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SESQUITERPENE-DERIVED METABOLITES FROM THE DEEP WATER MARINE SPONGE *POECILLASTRA SOLLASI*

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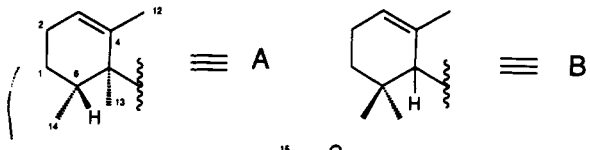
ABSTRACT.—Six sesquiterpene-derived compounds, **1–6**, which we call sollasins a–f, have been isolated from a deep water specimen of the sponge *Poecillastra sollasi*. The structures were elucidated by comparison of spectral data to related metabolites and confirmed using spectroscopic methods. The compounds inhibit the growth of the pathogenic fungi *Candida albicans* and *Cryptococcus neoformans* and the P-388 and A-549 tumor cell lines. Compounds **3** and **4** show weak inhibition of binding of [^{125}I] angiotensin II to rat aorta smooth muscle cell membranes.

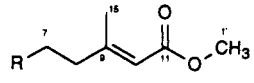
Bioassay-guided purification of marine extracts has great promise for the discovery of therapeutically useful natural products. As part of our continuing research aimed at the discovery of biologically active metabolites from deep water marine organisms, this paper describes the isolation and structure elucidation of six new sesquiterpene-derived compounds, **1–6**, which we call sollasins a–f, respectively. Sesquiterpenoid compounds with rearranged monocyclofarnesane skeletons have been isolated from fungal fermentation broths (1), liverworts (2), and sponges. Examples of the sponge-derived compounds are the microcionins from *Microciona toxystilla* (3) and compounds **7–10**, which have been reported from a sponge of the genus *Pachastrella* by Rinehart and Patil (4).

RESULTS AND DISCUSSION

A sample of the sponge *Poecillastra sollasi* Van Soest & Stentoft [Porifera, Demospongiae, Choristida, Pachastrellidae) (5)] was collected in October 1988 from Little Inagua Island, Bahamas at a depth of 1230 feet using the Johnson-Sea-Link manned submersible. Compounds **1–6** were purified by repeated chromatography of a nonpolar extract on Si gel stationary phases. Examination of the ^{1}H and ^{13}C nmr (Tables 1 and 2) and mass spectra of compounds **1–5** suggested that they were closely related to each other and to compounds **7–10**, previously reported from a *Pachastrella* sp. (4).

Compound **1** was separated from a small amount of its exo isomer via vacuum cc on an $AgNO_3$-impregnated Si gel stationary phase. The ^{13}C-nmr and mass spectra suggested a formula of $C_{16}H_{26}O_2$, which requires four unsaturation equivalents. The nmr indicated the presence of an α,β-unsaturated methyl ester (δ 167.3 s, 161.6 s, 114.6 d, 50.6 q) and a trisubstituted olefin (δ 124.7 d, 139.0 s). No further unsaturation was suggested by the nmr or ir data; therefore **1** must contain one ring. Comparison of the nmr data to those reported for compounds **7–10** suggested the presence of a monocyclofarnesyl-like carbon skeleton in **1**. Although the overall multiplicities observed in the ^{13}C spectrum were comparable, the chemical shifts were significantly different. A methyl doublet was observed at δ 0.85, which was consistent with a rearrangement of one of the methyl groups located on C-6 in compounds **7–10** to C-5 in **1**. Comparison of the ^{1}H- and ^{13}C-nmr chemical shifts to those reported for ageline A (6), which has a similar ring system, further supported this assignment, as did a ^{1}H-^{13}C HMBC experiment (Table 3). The relative stereochemistry at C-5 and C-6 was assigned by comparison of the ^{1}H chemical shifts of the H-13 and H-14 methyl





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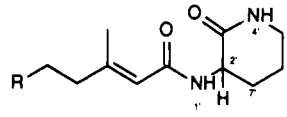

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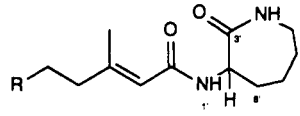
7 R=B



