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BIOLOGICALLY ACTIVE γ-LACTONES AND METHYLKETOALKENES FROM *LINDERA BENZOIN*

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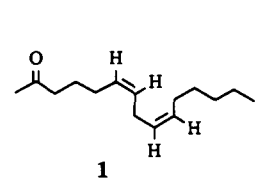
ABSTRACT.—Brine shrimp lethality-directed fractionation of the 95% EtOH extract of ripe berries from *Lindera benzoin* led to the isolation of three new C_{21} alkane-alkene γ-lactones designated isolinderanolide [**4**], isolinderenolide [**5**], and linderanolide [**10**] as well as the known series of C_{17} and C_{19} obtusilactones (isoobtusilactone A [**6**], obtusilactone A [**7**], isoobtusilactone [**8**], and obtusilactone [**9**]) previously isolated from *Lindera obtusiloba*. The novel (6*Z*,9*Z*,12*Z*)-pentadecatrien-2-one [**2**], the known (6*Z*,9*Z*)-pentadecadien-2-one [**1**], and the known (+)-(*Z*)-nerolidol [**3**] were also isolated as bioactive compounds. The structural elucidation and biological activities of these compounds are reported.

As a part of our continuing interest in the isolation of antitumor and pesticidal compounds from higher plants, the Indiana plant *Lindera benzoin* (L.) Blume (Lauraceae), spicebush, was investigated using the in-house brine shrimp (*Artemia salina*) lethality bioassay as a guide to fractionation (1). Parts of this plant have been used as a medicinal tea, a substitute for allspice (2,3) and as an insect repellent. Fresh leaves, when crushed and rubbed on the skin, repel mosquitoes; additionally, the leaves appear to be resistant to insect infestation (JLM, personal observations in the field). An Et_2O extract of the twigs displayed potent antifeedant activity against the fall armyworm, *Spodoptera frugiperda* (4). The single previous phytochemical study of *Lin. benzoin* reported the isolation of an alkaloid, laurotetanine, from the pulverized stems (5); however, a series of cytotoxic obtusilactones were previously isolated from the related species, *Lindera obtusiloba* from Japan (6–9). Due to the absence of previous studies focusing on bioactive compounds from *Lin. benzoin*, screening and fractionation were initiated. Bioactivity-directed fractionation of the berries led to the isolation of ten bioactive compounds, four of which are new to the literature; these compounds are potentially involved in the chemical ecology of this plant.

RESULTS AND DISCUSSION

Initial screening of the 95% EtOH extract and subsequent solvent partitions (CH_2Cl_2/H_2O followed by 90% MeOH/hexane partition of the CH_2Cl_2 residue) of leaves and twigs as well as fresh ripe berries of *Lin. benzoin* indicated that the 90% MeOH partition residue of the berries was the most lethal to brine shrimp (see Experimental); hence, the extract of the berries was subjected to further bioactivity-directed fractionation. Chromatographic separations over Si gel of the 90% MeOH residue (LC_{50} 17.7 ppm), using a vacuum column and a Michel-Miller column with continued purification of the most toxic fractions using a Chromatotron, led to the isolation of the brine-shrimp-toxic compounds **1–9**. Fractionation of the hexane partition residue provided compound **10**. The activities of all fractions were monitored with the brine shrimp assay at each step. Upon isolation, the active compounds were further tested for activity in the potato disc assay (10); an in vitro panel of the human solid tumor cell lines A-549 lung carcinoma (11), MCF-7 breast carcinoma (12), and HT-29 colon adenocarcinoma (13); and a variety of insects (Table 1). Compounds **1–3** showed comparable toxicity to brine shrimp; however, while compound **2** showed marginal cytotoxicity to the human tumor cell lines MCF-7 and HT-29, compounds **1** and **3** were inactive. Compounds **4–10** showed significant brine shrimp lethality, with com-



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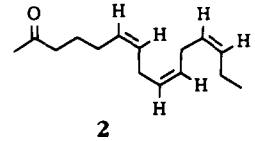

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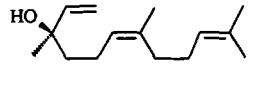

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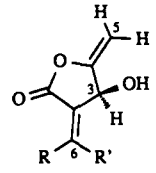
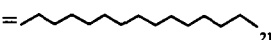
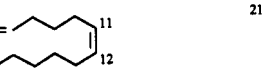
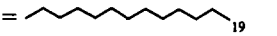
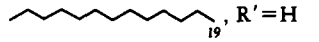


4 R=H, R'= 

5 R=H, R'= 

6 R=H, R'= 

7 R=  , R'=H

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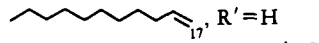
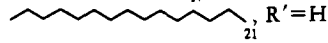
9 R=  , R'=H

10 R=  , R'=H

pounds **8** (isoobtusilactone) and **9** (obtusilactone) giving LC_{50} values on a par with the antitumor standard adriamycin (Table 1). Compounds **4**, **5**, and **9** (considered representative of the obtusilactone series) were tested in the potato disc assay and showed significant inhibition of crown gall tumors. The potato disc assay was previously shown to have significant correlation to in vivo 3-PS antitumor activity (14,15). In spite of the potent activities in the brine shrimp and potato disc assays, compounds **4–10** displayed only marginal cytotoxicity to the human tumor cell lines in culture. Insecticidal screening of compounds **4–9** showed that **4** caused a 20% mortality at 12 ppm to the southern corn rootworm; however, the other compounds were inactive at the doses tested (compounds **1–3** were not tested).

The structures of compounds **1–10** were established by spectrometric methods including ^{1}H nmr (COSY), ^{13}C nmr (APT), ms, and Ft-ir. Compounds **2**, **4**, **5**, and **10** are new to the literature. Compound **3** was previously isolated from *Lindera umbellata* (16), **6–9** were previously isolated from *Lin. obtusiloba* (6,7), and **1** was previously identified by gc-ms (but without a rigorous structural proof) from *Neolitsea sericea* (Lauraceae) and *Humulus lupulus* (Moraceae) (17,18).

Compounds **1** and **2** were determined to be (6*Z*,9*Z*)-pentadecadien-2-one and (6*Z*,9*Z*,12*Z*)-pentadecatrien-2-one, respectively. Initial analysis of spectral data indicted that **1** and **2** were a pair of closely related low mol wt compounds differing by only 2 mass units. Cims gave an $[MH]^+$ peak at *m/z* 223 for compound **1** and *m/z* 221 for compound **2**. Both compounds showed a loss of 18 mu in cims, indicating the presence of oxygen. The nature of the similarities between **1** and **2** was most apparent from their ^{1}H-nmr spectra.

