# Sesquiterpenoids from *Pulicaria canariensis* and Their Cytotoxic Activities[#]

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Thirteen new sesquiterpenes, pulicanadiene A (**1**), B (**2**), and C (**3**), pulicanone (**4**), pulicanol (**5**), pulicanarals A (**6**), B (**7**), and C (**8**), pulicanadienals A (**9**) and B (**10**), pulicanadienol (**11**), and pulioplopanones A (**12**) and B (**13**), and seven known compounds, stigmasterol, ergosterol peroxide, calenduladiol, 7,4′-di-*O*-methyldihydrokaempferol, 5,7-dihydroxy-3,3′,4′-trimethoxyflavone, dihydroquercetin 7,3′-dimethyl ether, and 6,15α-epoxy-1$\beta$,4$\beta$-dihydroxyeudesmane, were isolated from *Pulicaria canariensis*. Compound **4a** showed cytotoxicity on the human myeloid leukemia cell line HL-60. The cytotoxicity was caused by induction of apoptosis as determined by microscopy of nuclear changes, activation of caspases, and the cleavage of poly(ADP-ribose) polymerase-1.

The genus *Pulicaria* (Compositae) is represented in the Canary Islands by three species distributed in the more easterly islands, namely, *P. crispa* Forssk., *P. burchardii* Hutch., and *P. canariensis* Bolle. The last two are endemic, with *P. canariensis* having two subspecies, *canariensis* and *lanata*.[1] Several species of this genus have been used as insect repellents[2] and in the treatment of dysentery.[3,4] This genus has been the subject of several chemical investigations, giving rise to the isolation of flavonoids,[5] sesquiterpenes,[6,7] and triterpenes.[8] As a part of our continuing search for novel, plant-derived cancer chemotherapeutic agents,[9,10] we have investigated an ethanolic extract of the aerial part of *P. canariensis* Bolle subsp. *canariensis*, leading to the isolation of 13 new sesquiterpenes, pulicanadienes A (**1**), B (**2**), and C (**3**), pulicanone (**4**), pulicanol (**5**), pulicanarals A (**6**), B (**7**), and C (**8**), pulicanadienals A (**9**) and B (**10**), pulicanadienol (**11**), and pulioplopanones A (**12**) and B (**13**), and seven known compounds, stigmasterol,[11] ergosterol peroxide,[12] calenduladiol,[13] 7,4′-di-*O*-methyldihydrokaempferol,[14] 5,7-dihydroxy-3,3′,4′-trimethoxyflavone,[15] dihydroquercetin 7,3′-dimethyl ether,[16] and 6,15α-epoxy-1$\beta$,4$\beta$-dihydroxyeudesmane.[17] The structures of the known compounds were confirmed by comparison of their spectroscopic properties with published data. The structure elucidation of the new compounds was carried out by extensive spectral data interpretation as well as by chemical transformation. To assess whether these compounds display cytotoxic properties, we also studied their effects on the viability of the human promyelocytic cell line HL-60, using the colorimetric MTT procedure.

## Results and Discussion

Pulicanadiene A (**1**) was isolated as an amorphous powder, $[\alpha]^{25}_{D}$ 21.4° (*c* 0.014, $CHCl_3$), and its molecular formula was determined as $C_{19}H_{28}O_6$ by HREIMS (*m/z* 352.1877 $[M]^+$). The $^1H$ and $^{13}C$ NMR spectra of compound **1** (Table 1) carried out at 25 °C presented poor resolution owing to the existence of a conformational equilibrium,[18] but heating to 50 °C sharpened most lines and allowed assignment of the signals. Thus, characteristic signals for three methyl groups were observed at $\delta_H$ 0.97 (3H, d, $J$ = 4.5 Hz, $CH_3$-13), 1.06 (3H, d, $J$ = 5.6 Hz, $CH_3$-12), and 1.84 (3H, s, $CH_3$-15), two acetoxy groups at $\delta_H$ 2.00 (3H, s, OAc) and 2.05 (3H, s, OAc), two olefinic protons at $\delta_H$ 5.18 (1H, d, $J$ = 8.9 Hz, H-5) and 6.78 (1H, t, $J$ = 8.0 Hz, H-1), and two signals at $\delta_H$ 5.77 (1H, d, $J$ = 9.1 Hz, H-6) and 5.63 (1H, br s, H-8) corresponding to the geminal protons to the acetoxy groups. The $^{13}C$ NMR and DEPT spectra of **1** disclosed 19 carbons, which were indicative of an α,$\beta$-unsaturated carboxylic acid ($\delta_C$ 173.0, s, C-14; 137.4, s, C-10; 141.6, d, C-1), two oxygenated methines ($\delta_C$ 70.0, d, C-6; 70.1, d, C-8), an isopropyl group ($\delta_C$ 26.7, d, C-11; 22.4, q, C-12; 21.8, q, C-13), in addition to two acetoxy groups, three methylenes, two other methines, and one other quaternary carbon. These NMR data were closely comparable to those of the known germacrane diacetoxy tovarol (**14**)[19] and suggested that compound **1** is also a germacrane.[15] The IR absorption at 3200−3067 and 1693 $cm^{-1}$ confirmed the presence of an α,$\beta$-unsaturated carboxylic acid. In fact, methylation with diazomethane yielded **1a**, in the $^1H$ NMR spectrum (Table 1) of which appears a new singlet at $\delta_H$ 3.73 (3H, s, COOMe). Comparison of **1a** and **14** led us to conclude that acetoxy groups could be placed at C-6 and C-8 in **1a**. The $^1H$ and $^{13}C$ NMR spectral features found in both compounds suggest that they are similar. This was deduced from the following 2D NMR data of **1a**, in which the α,$\beta$-unsaturated acid group was supported by the HMBC correlations H-1 with C-10 and C-14; $H_2$-9 with C-1, C-8, C-10, and C-14; $CH_3$-15 with C-3, C-4, and C-5; $CH_3$-12 with C-7; and $CH_3$-13 with C-7.

[#] Dedicated to the memory of Professor Antonio G. González.

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**Chart 1**

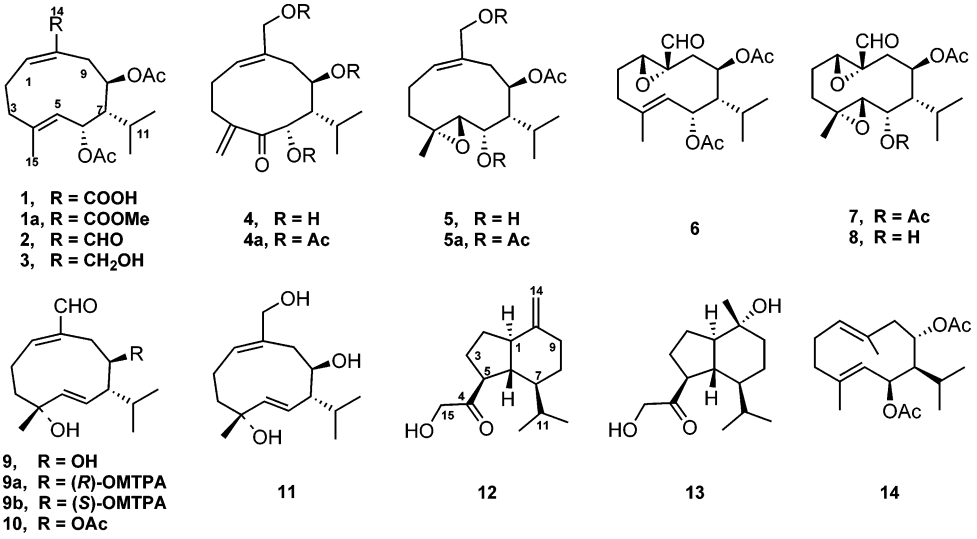


**Table 1.** $^1$H and $^{13}$C NMR Data for Compounds **1**, **1a**, **2**, and **3** (benzene-$d_6$ at 50 °C, $J$ values (Hz) in parentheses)

