# Cytotoxic Sesquiterpene Lactones from *Eupatorium kiirunense,* a Coastal Plant of Taiwan

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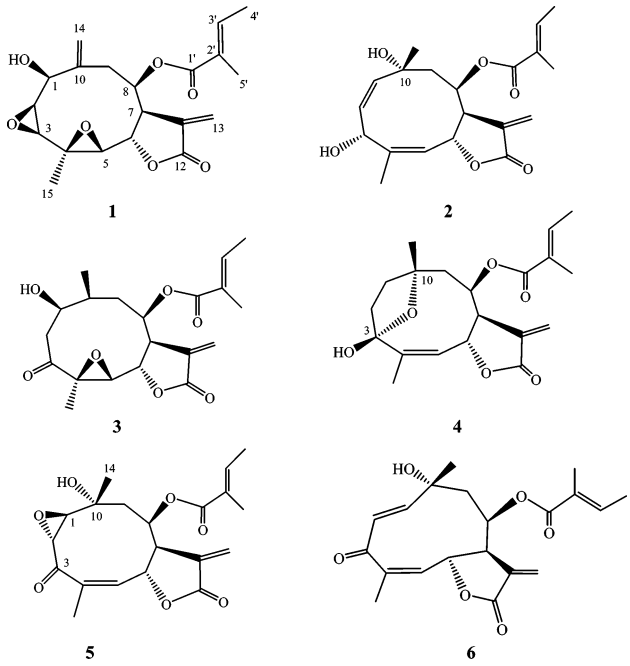
Phytochemical investigation of *Eupatorium kiirunense* has resulted in the isolation of eight new sesquiterpene lactones, constituted by five germacranolides, eupakirunsins A−E (**1**−**5**), and three heliangolides, eupaheliangolide A (**6**), 15-acetoxyheliangin (**7**), and 3-*epi*-heliangin (**8**), in addition to the known heliangin (**9**) and 8,10-epoxy-9-acetoxythymol angelate (**10**). The structures of the new compounds were established through detailed analysis of their spectroscopic data. Compounds **6**, **8**, and **9** exhibited cytotoxicity against human oral epidermoid (KB), cervical epitheloid (Hela), and liver (hepa59T/VGH) carcinoma cells.

Plant species in the genus *Eupatorium* (Asteraceae) have been used for many decades as antimalarial,[1] antibacterial,[2] antifungal,[3] anti-inflammatory,[4] hepatoprotective,[5] and immunostimulant[6] agents. A number of bioactive natural products, mainly sesquiterpene lactones, have been reported as constituents of *Eupatorium* species, and the genus is a promising bioresource for the development of potential drugs and value-added products.[7] Many sesquiterpene lactones isolated from *Eupatorium* have been proven to possess cytotoxic effects,[8,9] and some have antileukemic activity.[10,11] In the course of our search for bioactive leads from the local flora,[12] a phytochemical investigation of *Eupatorium kiirunense* Kitam. C. H. Ou & S. W. Chung was carried out. We report herein the isolation of five new germacranolides, eupakirunsins A−E (**1**−**5**), and three new heliangolides, eupaheliangolide A (**6**) 15-acetoxyheliangin (**7**), and 3-*epi*-heliangin (**8**), along with the known heliangin (**9**)[13] and 8,10-epoxy-9-acetoxythymol angelate (**10**).[14] Structures of the isolated compounds were established through detailed analysis of their spectroscopic data, especially 2D NMR methods. The cytotoxic activity of the isolated compounds against human oral epidermoid (KB), cervical epitheloid (Hela), and liver (hepa59T/VGH) carcinoma cells was also evaluated.




## Results and Discussion

The HRESIMS and FABMS of **1** exhibited quasimolecular ion peaks at *m/z* 358.1421 ([M − $H_2O$]$^+$) and at *m/z* 377 [M + H]$^+$, respectively, corresponding to the molecular formula $C_{20}H_{24}O_7$. The IR spectrum displayed absorption bands diagnostic of hydroxyl (3433 cm$^{-1}$), α,*β*-unsaturated-*γ*-lactone (1766 cm$^{-1}$), α,*β*-unsaturated ester (1707 cm$^{-1}$), and double-bond (1649 cm$^{-1}$) functionalities. The $^1$H NMR spectrum (Table 1) revealed two narrowly split signals at $\delta_H$ 6.28 and 5.48 (each d, $J$ = 3.3 Hz), together with a $CH_2$ signal at $\delta_C$ 122.6 and a carbonyl resonance at $\delta_C$ 169.4, and suggested the presence of an α-methylene-*γ*-lactone, commonly encountered in sesquiterpenes of *Eupatorium* species.[7] Another exomethylene group resonated at $\delta_C$ 120.4, and an olefinic $CH_2$ singlet at $\delta_H$ 5.06 was assigned to H-14. The carbonyl signal at $\delta_C$ 167.3 was attributed to an α,*β*-unsaturated acyl group, identified as a tigloyl unit from observation of two vinyl methyls at $\delta_H$ 1.77 (s, H-5′) and 1.75 (d, $J$ = 6.6 Hz, H-4′), together with an olefinic proton at $\delta$ 6.73 (q, $J$ = 6.3 Hz). The presence of a tigloyl ester was also supported by signals at $\delta_C$ 128.1 (C-2′), 138.4 (C-3′), 14.5 (C-4′), and 12.1 (C-5′), along with a base peak at *m/z* 83 [$C_5H_7O$]$^+$ in the EIMS.[15] The oxygenated proton at $\delta_H$ 4.35 showed an HMQC correlation to $\delta_C$ 76.3 (C-1) in addition to a HMBC correlation with the exomethylene at $\delta_C$ 120.4 (C-14) and was therefore attached to the hydroxyl-bearing C-1. Taking into account two carbonyls (from a lactone and an ester), three double bonds, and a lactone ring, there must be three additional rings to account for nine degrees of unsaturation allowed by the molecular formula in addition to two more oxygen atoms. Since the methyl at $\delta$ 1.67 (3H, s, H-15) had HMBC correlations with both the oxygenated quaternary at $\delta_C$ 66.3 (C-4) and the oxygenated CH resonance at $\delta_C$ 65.2 (C-3), along with the presence of two oxygenated CH resonances at $\delta_C$ 51.7 and 49.8, this implied the occurrence of two epoxy rings in close proximity to C-15.