The 500 MHz ^{1}H-nmr spectrum of compound **1** showed the presence of two methyl groups; one as a triplet integrating for three protons at δ 0.87 characteristic of splitting from a adjacent methylene, and the other as a singlet integrating for three protons at δ 2.11 diagnostic of a methyl adjacent to a carbonyl group. Additionally, the presence of two double bonds and eight aliphatic methylenes was evidenced by a complex olefinic multiplet at δ 5.35 integrating for four protons and a series of peaks with first order splitting upfield. APT ^{13}C nmr of compound **1** confirmed the presence of two methyl groups (δ 14.13 and δ 29.97), two double bonds (4 methine carbons at δ 127.55, 128.83, 129.07, and 130.36), and eight aliphatic methylenes (see physical data). The presence of a methyl ketone was evident by the characteristic chemical shifts of the methyl at δ 29.97 (^{1}H nmr δ 2.11, s) and methylene at δ 43.05 (^{1}H nmr δ 2.41,

TABLE 1. Biological Activities of Compounds **1–10**.

Compound	Brine Shrimp[a] LC_{50} (ppm)	Potato Disc % inhibition	Cell Culture (ED_{50} μg/ml)			Corn rootworm[b] (*Diabrotica undecipunctata*) % mortality (12 ppm)
			A-549	MCF-7	HT-29	
1	2.9 (1.8/4.4)	—	>10	>10	>10	—
2	3.0 (2.0/4.5)	—	>10	5.15	3.01	—
3	5.75 (3.6/8.9)	—	>10	>10	>10	—
4	0.96 (0.65/1.35)	44.9%	4.57	3.12	3.07	20
5	0.52 (0.33/0.83)	41.4%	3.01	2.58	1.90	0
6	0.72 (0.45/1.08)	—	3.75	2.31	2.48	0
7	0.89 (0.57/1.3)	—	>10	5.12	2.93	0
8	0.065 (0.01/0.12)	—	>10	4.60	2.92	0
9	0.035 (0.02/0.05)	64.2%	3.32	4.58	3.26	0
10	5.23 (1.89/8.08)	—	9.28×10^{-1}	73.6	30.5	—
Adriamycin	0.088 (0.01/0.6)	—	1.8×10^{-3}	5.1×10^{-2}	1.2×10^{-2}	—

[a]95% confidence intervals are given in parentheses.

[b]Compounds **4–9** were inactive (0% mortality) against Southern armyworm (*Spodoptera eridania*), two-spotted spider mite (*Tetranychus urticae*), and melon aphid (*Aphis gossypii*).

t) flanking the ketone carbonyl; however, the carbonyl carbon was not observed in the APT spectrum, perhaps due to the small amount of sample and short delay time. The molecular formula for a linear C_{15} methyl ketone derived from ^{1}H- and ^{13}C-nmr data was consistent with the mol wt of 222 derived from cims ($[MH]^+$ *m/z* 223). Positions of the two carbon-carbon double bonds along the C_{15} chain were determined by analysis of the 500 MHz ^{1}H-nmr COSY spectrum and 1D proton splitting patterns.

Two connectivity networks in **1** were established by COSY (Figure 1). The first network showed the linear connectivity of the terminal methyl triplet through four aliphatic methylenes to an olefinic proton. Correlation cross peaks were seen connecting, in turn, the methyl triplet at δ 0.87, a four proton multiplet at δ 1.26 (2 CH_2), a pentet at δ 1.32 (CH_2), a doublet of triplets at δ 2.02 (CH_2), and an olefinic multiplet at δ 5.35. The second network showed a connectivity chain from the terminal methyl ketone through three aliphatic methylenes to an olefinic proton. Correlation cross peaks were seen connecting, in turn, the olefinic multiplet at δ 5.35, a doublet of triplets at δ 2.06 (CH_2), a pentet at δ 1.63 (CH_2), and a triplet at δ 2.41 (CH_2). The δ 2.41 triplet assigned to the methylene adjacent to the ketone carbonyl showed a cross peak to the three proton singlet at δ 2.11 assigned to the terminal methyl ketone. The two coupling networks were linked by one methylene group as seen by a cross peak from the olefinic multiplet at δ 5.35 to the apparent triplet at δ 2.74 (CH_2). Compound **1** was thus established to be 6,9-pentadecadien-2-one.

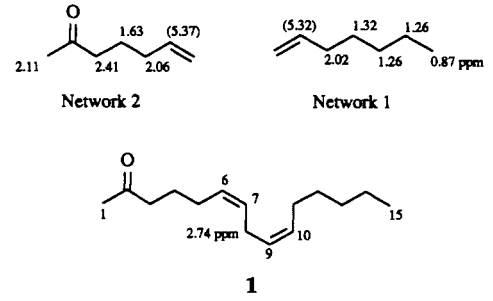


FIGURE 1. Connectivity networks for compound **1**.

The configurations of both double bonds of **1** were determined by decoupling the pair of methylenes flanking the double bonds (δ 2.04, H-5 and H-11) and decoupling the methylene flanked by the double bonds (δ 2.74, H-8). The resulting simplified olefinic region showed coupling constants of 10.7 and 10.8 Hz, indicating that both Δ^6 and Δ^9 are in the cis (*Z*) configuration (see physical data for a complete assignment).

Compound **2**, 2 mass units less than compound **1** as seen by cims ($[MH]^+$ *m/z* 221), showed many similarities to **1** as evidenced in the 500 MHz ^{1}H-nmr spectrum. The presence of the methyl ketone and three-carbon methylene chain attached to a double bond at H-6, as in compound **1**, was established by a methyl singlet at δ 2.11 (H-1) and methylene triplet at δ 2.41 (H-3) surrounding the carbonyl group, with the methylene at δ 2.41 coupled to the pentet at δ 1.63 (H-4), which was coupled to a multiplet at δ 2.05, which was further coupled to the olefinic region at δ 5.37 (see physical data). The four-proton multiplet at δ 2.05 was, as in **1**, assigned to the two methylene groups flanking the double bonds. In contrast to **1**, compound **2** contained a series of three double bonds, rather than two, isolated by single methylenes as seen by a complex multiplet at δ 5.37 (6H) and two overlapping triplets at δ 2.77 and 2.78 (4H). The remainder of the ^{1}H-nmr spectrum consisted of a terminal methyl triplet at δ 0.95 assigned to H-15. That the series of double bonds was terminated by an ethyl group rather than a

pentanyl group as in **1** was established by the collapse of the δ 0.95 methyl triplet into a singlet upon decoupling at δ 2.05 (4H, H-5 and H-14). Additionally, this caused the collapse of the δ 1.63 pentet (H-4) into a triplet, confirming its assignment adjacent to H-5, and a simplification of the olefinic region, confirming the placement of the three double bonds across carbons 6–7, 9–10, and 12–13. Decoupling at δ 2.77 (methylene protons at 8 and 11) caused the olefinic region to simplify into two doublets at δ 5.32 (10.5 Hz) and δ 5.36 (10.5 Hz) assigned to H-9 and H-10, two doublets of triplets at δ 5.33 (10.7 Hz, 7.2 Hz) and δ 5.38 (10.7 Hz, 7.1 Hz) assigned to H-6 and H-13, and two doublets of triplets at δ 5.29 (10.7 Hz, 1.4 Hz) and δ 5.37 (10.7 Hz, obscured) assigned to H-7 and H-12. From the olefinic coupling constants on the order of 10 Hz, all three double bonds were determined to be cis (*Z*). Compound **2** was thus established as (6*Z*,9*Z*,12*Z*)-pentadecatrien-2-one.

