, the detector temperature 200 °C, and the column temperature program set to 120 °C for 22 min and then ramped at 16 °C/min to 190 °C. (*Z*,*Z*)-1: ^{1}H NMR (acetone-d_6) δ 4.76 (q, *J* = 6.6 Hz, 2 H), 1.62 (d, *J* = 6.6 Hz, 6 H), 1.46 (s, 6 H); ^{13}C NMR (acetone-d_6) δ 147.17 (s), 111.65 (s), 89.41 (d, *J* = 159 Hz), 26.19 (q, *J* = 127 Hz), 10.25 (q, *J* = 127 Hz); IR (film) 2925 (s), 1690 (s); high resolution mass spectrum for $C_9H_{14}O_2$ calcd 154.09938, found 154.1035. (*E*,*Z*)-2: ^{1}H NMR (acetone-d_6) δ 4.94 (q, *J* = 7.3 Hz, 1 H), 4.89 (q, *J* = 6.9 Hz, 1 H), 1.69 (d, *J* = 7.3 Hz, 3 H), 1.68 (d, *J* = 6.9 Hz, 3 H), 1.43 (s, 6 H); ^{13}C NMR (acetone-d_6) δ 147.14 (s), 146.95 (s), 110.50 (s), 97.64 (d, *J* = 160 Hz), 93.86 (d, *J* = 156 Hz), 26.01 (q, *J* = 127 Hz), 11.23 (q, *J* = 127 Hz), 11.06 (q, *J* = 127 Hz); IR (film) 2915 (s), 1672 (s); high resolution mass spectrum for $C_9H_{14}O_2$ calcd 154.09938, found 154.0994.

(3*Z*,8*Z*)-8-Ethylidene-3,6,6-trimethyl-1,2,5,7-tetraoxaspiro[3.4]octane (4): ^{1}H NMR (acetone-d_6; −80 °C) δ 5.99 (q, *J* = 6.6 Hz, 1 H), 5.39 (q, *J* = 7.0 Hz, 1 H), 1.97 (s, 3 H), 1.69 (d, *J* = 7.0 Hz, 3 H), 1.67 (s, 3 H), 1.42 (d, *J* = 6.6 Hz, 3 H).

(3*Z*,8*E*)-8-Ethylidene-3,6,6-trimethyl-1,2,5,7-tetraoxaspiro[3.4]octane (5): ^{1}H NMR (acetone-d_6; −65 °C) δ 5.01 (q, *J* = 6.6 Hz, 1 H), 4.30 (q, *J* = 7.0 Hz, 1 H), 1.97 (s, 3 H), 1.66 (s, 3 H), 1.171 (d, *J* = 7.0 Hz, 3 H), 1.166 (d, *J* = 6.6 Hz, 3 H); ^{13}C NMR (acetone-d_6; −65 °C) δ 120.37, 112.19, 110.85, 83.26, 80.49, 28.03, 27.56, 11.93, 9.40.

(3*E*,8*Z*)-8-Ethylidene-3,6,6-trimethyl-1,2,5,7-tetraoxaspiro[3.4]octane (6): ^{1}H NMR (acetone-d_6; −65 °C) δ 6.03 (q, *J* = 6.6 Hz, 1 H), 5.33 (q, *J* = 7.7 Hz, 1 H), 2.07 (d, *J* = 7.7 Hz, 3 H), 1.51 (s, 3 H), 1.49 (d, *J* = 6.6 Hz, 3 H), 1.35 (s, 3 H).

***cis*-4,7-Dihydro-2,2,4,7-tetramethyl-1,3-dioxolo[4,5-*d*]-*o*-dioxin (7):** ^{1}H NMR (acetone-d_6; −80 °C) δ 5.05 (dq, *J* = 1.4, 6.6 Hz, 1 H), 4.80 (dq, *J* = 1.4, 6.2 Hz, 1 H), 1.58 (s, 3 H), 1.55 (s, 3 H), 1.22 (d, *J* = 6.2 Hz, 3 H), 1.17 (d, *J* = 6.6 Hz, 3 H); (acetone-d_6; room temperature) δ 4.81 (q, *J* = 6.6 Hz, 2 H), 1.56 (s, 3 H), 1.52 (s, 3 H), 1.29 (d, *J* = 6.6 Hz, 6 H).