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**Table 1.** $^1$H NMR Data ($CDCl_3$, 300 MHz) of Compounds **1**–**8**[a]

| position | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| 1 | 4.35 d (5.4) | 5.82 d (5.5) | 3.70 d (5.3) | 2.30 m (2H) | 3.24 d (2.1) | 6.94 d (17.1) | 2.80 t (4.3) | 2.58 dd (10.0, 5.5) |
| 2 | 2.97 d (5.4) | 6.32 dd (5.5, 1.5) | 2.62 d (5.3) | 2.29 overlap | 3.70 d (2.1) | 6.23 d (17.1) | 2.49 t (4.3) | 2.37 ddd, (12.3, 10.0, 5.0) |
| | | | 2.73 d (5.4) | 1.94 br s | | | 1.78 m | 1.69 m |
| 3 | 3.35 s | 5.10 t (3.3) | | | | | 4.60 m | 4.99 dd (12.5, 5.5) |
| 5 | 2.35 d (9.0) | 5.67 overlapped | 1.65 d (10.2) | 5.60 d (6.1) | 6.38 d (5.6) | 5.85 d (9.0) | 5.58 d (8.5) | 5.31 d (11.0) |
| 6 | 4.80 dd (9.0, 8.5) | 5.96 dd (11.0, 8.5) | 4.8 t (10.2) | 5.55 br d (6.1) | 5.66 d (5.6) | 5.45 d (9.0) | 6.10 m | 5.59 dd (11.0, 2.0) |
| 7 | 3.15 dd (8.5, 3.3) | 3.42 m | 2.85 dd (10.2, 2.8) | 3.14 br d (2.0) | 3.30 m | 3.57 m | 2.90 m | 2.90 br s |
| 8 | 5.45 overlapped | 5.11 t (3.3) | 5.78 d (2.4) | 5.47 m | 5.42 m | 5.34 t (6.0) | 5.19 br s | 5.20 t (3.5) |
| 9 | 2.90 t (7.5) | 2.37 m | 2.47 dd (14.0, 3.0) | 2.11 dd (14.0, 6.6) | 2.01 dd (13.0, 3.9) | 2.52 dd (13.9, 6.0) | 2.50 dd (14.3, 4.2) | 2.81 dd (15.2, 4.5) |
| | | | 1.58 m | 1.95 m | 1.80 m | 1.97 m | 1.73 m | 1.29 dd (15.2, 2.5) |
| 10 | | | 2.61 m | | | | | |
| 13 | 6.28 d (3.3) | 6.30 d (2.0) | 6.16 d (2.8) | 6.25 d (1.5) | 6.35 d (1.5) | 6.31 d (1.5) | 6.38 d (1.6) | 6.38 d (2.0) |
| | 5.48 d (3.3) | 5.70 d (2.0) | 5.46 d (2.8) | 5.71 d (1.5) | 5.86 d (1.5) | 5.79 d (1.5) | 5.80 d (1.6) | 5.78 d (2.0) |
| 14 | 5.06 s | 1.40 s | 1.30 d (6.6) | 1.51 s | 1.26 s | 1.50 s | 1.47 s | 1.49 s |
| 15 | 1.67 s | 1.94 br s | 1.27 s | 1.85 br s | 1.95 br s | 1.94 br s | 6.66 d (10.5) | 1.81 s |
| | | | | | | | 5.60 d (10.5) | |
| 3′ | 6.73 q (6.3) | 6.80 q (6.5) | 6.84 q (6.2) | 6.70 q (6.8) | 6.83 q (6.4) | 6.71 q (6.9) | 6.87 q (6.5) | 6.84 dq (6.5, 1.5) |
| 4′ | 1.75 d (6.3) | 1.78 d (6.5) | 1.80 d (6.2) | 1.75 d (6.8) | 1.78 d (6.4) | 1.75 d (6.9) | 1.80 d (6.5) | 1.80 d (6.5) |
| 5′ | 1.77 s | 1.80 s | 1.82 s | 1.50 s | 1.62 s | 1.69 s | 1.53 s | 1.88 d (1.5) |
| OAc | | | | | | | 2.11 s | |

[a] Chemical shifts in ppm, *J* values in Hz are in parentheses. Assignments were made using HMQC and HMBC techniques.

