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Terpenoids from *Melampodium perfoliatum*

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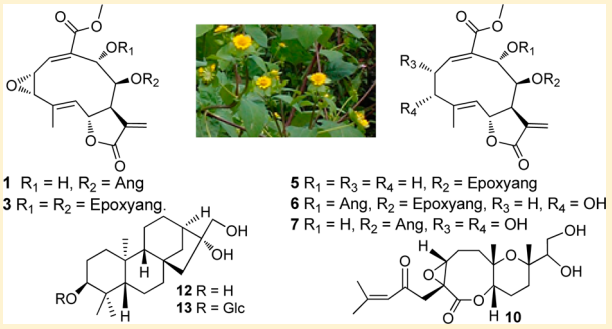
Supporting Information

ABSTRACT: The phytochemical study of the aerial parts of *Melampodium perfoliatum* afforded six melampolides (**1**, **3**, **5**−**8**), a eudesmanolide (**9**), two diterpene lactones (**10**, **11**), and two ent-kaurane derivatives (**12**, **13**), together with the known melampodin (**2**) and polymatin A (**4**). The structures of the compounds were elucidated by physical data analysis and chemical reactions. Compounds **2**, **4**, **5**, and **10** exhibited dose-dependent anti-inflammatory activity on the 12-*O*-tetradecanoylphorbol-13-acetate-induced ear edema model, with ID_{50} values of 1.14, 0.56, 1.15, and 1.49 μmol/ear, respectively, compared to the reference compound indomethacin (0.24 μmol/ear).



The genus *Melampodium* (Asteraceae, Heliantheae, Melampodinae) comprises 40 annual and perennial species widespread in tropical and subtropical regions of Mexico and Central America, with five species found in southwestern United States and three in Colombia and Brazil.[1] This genus can be characterized by the presence of melampolide-type germacranolides;[2] however, flavonoids[3] and some diterpenoids[4,5] have also been reported. Melampolides are known to present a wide variety of biological activities including antitumor,[6] antidiabetic,[7] anti-inflammatory,[8] and fungicidal.[9] The aim of this paper is to contribute to the chemistry of the genus studying the aerial parts of *Melampodium perfoliatum* and to search for anti-inflammatory compounds. In previous studies, Bohlmann and co-workers[5] reported the presence of kaurenoic acid derivatives, polyacetylenes, and enhydrin in the roots of *M. perfoliatum*, and the effect of its crude syrups on fall armyworm was investigated by Fischer and co-workers.[10] Herein the isolation and characterization of 11 new terpenoids—six melampolides (**1**, **3**, **5**−**8**), one eudesmanolide (**9**), two diterpene lactones (**10**, **11**), and two *ent*-kaurene derivatives (**12**, **13**)—in addition to the known melampodin (**2**)[11,12] and polymatin A (**4**)[13] are reported. The structures of these compounds were defined by physical data analysis and chemical reactions. The anti-inflammatory activity of extracts and more abundant compounds **2**−**5**, **8**, **10**, and **12** was evaluated using the 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced edema model of acute inflammation.

RESULTS AND DISCUSSION

Perfoliatin A (**1**) has a molecular formula of $C_{21}H_{24}O_8$ according to its HRDARTMS and ^{13}C NMR data. The IR spectrum showed the presence of hydroxy (3480 cm^{-1}), conjugated γ-lactone (1764 cm^{-1}), and α,β-unsaturated ester (1715 cm^{-1}) fuctionalities. Its NMR data (Table 1) were similar to those of melampodin (**2**).[11] The resonances at δ_H 6.23 (d, J = 3.2 Hz, H-13a), 5.66 (d, J = 3.2 Hz, H-13b), 2.59 (dd, J = 10.0, 1.6 Hz, H-7), and 5.21 (t, J = 10.0 Hz, H-6) and at δ_C 120.9 (CH_2, C-13), 169.0 (C, C-12), 134.4 (C, C-11), 50.7 (CH, C-7), and 75.7 (CH, C-6) were indicative of an α-methylene-γ-lactone moiety. An angeloyloxy residue was deduced from the resonances of two methyl groups at δ_H 1.91 (quint, J = 1.2 Hz, H-5′) and 1.99 (dq, J = 7.2, 1.2 Hz, H-4′) coupled to that of a methine proton at δ_H 6.13 (qq, J = 7.2, 1.2 Hz, H-3′). In the HMBC spectrum, a correlation of H-8 (δ_H 6.27, dd, J = 8.4, 1.6 Hz) with C-1′ (δ_C 167.4) permitted the assignment of this ester group at C-8. Correlations of H-2 (δ_H 3.62, dd, J = 3.6, 2.4 Hz) and H-3 (δ_H 3.63, brd, J = 3.6 Hz) with C-4 and C-1 defined the 2,3-epoxide function, and cross-peaks of H-1 and H-9 with C-14 established a C-14 methoxycarbonyl group. A hydroxy group was assigned to C-9 by the COSY correlation between H-9 (δ_H 4.04, d, J = 8.4 Hz) and H-8. The 1(10)*E*,4*E*-cyclodecadiene, i.e., melampolide skeleton, was inferred from the NOESY correlations of H-1 with the C-14 methoxy group and H-5 and of H_3-15 with H-6. In the same spectrum, correlations of H-8 with H-7, as well as their small coupling constant $J_{7,8}$ = 1.6 Hz, indicated that they are cofacial and α-oriented, based upon the biogenetic considerations regarding the orientation of H-7.[14] The large $J_{6,7}$ = 10.0 Hz and $J_{8,9}$ = 8.4 Hz coupling constants defined the β-orientation of H-6 and H-9 and disclosed a *trans*-fused lactone ring. Compound **1** has, therefore, the same relative configuration as described for melampodin (**2**),[11,12] thus possessing a 10-membered ring system in which the preferred conformation has C-14 and C-15 in an *anti*-arrangement, typical of melampolides.[15] Additionally, the NOESY spectrum

