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Sesquiterpenoids from *Pittocaulon filare*

Amira Arciniegas,[†] Karina González,[†] Ana-L. Pérez-Castorena,*[,†] Antonio Nieto-Camacho,[†] José-L. Villaseñor,[‡] and Alfonso Romo de Vivar[†]

[†]Instituto de Química, Universidad Nacional Autónoma de México, Circuito Exterior, Ciudad Universitaria, Coyoacán 04510, D.F., México

[‡]Instituto de Biología, Universidad Nacional Autónoma de México, Circuito Exterior, Ciudad Universitaria, Coyoacán 04510, D.F., México

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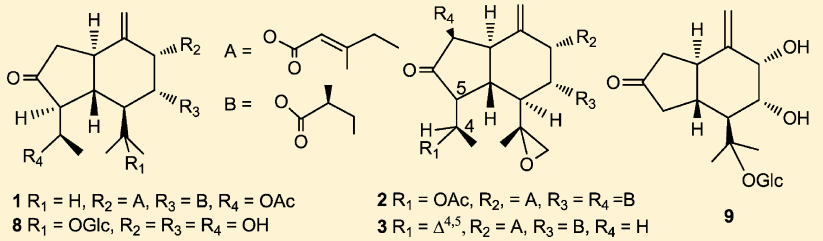

ABSTRACT: The phytochemical study of *Pittocaulon filare* afforded three oplopanes (**1**−**3**), a eudesmane (**6**), and three oplopane glucosides (**7**−**9**), one of them reported as its acetyl derivative (**7a**), together with several known compounds. The structures of the compounds were elucidated by spectroscopic analysis and chemical reactions. The anti-inflammatory activity of compounds **1**−**5** was determined using the 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced ear edema model, and the effect of compounds **1**−**4** on the recruitment of neutrophils was evaluated using the myeloperoxidase test. Compounds **1** and **2** were the more active anti-inflammatory agents, with lower ID_{50} values (0.17 and 0.18 μmol/ear, respectively) than indomethacin (0.24 μmol/ear), but they had a lesser effect on the inhibition of neutrophil infiltration than both indomethacin and compound **3**, indicating that the tested compounds do not have the same ability to inhibit edema and to prevent cell infiltration.

The genus *Pittocaulon* (Asteraceae, Senecioneae, Tussilagininae) groups five resinous species, with deciduous leaves and thick stems, segregated from the genus *Senecio*.[1,2] The plants of the genus *Pittocaulon* are endemic to Mexico and traditionally used to cure rheumatism.[3] Previous phytochemical studies have shown the presence of pyrrolizidine alkaloids in all five species of the genus (*P. praecox*, *P. velatum*, *P. bombycophole*, *P. filare*, and *P. hintonii*),[4] while sesquiterpenoids, mainly with an eremophilane skeleton, have been found in *P. praecox*,[5−7] *P. bombycophole*,[8] and *P. velatum*.[9] The aim of this paper was to contribute to the chemistry of the genus by studying the nonalkaloidal metabolites of *Pittocaulon filare* and to search for anti-inflammatory agents. The isolation of the new oplopanes **1**−**3**, the eudesmane **6**, and the three oplopane glucosides **7**−**9** including the acetyl derivative **7a** is reported. In addition, five known sesquiterpenoids and two phenylpropanoids were isolated. The anti-inflammatory activity of compounds **1**−**5** was evaluated, and the most active compounds (**1**−**4**) were tested in the myeloperoxidase (MPO) assay to determine the effect of compounds **1**−**4** on the recruitment of inflammatory cells, such as neutrophils.

RESULTS AND DISCUSSION

Compound **1** has the molecular formula $C_{28}H_{42}O_7$ according to its HRESIMS and ^{13}C NMR data. The IR spectrum showed evidence of cyclopentanone (1745 cm^{-1}) and ester group (1727 and 1715 cm^{-1}) functionalities. The ^{13}C NMR data (Table 1) exhibited four carbonyl carbons (a ketone carbonyl at δ_C 213.8 and three ester carbonyl carbons at δ_C 175.5, 170.8, and 165.7) and four vinylic carbons (δ_C 162.7, 143.3, 114.1, and 111.6), which accounted for six of the eight indices of hydrogen deficiency, thus indicating a bicyclic skeleton. The ^1H NMR data (Table 1) contained, in agreement with an oplopane structure, the resonances of the C-14 exocyclic methylene protons (δ_H 5.20 and 4.88) and the protons of methyl groups H_3-12 (δ_H 1.14), H_3-13 (δ_H 0.89), and H_3-15 (δ_H 1.24), assigned on the basis of the 2D NMR spectroscopic data. The ^1H NMR data also showed the resonances of hydrogen atoms geminal to three ester groups. The COSY experiment permitted assignment of these resonances to H-9 (δ_H 5.72) by its allylic correlations with H_2-14, to H-4 (δ_H 5.09) by its cross-peaks with H-5 and H_3-15, and to H-8 (δ_H 5.06) by its coupling with H-9 and H-7. HMBC correlations of H_3-2′ and H-4 with C-1′ indicated the presence of an acetoxy group at C-4, the correlations of H-2″, H-3″, and H-8 with C-1″ located the 2-methylbutanoyloxy group at C-8, and the correlations of H-2‴ and H-9 with C-1‴ placed the 3-methylpentenoyloxy group at C-9. The NOESY experiment of **1** exhibited cross-peaks between H-1, H-5, and H-7, indicating that they are

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Table 1. NMR Spectroscopic Data (500 MHz, $CDCl_3$) for Compounds 1−3