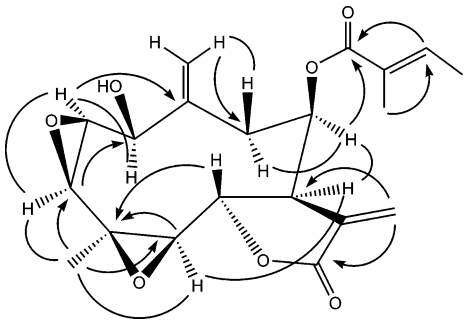
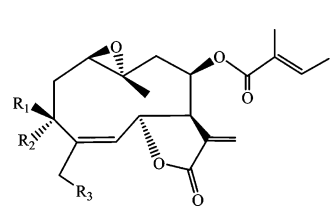
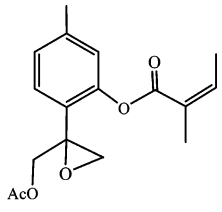


**Figure 1.** Selected HMBC (arrows) and NOESY (curves) correlations of **1**.

The epoxy rings were placed at the 4,5- and 2,3-positions as a result of the observed correlations between H-7/C-5; H-6/C-4; H-5/C-3; H-3/C-1,C-5; and H-2/C-10 in the HMBC spectrum (Figure 1). The magnitudes of $J_{5,6}$ (10.4 Hz) and $J_{6,7}$ (8.5 Hz) together with the small $J_{7,8}$ (3.3 Hz) were in accordance with *trans*-fusion of the lactone ring and α-orientation of both H-5 and H-8.[16–18] The proposed relative stereochemistry of **1** at positions 1−5 was determined by NOESY correlations (Figure 1) between H-7/H-5 and H-8; H-5/H-15, H-2; H-3/H-15, H-1; and H-2/H-1, proving the *β*-orientation of the hydroxyl at C-1 as well as the two epoxy rings. On the basis of the above discussion, eupakirunsin A was assigned as 8*β*-tigloyloxy-1*β*-hydroxy-2*β*,3-epoxy-4*β*,5-epoxy-6*βH*,7α*H*-germacra-10(14),11(13)-dien-6,12-olide (**1**).



**7** $R_1$ = OH, $R_2$ = H, $R_3$ = OAc
**8** $R_1$ = H, $R_2$ = OH, $R_3$ = H
**9** $R_1$ = OH, $R_2$ = H, $R_3$ = H



**10**

The molecular formula of **2** was calculated as $C_{20}H_{26}O_6$ from its HREIMS and FABMS data. The $^1$H NMR spectrum (Table 1) exhibited two narrow doublets at *δ* 6.30 and 5.70 (each d, $J$ = 2.0 Hz), typical of an α-methylene-*γ*-lactone, in addition to signals of a tigloyl group at *δ* 6.80 (1H, q, $J$ = 6.5 Hz), 1.78 (3H, d, $J$ = 6.5 Hz), and 1.80 (3H, s). An olefinic signal at *δ* 5.67 (H-5) was observed along with two vicinal-coupled protons at *δ* 5.82 (d, $J$ = 5.5 Hz, H-1) and 6.32 (dd, H-2). Four olefinic carbons at *δ* 139.4, 131.1, 127.5, and 139.6 suggested the presence of two double bonds in the sesquiterpene skeleton. In the HMBC spectrum, the methyl protons at *δ* 1.94 (H-15) were correlated with the signal at *δ* 131.1 (C-5), whereas the signal at *δ* 5.96 (H-6) was correlated to a resonance at *δ* 139.5 (C-4), indicating 4,5-unsaturation. The quaternary oxygenated carbon at *δ* 87.1 (C-10) showed correlations to *δ* 5.11 (H-8) and the olefinic signal at $\delta_H$ 6.32 (H-2), whereas another correlation was observed between the further olefinic resonance at *δ* 5.82 (H-1) and the $CH_2$ signal at $\delta_C$ 43.6 (C-9), thus verifying the attachment of a hydroxyl to C-10 and 1,2-unsaturation. An oxygenated methine at *δ* 5.10 was assigned to H-3 on the basis of HMBC correlations to C-5, C-15, and C-1. A germacra-1, 4-dienolide could be proposed with two hydroxyls at C-3 and C-10, and a tigloyloxy group at C-8 was confirmed through a COSY experiment that showed connectivities

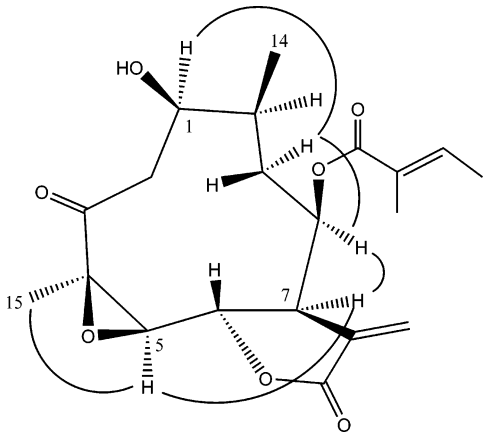


**Figure 2.** Selected NOESY correlations of **3**.

between H-1/H-2/H-3 and H-5/H-6/H-7/H-8 and H-9, in addition to H-3′/H-4′. The magnitude of the $J_{7,13}$ allylic coupling constant (2.0 Hz) and the broadening of the signal assigned to H-15 due to coupling with H-5 were in good agreement with *Z*-geometry of the double bond between C-4/C-5,[19−21] and this was supported by the strong NOESY correlation between H-15/H-5. The small value of $J_{1,2}$ (5.5 Hz) favored the *Z*-geometry of the 1,2-double bond, which differed from the corresponding value of a similar lactone possessing a 1,2-double bond in the *E*-form with a larger $J_{1,2}$ (15.7 Hz).[22] The NOESY correlations between H-3/H-6; H-6/H-14; and H-7/H-8 agreed with the α-orientation of the hydroxyl at C-3, the *β*-orientation of the tigloyloxy group at C-8, and the *trans*-fused lactone ring. The assignment of the stereochemistry at C-6, -7, and -8 was based on the same argument used previously for **1**. Consequently, the structure of eupakirunsin B (**2**) was deduced as 8*β*-tigloyloxy-3α,10α-dihydroxy-6*βH*,7α*H*-germacra-1*Z*,4*Z*,11(13)-trien-6,12-olide.

