Lupane Triterpenes with a δ-Lactone at Ring E, from *Lippia mexicana*

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Three new lupane-type triterpenes, lippiolide (**1**), lippiolidolic acid (**2**), and lippiolic acid (**3**), were isolated from aerial parts of *Lippia mexicana.* Compounds **1** and **2** exhibited a δ-lactone at ring E. The known cycloartane triterpene **5** was also isolated. The structures of these compounds were established on the basis of spectroscopic data and chemical reactions, and the structure of compound **1** was confirmed by X-ray diffraction analysis. Anti-inflammatory activity of compounds **1**, **3**, and **5** was evaluated in the TPA-induced ear mouse edema model. Lupanes **1** and **3** were more active than cycloartane **5**.

Lippia Houst. is a genus of the Verbenaceae including about 200 species of herbs, shrubs, and small trees, many of which are aromatic. As a consequence of this characteristic, the chemical studies of *Lippia* species have been focused on the composition of their essential oils, and a number of these plants are used as seasoning for food and beverages; *Lippia graveolens* H. B. K., known as Mexican oregano, is an example.[1−3] Although the studies on nonvolatile compounds of these plants are few, flavonoids,[4−7] phenylpropanoid and phenylethanoid glycosides,[7] napthoquinones,[8] iridoid glycosides,[5,9] sesquiterpenes,[4,5,7,10] and triterpenes[4,6,7,11] have been isolated from members of this genus. Moreover, in folk medicine, *Lippia* species have been used mainly as remedies for respiratory and gastrointestinal disorders, but also to treat malaria, hypertension, diabetes, syphilis, and hepatic diseases and as sedatives, stimulants, abortifacients, analgesics, and anti-inflammatories.[3]

As part of ongoing research on the chemistry of Mexican plants, we carried out a study of *Lippia mexicana* G. L. Nesom. (Verbenaceae), an evergreen tree endemic to Central Mexico, for which no chemical investigations have been reported. Our study resulted in the isolation of three new lupane-type triterpenes, named lippiolide (**1**), lippiolidolic acid (**2**), and lippiolic acid (**3**). The known triterpene cycloart-23-ene-3β,25-diol (**5**) was also isolated. The anti-inflammatory activity of compounds **1**, **3**, and **5** was evaluated in the TPA-induced ear mouse edema model.

Compound **1** was isolated as colorless crystals. The molecular formula $C_{30}H_{46}O_4$ (8 degrees of unsaturation) was deduced from the $[M + H]^+$ peak at *m/z* 471.3477 in the HRFABMS. The IR spectrum showed absorptions at 3591, 3433 (OH), 1726 (δ-lactone), and 1653 (double bond) cm^{-1}. The ^{13}C NMR spectrum showed 30 signals identified by DEPT and HSQC spectra as six methyls (one vinylic), 11 methylenes (one sp^2 and one oxymethylene), five methines (one oxymethine), and eight nonprotonated carbons (one sp^2, one hemiketalic, and one corresponding to the lactone carbonyl). These data suggested that **1** was a pentacyclic triterpene. The ^1H NMR signals of a terminal methylene (δ 5.06, br s and 5.01, br t, $J = 1$ Hz) correlated in the COSY spectrum with a methyl signal at δ 1.77, thus indicating the presence of an isopropenyl group and suggesting a lupane skeleton for **1**. Therefore, the mentioned signals were assigned to H-29a, H-29b, and H-30, respectively. These signals allowed us to locate the δ-lactone at ring E by their HMBC correlations with C-20 (δ 144.1) and with a lactone ring oxymethine carbon (δ 87.2), which was attributed to C-19 on the basis of the H-19 (δ 4.52, d, $J = 10$ Hz) correlations with the δ-lactone carbonyl (δ 170.3, C-21), as well as those with C-13, C-18, C-20, C-29, and C-30. The above was confirmed by the HMBC correlations of H-22α and H-22β with C-16, C-17, C-18, C-21, and C-28. In addition, a *W*-coupling ($J = 1$ Hz) between H-22α and CH_3-28 was observed in the COSY spectrum. On the other hand, the other two oxygen atoms of **1** were part of a hemiketal, whose presence was indicated by the ^{13}C NMR signal at δ 98.2. The HMBC correlations of the last signal with H-1a, H-2a, CH_3-23, and CH_3-24 identified the hemiketalic carbon as C-3. An ether bridge between C-3 and C-25 was established by the HMBC correlations of C-3 with one of the protons of a oxymethylene (δ 3.71), which was identified as the *pro-R* H-25b because of its correlations with C-5 and C-10 in the HMBC spectrum and its *W*-coupling ($J = 3$ Hz) with H-5, observed in the COSY spectrum. The *pro-S* oxymethylene proton, H-25a (δ 4.24), was *W*-coupled with H-1α. The same functionalization at ring A has been found in a series of oleanane-type triterpenes isolated from the Verbenaceae plants *Lippia turbinata*[11] and *Lantana camara.*[12] The relative configurations at various centers of **1** were established by interactions observed in the NOESY spectrum (Figure 1). Thus, the structure of **1** was established as 3β,25-epoxy-3α-hydroxy-19,21-*seco*-lup-20(29)-en-21,19-lactone, and it was confirmed by X-ray analysis (Figure 2). Compound **1** was named lippiolide.