| | **1** | | **1a** | | **2** | | **3** | |
|---|---|---|---|---|---|---|---|---|
| position | $\delta_C$ | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ | $\delta_H$ |
| 1 | 141.6 | 6.78 t (8.0) | 138.6 | 6.60 t (8.0) | 150.4 | 5.86 t (8.4) | 126.7 | 5.34 t (8.5) |
| 2 | 26.7 | 2.10 m<br>2.35 m | 29.6 | 2.12 m<br>2.32 m | 27.1 | 1.70 m<br>1.80 m | 26.0 | 1.70 m<br>2.01 m |
| 3 | 36.5 | 1.80 m<br>2.10 m | 37.0 | 1.80 m<br>2.12 m | 36.8 | 1.70 m | 31.0 | 1.40 m |
| 4 | 132.2 | | 132.8 | | 136.6 | | 136.1 | |
| 5 | 123.7 | 5.18 d (8.9) | 123.3 | 5.17 d (9.1) | 124.3 | 5.11 d (8.6) | 127.0 | 5.11 m |
| 6 | 70.0 | 5.77 d (9.1) | 69.9 | 5.77 d (9.2) | 70.0 | 6.02 dd (1.8, 8.8) | 71.0 | 6.13 d br (5.9) |
| 7 | 52.2 | 1.80 m | 53.0 | 1.80 m | 52.6 | 1.80 m | 50.0 | 1.92 dd br (3.3, 13.4) |
| 8 | 70.1 | 5.63 s br | 70.6 | 5.60 s br | 70.1 | 6.09 s br | 71.1 | 5.76 t (9.2) |
| 9 | 31.6 | 2.35 m<br>2.66 d (11.2) | 32.0 | 2.32 m<br>2.66 dd (4.0, 13.6) | 30.2 | 2.34 s br<br>2.61 dd (5.3, 14.0) | 30.0 | 2.50 m<br>2.68 m |
| 10 | 137.3 | | 137.4 | | 143.6 | | 135.0 | |
| 11 | 26.7 | 2.10m | 26.5 | 2.12 m | 26.7 | 1.80 m | 27.0 | 2.12 t (6.3) |
| 12 | 22.4 | 1.06 d (5.6) | 22.2 | 1.02 d (5.3) | 22.6 | 1.28 d (6.3) | 19.9 | 1.13 d (6.8) |
| 13 | 21.8 | 0.97 d (4.5) | 21.7 | 0.97 d (5.3) | 21.6 | 1.09 d (6.3) | 21.4 | 0.97 d (6.9) |
| 14 | 173.0 | | 168.7 | | 193.6 | 9.26 s | 69.7 | 4.12 d br (17.8) |
| 15 | 20.6 | 1.84 s | 20.5 | 1.83 s | 20.6 | 1.98 s | 20.0 | 1.52 s |
| OMe | | | 50.8 | 3.73 s | | | | |
| OAc | 18.0 | 2.00 s | 20.4 | 1.99 s | 17.9 | 1.77 s | 21.5 | 1.78 |
| | 20.6 | 2.05 s | 20.5 | 2.05 s | 20.5 | 1.72 s | 21.5 | 1.85 |
| | 169.1 | | 168.7 | | 169.0 | | 170.2 | |
| | 169.1 | | 168.7 | | 169.0 | | 170.2 | |

NOESY correlations between H-6 ($\delta_H$ 5.77) and $CH_3$-15, H-7 clearly showed that these protons were on the same face. Correlations were also observed between H-8 ($\delta_H$ 5.63) and $CH_3$-12, $CH_3$-13, and H-5. Thus, the C-6 acetoxy and C-7 isopropyl groups were deduced to be α correlated. This was also confirmed when similar correlations were observed in a NOESY experiment at −50 °C. From the above-mentioned spectra it could be deduced that the preferred conformers in solution maintain a similar orientation in space, and therefore compound **1** was assigned the structure (1*E*,4*E*)-6α,8*β*-diacetoxy-7*βH*-germacra-1(10),4-dien-14-oic acid, which was unequivocally confirmed by an X-ray crystallographic analysis of **1a** (Figure 1).

Pulicanadiene B (**2**) was obtained as a colorless oil, with $[\alpha]^{25}_D$ 35.7° (*c* 0.042, $CHCl_3$). The molecular formula of **2** was determined as $C_{19}H_{28}O_5$, one oxygen atom less than obtained for **1**, on the basis of its HRFABMS data (*m/z* 359.1874 $[M + Na]^+$). Both the $^1$H and $^{13}$C NMR (Table 1) spectral data of compound **2** were close to those of **1**, but differences were evident between these compounds since the carboxyl group at C-14 of **1** is replaced with an aldehyde group in **2**, $\delta_H$ 9.26 (1H, s, H-14). Therefore, the structure of **2** was elucidated as (1*E*,4*E*)-6α,8*β*-diacetoxy-7*βH*-germacra-1(10),4-dien-14-al.

Pulicanadiene C (**3**) was also isolated as a colorless oil with $[\alpha]^{25}_D$ −75.0° (*c* 0.008, $CHCl_3$). The molecular formula

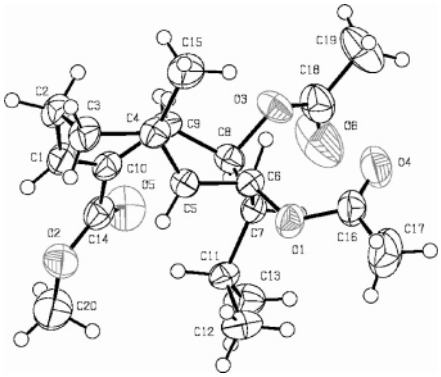


**Figure 1.** ORTEP drawing of compound **1a**.

**Table 2.** $^1$H and $^{13}$C NMR Data for Compound **4a** ($CDCl_3$, *J* values (Hz) in parentheses)

| position | $\delta_C$ | $\delta_H$ |
|---|---|---|
| 1 | 131.9 | 5.59 dd (5.7, 11.4) |
| 2 | 23.3 | 2.00 m |
| 3 | 32.6 | 2.07 m |
| | | 2.94 d br, (12.7) |
| 4 | 145.4 | |
| 5 | 198.7 | |
| 6 | 72.9 | 5.95 s |
| 7 | 45.5 | 2.54 dd (3.4, 10.5) |
| 8 | 69.6 | 5.41 dt (3.5, 10.6) |
| 9 | 30.0 | 2.32 dd (3.5, 8.9) |
| | | 2.44 td (4.3, 12.6) |
| 10 | 132.6 | |
| 11 | 27.4 | 2.27 m |
| 12 | 21.6 | 1.12 d (6.9) |
| 13 | 17.6 | 0.75 d (6.9) |
| 14 | 69.3 | 4.51 s |
| 15 | 124.1 | 5.66 s |
| OAc | 20.8 | 2.03 s |
| | 20.9 | 2.04 s |
| | 21.1 | 2.19 s |
| | 169.7 | |
| | 169.9 | |
| | 170.6 | |

$C_{19}H_{30}O_5$ was determined from EIMS and HREIMS analysis. The IR spectrum of **3** showed absorption bands at 3414 and 1728 $cm^{-1}$ due to hydroxyl and acetoxy functions. The $^1$H NMR (Table 1) spectrum of **3** showed signals analogous to those of **2** with the exception that the C-14 is replaced with a hydroxymethyl group, $\delta_H$ 4.12 (2H, d, $J$ = 11 Hz, $H_2$-14), in **3**. Thus, **2** was treated with sodium borohydride ($NaBH_4$) in methanol to give **3** as the major product. The IR and $^1$H NMR spectra of both compounds were superimposable. On the basis of the above spectral and chemical evidence, **3** was assigned as (1*E*,4*E*)-6α,8*β*-diacetoxy-7*β*H-germacra-1(10),4-dien-14-ol.

Pulicanone (**4**) was purified as its triacetate (**4a**) by treatment with acetic anhydride ($Ac_2O$) in pyridine: **4a** [colorless oil, $[\alpha]^{25}_D$ 4.9° (*c* 0.081, $CHCl_3$)]. The IR spectrum showed the presence of acetoxy functions (1741 $cm^{-1}$) and a conjugated carbonyl group (1694 $cm^{-1}$). In the UV spectrum of **4a**, an absorption maximum was observed at 225 nm, suggestive of an α,*β*-unsaturated carbonyl group. The $^1$H and $^{13}$C NMR (Table 2) spectra of **4a** showed signals assignable to the methyl groups of an isopropyl group at $\delta_H$ 0.75 (3H, d, $J$ = 6.9 Hz, $H_3$-13) and 1.12 (3H, d, $J$ = 6.9 Hz, $H_3$-12) and three olefinic protons at $\delta_H$ 5.59 (1H, dd, $J$ = 5.7, 11.4 Hz, H-1) and 4.51 (2H, s br, $H_2$-14). In the $^1$H−$^1$H COSY spectrum of **4a**, H-6 ($\delta_H$ 5.95) correlated to H-7 ($\delta_H$ 2.54) and the latter to H-8 ($\delta_H$ 5.41), suggesting that the acetoxy groups are located at C-6 and C-8. To determine the position of the carbonyl group, the HMBC spectrum of

