Journal of the Chilean Chemical Society
versión On-line ISSN 0717-9707

J. Chil. Chem. Soc. v.51 n.4 Concepción dic. 2006

http://dx.doi.org/10.4067/S0717-97072006000400003
J. Chil. Chi. Soc., 51, N°.4 (2006), p.1010-1014

SYNTHESIS AND CONFORMATIONAL ANALYSIS OF LEPTOCARPIN DERIVATIVES. INFLUENCE OF MODIFICATION OF THE OXIRANE RINGON LEPTOCARPIN'S CYTOTOXIC ACTIVITY.

ROLANDO MARTÍNEZ[1,*], VICTOR KESTERNICH[2], HÉCTOR CARRACSO[3], CAROLINA ÁLVAREZ-CONTRERAS[1], CAROLINA MONTENEGRO[1], RICARDO UGARTE[1], ELENA GUTIÉRREZ[1], JOSÉ MORENO[1], CARLOS GARCÍA[1], ENRIQUE WERNER[4] and JUAN CÁRCAMOS[4].

[1] *Instituto de Química,Universidad Austral de Chile. Valdivia, Chile.*
[2]*Departamento de Química, Universidad Católica del Norte. Antofagasta, Chile.*
[3]*Departamento Química Universidad Andrés Bello, Viña del Mar. Chile.*
[4]*Instituto de Bioquímica, Universidad Austral de Chile. Valdivia. Chile.*

ABSTRACT

The reaction in acidconditions of Leptocarpin **1**, a compound with antitumor activity, formed two new isomeric products, 8β-angeloyl-1β,3β-dihydroxy-4,10-dimethyl,-$\Delta^{11(13)}$ methylen-4*Z*,9*Z*-dieneheliangol-6,12-olide **2** and 8β-angeloyl-1β,3β-dihydroxy-4-methyl-$\Delta^{11(13)}$,$\Delta^{11(14)}$-dimethylen-4Z-eneheliangol-6,12-olide **3**, whose structures reported in this study were established by spectroscopy (^{1}H-NMR, ^{13}C-NMR, MS and IR) and confirmedthrough ROESY experiments and theoretical studies by molecular mechanics. The *in vitro* cytotoxicity of these isomeric compounds was less active than leptocarpin, showing the importance of the oxirane ring in the biological activity. Cytotoxic activity was measured in six cancer cell lines.

Keywords: Conformational analysis; Heliangolide; Sesquiterpene lactones; Cytotoxicity.

INTRODUCTION

"Palo negro" is a native shrub of southern Chile, originally called Cüdu-mamëll (in Mapudungun) by the Mapuche people of Chile. Its scientific name is *Leptocarpha rivularis*[1] and belongs to the Compositae family, to the Heliantheae tribe, presently classified as Asteraceae[2]. The secondary metabolites that characterize this family are acetylenics, sesquiterpene and sesquiterpene lactones. They also contain essentials oils, flavonoids, and triterpenes but not tannins, iridoids nor amino acids nonprotein[3]. The extracts of plants containing antineoplasic activity have been profusely studied in the last decades[4-10]. A high percentage of the evaluated sesquiterpenes are potential inhibitors of cellular growth in numerous tumor models[11-14] and it has been suggested that the high cytotoxicity of some lactones may be due to their capacity to inhibit the synthesis and/or the transcription of DNA[15]. On the other hand, the protein synthesis is also affected[16-17] and it has been dionstrated that the activity of sesquiterpene lactones-α,β-unsaturated is associated with the selective alkylation through a Michael-type reaction of biological nucleophiles such as L-cysteine or enzymes that control the cellular division. It is likely that these lactones inhibit the selective incorporation of amino acids[18-22]. The study of the effect of leptocarpin (Figure 1), a heliangolide type lactone isolated from *Leptocarpha rivularis*[23], on the macro-molecule synthesis of DNA, RNA and proteins in HeLa cell cultures[24], using Alonso's method25 showed that leptocarpin and its corresponding acetylated derivative inhibit approximately 50% of the incorporation of 35S-Methionine without affecting the incorporation of (methyl-^{3}H)-Thymidine and (5,6-^{3}H)-Uridine at a concentration of 3.0 x 10^{-5} M. However, at concentrations lower than 6.5 x 10^{-6} M, it shows cytostatic effects. The reduction of exocyclic double bond in $\Delta^{11,13}$Causes inactivation of the biological activity of leptocarpin.[26]

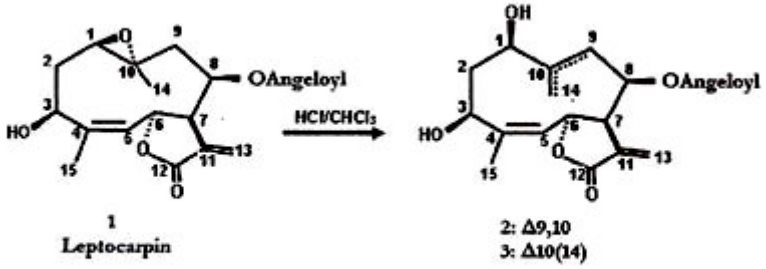

Figure 1. Acid treatment of leptocarpin with a saturated Chloroformic solution of HCl.