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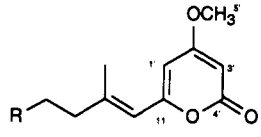
8 R=B



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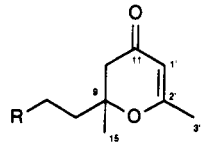
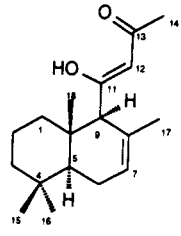
9 R=B



5 R=A
10 R=B



6

groups with those reported for ageline A and microcionin 2. H-13 and H-14 were observed at δ 0.85 and 0.86, respectively, in **1** and at δ 0.85 and 0.85 in ageline A. In microcionin 2, which has the opposite stereochemistry at C-6, the methyl groups were

TABLE 1. ^{1}H-nmr Data for Compounds **1–5**.[a]

Proton	Compound				
	1	**2**	**3**	**4**	**5**
H-1	1.42 (2H, m)	1.42 (2H, m)	1.41 (2H, m)	1.39 (2H, m)	1.42 (2H, m)
H-2	1.93 (2H, m)	1.94 (2H, m)	1.91 (2H, m)	1.87 (2H, m)	1.92 (2H, m)
H-3	5.40 (brs)	5.42 (brs)	5.38 (brs)	5.36 (brs)	5.40 (brs)
H-6	1.66 (m)	1.67 (m)	1.63 (m)	1.64 (m)	1.64 (m)
H-7	1.52 (2H, m)	1.50 (2H, m)	1.47 (2H, m)	1.47 (2H, m)	1.45 (2H, m)
H_a-8	1.77 (m)	1.64 (m)	1.82 (m)	1.76 (m)	1.44 (2H, m)
H_b-8	2.07 (m)	2.00 (m)	2.03 (m)	2.01 (m)	—
H_a-10	5.61 (brq, 1.1)	5.61 (brs)	5.61 (brs)	5.69 (brs)	2.30 (m)
H_b-10	—	—	—	—	2.47 (m)
H-12	1.58 (3H, dt 1.1, 2.3)	1.58 (3H, brs)	1.58 (3H, brs)	1.53 (3H, dt 1.1, 2.3)	1.56 (3H, dt 1.1, 2.3)
H-13	0.86 (3H, s)	0.86 (3H, s)	0.86 (3H, s)	0.80 (3H, s)	0.86 (3H, s)
H-14	0.85 (3H, d 7.0)	0.85 (3H, d 7.6)	0.85 (3H, d 6.4)	0.79 (3H, d 6.0)	0.85 (3H, d 6.8)
H-15	2.14 (3H, d 1.1)	2.14 (3H, brs)	2.14 (3H, brs)	2.09 (3H, brs)	1.32 (3H, s)
H-1′	—	6.35 (d, 4.8)	6.78 (d, 5.9)	5.71 (d, 2.1)	5.26 (s)
H-2′	3.66 (3H, s)	4.30 (ddd, 10.7, 5.9, 5.5)	4.53 (ddd, 10.9, 5.9, 1.2)	—	—
H-3′		—	—	5.34 (d, 2.1)	1.93 (3H, s)
H-4′		6.19 (brs)	6.61 (t, 6.1)		
H-5′		3.38 (2H, t 5.8)	3.21 (2H, m)		
H_a-6′		1.92 (2H, m)	1.33 (m)	3.71 (3H, s)	
H_b-6′		—	1.76 (m)		
H_a-7′		1.42 (m)	1.95 (2H, m)		
H_b-7′		2.58 (m)	—		
H_a-8′			1.40 (m)		
H_b-8′			1.80 (m)		

[a]Table entries are δ, multiplicity, *J* in Hz.

TABLE 2. ^{13}C-nmr Data for Compounds **1–5**.

