JOURNAL OF NATURAL PRODUCTS

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J. Nat. Prod., **1993**, 56 (4), 583-593• DOI: 10.1021/np50094a019 • Publication Date (Web): 01 July 2004

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Journal of Natural Products
Vol. 56, No. 4, pp. 583-593, April 1993

STRUCTURE AND STEREOCHEMISTRY OF PECTINOLIDES A–C, NOVEL ANTIMICROBIAL AND CYTOTOXIC 5,6-DIHYDRO-α-PYRONES FROM *HYPTIS PECTINATA*[1]

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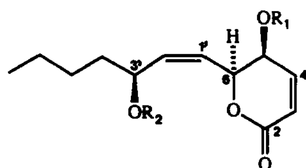
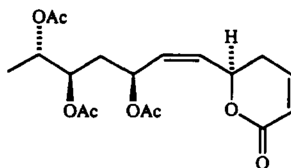
ABSTRACT.—By bioactivity-directed fractionation, three new antimicrobial and cytotoxic 5,6-dihydro-α-pyrones, pectinolides A–C, have been isolated from *Hyptis pectinata* (Lamiaceae). The absolute stereochemistry of pectinolide A [1] was established as 6*S*-[(3*S*-acetyloxy)-1*Z*-heptenyl]-5*S*-(acetyloxy)-5,6-dihydro-2*H*-pyran-2-one, on the basis of spectral, chiroptical, and chemical evidence. The structures of pectinolides B [2] and C [3] were determined as the monodeacetylated forms of 1 by comparison of their spectral data and chemical correlation with the prototype compound. *Staphylococcus aureus* and *Bacillus subtilis* were sensitive to pectinolide A [1] in the concentration range of 6.25–12.5 μg/ml. Compounds 1–3 exhibited significant cytotoxic activity (ED_{50}<4 μg/ml) against a variety of tumor cell lines.

Hyptis pectinata (L.) Poit. (Lamiaceae), popularly known as "hierba del burro" and with the Mexican Indian name of "xoltexnuk", is a very common herbaceous plant valued for its medicinal qualities and for its smell and taste in the regional cuisine of the southeastern region of Mexico (1). Formulations of the plant are used in popular medicine as a multipurpose remedy in the treatment of fevers, certain skin diseases, gastric disturbances (1), rhinopharyngitis, and lung congestion (2). Despite the widespread use of this herb, only two previous phytochemical investigations have been conducted. The first 5,6-dihydro-α-pyrone to be isolated from the Lamiaceae was hyptolide [4] from the leaves of this odoriferous plant (3). Gc-ms analysis of its essential oil has allowed the quantification of a large amount of thymol, which probably accounts for the antiseptic properties of *H. pectinata* oil (2). Extracts derived from the aerial parts of this





1 $R_1=R_2=Ac$
2 $R_1=Ac, R_2=H$
3 $R_1=H, R_2=Ac$

4

[1]Paper No. 5 in the series, "Chemical Studies on Mexican *Hyptis* species," and paper No. 1 in the series, "Biologically Active Natural Products from Mexican Medicinal Plants." Dedicated to the memory of Dr. A. Héber Muñoz (ENCB, Instituto Politécnico Nacional).

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plant were recently found to demonstrate antimicrobial activity against Gram-positive bacteria in qualitative biological assays (4).

In the course of our research directed toward the isolation and structural elucidation of bioactive constituents from economic and medicinal Mexican plants of the Lamiaceae (5), we now describe the structure and stereochemistry of pectinolides A [**1**], B [**2**], and C [**3**], three bioactive 6-substituted 5,6-dihydro-α-pyrones which are the major antimicrobial and cytotoxic constituents of *H. pectinata*.

RESULTS AND DISCUSSION

In an initial study, the $CHCl_3$ extract of *H. pectinata* leaves was found to show inhibitory activity against several microorganisms (4). In addition, this extract was cytotoxic (ED_{50} 2.2 μg/ml) when tested in the in vitro P-388 murine lymphocytic leukemia assay system. The antimicrobial activity has been traced to the mixture of pectinolides A [**1**], B [**2**], and C [**3**] by bioassay-directed fractionation using the agar well diffusion method (6). These novel antimicrobial compounds were tested for cytotoxicity in several tumor cell lines and demonstrated significant activities (ED_{50}<4 μg/ml).

Pectinolide A [**1**], the major active component, exhibited a molecular formula of $C_{16}H_{22}O_6$, based on its hreims data. The uv (λ max 208 nm) and ir (ν max 1730 cm^{-1}) spectra were in accord with the presence of an α,β-unsaturated δ-lactone (5,7). The ^{1}H-nmr spectrum (Table 1) indicated the presence of two acetoxy substituents (δ 2.05 and 2.10). The characteristic resonances of the vinylic protons on C-3 (δ 6.24) and C-4 (δ 6.96) in the lactone ring, as part of an ABX spin-system with H-5 (δ 5.19), indicated the substitution at C-5 by one of the acetyloxy functionalilties (7). The ^{13}C-nmr spectrum (Table 2) of **1**, assisted by off-resonance, APT, and ^{1}H-^{13}C HETCOR techniques, was in full agreement with the presence of a 3-(acetyloxy)-1-heptenyl moiety at C-6 (8). The discernible 10.5 Hz coupling constant for the two sets of olefinic protons at C-1′ (δ 5.73) and C-2′ (δ 5.62) demonstrated the cis configuration of the exocyclic double bond.

The absolute stereochemistry of pectinolide A [**1**] was determined as follows. The pseudo-equatorial orientation of the side chain at C-6 and the axial configuration for the acetyloxy group at C-5 were established by the H-5, H-6 coupling constant value (J = 2.9 Hz). This spectroscopic evidence was used, together with the positive sign of the cd curve ($\Delta\epsilon_{265} = +2.4$), to assign an *S* configuration to the chiral center at C-6 (5,7). Ozonolysis of **1** yielded 2-acetyloxyhexanoic acid as the major product, which was saponified to afford (+)-2-hydroxyhexanoic acid. The absolute *S* configuration for this α-hydroxy acid derived from **1** was correlated with the weak negative long-wave length cd maxima ($\Delta\epsilon_{244} = -0.01$) and the positive Cotton effect ($\Delta\epsilon = +1.58$) at 209 nm (9). Accordingly, these chiroptical and chemical results provided conclusive evidence for the formulation of pectinolide A as 6*S*-[(3*S*-acetyloxy)-1*Z*-heptenyl]-5*S*-(acetyloxy)-5,6-dihydro-2*H*-pyran-2-one [**1**].