***trans*-4,7-Dihydro-2,2,4,7-tetramethyl-1,3-dioxolo[4,5-*d*]-*o*-dioxin (8):** ^{1}H NMR (acetone-d_6; −65 °C) δ 5.10 (q, *J* = 5.9 Hz, 2 H), 1.59 (s, 6 H), 1.18 (d, *J* = 5.9 Hz, 6 H).

(*Z*)-5-Ethylidene-2,2-dimethyl-1,3-dioxolan-4-one (9). This compound was isolated by preparative gas chromatography from the decomposition of 1,2-dioxetane 4: ^{1}H NMR (acetone-d_6) δ 5.55 (q, *J* = 7.3 Hz, 1 H), 1.73 (d, *J* = 7.3 Hz, 3 H), 1.62 (s, 6 H); ^{13}C NMR (acetone-d_6) δ 162.90 (s), 140.32 (s), 111.59 (s), 105.17 (d, *J* = 162 Hz), 26.75 (q, *J* = 125 Hz), 10.71 (q, *J* = 127 Hz); IR (film) 3000 (m), 1790 (s).

(*E*)-5-Ethylidene-2,2-dimethyl-1,3-dioxolan-4-one (10). This compound was purified by recrystallization from acetone: ^{1}H NMR (acetone-d_6) δ 4.59 (q, *J* = 7.0 Hz, 1 H), 1.95 (s, 6 H), 1.25 (d, *J* = 7.0 Hz, 3 H); ^{13}C NMR (acetone-d_6) δ 172.78 (s), 111.13 (s), 82.18 (d, *J* = 153 Hz), 81.15 (s), 28.33 (q, *J* = 129 Hz), 13.70 (q, *J* = 131 Hz); IR (CCl_4) 3000 (w), 1770 (s); high resolution mass spectrum for $C_7H_{10}O_3$ calcd 142.0630, found 142.0627.

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Supplementary Material Available: ^{1}H NMR spectra from which the data in eq 1 and 2 were derived (4 pages). Ordering information is given on any current masthead page.

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Metabolites of the Antarctic Sponge *Dendrilla membranosa*

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Studies by Dayton et al.[1] of the benthic community at McMurdo Sound, Antarctica, revealed that the sponge *Dendrilla membranosa* was extremely slow growing and was never observed to be eaten. Dayton concluded that

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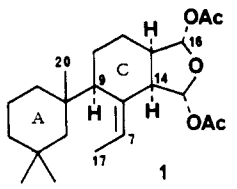
D. membranosa, which has neither apparent spicules nor mucus, was chemically defended. In this paper we report the isolation and identification of 9,11-dihydrogracilin A (1) and membranolide (2), two metabolites related to the spongian group of diterpenes, that are likely candidates to be defensive constituents of *D. membranosa*.



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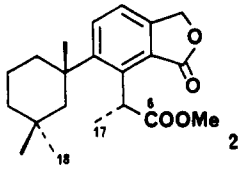

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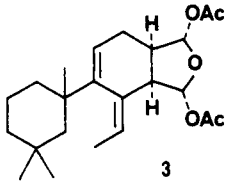

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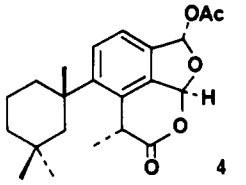

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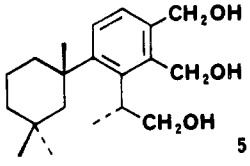

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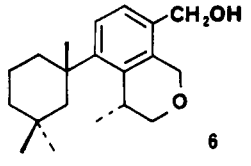

6

Two collections of *D. membranosa* were obtained and were subsequently extracted separately. The dichloromethane soluble material from a 2-propanol extract of the smaller collection was chromatographed on silica gel to obtain 9,11-dihydrogracilin A (1, 2.3% dry weight) as the major metabolite. Similar treatment of the larger collection resulted in the isolation of membranolide (2, 0.2% dry weight).