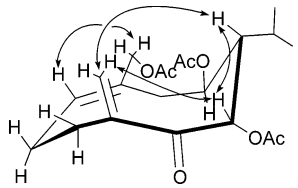


**Figure 2.** Selected ROESY correlations for compound **4a**.

**Table 3.** $^1$H and $^{13}$C NMR Data for Compound **5a** ($CDCl_3$, *J* values (Hz) in parentheses)

| position | $\delta_C$ | $\delta_H$ |
|---|---|---|
| 1 | 135.0 | 5.64 t (7.5) |
| 2 | 24.4 | 2.36 m |
| 3 | 38.4 | 2.19 m |
| | | 1.25 m |
| 4 | 58.8 | |
| 5 | 66.5 | 2.87 d (6.6) |
| 6 | 73.5 | 4.89 d (6.8) |
| 7 | 47.5 | 1.63 d (9.2) |
| 8 | 72.1 | 5.46 dd (4.7, 12.1) |
| 9 | 29.6 | 2.63 m |
| | | 1.27 m |
| 10 | 129.3 | |
| 11 | 26.2 | 1.86 m |
| 12 | 22.9 | 1.12 d (6.5) |
| 13 | 21.2 | 0.92 d (6.6) |
| 14 | 63.5 | 4.62 d (12.26) |
| | | 4.57 d (12.12) |
| 15 | 16.3 | 1.17 s |
| OAc | 20.8 | 2.00 s |
| | 21.0 | 2.07 s |
| | 21.1 | 2.09 s |
| | 169.8 | |
| | 169.8 | |
| | 170.8 | |

**4a** was useful. Thus, connectivities observed in the signals at $\delta_H$ 4.51 ($H_2$-14) and 5.95 (H-6) correlated with the carbonyl and helped situate that carbonyl group at C-5. The relative stereochemistry of **4a** was determined by a ROESY experiment (Figure 2) in which correlations were observed between H-7 ($\delta$ 2.54), H-6 ($\delta$ 5.95), and $H_2$-14 ($\delta$ 4.51). Thus, the C-6 acetoxy and C-7 isopropyl groups, as in **1**, possess an α-orientation. Therefore, the structure of **4** was determined to be (1*E*)-6α,8*β*,14-trihydroxy-7*β*H-germacra-1(10),4(15)-dien-5-one.

Pulicanol (**5**) was isolated as a colorless oil. The EIMS of **5** showed a molecular ion at *m/z* 312 $[M]^+$. The molecular formula $C_{17}H_{28}O_5$ of **5** was determined from molecular ion measurement. The IR spectrum of **5** showed absorption bands at 3404, 1736, 1647, and 897 $cm^{-1}$ assigned to hydroxyl, acetoxy, and olefinic functions, respectively. The $^1$H NMR spectrum of **5** showed the presence of three methyls at $\delta_H$ 0.95 (3H, d, $J$ = 6.4 Hz, $CH_3$-13), 1.12 (3H, d, $J$ = 6.4 Hz, $CH_3$-12), and 1.16 (3H, s, $CH_3$-15), one acetoxy group at $\delta_H$ 2.15 (3H, s, OAc), an olefinic proton at $\delta_H$ 5.45 (1H, t, $J$ = 6.8 Hz, H-1), one hydroxy methylene group at $\delta_H$ 4.29 (1H, d, $J$ = 11.8 Hz, $H_a$-14) and 4.12 (1H, d, $J$ = 11.8 Hz, $H_b$-14), and three oxygenated methine protons at $\delta_H$ 2.82 (1H, d, $J$ = 7.7 Hz, H-5), 3.51 (1H, m, H-6), and 5.18 (1H, dd, $J$ = 5.2, 12.0 Hz, H-8). To obtain a better resolution in the $^1$H and $^{13}$C NMR spectra, acetylation ($Ac_2O$−pyridine) of **5** yielded an acetoxy derivative (**5a**). The $^{13}$C NMR spectrum (Table 3) of **5a** disclosed 21 carbons, which were indicative of an oxygenated methylene ($\delta_C$ 63.5, t, C-14), an epoxide functional group ($\delta_C$ 66.5, d, C-5; 58.8, s, C-4), two oxygenated methines ($\delta_C$ 73.5, d, C-6; 72.1, d, C-8), and an isopropyl group ($\delta_C$ 26.2, d, C-11; 22.9, q, C-12; 21.2, q, C-13). An analysis of 2D NMR data revealed that **5a** possesses the same skeleton as **3**, with a

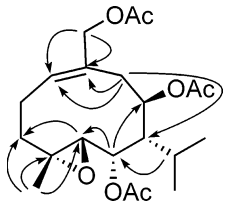


**Figure 3.** Selected HMBC (H to C) correlations for compound **5a**.

methyl-bearing trisubstituted epoxide occurring in **5a** instead of the methyl-bearing trisubstituted olefin in **3**. HMBC correlations (Figure 3) of $CH_3$-15 with C-4 and C-5, H-5 with C-3 and C-4, and H-7 with C-5, C-6, C-8, C-12, and C-13 helped position the methyl-bearing trisubstituted epoxide. The relative stereochemistry of **5a** was deduced from a 2D ROESY experiment, which indicated correlations of $CH_3$-15 ($\delta_H$ 1.17) with H-7 and H-6, clearly showing that these protons were on the same face, while H-5 ($\delta_H$ 2.87) correlated with H-8 on the opposite side of the molecule, indicating that the 4,5-epoxide ring was in *trans* form. Due to its large size, the conformational movement of this 10-membered ring is probably restricted.[20] The above confirmed the structure of **5** as (1*E*)-4α,5*β*-epoxy-8*β*-acetoxy-7*βH*-germacra-1(10)-ene-6α,14-diol.

Pulicanaral A (**6**) was isolated as colorless needles, mp 183−184 °C and $[\alpha]^{25}_D$ 11.1° (*c* 0.108, $CHCl_3$), and its molecular formula was determined as $C_{19}H_{28}O_6$ by HREIMS (*m/z* 352.1905 $[M]^+$). The $^{13}C$ NMR spectrum (Table 4) gave a total of 19 separate carbon resonances (five methyls, three methylenes, seven methines, and four quaternary carbons), in agreement with the molecular formula. The $^1H$ NMR spectrum (Table 4) of compound **6** displayed signals for three methyl groups at $\delta_H$ 0.97 (3H, d, $J$ = 6.6 Hz, $CH_3$-13), 1.32 (3H, d, $J$ = 6.6 Hz, $CH_3$-12), and 1.45 (3H, s, $CH_3$-15), two acetoxy groups at $\delta_H$ 2.00 (3H, s, OAc) and 2.08 (3H, s, OAc), one olefinic proton at $\delta_H$ 5.43 (1H, d, $J$ = 7.2 Hz, H-5), and one aldehyde proton at $\delta_H$ 9.28 (1H, s, H-14). The $^1H$ NMR spectra of **6** and **2** were similar with regard to their functional group analysis. However, compound **6** has only one olefinic proton. This led us to conclude that one of the two double bonds is epoxidized. In fact, its $^{13}C$ NMR spectrum showed signals ($\delta_C$ 63.9, d, C-1; 61.1, s, C-10) corresponding to an epoxide ring. In the HMBC spectrum of **6**, the signals at $\delta_H$ 9.28 (H-14) correlated with the oxygenated quaternary carbon ($\delta_C$ 61.1, C-10), which indicated that an epoxide ring was attached at C-1 (10). To establish the relative stereochemistry of **6**, a ROESY experiment was carried out. A correlation was observed between H-1 and H-8, while correlations observed between H-6, H-7 and $CH_3$-15 suggested that they have an opposite orientation. From these data together with an X-ray diffraction analysis (Supporting Information) the stereochemistry of the substituents was observed as well as the *trans* relationship between the methyl group at C-4 and the H at C-5 and of the aldehyde group at C-10 and the H at C-1. From these data the new compound **6** was deduced to be (4*E*)-1*β*,10α-epoxy-6α,8*β*-diacetoxy-7*βH*-germacra-4-en-14-al.

Compound **7**, pulicanaral B, was purified as colorless needles by recrystallization from chloroform and methanol and has the molecular formula $C_{19}H_{28}O_7$ as determined by HRFABMS (*m/z* 391.1738 $[M + Na]^+$) and NMR spectroscopy. The $^1H$ NMR data of **7** were very similar to those of **6**, suggesting that the two compounds are closely related in structure. The major difference is that **7** has an additional epoxide functional group. A detailed comparison of the $^1H$ and $^{13}C$ NMR signals of **6** and **7** (Table 4) revealed that the signals due to H-5 ($\delta_H$ 5.43, d, $J$ = 7.2 Hz), C-4 ($\delta_C$ 133.6), and C-5 ($\delta_C$ 129.7) of **6** have been replaced by signals due to an epoxide group [H-5 ($\delta_H$ 3.04, d, $J$ = 6.7 Hz), C-4 ($\delta_C$ 58.0), C-5 ($\delta_C$ 66.2)]. The structure was shown to be 1*β*,10α;4α,5*β*-diepoxy-6α,8*β*-diacetoxy-7*βH*-germacra-14-al, and again, only the relative configuration could be determined by an X-ray diffraction analysis (Supporting Information).

Compound **8**, pulicanaral C, was obtained as a colorless oil. The $^1H$ and $^{13}C$ NMR spectra (Table 4) were similar to those of **7**, with the exception of the absence of an acetoxy signal since the oxygenated methine proton of **7** at $\delta_H$ 4.87 (1H, dd, $J$ = 1.0, 6.7 Hz, H-6) was displaced to $\delta_H$ 3.51 (1H, dd, $J$ = 1.2, 6.7 Hz, H-6) in **8** and one of two acetyl methyl signals in **7** disappeared in **8**. Acetylation ($Ac_2O$−pyridine) of **8** gave a diacetoxy derivative whose physical and spectroscopical constants were identical with those of **7**.