	1		2[a]		3[b]	
position	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1	2.69 m	41.4	2.64 m	45.3	2.73 m	45.6
2a	2.46 ddd (16.0, 6.0, 1.5)	42.4	5.49 d (4.0)	71.3	5.78 d (4.0)	71.8
2b	2.17 m					
3		213.8		206.5		199.3
4	5.09 qd (6.5, 3.5)	69.8	5.20 m	68.5	6.33 qd (7.5, 1.0)	139.0
5	2.44 dd (11.0, 3.5)	57.4	2.75 dd (10.4, 3.2)	55.9		138.3
6	1.55 q (11.0)	44.9	under 6‴	41.1	2.69 m	41.5
7	2.15 m	48.8	1.95 m	48.8	2.28 m	48.3
8	5.06 dd (3.0, 2.5)	72.9	5.13 m	72.8	5.42 m	73.5
9	5.72 d (3.0)	72.3	5.74 d (3.2)	72.6	6.02 brs	72.4
10		143.3		137.0		138.0
11	2.25 dhept (11.0, 7.0)	27.9		55.1		55.6
12a	1.14 d (7.0)	23.0	2.84 d (4.0)	52.7	2.41 d (3.0)	50.8
12b			2.70 d (4.0)		2.13 d (3.0)	
13	0.89 d (7.0)	15.8	1.29 s	16.7[c]	1.28 s	21.9
14a	5.20 d (1.5)	111.6	5.31 d (2.0)	116.1	5.27 brs	113.8
14b	4.88 d (1.5)		4.90 d (2.0)		5.10 brs	
15	1.24 d (6.5)	15.9	1.25 d (6.4)	15.6	2.10 dd (7.5, 1.0)	15.0
1′		170.8		170.7		
2′	2.10 s	21.3	2.11 s	21.3		
1″		175.5		176.0		175.3
2″	2.32 brsext (7.5)	41.4	2.39 brsext (7.2)	41.1	2.36 brsext (7.0)	41.5
3″a	1.70 dquint (14.0, 7.5)	26.2	1.72 m	26.3	1.72 m	26.8
3″b	1.43 ddq (14.0, 7.5, 7.0)		1.48 m		1.40 ddq (13.5, 7.5. 70)	
4″	0.90 t (7.5)	11.7	0.89 t (7.2)[d]	11.6	0.84 t (7.5)	11.7
5″	1.11 d (7.5)	16.7	1.17 d (7.2)	16.2[c]	1.17 d (7.0)	16.8
1‴		165.7		165.2		165.5
2‴	5.66 qt (1.5, 1.0)	114.1	5.63 sext (1.2)	113.8	5.80 qt (1.5, 1.0)	114.2
3‴		162.7		163.3		163.4
4‴	2.18 qd (7.5, 1.0)	33.8	2.14 qd (7.2, 1.2)	33.7	1.75 m	33.8
5‴	1.08 t (7.5)	11.9	1.08 t (7.2)	11.8	0.72 t (7.5)	11.7
6‴	2.16 d (1.5)	16.7	2.14 d (1.2)	18.9	2.13 d (1.0)	18.9
1⁗				174.7		175.1
2⁗			2.39 brsext (7.2)	41.1	2.20 brsext (7.0)	41.1
3⁗a			1.72 m	26.7	1.59 ddq (13.5, 7.5, 7.0)	27.1
3⁗b			1.48 m		1.26 ddq (13.5, 7.5, 70)	
4⁗			0.92 t (7.2)[d]	11.6	0.76 t (7.0)	11.5
5⁗			1.16 d (7.2)	16.3[c]	0.97 d (7.0)	16.7

[a]400 MHz. [b]C_6D_6. [c,d]Interchangeable signals.

cofacial and on the side opposite from H-6, H-8, and H-9. Additionally, the interaction between H-2‴ and H-4‴ indicated the *E* configuration of the C-2‴ double bond.

The structure of **1** was confirmed by X-ray crystallographic analysis (Figure 1), and its (1*R*, 4*R*, 5*S*, 6*R*, 7*S*, 8*R*, 9*S*, 2″*S*) absolute configuration was established by the negative Cotton effect observed in the electronic circular dichroism (ECD) spectrum for the n → π electronic transition (λ 302 nm, $\Delta\varepsilon$ = −1.45) that was similar to the n → π electronic transition reported for tussilagone (**11**) with known absolute configuration.[10]

Compound **2** was obtained as colorless prisms (petroleum ether−acetone), mp 105−107 °C, and has the molecular formula $C_{33}H_{48}O_{10}$, as determined by HRESIMS and ^{13}C NMR data. The ^1H NMR data for compound **2** were similar to the data for compound **1**. However, H-2 appeared as an oxymethine proton (δ_H 5.49, d, J = 4.0 Hz) and the resonances of an additional 2-methylbutanoyloxy group were observed between δ_H 0.92 and 2.40. The attachment of this ester group

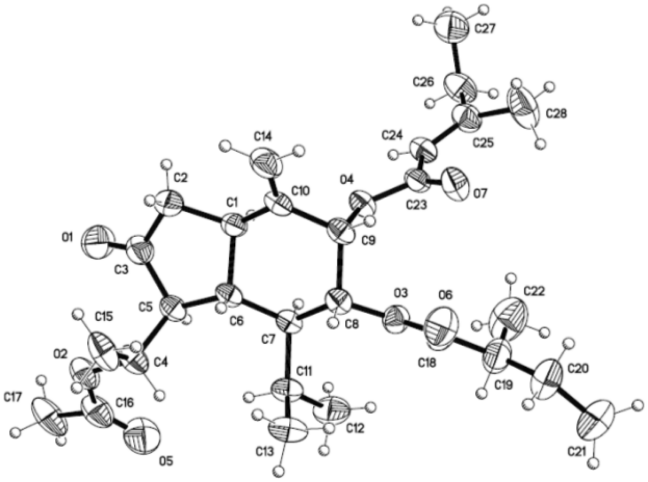

Figure 1. ORTEP drawing of compound **1**.

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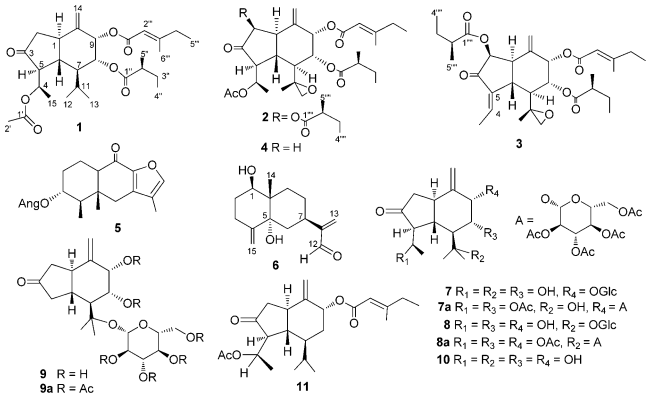

at C-2 was deduced from the cross-peak between H-2 and C-1‴ (δ_C 174.7) observed in the HMBC experiment. Additionally, the resonances of an 11,12-epoxide function were present, CH_2-12 resonated as an oxymethylene (δ_H 2.84 and 2.70, δ_C 52.7), and C-11 resonated as a nonprotonated carbon (δ_C 55.1). In the HMBC experiment, the interactions between H-4 and C-1′, H-8 and C-1″, and H-9 and C-1‴ indicated that the remaining substituents had the same locations as in compound **1**. The relative configuration of compound **2** was confirmed by X-ray crystallographic analysis (Figure 2), and the negative

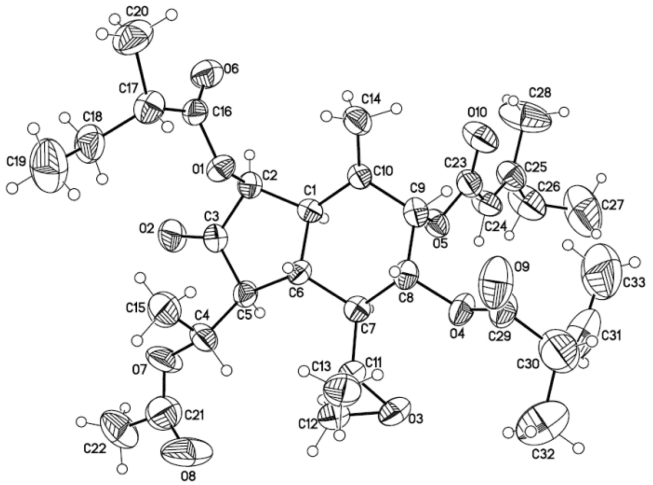

Figure 2. ORTEP drawing of **2**.