The empirical formula of **3**, $C_{20}H_{26}O_7$, and its IR absorption bands at 1768 and 1709 $cm^{-1}$ suggested a sesquiterpene lactone ester. In addition to $^1H$ NMR signals diagnostic of α*β*-unsaturated-*γ*-lactone and tigloyl ester functionalities (Table 1), two methyl signals were observed at *δ* 1.30 (d, $J$ = 6.6 Hz, H-14) and 1.27 (s, H-15), in agreement with a germacranolide bearing a tigloyl ester. The methyl protons at *δ* 1.30 showed an HMBC correlation to an oxygenated *C*H at $\delta_H$ 76.6 that was connected to a proton at $\delta_H$ 3.70 (H-1). The latter proton revealed a cross-peak to a carbonyl signal at *δ* 208.0 (C-3), which in turn was correlated to the methyl proton signal at *δ* 1.30 (H-14), hence suggesting the placement of a hydroxyl group at C-1 and a carbonyl at C-3. To satisfy seven oxygen and eight degrees of unsaturation indicated in the molecular formula, and to assign the oxygenated signals at $\delta_C$ 41.9 and 50.7, an additional epoxy ring was proposed at the 4,5-position. This was corroborated from the HMBC correlations between H-7/C-5; H-6/C-4; and H-15/C-5. The long-range correlation between the ester carbonyl at $\delta_C$ 167.2 and the proton at $\delta_H$ 5.78 (d, $J$ = 2.4 Hz, H-8) was used to locate the *β*-oriented tigloyloxy group at C-8. In the NOESY spectrum, the absence of a correlation between H-1/H-14 and the presence of correlations between H-7/H-9α and H-9α/H-1 proved the *β*-orientation of the hydroxyl group at C-1 (Figure 2). On the other hand, NOESY correlations between H-5/H-7/H-8 and H-5/H-15 indicated the *β*-orientation of the 4,5-epoxy ring as well as the *β*-configuration of the tigloyloxy group at C-8. The structure of eupakirunsin C (**3**) was assigned as 8*β*-tigloyloxy-1*β*-hydroxy-3-oxo-4*β*,5-epoxy-6*βH*,7α*H*-germacra-11(13)-en-6,12-olide.

The NMR spectral data of **4** (Tables 1 and 2) indicated the presence of a germacranolide related to **2** with a tigloyl ester at C-8 and a double bond between C-4/C-5 in the *Z*-form. In the HMBC spectrum, both proton signals at *δ* 5.47 (H-8) and 1.51 (H-14) showed correlations to a quaternary oxygenated carbon at *δ* 83.4 assigned to C-10. On the other hand, the vinylic methyl at $\delta_H$ 1.85 (br s, H-15) exhibited long-range correlations with the olefinic *C*H at $\delta_C$ 128.2 (C-5) as well as a carbon at *δ* 106.6 assigned to C-3, which suggested a hemiketal linkage between C-3/C-10. Moreover, the COSY spectrum showed correlations between the two vicinal methylene protons at positions C-1 and C-2 and the HMBC spectrum revealed correlations between H-2/C-4,C-10 and H-1/C-3. The molecular formula, $C_{20}H_{26}O_6$, implied the presence of eight degrees of unsaturation and was consistent with the formation of a C-3/C-10 ether linkage as a part of an additional tetrahydrofuran ring. The spectral data of **4** resembled those reported for 1-deoxyniveusin A[23] except that the angeloyl ester was replaced by a tigloyl moiety in **4**. Therefore, eupakirunsin D (**4**) was assigned as 8*β*-tigloyloxy-3*β*-hydroxy-3α,10α-epoxy-6*βH*,7α*H*-germacra-4*Z*,11(13)-dien-6,12-olide.

In the case of lactone **5**, the HMBC spectrum showed correlations between a carbonyl resonance at $\delta_C$ 193.7 (C-3) and the broad singlet at $\delta_H$ 1.95 (H-15) and a methine signal at $\delta_H$ 3.24 (H-1), whereas the methine signal at $\delta_H$ 3.70 (H-2) was correlated to the quaternary oxygenated carbon at $\delta_C$ 70.1. The fact that the latter two methines at $\delta_H$ 3.24 and 3.70 were directly correlated to two CH signals at $\delta_C$ 58.2 and 65.7 (C-1 and C-2, respectively) was in accordance with the presence of a carbonyl at C-3 and a C-1,C-2-epoxy ring. The NOESY spectrum correlations between H-2/H-6,$H_\beta$-9,H-14; H-7/H-8; and H-15/H-5, together with the absence of any NOE interactions between H-1/H-2,H-14, were used to establish the relative stereochemistry of H-8 (α), H-1 (α), and H-2 (*β*). The relative downfield shift of H-5 (*δ* 6.38) could be explained by the presence of a conformation in which the carbonyl (C-3) was nearly in plane with the conjugated double bond.[24] Because of the close similarity between the data of **5** and those of the angeloyl ester of 3-dehydrotifruticin,[25] it was deduced that the lactone skeletons of both compounds are the same, and hence eupakirunsin E (**5**) was assigned the structure 8*β*-tigloyloxy-1*β*,2α-epoxy-3-oxo-6*βH*,7α*H*-germacra-4*Z*,11(13)-dien-6,12-olide.

