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MAJORENOLIDE AND MAJORYNOLIDE: A NEW PAIR OF CYTOTOXIC AND PESTICIDAL ALKENE-ALKYNE δ-LACTONES FROM *PERSEA MAJOR*

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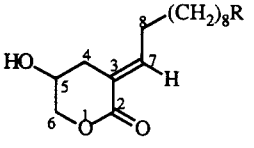
ABSTRACT.—From the EtOH extract of the bark of *Persea major*, two bioactive compounds, majorynolide [**1**] and majorenolide [**2**], were isolated by activity-directed fractionation using brine shrimp. Their structures have been elucidated on the basis of spectral data as a pair of new alkene-alkyne δ-lactones, each with an exocyclic alkylidene methine carbon. Both **1** and **2** are moderately cytotoxic, and **2** is selectively pesticidal.

In the course of screening plant extracts for antitumor and pesticidal activities, the CH_2Cl_2 (F003) and the aqueous MeOH (F005) partition fractions from an EtOH extract (F001) of the powdered bark of *Persea major* Mill. (Lauraceae), a tree from east-central Brazil, were found to exhibit potent bioactivities (Tables 1 and 2). By chromatographic separation of F003, two compounds, majorynolide [**1**] and majorenolide [**2**], were isolated while monitoring the fractionation with the brine shrimp lethality test (BST). Both compounds showed cytotoxic activities in human tumor cells, and **2** was insecticidal to certain insects (Tables 1 and 2).

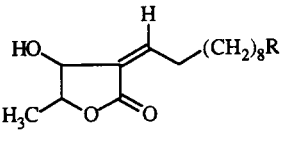
In this paper we describe the identification of **1** and **2** as a new δ-lactonic alkene-alkyne pair each with an exocyclic α-alkylidene methine carbon and a γ-hydroxyl. Terminal alkene-alkyne pairs of lactonic compounds have been previously found in plants of the Lauraceae (1–5). However, these compounds, such as the litsenolides **3** and **4** from *Litsea japonica* (Thunb.) Juss. (1), are γ-lactones with a terminal alkene or alkyne side chain. Compounds **1** and **2** appear to represent a new class of bioactive heterocycles, with the methine carbon as a potential pharmacophore for alkylations.

RESULTS AND DISCUSSION

Majorynolide [**1**] is a colorless crystalline compound with low mp (39°). Its hrms showed a molecular ion peak $[M]^+$ at *m/z* 278.1881 in agreement with the molecular formula $C_{17}H_{26}O_3$ (calcd 278.1881). The ^{13}C-nmr spectra of **1** revealed the presence of a terminal triple bond at δ 84.68 ($^2J = 48$) and δ 68 ($^1J = 252$). This functionality was confirmed by the ^{1}H-nmr spectra which showed an acetylene proton at δ 1.94 (1H, t, $J = 2.0$), and the ir spectrum which indicated a sharp peak at 3279 cm^{-1}. The ^{13}C-nmr signals at δ 77.27 (d, $J = 153.6$), 64.51 (t, $J = 144.4$) and 26.73 (t, $J = 135$) and ^{1}H-nmr signals at δ 4.66 (1H, dddd, $J = 8.4, 6.3, 5.3, 3.1$), 3.90 (1H, dd, $J = 12.2, 3.1$), 3.65 (1H, dd, $J = 12.2, 5.3$), 2.88 (1H, ddd, $J = 16.9, 8.4, 2.9$), and 2.68 (1H, ddd, $J = 16.9, 6.3, 2.9$), indicated the presence of an O-CH_2-CHOH-CH_2 group. This was substantiated by ^{1}H-nmr decoupling experiments in which the peaks at δ



1 R=CH CH
2 R=CH=CH_2



3 R=C≡CH
4 R=CH=CH_2

TABLE 1. Brine Shrimp, Potato Disc, and Cytotoxicity Bioassays of Fractions and Compounds from *Persea major*.

