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# SYNTHETIC DITERPENE DERIVATIVES FROM KAURA-9(11), 16-DIEN-19-OIC ACID: CYTOSTATIC AND CYTOTOXIC ACTIVITY AGAINST HUMAN CANCER CELL LINES

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Diterpenes **1** and **2** were synthesized via epoxidation and rearrangements of (-)-kaura-9(11), 16-dien-19-oate, isolated from species of espeletia (Frailejón). Evaluated at NIH, Developmental Therapeutics Program (DTP), compound **1** showed $GI_{50}$ values of 51.6 nM against CNS SF-539 ($LC_{50}$ = 100 μM, $lg(GI_{50})$ = –7.29). Additionally, compound **2** showed $GI_{50}$ at 4.17 μM against breast cancer T47D ($LC_{50}$ = 39 μM, $lg(GI_{50})$ = –5.38).

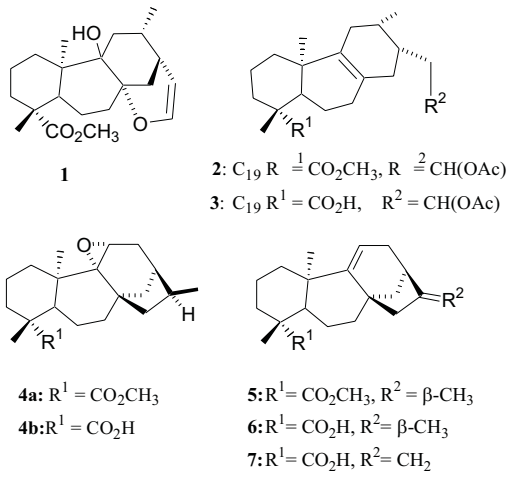
The species of *Espeletiinae* are plants of the *Asteraceae* family that grow at altitude above 2500 m in the Andes of Venezuela, Colombia, and Ecuador. In recent years, there is increasing interest in the medicinal properties of espeletia (Frailejón) species. These plants are used for the treatment of asthma in folk medicine of the Venezuela Andes. One current task in the chemistry of natural products is the development of new bioactive compounds. In this context, a series of modifications were performed (Scheme 1) on the molecule of grandiflorenic acid or (-)-kaura-9(11), 16-dien-19-oic acid (compound **7**) as starting material, which was obtained from the Venezuelan species of espeletia using the synthetic approach developed by Nakano et al. [1]. The goal was to obtain compounds for biological testing and for the investigation of structural requirements for antitumor activity. Hydrogenation of grandiflorenic acid (**7**) with platinum oxide in methanol yielded compound **6**. Esterification of (-)-kaura-9(11),16-dien-19-oic acid (**6**) led to compound **5** with a good yield. Then, compound **5** was converted into the new ring systems (compounds **1** and **2**) via epoxidation and rearrangements according to Schemes 2 and 3.

Epoxidation of compound **5** with MCPBA took place stereoselectively at the more readily accessible site and yielded exclusively 9,11-α-epoxide compound **4a**; epoxidation of compound **5** with *m*-chloroperbenzoic acid in the presence of N-nitrosomethylurea yielded compound **1** (Scheme 2). On rupture with boron trifluoride-diethyl ether ($BF_3$-$Et_2O$) in acetic anhydride, epoxide **4a** yielded acylal compound **2** (Scheme 3). Compound **6** was epoxidated to yield compound **4b**. On rupture with $BF_3$-$Et_2O$ in acetic anhydride, epoxide **4b** yielded acylal compound **3** (Scheme 3). All compounds **1**, **2** , **3**, and **4a** had physical and spectroscopic properties identical to those reported by Nakano et al. [1 – 3].

## *Experimental*

**Chemistry**. The melting points were measured on a Kofler hot-stage apparatus without corrections. The IR spectra were recorded with a Nicolet 5DXC FT-IR spectrometer. The NMR spectra were obtained using solutions in $CDCl_3$ on a Bruker Avance-500 spectrometer. The mass spectra were obtained with a Kratos MS 25 RFA spectrometer at 70 eV using a direct inlet system. Rotations were measured in chloroform solutions at 25°C with Perkin-Elmer Model 341 polarimeter (at concentrations expressed in g/100 ml). The column chromatography was performed using silica gel 60 (Merck, 70 – 230 mesh) in a Byotage column chromatography system. All organic extracts were dried over anhydrous sodium sulfate and evaporated under reduced pressure at temperatures below 60°C. Diethyl ether, tetrahydrofuran (THF), dichloromethane (DCM), and benzene were freshly distilled before use.