The UV absorption of **6** revealed a maximum absorption at $\lambda_{max}$ 249 nm attributable to a dienone chromophore.[26] The carbonyl signal at $\delta_C$ 197.1 together with the two sets of olefinic carbon signals at $\delta_C$ 160.6, 129.6 and 139.1, 137.4, and the HMBC correlations between the carbonyl signal ($\delta_C$ 197.1) and the olefinic proton resonances at $\delta_H$ 6.94 (H-1) and 5.85 (H-5), inferred the presence of a carbonyl at C-3 with α,*β*-unsaturation on each side (cross conjugation). The large value of $J_{1,2}$ (17.1 Hz) was consistent with the *E*-geometry of the C-1/C-2 double bond. The configuration of the lactone ring, the tigloyl ester at C-8, the hydroxyl at C-10, and geometry of the C-4/C-5 double bond were similar to those of **2** and **5**. On the basis of its collective spectral data as well as comparison with closely related sesquiterpene lactones,[25,27] eupaheliangolide A (**6**) was assigned as 8*β*-tigloyloxy-10α-hydroxy-3-oxo-6*βH*,7α*H*-helianga-1*E*,4*Z*,11(13)-trien-6,12-olide. It is worth noting that the corresponding angelate ester of **6**, with *Z*-geometry at C-1/C-2, was synthesized from deoxytifruticin by $MnO_2$ oxidation,[22] whereas the corresponding

**Table 2.** $^{13}C$ NMR Data ($CDCl_3$, 75 MHz) of Compounds **1**–**8**[a]

| carbon | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| 1 | 76.3 d | 127.5 d | 76.6 d | 38.1 t | 58.2 d | 160.6 d | 60.4 d | 59.4 d |
| 2 | 49.8 d | 139.6 d | 46.2 t | 37.5 t | 65.7 d | 129.6 d | 33.9 t | 34.4 t |
| 3 | 65.2 d | 77.0 d | 208.0 s | 106.6 s | 193.7 s | 197.1 s | 69.2 d | 66.5 d |
| 4 | 66.3 s | 139.5 s | 41.9 s | 141.5 s | 137.2 s | 139.1 s | 139.7 s | 141.7 s |
| 5 | 51.7 d | 131.1 d | 50.7 d | 128.2 d | 141.5 d | 137.4 d | 129.6 d | 124.3 d |
| 6 | 77.0 d | 74.7 d | 78.4 d | 72.4 d | 75.3 d | 76.2 d | 76.2 d | 72.9 d |
| 7 | 48.5 d | 47.9 d | 53.0 d | 49.9 d | 49.9 d | 43.3 d | 48.1 d | 48.7 d |
| 8 | 68.3 d | 77.0 d | 65.8 d | 75.8 d | 73.5 d | 74.8 d | 73.6 d | 75.9 d |
| 9 | 37.9 t | 43.6 t | 40.9 t | 40.9 t | 42.4 t | 48.5 t | 43.6 t | 43.3 t |
| 10 | 140.8 s | 87.1 s | 44.3 d | 83.4 s | 70.1 s | 72.0 s | 58.6 s | 56.9 s |
| 11 | 134.0 s | 139.4 s | 133.7 s | 136.7 s | 135.5 s | 136.1 s | 136.8 s | 136.8 s |
| 12 | 169.4 s | 169.0 s | 169.5 s | 170.0 s | 169.0 s | 169.9 s | 169.4 s | 169.0 s |
| 13 | 122.6 t | 124.2 t | 120.2 t | 122.9 t | 125.1 t | 124.7 t | 125.4 t | 125.2 t |
| 14 | 120.4 t | 31.6 q | 13.9 q | 28.4 q | 26.0 q | 28.9 q | 19.7 q | 18.5 q |
| 15 | 18.5 q | 20.6 q | 13.8 q | 22.5 q | 20.3 q | 19.8 q | 66.7 t | 17.0 q |
| 1′ | 167.3 s | 166.6 s | 167.2 s | 167.0 s | 167.1 s | 167.0 s | 166.6 s | 166.4 s |
| 2′ | 128.1 s | 128.1 s | 128.0 s | 128.0 s | 127.7 s | 127.6 s | 127.7 s | 127.7 s |
| 3′ | 138.4 d | 138.9 d | 138.9 d | 138.4 d | 139.7 d | 139.4 d | 139.1 d | 139.3 d |
| 4′ | 14.5 q | 14.6 q | 14.6 q | 14.5 q | 14.6 q | 14.6 q | 14.6 q | 12.0 q |
| 5′ | 12.1 q | 12.0 q | 12.2 q | 12.0 q | 11.9 q | 12.0 q | 11.2 q | 14.6 q |
| OAc | | | | | | | 170.5 s | |
| | | | | | | | 20.9 q | |

[a] DEPT and HMQC spectra were used for assignments.

isobutyl esters of both **5** and **6** were isolated previously from *Greenmaniella resinosa*.[24]

The $^1H$ NMR spectroscopic data of **7** were very close to those reported for heliangin[13] except for the absence of a signal attributable to a methyl group at C-15 and the appearance of an oxygenated $CH_2$ at $\delta_C$ 66.7, corresponding to two geminal protons at $\delta_H$ 6.66 and 5.60 (each 1H, d, $J$ = 10.5 Hz, H-15). An acetyl ester was evident from the methyl signal at $\delta_H$ 2.11 and a carbonyl signal at $\delta_C$ 170.5, together with a methyl carbon signal at $\delta_C$ 20.9. The HMBC correlation between the acetyl carbonyl and each of the two protons at $\delta_H$ 6.66 and 5.60 confirmed the attachment of the acetyloxy group at C-15. Consequently, **7** was assigned the structure 8$\beta$-tigloyloxy-3$\beta$-hydroxy-15-acetoxy-1$\beta$,10α-epoxy-6$\beta H$,7α$H$-helianga-4$Z$,11(13)-dien-6,12-olide, or 15-acetoxyheliangin. This was confirmed by comparison with the data of the isolated heliangin (**9**) as well as the data reported for the 15-acetoxy derivative of the corresponding angelate ester, 15-acetyloxyleptocarpin, previously isolated from *Tithonia rotundifolia*[28] and *T. longiradiata*.[29]