**Table 4.** $^1H$ and $^{13}C$ NMR Data for Compounds **6**−**8** ($CDCl_3$, $J$ values (Hz) in parentheses)

| | **6** | | **7** | | **8** | |
|---|---|---|---|---|---|---|
| position | $\delta_C$ | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ | $\delta_H$ |
| 1 | 63.9 | 3.34 d (11.6) | 62.3 | 3.50 d (11.2) | 62.5 | 3.45 d (11.5) |
| 2 | 23.7 | 1.75 m | 23.1 | 1.70 m | 23.0 | 1.75 m |
| | | 2.38 m | | 2.38 m | | 2.40 m |
| 3 | 35.9 | 2.33 m | 35.7 | 1.29 m | 35.8 | 1.30 m |
| | | 2.44 m | | 2.22 ddd (3.5, 7.1, 13.8) | | 2.20 ddd (3.5, 7.0, 13.5) |
| 4 | 133.6 | | 58.0 | | 58.0 | |
| 5 | 129.7 | 5.43 d (7.2) | 66.2 | 3.04 d (6.7) | 67.7 | 3.03 d (6.7) |
| 6 | 71.2 | 5.54 d (6.1) | 72.5 | 4.87 dd (1.0, 6.7) | 70.2 | 3.51 dd (1.2, 6.7) |
| 7 | 51.4 | 1.58 d br (9.3) | 47.9 | 1.52 m | 49.7 | 1.54 m |
| 8 | 68.8 | 5.40 dd (4.2, 12.6) | 69.2 | 5.44 dd (4.6, 12.1) | 70.0 | 5.20 dd (4.6, 12.6) |
| 9 | 34.6 | 2.79 t (12.8) | 33.3 | 2.84 t (12.6) | 34.5 | 3.01 t (12.6) |
| | | 1.43 d (4.20) | | 1.55 m | | 1.54 m |
| 10 | 61.1 | | 61.8 | | 61.5 | |
| 11 | 26.3 | 1.96 m | 26.3 | 1.95 m | 26.3 | 1.90 m |
| 12 | 22.0 | 1.32 d (6.6) | 21.9 | 1.29 d (6.7) | 22.1 | 1.31 d (6.6) |
| 13 | 21.1 | 0.97 d (6.6) | 21.0 | 0.96 d (6.7) | 21.1 | 0.99 d (6.6) |
| 14 | 199.7 | 9.28 s | 199.8 | 9.38 s | 199.3 | 9.40 s |
| 15 | 16.4 | 1.45 s | 16.7 | 1.10 s | 16.1 | 1.13 s |
| OAc | 20.9 | 2.00 s | 20.8 | 2.06 s | 21.0 | 2.12 s |
| | 21.1 | 2.08 s | 20.9 | 2.06 s | 173.0 | |
| | 169.8 | | 169.7 | | | |
| | 170.4 | | 169.8 | | | |

**Table 5.** $^1$H and $^{13}$C NMR Data for Compounds **9**−**11** ($CDCl_3$, *J* values (Hz) in parentheses)

| | **9** | | **10** | | **11** | |
|---|---|---|---|---|---|---|
| position. | $\delta_C$ | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ | $\delta_H$ |
| 1 | 163.7 | 6.58 dd (4.4, 11.9) | 161.6 | 6.41 dd (5.8, 10.5) | 134.0 | 5.36 dd (2.5, 9.0) |
| 2 | 25.0 | 2.11 m | 25.2 | 2.04 m | 23.1 | 1.80 m |
| | | 2.64 q (12.0) | | 2.53 m | | 2.26 d (10.2) |
| 3 | 41.0 | 1.67 dd (11.0, 14.0) | 39.9 | 1.74 m | 41.2 | 1.50 dd (10.8, 12.8) |
| | | 2.01 m | | 1.98 m | | 1.78 m |
| 4 | 73.6 | | 73.6 | | 74.1 | |
| 5 | 139.7 | 5.00 d (15.3) | 138.6 | 5.20 d (15.3) | 140.7 | 5.17 d (15.3) |
| 6 | 122.2 | 5.22 dd (10.7, 15.3) | 121.4 | 5.25 dd (10.0, 15.4) | 120.0 | 5.22 dd (9.4, 15.3) |
| 7 | 55.7 | 1.56 m | 52.8 | 1.60 m | 55.7 | 1.98 m |
| 8 | 66.7 | 3.76 m | 70.3 | 5.00 m | 68.6 | 3.85 dd (3.3, 6.2) |
| 9 | 32.1 | 2.83 dd (4.8, 15.3) | 31.9 | 2.79 dd (3.4, 15.0) | 35.6 | 2.10 m |
| | | 2.40 d (15.3) | | 2.51 dd (3.4, 15.0) | | 2.88 d br (14.9) |
| 10 | 139.7 | | 157.6 | | 150.0 | |
| 11 | 26.6 | 2.17 ddd (2.7, 6.8, 6.8) | 27.5 | 2.16 m | 27.3 | 2.01 m |
| 12 | 21.7 | 0.81 d (6.9) | 21.4 | 0.80 d (6.8) | 21.8 | 0.87 d (6.4) |
| 13 | 16.4 | 0.79 d (6.9) | 16.8 | 0.79 d (6.8) | 16.7 | 0.85 d (6.4) |
| 14 | 199.5 | 9.39 s | 194.8 | 9.42 s | 69.8 | 4.03 d (12.0) |
| | | | | | | 4.10 d (12.0) |
| 15 | 29.8 | 1.32 s | 29.6 | 1.34 s | 30.0 | 1.29 s |
| OAc | | | 171.2 | | | |
| | | | 21.2 | 2.12 s | | |

On the basis of the above evidence, we assigned **8** as 1*β*,-10α;4α,5*β*-diepoxy-8*β*-acetoxy-6α-hydroxy-7*βH*-germacra-14-al.

Pulicanadienal A (**9**), obtained as a colorless oil, has a molecular formula of $C_{15}H_{24}O_3$ as determined by HR-FABMS (*m/z* 275.1645 $[M + Na]^+$). The IR absorption of **9** (3410 and 1660 $cm^{-1}$) indicated the presence of a hydroxyl and an α,*β*-unsaturated carbonyl functional group. The $^1$H NMR spectrum (Table 5) of **9** showed signals for an oxygenated methine proton at $\delta_H$ 3.76 (1H, m, H-8), two methyl groups on a methine carbon at $\delta_H$ 0.81 (3H, d, $J$ = 6.9 Hz, $CH_3$-12) and 0.79 (3H, d, $J$ = 6.9 Hz, $CH_3$-13), an angular methyl group at $\delta_H$ 1.32 (3H, s, $CH_3$-15), three olefinic protons at $\delta_H$ 6.58 (1H, dd, $J$ = 4.4, 11.9 Hz, H-1), 5.22 (1H, dd, $J$ = 10.7, 15.3 Hz, H-6), and 5.00 (1H, d, $J$ = 15.3 Hz, H-6), and one aldehyde proton at $\delta_H$ 9.39 (1H, s, H-14). The $^{13}$C NMR spectrum and the corresponding DEPT spectrum exhibited 15 carbon signals, consisting of three methyl, three methylene, seven methine, and two quaternary carbons atoms, suggesting the presence of a germacrane-type sesquiterpene skeleton. The $^{13}$C NMR spectrum also established that two olefinic bonds (one disubstituted and one trisubstituted) were present and a hydroxyl group was located on the carbon totally substituted (a singlet carbon at $\delta_C$ 73.6). The COSY experiment disclosed two partial structures, $CHCH_2CH_2$ and CHCH-$CHCHCH_2$, corresponding to the C-1, C-2, C-3 and C-5, C-6, C-7, C-8, C-9 fragments. HMBC correlations of H-1 with C-10, C-14, and C-3; H-5 with C-4 and C-6; H-6 with C-5, C-4, and C-8; H-7 with C-8, C-12, and C-13; H-14 with C-1, C-9, and C-10; and $CH_3$-15 with C-4 and C-5 were observed. The stereochemistry of the C-5, C-6 double bond was determined to be *E* by considering the coupling constant ($J_{5,6}$ = 15.3 Hz). The relative stereochemistry of **9** was determined from the NOESY spectrum (Figure 4). Important correlations were observed between H-8, H-6, $CH_3$-12, and $CH_3$-13 as well as between H-14, H-1, and $CH_3$-15, leading to the conclusion that the stereochemistry of the C-1, C-10 double bond is *E*. Correlations were also observed between $CH_3$-15, H-5, and H-7. The absolute configuration of C-8 (Figure 5) was determined using the modified Mosher ester procedure.[21] Compound **9** was treated with (*S*)-(+) and (*R*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride in anhydrous pyridine at room temperature for 24 h, to afford the (*R*)- and (*S*)-MTPA ester derivatives **9a** and **9b**, respectively. Negative values ($\Delta\delta_{S-R}$) were obtained for H-7, H-11, H-6, and H-5, indicating that the absolute configuration at C-8 is *R*. Thus the structure of **9** may be formulated as (1*E*,5*E*,8*R*)-4α,8*β*-dihydroxy-7*βH*-germacra-1(10),5-dien-14-al.