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Chart 1

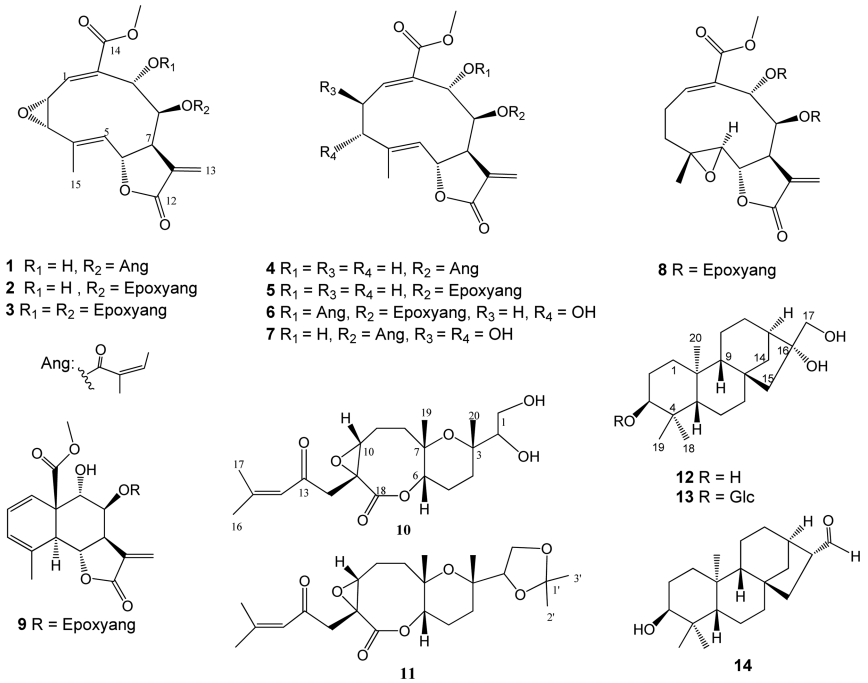

Table 1. NMR Spectroscopic Data (400 MHz, $CDCl_3$) for Compounds 1, 3, and 5

	1		3		5[a]	
position	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1	6.92, d (2.4)	134.8, CH	7.02, d (2.0)	139.2, CH	6.86, dd (10.5, 7.5)	144.5, CH
2a	3.62, dd (3.6, 2.4)	56.3, CH	3.64, dd (3.6, 2.4)	56.5, CH	2.50, dddd (13.5, 7.5, 6.0, 2.5)	25.8, CH_2
2b					2.26, dddd (13.5, 12.5, 10.5, 2.5)	
3a	3.63, brd (3.6)	60.3, CH	3.77, d (3.3)	60.6, CH	2.37, ddd (13.5, 6.0, 2.5)	36.8, CH_2
3b					2.04, brt (13.5)	
4		131.3, C		135.5, C		137.8, C
5	5.32, dq (10.0, 1.6)	122.9, CH	5.33, dq (10.4, 1.2)	122.4, CH	4.95, brd (10.5)	126.4, CH
6	5.21, t (10.0)	75.7, CH	5.26, t (10.4)	75.2, CH	5.09, t (10.5)	73.1, CH
7	2.59, dd (10.0, 1.6)	50.7, CH	2.72, dd (10.4, 1.2)	50.0, CH	2.64, brd (10.0)	51.3, CH
8	6.27, dd (8.4, 1.6)	70.8, CH	6.78, dd (8.8, 1.2)	69.2, CH	6.29, dd (8.5, 2.0)	71.0, CH
9	4.04, d (8.4)	72.0, CH	5.40, d (8.8)	72.8, CH	3.96, m	75.3, CH
10		134.5, C		127.3, C		133.5, C
11		134.4, C		134.0, C		134.8, C
12		169.0, C		168.6, C		169.1, C
13a	6.23, d (3.2)	120.9, CH_2	6.27, d (3.2)	121.2, CH_2	6.24, d (3.5)	121.0, CH_2
13b	5.66, d (3.2)		5.72, d (3.2)		5.64, d (3.5)	
14		166.7, C		165.3, C		166.9, C
15	2.09, d (1.6)	17.2, CH_3	2.18, d (1.2)	17.4, CH_3	1.91, d (1.0)	16.8, CH_3
1′		167.4, C		168.6, C		169.7, C
2′		126.6, C		59.2, C		59.6, C
3′	6.13, qq (7.2, 1.2)	140.4, CH	3.06, q (5.2)	60.0, CH	3.05, q (5.5)	59.9, CH
4′	1.99, dq (7.2, 1.2)	15.9, CH_3	1.22, d (5.2)	13.7, CH_3	1.28, d (5.5)	13.7, CH_3
5′	1.91, quint (1.2)	20.5, CH_3	1.52, s	19.3, CH_3	1.57, s	19.2, CH_3
1″				169.6, C		
2″				59.5, C		
3″			3.02, q (5.2)	60.0, CH		
4″			1.16, d (5.2)	13.3, CH_3		
5″			1.48, s	18.7, CH_3		
OCH_3	3.85, s	52.8, CH_3	3.83, s	52.9, CH_3	3.81, s	52.9, CH_3

[a]500 MHz.

also showed cross-peaks between H_3-15, H-9, and H-3, indicating the α-orientation of the epoxide function. The absolute configuration of melampodin (**2**) has been reported,[12] and since the ECD spectrum of **1** was similar to that of **2**, the

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positive Cotton effect at λ 245 nm permitted definition of the absolute configuration of perfoliatin A as [2*R*, 3*S*, 6*R*, 7*S*, 8*S*, 9*S*, 1(10)*E*, 4*E*].

Perfoliatin B (**3**) has a molecular formula of $C_{26}H_{30}O_{11}$ as determined by HRDARTMS and ^{13}C NMR data. The IR and NMR data for compound **3** were similar to those of compound **1** except for the presence of typical NMR resonances (Table 1) of two 2,3-epoxyangeloyloxy units. In the HMBC spectrum, correlations between H-8 (δ_H 6.78, dd, J = 8.8, 1.2 Hz) and H-9 (δ_H 5.40, d, J = 8.8 Hz) with C-1′ and C-1″ permitted the assignment of these ester groups to C-8 and C-9, respectively. The NOESY spectrum of perfoliatin B confirmed the same relative configuration as for compounds **1** and **2**, and since the ECD spectrum showed the same pattern as that of melampodin (**2**), the absolute configuration of **3** could be defined as [2*R*, 3*S*, 6*R*, 7*S*, 8*S*, 9*S*, 1(10)*E*, 4*E*].

Perfoliatin C (**5**) showed a molecular formula of $C_{21}H_{26}O_8$ by analysis of its HRDARTMS and ^{13}C NMR data. In the ^1H NMR data of compound **5** (Table 1) the resonances of the 2,3-epoxy group, present in melampodin, were replaced by those of H_2-2 (δ_H 2.50, dddd, J = 13.5, 7.5, 6.0, 2.5 Hz, H-2a and 2.26, dddd, J = 13.5, 12.5, 10.5, 2.5 Hz, H-2b) and H_2-3 (δ_H 2.37, ddd, J = 13.5, 6.0, 2.5 Hz, H-3a and 2.04, brt, J = 13.5 Hz, H-3b). The NOESY spectrum of **5** exhibited essentially the same correlations as those of compounds **1** and **3**; therefore its relative configuration should be the same. The [6*R*, 7*S*, 8*S*, 9*S*, 1(10)*E*, 4*E*] absolute configuration was deduced from the similarity of the ECD spectrum of **1**, perfoliatin C, with that of melampodin.

Perfoliatin D (**6**) and perfoliatin E (**7**) showed IR and NMR spectroscopic data closely related to those of **5**, and their molecular formulas were established as $C_{26}H_{32}O_{10}$ and $C_{21}H_{26}O_9$, respectively, by HRDARTMS and ^{13}C NMR data. In perfoliatin D, additional resonances of an angeloyloxy residue and of an oxymethine at δ_H 4.60 (brd, J = 4.4 Hz) were observed. The angeloyloxy moiety was assigned to C-9 by analysis of the HMBC spectrum, and the oxymethine to C-3 by the COSY correlation between H-3 (δ_H 4.60, brd, J = 4.4 Hz) and H_2-2 (δ_H 2.86, m). Oxymethine resonances in the NMR spectrum of **7** at δ_H 5.07 (dd, J = 6.0, 5.2 Hz), 4.65 (d, J = 5.2 Hz), and 4.85 (d, J = 8.4 Hz) were assigned to H-2, H-3, and H-9, respectively, on the basis of COSY and HMBC correlations. Similarly, an angeloyloxy group was located at C-8. In the NOESY spectrum of perfoliatin D (Figure 1), a

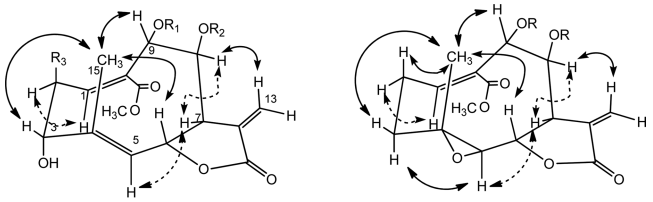

Figure 1. NOESY correlations of compounds **6**–**8**.

correlation of H-3 with H_3-15 indicated the α-orientation of the hydroxy group. In perfoliatin E, NOESY correlations of H-1 with H-2 and of H-3 with H_3-15 defined the β- and α-orientations of the hydroxy groups at C-2 and C-3, respectively. Additional NOESY correlations (Figure 1) showed that **6** and **7** had similar relative configurations to compounds **1**–**3** and **5**. Nevertheless, the ECD spectrum of these compounds did not show the same pattern as that of melampodin; therefore their absolute configurations were not defined.

