Gastroprotective Effect and Cytotoxicity of Natural and Semisynthetic Labdane Diterpenes from *Araucaria araucana* Resin

Guillermo Schmeda-Hirschmann[a,*], Luis Astudillo[a], Beatriz Sepúlveda[a], Jaime A. Rodríguez[b] , Cristina Theoduloz[b], Tania Yáñez[b], and José Antonio Palenzuela[c]

[a] Laboratorio de Química de Productos Naturales, Instituto de Química de Recursos Naturales, Universidad de Talca, Casilla 747, Talca, Chile. Fax: +56 71 20 04 48. E-mail: schmeda@utalca.cl

[b] Laboratorio de Cultivo Celular, Facultad de Ciencias de la Salud, Universidad de Talca, Casilla 747, Talca, Chile

[c] Instituto Universitario de Bio-Orgánica "Antonio González", Universidad de La Laguna, 38206 La Laguna, Tenerife, Spain

* Author for correspondence and reprint requests

Z. Naturforsch. **60 c**, 511–522 (2005); received November 24, 2004/January 28, 2005

The resin of the tree *Araucaria araucana* (Araucariaceae) is used by the Mapuche Amerindians in southern Chile and Argentina to treat ulcers and has been shown to display a gastroprotective effect in animal models. A study was undertaken to isolate, identify and assess the gastroprotective effect of the resin constituents and its semisynthetic derivatives as well as to evaluate the cytotoxicity of the products in cell cultures. Eleven diterpenes (ten labdane and a pimarane) were isolated from a resin sample collected in Chile. The labdane derivatives 15-acetoxylabd-8(17)-en-19-ol as well as 15,19-diacetoxylabd-8(17)-en are reported for the first time as natural products. Six diterpenes previously described from other plant sources are reported for the first time for the *A. araucana* resin. The structure of all compounds was elucidated by spectroscopic means. Some 24 diterpenes isolated/prepared in amounts over 10 mg were evaluated for gastroprotective effects in the ethanol/HCl-induced ulcer model in mice at 100 mg/kg. The highest gastroprotective activities were provided by 15-hydroxyimbricatolal, 15-acetoxyimbricatolal, 15-acetoxylabd-8(17)-en-19-oic acid methyl ester and 15-acetoxy-19-labdanoic acid, all of them being as active as the reference drug lansoprazole at 20 mg/kg. The cytotoxicity of 30 diterpenes as well as lansoprazole was assessed towards human lung fibroblasts (MRC-5) and 26 compounds were evaluated on the human gastric epithelial cell line AGS by means of the neutral red uptake assay. A concentration-dependent cell viability inhibition was found with IC_{50} values ranging from 27 up to > 1000 μM. The relationship between the cytotoxicity data and lipophilicity of the products is also discussed.

Key words: Araucaria araucana, Gastroprotective Activity, Diterpenes

Introduction

The large tree *Araucaria araucana* (Molina) K. Koch (syn.: *Araucaria imbricata* Pav.) is an endemic gymnosperm occurring in the southern Andes both in Argentina and Chile. The tree, known as "pehuen" was very important for the Mapuche culture, both for its edible seeds and the medicinal properties of the resin. The resin was used to heal wounds and ulcers.

Gastric and duodenal ulcers are digestive diseases that affect, with different degrees of intensity, the 8 to 10% of the population living in the industrialized countries. In 2002, the stomach cancer and peptic ulcer disease represented 1.5 and 0.5% of the total cause of deaths in the world, respectively (WHO, 2003). The pharmacological therapeutics of gastric ulcer have evolved from the use of antacids, anticholinergic drugs and H_2 receptor-blockers to the more recent antisecretory drugs that inhibit the gastric H^+-K^+ ATPase (Brunton, 1996). In the last decades, many efforts have been done in order to discover and/or develop new anti-ulcer drugs from natural sources. Some plants originate anti-ulcer drugs such as carbenoxolone from *Glycyrrhiza glabra*, solon from sophoradin and gefarnate from cabbage among others (Lewis and Hanson, 1991).

Several terpenes or their derivatives have been shown to possess gastroprotective activity in different models of induced gastric lesions in animals (Lewis and Hanson, 1991; Souza-Brito *et al.*, 1998; Schmeda-Hirschmann *et al.*, 2002). In a review, Singh *et al.* (1999) summarized the biological activ-

0939–5075/2005/0700–0511 $ 06.00

ities reported for labdane diterpenoids. They comprise anti-inflammatory, cytotoxic, antimicrobial, cardiotonic effect and enzyme inhibition activity. The cytotoxic effect of natural and semisynthetic labdanes from *Croton oblongifolius* has been reported by Sommit *et al.* (2003). Evaluation of a series of derivatives is required to relate the activity of a compound with its structure, as shown by Dimas *et al.* (1998) with the cytotoxicity of labdanes in human leukaemia lines *in vitro*.

Previous chemical studies on the terpenes of *A. araucana* reported the isolation of geraniolone, limonene, (-)-trachylobane, (-)-kaurene, (-)-atisirene, (+)-hibaene, (-)- isokaurene and (-)-isoatisirene from the essential oil of the plant. The labdane diterpene imbricatolic acid, its acetate, imbricatadiol, imbricatolal and its acetate were obtained from the resin (Hegnauer, 1986; Weissmann *et al.*, 1965). From a Chilean resin sample, Garbarino *et al.* (1987) isolated 15-acetoxyimbricatolal, 15-acetoxyimbricatolic acid, 15-hydroxyimbricatolal, 15-hydroxyimbricatolic acid and 15-formyloxyimbricatolal.

Taking into consideration that diterpenes have been reported to be active as gastroprotectors we now report the effect of natural and semisynthetic diterpenes from the resin of *A. araucana* on ethanol/HCl-induced gastric lesions in mice. Furthermore, the compounds were assessed for cytotoxic effect in AGS cells and fibroblasts.

Materials and Methods

Plant material

A resin sample was collected from a 15-year-old *A. araucana* tree grown in Santiago de Chile from seeds gathered nearby the Parque Nacional Conguillio, IX Región, Chile. A second resin collection from mature trees (3 kg) was carried out nearby the Parque Nacional Conguillio, IX Región, Chile, for the preparative isolation of the main diterpenes to be used for structural modifications by standard chemical reactions. A voucher herbarium specimen (N° 2803) is deposited at the Herbario de la Universidad de Talca.

Isolation of the resin diterpenes

The resin collected in Santiago de Chile (34 g) was dissolved in CH_2Cl_2 (DCM, 0.5 l) and acetone (0.5 l), filtered and taken to dryness affording the crude resin extract (23.5 g). The DCM/acetone soluble fraction was chromatographed on silica gel with a petroleum ether (PE) to EtOAc gradient. Some 20 fractions of 500 ml each were collected and pooled together according to the TLC pattern in 8 fraction groups. From the fractions 1, 2 (PE and PE/EtOAc 9:1 v/v), after preparative TLC (PE/EtOAc 7:3 v/v, silica gel) some 50 mg of compound **6** (Rf 0.81), 15 mg of compound **7** (Rf 0.60) and 10 mg of compound **11** (Rf 0.54) were obtained. Fraction 3 was repurified by CC on silica gel, affording 4.8 g of compound **8** (Rf 0.63), 1.0 g of compound **2** and 15 mg of compound **33**.

Fraction 4 yielded after repeated CC on silica gel and recrystallization some 2.5 g of compound **3** (Rf 0.30) and 20 mg of compound **14**. The pooled fractions 5 and 6 (PE/EtOAc 70:30 v/v to 30:70 v/v) were repurified by CC. Some 3.5 g of compound **3**, 2 g of compound **8** and 10 mg of compound **6** were obtained. Fraction 7 (300 mg) (PE/EtOAc 2:8 v/v) after repeated CC and preparative TLC on silica gel afforded 25 mg of compound **6**, 20 mg of compound **5** and 15 mg of compound **32**. Fraction 8, eluted with PE/EtOAc 1:9 v/v to EtOAc (4 g), yielded after CC 0.7 g of compound **2**, 2.5 g of compound **3** and 8 mg of compound **15**.