In this paper we report the hiisynthesis of two sesquiterpene lactones leptocarpin derivatives that were designed to prove the importance of the oxirane ring in the biological activity of leptocarpin on cell uptake and cytotoxicity. These studies have provided fundamental data to asses whether leptocarpin could be a promising anticancer drug.

EXPERIMENTAL

Experimental procedures.

The ^{1}H-NMR spectra were taken in a spectrometer Brüker Avance DRX-400 at

400 MHz in $CDCl_3$. and ^{13}C NMR (100 MHz).The IR spectra were obtained in a Perkin Elmer 200 spectrometer. The mass spectra (low resolution) were measured in a Vg-Micromass ZAB-2F spectrometer.

General Extraction Details.

Leptocarpin (**1)**, is isolated and purified through usual laboratory techniques, extraction by ethyl acetate or ethanol and the obtained extract is purified using technical chromatographic methods27 (silica-gel columns and solvents mixes of increasing polarity).

Hiisynthesis of compounds 2 and 3.

The transformation of Leptocarpin was obtained through the acid treatment of the leptocarpin with saturated chloroformic solution with HCl, to mimick what would happen when the drug encounters the gastric acid pH (Figure 1).Five hundred milligrams of leptocarpin were treated with a saturated solution of HCl in $CHCl_3$, shaking during 2 hours in an ice bath at 0ºC. It was concentrated in a rotary evaporator, washed with a saturated solution of NaHCO3, and then with distilled water. The latter was extracted with chloroform and dried with anhydrous Na_2SO_4. The products were separated and purified by preparative chromatography in silica-gel, eluted in a mixture of hexane/ethyl acetate (8:2), yielding 205 mg of recovered leptocarpin and 72 mg of a mixture of **2** and **3.** Attipts of purification by HPLC (Grad. Acetonitrile/H_2O) failed. Analytical samples were obtained by several HPLC runs and preparative chromatography on Si-gel. The analysis of mixtures28 by H-NMR revealed 6.9% of **3.**

***8β-angeloyl-1β,3β-dihydroxy-4,10-dimethyl,-$\Delta^{11(13)}$ methylen-4Z,9Z-dieneheliangol-6,12-olide*(2).**

IR $\nu^{Nujol}cm^{-1}$: 3450-3400 GRATIS 3450-3400 (broad), 1772, 1736, 1659. **MS** m/z (rel.int.): 362 M+ (6), 99 (10), 83 (98), 71 (12), 55 (100)**.^{1}H-NMR**, d: 1.80 (H-15,d, J=1.5Hz); 1.83 (H-14,d, J= 1.5Hz); 1.90 (H-5', d , J= 1.5Hz); 1.98 (H-4´, d, J=7.0Hz);2.19 (H-2, m); 3.12 (H-7, m); 4.53 (H-3, m); 4.80 (H-1, dd, J= 1.3, 3.5Hz); 5.30 (H-9, d, J= 9.0Hz); 5.42 (H-5, dd, J= 1.5, 3.5Hz); 5.80 (H-6, ddJ= 3.5, 10Hz); 5.95 (H-8, m, J= 3.97 and 9.24);6.10 (H-13a, d, J= 1.^{1}Hz); 6.12 (H-3', dd, J= 1.5, 7.0Hz); 6.25 (H-13b, d, J= 1.^{1}Hz).**^{13}C-NMR** : δ:C-4', 16.89; C-14 20.71; C-15, 23.29; C-5', 26.86; C-2, 41.08; C-7, 50.25; C-8, 75.02; C-3, 76.68; C-1, 77.00; C-6, 77.32; C-9, 121.87; C-13, 122.81; C-3', 122.85; C-5,127.13; C-11, 135.96; C-2', 139.82; C-4, 144.29; C-10, 146.29; C-1'167.16; C-12, 169.80

8β-angeloyl-1β,3β-dihydroxy-4-methyl-$\Delta^{11(13)}$,$\Delta^{11(14)}$-dimethylen-4Z-eneheliangol-6,12-olide (**3**)

IR $\nu^{Nujol}cm^{-1}$: 3445-3390 GRATIS 3445-3390 (broad), 1768, 1757, 1659. **MS** m/z (rel.int.): 362 M+ (4), 182 (4) 99 (14), 83 (89), 71 (12), 55 (100).**^{1}H-NMR**, δ: 1.80 (H-15, d, J= 1.5Hz); 1.81 (H-5, d, J=1.5Hz); 1.96 (H-4', d,

J=7.0Hz); 2.08 (H-2, m); 2.55 (H-9, m);2.90 (H-9´, m); 3.20 (H-7, m); 4.10 (H-3, dd,J= 8.0, 0.^{3}Hz); 4.50 (H-1, dd, J= 1.3, 3.5Hz); 5.12 (H-14, d, J= 1.^{3}Hz); 5.16 (H-8, m); 5.32 (H-5´, d, J= 1.5H; 5.50 (H-14´, d, J= 1.^{3}Hz); 5.75 (H-13a, d, J= 1.^{3}Hz); 6.10 (H-3', m); 6.30 (H-6, m, J= 0.8, 3.0Hz); 6.33 (H-13b, d, J= 1.^{3}Hz). **^{13}C-NMR** : δ: C-5', 18.92; C-15, 20.29; C-4', 22.89; C-9, 37.68; C-2, 41.44; C-7, 47.04; C-3, 76.68; C-1, 76.99; C-6, 77.20;C-8, 77.31; C-14, 117.90; C-13, 124.31; C-3', 126.31; C-5, 127.10; C-11, 137.41; C-2', 140.17; C-4, 141.71; C-10, 143.78; C-1', 166.68; C-12, 169.73.