Cotton effect observed in the ECD spectrum for the n → π electronic transition (λ 319 nm, Δε = −16.0)[10] established its (1*R*, 2*S*, 4*R*, 5*S*, 6*R*, 7*S*, 8*R*, 9*S*, 11*S*, 2″*S*, 2‴*S*) absolute configuration.

Compound **3** was obtained as a colorless oil, and its molecular formula was established as $C_{31}H_{44}O_8$ by HRFABMS and ^{13}C NMR data. The ^1H and ^{13}C NMR data for this compound were similar to those for compound **2** except for the resonances of a trisubstituted double bond at C-4 (δ_C 139.0 and 138.3, δ_H 6.33, qd, *J* = 7.5, 1.0 Hz). The *Z* configuration of this double bond was defined by the NOESY interactions of H-4 with H-6 and H_2-12. In the same experiment, correlations of H-1 with H-2 and H-7 indicated that they are cofacial and on the opposite side from H-6, H-8, and H-9. Finally, the similarity of its ECD spectrum to that of the acetic acid elimination product of tussilagone (**11**)[10] indicated the (1*R*, 2*S* 6*R*, 7*S*, 8*R*, 9*S*, 11*S*, 2″*S*, 2‴*S*) absolute configuration of compound **3**.

Compound **6** has the molecular formula $C_{15}H_{22}O_3$, as deduced from ^{13}C NMR and HRFABMS data. Its IR absorption bands at 3495 and 1677 cm^{-1} indicated the presence of hydroxy and conjugated carbonyl groups. The ^1H NMR data showed the characteristic resonances of a formyl group, two vinylic methylenes, and a hydroxy methine. Analysis of the HMBC spectrum permitted assignment of the formyl group at C-12 by the correlation of its proton (δ_H 9.55) with C-11 and of the C-13 (δ_H 6.32, d, *J* = 0.8 Hz and 6.03, s) and C-15 (δ_H 4.84, t, *J* = 2.0 Hz and 4.68, t, *J* = 2.0 Hz) vinylic methylenes by their long-range interactions with C-7 and C-11 and with C-5 and C-3, respectively. The hydroxy group was located at C-1 by the cross-peaks of H-1 (δ_H 4.11) with H_2-2 in the COSY spectrum. The eudesmane skeleton was deduced from the correlations observed in the HMBC spectrum, between the H_3-14 singlet with C-1 and C-9. The ^{13}C NMR data showed the resonances of a formyl carbonyl (δ_C 194.7), four vinylic carbons (δ 154.8, 150.2, 133.4, and 108.8), and two oxygenated carbons (C-1, δ_C 72.8 and C-5, δ_C 75.9), in agreement with structure **6**. The relative configuration of **6** was determined by the NOESY interactions of H-3α and H-1α, of H-3β and H-15a, and of H-6β and H_3-14, considering that on biogenetic grounds CH_3-14 is β-oriented.[12] In addition, the resonances of the α-axial protons H-1 (δ_H 4.11, dd, *J* = 11.6, 4.8 Hz), H-3 (δ_H 2.71, dddt, *J* = 13.6, 13.6, 5.6, 2.0 Hz), and H-7 (δ_H 3.11, ttd, *J* = 12.8, 3.6, 0.8 Hz) are deshielded (Δδ 1.0, 0.53, and 1.23 ppm, respectively) compared to β-dictyopterol,[13] which has a hydrogen atom at C-5, indicating that they are supporting the 1,3-diaxial deshielding effect of the α-hydroxy group at C-5.

The methanolic extract of *P. filare* afforded a complex mixture of glucosides (G1) free of acetoxy groups according to its ^1H NMR spectrum. This mixture was acetylated to facilitate its separation, producing the acetyl derivatives **7a**, **8a**, and **9a**. Compound **7a** has the molecular formula $C_{33}H_{46}O_{16}$ according to its HRFABMS and ^{13}C NMR data. The ^1H NMR data for this compound (Table 2) were clearly those of an oplopane, similar to those of compounds **1**−**4**, with the additional resonances of a peracetylated sugar moiety. This sugar was identified as β-D-glucose by the axial−axial coupling constants of H-1′, H-2′, H-3′, and H-4′ and corroborated when the initial G1 mixture was hydrolyzed to obtain D-glucose ($[\alpha]^{25}_D$ +49, *c* 0.3, H_2O). The ^1H NMR data also showed the resonances of two hydrogen atoms geminal to ester groups, assigned to H-4 (δ_H 5.63, dq, *J* = 6.5, 3.5 Hz) and H-8 (δ_H 5.10, dd, *J* = 3.2, 2.4 Hz) by their HMBC interactions with their respective acetoxy carbonyl carbons. In the ^{13}C NMR spectrum, 14 resonances were assigned to the peracetylated sugar, and the remaining 19 resonances indicated a diacetoxyoplopane (Table 2). The attachment of the sugar to C-9 was established by the HMBC correlation between H-1′ and C-9. The presence of a tertiary C-11 alcohol moiety was deduced by the multiplicity of the H_3-12 (δ_H 1.45 s) and H_3-13 (δ_H 1.18, s) resonances. Finally, the cross-peaks between H-1, H-5, and H-7 and of H-8 with H-6 and H-9 in the NOESY spectrum, in addition to the negative Cotton effect (λ 302 nm, Δε = −18.10)[10] observed in the ECD spectrum, permitted the definition of the (1*R*, 4*R*, 5*S*, 6*R*, 7*S*, 8*R*, 9*S*) absolute configuration of compound **7a**. To obtain the natural compound, **7a** was saponified, but the reaction conditions led to decomposition of compound **7**.