9,11-Dihydrogracilin A (1) was obtained as an oil. The molecular formula $C_{23}H_{36}O_5$ was determined by a combination of chemical ionization mass spectrometry to obtain the molecular weight coupled with high resolution mass measurement of a fragment ion. The infrared spectrum contained a strong ester band at 1740 cm^{-1} which was assigned to two acetate groups. The ^{13}C NMR spectrum (Table I) contained signals for two acetate groups at δ 170.2 (s), 170.0 (s), 21.3 (q), and 21.2 (q), two acetal carbons at 103.0 (d) and 100.7 (d), and two olefinic carbons at δ 133.9 (s) and 130.1 (d). The five oxygen atoms were therefore incorporated in a diacetoxy diacetal system similar to that found in gracilin A (3)[2] and related molecules.[3] Analysis of a ^1H NMR COSY spectrum indicated that the H-15 acetal proton signal at δ 6.44 (d, 1 H, J = 5.5 Hz) was coupled to an allylic H-14 signal at 3.14 (dd, 1 H, J = 7.5, 5.5 Hz) that was in turn coupled to the H-13 signal at δ 2.36 (ddd, 1 H, J = 9, 7.7, 7.5, Hz). The H-16 acetal signal at δ 5.97 (s, 1 H) is not coupled to H-13 but a 4% nuclear Overhauser enhancement confirms their proximity. The coupling constants ($J_{13,16}$ = 0 Hz, $J_{13,14}$ = 7.5 Hz) indicated that H-13 was cis to H-14 and trans to H-16. The ethenyl group at C-8 gave rise to ^{13}C NMR signals at δ 133.9 (s, C-8), 130.1 (d, C-7), and 15.9 (q, C-17) and ^1H NMR signals at δ 5.65 (q, 1 H, J = 6.9 Hz) and 1.65 (d, 3 H, J = 6.9 Hz).

Table I. ^{13}C NMR Spectral Data ($CDCl_3$) for 9,11-Dihydrogracilin A (1), Membranolide (2), Gracilin A (3),[2] and Aplysulphurin (4)[5a]

C no.	1	2	3	4
1	36.2 (2)	40.8 (2)	38.3	38.6
2	19.2 (2)	19.9 (2)	19.3	19.1
3	39.0 (2)	39.4 (2)	39.0	39.4
4	31.1 (0)	31.6 (0)	31.2	31.7
5	50.3 (2)	50.8 (2)	52.7	50.8
6		173.7 (0)		170.7
7	130.1 (1)	40.4 (1)	122.8	41.6
8	133.9 (0)	141.7 (0)	134.6	131.9
9	46.6 (1)	147.9 (0)	155.1	148.7
10	39.0 (0)	39.2 (0)	38.3	38.9
11	23.1[a] (2)	132.9 (1)	124.1	129.2
12	23.0[a] (2)	12.0 (1)	25.9	122.3
13	46.0 (1)	145.8 (0)	46.4	133.3
14	51.9 (1)	124.5 (0)	57.7	137.8
15	100.7 (1)	170.8 (0)	103.7	100.3
16	103.0 (1)	68.3 (2)	106.2	101.9
17	15.9 (3)	17.6 (3)	17.7	17.3
18	27.5 (3)	27.3 (3)	27.4	27.5
19	36.0 (3)	32.6 (3)	35.7	32.5[a]
20	24.0 (3)	32.6 (3)	25.5	32.7[a]
CH_3COO	170.2 (0), 170.0 (0)			169.6
	21.2 (3), 21.3 (3)			20.8
OCH_3		52.0		

[a] ^{13}C NMR assignments of 1 and 2 were made by one-, two-, and three-bond 2D ^1H–^{13}C correlation experiments. Some signals of 4 (δ 19.1 t, 50.8 t, 31.7 s, 38.9 s, 38.6 t, 39.4 t) have been reassigned to conform with assigments of 2. Figures in parentheses refer to the number of attached hydrogens deduced from DEPT experiments. Values with the same superscript within each column may be interchanged.