Pectinolides B [**2**] and C [**3**] were found to have a molecular formula of $C_{14}H_{20}O_5$, as determined by hrms. The uv (λ max 204 nm) and ir (ν max 1735 cm^{-1}) revealed that both have a common chromophore, which indicated the same 5,6-dihydro-α-pyrone nucleus of pectinolide A [**1**]. The major difference from **1** in the ir spectrum was the presence of a broad OH absorption. The ms showed that **2** and **3** differ from **1** in having a molecular ion (*m/z* 268) with the difference of 42 mass units. According to these spectral data, it was evident that **2** and **3** were deacetylated isomeric forms of **1**. On acetylation, both compounds afforded the same derivative which was identical (gc-ms, tlc, nmr) to pectinolide A [**1**]. ^{1}H- and ^{13}C-nmr spectra (Tables 1 and 2) of **2** and **3** were quite comparable to those obtained for **1**. After measuring the ^{1}H-^{1}H COSY and ^{1}H-^{13}C HETCOR, the OH group was placed at C-3′ in pectinolide B [**2**]. The H-3′ signal

TABLE 1. ^{1}H-Nmr Assignments for Pectinolides A [**1**], B [**2**], and C [**3**] and Derivatives **7**, **9–11**, **14**, and **15**.[a]

Proton	Compound								
	1	**2**	**3**	**7**	**9**	**10**	**11**	**14**	**15**
H-3	6.24 d (9.7)	6.22 d (9.8)	6.08 d (9.8)	6.22 d (9.7)	6.25 d (9.6)	6.24 d (9.7)	6.25 d (9.7)	4.98 d (5.13)	2.62 dd (17.3, 6.1) 2.67 dd (17.3, 3.6)
H-4	6.96 dd (9.7, 5.7)	6.98 dd (9.8, 5.6)	7.01 dd (9.8, 5.4)	7.12 dd (9.7, 6.1)	7.08 dd (9.6, 6.1)	6.95 dd (9.7, 6.0)	6.95 dd (9.7, 6.0)	4.72 dd (5.13, 2.6)	4.12 ddd (6.1, 5.4, 3.6)
H-5	5.19 dd (5.7, 2.9)	5.26 dd (5.6, 3.0)	4.12 dd (5.4, 3.0)	5.41 dd (6.1, 2.4)	5.41 dd (6.1, 2.2)	5.27 dd (6.0, 2.6)	5.26 dd (6.0, 2.6)	5.30 dd (2.6, 1.7)	4.45 t (5.4)
H-6	5.60 dd (8.1, 2.9)	5.54 dd (8.1, 3.0)	5.35 dd (8.0, 3.0)	4.58 dd (9.5, 2.4)	4.55 dd (9.3, 2.2)	4.54 dd (9.6, 2.6)	4.57 dd (9.6, 2.6)	6.11 dd (7.9, 1.7)	4.98 dd (8.7, 5.4)
H-1′	5.73 dd (10.5, 8.1)	5.64 ddd (11.1, 8.1, 0.9)	5.82 dd (11.2, 8.0)	3.92 d (9.5)	3.08 dd (9.3, 0.7)	5.44 dd (9.6, 2.1)	5.39 dd (9.6, 2.3)	5.65 dd (10.7, 7.9)	5.72 dd (10.1, 8.7)
H-2′	5.62 dd (10.5, 10.1)	5.76 dd (11.1, 7.3)	5.66 dd (11.2, 9.2)	3.99 d (6.4)	4.06 dd (5.6, 0.7)	5.46 dd (7.5, 2.1)	5.45 dd (7.6, 2.3)	5.56 dd (10.7, 9.3)	5.40–5.56 m[b]
H-3′	5.35 ddd (10.1, 7.3, 6.6)	4.39 qd (7.3, 0.9)	5.44 ddd (9.2, 6.8, 5.8)	5.09 ddd (6.4)	5.17 ddd (6.4)	5.01 ddd (8.2, 7.5, 4.5)	5.10 ddd (8.5, 7.6, 4.8)	5.61 ddd (9.3, 7.0, 6.5)	5.40–5.56 m[b]
H-4′	1.70 m[b]	1.60 m[b]	1.68 m[b]	1.68 m[b]	1.68 m[b]	1.70 m[b]	1.60–1.75 m[c]	1.63 m[b]	1.68 m[b]
H-5′	1.54 m[b]	1.50 m[b]	1.55 m[b]	1.52 m[b]	1.52 m[b]	1.60 m[b]	1.60–1.75 m[c]	1.58 m[b]	1.57 m[b]
H-6′	1.29 m[b]	1.29 m[b]	1.29 m[b]	1.29 m[b]	1.29 m[b]	1.29 m[b]	1.29 m[b]	1.24 m[b]	1.30 m[b]
H-7′	0.90 t (6.9)	0.90 t (7)	0.90 t (6.9)	0.90 t (6.9)	0.90 t (6.9)	0.89 t (6.9)	0.89 t (7)	0.91 t (6.9)	0.90 t (7)
5-OAc	2.09 s	2.10 s	—	2.10 s	2.12 s	2.10 s	2.10 s	2.12 s	—
1′-OAc	—	—	—	—	—	2.10 s	2.11 s	—	—
2′-OAc	—	—	—	—	—	2.06 s	2.06 s	—	—
3′-OAc	2.05 s	—	2.04 s	2.12 s	—	2.04 s	—	2.00 s	2.04 s
3′-CH_2Cl	—	—	—	—	4.11 s[b]	—	4.05 s[b]	—	—
4-OMe	—	—	—	—	—	—	—	—	3.30 s
Py-2″, -6″	—	—	—	—	—	—	—	8.95 d[c]	—
Py-3″, -5″	—	—	—	—	—	—	—	7.55 m[c]	—
Py-4″	—	—	—	—	—	—	—	7.92 m[b]	—

[a]Measured at 300 MHz in $CDCl_3$ (δ TMS = 0). Assignments confirmed by 2D-COSY.
[b]Signal integration for 2H.
[c]Signal integration for 4H.