Carbon	Compound				
	1	**2**	**3**	**4**	**5**
C-1	26.9 t	27.0 t	27.0 t	27.0 t	26.9 t
C-2	25.5 t	25.5 t	25.5 t	25.5 t	25.5 t
C-3	124.7 d	124.5 d	124.5 d	124.6 d	124.7 d
C-4	139.0 s	139.0 s	139.1 s	139.0 s	138.8 s
C-5	40.5 s	40.4 s	40.4 s	40.5 s	40.0 s
C-6	33.3 d	33.3 d	33.3 d	33.3 d	33.3 d
C-7	34.4 t	34.5 t	34.5 t	34.7 t	29.7 t
C-8	35.8 t	35.5 t	35.5 t	36.5 t	33.5 t
C-9	161.6 s	156.0 s	155.9 s	151.3 s	83.1 s
C-10	114.6 d	117.2 d	117.3 d	116.3 d	45.8 t
C-11	167.3 s	167.3 s	166.2 s	160.4 s	192.6 s
C-12	19.1 q	19.1 q	19.1 q	19.1 q	18.8 q
C-13	21.0 q	20.9 q	21.0 q	21.0 q	21.1 q
C-14	15.7 q	15.8 q	15.8 q	15.8 q	15.7 q
C-15	19.1 q	18.7 q	18.6 q	19.6 q	23.2 q
C-1′	—	—	—	100.7 d	103.2 d
C-2′	50.6 q	50.4 d	51.9 d	171.4 s	171.9 s
C-3′		172.0 s	176.0 s	87.4 d	21.4 q
C-4′		—	—	164.5 s	
C-5′		41.7 t	42.1 t	55.7 q	
C-6′		21.0 t	28.9 t		
C-7′		27.4 t	27.9 t		
C-8′			31.8 t		

TABLE 3. Long Range ^{1}H-^{13}C Correlations from HMBC Nmr Experiments for Compounds **1–5**.

Proton	Compound				
	1	**2**	**3**	**4**	**5**
H-1 . . .	C-2,C-3,C-5,C-6		C-3	C-2,C-14	C-6
H-2 . . .	C-1,C-3,C-4,C-6				
H-3 . . .	C-1,C-2,C-5,C-12	C-5,C-12			C-1,C-5,C-12
H-6 . . .	C-1,C-2,C-4,C-5 C-7,C-13,C-14			C-13	
H-7 . . .	C-4,C-5,C-6, C-8,C-13		C-4,C-5	C-13	
H-8 . . .	C-7,C-9,C-10,C-15	C-9,C-10			
H_a-10 . .	C-8,C-11,C-15	C-8,C-9,C-11,C-15		C-8,C-9,C-11, C-15,C-1′	C-8,C-9,C-11,C-15
H_b-10 . .	—	—	—	—	C-8,C-9,C-11,C-15
H-12 . . .	C-3,C-4,C-5	C-3,C-4,C-5		C-3,C-4,C-5	C-3,C-4
H-13 . . .	C-4,C-5,C-7	C-4,C-5,C-7	C-6,C-7	C-4,C-5,C-6,C-7	C-5,C-6,C-7
H-14 . . .	C-1,C-5,C-6	C-1,C-5,C-6	C-1,C-6	C-1,C-6	C-1,C-5,C-6
H-15 . . .	C-8,C-9,C-10	C-8,C-9,C-10	C-8,C-9,C-10	C-8,C-9,C-10	C-8,C-9,C-10
H-1′ . . .	—	C-11,C-2′,C-3′	C-11,C-2′,C-3′	C-10,C-11,C-2′,C-3′	C-10,C-11,C-2′,C-3′
H-2′ . . .	C-11	C-11,C-3′,C-6′,C-7′	C-11,C-3′,C-7′,C-8′	—	—
H-3′ . . .	—	—	—	C-1′,C-2′,C-4′	C-1′,C-2′
H-4′ . . .	—	—	C-2′,C-3′,C-5′	—	—
H-5′ . . .	—	C-3′,C-6′,C-7′	C-3′,C-6′,C-7′	C-2′	—
H_a-6′ . .	—	—		—	—
H_b-6′ . .	—	—		—	—
H-7′ . . .	—	C-2′,C-3′,C-5′,C-6′		—	—
H_a-8′ . .	—	—		—	—
H_b-8′ . .	—	—		—	—

observed at δ 1.09 and 0.99. The $\Delta^{9,10}$ olefin was determined to be *E* based upon a correlation observed between H-10 (δ 5.61) and H_a-8 and H_b-8 (δ 2.07, 1.72) in the 2D ^{1}H-^{1}H noesy experiment. No correlation was observed between H-10 and the H-15 methyl protons.