Fraction or Compound	BST[a] LC_{50} (ppm)	PD[b] % tumor inhibition	A-549[c] ED_{50} (μg/ml)	MCF-7[d] ED_{50} (μg/ml)	HT-29[e] ED_{50} (μg/ml)
F003	2.60	37	3.71	2.79	3.90
1	0.89	46	4.64	9.56	13.82
2	0.36	86	1.70	2.58	2.61

[a]Brine shrimp lethality test.
[b]% Inhibition of crown gall tumors on discs of potato tubers.
[c]Human lung carcinoma.
[d]Human breast carcinoma.
[e]Human colon adenocarcinoma.

3.90 and 3.65 became doublets and the ddd at δ 2.88 and 2.68 became dd when irradiating the peak at δ 4.66. The ^{13}C-nmr signal at δ 170.73 and the ir peak at 1745 cm^{-1} indicated an α,β-unsaturated lactone. The ^{13}C nmr exhibited double bond carbons at δ 141.48 (d, J = 159.2) and 125.69 (s), and the ^{1}H nmr showed an olefinic proton at δ 6.75 (1H, tt, J = 7.6, 2.9). The splitting pattern of the olefinic proton ruled out the possibility that the double bond was endocyclic. Therefore, the partial structure of **1** included an exocyclic α,β-unsaturated 4-OH-δ-lactone. This was further confirmed by COSY and HETCOR experiments.

TABLE 2. Pesticidal Activities[a] of Fractions and Compounds from *Persea major*.

Fraction or Compound	% Mortalities/ppm in pests[b]		
	ML	2SSM	MA
F003	100/10	0/5000	0/5000
F005	80/1	90/5000	100/5000
1	0/10	0/400	0/400
2	20/10	0/400	80/10

[a]No pesticidal activity was seen with blowfly larvae (1%), southern corn rootworm (300 ppm), southern army worm (5000 ppm), and *Haemonchus contortus* (a nematode) (0.1%)
[b]ML = mosquito larvae, 2SSM = Two spotted spider mite, MA = melon aphid.

Because there was no Me signal evident in either the ^{1}H- or ^{13}C-nmr spectra, the side chain of **1** was assumed to be unbranched. The geometry of the exocyclic double bond of **1** was clarified by an nOe experiment (Table 3), which indicated that the nOe effect could only be observed between H-8 and H-4. In other words, the vinyl proton is cis to the carbonyl group.

The hrms of **2** showed a molecular ion peak $[M]^+$ at m/z 280.2041, which indicated the molecular formula $C_{17}H_{28}O_3$ (calcd 280.2038). The ^{13}C-nmr showed another double bond at δ 139.18 and 144.55. ^{1}H nmr (C_6D_6) indicated additional olefinic protons at δ 5.81 (1H, ddt, J = 17.1, 10.3, 6.7), 5.6 (1H, ddt, J = 17.1, 2.1, 1.7), and 5.01 (1H, ddt, J = 10.3, 2.1, 1.2). The spectral signals for an acetylene group, as seen in **1**, were absent in the spectra of **2**. All other signals were very close to those of **1**, but in the ^{1}H nmr the H-16 signal had moved to δ 2.01 and in the ^{13}C nmr the C-16 signal had

TABLE 3. NOe Results of Compound **1** in C_6D_6.

Protons Saturated	% NOe of Observed Protons				
	4′	4	5	7	8
4′	—	34.4	14.3	—	11.7
4	18.4	—	—	—	3
7	—	—	—	—	—
8	13.6	4.4	—	10.5	—

moved to δ 34.19. Therefore, **2** must contain a terminal double bond with other parts of the structure the same as in **1**. This structure of **2** was further confirmed by its COSY experiment. The following correlations were detected: the olefinic proton at H-7 correlated with the methylene protons H-4 and H-4′, which also connected with the methine proton at H-5; the olefinic protons at H-17 and at H-18 connected with each other and also connected with the methylene protons at H-16.

Hydrogenation of **1** over Lindlar's catalyst (6) gave a major derivative that was identical with **2** on tlc and glc. Its eims matched with **2** at *m/z* $[M]^+$ 280, and ^1H nmr matched with 2, showing the olefinic protons at δ 5.81, 5.60, and 5.01 with the same splitting pattern as seen in **2**. It is known that the cis vinyl proton of the cisoid enone system usually appears 0.3–0.9 ppm downfield from that of the corresponding trans proton (7). Because the olefinic proton H-7 of **2** also appeared at δ 6.75, the geometry of the exocyclic double bond proton is cis to the carbonyl group. Thus, the structure of **2** is identified as illustrated.