Compounds **2**, **3**, **5**–**8**, **11**, **14**, **15**, **32** and **33** were isolated from the resin collection by standard chromatographic methods, and their structures elucidated by spectroscopic means and chemical correlations. Thus, diacetate **6** could also be obtained by acetylation of diol **1** which in turn could be obtained by $LiAlH_4$ reduction of acid **3**. Acetylation of monoacetate **5** also gave **6**.

From the DCM/acetone soluble part of the resin collected nearby the Parque Nacional Conguillio, three main constituents were isolated by column chromatography on silica gel and repeated recrystallization. The products were used to prepare the semisynthetic derivatives. The w/w yields of the main compounds from the resin sample were as follows: imbricatolic acid (**3**) 25%, 15-hydroxyimbricatolal (**2**) 5% and 15-acetoxyimbricatolic acid (**8**) 20%. The structures are presented in Fig. 1.

Melting points were determined on a Koffler hot stage apparatus (Electrothermal 9100) and were uncorrected. Optical rotations were obtained for solutions in $CHCl_3$ (concentrations expressed in g/100 ml) on a Jasco DIP 370 polarimeter. IR spectra were recorded on a Perkin Elmer 1330 and/or a Bruker FT-IR instrument. ^1H NMR spectra were recorded at 400 MHz, ^{13}C NMR at 100.6 MHz in $CDCl_3$ as solvent. TLC spots were visualized by spraying the chromatograms with

H_2SO_4/ethanol (10:90) and heating at 110 °C for 3 min. Column chromatography was performed over Merck Kieselgel 60, particle size 0.063–0.200 mm. Mass spectra (MS) ionization energy was 70 eV. Diethyl ether and THF were distilled from sodium while pyridine and dichloromethane were distilled from calcium hydride under N_2 atmosphere. All reactions were carried out under an inert dry nitrogen atmosphere. Benzene was distilled from calcium chloride and kept over sodium.

Preparation of derivatives

Compound **1** was obtained in 94% yield by $LiAlH_4$ reduction of the methyl ester **4**, obtained in turn by treatment of **3** with diazomethane (90% yield). The 13*R*-form of compound **1** was reported from *Araucaria imbricata* (Bruns, 1968) while the 13*S*-isomer is a constituent of *Juniperus communis* and *J. formosana* (Kuo and Yu, 1996). Compound **4** was prepared by methylation of **3** by Gough and Mills (1970). Compounds **1** and **4** were previously isolated from the resin of *Pinus elliottii* by Spalding *et al.* (1971).

The following compounds were obtained by methylation with diazomethane of the corresponding acids: **9** (93%) from **8** and **10** (5%) from **4**. The ^{1}H NMR data of compound **9** was reported by Weissmann *et al.* (1965).

Dialdehyde **11** was obtained by PCC oxidation of the natural product **2** in 95% yield, whereas **12** was prepared by PCC oxidation of **3** (96%). Compound **13** was obtained by treating **3** with formic acid and acetic anhydride (90% yield). Compound **13** is related to 15-formyloxyimbricatolal described by Garbarino *et al.* (1987) but with the aldehyde function at C-19. The use of Jones reagent transformed **3** into the diacid **15**. Compound **15** (junicedric acid) was reported by Su *et al.* (1996) as a constituent of *Cryptomeria japonica* leaves.

Compound **16** was obtained by acetalization of **12** with $HOCH_2CH_2OH$ and *p*TsOH in benzene (76% yield). Compound **17** was prepared from the natural compound **8** with $SOCl_2$ in dry benzene/morpholine (80% yield). Compound **18** was prepared from **3** by reaction with benzoyl chloride in dry benzene/TEA (75% yield). Product **19**, containing the propyloxy group, was obtained treating **3** with propionyl chloride in dry benzene/TEA (78% yield).

The following compounds were obtained by hydrogenation using Pd/C as catalyst. The yields are given in parentheses: compound **20** from the natural product **3** (95%), **21** from **4** (95%), **22** from **9** (92%) and **23** (98%) from **8**. The stereochemistry of the methyl group at C-8 (H-17) was deduced from NOESY experiments in compound **23**, which showed a clear interaction between H-17 and H-20. Therefore, the C-17 methyl group was established for compounds **20**–**23** as having β-configuration. Compound **24** was obtained by treating **3** with HBr in acetic acid (89% yield). Treatment of **24** with diazomethane afforded the corresponding methyl ester **25**. The derivative **26** was obtained from **24** by Jones oxidation (13% yield) whereas **27** was prepared by treating **24** with $SOCl_2$ in dry benzene/morpholine (85% yield). Compound **28** was obtained in 85% yield by epoxidation of **24** with *m*-chloroperbenzoic acid. Under the same conditions, compound **3** afforded the derivative **29** (85%), compound **1** yielded **30** (80%) and **8** yielded the product **31** (88% w/w yield). The stereochemistry of the epoxy function at C-8,17 of compounds **29**–**31** was deduced from ROESY experiments showing clearly a strong interaction between the methyl group at C-20, H-11 and the epoxidic proton. The structures of compounds **1**–**33** are shown in Fig. 1.

The ^{1}H NMR data of compounds **5**, **6**, **10**, **12**–**14** and **16**–**31** are presented in Table I. The ^{13}C NMR data of the compounds **5**, **6**, **10**, **12**–**14**, **16**–**19** are summarized in Table IIa, and of **20**–**25** as well as of **27**–**31** are presented in Table IIb. All compounds prepared in this work exhibit spectroscopic data in agreement with the proposed structures. The MS- and FT-IR-data can be ordered directly from the authors.

Labd-8(17)-en-15,19-diol (imbricatadiol) (**1**): Colorless crystals, m.p. 92–95 °C. – $[\alpha]_D^{20}$ 65.32° (c = 0.274, $CHCl_3$). – MS (EI): m/z = 308.271 (calcd. for $C_{20}H_{36}O_2$: 308.271).

15-Hydroxylabd-8(17)-en-19-al (15-hydroxyimbricatolal) (**2**): Colorless resin. – $[\alpha]_D^{20}$ – 0.50° (c = 0.56, $CHCl_3$). – MS (EI): m/z = 306.256 (calcd. for $C_{20}H_{34}O_2$: 306.256).

15-Hydroxylabd-8(17)-en-19-oic acid (imbricatolic acid) (**3**): Colorless crystals, m.p. 102–105 °C. – $[\alpha]_D^{20}$ 0.10° (c = 0.16, $CHCl_3$). – MS (EI): m/z = 322.250 (calcd. for $C_{20}H_{34}O_3$: 322.250).

15-Hydroxylabd-8(17)-en-19-oic acid methyl ester (imbricatolic acid methyl ester) (**4**): Colorless resin. – $[\alpha]_D^{20}$ 3.78° (c = 2.38, $CHCl_3$). –MS (EI): m/z = 336.512 (calcd. for $C_{21}H_{36}O_3$: 336.512).

15-Acetoxylabd-8(17)-en-19-ol (**5**): Colorless resin. – $[\alpha]_D^{20}$ 5.27° (c = 0.15, $CHCl_3$). – MS (EI): m/z = 350.539 (calcd. for $C_{22}H_{38}O_3$: 350.539).

15,19-Diacetoxylabd-8(17)-en (**6**): Colorless resin. – $[\alpha]_D^{20}$ 27.78° (*c* = 1.80, $CHCl_3$). –MS (EI): *m/z* = 392.576 (calcd. for $C_{24}H_{40}O_4$: 392.576).

15-Acetoxylabd-8(17)-en-19-al (15-acetoxyimbricatolal) (**7**): Colorless resin. – MS (EI): *m/z* = 348.523 (calcd. for $C_{22}H_{36}O_3$: 348.523).