Perfoliatin F (**8**) showed a molecular formula of $C_{26}H_{32}O_{11}$ on the basis of HRDARTMS and ^{13}C NMR spectra. ^1H and ^{13}C NMR spectroscopic data of **8** were comparable to those of **5**, with the exception of the resonances of H-5 (δ_H 2.67, d, J = 9.5 Hz), C-5 (δ_C 62.7), H_3-15 (δ_H 1.71, s), C-15 (δ_C 17.4), and C-4 (δ_C 59.2), assigned to a 4,5-epoxide function and those of a second epoxyangeloyloxy group located at C-9 by the HMBC cross-peaks. The NOESY spectrum (Figure 1) showed correlations of H-1 with H-2α (δ_H 2.45, dddd, J = 13.5, 7.0, 7.0, 2.0 Hz), of H-5 with H-7 and H-3α (δ_H 1.24, brd, J = 13.5 Hz), of H-9 with H-6 and H-2β (δ_H 2.99, m), and of H_3-15 with H-9 and H-6, indicating the *trans*-4,5-epoxide function and a relative configuration similar to that of melampodin. The ECD spectrum of perfoliatin F exhibited the same pattern as that of compound **2**; therefore it possesses a [4*R*, 5*R*, 6*R*, 7*S*, 8*S*, 9*S*, 1(10)*E*] absolute configuration.

De-*O*-acetylmeleucanthin (**9**) exhibited a molecular formula of $C_{21}H_{24}O_8$ according to its HRDARTMS and ^{13}C NMR data. The IR spectrum revealed the presence of hydroxy (3522 cm^{-1}), γ-lactone (1770 cm^{-1}), and ester (1713 cm^{-1}) functionalities. Analysis of the 1D and 2D NMR spectra showed the presence of an α-methylene-12,6-γ-lactone moiety (Table 2), as in compounds **1**–**8**. The olefinic methines H-1 at δ_H 5.29 (brd, J = 9.5 Hz), H-2 at δ_H 5.93 (dd, J = 9.5, 5.5 Hz,), and H-3 at δ_H 5.69 (d, J = 5.5 Hz) were assigned by the cross-peaks observed in the COSY experiment. An epoxyangeloyloxy moiety at C-8 (δ_C 72.5) was deduced by the HMBC correlation between H-8 (δ_H 5.67, t, J = 3.0 Hz) and C-1′ (δ_C 168.9), and a hydroxy group was assigned to C-9 (δ_C 70.7) by the COSY correlation between H-9 (δ_H 4.39, d, J = 3.0 Hz) and H-8. The eudesmanolide skeleton was deduced from the HMBC correlations of H-1 and H-9 with C-5 (δ_C 42.5) and of H-5 (δ_H 3.04, d, J = 11.5 Hz) with the C-14 carbonyl carbon (δ_C 171.3). The relative configuration of **9** was defined by the NOESY correlations of H-7 with H-5 and H-8 that suggested their α-orientation, based upon biogenetic considerations regarding the orientation of H-7.[14] H-8 ($J_{7,8}$ = 3.0 Hz) and H-9 ($J_{8,9}$ = 3.0 Hz) should be α- and β-equatorially oriented, respectively, as defined by correlations of H-9 with H-1 and H-8. The coupling constants of the CH_2-13 (d, J = 3.0 Hz)[16] and the $J_{6,7}$ value of 11.5 Hz indicated a *trans*-fused γ-lactone moiety with a dihedral angle between H-6 and H-7 of around 170° reminiscent of the β-axial orientation of H-6. The C-14 methoxycarbonyl group was considered β-oriented on biogenetic grounds.[14]

Compound **10** was obtained as a colorless oil. Its molecular formula, $C_{20}H_{30}O_7$, was assigned on the basis of HRDARTMS and ^{13}C NMR spectroscopic data. The IR absorption bands at 3468, 1712, 1685, and 1619 cm^{-1} were indicative of hydroxy, carbonyl, and conjugated carbonyl functionalities. In the ^{13}C NMR spectrum, the signals of two carbonyls and two vinylic carbons accounted for three of the six indices of hydrogen deficiency, indicating a tricyclic structure. The ^1H NMR spectrum showed a vinylic proton (δ_H 6.08, hept, J = 1.2 Hz, H-14), which correlated in the HMBC spectrum with C-16 (δ_C 20.9), C-17 (δ_C 27.8), and C-13 (δ_C 196.6). Correlations of the later with H_2-12 (δ_H 3.60, d, J = 17.2 Hz, H-12a and 2.38, d, J = 17.2 Hz, H-12b), observed in the same experiment, defined the presence of a methylpentenoyl moiety ($(CH_3)_2$-C=CHCOCH_2–). The NMR signals at δ_C 172.1 (C-18) and δ_H

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Table 2. NMR Spectroscopic Data (400 MHz, $CDCl_3$) for Compounds 6−8

	6		7		8[a]	
position	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1	7.06, dd (9.6, 8.0)	145.5, CH	6.94, d (6.0)	143.9, CH	7.17, dd (10.5, 7.5)	149.9, CH
2a	2.86, m	32.2, CH_2	5.07, dd (6.0, 5.2)	74.7, CH	2.99, m	24.6, CH_2
2b					2.45, dddd (13.5, 7.0, 7.0, 2.0)	
3a	4.60, brd (4.4)	74.0, CH	4.65, d (5.2)	76.3, CH	2.36, ddd (13.5, 7.0, 2.0)	35.3, CH_2
3b					1.24, brd (13.5)	
4		141.3, C		141.3, C		59.2, C
5	5.54, brd (11.2)	123.2, CH	5.30, brd (10.8)	125.8, CH	2.67, d (9.5)	62.7, CH
6	5.25, t (10.4)	74.9, CH	5.26, t (10.8)	75.2, CH	4.29, t (9.5)	75.8, CH
7	2.88, m	50.7, CH	2.74, brd (10.8)	51.6, CH	3.02, m	45.5, CH_2
8	6.79, dd (8.4, 1.6)	70.7, CH	6.33, dd (8.4, 1.6)	71.5, CH	6.81, dd (8.5, 1.0)	70.7,CH
9	5.36, d (8.4)	70.7, CH	4.85, d (8.4)	71.3, CH	5.90, d (8.5)	71.6, CH
10		131.6, C		135.5, C		129.8, C
11		134.2, C		134.8, C		133.2, C
12		169.1, C		169.2, C		167.9, C
13a	6.29, d (3.2)	121.9, CH_2	6.32, d (1.6)	121.3, CH_2	6.34, d (3.0)	122.9, CH_2
13b	5.79, d (3.2)		5.68, d (1.6)		5.85, d (3.0)	
14		165.6, C		166.9, C		165.2, C
15	2.01, brs	15.9, CH_3	2.03, d (1.2)	18.3, CH_3	1.71, s	17.4, CH_3
1′		168.5, C		167.7, C		168.5, C
2′		59.4, C		127.0, C		59.3, C
3′	3.02, q (5.2)	58.9, CH	6.13, qq (7.2, 1.2)	139.9, CH	3.04, m	60.0, CH
4′	1.16, d (5.2)	13.7, CH_3	1.99, dq (7.2, 1.2)	15.9, CH_3	1.19, d (5.5)	13.4, CH_3
5′	1.43, s	19.1, CH_3	1.88, quint (1.2)	20.6, CH_3	1.47, s	18.8, CH_3
1″		166.8, C				169.5, C
2″		126.5, C				59.6, C
3″	6.17, qd (6.0, 1.2)	141.2, CH			3.04, m	60.0, CH
4″	1.95, dq (6.0, 1.2)	15.9, CH_3			1.18, d (5.5)	13.5, CH_3
5″	1.81, quint (1.2)	20.5, CH_3			1.52, s	19.1, CH_3
OCH_3	3.79, s	52.2, CH_3	3.82, s	52.5, CH_3	3.83, s	52.6, CH_3

[a]500 MHz.