The spectroscopic data of **8** (Tables 1 and 2) were quite similar to those of **9** with respect to the α,$\beta$-unsaturated lactone, the $\beta$-tigloyloxy ester group at C-8, and the $Z$-form of the C-4/C-5 double bond, in addition to the 1,10-epoxy ring. The significant differences detected in the NMR data of **8** were the relative upfield shifts of both the C-3 and C-15 signals. The chemical shift of C-3 appearing at $\delta$ 72.1 in **9** was replaced by a signal at $\delta$ 66.5 in **8**, while the C-15 signal at $\delta$ 23.0 was replaced by a resonance at $\delta$ 17.0. This was accompanied by a slight downfield shift of H-3 ($\Delta\delta$ 0.54) with a change of its multiplicity (from a singlet to a doublet of doublets). The signal of H-3 at $\delta$ 4.99 (dd, $J$ = 12.5, 5.5 Hz) revealed HMBC correlations to C-1 ($\delta$ 59.4), C-15 ($\delta$ 17.0), and C-5 ($\delta$ 124.3). The chemical shift of C-15 ($\delta$ 17.0) was in accordance with the reported values for similar compounds with the $Z$-form of the C-4/C-5 double bond as well as an α-orientation of a hydroxyl substituent at C-3.[19,21] The strong NOESY correlation between H-3/H-6$\beta$ verified the $\beta$-orientation of H-3 and the α-orientation of the hydroxyl group at C-3, opposite to that of heliangin (**9**). A further confirmation of the configuration at C-3 between **9** and **8** was obtained by measuring their optical activity, and these were significantly different.

**Table 3.** Cytotoxicity of Compounds **1**–**9** against Human Tumor Cells ($ED_{50}$, $\mu$g/mL)[a]

| compound | KB[b] | Hela[c] | hepa59T/VGH[d] |
|---|---|---|---|
| **1** | 5.5 | 8.9 | 4.4 |
| **2** | (−)[e] | (−) | (−) |
| **3** | 8.1 | 7.1 | 8.6 |
| **4** | 4.8 | 5.8 | 5.0 |
| **5** | (−) | (−) | (−) |
| **6** | 3.5 | 3.3 | 3.5 |
| **7** | 4.3 | 7.5 | 7.0 |
| **8** | 3.2 | 3.1 | 4.4 |
| **9** | 1.8 | 4.4 | 2.0 |
| doxorubicin | 0.15 | 0.14 | 0.19 |

[a] The concentration that inhibits 50% of the growth of human tumor cell lines after 72 h exposure according to the method described in the Experimental Section. [b] Oral epidermoid carcinoma. [c] Human cervical epitheloid carcinoma. [d] Human liver carcinoma. [e] $ED_{50}$ > 20 $\mu$g/mL.

Therefore, the structure of **8** was determined as 8$\beta$-tigloyloxy-3α-hydroxy-1α,10-epoxy-6$\beta H$,7α$H$-helianga-4$Z$,11(13)-dien-6,12-olide, or 3-*epi*-heliangin.

Compound **9** was obtained as colorless prismatic crystals. The $^1H$ NMR spectral data were consistent with those reported for heliangin.[13] The structure was confirmed by $^{13}C$ NMR spectroscopic analysis, which has not been reported previously.

All compounds were evaluated for cytotoxic activity against human KB, Hela, and Hepa carcinoma cells. Table 3 shows that compounds **6**, **8**, and **9** exhibited significant activity against three tumor cell lines. Compounds **1**, **3**, **4**, and **7** showed low activity, while compounds **2** and **5** were inactive.

## Experimental Section

**General Experimental Procedures.** The melting point was taken on a Buchi mp B-540 apparatus and is uncorrected. Optical rotations were recorded on a JASCO DIP-1000 polarimeter. IR and UV spectra were measured on Hitachi T-2001 and U-3210 spectrophotometers, respectively. The $^1H$, $^{13}C$ NMR, COSY, HMQC, HMBC, and NOESY spectra were recorded on a Bruker FT-300 or a Varian Unity INOVA 500 FT-NMR spectrometer at 500 MHz for $^1H$ and 125 MHz for $^{13}C$, respectively, using TMS as internal standard. The chemical shifts are given in $\delta$ values (ppm) and coupling constants in Hz. Low-resolution EIMS and FABMS were

recorded on a VG Quattro 5022 mass spectrometer, and HREIMS were measured on a JEOL JMS-SX 102 mass spectrometer. Silica gel 60 (Merck) was used for column chromatography, and precoated silica gel plates (Merck, Kieselgel 60 F-254, 1 mm) were used for preparative TLC. Sephadex LH-20 (Amersham Pharmacia Biotech AB, Uppsala, Sweden) was used.

**Plant Material.** *Eupatorium kiirunense* Kitam. C. H. Ou & S. W. Chung was collected from the northern coast of Taipei County, Taiwan, in October 2003. This material was identified by one of the authors (Y.C.S). A voucher specimen (TP300-2) has been deposited in the Institute of Marine Resources, National Sun Yat-sen University, Kaohsiung, Taiwan. The leaves were collected and dried at room temperature.