Compound **8a** showed a molecular formula of $C_{35}H_{48}O_{17}$, as deduced from its HRFABMS and ^{13}C NMR data, indicative of one acetoxy group more than compound **7a**. The ^1H NMR data exhibited the resonances of a peracetylated β-glucose residue and of an oplopane aglycone moiety with three acetoxy

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Table 2. NMR Spectroscopic Data (500 MHz, $CDCl_3$) for Compounds 7a, 8a, and 9a

	7a		8a		9a[a]	
position	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1	2.85 m	41.0	2.99 m	40.9	2.93 m	42.7
2a	2.51 ddd (15.5, 6.0, 1.0)	42.5	2.56 ddd (16.0, 6.5, 1.0)	43.1	2.57 brdd (17.6, 6.8)	41.6
2b	2.12 brd (15.5)		2.12 dd (16.0, 2.0)		2.12 m	
3		214.9		214.1		216.1
4	5.63 qd (6.5, 3.5)	70.4	5.42 qd (6.5, 3.5)	69.3		
5a	2.63 dd (9.0, 3.5)	58.5	2.70 dd (10.0, 3.5)	57.9	2.75 dd (18.0, 5.2)	48.1
5b					2.09 m	
6	1.66 dt (12.0, 9.0)	41.7	2.01 m	40.1	1.99 m	38.3
7	2.10 m	56.9	2.17 dd (9.5, 2.0)	55.2	1.99 m	55.5
8	5.10 dd (3.2, 2.4)	72.4	5.48 t (2.5)	71.4	5.24 t (2.8)	71.7
9	4.43 brs	77.6	5.50 brs	71.1	5.61 dd (2.8, 2.0)	72.4
10		144.4		143.4		143.4
11		71.9		79.5		80.1
12	1.45 s	32.4	1.46 s	26.3	1.33 s	25.5
13	1.18 s	24.4	1.35 s	25.4	1.26 s	23.6
14a	5.29 brs	111.1	5.11 t (2.5)	110.8	5.07 t (2.0)	110.6
14b	4.93 brs		4.98 t (2.5)		4.93 t (2.0)	
15	1.16 d (6.5)	15.3	1.22 d (7.5)	15.2		
1′	4.65 d (8.0)	99.5	4.80 d (8.0)	95.1	4.69 d (8.0)	94.9
2′	5.01 dd (9.5, 8.0)	71.3	4.92 dd (9.5, 8.0)	71.5	4.89 dd (9.2, 8.0)	71.5
3′	5.22 t (9.5)	72.8	5.20 t (9.5)	73.0	5.21 t (9.2)	72.9
4′	5.07 t (9.5)	70.4	5.04 t (9.5)	68.3	5.02 t (9.2)	68.3
5′	3.68 ddd (9.5, 5.0, 2.5)	72.0	3.65 ddd (9.5, 5.0, 2.5)	71.6	3.66 dt (9.2, 4.0)	71.7
6′a	4.18 dd (12.0, 5.0)	62.0	4.16 dd (12.0, 2.5)	61.9	4.15 d (4.0)	62.0
6′b	4.13 dd (12.0, 2.5)		4.08 dd (12.0, 5.0)		4.15 d (4.0)	

[a]400 MHz. ^{1}H NMR CH_3COO **7a**: 2.08 s, 2.07 s, 2.05 s, 2.04 s, 2.03 s, 2.00 s; **8a**: 2.10 s, 2.07 s, 2.06 s, 2.05 s, 2.02 s, 2.01 s, 2.00 s; **9a**: 2.11, 2.07, 2.05, 2.04, 2.02, 2.00; ^{13}C NMR CH_3COO **7a**: δ 171.1, 170.5, 170.2, 169.6, 169.4, 169.2; CH_3COO: δ 21.5, 21.4, 21.1, 20.7, 20.6, 20.5; CH_3COO **8a**: 170.5, 170.4, 170.3, 170.3, 170.0, 169.9, 169.3; CH_3COO: 21.4, 20.3, 20.2, 20.8, 20.7, 20.6, 20.6; CH_3COO **9a**: 170.5, 170.3, 170.3, 169.8, 169.3, 169.0; CH_3COO: 21.2, 20.9, 20.7, 20.6, 20.9, 20.5.

groups. These groups were located at C-4, C-8, and C-9 by the correlations of H-4, H-8, and H-9 with their respective acetoxy carbonyl carbons, observed in the HMBC spectrum. In the same spectrum, the cross-peak between H-1′ and C-11 defined the attachment of the glucose moiety at this carbon. The (1*R*, 4*R*, 5*S*, 6*R*, 7*S*, 8*R*, 9*S*) absolute configuration of compound **8a** was determined by the negative Cotton effect observed in the ECD spectrum for the n → π electronic transition (λ 302 nm, $\Delta\varepsilon$ = −8.98).[10] Hydrolysis of **8a** afforded the new aglycone 4,8,9,11-tetrahydroxy-3-oxo-10(14)-oplopene (**10**) and D-glucose ($[\alpha]^{25}_D$ + 52 (*c* 0.2, H_2O)), and compound **8** was obtained by its saponification. Compound **8** showed the molecular formula $C_{21}H_{34}O_{10}$, as determined by HRFABMS and ^{13}C MNR data. The ^{1}H NMR data were free of acetyl group resonances and showed that the resonances of H-4, H-8, H-9, H_2′, H-3′, H-4′, and H-6′ were shielded compared to the respective resonances in compound **8a**. Therefore, the natural compound is (1*R*,4*R*,5*S*,6*R*,7*S*,8*R*,9*S*)-4,8,9,11-tetrahydroxy-11-*O*-β-D-glucopyranosyl-10(14)-oplopen-3-one (**8**).

Compound **9a** showed the molecular formula $C_{31}H_{42}O_{15}$ according to its HRFABMS and ^{13}C NMR data. The ^{1}H NMR data were similar to the ^{1}H NMR data for compound **8a**, with the resonances of a peracetylated β-glucose moiety and of two acetoxy groups but with the absence of the resonances of H_2-4 and H_3-15 (Table 2). The HMBC data defined the attachment of the sugar to C-11 and of the acetoxy groups to C-8 and C-9. In agreement with the above, the ^{13}C NMR data exhibited only 31 carbon resonances, including the 14 of the peracetylated glucose. Moreover, the resonances of the C-5 methylene protons (δ_H 2.75 and 2.09) confirmed the absence of CH_2-4 and CH_3-15 in structure **9a**. The (1*R*, 6*R*, 7*S*, 8*R*, 9*S*) absolute configuration of compound **9a** was established by the negative Cotton effect ($\Delta\varepsilon$ = −12.3) at λ 296 nm observed in its ECD spectrum, similar to that reported for the 5α-androstan-16-one[14] with known absolute configuration. Compound **9** was obtained by saponification of **9a** and showed the molecular formula $C_{19}H_{30}O_9$, as deduced from the HRFABMS and ^{13}C NMR data. The NMR spectroscopic data of **9** exhibited the resonances of β-glucose and those of the oxymethines C-8 and C-9 free of acetyl groups, which was in agreement with structure **9**.

The known compounds abrotanifolon (**4**),[8,15] 3α-angeloyloxy-9-oxofuranoeremophilane (**5**),[16] (7*S**)-opposit-4(15)-ene-1β,7-diol,[17] opposit-4(15)-ene-1β,11-diol,[18] 4α,15-epoxyeudesmene-1β,6α-diol,[19] 4(15)-eudesmene-1β,6α-diol,[20] 4-allyl-2,6-dimethoxyphenol glucoside,[21] syringin,[22] and 4-hydroxybenzyl methyl ether[23] were also obtained and identified by comparison of their physical and spectroscopic features with the data reported in the literature.