Compound **3** was determined through ^{1}H- and ^{13}C-nmr, ms, and ir spectral data and optical rotation to be the known sesquiterpene, (+)-(*Z*)-nerolidol. Comparison of spectral data to literature values (19) as well as tlc and ^{1}H-nmr comparison to a commercially available sample confirmed this identification. Brine shrimp bioassay of the commercially available cis-trans mixture of nerolidol showed an LC_{50} of 3.7 ppm (2.4/5.5 ppm), not significantly different from and within the 95% confidence intervals of compound **3** (Table 1).

Compounds **4–10** were determined to be a series of C_{17}, C_{19}, and C_{21} alkane-alkene γ-lactones. An extensive number of γ-lactones previously have been isolated from six genera of the Lauraceae, namely, from *Actinodaphne lancifolia* (20,21), *Clinostemon mahuba* (22), *Lin. obtusiloba* (6–9), *Litsea japonica* (23), *Machilus thunbergii* (24), *Nectandra rubra* (25), and now *Lin. benzoin*. A similar series of alkane, alkene, and alkyne δ-lactones have been isolated from *Persea major* (Lauraceae) (26,27). Spectral analysis of compounds **4–10** established that the γ-lactones of *Lin. benzoin* belong to the obtusilactone series with an exocyclic methylene at position 4 of the γ-lactone and a β-hydroxy at H-3 (6–8). Ft-ir (neat) provided the first evidence of the overall similarity of the seven compounds with characteristic absorption bands of a hydroxyl (3400 cm^{-1}), an alkane chain (2926 and 2854 cm^{-1}), a γ-lactone carbonyl (1780 cm^{-1}), and alkenes (1680 and 1670 cm^{-1}). The carbonyl stretch was consistent for the lauraceous γ-lactones with an exocyclic methylene at position 4 (1780 cm^{-1}) rather than a methyl group (1755 cm^{-1}) (22). Compounds **4**, **5**, and **10** are C_{21} alkane-alkene γ-lactones new to the literature; however, **6–9** were previously isolated from the leaves of *Lin. obtusiloba*. The known compounds isoobtusilactone A [**6**], obtusilactone A [**7**], isoobtusilactone [**8**], and obtusilactone [**9**] were identified by analysis of their spectral data and comparison with literature values (6,7). The structures of **4**, **5**, and **10** were elucidated from ^{1}H- and ^{13}C-nmr, ms, and Ft-ir spectral data.

High resolution cims of compound **4** gave an $[MH]^{+}$ peak at *m/z* 337.2740 consistent with the molecular formula $C_{21}H_{36}O_3$ (calcd *m/z* 337.2743 for $[MH]^{+}$), which requires four rings or double bond equivalents. The 125 MHz APT ^{13}C-nmr spectrum (Table 2) showed 14 aliphatic methylenes and one terminal methyl (δ 14.14) which constructed an alkane chain, as well as two peaks with distinct chemical shifts indicating the presence of a secondary hydroxy group methine carbon (δ 66.31) and an ester carbonyl (δ 166.82). The APT spectrum also revealed the presence of two additional quaternary carbons at δ 157.55 and 127.21, which are on the ring end of the exocyclic methylene (δ 91.35) and methine (δ 150.17) double bonds of the lactone ring. The four units of unsaturation are thus accounted for as a γ-lactone with an exocyclic methylene and an exocyclic methine double bond.

Interpretation of the 500 MHz ^{1}H-nmr spectrum of compound **4** (Table 3) revealed a coupling network consistent with the structure illustrated. Two doublet of doublets

TABLE 2. ^{13}C-nmr Chemical Shifts (δ) of γ-Lactones **4–10** in $CDCl_3$ at 125.697 MHz.

Carbon	APT	Compound						
		4	**5**	**6**	**7**	**8**	**9**	**10**
C-1	C	166.82	166.65	166.77	166.09	166.49	165.10	165.17
C-2	C	127.21	127.28	127.20	126.72	127.20	126.73	126.78
C-3	CH	66.31	66.38	66.35	68.89	66.46	68.88	68.90
C-4	C	157.55	157.55	157.56	157.40	157.54	157.39	157.50
C-5	CH_2	91.35	91.35	91.35	90.31	91.36	90.31	90.33
C-6	CH	150.17	150.18	150.20	151.34	150.15	151.31	151.43
C-7	CH_2	29.70	29.77	29.72	29.71	29.74	29.47	29.68
C-8	CH_2	29.69	29.66	29.70	29.69	29.45	29.46	29.67
C-9	CH_2	29.67	29.54	29.68	29.68	29.45	29.37	29.66
C-10	CH_2	29.66	27.26	29.66	29.65	29.39	29.28	29.64
C-11	CH_2[a]	29.65	130.26 CH[a]	29.63	29.54	29.35	29.14	29.62
C-12	CH_2[a]	29.62	129.24 CH[a]	29.51	29.41	29.12	28.94	29.60
C-13	CH_2	29.51	27.01	29.39	29.39	28.93	28.71	29.49
C-14	CH_2	29.39	29.45	29.38	29.30	28.33	28.34	29.36
C-15	CH_2	29.38	29.35	29.37	28.72	33.82	33.83	29.34
C-16	CH_2[a]	29.37	29.34	28.32	28.36	139.13 CH[a]	139.16 CH[a]	29.25
C-17	CH_2	29.36	28.99	31.93	31.95	114.09	114.06	28.67
C-18	CH_2	28.31	28.23	22.71	22.73			28.30
C-19	CH_2[a]	31.93	31.92	14.15 Me[a]	14.18 Me[a]			31.90
C-20	CH_2	22.70	22.71					22.67
C-21	Me	14.14	14.16					14.11

[a]Change from CH_2 to CH or Me in compounds **5–9**.