Compound **2** was obtained as a colorless oil. The ir (ν max cm^{-1} 3300 br, 1645 br) and uv (λ max 215 nm, ε = 18,900) spectra suggested the presence of an α,β-unsaturated amide in **2**. Hreims and ^{13}C nmr suggested a molecular formula of $C_{20}H_{32}N_2O_2$, requiring six sites of unsaturation. Comparison of the ^{1}H and ^{13}C nmr with those of **1** indicated that the sesquiterpene portion was identical (Tables 1 and 2). The ^{13}C nmr showed the presence of an additional amide carbon observed at 172.0 ppm. No further unsaturation was indicated by nmr or ir, which suggested the presence of two rings in **2**. Comparison of the nmr spectral data with those reported for **7** suggested the presence of a cyclized ornithine moiety in **2**. One-bond and multiple-bond ^{1}H-^{13}C correlations observed via the HMQC and HMBC experiments, respectively, confirmed this assignment. The relative stereochemistry of the terpene portion was assigned based upon comparison of the nmr data to those of **1**. ^{1}H homonuclear decoupling experiments allowed us to determine the scalar coupling constants for H-2′–H_a-7′ and H-2′–H_b-7′ as 10.7 and 5.9 Hz, respectively, which suggested that the H-2′ proton is axial.

Compound **3** was isolated as a colorless oil. The ir (ν max cm^{-1} 3390, 1645, 1620) and uv (λ max 222 nm, ε = 21,772) spectra once again suggested the presence of an α,β-unsaturated amide. Hreims suggested a formula of $C_{21}H_{34}N_2O_2$ which differs from **2** in the presence of an additional methylene group. Comparison of the ^{1}H and ^{13}C data to those of **1** indicated that the sesquiterpene portion was identical to that of **1** and **2** and that the additional methylene group should be part of the amide unit. Comparison of the nmr spectral data observed for the remaining unassigned atoms with those reported for compounds **8** and bengamide e (7), both of which contain a cyclized lysine unit, led us to suggest the presence of the same moiety in **3**. Long range ^{1}H-^{13}C correlations observed via the proton-detected HMBC experiment confirmed this assignment

(Table 3). Homonuclear decoupling experiments defined the scalar coupling constants for H-2′–H_a-8′ and H-2′–H_b-8′ as 1.2 and 10.9 Hz, respectively, which suggested that H-2′ is in a pseudoaxial configuration.

Compound **4** was obtained as a yellow oil. The hrms and ^{13}C-nmr data suggested a formula of $C_{20}H_{28}O_3$ for **4**, requiring seven unsaturation equivalents. Comparison of the ^{1}H- and ^{13}C-nmr data to those of **1–3** suggested that atoms 1–10 of the terpene unit were also present in **4**. The remaining sp^2 carbon resonances observed at δ 171.4 s, 164.5 s, 160.4 s, 100.7 d, and 87.4 d and the sp^3 resonance observed at 55.7 q are consistent with the presence of a 4-methoxy-6-alkylpyran-2-one (8) as found in **9**. All of the correlations observed in the ^{1}H-^{13}C HMBC experiment confirmed this assignment (Table 3) and allowed for a bond to be placed between C-10 and C-11. The optical rotation of **4** is opposite in sign to that observed for **1**, suggesting that it may have the opposite absolute configuration. The absolute configuration has not been determined for any of the compounds.