General Cell Culture Details.

The studies on biological activity took place on cell cultures[29], such as NSO-2, CHO, P-815, HeLa, J 774.2, 4 FIO-67, SK-Hep-1. The cell lines were rioved from liquid nitrogen, thawed and cultivated in RPMI 1640 culture medium (Chi.Sigma, USA) at 37ºC in a humid atmosphere with 5% of CO_2. The culture was placed in 40 mL cell culture flasks. Once the cultures dionstrated a suitable growth, the cells were obtained by centrifugation (in the case of the adherent cultures, these were treated previously with Trypsin to detach the cells from the flasks). Following the centrifugation, the supernatant was discarded, the cells were suspended in 3 mL of RPMI 1640 medium with 10% of fetal bovine serum and antibiotics, counted, assessed for viability and incubated at a concentration of $2.5x10^5$ live cells/mL[30-34].

Cell Uptake Studies.

The experiments of ^{3}H-Thymidine, ^{3}H-Uridine and ^{3}H-Leucine uptake to DNA, RNA and proteins in the different cell lines with and without the studied compounds were carried out using 200 mL cell suspensions, plated out and incubated for 24 h at 37°C, in 5 % CO_2. Then, sesquiterpene lactones were added and incubated for further 72 h. Before culture termination, 0.2 μCi of ^{3}H-Thymidine, ^{3}H-Uridine and ^{3}H-Leucine were added to each well respectively. After incubation, the cells were harvested onto a crystal filter paper using Multimash Harvested Dynatech Systi and processed for liquid scintillation counting (scintillation counter Packard instrument)[35]. The liquid scintillation contained 0.5% of 2.5-difeniloxazol (PPO, Sigma Chiical Co) and 0.025% of 2,2'-fenilen bis-(5-feniloxazol (POPOP, Sigma Chiical Co), in suitable solvent. The results were obtained in triplicate, expressed in counts per minute (cpm) and plotted using the program Graph Pad Prism 4.0.

Cytotoxicity Experiments.

Leptocarpin and its synthetic products were assayed for cytotoxic activity against SK-Hep-1 and HeLa cells.Cells (2.5×10^5 cells/mL) were cultivated previously on plastics plates of 35 mm (Falcon) in the culture medium described. Cells were allowed to proliferate for 24 h and then treated with leptocarpin and its synthetic products **2+3**. The cultures were treated with trypsin at different times post treatment, suspended in 1mL of phosphate buffered saline (PBS) and the number of cells alive and dead was determined in aliquots of the cellular suspension. A mixture of 100 μL of cells suspension and 900 μL of trypan blue 0.1 % in PBS were incubated for five minutes. The total number of cells and the number of dead cells and stained with the vital dye were determined using a Neubauer

chamber (Assisant, Thoma model, Germany) under a microscope (Baush and Lomb, model 313364, Rochester, NY/USA).

Interestingly, for a same concentration of leptocarpin, a suspension of 2.25 x 10^5 cells (100 µL) showed 66% cellular death, while with a suspension of 1.25 x 10^5 cells (50 µL) an 83% of inhibition was achieved. The treatment with **2**+**3** compounds, showed a cellular death of 34% and 8% respectively under the same conditions. (Table 5, Figure 8) respectively).

RESULTS AND DISCUSSION

Design and Synthesis of Derivatives.

The Figure 1 shows the target products of the synthesis used in this study.

The opening of the oxirane ring was designed to prove the importance of this structural characteristic in the biological activity and hence to dionstrate if the human gastric acid pH is able to alter the leptocarpin structure. In order to determine the stereochiical course of the reaction, leptocarpin **1**, was added to chloroform saturated with HCl at 0ºC for two hours.Analysis of the NMR spectrum of the crude product showed the presence of a significant number of impurities in addition to signals arising from **2** and **3**. Purification of the crude product by silica gel column chromatography allowed to recover non reacted material and to isolate a mixture of **2** and **3**. Purification of this mixture to obtain analytical samples of **2** and **3** by HPLC was difficult due to the close elution of these products and therefore, the isolation of pure analytical samples from the mixture required multiple cycles of HPLC and preparative TLC.

The structures were determined by H-RMN, ^{13}C-RMN and mass spectrometry, like the isomeric heliangolides ones, 8β-angeloyl-1β,3β-dihydroxy-4,10-dimethyl,-$\Delta^{11(13)}$ methylen-4*Z*,9*Z*-dieneheliangol-6,12-olide (**2)** and 8β-angeloyl-1β,3β-dihydroxy-4-methyl-$\Delta^{11(13)}$,$\Delta^{11(14)}$-dimethylen-4Z-eneheliangol-6,12-olide (**3)**. The conformational analysis took place in $CDCl_3$ solution through ROESY experiments, being **2** of CC (Chair-Chair) type and **3** of TT type (Twist-Twist) which agrees with the theoretical studies of molecular mechanics using Hyperchi MM2 program. (Figure 2).