**Scheme 1**




**1**

**2**: $C_{19}$ R $\overset{1}{=}CO_2CH_3$, R $\overset{2}{=}CH(OAc)$

**3**: $C_{19}$ $R^1 = CO_2H$, $R^2 = CH(OAc)$

**4a:** $R^1 = CO_2CH_3$

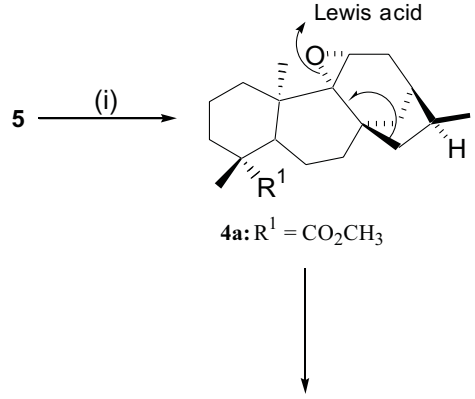
**4b:** $R^1 = CO_2H$

**5:** $R^1 = CO_2CH_3$, $R^2 = \beta\text{-}CH_3$

**6:** $R^1 = CO_2H$, $R^2 = \beta\text{-}CH_3$

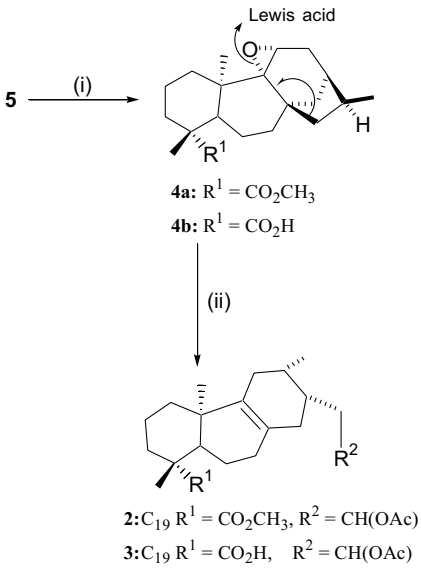
**7:** $R^1 = CO_2H$, $R^2 = CH_2$

**Scheme 2**




**4a:** $R^1 = CO_2CH_3$

**Scheme 3**




Compounds **1**, **2** , **3**, **4a**, **4b**, **5**, and **6** were prepared according to a procedure previously described by Nakano et al. [1].

**Compound 1**. Yield, 36 %; MS–GC (*m*/z): 334 (M+); 1H NMR (500 MHz) in $CDCl_3$ ($\delta_H$, ppm): 0.87 (3H, d, J 6 Hz, $CH_3$), 0.98 (3H, s, $CH_3$), 1.25 (3H, s, $CH_3$), 3.90 (3H, s, $CH_3O$), 5.01 (1H, ddd, J 7, 6 and 3 Hz, O-CH=CH), and 6.90 (1H, d, J 7 Hz, O-CH=CH), IR, $\nu_{max}$ 3550 (OH), 1740 (ester CO) and 1250 $cm^{-1}$ (CO); found (%): C 70.50, H 8.96; anal. calcd. for $C_{20}H_{30}O_4$ (%): C 71.82, H 9.04.

**Compound 2**. Yield, 43 %; m.p., 119°C; $[\alpha]_D^{21}$, –214° (*c*, 1.08; $CHCl_3$); found (%): C 68.73, H 8.77; anal. calcd. for $C_{25}H_{38}O_6$ (%): C 69.09, H 8.81.

**Compound 3**. Yield, 70 %; m.p.. 155°C; $[\alpha]_D^{21}$, –263° (*c*, 1.0; $CHCl_3$); MS–GC (*m*/z): 420 (M+); found (%): C 68.23; H 8.60; anal calcd. for $C_{24}H_{36}O_6$ (%): C 68.54; H 8.63.

**Compound 4a**. Yield, 83 %; MS–GC (*m*/z): 332 (M+); 1H NMR (500 MHz) in $CDCl_3$ ($\delta_H$, ppm): 0.65 (3H, s, $CH_3$), 0.95 (3H, d, J 6.5 Hz, $CH_3$), 1.19 (3H, s, $CH_3$), 3.00 (1H, bs, 11-H), 3.63 (3H, s, $CH_3$-O); found (%): C 75.20, H 9.83; anal calcd. for $C_{21}H_{32}O_3$ (%): C 75.86, H 9.70.