Compound **5** was isolated as a colorless oil. Hreims suggested a formula of $C_{18}H_{28}O_2$ for **5**, which requires five unsaturation equivalents. The ^{13}C-nmr spectrum suggested the presence of one trisubstituted olefin (124.7 d, 138.8 s) and a β-diketone trapped in the enol form (192.6 s, 103.2 d, 171.9 s). This accounted for three of the sites of unsaturation and suggested the presence of two rings. Inspection of the nmr data for atoms 1–6 and 12–14 suggested the presence of the same carbocyclic ring system found in compounds **1–4**. A large ion observed at *m/z* 123 in the eims was consistent with a 4,5,6-trimethyl-3-cyclohexene moiety as found in metabolites **1–4**. The chemical shifts of C-7 and C-8 were shifted upfield from those observed for **1–4**, but long range ^{1}H-^{13}C correlations (Table 3) confirmed their assignment. The remaining atoms have been assigned as a 2-methyl-5-(*H*)-6,6-dialkylpyran-4-one based upon comparison of the nmr data to the related compound **10** reported by Rinehart and Patil (4) and the observed long range ^{1}H-^{13}C correlations (Table 3). The base ion in the mass spectrum is observed at *m/z* 125 which is consistent with the presence of a 2,6-dimethyl-5-(*H*)-pyran-4-one unit. The terpenoid and pyranone partial structures were attached as shown based upon long range ^{1}H-^{13}C correlations observed between H-15 and C-8, C-9 and C-10. The stereochemistry at C-9 has not been determined.

Compound **6** was isolated as a colorless oil. ^{13}C nmr and hreims suggested a molecular formula of $C_{18}H_{28}O_2$ for **6**. An exchangeable singlet observed at δ 15.8 ppm in the ^{1}H-nmr spectrum, the ^{13}C-nmr resonances observed at δ 193.5 s (C-11), 103.9 d (C-12), and 191.4 s (C-13), and a broad intense ir absorption at 1603 cm^{-1} were indicative of an enolized β-diketone. This could be further defined as a 2,4-butanedione unit based upon long range ^{1}H-^{13}C correlations observed between the methyl protons observed at δ 2.05 (3H, s) and the carbons observed at δ 191.4 (C-13) and 103.9 (C-12). The remaining atoms were defined as a drimane skeleton based upon ^{1}H-^{1}H COSY, ^{1}H homonuclear decoupling data, ^{1}H-^{13}C HMQC, and HMBC data as well as comparison to the nmr data reported for the drimane portion of sulfircin (9) (Table 4). The butane dione unit was placed at C-9 of the drimane skeleton based upon the following long range ^{1}H-^{13}C correlations: 11-O*H* to C-9; H-9 to C-11 and H-9 to C-12. The relative stereochemistry of **6** has been assigned to be the same as that reported for sulfircin based upon comparison of the nmr chemical shifts.

Sollasins a–e, **1–5**, are closely related to metabolites **7–10** reported from the related sponge *Pachastrella* sp. and may result from a methyl rearrangement of these metabolites. A small quantity (4.8 mg) of compound **10** was also present in the sample analyzed in this study. Compounds **3** and **4** were found to inhibit the binding of [^{125}I] angiotensin II to rat aorta smooth muscle cell membranes with IC_{50} values of 63 ± 21 μM and 42 ± 20 μM, respectively. Compounds **2**, **3**, and **5** inhibited the growth of the

TABLE 4. ^1H and ^{13}C nmr Data for Compound **6**.

Position	^{13}C δ, mult	^1H δ mult (*J* in Hz)	Long range ^1H-^{13}C correlations observed between H-# and C-#
1	40.8 t	a 1.14 m	
		b 1.70 m	
2	18.7 t	a 1.41 m	C-1,C-3,C-10
		b 1.52 m	C-1
3	42.1 t	a 1.17 m	C-2
		b 1.43 m	C-4,C-5
4	33.1 s	—	—
5	49.8 d	1.21 m	C-6,C-10,C-15,C-16,C-18
6	23.6 t	a 1.94 m	C-7,C-8
		b 1.99 m	C-5,C-7,C-8
7	124.8 d	5.58 d (3.7)	C-5,C-6,C-9,C-17
8	130.2 s	—	—
9	64.9 d	2.66 s	C-7,C-8,C-10,C-11,C-12,C-18
10	37.0 s	—	—
11	193.5 s	—	—
12	103.9 d	5.44 s	C-11,C-13
13	191.4 s	—	—
14	25.3 q	2.05 3H, s	C-12,C-13
15	33.4 q	0.86 3H, s	C-3,C-4,C-5,C-16
16	22.0 q	0.90 3H, s	C-3,C-5,C-15
17	21.5 q	1.58 3H, s	C-7,C-8,C-9
18	15.1 q	0.95 3H, s	C-1,C-5,C-9,C-10
11-*OH*	—	15.8 bs	C-9