The HRFABMS of compound **2** exhibited a $[M + H]^+$ peak at *m/z* 471.3476, indicative of the molecular formula $C_{30}H_{46}O_4$ (8

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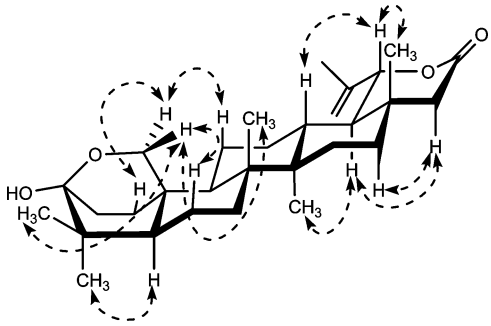

Figure 1. Key NOESY interactions of compound **1**.

degrees of unsaturation). The IR spectrum exhibited a band for a carboxylic acid at 3650−2450 cm^{-1} and a broad band at 1732 cm^{-1}, whose second derivative showed bands for the acid carbonyl (1705 cm^{-1}), δ-lactone (1735 cm^{-1}), and double bonds (1637 cm^{-1}). Analysis of the ^1H, ^{13}C, DEPT, and 2D NMR spectra indicated that, like **1**, compound **2** had a δ-lactone at ring E, an isopropenyl group at C-19, and the same structure as **1** at rings B to D (Tables 1 and 2). NOESY interactions between H-19 and CH_3-28 revealed α-equatorial disposition of the isopropenyl group. Thus, the differences between **1** and **2** were in ring A. The presence of a second isopropenyl group in **2** was deduced from the signals at δ 4.83, 4.66, and 1.73 (3H), which were assigned to H-23a, H-23b, and CH_3-24 on the basis of the HMBC correlations of H-23a and H-23b with C-24 and C-5 and those of H-5 (δ 1.94) with C-4, C-10, C-23, C-24, and C-25. This placed the isopropenyl group at C-5. On the other hand, the ^{13}C NMR signal at δ 178.0 confirmed the presence of a carboxylic acid. This group was located at C-3 by the HMBC correlations of C-3 with the protons of CH_2-1 (δ 1.61, 2H) and CH_2-2 (δ 2.38 and 2.17) and those of H-2a and H-2b with C-2, C-5, C-9, C-10, and C-25. From these data the structure of lippiolidolic acid (**2**) was determined to be 3,4;19,21-di-*seco*-lupa-4(23),20(29)-diene-21,19-lacton-3-oic acid.