15-Acetoxylabd-8(17)-en-19-oic acid (15-acetoxyimbricatolic acid) (**8**): Colorless resin. – $[\alpha]_D^{20}$ 2.63° (*c* = 1.9, $CHCl_3$). – MS (EI): *m/z* = 364.522 (calcd. for $C_{22}H_{36}O_4$: 364.522).

15-Acetoxylabd-8(17)-en-19-oic acid methyl ester (**9**): Colorless resin. – $[\alpha]_D^{20}$ 2.86° (*c* = 0.28, $CHCl_3$). – MS (EI) *m/z* = 378.549 (calcd. for $C_{23}H_{38}O_4$: 378.549).

15-Methoxylabd-8(17)-en-19 oic acid methyl ester (**10**): Colorless resin. – MS (EI): *m/z* = 350.539 (calcd. for $C_{22}H_{38}O_3$: 350.539).

Labd-8(17)-en-15,19-dial (**11**): Colorless resin. – $[\alpha]_D^{20}$ – 40.0° (*c* = 0.16, $CHCl_3$). – MS (EI): *m/z* = 304.471 (calcd. for $C_{20}H_{32}O_2$: 304.471).

Labd-8(17)-en-15-al-19-oic acid (**12**): Colorless resin. – $[\alpha]_D^{20}$ 2.8° (*c* = 2.51, $CHCl_3$). – MS (EI): *m/z* = 320.470 (calcd. for $C_{20}H_{32}O_3$: 320.470).

15-Formyloxylabd-8(17)-en-19-oic acid (**13**): Colorless resin. – $[\alpha]_D^{20}$ – 7.5° (*c* = 0.15, $CHCl_3$). – MS (EI): *m/z* = 350.246 (calcd. for $C_{21}H_{34}O_4$: 350.246).

19-Hydroxylabd-8(17)-en-15-oic acid (**14**): Colorless resin. – MS (EI) *m/z* = 322.250 (calcd. for $C_{20}H_{34}O_3$: 322.250).

Labd-8(17)-en-15,19-dioic acid (junicedric acid) (**15**): Colorless resin. – $[\alpha]_D^{20}$ 33.5° (*c* = 0.70, $CHCl_3$). – MS (EI): *m/z* = 336.469 (calcd. for $C_{20}H_{32}O_4$: 336.469).

15-(1,3-Dioxolan)-labd-8(17)-en-19-oic acid (**16**): Colorless resin. – $[\alpha]_D^{20}$ – 4.7° (*c* = 0.34, $CHCl_3$). – MS (EI): *m/z* = 364.522 (calcd. for $C_{22}H_{36}O_4$: 364.522).

15-Acetoxylabd-8(17)-en-19-oic acid morfolinamide (**17**): Colorless resin. – $[\alpha]_D^{20}$ 6.96° (*c* = 0.33, $CHCl_3$). – MS (EI): *m/z* = 433.318 (calcd. for $C_{26}H_{43}NO_4$: 433.318).

15-Benzyloxylabd-8(17)-en-19-oic acid (**18**): Colorless resin. – $[\alpha]_D^{20}$ 83.3° (*c* = 0.18, $CHCl_3$). – MS (EI): *m/z* = 426.278 (calcd. for $C_{27}H_{38}O_4$: 426.278).

15-Propionyloxylabd-8(17)-en-19-oic acid (**19**): Colorless resin. – $[\alpha]_D^{20}$ 2.63° (*c* = 1.9, $CHCl_3$). – MS (EI): *m/z* = 378.277 (calcd. for $C_{23}H_{38}O_4$: 378.277).

15-Hydroxylabd-19-oic acid (**20**): Colorless resin. – $[\alpha]_D^{20}$ 4.0° (*c* = 0.15, $CHCl_3$). – MS (EI): *m/z* = 324.501 (calcd. for $C_{20}H_{36}O_3$: 324.501).

15-Hydroxy-19-labdanoic acid methyl ester (**21**): Colorless resin. – MS (EI): *m/z* = 338.528 (calcd. for $C_{21}H_{38}O_3$: 338.528).

15-Acetoxy-19-labdanoic acid methyl ester (**22**): Colorless resin. – MS (EI): *m/z* = 380.528 (calcd. for $C_{23}H_{40}O_4$: 380.565).

15-Acetoxy-19-labdanoic acid (**23**): Colorless resin. – $[\alpha]_D^{20}$ 26.1° (*c* = 0.18, $CHCl_3$). – MS (EI): *m/z* = 366.538 (calcd. for $C_{22}H_{38}O_4$: 366.538).

15-Acetoxy-8-labdene-19-oic acid (**24**): Colorless resin. – $[\alpha]_D^{20}$ 1.11° (*c* = 0.18, $CHCl_3$). – MS (EI): *m/z* = 364.522 (calcd. for $C_{22}H_{36}O_4$: 364.522).

15-Acetoxy-8-labdene-19-oic acid methyl ester (**25**): Colorless resin. – MS (EI): *m/z* = 378.549 (calcd. for $C_{23}H_{38}O_4$: 378.549).

8-Labdene-15,19-dioic acid (**26**): Colorless resin. – MS (EI): *m/z* = 336.469 (calcd. for $C_{20}H_{32}O_4$: 336.469).

15-Acetoxy-8-labdene-19-oic acid morfolineamide (**27**): Colorless resin. – $[\alpha]_D^{20}$ 28.3° (*c* = 0.12, $CHCl_3$). – MS (EI): *m/z* = 433.628 (calcd. for $C_{26}H_{43}O_4N$: 433.628).

15-Acetoxy-8,9-epoxylabdane-19-oic acid (**28**): Colorless resin. – $[\alpha]_D^{20}$ – 6.11° (*c* = 0.18, $CHCl_3$). – MS (EI): *m/z* = 380.521 (calcd. for $C_{22}H_{36}O_5$: 380.521).

15-Hydroxy-8,17-epoxylabdane-19-oic acid (**29**): Colorless resin. – $[\alpha]_D^{20}$ 12.75° (*c* = 0.78, $CHCl_3$). – MS (EI): *m/z* = 338.458 (calcd. for $C_{20}H_{34}O_4$: 338.485).

15,19-Dihydroxy-8,17-epoxylabdane (**30**): Colorless resin. – MS (EI): *m/z* = 324.501 (calcd. for $C_{20}H_{36}O_3$: 324.501).

15-Acetoxy-8,17-epoxylabdane-19-oic acid (**31**): Colorless resin. – $[\alpha]_D^{20}$ 11.2° (*c* = 2.64, $CHCl_3$). – MS (EI): *m/z* = 380.521 (calcd. for $C_{22}H_{36}O_5$: 380.521).

Lipophilicity

The lipophilicity of the compounds was calculated using the Chem Office 2002 version 8.0 software. The parameter is presented as log *P* (Table IV).

Animals

Fasted Swiss albino mice weighing 30 ± 3 g were used. Fasting (24 h) prior to ulcerogenic assays was used because the reference compound (lansoprazole), the diterpenes and their derivatives were administered orally. The animals were fed on certified Champion diet with free access to water under standard conditions of 12 h dark-light period, 50% relative humidity and 22 °C room temperature.