Both majorynolide [**1**] and majorenolide [**2**] were active in bioassays showing brine shrimp lethality (8), inhibition of crown gall tumors on potato discs (9), and cytotoxicities to human tumor cells (Table 1). However, neither **1** nor **2** fully explains the spectrum of biological activities of the crude extracts (Tables 1 and 2), and the data suggest that additional bioactive compounds may be present. The activities of **2** are at least two or more times those of **1**; thus, the ethylene group enhances the biological effects more than the acetylene group.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Optical rotations were measured in a 1-dm cell on a Perkin-Elmer 241 polarimeter. Nmr spectra were determined in $CDCl_3$ or C_6D_6 on a Chemagnetic A-200 spectrometer. COSY and HETCOR nmr spectra were determined on a Varian XL-200A or a Varian VXR-500S spectrometer. ^1H-nmr decoupled spectra were obtained on a Varian XL-200 spectrometer. NOe's were measured on a Varian VXR-500S spectrometer. Low resolution cims and eims were recorded on the Finnigan 4000. Mass measurements (hrms) were determined on the Kratos MS-50. Ir spectra were determined on a Perkin-Elmer 1600 series FTIR in KBr or CCl_4. Uv spectra were obtained on a Beckman DU-7 spectrometer in MeOH. Mp's were determined in capillaries on a Mel-temp apparatus and were uncorrected.

PLANT MATERIAL, EXTRACTION, AND ISOLATION.—The bark of *P. major* was collected in Brazil under the auspices of Dr. Robert E. Perdue, Medicinal Plant Laboratory, USDA, Beltsville, Maryland, where voucher specimens (B-824500) are maintained. The dried powder (700 g) was extracted with 95% EtOH. The residue of the EtOH extract (F001, 80 g) was partitioned between H_2O and CH_2Cl_2, and the latter yielded, upon removal of solvent, 10.2 g of residue (F003) which was bioactive (Tables 1 and 2). The residue of F003 was subjected to Si gel cc using hexane, CH_2Cl_2, and MeOH gradient mixtures of increasing polarity. The fractions that showed activities to brine shrimp were combined (3.9 g) and further separated over Si gel on a Chromatotron with hexane-Me_2CO (3:1). Majorynolide [**1**] (52 mg) was obtained. After repeating the Chromatotron several times, majorenolide [**2**] (38 mg) was obtained.

MAJORYNOLIDE [**1**].—Colorless needle-shaped crystals from hexane/CH_2Cl_2: mp 39°; $[\alpha]_D$ −17° (c = 0.1, CH_2Cl_2); uv λ max (MeOH) 229 nm (log ε 3.92); ir ν max (KBr) 3378 (OH), 3279 (C≡CH),