The geometry of the olefinic bond was established by observation of nuclear Overhauser enhancements between H-7 and H-14 (17%) and CH_3-17 and H-9 (9%). The H-9 signal at δ 2.42 (dd, 1 H, J = 6.8, 4.2 Hz) was coupled to a signal at δ 1.85 (m, 2 H) that was assigned to the H-11 protons and both the H-13 and H-11 signals were coupled to an obscured multiplet at ~1.56 assigned to the H-12 protons. Comparison of the ^1H NMR and ^{13}C NMR data of 1 and 3 strongly suggested that ring A was identical in both molecules. A COLOC experiment[4] revealed correlations from two tertiary methyl ^1H signals (0.89 s, 0.96 s) to the C-4 signal (31.1 s). The observation of a nuclear Overhauser enhancement between H-15 and CH_3-20 requires the stereochemistry shown at C-9, C-14, and C-15 and an axial disposition of ring A relative to ring C. As was the case for gracilin A (3), the stereochemistry at C-10 could not be determined by spectroscopic measurements.

Membranolide (2) was obtained as an oil. The molecular formula, $C_{21}H_{28}O_4$, was determined by high resolution mass measurement. The ^1H NMR spectrum contained signals at δ 0.47 (s, 3 H, H_3-18), 0.94 (s, 3 H, CH_3-19), 1.37 (s, 3 H, CH_3-20), 1.53 (d, 1 H, J = 14 Hz, H-5), 1.75 (d, 3 H, J = 7 Hz, CH_3-17), 2.08 (br d, 1 H, J = 14 Hz, H-5), 4.61 (q, 1 H, J = 7 Hz, H-7), 7.31 (d, 1 H, J = 8.3 Hz, H-12), and 7.81 (d, 1 H, J = 8.3 Hz, H-11) that were reminiscent of the corresponding signals in the spectrum of aplysulphurin (4)[5] and macfarlandins A and B.[6] The anomalous high field chemical shift (δ 0.47 s) of the C-4 axial methyl signal in ring A of 2 is characteristic of (1′,3′,3′-trimethylcyclohexyl)benzenes and has been noted previously.[7]

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The remaining prominent ^1H NMR signals were at δ 3.68 (s, 3 H) and 5.21 (s, 2 H) assigned to a methyl ester and an $ArCH_2OCO$ group that must be incorporated into a lactone ring since the carbonyl signal is at δ 170.8 in the ^{13}C NMR spectrum (Table I). A two-dimensional ^{13}C–^1H correlation experiment (COLOC)[4] revealed three-bond couplings from H-16 to C-12, C-14 and C-15, from COO-CH_3 to C-6, from H-12 to C-14, from H-11 to C-13, and C-8 and from CH_3-20 and H-5 to C-9. These data require that the lactone ring be attached to the aromatic ring with the methylene group at C-13 and the carbonyl group at C-14. Although the stereochemistry at C-7 is not rigid as in aplysulphurin (4) it is significant that membranolide shows similar nuclear Overhauser enhancements between H_3-20 and H-7 and between CH_3-18 and CH_3-17 suggesting that both molecules have the same geometry at C-7 and adopt similar conformations in solution. The relative stereochemistry of membranolide (2) was determined by reduction of 2 with lithium aluminum hydride in ether to obtain the triol 5 that was dehydrated using *p*-toluenesulfonic acid in benzene to obtain the known isobenzopyran 6[5] as the only product. Membranolide (2) must therefore have the 7*R**,10*S** relative stereochemistry.