**Testing against human cancer cell lines**. The cytotoxic activities of compounds **1**, **2** , and **3** were evaluated at the National Cancer Institute, National Institute of Health, and the Development Therapeutics Program (DTP). This 3-cell line, one-dose assay has been used by DTP and has proven to be an effective pre-screen [4]. The evaluation of potential chemotherapeutic activity was performed on compounds **1**, **2** , and **3** at DTP against a panel of 3 cell lines $MCF_7$ (breast), NCI-$H_{460}$ (lung), and SF-268 (CNS) according to a standard protocol (Table 1) [5].

*Results and discussion*

Compounds **1** and **2** passed DTP criteria for activity. In this assay, samples were scheduled automatically for evaluation against the full panel of 60 tumor cell species selected from nine cancer types (leukemia, nonsmall cell lung (NSCL), colon, CNS, melanoma, ovarian, renal, prostate, and breast) according to standard protocol (Table 2) [6]. In each test, dose response curves for each cell line were measured with five different drug concentrations starting from as high as 1.0E-04 M. A 48 h continuous drug exposure protocol was used and a sulforhodamine B protein assay was used to estimate cell viability or growth, and the concentration causing 50% cell growth inhibition ($GI_{50}$) was calculated.

The results of cytostatic and cytotoxic activity testing for compounds **1** and **2** are presented in Table 2. Synthetic diterpene compound **1** inhibited cell proliferations with $GI_{50}$ = 51.6 nM against CNS SF-539 ($LC_{50}$ = 100 μM, $lg(GI_{50}$ = –7.29). Compound **1** was active against all cell lines, with mean $lg(GI_{50})$ values ranging from –4.00 (melanoma LOX IMVI) to –7.29 (CNS SF-539); compound **1** showed activity with $GI_{50}$ = 2.07 μM against NSCL cancer EKVX ($LC_{50}$ = 100 μM, $lg(GI_{50})$ = –5.68). Cytotoxic effects evaluated as $LC_{50}$ became visible at somewhat higher concentrations: 95 μM for the 60 cell line panels.

Compound **2** (an acetyl derivative of diterpene) inhibited cell proliferations with $GI_{50}$ at 4.17 μM against breast cancer $T_{47}D$ ($LC_{50}$ = 39 μM, $lg(GI_{50})$ = –5.38). Compound **2** was active against all cell lines with average $lg(GI_{50})$ values ranging from –4.80 (NSCL cancer NCI-$H_{522}$) to –5.38 (breast cancer $T_{47}D$) and additionally showed $GI_{50}$ at 7.41 μM against CNS cancer SF-268 ($LC_{50}$ = 27.8 μM, $lg(GI_{50})$ = –5.13). Cytotoxic effects evaluated as $LC_{50}$ became visible at somewhat higher concentrations: from 26 μM to 50.0 μM for 60 cell line panels.

Compound **3** contained a carboxylic acid residue at C-19 position. This small structural modification produced a moderate increase in the growth percentage for 3 tumor cell lines in comparison with compound **2** (Table 1).

In conclusion, this study was an initial stage of biological evaluation of newly synthesized skeletal diterpene compounds **1** and **2** obtained from grandiflorenic acid. Both **1** and **2** show a promising activity against human cancer cell lines. Additionally, compound **3** presents a carboxylic acid at $C_{19}$ position; this structural change at $C_{19}$ position produced a moderate increase in growth percentage for 3 tumor cell lines in comparison with compound **2** at the same concentration.

Table 1

**Primary Anticancer Assay Using 3-Cell Line Panel for Compounds 1 – 3**

| Compound | Concentration | Percentage growth | | |
|---|---|---|---|---|
| | | Breast | NSCL | CNS |
| | | MCF7 | NCI-H460 | SF-268 |
| **1** | 1.00E-04 M | 20 | 37 | 95 |
| **2** | 1.00E-04 M | 3 | 11 | 39 |
| **3** | 1.00E-04 M | 199 | 187 | 140 |