1746 (α,β-unsaturated δ-lactone), 1687 (C=C), 1464, 1218, 1057 cm^{-1}; ^1H nmr (200 MHz, $CDCl_3$) δ 6.75 (1H, tt, J = 7.6, 2.9, H-7), 4.66 (1H, dddd, J = 8.4, 6.3, 5.3, 3.1, H-5), 3.90 (1H, dd, J = 12.2, 3.1, H-6), 3.65 (1H, dd, J = 12.2, 5.3, H-6′), 2.88 (1H, ddd, J = 16.9, 8.4, 2.9, H-4), 2.68 (1H, ddd, J = 16.9, 6.3, 2.9, H-4′), 2.19 (4H, m, H-8 and H-16) [which was split into two peaks at 1.99 (2H, dt, J = 5.0, 2.0, H-16) and 1.80 (2H, tddd, J = 7.6, 6.7, 1.6, 1.2, H-8) on the 500 MHz spectrometer in C_6D_6], 1.94 (1H, t, J = 2.0, H-18), 1.30–1.52 (14H, m, H-9 to H-15); ^{13}C nmr (50 MHz, $CDCl_3$) δ 170.73 (s, C-2), 141.48 (d, ^1J = 159.2, C-7), 125.69 (s, C-3), 86.78 (d, ^2J = 48, C-17), 77.27 (d, ^1J = 153.6, C-5), 68.03 (d, ^1J = 252.2, C-18), 64.51 (d, ^1J = 144.4, C-6), 30.22 (t, ^1J = 135, C-8), 29.25, 28.98, 28.63, 28.39, 28.01, (t, C -9, C-10, C-11, C-12, C-13, C-14, C-15; these assignments are not precise due to the proximity of signals), 26.73 (t, C-4), 18.35 (t, C-16); eims (70 eV) m/z $[M+1]^+$ 279 (10), 278 (5.34), 261 (0.14), 247 (0.72), 229 (0.19), 211 (0.25), 201 (1.34), 179 (1.90), 161 (2.09), 143 (1.27), 137 (5.63), 123 (7.78), 109 (14.45), 95 (44.92), 81 (76.66), 67 (100); cims (isobutane) $[M+1]^+$ m/z 279; fabms (glycerol) $[M+1]^+$ m/z 279; hreims mass measurement found m/z 278.1881, calcd 278.1881 for $C_{17}H_{26}O_3$.

MAJORENOLIDE [**2**].—Colorless oil: $[\alpha]_D$ −13° (c = 0.1, CH_2Cl_2); uv λ max (MeOH) 220 nm (log ε 3.96); ir ν max (CCl_4) 3406, 2925, 2848, 1755, 1675, 1556, 1457, 1436, 1212 cm^{-1}; ^1H nmr (500 MHz, $CDCl_3$) δ 6.75 (1H, tt, J = 7.6, 2.9, H-7), 5.79 (1H, ddt, J = 17.1, 10.3, 6.7, H-17), 4.97 (1H, ddt, J = 17.1, 2.1, 1.7, H-18), 4.91 (1H, ddt, J = 10.3, 2.1, 1.2, H-18), 4.64 (1H, dddd, J = 8.4, 6.3, 5.3, 3.1, H-5), 3.86 (1H, dd, J = 12.2, 3.1, H-6), 3.63 (1h, dd, J = 12.2, 5.3, H-6′), 2.86 (1H, dddt, J = 16.9, 8.4, 2.9, 1.2, H-4), 2.67 (1H, dddt, J = 16.9, 6.3, 2.9, 1.6, H-4′), 2.15 (2H, tddd, J = 7.6, 6.7, 1.6, 1.2, H-8), 2.01 (2H, tddd, J = 7.5, 6.7, 1.7, 1.2, H-16), 1.45–1.25 (14H, m, H-9–H-15); ^{13}C nmr (50 MHz, C_6D_6) δ 170.66 (s, C-2), 139.81 (d, C-17), 139.18 (d, C-7), 127.12 (s, C-3), 114.55 (t, C-17), 77.38 (d, C-5), 64.23 (d, C-6), 34.19 (t, C-16), 30.21 (t, C-8), 30.13, 29.84, 29.62, 29.48, 29.31, 28.35 (t, C-9–C-15), 26.62 (t, C-4); eims $[M+1]^+$ m/z 281 (10), 280 (5.20), 263 (3.22), 249 (2.70), 231 (1.96), 213 (2.48), 203 (5.71), 155 (11.60), 137 (1.96), 123 (15.80), 109 (34.86), 95 (70.52), 81 (86.89), 67 (100); cims (isobutane) $[M+1]^+$ m/z 281; hreims mass measurement found 280.2041, calcd 280.2038 for $C_{17}H_{28}O_3$.

PARTIAL HYDROGENATION OF **1**.—Majorynolide [**1**] (5 mg) was dissolved in a hexane and CH_2Cl_2 mixture (freshly distilled over NaH), and $Pd/CaCO_3$ (0.5 mg) and quinoline (0.1 μl freshly distilled over zinc metal) were added. Hydrogenation proceeded at room temperature until about 0.2 ml of hydrogen was absorbed.

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