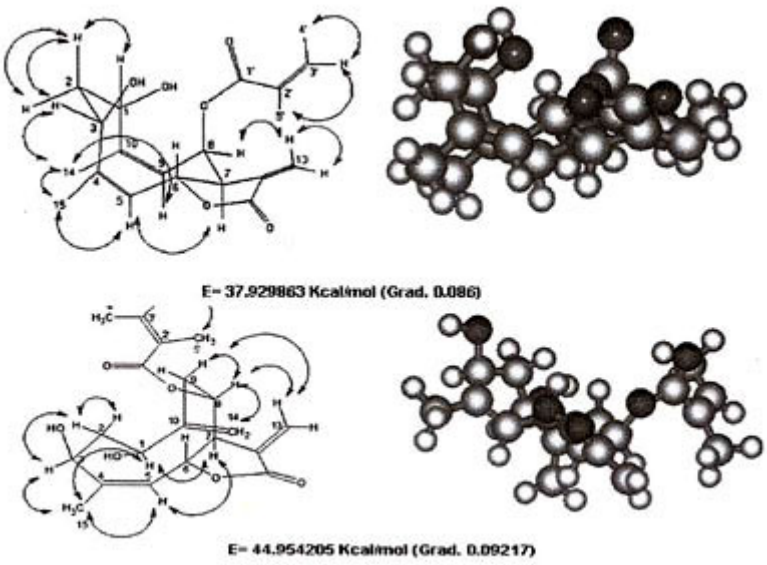

Figure 2. ROESY Experiments of 2 and 3.

From a theoretical point of view, leptocarpin under acid conditions can produce two types of cyclation products. First, an eudesmanolide type lactone by attack of the double bond Δ4,5 on C10 (6-Exo-Tet) producing the opening of the oxirane ring (Figure 3). The second product could be a guaianolide type lactone produced by the binding of carbons C5-C1 (5-Exo-Tet) as a consequence of the double bond Δ4,5 attack on C1[36]. Nevertheless, this did not happen and a possible interpretation could be that interatomic distances C5-C10 (3.94 Aº) and C5-C1 (3.26 Aº) could be too far to allow the cyclation in each case.

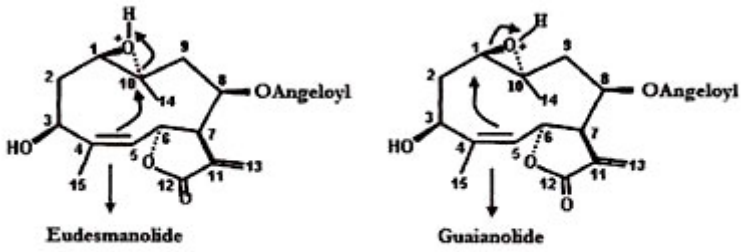

Figure 3. Structural types of sesquiterpene lactones expected from biogenetic theory by the acid treatment of Leptocarpin.

Contrary to the previous hypothesis, the acid treatment of leptocarpin (1) through the opening of the oxirane ring led to the formation of two heliangolide isomers, 8β-angeloyl-1β,3β-dihydroxy-4,10- dimethyl,-$\Delta^{11(13)}$ methylen-4*Z*,9*Z*-dieneheliangol-6,12-olide (**2)** and 8β-angeloyl-1β,3β-dihydroxy-4-methyl-$\Delta^{11(13)}$, $\Delta^{11(14)}$ -dimethylen-4Z-eneheliangol-6,12-olide (**3)**whose structures were confirmed by spectroscopy.

The conformational analysis of **2** and **3** was developed through ROESY experiments and molecular mechanics using the Hyperchi program. Thus, in ROESY experiments (Figure 2), they iphasize the interactions between protons H1-H2b, H2β-H3, H5-H7 and H15, H8-H13, H9-H14, and protons of methyl, H14-H15, and between protons of the angeloyl group H3´-H4´ and H3´-H15´. These interactions would be possible if **2** had a CC conformation as shown in Figure 2.

On the other hand, the study of Molecular Mechanics through Hyperchi MM2 program (Figure 2) is coincident with the ROESY experiment. Of all the possible conformations, the CC shows the least conformational energy value that corresponds to 37.929863 Kcal/mol (Grad. 0.086).

With respect to the conformational study of **3** it is clearly observed in ROESY experiment the interactions between protons H1-H7, H1-H15, H2a-H3, H3-H15, H15-H5, H5-H7, H8-H9a, H8-H13b, H8-H14 and between H9a-H13b. Finally, the proton interactions of the angeloyl group H3´-H4´and H3´-H15´are also observed. All these interactions agree with a conformation TT as shown in Figure 2.

The theoretical study by Molecular Mechanics of **3**, once again is consistent with the ROESY experiment since of all the possible conformations the most stable is TT conformation, with a conformational energy of 44.954205 Kcal/mol (Grad. 0.09217).

Solubility of compounds.