4.22 (dd, J = 8.0, 6.5 Hz, H-6) indicated the presence of a saturated 18,6-ζ-lactone unit.[17,18] In the HMBC spectrum, correlations of H-12 with C-18, C-11, and C-10; of H_2-8 with C-10, C-9, and C-7; and of H_3-19 with C-8, C-7, and C-6 established the connectivity of the ζ-lactone moiety and the attachment of the methylpentenoyl moiety to C-11. A 10,11-epoxide function was deduced from the chemical shifts of H-10 (δ_H 3.44, dd, J = 3.2, 2.4 Hz), C-10 (δ_C 62.8), and C-11 (δ_C 61.0). The tetrahydropyran portion was evidenced by the chemical shifts of C-3 (δ_C 85.7) and C-7 (δ_C 84.0), by the COSY correlations of H-6 with H-5 and of H-5 with H-4, and by the HMBC correlations of H-5 with C-6 and C-7 and of H_3-20 with C-3. Finally, the HMBC spectrum showed correlations of H_2-1 (δ_H 3.74, dd, J = 11.2, 6.8 Hz, H-1a and 3.66, dd, J = 11.2, 3.6 Hz, H-1b) with C-2 and C-3 and of H-2 (δ_H 3.56, dd, J = 6.8, 3.6 Hz) with C-3, C-4, and C-20, in accordance with structure **10**. The relative configuration of the tricyclic ring system in compound **10** was deduced by the NOESY correlations of H_3-19 with H-6, H_3-20, and H-9b and of H-10 with H-12b and H-9b, indicating that they are cofacial.

Compound **11** is an artifact emanating from the isolation and purification processes of compound **10**. The molecular formula $C_{23}H_{34}O_7$ was deduced from analysis of HRDARTMS and ^{13}C NMR spectroscopic data. The IR absorptions at 1712, 1686, and 1620 cm^{-1} indicated carbonyl group and double-bond functionalities, but no hydroxy groups. Its ^1H NMR spectrum was highly similar to that of compound **10**, except for the paramagnetic shift of H_2-1 (δ_H 3.99, dd, J = 8.0, 3.6 Hz and 3.77, dd, J = 8.0, 7.6 Hz) and H-2 (δ_H 4.08, dd, J = 7.6, 3.6 Hz) and for the signals attributable to the diastereotopic methyl groups of the acetonide moiety (δ_H 1.56, s, H_3-2′ and δ_H 1.42, s, H_3-3′). ^{13}C NMR data were in agreement with structure **11**.

Compound **12** was obtained as colorless prisms, mp 245−246 °C. The molecular formula $C_{20}H_{34}O_3$, determined by HRFABMS and ^{13}C NMR, indicated an index of hydrogen deficiency of four. In the ^{13}C NMR spectrum the resonances of three methyls, nine methylenes (one oxygenated), four methines (one oxygenated), and four nonprotonated carbons (one oxygenated) and the absence of resonances of carbonyl and olefinic groups suggested a tetracyclic structure of the kaurane type with a primary, a secondary, and a tertiary oxygenated carbon. In the ^1H NMR spectrum the oxymethylene resonances at δ_H 3.69 (d, J = 11.0 Hz) and 3.58 (d, J = 11.0 Hz) were assigned to H_2-17 by their correlations with C-15, C-16, and C-13 in the HMBC spectrum. Likewise, the oxymethine resonance at δ_H 3.32 (t, J = 3.0 Hz) was attributed to H-3 by its cross-peaks with C-1 and C-5, and a third hydroxy group was located at C-16 (δ_C 82.8) by the correlations of this carbon with H-17, H-14, and H-15. The α-equatorial orientation of H-3 was deduced from the small coupling constants of H_2-2/H-3 (3.0 Hz). The C-16 hydroxy group was assigned an α-orientation via comparison of the chemical shifts of C-16 and C-17 with reported data.[19,20] The NOESY interactions of H_3-20 and H_3-18α indicated that they are cofacial and on the opposite side from H-9 and H_3-19, which correlated with H-5 in the same spectrum. Thus, the

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structure of compound **12** is defined as 3*β*,16*α*,17-trihydroxy-*ent*-kaurane.[21] The (3*S*,5*S*,8*S*,9*R*,10*S*,13*R*,16*R*) absolute configuration of **12** was established by X-ray crystallographic analysis[22] (Figure 2).

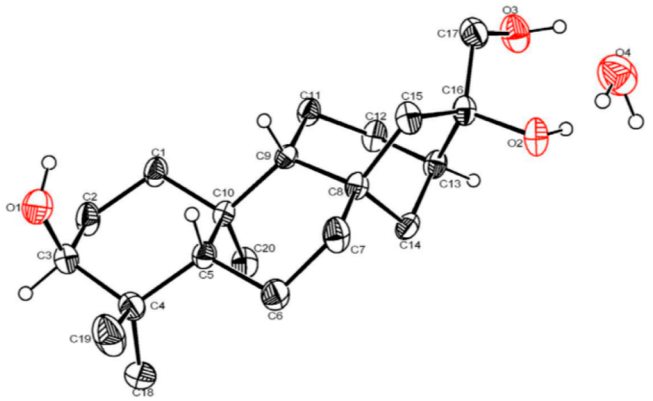

Figure 2. ORTEP drawing of compound **12**.

Compound **13** has a molecular formula of $C_{26}H_{44}O_8$ as determined by HRFABMS and ^{13}C NMR data. The NMR spectroscopic data of compound **13** were similar to those of **12** (Table 3), except for the signals of a sugar moiety. This sugar was identified as *β*-D-glucopyranose by the axial−axial coupling constants of H-1′, H-2′, H-3′, and H-4′ and by the isolation of D-glucose, $[\alpha]^{25}_D$ +56 (*c* 0.15, H_2O), following hydrolysis of **13**. In the ^{13}C NMR spectrum of compound **13**, C-3 appeared deshielded (δ_C 87.5) and correlated with the anomeric proton of the glucose (δ_H 4.29, d, *J* = 8.0 Hz) in the HMBC spectrum, indicating that compound **13** is the 3-*O*-*β*-D-glucopyranosyl derivative of **12**. Hydrolysis of glucoside **13** also afforded compound **14**, which exhibited a molecular formula of $C_{20}H_{32}O_2$ according to its HRDARTMS and ^{13}C NMR data. In the IR spectrum of **14** absorptions at 3476 and 1720 cm^{-1} indicated the presence of hydroxy and carbonyl groups. The ^1H NMR spectrum was similar to that of **12**, with the additional resonance of a formyl group at δ_H 9.65; this resonance was assigned to H-17 by its correlation with C-16 in the HMBC spectrum. As in compound **12**, H-3 showed (δ_H 3.42 t, *J* = 3.0 Hz) the same coupling constants, indicating its *α*-equatorial