at δ 4.93 (1H) and δ 4.72 (1H) showed 2.8 Hz splitting indicative of geminal coupling of protons in a methylene double bond. These protons lacked cis or trans coupling but showed long range coupling of 1.7 Hz and 1.4 Hz, respectively, to the single proton at δ 5.24. The olefinic geminal protons at δ 4.93 and 4.72 were thus assigned to the exocyclic methylene (H-5) evident from the APT spectrum. The single proton at δ 5.24 (H-3) was flanked by the exocyclic methylene on one side (1.7 Hz and 1.4 Hz coupling) and another double bond on the other side as evident from the 2.2 Hz coupling to the triplet of doublets (td) at δ 7.06 integrating for one proton. The triplet component of the td was attributed to 7.9 Hz vicinal coupling to two protons at δ 2.48 and 2.42. The olefinic proton at δ 7.06 (H-6) lacked cis or trans coupling and thus represented a single proton on a trisubstituted double bond (the exocyclic methine evident in the APT spectrum). The aliphatic protons at δ 2.48 (1H) and 2.42 (1H) were recognized as protons on the same carbon but in different environments and were located adjacent to the exocyclic methine (H-7). Both protons were split into doublet of doublet of doublet of triplets (dddt), each with 7.9 Hz coupling to the methine proton at δ 7.06, 14.94 Hz geminal coupling to each other, 1.0 Hz long range coupling, and 7.8 Hz coupling to an adjacent aliphatic methylene at δ 1.51. The peak at δ 1.51 (2H, H-8) was split into a crude pentet and was apparently linked to an alkane chain as evidenced by the aliphatic singlet at δ 1.24 (24H, positions 9–20) and the terminal methyl triplet (J = 7.0 Hz) at δ 0.87 (3H, H-21).

The 2D COSY ^{1}H-nmr (500 MHz) spectrum of compound **4** confirmed this coupling network and, in addition, showed a cross peak between the H-3 proton at δ 5.24 and the hydroxyl at δ 2.63; thus, the secondary -OH group was placed at position 3. The -OH was assigned the β configuration based on the levorotation ($[\alpha]_D = -49.5°$) of compound **4**. Dextrorotation is found in the γ-lactones of *Clinostemon mahauba* (22);

TABLE 3. ^{1}H-nmr (500 MHz) Data for C_{21} γ-lactones **4**, **5**, and **10** in $CDCl_3$.

Proton	Compound					
	4		**5**		**10**	
	δ (ppm)	coupling *J* (Hz)	δ (ppm)	coupling *J* (Hz)	δ (ppm)	coupling *J* (Hz)
H-3	5.24	bs	5.23	bd (7.6)	5.09	bd (8.1)
3-OH	2.63	bs	2.13	bd (7.6)	2.07	bd (8.1)
H-5	4.93	dd (2.8, 1.7)	4.93	dd (2.8, 1.6)	4.87	dd (2.8, 2.0)
H-5′	4.72	dd (2.8, 1.4)	4.70	dd (2.8, 1.4)	4.65	dd (2.8, 1.6)
H-6	7.06	td (7.9, 2.2)	7.06	td (8.0, 2.1)	6.67	td (7.9, 2.0)
H-7	2.48	dddt (14.9, 7.9, 1.0, 7.8)	2.48	dddt (14.7, 8.0, 1.0, 7.8)	2.78	dddt (15.0, 7.9, 1.6, 7.5)
H-7′	2.42	dddt (14.9, 7.9, 1.0, 7.8)	2.41	dddt (14.7, 8.0, 1.0, 7.8)	2.72	dddt (15.0, 7.9, 1.6, 7.5)
H-8	1.51	m (integrates for 2H)	1.51	p (7.8)	1.46	p (7.5)
H-9			1.34	m (integrates for 2H)		
H-10			1.99	m (integrates for total of 4H)		
H-11			5.35	dtt (10.9, 6.9, 1.3)		
H-12			5.30	ddt (10.9, 6.9, 1.3)		
H-13			1.99	m (integrates for total of 4H)		
			1.24			
H-14–20	1.24	s (integrates for 24H)	1.23	s (integrates for a total of 14H)	1.23	s (integrates for 24H)
H-21	0.87	t (7.0)	0.85	t (7.0)	0.85	t (7.0)

however, levorotation is found in the γ-lactones of *Lin. obtusiloba* (6,7) where the -OH group was determined to be β. Therefore, isolinderanolide [**4**] was assigned the structure as illustrated.

Compound **5**, a slight variation of isolinderanolide [**4**], has a cis double bond at position 11. High resolution cims gave an $[MH]^+$ peak at *m/z* 335.2584 consistent with the formula $C_{21}H_{34}O_3$ (calcd *m/z* 335.2586 for $[MH]^+$), which is two protons less than compound **4**. The presence of a double bond along the aliphatic side chain was evident in the APT ^{13}C-nmr spectrum of **5** by the emergence of two olefinic CH peaks at δ 130.26 and 129.24, while the number of aliphatic methylene peaks decreased from 14 in **4** to 12 in **5** (Table 2). ^{1}H-nmr data also indicated a double bond by the presence of a multiplet at δ 5.3 (2H) and a multiplet at δ 1.99 (4H), which was assigned to the methylenes flanking the double bond (Table 3). The flanking methylenes were assigned to the ^{13}C-nmr peaks at δ 27.26 and 27.01. Positioning of the double bond along the aliphatic chain and its configuration were resolved by ^{1}H-nmr spectrometry.

COSY ^{1}H nmr (500 MHz) of **5** confirmed the γ-lactone ring structure also present in **4** by correlation cross peaks both between the exocyclic methylene protons at δ 4.93 and 4.70 (H-5 and H-5′) and to the broad singlet at δ 5.23 (H-3), which additionally showed cross peaks to the -OH group at δ 2.13 (H-3) and to the exocyclic methine at δ 7.06 (H-6). The exocyclic methine (H-6) continued the coupling network with cross peaks to two aliphatic protons at δ 2.48 and 2.41 (H-7) and thus showed the link between the γ-lactone and the aliphatic side chain. The carbon-carbon double bond was established to be across carbons 11 and 12 of the aliphatic side chain by a series of correlation cross peaks connecting, in turn, the exocyclic methine (δ 7.06), the adjacent methylene (δ 2.48 and 2.41), a pentet at δ 1.51 (2H, H-8), a pentet at δ 1.34 (2H, H-9), and a multiplet at δ 1.99 (H-10) to the olefinic doublet of triplet of triplets (dtt) at δ 5.30 (1H, H-11). The dtt at δ 5.30 was coupled to another dtt at δ 5.35 (H-12), which showed a cross peak back to the flanking methylene at δ 1.99 assigned to H-13 (4H, overlapping the methylene at H-10). The remaining aliphatic chain from positions 14 to 21 was seen as two singlets at δ 1.24 and 1.23, integrating for 14 protons, and a terminal methyl triplet at δ 0.85 (Table 3).

The configuration of the double bond of **5** was determined by spin decoupling the two methylenes flanking the double bond (δ 1.99, H-10 and H-13). The resulting pair of doublets at δ 5.35 and 5.30 showed 10.9 Hz coupling indicative of a cis double bond. Compound **5** was determined to be a new C_{21}-Δ^{11}-alkene γ-lactone of the obtusilactone series and was named isolinderenolide.