Ethanol/HCl-induced lesions

The gastroprotective activity of the compounds was assessed in the ethanol/HCl-induced lesion model as described by Schmeda-Hirschmann *et al.* (2002). The protocols were approved by the Universidad de Talca Institutional Animal Care and Use Committee that follows the recommendations of the Canadian Council on Animal Care (Olfert *et al.*, 1993). Mice were randomly alloted into groups of eight animals each and fasted for 24 h with free access to water prior to the experiment. A unique dose of 100 mg/kg was selected because in a previous experiment we determined that the lesion index was not significant different when doses of 100 or 200 mg/kg were used. 50 min after oral administration of the compounds, lansoprazole (2-[[[3-methyl-4-(2,2,2-trifluoroethoxy)-2-pyridyl]methyl]sulfinyl]-1*H*-benzimidazole) (20 mg/kg) or 12% Tween 80 (10 ml/kg), all groups were orally treated with 0.2 ml of a solution containing 60% ethanol/0.3 M HCl (ethanol/HCl) for gastric lesion induction. Animals were sacrificed 1 h after the administration of ethanol/HCl, and the stomachs were excised and inflated by injection of saline (1 ml). The ulcerated stomachs were fixed in 5% formalin for 30 min and opened along the greater curvature. Gastric damage visible to the naked eye was observed in the gastric mucosa as elongated black-red lines, parallel to the long axis of the stomach similar to the ethanol/HCl-induced lesions in rats. The length (mm) of each lesion was measured, and the lesion index was expressed as the sum of the length of all lesions.

MRC-5 cell culture

The cytotoxic effect of the assayed compounds, expressed as cell viability, was assessed on a permanent fibroblast cell line derived from human lung (MRC-5) (ATCC CCL-171). MRC-5 fibroblasts were grown as monolayers in minimum essential Eagle medium (MEM), with Earle's salts, 2 mM L-glutamine and 2.2 g/l sodium bicarbonate, supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 IU/ml penicillin and 100 μg/ml streptomycin in a humidified incubator with 5% CO_2 in air at 37 °C. The cell passage was maintained between 10 and 16. The medium was changed every 2 days.

AGS cell culture

The cytotoxic effect of the assayed compounds, expressed as cell viability, was assessed on a permanent human epithelial gastric cell line (AGS) (ATCC CRL-1739). The AGS cells were grown as monolayers in Ham F-12 medium containing 1 mM L-glutamine and 1.5 g/l sodium bicarbonate, supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 IU/ml penicillin and 100 μg/ml streptomycin in a humidified incubator with 5% CO_2 in air at 37 °C. The cell passage was maintained between 42 and 48. The medium was changed every 2 days.

Cytotoxicity assay

Confluent cultures of MRC-5 as well as AGS cells were treated with medium containing diterpenes as well as with the reference compound lansoprazole at concentrations ranging from 0 up to 1000 μM. The products were first dissolved in DMSO and then in the corresponding culture medium supplemented with 2% FBS. The final content of DMSO in the test medium and controls was 1%. Cells were exposed for 24 h to the test medium with or without the compound (control). Each concentration was tested in quadruplicate together with the control and repeated three times in separate experiments. At the end of the incubation, the neutral red uptake (NR) assay was carried out as described by Rodríguez and Haun (1999). To calculate the IC_{50} values (concentration that produces a 50% inhibitory effect on the evaluated parameter) the results were transformed to percentage of controls and the IC_{50} values were graphically obtained from the dose-response curves.

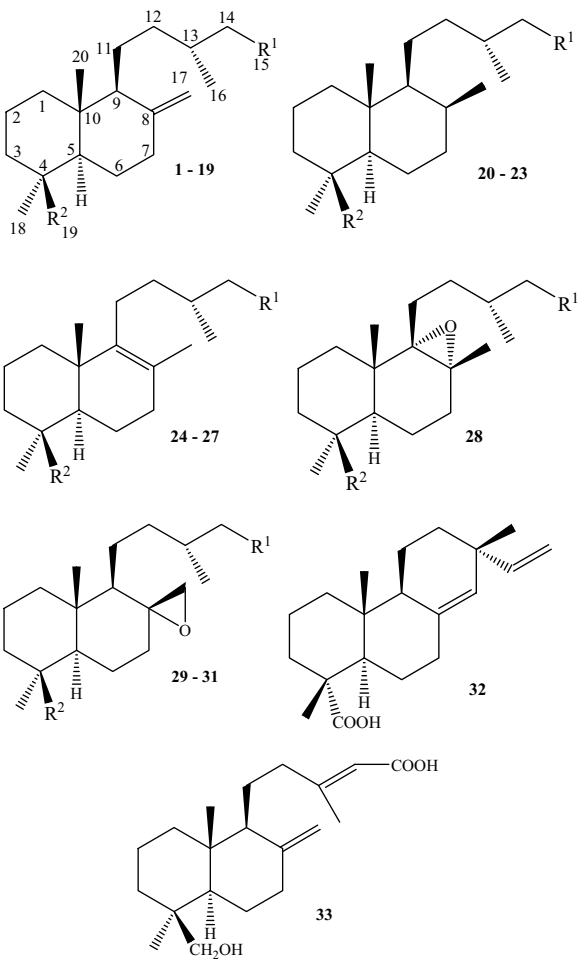
Statistical analysis

Results were expressed as the mean ± s.e.m. In all experiments, statistical differences between several treatments and their respective control were determined by one-way analysis of variance (ANOVA) and when the F value was significant, *post hoc* differences were determined by the Dunnett's multiple comparison test. The level of significance was set at $P < 0.05$. All statistical analyses were performed using the software Statistica 5.1 (StatSoft, Inc.).

Results and Discussion

Ten labdane and a pimarane diterpenes were isolated from a Chilean collection of *A. araucana* resin. In addition to the known diterpenes, 15-hydroxyimbricatolal (**2**), imbricatolic acid (**3**), 15-acetoxyimbricatolal (**7**), 15-acetoxyimbricatolic acid (**8**), labd-8(17)-en-15,19-dial (**11**), 19-hydroxylabd-8(17)-en-15-oic acid (**14**), junicedric acid (**15**), sandaracopimaric acid (**32**) and agatholic acid (**33**), two new labdane diterpenes were obtained (Fig. 1).

The high resolution mass spectrum of **5** calculated $C_{22}H_{38}O_3$ for the molecular ion at *m/z* 350. The ^{1}H NMR spectrum of **5** (Table I) was close to that of **3** differing mainly by the presence of a pair of doublets at δ 3.72 and δ 3.35 (J = 10.9 Hz) indicating a CH_2OH group instead of the COOH function at C-19. Furthermore, the m at δ 3.67 was shifted downfield (δ 4.07) and an additional s at δ 2.01 suggested acetylation of the C-15 hydroxy function. The ^{1}H NMR spectrum of **6** (Table I) was close to that of **5**. The main differences were the downfield shift of the pair of d at δ 3.85 and δ 4.22 (J = 10.9 Hz) and an additional methyl group at 2.01 indicating acetylation of the alcohol function at C-19. In the MS of **6** the molpeak at *m/z* 392 ($C_{24}H_{40}O_4$) clearly indicated an additional acetate and was in full agreement with the proposed structure. The ^{13}C NMR spectra of compounds **5** and **6** (Table II) supported the structures proposed as 15-acetoxylabd-8(17)-en-19-ol (**5**) and 15,19-diacetoxylabd-8(17)-en (**6**). Six diterpenes (compounds **1**, **11**, **14**, **15**, **32** and **33**) previously described from other plant sources are reported for the first time for the *A. araucana* resin. The semisynthetic derivatives **10**, **12**, **13**, **16**–**31** were not found in the Chemical Abstracts neither in the



Compound	R^1	R^2
1	CH_2OH	CH_2OH
2	CH_2OH	CHO
3	CH_2OH	COOH
4	CH_2OH	$COOCH_3$
5	CH_2OAc	CH_2OH
6	CH_2OAc	CH_2OAc
7	CH_2OAc	CHO
8	CH_2OAc	COOH
9	CH_2OAc	$COOCH_3$
10	CH_2OCH_3	$COOCH_3$
11	CHO	CHO
12	CHO	COOH
13	CH_2OCHO	COOH
14	COOH	CH_2OH
15	COOH	COOH
16	CH-OCH_2-CH_2-O-	COOH
17	CH_2OAc	CONMorf
18	CH_2OCOPh	COOH
19	$CH_2OCOC_2H_5$	COOH
20	CH_2OH	COOH
21	CH_2OH	$COOCH_3$
22	CH_2OAc	$COOCH_3$
23	CH_2OAc	COOH
24	CH_2OAc	COOH
25	CH_2OAc	$COOCH_3$
26	COOH	COOH
27	CH_2OAc	CONMorf
28	CH_2OAc	COOH
29	CH_2OH	CH_2OH
30	CH_2OH	COOH
31	CH_2OAc	COOH

Fig. 1. Structure of the labdane diterpenes from the resin of *A. araucana* and their derivatives.