Both 9,11-dihydrogracilin A (1) and membranolide (2) inhibited the growth of *Bacillus subtilis* at 100 μg/disk and membranolide (2) was also mildly active against *S. aureus*. We have not been able to assay the effects of either compound on the major Antarctic spongivores, the sea stars *Perknaster fuscus antarticus* and *Acodontaster conspicuus*.[1] However, there is an increasing body of circumstantial data to suggest that the spongian diterpenes are distasteful to all but specialized nudibranch predators.

Experimental Section

Two collections of *Dendrilla membranosa* were obtained from different sites [-40 m] at McMurdo Sound, Antarctica, and were stored separately in 2-propanol for 6 months. Each collection was examined separately as follows. The 2-propanol was decanted, the solvent evaporated, and the residue partitioned between dichloromethane and water. The dichloromethane extract was dried over sodium sulfate and the solvent evaporated. The smaller sample of *D. membranosa* (82-102B, 1.4 g dry weight) gave a brown oil (169 mg) that was purified by flash chromatography on silica gel (40–63 μm) using a hexane–ether gradient elution followed by LC on Partisil using 1:1 ether/hexane as eluant to obtain 9,11-dihydrogracilin A (1, 33 mg, 2.3% dry weight) as a colorless oil.

The larger sample of *D. membranosa* (85-102A, 30.1 g dry weight) gave a dichloromethane extract (1.77 g) that inhibited the growth of *S. aureus* and *B. subtilis*. The extract was chromatographed on Sephadex LH-20 using 1:1 dichloromethane/methanol as eluant and the active fractions were combined. The active material was purified by flash chromatography on silica gel using first a hexane–ethyl acetate gradient and then ether–hexane (1:1) to obtain membranolide (2, 65 mg, 0.2% dry weight) as a colorless oil.

9,11-Dihydrogracilin A (1): $[\alpha]_D$ -11.0° (*c* 1.3, $CHCl_3$); IR ($CHCl_3$) 1740, 1235, 990, 935 cm^{-1}; ^1H NMR (360 MHz, $CDCl_3$) δ 0.89 (s, 3 H), 0.96 (s, 3 H), 1.03 (s, 3 H), 1.16 (br d, 1 H, *J* = 14 Hz), 1.65 (d, 3 H, *J* = 6.9 Hz), 1.85 (m, 1 H), 2.08 (s, 3 H), 2.10 (s, 3 H), 2.36 (ddd, 1 H, *J* = 9, 7.7, 7.5 Hz), 2.42 (dd, 1 H, *J* = 6.8, 4.2 Hz), 3.14 (dd, 1 H, *J* = 7.5, 5.5 Hz), 5.65 (q, 1 H, *J* = 6.9 Hz), 5.97 (s, 1 H), 6.44 (d, 1 H, *J* = 5.5 Hz); ^{13}C NMR (50.3 MHz, $CDCl_3$) see Table I; EIMS, *m/z* (relative intensity) 272 (M^+ - 2 AcOH, 3), 208 (7), 148 (100), 125 (27), 83 (18), 69 (69), 57 (14); CIMS (NH_3), *m/z* (relative intensity) 410 ($M + NH_4^+$, 44), 290 ($M + NH_4^+$ - 2AcOH, 100); HRMS, obsd *m/z* 272.2122, $C_{19}H_{28}O$, (M - 2AcOH) requires 272.2141.

Membranolide (2): $[\alpha]_D$ -28.8° (*c* 2.25, $CHCl_3$); IR ($CHCl_3$) 1753, 1737, 1597 cm^{-1}; UV (MeOH) 211 nm (ϵ 20 300), 231 (5800), 285 (1600), 293 (1600); ^1H NMR (360 MHz, $CDCl_3$) δ 0.47 (s, 3 H), 0.94 (s, 3 H), 1.37 (s, 3 H), 1.53 (d, 1 H, *J* = 14 Hz), 1.75 (d, 3 H, *J* = 7 Hz), 2.08 (br d, 1 H, *J* = 14 Hz), 2.28 (br d, 1 H, *J* = 14 Hz), 3.68 (s, 3 H), 4.61 (q, 1 H, *J* = 7 Hz), 5.21 (s, 2 H), 7.31 (d, 1 H, *J* = 8.3 Hz), 7.81 (d, 1 H, *J* = 8.3 Hz); ^{13}C NMR (50.3 MHz, $CDCl_3$) see Table I; EIMS, *m/z* (relative intensity) 344 (M^+, 9), 313 (28), 312 (100), 297 (41), 284 (11), 269 (19), 230 (13), 229 (77), 228 (12), 219 (16), 213 (31), 201 (25), 200 (20), 189 (18), 187 (13), 83 (13), 69 (35), 57 (18), 55 (14); HRMS, *m/z* 344.1985, $C_{21}H_{28}O_4$ requires 344.1988.