Isoobtusilactone B, a C_{21} γ-lactone from *Lin. obtusiloba*, similarly contained a cis double bond along the aliphatic side chain; however, the double bond was determined to be across carbons 12 and 13 by oxidative degradation of the compound to nonanoic acid ($C_8H_{17}COOH$) (8). The COSY pulse sequence used above with **5** provided a convenient nondestructive means to determine double bond positioning.

Compound **10** was determined to be the *Z* configurational isomer at the exocyclic methine double bond of compound **4** (isolinderanolide). High resolution cims of **10** gave an $[MH]^+$ peak at *m/z* 337.2736 ($C_{21}H_{36}O_3$; calcd *m/z* 337.2743 for $[MH]^+$), consistent with the formula found for **4**, and low resolution cims confirmed the presence of a -OH group by the loss of 18 mu (*m/z* 319) and a carbonyl by the loss of 28 mu (*m/z* 309).

The ^{13}C-nmr data of **10** confirmed its identity as a C_{21} alkane γ-lactone of the current series by the presence of an ester carbonyl (δ 165.17), an exocyclic methylene double bond (δ 90.33, CH_2; δ 157.50, C), an exocyclic methine double bond (δ 150.17, CH; δ 127.21, C), and a secondary -OH group (δ 68.90) to construct the γ-lactone ring, and the presence of 14 aliphatic methylenes and a terminal methyl (δ 14.11) to

construct the alkane chain (Table 2). The δ 2.59 and 1.26 downfield shifts of C-3 and C-6, respectively, and the δ 1.02 upfield shift of C-5 are consistent with the chemical shift changes seen in the *Z* isomers of the *E,Z* pairs encountered so far (Table 2).

The ^{1}H-nmr spectrum of **10** confirmed its similarity to **4** by the presence of a terminal methyl triplet at δ 0.85 (H-21), an aliphatic peak at δ 1.23 (24H, positions 9–20), a β-aliphatic methylene at δ 1.46 (H-8), and a methylene at δ 2.78 and 2.72 (H-7 and H-7′) adjacent to the exocyclic methine to construct the alkane chain, and by the presence of a -OH group at δ 2.07 with coupling to the H-3 proton at δ 5.09, which was further coupled to the exocyclic methine proton at δ 6.67 and exocyclic methylene protons at δ 4.87 and 4.65 to construct the γ-lactone (Table 3). The characteristic upfield shift of the exocyclic methine proton (H-6; to δ 6.67) and the downfield shift and coalescence of the adjacent methylene protons (H-7; to δ 2.78 and δ 2.72), seen in compound **10** in comparison to compound **4**, are consistent with the *Z* isomers of the known obtusilactone *E,Z* pairs **6–9**. The negative optical rotation ([α]D −46.0) of **10** is consistent with the obtusilactone series of γ-lactones and, as it is the isomer of **4**, compound **10** was named linderanolide.

These bioactive compounds isolated from *Lin. benzoin* may be involved in the chemical ecology of this plant with insects. The widespread nerolidol [**3**], in addition to being a juvenile hormone mimic (28) and a potent gypsy moth larvae antifeedant (19), has been found in the pheromonal secretions of several insects, and the attractant and repellent activities of these secretions to various insects have been attributed to it (29,30). Additionally, Howard *et al.* (31) correlated nerolidol's toxicity to its deterrent activity to the leaf-cutting ant, *Atta cephalotes*. Although biological activity previously has not been associated with compound **1** or the novel compound **2**, similar methyl ketones such as (8*Z*)-pentadecen-2-one, 2-pentadecanone, and 2-tridecanone, and alkyl-dienes such as (6*Z*,9*Z*)-pentadecadiene have been found in the pheromonal secretions of several insects as well as in plants and have been suggested to play a role in chemical defense against insects (32–35). The obtusilactone series of γ-lactones has not been associated with modifying insect behavior; however, their potent toxicity in the brine shrimp assay and the significant toxicity of compound **4** to corn rootworm suggests that they may serve as insecticides for the plant. The presence of these potential defensive chemicals supports the hypothesis of Williams (36) that secondary metabolites have evolved to aid in the survival of the plant by attracting or repelling other organisms.

EXPERIMENTAL

PLANT MATERIAL.—The leaves and twigs of *Lin. benzoin* were collected in Branch County, Michigan, on September 30, 1988, and identified by Ralph T. McLaughlin. The fresh ripe berries were collected while still on the shrub October 14–15, 1989, in Tippecanoe County, Indiana, by Andrew T. McLaughlin. Identification of the collection was verified by Dr. J.W. Moser Jr. in the Department of Forestry and Natural Resources at Purdue University, and a voucher specimen was deposited in the Purdue Pharmacognosy Herbarium.

INSTRUMENTATION AND CHROMATOGRAPHIC MATERIAL.—Ft-ir spectra were recorded on a Perkin-Elmer 1600 series FTIR in $CHCl_3$ or neat. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. Low resolution ms was recorded on the Finnigan 4000 quadrupole mass spectrometer; high resolution ms was determined on the Kratos-50. High field nmr spectra were recorded in $CDCl_3$ on the Varian VXR-500s spectrometer at 499.843 MHz for ^{1}H and 125.697 MHz for ^{13}C. COSY and APT spectra were obtained using standard Varian pulse sequences. The following Si gel chromatographic supplies were used: Whatman LPS-1 for vacuum and mplc columns, Si gel 60 PF-254 (EM 7749) for chromatotron plates, and Si gel 60 F-254 (EM 5714) plates for tlc analysis.

BIOLOGICAL EVALUATIONS.—All partitions and chromatographic fractions were monitored for bioactivity using the brine shrimp lethality assay (1). The potato disc assay was performed as described previously (10). Pure active compounds were subsequently assayed for cytotoxicity to the human tumor cell lines A-549 (lung carcinoma), MCF-7 (breast carcinoma), and HT-29 (colon adenocarcinoma) at the Pur-

due Cell Culture Laboratory following established procedures (11–13). Insecticidal screenings of compounds **4–9** were run for southern corn rootworm, southern armyworm, two-spotted spider mite, and melon aphid at DowElanco, Greenfield, Indiana.

ISOLATION PROCEDURES.—The fresh berries (2.7 kg) of *Lin. benzoin* were blended with 95% EtOH in a Waring commercial blender and allowed to percolate for 1 week (5 liters 95% EtOH × 5). The dried EtOH residue (179 g; brine shrimp LC_{50} 95.7 ppm, 59/157 ppm) was partitioned between H_2O and CH_2Cl_2 to give an inactive interface (6.7 g), an inactive H_2O-soluble residue (110.5 g), and a brine shrimp toxic CH_2Cl_2-soluble residue (56.9 g; LC_{50} 62.5 ppm, 40/94 ppm) which was further partitioned between hexane and 90% aqueous MeOH to yield a hexane-soluble residue (31.0 g; LC_{50} 420 ppm, 272/650 ppm) and a 90% MeOH-soluble residue (23.0 g; LC_{50} 17.7 ppm, 11/27.3 ppm).