The anti-inflammatory response of compounds **1**−**5** was tested using the 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced acute inflammation model.[24] These compounds were assessed by measuring their effect on reducing the edema produced by the topical application of TPA on mouse ears, determined by the weight of the biopsies obtained 4 h after the administration of the samples. Compounds **1**−**4** displayed a dose-dependent anti-inflammatory effect. The higher activity was observed for compounds **1** and **2**, with lower ID_{50} values

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(0.17 and 0.18 μmol/ear, respectively) than indomethacin (0.24 μmol/ear), while for compounds **3** and **4**, the ID_{50} values were 0.55 and 0.29 μmol/ear, respectively. Neutrophils play an important role in acute inflammation, and because the edema and cell infiltration could be differentially modulated by distinct pharmacological agents,[25] neutrophil accumulation was monitored. The myeloperoxidase enzyme was used as a biochemical marker to quantify the skin neutrophils because the MPO content is directly related to neutrophil number.[26,27] The MPO activity was measured on the biopsies obtained 4 h after the application of compounds **1**−**4** in the TPA assay. The results revealed that the tested compounds do not have the same ability to inhibit edema and to prevent cell infiltration. Therefore, at doses of 1 μmol/ear, compound **3** showed a higher control of neutrophil infiltration with an MPO content of 11.07 ± 3.13%, close to that observed for indomethacin (8.83 ± 2.98%). For the most active anti-inflammatory compounds (**1** and **2**), the MPO content was 12.67 ± 3.67% and 19.05 ± 2.04% for **1** and **2**, respectively.

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 spectra were recorded on a Shimadzu UV 160U spectrometer. ECD spectra were obtained on a Jasco J-720 spectropolarimeter. IR spectra were recorded on a Bruker Tensor 27 spectrometer. 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 the internal standard. EIMS were determined on a Bruker Daltonics Analysis 3.2 mass spectrometer. FABMS were obtained on a JEOL JMS-SX102A mass spectrometer operated with an acceleration voltage of 10 kV, and samples were desorbed from a nitrobenzyl alcohol matrix using a 6 kV beam of xenon atoms. HRFABMS were performed at 10 000 resolution using electric field scans and poly(ethylene glycol) ions (Fluka 200 and 300) as reference material. HRESIMS were performed on a Bruker micrOTOF II mass spectrometer with mass resolution of 16 500 fwhm, mass interval 50−20 000 m/z, and speed 40 Hz. X-ray crystallographic analyses were performed on a Bruker Smart Apex CCD diffractometer with graphite-monochromated Mo Kα radiation (λ = 0.71073 Å). Structures were solved by direct methods using the program SHELXS. Non-hydrogen atoms were refined with anisotropic displacement parameters. Hydrogen atoms were included at calculated positions and were not refined. 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 200 °C in 10 min. Column chromatography (VCC) was carried out under vacuum on silica gel G 60 (Merck, Darmstadt, Germany). Flash chromatography (FCC) was performed on silica gel 230−400 (Macherey-Nagel, Germany). Analytical TLC was carried out on Si gel 60 GF_{254} or RP-18W/UV_{254} (10−40 μm, Macherey-Nagel, Germany), and preparative TLC was carried out on Si gel GF_{254} with a 2.0 mm layer thickness or RP-18W/UV_{254} with a layer thickness of 1.0 mm, using 10 × 20 cm plates.

Plant Material. The stems and roots of *Pittocaulon filare* (McVaugh) H. Rob. & Brettell were collected at km 17.5 Colima-Ticoman Rd., Colima, México, in March 2011, and authenticated by one of the authors, J.-L.V. A voucher specimen (Col. M. Harker and L. Hernández 4168) was deposited at the Herbario del Instituto de Botánica, Universidad de Guadalajara, México.

Extraction and Isolation. Dried and ground stems and roots (950 g) were extracted successively with petroleum ether and MeOH at room temperature. The petroleum ether extract (37 g) was fractionated by VCC eluted with a petroleum ether−acetone gradient system. The petroleum ether eluates afforded fraction A, and fractions B−D were obtained with mixtures of petroleum ether−acetone, 19:1, 9:1, and 4:1, respectively. Fraction A (6.5 g) was further purified using a VCC eluted with petroleum ether to obtain compound **1** (12 mg). Fraction B afforded by recrystallization (petroleum ether−EtOAc) 1.25 g of compound **5**.[16] Mother liquors of **5** (20 g) were purified by VCC using eluent mixtures of petroleum ether−acetone in increasing polarity to afford fractions B1 (eluted with petroleum ether), B2 (eluted with petroleum ether−acetone, 99:1), and B3 (eluted with petroleum ether−acetone, 19:1). Fraction B1 produced 2.1 g of compound **5**. Fraction B2 yielded a solid, which by crystallization (petroleum ether−EtOAc) gave 335 mg of compound **2**. Purification of the mother liquors of **2** (1.2 g) by FCC (petroleum ether−acetone, 19:1) gave **2** (25 mg), **3** (300 mg), and **5** (200 mg). Fraction B3 (6 g) was subjected to FCC (petroleum ether−acetone, 9:1) to afford 798 mg of **3**, 234 mg of **4**,[8,15] and 570 mg of **5**. Fraction C (8.5 g) was purified by VCC (petroleum ether−EtOAc, 4:1) to obtain compound **4** (132 mg) and a mixture, which by FCC (petroleum ether−EtOAc) followed by a preparative TLC (petroleum ether−acetone, 4:1, ×3) afforded compound (7*S**)-opposit-4(15)-ene-1β,7-diol[17] (15 mg). Fraction D (2.4 g) by VCC (petroleum ether−EtOAc mixtures in increasing polarity) produced fractions D1 (petroleum ether−EtOAc, 19:1) and D2 (petroleum ether−EtOAc, 9:1). Fraction D1 (204 mg), after two consecutive FCCs (petroleum ether−EtOAc, 7:3, and petroleum ether−acetone, 4:1), yielded compound **6** (9 mg). Fraction D2 (730 mg) was purified by FCC (petroleum ether−EtOAc, 3:2) followed by preparative RPTLC (MeOH−H_2O, 1:1, ×3) to obtain compounds 4α,15-epoxyeudesmene-1β,6α-diol[19] (8.3 mg) and opposit-4(15)-ene-1β,11-diol[18] (9 mg). The MeOH extract (140 g) was fractionated by VCC (EtOAc−MeOH gradient system) to obtain fractions F (EtOAc), G (EtOAc−MeOH, 19:1), and H (EtOAc−MeOH, 9:1). Fraction F (2.5 g), submitted to VCC (petroleum ether−acetone, 4:1), gave 20 mg of 4-hydroxybenzyl methyl ether[23] and F1. Purification of F1 (139 mg) by preparative TLC (petroleum ether−EtOAc, 4:1, ×3) produced compound 4(15)-eudesmene-1β,6α-diol[20] (15 mg). Fraction G (12 g) was separated by VCC (EtOAc−MeOH gradient system) to afford solids, which after crystallization (EtOAc) produced 4-allyl-2,6-dimethoxyphenol glucoside[21] (8 mg) and 800 mg of a complex mixture of glucosides G1, of which 750 mg was acetylated and 50 mg was hydrolyzed. Fraction H (5.4 g) by FCC (EtOAc−MeOH, 19:1) followed by a preparative RPTLC (MeOH−H_2O, 1:1, ×2) yielded syringin[22] (8 mg).