Compound **3** was isolated as colorless crystals. The $[M + H]^+$ peak at *m*/*z* 457.3678 in the HRFABMS indicated the molecular formula $C_{30}H_{48}O_3$. The ^{13}C NMR spectrum showed 30 signals, one of them (δ 176.3, C-3) together with the IR bands at 3650−2450 and 1710 cm^{-1} showing the presence of a carboxylic acid. In addition, the ^1H NMR signals at δ 4.83 (H-23a), 4.66 (H-23b), and 1.73 (CH_3-24) and their correlations in the HMBC spectrum revealed an isopropenyl group at C-5, thus indicating that compound **3** was also a 3,4-*seco*-lupane. Analysis of the NMR spectra of **3** indicated that it had the same structure as **2**, except for ring E. The ^1H and ^{13}C NMR spectra of **3** showed signals for a second isopropenyl group (CH_3-30: δ_C 20.9, δ_H 1.71; CH_2-29: δ_C 115.0, δ_H 4.81 and 4.66; C-20: δ_C 143.5), which was located at C-19 by the HMBC correlations of C-29 and C-20 with H-19 (δ 2.33, dd, $J = 12$, 5 Hz) and those of C-20 with H-18 (δ 1.41, t, $J = 12$ Hz) and of C-18 (δ 47.8) with H_3-28 (δ 1.02 s). The presence of an OH group at C-21 was suggested by the signal at δ 3.94 (ddd, $J =$ 8, 5, 1 Hz) and its correlations, in the HMBC spectrum, with C-17 (δ 41.6) and C-20 as well as those of C-21 (δ 77.2) with H-19, H-22α (δ 1.57), and H-22β (δ 1.52). Jones oxidation of **3** yielded ketone **4**, whose ^1H NMR spectrum showed signals for the protons α to the carbonyl group (H-19, H-22α, and H-22β), shifted to low field as compared to those of the same protons of **3**, thus confirming the OH group at C-21 in **3**. The β-orientation of this group was established by NOESY interactions of H-21 with CH_3-30, H-22α, H-22β (weak), and H-19 (weak) and by the absence of any interaction between H-21 and CH_3-28. Comparison of the NMR data of ring E with those of other 21β-hydroxy lupanes[13,14] gave further support to this assumption. Thus, the structure of lippiolic acid (**3**) was established as 21β-hydroxy-3,4-*seco*-lupa-4(23),20(29)-dien-3-oic acid.

Compound **5** was identified as cycloart-23-ene-3β,25-diol by comparison of its spectroscopic data with those reported in the literature.[15]

The anti-inflammatory effect of compounds **1**, **3**, and **5** on TPA-induced mouse ear edema was evaluated with doses of 1 μmol/ear. Results showed that the percentages of edema inhibition of **1** and **3** were almost double that of **5** (74.9, 74.5, and 41.2%, respectively). Solubility problems at concentrations above 1 μM/ear prevented determination of the IC_{50} of compound **3**, but that of **1** (IC_{50} 0.73 μM/ear) was 3 times higher than that of the reference compound, indomethacin (IC_{50} 0.24 μM/ear).

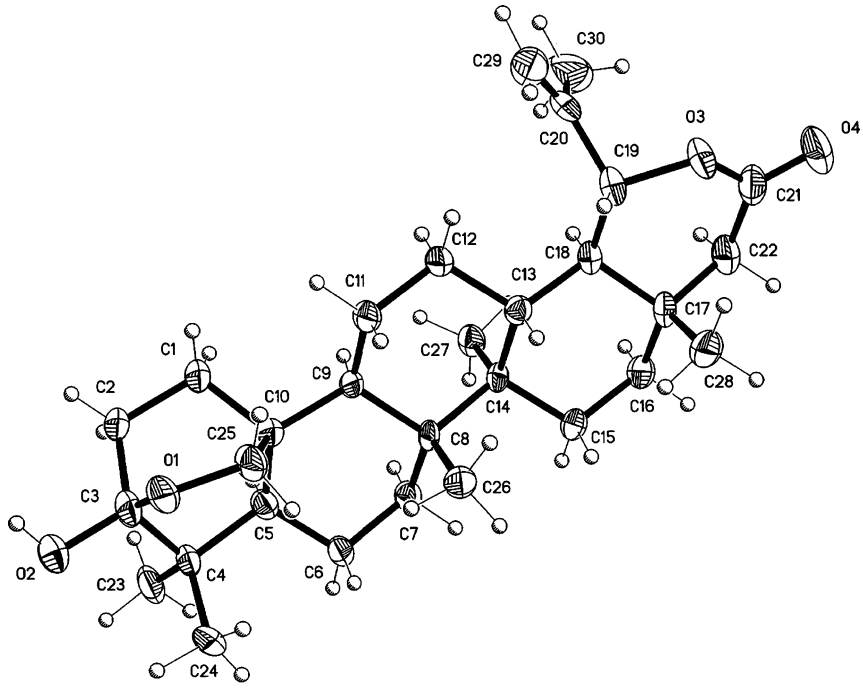

Figure 2. X-ray diffraction structure of compound **1**.