A portion of the bioactive 90% MeOH residue (10.5 × 2) was loaded onto a vacuum column (Whatman LPS-1 Si gel packed into a 350 ml sintered glass funnel; vacuum by water aspirator) and eluted in C_6H_6 with an increasing EtOAc gradient to yield a number of fractions (400 ml each). The fractions were combined, based on tlc similarities, into seven pools and assayed for toxicity. The first three pools (5.95 g total) were most toxic to brine shrimp, with LC_{50} values of 1.9 ppm (0.1/6.1 ppm), 2.4 ppm (1.5/3.7 ppm), and 2.5 ppm (1.7/3.4 ppm) sequentially; pool 4 (568 mg) had an LC_{50} of 7.8 ppm (3.2/11.3 ppm), while pools 5–7 (14.6 g total) had LC_{50} values > 100 ppm. Pools 1–3 were combined (5.9 g), loaded onto a Michel-Miller column (3 × 55 cm, Si gel), and eluted with 3% Me_2CO in hexane with an increasing Me_2CO gradient to yield 23 fractions (200 ml each) that were combined into eleven pools based on tlc and brine shrimp toxicity.

Michel-Miller column pool 2 (38 mg; LC_{50} 26.9 ppm, 18/40 ppm) consisting of three major components and a substantial polar baseline decomposition, as seen by tlc, was loaded onto a Chromatotron (2 mm Si gel rotor) and eluted with 2% EtOAc in hexane to yield 40 fractions of 20 ml each which were combined based on tlc and brine shrimp lethality to provide compound **1** (3.2 mg; LC_{50} 2.9 ppm, 1.8/4.0 ppm) and, after additional purification using the Chromatotron, compound **2** (2.5 mg; LC_{50} 3.0 ppm, 2.0/4.5 ppm). A majority of the fraction decomposed (17 mg).

Michel-Miller column pool 4 (61.5 mg; LC_{50} 27 ppm 18/40 ppm), which was a fragrant, clear, oily fraction, was loaded onto the Chromatotron (2 mm Si gel rotor) and eluted with 5% EtOAc in hexane to yield 40 fractions of 20 ml each, which were combined by tlc and toxicity similarities into seven fractions. Fraction 1 contained pure compound **3** (13.9 mg; LC_{50} 5.75 ppm, 3.6/8.9 ppm), which proved to be the sole bioactive component of pool 4.

Michel-Miller pool 6 (2.89 g; LC_{50} 4.7 ppm, 3.1/6.9 ppm) was loaded onto a Michel-Miller mplc column (3 × 55 cm) and eluted with 4% EtOAc in hexane with an increasing EtOAc gradient. Seventy fractions (10 ml) were collected, combined into 8 fractions, and assayed for toxicity. Fraction 3 (348.7 mg; LC_{50} 2.2 ppm) appeared as a single spot by tlc [hexane-EtOAc (8:2), Si gel]; however, upon chilling in the freezer, oil, crystals, and a transparent solid were separated into compound **4** (solid, oil at room temperature, 103 mg; LC_{50} 0.96 ppm, 0.7/1.4 ppm), compound **5** (oil, 113 mg; LC_{50} 0.52 ppm, 0.3/0.8 ppm), and compound **6** (white crystals, oil at room temperature, 82 mg; LC_{50} 0.7 ppm, 0.45/1.1 ppm). The adjacent fraction 4 (911 mg; LC_{50} 2.8 ppm) contained compounds **4–6**.

Michel-Miller pool 7 (970 mg; LC_{50} 0.61 ppm, 0.09/1.2 ppm), a reddish oil containing three major components, was loaded onto the Chromatotron (4 mm Si gel rotor) and eluted with 3% Me_2CO in hexane to yield, after additional separation via Chromatotron with a 2 mm Si gel rotor, pure compound **7** (36.1 mg; LC_{50} 0.89 ppm, 0.57/1.3 ppm), compound **8** (33.2 mg; LC_{50} 0.065 ppm, 0.01/0.12 ppm), and compound **9** (51 mg; LC_{50} 0.035 ppm, 0.02/0.05 ppm).

A portion of the hexane partition residue (27 g) was loaded onto a vacuum column (4.5 × 28 cm, Si gel) and eluted in hexane with an increasing EtOAc gradient to yield 16 fractions. Fractions 1–6 (20.5 g) and 10–15 (1.1 g) gave LC_{50} values > 1000 ppm. Fraction 7 (2.02 g, LC_{50} 35.1 ppm, 11/78 ppm) was placed in a freezer to yield compounds **4–6**, and a mother liquor (oil, 1.36 g, LC_{50} 72.8 ppm). Chromatotron resolution of the mother liquor (4 mm Si gel rotor) in hexane-EtOAc (9:1) produced active fraction 5 which, upon recrystallization from hexane in the freezer, yielded compound **10** (white crystals in freezer, oil at room temperature, 19 mg; LC_{50} 5.23 ppm, 1.89/8.08 ppm).

PHYSICAL DATA.—*(6Z,9Z)-Pentadecadien-2-one* [**1**].—Compound **1**: 3.2 mg; oil; cims (isobutane) *m/z* (% rel. int.), $[M+C_4H_9]^+$ 279 (17), $[M+C_3H_5]^+$ 263 (9.2), $[MH]^+$ 223 (100), $[MH-H_2O]^+$ 205 (71), 109 (19.8); ir ($CHCl_3$) ν max 3005, 2955, 2923, 2853, 1715, 1454, 1383, 1112, 1069 cm^{-1}; ^1H nmr (500 MHz, $CDCl_3$) δ 0.87 (3H, t, J = 7.0 Hz, H-15), 1.26 (4H, m, H-13, H-14), 1.32 (2H, p, J = 7.3 Hz, H-12), 1.63 (2H, p, J = 7.4 Hz, H-4), 2.02 (2H, dt, J = 6.8, 7.3 Hz, H-11), 2.05 (2H, dt, J = 7.1, 7.4 Hz, H-5), 2.11 (3H, s, H-1), 2.41 (2H, t, J = 7.4 Hz, H-3), 2.74 (2H, t, J = 7.1 Hz, H-8), 5.29 (1H, dtt, J = 10.7, 7.1, 1.5 Hz, H-7), 5.32 (1H, ddt, J = 10.8, 6.8, 1.6 Hz, H-10), 5.37 (1H, dtt, J = 10.8, 7.1, obscured Hz, H-9), 5.37 (1H, ddt, J = 10.7, 7.1, 1.6 Hz, H-6); ^{13}C nmr (125 MHz,

$CDCl_3$, APT) δ 14.13 (C-15 Me), 22.62, 23.63, 25.66, 26.52, 27.26, 29.36, 31.55 (CH_2 C-4, -5, -8, -11–14), 29.97 (C-1 Me), 43.06 (CH_2, C-3), 127.55, 128.83, 129.07, 130.36 (CH, C-6, -7, -9, -10).