Table 3. NMR Spectroscopic Data (500 MHz, CD_3OD) for Compounds 12−14

	12		**13**[a]			**14**[b]	
position	δ_H (*J* in Hz)	δ_C	δ_C[c]	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1a	1.49, m	34.5, CH_2	33.9, CH_2	1.48, m	35.3, CH_2	1.55, m	33.3, CH_2
1b	1.23, brt (13.5)			1.33, m		1.25, m	
2a	1.98, brt (13.5)	26.3, CH_2	26.4, CH_2	1.88, m	25.3, CH_2	1.96, brt (13.5)	25.3, CH_2
2b	1.48, m					1.53, m	
3	3.32, t (3.0)	76.8, CH	75.1, CH	3.35, m	87.5, CH	3.42, t (3.0)	76.0, CH
4		38.1, C	38.0, C		39.3, C		37.5, C
5	1.29, dd (12.0, 1.5)	50.0, CH	49.0, CH	1.35, m	50.4, CH	1.27, m	48.9, CH
6a	1.46, m	21.1, CH_2	20.5, CH_2	1.50, m	20.9, CH_2	1.48, m	20.3, CH_2
6b	1.37, m			1.39, m		1.32, m	
7a	1.60, m	43.2, CH_2	42.7, CH_2	1.57, m	43.1, CH_2	1.49, m	40.8, CH_2
7b	1.52, m						
8		45.8, C	44.9, C		45.8, C		45.9, C
9	1.10, d (7.0)	58.0, CH	57.0, CH	1.10, d (6.0)	57.9, CH	1.17, brd (7.5)	55.8, CH
10		40.3, C	39.6, C		40.2, C		39.1, C
11	1.59, m	19.3, CH_2	18.7, CH_2	1.61, m	19.3, CH	1.66, m	18.5, CH_2
12a	1.63, brd (9.0)	27.3, CH_2	26.9, CH_2	1.61, m	27.4, CH_2	1.66, m	31.1, CH_2
12b	1.53, m					1.56, m	
13	2.03, m	46.4, CH	46.1, CH	2.01, m	46.5, CH	2.56, m	37.8, CH
14a	1.93, brd (12.0)	38.2, CH_2	37.8, CH_2	1.92, d (11.2)	38.2, CH_2	1.89, brd (12.9)	37.7, CH_2
14b	1.59, m			1.60, m		0.84, brd (12.9)	
15a	1.51, d (14.5)	54.0, CH_2	54.0, CH_2	1.52, m	54.1, CH_2	1.73, dd (13.5, 5.7)	40.4, CH_2
15b	1.38, d (14.5)			1.38, m			
16		82.8, C	81.5, C		82.8, C	2.62, brd (5.7)	53.6, CH
17a	3.69, d (11.0)	66.7, CH_2	66.4, CH_2	3.69, d (11.6)	66.9 CH_2	9.65, s	203.8, C
17b	3.58, d (11.0)			3.58, d (11.6)			
18	0.83, s	22.6, CH_3	22.4, CH_3	0.84, s	22.7, CH_3	0.84, s	22.1, CH_3
19	0.90, s	29.1, CH_3	29.4, CH_3	0.99, s	29.1, CH_3	0.95, s	28.5, CH_3
20	1.06, s	18.3, CH_3	18.0,CH_3	1.11, s	18.5, CH_3	1.01, s	17.3, CH_3
1′				4.29, d (8.0)	106.4, CH		
2′				3.20, t (8.8)	75.8, CH		
3′				3.32, t (8.8)	78.3, CH		
4′				3.28, t (8.8)	71.7, CH		
5′				3.21, m	77.6, CH		
H6′a				3.82, dd (12.0, 2.5)	62.8, CH_2		
H6′b				3.65, dd (12.0, 5.0)			

[a]400 MHz. [b]$CDCl_3$. [c]Pyridine-d_5.

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orientation. The NOESY correlations for rings A, B, and C were similar to those of compound **12**, suggesting that **14** is the dehydration product of **12**. Finally, the NOESY correlation between H-16 and H-12 defined the α-orientation of the formyl group.

The known compounds melampodin (**2**)[11,12] and polymatin A (**4**)[13] were identified by comparison of their physical and spectroscopic features with reported data.

The phytochemical study of *M. perfoliatum* afforded melampolides and kaurane derivatives, as main secondary metabolites, in accordance with previous reports on species of the genus *Melampodium*.[2−5] Inspired by the anti-inflammatory activity reports displayed for this type of metabolites,[8,23] the anti-inflammatory activity of extracts and more abundant compounds **2**−**5**, **8**, **10**, and **12** was tested using the TPA-induced ear acute inflammation model.[24] Evaluations in primary screening were performed at doses of 1 mg/ear. Petroleum ether, acetone, and MeOH extracts displayed mild activity, with edema inhibitions of 54.61 ± 7.29%, 36.93 ± 2.66%, and 20.72 ± 2.25%, respectively. Compounds **3**, **8**, and **12**, with less than 50% of edema inhibition, were considered not active. Compounds **2**, **4**, **5**, and **10** exhibited a dose-dependent anti-inflammatory activity, with ID_{50} values of 1.14, 0.56, 1.15, and 1.49 μmol/ear, respectively, with activity lower than that of the reference compound, indomethacin (0.24 μmol/ear). The presence of the α-methylene-γ-lactone moiety seems to be one of the structural requirements for anti-inflammatory activity of sesquiterpene lactones,[25] and there is some evidence of a positive effect on activity by the presence of an acetoxy group at C-9 and of a methoxycarbonyl group at C-10.[26] Our results on the TPA model showed that the most active compound was polimatin A (**4**) (ID_{50} 0.56 μmol/ear), whose ability to inhibit NO production has been already reported.[26] The activity decreased with the epoxidation of the angeloyloxy group at C-8 in compound **5** (ID_{50} 1.15 μmol/ear), and the presence of a 2,3 epoxy moiety did not seem to affect the edema inhibition since compounds **2** and **5** had nearly the same ID_{50} values (ID_{50} 1.14 and 1.15 μmol/ear, respectively). In addition, when an epoxyangeloyloxy group at C-9 was present, there was loss of activity, as in compound **3**, suggesting that ester groups bigger than acetoxy at C-9 had a negative effect on edema inhibition.

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. Optical rotations were obtained on a PerkinElmer 343 polarimeter. UV and IR spectra were recorded on a Shimadzu UV 160U and a Bruker Tensor 27 spectrometer, respectively. ECD were obtained on a Jasco J-720 spectropolarimeter. X-ray diffraction analysis was performed on a Bruker D8 Venture diffractometer with Cu Kα radiation. 1D and 2D NMR spectra were obtained on a Bruker Avance III 400 MHz or a Varian-Unity Inova 500 MHz spectrometer with tetramethylsilane (TMS) as internal standard. For DARTMS a JEOL AccuTOF JMS-T100LC DART was used. FABMS data were obtained on a JEOL MStation JMS-700 mass spectrometer operated with an acceleration voltage of 10 kV, with samples desorbed from a nitrobenzyl alcohol matrix using 6 kV xenon atoms. HRFABMS were performed at 10 000 resolution using electric field scans and polyethylene glycol ions (Fluka 200 and 300) as the reference material. GC analysis was performed on an Agilent 6890 GC system with an AT5 column (30 m × 0.25 mm, 0.1 μm film thickness) using a temperature gradient starting at 100 °C, which was raised to a final temperature of 240 °C in 10 min. Column chromatography (VCC) was carried out under vacuum on silica gel G 60 (Merck, Darmstadt, Germany). Flash column chromatography (FCC) was performed on silica gel 230−400 (Macherey-Nagel, Düren, Germany). Analytical TLC was carried out on Si gel 60 GF_{254} or RP-18W/UV_{254} (10−40 μm, Macherey-Nagel, Düren, Germany), and preparative TLC on Si gel GF_{254} layer thickness 2.0 mm or RP-18W/UV_{254} layer thickness 1.0 mm, using 10 × 20 cm plates.

Plant Material. *Melampodium perfoliatum* (Cav.) Kunth was collected in Ozumba County, Mexico State, México, in September 2013 and authenticated by one of the authors (J.L.V.). A voucher specimen (MEXU 1369108) was deposited at the Herbario del Instituto de Biología, UNAM, México.