Table 2

**Results of *in vitro* Antitumor Screening for Compounds 1 and 2**

| Cell Line | Compound **1** | | | | Compound **2** | | | |
|---|---|---|---|---|---|---|---|---|
| | G % | $GI_{50}$ | $LC_{50}$ | $Log_{10} GI_{50}$ | G% | $GI_{50}$ | $LC_{50}$ | $Log_{10} GI_{50}$ |
| Leucemia | | | | | | | | |
| HL-60(TB) | 28 | 45.4 | > 100 | 4.34 | | | | |
| K-562 | 24 | 36.6 | > 100 | -4.44 | 7 | 17.5 | 50.0 | -4.76 |
| MOLT-4 | 32 | 24.5 | > 100 | -4.61 | | | | |
| RPMI-8226 | -4 | 7.84 | > 100 | -5.11 | | | | |
| SR | 16 | 17.1 | > 100 | -4.77 | | | | |
| N-SC Lung | | | | | | | | |
| A549 | 26 | 33.1 | > 100 | -4.48 | -14 | 14.4 | 50.0 | -4.84 |
| EKVX | 33 | 2.07 | > 100 | -5.68 | -48 | 12.3 | 50.0 | -4.91 |
| HOP-62 | 66 | 100 | > 100 | > 4.00 | -85 | 10.9 | 33.5 | -4.96 |
| HOP-92 | 22 | 22.0 | > 100 | -4.66 | -80 | 11.4 | 35.6 | -4.94 |
| NCI-H226 | 59 | 100 | > 100 | > 4.00 | -45 | 9.60 | 50.0 | -5.02 |
| NCI-H23 | 37 | 20.3 | > 100 | -4.69 | -100 | 9.44 | 28.7 | -5.02 |
| NCI-H322M | 38 | | | | -14 | 17.2 | 50.0 | -4.76 |
| NCI-H460 | 87 | 43.8 | > 100 | -4.36 | -98 | 75.4 | 27.1 | -5.12 |
| NCI-H522 | | 100 | > 100 | >4.00 | 6 | 15.7 | 50.0 | -4.80 |
| Colon | | | | | | | | |
| COLO 205 | 43 | 64.4 | > 100 | -4.16 | -88 | 8.25 | 30.5 | -5.08 |
| HCC-2998 | | | | | -100 | 9.32 | 28.6 | -5.03 |
| HCT-116 | 19 | 7.89 | > 100 | -5.10 | -43 | 9.54 | 50.0 | -5.03 |
| HCT-15 | 79 | 100 | > 100 | > 4.00 | -20 | 12.0 | 50.0 | -4.92 |
| HT29 | 33 | 11.0 | > 100 | -4.96 | 6 | 20.9 | 50.0 | -4.68 |
| KM-12 | 52 | | | | -39 | 10.6 | 50.0 | -4.97 |
| SW-620 | | 100 | > 100 | > 4.00 | -49 | 12.7 | 50.0 | -4.90 |
| CNS cancer | | | | | | | | |
| SF-268 | 59 | 100 | > 100 | >4.00 | -94 | 7.41 | 27.8 | -5.13 |
| SF-295 | -5 | 27.5 | > 100 | -4.56 | -59 | 9.22 | 43.2 | -5.04 |
| SF-539 | 26 | 0.51 | > 100 | -7.29 | -64 | 12.2 | 41.9 | -4.91 |
| SNB-19 | 89 | 100 | > 100 | > 4.00 | -52 | 10.3 | 48.4 | -4.99 |
| SNB-75 | 44 | 62.2 | > 100 | -4.21 | -36 | 16.8 | 50.0 | -4.78 |
| U251 | 81 | 100 | > 100 | > 4.00 | -79 | 8.29 | 33.5 | -5.08 |
| Melanoma | | | | | | | | |
| LOX IMVI | 84 | 100 | > 100 | > 4.00 | | | | |
| M14 | 41 | 50.3 | > 100 | -4.30 | -83 | 12.0 | 35.1 | -4.92 |
| SK-MEL-2 | 61 | 100 | > 100 | > 4.00 | -33 | 11.6 | 50.0 | -4.93 |
| SK-MEL-28 | 66 | 100 | > 100 | > 4.00 | -65 | 10.7 | 40.9 | -4.97 |
| SK-MEL-5 | 35 | 34.0 | > 100 | -4.47 | -99 | 9.12 | 28.7 | -5.04 |
| UACC-257 | 41 | 70.0 | > 100 | -4.15 | -70 | 10.2 | 38.3 | -4.99 |
| UACC-62 | 51 | 100 | > 100 | > 4.00 | -63 | 8.98 | 41.1 | -5.05 |
| Ovarian | | | | | | | | |
| IGROV1 | 83 | 100 | > 100 | > 4.00 | -36 | 12.0 | 50.0 | -4.92 |
| OVCAR-3 | -39 | 17.8 | > 100 | -4.75 | | | | |
| OVCAR-4 | 42 | 61.5 | > 100 | -4.21 | | | | |
| OVCAR-5 | 53 | 100 | > 100 | > 4.00 | -89 | 10.7 | 32.5 | -4.97 |
| OVCAR-8 | 68 | 100 | > 100 | > 4.00 | -62 | 11.3 | 42.6 | -4.95 |
| SK-OV-3 | 86 | 100 | > 100 | > 4.00 | 4 | 16.1 | 50.0 | -4.79 |
| Renal Cancer | | | | | | | | |
| 786-0 | 48 | 89.5 | > 100 | -4.05 | -87 | 9.91 | 32.3 | -5.00 |
| A498 | | | | | -75 | 13.4 | 38.4 | -4.87 |
| ACHN | 97 | 100 | > 100 | > 4.00 | -98 | 8.83 | 28.5 | -4.05 |
| CAKI-1 | 37 | 55.2 | > 100 | >-4.26 | -85 | 7.58 | 30.6 | -5.12 |
| RXF 393 | 35 | 66.8 | > 100 | -4.18 | -63 | 9.03 | 40.9 | -5.04 |
| SN12C | 63 | 100 | > 100 | >4.00 | -33 | 15.4 | 50.0 | -4.81 |
| TK-10 | | 100 | | | -100 | 8.80 | 28.0 | -5.06 |
| UO-31 | 57 | | > 100 | >4.00 | | | | |
| Prostata cancer | | | | | | | | |
| PC-3 | 41 | 38.0 | > 100 | -4.42 | -87 | 8.96 | 31.5 | -5.05 |
| DU-145 | -53 | 19.9 | 95.1 | -4.70 | -27 | 14.0 | 50.0 | -4.85 |
| Breast cancer | | | | | | | | |
| MCF7 | 16 | 25.7 | > 100 | -4.59 | -100 | 7.53 | 26.6 | -5.12 |
| NCI/ADR-RES | 68 | 100 | > 100 | > 4.00 | -100 | 9.06 | 28.3 | -5.04 |
| MDA-MB 231 | 44 | 65.9 | > 100 | -4.18 | -77 | 8.60 | 34.6 | -5.07 |
| HS 578T | -10 | 20.7 | > 100 | -4.68 | -33 | 16.2 | 50.0 | -4.79 |
| MDA-MB-435 | 21 | 24.0 | > 100 | -4.62 | -70 | 9.25 | 37.7 | -5.03 |
| BT-549 | 51 | 100 | > 100 | > 4.00 | -42 | 13.9 | 50.0 | -4.86 |
| T-47D | | | > 100 | | -61 | 4.17 | 39.4 | -5.38 |