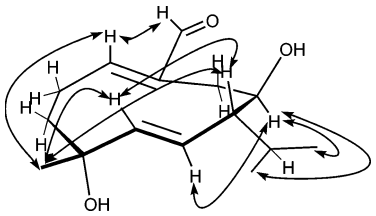


**Figure 4.** Selected NOESY correlations for compound **9**.

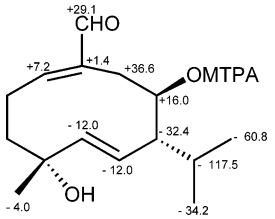


**Figure 5.** Δδ values [Δδ (in ppm) = $\delta_S - \delta_R$] obtained for (*S*)- and (*R*)-MTPA esters (**9a** and **9b**, respectively) of compound **9**.

Pulicanadienal B (**10**) was isolated as an amorphous solid, $[\alpha]^{25}_D$ 49.1° (*c* 0.059, $CHCl_3$), and its molecular formula was determined as $C_{17}H_{26}O_4$ by HRFABMS data (*m/z* 317.1718 $[M + Na]^+$). The $^1$H and $^{13}$C NMR (Table 5) spectral data of **10** were similar to those of **9** with the exception of signals for an acetoxy group. Thus, treatment of **9** with $Ac_2O$−pyridine gave a monoacetyl derivative whose physical and spectroscopic constants were identical with those of **10** and was determined as (1*E*,5*E*)-8*β*-acetoxy-4α-hydroxy-7*βH*-germacra-1(10),5-dien-14-al.

Pulicanadienol (**11**) was obtained as an amorphous solid, $[\alpha]^{25}_D$ 10.2° (*c* 0.049, $CHCl_3$). The IR spectrum showed the presence of hydroxyl groups (3335 $cm^{-1}$). The $^1$H NMR spectrum (Table 5) of **11** showed signals analogous to those of **9** with the difference that C-14 is replaced by a hydroxymethyl group, $\delta_H$ 4.03 (1H, d, $J$ = 12.0 Hz, H-14a), 4.10 (1H, d, $J$ = 12.0 Hz, H-14b). The relative stereochemistry of **11** was assigned as in **9** based upon the very close

**Table 6.** $^1$H and $^{13}$C NMR Data for Compounds **12** and **13** ($CDCl_3$, *J* values (Hz) in parentheses)

| | **12** | | **13** | |
|---|---|---|---|---|
| position | $\delta_C$ | $\delta_H$ | $\delta_C$ | $\delta_H$ |
| 1 | 51.7 | α, 1.95 m | 57.0 | α, 1.50 m |
| 2 | 27.3 | α, 1.73 m<br>β, 1.83 m | 25.4 | α, 1.50 m<br>β, 1.86 m |
| 3 | 29.4 | α, 1.95 m<br>β, 1.60 m | 29.5 | α, 1.96 m<br>β, 1.60 m |
| 4 | 212.4 | | 212.4 | |
| 5 | 50.8 | α, 2.66 ddd<br>(4.0, 5.8, 9.7) | 50.8 | α, 2.59 ddd<br>(5.0, 6.3, 10.6) |
| 6 | 52.0 | β, 1.86 m | 47.0 | β, 1.92 m |
| 7 | 49.0 | β, 1.25 m | 49.2 | β, 1.10 m |
| 8 | 26.3 | α, 1.70 m<br>β, 1.10 dq<br>(4.2, 12.9) | 22.8 | 1.60 m<br>1.10 m |
| 9 | 35.2 | α, 2.01 (m)<br>β, 2.37 dt<br>(4.0, 6.0) | 42.0 | 1.80 br d<br>(15.6)<br>1.41 m |
| 10 | 150.4 | | 73.0 | |
| 11 | 30.2 | 1.33 dt<br>(2.8, 6.9) | 30.0 | 1.29 m |
| 12 | 21.9 | 0.89 d<br>(6.8) | 21.9 | 0.89 d<br>(6.7) |
| 13 | 15.4 | 0.61 d<br>(6.8) | 15.3 | 0.66 d<br>(6.7) |
| 14 | 103.9 | 4.68 d<br>(1.0)<br>4.57 d<br>(1.0) | 20.4 | 1.21 s |
| 15 | 67.7 | 4.31 s | 68.0 | 4.30 t<br>(4.3) |

$^{13}$C NMR (Table 5) correlations between these compounds. This confirmed the structure of **11** as (1*E*,5*E*)-7β*H*-germacra-1(10),5-dien-4α,8β,14-triol.

Pulioplopanone A (**12**) exhibited the molecular formula $C_{15}H_{24}O_2Na$ by HRFABMS (*m/z* 259.1702 $[M + Na]^+$). It showed IR bands at 3489, 1710, and 1660 $cm^{-1}$ due to hydroxyl and carbonyl groups and a double bond. Its $^1$H NMR spectrum (Table 6) showed signals that corresponded to an exocyclic double bond at $\delta_H$ 4.57 (1H, d, $J = 1.1$ Hz, H-14a) and 4.68 (1H, d, $J = 1.1$ Hz, H-14b), a hydroxymethyl group at $\delta_H$ 4.31 (2H, s, $H_2$-15), and an isopropyl moiety at $\delta_H$ 0.61 (3H, d, $J = 6.8$ Hz, $H_3$-13), 0.89 (3H, d, $J = 6.8$ Hz, $H_3$-12), and 1.33 (1H, m, H-11), suggesting an oplopene skeleton.[22] From these data, the new compound **12** was deduced to be 15-hydroxy-10(14)-oplopen-4-one, and the structural model could be determined by a single-crystal X-ray diffraction analysis (Supporting Information).

Pulioplopanone B (**13**) exhibited a molecular formula of $C_{15}H_{26}O_3$, established from HREIMS (*m/z* 254.1901 $[M]^+$). Its $^1$H and $^{13}$C NMR spectra (Table 6) were similar to those of **12** except for the presence of a hydroxyl group attached to C-10. NOE effects between H-5 and $H_2$-15, H-7, and H-1 and between H-6 and $H_3$-14, observed in a NOESY experiment, suggested that OH-10 in **13** has an α-orientation and pointed to the same relative stereochemistry for **12** and **13**, leading to the conclusion that **13** is 10α,15-dihydroxyoplopan-4-one. A cinnamoyl derivative has been reported in the literature.[23]

Next, we investigated whether these sesquiterpenes possess cytotoxic activities against the HL-60 human tumor cell line. As determined by the MTT assay, all compounds tested were weakly cytotoxic to this cell line with 50% inhibitory concentration ($IC_{50}$) values (in μM) of 20 ± 7, 298 ± 22, 115 ± 18, 264 ± 6, and 242 ± 80, for **4a**, **5a**, **6**, **7**, and **12**, respectively. The triacetate of pulicanone (**4a**) was the most potent compound, with an $IC_{50}$ value of 6- and 15-fold lower than those of **6** and **5a**, respectively. To know if compound **4a** decreases cell viability through apoptosis activation, morphological changes characteristic of apoptotic cells (i.e., condensed and fragmented chromatin) were analyzed and quantified by fluorescent microscopy. The percentage of apoptotic cells increased in a dose−response manner in response to **4a**, as demonstrated in Figure 6. Furthermore, flow cytometric analysis of HL-60 cells incubated with **4a** (30 μM, 12 h) indicates that transition of cells from the diploid to hypodiploid (i.e., apoptotic cells) region occurs with the accumulation of cells in the G1 phase of the cell cycle (data not shown). In concert with induction of apoptosis, HL-60 cells also responded to **4a** with activation of caspase-3 and caspase-8 (Figure 7) and cleavage of PARP-1 (Figure 8).

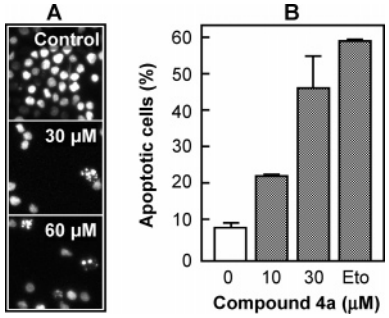


**Figure 6.** Induction of apoptosis in HL-60 cells in response to triacetoxy pulicanone **4a**. (A) Cells were cultured for 12 h in the absence (control) or presence of the indicated doses of compound **4a** and then stained with Hoeschst 33258 to evaluate nuclear chromatin condensation, i.e., apoptosis. (B) Quantitative analysis of apoptotic cells by fluorescence microscopy; etoposide (Eto, 10 μM) was used as a positive control. The results of a representative experiment are shown, and each point represents the average (±SE) of triplicate determinations.