pathogenic fungus *Candida albicans* with MICs of 125, 31.2, and 25 μg/ml, respectively. These compounds also inhibited the growth of the pathogenic fungus *Cryptococcus neoformans* with MICs of 31.2, 15.6, and 25 μg/ml, respectively. Compounds **2**–**4** were moderate inhibitors of the growth of a P-388 murine leukemia tumor cell line with IC_{50}s of 6.0, 8.4, and 5.5 μg/ml, respectively, and of the human A-549 lung tumor cell line with IC_{50}s of 17.0, 11.0, and 11.3 μg/ml, respectively.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Spectral data were measured on the following instruments: ir Perkin-Elmer 1310; uv/visible Perkin-Elmer Lamda 3B; nmr Bruker AM360 with the Aspect 3000 computer; ms Kratos MS-80RFA ei 70 eV (Chemical Instrumentation Center, Yale University); [α] Jasco DIP-360 Digital polarimeter. ^1H nmr chemical shifts are reported as δ values in ppm relative to $CDCl_3$ (7.26 ppm). ^{13}C nmr chemical shifts are reported as δ values in ppm relative to $CDCl_3$ (77.0 ppm). ^{13}C multiplicities were measured using the DEPT sequence, one-bond and multiple-bond ^1H-^{13}C connectivities were determined via the 2D proton-detected HMQC and HMBC experiments, respectively.

COLLECTION AND TAXONOMY.—The sample (DBMR Number: 8-X-88-3-005) was collected in October of 1988 from Little Inagua Island, Bahamas, on a rock slope at a depth of 1230 feet by the Johnson-Sea-Link I manned submersible. The sponge was amorphous, approximately 10 cm in diameter, pale green externally, and white internally. The sample corresponds most closely to *Poecillastra sollasi* as described by Van Soest and Stentoft (5). A voucher specimen is on deposit at the Harbor Branch Oceanographic Museum, Fort Pierce, Florida (catalog number 003:00595).

EXTRACTION AND CHROMATOGRAPHY.—The diced sponge (50 g) was extracted by blending with heptane (3 × 200 ml) followed by EtOAc (2 × 200 ml). These extracts were combined and dried under vacuum to obtain a pale yellow oil (890 mg). The extract was dissolved in heptane and deposited on a SiO_2 vacuum cc column (150 ml scintered glass funnel packed with Kieselgel 60H, 15 μ). The column was eluted with a step gradient from 0 to 100% EtOAc using 10% increments. The volume of fractions 1–11 was 100 ml each. The column was then washed with two additional 100-ml volumes of EtOAc, yielding fractions

12 and 13. Fraction 5 contained **4** (210 mg, 0.42% wet wt), fraction 8 contained **3** (156 mg, 0.31% wet wt), and fractions 12 and 13 contained **2** (203 mg, 0.41% wet wt). Fraction 3 (101 mg) was separated by hplc on a SiO_2 column (Whatman Partisil 10, 1 × 50 cm) with heptane-EtOAc (79:1) to yield **6** (7.6 mg, 0.02% wet wt) and a mixture of isomers **1** and what may be a small amount of its C-4/C-12 exo isomer. The isomeric mixture was separated by vacuum cc on $AgNO_3$-impregnated SiO_2 prepared by suspending 23.75 g SiO_2 in a solution of 1.25 g $AgNO_3$ in 70 ml H_2O. The H_2O was removed on a rotary evaporator, and the gel was dried at 110° for 12 h. Fraction 4 (132 mg) was separated by hplc (Whatman Partisil 10, 1 × 50 cm) with heptane-EtOAc (3:1) to yield **5** (20.3 mg, 0.04% wet wt) and **10** (4.8 mg, 0.01% wet wt).