Extraction and Isolation. Dried and ground aerial parts (2 kg) were extracted successively with petroleum ether, acetone, and MeOH at room temperature. The petroleum ether extract (14 g) was fractionated by VCC eluted with a petroleum ether−EtOAc gradient system. Fractions A−E were obtained with mixtures (19:1, 4:1, 7:3, 1:1) and EtOAc, respectively. Fraction A afforded 150 mg of a mixture of β-sitosterol and stigmasterol. Fraction B (430 g) was further purified using an FCC eluted with petroleum ether−acetone (4:1) to obtain mixtures B_1 and B_2. Mixture B_1 (46 mg) was purified by preparative TLC [CH_2Cl_2−acetone (49:1) × 4] to obtain polymatin A (**4**, 10 mg). Mixture B_2 (28 mg), by preparative TLC (CH_2Cl_2−acetone, 19:1 × 3), afforded de-*O*-acetylmeleucanthin (**9**, 12 mg). Fraction C (712 mg) was subjected to FCC (petroleum ether−acetone, 3:1) to yield fractions C_1 and C_2. Fraction C_1 (113 mg) was purified by preparative TLC [petroleum ether−acetone (4:1) × 3] to afford C_{1a} and C_{1b}; C_{1a} (32 mg) was purified by preparative RPTLC (MeOH−H_2O, 1:1) to afford perfoliatin A (**1**, 5 mg), while C_{1b} (52 mg), by preparative RPTLC (MeOH−H_2O, 1:1), yielded perfoliatin D (**6**, 5 mg) and perfoliatin F (**8**, 4 mg). Preparative RPTLC (MeOH−H_2O, 1:1) of fraction C_2 (68 mg) afforded 10 mg of **8**. Fraction D (860 mg) was purified by two successive FCCs eluted with petroleum ether−acetone (7:3) to afford melampodin (**2**, 25 mg) and mixture D_1. Mixture D_1 was purified by preparative TLC (petroleum ether−acetone, 7:3) to afford perfoliatin C (**5**, 40 mg). Fractions E (1.7 g) after two successive FCCs eluted with petroleum ether−acetone (7:3) and CH_2Cl_2−acetone (4:1), respectively, afforded compound **10** (15 mg) and a less polar compound (**11**, 9 mg) formed during the purification process. The acetone extract (17 g) was fractionated by VCC using a petroleum ether−acetone gradient system as eluent. Fractions eluted with petroleum ether−acetone (19:1) yielded mixture F, the fraction eluted with petroleum ether−acetone (17:3) afforded mixtures G and H, and those obtained with petroleum ether−acetone (7:3) yielded mixture J. From mixture F, 120 mg of a mixture of β-sitosterol and stigmasterol was isolated. Mixture G (1.1 g) was purified by FCC eluted with petroleum ether−acetone (4:1) to obtain mixtures G_1 and G_2. Mixture G_1 (180 mg), by FCC (CH_2Cl_2−acetone 19:1) followed of preparative TLC (CH_2Cl_2−acetone, 19:1), afforded **5** (20 mg). Mixture G_2 (352 mg) was subjected to two successive FCCs eluted with CH_2Cl_2−acetone (19:1) and petroleum ether−acetone (7:3) to obtain polymatin A (**4**, 5 mg) and perfoliatin B (**3**, 5 mg). Mixture H (1.6 g) yielded, after purification by FCC (petroleum ether−acetone, 7:3), compound **12** (35 mg) and mixture H_1. Purification of H_1 via FCC (CH_2Cl_2−acetone, 19:1) afforded **2** (40 mg). Mixture J (690 mg) after two consecutive FCCs (CH_2Cl_2−acetone, 4:1, and petroleum ether−acetone, 1:1), followed of a preparative RPTLC (MeOH−H_2O, 1:1), gave perfoliatin E (7, 5 mg). The MeOH extract (40 g) was fractionated in a VCC using as eluent an EtOAc−MeOH gradient system. Fractions eluted with EtOAc afforded 250 mg of β-sitosterylglucopyranoside and fraction K (1.2 g). Purification of K via FCC (EtOAc−MeOH, 49:1) yielded 35 mg of compound **13**. Sucrose (120 mg) was also obtained from fractions eluted with EtOAc−MeOH (7:3) from the main column.

Perfoliatin A (1): white, amorphous powder; $[\alpha]^{25}_D$ +109 (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 207 (4.43) nm; ECD (0.19×10^{-4} M, MeOH) $[\phi]_{214}$ −9262, $[\phi]_{246}$ +2862; IR ($CHCl_3$) ν_{max} 3480, 1764, 1715 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 1; DARTMS *m*/*z* 405 $[M + H]^+$; HRDARTMS *m*/*z* 405.15480 $[M + H]^+$ ($C_{21}H_{25}O_8$ requires 405.15494).

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*Melampodin (**2**):* colorless prisms (hexane−acetone); mp 210−212 °C; $[\alpha]^{25}_{D}$ +151 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 207 (4.41) nm; ECD (0.23 × 10^{-4} M, MeOH) $[\phi]_{214}$ −13 318, $[\phi]_{245}$ +4121; IR ($CHCl_3$) ν_{max} 3486, 1764, 1720 cm^{-1}; DARTMS *m*/*z* 421 $[M + H]^+$; HRDARTMS *m*/*z* 421.15037 $[M + H]^+$ ($C_{21}H_{25}O_9$ requires 421.14986).

*Perfoliatin B (**3**):* white, amorphous powder; $[\alpha]^{25}_{D}$ +35 (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 206 (4.18) nm; ECD (0.46 × 10^{-4} M, MeOH) $[\phi]_{214}$ −8819, $[\phi]_{248}$ +4132; IR ($CHCl_3$) ν_{max} 1772, 1747, 1715 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 1; DARTMS *m*/*z* 519 $[M + H]^+$; HRDARTMS *m*/*z* 519.18708 $[M + H]^+$ ($C_{26}H_{31}O_{11}$ requires 519.18664).

*Polymatin A (**4**):* colorless gum; $[\alpha]^{25}_{D}$ +35 (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 207 (4.38) nm; ECD (0.20 × 10^{-4} M, MeOH) $[\phi]_{216}$ −6035, $[\phi]_{247}$ +486; IR ($CHCl_3$) ν_{max} 3469, 1767, 1717 cm^{-1}; DARTMS *m*/*z* 391 $[M + H]^+$; HRDARTMS *m*/*z* 391.17522 $[M + H]^+$ ($C_{21}H_{27}O_7$ requires 391.17568).

*Perfoliatin C (**5**):* white, amorphous powder; $[\alpha]^{25}_{D}$ +11 (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 206 (4.11) nm; ECD (0.49 × 10^{-4} M, MeOH) $[\phi]_{213}$ −4641, $[\phi]_{246}$ +436; IR ($CHCl_3$) ν_{max} 3499, 1764, 1747, 1718 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 1; DARTMS *m*/*z* 407 $[M + H]^+$; HRDARTMS *m*/*z* 407.17049 $[M + H]^+$ ($C_{21}H_{27}O_8$ requires 407.17059).

*Perfoliatin D (**6**):* white, amorphous powder; $[\alpha]^{25}_{D}$ −16 (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 205 (4.03) nm; ECD (0.48 × 10^{-4} M, MeOH) $[\phi]_{221}$ −4335, $[\phi]_{253}$ +52; IR ($CHCl_3$) ν_{max} 3485, 1764, 1715 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 2; DARTMS *m*/*z* 505 $[M + H]^+$; HRDARTMS *m*/*z* 505.20773 $[M + H]^+$ ($C_{26}H_{33}O_{10}$ requires 505.20737).

*Perfoliatin E (**7**):* white, amorphous powder; $[\alpha]^{25}_{D}$ −4 (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 205 (3.90) nm; ECD (0.47 × 10^{-4} M, MeOH) $[\phi]_{212}$ −1141, $[\phi]_{222}$ −1013, $[\phi]_{250}$ ~0; IR ($CHCl_3$) ν_{max} 3489, 1765, 1715 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 2; DARTMS *m*/*z* 423 $[M + H]^+$; HRDARTMS *m*/*z* 423.16532 $[M + H]^+$ ($C_{21}H_{27}O_9$ requires 423.16551).

*Perfoliatin F (**8**):* white, amorphous powder; $[\alpha]^{25}_{D}$ −43 (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 208 (4.20) nm; ECD (0.38 × 10^{-4} M, MeOH) $[\phi]_{213}$ −7768, $[\phi]_{248}$ +998; IR ($CHCl_3$) ν_{max} 1773, 1758, 1714 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 2; DARTMS *m*/*z* 521 $[M + H]^+$; HRDARTMS *m*/*z* 521.20196 $[M + H]^+$ ($C_{26}H_{33}O_{11}$ requires 521.20229).