Table 1. ^{1}H NMR Spectroscopic Data (500 MHz, $CDCl_3$) for Compounds **1**−**4**

	lippiolide (**1**)		lippiolidolic acid (**2**)	lippiolic acid (**3**)[a]	**4**
position	δ, mult. (J in Hz)	HMBC	δ, mult. (J in Hz)		
1a	2.17, m	2, 3, 5, 10, 25	1.61, m	1.58, m	1.61, m
1b	1.13, dt (13, 3)		1.61, m	1.56, m	1.61, m
2a	2.15, m	1, 3, 10	2.38, m	2.31, m	2.38, m
2b	1.67, m		2.17, m	2.12, m	2.20, m
5	1.19, m	1, 3, 4, 6, 10, 23, 24, 25	1.94, dd (13, 2.5)	1.94, dd (12.5, 2.5)	1.95 m
6a	1.57, m	7	1.74, m	1.78, m	1.79, m
6b	1.50, m		1.40, m	1.35, m	1.39, m
7a	1.46, m	5, 9	1.37, m	1.41, m	1.39, m
7b	1.27, m	26	1.37, m	1.36, m	1.39, m
9	1.42, m	8, 10, 11, 12, 25, 26	1.47, m	1.49, m	1.51, dd (12.5, 3)
11a	1.57, m		1.39, m	1.36, m	1.38, m
11b	1.02, m		1.25, m	1.26, m	1.31, m
12a	1.66, m		1.68, m	1.67, m	1.62, m
12b	0.96, m		0.97, m	1.10, m	1.14, m
13	1.74, td (10.5, 3)	18	1.75, m	1.82, td (12, 3.5)	1.93, m
15a	1.66, m	14, 27	1.72, m	1.69, m	1.76, m
15b	1.19, m	14	1.15, m	1.04, m	1.15, m
16a	1.45, m	17, 22, 28	1.45, m	1.48, m	1.64, m
16b	1.34, ddd (13.5, 4.5, 3)	14, 18	1.36, m	1.34, m	1.59, m
18	1.79, t (10)	13, 17, 22, 28	1.77, m	1.41, t (12)	1.97, m
19	4.52, d (10)	13, 18, 20, 21, 29, 30	4.54, d (10)	2.33, dd (12, 5)	2.68, d (11.5)
21				3.94, ddd (8, 5, 1)	
22α	2.10, dd (17, 1)	16, 17, 18, 21, 28	2.11, dd (17, 1)	1.57, m	2.19, dd (17, 1)
22β	2.33, d (17)	13, 16, 17, 18, 21, 28	2.34, d (17)	1.52, m	1.97, d (17)
23a	1.02, s	3, 4, 5, 24	4.83, t (1.5)	4.83, br s	4.86, t (1.5)
23b			4.66, d (1.5)	4.66, d (1.5)	4.66, t (1.5)
24	0.97, s	3, 4, 23	1.73, s	1.73, s	1.74, s
25a	4.24, dd (9, 3)	1, 5	0.85, s	0.83, s	0.86, s
25b	3.71, dd (9, 2)	3, 5, 10			
26	0.956, s	7, 8, 9, 14	1.09, s	1.09, s	1.12, s
27	0.96, s	13, 14, 15	1.00, s	0.96, s	1.06, s
28	1.017, s	16, 17, 22	1.03, s	1.02, s	0.92, s
29a	5.06, br s	19, 20, 30	5.07, br s	4.81, d (2)	4.94, t (1.5)
29b	5.01, br t (1)	19, 30	5.03, t(1)	4.69, br s	4.81, br s
30	1.77, br s	19, 20, 29	1.78, s	1.71, s	1.71, s

[a] Determined in $CDCl_3$−DMSO-d_6; CO_2H signal at δ 11.4 br.