(6Z,9Z,12Z)-Pentadecatrien-2-one [**2**].—Compound **2**: 2.5 mg; oil; cims (isobutane) *m/z* (% rel. int.), $[MH]^+$ 221 (95), $[MH - H_2O]^+$ 203 (100), 163 (9), 109 (16.8); ir ($CHCl_3$) ν max 3005, 2954, 2918, 2852, 1715, 1637, 1448, 1112, 1069 cm^{-1}; ^1H nmr (500 MHz, $CDCl_3$) δ 0.95 (3H, t, $J = 7.6$ Hz, H-15), 1.63 (2H, p, $J = 7.4$ Hz, H-4), 2.05 (4H, m, H-5, H-14), 2.11 (3H, s, H-1), 2.41 (2H, t, $J = 7.4$ Hz, H-3), 2.77 (2H, t, $J = 5.7$ Hz, H-11), 2.78 (2H, t, $J = 5.9$ Hz, H-8), 5.29 (1H, ddt, $J = 10.7$, 5.9, 1.4 Hz, H-7), 5.32 (1H, dt, $J = 10.5$, 5.7 Hz, H-10), 5.33 (1H, dtt, $J = 10.7$, 7.2, obscured Hz, H-13), 5.36 (1H, dt, $J = 10.5$, 5.9 Hz, H-9), 5.37 (1H, dtt, $J = 10.7$, 5.7, 1.7 Hz, H-12), 5.38 (1H, dtt, $J = 10.7$, 7.2, obscured Hz, H-6).

(+)-(Z)-Nerolidol [**3**].—Compound **3**: 13.9 mg; oil; cims (isobutane) *m/z* (% rel. int.) $[MH]^+$ 223 (0.08), $[MH - H_2O]^+$ 205 (100), 149 (12), 137 (18), 135 (5), 123 (5), 121 (14), 109 (7), 95 (7), 81 (20); cims (NH_3) *m/z* (% rel. int.), $[M - NH_4]^+$ 240 (12), $[M + NH_4 - H_2O]^+$ 222 (81), $[M - NH_4 - H_2O]^+$ 205 (100); ir ($CHCl_3$) ν max 3389, 2967, 2925, 2856, 1665, 1640, 1450, 1411, 1375, 1109, 994, 920 cm^{-1}; ^1H nmr (500 MHz, $CDCl_3$) δ 1.26 (3H, s, H-3), 1.53 (6H, s, H-7 and H-12), 1.5–1.64 (3H, m, H-4, 3-OH), 1.66 (3H, bs, H-11), 1.94–2.06 (6H, m, H-5, -8, -9), 5.04 (1H, dd, $J = 10.8$, 1.3 Hz, H-1), 5.06 (1H, tm, $J = 6.9$, 1.5 Hz, H-6), 5.12 (1H, tm, $J = 7.0$, 1.4 Hz, H-10), 5.19 (1H, dd, $J = 17.4$, 1.3 Hz, H-1), 5.90 (1H, dd, $J = 10.8$, 17.4 Hz, H-2); ^{13}C nmr (125 MHz, $CDCl_3$) δ 16.09 (Me, C-11), 22.78 (CH_2, C-5), 25.77 (Me, C-3), 26.69 (CH_2, C-9), 27.94 (Me, C-7 and C-12), 39.74 (CH_2, C-8), 42.07 (CH_2, C-4), 73.54 (C, C-3), 111.64 (CH_2, C-1), 124.15 (CH, C-10), 124.18 (CH, C-6), 131.65 (C, C-11), 135.79 (C, C-7), 144.96 (CH, C-2); $[\alpha]_D$ +12.72° ($c = 0.0035$, $CHCl_3$). Spectral data are consistent with published values (19).

Isolinderanolide [**4**].—Compound **4**: 103 mg; oil at room temperature; cims (isobutane) *m/z* (% rel. int.), $[MH]^+$ 337 (36), $[MH - H_2O]^+$ 319 (9), $[MH - CO]^+$ 309 (34), $[MH - CO_2]^+$ 293 (100), 265 (68), 140 (22), 135 (13), 126 (33); hrcims found 337.2740 (calcd for $[MH]^+$ of $C_{21}H_{36}O_3$, 337.2743); ir (neat) ν max 341, 2921, 2852, 1789, 1769, 1682, 1672, 1469, 1276, 1186, 1032, 950, 855, 830, 719 cm^{-1}; ^1H nmr (500 MHz, $CDCl_3$) see Table 3; ^{13}C nmr (125 MHz, $CDCl_3$) see Table 2; $[\alpha]_D$ −49.5° ($c = 0.002$, $CHCl_3$).

Isolinderenolide [**5**].—Compound **5**: 113 mg; oil at room temperature; cims (isobutane) *m/z* (% rel. int.), $[MH]^+$ 335 (100), $[MH - H_2O]^+$ 317 (85), $[MH - CO_2]^+$ 291 (9); hrcims found 335.2584 (calcd 335.2586 for $[MH]^+$ of $C_{21}H_{34}O_3$); ir (neat) ν max 3428, 3005, 2925, 2854, 1789, 1770, 1682, 1670, 1464, 1273, 1154, 1063, 1027, 950, 855, 722 cm^{-1}; ^1H-nmr (500 MHz, $CDCl_3$) see Table 3; ^{13}C-nmr (125 MHz, $CDCl_3$) see Table 2; $[\alpha]_D$ −22.3° ($c = 0.0026$, $CHCl_3$).

Isoobtusilactone A [**6**].—Compound **6**: 82 mg; oil at room temperature; white crystals at −10°; cims (isobutane) *m/z* (% rel. int.) $[MH]^+$ 309 (100), $[MH - H_2O]^+$ 291 (52); hrcims found *m/z* 309.2430 (calcd 309.2429 for $[MH]^+$ of $C_{19}H_{32}O_3$); ir (neat) ν max 3390, 3296, 2953, 2917, 2848, 1779.8, 1752, 1671, 1470, 1279, 1079, 1037, 946, 873 cm^{-1}; ^1H nmr (500 MHz, $CDCl_3$) δ 0.85 (3H, t, $J = 6.5$ Hz, H-19), 1.23 (20H, s, H-9 to -18), 1.50 (2H, p, $J = 7.5$ Hz, H-8), 2.40 (1H, dddt, $J = 14.8$, 8.0, 1.0, 7.5 Hz, H-7), 2.46 (1H, dddt, $J = 14.8$, 8.0, 1.0, 7.5 Hz, H-7′), 2.46 (OH, bd overlapping with H-7′, $J = 6.1$ Hz, OH-3), 4.70 (1H, dd, $J = 1.4$, 2.8 Hz, H-5), 4.92 (1H, dd, $J = 1.7$, 2.9 Hz, H-5′), 5.22 (1H, bd, $J = 6.1$ Hz, H-3), 7.05 (1H, td, $J = 8.0$, 2.1 Hz, H-6); ^{13}C nmr (125 MHz, $CDCl_3$) see Table 2; $[\alpha]_D$ −4.8° ($c = 0.0025$, $CHCl_3$). Spectral data agree with published values (7).