Acetylation of the Glucoside Mixture. Mixture G1 (750 mg) was acetylated overnight at room temperature in an excess of anhydrous pyridine−Ac_2O. The reaction mixture was diluted with H_2O, washed successively with 5% HCl, a saturated $NaHCO_3$ solution, and H_2O, and dried with anhydrous Na_2SO_4, to afford 790 mg of yellow oil. Purification of this oil by FCC (petroleum ether−EtOAc, 3:2) gave fractions G11, G12, and G13. Fraction G11 (55 mg) produced compound **7a** (9 mg) by preparative TLC (petroleum ether−EtOAc 3:2, ×3). Fraction G12 (250 mg) afforded by FCC (petroleum ether−acetone, 7:3) solids, which by crystallization yielded 102 mg of compound **8a**. Fraction G13 (42 mg) was submitted to a preparative TLC (CH_2Cl_2−MeOH, 49:1, ×5) to produce compounds **8a** (7 mg) and **9a** (8 mg).

Saponification of Compounds 8a and 9a. Compound **8a** (25 mg) and K_2CO_3 (35 mg) in MeOH (5 mL) were refluxed for 3 h, and the reaction mixture was evaporated and purified by FCC eluted with CH_2Cl_2−MeOH (4:1) followed by three successive preparative TLCs (CH_2Cl_2−MeOH, 9:1) to afford 1.5 mg of compound **8** as an amorphous powder. Compound **9a** (6 mg) and K_2CO_3 (7.3 mg) in MeOH (3 mL) were refluxed for 3 h, and the reaction mixture was evaporated and purified by FCC eluted with CH_2Cl_2−MeOH (4:1) to afford 3.0 mg of compound **9** as an amorphous powder.

*(1R,4R,5S,6R,7S,8R,9S,2″S,2‴E)-4-Acetoxy-8-(2″-methylbutanoyloxy)-9-(3‴-methyl-2‴-pentenoyloxy)-10(14)-oplopen-3-one (**1**):* colorless prisms (petroleum ether−acetone); mp 115−117 °C; $[\alpha]^{25}_D$ −14 (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 219 (3.41) nm; ECD (0.28 × 10^{-3} M, MeOH) λ_{max} ($\Delta\varepsilon$) 302 (−1.45); IR ($CHCl_3$) ν_{max} 1745, 1727, 1715 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 1; EIMS m/z 490 $[M]^+$ (1), 432 (1), 389 (10), 377 (6), 97

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(100); HRESIMS m/z 529.2564 $[M + K]^+$ ($C_{28}H_{42}O_7K$ requires 529.2562).

*(1R,2S,4R,5S,6R,7S,8R,9S,11S,2″S,2‴E,2⁗S)-4-Acetoxy-11,12-epoxy-2,8-di(2″-methylbutanoyloxy)-9-(3‴-methyl-2‴-pentenoyloxy)-10(14)-oplopen-3-one (**2**):* colorless prisms (petroleum ether−acetone); mp 105−107 °C; $[\alpha]^{25}_D$ −69 (c 0.2, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 220 (4.17) nm; ECD (0.33×10^{-4} M, MeOH) λ_{max} ($\Delta\varepsilon$) 319 (−16.0), 221 (−8,8); IR ($CHCl_3$) ν_{max} 1762, 1728, 1647 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 1; HRESIMS m/z 627.3127 $[M + Na]^+$ ($C_{33}H_{48}O_{10}Na$ requires 627.3140).

*(1R,2S,4Z,6R,7S,8R,9S,11S,2″S,2‴E,2⁗S)-11,12-Epoxy-2,8-di(2″-methylbutanoyloxy)-9-(3‴-methyl-2‴-pentenoyloxy)-10(14)-oplopen-3-one (**3**):* colorless oil; $[\alpha]^{25}_D$ −115 (c 0.2, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 221 (4.27) nm; ECD (0.29×10^{-4} M, MeOH) λ_{max} ($\Delta\varepsilon$) 351 (−8.7), 250 (+75), 219 (−14.5); IR ($CHCl_3$) ν_{max} 1740, 1646 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 1; HRESIMS m/z 567.2925 $[M + Na]^+$ ($C_{31}H_{44}O_8Na$ requires 567.2928).

*1,5-Dihydroxy-4(15),11(13)-eudesmadien-12-al (**6**):* colorless needles (petroleum ether−acetone); mp 132−135 °C; $[\alpha]^{25}_D$ +58 (c 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 218 (3.87) nm; IR ($CHCl_3$) ν_{max} 3495, 1677 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 9.55 (1H, s, H-12), 6.32 (1H, d, J = 0.8 Hz, H-13a), 6.03 (1H, s, H-13b), 4.84 (1H, t, J = 2.0 Hz, H-15a), 4.68 (1H, t, J = 2.0 Hz, H-15b), 4.11 (1H, dd, J = 11.6, 4.8 Hz, H-1), 3.11 (1H, ttd, J = 12.8, 3.6, 0.8 Hz, H-7), 2.71 (1H, dddt, J = 13.6, 13.6, 5.6, 2.0 Hz, H-3α), 2.16 (1H, ddd, J = 13.6, 4.6, 2.0 Hz, H-3β), 1.90 (1H, brdd, J = 13.2, 5.0 Hz, H-9a), 1.85 (1H, m, H-2a), 1.75 (1H, t, J = 13.2 Hz, H-6a), 1.66 (1H, m, H-9b), 1.63 (1H, m, H-8a), 1.58 (2H, m, H-2b, H-8b), 1.55 (1H, m, H-6b), 0.82 (3H, s, H_3-14); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 194.7 (CH, C-12), 154.8 (C, C-11), 150.2 (C, C-4), 133.4 (CH_2, C-13), 108.8 (CH_2, C-15), 75.9 (C, C-5), 72.8 (CH, C-1), 42.1 (C, C-10), 35.9 (CH_2, C-6), 31.2 (CH, C-7), 30.5 (CH_2, C-9), 29.7 (CH_2, C-2, C-3), 25.4 (CH_2, C-8), 12.8 (CH_3, C-14); EIMS m/z 250 $[M]^+$ (20), 217 (30), 199 (20), 44 (100); HRFABMS m/z 251.1644 $[M + H]^+$ ($C_{15}H_{23}O_3$ requires 251.1647).