Table 2. ^{13}C NMR Spectroscopic Data (δ, mult., 125 MHz, $CDCl_3$) for Compounds **1**−**4**

position	lippiolide (**1**)	lippiolidolic acid (**2**)	lippiolic acid (**3**)[a]	**4**
1	35.2, CH_2	33.9, CH_2	33.9, CH_2	33.9, CH_2
2	29.5, CH_2	28.1, CH_2	28.1, CH_2	28.1, CH_2
3	98.2, C	178.0, C	176.0, C	178.6, C
4	40.4, C	147.2, C	147.4, C	147.4, C
5	49.7, CH	50.3, CH	50.0, CH	50.5, CH
6	19.6, CH_2	24.4, CH_2	24.4, CH_2	24.5, CH_2
7	31.7, CH_2	32.5, CH_2	32.4, CH_2	32.5, CH_2
8	40.0, C	40.7, C	40.3, C	40.72, C
9	44.8, CH	40.5,CH	40.4,CH	40.67,CH
10	35.4, C	39.2, C	38.9, C	39.2, C
11	22.1, CH_2	21.5, CH_2	21.1, CH_2	21.3, CH_2
12	25.9, CH_2	25.5, CH_2	24.5, CH_2	25.3, CH_2
13	37.2, CH	37.1, CH	37.4, CH	37.3, CH
14	42.0, C	42.7, C	42.8, C	43.1, C
15	26.2, CH_2	26.1, CH_2	26.9, CH_2	27.0, CH_2
16	35.4, CH_2	35.5, CH_2	35.5, CH_2	34.8, CH_2
17	33.6, C	33.6, C	41.6, C	37.9, C
18	40.8, CH	41.0, CH	47.8, CH	47.0, CH
19	87.2, CH	87.4, CH	59.4, CH	59.1, CH
20	144.1, C	144.1, C	148.2, C	143.5, C
21	170.3, C	170.5, C	77.2, CH	217.7, C
22	46.6, CH_2	46.6, CH_2	49.5, CH_2	55.4, CH_2
23	26.9, CH_3	113.7, CH_2	112.9, CH_2	113.6, CH_2
24	18.3, CH_3	23.2, CH_3	23.1, CH_3	23.2, CH_3
25	67.9, CH_2	20.1, CH_3	19.8, CH_3	20.1, CH_3
26	16.3, CH_3	15.9, CH_3	15.7, CH_3	16.0, CH_3
27	14.4, CH_3	14.7, CH_3	14.2, CH_3	14.5, CH_3
28	17.7, CH_3	17.7, CH_3	19.4, CH_3	18.7, CH_3
29	116.9, CH_2	117.0, CH_2	110.9, CH_2	115.0, CH_2
30	18.7, CH_3	18.7, CH_3	19.6, CH_3	20.9, CH_3

[a] Determined in $CDCl_3$−DMSO-d_6.

Experimental Section

General Experimental Procedures. Melting points (uncorrected) were determined on a Fisher-Johns apparatus. Column chromatography (CC) was performed on silica gel 60 (Merck G) and assisted with vacuum. TLC was carried out on precoated Macherey-Nagel Sil G/UV_{254} plates of 0.25 mm thickness. Preparative TLC was carried out on precoated Macherey-Nagel Sil G-/UV_{254} plates of 2.0 mm thickness. Optical rotations were measured on a Perkin-Elmer 343 polarimeter. The IR spectra were recorded on a Bruker Tensor 27 spectrometer. ^{1}H and ^{13}C NMR spectra were recorded either on a Varian Unity Plus 500 (^{1}H at 500 MHz; ^{13}C at 125 MHz) or on a Varian Unity (^{1}H at 300 MHz; ^{13}C at 75 MHz) spectrometer, with TMS as internal standard. EIMS and ESIMS were recorded on a JEOL JMS-AX505HA or on an ESI ion trap Bruker Esquire 6000 mass spectrometer. HRFABMS (polyethylene glycol 600 as standard) were measured on a JEOL JMS-SX102A spectrometer.