Table I. ^{1}H NMR data of compounds **5**, **6**, **10**, **12**–**14** and **16**–**31** (400 MHz, $CDCl_3$, δ-values).

	H											
Compound	15	16	17	18	19	20	OH	OMe	OAc	1′	2′	3′
5	4.07 m	0.88 d (6.3)	4.77 d (0.9), 4.45 br s	0.95 s	3.72 d (10.9) 3.35 d (10.9)	0.62 s	–	–	2.01 s	–	–	–
6	4.10 m	0.88 d (6.3)	4.80 s, 4.48 br s	0.95 s	4.22 d (10.9) 3.85 d (10.9)	0.67 s	–	–	2.01 s (6 H)	–	–	–
10	3.38 m	0.88 d (6.4)	4.82 s, 4.48 s	1.17 s	–	0.49 s	–	3.32 s, 3.60 s	–	–	–	–
12	9.73 t (2.4)	0.94 d (6.6)	4.81 br s, 4.44 br s	1.21 s	–	0.56 s	10.08 br s	–	–	–	–	–
13	4.13 m	0.86 d (6.4)	4.77 s, 4.42 s	1.18 s	–	0.53 s	–	–	–	8.00 s	–	–
14	-	0.96 d (6.4)	4.79 s, 4.47 s	0.98 s	3.76 d (10.9) 3.37 d (10.9)	0.63 s	5.75 br s	–	–	–	–	–
16	4.80 m	0.93 d (6.4)	4.80 s, 4.48 s	1.20 s	–	0.56 s	–	–	–	3.93 m	3.77 m	–
17	4.10 m	0.92 d (6.4)	4.84 s, 4.50 s	1.32 s	–	0.71 s	–	–	2.06 s	3.5–3.7 m	3.5–3.7 m	–
18	4.44 m	1.00 d (6.3)	4.87 s, 4.54 s	1.27 s	–	0.63 s	–	–	–	7.58 dd (7.4, 7.8), 2 H	7.59 dd br (7.4), 1 H	8.09 dd (7.8, 1.5) 2 H
19	4.02 m	0.83 d (6.3)	4.76 s, 4.41 s	1.16 s	–	0.51 s	11.00 s	–	–	2.24 q (7.5)	1.06 t (7.5)	–
20	3.65 m	0.89 d (6.4)	0.88 d (7.2)	1.19 s	–	0.74 s	5.59 br s	–	–	–	–	–
21	3.59 m	0.86 d (6.4)	0.84 d (7.2)	1.10 s	–	0.58 s	2.42 s	3.56 s	–	–	–	–
22	4.00 m	0.88 d (6.4)	0.86 d (7.6)	1.10 s	–	0.59 s	–	3.56 s	1.96 s	–	–	–
23	4.06 m	0.88 d (6.4)	0.87 d (7.2)	1.20 s	–	0.74 s	9.40 br s	–	2.01 s	–	–	–
24	4.06 m	0.92 d (6.4)	1.51 s	1.20 s	–	0.80 s	–	–	1.99 s	–	–	–
25	3.95 m	0.80 d (6.4)	1.42 s	1.05 s	–	0.62 s	–	3.48 s	1.89 s	–	–	–
26	-	1.01 d (6.6)	1.58 br s	1.27 s	–	0.86 s	–	–	–	–	–	–
27	4.1 m	0.85 d (6.4)	1.55 s	1.25 s	–	0.83 s	–	–	2.00 s	3.6 m	3.6 m	–
28	4.10 m	0.92 d (5.9)	1.32 s	1.21 s	–	0.97 s	–	–	2.06 s	–	–	–
29	3.62 m	0.84 d (6.4)	2.69 d (3.0), 2.50 d (3.0)	1.23 s	–	0.69 s	–	–	–	–	–	–
30	3.59 m	0.84 d (6.4)	2.69 d (4.1), 2.45 d (4.1)	0.97 s	3.71 d (11), 3.39 d (11)	0.75 s	–	–	–	–	–	–
31	4.00 m	0.78 d (6.2)	2.62 d (3.0), 2.44 d (3.0)	1.17 s	–	0.63 s	–	–	1.95 s	–	–	–

Table IIa. ^{13}C NMR data of compounds **5**, **6**, **10**, **12**–**14** and **16**–**19** (100 MHz, $CDCl_3$, δ-values).

C	5	6	10	12	13	14	16	17	18	19
1	39.0 t	38.9 t	39.2 t	39.1 t	39.1 t	41.4 t	40.5 t	40.6 t	39.2 t	39.5 t
2	19.0 t	18.9 t	20.0 t	19.9 t	19.9 t	18.9 t	19.9 t	21.2 t	19.9 t	20.3 t
3	35.4 t	36.0 t	38.8 t	37.9 t	38.0 t	38.6 t	39.7 t	40.4 t	38.8 t	39.2 t
4	38.6 s	38.9 s	44.3 s	44.2 s	44.1 s	38.8 s	44.2 s	46.9 s	44.2 s	44.6 s
5	57.3 d	57.3 d	56.6 d	56.3 d	56.6 d*	57.2 d	56.5 d	61.4 d	56.5 d	56.9 d
6	24.4 t	24.5 t	26.3 t	26.0 t	26.0 t	24.4 t	26.0 t	26.9 t	26.1 t	26.4 t
7	38.8 t	38.6 t	38.3 t	38.7 t	38.7 t	38.9 t	38.7 t	39.8 t	38.0 t	38.3 t
8	148.3 s	148.1 s	148.4 s	148.0 s	148.1 s	148.1 s	148.1 s	148.7 s	148.2 s	148.6 s
9	56.3 d	56.3 d	56.4 d	56.4 d	56.4 d*	56.3 d	56.3 d	58.4 d	56.4 d	56.8 d
10	39.6 s	39.6 s	36.4 s	40.5 s	40.5 s	39.6 s	40.5 s	41.3 s	40.6 s	40.9 s
11	20.9 t	21.0 t	21.1 t	21.2 t	21.0 t	21.1 t	21.0 t	21.4 t	21.1 t	21.4 t
12	35.9 t	36.3 t	36.4 t	36.1 t	36.0 t	35.8 t	36.6 t	36.6 t	36.1 t	36.5 t
13	30.5 d	30.6 d	30.6 d	29.0 d	30.5 d	30.9 d	30.0 d	31.0 d	29.0 d	29.4 d
14	35.1 t	35.2 t	40.3 t	50.7 t	35.1 t	35.4 t	39.1 t	35.6 t	35.3 t	35.6 t
15	63.1 t	63.0 t	71.2 t	203.2 d	62.5 t	179.1 s	103.8 d	63.5 t	63.6 t	63.3 t
16	19.7 q	19.7 q	19.9 t	20.1 q	19.6 q	19.9 q	20.1 q	20.2 q	19.9 q	20.2 q
17	106.4 t	106.7 t	106.3 t	106.4 t	106.3 t	106.6 t	106.4 t	106.6 t	106.4 t	106.8 t
18	27.1 q	27.6 q	28.8 q	28.8 q	28.9 q	27.1 q	29.0 q	28.5 q	30.6 q	30.9 q
19	64.9 t	66.8 t	177.8 s	184.3 s	183.1 s	64.9 t	184.0 s	176.5 s	184.3 s	184.7 s
20	15.2 q	15.2 q	12.5 q	12.7 q	12.7 q	15.3 q	12.7 q	15.7 q	12.8 q	13.2 q
OAc	171.3 s	171.3 s, 171.1 s	–	–	–	–	–	171.0 s	–	–
	21.0 q	21.0 q, 21.0 q	–	–	–	–	–	21.5 q	–	–
OMe	–	–	51.1 q	–	–	–	–	–	–	–
	–	–	58.6 q	–	–	–	–	–	–	–
R					Formyl				Benzyl	Propyl
C-1′	–	–	–	–	161.1 d	–	64.7 t	47.1 t	132.8 d	28.0 t
C-2′	–	–	–	–	–	–	64.6 t	67.4 t	130.5 s	9.6 q
	–	–	–	–	–	–	–	–	129.5 d 2C	–
	–	–	–	–	–	–	–	–	128.3 d 2C	–
C = O	–	–	–	–	–	–	–	–	166.7 s	175.0 s

* Could be interchangeable.