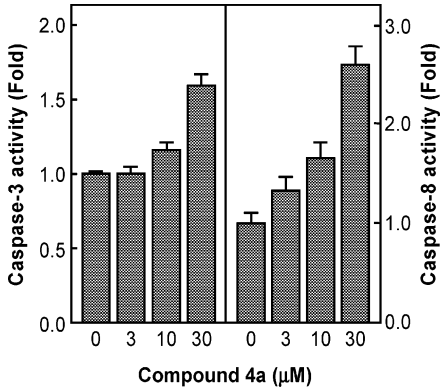


**Figure 7.** Triacetoxy pulicanone **4a** induces caspase-3/8 activation. HL-60 cells were cultured in the presence of increasing concentrations of compound **4a** and harvested at 12 h. Total cell lysates were obtained and used to determine caspase-3 (left panel) and caspase-8 (right panel) activities. The results of a representative experiment performed in duplicate are shown and expressed as *x*-fold increase of caspase activity compared with control.

## Experimental Section

**General Experimental Procedures.** Melting points were determined on a Büchi B-540 apparatus and are uncorrected. Optical rotations were recorded in a Perkin-Elmer model 343 polarimeter, and UV spectra were recorded using a JASCO model V-560 spectrophotometer. $^1$H NMR and $^{13}$C NMR spectra were obtained on Bruker model AMX-400 and AMX-500 spectrometers with standard pulse sequences operating at 400 and 500 MHz in $^1$H NMR and 100 and 125 MHz in $^{13}$C NMR, respectively. $CDCl_3$, $C_5D_5N$, and DMSO-$d_6$ were used as solvents. EIMS, HREIMS, and HRFABMS were taken on a Micromass model Autospec (70 eV) spectrometer. Column chromatography was carried out on silica gel 60 (Merck 230−

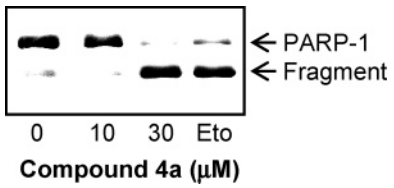


**Figure 8.** Triacetoxy pulicanone **4a** induces poly(ADP-ribose)polymerase-1 (PARP-1) cleavage in HL-60 cells. The indicated concentrations of compound **4a** were added to the cells for 12 h, and total cell lysates were then analyzed by immunoblotting with an anti-PARP-1 antibody. Etoposide (Eto, 10 $\mu$M) was included as a positive control.

400 mesh), and preparative TLC on silica gel 60 $PF_{254+366}$ plates (20 × 20 cm, 1 mm thickness).

**Plant Material.** *Pulicaria canariensis* was collected in Playa Quemada (Lanzarote, Canary Islands) in August 1998. The plant material was identified by Professor Rosa Febles, and a voucher specimen has been deposited at the Herbarium of the Viera y Clavijo Botanical Garden in Gran Canaria (No. 20146).

**Extraction and Isolation.** The aerial parts of the plant (1.5 kg) were extracted with EtOH (3 L, four times). The solvent was concentrated under reduced pressure. The residue (90 g) obtained after removing EtOH was fractionated by silica gel flash column chromatography using hexane and EtOAc mixtures of increasing polarity to yield four fractions. Fraction 1 (500 mg) eluted with hexane−EtOAc (4:1) was concentrated under reduced pressure and recrystallized from hexane−EtOAc to obtain the known compounds stigmasterol (180 mg), 7,4′-di-*O*-methyldihydrokaempferol (26 mg), and calenduladiol (41 mg), respectively; the liquid supernatant was chromatographed by medium-pressure liquid chromatography (MPLC) with the mobile phase hexane−EtOAc (4:1), furnishing after purification by preparative TLC with hexane−EtOAc (9:1) the new sesquiterpenes **6** (45 mg), **2** (48 mg), **7** (35 mg), and **1** (24 mg). Fraction 2 (425 mg) eluted with hexane−EtOAc (7:3) and treated in the same way as fraction 1 was then recrystallized from hexane−EtOAc to yield 5,7-dihydroxy-3,3′,4′-trimethoxyflavone (150 mg). The liquid supernatant was chromatographed on MPLC using hexane−EtOAc (7:3) as eluant, and subsequent purification by preparative TLC using benzene−EtOAc (4:1) gave **3** (9 mg), ergosterol peroxide (178 mg), **10** (12 mg), and a mixture which, after acetylation with $Ac_2O$−pyridine and MPLC in hexane−EtOAc (9:1), yielded **4a** (30 mg). Fraction 3 (200 mg), eluted with hexane−EtOAc (3:2), was subjected to column chromagraphy using hexane−EtOAc (4:1) to give dihydroquercetin 7,3′-dimethyl ether (18 mg) and the semipure compound **9**. This was finally purified by preparative TLC developed with benzene−acetonitrile (4:1), to afford pure compound **9** (42 mg). Fraction 4 (300 mg) eluted with hexane−EtOAc (1:1) was chromatographed by MPLC with hexane−EtOAc (3:1) and finally purified by preparative TLC developed with hexane−EtOAc (1:1) to afford compounds 6,15α-epoxy-1$\beta$,4$\beta$-dihydroxyeudesmane (45 mg), **11** (9 mg), **13** (8 mg), **8** (25 mg), **5** (40 mg), and **12** (68 mg).

**Pulicanadiene A (1):** amorphous solid; $[\alpha]^{25}_D$ 21.4° (*c* 0.014, $CHCl_3$); UV (EtOH) $\lambda_{max}$ (log $\epsilon$) 206 (3.2), 250 (2.3), 273 (1.8) nm; IR (KBr) $\nu_{max}$ 3067, 2937, 1731, 1693, 1554, 1430, 1372, 1245, 1023, 960 $cm^{-1}$; $^1H$ and $^{13}C$ NMR, see Table 1; EIMS *m/z* 352 ($M^+$, 1), 316 (1), 292 (1), 281 (0.5), 274 (1), 250 (12), 233 (15), 232 (66), 217 (24), 203 (12), 189 (100), 187 (41), 176 (16), 171 (17), 161 (19), 145 (89), 143 (44), 131 (40), 123 (56), 105 (34), 91 (49), 81 (42), 69 (30), 55 (30); HREIMS *m/z* 352.1877 $[M]^+$ (calcd for $C_{19}H_{28}O_6$ 352.1886).

**Methylation of Compound 1.** To compound **1** (4 mg) in diethyl ether (5 mL) was added an excess of freshly prepared diazomethane in diethyl ether, and the mixture was kept at room temperature for 12 h. Concentration of the reaction mixture under vacuum furnished **1a** (4 mg), as colorless needles: mp 162−163 °C; $[\alpha]^{25}_D$ 3.2° (*c* 0.062, $CHCl_3$); UV (EtOH) $\lambda_{max}$ (log $\epsilon$) 212 (2.0), 251 (2.1) nm; IR (KBr) $\nu_{max}$ 2930, 2360, 2341, 1732, 1435, 1371, 1246, 1215, 1021, 963 $cm^{-1}$; $^1H$ and $^{13}C$ NMR, see Table 1, $^1H$ NMR ($CDCl_3$, at −50 °C, 500 MHz) $\delta$ 6.64 (1H, t, $J$ = 7.9 Hz, H-1), 5.69 (1H, d br, $J$ = 11.0 Hz, H-6), 5.65 (1H, m, H-8), 5.14 (1H, d, $J$ = 10.9 Hz, H-5), 3.74 (3H, s, OMe), 2.49 (1H, dd, $J$ = 5.2, 13.9 Hz, H-9a), 2.32 (2H, m, overlapped signals, H-2a and H-3a), 2.11 (3H, s, OAc), 2.08 (1H, dd, $J$ = 13.9, 10.8 Hz, H-9b), 2.00 (3H, s, OAc), 1.98 (2H, m, overlapped signals, H-2b and H-3b), 1.93 (3H, s, $CH_3$-15), 1.72 (1H, d, $J$ = 11.4 Hz, H-7), 1.57 (1H, m, H-11), 0.97 (3H, d, $J$ = 6.2 Hz, $CH_3$-12), 0.95 (3H, d, $J$ = 6.2 Hz, $CH_3$-13); EIMS *m/z* 366 ($M^+$, 2), 307 (2), 306 (2), 274 (4), 264 (12), 247 (13), 246 (58), 231 (23), 214 (16), 203 (100), 187 (78), 171 (30), 145 (37), 143 (59), 131 (37), 123 (59), 105 (28), 91 (34), 81 (38), 79 (25), 69 (20), 55 (22); HREIMS *m/z* 366.2019 $[M]^+$ (calcd for $C_{20}H_{30}O_6$ 366.1733).