Conversion of Membranolide (2) into Isobenzopyran 6. A solution of membranolide (2, 19 mg, 0.055 mmol) in dry ether (2.0 mL) was added to a stirred suspension of lithium aluminum hydride (24 mg) in dry ether (4.0 mL) under an atmosphere of dry nitrogen. The mixture was heated under reflux for 1 h, cooled to 0 °C, and quenched with ethyl acetate, followed by 2 M hydrochloric acid (3 mL). The mixture was extracted with ethyl acetate (2 × 10 mL), and the combined organic extracts were washed with dilute sodium bicarbonate solution (2 mL), dried over Na_2SO_4, and evaporated to give a clear oil. The oil was purified by chromatography on silica gel (1:1 ethyl acetate/hexanes, then 1:10:10 2-propanol/ethyl acetate/hexanes) to obtain the triol 5 (9.3 mg, 52%): IR ($CHCl_3$) 3600–3400 (br), 3010, 2880, 1600 cm^{-1}; UV (MeOH) 218 nm (δ 7500), 269 nm (290), 270 nm (190); ^1H NMR ($CDCl_3$) δ 0.37 (s, 3 H), 0.90 (s, 3 H), 1.43 (s, 3 H), 1.44 (d, 3 H, *J* = 6.8 Hz), 2.16 (d, 1 H, *J* = 14.5 Hz), 2.28 (bd, 1 H, *J* = 15 Hz), 3.86 (dd, 1 H, *J* = 11, 7.9 Hz), 3.94 (dd, 1 H, *J* = 11, 8.1 Hz), 4.13 (ddq, 1 H, *J* = 8.1, 7.9, 6.8 Hz), 4.57 (d, 1 H, *J* = 11.9 Hz), 4.79 (d, 1 H, *J* = 12.6 Hz), 4.82 (d, 1 H, *J* = 11.9 Hz), 4.85 (d, 1 H, *J* = 12.6 Hz), 7.18 (d, 1 H, *J* = 8.3 Hz), 7.42 (d, 1 H, *J* = 8.3 Hz); EIMS, (relative intensity) 302 ($M^+ - H_2O$, 20), 284 (17), 273 (16), 272 (58), 271 (100), 257 (15), 243 (43), 42 (18), 241 (88), 176 (38), 175 (25), 171 (21), 161 (22), 157 (26), 151 (27), 69 (32), 55 (16); HRMS, *m/z* 302.2242, $C_{20}H_{30}O_2$ requires 302.2245.

A solution of the triol 5 (0.8 mg) in benzene (0.5 mL) was stirred with a small crystal of *p*-toluenesulfonic acid at 30 °C for 19 h. TLC showed two spots due to the presence of a trace of starting material and a single new nonpolar compound. The mixture was eluted through a short column of silica gel with 1:1 ethyl acetate/hexanes to afford the isobenzopyran 6 (0.2 mg), which had identical IR and NMR data with those reported by Karuso et al.[5]

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PD 125375, a Novel Metabolite Coproduced with Tomaymycin

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In our search for new antitumor agents, a *Streptomyces* sp. was found that produces the known antibiotic tomaymycin (**1a**)[1] and oxotomaymycin (**1b**).[2] During the course of isolating these two compounds, a third metabolite, PD

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