**Extraction and Isolation.** The dried leaves (2 kg) were reduced to a coarse powder and extracted with acetone three times, and the combined extract was evaporated under a vacuum. The resulting crude extract (120 g) was separated on Sephadex LH-20 using MeOH for elution to produce four fractions, $L_1$−$L_4$. $L_4$ (24 g) was fractionated on a silica gel column using *n*-hexane−EtOAc (100:0 to 0:100), to afford 29 fractions (F1−F29). Separation of fraction F22 (1.2 g) was effected on Sephadex LH-20, eluting with $CH_2Cl_2$−MeOH (1:1), to produce five fractions (F22-A to F22-E). Fraction F22-D (350 mg) was chromatographed on a silica gel column using *n*-hexane−EtOAc (4:1 to 2:1) to yield seven fractions (F22-D-a to F22-D-g). Fraction F22-D-b (38 mg) was subjected to NP-HPLC using *n*-hexane−$CH_2Cl_2$−MeOH (20:20:1) for elution and UV detection (at 254 nm) to afford **1** (6 mg). Fraction F22-D-d (128 mg) was further fractionated by NP-HPLC using *n*-hexane−$CH_2Cl_2$−MeOH (11:11:1) to yield **4** (13 mg). Fraction F23 (1.9 g) was separated on Sephadex LH-20, eluting with $CH_2Cl_2$−MeOH (1:1), to produce two fractions (F23-A and F23-B). Fraction F23-A (1.2 g) was separated on silica gel using *n*-hexane−EtOAc (3:1 to 1:2) to give five fractions (F23-A-1 to F23-A-5). Fraction F23-A-2 (19 mg) was subjected to NP-HPLC using *n*-hexane−EtOAc−$CHCl_3$ (75:20:5) for elution to afford **2** (10 mg). Fraction F23-A-3 (50 mg) was purified using NP-HPLC with *n*-hexane−EtOAc−$CHCl_3$ (75:20:5) for elution to yield 8,10-epoxy-9-acetoxythymol angelate [14] (**10**, 12 mg). Fraction F23-A-4 (230 mg) was purified using NP-HPLC with *n*-hexane−EtOAc−$CHCl_3$ (75:20:5) for elution to yield **5** (8 mg) and **6** (36 mg). Fraction F23-A-5 (65 mg) was purified using NP-HPLC with *n*-hexane−EtOAc−$CHCl_3$ (75:20:5) to produce heliangin (**9**, 50 mg). Fraction F24 (1.3 g) was fractionated on a silica gel column using *n*-hexane−EtOAc (3:1 to 1:1) to yield eight fractions (F24-A to F24-H). Fraction F24-H (68 mg) was repeatedly chromatographed on silica gel using *n*-hexane−EtOAc (3:1) to provide **8** (8 mg). Part of fraction F25 (1.3 g) was purified over Sephadex LH-20 using $CH_2Cl_2$−MeOH (1:1) to give **3** (10 mg). Fraction F26 (1.6 g) was fractionated by NP-HPLC using $CHCl_3$−MeOH (2:1) to give seven fractions, F26-A to F26-G. Fraction F26-C (600 mg) was rechromatographed on a silica gel column using *n*-hexane−EtOAc (3:1 to 1:3), then further purified by NP-HPLC using *n*-hexane−$CH_2Cl_2$−MeOH (10:10:1) for elution, to afford **7** (17 mg).

**Eupakirunsin A (1):** gum; $[\alpha]^{25}_D$ −58.3° (*c* 0.5, acetone); IR ($CH_2Cl_2$) $\nu_{max}$ 3433, 2935, 1766, 1707, 1649 cm$^{-1}$; $^1$H NMR (300 MHz, $CDCl_3$), Table 1; $^{13}$C NMR (75 MHz, $CDCl_3$), Table 2; FABMS *m/z* 377 $[M + 1]^+$; EIMS *m/z* 376 $[M]^+$, 276 $[M - C_5H_7O - OH]^+$, 260 $[M - C_5H_7O_2 - OH]^+$, 83 $[C_5H_7O]^+$ (100); HREIMS *m/z* 358.1421 (calcd for $C_{20}H_{22}O_6$, 358.1417).

**Eupakirunsin B (2):** white powder; $[\alpha]^{25}_D$ −37.8° (*c* 0.15, acetone); IR ($CH_2Cl_2$) $\nu_{max}$ 3439 br, 1752, 1710, 1647 cm$^{-1}$; $^1$H NMR (300 MHz, $CDCl_3$), Table 1; $^{13}$C NMR (75 MHz, $CDCl_3$), Table 2; FABMS *m/z* 386 $[M + 1 + Na]^+$; EIMS *m/z* 362 $[M]^+$, 261 $[M - C_5H_7O - H_2O]^+$, 245 $[M - H_2O - C_5H_7O_2]^+$, 83 $[C_5H_7O]^+$, 69 $[C_4H_5O]^+$ (100); HRESIMS *m/z* 362.1725 $[M]^+$ (calcd for $C_{20}H_{26}O_6$, 362.1722).

**Eupakirunsin C (3):** white powder; $[\alpha]^{25}_D$ −43.1° (*c* 0.3, acetone); IR ($CH_2Cl_2$) $\nu_{max}$ 3489 br, 2982, 1768, 1709, 1645 cm$^{-1}$; $^1$H NMR (300 MHz, $CDCl_3$), Table 1; $^{13}$C NMR (75 MHz, $CDCl_3$), Table 2; FABMS *m/z* 401 $[M + Na]^+$; EIMS *m/z* 378 $[M]^+$, 278 $[M - C_5H_7O - OH]^+$, 83 $[C_5H_7O]^+$ (100); HREIMS *m/z* 378.1675 $[M]^+$ (calcd for $C_{20}H_{26}O_7$, 378.1671).