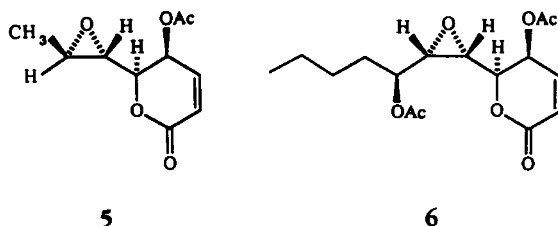
TABLE 2. ^{13}C-Nmr Spectra of Pectinolides A [1], B [2], and C [3] and Derivatives 7, 10, 11, 14, and 15.[a]

Carbon	Compound							
	1[b]	2[b]	3[b]	7	10[b]	11[b]	14	15
C-2	162.12	162.35	163.38	162.39	160.64	160.68	169.52	171.25
C-3	124.99	124.62	122.26	124.89	125.36	125.43	87.01[c]	33.82
C-4	140.01	140.48	144.83	140.83	139.32	139.33	88.18[c]	84.27
C-5	64.24	63.94	63.12	61.36	59.54	59.49	74.22	76.77
C-6	75.04	74.84	77.65	76.90	75.53	75.67	73.86	71.05
C-1′	133.17	122.77	126.29	68.20[c]	66.80	66.55	132.25	131.71
C-2′	126.18	129.24	133.25	69.93[c]	70.72	70.59	126.91	129.98
C-3′	69.42	68.32	70.28	75.61	71.73	73.99	70.07	65.88
C-4′	34.01	36.83	34.09	30.54	30.25	30.13	34.36	34.75
C-5′	27.20	27.43	27.01	27.31	27.03	27.00	27.11	27.16
C-6′	22.40	22.59	22.33	22.48	22.29	22.28	22.46	22.51
C-7′	13.88	13.96	13.79	13.92	13.84	13.90	13.97	13.95
Me-*CO*-	170.25	169.85	170.87	171.90	170.22	170.05	170.25	171.40
	169.78	—	—	170.38	169.98	169.73	170.01	—
	—	—	—	—	169.63	169.32	—	—
	—	—	—	—	169.25	—	—	—
$ClCH_2$*CO*-	—	—	—	—	—	166.88	—	—
Cl*CH_2*CO-	—	—	—	—	—	40.79	—	—
*Me*CO-	21.07	20.52	21.05	21.21	20.85	20.92	21.22	21.34
	20.46	—	—	20.72	20.55	20.62	20.81	—
	—	—	—	—	20.52	20.57	—	—
	—	—	—	—	20.51	—	—	—
*Me*O-	—	—	—	—	—	—	—	57.19
Py-2″, -6″	—	—	—	—	—	—	149.86	—
	—	—	—	—	—	—	149.72	—
Py-3″, -5″	—	—	—	—	—	—	125.64	—
	—	—	—	—	—	—	125.61	—
Py-4″	—	—	—	—	—	—	141.08	—
	—	—	—	—	—	—	140.99	—

[a]Measured at 75.4 MHz in $CDCl_3$ ($\delta_{TMS} = 0$).
[b]Assignments confirmed by HETCOR.
[c]Assignments may be interchanged.

had been shifted significantly upfield ($\delta_{(H\text{-}3')2}$ 4.39 − $\delta_{(H\text{-}3')1}$ 5.35 = −0.96), the H-2′ signal (δ 5.76) was shifted slightly downfield (Δδ = 0.14), and there was a diamagnetic shift observed for C-3′ (Δδ = −1.1). Similarly, the data obtained for pectinolide C [**3**] supported the placement of the OH group at C-5. The signal for H-5 was shifted 1.07 ppm upfield, and the C-5 was shifted 1.12 ppm upfield, relative to the same resonances in compound **1**. Application of the Horeau method (10) to pectinolide B [**2**] confirmed the *S* configuration for the chiral center C-3′ at the side chain of the three new 5,6-dihydro-α-pyrones from *H. pectinata*.

The structural resemblance of pectinolides A [**1**], B [**2**], and C [**3**] with asperlin [**5**] and its congeners, antimicrobial metabolites from *Aspergillus* (11), led us to propose a partial synthesis of analogous compounds. The route selected to introduce an oxirane ring on the side chain of pectinolide A [**1**] by treatment with *m*-CPBA should afford the same sequence of oxidation present in asperlin [**5**] at the α-pyrone nucleus and the C-1′ and C-2′ chiral centers. The expected product **6**, which would arise from epoxidation of the less hindered *re-si* face of the double bond at the side chain, is anticipated to afford, by acid-catalyzed hydrolysis, the threo diol **7** (5). This product, in turn, would show

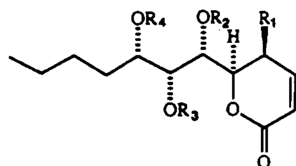


the same absolute stereochemistry described for the chiral centers on the side chain of the related α-pyrone boronolide [**8**] (12).

Attempts to obtain derivative **6** by changing the reaction conditions during treatment with *m*-CPBA were unsuccessful. Complex mixtures of polar compounds were produced on longer reaction times or at higher temperature, from which derivatives **7** and **9** could be isolated as the major products. These compounds clearly derived from the hydrolysis of the epoxide intermediate **6**. On acetylation, they afforded **10** and **11**, respectively. Gc-ms analysis of the oxidation mixture of pectinolide A [**1**] allowed the identification of two additional minor products, **12** and **13**. The eims and cims of these derivatives were similar to those obtained for **7** and suggested that both derivatives were transesterification subproducts of **7**. Acetylation of the reaction mixture composed by the three isomers (gc: Rt 14.9, 15.3, and 17.0 min) afforded a single product identical by gc-ms to derivative **10**.