*(1R,4R,5S,6R,7S,8R,9S)-4,8-Diacetoxy-9,11-dihydroxy-9-O-β-D-(2′,3′,4′,6′-O-tetraacetyl)glucopyranosyl-10(14)-oplopen-3-one (**7a**):* colorless oil; $[\alpha]^{25}_D$ −32 (c 0.2, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 204 (3.92) nm; ECD (0.60×10^{-4} M, MeOH) λ_{max} ($\Delta\varepsilon$) 302 (−18.10); IR ($CHCl_3$) ν_{max} 3543, 1746 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 2; HRFABMS m/z 699.2853 $[M + H]^+$ ($C_{33}H_{47}O_{16}$ requires 699.2864).

*(1R,4R,5S,6R,7S,8R,9S)-4,8,9,11-Tetrahydroxy-11-O-β-D-glucopyranosyl-10(14)-oplopen-3-one (**8**):* white, amorphous powder; $[\alpha]^{25}_D$ −62 (c 0.1, MeOH); IR (film) ν_{max} 3395, 1735 cm^{-1}; ^1H NMR (MeOH-d_4, 400 MHz) δ 5.16 (1H, brs, H-14a), 4.82 (1H, brs, H-14b), 4.47 (1H, d, J = 8.0 Hz, H-1′), 4.36 (1H, brs, H-9), 4.02 (1H, brq, J = 6.0 Hz, H-4), 3.99 (1H, t, J = 2.5 Hz, H-8), 3.83 (1H, dd, J = 12.0, 2.0 Hz, H-6′a), 3.63 (1H, dd, J = 12.0, 5.2 Hz, H-6′b), 3.51 (1H, m, H-1), 3.35 (1H, m, H-3′), 3.33 (1H, m, H-4′), 3.22 (1H, ddd, J = 9.2, 5.2, 2.0 Hz, H-5′), 3.07 (1H, dd, J = 9.2, 8.0 Hz, H-2′), 2.52 (1H, brd, J = 8.0 Hz, H-5), 2.43 (1H, brdd, J = 17.6, 7.6 Hz, H-2a), 2.30 (1H, dd, J = 10.8, 2.8 Hz, H-7), 2.12 (1H, m, H-6), 2.01 (1H, dd, J = 17.2, 11.2 Hz, H-2b), 1.41 (3H, s, H_3-12), 1.28 (3H, s, H_3-13), 1.27 (3H, d, J = 6.0 Hz, H_3-15); ^{13}C NMR (MeOH-d_4, 100 MHz) δ 222.1 (C, C-3), 152.3 (C, C-10), 108.2 (CH_2, C-14), 98.1 (CH, C-1′), 81.3 (C, C-11), 78.5 (CH, C-3′), 77.7 (CH, C-5′), 77.5 (CH, C-4), 75.2 (CH, C-2′), 74.5 (CH, C-8), 71.7 (CH, C-4′), 63.0 (CH_2, C-6′), 59.1 (CH, C-5), 55.3 (CH, C-6), 53.1 (CH, C-7), 43.8 (CH_2, C-2), 42.8 (CH, C-1), 30.7 (CH_3, C-13), 26.3 (CH_3, C-12), 17.3 (CH_3, C-15); HRFABMS m/z 446.2153 $[M]^+$ ($C_{21}H_{34}O_{10}$ requires 446.2152).

*(1R,4R,5S,6R,7S,8R,9S)-4,8,9-Triacetoxy-11-hydroxy-11-O-β-D-(2′,3′,4′,6′-O-tetraacetyl)glucopyranosyl-10(14)-oplopen-3-one (**8a**):* colorless needles (EtOAc); mp 204−206 °C; $[\alpha]^{25}_D$ −57 (c 0.2, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 203 (3.51) nm; ECD (0.22×10^{-3} M, MeOH) λ_{max} ($\Delta\varepsilon$) 302 (−8.98); IR ($CHCl_3$) ν_{max} 1743 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 2; HRFABMS m/z 741.2959 $[M + H]^+$ ($C_{35}H_{49}O_{17}$ requires 741.2970).

*(1R,6R,7S,8R,9S)-8,9,11-Trihydroxy-4,15-dinor-11-O-β-D-glucopyranosyl-10(14)-oplopen-3-one (**9**):* white, amorphous powder; $[\alpha]^{25}_D$ −83 (c 0.1, MeOH); IR (film) ν_{max} 3370, 1737 cm^{-1}; ^1H NMR (MeOH-d_4, 400 MHz) δ 5.16 (1H, brs, H-14a), 4.86 (1H, brs, H-14b), 4.54 (1H, d, J = 7.6 Hz, H-1′), 4.30 (1H, dd, J = 3.2, 2.0 Hz, H-9), 3.93 (1H, dd, J = 5.6, 3.2 Hz, H-8), 3.82 (1H, dd, J = 12.4, 2.0 Hz, H-6′a), 3.64 (1H, dd, J = 12.4, 5.2 Hz, H-6′b), 3.35 (1H, m, H-3′), 3.33 (1H, m, H-4′), 3.25 (1H, ddd, J = 9.0, 5.2, 2.0 Hz, H-5′), 3.12 (1H, dd, J = 9.2, 7.6 Hz, H-2′), 2.98 (1H, m, H-1), 2.67 (1H, dd, J = 18.0, 6.8 Hz, H-5a), 2.42 (1H, brdd, J = 17.6, 6.8 Hz, H-2a), 2.20 (1H, dd, J = 18.0, 6.0 Hz, H-5b), 2.15 (1H, m, H-7), 2.12 (1H, dd, J = 17.6, 7.6 Hz, H-2b), 1.82 (1H, m, H-6), 1.41 (3H, s, H_3-12), 1.32 (3H, s, H_3-13); ^{13}C NMR (MeOH-d_4, 100 MHz) δ 219.8 (C, C-3), 150.5 (C, C-10), 109.2 (CH_2, C-14), 98.0 (CH, C-1′), 82.2 (C, C-11), 78.3 (CH, C-3′), 77.8 (CH, C-5′), 75.2 (CH, C-9, C-2′), 74.0 (CH, C-8), 71.9 (CH, C-4′), 62.8 (CH_2, C-6′), 55.9 (CH, C-7), 49.0 (CH_2, C-5), 44.0 (CH, C-1), 42.2 (CH_2, C-2), 41.3 (CH, C-6), 25.9 (CH_3, C-12), 24.6 (CH_3, C-13); HRFABMS m/z 402.1888 $[M]^+$ ($C_{19}H_{30}O_9$ requires 402.1890).