Dictionary of Natural Products on CD-ROM (2004). The spectroscopic data are presented in this report.

Compound **2** (15-hydroxyimbricatolal) was previously reported from *Araucaria imbricata* (Bruns, 1968). Imbricatolic acid (**3**) was previously isolated from the resin of *A. araucana* by Garbarino *et al.* (1987). 15-Acetoxyimbricatolal (**7**) was one of the main compounds from a sample of *A. araucana* resin (Garbarino *et al.*, 1987) and was also described by Weissmann *et al.* (1965) and Bruns (1968).

Compound **8** was reported by Su *et al.* (1994) together with **3** and **4** from the Japanese cedar, *Cryptomeria japonica*.

Sandaracopimaric acid (**32**) was previously reported by Sakar and San Feliciano (1994) as a constituent of *Juniperus foetidissima* berries. The compound was also described as a constituent of *Cryptomeria japonica* (Su *et al.*, 1996). Agatholic acid (**33**) has been described as a constituent of *Araucaria angustifolia* (Campello and Fonseca, 1975).

In the last years, some reports on the gastroprotective activity of diterpenes belonging to different structural skeletons have been published. Most of the work has been focused on the clerodane diterpenes from the Brazilian Euphorbiaceae *Croton cajucara*. *Trans*-dehydrocrotonin (DHC) showed the best gastroprotective activity at 100 mg/kg in different models of induced gastric lesions in rats, reducing the occurrence of lesions by nearly 50% (Souza-Brito *et al.*, 1998). Under the same conditions, *trans*-crotonin prevented the gastric lesions by 51% (Hiruma-Lima *et al.*, 2002). Furthermore, pretreatment with DHC at 100 mg/kg decreased the ulcerative index by 50% in the ethanol/HCl-induced gastric lesions in mice (Rodríguez *et al.*, 2004). DHC derivatives exhibited better activity than DHC at 100 mg/kg reducing the gastric lesions up to 89%. Some structure-activity relationships of *C. cajucara* diterpenes have been pub-

Table IIb. ^{13}C NMR data of compounds **20**–**25** and **27**–**31** (100 MHz, $CDCl_3$, δ-values).

C	**20**	**21**	**22**	**23**	**24**	**25**	**27**	**28**	**29**	**30**	**31**
1	39.2 t	39.4 t	39.7 t	39.7 t	37.3 t	37.3 t	37.6 t	37.3 t	39.4 t	39.3 t	39.3 t
2	18.8 t	18.8 t	18.2 t	18.8 t	19.4 t	19.4 t	20.5 t	18.5 t	19.3 t	18.2 t	19.2 t
3	37.9 t	38.1 t	38.2 t	37.9 t	37.4 t	37.6 t	40.3 t	35.9 t	37.8 t	35.3 t	37.7 t
4	43.8 s	43.9 s	43.8 s	43.8 s	43.7 s	43.7 s	46.1 s	43.9 s	44.0 s	40.2 s	43.9 s
5	53.2 d	53.1 d	53.0 d	53.1 d	53.5 d	53.4 d	57.4 d	54.8 d	55.7 d	55.7 d	55.7 d
6	22.9 t	22.9 t	22.8 t	22.8 t	20.7 t	20.7 t	22.1 t	19.9 t	23.0 t	21.8 t	23.3 t
7	35.0 t	34.9 t	34.9 t	35.0 t	35.3 t	35.2 t	35.1 t	29.9 t	39.1 t	38.9 t	39.1 t
8	30.0 d	30.2 d	30.2 d	30.3 d	126.3 s	126.1 s	125.9 s	64.6 s	59.0 s	58.9 s	58.8 s
9	57.5 d	57.5 d	57.5 d	57.5 d	139.4 s	139.3 s	140.4 s	71.6 s	53.9 d	54.6 d	53.8 d
10	38.9 s	38.6 s	38.6 s	38.9 s	39.7 s	39.4 s	40.2 s	39.1 s	41.0 s	38.6 s	40.9 s
11	19.0 t	19.0 t	19.0 t	19.0 t	25.6 t	25.5 t	25.7 t	29.8 t	19.5 t	19.3 t	19.3 t
12	35.9 t	35.8 t	35.5 t	35.5 t	34.2 t	34.2 t	35.4 t	35.3 t	36.8 t	36.7 t	36.7 t
13	29.2 d	29.2 d	29.2 d	29.0 d	30.9 d	30.8 d	31.0 d	31.3 d	30.0 d	30.0 d	30.2 d
14	39.8 t	39.7 t	35.1 t	35.2 t	37.1 t	37.1 t	38.0 t	35.3 t	39.6 t	39.7 t	35.3 t
15	61.1 t	61.1 t	62.9 t	63.1 t	63.0 t	62.7 t	63.0 t	62.9 t	60.9 t	60.8 t	63.0 t
16	20.0 q	20.0 q	19.8 q	19.9 q	19.3 q	19.2 q	19.7 q	19.3 q	19.6 q	19.6 q	19.5 q
17	14.9 q	14.9 q	19.0 q	14.9 q	19.6 q	19.5 q	19.8 q	21.1 q	50.4 t	50.6 t	50.2 t
18	29.0 q	28.8 q	28.8 q	29.02 q	28.6 q	28.2 q	27.6 q	28.5 q	29.0 q	27.0 q	28.9 q
19	183.5 s	178.1 s	177.9 s	184.4 s	184.5 s	177.7 s	176.8 s	183.7 s	183.2 s	64.9 t	183.3 s
20	14.4 q	14.2 q	14.2 q	14.3 q	17.8 q	17.6 q	19.3 q	14.5 q	12.9 q	15.2 q	12.9 q
OAc	–	–	171.0 s	171.3 s	171.3 s	170.7 s	171.1 s	171.3 s	–	–	171.3 s
	–	–	20.9 q	21.0 q	21.0 q	19.5 q	21.0 q	21.0 q	–	–	20.9 q
OMe	–	51.1 q	51.0 q	–	–	50.8 q	–	–	–	–	–
C-1′	–	–	–	–	–	–	46.7 t	–	–	–	–
C-2′	–	–	–	–	–	–	67.0 t	–	–	–	–

lished by Melo *et al.* (2003). Crotonin, another diterpene from *C. cajucara* and its derivatives inhibited induced gastric lesions in animals by 80% when administered at 100 mg/kg (Albino de Almeida *et al.*, 2003).

Other diterpenes with an anti-ulcerogenic effect include clerodane cordatin from *Aparisthmium cordatum* (Hiruma-Lima *et al.*, 2000), geranylgeranylacetone with an cytoprotective effect against indomethacine-induced lesions (Tomisato *et al.*, 2000), trichorabdal A from *Rabdosia trichocarpa* (Kadota *et al.*, 1997), the monosodium salt of 12-sulfodehydroabietic acid (Onoda *et al.*, 1990), aparisthman (Hiruma-Lima *et al.*, 2001), centipedic acid and 12-acetoxy-hawtriwaic acid lactone (Guedes *et al.*, 2002) and solidagenone (Schmeda-Hirschmann *et al.*, 2002).