The ^{1}H-nmr analysis of **7** (Table 1) demonstrated the establishment of hydrogen bonding between the vicinal OH groups at C-1′ and C-2′ by the absence of the ^{1}H-^{1}H spin interactions due to the dihedral angle of 90° subtended by these nuclei in the more stable conformation. Comparison of the coupling constants and nOe's between the protons in the C-1′ to C-4′ segment of derivative **10** and boronolide [**8**] indicated that the relative stereochemistry in the side chain was the same for both compounds. Vicinal coupling constants (^3J) for H-1′, H-2′ ($\phi = 74°$, $J_{calcd} = 2.09$) and H-2′, H-3′ ($\phi = 138°$, $J_{calcd} = 7.5$) were calculated with a multiparamagnetic extension of Karplus equation (13,14) and provided support for the *R* stereochemistry at C-1′ and C-2′ in compound **10**.

The highly stable bis (pyridine) osmium complex **14** was prepared by treatment of **1** with OsO_4 in pyridine solution. It is important to note that exclusive conjugate addition occurred, and its α orientation could be deduced from the strong paramagnetic γ-gauche effect observed for H-6 ($\delta_{(H\text{-}6)\mathbf{14}}$ 6.11 − $\delta_{(H\text{-}6)\mathbf{1}}$ 5.60 = 0.51). Finally, the basic catalyzed methanolysis of **1** afforded derivative **15** as the only major product. The ob-



7 R_1=OAc, R_2=R_3=H, R_4=Ac
8 R_1=H, R_2=R_3=R_4=Ac
9 R_1=OAc, R_2=R_3=H, R_4=$COCH_2Cl$
10 R_1=OAc, R_2=R_3=R_4=Ac
11 R_1=OAc, R_2=R_3=Ac, R_4=$COCH_2Cl$
12 R_1=OAc, R_2=R_4=H, R_3=Ac
13 R_1=OAc, R_2=Ac, R_3=R_4=H

served coupling constant values for the methylene at C-3 ($J_{3\beta\text{-}4}$ = 6.1 Hz and $J_{3\alpha\text{-}4}$ = 3.6 Hz) were taken as evidence for the axial orientation of the MeO substituent at C-4.

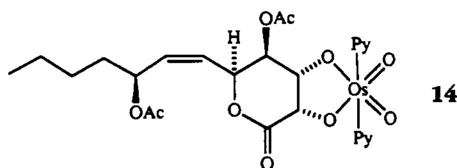
Pectinolide A [**1**] had the highest antimicrobial activity of all the compounds tested in this study. As summarized in Table 3, compound **1** showed significant activity against the Gram-positive bacteria. *Staphylococcus aureus* and *Bacillus subtilis* were sensitive to pectinolide A [**1**] in the concentration range of 6.25–12.5 μg/ml. However, its natural monodeacetylated derivatives, pectinolides B [**2**] and C [**3**], were much less active, with an MIC of 12.5–25 μg/ml against *B. subtilis* and a value of 100 μg/ml against *S. aureus*. On the other hand, hyptolide [**4**], boronolide [**8**], and deacetylepiolguine [**16**] (4), related natural α-pyrones, were inactive at concentrations below 100 μg/ml. Derivatives **7** and **10** showed a reduction in the Gram-positive antibacterial activity with an MIC of 50–100 μg/ml. Compounds **14** and **15** failed to have any antimicrobial activity. These results suggested that the presence of an acetyloxy group at C-5 and the possible hydrophobic properties conferred by the 3-acetyloxy-1-heptenyl residue at C-6 on the 5,6-dihydro-α-pyrone nucleus play roles in the antimicrobial activity.

The cytotoxic potential of the pectinolides and their derivatives was evaluated with a number of cultured cell lines. As indicated in Table 4, the three isolates **1**–**3** from the antimicrobial fraction demonstrated general nonspecific cytotoxic activity. The intensity of the responses displayed by pectinolides A–C were similar to each other and approximately 5- to 10-fold more intense than that demonstrated by derivatives **7** and **10**. Pectinolide A [**1**] was 2- to 5-fold more active than the structurally related 5,6-dihydro-α-pyrones **4** and **16**. It is evident that the cytotoxicity of pectinolides A [**1**], B [**2**], and C [**3**] is due to the α,β-unsaturated δ-lactone nucleus, and it appears that an acetyloxy group at C-5 may increase this biological activity.

TABLE 3. Antibacterial Activity of Pectinolides A [**1**], B [**2**], and C [**3**] and Derivatives **7**, **10**, **11**, **14**, and **15**.

Compound	Zone of inhibition, mm (MIC, μg/ml)				
	Bacillus subtilis	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Candida albicans*
1	10 (6.25)	8 (12.5)	—[a] (200)	— (200)	— (250)
2	6 (25)	4 (100)	— (300)	— (300)	— (300)
3	8 (12.5)	4 (100)	— (>500)	— (>500)	— (>500)
4	5 (100)	— (>100)	— (>500)	— (>500)	— (>500)
7	7 (50)	4 (75)	— (300)	— (300)	— (200)
10	5 (50)	4 (100)	— (300)	— (200)	— (500)
11	7 (50)	5 (75)	— (300)	— (300)	— (200)
14	— (>100)	— (>100)	— (>500)	— (>500)	— (>500)
15	— (>100)	— (>100)	— (>500)	— (>500)	— (>500)
Standard[b]	13 (3.12)	9 (6.25)	NT[c]	NT	7 (NT)

[a]No zone of inhibition observed below 100 μg/ml.
[b]Antibiotic standards: streptomycin sulfate for bacteria and nystatin for *C. albicans*.
[c]Not tested.