Leptocarpin (**1)** and its derivative compounds (**2)**, (**3)** are soluble in dimethyl sulfoxide (DMSO) but insoluble in water. Aqueous DMSO is often used for the dilution of poorly water soluble compounds to in vitro cytotoxicity assays37, up to a final concentration less than 5% of DMSO. Compounds **1**, **2** and **3** were soluble in 0.1% aqueous DMSO, which allowed us to perform *in vitro* biological studies on these compounds.The biological activity studies were carried out with the product mixture **2** and **3**.

Cell Uptake Studies.

The effect of leptocarpin and its acidic derivatives mixture on DNA, RNA and protein synthesis in five cancer cell lines,P-815, NSO-2, J 774.2, 4FIO-67, CHO, was examined by ^{3}H-thymidine (Table 1, Figure 4), ^{3}H-uridine (Table 2, Figure 5) and ^{3}H-leucine uptake assays (Table 3, Figure 6), respectively.

Table 1. (^{3}H)-Thymidine uptake by different cell lines.

Cell lines	RPMI(cpm)	Lep (cpm)	**2+3** Mixture (cpm)
P-815	80437 ± 3061	75414 ± 2734	75414 ± 2734
NSO-2	23234 ± 1778	23717 ± 2736	22250 ± 1713
J 774.2	41134 ± 1698	29843 ± 2876	22250 ±

4FIO -67	20733 ± 2481	15454 ± 2118	1713 17416 ± 2478
CHO	29775 ± 664.7	29970 ± 1045	30303 ± 1531

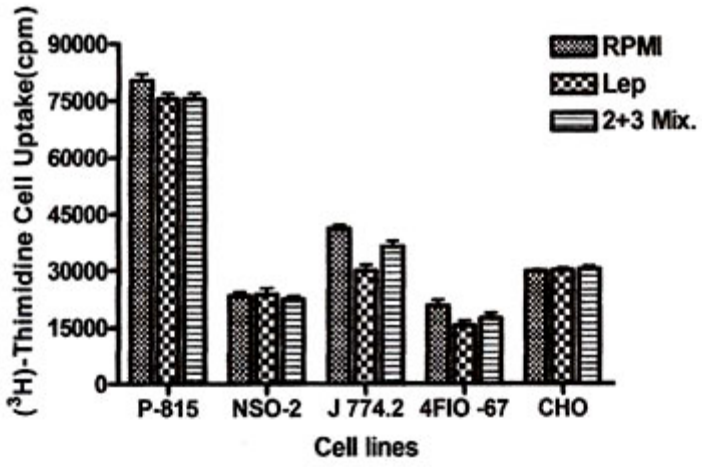

Figure 4. (^{3}H)-Thymidine uptake by five cell lines in the presence of Leptocarpin or synthetic products 2+3 at $3.0x10^{-5}$ M.

Table 2.(^{3}H)-Uridine uptake by different cell lines.

Cell lines	RPMI (cpm)	Lep (cpm)	**2+3** Mixture (cpm)
P-815	65416 ± 1416	65007 ± 2298	61765 ± 1835
NSO-2	36055 ± 2683	35852 ± 1798	33996 ± 1554
J 774.2	28115 ± 2543	28029 ± 1229	27714 ± 1932
4FIO -67	27328 ± 2565	27501 ± 8808	27260 ± 2607
CHO	5369 ± 691.4	5729 ± 592.6	5632 ± 531.1

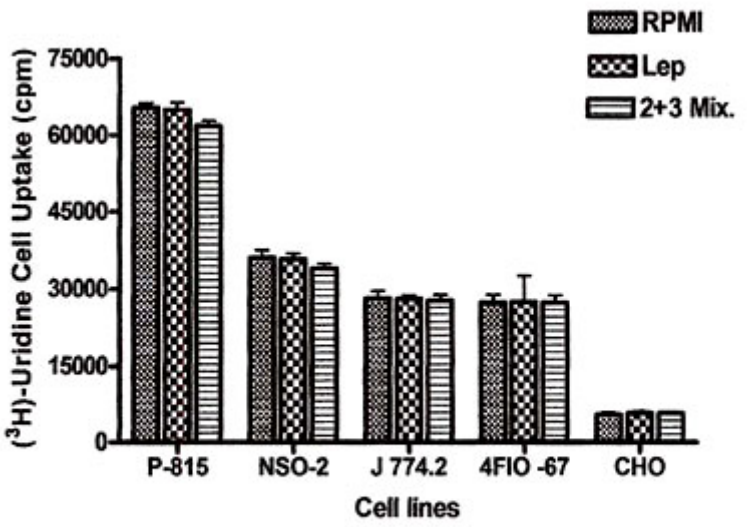

Figure 5. (^{3}H)-Uridine uptake by five cell lines in the presence of Leptocarpin or synthetic products 2+3 at $3.0x10^{-5}$ M.

Table 3. (^{3}H)-Leucine uptake by different cells lines.