*(1R,6R,7S,8R,9S)-8,9-Diacetoxy-11-hydroxy-4,15-dinor-11-O-β-D-(2′,3′,4′,6′-O-tetraacetyl)glucopyranosyl-10(14)-oplopen-3-one (**9a**):* colorless oil; $[\alpha]^{25}_D$ −74 (c 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 204 (3.62) nm; ECD (0.15×10^{-3} M, MeOH) λ_{max} ($\Delta\varepsilon$) 204 (−5.54), 296 (−12.3); IR ($CHCl_3$) ν_{max} 1746 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 2; HRFABMS m/z 655.2603 $[M + H]^+$ ($C_{31}H_{43}O_{15}$ requires 655.2602).

*Crystal data for compound **1** (ref 28):* $C_{28}H_{42}O_7$, M_r 490.61, monoclinic, space group C_2, a = 28.9743(15) Å, α = 90°, b = 6.5896(3) Å, β = 113.665(1)°, c = 16.3429(8) Å; γ = 90°, V = 2857.9(2) Å^3, Z = 4, D_c = 1.140 Mg/m^3, $F(000)$ = 1064; crystal dimensions/shape/color 0.472 × 0.147 × 0.040 mm/prism/colorless. Reflections collected 14 120, independent reflections 6416; final R indices $[I > 2\sigma(I)]$ R_1 = 0.0660, wR_2 = 0.1338; R indices (all data) R = 0.1266, wR_2 = 0.1581. Remarks: main residue disorder 46%.

*Crystal data for compound **2** (ref 28):* $C_{33}H_{48}O_{10}$, M_r 604.71, orthorhombic, space group $P2_12_12_1$, a = 8.4016(8) Å, α = 90°, b = 19.887(2) Å, β = 90°, c = 20.501(2) Å; γ = 90°, V = 3425.5(5) Å^3, Z = 4, D_c = 1.173 Mg/m^3, $F(000)$ = 1304; crystal dimensions/shape/color 0.36 × 0.08 × 0.05 mm/prism/colorless. Reflections collected 38 107, independent reflections 6271; final R indices $[I > 2\sigma(I)]$ R_1 = 0.0723, wR_2 = 0.1298; R indices (all data) R = 0.1381, wR_2 = 0.1539.

Hydrolysis of the Glucoside Mixture G1 and of Compound 8a. Mixture G1 (50 mg) was heated at 70 °C for 30 min with 2 M HCl. The reaction mixture was neutralized with 2 M KOH and washed with CH_2Cl_2, and the aqueous layer was evaporated to obtain an amber residue (17 mg). The residue (2 mg) was silylated with HMDS−TMCS−pyridine (3:1:9) and analyzed by GC to identify glucose as the sugar present in the mixture. The remaining 15 mg was purified by FCC (EtOAc−MeOH, 7:3) to obtain D-glucose (8.5 mg): $[\alpha]^{25}_D$ +49 (c 0.3, H_2O) (lit.[29] $[\alpha]^{25}_D$ +52).

Compound **8a** (80 mg) was heated at 70 °C for 2 h with 2 M HCl. The reaction mixture was neutralized with 2 M KOH and evaporated, and the residue was purified through a VCC column using an EtOAc−MeOH mixture in increasing gradient of polarity to obtain compound **10** (7 mg) and D-glucose (6.5 mg): $[\alpha]^{25}_D$ +52 (c 0.2, H_2O).

*4,8,9,11-Tetrahydroxy-3-oxo-10(14)-oplopene (**10**):* colorless needles (acetone); mp 147−150 °C; $[\alpha]^{25}_D$ −49 (c 0.2, MeOH); IR (Nujol) ν_{max} 3390, 1735 cm^{-1}; ^1H NMR (MeOH-d_4, 400 MHz) δ 4.97 (1H, brs, H-14a), 4.73 (1H, brs, H-14b), 4.22 (1H, d, J = 2.8 Hz, H-9), 3.64 (1H, dq, J = 9.6, 6.0 Hz, H-4), 3.39 (1H, dd, J = 10.4, 2.8 Hz, H-8), 2.71 (1H, ddd, J = 18.6, 10.8, 6.8 Hz, H-1), 2.38 (1H, dd, J = 18.0, 6.8 Hz, H-2a), 2.13 (1H, dd, J = 18.0, 10.8 Hz, H-2b), 1.98 (1H, dd, J = 12.0, 10.4 Hz, H-7), 1.69 (1H, dd, J = 14.0, 9.6 Hz, H-5), 1.39 (3H, s, H-12), 1.27 (3H, d, J = 6.0 Hz, H-15), 1.17 (1H, m, H-6), 1.17 (3H, s, H-13); ^{13}C NMR (MeOH-d_4, 100 MHz) δ 214.4 (C, C-3), 150.7 (C, C-10), 108.3 (CH_2, C-14), 79.2 (CH, C-9), 76.7 (C, C-11), 75.7 (CH, C-8), 69.1 (CH, C-4), 59.4 (CH, C-5), 49.3 (CH, C-7), 47.2 (CH, C-6), 41.3 (CH_2, C-2), 40.1 (CH, C-1), 31.6 (CH_3, C-12), 20.2 (CH_3, C-15), 19.1 (CH_3, C-13); EIMS m/z 284 $[M]^+$ (5), 266 (50), 208 (90), 190 (85), 97 (100); HRFABMS m/z 284.1627 $[M]^+$ ($C_{15}H_{24}O_5$ requires 284.1624).

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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 and were fed laboratory diet and H_2O *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 compounds **1**−**5** as previously reported.[24] Acetone−CH_2Cl_2 (1:1 mixture) was used as the solvent in the topical application of the tested compounds.

Myeloperoxidase Assay. Tissue MPO activity was measured for compounds **1**−**4** in biopsies taken from ears 4 h after product administration using an adapted method of Bradley et al.[26] and Suzuki et al.,[27] as previously reported.[30]

Statistical Analysis. All data are presented as the percentage mean ± standard error of the mean (SEM). The statistical analysis used Student's *t* test, whereas analysis of variance (ANOVA) followed by the Dunnett 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. Pearson's correlation coefficient was calculated for the edema and MPO activity of all biopsies showing a positive correlation with $p < 0.001$.

■ ASSOCIATED CONTENT

Supporting Information

^1H, ^{13}C, and 2D NMR spectra of compounds **1**−**3**, **6**, **7a**, **8**, **8a**, **9**, **9a**, and **10**; the ^1H NMR spectrum of the glucoside mixture G1, a table of primary anti-inflammatory screening of compounds **1**−**5** and ID_{50}, and a figure of the comparative effect of compounds **1**−**4** and indomethacin on myeloperoxidase levels in mouse ear edema induced by TPA are available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*(A.-L. Pérez-Castorena) Tel: (5255) 56-224412. Fax: (5255) 56-162217. E-mail: alperezc@unam.mx.

Notes

The authors declare no competing financial interest.

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