Plant Material. Aerial parts of *Lippia mexicana* were collected in Huiramba, State of Michoacán, México, in June 2006. A voucher specimen of the plant (M. Martínez 6678) was identified by Dr. Mahinda Martínez and deposited at the Herbarium of the Universidad Autónoma de Querétaro.

Extraction and Isolation. Dried and ground leaves, flowers, and stems of *L. mexicana* (866.2 g) were extracted with Me_2CO and then with MeOH to obtain 53.2 and 50.6 g of extracts, respectively. These extracts showed similar profiles by TLC; therefore, they were combined and fractioned by CC (column A) eluted with mixtures of hexane−EtOAc of increasing polarity to give seven fractions (1:0, A1; 19:1, A2; 9:1 to 17:3, A3; 17:3 to 4:1, A4; 4:1 to 7: 3, A5; 3:2 to 2:3, A6; 0:1, A7). Fraction A3 (9.37 g) was subjected to CC (column B) eluted with hexane−EtOAc (9:1 to 1:1) to obtain fractions B1−B5. Fraction B2 (3.24 g; eluted with hexane−EtOAc, 9:1) was purified by two successive CC runs, eluted with hexane−EtOAc (9:1) and hexane−Me_2CO (19:1), respectively, to obtain 48.6 mg of **5**. Compound **1** (535 mg) from fraction A4 was crystallized from EtOAc−hexane.

The mother liquors of **1** and fraction A5 were combined (10.33 g) and separated by silica gel CC (column C, hexane−EtOAc, 9:1 to 1:1) to give fractions C1−C7. Fractions C3−C5 (3.81 g; eluted with hexane−EtOAc, 17:3, 4:1, and 3:1) were purified by CC (column D, hexane−*i*PrOH, 24:1) to obtain fractions D1−D6. Crystallization of fraction D3 afforded **1** (338.7 mg). The mother liquors of **1** and fraction D2 (605 mg) were subjected to silica gel CC (CH_2Cl_2−Me_2CO, 97:3) to obtain fractions E1−E6. Compound **1** (92.6 mg) was isolated from fraction E4. Fraction E2 gave **2** (42.5 mg) after purification by silica gel CC (hexane−Me_2CO, 17:3) followed by preparative TLC (hexane−Me_2CO, 3:1) and crystallization in EtOAc−hexane. Fraction A6 (6.83 g) was fractioned by CC (column G; hexane−EtOAc, 4:1 to 1:1) to obtain fractions G1−G8 (4:1 G1−G4; 3:1 G5; 7:3 G6; 3:2 G7; 1:1 G8). Compound **3** (17 mg) was isolated from fraction G2 by crystallization in EtOAc−hexane, and its mother liquors were purified by CC (hexane−EtOAc, 17:3) to obtain 10.2 mg of **3**. Fraction G3 (1.45 g) was subjected to CC eluted with hexane−*i*PrOH (97:3) to obtain 403.3 mg of **1**, for a total of 1.37 g of compound **1**.

Lippiolide (1): colorless crystals (EtOAc−hexane); mp 244−246 °C; $[\alpha]^{20}_D$ +133 (*c* 0.208, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3591, 3433, 1726, 1653, 914 cm^{-1}; ^1H NMR and ^{13}C NMR ($CDCl_3$) see Tables 1 and 2; EIMS *m/z* 470 $[M]^+$ (100), 455 (13), 439 (12), 397 (43), 261 (10), 235 (16), 209 (11), 175 (14), 161 (15), 121 (25), 107 (33), 93 (27), 69 (28); HRFABMS *m/z* 471.3477 $[M + H]^+$ (calcd for $C_{30}H_{47}O_4$, *m/z* 471.3474).

Lippiolidolic Acid (2): amorphous solid; $[\alpha]^{20}_D$ +70 (*c* 0.160, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3650−2450, 1735, 1705, 1637 cm^{-1}; ^1H NMR and ^{13}C NMR ($CDCl_3$) see Tables 1 and 2; ESIMS *m/z* 493 $[M + Na]^+$, 509 $[M + K]^+$; HRFABMS *m/z* 471.3476 $[M + H]^+$ (calcd for $C_{30}H_{47}O_4$, *m/z* 471.3474).