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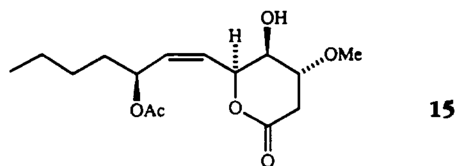

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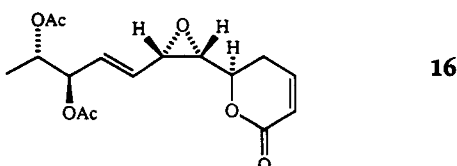

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EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mp's were taken in capillaries on a Büchi 530 apparatus and were uncorrected. Ir and uv were obtained on Perkin-Elmer Model 599B and Model 330 spectrophotometers, respectively. The nmr spectra were recorded on a Varian VXR-300S instrument with TMS as internal standard. Ms was determined on a Hewlett-Packard Model 595B spectrometer; in eims, the ionization voltage was 70 eV and the temperature of the ion source was 260°. Positive chemical ionization was performed using isobutane generating an internal source pressure of 0.2 torr; the ionization voltage was 90 eV, and the temperature of the ion source was 150°. For gc-ms analyses, the chromatograph was equipped with a fused silica capillary column (25 m × 0.32 mm i.d.) coated with methylsilicone (0.52 μm); He carrier at 1.5 ml/min; injection volume 1 μl; oven temperature program 50° for 1 min, subsequently increasing at 10°/min to 280°. Optical rotations were measured with a JASCO DIP-360 digital

TABLE 4. Cytotoxicity Data of Pectinolides A [**1**], B [**2**], and C [**3**] and Related Compounds **4**, **7**, **8**, **10**, **15**, and **16**.[a]

Compound	Cell line[b]										
	BC1	HT	Lu1	Mel2	Col2	KB	KB-V	P-388	A431	LNCaP	ZR75-1
1	1.0	1.7	0.9	0.7	1.0	1.8	1.8	0.9	1.4	0.7	3.6
2	2.5	2.3	3.8	2.2	1.1	1.4	2.0	0.1	0.6	0.9	1.6
3	2.0	1.8	2.3	3.3	1.6	1.7	3.2	2.2	0.8	0.8	1.9
4	2.4	9.6	4.9	12.2	4.7	3.6	4.5	1.6	3.7	7.5	6.4
7	9.9	6.7	8.8	6.0	5.1	5.9	>20	0.5	4.5	4.5	6.2
8	2.8	4.5	3.9	3.3	4.0	4.0	8.6	1.0	6.5	3.2	8.7
10	7.0	8.4	7.6	4.5	4.9	4.4	6.3	0.4	2.6	1.9	7.8
15	>20	>20	>20	>20	>20	>20	>20	>5	>20	>20	>20
16	4.8	5.7	3.3	4.1	3.0	2.9	3.4	0.4	1.9	1.8	4.7

[a]Results are expressed as ED_{50} values (μg/ml).

[b]BC1, human breast cancer; HT (HT-1080), human fibrosarcoma; Lu1, human lung cancer; Mel2, human melanoma; Col2, human colon cancer; KB, human nasopharyngeal carcinoma; KB-V, vinblastine resistant KB; P-388, murine lymphocytic leukemia; A431, human epidermoid carcinoma; LNCaP, hormone dependent human prostate cancer; ZR75-1, hormone dependent human breast cancer.

polarimeter. Cd was obtained on a JASCO-600 CD spectropolarimeter. Analytical and preparative tlc were performed on Si gel 60 F_{254} Merck Plates, and the spots were visualized under uv light or by spraying with 10% solution of $Ce(SO_4)_2$ in 2 N H_2SO_4, followed by heating at 110°. Preparative cc was done on Si gel 60 Merck (70–230 mesh). Semi-preparative hplc was performed on a silica column (10 μm, 10 × 250 mm) using an ISCO system incorporating a Model 2350 pump and a refractive index detector ERC-7522.

PLANT MATERIAL.—The aerial parts of *H. pectinata* were collected in November 1987, Km 9 Xalapa-Puerto de Veracruz, estado de Veracruz, México. A voucher specimen (M-21853) is deposited at the National Herbarium , Instituto de Biología, Universidad Nacional Autónoma de México.

EXTRACTION AND BIOASSAY-GUIDED ISOLATION PROCEDURES.—The dried and finely powdered aerial parts of the plant (3.7 kg) were extracted by maceration with $CHCl_3$ at room temperature. After filtration, the solvent was removed under vacuum to yield 200 g of a dark residue (P-388, ED_{50} 2.2 μg/ml). This crude extract showed strong activity (7–10 mm zone of growth inhibition) against Gram-positive bacteria when tested for antimicrobial activity by the agar hole-plate diffusion assay method (4). The extract was fractionated by chromatography on a Si gel (2.5 kg) column, using a $CHCl_3/Me_2CO$ gradient elution system and collecting 150 fractions of 600–800 ml each. The eluates were combined into five pools (I–V) on the basis of similar tlc profiles. Antimicrobial and cytotoxic activities were found in fractions 15–57 (pool II) which were eluted with $CHCl_3$.

Pool II (23.79 g; P-388, ED_{50} 2.0 μg/ml) was subjected to cc over Si gel (250 g). The elution was accomplished with hexane-$CHCl_3$ (3:1), and a total of 150 fractions (250 ml each) were collected. After antimicrobial assay, the active fractions (74–134; 9–11 mm zone of growth inhibition) were treated with activated charcoal to afford 16 g of a yellowish oil, which was further rechromatographed over Si gel (400 g) eluting with hexane-$CHCl_3$ (3:1). Antibacterial activity was identified with a single compound [R_f 0.52, solvent $CHCl_3$-hexane-Me_2CO (7:2:1)].The fractions containing this spot were bulked and afforded upon evaporation 5.6 g (0.1513% dry wt) of pectinolide A [**1**], which was closely followed in the chromatographic elution by two additional uv-active compounds. The fractions that were abundant in these constituents were combined to make a single pool (523 mg) and subjected to preparative tlc with $CHCl_3$-Me_2CO (9:1). The minor tlc band [R_f 0.55; $CHCl_3$-Me_2CO (4:1)] contained 58 mg (0.0015% dry wt) of pectinolide B [**2**]. The major tlc band [R_f 0.50; $CHCl_3$-Me_2CO (4:1)] afforded 100 mg (0.0027% dry wt) of pectinolide C [**3**].