Compound **1**.—Methyl 3-methyl-5-(1,2,6-trimethyl-2-cyclohexen-1-yl)-2-pentenoate: colorless oil; $[\alpha]^{24}D$ +12.2 ($CHCl_3$, c = 1.01); uv (EtOH) λ max 216 nm (ε = 12,880); ir (thin film) ν max cm^{-1} 2920, 1715, 1640 br, 1430, 1378, 1353, 1278, 1221, 1143, 920, 864, 846, 800; ^1H nmr see Table 1; ^{13}C nmr see Table 2; hreims observed *m/z* 250.1921 ($C_{16}H_{26}O_2$ requires 250.1934); eims *m/z* $[M]^+$ 250, $[M-Me]^+$ 235.

Compound **2**.—3-Methyl-*N*-(2-oxo-3-piperidinyl)-5-(1,2,6-trimethyl-2-cyclohexen-1-yl)-2-pentenamide: colorless solid; $[\alpha]^{24}D$ +29.8 ($CHCl_3$, c = 2.14); uv (EtOH) λ max 215 nm (ε = 18,900); ir (thin film) ν max cm^{-1} 3300 br, 2940, 2880, 1645 br, 1525, 1492, 1469, 1450, 1361, 1330, 1255, 1205, 1180, 1107, 1078, 980, 849; ^1H nmr see Table 1; ^{13}C nmr see Table 2; hreims observed *m/z* 332.2448 ($C_{20}H_{32}N_2O_2$ requires 332.2466); eims *m/z* $[M]^+$ 332, $[M-Me]^+$ 317, $[M-C_9H_{15}]^+$ 209.

Compound **3**.—*N*-(Hexahydro-2-oxo-1*H*-azepin-3-yl)-3-methyl-5-(1,2,6-trimethyl-2-cyclohexen-1-yl)-2-pentenamide: colorless oil; $[\alpha]^{24}D$ +10.0 ($CHCl_3$, c = 1.26); uv (EtOH) λ max 222 nm (ε = 21772); ir (thin film) ν max cm^{-1} 3390 br, 2910, 1645 br, 1620, 1472, 1430, 1365, 1330, 1170; ^1H nmr see Table 1; ^{13}C nmr see Table 2; hreims observed *m/z* 346.2610 ($C_{21}H_{34}N_2O_2$ requires 346.2622); eims *m/z* $[M]^+$ 346, $[M-Me]^+$ 331, $[M-C_9H_{15}]^+$ 223.

Compound **4**.—4-Methoxy-6-(1-methyl-4-(1,2,6-trimethyl-2-cyclohexen-1-yl)-1-butenyl)-2*H*-pyran-2-one: yellow oil; $[\alpha]^{24}D$ −22.1 ($CHCl_3$, c = 2.13); uv (EtOH) λ max 226 nm (ε = 23,510), 308 (ε = 11,579); ir (thin film) ν max cm^{-1} 2950, 2916, 1718, 1633, 1605, 1544, 1446, 1403, 245, 159, 1032, 990, 950, 804; ^1H nmr see Table 1; ^{13}C nmr see Table 2; hreims observed *m/z* 316.2055 ($C_{20}H_{28}O_3$ requires 316.2039); eims *m/z* $[M]^+$ 316, $[M-Me]^+$ 301, $[M-C_9H_{15}]^+$ 193, $[C_9H_{15}]^+$ 123.