**Notes**: G% = percentage growth at –4.8 M log concentration; lg($GI_{50}$), μM; $LC_{50}$, μM; ND = not determined.

*Acknowledgements*

The author is grateful to Dr. Alfredo Usubillaga for kindly providing grandiflorenic acid(Instituto de Investigacion Quimica, Facultad de Farmacia Universidad de los Andes, apartado 143, Merida, Venezuela) and M. Sc. M. Gomez, and M. Sc. S. Pekerar for measurements of the MS–GC and NMR spectra (Laboratorio Nacional de Analisis Quimico No Lab. 1998003690). The author also wish to thank Dr. V. L. Narayanan (Drug Synthesis and Chemistry Branch, National Cancer Institute, Bethesda, MD, USA) for performing the cytostatic and cytotoxic screening.

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## ИСКУССТВЕННЫЕ ДИТЕРПЕНОВЫЕ ПРОИЗВОДНЫЕ (-)-КАУРА-9(11),16-ДИЕНОАТА-19: СИНТЕЗ И ПРОТИВООПУХОЛЕВАЯ АКТИВНОСТЬ В ОТНОШЕНИИ КЛЕТОК РАКА ЧЕЛОВЕКА

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Искусственные дитерпены (**1** и **2**) получены с помощью реакций эпоксидирования и перегруппировки из (-)-каура-9(11),16-диеноата-19, выделенного из эспелетии (Frailejon), произрастающей в Венесуэльских Андах. Испытания на противоопухолевую активность в отношении ряда стандартных раковых клеток человека *in vitro* показали, что соединение **1** обеспечивает подавление роста клеток рака мозга (CNS SF-539, $GI_{50}$ = 51.6 nM, $LC_{50}$ = 100 mM, $lg(GI_{50})$ = –7.29), а соединение **2** эффективно в отношении рака груди (T47D, $GI_{50}$ = 4.17 mM, $LC_{50}$ = 39 mM, $lg(GI_{50})$ = –5.38).