Pool III (22.6 g) was applied to a column of Si gel (600 g) and developed with a gradient of hexane/$CHCl_3$, collecting 100 fractions of 150 ml each. Fractions 30–45, eluted with hexane-$CHCl_3$ (1:1), afforded 42 mg (0.0011% dry wt) of hyptolide [**4**]. From elution with $CHCl_3$ (fractions 71–86), betulinic acid (5 g; 0.1351% dry wt) was isolated as white crystals (mp 321–332°). Finally, pool IV left a residue that was recrystallized from MeOH to yield 27.3 g (0.7378% dry wt) of ursolic acid (mp 282–284°). Both triterpenoids, as well as their Me ester derivatives, were identical to standard samples (15).

ANTIMICROBIAL SCREENING.—The crude extract, all fractions, and pure compounds were routinely evaluated for qualitative antimicrobial activity using the agar hole-plate diffusion assay (4,6), against the following microorganisms: *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (ATCC 8739), *Pseudomonas aeruginosa* (ATCC 9027), and *Candida albicans* (ATCC 10231). Agar plates (15 ml) containing 1 ml (10^6 bacteria/ml) of an overnight broth culture were prepared. Holes having a diameter of 11 mm were made and filled with 100 μl of the test solution. The crude extract and fractions were assayed as aqueous suspensions in 1% Tween-80 at a concentration of 20 mg/ml. Isolated compounds and pure derivatives were tested at 1 mg/ml. For each organism, the experiment was performed in duplicate, and streptomycin sulfate (1 mlg/ml) and nystatin (3 mg/ml) were used as positive controls. The plates were incubated for 24 h at 37°, except for *C. albicans*, which was incubated at 25°. The diameters of the resultant inhibition zones were measured.

QUANTITATIVE ANTIMICROBIAL ASSAY.—Determination of the MIC was accomplished by the twofold serial dilution technique (4). A solution of the test compound (0.5 ml, 1 mg/ml) dissolved in MeOH-H_2O (1:1) was added to 4.5 ml of nutrient broth. Doubling serial dilutions were aseptically prepared from this broth with concentrations ranging from 100 to 0.2 μg/ml. Each dilution was inoculated with 10 μl of a suspension of *S. aureus* or *B. subtilis* to a final concentration of 10^6/ml. Each organism was tested in duplicate, and control broths containing solvent or streptomycin were also prepared. After overnight incubation at 37°, the MIC was read. The quantitative antimicrobial activity against *E. coli, P. aeruginosa,* and *C. albicans* was accomplished using the agar diffusion technique (16).

CYTOTOXICITY ASSAYS.—The cytotoxic potential of the extract and isolates derived from *H. pectinata* was evaluated essentially by established procedures. In brief, the cultured cell lines BC-1 (human breast cancer), HT-1080 (human fibrosarcoma), Lu-1 (human lung cancer), Mel-2 (human melanoma), Col-2 (human colon cancer), KB (human nasopharyngeal carcinoma), KB-V (vinblastine-resistant KB), A-

431 (human epidermoid carcinoma), LnCaP (hormone dependent human prostate cancer), ZR-75-1 (hormone dependent human breast cancer), and P-388 (murine lymphocytic leukemia) (at log growth-phase) were treated with five concentrations (20.0–0.032 μg/ml) of the test sample in DMSO and incubated for periods of 48 (P-388, HT-1080, Lu-1) or 72 h (all other cell lines) (37°, 5% CO_2, 100% humidity). The quantity of cells in each well was then determined by straining with sulforhodamine B. The results were expressed as ED_{50} values.

Pectinolide A [**1**].—Oil: [α]D +202° (*c* = 0.15, MeOH); uv λ max (MeOH) (log ε) 208 nm (3.96); ir ν max (neat) 2925, 2850, 2750, 1730, 1450, 1420, 1360, 1225, 1060, 1020, 940, 818 cm^{-1}; hreims *m/z* $[M]^+$ 310.1418 (calcd for $C_{16}H_{22}O_6$, 310.1416); eims *m/z* (%) $[M]^+$ 310 (0.2), $[M-HOAc]^+$ 250 (0.1), $[M-2(CH_2CO)]^+$ 226 (0.1), $[250-CH_2CO]^+$ 208 (5), 155 (3), 151 (10), 126 (10), 125 (9), $[155-CH_2CO]^+$ 113 (5), $[126-CH_2CO]^+$ 84 (16.2), 55 (12.2), 43 (100), 41 (8.8); cims *m/z* (%) $[MH]^+$ 311 (100), $[MH-HOAc]^+$ 251 (58); ^1H nmr see Table 1; ^{13}C nmr see Table 2; cd (*c* = 0.13, MeOH) Δε (nm) 0 (310), +2.45 (265), +1.20 (246), 0 (239), +24.7 (214).

Pectinolide B [**2**].—Oil: [α]D +89.6° (*c* = 0.57, MeOH); uv λ max (MeOH) (log ε) 204 nm (4.23); ir ν max ($CHCl_3$) 4375, 3038, 3018, 2929, 1735, 1455, 1375, 1249, 1066, 976, 815; hreims *m/z* $[M]^+$ 268.1311 (calcd for $C_{14}H_{20}O_5$, 268.1310); eims *m/z* (%) $[M]^+$ 268 (0.3), 250 (0.5), 208 (2.87), 167 (8.19), 155 (4.70), 151 (30.95), 149 (22.20), 126 (11.05), 125 (100), 113 (6.5), 97 (10.67), 96 (5.6), 95 (10.92), 85 (10.5), 84 (51.62), 83 (44.96), 57 (46.63), 55 (50); cims *m/z* (%) $[MH]^+$ 269 (100), $[MH-H_2O]^+$ 271 (47), $[MH-HOAc]^+$ 209 (68); ^1H nmr see Table 1; ^{13}C nmr see Table 2; cd (*c* = 0.02, MeOH) Δε (nm) 0 (310), +0.34 (285), +1.27 (267), +0.48 (247).