Some 24 products isolated/prepared in amounts over 10 mg were evaluated for gastroprotective effects in the ethanol/HCl induced ulcer in mice at 100 mg/kg (Table III). When considering compounds **1**–**5**, **7**–**9** and **11**–**12**, bearing an exomethylene at 8,17, the highest gastroprotective effect was observed for compounds **2** and **9**. If there is a free OH function at C-15 the best effect was found for the aldehyde **2**. If the OH at C-15 is acetylated, the highest activity was found for compound **9** with a methyl ester at C-19. However, compounds **7**–**9** presented similar gastroprotective effects. When there is an aldehyde function at C-15, the best effect was observed for the dialdehyde **11**. In the compounds **20** and **23**, no differences were detected if the OH group at C-15 is free or acetylated.

Epoxidation at 8,17 (compounds **30**, **31**) decreased the gastroprotective effect. No differences were observed if there is a free OH function at C-15 or if the OH is acetylated. When epoxidation takes place at 8,9 (compound **28**) the gastroprotective effect was much higher than when the epoxy group is placed at 8,17 suggesting a relation with the polarity of the compounds. Epoxidation of **24** (8,9-en) to the derivative **28** afforded a more active product.

At 100 mg/kg, the naturally occurring labdanes **2**, **5** and **7** were active as gastroprotectors with a similar effect as lansoprazole at 20 mg/kg reducing the lesions by 78, 69 and 73%, respectively. The compounds **9**, **20**, **23** and **28** were the best semisynthetic products protecting the gastric mucosa by 79, 66, 73 and 69%, respectively. Further studies have to be undertaken to confirm the observed

Table III. Gastroprotective effect of the diterpenes **1**–**9**, **11**–**13**, **16**–**20**, **23**–**25**, **27**, **28**, **30** and **31** (100 mg/kg) and lansoprazole (20 mg/kg) on ethanol/HCl-induced gastric lesions in mice.

Compound	Lesion index [mm]
1	14.4 ± 2.5*
2	9.2 ± 1.7*
3	16.6 ± 1.2*
4	13.5 ± 3.4*
5	13.1 ± 2.5*
6	23.1 ± 3.5*
7	11.2 ± 3.4*
8	14.5 ± 3.8*
9	8.9 ± 3.6*
11	14.0 ± 3.7*
12	23.4 ± 1.2*
13	15.1 ± 2.9*
16	19.7 ± 3.6*
17	18.0 ± 1.9*
18	20.4 ± 3.5*
19	16.3 ± 4.8*
20	14.4 ± 2.4*
23	11.3 ± 2.9*
24	18.5 ± 2.2*
25	16.2 ± 3.2*
27	23.7 ± 2.1*
28	13.2 ± 2.7*
30	19.2 ± 3.9*
31	20.8 ± 2.9*
Lansoprazole	12.6 ± 2.5*
Control	42.0 ± 3.7

Results are expressed as means ± s.e.m., $n = 8$.
* Significant difference from corresponding control (ANOVA followed by Dunnett's test). $P < 0.01$ compared to the controls.

Table IV. Lipophilicity of diterpenes **1**–**13**, **16**–**25**, **27**–**33** and cytotoxicity towards human gastric epithelial cells (AGS) and fibroblasts after treatment with the compounds for 24 h. The endpoint was assessed by means of the neutral red uptake assay.

Compound	Lipophilicity (log *P*)	IC_{50} [μM]	
		AGS	Fibroblasts
1	4.75	41	69
2	4.61	176	296
3	4.88	134	280
4	5.14	30	39
5	4.97	214	116
6	5.20	52	72
7	4.84	–	144
8	5.11	101	186
9	5.37	45	43
10	5.50	–	132
11	4.14	63	86
12	4.41	138	173
13	4.99	184	131
16	5.11	214	299
17	4.53	46	48
18	7.00	58	60
19	5.76	183	202
20	5.30	77	109
21	5.56	27	29
22	5.79	46	36
23	5.53	82	91
24	4.90	197	204
25	5.17	41	87
27	4.32	66	137
28	3.96	> 1000	889
29	3.81	288	305
30	3.68	> 1000	> 1000
31	4.04	280	405
32	5.31	–	51
33	4.36	–	141
Lansoprazole	–	162	306

Each IC_{50} value represents the mean of three different experiments in quadruplicate. The s.e.m. of IC_{50} values never exeeds 5% from the mean.
–: not determined.

trend including derivatives with oxygen functions in rings A and B.

Considering the cytotoxicity results for compounds **1**–**13**, **16**–**19** it can be noticed that the highest cytotoxicity was observed for compounds **1**, **4**, **6**, **9** and **17** (Table IV). In the compound series **1**–**4** with a free OH group at C-15, the highest cytotoxicity was found for compounds **1** and **4**, with an OH or $COOCH_3$ function at C-19. When the OH at C-15 is acetylated, the trend is similar with the highest effect for the less polar derivatives. Since the cytotoxicity of derivatives **4** and **21**, **9** and **22**, **9** and **25** is similar, this suggests that the double bond at 8,17 or 8,9 is not a requisite for this effect. Epoxidation at 8,9 or 8,17 reduces the cytotoxicity as can be seen when comparing the compounds pairs **1** and **29**, **3** and **30**, and **8** and **31**.

While the gastroprotective effect of other diterpenes such as DHC and crotonin at 100 mg/kg ranges between 50–80% (Souza-Brito *et al.*, 1998; Albino de Almeida *et al.*, 2003), their cytotoxicity was 240–360 and 200–500 μM, respectively (Rodríguez and Haun 1999; Albino de Almeida *et al.*, 2003). In the *Araucaria* diterpenes, the compounds **28** and **30** presented a strong gastroprotective effect (68 and 54%, respectively) with cytotoxicities higher than 1000 μM. Considering that simple structural modifications led to lower cytotoxicity maintaining the gastroprotective effect, selective modifications can afford labdane diterpenes with a better potential as anti-ulcer drugs.

The lipophilicity of the compounds assessed for gastroprotective effect ranged between 3.68 (compound **30**) to 7.0 (compound **18**). The best gastroprotective effect was found for the compounds **2**, **7**, **9** and **23** with lipophilicity values of 4.61, 4.84, 5.37 and 5.53, respectively. The comparison of the cytotoxicity with lipophilicity data indicates that the most cytotoxic products towards the AGS cells in the **1**–**19** series were the compounds **1**, **4**, **6**, **9** and **17** with IC_{50} values in the range 30–46 μM and lipophilicities of 4.53–5.37. The lipophilicity of the evaluated compounds is presented in Table IV. Rosenkranz *et al.* (1992) have reported that chemicals with higher lipophilicities show increased cellular toxicities. With a few exceptions our results exhibit a good correlation between both variables.

Although the mode of action of diterpenes has not been well established, this compounds seem to protect the gastric mucosa mainly through mechanisms that enhance the defensive factors of the stomach. The gastroprotective activity of diterpenes and their derivatives, observed in different models of induced gastric lesions in animals, has been explained by mechanisms that include stimulation of prostaglandin synthesis, increase of mucus production and suppression of gastric acid secretion (Hiruma-Lima *et al.*, 2002). Studying the gastroprotective mechanism of some derivatives from the anti-ulcerogenic diterpene solidagenone, using a gastric epithelial cell culture, we have observed that some of them stimulate the intracellular GSH content while others protect against damage induced by sodium taurocholate or increase the prostaglandin cell content (Rodríguez *et al.*, 2005). Currently, we are performing different experiments in order to determine the mode(s) of action of the gastroprotective activity displayed by the diterpenes and their derivatives obtained from *A. araucana.*

Acknowledgements

Financial support by FONDECYT, Grant Nº 1030583 and the Programa de Productos Bioactivos (Universidad de Talca) is gratefully acknowledged. Beatriz Sepúlveda thanks the Universidad de Talca for a Doctoral grant. We are grateful to the Agencia Española de Cooperación Internacional (AECI).