Cell lines	RPMI (cpm)	Lep (cpm)	**2+3** Mixture (cpm)
P-815	54343 ± 3922	26237 ± 1836	39477 ± 1300
NSO-2	29952 ± 3331	8650 ± 1643	23917 ± 3184
J 774.2	9964 ± 1651	4167 ± 294.3	7153 ± 860.6
4FIO -67	22022 ± 1933	11650 ± 1587	18343 ± 1027
CHO	6110 ± 871.2	2811 ± 215.9	3999 ± 174.9

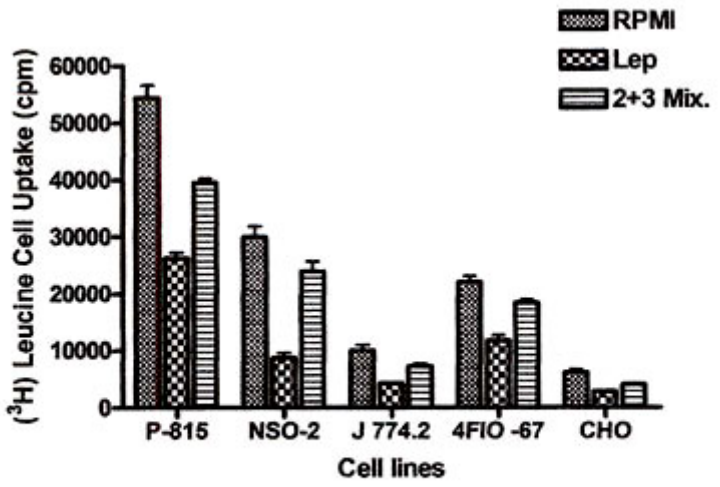

Figure 6. (^{3}H)-Leucine uptake by five cell lines in the presence of Leptocarpin or synthetic products 2+3 at $3.0x10^{-5}$ M.

The results show that in all cancer cell lines investigated, the ^{3}H-thymidine and ^{3}H-uridine uptake were not affected by leptocarpin or its derivatives. Leptocarpin, however and clearly at a lesser extent its derivatives **2** and **3,** inhibited the ^{3}H-leucine incorporation in every cancer cell lines examined. On the other hand, an increase in the concentration of leptocarpin or its derivatives had no effect on the thymidine and uridine uptakes.

Cytotoxicity Studies.

Leptocarpin (**1)** was assayed for cytotoxic activity against human adenocarcinoma cell lines (SK-Hep-1).One hundred µl of cells suspension were mixed with 900 µL of 0.1% trypan blue in PBS and incubated with leptocarpin for five minutes. The total cell count and the number of cells stained with trypan blue dye were determined using a Neubauer Chamber.

The effect of the Leptocarpin dose,in human liver adenocarcinoma cells SK-Hep-1, at different times of incubation was studied over a 48 hours period. Leptocarpin at 5.0x10^{-5} M induced a riarkable cytotoxic effect (12% of cell viability at 24 h); at 5.0x^{-4} M it was cytotoxic showing cytolisis and cellular death a few hours following treatment (2.73% of cell viability at 24 h). At a concentration of 5.0x10^{-6} M it showed a cytostatic effect with IC50 value of 5 µM at 48 hours of treatment (Table 4 and Figure 7). The dose-response curves for the treatment of SK-Hep-1 cells with leptocarpin (**1)** for 24, 36 and 48 h are shown in Figure 7. It is clear that longer exposure times lead to increased cell death with very little reduction in viability observed in the cells treated just for 24 h with 0.1% DMSO and leptocarpin 5x10^{-6} M. The cells treated with leptocarpin 5x10^{-5} M and 5x^{-4} M for 24 to 48 h showed similar levels of viability with very little cellular survival (12% and 2.73% of viability respectively at 24 h treatment). The cells treated for 48 h displayed 50% viability at a concentration of 5x10^{-6} M of leptocarpin (**1)**.

Table 4. Cytotoxicity of Leptocarpin on human adenocarcinome SK-Hep-1 cells (1.8x10^{5} cells).

Treatment hours	0.1% Aqueous DMSO/Viability	Lep 5.0x10^{-6} M/Viability	Lep 5.0x10^{-5} M/Viability	Lep 5.0x^{-4} M/Viability
24	89.9±1.88	86.2±2.19	312.0±1.06	2.73±0.21
36	91.4±2.61	56.1±1.62	7.56±0.58	1.50±0.26
48	90.1±2.84	46.9±1.81	2.56±0.50	0.93±0.15

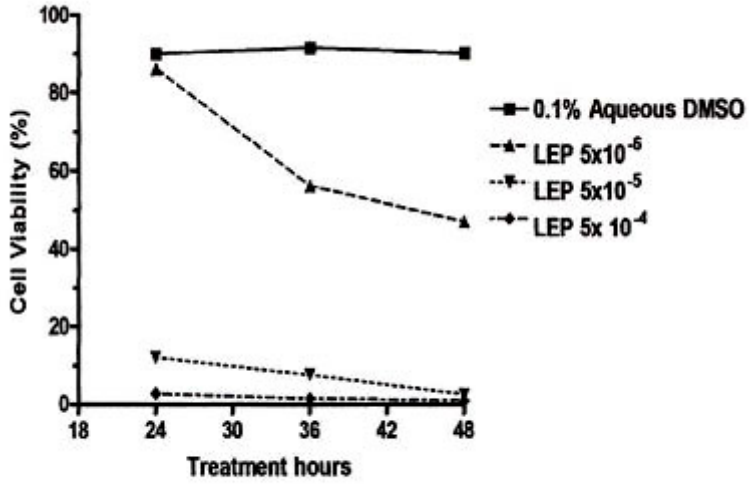

Figure 7. Kinetic of the effect of different concentrations of leptocarpin 1 on SK-Hep-1 cells viability.