Pectinolide C [**3**].—Oil: [α]D +80.99° (*c* = 0.76, MeOH); uv λ max (MeOH) (log ε) 204 nm (3.96); ir ν max ($CHCl_3$) 3475, 3088, 3018, 2929, 1735, 1435, 1423, 1374, 1249, 1066, 1030, 976, 876 cm^{-1}; hreims *m/z* $[M]^+$ 268.1312 (calcd for $C_{14}H_{20}O_5$, 268.1310); eims *m/z* (%) $[M]^+$ 268 (0.3), 250 (0.32), 209 (10.63), 208 (3.85), 191 (3.14), 155 (5.80), 151 (18.75), 126 (10.14), 125 (10), 113 (7.85), 97 (8.58), 96 (4.67), 95 (9.87), 85 (14.14), 84 (95.48), 83 (42.89), 81 (19.32), 57 (27.21), 56 (13.97), 55 (49.41) 43 (100); cims *m/z* (%) $[MH]^+$ 269 (100), $[MH-H_2O]^+$ 271 (67), $[MH-HOAc]^+$ 209 (59); ^1H nmr see Table 1; ^{13}C nmr see Table 2; cd (*c* = 0.04, MeOH) Δε (nm) + 0.38 (285), +1.24 (264), −1.16 (243), +16.32 (213).

Hyptolide [**4**].—White needles: mp 86–88°; co-tlc with authentic sample; ir and nmr spectra identical to those reported (3).

OZONOLYSIS OF PECTINOLIDE A [**1**].—An excess of ozone was bubbled for 2 h through a solution of compound **1** (200 mg) in dry $CHCl_3$ (20 ml) at 0°. While the solution was still cold, 10 ml of H_2O_2 (30%) were added. The mixture was stirred at room temperature overnight. The layers were separated, and the H_2O layer was washed with Et_2O. The organic portions were combined, and the solvent was removed at reduced pressure. The major ozonolysis product was purified by isocratic normal phase hplc using 1% MeOH in $CHCl_3$ as mobile phase delivered at a flow rate of 5 ml/min to give 35.6 mg of 2-acetyloxy-hexanoic acid: ir ν max ($CHCl_3$) cm^{-1} 3010, 2990, 2910, 1730, 1710, 1440, 1240, 1100; ^1H nmr ($CDCl_3$) δ 0.90 (3H, t, *J* = 7 Hz), 1.38 (4H, m), 1.85 (2H, m), 2.09 (3H, s), 4.95 (1H, t, *J* = 6.0 Hz), 9.45 (1H, s, OH); ^{13}C nmr ($CDCl_3$) δ 13.90 (C-6), 20.70 (Ac-), 22.35 (C-5), 27.30 (C-4), 30.76 (C-3), 72.18 (C-2), 176.00 (C-1); eims *m/z* (%) $[M]^+$ 174 (0.5), $[M-CO_2H]^+$ 129 (76), $[M-HOAc]^+$ 114 (18), 69 (30), 57 (10), 55 (90), 43 (100); cims *m/z* (%) $[MH]^+$ 175 (45), $[MH-H_2O]^+$ 157 (19), $[MH-HOAc]^+$ 115 (100).

HYDROLYSIS OF 2-ACETYLOXY-HEXANOIC ACID.—The ozonolysis product (35 mg) was dissolved in 3 ml of 2% NaOH and stirred for 24 h at room temperature. The reaction mixture was washed with Et_2O, and HCl was added to the aqueous layer until it reached pH 4. The resultant precipitate was filtered and recrystallized from Me_2CO to yield 19.8 mg of crystalline 2-hydroxyhexanoic acid: mp 60°–62°; [α]D + 14.8° (*c* = 1.85, MeOH); cd (*c* = 0.6, MeOH) Δε (nm) +0.12 (190), +1.58 (209), +0.19 (230), 0 (238), −0.01 (244), identical (gc-ms, ir, nmr) to a commercial sample.

APPLICATION OF HOREAU METHOD TO PECTINOLIDE B [**2**].—A solution of 15 mg of **2** and 83 mg of racemic 2-phenylbutyric anhydride in 0.5 ml of pyridine was allowed to stand over the weekend at room temperature. H_2O (1 ml) was added, and the mixture was left to stand for 30 min. The hydrolysis mixture was extracted three times with Et_2O. The organic phase was back-extracted twice with 10% Na_2CO_3 solution. The aqueous alkaline solution was washed with $CHCl_3$, acidified with 1 N HCl, and extracted once again with C_6H_6. This extract was dried, evaporated, and weighed before measuring the optical rotation. Tlc using C_6H_6-dioxane-HOAc (50:50:2) on Si gel showed this material to be (−)-*R*-2-phenylbutanoic acid (ir, nmr), [α]D −2.57° (*c* = 2.9, C_6H_6).

OXIDATION OF PECTINOLIDE A [**1**] WITH *m*-CPBA.—Compound **1** (170 mg) was dissolved in $CHCl_3$ (25 ml) and treated with *m*-CPBA (135 mg) at room temperature. After shaking for 2 days, 0.3 ml

of H_2O_2 (30%) was added and the mixture was stirred for 6 h. Usual workup of the reaction gave an oily residue (168 mg), which was separated by cc on Si gel. Elution with $CHCl_3$-Me_2CO (4:1) yielded 14.6 mg of product **9** (R_f 0.56), which was followed in the chromatographic elution by 141.9 mg of compound **7** (R_f 0.47). Fractions eluted with $CHCl_3$-Me_2CO (3:2) afforded 7.5 mg of a mixture of derivatives **7**, **12**, and **13**. An aliquot (2 mg) of this mixture was converted into its TMSi derivative by treatment with Sigma-Sil A (0.1 ml) and analyzed by gc-eims to give three peaks, R_t 17.60, 19.14 (**7**), and 19.73 min. The remainder of this elution (5.5 mg) was dissolved in Ac_2O (0.1 ml) and pyridine (0.1 ml) and heated at 60° for 1 h. The reaction mixture was concentrated to dryness and directly analyzed by gc-cims to detect one peak, R_t 13.87 min.