Obtusilactone A [**7**].—Compound **7**: 36.1 mg; oil; cims (isobutane) *m/z* (% rel. int.) $[MH]^+$ 309 (100), $[MH - H_2O]^+$ 291 (17), $[MH - CO]^+$ 281 (42.5); ir (neat) ν max 3428, 2955, 2924, 2853, 1787, 1770, 1680, 1465, 1367, 1096, 1053, 966, 853, 813, 722 cm $^{-1}$; ^1H nmr (500 MHz, $CDCl_3$) δ 0.86 (3H, t, $J = 6.9$ Hz, H-19), 1.23 (20H, s, H-9 to -18), 1.46 (2H, p, $J = 7.5$ Hz, H-8), 2.75 (2H, two overlapping dddt, $J = 15.0$, 7.8, 1.6, 7.5 Hz, H-7), 4.65 (1H, dd, $J = 1.7$, 2.8 Hz, H-5), 4.87 (1H, dd, $J = 2.0$, 2.8 Hz, H-5′), 5.09 (1H, bs, H-3), 6.67 (1H, td, $J = 7.8$, 1.9 Hz, H-6); ^{13}C-nmr (125 MHz, $CDCl_3$) see Table 2; $[\alpha]_D$ −47.5° ($c = 0.0016$, $CHCl_3$). Spectral data agree with published values (7).

Isoobutasilactone [**8**].—Compound **8**: 33.2 mg; oil; cims (isobutane) *m/z* (% rel. int.) $[MH]^+$ 279 (64), $[MH - H_2O]^+$ 261 (100); ir (neat) ν max 3428, 3075, 2926, 2854, 1787, 1770, 1682, 1670, 1640, 1463, 1274, 1170, 1028, 951, 909, 856, 722 cm^{-1}; ^1H nmr (500 MHz, $CDCl_3$) δ 1.28 (10H, s, H-9 to H-13), 1.35 (2H, p, $J = 7.8$ Hz, H-14), 1.52 (2H, pd, $J = 7.2$, 1.8 Hz, H-8), 2.03 (2H, dddt, $J = 6.7$, 1.7, 1.2, 6.7 Hz, H-15), 2.13 (1H, bd, $J = 7.8$ Hz, 3-OH), 2.43 (1H, dddt, $J = 14.7$, 7.9, 0.8, 7.2 Hz, H-7′), 2.50 (1H, dddt, $J = 14.7$, 7.9, 0.8, 7.2 Hz, H-7), 4.72 (1H, dd, $J = 2.8$, 1.4 Hz, H-5′), 4.93 (1H, ddt, $J = 10.2$, 2.2, 1.2 Hz, H-17′), 4.96 (1H, dd, $J = 2.8$, 1.7 Hz, H-5), 4.99 (1H, ddt, $J = 17.1$, 2.2, 1.7 Hz, H-17), 5.26 (1H, bs, H-3), 5.81 (1H, ddt, $J = 17.1$, 10.2, 6.7 Hz, H-16), 7.09

(1H, td, $J=7.9$, 2.2 Hz, H-6); ^{13}C nmr (125 MHz, $CDCl_3$) see Table 2; $[\alpha]D$ $-43.0°$ ($c=0.003$, $CHCl_3$). Spectral data agree with published values (7).

Obtusilactone [**9**].—Compound **9**: 51 mg; oil; cims (isobutane) *m/z* (% rel. int.) $[MH]^+$ 279 (100), $[MH-H_2O]^+$ 261 (84.6); ir (neat) ν max 3428, 3075, 2926, 2854, 2786, 1767, 1680, 1640, 1464, 1367, 1263, 1096, 1053, 965, 909, 854, 813, 757, 722 cm^{-1}; ^1H-nmr (500 MHz, $CDCl_3$) δ 1.28 (10H, s, H-9 to H-13), 1.36 (2H, p, $J=7.6$ Hz, H-14), 1.49 (2H, p, $J=7.5$ Hz, H-8), 2.04 (2H, dddt, $J=6.7$, 1.6, 1.2, 7.6 Hz, H-15), 2.16 (1H, bd, $J=7.5$ Hz, 3-OH), 2.75 (1H, dddt, $J=15.1$, 7.9, 1.7, 7.5 Hz, H-7'), 2.79 (1H, dddt, $J=15.1$, 7.9, 1.7, 7.5 Hz, H-7), 4.70 (1H, dd, $J=2.8$, 1.6 Hz, H-5'), 4.89 (1H, dd, $J=2.8$, 2.0 Hz, H-5), 4.93 (1H, ddt, $J=10.2$, 2.2, 1.2 Hz, H-17'), 4.99 (1H, ddt, $J=17.2$, 2.2, 1.6 Hz, H-17), 5.12 (1H, bs, H-3), 5.81 (1H, ddt, $J=17.2$, 10.2, 6.7 Hz, H-16), 6.68 (1H, td, $J=7.9$, 2.0 Hz, H-6); ^{13}C nmr (125 MHz, $CDCl_3$) see Table 2; $[\alpha]D$ $-45.0°$ ($c=0.0024$, $CHCl_3$). Spectral data agree with published values (6).

Linderanolide [**10**].—Compound **10**: 19 mg; oil at room temperature, white crystals at $-10°$; cims (isobutane) *m/z* (% rel. int.) $[MH]^+$ 337 (100), $[MH-H_2O]^+$ 319 (89), $[MH-CO]^+$ 309 (50.4), $[MH-CO-H_2O]^+$ 291 (9.7); hrcims 337.2736 (calcd 337.2743 for $[MH]^+$ of $C_{21}H_{36}O_3$); ir (neat) ν max 3395, 2920, 2850, 1779, 1770, 1671, 1469, 1275, 1033, 948, 863, 718 cm^{-1}; ^1H nmr (500 MHz, $CDCl_3$) see Table 3; ^{13}C nmr (125 MHz, $CDCl_3$) see Table 2; $[\alpha]D$ $-46.0°$ ($c=0.01$, $CHCl_3$).

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