*De-O-acetylmeleucanthin (**9**):* white, amorphous powder; $[\alpha]^{25}_{D}$ +238 (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 206 (4.04), 269 (3.50) nm; ECD (0.5 × 10^{-4} M, MeOH) $[\phi]_{215}$ −4468, $[\phi]_{273}$ +4497; IR ($CHCl_3$) ν_{max} 3522, 1770, 1713 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 6.16 (1H, d, *J* = 3.0 Hz, H-13a), 5.93 (1H, dd, *J* = 9.5, 5.5 Hz, H-2), 5.69 (1H, brd, *J* = 5.5 Hz, H-3), 5.67 (1H, t, *J* = 3.0 Hz, H-8), 5.42 (1H, d, *J* = 3.0 Hz, H-13b), 5.29 (1H, d, *J* = 9.5 Hz, H-1), 4.55 (1H, t, *J* = 11.5 Hz, H-6), 4.39 (1H, d, *J* = 3.0 Hz, H-9), 3.72 (3H, s, OCH_3), 3.36 (1H, dq, *J* = 11.5, 3.0 Hz H-7), 3.04 (H, d, *J* = 11.5 Hz, H-5), 3.03 (1H, q, *J* = 5.5 Hz H-3′), 2.03 (3H, brs, H-15), 1.53 (3H, s, H-5′), 1.25 (3H, d, *J* = 5.5 Hz H-4′); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 171.3 (C, C-14), 169.3 (C, C-12), 168.9 (C, C-1′), 139.0 (CH, C-4), 133.9 (C, C-11), 125.7 (CH, C-2), 122.3 (CH, C-1), 119.6 (CH_2, C-13), 117.4 (CH, C-3), 77.2 (CH, C-6), 72.5 (CH, C-8), 70.7 (CH, C-9), 60.3 (CH, C-3′), 59.3 (C, C-2′), 54.1 (C, C-10), 52.7 (CH_3, OCH_3), 44.8 (CH, C-7), 42.5 (CH, C-5), 29.7 (CH_3, C-15), 19.0 (CH_3, C-5′), 13.6 (CH_3, C-4′); DARTMS *m*/*z* 405 $[M + H]^+$; HRDARTMS *m*/*z* 405.15455 $[M + H]^+$ ($C_{21}H_{25}O_8$ requires 405.15494).

*3,7:10,11-Diepoxy-1,2-dihydroxy-13-oxo-14-phyten-18,6-olide (**10**):* colorless oil; $[\alpha]^{25}_{D}$ −20 (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 205 (3.65), 235 (3.78) nm; IR ($CHCl_3$) ν_{max} 3468, 1712, 1685, 1619 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 6.08 (1H, hept, *J* = 1.2 Hz, H-14), 4.22 (1H, dd, *J* = 8.0, 6.5 Hz, H-6), 3.74 (1H, dd, *J* = 11.2, 6.8 Hz, H-1a), 3.66 (1H, dd, *J* = 11.2, 3.6 Hz, H-1b), 3.60 (1H, d, *J* = 17.2 Hz, H-12a), 3.56 (1H, dd, *J* = 6.8, 3.6 Hz, H-2), 3.34 (1H, dd, *J* = 3.2, 2.4 Hz, H-10), 2.44 (2H, m, H-9), 2.38 (1H, d, *J* = 17.2 Hz, H-12b), 2.16 (1H, m, H-4a), 2.13 (3H, d, *J* = 1.2 Hz, H-16), 2.04 (1H, m, H-5a), 1.98 (1H, m, H-5b), 1.90 (3H, d, *J* = 1.2 Hz, H-17), 1.86 (2H, m, H-8), 1.69 (1H, m, H-4b), 1.41 (3H, s, H-19), 1.21 (3H, s, H-20); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 196.6 (C, C-13), 172.1 (C, C-18), 157.2 (C, C-15), 123.3 (CH, C-14), 85.7 (C, C-3), 84.0 (C, C-7), 82.3 (CH, C-6), 76.5 (CH, C-2), 63.4 (CH_2, C-1), 62.8 (CH, C-10), 61.0 (C, C-11), 49.6 (CH_2, C-12), 34.8 (CH_2, C-4), 31.4 (CH_2, C-8), 27.8 (CH_3, C-17), 26.4 (CH_2, C-5), 22.6 (CH_2, C-9), 22.3 (CH_3, C-20), 22.1 (CH_3, C-19), 20.9 (CH_3, C-16); DARTMS *m*/*z* 383 $[M + H]^+$; HRDARTMS *m*/*z* 383.20673 $[M + H]^+$ ($C_{20}H_{31}O_7$ requires 383.20698).

*1,2-Acetonide-3,7:10,11-diepoxy-1,2,dihydroxy-13-oxo-14-phyten-18,6-olide (**11**):* colorless oil; $[\alpha]^{25}_{D}$ −24 (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 205 (3.37), 235 (3.73) nm; IR ($CHCl_3$) ν_{max} 1712, 1686, 1620 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 6.06 (1H, hept, *J* = 1.2 Hz, H-14), 4.47 (1H, t, *J* = 6.8 Hz, H-6), 4.08 (1H, dd, *J* = 7.6, 3.6 Hz, H-2), 3.99 (1H, dd, *J* = 8.0, 3.6 Hz, H-1a), 3.77 (1H, dd, *J* = 8.0, 7.6 Hz, H-1b), 3.70 (1H, d, *J* = 17.2 Hz, H-12a), 3.28 (1H, dd, *J* = 2.8, 2.4 Hz, H-10), 2.51 (1H, m, H-9a), 2.37 (1H, d, *J* = 17.2 Hz, H-12b), 2.30 (1H, m, H-9b), 2.17 (1H, m, H-5a), 2.13 (3H, d, *J* = 1.2 Hz, H-16), 2.04 (1H, m, H-5a), 2.01 (1H, m, H-8a), 1.95 (2H, m, H-4a, H-5b), 1.89 (3H, d, *J* = 1.2 Hz, H-17), 1.85 (1H, m, H-8b), 1.67 (1H, m, H-4b), 1.56 (3H, s, H-2′), 1.42 (3H, s, H-3′), 1.33 (3H, s, H-19), 1.22 (3H, s, H-20); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 196.8 (C, C-13), 171.7 (C, C-18), 156.8 (C, C-15), 123.2 (CH, C-14), 109.4 (C, C-1′), 84.1 (C, C-7), 83.3 (C, C-3), 80.7 (CH, C-2), 79.0 (CH, C-6), 65.7 (CH_2, C-1), 63.4 (CH, C-10), 61.4 (C, C-11), 50.2 (CH_2, C-12), 33.3 (CH_2, C-4), 31.9 (CH_2, C-8), 27.7 (CH_3, C-17), 26.3 (CH_3, C-2′), 25.8 (CH_2, C-5), 25.2 (CH_3, C-3′), 22.9 (CH_2, C-9), 22.5 (CH_3, C-20), 22.3 (CH_3, C-19), 20.9 (CH_3, C-16); DARTMS *m*/*z* 423 $[M + H]^+$; HRDARTMS *m*/*z* 423.23836 $[M + H]^+$ ($C_{23}H_{35}O_7$ requires 423.23828).

*(3S,5S,8S,9R,10S,13R,16R)-3,16,17-Trihydroxy-ent-kaurane (**12**):* colorless prisms (MeOH); mp 245−246 °C; $[\alpha]^{25}_{D}$ −28 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3263 cm^{-1}; ^1H NMR and ^{13}C NMR see Table 3; HRFABMS *m*/*z* 322.2511 $[M]^+$ ($C_{20}H_{34}O_3$ requires 322.2508).

*3-O-β-D-Glucopyranosyl-3,16,17-trihydroxy-ent-kaurane (**13**):* white, amorphous powder; $[\alpha]^{25}_{D}$ −5 (*c* 0.1, MeOH); ^1H NMR and ^{13}C NMR see Table 3; HRFABMS *m*/*z* 507.2926 $[M + Na]^+$ ($C_{26}H_{44}O_8Na$ requires 507.2934).

*3β-Hydroxy-ent-kaurane-17-al (**14**):* white, amorphous powder; $[\alpha]^{25}_{D}$ −68 (*c* 0.1, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3476, 1720 cm^{-1}; ^1H NMR and ^{13}C NMR see Table 3; DARTMS *m*/*z* 304 $[M]^+$; HRDARTMS *m*/*z* 305.24897 $[M + H]^+$ ($C_{20}H_{33}O_2$ requires 305.24805).