**Eupakirunsin D (4):** white powder; $[\alpha]^{25}_D$ −51° (*c* 0.23, acetone); IR ($CH_2Cl_2$) $\nu_{max}$ 3468 br, 2968, 1763, 1707, 1651 cm$^{-1}$; $^1$H NMR (300 MHz, $CDCl_3$), Table 1; $^{13}$C NMR (75 MHz, $CDCl_3$), Table 2; FABMS *m/z* 363 $[M + 1]^+$; EIMS *m/z* 362 $[M]^+$, 279 $[M - C_5H_7O]^+$, 262 $[M - C_5H_7O - OH]^+$, 83 $[C_5H_7O]^+$ (100), 55 $[C_4H_7]^+$; HREIMS *m/z* 362.1728 (calcd for $C_{20}H_{26}O_6$, 362.1724).

**Eupakirunsin E (5):** gum; $[\alpha]^{25}_D$ −36.8° (*c* 0.15, acetone); IR ($CH_2Cl_2$) $\nu_{max}$ 3530 br, 2924, 1767, 1714 cm$^{-1}$; $^1$H NMR (300 MHz, $CDCl_3$), Table 1; $^{13}$C NMR (75 MHz, $CDCl_3$), Table 2; FABMS *m/z* 377 $[M + 1]^+$; EIMS *m/z* 376 $[M]^+$, 276 $[M - C_5H_7O - OH]^+$, 83 $[C_5H_7O]^+$ (100); HREIMS *m/z* 358.1421 $[M - H_2O]^+$ (calcd for $C_{20}H_{22}O_6$, 358.1417).

**Eupaheliangolide A (6):** white powder; $[\alpha]^{25}_D$ −129° (*c* 0.6, acetone); UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 249 (2.7), 203 (3.8) nm; IR ($CH_2Cl_2$) $\nu_{max}$ 3470 br, 1765, 1711 and 1652 cm$^{-1}$; $^1$H NMR (300 MHz, $CDCl_3$), Table 1; $^{13}$C NMR (75 MHz, $CDCl_3$), Table 2; FABMS *m/z* 361 $[M + 1]^+$; EIMS *m/z* 360 $[M]^+$, 260 $[M - C_5H_7O - OH]^+$, 83 $[C_5H_7O]^+$ (100), 55 $[C_4H_7]^+$; HRESIMS *m/z* 383.1471 (calcd for $C_{20}H_{24}O_6Na$, 383.1471).

**15-Acetoxyheliangin (7):** white powder; $[\alpha]^{25}_D$ −42.8° (*c* 0.3, acetone); IR ($CH_2Cl_2$) $\nu_{max}$ 3489 br, 2918, 1751, 1714, 1651 cm$^{-1}$; $^1$H NMR (300 MHz, $CDCl_3$), Table 1; $^{13}$C NMR (75 MHz, $CDCl_3$), Table 2; FABMS *m/z* 421 $[M + 1]^+$, 402 $[M - H_2O]^+$; EIMS *m/z* 420 $[M]^+$, 319 $[M - C_5H_7O - H_2O]^+$, 260 $[M - C_5H_7O - OH - AcOH]^+$, 83 $[C_5H_7O]^+$ (100), 55 $[C_4H_7]^+$; HREIMS *m/z* 420.1775 (calcd for $C_{22}H_{28}O_8$, 420.1778).

**3-*Epi*-heliangin (8):** gum; $[\alpha]^{25}_D$ −45.2° (*c* 0.3, acetone); IR ($CH_2Cl_2$) $\nu_{max}$ 3456 br, 2920, 1761, 1712, 1651 cm$^{-1}$; $^1$H NMR (300 MHz, $CDCl_3$), Table 1; $^{13}$C NMR (75 MHz, $CDCl_3$), Table 2; FABMS *m/z* 385 $[M + Na]^+$, 363 $[M + 1]^+$; EIMS *m/z* 362 $[M]^+$, 344 $[M - H_2O]^+$, 262 $[M - C_5H_7O - OH]^+$, 83 $[C_5H_7O]^+$ (100); HREIMS *m/z* 362.1720 (calcd for $C_{20}H_{26}O_6$, 362.1722).

**Heliangin (9):** colorless prisms; mp 237−238 ° C; $[\alpha]^{25}_D$ −91° (*c* 0.25, acetone); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 60.8 (d, C-1), 32.5 (t, C-2), 72.1 (d, C-3), 141.9 (s, C-4), 126.2 (d, C-5), 74.4 (d, C-6), 48.4 (d, C-7), 76.4 (d, C-8), 43.4 (t, C-9), 58.9 (s, C-10), 137.5 (s, C-11), 169.8 (s, C-12), 124.9 (t, C-13), 19.8 (q, C-14), 23.0 (q, C-15), 166.8 (s, C-1′), 127.9 (s, C-2′), 139.0 (d, C-3′), 12.0 (q, C-4′), 14.7 (q, C-5′); FABMS *m/z* 385 $[M + Na]^+$, 363 $[M + 1]^+$; EIMS *m/z* 362 $[M]^+$, 344 $[M - H_2O]^+$, 262 $[M - C_5H_7O - OH]^+$, 83 $[C_5H_7O]^+$ (100).

**Cytotoxicity Assay.** The tumor cells for assay were cultured in RPMI-1640 medium, and growth inhibition was determined using the methylene blue staining method.[30] The $ED_{50}$ value was defined by a comparison with the untreated cells as the concentration of test sample resulting in 50% reduction of absorbance.

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**Supporting Information Available:** The $^1$H NMR data for **9** and NOESY spectra in $CDCl_3$ for compounds **2**, **3**, and **5**. This material is available free of charge via the Internet at http://pubs.acs.org.

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