**Pulicanadiene B (2):** colorless oil; $[\alpha]^{25}_D$ 35.7° (*c* 0.042, $CHCl_3$); UV (EtOH) $\lambda_{max}$ (log $\epsilon$) 209 (3.2), 222 (2.7), 251 (2.1) nm; IR (KBr) $\nu_{max}$ 2932, 1732, 1687, 1433, 1372, 1243, 1141, 1023, 960 $cm^{-1}$; $^1H$ and $^{13}C$ NMR, see Table 1; EIMS *m/z* 277 (35), 235 (10), 217 (74), 216 (72), 199 (79), 187 (82), 173 (93), 150 (62), 145 (94), 131 (41), 123 (83), 109 (38), 107 (100), 95 (39), 93 (33), 91 (33), 84 (45), 55 (49); HRFABMS *m/z* 359.1874 $[M + Na]^+$ (calcd for $C_{19}H_{28}O_5Na$ 359.1834).

**Pulicanadiene C (3):** colorless oil; $[\alpha]^{25}_D$ −75.0° (*c* 0.008, $CHCl_3$); UV (EtOH) $\lambda_{max}$ (log $\epsilon$) 205 (3.2), 271 (1.9) nm; IR (KBr) $\nu_{max}$ 3414, 3020, 2928, 2854, 1728, 1502, 1411, 1381, 1332, 1219, 1159, 1107, 1028, 766 $cm^{-1}$; $^1H$ and $^{13}C$ NMR, see Table 1; EIMS *m/z* 338 ($M^+$, 1), 290 (10), 279 (10), 241 (13), 218 (48), 203 (25), 200 (78), 193 (11), 187 (100), 178 (59), 175 (99), 161 (44), 157 (71), 149 (45), 143 (46), 135 (38), 131 (35), 122 (28), 109 (23), 105 (30), 97 (33), 95 (26), 91 (25), 83 (36), 81 (30), 69 (33), 57 (45), 55 (95); HREIMS *m/z* 338.2019 $[M]^+$ (calcd for $C_{19}H_{30}O_5$ 338.2093).

**Reduction of 2.** Compound **2** (5.0 mg) in methanol (5 mL) was treated with excess $NaBH_4$ at room temperature for 30 min. The reaction was worked up by addition of $H_2O$ and was extracted with $CH_3Cl$. The concentrated dried $CH_3Cl$ extract was separated by preparative TLC (benzene−AcOEt, 80:20) and furnished **3** (1.2 mg).

**Triacetoxy pulicanone (4a):** colorless oil; $[\alpha]^{25}_D$ 4.9° (*c* 0.081, $CHCl_3$); UV (EtOH) $\lambda_{max}$ (log $\epsilon$) 205 (3.2), 223 (2.7), 271 (3.1) nm; IR (KBr) $\nu_{max}$ 2960, 2926, 2851, 1741, 1694, 1463, 1438, 1373, 1233, 1123, 1053, 1025, 935, 852, 666 $cm^{-1}$; $^1H$ and $^{13}C$ NMR, see Table 2; EIMS *m/z* 394 ($M^+$, 0.5), 334 (12), 292 (17), 274 (78), 249 (24), 232 (62), 214 (85), 205 (94), 189 (95), 171 (42), 161 (47), 149 (68), 137 (41), 136 (44), 107 (74), 96 (65), 69 (61), 57 (100); HREIMS *m/z* 394.1982 $[M]^+$ (calcd for $C_{21}H_{30}O_7$ 394.1991).

**Pulicanol (5):** colorless oil; IR (KBr) $\nu_{max}$ 3404, 2929, 1736, 1647, 1470, 1375, 1243, 1088, 1023, 963, 897 $cm^{-1}$; EIMS *m/z* 312 ($M^+$, 2), 279 (11), 252 (4), 234 (5), 216 (4), 173 (14), 171 (16), 167 (37), 149 (87), 133 (32), 129 (33), 123 (46), 121 (50), 111 (56), 105 (48), 97 (87), 95 (91), 91 (57), 85 (70), 83 (100), 57 (89), 55 (96); $^1H$ NMR ($CDCl_3$, 400 MHz) $\delta_H$ 5.45 (1H, t, $J$ = 6.8 Hz, H-1), 5.18 (1H, dd, $J$ = 5.2, 12.0 Hz, H-8), 4.29 (1H, d, $J$ = 11.8 Hz, H-14a), 4.12 (1H, d, $J$ = 11.6 Hz, H-14b) 3.51 (1H, m, H-6), 2.82 (1H, d, $J$ = 7.7 Hz, H-5), 2.53 (2H, d, $J$ = 12.4 Hz, $H_2$-9), 2.34 (2H, m, $H_2$-3), 2.15 (3H, s, OAc), 2.10 (1H, m, $H_2$-3), 1.80 (1H, m, H-11), 1.59 (1H, m, H-7), 1.16 (3H, s, $CH_3$-15), 1.12 (3H, d, $J$ = 6.4 Hz, $CH_3$-12), 0.95 (3H, d, $J$ = 6.4 Hz, $CH_3$-13).

**Acetylation of 5.** Compound **5** (20 mg) was dissolved in pyridine (3 mL) and acetic anhydride (3 mL), and the solution was stirred for 16 h at room temperature. The product was dried under vacuum to furnish **5a** (15 mg) as a colorless oil; $[\alpha]^{25}_D$ −6.9° (*c* 0.188, $CHCl_3$); UV (EtOH) $\lambda_{max}$ (log $\epsilon$) 214 (2.4) nm; IR (KBr) $\nu_{max}$ 2930, 2874, 1739, 1653, 1597, 1458, 1371, 1238, 1130, 1078, 1026, 962, 817 $cm^{-1}$; $^1H$ and $^{13}C$ NMR, see Table 3; EIMS *m/z* 336 (6), 294 (8), 276 (5), 234 (21), 216 (32), 194 (26), 191 (28), 175 (45), 173 (37), 163 (25), 150 (29), 145 (33), 139 (46), 131 (50), 119 (33), 107 (49), 91 (47), 84 (100), 81 (40), 69 (28), 67 (28), 57 (22), 55 (35); HRFABMS *m/z* 419.2009 $[M + Na]^+$ (calcd for $C_{21}H_{32}O_7Na$ 419.2046).

**Pulicanaral A (6):** colorless needles; mp 183−184 °C; $[\alpha]^{25}_D$ 11.1° (*c* 0.108, $CHCl_3$); UV (EtOH) $\lambda_{max}$ (log $\epsilon$) 209 (3.1), 272 (1.9) nm; IR (KBr) $\nu_{max}$ 2928, 1732, 1716, 1375, 1243, 1025, 935 $cm^{-1}$; $^1H$ and $^{13}C$ NMR, see Table 4; EIMS *m/z* 352 ($M^+$,

0.5), 337 (0.6), 292 (6), 249 (10), 232 (68), 203 (21), 189 (66), 175 (31), 171 (20), 166 (28), 161 (45), 153 (47), 145 (33), 143 (100), 135 (22), 129 (58), 109 (46), 107 (28), 105 (28), 95 (42), 84 (56), 81 (61), 69 (36), 55 (50); HREIMS *m/z* 352.1905 $[M]^+$ (calcd for $C_{19}H_{28}O_6$, 352.1886).

**Pulicanaral B (7):** colorless needles; mp 205−210 °C; $[\alpha]^{25}_D$ −60.0° (*c* 0.005, $CHCl_3$); UV (EtOH) $\lambda_{max}$ (log $\epsilon$) 208 (2.3) nm; IR (KBr) $\nu_{max}$ 2932, 1737, 1715, 1432, 1373, 1244, 1130, 1090, 1033, 894, 767 $cm^{-1}$; $^1H$ and $^{13}C$ NMR, see Table 4; EIMS *m/z* 309 (3), 308 (1), 297 (4), 279 (3), 266 (6) 255 (27), 248 (4), 237 (12), 223 (15), 220 (18), 219 (28), 209 (10), 207 (13), 205 (27), 195 (34), 191 (14), 187 (11), 177 (44), 166 (27), 163 (28), 159 (14), 151 (24), 149 (47), 141 (30), 139 (66), 127 (50), 123 (44), 109 (59), 99 (76), 97 (100), 95 (74), 85 (58), 83 (100), 81 (83), 71 (53), 69 (60), 55 (90); HRFABMS *m/z* 391.1738 $[M + Na]^+$ (calcd for $C_{19}H_{28}O_7Na$ 391.1733).

**Pulicanaral B (8):** colorless oil; IR (KBr) $\nu_{max}$ 3462, 2964, 2925, 2856, 1719, 1597, 1455, 1382, 1255, 1074 $cm^{-1}$; $^1H$ and $^{13}C$ NMR, see Table 4; EIMS *m/z* 326 ($M^+$, 0.2), 279 (15), 251 (6), 237 (6), 223 (8), 219 (10), 205 (7), 195 (5), 191 (13), 177 (13), 167 (27), 163 (19), 149 (90), 139 (28), 137 (31), 123 (42), 111 (41), 109 (68), 97 (100), 95 (79), 83 (72), 81 (66), 69 (61), 55 (77).

**Acetylation of 8.** Compound **8** (4.0 mg) was dissolved in pyridine (1 mL) and acetic anhydride (1 mL), and the solution was stirred for 18 h at room temperature. The product was dried under vacuum to furnish **7** (3.5 mg).