The ability of leptocarpin (**1)** to inhibit the growth of human tumor cell lines was also assessed. HeLa cell suspensions of $2.25x10^5$ and $1.25x10^5$ cells exposed to a leptocarpin concentration of $3.0x10^{-5}$ M showed 34% and 16% cell viability, respectively, dionstrating the effectiveness of leptocarpin in the primary stages of a tumor. Using compounds **2** and **3** the results were 66% and 92% cell viability for the same cell suspensions. (Table 5, Figure 8).

Table 5. Cytotoxic effect of Leptocarpin $3.0x10^{-5}$ M on two different HeLa cell suspensions.

HeLa Cells	RPMI/Viability	Lep/Viability	2+3 Mixture/Viability
$2.25x10^5$	96.17±2.12	33.83±3.24	66.03±2.25
$1.25x10^5$	96.03±1.06	15.87±2.80	92.2±1.83

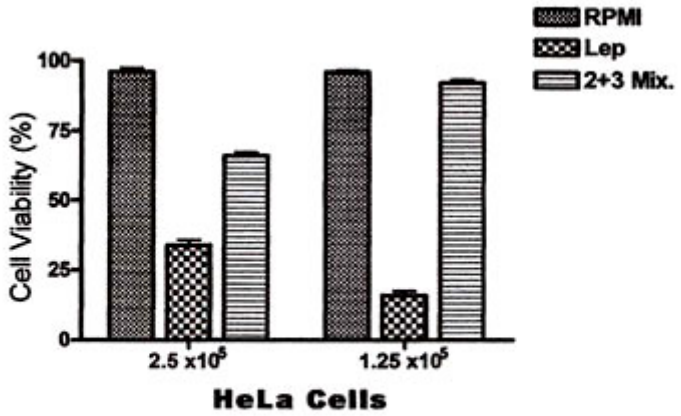

Figure 8. Effect of the treatment size dependence for two samples of HeLa cell suspensions with Leptocarpin and 2+3 compounds $3.0x10^{-5}$ M.

CONCLUSIONS

The transformation of leptocarpin is a relative straightforward process without any major technical problis for its synthesis, except for the separation and purification of the products.

DNA and RNA synthesis are not affected by Leptocarpin and the synthetic products therefore, its biological activity is not at this level. Leptocarpin produces a marked effect on the protein biosynthesis at a different extent in cancerous cellular lines and on the other hand, the synthetic products **2+3** have very low effect in comparison to leptocarpin thus dionstrating the importance of the oxirane ring for the biological activity.

The effectiveness of the results could determine that the leptocarpin is an antineoplasic agent of natural origin which can be used in different types of neoplasia.

The behaviour of Leptocarpin in an acid environment allows us to think that this molecule is sensitive to acid conditions and therefore as a possible future drug, it should not be administered orally, unless it is protected from gastric acid; this is why it is transformed into heliangolides structures with decreased biological activity.

Acknowledgments

This research was supported by the DID Universidad Austral de Chile, grant S-2003-44 and S-200522.

REFERENCES

1. Marticorena, C; Quezada, M. Catalogo de la flora vascular de Chile. Gayana

Botánica 42; Universidad de Concepción, 1985;pp 1-157. [Links]

2. Muñoz, C. Sinopsis de la flora chilena. 2th ed.;Ed. Universidad de Chile. 1966; pp 1- 500. [Links]

3. Muñoz, O. Química de la flora de Chile.Ed. Univ. Chile. 1992; pp 1-351. [Links]

4. Lee, K.H.; Huang, E.S.; Piantadosi, C.; Pagano, J.S.; Geissman, T.A. *Cancer Res*. **31**, 1649-1654 (1971). [Links]

5. Lee, K.H.; Ibuka, T.; Huang, H.C.; *J Pharm Sci*. **64**, 1077-1078 (1975). [Links]

6. Woerdenbag, H.J. ; Meijer, C. ; Mulder, N.H.; de Vries, E.G.; Hendriks, H.; Malingre, T.M. *Planta Med*. **2**, 112-114 (1986). [Links]

7. Woerdenbag, H.J.; Listra, W.; Hendriks, H.; Malingre, T.M.; Konings, A.W. *Planta Med.***53**, 318-322 (1987). [Links]

8. Woerdenbag, H.J.; Hendriks, H.; Malingre, T.M.; Van Stralen, R.; Van den Berg K.J.; Konings, A.W.T. *Phytother.Res*. **2,3**, 109-114 (1988). [Links]

9. Woerdenbag, H. J., Listra W.; Malingre T.M.; Konings A.W. *Br. J. Cancer.***59**, 68-75 (1989). [Links]

10. Woerdenbag, H. J.; Van der Linde, J.C.C.; Kampinga, H.H.; Malingre, T.M.; Konings, A.W.T. *Biochi. Pharmacol.***38,** 2279-2283 (1989). [Links]