*Crystal Data for Compound **12**.*[20] $C_{20}H_{34}O_3 \cdot H_2O$, M_r 340.49, monoclinic, space group $P2_1$, *a* = 11.2505(4) Å, α = 90°, *b* = 7.3824(2) Å, β = 93.6665(14)°, *c* = 11.4285(4) Å; γ = 90°, *V* = 947.26(5) $Å^3$, *Z* = 2, D_c = 1.194 Mg/m^3, *F*(000) = 376; crystal dimensions/shape/color 0.269 × 0.132 × 0.050 mm^3/prism/colorless. Reflections collected 14 893, independent reflections 3405; final *R* indices [$I > 2\sigma(I)$] R_1 = 0.0502, wR_2 = 0.1345; *R* indices (all data) *R* = 0.0530, wR_2 = 0.1383. Absolute structure parameter: −0.1(3).

Hydrolysis of Compound 13. Compound **13** (15 mg) was refluxed for 4 h with MeOH (1 mL) and 2 N HCl (1 mL). The MeOH was evaporated, the reaction mixture was extracted with $CHCl_3$, the aqueous layer was evaporated to obtain an amber residue (4 mg), and 0.5 mg of this was silylated using silylating mixture Fluka 1 (85434) and analyzed by GC to identify glucose as the sugar present. The remaining 3.5 mg was purified by FCC (EtOAc−MeOH, 7:3) to obtain D-glucose ($[\alpha]^{25}_{D}$ +56 (*c* 0.15, H_2O)). The organic layer (9 mg) was purified by FCC (CH_2Cl_2−acetone, 19:1) to obtain compound **14** (3 mg).

Evaluation of the Anti-inflammatory Activity. *Animals.* Male CD-1 mice weighing 25−30 g were maintained in standard laboratory conditions in the animal house (temperature 27 ± 1 °C) in a 12/12 h light−dark cycle, being fed laboratory diet and water *ad libitum*, following the Mexican official norm MON-062-Z00-1999.

TPA-Induced Edema Model. The TPA-induced ear edema assay in mice was performed for extracts and isolated compounds as previously reported.[24] Purity of tested compounds was not less than 98% by HPLC. Indomethacin was used as reference compound. In the primary

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screening extracts and compound were evaluated at doses of 1 mg/ear. Reported ID_{50} values are the average of five independent experiments.

Statistical Analysis. All data were represented as percentage mean ± standard error of mean (SEM). The statistical analysis used Student's *t* test, whereas analysis of variance (ANOVA) followed by Dunnett's test was used to compare several groups with a control. Values of $p \leq 0.05$ and $p \leq 0.01$ were considered to be significant.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.6b00321.

Comparative ECD of compounds **1**, **3**, **5**, and **8** with that of melampodin (**2**); ^{1}H, ^{13}C, and 2D NMR spectra of compounds **1**, **3**, and **5**−**14**; and ^{1}H and ^{13}C NMR spectra of compounds **2**−**4** (PDF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Blöch, C.; Weiss-Schneeweiss, H.; Schneeweiss, G. M.; Barfuss, M. H. J.; Reberning, C. A.; Villaseñor, J. L.; Stuessy, T. F. *Mol. Phylogenet. Evol.* **2009**, *53*, 220−233.

(2) Seaman, F. C.; Fischer, N. H.; Stuessy, T. F. *Biochem. Syst. Ecol.* **1980**, *8*, 263−271.

(3) Bohm, B. A.; Stuessy, T. F. *Biochem. Syst. Ecol.* **1991**, *19*, 677−679.

(4) Quijano, L.; Fronczek, F. R.; Fischer, N. H. *Phytochemistry* **1985**, *24*, 1747−1753.

(5) Bohlmann, F.; Zdero, C. *Chem. Ber.* **1976**, *109*, 1670−1672.

(6) Ma, G.; Khan, S. I.; Benavides, G.; Schühly, W.; Fischer, N. H.; Khan, I. A.; Pasco, D. S. *Cancer Chemother. Pharmacol.* **2007**, *60*, 35−43.

(7) Serra-Barcelona, C.; Coll, A. M. V.; Cabrera, W. M.; Habib, N. C.; Honoré, S. M.; Catalán, C. A. N.; Grau, A.; Genta, S. B.; Sánchez, S. S. *Chem.-Biol. Interact.* **2014**, *209*, 35−47.

(8) Hwang, D.; Fischer, N. H.; Jang, B. C.; Tak, H.; Kim, J. K.; Lee, W. *Biochem. Biophys. Res. Commun.* **1996**, *226*, 810−818.

(9) Inoue, A.; Tamogami, S.; Kato, H.; Nakazato, Y.; Akiyama, M.; Kodama, O.; Akatsuka, T.; Hashidoko, Y. *Phytochemistry* **1995**, *39*, 845−848.

(10) Smith, C. M.; Kester, K. M.; Fischer, N. H. *Biochem. Syst. Ecol.* **1983**, *4*, 377−380.

(11) Fischer, N. H.; Wiley, R.; Wander, J. D. *J. Chem. Soc., Chem. Commun.* **1972**, 137−139.

(12) Neidle, S.; Rogers, D. *J. Chem. Soc., Chem. Commun.* **1972**, 140−141.

(13) Le Van, N.; Fischer, N. H. *Phytochemistry* **1979**, *18*, 851−854.

(14) Richards, J.; Hendrickson, J. *Biosynthesis of Terpenes, Steroids and Acetogenins*; W. A. Benjamin Inc.: New York, 1964; Vol. *8*, pp 225−237.

(15) Fischer, N. H.; Olivier, E. J.; Fischer, H. D. In *Progress in the Chemistry of Natural Products*; Herz, W., Grisebach, H., Kirby, G. W., Eds.; Springer-Verlag: Weinheim, 1979; Vol. *38*, p 90.

(16) Catalán, C. A. N.; Cuenca, M. R.; Hernández, L. R.; Joseph-Nathan, P. *J. Nat. Prod.* **2003**, *66*, 949−953.

(17) Bohlmann, F.; Jakupovic, J.; Dhar, A. K.; King, R. M.; Robinson, H. *Phytochemistry* **1981**, *20*, 1081−1083.

(18) Quijano, L.; Fischer, N. H. *Phytochemistry* **1984**, *23*, 829−831.

(19) Li, X.; Zhang, D.; Onda, M.; Konda, Y.; Iguchi, M.; Harigaya, Y. *J. Nat. Prod.* **1990**, *53*, 657−661.

(20) Hirono, S.; Chou, W.-H.; Kasai, R.; Tanaka, O.; Tada, T. *Chem. Pharm. Bull.* **1990**, *38*, 1743−1744.

(21) IUPAC. *Pure Appl. Chem.* **1999**, *71*, 587−643.

(22) Crystallographic data of structure **12** (CCDC 1511101) has been deposited with the Cambridge Crystallographic Data Centre. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: + 044(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).

(23) Hueso-Falcon, I.; Cuadrado, I.; Cidre, F.; Amaro-Luis, J. M.; Ravelo, A. G.; Esteves-Braun, A.; De las Heras, B.; Hortelano, S. *Eur. J. Med. Chem.* **2011**, *46*, 1291−1305.

(24) Arciniegas, A.; Pérez-Castorena, A. L.; Nieto-Camacho, A.; Villaseñor, J. L.; Romo de Vivar, A. *J. Mex. Chem. Soc.* **2009**, *53*, 229−232.

(25) Dirsch, V. M.; Stuppner, H.; Ellmerer-Müller, E. P.; Vollmar, A. M. *Bioorg. Med. Chem.* **2000**, *8*, 2747−2753.

(26) Hong, S. S.; Lee, S. A.; Han, X. H.; Lee, M. H.; Hwang, J. S.; Park, J. S.; Oh, K.- W.; Han, K.; Lee, M. K.; Lee, H.; Kim, W.; Lee, D.; Hwang, B. Y. *Chem. Pharm. Bull.* **2008**, *56*, 199−202.

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