**Pulicanadienal A (9):** colorless oil; $[\alpha]^{25}_D$ 210° (*c* 0.020, $CHCl_3$); UV (EtOH) $\lambda_{max}$ (log $\epsilon$) 212 (2.9), 253 (2.2) nm; IR (KBr) $\nu_{max}$ 3406, 2957, 2926, 2870, 1728, 1660, 1623, 1440, 1370, 1272, 1142, 1060, 987, 669 $cm^{-1}$; $^1H$ and $^{13}C$ NMR, see Table 5; EIMS *m/z* 234 (28), 219 (9), 216 (15), 205 (10), 201 (8), 194 (7), 191 (15), 187 (9), 176 (26), 173 (14), 169 (11), 161 (11), 151 (76), 145 (27), 133 (22), 131 (22), 123 (100), 122 (62), 119 (18), 117 (15), 107 (53), 105 (39), 95 (43), 93 (62), 91 (43), 81 (53), 69 (54), 67 (46), 55 (58); HRFABMS *m/z* 275.1645 $[M + Na]^+$ (calcd for $C_{15}H_{24}O_3Na$ 275.1623).

**Preparation of the (*R*)- and (*S*)-MTPA Ester Derivatives of 9.** Two portions (1.5 mg) of compound **9** were treated with (*R*)-(+)- and (*S*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (8 $\mu$L) in anhydrous pyridine (0.5 mL) at room temperature overnight. When the reaction was completed, the residue was concentrated and purified on silica gel by preparative TLC eluting with $CHCl_3$−$Et_2O$−MeOH (14:6:1), affording the (*S*)- and (*R*)-MTPA ester derivatives (**9a** and **9b**) of **9**, respectively. $^1H$ NMR data of **9a** (500 MHz, $CDCl_3$): $\delta_H$ 9.420 (1H, s- H-14), 6.484 (1H, t, $J$ = 7.8 Hz, H-1), 5.360 (1H, m, H-8), 5.322 (1H, d, $J$ = 15.4 Hz, H-5), 5.120 (1H, dd, $J$ = 11.1, 15.4 Hz, H-6), 2.684 (1H, dd, $J$ = 6.7, 14.3 Hz, H-9a), 2.610 (1H, dd, $J$ = 4.0, 14.3 Hz, H-9b), 2.170 (1H, dd, $J$ = 3.0, 8.9 Hz, H-7), 1.471 (1H, td, $J$ = 2.8, 6.9 Hz, H-11), 1.310 (3H, s, $CH_3$-15), 0.730 (3H, d, $J$ = 6.9 Hz, $CH_3$-13), 0.632 (3H, d, $J$ = 6.8 Hz, $CH_3$-12); $^1H$ NMR data of **9b** (500 MHz, $CDCl_3$) $\delta_H$ 9.347 (1H, s- H-14), 6.466 (1H, t, $J$ = 8.3 Hz, H-1), 5.352 (1H, d, $J$ = 15.1 Hz, H-5), 5.320 (1H, m, H-8), 5.151 (1H, dd, $J$ = 10.7, 15.1 Hz, H-6), 2.680 (1H, dd, $J$ = 7.7, 14.7 Hz, H-9a), 2.518 (1H, d br, $J$ = 14.1 Hz, H-9b), 2.251 (1H, m, H-7), 1.765 (1H, m, H-11), 1.321 (3H, s, $CH_3$-15), 0.815 (3H, d, $J$ = 7.0 Hz, $CH_3$-13), 0.784 (3H, d, $J$ = 6.7 Hz, $CH_3$-12).

**Pulicanadienal B (10):** amorphous solid; $[\alpha]^{25}_D$ 49.1° (*c* 0.059, $CHCl_3$); UV (EtOH) $\lambda_{max}$ (log $\epsilon$) 214 (3.0), 251 (2.1) nm; IR (KBr) $\nu_{max}$ 3459, 2922, 1732, 1684, 1630, 1375, 1256, 1026, 880, 803 $cm^{-1}$; $^1H$ and $^{13}C$ NMR, see Table 5; EIMS *m/z* 317 ($M^+$, 2), 165 (1), 154 (8), 136 (13), 109 (12), 97 (17), 95 (31), 81 (35), 71 (31), 69 (66), 57 (81), 55 (100); HRFABMS *m/z* 317.1718 $[M + Na]^+$ (calcd for $C_{17}H_{26}O_4Na$ 317.1729).

**Acetylation of 9.** Compound **9** (5.0 mg) was dissolved in pyridine (1 mL) and acetic anhydride (1 mL), and the solution was stirred for 18 h at room temperature. The product was dried under vacuum to furnish **10** (4.0 mg).

**Pulicanadienol (11):** amorphous solid; $[\alpha]^{25}_D$ 10.2° (*c* 0.049, $CHCl_3$); UV (EtOH) $\lambda_{max}$ (log $\epsilon$) 212 (3.0) nm; IR (KBr) $\nu_{max}$ 3335, 2957, 2926, 2871, 1661, 1625, 1449, 1384, 1368, 1244, 1174, 1037, 984, 891 $cm^{-1}$; $^1H$ and $^{13}C$ NMR, see Table 5; EIMS *m/z* 236 (5), 218 (26), 203 (11), 202 (5), 201 (10), 189 (12), 187 (12), 185 (9), 175 (56), 160 (17), 159 (25), 157 (30), 145 (44), 133 (42), 131 (41), 123 (39), 107 (58), 105 (69), 95 (55), 93 (64), 91 (72), 81 (79), 69 (79), 57 (84), 55 (100); HREIMS $[M - H_2O]^+$ *m/z* 236.2155 (calcd for $C_{15}H_{24}O_2$ 236.1776).

**Pulioplopanone A (12):** colorless needles; mp 141−142 °C; $[\alpha]^{25}_D$ −40.0° (*c* 0.005, $CHCl_3$); UV (EtOH) $\lambda_{max}$ (log $\epsilon$) 209 (2.7), 274 (1.2) nm; IR (KBr) $\nu_{max}$ 3489, 2960, 2939, 2876, 1710, 1660, 1602, 1405, 1280, 1230, 1055, 893, 748 $cm^{-1}$; $^1H$ and $^{13}C$ NMR, see Table 6; EIMS *m/z* 236 ($M^+$, 12), 205 (100), 187 (74), 177 (35), 135 (17), 121 (55), 107 (18), 95 (39), 93 (27), 91 (18), 79 (24), 69 (17), 67 (15), 55 (13); HRFABMS *m/z* 259.1702 $[M + Na]^+$ (calcd for $C_{15}H_{24}O_2Na$ 259.1703).

**Pulioplopanone B (13):** colorless oil; $[\alpha]^{25}_D$ 33.3° (*c* 0.012, $CHCl_3$); IR (KBr) $\nu_{max}$ 3401, 2930, 2871, 2360, 2342, 1718, 1459, 1369, 1263, 1126, 1053 $cm^{-1}$; $^1H$ and $^{13}C$ NMR, see Table 6; EIMS *m/z* 254 ($M^+$, 2), 236 (5), 224 (15), 223 (96), 205 (35), 187 (43), 177 (100), 169 (20), 147 (9), 135 (13), 121 (33), 107 (19), 95 (34), 81 (29), 69 (25), 55 (15); HRFABMS $[M]^+$ *m/z* 254.1901 (calcd for $C_{15}H_{26}O_3$ 254.1882).

**Cell Culture and Cytotoxicity Assays.** HL-60 cells were cultured in suspension in RPMI-1640 medium supplemented with 10% (v/v) heat-inactivated fetal bovine serum, penicillin (100 units/mL), and streptomycin (100 $\mu$g/mL) in a humidified atmosphere of 95% air and 5% $CO_2$ at 37 °C. Cells were maintained at a density of $<1 \times 10^6$ cells/mL. Cytotoxicity studies were performed using the MTT assay as described.[9]

**Apoptosis Quantitation by Fluorescence Microscopy.** Quantitation of apoptotic cells was performed as described previously.[10]

**Assay of Caspase Activity.** Activity of caspase was determined as described previously[24] from cytosolic lysates, using the specific colorimetric substrate DEVD-*p*NA (caspase-3) or IETD-*p*NA (caspase-8).

**Analysis of PARP-1 Hydrolysis by Immunoblotting.** PARP-1 cleavage was analyzed by Western blot assay as described[10] using a PARP-1 monoclonal antibody which recognizes the native form (116 kDa) and the hydrolyzed form (85 kDa).

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**Supporting Information Available:** $^1H$ NMR spectra of compound **1**, **1a** (at 50 °C, 25 °C, −50 °C), **5a**, **6**, **7**, **9**, and **12**. Enlargement of the NOESY experiment performed at 50 and −50 °C, and X-ray diffraction data for compounds **1a**, **6**, **7**, and **12**. These materials are available free of charge via the Internet at http://pubs.acs.org.

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