Compound **7**.—Oil: ir ν max ($CHCl_3$) 3540, 1736, 1374, 1249, 1066 cm^{-1}; eims *m/z* (%) 345 (1.19), 327 (5.82), 285 (2.24), 327 (5.82), 216 (16.36), 189 (14.93), 185 (11.26), 159 (65.36), 156 (13.25), 155 (54.87), 143 (12.47), 139 (17.92), 138 (18.05), 129 (13.94), 126 (57.61), 125 (25.23), 115 (17.92), 110 (14.62), 109 (14.59), 108 (11.5), 97 (100), 83 (34), 77 (50), 55 (45), 43 (94); eims TMSi-derivative, *m/z* (%) 359 (3), 333 (2), 257 (6), 231 (100), 155 (3), 129 (13); ^{1}H nmr see Table 1; ^{13}C nmr see Table 2.

Compound **9**.—Oil: ir ν max ($CHCl_3$) 3540, 1730, 1374, 1250, 815 cm^{-1}; eims *m/z* (%) $[M+2-H_2O]^+$ 382 (1.6), $[M+1-H_2O]^+$ 381 (2.3), $[M-H_2O]^+$ 380 (4.9), $[M-Cl]^+$ 343 (5.2), $[M+2-HOAc]^+$ 3409 (3.2), $[M-HOAc]^+$ 338 (9.8), 269 (17.4), 185 (3), 171 (53), 139 (25), 129 (7), 126 (11), 125 (7), 115 (7), 111 (42), 97 (61), 83 (23), 55 (27), 43 (100); ^{1}H nmr see Table 1.

Compound **12**.—Gc-eims TMSi-derivative (R_t 19.73 min): *m/z* (%) 333 (2), 257 (3), 231 (6), 159 (15), 155 (3).

Compound **13**.—Gc-eims TMSi-derivative (R_t 17.60 min): *m/z* (%) 401 (13), 333 (1), 261 (7), 227 (6), 159 (76), 155 (2).

ACETYLATION OF DERIVATIVE **7**.—Compound **7** (80 mg) was dissolved in Ac_2O/pyridine (1:4) (5 ml) and maintained at room temperature for 24 h. The reaction mixture was diluted with H_2O, extracted with CH_2Cl_2, washed with saturated aqueous $KHSO_4$ and dried with anhydrous Na_2SO_4. Cc on Si gel [hexane-EtOAc, (4:1)] yielded 48.8 mg of product **10**: white solid; mp 75–76°; ir ν max ($CHCl_3$) 1730, 1365, 1210, 1080, 1020 cm^{-1}; gc R_t 13.8 min; eims *m/z* (%) 300 (31), 240 (37), 201 (31), 198 (13), 197 (12), 180 (15), 168 (23), 159 (24), 157 (42), 156 (11), 155 (11), 138 (34), 137 (46), 125 (90), 115 (37), 102 (11), 97 (28), 43 (100); cims *m/z* (%) $[MH]^+$ 429 (43), $[MH-HOAc]^+$ 369 (100), 327 (13), 309 (6); ^{1}H nmr see Table 1; ^{13}C nmr see Table 2.

ACETYLATION OF DERIVATIVE **9**.—Compound **9** (10 mg) was dissolved in Ac_2O/pyridine (1:4) (2 ml) under the same working conditions as for product **7**. Tlc [hexane-EtOAc (3:1)] yielded 6.8 mg of product **11**: oil; ir ν max ($CHCl_3$) 1730, 1455, 1230, 1110, 1060 cm^{-1}; cims *m/z* (%) $[MH+2]^+$ 465 (3.99), $[MH+1]^+$ 464 (2.41), $[MH]^+$ 463 (11.15), $[MH+2-HOAc]^+$ 405 (2.05), $[MH-HOAc]^+$ 403 (5.87), 369 (100), 327 (58.30), 309 (12.45), 285 (1.6), 267 (19.3), 207 (14.2); ^{1}H nmr see Table 1; ^{13}C nmr see Table 2.

OXIDATION OF PECTINOLIDE A [**1**] WITH OsO_4.—To 60 mg of compound **1** in 1 ml of anhydrous pyridine was added 50 mg of OsO_4 in 1 ml of pyridine. The mixture was stirred at 25° for 3 days, and the solvent was removed at reduced pressure. The crude product was purified by tlc to afford 53.4 mg of **14**: amorphous brown solid; mp 56–58°; ir ν max (KBr) 2940, 2920, 1735, 1605, 1445, 1365, 1220, 1060, 1010, 945, 830, 750, 680, 600 cm^{-1}; ^{1}H nmr see Table 1; ^{13}C nmr see Table 2.

HYDROLYSIS OF PECTINOLIDE A [**1**].—Compound **1** (50 mg) was dissolved in MeOH/H_2O (70%) (10 ml) containing 0.1 M KOH (3 ml) and stirred for 3 h. Usual workup of the reaction gave an oily residue which was separated by tlc to afford 15.8 mg of **15** [R_f 0.45; $CHCl_3$-Me_2CO (4:1)]: oil; ir ν max ($CHCl_3$) 3470, 1735, 1430, 1030, 970 cm^{-1}; cims *m/z* (%) $[MH]^+$ 343 (100), $[MH-H_2O]^+$ 321 (30), $[MH-MeOH]^+$ 311 (26), $[MH-HOAc]^+$ 283 (73); ^{1}H nmr see Table 1; ^{13}C nmr see Table 2.

ACKNOWLEDGMENTS

We gratefully acknowledge SYNTEX S.A. de C.V. and Consejo Nacional de Ciencia y Tecnología for sponsoring the MS program of L. Hernández and M.J. Villavicencio through "Programa de Fortalecimiento del Posgrado Nacional y Participación del Sector Productivo." This research was also partially supported by Projects PADEP No. FQ-9127 and FQ-9204 from the Coordinación General de Estudios de Posgrado (UNAM) and by a grant from CONACyT through "Programa de Fortalecimiento del Posgrado Nacional" (Convenio No. PFPN/112/91). We thank Dr. A. Kjaer (The Technical University of Denmark) for an authentic sample of hyptolide and Dr. D.E.A. Rivett (Rhodes University, South Africa) for a sample of boronolide. The authors are also indebted to the staff of the nmr and ms laboratories from Instituto de

Química (UNAM), especially to Federico del Río, for technical assistance. Finally, we express our grateful recognition to Dr. Andrés Hernández (Universidad Autónoma Metropolitana, México) for the cd analysis.

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Received 31 August 1992