11. Woynarowsky, J.M.; Konopa, J. *Mol. Pharmac.*, **197**, 97-102 (1980). [Links]

12. Hoffmann, H.M.R.; Rabe Jürgen. *Angew.chi..Int. Ed. Engl*., **24**, 94-110 (1985). [Links]

13. Giordano, O.S.; Pestchanker, M.J.; Guerreiro, E.; Saad, J.R.; Enriz, R.D.; Rodriguez, A.M.; Jauregui, E.A.; Guzmán, J.; María, A.O.M.; Wendel, G.H. *J. Med. Chi.*, **35**, 2452-2458 (1992). [Links]

14. Dirsch, V.M.; Stuppner, H.; Vollmar, A.M *Planta Med*., **67**, 557-559 (2001). [Links]

15. Müller, S.; Murillo, R., Castro, V.; Brecht, V.; Merfort, I.*J. Nat. Prod*., **67**, 622-630 (2004). [Links]

16. Lee, K.H.; Hall I.H.; Mar, E.C; Starnes, C.O.; ElGebaly, S.A.; Wadell, T.G.; Hadgraft, R.I.; Ruffner, C.G.; Weidner I. *Sci*., **196**, 533-536 (1977). [Links]

17. Voynova, E.; Dimitrova, S.; Naydenova, E.; Karadjov, P. *Acta Physiol.*

Pharmacol Bulg., **17**, 50-52 (1991). [Links]

18. Kupchan, S.M.; Eakin, M.A.; Thomas; A.M.*J. Med. Chi*., **14**, 1147-1152 (1971). [Links]

19. Cassady, J.M.; Byrn, S.R.;Stamos, I.K.; Evans, S.M.; McKenzie A. *J. Med. Chi*., **21**, 815 - 819 (1978). [Links]

20. Liou, Y.; Hall, I.H.; Lee, K.; Williams, W.L.; Chamey, S.G. *Biochim. Biophys.*, **739**, 190-196 (1983). [Links]

21. Ginanneschi, M.; Chelli, M.;Papini, A.M.; Pinzani D.; Rapi. G. *Magnetic Res. Chi*.,**34**, 95-97 (1996). [Links]

22. Quintero A.; Pelcastre, A.; Solano, J. *J. Pharmaceut Sci.*, **2**, 108-112 (1999). [Links]

23. Martínez, R.; Ayamante, I.; Núñez, J.; Romo de Vivar, A. *Phytochi.*, **18**, 1527-1528 (1979). [Links]

24. Martínez, R.; Kesternich, V.; Gutiérrez, E.; Dölz, H.; Mansilla, H. *Planta Med*., **61**, 188-189 (1995). [Links]

25. Alonso, M.A.; Carrasco, L. *Eur J Biochi*., **109**, 535-540 (1980). [Links]

26. Martínez, R.; Kesternich, V.; Carrasco, H.; Bustos, C.; Fernandez, S. *Bol. Soc. Chil. Quim*., **43**, 7-12 (1998). [Links]

27. Martínez J. Rolando, Kesternich M Victor. Chilean Pat. Nº 40787.- Procedimiento para obtener el principio activo denominado "Leptocarpina" a partir de Leptocarpha rivularis, la cual presenta actividad citotóxica y antineoplásica. (2000). [Links]

28. Nathan, P.J.; Díaz, E. Introducción a la Resonancia Magnética Nuclear. Limusa. México, 1980, pp 127-147. [Links]

29. García, C. Síntesis de intermediarios de Leptocarpina y evaluación de la actividad biológica de leptocarpina y del producto de síntesis biomimética. Tesis de Grado Prof. de Biol., Quim. y Cs. Nat. Universidad Austral de Chile. Valdivia. 1997, pp 1-84. [Links]

30.Earle, W.R. *J. Natl Cancer Inst*., **4**, 165-212 (1943). [Links]

31. Eagle, H. *Proc. Soc. Exp. Biol. Med*., **89**, 362-364 (1955). [Links]

32. Geran, R. I.; Greenberg, N.H.; Macdonald M.M.; Schumacher A.H.;Abbott, B.J. *Cancer Chiother. Rep*., **3**, 51-61 (1972). [Links]

33. Geran, R.I.; Greenberg, N.H.; Macdonald, M.M.; Schumacher A.H.; Abbott, B. *Cancer Chiother. Reports* (Part 3), **3**, 1-103 (1972). [Links]

34. UKCCCR guidelines for the use of cancer cell lines in cancer research. *Br. J. Cancer*., **82**, 1495-1509 (2000). [Links]

35. Terrazas, L.I.; Bojalil, R.; Govezensky, T.; Sarralde, C. *J.Parasitol*., **84**, 74-78 (1998). [Links]

36.Baldwin, Jack E.; Thomas, Richard C.; Kruse, Lawrence I.; Silberman Lee.*J. Org. Chi*. , **42**, 3846-3852 (1977). [Links]

37. Waern, J.B.; Dillon,C.T; Harding, M.M. *J. Med. Chi*., **48**, 2093-2099 (2005). [Links]

*Corresponding author.Phone: 56 63 221905 GRATIS 56 63 221905 . Fax: 56 63 221597. E-mail: rmartin2@uach.cl

Paicaví 170, Depto. 19
P.O. Box 2613, Concepción, Chile

Phone 41-2227815 GRATIS 41-2227815 , Fax 41-2235819

e-Mail

schgjournal@entelchile.net