Compound **5**.—2,3-Dihydro-2,6-dimethyl-2-(2-(1,2,6-trimethyl-2-cyclohexen-1-yl)-ethyl)-4*H*-pyran-4-one: colorless oil; $[\alpha]^{24}D$ +57.6 ($CHCl_3$, c = 0.75); uv (heptane) λ max 266 nm (ε = 14,723); ir (thin film) ν max cm^{-1} 2950, 2900, 1650, 1595, 1423, 1370, 1340, 1232, 1140, 1050, 1025, 980, 872, 840, 790; ^1H nmr see Table 1; ^{13}C nmr see Table 2; hreims observed *m/z* 276.2075 ($C_{18}H_{28}O_2$ requires 276.2090); eims *m/z* $[M]^+$ 276, $[M-C_9H_{15}]^+$ 153, $[C_9H_{15}]^+$ 123.

Compound **6**.—4-(1,4,4a,5,6,7,8,8a-Octahydro-2,5,5,8a-tetramethyl-1-naphthalenyl)-butane-2,4-dione: colorless oil; $[\alpha]^{24}D$ +92.5 ($CHCl_3$, c = 0.28); uv (heptane) λ max 276 nm (ε = 10,602); ir (thin film) ν max cm^{-1} 2940, 2915, 2820, 1603 (br), 1433 (br), 1380, 1358, 1303, 1279, 1260, 1217, 1202, 1147, 1100, 1086, 1055, 1048, 1030, 979, 949, 878, 850, 825, 803, 778; ^1H nmr see Table 4; ^{13}C nmr see Table 4; hreims observed *m/z* 276.2083 ($C_{18}H_{28}O_2$ requires 276.2090).

BIOLOGICAL METHODS.—*Antimicrobial assays.*—Minimum inhibitory concentrations (MICs) were determined by standard microdilution broth techniques (10) in a total volume of 50 μl. The growth media used were as follows: *Ca. albicans* (ATCC 44506), Sabouraud dextrose broth; *Cr. neoformans* (ATCC 32045), Emmon's modification of Sabouraud dextrose broth. Plates were incubated at 37° for either 24 h (bacteria and *Ca. albicans*) or 48 h (*Cr. neoformans*). The MIC was determined as the lowest concentration of the drug which completely inhibited growth.

Antitumor assays.—The antitumor assays were run using standard protocols in 96-well plates and MTT to detect cytotoxicity (11).

{^{125}I} Angiotensin II receptor binding in rat aorta smooth muscle cell membranes.—Binding of [^{125}I] angiotensin II to rat aorta smooth muscle cell membranes was measured as previously described with modifications (12). Frozen cells (in vials containing approximately 1×10^8 cells) were thawed and homogenized by Polytron (setting 8 for 8 sec) in 20 ml 20 mM $NaHCO_3$. The homogenate was diluted to a concentration of 5×10^7 cells/10 cc 20 mM $NaHCO_3$. Aliquots of 10 ml were centrifuged for 30 min at 20,000 rpm. The resulting pellet was frozen at −70° or homogenized with a Teflon pestle in 20 ml of assay buffer consisting of 50 mM Tris-HCl pH 7.4, 125 mM NaCl, 6.5 mg $MgCl_2$, 1 mM EDTA, 2 mg/ml BSA, and 0.5 cc/100 ml buffer of leupeptin, pepstatin A, bestatin, and amastatin (each at a final concentration of 1 μg/ml). Each pellet was diluted in 100 ml of the assay buffer. Assays were run in a total volume of 0.25 ml in plastic tubes. Tubes in duplicate contained 0.025 ml [^{125}I] angiotensin II at a final concentration of 0.88 nM,

0.025 ml assay buffer, unlabeled angiotensin II at a final concentration of 1 μM, or drug, to determine total, nonspecific, and drug-dependent binding, respectively.

The reaction was initiated by the addition of 0.200 ml membrane suspension (75–125 μg/ml protein) and continued for 3 h at 25°. Bound radioactivity was isolated by filtration (Brandel cell harvester, Gaithersburg, MD) onto Whatman GF/C glass fiber filters. Unbound radioactivity was removed by two 5-ml washes of 5 mM Tris-HCl, pH 7.4. Filters were counted in a Genesys gamma counter. Data calculations for the amount of radioactivity bound were performed using non-linear regression analysis (RS/1, Bolt, Beranek and Newman, Boston, MA).

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