Albino de Almeida A. B., Melo P. S., Hiruma-Lima C. A., Gracioso J. S., Carli L., Nunes D. S., Haun M., and Souza-Brito A. R. (2003), Antiulcerogenic effect and cytotoxic activity of semi-synthetic crotonin obtained from *Croton cajucara* Benth. Eur. J. Pharmacol. **472**, 205–212.

Bruns K. (1968), Diterpene. V. Über die C13-Konfiguration der Diterpene aus *Araucaria imbricata* Pavon (Araucariaceae). Tetrahedron **24**, 3417–3423.

Brunton L. (1996), Agents for control of gastric acidity and treatment of peptic ulcers. In: The Pharmacological Basis of Therapeutics, 9th ed. (Hardman J., Limbird L., Molinoff P., Ruddon R., and Goodman A., eds.). Mc Graw Hill, New York, pp. 901–916.

Campello J. and Fonseca S. F. (1975), Diterpenes from *Araucaria angustifolia*. Phytochemistry **14**, 2299–2300.

Dictionary of Natural Products on CD-ROM (2004), version 12:2. Chapman and Hall/CRC, Boca Raton, FL, U.S.A.

Dimas K., Demetzos C., Marsellos M., Sotidiadou R., Malamas M., and Kokkinopoulos D. (1998), Cytotoxic activity of labdane type diterpenes against human leukemic cell lines *in vitro.* Planta Med. **64**, 208–211.

Garbarino J., Oyarzún M., and Gambaro V. (1987), Labdane diterpenes from *Araucaria araucana.* J. Nat. Prod. **50**, 935–936.

Gough L. J. and Mills J. S. (1970), The occurrence of imbricatolic acid in *Cupressus* resins. Phytochemistry **9**, 1093–1096.

Guedes M. M., Cunha A. N., Silveira E. R., and Rao V. S. (2002), Antinociceptive and gastroprotective effects of diterpenes from the flower buds of *Egletes viscosa*. Planta Med. **68**, 1044–1046.

Hegnauer R. (1986), Chemotaxonomie der Pflanzen, Band 7. Birkhäuser Verlag, Basel, pp. 462–554.

Hiruma-Lima C. A., Gracioso J. D., Toma W., de Paula A. C., de Almeida A. B., Brasil D. D., Muller A. H., and Souza-Brito A. R. (2000), Evaluation of the gastroprotective activity of cordatin, a diterpene isolated from *Aparisthmium cordatum* (Euphorbiaceae). Biol. Pharm. Bull. **23**, 1465–1469.

Hiruma-Lima C. A., Gracioso J. S., Toma W., Almeida A. B., Paula A. C., Brasil D. S., Muller A. H., and Souza-Brito A. R. (2001), Gastroprotective effect of aparisthman, a diterpene isolated from *Aparisthmium cordatum*, on experimental gastric ulcer models in rats and mice. Phytomedicine **8**, 94–100.

Hiruma-Lima C. A., Toma W., de Souza Gracioso J., de Almeida A. B. A., Batista L. M., Magri L., de Paula A. C. B., Soares F. R., Nunes D. S., and Souza-Brito A. R. M. (2002), Natural *trans*-crotonin: the antiulcerogenic effect of another diterpene isolated from the bark of *Croton cajucara* Benth. Biol. Pharm. Bull. **25**, 452–456.

Kadota S., Basnet P., Ishii E., Tamura T., and Namba T. (1997), Antibacterial activity of trichorabdal A from *Rabdosia trichocarpa* against *Helicobacter pylori.* Zentralbl. Bakteriol. **286**, 63–67.

Kuo Y. H. and Yu M. T. (1996), Three labdane-type diterpenes from the bark of *Juniperus formosana* Hay. var. *concolor* Hay. Chem. Pharm. Bull. **44**, 1242–1244.

Lewis D. A. and Hanson D. (1991), Anti-ulcer drugs of plant origin. In: Progress in Medicinal Chemistry (Ellis G. P. and West G. B., eds.). Elsevier Science Publishers B. V., Amsterdam, Vol. 28, pp. 201–231.

Melo P. S., Duran N., Hiruma-Lima C. A., Souza-Brito A. R., and Haun M. (2003), Comparison of the gastroprotective effect of a diterpene lactone isolated from *Croton cajucara* with its synthetic derivatives. J. Ethnopharmacol. **87**, 169–174.

Olfert E. D., Cross B. M., and McWilliam A. A. (1993), Guide to the care and use of experimental animals. Canadian Council on Animal Care, Vol. 1 Ottawa, Ontario, p. 213.

Onoda Y., Magaribuchi T., and Tamaki H. (1990), Effects of the new anti-ulcer agent 12-sulfodehydroabietic acid monosodium salt on duodenal alkaline secretion in rats. Arzneim. Forsch. **40**, 576–578.

Rodríguez J. A. and Haun M. (1999), Cytotoxicity of *trans*-dehydrocrotonin from *Croton cajucara* (Euphorbiaceae) on V79 cells and rat hepatocytes. Planta Med. **65**, 522–526.

Rodríguez J. A., Hiruma-Lima C., and Souza-Brito A. R. M. (2004), Antiulcer activity and subacute toxicity of *trans*-dehydrocrotonin from *Croton cajucara*. Hum. Exp. Toxicol. **23**, 455–461.

Rodríguez J. A., Theoduloz C., Sánchez M., Yáñez T., Razmilic I., and Schmeda-Hirschmann G. (2005), Gastroprotective activity of a new semisynthetic solidagenone derivative. J. Pharm. Pharmacol. **57**, 265–271.

Rosenkranz H. S., Matthews E. J., and Klopman G. (1992), Relationships between cellular toxicity, the maximum tolerated dose, lipophilicity and electrophilicity. ATLA-Altern. Lab. Anim. **20**, 549–562.

Sakar M. K. and San Feliciano A. (1994), Diterpenoids of *Juniperus foetidissima* unripe berries. Fitoterapia **LXV**, 304–306.

Schmeda-Hirschmann G., Rodríguez J. A., and Astudillo L. (2002), Gastroprotective activity of the diterpene solidagenone and its derivatives on experimentally induced gastric lesions in mice. J. Ethnopharmacol. **81**, 111–115.

Singh M., Mahesh P., and Sharma R. P. (1999), Biological activity of labdane diterpenes. Planta Med. **65**, 2–8.

Sommit D., Petsom A., Ishikawa T., and Roengsumran S. (2003), Cytotoxic activity of natural labdanes and their semi-synthetic modified derivatives from *Croton oblongifolius.* Planta Med. **69**, 167–170.

Souza-Brito A. R., Rodríguez J. A., Hiruma-Lima C., Haun M., and Nunes D. (1998), Antiulcerogenic activity of *trans*-dehydrocrotonin from *Croton cajucara*. Planta Med. **64**, 126–129.

Spalding B. P., Zinkel D. F., and Roberts D. R. (1971), Gymnospermae. Pinaceae. New labdane resin acids from *Pinus elliottii.* Phytochemistry **10**, 3289–3292.

Su W. C., Fang J. M., and Cheng Y. S. (1994), Labdanes from *Cryptomeria japonica*. Phytochemistry **37**, 1109–1114.

Su W. C., Fang J. M., and Cheng Y. S. (1996), Diterpenoids from leaves of *Cryptomeria japonica.* Phytochemistry **41**, 255–261.

Tomisato W., Takahashi N., Komoto C., Rokutan K., Tsuchiya T., and Mizushima T. (2000), Geranylgeranylacetone protects cultured guinea pig gastric mucosal cells from indomethacin. Dig. Dis. Sci. **45**, 1674–1679.

Weissmann G., Bruns K., and Grützmacher H. F. (1965), Terpene aus dem Harz von *Araucaria imbricata* Pavon (*A. araucana*). Tetrahedron Lett. **51**, 4623–4626.

WHO (2003), The World Health Report 2003, pp. 154–159.