Lippiolic Acid (3): colorless crystals; mp 246−248 °C, $[\alpha]^{20}_D$ +57 (*c* 0.165, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3650−2450, 1710, 1637 cm^{-1}; ^1H NMR and ^{13}C NMR ($CDCl_3$−DMSO-d_6) see Tables 1 and 2; EIMS *m/z* 456 $[M]^+$ (18), 438 (16), 412 (12), 383 (19), 357 (100), 261 (24), 175 (34), 135 (43), 109 (72), 84 (82), 28 (83); HRFABMS *m/z* 457.3678 $[M + H]^+$ (calcd for $C_{30}H_{49}O_3$, *m/z* 457.3682).

Oxidation of Compound 3. Jones reagent was added dropwise (until persistence of orange color) to a solution of **3** (10.2 mg) in Me_2CO (2 mL) at 0 °C. The reaction was worked up as usual to obtain 5.5 mg of **4** after crystallization from EtOAc−hexane. Compound **4**: colorless crystals; mp 175−177 °C; IR ($CHCl_3$) ν_{max} 3600−2400, 1734, 1642 cm^{-1}; ^1H NMR and ^{13}C NMR ($CDCl_3$) see Tables 1 and 2; EIMS *m/z* 454 $[M]^+$ (57), 439 (7), 381 (51), 373 (49), 355 (100), 337 (17), 107 (41), 81 (37), 69 (36), 55 (35), 43 (22).

X-ray Crystallographic Data of Lippiolide (1). Data were collected from a colorless needle (0.486 × 0.102 × 0.076 mm) at 100(2) K on a Bruker Smart Apex CCD diffractometer with monochromated Mo Kα radiation (λ 0.71073 Å): $C_{32.55}H_{51.96}O_4$, (formula weight sum 507.34); hexagonal, space group $P6_5$; $a = 20.278(2)$ Å, $b = 20.278(2)$ Å, $c = 11.852(2)$ Å; $\alpha = \beta = 90°$, $\gamma = 120°$; $V = 4220.5(8)$ $Å^3$; $Z = 6$; $D_{calc} = 1.198$ Mg/m^3; $F(000) = 1676$. A total of 35 168 reflections were collected in the range $2.01° < \theta < 27.94°$, with 3530 independent reflections [R(int) = 0.0916], completeness to θ_{max} was 99.7%. The structure was solved by direct methods and refined by full matrix least-squares on F^2, with anisotropic temperature factors for non-hydrogen atoms converging at final R indices [$I > 2\sigma(I)$], $R_1 = 0.0670$, $wR_2 = 0.1453$. Hydrogen atoms, except those bonded to oxygen, were included at calculated positions and were not refined. Crystallographic data for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre, CCDC 795689. Copies of the data can be obtained, free of charge, on application to the director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).

12-*O*-Tetradecanoylphorbol 13-Acetate (TPA)-Induced Ear Edema. Anti-inflammatory activity was evaluated as previously described.[16] Groups of five male CD-1 mice weighing 25−30 g were anaesthetized with sodium pentobarbital. TPA (2.5 μg) dissolved in EtOH (10 μL) was topically applied to both sides of the right ear of the mice (5 μL each side). The left ear received only EtOH (10 μL). After 10 min, doses of 0.1 to 1.0 μM of the test compounds, or indomethacin as reference, dissolved in 20 μL of EtOH−CH_2Cl_2 (1:1) were applied to the right ear (10 μL each face). Control animals received only vehicle (20 μL). Four hours later the animals were sacrificed by cervical dislocation, and a plug (7 mm diameter) was removed from each ear. The edematous response was measured as the weight difference between the two punches. The percent of inhibition was calculated by the following equation: $\% = [(A - B)/A]100$; A = edema induced by TPA; B = edema induced by TPA plus sample. Data were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's test. The IC_{50} values (μM/ear, determined using Me_2CO−CH_2Cl_2, 1:1, as vehicle) were estimated from the linear regression equation.

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Supporting Information Available: IR and NMR spectra of compounds **1**−**4**, a table with the HMBC correlations of **2**−**4**, and the X-ray data of **1** are available free of charge via the Internet at http://pubs.acs.org.

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